

Synergy H1 Training

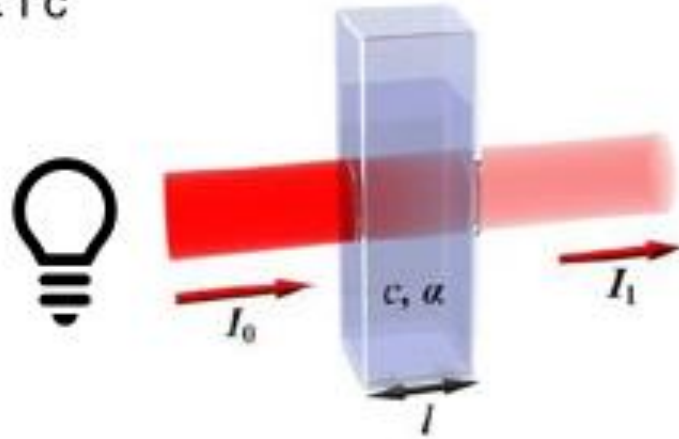


Microplate Reader and Absorbance

微量盤分析儀與吸光值

比爾定律 Beer's law

當光穿透樣品溶液時，光的吸收度(A)與吸收係數(ϵ)、光徑長(l)、濃度(c) 三者均呈正比： $A = \epsilon l c$



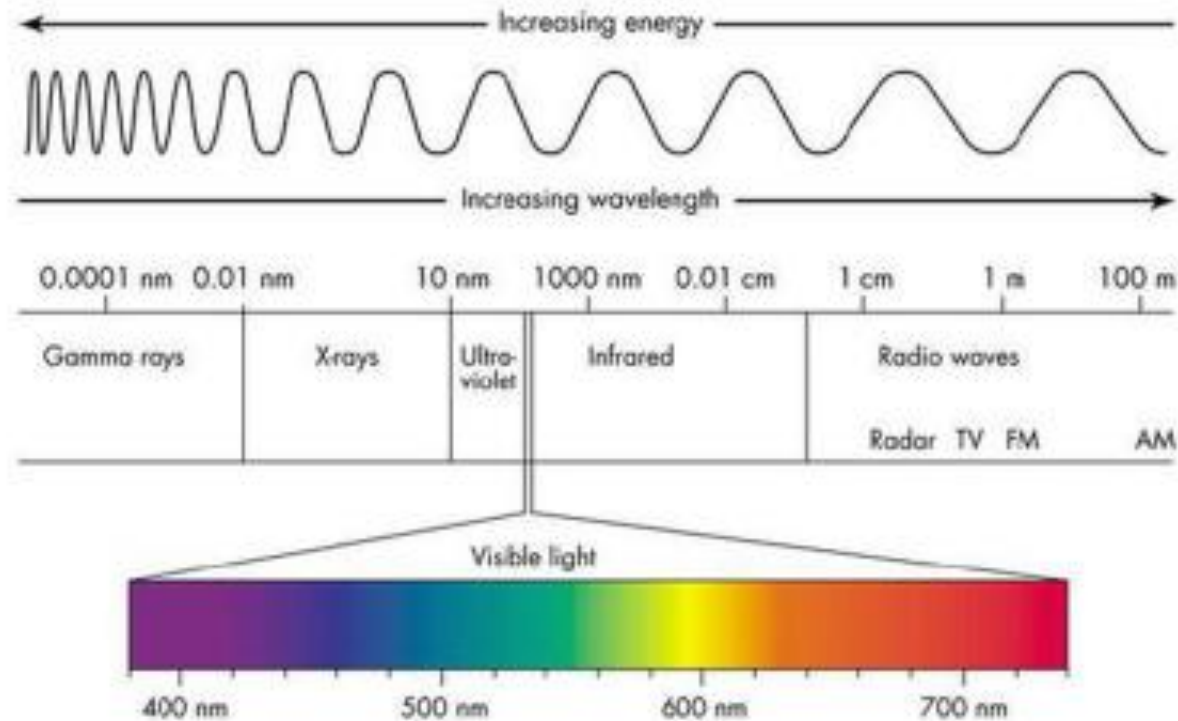
原本光入射線強度 I_0 ，穿透光線強度變為 I_1 ，此時光的穿透度為T (Transmittance)
定義光的被吸收度A

