# TROKEY 20 YEARS

thermo scientific

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# Basics of Flow Cytometry & Attune NxT

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20220908 Taqkey Science 張政暐

# Basics of Flow Cytometry





# What is Flow Cytometry?



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Performed using **single cell** suspensions in fluidics



#### **Measurement of cell properties**



Cells can be measured based on **size**, **shape**, **granularity**, **and light scattering** properties



**Fluorochromes** are used to label the cell's physical properties to help provide measurements



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Flow Cytometer – a different kind of "microscope" to observe cells



#### **Flow Cytometer**





# Flow Cytometer Components





#### Forward scatter (FSC)



Complexity

Size

Side scatter (SSC)



Fluorescence

Phenotype

invitrogen

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https://www.thermofisher.com/tw/en/home/life-science/cell-analysis/cell-ana

# Fluidics: 輸送細胞

The **fluidics system** of a flow cytometer is responsible for transporting sample from the sample tube to the flow cell.





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 https://www.thermofisher.com/tw/en/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/flow-cytometry-basics/flow-cytometry-fundamentals/fluidics-flow-cytometer.html

# Optics: 獲取資訊

The components of the **optical system** include excitation light sources, lenses, and filters used to collect and move light around the instrument and the detection system that generates the photocurrent.





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https://www.thermofisher.com/tw/en/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/flow-cytometry-basics/flow-cytometry-fundamentals/optics-flow-cytometer.html

# Electronics: 記錄資訊

The **electronics** are the brains of the flow cytometer. Here, the photocurrent from the detector is digitized and processed to be saved for subsequent analysis.







# Signal Pulse



# Data of Flow Cytometry



File format: FCS3.0, FCS3.1

Flow Cytometry Standard (FCS)

https://isac-net.org/page/Data-Standards

Cell	FSC	SSC	FITC	PE	APC	
1	91.3	27.8	62.0	78.9	83.4	73.1
2	93.0	44.9	73.8	47.7	19.2	29.0
3	39.5	75.7	23.3	68.3	49.2	53.7
4	76.5	3.9	12.3	76.1	72.5	70.0
5	98.8	92.8	63.2	52.3	24.2	11.4
6	48.6	46.5	93.7	52.9	74.8	87.0
7	87.7	29.2	4.1	6.9	48.7	57.7
8	54.4	26.5	68.1	72.1	12.7	80.1
9	91.5	80.8	63.8	71.6	15.0	89.9
	19.8	63.9	69.4	46.7	43.9	25.7







Dot plot



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# The Visible Light Spectrum





# Fluorescence





# **Channels for Fluorochrome**

#### Fluorochrome: PE Channel: Ex 488, Em 574/26



#### Fluorochrome: APC Channel: Ex 637, Em 670/14







# **Fluorescent Reagents**

#### What type of applications are you using in flow cytometry?



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https://www.thermofisher.com/tw/en/home/life-science/cell-analysis/flow-cytometry/flow-cytometry-assays-reagents.html

### Fluorescent Antibody

Antibody: Specificity 專一性



#### Fluorescence: Identity 辨識度







# Cell Characteristics by Flow Cytometry



# **Applications of Flow Cytometry**



# **Applications of Flow Cytometry**

- Phenotype Analysis (Cell Surface Antigens/Markers)
- DNA cell cycle analysis
- Membrane potential
- Ion flux (Calcium)
- Cell viability/Apoptosis
- Intracellular protein staining
- pH changes
- Cell tracking and proliferation (CFSE, Ki-67)
- Redox state
- In vivo CTL
- FRET
- Phospho-Flow cytometry

- Total protein
- Fluorescent proteins
- Lipids
- Membrane fusion
- Microparticles
- Phagocytosis
- Enzyme activity
- Oxidative metabolism
- DNA synthesis
- DNA degradation
- Gene expression (RNAFlow)
- Rare events
- The list goes on...If it glows, it probably flows



# Flow Cytometry產品諮詢與技術服務



歡迎與當區業務 巧盈 聯絡取得相關訊息~



# Attune NxT Flow Cytometer





# Attune<sup>™</sup> NxT Acoustic Focusing Flow Cytometer

#### Small in size, big in performance



# **Acoustic Focusing**



#### End-on view of capillary





# **Acoustic Focusing**

#### High sample input flow rates allow for more sample flexibility



#### 傳統流體動力聚焦

聲波輔助聚焦



### **Comparable Results at All Flow Rates**



# Flat-Top Lasers



#### Gaussian laser

#### Flat-Top laser



- Stable laser alignment
- Corrects for fluidic instabilities
- Minimized downtime



# Lasers & Optics



Color	Wavelength (nm)	Power (mW)
Violet	405	50
Blue	488	50
Green	532	100
Yellow	561	50
Red	637	100

- Five laser colors available
- From 1 to 4 lasers, 4 to 14 fluorescence channels
- Field upgradable!

