



Basics of Flow Cytometry & Attune Flow Cytometer

20241112

Taqkey Science

張政暉

thermo
scientific

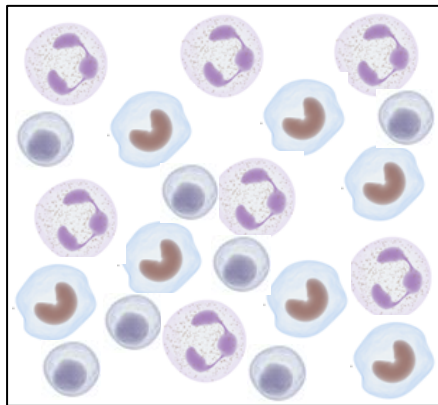
Authorized Distributor

Basics of Flow Cytometry

Flow Cytometer – a different kind of “microscope” to observe cells

Microscope

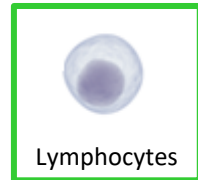
Lysed human whole blood



Granulocytes

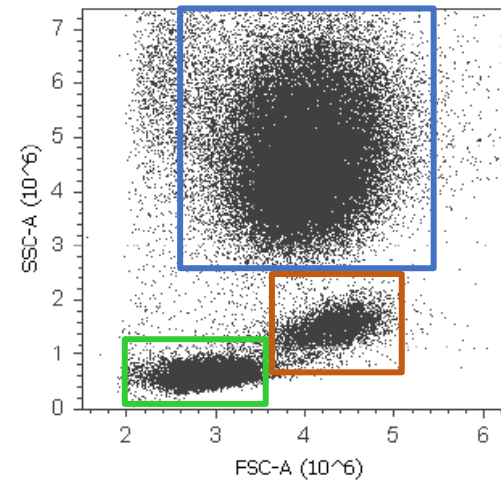


Monocytes



Lymphocytes

Flow Cytometer



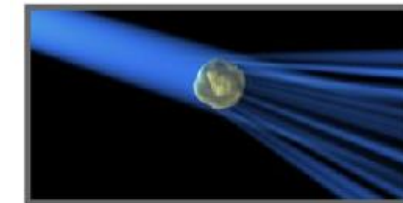
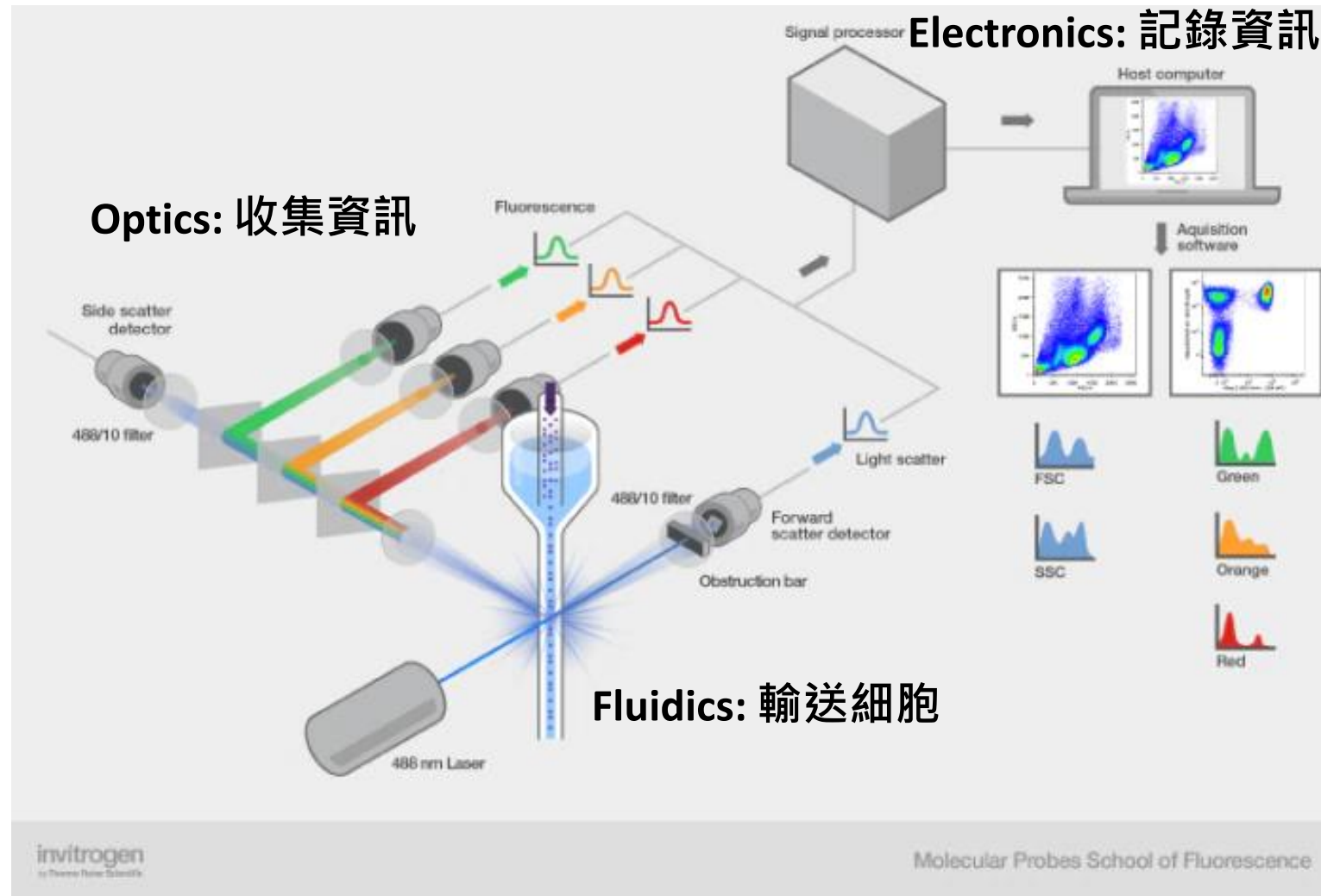
Advantage