$$A = -\log T = -\log \frac{I_1}{I_0}$$

OD值 Optical Density

每種物質有其特定的吸收波長

一物質在某特定波長及固定距離下對於該光源的吸收值



濃度計算範例

$$A = \epsilon l c$$

利用分光光度計在260 nm直接定量

若OD(吸光值) = 0.8 , 已知 ϵ (常數) = 0.02 mg/ml l (光徑) = 1 cm

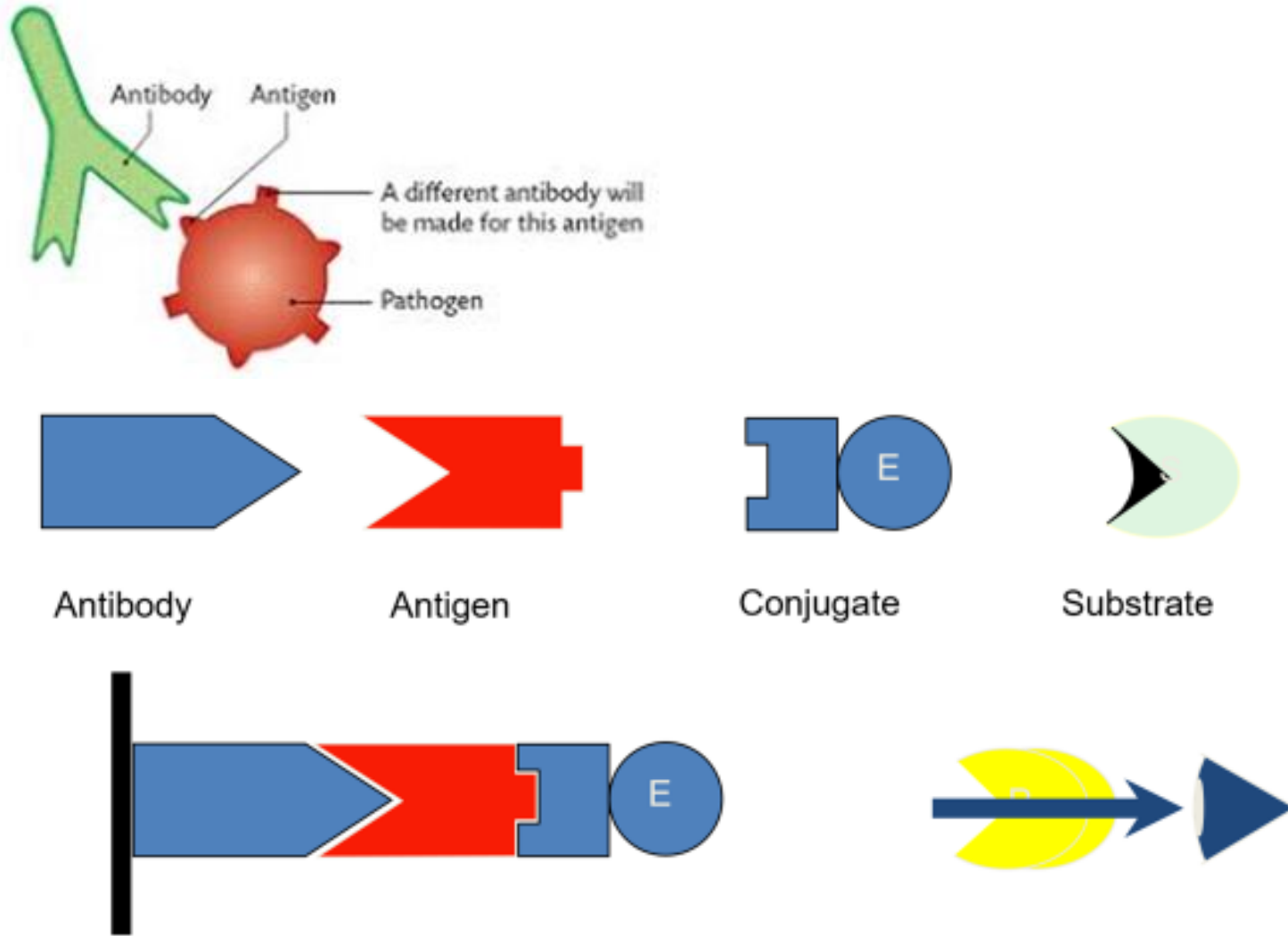
公式 $A = \epsilon l c$

因此 $0.8 = 0.02 \times 1 \times c$

$C = 0.8 \times 50 \mu\text{g/ml} = 40 \mu\text{g/ml}$

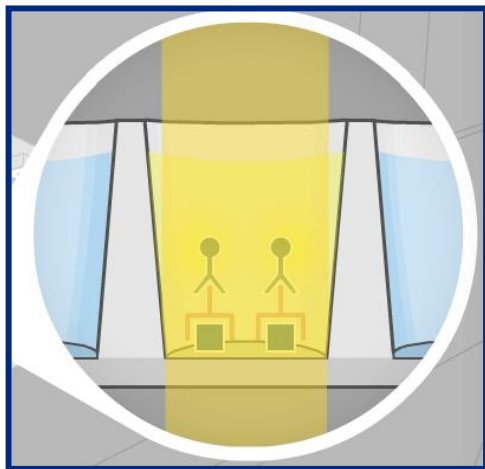
ELISA檢測原理

- 專一性抗原抗體反應，依據反應物顯色深淺進行定性或定量分析

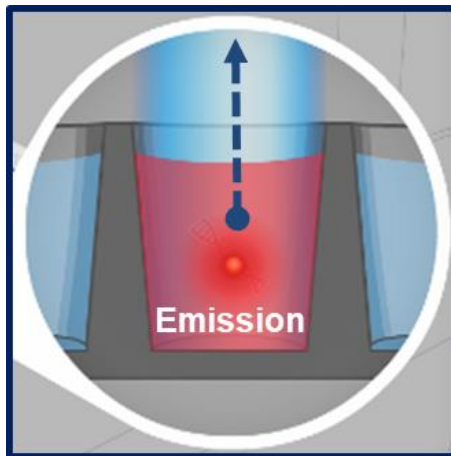


偵測方式

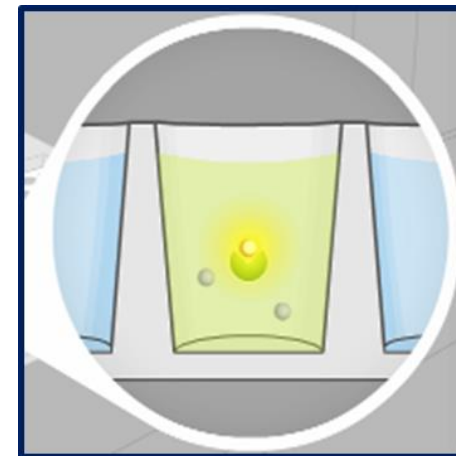
- 吸收光
- 螢光
- 冷光



Absorbance
吸收光

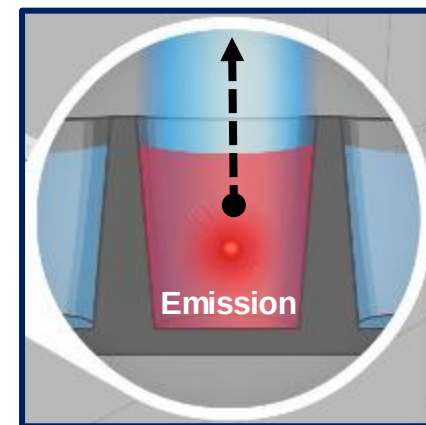
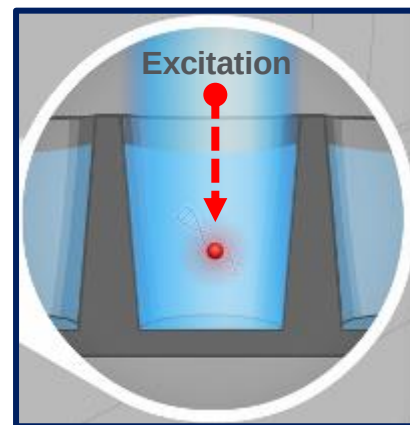
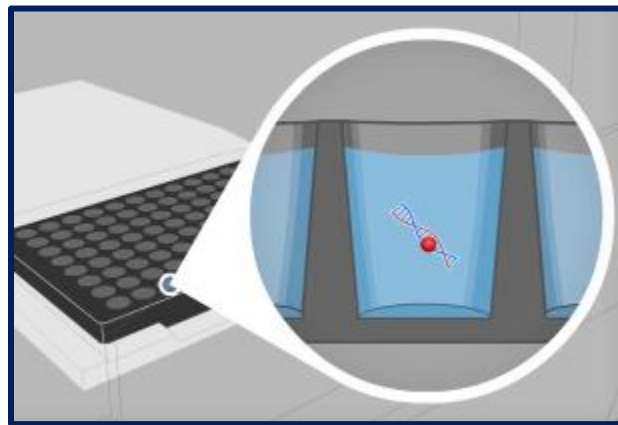
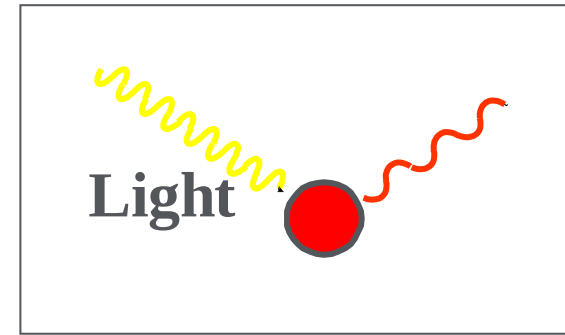
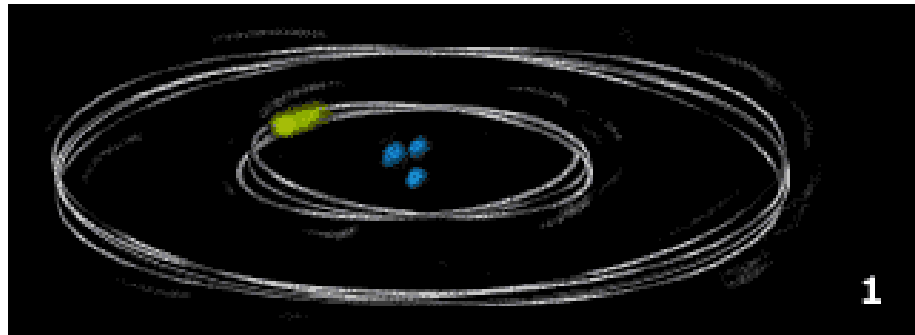


Fluorescence
螢光



Luminescence
冷光

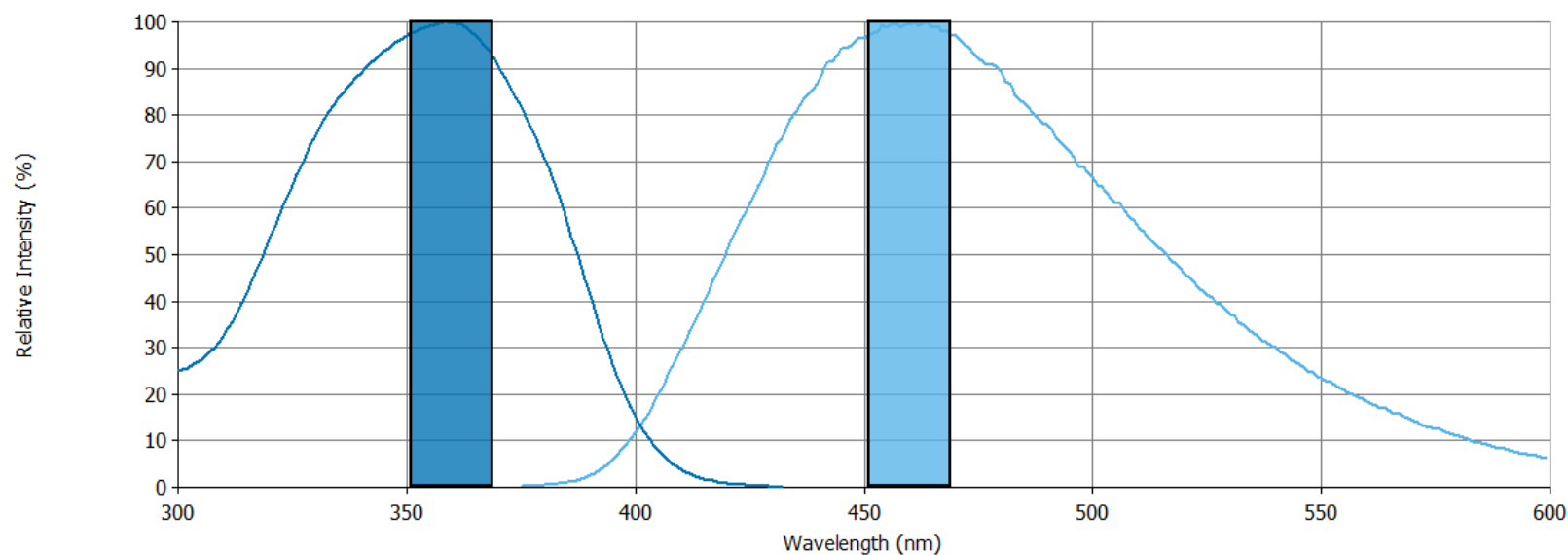
螢光原理與應用



Unit : **RFU** (**R**elative **F**luorescence **U**nits)

螢光原理與應用

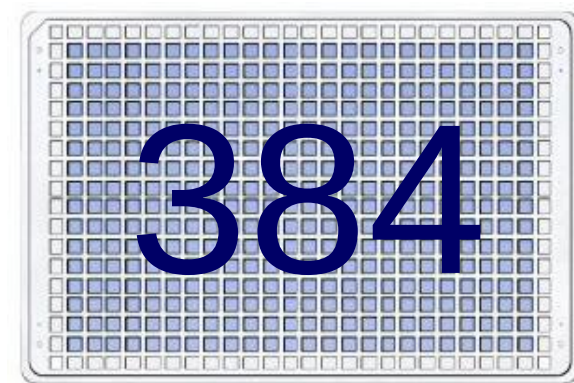
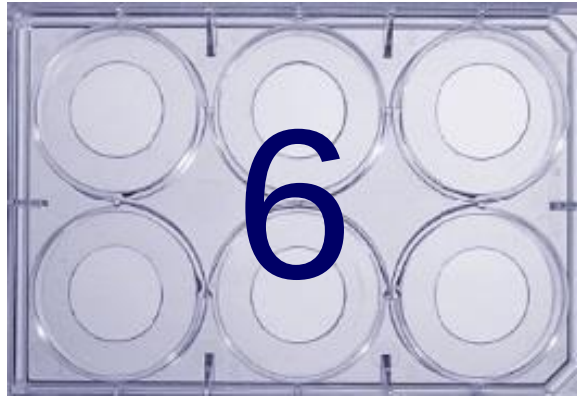
- Excitation 激發光
 - 光源激發螢光分子
- Emission 散射光
 - 螢光分子產生散射光
- 儀器偵測的是?
 - Emission激發光的數值
- Bandpass?
 - 波長可通過的範圍
- 460/40代表?
 - 440~480 nm範圍可通過濾鏡