# Attune NxT Configurations



#### A24864 A28995 A24861 A24863 A24862 A29002 A28997 A24860 A28999 A28993 A24859 A29003 A29004 A29001 A24858 Cat. No Detectors 4 7 7 8 9 10 10 11 10 11 12 14 14 14 Channel Emission filter (nm) BL1 BL2 695/40 695/40 695/40 695/40 695/40 695/40 695/40 695/40 695/40 695/40 695/40 695/40 BL3 695/40 695/40 780/60 780/60 780/60 780/60 BL4 GL2 620/15 620/15 620/15 620/15 695/40 695/40 695/40 695/40 780/60 780/60 780/60 780/60 620/15 620/15 620/15 620/15 620/15 695/40 695/40 695/40 780/60 695/40 780/60 780/60 780/60 780/60 670/14 670/14 670/14 670/14 670/14 670/14 670/14 670/14 720/30 720/30 720/30 720/30 720/30 720/30 720/30 720/30 780/60 780/60 780/60 780/60 780/60 780/60 780/60 780/60 440/50 450/40 440/50 440/50 440/50 450/40 450/40 440/50 440/50 VL2 512/25 525/50 512/25 512/25 512/25 525/50 525/50 512/25 512/25 VL3 660/20 710/50 710/50 660/20 710/50 710/50 710/50 710/50 660/20 VL5 710/50 710/50 710/50 780/60 780/60 780/60

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# Syringe Pump & Sample Concentration



#### Why is stable fluid delivery important?

• If the count fluctuates rapidly, the precision of the absolute count will also fluctuate rapidly. If one wants reliable counts, then stable fluid delivery cannot be compromised.



# CyKick (MAX) Autosampler

#### CytKick 與 CytKick MAX自動上樣機

- ▶ 可選用96孔,384孔,與深孔盤
- ▶ 與手動上樣相當的分析品質
- ▶ 抽吸式樣品均質化,均勻不傷細胞
- ▶ 隨時切換手動與盤式上樣
- ▶ 關機自動清洗

#### CytKick MAX自動上樣機

- ▶ 可選用24孔離心管(1.5-2 mL)上樣架
- ▶ 96孔盤式上樣總時間 < 42 mins</p>
- ▶ 96孔盤與24孔離心管上樣架可被動降溫







### Attune NxT Software



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# Attune NxT Configurations, NTOU



FSC: Forward scatter SSC: Side scatter

Excitation Laser	Emission Filter (nm)	Channel	Common Fluorophores	Fluorescent Proteins/Compounds	
Blue-488 nm	530/30	BL1	Alexa Fluor 488, FITC	eGFP, Emerald, eYFP	
	574/26	BL2	Alexa Fluor 546, PE( <mark>phycoerythrin</mark> ), Nile Red(N)	eYFP, mCitrine, Venus	
	695/40	BL3	PE-Alexa Fluor 700, PE-Cy5.5, PerCP, PerCP-Cy5.5	*chlorophyll	
	780/60	BL4	PE-Cy7, PE-Alexa Fluor 750		
	670/14	RL1	APC, Alexa Fluor 647	phycocyanin	
Red-637 nm	720/30	RL2	Alexa Fluor 680, Alexa Fluor 700, APC-Alexa Fluor 700		
	780/60	RL3	APC-Alexa Fluor 750, APC-Cy7		



# Flow Cytometry Learning Resource



#### Flow Cytometry Learning Center

Learn more about flow cytometry applications, techniques, and basic principles.



#### Molecular Probes<sup>™</sup> School of Fluorescence—Flow Cytometry Basics

Learn how a flow cytometer works including the fluidics, optics and electronics. This is a free resource to help you get started with flow cytometry, which can be a complex and challenging application.

#### Flow Cytometry Resource Library

Curated collection of scientific application notes, publications, videos, webinars, and scientific posters for flow cytometry.



- Flow Cytometry Application Notes, Scientific Posters, and BioProbes Articles Various applications, providing the conditions and reagents used to achieve the results. Scientific posters presented by our R&D scientists at key conferences. Articles from BioProbes Journal.
- **T Cell Stimulation and Proliferation eLearning Course** Modular, animated, and narrated eLearning course on T cell activation and the methods used to measure T cell function. Knowledge checks and a practical application session.
- Flow Cytometry Educational Videos & Webinars Media for researchers interested in flow cytometry.
- Flow Cytometry Research Tools Fluorescence SpectraViewer, flow cytometry panel design tool, antibodies search tool, mobile apps and more.
- Flow Cytometry Protocols Step-by-step instructions for successful fluorescence-based assays to measure cell proliferation, viability, and vitality using your flow cytometer.
- The Molecular Probes<sup>™</sup> Handbook—A Guide to Fluorescent Probes and Labeling Technologies Extensive references and technical notes. Contains 3,000 technology solutions representing a wide range of biomolecular labeling and detection reagents.





# Attune NxT, BRXX, 海洋大學