- Quantitative
- Rare population
- Multiple phenotype

Disadvantage

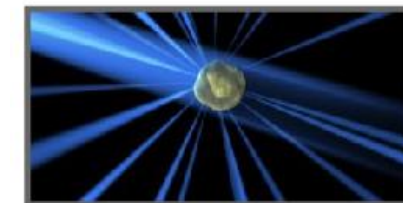
- Lost structure information of tissue/cell

Flow Cytometer Components



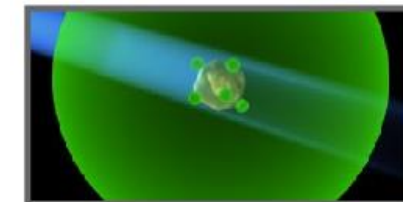
Size

Forward scatter (FSC)



Complexity

Side scatter (SSC)

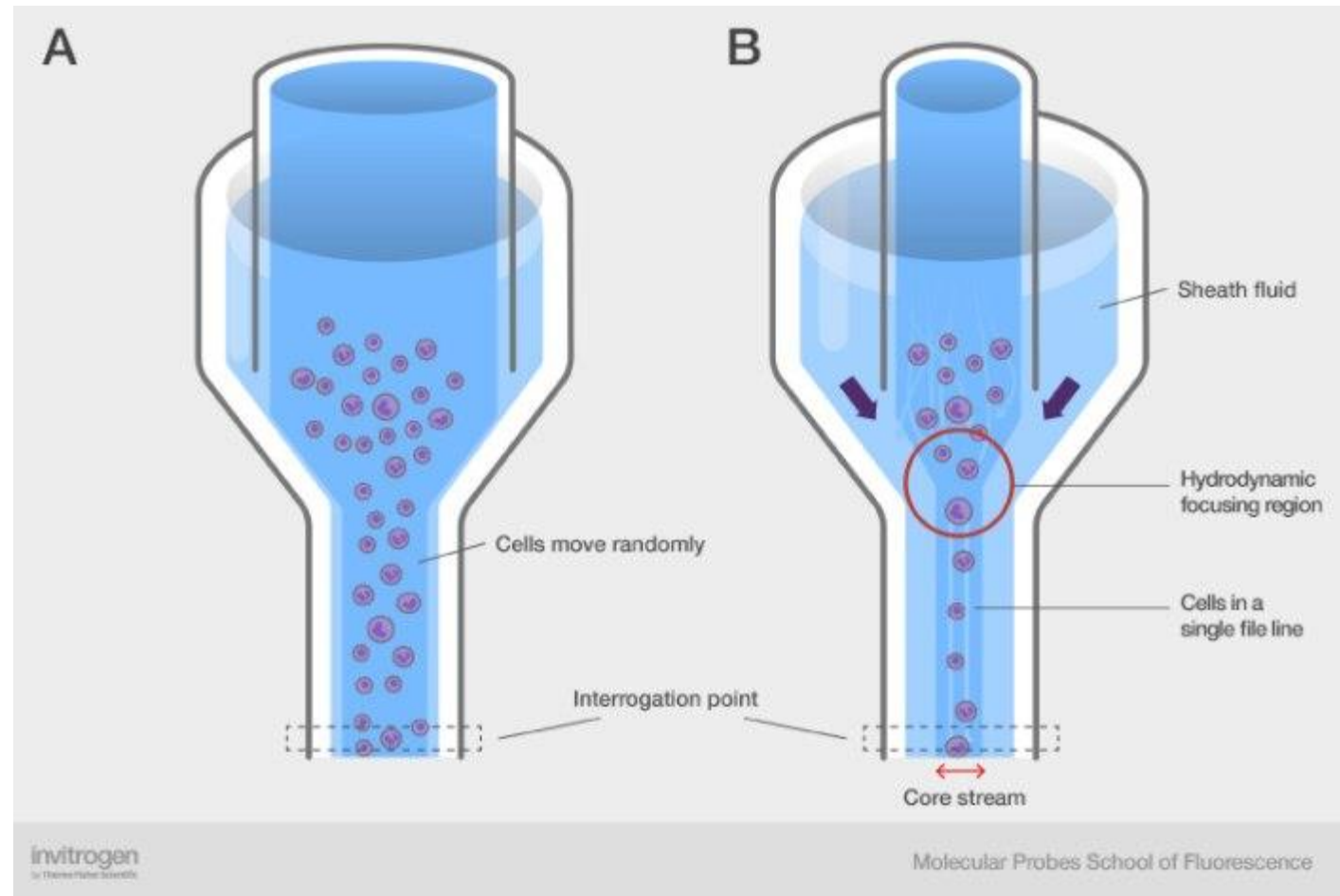


Phenotype

Fluorescence

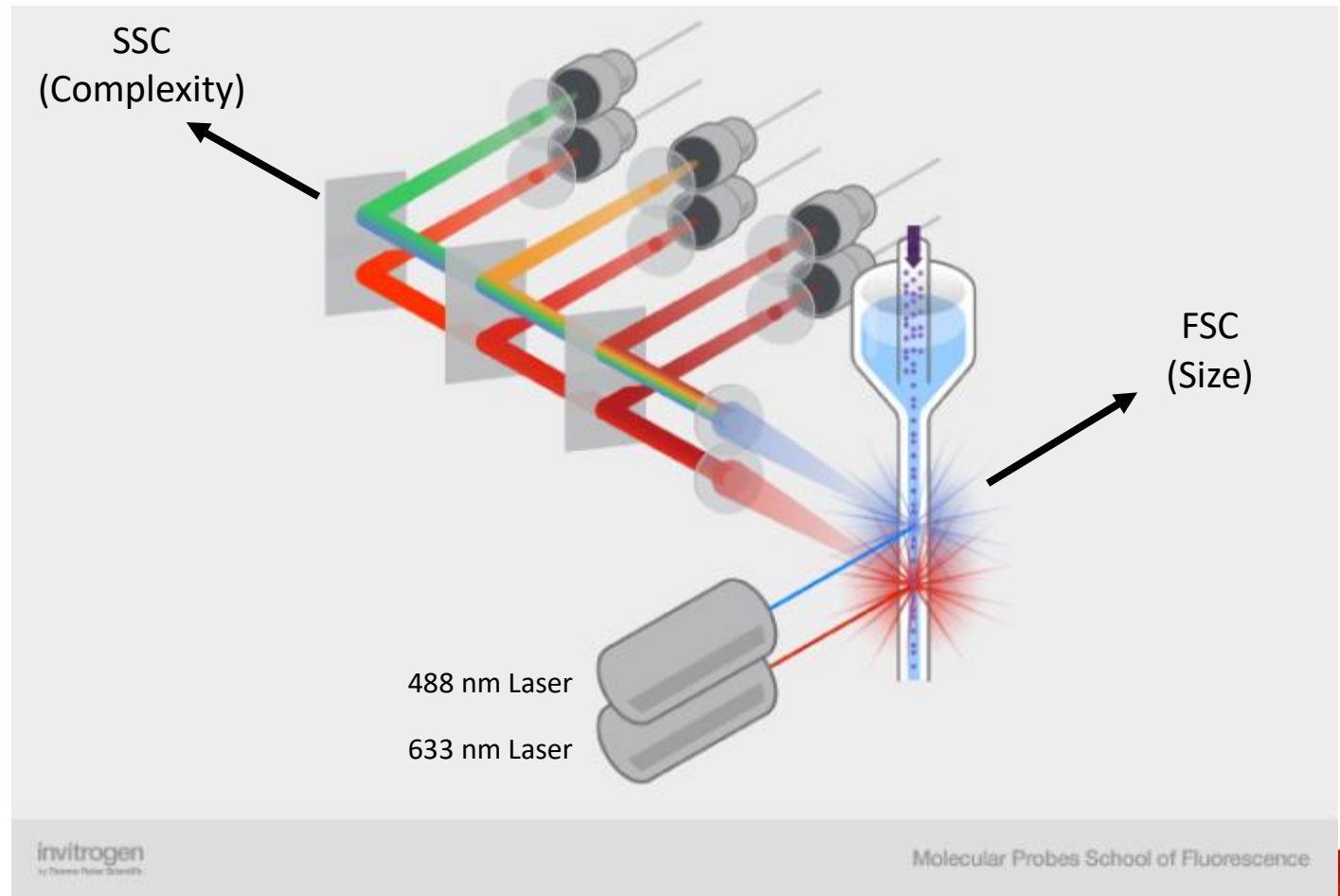