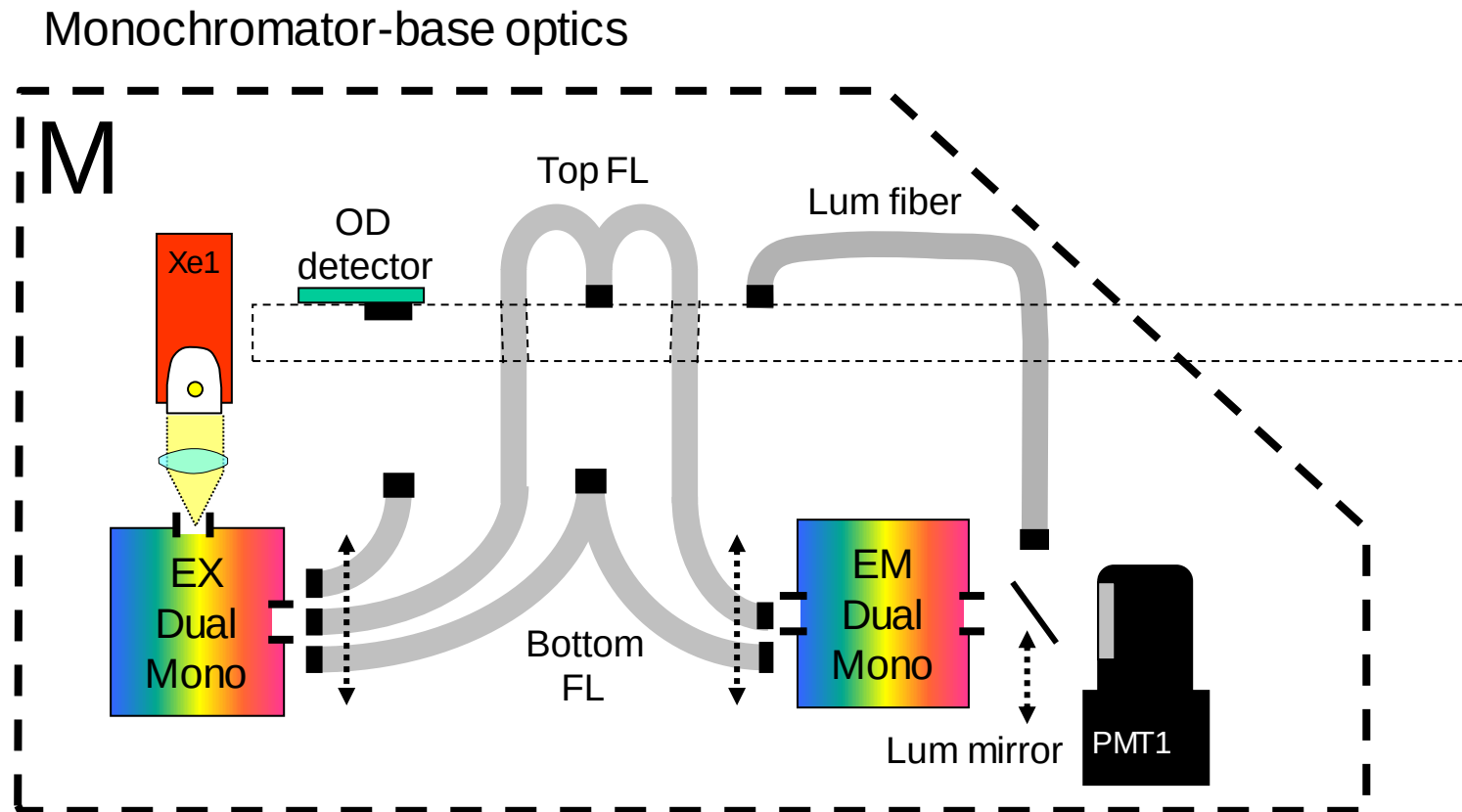


Synergy H1

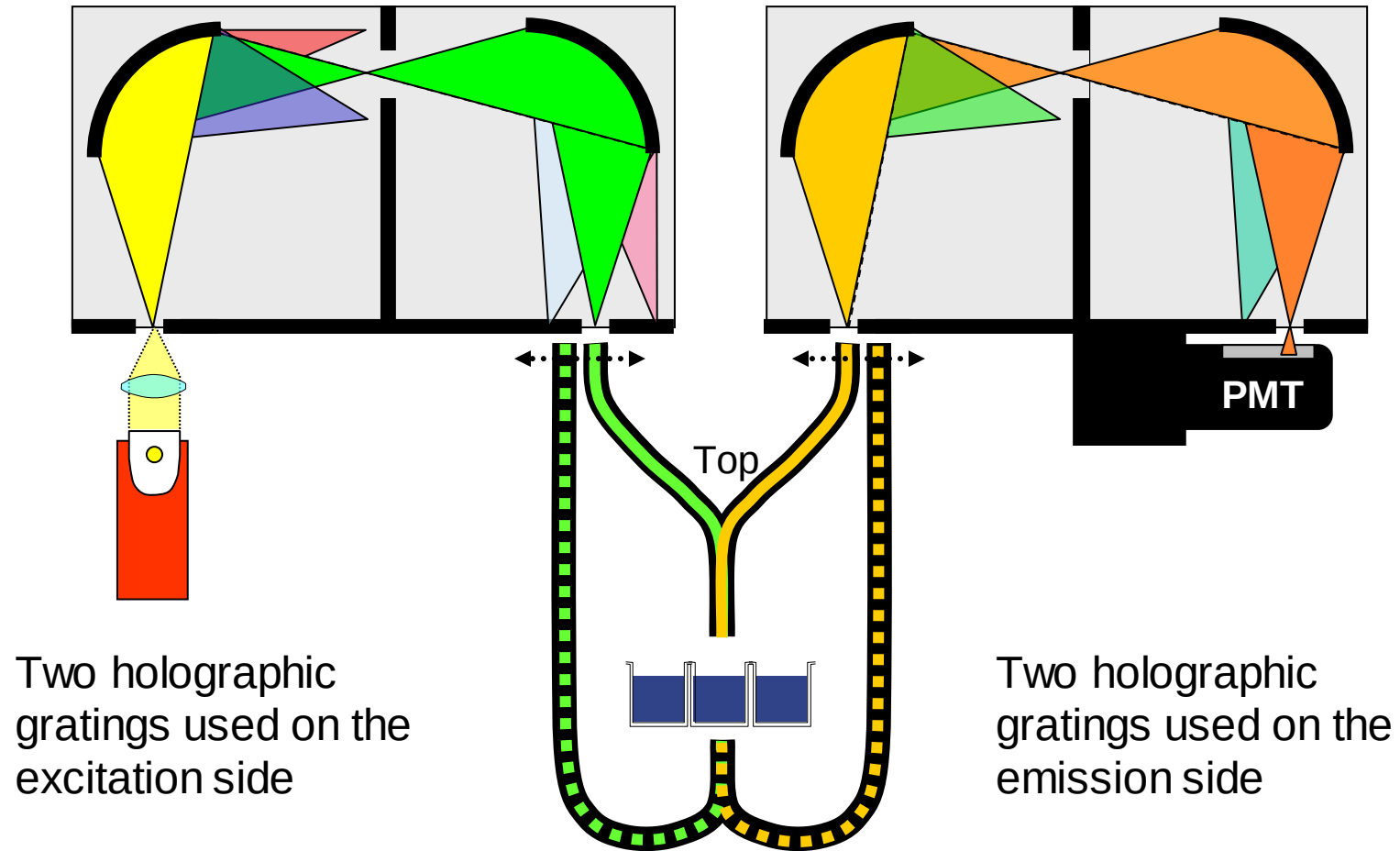


微量盤類型

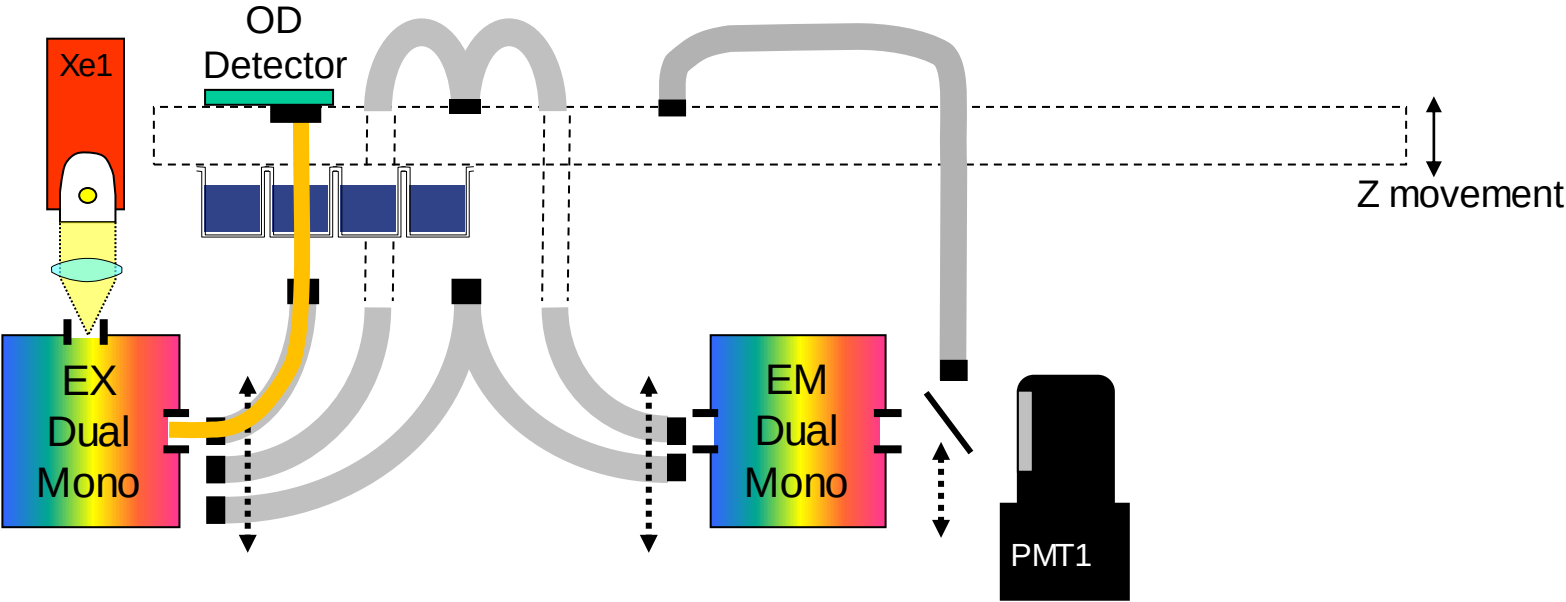




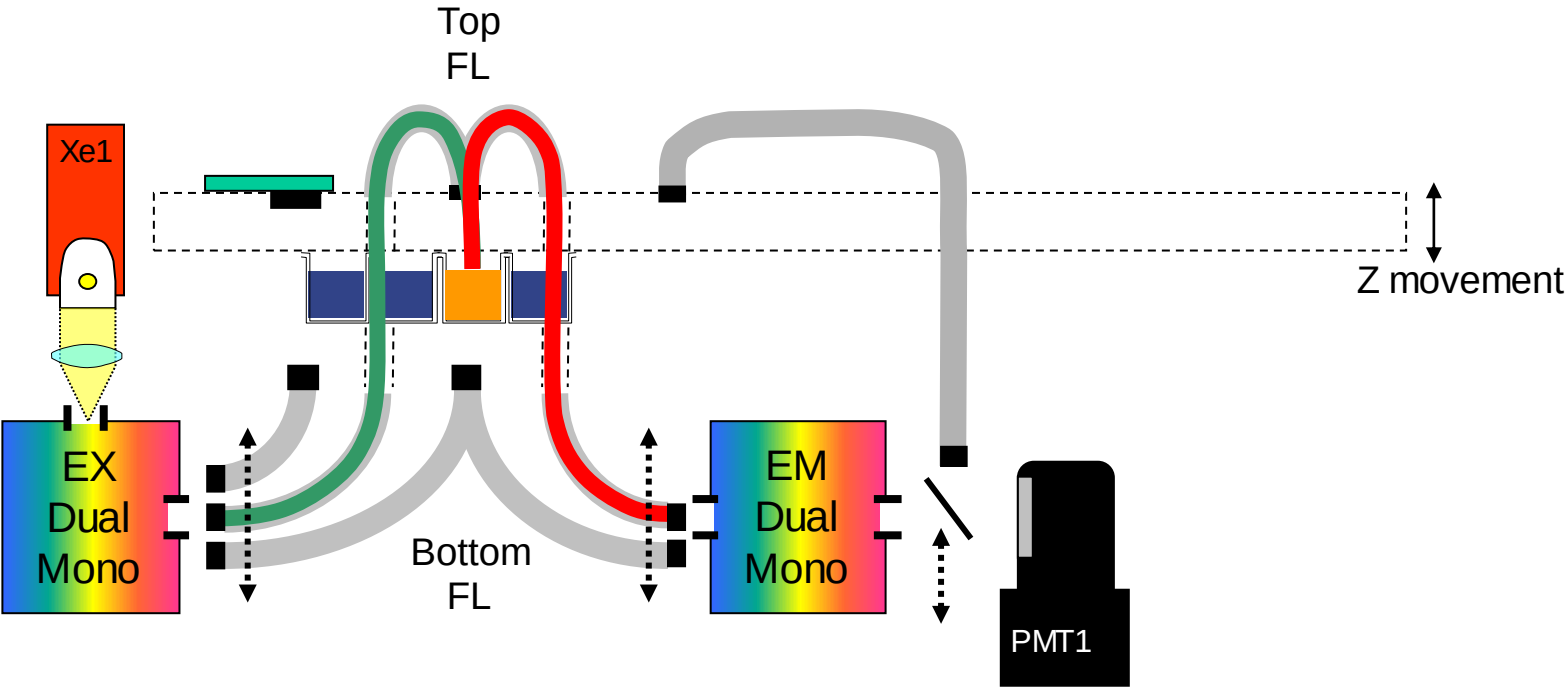
Quadruple Grating Optical Architecture



