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Attune™ Cytometric Software

USER GUIDE

For data acquisition and analysis using the Attune™ NxT and Attune™ CytPix™ Flow Cytometers

Publication Number MAN0026553

Revision A.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Manufacturer: Life Technologies Holdings Pte Ltd | Block 33 | Marsiling Industrial Estate Road 3 | #07-06, Singapore 739256

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L.0	21 June 2021	Change the name of the SW to Attune Cytometric Software and update the title of the user guide. Add information about Attune™ CytPix™ Flow Cytometer. Update the UI screens to reflect the new instrument and software functions.
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F.0	29 December 2016	Update Experiment tab, Statistics tab, and Collection panel functions, update various context menus; add Overlay tab, Overlay View, Attune™ Database Utility sections.
E.0	20 May 2016	Add new instrument functions and filter configurations.
D.0	13 November 2015	Basis for this revision

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About this guide

This user guide describes how to use the Attune™ Cytometric Software to acquire and analyze data using the Attune™ NxT Flow Cytometer or the Attune™ CytPix™ Flow Cytometer and assumes that you have a working knowledge of basic Microsoft™ Windows™ operation.

IMPORTANT! For instructions on using the Attune™ NxT Flow Cytometer, see the *Attune™ NxT Flow Cytometer User Guide* (Pub. No. MAN0026547) and the *Attune™ NxT Flow Cytometer Quick Reference Guide* (Pub. No. 100024233).

For instructions on using the Attune™ CytPix™ Flow Cytometer, see the *Attune™ CytPix™ Flow Cytometer User Guide* (Pub. No. MAN0019440).

Conventions

Text and keyboard conventions

The following table lists the text and keyboard conventions used in the *Attune™ Cytometric Software User Guide.* For safety alert words and symbols used in this document, see page 12.

Convention	Use
Italics	*Italic* text highlights new or important terms on their first appearance in the user guide. It is also used for emphasis and for user guide or reference titles. For example: *Experiment Explorer* lists *Experiments* in a hierarchal view and functions as an interface for creating new Experiments and recording data.
Bold	**Bold** text indicates user action. For example: Click **Run**.
▶	Right arrow symbol (▶) indicates a menu choice and separates successive commands you select from a drop-down or shortcut menu. For example: Select **Show Events**▶**All Events**.
Ctrl+X	When used with key names, a plus sign means to press two keys simultaneously. For example: Click **Ctrl+P**.

Clicking

Unless explicitly stated, clicks are left mouse button clicks. If you have transposed the mouse buttons, the primary click is the left click, even though it can be physically swapped.

User attention symbols

The following attention symbols are used in the *Attune™ Cytometric Software User Guide*. For safety alert words and symbols used in this document, see page 12.

Symbol	Use
	Note: Describes important features or instructions, and highlights tips that can save time and prevent difficulties.
	IMPORTANT! Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

Other Attune™ user guides

Guide	Pub. No.
Attune™ Cytometric Software User Guide	MAN0026553
Attune™ NxT Flow Cytometer Quick Reference Guide	100024233
Attune™ NxT Flow Cytometer User Guide	MAN0026547
Attune™ NxT Flow Cytometer Maintenance and Troubleshooting Guide	100024234
Attune™ NxT Flow Cytometer Site Preparation Guide	100024428
Attune™ NxT External Fluid Supply User Guide	100038577
Attune™ NxT External Fluid Supply Quick Reference Guide	100037944
Attune™ NxT Auto Sampler User Guide	100032905
Attune™ CytPix™ Flow Cytometer User Guide	MAN0019440
Attune™ CytPix™ Flow Cytometer Site Preparation Guide	MAN0019443
CytKick™ and CytKick™ Max Autosampler User Guide	MAN0018351

Additional resources are available on the Flow Cytometry Technical Resources page at **thermofisher.com/flowresources**, where you can find protocols, application notes, and tutorials.

Safety information

Note: See **"Appendix E: Safety"** for the complete the chemical or instrument safety information.

Safety alert words

Four safety alert words appear in this user guide at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a specific level of observation or action, as defined below:

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, could result in minor or moderate injury. It is also used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for **IMPORTANT!** safety alerts, each safety alert word in this document appears with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instruments (see **"Symbols on instruments"**).

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Thermo Fisher Scientific are available to you free 24 hours a day. For instructions on obtaining SDSs, see **"Obtaining SDSs"**.

IMPORTANT! For the SDSs of chemicals not distributed by Thermo Fisher Scientific contact the chemical manufacturer.

1. Attune™ Cytometric Software

Attune™ Cytometric Software overview

The functions of the Attune™ NxT Flow Cytometer and the Attune™ CytPix™ Flow Cytometer are controlled by the Attune™ Cytometric Software. The software is preinstalled to the computer workstation supplied with the instrument, and the Attune™ Software icon (i.e., shortcut) is placed on the computer desktop and under **Start ▶ ThermoFisher ▶ Attune™ Software.**

Note: To reinstall the Attune™ Cytometric Software for recovery, you must have *Administrator* access (see page 24). For instructions to reinstall the Attune™ Cytometric Software, see page 15.

About the software

The Attune™ Cytometric Software is a flexible data acquisition and analysis tool that uses a browser view to:

- Design and perform experiments
- Define independent instrument settings and optimize data collection
- Carry out instrument performance checks and track instrument performance
- Acquire and record data
- Manage and process recorded data

System requirements

A PC workstation running the Attune™ Cytometric Software on a Windows® 10 64-bit platform is provided with the instrument.

Note: We recommend that you set the text to small (100%) in the Windows display options (96 dpi) for the best display of the software application.

Supported files

- The Attune™ Cytometric Software records and outputs data in FCS (Flow Cytometry Standard) 3.1.
- The Attune™ Cytometric Software can export data in FCS 3.0 and 3.1 formats.
- FCS formats 3.0 and 3.1 are supported for data analysis.

Software layout and Ribbon bar menu system

Software layout

The Attune™ Cytometric Software has three main screens, *Login*, *Main Menu*, and *Attune™ Desktop*.

- *Login* – Used for signing into the software. The Login screen is the first screen presented after the main application startup (page 19).
- *Main Menu* – Used for running and reviewing performance tracking tests, creating new experiments, and accessing the current and stored experiments. Main Menu is the first screen presented after a successful login. For more information, see page 26.
- *Attune™ Desktop* – Used for controlling the Attune™ NxT and Attune™ CytPix™ Flow Cytometers to run samples, generate data, and analyze results. Attune™ Desktop is the main application window. For more information, see page 29.

Ribbon bar menu system

The Attune™ Cytometric Software uses the Microsoft™ Ribbon interface. The *Ribbon bar* consolidates related functionality by organizing control elements in logical groups under contextual tabs. Each *Ribbon tab* relates to a type of activity.

The availability of the Ribbon tabs in the Ribbon bar is dependent on the context of the software, i.e., they are contextual tabs that are displayed only when they are needed by the user. Objects that are not available as a function in a specific context are shaded gray in the tab.

For more information, see page 33.

Note: Only a limited set of Ribbon tabs are available on the Main Menu (page 26) when running Performance Tests (page 353).
On the Login screen (page 19), only the File tab (page 49) is available.

Context menus

The Attune™ Cytometric Software interface also allows the use of *context menus* (also called contextual, shortcut, and pop-up menu), which appear upon user interaction with a right-click mouse operation. A context menu offers a limited set of choices that are available in the current state, or context, of the software and the available choices are actions related to the selected object.

Keyboard shortcuts

Several keyboard shortcuts are available for use in the Attune™ Cytometric Software.

Keyboard shortcut	Function
Ctrl + N	New Experiment
Ctrl + C	Copy Workspace elements (plots, gates, stats boxes)
Ctrl + V	Paste Workspace elements (plots, gates, stats boxes)
Ctrl + X	Delete Workspace elements (plots, gates, stats boxes)
Ctrl + A	Select all (objects on Workspace)
Ctrl + P	On demand printing
Ctrl + S	Save as template
Ctrl + space bar	Select entire column on Heat map
Shift + space bar	Select entire row on Heat map
Ctrl + mouse wheel	Zoom in/out of Workspace or Overlay view (or galleries)

Attune™ Cytometric Software Installation

The Attune™ Cytometric Software is preinstalled to the computer workstation supplied with the Attune™ NxT and Attune™ CytPix™ Flow Cytometers.

The Attune™ Cytometric Software shortcut is placed on the computer desktop and under **Start ▶ ThermoFisher ▶ Attune™ Software.**

However, if you need to reinstall the software for recovery purposes or install a newer version of the software, follow the instructions below.

Note: You have the option to install the Attune™ Cytometric Software with or without the Security, Auditing, and e-Signature functions (i.e., the "SAE module"), which allow you to configure the software to meet specific requirements for security, audit, and e-Signature (21 CFR Part 11) (see "Appendix C: SAE Administrator Console", page 576).

A license control mechanism in the form of a DESkey device is required for the operation of the Attune™ Cytometric Software after installation. The type of the DESkey device required depends on the installation option selected (i.e., with or without SAE functions) and the number of licenses purchased (single or multiple users).

Guidelines for installation

- **IMPORTANT!** Ensure that all data is backed up to an external storage device before installation.
- The instrument must be powered on and connected to the computer for the firmware updater to run at the end of the installer.
- **DO NOT** update the firmware if the instrument is in a sleep state. The indicator lights on the front of the instrument will fade in/fade out in multiple colors during the sleep state. Power cycle (turn on and off) the instrument before running the firmware updater.
- **DO NOT** launch the software application until all installation steps have been completed.
- **DO NOT** run any other applications while completing these steps.
- No change to existing login credentials will occur during the software upgrade.
- If installing the Attune™ Cytometric Software with the Security, Auditing, and e-Signature option, ensure that the computer on which the SAE server is installed is configured with a static IP address. Consult your network administrator for help with checking the IP address configuration.

Install the Attune™ Cytometric Software

1. Insert the DESkey USB device appropriate for your installation type (i.e., with or without SAE functions) into the computer.
2. Restart or power on the computer and the cytometer.
3. Log into Windows as:

   User: INSTR-ADMIN
   Password: INSTR-ADMIN

   Note: This is the default administrator account. If your instrument is on a network, ensure that the administrator privileges have not been removed by your local IT department.

4. Unzip (select **extract all files**) the **AttuneNxT_XX.zip** file to the desktop.

   The XX in the zip file denotes the Attune™ Cytometric Software version and must be version 5.0 or newer for installation with the Security, Auditing, and e-Signature functions.
5. To install the software, double-click **SetupAttune.exe** in the **Attune NxT XX** folder.
6. In the installer, select **Attune™ Software (**or **Attune™ Software with Security, Audit, E-Signatures**, if SAE is required), then click **Install**.

7. When the **Installation completed successfully** message is displayed, click **Exit** to close the installer.

Note: When signing into the Attune™ Cytometric Software for the first time after installation, you must sign in as an Administrator. The default username and password for the Administrator are both admin. After you sign in for the first time, the software will prompt you to change the password.

Update the instrument firmware

After software installation is completed, you must update the instrument firmware. The firmware updater utility launches automatically if the instrument is powered on and connected.

IMPORTANT! DO NOT update the firmware if the instrument is in a sleep state. The indicator lights on the front of the instrument will fade in/fade out in multiple colors during the sleep state. **Power cycle** (turn on and off) the instrument before running the firmware updater. The firmware update process takes less than 15 minutes.

1. On the instrument firmware utility dialog, click **Update Firmware** to update the firmware, then click **OK** to confirm the request to update instrument firmware.
2. After the firmware update is completed, the **Firmware Update Complete** dialog is displayed. Click **Close** to close the dialog to exit the firmware update utility.
3. **Power cycle** the instrument (turn off, then on) to complete the firmware update.
4. Launch the software using login credentials used in earlier software versions.

Note: If the firmware updater is not completed as part of the installation, you will be prompted to update the firmware the first time the application is launched when the instrument is powered up and connected.

2. Startup, Login, and Main Menu

Main application startup

Main application startup

1. To launch the Attune™ Cytometric Software, double-click the Attune™ Software shortcut icon on the desktop.

   Alternatively, select **Start ▶ ThermoFisher ▶ Attune™ Software**.

IMPORTANT! A license control mechanism in the form of a DESkey device is required for the operation of the Attune™ Cytometric Software.

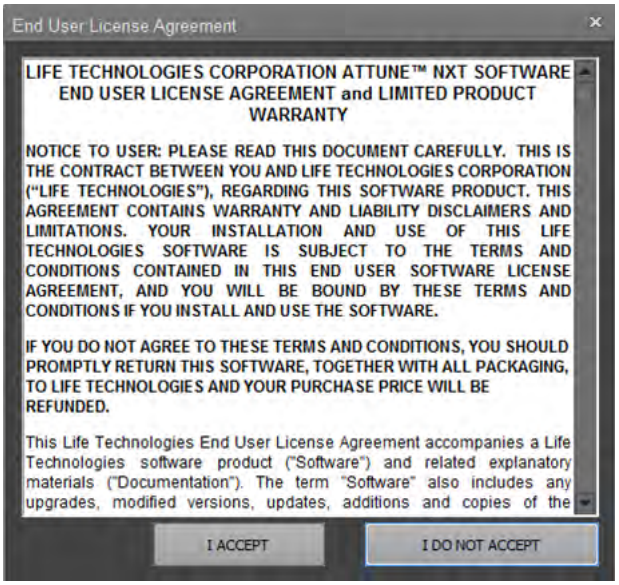
- If a valid license mechanism is detected when the software is started, the End User License Agreement (EULA) will be displayed on first use of the software (page 18).
- If a valid DESkey device is not present, the software will display the appropriate warning message.

End User License Agreement (EULA)

The End User License Agreement (EULA) is displayed when:

- The Attune™ Cytometric Software is first installed.
- A new version of the Attune™ Cytometric Software is installed.
- A new Windows user is attempting to open the Attune™ Cytometric Software.

Upon installation of the Attune™ Cytometric Software, the EULA can also be found with the Licenses directory contained within the application's installation folder.



Click **I ACCEPT** to accept the terms of the agreement and launch the software.

Click **I DO NOT ACCEPT** to reject the terms of agreement. The EULA dialog is closed and the software is not launched.

SAE module

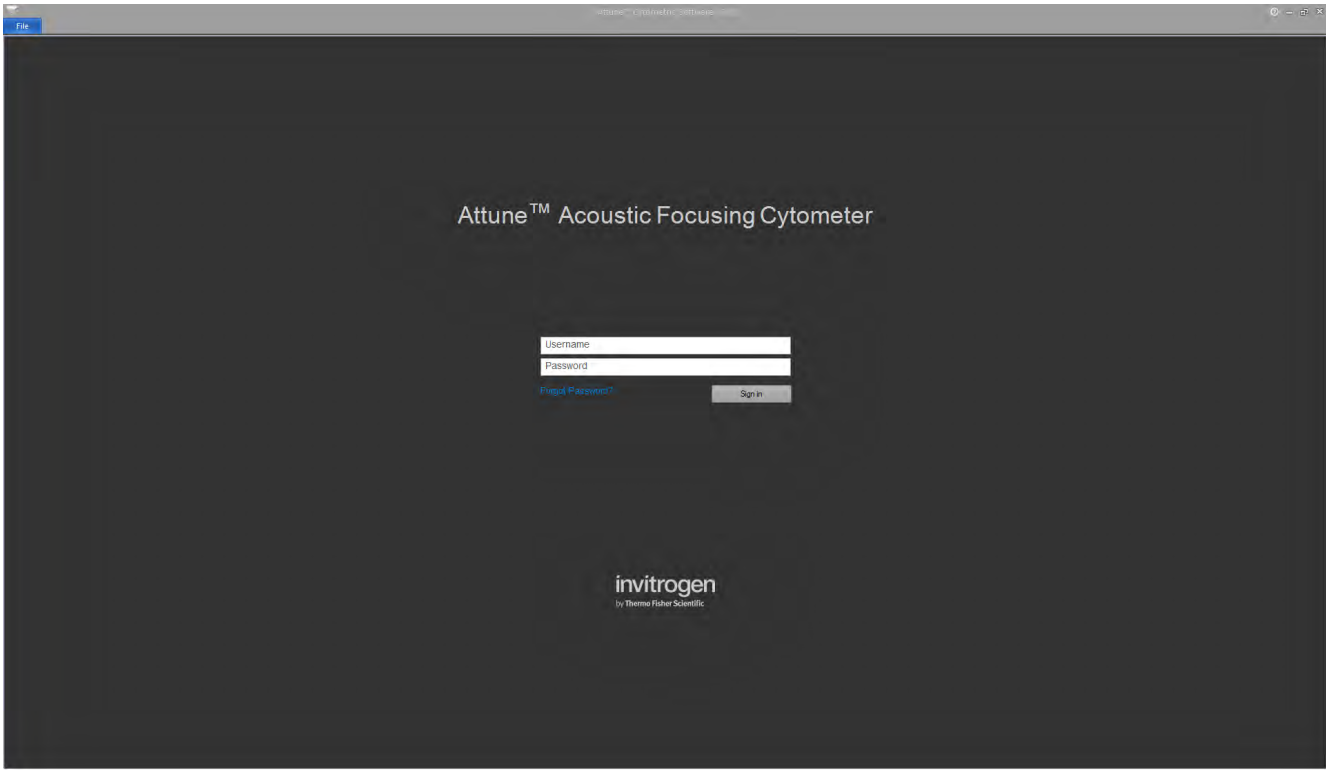
The Attune™ Cytometric Software also has an SAE ("Security, Auditing, and Electronic Signature") module, which allows you to configure the software to meet specific requirements for security, audit, and e-Signature (21 CFR Part 11).

- Use of the SAE mode requires an SAE-specific DESkey device.
- When the SAE mode is enabled (see "Enable the SAE mode", page 502), the SAE-specific DESkey is checked upon login when signing into the software as an SAE user.

Note: For more information on the SAE module and how to configure it to meet specific requirements for security, audit, and e-Signature, see "Appendix C: SAE Administrator Console", page 576.

Login screen

The *Login* screen is the first screen that is displayed after the splash screen when you start the Attune™ Cytometric Software. Each user is required to sign in before being allowed to use the software. The Login screen also displays the current Performance Test status (see "Performance Test Status", page 20).



Sign in button

By default, the Sign in button is a standard button (see "Sign in – Standard mode (page 20).

When the application is set to SAE ("Security, Auditing, and e-Signature") mode, the Sign in button is a split button (see "Sign in – SAE mode", page 21).

Performance test status

Performance test is a comprehensive set of procedures to monitor the daily performance of the cytometer. The *Performance test status* for the day is displayed below the Sign in text fields on the Login screen.

- The Performance test status includes a message and an icon that indicate the date and the results of the last performance test (see below). If no baseline has ever been run, no message is displayed.

Icon	Message
	Performance test has been completed successfully today.
	Performance test has not been completed today.
	Performance test has not been completed successfully today.

- For more information on Performance test procedures, see "Performance Testing", page 353.

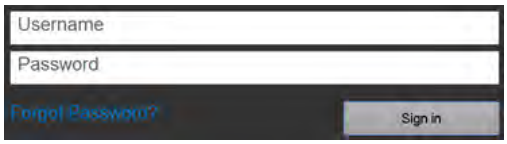
  For more information on reports detailing the performance test results, see "Performance Test Reports", page 363.

Note: Performance test status is also displayed on the Main Menu below the Main Menu buttons. For more information, see "Main Menu", page 26.

Sign in – Standard mode

1. To sign in the Attune™ Cytometric Software, type a valid username and password in the appropriate text box fields, then click **Sign In**.

   Alternatively, you can press the **Enter** key on the computer keyboard after typing the username and password.



2. If the correct username and password are entered, *Main Menu* is displayed (page 26).

   If an invalid username or password is entered, the warning banner displays "Invalid username or password" (see page 23).

IMPORTANT! When signing into the Attune™ Cytometric Software for the first time after installation, you must sign in as an *Administrator*.

The default username and password for the Administrator are both admin.

For more information on other available account types, see "Account types", page 24.

Sign in – SAE mode

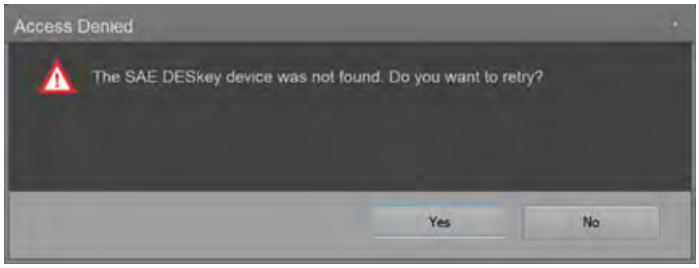
To sign in to the Attune™ Cytometric Software in the SAE ("Security, Auditing, and Electronic Signature") mode, you must have an SAE-specific DESkey device installed and the SAE module enabled and configured to allow sign in using SAE credentials. When signing in, you must have the appropriate SAE user credentials, and meet the SAE account rules for signing in (see "SAE account rules", page 22).

1. To sign in to the Attune™ Cytometric Software using your SAE user credentials, type your valid SAE username and password in the appropriate text box fields, then click the main **Sign In** button.

   Alternatively, you can press the **Enter** key on the computer keyboard after typing the username and password.

2. To sign in to the SAE-enabled Attune™ Cytometric Software with a local account, click the **drop-down arrow** on the **Sign In** button, then select **Sign in with local account**.

Note: If the SAE specific DESkey is not present, you will not be able to sign in and the software displays the "Access Denied" warning dialog.



- Click **Yes** to retry the sign in attempt. If the SAE DESkey device is detected, you will be able to sign in as an SAE user.
- Click **No** to close the dialog without signing in as an SAE user.
- If the Yes button is pressed, the dialog will persist as long as the SAE Deskey device check fails.

3. To sign in to the Attune™ Cytometric Software in the SAE ("Security, Auditing, and To sign in to the SAE-enabled Attune™ Cytometric Software with a local account, click the **drop-down arrow** on the **Sign In** button, then select **Sign in with local account**.

IMPORTANT! When signing into the Attune™ Cytometric Software after the SAE mode is enabled, you must use your SAE account username and password, and not your Attune™ instrument username and password.

In cases where the SAE server is unavailable or you need service repair of your instrument, you can sign in to your local Attune™ Software account using the **Sign in with local account** dropdown. This option is only available if you have an Administrator, System Administrator, or Service account.

Users and Advanced Users are not allowed to login to the local account once SAE is enabled.

Note that the data from local User and Advanced User accounts become inaccessible after the SAE mode is enabled. We recommend that you export all local User and Advanced User data before enabling the SAE mode.

4. Upon successful sign in, the following steps are performed:
   - If the specified SAE account password has expired, a mandatory change password screen is displayed.
   - If SAE account is configured to show the password expiry reminder X number of days before password expiry, the Password expiry reminder is displayed.
   - If the correct username and password are entered, the user login is completed and the *Main Menu* is displayed (see page 26).
   - If an invalid username or password is entered, the warning banner displays "Invalid username or password" (see page 23).
   - A login audit record is generated (see Note below)
   - If it is the first time sign in on the specific Attune™ instrument, a local Attune™ instrument account is created after the successful SAE account sign in. This account is flagged as an SAE user account and is used to allow sign in, if the SAE server is offline.

Note: In the SAE mode, all successful login events (both local and with SAE credentials) are audited as actions on the SAE server as "Sign In Success" along with the username and role. Local Attune user logins are noted with a comment "This is a non-SAE Attune user account."

All sign in failures (both local and SAE credentials) are audited as actions on the SAE server as "Sign In Failure" along with the username and role. Local Attune user logins are noted with a comment "This is a non-SAE Attune user account."

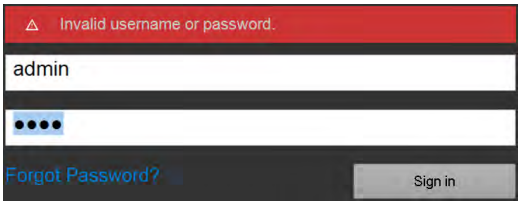
SAE account rules

Any user with an SAE account can sign in to the instrument if the following conditions are met:

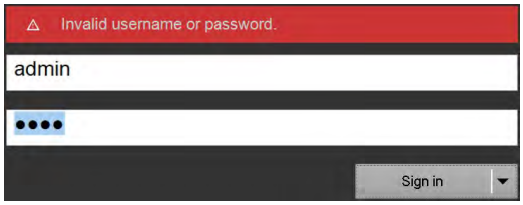
1. User provides valid user credentials.
2. The SAE account is not disabled or suspended by the SAE Administrator.
3. The SAE account is not locked out (due to too many failed sign in attempts).
4. The SAE server is online unless offline sign in has been configured.

Invalid username or password

- If you enter an invalid username or password, the warning banner on the Login screen displays "Invalid username or password". You must then enter the username or the password correctly or the warning banner is displayed again.



Warning banner for sign in attempt with invalid username or password (local Attune™ account)



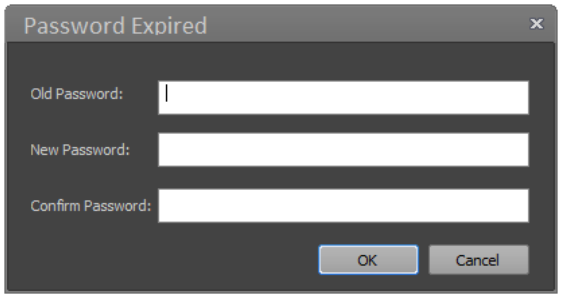
Warning banner for sign in attempt with invalid username or password (SAE account)

- If you attempt to login to an account that has been disabled (i.e., set to inactive) or manually suspended, the warning banner displays "Your account has been disabled. Contact administrator."

Note: SAE user accounts cannot be modified using the Attune™ Cytometric Software (i.e., disabled, deleted, password reset or changed, etc.). All SAE user accounts are managed by the SAE administrator using the SAE Administrator Console (see "Roles tab" in "Appendix C: SAE Administrator Console", page 581).

Expired password

If your password has expired, the Attune™ Cytometric Software will prompt you to change your password. You must then enter your old password, your new password, and a confirmation of the new password.



Note: The *Password Expired dialog* is displayed only if you successfully sign in with the correct credentials. If any updates to the password are required, you must complete them before the Main Menu is displayed.

- If the new password and confirmation password do not match, you will be prompted to enter the new password again.
- If the new password that does not meet password complexity requirements, you must choose another password. The new password must contain at least one uppercase, one lowercase, one numeric, or one non-alpha-numeric character, and must be at least 4 characters long.
- If you enter a password that has been previously reused within the disallowed time frame as specified by the System Administrator, you will be prompted to choose another password.
- For more information on user passwords for local Attune™ instrument users, see "User Management", page 522.

  For more information on user passwords for SAE users, see "System tab" in "SAE Administrator Console", page 591.

Account types

Default local Attune™ accounts

Upon installation, the Attune™ Cytometric Software has three types of default accounts for local Attune™ users, *System Administrator*, *Administrator*, and *Service*.

System Administrator

System Administrator is the highest level account and can define the system security policy for username length, password length, password expiration and lock-out, and the auto lock out time due to system inactivity. The default system administrator account cannot be deleted.

Administrator

Administrators have full access to the software and can perform a variety of tasks, including managing user accounts, running system diagnostic tests, system decontamination, performance tests and baseline calculations. However, they cannot set system security policy. The default administrator account cannot be deleted.

Service

Service is a special type of account that gives access to service-only features of the software. The Service account cannot be deleted and is only accessible by Thermo Fisher Scientific service team.

Note: For the permissions assigned to each account type, see "Account permissions", page 25.

For information on how to create additional local Attune™ accounts or delete existing ones, see "User Management", page 522.

Local Attune™ user accounts

Users can access and modify their own Workspace and Experiments, and run Performance tests, but they cannot run any advanced functions available to other account types. User accounts must be created by the System Administrator or Administrator upon successful installation of the Attune™ Cytometric Software and the initial sign in.

For more information on how to create additional User accounts or delete existing ones, see "User Management", page 522.

Default usernames and passwords (local Attune™ accounts)

For the default usernames and passwords for local Attune™ users, see the following table. Note that each account will be forced to reset their password upon first login.

	Default username	Default password
System Administrator	sysadmin	sysadmin
Administrator	admin	admin
User	Set by System Administrator or Administrator when the account is created.*	Set by default to the username created by System Administrator or Administrator.

*See "User Management", page 522.

SAE user accounts

In the SAE module, the Attune™ Cytometric Software has four default SAE user account types, *Administrator*, *Advance User*, *User*, and *Reviewer*. In addition, there is the *No Privileges Role*, which is for internal use only by the SAE Administrator Console when you set up user repositories. For more information, see "Roles tab", page 581).

For the permissions assigned to each default SAE account type, see "SAE account permissions", page 583.

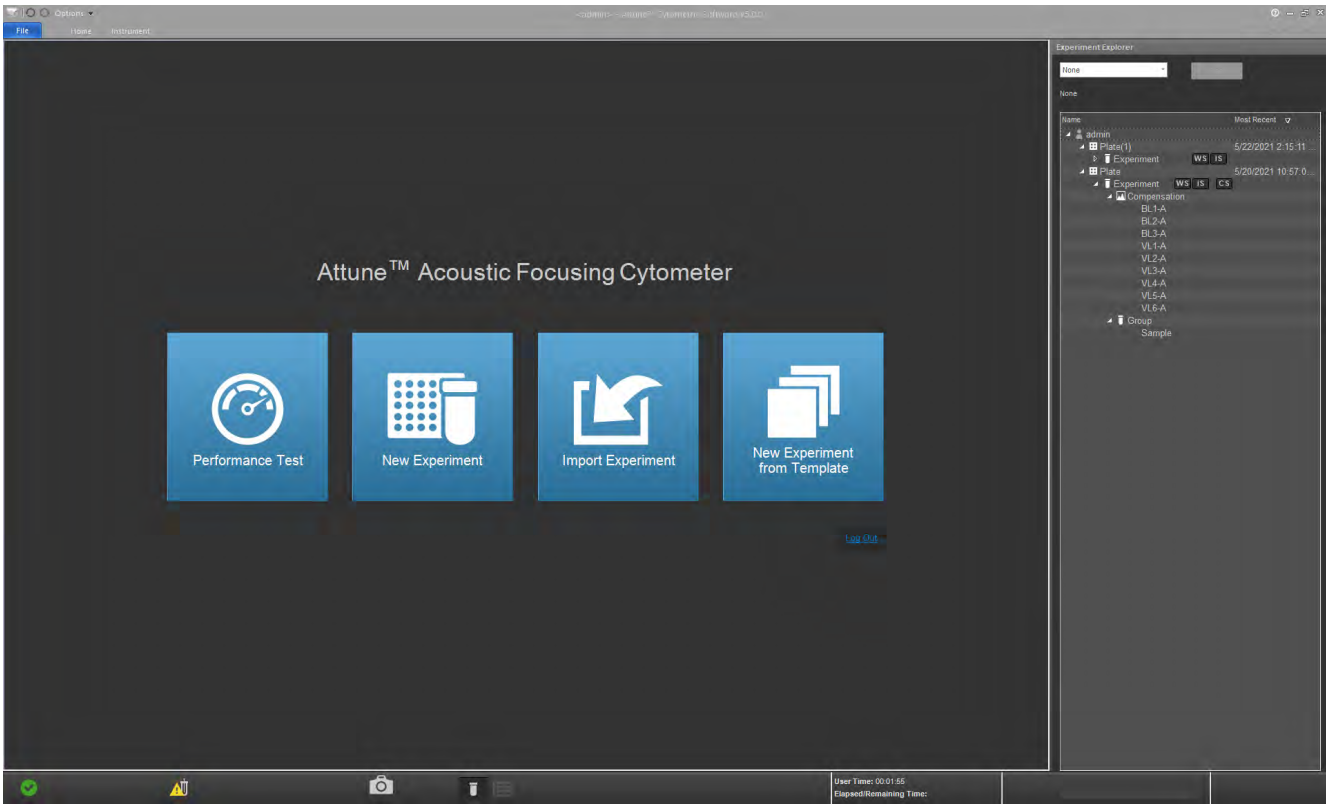
Account permissions For the permissions assigned to each account type, see the following table.

Permission	Description	System Admin.	Admin.	Adv. User	User	SAE User
Adjust Laser Delays	Allows user to adjust the laser delay values for the system		✓			
Advanced Instrument Settings	Allows user to adjust width threshold, window extensions, and area scaling factor within an Experiment		✓	✓		*
Allow Local Login in SAE Mode	Allows a local Attune™ user to login when the SAE mode is enabled	✓	✓			NA
Change Password	Allows a user to change their password	✓	✓	✓	✓	✓
Configure Device	Allows a user to setup a device to connect with Connect™ Cloud-based platform	✓	✓			*
Configure SAE	Allows a user to enable/disable SAE	✓	✓			✓
Create or Edit Global Keyword Access	Allows a user to set and edit global keywords		✓			*
Create Plate Definition Access	Allows a user to create and/or duplicate custom plate definitions		✓	✓		*
Database Utility Full Access	Allows a user to access the database utility to schedule database and data backups	✓	✓			*
Delete Report Column Access	Allows a user to delete plate definitions	✓	✓			*
Edit Filters	Allows a user to edit filter configuration filters		✓	✓		*
Edit Plate Definition Access	Allows a user to edit plate definitions		✓	✓		*
Edit Security Policy	Allows a user to set system security policy (i.e., username and password policies)	✓				*
Edit User	Allows a user to modify a user's profile	✓	✓	✓	✓	NA
Export Service Logs	Allows a user to export application logs	✓	✓	✓	✓	✓
Manage User Accounts	Allows a user to create and edit user accounts	✓	✓			*
Modify Instrument Configuration	Allows a user to modify instrument configuration		✓	✓		*
Run Baseline Calculations	Allows a user to run and reset a baseline		✓	✓		*
Run Performance Test	Allows a user to run a performance test		✓	✓	✓	*
Run System Decontamination	Allows a user to perform a system decontamination		✓	✓		*
Run System Tests	Allows a user to run system diagnostic tests		✓	✓		*

* SAE user privileges are managed by the SAE administrator using the SAE Administrator Console (see "Roles tab" in "Appendix C: SAE Administrator Console", page 581).

Main Menu

After you sign in successfully to the Attune™ Cytometric Software, the *Main Menu* is displayed. The Main Menu contains the buttons for the most frequently used commands (Performance Test, New Experiment, Import Experiment, and Templates), the *Experiment Explorer* panel, the *Performance test status* bar, and the *Log Out* hyperlink control. If the software is in the Automation mode (page 65), all buttons and the hyperlink are disabled.



Performance test

Performance Test initializes the *Performance Test setup* screen within the Performance Test module, which allows you to perform the baseline and performance test and view the baseline and performance test reports (see "Performance Testing", page 353).

New Experiment

New Experiment launches the *New Experiment dialog*, which allows you to create a Plate or Tube-based Experiment, or an Experiment using imported files (see "New Experiment dialog", page 399).

Import Experiment

Import Experiment launches the *File Open (Import) dialog* (page 535), which allows you to import pre-existing Plate or Tube-based Experiments.

Templates

Templates launches the *New Experiment from Template dialog*, which allows you to create a new Experiment form an existing template stored in the database within your account (see "New Experiment from Template dialog", page 405).

Performance test status

Performance test is a comprehensive set of procedures to monitor the daily performance of the cytometer. The *Performance test status* for the day is displayed below the sign in text fields in the Login screen.

The performance test status includes a message and an icon that indicate the date and the results of the last performance test (see below). If no baseline has ever been run, no message is displayed.

Icon	Message
	Performance test has been completed successfully today
	Performance test has not been completed today
	Performance test has not been completed successfully today
	Bead lot has expired (see **Note** below)

- For more information on performance test procedures, see “Performance Testing”, page 353.
- For more information on reports detailing the performance test results, see “Performance Test Reports”, page 363.

Note: If the baseline calculations have expired, the performance test status bar displays the “Bead lot has expired” message. For more information on baseline calculations, see “Setting up and running a Baseline”, page 355, and “Baseline results”, page 358.

Log Out

Clicking **Log Out** logs out the current user and the software returns to the Login screen (page 19).

Note: The Log Out link is only enabled when the instrument is not acquiring (see “Acquisition Status Indicators”, page 222).

Main Menu ribbon bar

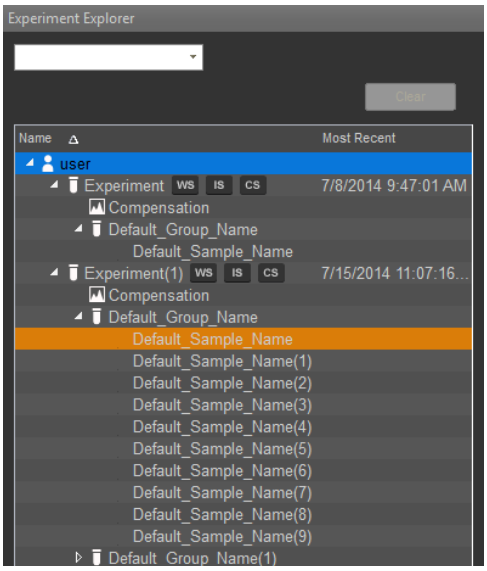
Main Menu provides a limited set of options for the user to perform, and contains only the File (page 49), Home (page 50), and Instrument ribbon tabs (page 61).

- The Main Menu ribbon bar is minimized by default. The minimized or expanded state of the Main Menu ribbon bar persists on a per user basis.
- When the Main Menu ribbon is expanded, the Home tab is selected by default.

Experiment Explorer

Experiment Explorer is used to create, view, and manage *Experiments*, and it is described in detail in "Experiment Explorer", page 189.

The Experiment Explorer panel cannot be undocked or resized.

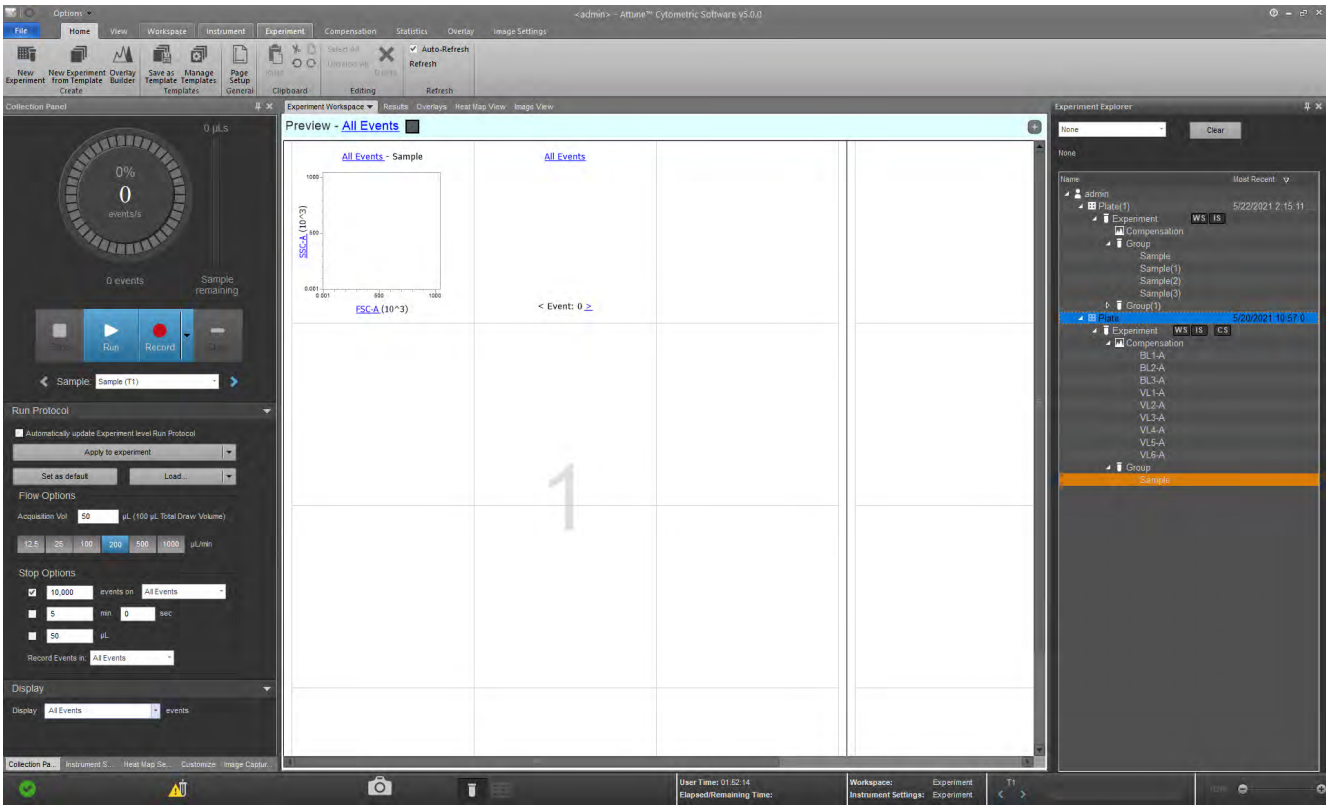


3. Attune™ Desktop

Attune™ Desktop overview

Attune™ Desktop is the main application window, and it is used for controlling the Attune™ NxT and Attune™ CytPix™ Flow Cytometers to run samples, generate data, and analyze results. It consists of the sections listed below.

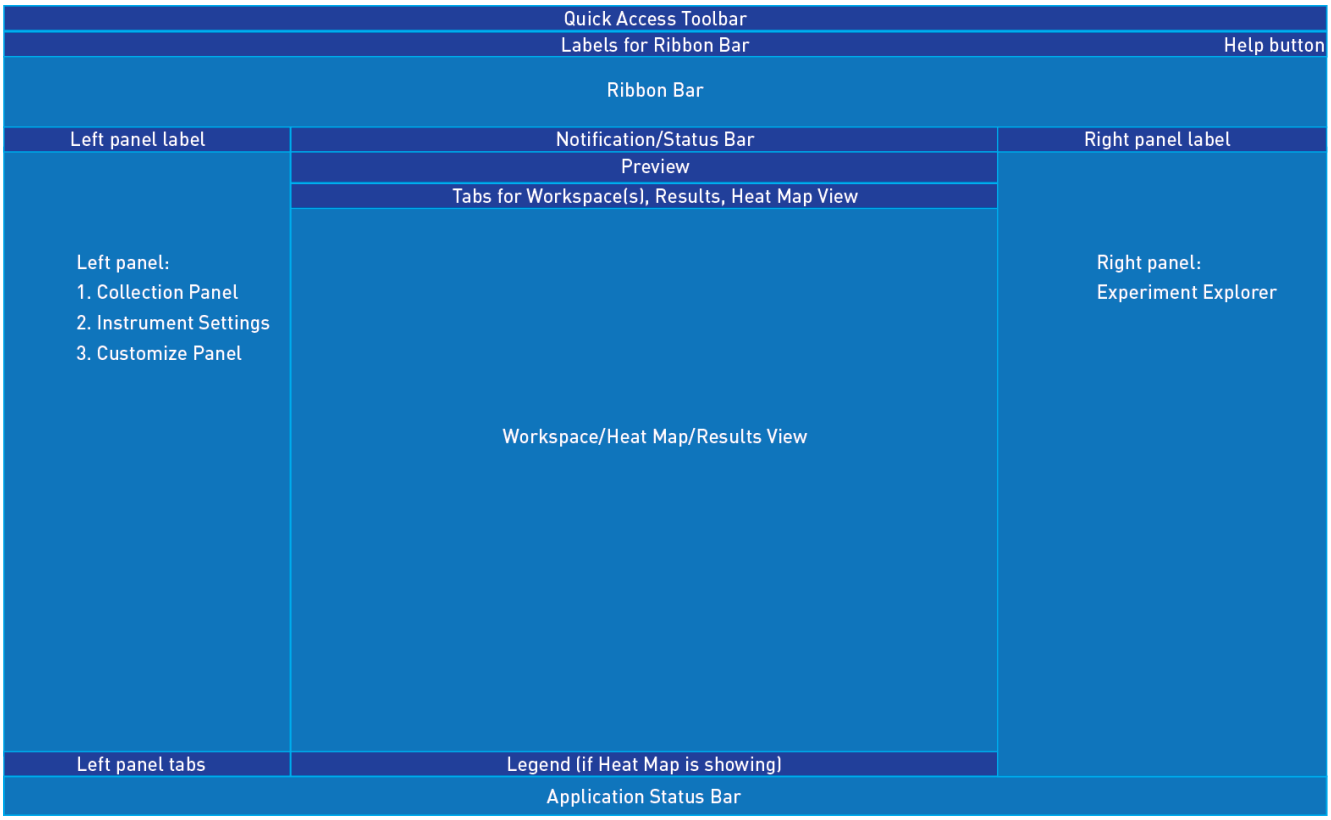
- *Application title bar* (page 31) contains the Application button, Quick Access toolbar, Application name, and Window Size buttons.
- *Ribbon bar* (page 33) is organized into a series of tabs that allow access to the main functions of the Attune™ Cytometric Software.
- *Main application workspace* (page 36) accommodates the Application area, the adjustable docking panels that contain controls grouped by functionality, the Experiment Explorer, and the Status notification display.
- *Application status bar* (page 41) displays various icons depicting the status of the instrument and the experiment, any alerts relating to the instrument, as well as the progress bar for instrument functions, loading and refreshing. It also contains the Size slider (page 47).



Attune™ Desktop layout

The layout of the Attune™ Desktop follows Microsoft™ Windows 7 and Microsoft™ Office 2010 styles; it uses the ribbon menu system and adjustable docking panels that contain controls grouped by functionality.

The schematic below represents the default layout of the Attune™ Desktop, showing the default locations of the various panels and toolbars. Note that the contents of the Attune™ Desktop changes depending on the context of the software.



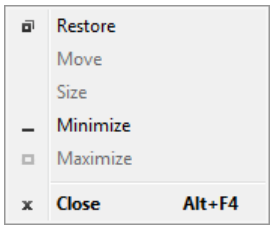
Note: *Application panels* are dockable panels that contain controls grouped by functionality. For more information, see "Application panels", page 36.

Application title bar

Application title bar layout

The *Application title bar* is displayed at the top of the application window, and contains the Application button, Quick Access toolbar, Application name, and Window size buttons.

- **Application button** opens the standard Microsoft™ Windows application size menu.



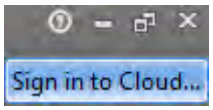
  You can also open the application size menu by right-clicking anywhere on the application title bar.

- **Quick Access toolbar** (page 32) provides easy access to frequently used commands and can be customized to include any commands within the ribbon menu system.

- **Application name** shows the version number of the Attune™ Cytometric Software and the currently logged in username. If these names are too long, they are truncated and ellipses are used.
- **Window size buttons** are the standard buttons used in Microsoft™ Windows applications for minimizing, maximizing, and restoring the open application window.

- **Help button** to the left of the Window size buttons opens the *Attune™ Cytometric Software User Guide* (Pub. No. MAN0026553).

- **Cloud sign in** (page 33) button at the bottom right of the Application title bar allows you to add the device (instrument or software) to your Connect account, which enables the export and import of files from the Connect cloud-based platform.



Quick Access toolbar

The *Quick Access toolbar* is a customizable toolbar containing frequently used commands that are independent of the tab currently displayed.

By default, the Quick Access Toolbar is located above the Ribbon bar. However, you can also choose to display it below the Ribbon bar using the Customize Quick Access Toolbar drop-down list.

Default buttons

By default, the Quick Access Toolbar contains the Undo and Redo commands, a link to the Main Menu, a link to the Options menu, and the Quick Print command.

Undo: Negates the last undoable action performed and brings the application to its previous state. The Undo button is enabled only if there is an undoable action available.

Redo: Reverses the Undo or advances the application to a more current state. The Redo button is enabled only if there is a redo action available.

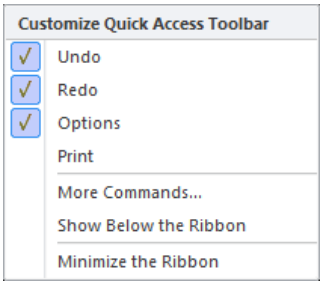
Main Menu: Navigates to the Main Menu (page 26). The Main Menu button is enabled only if a user is logged in, and the instrument is not acquiring.

Options: Opens the Options dialog (page 472), which allows you to customize the Attune™ Cytometric Software by configuring your personal settings and changing the default options. The Options button is enabled only if a user is logged in.

Customize Quick Access Toolbar drop-down menu: Opens the *Customize Quick Access Toolbar dropdown menu*.

Customize Quick Access toolbar menu

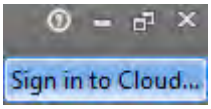
The arrow next to the Quick Access buttons opens the *Customize Quick Access Toolbar dropdown menu*. The dropdown list includes the following options:



- **Show Quick Access Toolbar Below the Ribbon/Above the Ribbon:** Toggles the Quick Access Toolbar to show below or above the Ribbon bar; the text on the menu item toggles between *Show Below the Ribbon* and *Show Above the Ribbon* depending on its location.
- **Minimize the Ribbon:** Checking and un-checking this menu item toggles the Ribbon bar between minimized and restored. When the Ribbon bar is minimized, the tabs are still active. Changing to another tab displays the Ribbon bar.

Note: Options to customize the Quick Access Toolbar, to show the toolbar above or below the ribbon, and to minimize the ribbon are also available through the Ribbon context menu. For more information, see "Ribbon context menu", page 35.

Sign In To Cloud

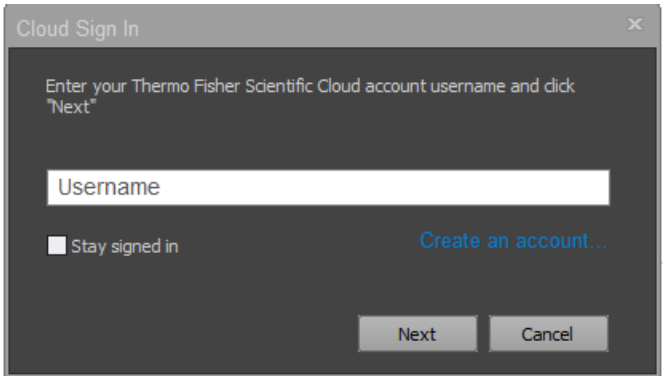


Sign in to Cloud button at the top right of the screen allows you to add the device (instrument or software) to your Connect account, which is Thermo Fisher's cloud-based platform.

When signed to your Connect account, you can export and import of files to and from the Connect cloud-based platform view performance data from a registered device.

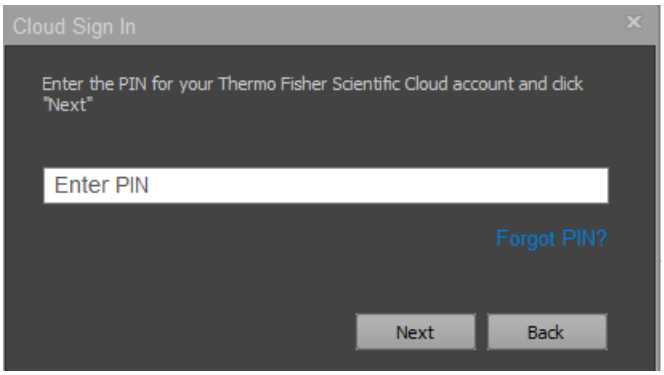
Note: The **Sign in to Cloud** button is only enabled if the device (instrument or software) is registered with the Connect platform. For more information on how to register your instrument or software, see page 517.

- If no user is signed into the Connect cloud-based platform, the button displays "**Sign in to Cloud…**".
- Click the **Sign in to Cloud…** button to open the *Cloud Sign In dialog* (page 457), where you are prompted to enter your Thermo Fisher Cloud username to sign in to your Connect account.



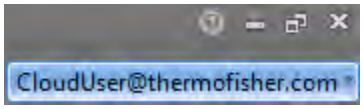
- If you have not yet used your Connect account on the Attune™ Software but the instrument is registered with the Connect platform, the software displays the *Link Account dialog* to link your account to the software for the first time.

- If you have accessed the software before and your account is already linked, you are prompted to enter your four-digit PIN to link your Thermo Fisher Cloud account to the Attune™ Software.

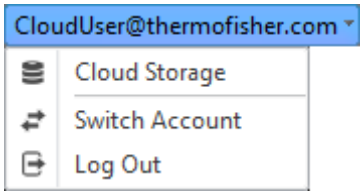


Note: For more information, see the *Cloud Sign In dialog* (page 457).

- After you sign in to your Connect account, the "Sign in to Cloud..." button becomes a dropdown button that displays your Connect username as the main button text.



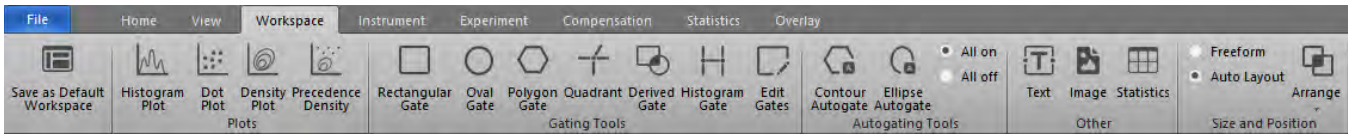
- The dropdown menu has three options:



  - **Cloud Storage:** Opens the *Connect Storage dialog* (page 460), which displays the available storage on your Connect account.
  - **Switch Account:** Opens the *Current Attune User Password Required dialog* (page 457) to verify your Connect account password. If the password is correct, the dialog closes and the *Connect Sign in dialog* (page 457) opens.
  - **Log Out:** Allows you to log out of your Connect account. When you log out of your account, the *Remember me* settings set in the Connect Sign in dialog (page 457) are cleared.

Ribbon bar

The *Ribbon bar* is organized into a series of tabs which represent the main functions of the Attune™ Cytometric Software.



Ribbon tabs

The availability of the tabs in the Ribbon bar is dependent on the context of the software; they are displayed only when the user needs them. Objects that are not available as a function in a specific context are shaded gray in the tab.

Depending on the context of the application and the instrument type (Attune™ NxT or Attune™ CytPix™ Flow Cytometer), the Ribbon bar contains one or more of the following tabs:

- *File tab* (page 49)
- *Home tab* (page 50)
- *View tab* (page 53)
- *Workspace tab* (page 55)
- *Instrument tab* (page 61)
- *Experiment tab* (page 66)
- *Compensation tab* (page 70)
- *Statistics tab* (page 74)
- *Overlay tab* (page 78)
- *Image Settings tab* (page 79)
- *SAE tab* (page 81)

Note: When performing a Performance Test, only a limited set of tabs are available on the Main Menu.

On the Login screen, only the File tab is available.

SAE tab is available only when an SAE user is signed in.

Image Settings tab is available only for Attune™ CytPix™ Flow Cytometers.

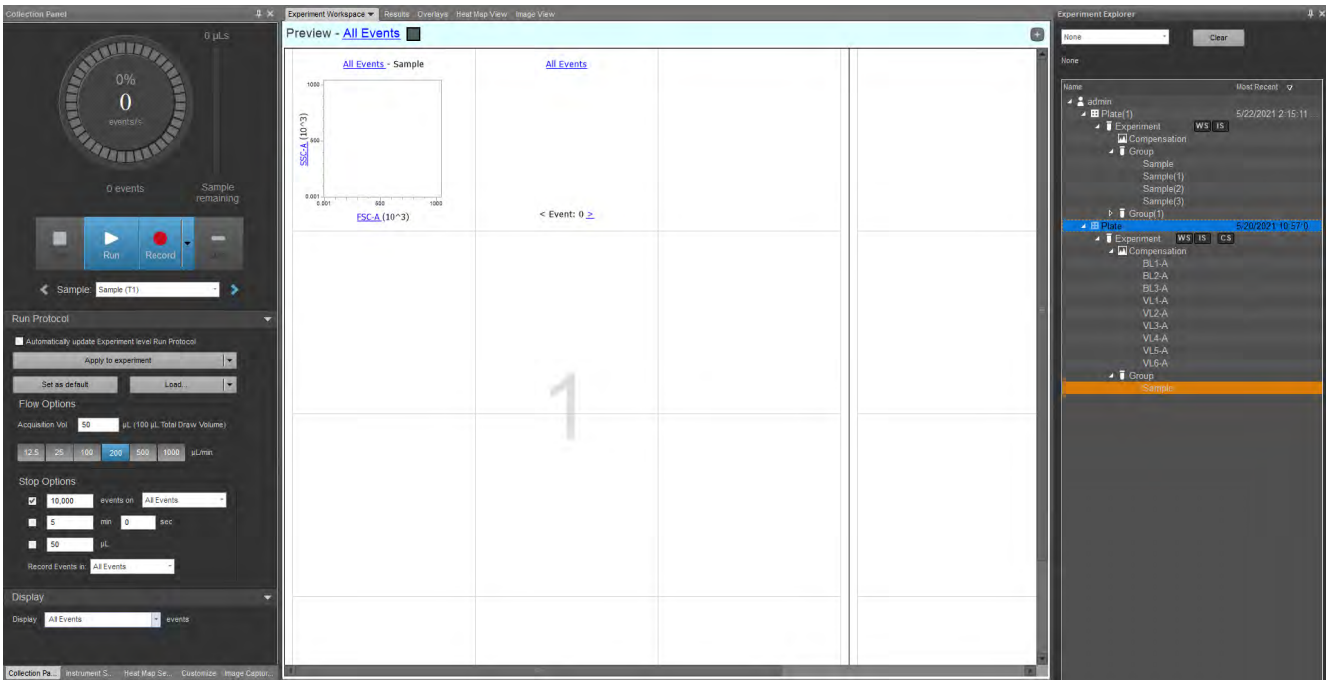
Ribbon context menu

Right-clicking on a control in the Ribbon bar brings up the *Ribbon context menu*, which contains the options listed below.

- **Show Quick Access Toolbar Below the Ribbon/Above the Ribbon:** Toggles the Quick Access Toolbar to show below or above the Ribbon bar; the text on the menu item toggles between *Show Below the Ribbon* and *Show Above the Ribbon* depending on its location.
- **Minimize the Ribbon:** Checking and un-checking this menu item toggles the Ribbon bar between minimized and restored. When the Ribbon bar is minimized, the tabs are still active. Changing to another tab displays the Ribbon bar.

Main application workspace

The *Main application workspace* accommodates the *Application area*, the *Application panels*, the *Experiment Explorer*, and the *Status notification display*. The contents of this area change depending on the context of the software.



Application area

The *Application area* displays the analysis objects (plots, gates, and statistics) that are associated with the current or a saved Sample or Experiment. Text boxes and image files can also be displayed in the Application area. The Application area contains the following *Application tabs*:

- Workspace view (page 82)
- Heat Map view (page 138)
- Sample List view (page 146)
- Results view (page 151)
- Overlay view (page 156)
- Image view (page 180)

Application panels

Application panels are adjustable docking panels that contain instrument and software controls that are grouped by functionality. The Main Application Workspace contains the following Application panels:

- Experiment Explorer (189)
- Collection (page 221)
- Instrument Settings (page 250)
- Customize (page 276)
- Image Capture Settings (page 319)
- FCS Information (page 326)

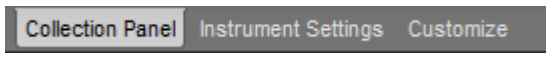
Note: The Application tabs and the Application panels are displayed in the Main Application Workspace as described above, except in the following application-specific modules:

- Login screen – All panels are hidden.
- Main Menu – All panels are hidden except for Experiment Explorer, which cannot be undocked or resized.
- Performance Test/Baseline Test – All panels are hidden.
- Image view tab and Image Capture Settings panel are available only when using an Attune™ CytPix™ Flow Cytometer or when an Experiment that was created with an Attune™ CytPix™ Flow Cytometer is active.

Docking behavior

- By default, the Collection, Instrument Settings, and Customize panels are docked to the left of the Application area in the order listed.
- The Experiment Explorer is by default docked to the right of the Application area (see the schematic above).
- The FCS Information panel is set as a floating panel by default (i.e., with its "Dockable" attribute deselected; see page 40).
- The Workspace, Results, and Heat Map view tabs are docked to the Application area. By default, they appear in the order listed when docked.
- You can re-order all tabs except the Workspace tab, which always appears first in the list. To reorder the tabs, click a tab and drag it to different position.
- When multiple panels are docked together to the left or the right of the Application area, the name of the currently displayed panel appears in the *container title bar* (i.e., the title bar immediately above the panel). When docked to the Application area, no title bar is displayed.
- When docked to the Application area, tabs for each docked panel appear on the top edge of the Application area.

  When docked to the left or right of the Application area, docked panels are displayed as tabs on the bottom of the panel, unless only one panel is located within the *panel container*.
- The tab containing the currently open panel is highlighted.

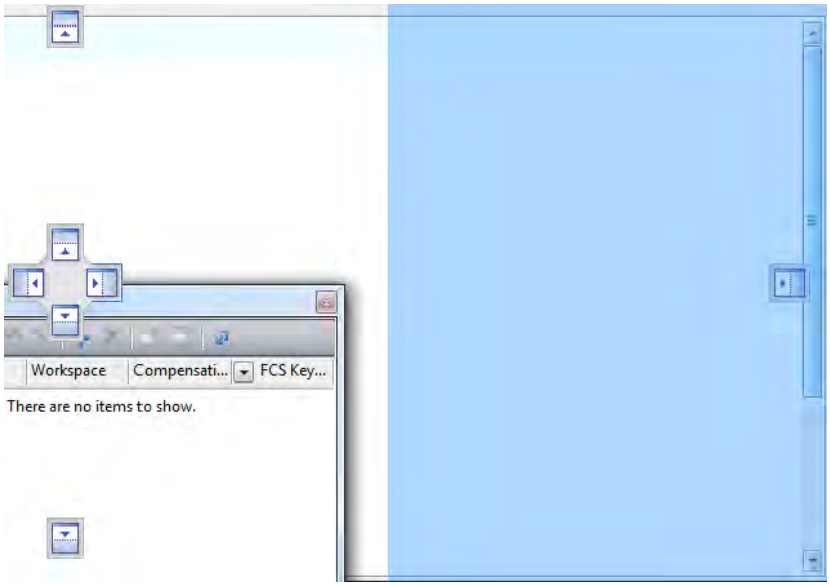


- Application panels that are docked to the Application area will display as tabs.
- The layout of dockable panels persists for each user.

Docking and undocking panels

1. To undock an Application Panel, click and drag the title bar of the panel container. While dragging, docking icons will be displayed in the center, left, and right of the Application area to show where the panel can be docked.

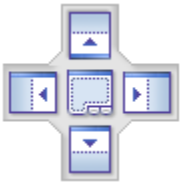
2. Hover over one of the docking icons to display the indicator showing where the panel will be docked (see image below).



3. Release the panel on the desired docking icon to dock it to the area indicated.

Docking and undocking tabs

1. To undock an Application tab, select and drag the individual tab.
2. Hover over an existing panel to display the docking icon, which allows you to dock the selected tab to any of four sides of the panel or to add it as a tab to the full-size panel.



3. Release the tab on the desired docking icon to dock it to the left or right of the Main Application Workspace or to any of the four docking points of another panel.

   You can also drop a selected panel onto the title bar of an existing panel to dock it as a full-size panel, which will be added as a tab.

Note: Heat Map and Results tabs can be docked anywhere on the Main Application Workspace. All other tabs and panels are limited to left and right only docking rules.

Floating panels

- Double-clicking on the title bar of a docked panel or tab results in panel to be floated to its default position.
- If a panel has been docked from a floating position, it returns to the same floating position when re-floated.
- If multiple floating panels are docked together before docking the entire container to the left or right of the Main Application Workspace, clicking the title bar of the docked container re-floats the container in its entirety to its previous position.
- Double clicking on floating container title bar or tab results in that container or panel to be re-docked to its last docked position.

Auto Hide

Auto Hide option reduces a panel to a tab icon when the focus is lost by clicking anywhere outside the menu.

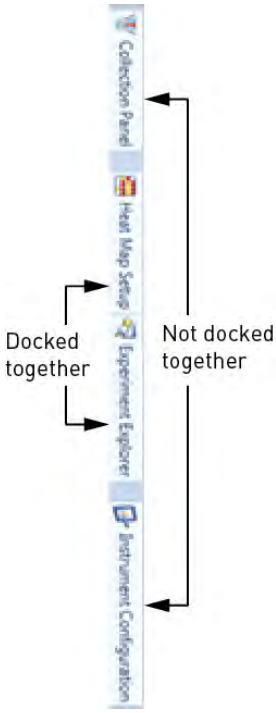
Push Pin icon is used for turning the Auto Hide option on and off, and it is available only when a panel is docked. The Push Pin icon changes to display the current state of the panel.

Alternatively, you can turn the Auto Hide option on and off using the *Title bar context menu* (see page 40).

Panel pinned – Auto Hide is turned off.

Panel unpinned – Auto Hide is turned on

- When hidden, the panel name appears as a tab on the side of the Main Application Workspace where the panel was docked.
- When multiple panels that are docked together, hiding one panel also hides the others with which it was docked. All hidden panels then appear together as tabs on the side of the Main Application Workspace where they were docked.
- Clicking on a tab only displays the selected panel.
- Clicking on the Push Pin of an open panel restores all panels with which it was docked.
- When hiding multiple panels on the same side of the Main Application Workspace, panels that ware docked together will be grouped together and be separated from other panels as shown on the right.



Close

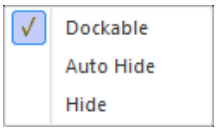
Clicking the **Close icon** (✕) closes the current panel. This deselects the current panel in the *Show Panels* group of the *View ribbon tab* (see page 54).

Alternatively, you can select the Hide option using the *Title bar context menu* (see below).

- The only way do re-display a closed panel is to reselect the relevant check box in the Show Panels group of the View ribbon bar (page 54).
- If multiple panels are docked together and the panel is docked, the close icon only hides the current panel. If the panel is undocked the entire panel is closed.

Title bar context menu

Right-clicking on the title bar of a panel displays the *Title bar context menu*, which contains the options listed below.



The current state of the panel is indicated in the context menu by a check mark.

- **Dockable:** Allows the docking of a panel. A panel cannot be docked unless this option is checked.

  Unchecking the Dockable option for a docked panel floats that panel, which cannot be re-docked until the Dockable option is re-selected.

  Dockable option is not available when the Auto Hide option is selected, because a panel must be docked to allow auto-hiding.
- **Auto Hide:** Reduces a panel to a tab icon when the focus is lost by clicking anywhere outside the menu. This has the same action as selecting the *Push Pin icon* in the panel header (see page 39).

  The *Auto Hide* option is only available when the panel is docked, not floating.
- **Hide:** Hides the current panel. When hidden, the panel name appears as a tab on the side of the Main Application Workspace where the panel was docked.

  Selecting the Hide option from the context menu deselects the current panel in the *Show Panels* group of the *View ribbon bar* (see page 54).

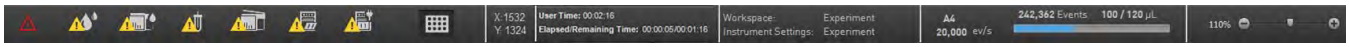
  The only way re-display a hidden panel is to reselect the relevant check box in the Show Panels group of the View ribbon bar.

  If multiple panels are docked together, the Hide option only hides the current panel.

Application status bar

The *Application status bar* indicates the status of the cytometer and its peripherals and provides feedback on the system using indicator icons. It also contains additional controls to navigate between samples.

The configuration of the Application status bar depends on whether the instrument is acquiring (top) or whether it used for analysis (bottom).



The Application status bar is described in parts as listed below:

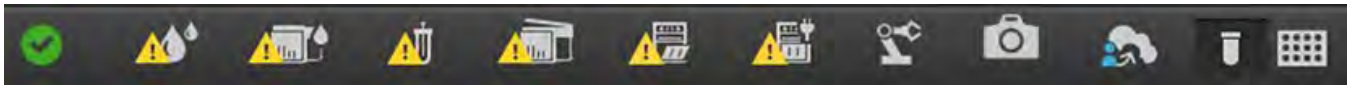
- *Instrument status and alerts* (page 41)
- *Camera status* (page 43)
- *Connect status* (page 43)
- *Tube and Plate modes* (page 43)
- *Time* (page 44)
- *Coordinates* (page 44)
- *Workspace and Instrument Settings* (page 45)
- *Acquisition* (page 45)
- *Analysis* (page 46)
- *Size slider* (page 47)

Note: Clicking indicator icons does not affect the instrument. They are only used for providing feedback on the status of the system.

Instrument status and alerts

The image below shows the fixed positions of *Instrument Status and Alerts* indicator icons on the status bar. If a specific indicator icon is not displayed, then a gap is left in its position.

Note that some of the indicator icons that are displayed depend on the instrument model and system configuration (for example, camera connected/disconnected icon is available only when an Attune™ CytPix™ instrument is in use).



Instrument status icons

Instrument Idle: The Instrument Idle icon is displayed when the instrument is connected and there are no alarms, or if an instrument has never been connected. The tooltip displays “Idle”.

Instrument Busy: The Instrument Busy icon is displayed when the instrument is connected and it is not idle. The tooltip displays “Instrument busy”.

Alarm: The Instrument Status Alarm icon is displayed when there is an instrument error state. The tooltip displays the relevant error message. If multiple error messages are present, each error message is separated by a comma.

Instrument alert icons

Instrument Fluid: The Instrument Fluid icon is displayed when a fluid level warning is given. The tooltip may contain more than one message concerning the fluidics (see below), using commas between messages.

- "Shutdown solution tank low"
- "Wash tank low"
- "Focusing fluid tank low"
- "Waste tank full"
- "Auto sampler focusing fluid tank low"
- "Auto sampler waste tank full"

Plate Leak Detected: The Plate Leak Detected icon is displayed when a leak in the Auto Sampler has been detected. Tooltip displays "Auto Sampler leak detected".

Instrument Leak Detected: The Instrument Leak Detected icon is displayed when a leak in the instrument has been detected. Tooltip displays "Instrument Leak Detected".

If a leak is also detected in the Auto Sampler, the Tooltip displays "Instrument Leak and Auto Sampler Leak Detected".

Tube Lifter Down: The Instrument Tube Lifter icon is displayed when the tube lift is not in the up position. If the tube lifter is down, the Tooltip displays "Tube lifter is down".

If the tube lifter is in the intermediate state, the Tooltip displays "Tube lifter is intermediate".

Instrument Cover Open: The Instrument Top Cover icon is displayed when the top cover of the cytometer is not properly closed. Tooltip displays "Top cover is open".

Auto Sampler Door Open: The Auto Sampler Door Open icon is displayed when the Auto Sampler door is not properly closed. Tooltip displays "Auto Sampler door is open".

Connection status icons

Cytometer Not Connected: The Cytometer Not Connected icon is displayed if the cytometer is not connected. The tooltip displays "Attune not connected".

If both the cytometer and the Auto Sampler are not connected, the Tooltip displays "Attune and Auto Sampler are not connected".

Auto Sampler Not Connected: The Auto Sampler Not Connected icon is displayed if the Auto Sampler is not connected. The Tooltip displays "Auto Sampler is not connected".

Note: The connection status icons are only displayed if the software has previously been connected to an instrument as indicated by the database flag, and the conditions described above are met.

Automation Mode icon

Automation Mode: The Automation Mode icon is displayed when connected to an instrument and Automation Mode is enabled (page 65). The Tooltip displays “Automation mode”.

Camera status

The Camera status icons are displayed only if an Attune™ CytPix™ Flow Cytometer is in use.

Camera connected: If the Attune™ CytPix™ camera is connected and functioning properly, the Camera connected icon is displayed.

Camera is not connected: If the Attune™ CytPix™ camera is not functioning properly or the SBC network connection has failed, the Camera is not connected icon is displayed.

Connect status

The Connect status icons are displayed only if the software/instrument has been registered to a Connect cloud-based platform account.

Registered: If the instrument is registered, the Connect icon is displayed.

Registered and Signed in: If the instrument is registered and the user is signed in to their Connect account, the Connect icon is displayed with the user icon.

Connect connection error: If the instrument is registered with a Connect account, but the connection cannot be established, the Connect icon is displayed with the warning icon.

Tube and Plate modes

Tube mode – Plate present: When the Experiment is set to Tube mode and the Auto Sampler contains a plate, both the Tube and Plate icons are displayed and the Tube icon is selected. The Tooltip displays “Tube mode”.

Tube mode – Plate absent: When the Experiment is set to Tube mode and the Auto Sampler does not contain a plate, the Tube icon is displayed and the Plate icon is greyed out. The Tooltip displays “Tube mode”.

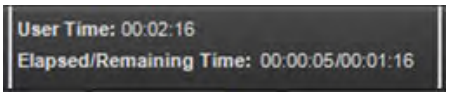
Plate mode – Plate present: When the Experiment is set to Plate mode and the Auto Sampler contains a plate, the Plate icon is selected and the Tube icon is greyed out. The Tooltip displays “Plate mode, plate present in Auto Sampler”.

Plate mode – Plate absent: When the Experiment is set to Plate mode and the Auto Sampler does not contain a plate, the Plate icon is selected, but it is greyed out. The Tooltip displays “Plate mode, no plate in the Auto Sampler”.

Note: When connected to an instrument, either the Tube or the Plate icon is always displayed.

Time

The *Time* panel displays the *User time* and *Elapsed/Remaining time.*



- **User Time:** User time shows the duration a user has been logged on to the instrument in the HH:MM:SS format. The time resets after the user logs out.
- **Elapsed/Remaining Time:** When an Experiment (Plate, Tube, or Manual well) is processing (Run or Record), the Status bar displays the Elapsed and Remaining Time in the HH:MM:SS format.

  The Elapsed Time counter only increments while processing. If the Experiment is paused, the Elapsed Time counter continues to increment.

  If the Experiment is paused, the Remaining Time counter also pauses.

  When the Experiment stops, the Elapsed/Remaining Time counter also stops and the time persists in the Status bar until another run is initiated (Tube or Plate, Run or Record) or the active Experiment is changed (this includes going to another application view such as the Main Menu, Login, etc.).

  When the counter is reset or cleared, the time will be blank.

Note: If the timer reaches 99:59:59 for the User time or the Elapsed/ Remaining time, the displayed timer stops and remains at this value. However, the time recorded in the log file displays the correct time.

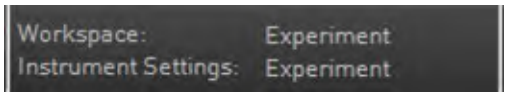
- The Run Protocol times for the Remaining Time counter are calculated as follows:
  - If the stop criteria are based on Event, the estimated Remaining Time defaults to the maximum possible Run time for the Normal well, Tube, or Manual well, given the specified Acquisition volume and Flow rate.
  - For stop on Volume, the time is calculated based on the time it takes to process the given volume at the specified Flow rate.
  - For stop on Time, the time for a given Normal well, Tube, or Manual well is based on the specified Stop time.
  - While processing a Normal well, Tube, or Manual well, the estimated time remaining is decremented based on the current rate towards completion of the Well.
  - For Plate experiments, additional time is added to the estimate of each well based on the number of rinses, number of mixes, Wait before record options, Mix mode, Aspiration rate, and Screening mode parameters (these time constants are model dependent).
  - For Plate experiments, the estimated Remaining Time is recalculated after each well.

Coordinates

The *Coordinates* panel shows the X and Y coordinates of the selected gate as described in “Moving and Resizing Gates”, page 100.

Workspace and Instrument Settings

The *Workspace and Instrument Settings* panel displays the open Workspace and the Instrument settings for the open sample.



- **Workspace:** Workspace displays the Experiment-level workspace name during acquisition. Depending on the Workspace selected from the Workspace dropdown (page 83) for the active Sample, this is “Experiment”, “Group”, or “Sample”.
- **Instrument Settings:** Instrument settings displays the lowest level instrument settings, either “Experiment” or “Sample”.

Acquisition

The *Acquisition status* panel is visible only during acquisition and provides information and feedback on the following parameters:

- Well location for a Plate or the Tube ID

- Calculated event rate (number of events/acquisition time)

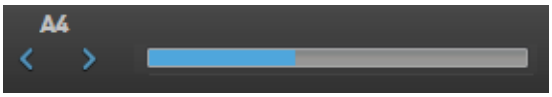
- Number of events acquired at the current time

- Current acquired volume and the total available volume

- Acquisition progress bar showing the progress towards the *Stop condition*, where 100% activates the stop condition

Analysis

The *Analysis status* panel is visible only when an active Experiment is open for analysis and the instrument is not acquiring. It consists of sample location (well location or Tube ID), navigation buttons, and the Analysis progress bar.



- Well location for a Plate or the Tube ID is displayed for active Experiments.
- The navigation buttons allow navigation between Samples for the current Experiment.

- The *Left navigation button* is disabled when the first Sample in an Experiment is active.
- If the last Tube sample in an Experiment is active, and the 400 Sample limit has not been reached, the *Right navigation button* will be active and add a new Tube sample, if clicked. The Tooltip for the button will display “Create new Sample”.

  In all other cases when the navigation buttons are enabled, the Tooltip displays either the previous or the next sample name as appropriate.
- If the 400 Sample limit has been reached, the right button will be disabled.
- The Analysis progress bar is displayed during instrument functions and while loading or refreshing data.
- If multiple operations requiring a progress bar occur simultaneously, the progress bar displays the operation requiring the longest time to be completed.
- In the collapsed state, the priority for display is: instrument function, refreshing, loading. As operations are completed, remaining operations are brought to the foreground.
- The Analysis Progress bar is not displayed for tasks that take less than 3 seconds to complete.

Size slider

The *Size slider* adjusts the size of a selected panel on the Main Application Window.

- The Size slider is contextual for the current view. If a panel that is not zoomable is in focus, the Size slider is disabled. The views for which this is applicable are:
  - Workspace
  - Preview
- The zoom settings persist per Experiment.
- Size adjustments apply only to the screen display and do not affect the print view.
- The scale of the slider runs from 10% to 400% magnification, with tick marks shown at 10%, 100%, and 400% magnification.
- The scale shows the current scale value rounded to the nearest percent.
- Click the **+ button** to increase the magnification to the nearest whole 10%. For example, from 93% to 100%.
- Click the **– button** to decrease the magnification to the nearest whole 10%. For example, from 93% to 90%.
- Drag the slider caret to adjust the plot size according to its position. The minimum drag rate is a single percent.
- A left mouse click on the scale, lower to upper tick, sets the caret to match the horizontal position.

Note: You can also adjust the Size slider by holding the **Ctrl** key down and turning the mouse wheel. This adjusts the view that is currently in focus or underneath the mouse cursor at the time you make the adjustment.

4. Ribbon tabs

Overview

Ribbon tabs

Ribbon tabs are contextual tabs that consolidate related software and instrument functionality in the Ribbon bar.

The availability of the Ribbon tabs in the Ribbon bar is dependent on the context of the software, i.e., the tabs are displayed only when the user needs them. Similarly, objects and controls that are not available as a function in a specific context are shaded gray in the tab.

Depending on the context of the application and the instrument type (Attune™ NxT or Attune™ CytPix™ Flow Cytometer), the Ribbon bar contains one or more of the following tabs:

- *File* (page 49)
- *Home* (page 50)
- *View* (page 53)
- *Workspace* (page 55)
- *Instrument* (page 61)
- *Experiment* (page 66)
- *Compensation* (page 70)
- *Statistics* (page 74)
- *Overlay* (page 78)
- *Image Settings* (page 79)
- *SAE* (page 81)

Note: When performing a Performance Test, only a limited set of tabs are available on the Main Menu.

On the Login screen, only the File tab is available.

SAE tab is available only when an SAE user is signed in.

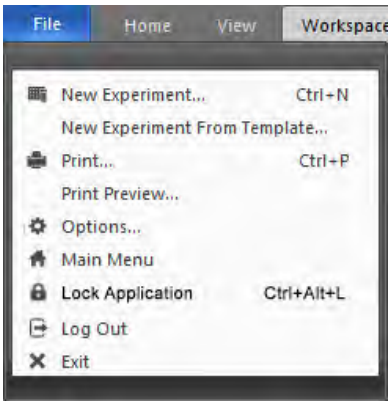
Image Settings tab is available only for Attune™ CytPix™ Flow Cytometers or when an Experiment that was created with an Attune™ CytPix™ Flow Cytometer is active.

File tab

File menu

File tab is unique amongst Ribbon bar tabs in that it functions as a dropdown menu.

Clicking **File** tab opens the *File menu*, which provides the following options:



New Experiment: Opens the *New Experiment* dialog (page 399), which allows you to create a tube only Experiment, a Plate Experiment, or an Experiment using imported files. The New Experiment option is enabled only when the instrument is not acquiring.

New Experiment from Template: Opens the *New Experiment from Template* dialog (page 405), which allows you to create a new Experiment from an existing template with pre-defined settings. This option is enabled only when the instrument is not acquiring.

Print: Opens the *Print* dialog (page 417), which is a modified Windows® Print dialog containing the standard Windows® print commands as well as the option to select Workspace, Heat Map, Results, Overlays, and Reports for printing. The Print dialog option is enabled only when the instrument is not acquiring. It is not enabled on the Main Menu (page 26).

Print Preview: Opens the *Print Preview* dialog (page 417), which displays the document as it will appear when printed and contains the controls for various printing options. The Print Preview option is enabled only when the instrument is not acquiring.

Options: Opens the *Options* dialog (page 472), which is used to customize the Attune™ Cytometric Software. Some options are user-specific, while others are application-specific (i.e., global to all users) and customizable only by an authorized user. This option is enabled only when the instrument is not acquiring.

Main Menu: Navigates to the starting Main Menu (page 26). This option is enabled only when the instrument is not acquiring.

Lock Application: Locks the Attune™ Cytometric Software. This option is visible only if the Attune™ Cytometric Software is configured to use the SAE mode. This option is enabled when a user is signed in. If no user is signed in, the command is disabled.

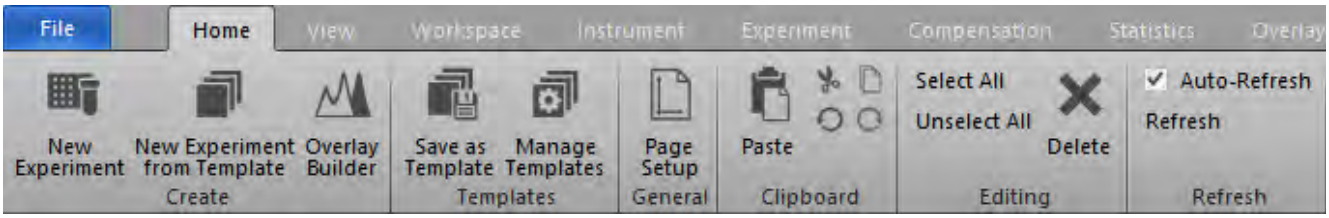
Log Out: Closes the software and logs out the current user, then displays the *Login* screen (page 19). During Log Out, all FCS files that have not been backed up are backed up automatically. The Log Out option is enabled only when the instrument is not acquiring.

Exit: Closes the Attune™ Cytometric Software. This option is enabled only if the instrument is idle.

Home tab

Home tab provides Experiment setup and Output options, and it is organized into six functional groups:

- *Create* (page 50)
- *Templates* (page 50)
- *General* (page 50)
- *Clipboard* (page 51)
- *Editing* (page 52)
- *Refresh* (page 52)



Create group

Create group allows you to create a new Experiment.

New Experiment: Opens the *New Experiment* dialog (page 399), which allows you to create a Tube-only Experiment, a Plate Experiment, or an Experiment using imported files. This button is always visible and enabled except during acquisition.

New Experiment from Template: Opens the *New Experiment from Template* dialog (page 405), which allows you to create a new Experiment from an existing template with pre-defined settings. This button is always visible and enabled except during acquisition.

Overlay Builder: Opens the *Overlay builder* dialog (page 172), which allows you to select the samples and plots for inclusion in an Overlay plot.

This button is only visible when the active experiment contains one or more samples and the experiment level workspace contains at least one plot. This button is not available during acquisition and it is disabled when a Compensation control is active.

Templates group

Templates group allows you to save an active Experiment as a template.

Save as Template: Opens the *Save as Template* dialog (page 428), which allows you to save the current Experiment as a template. This button is disabled on the Main Menu, Performance Test, and Instrument Configuration screens.

Manage Templates: Opens the *Manage Templates* dialog (page 430), which allows you to edit, import, export, and delete templates. This button is disabled when acquisition is in progress.

General group

General group allows you to customize the page layout and save an active Plate or Tube Experiment Workspace as a PDF.

Page Setup: Opens the *Page Setup* dialog (page 414), which allows you to customize the page layout (page size and orientation). This button is only available when Workspace, Reports, Results, Heat Map, or Overlay tab is active.

Clipboard group

The *Clipboard group* allows you to cut, copy, and paste selected objects and text in the work area, and to Undo and Redo previous actions.

Paste: Pastes the clipboard item into the current Workspace, Overlay gallery, Run Protocol, or Report. This option is only available when an item has previously been copied or cut into clipboard memory.

Cut: Removes the selected item from the Workspace, Overlay gallery, or Report and retains it in memory until another item is cut or copied into memory. This option is only available when a Workspace, Overlay gallery, or Report item is selected.

Copy: Retains the selected item in memory for pasting until another item is cut or copied in to memory. Workspace objects (including gates), Run Protocols, Overlays, Gallery plots, Report objects and Results can be copied into memory for pasting.

- Selecting Copy does not remove the item from the Workspace.
- When only a single statistics box, text box, or image is selected on the Workspace, the object is copied as a picture (enhanced metafile) to the Windows™ clipboard and as an HTML formatted object.

  The object (stats box, text box, or image) can be pasted to an external application as a picture (enhanced metafile) or as HTML (CF_HTML).
- When multiple workspace objects are selected, the selected objects are copied to the clipboard as an HTML formatted object and can be pasted to an external application as an HTML formatted object.

  When objects are pasted as HTML formatted objects, the individual elements are pasted to the external application in the order they are selected.
- If plots are within the selection, the plots are copied as a picture (enhanced metafile) to the Windows™ clipboard and can be pasted as a picture to an external application.
- When Workspace objects are copied to the clipboard as HTML formatted objects, temporary files are created in:

  C:\Users\<username>\AppData\Local\Temp\AttuneNxTClipboard

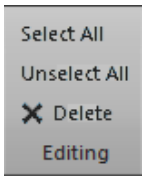
  When the application closes, these temporary files are deleted.

Undo: Negates the last undoable action in the order performed until the undo stack is empty. The Undo button is enabled only if there is an undoable action available. External functions including Save, Import, Export, or Print commands cannot be undone.

Redo: Reverses the Undo action in the order performed until all available tasks have been carried out. This option is only available when an Undo action has been successfully performed.

Editing group

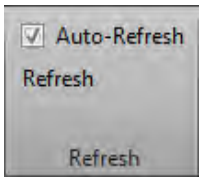
The *Edit group* contains shortcuts for selecting, unselecting, and deleting any of the objects in the work area.



- **Select All:** Selects and highlights all objects on the current Workspace, Results, or Heat Map. This button is available if a Workspace, Results, or Heat Map is open and one or more items are present.
- **Unselect All:** Deselects all previously selected objects on the current Workspace. This button is available if a Workspace is open and one or more items are currently selected.
- **Delete:** Deletes the selected item. This button is available when a Workspace object or a Heat Map location is selected.

Refresh group

The *Refresh group* contains options that allow the *Auto-Refresh* function to be turned off for inactive Workspaces, Heat Maps, and Results, thus requiring them to be manually refreshed.

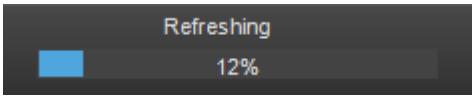


- **Auto-Refresh checkbox:** When **Auto-Refresh** is selected, all statistics in the Results tab are updated automatically in the background as the Workspace is modified. The Heat Map for all wells and tubes are also updated automatically.

  Un-checking the Auto-Refresh checkbox turns off the automatic updates. However, printing and exporting statistics force an automatic refresh of all out-of-date items.

  The Auto-Refresh checkbox is enabled and checked by default.
- **Refresh:** Forces a refresh of all out-of-date items on the Results, Heat Map items, and Overlays. The Refresh button is always enabled.

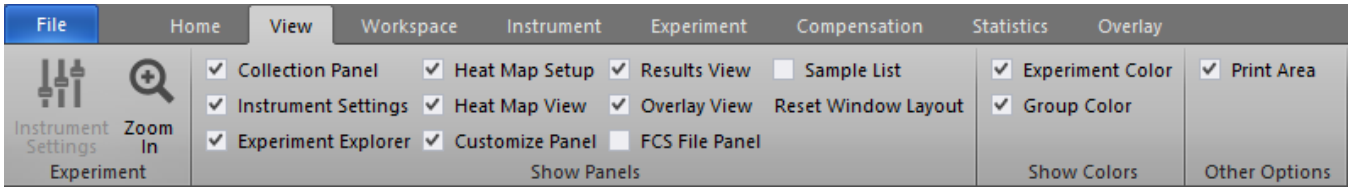
  You can also access the Refresh button using the keyboard shortcut **F5**.



View tab

View tab provides options to adjust the instrument settings and to control the overall look of the software. It is organized into four functional groups:

- *Experiment* (page 53)
- *Show Panels* (page 54)
- *Show Colors*
- *Other Options* (page 54)



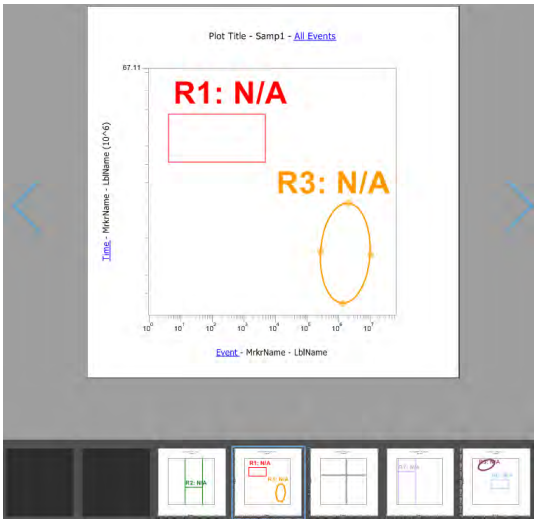
Experiment group

The *Experiment group* allows you to view and adjust instrument settings for an Experiment and to zoom in and out.

Zoom In: Displays all plots present in the *filmstrip area* of the Workspace view (page 91) and allows you to zoom in (maximize) each plot. This is a toggle button (see "Zoom Out") and is always visible; it is enabled when the Workspace view is active and contains plots.

The plots are displayed in the filmstrip area in the order that they were created. When you press the Zoom In button, the plots are maximized as follows:

- If no plots are selected, the first plot is maximized.
- If one plot is selected, the selected plot is maximized.
- If more than one plot is selected, the first plot created is maximized.

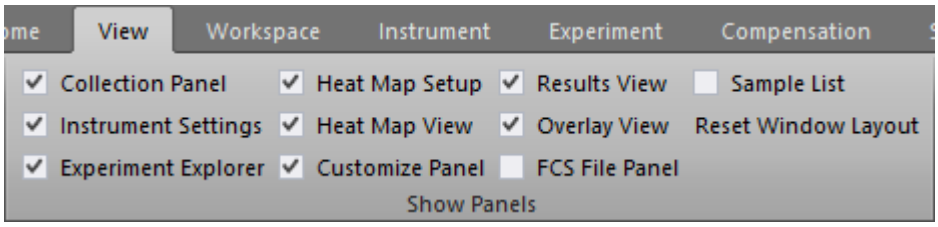


Zoom Out: Closes the Zoom In function and restores the normal Workspace view. This is a toggle button (see "Zoom In") and is always visible; it is enabled when the Workspace view is in the zoomed-in state.

Note: Alternatively, click the **Close** icon (✕) to restore the normal Workspace view.

Show Panels group

The *Show Panels group* allows you to open or close the following panels and views in the Main Application Workspace (page 36).



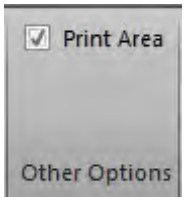
- *Collection Panel* (page 221)
- *Instrument Settings* (page 250)
- *Experiment Explorer* (page 189)
- *Heat Map Setup* (page 267)
- *Heat Map View* (page 138)
- *Customize Panel* (page 276)
- *Results View* (page 146)
- *Overlay View* (page 156)
- *FCS File Panel* (page 326)
- *Sample List View* (page 146)

- All Show Panels options are always visible and enabled. For the default positions and display status of the panels and views, see "Docking behavior" under "Main Application Workspace" on page 37.
- To close a panel or view, deselect its checkbox.
- To open a panel or view, select its checkbox. Selecting a checkbox restores the panel or view to the state it was in when it was last closed.
- If a panel or view is hidden (page 39), but not closed, its checkbox remains selected.
- By default, the FCS File Panel and Sample List are deselected, and all other panels and views are selected.
- Any changes you make for the Show Panels options persist for the next time you sign in.
- Click **Reset Windows Layout** to restore all panels and views to their default states.

Note: When hidden, the panel or view name appears as a tab on the side of the Main Application Workspace where the panel was docked.

Other Options group

The *Other Options group* contains the display options for the print area and for events.



The Print Area option is always visible and enabled.

Print Area

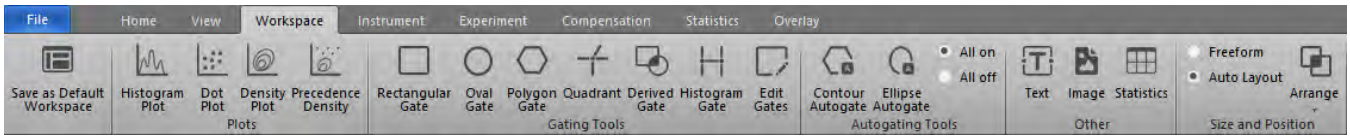
Allows you to display the Workspace view (page 82) as it will be printed on a page.

- Select the **Print Area** checkbox to display the page boundaries as gray lines drawn under an object.
- Deselect the **Print Area** checkbox to restore the normal Workspace view.

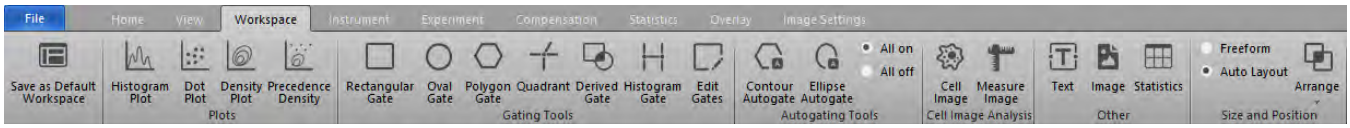
Workspace tab

Workspace tab provides tools for adding objects to a Workspace and aligning objects on the Workspace.

When using the Attune™ CytPix™ instruments or when the experiment supports imaging, the Workspace tab also contains the Cell Image Analysis tools.



Workspace tab (Attune™ NxT Flow Cytometer)



Workspace tab with Cell Image Analysis tools (Attune™ CytPix™ Flow Cytometer)

The Workspace tab is organized into the following functional groups:

- *Save as Default Workspace* (page 55)
- *Plots* (page 56)
- *Gating Tools* (page 57)
- *Autogating Tools* (page 58)
- *Cell Image Analysis* (page 59)
- *Other* (page 59)
- *Size and Position* (page 60)

Note: Cell Image Analysis group is available only for Attune™ CytPix™ Flow Cytometers or when the experiment supports imaging. This group is not visible for experiments created with Attune™ NxT Flow Cytometers, which do not have imaging capability.

Note: All options on the Workspace tab are disabled when a *Compensation Control Sample* is being analyzed.

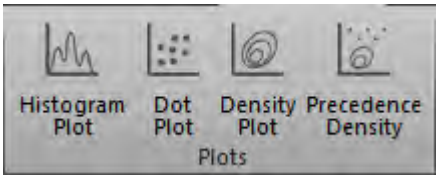
Save as Default Workspace option

Save as Default Workspace: Saves the active Workspace as the default for the current user. Any new Experiments and Plates will use this Workspace as the default Experiment Workspace.

The Save as Default Workspace option is disabled when the Workspace view (page 82) is not in focus.

Plots group

Plots group is used for creating plots of various types. Plots group controls are enabled when the Workspace view (page 82) is in focus and at least two parameters are active, except for the Histogram plot control, which requires only one active parameter.



Plots group contains the following controls. For more information on plot types, see "Plots" under "Workspace view" (page 95).

Histogram Plot: Adds a new single-parameter *Histogram plot* to the current Workspace. The Histogram plot displays the default parameter plotted against count (page 95).

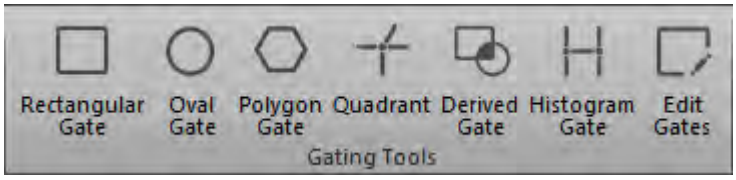
Dot Plot: Adds a new *Dot plot* to the current Workspace. The Dot plot displays two-parameter data where each axis represents the signal intensity of one parameter. Different colors are used to represent the parent gate of events that fall within bins on the plot (page 96).

Density Plot: Adds a new *Density plot* to the current Workspace. The Density plot displays two-parameter data where the colors represent the collection of events with the same intensity and each axis represents the signal intensity of one parameter (page 96).

Precedence Density Plot: Adds a new *Precedence Density plot* to the current Workspace. The Precedence Density plot is a combination of Dot and Density plots, where a gradient is used to indicate the number of events within each of the plot bins and color is used to display the parent gate of events present (page 96).

Gating Tools group

Gating Tools allow you to isolate a region in a selected plot for analysis. Gating Tools are available when the Workspace view (page 82) is displayed, unless otherwise specified.



- Dual-parameter gate types are enabled when the current Workspace contains at least one dual-parameter plot (i.e., Dot plot, Density plot, Precedence Density plot).
- Single-parameter gate types are enabled when the Workspace contains at least one Histogram plot.
- A maximum of 128 gates can be created on plots on a Workspace, except Quadrant gates, of which at least 1,000 can be created. The Gating Tools controls are disabled when the Workspace reaches the maximum number of gates allowed.

The Gating Tools group contains the following controls. For more information on gates, including instructions for creating and deleting gates, see "Gates" under "Workspace view" (page 97).

Rectangular Gate: Allows you to draw a *Rectangular gate* on a dual-parameter plot as described on page 101.

Oval Gate: Allows you to draw an *Oval gate* on a dual-parameter plot as described on page 102.

Polygon Gate: Allows you to draw a *Polygon gate* on a dual-parameter plot as described on page 103.

Quadrant Gate: Allows you to draw a *Quadrant gate* on a dual-parameter plot as described on page 104.

Derived Gate: Displays the *Derived Gate dialog*, which allows you to create gates based on Boolean operators (i.e., *Derived gates*) as described on page 421.

Derived Gate control is enabled when the Workspace view is displayed and the current Workspace has at least one gate present.

Histogram Gate: Allows you to draw a *Histogram gate* on a Histogram plot as described on page 106.

Edit Gates: Displays the *Edit Gates dialog*, which allows the editing of gate color, parent gate, gate math expression, the z-order in which gates are painted as described on page 424.

Edit Gates control is available when the Workspace view is displayed and the current Workspace has at least one gate present.

Autogating Tools group

Autogating Tools allow you to create auto region bounding rectangles in a selected plot, which determine the autogate target area when identifying populations based on autogate settings. If the criteria defined in autogate settings are met, the target area becomes autogated (see "Autogate gating rules", page 111). Autogates function in the same way as regular gates to allow you to isolate a region for analysis.

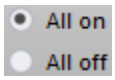
Autogating Tools are available when the Workspace view (page 82) is displayed, unless otherwise specified.

- Autogating Tools are enabled when the current Workspace contains at least one dual-parameter plot (i.e., Dot plot, Density plot, Precedence Density plot). If the Workspace does not contain any plots, then all buttons in the Autogating Tools group are disabled.
- A maximum of 128 gates, including autogates, can be created on plots on a Workspace, except Quadrant gates, of which at least 1,000 can be created. The Autogating Tools controls are disabled when the Workspace reaches the maximum number of gates allowed.

The Autogating tools group contains the following controls:

Contour Autogate: Allows you to create a *Contour auto region* on a dual-parameter plot as described on page 107.

Ellipse Autogate: Allows you to create an *Ellipse auto region* on a dual-parameter plot as described on page 109.



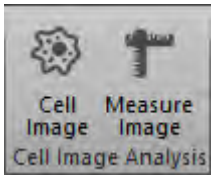
All on and **All off:** The **All on** button turns all autogated and un-autogated regions in the currently active Workspace ON. The default state for all autogates is to be in an ON state with the All on button selected.

The **All off** button turns all autogated and un-autogated regions in the currently active Workspace OFF.

The All on and All off button states persist with the Workspace.

Cell Image Analysis group

Cell Image Analysis group allows you to add a new cell image container to the Workspace and to perform size measurements on the image displayed in the image container.



The Cell Image Analysis tools are available only when using an Attune™ CytPix™ Flow Cytometer or when the experiment supports imaging. This group is not visible for experiments that were created using an Attune™ NxT Flow Cytometer, which does not have imaging capability.

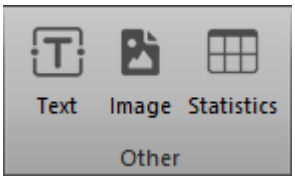
The Cell Image Analysis group contains the following controls:

Cell Image: Adds a new *Cell Image Container* to the current Workspace, which allows you to add a cell image specific to the sample (page 119). Cell image containers act like plots and can be batch printed with the Workspace (cell images cannot be printed from the Image view Image Gallery).

Measure Image: Allows you to draw a measurement tool on a cell image container to measure the area of the cell in the image (page 121). This control is only enabled if the Workspace contains a cell image container.

Other group

Other group is used to insert text and images into the Workspace, and to display the statistics that correspond to a selected plot in the Workspace.



The Other group contains the following controls, which are available when the Workspace view is in focus.

Text: Adds a new *Text box* to the current Workspace.

- The Text box displays the default text "New Text Box", which can be edited in place without opening a separate dialog box.
- The style and border of the Text box can be customized as described in "Customize Text Box Options" on page 290.

Image: Opens a standard Windows file selection dialog for selecting an image file to add to the Workspace.

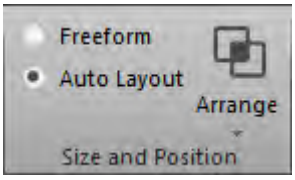
- The image file cannot be larger than one page and will be scaled to fit on the page preserving the aspect ratio, if necessary.
- The image types available for insertion include JPG, GIF, BMP, PNG, TIF, and Windows enhanced metafile.

Statistics: Adds a new *Statistics box* to the current Workspace.

- If no plot is selected, clicking on the Statistics button inserts a *Workspace statistics table* in the default Workspace location.
- If one or more plots are selected, then one *Plot Statistics box* is added to each selected plot immediately below the relevant plots as described in "Workspace statistics", page 115.

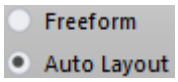
Size and Position group

The *Size and Position group* provides tools that assist in creation and customization of Workspaces, and contains the buttons listed below.



Layout

Layout controls consist of two radio buttons, which control the view mode of the Workspace layout as described in "Workspace view modes" on page 88.



- **Auto Layout**: In this mode, objects are placed into grid slots. The number of available slots per page is defined by the *Grid Size* options (page 60). Auto Layout is the default mode.
- **Freeform:** In this mode, objects can be freely resized and moved anywhere on the Workspace. There is no automatic arrangement or alignment of objects on the page.

Arrange

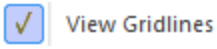
Arrange opens the *Arrange Tools* dropdown menu, which contains the tools to arrange, align, and size multiple Workspace objects.

- The Arrange Tools commands are available only when multiple items are selected on the Workspace, unless otherwise stated.
- The View Gridlines and Grid Size options are only enabled in the Auto Layout mode; all other options are enabled only in the Freeform mode.

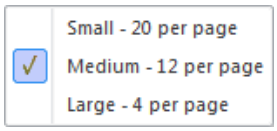
Grid options

Grid options are enabled only when the Auto Layout mode is selected using the Layout Radio Buttons (page 60).

View Gridlines: Selecting this option displays the lines that form the grid onto which the Workspace objects are snapped. By default, the gridlines are displayed.



Grid Size: Opens the *Grid Size dropdown* menu, which contains the options to define the number of grid slots available per page. A check mark appears next to the currently selected size.



The default selection is 12 grid slots per page.

You can select a larger grid size allowing only 4 items per page, or a smaller grid size allowing up to 20 items per page at the default sizes.

Instrument tab

Instrument tab allows you to view and export instrument performance and maintenance data, to update instrument configuration, to view and modify system instrument settings, and to perform instrument maintenance tasks. It is organized into five functional groups:

- *Setup* (page 61)
- *System History* (page 62)
- *Functions* (page 62)
- *Service* (page 64)
- *Automation* (page 65)

Note: Most of the buttons in the Instrument tab are disabled in Automation mode (page 65), with a few becoming enabled when Plate Acquisition is paused in Automation mode.

Setup group

The *Setup group* allows you to view instrument data and manage configuration files.

Configuration: Opens the *Configuration* module (page 331), which allows you to manage instrument configuration files and filters. This button is always visible and enabled except during acquisition.

System Instrument Settings: Opens the *System Instrument Settings* dialog (page 265), which allows you to modify Area scaling factor (ASF) and Window extension settings.

This button is visible only to users that have privileges to modify System Instrument Settings.

Access to System Instrument Settings is disabled when the instrument is acquiring a sample or a plate, running Baseline/PT, while paused, and when set to automation mode.

Laser Power: Allows users with Service accounts to turn the lasers on and off. This button is available only for Service accounts (i.e., Thermo Fisher Scientific service team).

System History group

The *System History group* allows you to view System Log and Performance History report.

System Log: Opens the *System Log* dialog (page 462), which allows you to view system transactions. The information displayed in the System Log depends on the type of account that you have: service, administrator, system administrator, or general user account. This button is always visible and enabled.

Performance History: Opens the *Performance History* report (page 364), which allows you to view the pass/fail status of all Performance Tests. This button is always visible and enabled except during acquisition.

Functions group

The *Functions* group allows you to initiate the instrument functions.

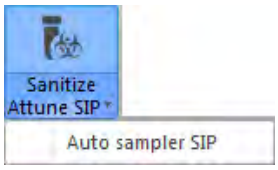
Stop: Allows you to stop any acquisition or instrument function, except the *Calibrate*, *Self Test*, and *Rinse* instrument functions. This button is always visible and enabled.

- If you click **Stop** during the following instrument functions, you must run Startup before running the instrument again:
  - *Startup* (page 63)
  - *Unclog* (page 63)
  - *Shutdown* (page 63)
  - *Deep Clean* (page 63)
  - *Decontaminate System* (page 63)
- If you click **Stop** during the Rinse function, the instrument ignores the Stop command.
- If you click **Stop** while running a Plate Sample, the instrument stops the acquisition and aborts the Plate.
- If you click **Stop** during all other instrument functions, the instrument forces a Rinse before performing any other operation.
- Clicking **Stop** when the instrument is in the Automation mode toggles the Automation mode to 'OFF'.

Recover Sample: Allows you to recover unused sample from the sample loop. This button is enabled when the instrument status is idle (i.e., not actively acquiring) and there is enough sample within the sample loop or enough preloaded sample to recover. When the button is pressed, the remaining sample is returned into a tube (from the sample loop) or back into the sample well (preloaded sample in a plate).

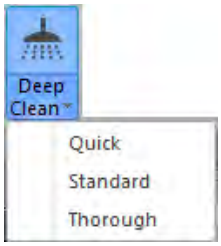
Rinse: Opens the *Rinse* dialog, which allows you to rinse the Sample lines. This button is always visible; it is enabled when the instrument status is idle and not actively acquiring a plate.

Sanitize Attune SIP : Opens the *Sanitize* dialog, which allows you to sanitize the instrument SIP (sample injection port) and sample lines, or the Auto Sampler SIP and Sample lines. This button is always visible; it is enabled when the instrument status is idle and not actively acquiring.



This is a split button: Click **Sanitize Attune SIP** or select **Auto Sampler SIP** from the dropdown menu.

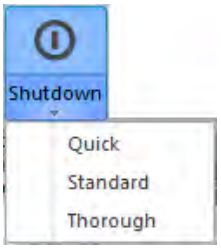
Deep Clean: Opens the *Deep Clean* dialog, which allows you to thoroughly wash the system sample lines and flow cell. This button is always visible; itis enabled when the instrument status is idle and not actively acquiring. It is disabled when a Plate run is paused.



This is a split button: Click **Deep Clean** to perform a standard clean cycle, or select **Quick**, **Standard**, or **Thorough** from the dropdown menu.

Startup: Opens the *Startup* dialog, which allows you to prime the system fluidics. This button is always visible; it is enabled when the instrument status is idle and not actively acquiring, or when the instrument is in the "sleep" state. It is disabled when a Plate run is paused.

Shutdown: Opens the *Shutdown* dialog, which allows you to sanitize and shut down the instrument. This button is always visible; it is enabled when the instrument status is idle and not actively acquiring. It is disabled when a Plate run is paused.



This is a split button: Click **Shutdown** to perform a standard shutdown cycle, or select **Quick**, **Standard**, or **Thorough** from the dropdown menu.

De-bubble: Opens the *De-bubble* dialog, which allows you to clear the sample lines and flow cell of bubbles. This button is always visible; it is enabled when the instrument status is idle.

Unclog: Opens the *Unclog* dialog, which allows you to unclog the Sample lines. This button is always visible; it is enabled when the instrument status is idle and not actively acquiring.

Decontaminate System: Opens the *Decontaminate* dialog, which allows you to decontaminate all system fluid lines. This button is always visible; it is enabled when the instrument status is idle and not actively acquiring. It is disabled when a Plate run is paused.

If an Attune™ Auto Sampler is connected to the system and powered on, Decontaminate System initiates a decontamination cycle for the cytometer and the Auto Sampler.

For detailed instructions on instrument decontamination, refer to the *Attune™ NxT Flow Cytometer Maintenance and Troubleshooting Guide* (Pub. No. 100024234), available for download at **thermofisher.com**.

Calibrate Auto Sampler: Opens the *Auto Sampler Calibration* dialog, which allows you to calibrate the Attune™ Auto Sampler. Calibrate Auto Sampler button is visible only when there is an auto sampler connected to the instrument. The button is enabled when the instrument status is idle and not actively acquiring; it is disabled when a Plate run is paused.

Note: Auto Sampler calibration automatically occurs every 30 days in Attune™ Cytometric Software versions 2.5 or greater.

Self Test (System Test): Opens the *System Test* dialog, which allows you to run system diagnostics. This button is visible only to Service accounts; it is enabled when the instrument status is idle and not actively acquiring. It is disabled when a Plate run is paused.

Service group

The *Service group* contains functions for servicing the instrument.

Laser Delays and Calibrate PZT functions are available only to users with Service accounts (i.e., Thermo Fisher Scientific service team).

Export Logs for Service function is available to all users.

Export Logs for Service: Opens the *Export Logs for Service* dialog (page 447), which allows you to export selected logs to a single zip file based on the specified date range. This button is enabled, if the instrument status is idle or when a Plate run is paused. It is disabled, when moving between wells.

Test Plate: Opens the *Test Plate* dialog (page 451), which allows you to validate a plate to ensure that the autosampler probe position is in the correct location in all four corners of the plate.

This button is only visible if the system is connected to or set to a CytKick™ Autosampler and the autosampler is powered on.

This button is enabled if the instrument status is idle and an autosampler is connected and powered on. This function will be disabled if the instrument is paused or set to automation mode.

Automation group

The *Automation group* allows you to put the application under the control of external software to allow the automated loading and running of plates. This group is only visible if a TCP/IP Communication port has been specified in the command line (i.e., attune.exe /p:8225) or in the *Configuration options* dialog (see "Automation Communication Settings", page 516).

Connect Robot: Sets the software in *Automation mode*, which puts the application under the control of external software. This button is only enabled if there is an Auto Sampler present and the instrument status is idle.

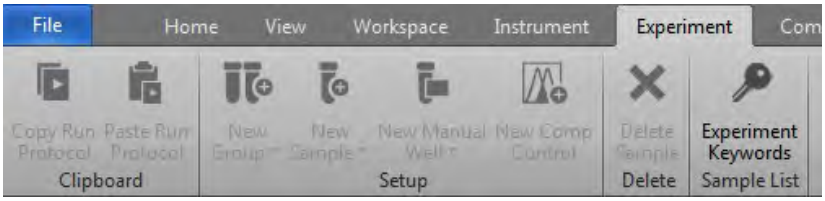
- When the Connect Robot button is toggled ON, the software is set to Automation mode and the button is highlighted blue.
- While in Automation mode:
  - All fluid functions are disabled, the Experiment cannot be changed, and navigating away from the Workspace view (i.e., Attune™ desktop) is disabled:
  - All File Menu commands except for Exit are disabled.
  - The Instrument ribbon setup buttons are all disabled unless a plate run is paused.
- If the instrument is disconnected from the external device, the toggle state is automatically turned OFF and the Connect Robot button is disabled.
- When the Connect Robot button is toggled off, the software accepts normal user inputs as described throughout the document.

Map Plates: Opens the *Plate Mapping dialog* (page 434), which allows you to map defined templates to barcodes to be used while in Automation mode.

Experiment tab

Experiment tab provides tools to define the Experimental layout for tubes and plates. The Experiment tab is visible only when the *Heat Map view* (page 138) is active.

Any changes that you make to the Experiment layout in the Experiment tab are automatically reflected in real-time in the *Experiment Explorer* (page 189).



Experiment limits

Plate-based Experiments can contain Samples in wells and/or tubes. Unless otherwise specified, in this section *Sample* is used to mean Samples in tubes and in wells.

All Samples on a Plate Experiment must total ≤ 400 wells and tubes. This limits the number of Tube samples per plate to:

- ≤ 304 Tube samples for 96-well plates
- ≤ 16 Tube samples for 384-well plates

Changes made to the Experiment within the Heat Map view will be automatically reflected within the Experiment Explorer.

Experiment tab controls

Copy Run Protocol: Copies the Run Protocol for the selected samples to the clipboard. This button is always visible, but it is enabled only when samples are selected.

- If you select multiple samples that have different Run Protocols, the software copies all protocols and maintains the relative positions of the selected samples.

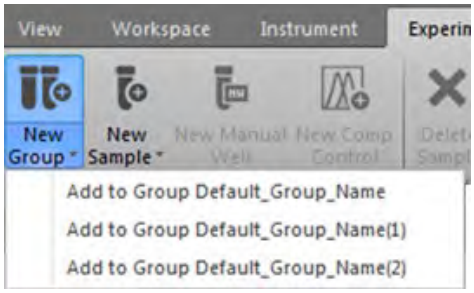
Paste Run Protocol: Pastes Run Protocol from the clipboard into the selected samples. This button is always visible, but it is enabled only when one or more Run Protocols are copied to the clipboard and samples are selected.

- If you copy only one Run Protocol to the clipboard, the software pastes that Run Protocol into all selected samples.
- If you copy multiple Run Protocols to the clipboard, this button is active only when the same array of samples is selected. The software pastes each unique Run Protocol into the selected sample of the same position in the array.
- If the Run Protocol is copied from and pasted into plates of different types, the software checks all entries for validity following rules set in "Run Protocol Options" (page 238).

  For example, if the pasted well volume is greater than allowed in the selected well, the software defaults to the maximum volume allowed.

New Group: Adds the selected Samples to a new or existing *Group*. This button is always visible, but it is enabled only when samples are selected. The selected samples can be un-programmed or marked as part of the Experiment.

The New Group button is a split button that includes a dropdown menu option.

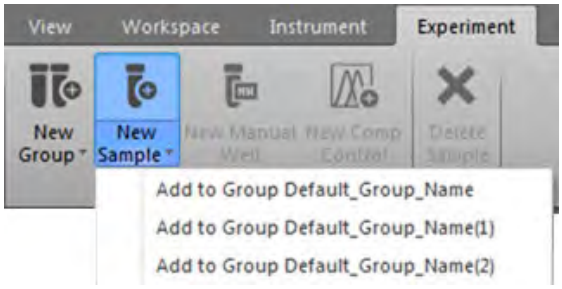


- To create a new Group and add the selected Samples to it, click the main portion of the **New Group** button.
- To add the selected Samples to an existing Group, select the Group from the **New Group dropdown** menu.

Note: Each Experiment that has Samples assigned consists of one or more Groups. Each group can contain up to 400 Samples, each of which can be derived from a well within a plate or from a tube.

New Sample: Marks selected blank tubes or wells as Samples and adds these new Samples to a Group. This button is always visible, but it is enabled only when blank tubes and/or wells are selected.

The New Sample button is a split button that includes a dropdown menu option.

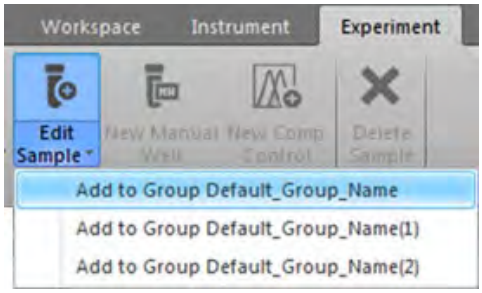


- To add the new Samples to the most recently created Group, click the main portion of the **New Sample** button. If no groups exist, the software creates a new Group.
- To add the new Samples to an existing Group, select the Group from the **New Sample dropdown** menu.

Note: The New Sample button is a toggle button, which changes to "Edit Sample" when one or more wells and/or tubes already marked as Samples are selected (see "Edit Sample", page 68).

Edit Sample: Moves selected Samples to a different Group. This toggle button (see "New Sample" above) is always visible, but enabled only when Samples are selected.

The Edit Sample button is a split button that includes a dropdown menu option.

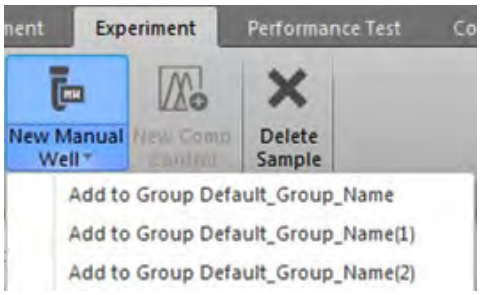


- To move the selected Samples to a different Group, select the Group from the **Edit Sample** dropdown menu.
- If your selection also includes new Samples, the button remains **Edit Sample.** New Samples are added to the same Group selected for the existing Samples.

New Manual well: Creates a new Sample in the selected wells marked as Manual wells in the most recently created Group. If no Groups exist, a new Group is created and the Manual Well samples are added to this group.

This button is always visible, but it is enabled only when one or more wells are selected. The button is disabled for Tube-based Experiments or if Tube locations are selected on the Heat Map in a Plate-based Experiment.

The Edit Sample button is a split button that includes a dropdown menu option.



- The software marks Manual wells with a "MW" icon MW in the top left corner of the well.

- The tooltip for the Manual well button reads "Defines a well that has both 'Run' and 'Record' modes."

New Comp Control: Opens the *Compensation Setup dialog* (page 379).

- Compensation Setup dialog allows you to select the compensation source from wells or tubes. If blank wells are selected before clicking **New Comp Control**, the compensation dialog box automatically selects wells as the compensation source and the selected wells are pre-populated with the Compensation controls. By default, all possible Compensation controls are selected. Note that each parameter selected for compensation needs to have an associated well location before the dialogue box will close.
- Compensation wells are indicated within the software by the "CS" icon in the top left corner of the well (shown below). The well is also labeled with the fluorescence channel or unstained control mapped to the compensation well.

Delete Sample: Deletes the selected Samples, which returns the Samples to the undefined state and removes all associated sample data (Workspaces, Run Protocols, and FCS files). This button is always visible, but it is enabled only when Samples marked as part of an Experiment are selected.

Experiment Keywords: Opens the *Experiment Keywords dialog* (page 445), which allows you to add, remove, or edit experiment keywords. This button is only enabled when an Experiment is active, the Workspace is in view, and the system is not acquiring, paused, or in the Automation mode.

Hot keys

The following hot keys can be used alone or in combination to copy and paste, Run Protocols and delete selected samples.

- **Ctrl+C** combination on the keyboard copies the selected Run Protocols to the clipboard as described in "Copy Run Protocol" (page 66).
- **Ctrl+V** combination on the keyboard pastes the Run Protocol in the clipboard to the selected sample as described in "Paste Run Protocol" (page 66).
- **Delete** key on the keyboard deletes selected samples as described in "Delete Sample" (page 69).

Compensation tab

Compensation tab contains controls for setting compensation, selecting the compensation source, and changing the view of Compensation samples.

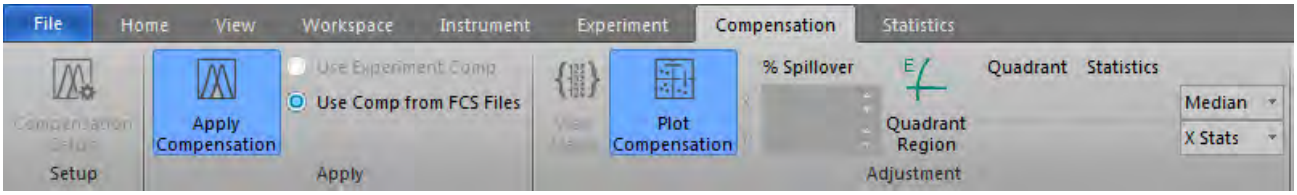
The contents of the Compensation ribbon tab vary depending on whether a *Sample Workspace* or a *Compensation Workspace* is displayed (see below).

Depending on the context, the Compensation tab contains the following functional groups:

- *Setup* (page 70)
- *Apply* (page 71)
- *Adjustment* (page 72)

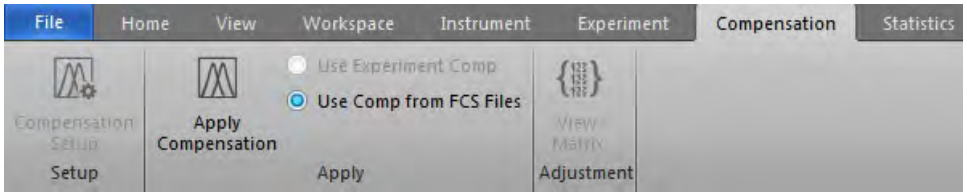
Compensation tab on Compensation Workspace

The Compensation tab fully expanded on a Compensation Workspace is shown below.



Compensation tab on Sample Workspace

The Compensation tab fully expanded on a Sample Workspace is shown below.



Note: For more information on the compensation functions of the Attune™ Cytometric Software, see "Compensation" on page 378.

Setup group

Setup group contains the *Compensation Setup* button. When the Compensation Workspace is active, additional controls are visible that allow you to select *Histogram View*. The Setup group is always visible.

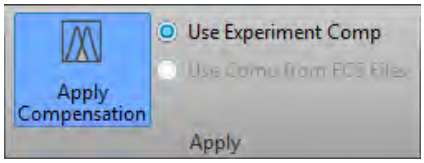
Compensation Setup: Opens the *Compensation Setup dialog* as described on page 379. The Compensation Setup button is always visible, but it is enabled only when a Plate or Tube Experiment is active.

Apply group

Apply group includes controls to turn compensation on and off for the selected Workspace and to choose between Experiment Compensation or compensation loaded from the FCS headers. The Apply group is only visible on a Sample Workspace.

Apply Compensation: When selected, all data shown on the Workspace and used for calculating statistics is compensated. When deselected, all data shown on the Workspace and used for calculating statistics is the raw uncompensated data.

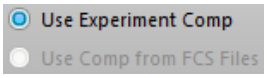
- The Apply Compensation button is ON by default, if compensation is available. The button state persists on a per Experiment basis.



- If Experiment-level compensation or FCS file compensation do not exist, **Apply Compensation** opens the *Compensation Setup dialog* (page 379).

Note: The Apply Compensation button is a state button; it remains ON until turned OFF. When active, the selected compensation option is applied. If an alternative radio button is selected, the settings are updated automatically.

Use Experiment Comp/Use Comp From FCS Files



Use Experiment Comp and **Use Comp from FCS Files** radio controls are only visible and enabled when a Sample is active. These options are disabled on the Compensation Workspace and when compensation is not available from either the Experiment or the selected FCS file.

The button state persists on a per Experiment basis except for Samples where the selected compensation is invalid.

Use Experiment Comp: The *Use Experiment Compensation* option uses the *Compensation Matrix* defined using Compensation controls or an imported compensation settings file. This is the default selection when Experiment-level compensation exists.

Use Comp From FCS Files: The *Use Compensation From FCS Files* option uses the Compensation Matrix values stored within the FCS header. This is the default when Experiment compensation does not exist

- If an FCS file does not contain a Compensation Matrix and the Use Comp From FCS Files selection is set, then Experiment Compensation option is used for that Sample, if available.
- If an Experiment-level compensation is incompatible with an FCS file in the Experiment (due to importing an FCS file), then Use Compensation From FCS Files option is used for that Sample, if available.
- In these cases, the invalid option is disabled. If the selected option is disparate to the Compensation used for a selected Sample (due to the Sample-level override), the buttons display the indeterminate state.

Adjustment group

Adjustment group includes controls to view the *Compensation Matrix* and to enable the *Plot Compensation* function.

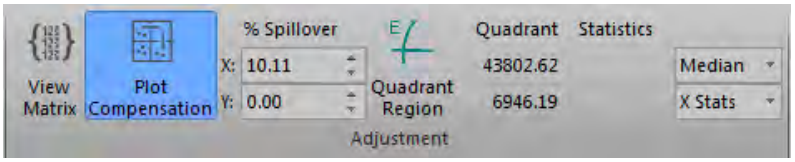
View Matrix: Opens the *Matrix* dialog (page 393), which shows the active compensation associated with the current Experiment or file. The View Matrix button is always visible, but it is only enabled when a Plate or Tube Experiment is active and either Experiment-level compensation settings exist or there is an active Sample containing an FCS file.

Plot Compensation: Enables *On-Plot Compensation Adjustment* (page 396) and controls the visibility of the *Compensation Adjustment Tools* (below).

- The Plot Compensation button is deselected (OFF) by default.
- The button is enabled and visible only when **Apply Compensation** is selected. It is not visible in the Compensation Workspace, unless the **View Results** button is selected.
- Any changes made to compensation persist for both the Experiment-level compensation and the FCS compensation.
- The Plot Compensation button is a state button; it remains ON until it is turned OFF or if the selection of Workspace or the Sample changes.

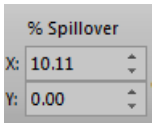
Compensation adjustment tools

- When **Plot Compensation** is turned **ON**, a collection of compensation adjustment tools are displayed on the Compensation ribbon tab.



- The Compensation Adjustment tools allow the manual adjustment of a selected plot, the ability to add a Quadrant gate to a plot, and the display of Quadrant statistics as described below.
- When **Plot Compensation** is turned **OFF**, these controls are not visible.

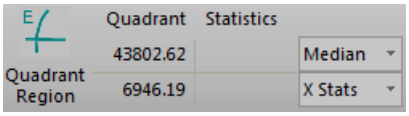
% Spillover Adjustment: These tools consist of two controls that allow adjustment of the compensation or the spillover matrix.



- These controls are only enabled when a dual-parameter plot with two fluorescence parameters is selected in a Sample Workspace or on the Compensation Workspace when "View Results" is turned ON.
- The top control corresponds to the X-axis and the bottom control corresponds to the Y-axis. The up/down arrows of the control or the keyboard move the spillover matrix value by increments of 1 with each click or continuously by maintaining the click.
- Pressing the **Shift** while clicking on the arrows or when using the keyboard up/down arrow buttons moves the value by increments of 0.1.
- The textbox accepts numbers from 0 to 100 and displays 2 decimal places.

Quadrant Gate: Allows the insertion of a Quadrant gate on a dual-parameter plot as described on page 57.

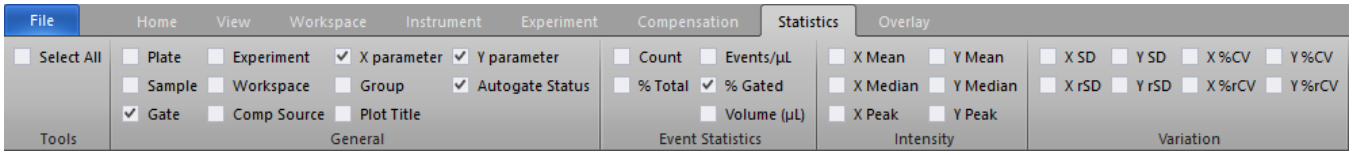
Quadrant Statistics: Allows the selection of the mean or the median values and the Y-axis or X-axis statistics.



- By default, the median and the X-axis statistics are selected.
- The statistics values are displayed within a representation of a quadrant gate. When selected, the X-axis statistics are shown in the lower left and lower right quadrants, and the Y-axis statistics are shown in the lower left and upper left quadrants.
- The values shown are based on the quadrant set on the currently selected plot.
- For the statistics to be displayed, a single dual-parameter plot containing only fluorescence parameters (not scatter) must be selected, and the plot must display a quadrant. In addition, an FCS file that can be compensated must be present. If these requirements are not met, the control does not show any statistics.
- If a statistics value cannot be calculated, the respective quadrant will display “N/A” (i.e., not applicable).

Statistics tab

Statistics ribbon tab allows you to select which statistic is displayed on the Plots, Workspaces, and the Results view. The statistics are grouped within the ribbon according to their statistical type (*general*, *event statistics*, *intensity*, and *variation*).



The Statistics tab displays the following functional groups:

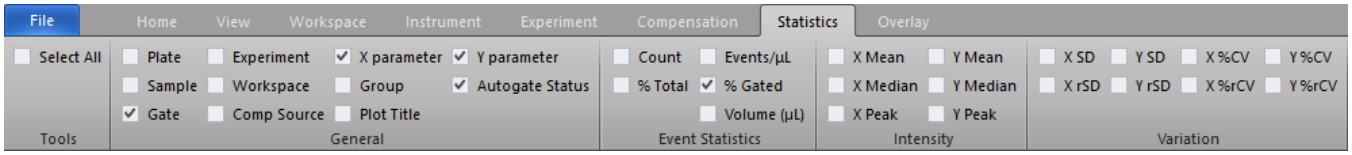
- *Results* (page 75)
- *Tools* (page 76)
- *General* (page 76)
- *Event Statistics* (page 77)
- *Intensity* (page 77)
- *Variation* (page 77)

Note: The Statistics tab behaves differently (as explained below) depending on which Application tab is in focus or which object within a Workspace is selected. The entire Statistics tab is disabled when the *Heat Map view* is in focus (page 138).

Statistics tab behavior

Behavior in Workspace view

When the *Workspace view* (page 82) is in focus and a statistics table is **not** selected, only a single statistic can be selected at a time. This selection applies to the statistic displayed on all plots. In the Workspace view, the Results group (page 75) is not visible and the General group (page 76) is not enabled.



- **Plot statistics:** By default, all gates show the *% Gated* statistic. The selection persists on a per Workspace basis.

  When a statistic option is checked (i.e., selected), all plots with gates are automatically updated to display it. Selecting a new statistic automatically un-checks the previously selected one.

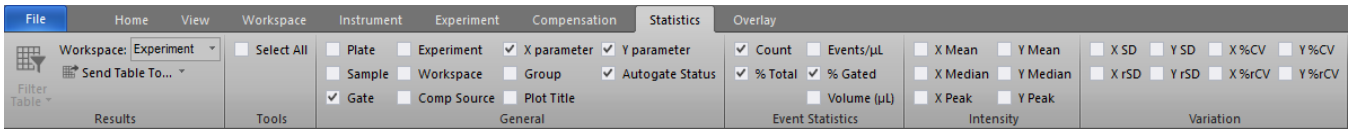
- **Statistics tables:** Both individual Plot statistics and Workspace statistics can be selected at the same time. When multiple statistics tables are selected, the selection and deselection of statistics in the ribbon applies to all tables.

  Where a statistic is selected in one statistics table and not in another, the checkbox will be in the indeterminate state.

  The selection or deselection of the Experiment name, Group name, Sample name, and plot title will also update the selections in the *Customize statistics* panel.

Behavior in Results view

- When the *Results view* (page 146) is in focus, all statistics can be checked and unchecked. The Results group (below) is only available when the Results view is in focus.



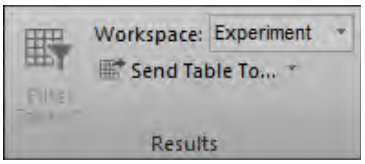
- A statistical value is displayed in the Results table when the associated checkbox is checked in the Statistics ribbon. Unchecking the checkbox removes the entire column from the Results table.

Persistence of selections

The persistence of selections in the Statistics tab is context dependent.

- For Workspace selections, the selections for on-plot statistics and for each statistics box persist on a Workspace level.
- For the Results view, all selections (including the filter selection) persist on an Experiment-level.

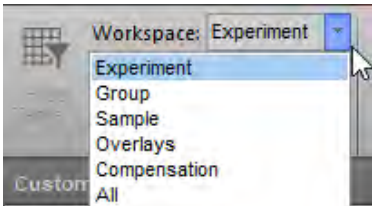
Results group



The *Results group* contains the controls to turn the *Results table filter* ON and OFF, to edit the filter criteria, to select the Workspace on which the Results table is based, and to select where to send the *Results table* (page 152).

The Results group is only visible when the Results view is in focus.

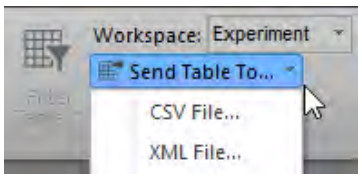
Workspace filter dropdown



The *Workspace filter* dropdown allows you to select the Workspace on which the Results table is based.

The options include **Experiment**, **Group**, **Sample**, **Overlays**, and **All**. When **All** is selected, all options are displayed. By default, the **Experiment** is selected.

Send Table To...dropdown



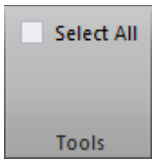
The *Send Table To…* function exports all results that are displayed in the Results view, including all relevant statistics for the region and gate type, and filters; grouping is not included.

The **Send Table To…** dropdown allows you to export the results from your experiment as a **CSV file** or as an **XML file**.

By default, all experimental metadata, statistics, and keywords are exported as an XML file.

Tools group

The *Tools group* allows you to enable all statistics with a single click.



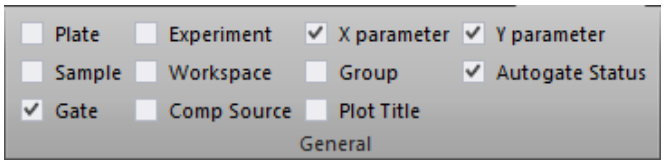
Select All: Enables all statistics. The Select All checkbox is enabled only when the Results view is in focus or if a Statistics box is selected on the Reports view or the Workspace view. The checkbox is disabled for all other instances.

When **Select All** is checked, then all statistics are checked. If any statistics are unchecked, Select All checkbox becomes unchecked.

Deselecting the checkbox unchecks all statistics.

General group

The statistics in the *General group*, when checked, are displayed on plots and in the Results table.

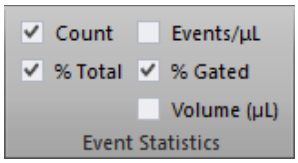


If a statistics table is not selected, this group is disabled on the Workspace view.

- **Plate**: Displays the Plate name (enabled for plate experiments only)
- **Experiment:** Displays the Experiment name.
- **Group:** Displays the Group name.
- **Sample:** Displays the Sample name.
- **Workspace:** Displays the Workspace from where the statistics is calculated.
- **Comp Source**: Displays the Compensation Source for the Results, either calculated in the Experiment or embedded with FCS files
- **Plot Title:** Displays the plot title for all gates.
- **Gate:** Displays the name of the gate for which the statistics is calculated.
- **X Parameter:** Displays the X parameter name as shown on the plot that contains the gate.
- **Y Parameter:** Displays the Y parameter name as shown on the plot that contains the gate. If the Y parameter is from a Histogram plot, *Count* or *% of max* is displayed as the parameter name, depending on what is selected for that plot.
- **Autogate Status:** Displays the status of an autogate.

Event Statistics group

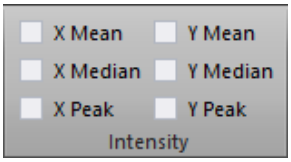
The statistics in the *Event Statistics group*, when checked, are displayed on plots and in the Results table.



- **Count:** Displays the number of events in gates.
- **% Total:** Displays the percentage of total events.
- **Events/µL (Concentration):** Displays the number of events per µL.
- **% Gated:** Displays the percentage of events within the gate.
- **Volume (µL):** Displays the volume of sample that the data is derived from.

Intensity group

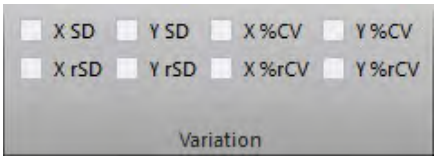
The statistics in the *Intensity group*, when checked, are displayed on plots and in the Results table.



- **X Mean:** Displays the arithmetic mean for the X-axis of a gate.
- **Y Mean:** Displays the arithmetic mean for the Y-axis of a gate. When the gate is on a single-parameter plot, the entry on the Results table is "N/A".
- **X Median:** Displays the median for the X-axis of the gate.
- **Y Median:** Displays the median for the Y-axis of the gate. When the gate is on a single-parameter plot the entry on the Results table is "N/A".
- **X Peak:** Displays the peak channel (mode) for the X-axis of the gate.
- **Y Peak:** Displays the peak channel (mode) for the Y-axis of the gate. When the gate is on a single-parameter plot, the entry on the Results table is "N/A".

Variation group

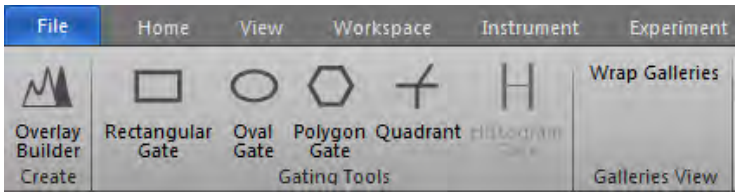
The statistics in the *Variation group*, when checked, are displayed on plots and in the Results table.



- **X SD:** Displays the standard deviation for the X-axis of a gate.
- **Y SD:** Displays the standard deviation for the Y-axis of a gate. When the gate is on a single-parameter plot, the entry on the Results table is "N/A".
- **X %CV:** Displays the %CV (percentage coefficient of variation) statistics for the X-axis of a gate.
- **Y %CV:** Displays the %CV (percentage coefficient of variation) statistics for the Y-axis of a gate. When the gate is on a single-parameter plot, the entry on the Results table is "N/A".
- **X rSD:** Displays the robust standard deviation statistics for the X-axis of a gate.
- **Y rSD:** Displays the robust standard deviation statistics for the Y-axis of a gate. When the gate is on a single-parameter plot, the entry on the Results table is "N/A".
- **X %rCV:** Displays the percentage robust CV (coefficient of variation) statistics for the X-axis of a gate.
- **Y %rCV:** Displays the percentage robust CV (coefficient of variation) statistics for the Y-axis of a gate. When the gate is on a single-parameter plot, the entry on the Results table is "N/A".

Overlay tab

Overlay ribbon tab contains the tools used for creating *Overlays*, which are used for comparing data by superimposing selected plots. Each Overlay consists of an *Overlay plot* and an associated *Overlay gallery* (page 156).



Overlay tab contains three functional groups:

- *Create* (page 78)
- *Gating Tools* (page 78)
- *Galleries View* (page 78)

Note: The Overlay plots and the associated Overlay galleries are displayed in the *Overlay view* (page 156).

Create group

Create group contains the *Overlay Builder* button.

- The **Overlay Builder** launches the *Overlay Builder* (page 172), which allows you to select the samples and plots for inclusion in an Overlay plot.
- The Overlay Builder is only enabled when at least one plot exists on the Experiment Workspace and the instrument is not acquiring. It is disabled when a Compensation control is active.

Gating Tools group

Gating Tools group contains the buttons used for inserting Rectangle, Oval, Polygon, Quadrant, and Histogram gates into Overlay plots. These buttons are only enabled if at least one Overlay plot exists.

Rectangular Gate: Allows you to draw a *Rectangular gate* on a dual-parameter plot.

Oval Gate: Allows you to draw an *Oval gate* on a dual-parameter.

Polygon Gate: Allows you to draw a *Polygon gate* on a dual-parameter.

Quadrant Gate: Allows you to draw a *Quadrant gate* on a dual-parameter.

Histogram Gate: Allows you to draw a *Histogram gate* on a Histogram plot.

Note: The buttons within the Gating Tools group are highlighted when selected. They revert to their standard color when you click in the Overlay plot to create a gate, select another button, or press the **ESC** key.

Galleries View group

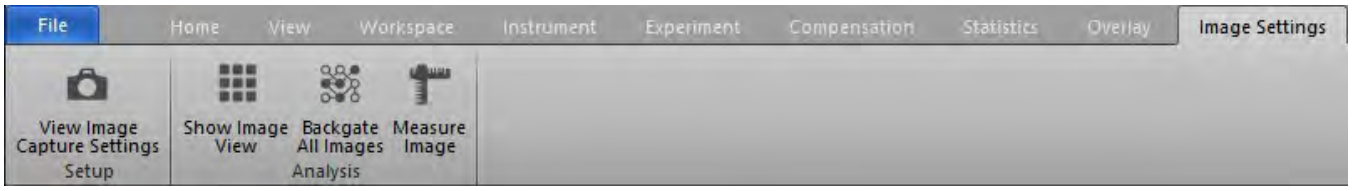
Galleries View group contains the *Wrap Galleries* button.

When **Wrap Galleries** is selected, the plots within the view all fit within the width of the gallery view area. If there are too many plots to be shown vertically, vertical scroll bars are shown.

When the gallery is wrapped, the *Wrap Galleries* button is highlighted in blue. The button is only enabled if the gallery plot view or the overlay view area is in focus.

Image Settings tab

Image Settings ribbon tab provides options to set up cell image capture and acquisition settings, view captured Images, turn on image backgating, and add measurement tools for image analysis.



The Image Settings tab is only visible when the Attune™ desktop is in view and one of the following criteria are met:

- An Attune™ CytPix™ Flow Cytometer is in use or is selected as the active instrument type in Configuration options (page 515)
- When an Experiment that was created with an Attune™ CytPix™ Flow Cytometer is active.

The Image Settings tab is not visible on the Main Menu, Login screen, Performance test views, or Filter Configuration views.

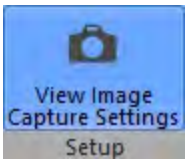
The Image Settings tab is not available for Attune™ NxT Flow Cytometers (unless viewing an Experiment that was created using an Attune™ CytPix™ Flow Cytometer).

The Image Settings tab contains two functional groups:

- *Setup* (page 79)
- *Analysis* (page 80)

Setup group

The *Setup group* contains the *View Image Capture Settings* button.

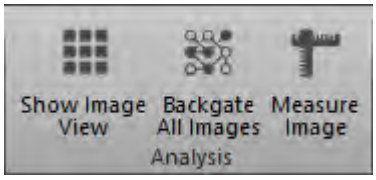


- The **View Image Capture Settings** toggles the display of the *Image Capture Settings panel* (page 172), which allows you to adjust settings that affect the capture of images.
- When the Image Capture Settings panel is displayed, the View Image Capture Settings button will be in the ON state (blue).

  When the panel is closed, the View Image Capture Settings button will be in the OFF state.
- By default, when the instrument model is changed and set to Attune™ CytPix™ Flow Cytometer and the first experiment is activated, the View Image Capture Settings button is in the ON state. After this, the ON/OFF state of the button will persist with user settings.

Analysis group

The *Analysis group* allows you to show and hide the Image View, to backgate images, and to measure images.



- **Show Image View** toggles the display of the Image View tab (page 180).

  When the Show Image View tab is displayed, the Show Image View button will be in the ON state (blue).

  When the tab is closed, the Show Image View button will be in the OFF state.

  By default, when the instrument model is changed and set to Attune™ CytPix™ Flow Cytometer and the first experiment is activated, the Show Image View button is in the ON state. After this, the ON/OFF state of the button will persist with user settings.

- **Backgate All Images** toggles the backgating of all imaged events on Workspace plots. Backgating allows you to identify the events on the plot in Workspace view that are associated with the captured cell images.

  By default, backgated events appear on the associated plot in red. For more information, see "Image backgating" (page 118).

  When image backgating is applied, the Backgate All Images button will be in the ON state (blue). Otherwise, the button will be displayed in its OFF state.

  Image backgating persists at the Workspace level for an Experiment, Group, or Sample.

  The Backgate All Images button is enabled only if the selected Sample's listmode specification contains the "ImageFlag" and "Event" parameters, and the workspace is in focus/active.

- **Measure Image** allows you to insert a measurement ellipse on the active image in the Image view, which allows you to measure the area of the cell in the image (page 187).

  While using the measurement tool to insert the measuring ellipse, the button will be displayed in the ON state (blue).

SAE tab

The *SAE* ribbon tab provides tools to manage signatures (e-Signing) and to access the *SAE Administrator Console* (page 577), where you can configure the Security, Auditing, and e-Signature (SAE) settings. SAE tab is available only when an SAE user is signed in.

The SAE tab is divided into two functional groups:

- *Signing* group (page 81)
- *Other group* (page 81)

The SAE tab is only visible on the Main Menu and Workspace views. During acquisition, when the plate is paused, or when in automation mode, all buttons are disabled.

Signing group

The *Signing group* allows you to request e-Signatures for the active experiment, view pending signature requests, sign the active experiment, and view signed records in the e-Signature Record Report History.

Request Signatures: Opens the *Request Signatures* dialog (page 467), which allows you to request signatures for the active experiment.

This button is only enabled if e-Signatures are enabled from the SAE Administrator Console, actions are set up that require signatures, and an experiment is active.

View Pending Signatures: Opens the *Sign Records* dialog (page 469), which allows you to view and sign pending signature requests.

This button is only enabled if e-Signatures are enabled from the SAE Administrator Console.

Sign Record: Opens the *Sign Experiment* dialog (page 470), which allows you to sign the active experiment.

This button is only enabled if e-Signatures are enabled from the SAE Administrator Console and an experiment is active.

View Signed Record: Opens the *e-Signature Record Report History* dialog (page 471), which allows you to view and print signed records.

Other group

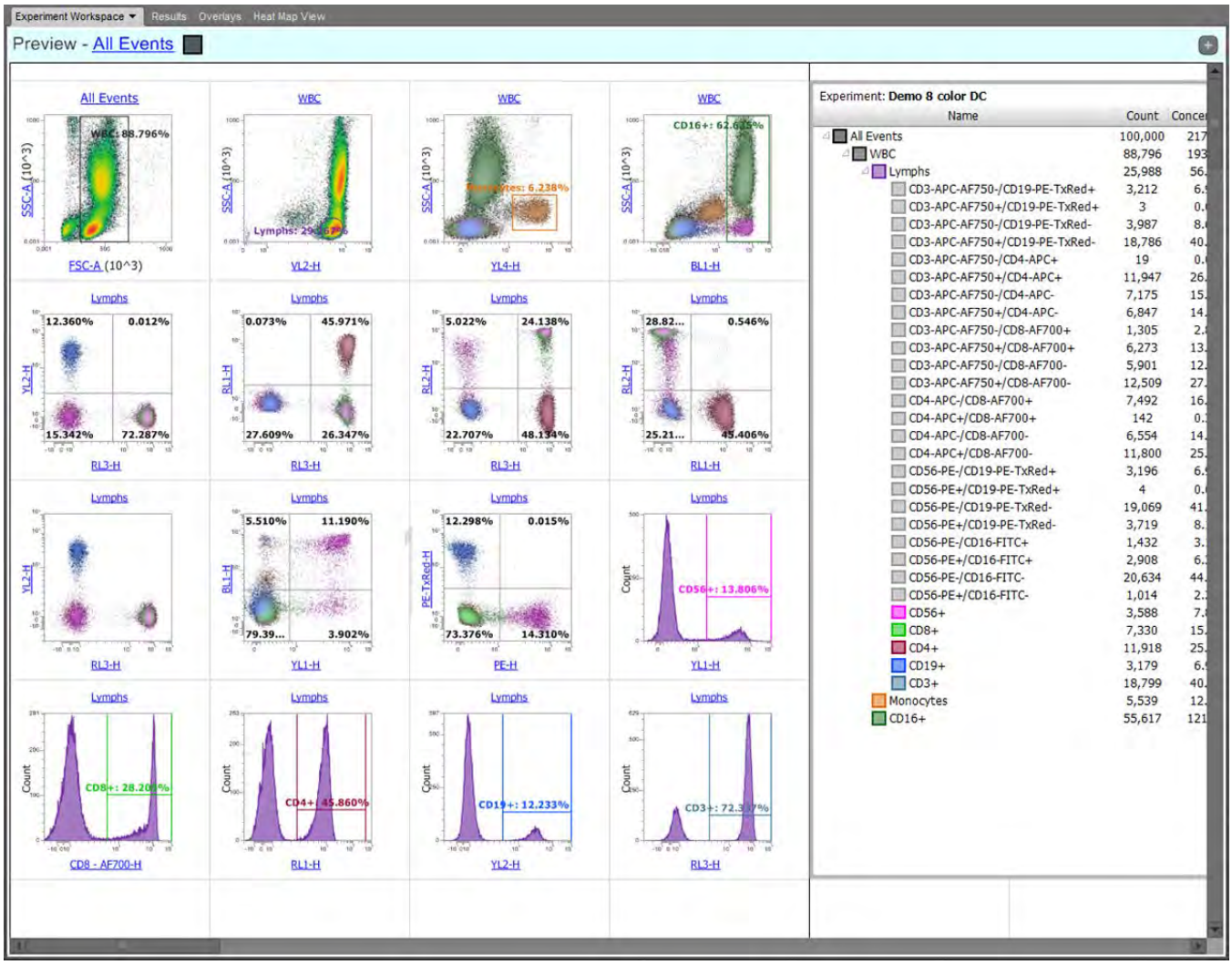
The *Other group* only contains the *View SAE Console* button, which opens the SAE Admin Console to configure Security, Auditing, and e-Signature settings, and to view auditing and signature records.

View SAE Console: Opens the *SAE Administrator Console* (page 577) using the system's default web browser. The SAE Administrator Console allows a user with SAE administrative privileges to configure Security, Auditing, and e-Signature settings, view application action records, application object records, and signature history, and to set up advanced settings including email notification, LDAP user management synchronization, and SAE archive settings.

5. Workspace view

Overview

The *Workspace view* displays the plots, gates, and statistics associated with the current sample, as well as text boxes and image files. It also contains the *Preview panel* (page 91), which is displayed by default as a minimized bar.



The Workspace view contains the following elements:

- Workspace (page 84)
- Workspace objects (page 85)
- Previews (page 91)
- Plots (page 95)
- Gates (page 98)
- Workspace statistics (page 115)
- Workspace images and text (page 117)
- Cell image container (page 119)

You can access Workspace view and Workspace object context menus when you right-click on any empty area (i.e., white space) within the Workspace view or when you right-click on Workspace objects, which are described on pages 122–137.

Workspace behavior

- The Workspace view is always the first tab of the *Main Application area* (page 36). It cannot be undocked from the main application and the associated ribbon bar (see "Docking behavior", page 37).
- The Workspace view can display one of three independent workspaces, the *Sample Workspace*, *Group Workspace*, or the *Experiment Workspace* (page 84)
- The Workspace can be displayed in two different modes, *Freeform* or *Auto Layout* (page 88). These allow automatic or user-specified placement of objects on the Workspace depending on the mode selected.
- The Workspace acts as a single worksheet that is split into "pages" when printed. The page breaks can be visualized by selecting **Show print area** in the *View tab* (page 53).
- Changes to the Workspace are saved automatically if the active Workspace changes, if the program is exited, if the active sample changes, prior to printing, or if one minute has elapsed since the last Workspace save action.
- When a Compensation sample is active, all Workspace functionality is disabled except as specified in "Compensation", page 378.
- The Workspace view will always move to the front of any docked panels at the beginning of acquisition for each sample. The Experiment Workspace will become active and the dropdown menu items will become disabled.

Note: The Preview panel automatically displays previews of all possible permutations of the Histogram and Precedence Density plots based on the parameters selected in the Workspace tab, and allows you to quickly add these plots to the Workspace as described in "Previews" on page 91.

Workspace selection

Workspace levels

The Workspace view can display one of three independent workspaces, the **Sample Workspace**, the **Group Workspace**, or the **Experiment Workspace**.

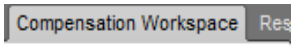
- Experiment Workspace is a Workspace that is common to all members of the current Experiment.
- Group Workspace, when created, is common to all members of the current Group.
- Sample Workspace is unique to each Sample.

Workspace view label

The Workspace view tab on the Main Application area is labeled according to the level of Workspace displayed: Sample Workspace, Group Workspace, or Experiment Workspace.

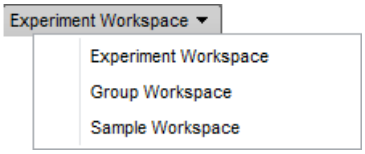
- When a Compensation sample is open, the Workspace is labeled "Compensation Workspace", and the dropdown selection menu is not present.

- By default, the Experiment Workspace is displayed when the Sample is first displayed. Group and Sample-level Workspaces are created upon first use.

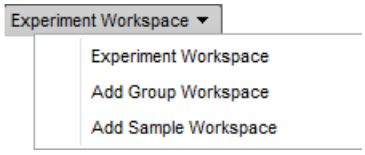


Workspace selection menu

- The **Workspace selection dropdown** menu on the Workspace view tab allows you to select level of Workspace displayed:
  - Experiment Workspace
  - Group Workspace
  - Sample Workspace



- If the Group or Sample Workspaces do not exist, the workspace label is displayed as "Add Group Workspace" or "Add Sample Workspace".



- If you select a Group Workspace, that Workspace is displayed. If a Group Workspace does not exist for the current Group, a default group-level Workspace is created.
- If you select the Sample Workspace option, the Sample Workspace for that sample is displayed. If a Sample Workspace does not exist for the current Sample, a default Sample-level Workspace is created.
- If you select the Experiment Workspace, the current Experiment Workspace is displayed.
- When a new Group Workspace is created, the Experiment Workspace is used as a template, and all plots, gates, and statistics are reproduced.
- When a new Sample Workspace is created, the Group Workspace is used as a template. If the Group Workspace does not exist, then the Sample Workspace will use the Experiment Workspace as a template.
- When a new Group or Sample Workspace is created, an indicator is displayed in the Experiment Explorer as described in "Experiment Explorer", page 189.

Workspace objects

Create Workspace objects

- The Workspace can display plots, gates, statistics, text boxes, and images (i.e., Workspace objects), all of which can be created using the options available on the **Workspace ribbon tab** (page 53).

  If you are using an Attune™ CytPix™ Flow Cytometer or the current Experiment supports imaging, you can add **Cell Image Containers** to the Workspace, which act like plots and allow you to add cell images specific to the sample (page 119).

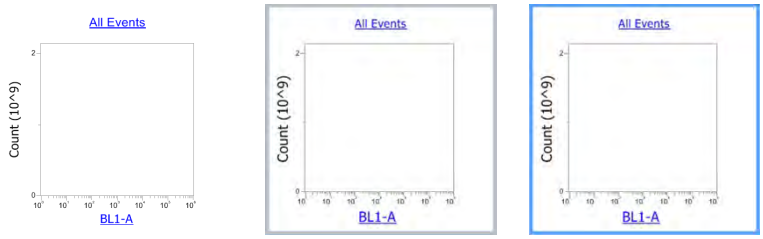
  You can use the **Workspace view context menu** (page 122) to add the Workspace objects onto the Workspace.
- The **Plot context menu** (page 125) allows you to add plot-specific statistics.
- The **Preview panel** allows you to add plots to the Workspace (page 92).
- In the **Freeform** mode (page 88), you can add Workspace objects (i.e., plots, text boxes, images, or statistics) of any size to the Workspace. These objects can be freely resized and moved on the Workspace. There is no automatic arrangement or alignment of objects.
- In the **Auto Layout** mode (page 88), the Workspace is split into grid slots. By default, new Workspace objects are placed into the next available grid space and are sized to one grid square.
- Single plots that are added using the Previews option (page 92) or the daughter plot option (page 128) are inserted at next available space of sufficient size after the last workspace object. Objects are created using the default size.

Select Workspace objects

- You can select Workspace objects by clicking within the **gray bounding rectangle** that is displayed when you move the mouse pointer over the object.

  When you select a Workspace object by clicking within the gray bounding rectangle, it is displayed with a **light blue selection rectangle**.

  Non-selected Workspace objects do not have a selection rectangle displayed.

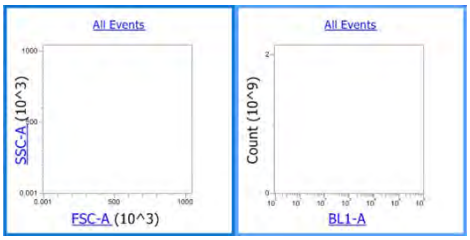


- You can select multiple objects within the Workspace by clicking on the desired Workspace objects while holding down the **Ctrl** key.

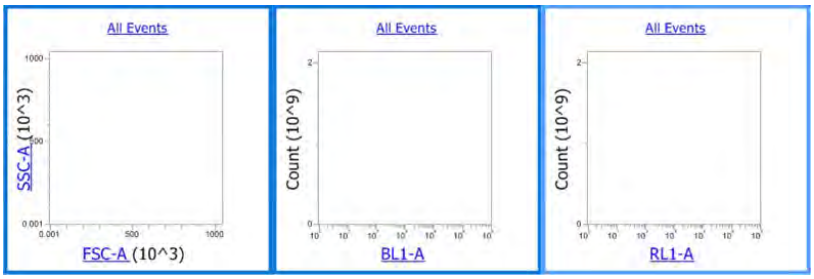
  Alternatively, you can drag a selection rectangle around several objects with the mouse.

  Using the **Ctrl+A** key combination on the keyboard selects all Workspace objects on the current Workspace.

  Objects which are part of the multi-select are bounded by **dark blue multi-select rectangles**.



- When multiple objects are selected (either individually clicked or selected by dragging the selection rectangle), the last selected object remains light blue to designate it as the **reference item for align options.**

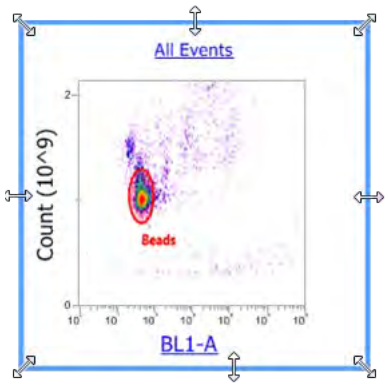


Resize Workspace objects

- You can resize single or multiple Workspace objects by selecting the object and then moving the mouse pointer over the edge of the bounding rectangle.

  Vertical, horizontal, and diagonal resize cursors are displayed as you move the mouse pointer over the appropriate area.

  The resize cursors are also displayed when the mouse pointer is hovered over the bounding rectangle of non-selected objects; when clicked, this selects the object and begins resizing.



- Once the resize cursor is displayed, the object can be dragged to resize in the direction indicated by the arrow cursor. All selected objects are resized in the same dimension and magnitude.
- Dual parameter plots and images always maintain their aspect ratio when resized.

  Histograms can be stretched horizontally. They can be stretched vertically, but the width of the object must always be greater than or equal to its height.

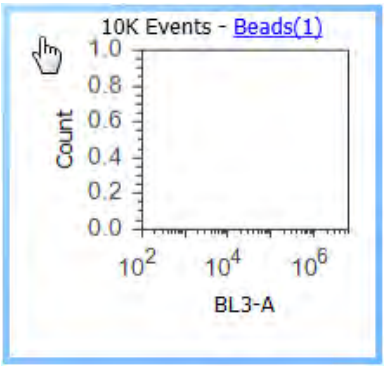
  Text boxes and statistics boxes can be stretched in any direction and any ratio.
- Pressing the **Shift** key while dragging diagonal resizing cursors resizes the selected object and maintains its aspect ratio.
- When multiple Workspace objects are selected, resizing options are determined by the object being manipulated. All other objects in the selection are bound by the resizing constraints described above.
- If mixed object types are selected when manipulating an object whose aspect ratio is not limited, histogram and dual parameter plots are resized within the aspect ratio constraints described above.

  Where necessary, requirements for width take precedence over height to ensure that the resizing constraints are met.

Move Workspace objects

- Moving the pointer over a Workspace object displays the **select cursor** in the shape of a pointing finger, except when the pointer is over a line or the select point of a gate.

  In such cases, **resize**, **move**, or **rotate cursors** are displayed as described on page 100.
- When the select cursor is displayed, you can click and drag single or multiple selected Workspace objects to a new position.



Delete, format, or duplicate Workspace objects

Delete Workspace objects:

- You can delete selected Workspace objects by pressing the **Delete** key on the keyboard.
- Alternatively, you can right-click on the object to open the *object context menu* and selecting the **Delete** option.

Format Workspace objects

- You can format the selected Workspace object using the *Customize panel options* (page 276).
- Alternatively, you can right-click on the object to open the object context menu and selecting the desired formatting option.

Duplicate Workspace objects

- You can duplicate Workspace objects by selecting one or more objects and dragging them while pressing the **Ctrl** key on the keyboard.
- Objects can also be duplicated using the keyboard combinations **Ctrl+C** (copy) followed by **Ctrl+V** (paste).
- Alternatively, you can copy the object using the **Copy** command on the object's context menu and then pasting it using the keyboard combination **Ctrl+V**.

Paste Workspace objects

- Selecting the **Ctrl+V** keyboard combination pastes the object at same location as the original. As each new object is pasted, it is inserted cascading downwards and to the right of the original location.
- Objects can be cut or copied from any Workspace and pasted to any other Workspace.
- Plots are pasted with any gates present. If a gated plot is copied to a new location where the parent gate does not exist, the pasted plot will not be gated and will display all events.
- If a Plot statistics box is pasted to a new Workspace and the target Workspace does not contain a copy of the original plot, the statistics box will update to show all gates present in the current Workspace. The Plot axis and Gate fields will be removed from the statistics box header (if originally present).
- Pasted objects are selected by default after the paste action has completed.

Workspace view layout

Resize the Workspace view

There are two ways to resize the Workspace view display:

- When the mouse cursor is over the Workspace or when the Workspace is in focus, turn the **mouse scroll wheel** while holding down the **Ctrl** key to zoom in or out.
- Use the **Size slider** on the *Application status bar* (page 47) to resize the Workspace view when the Workspace is in focus.

Workspace view modes

The Workspace can be displayed in two different modes, *Freeform* or *Auto Layout*. These allow automatic or user-specified placement of objects on the Workspace depending on the mode selected.

The Workspace view mode is controlled by the **Layout radio buttons** available on the Workspace ribbon tab (page 60).

Freeform mode

Freeform mode allows you to add plots, text boxes, images, or statistics of any size anywhere on the Workspace. These objects can be freely resized and moved on the Workspace. There is no automatic arrangement or alignment of objects.

- To add a custom-size object to the Workspace, select the desired object type from the Workspace ribbon tab (page 53) and then draw the area in the Workspace where you want to insert the selected object. When you let go of the mouse button, the object is inserted into the area drawn.
- Selecting an object type and then single-clicking on the Workspace places the default-sized object at the position clicked.

  The default size for new plots, statistics boxes, and text boxes in the Freeform mode is 240 × 240 pixels with objects arranged in 3 columns × 4 rows per page.
- In this mode, objects can be moved and positioned anywhere within the Workspace, and they can be positioned across multiple pages.
- You cannot print a Workspace in the Freeform mode.

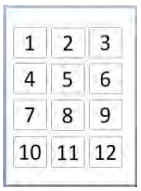
Auto Layout mode

In the Auto Layout mode, the Workspace is split into grid slots, which contain the Workspace objects of fixed size and placement. Auto Layout is the default option.

Layout behavior

- Only one object can appear in each grid slot. By default, new Workspace objects are placed into the next available grid space and are sized to one grid square.
- When switching from Freeform to Auto Layout mode, any objects present on the Workspace are placed into grid positions in the following order:
  - Plots based in Workspace insertion order
  - Plots statistics based on Plot insertion order
  - Workspace statistics
  - Text boxes
  - Images
- When switching from Auto Layout to Freeform mode, objects remain in their current position.
- The Workspace is initially setup as one page view. Additional pages are added automatically as existing pages are filled and as the Workspace zoom is changed.

Grid size



- The number of available grid slots per Workspace page is defined by **Grid Size** options on the Workspace ribbon tab (page 60).
- By default, the grid size is set to 12 grid slots per page.
- You can select a larger grid size allowing only 4 items per page, or a smaller grid size allowing up to 20 items per page at the default sizes.

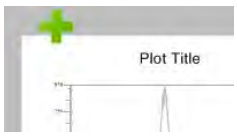
Object size

- By default, objects on Workspace are sized to one grid square when the Workspace view is in the Auto Layout mode.
- Objects on the Workspace can be resized, but they have limited resize options. When an object is resized, the top left corner of the object is pinned to its current position. Objects cannot be resized across pages or resized to occupy more than one page.
- The resize options available for various Workspace objects are listed below, where the sizes are defined as (Rows × Columns).
  - **Dual parameter plots** are constrained to remain square and can be resized to take occupy 1, 4 (2 × 2), 9 (3 × 3) or 16 (4 × 4) squares.
  - **Histogram plots** are either constrained to remain square and occupy 1, 4 (2 × 2), 9 (3 × 3), or 16 (4 × 4) grid spaces, or they can be made rectangular and occupy 2 (1 × 2), 3 (1 × 3), 4 (1 × 4 or 2 × 2), 6 (2 × 3), 8 (2 × 4), 12 (3 × 4), or 16 (4 × 4) squares.
  - **Statistics boxes** and **images** can be resized up to the maximum number of grids (5 × 4) per page. For images, the aspect ratio is maintained within the grid containers.
- When increasing grid sizes (from more slots per page to less slots per page) or changing page orientation, any object that exceed the allotted slots in the horizontal and/or vertical directions is resized to 1 × 1.

Move objects in Auto Layout mode

- Object arrangement precedence is dictated by grid position and page number. The position with the highest precedence is the top left corner of the first page, and the precedence decreases from left to right, top to bottom.

  The grid position of objects larger than 1 × 1 is dictated by the top, leftmost position occupied by that object.



- When moving an object, a gray box indicates the grid location into which the object will be inserted or expanded.
- When multiple objects are selected, only the object with the highest precedence is displayed, and a plus icon indicates that multiple objects have been selected.
- If the objects have been selected using the **Ctrl+click** method, then the first selected object is displayed while dragging.

  If the objects have been selected by dragging a selection area, then the object with the highest precedence based on Workspace location (top to bottom, left to right) is displayed.
- After objects have been resized or moved, the selection indicators are maintained on the selected objects.

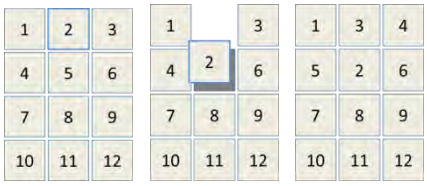
Rules for rearranging objects in Auto Layout mode

When a resized object causes another object to be displaced or a deleted object results in a blank grid slot, the objects on the Workspace are rearranged according to the rules listed below.

1. The displacing objects are always inserted in the position indicated by the gray box.
2. Displaced objects are always placed in the highest precedence available grid position as defined above.
3. The size of the object cannot span more than one page
4. Objects will always fill blank positions whenever possible.

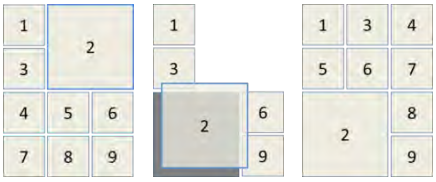
- **Example 1:** Plot 2 is a dot plot and it is dragged over Plot 5.

  Plot 3 moves to fill position 2, Plot 4 moves to fill position 3, and Plot 5 moves to fill position 4. Plot 2 takes the position of Plot 5.



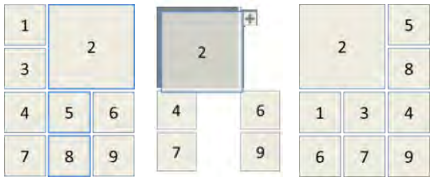
- **Example 2:** Plot 2 is a dot plot occupying 4 grid positions and it is dragged over Plot 4.

  Plots 3, 4, 5, 6, 7, 8, and 9 move to refill the displaced space.



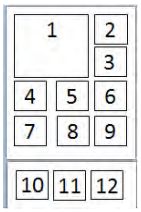
- **Example 3:** Plot 2 (a dot plot occupying 4 grid positions) along with Plots 5 and 8 are dragged over position 1.

  Plots 1, 3, 4, 6, 7, and 9 move to refill the displaced spaces.



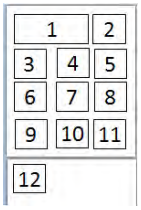
- **Example 4:** Plot 1 is a dot plot and it is resized to be 4 grid slots.

  Plots 2 and 3 automatically re-arrange to optimally fill the space. Plots 10–12 are pushed onto the next page, which appears below the current page.



- **Example 5:** Plot 1 is a histogram and it is resized to 2 grid slots.

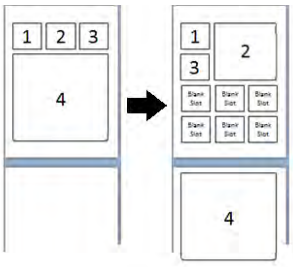
  Plots automatically re-arrange as required, and Plot 12 is pushed onto the next page, which appears below the current page.



- **Example 6:** Plot 2 is dot plot and it is resized to be 4 grid slots.

  Plots automatically re-arrange as required, and Plot 4 is pushed onto the next page, which appears below the current page.

  Because there are no other plots available to occupy them, blank slot(s) is left on the original page.



Previews

Overview

The *Preview panel* of the Workspace displays all permutations of Histogram (page 95) and Precedence Density plots (page 96) based on the parameters selected in the *Workspace ribbon tab* (page 55). This provides an easy way of adding plots to the current Workspace.

Access the Preview panel

The Preview panel is located at the top portion of the Workspace view. By default, the panel is displayed as a minimized bar.

- When opened, the Preview panel occupies 50% of the vertical space of the Workspace view. The size of the panel cannot be changed.
- The Preview panel cannot be opened during acquisition. The panel automatically closes when **Tube** or **Plate acquisition** buttons are selected on the *Collection panel* (page 221).
- The Preview panel is disabled in the Compensation Workspace.
- When the Preview panel is minimized, the **Expand button** is shown in the collapsed panel. The panel can be expanded by clicking anywhere, except for the *gate hyperlink* on the title bar of the collapsed Preview panel. The expanded setting of the Preview panel does not persist between user sessions.

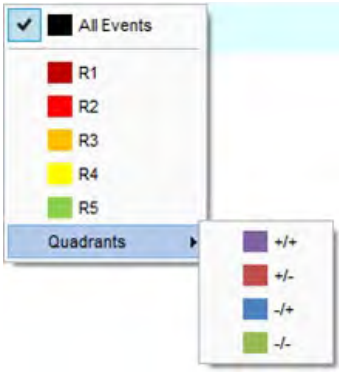
- When the Preview panel is expanded, the **Collapse button** is shown on the panel. The panel can be collapsed by clicking anywhere on the title bar, except for the gate hyperlink of the expanded Preview panel or when the panel is closed automatically as described above.

- When the Preview panel is disabled during acquisition or in the Compensation Workspace, the hyperlink control and the Expand/Collapse buttons are also disabled.

Preview panel title bar

- The Preview panel title bar displays "Previews – <Gate Name> <Gate Color>" where <Gate Name> is the name of the selected gate used to filter the Preview plots. The gate name is a hyperlink control.

- Clicking on the **Gate Name** and/or **Gate Color** on the Preview panel title bar opens a menu of all available gates and an option for **All Events** at the top of the list. Quadrant gates are shown in a submenu under the title **Quadrants**.



Preview plots

Layout of Preview plots

- The preview plots are arranged in a grid organized such that all plots share the same X parameter within a column and the same Y parameter within a row.

- The single-parameter Histogram plots are displayed in the first row.
- Dual-parameter plots show all possible combinations of available parameters.
- The plot order is based on parameter order, and starts with the first parameter in Column 1, Row 1 and ends with the Nth parameter in Column N, Row N.
- Self vs. Self dual parameter plots are not shown in the Preview panel. They are represented by white spaces that form a diagonal from the top left to the bottom right of the panel.
- The default scale for Preview plots is set to logarithmic.

Resize Preview plots

Turning the **mouse scroll wheel** while holding down the **Ctrl** key resizes the Preview.

Add plots to Workspace – single plot selection

- Plots are added sequentially to the currently displayed Workspace and their inserted position depends on the Workspace view mode (page 88).
- Plots can be added to the Workspace multiple times.
- The indicator for plot addition persists with the Workspace.
- Single clicking a plot adds it to the Workspace in the order described in “Create Workspace objects”, page 85.

Add plots to Workspace – group selection

Column header selection buttons

- Each column in the Previews area has an *Add column* button labeled with the parameter name positioned at the top of the column as a column header.
- By default, the parameter name is specified in the *Options dialog* (page 476).
- Clicking **Add column** adds the entire column of plots (excluding Histograms) to the Workspace area as described in "Create Workspace objects", page 85.

Row header selection buttons

- Each column in the Previews area has an *Add row* button labeled with the parameter name positioned at the beginning of the row as a row header.
- By default, the parameter name is specified in the *Options dialog* (page 476).
- Clicking **Add row** adds the entire row of plots (both Histograms and dual-parameter plots) to the Workspace area as described in "Create Workspace objects", page 85.

Select All split button

- Positioned at the top left corner of the Previews area is a split button, which can add the plots in either the upper right triangle or lower left triangle of the Previews area to the Workspace.
- Clicking the **top right** button adds the plots in the upper right triangle.
- Clicking the **lower left** button adds the plots in the lower right triangle.
- Plots are added to the Workspace as described in "Create Workspace objects", page 85.

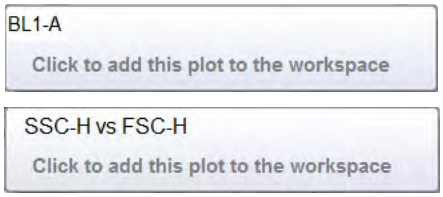
Mouse over behavior

Mouse over plot

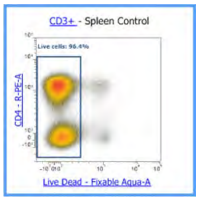
- When the mouse pointer is hovered over a plot, a tooltip is displayed with the plot name and required action to add the plot to the Workspace.

  For single-parameter plots, the tooltip includes the X-parameter label.

  For dual parameter plots the tooltip includes the X-parameter and Y-parameter labels in the format <Y Parameter> vs <X Parameter>.

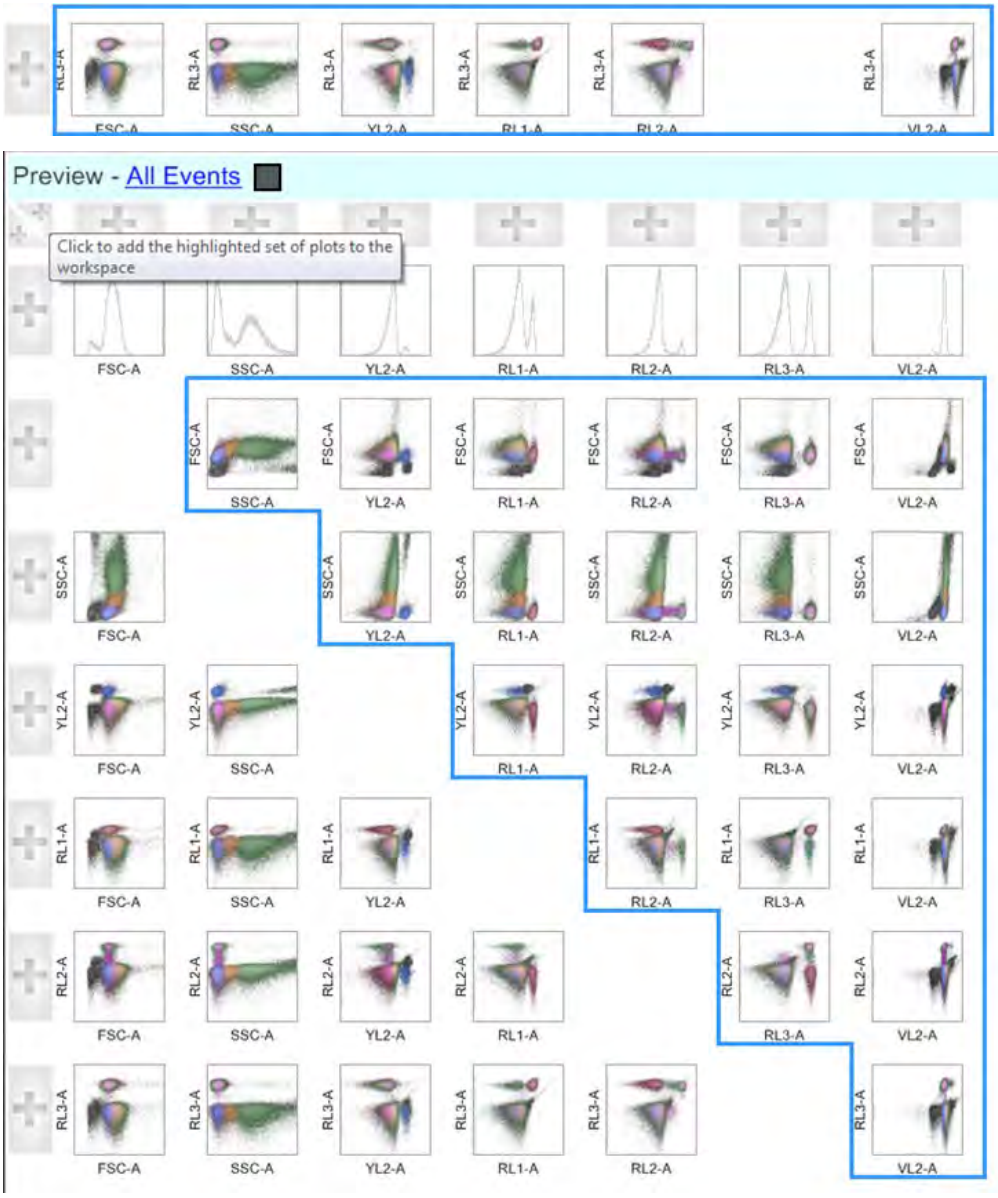


- When the mouse pointer is hovered over a plot, a bounding rectangle as described in "Select Workspace objects" (page 85) is shown around the plot.

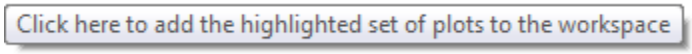


Mouse over buttons

- When the mouse pointer is hovered over *Group selection* buttons (i.e., column header selection, row header selection, or the *Select All* split buttons; page 93), the plots that will be added when the button is clicked are highlighted in an outline as described in "Select Workspace objects", page 85.



- All group selection buttons display the following tooltip when the mouse pointer is hovered over them:



Plots

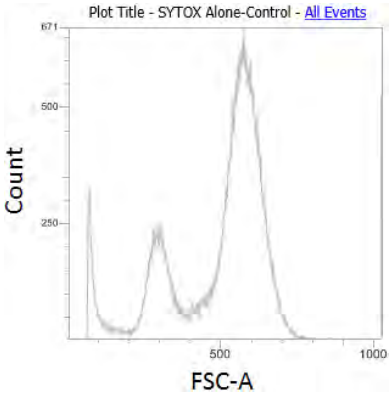
Overview

The Attune™ Cytometric Software can display *Histogram*, *Dot*, *Density*, and *Precedence Density plots* on the Workspace.

- Plots are created using the Plots group options present on the Workspace ribbon tab. The buttons within the Plots group are enabled when the Workspace view is in focus and at least two parameters are active, except for the Histogram plot button, which requires only one active parameter.
- Plot options such as titles, axis labels, axis scaling, resolution, and colors used to display data points can be customized using the *Customize panel* (page 277). Available customization options depend on the plot type selected.
- Default plot options are set using the *Options dialog* (page 480).
- Dual-parameter plots (i.e., Dot, Density, and Precedence Density plots) display FSC values on the x-axis and SSC values on the y-axis by default. Histogram plots display FSC values on the x-axis by default.
- If FSC and/or SSC parameters are not selected for collection, the next parameter selected in the *Parameters* section of the *Instrument Settings panel* (page 253) is used. The *Time* and *Event* parameters are only used if no other parameters are being collected.
- If the parameters that exist in the Workspace are disabled prior to recording data, a watermark on the plot states "Parameter(s) not available in dataset". Any daughter plot also displays the watermark. The watermark does not appear until data is displayed or collected on the Workspace.
- Gates can be manipulated on top of the watermark. The plot scales are set to the last seen valid scale. Gate statistics from plots that do not have available parameters are displayed as "N/A".

Histogram plot

A Histogram plot is a graphical representation of single-parameter data and shows the relative number and distribution of events.

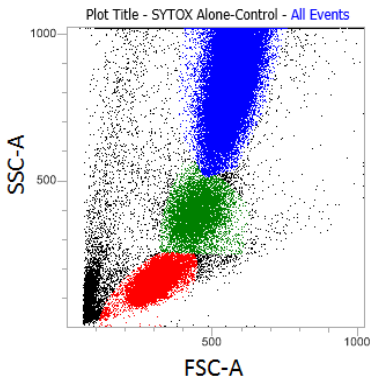


In a Histogram plot, the horizontal axis corresponds to the signal intensity of the selected parameter while the vertical axis represents the number of events (count).