Fluidics: 輸送細胞

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



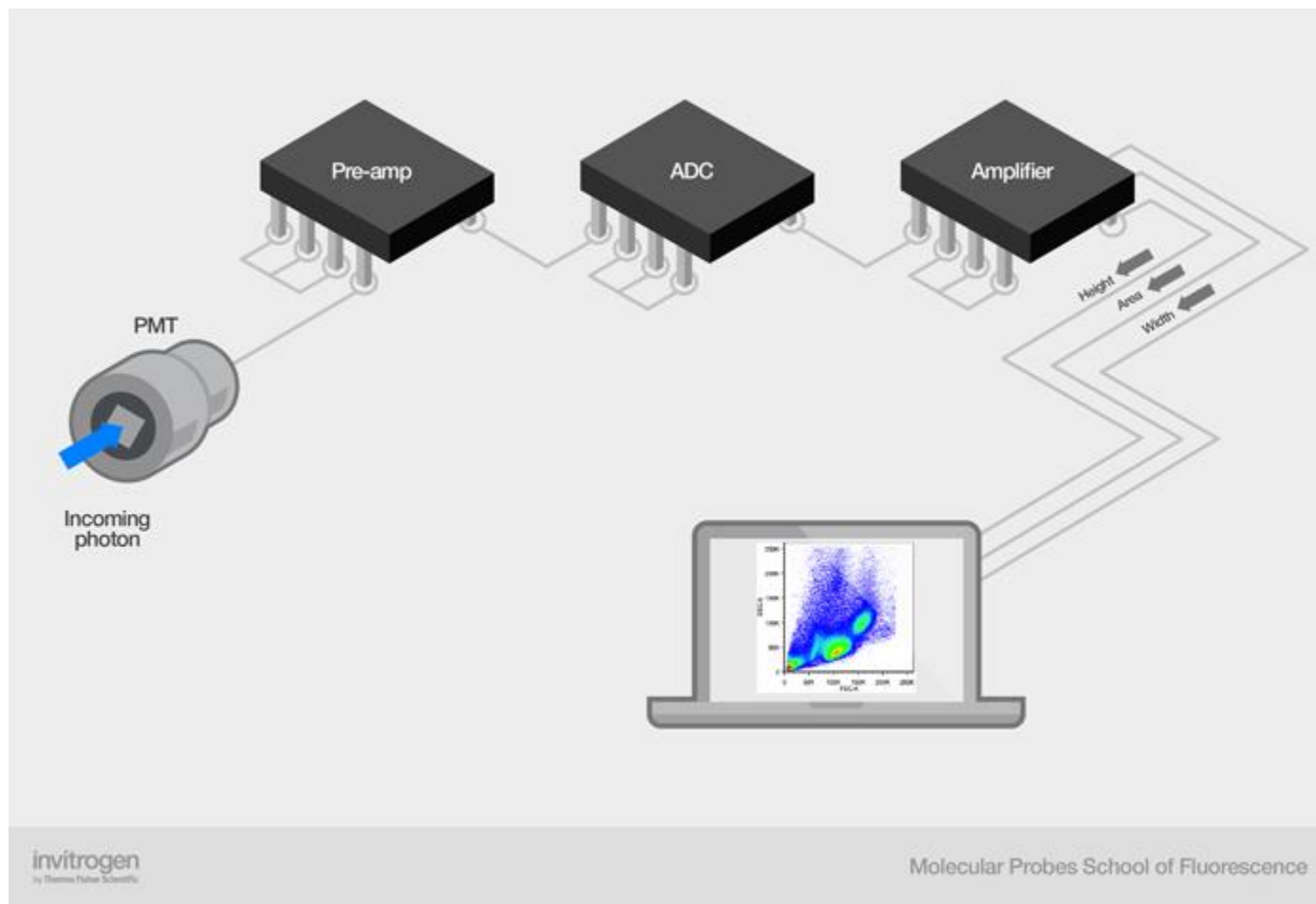
Optics: 獲取資訊

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.