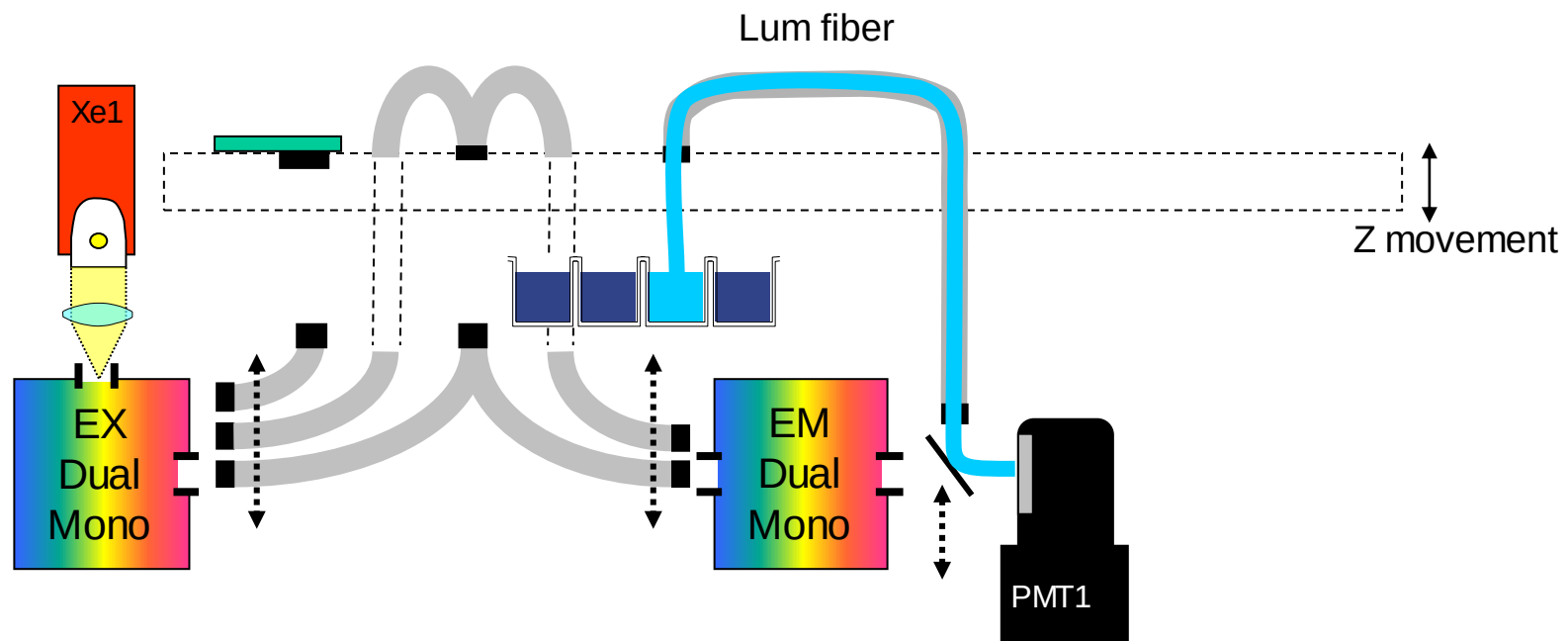
吸收光偵測路徑



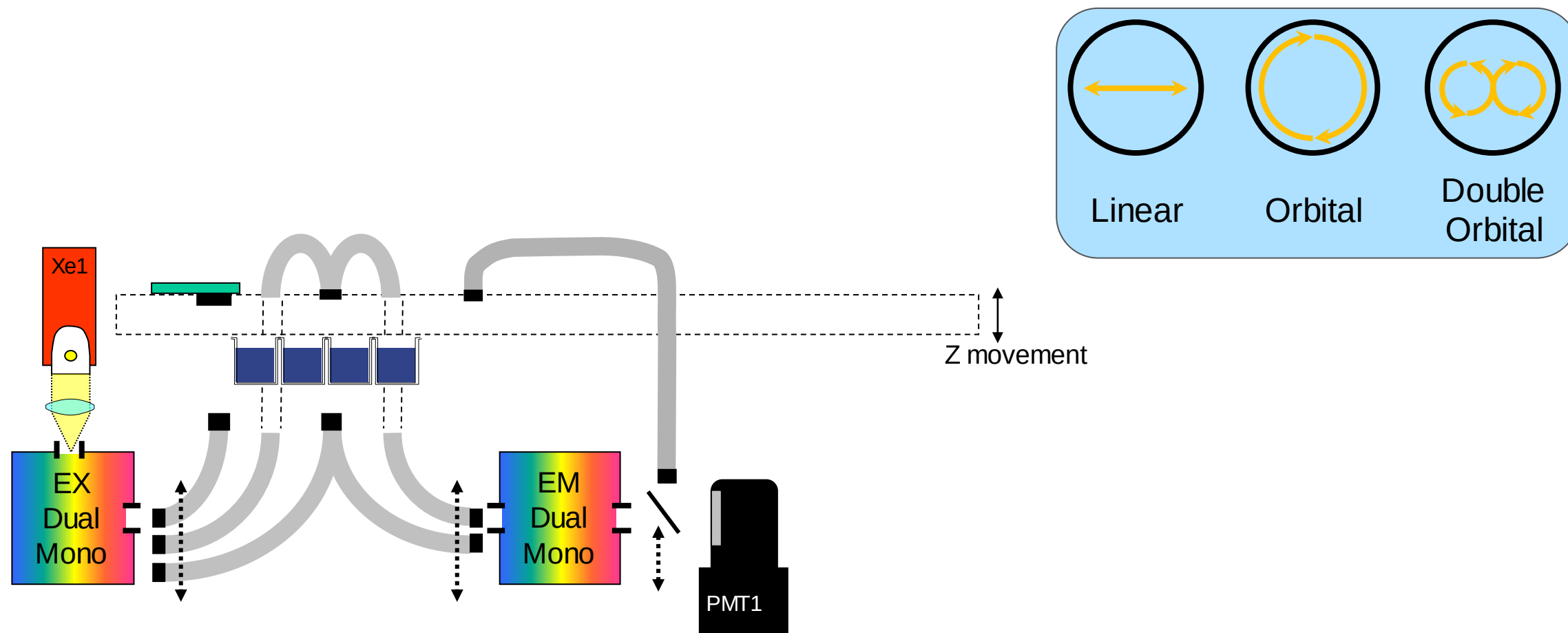
螢光偵測路徑



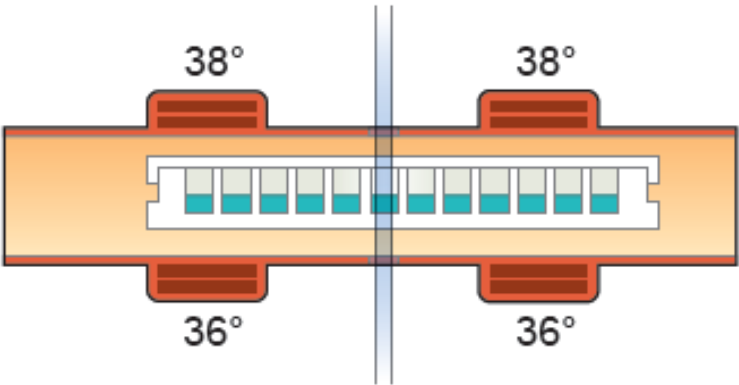
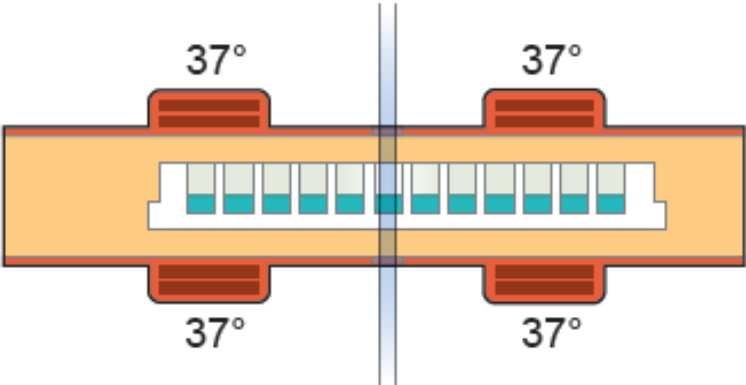
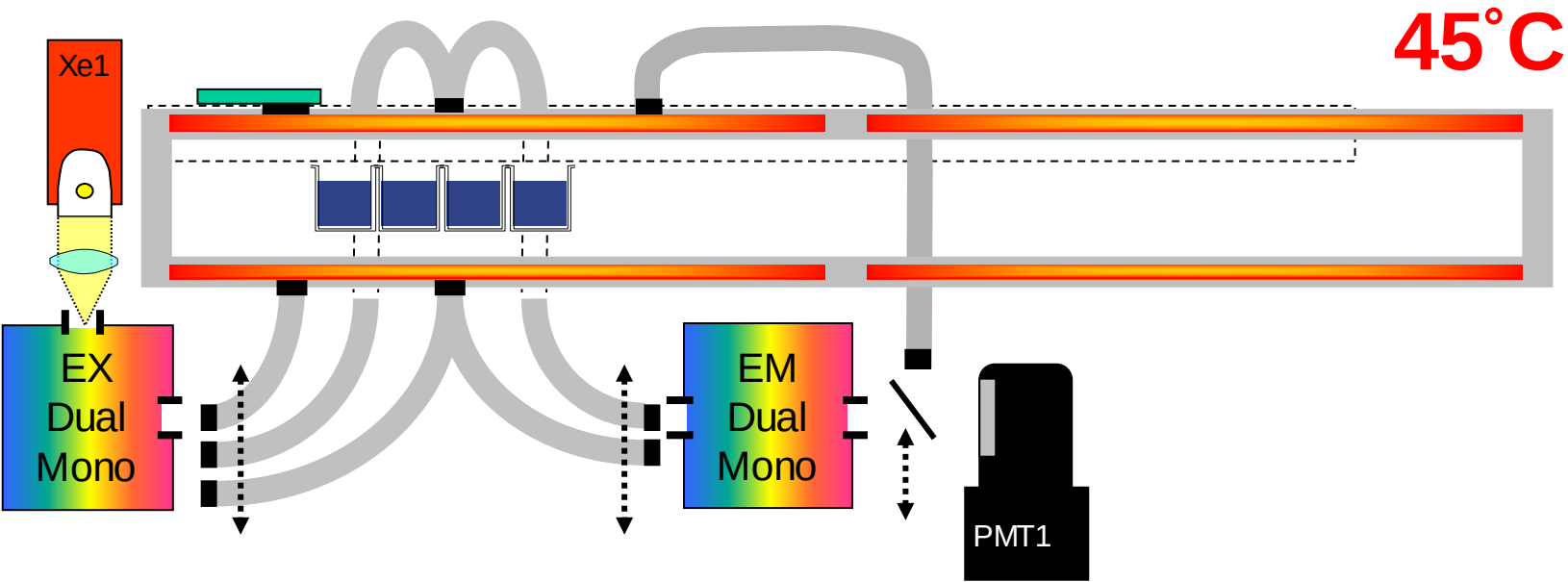
冷光偵測路徑