- Plot customization options available for Histograms are Resolution, Normalize Count, Use Shading, and Line Width.
- The maximum plot resolution is 1024 channels, which is also the default resolution.
- The default line color for an ungated plot is set according to the selection in *Plot Options* in the *Options dialog* (page 480).
- The line color for a histogram plot that is based on a parent gate is determined by the parent gate's color.

Dot plot

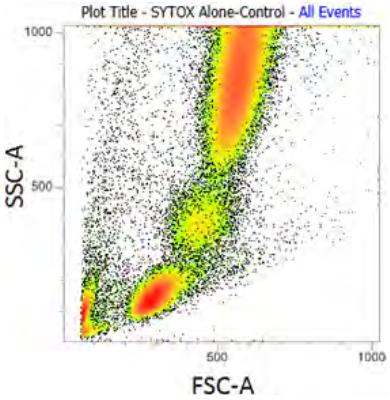
A Dot plot is a graphical representation of two-parameter data where each axis represents the signal intensity of one parameter. Each dot in the plot corresponds to one or more events detected above the threshold. Different colors are used to represent the parent gate of events that fall within bins on the plots.



- Plot customization options available for Dot plots are Resolution and % of Events displayed.
- The maximum plot resolution is 1024 × 1024 channels. The default plot resolution is set at 256 × 256 channels.
- Where an event is in multiple gates, the precedence order determines which color is displayed. Where events from multiple gates fall within a single bin, the highest precedence gate color is displayed.
- Ungated events are painted black by default. The default dot color for ungated events can be modified in *Plot Options* in the *Options dialog* (page 480).

Density plot

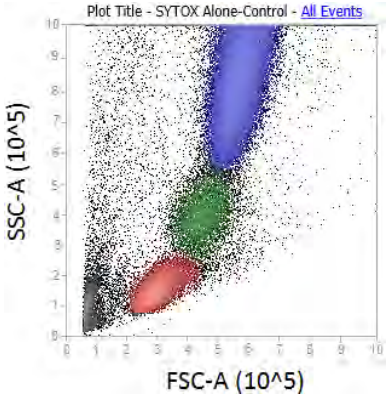
A Density plot is a graphical representation of two-parameter data where the colors represent the collection of events with the same intensity and each axis represents the signal intensity of one parameter.



- Plot customization options available for Density plots are Resolution, Color, Color Mode, and % of Events displayed.
- The maximum plot resolution is 1024 × 1024 channels. The default plot resolution is set at 256 × 256 channels.

Precedence Density plot

A Precedence Density plot uses a combination of Dot and Density display, where a gradient indicates the number of events within each of the plot bins and color is used to display the parent gate of events present.

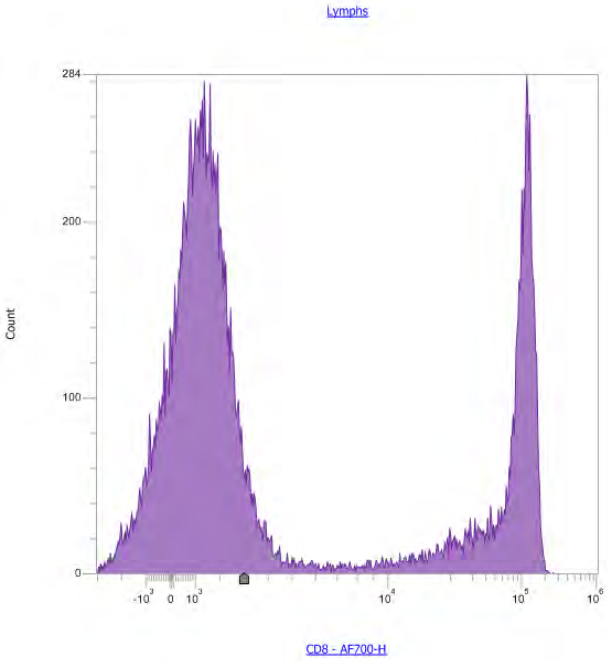


- Plot customization options available for Precedence Density plots are Resolution, Color Mode, and % of Events displayed.
- The maximum dot plot resolution is 1024 × 1024 channels. The default plot resolution is set at 256 × 256 channels.

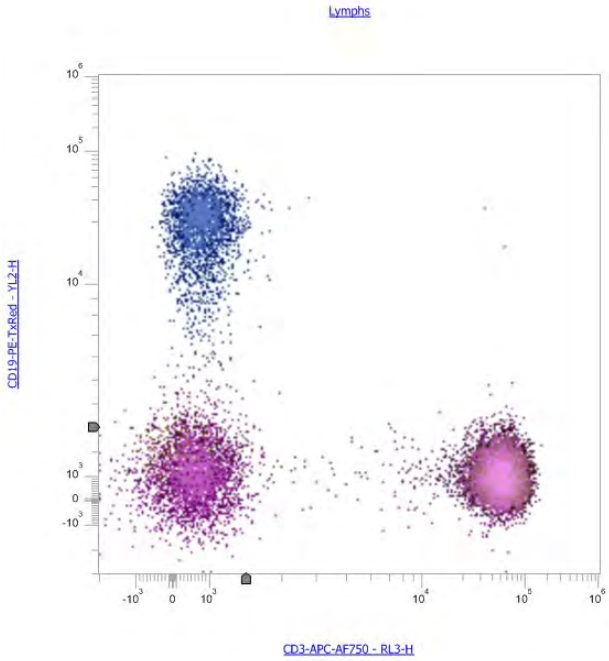
Adjust plots

When you select individual plots, additional controls become available, depending on the selected plot and system status.

- When **Plot Compensation** state button on the Compensation ribbon tab (page 53) is turned **ON**, you can adjust compensation directly on the selected plot (page 396).
- If the selected plot has an axis where the scale is set to HyperLog™, a HyperLog™ transition value slider ( ) becomes available on each axis that is set to HyperLog™.



Histogram with HyperLog™ slider



Dual Parameter plot with HyperLog™ slider

- As you move the slider, the transform value is applied to the selected parameter on the selected plot (no other plots are updated). The value does not update in the customize panel when moving the slider.
- The slider position can be moved from 1 to 100,000.
- When you release the slider, the transition value is applied to all plots where the parameter is used, and the axis scaling changes to HyperLog™.
- The new transform value is reflected in the Plot Customize panel for the selected plot and axis.
- The slider transform value applied to the data is linked to the axis value where the slider needle is depressed, and the axis tick marks are updated based on the newly applied transform.
- The slider needle is not displayed on plots when the workspace is printed, a plot is copied to the clipboard, or the plot is exported as an image.

Gates

Overview

Gates allow you to identify and analyze subsets of data (i.e., populations) by isolating a region in a selected plot. They can also be used to limit the number of events collected or stored.

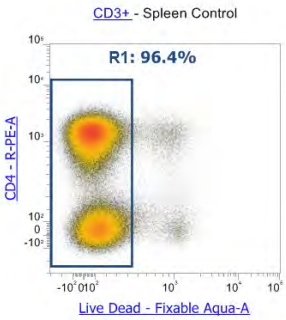
After defining gates, you can combine them to create gates based on Boolean operators (i.e., Derived gates). You can use gated populations to generate statistics, display them in a hierarchal view, and create subsets within defined populations (i.e., daughter plots and associated statistics).

Create and delete gates

- You can create gates on a selected plot using the *Gating Tools* available on the Workspace ribbon tab. Alternatively, you can use the *Plot context menu* (page 126) to create gates.
- The following types of gates can be added on plots on a Workspace:
  - *Rectangular gate* (page 101)
  - *Oval gate* (page 102)
  - *Polygon gate* (page 103)
  - *Quadrant gate* (page 104)
  - *Bent Quadrant gate* (page 105)
  - *Histogram gate* (page 106)
  - *Contour autogate* (page 107)
  - *Ellipse autogate* (page 109)
  - *Derived gate* (page 112)
- Autogates are defined by auto region bounding rectangles, which determine the autogate target area when the underlying data meet defined autogate criteria. If these criteria are met, an autogate of the specified type (contour or ellipse) is generated (see "Autogate gating rules", page 111).
- Gate creation defaults, including autogates, are set in *Gate Options* in the *Options dialog* (page 477).
- You can create a maximum of 128 gates on plots on a Workspace, except for Quadrant gates, of which at least 1000 can be created.
- You can create a maximum of 128 gating equations. A gating equation is generated when a new rectangle, polygonal, oval, autogate, or histogram gate is created, or when the Derived Gate option is used to create a compound gate equation.
- The maximum number of gates present in an individual gate equation is 28.
- When the Workspace reaches the maximum number of gates allowed, the relevant gate creation buttons on the Workspace ribbon tab and the options on the Plot context menu become disabled.
- To delete a gate, select it, and press the **Delete** key on the keyboard or the **Delete** button on the Home tab.
- When a gate is deleted, any Derived Gate that includes the deleted gate is modified to exclude the deleted gate.

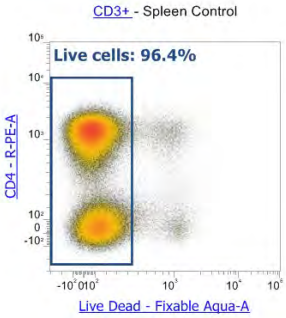
  If the gate equation includes only the deleted gate, then the entire gate equation is deleted.
- Gate name, appearance, and position can be customized using the *Customize panel* as described in "Customize Gate options" (page 293).

Gate name



- By default, each new gate is automatically assigned a unique name, which consists of the letter R followed a number. The number increases incrementally for each new gate created, ensuring that each gate has a unique name (e.g., R1, R2,…).
- For Quadrant gates, the default gate name consists of the letter Q followed by an incrementally increasing number until a unique gate name is achieved (e.g., Q1, Q2,…).
- The gate name and the associated statistic (optional) are displayed adjacent to the gate on the relevant plot on separate lines. The font size auto-adjusts to optimally display the text.
- The display of a statistic is selected on the **Statistics ribbon tab** (page 74) along with the desired statistic. Only one statistic can be shown and this applies to all plots. If a statistical value cannot be calculated, "N/A" is displayed instead of a numerical value.
- To change the automatically assigned gate name, manually enter a name in the **Name** field in the Customize panel or select one of the pre-defined names from the **Quick Select** dropdown menu (page 299).

  Alternatively, double-click on the name of a selected gate to enter the name on plot edit mode



- To reposition the gate name, first select the gate, then placing the cursor over the name. When the cursor changes to the standard precision select pointer (i.e., cross-hair), click and drag the gate name to a new position. You cannot drag the gate name outside the plot.
- You can move the gate name independently of the gate, but moving the gate keeps the gate name in the same relative position.

Gate color

- When a gate is created, the gate color is automatically assigned.
- There are ten default colors defined for gates. As a new gate is created, the next available color is used. When an eleventh gate is created, the first color will be re-used. This process continues as each new gate is created.
- The gate color and the opacity of the fill color can be customized in the Customize panel as described in "Customize Gate options" (page 293).

Move and resize gates

You can move and resize any gate. When you move or resize a gate, all populations derived from that gate are affected and statistics are automatically updated.

- When you click on a gate to select it, the gate displays the *control points*, which are used for resizing or rotating the gate.
- When you move the mouse over the gate or the handle, the cursor changes its shape to indicate its action mode (move, resize, or rotate).

  Move: Used to move the selected object in any direction.

  Resize: Used to resize a single dimension (vertical or horizontal).

  Diagonal resize: Used to resize two dimensions simultaneously.

  Rotate: Used for freely rotating the selected object around a pivot point. Rotate function is available only for polygon and rectangular gates.

- To move a selected gate, grab it between the handles as indicated by the Move cursor, and then drag it to a new position. For opaque gates, you can also grab the center area of the gate to move it.
- You cannot move or resize autogates when they are ON. When the autogates are OFF or in the ON – [NO FIT] state, the underlying bounding box can be moved and resized.
- A Quadrant gate displays a move cursor over the *intercept point*. For opaque quadrants, the center is not selectable for moving the gate.
- You cannot move a gate or quadrant intercept point outside of the plot axes, although a gate may exist outside the visible plot area, if an area that does not include the location of the gate was zoomed on the plot.

Note: It is also possible for a gate to exist outside of the plot limits, if the scale type or scale range of a plot has been modified after the gate has been defined (see "Off-plot Gates", page 113).

- When the rotate icon is displayed on a selected gate, you can rotate the gate by dragging the indicated rotate control point.
- When a control point of a selected gate is being moved, the following X and Y coordinates are displayed in the *Application status bar*:
  - **Polygon gate:** Coordinates of the point being moved are displayed.
  - **Histogram gate:** Coordinates of the point being moved are displayed.
  - **Rectangular gate:** Coordinates of the point being moved are displayed.

    If moving the top or right boundary line, the coordinates for the upper right control point are displayed.

    If moving the lower or left boundary line, the coordinates of the lower left control point are displayed.
  - **Oval gate:** Coordinates of the center point are displayed.
  - **Autogates (Contour and Ellipse):** Coordinates of the auto region bounding box are displayed.
  - **Quadrant gate:** Coordinates of the intercept are displayed.
- When moving an entire Rectangular, Polygon, or Oval gate, or a broken quadrant, no coordinates are displayed.

Rectangular gate

To insert a *Rectangular gate* into a selected dual parameter plot, click the **Rectangular gate** button on the Workspace ribbon tab.

Alternatively, select **Add Gate ▶ Rectangular** from the Plot context menu.

- To insert the gate, click the **Rectangular gate** button to select, then click on the desired dual parameter plot, which defines a corner of the rectangle.

  To draw the gate, drag out a rectangle from this point.
- When creating a Rectangular gate, the Application status bar displays the X and Y coordinates of the point being dragged.
- Select the **Rectangular gate** button, then click on a dual parameter plot to insert a default sized Rectangular gate centered on the point where the plot was clicked.

  The width of the Rectangular gate is 40% of the plot width and its default height is 20% of the plot width.
- On a single parameter plot, a no entry cursor (⃠) indicates that a Rectangular gate cannot be created.
- Rectangular gates have eight control points.
- You can drag the center of each line forming the rectangle perpendicular to the line to resize the gate.

- You can drag the four corners of the rectangle, with the opposite corner being the anchored point. Its new position is determined by the rectangle formed by the anchored point and the final position where the mouse button is released.
- When you drag the rectangle, but not on a control point, the whole gate is dragged while maintaining its shape. For opaque gates, the center area is also selectable for moving the gate.

Note: If you are using an Attune™ CytPix™ Flow Cytometer, you can use Rectangular gates to set Image gates, which allow you filter the cell images displayed in Image view. See "Image gate" on page 185 for more information.

Oval gate

To insert an *Oval gate* into a selected dual parameter plot, click the **Oval gate** button on the Workspace ribbon tab.

Alternatively, select **Add Gate ▶ Oval** from the Plot context menu.

- To insert the gate, click the **Oval gate** button to select, then click on the desired dual parameter plot, which defines a corner of the bounding box for the ellipse.

  To draw the gate, drag out an ellipse from this point.
- When an Oval gate is being created, the Application status bar displays the X and Y coordinates of the upper right corner of the bounding rectangle.
- Select the **Oval gate** button, then click on a dual parameter plot to insert a default sized Oval gate centered on the point where the plot was clicked.

  The default width of the Oval gate is 40% of the plot width and its default height is 20% of the plot height.

  The default Oval gate is created with the major axis horizontal.
- On a single parameter plot, a no entry cursor (⃠) indicates that an Oval gate cannot be created.
- Oval gates have four control points. These represent the major and minor axis of the ellipse.
- Drag the control point in the direction of an axis to resize that axis.

- When you drag on the ellipse, but not on a control point, the whole gate is dragged while maintaining its shape.

  For opaque gates, the center area is also selectable for moving the gate.

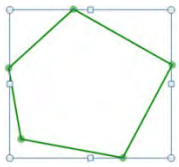
Polygon gate

To insert a *Polygon gate* into a selected dual parameter plot, click the **Polygon gate** button on the Workspace ribbon tab.

Alternatively, select **Add Gate ▶ Polygon** from the Plot context menu.

- To insert the gate, click the **Polygon gate** button to select, then click on a dual parameter plot, which determines where the first point of the gate is placed. Continue to click on the plot to add additional points to the polygon to draw the gate around the population of interest. To complete the process, click on the original point or double-click when inserting the last point.
- If there are at least three points, you can also complete the polygon by right clicking, which adds a line from the last to first point.

  If you right click when only two points have been defined, the gate is removed from the plot.
- The maximum allowable number of defined points on a Polygon gate is 40.
- When setting the initial point of the polygon, a no-entry cursor (⊘) is displayed when hovering outside a plot window. On a single parameter plot, a no entry cursor indicates that a Polygon gate cannot be created.
- When a Polygon gate is being drawn, the Application status bar displays the X and Y coordinates of the cursor as each point is created.
- When creating a point of the polygon (other than the initial point), if you move the cursor outside of the plot but remain within the application window (or child windows), a precision select pointer (i.e., cross-hair) continues to be displayed. If you click while the cursor is outside the plot, a control point is created at the coordinate closest to the clicked point.
- If you click outside the application window (or application child windows), the current gate being created is removed and the create mode is cancelled.
- Clicking when the no-entry cursor is displayed to create the first point of the polygon cancels the create mode without creating the point.
- Each vertex of the polygon is a control point. A bounding rectangle is displayed when the completed Polygon gate is selected.



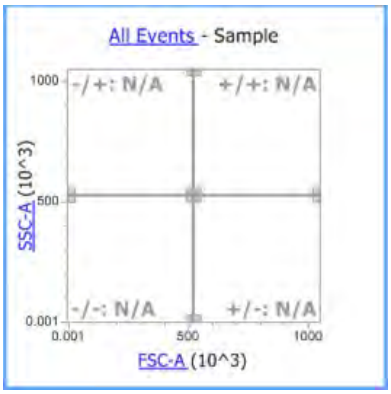
- Dragging a vertex moves that vertex only within the polygon.
- Dragging on a line within the polygon or the bounding rectangle moves the gate while maintaining the shape of the gate.
- Double-click on a line within the polygon to insert a new vertex.
- Drag the line that forms the bounding rectangle perpendicular to the line to resize the gate.
- You can drag the four corners of the bounding rectangle, with the opposite corner being the anchored point.
- When you drag the bounding rectangle, but not on a vertex control point, or when you drag the line between vertex control points, the whole gate is dragged while maintaining its shape. For opaque gates, the center of the Polygon gate is also selectable for moving the gate.
- The default Polygon gate is defined by a bounding rectangle with 5 control points in the center of the plot. The width of the default bounding rectangle is 40% of the plot width and its default height is 20% of the plot height.

Quadrant gate

To insert a Quadrant gate into a selected dual parameter plot, click the **Quadrant gate** button on the Workspace ribbon tab.

Alternatively, select **Add Gate ▶ Quadrant** from the Plot context menu.

- Only a single Quadrant gate can be created on a plot.
- Quadrant gates are formed from two divider lines (one vertical, one horizontal). To insert the gate, click the **Quadrant gate** button, then click on the desired dual parameter plot, which determines where the quadrant intercept point is placed.
- On a single parameter plot, a no entry cursor (⃠) indicates that a Quadrant gate cannot be created.
- After you have created the gate, you can edit the placement of the gate using the control point displayed on the quadrant intercept.
- By default, quadrants are added to a plot with the center point at the location that the plot is clicked.
- The quadrant is added orthogonally.
- By default, quadrants have five control points; one situated at the divider intercept point and one situated at each end of the two dividers.
- While a control point is being moved, the X and Y coordinates of the intercept point are displayed in the status bar.
- The quadrant intercept cannot be moved outside of the plot axes.
- The quadrant will remain orthogonal until an end point is moved (or the Bendy Quad mode is selected in the Quadrant gate customize panel).



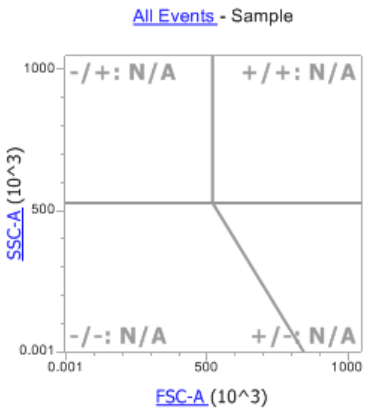
- If an end point is not moved, moving the center point moves the quadrant while maintaining the orthogonal nature of the quadrant gate.
- When an end point is moved, the quadrant becomes bendy and the Bendy Quad button is selected in the Quadrant gate customize panel.
- Any time a quad vertex is clicked with the left mouse button, the coordinates are displayed in the Status bar.

Bent Quadrant gate

To insert a Quadrant gate into a selected dual parameter plot, click the **Quadrant gate** button on the Workspace ribbon tab, then select the **Bent Quadrant** option.

Alternatively, select **Add Gate ▶ Quadrant** from the Plot context menu (page 126), then select the **Bent Quadrant** option.

- In a Bent Quadrant gate, moving the center point only moves the center point while the end points remain at their original positions.
- The coordinates of the end points of a quadrant are extrapolated from the center point to a limit of PnR for positive coordinates and –PnR for negative coordinates. You can only move an end point within these limits.
- You cannot move an end point from its corresponding border (i.e., the left end point will remain on the left boundary of the plot, the top on the top, the right on the right, and the bottom on the bottom) where the coordinate of that border is fixed at its limits (for example, the left end point has an x-coordinate of –PnR).



- For a Bent Quadrant gate, the status bar displays the following coordinates:
  - Top X: User defined within plot limits for X axis
  - Top Y: Plot's Y Max
  - Right: Plot's X Max
  - Right Y: User defined within plot limits for Y axis
  - Left X: Plot's X Min
  - Left Y: User defined within plot limits for Y axis
  - Bottom X: User defined within plot limits for X axis
  - Bottom Y: Plot's Y Min
  - Center Points: User defined within plot scale space (visible and not visible) (i.e., Linear, Log, or Hyperlog™)
- When the plot scale and range is changed where the center point is outside the visible bounds of the plot, the quadrant is displayed in red to indicate that the quadrant center point is outside the visible plot space. If the range and scale are changed so that the center point is visible, it is rendered without red and with the center point in its specified location.
- If the range or scale is changed such that a quadrant vertex is no longer on its corresponding plot border, it is drawn with a dashed line.

Histogram gate

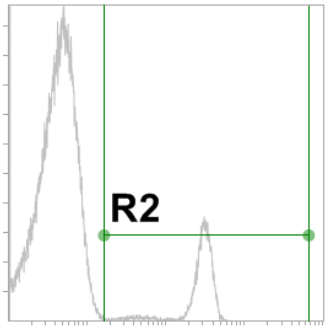
To insert a Histogram gate into a selected single parameter plot, click the **Histogram gate** button on the Workspace ribbon tab.

Alternatively, select **Add Gate ▶ Histogram** from the Plot context menu.

- To insert the gate, click the **Histogram gate** button, then click on the desired single parameter plot and drag out a line to determine the full gate size.
- When a Histogram gate is being created, the Application status bar displays the X and Y coordinates of the point being dragged.
- Selecting the Histogram gate button and clicking on a single parameter plot inserts a default sized Histogram gate centered on the point where the plot was clicked.

  The default width of the Histogram gate is 40% of the plot width. Full height vertical lines are used to mark the upper and lower limits of the gate.
- On a dual parameter plot, a no entry cursor (⃠) indicates that a Histogram gate cannot be created.
- Histogram gates have control points situated at each end of the gate. When the gate is selected, control points are displayed on each boundary.
- Dragging the control points or the vertical boundary line moves the end points in the direction of the appropriate axis.

  Dragging the horizontal line, but not on a control point, allows the whole gate to be moved.
- The Histogram gate cannot be moved outside the plot axes. For opaque gates, the center is also selectable for moving the gate.



- When required, the default position of the Histogram gate is the center point of the gate set to the center of the plot.

Note: If you are using an Attune™ CytPix™ Flow Cytometer, you can use Histogram gates to set Image gates, which allow you filter the cell images displayed in Image view. See “Image gate” on page 185 for more information.

Contour autogate

To insert a Contour autogate into a selected dual-parameter plot, click the **Contour Autogate** button on the Workspace ribbon tab.

This allows you to draw a rectangular region (auto region bounding rectangle), which becomes the basis of the Contour autogate target area.

Alternatively, select **Add Gate ▶ Contour Autogate** from the Plot context menu.

Note: Autogates are set if the criteria for population identification are met within a defined auto region bounding rectangle (see "Autogate gating rules", page 111).

- To insert the Contour auto region bounding rectangle, click the **Contour Autogate** button, then click on the desired dual parameter plot and drag out a line to determine the full gate size.
- When a Contour autogate is being created, the Application status bar displays the X and Y coordinates of the point being dragged.
- Selecting the Contour Autogate button and clicking on a plot inserts a default sized Contour auto region bounding rectangle that is centered on the point where the plot was clicked.

  The default width and height of an autogating region is 20% of the plot width and height.
- A no entry cursor (⃠) indicates that a Contour autogate cannot be created in that area of the Plots view.
- When the Contour auto bounding region is first created, it is in the *un-autogated* state, which is indicated by dashed lines that make up the rectangle (see "Contour auto region states", page 108).

  If the autogating criteria are met when the gate is ON, then the auto region bounding rectangle automatically forms an autogate.
- When a successful autogate forms in the ON state, a smaller bounding rectangle that reflects the new Contour autogate is created, and the Application status bar displays the X and Y coordinates of the new bounding rectangle.

  However, the coordinates of the bounding box for an autogate that is in the ON – [NO FIT] or the OFF state reflect the original, larger auto region bounding box.
- Contour auto region bounding rectangles have eight control points.
- If the autogate is OFF or in the ON – [NO FIT] state, you can drag the center of each line forming the rectangle perpendicular to the line to resize the auto region.

  If the autogate is in the ON state, you cannot resize the auto region.
- You can drag the four corners of the rectangle, with the opposite corner being the anchored point. Its new position is determined by the rectangle formed by the anchored point and the final position where the mouse button is released.
- When you drag the rectangle, but not on a control point, the whole auto region is dragged while maintaining its shape. For opaque gates, the center area is also selectable for moving the gate.
- To delete a gate, select it, then press the **Delete** key or select **Delete** in the right-click menu after the gate is selected.

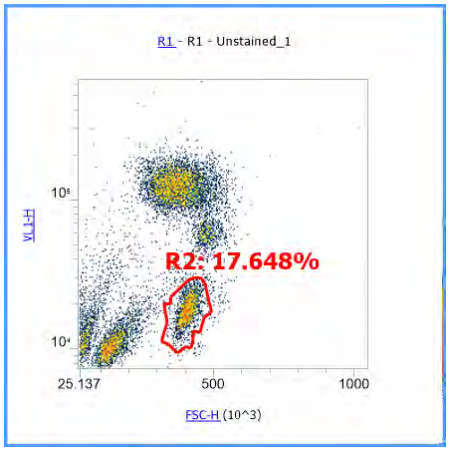
Note: Auto region default settings are dictated by the setting in the Gate options (page 486).

Contour auto region states

State: Autogate ON

Visualisation: Solid line smoothed polygon

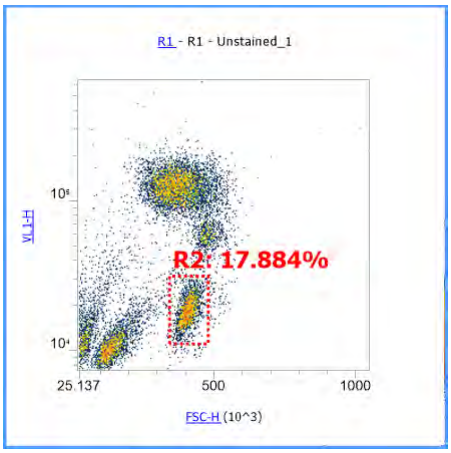
Manipulation options: Control points displayed when selected. Size and position are not manipulatable.



State: Autogate OFF

Visualisation: Dashed line polygon

Manipulation options: Control points displayed when selected. Size and position are manipulatable like a rectangular gate.

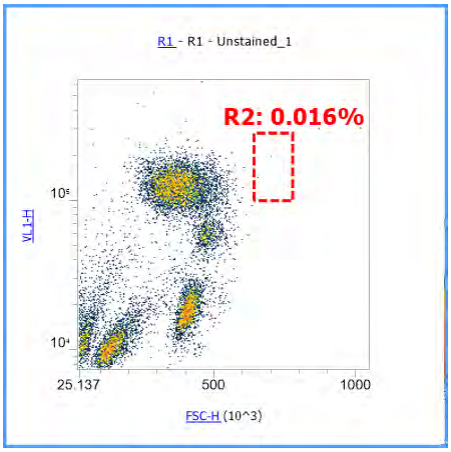


State: Autogate ON – [NO FIT]

Visualisation: Dashed line rectangle

Manipulation options: Control points displayed when selected. Size and position are manipulatable based on rectangular region rules.

Note: After manipulation, the Auto Region will attempt to autogate in this state. If successful, its state changes to ON.



Ellipse autogate

To insert an Ellipse autogate into a selected dual-parameter plot, click the **Ellipse Autogate** button on the Workspace ribbon tab.

This allows you to draw a rectangular region (auto region bounding rectangle), which becomes the basis of the Ellipse autogate target area.

Alternatively, select **Add Gate ▶ Ellipse Autogate** from the Plot context menu.

Note: Autogates are set if the criteria for population identification are met within a defined auto region bounding rectangle (see "Autogate gating rules", page 111).

- To insert the Ellipse auto region bounding rectangle, click the **Ellipse Autogate** button, then click on the desired dual parameter plot and drag out a line to determine the full gate size.
- When an Ellipse Autogate is being created, the Application status bar displays the X and Y coordinates of the point being dragged.
- Selecting the Ellipse Autogate button and clicking on a plot inserts a default sized Ellipse auto region bounding rectangle that is centered on the point where the plot was clicked.

  The default width and height of an autogating region is 20% of the plot width and height.
- A no entry cursor (⃠) indicates that an Ellipse autogate cannot be created in that area of the Plots view.
- When the Ellipse auto bounding region is first created, it is in the *un-autogated* state, which is indicated by dashed lines that make up the rectangle (see "Ellipse auto region states", page 110).

  If the autogating criteria are met when the gate is ON, then the auto region bounding rectangle automatically forms an autogate.
- When a successful autogate forms in the ON state, a smaller bounding rectangle that reflects the new Ellipse autogate is created, and the Application status bar displays the X and Y coordinates of the new bounding rectangle.

  However, the coordinates of the bounding box for an autogate that is in the ON – [NO FIT] or the OFF state reflect the original, larger auto region bounding box.
- Ellipse auto region bounding rectangles have eight control points.
- If the autogate is OFF or in the ON – [NO FIT] state, you can drag the center of each line forming the rectangle perpendicular to the line to resize the auto region.

  If the autogate is in the ON state, you cannot resize the auto region.
- You can drag the four corners of the rectangle, with the opposite corner being the anchored point. Its new position is determined by the rectangle formed by the anchored point and the final position where the mouse button is released.
- When you drag the rectangle, but not on a control point, the whole auto region is dragged while maintaining its shape. For opaque gates, the center area is also selectable for moving the gate.
- To delete a gate, select it, then press the **Delete** key or select **Delete** in the right-click menu after the gate is selected.

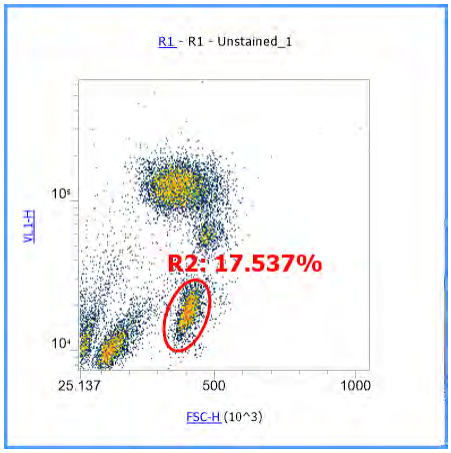
Note: Auto region default settings are dictated by the setting in the Gate options (page 486).

Ellipse auto region states

State: Autogate ON

Visualisation: Solid line smoothed ellipse

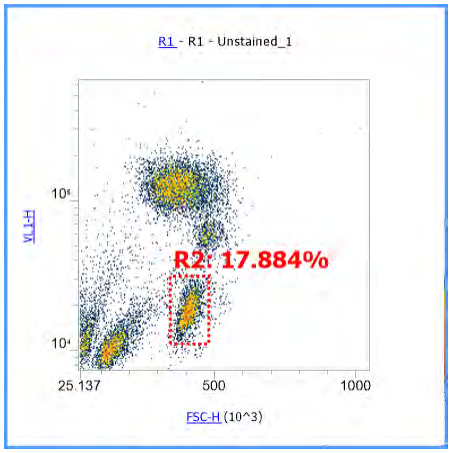
Manipulation options: Control points displayed when selected. Size and position are not manipulatable.



State: Autogate OFF

Visualisation: Dashed line ellipse

Manipulation options: Control points displayed when selected. Size and position are manipulatable like a rectangular gate.

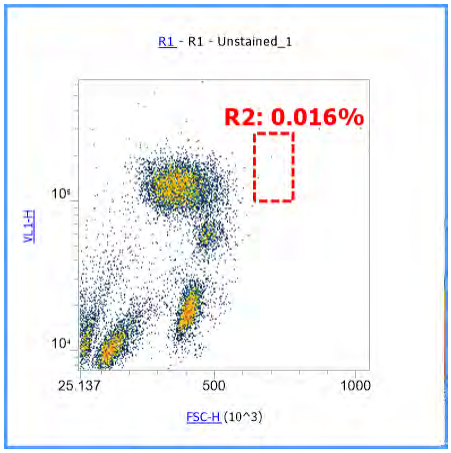


State: Autogate ON – [NO FIT]

Visualisation: Dashed line rectangle

Manipulation options: Control points displayed when selected. Size and position are manipulatable based on rectangular region rules.

Note: After manipulation, the Auto Region will attempt to autogate in this state. If successful, its state changes to ON.



Autogate gating rules

Autogates are defined by auto region bounding rectangles, which determine the autogate target area for underlying data based on autogate settings. If the criteria defined in autogate settings are met, the auto region of the specified type (contour or ellipse) is created within the bounding rectangle.

The following table describes the potential auto region states at any given time after creation.

State	Description
Autogate ON	Auto region has been successfully autogated around a population or populations. The auto region is represented as the appropriate autogate type (Ellipse or Contour). ON indicates that the auto region will attempt to autogate under any of the re-autogate scenarios described in this section.
Autogate ON – [NO FIT]	Auto region has not been successfully autogated. The auto region is represented as a rectangular region type with a dashed boundary line. ON indicates that the auto region will attempt to autogate under any of the re-autogate scenarios described in this section.
Autogate OFF	Auto region has not been successfully autogated. The auto region is represented as a rectangular region with a dotted boundary line. OFF indicates the auto region will **not** attempt to autogate under any of the re-autogate scenarios described in this section.

Re-autogating

An auto region with the Autogate ON setting will re-attempt to autogate based on its auto region bounding rectangle co-ordinates and its current autogate settings when new data are acquired, when FCS files are imported in a shared workspace, or when present in an imported workspace and applied to underlying data.

Derived gate

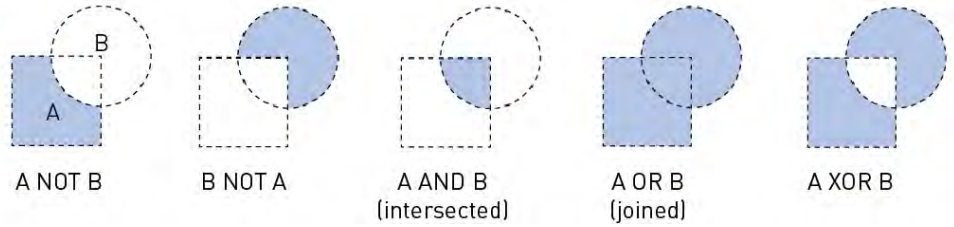
Derived gates are gates based on Boolean operators applied to existing gates. You can create a derived gate when there is at least one gate present in the Workspace, with any or no plot selected.

- You can create a Derived gate through the *Derived Gate dialog* (page 421).

  To open the Derived Gate dialog, click the **Derived Gate** button on the Workspace ribbon tab.

  Alternatively, select **Add Gate▶Derived Gate** from the Plot context menu.

- The Boolean operators available for creating Derived gates are blank, AND, OR, XOR, AND NOT, and OR NOT.

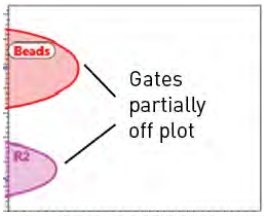


- For more information on creating Derived gates, see "Derived Gate dialog" (page 421).

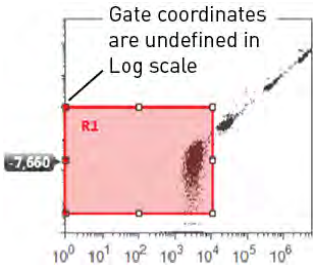
Off-plot gates

It is also possible for a gate to exist outside of the plot limits, if the scale type or scale range of a plot has been modified after the gate has been defined.

- If the gate is partially off the visible plot scale range and the gate coordinates exist within the plot scale type, the displayed gate is clipped at the plot axis or plot boundary.

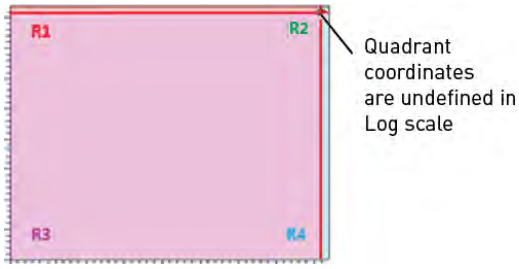


- If the gate is partially off the visible plot scale range and the gate coordinates do not exist within the plot scale type (e.g., changing from Linear scale to Log scale with negative coordinates), the displayed gate is shown with the undefined gate coordinates and the vertices depicting the undefined coordinates are filled with red.

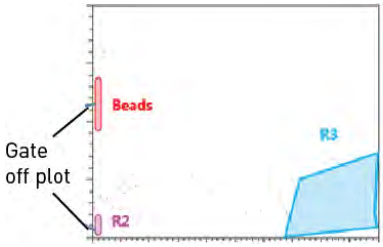


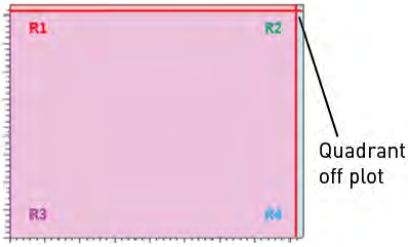
  Hovering over the vertices depicting undefined coordinates displays a tooltip that states "Coordinate exists beyond the limits of the selected scale type". These vertices are always shown until the gate is moved so that all vertices exist within the defined space or the scale type is changed to allow these points to be displayed in the plot.

- For a Quadrant gate, the quadrant is displayed away from the plot axis so that the presence of the off-plot gate can clearly be seen and a single intercept point depicting the undefined coordinates is filled with red.



- Dragging the gate away from plot axis moves the gate into the defined space.
- If the gate is completely off plot, the gate is displayed adjacent to the plot axis with a default size for the off-plot parameter, so that the presence of the off-plot gate can be ascertained.





Gate behavior

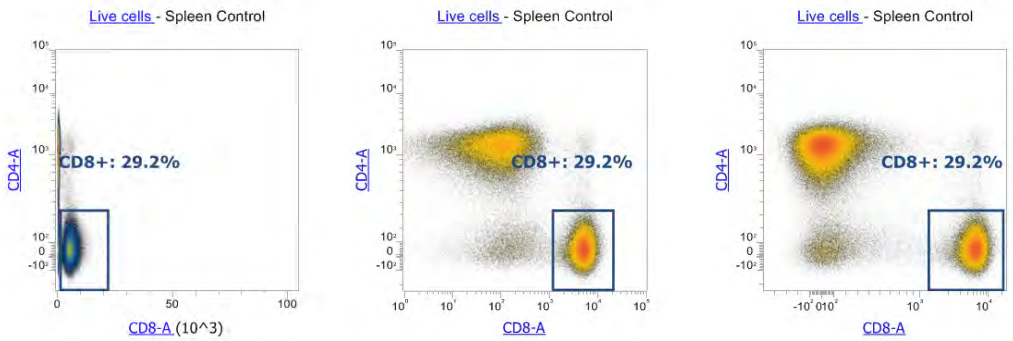
When changing plot parameter number or range, the gate is resized or deleted according to the rules described below. When the plot scale range, scale type, axes' parameters, or plot type is changed, the gates are resized or deleted as described below.

Changing Plot Scale Range

- If the plot scale range is changed on any axis, all gates remain fixed in their specified locations.
- If the change in scale range results in a gate being off plot, the gate behaves as described above in "Off-plot gates", page 113.

Changing Plot Scale Type

- If the plot scale type is changed (Linear, Log, HyperLog™) on any axis, the coordinates of the vertices for all gates (excluding oval gates) remain fixed and their connecting lines are redrawn as necessary.



Linear Log HyperLog™

- For oval gates, the coordinates of the bounding rectangle remain fixed and the perimeter ellipse and center position are redrawn as necessary.
- If the change in scale type results in a gate being off plot, the gate behaves as described above in "Off-plot gates", page 113.

Changing Plot Parameter

- When the parameter of any axis is changed, including using the *Swap Axes* option in the Plot context menu (page 127), all gates are maintained on the plot in their specified locations.
- If the change in parameter results in a change in scale type or scale range, the gates will conform to the rules defined for changing the plot scale or scale range as described above.

Changing Plot Type

- When a plot type is changed, a gate may or may not be deleted.
- When changing from a single parameter to a dual parameter plot or vice versa, all gates are removed from the plots.
- When changing from any dual parameter plot another type of dual parameter plot, the gates are maintained at their specified locations.

Workspace statistics

Overview The Attune™ Cytometric Software generates statistics from acquired events. Statistics can be displayed for any parameter and calculated for any defined population. As the events are acquired, statistics are updated in real time.

- To add a new **Statistics box** to the current Workspace, click the **Statistics** button on the Workspace ribbon tab (page 59).

  Alternatively, select **Insert ▶ Statistics** from the Workspace view context menu (page 122) or the Plot context menu (page 125).

Experiment: **In Vivo T cell Proliferation**
Group: **Group**
Sample: **Spleen Control**
Time Recorded: **14:58:49**

Name	Gate	Count	%Total	%Gated
All Events	All Events	2,239,344	100.0	100.0
Cells	Cells	1,183,954	52.9	52.9
singlets	singlets	1,160,149	51.8	98.0
CD3+	CD3+	500,075	22.3	43.1
Live cells	Live cells	482,357	21.5	96.5
CD8+	CD8+	141,087	6.3	29.2
R7	R7	9	0.0	0.0
R8	R8	45	0.0	0.0

- The statistics available are as follows: Plate, Experiment, Group, Sample, Workspace, Plot Name, Gate, X parameter, Y parameter, event count, concentration, % Total, % Gated, X mean, Y mean, X median, Y median, X mode, Y mode, X SD, Y SD X % CV, Y % CV, X rSD, Y rSD, X r% CV, and Y r% CV.
- You can customize the Statistics box using the **Customize Statistics Box Options** available in the Customize panel (page 291).
- When the statistics container is not large enough to hold all columns and rows of the Statistics table, indicators reveal that more data are present to the right or below the viewable area. To view any non-visible results, you must expand the results area.

Experiment: **In Vivo T cell Proliferation**
Group: **Group**
Sample: **Spleen Control**
Time Recorded: **14:58:49**

Name	Gate	Count	%Total	%Gated
All Events	All Events	2,239,344	100.0	100.0
Cells	Cells	1,183,954	52.9	52.9
singlets	singlets	1,160,149	51.8	98.0
CD3+	CD3+	500,075	22.3	43.1
Live cells	Live cells	482,357	21.5	96.5
CD8+	CD8+	141,087	6.3	29.2
R7	R7	9	0.0	0.0
R8	R8	45	0.0	0.0

- Double-clicking the Statistics table allows you to scroll to see the contents that are outside the visible area. In this mode, the right-click context menu also includes an option to export the statistics of the selected table.

Workspace statistics

- If no Workspace object is selected, clicking on the **Statistics** button on the Workspace ribbon tab or selecting **Insert ▶ Statistics** from the Workspace view context menu inserts a *Workspace statistics table* that displays results from all gates.

Experiment: **Whole Blood 1-4000 dilution**
Group: **Whole Blood**
Sample: **T6**
Time Recorded: **09:23:04**

Name	Count	Concentration	%Total	%Gated
All Events	57,545	30.4	100.0	100.0
PLT + WBC	57,545	30.4	100.0	100.0
Platelets	53,992	28.5	93.8	93.8
WBCs	3,430	1.8	6.0	6.0
-/+	84	0.0	0.1	0.1
+/+	2,456	1.3	4.3	4.3
-/-	53,893	28.5	93.7	93.7
+/-	1,112	0.6	1.9	1.9
RBC1	0	0.0	0.0	0.0
Noise	0	0.0	0.0	0.0

- The gates in the Workspace statistics table are displayed in the order specified by the *gate hierarchy*. Gates at the same hierarchical level are displayed in the order they are created. The color of the gate is displayed as a colored square to the left of the gate name.
- The *All Events statistic node* is displayed at the top of the hierarchy.

  The All Events node in the Statistics table only shows results for the following statistics: %gated, %Total, and Event Count.

  All other statistics for the All Events node display "N/A".

Individual plot statistics

- If a single plot is selected, clicking the **Statistics** button on the Workspace ribbon tab or selecting **Insert ▶ Statistics** from the Plot context menu creates a *Plot statistics box*, which displays the results from the gates present on the selected plot.

Parameters: **Blue 488 nm SSC - SSC-H vs Violet 405 nm SSC - VL1-H**
Gate: **All Events**
Experiment: **Whole Blood 1-4000 dilution**
Group: **Whole Blood**
Sample: **T6**
Time Recorded: **09:23:04**

Name	Count	Concentration	%Total	%Gated
All Events	57,545	30.4	100.0	100.0
PLT + WBC	57,545	30.4	100.0	100.0
RBC1	0	0.0	0.0	0.0
Noise	0	0.0	0.0	0.0

- The statistics box for the selected plot also displays the All Events statistic node as well as the results from any gate present on the plot.
- The individual plot statistics box displays the results for the gates on the current plot and any upstream gates.

Multiple plot statistics

- If multiple plots are selected, clicking the **Statistics** button on the Workspace ribbon tab or selecting **Insert ▶ Statistics** from the Plot context menu creates a Plot statistic box for each plot selected.
- Each statistic box contains the All Events statistics row, and only the statistics for the gates present on the selected plots as well as their parent gates.

Workspace images and text

Workspace images

You can add an image to the Workspace by selecting the *Image button* on the Workspace ribbon tab (page 59).

- The image types available for insertion include JPG, GIF, BMP, PNG, TIF and Windows enhanced metafile. The image file cannot be larger than one page and will be scaled to fit on the page preserving the aspect ratio, if necessary.
- Workspace images can be resized as described in "Resizing Workspace objects", page 86.

Workspace text

You can add a Text box to the Workspace by selecting the *Text box button* on the Workspace ribbon tab (page 59).

- The Text box displays the default text "New Text Box", which can be edited in place without opening a separate dialog box. The Text box can contain at up to 500 characters.
- The style and border of the Text box can be customized as described in "Customize Text Box Options" on page 290.
- If the Text box container is not large enough to hold all text, indicators reveal that more text is present to the right or below the viewable area. To view any non-visible text, you must expand the Text box.

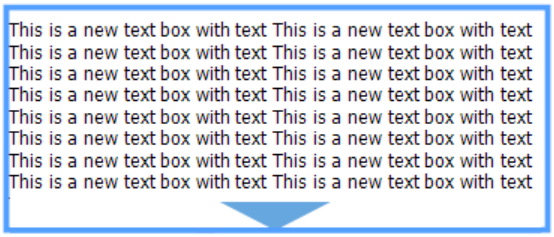
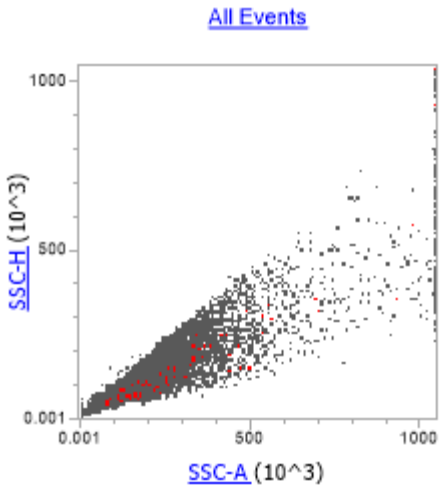


Image backgating

Image backgating allows you to identify the event that is associated with the captured cell image on a Dot plot or a Precedence Density plot in Workspace view.

When image backgating is applied, the events that have a corresponding image (ImageFlag parameter value of 1) are automatically identified in the plot in red.



By default, backgated events appear on the associated plot in red, but you can select a different color for backgated events using the Image Options dialog (page 498).

The backgated image events are always be maintained as the top-most events in the precedence order of backgated events (i.e., they are painted on top of other events).

Image backgating persists at the Workspace level for an Experiment, Group, or Sample.

You can use the following methods to backgate images:

- Right-click on the cell image you want to backgate in Image View, then select **Show Image on Plots.** You can select the Active image or any image from the Image Gallery.

  To backgate multiple images, select the images you want to backgate in the Image Gallery, then right-click and select **Show Images on Plots**.

  To undo the backgating of an image, right-click on the backgated image, then select **Clear Image on Plots**.

  To undo all backgates on a plot, right-click on the Active image or on any image in the Image Gallery, then select **Clear All Images on Plots**.

- Click **Backgate All Images** in the Image Settings ribbon tab (page 79) to backgate all imaged events on the corresponding Workspace plots.

  To undo the backgating of all images, click **Backgate All Images** again.

  When enabled, the Backgate All Images button will be in the ON state (blue). When disabled, the button will be displayed in its OFF state.

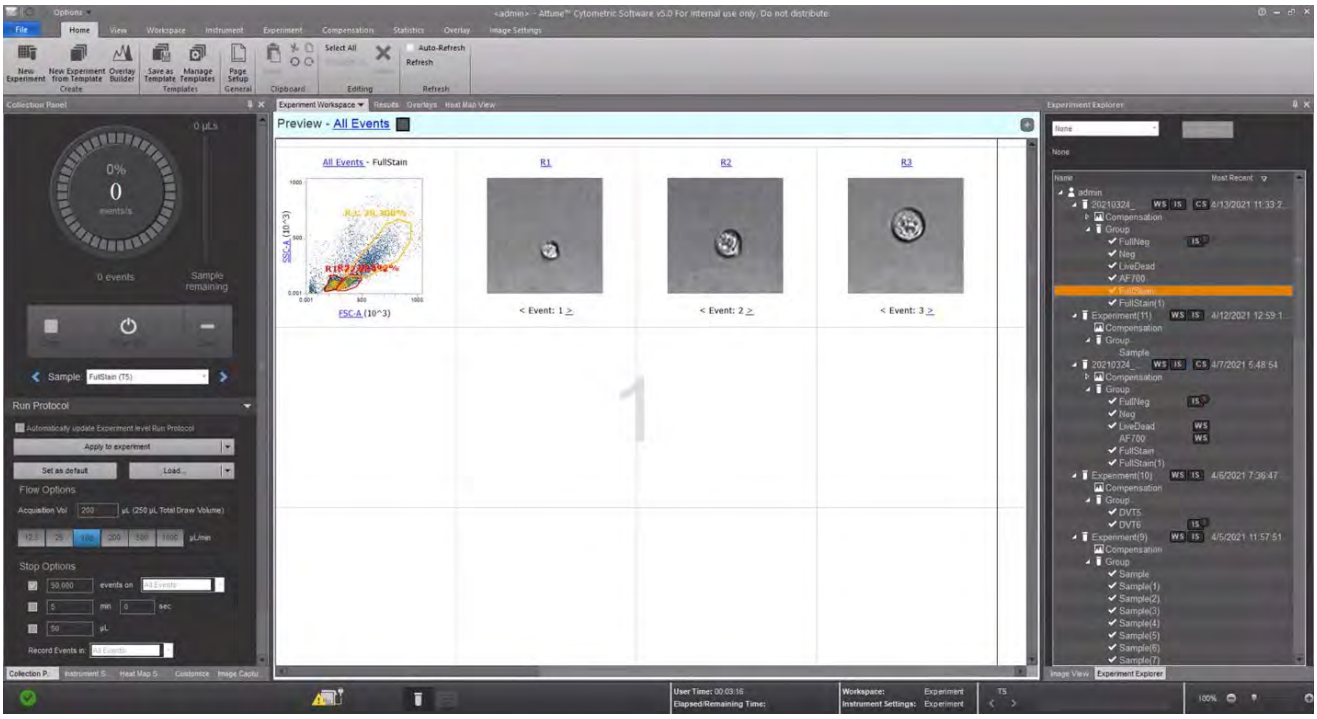
  The Backgate All Images button is enabled only if the selected Sample's listmode specification contains the “ImageFlag” and “Event” parameters.

Cell Image Container

Overview *Cell Image Containers* allow you to add cell images specific to the sample to the current Workspace. They act like plots and can be batch printed with the Workspace (cell images cannot be printed from the Image view Image Gallery).

Note: Cell image containers are available only when using an Attune™ CytPix™ Flow Cytometer or if the active Experiment was created with an Attune™ CytPix™ Flow Cytometer.

In the following example, the Workspace view contains a dot plot with three gated populations (R1, R2, R3) and three cell image containers. The cell image containers each have been gated to contain cell images from within one of the three gates (see "Population menu", page 120).



Note: For the Cell Image Container context menu, see page 137.

Population menu

- The Cell Image Container displays a gate hyperlink that allows you to filter the images displayed in the container.

  By default, the gate hyperlink is set to **All Events**.

- Clicking the gate hyperlink displays the gate context menu where you can select a gate to only allow images within the gate to be displayed.

- You can select any gate type. Quadrant gates are displayed in a sub menu.
- The active gate is indicated by the check mark to the left of the gate name.
- When you select a gate, the cell image container only allows the selection of images where the events in the gate have a corresponding image.
- If the gate does not contain any images, the image container display “No images are available”.
- When a gate is moved or the plot/gate is deleted, the cell image container updates accordingly. If the gate equation is changed, the cell image container can only display images within the gating hierarchy.

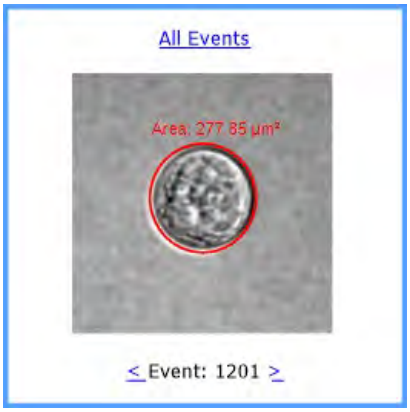
Navigation

- The Cell Image Container displays the event ID as a label under the image.

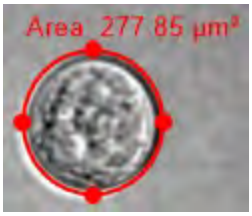
- The image container allows the image to be changed within the selected gate using the < and > hyperlinks to the left and right of the event label that shows the active event ID of the image.
- The < hyperlink is disabled when the first available image in the selection is displayed and the > hyperlink is disabled when the last image within the gate selection is displayed.
- The selected images index persists as part of the image container report object. The image index is the index of the image within a vector of images; it is **not** the event ID.
- If the image index does not exist when opening another sample, it defaults to the first image within the available images for the selected gate.

Measure image tool

The *Measure image* tool allows you to measure the area of an imaged cell by drawing an ellipse in the Cell Image Container that encircles the imaged cell.



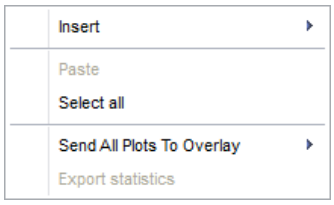
- When the **Measure** option is selected in the cell image container context menu (page 137) or the **Measure Image** tool is selected in Cell Image Analysis group of the Workspace tab, the measure image insert cursor is displayed.
- You can resize the ellipse using the handles that become visible when the ellipse is selected to stretch and rotate it.
- To delete the measurement tool, click on the ellipse, then select from the keyboard or the context menu.
- The measured area is based on a conversion factor of micrometers per pixel.



Workspace view context menu

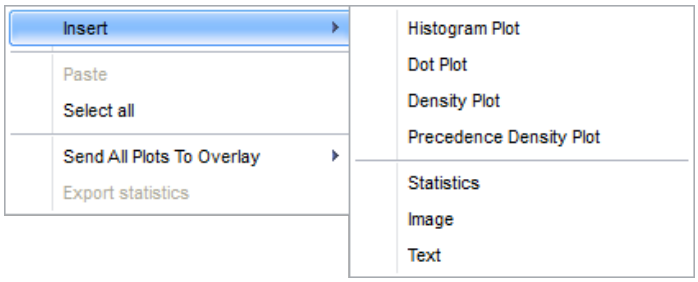
Overview

The *Workspace view context menu* is displayed when you right-click on any empty area (i.e., white space) within the Workspace view. It contains the tools to insert plots, statistics, images, and text to the Workspace, to paste and select Workspace objects, and to export statistics.



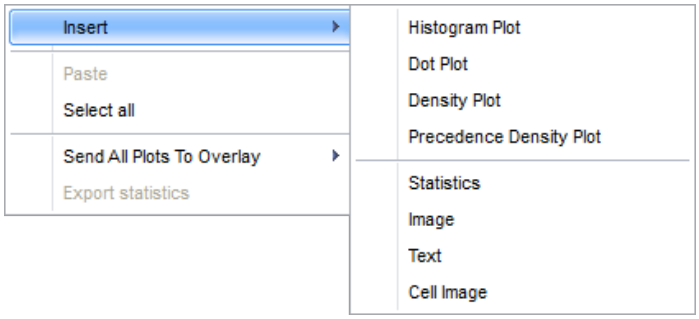
Insert

Selecting the **Insert** option displays a sub-menu, allowing you to add Histogram Plot, Dot Plot, Density Plot, Precedence Density Plot, Statistics, Image, and Text objects to the current Workspace.



These menu options have the same effect as pressing the respective buttons on the Workspace ribbon tab as described on page 55.

If you are using an Attune™ CytPix™ Flow Cytometer or when an Experiment that was created with an Attune™ CytPix™ Flow Cytometer is active, the Insert sub menu also includes an option to insert Cell Image Container to the current Workspace.



Paste and Select all

Paste

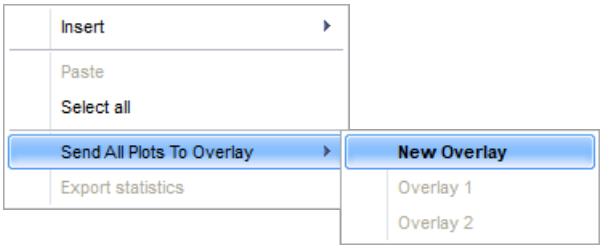
- **Paste** inserts the object currently in the clipboard to the Workspace at the location clicked. Paste option is only available if a Workspace object has been copied or cut to the clipboard.
- Clicking the **Ctrl+V** keyboard combination pastes the object at same location as the original. As each new object is pasted, it is inserted cascading downwards and to the right of the original location.
- Objects can be cut or copied from any Workspace and pasted to any other Workspace.
- Plots are pasted with any gates present. If a gated plot is copied to a new location where the parent gate does not exist, the pasted plot will not be gated and will display all events.
- If a Plot statistics box is pasted to a new Workspace and the target Workspace does not contain a copy of the original plot, the statistics box will update to show all gates present in the current Workspace. The Plot Axis and Gate fields will be removed from the statistics box header (if originally present).
- Pasted objects are selected by default after the paste action has completed.

Select all

- **Select all** selects all objects on the Workspace.

Send All Plots to Overlay

- **Send All Plots to Overlay** sends all Workspace plots to a selected *Overlay* (page 156).



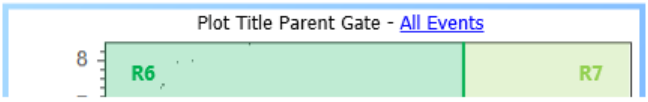
The options depend on the type of plot present on the Workspace and the Overlay plots already present in the Overlay view.

- The *New Overlay* option is always present and enabled unless the maximum number of Overlays have already been created.
- The names of any Overlay plots already present in the Overlay application are also displayed.
- If both Histogram and dual parameter plots (Dot plot or Density) are present on the Workspace, the only option is to send plots to a new Overlay. This will send histogram plots to a new Overlay and dual parameter plots to another new Overlay.
- If only Histogram plots or dual parameter plots are present on the Workspace, you can either create a new Overlay or send the plots to an existing Overlay that contains plots with the same number of parameters.

  Overlay plot names in the context menu will be disabled for Overlay plots with non-matching number of parameters.
- The Overlay name in the context menu will also be disabled if the number of plots to be sent exceeds the allowable number of plots in the specific Overlay.

Plot title hyperlink

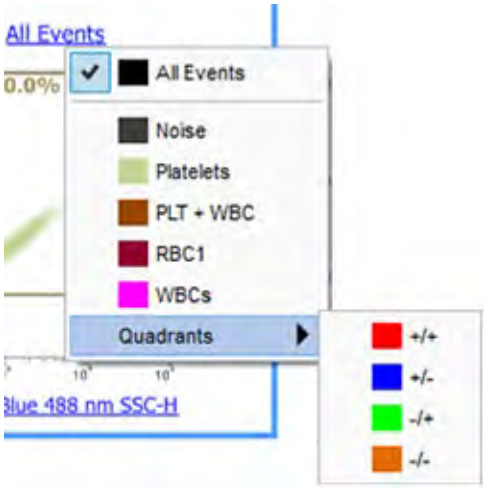
If **Include** option is checked in the *Customize Plot* options (page 277), the plot title displays the current parent gate of the plot as a hyperlink. This option is checked by default.



- Clicking the **plot title hyperlink** displays a dropdown menu that contains a list of all available gates, including any derived gates.

  Any gates present on the currently selected plot and their daughter gates are not included in the list of gates.

  Quadrant gates are displayed in a submenu.



- You can select one gate from the dropdown menu to be used as the parent gate of the current plot. The hyperlink then updates to display the currently selected gate name.

Plot context menu

Overview

The *Plot context menu* is displayed when you right-click on any empty area (i.e., white space) within the plot boundary, but not on another active area (gate, plot title, or plot axis).

The Plot context menu contains the tools for gate creation and customization, to cut, copy, paste, and delete selected items, and save all selected items as a new graphics file.

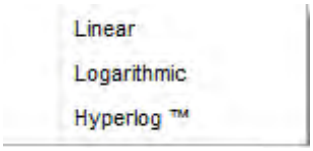
Customize

Selecting **Customize** from the dropdown menu displays the *Plot Customize panel*, which allows you to format different properties of the selected plot as described in "Customize Plot Options" on page 277.

- If the panel is closed, it will be opened and positioned in the left docking panel.
- If the panel is docked and not in the foreground, it will be brought to the front of the docked panels.

Scale

Scale sets all parameter axes to the selected scale. Available scale types are Linear, Logarithmic, and HyperLog™.



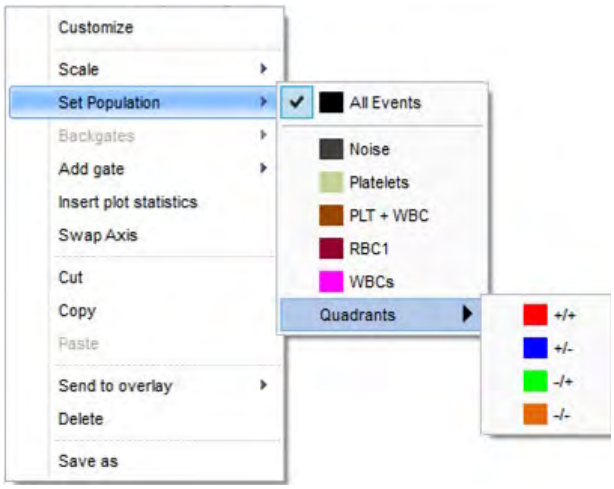
- The Scale option is available only when single or multiple plots are selected. If images or statistics objects are also selected, this option is not available.
- On dual-parameter plots (Dot or Density plots) the X and Y-axes are modified.

  On single parameter plots (i.e., Histograms), the selection is only applied to the X-axis.

Note: The Hyperlog™ scale uses log-linear hybrid transformations to display compensated flow cytometry data that frequently contain negative values due to compensation. Logarithmic transformations cannot properly handle negative values, and poorly display normally distributed cell types.

Set population

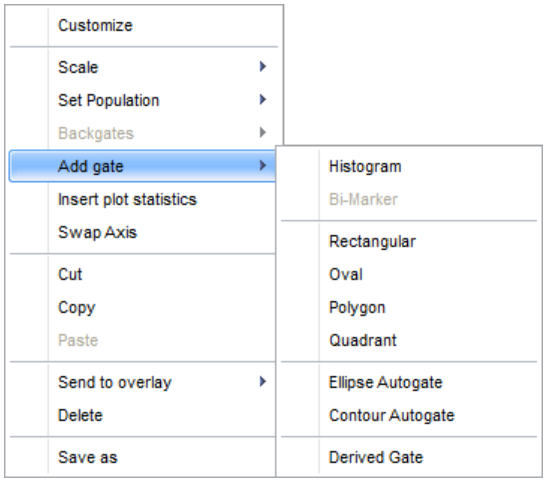
Set population allows you to choose to limit the data displayed on a plot to a given gate. It makes the plot a daughter of the selected upstream gate.



- A single gate can be selected from the list of available gates to be used as a parent gate for the selected plot. The selected gate is indicated by a tick mark in the list. It makes the plot a daughter of the upstream gate.
- The list of gates includes the All Events option and any available derived gates.
- The list does not include any gates present on the selected plot or any gates derived from those gates.
- The Set population option is also available when multiple plots are selected. In such cases, the available gate list excludes the gates that are present on any of the selected plots or any gates derived from those gates. The selected gate is used as a parent gate for all selected plots.

Add gate

Add gate allows you to create a new gate or a new derived gate in the default position on the currently selected plot. This option is available only when a single plot is selected.



- The Histogram option is only available for Histogram plots.

  Rectangular, Oval, Polygon, Ellipse Autogate, Contour Autogate, and Quadrant options are available only for dual parameter plots.

  Selecting these options creates the selected gate type in the default position described in "Create and delete gates", page 98.
- The Derived Gate option opens the Derived Gate dialog (see page 421).

Insert plot statistics

Insert plot statistics inserts a *Plot statistics box* for each selected plot as described on page 116. This option is available when single or multiple plots are selected.

Cut, Copy, and Delete

Cut

Selecting **Cut** copies the selected items to the clipboard and removes them from the Workspace.

Copy

Selecting **Copy** copies the selected items to the clipboard, but keeps the original items on the Workspace.

Delete

Selecting **Delete** permanently removes all selected items from the Workspace.

Send to Overlay

Send to Overlay sends all selected plots to a selected *Overlay* (page 156). The available options depend on the type of plots selected and the Overlay plots already present in the Overlay view.

- The **New Overlay** option is always present and enabled unless the maximum number of Overlays have already been created.
- The names of any Overlay plots already present in the Overlay application are also displayed.
- If both Histogram and dual parameter plots (Dot plot or Density) are selected, the only option is to send plots to a new Overlay. This will send histogram plots to a new Overlay and dual parameter plots to another new Overlay.
- If only Histogram plots are present or only dual parameter plots are selected, you can either create a new Overlay or send the plots to an existing Overlay that contains plots with the same number of parameters.
- Overlay plot names in the context menu will be disabled for Overlay plots with non-matching number of parameters. The Overlay name in the context menu will also be disabled if the number of plots to be sent exceeds the allowable number of plots in the specific Overlay.

Save As

Selecting **Save As** displays the *File Save (Export) dialog* as described on page 532, which allows you to send all selected items to a new graphics file.

- The layout of the graphics file will replicate the layout of the selected items on the current Workspace.
- The selection area for saving may only cover one page.
- The available image file formats are PDF, BMP, JPG, TIFF, GIF, PNG, and Windows Metafile. The default format is JPG.

Swap Axes

Selecting **Swap Axes** flips the axes of the selected plot, so that the X parameter becomes the Y parameter, and the Y parameter becomes the X parameter.

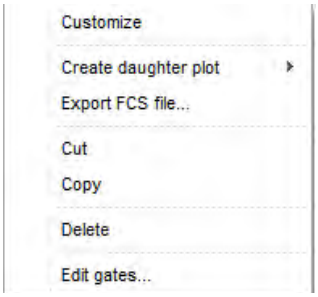
The Swap Axes option is only visible if at least one dual parameter plot is selected.

Gate context menu

Overview

The *Gate context menu* is displayed when you right-click on any part of a gate, on the gate boundary line, or a control point.

The Gate context menu contains the tools for customizing and editing gates, creating daughter plots, exporting FCS file, as well as for cutting, copying, and deleting selected gates.



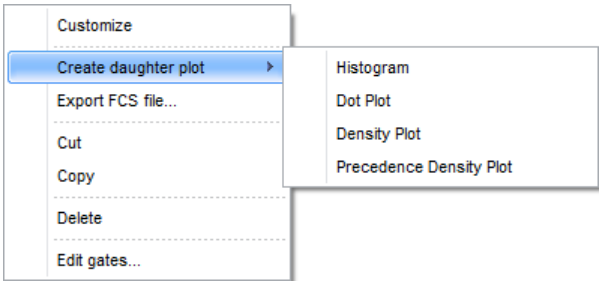
Customize

Customize displays *Gate Customize panel,* which allows you to format different properties of the selected gate as described in "Customize Gate options" on page 293.

- If the panel is closed, it will be opened and positioned in the left docking panel.
- If the panel is docked and not in the foreground, it will be brought to the front of the docked panels.

Create daughter plot

Create daughter plot displays a sub-menu, which allows you to choose the type of plot to display. This creates a new plot gated on the currently selected gate.



- Properties of the newly created daughter plot are based on the defaults set in the *Plot Options dialog* (page 480) and not those of the parent plot.
- If you select a dual parameter plot type (Dot plot, Density plot, or Precedence Density plot), the new plot displays parameter 1 on the X-axis (normally FSC) and parameter 2 (normally SSC) on the Y-axis. If FSC and/or SSC are not being collected, the appropriate parameter 1 and 2 will be used as described in "Plots" on page 91.
- If you select the Histogram option, the new plot displays parameter 1 (normally FSC) on the X-axis and Count on the Y-axis.
- If the Workspace is in Freeform mode (page 88), the new plot is created in the default position as described "Create Workspace objects", page 85.
- If the Workspace is in Auto Layout mode (page 88), a new plot of the same size as the parent is inserted in the next free grid location available on the Workspace.

Export FCS file

Export FCS file opens the *File Save* dialog to save a new FCS file containing only the events within the selected gate.

- This option is only enabled if the active sample has data and acquisition is not in progress.
- This option is disabled for quadrants.
- If there are any values for any of the keywords that have changed, a dialog is displayed allowing you to update the keywords.
  - If you select **Ignore**, the keywords are not updated (except for originality keywords).
  - If you select **Cancel**, the gated FCS data is not exported.
  - If you select **Update keywords** option, the keywords that have changed are updated.
- In all cases, the following keywords are updated:
  - $ORIGINALITY: Set to NonDataModified
  - $LAST_MODIFIED: The time a data file is appended or updated.
  - $LAST_MODIFIER: The name of the currently logged in user.
  - $TOT: Updated to reflect the number of events exported within the gate.

Cut, Copy, and Delete

Cut

Cut copies the selected gate to the clipboard and removes it from the plot.

When a gate is removed from a plot, any Derived Gate equations that include the removed gate are modified to exclude that gate. If the Derived gate includes only the gate that was removed, then the entire Derived Gate equation is deleted.

The cut gate can be pasted as described on page 87.

Copy

Copy copies the selected gate to the clipboard, but keeps the original gate on the plot.

Delete

Delete permanently deletes the currently selected gate and removes the gate from any Derived Gate equations and plot gates. If the gate equation includes only the deleted gate, then the entire gate equation is deleted.

Edit gates

Edit gates displays the *Edit gates dialog* (page 424).

This dialog provides a list of all available gates on the active Workspace and allows you to edit the gate color, gate math expression, and the order in which the gates are displayed in the dialog, to backgate all plots, and to delete gates.

Plot axis context menu

Overview

The *Plot axis context menu* is displayed when you right click on a plot scale or parameter name.

The Plot axis context menu contains the tools for creating and customizing plots and gates, to cut, copy, paste, and delete selected items, and save all selected items as a new graphics file.

Customize

Customize displays the *Plot Customize panel,* which allows you to format different properties of the selected plot as described in "Customize Plot Options" on page 277.

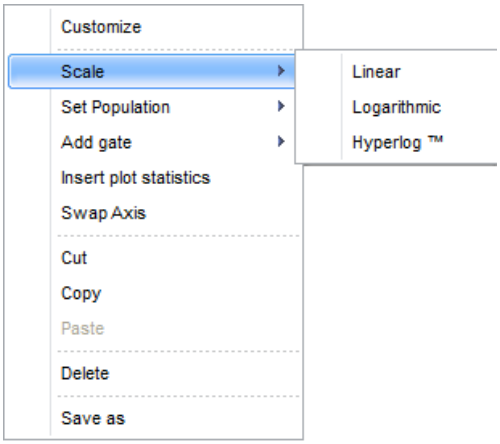
- If the panel is closed, it will be opened and positioned in the left docking panel.
- If the panel is docked and not in the foreground, it will be brought to the front of the docked panels.

Scale

Scale sets all parameter axes to the selected scale. Available scale types are Linear, Logarithmic, and HyperLog™.

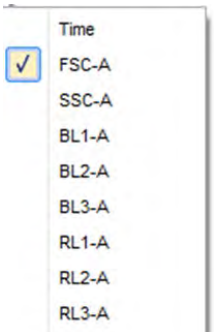
- The Scale option is available only when single or multiple plots are selected. If images or statistics objects are also selected, this option is not available.
- On dual-parameter plots (Dot or Density plots) the X and Y-axes are modified.

  On single parameter plots (i.e., Histograms), the selection is only applied to the X-axis.



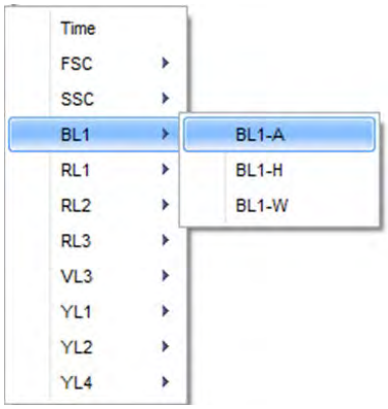
Parameter

Parameter allows you to choose the parameters to display on a selected plot axis. This option is visible and enabled when single or multiple plots are selected. If images or statistics objects are also selected, this option is not visible.



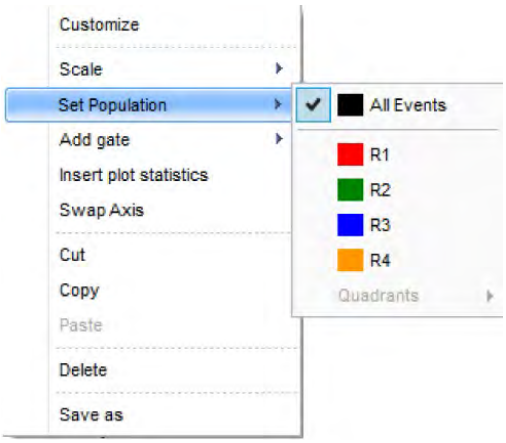
- The options displayed in the Parameter submenu depend on the number of parameters present in the current sample.

  If no dataset is present (i.e., the sample is empty), then the parameter list is based on the available and enabled parameters within the *Instrument Settings* (page 253).
- If 18 or less parameters are selected in the current sample, the Parameter submenu displays all the parameters.
- The currently displayed parameter appears checked in the menu, and you can select any of the available parameters to display. The appropriate plot axis will be updated on all selected plots to display the chosen parameter.
- If more than 18 parameters are selected in the current sample, the context menu displays the parameter names with the parameter type displayed as a sub-menu.



Set population

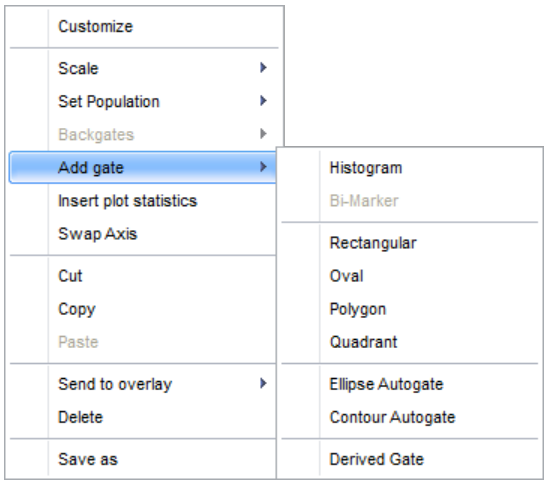
Set population allows you to choose to limit the data displayed on a plot to a given gate. It makes the plot a daughter of the selected upstream gate.



- A single gate can be selected from the list of available gates to be used as a parent gate for the selected plot. The selected gate is indicated by a tick mark in the list. It makes the plot a daughter of the upstream gate.
- The list of gates includes the All Events option and any available derived gates.
- The list does not include any gates present on the selected plot or any gates derived from those gates.
- The Set population option is also available when multiple plots are selected. In such cases, the available gate list excludes the gates that are present on any of the selected plots or any gates derived from those gates. The selected gate is used as a parent gate for all selected plots.

Add gate

Add gate allows you to create a new gate or a new derived gate in the default position on the currently selected plot. This option is available only when a single plot is selected.



- The Histogram option is only available for Histogram plots; Rectangular, Oval, Polygon, Ellipse Autogate, Contour Autogate, and Quadrant options are available only for dual parameter plots. Selecting these options creates the selected gate type in the default position described in "Creating and deleting gates" on page 98.
- The Derived Gate option opens the Derived Gate dialog described on page 421.

Insert plot statistics

Insert plot statistics inserts a *Plot statistics box* for each of the selected plots as described on page 116. This option is available when single or multiple plots are selected.

Swap axis

Swap axis flips the axes of the selected plot, so that the X parameter becomes the Y parameter, and the Y parameter becomes the X parameter.

The Swap Axis option is only visible if at least one dual parameter plot is selected.

Cut and Copy

Cut

Cut copies the selected items to the clipboard and removes them from the Workspace.

Copy

Copy copies the selected items to the clipboard, but keeps the original items on the Workspace.

Paste and Delete

Paste

Paste inserts the gate currently in the clipboard to the Workspace. The new gate will be assigned the next available gate name as described in "Gate name" on page 99.

The size and position of the pasted gate will be identical to the location and size of the original gate when it was copied to the clipboard, even if the location is outside the current plot area.

The Paste option is available only if a single plot is selected and if a gate type relevant to the current plot type has previously been copied into the clipboard.

If **Smart gating** is enabled, the new gate name is created as described on page 299.

Delete

Selecting *Delete* permanently removes all selected items from the Workspace.

Send to Overlay

Send to Overlay sends all selected plots to a selected *Overlay* (page 156).

The options available in the dropdown depend on the type of plots selected and the Overlay plots already present in the Overlay view.

- The **New Overlay** option is always present and enabled unless the maximum number of Overlays have already been created.
- The names of any Overlay plots already present in the Overlay application are also displayed.
- If both Histogram and dual parameter plots (Dot plot or Density) are selected, the only available option is **New Overlay**. This sends histogram plots to a New Overlay and dual parameter plots to another New Overlay.
- If only Histogram plots are present or only dual parameter plots are selected, you can either create a New Overlay or send the plots to an existing Overlay that contains plots with the same number of parameters.
- Overlay plot names in the context menu are disabled for Overlay plots with non-matching number of parameters. The Overlay name in the context menu is also disabled if the number of plots to be sent exceeds the allowable number of plots in the specific Overlay.

Save As

Save As displays the *File Save (Export) dialog* (page 532), which allows you to send all selected items to a new graphics file.

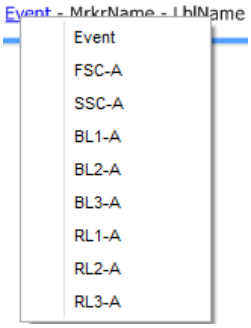
- The layout of the graphics file will replicate the layout of the selected items on the current Workspace.
- The selection area for saving may only cover one page.
- The available image file formats are PDF, BMP, JPG, TIFF, GIF, PNG, and Windows Metafile. The default format is JPG.

Plot parameter hyperlink

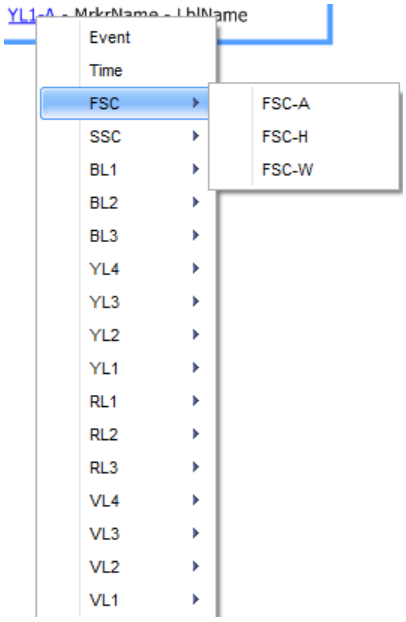
The plot parameter name, the X- and Y-axes of a dual parameter plot, or the X-axis of a Histogram plot are hyperlinks.

- Clicking on a parameter name displays the list of available parameters and calculated parameters. You select a parameter to apply to the selected axis from this list.
- The options displayed in the parameter name context menu depend on the number of parameters present in the current sample. Only parameters that are part of the dataset and selected in the *Workspace filters* (page 75) are listed.

  If no dataset is present (i.e., the sample is empty), then the parameter list is based on the available parameters within the *Instrument Settings* (page 253).
- If 18 or less parameters are selected in the current sample, the Parameter submenu displays all the parameters present in the current sample.



- The currently displayed parameter appears checked in the menu, and you can select any of the available parameters to display. The appropriate plot axis will be updated on all selected plots to display the chosen parameter.
- If more than 18 parameters are selected in the current sample, the parameter name context menu displays the parameter names with the parameter type displayed as a sub-menu.
- If only one parameter type exists, the parameter selection will remain in the main menu.
- If multiple plots are selected, the chosen parameter is applied to the selected axis of all selected plots. Any Y-parameter selection is not applied to any Histogram plots that are part of the multi-select.

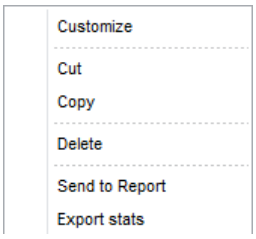


Statistics context menu

Overview

The *Statistics context menu* is displayed when you right-click a Statistics box on the Workspace (page 115).

The Statistics context menu contains the tools for customizing the Statistics box, to cut, copy, and delete the selected Statistics box, and to Export the selected Statistics as a single file.



Customize

Customize displays the *Statistics Box Customize panel,* which allows you to customize the statistic style and formatting of the selected Statistics box as described in "Customize Statistics Box Options" on page 291.

- If the panel is closed, it will be opened and positioned in the left docking panel.
- If the panel is docked and not in the foreground, it will be brought to the front of the docked panels.

Cut, Copy, and Delete

Cut

Cut copies the selected items to the clipboard and removes them from the Workspace. Cut items can be pasted as described on page 87.

Copy

Copy copies the selected items to the clipboard and keeps the original items on the Workspace. Copied items can be pasted as described on page 87.

Delete

Delete permanently removes all selected items from the Workspace.

Export stats

Export stats opens the *Save As* which allows you to specify a file name and location to export the selected statistics table to a CSV file.

This option is only enabled if the statistics table is activated by double clicking the table.

Image context menu

Overview

The *Image context menu* is displayed when you right-click on an image on the Workspace (page 115).

The Image context menu contains the tools to cut, copy, and delete the selected image.

Cut, Copy, and Delete

Cut

Cut copies the selected images to the clipboard and removes them from the Workspace. Cut images can be pasted as described on page 87.

Copy

Copy copies the selected images to the clipboard and keeps the original images on the Workspace. Copied images can be pasted as described on page 87.

Delete

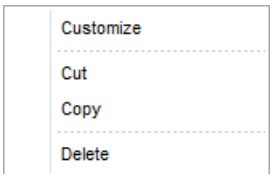
Delete permanently removes all selected images from the Workspace.

Text box context menu

Overview

The *Text box context menu* is displayed when you right-click on a Text box on the Workspace (page 115).

The Text box context menu contains the tools to cut, copy, and delete the selected image.



Cut, Copy, and Delete

Cut

Cut copies the selected Text box to the clipboard and removes it from the Workspace. Cut Text box can be pasted as described on page 87.

Copy

Copy copies the selected Text box to the clipboard and keeps the original Text box on the Workspace. Copied Text box can be pasted as described on page 87.

Delete

Delete permanently removes all selected Text boxes from the Workspace.

Text box text context menu

Overview

The *Text box text context menu* is displayed when you right-click on text within the edit area of a text box while the Text box is being edited or text is selected.

The Text box text context menu contains the tools to cut, copy, and delete the selected text.

Cut, Copy, and Delete

Cut

Cut copies the selected text to the clipboard and removes it from the Text box. Cut text can be pasted as described on page 87.

Copy

Copy copies the selected text to the clipboard and keeps the original text in the Text box. Copied text can be pasted as described on page 87.

Delete

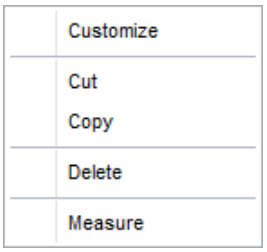
Delete permanently removes all selected text from the Text box.

Cell Image Container context menu

Overview

The *Cell Image Container context menu* is displayed when you right-click on a cell image container.

The Cell Image Container context menu contains the tools to customize, cut, copy, delete, and measure the selected cell image.



Customize

Customize displays the *Image Adjustment Customize panel,* which allows you to adjust the Brightness and Contrast of the image in the selected Cell image container as described in "Customize Image Adjustment options" on page 317.

- If the panel is closed, it will be opened and positioned in the left docking panel.
- If the panel is docked and not in the foreground, it will be brought to the front of the docked panels.

Cut, Copy, and Delete

Cut

Cut copies the selected text to the clipboard and removes it from the Text box. Cut text can be pasted as described on page 87.

Copy

Copy copies the selected text to the clipboard and keeps the original text in the Text box. Copied text can be pasted as described on page 87.

Delete

Delete permanently removes all selected text from the Text box.

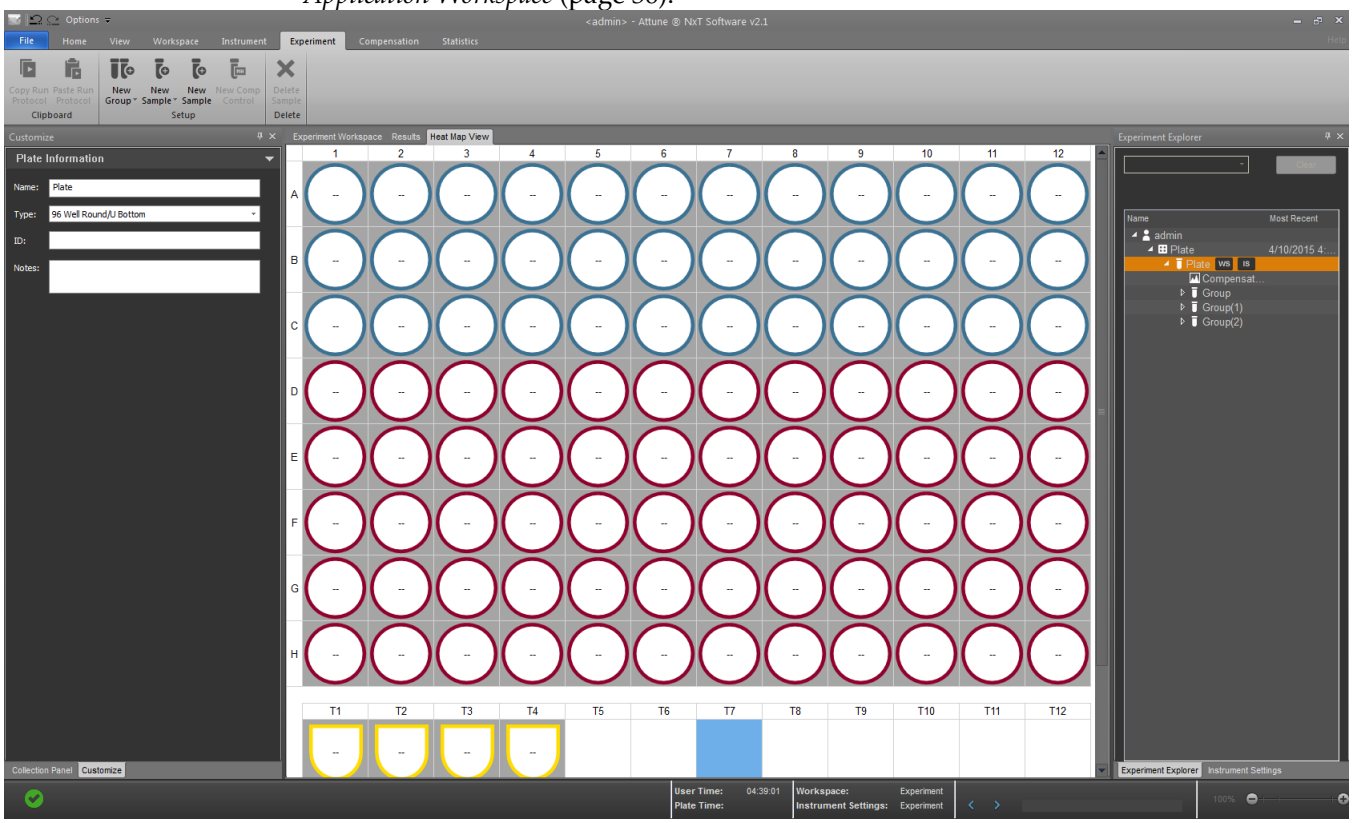
Measure

Measure you to draw a measurement tool on a cell image container to measure the area of the cell in the image (page 121).

6. Heat Map view

Overview

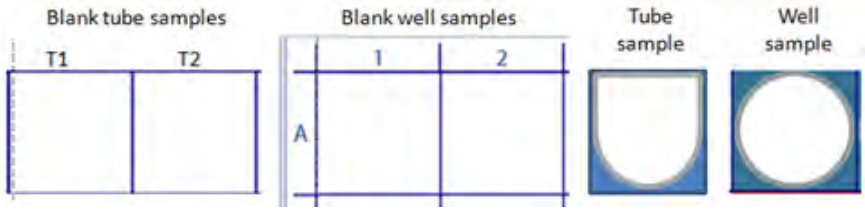
The *Heat Map view* provides a graphical method for setting up and analyzing plate- and tube-based experiments. By default, the Heat Map view is docked to the *Main Application Workspace* (page 36).



Display of Samples

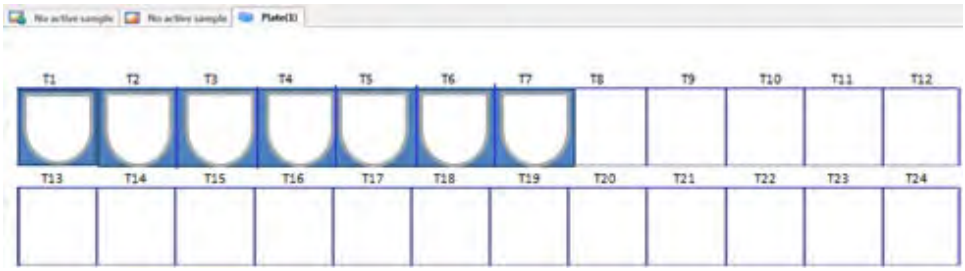
Overview

- The Heat Map displays both *Well* and *Tube samples*.
- A tube- or plate-based experiment may contain a total of 400 Samples.
- Well samples are created in a table as part of a 96- or 384-well plate and Tube samples are created by row.
- Blank sample spaces are displayed as white squares and Samples marked as part of an experiment show the experiment and group color as described in "Experiment/Group labels" on page 142.



Tube Experiment

- Tube samples for a new tube experiment are displayed in rows of 12 until 205 Samples are reached, at which point the view will switch to 24 Samples per row.
- Each Tube sample is labeled with its location on the Heat Map, beginning with T1 and continuing to T400, which is the limit to the number of Samples a tube- or plate-based experiment may contain.

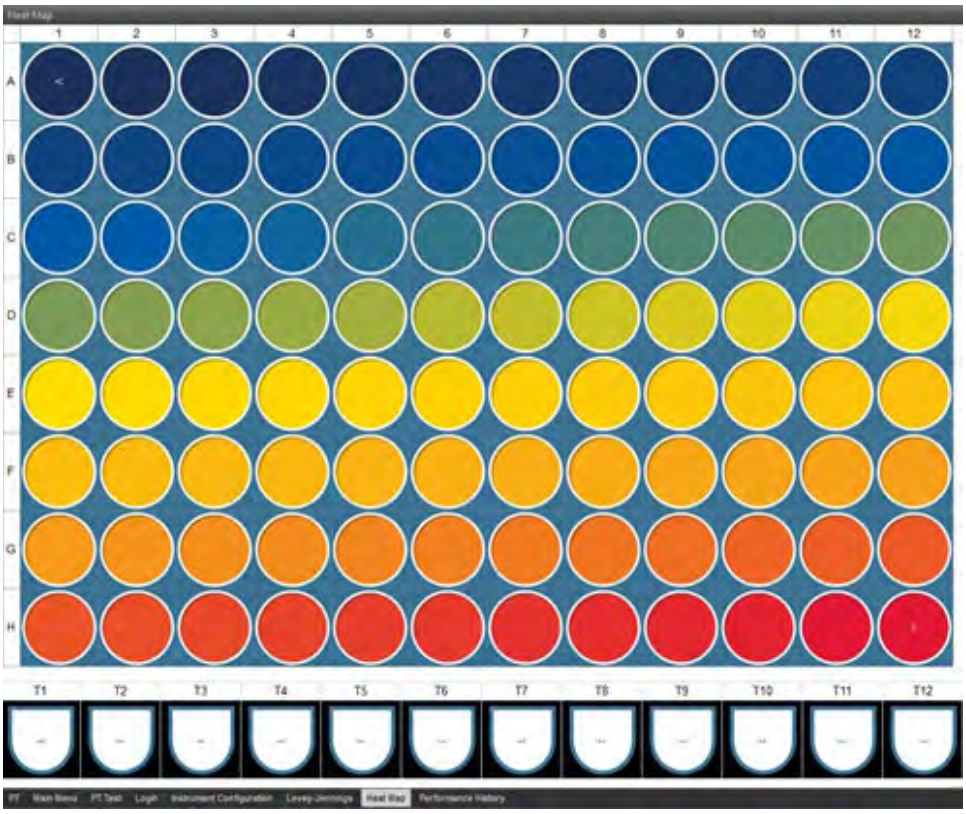


- The Heat Map for a Tube experiment contains the number of Tube samples that were originally created using the *New Experiment dialog* (page 399).

  Any remaining tube spaces in a Tube sample row and the entire row below it are left as *blank Tube samples*.
- As new Tube samples are generated after the initial experiment creation, the Heat Map continues to retain a complete row of blank Tube samples until the total Sample limit is reached.

Plate Experiment

- Heat Map for a new Plate Experiment is displayed with an empty table of 96 or 384 wells (depending on the plate type selected), plus the number of Tube samples created in the New Experiment dialog (page 399).



- 96-well Plate Experiments can contain up to 304 Tube samples (for a total of 400 Samples per experiment). Any remaining Samples are shown as blank Tube samples.
- 384-well Plate Experiments can contain up to 16 Tube samples (for a total of 400 Samples per experiment). Any remaining Samples are shown as blank Tube samples.
- Any Tube samples displayed in the Heat Map for a Plate Experiment are followed by the required number of blank Tube samples to complete the row, plus an entire row of Blank samples.
- If the Plate Experiment contains no Tube samples, then the Heat Map displays one complete row of blank Tube samples following Well samples.
- As new Tube samples are generated after the initial experiment creation, the Heat Map continues to retain a complete row of blank Tube samples until the total Sample limit is reached.

Note: Compensation samples in tubes are not shown in the Heat Map. Therefore, they do not count towards the 400 Sample limit.

Navigation and selection

- Double-clicking a Sample in the Heat Map view loads the Sample using the *Experiment-level Workspace*.
- Multiple Samples can be selected at once. The following methods can be used alone or in combination to select the desired Tube and/or Well samples.
  - Use left-click and drag to lasso a selection of Tube and/or Well samples.
  - Click while holding **Ctrl** key to select non-contiguous Tube and/or Well samples.
  - Click while holding **Shift** key to select all wells between the selected points, by row order.
  - Click the header of a row to select that entire row.

    Alternatively, you can use the **Shift+Space** combination to select the entire row, if the row contains at least one selected Sample.
  - Click the header of a column to select that entire column.

    Alternatively, you can use the **Ctrl+Space** combination to select the entire column, if the column contains at least one selected Sample.
  - Click the top left corner of a plate diagram to select the entire plate.

    Alternatively, you can use the **Ctrl+A** combination to select the entire plate, if the plate contains at least one selected Sample.

Set up an Experiment

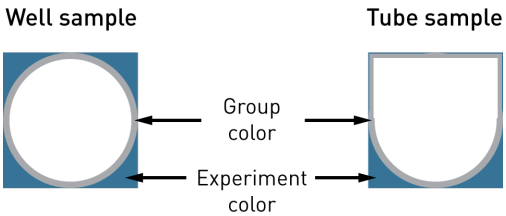
Experiment/Group labels

Use the **New Group** ( ), **New Sample** ( ), and **Edit Sample** ( ) buttons to create Samples and organize them into groups.

- Tube- and Well-based Samples have different shape to help identify them more easily.
- Each Experiment has a unique identifying color that fills the area around the Sample that belongs to it. This color differentiates the Sample belonging to that Experiment from Samples in other Experiments.

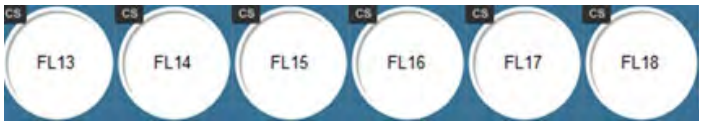
  You can change the Experiment color using the *Experiment information* options in the *Customize panel* (page 307) while in the Heat Map view.
- Each Group has a unique identifying color that is marked around the inside of the Experiment color identifier.

  You can change the Group color from the *Group information* options in the *Customize panel* (page 308) while in the Heat Map view.



Compensation wells

- You can map Well samples as Compensation samples using the *Compensation Setup dialog* as described on page 379.
- Well samples that are mapped as Compensation samples are tagged with a Compensation sample icon CS and the name of the parameter they represent. These samples display the Experiment color as the Group color.



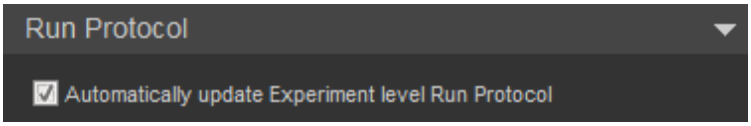
Manual wells

- You can map Well samples as Manual wells using the *New Manual Well* button in the Experiment tab (page 66).
- Well samples that are mapped as Manual wells are tagged with a manual well icon MW.

Run Protocol

- You can set up Run Protocols using the *Collection panel* (page 221). Each Sample can have a unique Run Protocol.

Note: By default, the "Automatically update Experiment-level Run Protocol" option is selected in the Run Protocol (page 240). You must deselect this option for each sample to have a unique run protocol.

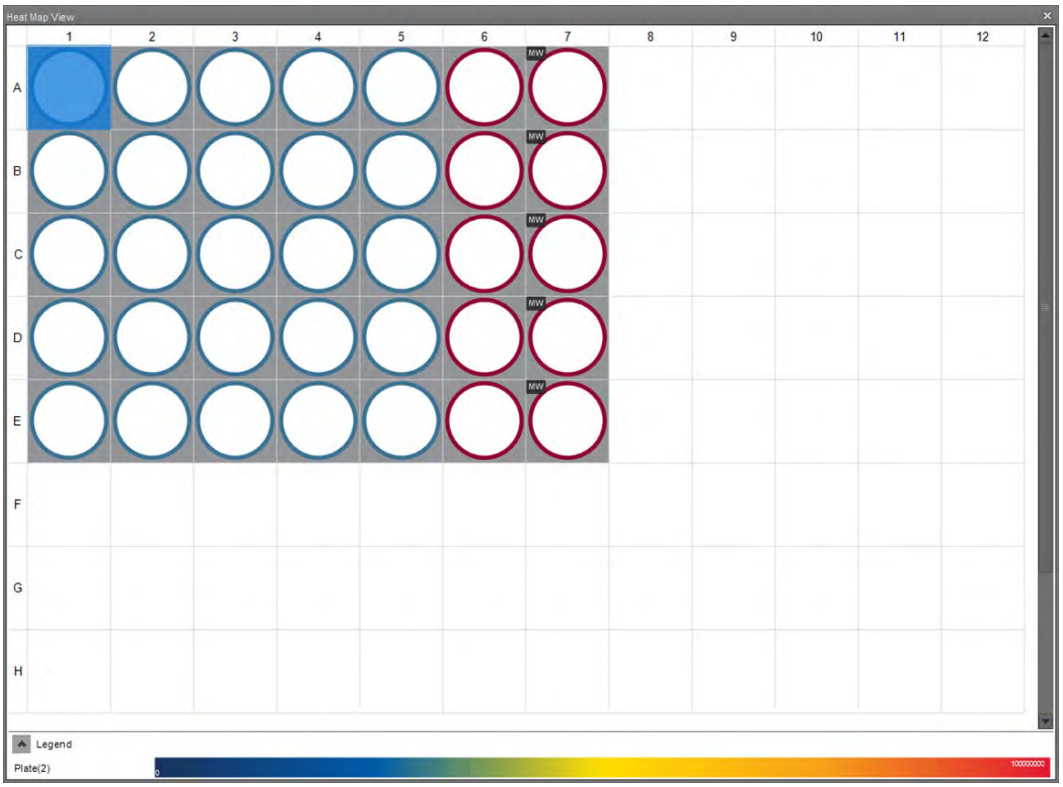


- You can and paste Run Protocols using the **Copy Run Protocol** and **Paste Run Protocol** buttons.

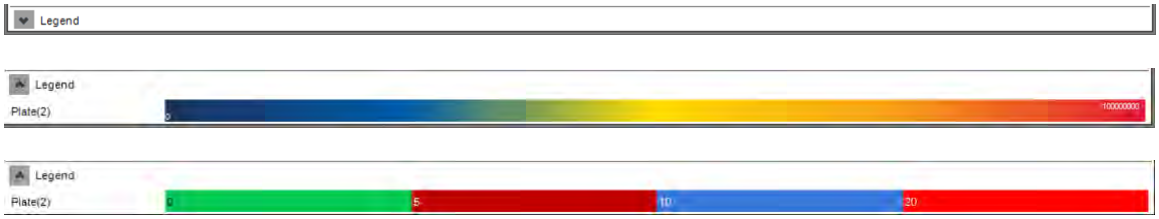
  Alternatively, you can use the hot keys to copy and paste the selected Run Protocols.
- If multiple wells with different Run Protocols settings are selected, you can edit individual fields that are in common without altering other settings.
- Fields with all values in common display the selected value. Fields with differing values are displayed as blank, but are active.

Legend

The legend for the Experiment shows the display mode, color key, and the range that is specified during Heat Map setup (page 267).



- The legend is attached to the bottom of the Heat Map panel and can be minimized, but not closed. It is automatically resized horizontally if the Heat Map panel size is changed.
- The legend displays the Experiment name in a column on the left, and the Heat Map or Threshold colors on the right.
- In the **Threshold display** mode, transition values are shown just to the right side of the transition point (see "Display of results", page 145).
- In **Gradient display** (i.e., Heat Map display) mode, minimum and maximum values are always displayed on the legend (see "Display of results", page 145).
- The value and location of any transition points that have been added by the user will be displayed on the legend just to the right side of the transition point.



Minimized

Gradient mode

Threshold mode

Analyze results

Display of results

Based on the Heat Map settings specified in the *Heat Map Setup panel* (page 267), each Sample with saved data displays a color and, if it is set, the numerical value of the statistic selected for that Sample.

Threshold display mode

If the **Threshold display mode** is selected on the Heat Map Setup panel, each Sample in the Heat Map view displays the appropriate color depending on whether the signal from the Sample falls above or below the set threshold value.

Heat Map display mode (i.e., Gradient mode)

If the **Heat Map display mode** (i.e., Gradient mode) is selected on the Heat Map Setup panel, each Sample in the Heat Map view displays the color that corresponds to the appropriate signal range set during Heat Map setup.

- If the values fall below the minimum range set for the sample, the Heat Map shows the color representing the minimum value and the '<' symbol.
- If the values exceed the maximum range set for the sample, the Heat Map shows the color representing the maximum value and a '>' symbol.
- If statistics are selected for display on the Heat Map, the < and > symbols are hidden by the displayed statistic.

Tooltips

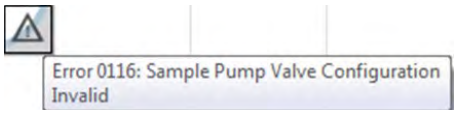
- Hover the mouse pointer over a well or a tube to display a tooltip with the following information:
  - Gate
  - Statistic
  - Sample
  - Group
  - Time recorded
  - Location
- Hover the mouse pointer over Blank sample spaces to display a tooltip that shows the Heat Map location (i.e., plate row and column or tube number).
- Hover the mouse pointer over Compensation samples to display a tooltip that shows the compensation parameter and the Heat Map location (i.e., plate row and column or tube number).
- Any fields on the tooltip which are not applicable is left blank.

Indicators

- If an error causes the Sample run to terminate prematurely, the *error icon* is displayed in the Heat Map location of the Sample.

  On mouse-over, a tooltip is displayed providing more information about the error that was encountered.
- If a bubble is detected during acquisition, the *Bubble detected icon* is displayed in the Heat Map location of the Sample.

- If an error occurs during acquisition, the *Generic Error icon* is displayed in the Heat Map location of the Sample.

7. Sample List view

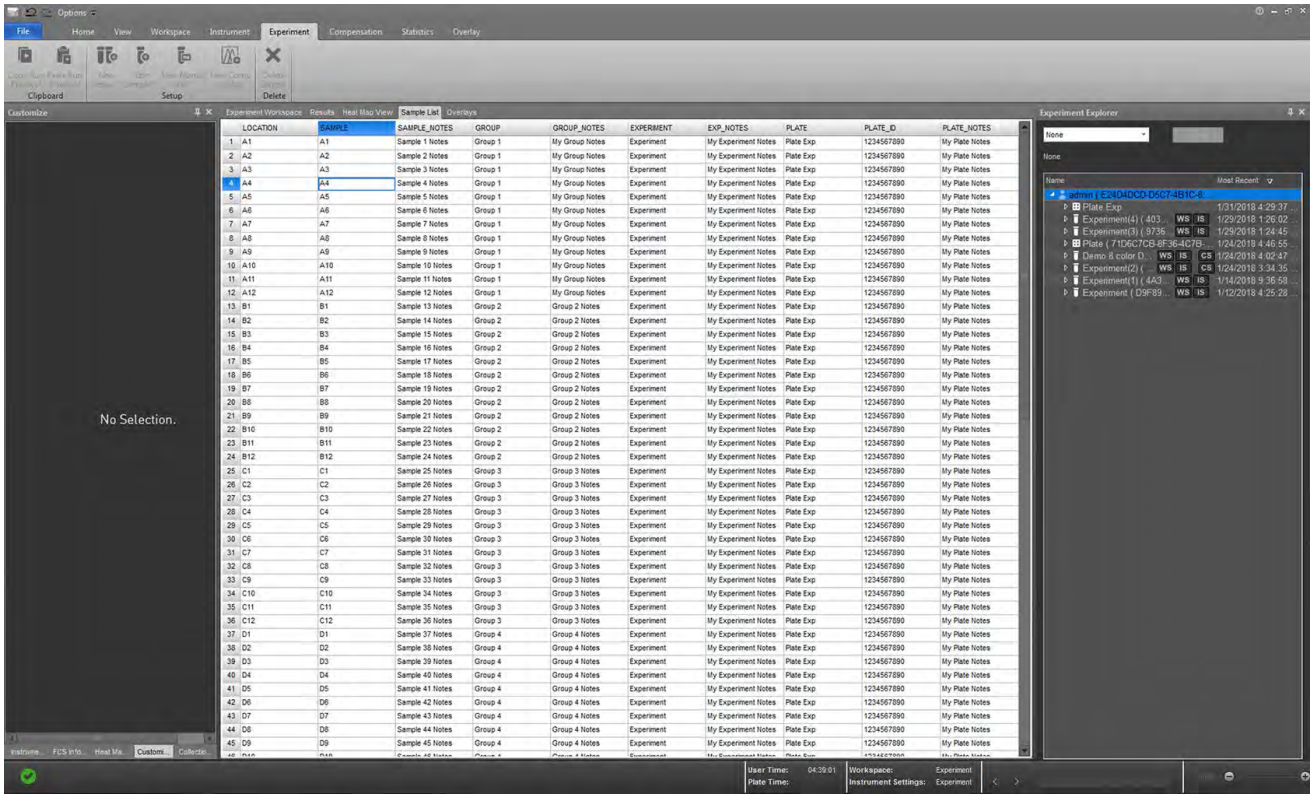
Overview

The *Sample List* is a grid view of the Samples in the current Experiment. It displays a table of all Samples in the active Experiment with columns for the following values:

- Location
- Sample name
- Sample notes
- Group name
- Group notes
- Experiment name
- Experiment notes
- Plate name
- Plate notes
- Plate ID

Additional columns for Experiment keyword are also displayed for all keywords listed in the *Experiment Keywords dialog* (page 445).

By default, the Sample List view is docked to the *Main Application Workspace* (page 36) and is closed. You can open the Sample List View by selecting it in the Show Panels group of the View tab (page 54).



Sample List properties

Edit values in the Sample List

- You can edit the following sample information in the Sample List:

  Sample name, Sample notes, Group name, Group notes, Experiment name, and Experiment notes
- You cannot edit the Location information (T1, T2, T3... etc; A1, A2, A3… etc).
- Modifications to Group name apply to all Samples within the same Group.
- Modifications to Experiment name apply to all Samples within the Experiment.
- When the Sample List view for a specific Experiment is in view, additional columns for Experiment keyword are displayed for all keywords listed in the *Experiment Keywords dialog* (page 445).

  The title of each column is the name of the keyword.

  When creating a new Experiment, if a default value is specified, that value is populated for the given keyword.

  You can edit the keyword fields for each Sample, or you can leave them blank.
- All changes to the Names and or Notes made in the Sample List are propagated throughout the application (i.e., Customize panel, Collection panel, Status bar, Experiment Explorer, and the Stats cache are all updated).
- Name validation occurs when you click elsewhere or when you press the **Enter** key. For the characters and names allowed when editing a field in the Sample List, see "Rules for editing the Sample List".

Rules for editing the Sample List

- Name fields within each grouping must be unique. If names are not unique, a warning message is displayed and the cell reverts to the last unique name used.
- Sample, Group, Experiment, and Plate names must be less than or equal to 50 characters long. These fields cannot be blank.
- The Sample notes, Group notes, and Experiment notes allow up to 500 characters. Note fields can be left blank.
- Names cannot contain the following illegal characters: /:*?"<>|&.
- Names cannot end with a "period" character and cannot be any of the following words: "CON", "PRN", "AUX", "CLOCK$", "NUL", "COM1", "COM2", "COM3", "COM4", "COM5", "COM6", "COM7", "COM8", "COM9", "LPT1", "LPT2", "LPT3", "LPT4", "LPT5", "LPT6", "LPT7", "LPT8", "LPT9".
- If a Sample, Group, Experiment, or Plate name contains one of these invalid characters or names, the illegal character or name is not accepted and a warning message is displayed. In the case of the invalid name, the cell reverts to the last good name used.
- When a column is set to a number type, the cell only accepts digits, a negative character, thousands separator, and the decimal character as per system locale settings. The number of decimal digits allowed is determined by the limit specified in the keyword definition. A number field can be left blank.

  Note: The number fields allow only up to 16 decimals of precision as per the IEEE Standard for Floating-Point Arithmetic (IEEE 754).

- If a non-numeric value is entered into a number field, it will revert to the last permitted value in that field upon validation (i.e., pressing **Enter**, tabbing off the cell, or clicking elsewhere).
- Numeric value formats displayed in the Sample List are determined by the system locale settings.

Navigation in the Sample List

You can navigate the Sample List using the keyboard.

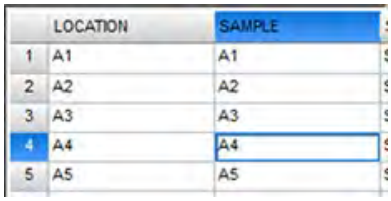
- Press the **Tab** key to move from cell to cell from right to left, and to jump to the next row when you reach the last column.
- Press **Enter** to validate the data in the current cell and move to the cell in the next row. When you reach the last row, press **Enter** to jump to the first cell in the next column.
- The **up** and **down arrow** keys move the cell up and down. If the cell is in Edit mode, the cell data is validated before advancing to the next well.
- The **left** and **right arrow** keys move the active cell left and right. If the cell is in Edit mode, the **left** and **right arrow** keys move the cursor position in the selected text.
- **Page up** and **page down** scroll the active view up and down a page at a time.
- To enter a cell into Edit move, enter any unicode character in that cell.

  Alternatively, press **F2** to enter the cell into Edit mode.
- You can only edit cells that are in editable columns.

Selection in the Sample List

Selection in the Sample List works similar to selection in Microsoft® Excel:

- To select a row of cells, click on the **row header** or use the **Shift+Space** keyboard combination.
- To select a column of cells, click on the **column header** or use the **Ctrl+Space** keyboard combination.
- To select all cells in the Sample List, click the **top left corner** of the table or use the **Ctrl+A** keyboard combination.
- Selected cells are highlighted in blue.
- The active cell is shown with a blue border.
- The row and column headers corresponding to selected cells are highlighted with a blue gradient to indicate which rows and columns have selected cells.



Multi-selection in the Sample List

You can select multiple cells in the Sample List as follows:

- Single-click a cell to select it.
- Ctrl-click additional cells to select those cells. Focus is set to the last selected cell.
- Shift-click to select the range of cells that fall within the rows/columns defined from the first cell to the last cell selected.
- Alternatively, click on a cell and drag the mouse to select multiple cells in the Sample List. The selected cells are defined by the row/column rectangle created between the start point (last clicked cell) and the cell under the current mouse position. The selection is actively updated while dragging the mouse.
- To select additional blocks of cells, Ctrl-click, then follow by Shift-click or click and drag.
- You can also select multiple cells using the keyboard arrows to select a cell, then using the Shift key along with the left/right/up/down arrows or page up/page down keys to select the range of cells defined by the row/column rectangle created between the start point (last clicked cell) and the current selected cell.
- If the Shift key is not pressed, only a single cell is selected and navigation occurs as described above.
- Press **Ctrl+Shift + an arrow key** to select all cells from the last clicked cell to the limit of the Sample List in the direction the arrow key.

Copy and Paste

You can copy content from the Sample List to the clipboard, and then paste it elsewhere in the Sample List or to an external spreadsheet such as Microsoft® Excel.

Similarly, you can copy content from Microsoft® Excel, and then paste it into the Sample List.

Copy and paste in the Sample List works similar to Microsoft® Excel:

- You can select, copy, and paste multiple columns and rows.
- You can paste only into the editable cells in the Sample List. New Samples must be added using the Import Sample List option in the Plate or Experiment context menus (page 203 and page 207, respectively).
- To copy the selected cells, use the keyboard shortcut **Ctrl+C** or the right-click context menu Copy option (page 150).
- If multiple cells are selected, the destination range must be the same as the source range (i.e., the number of rows and columns must match).
- When pasting contents from clipboard to cells in the Sample List that are part of a set (i.e., Group, Group note, Experiment, Experiment note, Plate, Plate note, or Plate ID), the items are pasted from top to bottom and left to right.
- The last item pasted in the set is applied to the set.
- If the clipboard contents include invalid characters or illegal names (i.e., system reserved names or names ending in periods), the contents are not pasted into cells where those entries are invalid.
- When pasting into a numeric keyword column and the allowable number of decimal places is exceeded, the number is rounded to the allowable number of decimal places.
- You cannot paste a non-numeric value into a numeric keyword cell.

Print the Sample List

To print the Sample List table, use the print command **(Ctrl+P)** when the Sample List view is in focus.

Alternatively, select **Print ▶ Sample List** from the File tab.

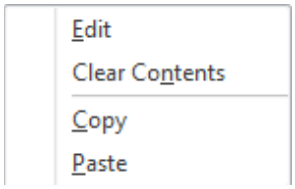
- When printing the Sample List view, the Sample List table is printed from left to right using the current page layout settings (orientation and paper size).
- Columns that do not fit on the same page are printed on the next page.
- Column headers are printed on each page.
- Cell selection does not show up when printed. The column and row header gradients are not printed.

Sample List context menu

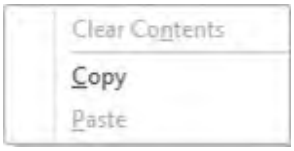
Sample List context menus

To display the Sample List context menu, right-click on a cell selected in the Sample List.

- The Sample List context menu has the following options:



Single cell context menu



Multi-cell selection context menu

- **Edit:** Sets the cell to Edit mode. This option is only enabled when the instrument is not acquiring, or the instrument is paused. You can also enter cells into Edit mode by double-clicking on them.
- **Clear Contents:** Deletes the contents of the selected cells. This is only enabled for Sample notes, Group notes, Experiment notes, or Plate notes, and the instrument is not acquiring, or the instrument is paused.
- **Copy:** Copies the contents of the selected cells to the clipboard. This option is only enabled when the instrument is not acquiring, or the instrument is paused.
- **Paste:** Pastes the clipboard contents to the selected cells. This option is only enabled when there is content in the clipboard to paste, the instrument is not acquiring or paused, and the instrument is not in automation mode.

8. Results view

Overview

The *Results view* displays results from all Samples consolidated into a single table (i.e., the *Results table*).

Experiment Workspace | Results | Overlays | Heat Map View

Drag a column header here to group by that column.

Group	Workspace	Compensation Source	X Parameter	Gate	Y Parameter	Count	Volume	Concentration	%Total	%Gated	X Mean	Y Mean	X Median	Y Median	X Peak	Y Peak	X SD	Y SD	X %CV	Y %CV	X rSD	Y rSD
Group	Experiment	None	FSC-A	R1	SSC-A	13841	55	251.655	70.448	70.448	438284	226247	436047	214784	4086...	185418	72377.38	77395.62	16.51	34.21	71321.95	7664
Group	Experiment	None	FSC-A	All Events	SSC-A	19647	55	357.218	100....	100.000	458639	264860	438163	219773	4086...	185418	199578....	186772.82	43.52	70.52	112954....	10959
Group	Experiment	None	SSC-A	R2	SSC-H	13795	55	250.818	70.214	99.668	225783	145644	214352	141084	1854...	129914	77050.31	42041.68	34.13	28.87	76201.19	4189
Group	Experiment	None	SSC-A	All Events	SSC-H	19647	55	357.218	100....	100.000	264860	N/A	219773	N/A	1854...	N/A	186772....	N/A	70.52	N/A	109596....	
Group	Experiment	None	SSC-A	R1	SSC-H	13841	55	251.655	70.448	70.448	226247	145621	214784	141033	1854...	129913	77395.62	42012.88	34.21	28.85	76643.01	4180
Group	Experiment	None	BL2-A	R3		7142	55	129.855	36.352	51.772	398075	N/A	267276	N/A	3576...	N/A	376090....	N/A	94.48	N/A	355288....	
Group	Experiment	None	BL2-A	All Events		19647	55	357.218	100....	100.000	203052	N/A	10094	N/A	-555	N/A	334935....	N/A	164.95	N/A	14213.69	
Group	Experiment	None	BL2-A	R2		13795	55	250.818	70.214	70.214	207234	N/A	9586	N/A	-984	N/A	335149....	N/A	161.73	N/A	13027.61	
Group	Experiment	None	RL1-A	R4		1	55	0.018	0.005	0.007	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Group	Experiment	None	RL1-A	All Events		19647	55	357.218	100....	100.000	1635	N/A	1369	N/A	-33	N/A	1543.37	N/A	94.42	N/A	699.79	
Group	Experiment	None	RL1-A	R2		13795	55	250.818	70.214	70.214	1413	N/A	1340	N/A	1333	N/A	534.55	N/A	37.83	N/A	490.74	
Group	Experiment	None	RL1-A	-/+	BL2-A	7139	55	129.800	36.336	51.751	1373	398224	1301	267402	1333	357691	509.88	376098.32	37.13	94.44	468.50	35546
Group	Experiment	None	RL1-A	+/+	BL2-A	3	55	0.055	0.015	0.022	4694	44414	4513	22098	4513	10534	323.65	49009.43	6.89	110.35	16.31	1714
Group	Experiment	None	RL1-A	-/-	BL2-A	6644	55	120.800	33.817	48.162	1449	2364	1386	1973	1370	-984	536.61	1748.12	37.03	73.93	506.31	121
Group	Experiment	None	RL1-A	+/-	BL2-A	9	55	0.164	0.046	0.065	4991	3795	4781	3257	4781	1479	819.68	1600.78	16.42	42.18	238.70	133
Group	Experiment	None	RL1-A	All Events	BL2-A	19647	55	357.218	100....	100.000	1635	203052	1369	10094	-33	-555	1543.37	334935.25	94.42	164.95	699.79	1421
Group	Experiment	None	RL1-A	R2	BL2-A	13795	55	250.818	70.214	70.214	1413	207234	1340	9586	1333	-984	534.55	335149.71	37.83	161.73	490.74	1302
Group	Experiment	None	BL1-A	R5		11514	55	209.345	58.604	83.465	445260	N/A	142739	N/A	4333	N/A	470956....	N/A	105.77	N/A	205374....	
Group	Experiment	None	BL1-A	All Events		19647	55	357.218	100....	100.000	361489	N/A	39461	N/A	-1464	N/A	455263....	N/A	125.94	N/A	57618.28	
Group	Experiment	None	BL1-A	R2		13795	55	250.818	70.214	70.214	371900	N/A	38722	N/A	-2043	N/A	460751....	N/A	123.89	N/A	55311.36	
Group	Experiment	None	RL1-A	-/+(1)	BL1-A	12175	55	221.364	61.969	88.257	1435	421255	1360	95229	1333	4333	535.44	468823.55	37.31	111.29	496.67	13622
Group	Experiment	None	RL1-A	+/+(1)	BL1-A	1	55	0.018	0.005	0.007	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Group	Experiment	None	RL1-A	-/-(1)	BL1-A	1619	55	29.436	8.240	11.736	1242	970	1201	1761	1246	-2043	476.03	2364.69	38.32	243.89	421.06	91
Group	Experiment	None	RL1-A	+/-(1)	BL1-A	0	55	0.000	0.000	0.000	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Group	Experiment	None	RL1-A	All Events	BL1-A	19647	55	357.218	100....	100.000	1635	361489	1369	39461	-33	-1464	1543.37	455263.76	94.42	125.94	699.79	5761
Group	Experiment	None	RL1-A	R2	BL1-A	13795	55	250.818	70.214	70.214	1413	371900	1340	38722	1333	-2043	534.55	460751.03	37.83	123.89	490.74	5531
Controls	Experiment	None	FSC-A	R1	SSC-A	10046	99	101.475	64.426	64.426	422597	231820	420499	220605	4045...	205752	81195.27	79532.81	19.21	34.31	88236.94	7979
Controls	Experiment	None	FSC-A	All Events	SSC-A	15593	99	157.505	100....	100.000	394600	239119	397292	208572	4045...	156638	180283....	159317.94	45.69	66.63	156065....	11016
Controls	Experiment	None	SSC-A	R2	SSC-H	9987	99	100.879	64.048	99.413	231039	147811	219970	143270	2057...	130079	78992.54	43165.74	34.19	29.20	79104.12	4430
Controls	Experiment	None	SSC-A	All Events	SSC-H	15593	99	157.505	100....	100.000	239119	N/A	208572	N/A	1566...	N/A	159317....	N/A	66.63	N/A	110164....	
Controls	Experiment	None	SSC-A	R1	SSC-H	10046	99	101.475	64.426	64.426	231820	147744	220605	143222	2057...	130079	79532.81	43093.64	34.31	29.17	79797.98	4422
Controls	Experiment	None	BL2-A	R3		3	99	0.030	0.019	0.030	263086	N/A	31736	N/A	18136	N/A	412543....	N/A	156.81	N/A	20163.36	
Controls	Experiment	None	BL2-A	All Events		15593	99	157.505	100....	100.000	1165	N/A	879	N/A	-45	N/A	6773.41	N/A	581.33	N/A	502.60	
Controls	Experiment	None	BL2-A	R2		9987	99	100.879	64.048	64.048	1044	N/A	869	N/A	1045	N/A	7412.37	N/A	709.80	N/A	398.82	
Controls	Experiment	None	RL1-A	R4		193	99	1.949	1.238	1.933	20958	N/A	8144	N/A	6171	N/A	106248....	N/A	506.95	N/A	2235.76	
Controls	Experiment	None	RL1-A	All Events		15593	99	157.505	100....	100.000	2994	N/A	1737	N/A	-48	N/A	25802.92	N/A	861.70	N/A	908.83	
Controls	Experiment	None	RL1-A	R2		9987	99	100.879	64.048	64.048	2148	N/A	1646	N/A	1654	N/A	14990.60	N/A	697.99	N/A	661.24	
Controls	Experiment	None	RL1-A	-/+	BL2-A	2	99	0.020	0.013	0.020	1705	385561	1705	385561	1709	31736	6.36	500383.41	0.37	129.78	6.67	52458
Controls	Experiment	None	RL1-A	+/+	BL2-A	1	99	0.010	0.006	0.010	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Controls	Experiment	None	RL1-A	-/-	BL2-A	9550	99	96.465	61.245	95.624	1696	923	1611	848	1654	964	674.38	415.71	39.76	45.04	619.73	37
Controls	Experiment	None	RL1-A	+/-	BL2-A	434	99	4.384	2.783	4.346	11919	1900	5833	1883	4401	2055	71135.82	633.87	596.82	33.36	1781.34	53
Controls	Experiment	None	RL1-A	All Events	BL2-A	15593	99	157.505	100....	100.000	2994	1165	1737	879	-48	-45	25802.92	6773.41	861.70	581.33	908.83	50
Controls	Experiment	None	RL1-A	R2	BL2-A	9987	99	100.879	64.048	64.048	2148	1044	1646	869	1654	1045	14990.60	7412.37	697.99	709.80	661.24	39
Controls	Experiment	None	BL1-A	R5		15	99	0.152	0.096	0.150	74138	N/A	4077	N/A	4106	N/A	269577....	N/A	363.62	N/A	407.71	
Controls	Experiment	None	BL1-A	All Events		15593	99	157.505	100....	100.000	1094	N/A	809	N/A	-25	N/A	8738.21	N/A	798.70	N/A	462.57	
Controls	Experiment	None	BL1-A	R2		9987	99	100.879	64.048	64.048	994	N/A	779	N/A	669	N/A	10494.33	N/A	1055....	N/A	345.45	
Controls	Experiment	None	RL1-A	-/+(1)	BL1-A	20	99	0.202	0.128	0.200	3291	55413	3115	3124	3115	2792	1200.52	233766.29	36.48	421.86	1340.27	43

Results table

Results table

The Results table shows the statistics associated with gates from the selected Workspaces for **all Samples** within an Experiment.

- Results table displays only the gates for the Workspace. Each gate created in the Workspace generates a *results row* in the Results table.
- If **Auto-Refresh** is enabled, then results for all files in the Experiment are displayed; otherwise, only results for the processed files are displayed.

Primary table

- Primary table only displays the statistics selected in the Statistics tab (page 74), with each statistic arranged in a separate column in the table.
- By default, columns automatically adjust their width to fit the widest text in the column. The width of a column can be also manually adjusted by dragging the right column divider (i.e., the divider on the right of the column).

  Once a column width has been manually adjusted, it does not automatically adjust to fit any new text in the column. Double-clicking the right column divider sets that column back to auto-adjust its width.
- By default, the columns for the primary table are added in the following order:

  Plate, Experiment, Group, Sample, Workspace, Plot Title, Gate, X parameter, Y parameter, Count, Concentration, % Total, % Gated, X mean, Y mean, X median, Y median, X mode, Y mode, X SD, Y SD, X %CV, Y %CV, X rSD, Y rSD, X %rCV, Y %rCV, and Autogate Status.

  Plate, Experiment, Group, and Sample columns display their respective names, and the Workspace column displays the source.
- All columns are added to the table in the order the checkboxes are arranged on the Statistics tab, left to right then top to bottom within a Ribbon group. You can re-order the columns by dragging and dropping their headers.
- When a new checkbox is selected, the respective column is inserted in the correct position in the table.
- You can remove the columns from the table by un-selecting them in the Statistics tab or by dragging a column header outside of the column header area. When dragging a column header, an “X” indicates that it will be removed.
- If a statistical value cannot be calculated, “N/A” is displayed instead of a numerical value.
- If a value is not applicable to the row (e.g., Y values for a histogram gate), it is left blank.
- The table can be sorted by any column in an ascending or descending order (case insensitive) by clicking the column title.

Select rows

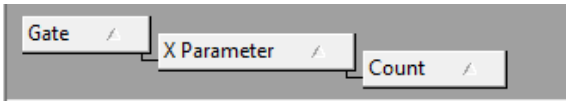
- Left or right mouse click on a Results row selects the entire row.
- Hold down the **Ctrl** or **Shift** keys to select multiple rows.
- **Ctrl+A** combination selects all the rows in the table.
- **Ctrl+Shift+End** combination selects all rows from the currently selected row to the end of the table.
- **Ctrl+Shift+Home** combination selects all rows from the currently selected row to the top of the table.

Group results

Group results by column headers

- Results can be grouped by the values displayed in the column headers by dragging a column header from the Results table onto the *group control* labeled "Drag a column header here to group by that column".

- You can place more than one header in the "Drag a column header …" group control, which will group results by all the headers placed there. The headers will be linked by arrows denoting the sort order:



- You can change the sort order by dragging a header in a different position on the group control.
- Clicking on a header arrow ( ) toggles the sorting order between ascending and descending within the column.
- To collapse the information displayed in the groups, click the button; to expand it, click the button.

Add and remove column headers

- When a header is dragged, two hot divider arrows indicate where the column header will be placed on the "Drag a column header …" group control.

- To remove a header from the grouping, drag it back to its position in the Results table. When a column header is dragged back to the Results table, two hot divider arrows indicate where the header will be placed.

  Alternatively, drag the header off the table to remove it from the group control.

Moving versus copying headers

- Dragging a header onto the "Drag a column header …" group control removes the header from the table and places it in the group control.

  Dragging the header back to the table removes it from the group control and places it back in the table.
- If you hold down the **Ctrl** key while dragging a header, the header is copied rather than moved.

Examples of grouped results

Example of results grouped by Gate

Gate

Group	Sample	Count	Volume	Concentration	%Total	%Gated
Gate: All Events						
Group	Spleen Control	2239344	212.0	10562.9	100.0	100.0
Group	Spleen Control	2239344	212.0	10562.9	100.0	100.0
Group	Spleen Control	2239344	212.0	10562.9	100.0	100.0
Group	Spleen Control	2239344	212.0	10562.9	100.0	100.0
Group	Spleen Control	2239344	212.0	10562.9	100.0	100.0
Group	Spleen Control	2239344	212.0	10562.9	100.0	100.0
Group	Spleen Control	2239344	212.0	10562.9	100.0	100.0
Group	Spleen Control	2239344	212.0	10562.9	100.0	100.0
Group	Spleen Control	2239344	212.0	10562.9	100.0	100.0
Group	Spleen Control	2239344	212.0	10562.9	100.0	100.0
Gate: CD3+						
Group	Spleen Control	500075	212.0	2358.8	22.3	43.1
Group	Spleen Control	500075	212.0	2358.8	22.3	22.3

Example of results grouped by Gate and X Parameter

Experiment Workspace | Results | Overlays | Sample List | Heat Map View

Gate

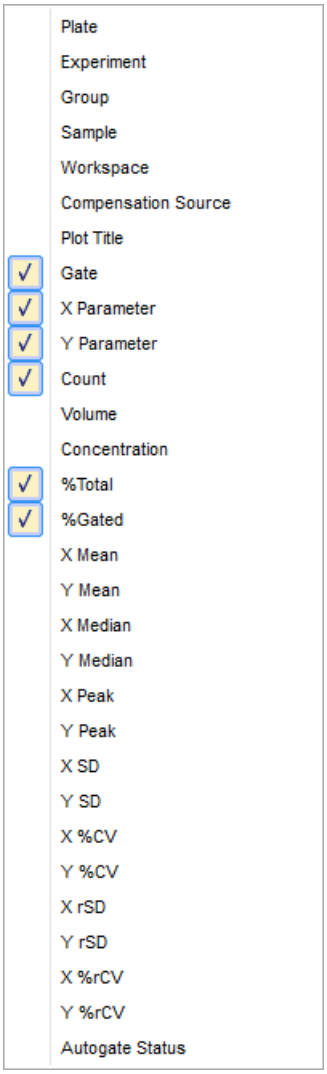
X Parameter

G.	Sample	Count	Volume	Concentration	%Total	%Gated
Gate: All Events						
X Parameter: BL1 - FJComp-BL1-A						
G..	Spleen Control	2239344	212.0	10562.9	100.0	100.0
X Parameter: FSC - FSC-A						
G..	Spleen Control	2239344	212.0	10562.9	100.0	100.0
X Parameter: FSC - FSC-H						
G..	Spleen Control	2239344	212.0	10562.9	100.0	100.0

Column header context menu

Right-click on a column header to display the *column header context menu*.

- The column header context menu contains all the statistics that can possibly be displayed in the Results table.
- The statistics that are already present in the table are checked in the context menu.
- To add new columns to the Results table, check the corresponding item in the column header context menu.
- To remove columns from the Results table, un-check the corresponding item in the column header context menu.
- The column header context menu can be displayed when there are no columns in the table or when you click on a blank area of the header.



9. Overlay view

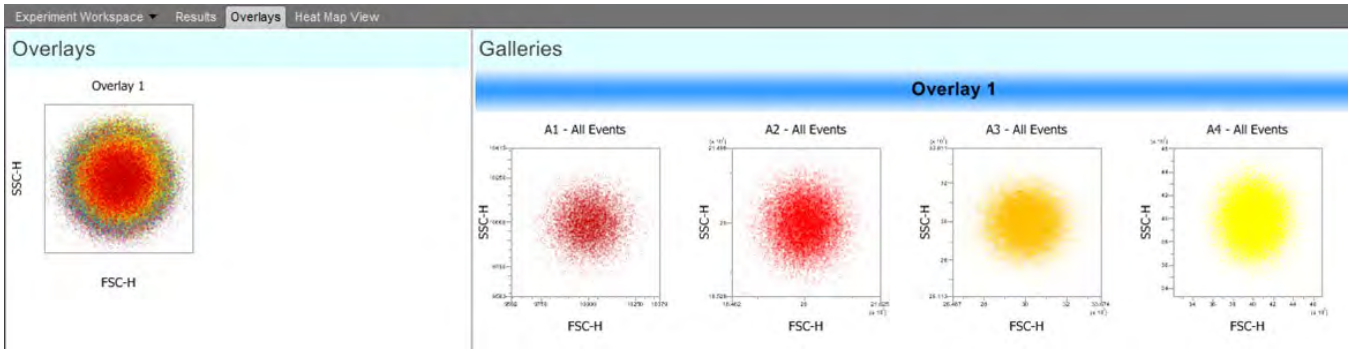
Overview

Overlays provide a graphical method for comparing Experiment data, in which selected plots are superimposed for a direct visual comparison of overlapping data.

- Each Overlay consists of an *Overlay plot* (page 158) and an associated *Gallery* containing the Source plots for the Overlay (i.e., *Gallery plots;* page 161).
- Overlays are viewed and modified in the *Overlay view tab*.

Overlay view tab

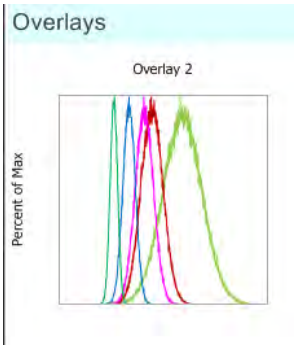
Overlay view tab is displayed in the Application Area on the Main Application Workspace (page 36) and allows comparison of Histogram or dual-parameter plots.



- The *Overlay view* is divided vertically into two windows by a splitter bar.
  - The left window of the Overlay view is the *Overlays area* (page 157)
  - The right window is the *Galleries area* (page 157)
- The docking behavior of the Overlay view tab is described on page 78.
- There is no limit to the number of Overlays that can be added to the Overlay view.
- When the Customize Overlay panel (page 310) is visible, the panel contents are updated based on the selected plots. The customize panel allows you to customize the appearance of Overlay and Gallery plots.

Overlays area

The *Overlays area* displays the Overlay plots (page 158).

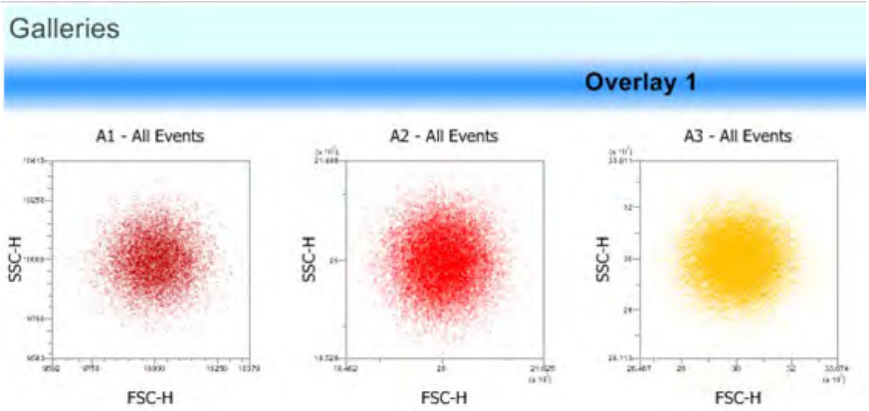


- To resize the Overlays area slide the splitter bar to the left or to the right.
- Plots within the Overlays area automatically wrap, when needed. By default, the divider is set so that only one Overlay plot is shown per row.

  When required, vertical and horizontal scroll bars become available on the Overlays area to allow the viewing of all existing plots.
- When new plots are added to an existing Overlay or if a new Overlay is created by sending plots from the Workspace, the most recently created and/or modified Overlays are selected when viewing the Overlay view tab.
- When one or more Overlay plots are selected, the titles for the corresponding galleries are highlighted in blue.
- Overlay plots are displayed 240 × 240 pixels. If legends are enabled, the Overlay plots are resized to 240 × 480 pixels (H × W).

Galleries area

The *Galleries area* contains the *Galleries* of Source plots (i.e., *Gallery plots*; page 161) for each Overlay plot displayed in the Overlays area.



- Each Gallery starts on a new line and has the same title as the Overlay whose Source plots it contains. The Gallery title is displayed above the plots and is always visible, even when the plots are horizontally scrolled.
- Horizontal and vertical scroll bars are available, when needed.
- The Galleries area can be resized by sliding the splitter bar to the left or to the right.
- Selecting any plot within a Gallery highlights the Gallery title in blue.
- To resize the Plots within the Galleries area, use the **Size Slider** in the bottom right corner of the on the *Application Status Bar* (page 47) or hold down **Ctrl** on the keyboard and turn the **mouse scroll wheel**.
- Gallery plots are displayed in 240 × 240 pixels. If legends are enabled, the Gallery plots are resized to 240 × 480 pixels (H × W).

Note: Galleries view cannot be printed.

Overlay plots

Overlay plots allow the visualization and comparison of overlapping data by superimposing the selected Histogram or of dual-parameter plots. The Overlay plots are displayed in the Overlays area (page 157).

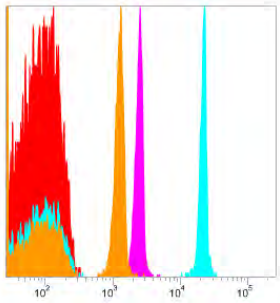
Overlay plot modes

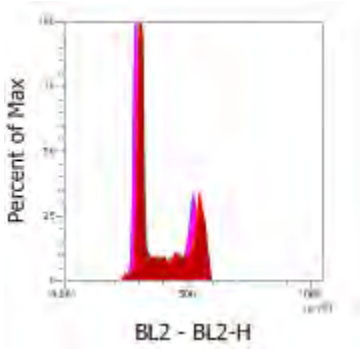
Overlays plots have two display modes: *Overlay mode* and *3D mode*.

To switch between Overlay and 3D modes, select or deselect the **View 3D Mode** checkbox within the *Customize Overlay panel* (page 310). The selected mode only applies to the currently selected Overlay.

Overlay mode

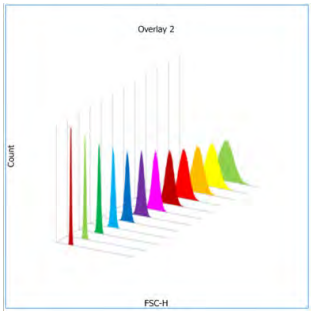
- The Overlay mode is the default display mode, where the overlay plots are shown superimposed over one another.

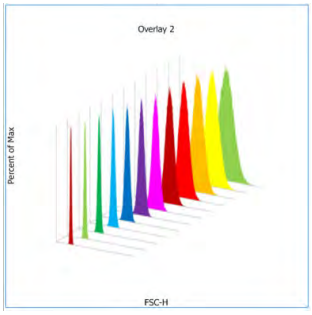




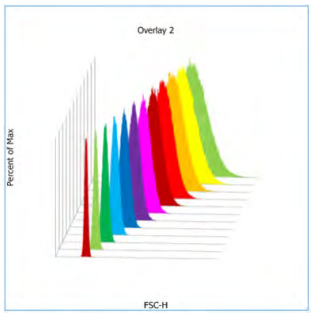
3D mode

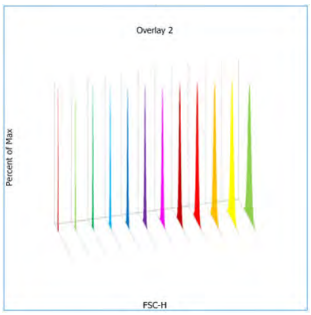
- In the 3D mode, plots are displayed three dimensionally at a user-specified angle and are separated by a set distance. The distance between the plots is calculated based on the number of plots being overlaid.

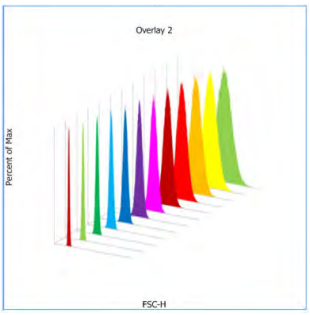




- In this mode, you can rotate the plot by selecting the desired angle from the **Angle (deg)** dropdown control on the Customize Overlay panel (page 314).







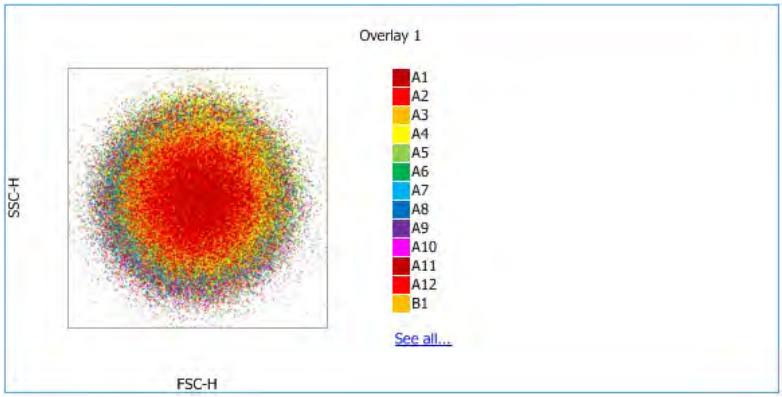
Overlay plot characteristics

Plot title

- Overlay plot titles are defined using the **Customize Text** controls on the Customize Overlay panel (page 310).

Legend

- By default, the legend is not displayed. However, you may choose to display the legend by checking the **Show Legend** checkbox in the Customize Overlay panel (page 311). The option to display the legend may also be set using the Overlay Builder described on page 179.
- By default, the legend displays the name of each sample in the overlay. The Parameter name may also be displayed in the legend with or without the Sample name using the Customize Overlay panel (page 311).



- The color associated with each sample in the legend corresponds to the data color of that sample on each plot.

  Name labels of dual parameter plots in the legend are represented by solid colored rectangles.

  Name labels of single parameter plots in the legend are represented by unfilled rectangles with colored borders unless the single parameter plot is itself filled. In that case, the legend is also displayed as filled.

- If all the plots in the Overlay cannot be represented on the legend due to insufficient space, a hyperlink titled "See All" is displayed below the last legend item shown.
- Click the **See All** hyperlink to display a floating list that shows all the plots on the Overlay.
- Click the **close button** (**X**) on the floating list to close it.

Axis labels

- In the Overlay mode, an axis label is displayed only if it is the same for all the plots in the Overlay. If an overlay is created from plots where a parameter is different for one of the axes, no axis label is displayed for that axis.
- To edit the axis labels on the Overlay plot, use the **Customize Text** controls on the Customize Overlay panel (page 316).

Axis tick marks

- For the selected Overlay plots, you can specify the presence of X- and/or Y-axis tick marks by checking the **Show X-Axis Ticks** and **Show Y-Axis Ticks** checkboxes on the Customize Overlay panel (page 311).
- By default, both the X-axis and Y-axis tick mark display options are enabled.
- Changing tick mark display options for an Overlay plot updates all the plots in the associated gallery.
- By default, the tick marks are displayed on Overlay plots when all the Source plots have the same scale and range. If the scale ranges are different, the tick marks are not displayed, even if they are enabled on the Customize Overlay panel.

Gates

- To add Gates to Overlay plots in Overlay mode, use the **Gating Tools** on the Overlay ribbon tab (page 78).
- When a gate is drawn on an Overlay plot, the same gate is drawn on all the plots in the associated Gallery.

Re-ordering Plots

- To reorder Plots in the Overlay mode, drag and drop the selected plots.

  When you reorder Overlay plots, their respective galleries are also re-ordered to match the order of the Overlay plots.
- When dragging plots, a blue vertical line indicates the insertion location of the plots.
- When you select and drag multiple plots, they are grouped together and can be moved and inserted as a group.
- If more than 3 plots are selected, a plus (+) icon is shown next to the plot images indicating the presence of additional plots being dragged.
- You can move plots only when and where the *insert indicator* appears; otherwise the plots return to their original positions when released.

Gallery plots

Gallery plots are the Source plots that are overlaid to generate the Overlay plot. Gallery plots are displayed in the Galleries area (page 157).

Gallery plot characteristics

Plot title

- Gallery plot titles are defined using the **Customize Text** controls on the Customize Overlay panel (page 316).

Axes labels

- Gallery plot axis labels can be edited using the **Customize Text** controls on the Customize Overlay panel (page 316).

Axis tick marks

- By default, both the X-axis and Y-axis tick mark display options are enabled.
- Tick mark visibility for the selected Gallery plots can be changed using the **Show X-Axis Ticks** and **Show Y-Axis Ticks** checkboxes on the Customize Overlay panel (page 311).

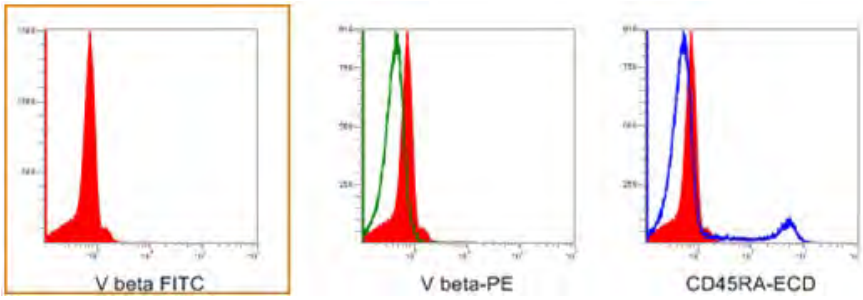
Order of plots in the Galleries area

- The order of the plots within a Gallery (left to right) determines the z-order (front to back) of plots in its corresponding Overlay plot.
- Plots can be rearranged within each Gallery by dragging and dropping. When plots are re-arranged in a Gallery, they are re-arranged in the respective Overlay plot as well.
- When dragging plots, a vertical blue line highlights the insertion location of the plots.
- When multiple plots are selected and dragged, they are grouped together and moved and inserted as a group.
- If more than 3 plots are selected, a plus (+) icon is shown next to the plot images indicating the presence of additional plots being dragged.
- Plots can only be moved when and where the *insert indicator* appears; otherwise they return to their original positions when released.

Note: Gallery plots cannot be printed.

Control plot

- Within the Galleries view, one gallery plot for each single parameter overlay can be selected to be a *Control plot*.
- The Control plot is displayed as an overlay on all single parameter plots present for a specific overlay in the gallery. The specified control plot is shown with an orange dashed line around its perimeter.
- By default, a Control plot is not defined.



- The Control plot is selected by clicking the **Set as Control** button on the Customize Overlay panel (page 315).
- If there is already a Control plot, defining a different plot as a new Control plot replaces the previous plot as the control.
- Selecting the **Move to Front** option on the Customize Overlay panel (page 315) displays the Control plot on each Gallery plot in front of the data plot.

  By default, this option is not selected and the data plots are displayed in front of the Control plot.
- When a Control plot has been defined, the software allows comparison statistics to be calculated (see "Overlay Calculations", below).

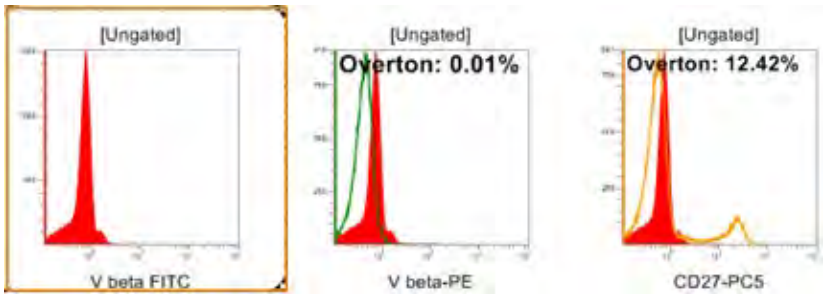
Overlay calculations

- When a Control plot has been defined, the data sets can be compared statistically through selection of the desired **Calculation method** radio buttons on the Customize Overlay panel (page 316).

  The available options are *Overton* (below) and the *Kolmogornov-Smironov (K-S)* (page 163) methods. Both methods are used to determine the statistical difference between two samples.

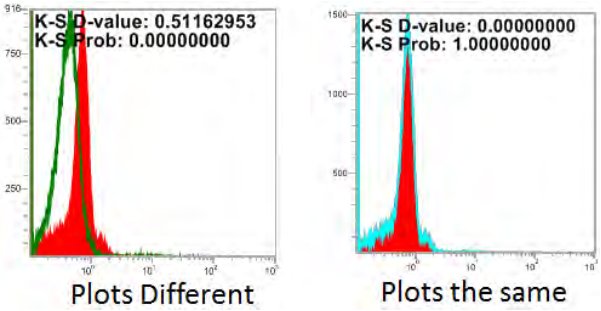
Overton method

- Selecting the **Overton** option compares the control sample and each Gallery plot using the Overton's cumulative statistics calculation (page 628).
- The resulting value indicates the percent difference between the test sample and the control sample and is displayed on each test plot. The calculations are not shown on the plot set as the Control plot.



Kolmogornov-Smironov (K-S) method

- **KS** option compares the control and test sample using the Kolmogorov-Smirnov test (page 628) to determine the probability that the two plots are the same. Results are displayed on the plot.

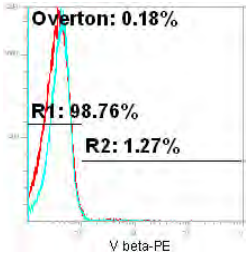


- The lower the calculated *D-value*, the more likely that the plots are the same. The lowest possible D-value is 0, in which case the plots are identical.
- The *Probability value* is a value between 0 and 1. The higher the Probability value, the greater is the probability that the plots are the same.

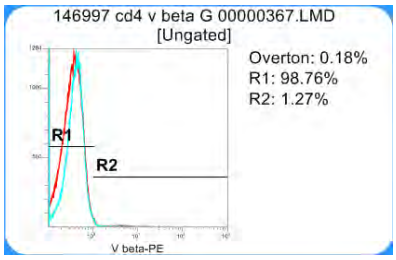
Note: For more information on the *Overton cumulative statistics calculation* and the *Kolmogornov-Smironov (K-S) test*, see "Appendix B: Technical Reference", page 554).

Display of statistics

- The Overlay calculation statistics (page 162) and the Overlay gate statistics (page 310) can be displayed on the plot or to the side of the plot. By default, they are displayed on the plot.



- When the **Show legend** option (page 311) is selected, statistics are displayed on the side of the plot as a legend.



- If all the plots and statistics cannot be shown on the legend due to insufficient space, a hyperlink titled "See All" is displayed below the last legend item shown. Clicking the **See All** hyperlink displays a floating list that shows all the plots on the Overlay. Clicking the **close button** (**X**) on the floating list closes it.
- Overton statistics always display two decimal places on the plot. All other statistics show decimal places as specified in the *Options dialog* (page 492).

Gates

- Gates are created on a selected plot using the *Gating Tools* available on the *Workspace ribbon tab* (page 57).
- Gates created on a Gallery plot are displayed on all the plots in the Gallery and on the related Overlay plot, if the Overlay plot is in Overlay mode and not in 3D mode (page 158).
- Gates are not displayed on Overlay plots that are in the 3D mode (page 158).
- Gates created on an Overlay plot in the Overlay mode are reflected on all the plots in its Gallery.
- The maximum number of gates for an Overlay is 5.

  If you attempt to add a gate after the maximum number of gates on that plot has already been reached, a *no entry symbol* (⃠) is displayed, indicating that the additional gate cannot be created.
- Only one Quadrant gate can be drawn per Overlay. A Quadrant counts as a single gate, thus allowing 4 additional, non-Quadrant gates on the same plot.
- Autogates are not supported in Overlay plots.
- Gates are drawn in black with no fill and are assigned default names in the order of their creation.
- When a gate is moved or resized on any plot in the Overlay, it is updated accordingly in all the rest of the plots in the same Overlay.
- Statistics are shown on the Gallery plot as specified in "Display of statistics", page 163; they are not shown on the Overlay plot.
- The default statistic displayed is *%Gated* (page 77). You can change the statistics displayed by selecting the desired one from the *Statistics ribbon tab* (page 74).
- The decimal places for the statistics are as specified in the Options dialog (page 492). The displayed statistics persist on a per Experiment basis.
- If the statistic cannot be calculated, N/A is displayed.
- Gates on the Overlay tab do not affect the Source plots on the Workspace and do not count towards the maximum numbers of gates.

Overlay properties

Overlay settings

Overlay title

- The *Overlay title* is unique for each Overlay, and it distinguishes the Overlay plot and its respective Gallery from other existing Overlays.
- The default title for a new Overlay is "*Overlay n*", where *n* is a numerical suffix (first available integer) to ensure that the title is unique. If an Overlay is deleted or renamed, the number designation is re-used.

Overlay plot colors

- By default, all dual-parameter plots (i.e., Dot, Density, and Precedence Density plots) in an Overlay are displayed in a solid color.
- By default, all single-parameter plots are displayed as a solid line, without fill.
- The color of a plot in an Overlay is determined by the defaults specified in the *Color and Themes* tab of the *Options dialog* (page 479).

  Each new plot in the Overlay is assigned the next available color listed in the *Gates and sample Overlays* group.

Range

You cannot change the range of individual plots on the Overlays tab.

Parameter

You cannot change parameters of plots on the Overlays tab.

Persistence of Overlay settings

All plots, options, and settings defined on the Overlays tab persist at the Experiment level.

Add plots to Overlays

- To send a Source plot from a Workspace to a new or existing Overlay:
  - Right-click on any empty area (i.e., white space) within the Workspace view to open the *Workspace view context menu.*

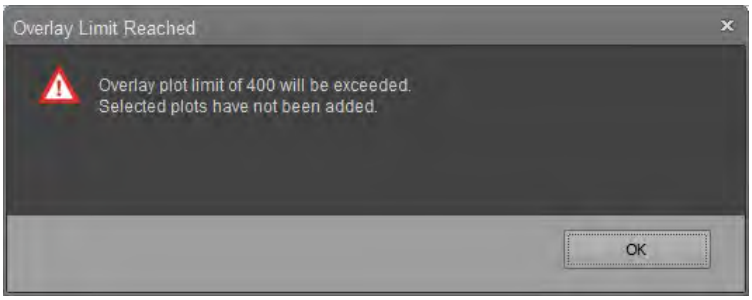
    From here, select **Send All Plots to Overlay** (page 122) to overlay all plots within the Workspace of the single Sample on top of each other.
  - Right-click on any empty area within the boundary of a single plot to open the *Plot context menu*.

    From here, select **Send to Overlay** (page 125) and select the appropriate overlay from the dropdown menu. The selected plot is added to the selected overlay.
  - Alternatively, click the **Overlay Builder** button on the Overlay ribbon tab to create an overlay using *Overlay Builder* (page 172).

- Each plot is added to the selected Overlay plot and to its respective Gallery.
- By default, plots added to a new Overlay are arranged in the order of addition.

  When new plots are added to an existing Overlay, they are added to the end of the Gallery.
- By default, New Overlay plots are displayed in the Overlay mode (page 158).
- Gates present on the Source plots in the Workspace are not added to the Overlay.

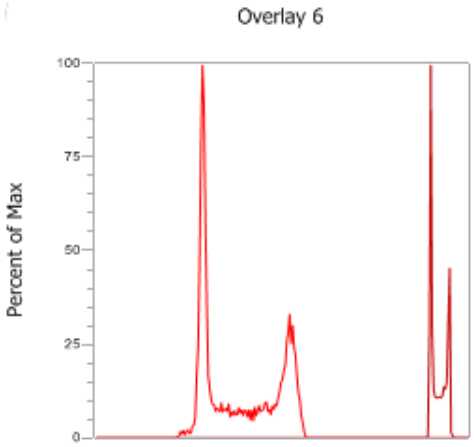
- Range and scale settings of the Source plots are applied to the Overlay plots. The scale setting of the newly added Source plot always takes precedence over any existing scaling set in the Overlay.
- An Overlay can contain a maximum of 400 Source plots. If the addition of new plots would cause the Overlay to exceed the 400 plot limit, the software displays a warning dialog.



Clicking **OK** closes the dialog without adding the new plots to the Overlay.

Compare plots with different scale and range settings

- Plots with different scale settings (i.e., linear, log, Logicle, or HyperLog™) or different scales of the same type can be compared on the same Overlay.



- Plots with different range settings can be compared on the same Overlay. When comparing plots with different range settings, axis labels behave as described on page 166.

Note: The comparing plots with different scale and range, the settings of individual plots remain unchanged.

Select plots

Select a single plot

- To select single Overlay or Gallery plots, click on the desired plot.

  The selected plot is surrounded by a **light blue selection rectangle**.

Select multiple plots

- To select multiple plots, click on the desired plots while holding down the **Ctrl** key.

  Objects which are part of the multi-select are bounded by **dark blue multi-selection rectangles**.
- Alternatively, drag a selection rectangle using the mouse to select several plots within the same area.

  When drawing a selection rectangle, the selection process must be initiated outside a currently selected plot to avoid dragging the current plot to a new position.
- To deselect a single plot, click on the selected plot while holding down the **Ctrl** key. To deselect all plots, click on an object other than the plot.

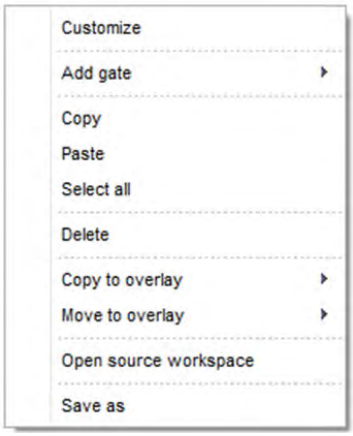
Select all plots

- To select all plots in the Overlays area, press the **Ctrl+A** keys when an Overlay plot is selected.
- To select all Gallery plots for the selected Overlays, press the **Ctrl+A** keys when a Gallery plot is selected.

Move and copy plots between Overlays

- To move a plot, right-click the plot in the Gallery to open the *Gallery plot context menu*, then select **Move To Overlay** (page 170).

  Alternatively, plots can be moved from one Overlay to another by the cutting and pasting, using the standard **Ctrl+X** (cut) and **Ctrl+V** (paste) key combinations.
- You can copy a plot from one Overlay to another by using **Copy** and **Paste** options or the **Copy to Overlay** option on the *Overlay plot context menu* (page 169) or by using the standard **Ctrl+C** (copy) and **Ctrl+V** (paste) key combinations..



Copy plot images

You can copy images of the selected plots in the Overlay view to the clipboard for pasting in other applications. This is accomplished by using the **Copy** option on the *Home ribbon tab* (page 50) or using the standard **Ctrl+C** key combination.

Resize plots

- Overlay plots within the Overlay area and Gallery plots within the Gallery area have a uniform size. You can resize Overlay and Gallery plots independently.
- Turn the **mouse scroll wheel** while holding down the **Ctrl** key to resize the Overlay or Gallery plots, when dragging is not currently taking place.
- If either an Overlay plot or a Gallery plot is selected, you can use the **Size Slider found on the bottom right** on the *Application Status Bar* (page 47) to resize the currently selected plot type.

Overlay plot links to Source plot

Link to Live Data of the Source Plot

- Overlay plots are automatically updated to reflect the changes made to them in the Workspace, except for the gates created on Source plots in the Workspace area, which are not displayed on the Overlay plots.
- If a single-parameter plot is changed to a dual-parameter plot or vice versa, the plot is removed from its respective Overlays.

Source Plot Not Available

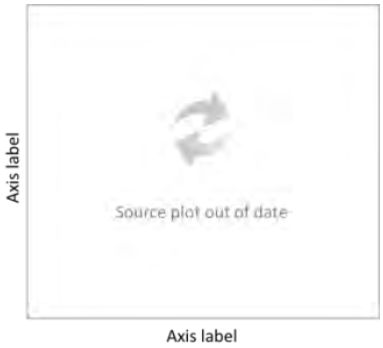
- When Source plots are deleted from the Workspace or a sample is deleted, their corresponding plots are removed from the Gallery and Overlay plots.

Empty Overlays

- If all plots in the same Gallery are deleted, the Overlay itself is automatically deleted.

Auto-Refresh

- When **Auto-Refresh** is selected on the *Home ribbon tab* (page 50), all plots and statistics in the Overlays tabs are updated automatically in the background as the Workspace is modified.
- If Auto-Refresh is disabled or the system has not yet retrieved the image, the Overlays only show data from source locations that are up-to-date.
- When Auto-Refresh is disabled, the Gallery plots for the source locations that need to be refreshed display the *out-of-date watermark* stating "Source plot out of date" on the plot.



The Gallery plots for the source locations that do not contain data are left blank.

- Only Gallery plots containing data (no watermark) are shown in the Overlay plot.

Overlay Plot Context Menu

Overview

The *Overlay Plot context menu* is displayed when an area within the plot boundary is right clicked.

The Overlay Plot context menu contains commands for customizing the Overlay, adding gates to Overlay plots, for cutting, copying, pasting, and deleting selected plots, for sending selected plots to a selected Overlay, and saving all selected items as a new graphics file.

Overlay Plot context menu options

Customize

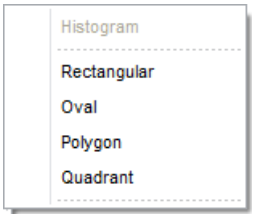
- **Customize** opens the *Customize panel,* which allows you to modify the appearance of the selected Overlay plot as described in "Customize Overlay Options", page 310.
- If the panel is closed, it will be opened and brought to the front of other open panels.

Add gate

- Select **Add gate** to create of a new gate in the default position on the currently selected plot.

  If multiple plots are selected, this option adds a gate to a single plot.

  The Histogram option is the only available for Histogram plots.

  Rectangular, Oval, Polygon, and Quadrant options are available only for dual-parameter plots.
- Selecting these options creates the selected gate type in the default position as described in "Create and delete gates" on page 98.
- The maximum number of gates allowed is 5, as described on page 160.
- The Quadrant option is unavailable if a Quadrant gate has been created on the current plot; all other options are unavailable once the gate limit has been reached.
- The gate set on the selected Overlay plot appears on all Gallery plots for the current Overlay and on the Overlay plot.

Copy

Copy copies the selected objects to the clipboard, keeping the original objects on the Workspace.

Paste

Paste inserts the objects currently in the clipboard onto the current Overlay.

Select all

Select all option selects all Overlay plots.

Delete

Delete removes all selected Overlay plots and deletes all associated Gallery plots.

Save As

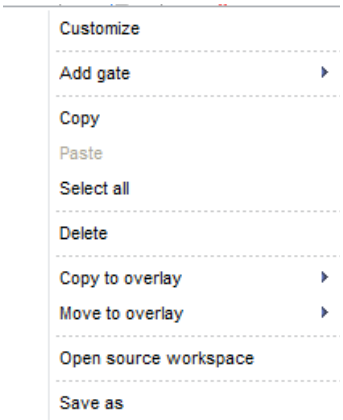
- **Save As** displays the *File Save (Export) dialog* as described on page 532, which allows you to send all selected items to a new graphics file.
- The layout of the graphics file replicates the layout of the selected items on the current Workspace, and the plot contents are based on the options selected in the *Export Options* menu as described on page 490.
- The selection area for saving can only cover one page.
- The available image file formats are, BMP, JPG, TIFF, GIF, PNG, and Windows Metafile (EMF). The default format is JPG.

Gallery Plot Context Menu

Overview

The *Gallery Plot context menu* is displayed when any within the Gallery plot boundary is right clicked.

The Gallery Plot context menu contains the commands for customizing the Overlay, adding gates to Overlay plots, for cutting, copying, pasting, and deleting selected plots, for sending selected plots to a selected Overlay, and saving all selected items as a new graphics file.

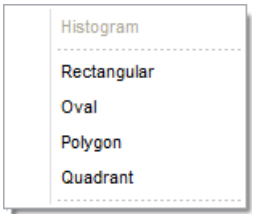


Gallery Plot context menu options

Customize

- **Customize** displays the *Customize Overlay options panel*, which allows you to customize the appearance of the selected Gallery plot as described on page 310.
- If the panel is closed, it opens and is displayed in front of other open panels.

Add gate



- **Add gate** creates a new gate in the default position in all gallery plots as described on page 98.
- The Histogram option is only available for Histogram plots.

  Rectangular, Oval, Polygon, and Quadrant options are available only for dual-parameter plots.
- The maximum number of gates allowed is 5, as described on page 160.
- The Quadrant option is unavailable if a Quadrant gate has been created on the current plot; all other options are unavailable once the gate limit has been reached.

Copy

Selecting **Copy** copies the selected plots to the clipboard, while keeping the original plots on the Workspace.

Paste

Selecting **Paste** inserts the previously copied Gallery plots onto the current Overlay plot. The plots are appended at the end of the existing plots.

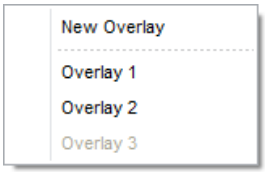
Select all

Select all option selects all Gallery plots for the current Overlay plots.

Delete

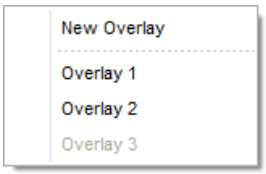
Selecting **Delete** removes selected Gallery plots and removes them from the Overlay.

Copy to Overlay



- **Copy to Overlay** sends a copy of selected plots within a Gallery to a selected Overlay, while retaining the original plots in the Gallery.
- A new Overlay can be created by selection of **New Overlay** or send the plots to an existing Overlay that contains plots with the same number of parameters.
- Overlays with non-matching number of parameters are listed in the context menu, but they cannot be selected.
- Similarly, if the selected number of plots would cause an Overlay to exceed the allowable number of plots, that Overlay cannot be selected from the menu.

Move to Overlay



- **Move to Overlay** moves the selected plots within a Gallery to a selected Overlay. The Gallery does not retain the plot that is moved.
- A plot can be moved to either a **New Overlay** or an existing overlay.
- Overlays with non-matching number of parameters are listed in the context menu, but they cannot be selected.
- Similarly, if the selected number of plots would cause an Overlay to exceed the allowable number of plots, that Overlay cannot be selected from the menu.

Save As

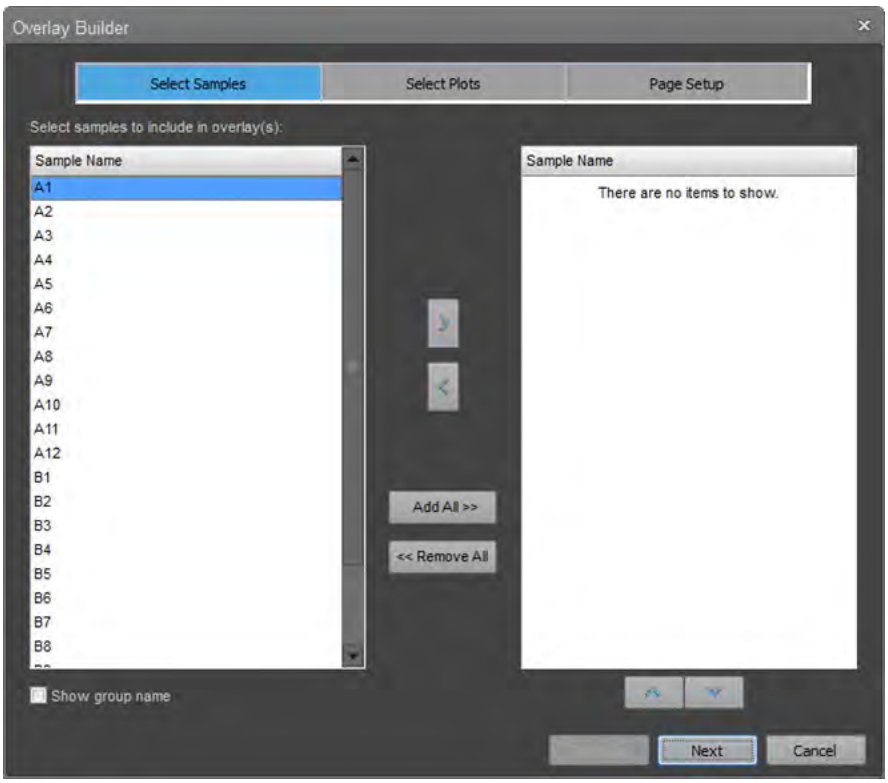
- Selecting **Save As** displays the *File Save (Export) dialog* (page 532), which allows you to send all selected items to a new graphics file.
- The layout of the graphics file replicates the layout of the selected items on the current Workspace, and the plot contents are based on the options selected in the *Export Options* menu as described on page 490.
- The selection area for saving may only cover one page.
- The available image file formats are, BMP, JPG, TIFF, GIF, PNG, and Windows Metafile (EMF). The default format is PNG.

Overlay Builder

Overview The *Overlay Builder* tool guides you through the process of creating new Overlay plots within a single experiment. The Overlay builder allows you to select samples and plots for a new Overlay and determine how to display the selected plots.

Note: Overlay of plots from different experiments is not permitted.

- To launch the Overlay Builder, click the **Overlay Builder** button on the Overlay ribbon tab (page 78). Overlay Builder is only enabled when at least one plot exists on the Experiment Workspace and the instrument is not acquiring.



- The Overlay Builder consists of three tabs, listed below, that are displayed sequentially to guide you through the process of building a New Overlay.
  - *Select Samples* (page 173)
  - *Select Plots* (page 175)
  - *Page Setup* (page 177)
- The top portion of the Overlay Builder contains the *progress indicator*, which shows the current page highlighted.

- **Next** moves to the next tab of the Overlay Builder.

  This button is not displayed on the last tab of the builder (Page Setup tab), where it is replaced by the Finish button.

- **Previous** returns to the previous tab of the builder.

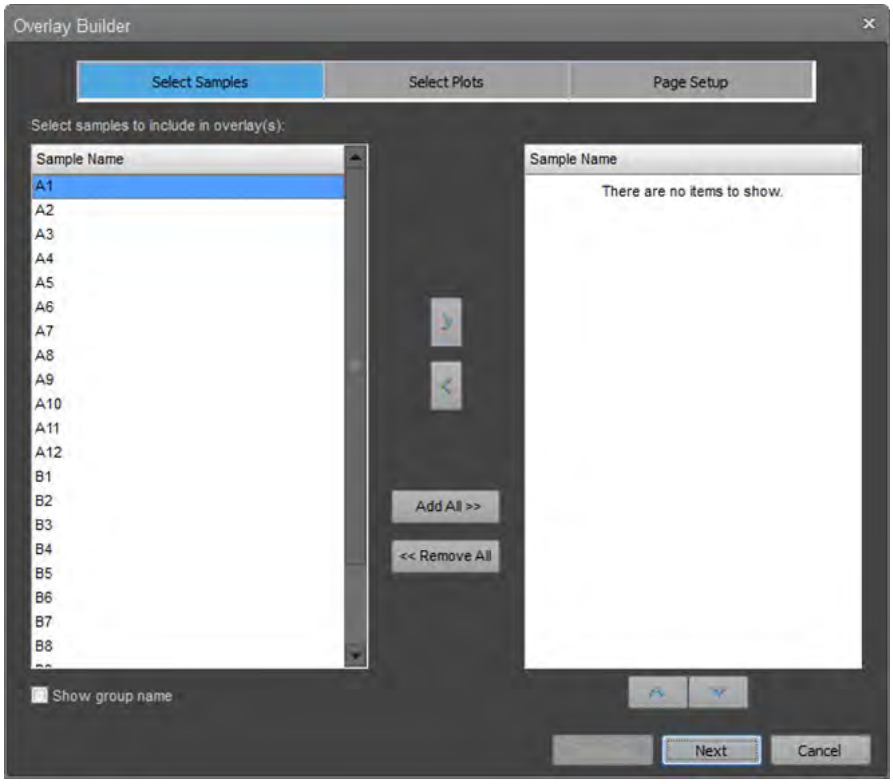
  This button is not active on the first tab of the builder (Select Samples tab).
- **Finish** closes the Overlay Builder and creates a new Overlay based on the selections made in the Overlay Builder.
- **Cancel** closes the Overlay Builder without creating Overlay plots. Any changes made with the builder are discarded.

  This button is available on all pages of the builder.
- The default title for a new Overlay is "*Overlay n*", where n is a numerical suffix (first available integer) that ensures that the title is unique.

  Gallery plot titles display the sample name by default.

Select and Order samples

The **Select Samples** tab of the Overlay Builder allows you to select of sample for inclusion in the New Overlay and reorder the selected samples, if desired.



Sample lists

- The **Available Samples panel** on the left contains a list of all samples in the current Experiment based on the Experiment hierarchy.

  By default, this list only displays the sample name.

  Check the **Show group name** to display the group name in front of the sample name (e.g. group1\sample1).
- The *Selected Samples panel* on the right contains a list of the samples that are selected to be displayed in the Overlay plots. By default, this list is empty.
- Sample names only appear in one list at a time.
- Use the **arrow** buttons (page 174) located between the two panels to transfer samples between the two lists.

- You can select multiple samples in a list simultaneously using one of the following methods:
  - To select non-consecutive samples in a list, press and hold down the **Ctrl** key, then click each **sample** that you want to select.
  - To select a consecutive group of samples in a list, click the **first sample**, press and hold down the **Shift** key, then click the **last sample**.
  - Use the **Ctrl+A** key combination to select all samples in the currently active list.
- Vertical and horizontal scroll bars will appear in each list as needed.

Arrow buttons

- Click the **right arrow** to transfer all selected samples from the Available Samples panel to the Selected Samples panel.

  The right arrow button is only enabled when one or more samples are selected in the Available Samples list.
- Click the **left arrow** to transfer all selected samples from the Selected Samples panel to the Available Samples panel.

  The left arrow button is only enabled when one or more samples are selected in the Selected Samples list.

Add All button

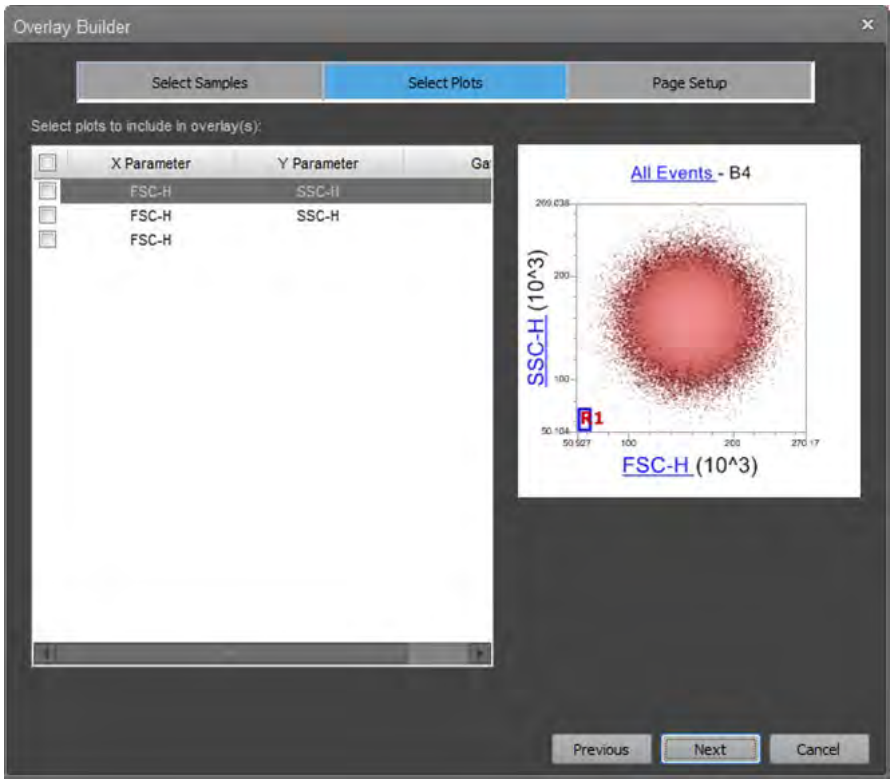
- Click **Add All** to transfer all samples from the Available Samples panel to the Selected Samples panel.

Remove All button

- Click **Remove All** to transfer all samples from the Selected Samples list to the Available Samples panel.

Select Plots

The Select Plots tab allows you to select plots to display them on an Overlay plot.



- The select plots tab contains the **Plot Selection Table** (left panel) and a **preview image** of the currently selected plot (right panel).

Plot Selection Table

- The Plot Selection Table lists all plots present on the Experiment-level Workspace of the current Experiment in the order they were created.

  The Plot Selection Table consists of X parameter, Y parameter name, and parent gate name columns, and an additional checkbox column to the left of the plot title used for selecting the plots of interest.

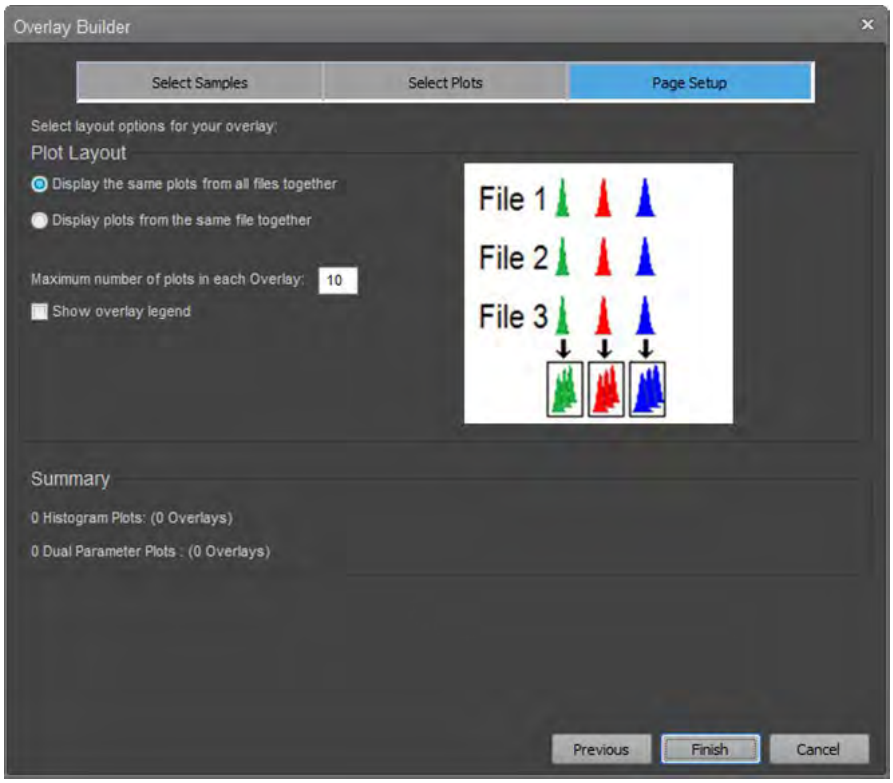
- To display one or more plots on an Overlay plot, select the relevant **checkboxes** next to the plot of interest.
- To display all plots listed in the plot selection list, click the **column heading checkbox.**
- Uncheck the **column heading checkbox** to deselect all individual plot s.
- If one or more of the individual plot checkboxes are deselected, the column heading checkbox is also unchecked.
- By default, no plots are selected for overlay and all checkboxes are deselected.
- Vertical and horizontal scroll bars will appear in the list as needed.