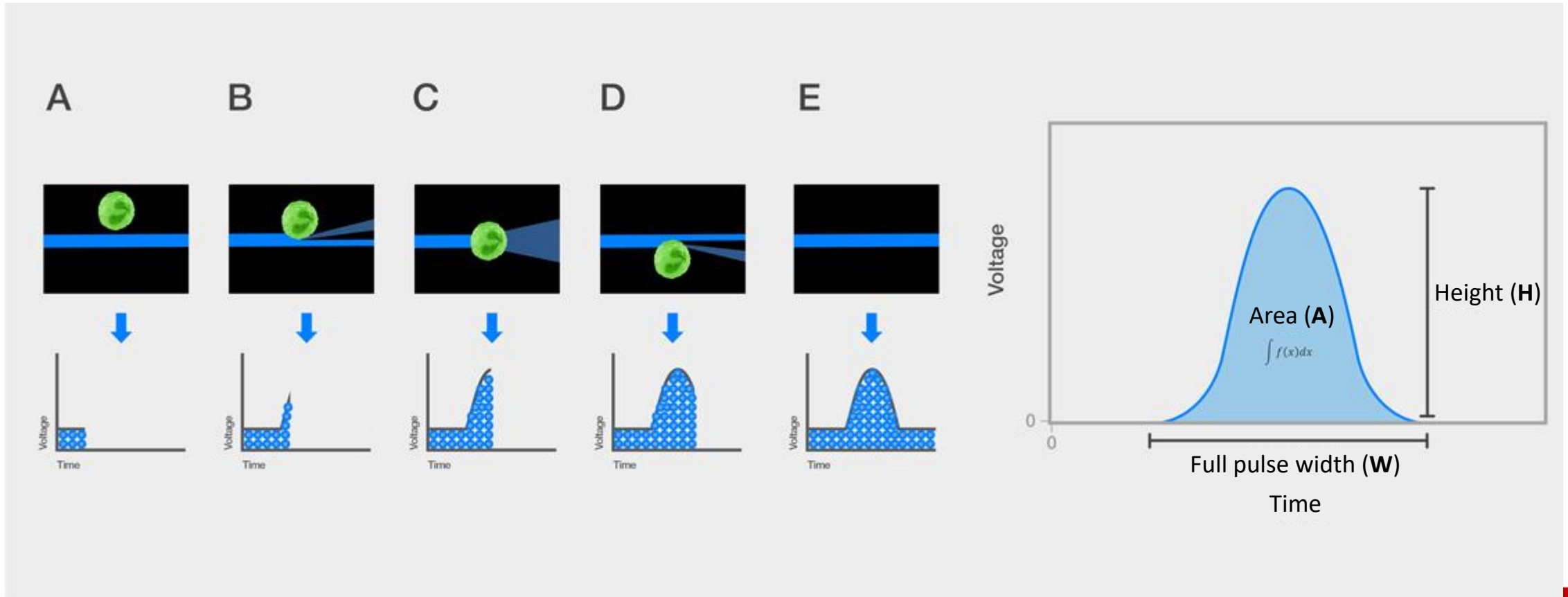


Electronics: 記錄資訊

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

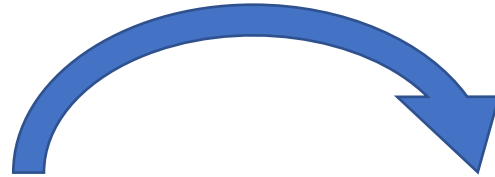


Data of Flow Cytometry

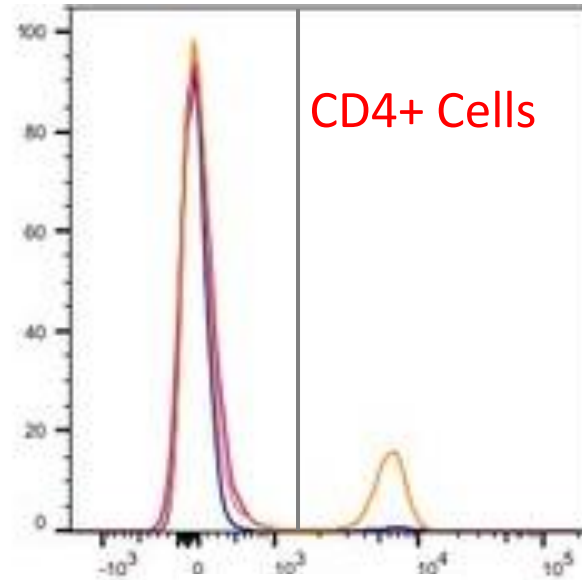