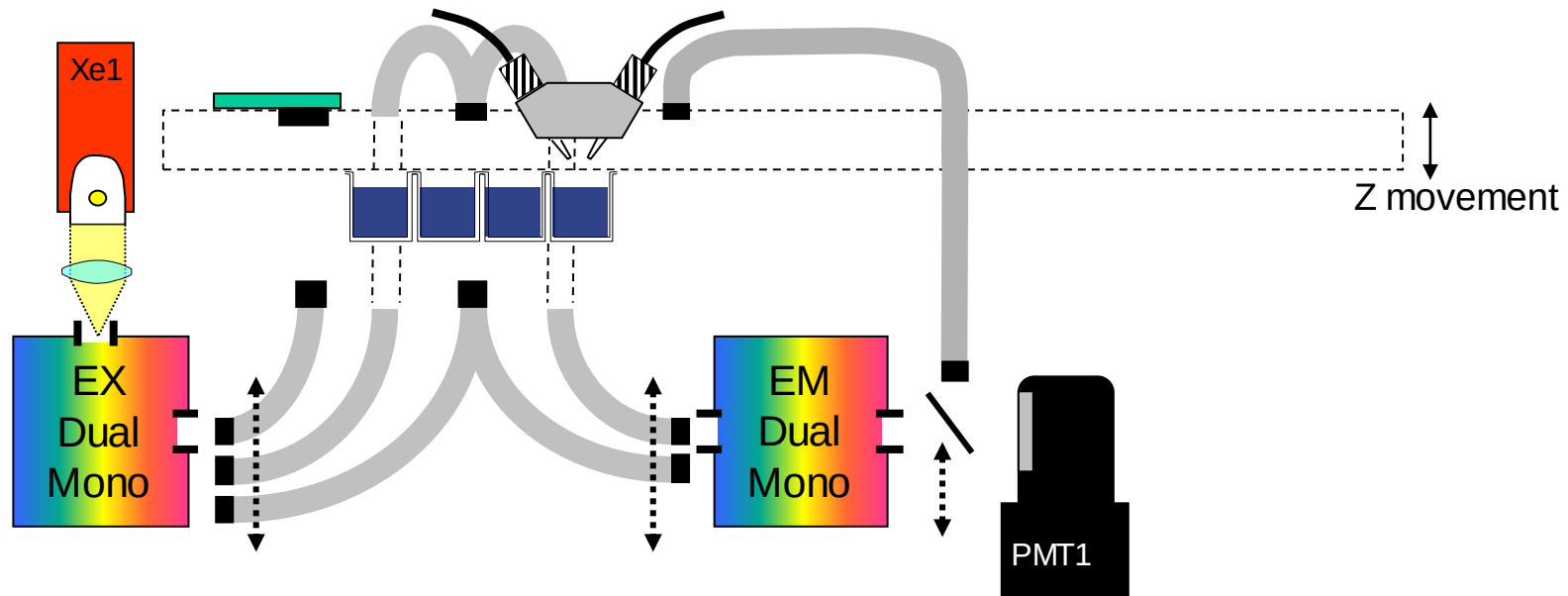
震盪功能



溫度控制: 溫度梯度防止水氣凝結



分注器



Number of Injectors:	2
Supported labware:	6- to 384-well microplates, Petri and cell culture dishes
Volume range:	5 – 1000 μ L in 1 μ L increments
Dead volume:	<1.1 mL with back flush (Synergy and Cytation) <1.65 mL with back flush (Lionheart FX)
Dispense accuracy:	+1 μ L or 2%
Dispense precision:	<2% at 50 - 200 μ L



應用

- ELISA
- Toxicity & drug screening
- Protein quantitation
- Nucleic acid quantitation
- Chemical measurements
- Cell viability, proliferation & death
- Gene expression

儀器規格

- 吸收光: 230 - 999 nm
- 螢冷光: 濾鏡式 或 全波長式 250-700nm
- 0 - 4.0 OD
- 溫度控制
- 震盪功能

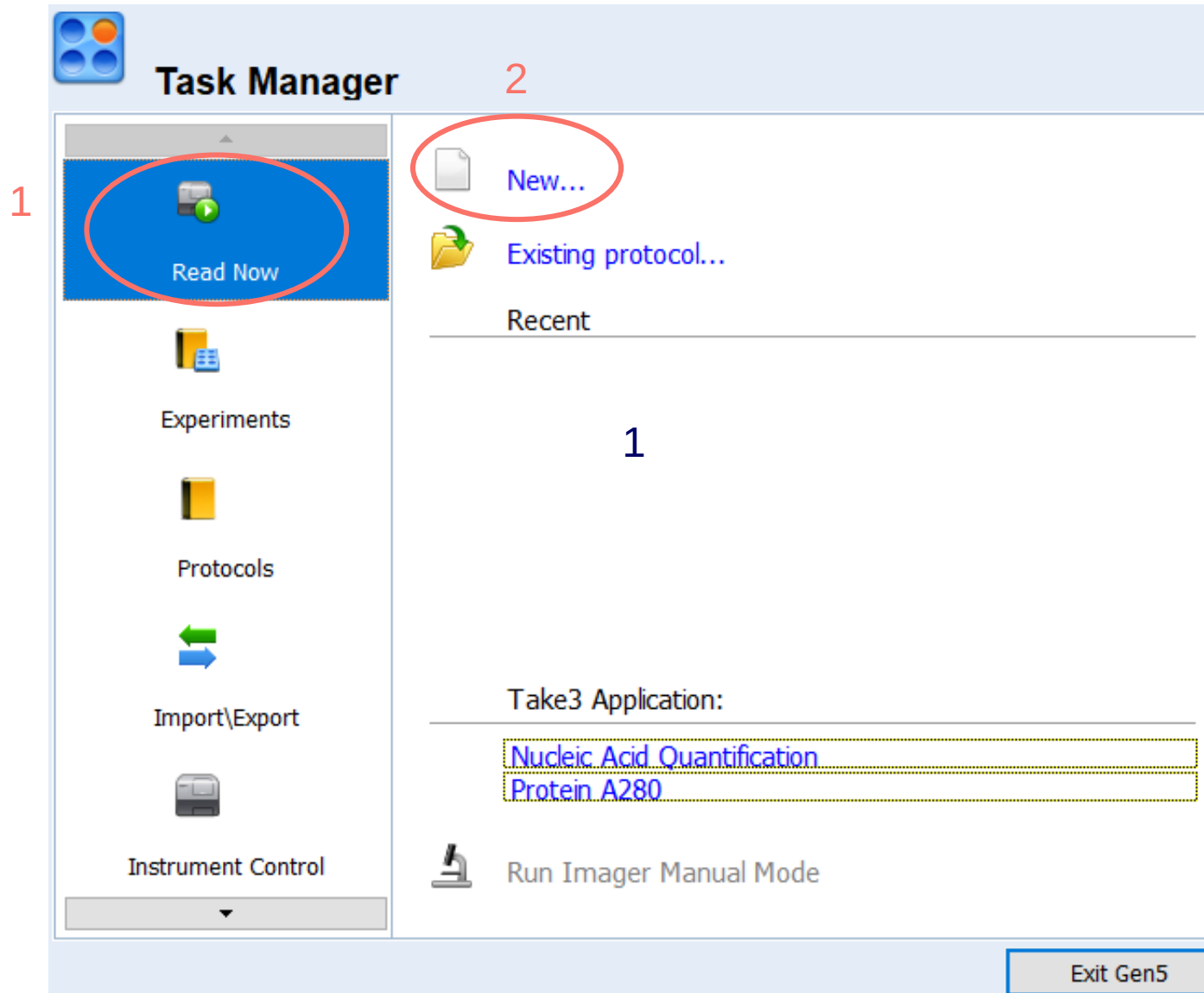
Gen5 分析軟體



Gen5 軟體功能

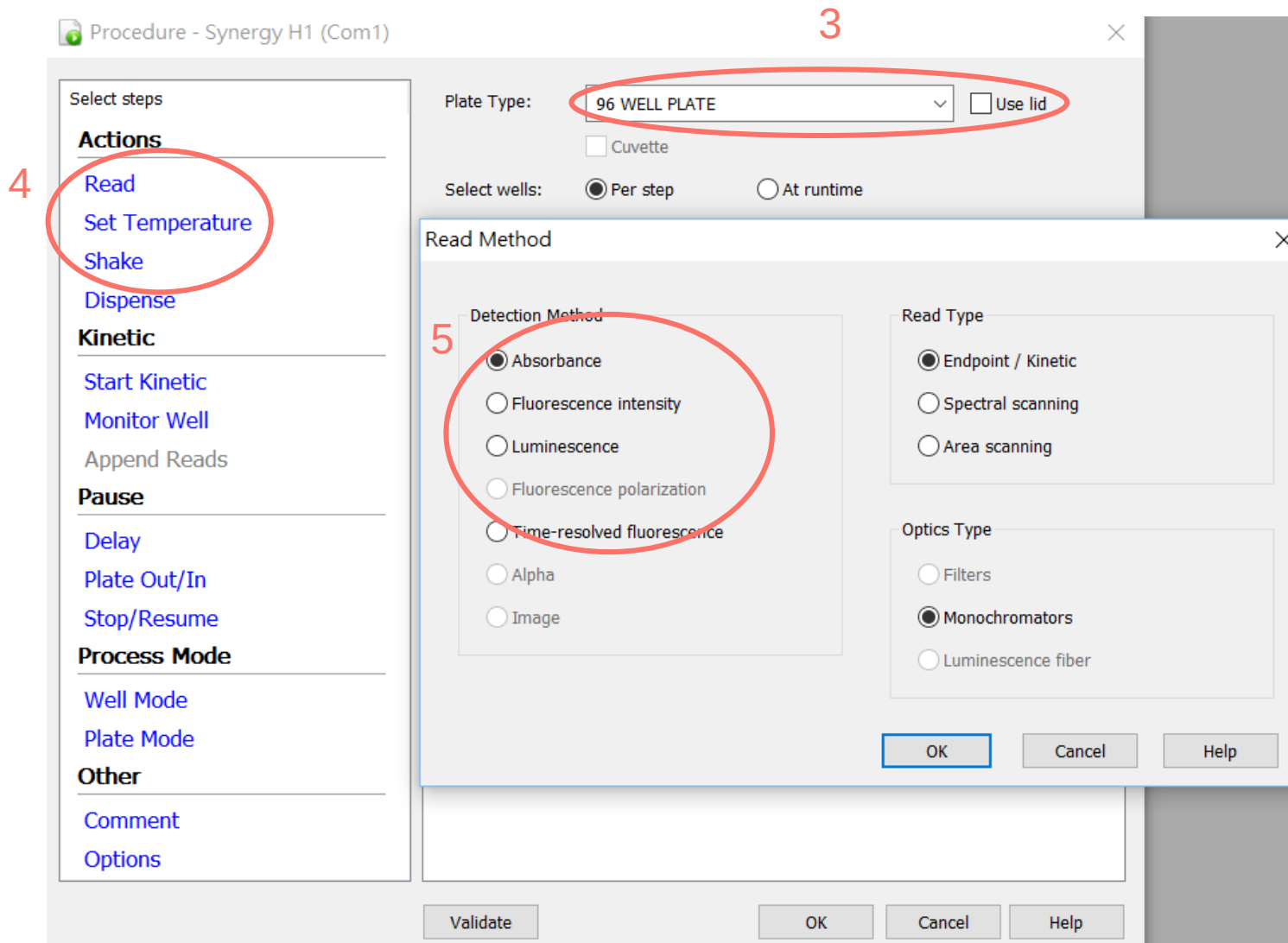
- 盤型
- 設定波長、溫度、震盪
- Plate Layout: 樣本佈局
- Data Reduction: 讀值運算及標準曲線
- 匯出報告

Gen5 軟體功能



- Experiment
- Protocol

Gen5 軟體功能



Gen5 軟體功能

Read Step

Step Label: Full Plate

Wavelengths

☐ 1 ☒ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

☐ Wavelength Switching per Well

Read Speed: Normal Edit

☐ Pathlength Correction Edit

吸收光

Read Step

Step Label: Full Plate

Wavelengths

☐ 1 ☐ 2 ☒ 3 ☐ 4

Excitation:

Emission:

Optics Position: Top Top Top

Gain:

Options... Options... Options...