Plot Preview

- The preview section of the overlay builder will use plots from the experiment workspace regardless of what workspace is active.
- Click on a single row in the plot selection list to highlight the selected row and display a preview of the selected plot in the preview area.
- The preview displays the selected plot with data from the first selected sample together with any gates and labels that are present on the plot.
- You cannot format plots or modify gate positions on the preview image.

Page Setup

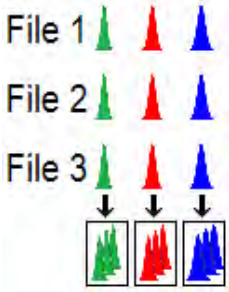
Page Setup allows you to set the display options for the selected plots.



- The Plot Layout section contains options to control how selected plots from selected files are added to new Overlay plots.

Display the same plots from all files together

- The **Display the same plots from all files together** option is the default option. It creates overlay plots that include data from all samples (multiple files) within an experiment.



- When the **Display the same plots from all files together** option is selected, Overlay plots are created according to the following rules:
  - Selected Histogram plots are placed in an Overlay plot based on the X-axis parameter. Each parameter is contained in a different Overlay plot.

    If only one histogram plot is selected for overlay, only one histogram overlay plot is generated.

    If multiple plots are selected, multiple histogram overlay plots are generated.
  - If the number of plots set in the **Maximum number of plots** (page 179) is exceeded at any time, additional Overlay plots are created to contain the remaining single-parameter Histogram plots from each file.

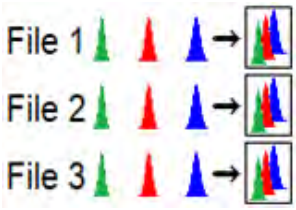
- All selected dual-parameter plots are contained in an Overlay plot based on like X- and Y-axis parameters. Each parameter combination is placed in a different Overlay plot.

  If only one dual parameter plot is selected for overlay, only one overlay plot of dual parameter data is generated.

  If multiple plots are selected, multiple dual parameter overlay plots are generated.
- If the number of plots set in the **Maximum number of plots** (page 179) is exceeded at any time, additional Overlay plots are created to contain the remaining dual-parameter Histogram plots from each file.

Display plots from the same file together

- When the **Display plots from the same file together** option selected, each Overlay plot generated contains plots from a single Sample (one FCS file) within the Experiment and not multiple Samples. This option is useful if fluorescence emission is compared between parameters, for example spillover of FITC into PE.



- When the **Display plots from the same file together** is selected, Overlay plots are created according to the following rules:
  - All single-parameter Histograms specific for the Sample are placed in a single Overlay plot.
  - If the number of plots set in the **Maximum number of plots** (page 179) is exceeded at any time, additional Overlay plots are created to contain the remaining single-parameter Histogram plots from the same file.
  - All selected dual-parameter plots specific for the Sample are placed in a single Overlay plot.
  - If the number of plots set in the **Maximum number of plots** (page 179) is exceeded at any time, additional Overlay plots are created to contain the remaining dual-parameter Histogram plots from the same file.

Maximum number of plots

- The **Maximum number of plots** edit box allows you to specify the maximum number of plots to be added to an Overlay plot by the Overlay Builder. If the requested plots exceed this number, new Overlay plots are created as required.
- The default number of plots in an Overlay is 10 plots.

  The minimum number of plots is 1.

  The maximum number of plots is 400.

  If you attempt to enter a number outside this range, the number is automatically adjusted to the closest limit.
- Only numeric characters are allowed.
- If the number entered is greater than the number of samples or plots, then the Overlay is created with the number of samples or plots.

Show Overlay legend

- If the **Show Overlay legend** option is checked, Overlay plots are created with the legend panel displayed as described on page 159.

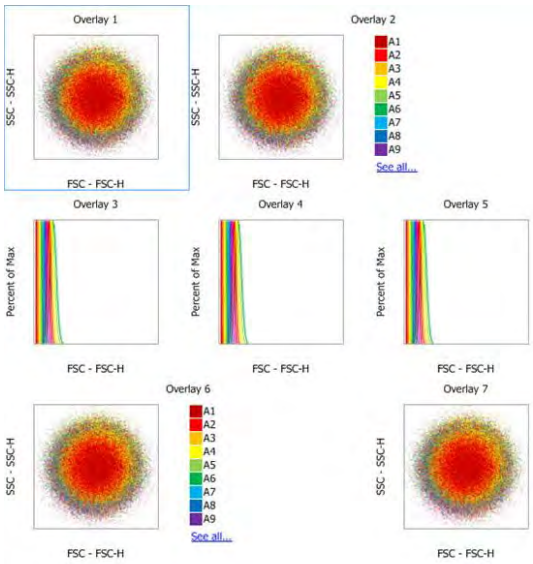
Number of plots indicator

- The **Number of plots indicator** shows the number of Source plots and Overlay plots created for both Histogram and dual parameter plots.

Print Overlays

The print command **(Ctrl+P)** and previews are enabled when either the Overlay plot or Gallery view is in focus.

- The Overlays are printed in the order they appear on the Overlays tab using the current page layout settings (orientation and paper size).



- Gallery plots are not printed.
- The zoom setting of the Overlays affects the printed size of the overlay plot. The number of Overlays per page is based on the zoom and the page layout settings.
- Before the Overlays are printed, the statistics are refreshed.

10. Image view

Overview

Image view allows you to display the cell images that are collected from a sample during acquisition. In addition, you can:

- Select from which population the images are captured using an Image gate (page 185)
- Backgate displayed images to identify the associated events on the corresponding plot in Workspace view (page 186)
- Measure the area of imaged cells (page 187)

Note: Image view is only available when using an Attune™ CytPix™ Flow Cytometer or if the active Experiment was created with an Attune™ CytPix™ Flow Cytometer.

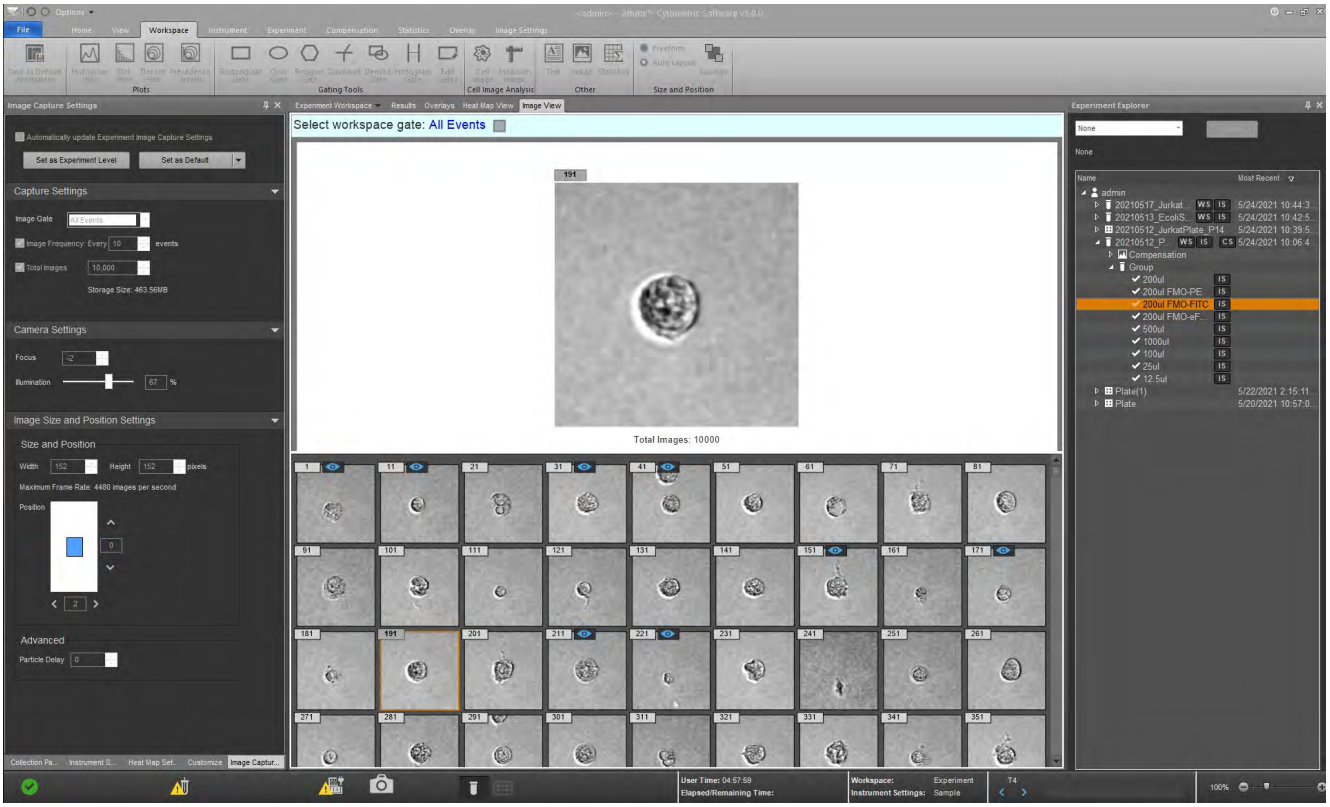


Image View tab

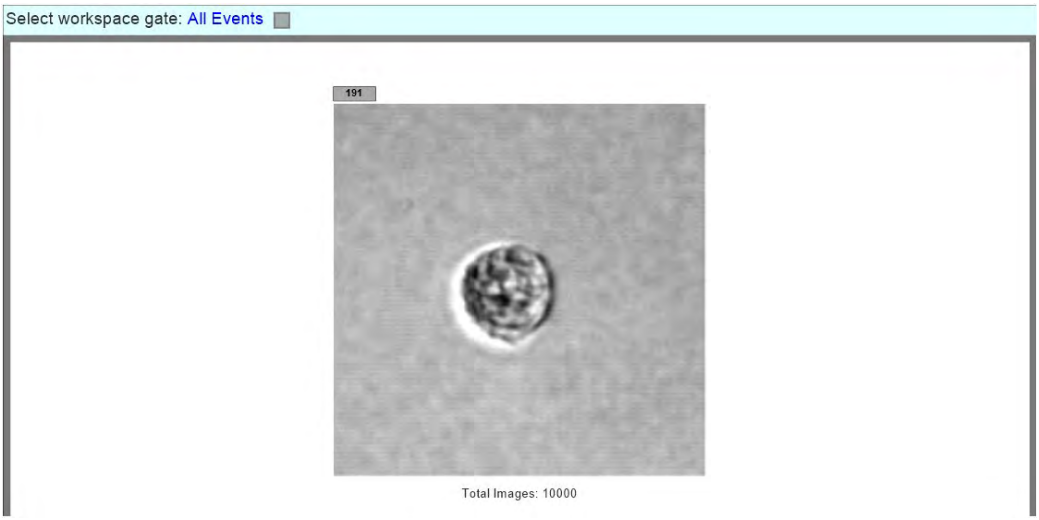
- Image view tab is displayed in the Application area on the Main Application Workspace (page 36).
- The Image view tab is only visible when the Attune™ desktop is in view. It is not visible on the Main menu, Login screen, Performance test views, or Filter configuration views.
- By default, the Image view tab is docked to the center application window.

  The Image view tab can be undocked, center docked (with main application view windows), left docked, or right docked. The tab's docked state, size, and position persists as part of the user settings.
- You can toggle the Image view on and off using the **View Images** toggle button in the Image Settings ribbon tab (page 79).
- Image view tab consists of an Active image area and an Image gallery that contains all the captured images.
- The Active image area is separated from the Image gallery using a splitter window control that allows resizing of the Active image and the Image gallery areas.

1. Active image
2. Image gallery

Active image

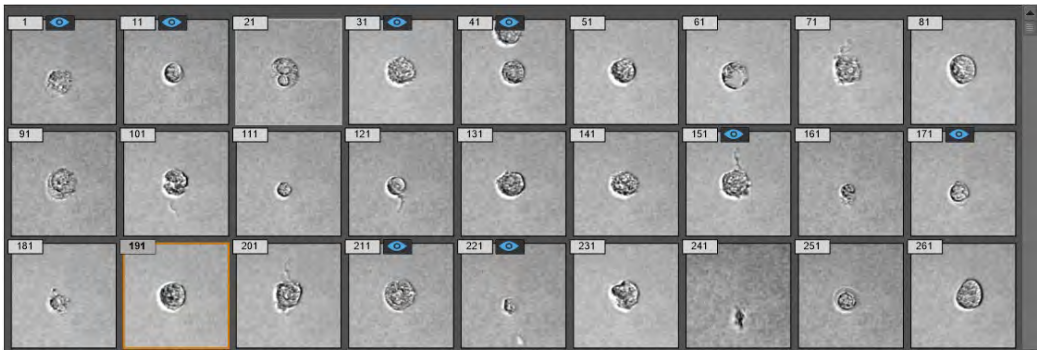
The active image is displayed in the center of the main active image area.



- By default, the active image area displays the first image that is acquired. If the active sample or the image gate does not contain any images, the image container displays the message "No images are available".
- The active image maintains its aspect ratio and fills the available size of active image area when the active image is resized.
- The active image allows additional controls using the Customize panel, right-click context menu options, and image analysis tools.
- The active image area in Image view contains a hyperlink that allows you to select a gate from the Workspace to filter the images displayed in the Image view tab (see “Image gate”, page 185).

Image gallery

The Image gallery displays all the images that are contained within a selected gate for the active workspace.



- The gallery displays up to at 20 images, which are arranged in the image acquisition event order.

  When the splitter window is adjusted to change the area available to the Active Image and the Image Gallery, the number of images per row in the Image Gallery is dynamically updated based on the available area.

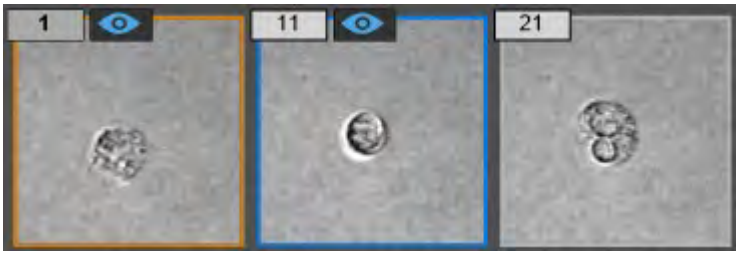
  The minimum number of images per row is 1.
- The gallery area contains vertical scroll bars to allow additional images to be visible in rows.
- The images shown in the gallery display the event ID in the top left of the image.
- In the event an image is missing, the "parameter not available" icon serves as a placeholder and the event ID is displayed in the top left of the image.

Navigation and selection

- You can navigate on the image gallery using the up, down, left, and right arrow keys on the computer keyboard.
- Page up and page down keys scroll the view by one page up or down.
- The mouse scroll wheel moves the view up and down as a line per click of the mouse.
- You can select any image in the gallery to make that image the active image.

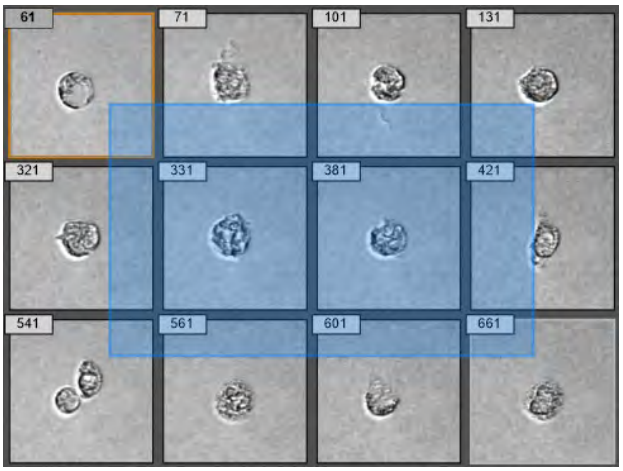
  The active selected image is indicated with an orange border around it (image 1 in the following example).

  A selected image that is not the active image is indicated with a blue border around it (image 11 in the example).



Images that have been backgated (page 186) are indicated with an "eye" icon (images 1 and 11 in the example).

- You can select multiple images using one of the following methods:
  - Left-click and drag to select all images within the defined rectangle.



  - Click while holding Ctrl to select or deselect non-contiguous images.
  - Press Ctrl+A to select all images in the gallery.
- When multiple images are selected, the images that are selected are indicated with a blue border around them.

  The active image within the selection is still indicated with an orange border around it (image 51 in the following example).

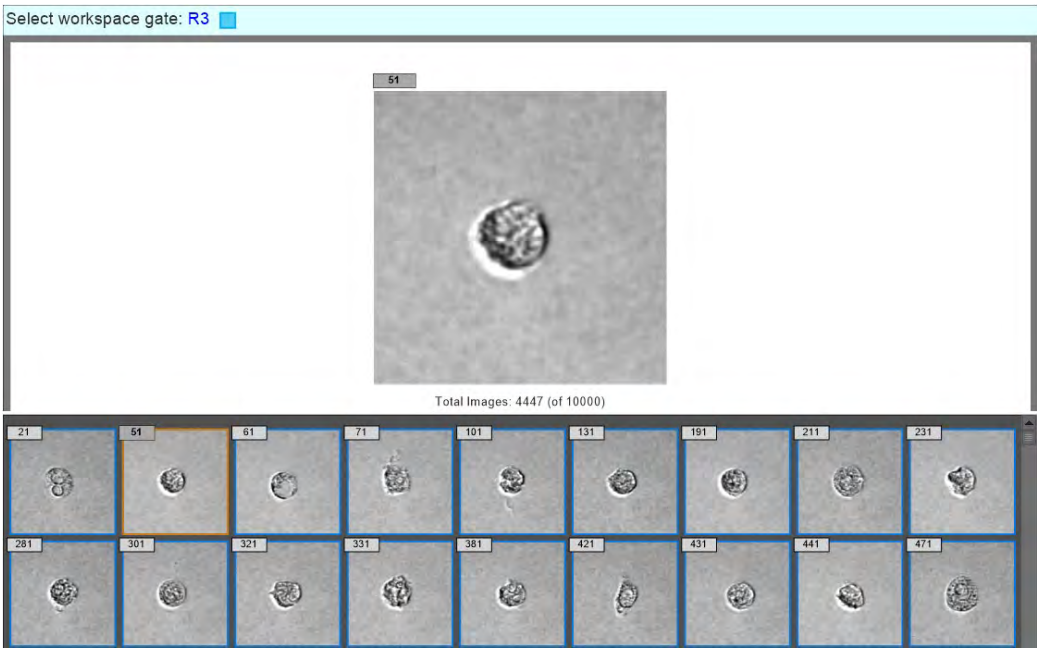
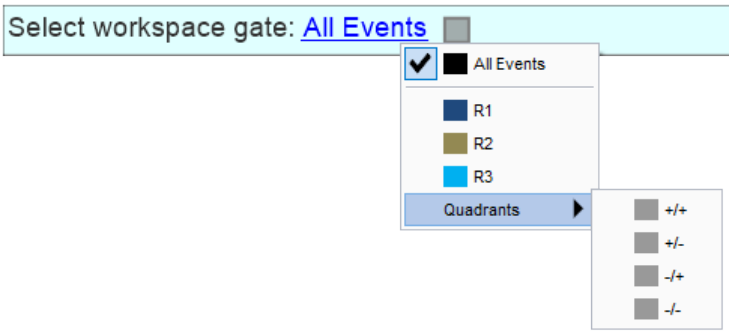


Image gate

The active image area in the Image view contains a *Select workspace gate* hyperlink that allows you to select from which population the images are captured (Active image and Image Gallery images) using an Image gate.

- When the **Select workspace gate** hyperlink is selected, the dynamic gate context menu is displayed.



- The gate context menu displays a list of all available gates from the active Workspace and includes the All Events option as well as any derived gates.
- You can only select a single gate from the gate context menu. The selected gate is used to filter the images and is indicated by a check mark.
- When you select a gate, only the images of events that are contained within the selected gate are displayed in the Image view.
- If the source gate is moved, the images in the image filter are updated to reflect the event images contained within the moved gate.
- If the source gate is deleted, the image filter reverts to All Events.
- The image gate feature is available only if the selected sample's listmode specification contains both the event and imageflag parameters. Otherwise, it reverts to All Events.
- When available, the image gate function applies to both a recorded file and empty sample as long as the sample is contained within an experiment that supports imaging and the active instrument model is set to an imaging cytometer model (i.e., an Attune™ CytPix™ instrument).

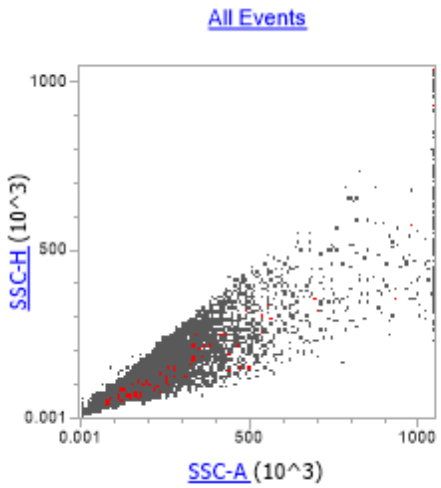
Image backgating

Image backgating allows you to identify the event that is associated with the captured cell image on a Dot plot or a Precedence Density plot in Workspace view (page 118).

Note: Image backgating is only available when using an Attune™ CytPix™ Flow Cytometer or if the active Experiment was created with an Attune™ CytPix™ Flow Cytometer.

- To backgate images in Image view, right-click on the cell image you want to backgate in Image View, then select **Show Image on Plots.** You can select the Active image or any image from the Image Gallery.

  To backgate multiple images, select the images you want to backgate in the Image Gallery, then right-click and select **Show Images on Plots**.
- By default, backgated events appear on the associated Workspace plot in red, but you can select a different color for the backgated events using the Image Options dialog (page 498).



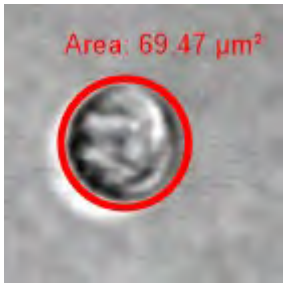
- The images that have an image backgate applied display an “eye” icon.

- To undo the backgating of an image, right-click on the backgated image, then select **Clear Image on Plots**.

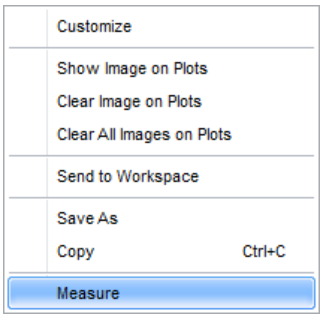
  To undo all backgates on a plot, right-click on the Active image or on any image in the Image Gallery, then select **Clear All Images on Plots**.

Measure image tool

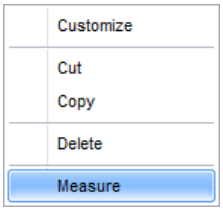
The *Measure image* tool allows you to measure the area of an imaged cell by drawing an ellipse on the active image that encircles the imaged cell.



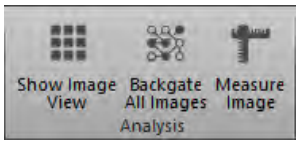
- You can select the Measure image tool by one the following methods:
  - Right-click on the active image in the Image view tab, then select **Measure** from the active image context menu.



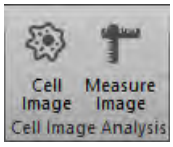
  - Right-click on a cell image container in the Experiment Workspace, then select **Measure** from the cell image container context menu.



  - Click **Measure Image** in the Analysis group of the Image Settings ribbon tab.



  - Click **Measure Image** in the Cell Image Analysis group of the Workspace ribbon tab.



- You can resize the ellipse using the handles that become visible when the ellipse is selected to stretch and rotate it.
- To delete the measurement tool, click on the ellipse, then select from the keyboard or the context menu.
- The measured area is based on a conversion factor of micrometers per pixel.

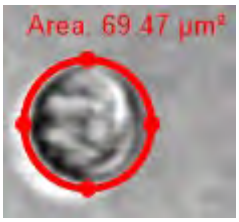
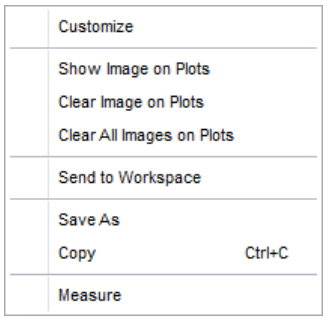


Image view context menus

Active image context menu



The *Active image context menu* is displayed when you right-click on the active image in the Image view tab.

The Active image context menu contains the following options:

- **Customize:** Opens the Image Adjustment customize panel, which allows you to adjust the brightness and contrast of the image (page 317).
- **Show Image on Plots:** Identifies the event that is associated with the image on the Workspace plots. By default, the backgated imaged event is shown in red.
- **Clear Image on Plots:** Removes the backgate associated with the image from the Workspace plots.
- **Clear All Images on Plots:** Removes all backgates associated with all images from the Workspace plots.
- **Send to Workspace:** Sends the selected image to the active Workspace.
- **Save As:** Opens the File Save (Export) dialog, which allows you to save the selected image in the desired location (page 532). The Save As option is disabled if acquisition is in progress.
- **Copy:** Copies the selected image to the windows clipboard. You can also press **Ctrl+C** on the keyboard to copy the selected image.
- **Measure:** Allows you to add the Measure Image tool to the selected image (page 187).

Image gallery context menu

The *Image Gallery context menu* is displayed when you right-click on the images displayed in the Image Gallery in the Image view tab.

The Image Gallery context menu contains the following options:

- **Customize:** Opens the Image Adjustment customize panel, which allows you to adjust the brightness and contrast of the selected images (page 317).
- **Send to Workspace:** Sends the selected images to the active Workspace.
- **Save As:** Opens the File Save (Export) dialog, which allows you to save the selected images in the desired location (page 532). The Save As option is disabled if acquisition is in progress.
- **Copy:** Copies the selected images to the windows clipboard. You can also press **Ctrl+C** on the keyboard to copy the selected images.
- **Delete:** Opens the Confirm deletion dialog, which allows you to delete the selected images. The Delete option is disabled if acquisition is in progress.

Measure image tool context menu

The *Measure image tool context menu* is displayed when you right-click on the image measurement tool.

The Measure image tool context menu contains only the **Delete** option, which deletes the selected measurement tool.

11. Experiment Explorer

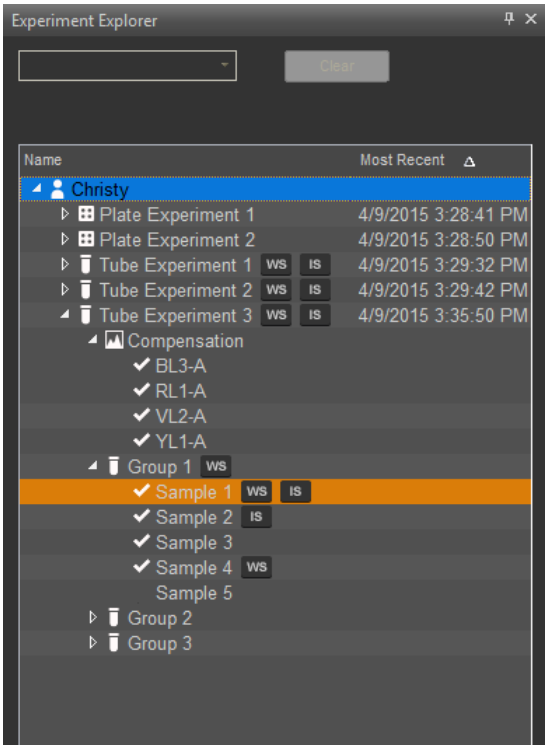
Overview

Experiment Explorer functions as an interface for creating, viewing, and managing *Experiments*.

Experiment Explorer is displayed both on the Main Menu (page 26) and the Attune™ Desktop (page 29). By default, it is docked on the right side of the application window.

- Files view section (page 190) displays the User folders, which contain all Experiment files in a hierarchal view.
- The Files view section can contain multiple folders, each with multiple Experiments.
- Each Experiment can have more than one *Group*, each with one or more *Samples*.

  Each Sample is associated with its own FCS file.



Note: When the Attune™ Cytometric Software is in the SAE mode, you can display application objects IDs in the Experiment Explorer (see "Display ID", page 198).

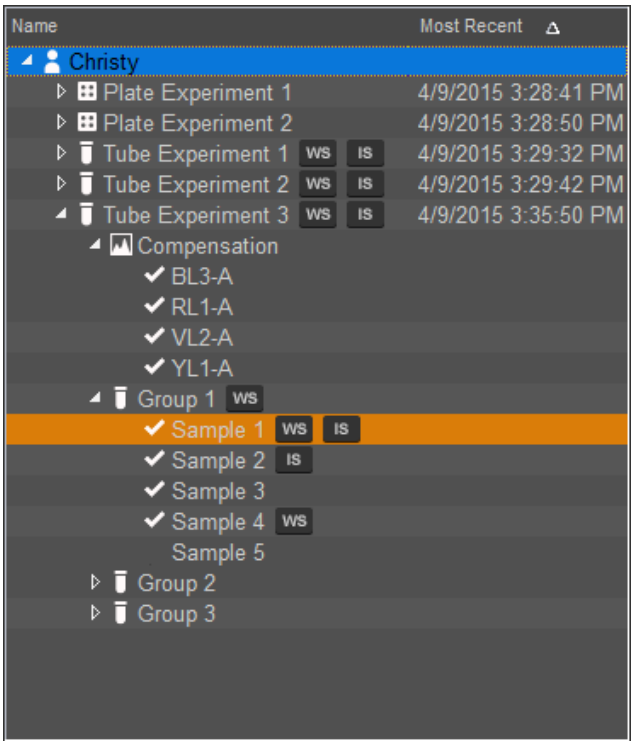
Experiment Explorer icons

Experiment Explorer uses the following icons to indicate the various elements and their conditions within the Files view.

Icon	Indication	Icon	Indication
	User		Compensation
	Plate Experiment node		Read-only compensation
	Tube Experiment node	WS	Workspace badge
	Group node	IS	Instrument Settings badge
	Sample with recorded data	CS	Compensation Settings badge

Files view

Organization Files view shows all Experiments and Samples generated by the current user or the Samples selected as a result of a filter being applied.



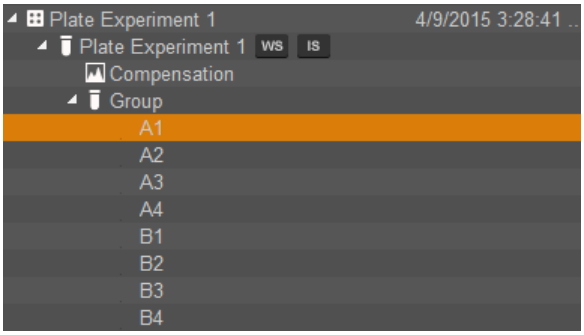
- By default, the *User folder* is displayed on top of the files list and bears the name of the user currently logged in.
- The files in the folders are arranged in a tree structure, which displays the hierarchy of Plates, Experiments, Groups, and Samples.

  The tree structure varies for Plate and Tube-based Experiments (see "Experiment hierarchy", page 191).
- Badges next to individual nodes or files in the hierarchy indicate the presence of Workspaces (WS) and Instrument Settings (IS).
- You can use the *folder context menu* (page 200) to add a new Plate or Tube Experiment to any folder.
- When you create a new Tube or Plate Experiment, the Experiment hierarchy is expanded by default.
- When items are selected in the Experiment Explorer, the entire row is highlighted as specified.

Experiment hierarchy

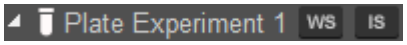
Plate hierarchy

The Experiment Explorer displays the Plate Experiments in a defined hierarchy with a tree structure.

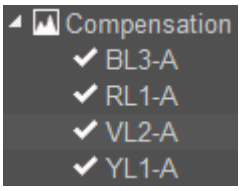


- When you create a new Plate, the software automatically creates a Plate Experiment containing a Compensation node. However, the software does not create any Groups or Samples for new Experiments, unless specified in the *New Experiment dialog* (page 399).
- A Plate can contain one Plate Experiment, which has an associated Workspace, as well as Instrument and Compensation settings.

  Badges to the right of the Experiment name indicate the presence of Experiment-level Workspace (WS) and Instrument (IS) settings.



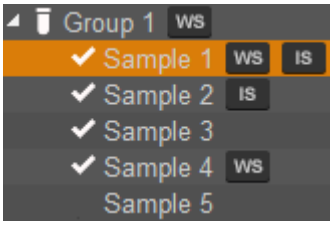
- The Experiment-level compensation settings can have multiple associated Compensation samples, which are displayed in laser and detector order.
- When compensation is recorded for a Compensation sample, the check mark icon appears to the left of the Compensation sample name, indicating that the Sample has recorded data.



  If Compensation has not been recorded for a Sample, then no icon will appear.

- Each Experiment that has assigned Samples consists of one or more Groups. A Workspace badge to the right of the Group name indicates the presence of Group-level Workspace.

  Each group may contain up to 400 Samples. Samples can be derived from a well within a Plate or a Tube.

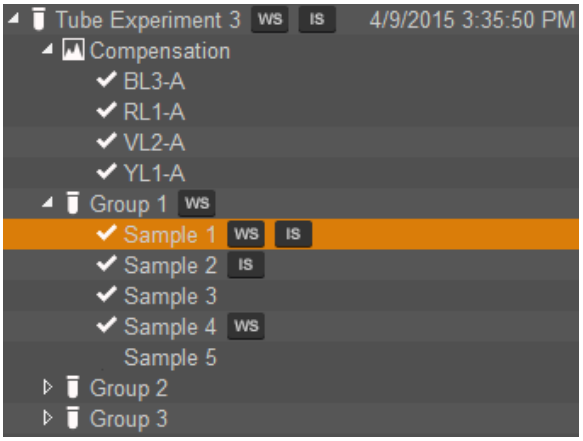


- Each Sample can have a Sample-level Workspace. A Sample can also have Sample-level Instrument settings used instead of the Experiment defaults. Badges to the right of the Sample name indicate the presence of Sample-level Workspace (WS) and Instrument (IS) settings.
- When the Sample is recorded, the check mark icon appears to the left of the Sample name, indicating that the Sample has recorded data.

  If the Sample has not been recorded, then no icon appears.

Tube hierarchy

The Experiment Explorer displays the Tube Experiments in a defined hierarchy with a tree structure.

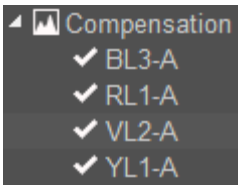


- When you create a new Tube Experiment, the software automatically creates a Compensation node in the hierarchy. However, the software does not create any Groups or Samples for new Experiments, unless specified in the *New Experiment dialog* (page 399).
- Each Tube Experiment has an associated Workspace, as well as Instrument and Compensation settings.

  Badges to the right of the Experiment name indicate the presence of Experiment-level Workspace (WS) and Instrument (IS) settings.

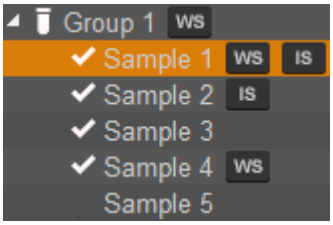
- The Experiment-level compensation settings can have multiple associated Compensation samples, which are displayed in laser and detector order.
- When compensation is recorded for a Compensation sample, the check mark icon appears to the left of the Compensation sample name, indicating that the Sample has recorded data.

  If compensation has not been recorded for a Sample, then no icon appears.

- Each Experiment that has assigned Samples consists of one or more Groups. A Workspace badge to the right of the Group name indicates the presence of Group-level Workspace.

  Each group can contain up to 400 Samples. Samples can be derived from a well within a Plate or a Tube.
- Each Sample can have a Sample-level Workspace. A Sample can also use Sample-level Instrument settings instead of the Experiment defaults. Badges to the right of the Sample name indicate the presence of Sample-level Workspace (WS) and Instrument (IS) settings.

- When the Sample is recorded, the check mark icon appears to the left of the Sample name, indicating that the Sample has recorded data.

  If the Sample has not been recorded, then no icon appears.

Experiment Explorer behavior

Selection indicators

Colors are used to indicate various states of selection within the Experiment Explorer. When items are selected, the entire selected row in the Files view, including the name and date columns, is highlighted.

- **Mouse over:** When the mouse is moved over an item in the Experiment Explorer, the item is highlighted in gray.
- **Selected item:** When an item is selected in the Experiment Explorer, the item is highlighted in dark blue.

  You can select multiple items at once by holding the **Ctrl** or the **Shift** key while clicking. Items which are multi-selected are highlighted in light blue, except the last selected item, which is highlighted in dark blue.
- **Active item:** When an item is loaded into the current Workspace, the Sample name in Experiment Explorer is highlighted in orange.

Selection

- Double-click on the Plate node or Plate or Tube Experiment node to set the focus to the *Heat Map view* (page 138) for that Experiment.

  If another Experiment was already open, this action opens the first Sample within the selected Experiment.

  If no Sample exists within the selected Experiment (if Plate), then double-clicking the node opens the Experiment-level Workspace and sets the Experiment node as active. The Workspace dropdown menu is disabled.
- Double-click on a Group node to activate the first sample within the Group.

  If another Experiment is already open, this action opens the first Sample within the selected Group.

  If no Sample exists within the selected Group, then double-clicking the node opens the Experiment-level Workspace and sets the Experiment node as active. The Workspace dropdown menu is disabled.
- Double-click on the Compensation node or on any Compensation sample to set the focus to the Compensation Workspace for the selected Sample. The *Compensation tab* becomes available in the Ribbon bar (page 66).
- If you double-click the main Compensation node and *Compensation controls* exist, the first Compensation control Sample is selected and the focus is set to the *Workspace view* (page 82).

  If no Compensation controls exist, double-clicking on the Compensation node opens the *Compensation Setup dialog* described on page 379.

  Double-clicking the Compensation node showing the "read-only" icon ( ) opens the *Compensation Matrix* (page 393) for that Experiment.
- Double-clicking on a Sample opens the Sample using the currently selected Workspace and loads the data, if present.

  If a Sample does not have the selected Workspace, it reverts to the next Workspace in the hierarchy (Sample→Group, or Group→Experiment).

  If a new Experiment is loaded, the Workspace is set to the Experiment-level Workspace.

- During acquisition, the active Sample cannot be changed
- During acquisition, double-clicking on the active Sample's Plate node, or the Plate or Tube Experiment node sets the focus to the Heat Map view for the active Experiment.
- The double-clicking on any other node or object does not have an effect.

Drag and drop

Workspace (WS)

A Workspace (WS) badge can be dragged from an Experiment, Group, or Sample, and dropped to another Experiment, Group, or Sample.

- To apply the Workspace from one location (Experiment, Group, or Sample) to another location, click on the WS badge, then drag the badge to the destination location while continuing to hold on the badge. Release the WS badge at the new location to apply the Workspace to the location.
- Dragging and dropping a WS badge within the Experiment Explorer creates a copy of the Workspace at the dropped location. The copy of the Workspace has contents identical to the original, but it is not linked to it.
- Experiment-level WS badge can be dragged to another Experiment, Group, or Sample, which copies the Workspace to the Experiment, Group, or Sample.
- Group-level WS badge can be dragged to another Experiment, Group, or Sample, which copies the Workspace to that respective level.
- A Sample-level WS badge can be dragged to another Experiment, Group, or Sample, which copies the Workspace to that respective level.

Instrument Settings (IS)

An Instrument Settings (IS) badge can be dragged from an Experiment or Sample to another Experiment, Group, or Sample.

- To apply the Instruments Settings from one location (Experiment, Group, or Sample) to another location, click on the IS badge, then drag the badge to the destination location while continuing to hold on the badge. Release the IS badge at the new location to apply the instrument settings to the new location
- Experiment-level IS badge can be dragged to another Experiment or empty Samples, which updates the IS for the Experiment or the Samples.

  If the Experiment contains acquired Samples that have Experiment-level IS, the Samples are updated to display the Sample IS indicator.
- Experiment-level IS badge can also be dragged to a Group folder to apply the Experiment IS to all unrecorded Samples within that Group.

  If this is done within the same Experiment, any Sample-level IS that previously existed within that Group is cleared.
- Experiment-level IS badge cannot be dragged to an Experiment that has existing compensation.
- If the Experiment contains acquired Samples that already have Sample-level IS, dragging the IS badge has no effect on these Samples.
- Sample-level IS badge can be dragged to other Experiments or empty Samples.
- Sample-level IS badge can be dragged to a Group folder to apply the IS to all unrecorded Samples within that Group.

- Dragging a Sample-level IS badge to an Experiment updates the Experiment IS. When this is done, the Sample-level IS badge is removed from the Sample, which is set to use the Experiment-level IS. All other Sample-level IS badges remain regardless of their similarity to the current Experiment-level IS.
- Sample-level IS badge cannot be dragged to a Sample with a recorded FCS file.
- The IS badge cannot be dropped on an Experiment, Group, or Sample, where the instrument configuration does not match. In this case, a warning dialog is displayed with the message: "The instrument settings are not supported by the current instrument configuration and cannot be imported".

Compensation Settings (CS)

A Compensation Settings (CS) badge can be dragged from one Experiment to another Experiment.

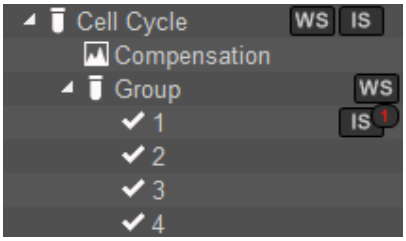
- To apply the Compensation Settings from one Experiment to another, click on the CS badge, then drag the badge to the destination Experiment in the Experiment Explorer while continuing to hold on the CS badge. Release the CS badge at the new location to apply the Compensation and Instrument settings from the original Experiment to the new Experiment.
- The CS badge is only shown next to an Experiment, if the Experiment has compensation defined.
- The CS badge can be dragged from one Experiment to another, which copies the Compensation Settings and the corresponding Instrument Settings to that Experiment.
- When Compensation Settings are applied to another Experiment via drag and drop, they are applied as read-only compensation based on an XML file. This is indicated in the software by a padlock icon adjacent to the Compensation Control.
- The original FCS files recorded for the Compensation Controls are not viewable from the Experiment in which the settings were copied; only the compensation values are visible in the Compensation Matrix (page 393)
- The Compensation Settings can only be applied to another Experiment, if the instrument models match and the target Experiment does not already have compensation defined or recorded.
- The rules for drag and drop of the Compensation Settings badge follow the same guidelines described for dragging an IS badge to an Experiment-level IS.

Instrument Settings badge identification

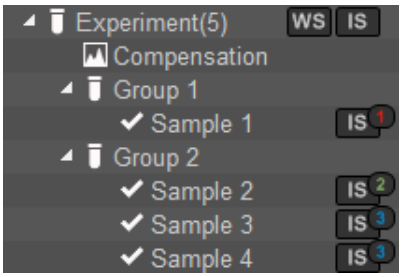
The Instrument Settings (IS) badge is used to identify Samples that have Sample-level Instrument Settings. To provide additional information as to whether Samples within an Experiment have different Instrument Settings, a number is placed with the IS badge to show which Samples have the same Instrument Settings.

- When samples display an IS badge, they are compared with each other to determine whether the Instrument Settings XML is different.
- If the Sample-level IS is different than the Experiment-level IS, the Sample IS displays an additional badge with a colored number to indicate that the Instrument Settings are different (for example, IS1, IS2, etc.).

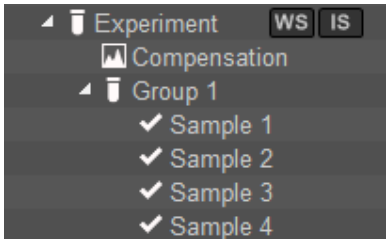
- If the Instrument Settings are the same as the experiment level IS, the IS badge is displayed without the additional colored number badge.
- Each Sample is compared to the Experiment-level Instrument Settings and to the other Sample Instrument Settings (this also applies when pasting IS settings via the Heat Map or when dragging and dropping the IS badges in the Experiment Explorer).
- Each Sample with new or unique Instrument Settings is assigned an IS badge that includes a new number (base 1), which is incremented sequentially. All Instrument Settings that are shared display the same colored number badge.



Sample 1 has unique, Sample-level IS that is different than the Experiment-level IS.



Samples 1, 2, and 3 have unique IS



Samples 1, 2, 3, 4 have the same IS

- If a Sample-level IS with a number has the badge dragged to the Experiment-level in the Experiment Explorer, the Sample-level IS number is removed so that only the IS badge is displayed (that Sample IS now equals the Experiment-level IS), and the remaining badges are renumbered.

Drag and drop from Experiment Explorer to Overlays

You can select Samples to drag and drop on to plots within the Overlay gallery view (page 156).

- When Samples are dragged from the Experiment Explorer to a plot within the Overlay gallery view, new plots are added to the gallery corresponding to the selected Samples and plot.
- The plot into which the dragged Samples are dropped is used to set the gallery plot customization for the newly added plots.

Drag and drop FCS files

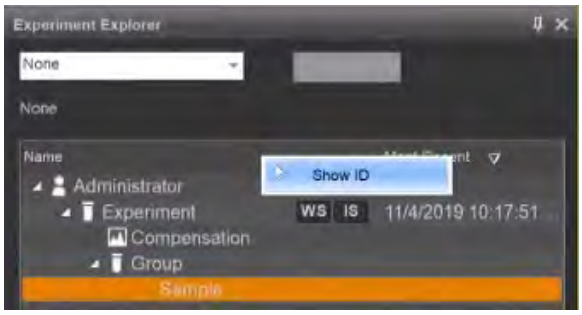
You can import a Sample by dragging an FCS file onto an empty Sample in the Experiment Explorer.

- If the drag and drop operation is valid, the drop icon (a + sign on top of the arrow) is displayed.
- If the Sample already has data, Acquisition is in progress, Automation mode is enabled, or the instrument is paused, the drop option is disabled and the drop icon is displayed (the no entry cursor icon is displayed).
- When the FCS file is dropped onto the empty Experiment Explorer item, the FCS file is imported as though it was imported using the Import FCS File command in the Sample context menu (page 214). The Sample name in the Experiment Explorer is updated to the name of the FCS file after the import.
- Only a valid FCS file can be imported.

Display ID

When the Attune™ Cytometric Software is in the SAE mode, you can display the ID of application objects in the Experiment Explorer.

- Application objects are the domain objects that are of interest for auditing and e-signing when the Attune™ Cytometric Software is in the SAE mode (see "Appendix C: SAE Administrator Console", page 576).
- Examples of application types in the Attune™ Cytometric Software are Plate Experiment, Tube Experiment, Template, Filter Configuration, and Filter Definition.
- The GUID is the unique ID assigned each an application object. It is an immutable property and cannot be changed.
- To display the GUID of application objects in Experiment Explorer, right-click on the column header in Experiment Explorer, then select **Show ID**.

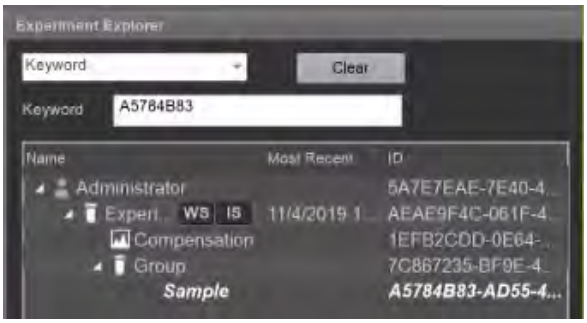


The GUID for the application objects are displayed in the ID column of Experiment Explorer.

- When the ID column is shown on the Experiment Explorer, you can use the GUID to search for specific application objects.

  To do this, select **Keyword** in the Search dropdown, enter the GUID of the application object you wish to search, then click **Enter**.

  If found, the application object you have searched appears bolded and italicized in the Experiment Explorer.



Experiment Explorer context menus

Overview

Experiment Explorer has context menus associated with different levels of the hierarchy in the tree structure. You can access each context menu by right-clicking the appropriate object in the tree structure.

- The Experiment Explorer context menus include the following:
  - *User context menu* (page 200)
  - *Plate context menu* (page 202)
  - *Experiment context menu* (page 206)
  - *Group context menu* (page 210)
  - *Sample context menu* (page 213)
  - *Compensation node context menu* (page 217)
  - *Compensation control context menu* (page 218)
- Unless indicated otherwise, all Experiment Explorer context menu items are always visible and enabled.
- When a combination of different objects is selected in the Experiment Explorer (e.g. Experiment, Group, and Samples), the right-click context menus are not available.

Validation of name fields

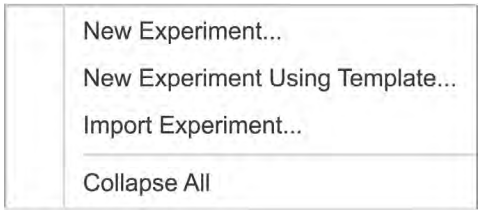
Context menu options that allow you to create or edit names of items follow the validation rules outlined below:

- **Duplicate** context menu option duplicates the selected object and displays the name in a textbox in the Edit mode.
- **Rename** context menu option displays the current name of the selected object in a textbox in the Edit mode.
- You can rename a node by pressing the **F2** key to enter the Edit mode.
- Click anywhere outside the edit textbox or press **Enter** to validate the entered name and close the Edit mode.
- If a valid name is present in the textbox, the name is updated.
- Leading and trailing spaces are removed on validation. Consecutive spaces are converted to single spaces on validation.
- If you attempt to enter invalid characters into the edit textbox, a warning dialog indicates the error condition and the invalid characters do not appear in the textbox.
- If an invalid name is present when focus is lost, then the name reverts to the previous valid name.
- If the entered name already exists, when focus is lost, a warning dialog indicates that the entered name already exists and the textbox remains in Edit mode.

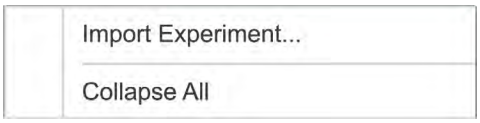
User and Shared folder context menu

Right-click on the **User folder heading** to display the *User folder* or the *Shared folder context menu*, which allows you to create New Experiments from scratch or using a Template, or to import pre-defined Experiments.

During acquisition, only the *Collapse All* option is enabled.



User folder context menu



Shared folder context menu

New Experiment

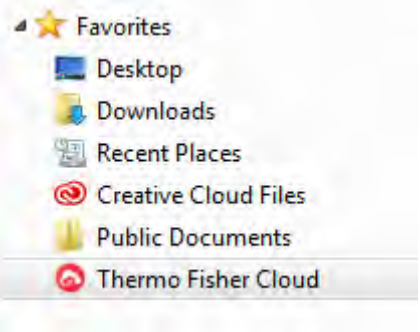
- **New Experiment** opens the *New Experiment dialog* (page 399), which allows you to create a new Plate or Tube Experiment or to import FCS files into a new Analysis Experiment.
- The New Experiment option is not available on any Shared folder context menus. It is disabled if a Plate is paused or if the application is in the Automation mode.

New Experiment Using Template

- **New Experiment Using Template** opens the *New Experiment from Template Dialog* (page 405), which allows you to select a Plate- or Tube-based Experiment as a template for a new Experiment.
- The New Experiment Using Template option is disabled if a Plate is paused or if the application is in the Automation mode.

Import Experiment

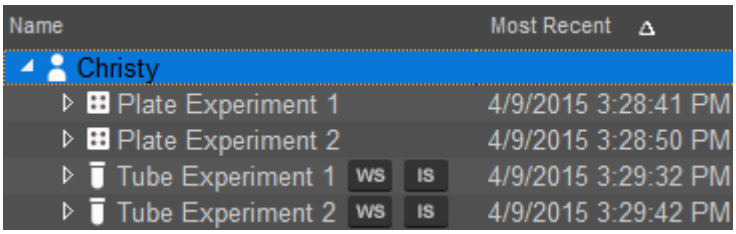
- **Import Experiment** opens the *File Open (Import) dialog* (page 535), which allows you to import pre-existing Plate- or Tube-based Experiments.
- The Import Experiment option is not available during acquisition.
- If the device (instrument or software) has been registered to a Connect account, the File Open dialogue includes an icon for the Thermo Fisher Cloud listed under Favorites. Clicking on this icon opens a virtual folder listing all files and folders included in your Connect account. You can import the Experiment from the Connect account by selecting the desired location in the Connect account during Experiment import.



- Connect account contents are only visible when viewed through the File Open (Import) and File Save (Export) dialogues from the Attune™ Cytometric Software.

Collapse All

- **Collapse All** collapses all expanded nodes down to the Plate or Tube Experiment-levels for the current Folder.



The Collapse All option is always available.

Plate context menu

Right-click on a **Plate icon** or **Plate name** in the User folder to display the *Plate context menu*.

During acquisition, all options in Experiment context menus are disabled.

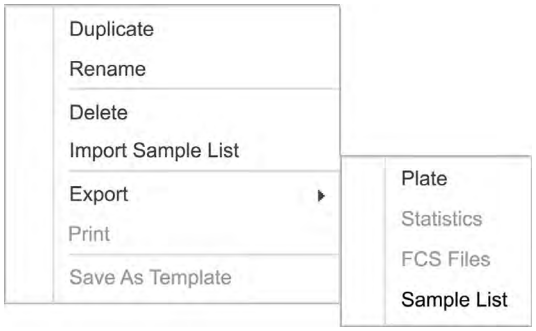


Plate context menu in User folder

Plate context menu in Shared folder

Duplicate

- **Duplicate** allows you to duplicate the selected plates and their full hierarchy (i.e., Experiment, Compensation, Groups, and Samples).
- The Duplicate option is enabled when single or multiple plates are selected.

  This option is disabled if the Plate Experiment Instrument settings do not match the system configuration
- When a Plate Experiment is duplicated, all Workspaces (Experiment-, Group-, and Sample-level), Run Protocols, and annotations are preserved and all Experiment and Sample-level keywords are duplicated.

  Only the Experiment-level Instrument Settings are preserved. Sample-level Instrument settings and FCS files are not duplicated.
- If Compensation settings are available, the newly created Plate contains Experiments with empty Compensation wells or Tubes as defined in the source Experiment.
- The newly created Plate must have a unique name. When a Plate is duplicated, it is automatically assigned a name, which consists of the same name as the current Plate plus an incrementing numerical suffix. The numerical suffix starts at 1 and increments until a unique identifier is achieved.
- The Plate name appears in an edit textbox ready for editing. The newly entered name is validated as described on page 199.

Rename Plate

- **Rename** opens an edit textbox containing the name of the current Plate in the edit mode.
- The Rename option is enabled when a single plate is selected.
- Clicking anywhere outside the edit textbox validates the Plate name as described on page 199.
- You can also rename a selected Plate by pressing **F2** to enter the editing mode.

Delete Plate

- **Delete** opens the *Deleted Items dialog* (page 220), which allows you to delete the selected Plates.
- The Delete option is enabled when single or multiple Plates are selected.

Import Sample List

Opens the *File Open (Import) dialog* (page 535) with the extension set as CSV by default (*.csv), which allows you to import a Sample List from an existing CSV file. Other file formats are not supported.

- The Import Sample List option is disabled if the Experiment contains any recorded data, multiple nodes of the same level are selected in Experiment Explorer, acquisition is in progress, or the Automation mode is enabled.

  When the Sample List is imported, the software looks for the following columns in the CSV file:

  - LOCATION
  - SAMPLE
  - SAMPLE_NOTES
  - GROUP
  - GROUP_NOTES
  - EXPERIMENT
  - EXP_NOTES
  - PLATE
  - PLATE_ID
  - PLATE_NOTES

  LOCATION column is required for import and must contain data in the format A1, A2, A3,... etc. (for a Plate Experiment) or T1, T2, T3,… etc. (for a Tube Experiment). All other columns are optional.

  PLATE, PLATE_ID, and PLATE_NOTES columns are only created when importing into a Plate Experiment. These columns are ignored when importing the Sample List into a Tube Experiment.

- When importing the CSV sample list, the *Map Sample List* dialog (page 440) is displayed, which allows you to create and map Samples based on Location (i.e., a Sample mapped to A1 in the Sample List will be mapped to A1 on the Heat Map).

  Additional keywords are mapped based on the keywords defined for the Experiment. If the Experiment does not have the custom keywords included in the Sample List CSV file, the custom keywords in the file are not added to the Sample List in the Experiment.

- If Samples already exist within the Experiment or Plate (i.e., Samples are assigned to a location), a prompt to update the Sample information is displayed (page 442).

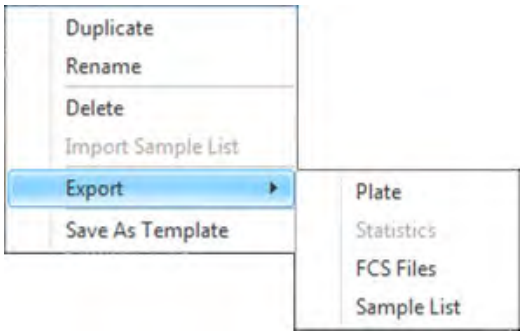
- After the existing Samples are updated, the import process continues to process the addition of new Samples, as necessary.

- The Sample names created within each Group during the import process must be unique, and Sample, Group, Experiment, and Plate names must be less than or equal to 50 characters long.

- Names cannot contain the following illegal characters /:*?"<>|& and end with a period. Names also cannot be any of the following words: CON, PRN, AUX, CLOCK$, NUL, COM1, COM2, COM3, COM4, COM5, COM6, COM7, COM8, COM9, LPT1, LPT2, LPT3, LPT4, LPT5, LPT6, LPT7, LPT8, and LPT9.

Export Plate

Selecting this option displays a sub menu containing Export Plate, FCS files, and Sample List as options. The Export option is enabled when single or multiple Plates are selected.



- **Export ▶ Plate** opens the *File Save (Export) dialog* (page 532), which allows you to save the selected Plates in the desired location.
- **Export ▶ FCS Files** opens the *File Save (Export) dialog* (page 532), which allows you to save the selected FCS files using the required FCS version in the desired location.

  All FCS files within the selected Experiments are exported. If a Sample within the selected Experiment has no FCS file, then no exported FCS file is created for that Sample.

  If the Experiment compensation settings are different from the Compensation settings contained within the FCS file, the target and label names have been changed, or Experiment or custom keywords have been changed, a prompt to update the keywords is displayed.

  The Export ▶ FCS Files option is disabled during data acquisition (Run or Record) or if the selected Experiment does not contain FCS data files.
- **Export ▶ Sample List** opens the *File Save (Export) dialog* (page 532), which allows you to save the Sample List in the desired location.

  The Export ▶ Sample List option is disabled if acquisition is in progress or the Automation mode is enabled. In both cases, this option is enabled when a plate is paused. If multiple nodes of the same level are selected, this option is also disabled.

  When the Sample List is exported, a CSV file is created that contains a list of all Samples in the Experiment (Compensation controls are not included in the Sample List) with the following columns:

  - LOCATION
  - SAMPLE
  - SAMPLE_NOTES
  - GROUP
  - GROUP_NOTES
  - EXPERIMENT
  - EXP_NOTES
  - PLATE
  - PLATE_ID
  - PLATE_NOTES

PLATE, PLATE_ID, and PLATE_NOTES columns are only created when exporting from a Plate or Plate Experiment. These columns are not created when exporting the Sample List from a Tube Experiment.

All user created keywords are also included in the export set, and the user defined keyword names are used as the column headers for the user defined keywords.

If a field is blank, it is left empty in the resulting CSV file.

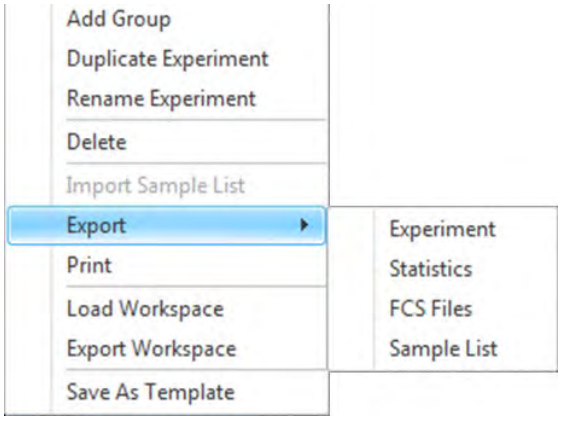
For Tube samples, the location information is based on Tube position in the Heat Map and is designated as T1, T2, etc.

Save As Template

Save As Template opens the *Save As Template dialog* (page 428), which allows you to name the current Plate and save it as a template in database for future re-use.

Experiment context menu

- Right-click on a **Tube Experiment** in the User or Shared folder to display the *Tube Experiment context menu*.
- Right-click on a **Plate Experiment node** in the User or Shared folder to display the *Plate Experiment context menu*.
- During acquisition, all options in Experiment context menus are disabled.



Tube Experiment

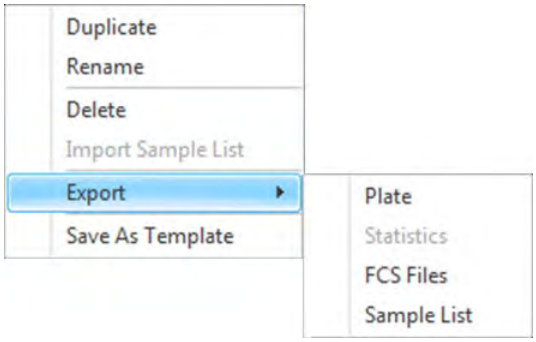


Plate Experiment

Add Tube Group

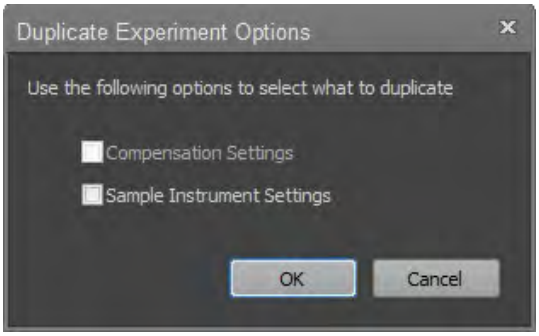
- **Add Tube Group** opens the *New Group dialog* (page 405), which allows you to add a Group to the end of the currently selected Experiment.
- You can create up to 100 Groups for each Experiment.
- Add Tube Group option is only enabled when a single Experiment is selected.

Duplicate Experiment

- **Duplicate Experiment** duplicates the selected Experiments with their associated Groups and Samples.
- The Duplicate Experiment option is visible only for Tube Experiments, and it is enabled only when single or multiple Experiments are selected.

  This option is disabled if the Experiment Instrument settings do not match the system configuration
- When an Experiment is duplicated, all Workspaces (Experiment-, Group-, and Sample-level), Run Protocols, and annotations are preserved.

  Experiment-level Instrument Settings (IS) are preserved. If Sample-level IS exists in the Experiment, a duplicate Experiment options menu provides the option to duplicate Sample-level IS and Compensation Settings.



FCS files are not duplicated.

All Experiment- and Sample-level keywords are duplicated.

- If Compensation Settings have not been duplicated, the newly created Experiment contains a Compensation node with empty Samples as defined in the source Experiment.

  If the Compensation Settings have been duplicated, the newly created Experiment contains a Compensation node with locked Compensation controls matching the compensation values in the source Experiment.
- The newly created Experiment must have a unique name. Once an Experiment is duplicated, it is automatically assigned a name, which consists of the same name as the current Experiment plus an incrementing numerical suffix. The numerical suffix starts at 1 and increments until a unique identifier is achieved.

Rename Experiment

- **Rename** opens an edit textbox containing the name of the current Experiment in the edit mode.
- The Rename option is enabled when a single Experiment is selected.
- Clicking anywhere outside the edit textbox validates the Experiment name as described on page 199.
- You can also rename an Experiment by first selecting it, then pressing **F2** to enter the editing mode.

Delete

- **Delete** opens the *Deleted Items dialog* (page 220), which allows you to delete the selected Experiments.
- The Delete option is enabled when single or multiple Experiments are selected.

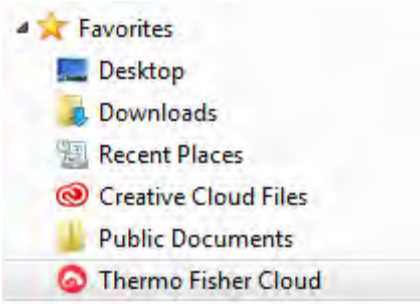
Import Sample List

- **Import Sample List** opens the *File Open (Import) dialog* (page 535) with the extension set as CSV by default (*.csv), which allows you to import a Sample List from an existing CSV file as described for Import Sample List in Plate context menu (page 203). Other file formats are not accepted.

Export

- **Export** displays a sub-menu containing *Experiment*, *Statistics*, *FCS Files,* and *Sample List* as options.
- The Export option is enabled when single or multiple Plates are selected.
- **Export ▶ Experiment** opens the *File Save (Export) dialog* (page 532), which allows you to save the selected Experiments in the desired location.

  The Export ▶ Experiment option is visible and enabled only for Tube Experiments.
- If the device (instrument or software) has been registered to a Connect account, the File Open dialogue includes an icon for the Thermo Fisher Cloud listed under Favorites. Clicking on this icon opens a virtual folder listing all files and folders included in your Connect account. You can save the Experiment to your Connect account by selecting the desired location in the Connect account during Experiment export.



Connect account contents are only visible when viewed through the File Open (Import) and File Save (Export) dialogues from the Attune™ Cytometric Software.

- **Export ▶ FCS Files** opens the *File Save (Export) dialog* (page 532), which allows you to save the selected FCS files using the required FCS version in the desired location.

  All FCS files within the selected Experiments are exported. If a Sample within the selected Experiment has no FCS file, then no exported FCS file is created for that Sample.

  If the Experiment compensation settings are different from the Compensation settings contained within the FCS file, the target and label names have been changed, or Experiment or custom keywords have been changed, a prompt to update the keywords is displayed.

  The Export ▶ FCS Files option is disabled during data acquisition (Run or Record) or if the selected Experiment does not contain FCS data files.

- **Export ▶ Sample List** opens the *File Save (Export) dialog* (page 532), which allows you to save the Sample List in the desired location.

  The Export ▶ Sample List option is disabled if acquisition is in progress or the Automation mode is enabled. In both cases, this option is enabled when a plate is paused. If multiple nodes of the same level are selected, this option is also disabled.

  When the Sample List is exported, a CSV file is created that contains a list of all Samples in the Experiment (Compensation controls are not included in the Sample List) with the following columns:

  - LOCATION
  - SAMPLE
  - SAMPLE_NOTES
  - GROUP
  - GROUP_NOTES
  - EXPERIMENT
  - EXP_NOTES
  - PLATE
  - PLATE_ID
  - PLATE_NOTES

  PLATE, PLATE_ID, and PLATE_NOTES columns are only created when exporting from a Plate or Plate Experiment. These columns are not created when exporting the Sample List from a Tube Experiment.

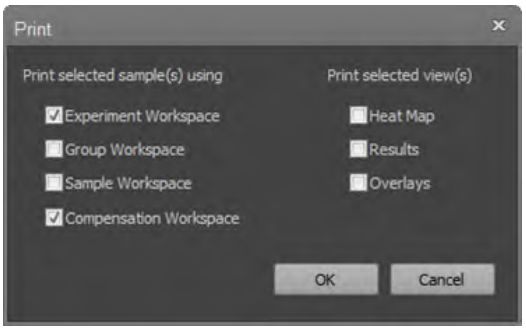
  All user created keywords are also included in the export set, and the user defined keyword names are used as the column headers for the user defined keywords.

  If a field is blank, it is left empty in the resulting CSV file.

  For Tube samples, the location information is based on Tube position in the Heat Map and is designated as T1, T2, etc.

Print

- **Print** opens the *Batch Print dialog* (page 418), which allows you to select a Workspace (Experiment, Group, or Sample) or view (Heat Map, Results, or Overlays) to print.



- The Print option is only enabled when a single Experiment is selected. It is disabled during acquisition.

Load Workspace

- **Load Workspace** opens the *File Open (Import) dialog* (page 535), which allows you to select a Workspace (.aws file) to import.
- The selected Workspace will replace the existing Experiment-level Workspace.
- The Load Workspace option is only enabled when a single Experiment is selected.

Export Workspace

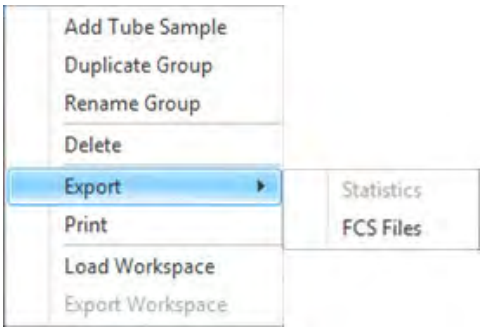
- **Export Workspace** opens the *File Save (Export) dialog* (page 532) which allows you to select a name and location to save the Workspace file.
- The Export Workspace option is only enabled when a single Experiment is selected.

Save As Template

Save As Template opens the *Save As Template dialog* (page 428), which allows you to name the current Experiment and save it as a Template in the database for future reuse.

Group context menu

Right-click on a **Group node** within the User folder to display the *Group context menu*. During acquisition, all options in Group context menus are disabled.



Group context menu in User folder

Add Tube sample

- **Add Tube sample** adds a Tube sample to the selected Group.
- You can create up to 400 Samples for each Experiment. The option is disabled if there are already 400 Samples within the Experiment.
- This option is only enabled when a single Group is selected.

Duplicate Group

- **Duplicate** allows you to duplicate the current Group within the current Experiment, creating an exact duplicate with the same number of Tubes and Sample-level Workspaces. If a Group includes Samples with recorded data, the duplicated Samples do not include the data.
- When a Group is duplicated, all Samples and their corresponding keyword values are duplicated. The FCS files are not duplicated.
- If duplicating a Group results in the creation of over 400 Samples for that Experiment, the new Group is created with as many Samples as possible until the 400 Sample limit is reached.
- The Duplicate option is available for Tube Experiments and for Groups within Plate Experiments that contain Tube samples. It is enabled only when a single Group is selected.

  Groups composed of only Well samples cannot be duplicated.

  If a Group includes both Well and Tube samples, the Duplicate option will only duplicate the Tube samples within the Group.
- The newly created Group must have a unique name. Once a Group is duplicated, it is automatically assigned a name, which consists of the same name as the current Group plus an incrementing numerical suffix. The numerical suffix starts at 1 and increments until a unique identifier is achieved.

Rename Group

- **Rename** opens an edit textbox containing the name of the current Group in the edit mode.
- The Rename option is enabled when a single Group is selected.
- Clicking anywhere outside the edit textbox validates the Group name as described on page 199.
- You can also rename a Group by first clicking on the Group name to select it, then pressing **F2** to enter the editing mode.

Delete

- **Delete** opens the *Deleted Items dialog* (page 220), which allows you to delete the selected Groups.
- The Delete option is only enabled when single or multiple Groups are selected in the User folder.
- If the deleted Group contains the active Sample, the software displays the Experiment-level Workspace.
- You can also use the keyboard **Delete** key to delete selected items.

Export

- **Export** displays a sub-menu containing *Statistics* and *FCS Files* as options.
- **Export ▶ Statistics** opens the *Export Statistics dialog* (page 420), which allows you to select Experiment-level, Group-level, or Sample-level Workspace statistics to export.

  Statistics for all Samples within the selected Experiments are exported except Samples with no data.

  Statistics can be exported as a single file (statistics for all Samples are combined into a single CSV file) or as individual files (statistics for all Samples are exported into separate CSV files) (page 420).

  The Export ▶ Statistics option is always enabled, except during acquisition.
- **Export ▶ FCS Files** opens the *File Save (Export) dialog* (page 532), which allows you to save the selected FCS files using the required FCS version in the desired location.

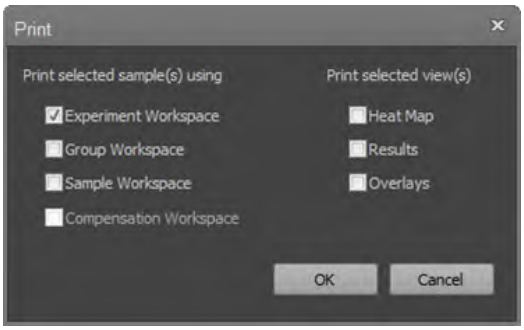
  All FCS files in the selected Group are exported. If a Sample within the selected Group has no FCS file, then no exported FCS file is created for that Sample.

  If the Experiment compensation settings are different from the Compensation settings contained within the FCS file, a prompt to update the compensation keyword is displayed.

  The Export ▶ FCS Files option is disabled during data acquisition (Run or Record).

Print

- **Print** opens the *Batch Print dialog* (page 418), which allows you to select Workspaces (Experiment, Group, and Sample) or views (Heat Map, Results, and Overlays) to print.
- The option to print the Compensation Workspaces is disabled, which can only be printed using the Batch Print dialogue from the Compensation node (page 219).



- The Print option is only enabled when single or multiple Groups within the same Experiment are selected. It is disabled during acquisition.

Load Workspace

- **Load Workspace** opens the *File Open (Import) dialog* (page 535), which allows you to select a Workspace file (*.aws) to import.
- If a Workspace file is selected, it will replace the existing Group-level Workspace.
- The Load Workspace option is only enabled when a single Group is selected.

Export Workspace

- **Export Workspace** opens the *File Save (Export) dialog* (page 532) which allows you to select a name and location to save the Workspace file
- This option is only enabled if a Group-level Workspace exists for the selected Group.
- The Export Workspace option is only enabled when a single Group is selected. It is disabled when multiple Groups are selected.

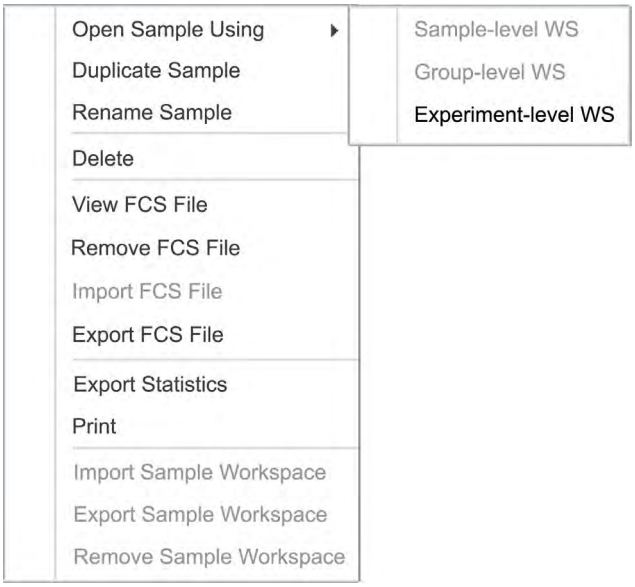
Remove Group Workspace

- **Remove Group Workspace** removes the Group-level Workspace of the selected Groups.
- This option is only available if a Group-level Workspace is present in at least one of the selected Groups.

Sample context menu

Right-click on a **Tube sample** or **Plate sample** in the User folder to display the *Sample context menu*.

During acquisition, all options in the Sample context menu are disabled.



Tube sample context menu

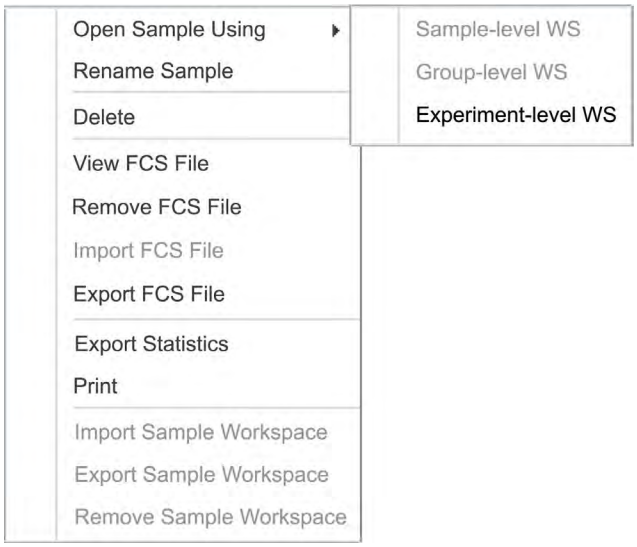


Plate Sample context menu

Open Sample Using

- **Open Sample Using** opens a secondary menu with the choice to open the selected data file using the Sample-, Group-, or the Experiment-level Workspace.
- This option enabled only when a single Sample is selected.
- The Group- and Sample-level Workspace options are enabled only if the selected Sample has a Group or Sample Workspace, respectively.

Duplicate Sample

- **Duplicate** creates a duplicate of the current Tube sample within the current Group. The Sample-level Workspace is copied, if present. All Sample-level keywords are duplicated. The Sample-level Instrument settings and the FCS file are not duplicated.
- The **Duplicate Sample** option is available only for Tube samples, and it is enabled only when a single Sample is selected.
- You can create up to 400 Samples for each Experiment. If the 400 Sample limit has been reached for the Experiment, this option is disabled.
- Each new Sample is created at the end of the current Group and must have a unique name. After a Sample is duplicated, it is automatically assigned a name, which consists of the same name as the current Sample plus an incrementing numerical suffix. The numerical suffix starts at 1 and increments until a unique identifier is achieved.
- The Sample name appears in an edit textbox ready for editing. The newly entered name is validated as described on page 199.

Rename Sample

- **Rename** opens an Edit textbox with the name of the current Sample in edit mode.
- The Rename option is enabled when a single Sample is selected.
- Clicking anywhere outside the edit textbox validates the Sample name as described on page 199.
- You can also rename a node by first clicking on the node to select it, then pressing **F2** to enter the editing mode.

Delete Sample

- **Delete** opens the *Deleted Items dialog* (page 220), which allows you to delete the selected Samples.
- The Delete option is enabled when single or multiple Samples are selected. It is disabled if a Plate is paused or the application is in the Automation mode.
- If the active Sample is deleted, the Sample above it is activated. If there are no Samples above the deleted Sample, then the Sample below it is activated. If there are no Samples, the Experiment-level Workspace is displayed. If the Experiment is deleted, the software displays the main menu.
- You can also use the keyboard **Delete** key to delete selected items.

View FCS File

- **View FCS File** displays the FCS Sample information for the active Sample in the *FCS Sample Information* panel (page 326).
- This option is only enabled for the active Sample.

Remove FCS File

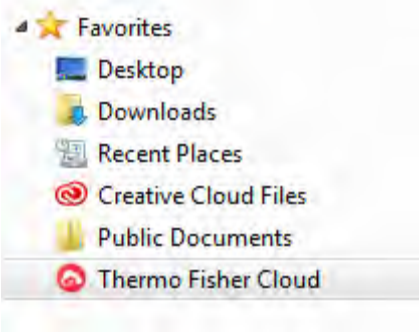
- **Remove FCS File** opens the *Deleted Items dialog* (page 220).
- Confirming the Delete command removes the data file from the selected Samples and sends it to the recycle bin.

  Empty Sample entries are retained in the hierarchy, if the data files are removed.
- The Remove FCS File option is visible and enabled if one or more of the selected Samples have data present. It is disabled if a Plate is paused or the application is in the Automation mode.

Import FCS File

- **Import FCS File** opens the *File Open (Import) dialog* (page 535), which allows you to select a single data file to import into the selected Sample.
- The Import FCS File option is visible but disabled if the selected Sample contains FCS data. It is disabled during data acquisition (Run or Record), if a Plate is paused, or if the application is in the Automation mode.
- This option is not available when multiple Samples are selected.

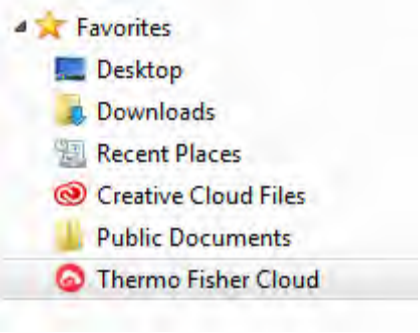
- If the device (instrument or software) has been registered to a Connect account, the File Open dialogue includes an icon for the Thermo Fisher Cloud listed under Favorites. Clicking on this icon opens a virtual folder listing all files and folders included in your Connect account. You can save the FCS file to your Connect account by selecting the desired location in the Connect account during FCS File import.



  Connect account contents are only visible when viewed through the File Open (Import) and File Save (Export) dialogues from the Attune™ Cytometric Software.

Export FCS File

- **Export FCS File** allows you to save the selected FCS file in the desired location.
- If a single Sample is selected for export, the Export FCS File option opens the *File Save (Export) dialog* (page 532), which allows you to save the FCS file associated with the selected Sample using a custom name.
- If multiple Samples are selected for export, the Export FCS File option opens the *File Browser dialog* on page 537, which allows you to create or to select a destination folder for exporting multiple FCS files.
- If the device (instrument or software) has been registered to a Connect account, the File Open dialogue includes an icon for the Thermo Fisher Cloud listed under Favorites. Clicking on this icon opens a virtual folder listing all files and folders included in your Connect account. You can save the FCS File to your Connect account by selecting the desired location in the Connect account during Experiment export.



  Connect account contents are only visible when viewed through the File Open (Import) and File Save (Export) dialogues from the Attune™ Cytometric Software.
- When exporting FCS files, you have the option to choose the FCS file name and the FCS version (FCS 3.1 or FCS 3.0). Alternatively, you can save the FCS files in the form of a CSV (.csv) file.

- If the Experiment compensation settings are different from the Compensation settings contained within the FCS file, the target and label names have been changed, or Experiment or custom keywords have been changed, a prompt to update the keywords is displayed.

  Select **Update all FCS Keywords** to update the keywords in the FCS file (for example, update the Sample name).

  Select **Ignore** to close the prompt without updating the keywords.
- If a selected Sample has no FCS file, the Export FCS file option is not displayed.
- The Export FCS Files option is disabled during data acquisition (Run or Record).

Export Statistics

- **Export Statistics** opens the *Export Statistics dialog* (page 420), which allows you to select Experiment-level, Group-level, or Sample-level Workspace statistics to export.
- Statistics for all selected Samples that contain data are exported.
- The Export Statistics option is visible if one or more of the selected Samples has an associated data file. It is disabled during acquisition.

Print

- **Print** opens the *Batch Print dialog* (page 418), which allows you to select Workspaces (Experiment, Group, and Sample) or views (Heat Map, Results, and Overlays) to print.
- The Print option is only enabled when single or multiple Samples within the same Experiment are selected. It is disabled during acquisition.

Import Sample Workspace

- **Import Sample Workspace** opens the *File Open (Import) dialog* (page 535), which allows you to select a Workspace file (*.aws) to import.
- If a Workspace file is selected for import, the selected Workspace will replace the existing Sample-level Workspace.
- The Load Workspace option is only enabled when a single Sample is selected.

Export Sample Workspace

- **Export Workspace** opens the *File Save (Export) dialog* (page 532) which allows you to select a name and location to save the Workspace file
- This option is only enabled when a single Sample is selected. It is not visible if the Sample does not contain a Sample-level Workspace.

Remove Sample Workspace

- **Remove Workspace** removes the Sample-level Workspace of the selected Samples.
- If the Sample-level Workspace that is removed is also the active Workspace, the Workspace view returns to the Experiment-level Workspace when the Sample-level Workspace is removed.
- This option is only visible if a Sample-level Workspace is present in at least one of the selected Samples.

Compensation node context menu

Right-clicking on the main **Compensation node** displays the *Compensation node context menu*. During acquisition, all options in this context menu are disabled.

Compensation node context menu for Experiments without recorded Compensation

Compensation node context menu for Experiments with recorded Compensation

Compensation Setup

- **Compensation Setup** opens the *Compensation Setup dialog* (page 379), which allows you to choose the Compensation Source, Compensation Measurement, Background Fluorescence Mode, and the required compensation parameters.
- This dialog also allows you to make modifications to an existing Compensation Setup.
- The Compensation Setup option is disabled if a Plate is paused or the application is in the Automation mode. It is not visible in any Shared folder.

Import Compensation

- **Import Compensation** opens the *File Open (Import) dialog* (page 535), which allows you to select a Compensation Settings file (*.acs) to import.
- Importing compensation creates Compensation controls with a read-only attribute ( ) (see Read-only or file-based compensation).
- The Import Compensation option is not enabled if any Compensation control nodes exist.

Read-only or file-based compensation

- Read-only or file-based compensation is a Compensation Setup based entirely on the underlying Compensation XML. Read-only Compensation Controls are displayed in the Experiment Explorer with the “read-only” icon ( ).
- A Compensation Setup cannot include both read-only controls and Sample-based controls, where an FCS file is used to determine the spillover value.
- You cannot modify read-only Compensation controls to add additional Compensation controls or to remove existing Compensation controls. You must remove the entire compensation to alter the number of controls or use Samples as Compensation controls.
- You can modify the read-only Compensation controls using any of the post-acquisition compensation modification options, including modifying the spillover values by directly typing in the Compensation matrix or using the Plot compensation modification option.
- Double clicking on any read-only control displays the *Compensation Matrix dialog* (page 393).

Export Compensation

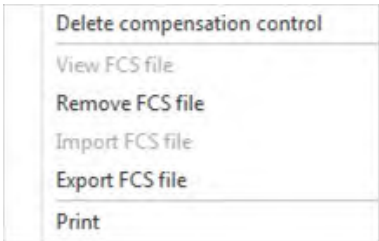
- **Export Compensation** opens the *File Save (Export) dialog* (page 532), which allows you to select a name and location to save the Compensation Settings file (*.acs).
- This option is enabled only when all Compensation samples have been acquired.

Remove Compensation

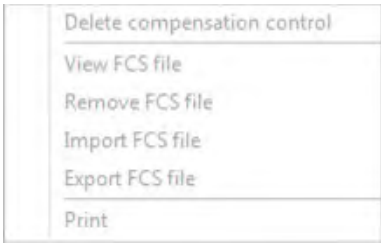
- **Remove Compensation** removes the Compensation Settings and existing Compensation samples and opens the *Deleted Items dialog* (page 220). If the Compensation consists of read-only controls, these are also removed.
- The Remove Compensation option is disabled if a Plate is paused or the application is in the Automation mode.

Compensation control context menu

Right-clicking on a **Compensation control** displays the *Compensation control context menu*. During acquisition, all options in this context menu are disabled. If the compensation is a read-only (i.e., imported file), the Compensation control context menu options are all disabled.



Compensation control context menu for controls with recorded Compensation



Compensation control context menu for read-only Compensation controls

Delete Compensation control

- **Delete Compensation control** opens the *Deleted Items dialog* (page 220), which allows you to delete the selected Compensation control.
- This option is disabled if a Plate is paused or if the application is in the Automation mode.
- You can also use the keyboard **Delete** key to delete selected items.

View FCS File

- **View FCS File** opens the *FCS Sample Information panel* (page 326), which displays the FCS metadata (the text segment portion) for the selected Compensation control.
- This option is enabled only for the active Compensation control containing data.

Remove FCS File

- **Remove FCS File** opens the *Deleted Items dialog* (page 220).
- Confirming the Delete command removes the data file from the selected Compensation controls and sends the files to the recycle bin. Empty Compensation controls are retained in the hierarchy, if the files are removed.
- This option is enabled if one or more of the selected Compensation controls have data present. It is disabled, if a Plate is paused or if the application is in the Automation mode.

Import FCS File

- **Import FCS File** opens the *File Open (Import) dialog* (page 535), which allows you to select a data file to import.
- Upon import of an FCS file as a Compensation control, the Compensation Matrix is recalculated.
- The import FCS File option is visible but disabled if the selected Compensation control contains FCS data. It is disabled during data acquisition (Run or Record), if a Plate is paused, or if the application is in the Automation mode.
- This option is not visible when multiple Compensation controls are selected.

Export FCS File

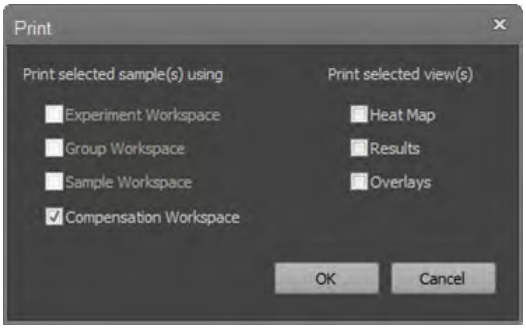
- **Export FCS File** allows you to save the selected FCS file in the desired location.
- If a single Compensation sample is selected for export, the Export FCS File option opens the *File Save (Export) dialog* (page 532), which allows you to save the FCS file associated with the selected Sample using a custom name.
- If multiple Compensation samples are selected for export, this option opens the *File Browser dialog* (page 537), which allows you to create or select a destination folder for exporting multiple FCS files.

  In this case, the FCS files that are saved are automatically named using the Experiment and the Compensation parameter name with an FCS extension.
- If a selected Sample has no FCS file, then no exported FCS file is created for that Sample.

Print

- **Print** option opens the *Batch Print dialog* (page 418), which allows you to select the Compensation Workspace and the Heat Map, Results, and Overlays views to print.

  The options to print the Experiment, Group, or Sample Workspaces are disabled.



- The Print option is only enabled when single or multiple Samples within the same Experiment compensation are selected. It is disabled during acquisition.

Deleted items dialog

The *Deleted items dialog* appears when you select **Delete** from the context menus on the Experiment Explorer or the Heat Map view, or press the **Delete key** on the keyboard to delete Tube samples, Samples, Compensation samples, Groups, Experiments, or Plates.

- All relevant prompts occur before the deletion process begins.
- Click **Yes** to move the selected FCS files to the recycle bin and remove the items from the Experiment Explorer.
- Click **Cancel** or **X** to close the dialog box (if the last item in the list) without performing an action.

Do this for all other cases (x found) checkbox

- This checkbox appears only if multiple Experiments, multiple Groups, or multiple Samples are selected. It is unchecked by default.
- The warning displayed by the checkbox states "Do this for all other selected items (x found)", where x is the number of selections.
- If checked, all other selected items selected are subjected to the same command.
- If left unchecked, the dialog reappears for each selected item in the queue.

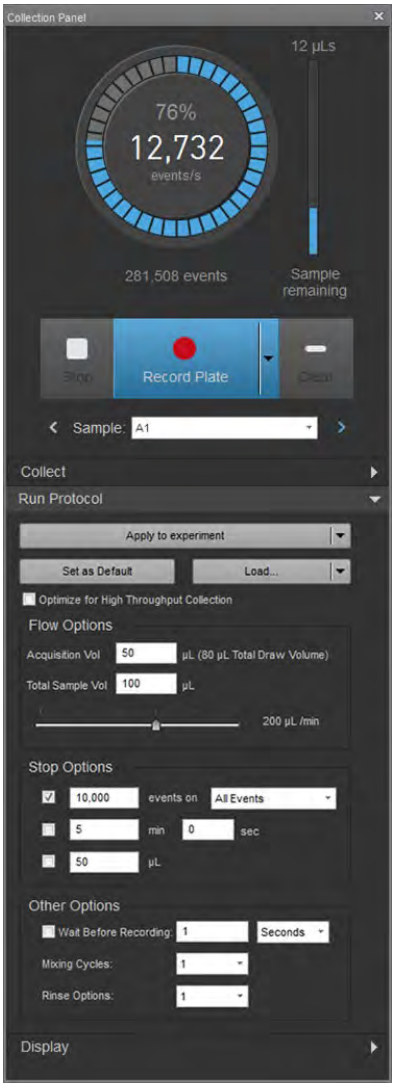
12. Collection panel

Overview

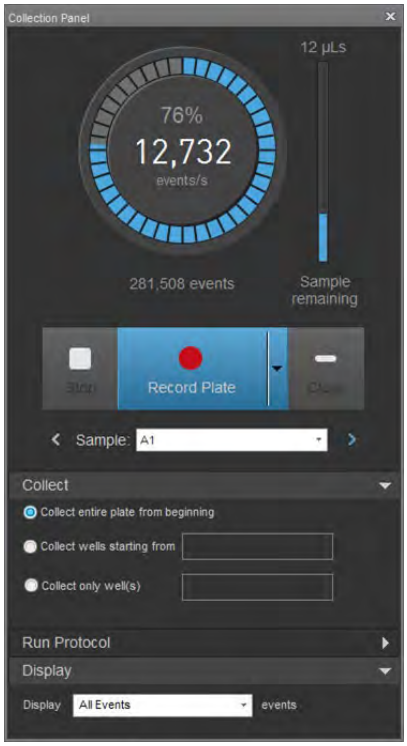
The Collection panel is used for the acquisition of samples. The appearance of the Collection panel varies depending on the sample source (i.e., Plate or Tube) and the setup of the Experiment. By default, it is docked to the left of the Main Application area and organized into four functional groups:

- *Acquisition status* (page 222)
- *Collection controls* (page 223)
- *Collect and Display options* (page 231)
- *Run Protocol* (page 238).

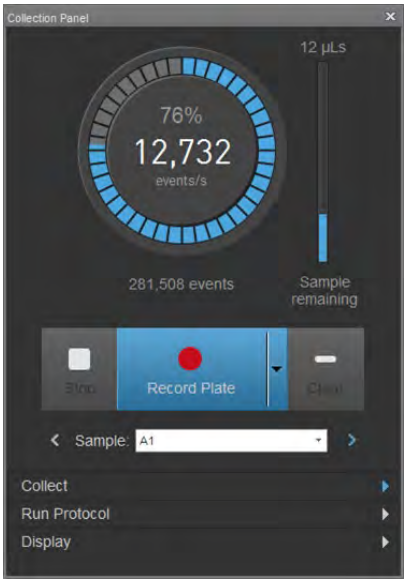
To expand or collapse the groups, click on the group header. By default, only the Run Protocol group is expanded.



Run Protocol expanded (Default)



Collect and Display expanded



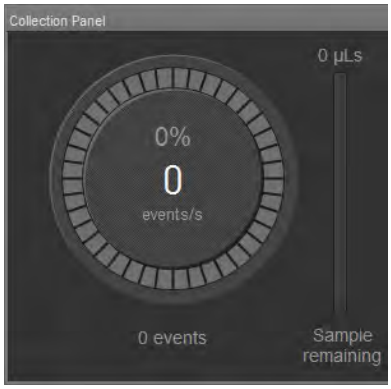
All collapsed

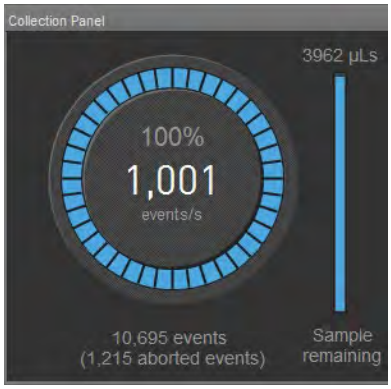
Note: Access to Collection panel features may be restricted based on account permissions as described in "Options dialog – User Management options" (page 508) for local Attune™ Users and SAE Administrator Console (see "Roles tab", page 581) for SAE Users.

Acquisition status

Acquisition status indicators

The *acquisition status indicators* display information about the current acquisition status of the instrument.





- **Events per second (events/s)** displays the number of events currently being collected per second. This field is set to zero and does not change unless the system is acquiring data.
- **Progress dial** displays the progress of the current acquisition related to stop conditions set in the **Stop options** (page 246). The **percent progress** is displayed inside the dial above the events per second field.
- **Events** displays the total number of events collected. This value is reset to zero at the start of an acquisition, the start of recording the current sample, when the **Clear** button is clicked, or if a gate is moved and the stop condition is set to stop on a certain number of gated events.
- **Aborted events** displays the total number of aborted events. This value is reset to zero at the start of an acquisition, at the start of recording, or when the clear button is clicked. It is only displayed if the **Exclude coincidence checkbox** is checked (page 259).
- If **Complete Stop Condition** is selected (page 237), the progress dial and the events field resume from where they left off.
- If multiple stop conditions are selected, the progress dial displays the condition that has progressed furthest towards being met.
- When a sample is run but not recorded, the progress dial does not increment.

Sample remaining indicator

- The **sample remaining indicator** displays the remaining volume of the current sample that is being acquired.
- The **progress bar** shows the amount of sample remaining in relation to the acquisition volume set as part of the Run Protocol.

Collection controls

Overview

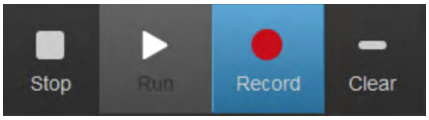
The *collection controls* allow you to prompt the Attune™ instrument to run your samples and record flow cytometric data.

- The collection controls are contextual; the options that are displayed depend on the tube or the type of well that is being acquired. Controls that are not available as a function in a specific context are not shown or shaded gray on the panel.
- If a plate is being acquired, the default controls are the *Plate Collection Controls* (see "Collection controls – Plate", page 226).
- If a tube is being acquired, only the *Tube Collection Controls* are available as described in "Collection controls – Tube and Manual wells", below.

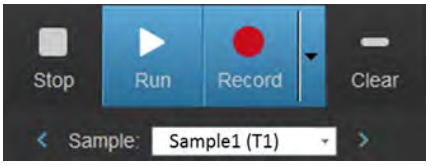
Collection controls – Tube and Manual wells

Overview of collection controls for Tube and Manual Well samples

The collection controls for Tube and Manual Well samples are similar. However, for Manual Well samples, the Run/Record split button has an additional acquisition task dropdown, and the collection controls include the Sample navigation and Sample dropdown buttons below the basic controls.



Collection controls for Tube samples



Collection controls for Manual Well samples

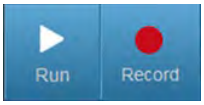
- **Run**, **Record**, **Stop**, and **Clear** buttons are the base set of buttons.
- For Manual Well samples, the **Run/Record** split button has an additional dropdown control that allows the navigation through acquisition tasks. The acquisition task dropdown is not available for Tube samples.
- When any button within the *Functions group* of the *Instrument tab* (page 62) is clicked, all plate collection control buttons are disabled and grayed out until the process is completed, except the navigation buttons, the acquisition task dropdown (for Manual Well samples only), and the Save button.
- **Run** and **Record** buttons are disabled for any active sample where the sample instrument settings do not match the system instrument configuration and for any experiment where the experiment instrument settings do not match the system instrument configuration (e.g., RB vs. RBVY).

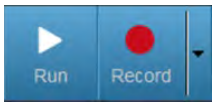
Buttons displayed

Collection controls for Tube samples are contextual; the buttons that are displayed depend on the acquisition task and the type of Sample being acquired. The collection control buttons that are available are described below.

Middle buttons

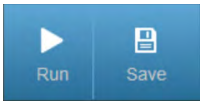
The buttons that occupy the middle section of the collection controls are used for running and recording Samples, and for saving data.

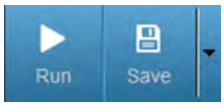




Run/Record: Clicking **Run** (on the left) starts the acquisition of the selected Sample; clicking **Record** (on the right) initiates the recording of the selected Sample.

- Run/Record split button is displayed by default when the system is ready for acquisition.
- The acquisition task dropdown is displayed only for Manual Well samples.

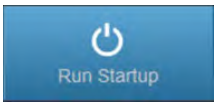




Run/Save: Clicking **Run** re-runs the selected Sample; clicking **Save** saves the data to the FCS file for the selected Sample. If the Sample has existing data, you can append or overwrite the existing data.

- Run/Save split button is displayed when a Run mode acquisition has been completed for a Manual Well (i.e., end of Sample is reached or **Stop** is clicked).
- The acquisition task dropdown is displayed only for Manual Well samples.

Note: The buffer size is determined by the number of events set in the Display Events group. By default, this is set to "All Events".



Run Startup: Initiates the *Startup* function.

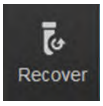
- Run Startup button is displayed if Startup function has not been run prior to running samples or an error state requires Startup to be performed.

Left buttons

The buttons that appear on the left side of the collection controls are used for stopping sample acquisition and for recovering unused samples.

Stop: Stops the acquisition or recording of the current sample immediately.

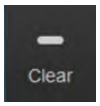
- Stop button is displayed by default when the system is in acquisition state (i.e., running or recording Sample) and while running the Startup function.



Recover: Recovers the unused sample to a tube.

- **Recover** button is displayed when the instrument is idle and there is a sample available to recover.
- Clicking **Recover** returns the unused sample to a tube.

Right button



Clear: Clears the most recent data in the memory (see below).

- Clear button is displayed by default when the system is in acquisition state. It is also available post-acquisition when an existing sample has been overwritten in the Run mode.
- Clicking **Clear** during acquisition clears the data in memory and displays fresh data.
- Clicking **Clear** post-acquisition discards the most recent data and retains the original data.

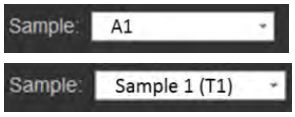
Navigation buttons and Sample dropdown

Previous: Allows navigation through previous tasks and through Samples pre- and post-acquisition.

- This navigation button is available for both well and Tube samples, and can navigate from well to Tube samples, and from tube to Well samples.
- The Previous button is active when the current sample is not the first sample in the task list. It is disabled during acquisition.
- The button tooltip for Well samples before recording has the following form: "<phase> (sample location)", where <phase> is Record Plate.
- Once the plate is recorded, the tooltip for the wells is: "<well location>". For example, "A1".
- The tooltip for Tube samples has the form: "<sample name> (tube location)", where (tube location>) is T1 through T399. For example, "Sample 1 (T1)".

Next: Allows navigation through samples pre- and post-acquisition, and through steps within each acquisition task.

- This navigation button is available for both well and Tube samples, and can navigate from well to Tube samples, and from tube to Well samples.
- The Next button is active when the current sample is not the last well of the plate. However, it is active for last Tube sample and creates a new Tube sample, if clicked. The button is disabled during acquisition.
- The button tooltip for Well samples before recording has the format: "<phase> (sample location)", where <phase> is Record Plate.
- Once the plate is recorded, the tooltip for the wells is: "<well location>". For example: "A1"
- The tooltip for Tube samples has the form: "<sample name> (tube location)", where (tube location) is T1 through T400. For example, "Sample 1 (T1)".
- If a tube new sample will be created by clicking **Next**, then the tooltip states: "New Sample".



Sample dropdown: Allows the selection of any sample within the current acquisition task. The dropdown is disabled during acquisition.

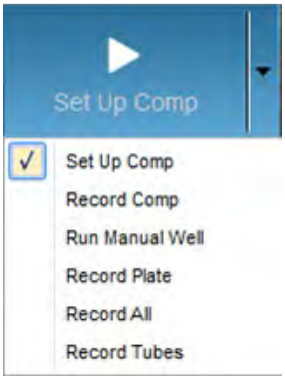
- For Sample wells, only the well location is displayed. For example, "A2".
- For Tube samples, the name will be: "<sample name> (tube location)". For example, "Sample 1 (T1)".

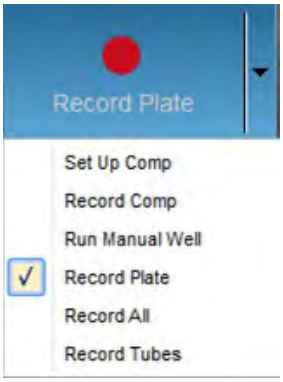
Collection controls – Plate

Overview of plate collection controls

Plate collection controls are displayed in an array of three buttons that execute the basic commands for acquisition. The Sample navigation and Sample dropdown buttons are arranged below the basic controls.

- The state and the label of the buttons depend on the setup of the Plate, specifically the presence of Compensation samples, Manual Wells, and Well samples.
- The *Setup*, *Run*, and *Record* buttons are disabled for:
  - Any active Sample where the Sample-level Instrument Settings do not match the System Instrument Configuration.
  - Any Experiment where the Experiment-level instrument settings do not match the System Instrument Configuration (e.g. RB vs RBVY).
- The processing of a Plate is divided into distinct tasks. The dropdown arrow adjacent to the center button opens the *acquisition task list*, which allows you to select an acquisition task to perform. The acquisition task list is available only for Plate samples.





The available tasks are:

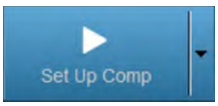
- Set-up Compensation
- Record Compensation for the current Experiment
- Run and/or Record Manual wells for the current Experiment
- Record Plate
- Record All
- Record Tubes

Buttons displayed

Plate collection control buttons and their functions are described in this section. The plate collection controls are contextual; the buttons that are displayed depend on the acquisition task and the type of well that is being acquired. Buttons not needed in a specific context are not shown or they are disabled and shaded gray.

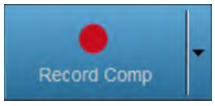
Middle buttons

The buttons that occupy the middle section of the collection controls are used for running and recording samples and saving data. The labels and the visibility and functionality of the buttons depend on the plate setup and acquisition context.



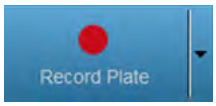
Set Up Comp: Initiates acquisition of compensation wells in the run mode.

- **Set Up Comp** button is displayed by default if the plate contains Compensation wells and a Compensation well is active. It is also displayed when **Set Up Comp** is selected from acquisition task menu.
- When the mouse pointer is hovered over the button, a tooltip states: "Allows optimization of Compensation control voltages".



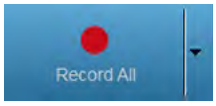
Record Comp: Initiates recording of data from all compensation wells defined in the "Collect options" (page 232).

- Record Comp button is displayed when **Record Comp** is selected from the acquisition task menu or after completing the Set Up Comp phase.



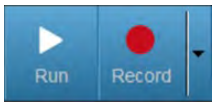
Record Plate: Initiates recording of data from all Sample wells defined in the "Collect options" (page 232).

- Record Plate button is visible and enabled by default when the system is ready for recording wells and the plate contains only Well samples.
- It is also displayed when **Record Plate** is selected from the acquisition task menu or after any compensation tasks have been completed.



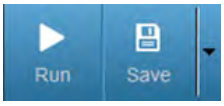
Record All: Opens the first Compensation control or Sample well within the active Plate Experiment, and automates the recording of all Compensation controls and Sample wells within the active Plate Experiment as defined in the "Collect options" (page 232).

- When **Record All** is selected, you cannot manually adjust voltage settings for the Compensation controls or Manual wells.



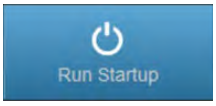
Run/Record: Clicking **Run** (on the left) runs the selected Sample; clicking **Record** (on the right) records the selected Sample.

- Run/Record split button is displayed for Tube samples, when a Manual well is selected, when **Run Manual Well** is selected from the acquisition task menu, or after the completion of any compensation tasks (if present).



Run/Save: Clicking **Run** re-runs the same Sample; clicking **Save** saves the data to the FCS file for the sample that has just been run. If the Sample has existing data, you can append or overwrite the existing data.

- Run/Save split button is displayed after running a Tube sample or a Manual well.

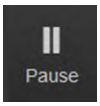


Run Startup: Initiates the *Startup* function.

- Run Startup button is displayed if the Startup function has not been run before running Samples or an error state requires Startup to be performed.

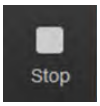
Left buttons

The buttons that appear on the left side of the collection controls are used for stopping and pausing sample acquisition, and for recovering pre-loaded samples. The visibility and functionality of the buttons depend on the acquisition context.



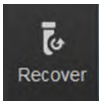
Pause: Pauses the acquisition or recording of the wells **after** the current well has finished. Pressing **Pause** while a well is being acquired does not temporarily stop the acquisition of the well.

- While recording the last sample the Pause button becomes a Stop button.



Stop: Stops the acquisition or recording of the wells immediately.

- Stop button is displayed by default during Compensation setup and while recording compensation. It is also available during Startup.
- While stopped, the Stopped button will be replaced with a Recover Sample button if there is sample to recover.

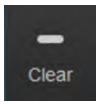


Recover: Recovers the unused sample to a tube or to a well, depending on when the **Stop** button was pressed.

- Recover button is displayed when the instrument is idle and there is sample available to recover.
- Clicking **Recover** returns a pre-loaded sample back to a well or the unused sample to a tube.

Right button

The right side of the collection controls only displays the Clear button.



Clear: Clears the data of the current sample and displays fresh data.

- Clear button is always displayed. It is enabled during running or recording of all samples and wells.

Navigation buttons

The Previous and Next navigation buttons are available for both Well and Tube samples and can navigate from Well to Tube samples and from Tube to Well samples.

Previous: Allows navigation through previous tasks and through Samples pre- and post-acquisition.

- The Previous button is active when the current sample is not the first sample in the task list. The Previous button is disabled during acquisition.
- The tooltip for Tube samples has the form: “<sample name> (tube location)”, where (tube location>) is T1 through T399. For example, “Sample 1 (T1)”.

Next: Allows navigation through Samples pre- and post-acquisition, and through steps within each acquisition task.

- The Next button is active when the current Sample is not the last well of the plate. However, it is active for last Tube sample and creates a new Tube sample, if clicked. The Next button is disabled during acquisition.
- After the Plate is recorded, the tooltip for the wells is: “<well location>”.
  For example: “A1”
- The tooltip for Tube samples has the form: “<sample name> (tube location)”, where (tube location) is T1 through T400. For example, “Sample 1 (T1)”.
- If a tube new Sample is created by clicking **Next**, then the tooltip states: “New Sample”.

Sample dropdown

Sample dropdown: Allows the selection of any sample within the current acquisition task. The dropdown is disabled during acquisition.

- For Sample wells, only the well location is displayed. For example, “A2”.
- For Tube samples, the name will be: “<sample name> (tube location)”.
  For example, “Sample 1 (T1)”.

Button availability

- The plate collection control buttons are contextual; the buttons that are displayed depend on the acquisition task and the type of well that is being acquired.
- Record Plate, Stop, and Clear buttons are the base set of buttons for plates.
- Buttons are grayed out when they are not available in a specific context.
- All plate collection control buttons are disabled if the instrument is not ready for acquisition, between wells, and after **Stop** has been clicked but the acquisition of the current sample has not been completed.
- When any button within the *Functions group* of the *Instrument tab* (page 62) is clicked, all plate collection control buttons are disabled and grayed out until the process is completed, except the navigation buttons, the acquisition task dropdown, and the Save button.
- A “Bubble detected error” does not change the enabled/disabled state of a button.

Current well indicator

- The location of the current well and the sample type that is being analyzed is displayed above the collection control buttons.
- The indicator has the format: <well location> (Compensation designation)

  For example:

  - A1 = sample in well A1
  - A1 (M) = sample in Manual well A1
  - A1 (UC) = Unstained Control sample in well A1
  - A1 (BL1) = BL1 Compensation sample in well A1

Set Up Compensation workflow

Click **Set Up Comp** to initiate the acquisition of Compensation wells.

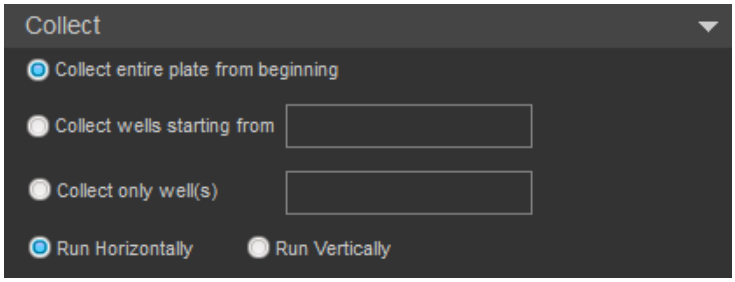
- The order in which Compensation controls are acquired from a plate is in the same order they are listed in the Experiment Explorer:

  Unstained control, Blue laser controls, Red laser controls, Violet laser controls, and Yellow laser controls.
- Acquisition of the Compensation control continues until either **Stop** is clicked or the acquisition volume as defined by the Run Protocol (page 238) is exhausted.
- The current well remains active until the navigation buttons are used to move onto the next well within the compensation setup task or a different task is selected from the acquisition task dropdown list.
- To initiate the acquisition of the next Compensation control, you must click **Set Up Comp** again.
- Clicking the **Next** button on the last sample within the compensation setup task advances the task button to **Record Comp**.

Collect and Display options

Collect group overview

Collect group allows you to control which wells are collected during acquisition. The options in this group are available only for Plate Experiments and are not displayed for Tube samples.



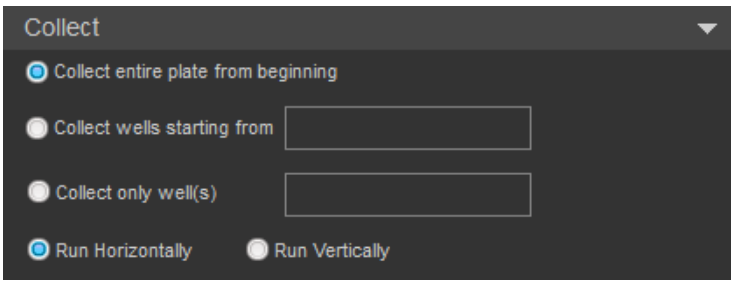
- Collect group contains three options for which wells to collect:
  - Collect entire plate from beginning
  - Collect wells starting from
  - Collect only well(s)

  In addition, there is a selection for the Run Order, which determines the order of collection (page 235).
- By default, the Collect group is collapsed with the "Collect entire plate from beginning" option selected.
- The display state of Collect options persists as part of the Experiment.
  Collect options are also saved when duplicating, exporting, or saving a Plate as a Template, or when creating a Plate from a Template.
- If a combination of Tube and Well samples is selected in the Heat Map or in the Experiment Explorer, the options in the Collect group are disabled.
- Wells can be selected in the Heat Map to populate the "Collect wells starting from" text box.
- If the Collection panel is in the Resume state and the Collect options are modified, the Collection panel will be reset to the pre-acquisition state.
- The order of collection is based on the selected **Run Order** option (page 235).

Collect options

Collect entire plate from beginning

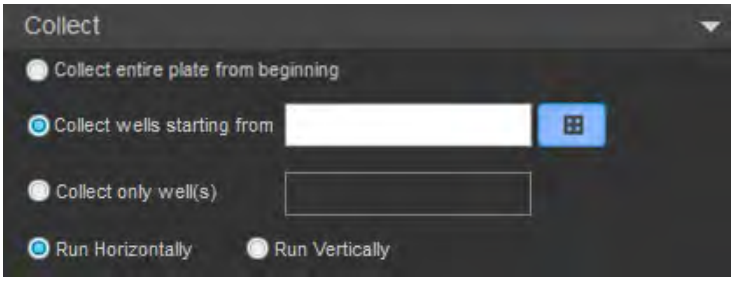
Collect entire plate from beginning collects all samples on a plate.



- This option is only available for Plate Experiments, and it is the default option.
- If a plate layout is specified, acquisition starts at well A1 and proceeds in the selected Run Order (page 235).
- The buttons that occupy the middle section of the collection controls are used for running and recording samples, and for saving data (see page 227).

Collect wells starting from

Collect wells starting from collects all samples starting at the specified well.



- To specify the starting well, enter its location in the textbox field. The entry format is the row letter followed by the column number (e.g., A1). Any letter typed is automatically capitalized.
- Alternatively, you can specify the starting well directly from the Heat Map by selecting the Heat Map interaction control (page 234) to the right of the textbox.

  When the Heat Map interaction control is toggled on, the Heat Map of the Plate Experiment is displayed (if not already in view), allowing you to select the starting well directly from the Heat Map.

  When the starting well is selected on the Heat Map, the “Collect wells starting from” textbox automatically updates to display the “starting from” location.

  When the Heat Map interaction control is toggled off, the selection on the Heat Map does not update the textbox contents.

- The well location entered must be a valid location and entered in the correct format. If you make an invalid entry, a warning describes the invalid entry, and the acquisition control buttons are disabled until the invalid entry is corrected. The acquisition button status indicator updates according to the well type selected.

- Invalid entries include using incorrect format (e.g., 1A), specifying a location not mapped to the selected well, entering a location not on the selected plate type (e.g., H13 on a 96-well plate), using a character that is not alphanumeric.
- Select the order of collection using the **Run Order** options (page 235).

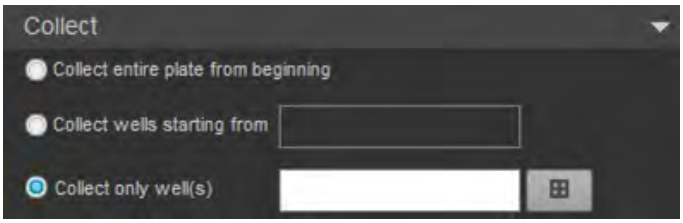
When **Run Vertically** is selected, the wells are processed from the top of the plate to the bottom of the plate, and left to right, where the collection boundary on the left is the column selected as the **Collect wells starting from**.

For example, if you select D3 as Collect all wells starting from, then E–H 1–2 are not collected, because column 3 defines the left most collection boundary.

- If a collect plate sequence is stopped, the location is automatically updated to the next well to run.

Collect only well(s)

The **Collect only well(s)** option allows collection from the specified wells.



- To specify the wells you wish to collect, enter their location in the textbox field.

  The entry format is one or more well locations separated by a comma or a hyphen. A well location comprises a row letter followed by one or two digits for the column number (e.g., B11). Any letter typed is automatically capitalized. You can use commas and hyphens for multiple entries (see below), but all other none-alphanumeric characters are invalid.

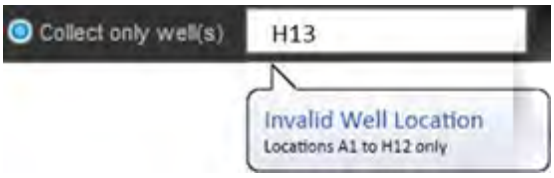
- Alternatively, you can specify the Wells you wish to collect directly from the Heat Map by selecting the Heat Map interaction control (page 234) to the right of the textbox.

  When the Heat Map interaction control is toggled on, the Heat Map of the Plate Experiment is displayed (if not already in view), allowing you to select the wells you wish to collect directly from the Heat Map as an alternative to entering the well location in the textbox.

To select multiple wells, click and drag the wells on the Heat Map or press the **Ctrl** key while clicking to select non-adjacent wells.

The "Collect only well(s)" textbox is automatically updated to reflect the selected wells.

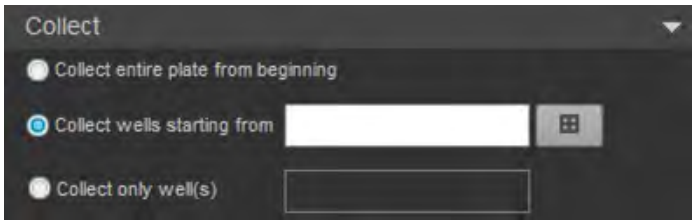
- You can enter multiple wells by using a hyphen to indicate a range of wells and a comma to separate individual wells and ranges. White spaces are ignored. For example: A1, B1– C12, D3.
- Where a range spans multiple rows, it is interpreted as including all wells in between. For example: B2– C6 on a 96-well plate is interpreted as comprising the wells B2 to B12, C1 to C6, inclusive.
- The well location entered must be a valid location and entered in the correct format. If you make an invalid entry, a warning describes the invalid entry, and the acquisition control buttons are disabled until the invalid entry is corrected. The acquisition button status indicator updates according to the well type selected.



- Invalid entries include using incorrect format (e.g., 1A), specifying a location not mapped to the selected well, entering a location not on the selected plate type (e.g., H13 on a 96-well plate), using a character that is not alphanumeric.
- The order of collection is based on the order of entry of well locations. All ranges are processed left to right, then top to bottom.
- When **Collect only well(s)** is selected, the Run Order options are disabled and the wells are collected in the order they are specified in the edit control, where all ranges are processed left to right.

Heat Map Interaction

When the **Collect well starting from** or the **Collect only well(s)** option is selected, a Heat Map interaction control becomes visible.



- When the **Heat Map interaction** button is clicked, the Heat Map is brought to the foreground.
- The textbox updates based on the wells that are selected on the Heat Map.
- For the Collect wells starting from option, you can select only a single well. Each time the Heat Map is clicked, the selected well is updated.
- For the Collect only well(s) option, you can select multiple wells by clicking and dragging to highlight the wells on the Heat Map or by pressing the **Ctrl** key while clicking to select non-adjacent wells.

Run Order

The Run Order specifies the order in which the wells are collected.

- When **Run Horizontally** is selected, wells are processed from left to right, row by row.
- When **Run Vertically** is selected, wells are processed from top to bottom, column by column.

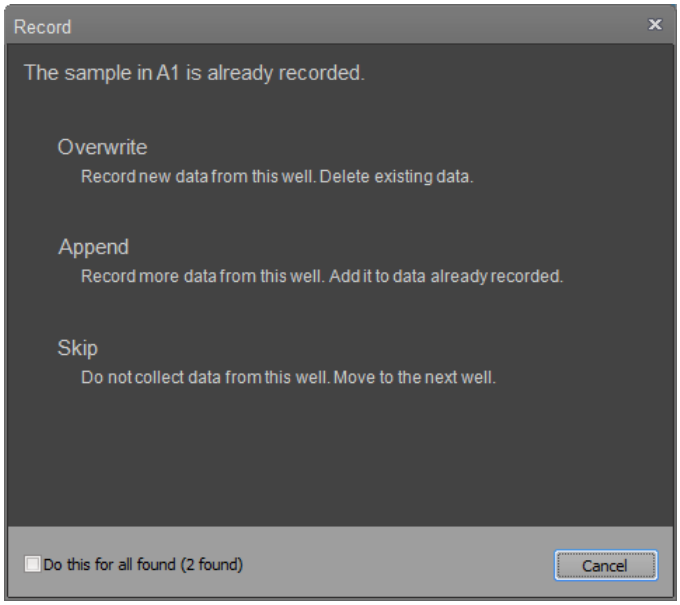
  When **Collect wells starting from** is selected, the wells before the starting well are not collected and the well selected as the starting well defines the boundary column or row, depending on the Run Order selected.

  For example, for a plate with samples in A1–B12 with A3 as the starting well (Collect wells starting from: A3), selecting Run Horizontally results in wells A3–B12 being collected, whereas selecting Run Vertically results in wells A3–A12 and B3–B12 being collected.
- This setting only applies to the **Collect entire plate from beginnin**g or **Collect wells starting from** options.
- When the **Collect only well(s)** is selected, the Run Order options are disabled and the wells are collected in the order they are specified in the edit control, where all ranges are processed left to right.
- Changing the collect options does not change the selected Run Order.

Record and Save dialogs

Record dialog for Plate sample well

When **Record** is selected for a Plate sample well that has already been collected, then the *Plate Record dialog* is displayed.



The dialog heading indicates the well location and the dialog offers the following clickable options:

- **Overwrite:** Discards the data already stored in the FCS file and collects new data. Only new data is used for determining the stop condition.
- **Append:** Appends the new data to the data already stored in the FCS file. Only the new data is included in stop condition determination.

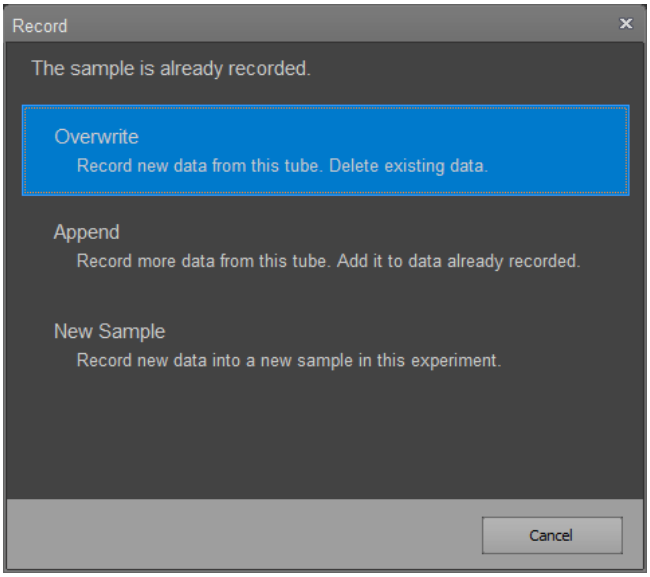
  The Append option is not available if the existing data file was generated in another instrument or using a different laser configuration.
- **Skip:** Skips the well without recording.
- **Do this for all found:** Applies the selection to the indicated well and all the subsequent wells in the list that have already been collected. Otherwise the selection only applies to the well indicated in the dialog header. If there are no further wells for selection, then the next selection closes this dialog.
- **Cancel:** Closes the dialog with no action taken and the collection does not occur.

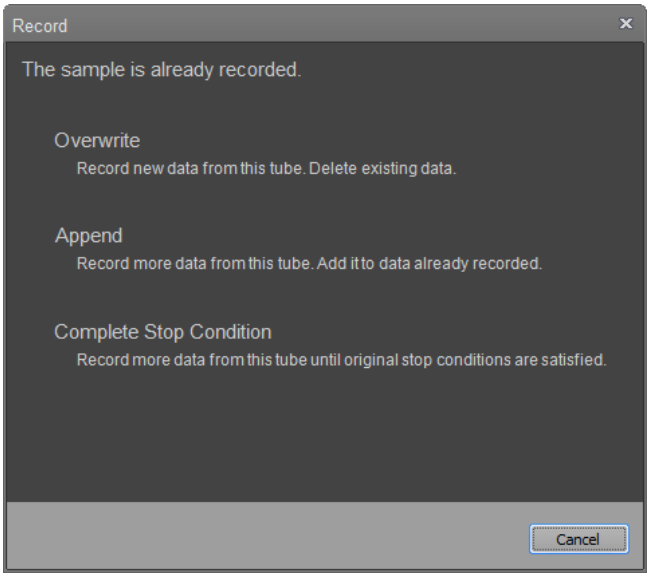
Note: The Run protocol for the Sample cannot be changed when overwriting, appending data, or skipping the Sample using this dialog.

Record/Save dialogs for Tube sample

When **Record** or **Save** is selected for a Tube sample that already has saved data associated with it, then one of the two dialogs is displayed with options to overwrite, append, complete stop condition, new sample, or cancel.

The dialog displayed depends on the user's actions and the stop conditions. If the stop condition has already been met, the dialog on the left is displayed. If the stop condition has not yet been met, then the dialog on the right is displayed.





- **Overwrite:** Discards the data already stored in the FCS file and collects new data. Only new data is used for determining the stop condition.
- **Append:** Appends the new data to the data already stored in the FCS file. Only the new data is included in stop condition determination.

  The Append option is not available if the existing data file was generated in another instrument or using a different laser configuration.
- **New Sample:** Selecting this option creates a new sample in the current experiment. Data is collected for that sample using the default settings.

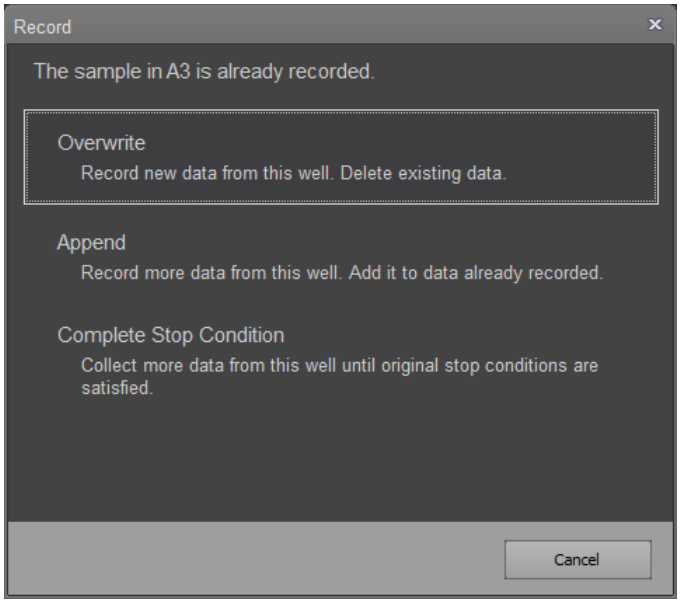
  The New Sample option is enabled when "Complete stop option" is not available. It is disabled on Compensation control samples.
- **Complete Stop Condition:** Appends the new data to the data already stored in the FCS file. All data is included in stop condition determination.

  The Complete Stop condition option is available only if the stop condition has not already been met, the recording was not manually stopped, and the same sample is still being run (i.e., samples have not been switched).
- **Cancel:** Closes the dialog and leaves the operation in the Run mode.

Note: The Run protocol for the Sample cannot be changed when overwriting, appending data, or skipping the Sample using this dialog.

Record dialog for Manual well

When **Record** is selected for a Sample well that has already been collected, then the *Plate Record dialog* is displayed.



The dialog heading indicates the well location and the dialog offers the following clickable options:

- **Overwrite:** Discards the data already stored in the FCS file and collects new data. Only new data will be used for determining the stop condition.
- **Append:** Appends the new data to the data already stored in the FCS file. Only the new data is included in stop condition determination.

  This option is not available if the existing data file was generated in another instrument or using a different laser configuration.
- **Complete Stop Condition:** Appends the new data to the data already stored in the FCS file. All data is included in stop condition determination.

  This option is available only if the stop condition has not already been met, the recording was not manually stopped, and the same sample is still being run (i.e., samples have not been switched).

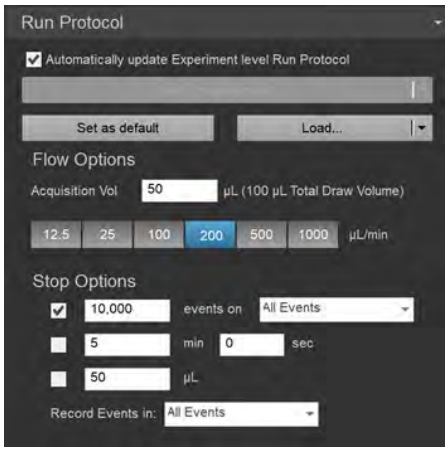
  This option is not available if the existing data file was generated in another instrument or using a different laser configuration.
- **Cancel:** Closes the dialog with no action taken and the collection will not occur.

Note: The Run protocol for the Sample cannot be changed when overwriting, appending data, or skipping the Sample using this dialog.

Run Protocol

Overview *Run Protocol* group allows you to define the collection criteria, including the Recording and Stop options. It is also used for determining Compensation Setup options, when available. By default, the Run Protocol group is expanded.

The options displayed in this group depend on the type of sample being run and the mode of collection. The examples below show Run Protocol options for Tubes (left), Normal and Manual wells (middle), and Compensation wells (right).



Tube

Normal and Manual well

Compensation well

- Any changes made in the Run Protocol automatically apply to all active Samples, if the "Automatically update Experiment level Run Protocol" checkbox (page 240) is checked.
- If the "Automatically update Experiment level Run Protocol" checkbox is unchecked, any changes made only apply to the active Sample.
- The Run Protocols for Samples with recorded data remain unchanged. Samples with recorded data get their own Run Protocols that are not linked to the Experiment Run Protocol.

Settings buttons

Settings buttons consist of *Apply to Experiment/Group* split button, *Set as default* button, and the *Load/Export* split button. When Run Protocol settings are modified, the changes are applied to the active Sample automatically. These buttons allow you apply the settings to other members of the current Group or Experiment.

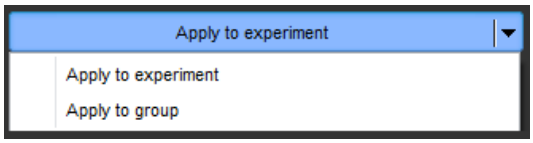
Automatically update Experiment level Run Protocol

When checked, this option automatically applies the changes made in Run Protocol settings to all Samples in the Experiment that do not contain data and to all new Samples.

- By default, the "Automatically update Experiment level Run Protocol" is unchecked. Its checked/unchecked state persists as part of the user's settings.
- If the active Sample has an associated FCS file and the Run Protocol does not match the Experiment level Run Protocol, this checkbox is disabled.
- The "Automatically update Experiment level Run Protocol" option does not apply to Compensation Controls and is disabled when a Compensation Control is active

Apply to Experiment/Group split button

The main part of the split button contains the *Apply to Experiment* option. Clicking the dropdown arrow adjacent to the main button displays the *Apply to Group* option.

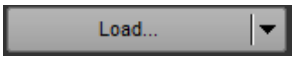


- Click **Apply to Experiment** on the main button to apply the current Run Protocol to all Samples in the current Experiment. Samples that do not have associated FCS files are updated to use the Experiment level Run Protocol.
- Select **Apply to Group** from the dropdown to apply the current Run Protocol to all Samples of the selected Group.
- When the "Automatically update Experiment level Run Protocol" is selected, this split button is disabled.
- These options are disabled if a Compensation Control is active.

Set as Default

Set as Default sets the current Run Protocol to be used by default on all future Plate or Tube Experiments run by the current user.

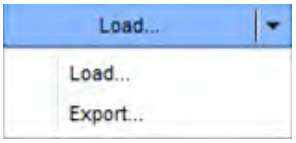
Load/Export split button



- **Load** button opens the *File Open (Import)* dialog (page 535).

  Using the File Open (Import) dialog, you can select a Run Protocol from a saved Run Protocol file (*.arp).

  For a sample with FCS data selecting the load option has no effect and does not launch the database browser.



- Click on the **arrow** to display the dropdown list with the Load and Export options.
- **Export** option opens the *File Save (Export)* dialog (page 532), which allows you to export and save the current Run Protocol with a user defined name as a Run Protocol file (*.arp).

Optimize for high throughput collection

Optimize for high throughput collection option optimizes the Run Protocol for high-throughput data collection.

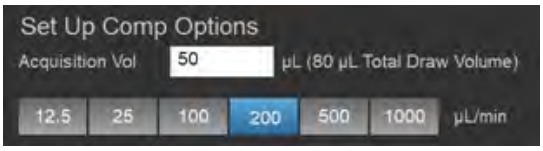
- This option is available only when an autosampler is connected to the Attune™ instrument.
- The Optimize for High-Throughput Collection option is visible and enabled only for Plate collection (Normal well). It is visible but disabled for wells marked as Manual wells.
- When selected, the **Optimize for High-Throughput Collection** option optimizes the Run protocol for high-throughput data collection as follows:

Run protocol parameter	Attune™ NxT Auto Sampler and CytKick™ Autosampler	CytKick™ Max Autosampler
Stop volume	40 µL	20 µL
Acquisition volume	40 µL	20 µL
Flow rate	500 µL/minute	1000 µL/minute
Mixing cycles	1	1
Rinse between samples	1	1
Wait before recording	Unchecked	Unchecked

- If you manually change any of these settings, the checkbox is unchecked.
- If you manually adjust all settings to the conditions shown above, the checkbox becomes checked.

Set Up Comp options

Set Up Comp options are displayed only when a Compensation well is selected. The criteria defined here are used during the set-up of Compensation wells.



Acquisition volume

- *Acquisition volume* option allows you to specify the volume of sample that can be used during acquisition of a Compensation well in the Setup mode.
- The default Acquisition volume is 50 µL.
- The number in the Acquisition volume is validated on entry and must be an integer. If the number entered is outside the allowed range, it will be automatically adjusted to the nearest allowable number.
- The default, minimum and maximum values are dependent on the type of plate being used.
- The total draw volume (acquisition volume + dead volume) is displayed next to the acquisition volume field. This is total volume of sample drawn from the well and includes the dead volume.
- For Compensation samples, the sum of the total draw volumes for the Setup Flow options' and Recording options' acquisition volume cannot exceed the total allowable acquisition volume for the well. These limits are calculated by the software and the number entered is automatically adjusted to the maximum allowable number.

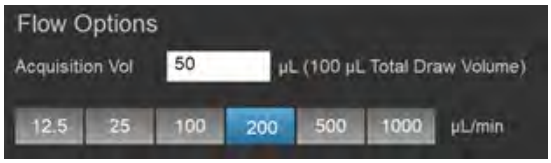
Sample flow rate

The *Sample Flow Rate* option controls the rate of delivery of the sample during the acquisition of Compensation samples in the Run mode.

- The flow rate values are set using the flow rate buttons. Available flow rates are:
  - 1000 µL/minute
  - 500 µL/minute
  - 200 µL/minute
  - 100 µL/minute
  - 25 µL/minute
  - 12.5 µL/minute.
- The default flow rate is 200 µL/minute.

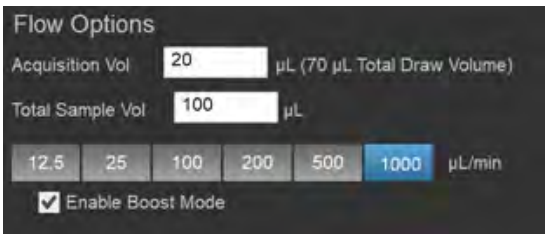
Flow/Recording Flow options

Flow/Recording Flow options are displayed for all sample types (Tube or Well samples). These settings are used when collecting data from these samples.



Tube Flow options

Well Flow options



High-Throughput Flow options

Recording Flow options
(for Compensation wells only)

Note: For Tubes and Manual wells, the group title displays *Flow options*. For Compensation wells, the group title displays *Recording Flow options*.

Acquisition Volume

- **Acquisition Volume** allows you to specify the volume of sample that can be used during the acquisition of the well or the tube.

- The default Acquisition volume is 50 µL.
- The number is validated on entry for all fields and must be an integer.
- The total draw volume (acquisition volume + dead volume) is displayed next to the acquisition volume field.
- When the acquisition volume is changed and **Automatically update Experiment level Run Protocol** or **Apply to Experiment** is selected, the changes only apply to Samples of the same type (such as wells or tube samples).

Note: The sample dead volume from wells is 30 µL for sample flow rates of up to 500 µL/minute and 50 µL for sample flow rates of 1000 µL/minute.

Total Sample Volume

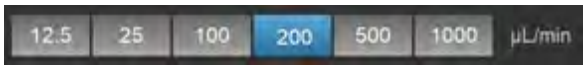
- *Total Sample Volume* field is only displayed for Plate samples. It allows you to specify the amount of sample present in the selected well of a plate.

- The total draw volume auto-populates the total sample volume field, but you can edit the volume to be any number greater than the total draw volume.
- If the total sample volume field already contains a value greater than the total draw volume, the total draw volume field does not update this field.
- For Compensation wells, the sum of the volumes entered into the Acquisition volume text boxes in Setup Flow options and Record Flow options **cannot exceed the total allowable acquisition volume** for the well. These limits are calculated by the software and the number entered is automatically adjusted to the maximum allowable number.

Note: Setting the Total Sample Volume correctly ensures that Well samples are mixed adequately.

Sample flow rate

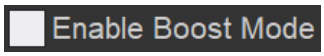
- Sample Flow Rate buttons control the rate of delivery of the sample during acquisition of Sample wells and Tube samples in the Record mode.



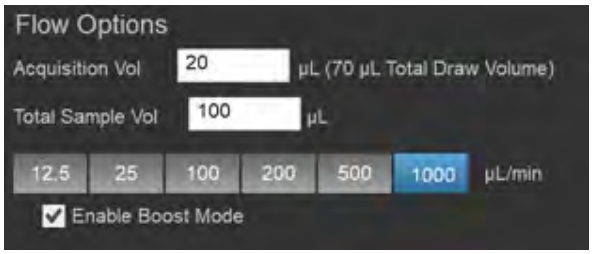
- You can select the following Sample flow rates:
  - 1000 µL/minute
  - 500 µL/minute
  - 200 µL/minute
  - 100 µL/minute
  - 25 µL/minute
  - 12.5 µL/minute.
- The default flow rate is 200 µL/minute.
- The sample dead volume from tubes is 50 µL for flow rates up to 200 µL/minute; 60 µL for 500 µL/minute, and 75 µL for 1000 µL/minute.

Enable Boost Mode

Enable Boost Mode allows the processing of the Samples with reduced boost volume for high-throughput acquisition, which reduces the processing time for a standard 96-well plate from 45 minutes to 22 minutes.



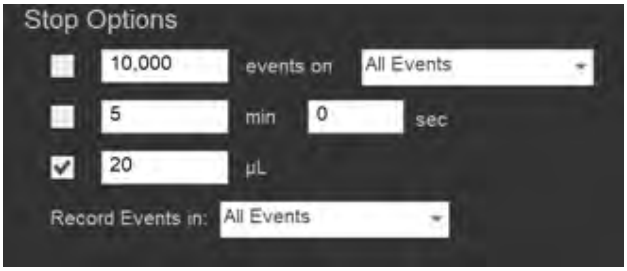
- The Enable Boost Mode option is available only when a CytKick™ Max Autosampler is connected to the Attune™ instrument.
- When available, you can set the **Enable Boost Mode** in the **Collection ▶ Run protocol** panel under **Flow options**.



- The Enable Boost Mode option is visible only for Normal wells and Compensation wells in Plate Experiments. However, it is enabled only when the flow rate is set to 500 µL/minute or 1000 µL/minute.
- The Enable Boost Mode option is not available for Manual wells or Tube samples.

Stop options

Stop options allow you to specify when the collection of data will end. If multiple conditions are selected, the acquisition ends when any one of the selected stop conditions are met. If no option is selected, the sample will continue recording until the acquisition volume is exhausted.



Event count stop

Event count stop is used for stopping acquisition when the specified number of events has been collected within the specified gate.

- The number field allows you to enter a number between 1 and 20,000,000. The default value is 10,000. The number is validated on entry and must be an integer.
- The dropdown list displays all gates in the currently active Workspace for tubes or the Experiment Workspace for plates. An additional option, *All Events*, is always available and is the default selection.
- The following actions can result in recounting the data or changes in Event count stop.
  - If the stop gate or a dependent gate (i.e., a gate hierarchically upstream of the stop gate) is moved during acquisition, then all data is recounted. This does not occur during actual gate movement but on release of the gate to a new position.
  - If the combination of gates in the hierarchy on which the stop gate is dependent is changed during acquisition, then all data is recounted.
  - Renaming the stop gate or a dependent gate does not alter the event count or the stop condition. If the stop gate is renamed, the Events dropdown is updated to show the new name when the new name is validated.
  - If the stop gate is deleted, then “All Events” option is selected and the stop option remains checked. If a dependent gate is deleted, then the stop option remains unchanged. In both cases, the data will be recounted.
  - If a stop gate is un-deleted as part of an undo operation, then the previous state of the Event count stop option is restored. If a stop gate or a dependent gate is un-deleted, then the data is recounted.
- If recounting the data results in the stop condition being met, then the recording stops, all data is displayed, and the statistics are back-tracked to the stop event that caused the stop condition to be reached.

Time stop

Time stop is used for stopping acquisition when the specified time has elapsed.

- You can enter a number between 0 and 59 into the **minutes** and **seconds** fields. The number is validated on entry and must be an integer. If a number higher than 59 is entered, it is automatically adjusted to 59.
- The default stop time is 5 minutes 0 seconds.

Volume stop

Volume stop allows you to specify a delivered volume at which acquisition will terminate.

- The allowable range depends on the sample source, plate or tube. The number is validated on entry and must be an integer.
- If a number higher than the allowable maximum is entered, the entry is automatically adjusted. The minimum value is 1.
- For a plate, the maximum value is the entered acquisition volume.
- For a tube the maximum value is 4,000 µL.
- The default stop volume is 50 µL.

Record Events in:

Record Events in allows you to set a gate where only the events that are within that gate at the end of the Sample recording are saved in the FCS file.

- The storage gate dropdown displays a list of all gates on the Experiment-level Workspace.
- By default, All Events is selected.
- The specified gate persists as part of the Run Protocol settings.
- As the data is acquired, **all events are displayed** but only the events within the specified gate are saved (data exclusion only occurs on the writing of the FCS file).

Other options

Other options contain *Wait Before Recording*, *Mixing Cycles*, and *Rinse Options*. These options are visible when at least one well is selected.

Wait Before Recording

Wait Before Recording allows you to set a delay for the start of acquisition.

- When this option is enabled, the data at the start of acquisition is discarded. After the wait period or once the events count has been exceeded, data collection resumes.
- The dropdown list allows you to select Seconds, Events, or Volume (µL) to be the deciding factor for the start of recording the data.
- You can enter a number between 0 and 1,000,000 for Events, 0 to 30 seconds for Time, or 0 to 100 µL for Volume.
- By default, this option is unchecked and set at 1 second.

Mixing Cycles

Mixing Cycles allows you to select the number of mix cycles to perform on the selected wells. This option is not displayed for Tube samples.

- The dropdown list allows you to select between 0 and 10 mixing cycles. The default value is 1.
- The recommended number of mixing cycles is two or less to minimize the potential for creating bubbles or froth from over-mixing the samples.

Rinse Options

Rinse Options allows you to select the number of rinse cycles to perform between Well samples. This option is not displayed for Tube samples.

- The dropdown list allows you to select between 0 and 10 rinses. The default setting is 1.

Mix Mode

Mix Mode allows you to select between **Standard** and **Gentle** mix modes.

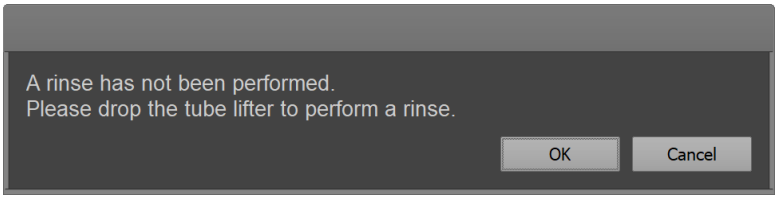
- The Mix Mode selection is available only when a CytKick™ Max Autosampler is connected to the Attune™ instrument.
- When available, you can set the **Mix Mode** in the **Collection ▶ Run protocol** panel under **Other options**.

- The Mix Mode option is available only when a Plate well is selected. It is not available for Tube samples.
- When the option is set as **Standard**, the Sample is mixed using the normal mixing speed.
- When the option is set to **Gentle**, the Sample is mixed and aspirated at a slower speed.

Note: For fragile cells, viscous samples, or samples prepared in viscous buffers, use the **Gentle** mix mode.

Rinse reminder dialog

Rinse reminder dialog is displayed if you have acquired a sample from a tube and have not dropped the tube lifter to initiate an automatic rinse within 3 minutes of sample acquisition.

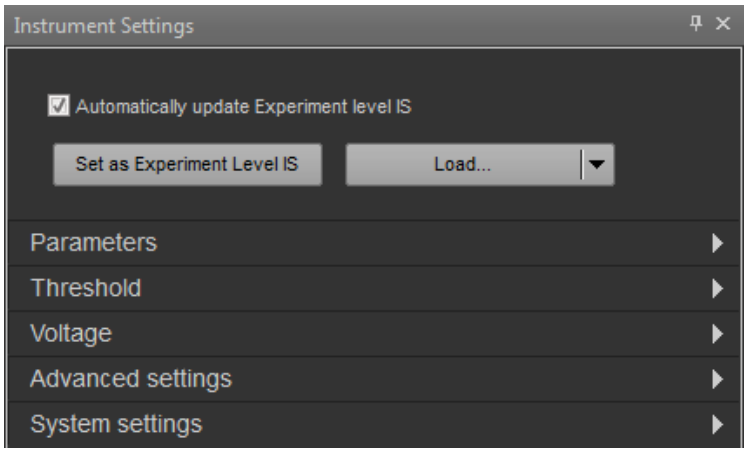


- Click **OK** to close the dialog. The dialog is presented again after another 3 minutes if the tube lifter was not dropped.
- Drop the tube lifter to automatically close the dialog and perform an automatic rinse.
- If you run another acquisition from the same tube within the 3 minutes, the timer is stopped and reset at the end of acquisition.

13. Instrument Settings panel

Overview

Instrument Settings panel allows you to edit acquisition parameters, create custom parameters, define threshold and voltage settings, and edit advanced instrument settings. By default, it is docked to the left of the Main Application area (page 36).



The Instrument Settings panel is organized into six functional groups in the following order:

- *Default and Load options* (page 251)
- *Parameters* (page 253)
- *Threshold* (page 257)
- *Voltage* (page 260)
- *Advanced settings* (page 262)
- *System settings* (page 265)

The Instrument Settings options are contextual; controls that are not available in a specific context are not shown or shaded gray on the panel.

General panel properties

- The docking properties of the Instrument Settings panel are described on page 37.
- If the instrument settings for the active sample do not match the system instrument configuration, the instrument settings panel displays the following message:

  "Instrument settings cannot be displayed for the selected sample. The instrument configuration is not supported".
- The same warning message is displayed for any Experiment where the instrument settings for the Experiment do not match the system instrument configuration (e.g., RB vs. RBVY). Contents of other instrument settings panels are not displayed.
- Typing into the textbox and pressing **Enter** updates the value in the textbox. Pressing **Tab** updates and moves to the next item specified.

Default and Load options

Overview

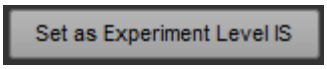
Default and *Load options* consist of the three controls that lie at the top of the Instrument Settings panel. They allow automatic updating of Experiment-level instrument settings, one-time updating of Experiment-level instrument settings, and saving of instrument settings as default.

Automatic update option

Automatically update experiment instrument settings checkbox enables automatic update of Experiment-level instrument settings to the previously recorded Sample-level instrument settings for each Sample when instrument settings are changed.

- The checkbox works for Tube samples only, and it is checked by default.
- Compensation samples (Tube or Well) always update the Experiment-level instrument settings. When Compensation samples are active, the checkbox is automatically checked and disabled (only when running compensation).
- Behavior, if the checkbox is checked:
  - If the instrument settings are modified, the Experiment-level instrument settings are automatically updated.
  - If the instrument settings are modified on subsequent Samples, the Experiment-level instrument settings are automatically updated.
  - If any Sample has recorded data, modification of the Experiment-level instrument settings result in the recorded Samples displaying the Instrument Settings badge (IS), which indicates that the Sample now has Sample-level instrument settings independent of the Experiment. The Sample-level instrument settings reflect the instrument settings used at the time the Sample was recorded
  - Changes to Instrument Settings after a Sample has been recorded that alters the state of the IS badge is a change to the Parameter label (target and label) after the Sample is recorded.
- Behavior, if the checkbox is unchecked:
  - If the active Sample's instrument settings are modified, the modified settings do not update the Experiment-level IS. Any additional changes to the instrument settings only apply to the active Sample.
  - The Sample displays the Instrument Settings badge (IS), which indicates that a Sample-level instrument settings has been created independent of the Experiment-level instrument settings.
  - When the checkbox is unchecked, any modification of the instrument settings of the active Sample only affect the active Sample.
  - If a Sample-level instrument settings is created for a Sample, the checkbox is unchecked and disabled until the Sample's settings are applied to the Experiment either by using the "Set as Experiment Level IS" button or by dragging the IS badge to the Experiment level.

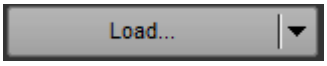
Set as Experiment IS



- Clicking **Set as Experiment Level IS** for Tubes saves the Instrument Settings of the current active Sample as the Experiment-level Instrument Settings.
- Experiment-level Instrument Settings are indicated by the IS badge (IS), which is displayed to the right of the Experiment name within the Experiment Explorer.
- Subsequent Samples in this Experiment will have these Instrument Settings as default.
- If the Experiment contains Samples with recorded data, those Samples display the IS badge

Save as Default split button

- The *Save as Default button* allows the Instrument Settings to be saved as a default.
- The split button is disabled during tube acquisition, well acquisition, or while processing a plate.
- **Save as Default:** Saves all changes to the Instrument Settings as default Instrument Settings to be used for all new Experiments. This includes all enabled and selected parameters and measurements, targets, fluorophores, thresholds, voltages, custom parameters, and advanced settings.
- **Load:** Opens the *File browser* dialog (page 537).

  Using the File browser dialog, you can select an Instrument Settings file (*.ais) from a saved location

  The Load button is available only when the Experiment does not contain data.
- **Export:** Opens the *File Save (Export) dialog* (page 532).

  Using the File Save (Export) dialog, you can save the current Instrument Settings with a user-defined name in the specified directory.

  You will be prompted for confirmation before overwriting an existing Instrument Settings file.

Parameters

Overview

Parameters group allows you to select the parameters to be recorded within the Experiment, based on the selected instrument configuration.

- Parameters group contains the following controls:
  - *Configuration dropdown* (page 254)
  - *Parameter selector checkboxes* (page 255)
  - *Target combo box* (page 256)
  - *Fluorophore combo box* (page 256)
- Parameters are listed in the following order:
  - Scatter channels (FSC, SSC)
  - Blue laser channels (BL)
  - Red laser channels (RL)
  - Violet laser channels (VL)
  - Yellow laser channels (YL)
- Only lasers and channels within the installed laser configuration are available in the Parameters options. The list for possible configurations and their respective laser and channel order is shown in the following table.

B	BR	BY	BG	BV	BV6	BRY	BRG	BVY	BVG	BRV	BRV6	BRVY	BRVG	BRV6Y
FSC of B	FSC of B	FSC of B	FSC of B	FSC of B	FSC of B	FSC of B	FSC of B	FSC of B	FSC of B	FSC of B	FSC of B	FSC of B	FSC of B	FSC of B
SSC of B	SSC of B	SSC of B	SSC of B	SSC of B	SSC of B	SSC of B	SSC of B	SSC of B	SSC of B	SSC of B	SSC of B	SSC of B	SSC of B	SSC of B
BL1	BL1	BL1	BL1	BL1	BL1	BL1	BL1	BL1	BL1	BL1	BL1	BL1	BL1	BL1
BL2	BL2	BL2	BL2	BL2	BL2	BL2	BL2	BL2	BL2	BL2	BL2	BL2	BL2	BL2
BL3	BL3	BL3	BL3	BL3	BL3	BL3	BL3	BL3	BL3	BL3	BL3	BL3	BL3	RL1
BL4	BL4	YL1	GL1	BL4	VL1	RL1	RL1	VL1	VL1	BL4	RL1	RL1	RL1	RL2
	RL1	YL2	GL2	VL1	VL2	RL2	RL2	VL2	VL2	RL1	RL2	RL2	RL2	RL3
	RL2	YL3	GL3	VL2	VL3	RL3	RL3	VL3	VL3	RL2	RL3	RL3	RL3	VL1
	RL3	YL4	GL4	VL3	VL4	YL1	GL1	VL4	VL4	RL3	VL1	VL1	VL1	VL2
				VL4	VL5	YL2	GL2	YL1	GL1	VL1	VL2	VL2	VL2	VL3
					VL6	YL3	GL3	YL2	GL2	VL2	VL3	VL3	VL3	VL4
						YL4	GL4	YL3	GL3	VL3	VL4	VL4	VL4	VL5
								YL4	GL4	VL4	VL5	YL1	GL1	VL6
											VL6	YL2	GL2	YL1
												YL3	GL3	YL2
												YL4	GL4	YL3

- If you modify the instrument configuration, target, or fluorophore for any sample post-recording, a *notification badge* ( ) is displayed adjacent to the affected parameter or the instrument configuration dropdown (page 256), and a notification key becomes visible at the bottom of the panel.
- Clicking the notification button reverts the change to the value stored within the FCS file.

Configuration dropdown

Configuration dropdown allows you to select a filter configuration and update the choice of fluorophore for each enabled parameter for the selected configuration.

- The configuration dropdown list is linked to the filter configuration manager and contains all available configurations saved by the current user.
- The configuration selected from the dropdown list updates the choice of fluorophores for each enabled parameter. The configuration dropdown is always active, even after a sample from the Experiment has been recorded.

Update all samples

Update all samples allows you to apply the change of target and label names to all Samples within an Experiment or Group (rather than only to the active sample, which is the default).

- Click **Update all samples**, then select from the following options in the dropdown menu:
  - Apply to Experiment
  - Apply to Group
- **Apply to Experiment:** Applies all the modifications to all Samples in the Experiment. This option keeps the IS/badge state of all existing samples unchanged as the Experiment-level IS are updated.
- **Apply to Group:** Applies all modifications to all Samples in the Group and updates the IS/badge state of all existing Samples according to the following criteria:
  - Any Sample with an IS badge remains unchanged (i.e., the badge persists)
  - If the Auto Update Experiment IS is checked and the active Sample does not have data, any Sample within the Group that does not have an IS badge remains unchanged (i.e., no IS badge).
  - If the Auto Update Experiment IS is unchecked or the active Sample has data, any Sample that did not have an IS badge is assigned an IS badge.
- The Update all samples option is disabled during acquisition and while processing a Plate. While paused, the button is enabled.

Parameter selector

Parameter selector checkboxes allow you to select parameters (including **Event number**) and their respective measurements for any channel available in the selected configuration.

- By default, all parameters are selected except for the Event number parameter.
- Selecting the **Enable** checkbox enables all available channels with a single click.
- **Area**, **Height**, and **Width** measurements can be enabled for all channels with a single click by selecting the respective single-click checkbox (**A**, **H**, **W**).
- If all parameters or measurements are enabled using a single-click checkbox, then all related checkboxes are selected in that column.

  If any of those checkboxes are deselected, then the respective single-click checkbox is unchecked.
- Deselecting a **channel selector** checkbox unchecks all measurement checkboxes for that channel and disables the **Target** and **Fluorophore** fields.

  The channel selector checkbox is selected when at least one measurement is checked for that channel.

  If all measurements for a channel are unchecked, the channel selector checkbox is unselected and the Target and Fluorophore fields are disabled.
- Time parameter cannot be deselected.
- Parameters cannot be enabled or disabled in the active sample (wells or tubes) after that sample has been recorded and contains an FCS file.
- Parameters can be enabled or disabled during acquisition and prior to recording a sample. When parameters are enabled or disabled, the data will be cleared.
- Any parameter that is used as a Compensation control is disabled.

Target textbox

- **Target** textbox allows you to enter the target name of the fluorophore in the text field or leave it blank. The Target field is available for scatter and fluorescence parameters only.
- You can update the target name pre- or post-recording.
- Any changes made in the Target field updates the parameter name used in the Workspace.
- Entries made in the Target textbox are used by the *Smart Gate Naming* tool as described in "Customize Gate options", page 299.
- You can enter up to 50 characters into the Target textbox. Characters exceeding this limit cannot be entered. Any ASCII character is valid.

  The tooltip for Target textbox reads "Example: CD3".
- Any changes made to the target name post-recording only applies to the active sample, unless **Update all samples** button is clicked, which applies the target name to all samples within the Experiment.
- The tooltip for the Update all samples button displays "Updates Target and Fluorophore names for all samples within experiment."

Fluorophore textbox

- The **Fluorophore** textbox allows you to enter the fluorophore name (i.e., label name) in the text field or leave it blank. The Fluorophore field is available for scatter and fluorescence parameters only.
- You can update the fluorophore name pre- or post-recording.
- You can enter up to 50 characters into the textbox. Characters exceeding this limit cannot be entered. Any ASCII character is valid.

  The tooltip for Fluorophore textbox reads "Example: FITC".
- Any changes made to the fluorophore name post-recording only applies to the active sample, unless **Update all samples** button is pressed, which applies the fluorophore name to all Samples within the Experiment.

Notification badge

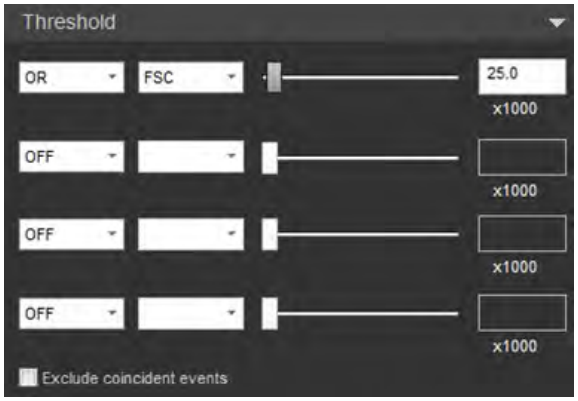
If you modify the instrument configuration selection, target, or fluorophore for any sample post-recording, a *notification badge* is displayed adjacent to the affected parameter or the instrument configuration dropdown (if modified).

- Clicking the notification button restores the target or fluorophore to the original value stored within the FCS file.
- Clicking the button next to the instrument configuration combo box restores the selection to the configuration used at the time the file was recorded.

Threshold

Overview

Threshold settings allow you to set the minimum signal level for up to four channels (i.e., detector) to eliminate unwanted events and reduce noise. You can also combine the thresholds using Boolean operators (see *Logic selector*, page 257).



- Threshold settings contain the following controls in the order listed:
  - *Logic selector combo box* (page 257)
  - *Channel selector combo box* (page 258)
  - *Threshold adjustment slider* (page 258)
  - *Threshold signal textbox* (page 259)
  - *Exclude coincident events checkbox* (page 259)
- A new value for the threshold signal is applied when the slider bar is moved, if the threshold logic or channel is changed, when the focus is lost from the threshold textbox, or when the **Enter** key is pressed.
- When a new threshold value is applied, existing data are cleared, and the software records and analyzes only the events with parameter values above the set threshold.
- Adjustments made to threshold with the slider bars dynamically refresh the data on the Workspace.

Logic selector

Logic selector allows you to select up to four channels to apply threshold(s) and to combine the thresholds using Boolean operators.

- Each logic selector dropdown includes three options: **AND**, **OR**, and **OFF**.
- If the **OFF** option is selected for a channel, the detector is ignored for threshold purposes, and the channel selector, slider, and textbox are grayed out.
- The **AND** and **OR** selections follow Boolean logic rules.
  - If the **AND** operator is selected, all the set thresholds must be met before the data is collected.
  - If the **OR** operator is selected, data collection begins when at least one of the thresholds is met.
- Since the selected logic operator determines the enabled/disabled state for the rest of the controls, the Logic selector combo boxes are available before the other threshold controls.

Channel selector

Channel selector allows you to select the channels to apply threshold.

- The first dropdown list includes all enabled channels selected in the Parameters group (excluding Time and Event) (page 255).
- Each subsequent dropdown list shows only the remaining available parameters. For example, if FSC is used as the first threshold, then all other dropdowns will not have FSC as an option.
- Threshold is only applied to parameters that are visible, allowing up to four threshold permutations.
- Default Threshold settings are: **OR** (selected in the first Logic combo box), **FSC** with a value of 25 (× 1000), and **OFF** on the second, third, and fourth logic dropdowns (i.e., not used for threshold).
- When other Threshold settings are added, their default value will be 10 (× 1000).

Threshold adjustment slider bar

Threshold adjustment slider bar allows you to adjust the numerical threshold setting.

- Adjusting this slider dynamically updates the value in the threshold textbox accordingly. While adjusting the slider bar, a tooltip displays the full threshold value (i.e., the textbox value × 1000).
- The lower limit is 0.1. The upper limit is 1/1000 of the range set for the height measurement of the selected channel.
- Moving the slider bar adjusts the threshold setting by increments of 10.

  When starting at the lower limit of 0.1, the next level will be 10, after which the threshold will increase in steps of 10.
- You can use the **up/right** and **down/left arrow** keys can also be used to adjust the slider bar in 1 unit (× 1000) increments. The up/right arrow keys increase the value, and the down/left arrow keys decrease the value.
- When the textbox or slider bar is in focus, you can use the **mouse scroll wheel** to adjust the slider bar position.

Threshold textbox

Threshold textbox allows you to type in the numeric value for a threshold setting. The software automatically multiplies the input value by 1000.

- Only numeric values are allowed in the textbox. One decimal place is always displayed. If you do not enter a decimal place, it will be automatically added with a zero in the tenths place.
- Changing the value in the textbox automatically updates the position of the slider after the textbox loses focus, the **Enter** key is pressed, the **up/right** and **down/left arrow** keys are pressed, or when the value is changed using the **mouse scroll wheel**.
- The accepted range of values is 0.1 for the lower limit, and 1/1000 of the allowed maximum for the height measurement for the upper limit (2^{20} by default). The resolution of entry is 0.1.
- When a number is entered that is greater than the maximum allowed value for the parameter, the focus is set to the entire text to allow editing (instead of defaulting to maximum).
- If the values are deleted from the textbox and the textbox loses focus, then the previous value is restored to the textbox.

Exclude coincident events check box

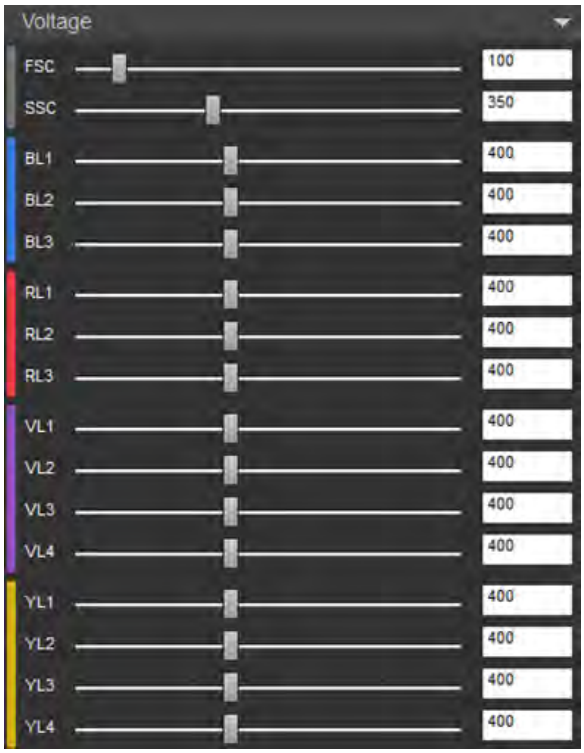
Exclude coincident events check box removes coincident events.

- When checked, the coincident events are removed from the data stream, and the number of aborted coincident events is counted in the FCS file. Aborted coincident events are annotated using the $ABRT keyword.
- When checked, the number of aborted coincident events is updated in real-time in the Collection Panel.
- By default the checkbox is unchecked.

Voltage

Overview

Voltage settings allow you to adjust voltages for each channel. The number of channels shown depends on the configuration of the instrument.



- Voltage settings consist of the following controls:
  - *Voltage adjustment slider bar* (page 261)
  - *Voltage value textbox* (page 261)
- The default voltages are set to zero, which are automatically updated to the voltages from the first successful Baseline Performance Test results.
- You can adjust the PMT voltage for an available channel using the slider bar or the textbox for that channel.

  If a parameter is deselected within the Parameters group (page 255), then the deselected channel is grayed out.
- Adjustments made to voltages with the slider bars dynamically refresh the data on the Workspace. However, adjustments are not applied until the user stops pressing the mouse button when using the slider.
- Adjustments made using the textboxes automatically refresh the data on the Workspace when you click elsewhere on the screen or press the **Enter** key.
- When Compensation samples are run, the respective channel is highlighted with a light blue color.
- When a compensation channel is recorded or the Compensation sample contains an FCS file, all controls for other enabled fluorescence channels are disabled and grayed out for the remaining samples in the Experiment.

Note: This ensures that the voltages used in the compensation calculation are not modified and the parameters are not disabled.

- If the compensation settings are removed from the Experiment, the parameter and voltage controls for the fluorescence channels are re-enabled.

Voltage adjustment slider bar

Voltage adjustment slider bar allows you to adjust the PMT voltages for available channels.

- Adjusting the voltage slider bar dynamically updates the value in the voltage textbox accordingly. However, adjustments are not applied until the user stops pressing the mouse button when using the slider.
- The range of the voltage slider bar is from 1 to 1000 (left to right).
- Moving the voltage slider bar adjusts the PMT voltage by increments of 20.
- When the voltage slider or textbox is in focus, you can use the **mouse scroll wheel** or the **up/right** and **down/left arrow** keys to adjust the slider bar position by increments of 10. The up/right arrow keys increase the value, and the down/left arrow keys decrease the value.

Voltage value textbox

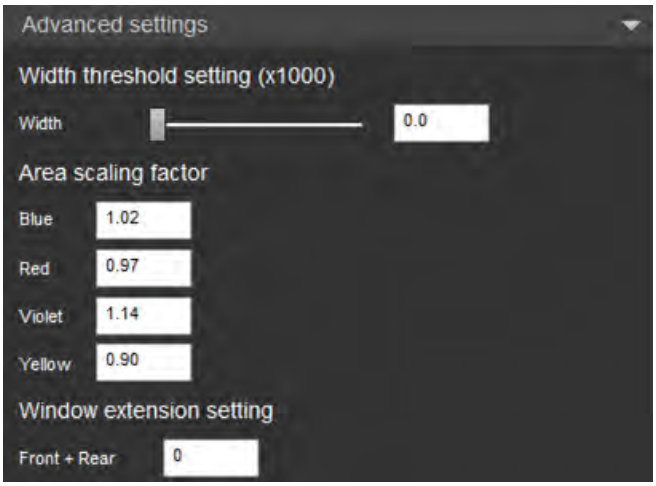
Voltage value textbox allows you to directly enter the desired voltage setting for a specific channel.

- Only numeric values are allowed in the textbox. Non-numeric characters are not registered.
- The accepted range of values is 1–1000 mV for all channels. The resolution of entry is 1 mV.
- Changing the value in the textbox automatically updates the position of the slider after the textbox loses focus, the **Enter** key is pressed, the **up/right** and **down/left arrow** keys are pressed, or when the value is changed using the mouse scroll wheel.
- Adjustments to the textbox refresh the data on the Workspace once focus is lost or **Enter** is pressed.
- When a number is entered that is greater than the maximum allowed value for the parameter, the focus is set to the entire text to allow editing (instead of defaulting to maximum).
- If the values are deleted from the textbox and the textbox loses focus, then the previous value is restored to the textbox.
- During compensation, the fluorescence channels are highlighted with a light blue color.

Advanced settings

Overview *Advanced Instrument Settings* allows you to adjust the following settings, if you have the appropriate permissions (see Note below):

- *Width threshold setting* (page 263)
- *Area scaling factors (ASF)* (page 263)
- *Window extension setting* (page 264)

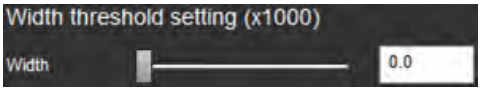


- If you do not have permissions to any of the functions within this section, the Advanced settings section is not visible.
- Adjustments to the slider bar dynamically refresh the data on the Workspace. Adjustments to the textboxes update the data once you click elsewhere on the screen or when you press the **Enter** key.
- If the values are reset to default, the data is cleared and new values take effect.
- If you modify the area scaling factor (ASF) for any laser or change the window extension setting, a *notification button* (page 264) is displayed next to the affected setting, and a notification key is shown at the bottom of the panel.
- Changes made to Advanced settings persist for the current user and the current experiment. New experiments will use the default system settings.
- Changes made to Advanced settings for one user are not applied to other users.

Note: The Advanced settings options are available only to users with Advanced User and Administrator level permissions. For more information, see "Account permissions", page 25.

Width threshold setting

Width threshold setting sets the threshold above which the width measurement is determined. It consists of a slider and a textbox.



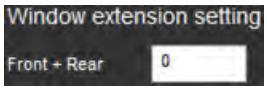
- The width threshold setting is set per Experiment. When it is saved as default instrument settings, it is used for new Experiments.
- The range of the width threshold setting is from 01 to 1048.5 (left to right), with a resolution of 0.1. The entered values are in thousands. The default value is 1.
- Moving the slider bar adjusts the value by increments of 10.
- Only numeric values are allowed in the textbox. One decimal place is always displayed. If you do not enter a decimal place, it is automatically added with a zero in the tenths place.
- When the slider or text box is in focus, you can use the **mouse slider wheel** or the **up/right** and **down/left arrow** keys to adjust the slider bar position by increments of 5.
- Changing the value in the textbox automatically updates the position of the slider after the textbox loses focus, the **Enter** key is pressed, the **up/right** and **down/left arrow** keys are pressed, or when the value is changed using the mouse scroll wheel.
- If the values are deleted from the textbox and the textbox loses focus, then the previous value is restored to the textbox.

Area scaling factor (ASF)

Area scaling factor (ASF) option sets the value to scale height and area to equivalent values. Area scaling is calculated during the Performance Test and is automatically applied to new Experiments created after Performance Test is completed.

- The ASF can be set per Experiment or it can be used for all new Experiments when saved as default Instrument Settings.
- The ASF can be set for each configured laser and only configured lasers are listed.
- The ASF values are updated after each successful Performance Test. The default values are set by the most recent, successful Performance Test. If no Performance Test or Baseline has been run, the default values are set to 1.0
- The ASF values range from 0.1 to 10.
- Only numeric values are allowed in the textbox. Non-numeric characters are not registered.
- If the System Settings update the Area scaling factor settings, the ASF values are updated to the System Settings.
- If the ASF value is modified in the text box for a specific laser, the System Setting is overridden such that the ASF value for that modified laser will not automatically update based on each successful Performance Test.
- If the ASF values are deleted from the textbox and the textbox loses focus, the previous value is restored to the textbox.
- If you do not have permission to access the Advanced Instrument Settings, the ASF value used is set by the System Instrument Settings.

Window extension setting



The **Front + Rear window extensions** can be configured for an Experiment and saved as part of the default Instrument Settings.

- The value ranges from –40 to 75. The default value is 0.
- Only integer values are allowed in this text box.
- The window extension value input in the text box corresponds to a single extension and the full added extension is 2X of the input value.
- If the value is deleted from the text box, and the text box loses focus then the previous value will be restored to the text box.
- If the value is modified, the system window extension setting is overridden such that the value will not automatically update based on each successful Performance Test.

Notification button

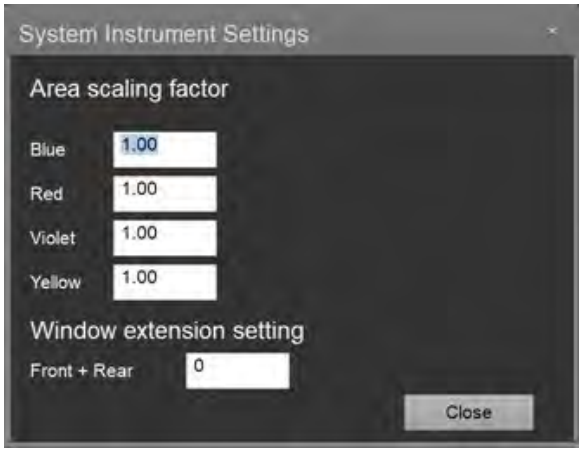
If you modify the area scaling factor (ASF) value for any laser or change the window extension setting, a *notification badge* is displayed adjacent to the affected setting.

- Clicking the notification button next to the area scaling factor (ASF) restores the affected setting to the System Setting value for the corresponding laser.
- Clicking the notification button next to the window extension setting restores the setting to the System Setting value.

System Instrument settings

Overview *System Instrument Settings* allows you to adjust the following system settings, if you have the appropriate permissions (see Note below):

- *Area scaling factor (ASF)* (page 266)
- *Window extension setting* (page 266)



- If you do not have permissions to any of the functions within this section, the System Instrument Settings section is not visible.
- If the values are reset to default, the data is cleared and new values take effect.
- If you modify the Area scaling factor (ASF) value for any laser or change the window extension setting, a *notification badge* is displayed adjacent to the affected setting, and a notification key becomes visible at the bottom of the panel (page 266).
- Changes made to System Settings are applied to each new Experiment created after the values have been changed. These changes persist until the next Performance Test is completed or until the System Settings Values are changed by an authorized user.
- Changes made to System Settings are applied as default to all user accounts after the values are changed.
- The System settings are updated after each successful Performance Test.

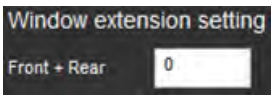
Note: The System Instrument Settings options are available only to users with Administrator level permissions. For more information, see "Account permissions", page 25.

Area scaling factor (ASF)

Area scaling factor (ASF) option sets the value to scale height and area to equivalent values. Area scaling is calculated during the Performance Test.

- The ASF setting applies to all Experiments and Users, where the Advanced Setting ASF value has not been set (page 263).
- The ASF can be set for each configured laser and only configured lasers are listed.
- The ASF values are updated after each successful Performance Test. The default values are set by the most recent, successful Performance Test.
- If no Performance Test or Baseline has been run, the default values are set to 1.
- The values range from 0.1 to 10.
- Only numeric values are allowed in the textbox. Non-numeric characters are not registered.
- If the values are deleted from the textbox and the textbox loses focus, then the previous value is restored to the textbox.

Window extension setting



The **Front + Rear window extensions** can be configured for an Experiment and saved as part of the default instrument settings.

- The value ranges from –40 to 75. The default value is 0.
- Only integer values will be allowed in this text box.
- The window extension value input in the text box corresponds to a single extension and the full added extension is 2X of the input value.
- If the value is deleted from the text box, and the text box loses focus then the previous value will be restored to the text box.
- The window extension value is reset to 0 after each successful Performance Test.

Notification icon

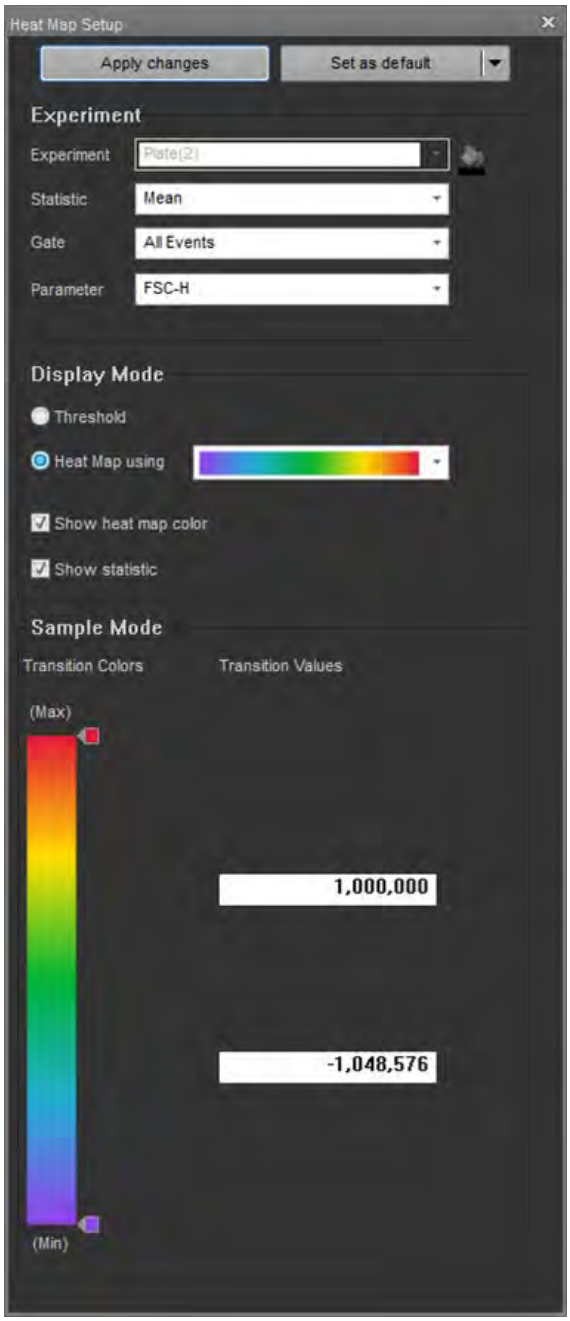
If you modify the Area scaling factor (ASF) value for any laser or change the Window extension setting, a *notification badge* is displayed adjacent to the affected setting.

- Clicking the notification button next to the Area scaling factor (ASF) restores the affected setting to the most recent successful Performance Test value.
- Clicking the notification button next to the Window extension setting restores the setting to its default value of 0.

14. Heat Map Setup panel

Overview

Heat Map Setup panel allows you to select the statistic, gate, and parameter for visualizing the data from the current Experiment in the *Heat Map view* (page 138), and to specify the **display mode** and **transition values** to help you analyze the data at a glance.



- In the **Threshold display mode**, each well and/or tube is assigned a color based on whether the selected statistic for that sample falls above or below the set threshold value.
- In the **Heat Map display mode**, individual values in each well and/or tube are represented as colors on a gradient.
- By default, the Heat Map Setup panel is docked to the left of the Main Application Area. The docking properties of the panel are described on page 37.
- The Heat Map Setup panel is organized into four functional groups in the following order:
  - Apply, Save, and Load options (page 268)
  - Experiment (page 269)
  - Display mode (page 271)
  - Sample value (page 272)
- Sample value options on the Heat Map Setup panel are contextual; the controls displayed depend on whether you select Threshold or the Heat Map in the Display mode options (page 271).
- By default, the Heat Map Setup panel displays the sample value controls for the Threshold mode.

Note: For information on the Heat Map view, including Sample display and Experiment Setup, refer to "Heat Map View" on page 138.

Apply, Save, and Load options

Overview

Apply, Save, and Load options consist of the two controls that lie at the top of the Heat Map Setup panel.

Apply changes

- **Apply changes** applies the current Heat Map settings to all samples in the current Experiment.
- This option is active if the Heat Map settings of the current Experiment have been changed.
- If the Heat Map Setup panel loses focus after a change has been made, the new settings are automatically applied to the Heat Map.

  The Status Notification Display located above the Main Application Workspace (page 36) then displays "The Heat Map settings have been updated" and provides an **Undo** option.

Set as default split button

Set as default button is a split button that allows you to apply the Heat Map settings to the current Experiment, set them as default, or save (export) and retrieve them for other Experiments.

The main button on the left is the **Set as default** command and the right side is a dropdown containing **Load** and **Export** options.