File format: FCS3.0, FCS3.1

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

Flow Cytometry Standard (FCS)
<https://isac-net.org/page/Data-Standards>

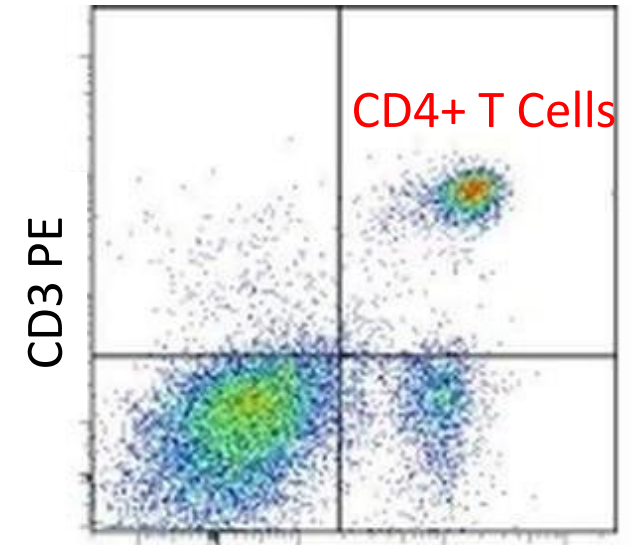


1 marker



CD4 FITC