☐ Wavelength Switching per Well

Read Speed: Normal Edit

Time resolved options: Edit

Read Height: mm Auto-Adjust...

螢光

Read Step

Step Label: Full Plate

Wavelengths

☒ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Excitation:

Emission:

Optics Position:

Gain: Options...

☐ Wavelength Switching per well

Integration Time: MM:SS.ss Edit

Read Height: mm Auto-Adjust...

OK Cancel

冷光

Gen5 軟體功能

Temperature Step ✕

☐ Incubator Off

☒ Incubator On

Temperature: °C

☒ Preheat before continuing with next step

設定溫度

Shake Step ✕

Shake Mode: ▾

Duration: ☐ Continuous Shake

Linear Frequency: Slower Faster

Orbital Speed: ☒ Slow ☐ Fast

設定震盪

Gen5 軟體功能

Procedure - Synergy H1 (Com1) ✕

Select steps

Actions

Read

Set Temperature

Shake

Dispense

Kinetic

Start Kinetic

Monitor Well

Append Reads

Pause

Delay

Plate Out/In

Stop/Resume

Process Mode

Well Mode

Plate Mode

Other

Comment

Options

Plate Type: 96 WELL PLATE ▼ ☐ Use lid

☐ Cuvette

Select wells: ☒ Per step ☐ At runtime

Description	Comments
Temperature: Setpoint 37 °C	
Shake: Linear for 0:05	
Read: (A) 450	

依據步驟順序由上而下排列

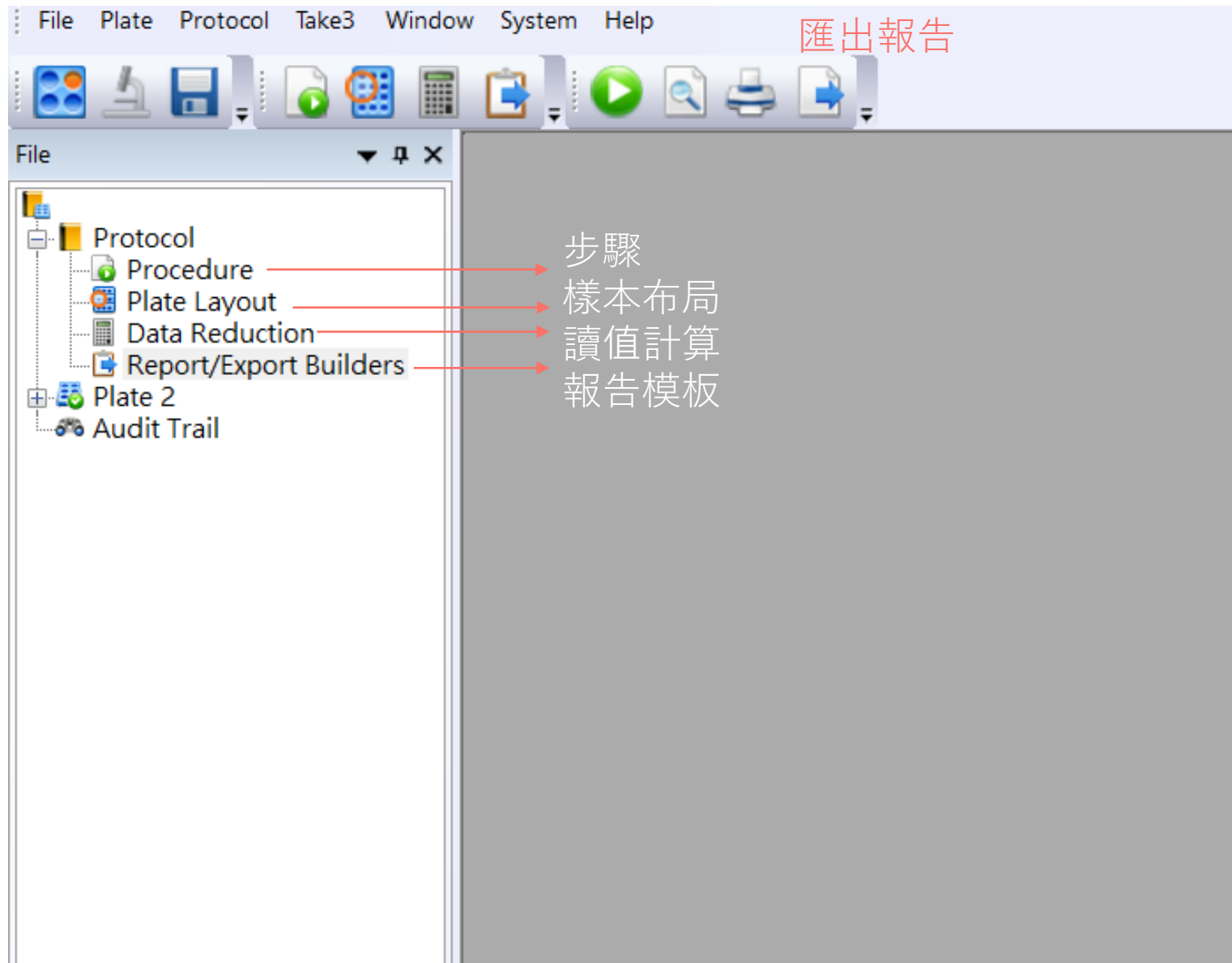
Validate

OK

Cancel

Help

Gen5 軟體功能



Gen5 軟體功能

Plate Layout

Select a Well ID in the list on the left, then assign to the matrix.

Add... **Delete**

- <Empty>
- BLK (x2)
- STD (x10)
 - 0 (x2)
 - 10 (x2)
 - 25 (x2)
 - 50 (x2)
 - 100 (x2)
- Sample
 - SPL1 (x2)
 - SPL2 (x2)
 - SPL3 (x2)
 - SPL4 (x2)
 - SPL5 (x2)
 - SPL6 (x2)
 - SPL7 (x2)
 - SPL8 (x2)
 - SPL9 (x2)
 - SPL10 (x2)
 - SPL11 (x2)
 - SPL12 (x2)
 - SPL13 (x2)
 - SPL14 (x2)
 - SPL15 (x2)
 - SPL16 (x2)
 - SPL17 (x2)
 - SPL18 (x2)
 - SPL19 (x2)
 - SPL20 (x2)
 - SPL21 (x2)
 - SPL22 (x2)
 - SPL23 (x2)
 - SPL24 (x2)
 - SPL25 (x2)
 - SPL26 (x2)
 - SPL27 (x2)
 - SPL28 (x2)
 - SPL29 (x2)

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	STD1 0	STD2 10	STD3 25	STD4 50	STD5 100	SPL1	SPL2	SPL3	SPL4	SPL5	SPL6
B	BLK	STD1 0	STD2 10	STD3 25	STD4 50	STD5 100	SPL1	SPL2	SPL3	SPL4	SPL5	SPL6
C	SPL7	SPL8	SPL9	SPL10	SPL11	SPL12	SPL13	SPL14	SPL15	SPL16	SPL17	SPL18
D	SPL7	SPL8	SPL9	SPL10	SPL11	SPL12	SPL13	SPL14	SPL15	SPL16	SPL17	SPL18
E	SPL19	SPL20	SPL21	SPL22	SPL23	SPL24	SPL25	SPL26	SPL27	SPL28	SPL29	SPL30
F	SPL19	SPL20	SPL21	SPL22	SPL23	SPL24	SPL25	SPL26	SPL27	SPL28	SPL29	SPL30
G	SPL31	SPL32	SPL33	SPL34	SPL35	SPL36	SPL37	SPL38	SPL39	SPL40	SPL41	SPL42
H	SPL31	SPL32	SPL33	SPL34	SPL35	SPL36	SPL37	SPL38	SPL39	SPL40	SPL41	SPL42

Serial Assignment

Replicates: 1

☐ Next

☐ Auto Select Next ID

Import **Export** **Undo** **Print**

OK **Cancel** **Help**

Gen5 軟體功能

Data Reduction

Data Reduction Tools

- Transformations
 - Blank
 - Normalize
 - Ratio
 - Delta
 - Custom
 - Data Reduction Variable
- Curve Analysis**
 - Standard Curve**
 - Custom - PLA
- Qualitative Analysis / QC
 - Replicate Rejection
 - Cutoff
 - Validation
 - ZPrime

Description	Data Out	Comments
Transformation	Blank 562	Blank Subtraction
Curve Analysis	Curve, Conc	

Curve Analysis

Curve:

Data In | **Curve Fit** | Data Out

Curve Fit Method

- ☒ Linear Regression
- ☐ Nonlinear Regression 4 Parameter
- ☐ Polynomial Regression Degree: 2
- ☐ Point to Point
- ☐ Spline Smoothing Factor: 0

Axis Transformation

X: ☒ None ☐ Log

Y: ☒ None ☐ Log

Formula:

Y Axis Data

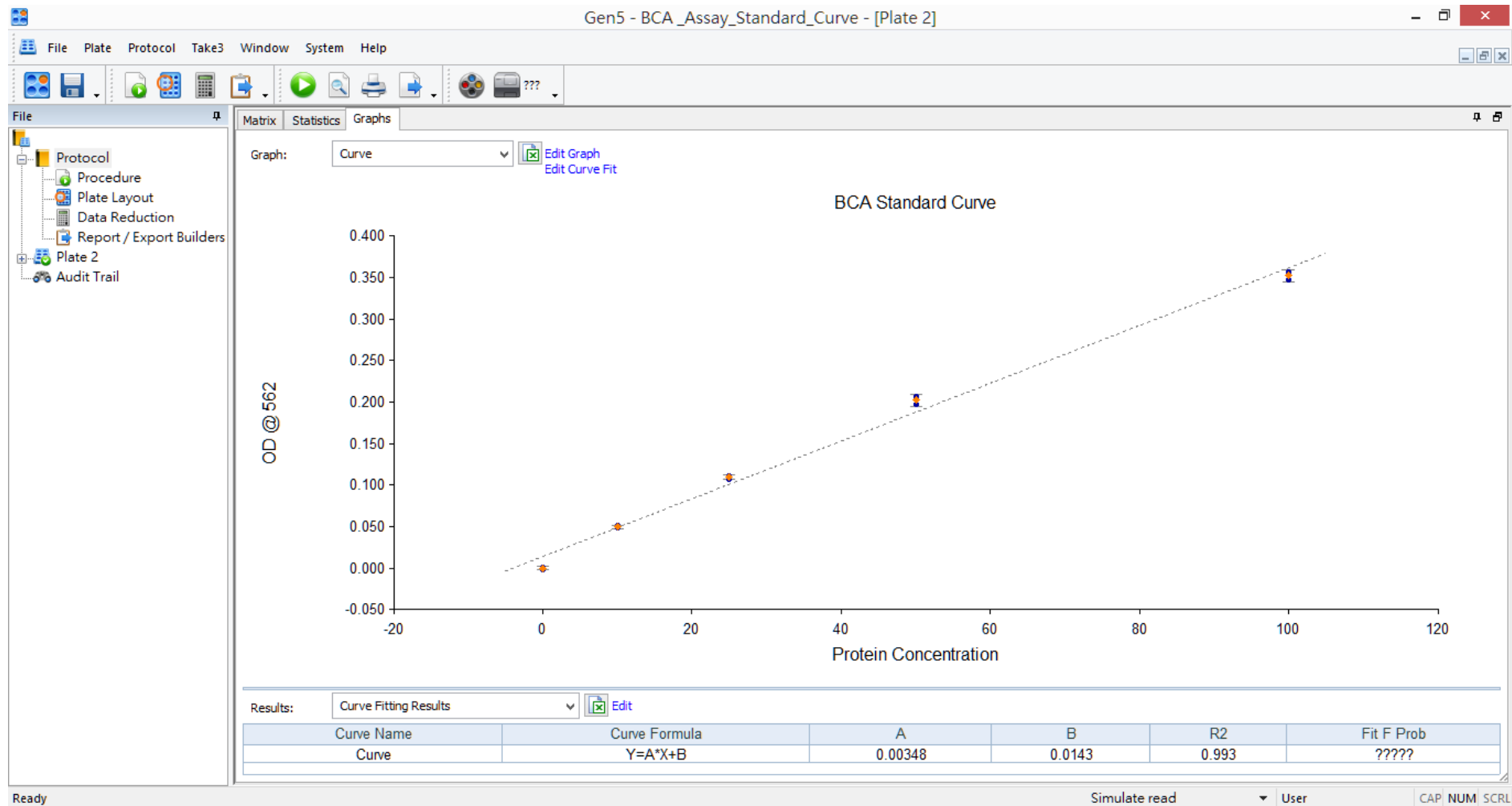
- ☒ Use average of replicates
- Weighting: None Edit
- Extrapolation Factor: 1.1

Parameter Constraints

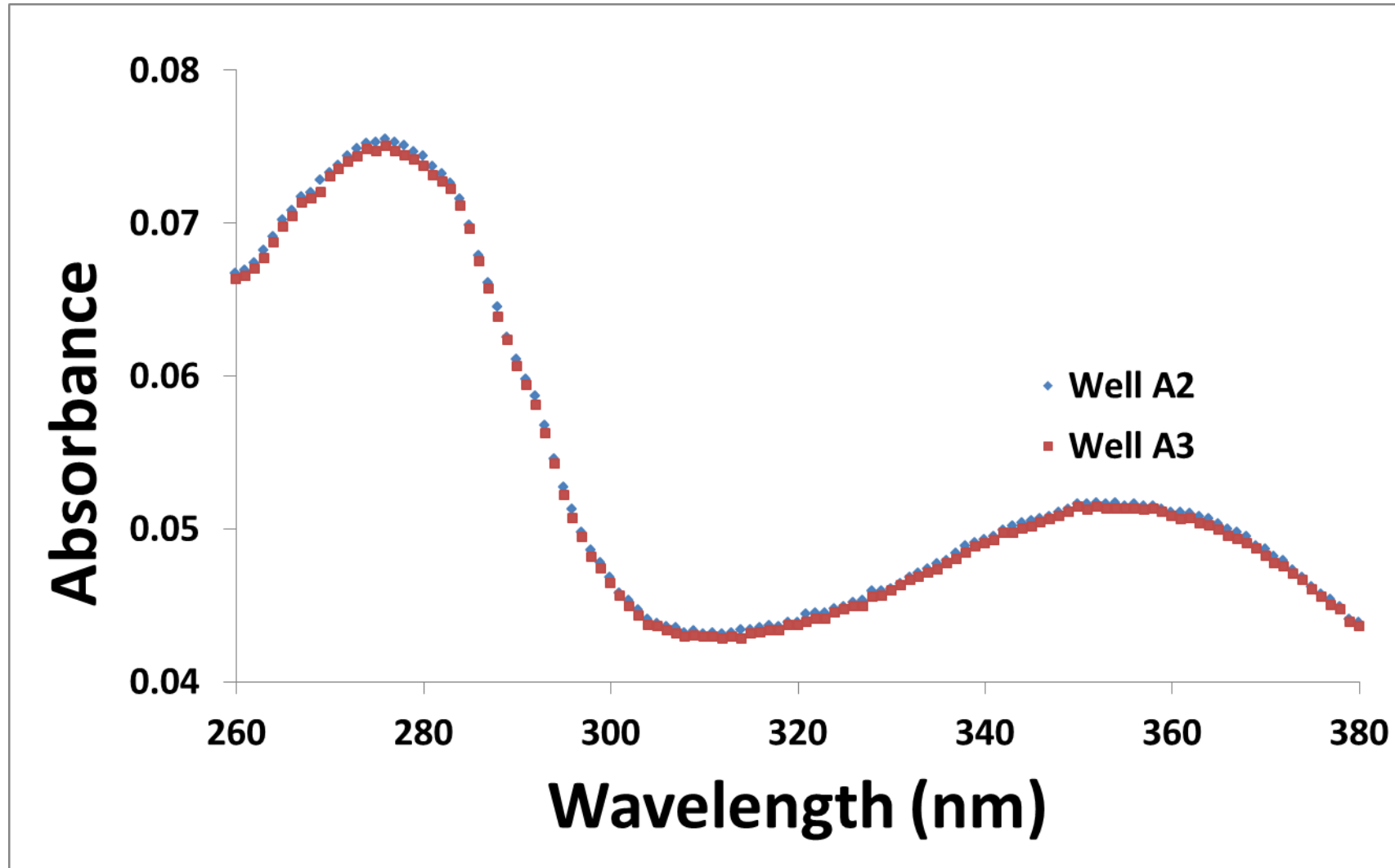
- ☒ None (Estimate all)
- ☐ Y Axis Intercept: 0
- ☐ Advanced Edit