- **Set as default:** Saves the current Heat Map setting as the default Heat Map settings for all new Experiments.
- **Load:** Opens the *File Browser dialog* (page 537).

  Using the File Browser dialog, you can select a Heat Map settings file from within any experiment in your folder, from available Shared folders, or from available default instrument settings files.
- **Export:** Opens the *File Save (Export) dialog* (page 532).

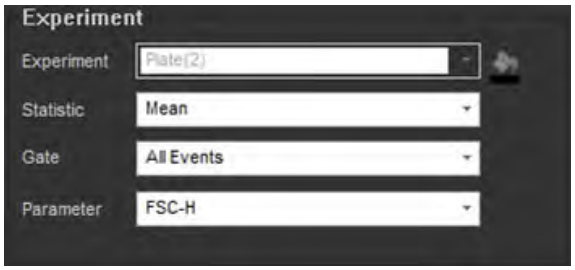
  Using the File Save (Export) dialog, you can save the current Heat Map settings with a user-defined name in your own folder or any Shared folders in the database.

  You will be prompted for confirmation before overwriting an existing Heat Map settings file.

Experiment

Overview

Experiment options allow you to select the statistics, populations (all events or specific gated populations), and the parameter for the current Experiment to display in the Heat Map view.
It also contains the control for assigning a unique indicator color for the current Experiment.



Experiment dropdown

Experiment dropdown list displays the name of the Experiment defined within the current plate and any associated tubes.

- The Experiment dropdown control is disabled as each plate is limited to a single Experiment.
- The control is labeled with the name of the currently open Experiment. The default label is **Experiment**.
- Default settings are loaded the first time the Experiment Heat Map is selected.

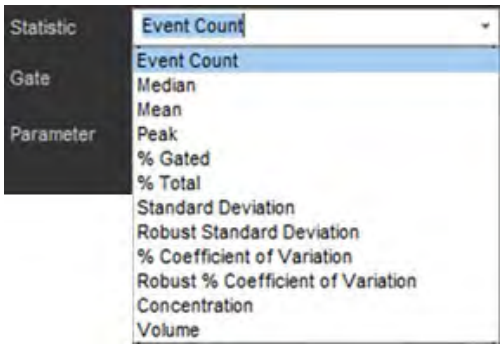
Experiment color button

Experiment color button opens the *color picker dialog*, which allows you to select the indicator color for the current Experiment.

- By default, the Experiment color dropdown button shows the currently selected color for the Experiment.
- When applied, the selected color is reflected in the Heat Map view (page 138).

Statistic dropdown

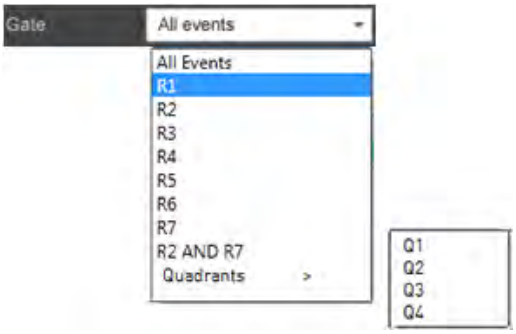
Statistic dropdown displays the list of available statistics and allows you to select the statistic of interest to display in the Heat Map view.



- The statistic dropdown control is labeled with the name of the currently selected statistic. The default statistic is **Event Count**.
- The dropdown list includes the following statistics:
  - Event Count
  - Median
  - Mean
  - Peak
  - % Gated
  - % Total
  - Standard Deviation
  - Robust Standard Deviation
  - % Coefficient of Variation
  - Robust % Coefficient of Variation
  - Concentration
  - Volume

Gate dropdown

Gate dropdown list allows you to select the populations of interest (all events or specific gated populations).



- The dropdown list contains all gates in the Experiment-level Workspace (page 84), including quadrant and derived gates.
- The default population is **All Events**.
- Only gates from the Experiment-level Workspace are displayed.
- Selecting a gate sets the parent gate for the Heat Map display.
- If the default Heat Map or loaded Heat Map refer to a gate that is not available on the Experiment workspace, the gate selection defaults to All Events.

Parameter dropdown

Parameter dropdown allows you to select the parameter to display in the Heat Map view for the current Experiment.

- The Parameter dropdown is available only for intensity related statistics, Mean, Median, Standard Deviation (SD), Robust SD, Coefficient of Variation (CV), and Robust CV.

  It is hidden when Event Count, Percentage of Parent, Percentage of Total, or Concentration is selected in the Statistic dropdown list (page 269).
- The dropdown list contains only the parameters on the plot for the gate selected in the Gate dropdown.
- If **All Events** is selected in the Gate dropdown, the parameters are not filtered and all enabled parameters are displayed by default.
- If the gate is derived from a Histogram plot (i.e., a single parameter plot), then the parameter list only contains the parameter that is on the selected plot.

  Selecting the **More** displays all available parameters.
- For a dual-parameter plot, the parameter list is filtered to show the two parameters that are on the plot from which the gate is derived.

  Selecting the **More** option shows all parameters.

Display mode

Overview

Display mode options allow you to choose between the **Threshold** and **Heat Map** display modes and control the display of the colors used in the Heat Map view.

You can select the Threshold or the Heat Map mode by clicking the appropriate radio button.

The default option is Threshold mode.

Note: Threshold display mode is a visual aid to determine at a glance whether the signal from a well falls above or below a set value; it does not affect data collection. As such, it is different from the *Threshold settings*, which sets the minimum signal level for each detector to eliminate unwanted events and reduce noise. The threshold value set in Threshold settings instructs the software to record and analyze only the events with parameter values above the set threshold.

Display mode options

Threshold mode

- **Threshold** radio button selects the Threshold mode and displays each well in user-defined colors based on whether the signal from the well and/or tube falls above or below the set threshold value.
- By default, only a single threshold is defined. However, you can create up to 10 signal threshold levels using the **Sample value** options (page 272).
- When **Threshold** mode is selected, choosing a color gradient from the dropdown switches the radio button to the **Heat Map** mode.

Heat Map mode

- **Heat Map** radio button allows you to represent the data from each sample well as colors on a gradient selected from the **color gradient dropdown** list.

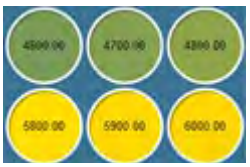
- There are six gradients available in the color gradient dropdown list, which are not editable. The default option is the first color range in the list.
- By default, only the minimum and maximum transition points are defined in Heat Map. However, you can create up to 10 transition points using the **Sample value** options (page 274).

Show heat map color

- **Show heat map color** toggles the heat map color displayed on the Heat Map on and off.
- This option is selected by default (i.e., the Heat Map color is turned on).

Show statistic

- When selected, the **Show statistic** option overlays the selected statistic centered in the wells and/or tubes.
- The background color includes the Experiment color (if selected) and the Heat Map color (if selected).

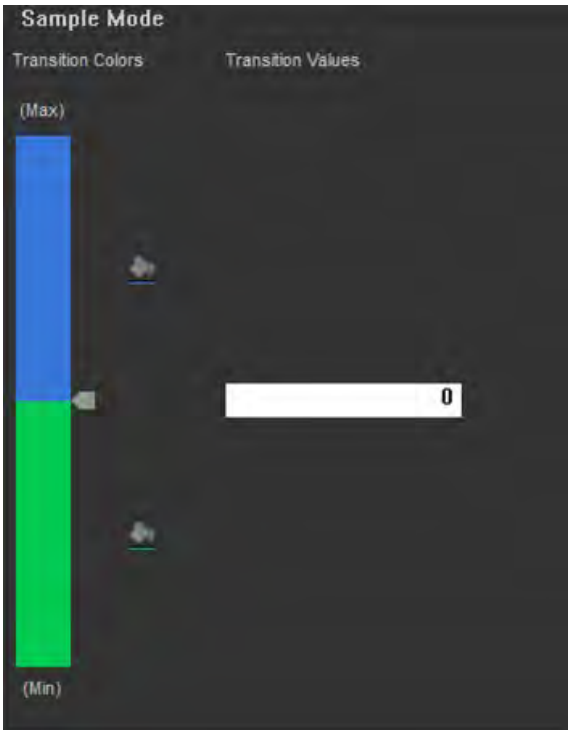


Sample mode – Threshold

Overview

Sample mode options allow you to add and remove color transition points and set the data level and the associated color.

- Clicking the **Threshold** radio button in Display mode options (page 271) displays the sample value controls for the Threshold mode.
- In the Threshold mode, each well and/or tube is assigned a color based on whether the selected statistic for that sample falls above or below the set threshold value.
- Threshold mode is the default option for the sample value controls.



Transition colors

- The color of the well in the Heat Map view (page 138) changes, if the threshold signal level is exceeded. It will not change if the value is the same or less than the transition value.
- By default, if the selected statistic is above the threshold, the well appears green; if it is below this level, the well appears blue.
- Clicking the **color picker** button next to a color block opens the standard color picker dialog, which allows you to define a different color for that block.

Transition values

- By default, a single transition point (i.e., threshold) is set in the Threshold display mode. The default transition value is 10.
- You can enter the data value for a transition point directly in the **transition value** edit box.

- Transition value edit boxes accept only numerical characters. Decimal values are shown only if you enter them.
- Each transition value edit box allows manual entry of a number based on the statistic being displayed.
  - Percentage of count values: 0 to 100%.
  - Percent CV values: ± 1000%
  - Events: 1 to 100,000,000
  - Intensity: minimum to maximum allowed for the selected parameter
- If the entry for the transition point exceeds the maximum value allowed, it will default to the maximum; if the value is lower than the minimum value allowed, then it will default to the minimum.
- If the statistic is changed, then the threshold returns to the default transition point set at 10.

Create additional transition points

- You can add additional transition points by clicking on any given colored block sector. This inserts a new threshold bisecting the selected sector and adds an additional color block, which adopts the next color in the automatic color sequence.
- A transition value edit box is automatically created for each new level, and a color picker is associated with each new color block.
- You can create up to 10 levels. If you attempt to create more than 10 transition points, a warning message is displayed below the color bar.
- Clicking to create an additional transition point of a higher value creates a transition point at double the value of the first point.

  For example, if the first transition point is set at 100, the new one will be set to 200.
- Clicking to create an additional transition point of a lower value creates a transition point at half the value of the first point.

  For example, if the first transition point was set at 100, the new one will be set to 50.
- Clicking to create an additional transition point between two existing points creates a transition point half way between the two existing values.

  For example, if you click between the transition points set at 100 and 200, the new transition point value will be set to 150.
- Clicking and dragging an indicator arrow from the color bar by more than 20 pixels displays a red **X** icon, and when the mouse button is released, the selected transition point and the associated color picker and transition value edit box are removed.

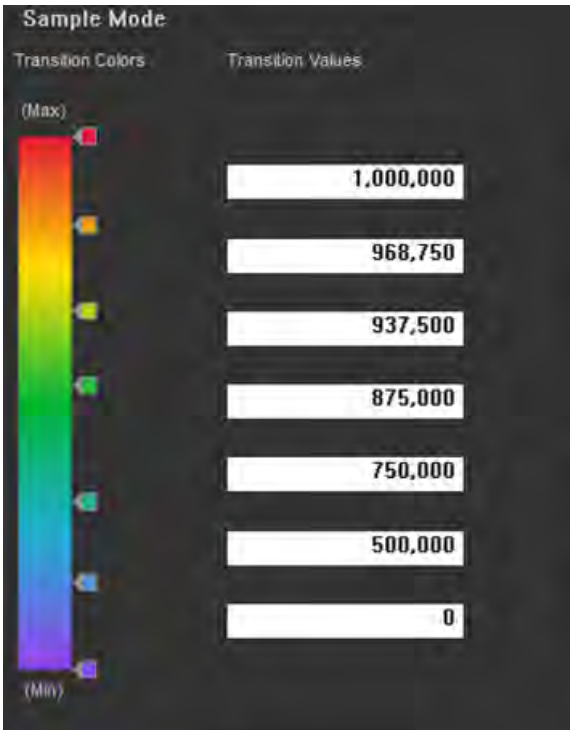
- There must always be one transition point defined. The last point cannot be removed.

Sample mode – Heat Map

Overview

Sample mode options allow you to add and remove color transition points and set the data level and the associated color.

- Clicking the **Heat Map** radio button in Display mode options and selecting the transition colors from the **color gradient dropdown** list (page 271) displays the sample value controls for the Heat Map display mode (i.e., gradient mode).
- In the Heat Map mode, each well and/or tube displays the appropriate color from the selected gradient depending on whether the signal from the well falls within the set boundaries.



Transition colors

- The color of the well in the Heat Map view (page 138) changes depending on where the result is in comparison to the selected color gradient.
- The Transition color gradient is selected using the **color gradient** dropdown list as described in "Display mode options", page 271.

- Colored indicator arrows are displayed at the minimum and maximum points by default and any added transition points in between. The color displayed indicates the transition point color.

- Transition points can be moved up and down to adjust the color assigned to that transition point by clicking and dragging the indicator arrows with the mouse.

  The associated textbox will be highlighted in light blue to distinguish which transition point is being adjusted.

Transition values

- By default, minimum and maximum transition points are defined in the Heat Map display mode.
- The default minimum transition point is 0 and the default maximum transition point is 10, 000, from bottom to top respectively.

  The default maximum for statistics containing a percentage is 100%.
- You can enter the data value for a transition point directly in the **transition value** edit box.
- Transition value edit boxes accept only numerical characters. Decimal values are shown only if you enter them.

- Each transition value edit box allows manual entry of a number based on the statistic being displayed.
  - Percentage of count values: 0 to 100%.
  - Percent CV values: ± 1000%
  - Events: 1 to 100,000,000
  - Intensity: minimum to maximum allowed for the selected parameter
- If the entry for the transition point exceeds the maximum value allowed, it will default to the maximum; if the value is lower than the minimum value allowed, then it will default to the minimum.
- If the statistic is changed, the minimum and maximum values revert to the appropriate default based on that statistic. Any middle transition points that existed are also removed.

Create additional transition points

- You can add additional transition points by clicking on the color bar. This inserts a new transition point at the selected point and adds an additional indicator arrow, which adopts the color of the point selected.
- A transition value edit box is automatically created for each new transition point.
- You can create up to 10 levels. If you attempt to create more than 10 transition points, a warning message is displayed below the color bar.
- Clicking to create an additional transition point between two existing points creates a transition point half way between the two existing values.

  For example, if you click between the transition points set at 100 and 200, the new transition point value will be set to 150.
- The transition value entered must be in ascending order. If an incorrect value is entered, a warning balloon is displayed.
- Clicking and dragging an indicator arrow from the color bar by more than 20 pixels displays a red **X** icon, and when the mouse button is released, the selected transition point and the associated transition value edit box are removed.

- The minimum and maximum points cannot be removed.

15. Customize panel

Overview

The *Customize panel* displays context sensitive formatting options relevant to objects selected on the Workspace or the Heat Map view.

Using the Customize panel, you can format the following objects:

- *Plots* (page 277)
- *Text boxes* (page 290)
- *Statistics boxes* (page 291)
- *Gate styles and labeling* (page 293)
- *Cell Image Container - Image Adjustment* (page 317)

Customize panel behavior

- To display the Customize panel, right-click an object to open its context menu, then select **Customize**.
- The formatting options in the Customize panel are context-sensitive and dynamically update depending on the currently selected objects.
- When a Compensation sample is active, all customize functionality is disabled except those specified in "Compensation", page 378.
- You can select multiple objects of the same type (e.g., plots), but not multiple objects of different types (e.g., plots and text boxes).

  If you select multiple objects of different types, the Customize panel displays a warning message stating:

  "Customize options are available only when similar objects are selected."

Customize Plot options

Overview

Customize Plot options allow you to customize properties for the following plot types: *Histogram*, *Dot*, *Density*, and *Precedence Density*.

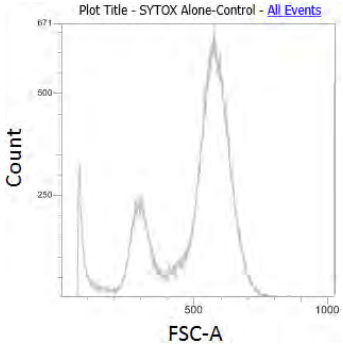
Note: To set default options for plots, use the *Options dialog* (page 480).

Plot types

Histogram plot

A *Histogram plot* is a graphical representation of single-parameter data and shows the relative number and distribution of events.

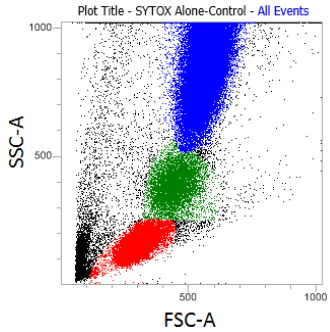
In a Histogram plot, the horizontal axis corresponds to the signal intensity of the selected parameter while the vertical axis represents the number of events (count).



Dot plot

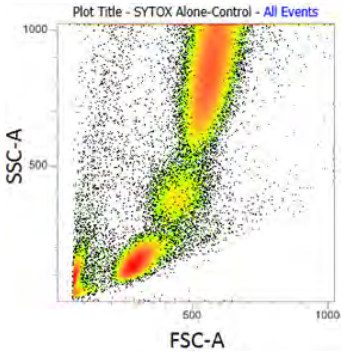
A *Dot plot* is a graphical representation of dual-parameter data, where each axis represents the signal intensity of one parameter.

Each dot in the plot corresponds to one or more events detected above the threshold. Different colors are used to represent the parent gate of events that fall within bins on the plots.



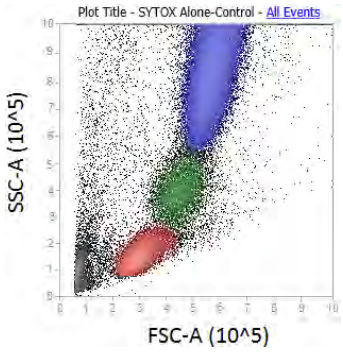
Density plot

A *Density plot* is a graphical representation of dual-parameter data, where the colors represent the collection of events with the same intensity and each axis represents the signal intensity of one parameter.



Precedence Density plot

A *Precedence Density plot* is a combination of Dot and Density display, where a gradient is used to indicate the number of events within each of the plot bins and color is used to display the parent gate of events present.

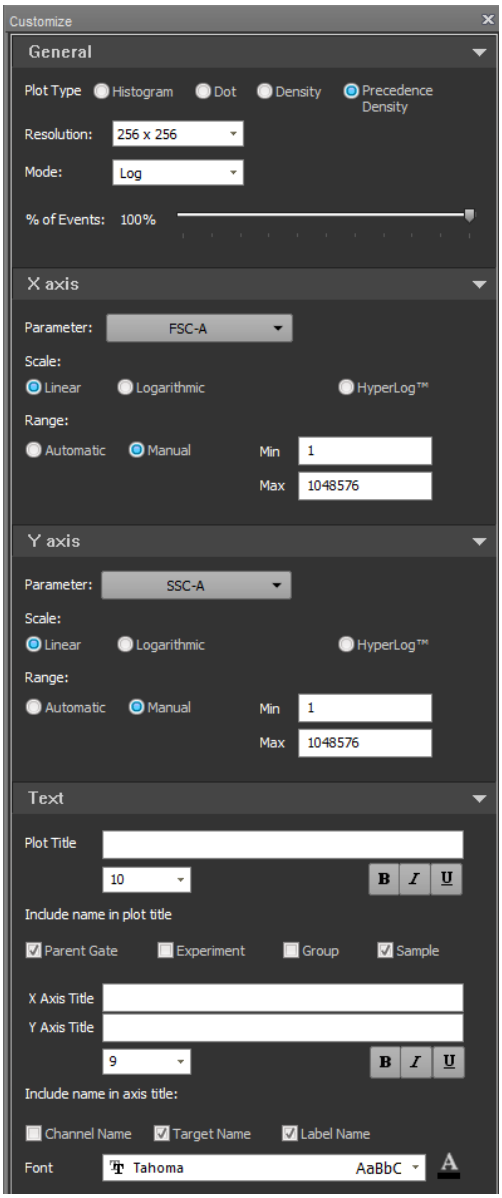


Customize panel behavior for plots

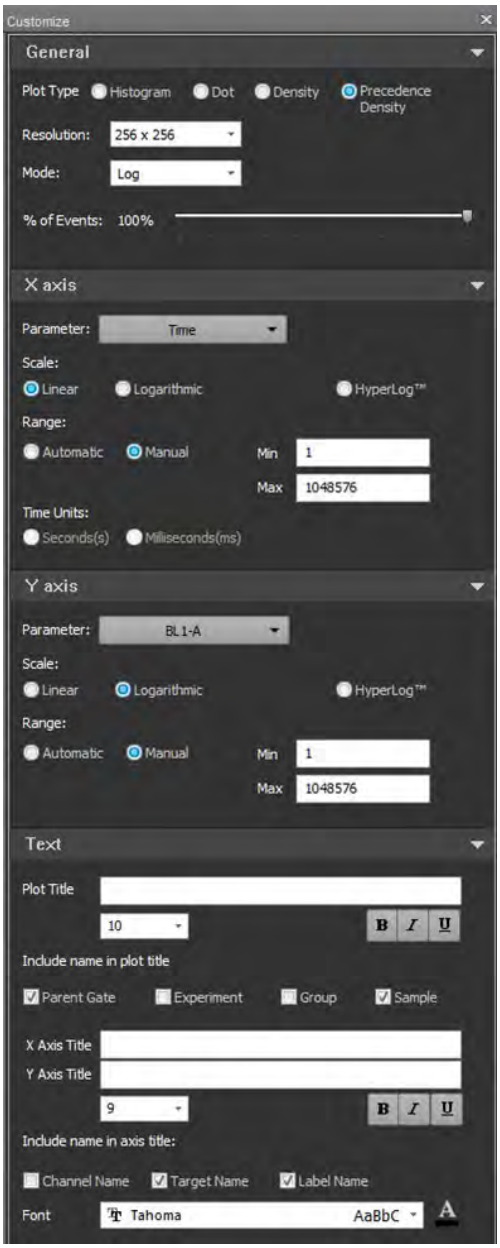
The Customize Plot options are displayed when one or more plots are selected. They are arranged into four groups:

- *General* (page 279)
- *X-axis* (page 284)
- *Y-axis* (page 287)
- *Text* (page 288)

- The options displayed in each group vary, depending on the plot types and options selected. The example images below show the options available for Density plots when scatter or time and fluorescent parameters are selected.
- Each field in the panel displays the current settings for the selected plot. If multiple plots with differing values are selected, the value fields show the indeterminate state.



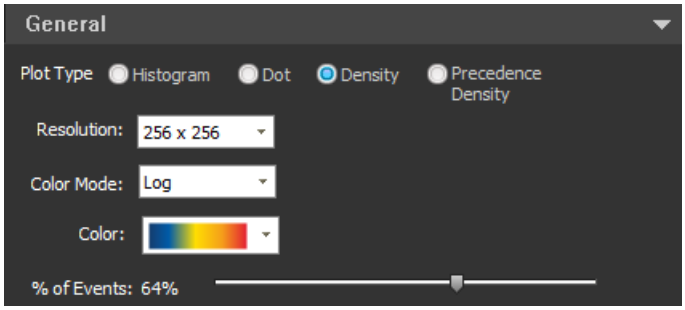
Customize Plot options for Density plots (scatter parameters)



Customize Plot options for Density plots (time and fluorescent parameters)

General group

General group controls the plot type and general plot characteristics (such as plot resolution).

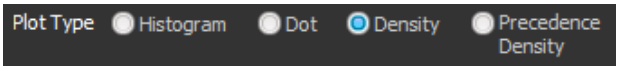


General group for Density plots

Note: The customization options that appear in the General group vary depending on the plot types selected. This section describes all possible options available in the General group. For illustrations of the plot-specific options, see page 283.

Plot Type radio buttons

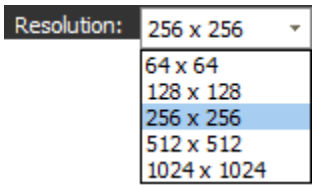
Plot Type radio buttons allow you to assign a plot type for the selected plots.



- The Plot Type option applies to all plot types and it is displayed when any plot is selected.
- If one or more plots of the same type are selected, the radio button for that plot type is selected.
- If multiple plot types are selected, all radio buttons are deselected.
- Selecting a radio button changes all selected plots to the selected plot type.
- If the current Workspace is a Compensation Workspace, the Histogram radio button is disabled when Density, Dot, or Precedence Density plots are selected. Similarly, if Histogram plots are selected, the Density, Dot, and Precedence Density radio buttons are disabled.

Resolution dropdown menu

Resolution dropdown menu allows you to select a channel resolution for the selected plots.



- Resolution option applies to all plot types and it is displayed when any plot is selected.
- If a single plot is selected, or if multiple plots with the same resolution (including a mixture of plot types) are selected, the Resolution dropdown menu displays the resolution of the currently selected plots.
- If multiple plots of any type with different resolutions are selected, the Resolution field is empty (blank).
- The maximum channel resolution is 1024 × 1024 channels for Dot, Density, and Precedence Density plots, and 1024 channels for Histogram plots.

- If a Histogram plot is selected along with two- parameter plots (Dot, Density, and/or Precedence Density plots), the Resolution dropdown menu for dual-parameter plots is displayed.
- If a Histogram plot is among the selected plots, the resolution is applied to the X-axis of the Histogram plot.
- The default resolutions are:
  - Histogram – 1024 channels
  - Dot – 256 × 256 channels
  - Density – 256 × 256 channels
  - Precedence Density – 256 × 256 channels

  The defaults can be modified in *Plot Options* in the *Options dialog* (page 480).

Color dropdown menu

The **Color** dropdown menu allows you to select a color scheme for Density plots.

- Color option is displayed when one or more Density plots are selected.
- If a single Density plot is selected, or if multiple Density plots with the same color scheme are selected, the Color field displays the density color scheme of the currently selected plots.
- If multiple Density plots with different color schemes are selected, the Color field is empty (blank).
- You can select a different color scheme from the dropdown menu. The selected color is applied to all selected Density plots.
- The default color scheme can be modified in *Plot Options* in the *Options dialog* (page 480).

% of Events slider

The **% of Events** slider adjusts the percentage of events displayed on the selected plots. The sample data is taken from the entire data file.

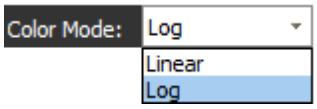
- % of Events option applies only to dual-parameter plots (Dot, Density, and Precedence Density plots), and is displayed when one or more dual-parameter plots are selected.
- % of Events option is not displayed when only Histogram plots are selected. If Histograms plots are selected along with two- parameter plots, adjusting the slider has no effect on the Histogram plots.
- The current value for the slider setting is shown to the left of the slider and it is updated as the slider is moved.
- When using the mouse, you can adjust in 1-unit increments.

  When using the arrow keys, you can adjust in 5-unit increments.

  Clicking the bar to the left or right of the slider moves the slider by 25 units.

Color Mode dropdown menu

Color Mode dropdown menu allows you choose **Linear** or **Log** binning of the data to the selected color palette for Density and Precedence Density plots.



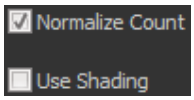
- Color Mode option applies only to Density and Precedence Density plots and it is displayed when one or more Density and/or Precedence Density plots are selected.
- If a single Density or Precedence Density plot is selected, or if multiple Density and/or Precedence Density plots with the same color mode are selected, the Mode field displays the color mode of the currently selected plots.
- If multiple Density and/or Precedence Density plots with different color modes are selected, the Color Mode field is empty (blank).
- You can select a different color mode from the dropdown menu. The selected mode is applied to all selected Density and/or Precedence Density plots.

Note: The *linear mode* bins data by assigning a color index for each density pixel linearly such that each increment is determined by dividing the range (Z_{max}–Z_{min}) by the number of color steps.

The l*og mode* bins data by assigning a color index for each density pixel logarithmically such that each increment is determined by dividing the logarithmic range (log Z_{max}–log Z_{min}) by the number of color steps. The index is then determined as int((log Z_{val} – log Z_{min})/increment).

Normalize Count

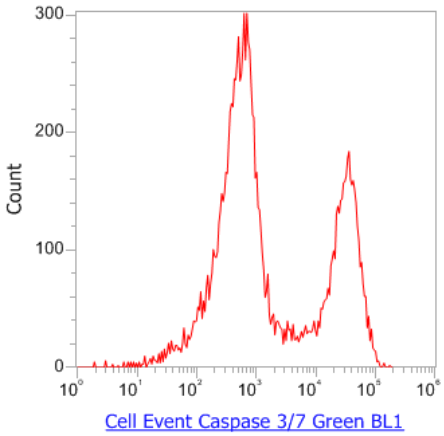
Normalize Count checkbox controls the scaling for the selected Histogram plots.



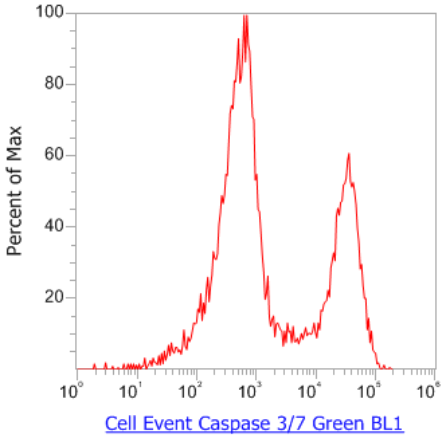
- Normalize Count option applies only to Histogram plots and it is displayed when one or more Histogram plots are selected.
- When the checkbox is unchecked, the scale label is **Count**.
- When the checkbox is checked, the Y-axis scaling is changed to scale the plot to the Histogram peak value.

  The scale displayed is a percentage scale from 0 to 100%, where 100% is the Histogram peak value.

  The scale label is **Percent of Max.**

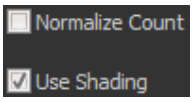


Normalize Count unchecked



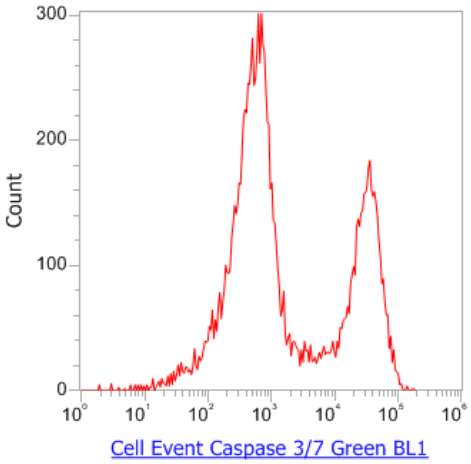
Normalize Count checked

Use Shading

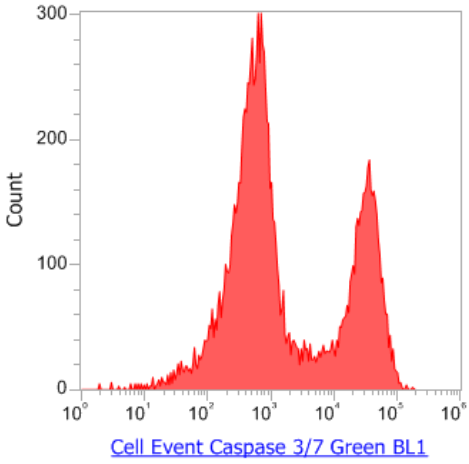


Use Shading checkbox controls the shading for the selected Histogram plots.

- Use Shading option applies only to Histogram plots and it is displayed when one or more Histogram plots are selected.
- The color used for shading is the same as that used for the line color (at 64% opacity). The color and the opacity cannot be adjusted.
- When the checkbox is unchecked, shading is removed.
- When the checkbox is checked, shading is added.



Use Shading unchecked



Use Shading checked

Line Width

Line Width menu controls the line thickness for the selected Histogram plots.

- Line Width option applies only to Histogram plots and it is displayed when one or more Histogram plots are selected.
- You can select or enter a number from **1** to **5**.
- If you enter an out-of-range number, the software corrects it to the nearest valid number. Non-numeric characters are not permitted.

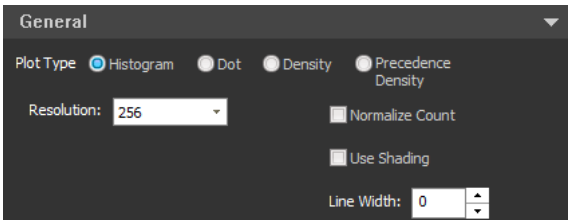
General group – plot-specific options

This section illustrates the customization options available in the General group when single or multiple plot types are selected.

Each field in the panel displays the current settings for the selected plot. If multiple plots with differing values are selected, the value fields show the indeterminate state.

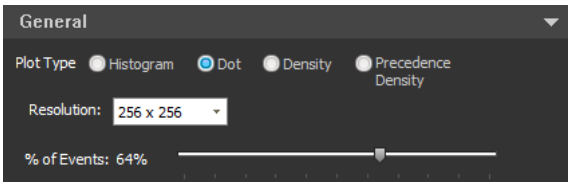
Histogram only

- Plot Type (page 279)
- Resolution (page 279)
- Normalize Count (page 281)
- Use Shading (page 281)
- Line Width (page 282)



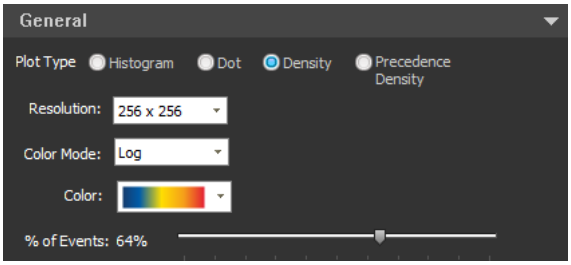
Dot only

- Plot Type (page 279)
- Resolution (page 279)
- % of Events (page 280)



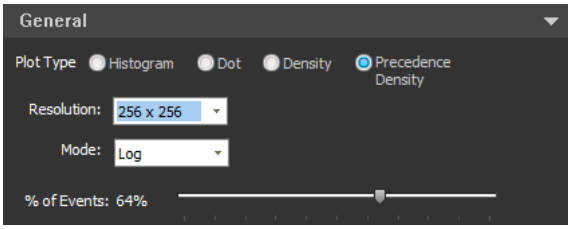
Density only

- Plot Type (page 279)
- Resolution (page 279)
- Mode (page 281)
- Color (page 280)
- % of Events (page 280)



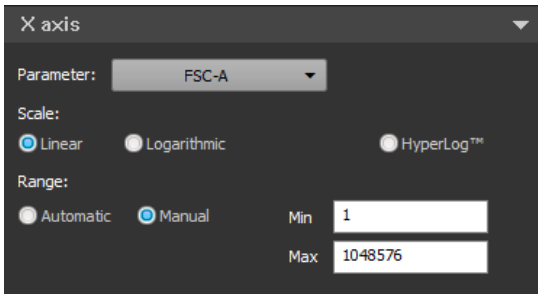
Precedence Density only

- Plot Type (page 279)
- Resolution (page 279)
- Mode (page 281)
- % of Events (page 280)



X-axis group

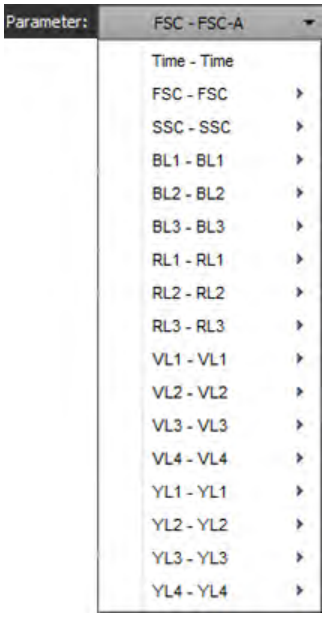
The *X-axis group* allows you to customize the X axes of selected plots.



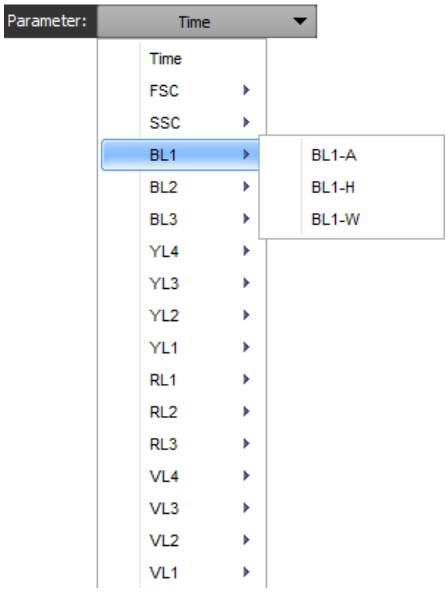
- All customization options in the X-axis group apply to all plot types and are displayed when any plot is selected.
- The options that appear in the X-axis group vary depending on the scale selected (page 285).

Parameter dropdown menu

Parameter dropdown menu allows you to select any enabled parameter for the X-axis. All parameters in the FCS file.

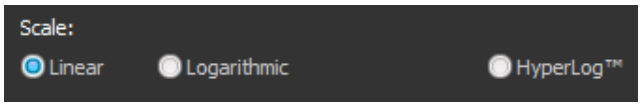


- If a single plot is selected, or if multiple plots with the same X-axis parameter (including a mixture of plot types) are selected, the Parameter dropdown menu displays the parameters of the currently selected plots.
- If multiple plots of any type with different X-axis parameters are selected, the Parameter field is empty (blank).
- If the current Workspace is a Compensation Workspace, the Parameter dropdown menu is not available for dual-parameter or Histogram plots.
- To enable the Parameter dropdown menu, you can change the parameters of the gating plot within a Compensation Workspace.
- If more than 18 parameters are selected in the current Sample and if two or more measurements are enabled for the parameter, the dropdown menu includes a submenu of parameter types. Calculated parameters are always displayed in the primary menu.
- Selected plots are updated to display the selected parameter on the X-axis.



Scale options

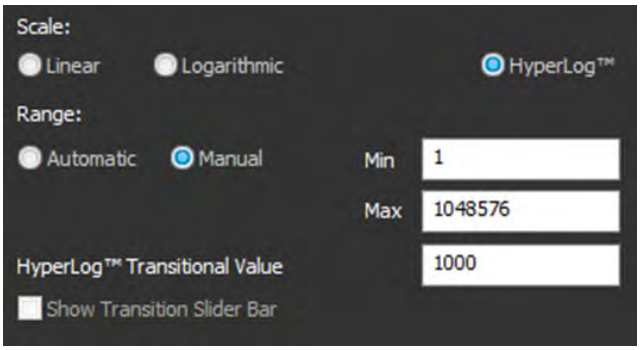
- **Scale** options allow you to select the X-axis scaling method for the selected plots: **Linear**, **Logarithmic**, and **HyperLog™**.



Note: The Hyperlog™ scale uses log-linear hybrid transformations to display compensated flow cytometry data that frequently contain negative values due to compensation. Logarithmic transformations cannot properly handle negative values, and poorly display normally distributed cell types.

HyperLog™ scale

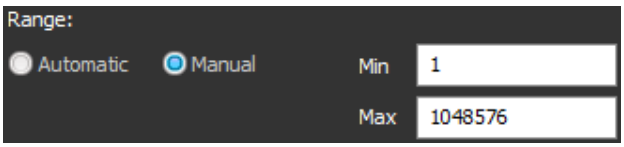
HyperLog™ is an alternative bi-exponential scaling. When the **HyperLog™** scale is selected, an additional **Transitional Value** control becomes available.



- To adjust the width of the linear area of the plot, enter a numeric value in the **HyperLog™ Transitional Value** field. Enter a value from **0** to **1,000,000**; decimal places are not permitted.
- The Transitional Value is applied to the selected parameter.
- The Transitional Value changed on one plot changes the Transitional Value on another plot with the same parameter that is also set to HyperLog™ scale.

Range options

Range options allow you to adjust the scale range of a plot's axis.



- **Automatic:** Sets the minimum and maximum values automatically and adjusts the upper value to the highest channel with data present.
  - For axes set to Log scaling, the minimum value is set at 1. For axes in Linear or HyperLog™ scaling, the minimum value is set to the lowest value in the data set.
  - Data ranges are limited by the parameter range set in the FCS file ($PnR) (see Note below).
- **Manual:** Allows you can enter the minimum and maximum values manually. The allowable range depends on the type of scale selected.
  - **For Linear and HyperLog™ scaling**: The default manual range is ± the maximum range set in the FCS file ($PnR) (see Note below).

    The allowable minimum range is -2^{31} to $2^{31}-2$.

    The allowable maximum range is $-2^{31}+1$ to $2^{31}-1$.
  - **For Log scaling**: The default manual range is 1 to the maximum value set in the FCS file ($PnR) (see Note below).

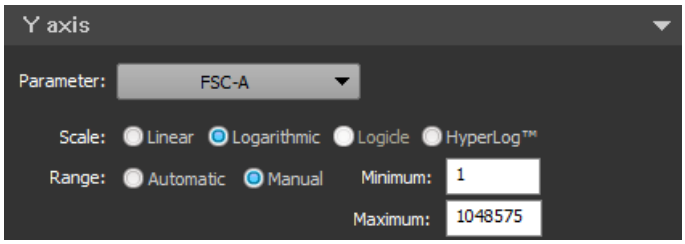
    The allowable minimum range is 1 to $2^{31}-2$.

    The allowable maximum range is 2 to $2^{31}-1$.
  - If **Time** is selected from the **Parameter** dropdown menu (page 284), the Range fields are based on milliseconds.
  - If you enter an out-of-range number in the Minimum or Maximum fields, the software adjusts it to the minimum or maximum value, respectively. Non-numeric characters are not permitted.

Note: $PnR is the range for the selected parameter n (page 550). By default, this is set to 2^{26} for Event count and Time parameters, 2^{20} for all Height and Area measurements, and 2^{10} for all Width measurements.

Y-axis group

The *Y-axis group* allows you to customize the Y-axes of selected plots.



- All customization options in the Y-axis group apply to all plot types and are displayed when any plot is selected.
- The options that appear in the Y-axis group vary depending on the scale selected (see Scale options, below).

Parameter dropdown menu

Parameter dropdown menu allows you to select any enabled parameter for the Y-axis. All parameters in the FCS file, including any calculated parameters, are available.

The Parameter dropdown menu for the Y-axis behaves the same as the dropdown menu for the X-axis, except when a Histogram plot is selected.

- If a Histogram plot is selected, the Y-axis parameter is fixed as **Count** and cannot be changed unless the plot is changed to a dual-parameter plot type.
- If a Histogram plot is selected and the Normalize Count checkbox is checked, the Y-axis parameter is fixed as **Percent of Max**.
- If Histograms plots are selected along with dual-parameter plots, the Parameter dropdown menu selection has no effect on the Histogram plots.

Scale options

Scale options allow you to select the Y-axis scaling method for the selected plots: **Linear**, **Logarithmic**, and **HyperLog™**.

- **Scale** radio buttons for the Y-axis behave the same as the options for the X-axis, except when a Histogram plot is selected:
  - If a Histogram plot is selected, the HyperLog™ scaling option is disabled.
  - If Histograms plots are selected along with two-parameter plots, all scale options are enabled; any changes to the HyperLog™ scaling option has no effect on the Histogram plots.

Range options

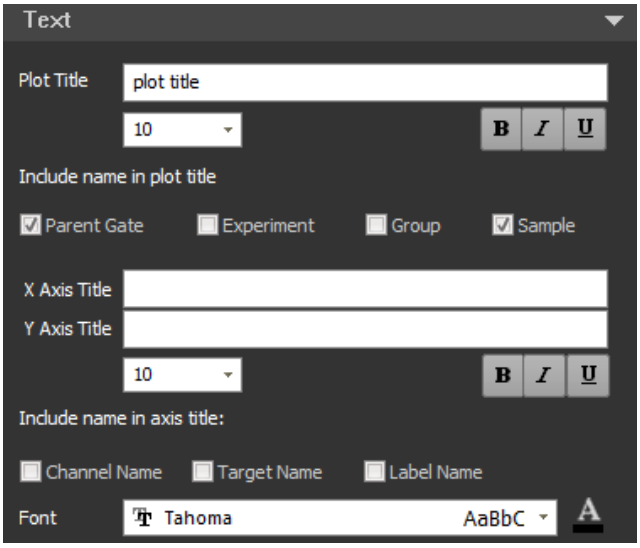
Range options allow you to adjust the scale range of a plot's axis. **Range** options for the Y-axis behave the same as the options for the X-axis, except when a Histogram plot is selected:

- If a Histogram plot is selected, the Y-axis is set to **Count**, the minimum value can be 0 to 2^{31}–2, and the maximum value can be 1 to 2^{31}–1.
- For **Percent of Max**, the minimum value can be 1 to 99, and the maximum value can be 2 to 100.

Text group (plots only)

The *Text group* allows you to customize the plot text boxes. All customization options in this group apply to all plot types and are displayed when any plot is selected.

The options in the plot Text group are disabled when items other than plots are selected in the Workspace or a Compensation Workspace is displayed.



Plot title options

Plot title options allow you to enter text and select font formats for the plot title. The formatted text appears at the top of all selected plots.

- You can enter up to 50 characters in the **Plot title** field; any character is permitted. If the plot title is too large to fit above the selected plots, the software truncates the title.
- In the **font size** field, you can select a font size from the dropdown menu or enter a number from **6** to **72**.
- You can select the **bold**, **italics**, and/or **underline** options to format the font.
- You can include the Experiment name, Group name, Sample name, and/or parent gate by checking the appropriate **Include** checkboxes. The software appends your selections to the plot title, separated by hyphens.

X-axis and Y-axis title options

The **X-** and **Y-axis title** options allow you to enter text and select font formats for the X- and Y-axis titles. The formatted text appears as the X- and Y-axis labels for all selected plots.

- You can enter up to 50 characters in the X-axis and Y-axis title fields; any character is permitted. If an axis title is too large to fit in the selected plots, the software truncates the title.
- For the X-axis:
  - The tick channel labels change from displaying the full channel number to thousands or millions, as appropriate.
  - The tick channel labels display exponential numbers if the channel number entered is >10,000.
  - The size of the labels cannot be changed.
  - Parameter names are appended with (10^3) or (10^6) to indicate channel scaling.
- For the Y-axis:
  - If Histogram plots are selected, the Y-axis title defaults to **Count** if all Histogram plots are the same and none are normalized.

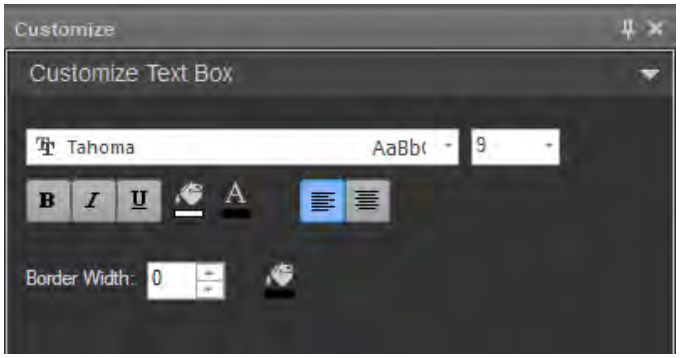
    If Histogram plots are all set to Normalize Count, the title defaults to **Percent of Max**.
  - The tick channel labels display exponential numbers if the channel number entered is >10,000.
  - The size of the labels cannot be changed.
  - Parameter names are appended with (10^n) to indicate channel scaling.
- In the X-axis and Y-axis font size fields, you can select a **font size** from the dropdown menu or enter a number from **6** to **72**.
- You can select the **bold**, **italics**, and/or **underline** options to format the font.
- You can include the wavelength, channel name, target name, and/or fluorophore name by checking the appropriate **Include** checkboxes. The software appends your selections to the axis labels, separated by hyphens.
  - If you do not check any Include checkboxes, the axis label defaults to the parameter name specified in the *Display Parameter Name As* setting in the General section of the *Options dialog* (page 477).

    For example, if $PnS (Stain Name) is selected, axis label defaults the parameter name to the stain name (for example, CD4-FITC).
  - If the target and fluorophore names are selected but unavailable (i.e., the names are from third party support/imported FCS files), the default parameter or channel name included in the imported FCS file are used.
  - Include options are not applied to the Y-axis of Histogram plots when the name is set to **Count** or **Percent of Max** as described above.

Customize Text box options

Text box options

Customize Text box group allows you to customize the font style and borders of text boxes. These options are displayed when one or more text boxes are selected in a Workspace.



Style options

Style options allow you to select the font type, size, and formatting for all selected text boxes.

- You can select a **font type** from the dropdown menu. The dropdown menu includes all fonts installed on the system. The default font is Tahoma.
- You can select a **font size** from the dropdown menu or enter a number from **6** to **72**.
- You can select the **bold**, **italics**, and/or **underline** options to format the font.
- You can select to **left-align** or **center** the text.
- You can select **colors** for the text and for the Text box fill. The software uses the standard color picker dialog.

Border options

Border options allow you to select the border width and color for all selected text boxes.

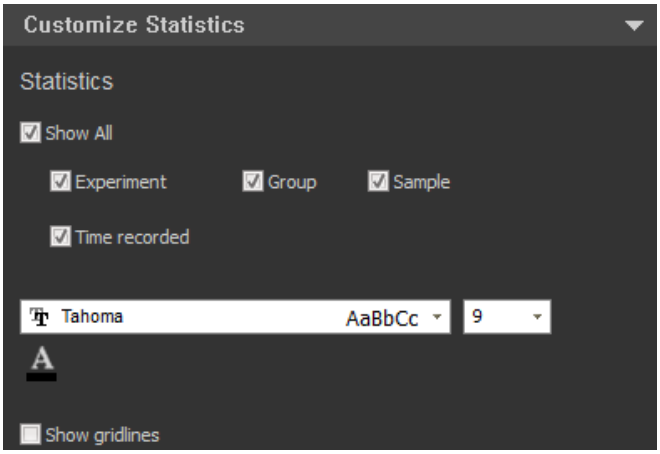
- You can select a **border width** or enter an integer from **1** to **5**.
- You can select a **color** for the border. The software uses the standard color picker dialog.

Customize Statistics box options

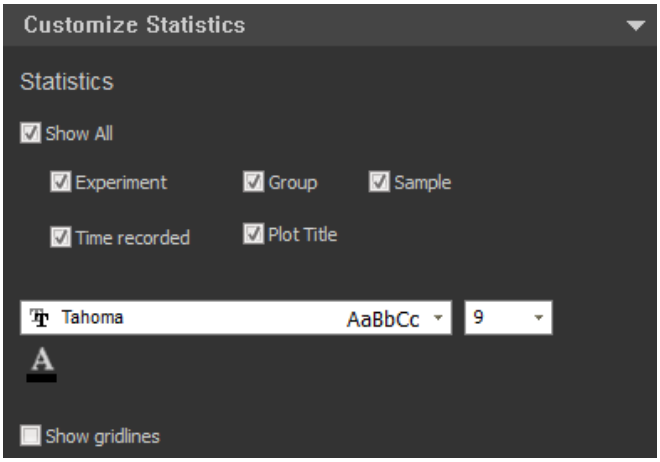
Statistics box options

Customize Statistics box group allows you to customize the formatting of statistics boxes and the statistics they displayed.

- The customization options are displayed when one or more Sample-level or Plot-level statistics boxes are selected in a Workspace. The options vary slightly between sample- and Plot-level statistics boxes.
- The following option is displayed when one or more **Sample-level** statistics boxes are selected:



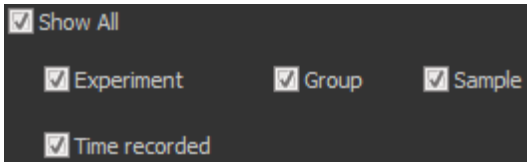
- The following option is displayed when one or more **Plot-level** statistics boxes are selected:



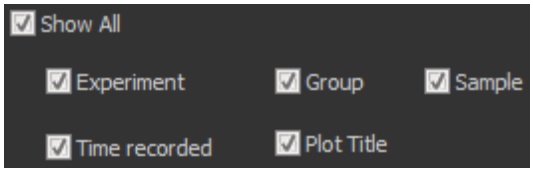
Header options

Header options allow you to select which elements are automatically added to the headers of all selected statistic boxes.

- For Sample-level statistics boxes, you can include the **Experiment**, **Specimen**, and **Sample names** and the **Time Recorded** by checking the appropriate checkboxes.



- For Plot-level statistics boxes, you can include the **Experiment**, **Group**, and **Sample** names, the **Time Recorded**, and the **Plot title** by checking the appropriate checkboxes.



- Checking the **Show All** checkbox selects all available elements.
- The software includes your selections in the statistics box header.

Formatting options

Formatting options allow you to select the font type, size, and formatting for all selected statistics boxes.

- You can select a **font type** from the dropdown menu. The dropdown menu includes all fonts installed on the system. The default font is Tahoma.
- You can select a **font size** from the dropdown menu or enter a number from **6** to **72**.
- You can select the **bold**, **italics**, and/or **underline** options to format the font.
- You can select a **color** for the text. The software uses the standard color picker dialog.
- You can display a border around the box and gridlines inside the box by checking the **Show Gridlines** checkbox. The border and gridline colors and widths cannot be adjusted.

Customize Gate options

Overview

Customize Gate options allow you to customize the properties of the following gate types:

- Rectangle, Oval, and Polygon
- Contour Autogate and Ellipse Autogate
- Histogram
- Quadrant

Note: To set default options for gates, use the *Gate Options dialog* (page 486).

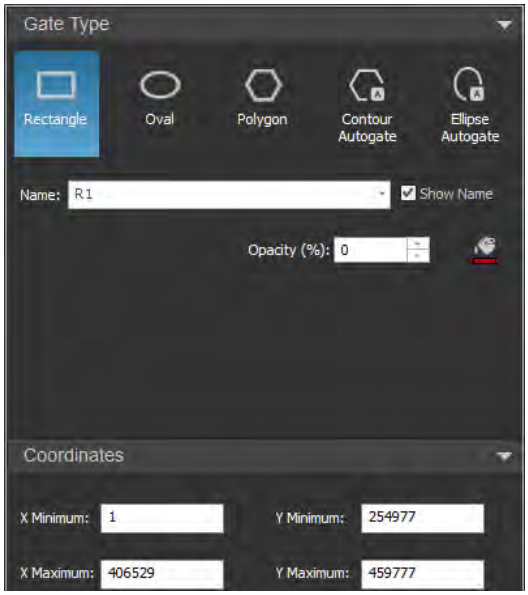
Customize panel behavior for gates

- The Customize Gate options are displayed only when a gate is selected.
- The Customize Gate options are arranged into the following groups:
  - *Gate Type* (page 294)
  - *Name options* (page 299)
  - *Coordinates* (page 300)
  - *Autogating* (available only for Contour and Ellipse Autogates) (page 302)
- Each group is contained in a group box that is collapsible.
- The options displayed in each group vary, depending on the gate types and options selected.
- The values displayed in any field are the current settings for the selected gate.
- When a Compensation sample is active, all gate customization options are disabled, except for the options specified in “Compensation”, page 378.

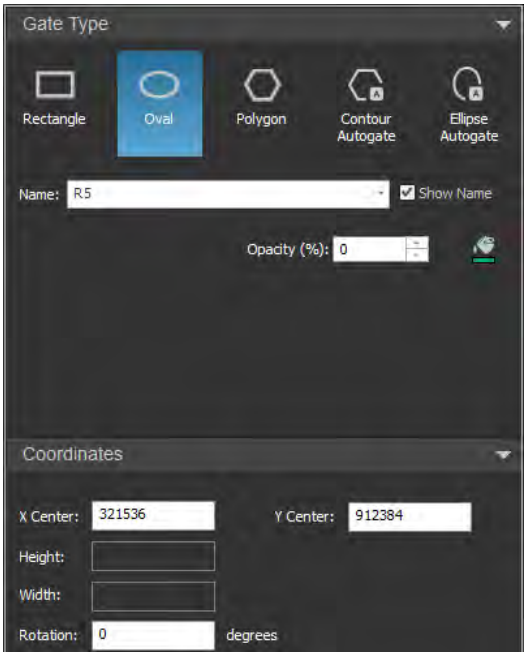
Gate Type group

Gate Type group controls the gate type and general gate characteristics (such as opacity and color). The customization options that appear in the Gate Type group vary, depending on the gate types selected.

Rectangle gates

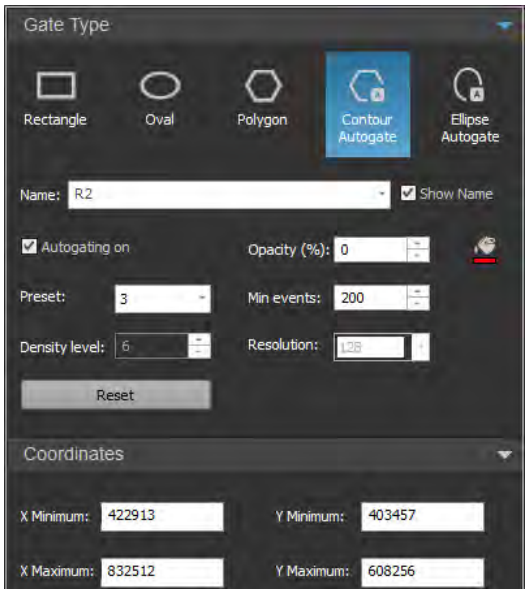


Oval gates



Polygon gates

Contour Autogate



Ellipse Autogate

Histogram gates

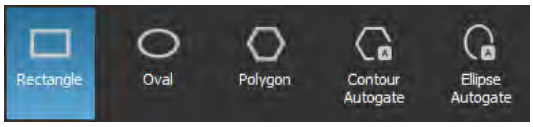
Quadrant gates

Gate Type buttons

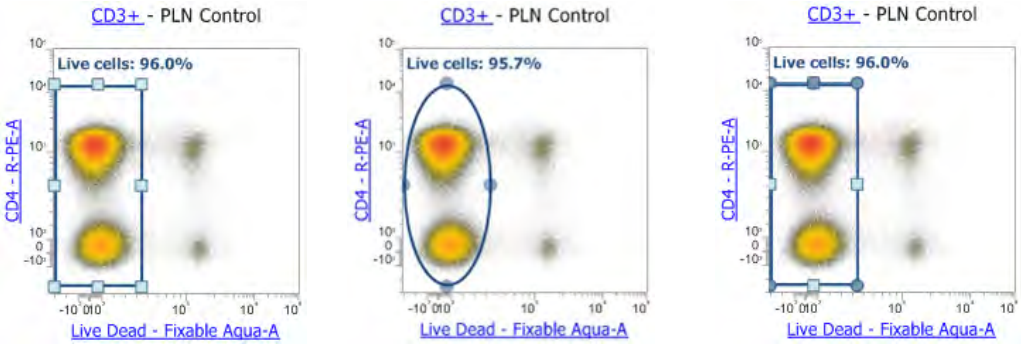
The Gate Type buttons allow you to change the gate type of the selected gate on a plot. The button for the currently selected gate type is highlighted.

Rectangle, Oval, and Polygon gates

When a **Rectangle**, **Oval**, **Polygon gate**, **Countour Autogate**, or **Ellipse Autogate** is selected, all gate type buttons are displayed together. The button for the selected gate type is highlighted.

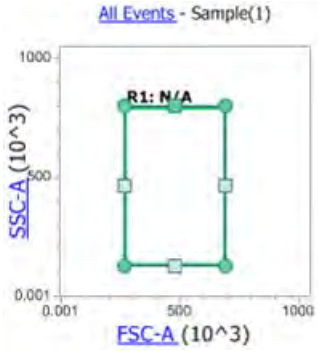


- To change the gate type of the selected gate, click the desired **Gate Type** button.
- The following figures show the same area of the plot with different gate types.



Rectangle gate | Oval gate | Polygon gate

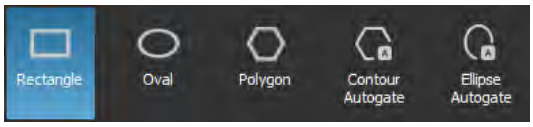
- When you switch to a different gate type, the software:
  - Creates a new gate with the same boundaries as the original gate (X minimum, X maximum, Y minimum, and Y maximum)
  - Sizes the new gate according to the original gate's size and position.
- When you switch to a Polygon gate, the software creates a 5-point polygon in the shape of the bounding rectangle as shown in the following example.



Note: For a description of the behaviors when you switch to and from a Contour Autogate or Ellipse Autogate to other gate types, see "Contour Autogate and Ellipse Autogate", page 297.

Contour Autogate and Ellipse Autogate

When a **Rectangle**, **Oval**, **Polygon gate**, **Countour Autogate**, or **Ellipse Autogate** is selected, all gate type buttons are displayed together. The button for the selected gate type is highlighted.



To change the gate type of the selected gate, click the desired **Gate Type** button.

The following tables describe the behavior when a Contour or Ellipse Autogate is switched to another gate type and vice versa.

Conversion of Contour Autogate to another gate

New gate	Behavior
Rectangular gate	Bounding box of the autogated ellipse generates a new gate.
Oval gate	
Polygon gate	Polygon vertices persist.
Ellipse autogate	New gate is generated based on polygon vertices. Smoothing vertices and other smoothing behaviors are applied.

Conversion of other gates to Contour Autogate

Old gate	Behavior
Rectangular gate	Bounding box of the Rectangle gate generates a Contour autogate bounding box.
Oval gate	Bounding box of the Oval gate generates a Contour autogate bounding box.
Polygon gate	Bounding box of the Polygon gate generates a Contour autogate bounding box.
Ellipse autogate	A Contour autogate is generated based on the Ellipse autogate bounding box and polygon vertices with same scanning region. Smoothing behavior is **not** applied.

Conversion of Ellipse Autogate to another gate

New gate	Behavior
Rectangular gate	Bounding box of the autogated polygon generates a new gate.
Oval gate	
Polygon gate	New gate is generated based on polygon vertices. Smoothing vertices and other smoothing behaviors are **not** applied.
Contour autogate	

Conversion of other gates to Ellipse Autogate

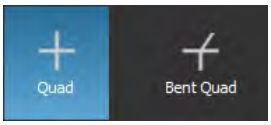
Old gate	Behavior
Rectangular gate	Bounding box of the Rectangle gate generates an Ellipse autogate bounding box.
Oval gate	Bounding box of the Oval gate generates an Ellipse autogate bounding box.
Polygon gate	Bounding box of the Polygon gate generates an Ellipse autogate bounding box.
Contour autogate	An Ellipse autogate is generated based on the Contour autogate bounding box and polygon vertices with same scanning region.

Histogram gates

When a **Histogram gate** is selected, only the Histogram button is displayed. You cannot change a Histogram gate to a different gate type.

Quadrant gates

When a **Quadrant gate** is selected, you can choose between the **Orthogonal** and **Bent Quadrant** gates.



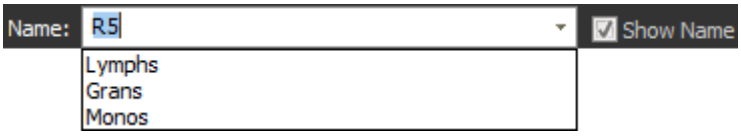
- Orthogonal mode maintains the quadrant orthogonally as the center point is moved.

  In the Bent mode, all vertices move independently of each other.
- The Quadrant mode selection is mutually exclusive. Selecting the Orthogonal Quadrant option deselects the Bent Quadrant option and vice versa.
- If the end points of a quadrant gate are moved on the Workspace or Filmstrip view, the quadrant gate is automatically set as a Bent Quadrant gate.

Name options

Name field

Name field displays the name of the currently selected gate. The Name options allow you to assign a gate name and show or hide the gate name on the plot.

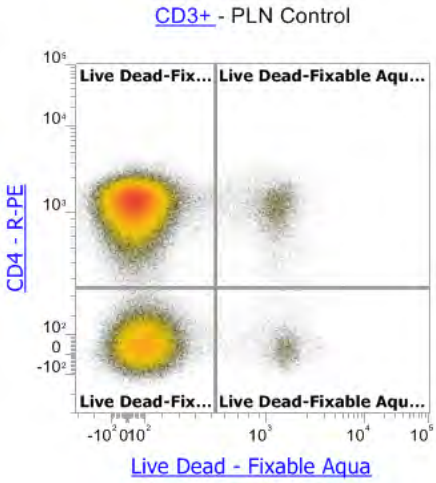


- You can enter up to 50 characters in the Name field; any character is permitted.

  Alternatively, you can select a gate name form the **Quick Select** dropdown menu. The dropdown menu options are set in *Gate Options* in the *Options dialog* (page 477).
- The gate name must be unique. If you enter a duplicate name, the software appends a numerical suffix in parentheses; for example, **GateName (2)**.
- You can show or hide the gate name on the plot by checking or unchecking the **Show Name** check box.
- For Quadrant gates, four Name fields are displayed (one for each quadrant).
- For Quadrant gates, you can select the **Use smart gate naming** option (see below) instead of entering the gate names.

Smart Gate Naming

Smart Gate Naming option allows you to select and deselect automatic gate naming. This option is available only for Quadrant gates.



- If you check the **Use smart gate naming** check box, the software automatically names the Quadrant gates, using the options specified in *Gate Options* in the *Options dialog* (page 477).
- For Quadrant gates, the four gate names are generated (one for each quadrant).
- Smart names can be up to 50 characters; any character is permitted.
- Smart names must be unique. If a duplicate name is generated, the software appends a numerical suffix in parentheses; for example, **GateName (2)**.
- When you edit a smart name, **Use smart gate naming** is automatically unchecked. You must enter the gate names manually, as described in **Name field** above.
- When you uncheck **Use smart gate naming**, you must enter the gate names manually, as described in **Name field** above.

Note: Smart Gate Naming option is available only for Quadrant gates.

Coordinates group

The customization options that appear in the Coordinates group vary depending on the gate type selected. This section describes all possible options available in the Coordinates group.

Note: Coordinate options are not available for Polygon gates.

Coordinates group for Rectangular gates, Contour Autogates, and Ellipse Autogates

For a Rectangular gate or the bounding box of Contour or Ellipse autogates, you can enter **X** and **Y minimum** and **maximum coordinates**.

- Non-numeric characters are not permitted.
- The allowable X and Y coordinates depend on the type of scale selected.
  - **Linear and HyperLog™ scaling:**

    The allowable minimum range is -2^{31} to $2^{31}-2$.

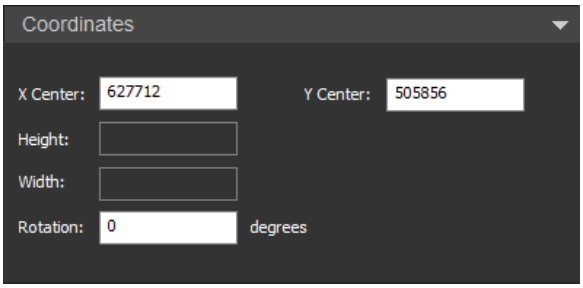
    The allowable maximum range is $-2^{31}+1$ to $2^{31}-1$.
  - **Log scaling:**

    The allowable minimum range is 1 to $2^{31}-2$.

    The allowable maximum range is 2 to $2^{31}-1$.
- If you enter an out-of-range number, the software defaults to the minimum or maximum value in the allowable range.

Size and Coordinates group for Oval gates

For an Oval gate, you can enter **center point coordinates**, and the **height**, **width**, and **rotation** values.



- Non-numeric characters are not permitted.
- The allowable X and Y coordinates depend on the type of scale selected:
  - **Linear and HyperLog™ scaling:** The allowable range is -2^{31} to $2^{31}-1$.
  - **Log scaling:** The allowable range is 1 to $2^{31}-1$.
- The allowable height range is -2^{31} to $2^{31}-1$.
- The allowable width range is -2^{31} to $2^{31}-1$.
- The allowable rotation range is –360 to 360.
- If you enter an out-of-range number, the software defaults to the minimum or maximum value in the allowable range.

Coordinates group for Histogram gates

For a Histogram gate, you can enter **left** and **right boundary coordinates**.

- Non-numeric characters are not permitted.
- The allowable coordinates depend on the type of scale selected:
  - **Linear and HyperLog™ scaling:**

    The allowable minimum range is-2^{31} to $2^{31}-2$.

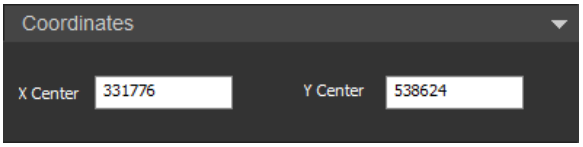
    The allowable maximum range is $-2^{31}+1$ to $2^{31}-1$.
  - **Log scaling:**

    The allowable minimum range is 1 to $2^{31}-2$.

    The allowable maximum range is 2 to $2^{31}-1$.
- If you enter an out-of-range number, the software defaults to the minimum or maximum value in the allowable range.

Coordinates group for Quadrant gates

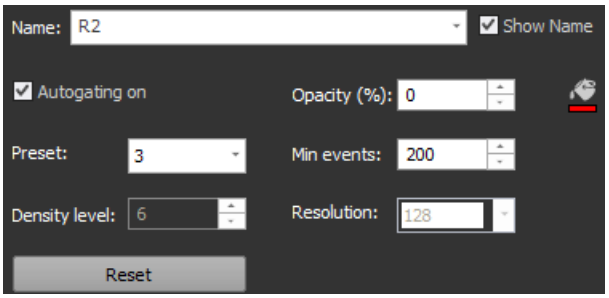
For a Quadrant gate, you can enter the **center point coordinates**.



- Non-numeric characters are not permitted.
- The allowable X and Y coordinates depend on the type of scale selected:
  - **Linear and HyperLog™ scaling:** The allowable range is -2^{31} to $2^{31}-1$.
  - **Log scaling:** The allowable range is 1 to $2^{31}-1$.
- If you enter an out-of-range number, the software defaults to the minimum or maximum value in the allowable range.

Autogating options

Autogating options in the Customize Gate Options panel allow you to customize settings for the selected Contour or Ellipse Autogate.



Customize Autogating options are available only when a Contour Autogate or an Ellipse Autogate is selected from a plot in the Workspace.

Note: To set default Autogating options, use the Options dialog ▶ Gate Options tab (page 486).

Autogating on

Autogating on checkbox allows you to turn the autogating function for the selected auto region on or off. This control is enabled when an auto region is selected from a plot in the Workspace.

- **Autogating on** control sets the selected auto region to the following state, depending on whether it is checked or unchecked:

Previous state	New state when checked	New state when unchecked
Autogate ON	Autogate ON	Autogate OFF
Autogate ON – [NO FIT]	Autogate ON – [NO FIT][1]	Autogate OFF
Autogate OFF	Autogate ON – [NO FIT]	Autogate OFF

[1] An autogate that is ON but with NO FIT becomes an ON autogate at this point, if the criteria for a successful autogate are met.

Reset

Reset button is enabled when an auto region is selected. When clicked, autogates will revert to the global default settings set in the Gate Options menu.

Preset

Preset dropdown allows you to set the **Preset** for individual autogates selected from a plot in the Workspace. A Preset consists of Density Level and Resolution settings.

- The Preset dropdown has the following options:
  - **1** (Density level: 30, Resolution: 128)
  - **2** (Density level: 10, Resolution: 128)
  - **3** (Density level: 6, Resolution: 128)
  - **4** (Density level: 1, Resolution: 128)
  - **Custom** (By default, Density level: 5 and Resolution: 64, but you can change the custom settings using the **Density level** and **Resolution** controls)
- When a Preset is selected for an autogate, the Density level and Resolution controls display the values that correspond to the Preset choice for that autogate.
- By default, Preset is set at 3. However, you can change the default Preset in the Options dialog ▶Gate Options tab (page 486).

Min events

Sets the **Min events** value for the selected autogate, which is the number of events that must be contained within the auto region for the population to be autogated.

- By default, Min events is set to 200.
- The range of values for Min events is 1 to 9,999,999.
- If you enter a Min events value that is outside the numeric limits, the value defaults to the maximum allowed number, if the value exceeds the upper limit, or to the minimum allowed value, if the entered value is less than the lower limit.

Density level

Sets the default **Density level** for the selected autogate.

- The Density level field is populated the value that corresponds to the selected Preset option (see Preset, above).
- The range for Density level is 1 to 100.
- The Density level control is disabled unless the **Custom** option is selected in the Preset dropdown.

Resolution

Sets the default **Resolution** for the selected autogate.

- The Resolution field is populated with the value that corresponds to the selected Preset option (see Preset, above).
- The Resolution dropdown has the following options: **64**, **128**, **256**, **512**, **1024**.
- The Resolution control is disabled unless the **Custom** option is selected in the Preset dropdown.

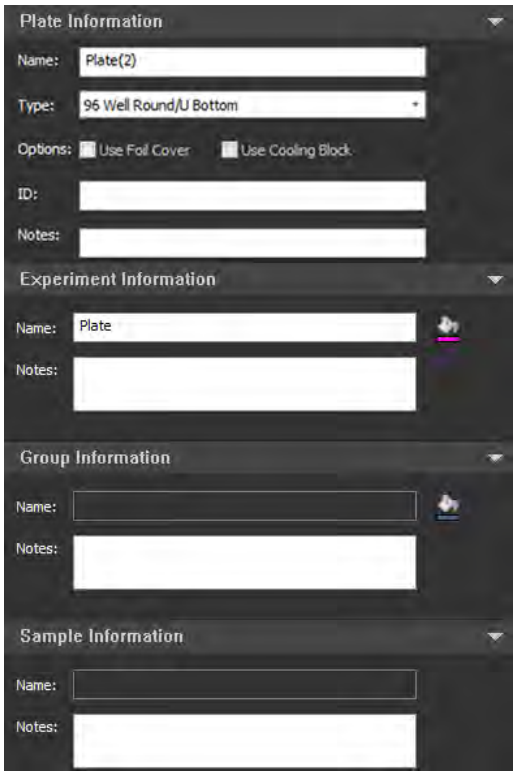
Customize Experiment (Plate or Tube) options

Overview

Customize Plate Experiment and *Customize Tube Experiment options* allow you to customize the following:

- Plate information (available only for Plate Experiments)
- Experiment information
- Group information
- Sample information

Customize panel for Plate Experiments



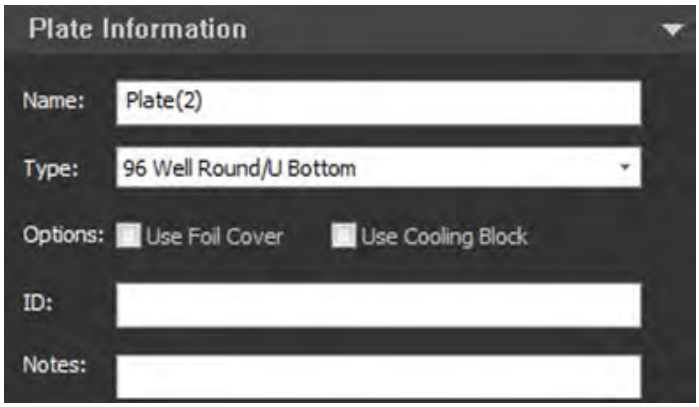
- Customize Plate options are available only when a Plate Experiment is displayed and the *Heat Map view* (page 138) is in focus.
- The Customize Plate options are arranged into the following groups:
  - *Plate information* (page 305)
  - *Experiment information* (page 307)
  - *Group information* (page 308)
  - *Sample information* (page 309)
- Each group is contained in a group box that is collapsible.
- The values displayed in any field are the current settings.
- Plate information is automatically saved and applied to all Samples in the Plate, including any Tubes added to the Plate Experiment, after the focus is lost from the selected field.
- Experiment information is applied to the selected Experiment after the focus is lost from the selected field.
- Group information is automatically saved in the selected Group after the focus is lost from the selected field.
- Sample information is automatically saved and applied to all selected Samples after the focus is lost from the selected field.

Customize panel behavior for Tubes

When a Tube Experiment is loaded, the customizations options are the same as the options available for Plate Experiments, but the Plate information options (page 305) are not displayed.

Plate Information

Plate Information options are available only when a Plate Experiment is displayed in the application and the Heat Map view (page 138) is in focus.



Note: Plate information options are not available when a Tube Experiment is loaded.

Name

- The **Name** field displays the current name of the Plate.
- To modify the name of the selected Plate, enter the new name in the **Name** field. The entered name is automatically saved and applied to the Plate once the focus is lost from the Name field.
- The Plate Name field in the Experiment Explorer (page 191) is automatically updated when the new name is entered and validated by the database.
- You can enter up to 50 alpha-numeric characters in the **Name** field.
- Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning balloon indicates the error condition, and the invalid characters do not appear in the Name field.

  The following characters are not allowed: \ / : * ? ″ < > | %.
- If an invalid name is present in the Name field when focus is lost, the name reverts to the previous valid name.
- Duplicate names are not permitted. If the entered name already exists, a numerical suffix in parentheses is added to the Plate name when the focus is lost; for example, **Plate(2)**.

  If the addition of the suffix causes the Plate name to exceed the 50-character limit, the entered name is truncated.

Plate Type

- The Plate Type dropdown list allows you to select the type of micro-titer plate (U-, V-, or flat-bottom) that is being used for the current Plate (96- or 384-well). The following options are available:
  - 96 Well Round/U Bottom
  - 96 Well Flat Bottom
  - 96 Well Conical/V Bottom
  - 96 Deep Well Round/U Bottom
  - 96 Deep Well Conical/V Bottom
  - 384 Well Round/U Bottom
  - 384 Well Flat Bottom
  - 384 Well Conical/V Bottom
  - 384 Deep Well Round/U Bottom
  - 384 Deep Well Conical/V Bottom
- You cannot switch between 96- and 384-well plates. The dropdown list is limited to 96- or 384-well plate options based on the original Plate selected when creating the new Experiment as described in "Creating a Plate Experiment", page 402.

Options

- **Use Foil Cover** allows you to set up a Plate Experiment using a foil cover to protect the sample plate from condensation or evaporation.

  When the **Use Foil Cover** option is enabled, the autosampler disables the probe collision sensor, which allows the use of a foil cover on the plate.
- **Use Cooling Block** allows you to set up a Plate Experiment using a cooling block. When selected, the autosampler accounts for the extra height that the cooling block adds to the plate specification.
- **Use Foil Cover** and **Use Cooling Block** options are available only when a CytKick™ Max Autosampler is connected to the Attune™ instrument. Otherwise, they are not visible.
- When available, you can also set the **Use Foil Cover** and **Use Cooling Block** options in the New Experiment dialog (page 399).

Plate ID

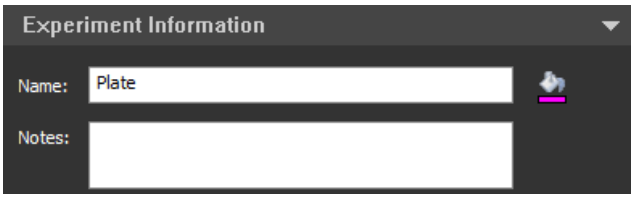
- The Plate ID field allows 50 characters to be entered to identify the selected Plate. Any printable character can be entered.
- The Plate ID is applied to the Plate and is used by any Tubes added to the Experiment.

Notes

- You can enter up to 500 characters in the Notes field to provide relevant information about the Plate; any printable character is permitted.
- The notes are saved with all Samples in the Plate, including any Tube Experiments added to the Plate.
- Two lines of space are given by default. If you enter more text than can fit in the available space, a vertical scroll bar and the "…" button is displayed adjacent to the text field.
- Clicking the **… button** opens the *Notes dialog*, which contains an editable text field that allows up to 500 characters to be displayed and edited. You can enter any printable character.

Experiment information

Experiment information options are available when the Heat Map view (page 138) is in focus and any Sample is selected.



Name

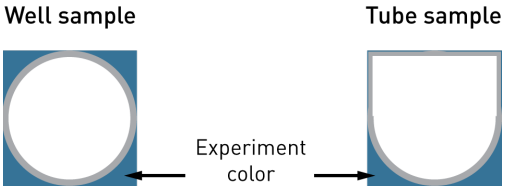
- The **Name** field displays the name of the currently selected Experiment.
- You can modify the name of the selected Experiment by entering the desired name in the **Name** field. The entered name is automatically saved and applied to the Experiment once the focus is lost from the Name field.
- The Experiment Name field in the Experiment Explorer (page 190) is updated automatically when the new name is entered and validated by the database.
- You can enter up to 50 alpha-numeric characters in the **Name** field. Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning balloon indicates the error condition, and the invalid characters do not appear in the Name field. The following characters are not allowed: \ / : * ? ″ < > | %.
- If an invalid name is present when focus is lost, then the name reverts to the previous valid name.
- Duplicate names are not permitted. If the entered name already exists, a numerical suffix in parentheses is added to the Experiment name when the focus is lost; for example, **Experiment(2)**.

  If the addition of the suffix causes the name to exceed the 50-character limit, the entered name is truncated.

Experiment color picker

- Clicking the **color icon** opens a standard color picker dialog, which allows you to customize the color for the current Experiment.

- The Experiment color appears on the Heat Map view.



Notes

- The Notes field for the Experiment information works the same way as the Notes field for the Plate information (page 306).

Group information

Group information options are available when the Heat Map view is in focus and any Samples belonging to the same Group are selected.

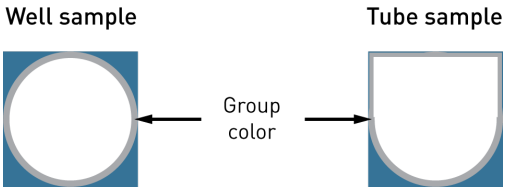
Name

- The **Name** field displays the name of the currently selected Group.
- You can modify the name of the selected Group by entering the desired name in the **Name** field. The entered name is automatically saved and applied to the selected Group once the focus is lost from the Name field.
- The Group Name field in the Experiment Explorer (page 190) is updated automatically when the new name is entered and validated by the database.
- You can enter up to 50 alpha-numeric characters in the **Name** field. Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning balloon indicates the error condition, and the invalid characters do not appear in the Name field. The following characters are not allowed: \ / : * ? ″ < > | %.
- If an invalid name is present when focus is lost, then the name reverts to the previous valid name.
- Duplicate names are not permitted. If the entered name already exists, a numerical suffix in parentheses is added to the Group name when the focus is lost; for example, **Group(2)**.

  If the addition of the suffix causes the name to exceed the 50-character limit, the entered name is truncated.

Group color picker

- Clicking the **color icon** opens a standard color picker dialog, which allows you to customize the color for the current Group.
- The Group color appears on the Heat Map view.



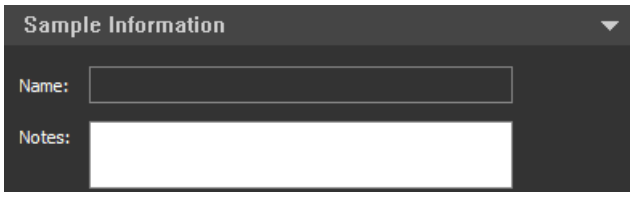
- This option is disabled when the selected Well or Tube is a Compensation control. When multiple Wells are selected, the Group color is not applied to Compensation controls.

Notes

- The Notes field for the Group information works the same way as the Notes field for the Plate information (page 306).

Sample information

Sample information options are available when the Heat Map view is in focus and a Tube or a Well sample is selected.



- The Name field is hidden if multiple Samples are selected in the Heat Map view.
- The Notes section is enabled and visible when multiple Tube or Well samples are selected.

Name

- The **Name** field displays the name of the currently selected Sample.
- You can modify the name of the selected Sample by entering the desired name in the **Name** field. The entered name is automatically saved and applied to the Sample once the focus is lost from the Name field.
- The Sample Name field in the Experiment Explorer (page 190) is updated automatically when the new name is entered and validated by the database.
- You can enter up to 50 alpha-numeric characters in the **Name** field. Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning balloon indicates the error condition, and the invalid characters do not appear in the Name field. The following characters are not allowed: **\ / : * ? ” < > | %**.
- If an invalid name is present when focus is lost, then the name reverts to the previous valid name.
- Duplicate names are not permitted. If the entered name already exists, a numerical suffix in parentheses is added to the Sample name when the focus is lost; for example, **Sample(2)**.

  If the addition of the suffix causes the name to exceed the 50-character limit, the entered name is truncated.

Notes

- The Notes field for the Sample information works the same way as the Notes field for the Plate information (page 306).

Customize Overlay options

Overview The *Customize* menu displays options for editing overlay plots when Overlay or Gallery plots are selected. The options displayed depend on whether Overlay plots or Gallery plots are selected.

You can customize the appearance of Overlay and Gallery plots in an Overlay using options available in the Customize menu.

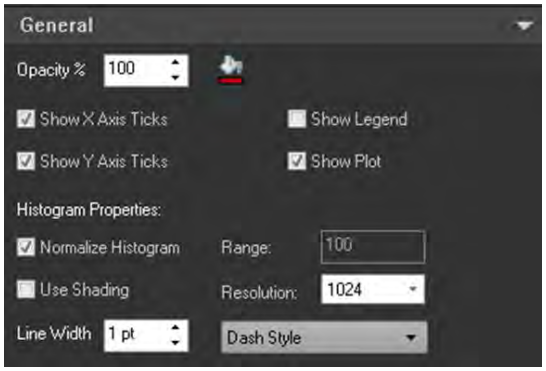
Overlay plots selected

Gallery plots selected

- The Customize options are arranged into the following functional groups:
  - *General options* (page 311)
  - *3D options* (available only for Overlay plots) (page 314)
  - *Calculations* (available only for Gallery plots) (page 315)
  - *Text* (page 316)
- Each functional group is contained in a collapsible group box.

General options

General options control line style and plot display options. They are available for both Overlay plot and Gallery plots.



Opacity

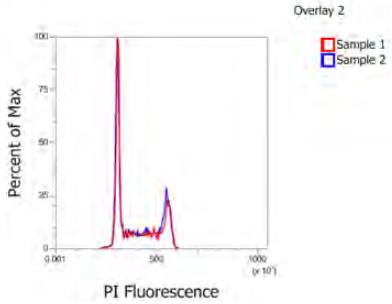
- The **Opacity** option controls the opacity of the line color for Gallery or Overlay Histograms or the data color for dual-parameter plots.
- To change the **Opacity,** enter a number between **20** and **100** into the **Opacity text field**.
- If Histograms are filled, the opacity option applies to both the histogram line and the fill of the histogram.

Data Color

- Click **Data Color** to open the color picker dialog, which allows you to choose the line color for the selected Histograms or the data color for selected dual-parameter plots.
- This option is available when any individual or a combination of gallery plots is selected.
- The selected color is applied to all selected plots.

Show Legend

- Select the **Show Legend** option to display the legend for an Overlay plot or displays the plot statistics as a legend for a Gallery plot.
- The legend appears to the right of the plot.



Show X-Axis and Show Y-Axis Ticks

- By default, **Show X-Axis Ticks** and **Show Y-Axis Ticks** options are checked.
- When the **Show X-Axis Ticks** option is checked, the X-axis tick marks are displayed on selected plots.
- When the **Show Y-Axis Ticks** option is checked, the X-axis tick marks are displayed on selected plots.
- When the options are unchecked, the corresponding tick marks are not shown on the selected plots.

Line Width

- The **Line Width** option is only active when Histogram plots in the Gallery or Histogram-based Overlay plots are selected.

  This option is unavailable when only dual-parameter plots are selected.

  If a mixture of plot types are selected (Histograms and dual-parameter plots), this option is available, but only applies to Histogram plots.
- The **Line Width text box** allows adjustment of the line width of selected histogram plots. You can directly enter an integer between 1 and 5 in the textbox.

  Alternatively, you can use the **up** and **down arrows adjacent to the text box** to increase or decrease the line width between **1** and **5**.
- If you enter a line width value that is outside the allowed range, the number is automatically adjusted to the nearest limit.
- If you select different line widths for individual gallery plots, the line width textbox initially appears blank with the edit box active.
- Click the **up arrow** to increase the line width in steps of 1 for each gallery plot and the overlay plot.
- Click the **down arrow** to decrease the line width in steps of 1 for each gallery plot and the overlay plot.
- The up and down arrow buttons have no effect if the edit field is blank.

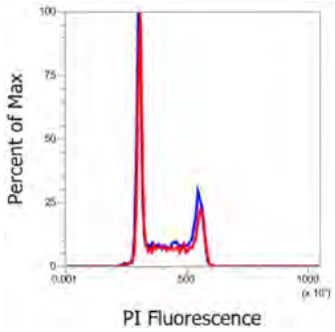
Dash Style

- The **Dash Style** option is only available when Histogram Gallery plots or Histogram Overlay plots are selected.
- The option is not visible when only dual-parameter plots are selected.
- If you select a mixture of plot types (Histograms and dual-parameter plots), this option is visible, but only applies to the selected histogram plots.
- The **Dash Style** dropdown displays a list of available dash styles (solid, dash, dots, dash dot, dash dot dot)

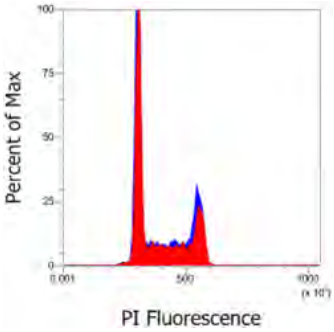
- The dash style selected from the dropdown list applies to all selected Gallery plots or individual members of Overlay plots.
- The default dash type is a solid line.

Use Shading

- **Use Shading** checkbox controls the shading for the selected Histogram plots.
- This option applies only to Histogram Overlay plots or Gallery plots.



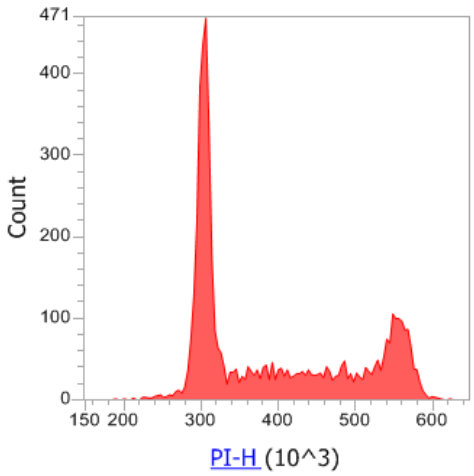
Use Shading - OFF



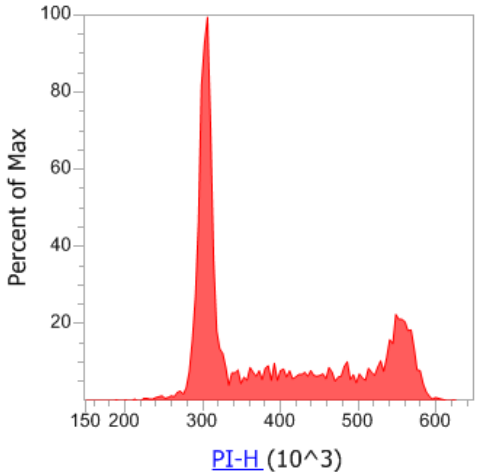
Use Shading - ON

Normalize Count

- **Normalize Count** checkbox controls the scaling for the selected Histogram plots.
- This option applies only to Histogram plots, and it is only displayed when one or more Histogram plots are selected.
- When the **Normalize Count** is unchecked, the scale label is **Count**.
- When the **Normalize Count** is checked, the Y-axis scaling is changed to scale the plot to the Histogram peak value.
- The scale displayed is a percentage scale from 0 to 100%, where 100% is the Histogram peak value.
- The scale label is **Percent of Max.**



Normalize Count - OFF



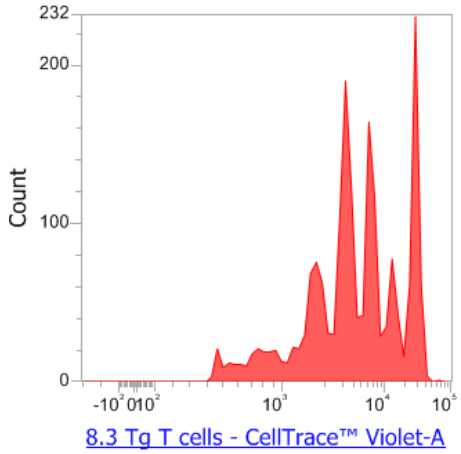
Normalize Count - ON

Range

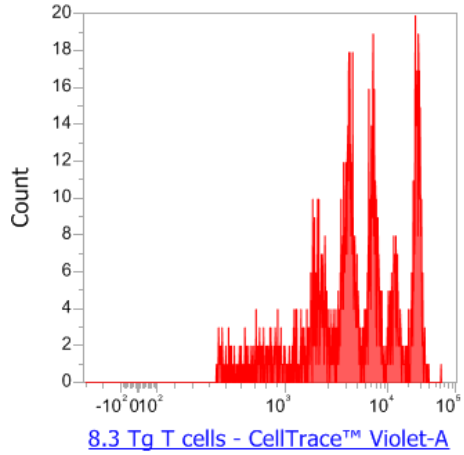
- **Range** sets the upper y-axis range for the selected histograms. The lower end of the range is always set to 0.
- To change the **Range**, enter the desired value in the **Range textbox**. Range will not be automatically set and must be manually adjusted.
- The **Range** option control is only enabled when the normalize histogram checkbox is unchecked.

Resolution

- **Resolution** determines the resolution for the selected histograms and sets the bin size for drawing the histogram data.
- The available resolutions are **64**, **128**, **256**, **512**, and **1024**.
- The default resolution is 1024.



Resolution: **64**

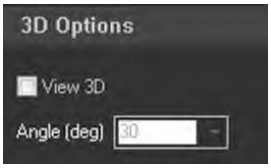


Resolution: **1024**

3D Options

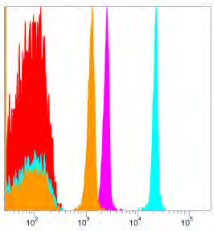
3D Options allow you to display Overlay plots three dimensionally and set the view option for 3D plots.

3D options are available only when one or more Overlay plots are selected. You cannot view Gallery plots in the 3D mode.

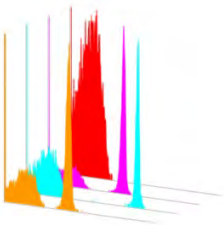


View 3D

- Select **View 3D** to display selected Overlay plots in the *3D mode*.



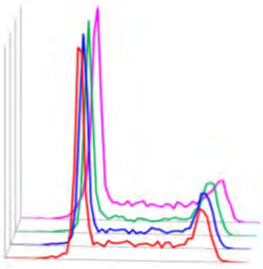
Overlay mode



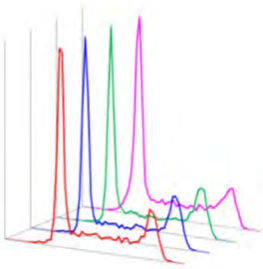
3D mode

Angle(deg)

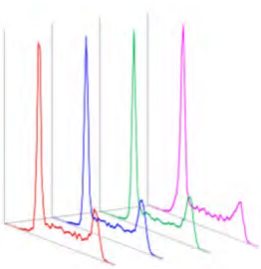
- **Angle(deg)** determines the viewing angle of the Overlay plot in the 3D mode.
- The **Angle (Deg) dropdown** becomes active only when **View 3D** is selected.
- **Angle(deg)** dropdown displays a list of angles (5, 15, 30, 45, 60, 75 degrees). Increasing the angle rotates the 3D plot clockwise.



5 Degrees



30 Degrees



60 Degrees

Calculations

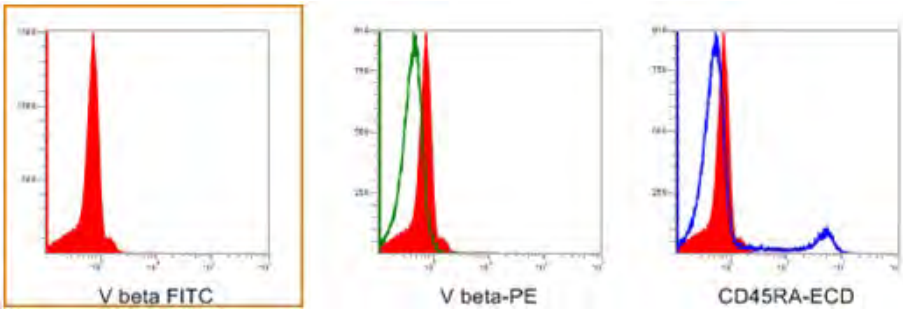
The **Calculations** functional group is displayed only for Gallery plots.

The Calculations options are available only for single-parameter plots; they are not displayed if a dual-parameter Gallery plot is selected.

Set as Control

- **Set as Control** option designates the selected plot as the "control plot" and overlays all Gallery plots with it to allow direct visual comparison of several "test plots" to one "control plot".

  The Set as Control button is available only when a single Gallery plot is selected. By default, Set as Control is deselected.

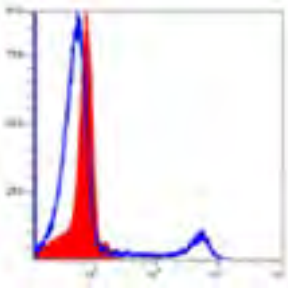


- The plot set as a control is always moved to the first position in the Gallery and highlighted in a dashed orange rectangle.
- To remove a control plot, click **Remove control**.

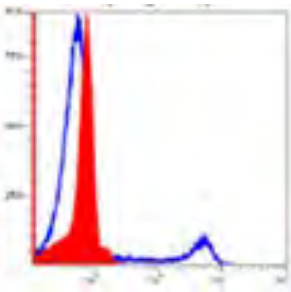
Move to Front

- Click **Move to Front** to display the control plot overlay on each Gallery plot in front of the data plot.

  Click the button again to move the control plot behind the selected plot in the Gallery view.
- Move to Front button is available only when the Set as Control option has been selected.
- By default, this option is not selected and the data plot is displayed in front of the control plot.



Move to Front - OFF



Move to Front - ON

Calculation Method Radio Buttons

- **Calculation Method** radio buttons are only available once a plot has been set as a control.
- By default, **None** is selected and no calculations are applied.
- The **Overton** option compares the control sample and each Gallery plot using Overton's cumulative statistics calculation (page 628).
- The **KS** option compares the control and test samples using the Kolmogorov-Smirnov test (page 628) to determine the probability that the two plots are the same.

Text

Text options allow you to customize the plot text on Overlay or Gallery plots.

Text options consist of the Text group and the Legend group controls.

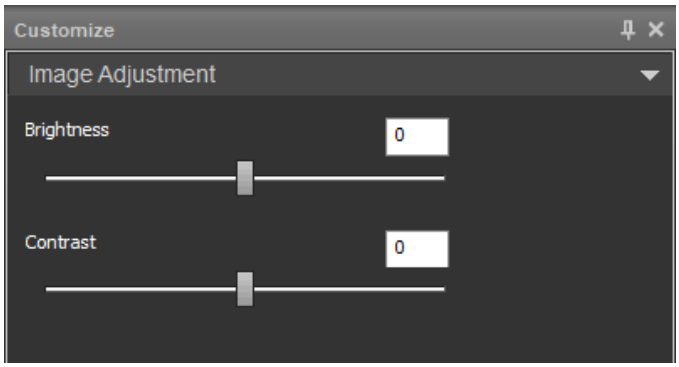
- The *Text group options* are the same as those described for the "Text group" in "Customize Plot Options", page 288.
- The *Legend group options* contain the sample name and Parameter name checkboxes.
- Select the **sample name** and/or the **Parameter name** checkbox to display the corresponding legend on the plot. You can select either one or both options for display
- You can edit the legend font size using the **Font size** control. Font size is selected from the Font size **dropdown menu**, or by entering a number between **6** and **72** into the **font size text field**.

Customize Image Adjustment options

Image Adjustment options

Customize Image Adjustment group allows you to adjust the Brightness and Contrast of the cell image in a Cell Image Container in the Workspace or the active image in the Image View tab.

The Image Adjustment options are not available for Workspace images, which are distinct from images in Cell Image Containers or Image View tab images.



- Slider controls and a corresponding edit control allow adjustment of brightness or contrast.
- Changes to brightness and contrast are applied to all images in the image gallery and view.
- The brightness control allows a value from –255 to 255.

  The contrast control allows a value from –100 to 100.
- For images in cell image containers on the Workspace, the contrast and brightness settings are applied on a per cell image basis and the setting persists with the Workspace.

  If multiple cell image containers are selected in the Workspace, the values displayed in the Image Adjustment customize panel depend on whether the values for all selected images are the same.

  If the brightness and contrast values are different for the selected images, the slider shows the value set at 0 and the edit control will be blank for the corresponding setting.

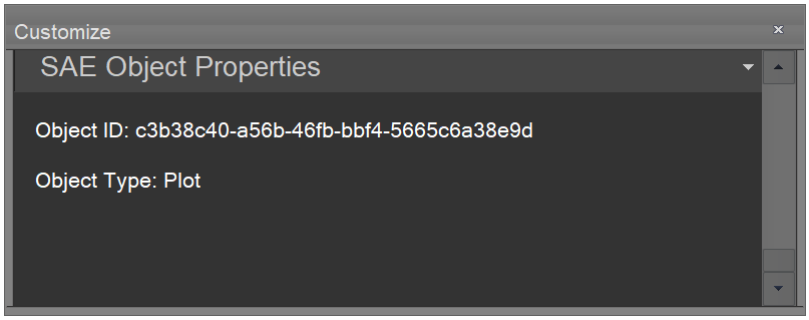
  If the brightness and contrast values match for the selected images, they show the actual value.

  If the value for contrast or brightness is changed, the new value is applied to all selected cell images.
- For images in the active image area and image gallery of the Image View tab, the change to brightness and contrast is set globally for all cell images. The brightness and contrast adjustment values persist with the Workspace.

SAE Object Properties

Overview When the Attune™ Cytometric Software is in the SAE mode, you can view the ID of workspace objects in the object's customize panel. Each customize panel will have an additional group where the "SAE Object Properties" for the selected workspace object are displayed.

- Each workspace object has a unique ID or GUID. This ID is an immutable property and cannot be changed. This ID is used by the SAE console to enable auditing of workspace objects.
- The workspace objects that have a corresponding GUID are workspace plots, workspace gate, workspace text boxes, workspace statistics boxes, workspace images, overlay plots, and overlay/gallery plots.
- The SAE Object Properties displays the Object ID and Object Type.
- You can copy the Object ID to the clipboard by double clicking on the text of the Object ID.



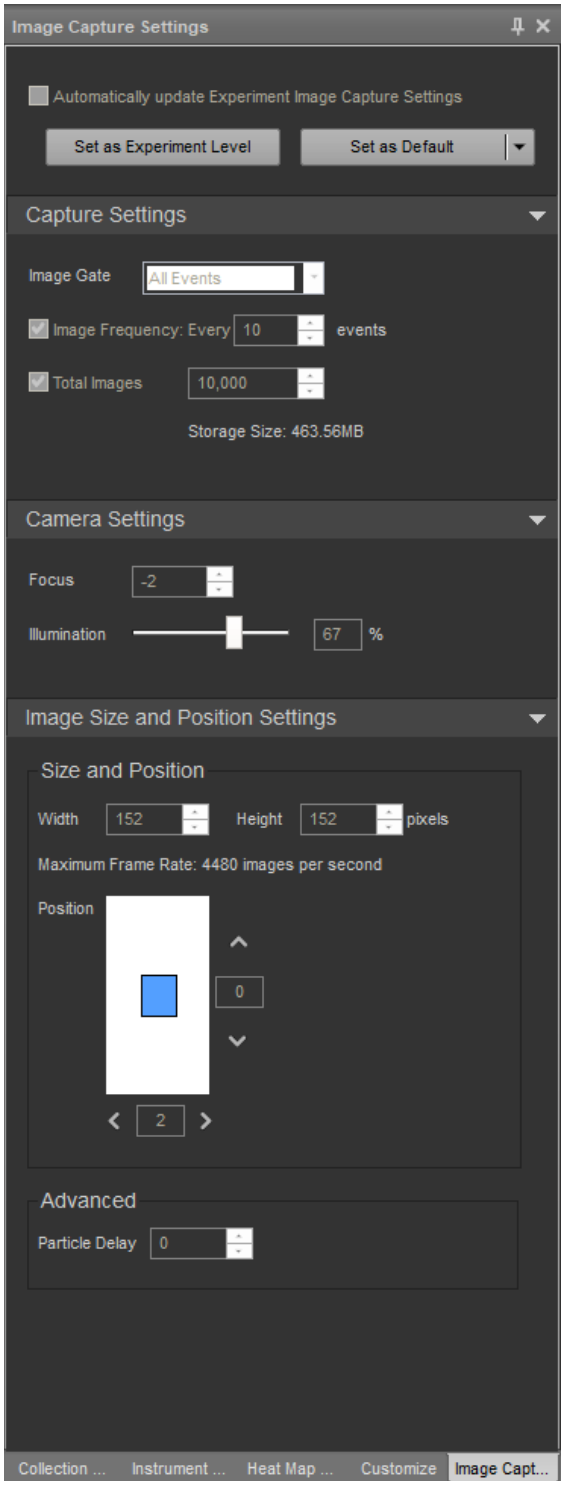
16. Image Capture Settings panel

Overview

Image Capture Settings panel allows you to adjust settings that affect the capture of images.

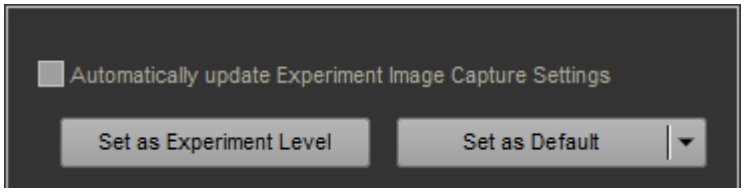
- Image Capture Settings panel is only available when using an Attune™ CytPix™ Flow Cytometer.
- By default, the panel is docked to the left of the Main Application Area. The docking properties of the panel are described on page 37.
- The panel contents are organized into the following groups:
  - *Settings options* (page 320)
  - *Capture Settings* (page 321)
  - *Camera Settings* (page 323)
  - *Image Size and Position Settings* (page 324)
- Each group can be collapsed and expanded, except General panel properties.

  By default, the groups are all expanded, unless otherwise specified below. The expanded or collapsed state persists on a per user basis.
- During Run mode, Image capture settings can be adjusted to optimize image capture, and they can be stored at the Sample or Experiment level.
- While recording or after data is recorded, the Image Capture Settings are disabled to reflect the state in which the sample was recorded.



Settings options

Settings options section of the Image Capture Settings panel contains three controls:



- Automatically Update Experiment Image Capture Settings check box
- Set as Experiment Level button
- Set As Default split button

Automatically Update

When selected, **Automatically Update Experiment Image Capture Settings** option automatically applies the changes to Capture, Camera, and Image Size and Position Settings to the Experiment-level settings.

This checkbox is disabled if the current active Experiment Explorer item is a Compensation node or a Sample with Sample-level Image Capture Settings.

Set as Experiment Level

Set as Experiment Level updates the appropriate Experiment-level Image Capture Settings with the settings of the active sample (Tube or Well).

Set all applicable samples (tube or well) in the Experiment to use the new Experiment-level Image Capture Settings.

There is an Experiment level Image Capture Settings for tubes and another one for plates.

All newly created samples use the Experiment-level Image Capture Settings until they are activated or edited, at which point a Sample-level Image Capture Setting is created for the applicable samples. This newly created setting is a copy of the Experiment-level Image Capture Settings.

Set as Default

Set as Default split button contains the following three options:

- **Set as Default** option sets the current Image Capture Settings as the default for all future Plate or Tube experiments run by the current user.
- **Load** option opens the standard File Open (Import) dialog, which allows you to import image capture settings from a saved Image Capture Settings file (*.aics) and update the current settings with the imported settings.
- **Export** option opens the standard File Save (Export) dialog, which allows you to export the current Image Capture Settings with a user defined name. If the name selected for the capture settings matches the name of another capture setting in the database, you will be prompted for confirmation before the existing image capture setting is overwritten.

Capture Settings

Capture Settings allows you to set image capture criteria for your sample and contains the Image Gate, Image Frequency, and Total Images controls. It also provides information about the Storage Size required for the captured images based on your capture criteria.

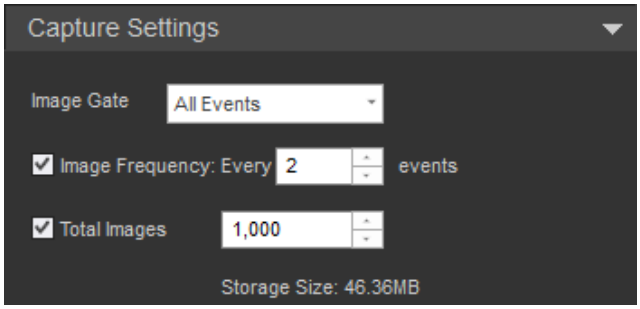
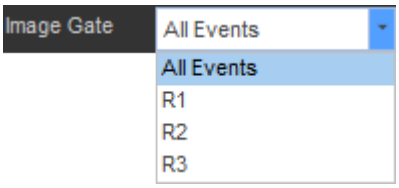


Image Gate

Image Gate dropdown allows you to set an image capture gate, so that the images are captured only from a specific subset of events on a Workspace plot. Events that are outside of the Image Gate are excluded from image collection.

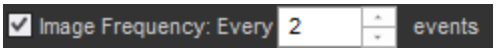


- Image Gate dropdown only allows Rectangle gates on dual-parameter plots and Histogram gates on single-parameter plots to be used as the image capture trigger gate. The default trigger gate is All Events.
- The coordinates of the trigger gate and the corresponding parameter define the image threshold that triggers the system to capture an image if a cell's signal falls within the trigger window's minimum and maximum values (inclusive).
- The image trigger window persists on a per Sample or per Experiment basis.
- If the trigger gate is moved during acquisition, the new trigger threshold values are sent to the instrument.

  If the trigger gate is deleted during acquisition, the trigger window defaults to All Events. In this case, any event triggers image acquisition.
- The image settings persist as part of the Experiment on a per Experiment or Sample basis.
- Image Gate does not allow you to set up population hierarchies; it only supports a top-level perspective.

Image Frequency

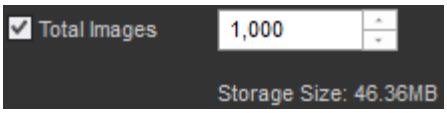
Image Frequency sets the image capture frequency such that every Nth event that exceeds the imaging trigger setting is imaged. This allows you to capture only a subset of reference images rather imaging all events and reduces the amount of data storage required for image collection.



- By default, Image Frequency setting is unchecked.
- By default, Image Frequency is set to every 10 events.
- You can set an Image Frequency value of between every 1 and 10,000 events.
- If the Image Frequency value is changed during a run, the new frequency setting is applied only to new events and will not affect any previously acquired images.
- While recording data or after the recording is completed, the image capture settings are disabled to reflect the state in which the sample was recorded.

Total Images and Storage Size

Total Images sets the maximum number of images to be acquired during a run (if selected). This allows you to limit the number of images collected and reduces the amount of data storage required for image collection.



- By default, Total Images setting is checked and set to 10,000 events.
- You can set a Total Images value of between every 1 and 30,000 events.
- If the Total Images value is changed during a run, the new setting is applied to the runtime total.

  If the Total Images value is changed such that the total number of imaged events is less than the number of currently captured events, no new images are acquired.
- While recording data or after the recording is completed, the image capture settings are disabled to reflect the state in which the sample was recorded.

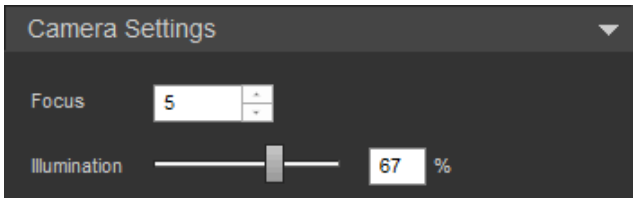
Storage Size displays the amount of storage needed to store the captured when the Total Images option is enabled.

- The Storage Size value is calculated from the total number of images and the image size (based on width and height specified in Image Size and Position Settings).
- If the number of images, image width, or image height are changed, the Storage Size is automatically recalculated and the new value is displayed.
- When the Total Images option is disabled, **Estimated Total Images** is displayed instead of Storage Size.

Note: By default, captured images are stored in the following location:
C:\Users\Public\Documents\Life Technologies\AttuneCytPix_Images
User with Administrator or System Administrator roles can select a different image storage path using the Attune™ Database Utility (page 538).

Camera Settings

Camera Settings allows you to control the camera focus and illumination.



Focus

Focus control allows you to adjust the focus of the camera used for image capture.

- Camera focus is pre-set to the optimal position with the default focus value of 0.
- To change the focus of the camera, enter a number into the focus edit field or adjust the value using the adjacent up and down controls.
- The up and down controls change the focus value by 1.
- The focus edit field only accepts integers between –1000 and 1000.
- If the entered focus value exceeds the limits, the focus edit field reverts to the last good value and a warning message instructs you to enter a number within the acceptable range.
- While recording or once data is recorded, the camera settings are disabled to reflect the state in which the sample was recorded.

Note: Although the focus is pre-set to the optimal position by service engineers, depending on cell size, cell type, or flow rate, you might choose to modify the focus value to suit the needs of your experiment.

Usually, a change of ± 10 units is sufficient to find the ideal focus position for most applications.

Illumination

Illumination slider bar and edit field allow you to adjust the camera illumination used for image capture.

- The default value for camera illumination is 100%.
- To change the camera illumination, enter a number into the illumination edit field or adjust the value using the illumination slider.
- The illumination edit field only accepts integers between 1 and 100.

  Clicking the keyboard up (or right) and down (or left) arrow buttons while the edit control is active changes the illumination value by 5 units.
- The illumination slider has a resolution of 1 unit.

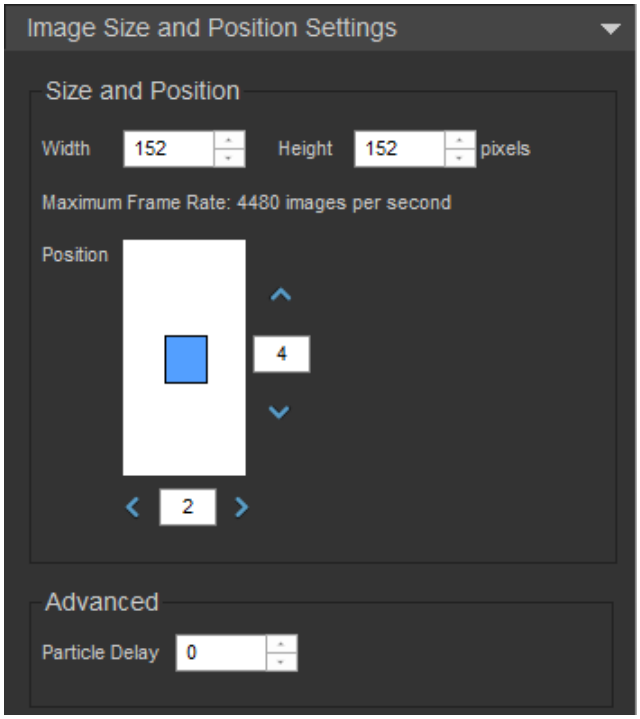
  Clicking the keyboard up (or right) and down (or left) arrow buttons when the slider control is active changes the illumination value by 1 unit.

  Clicking the keyboard Page Up and Page Down buttons while the slider control is active changes the illumination value by 5 units.
- If the entered focus value exceeds the limits, the illumination edit field reverts to the last good value and a warning message instructs you to enter a number within the acceptable range.
- While recording or once data is recorded, the camera settings are disabled to reflect the state in which the sample was recorded.

Image Size and Position Settings

Image Size and Position Settings allows you to adjust the size and position of the image window and to set particle delay. It also displays the image frame rate, which is calculated based on the height and width of the image window.

While recording or once data is recorded, the Image Size and Position Settings are disabled to reflect the state in which the sample was recorded.

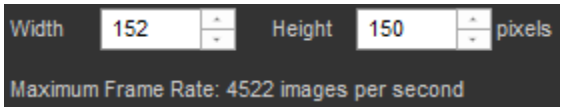


Note: Changing image capture settings is not instant; it takes 1–2 seconds for the camera to be cleared and streaming to restart. We do **not** recommend changing the image size and position incrementally with mouse clicks.

Size and Position

Size

Image size controls consist of **Width** and **Height** controls, which allow you to set the image dimensions.



- **Width** edit field allows you enter a value from 96 to 248 pixels for the width of the image window. The up and down buttons adjust the width by 8 pixels each click.
- **Height** edit field allows you enter a value from 96 to 248 pixels for the height of the image window. The up and down buttons adjust the height by 2 pixels each click.
- The default values for Width and Height are 152 pixels, which is the optimal size for the image window.
- If you change the image size and capture new images, all the images shown in Image Gallery will have the new aspect ratio as defined by new width and height.
- **Maximum Frame Rate** displays the maximum rate by which the system can trigger the camera for image capture, which is calculated based on the pixel size, image window, and system electronics.

Position

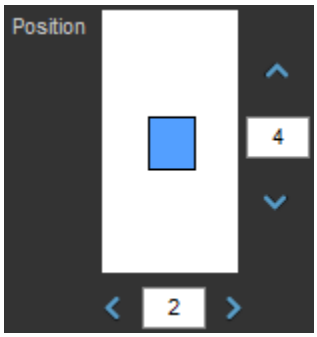


Image position controls allow you to adjust the position the image window within the scanning region. The controls consist of a blue rectangle that represents the image window within the scanning region and directional arrow controls and position edit fields to adjust the coordinates of the image window.

- The **blue rectangle** represents the image window and the **white rectangle** represents the scanning region that surrounds it.

  Changing the size of the image window using the **Width** and **Height** controls changes the size of the blue rectangle that represents it.
- The maximum and minimum size of the image window is 248 × 248 pixels and 96 × 96 pixels (width × height), respectively.

  96 × 96 pixels correspond to a field of view that is 29 × 29 µm and 248 × 248 pixels correspond to a field of view that is 74 × 74 µm.

  The default size of the image window is 152 × 152 pixels (46 × 46 µm field of view), which is also the optimal size for image collection.
- The size of the scanning region is fixed at 450 × 750 pixels (width × height). The scanning region adheres to the coordinate system where the coordinates are (-/-) on the top left and (+/+) on the bottom right.
- By default, the image window (blue rectangle) is centered around the coordinates 0 and 0.
- You can change the position of the image window within the scanning region using one of the following methods:
  - Drag and drop the blue rectangle in a new position within the scanning region.
  - Enter the new position coordinates into the X and Y coordinate fields.
  - Use the directional arrows to adjust the position of the image window by 1 pixel increments.

Advanced

Advanced group consists of the **Particle Delay** control, which allows you to adjust the image delay relative to the trigger event.

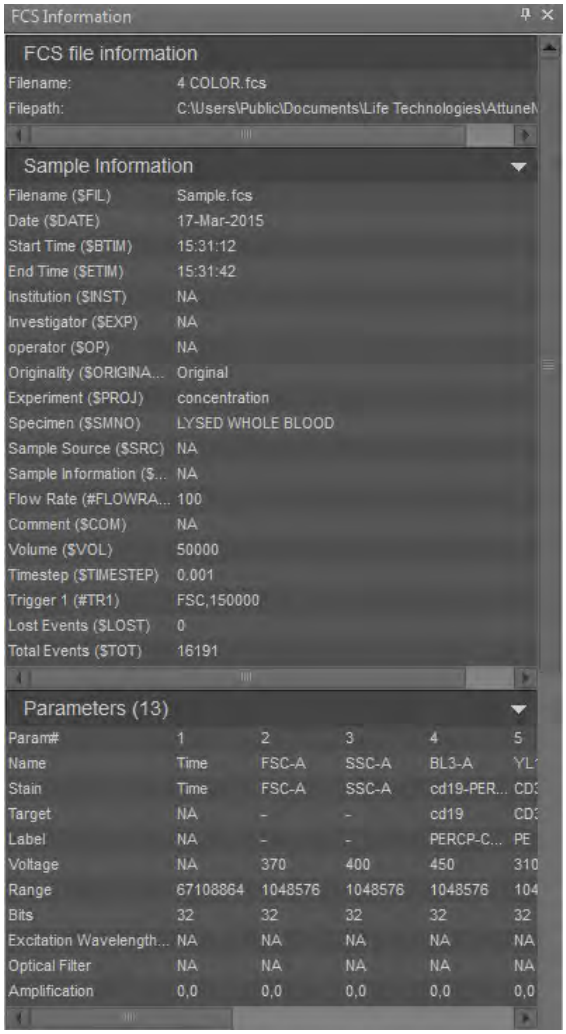
- By default, Particle Delay value is set to 0.
- To change the Particle Delay, enter a number into the particle delay edit field or adjust the value using the adjacent up and down controls.
- The up and down controls change the focus value by 1.
- The particle delay edit field only accepts integers between –100 and 100.

Note: Particle Delay changes the position of the particle to keep it within the image capture region. Size and Position settings move the image capture region within the scanning area. If the Particle Delay and Size and Position settings are mismatched, you will not be able to see and image the particle.

To reset the Position and Particle Delay settings, enter 0/0 for Position to center the image field in the scanning region and enter 0 for Particle Delay.

17. FCS Information panel

Overview



FCS Information panel displays the FCS metadata (the text segment portion) of the selected Sample.

- The panel is set as a floating panel by default (i.e., its "Dockable" attribute deselected), but it can be set as a dockable panel as described on page 40.
- The panel header displays the name of the selected Sample, and the complete file path is shown under the panel header.
- The panel contents are organized into the following groups:
  - *Sample information* (page 327)
  - *Parameters* (page 328)
  - *Compensation* (page 328)
  - *System information* (page 329)
  - *Data format* (page 330)
  - *Other* (page 330)
- Each group can be collapsed and expanded. By default, the groups are all expanded, unless otherwise specified below. The expanded or collapsed state persists on a per user basis.
- The metadata are displayed in a table where the first column is the FCS keyword and the second column is the keyword value.
- Only keywords and keyword values that are present within the FCS file are shown.
- If the panel contents exceed the visible area, vertical and horizontal scroll bars are shown as needed.

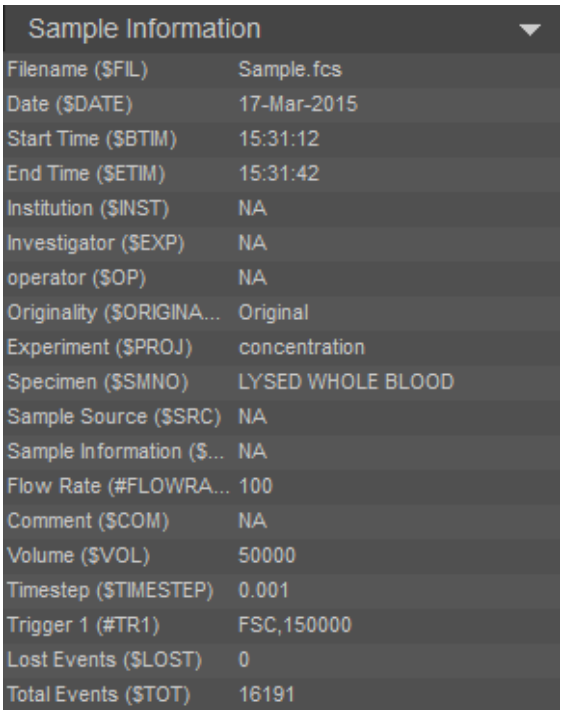
Note: The FCS keyword and keyword values are as described in "Appendix A: FCS File Reference", page 549.

FCS file information

Sample information group

The *Sample information group* contains keywords that describe the FCS file.

The keywords displayed in the Sample information group are listed in the following table and are shown in the sort order indicated in the table.



Sort	Keyword	Description
1	$FIL	Filename
2	GUID – BD Keyword	GUID
3	$DATE	Date
4	$BTIM	Start Time
5	$ETIM	End Time
6	$INST	Institution
7	$EXP	Investigator
8	$OP	Operator
9	$LAST_MODIFIER	Last Modified By
10	$LAST_MODIFIED	Last Modified
11	$ORIGINALITY	Originality
12	$PLATENAME	Plate Name
13	$PLATEID	Plate ID
14	$WELLID	Well Location

Sort	Keyword	Description
15	$PROJ	Experiment
16	$SMNO	Specimen
17	$SRC	Sample Source
18	$CELLS	Sample Information:
18	#FLOWRATE	Flow Rate
19	$COM	Comment
19	$VOL	Sample Volume
20	#TOTALVOLUME	Sample Volume
21	$TIMESTEP	Timestep
22	$TR	Trigger
23	$ABRT	Aborted Coincident Events
24	$LOST	Lost Events
25	$TOT	Total Events

Parameters group

The *Parameters group* contains the parameter description keywords (e.g., $PnN, $PnS, etc.) for each parameter.

The number of parameters (Parameters ($PAR)) is indicated above the table displaying the Parameter keywords.

Parameters (13)

Param#	1	2	3	4	5
Name	Time	FSC-A	SSC-A	BL3-A	YL1
Stain	Time	FSC-A	SSC-A	cd19-PER...	CD3
Target	NA	-	-	cd19	CD3
Label	NA	-	-	PERCP-C...	PE
Voltage	NA	370	400	450	310
Range	67108864	1048576	1048576	1048576	104
Bits	32	32	32	32	32
Excitation Wavelength...	NA	NA	NA	NA	NA
Optical Filter	NA	NA	NA	NA	NA
Amplification	0,0	0,0	0,0	0,0	0,0

The column headers indicate the parameter number and the row labels correspond to the Parameter description keywords as described in the following table.

Sort	Keyword	Description
1	$PnN	Channel Name
2	$PnS	Stain
3	#PnTarget	Target
4	#PnLabel	Label
5	$PnV	Voltage
6	$PnR	Range
7	$PnG	Gain
8	$PnB	Bits

Sort	Keyword	Description
9	$PnL	Excitation Wavelength
10	$PnO	Excitation Power
11	$PnF	Optical Filter
13	$PnE	Amplification
14	$PnP	% Emitted Light
14	$PnT	Detector Type
15	$PnD	Visualization Scale
16	$PnCALIBRATION	Calibration

Compensation group

The *Compensation group* displays the spillover values contained within the FCS file in a matrix view where the row and column headers are named using the $PnN values (i.e., Channel name).

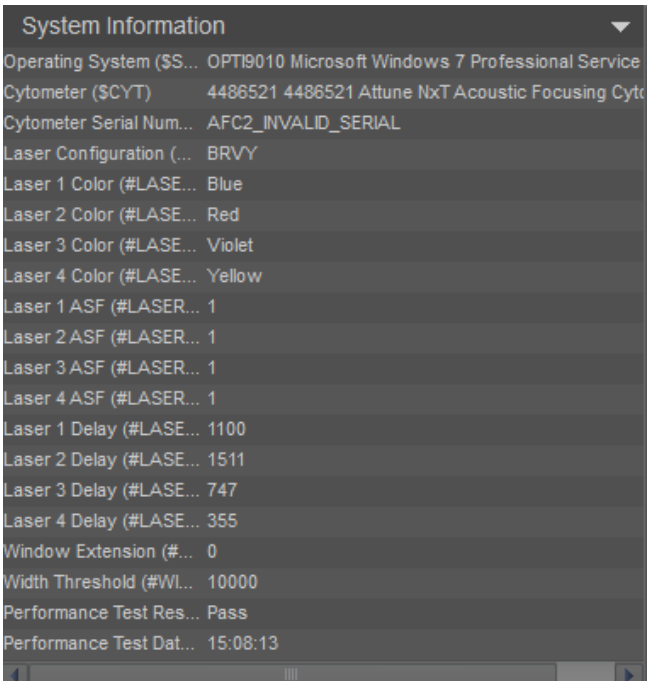
The keywords used to establish compensation ($Spillover, $Comp, Spill, Spillover, None) are shown next to the compensation group name.

Compensation ($SPILLOVER)

	BL3-A	YL1-A	RL1-A	VL2-A	BL3-H	YL1
BL3-A	100.00	0.04	3.20	0.02	0.00	0.0
YL1-A	63.74	100.00	0.07	0.04	0.00	0.0
RL1-A	2.48	0.01	100.00	0.01	0.00	0.0
VL2-A	0.30	0.01	0.08	100.00	0.00	0.0
BL3-H	0.00	0.00	0.00	0.00	100.00	0.0
YL1-H	0.00	0.00	0.00	0.00	63.74	100
RL1-H	0.00	0.00	0.00	0.00	2.48	0.0
VL2-H	0.00	0.00	0.00	0.00	0.30	0.0

System information group

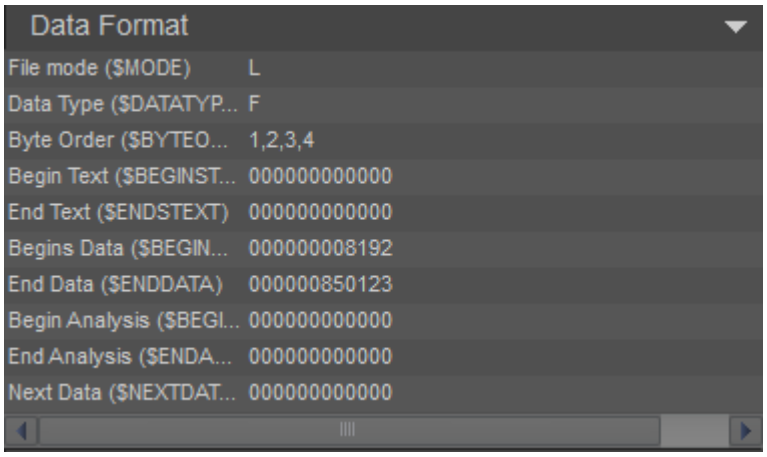
The *System information group* contains keywords that that pertain to the system.
For all custom keywords, keywords without the "#" sign will also be recognized.



Sort	Keyword	Description
1	$SYS	Operating System
2	$CYT	Cytometer
3	$CYTSN	Cytometer Serial Number
4	#LASERCONFIG	Laser Configuration
5	#LASER1COLOR	Laser 1 Color
6	#LASER2COLOR	Laser 2 Color
7	#LASER3COLOR	Laser 3 Color
8	#LASER4COLOR	Laser 4 Color
9	#LASER1ASF	Laser 1 ASF
10	#LASER2ASF	Laser 2 ASF

Sort	Keyword	Description
15	#LASER3ASF	Laser 3 ASF
16	#LASER4ASF	Laser 4 ASF
17	#LASER1DELAY	Laser 1 Delay
18	#LASER2DELAY	Laser 2 Delay
18	#LASER3DELAY	Laser 3 Delay
19	#LASER4DELAY	Laser 4 Delay
19	#WINEXT	Window Extension
20	#WIDTHTHRESHOLD	Width Threshold
21	#PTRESULT	Performance Test Result
22	#PTDATE	Performance Test Date

Data format group The *Data format group* contains keywords that describe the instrument. This group is collapsed by default.



Sort	Keyword	Description
	FCS Standard	FCS Version
1	$MODE	File Mode
2	$DATATYPE	Data Type
3	$BYTEORD	Byte Order
4	$BEGINSTEXT	Begin Text
5	$ENDSTEXT	End Text

Sort	Keyword	Description
6	$BEGINDATA	Begin Data
7	$ENDDATA	End Data
8	$BEGINANALYSIS	Begin Analysis
9	$ENDANALYSIS	End Analysis
10	$NEXTDATA	Next Data
6	$BEGINDATA	Begin Data

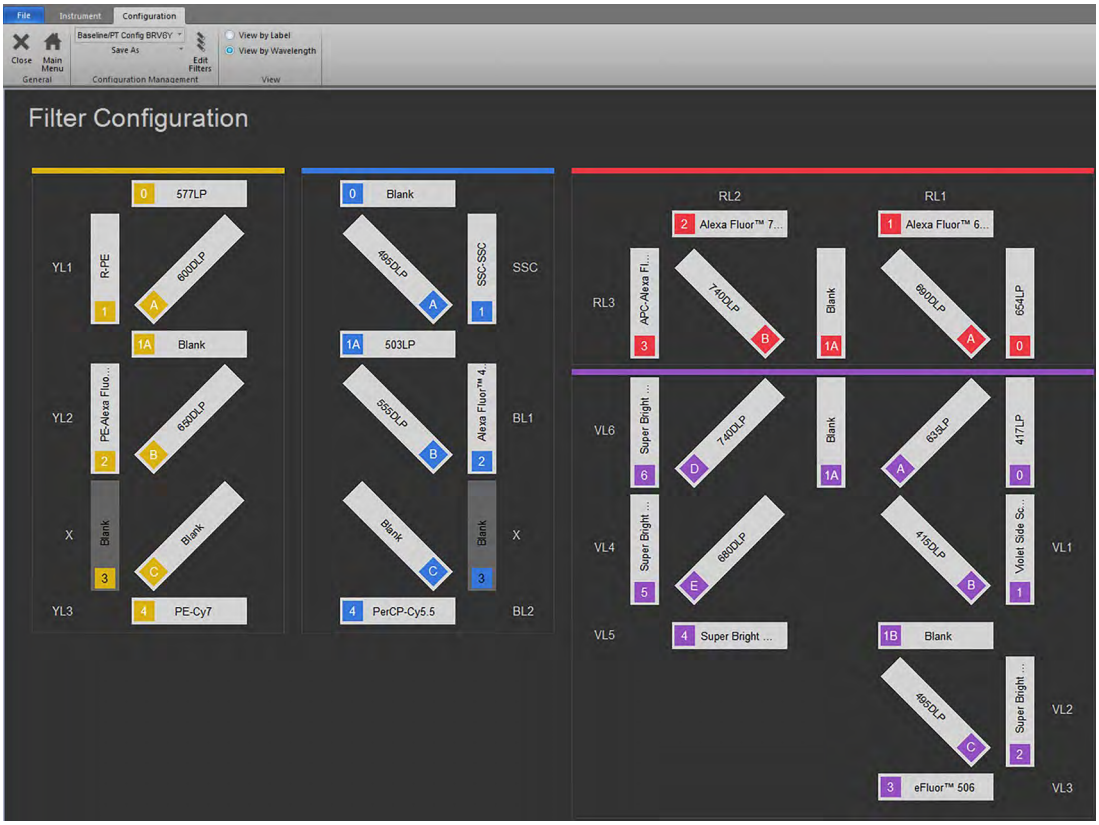
Other group The *Other group* contains all other keywords not captured within the other groups. This group is collapsed by default.

18. Filter configuration

Filter Configuration (FC) module

Overview

The *Filter Configuration (FC) module* of the Attune™ Cytometric Software consists of the *File* (page 49) and *Configuration ribbon tab* (page 332), and the *Filter Configuration View* (page 341).



Attune™ NxT BRV6Y configuration

The FC module is used to:

- Select view options for the filters (page 340)
- Manage filter labels (page 339)
- Create, edit, and delete filters (page 335)
- Select instrument configuration files (page 332)
- Save, load, export, and delete instrument configuration files (page 333)

Launch the FC module

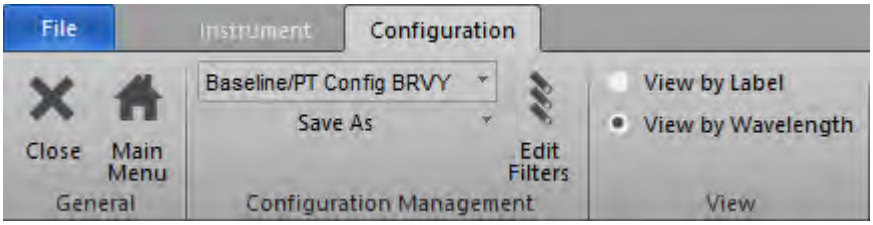
On the *Instrument ribbon tab* (page 61), click the **Configuration** button to launch the *Filter Configuration (FC) module*.

The Configuration button is always visible and enabled except during acquisition.

- When the FC module is opened, all other panels are hidden. The FC module is not resizable or floatable.
- The FC module contains only the File (page 49), Configuration (page 332), and Instrument (page 61) ribbon tabs. All other ribbons tabs are hidden.

Configuration ribbon tab

The *Configuration ribbon tab* allows you to manage the filter configuration and to select view options for the filters. It is visible only when the FC module is open.



The Configuration ribbon tab is organized into three functional groups:

- *General* (page 332)
- *Configuration Management* (page 332)
- *View* (page 340)

General group

General group allows you to close the IC module and to return to the Main Menu.

Close: Closes the IC module and returns to the previous view before the Instrument Configuration panel was opened. If you have unsaved configuration changes, the *Save As* dialog (page 333) appears.

Main Menu: Closes the IC module and returns to the Main Menu. If you have unsaved configuration changes, the *Save As* dialog (page 333) appears.

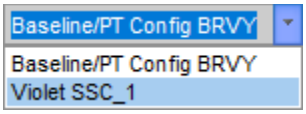
Configuration Management group

Configuration Management group allows you to select instrument configuration, to save, load, export, and delete filter configuration files, and edit the table of filters to be saved as a user-defined configuration.

Select configuration

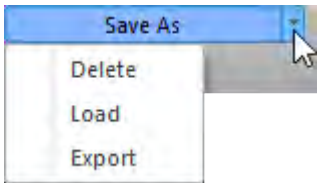
Select configuration dropdown menu displays the currently selected instrument configuration.

- The default filter configuration is the baseline configuration provided by the instrument manufacturer and is called **Baseline/PT Config <*CCC#C*>**, where **<C>** is the first letter of each laser color (**B**lue, **R**ed, **V**iolet, or **Y**ellow) and **#** corresponds to 4 or 6 channels detected off the Violet laser. An **X** for laser color indicates that one of the lasers is not present.
- The Baseline/PT Configuration is set by the service engineer at time of instrument installation.
- The file containing the default filter configuration cannot be overwritten or deleted.
- To change the filter configuration, select the file for the configuration of interest from the **Select configuration** dropdown menu. The Filter Configuration View (page 341) is automatically updated to display the filter information for the selected configuration.

  Only filter configurations that match the **Hardware/Virtual Laser Configuration** as defined in the *Options dialog* (page 352) are displayed.
- New configurations are user-account specific and can be shared with other users by saving the configuration file, then exporting it to a shared location for import into another user's account.

Save As



The Save As button is a split button used for performing the **Save As**, **Load**, **Export**, and **Delete** functions.

Save As: Allows you to save the changes to a new file, or overwrite an existing file, if you made changes to the current filter configuration file.

To save changes to a filter configuration file:

1. Click the main portion of the **Save As** button to open the *Save As* dialog.

2. To save the changes to:
   - A new file – Enter a file name in the text field.
   - An existing file – Select the file from the dropdown menu or enter the name of the existing file in the text field. You can enter up to 50 characters; the following characters are not permitted: \ / : * < > | ?.

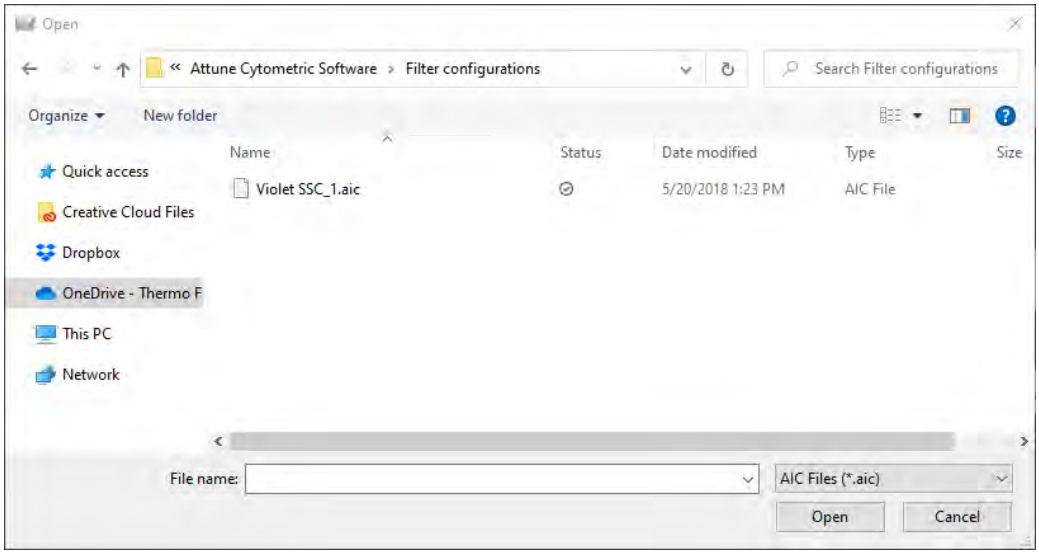
Note: If you select the default filter configuration file, the **Save** button is disabled.

3. Click **Save** (for a new file) or click **Overwrite** (for an existing file) to save the changes.

Alternatively, click **Discard** or **X** to close the *Save As* dialog without saving the changes.

Load: Allows you to import a saved filter configuration. To load (import) a filter configuration file:

1. Click the **arrow** next to the **Save As** button, then select **Load**. The *Open* dialog opens. The default location is the location of the last viewed folder.

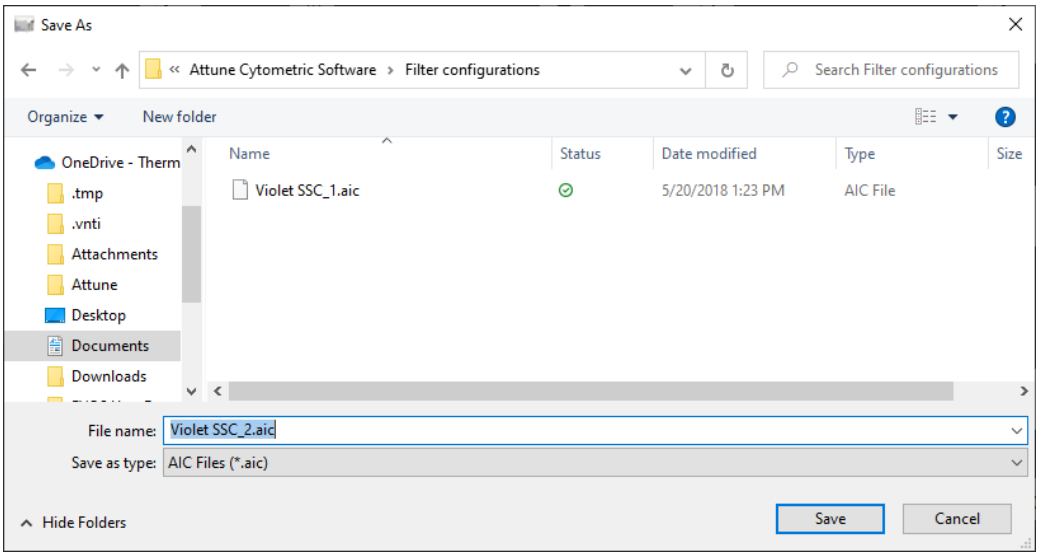


2. Select a file and then click **Import**.

   If you select a file that has the same name as a file in your own user list, the software appends an integer onto the file name upon loading.

Export: Allows you to export a saved filter configuration file. To export a filter configuration file:

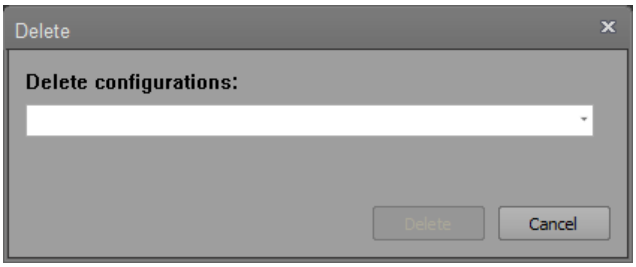
1. Click the **arrow** next to the **Save As** button, then select **Export** to open the *Save As* dialog. The *Save As* dialog automatically opens the location where you last saved files.



2. Browse to a save location, enter a file name, select the ***.ais** file type, then click **Save**.

Delete: Allows you to delete a filter configuration file. To delete a filter configuration file:

1. Click the **arrow** next to the **Save As** button, then select **Delete** to open the *Delete* dialog.



2. By default, the currently loaded filter configuration file is selected. Accept the default or select another file from the dropdown menu.

   The dropdown menu displays all available filter configuration files, except for the default filter configuration file.

3. Click **Delete** to delete the selected file and close the dialog box.

   Alternatively, click **Cancel** or **X** to close the *Delete* dialog without deleting the file.

Note: If you delete the currently loaded filter configuration file, the software updates the view to the default filter configuration file.

Edit Filters

Edit Filters button opens the *Edit Filters dialog* (page 336). The dialog contains the *Filters table*, the contents of which are based on the filter configuration selected from the **Select configuration** dropdown menu (page 332).

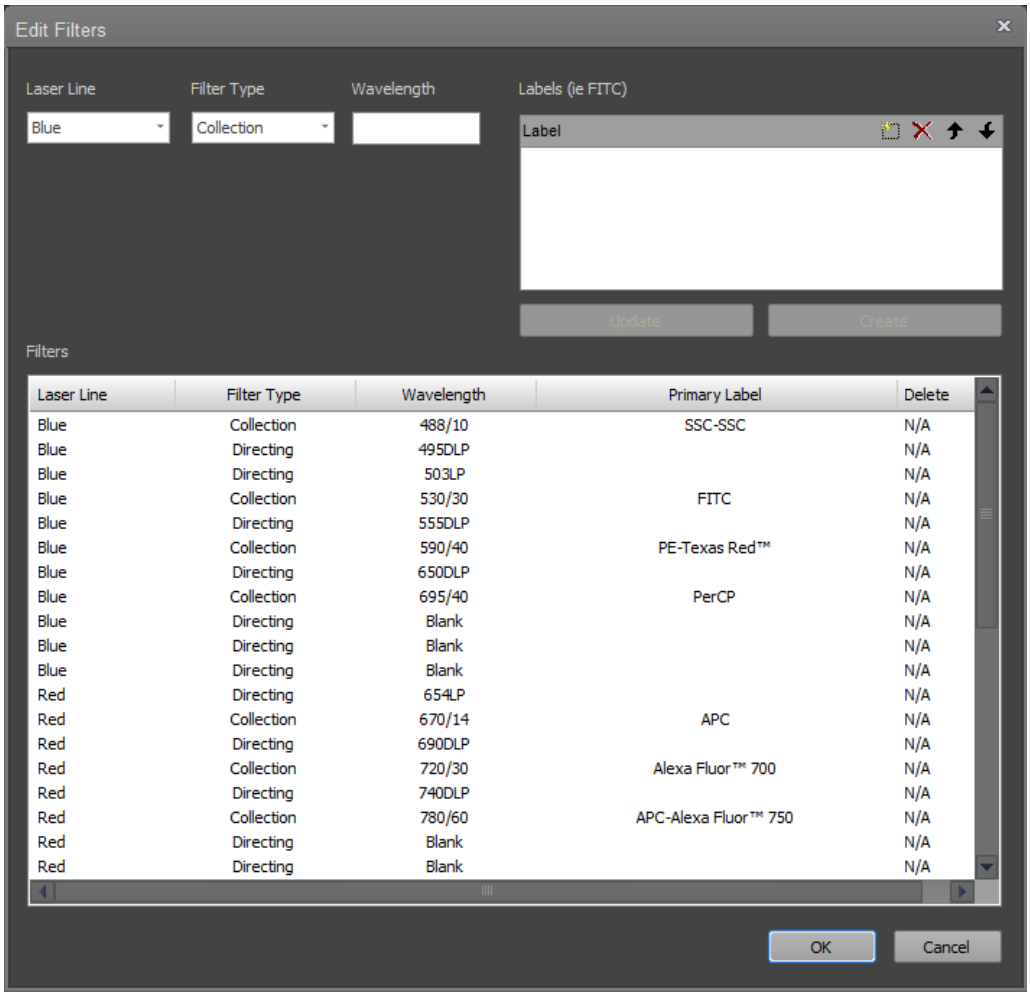
Using the Edit Filters dialog, you can:

- Define a new filter (page 337)
- Edit an existing filter (page 338)
- Delete an existing filter (page 339)
- Manage filter labels (page 339)

Note: Only *Advanced Users* and *Administrators* (page 25) can perform the tasks in the *Edit Filters* dialog.

Edit Filters dialog

Using the tools available in the Edit Filters dialog, authorized users can define new filter sets, change the filters that can be applied to each instrument channel, and manage filter labels.



Filters table

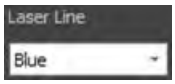
The Filters table displays the filters for the currently selected filter configuration, with each filter taking one row of the table.

- The columns in the table are listed from left to right as follows:
  Laser Line, Filter Type, Wavelength, Primary Label, and Delete button.
- By default, the filters are sorted by the laser line group, and the secondary sort order is wavelength.
- Authorized users can edit the contents of this table as described below. Changes to the table are saved to the user-defined filter configuration.

Edit Filters tools

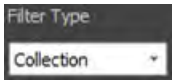
The upper portion of the Edit Filters dialog contains the tools for defining a new filter or editing an existing filter. It contains the following options:

- **Laser Line dropdown:** Allows you to select the laser to which the filter will be assigned. The default is the first laser in the configuration file.

  When an Attune™ system is detected or selected within the virtual mode, only the lasers which are installed in that instrument are available.

- **Filter Type dropdown:** Allows you to select the filter type. You can choose between **Band Pass**, **Long Pass**, **Short Pass**, and **Dichroic**. By default, Band Pass is selected.

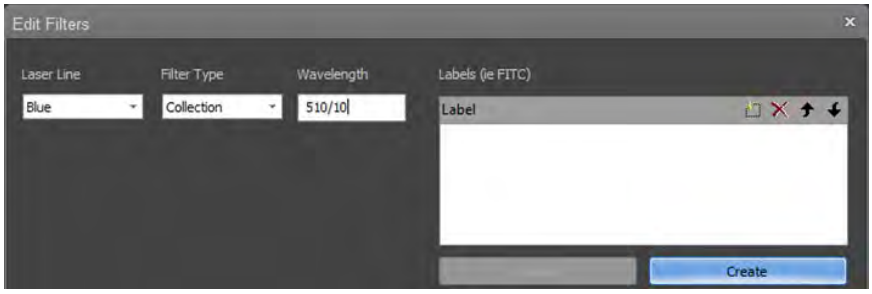
- **Wavelength:** Allows you to type in the filter specifications. You can enter up to 10 characters to the text field. By default, this field is blank.

- **Labels table:** Allows you to assign and manage filter labels using the tools available in the table header (see "Manage filter labels", page 339). You can enter up to 50 characters per line to save as a label for the selected filter. By default, this table is blank.

  Update and **Create** buttons are contextual; they are enabled only when the appropriate action has been taken in the Edit Filters dialog.

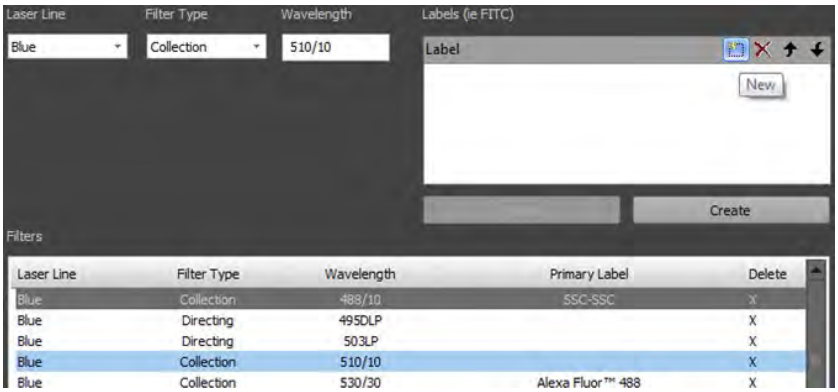
Define a new filter

1. Click **Edit Filters** to open the *Edit Filters* dialog.
2. In the **Laser Line** dropdown menu, select the laser to which you want to assign the new filter.
3. In the **Filter Type** dropdown menu, select the desired filter type.
4. In the **Wavelength** field, enter the filter specifications. You can enter up to 10 characters.

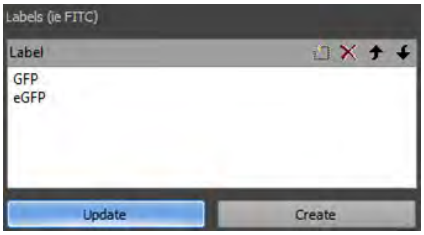


5. Click **Create**. The software adds the new filter to the **Filters** table.

6. To assign a *primary label* (i.e., the default display name) for the filter, select the new filter from the Filters table, then click **New** in the Labels table.



7. Type the name for the new filter in the Labels table, then click **Update**. You can create additional labels by repeating this process.



7. If needed, you can edit the label, or create a new label (see "Manage filter labels", page 339).
8. Click **OK** to apply the changes and close the *Edit Filters* dialog. The software saves the changes to the currently selected instrument configuration file. Changes will be lost if the **OK** button is not clicked before closing the menu.

   Alternatively, click **Cancel** to close the *Edit Filters* dialog without applying the changes.

Edit an existing filter

1. Click **Edit Filters** to open the *Edit Filters* dialog.
2. In the **Filters** table, select the filter to edit. The software populates the fields with the selected filter's information.
3. For user-defined filters, you can make the following changes:
   - In the **Laser Line** dropdown menu, select the laser to assign the filter to.
   - In the **Filter Type** dropdown menu, select the desired filter type.
   - In the **Wavelength** field, enter the filter specifications. You can enter up to 10 characters.

Note: The Laser Line, Filter Type, and Wavelength fields are disabled for the filters in the system default configuration.

4. Assign the primary label for the selected filter by selecting the label of interest in the **Labels** table, and then using the up arrow in the header to move it to the top of the list.

   If needed, you can edit the label, or create a new label (see "Manage filter labels", page 339).
5. Click **Update**.
6. Click **OK** to apply the changes and close the *Edit Filters* dialog. The software saves the changes to the currently selected instrument configuration file. Changes will be lost if the **OK** button is not clicked before closing the menu.

   To close the *Edit Filters* dialog without applying the changes, click **Cancel**.

Delete a filter

You can only delete filters that were created by a user; filters installed with the system cannot be deleted.

1. Click **Edit Filters** to open the *Edit Filters* dialog.
2. In the **Filters** table, select the filter to delete and click **X** in the Delete column.
3. Click **OK** to apply the changes and close the *Edit Filters* dialog. The software saves the changes to the currently selected instrument configuration file. Changes will be lost if the **OK** button is not clicked before closing the menu.

   To close the *Edit Filters* dialog without applying the changes, click **Cancel**.

Manage filter labels

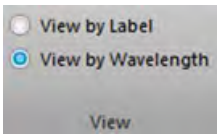
You can arrange, create, edit, and delete filter labels in the **Labels** table.

1. Click **Edit Filters** to open the *Edit Filters* dialog.
2. In the **Filters** table, select the filter to edit. The software populates the fields with the selected filter's information.
3. In the **Labels** table, perform the following tasks as needed:
   - Click on a label to select it. To select multiple labels at a time, press **Ctrl** or **Ctrl+Shift** when selecting the labels.
   - To arrange labels in the table, select a label, then use the up or down arrows in the header ( ) to move the selected label.

     The label at the top of the list is considered the *primary label* and will be used as the default display name for the selected filter.
   - To create a new label, click **Add** ( ) in the header or double-click the white space within the table, and then enter a label name. You can enter up to 50 characters; the following characters are not permitted: \ / : * < > | ?.
   - To edit an existing label, double-click the label and enter changes.
   - To delete a label, select it, and then click **Delete** ( ) in the header.

View group

View group is used for displaying the filters in the filter configuration by primary label or by assigned wavelength. It consists of **View by label** and **View by wavelength** radio buttons. Selecting one option deselects the other.



- The default selection is View by wavelength.
- The selection of View by label or View by wavelength is remembered as a user setting and persists on a per user basis.

Default filter label list

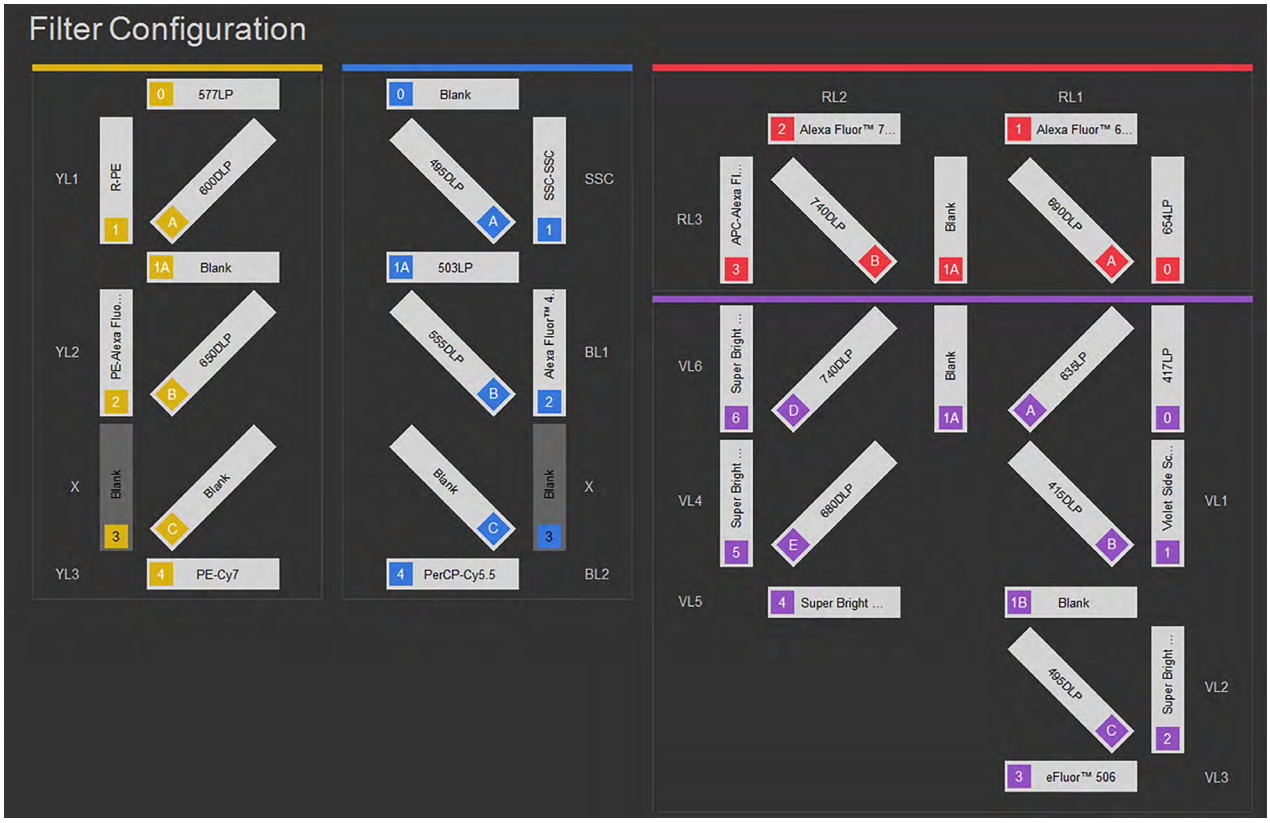
The following tables list the filter labels that are displayed for each channel for the available system default configurations. The naming convention for the system default filter configuration is as follows:

Baseline/PT Config <*CCCC#*>, where **<C>** is the first letter of each laser color (**B**lue, **G**reen, **Y**ellow, **R**ed, or **V**iolet) and **#** corresponds to 4 or 6 channels detected off the Violet laser. An **X** for laser color indicates that one of the lasers is not present.

Configuration		BXXX	BGXX	BYXX	BRXX	BV4XX	BV6XX	BGRX	BRV4X	BGV4X	BYRX	BYV4X	BRV6X	BYRV6	BGRV4	BYRV4
No. of detectors		4	7	7	7	7	9	10	10	11	11	11	12	14	14	14
Laser	Channel	Emission filter (nm)														
Blue	BL1	530/30	525/50	530/30	530/30	530/30	530/30	525/50	530/30	525/50	530/30	530/30	530/30	530/30	525/50	530/30
	BL2	574/26	590/40	590/40	574/26	574/26	574/26	590/40	574/26	590/40	574/26	590/40	574/26	695/40	590/40	590/40
	BL3	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40	695/40		695/40	695/40
	BL4	780/60			780/60				780/60		780/60					
Green	GL1		575/36					575/36		575/36					575/36	
	GL2		620/15					620/15		620/15					620/15	
	GL3		695/40					695/40		695/40					695/40	
	GL4		780/60					780/60		780/60					780/60	
Yellow	YL1			585/16							585/16	585/16		585/16		585/16
	YL2			620/15							620/15	620/15		620/15		620/15
	YL3			695/40							695/40	695/40		780/60		695/40
	YL4			780/60							780/60	780/60				780/60
Red	RL1				670/14			670/14	670/14		670/14		670/14	670/14	670/14	670/14
	RL2				720/30			720/30	720/30		720/30		720/30	720/30	720/30	720/30
	RL3				780/60			780/60	780/60		780/60		780/60	780/60	780/60	780/60
Violet	VL1					440/50	450/40		440/50	440/50		440/50	450/40	450/40	440/50	440/50
	VL2					512/25	525/50		512/25	512/25		512/25	525/50	525/50	512/25	512/25
	VL3					603/48	610/20		603/48	603/48		603/48	610/20	610/20	603/48	603/48
	VL4					710/50	660/20		710/50	710/50		710/50	660/20	660/20	710/50	710/50
	VL5						710/50						710/50	710/50		
	VL6						780/60						780/60	780/60		

Filter Configuration View

Overview The *Filter Configuration View* displays an interactive diagram of the filter array for the current instrument model, which allows you to assign appropriate filters to specific filter locations.



- Only detectors that are part of the active instrument model are displayed.
- The filter array reflects the **Hardware/Virtual Laser Configuration** as defined in the Configuration *Options dialog* (page 352) and can be used as a quick reference.
- Clicking one of the filter locations opens a dropdown menu, which contains all unassigned filters for that laser line and type. You can select any of these to assign it to that filter location.

  Filters marked as **BP** (Band Pass) and **LP** (Long Pass) can be selected at the detector output.
- Filters marked as **Dichroic** are only available in the positions that accept dichroic filters.
- Default Instrument Configuration panels for each laser configuration are shown on pages 342–351.

Note: Available laser configurations depend on the type of your instrument (Attune™ NxT or the Attune™ CytPix™ Flow Cytometer).

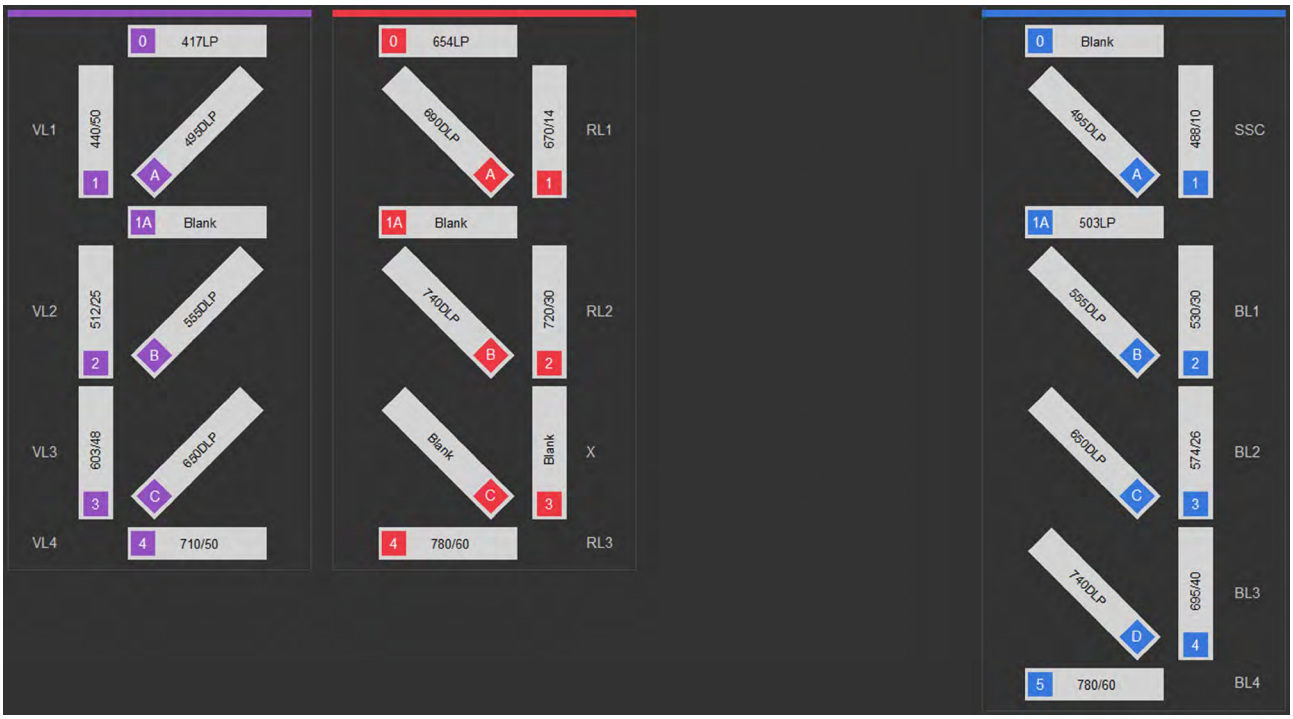
Default instrument configurations

The following images show the Instrument Configuration panel for each laser configuration that is available for both Attune™ NxT and Attune™ CytPix™ Flow Cytometers (page 342), for Attune™ NxT Flow Cytometers only (page 346), and for Attune™ CytPix™ Flow Cytometers only (page 350).