Histogram

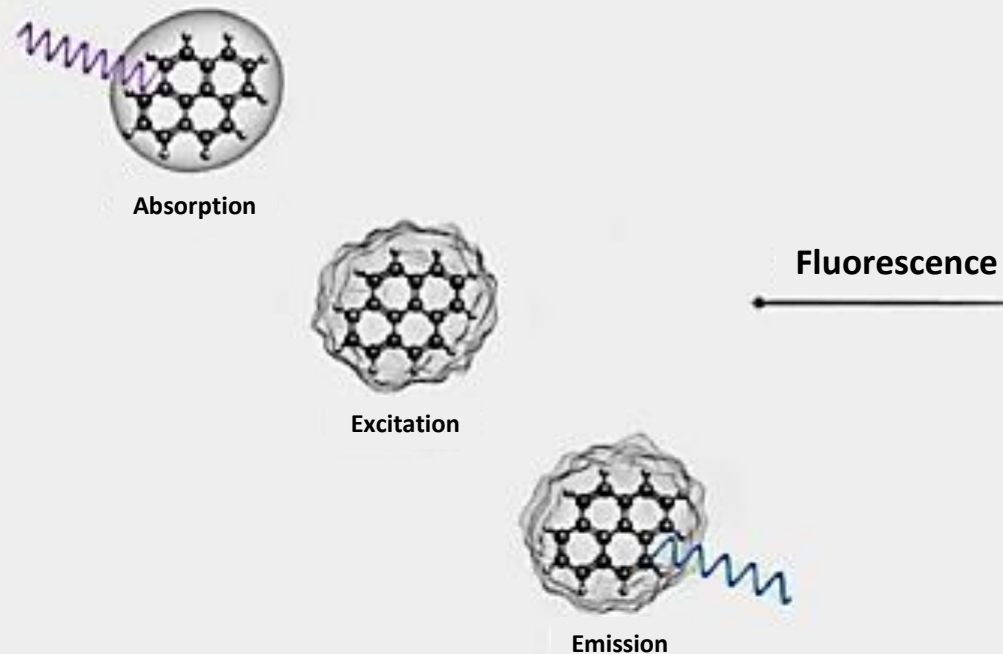
2 markers



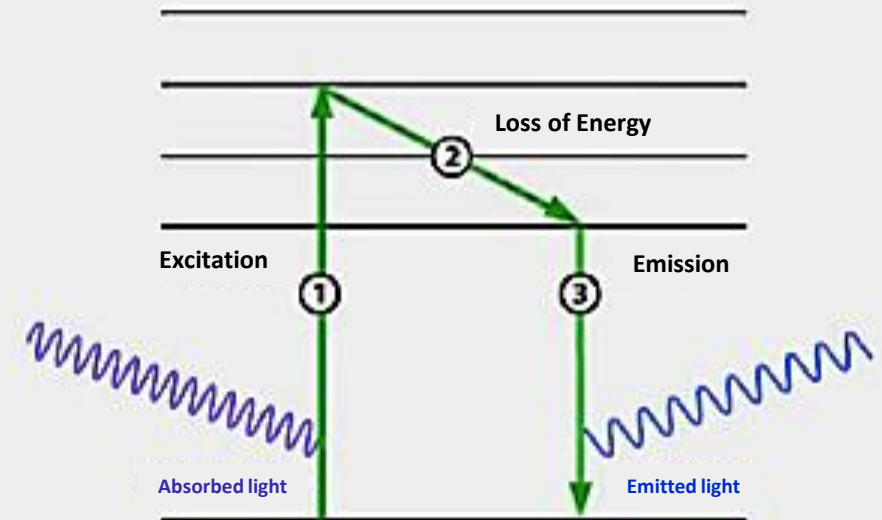
CD4 FITC
Dot plot

Fluorescence

Definition of Fluorescence



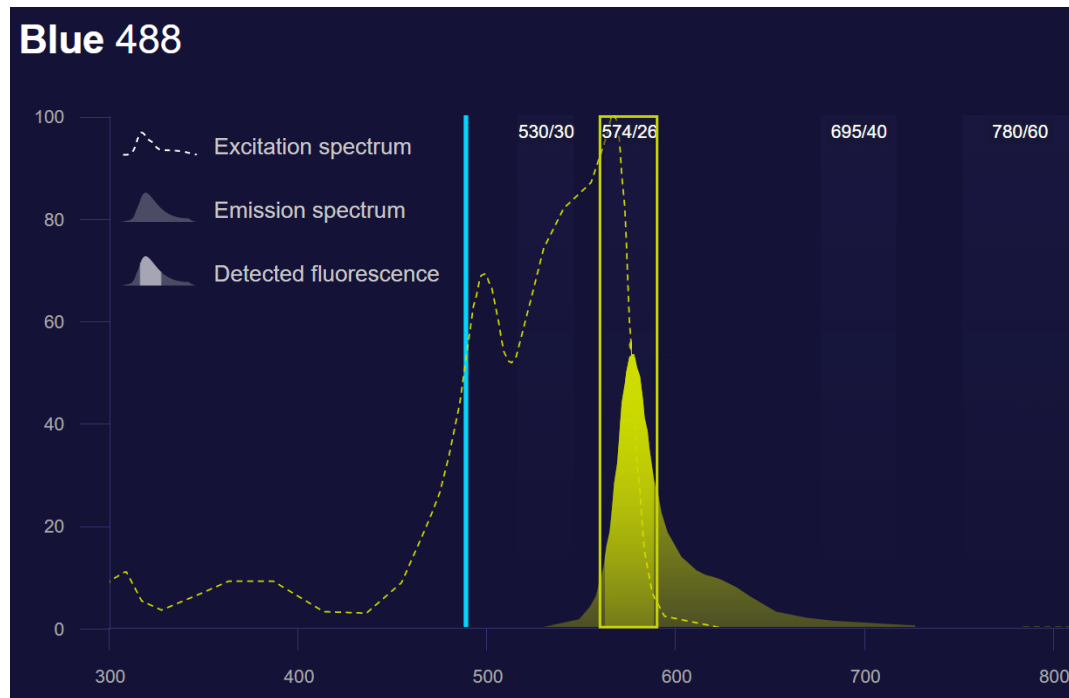