確定 取消 說明

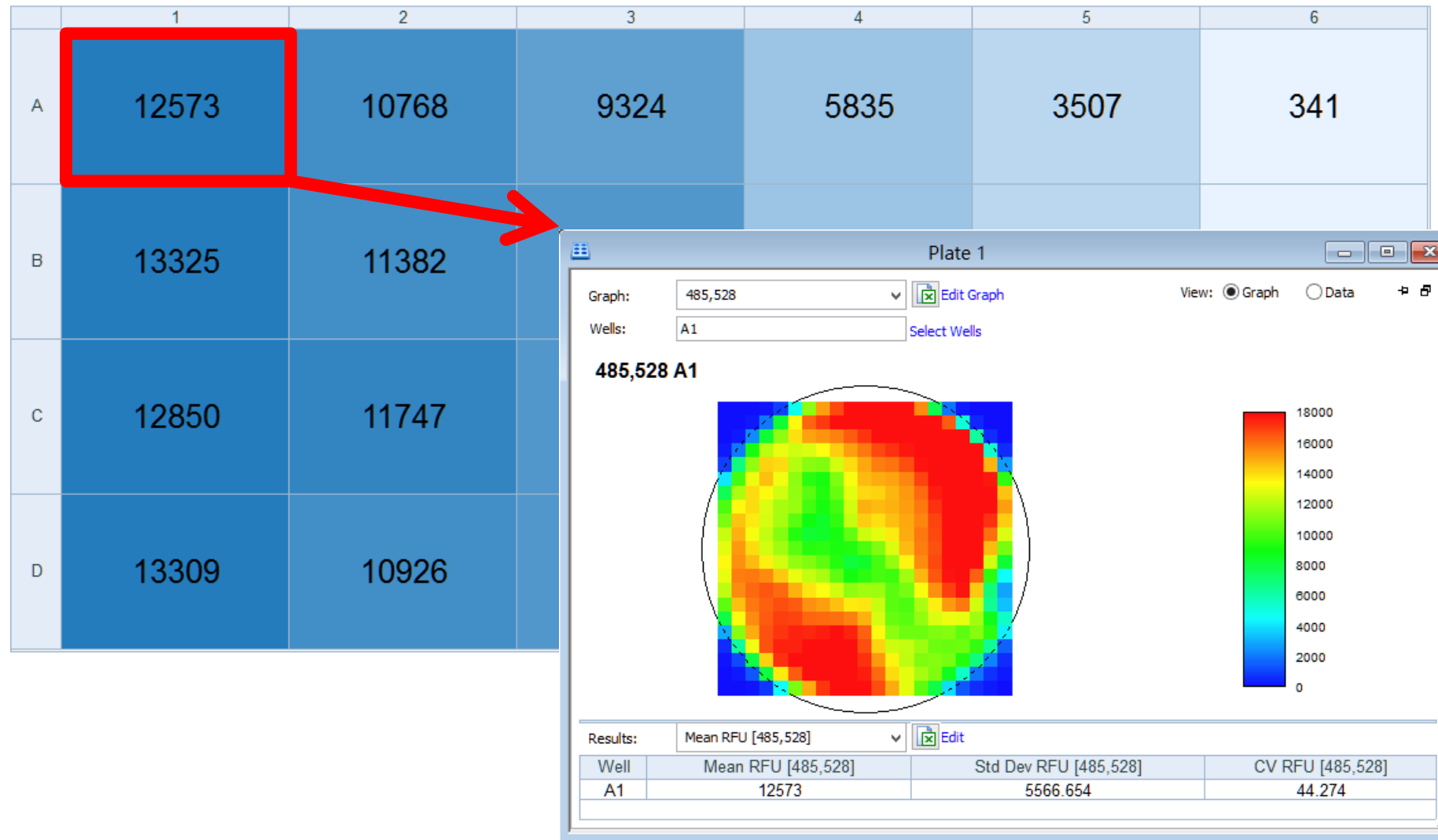
Gen5 軟體功能



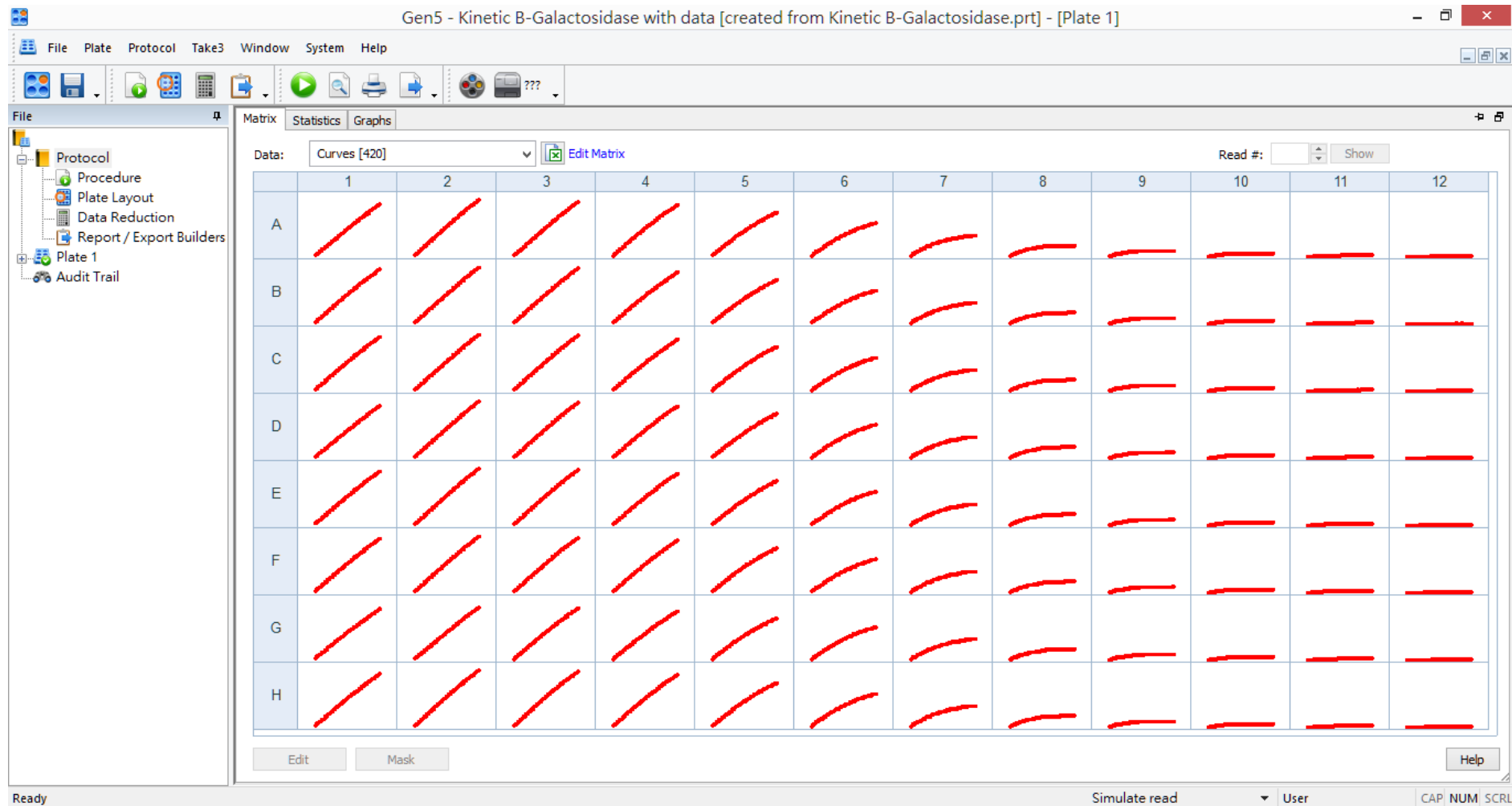
Gen5 軟體功能: 光譜掃描



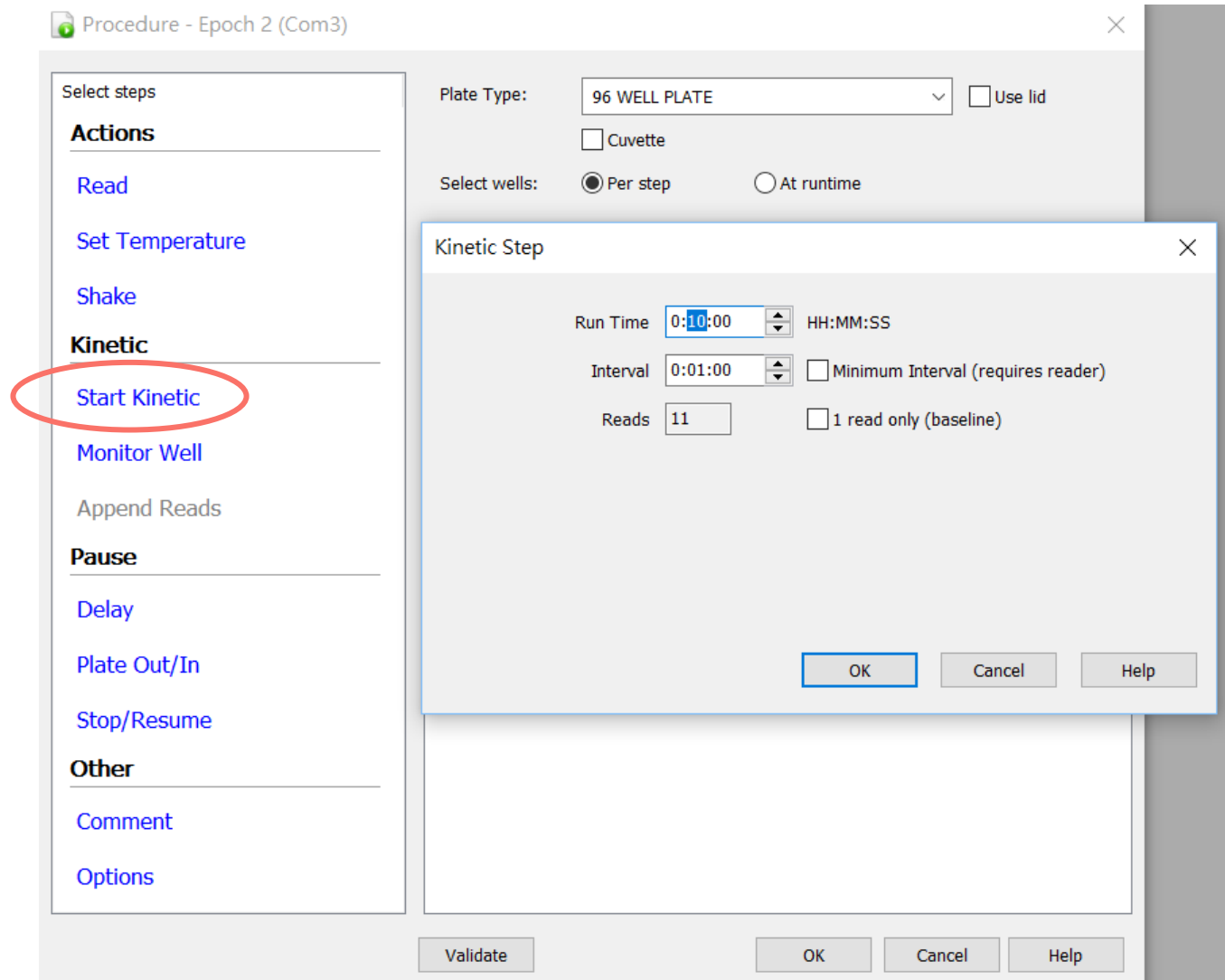
Gen5 軟體功能: 孔域掃描



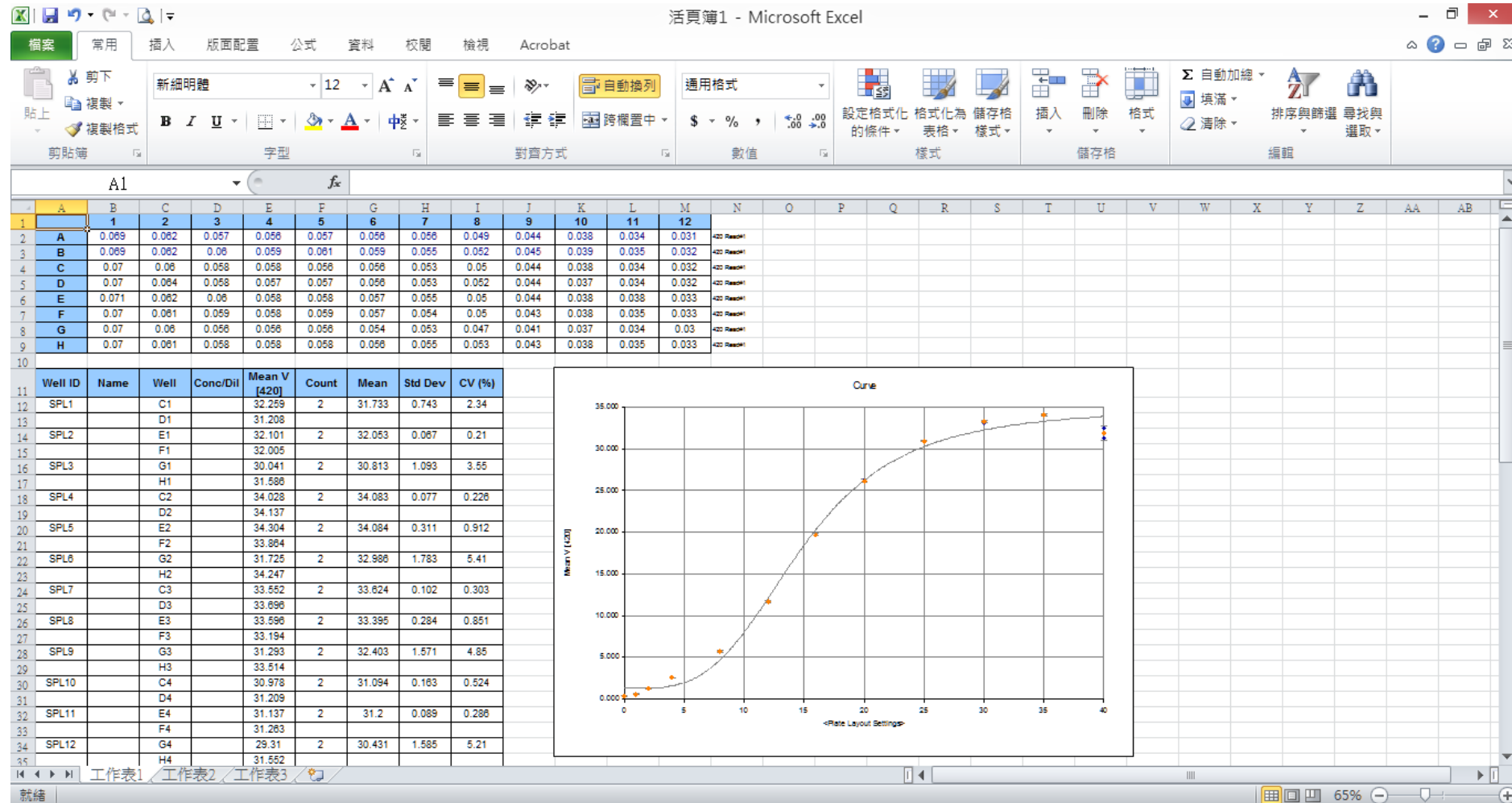
Gen5 軟體功能: Kinetic



Gen5 軟體功能: Kinetic



Gen5 軟體功能: 匯出報告



Q & A