Available for Attune™ NxT and Attune™ CytPix™ Flow Cytometers

BYRV4 (Blue, Yellow, Red, Violet)

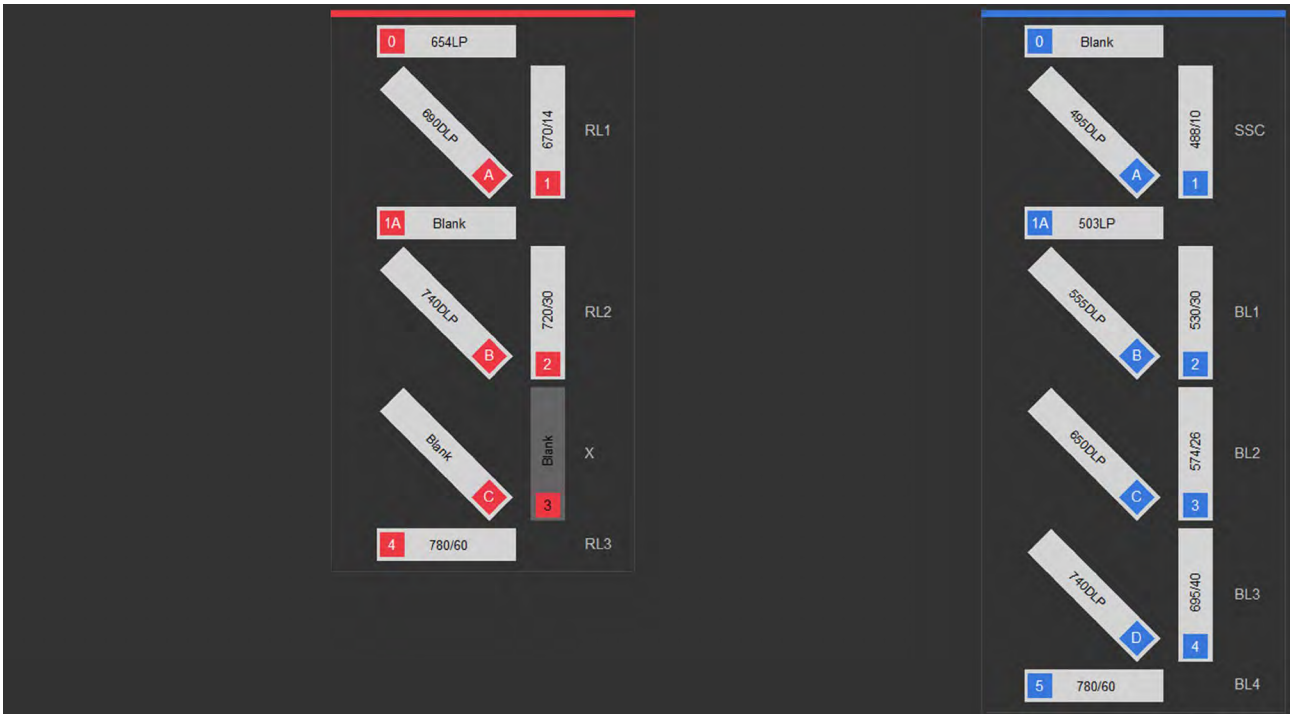
BRV4 (Blue, Red, Violet)



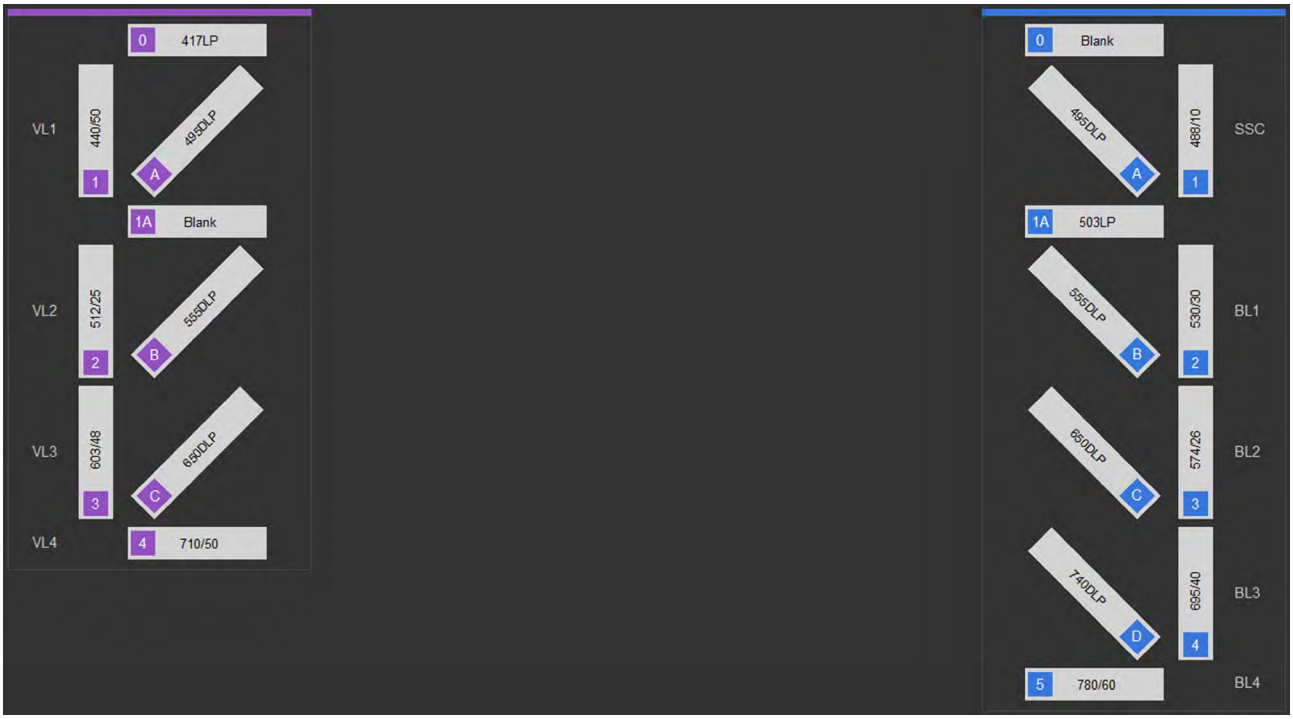
BYV4 (Blue, Yellow, Violet)

BYR (Blue, Yellow, Red)

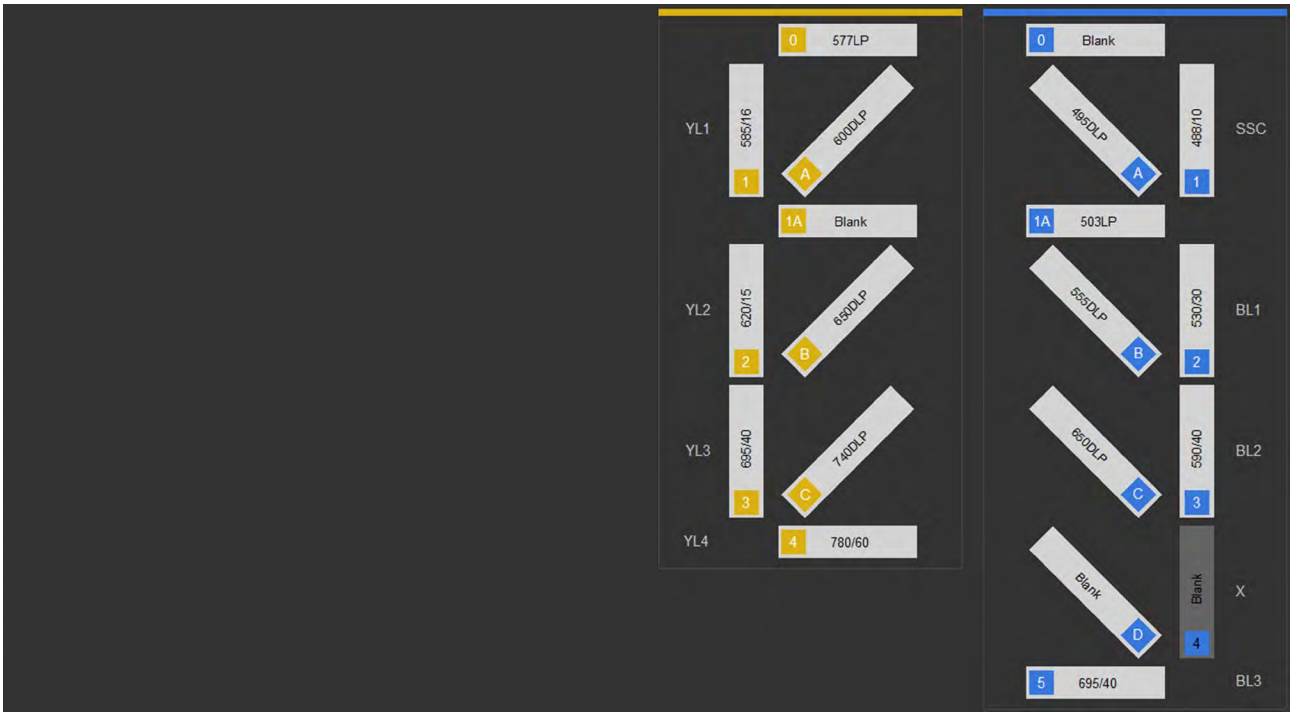
BR (Blue, Red)



BV4 (Blue, Violet)

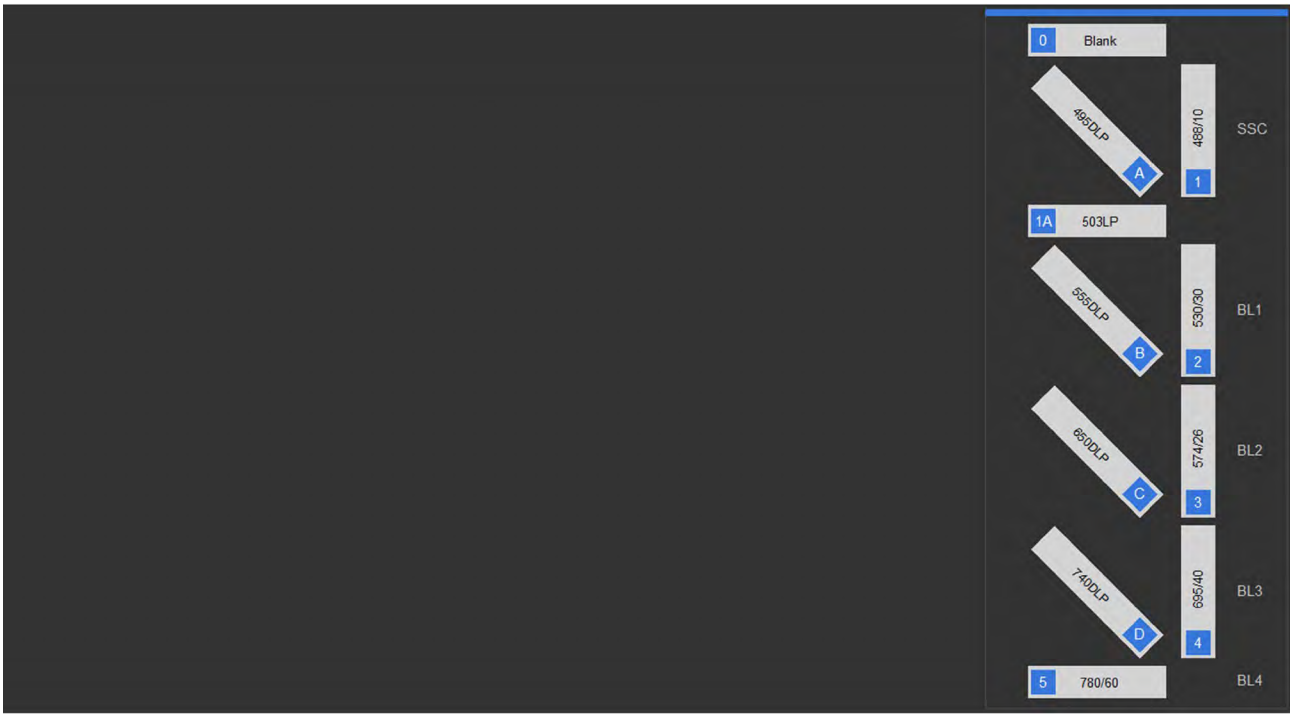


BY (Blue, Yellow)

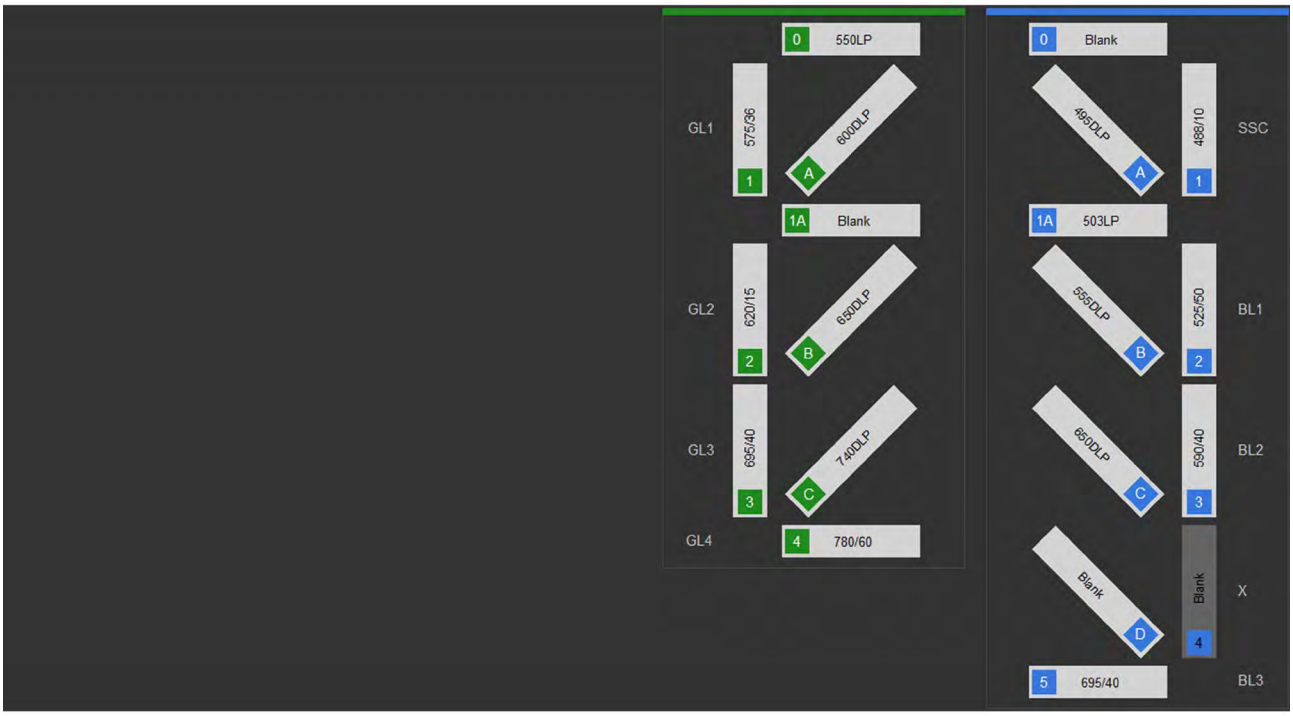


Available for the Attune™ NxT Flow Cytometer only

B (Blue)

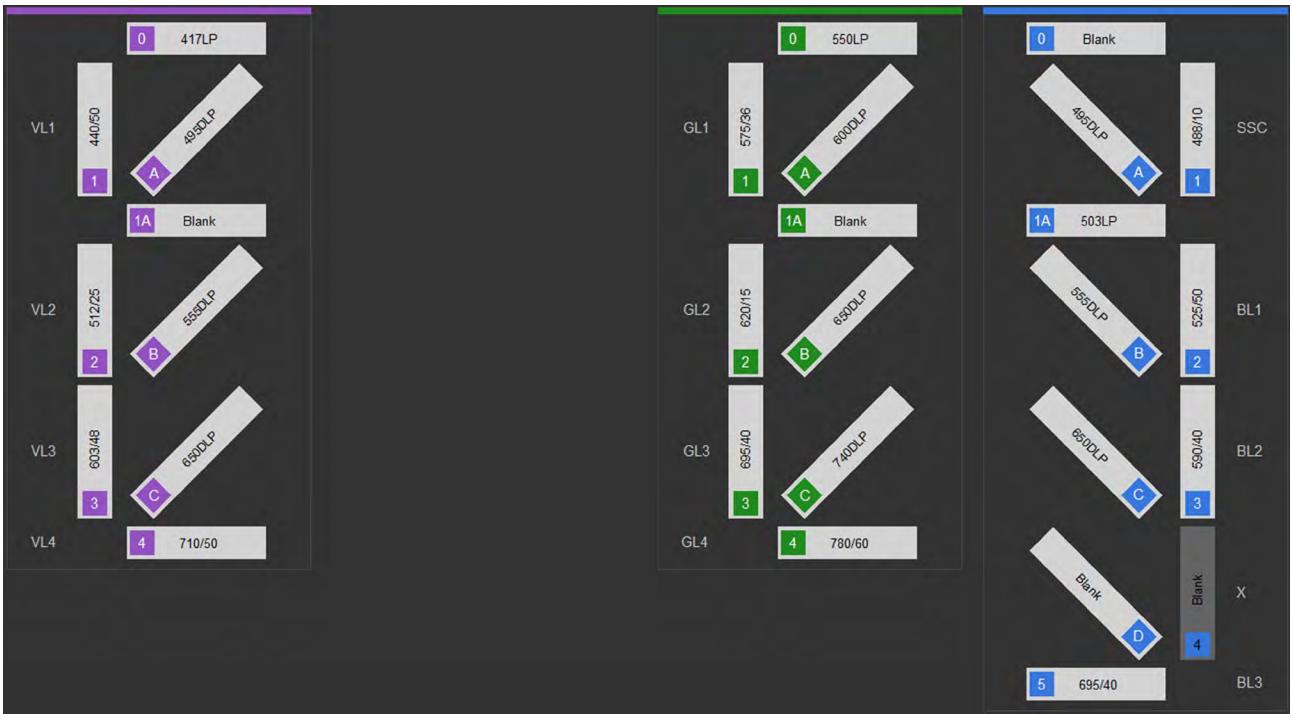


BG (Blue, Green)



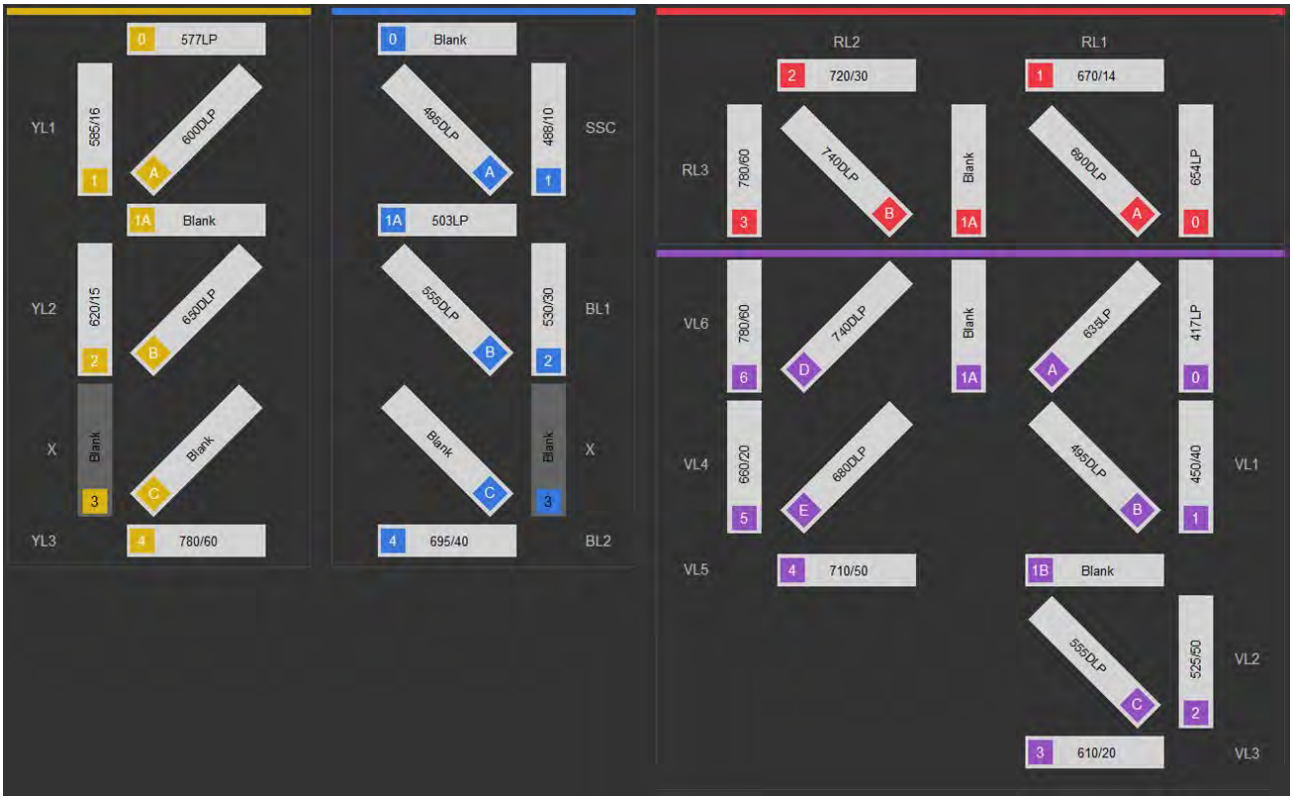
BGR (Blue, Green, Red)

BGV4 (Blue, Green, Violet)

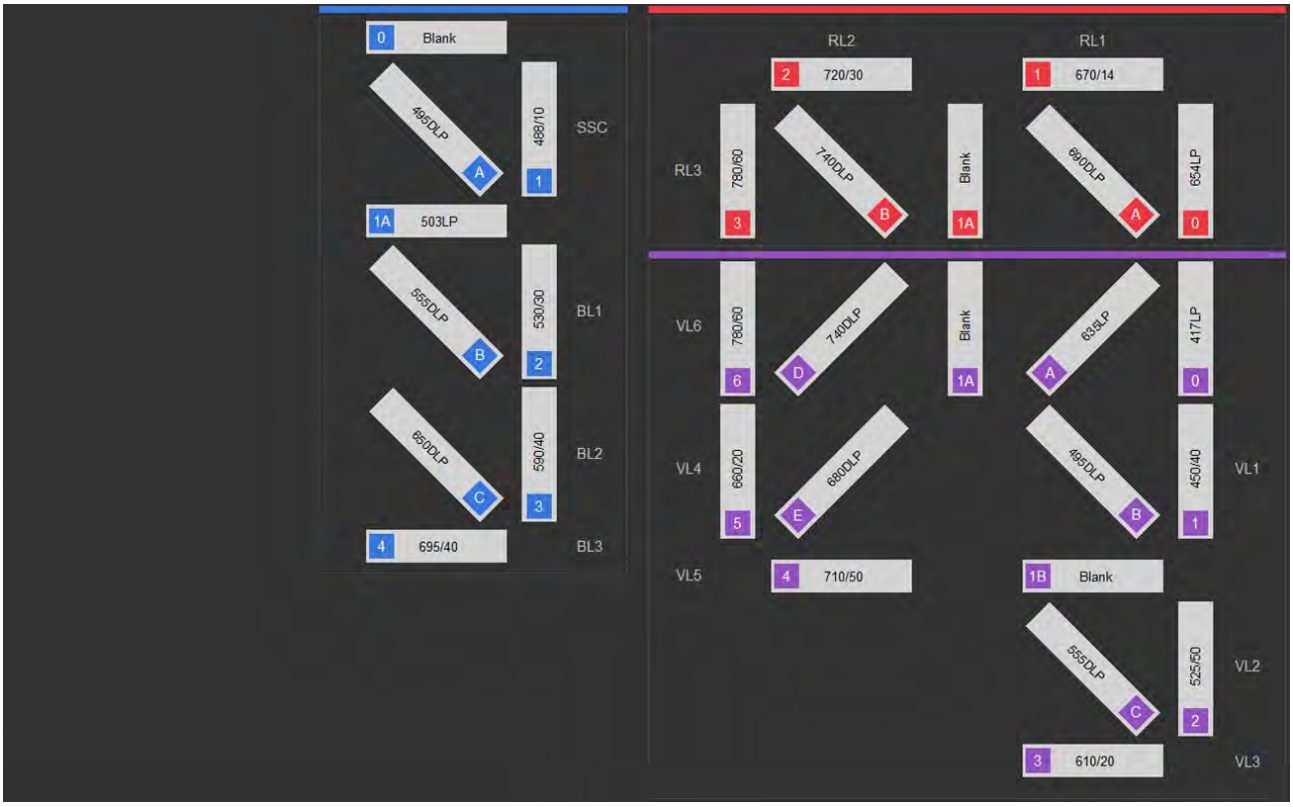


BGRV4 (Blue, Green, Red, Violet)

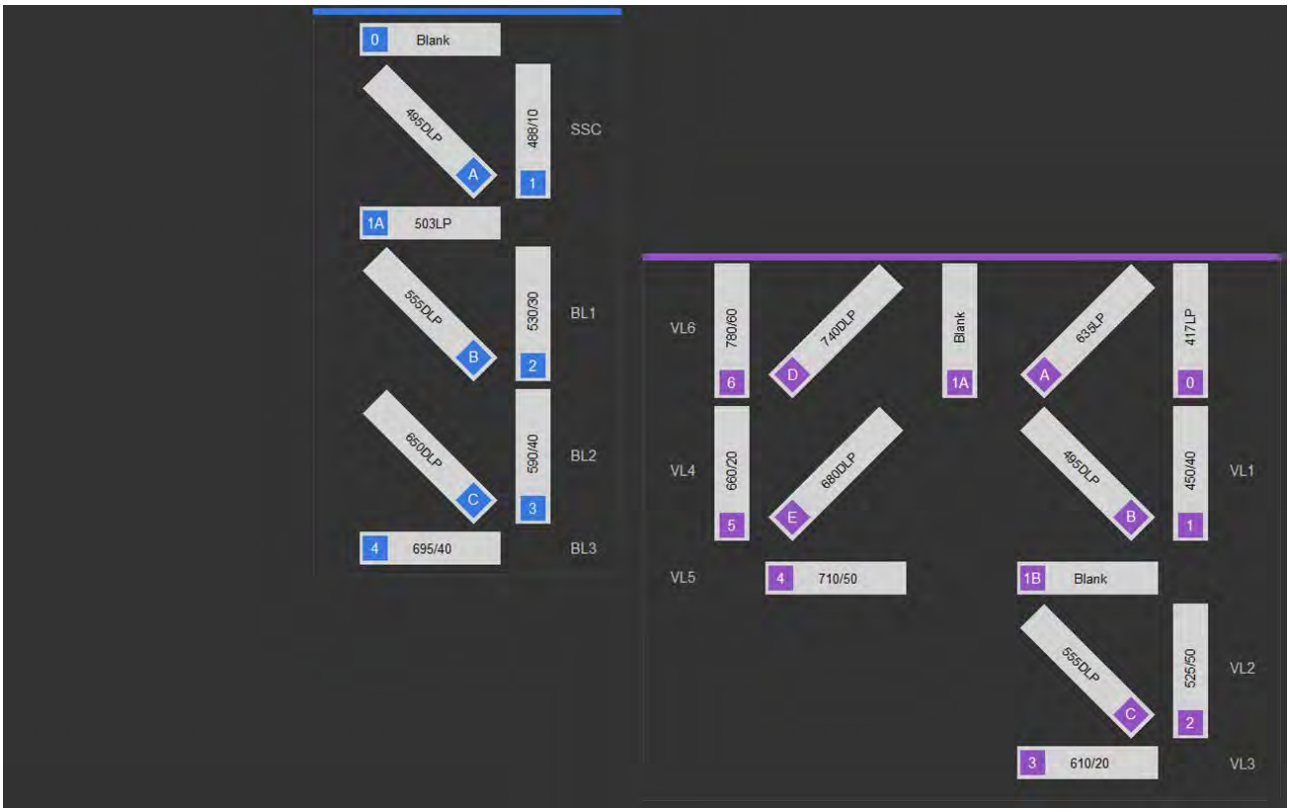
BYRV6 (Blue, Yellow, Red, Violet 6)



BRV6 (Blue, Red, Violet 6)

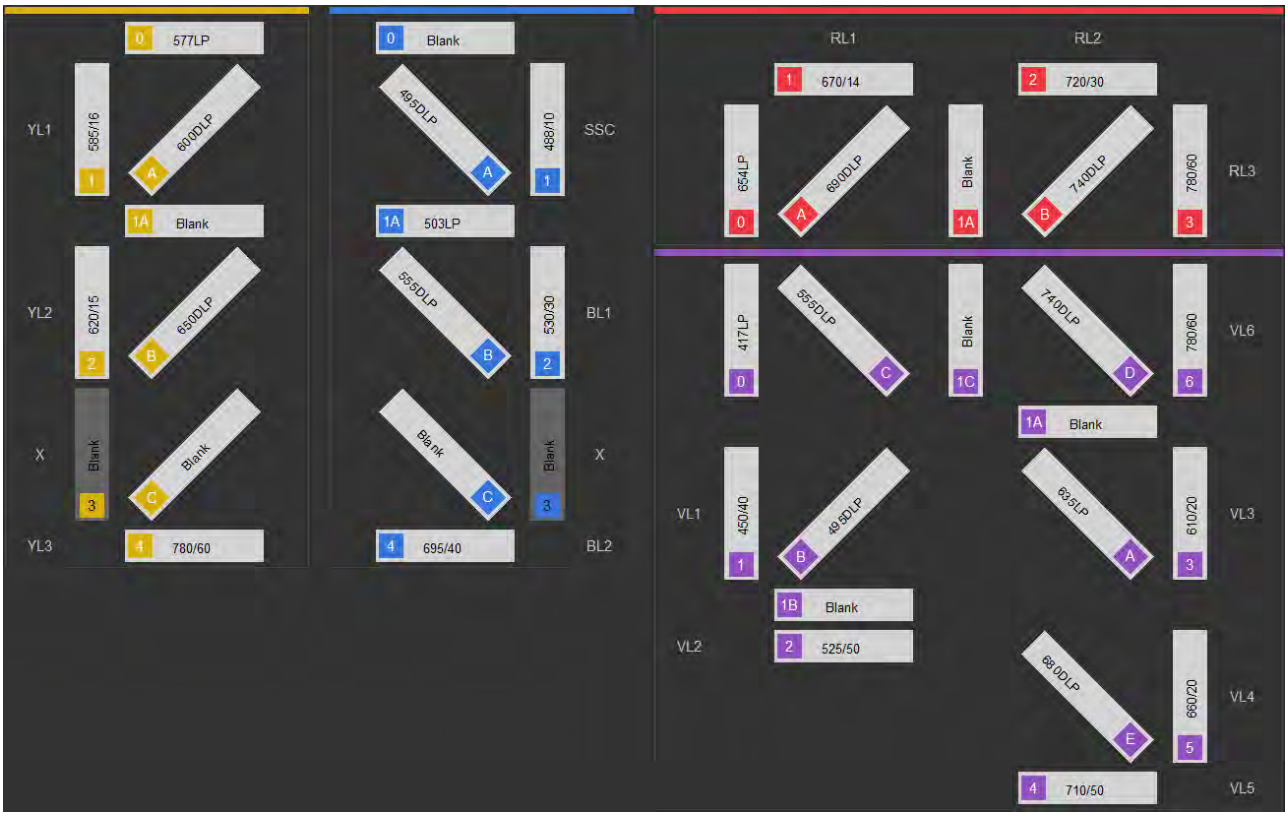


BV6 (Blue, Violet 6)

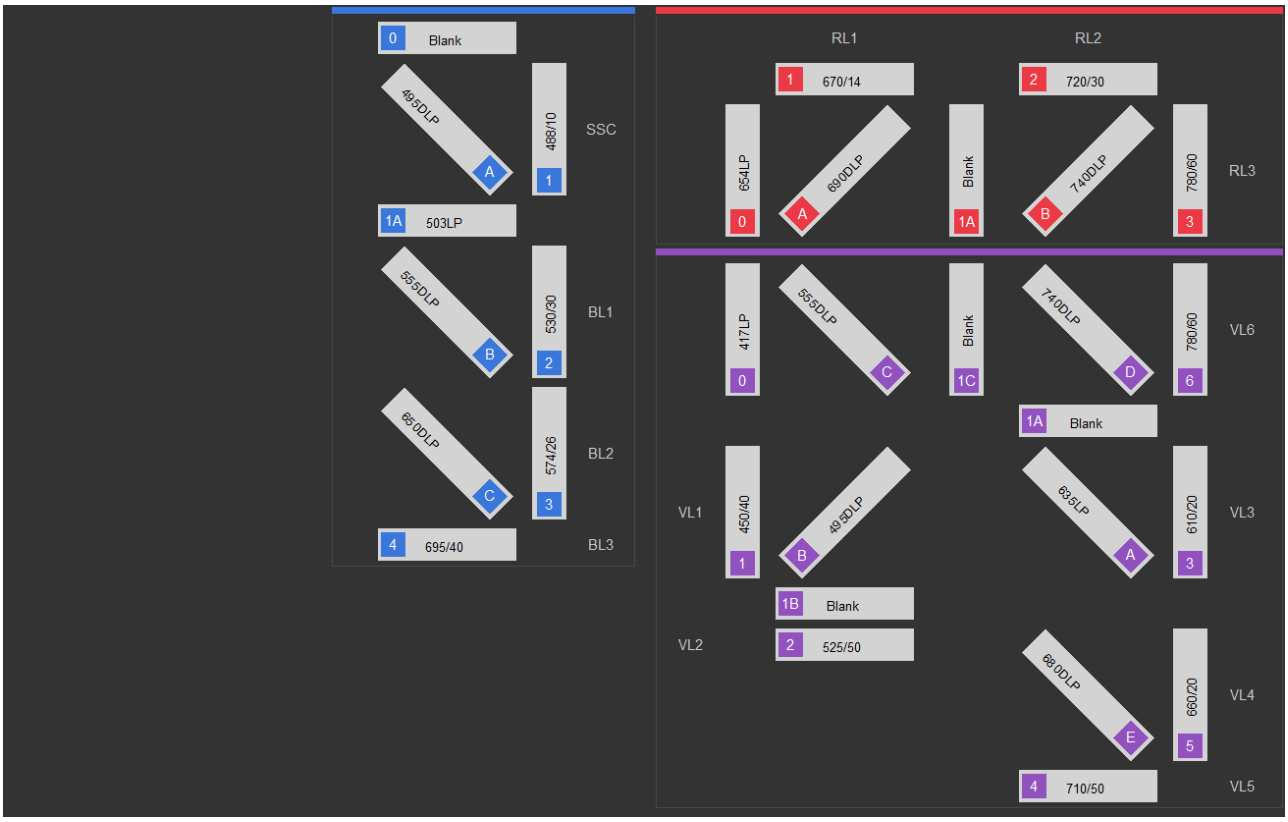


Available for the Attune™ CytPix™ Flow Cytometer only

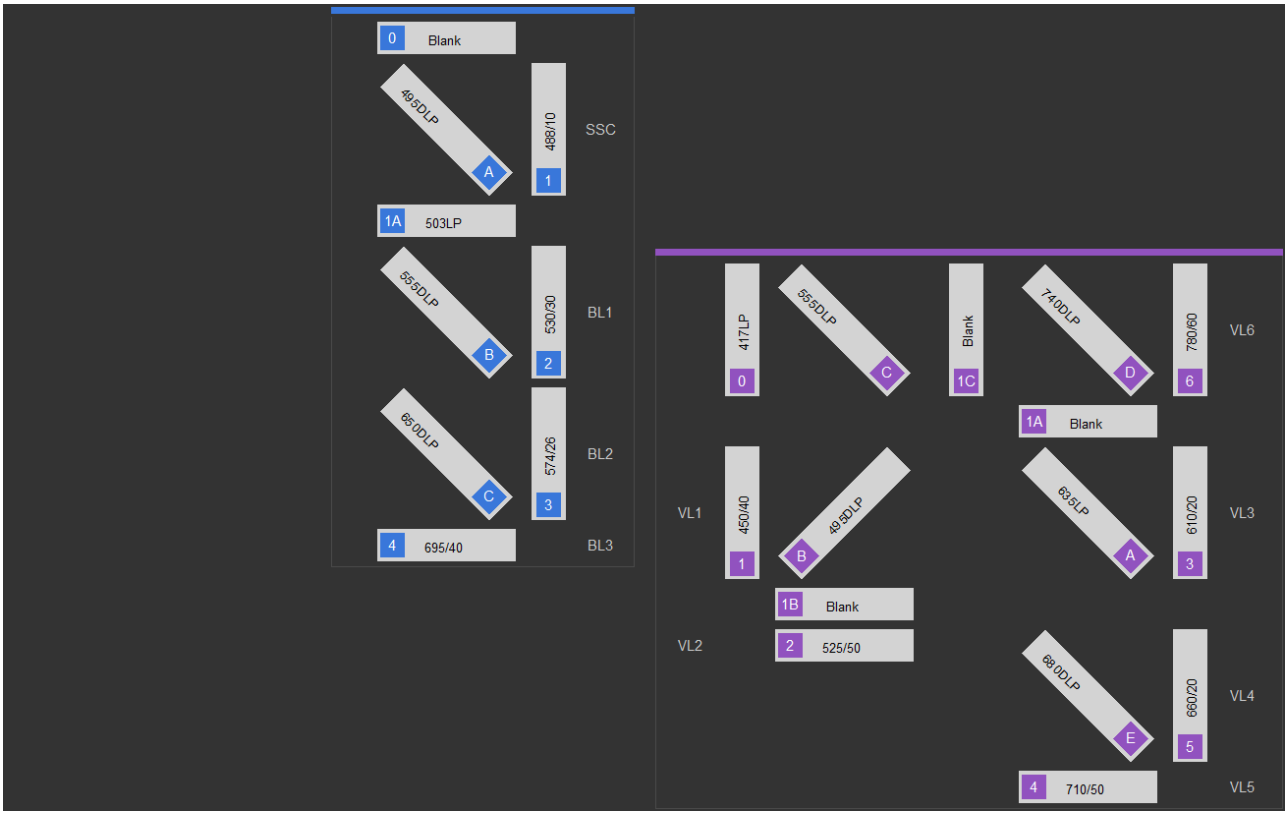
BYRV6 (Blue, Yellow, Red, Violet 6)



BRV6 (Blue, Red, Violet 6)



BV6 (Blue, Violet 6)

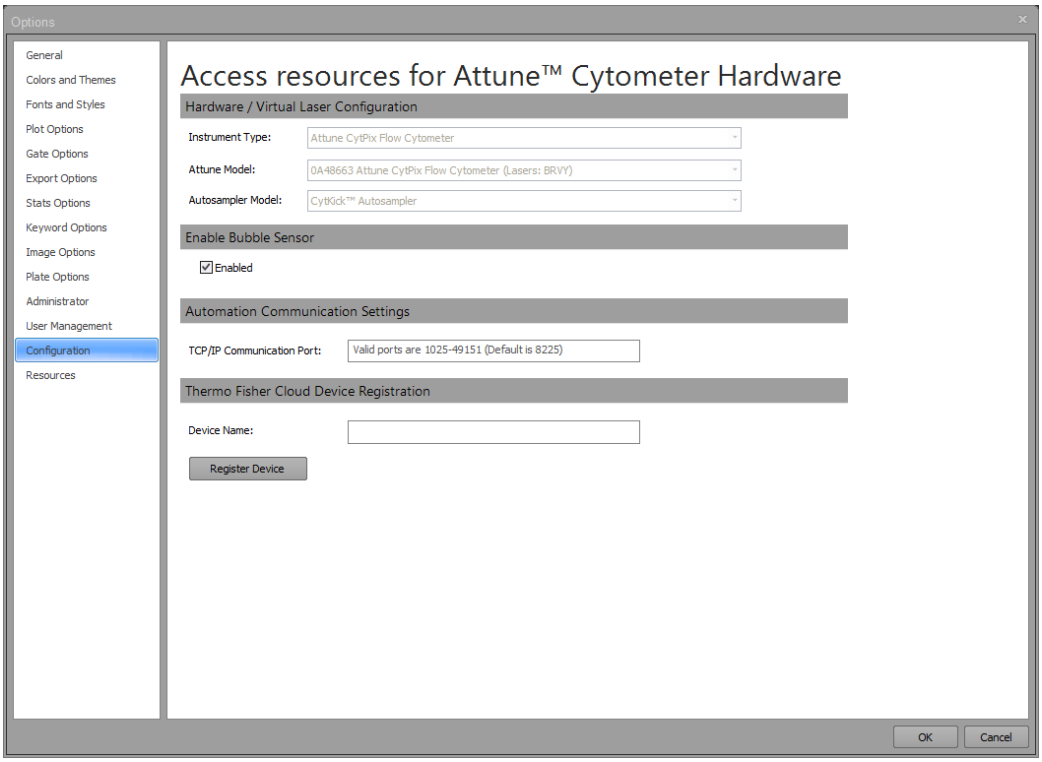


Note: In Attune™ CytPix™ Flow Cytometers, the orientation of the Violet 6 laser configuration is reversed to accommodate the image collection optics.

Configuration options

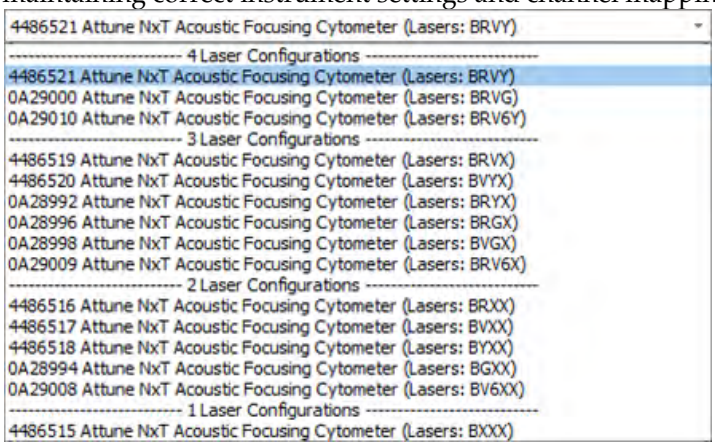
Overview

Configuration options allow you to create virtual configuration settings for working in analysis-only conditions, and to manage the bubble sensor and set the sample conservation mode property.



Hardware/ Virtual Laser Configuration

Hardware/Virtual Laser Configuration dropdown allows you select a virtual instrument configuration. This aids in the creation of Experiments when not connected to a cytometer by maintaining correct instrument settings and channel mapping.



- Clicking the **Hardware/Virtual Laser Configuration** combo box control opens the instrument configuration dropdown list, which is populated from the Attune™ NxT instrument configurations enumerated in the database.
- The available configurations are described in "Default instrument configurations" (pages 342–342).

19. Performance testing

Overview

Performance Tests

The Attune™ Cytometric Software uses Attune™ Performance Tracking Beads to define the cytometer's initial *Baseline status*. During this process, the median fluorescence intensity of each bead and the r%CV (robust percent coefficient of variation) are automatically measured in all fluorescence detectors. Software algorithms use this information to determine cytometer settings and provide target values for subsequent application-specific settings (see "Performance Test data", page 353).

After the Baseline values are established, you use the same lot of Attune™ Performance Tracking Beads to run the *Performance Tests*. The Performance Tests measure variation from the Baseline values to track the daily performance of the cytometer.

Performance Test data

The Attune™ Cytometric Software generates data for the parameters listed below to establish the initial Baseline status of the cytometer and measures their variation from the target values established by the Baseline calculations to track the daily performance of the instrument.

- PMTV (photomultiplier tube voltage)
- Delta PMTV
- Target MFI (target median fluorescence intensity)
- Measured MFI
- r%CV (robust percent coefficient of variation)
- Quantum Efficiency (Qr)
- Background (Br)
- Linearity
- Area Scaling Factor
- Laser Delay

Baseline and Performance Test (BL/PT) module

The *Baseline and Performance Test (BL/PT) module* of the Attune™ Cytometric Software is used to:

- Set up and run the Baseline, page 355
- Run a Performance Test, page 359
- View Baseline and Performance Test reports, page 363

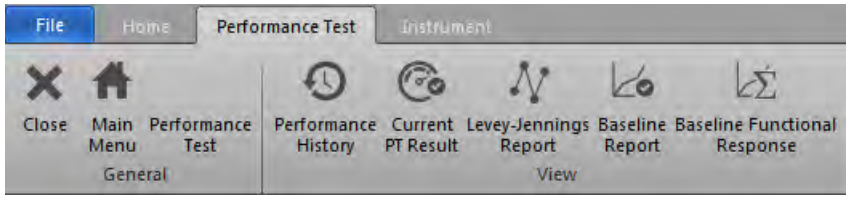
Note: For information on viewing the Baseline and Performance Test reports, see "Performance Test Reports", page 363.

Launch the BL/PT module

On the Main Menu (page 26), click **Performance Test** to open the BL/PT module, which contains the *Performance Test ribbon tab*.

Alternatively, click **Performance History** on the Instrument ribbon tab (page 61), when the instrument is connected.

- In the BL/PT module, only the File, Home, Performance Test, and Instrument ribbon tabs are available. All other tabs are hidden.



Note: The Performance Test ribbon tab is only visible when the BL/PT module is active.

- If no Baseline has previously been run, the Baseline setup screen is displayed; see "Setting up and running a Baseline", page 355.
- If a Baseline already exists, the Performance Test setup screen is displayed; see "Running a Performance Test", page 359.
- To close the BL/PT module and return to the previous view, click **Close**.
- To return to the Main Menu, click **Main Menu**.

Set up and run a Baseline

Baseline setup screen

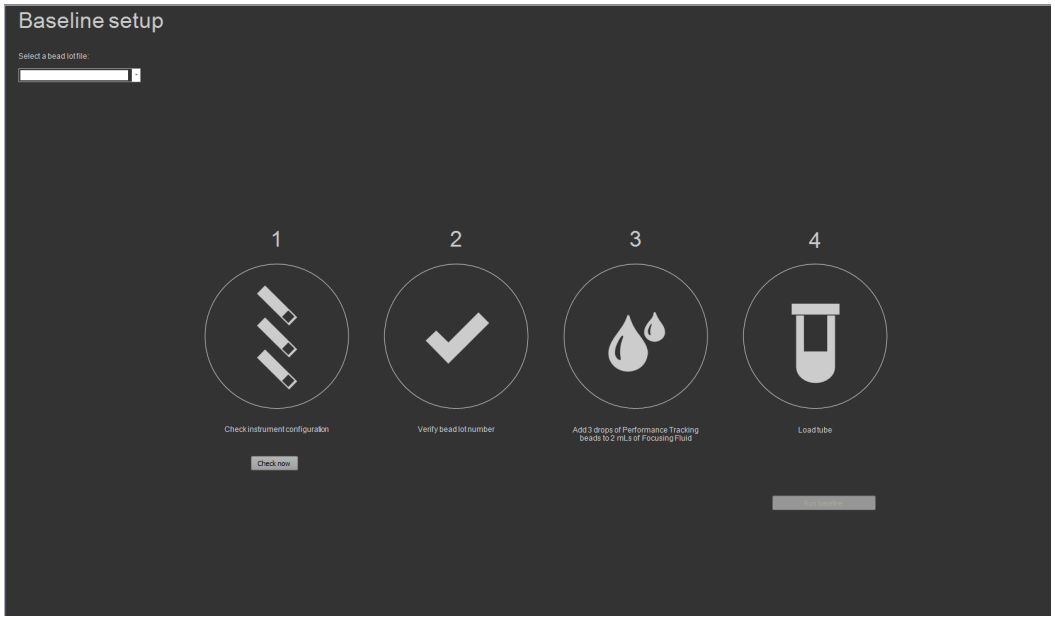
Open the BL/PT module (page 354) to view the *Baseline setup* screen.

Note: If a Baseline already exists, the *Performance Test setup* screen is displayed instead of the Baseline setup screen. On the Performance Test setup screen (page 359), you can run a Performance Test or reset the Baseline.

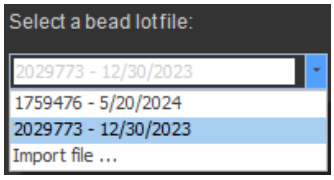
The Baseline setup screen provides general instructions for setting up a Baseline:

1) Check instrument configuration
2) Verify bead lot number
3) Add 3 drops bead solutions to 2 mL of focusing fluid
4) Load tube

For detailed procedures, see "Set up and run a Baseline", page 356



1. The Select bead lot file dropdown menu lists all bead lot files that have been imported.



2. Each file in the dropdown menu is listed in the following format:

   L/N: ### = Bead lot number

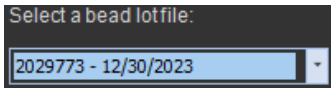
   DD/MM/YYYY = Expiration date

3. To set up a Baseline for another bead lot, select the bead lot file of interest from the dropdown menu.

   The software displays the bead lot number and expiration date for the currently selected bead lot.

Set up and run a Baseline

1. Select the bead lot file of interest from the **Select bead lot file** dropdown menu.



2. Verify that the bead lot number you have selected matches the bead lot number of the Attune™ Performance Tracking Beads you are using.

   The bead lot number is the **first seven digits** printed on the label (disregard the alpha numeric characters).

3. Prepare the beads by adding 3 drops of bead solution to 2 mL of Focusing Fluid.
4. Load the tube as described in the *Attune™ NxT Flow Cytometer User Guide* (Part. No. MAN0026547) or the *Attune™ CytPix™ Flow Cytometer User Guide* (Pub. No. MAN0019440).
5. Click **Run Baseline**.

> **Note:** If the *Startup* procedure has not been performed, the button displays **Run Startup** instead of **Run Baseline**. You must run the Startup procedure before proceeding.

6. If the bead lot is expired, a warning dialog appears. Click **Yes** to continue or click **No** to cancel the Baseline run.

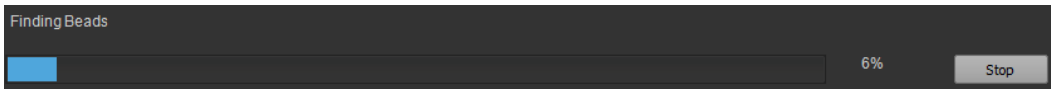
   If they are not already powered on, all lasers are powered on and will remain on at the completion of the test process.

Baseline screen

The *Baseline screen* provides progress information for the Baseline procedure.

- Four baseline steps are displayed above the progress bar as they occur:
  - "Finding Beads"
  - "Measuring Laser Delays"
  - "Adjusting PMTV to MFI Criteria"
  - "Gathering Final Statistics"

- Baseline procedure gathers statistics at flow rates of 100, 500, and 1000 µL/minute and reports results for 100 µL/minute.
- In the final step of the Baseline procedure, the instrument performs a fluid line calibration step.
  - Fluid line calibration requires a rinse to clear the fluid lines of sample.
  - Calibration compensates for fluid line volume tolerances and minimizes dead volume.
- The progress bar displays the percent completion (0–100%).



Note: The Baseline steps and progress bar are updated by the software in real time.

- Depending on the number of configured channels, the screen displays:
  - One scatter density plot (SSC-H vs. FSC-H)
  - Bivariate dot plots for each laser (for example, BL2-H vs. BL1-H)

  For more information on the plots, see "Plots" (page 95).
- To zoom on a plot, use the **Size slider** on the Application status bar (page 47). You cannot resize objects on the workspace. The plots do not get resized if the application window is resized.
- To stop the Baseline run, click **Stop**. The baseline procedure stops and the software returns to Baseline setup (page 355).

Baseline results

Baseline completion without errors

If the Baseline completes without errors, the software displays the *Baseline Results* screen (page 367) and calculates the system area scaling factor constant based on the results for each configured laser.

Baseline Test Results

Baseline test successful

Baseline 756080D - 6/17/2014 6/17/2014 3:50:03 PM

Channel	PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	591	300000	299368	1.50 %	0.000	0	0.000	0.79	1100	✓
SSC	330	300000	312932	3.02 %	0.000	0	0.000	0.79	1100	✓
BL1	428	300000	309464	1.18 %	0.036	49	1.000	0.79	1100	✓
BL2	337	300000	288903	1.03 %	0.050	73	0.965	0.79	1100	✓
BL3	386	300000	306699	1.68 %	0.045	14	1.000	0.79	1100	✓
RL1	371	300000	302229	3.73 %	0.047	33	0.997	0.90	1609	✓
RL2	400	300000	313139	3.66 %	0.006	95	1.000	0.90	1609	✓
RL3	465	300000	309675	3.98 %	0.035	84	0.999	0.90	1609	✓
VL1	297	300000	291541	1.03 %	0.008	2375	1.000	1.07	658	✓
VL2	348	300000	314794	1.27 %	0.011	215	0.998	1.07	658	✓
VL3	346	300000	307157	1.50 %	0.017	49	0.995	1.07	658	✓
VL4	414	300000	299723	2.29 %	0.004	168	0.986	1.07	658	✓
YL1	425	300000	306990	1.42 %	0.062	13	1.000	0.79	193	✓
YL2	390	300000	313169	1.51 %	0.028	19	0.971	0.79	193	✓
YL3	435	300000	310555	2.22 %	0.028	59	0.999	0.79	193	✓
YL4	456	300000	299706	2.94 %	0.006	74	1.000	0.79	193	✓

Baseline completion with errors

If the Baseline completes with errors, the software displays the *Errors Detected* dialog, which provides a list of the channel statistics that failed.

To go to the Baseline Test Results screen (page 367), click **Continue to results**.

Run a Performance Test

Performance Test setup screen

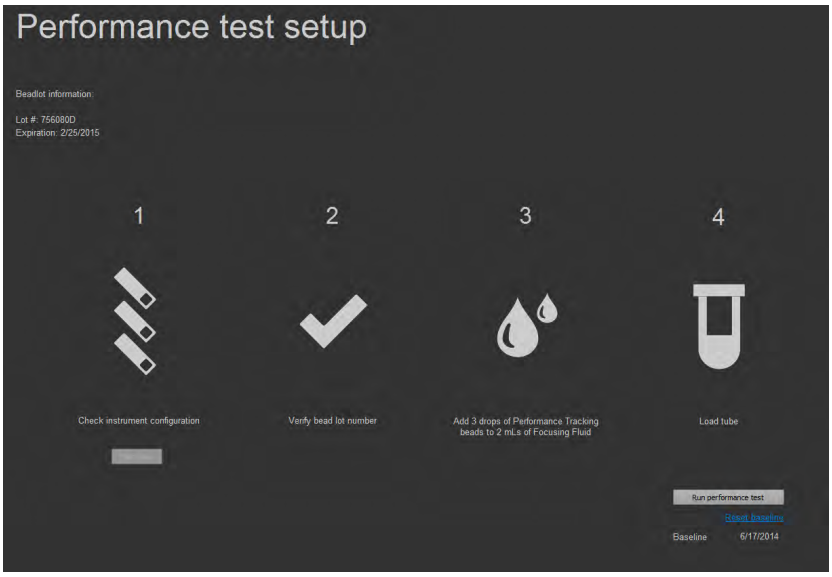
Launch the **BL/PT module** (page 354) to view the *Performance Test setup* screen.

Note: If no Baseline exists, the Baseline setup screen is displayed instead of the Performance Test setup screen. You must run the Baseline first; see "Setting up and running a Baseline", page 355.

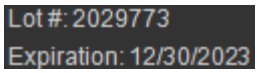
The Performance Test setup screen provides general instructions for setting up a Performance Test:

1) Check instrument configuration
2) Verify bead lot number
3) Add 3 drops bead solution to 2 mL Focusing Fluid
4) Load tube

For detailed procedures, see "Set up and run a Performance Test", page 360.



- The software displays the bead lot number and expiration date for the most recent bead lot used to set up a Baseline in the following format:

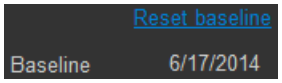
  L/N: ### = Bead lot number

  DD/MM/YYYY = Expiration date

  The bead lot number is the **first seven digits** printed on the label (disregard the alpha numeric characters).

- If the bead lot has expired, the lot number and expiration date are in contrasting color.
- The software displays the current Baseline information in the lower right of the screen.

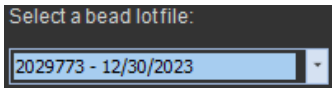
  To reset the current Baseline, click **Reset Baseline**, which navigates to the Baseline setup screen (page 355).

Note: The **Reset Baseline** hyperlink is enabled only if you are assigned the privileges to run the Baseline (see page 25).

Set up and run a Performance Test

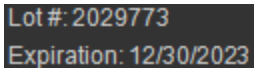
1. Select the bead lot file of interest from the **Select bead lot file** dropdown menu. For detailed procedures, see "Set up and run a Baseline", page 356.



   If needed, you can first select a different Baseline to run the Performance Test against, then click **Reset Baseline** to navigate to the Baseline setup screen.

> **Note:** The **Reset Baseline** hyperlink is enabled only if you are assigned the privileges to run the Baseline (see page 25).

2. Verify that the bead lot number you have selected matches the bead lot number of the Attune™ Performance Tracking Beads you are using.



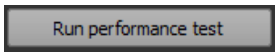
   The bead lot number is the **first seven digits** printed on the label (disregard the alpha numeric characters).

3. Prepare the Focusing Fluid by adding 3 drops of bead solution to 2 mL of Focusing Fluid.

   For more information, see the *Attune™ NxT Flow Cytometer User Guide* (Part. No. MAN0026547) or the *Attune™ CytPix™ Flow Cytometer User Guide* (Pub. No. MAN0019440).

4. Load the tube as described in the *Attune™ NxT Flow Cytometer User Guide* (Part. No. MAN0026547) or the *Attune™ CytPix™ Flow Cytometer User Guide* (Pub. No. MAN0019440)

5. Click **Run Performance Test**.



> **Note:** If the *Startup* procedure has not been performed, the button displays **Run Startup** instead of **Run Performance Test**. You must run the Startup procedure before proceeding.

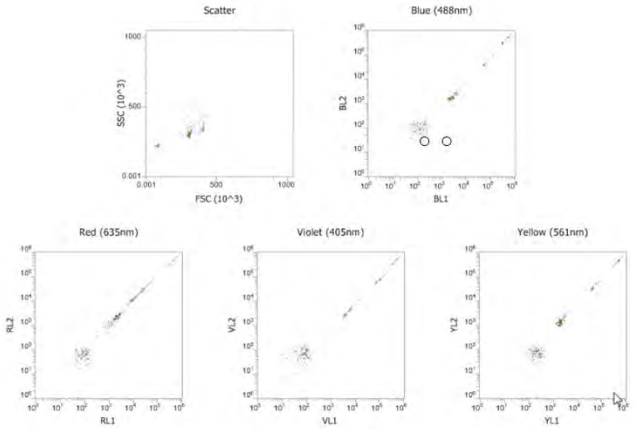
6. If the bead lot is expired, a warning dialog appears. Click **Yes** to continue or click **No** to cancel the Performance Test run.

   If they are not already powered on, all lasers are powered on and will remain on at the completion of the test process.

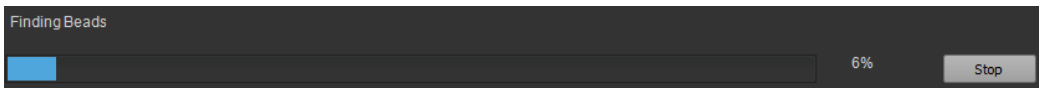
7. After the Performance Test is completed, click **Sanitize Attune SIP** (page 63) to run the SIP sanitize function before proceeding with your experiments.

Performance Test screen

The *Performance Test screen* provides progress information for the Performance Test procedure.



- Four Performance Test steps are displayed above the progress bar as they occur:
  - "Finding Beads"
  - "Measuring Laser Delays"
  - "Adjusting PMTV to MFI Criteria"
  - "Gathering Final Statistics"
- The progress bar displays the percent completion (0–100%).



Note: The Performance Test steps and progress bar are updated by the Attune™ Cytometric Software in real time.

- Depending on the number of configured channels, the screen displays:
  - One scatter density plot (SSC-H vs. FSC-H)
  - Bivariate dot plots for each laser (for example, BL2-H vs. BL1-H)

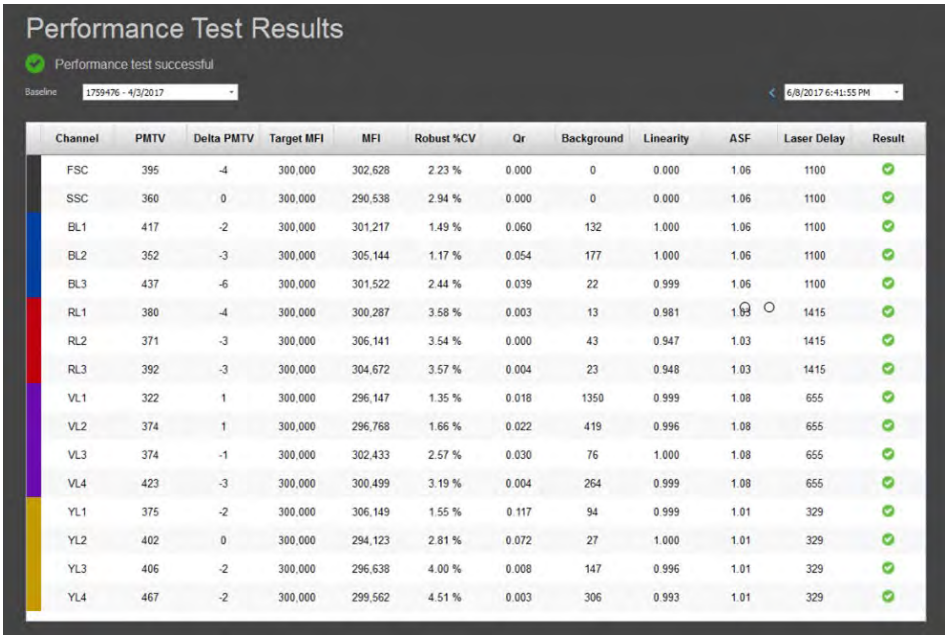
  For more information on the plots, see "Plots" (page 95).
- To zoom on a plot, use the **Size slider** on the Application status bar (page 47). You cannot resize objects on the workspace. The plots do not get resized if the application window is resized.
- To stop the Performance Test procedure, click **Stop**. The procedure stops and the software returns to Baseline setup (page 355).

Performance Test results

Performance Test completion without errors

If the Performance Test completes without errors, the software:

- Displays the *Performance Test Results* screen (page 370).



Channel	PMTV	Delta PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	395	-4	300,000	302,628	2.23 %	0.000	0	0.000	1.06	1100	✓
SSC	360	0	300,000	290,538	2.94 %	0.000	0	0.000	1.06	1100	✓
BL1	417	-2	300,000	301,217	1.49 %	0.060	132	1.000	1.06	1100	✓
BL2	352	-3	300,000	305,144	1.17 %	0.054	177	1.000	1.06	1100	✓
BL3	437	-6	300,000	301,522	2.44 %	0.039	22	0.999	1.06	1100	✓
RL1	380	-4	300,000	300,287	3.58 %	0.003	13	0.981	1.03	1415	✓
RL2	371	-3	300,000	306,141	3.54 %	0.000	43	0.947	1.03	1415	✓
RL3	392	-3	300,000	304,672	3.57 %	0.004	23	0.948	1.03	1415	✓
VL1	322	1	300,000	296,147	1.35 %	0.018	1350	0.999	1.08	655	✓
VL2	374	1	300,000	296,768	1.66 %	0.022	419	0.996	1.08	655	✓
VL3	374	-1	300,000	302,433	2.57 %	0.030	76	1.000	1.08	655	✓
VL4	423	-3	300,000	300,499	3.19 %	0.004	264	0.999	1.08	655	✓
YL1	375	-2	300,000	306,149	1.55 %	0.117	94	0.999	1.01	329	✓
YL2	402	0	300,000	294,123	2.81 %	0.072	27	1.000	1.01	329	✓
YL3	406	-2	300,000	296,638	4.00 %	0.008	147	0.996	1.01	329	✓
YL4	467	-2	300,000	299,562	4.51 %	0.003	306	0.993	1.01	329	✓

- Calculates the system area scaling factor constant based on the Performance Test results for each configured laser.
- Updates the default system values for the area scaling factor in the Instrument Settings panel (page 263).
- Updates the laser delay.

Note: Voltage settings are not updated or modified as a result of daily performance testing. For best results, optimize voltage settings for each experiment.

Note: Laser delays for each flow rate are set based on the Performance Test results. These values cannot be modified.

Performance Test completion with errors

If the Performance Test completes with errors, the software displays the *Errors Detected* dialog, which provides a list of the channel statistics that failed

To go to the Performance Test Results screen (page 370), click **Continue to results**.

Performance Test Reports

After running the Performance Tests, you can generate the following reports in the Attune™ Cytometric Software:

- Performance History
- Current PT Results
- Levey-Jennings Report
- Baseline Results

For information on viewing the Performance Test reports, see "Performance Test Reports", page 363.

20. Performance Test reports

Overview

Performance Tests

The Attune™ Cytometric Software uses Attune™ Performance Tracking Beads to define the cytometer's initial *Baseline status*. During this process, the median fluorescence intensity of each bead and the r%CV (robust percent coefficient of variation) are automatically measured in all fluorescence detectors. Software algorithms use this information to determine cytometer settings and provide target values for subsequent application specific settings (see "Performance Test data", below).

After the Baseline values are established, you use the same lot of Attune™ Performance Tracking Beads to run the *Performance Tests*. The Performance Tests measure variation from the Baseline values to track the daily performance of the cytometer.

Note: For information on running Performance Tests, see "Performance Testing", page 353.

Performance Test data

The Attune™ Cytometric Software generates data for the parameters listed below to establish the initial Baseline status of the cytometer and measures their variation from the target values established by the Baseline calculations to track the daily performance of the instrument.

- PMTV (photomultiplier tube voltage)
- Delta PMTV
- Target MFI (target median fluorescence intensity)
- Measured MFI
- r%CV (robust percent coefficient of variation)
- Quantum Efficiency (Qr)
- Background (Br)
- Linearity
- Area Scaling Factor
- Laser Delay

Performance Test Reports

After running the Performance Tests, you can generate the following reports in the Attune™ Cytometric Software:

- Performance History (page 364)
- Baseline Results (page 367)
- Current PT Results (page 370)
- Levey-Jennings Report (page 373)

Performance History Report

The Performance History Report shows the pass/fail status of all Performance Tests run against a selected Baseline test during a selected time period. The Performance History report is useful for gauging the overall "health" of the instrument over the selected time period.

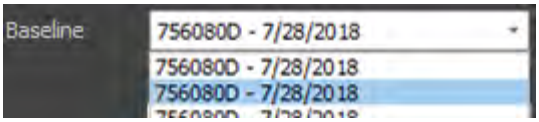
View the Performance History Report

On the Performance Test ribbon (available on the BL/PT module; page 354), click **Performance History**.

Alternatively, click **Performance History** on the Instrument ribbon tab (page 61) or on the Main Menu (page 27).

- By default, the Performance History Report is displayed for the most recent Baseline, which shows the *Pass/Fail status* of Performance Tests for the most recent 180-day period. For more information, see "Results plot", page 365.

- To view the report for Performance Tests associated with another Baseline and Bead lot, select the Baseline of interest from the **Baseline** dropdown menu.



Each Baseline in the dropdown menu is listed in the following format:

= Performance Test bead lot number

DD/MM/YYYY = date Baseline status was established

> **Note:** The Baseline dropdown menu is enabled only if more than one Baseline test has been run. The Baselines are ordered chronologically, with the most recent displayed first.

- By default, the Performance History Report shows the most recent 6-month data for the Performance Test series within the selected Baseline.

  To view the Performance History Report for a different data range, click the **calendar icon** on the **Date Range** tool to select the desired beginning and end dates.

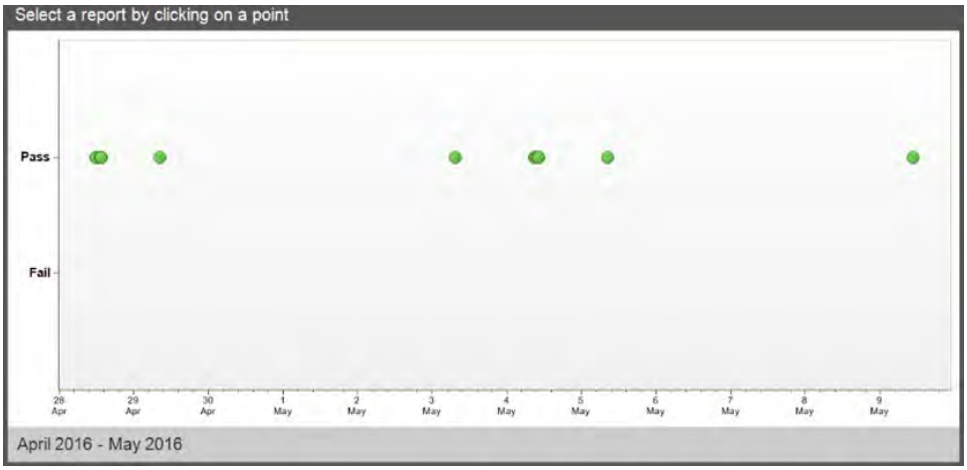
Note: By default, the range of the Performance History Report begins with the first Baseline and ends with the most recent Performance Test available for the selected Baseline.

If the default range exceeds 180 days, then the report shows data for the most recent 180-day time period.

Using the Date Range tool, you can select a date range of up to 180 days.

Results plot

The *Results plot* graphically summarizes the Pass/Fail status of the selected Baseline and associated Performance Tests within the selected date range.



- Each data point on the Results plot corresponds to a single date and the color of the data point indicates whether the result was a pass or fail.

Icon	Pass/Fail status
	Pass - All Performance Tests run on that date are within the limits set by the Baseline calculation.
	Fail - One or more Performance Tests run on that date deviate significantly from the target set by the Baseline calculation.

- If more than one result is available on a single date and the range is set such that the results overlap, the most recent result is displayed first.
- Hover over a data point to display the Pass/Fail status and the date of the Performance Test.

- Click on a data point within the plot to open the Baseline or Performance Test result for that date.
- Use the mouse wheel to zoom in on the X-axis, which displays a scroll bar when the zoom function is applied. The Y-axis cannot be zoomed.
- If the date range is changed, the zoom is reset to the default.

Note: The Pass/Fail status is based on the overall result of the Performance Tests for that date and the Baseline.

- If all Performance Tests run on that date meet the target values as determined by the Baseline test, the status is *Pass*.
- If one or more Performance Tests run on that date do not meet the Baseline test values, the status is *Fail*.

Print the Performance History Report

Select **File** ▶ **Print** to print the Performance History Report.

In addition to the Results plot, the printout includes a header containing the date range of the report and the instrument information.

Attune™ NxT Cytometer Performance History 7/28/2018 -7/28/2018

Cytometer: Attune NxT Acoustic Focusing Cytometer (Lasers: BRVY)	User: admin
Instrument Model Number: 4486521	Institution: Thermo Fisher Scientific
Instrument Serial Number: 1AFC200010913	Software: 2.7.873.0

Baseline Test Results

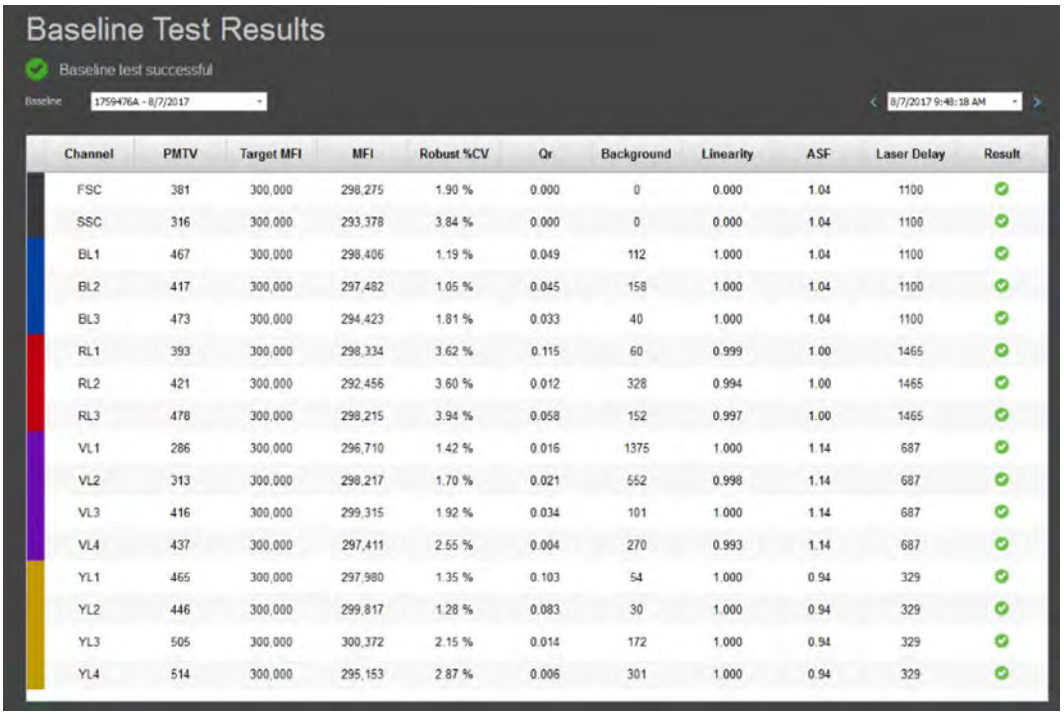
The *Baseline Test Result* displays the results of a selected Baseline.

When establishing a Baseline, the Attune™ Cytometric Software generates data for the parameters listed in "Performance Test data", page 363, to determine the initial status of the cytometer. These values are then used as a benchmark when tracking the daily performance of the instrument with Performance Tests.

View the Baseline Test Results

The Baseline Result report automatically appears after a Baseline is completed.

Alternatively, click **Baseline Results** on the Performance Test ribbon tab of the BL/PT module (page 354).



Channel	PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	381	300,000	298,275	1.90 %	0.000	0	0.000	1.04	1100	✓
SSC	316	300,000	293,378	3.84 %	0.000	0	0.000	1.04	1100	✓
BL1	467	300,000	298,406	1.19 %	0.049	112	1.000	1.04	1100	✓
BL2	417	300,000	297,482	1.05 %	0.045	158	1.000	1.04	1100	✓
BL3	473	300,000	294,423	1.81 %	0.033	40	1.000	1.04	1100	✓
RL1	393	300,000	298,334	3.62 %	0.115	60	0.999	1.00	1465	✓
RL2	421	300,000	292,456	3.60 %	0.012	328	0.994	1.00	1465	✓
RL3	478	300,000	298,215	3.94 %	0.058	152	0.997	1.00	1465	✓
VL1	286	300,000	296,710	1.42 %	0.016	1375	1.000	1.14	687	✓
VL2	313	300,000	298,217	1.70 %	0.021	552	0.998	1.14	687	✓
VL3	416	300,000	299,315	1.92 %	0.034	101	1.000	1.14	687	✓
VL4	437	300,000	297,411	2.55 %	0.004	270	0.993	1.14	687	✓
YL1	465	300,000	297,980	1.35 %	0.103	54	1.000	0.94	329	✓
YL2	446	300,000	299,817	1.28 %	0.083	30	1.000	0.94	329	✓
YL3	505	300,000	300,372	2.15 %	0.014	172	1.000	0.94	329	✓
YL4	514	300,000	295,153	2.87 %	0.006	301	1.000	0.94	329	✓

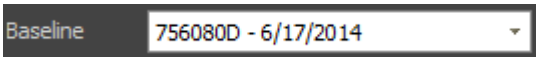
Baseline Results Report

- The overall Pass/Fail status of the current Baseline is displayed by the *Pass/Fail indicator*, which includes a message and an indicator icon.

Pass – Data from all channels meet the criteria specified by the instrument.

Fail – Data from one or more of the channels fail to meet the criteria specified by the instrument.

- To view the report for another Baseline run and Bead lot, select the Baseline of interest from the **Baseline history** dropdown menu.



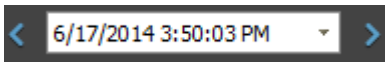
Each Baseline in the dropdown menu is listed in the following format:

= Performance Test bead lot number

DD/MM/YYYY = date Baseline status was established

Note: The Baseline dropdown menu is enabled only if more than one Baseline has been established. The Baselines are ordered chronologically, with the most recent displayed first.

- To view results from other Baseline calculations, select the desired test date from the **Baseline history** dropdown, or click the blue **arrows** to navigate through the Baseline reports.



Note: If there are multiple reports for a single day, the reports are displayed chronologically, with the most recent displayed first.

Baseline Results table

The Baseline Results table displays all statistics and calculations for all configured channels on the instrument.

Channel	PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	381	300,000	298,275	1.90 %	0.000	0	0.000	1.04	1100	✓
SSC	316	300,000	293,378	3.84 %	0.000	0	0.000	1.04	1100	✓
BL1	467	300,000	298,406	1.19 %	0.049	112	1.000	1.04	1100	✓
BL2	417	300,000	297,482	1.05 %	0.045	158	1.000	1.04	1100	✓
BL3	473	300,000	294,423	1.81 %	0.033	40	1.000	1.04	1100	✓
RL1	393	300,000	298,334	3.62 %	0.115	60	0.999	1.00	1465	✓
RL2	421	300,000	292,456	3.60 %	0.012	328	0.994	1.00	1465	✓
RL3	478	300,000	298,215	3.94 %	0.058	152	0.997	1.00	1465	✓
VL1	286	300,000	296,710	1.42 %	0.016	1375	1.000	1.14	687	✓
VL2	313	300,000	298,217	1.70 %	0.021	552	0.998	1.14	687	✓
VL3	416	300,000	299,315	1.92 %	0.034	101	1.000	1.14	687	✓
VL4	437	300,000	297,411	2.55 %	0.004	270	0.993	1.14	687	✓
YL1	465	300,000	297,980	1.35 %	0.103	54	1.000	0.94	329	✓
YL2	446	300,000	299,817	1.28 %	0.083	30	1.000	0.94	329	✓
YL3	505	300,000	300,372	2.15 %	0.014	172	1.000	0.94	329	✓
YL4	514	300,000	295,153	2.87 %	0.006	301	1.000	0.94	329	✓

- The configured channels are listed in the following order and color-coded based on the laser color.

  - Scatter channels (FSC and SSC)
  - Blue channels
  - Red channels
  - Violet channels
  - Yellow channels
  - Green channels

- The data for the following parameters is displayed for all configured channels in the order specified below.
  - PMTV (photomultiplier tube voltage)
  - Delta PMTV
  - Target MFI (target median fluorescence intensity)
  - Measured MFI
  - r%CV (robust percent coefficient of variation)
  - Quantum Efficiency (Q)
  - Background (B)
  - Linearity
  - Area Scaling Factor
  - Laser Delay
- The decimal places are only shown for r%CV, Q, Linearity, and Area Scaling Factor (2 decimal places for r%CV and Area Scaling Factor, and 3 decimal places for Q and Linearity).
- The Pass/Fail status for each configured channel is indicated by the status indicator icons shown below.

Pass - All statistics and calculations meet the criteria set by the instrument.

Fail - One or more of the calculations or statistics deviate significantly from the criteria set by the instrument.

Print the Baseline Results Report

Select **File** ▶ **Print** to print the Baseline Results Report.

In addition to the Baseline Results table, the printout includes a header containing the date range of the report and the instrument information.

Attune™ NxT Cytometer Baseline Report 7/28/2018 8:28:00 PM

Cytometer: Attune NxT Acoustic Focusing Cytometer (Lasers: BRVY)	User: admin
Instrument Model Number: 4486521	Institution: Thermo Fisher Scientific
Instrument Serial Number: 1AFC200010913	Software: 2.7.873.0

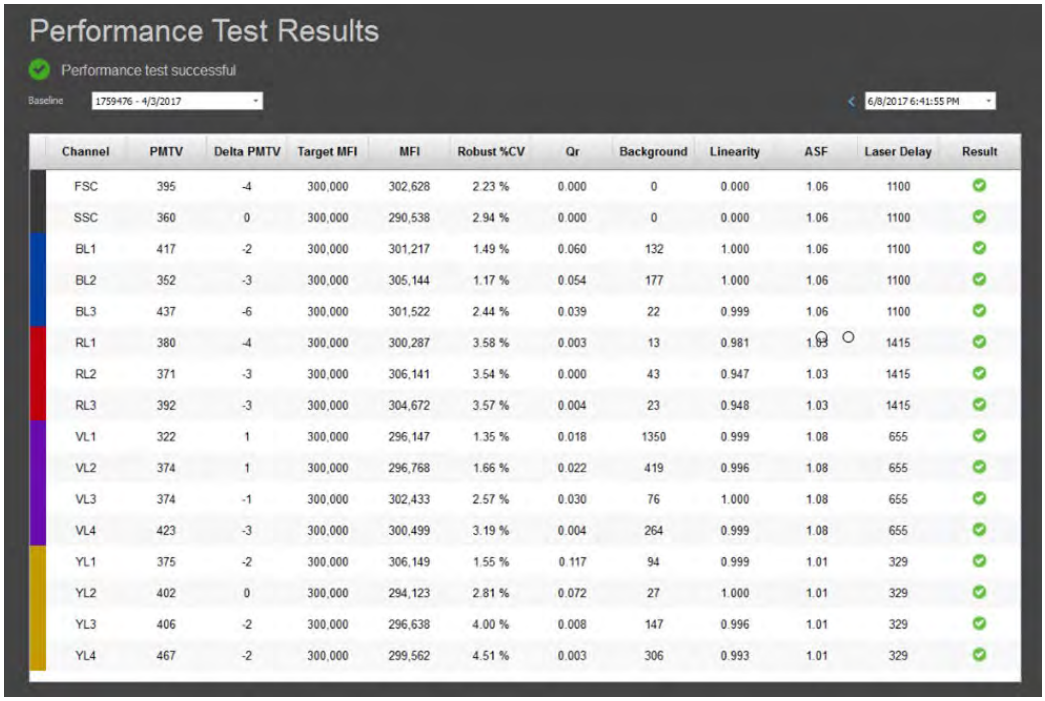
Current PT Results

Current PT Results displays the results of the current Performance Test and includes all statistics and calculations for all configured channels on the instrument.

View the Current PT Results

Current PT Results report appears automatically after a Performance Test is completed.

You can also access the most recent Performance Test report by clicking **Current PT Result** on the Performance Test ribbon tab of the BL/PT module (page 354).



Performance Test Results

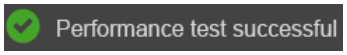
Performance test successful

Baseline 1759476 - 4/3/2017 6/8/2017 6:41:55 PM

Channel	PMTV	Delta PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	395	-4	300,000	302,628	2.23 %	0.000	0	0.000	1.06	1100	✓
SSC	360	0	300,000	290,538	2.94 %	0.000	0	0.000	1.06	1100	✓
BL1	417	-2	300,000	301,217	1.49 %	0.060	132	1.000	1.06	1100	✓
BL2	352	-3	300,000	305,144	1.17 %	0.054	177	1.000	1.06	1100	✓
BL3	437	-6	300,000	301,522	2.44 %	0.039	22	0.999	1.06	1100	✓
RL1	380	-4	300,000	300,287	3.58 %	0.003	13	0.981	1.03	1415	✓
RL2	371	-3	300,000	306,141	3.54 %	0.000	43	0.947	1.03	1415	✓
RL3	392	-3	300,000	304,672	3.57 %	0.004	23	0.948	1.03	1415	✓
VL1	322	1	300,000	296,147	1.35 %	0.018	1350	0.999	1.08	655	✓
VL2	374	1	300,000	296,768	1.66 %	0.022	419	0.996	1.08	655	✓
VL3	374	-1	300,000	302,433	2.57 %	0.030	76	1.000	1.08	655	✓
VL4	423	-3	300,000	300,499	3.19 %	0.004	264	0.999	1.08	655	✓
YL1	375	-2	300,000	306,149	1.55 %	0.117	94	0.999	1.01	329	✓
YL2	402	0	300,000	294,123	2.81 %	0.072	27	1.000	1.01	329	✓
YL3	406	-2	300,000	296,638	4.00 %	0.008	147	0.996	1.01	329	✓
YL4	467	-2	300,000	299,562	4.51 %	0.003	306	0.993	1.01	329	✓

Current PT Results Report

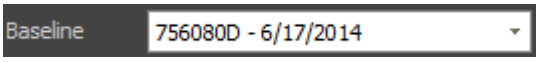
- The overall Pass/Fail status of the current Performance Test is displayed by the *Pass/Fail indicator*, which includes a message and an indicator icon.



Pass - All channels meet the criteria set by the Baseline calculation.

Fail - One or more of the channels fail to meet the criteria set by the Baseline calculation.

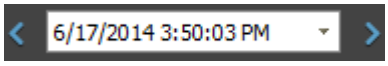
- To view the report for Performance Tests associated with another Baseline and Bead lot, select the Baseline of interest from the **Baseline** dropdown menu.



Each Baseline in the dropdown menu is listed by the Performance Test bead lot number followed by the date the Baseline status was established

Note: The Baseline dropdown menu is enabled only if more than one Baseline has been established. The Baselines are ordered chronologically, with the most recent displayed first.

- To view results from other Performance tests, select the desired test date from the **Performance test history** dropdown, or click the blue **arrows** to navigate through the Baseline reports and Performance Test reports.



Note: If there are multiple reports for a single day, the reports are displayed chronologically, with the most recent displayed first.

PT Results table

The PT Results table displays all statistics and calculations for all configured channels on the instrument.

Channel	PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	381	300,000	298,275	1.90 %	0.000	0	0.000	1.04	1100	✓
SSC	316	300,000	293,378	3.84 %	0.000	0	0.000	1.04	1100	✓
BL1	467	300,000	298,406	1.19 %	0.049	112	1.000	1.04	1100	✓
BL2	417	300,000	297,482	1.05 %	0.045	158	1.000	1.04	1100	✓
BL3	473	300,000	294,423	1.81 %	0.033	40	1.000	1.04	1100	✓
RL1	393	300,000	298,334	3.62 %	0.115	60	0.999	1.00	1465	✓
RL2	421	300,000	292,456	3.60 %	0.012	328	0.994	1.00	1465	✓
RL3	478	300,000	298,215	3.94 %	0.058	152	0.997	1.00	1465	✓
VL1	286	300,000	296,710	1.42 %	0.016	1375	1.000	1.14	687	✓
VL2	313	300,000	298,217	1.70 %	0.021	552	0.998	1.14	687	✓
VL3	416	300,000	299,315	1.92 %	0.034	101	1.000	1.14	687	✓
VL4	437	300,000	297,411	2.55 %	0.004	270	0.993	1.14	687	✓
YL1	465	300,000	297,980	1.35 %	0.103	54	1.000	0.94	329	✓
YL2	446	300,000	299,817	1.28 %	0.083	30	1.000	0.94	329	✓
YL3	505	300,000	300,372	2.15 %	0.014	172	1.000	0.94	329	✓
YL4	514	300,000	295,153	2.87 %	0.006	301	1.000	0.94	329	✓

- The configured channels are listed in the following order and color-coded based on the laser color.

  - Scatter channels (FSC and SSC)
  - Blue channels
  - Red channels
  - Violet channels
  - Yellow channels
  - Green channels

- The data for the following parameters are displayed for all configured channels in the order specified below.
  - PMTV (photomultiplier tube voltage)
  - Delta PMTV
  - Target MFI (target median fluorescence intensity)
  - Measured MFI
  - r%CV (robust percent coefficient of variation)
  - Quantum Efficiency (Q)
  - Background (B)
  - Linearity
  - Area Scaling Factor
  - Laser Delay
- The decimal places are only shown for r%CV, Q, Linearity, and Area Scaling Factor (2 decimal places for r%CV and Area Scaling Factor, and 3 decimal places for Q and Linearity).
- The Pass/Fail status for each configured channel is indicated by the status indicator icons shown below.

Pass - All statistics and calculations for the channel meet the criteria set by the Baseline calculation.

Fail - One or more of the statistics or calculations for the channel deviate significantly from the target set by the Baseline calculation.

Print the PT Results Report

Select **File** ▶ **Print** to print the PT Results Report.

In addition to the PT Results table, the printout includes a header containing the date range of the report, as well as the instrument information.

Cytometer: Attune NxT Acoustic Focusing Cytometer (Lasers: BRVG)	User: admin
Instrument Model Number: 0A29000	Institution: Thermo Fisher Scientific
Instrument Serial Number: 1AFC200010913	Software: 2.7.873.0

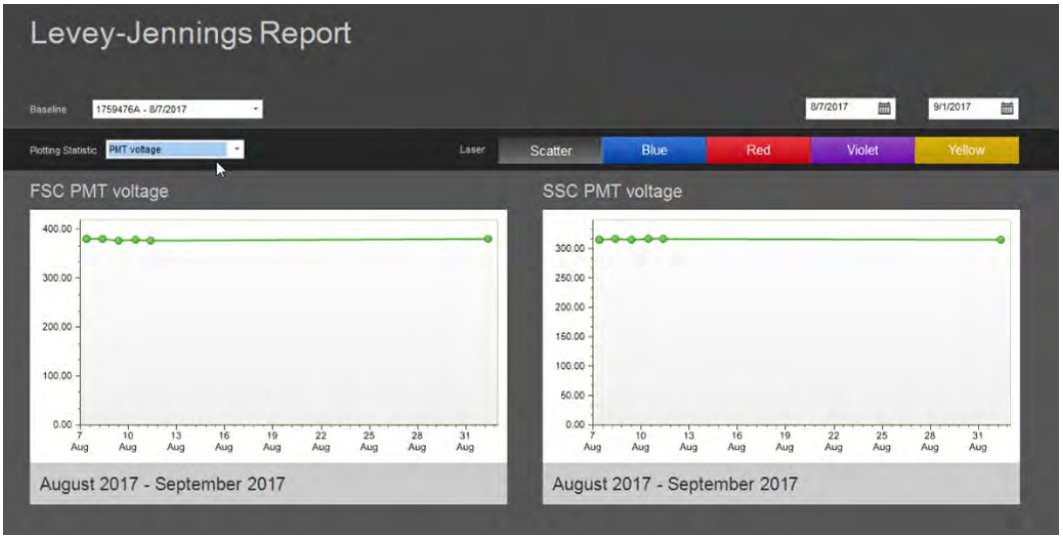
Levey-Jennings Report

The Levey-Jennings Report tracks the parameters listed in “Performance Test data”, page 363, for all configured channels to check for shifts and trends in cytometer performance and provides a visual indication of the cytometer performance over time.

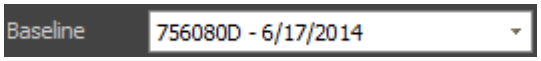
View the Levey-Jennings Report

To view the Levey-Jennings Report, click **Levey-Jennings Report** on the Performance Test ribbon tab of the BL/PT module (page 354).

The Levey-Jennings Report is displayed for the most recent Baseline, showing the data for the most recent 180-day time period.



- To view the Levey-Jennings Report for Performance Tests associated with another Baseline and Bead lot, select the Baseline of interest from the **Baseline** dropdown menu.



  Each Baseline in the dropdown menu is listed in the following format:

  ### = Performance Test bead lot number

  DD/MM/YYYY = date Baseline status was established

Note: The Baseline dropdown menu is enabled only if more than one Baseline has been established. The Baselines are ordered chronologically, with the most recent displayed first.

- To view the Levey-Jennings Report for a different data range, click the **calendar icon** on the **Date Range** tool to select the desired beginning and end dates.

Note: By default, the range of the Levey-Jennings Report begins with the first Baseline and ends with the most recent Performance Test available for the selected Baseline.

If the default range exceeds 180 days, then the report shows data for the most recent 180-day time period.

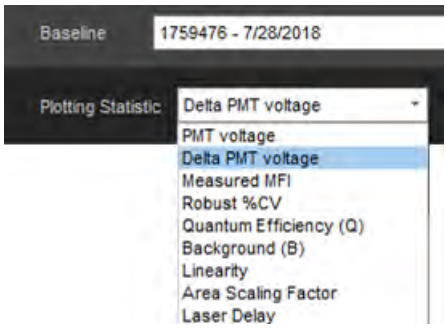
Using the Date Range tool, you can select a date range of up to 180 days.

If more than one result is available on a single date and the range is set such that the results overlap, the most recent result is displayed first.

Levey-Jennings plots

- The Attune™ Cytometric Software generates Levey-Jennings plots for the parameters listed in "Performance Test data", page 363.

  Select the parameter of interest from the **Plotting Statistic** dropdown menu to view the Levey-Jennings plot for that parameter.



- The **Laser** selection tool on the Levey-Jennings Report screen allows data to be shown for the scatter channels, blue channels, red channels, violet channels, and yellow channels.
- The buttons on the Laser selection tool are color-coded based on the laser color. To display the Levey-Jennings plot for the scatter/laser channel of interest, click the button for the desired channel.

Note: The software displays only the channels that were configured at the time that the Performance Test was run.

- The Levey-Jennings plots display the Pass/Fail status for the selected parameter within the selected date range. Each data point on the plot corresponds to a single date and the color of the data point indicates its status (see below).

  If more than one result is available on a single date and the range is set such that the results overlap, the most recent result is displayed first.

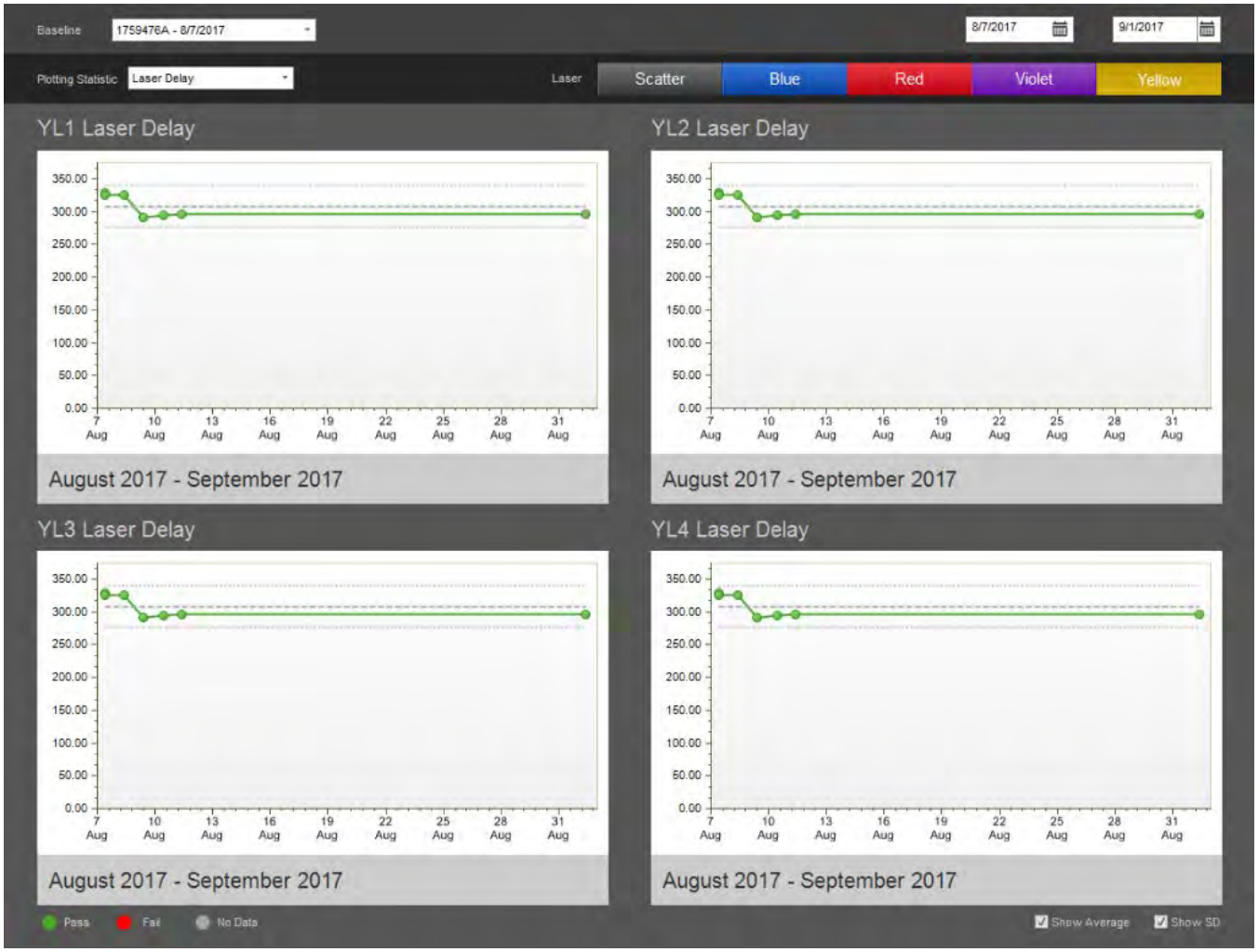
Icon	Pass/Fail status
	Pass – Performance of the selected channel for the selected parameter is within the limits set by the Baseline calculation.
	Fail - Performance of the selected channel for the selected parameter deviates significantly from the target set by the Baseline calculation.
	No data - There is no data for the selected channel or the parameter on that date.

- Hover over a data point to display the corresponding data and the date. The decimal places are only shown for r%CV, Q, Linearity, and Area Scaling Factor (2 decimal places for r%CV and Area Scaling Factor, and 3 decimal places for Q and Linearity).

200mV
10/31/2012

- Click on a data point within the Levey-Jennings plotto open the Baseline or Performance Test result for that date, depending on whether the data point represents the Baseline or the Performance Test.
- Use the mouse wheel to zoom in on the X-axis, which displays a scroll bar when the zoom function is applied. The Y-axis cannot be zoomed.
- If the date range is changed, the zoom is reset to the default.
- Select **Show Average** below the Levey-Jennings plot to display the average for the selected parameter and channel within the selected date range. The average appears as a dashed line.

- Select **Show SD** (standard deviation) to display the standard deviation for the data. Two standard deviations appear as dotted lines above and below the center line.



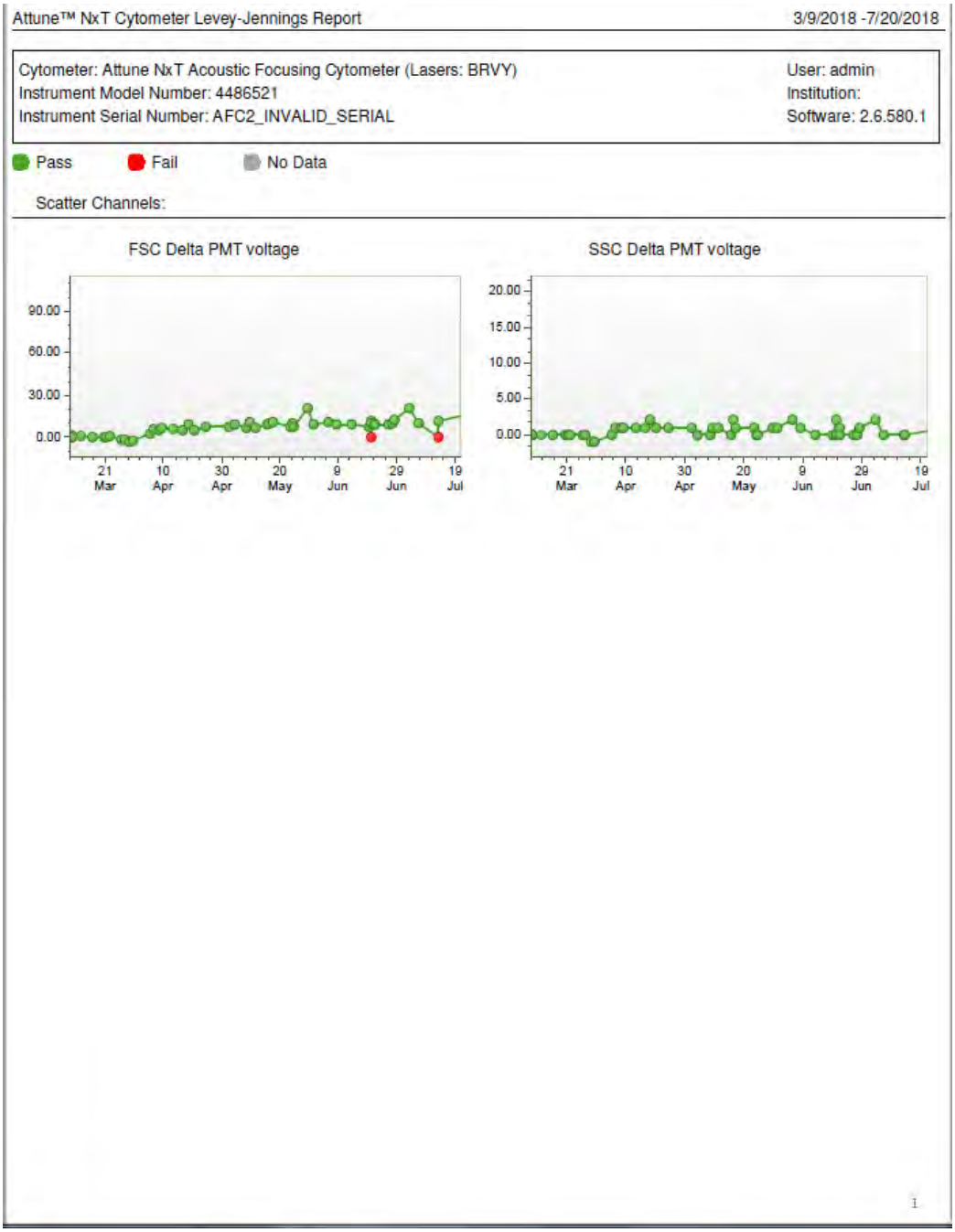
Print the report

Select **File** ▶ **Print** to print the report.

The printout of the Levey-Jennings Report includes a header containing the date range of the report and the instrument information, and the Levey-Jennings plots for all configured lasers.

The first page of the printout contains both scatter channels and the first laser channel. The remaining channels are printed individually on the subsequent pages.

The following examples show the first and second pages of a Levey-Jennings Report printout.



Attune™ NxT Cytometer Levey-Jennings Report 3/9/2018 -7/20/2018

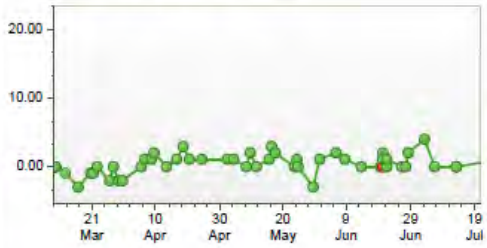
Cytometer: Attune NxT Acoustic Focusing Cytometer (Lasers: BRVY)
Instrument Model Number: 4486521
Instrument Serial Number: AFC2_INVALID_SERIAL

User: admin
Institution:
Software: 2.6.580.1

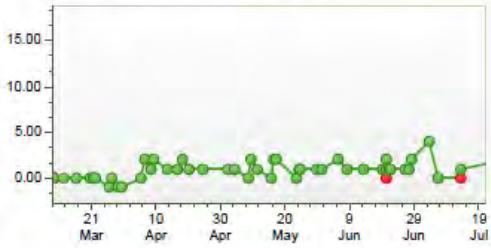
Pass Fail No Data

Blue Laser Channels:

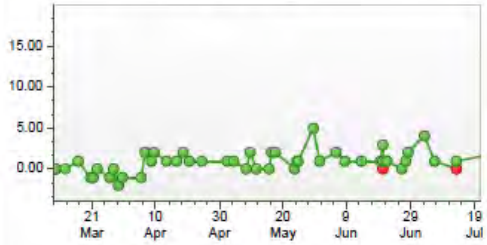
BL1 Delta PMT voltage



BL2 Delta PMT voltage



BL3 Delta PMT voltage



2

21. Compensation

Overview

Compensation

Fluorophores emit light over a range of wavelengths. Although optical filters limit the range of frequencies measured by a given detector, when two or more fluorophores are used in an experiment, there is often an overlap between the wavelength ranges. *Compensation* is the mathematical method used to correct the emission overlap from one fluorophore into the emission channel of another fluorophore.

The Attune™ NxT Cytometric Software calculates the Compensation settings automatically as it guides you through the process. This chapter details:

- Process of setting up Compensation (page 379)
- Working with the Compensation Workspaces (page 383)
- Modifying Compensation (page 393)

Compensation controls

- If the experiment requires compensation, prepare the necessary Compensation controls. You will need single-stained controls (i.e., compensation beads or cells) for each fluorophore you are using for compensation. Unless you select to use a negative gate or none, you will also need an unstained or isotype-labeled control.
- You can use Tubes or Plates as the source of your Compensation controls. The Compensation Setup dialog provides different options for setup based on your selection of the Compensation Source. For more information, see "Compensation Setup Dialog", page 379.

IMPORTANT! Once you click **Record** on any of the Compensation controls within the Compensation Setup, the Instrument Settings for all fluorescent channels will be shaded gray and cannot be changed. It is critical that you optimize voltages prior to recording any Samples or Compensation controls.

Compensation Setup

Initiate Compensation Setup

To initiate Compensation Setup, do one of the following:

- Click the **Compensation Setup** button on the *Compensation ribbon tab* (page 53).

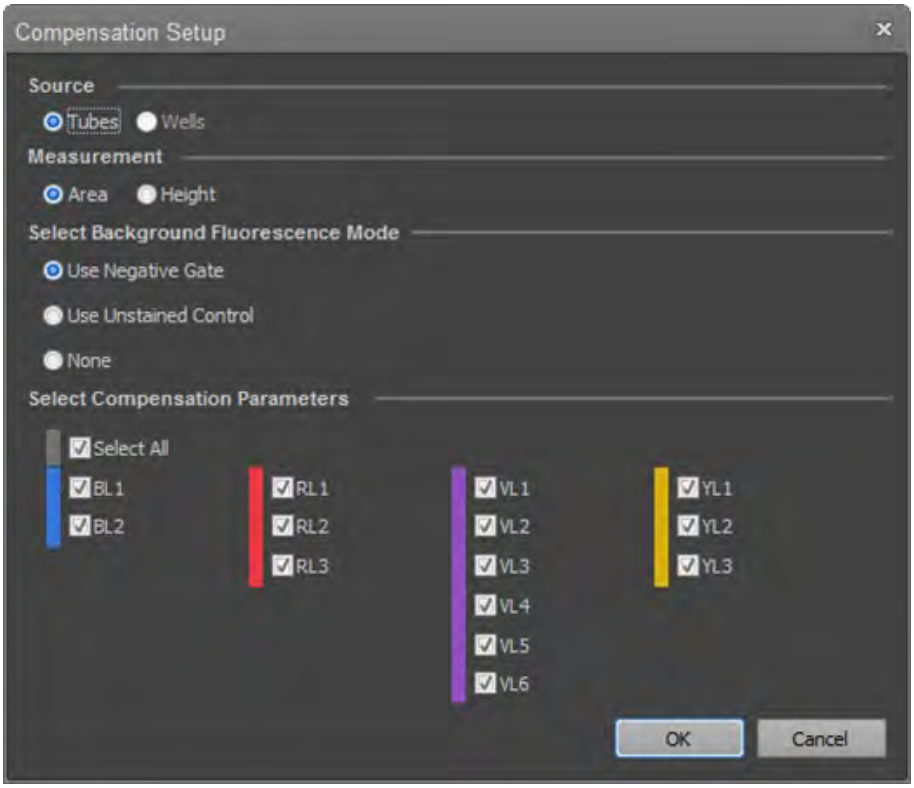
- Double-click on the **Compensation node** within an Experiment on the *Experiment Explorer* (page 189) when there are no Compensation controls present.

- Click on the **Compensation well** button on the *Experiment ribbon tab* to define the Compensation samples.

Each of these methods launches the *Compensation Setup dialog*.

Compensation Setup dialog

The *Compensation Setup dialog* allows you to choose the Compensation Source, the Compensation Measurement, the Background Fluorescence mode, and the required Compensation Parameters. This dialog also allows you to make modifications to an existing Compensation Setup.

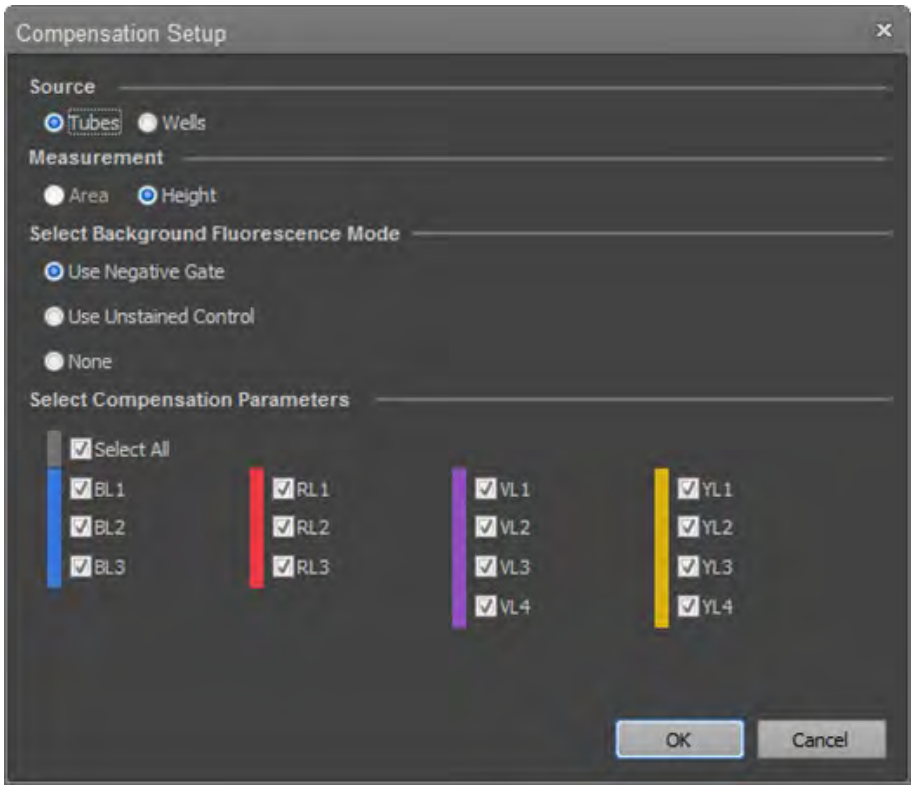


- The *Source* section shows the option for the source of Compensation controls, which is **Tubes** (page 380).
- The options and controls available in the remaining sections of the dialog (*Measurement*, *Background Fluorescence Mode*, and *Compensation Parameters*) are based on the Source selection.
- Once Compensation controls have been created and recorded, the Source selection cannot be changed and this option becomes disabled.

Compensation Control Source – Tubes

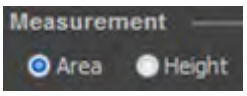
When **Tubes** are selected as the Compensation control source, the Compensation Setup dialog appears and contains the following controls:

- *Measurement* radio buttons (page 380)
- *Select Background Fluorescence Mode* radio buttons (page 381)
- *Select Compensation Parameters* check boxes (page 381)



Measurement

Measurement allows you select **Area** or **Height** as the parameter for calculating the compensation.

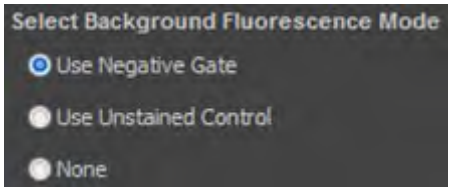


- By default, **Area** is selected. If no area measurements are selected in the Experiment Instrument Settings, the selection defaults to **Height**.
- When **Area** is selected, the resulting Compensation Workspace and controls use the area measurement as the parameter for calculating the compensation.

  When **Height** is selected, the resulting Compensation Workspace and controls use the height measurement as the parameter for calculating the compensation.
- If all the channels have both the height and area measurements enabled, the Measurement selection can be changed after the Compensation controls have been recorded; otherwise this option is disabled.
- If the Measurement selection is changed, the Workspaces are updated to use the new Measurement parameter (if the parameters are enabled) and the Compensation is recalculated.

Select Background Fluorescence Mode

Select Background Fluorescence Mode allows you to choose the source of the background fluorescence when calculating compensation.



- The available options are **Use Negative Gate**, **Use Unstained Control**, or **None**.
- By default, the **Use Negative Gate** is selected.
- **Use Unstained Control** adds Unstained Control as an additional Compensation control to the Compensation node in the Experiment.
- **Use Negative Gate** provides an additional Histogram gate or Rectangle gate on the Compensation Workspace for defining the negative population.
- When **None** is selected, compensation is calculated without correcting for background auto-fluorescence.
- If you change the Background Fluorescence Mode before recording any Compensation controls, the Compensation Workspaces are refreshed with the new background option and any changes are reset to the defaults.
- The Background Fluorescence Mode options are disabled once Compensation controls have been created and any Compensation controls have data recorded.

  If there is no recorded data, changing the Background Fluorescence Mode option updates the Compensation controls and Workspaces accordingly.

Select Compensation Parameters

Select Compensation Parameters contains checkbox controls that determine the parameters used for calculating compensation.

- The **Compensation Parameter** controls are visible and enabled only for the parameters that are selected in the Parameters section of the Instrument Settings Panel, as described on page 253.
- The checkboxes are arranged in columns grouped by laser color (Blue, Red, Violet, and Yellow) with a checkbox for each fluorescence detector assigned to each laser.
- By default, all enabled checkboxes are selected.

- When the **Select All** checkbox is selected, all enabled parameters are checked.

  When the **Select All** checkbox is deselected, all checkboxes are unchecked.

  Deselecting one or more of the parameters also deselects the Select All checkbox.
- You must select at least two Compensation Parameters.

  If less than two parameters are checked, the OK button is grayed out and a warning stating "At least two fluorescence parameters must be selected." is shown under the buttons.

- Selection of Compensation Parameters can be modified after controls have been created and recorded.

  Any controls that do not have data recorded can be deselected, which removes them from the Experiment Explorer.

  Additional controls that have not been assigned can be selected, which adds them to the Experiment Explorer.
- The checkboxes become disabled and remain checked for any controls that have recorded data.

OK and Cancel buttons

- Click **OK** to close the dialog box and create or update the Compensation control for each selected parameter within the Compensation node of the Experiment in the Experiment Explorer.

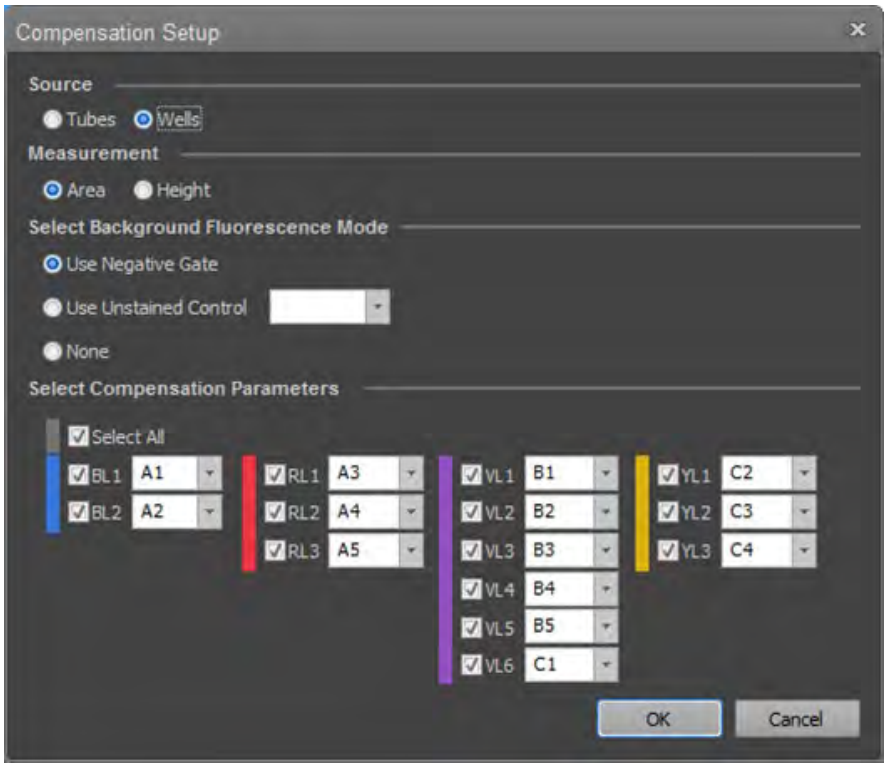
  The Compensation Workspace for the first control or the unstained control (if **Use Unstained Control** was selected) opens automatically.

  An *Identity Compensation matrix*, a matrix of N × N dimensions with ones on the main diagonal and zeros elsewhere, is created, where N is the number of compensation parameters.
- Click **Cancel** to close the dialog without making any changes.

Compensation Control Source – Wells

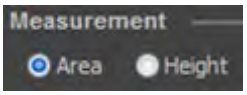
When **Wells** is selected as the Compensation control source, the Compensation Setup dialog appears and contains the following controls:

- *Measurement* radio buttons: Height, Area (page 383)
- *Select Background Fluorescence Mode* radio buttons: Negative gate, Unstained control, None (page 384)
- *Select Compensation Parameters* combo boxes (page 385)



Measurement

Measurement allows you select **Area** or **Height** as the parameter for calculating the compensation.



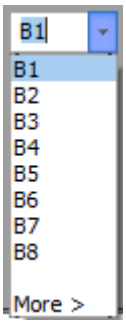
- By default, the **Area** is selected. If no area measurements are selected in the Experiment Instrument Settings, the selection defaults to Height.
- When **Area** is selected, the resulting Compensation Workspace and controls use the area measurement as the parameter for calculating the compensation.

  When **Height** is selected, the resulting Compensation Workspace and controls use the height measurement as the parameter for calculating the compensation.
- If all the channels have both the height and area measurements enabled, the Measurement selection can be changed after the Compensation controls have been recorded; otherwise this option is disabled.
- If the Measurement selection is changed, the Workspaces update to use the new Measurement parameter (if the parameters are enabled) and the Compensation is recalculated.

Select Background Fluorescence Mode

Select Background Fluorescence Mode allows you to choose the source of the background fluorescence when calculating compensation.

- The available options are **Use Negative Gate**, **Use Unstained Control**, or **None**. By default, the **Use Negative Gate** is selected.
- **Use Unstained Control** adds Unstained Control as an additional Compensation control to the Compensation node in the Experiment.
- The **Unstained Control combo box** in the Select Compensation Parameters section displays a list of all selected wells on the Heat Map and a More option to expand the list to include all wells in the plate that do not contain data.

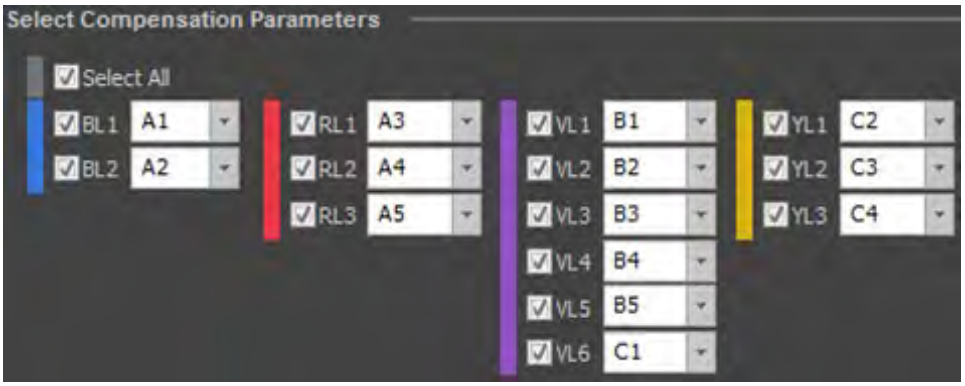


- The wells in the combo box dropdown are listed in the same order as the Plate run order (A1–A12, B1–B12, etc.). By default, the first selected well is set as the Unstained Control
- **Use Negative Gate** provides an additional Histogram gate or Rectangle gate on the Compensation Workspace for defining the negative population (see "Compensation Workspace", page 387).
- When **None** is selected, compensation is calculated without correcting for background auto-fluorescence.
- The Background Fluorescence Mode options are disabled once Compensation controls have been created and any Compensation controls have data recorded.

  If data has not been recorded, changing the Background Fluorescence Mode option updates the Compensation controls and compensation Workspaces accordingly.

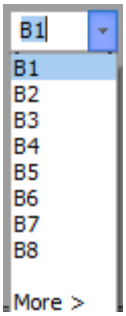
Select Compensation Parameters

Select Compensation Parameters contains checkbox controls that determine the parameters used for calculating compensation.



- The **Compensation Parameter** controls are visible and enabled only for the parameters that are selected in the Parameters section of the Instrument Settings Panel (page 253).
- The checkboxes are arranged in columns grouped by laser color (Blue, Red, Violet, and Yellow) with a checkbox for each fluorescence detector assigned to each laser.
- The checkbox labels use the display name set in the Options dialog (page 476). The display name can be modified to display the Parameter name (e.g., BL1-A), the Fluorophore name (e.g., FITC), or both (e.g., BL1-A – FITC) (page 476).
- When wells are selected prior to opening the Compensation Setup dialog, the corresponding Compensation control parameters are pre-selected on the Compensation Setup dialog and mapped to the wells in the order of well location (top to bottom, column by column).
- When wells are not preselected prior to opening the compensation setup dialog, the selected Compensation control parameters are mapped to the wells in the order of well location (top to bottom, column by column).
- To manually map a Compensation Parameter to a specific well, select the well location from the dropdown menu next to the selected parameter.
- The **Compensation Parameter** combo boxes contain a list of all selected wells and a More option to expand the list to include all wells in the plate that are unmapped.
- The wells in the combo box dropdown are listed in the same order as the Plate run order (A1–A12, B1–B12, etc.). If the list shows more than 25 well locations, a scroll bar is displayed, allowing you to view the entire list.

  If no wells are selected, the combo box list includes all unmapped wells.



- When the **Select All** checkbox is selected, all enabled parameters are checked.

  When the **Select All** checkbox is deselected, all checkboxes are unchecked.

  Deselecting one or more of the parameters also deselects the Select All checkbox.
- At least two Compensation Parameters must be selected for compensation to be calculated and applied to an experiment.

  If less than two parameters are checked, the OK button is grayed out and a warning stating "At least two fluorescence parameters must be selected." is shown under the buttons.

- Selection of Compensation Parameters can be modified after controls have been created and recorded.
  - To remove Compensation controls from the experiment, open the Compensation Setup dialog and deselect the parameter by unchecking the checkbox next to the parameter name. Only controls that do not have data can be removed.
  - To add additional controls to the compensation matrix, open the Compensation Setup dialog, select the appropriate parameter from the dialog, then choose the appropriate well from the dropdown menu next to the parameter name.

    You can designate only unmapped wells as Compensation control wells. The additional Compensation controls will be listed in the Experiment Explorer and the Heat Map after clicking **OK** and closing the compensation setup dialog.
- The checkboxes become disabled and remain checked for any controls that have recorded data. If the FCS file is removed, these controls will become enabled for deselection.

OK and Cancel buttons

- Clicking **OK** closes the dialog box and creates or updates the Compensation control for each selected parameter within the Compensation Node of the Experiment in the Experiment Explorer.

  The Compensation Workspace for the first control or the unstained control (if **Use Unstained Control** was selected) opens automatically following compensation setup.

  An *Identity Compensation matrix* is created before Compensation controls are recorded. This matrix is composed of all selected compensation parameters and has values of 0%, because compensation has not yet been measured and calculated.
- Clicking **Cancel** closes the dialog and any changes are reverted.

Compensation Workspace

Overview

Compensation Workspace is a special Workspace that contains all the necessary plots and gates to optimize Instrument Settings for the Compensation controls, to record Compensation controls, and to define the populations used when calculating compensation.

Customize the Compensation Workspace

The Compensation Workspace has a fixed landscape view where plot layout or size cannot be adjusted. The default plot attributes are as described in the *Options dialog* (page 480).

- The **Workspace Zoom** tool on the *View Ribbon tab* (page 53) is available for the Compensation Workspace. This tool displays all plots present in the filmstrip area of the Workspace view (page 91) and allows you to zoom in and out of each plot.

- Plot scale ranges for all parameters are set to **manual**, which allows you to enter the minimum and maximum values manually. The allowable range depends on the type of scale selected.

  The default maximum value for each parameter is determined by the range set in the FCS file. By default, the maximum is set to 2^{26} for Event and Time counts, to 2^{20} for Height and Area measurements, and to 2^{10} for Width measurements.

  The minimum value for logarithmic parameters is set to 1, and the minimum for linear parameters is set to 0.
- The plot attributes listed below can be customized as described in "Customize Plot Options" (page 277) and in "Workspace view" (page 82).
  - Resolution
  - X and Y parameters on gating plots and where specified below
  - Scale type and range
- The plot and axis titles cannot be modified.
- The gates used on the Compensation Workspace plots use the gate style and gate label style default settings as described in the Options dialog (page 477).
- Gates cannot be customized and their names cannot be modified.

Plot context menu

Plot context menu is displayed when you right-click on any empty area (i.e., white space) within the plot boundary, but not on another active area (gate, plot title, or plot axis).

- Plot context menu contains the following options:
  - Customize
  - Scale
  - Copy
  - Send to Overlay
  - Save As
- All options listed function as described in "Plot Context Menu", page 125.

Plot axis context menu

Plot axis context menu is displayed when you right-click on a plot scale or parameter name. The plot axis context menu options displayed depend on the plot axis selected.

- The X-axis parameter of fluorescence plots cannot be changed, and only the following options are displayed:
  - Customize
  - Scale
  - Copy
  - Send to Overlay
  - Save As

- For the Scatter plots and Y-axis of dual-parameter fluorescence plots, the parameter can be changed, and the following options are displayed:
  - Customize
  - Scale
  - Parameter
  - Copy
  - Send to Overlay
  - Save As

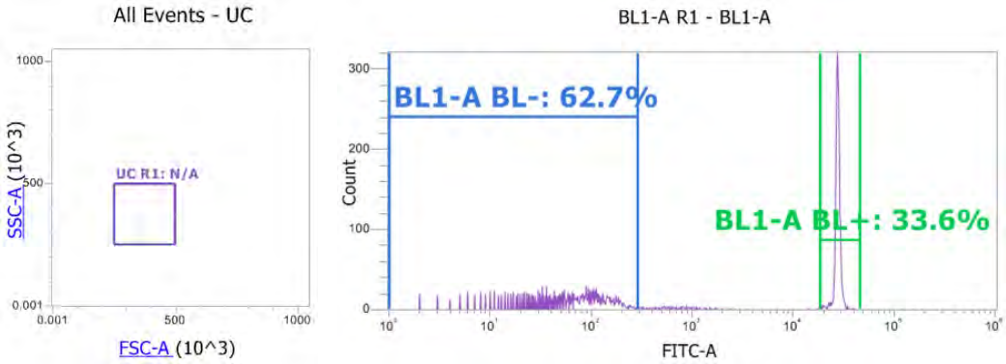
- All options listed function as described in "Plot Axis Context Menu", page 130.

Gate context menu

- The Gate context menu in the Compensation Workspace is available only for gates on dual-parameter scatter plots.
- Right-clicking on any part of a gate, on the gate boundary line, or a control point displays the Gate context menu.
- The context menu for dual-parameter scatter plot gates consists of a single option: Apply gate shape to all controls.
- This option applies the current scatter gate type, size, and location to the scatter gate on all Compensation controls within the current Experiment.
- Apart from the scatter gate described above, there is no gate context menu for compensation plots.

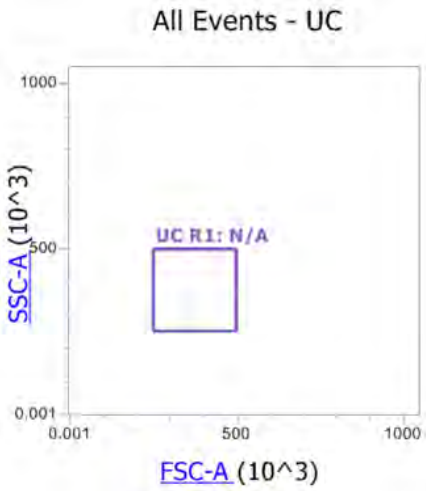
Compensation Workspace for Histogram View

The Workspace for the *Histogram View* consists of a *Gating plot* and a *Histogram Compensation plot* corresponding to the selected Compensation Parameter.

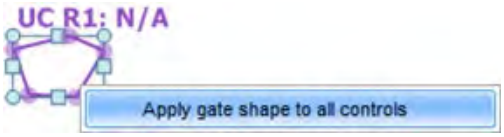


Gating plot

The *Gating plot* is an ungated dual-parameter density plot used to gate the events shown on the Compensation plot.



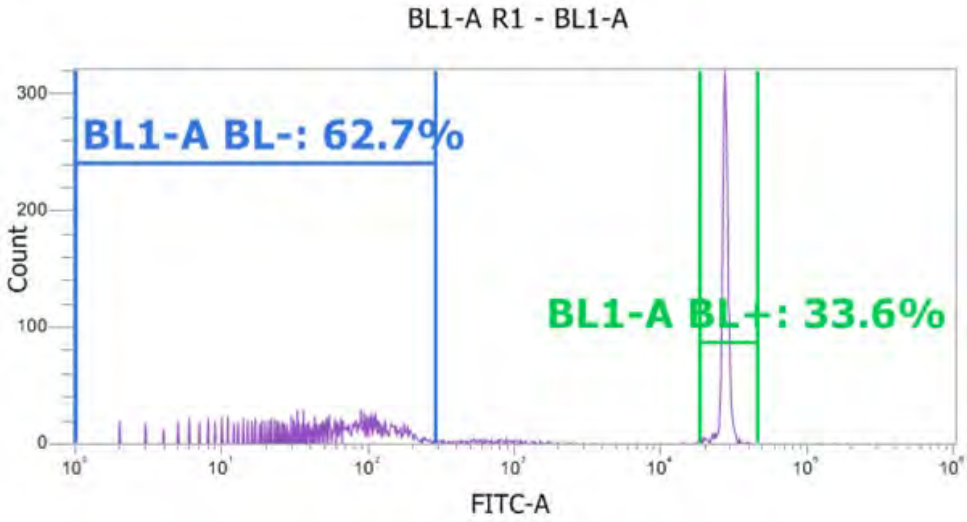
- By default, the parameters for the X- and Y-axes are set to FSC and SSC, respectively, and use the measurement specified in the Measurement section of the Compensation Setup dialog (page 379).
- The Gating plot type can be changed to Ellipse or Contour Autogate.
- The Gating plot can only be set to display *All Events*. The Plot title hyperlink, if shown, is disabled.
- The Parameter names on the Gating plot are hyperlink controls that link to the Parameter selection dropdown list (see "Plot Parameter Hyperlink", page 134). You can change the selected parameters for both the X- and Y-axes.
- The Gating plot contains a single 5-point polygon gate (drawn in a rectangle) named *Parent*. This gate cannot be deleted.
- The default position of the Parent gate has the following (X, Y) coordinates:
  - lower left corner coordinates (250000, 250000)
  - upper right corner coordinates (500000, 500000)
- You can move and resize the Parent gate as needed.
- Any adjustment made to the Parent gate automatically updates the Histogram plots and the calculated Compensation matrix (page 393).
- To apply the position of the Parent gate to other compensation controls, right-click on the gate and select **Apply gate shape to all controls**.



- Initially, when the Gating plot gate position is moved on the Workspace of the first Compensation control, it automatically applies to all Compensation control samples. After a gate is moved on subsequent Compensation control samples, moving the gate on the Workspace of the first control has no effect.

Histogram Compensation plot without Negative gate

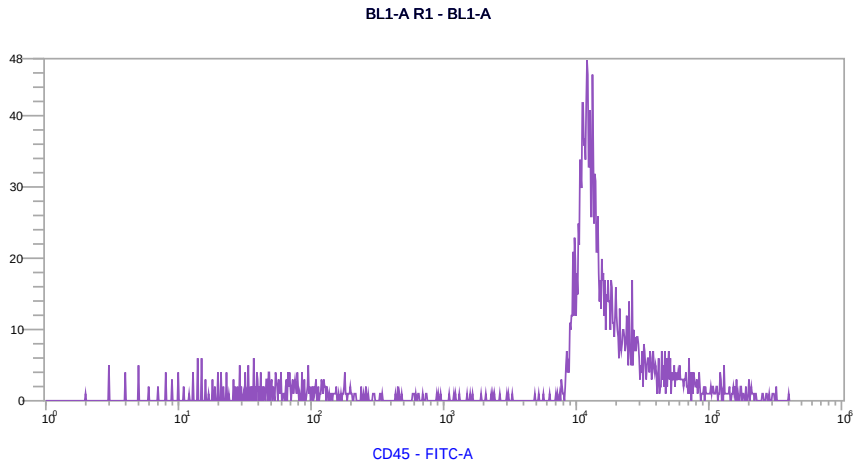
The *Histogram Compensation plot* is a single-parameter daughter plot derived from the Parent gate on the dual-parameter Gating plot (page 389). This plot type cannot be changed.



- The X-axis parameter on the plot is the currently selected Compensation control on the Experiment Explorer. This parameter cannot be changed.
- The Gate name hyperlink on the Plot title, if shown, is disabled.
- The Compensation plot contains a single Histogram gate, which is named in the following format: “<parameter channel name> +”. For example, BL1+.
- The default position of the Histogram gate has the following (X, Y) coordinates:
  - left coordinate (500,000)
  - right coordinate (750,000)
- You can move and resize the gate as needed.
- The Compensation Histogram gate cannot be deleted.
- Any adjustment made to the Histogram gate automatically updates the calculated Compensation matrix (page 393).

Histogram Compensation plot with Negative gate

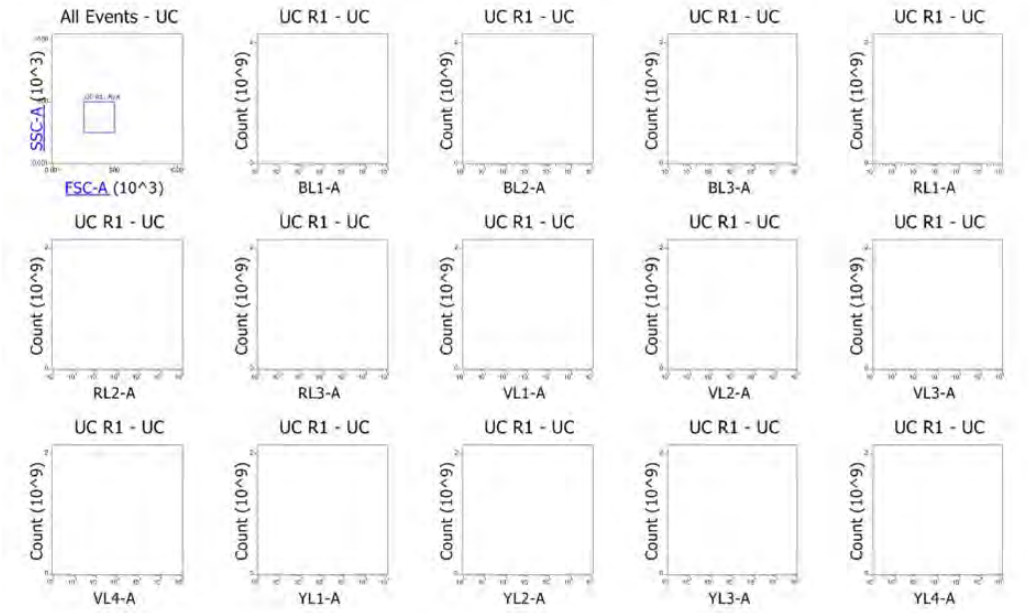
This Compensation plot is identical to the Histogram Compensation plot (page 390) with the exception that it contains an additional gate defining a negative population.



- When **Use Negative Gate** is selected in the Compensation Setup dialog (page 379), the Histogram plot contains an additional gate to define a negative population.
- The negative gate is named in the following format: “<parameter channel name> -”. For example, BL1-.
- The default position of the Negative gate has the following (X, Y) coordinates:
  - left coordinate (1)
  - right coordinate (1000)

Unstained Control Workspace

When **Use Unstained Control** is selected in the Compensation Setup dialog (page 379), an additional Control Workspace for the Unstained control is created.



- The Unstained Control Workspace contains a Gating plot as described on page 389 and Histogram plots for each Compensation parameter.
- The Histogram plots for Compensation parameters on the Unstained Control Workspace do not display any gates. All Histogram plots are gated on the Parent gate.
- The background signal is calculated based on the median of the gated data for each parameter; no additional gates are required.

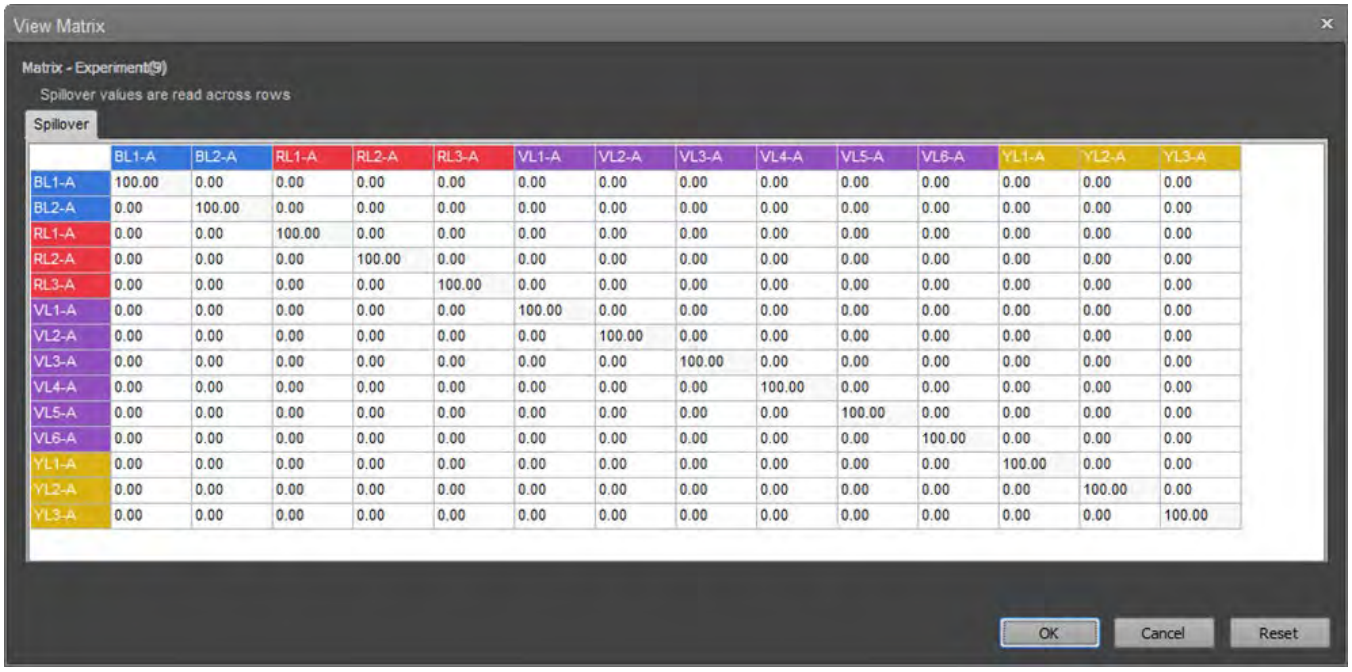
Matrix dialog

Overview The *Matrix dialog* shows the active compensation associated with the current Experiment or FCS file and allows you to manually edit or reset the *Spillover matrix* values.

- Click the **View Matrix** button on the *Compensation ribbon tab* (page 53) to launch the Matrix dialog.

  Alternatively, double-click the **Compensation node** on the Experiment Explorer when Compensation controls are present.

Note: *Spillover matrix* shows the amount of spillover from each fluorophore into each of the other fluorescent channels.



	BL1-A	BL2-A	RL1-A	RL2-A	RL3-A	VL1-A	VL2-A	VL3-A	VL4-A	VL5-A	VL6-A	YL1-A	YL2-A	YL3-A
BL1-A	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BL2-A	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RL1-A	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RL2-A	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RL3-A	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VL1-A	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VL2-A	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VL3-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
VL4-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
VL5-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
VL6-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
YL1-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
YL2-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00
YL3-A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

- The Matrix title indicates the source of the active compensation, which is the Experiment or the Sample name based on the selection made in the *Apply group* of the Compensation ribbon tab (page 71) for the current Sample.
- If the Matrix is opened by double-clicking the **Compensation node**, the compensation source is always the Experiment.

Matrix dialog table

Matrix dialog table shows the Spillover values for the current compensation in editable textboxes that are arranged in a table.

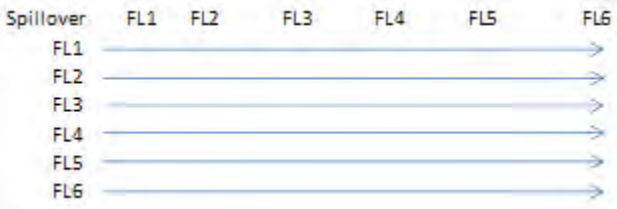
- The row and column labels use the Channel name ($PnN) by default.
- The row and column labels have a background color based on the laser color associated with the channel. If a channel color is not available for a parameter (i.e., non-Attune™ file), the default fill is white and the font color is black.
- When a textbox is selected, the column and row is highlighted at the point of their intersection in blue. The diagonal values of the matrix are shaded in light gray.

	BL1-A	BL2-A	BL3-A
BL1-A	100.00	0.00	0.00
BL2-A	0.00	100.00	0.00
BL3-A	0.00	0.00	100.00
RL1-A	0.00	0.00	

- In the Spillover matrix, the textbox controls accept numbers from 0% to 100%, and allow the use of two decimal places.

  The diagonal values cannot be adjusted in the Spillover matrix is shown.
- You can enter up to 7 total characters into the textboxes in the Spillover matrix, but all entered values are rounded to 2 decimal places when you click elsewhere or when you press the **Enter** key.

  Only numbers, decimals, and + and – signs can be input in the textboxes.
- The Spillover values in the table are written across rows. For example, the spillover of FL1 into FL2 is shown in row 1, column 2.



  In the example below, the spillover from FL1 (BL1-A) into FL2 (BL2-A) is 38.10%.

	BL1-A	BL2-A	BL3-A	RL1-A
BL1-A	100.00	38.10	5.86	0.01
BL2-A	0.51	100.00	24.18	0.00
BL3-A	0.22	25.40	100.00	13.92
RL1-A	0.06	0.04	0.01	100.00

- When the cursor is hovered over the table textboxes, tooltips are displayed. When the Spillover matrix is displayed, the tooltip reads:

  "%<Row Label> in <Column Label>". For example, %BL1 in BL2.

OK, Reset, and Cancel buttons

OK button

- **OK** accepts any changes made to the matrix and applies the updated compensation values to the dataset.
- If changes are made to the matrix and the **OK** button is not clicked, the changes will be lost.
- If the matrix values are adjusted during recording a Sample and a stop gate is set on a population, the stop gate condition recounts the events once **OK** has been clicked.

Reset

- **Reset** resets the compensation values to their default states as described below.
- If Experiment-level compensation is modified and Compensation controls are available, clicking **Reset** recalculates the compensation values based on the current Histogram gate positions on the Compensation Workspace.
- If any existing Experiment-level compensation settings are modified and Compensation controls are not available (for example, if the Experiment has been duplicated), clicking **Reset** reverts the Compensation matrix to the original Experiment-level calculated values.
- If FCS file compensation is modified, clicking **Reset** restores the compensation values to the values specified within the FCS data file.

Cancel

- **Cancel** undoes any changes made to the Spillover matrix and retains the existing values.

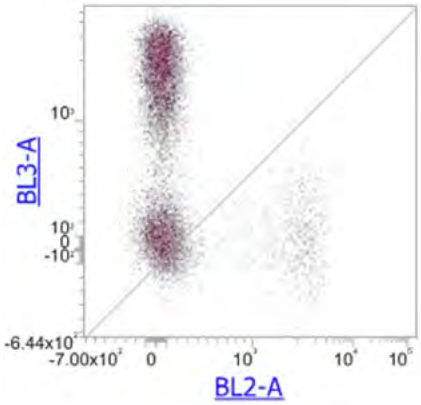
On-Plot Compensation Adjustment

Overview

- When **Plot Compensation** state button on the Compensation ribbon tab (page 53) is turned **ON**, you can drag event populations displayed on dual-parameter fluorescence plots to new positions, which changes the associated Compensation matrix values.

- When **Plot Compensation** is **ON**, hovering over a dual-parameter fluorescence plot displays a tooltip showing the compensation coefficients. The coefficients displayed are the spillover values.
- For the selected plot, a diagonal line from the lower left to the upper right distinguishes the two coefficient sections.

  Dragging a selected population above the line in a horizontal direction adjusts one coefficient, dragging below the line in a vertical direction adjusts the other coefficient.
- For Spillover values, the compensation coefficients can be dragged from 0% to 100%.



Manual on-plot compensation adjustment

- Click on the selected population and drag the population to the desired location on the plot.
- The sensitivity of the dragging action depends on where the plot is clicked.
  - Clicking and dragging the population closer to the diagonal line results in larger adjustments to the spillover values.
  - Clicking and dragging farther from the diagonal line makes finer adjustments to the spillover values.
  - The exact values of the changes depend on the scale of plot and the range in which a population is moved.
- Gate labels are not visible during the drag operation.
- Only the current plot is updated during dragging. When the dragging is complete, all plots and preview plots affected by the change are updated. The compensation adjustment is only registered into the undo stack once the dragging is complete.
- If compensation values are adjusted during recording a Sample and a stop gate is set on a population, the stop gate condition will recount the events once the mouse button has been released
- You can also adjust the compensation for the selected plot using the arrow keys. The **up** and **down arrow** keys adjust the Y-axis coefficient, and the **left** and **right arrow** keys adjust the X-axis coefficient. Each arrow key changes the compensation value by 1 unit. Holding down the arrow keys allows continual adjustment while the key is pressed.
- Holding **Shift+arrow** keys change the compensation value by 0.1 units.

Compensation acquisition workflow

Overview

Once the Tube or Well Compensation controls have been defined, they can be acquired. The expected workflow is to go through a round of Instrument Settings optimization to correctly set the voltages, thresholds, and gates, followed by a round of recording. At this point, the recorded Compensation controls are factored into the compensation calculation.

IMPORTANT! Once you have recorded all Compensation controls and calculated and applied the Compensation matrix, you cannot adjust the PMT voltages for experimental data.

Compensation acquisition from Tubes

- Compensation setup and acquisition from Tubes is performed manually.
- You can optimize the Instrument Settings for Compensation controls using the Unstained Control Workspace or the Workspace associated with the Compensation control sample.
- The optimization process for Instrument Settings is repeated for all Compensation controls.
- After the optimization of Instrument Settings is completed, load the first Compensation control, click on its corresponding Sample tube, then record using the Collection Panel controls (page 223). Repeat the process for each Compensation control.
- While recording, gates can be moved.
  - Adjust the Polygon parent gates so that the Parent gate includes the target population (page 389).
  - Adjust the Histogram gates to include the positive and negative populations (pages 390–391).
- The Compensation matrix is calculated as new Compensation controls are recorded. The calculation will only include the controls that have been recorded.

Compensation acquisition from Wells

- Compensation setup and acquisition from Wells is initiated using the **Set Up Comp** button in the Collection Panel (page 227).

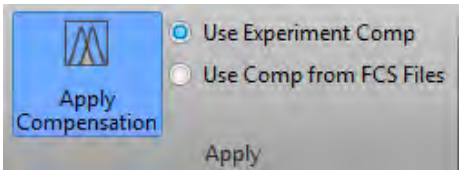
  Alternatively, you can acquire Compensation controls by selecting **Record All** on the Collection Panel menu.
- You can navigate between Compensation controls using the **Navigation buttons** in the Collection Panel (page 229).
- You can optimize each Compensation control using the Workspace associated with the Compensation control sample.
- When **Set Up Comp** is clicked, the Sample is acquired in the Set Up mode, allowing you to adjust the voltages and thresholds.
- During the **Set Up Comp** or **Record Comp** phases, you can adjust and set any gates on the gating plot and the corresponding histogram or dual parameter plot.
- The Compensation matrix is calculated as new Compensation controls are recorded. The calculation will only include the controls that have been recorded.

Compensation of instrument data

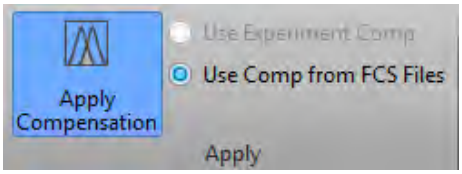
For Samples acquired from the instrument, the Compensation matrix is applied to both the Area and Height measurements (if available) regardless of whether the compensation was calculated using Area or Height measurements.

- After Compensation controls have been recorded, compensation is automatically applied to all Samples in the Experiment as indicated by the blue colored Apply Compensation button in the Compensation tab of the Ribbon bar.

- When an Experiment includes compensation and FCS files have been recorded, the **Use Experiment Comp** radio button becomes active.



- If FCS files have been imported into an Experiment, the compensation applied to the Sample is the compensation included in the FCS file itself. In this instance, the **Use Comp from FCS files** radio button becomes active.



22. New Experiment and Group dialogs

New Experiment dialog

The *New Experiment* dialog allows you to create New Experiments of the following types:

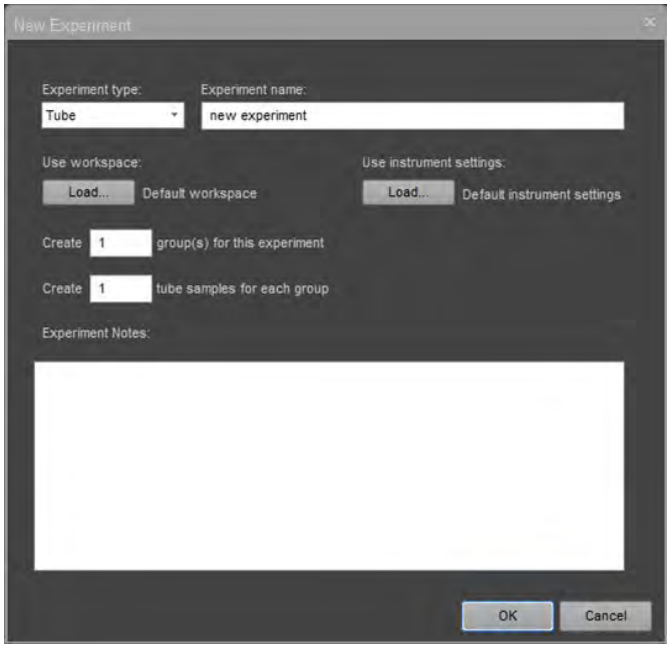
- Tube-only Experiment (page 400)
- Plate Experiment (page 402)

Launch the New Experiment dialog

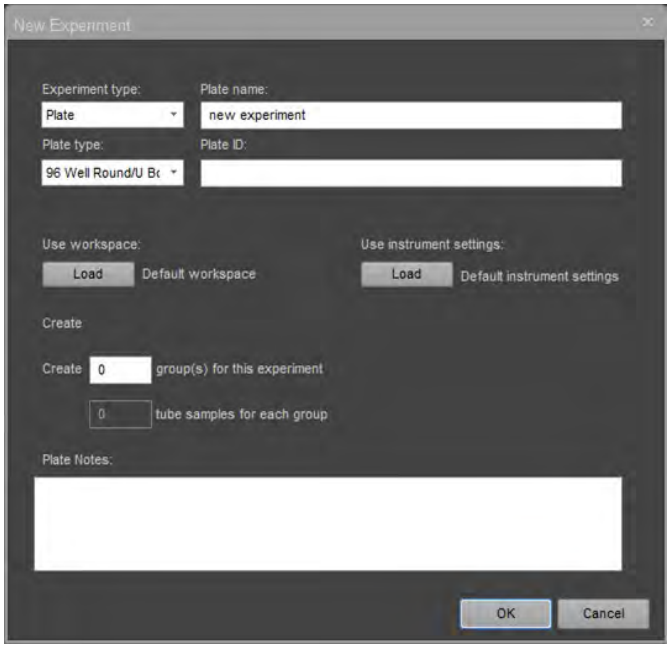
On the **Ribbon** bar, click **File** to open the File menu, then select **New Experiment**.

Alternatively, click the **New Experiment** button in the **Home** tab or use the keyboard shortcut **Ctrl+N**.

Note: The New Experiment option is enabled only when the instrument is not acquiring.



New Experiment dialog for Tube Experiments



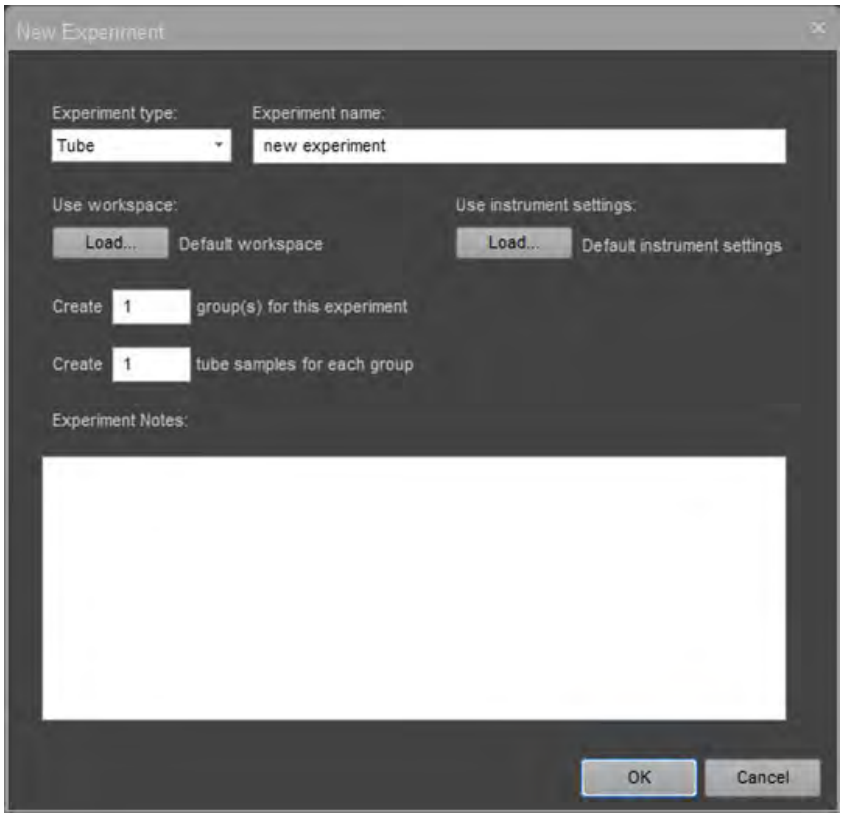
New Experiment dialog for Plate Experiments

Note: The contents of the New Experiment dialog changes, depending on the experiment type you select.

Create a Tube-only Experiment

1. On the **Ribbon** bar, click **File** to open the File menu, then select **New Experiment**.
2. From the Experiment type dropdown menu, select **Tube** (default selection).

   The contents of the New Experiment dialog changes to display the Tube Experiment options.

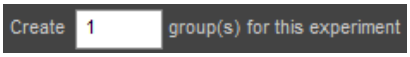
   

3. Accept the default Experiment name or enter a new name.

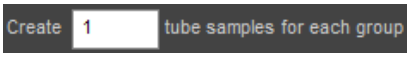
   - You can enter up to 50 characters; the following characters are not permitted: \ / : * ? ″ < > | %. The software automatically removes trailing or leading spaces.
   - Names cannot end with a period character or be any of the following system reserved names: CON, PRN, AUX, CLOCK$, NUL, COM1, COM2, COM3, COM4, COM5, COM6, COM7, COM8, COM9, LPT1, LPT2, LPT3, LPT4, LPT5, LPT6, LPT7, LPT8, LPT9.
   - Duplicate names are not permitted.
   - The default name for a Tube experiment is **Experiment**. If an Experiment using the default name already exists, a numerical suffix is added in parentheses; for example, **Experiment (2)**.

4. Accept the default **Workspace**.

5. Accept the default **Instrument Settings**.
6. Enter the number of Groups to create for the Experiment.



7. You can enter a number from **1** to **25**. The software adds the number of Groups to the Experiment Explorer.
8. The software creates a unique name for each Group with the default Group name set in the Options dialog ▶General tab and appends it with a numerical suffix; for example, **Group (2)**.
9. Enter the number of Tube samples to create for each Tube group.



10. You can enter a number from **1** to **400**. If the number of Groups times the number of Samples is >400, the number of Samples within a Group reverts to a number that is ≤400.
11. The software adds the number of Samples to Experiment Explorer.
12. The software creates a unique name for each Sample with the default Sample name set in the Options dialog ▶General tab and appends it with a numerical suffix; for example, **Sample (2)**.
13. *Optional:* Enter notes for the Experiment. You can enter up to 500 characters; any character is permitted.

14. Click **OK** to create the New Experiment and close the New Experiment dialog. The software opens the first Sample in the Experiment and the Experiment Workspace.

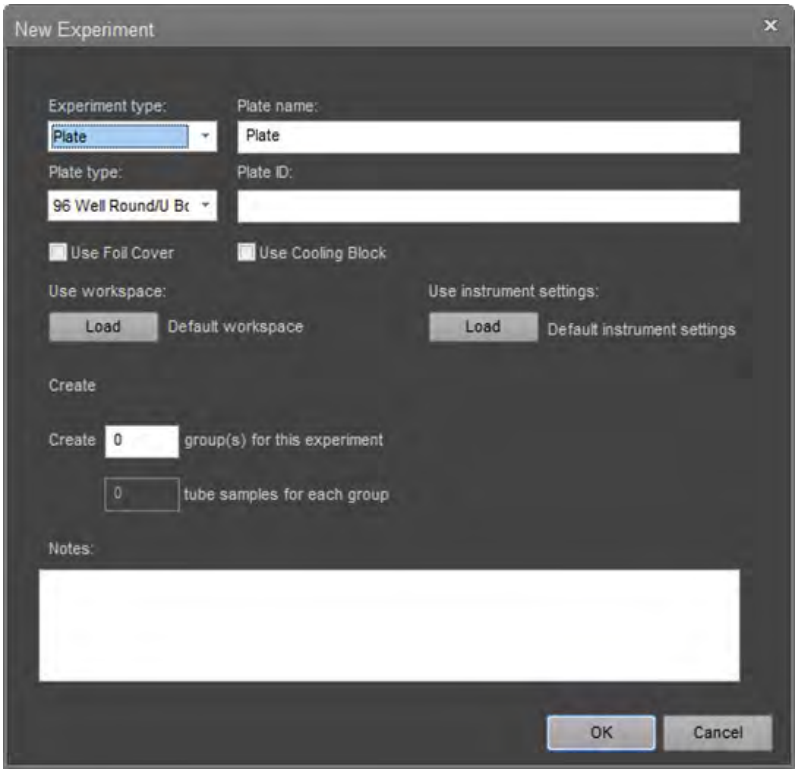
    To close the dialog without creating an Experiment, click **Cancel**.

Create a Plate Experiment

A *Plate Experiment* includes one Plate, and but it can also include Tube groups and Tube samples.

1. On the **Ribbon** bar, click **File** to open the File menu, then select **New Experiment**.
2. From the Experiment type dropdown menu, select **Plate**.

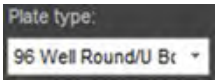
The contents of the New Experiment dialog changes to display the Plate Experiment options.



3. Accept the default plate name or enter a new name.

- You can enter up to 50 characters; the following characters are not permitted: \ / : * ? ″ < > | %. The software automatically removes trailing or leading spaces.
- Names cannot end with a period character or be any of the following system reserved names: CON, PRN, AUX, CLOCK$, NUL, COM1, COM2, COM3, COM4, COM5, COM6, COM7, COM8, COM9, LPT1, LPT2, LPT3, LPT4, LPT5, LPT6, LPT7, LPT8, LPT9.
- Duplicate names are not permitted.
- The default name for a Plate Experiment is **Plate**. If an Experiment using the default name already exists, a numerical suffix is added in parentheses to ensure a unique name; for example, **Plate (2)**.

4. From the **Plate type dropdown** menu, select the type of plate that you are using for the Experiment.



5. Enter the plate ID.

You can enter up to 50 characters; all characters are permitted. The software automatically removes trailing or leading spaces.

6. *Optional*: If you are using a foil cover to protect the sample plate from condensation or evaporation, select **Use Foil Cover**.

- When the **Use Foil Cover** option is enabled, the autosampler disables the probe collision sensor, which allows the use of a foil cover on the plate.

  When this option is enabled, the software displays the "Foil cover present" warning dialog at the beginning of the plate acquisition to ensure that the correct plate is used.
- Use Foil Cover selection persists as part of a Template, and when exporting or duplicating a Plate.

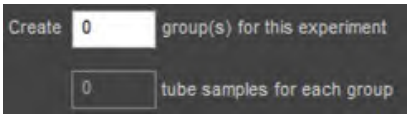
7. *Optional*: If you are using a cooling block, select **Use Cooling Block**.

- When **Use Cooling Block** option is selected, the autosampler accounts for the extra height that the cooling block adds to the plate specification.
- When available, this option is enabled only for standard 96-well round bottom plates (such as Thermo Scientific™ 96-well Microtiter™ Microplates, Cat. No. 2205). A standard plate is defined as a plate that is within the height range of 14.35 mm ± 0.76 mm.
- Use Cooling Block selection persists as part of a Template, and when exporting or duplicating a Plate.

Note: Use Foil Cover and Use Cooling Block options are available only when a CytKick™ Max Autosampler is connected to the Attune™ instrument. Otherwise, they are not visible.

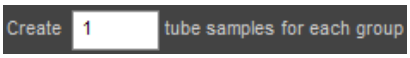
8. Accept the default **Workspace** or click **Load** to select a saved Workspace file.
9. Accept the default **Instrument Settings** or click **Load** to select a saved Instrument Settings file.

10. If you are including tubes, enter the number of **Tube Groups** to create for the Experiment.



- You can enter a number from **0** to **5**, depending on the plate type you have selected in step 4. The default is **0**.
- The software adds the number of Groups to Experiment Explorer.
- If zero Groups are entered, then the Tube Samples field is disabled.
- The software creates a unique name for each Group with the default Group name set in the Options dialog▶General tab and appends it with a numerical suffix; for example, **Group (2)**.

11. Enter the number of **Tube Samples** to create for each Tube Group.



- For a 96-well plate, you can enter a number from **1** to **304**. If the number of Groups times the number of Samples is >304, the number of Samples within a group will revert to a number that is ≤304.
- For a 384-well plate, you can enter a number from **1** to **16**. If the number of Groups time the number of Samples is >16, the number of Samples within a Group will revert to a number that is ≤16.
- The software adds the number of Samples to Experiment Explorer (page 190).
- The software creates a unique name for each Sample with the default Sample name set in the Options dialog▶General tab and appends it with a numerical suffix; for example, **Sample (2)**.

12. *Optional:* Enter notes for the Experiment. You can enter up to 500 characters; any character is permitted.

13. Click **OK** to create the New Experiment and close the New Experiment dialog. The software opens the Heat Map view (page 138).

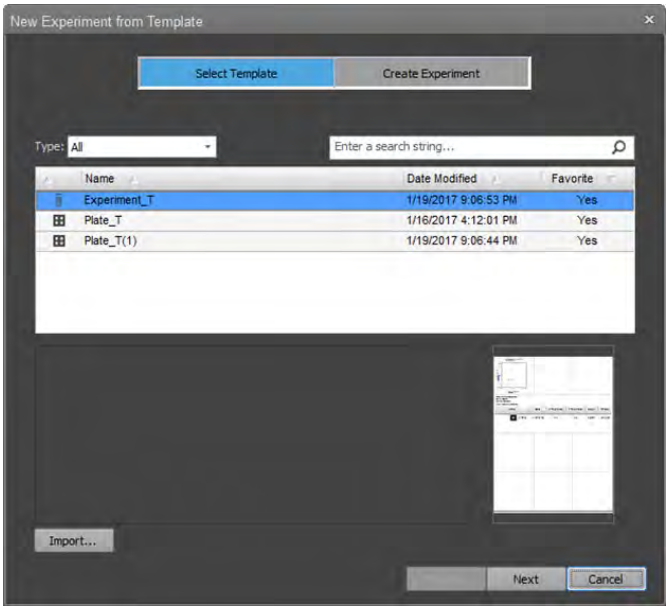
    To close the dialog without creating an Experiment, click **Cancel**.

New Experiment from Template dialog

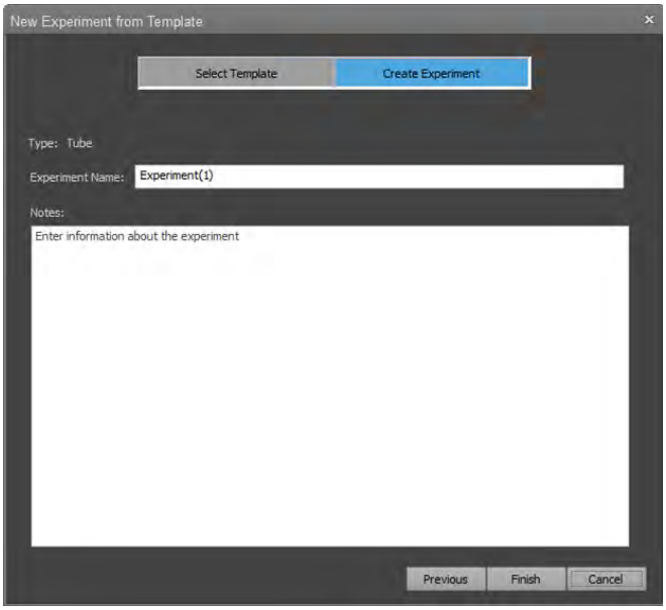
The *New Experiment from Template* dialog is a modal dialog that allows you to create a new Experiment from an existing template with pre-defined settings by guiding you to select an Experiment template and enter experiment information (such Experiment name, description, notes).

The template applies the template's Workspace, Instrument settings, Run Protocols, Heat Map settings, and defined compensation to the new experiment and creates a new experiment with the same number of mapped groups and samples.

Note: For Experiments created from a template, no FCS files are attached to any of the Samples.



New Experiment from Template – Select Template

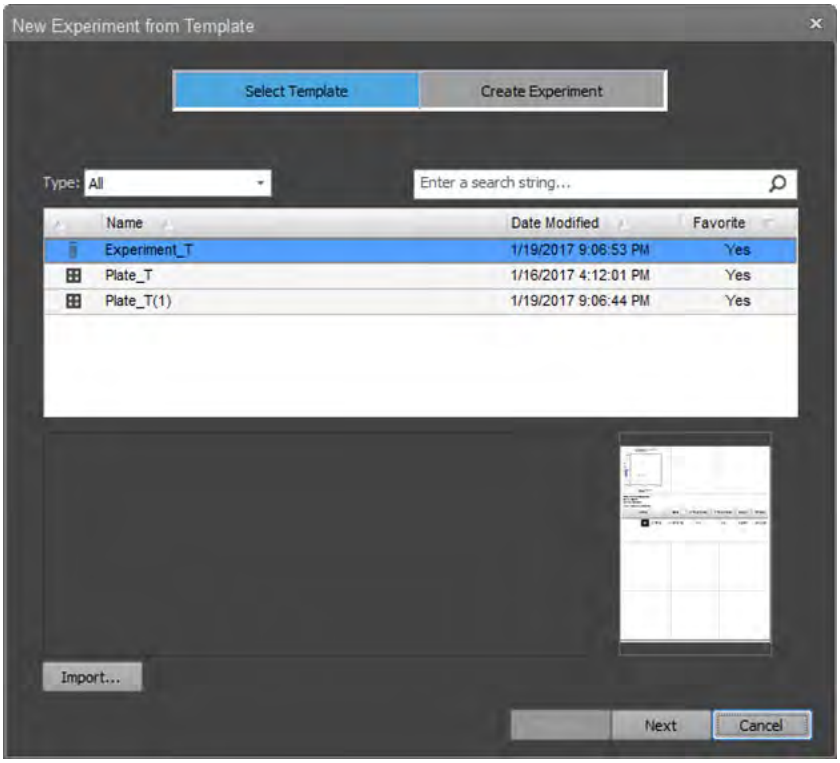


New Experiment from Template – Create Experiment

Launch the New Experiment from Template dialog

On the Ribbon bar, click **File** to open the File menu, then select **New Experiment from Template**.

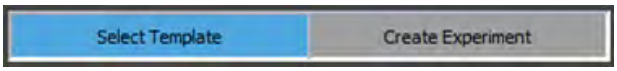
Alternatively, click the **New Experiment from Template** icon in the *Home* tab.



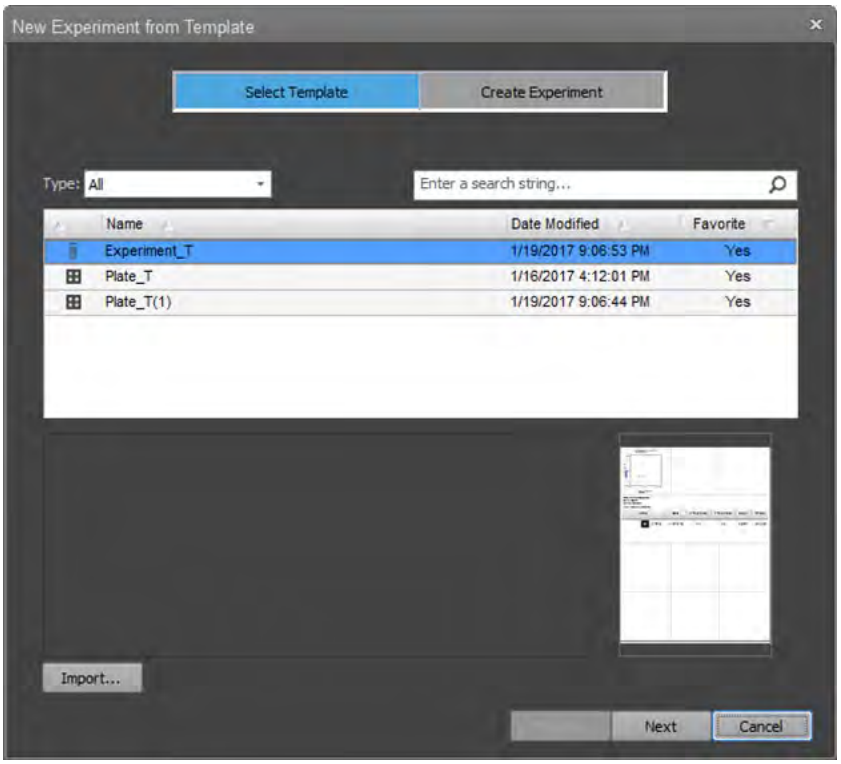
Select Template

The first step in creating a New Experiment from Template is template selection. In a template, the following Experiment settings are already defined: Workspace, Instrument settings, Run Protocols, Heat Map settings, Compensation, Overlays, Hierarchy, and the number of mapped Groups and Samples.

1. If it is not already selected, click **Select Template**.



Select Template tab of the New Experiment from Template dialog opens. The Select Template tab contains a list of available templates and allows you to filter the list by template type or by user-defined keywords found in the template name or description.



2. To filter the template list by template type, select the desired type from the **Type** dropdown. Available options are **All**, **Tube**, or **Plate**.

The template list displays only the type of template selected in the dropdown.

3. To search the template list by keywords found in the template name or description, type in the keyword in the search text box and press **Enter**.

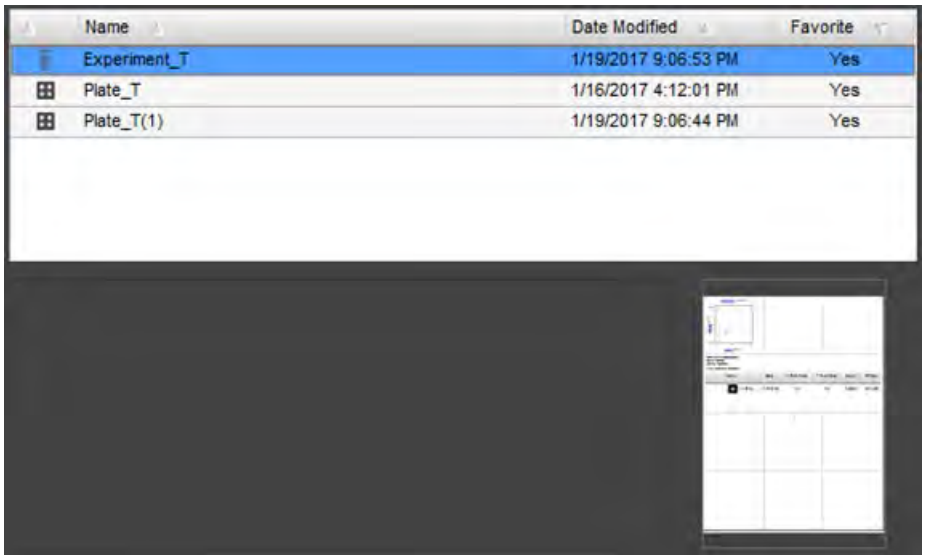
The template displays only the templates that contain the user-defined keyword in their name or description.

4. To sort the template listed by Type, Name, or Date Modified, click on the corresponding column heading.

5. To import a template that is not displayed in the template list, click **Import** to open the *File Open (Import) dialog* with the filter set to Attune Experiment Template (*.aet).

   Select the ***.aet** file that corresponds to the template you wish to add to the template list, then click **Open**. The imported template is displayed and selected in the template list.

6. To select a template from the template list, click the desired template in the list. The selected template will be highlighted in the list, the description field displays the template description (if available), and the thumbnail shows the thumbnail image that corresponds to your selected template.



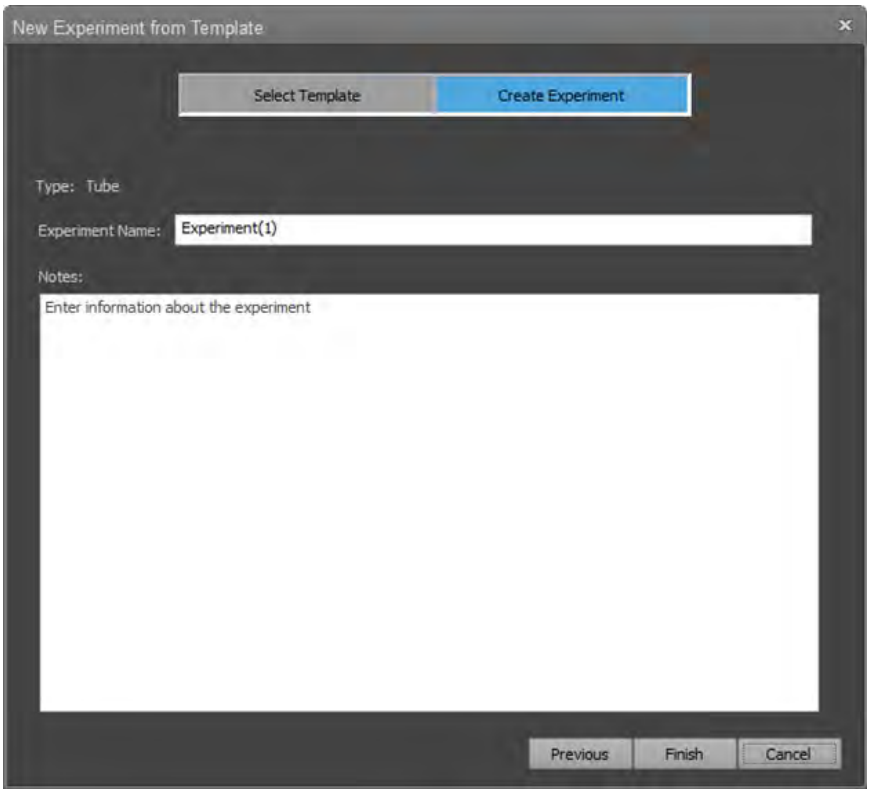
7. To select the template for your new experiment, select the desired template in the list, then click **Next**.

   Alternatively, click the **Create Experiment** tab after selecting the template from the list.

   Create Experiment tab of the New Experiment from Template dialog opens.

Create Experiment

The second step in creating a New Experiment from Template is the addition of Experiment name and Experiment notes in the Create Experiment tab.



- The Create Experiment tab displays the Experiment type selected in the Select Template tab (in this example, Tube experiment).
- By default, the Experiment Name is populated with Experiment or Plate (for Tube and Plate experiments, respectively).

1. To change the Experiment Name, click on the Experiment Name text box and type in the desired name.
   - You can enter up to 50 characters; the following characters are not permitted: \ / : * ? ” < > | %.
   - The software automatically removes trailing or leading spaces.
   - Names cannot end with a period character or be any of the following system reserved names: CON, PRN, AUX, CLOCK$, NUL, COM1, COM2, COM3, COM4, COM5, COM6, COM7, COM8, COM9, LPT1, LPT2, LPT3, LPT4, LPT5, LPT6, LPT7, LPT8, LPT9.
   - Duplicate names are not permitted. If the name is already used, the name is appended with "(#)", where # is the next available unique number. This number increments as necessary to assure that the name is unique. For example, Experiment(2), Experiment(3), Experiment(4), etc.

2. *Optional*: Enter notes for the Experiment. You can enter up to 500 characters; any character is permitted.

3. Click **Finish** to create the New Experiment and close the dialog.
   - For Tube-only Experiments, the software opens the first Sample in the Experiment and displays the Experiment Workspace (page 84).
   - For Plate Experiments, the software displays the Heat Map view (page 138).

   To go to the Select Template tab to select another template, click **Previous**.

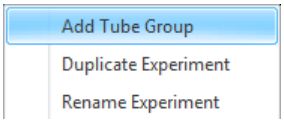
   To close the dialog without creating an Experiment, click **Cancel**.

New Group dialog

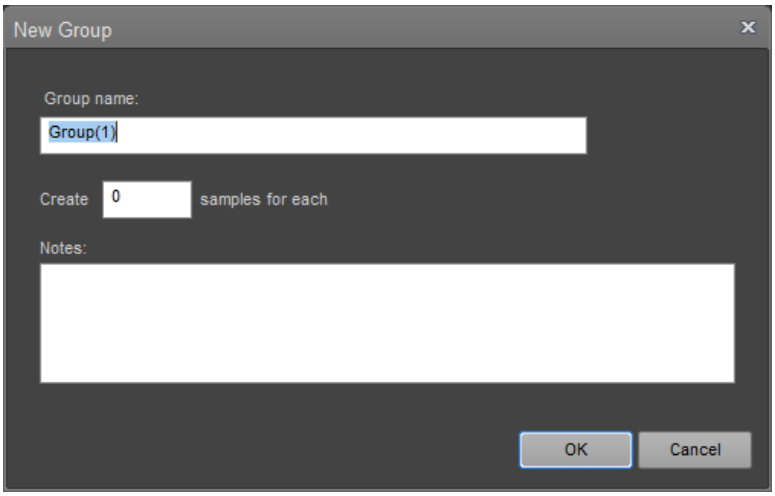
The *New Group* dialog allows you to create a new Tube group and specify the number of Samples to create for that Group.

Launch the New Group dialog

On the *Experiment Explorer*, right-click on an Experiment and select **Add Tube Group**.



New Group dialog opens.



Create a Group

1. Accept the default Group name or enter a new name.

- You can enter up to 50 characters; the following characters are not permitted: **\ / : * ? " < > | %**.
- The software automatically removes trailing or leading spaces.
- Names cannot end with a period character or be any of the following system reserved names:

  CON, PRN, AUX, CLOCK$, NUL, COM1, COM2, COM3, COM4, COM5, COM6, COM7, COM8, COM9, LPT1, LPT2, LPT3, LPT4, LPT5, LPT6, LPT7, LPT8, LPT9.
- Duplicate names are not permitted.
- The default name for a Group is **Group**. If a Group using the default name already exists, a numerical suffix is added in parentheses to ensure a unique name; for example, **Group (2)**.

2. Enter the number of Tube samples to create for the Group.

   - For a Tube-only Experiment, you can enter a number from **1** to **400**. If the number of Groups times the number of Samples is >400, the number of Samples within a Group will revert to a number that is ≤400.
   - For a 96-well plate, you can enter a number from **1** to **304**. If the number of Groups times the number of Samples is >304, the number of Samples within a Group will revert to a number that is ≤304.
   - For a 384-well plate, you can enter a number from **1** to **16**. If the number of Groups times the number of Samples is >16, the number of Samples within a Group will revert to a number that is ≤16.
   - The software adds the number of Samples to Experiment Explorer.

3. The software creates a unique name for each Sample with the default Sample name set in the Options dialog ▶ General tab and appends it with a numerical suffix; for example, **Sample (2)**.
4. *Optional:* Enter notes for the Experiment. You can enter up to 500 characters; any character is permitted.

5. Click **OK** to create the new Group and close the *New Group* dialog.

   To close the dialog without creating a Group, click **Cancel**.

23. Dialogs

Overview

This chapter describes the non-instrument dialogs that are displayed throughout the Attune™ Cytometric Software.

All dialogs are *modal*, unless otherwise specified.

Note: Modal dialogs appear when you are performing tasks in the main software windows. You must interact with the modal dialog before you can return to the main window.

Non-instrument dialogs

- Page Setup dialog (page 414)
- Print dialog (page 415)
- Print Preview dialog (page 417)
- Batch Print dialog – Experiment Explorer context menu (page 418)
- Export Statistics dialog – Experiment Explorer context menu (page 420)
- Derived Gate dialog (page 421)
- Edit Gates dialog (page 424)
- Confirm File Save dialog (page 427)
- Save as Template dialog (page 428)
- Manage Templates dialog (page 430)
- Plate Mapping dialog (page 434)
- Select Template dialog (page 437)
- Map Sample List Data dialog (page 440)
- Update Sample Information dialog (page 442)
- Create/Edit Keyword dialog (page 443)
- Experiment Keywords dialog (page 445)
- Export Logs for Service dialog (page 447)
- Test Plate dialog (page 451)
- Device Registration Options dialog (page 451)
- Connect Sign in dialog (page 457)
- Switch Account dialog (page 460)
- Connect Storage dialog (page 460)
- Maintenance Required dialog (page 461)
- System Log dialog (page 462)
- SAE signing dialogs (page 466)

Page Setup dialog

The *Page Setup* dialog allows you to customize the paper size and orientation.

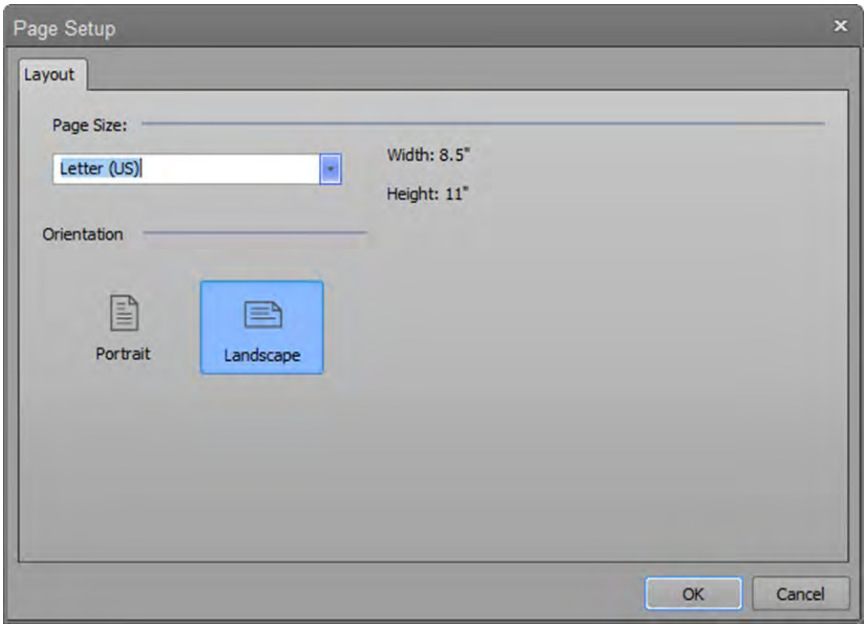
Launch the dialog

On the **Home ribbon tab** (page 50), click **Page Setup**.

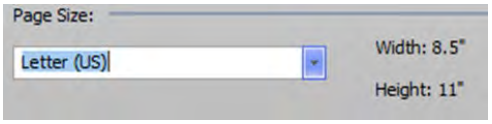
The Page Setup button is only available when the Workspace, Results, Heat Map, or Overlay views are active.

Layout tab

The Layout tab allows you to customize the page size and orientation.

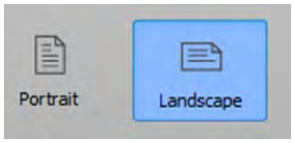


1. To select a page size for the printed document, select a size from the **Page Size** dropdown menu.



   The software displays the width and height dimensions for the selected size.

2. To select the orientation of the printed document, click the **Portrait** or **Landscape** icon.



3. To save the changes and close the dialog, click **OK**.

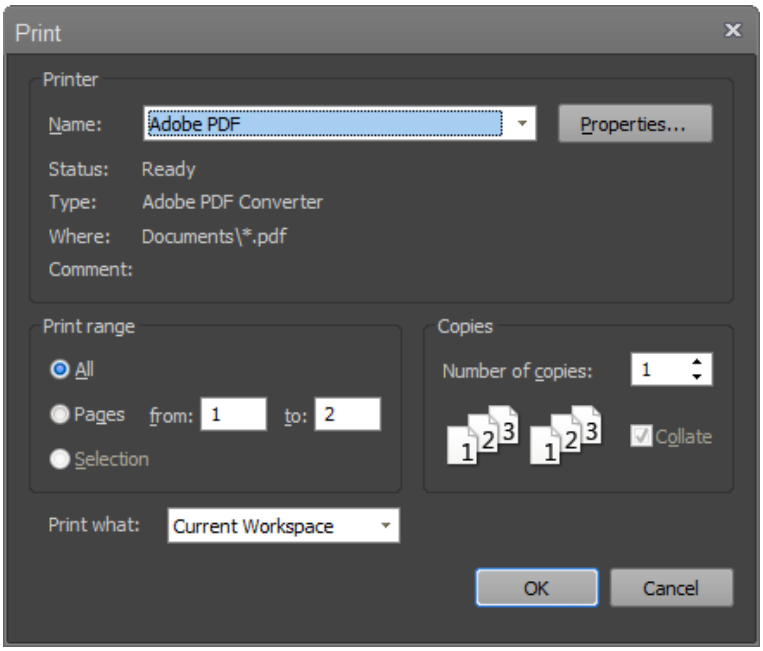
   To close the dialog without saving the changes, click **Cancel** or **X**.

Print dialog

The *Print* dialog is a modified Windows® Print dialog. All the controls on this dialog, except the *Print what combo box* and the print range, are standard Windows® functions and are not described here.

Launch the dialog

On the **File Ribbon tab** (page 49), select **File ▶ Print** or press **Ctrl+P** on the keyboard.



Print range

This is the standard print range control. However, the Print range options are disabled when the Print dialog is opened from the **Experiment Explorer context menu ▶ Batch Print dialog** (page 418). In this case, **All** is selected and the entire print queue is processed.

Print what options

- The **Print what options** are not displayed when the Print dialog is opened from the **Experiment Explorer context menu ▶ Batch Print dialog** (page 418).
- If the Print dialog is opened from within any of the standard application views (Workspace, Heat Map, Results, or Overlays), the **Print what dropdown** list displays the following options:
  - **Current Workspace:** Prints the currently active Workspace in the order specified in the Page setup dialog (page 414).
  - **Heat Map:** Prints the Heat Map and its legend scaled to one page width.
  - **Results:** Prints Results tables (both primary and secondary, if available) scaled to one page width.
  - **Overlays:** Prints Overlay plots and their corresponding legends (if enabled) with one plot per row. The Overlay galleries cannot be printed.
  - **Sample List:** Prints the Sample List as described in "Print the Sample List" (page 150).

- If the Print dialog is opened from the Performance Test module (page 363), the **Print what dropdown** list displays the following options:
  - **Selected PT Report:** Prints the currently active and visible Performance Test report (page 370). If no PT report is visible, the most recent PT report is printed.
  - **Levey-Jennings:** Prints the currently selected Levey-Jennings Report for all installed detectors. Only the selected date range and statistics are printed. If no Levey-Jennings is selected, the most recent Levey-Jennings plot is printed using the specified date criteria.
  - **Performance History:** Prints the performance history for the currently selected baseline is printed. If no baseline is selected, the most recent performance history is printed.
- Selecting one of the options changes the view to the selected view and sends the selected option to the print queue when the **OK** button is clicked.
- If any selection is not present or does not contain data, nothing will print.
- The default selection for the Print what dropdown is determined by the view currently in focus.

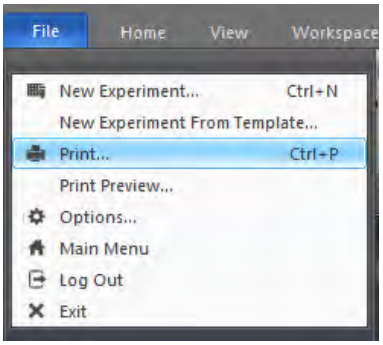
Print Preview dialog

The *Print Preview* dialog displays the document as it will appear when printed and allows you to set various printing options.

Launch the dialog

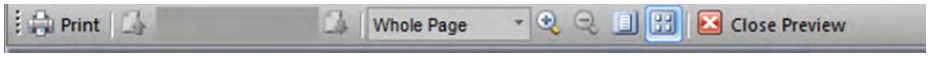
On the **File Ribbon tab** (page 49), select **File ▶ Print Preview**. The Print Preview option is enabled only when the instrument is not acquiring.

The Print Preview opens as a full screen modal dialog that displays the document as it will appear when printed.



Print Preview dialog

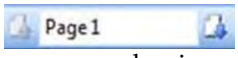
The controls to close the print preview, print the document, select portrait vs. landscape, open the page setup dialog, view the document full page width, view a whole page or multiple pages, and navigate are displayed in a command bar at the top of the dialog.



- To print the document, click **Print**, which opens a *Print* dialog that contains standard Windows® print functions.

- To navigate through the preview pages, click the **up** and **down arrows**.

  The current page number is displayed in the **Page <*n*>** box.



  Alternatively, navigate directly to a specific page by entering the page number in the **Page <*n*>** text box and press the **Enter** key.

- To resize the page view, you can select any of the following options:
  - Select a size from the **Page View** dropdown menu.

    By default, whole page is selected.

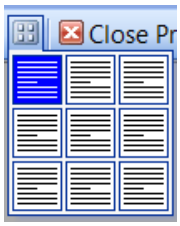
  - **Zoom In** or **Zoom Out** buttons allow the page view to zoom in and out.

    When the page zoom is at 500%, the Zoom In button is disabled.

    When the page zoom is at 10%, the Zoom Out button is disabled.

  - Click the **Page Size quick selection** button to set the view to the whole page.

  - To view up to 9 pages at one time, click the **View Multiple Page** icon to open a popup, then mouse-over the pages to view.

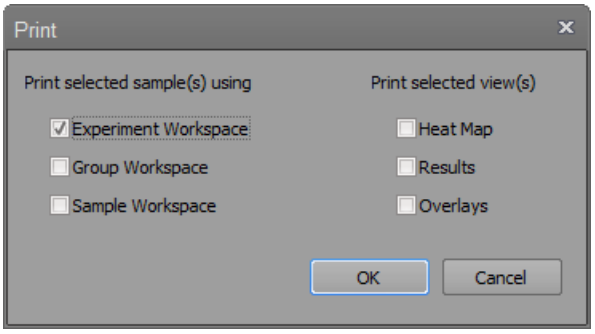


  - To close the dialog, click **Close Preview**.

Batch Print dialog

Launch the dialog

The *Print* dialog is displayed when the **Print** option is selected from the Experiment, Group, or Sample context menu in the Experiment Explorer or from the Heat Map context menu.



Print dialog options

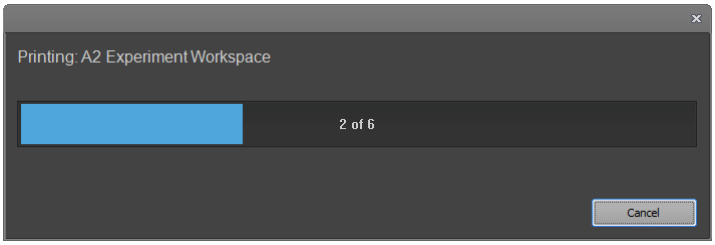
Print selected sample(s) using

- The options in this group allow you to select the Workspaces to use when printing the selected samples. Available options are:
  - Experiment Workspace
  - Group Workspace
  - Sample Workspace
- You can select multiple Workspaces. By default, the Experiment Workspace is selected.

Print selected view(s)

- The options in this group allow you to select the view to print. Available options are:
  - Heat Map
  - Overlay
  - Results

  By default, none of these options are selected.
- To begin printing, click **OK**, which opens the Print dialog (page 415).
- Before printing, the Heat Map, Overlays, and Results view are automatically refreshed to ensure that all statistics are up to date.
- During the batch print process, the progress of the print job is indicated on a dialog. The number of items in the print queue is the sum of Workspaces printed for each Sample and views selected to print.



- To cancel the print job, click **Cancel** on the progress dialog.

Print order

- The print order is:
  - Experiment Workspace *(<n>)*
  - Group Workspace *(<n>)*
  - Sample Workspace *(<n>)*
  - Results
  - Heat Map
  - Overlays

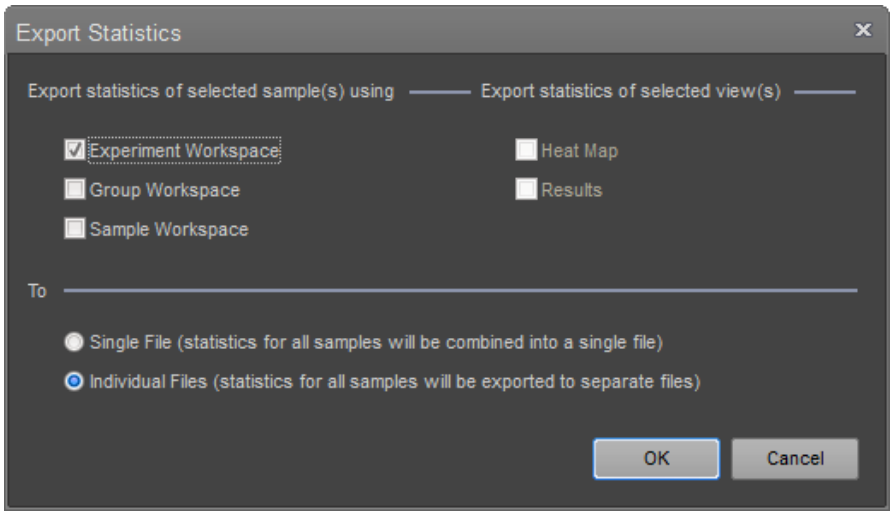
  where ***<n>*** is the sample index
- A page break is added between each new Workspace and each new Sample, and between the Heat Map, Results, and Overlays.
- Items are printed using the current page layout settings.
- The Heat Maps legend is scaled to the available print width.
- Result tables are scaled to the page width.
- The Results view is based on the currently selected filters and statistics.
- Overlays are printed in the order that they appear on the Overlay view (page 156). Gallery plots are not printed.

Export Statistics dialog

The *Export Statistics* dialog is displayed when **Export Statistics** is selected from the Plate, Sample, Group, or Experiment context menu of the Experiment Explorer (page 194).

Launch the dialog

- In the *Experiment Explorer* (page 189), right click on the desired Plate, Sample, Group, or Experiment icon to open the associated context menu, then select **Export Statistics**.



Export Statistics dialog

Export statistics of selected samples using

The options in this group allow you to select the Workspaces to use when exporting the selected Samples. You can select multiple Workspaces. Available options are:

- Experiment Workspace
- Group Workspace
- Sample Workspace

By default, the Experiment Workspace is selected. For Workspace statistics, the individual plot statistics are not exported.

To

The options in this group allow you to select the output. Available options are:

- **Single File:** Exports the statistics as a single CSV file. Results are collated into a single table for the Workspace statistics and the Results tab's primary table.
- **Individual Files:** Exports the statistics as multiple CSV files. The software creates a CSV file for each workspace and each selected sample. If the Heat Map and Results views are selected, the software creates additional files.
- To begin printing, click **OK**. When prompted, complete the:
  - *Save* dialog (page 532) for the **Single File** option.
  - *Open* dialog (page 535) for the **Individual Files** option.
- To close the dialog without printing, click **Cancel** or **X**

Derived Gate dialog

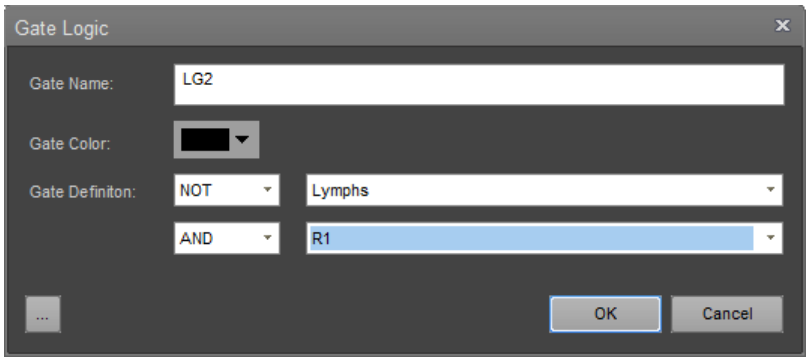
The *Derived Gate dialog* allows you to create gates based on Boolean operators (i.e., *Derived gates*).

Launch the dialog

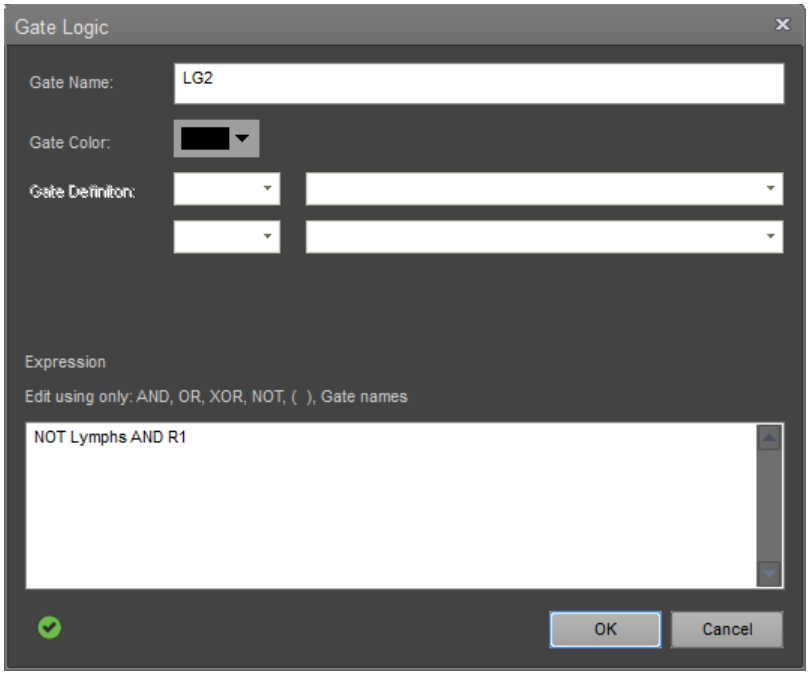
In the Workspace tab (page 57), click **Derived Gate** to open the Derived Gate dialog.

The Derived Gate button is enabled when the Workspace view is displayed and the current Workspace has at least one gate present.

Collapsed view



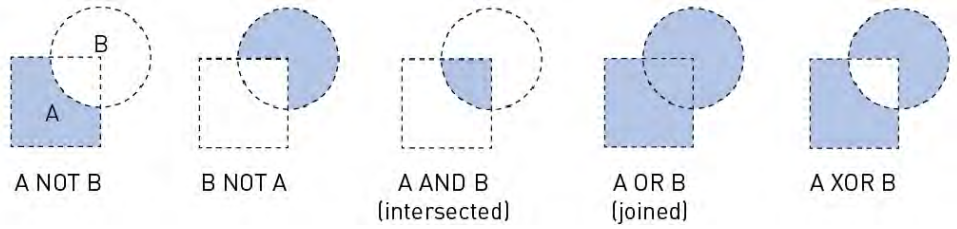
Expanded view



Note: The contents of the Derived Gate dialog changes depending on the context of the active Workspace.

Boolean operators

The Boolean operators available for creating Derived gates are blank, AND, OR, XOR, AND NOT, and OR NOT.



Derived Gate dialog

Gate name

Allows you to accept the default gate name, or enter a new name.

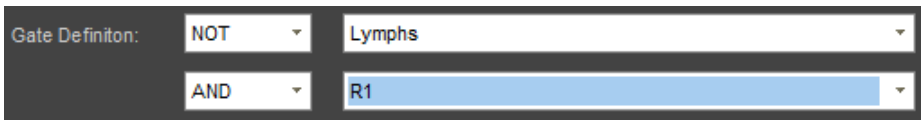
- You can enter up to 50 characters.
- Duplicate names and names that are only a whole number are not permitted.
- The default name for a gate is **LG1**. If a gate using the default name already exists, the next available integer is automatically used; for example, **LG2**.

Gate color

Allows you to select a gate color from the dropdown menu.

Gate definition

Allows you to create gate math expressions using Boolean operators.



- Select a gate from each of the two dropdown menus. The menus contain alphabetized lists of all gates on the active Workspace.
- From the first Boolean dropdown menu, select ***<blank>*** or **NOT**.
- From the second Boolean dropdown menu, select another Boolean operator ***<blank>***, **AND**, **OR**, **XOR**, **AND NOT**, or **NOT**.

Expression editor

- Click the **expander button** to expand the *Derived Gate dialog* and display the *Expression Editor*. If you made selections in the Gate Definition dropdown menus (above), they appear in the text box.

- Edit the text using only: **AND**, **OR**, **XOR**, **NOT**, ***<blank>***, and gate names. You can enter at least 1500 characters.
- All gate names must be entered in quotation marks (i.e., enter R1 as “R1”).
- As you enter text, the software validates the expression and indicates the status:

  = valid

  = invalid
- To save the changes and close the dialog, click **OK**.
- To close the dialog without creating gates, click **Cancel** or **X**.

Edit Gates dialog

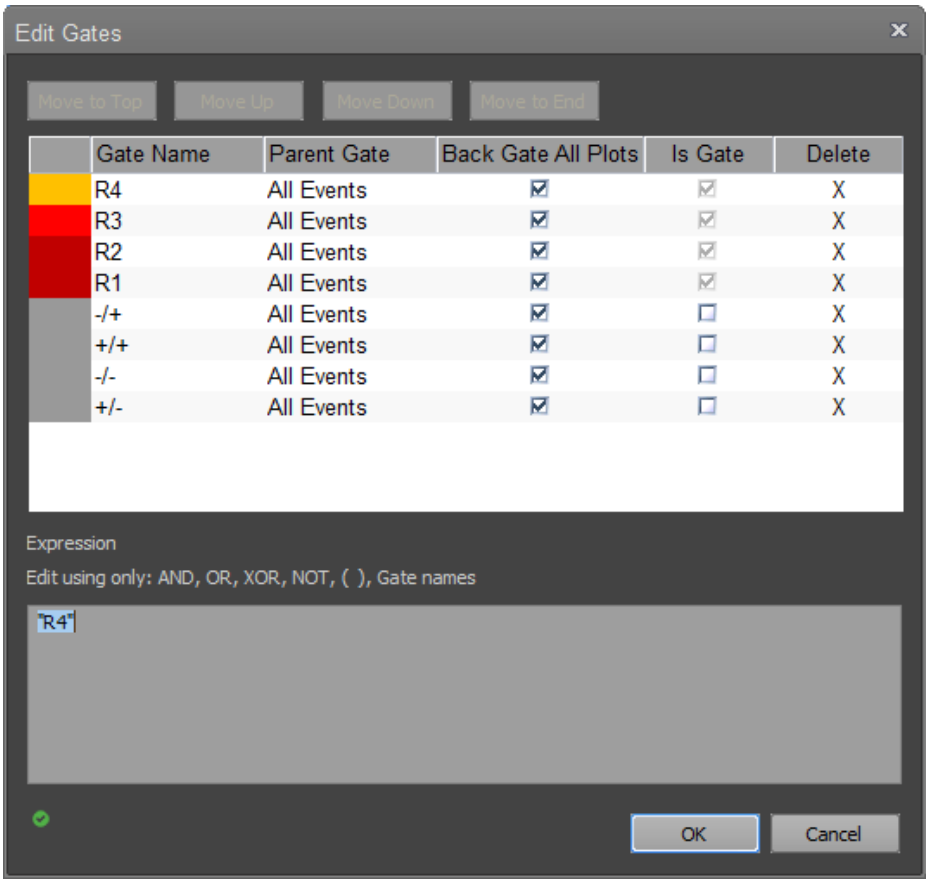
The *Edit Gates* dialog provides a list of all available gates in the active Workspace, and allows you to:

- Edit the gate color, gate math expression, and the order in which the gates are displayed in the dialog
- Delete gates

Launch the dialog

In the Workspace tab (page 53), click **Edit Gates**.

The Edit Gates button is available when the Workspace view is displayed and the current Workspace has at least one gate present.



Note: The contents of the *Edit Gates* dialog changes, depending on the context of the active workspace.

Edit Gates dialog

General properties

- By default, gates are added to the top of the list.
- Changes are applied to all gates after **OK** is clicked.
- To remove the gate name from the list and delete the gate from the plot, click **Delete**.
- You cannot sort the table or rearrange the columns.
- You can resize columns by selecting and dragging the column divider or by double clicking on the divider to auto-fit the column width to the contents.
- Vertical and horizontal scrollbars are displayed as necessary.

Reorder gates

- To reorder the gate list, select a gate, then drag and drop it to a different location within the list. Changing the gate list order does not change the gating hierarchy.
- Alternatively, select a gate, then click **Move to Top**, **Move Up**, **Move Down**, or **Move to End**, as necessary.

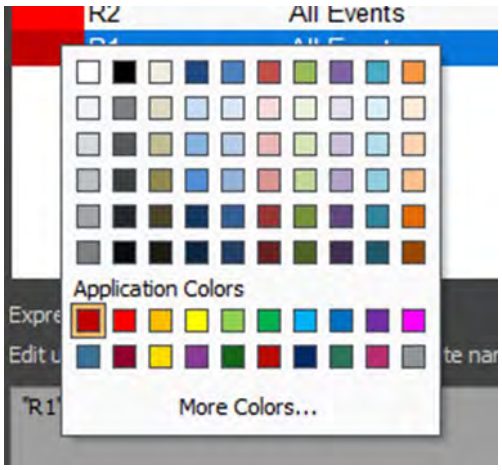
- To select multiple gates at one time, press **Ctrl** or **Shift** when selecting the gates. The order of the selected gates is maintained when they are moved.

Resize columns

- To resize a column, drag the column divider, or double-click the divider to auto-fit the column width to the contents.

Change gate color

- To change the gate color, select the **Color** cell to open the color picker.



Edit gate names

- To edit a gate name, select a name from the dropdown menu in the **Gate Name** cell, or double-click the gate name and enter new text.
- You can enter up to 50 characters.
- Duplicate names and names that are only a whole number are not permitted.
- The default name for a gate is LG1. If a gate using the default name already exists, the next available integer is automatically used; for example, LG2.

Backgate all plots

- The **Backgate all plots** option is checked by default for all newly created oval, rectangle, and polygon gates, if the **Backgate all plot**s option is checked in the Gate options dialog (page 486).
- When selected, backgating is applied in all dot and precedence density plots for the selected gate. This paints the events contained within the gate on these plot types based on the color of the gate.
- When de-selected, backgating is turned off for the selected gate on all plots.

Is Gate

- The "Is Gate" column as a checkbox control indicates whether a region has an associated gate.
- By default, histogram, oval, rectangle, and polygon regions are gates. Derived gates are always gates.
- When a region is associated with a gate, it cannot be removed and the checkbox is disabled.
- The software limits the number of total gates to 128. Because each quadrant contains four members, quadrants are created initially without an associated gate. For quadrant members that do not have an associated gate, the "Is Gate" checkbox is unchecked. When the "Is Gate" is checked for a quadrant member, a gate is created for that quadrant member and the checkbox is disabled.

Edit gate equation

- To edit a gate equation, select a derived gate, then enter the equation in the *Expression Editor*.

- Only derived gate equations can be edited; otherwise this feature is disabled. If you select a non-derived gate, the Expression Editor is disabled.
- Edit the text using only: **AND**, **OR**, **XOR**, **NOT**, ***<blank>***, and gate names. Gates names must be bounded by quotation marks ("gate name"). You can enter at least 1500 characters.
- As you enter text, the software validates the expression and indicates the status:

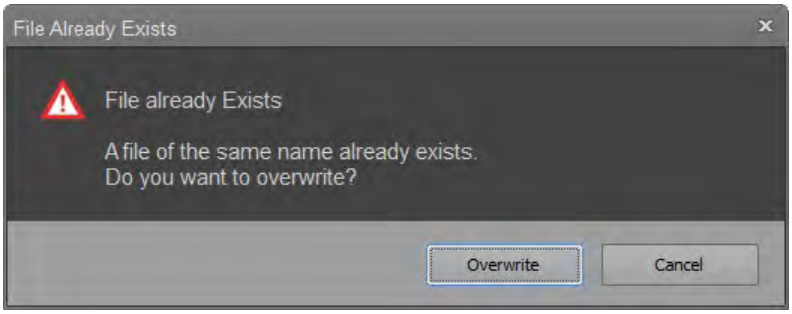
  = valid

  = invalid

- To save the changes and close the dialog, click **OK**.

Confirm File Save dialog

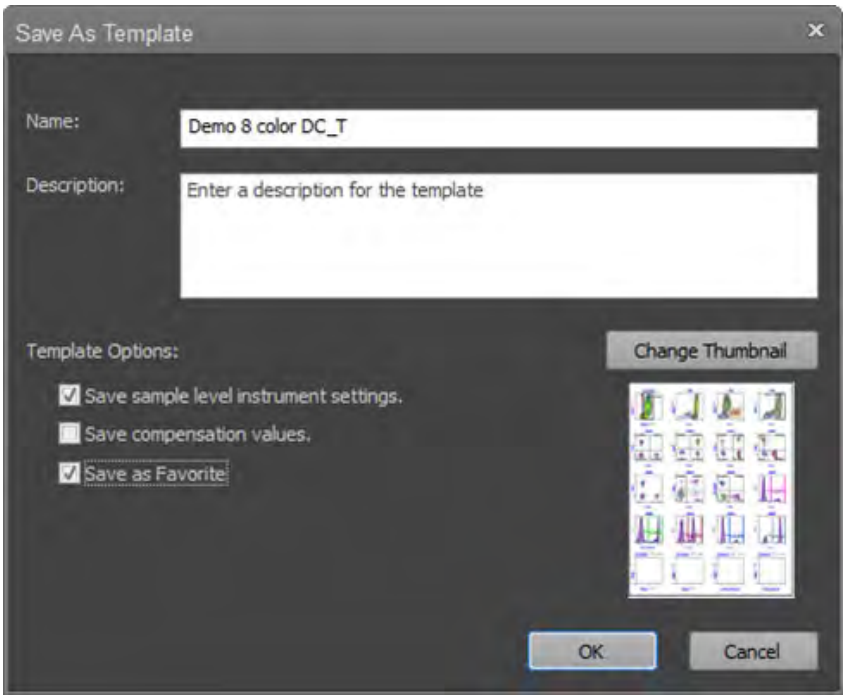
The *Confirm File Save* dialog helps to prevent exported files from being accidentally overwritten. The dialog is automatically displayed when you attempt to export a file to a selected export location that already contains a file of the same name.



- To replace an existing file with the current file, click **Overwrite**.
- If you are exporting a batch of files, and multiple duplicate file names exist in the export location, the **Do this for all other cases (x found)** check box is enabled, where **x** is the number of existing files.
- To overwrite all duplicate files in the export location, check the **Do this for all other cases (x found)** check box.
- To decide on a case-by-case basis for each file, leave the check box unchecked.
- The checked state applies only to the current export batch and is not persisted.
- To close the dialog without exporting any files, click **Cancel** or **X**.

Save as Template dialog

The *Save as Template* dialog allows you to save the current Experiment as a template so that the Experiment Workspace, Experiment Instrument settings, Run Protocols, Heat Map settings, and Experiment hierarchy are preserved for future Experiments.

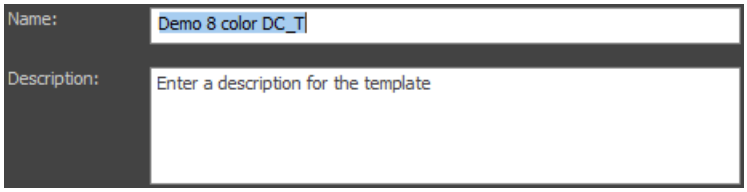


Launch the Save as Template dialog

Click the **Save as Template** icon in the Home tab (page 50) or use the keyboard shortcut **Ctrl+S**. This will save the active experiment as a template.

Alternatively, right-click on a **Tube** or **Plate Experiment**, then select the **Save As Template** option.

Name and Description fields



- By default, the **Name** text field shows the name of the current Experiment from which the user selected the “Save As Template” option appended with “_T”.

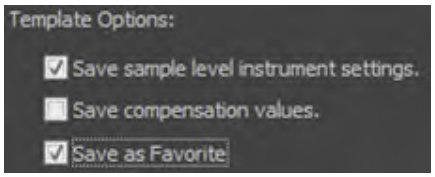
  If the default name is already in use, the name is checked for the suffix "(#)", where # is the next available integer.

  The template name field allows up to 50 printable characters.
- The **Description** field is optional and allows up to 500 printable characters.
- If the template name is in use when the OK button is pressed, a warning dialog indicates that the template already exists.
  - **Overwrite** saves the template and overwrites the existing template.
  - **Cancel** closes the dialog and returns to the Save as Template dialog.

Template Options

There are three options to save an Experiment as a Template:

- Save sample level instrument settings
- Save compensation settings values
- Save as Favorite.



- **Save sample level instrument settings** is the default option and specifies whether the template is saved with the sample level instrument settings.
- **Save as compensation settings values** option allows you to save the template with the compensation settings values (compensation XML). The option is deselected by default.

  If the experiment does not have compensation settings, this option is disabled.

  If a plate experiment contains Compensation controls mapped to wells, these wells will be removed if this option is selected.

  If the option is unchecked, the template maintains the compensation controls to be used in the template, but the compensation settings values are not carried over.
- **Save as Favorite** specifies that the template is set as a favorite. This option is unchecked by default.

  Templates that are marked as "Favorite" are tagged for easy identification within the Momentum Software when using the Attune™ NxT instrument integrated with the Orbitor RS2 or Orbitor RS3 Microplate Mover.

Template thumbnail

Displays a thumbnail image that corresponds to the selected template.

- By default, the template thumbnail is the first page of the experiment workspace of the experiment.
- You can select another template thumbnail by clicking the **Change Thumbnail** button, which opens the File Open browser and allows you to select images of the following formats: JPG, GIF, BMP, PNG, TIF, EMF.

Template attributes

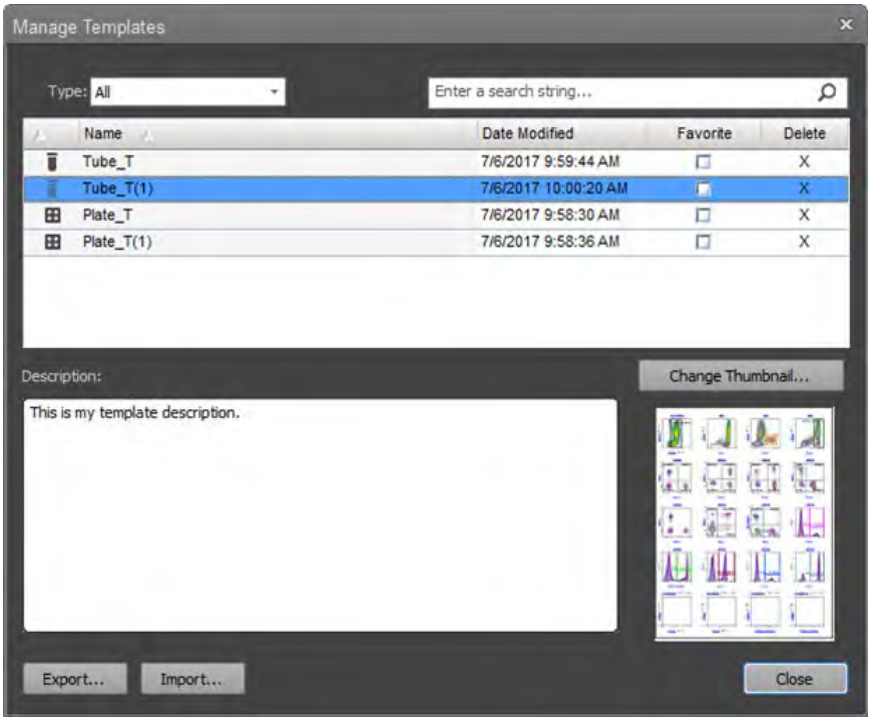
A template is saved with the following attributes:

- Experiment hierarchy/layout (numbers of Groups and Samples, types of Samples)
- Heat Map mapping (location of Tubes and/or Wells)
- Workspaces (Experiment, Group and Sample)
- Instrument settings (Sample-level are optional, if selected as described above)
- Run Protocols
- Compensation settings (optional, as described above)
- Heat Map settings
- Overlay settings
- Results View settings (selected statistics, sorting, grouping, column sizing, column order)
- Experiment keywords
- Sample level keyword values

Manage Templates dialog

Manage Templates dialog allows you to edit, import, export, and delete templates. It contains the following elements:

- Type filter (page 430)
- Keyword search (page 430)
- Template list (page 431)
- Description field (page 432)
- Thumbnails and Change Thumbnail button (page 432)
- Export button (page 432)
- Import button (page 432)



Type filter

Allows you to filter the templates by **type**. By default, the type filter is set to "All".

- When **All** is selected, all templates that are valid for the active instrument model (i.e., BRVY) are displayed.
- When the filter type is changed to **Tube**, only tube templates are displayed.
- When the filter type is changed to **Plate**, only plate templates are displayed.

Keyword search

Allows you to apply a search keyword to filter the list of displayed templates, where the experiment name or description contains the keyword.

- To filter the list of displayed templates, enter a keyword, then press **Enter** or click the **Search** button. This applies the search keyword as a filter, so that only the templates that contain the keyword in the name or the description field are displayed in the list.
- When a search keyword is applied, the search button displays "Clear".
- To remove the keyword filter, click **Clear**, or delete the text in the edit control and press **Enter**.

Template list

The template list is populated with the list of templates based on the active model and the filters applied by the experiment type and keyword search filters.

	Name	Date Modified	Favorite	Delete
	Tube_T	7/6/2017 9:59:44 AM	☐	X
	Tube_T(1)	7/6/2017 10:00:20 AM	☐	X
	Plate_T	7/6/2017 9:58:30 AM	☐	X
	Plate_T(1)	7/6/2017 9:58:36 AM	☐	X

- The template list has the following columns:
  - **Template type:** Displays an icon that corresponds to the experiment type (tube icon for tube experiment and plate icon for plate experiment).
  - **Template name:** Displays the name of the template.

    To edit a template name, double-click on the template name. Alternatively, press the F2 key, if only a single template is selected.

    You can enter up to 50 alpha-numeric characters for the template name.

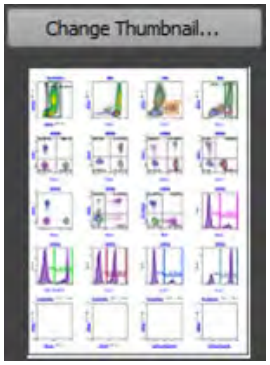
    The template name cannot end with a period character and cannot be any of the following words: "CON", "PRN", "AUX", "CLOCK$", "NUL", "COM1", "COM2", "COM3", "COM4", "COM5", "COM6", "COM7", "COM8", "COM9", "LPT1", "LPT2", "LPT3", "LPT4", "LPT5", "LPT6", "LPT7", "LPT8", "LPT9".
  - **Date Modified:** Displays the date the template was created or last modified. The date is displayed in system locale in the long time format (i.e., 6/15/2009 1:45:30 PM
  - **Favorite:** Favorite checkboxes allow you to designate a template as a favorite.
  - **Delete:** Deletes the template.
- To sort a column, click on the desired column heading. When sorting, a sort arrow indicates the sort direction (i.e., ascending or descending). Favorite and Delete columns are not sortable.
- To select a template, click on the row that displays the template you wish to select. You can select multiple templates.
- When a single template is selected in the template list, the Description field updates to display the template description (if entered) and the Thumbnail field displays the corresponding thumbnail.

  If multiple templates are selected, the description field and thumbnails are not displayed.
- Right-click a template to open the template list context menu with options to **Export** or **Delete** the template.

Description Displays the optional, user-defined template description (see "Save As Template dialog", page 428).

To edit the template description, click on the Description field and type in the desired description. You can enter up to 500 alpha-numeric characters.

Thumbnails Displays a thumbnail image that corresponds to the selected template.



- By default, the template thumbnail is the first page of the experiment workspace of the experiment.
- You can select another template thumbnail by clicking the **Change Thumbnail** button, which opens the File Open browser and allows you to select images of the following formats: JPG, GIF, BMP, PNG, TIF, EMF.

Export Allows you to export the selected dialog with your changes.

- Clicking **Export** when only a single template is selected opens the *File Save (Export) dialog* (page 532) with the "File name" set to the selected template name and the "Save as type" set to Attune Experiment Template (*.aet).

  Click **Save** on the File Save (Export) dialog to save the selected template to the specified location.
- Clicking **Export** when multiple templates are selected opens the *Folder Browser dialog* (page 537).

  Click **Select Folder** on the Folder Browser dialog to exports the selected templates to the selected folder with each template named as it was named in the Template list and given the .aet extension.

Import Allows you to import an Attune Experiment Template (*.aet) file to your list of templates.

- Clicking **Import** opens the *File Open (Import) dialog* (page 535) with the filter set to Attune Experiment Template (*.aet).
- Select a ***.aet** file to add the template to your list of templates and select the template in the Template list.
- You can import multiple templates. If you import multiple templates, the last imported template is selected in the Template list.

Delete

- Click the **X** in the Template list or right-click a template and select **Delete** from the context menu to delete the selected template.

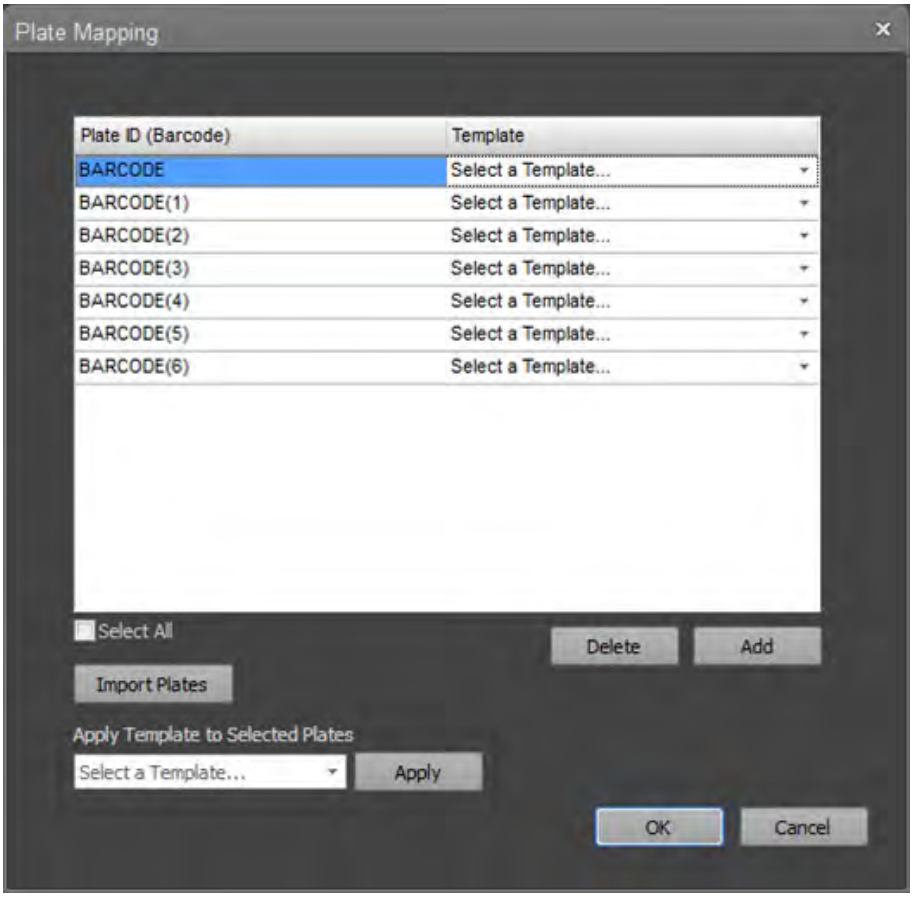
  When you click **X** or select the context menu **Delete** option, the software displays the *Confirm Delete dialog*.

  The dialog contains an option for **Do this for all other selected items (n found)**, where n is the number of remaining templates.
- Click **Yes** to delete the first selected template. The dialog will be presented again for each remaining selected template.
- Check the **Do this for all other selected items (n found)**, then click **Yes** to delete all selected templates.
- Click **Cancel** to return to the Manage Templates dialog without deleting any of the selected templates.

Plate Mapping dialog

The *Plate Mapping* dialog allows you to map templates to specific plates that are defined by barcodes in the Automation mode. The Plate ID (Barcode) to Template mappings are accessible by the automation software (such as the Thermo Scientific Momentum™ Software) to run the selection of plates.

To open the Plate Mapping dialog, click the **Map Plates** button in the Instrument ribbon tab (page 65).

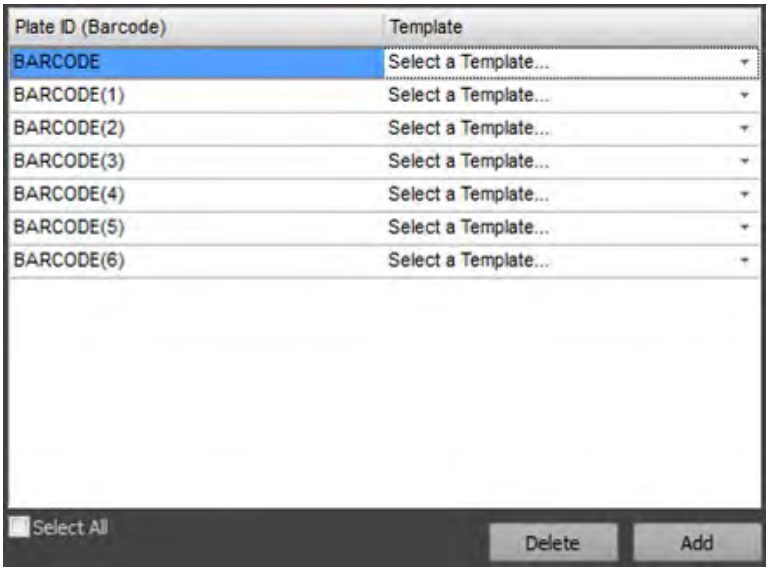


The Plate Mapping dialog contains the following controls:

- Plate ID (Barcode) to Template table (page 435)
- Select All checkbox (page 435)
- Delete and Add buttons (page 435)
- Import Plates (page 436)
- Apply Template to Selected Plates (page 436)
- OK and Cancel buttons (page 436)

Plate ID (Barcode) to Template table

The Plate ID (Barcode) to Template table allows you to select a template from a dropdown list to assign to the selected Plate ID (i.e., barcode).



- The Plate ID (Barcode) to Template table has the following columns:
  - **Plate ID (Barcode):** Displays the Plate ID (Barcode). By default, the Plate ID name is BARCODE.

    If the name is already in use, the name is checked for the suffix "(#)", where # is the next available integer. For example, BARCODE(2), BARCODE(3) etc.

    To edit a Plate ID name, double-click on the name. Alternatively, press the F2 key, if only a single template is selected.

    You can enter up to 50 alpha-numeric characters for the template name.

    The template name cannot end with a period character and cannot be any of the following words: "CON", "PRN", "AUX", "CLOCK$", "NUL", "COM1", "COM2", "COM3", "COM4", "COM5", "COM6", "COM7", "COM8", "COM9", "LPT1", "LPT2", "LPT3", "LPT4", "LPT5", "LPT6", "LPT7", "LPT8", "LPT9".
  - **Template:** Allows you to select a template to map to a Plate ID (Barcode).

    To map a template to a selected Plate ID, click **Select a Template…**, then select from the list of templates that have been marked as favorites.

    Alternatively, click **Select a Template…**, then select **Import/Browse...** at the top of the dropdown list, which opens the Select Template dialog (page 437).

Select All

Selects all Plate ID/Template rows in the Plate ID (Barcode) to Template table.

Uncheck the checkbox to deselect all rows except the last row in table.

Delete and Add buttons

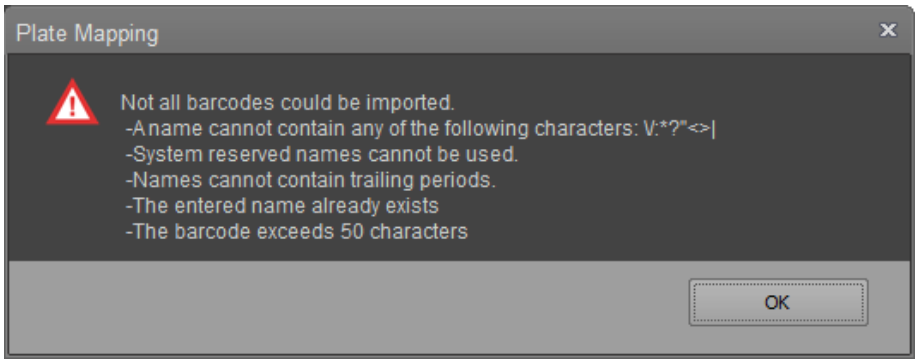
- **Delete:** Deletes all selected rows from the Plate ID (Barcode) to Template table.

- **Add:** Adds a new row to the Plate ID (Barcode) to Template table using the default Plate ID name.

Import Plates

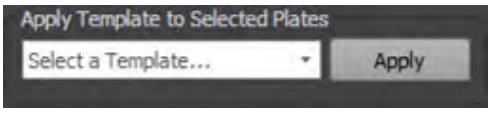
Opens the File Open (Import) dialog (page 535), which allows you to import a CSV file to add new barcodes to the Plate ID (Barcode) to Template table.

The CSV file is read from column by column and row by row and only names that are permitted are added. If an invalid name is detected, a dialog is displayed stating the reason that not all barcode names were imported:



Apply Template to Selected Plates

The Apply Template to Selected Plates function allows you to assign a template to the selected Plate ID/Template rows.



- Click **Select a Template…** to display and select from the list of templates that have been marked as favorites.

  Alternatively, click **Select a Template…**, then select **Import/Browse...** at the top of the dropdown list, which opens the Select Template dialog (page 437).
- Click **Apply** to apply the selected template to the selected rows.

OK and Cancel buttons

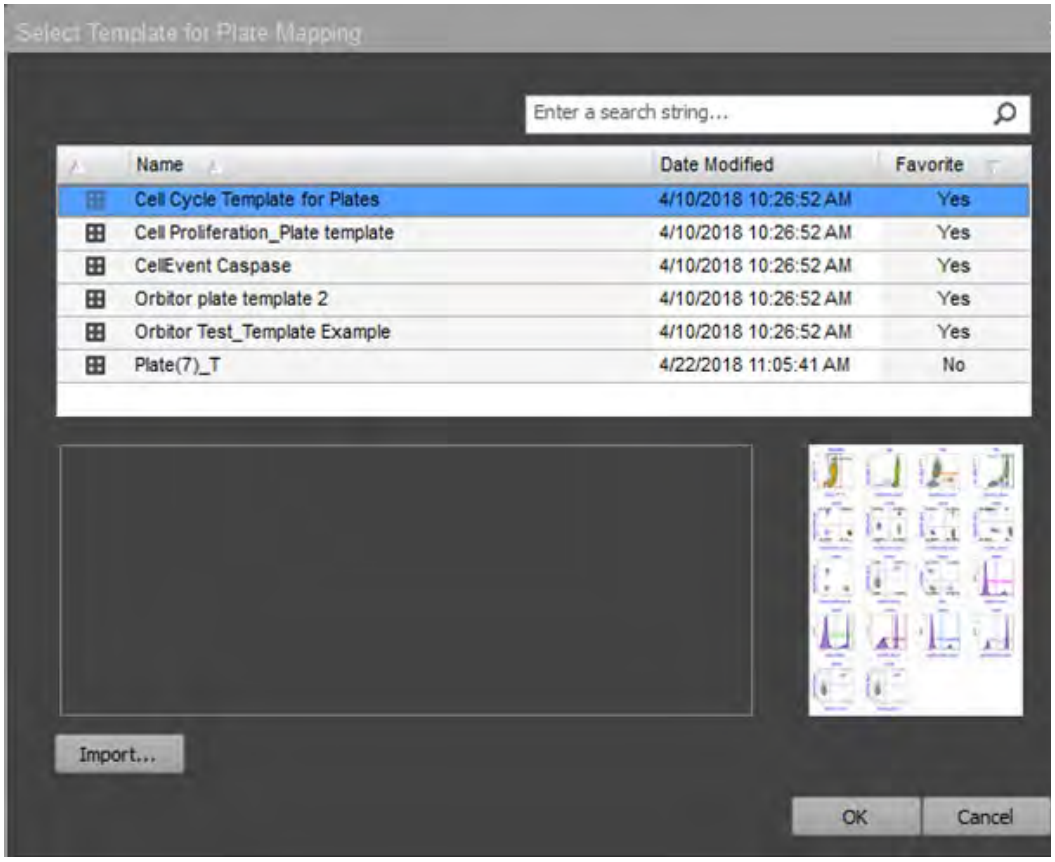
- Click **OK** to save the Plate ID (Barcode) to Template mappings in the database. The Plate ID (Barcode) to Template mappings are accessible by the automation software (such as the Thermo Scientific Momentum™ Software) to run the selection of plates.
- Click **Cancel** to close the dialog without saving the mappings.

Select Template dialog

The *Select Template* dialog is a modal dialog that allows you to select a Template to map to the selected Plate IDs (Barcodes).

The Select Template dialog only shows Plate templates for the active instrument model (e.g., BRVY). Tube templates are NOT displayed.

- Template Keyword search (page 437)
- Template list (page 438)
- Template description (page 438)
- Thumbnails (page 438)
- Import (page 439)
- OK and Cancel (page 439)



Template keyword search

Allows you to apply a search keyword to filter the list of templates displayed in the template list, where the experiment name or description contains the keyword.

- To filter the list of displayed templates, enter a keyword, then press **Enter** or click the **Search** button. This applies the search keyword as a filter, so that only the templates that contain the keyword in the name or the description field are displayed in the list.
- When a search keyword is applied, the search button displays "X".
- To remove the keyword filter, press the **X** button, or delete the text in the edit control and press **Enter**.

Template list The Template list is populated with a list of templates based on the active model and the filters applied by the Experiment type and keyword search filters.

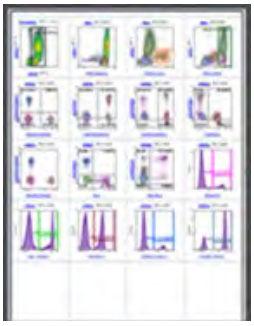
Name	Date Modified	Favorite
Cell Cycle Template for Plates	4/10/2018 10:26:52 AM	Yes
Cell Proliferation_Plate template	4/10/2018 10:26:52 AM	Yes
CellEvent Caspase	4/10/2018 10:26:52 AM	Yes
Orbitor plate template 2	4/10/2018 10:26:52 AM	Yes
Orbitor Test_Template Example	4/10/2018 10:26:52 AM	Yes
Plate(7)_T	4/22/2018 11:05:41 AM	No

- The Template list has the following columns:
  - **Template type:** Displays an icon that corresponds to the Experiment type (tube icon for Tube experiment and plate icon for Plate experiment). Only Plate experiment templates are listed in the "Select Template for Plate Mapping" dialogue.
  - **Template name:** Displays the name of the template.
  - **Date modified:** Displays the date the template was created or last modified. The date is displayed in system locale in the long time format (i.e., 6/15/2009 1:45:30 PM
  - **Favorite:** Shows whether the template was designated as a favorite.
- To sort a column, click on the desired column heading. When sorting, a sort arrow indicates the sort direction (i.e., ascending or descending). Favorite and Delete columns are not sortable.
- To select a template, click on the row for the template you wish to select. You can select multiple templates.
- When a single template is selected in the template list, the Description field updates to display the template description (if entered) and the Thumbnail field displays the corresponding thumbnail.

  If multiple templates are selected, the description field and thumbnails are not displayed.
- Right-click a template to open the template list context menu with options to **Export** or **Delete** the template.

Template description Displays the optional, user-defined template description (see "Save As Template dialog", page 428).

Thumbnails Displays a thumbnail image that corresponds to the selected template. By default, the template thumbnail is the first page of the experiment workspace of the experiment.



Import

Allows you to import an Attune Experiment Template (*.aet) file to your list of templates.

- Click **Import** to open the *File Open (Import) dialog* (page 535) with the filter set to Attune Experiment Template (*.aet).
- Select a ***.aet** file to add the template to your list of templates and select the template in the Template list.
- You can import multiple templates. If you import multiple templates, the last imported template is selected in the Template list.

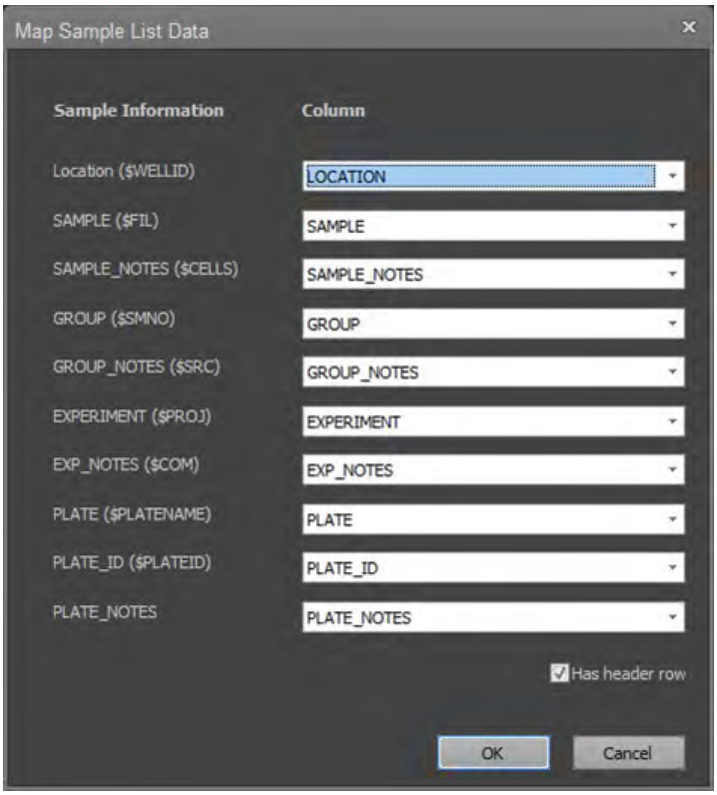
OK and Cancel buttons

- Click **OK** to close the dialog and assign the selected template to the Template list that has launched the dialog.
- Click **Cancel** to close the dialog. The template list in the Plate Mapping dialog reverts to its previously mapped template. The default is blank.

Map Sample List Data dialog

The *Map Sample List Data* dialog allows you to create Samples and map them based on location when you import a Sample List from a CSV file.

Note: *.xls files may not be imported as Sample Lists.



- When the Sample List is imported, the software looks for the following default system-defined columns/keywords:
  - LOCATION
  - SAMPLE
  - SAMPLE_NOTES
  - GROUP
  - GROUP_NOTES
  - EXPERIMENT
  - EXP_NOTES
  - PLATE
  - PLATE_ID
  - PLATE_NOTES
- The Map Sample List dialog also displays the keyword label and the corresponding control for each custom (user-defined) keyword.
- The additional keywords mapped from a CSV file are based on the existing Experiment keywords. If there are no custom keywords assigned in the Experiment, the dialog only displays the system-defined keywords and default sample list columns, regardless of the custom keywords present in the CSV file.
- If the column headers match the specified column names, they are automatically mapped to their respective fields.

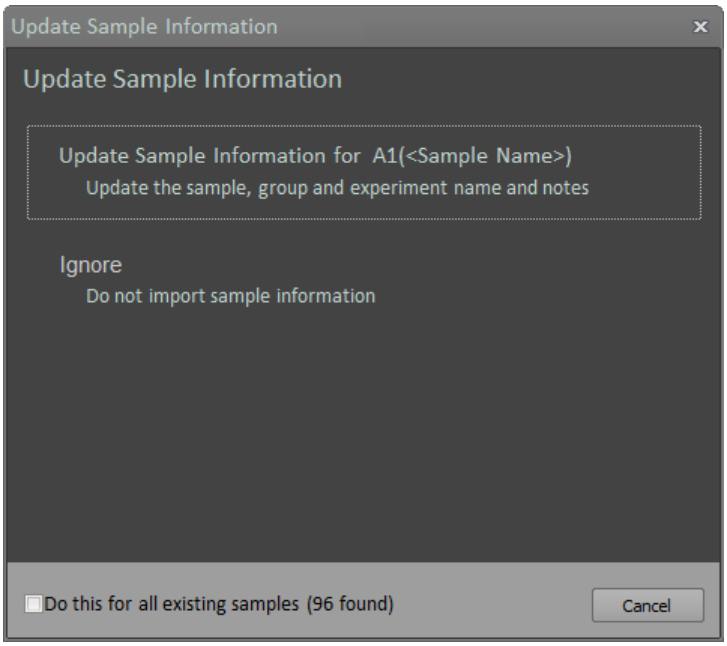
- The **Has header row** option allows the first row in the CSV file to be interpreted as a column header instead of actual row data. By default, this option is selected.

  - When checked, the first row of the CSV is interpreted as column names instead of actual row data, and the combo box controls in the dialog are populated with the column header values as parsed from the CSV file.
  - When unchecked, the first row is interpreted as actual row data, and the combo box controls are populated with generic column names (Column 1, Column2, etc.) with the number of columns corresponding to the number of columns in the CSV file.
  - The combo box controls also include a **None** option, which excludes the corresponding sample information from being read from the CSV file.
- Click **OK** to update the Experiment with the Sample List data in the CSV file using the column mapping specified in the dialog.

  Click **Cancel** or **X** to close the dialog without updating the Experiment and importing the Sample List data.

Update Sample Information dialog

The *Update Sample Information* dialog is displayed if you attempt to import a Sample List from an existing CSV file, but Samples already exist within the Experiment or Plate (i.e., Samples are assigned to a location).

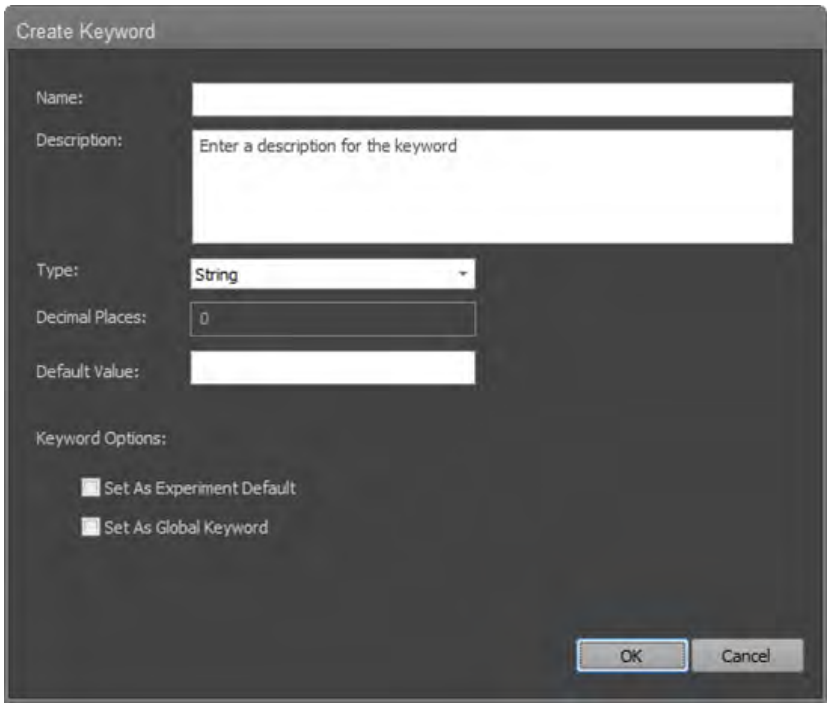


- Click **Update Sample Information** to update the Sample, Sample Notes, Group, Group Notes, Experiment, Experiment Notes, Plate, Plate ID, and Plate Notes for the specified Sample.
- Click **Ignore** to skip the import/update of the Sample information for the specified Sample.
- Check **Do this for all existing samples** to repeat the Update or Ignore action for every Sample in the Experiment or Plate.
- Click **Cancel** or the **X** button to close the dialog and prevent additional actions from being performed (any changes to existing Samples before this are not reverted).

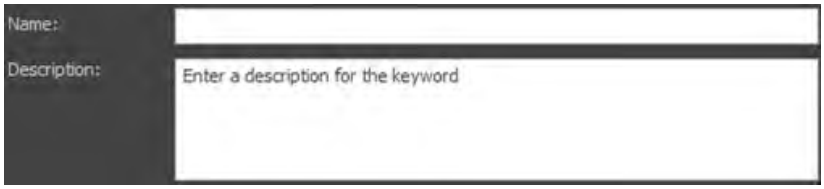
Create/Edit Keyword dialog

The *Create/Edit Keyword dialog* allows you to define new keywords to add to the global keyword options list (see "Keyword options", page 499).

- When the dialog is launched from Keyword options by clicking the **Create** button, the dialog title is "Create Keyword".
- When the dialog is launched from Keyword options by clicking the **Edit** button, the dialog title is "Edit Keyword".



Name and Description



- The **Name** field is required and allows up to 50 printable characters.
  - If the Name field is left blank when the OK button is pressed, a warning dialog prompts you to enter a name for the keyword.
  - If the keyword name is in use when the OK button is pressed, a warning dialog indicates that the keyword name already exists.
- The **Description** field is optional and allows up to 500 printable characters.

Type

Type dropdown allows you to select **String** or **Number** for the keyword type.

- If the keyword type is changed from String to Number or vice versa, a warning dialog prompts you to confirm the change.
  - Click **Yes** to confirm the change, close the warning dialog and the Edit Keywords dialog, and reset the keyword values.
  - Click **No** to close the warning dialog and return to Edit Keywords dialog.

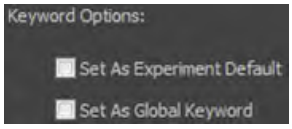
Default Value and Decimal Places

Default Value allows you set the keyword value automatically when creating Samples in an Experiment using the selected keyword.

- The Default Value is blank by default and can be left blank.
- The Default Value field allows up to 50 characters.
- When the **Type** is set as a **Number**, the Default Value only accepts the negative sign, numbers, thousands separators, and decimal places as per the locale setting.
- If the **Type** is set to **Numeric**, the **Decimal Places** field is enabled. The Decimal Places setting is used to format a numeric keyword for display in the Sample List table and sets the number of decimal places to include in the FCS file.
  - The default number of decimal places is set to 0.
  - The number of decimal places allows up to 30 decimals.

Keyword Options

Keyword Options allow the keyword to be set as an Experiment Default and as Global Keyword.



- **Set As Experiment Default** adds the selected keyword to the Experiment keyword list automatically when the Experiment is created.

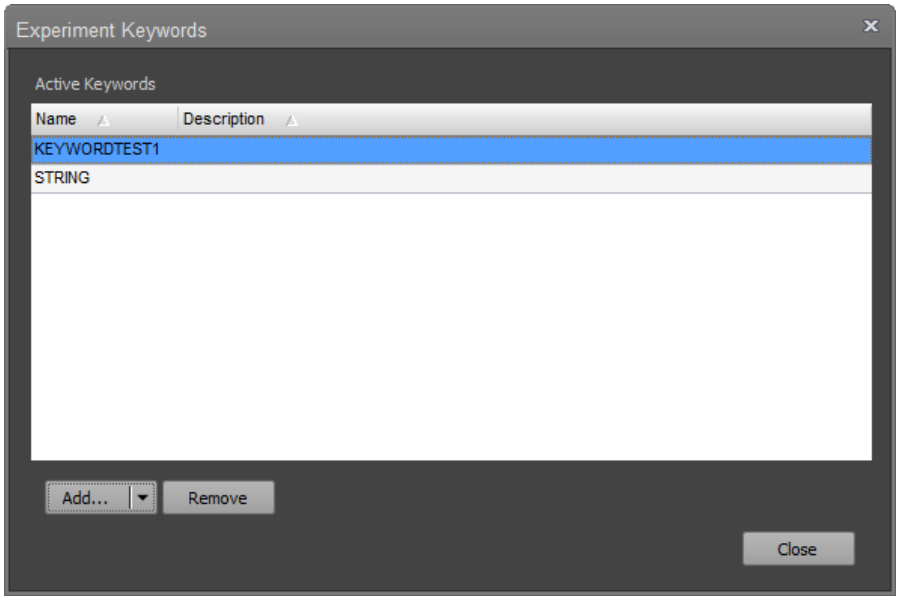
  All users have the option to set a keyword as an Experiment Default.
- **Set As Global Keyword** sets the custom keywords in the Keyword Options dialog list to global. Global keywords are included in each user account after creation.

  The Set As Global Keyword checkbox is only visible to Administrator accounts, and only they can edit or delete a global keyword. User-level accounts cannot delete a global keyword.

Experiment Keywords dialog

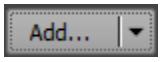
The *Experiment Keywords* dialog displays the Active Keywords list for the current Experiment and allows you to add, edit, and remove custom keywords for the Experiment.

The Active Keywords list has two columns: Name and Description.



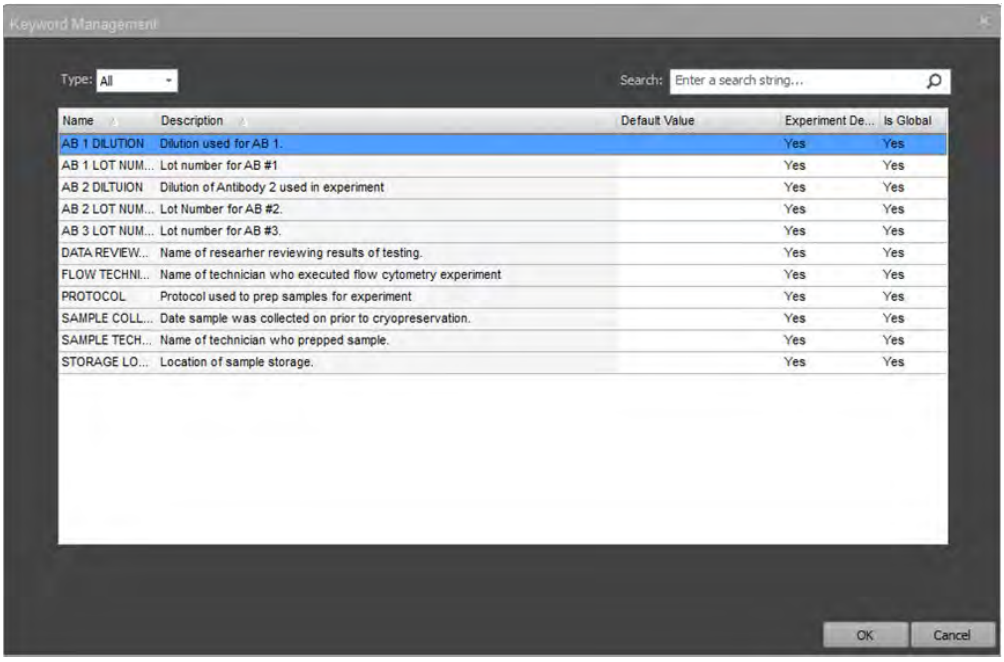
Note: The Experiment Keywords (including any local modifications made) persist within the Experiment. To add new keywords to the master list to be available for use in an Experiment, use the Keyword tab of the Options dialog (page 499) to edit or create user and global keywords.

Add/Edit The Add/Edit split button allows you to add and edit custom keywords for the active Experiment.



Add

- Click **Add** to open the *Select Keywords* dialog, which includes all the keywords defined in the account that are not already included in the active experiment.



- Keywords that are already part of the Experiment Keyword set are not displayed in the Select Keywords dialog.
- You can select multiple keywords, then click **OK** to copy them to the Experiment and display them in the Experiment Keywords dialog.
- Click **Cancel** or **X** to close the dialog without taking action.

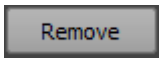
Edit

- Click the **Edit** portion of the Add/Edit split button to open the Edit Keyword dialog described in "Create/Edit Keyword dialog" (page 443).

- Changes made in the Edit Keywords dialog are only applied to the keywords within the active Experiment. Changes are not applied to the keyword of same name in the master list in the Keyword tab of the Options dialog (page 499).
- Click **OK** to apply the changes locally to the keyword in the active Experiment.
- Click **Cancel** or **X** to cancels any changes and close the dialog.

Remove

- Click **Remove** to remove any selected keywords from the Active Keywords list of the active Experiment.

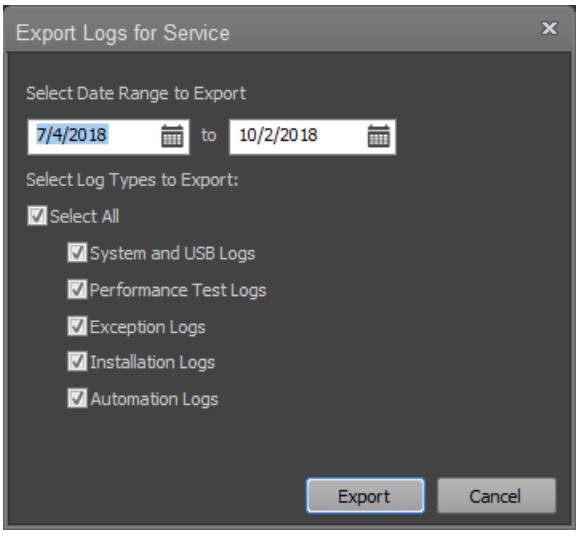


- You can select and remove multiple keywords at once.
- Keywords that are removed from the Active Keywords list are not removed from the master list in the Keyword tab of the Options dialog (page 499).

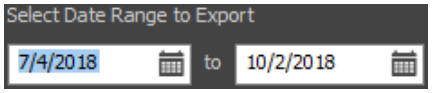
Export Logs for Service dialog

The *Export Logs for Service* dialog allows you to export selected logs to a single zip file based on a specified date range.

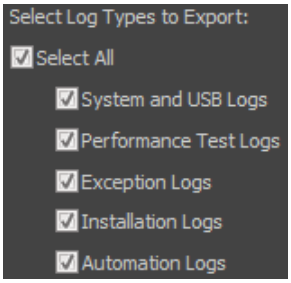
The dialog consists of two date pickers and a list of log types that you can select for export. By default, the date range is set to include the last 90 days of logs and all logs are selected.



- **Date picker** controls allow you to choose *to* and *from* dates. The start date must occur before the end date. If you enter an invalid date range, the instrument displays a warning.

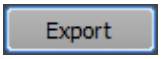


- **Select Log Types to Export** allows you to export all logs at once or select the individual log types you wish to export.
  - System and USB Logs (which include the system and USB logs)
  - Performance Test Logs (which include the logs, corresponding iteration logs, and FCS files)
  - Exception Logs (only zipped logs are included)
  - Installation Logs (installation and firmware logs)
  - Automation Logs



- **Export** opens the File Save (Export) Dialog (page 532), which allows you to save the selected logs as a zip file in the desired directory.

  The default file name for the zip file is "AttuneServiceLogs_YYYY-MM-DD_HH-MM-SS", where YYYYMMDD_HHMMSS is the date and time.

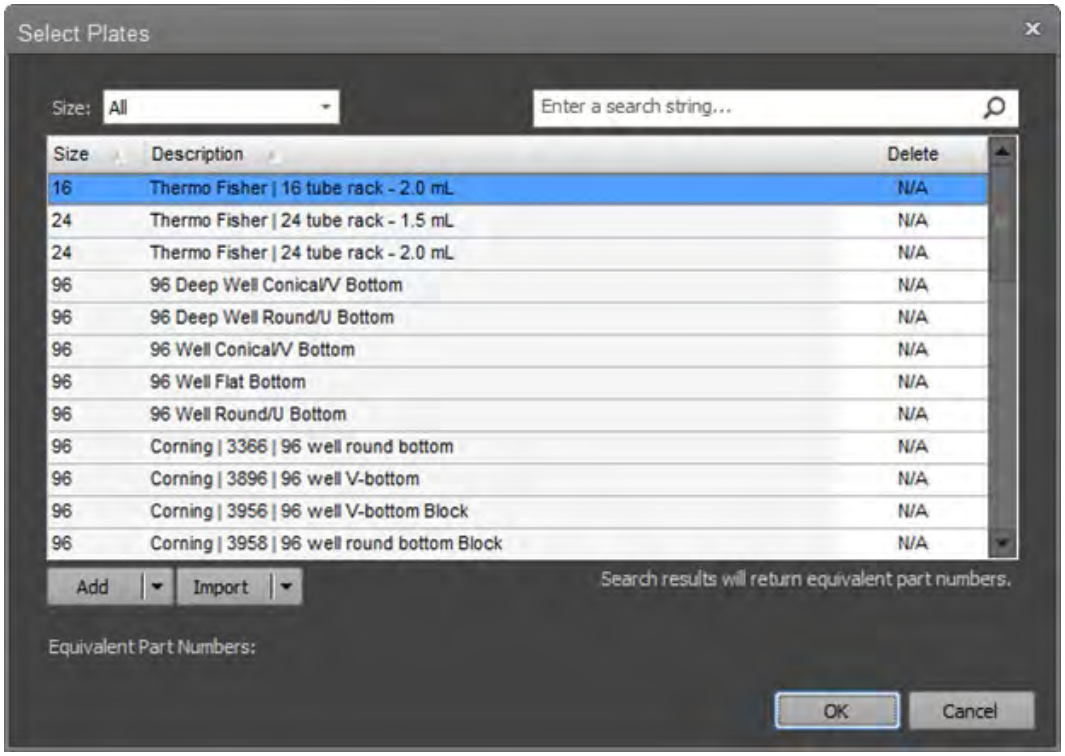


- **Cancel** or the **X** button closes the dialog with no further action.

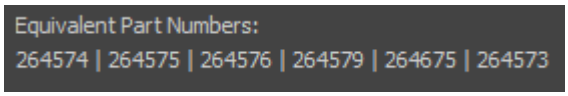
Select Plates dialog

The *Select Plates* dialog displays a list of all available plates in the database. You can add these plates to the **Quick Select Plates** list in the **Options ▶ Plate Options** dialog (page 499) or access them from the **New Experiment** dialog (page 399) or the **Customize Plate Experiment** panel (page 304).

The Select Plates dialog is available only when a CytKick™ Max Autosampler is connected to the Attune™ instrument.



- The plates list has columns for **Size**, **Description**, and **Delete**. To sort the plates list by Size or Description, click on the Size or Description column header.
- To search the available plates in the database, enter the search query in the **Search** field. You can search available plates based on **Size** or **Description**.
- When you perform a search, **equivalent part numbers** where the plate definitions match are displayed in the lower left of the dialog.



- To filter the available plate list by Size, select the desired option from the **Size** dropdown menu. Available options are **All**, **16**, **24**, **96**, and **384** (well plates).

- The **Delete** column is only visible for Administrator accounts when the dialog is launched from the Quick Select Plates list in the **Options ▶ Plate Options** dialog (page 499).

  When the mouse moves over the Delete column, the cursor changes to the hand cursor, if the plate can be deleted. You can only delete user-defined plates.

  System-defined plates display N/A in the Delete column and cannot be deleted.

  If a plate definition is in use as an existing Plate Experiment or Template, deletion is not allowed.
- To remove a Plate from the list of favorites, click the **X** in the Delete column next to the plate you wish to delete.
- **Add/Edit/Duplicate** split button allows you to add, edit, or duplicate a plate definition.

  Only Administrators, Advanced Users, and Service accounts can add, edit, or duplicate plate definitions. For users who do not have this permission, the button displays View and no dropdown menu is provided.
- To add a new plate definition to the plates list, click **Add** to open the **Create Plate** dialog (page 450).
- To edit a plate definition in plates list, select the **Edit** option on the Add/Edit/Duplicate split button to open the **Edit Plate** dialog (page 450).
- To duplicate a plate definition, select the Duplicate option on the Add/Edit/Duplicate split button. The Create Plate dialog opens with all the fields pre-populated to match those fields in the plate definition that was selected. However, the description field will be updated to have (#) appended to the description to create a unique name for the duplicated plate definition.
- **Import/Export** split button allows you to import or export a plate definition.

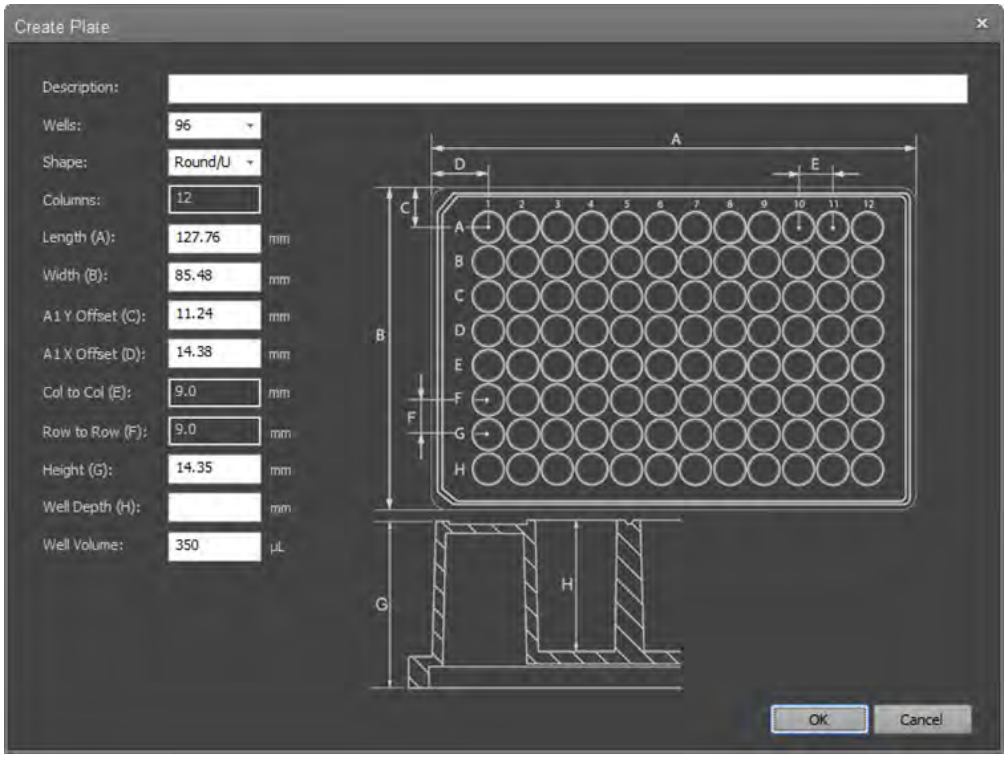
  This button is a split button only when launched from the Quick Select Plates list in the **Options ▶ Plate Options** dialog (page 499); otherwise, it is a standard button with the Import option only.
- To import a plate definition to the plates list, click **Import**. The **File Open (Import)** dialog opens, which allows you to import a file containing plate definitions (page 535).
- To export a plate definition, select the **Export** option on the Import/Export split button. The **File Save (Export)** dialog opens, which allows you to export the selected plate definitions (page 532).

Create/Edit Plate dialog

Create/Edit Plate

Create/Edit Plate dialog allows you to create custom plates with specific dimensions (such as plate height and well volume) to add to the database.

The Create/Edit dialog is available only when a CytKick™ Max Autosampler is connected to the Attune™ instrument.



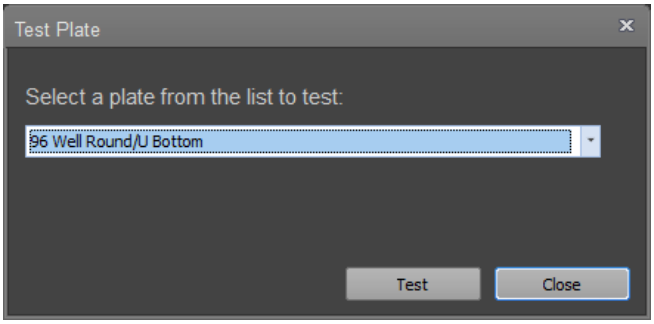
- If you have clicked **Add** in the **Select Plates** dialog (page 448) to launch the dialog, the dialog title is **Create Plate**.

  If you have clicked **Edit** in the **Select Plates** dialog to launch the dialog, the dialog title is **Edit Plate**.
- If a plate is used in an existing Experiment or Template, the Edit Plate dialog does not allow modification of any of the plate fields (they are read-only).
- The **Description** field must contain a unique description (it cannot be blank). The field allows 260 characters.
- New plate definitions have the following default values:
  - **Wells:** 96 (available options are 48, 96, and 384 wells)
  - **Shape:** Round/U bottom (available options are Round/U, Conical/V, and Flat/F bottom)
  - **Columns (read-only):** 6 for 48-well, 12 for 96-well, 24 for 384-well
  - **Length (A):** 127.76 mm
  - **Width (B):** 85.48 mm
  - **A1 Y Offset (C):** 11.24 mm for 48-well and 96-well, 8.99 mm for 384-well
  - **A1 X Offset (D):** 18.88 mm for 48-well, 14.38 mm for 96-well, 12.13 mm for 384-well

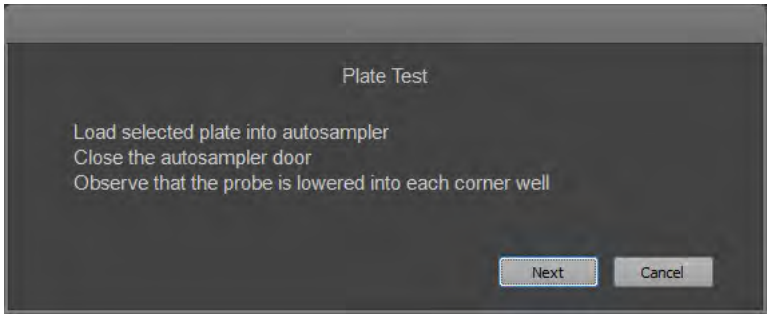
Test Plate dialog

The *Test Plate* dialog allows you to validate a plate to ensure that the autosampler probe position is in the correct location in all four corners of the plate.

The dialog consists of a plate selection dropdown and Test and Close buttons.



- **Plate Selection** dropdown list allows you to select a plate to test. The list of plates is based on the Quick Select plates definition.
- **Test** button initiates the test procedure and displays the test instructions.



Run the plate test

1. Click **Test Plate** button on the Instrument ribbon tab (page 64) to open the Test Plate dialog.

2. Select the plate you want to test from the **Plate Selection** list, then click **Test**.
3. Load the selected plate into the autosampler, close the autosampler door, then click **Next**.

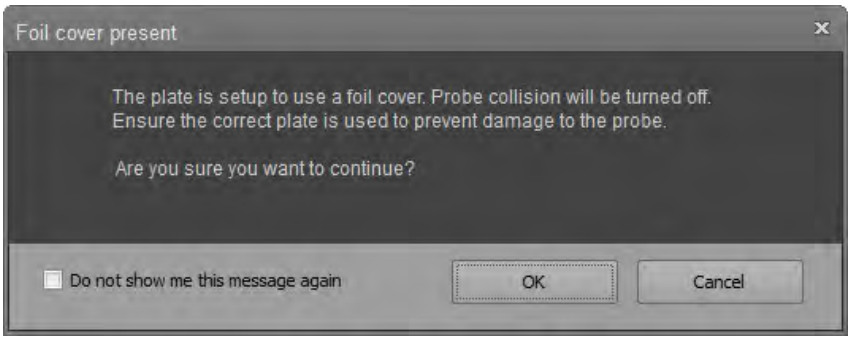
   During the test, the selected plate definition is sent to the instrument. The autosampler then iterates through each corner of the selected plate or tube rack and lowers the probe to the well depth as defined in the plate definition. The autosampler cycles through each corner of the plate three times.

   Note: You cannot cancel the Plate Test procedure once it has started.

4. At the completion of the test, the dialog displays the message "Test completed without errors!". If a probe collision error occurs, the error message is displayed instead of the successful completion message.
5. When the test is completed, click **Close** to close the Test Plate dialog.

Foil Cover Present dialog

When **Use Foil Cover** is enabled (page 403), the software displays the "Foil cover present" warning dialog at the beginning of a plate acquisition.

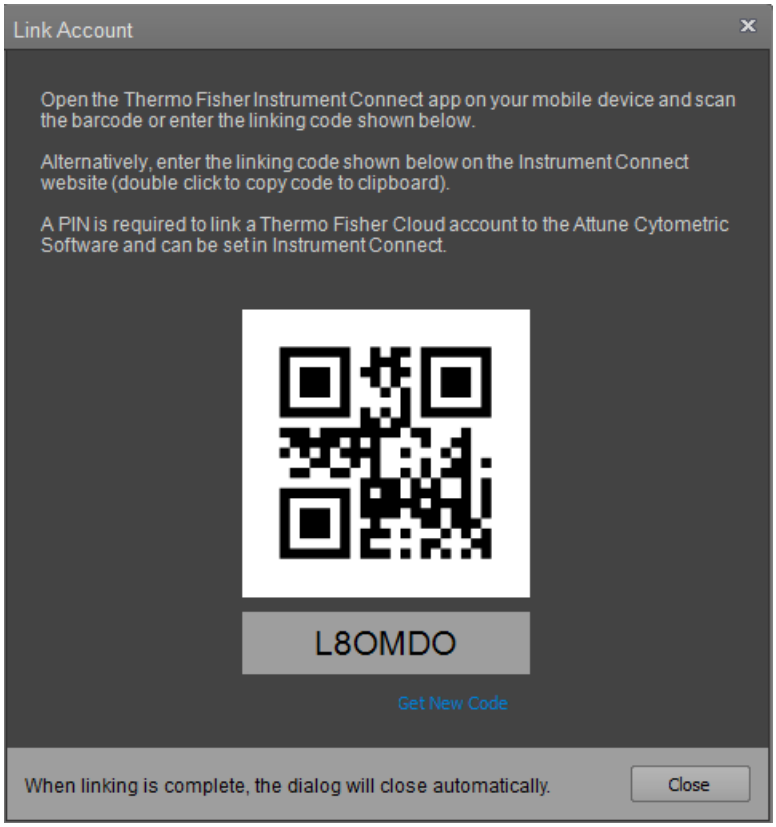


- The Foil cover present dialog is displayed when you click the **Setup Comp**, **Record Comp**, **Record All**, or **Record Plate** button on the Collection Panel, or if you attempt to **Run/Record** from a Manual well).

  The dialog is not displayed when you run a Tube sample or a Compensation sample from a Tube within a Plate Experiment.
- Click **OK** to close the dialog and proceed with acquisition.
- Click **Cancel** or the **X** button to abort the acquisition and close the dialog.
- Select **Do not show me this message again** to suppress dialog for the duration of the current session.

Link Account dialog

The *Link Account* dialog allows you to register the Attune™ Cytometric Software with the Connect™ cloud-based platform and link your instrument to your account on the Instrument Connect website.



Note: Only System Administrator and Administrator accounts can register the instrument with the Connect platform. After the device (instrument or software) is registered with the Connect platform, individual User accounts can link the registered instrument with their Connect accounts using the Cloud sign in feature. This allows a single instrument to be associated with multiple Connect accounts.

- The Link Account dialog provides two options to register the Attune™ Cytometric Software:
  - **Mobile:** Allows you to register the device software using a unique QR code with the QR code scanner within the *Instrument Connect* mobile application (page 454).
  - **Linking Code:** Allows you to register the device software using a unique linking code from the Connect platform to register the device software on the Connect website (page 455).
- If you click **Cancel** or the **X** button, the dialog closes without completing the device registration.

Link account with the QR code

To register the device software with a unique QR code using the QR code scanner within the *Thermo Fisher Instrument Connect* mobile application:

1. On the Options dialog ▶ Configuration tab (page 514), click **Register Device.** The software displays the *Starting the Attune Cloud Service* dialog as the instrument establishes a connection.

2. When the connection with the cloud service is established, the *Link Account* dialog prompts you to link your cloud account by using either the **QR code** option (for mobile app devices) or a **linking code** (for web or mobile apps).

3. If an error occurs while retrieving the QR code, a warning banner is displayed on the dialog that provides a description of the error.

   To request another linking code, click the **Get New Code** hyperlink under the QR code. A new QR code is generated and displayed on the dialog.
4. When you have the QR code, open the **Thermo Fisher Scientific Instrument Connect** application on your mobile device and scan the QR barcode image.
5. The Instrument Connect mobile application automatically sends the QR code to the Connect platform.
6. When registration is complete, the Attune™ Cytometric Software receives a successful response from the Connect platform, and the dialog automatically closes.

Link account with the linking code

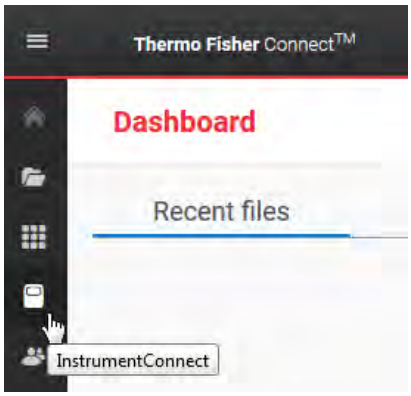
To register the device software using a unique linking code from the Connect platform:

1. On the Options dialog ▶ Configuration tab (page 514), click **Register Device.**
2. When the connection with the cloud service is established, double-click the **linking code** below the QR code to copy the linking code to the Windows® clipboard.

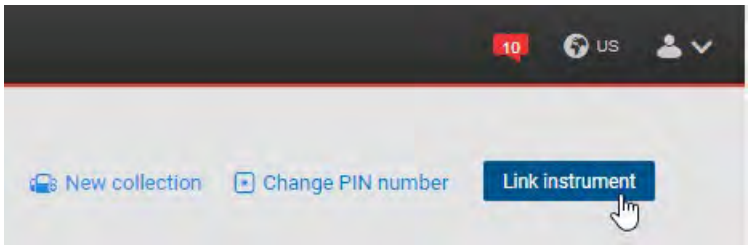
3. If an error occurs while retrieving the linking code, a warning banner is displayed on the dialog that provides a description of the error.

   To request another linking code, click the **Get New Code** hyperlink under the linking code. A new linking code is generated and displayed on the dialog.

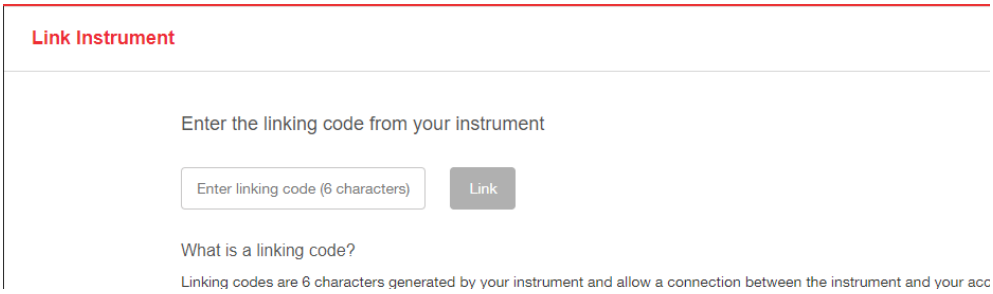
4. When you have the linking code, go to **thermofisher.com**, sign in to your Connect account, then click the **Instrument Connect** icon.



5. In the Instrument tab, click **Link instrument** to open the *Link instrument* dialog.

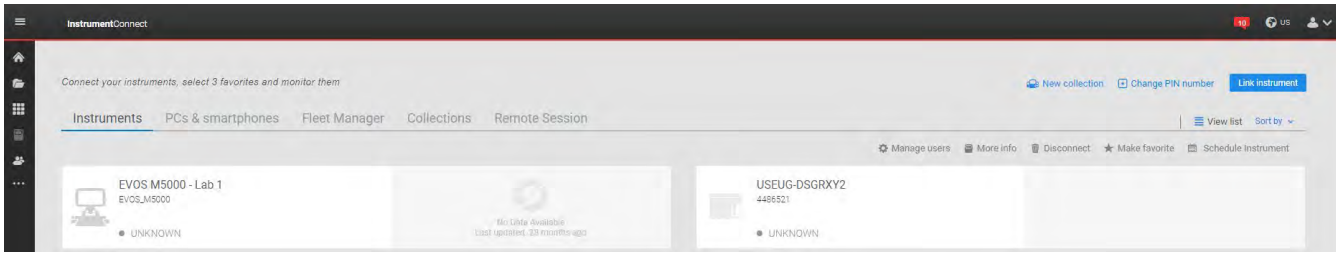


6. Enter the unique linking code you have copied on the Link Account dialog, then click **Send**.



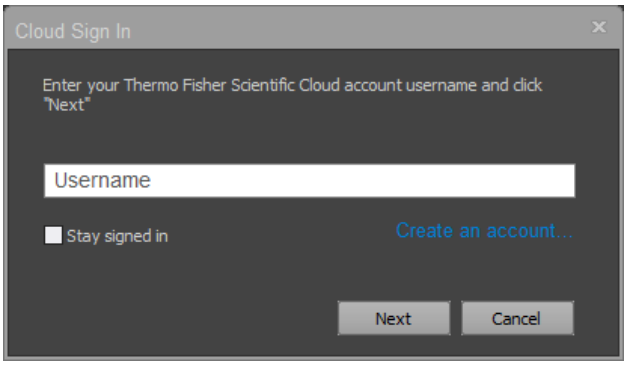
7. When registration is complete, the Attune™ Cytometric Software receives a successful response from the Connect platform, and the dialog automatically closes.

   The registered device is listed in the Instruments section of your Connect account.



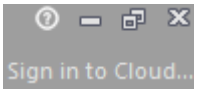
Cloud Sign In dialog

The *Cloud Sign In* dialog allows you to sign in to your Connect account, Thermo Fisher Scientific's cloud-based platform, with your account username and password.



Note: Only System Administrator and Administrator accounts can register the instrument with the Connect platform. After the device (instrument or software) is registered with the Connect platform, individual User accounts can link the registered instrument with their Connect accounts using the Cloud sign in feature. This allows a single instrument to be associated with multiple Connect accounts.

1. To sign in to your Connect account, click **Sign in to Cloud** button, located at the top right corner of the screen. The Cloud Sign In dialog opens.

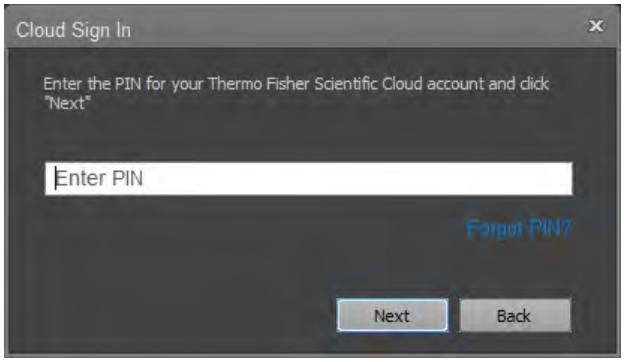


2. In the dialog, enter your Thermo Fisher Cloud username in the **Username** field, then click **Next**.
   - If you do not yet have a Connect account, click **Create an account…** hyperlink, which opens a web browser directed to the global registration site (www.thermofisher.com/global-registration/registration) to enable you to create an account.
   - If you wish to automatically sign in to the Connect platform the next time you sign in to the Attune™ Cytometric Software, select **Stay signed in**.

     If the checkbox is unchecked, every time you sign out of the Attune™ Cytometric Software, your sign in credentials are purged, which requires you to sign in the next time you wish to connect to the Connect platform.

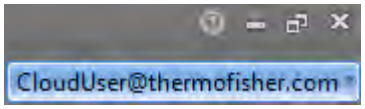
3. If you have not yet used your Connect account on the Attune™ Cytometric Software, the software displays the Link Account dialog (page 453), which allows you to link your account to the software for the first time.

4. If you were the user who had registered the instrument or if you had already linked the instrument to your Connect account, the software prompts you to enter your **four-digit PIN** to link their Thermo Fisher Cloud account to the Attune™ Cytometric Software.

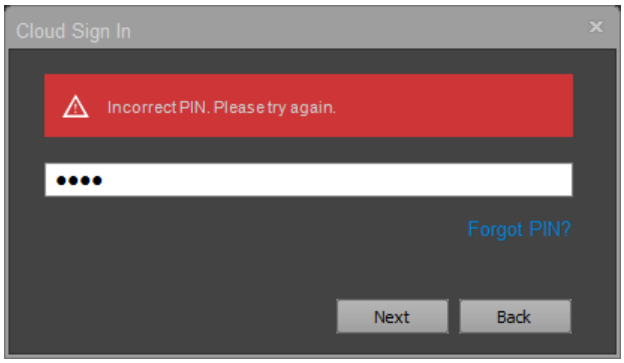


The **Forgot PIN?** hyperlink directs you to the web version of Instrument Connect application, where you can change or reset your PIN number.

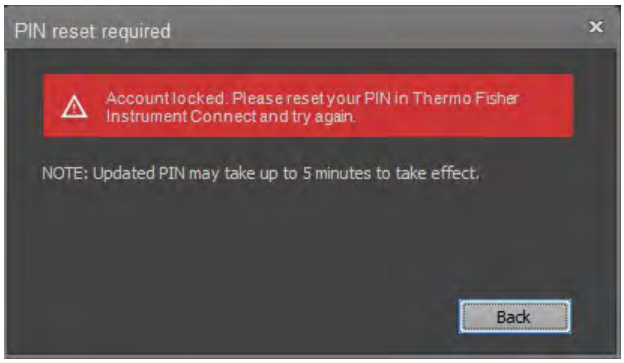
5. Providing a correct PIN number completes the account linking process and the Sign in to Cloud button located at the top right corner of the screen, becomes a dropdown button that displays your Connect username as the main button text.



6. If you provide a wrong PIN, you will be notified and asked to try again.



7. If you provide the wrong PIN 5 times in a row, your account will be locked and you will be required to reset your PIN in Instrument Connect before trying again.

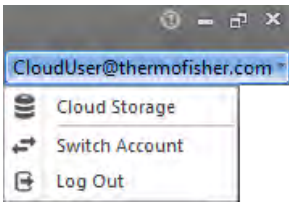


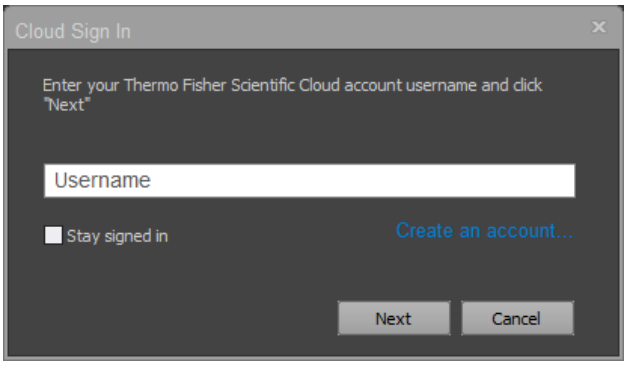
After you enter a new PIN on the Thermo Fisher Instrument Connect website or mobile app, the Incorrect PIN dialog message switches to the Enter PIN dialog.

Note: The account lock out is only a local lock that prevents the user from linking their cloud account to the Attune™ Cytometric Software; it does not completely lock the Thermo Fisher Clound account on the web.

Switch Account dialog

When you select **Switch Account** on the **Sign in to Cloud...** dropdown menu, the *Cloud Sign In dialog* (page 457) opens and allows you to sign in with a different username and for-digit PIN.



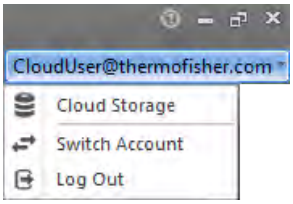


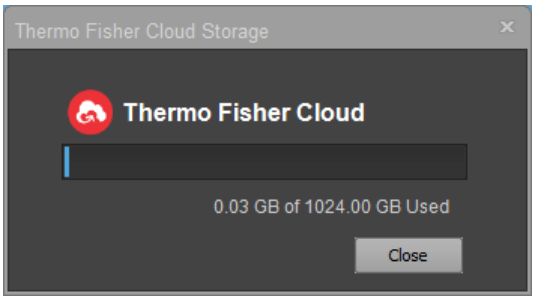
- The Switch account workflow is identical to that of Cloud Sign In, as described on page 457.
- When you switch to another Connect account successfully, the software automatically signs out of the old cloud account and clears the “Stayed sign in” setting.

Thermo Fisher Cloud Storage dialog

When you select **Cloud Storage** on the **Sign in to Cloud...** dropdown menu, the *Thermo Fisher Cloud Storage dialog* opens and shows the available storage on the Thermo Fisher Cloud account for the current user.

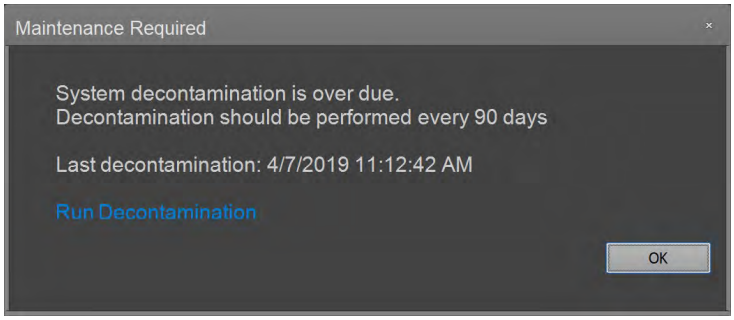
The progress bar on the dialog graphically displays the total used space.





Maintenance Required dialog

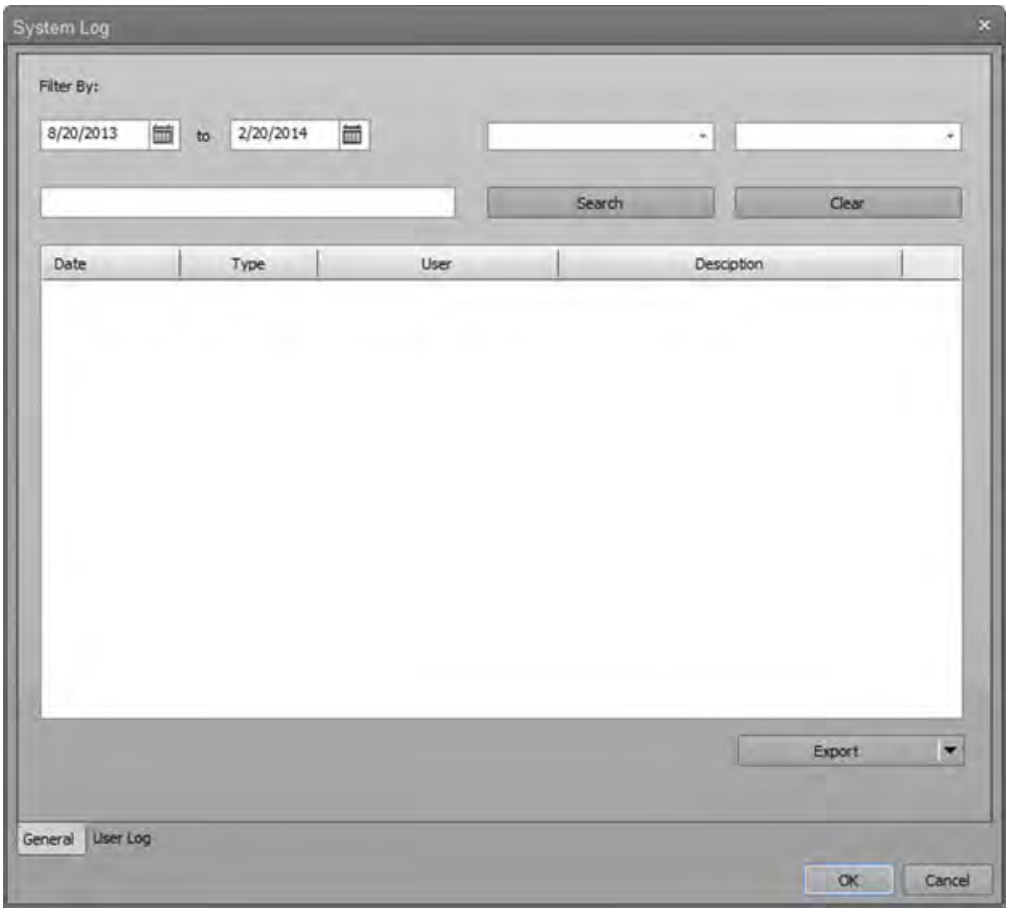
If there is overdue maintenance and the instrument is online and connected, the *Maintenance Required* dialog is displayed during the sign in and describes which maintenance procedure is overdue.



- To start the described maintenance procedure and close the dialog, click the **maintenance procedure hyperlink** displayed in the dialog. The hyperlink is displayed only for users who have permission to perform the maintenance procedure.
- To dismiss the dialog without performing the maintenance procedure, click **OK**. However, the dialog will be displayed at each subsequent sign in until the required maintenance procedure is performed.

System Log dialog

System Log dialog is displayed when the System Log button is clicked on the Instrument Ribbon tab (page 62).



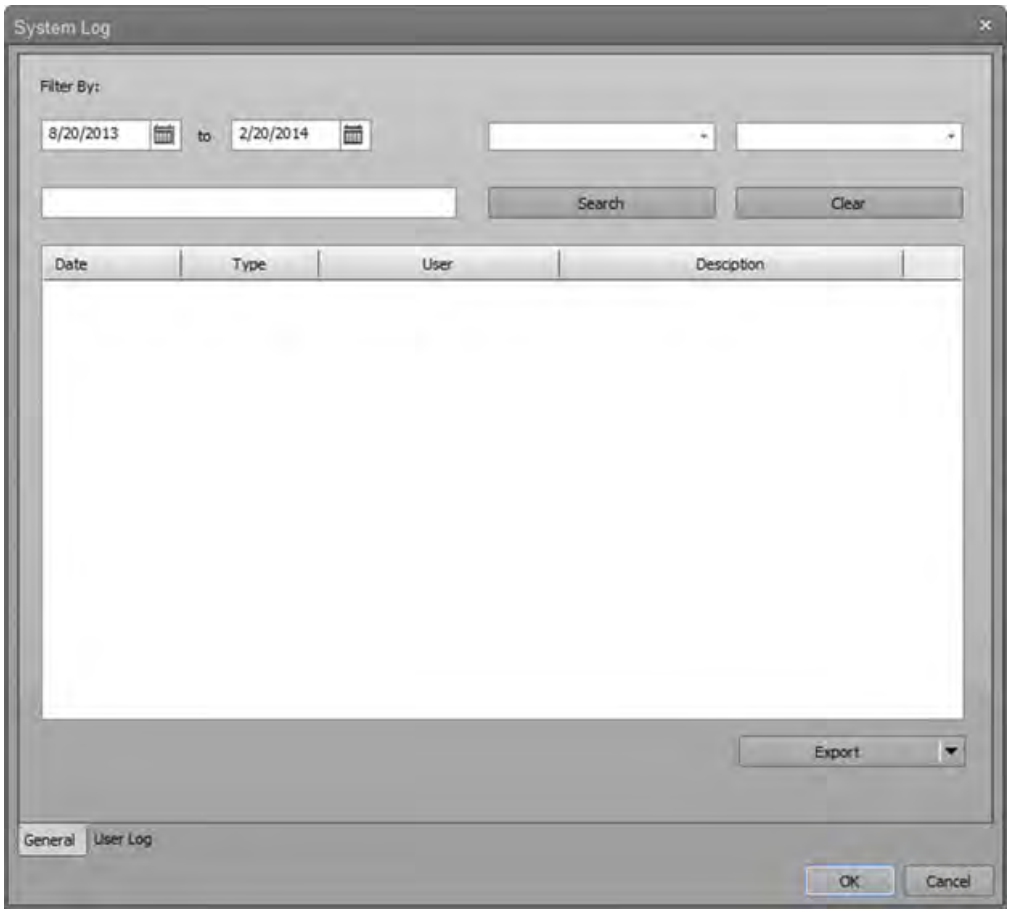
The System Log dialog allows users to view the system transactions based on the user account privileges described in "Account types" (page 24).

Depending on the current user's account type, the System Log dialog displays different information:

- For Service accounts, the User column and User combo box within the General tab, and the User Log tab are not visible.
- For System Administrator and Administrator accounts, the Service column within the General tab is not visible. Both System Administrator and Administrator accounts have access to the User log and can view all user level information.
- For general user accounts, the Service column, User column, and User filter within the General tab, and the User Log tab are not visible.

General tab

General tab of the System Log dialog allows you to view general system transactions based on the user account privileges described in "Account types" (page 24). It contains the filters and the log table.



Filters

Filters are available based on account type. For all filter types, the filter is applied to the list after the filter selection is made. The filters reset when **Clear** is clicked.

- **Date range:** Selects the date range for the system log entries.
- **Type combo box:** Contains the **Error**, **Service**, **Info**, **User**, and **All** options. By default, **All** is selected.
- **User combo box:** Contains all users of the system. Users are as their full name (last, first), and the options include **All Users**. By default, **All Users** is selected.
- **Search box and Search button:** Search box allows you to search through any column to find rows containing the phrase typed within the box.

  Clicking the **Search** button applies the search query to the list. If no text is entered in the search textbox, the Search button is disabled.
- **Clear:** Clears all filters and resets the filters to their defaults including the entered search text.

Log table

The columns within the Log table depend on the user type. At a minimum, the following columns exist: **Date**, **User**, **Type**, **Description**, and **Service**.

The log table contains the following information, depending on the user type.

Action	Type	Description	Service Value
Performance Test	Info	Result	N/A
Baseline	Info	Result	N/A
Functions (wash, rinse, decontaminate, etc.)	Info	Function and function cycle	N/A
Plate run time	Info	<Plate experiment name> HH:MM:SS	N/A
Sample run time	Info	<sample name> HH:MM:SS	N/A
Error messages that need customer intervention (low fluid level, etc.)	Info	Which Fluid low warning triggered	N/A
Error messages that generate an error dialog and error number	Error	Error number	The error description as retrieved from instrument API
Log in	User	Username login	N/A
Log out	User	Username logout	N/A
PZT calibration	Service	PZT calibration performed and outcome	Message: Resonance Power Value or Error message
PZT Acoustic Power Adjustment	Service	PZT acoustic power adjusted	Flow Rate: Value
Laser Delay Adjustment	Service	Laser Delay Adjusted for <color> laser	Value
System Test	Service	Outcome	Specific errors

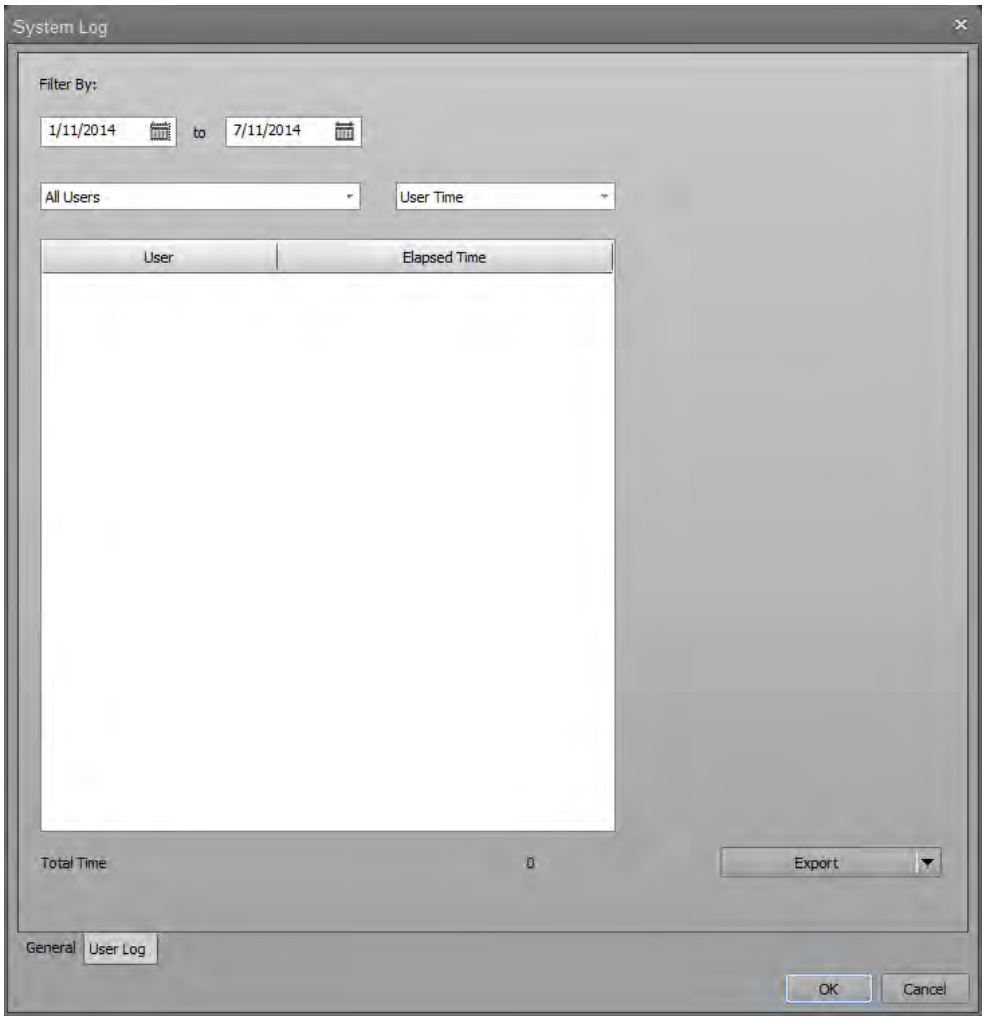
Export/Print split button

The **Export/Print** split button allows you to export or print the system log.

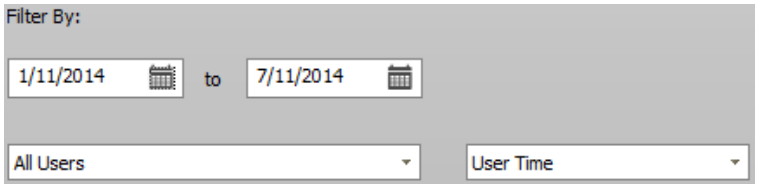
- Clicking the **Export** portion of the button opens the *File Save (Export)* dialog (page 532). The exported file is saved as an ALF file.
- The dropdown arrow of the split button allows you to select **Print**, which opens the *Print* dialog (page 415).

User Log tab

User Log tab of the System Log dialog shows the elapsed login time for any user within a specified date range.



Filters



- **Date range:** Selects the date range for the system log entries.
- **User combo box:** Contains all users of the system. Users are as their full name (last, first), and the options include **All Users**. By default, **All Users** is selected.
- **User Time combo box:** Contains the options to display elapsed **User Time** or **Sample Count** for each user listed in the Log Table. The default option is set to **User Time**.

Log table

The table displays columns according to the selection in the User Time combo box:

- When **User Time** is selected, the columns are **User** and **Elapsed Time**.
- When **Sample Count** is selected, the columns are **User** and **Sample Count**.

SAE signing dialogs

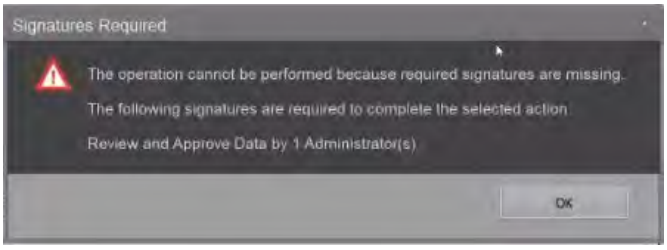
Overview

SAE Signing dialogs include *Request Signature* (page 467), *e-Signature Request* (page 468), *Pending Signatures* (page 469), *Sign Record* (page 470), and *e-Signature Record Report* (page 471) dialogs, which are displayed when the corresponding SAE Signing button is clicked on the SAE Ribbon tab (page 79).

You can configure the e-Signature settings (actions requiring signatures, number of signatures required for each action, reasons available for e-Signature, data to be signed) in the SAE Administrator Console e-Signature tab (page 603).

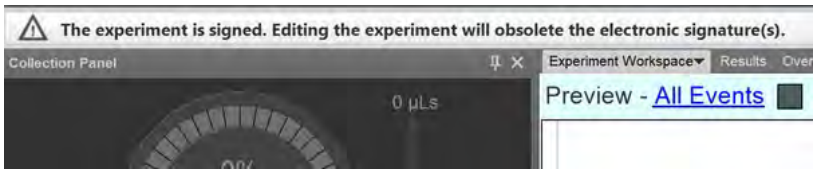
e-Signature workflow

- Actions that require signatures to be completed cannot be performed unless the required signatures are provided.
- *Request Signature* dialog (page 467) displays the Report of the active experiment and allows you to request signatures for the selected action. Until it is signed, the Report displays the watermark that states "Unsigned".
- If there are pending e-Signature requests, the *e-Signature Request* dialog (page 468) is displayed upon signing into the software. The e-Signature Request dialog contains a link to the Sign Records dialog.
- If you attempt to perform an action that has unmet signature requirements, the *e-Signatures Required* dialog is displayed. The dialog shows the number outstanding roles and meanings that need to be signed for before you can perform the action.



- The *Sign Records* dialog (page 469) allows you to view and sign pending signature requests. After the report has been signed, the Experiment Report removes the watermark and changes the report status to "Current".

  When all signatures are current, the notification bar on the Attune™ desktop states "The experiment is signed. Editing the experiment will obsolete the electronic signature(s)."



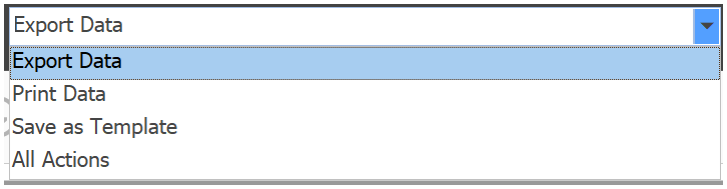
- Signatures represent the state of the experiment at the time of signing. If any modifications are made to the experiment after signing, the signatures become obsolete and the Experiment Report displays the watermark that states "Obsolete". In such cases, actions that require signatures cannot be performed unless new signatures are provided.
- *e-Signature Record Report History* dialog (page 471) allows you to view and print signed records.

Request Signature dialog

Request Signature dialog displays the Report of the active experiment and allows you to request signatures for the active experiment.

To view this dialog, click the **Request Signatures** button on the SAE Ribbon tab (page 79).

1. Select the action requiring the e-Signature from the dropdown menu.



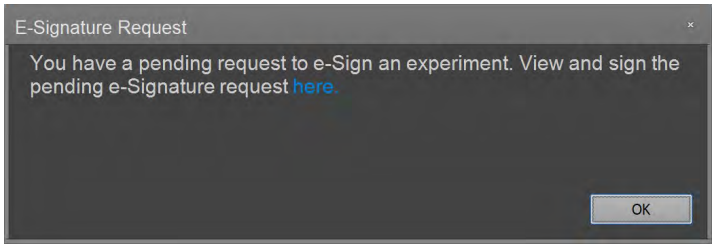
Note: Only actions that have been configured to require signatures are displayed in the dropdown menu (see "Select the actions that require e-Signature", page 605).

2. *(Optional)* Click **Print** to print the report for the experiment.
3. Click **Request Signatures** to send an e-Signature request.

e-Signature Request dialog

e-Signature Request dialog is displayed upon signing into the software if there is a pending signature request required of the user signing in.

The dialog is displayed each time a user signs in as long as there is a pending signature request.

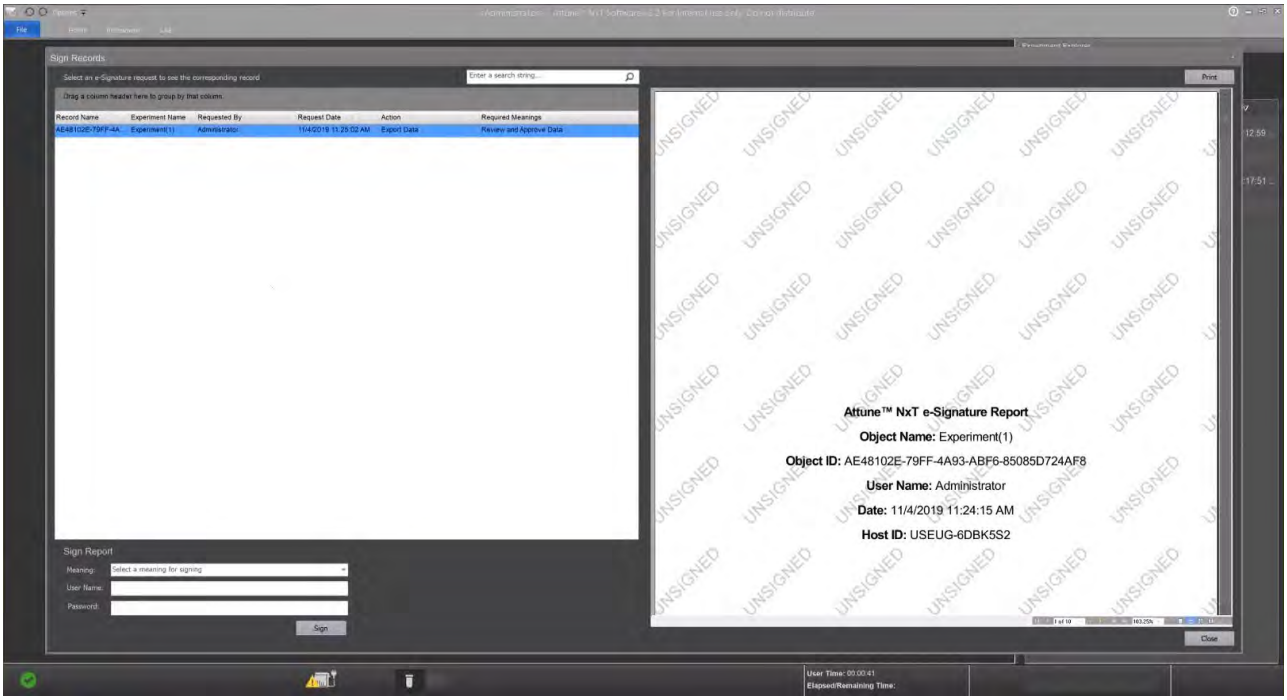


- Click the **here** hyperlink to close the dialog and open the Sign Records dialog (page 469).
- Click **OK** to close the dialog without taking any further action.

Sign Records dialog

Sign Records dialog allows you to view and sign pending signature requests.

To view this dialog, click the **View Pending Signatures** button on the SAE Ribbon tab (page 79).



- The pending signature request is shown in the *Pending Signatures* panel on the left, which lists the Record name, Experiment name, the requester, the request date, requested action, and required meanings for the requested signature.

Record Name	Experiment Name	Requested By	Request Date	Action	Required Meanings
CBE14949-76BA-4...	Experiment(1)	admin	3/20/2019 5:28:32 PM	Export Data	Review and Approved Data | Review and Approv...

1. Select the record you wish to sign for from the Pending Signatures panel.

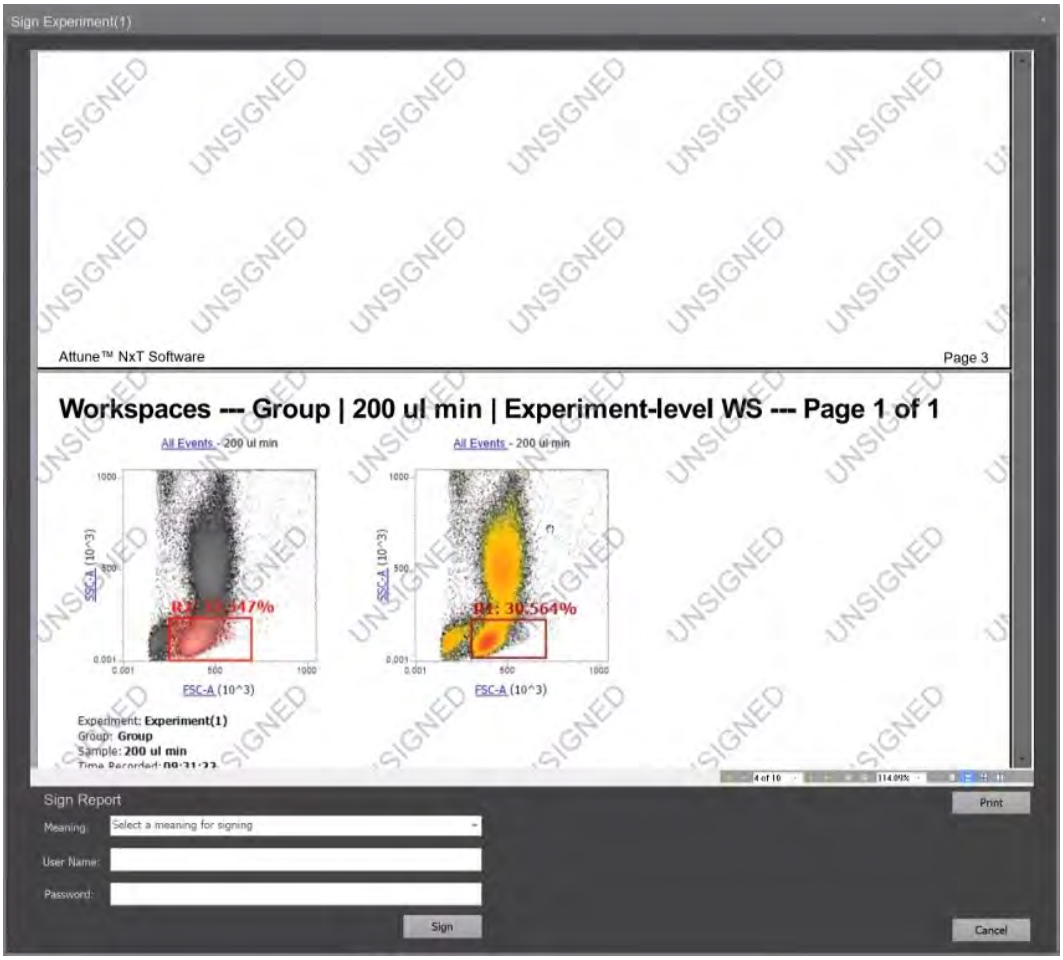
   The *Experiment Report* for the selected signature request is displayed on the right panel.
2. *(Optional)* Click **Print** to print the report for the experiment.
3. Select the **Meaning** for the signature request in the Sign Report panel (which is listed in the Required Meanings column of the Pending Signatures panel), then enter your **Username** and **Password**.

4. Click **Sign** to provide your e-Signature for the selected signature request.

Sign Experiment dialog

Sign Experiment dialog displays the Report of the experiment for which the signature is requested and allows you to sign the experiment.

To view this dialog, click the **Sign Record** button on the SAE Ribbon tab (page 79).



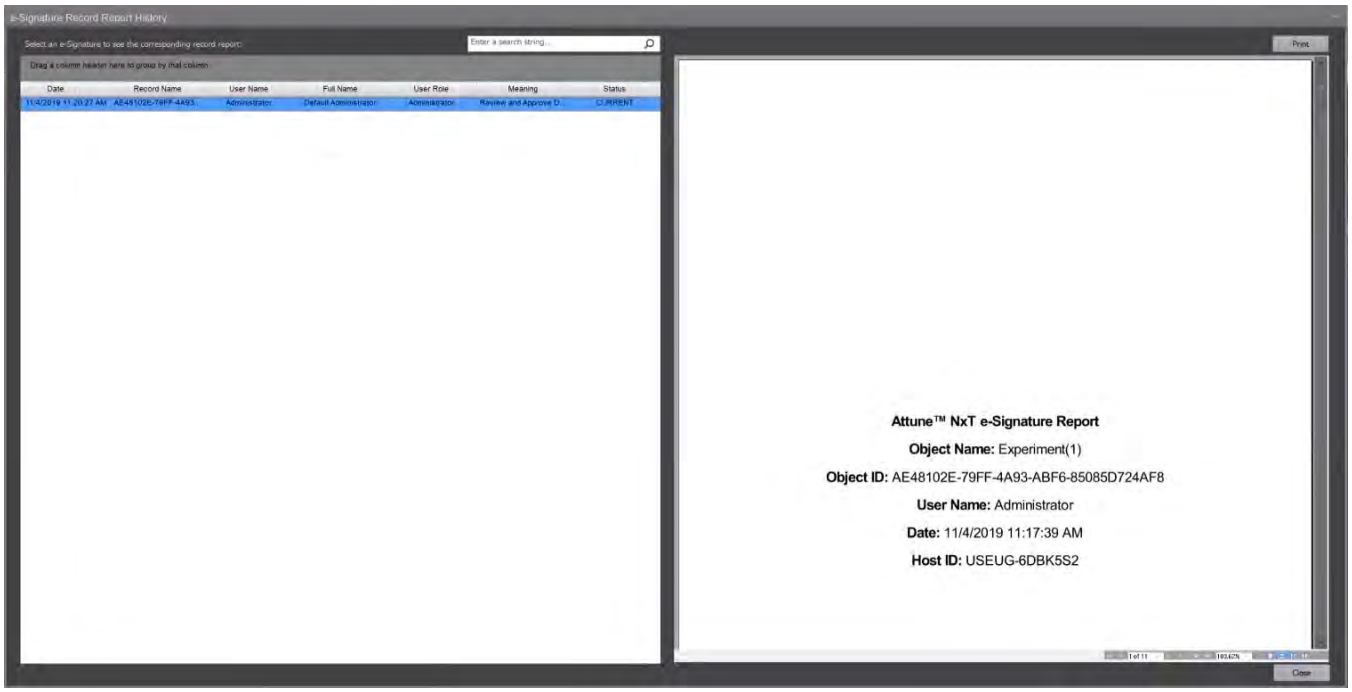
1. To sign the report for the experiment, select the **Meaning** for the signature, then enter your **Username** and **Password**.

2. *(Optional)* Click **Print** to print the report for the experiment.
3. Click **Sign** to provide your e-Signature for the active experiment report.

e-Signature Record Report History dialog

e-Signature Record Report History dialog allows you to view and print signed records.

To view this dialog, click the **View Signed Records** button on the SAE Ribbon tab (page 79).

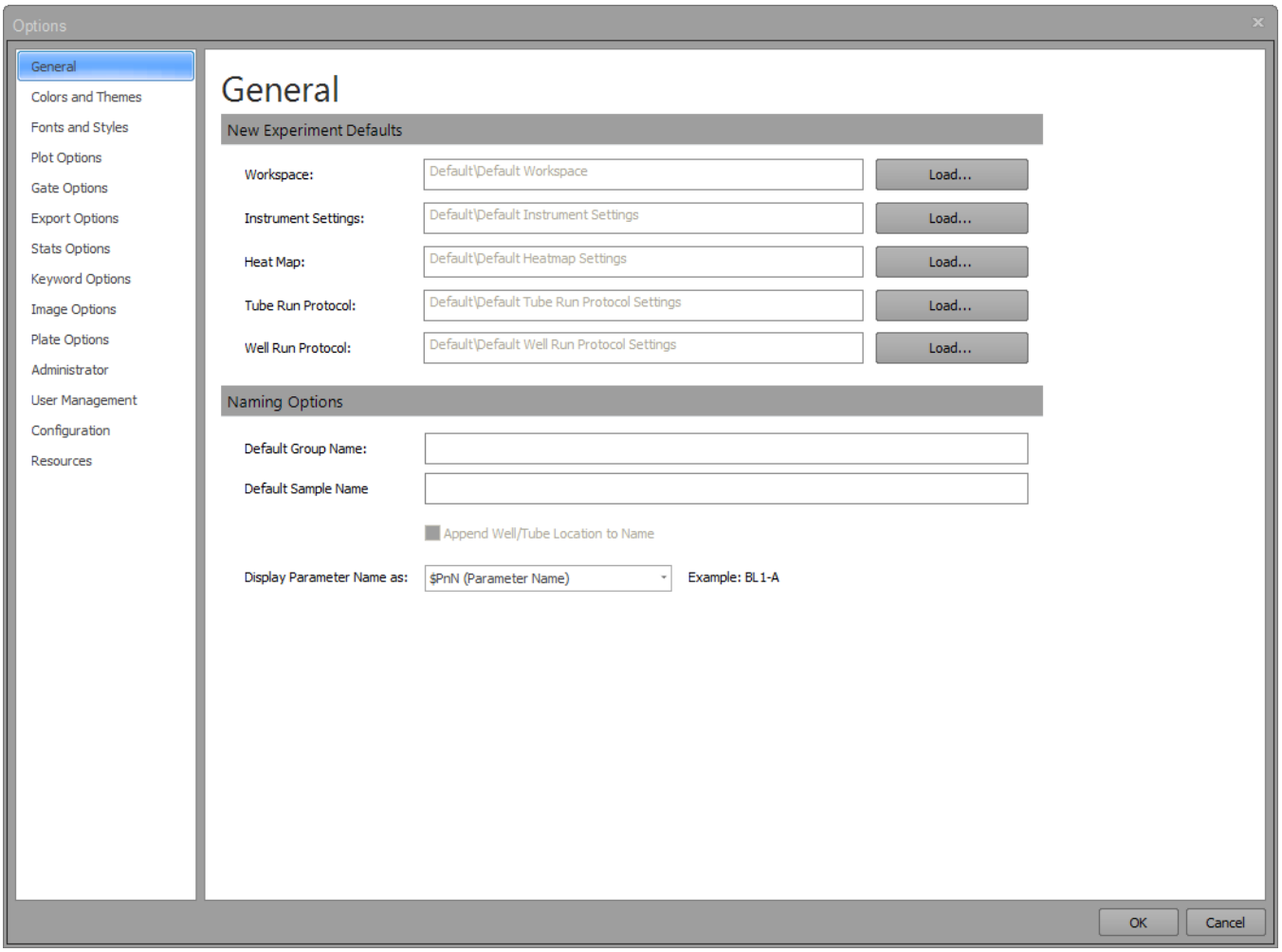


- The e-Signatures that are on record are shown in the *Signatures* panel on the left, which contains the Date, Record name, Username, Full name, User role, Meaning, and Status columns.
- To search for an e-Signature record, enter a search string into the search field, then click **Enter**.
- To sort e-Signature records, drag the column header to the area where it states "Drag a column header here to group by that column".
- To print an e-Signature report, select the report from the Signatures panel on the left, then click **Print** to print the report.

24. Options dialogs

Overview

The *Options dialogs* allow you to customize the Attune™ Cytometric Software by configuring your personal settings and changing the default options.



- To launch the Options dialog, click the **Options** button on the *Quick Access toolbar* (page 32). This option is enabled only when the instrument is not acquiring.

- The Options dialog is divided into tabs, which are listed on the left side of the window. Select a tab to open the tab content on the right side of the window.
- Some options are user-specific, while others are application-specific (i.e., global to all users) and customizable only by an authorized user.

  For example, the Plate Options tab (page 499) is available only when a CytKick™ Max Autosampler is connected to the Attune™ instrument. Similarly, the SAE Configuration controls (page 502) in the Administrator Options tab are only visible if the SAE-specific DESkey device is present.

- The Options dialog contains the following tabs.
  - *General options* (page 474)
  - *Colors and Themes* (page 479)
  - *Fonts and Styles* (page 480)
  - *Plot options* (page 483)
  - *Gate options* (page 486)
  - *Export options* (page 490)
  - *Stats options* (page 492)
  - *Keyword options* (page 495)
  - *Image options* (page 498)
  - *Plate options* (page 499)
  - *Administrator options* (page 500)
  - *User Management options* (page 508)
  - *Configuration* (page 514)
  - *Resources* (page 520)
- By default, the General tab is displayed upon opening the Options dialog.
- When you make changes in the options menu, click **OK** to save the changes and close the Options dialog.

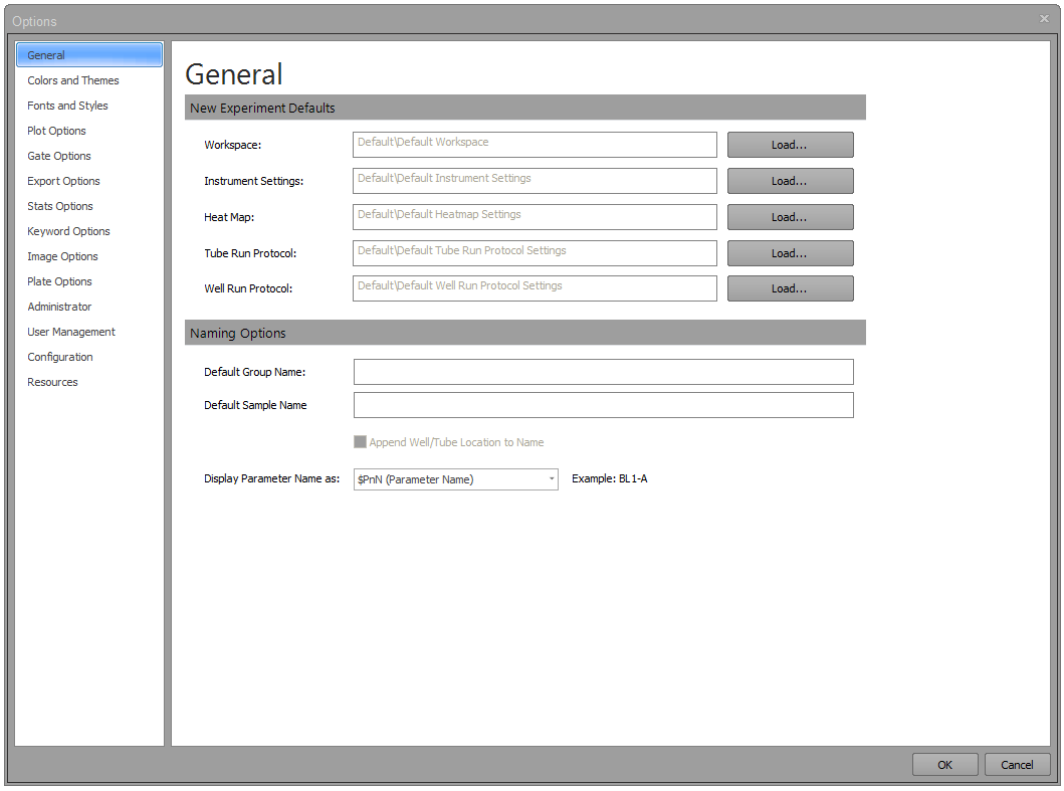
  Click **Cancel** or the **X** button in the top right corner to close the dialog and revert to the previous selections without applying the changes.
- When you switch between tabs or click **OK**, all fields are validated.

  Any invalid fields within the currently selected tab view are indicated on a field-by-field basis. You cannot switch views or complete the OK action until all errors are corrected.

General options

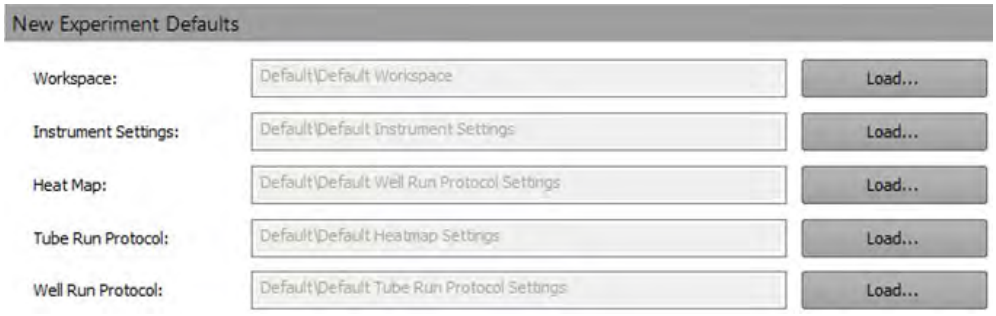
Overview

General options tab is selected by default when the Options dialog is first opened. It allows you to change the default Naming Options for Groups and Samples (page 476). The other options are not available.



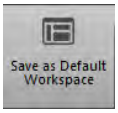
New Experiment Defaults

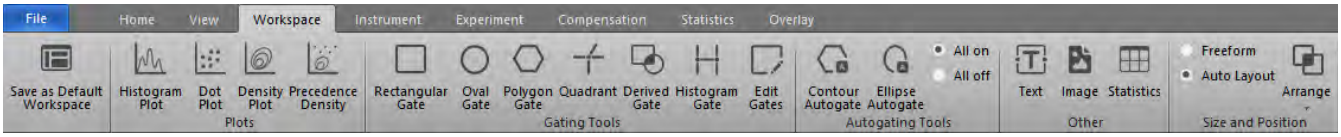
New Experiment Defaults indicates the location of default files for Experiment Workspace, Instrument Settings, Tube Run Protocol, Well Run Protocol, and Heat Map settings. No changes can be made to the defaults using this menu.



- **Workspace** indicates the default Experiment-level Workspace for each new Experiment. The **Load** button is inactive and cannot be clicked.

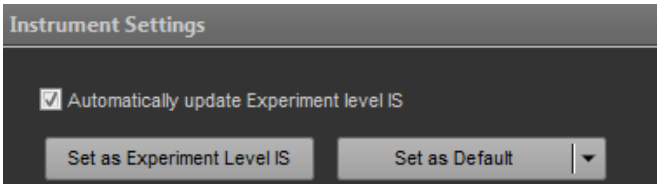
  To change the default Workspace, save a Workspace as default using the **Save as Default Workspace** button in the Workspace tab of the Ribbon bar. Default Workspaces are user-account specific.





- **Instrument Settings** indicates the default Experiment-level Instrument Settings (IS) for each new Experiment. The **Load** button is inactive and cannot be clicked.

  To change the default Experiment-level instrument settings, click **Set as Default** in the Instrument Settings panel (page 250).

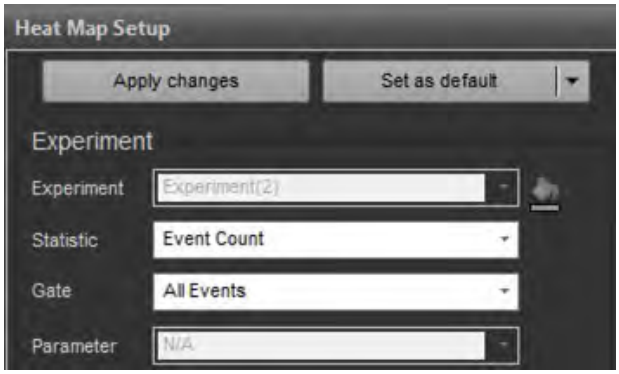


  If the instrument configuration is changed, the default is reset to the default configuration for the user.

  Note: Default Instrument Settings (IS) are user-account and instrument configuration specific. Changes made to the default IS of one user are not applied to the default IS of other users, except when the changes were made to system-level Instrument Settings by the Administrator.

- **Heat Map** indicates the default Heat Map Settings used for each new Experiment. The **Load** button is inactive and cannot be clicked.

  To change the default Heat Map, click **Set as default** in the Heat Map Setup panel (page 267).

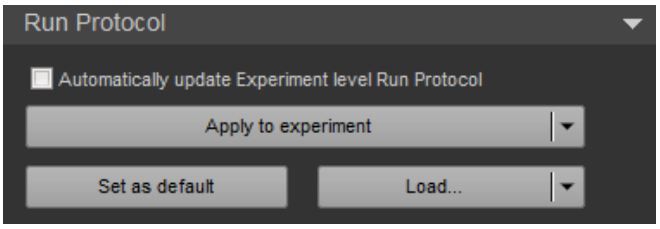


  If changes are made to the default Heat Map settings, the default setting is changed for only that user account.

  If an Experiment is created using a default Workspace that does not contain gates used to define the Heat Map settings, the Heat Map settings revert to “All Events” for statistics.

- **Tube Run Protocol** indicates the default Run Protocol to use for all new Tube Samples in new Plate or Tube Experiments. The **Load** button is inactive and cannot be clicked.

  To change the default Run Protocol, click **Set as default** in the Run Protocol section of the Collection Panel (page 239).



- **Well Run Protocol** indicates the default Run Protocol to use for all new Well Samples (including Manual Wells) in new Plate Experiments. The **Load** button is inactive and cannot be clicked.

  To change the default Run Protocol for a Plate Experiment, click Set as default in the Run Protocol section of the Collection Panel (page 239).

Naming Options

Naming Options allows you to define the default Group and Sample names. Changes made to the *Default Group Name* and *Default Sample Name* options are only applied to newly created Groups or Samples.

Default Group Name

- By default, the Default Group Name text field is blank, which sets the default Group name to *Group* when creating new Groups.
- You can enter a new name in the **Default Group Name** text field, which then becomes the default Group name for all new Groups.
- Duplicate names are not permitted. If the entered name already exists, a numerical suffix in parentheses is automatically added to the Group name; for example, **Group(2)**.
- You can enter up to 50 alpha-numeric characters in the text field.
- Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning dialog indicates the error condition, and the invalid characters do not appear in the text field.

  The following characters are not allowed: \ / : * < > | ?.

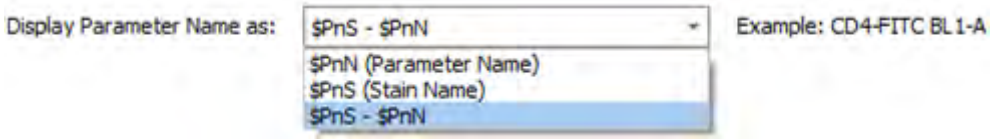
Default Sample Name

- By default, the Default Sample Name text field is blank, which sets the default Sample name to *Sample* when creating new Tube samples and to the Well location (e.g., A1) when creating new Well samples.
- You can enter a new name in the **Default Sample Name** text field, which then becomes the default Sample name for all new Samples.
- Duplicate names are not permitted. If the entered name already exists, a numerical suffix in parentheses is automatically added to the Sample name; for example, **Sample(2)**.
- You can enter up to 50 alpha-numeric characters in the text field.
- Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning dialog indicates the error condition, and the invalid characters do not appear in the text field.

  The following characters are not allowed: \ / : * < > | ?.

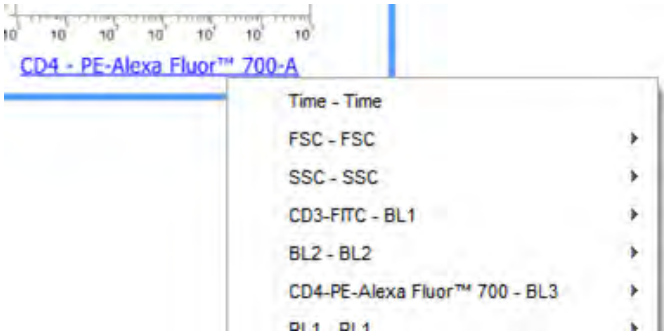
Display Parameter Name As

- By default, the "Display Parameter Name as" is set to $PnN (Parameter Name).
- Clicking on the **Display Parameter Name As** control opens a dropdown list that includes **$PnN** (Parameter Name), **$PnS** (Stain Name), and **$PnS–$PnN** options.

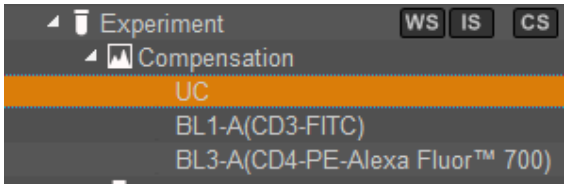


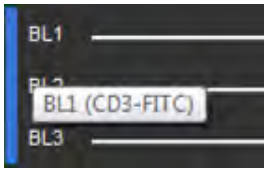
Option	Example label
$PnN (Parameter Name)	"Example: BL1-A"
$PnS (Stain Name)	"Example: FITC"
$PnS–$PnN	"Example: CD4-FITC BL1-A"

- Display Parameter As setting applies to all Experiments and affects the parameter name displayed in the Parameter dropdown menu for all plots, and the name displayed when the Axis Label checkboxes are all unchecked and no custom label has been entered.



- Display Parameter As setting also applies to the Tube or Well names for Compensation controls and to the name displayed in the tool tip for a parameter.

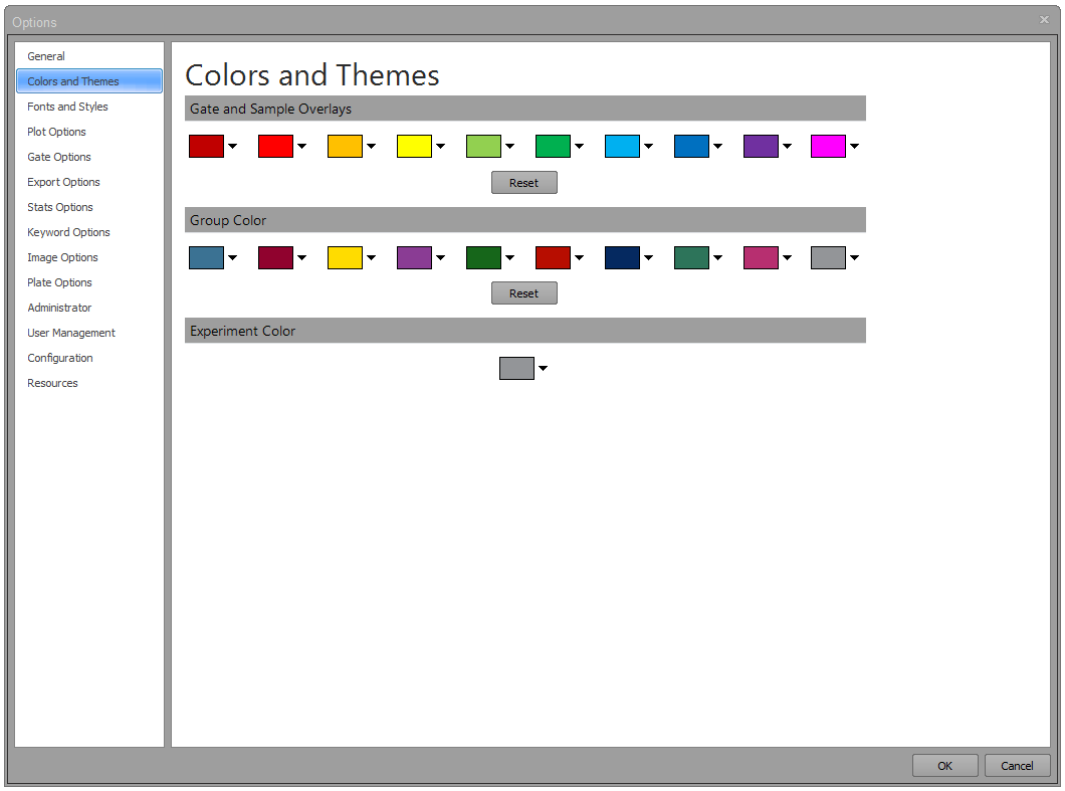




Colors and Themes

Overview

Colors and Themes allows you to define the default colors for gates, Overlays, and Experiment and group labels.



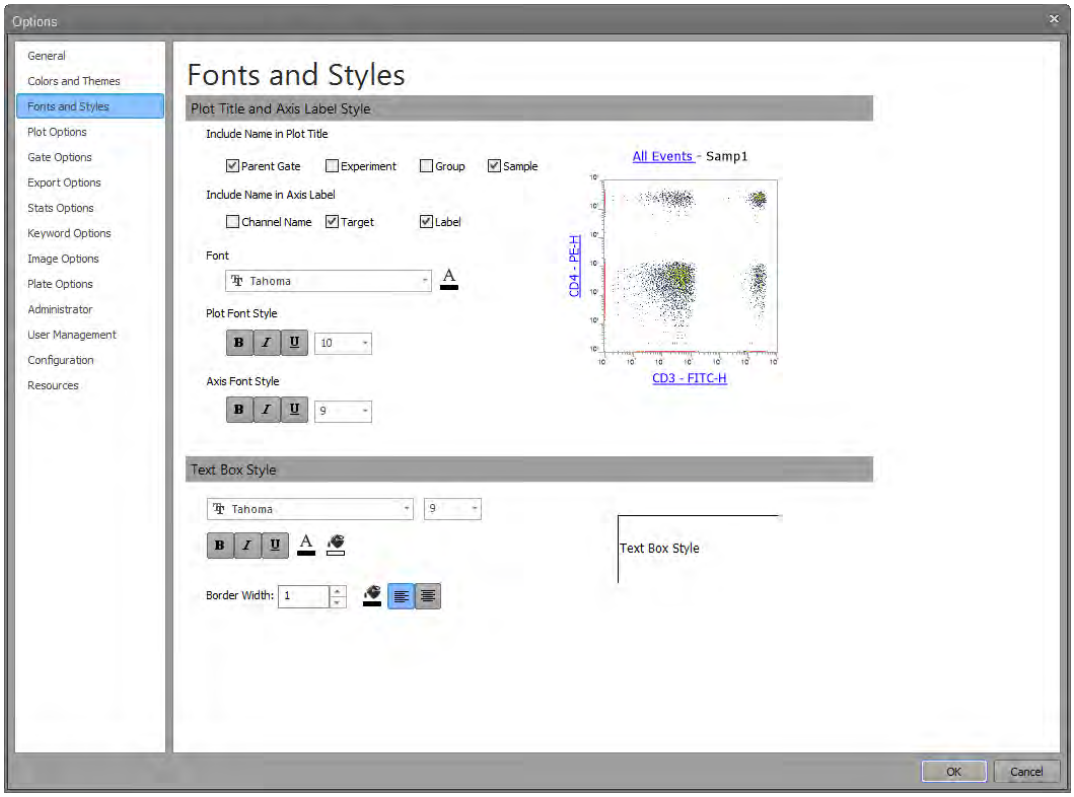
Colors and Themes options

- The Colors and Themes tabs contains three groups of controls titled “Gates and Sample Overlays”, “Group Color”, and “Experiment Color”.
- Gates and Overlays share the defaults, but the defaults for Experiment and Group labels are controlled separately.
- Each set of defaults contains 10 standard colors. You can modify each color by clicking on the **color**, which opens the standard color picker dialog.

  When a color is selected from the color picker dialog, the color displayed in the color dropdown is updated and the color picker dialog is closed.
- The Experiment color section contains a single color dropdown with the same behavior as described above.
- Changes made to the default colors only apply to newly created Gates, Groups, and Experiments after the change is accepted.
- The **Reset** button restores the colors to the original default application colors. This option is not available on the Experiment Color section.

Fonts and Styles

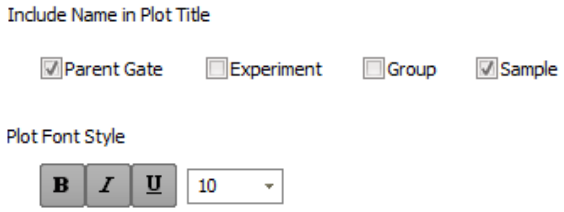
Overview *Fonts and Styles* allows you to define default display options for the text in plot labels and text boxes. The tab is divided into four sections: *Plot title*, *Axis label*, *Tick mark label*, and *Text box*.



- The current settings are displayed in the Previews area for plot and text objects on the right side of the dialog.
- Changes made in this section are applied only to newly created plots except the axis tick mark label. When **OK** is clicked, the axis tick label style is applied immediately and affects all past, present, and future instances.

Plot title style

Plot title style section allows you to modify the default settings for the naming format, font, and style of the plot title.



- Selecting the relevant **Include Name in Title** checkboxes allows you to include the Parent gate name, Experiment name, Group name, and/or Sample name in the default plot title. The software appends your selections to the default plot title, separated by hyphens.
- In the **font size** field, you can select a font size from the dropdown menu, or enter a number from **6** to **72**. The default size in 10 pt.
- You can select the **bold**, **italics**, and/or **underline** options to format the font. Selecting one choice deselects the other.

Axis label style

Axis label style section allows you to modify the default settings for the naming format, font, and style of plot axis labels.

- Selecting the relevant **Include Name in Label** checkboxes allows you to include the Channel name, Target name, and/or Label name in the default axis labels. The software appends your selections to the default axis labels, separated by hyphens. By default, the target name and label name are selected.
- In the **font size** field, you can select a font size from the dropdown menu, or directly enter a number. The default size in 9 pt.
- You can select the **bold**, **italics**, and/or **underline** options to format the font. Selecting one choice deselects the other.

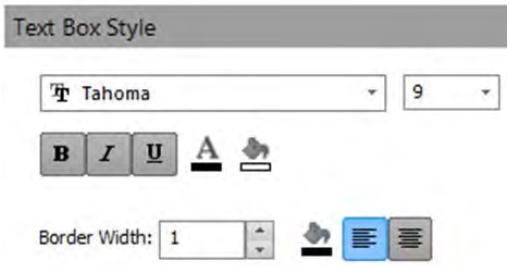
Font

Font style section allows you to set the font and font color for the plot title and axes labels.

- You can select a **font type** from the font dropdown menu. The dropdown menu includes all fonts installed on the system. The default font is Tahoma.
- Using the **font color** picker, you can define the default font color of the plot title. The software uses the standard color picker dialog. The default color is black.

Text box style

Text box style section allows you to modify the default settings for text boxes.

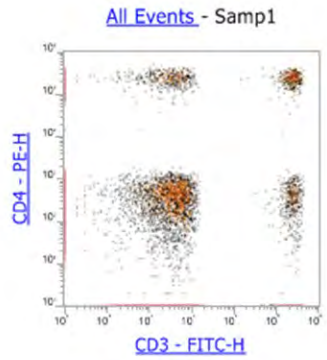


- You can select a **font type** from the font dropdown menu. The dropdown menu includes all fonts installed on the system. The default font is Tahoma.
- In the **font size** field, you can select a font size from the dropdown menu, or directly enter a number. The default size in 9 pt.
- You can select the **bold**, **italics**, and/or **underline** options to format the font. Selecting one choice deselects the other.
- Using the **font color** picker, you can define the default font color of the plot title. The software uses the standard color picker dialog. The default color is black.
- You can select to **left-align** or **center** the text. By default, text is left-aligned.
- You can select a **border width** for the text box, or enter an integer from **1** to **5**.
- You can select a **border color** and **text box fill color** using the appropriate color picker control. By default, border color is black and the text box is not filled.

Previews

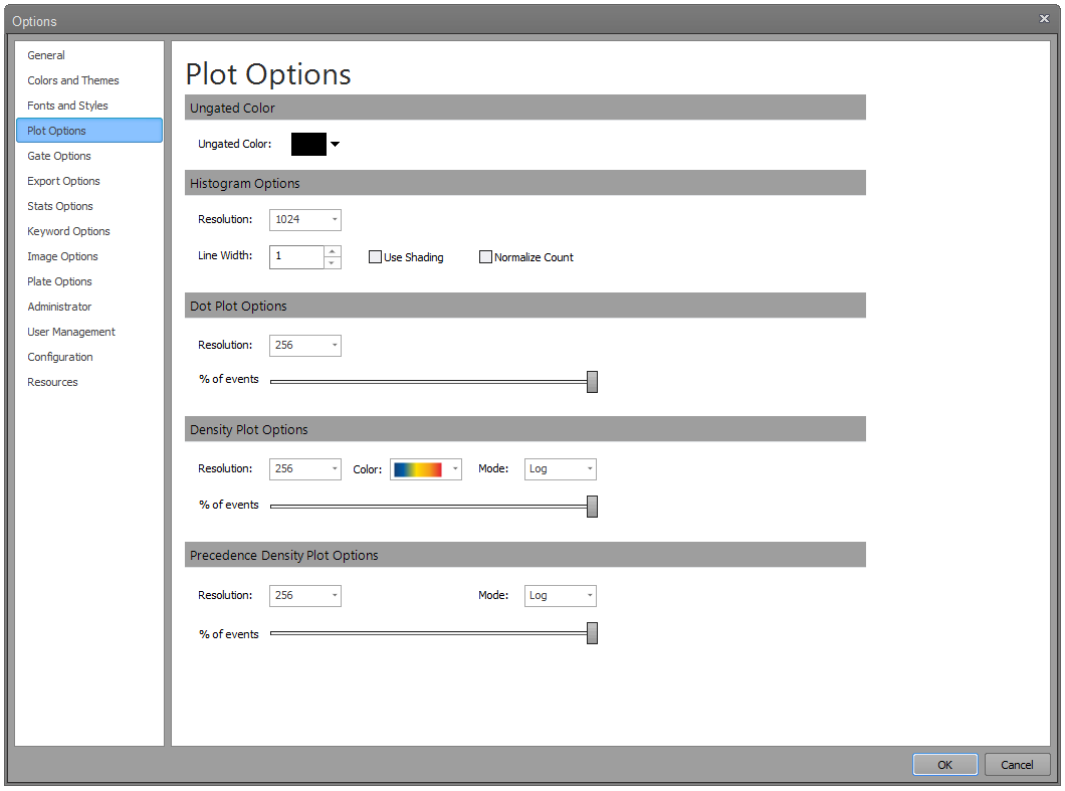
Previews section displays an example plot and an example text box reflecting the current selections made in the Fonts and Styles tab.

The previews are updated as the selections are changed.



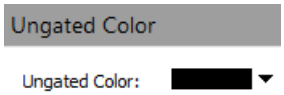
Plot options

Overview *Plot Options* tab is used to define display options for ungated plots (*Ungated color*) and for each type of plot: Histogram plot, Dot plot, Density plot, and Precedence Density plot. Changes made in the Histogram plot, Dot plot, Density plot, and Precedence Density plot sections are only applied to newly created plots.



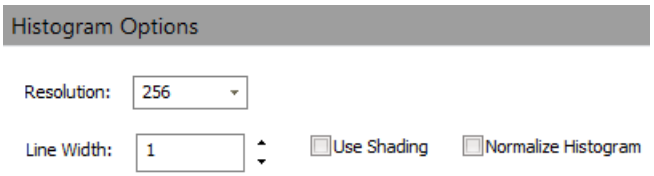
Ungated color

- By default, Ungated color for all plot types is black.



- You can modify the default color for ungated plots by clicking on the **Ungated color** dropdown button, which opens the standard color picker dialog.
- When a color is selected from the color picker dialog, the color displayed in the Ungated color dropdown is updated and the color picker dialog is closed.
- Changes made to Ungated color are applied immediately upon clicking **OK** and affect all past, present and future instances.

Histogram

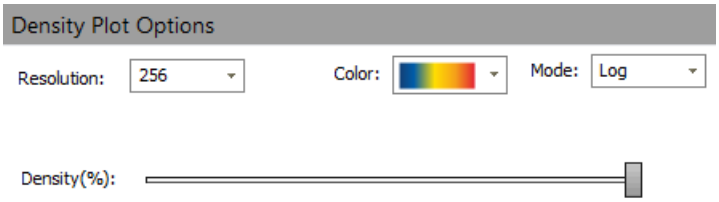


- The default resolution for Histograms is 1024. You can modify the resolution using the **Resolution** dropdown menu, which offers the following choices: 64, 128, 256, 512, and 1024.
- The default line thickness for Histograms is set at 1 pixel. You can modify the line thickness using the **Line Width** control. The minimum line thickness is 1 pixel and the maximum is 5 pixels.
- Selecting the **Use Shading** checkbox shades the area under the curve in the Histogram using the same color as the line color at 64% opacity. By default, Use Shading is deselected.
- Selecting the **Normalize Histogram** checkbox normalizes all new Histograms to the maximum peak. By default, Normalize Histogram is deselected.

Dot plot

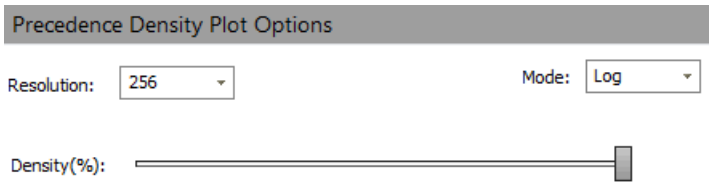
- The default resolution for Dot plots is 256 × 256. You can modify the resolution using the **Resolution** dropdown menu, which offers the following choices: 64 × 64, 128 × 128, 256 × 256, 512 × 512, and 1024 × 1024.
- You can modify the percentage of events displayed in a Dot plot from 0% to 100% (left to right) by using **% of Events** slider bar. The sample data is taken from the entire data file. By default, Dot plots display 100% of events.

Density plot



- The default resolution for Density plots is 256 × 256.
- You can modify the resolution by using the **Resolution** dropdown menu, which offers the following choices: 64 × 64, 128 × 128, 256 × 256, 512 × 512, and 1024 × 1024.
- Density plots do not use the Ungated color setting. The default color scheme for a Density plot is set to the first gradient available in the **Color** dropdown menu. You can modify the default color setting by selecting one of the gradient choices within the **Color** dropdown list.
- You can change the binning mode for Density plots by using the **Mode** dropdown menu, which offers a choice between **Log** and **Linear** modes. By default, the binning mode is set to Log.
- You can modify the percentage of events displayed in a Density plot from 0% to 100% (left to right) by using **% of Events** slider bar. The sample data is taken from the entire data file. By default, Density plots display 100% of events.

Precedence Density plot



- The default resolution for Precedence Density plots is 256 × 256.
- You can modify the resolution by using the **Resolution** dropdown menu, which offers the following choices: 64 × 64, 128 × 128, 256 × 256, 512 × 512, and 1024 × 1024.
- You can change the binning mode for Precedence Density plots by using the **Mode** dropdown menu, which offers a choice between **Log** and **Linear** modes. By default, the binning mode is set to Log.
- You can modify the percentage of events displayed in a Precedence Density plot from 0% to 100% (left to right) by using **% of Events** slider bar. The sample data is taken from the entire data file. By default, Precedence Density plots display 100% of events.

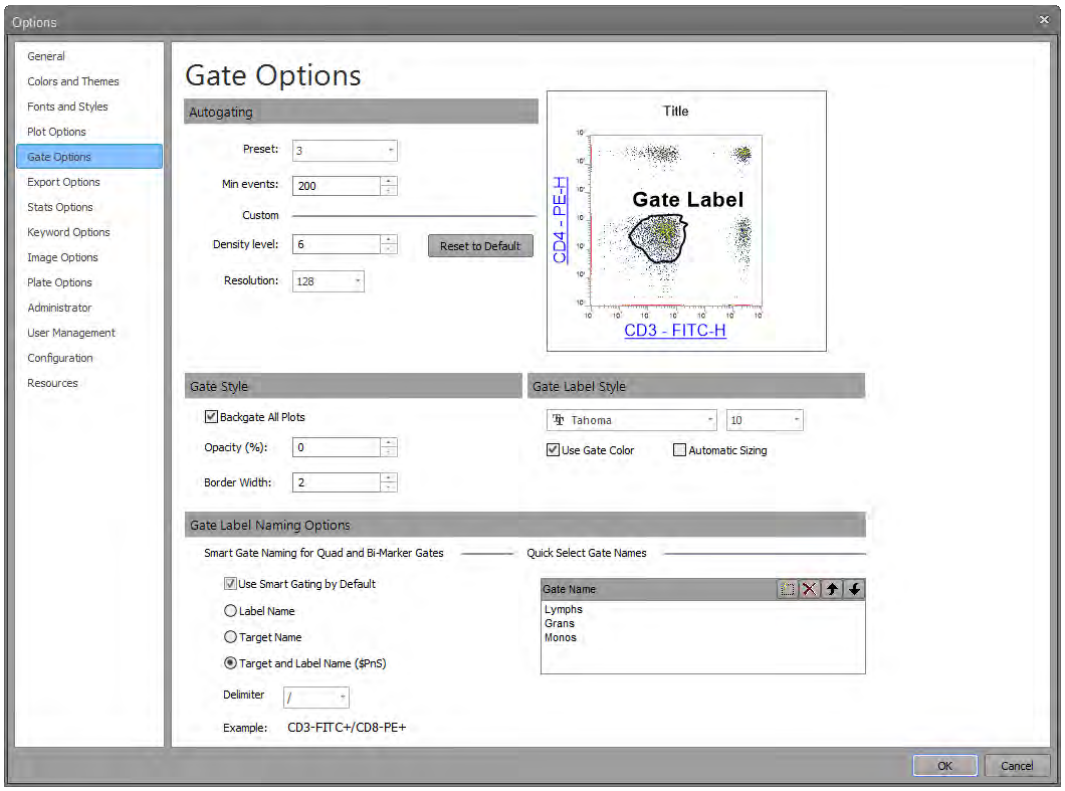
Note: The **Linear mode** bins data by assigning a color index for each density pixel linearly such that each increment is determined by dividing the range (Z_{max}–Z_{min}) by the number of color steps.

The **Log mode** bins data by assigning a color index for each density pixel logarithmically such that each increment is determined by dividing the logarithmic range (log Z_{max}–log Z_{min}) by the number of color steps. The index is then determined as int((log Z_{val} – log Z_{min})/increment).

Gate options

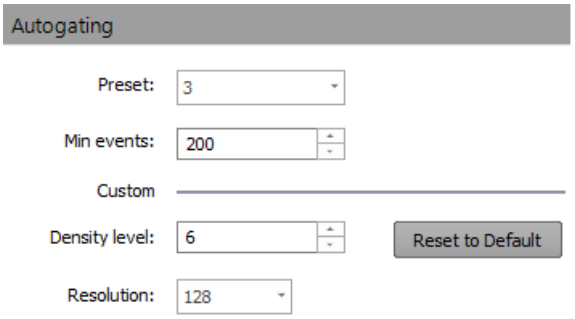
Overview

Gate Options dialog allows you to define the default settings for autogating, gate style, and gate label style, and for *Smart Gate Naming* and *Quick Select Gate Names* options.



Autogating

Autogating allows you to set default autogating preferences, which include default preset, the number of events that must be contained within a gate, and custom settings for setting the density level and plot resolution.



The Autogating section contains the Preset, Min events, Density level, and Resolution controls, and the Reset to Default button (page 487).

Note: The autogating preferences that are defined in Gate Options dialog are only applied when creating new autogates. Changes to these settings do not affect autogate settings of existing gates.

To change the settings of existing autogates, use the Customize panel ▶ Gate options (page 293).

Preset

Preset dropdown allows you to set the default **Preset** for new autogates. A Preset consists of Density Level and Resolution settings.

- The Preset dropdown has the following options:
  - **1** (Density level: 30, Resolution: 128)
  - **2** (Density level: 10, Resolution: 128)
  - **3** (Density level: 6, Resolution: 128)
  - **4** (Density level: 1, Resolution: 128)
  - **Custom** (By default, Density level: 5 and Resolution: 64, but you can change the custom settings using the **Density level** and **Resolution** controls)
- When a Preset is selected, the Density level and Resolution controls display the values that correspond to the Preset choice.
- By default, Preset is set at 3.

Min events

Sets the default **Min events** value for new autogates, which is the number of events that must be contained within a gate for the population to be autogated.

- If the Min events threshold is not reached and the gate is ON, the autogate remains in the Autogate ON – [NO FIT] mode.
- By default, Min events is set to 200.
- The range of values for Min events is 1 to 9,999,999.
- If you enter a Min events value that is outside the numeric limits, the value defaults to the maximum allowed number, if the value exceeds the upper limit, or to the minimum allowed value, if the entered value is less than the lower limit.

Density level

Sets the default **Density level** for the **Custom Preset** autogate option.

- The Density level field is populated the value that corresponds to the selected Preset option (see Preset, above).
- The Density value for the Custom Preset listed above is the default value from a clean installation or for a new user account. The last used Custom Pre-set setting persists for the actively logged in user.
- The range for custom Density level is 1 to 100.
- When a density level is selected that is different than the preset selected in the Preset dropdown, the Preset dropdown reverts to Custom.

Resolution

Sets the default **Resolution** for the **Custom Preset** autogate option.

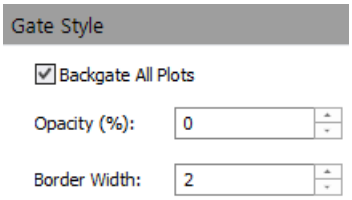
- The Resolution field is populated with the value that corresponds to the selected Preset option (see Preset, above).
- The Resolution value for the Custom Preset listed above is the default value from a clean installation or for a new user account. The last used Custom Pre-set setting persists for the actively logged in user.
- The Resolution dropdown has the following options: **64**, **128**, **256**, **512**, **1024**.
- When a density level is selected that is different than the preset selected in the Preset dropdown, the Preset dropdown reverts to Custom.

Reset to Default

Resets the Preset to 3 and Min events to 200.

Gate Style

Gate Style options include settings for gate opacity, gate border width, and an option to automatically add backgates on all plots.



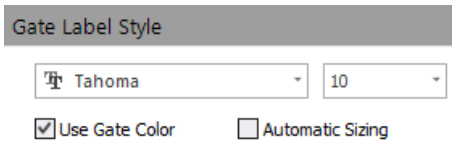
- By default, gate opacity is set at 0% and gate border width is set at 2 pixels.
- Using the **Opacity** control, you can set the gate opacity between 0% and 75%.

  Alternatively, you can directly type in the desired value in the text field.
- Using the **Border Width** control, you can set the gate border width between 1 and 5 pixels.

  Alternatively, you can directly type in an integer from **1** to **5** in the text field.
- Clicking **OK** after changing the gate opacity and gate border width options defines the new default settings for these options.
- Only newly created gates use the new default settings.

Gate Label Style

Gate Label Style options include controls for gate label font and font size.



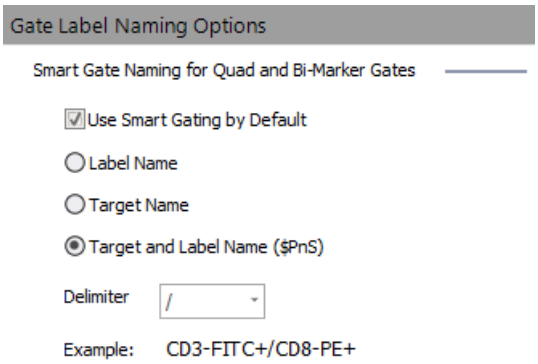
- **Font** dropdown menu allows you to select a font type. The dropdown menu includes all fonts installed on the system. The default font is Tahoma.
- You can select a font size from the **Font size** dropdown menu or enter a number from **6** to **72**.
- **Use Gate Color** checkbox sets the gate label color to match the gate color. By default, the checkbox is selected.

  When the checkbox deselected, the gate label color is black.
- **Automatic Sizing** checkbox enables the software to adjust the text size automatically to fit within the plot. By default, the checkbox is not selected.
- Any changes made within this section are applied immediately upon closing the dialog and affect all past, present and future instances.

Gate Label Naming

Gate naming options section includes settings for *Smart Gate Naming for Quad and Bi-Marker Gates* and *Quick Select Gate Names*.

Gate Label Naming Options



- **Use Smart Gate Naming** checkbox enables the software to automatically generate names for Quadrant gates. By default, the checkbox is selected.
- You can choose the format for smart gate naming by clicking the appropriate radio control button. Available options are **Target Name**, **Label Name**, and **Target and Label Name ($PnS)**.

  By default, smart gate naming format is set to **Target and Label name ($PnS)**.
- For Quadrant gates, the four gate names are generated (one for each quadrant).
- The **Delimiter** dropdown allows you to select a character to separate the names created for each quadrant of a Quadrant gate.
- An example of the concatenation of names and the delimiter is shown based on the selections made.
- Any changes made in this section are only applied to newly created gates.

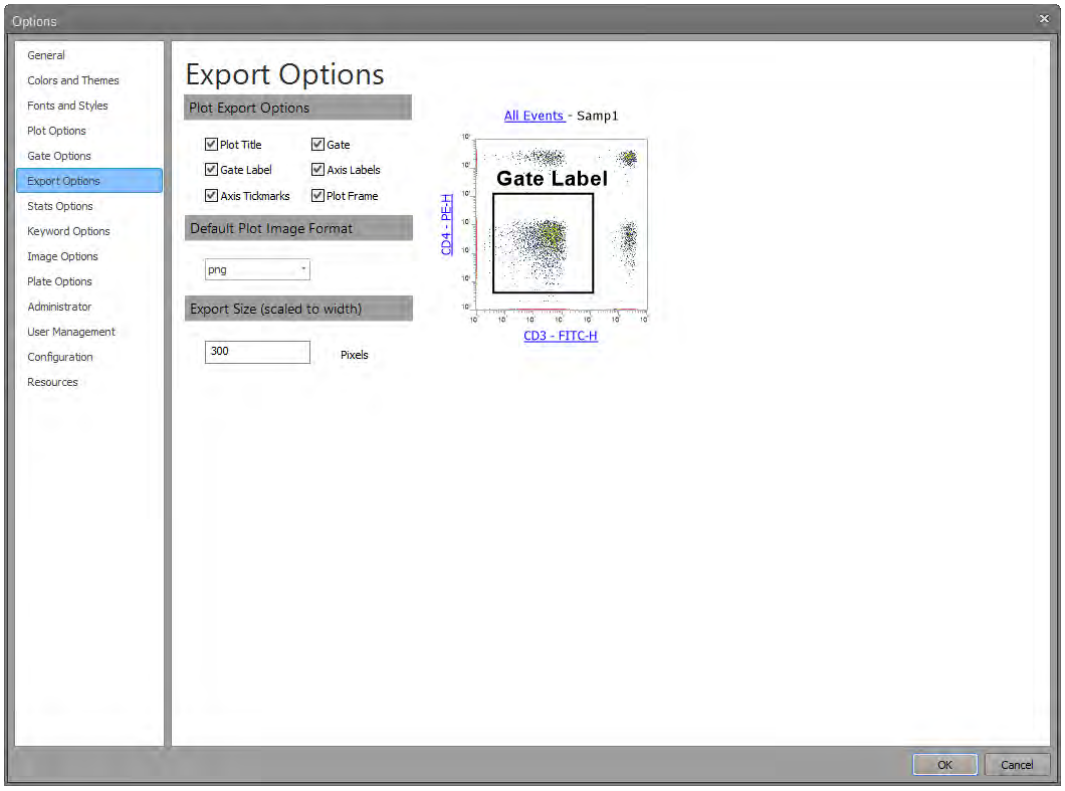
Quick Select Gate Names

- *Quick Select Gate Names* allows you define the gate names available in the *Name* dropdown menu on the *Customize Gate panel* (page 299)
- By default, the Quick Select Gate Names table contains the **Lymphs**, **Grans**, and **Monos** options.
- You can add additional names to the table by double-clicking on the whitespace of the list view or by clicking the **New** button in the table header.
- You can edit the names in the table by double-clicking on the name you wish to edit.
- Gate names can be up to 50 characters long and can include any character except the following: \ / : * < > | ?.
- Duplicate entries are not allowed.
- Upon validation, the software automatically removes leading and trailing spaces, and converts consecutive spaces to single spaces.
- If you attempt to enter invalid characters, a warning dialog indicates the error condition, and the invalid characters do not appear in the text field.
- You can modify the sort order of the names in the table by selecting a row and using the **Up** and **Down** arrows located in the table header.
- You can delete names from the table by selecting a row and using the **Delete** button located in the table header.
- Changes made to entries in the table are available immediately. Gate names are not affected on established Workspaces.

Export options

Overview

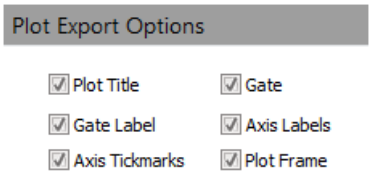
Export options tab allows you to define the default plot content that is copied to the Windows clipboard using the **Copy** command (when a plot is pasted outside the application) or when the plot is saved using the **Save As** command. In addition, it allows you to specify the default plot image format used when saving plots.



- Changes made in the Export Options section take effect immediately and applied to newly exported objects.

Plot export options

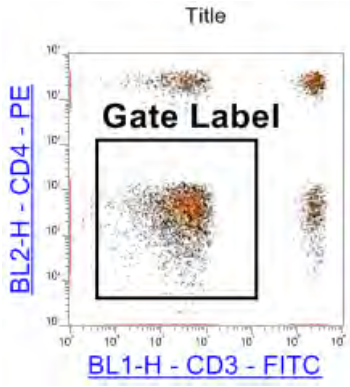
Plot export options allow you to define the default plot contents that are included when a plot is copied or saved. It consists of six checkbox controls, all of which are selected by default.



- When the Plot Export Option checkboxes are selected or deselected, a plot preview shows what plot content will be included when copying or saving a plot.

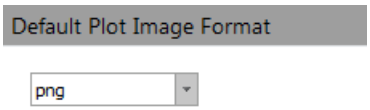
Plot export preview

When the Plot export option checkboxes are selected, the *Plot export preview* displays an example plot showing the plot content to be included when copying or saving a plot.



Default plot image format

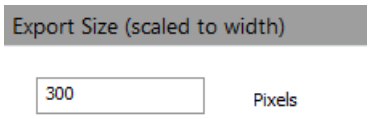
Default plot image format option sets the default format used when saving a plot.



- The combo box control for plot image format contains a list of available image formats. Available options are PNG, GIF, JPG, TIF, BMP, and EMF.
- By default, plot image format is set to PNG.

Export size (scaled to width)

Export size (scaled to width) option allows you to set the X-axis pixel size when exporting or copying objects.



- The x-axis pixel range for the export size is 100–5000.

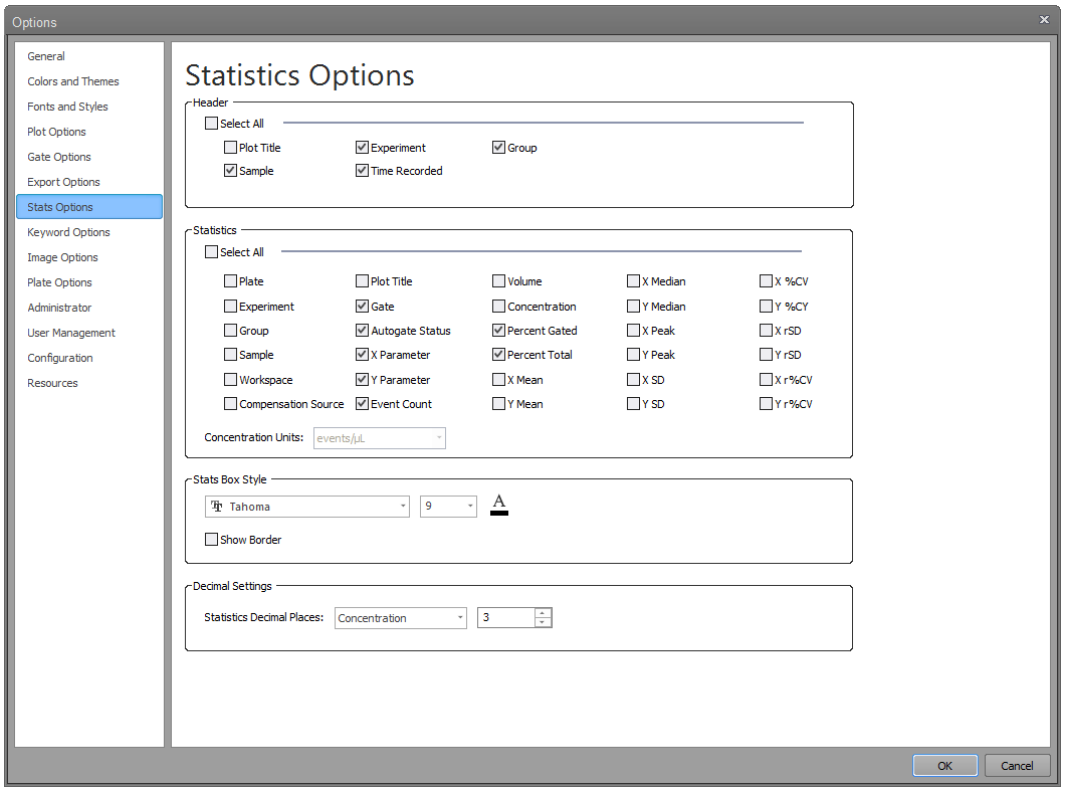
  The default pixel size is 400.
- When a pixel size value greater than 5000 is entered, the value defaults to 5000.

  When a value less than 100 is entered, the value defaults to 100.

Stats options

Overview

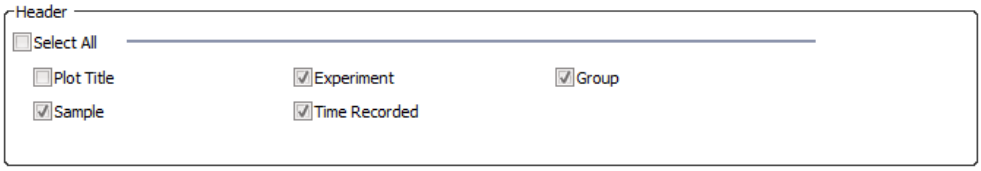
Stats options allow you to define default display preferences for statistics boxes in the Workspace and Results views. The tab is divided into four sections: *Header*, *Statistics*, *Stats Box Style*, and *Decimal Settings*.



- Changes made in this section are applied only to newly created statistics tables in the Workspace and newly created Experiments in the Results view, except Decimal Settings.
- Changes made to Decimal Settings are applied immediately upon clicking **OK** to close the dialog, and affect all past, present, and future instances.

Header

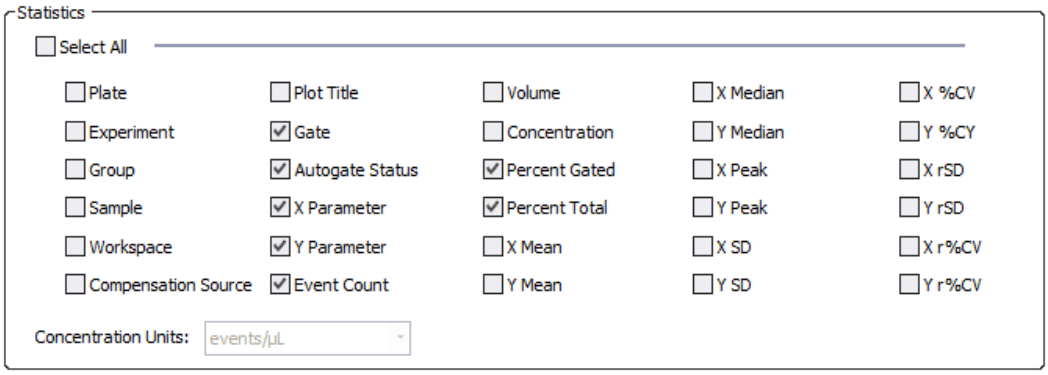
Header options allow you to define the default format for the statistics box headers.



- You can select to display **Plot Title**, **Experiment**, **Group**, **Sample**, and **Time Recorded** in the header area of statistics boxes on the Workspace. By default, all options are selected.
- Selecting/deselecting the **Select All** checkbox selects/deselects all other header checkboxes. Deselecting any of the checkboxes also deselects the Select All checkbox.

Statistics

Statistics allow you to determine which default statistics are displayed in statistics boxes on the Workspace.



- You can select the options you wish to display by default in the statistics boxes by selecting the relevant **Statistics** checkbox. The following options are available:

  Plate, Experiment, Group, Sample, Workspace, Compensation Source, Plot Title, Gate, X Parameter, Y Parameter, Event Count, Volume, Concentration, % Total, % Gated, X Mean, Y Mean, X Median, Y Median, X Mode, Y Mode, X SD, Y SD X % CV, Y % CV, X rSD, Y rSD, X r% CV, Y r% CV, and Autogate Status.

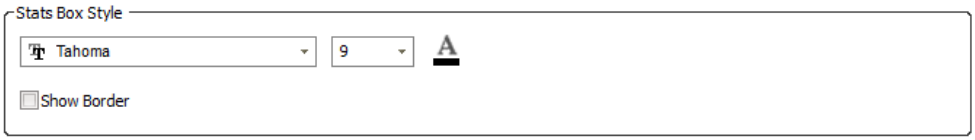
  You can change the statistics included in the statistics boxes at any time by selecting the statistics within the Ribbon bar when the Statistics box is selected.

  For more information on each option, see "Statistics tab", page 74.
- By default, Gate, X Parameter, Y Parameter, Event Count, % Gated, % Total, and Autogate Status are selected.
- Default settings are applied to Experiment-level and Plot-level statistics boxes.
- Selecting/deselecting the **Select All** checkbox selects/deselects all statistics checkboxes.

  If any of the statistics checkboxes is deselected, the Select All checkbox is also deselected.

Stats Box Style

Stats Box Style options contain the formatting tools for the display of statistics boxes.



- You can select a **font type** from the font dropdown menu. The dropdown menu includes all fonts installed on the system. The default font is Tahoma.
- In the **font size** field, you can select a font size from the dropdown menu, or directly enter a number. The default size in 9 pt.
- Using the **font color** picker, you can define the default font color of the plot title. The software uses the standard color picker dialog. The default color is black.

Decimal Settings

Decimal Settings options define the default decimal places displayed for each statistic. It contains a combo box control with a list of statistics and a control to select the number of decimal places for each statistic.

- Clicking the **Statistics** combo box opens the **Statistics dropdown** list, which contains the following options:

  All, Concentration, % Total, % Gated, Mean, Median, SD, %CV, rSD, r%CV, and Volume.

  Event Count cannot be adjusted as it does not show any decimal places.
- When a statistic is selected from the list, the **Decimal Places** control updates to the default value assigned to the selected statistic.

  The default number of decimals for each statistic is as follows:

Statistic	Decimals
Concentration	3
% Total	3
% Gated	3
Mean	0
Median	0
SD	2
% CV	2
rSD	2
r%CV	2
Volume	0

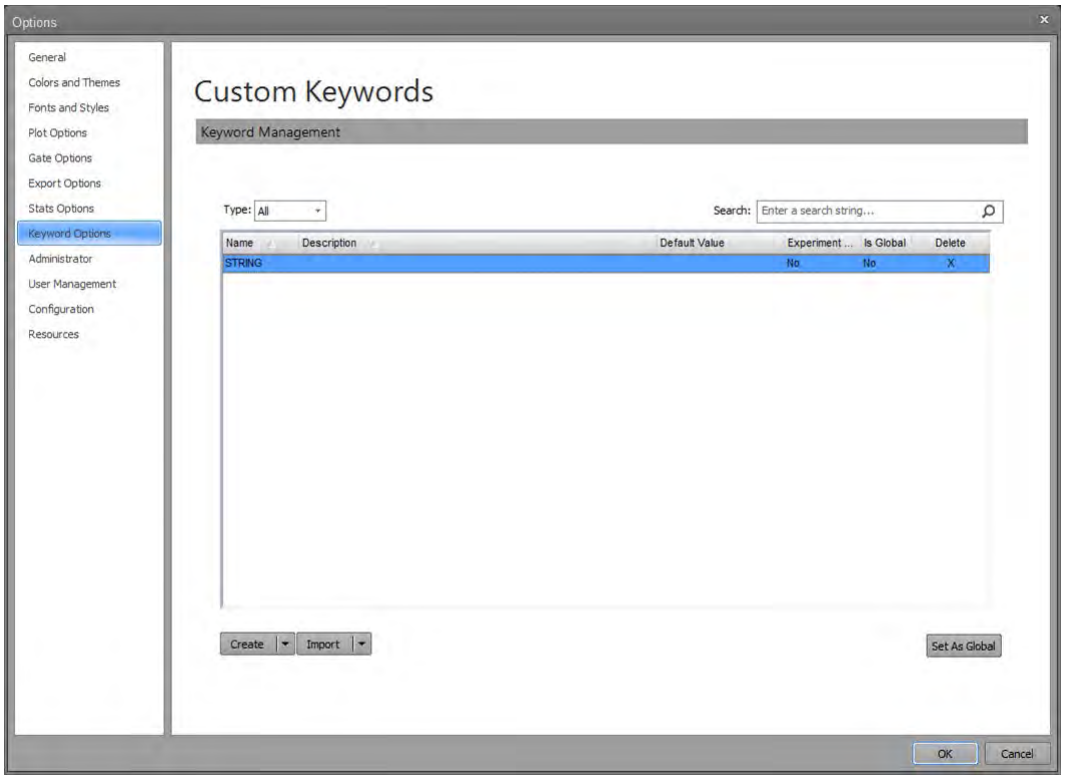
- You can modify the number of decimal places displayed using the **Decimal Places** spin box control. You can directly enter a number from **0** to **7**, or use the **up** and **down** buttons on the spin box control to modify the decimal places.

  Alternatively, you can use the **up** and **down keyboard arrows**, or the **mouse scroll wheel**.
- The text field only allows integer values. Entering a value greater than 7 defaults the value to 7.
- The decimal places assigned to each statistic are used throughout the software, including the Workspace statistics tables, the Results tab values, the Report statistics tables, on-plot statistics for gates, and on the Heat Map legend.
- When **All** is selected from the Statistic dropdown list, the value input in the spin box control applies to all statistics within the list.
- If the decimal places are different for each statistic, the spin box will be blank when **All** is selected.
- The number of decimal places specified here applies to all past, present, and future Experiments.
- The Count and Mode statistics only display integers without a decimal place.

Keyword options

Overview

Keyword options allow you to define custom keywords to provide additional information about the Sample.



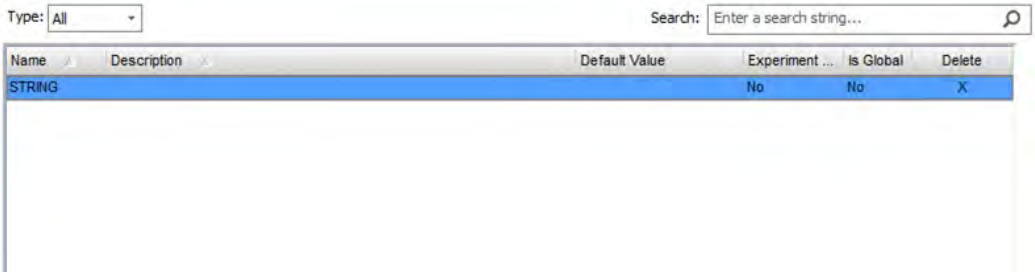
Keyword management

The Keyword options tab contains a table displaying a list of all user-defined keywords and controls to create, edit, import and export keywords.

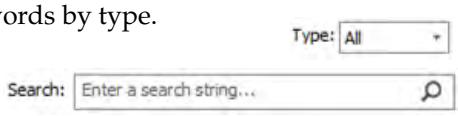
All users can create, edit and delete their own custom keywords and add them to their user list in keyword options.

Keywords list

You can filter the keywords or search for existing keywords in the list.



- Use the **Type** dropdown to filter existing keywords by type.
- Enter a search string into the **Search** field, then press **Enter** to search for existing keywords in the list.



Create/Edit

Create/Edit split button allows you to create custom keywords and edit existing keywords using the “Create/Edit Keyword dialog” (page 443).

- Click **Create** to open the “Create Keyword dialog” (page 443), then enter the name and description of the custom keyword you wish to create.
- Select an existing keyword, then click the **Edit** portion of the split button to open the “Edit Keyword dialog” (page 443).

  The Edit Keyword dialog is identical to the Create Keyword dialog, except the existing keyword values are populated.
- Only users with Administrator accounts can edit or delete a global keyword.

  Global keywords are displayed to all users, but the Edit keyword option is disabled for non-administrator users.
- Edits made to a global keyword apply to the keyword master lists of all users, but they do **not** affect keywords that are associated with existing Experiments.
- To change the keyword included in an Experiment, use the **Edit** option for the keyword from the Experiment Keyword dialog (page 445) within the selected Experiment.

Note: Once an Experiment is created, it gets its own set of keywords that persist with the Experiment. These keywords also persist through duplication, export/import, and as part of a Template.

Export/Import

Export/Import split button allows you to export the selected keywords to a file, or to import keywords that were previously exported.

- Select an existing keyword, then click **Export** to export it to a file.

  The default file name for exported keywords is "AttuneKeywords" and the default file extension is *.akw.
- If only a single keyword is selected, a single keyword will be exported. To export multiple keywords, select multiple keywords (or the entire list), then click **Export**.
- Click **Import** to open the “File Open (Import)” dialog (page 535), go to the appropriate folder, and import the desired keyword file (*akw).

  If the imported keyword is a duplicate of an existing keyword, the duplicate keyword is appended by a numeric character (e.g., (1)).

Set As Global

- *Set As Global* button allows users with Administrator accounts to set the custom keywords in the Keyword options list to global. Global keywords are included in the Keyword Options for all
user accounts.
- To set a custom keyword as global, select the keyword from the Keywords list, then click **Set As Global**.
- Alternatively, you can check the **Set As Global** checkbox for the desired keywords in the "Create/Edit Keyword dialog" (page 443)).
- The Set As Global button is only visible to users with Administrator accounts.

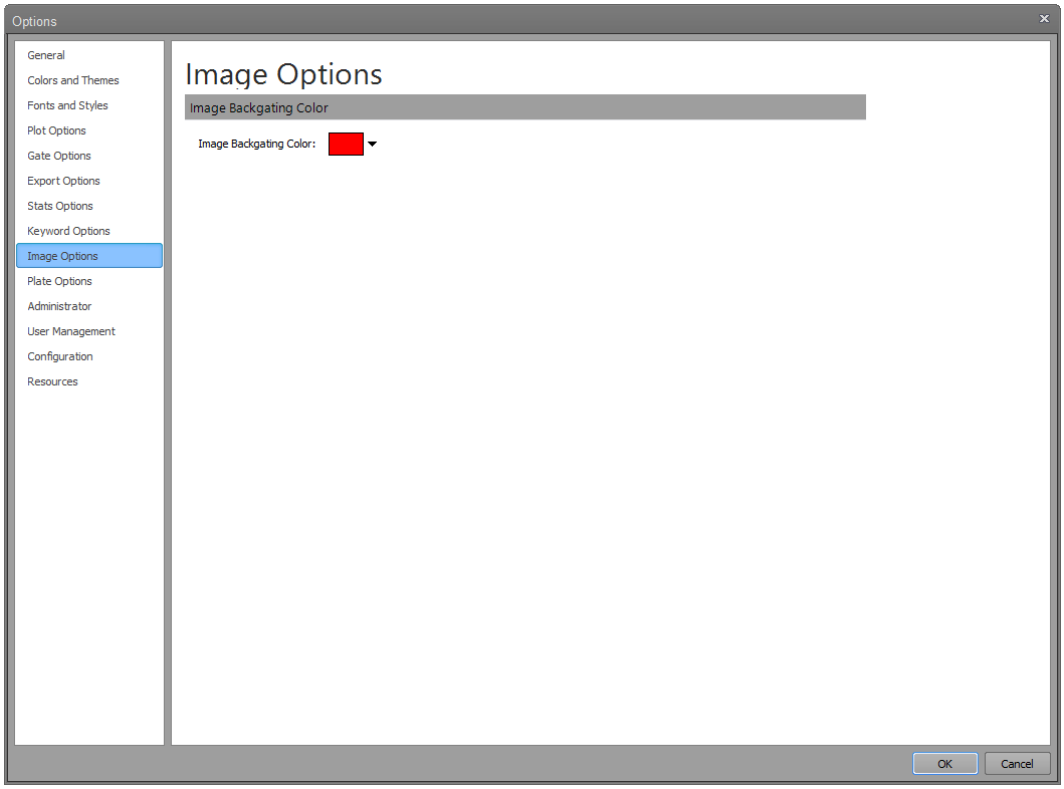
Delete global keywords

- Only users with Administrator accounts can delete a global keyword.
- For non-administrator users, the Keywords list displays **N/A** in the Delete column for global keywords.
- Deleting a global keyword removes that keyword from the keyword master lists of all users, but it does not update existing Experiments.

Image options

Image Options tab contains *Image Backgating Color* menu, which allows you to select the color with which the backgated imaged events are identified in the corresponding Workspace plots.

The Image Options tab is only available when using an Attune™ CytPix™ Flow Cytometer or when an Experiment that was created with an Attune™ CytPix™ Flow Cytometer is active.

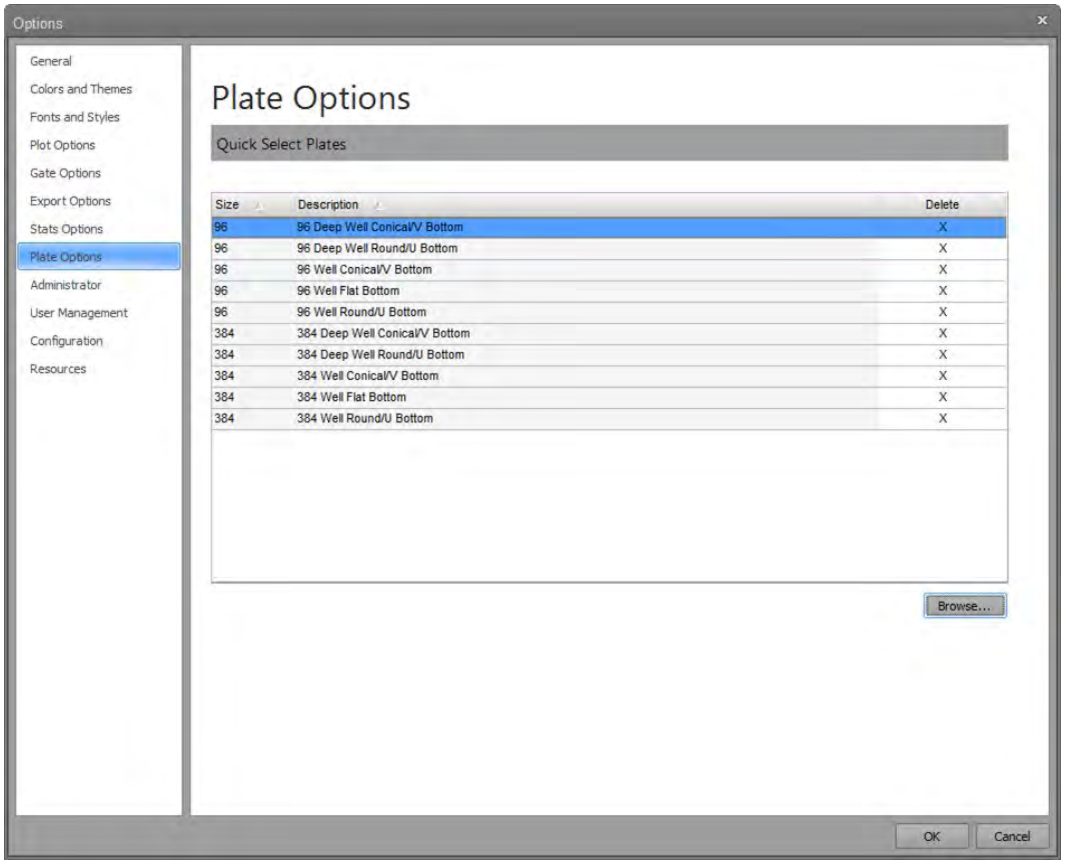


- You can modify the Image Backgating Color by clicking on the **color dropdown**, then select the new color from the standard color picker dialog.

  When a color is selected from the color picker dialog, the color displayed in the color dropdown is updated and the color picker dialog is closed.

Plate options

Plate options tab contains the *Quick Select Plate*s list, which allows you to designate selected plates as favorites. The selected plates become available in the Plate Type dropdown in the New Experiment dialog (page 399) and in the Customize Plate Experiment panel (page 304).



Note: The Plate Options dialog is available only when a CytKick™ Max Autosampler is connected to the Attune™ instrument.

- To access the dialog, click the **Options** button on the **Quick Access** toolbar, then select the **Plate Options** tab. The Options button is enabled only when the instrument is not acquiring.
- By default, the Quick Select Plates list includes the 10 defined plates available to all models. However, you can add additional plate definitions to the list using the **Select Plates** dialog (page 448).

  To launch the **Select Plates** dialog, click the **Browse** button.
- To sort the Quick Select Plates list, click on the column header. The default sort order is plate size, followed by description.
- When the mouse moves over the Delete column, the cursor changes to the hand cursor, if the plate can be deleted. To remove a Plate from the list of favorites, click the **X** in the Delete column next to the plate you wish to delete.

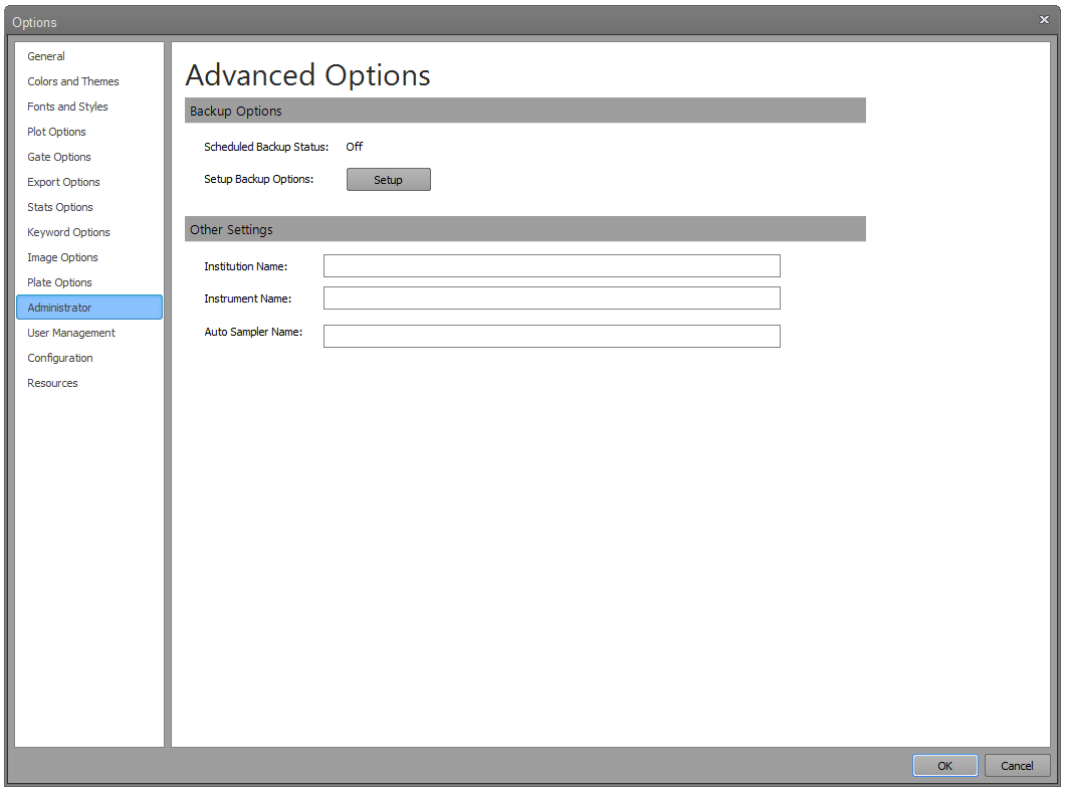
Administrator options

Overview

Administrator options allow users with Administrator account privileges to:

- Set system level FCS backup policy (“Backup Options”, page 501)
- Define institution and instrument names (“Other Settings”, page 501)
- Set up the software to access the SAE (Security, Auditing, e-Signature) features (“SAE Configuration”, page 502)

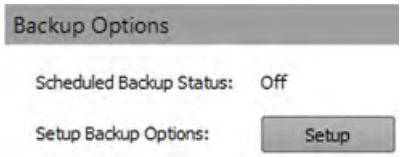
The Administrator tab is only visible to System Administrators, Administrators, and Service accounts.



Note: The SAE Configuration options are only available if the SAE-specific DESkey device is present to allow access to the 21 CFR Part 11 features (see “SAE mode”, page 19).

Backup Options

Backup Options section allows you to see the status of a scheduled backup and to setup backup options.



Scheduled Backup Status

Shows whether the scheduled backup function is turned on or off.

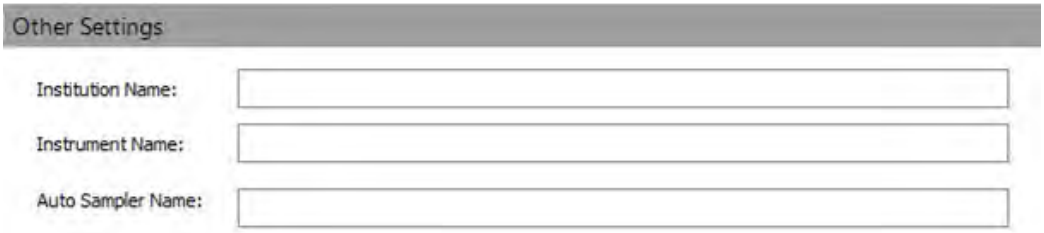
Setup Backup Options

- Click the **Setup** button to launch the *Attune™ Database Utility* (page 538).
- The database utility allows you to back up the data and database, to restore data and database, to schedule automated backups, or to reinstall the database.
- When Attune™ CytPix™ Flow Cytometer is in use, the database utility allows you to select the Image Storage Path for captured images.
- By default, the captured images are stored at:

  C:\Users\Public\Documents\Life Technologies\AttuneCytPix_Images

Other Settings

The *Other Settings* section includes settings for the Institution Name, Instrument Name, and Auto Sampler Name.



- You can enter up to 50 alpha-numeric characters in the Institution Name, Instrument Name, and Auto Sampler Name textboxes. The following characters are not allowed: \ / : * < > |.
- If invalid characters are entered, a warning dialog indicates the error condition and the invalid characters will not appear in the text field.
- Upon validation, the software automatically removes leading and trailing spaces.

Institution Name

- To define the Institution Name for the system, type in the name into the **Institution Name** textbox. The Institution Name setting is used to populate the $INST keyword in the FCS file TEXT segment (page 551). The institution name will be saved to each FCS file when entered in the textbox.
- By default, the Institution Name is blank.

Instrument Name

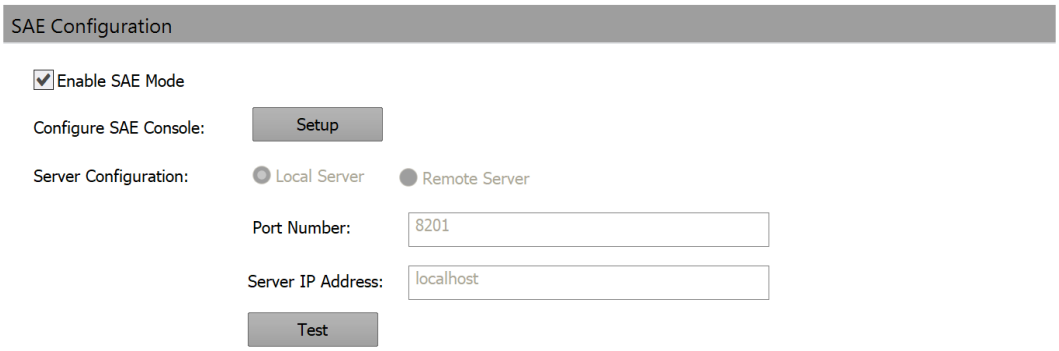
- To define the Instrument Name for the system, type in the name into the **Instrument Name** textbox.
- By default, the Instrument Name is blank.

Auto Sampler Name

- To define the Auto Sampler Name for the system, type in the name into the **Auto Sampler Name** textbox.
- The Auto Sampler Name is blank by default.

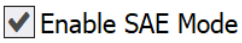
SAE Configuration

SAE Configuration options allow you to set up the software to access the SAE (21 CFR Part 11) features.



- The SAE Configuration options are only available if the SAE-specific DESkey device is present to allow access to the 21 CFR Part 11 features (see "SAE mode", page 19).
- To configure the software for the SAE mode, the SAE server must be running.

Enable SAE Mode checkbox



Enable SAE Mode checkbox is used to enable and disable the SAE mode for the Attune™ Cytometric Software.

- To enable the SAE mode, check **Enable SAE Mode**.

  Only SAE user accounts with the Administrative privilege that have permission to "Configure Security and Auditing" can enable the SAE mode.

  For detailed instructions on how to enable the SAE mode, see "Enable the SAE mode", page 504.
- To disable the SAE mode, uncheck **Enable SAE Mode**.

  Only local Attune™ users with Administrator or System Administrator account, or SAE user accounts with Administrator privileges can disable the SAE mode.

  For detailed instructions on how to disable the SAE mode as an SAE user, see "Disable SAE mode – SAE user", page 506.

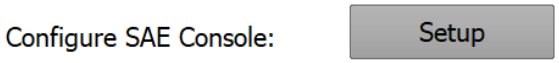
  For detailed instructions on how to disable the SAE mode as a local Attune™ administrator or system administrator, see "Disable SAE mode – local Attune™ administrator", page 507.

IMPORTANT! When signing into the Attune™ Cytometric Software after the SAE mode is enabled, you must use your SAE account username and password, and not your Attune™ NxT username and password.

Note that the data from local User and Advanced User accounts become inaccessible after the SAE mode is enabled. We recommend that you export all local User and Advanced User data before enabling the SAE mode.

Configure SAE Administrator Console

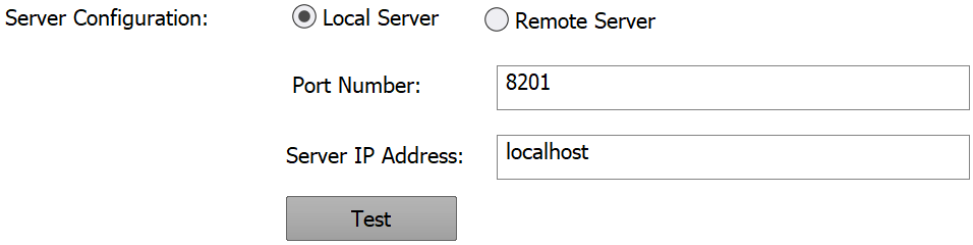
Configure SAE Administrator Console allows you to configure SAE Console settings.



- To configure the SAE Administrator Console, click **Setup** to open the SAE Administrator Console window in the default web browser, then sign in to the *SAE Administrator Console*.
- You can create new SAE users, new SAE user roles, set security policies, and configure auditing and e-Signature requirements in the SAE Server Console (see "Appendix C: SAE Administrator Console", page 576).

Server Configuration

Server Configuration allows you to configure the SAE server to be run on a local or remote server, and to set the Server IP Address and Port Number.

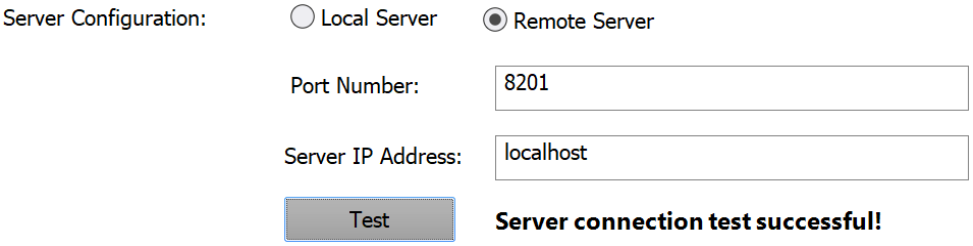


- The SAE Server Configuration options are only enabled if the SAE Mode is disabled (i.e., Enable SAE Mode is unchecked; see page 506).
- For both local and remote servers, the **Port Number** is set to 8201 by default and the **Server IP Address** is set to "localhost".

  You can set the Port Number to 1025–49151.
- When **Local Server** option is selected, only the Port Number is editable. In this case, the Server IP Address is set to read-only and displays "localhost".

  When the **Remote Server** option is selected, both the Server IP Address and Port Number can be changed.
- **Test** button allows you to test the connection to the Remote Server.

  When **Test** is clicked, the software attempts to connect to the Remote Server and performs a time check between the client and the server. For a successful connection, the time difference between the client and the server must be less than 5 minutes.
- If the test is successful, the "Server connection test successful!" message is displayed.



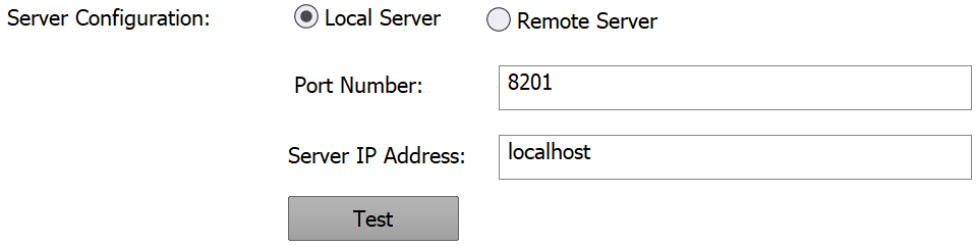
Rules for enabling the SAE mode

1. Only SAE User accounts with Administrative privilege that have permission to "Configure Security and Auditing" can enable the SAE mode.
2. Enabling the SAE mode requires a connection to the SAE server.
3. After the user provides credentials to enable the SAE mode, the following take place behind the scenes:
   i. An authentication request for the administrator is sent to the SAE server.
   ii. If the provided credentials are valid and the specified SAE user account is authorized to enable the SAE mode, the server returns a success status code.
   iii. Upon successful administrator authentication, an SAE user session for the specified administrator account is initiated and all relevant security, audit, and e-Signature configurations take effect.

Enable the SAE mode

1. To enable the SAE mode, check **Enable SAE Mode** (page 502).
2. When the **Enable SAE Mode** checkbox is checked, the software prompts you to perform a test with the proper IP address and port number.

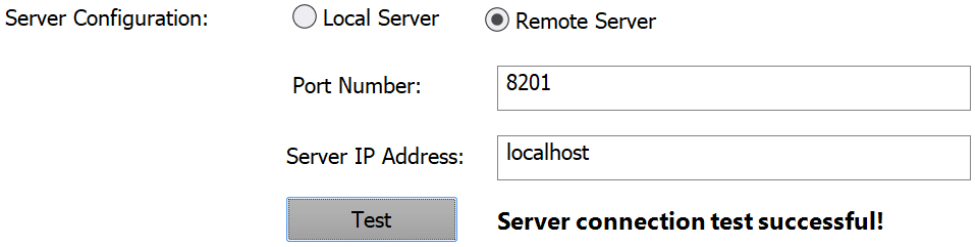
3. Click **OK** to close the dialog, then enter the **Port Number** and **Server IP Address**.



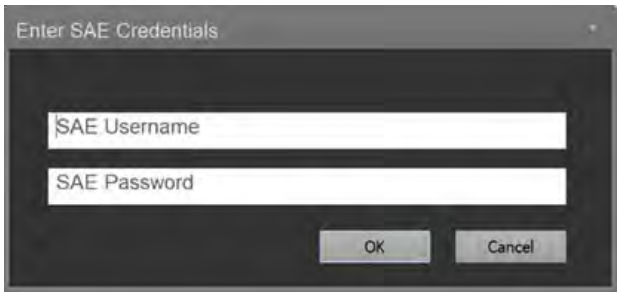
4. Click **Test** to test the connection to the server.

   The software attempts to connect to the server and performs a time check between the client and the server. For a successful connection, the time difference between the client and the server must be less than 5 minutes.

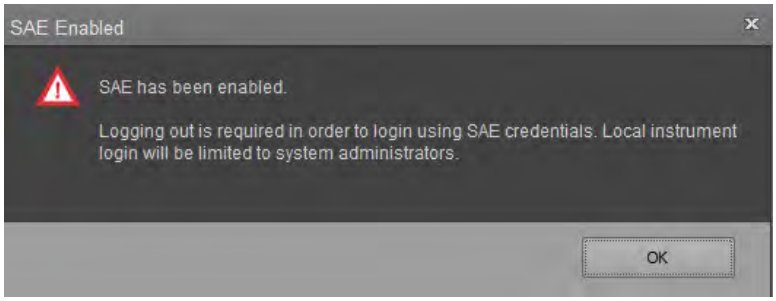
   If the test is successful, the "Server connection test successful!" message is displayed.



5. If the server can be reached and the test is successful, the *SAE Credentials dialog* prompts you to enter your SAE credentials.



6. If the credentials are correct, the *SAE Enabled dialog* is displayed.



If the server check fails, Unable to connect to authentication server dialog is displayed and the Enable SAE Mode checkbox is unchecked.

7. When the SAE mode is enabled, the Attune™ Cytometric Software displays the SAE mode sign in option (see "Sign in – SAE mode", page 21).

All relevant security, audit, and e-Signature configurations take effect immediately after enabling the SAE mode, and two action records appear in SAE Admin Console: "Enable Security" and "Login Success".

After the SAE mode is enabled

1. Only SAE user accounts can access the Attune™ instrument to perform runs (except Administrator, System Administrator, and Service users).
2. Users will not be able to sign in to the instrument via the local instrument profiles that were in use previously:
   i. The local instrument profiles and their associated data are not deleted via this action of enabling SAE.
   ii. The action of disabling SAE enables these local instrument profiles to be accessible again.

IMPORTANT! When the application is configured to the SAE mode, you can sign in to your local Attune™ Cytometric Software account only if you have an Administrator, System Administrator, and Service Account. Users and Advanced Users are not allowed to login to the local account once SAE is enabled.

Disable the SAE mode – SAE user

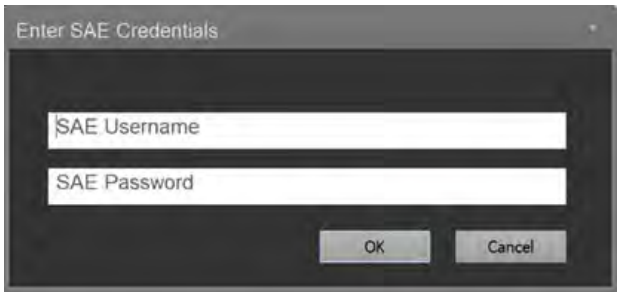
Rules for disabling the SAE mode as an SAE user

1. Only SAE user accounts that have SAE Administrator privileges can disable the SAE mode.
2. Disabling the SAE mode requires a connection to the SAE server.

   Note: Local Attune™ users with Administrator or System Administrator accounts can disable the SAE mode regardless of server status (page 507).
3. After the SAE user provides credentials to disable the SAE mode, the following take place behind the scenes:
   i. An authentication request for the SAE Administrator role is sent to the SAE server.
   ii. If the provided credentials are valid and the specified SAE user account is authorized to disable the SAE mode, the server returns a success status code.
   iii. Upon successful administrator authentication, the instrument logs out of the current SAE account, and all relevant security, audit, and e-Signature configuration no longer affect the instrument.

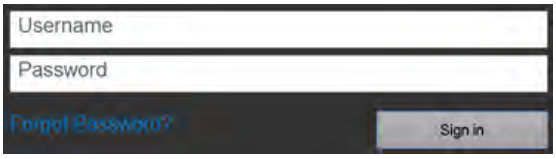
Disable the SAE mode

1. To disable the SAE mode, uncheck **Enable SAE Mode** (page 502).
2. If the server is online and can be reached, the *SAE Credentials dialog* prompts you to enter your SAE credentials.



3. The credentials are checked on the SAE server and only users with the permission to "Configure Security and Auditing" can disable SAE mode.

   If an error occurs while entering credentials, an error message is displayed in the SAE Credentials dialog.
4. If the entered credentials are correct, the SAE Credentials dialog closes and the SAE user is logged out of the application. The login screen displays the non-SAE mode sign in options (see "Sign in – Standard mode" page 20).



All relevant security, audit, and e-Signature configurations are disabled immediately after disabling the SAE mode, and two action records appear in SAE Admin Console: "Disable Security" and "Logout".

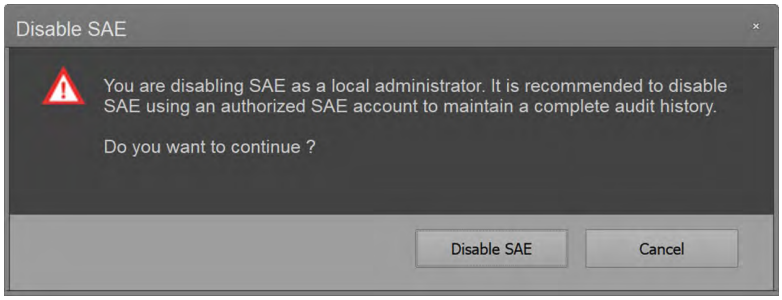
Disable SAE mode – local Attune™ administrator

Rules for disabling the SAE mode as a local Attune™ user

1. Only local Attune™ users with Administrator or System Administrator accounts can disable the SAE mode.
2. Local Attune™ users with Administrator or System Administrator accounts can disable the SAE mode regardless of server status.

Disable the SAE mode as a local Attune™ user

1. To disable the SAE mode, uncheck **Enable SAE Mode**.
2. When Enable SAE Mode is unchecked, a warning dialog is displayed to confirm that you want to disable the SAE.

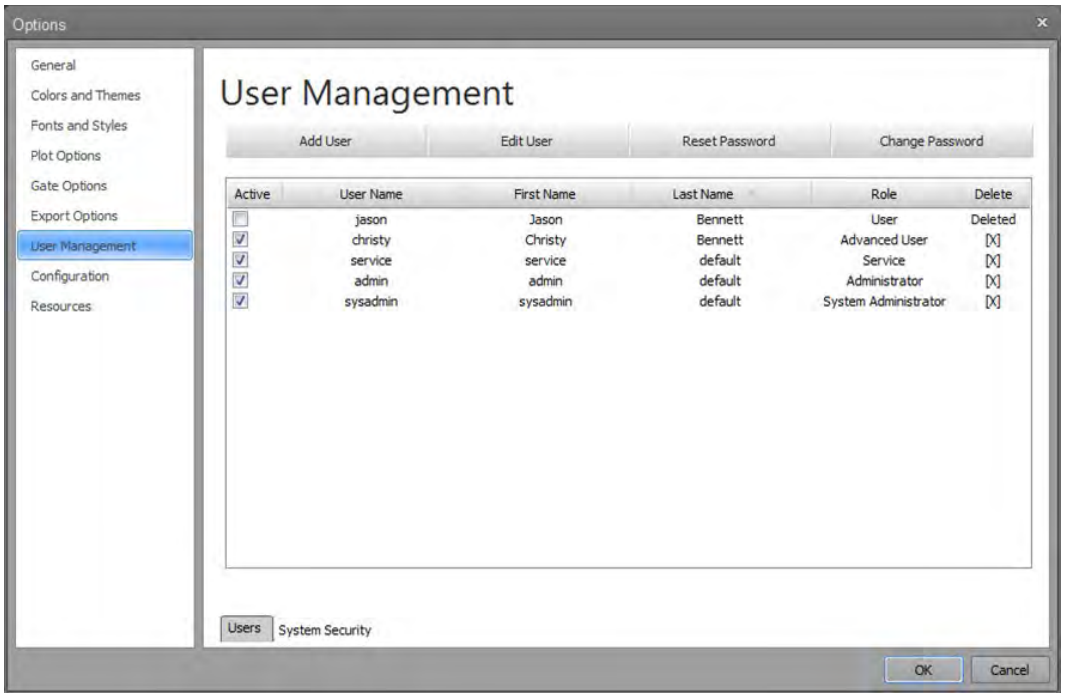


3. Click **Disable SAE** to disable the account or click **Cancel** to close the dialog without disabling the SAE mode.
4. When SAE is disabled by a local Attune™ user, the "Disable Security" event is audited on the SAE Server in Audit History ▶ Action Records page.

Note: To maintain a complete audit history, we recommend that you disable the SAE mode using an authorized SAE account (page 506).

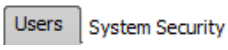
User Management options

Overview *User Management* allows authorized users to add, edit, and view local Attune™ user accounts and manage user account passwords.



Note: When the SAE mode is enabled (see "Enable the SAE mode", page 504), user management is controlled by the SAE Administrator Console (see "Appendix C: SAE Administrator Console", page 576).
For SAE specific permissions, see "SAE account permissions", page 583.

- The tabs at the bottom of the User Management indicate the menu selected.

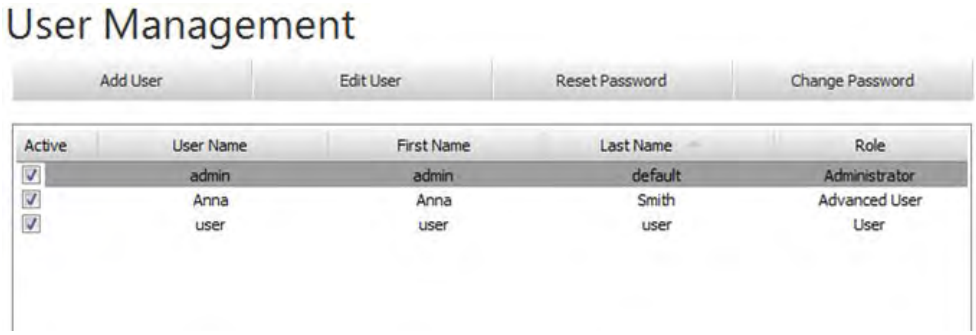
  For all account types except for the System Administrator, only the *Users* tab (page 509) is visible. System Administrators have access to define the *System Security* (page 512) setting within the System Security tab.
- The User Management tab provides users with different account types the privileges described in "Account types" (page 24).
- For Users, the Users tab (page 509) contains the controls for editing and viewing user's accounts and for changing the user account passwords.

  For Administrators, the Users tab (page 509) contains controls for adding users, editing and viewing accounts, and resetting or changing passwords.

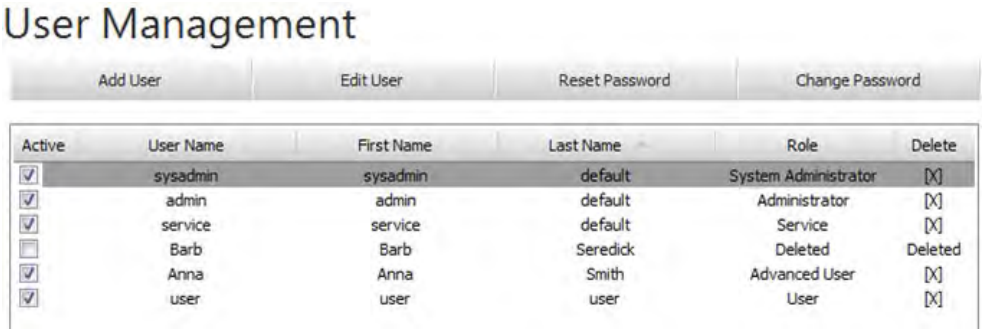
  For System Administrators, the Users tab (page 509) contains controls for adding and editing user accounts and for resetting or changing passwords. The System Administrator can also delete user accounts from the Users tab.
- User privileges for different account types are described in "Account permissions" (page 25).
- The settings defined in the User Management tab are global and they are stored in and retrieved from the database.

Users tab options

Users tab options consists of buttons across the top that allow authorized users to add and edit users and to reset or change passwords. The table of User Accounts below these buttons displays the database-derived information for all users.



Users tab – Administrator account view



Users tab – System Administrator account view

- Any changes made in the Users tab take effect immediately and are not reverted if **Cancel** is clicked on the Options dialog.

Add User

- The Add User button allows an authorized user to create a new user account. This button is enabled only for Administrators and System Administrators.
- Clicking **Add User** opens the *Add User dialog* (page 523).
- If SAE mode is enabled (page 504), new SAE user accounts can only be created using the SAE Administrator Console (see "Users tab" in "SAE Administrator Console", page 578).
- The Add User button is disabled if an SAE user is signed in.

Edit User

- The Edit User button allows an authorized user to modify a user account profile or the current user currently to modify their user profile.
- The Edit User button is enabled for all local Attune™ users.
- Administrators and System Administrators can edit any user account as described on page 525. Any user can edit their own account (page 525).
- Clicking **Edit User** opens the *Edit User dialog* (page 525).
- SAE users cannot edit their user profiles using the Attune™ software and must use the SAE Administrator Console (see "Users tab" in "SAE Administrator Console", page 578)).
- The Edit User button is disabled when signed in as an SAE user.

Reset Password

- The Reset Password button allows an authorized user to reset the password for any user account.
- The Reset Password button is enabled only for local Attune™ Administrator and System Administrator accounts.

  SAE users who have the SAE permission to "Configure Security and Auditing" cannot reset user passwords using the Attune™ software and must use the SAE Administrator Console (see "Users tab" in "SAE Administrator Console", page 578).
- Clicking **Reset Password** opens the *Reset Password dialog* (page 529).

Change Password

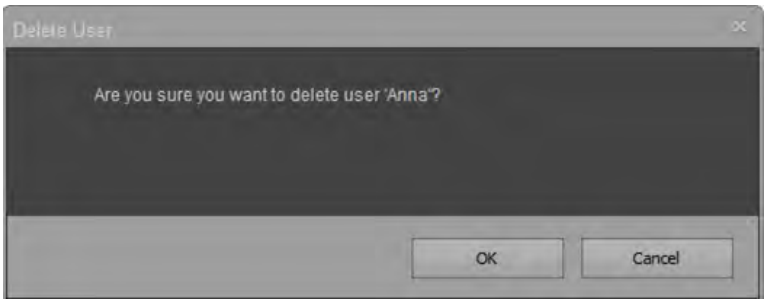
- The Change Password button allows the current users to change their own passwords. This button is enabled for all users.
- Clicking **Change Password** opens the *Change Password* dialog (page 530).
- Change Password feature is available for both SAE and local Attune™ users.

> **Note:** For local Attune™ users, the password policy applied to new and changing passwords is based on the system security policy for user passwords set in the "System Security" tab of "User Management options" (see page 512).
>
> For SAE users, the password policy applied to new and changing passwords is set based on the SAE security policy (see "System tab" in "SAE Administrator Console", page 591).

User Accounts Table

- The User Accounts table displays a list of all user accounts. It consists of columns for **Account Status**, **Username**, **First Name**, **Last Name**, **Roles**, and a column that includes a **Delete User** (**X**) button to remove specific user accounts.
- All selections in this table are single select. No multi selection is allowed.
- The table can be sorted by clicking on the column headers. When clicked, a sort indicator in the column heading specifies both the active sort column and the sort direction. Any single column can be used for sorting. Clicking on the same header reverses the sort direction.
- The default sort order is ascending by Last Name.
- Any user can see their own account listed in the User Accounts table. Only users with Administrator and System Administrator accounts can view other user accounts within the table.
- The **Account Status** (i.e., Active) column is only enabled for users with Administrator and System Administrator accounts to allow enabling and disabling of accounts. All other users can view the column, but the checkbox is disabled.
- When the **Account Status** checkbox is selected, the relevant account is enabled. When unchecked, the account is disabled.

- Disabling an account prevents a user from logging in. If a user with a disabled account attempts to log in, the software displays a warning message to inform that the account has been disabled.
- The delete user option is only enabled for System Administrators. For other users, the column is visible, but disabled.
- When the **Delete User** (**X**) button is clicked, the *Delete User dialog* prompts the user to confirm the deletion. Only a single user can be selected for deletion at a time.

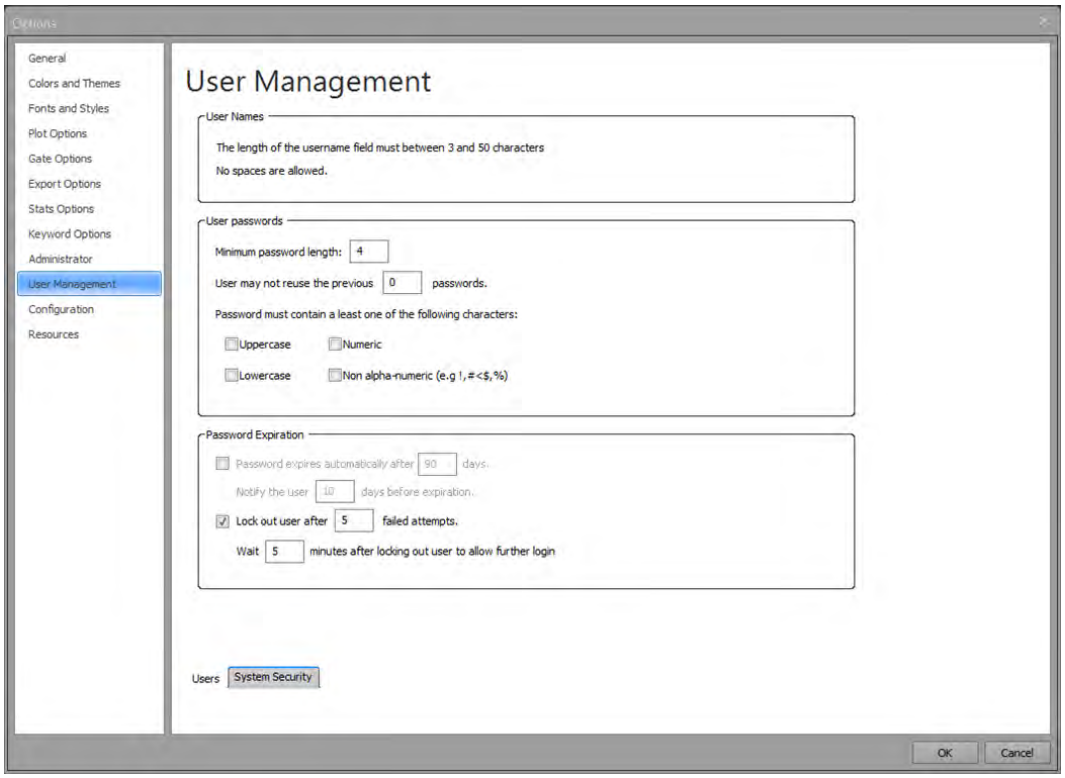


- Clicking **OK** deletes the account, closes the Delete User dialog, and updates the User Accounts table.

  Clicking **Cancel** closes the Delete User dialog without deleting the account.
- When an account is deleted, it is removed from the list of user accounts visible to Administrator accounts.
- Only System Administrators can view the full list of all users, where deleted user accounts are listed with the **Active** checkbox unchecked and disabled, and the **Roles** set to **Deleted**.
- When an account is deleted, the account is also set to a **Deleted** status, and the Active checkbox is unselected and disabled. The account remains in the list.
- A deleted account relinquishes the username, allowing the username to be reused.
- SAE user accounts and local service accounts cannot be set to inactive or be deleted. If a System Administrator account attempts to delete or disable an SAE user account, a warning dialog is displayed.

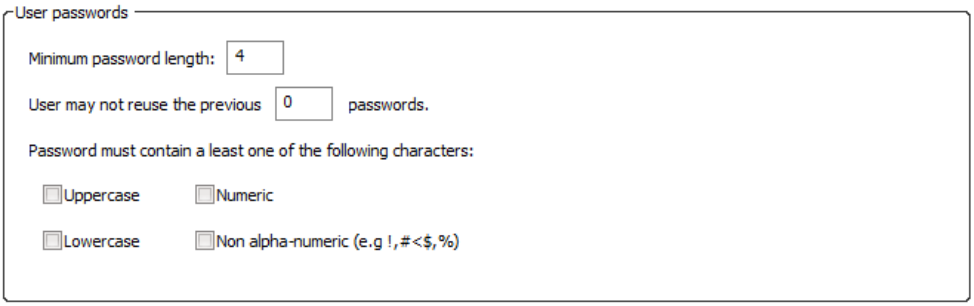
System Security tab

System Security tab allows the System Administrator to define the system security policy for user passwords and password expiration criteria. This tab is only accessible to System Administrator accounts. This tab provides controls to set requirements around.



User Passwords

User Passwords section provides options to set the minimum password length, to limit the reuse of passwords, and to set password complexity requirements.

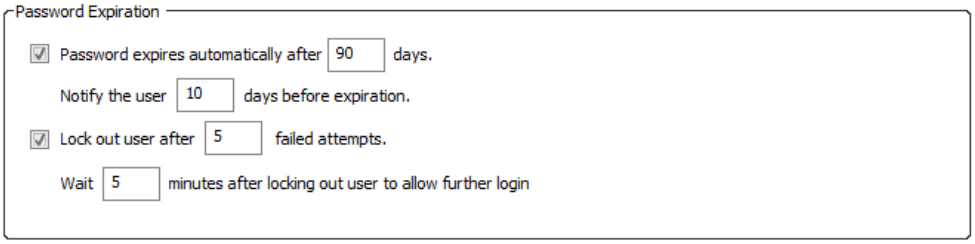


- The default **minimum password length** is 4 characters.
- The default for not allowing **reuse of previous passwords** is 0, which allows users to reuse their last password.
- The maximum allowed value is 10, which prohibits the use of the previous 10 passwords. If a value greater than 10 is entered, the value will default to 10.
- The **password complexity requirements** are unchecked by default. When selected, passwords are required to have at least one character from the selected complexity options.
- **Uppercase** selection requires that at least one uppercase alphabetic character is used in a password. Uppercase characters are defined from European languages (A through Z, with diacritic marks, Greek and Cyrillic characters).

- **Lowercase** selection requires that at least one lowercase alphabetic character is used in a password. Lowercase characters are defined from European languages (a through z, sharp-s, with diacritic marks, Greek and Cyrillic characters).
- **Numeric** selection requires that at least one numeric character is used in a password. A numeric character is defined as a base 10 digit (0 through 9).
- **Non-alpha-numeric** selection requires that at least one non-alpha-numeric character is used in a password. The non-alpha-numeric characters are: **~ ! @ # $ % ^ & * _ - + = ` | \ ( ) { } [ ] : ; " ' < > , . ? /**.
- Any Unicode character that is categorized as an alphabetic character but is not uppercase or lowercase may also be used. This includes Unicode characters from Asian languages.

Password Expiration

Password Expiration section is used to set a period to automatically force users to change their passwords, to notify users before their password expires, and to lock out users after a defined number of failed login attempts.

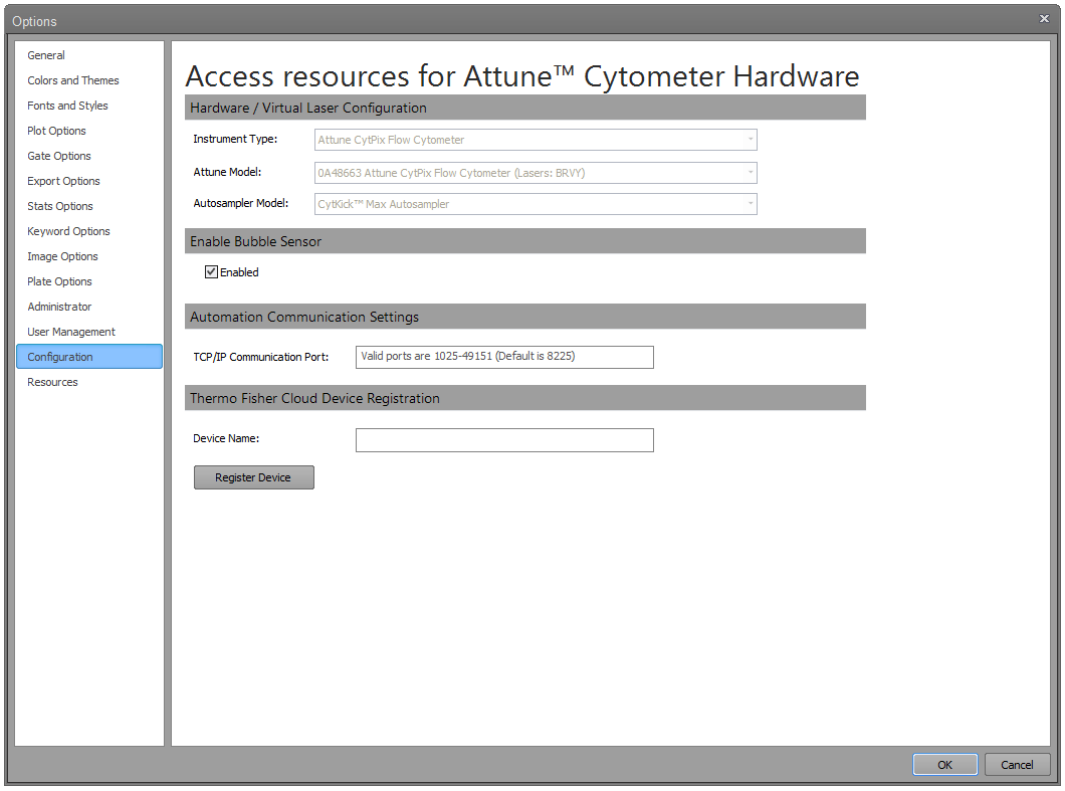


- For all number fields, if you enter a number that is lower than the minimum allowed, the number is adjusted to the minimum when you elsewhere or when you press **Enter**.
- If you enter a number that is higher than the maximum allowed, the number is adjusted to the maximum when you elsewhere or when you press **Enter**.
- Only integers are allowed.
- The default setting for password expiration is 90 days and deselected.
- The minimum value for password expiration is 30 days and the maximum is 365 days.
- The default setting for password reset notification is 10 days prior to expiration and deselected.
- The minimum value for notification is 1 day and the maximum is 10 days prior to password expiration.
- The default setting for password lockout for failed login attempts is 5 failed attempts and selected.
- The minimum value for password lockout is 3 failed attempts and the maximum is 10 failed attempts.
- The default setting for wait between failed login attempts is 5 minutes and selected.
- The minimum value for wait between failed login attempts is 1 minute and the maximum is 15 minutes.

Configuration

Overview

Configuration options allow you to set the offline instrument configuration when working in analysis-only conditions, and to manage the bubble sensor and automation port settings.



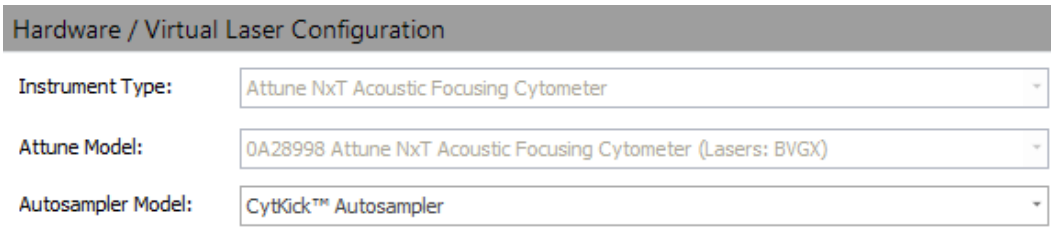
Configuration options tab contains the following controls:

- *Hardware/Virtual Laser Configuration* (page 515)
- *Enable Bubble Sensor* (page 515)
- *Automation Communication Settings* (page 516)
- *Thermo Fisher Cloud Device Registration* (page 517)

Hardware/ Virtual Laser Configuration

Hardware/Virtual Laser Configuration allows you select offline instrument type and model. This selection aids in the creation of Experiments when not connected to an Attune™ instrument by maintaining correct instrument settings, channel mapping, and run protocol options.

When the instrument is connected, the Instrument Type and Attune Model field are automatically selected based on the instrument and model in use.



Instrument Type

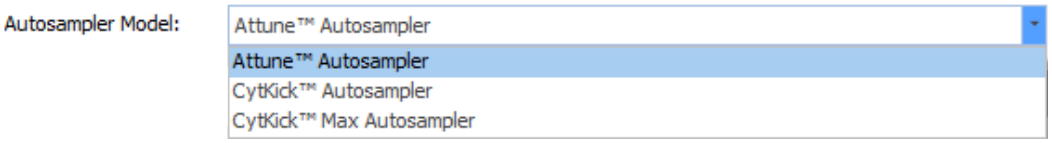
- **Instrument Type** opens the instrument type dropdown list, which contains the instrument configurations listed in the database.
- Available instrument types are **Attune NxT Acoustic Focusing Cytometer** and **Attune CytPix Flow Cytometer**.

Attune Model

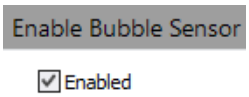
- **Attune Model** opens the instrument configuration dropdown list, which contains the instrument configurations listed in the database.
- The available models are organized by instrument type and the number of lasers in descending order. Within each grouping, models are listed in ascending order.
- When the system is offline, the instrument configuration dropdown remains disabled but visible to all user accounts except for the System Administrator and Service accounts, which will be able to modify and set the configuration.
- System Administrator and Service accounts can modify and set the configuration when the Attune™ instrument is offline. This resets the state of the dropdown so any user can modify the configuration (i.e., if either the System Administrator or Service modifies this, then the dropdown list will be re-enabled for all users).

Autosampler Model

- **Autosampler Model** opens the autosampler model dropdown list, which contains the instrument configurations listed in the database.
- Available autosampler models are **Attune™ Autosampler**, **CytKick™ Autosampler**, and **CytKick™ Max Autosampler**.



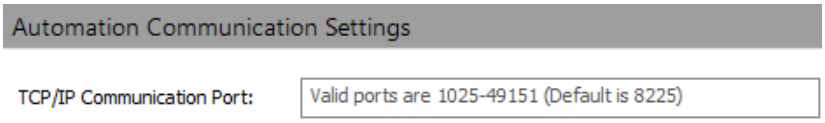
Enable Bubble Sensor



- **Enable Bubble Sensor** option enables the bubble sensor for the system.
- By default, the bubble sensor option is enabled. This setting is applied to all users.
- Only Administrator, System Administrator, and Service accounts can access the Enable Bubble Sensor option. For all other user types, this option is visible but disabled.

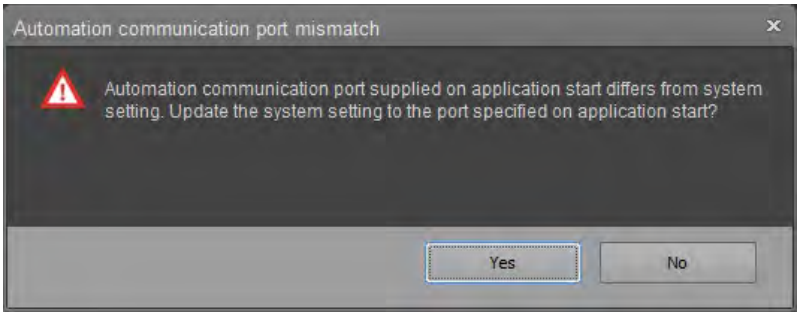
Automation Communication Settings

- **TCP/IP Communication Port** allows you to set the port used for communication to and from the automation software (such as the Thermo Scientific Momentum™ Software).



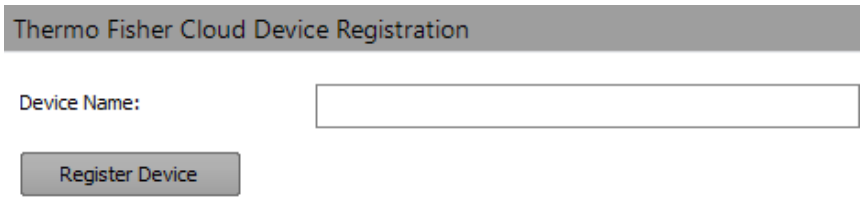
- The text box allows values from 1025 to 49151. If the input is outside this range, the text box resets to the last *good* setting, and a warning balloon displays the following text: "Invalid port specified. Valid ports are 1025-49151".
- To disable the communication port, leave the field blank.
- Click **OK** within the dialog box to set the TCP/IP Communication Port value. Otherwise the changes made to port settings are not preserved.

  Click **Cancel** to revert the port setting to the existing setting. The changes are only applied when the application is restarted.
- Only Administrator, System Administrator, and Service accounts can modify the TCP/IP Communication Port settings. For all other user types, this option is visible but disabled.
- When using the Orbitor RS3 Microplate Mover with Attune™ NxT and Attune™ CytPix™ instruments, the TCP/IP Communication Port must be set to 8225.
- If the application is started with the port command line argument and the value is different than the application setting, the command line value takes priority.
- When you sign in as an Administrator or System Administrator and there is an Automation communication port mismatch, the *Automation communication port mismatch dialog* allows you to update the application setting.



Thermo Fisher Cloud Device Registration

Thermo Fisher Cloud Device Registration allows the Administrator or System Administrator to register a device (Attune™ instrument or software) with the Thermo Fisher Connect cloud-based platform. The Device Name field and the Register Device buttons are only enabled when an Attune™ Administrator or System Administrator is signed into the Attune™ Cytometric Software.



- **Device Name** allows an Administrator or System Administrator to assign a name to the instrument used on the Connect platform when registering the instrument.

  By default, the device name uses the serial number of the instrument. If this is not available, the machine ID is used as the device name.

- **Register Device** opens the *Link Account* dialog (page 453).

  Only Administrators and System Administrators can register a device to the Connect platform. After the device is first registered, different users can log in and log out of their Connect account using the Cloud sign in function (page 33).

  If the device is already registered, the Register Device button is labeled **Unregister Device**.

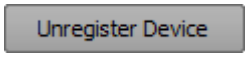
- After the instrument has been registered, the Device Name field displays the current device name and the *Update Device Name* button.

- To update the device, type in the new name into the Device Name field and click **Update Device Name**. Note that you must have an Administrator or System Administrator account to update the device name.

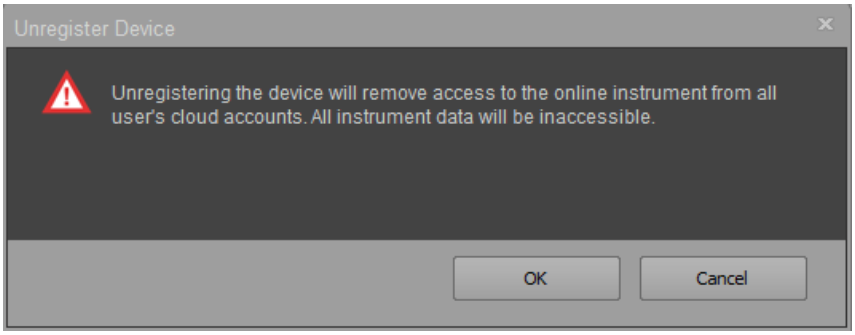
Unregister a device from the Thermo Fisher Cloud

You can completely unregister a device (Attune™ instrument or Attune™ Cytometric software) from the Thermo Fisher Connect cloud-based platform or dissociate the device from individual Connect accounts.

1. To completely unregister the device from all Connect accounts, click **Unregister Device**.

   The software displays a warning dialog informing that that unregistering the device removes the instrument from the Connect accounts of all users.

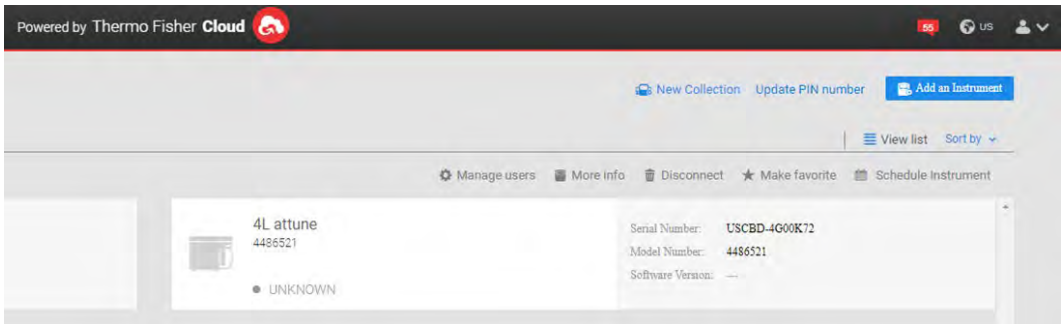
   Unregistration completely removes the instrument from the Connect platform as well as from all linked accounts and all data associated with them.

   The accounts that were linked to the unregistered Attune™ instrument still exist in the Connect platform and are active for any other devices to which they are connected, but the unregistered Attune™ instrument is no longer listed on the accounts.

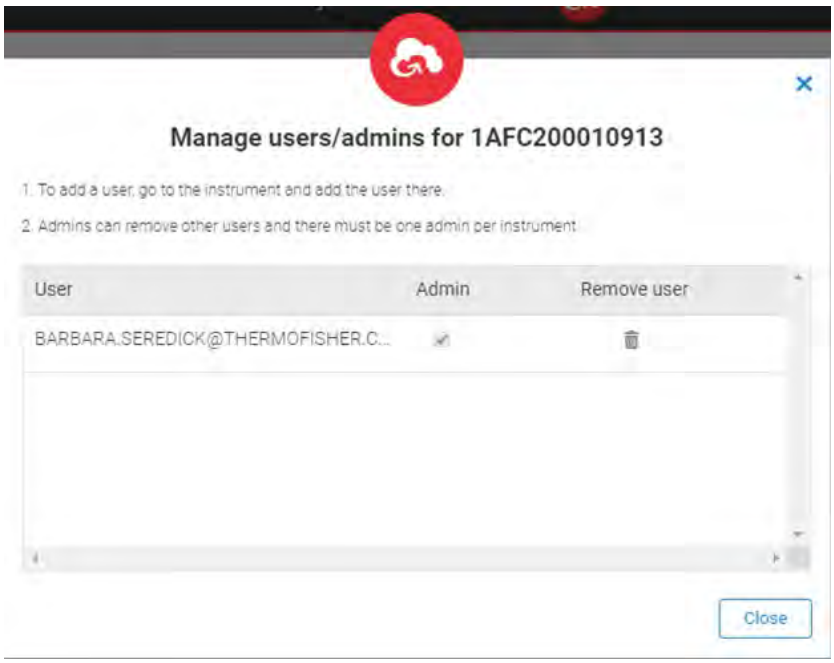
Remove individual users from a device on the Thermo Fisher Cloud

To remove an individual user from the device, dissociate the device from the Administrator or System Administrator's Thermo Fisher Connect cloud-based platform account online.

1. Login to the Administrator or System Administrator's Connect account.
2. Within the instrument dashboard, click on the device and select **Manage users**.



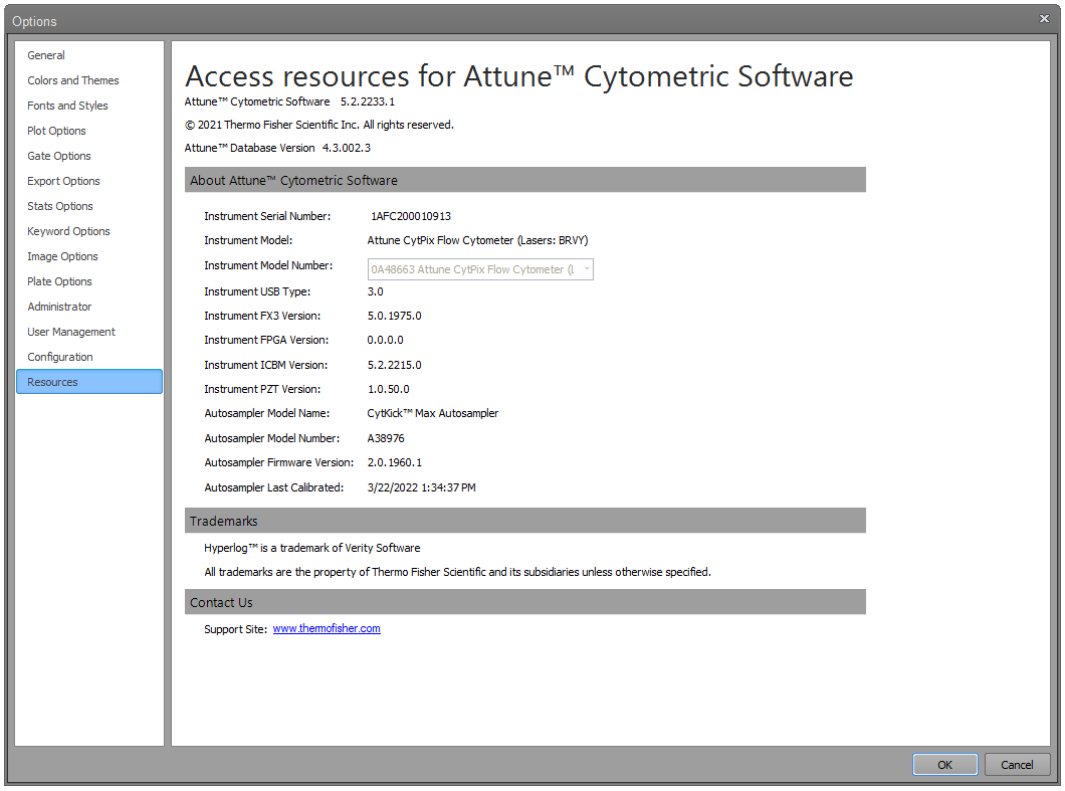
3. In the Manage users/admins dialogue, select the user account to dissociate from the instrument and click on the symbol to remove the user.



Resources

Overview

Resources tab provides information about the system including the software version, the instrument and auto sampler serial numbers, the instrument and auto sampler firmware versions, the system model, and other licensing and contact information.



Access resources

This section displays Attune™ Cytometric Software and database version information.

Attune™ Cytometric Software

The current Attune™ Cytometric Software version is shown including the software name and software revision number in the format A.B.C.D where A is the major build, B is the minor build, C is the build number and D is the revision.

Attune™ Database Version

The current Attune™ database version is read from the database and includes the MySQL version in the format MySQL #.#.## | Database #.#.#.####.

About Attune™ Cytometric Software

This section contains the instrument serial number, the instrument model and model number, the instrument firmware, the auto sampler serial number, the auto sampler firmware, and the last calibrated date and time of the auto sampler.

Instrument Serial Number

The instrument serial number is automatically retrieved from the instrument.

Instrument Model

- The content for instrument model is automatically populated upon connection to an instrument.
- If no instrument has been connected, the model selected in the *Configuration tab* (page 514) is shown.
- The instrument model field is updated based on the selection made for instrument model number.

Instrument Model Number

- The instrument model number combo box options are automatically populated from the database.
- Upon connection to an instrument, the appropriate model number is automatically selected from the instrument model number combo box options.
- The combo box is disabled by default. It is enabled only for users with Service accounts (page 25).

Instrument Firmware

- The content for instrument firmware is automatically populated upon connection to an instrument. If no instrument is connected, this field is left blank.
- The instrument firmware field displays the major, minor, and build revision number in the format ##.##.###.#.

Auto Sampler Serial Number

- The auto sampler serial number field is blank by default. A user with a Service account can enter an auto sampler serial number.
- The maximum length for a serial number is 50 characters. Any ASCII characters can be entered except **/ \ ? % * : | ″ < >**.

  Spaces are not permitted and cannot be entered.
- If a user attempts to enter an illegal character, a warning dialog indicates the error condition, and the invalid characters are not entered.

Auto Sampler Last Calibrated

- The section is automatically updated to show the date and time of the last auto sampler calibration. This value is read from the instrument.
- The data and time are displayed in the local date and time format.

Trademarks

This section contains information on any trademarks used within the software.

Contact Us

This section contains hyperlinks to Thermo Fisher Scientific websites.

25. User Management

The *User Management tab* of the Options dialog allows authorized users to perform the following user management functions:

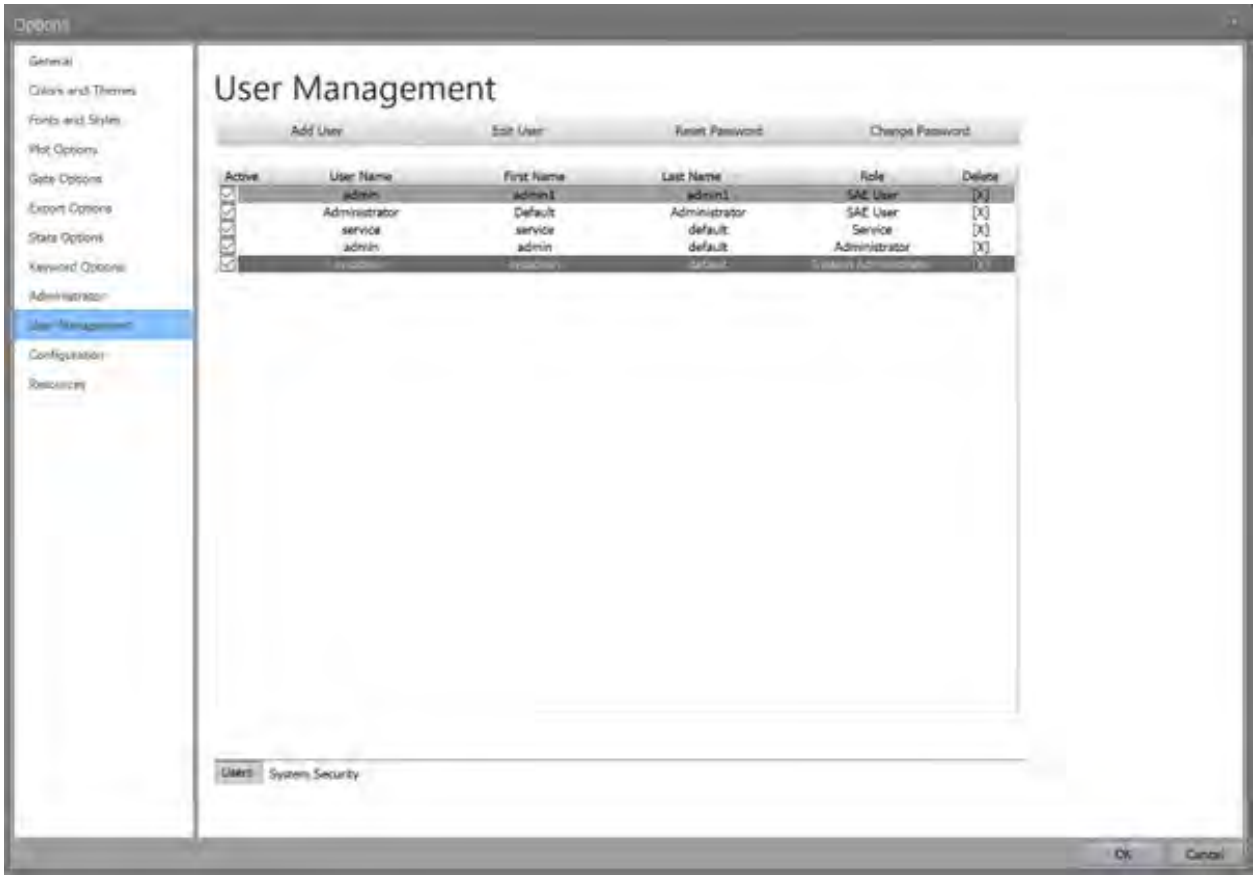
- Add or edit a local Attune™ account (page 523)
- Reset a password for a local Attune™ account (page 529)
- Change a password for a local Attune™ account (page 530)

Note: When the SAE mode is enabled (see "Enable the SAE mode", page 504), user management is controlled by the SAE Administrator Console (see "Appendix C: SAE Administrator Console", page 576).

For SAE specific permissions, see "SAE account permissions", page 583.

Access the User Management tab

1. In the *Quick Access* toolbar, click **Options Menu** button to open the Options dialog.
2. In the left pane, select the **User Management** tab.



User Management tab – System Administrator view

Add or edit an account

Add an account

Only *System Administrator* or *Administrator* accounts (page 24) can add new local Attune™ accounts to the software. To create a user profile to add a new account, see page 523.

Edit an account

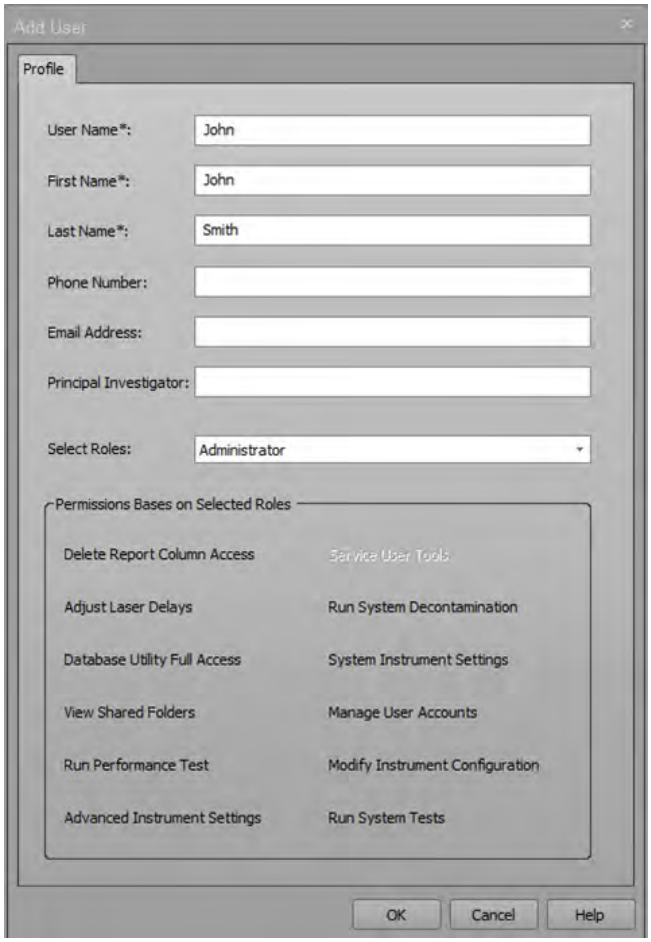
System Administrator or *Administrator* accounts can edit all local Attune™ accounts (except the *Service* account, which cannot be edited). *Users* can only edit their own accounts. To edit an existing user account, see page 525.

Create a user profile

Only System Administrator or Administrator accounts can create a local Attune™ user profile.

1. In the User Management tab of the Options dialog (page 509), click **Add User** to open the *Add User* dialog.

2. Click the **Profile** tab.

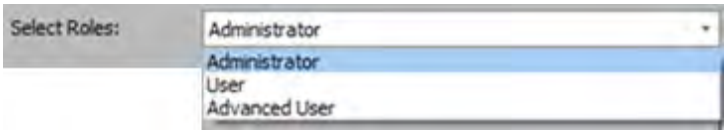


3. Enter the requested profile information:
   - **Username*:** Required field. Enter a unique username between 3 and 50 characters. Duplicate usernames are not permitted. Spaces and the following characters are not permitted: \ / : * ? ″ < > |

- **First Name*:** Required field. Enter up to 50 characters; the following characters are not permitted: **\ / : * ? ″ < > |**
- **Last Name*:** Required field. Enter up to 50 characters; the following characters are not permitted: **\ / : * ? ″ < > | %**
- **Phone Number:** Optional field. Enter up to 50 characters. The following characters are permitted: **+**, and the numerals **0** through **9**
  The **+** character can only be in the first position; for example: **+0123456789**
- **Email Address:** Optional field. Enter up to 100 characters; the following characters are not permitted: **\ / : * ? ″ < > | %**
  The email address must contain the **@** character and a domain name; for example, **email@address.com**.
- **Principal Investigator:** Optional field. Enter up to 100 characters; the following characters are not permitted: **\ / : * ? ″ < > | %**

Note: For all fields, the software automatically removes trailing, leading, or consecutive spaces.

4. Select an account type from the **Select Roles** dropdown menu: **User**, **Advanced User**, **Administrator.**



   - You cannot add a new Service account to the system.
   - Permissions assigned to the selected account are displayed in black text; permissions not assigned to the account are grey. None of the user account permissions can be modified.
   - For a list of account permissions, see "Account Types", on page 24.
5. Click **OK** to create the user account and close the dialog.

   To close the dialog without creating an account, click **Cancel** or **X**.

Note: For the permissions assigned to each local Attune™ account type, see "Account permissions", page 25.

For SAE specific permissions, see "SAE Account Permissions", page 583.

Edit a user profile

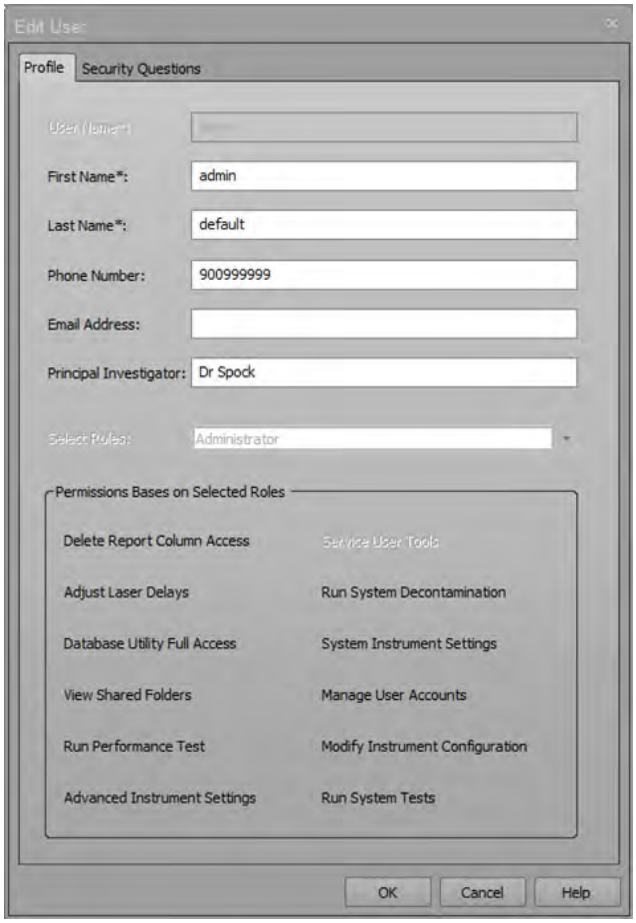
System Administrator or Administrator accounts can edit all local Attune™ accounts (except the Service account, which cannot be edited). Users can only edit their own local Attune™ accounts. SAE users cannot edit their user profiles using the Attune™ Cytometric Software and must use the SAE Administrator Console (page 576).

Note: For the permissions assigned to each local Attune™ account type, see "Account permissions", page 25.

For SAE specific permissions, see "SAE Account Permissions", page 583.

1. In the User Management tab of the Options dialog (page 509), click **Edit User** to open the *Edit User* dialog.

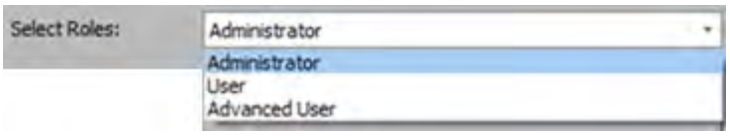
2. Click the **Profile** tab.



3. Edit the desired profile information fields as described in "Create a user profile" on page 523.

Note: For all fields, the software automatically removes trailing, leading, or consecutive spaces.

4. Select an account type from the **Select Roles** dropdown menu: **User**, **Advanced User**, or **Administrator**.



   - Only System Administrator or Administrator accounts can edit the account type.

     The **Select Roles** dropdown menu is disabled for all other users.
   - Permissions assigned to the selected account are displayed in black text; permissions not assigned to the account are grey. None of the user account permissions can be modified.
   - For a list of user account permissions, see "Account Types", page 24.
5. Click **OK** to save the changes and close the dialog.

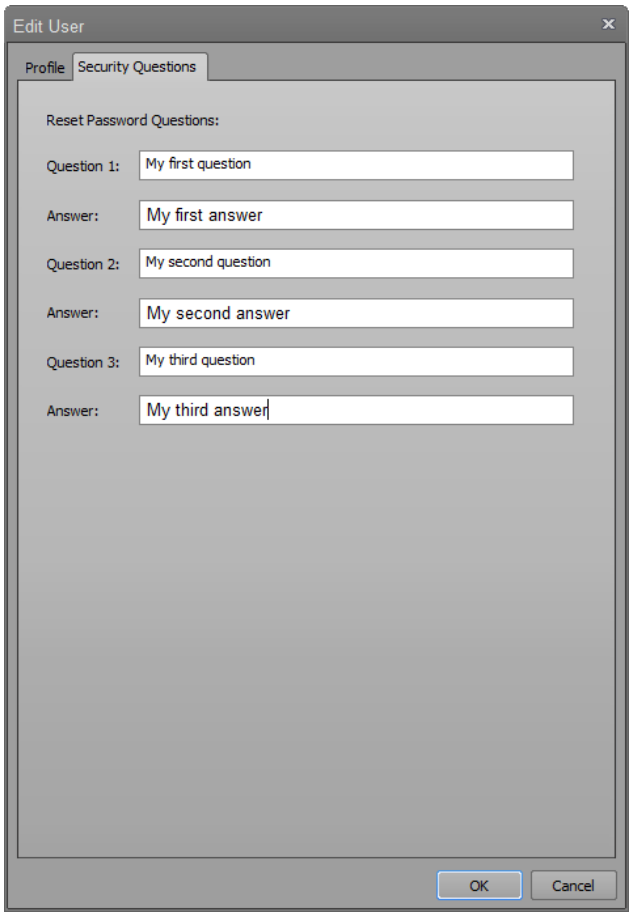
   To close the dialog without saving the changes, click **Cancel** or **X**.

Set password reminders

You can set password reminders only for your own local Attune™ account.

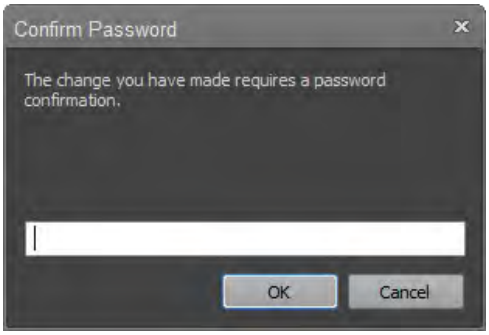
1. If it is not open already, click **Add User** or **Edit User** in the User Management tab (page 509) to open the User dialog.

2. Click the **Password Reminder** tab.



3. Enter up to three password reminder questions and answers, then click **OK**.
   - For each question and answer, you can enter up to 100 characters; any character is permitted.
   - To prevent unauthorized access to a user's account, the answers are displayed as bullet points.
   - You can reset a forgotten password by correctly answering the password reminder questions.

4. At the prompt, enter your password to confirm the password reminder changes, then click **OK**.



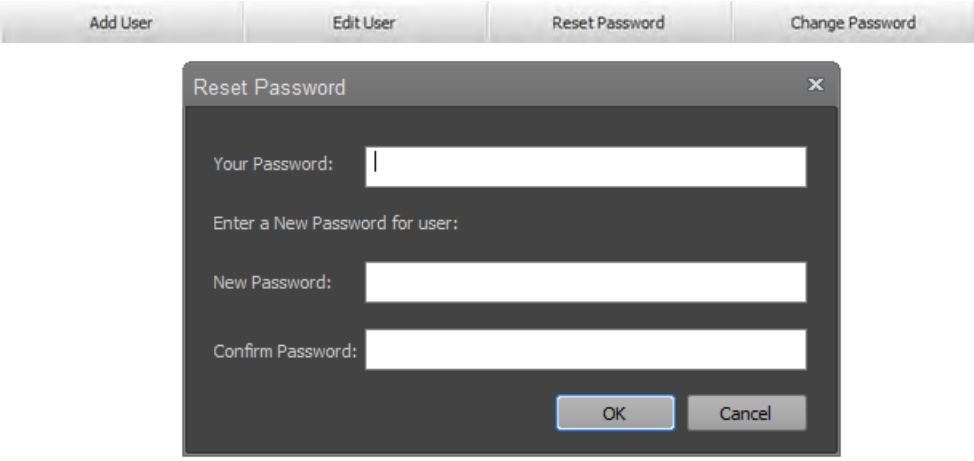
5. In the User dialog, click **OK** to save the changes and close the dialog.

   To close the dialog without saving the changes, click **Cancel** or **X**.

Reset a password

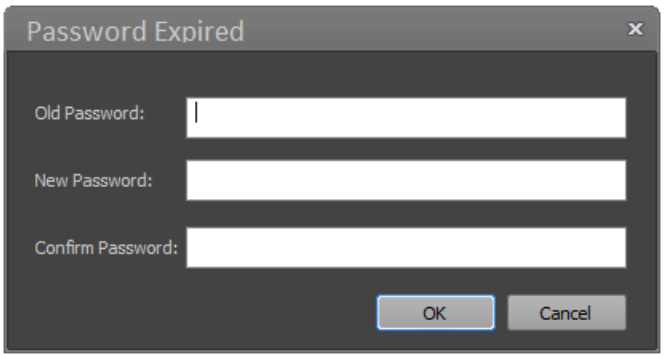
Reset Password provides System Administrators and Administrators the ability to reset the password for local Attune™ User and Advanced User accounts. Only System Administrator or Administrator accounts can reset a password. User and Advanced User accounts cannot reset the passwords of other accounts.

1. In the User Management tab (page 522), click **Reset Password** to open the *Reset Password* dialog.



2. Enter your System Administrator or Administrator password.
3. Enter a new password for "Username", which is the account whose password is to be reset.
4. Confirm the new password.
5. Click **OK** to save the changes and close the dialog.

   To close the dialog without saving the changes, click **Cancel** or **X**.
6. Provide the user with their new password. At next login, the user will be prompted to change the password.



Note: For local Attune™ users, the password policy applied to reset passwords is based on the system security policy for user passwords set in the "System Security" tab of "User Management options" (see page 512).

For SAE users, the password policy applied to reset passwords is set based on the SAE security policy (see "SAE Account Permissions", page 583).

Change a password

Change Password provides any user the option to change their own local Attune™ password.

1. In the User Management tab (page 522), click **Change Password** to open the *Change Password* dialog.

2. Enter your existing password.
3. Enter a new password, then confirm the new password.
4. Click **OK** to save the changes and close the dialog.

   To close the dialog without saving the changes, click **Cancel** or **X**.

Note: For local Attune™ users, the password policy applied to changing passwords is based on the system security policy for user passwords set in the "System Security" tab of "User Management options" (see page 512).

For SAE users, the password policy applied to changing passwords is set based on the SAE security policy (see "SAE Account Permissions", page 583).

26. File dialogs

Overview

This chapter describes the dialogs used for opening and saving files and folders, loading configuration files (Workspace, Instrument Settings, Heat Map, Run Protocols, Compensation Settings) stored within the database, and dialogs that are displayed when importing or exporting FCS files or invalid configuration files.

All dialogs are modal, unless otherwise specified.

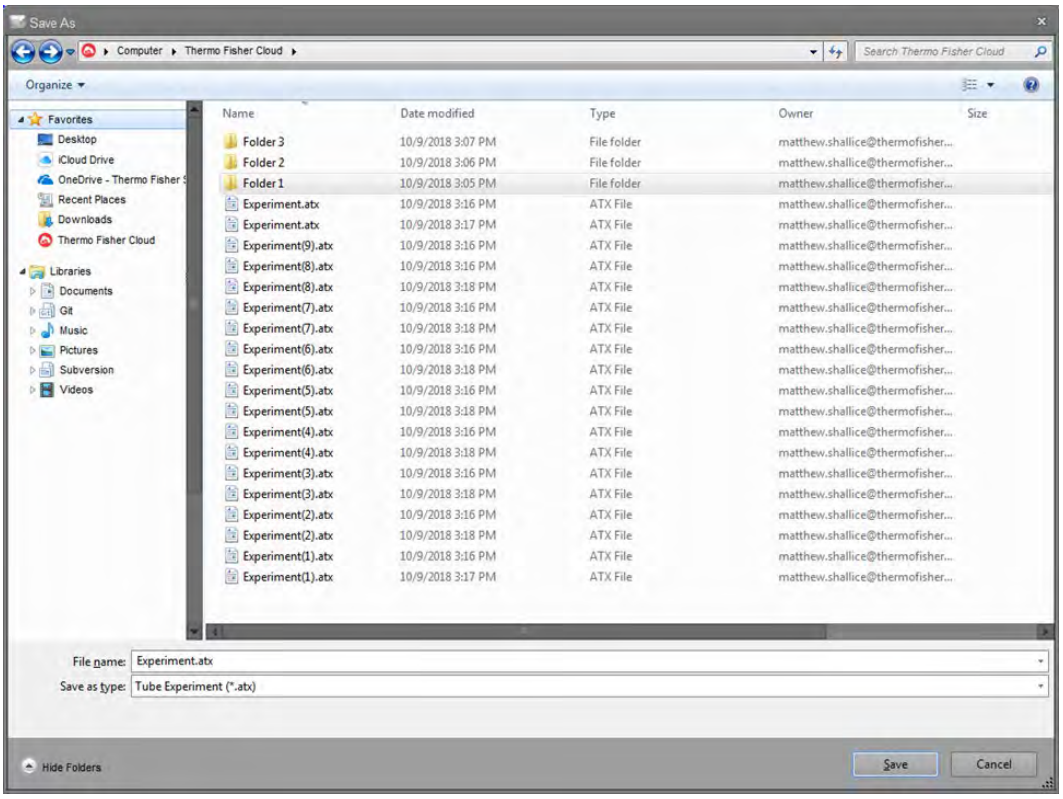
Note: Modal dialogs appear when you are performing tasks in the main software windows. You must interact with the modal dialog before you can return to the main window.

File dialogs

- *File Save (Export) dialog* (page 532)
- *File Open (Import) dialog* (page 535)
- *Folder Browser dialog* (page 537)

File Save (Export) dialog

Overview *File Save (Export)* dialog uses the standard Microsoft™ Windows browser, which allows you to save the selected files in the desired location.



Dialog behavior

- The File Save (Export) dialog opens to the last visited directory where files were saved.
- The default filter for FCS files is set to the FCS 3.1 version. Files can be saved as FCS 3.0 by selecting FCS 3.0 from the **Save as type** dropdown menu.
- When saving, the file extension is automatically added to each file type and the *Confirm File Save* dialog (page 427) is displayed, if required.
- If a name with invalid characters is entered or a name that results in a path length over 260 characters, a warning dialog indicating the error condition is displayed.
- Clicking **OK** on the warning dialog returns to the File Save (Export) dialog and the file is not saved.

File filters and default names

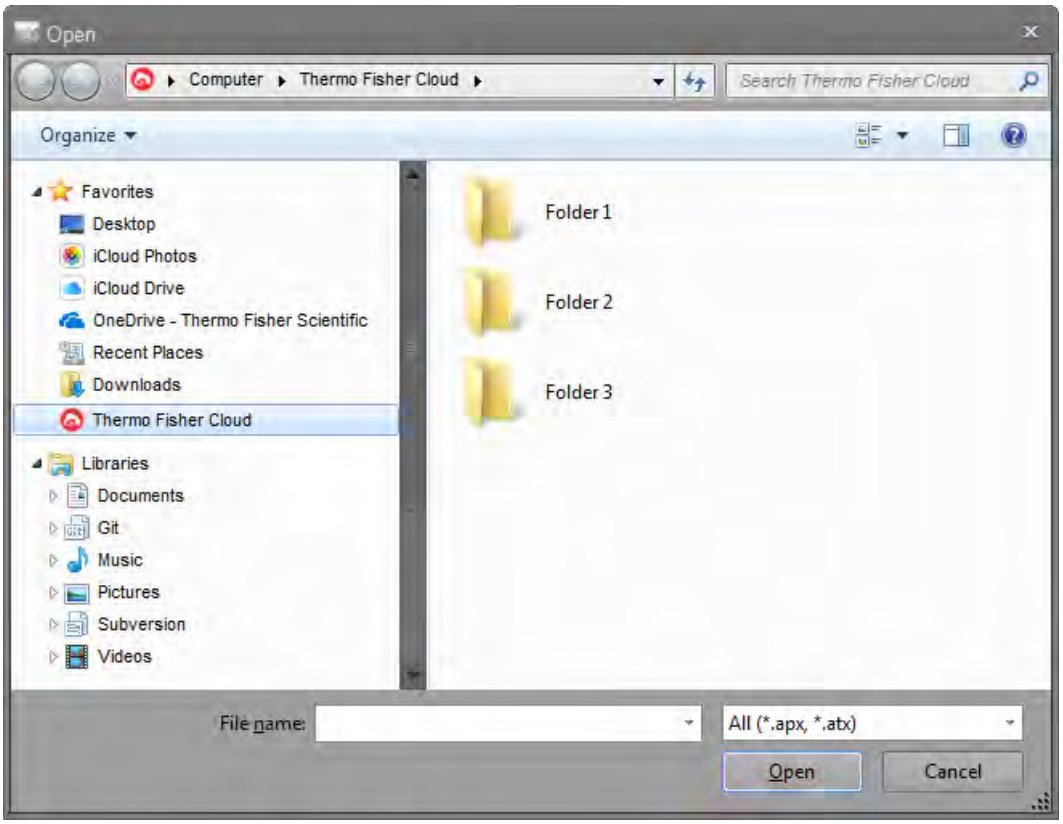
The file filters and default names depend on the source of the File Save (Export) dialog and are described in the following table.

Action/Source	Default Extension	Default File Name	Filter
Export FCS File	FCS	Name of File as saved by user	FCS 3.0 (*.fcs) FCS 3.1 (*.fcs)
Export Heat Map	AHM	Plate_Experiment Experiment	HeatMap File (*.ahm)
Export Run Protocol	ARP	Plate_Experiment_Group_Sample Experiment_Group_Sample	Run Protocol File (*.arp)
Export Workspace	AWS	Experiment-level: Plate_Experiment Group-level: Plate_Experiment_Group Sample-level: Plate_Experiment_Group_Sample	Workspace File (*.aws)
Export Instrument Settings	AIS	Experiment-level: Plate_Experiment Sample-level: Plate_Experiment_Group_Sample	Instrument Settings File (*.ais)
Export Template	AET	Tube or Plate	Template (*.aet)
Export Plate Experiment	APX	Plate	Plate Experiment (*.apx)
Export Tube Experiment	ATX	Experiment	Tube Experiment (*.atx)
Export Statistics (Single File Output)	CSV	Sample-level: Plate_Experiment_Group_Sample Results tab: Plate_Experiment Multiple files: "Blank"	CSV (*.csv)
Export Compensation Settings	ACS	Experiment-level: Experiment Sample-level: Plate_Experiment_Group_Sample	Compensation Settings (*.acs)
Send Table to XML	XML	Name of file as saved by user	XML (*.xml)
Print to PDF	PDF	Sample-level: Plate_Experiment_Group_Sample Results tab: Plate_Experiment Multiple files: "Blank"	PDF (*.pdf)
Save as Powerpoint	PPTX	Report: Plate_Experiment_Group_Sample	Powerpoint (*.pptx)
Save System Log	ALF	Long Date in system locale format	Log File (*.afl)
Export Gate to FCS	FCS	Sample_GateName	FCS 3.0 (*.fcs) FCS 3.1 (*.fcs)
Save as (to image)	PNG	Sample	PNG (*.png) GIF (*.gif) JPG (*.jpg) TIF (*.tif) BMP (*.bmp) PDF (*.pdf) Windows Metafile

Action/Source	Default Extension	Default File Name	Filter
Save as (cell image)	TIF-8	"Blank"	TIF-8 (*.tif) PNG (*.png) BMP (*.bmp) GIF (*.gif) JPG (*.jpg) EMF (*.emf)
Export Instrument Configuration	AIC	Instrument Configuration Name	AIC (*.aic)
Export Plates	APL	Multi-select: "Attune Plate Definitions" Single-select: <plate name>	APL (*.apl)
Sample List	CSV	Plate or Experiment name	CSV (*.csv)
Export Logs for Service	ZIP	AttuneServiceLogs_YYYY-MM-DD_HH-MM-SS (where YYYYMMDD_HHMMSS is the date and time)	ZIP (*.zip)

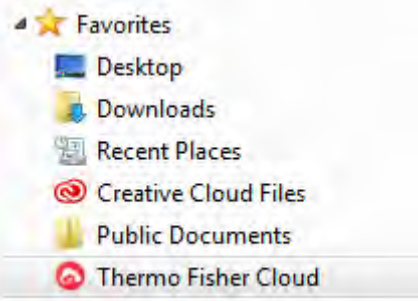
File Open (Import) dialog

Overview *File Open (Import)* dialog uses the standard Microsoft™ Windows File Open browser and is displayed when **Import** is selected from within the software.



Dialog behavior

- The File Open (Import) dialog opens to the last visited directory with open files.
- FCS files, Plate and Tube Experiments can be imported.
- If the device (instrument or software) has been registered to a Connect account, the File Open (Import) dialogue includes an icon for the Thermo Fisher Cloud listed under Favorites. Clicking on this icon opens a virtual folder listing all files and folders included in the Connect account of the user.



- Multiselect is enabled for FCS files when **Import FCS Files** is selected from the Experiment context menu on the Experiment Explorer (page 207), using the New Experiment Dialog's Analysis type experiment or Compensation Setup Dialog's "Files" source option. Multiselect is also available for Plate and Tube experiments. Multiselect is not enabled for the other file types.

- When FCS files are imported, additional options to update the experiment voltages and experiment compensation are provided.
- If the Instrument settings of the imported FCS file do not match the current Instrument configuration, no option to update the Experiment voltages or experiment compensation is provided.
- When multiple FCS files are selected, no option to update the experiment voltages or experiment compensation is provided

File filters

The file filters depend on the source of the File Open (Import) dialog command and are described in the following table.

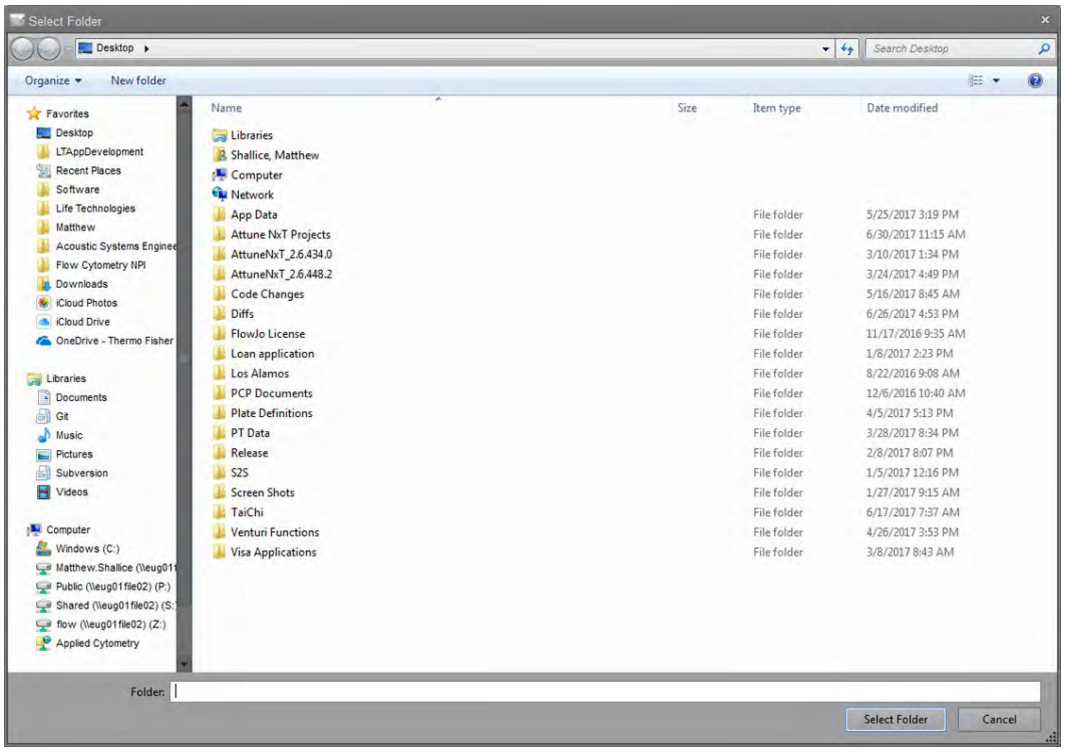
Action	Filter
Import FCS File	All (*.fcs,*.lmd)
Import Heat Map	Heat Map File (*.ahm)
Import Run Protocol	Run Protocol File (*.arp)
Import Workspace	Workspace File (*.aws)
Import Instrument Settings	Instrument Settings File (*.ais)
Import Template	Plate Template (*.aet)
Import Experiment files	All (*.apx, *.atx) **Note:** Plate Experiment (*.apx), Tube Experiment (*.atx)
Import Compensation Settings	Compensation Settings File (*.acs)
Import Bead Lot File	Bead Lot File (*.abl)
Import Instrument Configuration	Instrument Configuration (*.aic)

Folder Browser dialog

Overview

Folder Browser dialog is displayed when multiple files can be opened by selecting a folder or multi-selecting several files within a folder. Additionally, this dialog is used when a **Save** command requires the selection of a folder.

The style of the Folder Browser is the same as those used for the File Save (Export) and File Open (Import) browsers.



Dialog behavior

- The Folder Browser dialog opens to the last visited directory where files were open.
- When exporting, the file extension is automatically added to each file type and the *Confirm File Save* dialog (page 427) is displayed for each save action. .
- When exporting FCS files, the folder dialog includes the **Save as** option, which allows FCS files to be exported as FCS 3.1 or FCS 3.0 files.

File filters and default names

When exporting, file filters and default names are as described in the following table.

Action/Source	Default Extension	Default File Name	Filter
Import FCS Files	FCS	Name of File as saved by user	All (*.fcs,*.lmd)
Export FCS Files	FCS	Plate_Experiment_Group_Sample	FCS 3.1 (*.fcs)
Export Statistics (Multiple File Output)	CSV	Sample-level: Plate_Experiment_Group_Sample	CSV (*.csv)

27. Attune™ Database Utility

Overview

The Attune™ Database Utility is a data backup program that is provided with the Attune™ Cytometric Software. This utility program allows you to back up your data to a custom location, either on-demand or at a specified, scheduled frequency.

The Attune™ Database Utility is used for the following procedures:

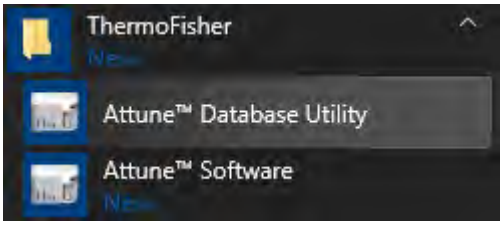
- **Backup** existing data and the database.
- **Restore** backed up data and database.
- **Schedule** an automate backup of the data and database.
- **Reinstall** the database.

Startup

Main application startup

The Attune™ Database Utility can be launched via the Windows "Start" menu or directly from the Attune™ Cytometric Software.

1. Select **Start ▶ All Programs ▶ ThermoFisher ▶ Attune ▶ Attune™ Database Utility**.



   Alternatively, launch the Attune™ Database Utility from within the Attune™ Cytometric Software using the **Options ▶ Administrator options ▶ Backup options** dialog as described on page 501.

2. The application opens to the Login screen (page 539).

Startup Errors

PostGreSQL Error

If the database application is missing (PostGreSQL), the utility will not start and will display an error dialog with the following message:

"PostgreSQL 9.3.5 was not found. Please install the PostgreSQL using the Attune™ installer."

Database Missing

If the Attune™ database is not present when starting the Attune™ Database Utility, the application launches in Recovery mode.

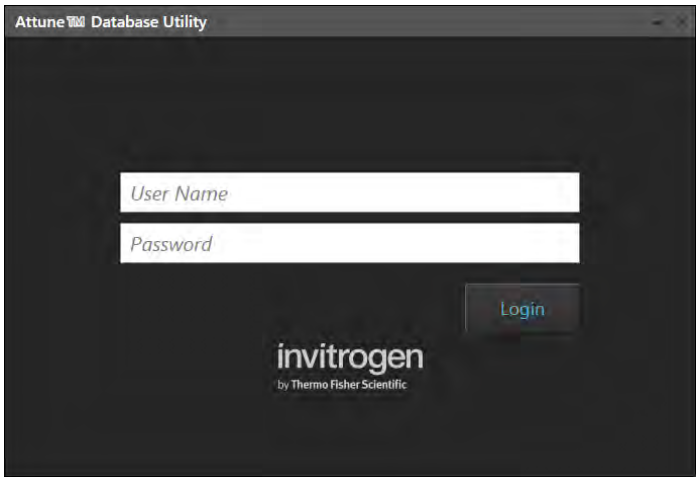
In Recovery mode, the application launches to the Restore page, which allows you to run the Restore or Reinstall database options. In this case, no login is required.

Login screen

Overview

The *Attune™ Database Utility Login* screen is the first screen that is displayed after the launch of the utility. Each user is required to sign in before being allowed to use the utility.

Depending on the type of user (i.e., Administrator, System Administrator, User, etc.) certain features are available in the utility.



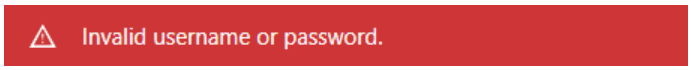
User sign in

1. To sign into the Attune™ Database Utility, type a valid username and password in the corresponding text box field and click **Login**.

   Alternatively, press the **Enter** key on the computer keyboard after typing the username and password.

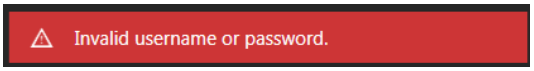
2. If the correct username and password are entered, the user login is completed and the *Attune™ Database Utility* opens.

   If an invalid username or password is entered, the warning banner displays "Invalid username or password" (see "Login Warnings").



Login warnings

- If an invalid username or password is entered, the warning banner displays "Invalid username or password". A valid username and password must be entered to proceed.



- If the security settings (page 512) have been set to lockout a user after a certain number of failed attempts the warning banner displays "Invalid username or password. Your account has been locked. Try again in X minutes."

- If an attempt is made to login to an account that has been disabled, the warning banner displays "Your account has been disabled. Contact an administrator."

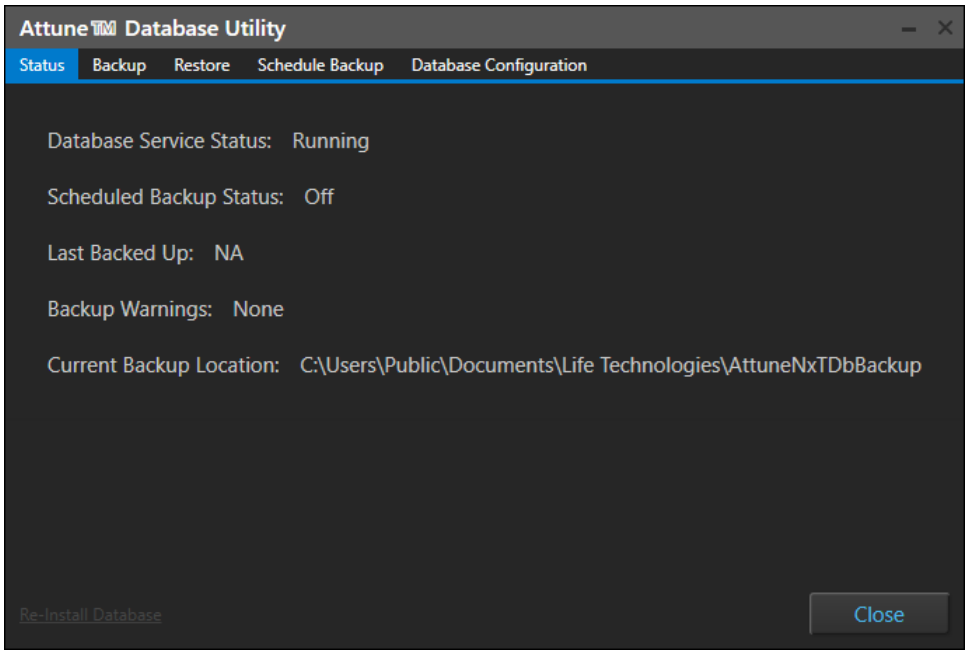
Main Application

Overview The main application of the Attune™ Database Utility consists of a dialog box that contains the following controls:

- *Status tab* (page 541)
- *Backup tab* (page 542)
- *Restore tab* (page 544)
- *Schedule Backup tab* (page 545)
- *Reinstall Database hyperlink* (page 546)
- *Database Configuration* (page 547)

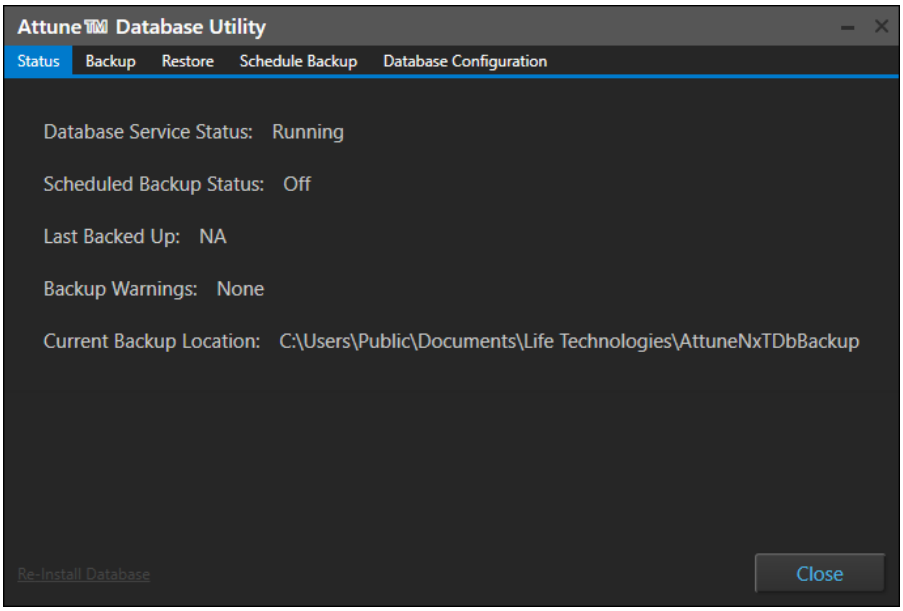
The visibility of the tabs is dependent on the user role. All users can see the Status tab and Backup/Restore tab. Only a System Administrator or Administrator can see the scheduled backup and restore tabs.

By default, Status tab is selected upon logging into the application.



Status tab

The Status tab displays the current status of the scheduled backup, the last backup time and date, the backup path, and any errors reported by the backup.



Database Service Status

- The *Database Service Status* checks to see if the database service is running in the background. Typically, this will display the status of "Running".
- If the backup service is "Stopped", an option to start the backup service appears as the Start Service button next to the text.

  Click the **Start Service** button to start the backup.
- Successful start changes the status to "Running" and hides the Start Service button.

Scheduled Backup Status

The *Scheduled Backup Status* shows the current ON/OFF state of the scheduled backup.

Last Backed Up

- The *Last Backed Up* displays the date and time of the last backup in system locale format with the time shown in 24 hour format.
- If no backup has occurred, the status displays NA.

Backup Warnings

The *Backup Warnings* displays any warnings or errors that have occurred while performing a scheduled backup.

If there are no warnings or errors, the status displays "None".

Possible Errors include:

- **Insufficient Disk Space** warns that the attempted backup or database restore would consume >90% of the free disk space.
- **Security Error** indicates that the user does not have permission to write files or folders at the destination.
- **Directory Not Found** indicates that the destination directories have been removed or renamed during backup or restore process.

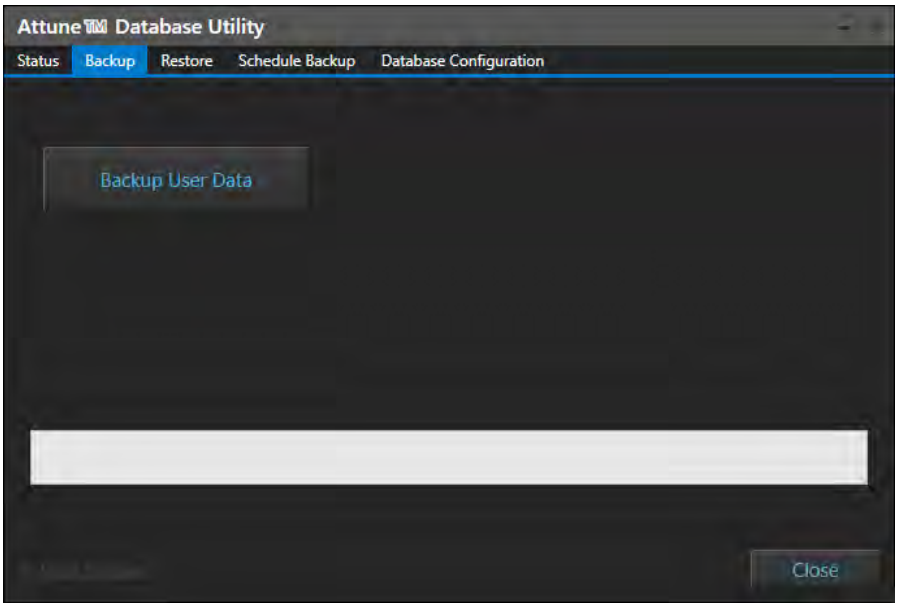
- **Path Too Long** indicates that the file path name exceeds the number of characters allowed by the system.
- **Other exceptions** lists the last of the backup fails due to unforeseen problems.

Current Backup Location

The *Current Backup Location* displays the current file path of the scheduled backup. If no path is specified, the status displays “NA”.

Backup tab

The *Backup tab* allows the user currently signed in to back up all user data and the database to the location specified by the user (“On demand” backup of user data).

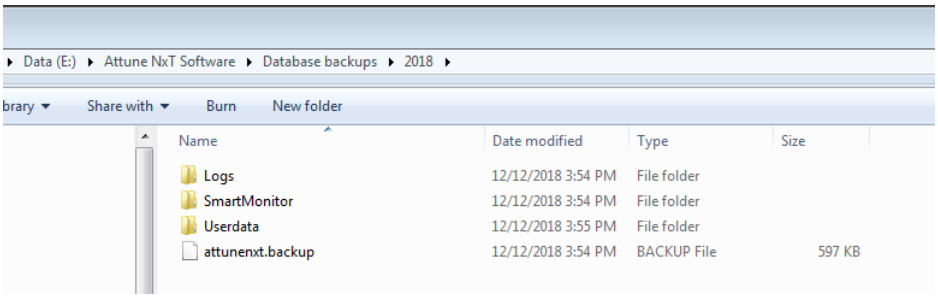


Backup User Data (“On demand” backup of user data)

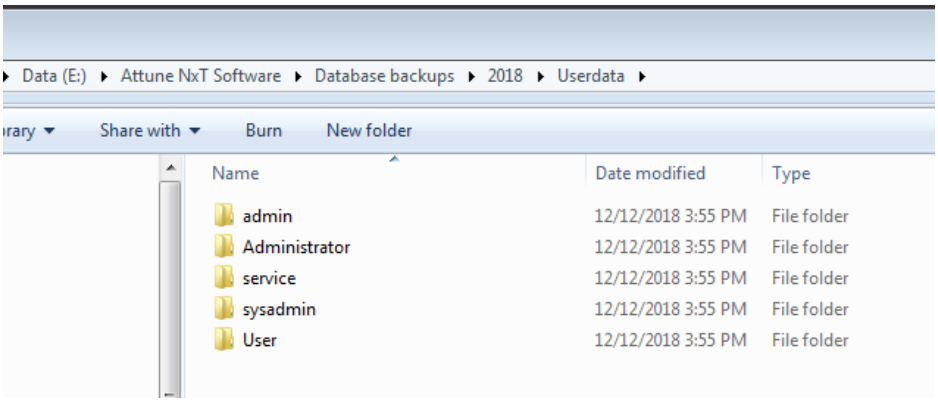
Click the **Backup User Data** button to open the file browser and select a location to back up the database and the corresponding data.

After confirming a location for the data backup, the database utility copies all user data located in **\Users\Public\Public Documents\Thermo Fisher\AttuneNxt\Userdata** and exports the database to the specified location.

- The database backup file that is created is called “attunenxt.backup” and includes information regarding the data structure.
- The data that are copied during the backup include copies of the Log files, Smart Monitor Data, and Userdata folders, which are in folders at the backup location.



- Within the Userdata folder are folders for each account created in the software, including the default Service, System Administrator (sysadmin) and Administrator (admin) accounts, as well as custom, user-created accounts.



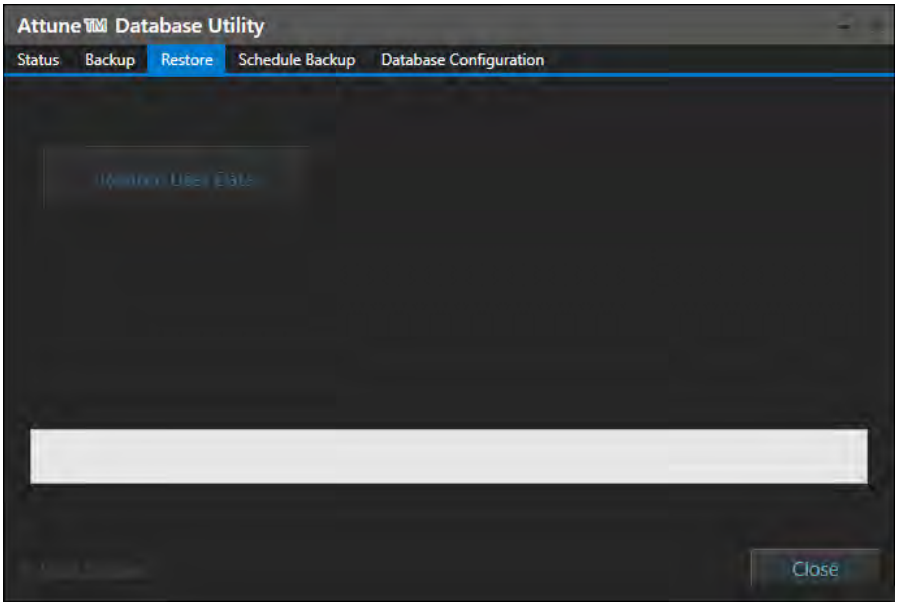
- Within the folder for each user profile are data for each user account, including saved FCS files, and data associated with Experiment and Plate files, including Instrument settings, Workspaces, Compensation settings, Run protocols, Heat Map settings, Print settings, Overlay settings, Sample List settings, and Results Table settings.
- After a database backup has been completed once, if the same database backup location is selected for a secondary backup, only the files that have changed since the last backup are recopied. Any data files that have not changed are not recopied to the backup location. The new backup file replaces the previously saved attunenxt.backup file.

Progress

A progress bar shows the progress of the backup operation as a percentage towards completion. The progress bar shows the current status as “Copying data” or “Backing up database”.

Restore tab

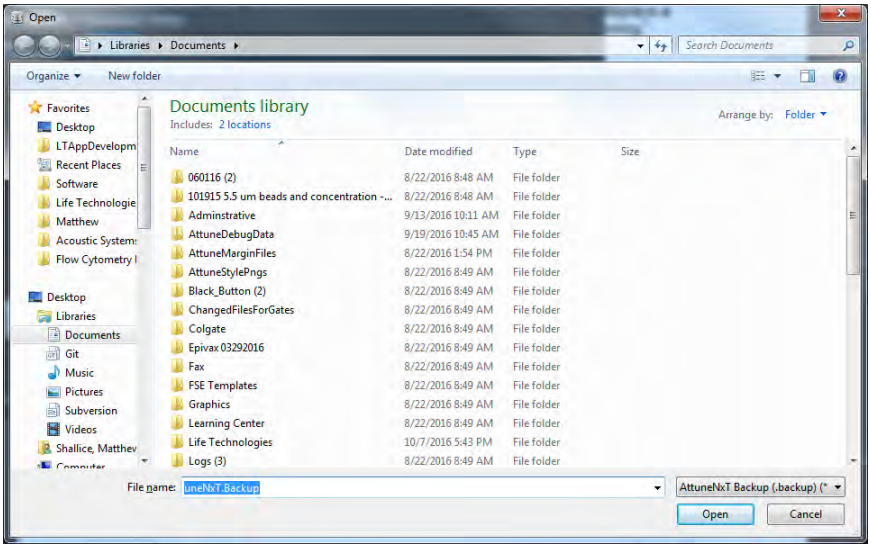
The *Restore tab* allows the user currently signed in to restore all user data and the database using a previously saved database.



Restore User Data

Restore user data cannot be completed when the Attune™ Cytometric Software is open. If the application is running, this option is disabled and displays a tooltip "This operation cannot be performed while the application is running."

1. Click **Restore User Data** to open the Open File dialog:



2. Select a backup file and click **Open** to initiate the restore operation.

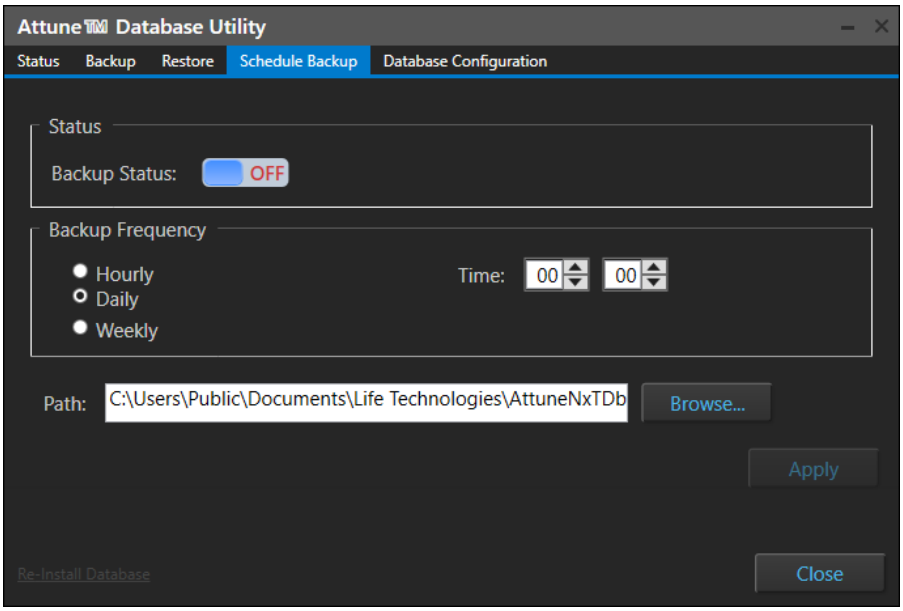
IMPORTANT! If the current database includes data that is not saved in the database backup file, new data is overwritten and lost as part of the database restoration process. To prevent data loss from occurring, export the data to a secondary location before initiating the database restoration.

Progress

A progress bar shows the progress of the backup operation as a percentage towards completion. The progress bar shows the current status as "Copying data" or "Restoring database".

Schedule Backup tab

The *Schedule Backup tab* allows an authorized user to set the backup policy and schedule. This tab is only visible to the System Administrator or Administrators.



Status

The *Status* group includes a toggle control to turn the backup on or off.

- By default the **Backup Status** option is set to OFF
- When turned ON, the backup will attempt to backup to the specified path and frequency.
- If an error occurs while performing a scheduled backup, the error is displayed on the Status tab (page 541).
- When turned OFF, no automatic backup will occur.
- If a valid path is not entered, a warning balloon is displayed if the user tries to turn the status ON: "Valid path is required to schedule an automatic backup."

Backup Frequency

The *Backup Frequency* sets the frequency of the scheduled backup. You can set the scheduled backup to run **Hourly**, **Daily**, or **Weekly**.

- By default, the frequency is set to **Daily**.
- The **start time** must be set to specify the time to run the backup. The time format is in 24 hour time as HH:MM.
- By default, the backup is scheduled to run at 00:00.
- When the **Hourly** option is selected, the time entry is not visible and the backup runs at the start of the hour (:00).
- When the **Weekly** option is selected, the day must be specified. By default, the day is set to Sunday.

Path

You can type the File Path of the backup directory directly into the textbox or click **Browse** to select it from the File path dialog.

If the File Path does not exist, the directory is automatically created when the application is closed.

Copied Files

The first time the database backup is completed, all data is copied to the backup location and an “attunenxt.backup” file is created.

Every time the scheduled backup occurs thereafter, only data that has changed since the last backup are copied; any data files that have not changed are not recopied to the backup location.

The new backup file replaces the previously saved “attunenxt.backup” file each time the scheduled backup is completed.

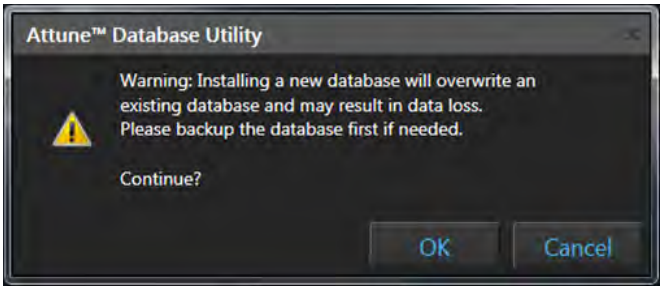
Reinstall Database

The *Reinstall Database* hyperlink is only visible to the System Administrator and Administrators. Reinstallation of the database replaces the existing database with a new, blank database.

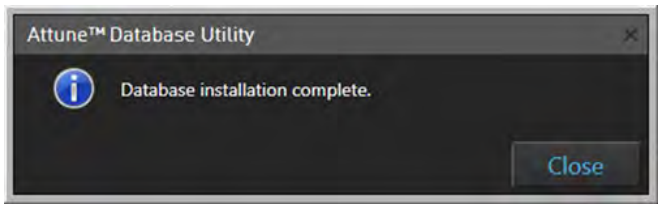
Note: Database reinstallation completely erases all data in the current database. However, it does not erase data saved in the previous backup.

IMPORTANT! If the backup scheduler is set to automatically back up user data at a specified time, the first scheduled backup that occurs after a database reinstallation overwrites all previously saved data. To prevent data loss from occurring, copy the Userdata folders and “attunenxt.backup” file to an alternate location before using the scheduled backup procedure.

1. Click the **Reinstall Database** hyperlink. A confirmation dialog opens.



2. Click **OK** to reinstall the Attune™ Database. Upon completion of the reinstall, the following confirmation dialog appears:



Note: If the Attune™ application is running, the Reinstall Database hyperlink is disabled and displays a tooltip “This operation cannot be performed while the application is running.”

Database Configuration tab

The *Database Configuration tab* allows an authorized user to set the Database Storage Options by selecting the Image Storage Path for images captured with the Attune™ CytPix™ Flow Cytometer.

This tab is only visible to the System Administrator or Administrators when an Attune™ CytPix™ Flow Cytometer is in use.

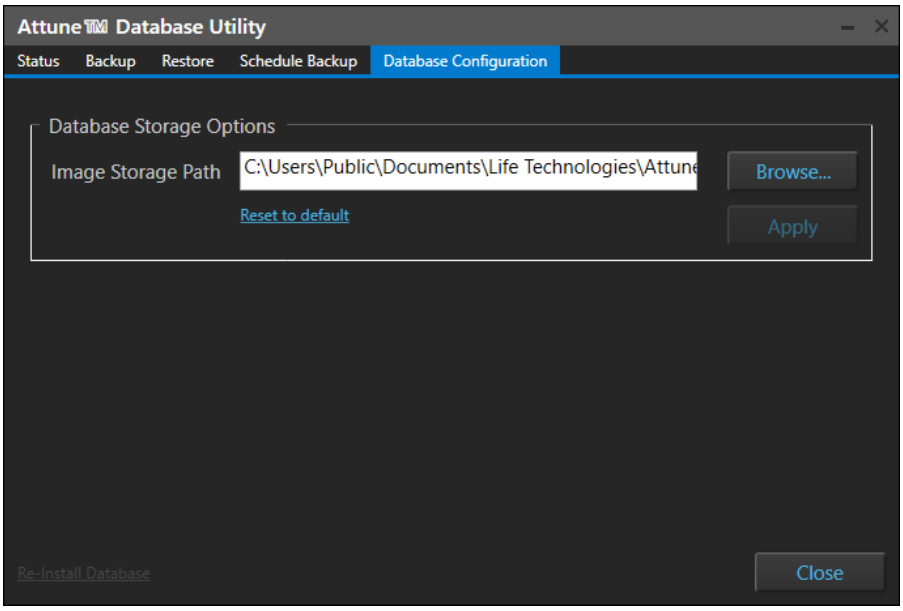


Image Storage Path

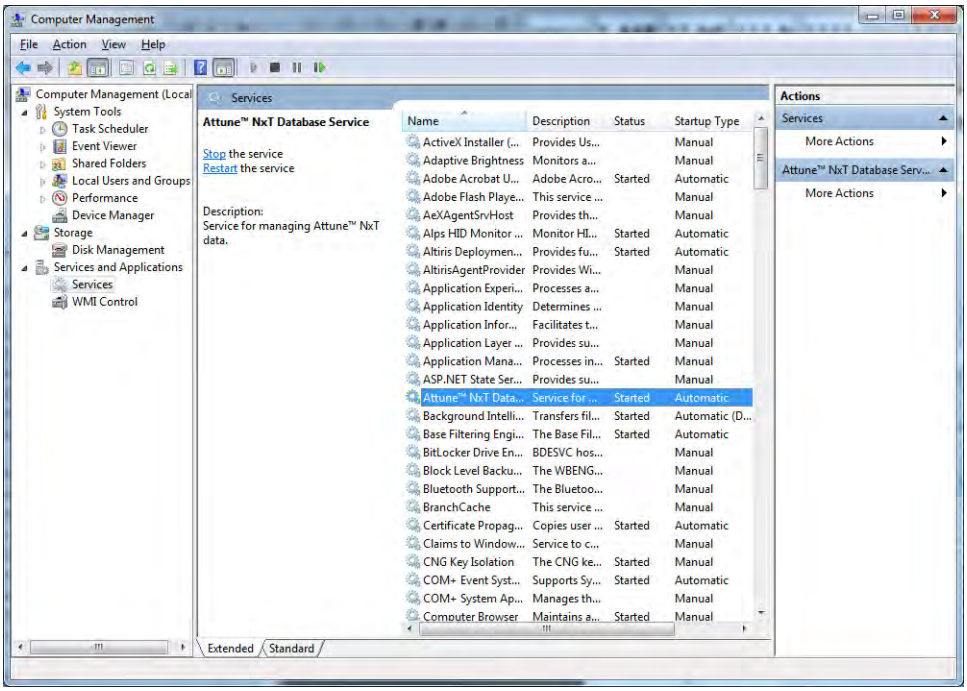
- You can type the File Path of the backup directory directly into the textbox or click **Browse** to select it from the File path dialog.
- If the File Path does not exist, the directory is automatically created when the application is closed.
- The default image storage path is:

  C:\Users\Public\Documents\Life Technologies\AttuneCytPix_Images
- To reset the image storage path, click **Reset to default**.

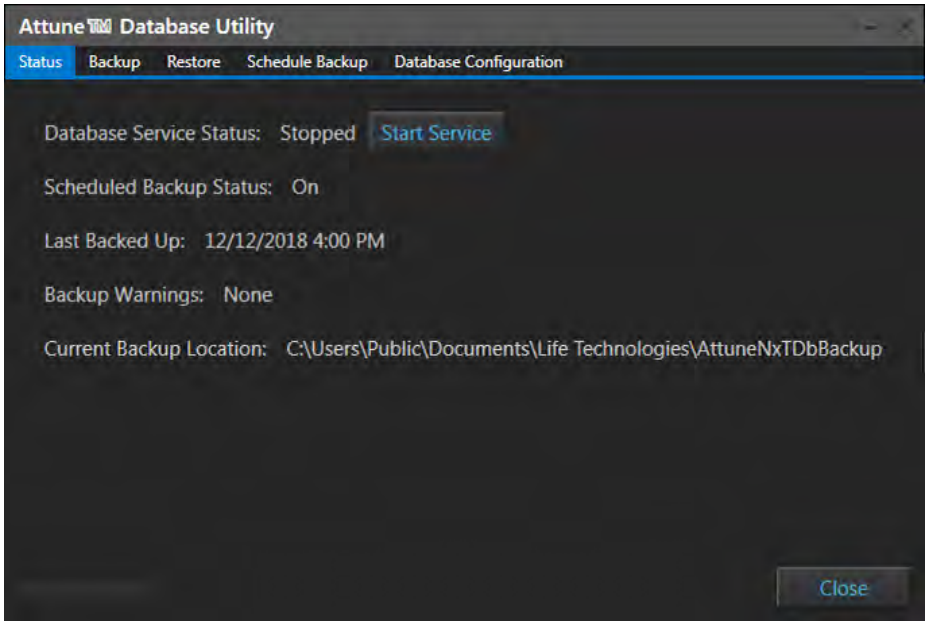
Attune™ Database Service

The Attune™ Database Service is a Windows® data backup service that runs in the background and performs the backup at the scheduled time. You can manage the service via the Windows® Management Console.

1. To access the Windows Management Console, select **Start**, then right-click **Computer ▶ Manage**.



2. You can start or stop the service from Windows Management Console or double-click the service to get more options.
3. After the service is stopped, you can start the service again from the Attune™ Database Utility application.



Appendix A: FCS file reference

Overview

This chapter describes the FCS keywords that are used when generating FCS files from data acquired from the Attune™ NxT Flow Cytometer.

- When in the Record mode, the FCS is recorded regardless of whether the stop criteria are met.
- For appended data sets, the time and event offsets (elapsed time from $BTIM in ms and $TOT of previous dataset; see below) are added to the time and event parameter values (when enabled) in the new data stream.
- The FCS files generated by the Attune™ Cytometric Software when recording or exporting conform to the FCS standard as described by the Data File Standards Committee of the International Society for Analytical Cytology (ISAC) for FCS 3.1.

FCS HEADER format

The *FCS HEADER* conforms to the FCS standard as described by the Data File Standards Committee of the International Society for Analytical Cytology (ISAC). An example table is displayed below.

Contents	Start by position	End byte position
FCS 3.1	00	05
ASCII(32) – space characters	06	09
ASCII-encoded offset to first byte of TEXT segment	10	17
ASCII-encoded offset to last byte of TEXT segment	18	25
ASCII-encoded offset to first byte of DATA segment	26	33
ASCII-encoded offset to last byte of DATA segment	34	41
ASCII-encoded offset to first byte of ANALYSIS segment	42	49
ASCII-encoded offset to last byte of ANALYSIS segment	50	57
ASCII-encoded offset to user-defined OTHER segment	58	beginning of next segment

FCS TEXT segment

- The delimiter used in the TEXT segment of the FCS file is **/** (ASCII 47). If the delimiter appears in a keyword or keyword value, it is immediately followed by a second delimiter **//**.
- For custom keywords, the **#** symbol precedes the keyword name instead of the **$** symbol.
- The FCS Keywords in the FCS TEXT segment are listed in alphabetical order.
- The parameter description keywords ($PnN, $PnR, etc.) in the FCS TEXT segment are numbered consecutively and are listed in the order the parameters are written to the file.

Required keywords

Keyword	Description	Source of Value
$BEGINANALYSIS	Byte-offset to the beginning of the ANALYSIS segment	This is set to "0"
$BEGINDATA	Byte-offset to the beginning of the DATA segment	By default, this is set at 8192. If the TEXT segment exceeds this byte offset, the value will be set to ensure correct alignment of the DATA segment. This byte-offset is a duplicate of the offset contained in the HEADER. If the DATA segment exceeds the first 99,999,999 bytes, the offset in the HEADER will be set to 0 and this value will take precedence.
$BEGINSTEXT	Byte-offset to the beginning of a supplemental TEXT segment	This is set to "0"
$BYTEORD	Byte order for data acquisition computer	This is set to "1,2,3,4"
$DATATYPE	Type of data in DATA segment (ASCII, integer, floating point)	This is set to "F"
$ENDANALYSIS	Byte-offset to the end of the ANALYSIS segment	This is set to "0"
$ENDDATA	Byte-offset to the end of the DATA segment	This is dynamically updated based on the offset of the last DATA byte.
$ENDSTEXT	Byte-offset to the end of a supplemental TEXT segment	This is set to "0"
$MODE	Data mode (list mode, histogram)	This is set to "L"
$NEXTDATA	Byte offset to next data set in the file	This is set to "0"
$PAR	Number of parameters in an event	From the total number of enabled parameters, including custom parameters, recorded in to the FCS file.
$PnB	Number of bits reserved for parameter number n	This is set to "32"
$PnE	Amplification type for parameter n	This is set to 0,0
$PnR	Range for parameter number n	By default this is set to 2^{26} for Event.* By default this is set to 2^{26} for Time.* By default this is set to 2^{20} for all Height measurements.* This is set to 2^{20} for all Area measurements.* By default this is set to 2^{10} for all Width measurements.*
$TOT	Total number of events in the data set	Number of events in the file (updated when appended).

* Data values transferred from the instrument are constrained to be within the $PnR limits.

Optional keywords

Keyword	Description	Source of Value
$ABRT	Events lost due to data acquisition electronic coincidence	From datastream when "Exclude coincident events" is selected in the Instrument Settings Panel as described on page 259.
$BTIM	Clock time at beginning of data acquisition	The system clock time at the time the recorded data stream commences.
$CELLS	Description of objects measured	From Sample information Notes field as described on page 309.
$COM	Comment	
$CYT	Type of flow cytometer	From the Attune™ model name and number as described on page 521.
$CYTSN	Flow cytometer serial number	From the cytometer serial number as described on page 521.
$DATE	Date of data set acquisition	The system clock date at the time the recorded data stream commences.
$ETIM	Clock time at end of data acquisition	The system clock time at the time the recorded data stream ends. For appended data, this is based on the time of the last addition to the data file.
$EXP	Name of investigator initiating the experiment	From the name of the PI, if specified, for the currently logged in user as specified on page 523.
$FIL	Name of the data file containing the data set	From the sample name at the time the data is recorded. This is the pathless filename and extension.
$INST	Institution at which data was acquired	From the institution name field as specified on page 523.
$LAST_MODIFIED	Timestamp of the last modification of the data set	The time a data file is appended or updated based on exporting the dataset. This is only added when the $ORIGINALITY is not set to Original.
$LAST_MODIFIER	Name of the person performing last modification of a data set	The name of the currently logged in user when a data file is appended or updated based on exporting the dataset. This is only added when the $ORIGINALITY is not set to Original.
$LOST	Number of events lost due to computer busy	From the data stream
$OP	Name of flow cytometry operator	From the name (first and last) of the currently logged in user
$ORIGINALITY	Information whether the FCS data set has been modified (any part of it)	For appended datasets, the keyword value is set to "Appended". For exported data where the TEXT segment or data segment is modified, the keyword value is set to "DataModified". When the dataset has not been modified, this is set to "Original".
$PLATEID	Plate identifier	From the "Plate ID" field s. If it is left blank, the keyword is the plate name.
$PLATENAME	Plate name	From the "Plate Name" field.
$PnF	Name of optical filter for parameter n	From the instrument configuration information for the specified detector.
$PnL	Excitation wavelength(s) for parameter n	From the instrument configuration information for the specified detector.
$PnS	Name used for parameter n	From the concatenation of the "target and label". When absent, the $PnS is equivalent to the $PnN.
$PnV	Detector voltage for parameter n	From the voltage for each detector at the time the file was recorded.
$PnN	Channel name for parameter n	From the channel name for the specified detector.
$PROJ	Name of the experiment project	From the experiment name.
$SMNO	Specimen (e.g., tube) label	From the group name.
$SPILLOVER	Fluorescence spillover matrix	From the Experiment-level spillover matrix state at the time the file is recorded or exported.
$SRC	Source of the sample (sample name, cell types)	
$SYS	Type of computer and its operating system	From the system attributes including computer type and operating system.
$TIMESTEP	Time step for time parameter	This will be set to 0.001 but should be provided by the instrument API.
$TR	Trigger parameter and its threshold	From the threshold settings set at the time a file was recorded in the format (Boolean_Channel, Boolean_Channel....).

Keyword	Description	Source of Value
$VOL	Volume of sample run during data acquisition	From the instrument API. Volume is updated to reflect total volume for all draws of a multidraw, and, if appended, the volume is totaled.
$WELLID	Well identifier	From the well location (i.e., A1, A2,...) for a Plate sample. For tubes recorded in a Plate Experiment, the tube location for the Tube sample is recorded (i.e., T1, T2...).

Custom keywords

Keyword	Description	Source of Value
#TOTALVOLUME	Volume of sample run during data acquisition	From the instrument API. Volume is updated to reflect total volume for all draws of a multidraw, and, if appended, the volume is totaled.
#PnTarget	The target name for parameter n	From the target field for each parameter within the Instrument Settings Panel.
#PnLabel	The label name for parameter n	From the label field for each parameter within the Instrument Settings Panel.
#FLOWRATE	The flow rate of the sample during data acquisition	From the instrument API or collection panel state.
#LASER1ASF	Area Scaling Factor used for channels recorded off of laser 1	Area scaling factor for detectors off of laser 1 at time of recording.
#LASER2ASF	Area Scaling Factor used for channels recorded off of laser 2	Area scaling factor for detectors off of laser 2 at time of recording.
#LASER3ASF	Area Scaling Factor used for channels recorded off of laser 3	Area scaling factor for detectors off of laser 3 at time of recording.
#LASER4ASF	Area Scaling Factor used for channels recorded off of laser 4	Area scaling factor for detectors off of laser 4 at time of recording.
#LASER1DELAY	Laser Delay of Laser 1	The laser delay setting for laser 1 at time of recording. Only recorded if laser 1 is present and turned on.
#LASER2DELAY	Laser Delay of Laser 2	The laser delay setting for laser 2 at time of recording. Only recorded if laser 2 is present and turned on.
#LASER3DELAY	Laser Delay of Laser 3	The laser delay setting for laser 3 at time of recording. Only recorded if laser 3 is present and turned on.
#LASER4DELAY	Laser Delay of Laser 4	The laser delay setting for laser 4 at time of recording. Only recorded if laser 4 is present and turned on.
#WINEXT	Window Extension	The window extension setting at time file is recorded
#PTRESULT	Performance Test Result	Performance Test status at time of recording (Pass/Fail)
#PTDATE	Date of most recent performance test	Date of most recent performance test at time of recording.
#WIDTH THRESHOLD	Width Threshold	The width threshold setting at the time of recording. Only needed, if width is a recorded measurement.
#LASER1COLOR	Laser 1 Color	Laser 1 Color at time of recording. Specified in terms of wavelength in units of µm.
#LASER2COLOR	Laser 2 Color	Laser 2 Color at time of recording. Specified in terms of wavelength in units of µm.
#LASER3COLOR	Laser 3 Color	Laser 3 Color at time of recording. Specified in terms of wavelength in units of µm.
#LASER4COLOR	Laser 4 Color	Laser 4 Color at time of recording. Specified in terms of wavelength in units of µm.
#LASERCONFIG	The laser configuration of the Attune™ Instrument	Laser configuration at time of file recording.
#DRAWnVOLUME	Acquisition volume of the nth draw in a multi-draw	Instrument API will provide this value upon completion of nth draw. Only recorded if the TIME acquisition parameter is enabled.

Keyword	Description	Source of Value
#DRAWnSTART	Acquisition start time in terms of the TIME parameter	Instrument API will provide this value upon start of nth draw. Only recorded if the TIME acquisition parameter is enabled.
#DRAWnSTOP	Acquisition stop time in terms of the TIME parameter	Instrument API will provide this value upon completion of nth draw. Only recorded if the TIME acquisition parameter is enabled.

Storage of user-defined keywords in the FCS file

User-defined keywords are stored as custom keywords when recording FCS files.

- When recording FCS files, the keyword values are written to the FCS file as customer keyword value pair, where the **#** symbol is added to the front of the name to indicate that it is a custom keyword.
- The custom keywords are also considered when exporting an FCS file and the user is provided with the option to update these keywords.

Storage of compensation values in the FCS file

- If an Experiment Compensation exists prior to recording a sample, the spillover values are recorded into the FCS file using the $Spillover keyword.
- Only the parameters that are included in the compensation calculation are included in the $Spillover keyword value.
- Compensation controls only contain the identity matrix.

Appendix B: Data management in Attune™ Cytometric Software

Overview

This section describes options for managing (saving and/or deleting) data in the Attune™ Cytometric Software. Note that it is critical for you to review all instructions completely before executing the steps outlined in this section. We recommend that you archive data prior to deletion.

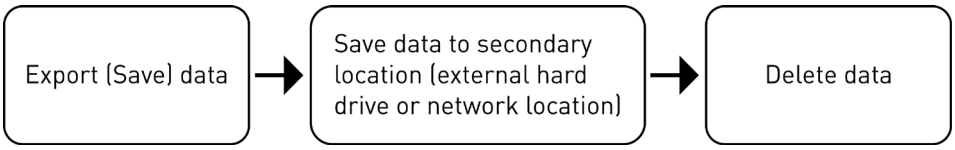


Figure 1. Data management workflow

Contents

- Saving User data
- Types of Data included in the Attune Software
- Choosing one or more data archiving methods
- Database backup
- How to Save and Export Plate and Tube Experiment Files
- How to Save and Export FCS Files
- How to Use the Attune Database Utility Program
- How to Restore Data using a Saved Database
- How to Copy a Database to a Different Computer
- Deletion of Experiment and Plate Files
- Reinstallation of a New Database
- Deleting a User Account to Remove Data
- Permanent Removal of old Baseline and Performance Test Data

Types of data in Attune™ Cytometric Software

There are many types of data associated with the Attune™ instrument and software (Figure 2). Users create "data" each time they create a Tube or Plate Experiment and when FCS files are recorded during sample acquisition. Data also refers to other inputs into the software, such as instrument management information that allow the cytometer to collect samples, retain performance data and log information. User management data is retained by the software and provides instructions to manage user accounts, security, and user preferences. Data saved from analysis of results include information stored in the Heat Map, Results table, and Overlay settings.

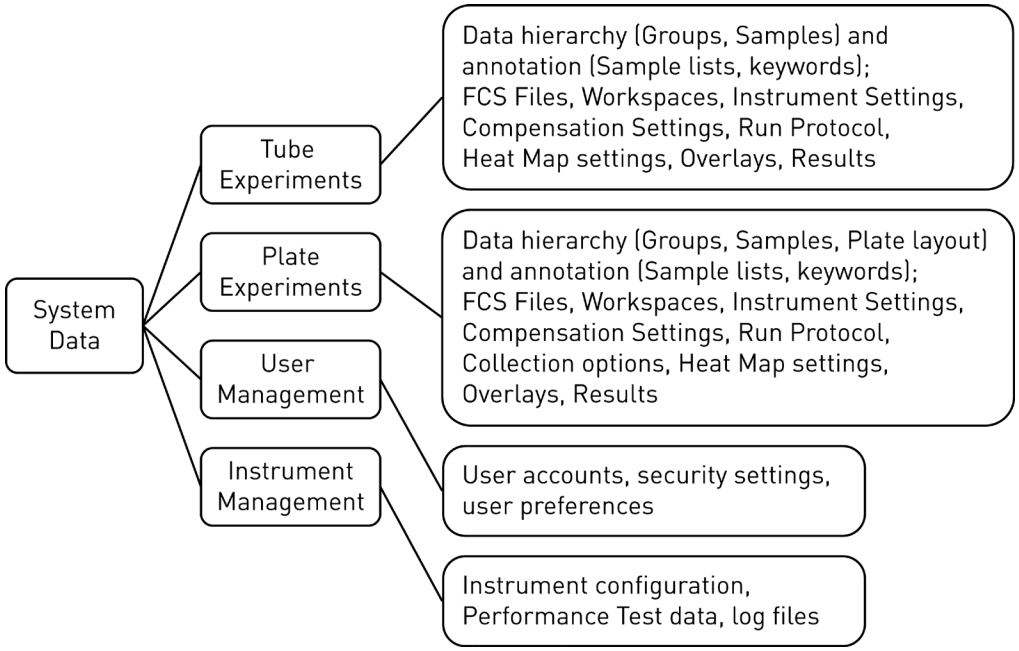


Figure 2. Data within the Attune™ Cytometric Software

Options for saving User data

There are many options for saving user data from the Attune™ Cytometric Software. You can export Tube and Plate experiment files (.apx and .atx) or individual FCS files (.fcs) from the software and store them in a secondary location. Alternatively, you can use the Attune™ Database Utility program to create a copy of all the experiments and associated data files in the software at the time the backup is created.

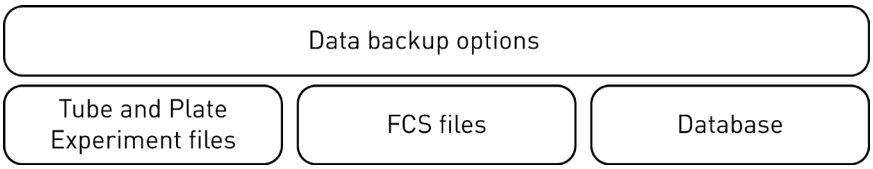


Figure 3. Options of saving User data from the Attune™ Cytometric Software

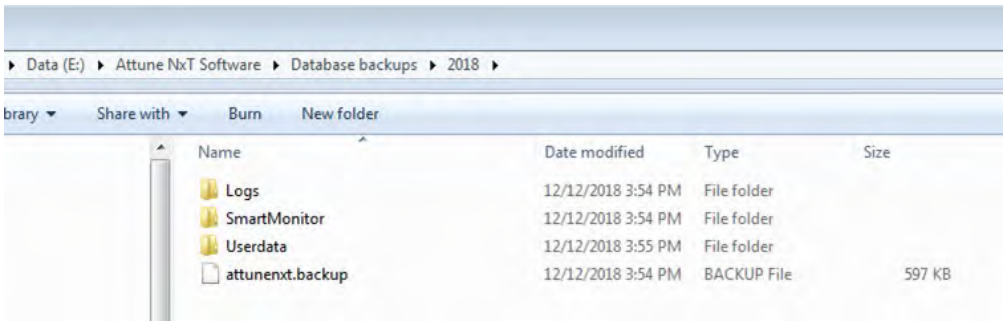
Choosing one or more data archiving methods

- When you export a Tube experiment file as an .atx file, the information saved includes all data associated with the Experiment: Groups and Samples, recorded FCS files, Run Protocol settings, Instrument settings, Workspace settings, Compensation files and settings, Heat Map settings, Sample lists, Overlays, Results table settings, and print settings.
- When you export a Plate experiment as an .apx file, the information that is saved includes the same information as an Experiment file, but also includes Plate layout information and the Plate collection settings.
- When you export FCS files, you can save the file as an FCS 3.0 or FCS 3.1 file data standard. The information that is saved includes the raw data acquired for the sample and associated sample information that is annotated using FCS file keywords (see "Appendix A: FCS file reference", page 549). Some keywords provide annotation and standardization about the sample (such as cytometer used for acquisition, time of acquisition, number of events, etc.), while others are Attune™ NxT custom keywords (including flow rate, etc.) or user defined custom keywords (such as dilution factor, sample ID, etc.). You can re-import FCS files individually into the Attune™ Cytometric Software or you can analyze them using other flow cytometry analysis software.
- When the Attune™ Database Utility program is used as a file backup system (see "Database backup", page 556), the information that is saved includes all data files used in the software as described above and also includes user management settings (profiles, user settings, security settings), instrument configuration settings, baseline and performance test data, and instrument data logs.

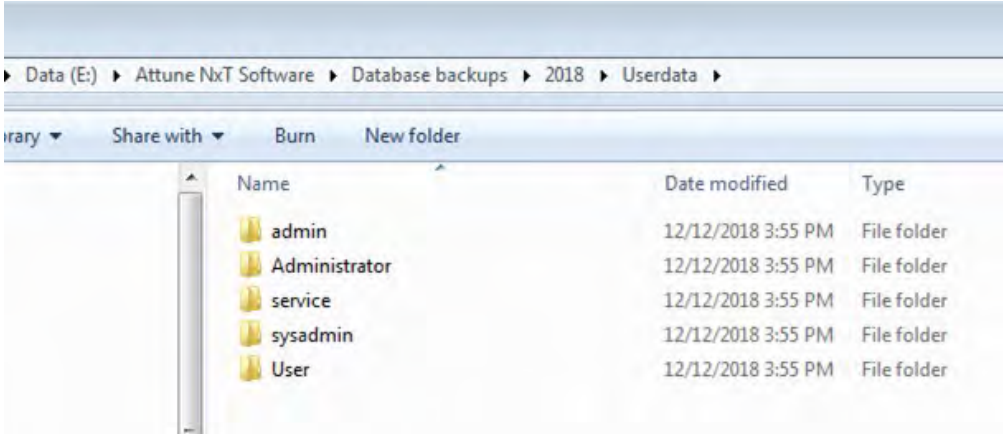
Database backup

The Attune™ Database Utility (page 538) is a data backup program that is provided with the Attune™ Cytometric Software. This utility program allows you to back up user data to a custom location, either on-demand or at a specified, scheduled frequency.

- The database utility copies all user data located in the "Users\Public\Public Documents\Thermo Fisher\AttuneNxt\Userdata" folder and exports the database to the specified location defined by the user.
- The database backup file that is created is called "attunenxt.backup" and includes information regarding the data structure. The data that are copied during the backup include copies of the Log files, Smart Monitor Data, and Userdata folders, which are located at the backup location.

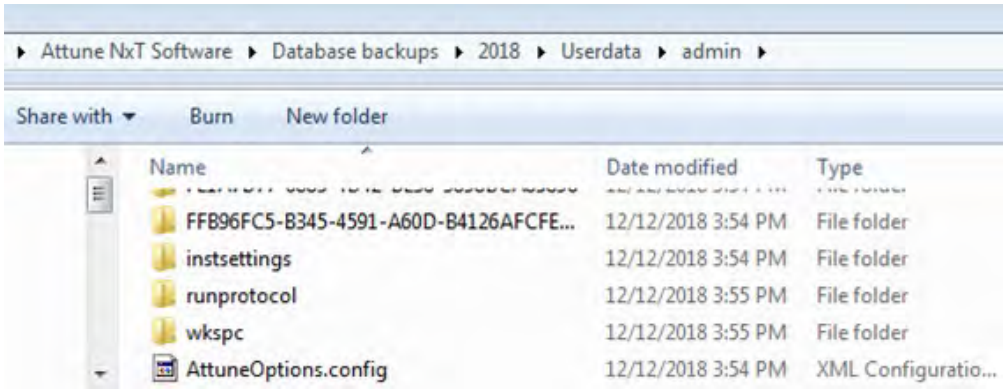


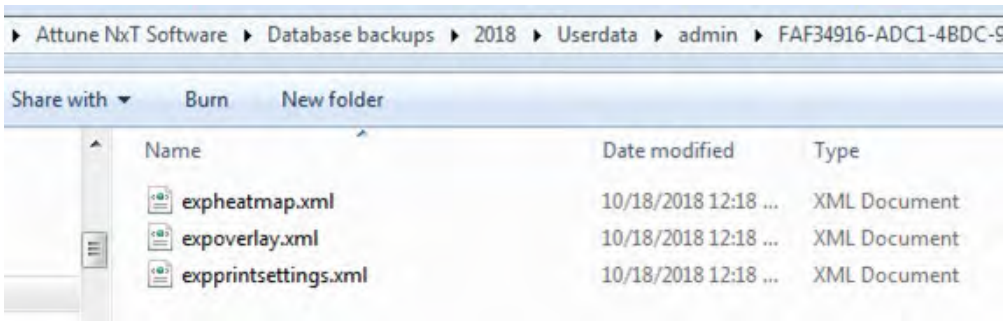
- Within the Userdata folder are folders for each account created in the software, including the default Service, System Administrator (sysadmin) and Administrator (admin) accounts, as well as custom, user-created accounts.



- Within the folder for each user profile are data for the user account, including saved FCS files, and data associated with Experiment and Plate files, including Instrument settings, Workspaces, Compensation settings, Run protocols, Heat Map settings, Print settings, Overlay settings, Sample List settings, and Results Table settings.

  The data are grouped into folders with easily identifiable names (Instrument settings, Run protocol settings, and Workspace settings) or saved in a folder with a Globally Unique Identifier label (GUID) that corresponds to the ID of the Sample or Experiment.

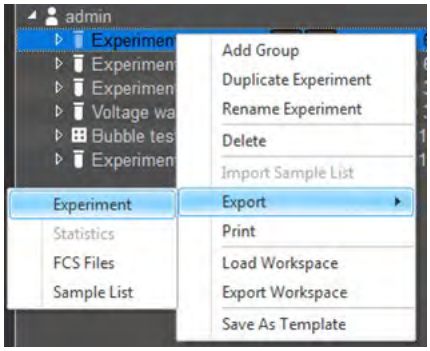


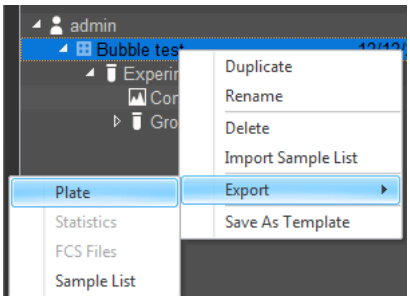


Back up data

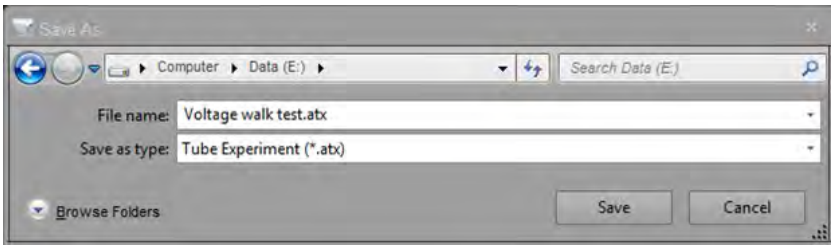
Save and Export Plate and Tube Experiment files

1. In the Experiment Explorer, right-click on the **Experiment** or **Plate name**, then select **Export ▶ Experiment** (or **Plate**).



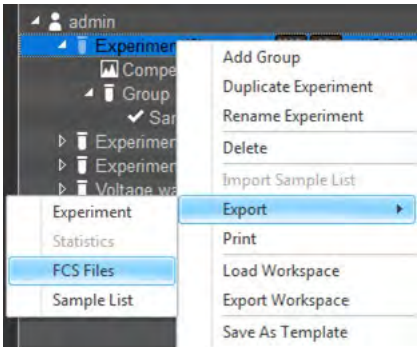


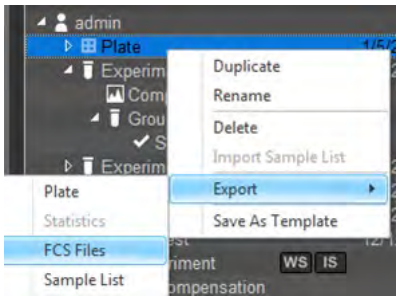
2. In the Save As dialogue, select the location to save the file, then click **Save**.



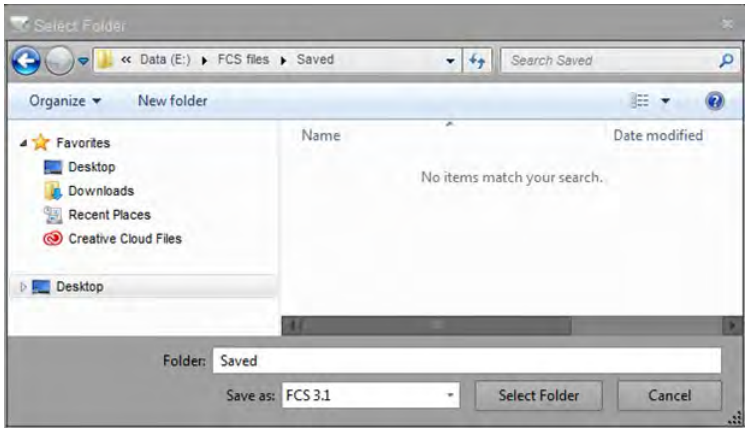
Save and Export FCS files

1. In the Experiment Explorer, right-click on the **Experiment** or **Plate name**, then select **Export ▶ FCS Files**.



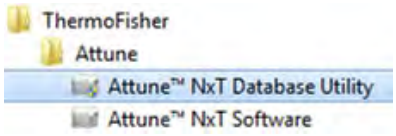


2. In the Save As dialogue, select the location to save the file, then click **Save**.

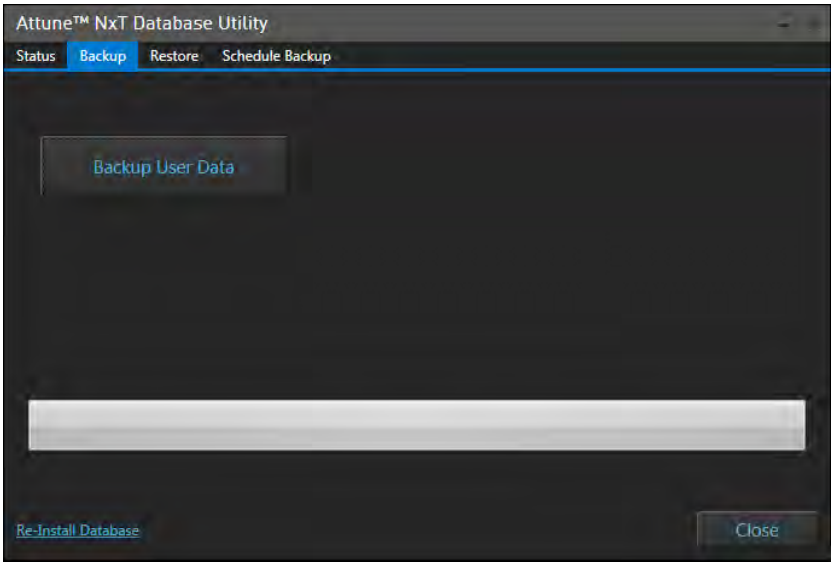


Back up data using the Attune™ Database Utility

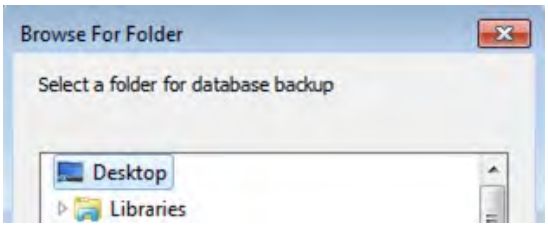
1. Close the Attune™ Cytometric Software.
2. Open the **Attune™ Database Utility** from the Start Menu, then sign in as **Administrator**.



3. On the **Backup** tab, click **Backup User Data**.



4. Select the desired location to save the database.



As the backup continues, the progress bar indicates that files are being copied. When the backup is complete, the dialogue states “Backup Complete”.

5. Click **Close** to exit the utility. A complete copy of all data associated with your Attune™ system has now been created.

Restore data

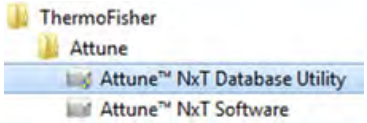
Restore data using the Attune™ Database Utility

You can use the Attune™ Database Utility to restore the software from a saved database. When you restore user data using a saved database, all data that is currently saved within the Attune™ Cytometric Software is replaced with data that was saved at the time the database backup was created. The database does not need to be created using the same version of software currently in use.

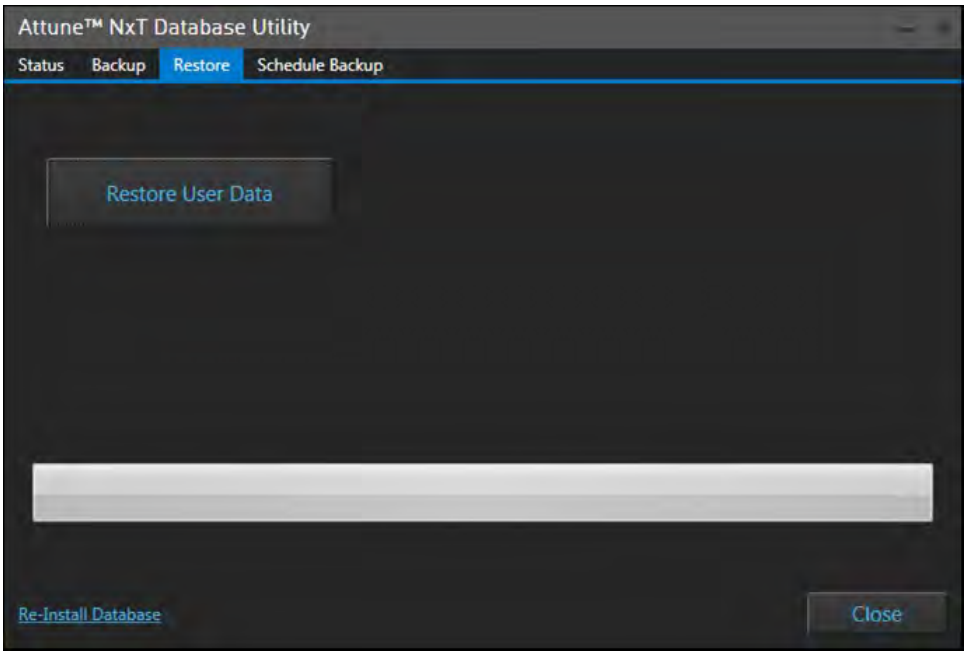
IMPORTANT! Restoration of user data from a saved database overwrites all data in the active, current instance of the Attune™ Cytometric Software.

Do **not** restore from a saved database without archiving data in the active instance of the Attune™ Cytometric Software.

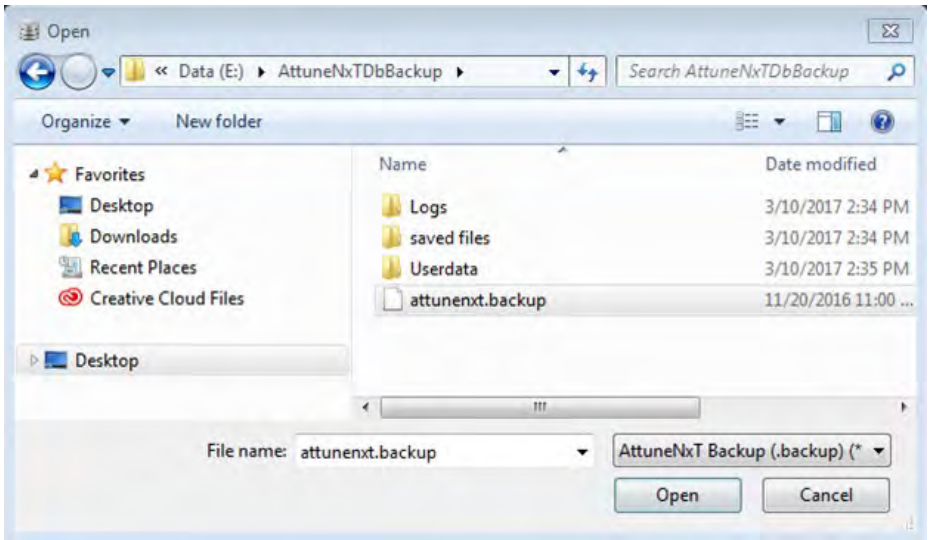
1. Close the Attune™ Cytometric Software.
2. Open the **Attune™ Database Utility** from Start Menu, then sign in as an **Administrator**.



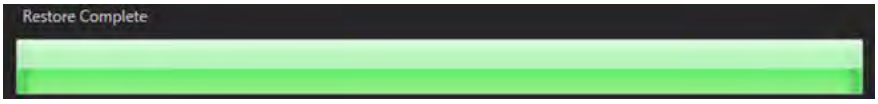
3. On the **Restore** tab, click **Restore User Data.**



4. In the Open dialog, select the correct database backup file (**attunenxt.backup**).



The database restoration begins immediately. As the restoration continues, the progress bar indicates the progress to completion. When the restoration is complete, the dialogue states “Restore Complete”.



5. Click **Close** to exit the utility. The Attune™ Cytometric Software now contains data, including Experiments, FCS files, user profiles, etc., from the database used for restoration.

Copy a database to a different computer

Workflow Copying an instance of the Attune™ Cytometric Software to a new computer is a 4-phase process that includes backing up data using the Attune™ Database Utility, copying all files to a new computer using an external hard drive or a network location, installing the Attune™ Cytometric Software on the new computer, and reinstalling the saved database.

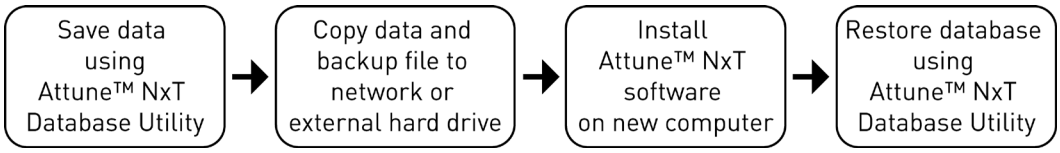
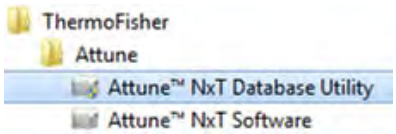


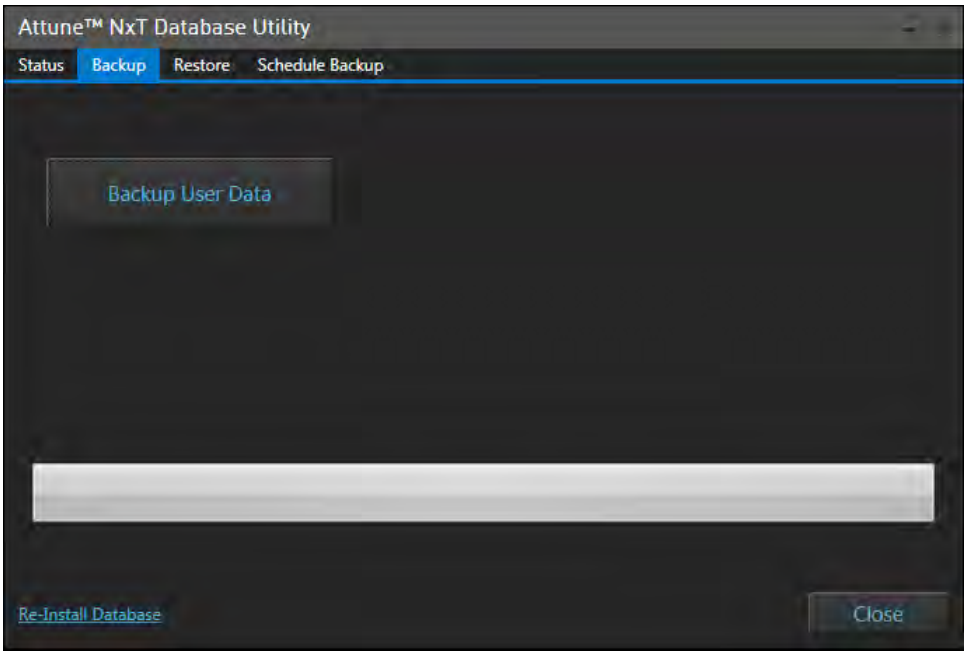
Figure 4. Workflow to copy an instance of the Attune™ Cytometric Software to another computer

Save data using the Attune™ Database Utility

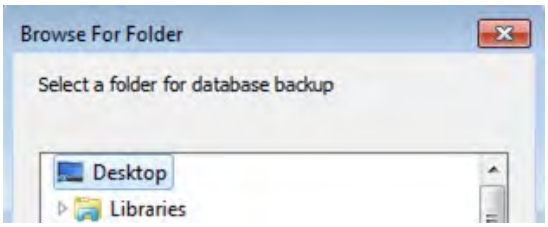
1. Close the Attune™ Cytometric Software.
2. Open the **Attune™ Database Utility** from the Start Menu, then sign in as **Administrator**.



3. On the **Backup** tab, click **Backup User Data**.



4. Select the desired location to save the database.

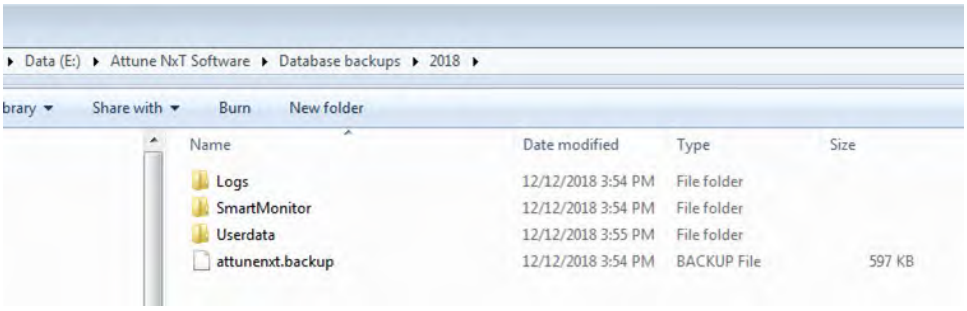


As the backup continues, the progress bar indicates that files are being copied. When the backup is complete, the dialogue states “Backup Complete”.

5. Click **Close** to exit the utility. A complete copy of all data associated with your Attune™ NxT system has now been created.

Copy data and backup file to network or external hard drive

1. Navigate to the location on the computer where the database file and associated data were saved.



2. **Copy all of the data (folders and attunenxt.backup file)** to another location (network location or external hard drive).

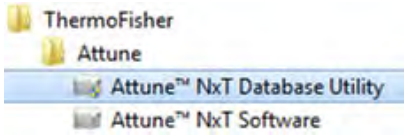
IMPORTANT! Failure to copy **all** data folders will cause database restoration to fail.

Install Attune™ Cytometric Software on new computer

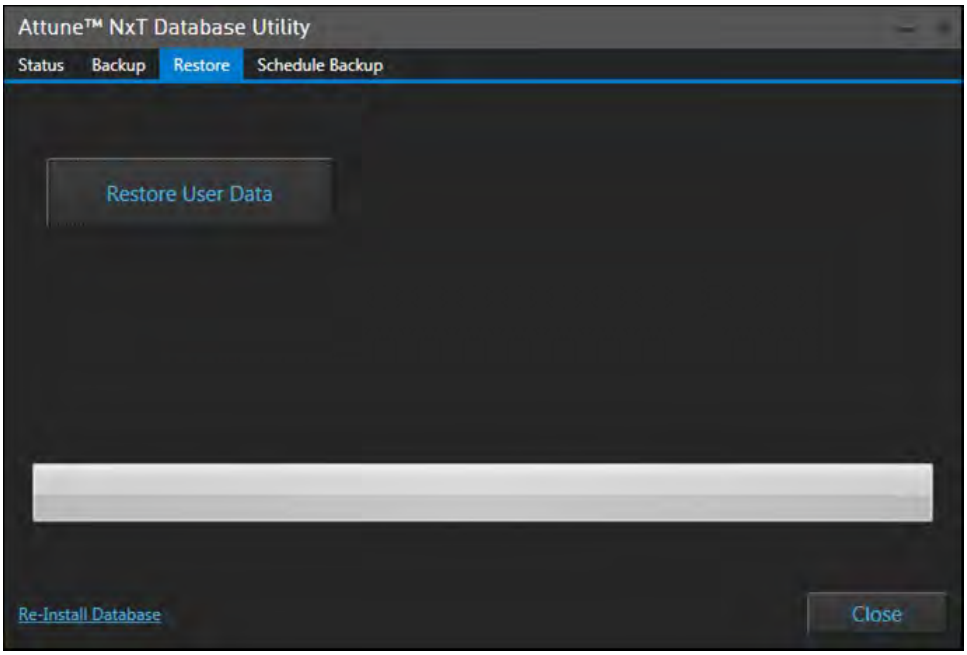
1. Install the Attune™ Cytometric Software on the secondary computer. For complete instructions, refer to the software release notes at **thermofisher.com/attune**.
2. If open, close the Attune™ Cytometric Software.

Restore database using the Attune™ Database Utility

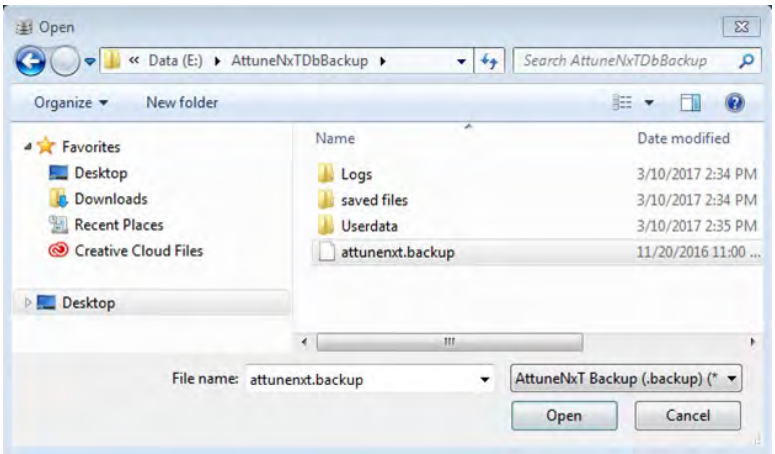
1. Copy all data folders and the attunenxt.backup file to the new computer.
2. Open the **Attune™ Database Utility** from Start Menu, then sign in as an **Administrator**.



3. On the **Restore** tab, click **Restore User Data.**



4. In the Open dialog, select the database backup file (**attunenxt.backup**) that was saved on the computer.



The database restoration begins immediately. As the restoration continues, the progress bar indicates the progress to completion. When the restoration is complete, the dialogue states “Restore Complete”.

5. Click **Close** to exit the utility. The Attune™ Cytometric Software now contains data, including Experiments, FCS files, user profiles, etc., from the database used for restoration.

Permanently remove saved data from the Attune™ computer

Data deletion options

The recommended method for deletion of data from the Attune™ Cytometric Software is to delete Tube or Plate Experiments from the Experiment Explorer within each user profile. When deleting files from individual user accounts is not possible, you can use one the other options described in this section.

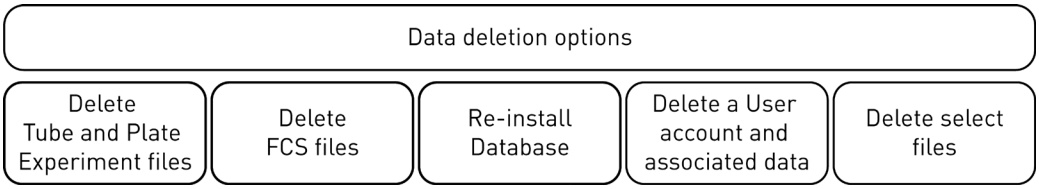
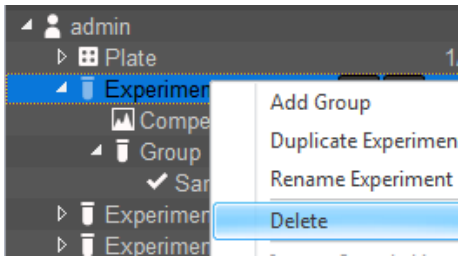


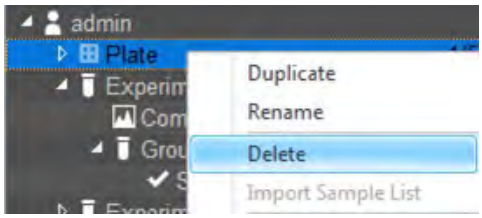
Figure 5. Options for permanently removing saved data from the Attune™ computer

Delete Tube and Plate Experiment files

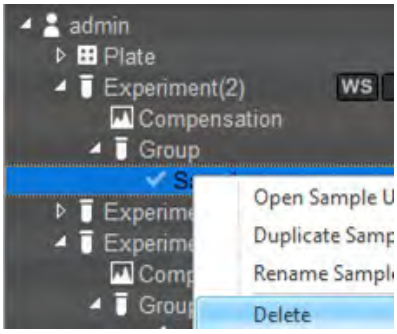
1. Sign in to the User account that includes the data you wish to delete.
2. Right-click on the **Experiment**, **Plate**, or **Sample** name, then select **Delete** from the context menu.



Delete Tube Experiment

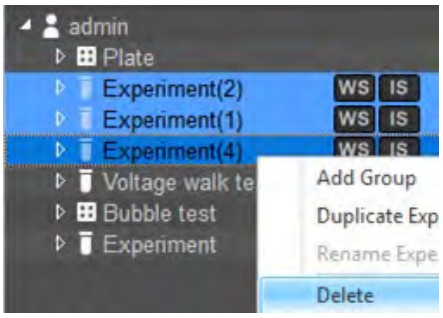


Delete Plate Experiment

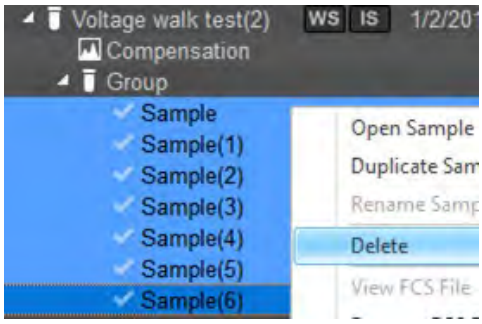


Delete Sample

3. *Optional*: To select multiple Tube Experiments, Plate Experiments, or FCS files for bulk deletion, select each file individually while holding the keyboard Ctrl key.

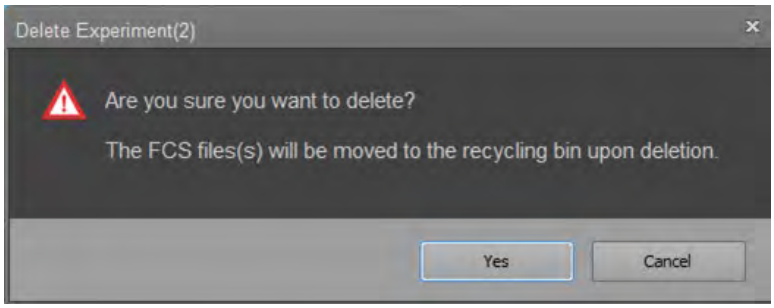


Delete multiple Tube Experiments

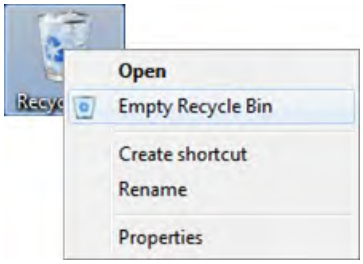


Delete multiple Samples

4. In the Delete dialog, click **Yes** to confirm the file deletion or click **Cancel** to close the dialog without deleting the selected files. The files that are deleted from the software are sent to the Recycle Bin of the computer.



5. Empty the Recycle Bin to remove the deleted files permanently from the computer. Alternatively, open the Recycle Bin and select individual files to delete permanently.

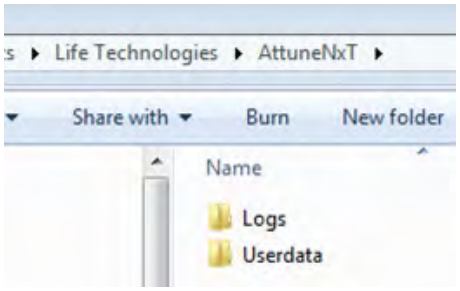


Reinstall a new database to erase existing data

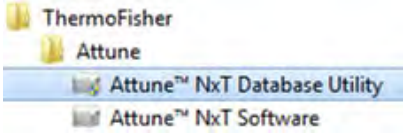
You can use the Attune™ Database Utility to reinstall a new, empty database. Installation of a new database completely erases all existing data and user accounts in the current, active instance of the Attune™ Cytometric Software.

IMPORTANT! Do **not** reinstall a new database without first saving and exporting your experiments and files from the Attune™ Cytometric Software.

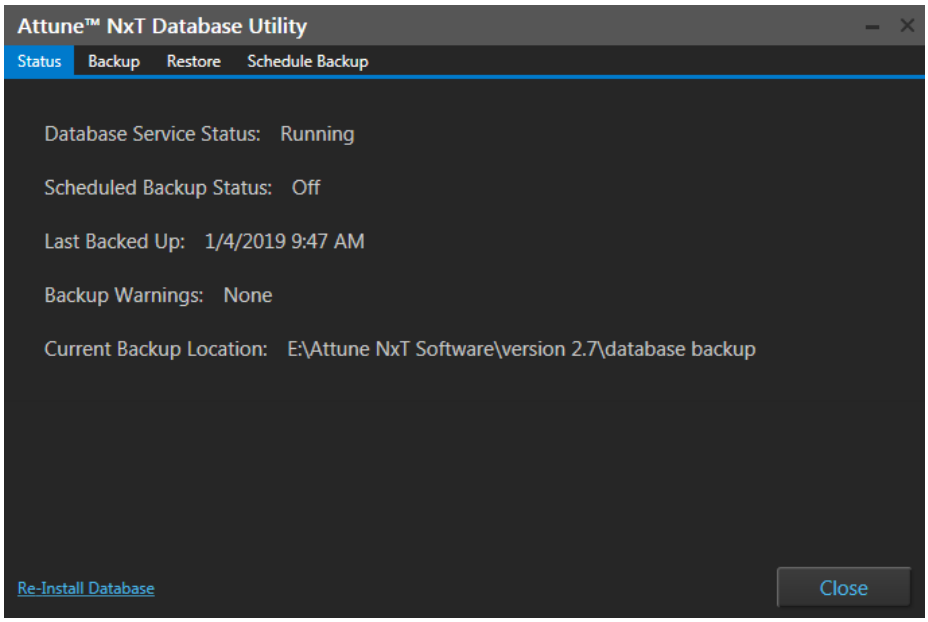
1. Close the Attune™ Cytometric Software.
2. Go to the **C:\Users\Public\Documents\Life Technologies\AttuneNxT** folder, then right-click on the **Userdata** folder and select **Delete** from the dropdown menu.



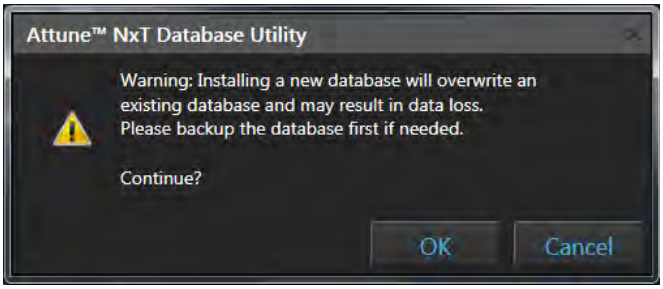
3. Open the **Attune™ Database Utility**, then sign in as an **Administrator**.



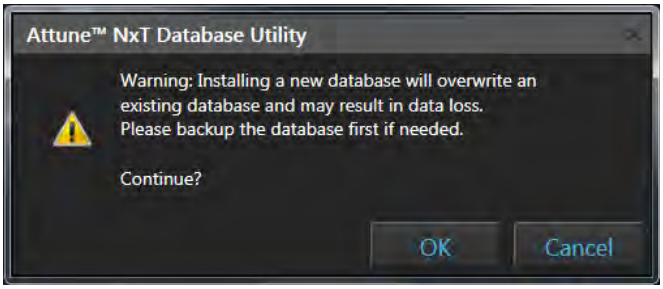
4. In the **Status** tab, select **Re-install Database** (in the bottom left corner).



5. Click **OK** to confirm your intent to install a new database or click **Cancel** to close the dialog without installing a new database.



6. When the new database installation is completed, click **Close**.



7. Open the Attune™ Cytometric Software. The new database does not include any data from the previously created accounts.

Note: Sign in to the Attune™ Cytometric Software using one of the default account settings (**sysadmin** or **admin**).

The default username for the Administrator account is **admin** and the default password is **admin**.

The default username for the System Administrator account is **sysadmin** and the default password is **sysadmin**.

Delete a user account to remove data

The recommended method for deletion of data from the Attune™ Cytometric Software is to delete Tube or Plate Experiments from the Experiment Explorer within each user profile. However, if a user account is no longer needed, you can delete the account to remove all files associated with it.

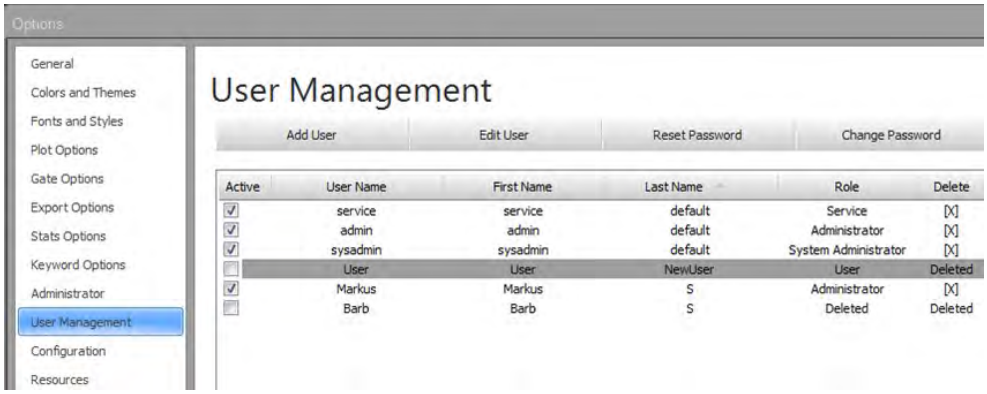
IMPORTANT! Deleting a user account permanently removes the user account and all associated files from the Attune™ Cytometric Software.
We do **not** recommend deletion of a user account without first backing up the user data to an external location.

1. Sign in to the Attune™ Cytometric Software as the **System Administrator** (sysadmin).

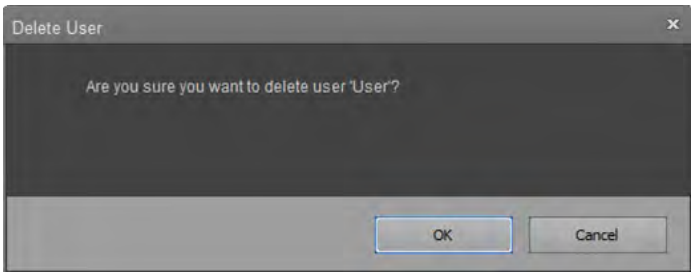
   Note: The default username for the System Administrator account is **sysadmin** and the default password is **sysadmin**.

2. In the **Options** dialog, select **User Management**.

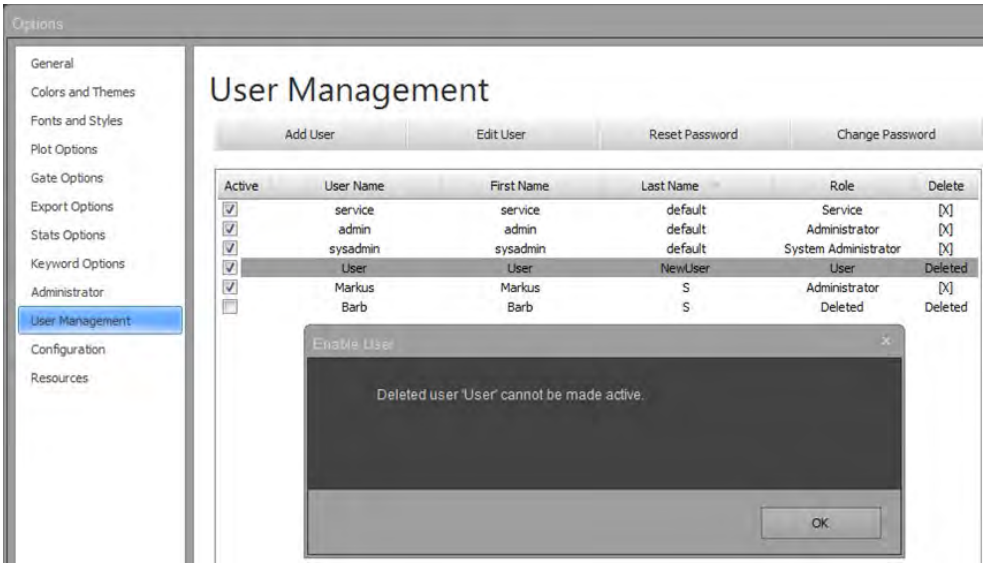
3. Select the user account you wish to delete, then click the **X** in the **Delete column**.



4. In the Delete User dialogue, click **OK** to confirm your intent to delete the selected user account or click **Cancel** to close the dialog without deleting the account.

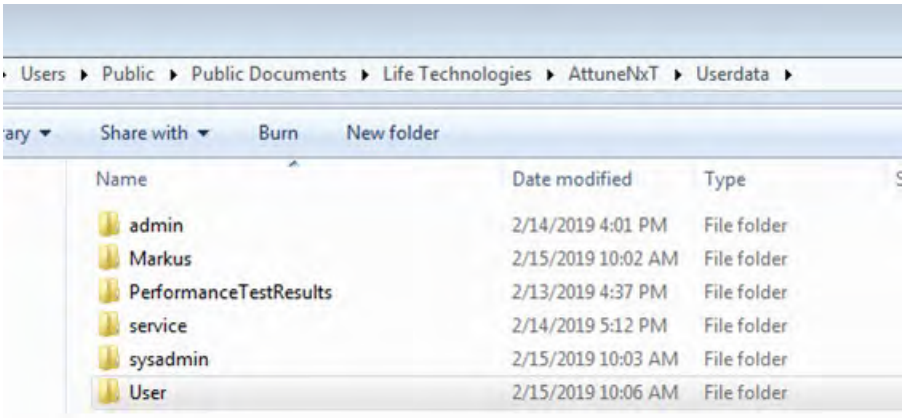


When the account has been deleted, the Active box will be unchecked and the user status cannot be re-enabled by selecting the Active box.

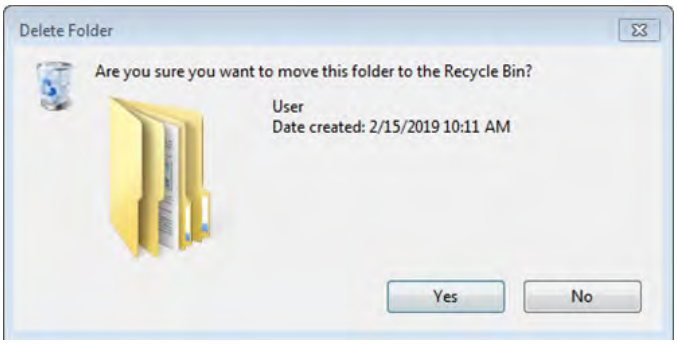


5. Although the user account has been deleted, all associated user data remains in the Attune™ NxT database until the data is manually deleted.

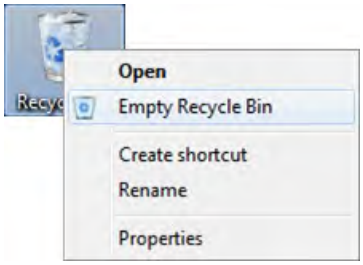
   To delete all associated data, go to **C:\Users\Public\Documents\Life Technologies\AttuneNxT\Userdata\X**, where X is the user account name for the deleted user account.



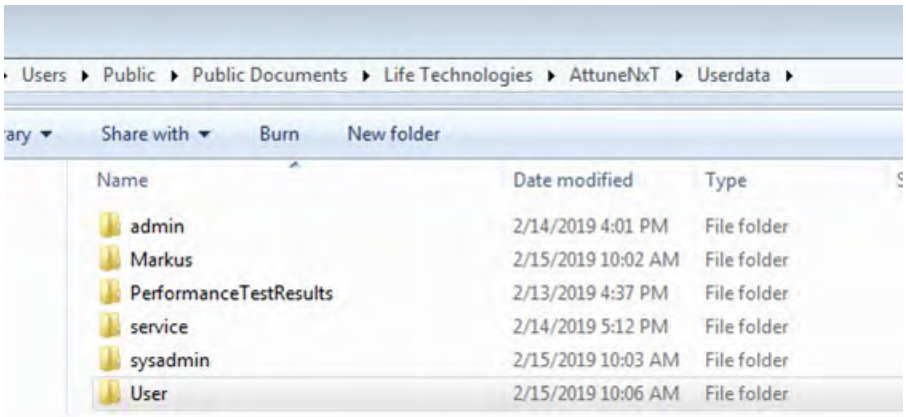
6. Right-click on the data folder for the deleted user account, then select **Delete** from the dropdown menu.
7. In the Delete Folder dialog, click **Yes** to confirm your intent to move the data folder to the Recycle Bin or click **No** to close the dialog without deleting.



8. Empty the Recycle Bin to remove the deleted files permanently from the computer. Alternatively, open the Recycle Bin and select individual files to delete permanently.



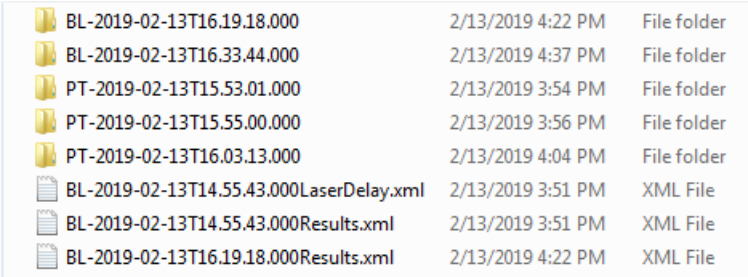
Note: When a user account is deleted, the folder corresponding to the deleted account will still be visible in C:\Users\Public\Documents\Life Technologies\AttuneNxT\Userdata. This User folder can be opened, but it will contain empty subfolders and no actual data.



Permanently remove old Baseline and Performance Test data

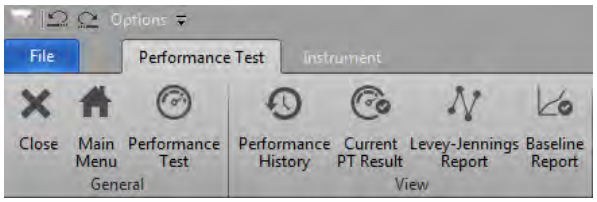
About Baseline and Performance Test data

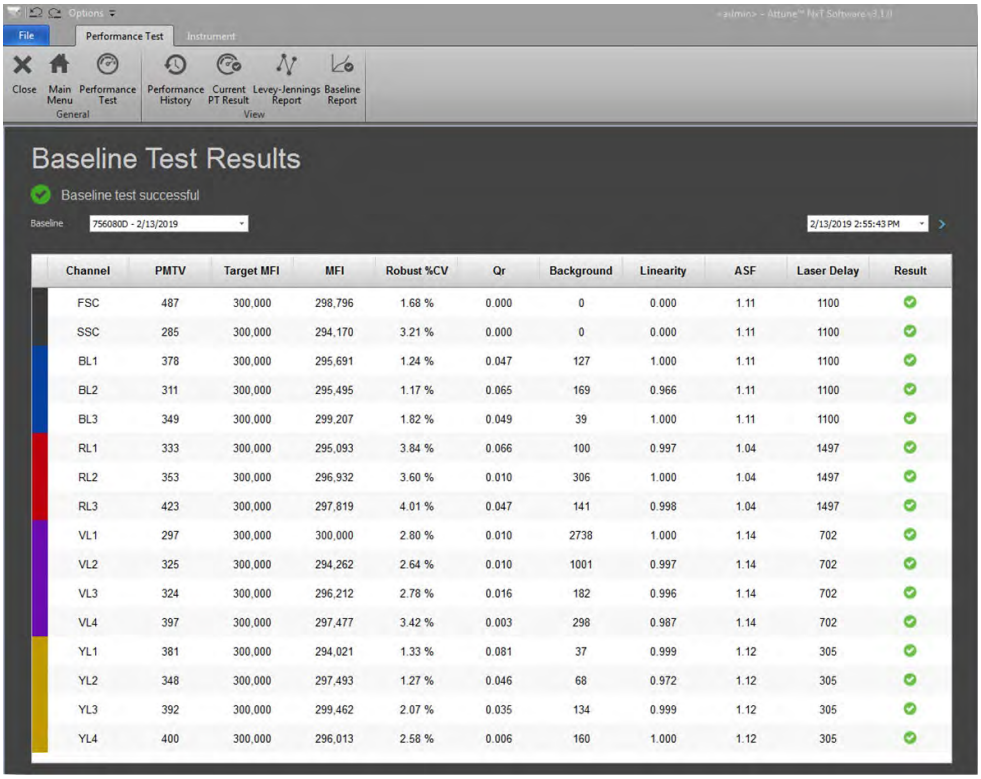
Baseline and Performance Test generate many data files including XML files associated with laser delay and test results, FCS files, CSV files, and log files annotating steps that occur during Baseline and Performance Test.



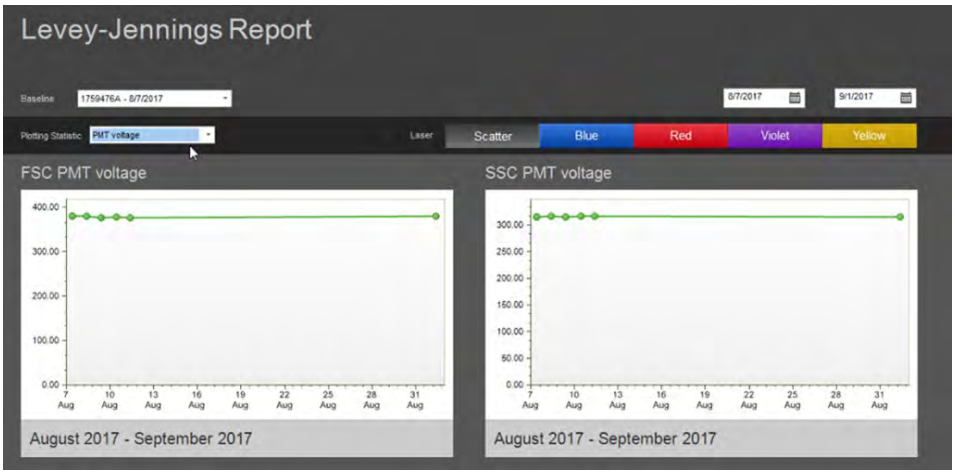
BL-2019-02-13T16.19.18.000	2/13/2019 4:22 PM	File folder
BL-2019-02-13T16.33.44.000	2/13/2019 4:37 PM	File folder
PT-2019-02-13T15.53.01.000	2/13/2019 3:54 PM	File folder
PT-2019-02-13T15.55.00.000	2/13/2019 3:56 PM	File folder
PT-2019-02-13T16.03.13.000	2/13/2019 4:04 PM	File folder
BL-2019-02-13T14.55.43.000LaserDelay.xml	2/13/2019 3:51 PM	XML File
BL-2019-02-13T14.55.43.000Results.xml	2/13/2019 3:51 PM	XML File
BL-2019-02-13T16.19.18.000Results.xml	2/13/2019 4:22 PM	XML File

Baseline and Performance Test data are stored in two locations in the Attune™ Cytometric Software: directly in the database and in the Public Documents folder. Within the Attune™ Cytometric Software, data are pulled directly from the database to view Baseline or Performance Test Results, Levey-Jennings data, Performance History, and the Baseline Report.





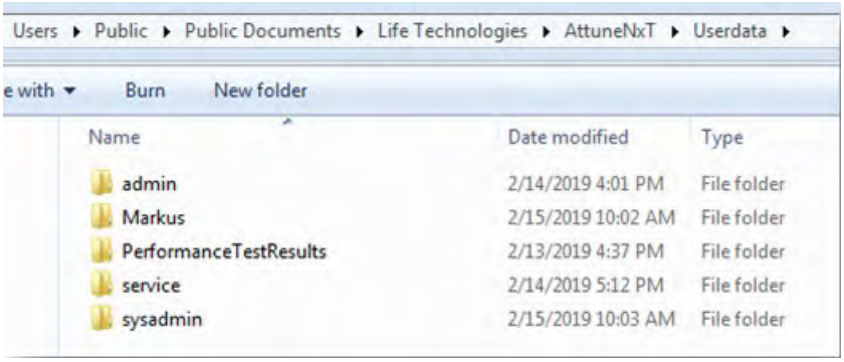
Channel	PMTV	Target MFI	MFI	Robust %CV	Qr	Background	Linearity	ASF	Laser Delay	Result
FSC	487	300,000	298,796	1.68 %	0.000	0	0.000	1.11	1100	✓
SSC	285	300,000	294,170	3.21 %	0.000	0	0.000	1.11	1100	✓
BL1	378	300,000	295,691	1.24 %	0.047	127	1.000	1.11	1100	✓
BL2	311	300,000	295,495	1.17 %	0.065	169	0.966	1.11	1100	✓
BL3	349	300,000	299,207	1.82 %	0.049	39	1.000	1.11	1100	✓
RL1	333	300,000	295,093	3.84 %	0.066	100	0.997	1.04	1497	✓
RL2	353	300,000	296,932	3.60 %	0.010	306	1.000	1.04	1497	✓
RL3	423	300,000	297,819	4.01 %	0.047	141	0.998	1.04	1497	✓
VL1	297	300,000	300,000	2.80 %	0.010	2738	1.000	1.14	702	✓
VL2	325	300,000	294,262	2.64 %	0.010	1001	0.997	1.14	702	✓
VL3	324	300,000	296,212	2.78 %	0.016	182	0.996	1.14	702	✓
VL4	397	300,000	297,477	3.42 %	0.003	298	0.987	1.14	702	✓
YL1	381	300,000	294,021	1.33 %	0.081	37	0.999	1.12	305	✓
YL2	348	300,000	297,493	1.27 %	0.046	68	0.972	1.12	305	✓
YL3	392	300,000	299,462	2.07 %	0.035	134	0.999	1.12	305	✓
YL4	400	300,000	296,013	2.58 %	0.006	160	1.000	1.12	305	✓



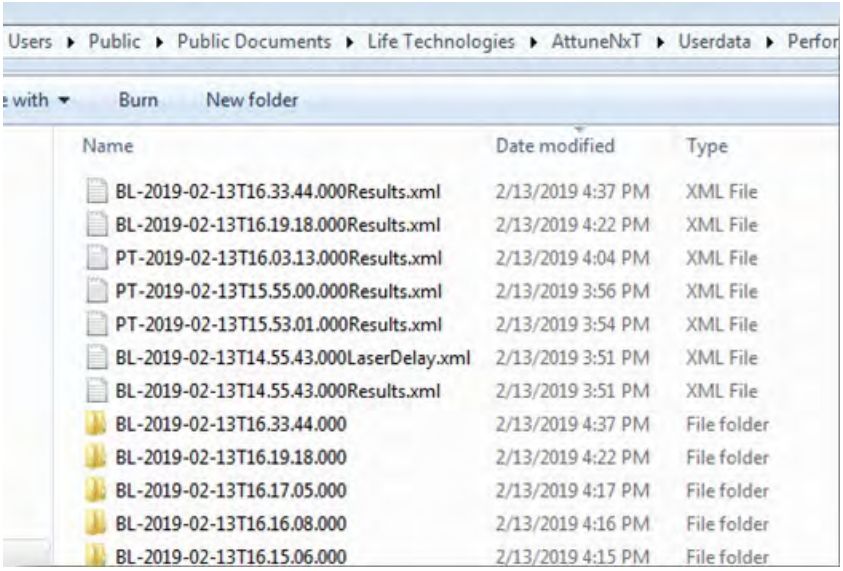
Over time, the PerformanceTestResults folder (X:\Users\Public\Documents\Life Technologies\AttuneNxT\Userdata) can become very large. To permanently remove the files contained within the PerformanceTestResults folder, follow the steps described in this section.

Remove Baseline and Performance Test Data in Attune™ Cytometric Software version 2.7 or earlier

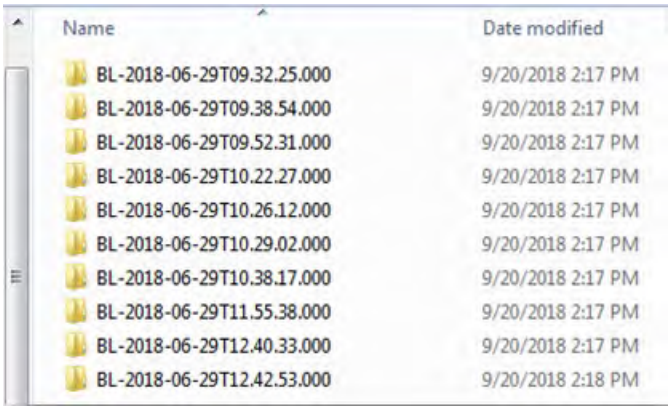
1. Navigate to the **PerformanceTestResults** folder (X:\Users\Public\Documents\Life Technologies\AttuneNxT\Userdata).



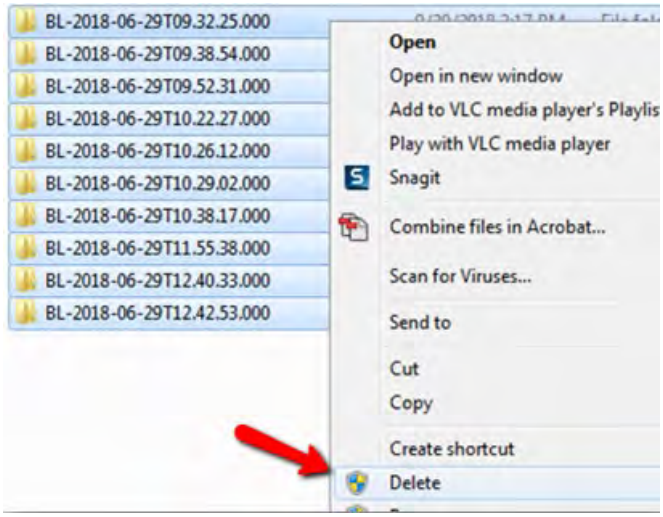
2. Within the PerformanceTestResults folder, organize data based on the "Date modified column" by clicking on the **Date modified column header**.



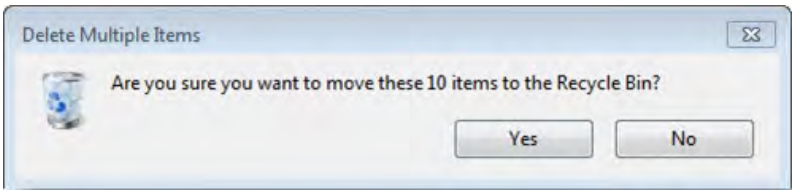
3. Select data to delete based on the date the data was created. We recommend keeping the last 3 months of data in the folder in case these files are needed for troubleshooting. In the following example, select the files created before 13 November 2018.



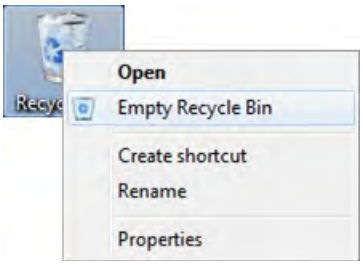
4. Multi-select the appropriate files to delete, right-click, then select **Delete** from the drop-down menu to delete the files.



5. Click **Yes** to confirm the deletion.



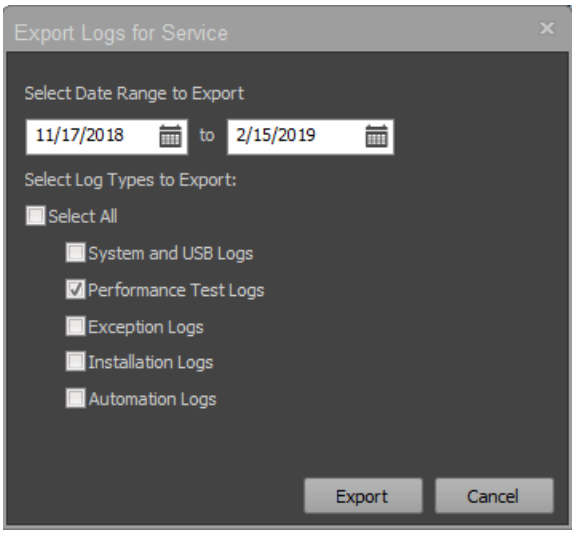
6. Empty the Recycle Bin of the computer to remove the deleted files permanently from the computer.



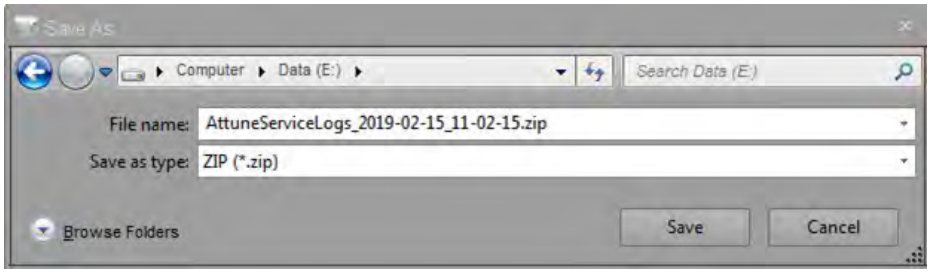
Remove Baseline and Performance Test Data in Attune™ Cytometric Software version 3.1

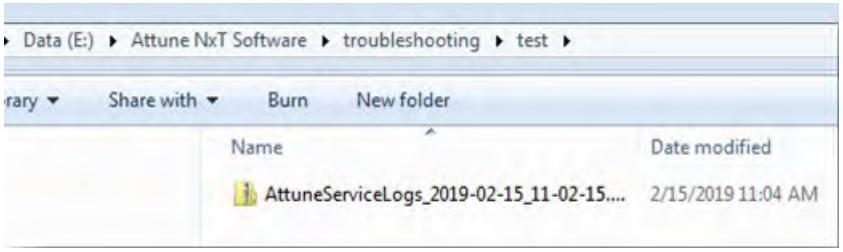
1. Open the Attune™ Cytometric Software and sign in to an administrator account.
2. Archive at least 3 months of Baseline and Performance Test Data before deletion. To do this, click **Export Logs for Service** on the Instrument tab of the Ribbon bar.

3. In the Export Logs for Service dialogue, select **Date Range to Export**, check the **Performance Test Logs** option, then click **Export**.

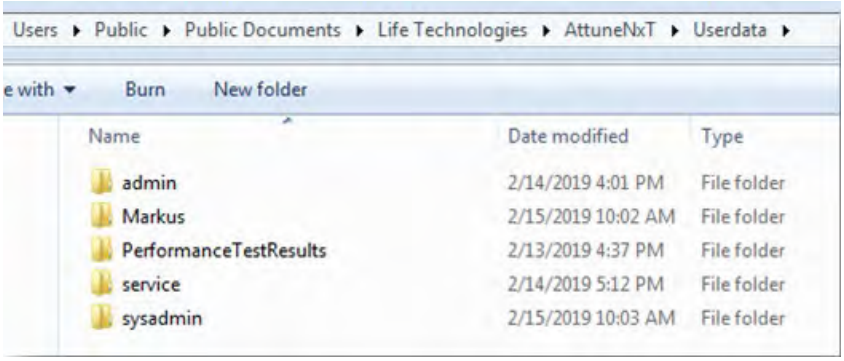


4. In the **Save as** dialogue, select the file to save the data, then click **Save**. All files required for troubleshooting are exported to the save location as a zip folder.

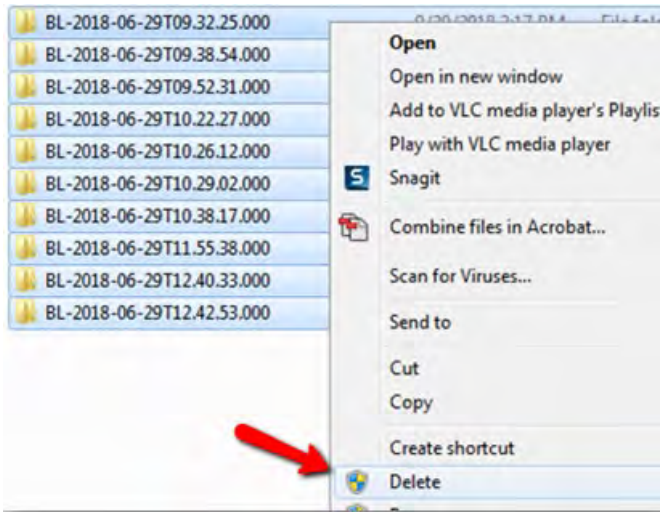




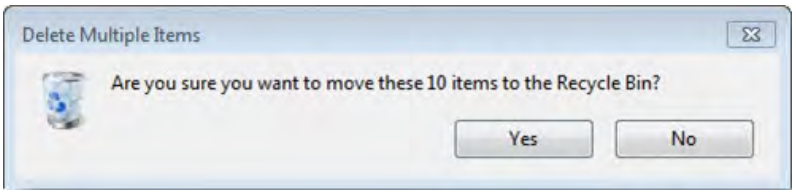
5. Navigate to the **PerformanceTestResults** folder (X:\Users\Public\Documents\Life Technologies\AttuneNxT\Userdata).



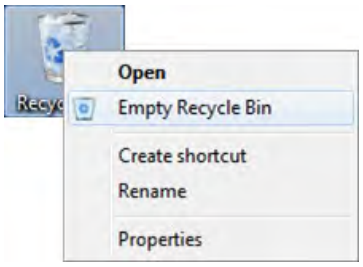
6. Open the PerformanceTestResults folder, multi-select all files within the folder, right-click, then select **Delete** from the drop-down menu to delete the files.



7. Click **Yes** to confirm the deletion.



8. Empty the Recycle Bin of the computer to remove the deleted files permanently from the computer.



Appendix C: SAE Administrator Console

SAE Module

Overview of the SAE Module

The *SAE Module* (Security, Audit, and Electronic Signature) is a client-server software configuration that is used to meet specific requirements for security, audit, and e-Signature (such as 21 CFR Part 11 compliance).

IMPORTANT! 21 CFR part 11 is a regulation that describes the criteria for acceptance by the FDA for electronic records and electronic signatures. Part 11 is composed of procedural and technical requirements. Procedural requirements are the standard operating procedures instituted by the end user, and technical requirements are the technical characteristics of the software used.

The SAE Module of the Attune™ Cytometric Software does not automatically guarantee 21 CFR part 11 compliance. Compliance is the consequence of the **end user's work process and systems used**. Attune™ Cytometric Software in the SAE mode enables 21 CFR part 11 compliance for the flow cytometry data collection and analysis steps within the workflow.

The SAE Module includes three components:

- **SAE Administrator Console**—Tool that is used by an SAE administrator to configure the SAE Module (see "SAE Administrator Console", page 577).
- **SAE server (server)**—Service that runs in the background and stores SAE settings, user accounts, audit records, and e-Signature records. By default, the SAE server is installed on the same computer as the SAE Administrator Console.
- **SAE screens (client)**—Screens that are displayed (sign in, audit, and e-Signature) in the application (such as the Attune™ Cytometric Software) and that require user input.

The SAE Module can be configured to provide the following functionality:

Function	Description
System Security	Controls user access to the Attune™ application. A default user account with the Administrator role is provided at installation that has access to both SAE Administrator Console and the Attune™ Cytometric Software. Other default SAE user roles include Advanced user, User, Reviewer, and No Privileges roles (page 583). You can set up additional SAE user accounts with specific permissions.
Auditing	Tracks actions performed by users, changes to SAE settings, and changes made to application objects (i.e., Experiments) (page 597). You can: • Enable or disable audits, select actions and application objects to be audited, specify the audit mode, and require users to provide a reason for changes made to auditable application objects. • View specific audit logs and generate printable audit records. • Archive the audit records or configure auto archive settings.
Electronic Signature (e-Signature)	Determines if users are required to fulfill signature requirements before performing specific functions. You can: • Configure e-signature so that a user can export data, print data, and save experiment as a template only if the required e-Signatures are provided. • Configure each e-Signature event to require multiple signatures and to require users with specific roles to sign.

SAE Administrator Console

Overview of the SAE Administrator Console

The *SAE Administrator Console* is used to configure the SAE Module, including user credential management (username and password complexity), users, user roles (security), auditing, and e-Signature features.

In the SAE Administrator Console, a software or instrument that is configured for the SAE Module is called an "application". An example application is the Attune™ Cytometric Software.

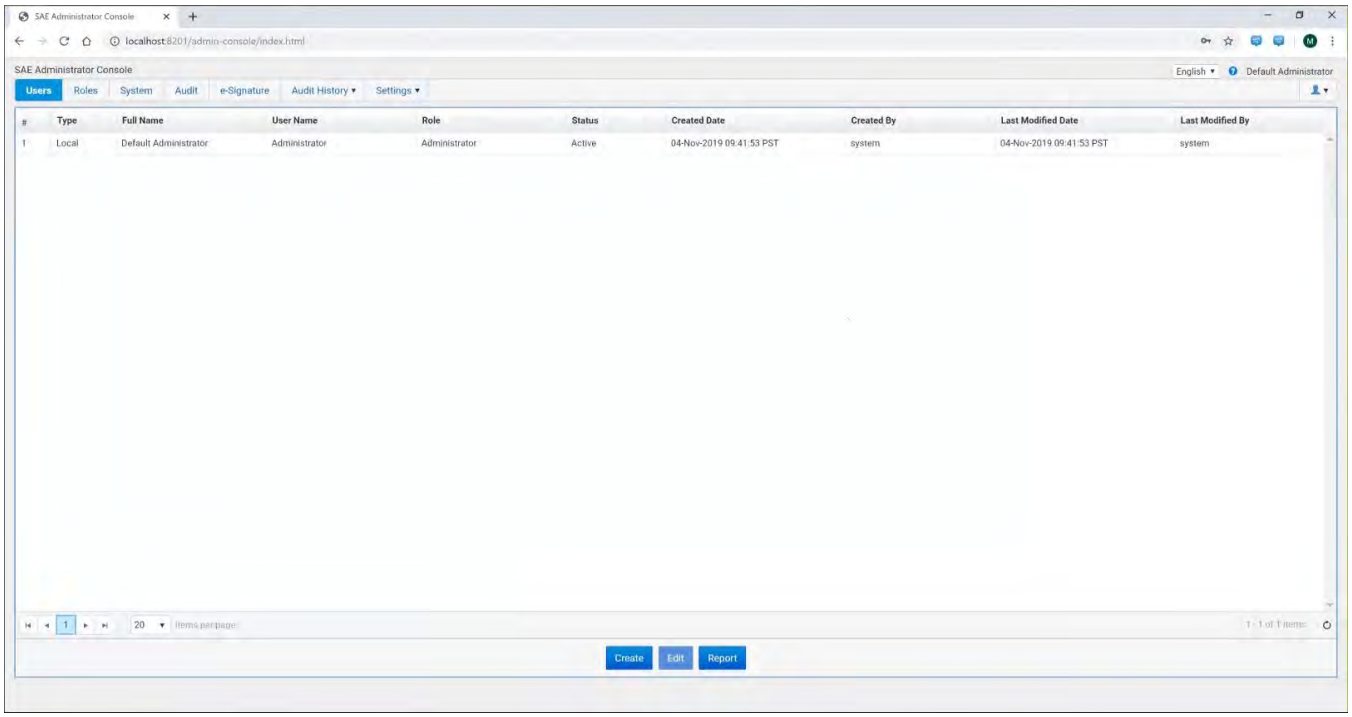
The SAE Administrator Console has an Attune™-specific security profile that defines application permissions, default user roles, auditable application objects, signature actions, signature application objects, and default signature meanings.

Changes made on the SAE Administrator Console are synchronized with the Attune™ Cytometric Software every 10 seconds. If the SAE Administrator Console is offline, the Attune™ Cytometric Software uses a cache to manage a local copy of the SAE Administrator Console server settings and a cache of all audit and e-Signature records that are created while the server is offline.

Note: If your SAE Administrator Console is configured to manage the SAE module for more than one application, you can create roles that specify permissions for more than one application (for example, Attune™ Cytometric Software and SeqStudio™ Genetic Analyzer). However, this section is focused solely on managing the SAE module for the Attune™ Cytometric Software.

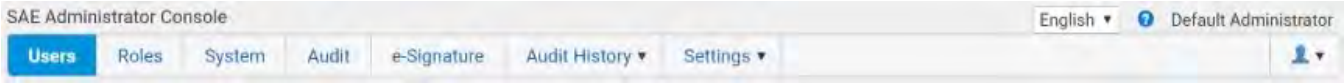
Open the SAE Administrator Console

To open the SAE Administrator Console from the Attune™ Cytometric Software, click **View SAE Console** on the SAE ribbon tab (page 79). The SAE Administrator Console opens in the system's default web browser.



SAE Administrator Console tabs

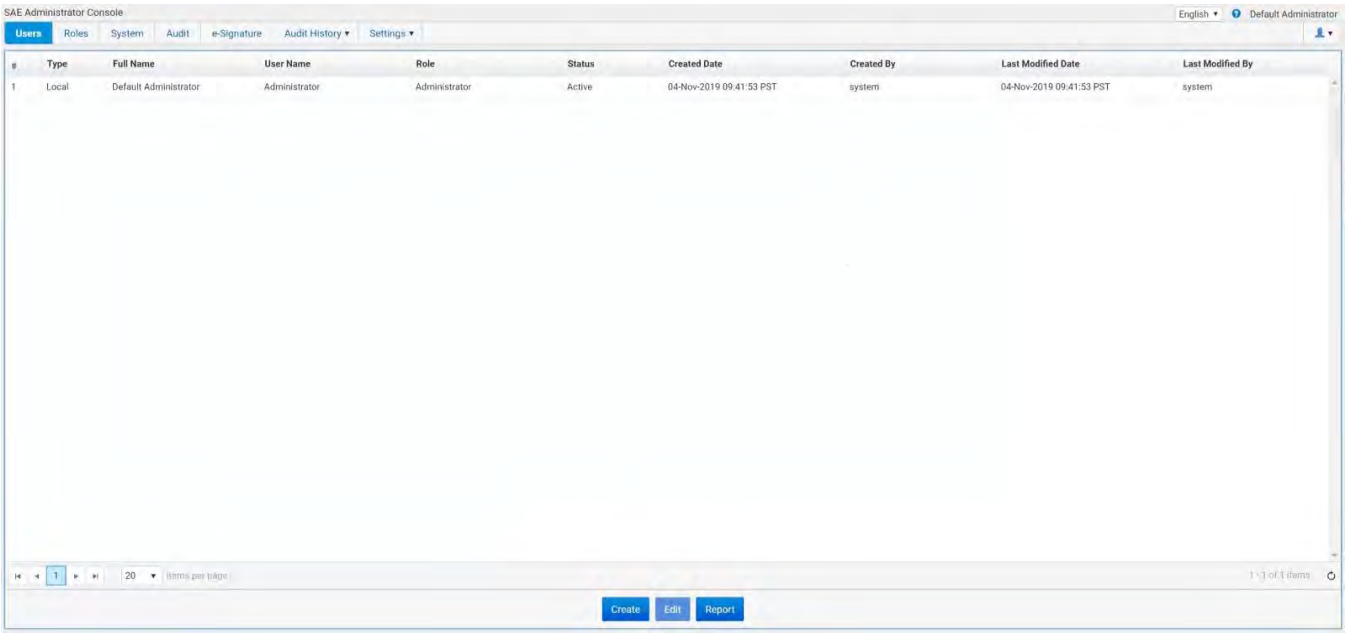
The SAE Administrator Console is organized into a series of tabs which represent the main functions of the SAE Module.



- *Users* (page 578)
- *Roles* (page 581)
- *System* (page 591)
- *Audit* (page 597)
- *e-Signature* (page 603)
- *Audit History* (page 606)
- *Settings* (page 619)

Users tab

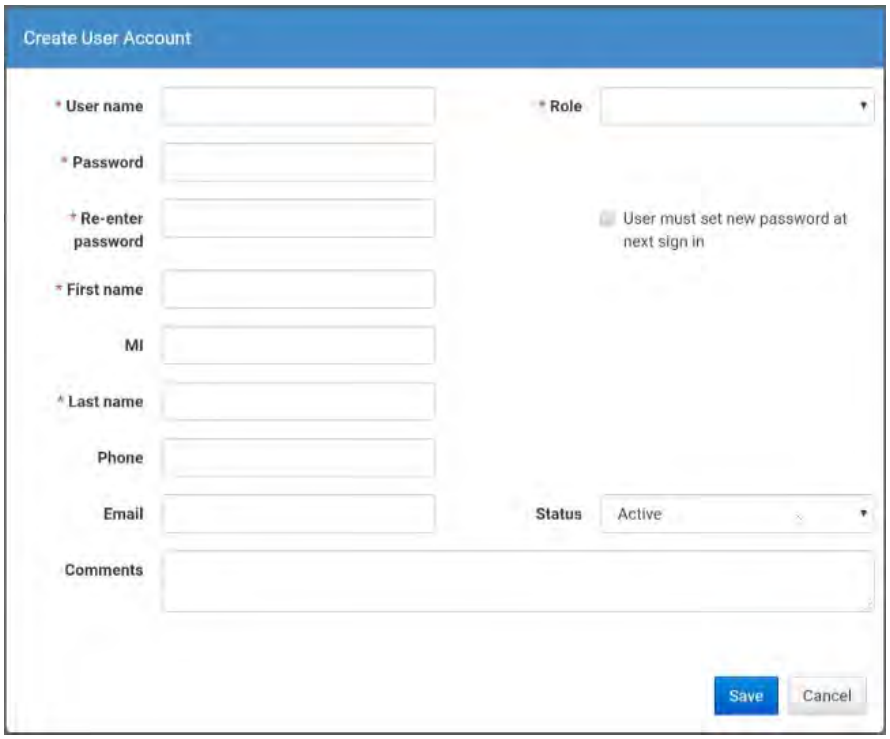
Users tab displays a list of the current SAE accounts in the SAE server, and allows the SAE Administrator to create additional SAE users, edit current users, and to create Users reports.



- The list of SAE users contains columns for the User number (#), Type, Full Name, Username, Role, Status, Created Date, Created By, Last Modified Date, and Last Modified By.
- The full name of the currently sign in user is displayed on the upper right corner of the SAE Administrator Console.
- There are three action buttons in the Users tab:
  - **Create**: Allows the SAE Administrator to create additional SAE users (see "Create an SAE user account", page 579).
  - **Edit**: Allows the SAE Administrator to edit the settings for the selected SAE user (see "Edit an SAE user account", page 580).
  - **Report**: Allows the SAE Administrator to create a PDF report that lists the current SAE accounts saved in the SAE server (see "Print or view a user report", page 580).

Create an SAE user account

1. In the SAE Administrator Console main screen, click the **Users** tab.
2. Click **Create** to open the *Create User Account* dialog.



3. Enter the **username**, **password**, **first name**, (optional) **middle initial**, and **last name**. The field limits are specified in the system security function settings (see "System tab", page 585).

> **Note:** First name, MI (middle initial), and last name are used to create the User Full Name, which is displayed as the name of the signed-in user. You cannot delete a user account or change the username after you save the user account.

4. *(Optional)* Deselect **User must set new password at next sign in**. By default, this option is selected. Users must specify a new password the first time they sign in to an application.

> **Note:** The user account password automatically expires after the number of days that are specified in the system security function settings (see "System tab", page 585).

5. Select the **Role** for the user account. Available default options are **Administrator**, **Advanced User**, **User**, and **Reviewer**.

   The **No Privileges Role** is for internal use by the SAE Administrator Console. Do not assign this role to a user account.

> **Note:** Each role grants specific SAE permissions to the user. You can also create a custom role with specific privileges in the Roles tab. For more information, see "Roles tab", page 581.

6. Leave the **Status** set to **Active**.
7. (*Optional*) Enter phone, email (for information only), and comments.
8. Click **Save**. The newly created SAE user is displayed in the SAE users list in the Users tab.

Edit an SAE user account

1. In the SAE Administrator Console main screen, click the **Users** tab.
2. Select a user account, then click **Edit** to open the *Edit User Account* dialog.
3. Edit the settings as desired. You cannot edit the username of an existing user.
4. Click **Save**.

Note: The Username cannot be changed after a user account is created and saved.

Activate a suspended SAE user account

1. In the SAE Administrator Console main screen, click the **Users** tab.
2. Select a user account, then click **Edit** to open the *Edit User Account* window.
3. Change the Status from **Suspended** to **Active**.
4. Click **Save**.

Disable (inactivate) an SAE user account

1. In the SAE Administrator Console main screen, click the **Users** tab.
2. Select a user account, then click **Edit** to open the *Edit User Account* window.
3. Change the Status from **Active** to **Inactive**.
4. Click **Save**.

Reset an SAE user password

1. In the SAE Administrator Console main screen, click the **Users** tab.
2. Select a user account, then click **Edit** to open the *Edit User Account* window.
3. Enter a replacement password for the user account, then re-enter the password for confirmation.
4. If you assigned the user account a temporary password, then select **User must set new password at next sign in** to require the user to enter a new password at sign in.
5. Click **Save**.

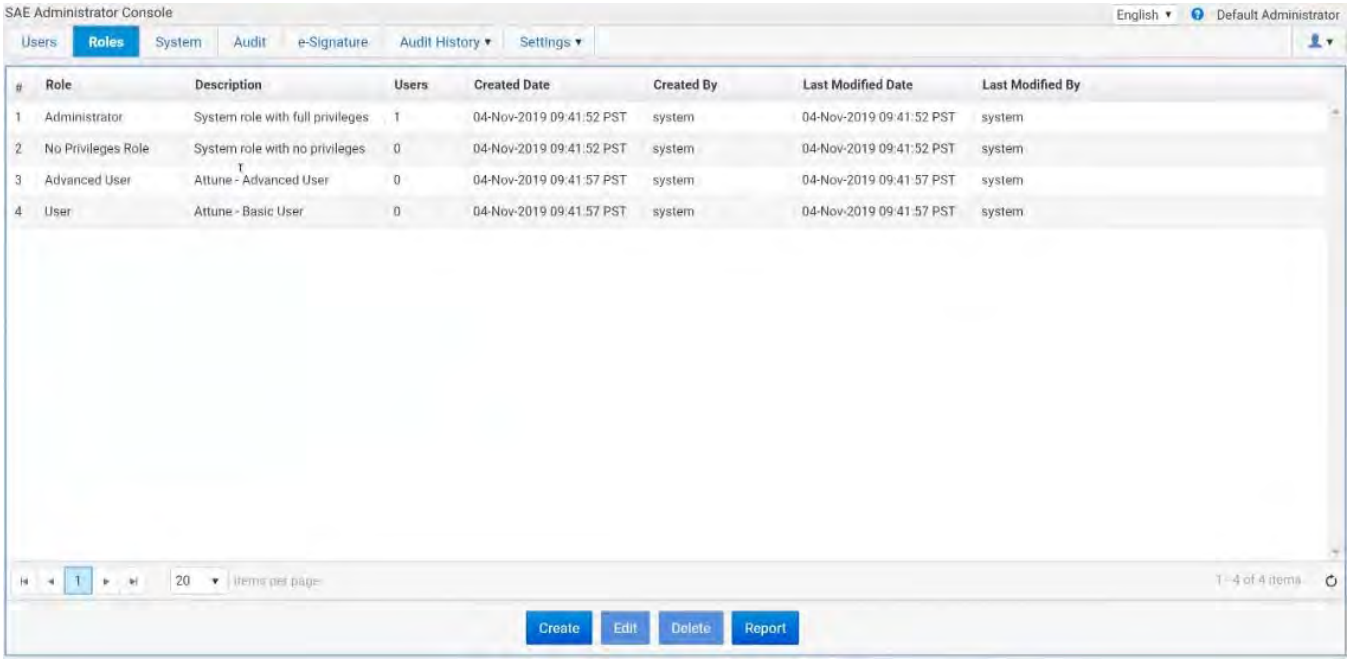
IMPORTANT! There is no way to recover a forgotten password. If the SAE Administrator forgets their password, the software must be reinstalled. Export all data before reinstalling the software. Otherwise, the data will be lost. For more information, see "Export configuration", page 624.

Print or view a user report

1. In the SAE Administrator Console main screen, click the **Users** tab.
2. Click **Report**. The user report opens in the default web browser.
3. View the report, then print or save the report as a PDF in the desired location.
4. Close the report.

Roles tab

Roles tab allows SAE Administrators to create, edit, and delete custom roles, and to create roles reports. SAE roles determine the SAE permissions that are associated with an SAE user account.



- The SAE module for the Attune™ Cytometric Software provides the following default SAE account types:
  - **Administrator:** System administrator role with full access to the software and full privileges, including the ability to modify SAE configuration and define the system security policy.
  - **Advanced User:** Similar to Attune™ advanced user.
  - **User:** Similar to Attune™ user.
  - **Reviewer:** Can only review Audit History and Performance Test Reports.
  - **No Privileges Role:** This role is for internal use only by the SAE Administrator Console to set up user repositories. Do not assign this role to a user account.
- For the permissions assigned to each default SAE account type, see "SAE account permissions", page 583.

- There are four action buttons in the Roles tab:
  - **Create**: Allows the SAE Administrator to create custom SAE roles that give granular permissions to the Attune™ Cytometric Software features (see “Create a role”, page 588).
  - **Edit**: Allows the SAE Administrator to edit the settings for the selected custom SAE role (see “Edit a role”, page 589).

    The default SAE roles (Administrator, Advanced User, User, Reviewer, and No Privileges) are non-modifiable and are fixed with the functions they can perform.
  - **Delete**: Allows the SAE Administrator to delete selected custom SAE roles (see “Delete a role”, page 589).
  - **Report**: Allows the SAE Administrator to create a PDF report that lists the available SAE roles (see “Print or view a user report”, page 590).

Note: Changes made to a role in the SAE Administrator Console are reflected in the client application (i.e., Attune™ Cytometric Software) within 10 seconds.

SAE account permissions

For the permissions assigned to each default SAE account type, see the following table. To create a custom role with specific privileges, see "Create a role", page 588.

Permission	Administrator	Advanced User	User	Reviewer
SAE Administrator Console permissions				
Security Configuration				
- Configure Security and Auditing	Yes	No	No	No
Audit History				
- View Action Records	Yes	No	No	Yes
- View System Configuration Records	Yes	No	No	Yes
- View Application Object Records	Yes	No	No	Yes
- View Instrument Run Records	Yes	No	No	Yes
Attune™ Cytometric Software permissions				
Acquisition Control				
- Copy and paste run protocols settings	Yes	Yes	Yes	No
- Modify collect options	Yes	Yes	Yes	No
- Modify run protocol settings	Yes	Yes	Yes	No
- Record over (overwrite) existing sample data	Yes	Yes	Yes	No
- Run and record samples/plates	Yes	Yes	Yes	No
Compensation				
- Apply compensation	Yes	Yes	Yes	No
- Create compensation	Yes	Yes	Yes	No
- Delete compensation	Yes	Yes	Yes	No
- Edit compensation channels	Yes	Yes	Yes	No
- Export compensation	Yes	Yes	Yes	No
- Import compensation	Yes	Yes	Yes	No
- Modify compensation values	Yes	Yes	Yes	No
Experiment Management				
- Change experiment and group colors	Yes	Yes	Yes	No
- Create experiments	Yes	Yes	Yes	No
- Create experiments from templates	Yes	Yes	Yes	No
- Create samples and groups	Yes	Yes	Yes	No
- Create templates	Yes	Yes	Yes	No
- Delete experiments	Yes	Yes	Yes	No
- Delete samples	Yes	Yes	Yes	No
- Delete templates	Yes	Yes	Yes	No
- Duplicate experiments	Yes	Yes	Yes	No
- Export experiments	Yes	Yes	Yes	No
- Export FCS files	Yes	Yes	Yes	No
- Export sample list	Yes	Yes	Yes	No
- Export templates	Yes	Yes	Yes	No
- Import experiments	Yes	Yes	Yes	No
- Import FCS files	Yes	Yes	Yes	No
- Import sample list	Yes	Yes	Yes	No

Permission	Administrator	Advanced User	User	Reviewer
Experiment Management, continued				
- Import templates	Yes	Yes	Yes	No
- Modify experiment annotation	Yes	Yes	Yes	No
- Modify templates	Yes	Yes	Yes	No
- Open experiments	Yes	Yes	Yes	No
- Remove FCS files	Yes	Yes	Yes	No
- Show/Hide experiment and group colors	Yes	Yes	Yes	No
- Update keywords in exported FCS files	Yes	Yes	Yes	No
Filter Configuration				
- Create and edit filters	Yes	Yes	No	No
- Manage filter configurations	Yes	Yes	No	No
- Modify filter mapping	Yes	Yes	No	No
- View filter configuration	Yes	Yes	Yes	No
Image Analysis Settings				
- Apply Image Filtering	Yes	Yes	Yes	No
- Delete Cell Images	Yes	Yes	Yes	No
- Export Cell Images	Yes	Yes	Yes	No
- Insert Cell Image Objects	Yes	Yes	Yes	No
- Process Cell Images	Yes	Yes	Yes	No
- Measure Image	Yes	Yes	Yes	No
Image Capture Settings				
- Modify camera control settings	Yes	Yes	Yes	No
- Modify camera ROI settings	Yes	Yes	Yes	No
- Modify image capture settings	Yes	Yes	Yes	No
Instrument Control				
- Run auto sampler calibration	Yes	Yes	Yes	No
- Run debubble and unclog	Yes	Yes	Yes	No
- Run deep clean and shutdown	Yes	Yes	Yes	No
- Run recover sample	Yes	Yes	Yes	No
- Run rinse, SIP sanitize	Yes	Yes	Yes	No
- Run startup	Yes	Yes	Yes	No
- Run system decontamination	Yes	Yes	No	No
- Run system self test	Yes	Yes	No	No
Instrument Settings				
- Copy and paste instrument settings	Yes	Yes	Yes	No
- Export instrument settings	Yes	Yes	Yes	No
- Import instrument settings	Yes	Yes	Yes	No
- Modify advanced instrument settings	Yes	Yes	No	No
- Modify parameter on/off states	Yes	Yes	Yes	No
- Modify parameter target and label names	Yes	Yes	Yes	No
- Modify system instrument settings	Yes	No	No	No

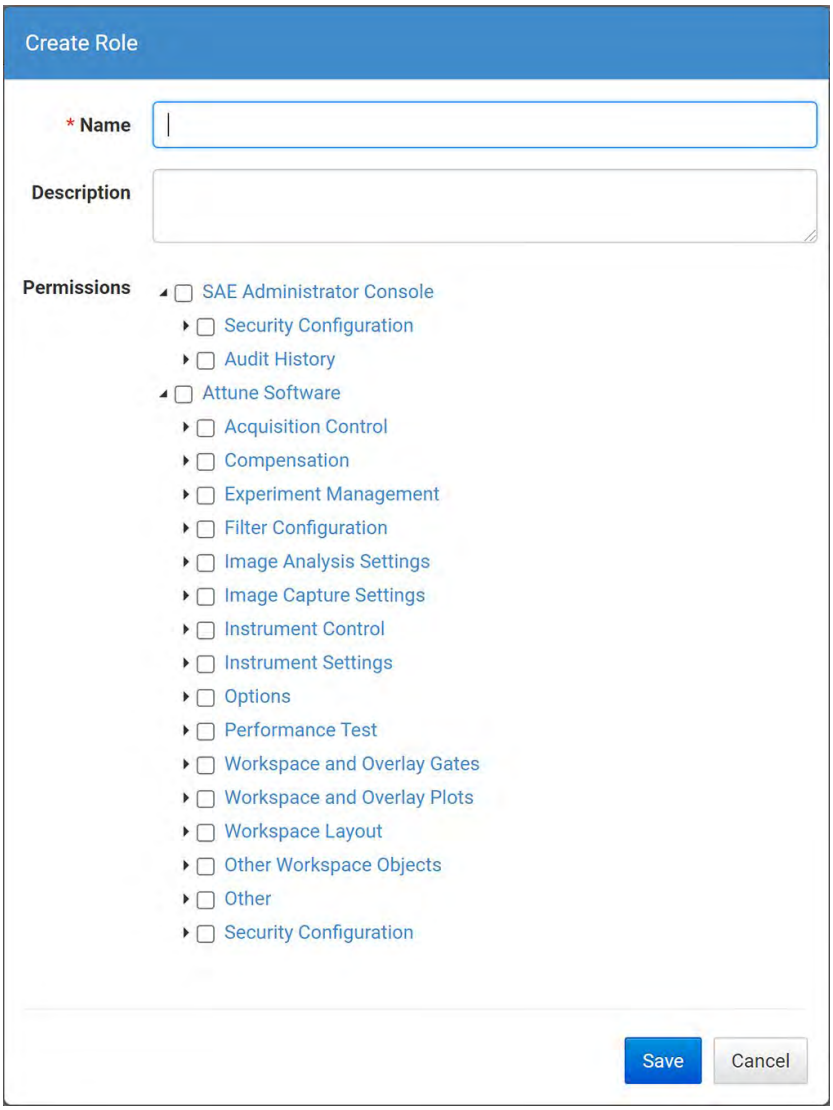
Permission	Administrator	Advanced User	User	Reviewer
Instrument Settings, continued				
- Modify thresholds	Yes	Yes	Yes	No
- Modify voltages	Yes	Yes	Yes	No
Options				
- Create and edit plates	Yes	Yes	No	No
- Create and edit user keywords	Yes	Yes	Yes	No
- Manage global keywords	Yes	No	No	No
- Modify administrator options	Yes	No	No	No
- Modify configuration options	Yes	No	No	No
- Modify default colors	Yes	Yes	Yes	No
- Modify default font options	Yes	Yes	Yes	No
- Modify default gate options	Yes	Yes	Yes	No
- Modify default plot options	Yes	Yes	Yes	No
- Modify default sample and group name	Yes	Yes	Yes	No
- Modify default statistics options	Yes	Yes	Yes	No
- Modify display options	Yes	Yes	Yes	No
- Modify export options	Yes	Yes	Yes	No
Performance Test				
- Reset baseline	Yes	Yes	No	No
- Run performance test	Yes	Yes	No	No
- View performance test report	Yes	Yes	Yes	Yes
Workspace and Overlay Gates				
- Change gate color	Yes	Yes	Yes	No
- Change gate coordinates	Yes	Yes	Yes	No
- Change gate name	Yes	Yes	Yes	No
- Change gate opacity	Yes	Yes	Yes	No
- Change gate type	Yes	Yes	Yes	No
- Create gates	Yes	Yes	Yes	No
- Delete gates	Yes	Yes	Yes	No
- Export gate to FCS file	Yes	Yes	Yes	No
- Modify autogate settings	Yes	Yes	Yes	No
- Modify gate equation	Yes	Yes	Yes	No
- Modify gate Z order	Yes	Yes	Yes	No
- Show/Hide gate name	Yes	Yes	Yes	No
Workspace and Overlay Plots				
- Change density plot color and mode	Yes	Yes	Yes	No
- Change legend text	Yes	Yes	Yes	No
- Change overlay opacity	Yes	Yes	Yes	No
- Change overlay plot color	Yes	Yes	Yes	No
- Change percent of displayed events	Yes	Yes	Yes	No
- Change plot axes labels	Yes	Yes	Yes	No

Permission	Administrator	Advanced User	User	Reviewer
Workspace and Overlay Plots, continued				
- Change plot parameters	Yes	Yes	Yes	No
- Change plot range	Yes	Yes	Yes	No
- Change plot resolution	Yes	Yes	Yes	No
- Change plot scale	Yes	Yes	Yes	No
- Change plot title	Yes	Yes	Yes	No
- Change plot types	Yes	Yes	Yes	No
- Create overlays	Yes	Yes	Yes	No
- Delete overlays	Yes	Yes	Yes	No
- Delete plots	Yes	Yes	Yes	No
- Format plot text	Yes	Yes	Yes	No
- Hide tick marks on overlays	Yes	Yes	Yes	No
- Insert plots	Yes	Yes	Yes	No
- Modify histogram properties	Yes	Yes	Yes	No
- Modify overlay 3D options	Yes	Yes	Yes	No
- Modify plot statistics	Yes	Yes	Yes	No
- Perform overlay calculations	Yes	Yes	Yes	No
- Reorder overlay plot members	Yes	Yes	Yes	No
- Save plot as image	Yes	Yes	Yes	No
- Show/Hide overlay plots	Yes	Yes	Yes	No
- Show legend on overlays	Yes	Yes	Yes	No
Workspace Layout				
- Align objects	Yes	Yes	Yes	No
- Change workspace grid size	Yes	Yes	Yes	No
- Modify workspace mode	Yes	Yes	Yes	No
- Move and resize objects	Yes	Yes	Yes	No
Other Workspace Objects				
- Copy objects to clipboard	Yes	Yes	Yes	No
- Delete images	Yes	Yes	Yes	No
- Delete statistics	Yes	Yes	Yes	No
- Delete text boxes	Yes	Yes	Yes	No
- Edit text box text	Yes	Yes	Yes	No
- Format statistics	Yes	Yes	Yes	No
- Format text box	Yes	Yes	Yes	No
- Insert images	Yes	Yes	Yes	No
- Insert statistics	Yes	Yes	Yes	No
- Insert text boxes	Yes	Yes	Yes	No
- Modify statistics box statistics	Yes	Yes	Yes	No

Permission	Administrator	Advanced User	User	Reviewer
Other				
- Export workspace	Yes	Yes	Yes	No
- Import workspace	Yes	Yes	Yes	No
- Modify heatmap settings	Yes	Yes	Yes	No
- Printing and page layout	Yes	Yes	Yes	No
- View system logs	Yes	Yes	Yes	No
- View user logs	Yes	No	No	No
Security Configuration				
- Perform e-signing	Yes	Yes	No	No

Create a role

1. In the SAE Administrator Console main screen, click the **Roles** tab.
2. Click **Create** to open the *Create Role* dialog.

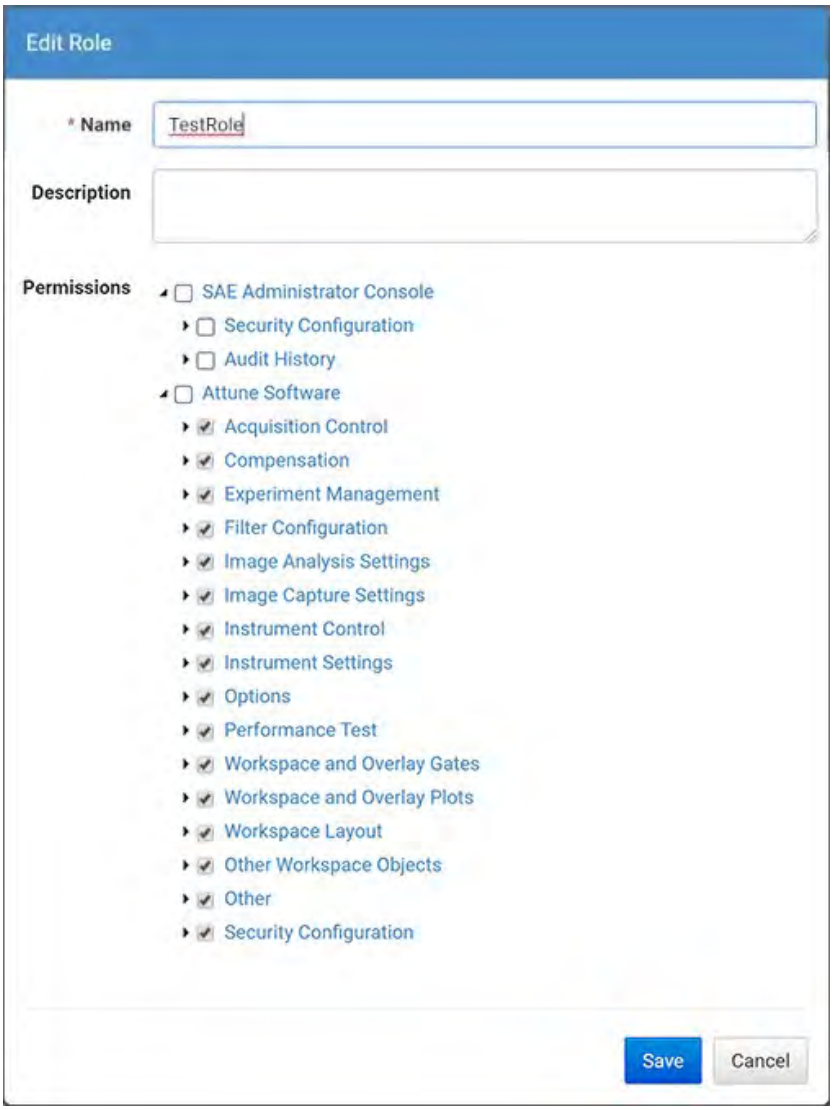


3. Enter the **Name** and *(optional)* **Description** for the new role.
4. Select the SAE permissions for the role.

   To select all SAE permissions in a category, select the checkbox next to the category.
5. Click **Save**. The newly created role is displayed in the SAE Roles list in the Roles tab and becomes available in the Role dropdown in the Create User Account dialog (page 579).

Edit a role

1. In the SAE Administrator Console main screen, click the **Roles** tab.
2. Select a role, then click **Edit** to open the *Edit Role* dialog.



Note: You cannot edit the default SAE roles (Administrator, Advanced User, User, Reviewer, and No Privileges).

3. Edit the settings as desired, then click **Save**.

Note: Changes made to a role in the SAE Administrator Console are reflected in the client application (i.e., Attune™ Cytometric Software) within 10 seconds.

Delete a role

1. In the SAE Administrator Console main screen, click the **Roles** tab.
2. Select a role, then click **Delete**.

Note: If an SAE user account is assigned to a role, that role cannot be deleted.

Print or view a role report

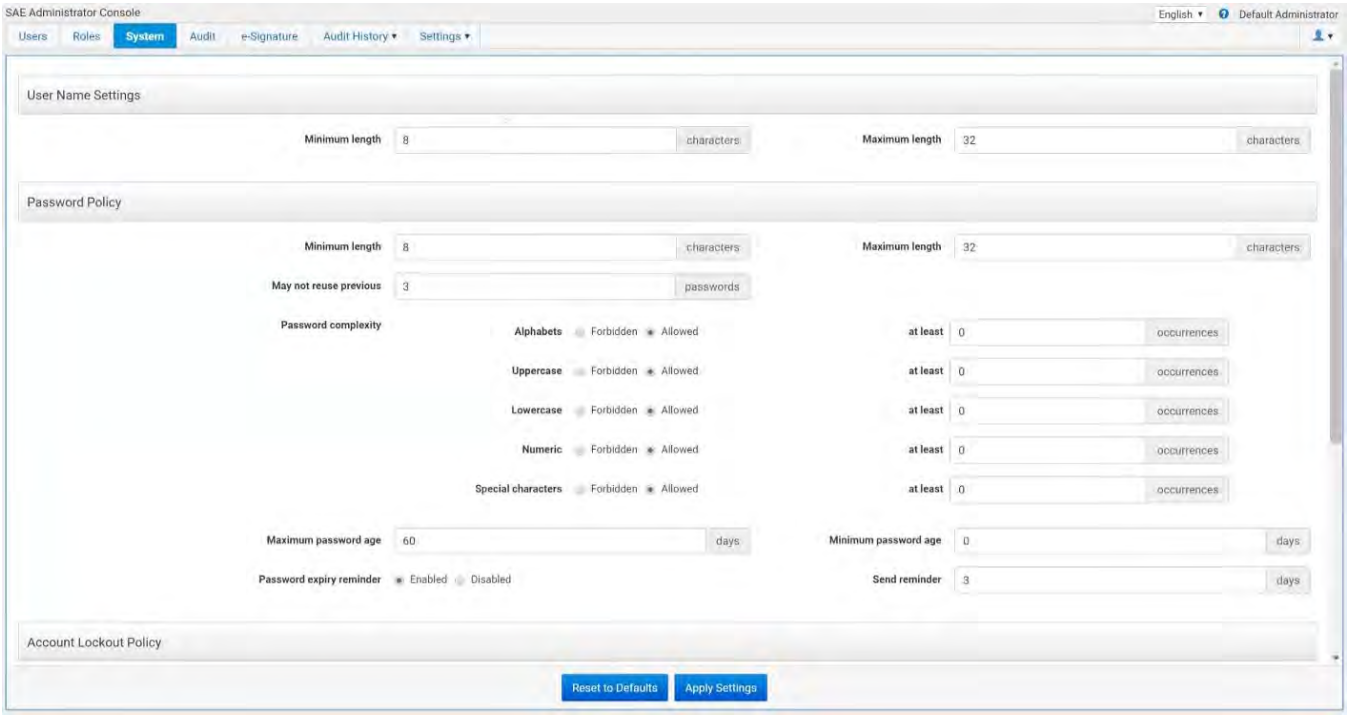
1. In the SAE Administrator Console main screen, click the **Roles** tab.
2. Click **Report**. The role report opens in the default web browser.
3. View the report, then print or save the report as a PDF in the desired location.
4. Close the report.

System tab

System tab allows the SAE Administrator to modify SAE configuration and define the system security policy. In this tab, the SAE Administrator can:

- Configure username settings (page 591)
- Set password policy (page 592)
- Define account lockout policy (page 593)
- Configure other system settings (page 594).

Access to the System tab is restricted to SAE users with the permission to "Configure Security and Auditing" (see "SAE account permissions", page 583).



Note: Settings in this screen affect all SAE user accounts. Settings are applied the next time that users sign in to an application.

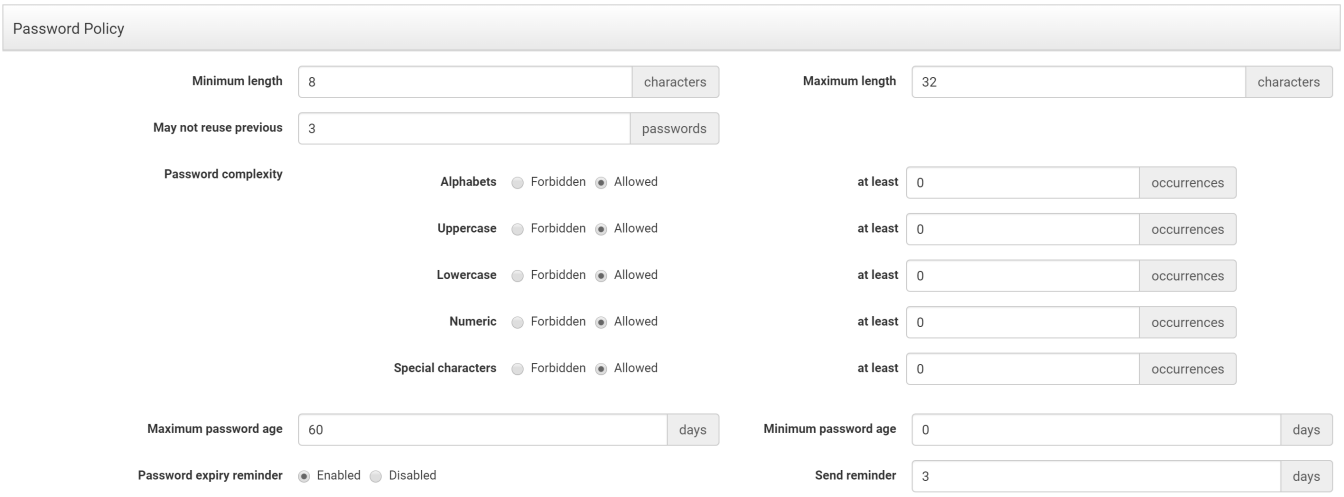
Username Settings

Username Settings allow the SAE Administrator to set a minimum and maximum length for SAE usernames.

- By default, minimum and maximum length for SAE usernames are set as 8 and 32 characters, respectively.
- The Attune™ Cytometric Software only allows a maximum of 50 characters for usernames. If a username with greater than 50 characters is chosen, the SAE user will not be able to login into the Attune™ Cytometric Software.

Password Policy

Password Policy provides options to configure minimum and maximum password length, password complexity requirements (inclusion of alpha, numeric, lower case, upper case and special characters), password age, and password expiration reminders settings.



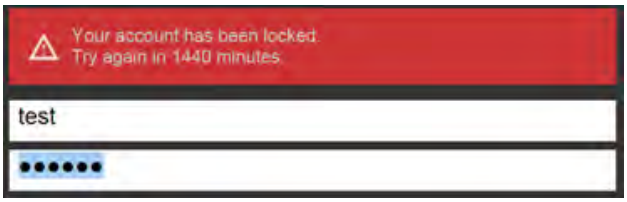
- The password policy is only applied to SAE user accounts and is considered when a user chooses to change their password in the Attune™ Cytometric Software (see "User Management – Change a password", page 530) or is required to change their password on login (see "Expired Password", page 23).
- If the password does not meet any of the password policy settings, a balloon tip is displayed specifying the password policy criteria.
- *Password Age* settings specify the maximum (password expiration) and minimum password age (time before a user can change their password), and the password expiration reminder settings.
- When the password reminder setting is enabled, the user is presented with a warning dialog when logging in telling them that their password will expire in a specified number of days (as set by the send reminder value).

Account Lockout Policy

Account Lockout Policy settings control the number of failed sign in attempts allowed as an SAE user before locking the user out for a specified time.

- By default, the Account Lockout feature is enabled and allows 3 invalid sign in attempts before locking the account (suspending) for a specified time (24 hours by default).

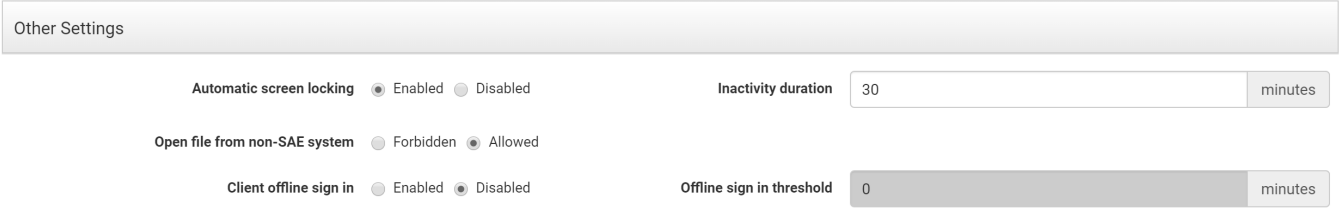
  You can set the lockout threshold from 1 to 999 attempts, and the lockout time from 1 to 99,999 minutes.
- The account lockout policy also includes a setting that allows the sign in attempt counter to automatically reset (enabled by default) after a specified time (24 hours by default and configurable between 1 and 99,999 minutes).
- If a user exceeds the sign in attempt limit, the user's account is suspended and a warning is displayed in the sign in screen.



Other Settings

Other Settings group allows the SAE Administrator to control the following settings:

- Automatic screen locking (due to inactivity) (page 594)
- Open files from non-SAE system (page 595)
- Client offline sign in (page 596)



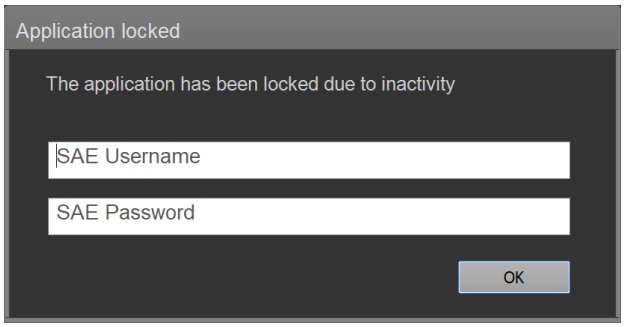
Automatic screen locking

Automatic screen locking allows the system to automatically lock out users after a specified period of inactivity, which is defined as no keyboard or mouse input in the client application software (i.e., Attune™ Cytometric Software).

- Automatic screen locking is enabled by default and set to 30 minutes of inactivity. You can set the inactivity duration from 1 to 99,999 minutes.
- When you sign in as an SAE user, your user session is actively managed to ensure that the system is in use by you. The software registers any movement of the mouse or keyboard input as activity.

> **Note:** You can also manually lock the Attune™ Cytometric Software by selecting **Lock Application** from the **File** menu or by entering **Ctrl+Alt+L** on the keyboard.

- When the SAE server is configured to automatically lock out users after a specified period of inactivity, the Application Locked dialog is displayed after the set period of inactivity, requiring you to enter your credentials to unlock the software.



- During acquisition, when the lockout dialog is displayed due to inactivity, the acquisition will continue uninterrupted.
- If you are signed in while the server is offline, automatic account lockout does not take effect.

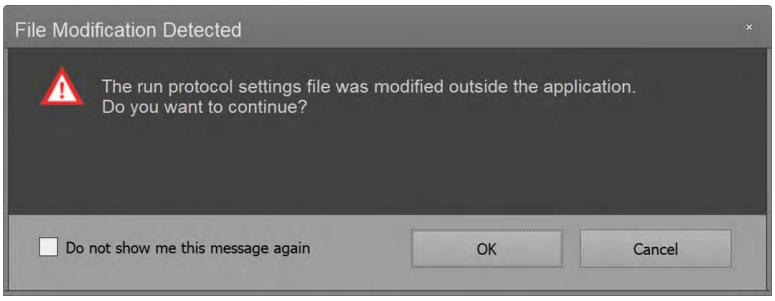
Open file from non-SAE system

When allowed, *Open file from non-SAE system* allows you to access and open files (such as FCS files, Workspace files, Run protocol files) from non-SAE systems. This function also allows you to open, duplicate, and export Experiments containing modifications that result in audit gaps.

Note: When an Experiment is opened, all associated files (such as FCS files, Workspace files, Run protocol files) are checked for any modifications by calculating file checksums and comparing them to the expected values in the database. A mismatch between the checksums from the opened file and the expected values results in an audit gap. When an audit gap is detected, the software displays the "File Modification Detected" warning dialog (see below).

- By default, "Open file from non-SAE system" is set to **Forbidden**.
- If you want to transfer data from a non-SAE Attune™ account into an SAE account, set "Open file from non-SAE system" to **Allowed**.
- For an initial migration step for files that do not have the SAE marking, the SAE Administrator could allow non-SAE files to be opened for set time period, then readjust the SAE marking requirement to not allow those files after the initial migration.
- When set to **Allowed**, "Open file from non-SAE system" also allows you to open an Experiment that has been altered outside of the application and continue with that Experiment. However, depending upon what has been changed in the Experiment data file, continuing with a tampered Experiment could result in an Audit gap.

  If there are audit gaps in the audit history, the Attune™ Cytometric Software displays the "File Modification Detected" warning dialog to notify you that you are trying to open an Experiment that has an invalid audit history and ask if you want to continue.



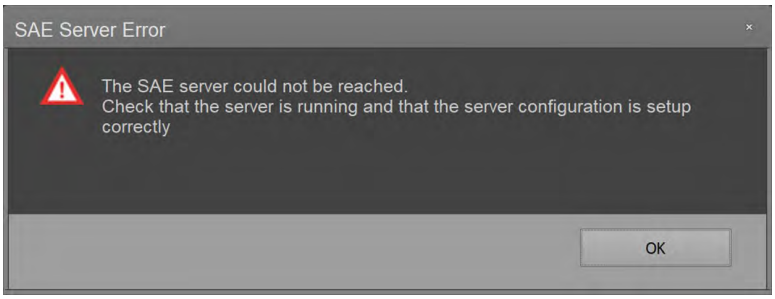
Client offline sign in

Client offline sign in settings control whether an SAE user can sign into the Attune™ Cytometric Software when the SAE server is offline or disconnected.

- Offline sign in is only available for SAE accounts that have signed into the software at least once while the server was online.

  If an account has not accessed the Attune™ Cytometric Software before, that account cannot perform offline sign in, even if the Client offline sign in option is enabled for the system.
- By default, Client offline sign in is disabled and set to allow an offline login threshold of 0 minutes.
- The *Offline sign in threshold* specifies the required elapsed time since the last disconnected state with the SAE server and when an SAE user can sign into the Attune™ Cytometric Software.

  When Client offline sign in is enabled, a value between 1 and 99,999 minutes must be specified for the Offline sign in threshold.
- If the Offline sign in threshold is exceeded, offline sign in cannot be performed, and the software displays the SAE Server Error message.

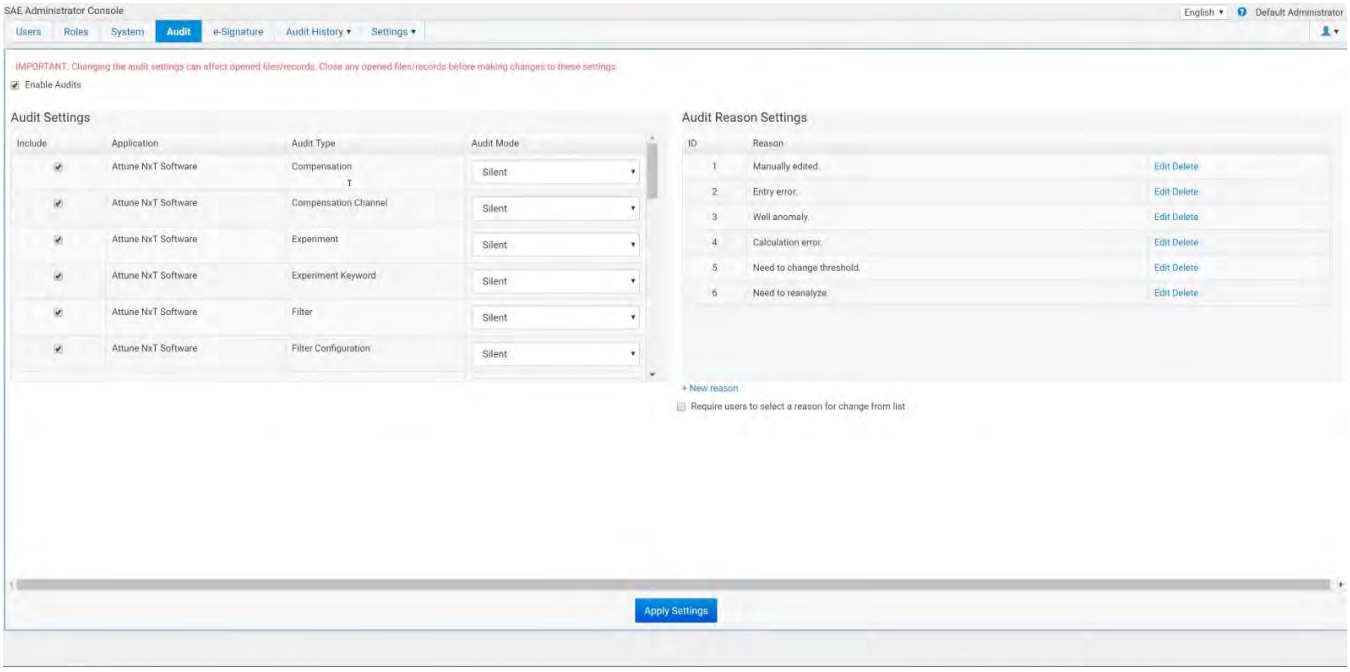


- A currently signed in user can remain signed in after the sign in threshold limit is exceeded.
- After an SAE user has signed in offline, the security configuration (FAC) that takes effect is based on the last connected state.
- Audited events, data audits and e-Signature records are generated and sent to server when the SAE server connection is restored. The audit data and e-Signature data are stored in an encrypted cache and synchronized with the server upon reconnection.
- Automatic account lockout, password reminder notification, and mandatory password change does not take effect under offline sign in.
- The following features cannot be performed under offline sign in:
  - **Disable SAE:** If a user attempts to disable the SAE mode while in the offline mode, the software displays the SAE Server Error message stating that the SAE mode cannot be disabled while in the offline mode.
  - **Change Password (for SAE user):** If a user attempts to change the password while in the offline mode, the software displays the SAE Server Error message stating that the password cannot be changed while in the offline mode.

Audit tab

Audit tab allows you to enable or disable audits, which track actions performed by users, changes to SAE settings, and changes made to application objects (i.e., Experiments). In the Audit tab, you can also control which actions and application objects are audited, and require users to provide a reason for changes made to auditable application objects.

IMPORTANT! Changing audit settings after Experiments have been created can result in audit gaps. Ensure that the audit settings are configured before using the Attune™ Cytometric Software in the SAE mode.

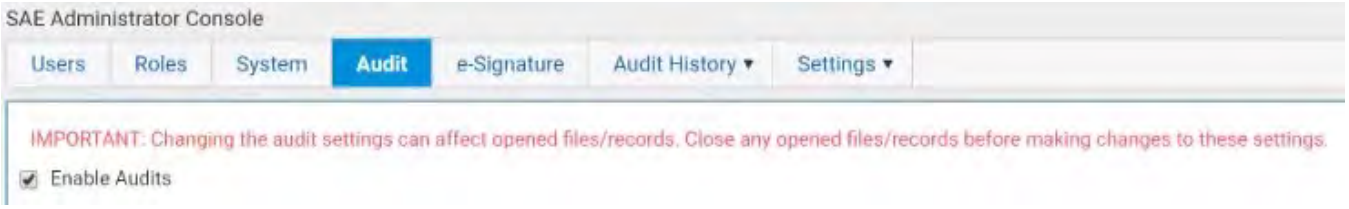


Audit tab has the following two functional groups:

- **Audit Settings:** Consists of the Enable Audits checkbox (page 598) and the Audit Settings panel (page 598)
- **Audit Reason Settings** (page 600)

Enable Audits checkbox

Enable Audits checkbox allows you to turn the audit function on or off (see "Enable or disable the audit function", page 601).

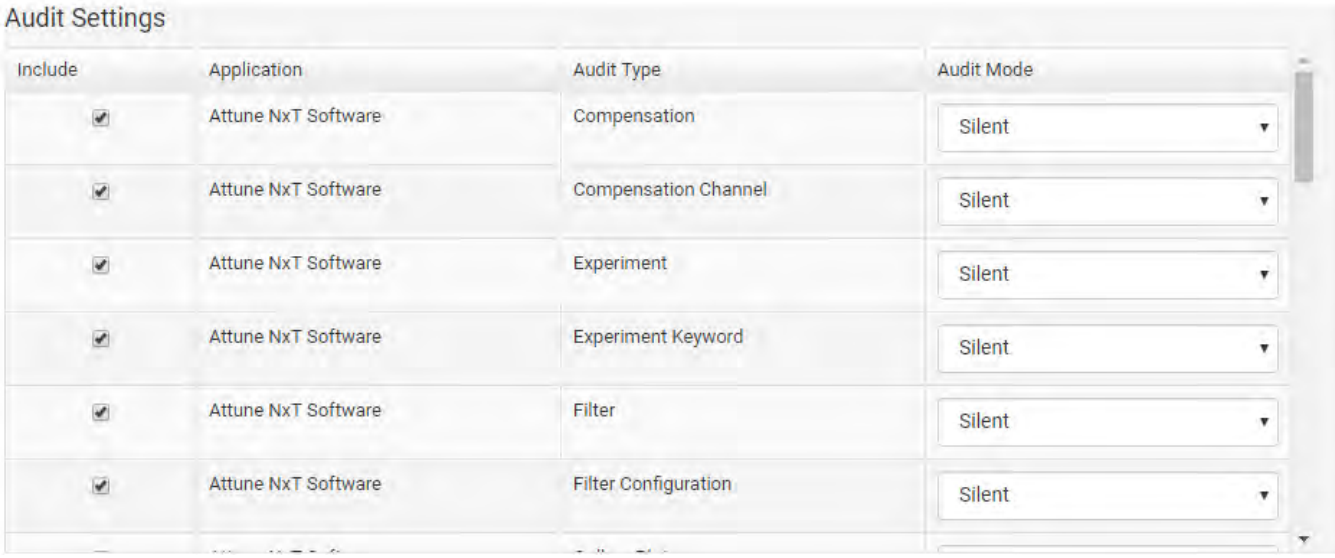


- When the SAE mode is enabled and **Enable Audits** checkbox in the Audit tab is checked, any change to an Experiment results in an audit transaction that is captured in the SAE console, where the old and new values of each Experiment attribute is recorded.
- You can disable the audit function in its entirety or for specific Audit types (see "Audit Settings panel", page 598).
- By default, auditing is enabled for all Audit Types.
- If you have permission to view Audit History, you can view the audit records in the *Audit History tab* (page 607).

Note: You cannot turn off auditing for Action Records (page 608) and System Configuration (page 610). Even if you unselect the **Enable Audits** checkbox, these are always audited.

Audit Settings panel

Audit Settings panel allows you to select or deselect which specific application objects are included in the audit, and the Audit mode (see "Select items to audit and set the Audit Mode", page 601).



- Audit Settings panel has four columns:
  - **Include:** Selects the items to include in the audit.
  - **Application:** Displays the application to which the audit applies.

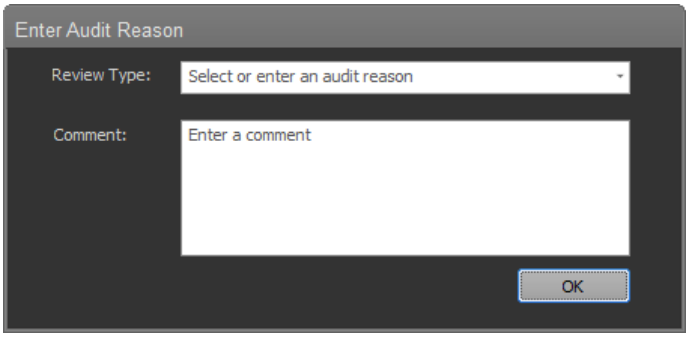
    If your SAE Administrator Console is configured to manage the SAE module for more than one application, you can audit more than one application (for example, Attune™ Cytometric Software and SeqStudio™ Genetic Analyzer).
  - **Audit Type:** Lists what is included in the audit.
  - **Audit Mode:** Allows you to select one of three audit modes:

Audit mode	Reason
Silent	The event is audited, no reason prompt is displayed.
Optional	The event is audited, a reason prompt is displayed, but you can cancel and continue without entering a reason.
Required	The event is audited, a reason prompt is displayed, and you must specify a reason.

- The default audit mode for Attune™ Cytometric Software application objects is **Silent**.
- For **Silent** auditing, audits take place silently with no user input. The changes are audited with the following default reasons (depending on Change type):

Change type	Reason
Create	User wants to create
Update	User wants to update
Delete	User wants to delete

- If an application object is set to audit using the **Optional** or **Required** mode, the *Enter Audit Reason dialog* is displayed each time a change is made that results in an audit.



Audit Reason Settings panel

Audit Reason Settings panel allows you to configure the audit reasons that are presented in the Enter Audit Reason dialog.

Audit Reason Settings

ID	Reason	
1	Manually edited.	Edit Delete
2	Entry error.	Edit Delete
3	Well anomaly.	Edit Delete
4	Calculation error.	Edit Delete
5	Need to change threshold.	Edit Delete
6	Need to reanalyze.	Edit Delete

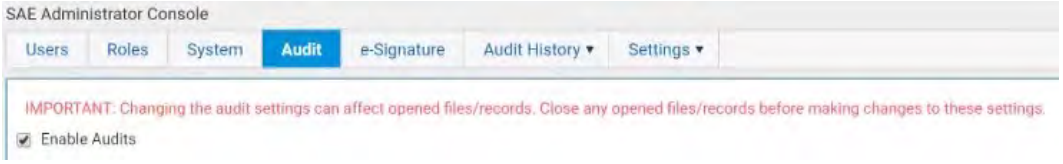
+ New reason

- The SAE console is preloaded with the following defined audit reasons:
  - Manually edited
  - Entry error
  - Well anomaly
  - Calculation error
  - Need to change threshold
  - Need to reanalyze
- You can edit or delete any existing reason by clicking **Edit** or **Delete**.
- You can create new audit reasons by clicking **New reason**.
- You can enforce the use of predefined reasons instead of allowing a user to manually type a reason by selecting the **Require users to select a reason for change from list** checkbox.

☐ Require users to select a reason for change from list

Enable or disable the audit function

1. In the SAE Administrator Console main screen, click the **Audit** tab.
2. Select or deselect **Enable Audits**. The role report opens in the default web browser.



> **IMPORTANT!** Changing the audit settings can affect opened files or records. Close any opened files or records before making changes to audit settings.

3. *(Optional)* Set or modify the **Audit Settings** and the **Audit Reason Settings**.

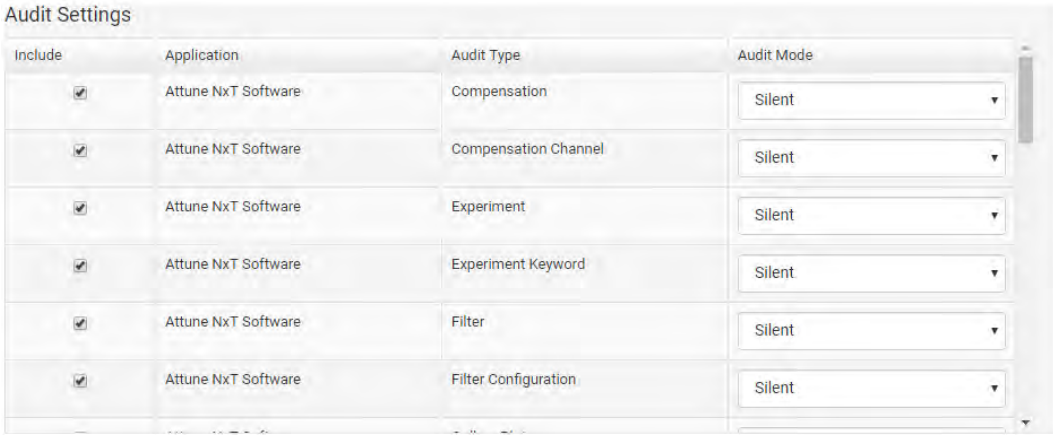
> **Note:** When the **Audit mode** is set to **Silent**, audit reasons are not available for user selection in an application.

4. Click **Apply Settings**.

> **Note:** You cannot turn off auditing for Action Records (page 608) and System Configuration (page 610). Even if you unselect the **Enable Audits** checkbox, these are always audited.

Select items to audit and set the Audit Mode

1. In the SAE Administrator Console main screen, click the **Audit** tab.
2. In the **Audit Settings** panel, select the items to audit.

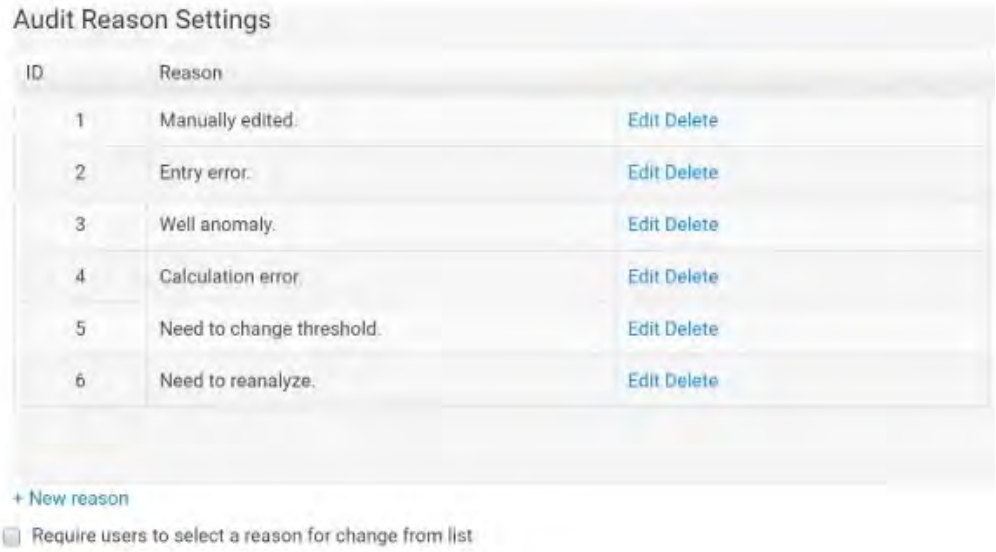


3. Select the **Audit Mode** for each item you include for auditing:
   - **Silent**: The event is audited, no reason prompt is displayed.
   - **Optional**: The event is audited, a reason prompt is displayed, but the user can cancel and continue without entering a reason.
   - **Required**: The event is audited, a reason prompt is displayed, and the user must specify a reason.
4. Click **Apply Settings**.

Configure audit reason settings

1. In the SAE Administrator Console main screen, click the **Audit** tab.

   Configure the audit reason settings in the Audit Reason Settings panel as described below.



2. *(Optional)* Select **Require users to select a reason for change from list** to require users to select a pre-defined audit reason from the Reason list.

Add new audit reason

1. Click **New Reason**.
2. Enter a reason for change, then click **Save**.
3. Click **Apply Settings**.

Edit an existing audit reason:

1. Click **Edit**.
2. Edit the reason for change, then click **Save**.
3. Click **Apply Settings**.

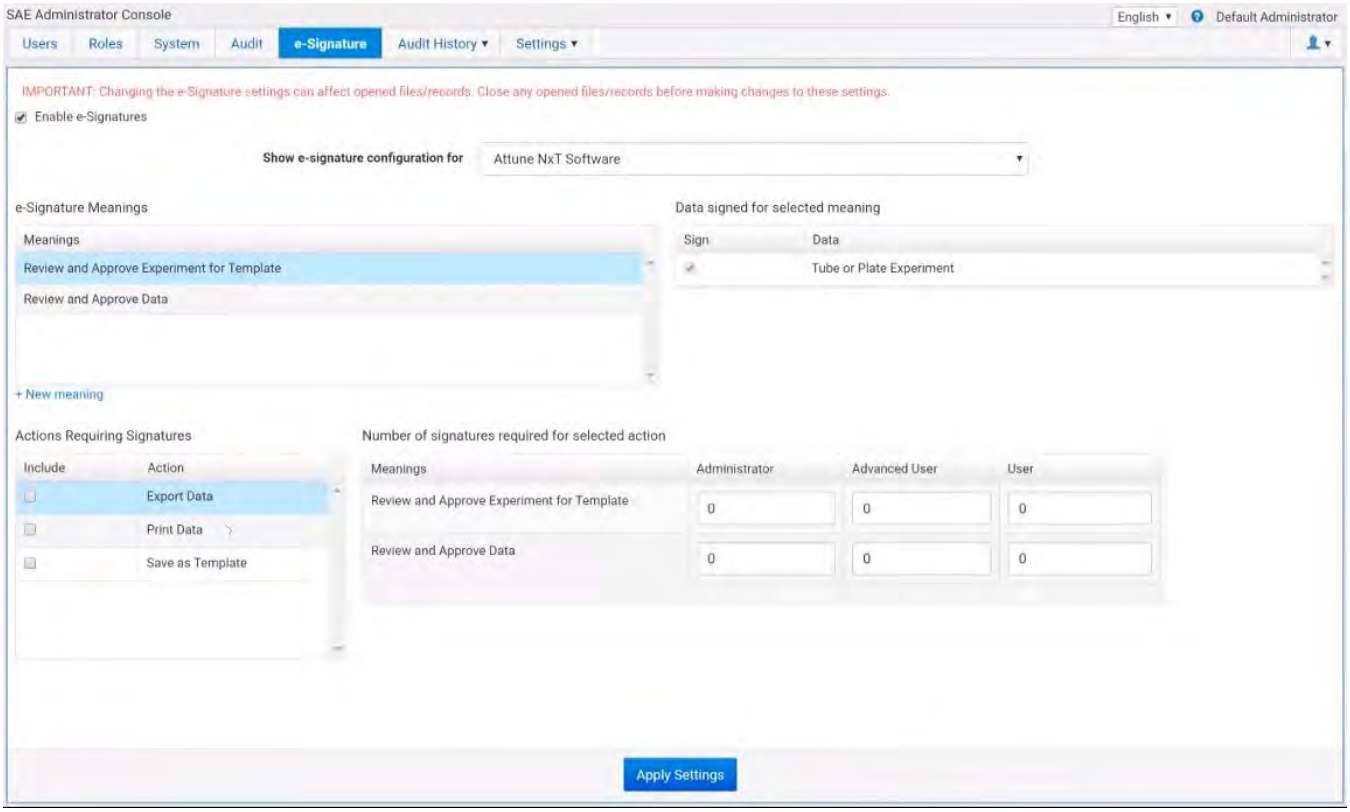
Delete an existing audit reason:

1. Click **Delete**.
2. Click **Delete** to confirm deletion of the audit reason or **Cancel** to exit the dialog.
3. Click **Apply Settings**.

Note: Deleting an audit reason also deletes its ID number. The deleted ID number is not reused for the next audit reason in the list.

e-Signature tab

e-Signature tab allows you to control the e-Signature rights of SAE roles, the actions requiring signatures, the reasons available for e-Signature, and the data to be signed.



- Actions that require signatures to be completed cannot be performed unless the required signatures are provided.
- Signatures represent the state of the Experiment at the time of signing. If any modifications are made to the Experiment after signing, actions that require signatures cannot be performed unless new signatures are provided.
- You can view the log of e-Signatures and the status of the signatures (Current or Obsolete) in the **Audit History ▶ Application Object Records ▶ e-Signature Records** tab (page 616).
- You can enable or disable the requirement for e-Signatures by selecting or unselecting the **Enable e-Signatures** checkbox (see "Enable or disable the e-Signature function", page 604).

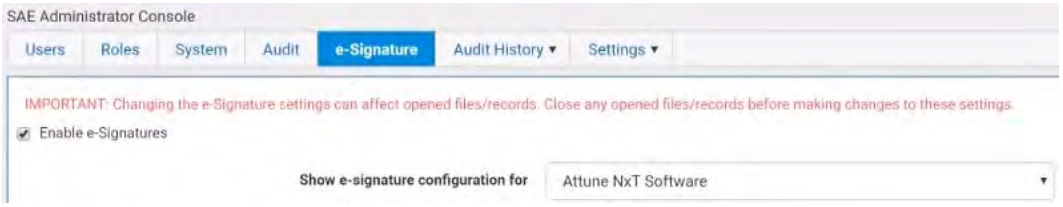
- There are three built-in **Actions Requiring Signatures** for the Attune™ Cytometric Software that you can select in e-Signature tab:
  - Export Data
  - Print Data
  - Save As Template

  By default, the **Include** checkboxes for these actions are unchecked (that is, they do not require e-Signatures unless the **Include** checkbox is checked).
- For instructions to include selected actions for e-Signature requirements, see "Select the actions that require e-Signature", page 605.
- The e-Signature Meanings are the stated reason for an e-Signature. There two built-in **e-Signature Meanings**:
  - Require and Approve Experiment for Template
  - Review and Approve Data

  If desired, you can add custom meanings (see "Configure the meanings of e-Signatures", page 606).
- You can set the number of signatures required for each selected action and for each required meaning in the **Number of signatures required for selected action** panel (see "Select the actions that require e-Signature", page 605).

Enable or disable the e-Signature function

1. In the SAE Administrator Console main screen, click the **e-Signature** tab.
2. Select **Enable e-Signature**.



> **IMPORTANT!** Changing the e-Signature settings can affect opened files or records. Close any opened files or records before making changes to e-Signature settings.

3. From the **Show e-Signature configuration for** dropdown, select **Attune™ Software**.

> **Note:** If your SAE Administrator Console is configured to manage the SAE module for more than one application, you can configure the e-Signature settings for more than one application (for example, Attune™ Cytometric Software and SeqStudio™ Genetic Analyzer).

4. *(Optional)* Set or modify the e-Signature Settings.
5. Click **Apply Settings**.

Select the actions that require e-Signature

1. In the SAE Administrator Console main screen, click the **e-Signature** tab.
2. In the **Actions Requiring Signatures** panel, select each action that requires an e-Signature.

Actions Requiring Signatures

Include	Action
☑	Export Data
☐	Print Data
☐	Save as Template

3. For each meaning of each selected action, enter the number of e-Signatures that are required from each SAE role before that action can be performed.

Number of signatures required for selected action

Meanings	Administrator	Advanced User	User
Review and Approve Experiment for Template	0	0	0
Review and Approve Data	0	0	0

4. Click **Apply Settings**.

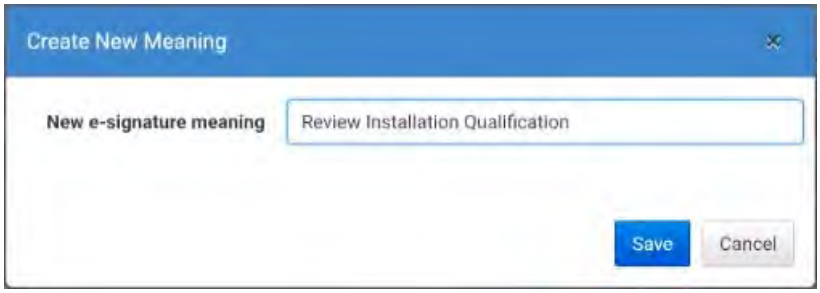
> **Note:** Actions that require signatures to be completed cannot be performed unless the required signatures are provided.

Configure the meanings of e-Signatures

Add an e-Signature meaning

1. In the SAE Administrator Console main screen, click the **e-Signature** tab.
2. In the **e-Signature Meanings** panel, click **New meaning**.

3. In the **Create New Meaning** dialog, enter an e-Signature meaning in the **Name** field, then click **Save**.



4. In the **e-Signature Meanings** panel, select a meaning from the **Meanings** list.
5. In the **Data signed for the selected meaning** list, select the item with which to associate the meaning.

6. Set the actions that require e-Signature and the number of e-Signatures that are required for that action (see "Select the actions that require e-Signature", page 605).
7. Click **Apply Settings**.

Delete an e-Signature meaning

1. In the SAE Administrator Console main screen, click the **e-Signature** tab.
2. In the **e-Signature Meanings** pane, select a meaning from the **Meanings** list, then click **Delete**.

> **Note:** Default meanings cannot be deleted.

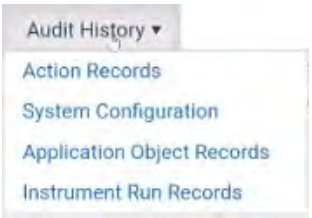
3. Confirm the deletion of the meaning, then click **OK**.
4. Click **Apply Settings**.

Audit History tab

When SAE mode is enabled and application auditing is enabled, any change to an experiment results in an audit transaction that is captured in the SAE console where the old and new values of each experiment attribute is recorded.

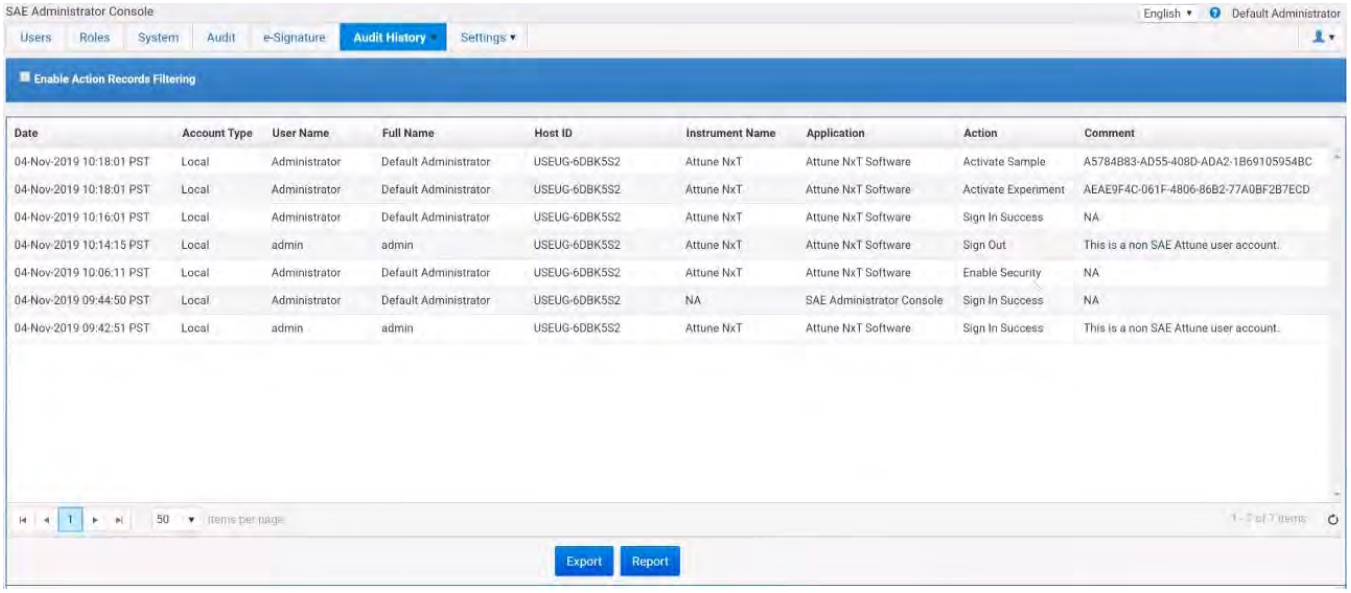
Audit History tab allows users with permission to view Audit History to access the audit logs for the auditable transactions. The permission to view the entire Audit History or specific audit logs is granted in the Roles tab (page 581).

- Use the Audit History dropdown to select the audit history to view.

  Audit logs available for viewing are:
  - *Action Records* (page 608)
  - *System Configuration Records* (page 610)
  - *Application Object Records* (page 612)
  - *Instrument Run Records* (this function is not used by the Attune™ Cytometric Software) (page 616)

  The following example shows the Audit History for Action Records.



- For instructions on how to view specific audit logs and generate printable audit records, see "View audit logs (audit history)", page 617.
- If desired, you can archive the audit records or configure auto archive settings in the SAE Administrator Console – Settings tab (page 619).

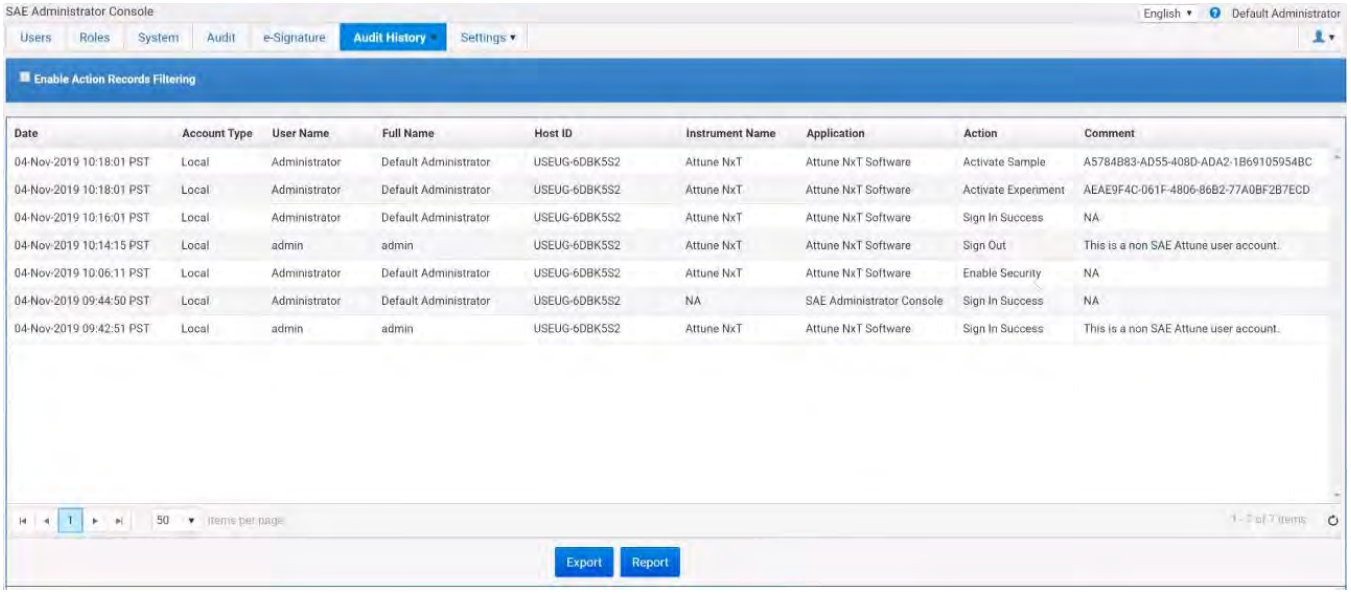
  Archiving audit records removes the records from the SAE Administrator Console and saves them in an internally specified location on the same computer on which the SAE Administrator Console is installed.

  Archived audit records remain accessible for viewing from the Settings tab of the SAE Administrator Console.

  For instructions on how to archive and restore audit records, see "Settings tab", page 619.

Action Records audit log

Action Records audit history allows you to view a log of auditable actions taken by any signed in user, including signing in and out of the application, audit events on an application (such activate, duplicate, delete), running instrument maintenance functions (Startup, Shutdown, Performance Test, etc.), as well as actions taken on the SAE Administrator Console.



Date	Account Type	User Name	Full Name	Host ID	Instrument Name	Application	Action	Comment
04-Nov-2019 10:18:01 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	Attune NxT	Attune NxT Software	Activate Sample	A5784B83-AD55-408D-ADA2-1B69105954BC
04-Nov-2019 10:18:01 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	Attune NxT	Attune NxT Software	Activate Experiment	AEAE9F4C-061F-4806-86B2-77A0BF2B7ECD
04-Nov-2019 10:16:01 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	Attune NxT	Attune NxT Software	Sign In Success	NA
04-Nov-2019 10:14:15 PST	Local	admin	admin	USEUG-6DBK5S2	Attune NxT	Attune NxT Software	Sign Out	This is a non SAE Attune user account.
04-Nov-2019 10:06:11 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	Attune NxT	Attune NxT Software	Enable Security	NA
04-Nov-2019 09:44:50 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	NA	SAE Administrator Console	Sign In Success	NA
04-Nov-2019 09:42:51 PST	Local	admin	admin	USEUG-6DBK5S2	Attune NxT	Attune NxT Software	Sign In Success	This is a non SAE Attune user account.

- Action Records are always audited and cannot be turned off.
- All items in the Action Records log are audited silently (see "Audit mode", page 598).
- Actions Records audit log contains the following columns:
  - Date
  - Account Type (Local or SAE)
  - Username
  - Full Name
  - Host ID
  - Instrument Name
  - Application Name
  - Action
  - Comment
- The Host ID is the PC name or instrument serial number.
- Application Name is the application for which the audit log is created.

> **Note:** If your SAE Administrator Console is configured to manage the SAE module for more than one application, the Action Records audit log can contain audit records for the configured applications (for example, Attune™ Cytometric Software and SeqStudio™ Genetic Analyzer).

- If the action was taken by a non-SAE account, the Comment column displays "This is a non-SAE Attune user account."

- If the audited action affects an application object (such as Experiment, Sample, etc.), the Comment column displays the GUID of the application object.

  The GUID is the unique ID assigned to each application object. It is an immutable property and cannot be changed.

  In the following example, the Comments column displays the GUID of the Sample and the Experiment listed as "Activated" in the Action column.

Action	Comment
Activate Sample	A5784B83-AD55-408D-ADA2-1B69105954BC
Activate Experiment	AEAE9F4C-061F-4806-86B2-77A0BF2B7ECD
Sign In Success	NA
Sign Out	This is a non SAE Attune user account.

- To filter the Actions Records audit log, check the **Enable Action Records Filtering** checkbox, then select the desired **Application** and **Action** from the corresponding dropdown controls.

  If desired, select or enter other filter criteria using the available controls.

  Click **Search** to apply the filter criteria to the Application Object Records.
- Click **Report** to generate a PDF file of the log.
- Click **Export** to generate a TXT file of the log.

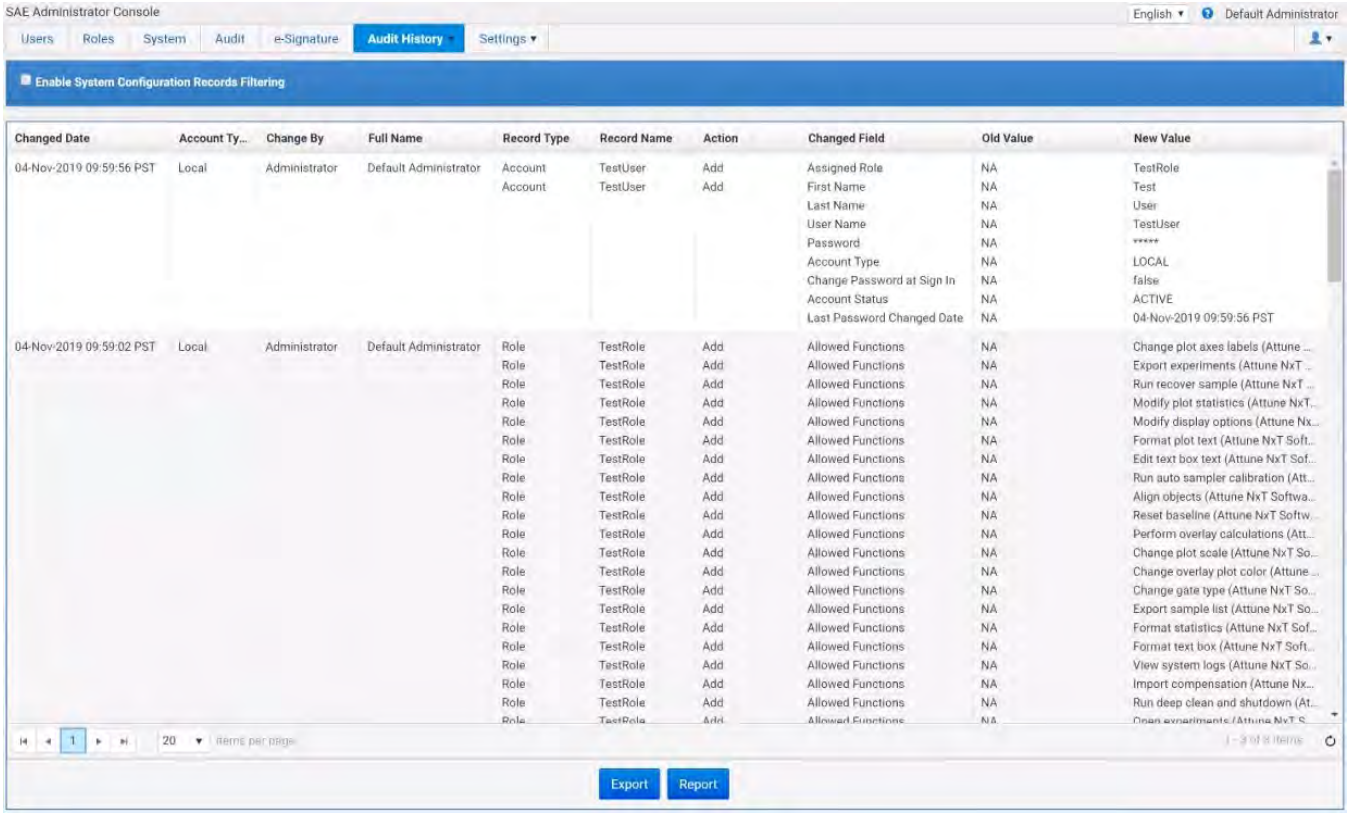
Auditable actions in the SAE console

Auditable actions in the SAE Administrator Console are:

- Enable or disable security, audit, and e-Signature
- Sign in or out of the SAE Administrator Console
- Import or export an SAE configuration
- Install an application profile
- Archive, purge, or restore audit records
- Manual Sync with LDAP Directory

System Configuration audit log

System Configuration audit history allows you to view a log of the system security, audit, and e-Signature configuration records.



- System Configuration is always audited and cannot be turned off.
- System Configuration audit log contains the following columns:
  - Changed Date
  - Account Type (Local or SAE)
  - Change By
  - Full Name
  - Record Type
  - Record Name
  - Action
  - Changed Field
  - Old Value
  - New Value
- The Action Field shows the auditable action taken by the user listed in the Change By and Full Name columns.
- The Record Type, Record Name, Changed Field, Old Value, and New Value columns display the changes made to the system configuration, and the old and new values for the changed configuration.

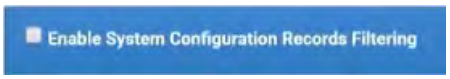
- When a change is made to the system configuration, such as add or delete users, create or assign roles, change system security settings, change audit and e-Signature configurations, it results in an audit change record.

  Only the attributes that are modified are logged and the old value vs. new value is recorded in the SAE console.

  In the following example, the System Configuration audit log shows that a new SAE Account with the account name TestUser is added to the application and the assigned role of the TestUser is TestRole.

Record Type	Record Name	Action	Changed Field	Old Value	New Value
Account	TestUser	Add	Assigned Role	NA	TestRole
Account	TestUser	Add	First Name	NA	Test
			Last Name	NA	User
			User Name	NA	TestUser
			Password	NA	*****
			Account Type	NA	LOCAL
			Change Password at Sign In	NA	false
			Account Status	NA	ACTIVE
			Last Password Changed Date	NA	04-Nov-2019 09:59:56 PST

- To filter the System Configuration Records audit log, check the **Enable System Configuration Records Filtering** checkbox, then select the desired **Record type** from the corresponding dropdown control.

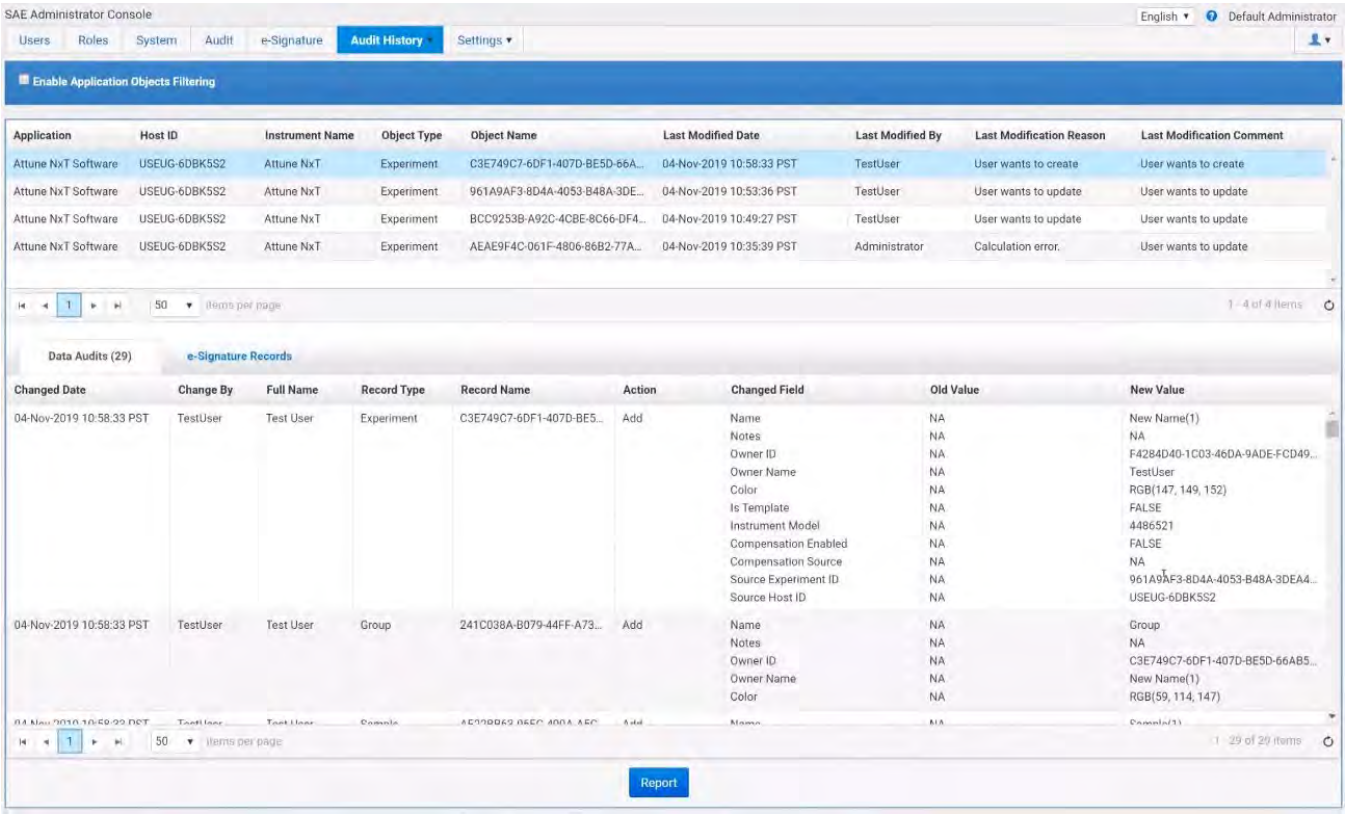
  

  If desired, select or enter other filter criteria using the available controls.

  Click **Search** to apply the filter criteria to the Application Object Records.
- Click **Report** to generate a PDF file of the System Configuration audit log.
- Click **Export** to generate a TXT file of the System Configuration audit log.

Application Objects Records audit log

Application Object Records audit history allows you to view a log of the application object records.



- The Audit History – Application Objects tab is divided into two panels:
  - **Application objects list** (upper panel; page 613)
  - **Data Audits and e-Signature Records tabs** (bottom panel; page 615 and page 616, respectively)

Application objects list

The Application objects panel is the upper panel in the Audit History – Application Objects tab, and it displays a list of the audited application objects.

Enable Application Objects Filtering

Application	Host ID	Instrument Name	Object Type	Object Name	Last Modified Date	Last Modified By	Last Modification Reason	Last Modification Comment
Attune NxT Software	USEUG-6DBK5S2	Attune NxT	Experiment	C3E749C7-6DF1-407D-BE5D-66A...	04-Nov-2019 10:58:33 PST	TestUser	User wants to create	User wants to create
Attune NxT Software	USEUG-6DBK5S2	Attune NxT	Experiment	961A9AF3-8D4A-4053-B48A-3DE...	04-Nov-2019 10:53:36 PST	TestUser	User wants to update	User wants to update
Attune NxT Software	USEUG-6DBK5S2	Attune NxT	Experiment	BCC9253B-A92C-4CBE-8C66-DF4...	04-Nov-2019 10:49:27 PST	TestUser	User wants to update	User wants to update
Attune NxT Software	USEUG-6DBK5S2	Attune NxT	Experiment	AEAE9F4C-061F-4806-86B2-77A...	04-Nov-2019 10:35:39 PST	Administrator	Calculation error.	User wants to update

- Application objects are auditable items such as plate setups, templates, or other items that you create in an application.
- Examples of application object types in the Attune™ Cytometric Software are Plate Experiment, Tube Experiment, Template, Filter Configuration, and Filter Definition.
- When an application object is created, updated, or deleted, an audit record is generated on the SAE console. An application object can have multiple data audit records as well as multiple e-Signature records.
- The Application objects list has the following columns:
  - Application
  - Host ID
  - Instrument Name
  - Object Type
  - Object Name
  - Last Modified Date
  - Last Modified By
  - Last Modification Reason
  - Last Modification Comment
- The Host ID is the PC name or instrument serial number.
- The Object Name is the GUID of the object. The GUID is a unique ID assigned each an application object. It is an immutable property and cannot be changed.
- Each application object record has a corresponding audit log consisting of all sub-application object components with their attribute value change log (old value vs. new value).

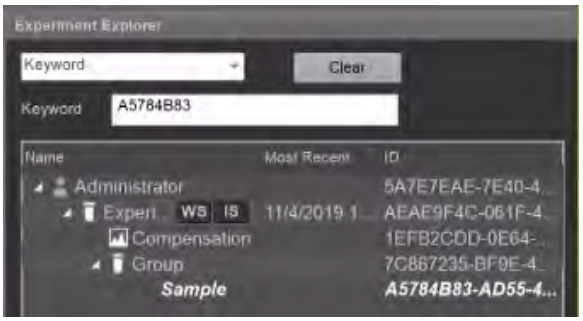
  If you have permission to view Application Object Records in Audit History, you can view the audit log in the **Data Audits** tab (page 615).
- If the e-Signature function is enabled in the e-Signature tab (page 603) and you have permission to view Application Object Records in Audit History, you can view the e-Signature log for the selected application object in the **e-Signature Records** tab (page 616).

- If an application object is deleted, the deleted object appears with a strike-through in the SAE server.

  In the following example, the second experiment from the top is deleted. As a result, the GUID of the deleted experiment is shown with a line over it (strike-through).

Application	Host ID	Instrument Name	Object Type	Object Name	Last Mo
Attune NxT Software	USEUG-6DBK5S2	Attune NxT	Experiment	AE48102E-79FF-4A93-ABF6-8508...	04-Nov-
Attune NxT Software	USEUG-6DBK5S2	Attune NxT	Experiment	~~961A9AF3-8D4A-4053-B48A-3DE...~~	04-Nov-
Attune NxT Software	USEUG-6DBK5S2	Attune NxT	Experiment	C3E749C7-6DF1-407D-BE5D-66A...	04-Nov-

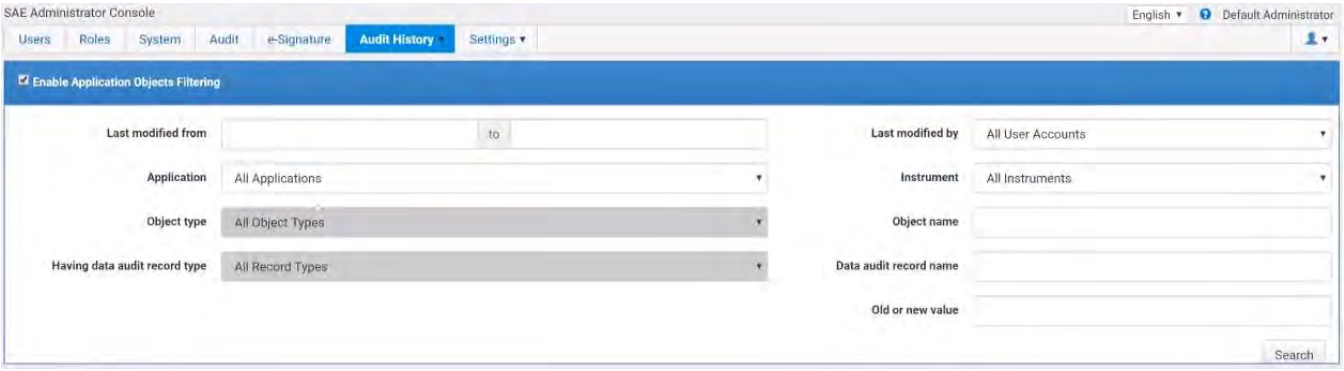
Note: When the Attune™ Cytometric Software is in the SAE mode, you can display the GUID of application objects in the Experiment Explorer, and perform a Keyword search using the objects GUID (see "Display ID", page 198).



- To display a list of items that are audited for your application, select **Enable Application Object Records Filtering**.

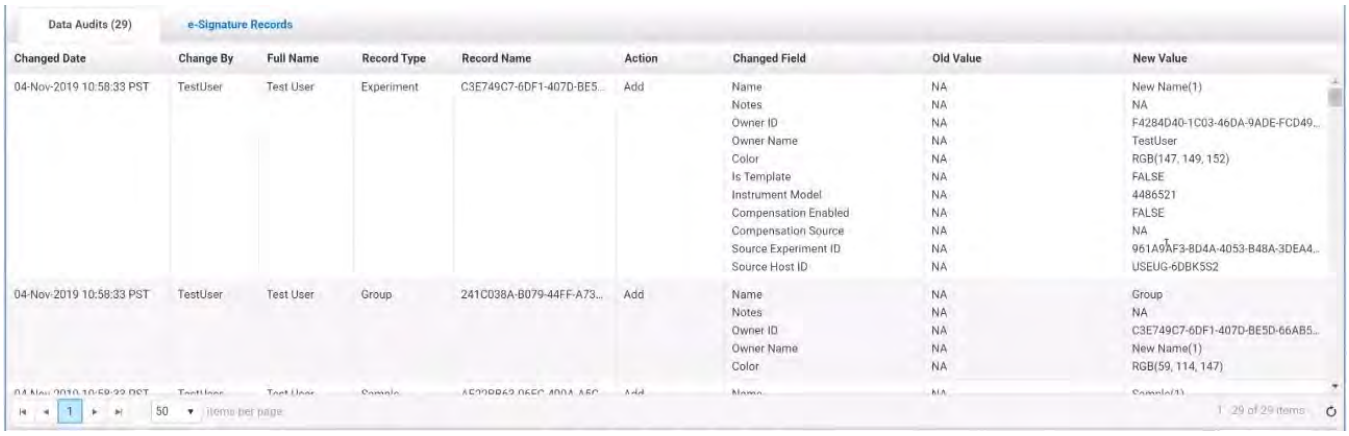
  In the Filtering panel, select **Attune™ Cytometric Software** for the **Application**, then select or enter the desired filter criteria using the available controls.

  Click **Search** to apply the filter criteria to the Application Object Records.



Data Audits tab

The **Data Audits** tab in the bottom panel contains the audit log corresponding to the selected application object.



Data Audits (29) | e-Signature Records

Changed Date	Change By	Full Name	Record Type	Record Name	Action	Changed Field	Old Value	New Value
04-Nov-2019 10:58:33 PST	TestUser	Test User	Experiment	C3E749C7-6DF1-407D-BE5...	Add	Name	NA	New Name(1)
						Notes	NA	NA
						Owner ID	NA	F4284D40-1C03-46DA-9ADE-FCD49...
						Owner Name	NA	TestUser
						Color	NA	RGB(147, 149, 152)
						Is Template	NA	FALSE
						Instrument Model	NA	4486521
						Compensation Enabled	NA	FALSE
						Compensation Source	NA	NA
						Source Experiment ID	NA	961A9AF3-8D4A-4053-B48A-3DEA4...
						Source Host ID	NA	USEUG-6DBK5S2
04-Nov-2019 10:58:33 PST	TestUser	Test User	Group	241C038A-B079-44FF-A73...	Add	Name	NA	Group
						Notes	NA	NA
						Owner ID	NA	C3E749C7-6DF1-407D-BE5D-66AB5...
						Owner Name	NA	New Name(1)
						Color	NA	RGB(59, 114, 147)

1 50 items per page 1 - 29 of 29 items

- The Data Audits tab has the following columns:
  - Changed Date
  - Change By
  - Full Name
  - Record Type
  - Record Name
  - Action
  - Changed Field
  - Old Value
  - New Value
- The Changed Field, Old Value, and New Value columns display the sub-application object components of an application object, and the new and old values of its attributes.

  When an application object is a first created, each of these attributes will display NA for the Old Value.

  When any of these attributes are changed after the object is created, it results in an audit change record. Only the attributes that are modified are logged and the old value vs. new value are recorded in the SAE console.

  The following example shows the Changed Field, Old Value, and New Value columns for a Plate Record.

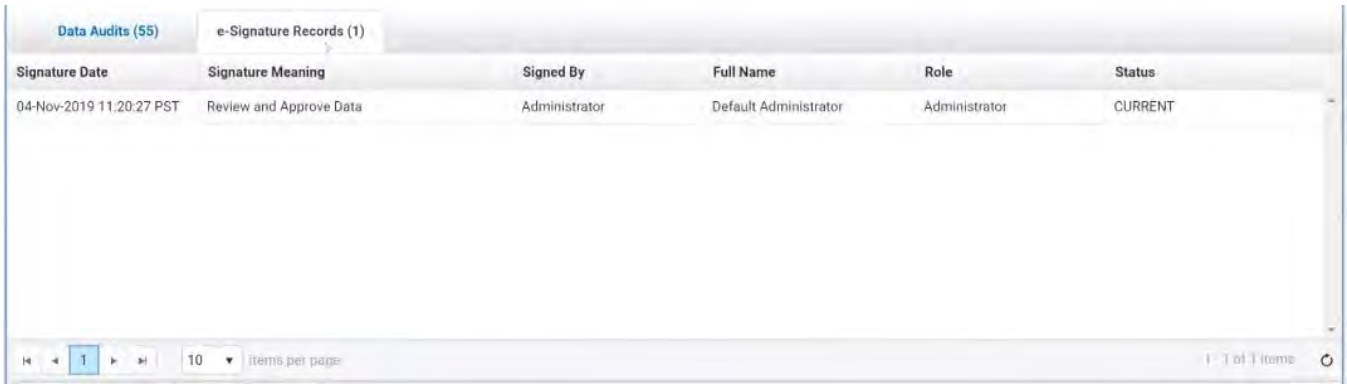
Changed Field	Old Value	New Value
Name	NA	Plate(2)
Notes	NA	NA
Owner ID	NA	B817D128-7609-42BD-9831-07412...
Owner Name	NA	Administrator
Description	NA	NA
Barcode (Plate ID)	NA	NA
Is Template	NA	FALSE
Instrument Model	NA	4486521
Run Orientation	NA	Horizontal
Collection Mode	NA	Only Wells - 1,2,3,4,5,6,7,13,14,15,1...
Collection Wells	NA	A1, A10, A11, A12, A2, A3, A4, A5, ...
Source Plate ID	NA	C2DEF134-4D36-4F0B-B771-A2C9...
Source Host ID	NA	USEUG-6DBK5S2

- Click **Report** to generate a PDF file of the Data Audits log.

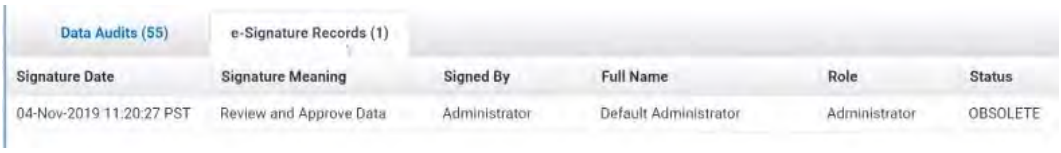
  Export is not supported for this audit log.

e-Signature Records tab

The **e-Signature Records** tab in the bottom panel displays the log of e-Signatures and the status of the signatures for the selected application object.



- The e-Signature Records tab has the following columns:
  - Signature Date
  - Signature Meaning
  - Signed By
  - Full Name
  - Role
  - Status (Current or Obsolete)
- Signatures represent the state of the Experiment at the time of signing. If any modifications are made to the Experiment after signing, the existing signature becomes obsolete.



- When the signatures become obsolete, actions that require signatures cannot be performed unless new signatures are provided.
- Click **Report** to generate a PDF file of the e-Signature Records log.

Instrument Run Records audit log

Instrument Run Records audit history allows you to view a log of the instrument run, including a summary of the run, the application objects used and the actions performed in the run, any changes made during the run, and a list outputs at the completion of the run.

IMPORTANT! Instrument Run Records audit log is not used by the Attune™ Cytometric Software. Therefore, the Instrument Run Records audit history is not available for the Attune™ Cytometric Software application.

View audit logs (audit history)

To view audit logs in the Audit History tab, you must have permission to view Audit History or specific audit logs, which is granted in the Roles tab (page 581).

View the Action Records audit log

All items in the action records log are audited silently.

1. In the SAE Administrator Console main screen, click the **Audit History** tab.
2. Select **Action Records** to view a log of the specified audit events.
3. To display a list of items that are audited for your application:
   a. Select **Enable Action Records Filtering**.
   b. Select your application from the **Application** list.
   c. In the **Action** field, click the dropdown arrow to display the list of auditable actions.
4. *(Optional)* Perform the following actions:
   - Specify other filtering settings.
   - Click **Report** to generate a PDF file of the log.
   - Click **Export** to generate a TXT file of the log.

View the System Configuration audit log

1. In the SAE Administrator Console main screen, click the **Audit History** tab.
2. **Select System Configuration** to view a log of the system security, audit, and e-Signature configuration records.
3. To display a list of items that are audited:
   a. Select **Enable System Configuration Records Filtering**.
   b. In the **Record Type** field, click the dropdown arrow to display the list of auditable system configuration objects.
4. *(Optional)* Perform the following actions:
   - Specify other filtering settings.
   - Click **Report** to generate a PDF file of the log.
   - Click **Export** to generate a TXT file of the log.

View the Application Object Records audit log

Application objects are auditable items such as plate setups, templates, or other items that you create in an application.

1. In the SAE Administrator Console main screen, click the **Audit History** tab.
2. Select **Application Object Records**.
3. To display a list of items that are audited for your application:
   a. Select **Enable Application Object Records Filtering**.
   b. Select your application from the **Application** list.
   c. In the **Having data audit record type** field, click the dropdown arrow to display the list of auditable objects.
4. *(Optional)* Perform the following actions:
   - Specify other filtering settings.
   - Click **Report** to generate a PDF file of the log.
   - Export is not supported for this audit log.

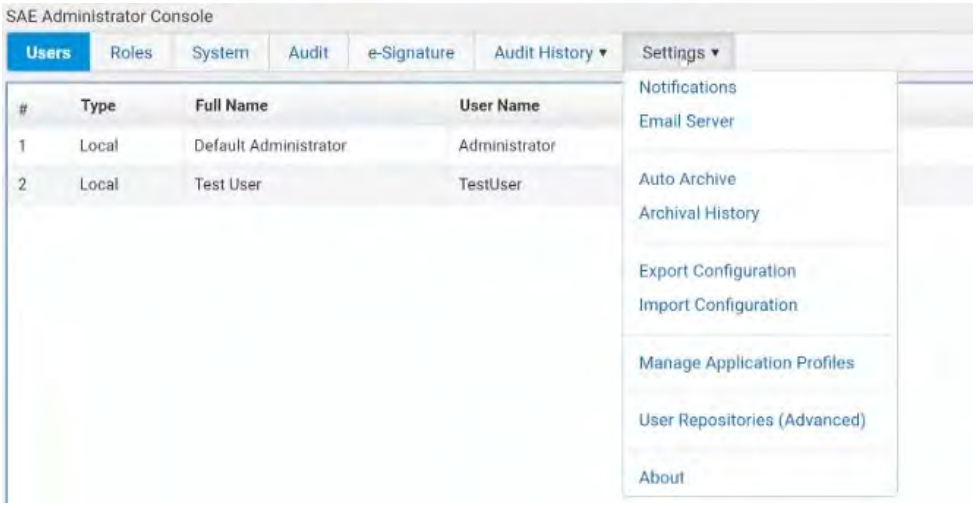
View the Instrument Run Records audit log

Audit records are listed when a run is complete.

1. In the SAE Administrator Console main screen, click the **Audit History** tab.
2. Select **Instrument Run Records**, then select the tab for the specific audit log you wish to view:
   - Run Summary
   - Application Objects
   - Action Records
   - Data Audit Records
   - Run Completion Outputs
3. To limit the records that are displayed, select **Enable Instrument Run Records Filtering**.
4. *(Optional)* Perform the following actions:
   - Specify other filtering settings.
   - Click **Report** to generate a PDF file of the log.
   - **Export** is not supported for this audit log.

Settings tab

Settings tab allows users that have permission to modify SAE Administrator Console to configure optional and advanced SAE Console settings, which are available as a dropdown menu from the **Settings** tab.



Using the Settings dropdown, you can view or configure the following options:

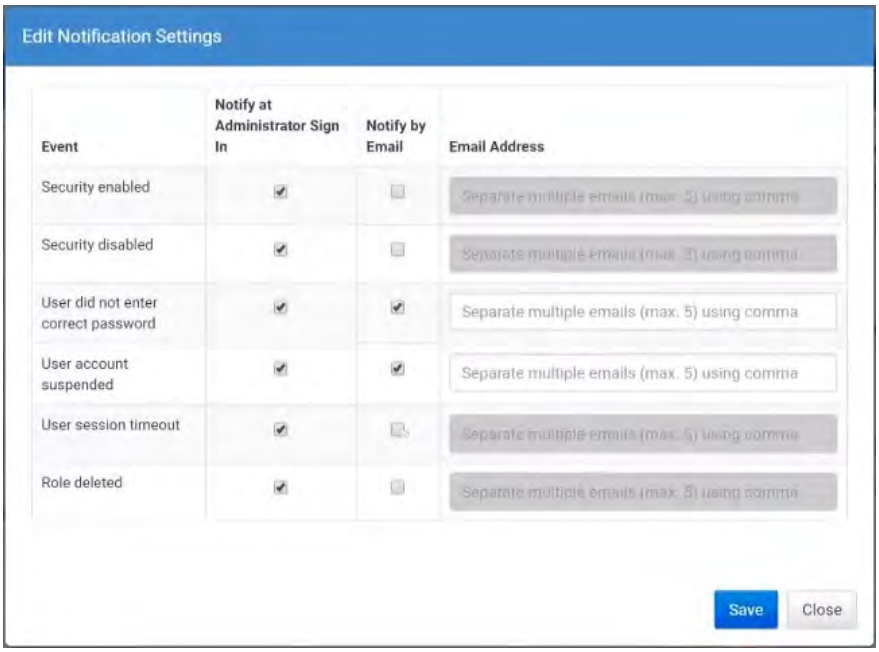
- **Notifications:** Allows you to specify when and how to be notified when specified events occur in the SAE (see "Set up SAE messaging notifications", page 620).
- **Email Server:** Allows you to configure the SMTP server so that the SAE Administrator Console can send email notifications (see "Configure the SMTP server for email notifications", page 621).
- **Auto Archive:** Allows you to archive audit records automatically (see "Auto archive audit records", page 622).
- **Archival History:** Allows you to:
  - Archive audit records manually (see "Archive audit records manually", page 622).
  - View or export archived audit records (see "View or export archived audit records", page 623).
  - Restore archived audit records (see "Restore archived audit records", page 624).
- **Export Configuration:** Allows you to export user, system security, audit, and e-Signature settings from the SAE Administrator Console (see "Export configuration", page 624).
- **Import Configuration:** Allows you to import settings from another installation of the SAE Administrator Console (see "Import configuration", page 624).
- **Manage Application Profiles:** Allows you to install a profile for the application for which you wish to configure the SAE module (see "Set up the SAE Administrator Console with application profiles", page 625).
- **User Repositories (Advanced):** Allows you to configure user repositories for SAE or external account access (see "Configure user repositories", page 625).
- **About:** Displays the SAE Administrator Console software version.

Set up SAE messaging notifications

You can specify when and how to be notified when specified events occur in the SAE. You have the option to be notified at sign in, to be notified by email when the event of interest occurs, or both.

IMPORTANT! You **must** configure the SMTP server before you set up email notifications (page 621).

1. In the SAE Administrator Console main screen, click the **Settings** tab, then select **Notifications**.

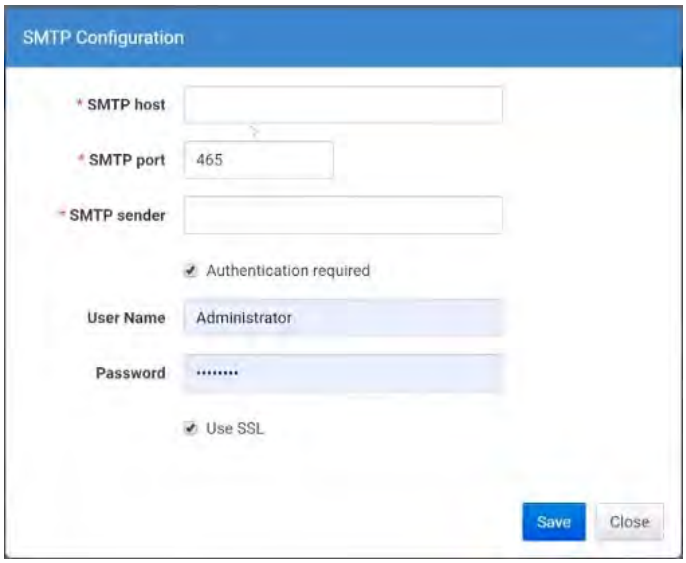


2. In the **Edit Notification Settings** dialog, select **Notify at Administrator sign in** for the events of interest.
3. *(Optional)* Select **Notify by Email**, then specify an email address.
4. Click **Save**.
   - If you have selected **Notify at Administrator sign in,** you will be notified at each sign in if an event of interest occurs while you were signed out.
   - If you have selected **Notify by Email**, an email will be sent to you when an event of interest occurs.

Configure the SMTP server for email notifications

Before you set up email notifications (page 620), you must configure the SMTP server so that the SAE Administrator Console can send email notifications.

1. In the SAE Administrator Console main screen, click the **Settings** tab, then select **Email Server**.



2. In the **SMTP Configuration** dialog, enter the following:
   - **SMTP host**, **SMTP port**, and **SMTP sender**

     Note: Select **Authentication required** if the SMTP server requires authentication.

   - **Username** and **Password**

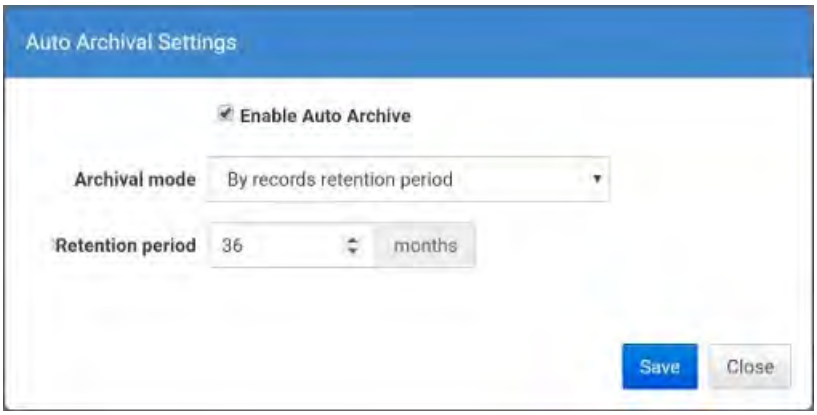
     Note: Select **Use SSL** if the SMTP server requires an encrypted channel connection.

3. Click **Save**.

Auto archive audit records

Archiving audit records removes the records from the SAE Administrator Console and saves them in an internally specified location on the same computer on which the SAE Administrator Console is installed.

1. In the SAE Administrator Console main screen, click the **Settings** tab.
2. Select **Auto Archive**.
3. In the **Auto Archival Settings** dialog, select **Enable Auto Archive**.

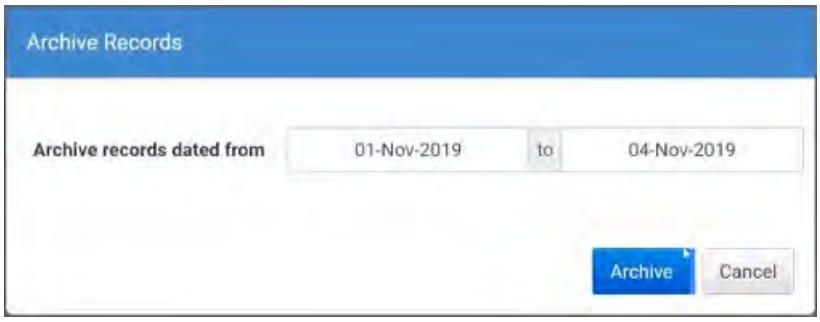


4. Select the **Archival mode** and associated settings:
   - By number of records or retention period
   - By number of records
   - By retention period
5. Click **Save**.

   The software periodically checks the audit record status and archives when the specified archive conditions are met.

Archive audit records manually

1. In the SAE Administrator Console main screen, click the **Settings** tab.
2. Select **Archival History**.
3. Click **Ad-hoc Archive**, select the start and end dates, then click **Archive**.

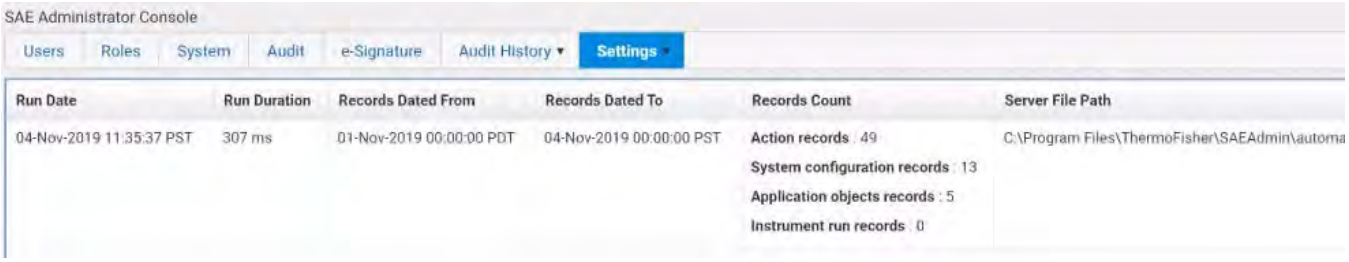


Note: Archived audit records (automatic or manual) are accessible for viewing in the SAE Administrator Console (see "View or export archived audit records", page 623).

View or export archived audit records

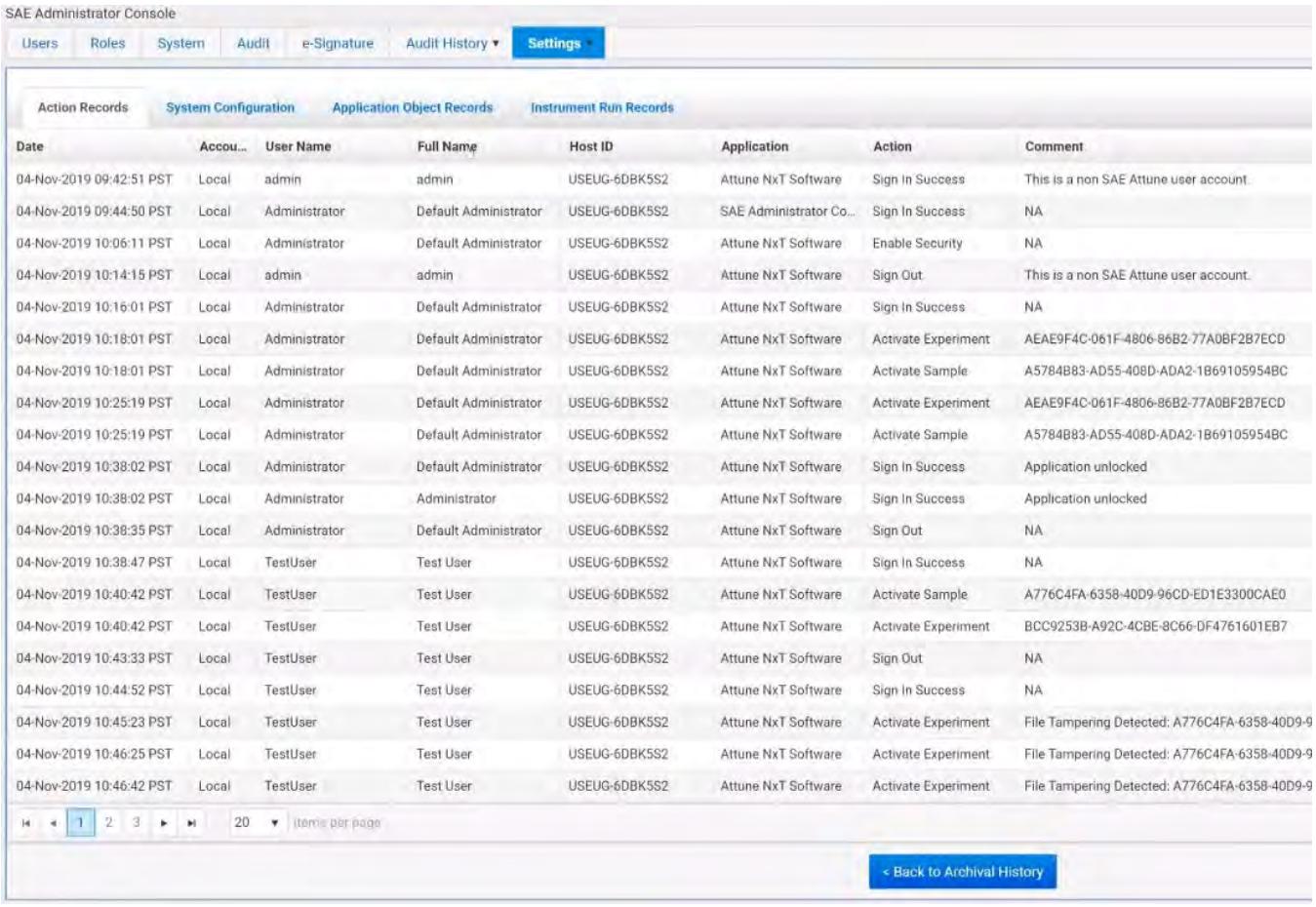
1. In the SAE Administrator Console main screen, click the **Settings** tab.
2. Select **Archival History**.

   The list of archived records is displayed in the **Settings tab ▶ Archival history** screen.



3. To view a record, select the record from the list, then click **View Archived Records**.

   Audit log for the selected record is displayed, arranged in **Action Records**, **System Configuration**, **Application Object Records**, and **Instrument Run Records** tabs.



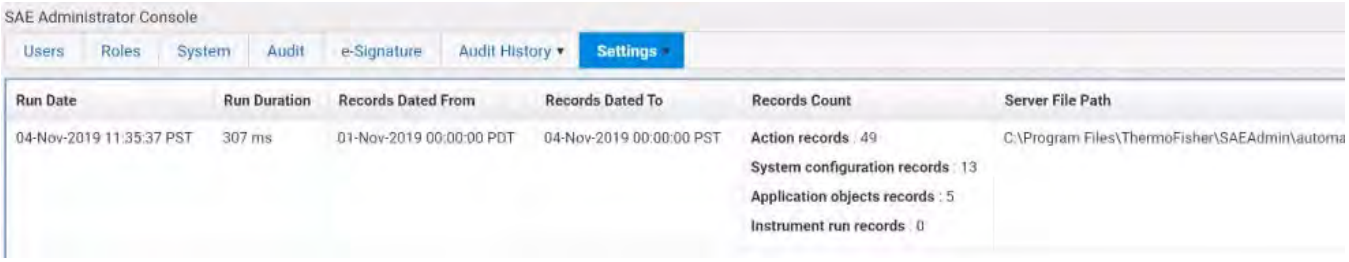
Date	Accou...	User Name	Full Name	Host ID	Application	Action	Comment
04-Nov-2019 09:42:51 PST	Local	admin	admin	USEUG-6DBK5S2	Attune NxT Software	Sign In Success	This is a non SAE Attune user account.
04-Nov-2019 09:44:50 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	SAE Administrator Co...	Sign In Success	NA
04-Nov-2019 10:06:11 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	Attune NxT Software	Enable Security	NA
04-Nov-2019 10:14:15 PST	Local	admin	admin	USEUG-6DBK5S2	Attune NxT Software	Sign Out	This is a non SAE Attune user account.
04-Nov-2019 10:16:01 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	Attune NxT Software	Sign In Success	NA
04-Nov-2019 10:18:01 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	Attune NxT Software	Activate Experiment	AEAE9F4C-061F-4806-86B2-77A0BF2B7ECD
04-Nov-2019 10:18:01 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	Attune NxT Software	Activate Sample	A5784B83-AD55-408D-ADA2-1B69105954BC
04-Nov-2019 10:25:19 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	Attune NxT Software	Activate Experiment	AEAE9F4C-061F-4806-86B2-77A0BF2B7ECD
04-Nov-2019 10:25:19 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	Attune NxT Software	Activate Sample	A5784B83-AD55-408D-ADA2-1B69105954BC
04-Nov-2019 10:38:02 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	Attune NxT Software	Sign In Success	Application unlocked
04-Nov-2019 10:38:02 PST	Local	Administrator	Administrator	USEUG-6DBK5S2	Attune NxT Software	Sign In Success	Application unlocked
04-Nov-2019 10:38:35 PST	Local	Administrator	Default Administrator	USEUG-6DBK5S2	Attune NxT Software	Sign Out	NA
04-Nov-2019 10:38:47 PST	Local	TestUser	Test User	USEUG-6DBK5S2	Attune NxT Software	Sign In Success	NA
04-Nov-2019 10:40:42 PST	Local	TestUser	Test User	USEUG-6DBK5S2	Attune NxT Software	Activate Sample	A776C4FA-6358-40D9-96CD-ED1E3300CAE0
04-Nov-2019 10:40:42 PST	Local	TestUser	Test User	USEUG-6DBK5S2	Attune NxT Software	Activate Experiment	BCC9253B-A92C-4CBE-8C66-DF4761601EB7
04-Nov-2019 10:43:33 PST	Local	TestUser	Test User	USEUG-6DBK5S2	Attune NxT Software	Sign Out	NA
04-Nov-2019 10:44:52 PST	Local	TestUser	Test User	USEUG-6DBK5S2	Attune NxT Software	Sign In Success	NA
04-Nov-2019 10:45:23 PST	Local	TestUser	Test User	USEUG-6DBK5S2	Attune NxT Software	Activate Experiment	File Tampering Detected: A776C4FA-6358-40D9-9
04-Nov-2019 10:46:25 PST	Local	TestUser	Test User	USEUG-6DBK5S2	Attune NxT Software	Activate Experiment	File Tampering Detected: A776C4FA-6358-40D9-9
04-Nov-2019 10:46:42 PST	Local	TestUser	Test User	USEUG-6DBK5S2	Attune NxT Software	Activate Experiment	File Tampering Detected: A776C4FA-6358-40D9-9

4. Click **Back to Archival History** to view the list of archived records again.
5. To export a record, select the record from the **Archival history** list, then click **Export**.

Restore archived audit records

1. In the SAE Administrator Console main screen, click the **Settings** tab.
2. Select **Archival History**.

   The list of archived records is displayed in the **Settings tab ▶ Archival history** screen.



3. To restore a record, select the record from the list, then click **Restore**.
4. To restore a ZIP file that was exported from the archival history, click **Restore (upload)**, then select the ZIP file.

Export configuration

1. In the SAE Administrator Console main screen, click the **Settings** tab.
2. Select **Export Configuration**.
3. In the **Export Configuration** dialog, select an export option:

Setting	Exports
All	SAE settings and SAE user accounts
Custom Users & Roles	- SAE user accounts with **Active** status - SAE roles and their associated permissions
Custom System & Roles	- SAE settings - SAE roles and their associated permissions

4. Click **Export**.

   The exported file (DAT format) downloads to the default location of the computer.

Import configuration

1. In the SAE Administrator Console main screen, click the **Settings** tab.
2. Select **Import Configuration**.
3. Click **Choose File** to select the DAT file with the desired configuration settings.
4. Select an import option.
5. Click **Import.**
6. If imported user accounts exist in the SAE Administrator Console, click **Skip** or **Overwrite** for each user account, then click **Confirm and Import**.

Set up the SAE Administrator Console with application profiles

An application profile contains default SAE Administrator Console settings for an application (such as the Attune™ Cytometric Software, SeqStudio™ Genetic Analyzer, etc.).

Before you can use the SAE Administrator Console to configure the SAE module for an application, you must install a profile for the application.

1. In the SAE Administrator Console main screen, click the **Settings** tab.
2. Select **Manage Application Profiles**.
3. Click **Install Application Profile**, then select the DAT file for the application profile.
4. Click **Verify Data File**.
5. Select **Install new application**, then click **Install**.

   The application name and settings are added to the SAE Administrator Console.

Configure user repositories

SAE user account information is stored in a "user repository". The SAE Administrator Console allows you to use **Internal**, **External LDAP**, or **Federated Repositories** (see "User repository overview", page 627).

1. In the SAE Administrator Console main screen, click the **Settings** tab.
2. Select **User Repositories (Advanced)**.
3. Select the **User repository definition:**

Setting	Exports
Internal User Repository	Allows SAE user accounts to sign in
External LDAP User Repository	Allows external user accounts to sign in
Federated Repositories	Allows SAE user accounts or LDAP accounts to sign in

Note: For more information on SAE Administrator Console user repositories, see "User repository overview", page 627.

4. If you selected **External LDAP User Repository** or **Federated Repositories**, click **Next**, then enter the required information (see "User repository settings", page 626).
5. Click the **Users** tab to display the list of accounts added to the SAE Administrator Console.
   - New LDAP accounts are listed as **External**, and **Role** is set to the default specified during account mapping. If no default was specified, accounts are set to **No Privileges Role**.
   - SAE user accounts that were previously created in the SAE Administrator Console are listed as **Local**.
   - If you selected LDAP, the **Status** for all accounts except for the default SAE Administrator account is set to **Inactive**.

6. Click **Test Connection** to synchronize the new accounts with the LDAP server.

   The SAE server also periodically syncs the LDAP accounts with the LDAP server if changes are made to the User repository definition or any setting on the LDAP server.

7. If needed, edit the user accounts to assign roles.

User repository settings

The following table lists the **External LDAP User Repository** and **Federated Repositories** settings.

Setting	Description
LDAP Server Configuration	
Host name, Port, and Use ssl	LDAP server name or IP address, port, and interface protocol.
Bind distinguished name, Bind password, Base distinguished name	LDAP server attributes required for access.
User Account Mapping	
Directory type	LDAP server configuration. Click **Set Defaults** after you select the **Directory type** to display typical default parameters for mapping to an LDAP system.
Username	Parameter that maps to the username in the LDAP system.
Default role assignment	The SAE role that will be assigned to all user accounts. You can change the role after the user accounts are imported into the SAE Administrator Console.
Username and other settings	Parameters that correspond to the username and other fields in the LDAP system.
Authentication verification	
Username and Password	LDAP username and password.

Sign-in with LDAP or federated user repositories

The following table lists the required credentials for User or Administrator sign in to the SAE Administrator Console with LDAP or federated user repositories.

User repository	User signs in with	Administrator signs in with
Internal	Internal (local) account: Username and password created in the SAE Administrator Console	• Username and password for the default SAE Administrator user account • Any SAE user account that has been assigned the SAE role of Administrator
External	External account: Username and password created in the LDAP user management system. **Note:** Local accounts are set to **Inactive**.	• Username (with local/ prefix) and password for the default SAE Administrator user account Example: local/Administrator • Any external account that has been assigned the SAE role of Administrator
Federated	The account type that they are assigned: • External account • Internal (local) account (with local/prefix) Example: local/Username	• Username (with local/ prefix) and password for the default SAE Administrator user account Example: local/Administrator • Any external account that has been assigned the SAE role of Administrator

User repository overview

SAE user account information is stored in a "user repository".

The SAE Administrator Console provides the following options for user repositories:

- **Internal**: Allows only SAE user accounts to sign in to an application. SAE user accounts are referred to as "local" accounts in the SAE Administrator Console.
  - SAE user accounts are created in the SAE Administrator Console and are identified as "local" in the **Users** tab.
  - User authentication is based on the accounts that are listed in the **Users** tab and the SAE settings that are specified in the **System** tab.
  - User permissions are determined by the roles that are configured in the SAE Administrator Console.
- **External LDAP**: Enables LDAP based authentication with an LDAP directory. Allows only external user accounts to sign in to an application.
  - User accounts are created in an LDAP (Lightweight Directory Access Protocol) user management system and are identified as "external" in the SAE Administrator Console **Users** tab.
  - User authentication is based on the accounts that are listed in the SAE Administrator Console **Users** tab and the external LDAP user repository. The SAE settings that are specified in the SAE Administrator Console **System** tab are not used.
  - User permissions are determined by the roles that are configured in the SAE Administrator Console.
  - All local user accounts except the default Administrator account are set to **Inactive**.
  - Passwords cannot be changed in the SAE Administrator Console.
- **Federated**: Allows internal (local) and external account sign-in to an application.
  - User accounts are created in the SAE Administrator Console or in an LDAP user management system.
  - User authentication is based on the respective internal or LDAP user repository.

Appendix D: Technical reference

Overton's cumulative statistics

The Overton Subtraction method determines the positive result by subtracting a control histogram from the sample histogram on a channel-by-channel basis. Any negative differences (where the control has a greater number of cells in a channel compared to the sample) are added to the positive differences in the lower channels.

The percent positive is then calculated as the sum of the positive differences for all channels divided by the total number of events.

For a complete description of the Overton Subtraction method, refer to "Modified Histogram Subtraction Technique for Analysis of Flow Cytometry Data W. Roy Overton Cytometry 9:619-626 (1988)".

Kolmogorov-Smirnov test

In statistics, the Kolmogorov–Smirnov test (K–S test) is a nonparametric test for the equality of continuous, one-dimensional probability distributions that can be used to compare a sample with a reference probability distribution (one-sample K–S test) or to compare two samples (two-sample K–S test).

The Kolmogorov–Smirnov statistic quantifies a distance between the empirical distribution function of the sample and the cumulative distribution function of the reference distribution, or between the empirical distribution functions of two samples. The null distribution of this statistic is calculated under the null hypothesis that the samples are drawn from the same distribution (in the two-sample case) or that the sample is drawn from the reference distribution (in the one-sample case).
In each case, the distributions considered under the null hypothesis are continuous distributions but are otherwise unrestricted.

The two-sample KS test is one of the most useful and general nonparametric methods for comparing two samples, as it is sensitive to differences in both location and shape of the empirical cumulative distribution functions of the two samples.

HyperLog™

Compensated flow cytometry data frequently contains negative values due to compensation, and cell populations do occur which have low means and normal distributions. Logarithmic transformations cannot properly handle negative values, and poorly display normally distributed cell types. Alternative transformations addressing this issue include the log-linear hybrid transformations and HyperLog™.

The HyperLog™ transform is a log-like transform that admits negative, zero, and positive values. The transform is a hybrid type of transform specifically designed for compensated data. One of its parameters allows it to smoothly transition from a logarithmic to linear type of transform that is ideal for compensated data (Cytometry A., 2005, 64:34-42).

Linear and log color binning reference

The **Linear mode** bins data by assigning a color index for each density pixel linearly such that each increment is determined by dividing the range (Z_{max}–Z_{min}) by the number of color steps.

The **Log mode** bins data by assigning a color index for each density pixel logarithmically such that each increment is determined by dividing the logarithmic range (log Z_{max}–log Z_{min}) by the number of color steps. The index is then determined as int((log Z_{val} – log Z_{min})/increment).

Appendix E: Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Symbols on the instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Standard safety symbols

Symbol	English	Français
	CAUTION! Risk of danger. Consult the manual for further safety information.	**MISE EN GARDE !** Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
	CAUTION! Risk of electrical shock.	**MISE EN GARDE !** Risque de choc électrique.
	CAUTION! Hot surface.	**MISE EN GARDE !** Surface chaude.
	CAUTION! Laser radiation.	**MISE EN GARDE !** Rayonnement laser.
	CAUTION! Moving parts.	**MISE EN GARDE !** Parties mobiles.
	CAUTION! Piercing hazard.	**MISE EN GARDE !** Danger de perforation.
	CAUTION! Potential biohazard.	**MISE EN GARDE !** Danger biologique potential.
	CAUTION! Ultraviolet light.	**MISE EN GARDE !** Rayonnement ultraviolet.

Safety alerts on the instrument

Symbol	English	Français
	CAUTION! Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**MISE EN GARDE !** Produits chimiques dangereux. Lire les fiches signalétiques (FS) avant de manipuler les produits.
	CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**MISE EN GARDE !** Déchets dangereux. Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l'élimination des déchets.
	DANGER! High voltage.	**DANGER !** Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Thermo Fisher Scientific qualified service personnel.	**AVERTISSEMENT !** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Thermo Fisher Scientific.
	CAUTION! Class 3B (III) visible and/or invisible laser and/or LED radiation present when open. Avoid exposure to beam.	**MISE EN GARDE !** Rayonnement laser ou DEL visible ou invisible de classe 3B, (III) présent en position ouverte. Éviter toute exposition au faisceau.

Location of safety labels

For the locations of safety labels found on the instruments, refer to the specific instrument user guide. For a list of other Attune™ user guides, including instrument user guides, see "Other Attune™ user guides", page 11.

Control and connection symbols

Symbol	English	Français
	On	On (marche)
	Off	Off (arrêt)
	Standby	En attente
	On/Off	On/Off (marche/arrêt)
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
	Terminal that can receive or supply alternating current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant de type alternatif

Environmental symbols

Symbol	English	Français
	Do not dispose of this product in unsorted municipal waste **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif. **CAUTION!** Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union requirements.
	Indicates conformity with Australian standards for electromagnetic compatibility.

Instrument safety

General

CAUTION! **Do not remove instrument protective covers.** If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Physical injury

CAUTION! **Moving and Lifting Injury.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide.

Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

CAUTION! **Moving Parts.** Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical safety

WARNING! **Fuse Installation.** Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

AVERTISSEMENT ! **Installation des fusibles.** Avant d'installer l'instrument, vérifier que les fusibles sont correctement insérés et que leur tension correspond à celle fournie par le circuit d'alimentation. Ne remplacer les fusibles que par des modèles du type et de la puissance spécifiés pour l'appareil. L'utilisation de fusibles inadaptés peut endommager le circuit électrique de l'instrument et provoquer un incendie.

WARNING! **Ensure appropriate electrical supply.** For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

AVERTISSEMENT ! **Veiller à utiliser une alimentation électrique appropriée.** Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.

WARNING! **Power Supply Line Cords.** Use properly configured and approved line cords for the power supply in your facility.

AVERTISSEMENT ! **Cordons d'alimentation électrique.** Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.

WARNING! **Disconnecting Power.** To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

AVERTISSEMENT ! **Déconnecter l'alimentation.** Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Overvoltage rating The Attune™ NxT Flow Cytometer and the Attune™ CytPix™ Flow Cytometer have an installation (overvoltage) category of II and are classified as portable equipment.

Cleaning and decontamination

CAUTION! **Cleaning and Decontamination.** Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

MISE EN GARDE ! **Nettoyage et décontamination.** Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risqué pas d'endommager l'appareil.

Instrument component and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Laser safety

WARNING! LASER HAZARD. Under normal operating conditions, the Attune™ NxT Flow Cytometer and the Attune™ CytPix™ Flow Cytometer are categorized as Class 1 laser products. However, removing the protective covers and (when applicable) defeating the interlock(s) may result in exposure to the internal Class 3B (III) laser. Lasers can burn the retina, causing permanent blind spots. To ensure safe laser operation:

- Never look directly into the laser beam.
- Do not remove safety labels, instrument protective panels, or defeat safety interlocks.
- The system must be installed and maintained by a Thermo Fisher Scientific Technical Representative.
- Remove jewelry and other items that can reflect a laser beam into your eyes or those of others.
- Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the laser protection is defeated for servicing.
- DO NOT operate the laser when it cannot be cooled by its cooling fan; an overheated laser can cause severe burns on contact.

The following table lists laser safety symbols and alerts that may be present on the instrument.

Alert
DANGER! Class 3B (III) visible and/or invisible laser and/or LED radiation present when open. Avoid exposure to beam.

Alerte
DANGER ! Rayonnement laser ou DEL visible ou invisible de classe 3B, (III) présent en position ouverte. Éviter toute exposition au faisceau.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture comply with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

Safety standards

Reference	Description
EU Directive 2014/35/EU	European Union "Low Voltage Directive"
EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*
IEC 60825-1 EN 60825-1	*Safety of laser products – Part 1: Equipment classification and requirements*
21 CFR 1040.10 and 1040.11 as applicable	U.S. FDA Health and Human Services (HHS) "Radiological health performance standards for laser products" and "Radiological health performance standards for specific purpose laser products"

EMC standards

Reference	Description
EU Directive 2014/30/EU	European Union "EMC Directive"
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 18 (47 CFR)	U.S. Standard "Industrial, Scientific, and Medical Equipment"
AS/NZS CISPR 11	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 3	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union "WEEE Directive"—Waste electrical and electronic equipment
Directive 2011/65/EU	European Union "RoHS Directive"—Restriction of hazardous substances in electrical and electronic equipment

Chemical safety

WARNING! **GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! **PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en oeuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.
- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du recipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)

- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

WARNING! **HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

Biological hazard safety

WARNING! **Potential Biohazard.** Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! **BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at:

  www.cdc.gov/biosafety
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at:

  www.access.gpo.gov/nara/cfr/waisidx_01/ 29cfr1910a_01.html
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at:

  www.cdc.gov

In the EU:

- Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at:
  www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Documentation and support

Obtaining support

Technical support

For the latest services and support information for all locations, go to **thermofisher.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical support (**thermofisher.com/support)**
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Note: When contacting customer support for instrument troubleshooting, provide the instrument model and the instrument serial number. Convey to the technical support any error messages that were displayed on your instrument and any troubleshooting that you have already performed (refer to *Attune™ Flow Cytometry Maintenance and Troubleshooting Guide*; Pub. no. 100024234).

Obtaining SDSs

Safety Data Sheets (SDSs) are available at **www.thermofisher.com/sds**.

IMPORTANT! For the SDSs of chemicals not distributed by Thermo Fisher Scientific, contact the chemical manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

IMPORTANT! Wiping the computer supplied with the Attune™ NxT Flow Cytometer (i.e., erasing the hard drive to remove all programs, files, and the operating system) voids the product warranty.