Jablonski Diagram Summary



Channels for Fluorochrome

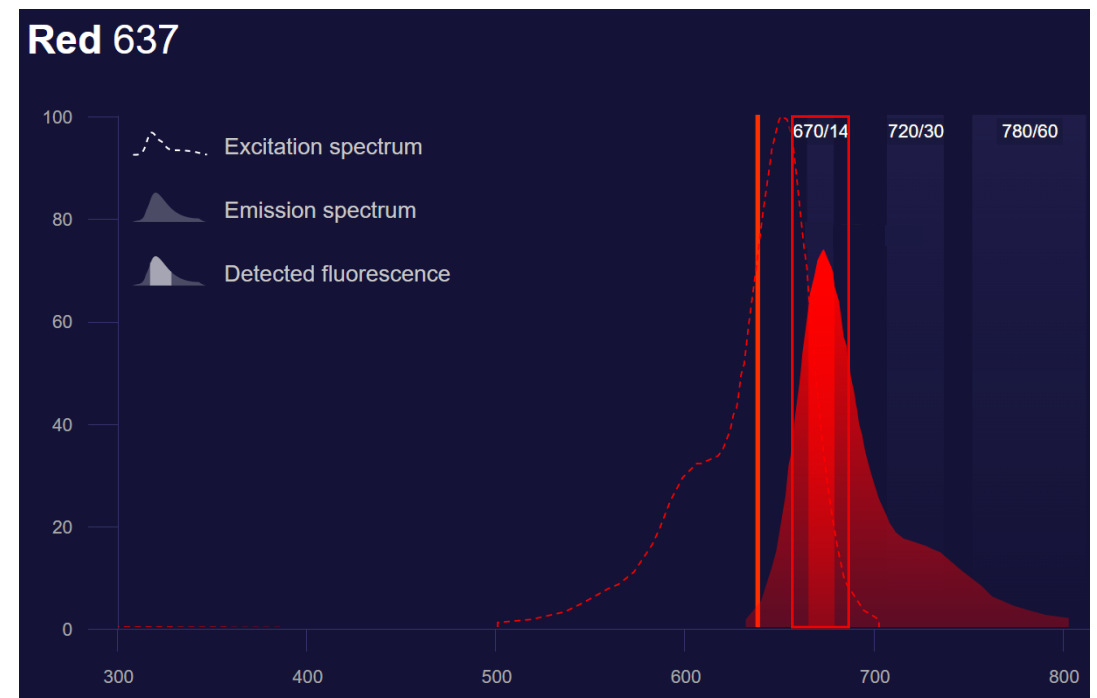
Fluorochrome: PE

Channel: Ex 488, Em 574/26



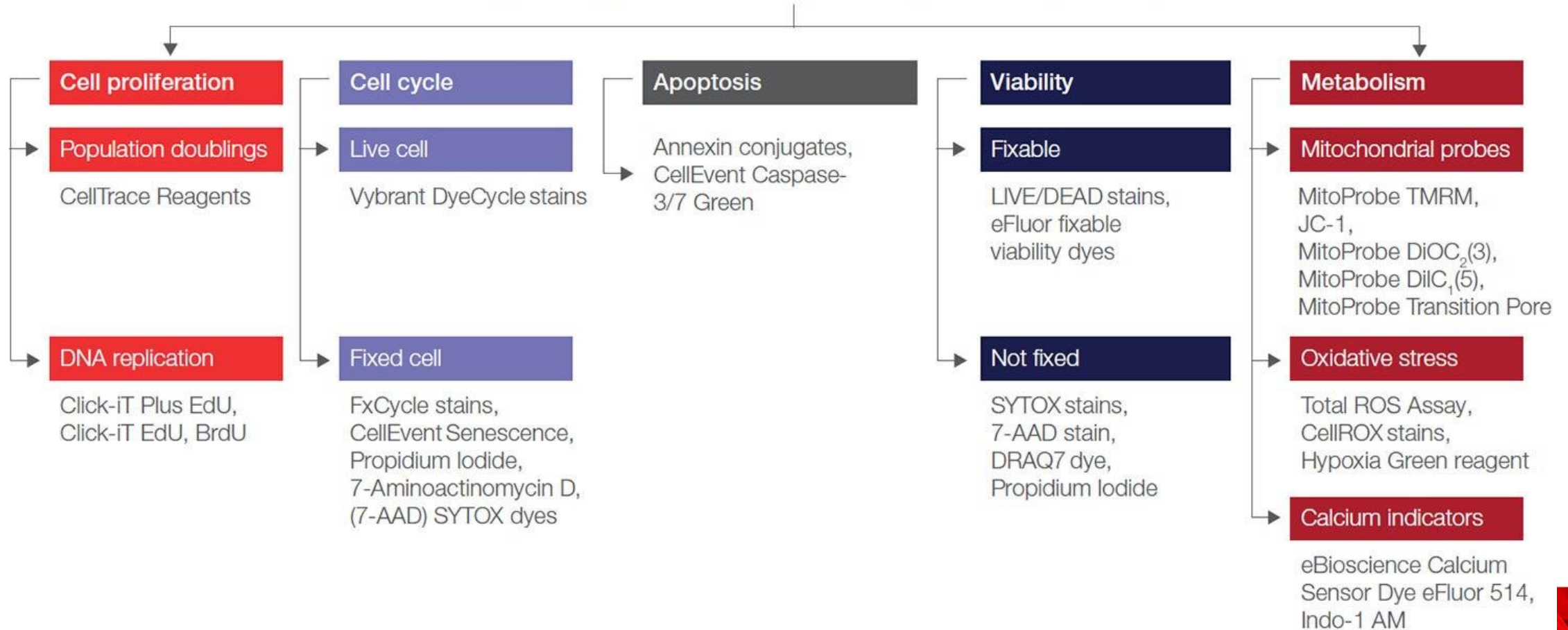
Fluorochrome: APC

Channel: Ex 637, Em 670/14



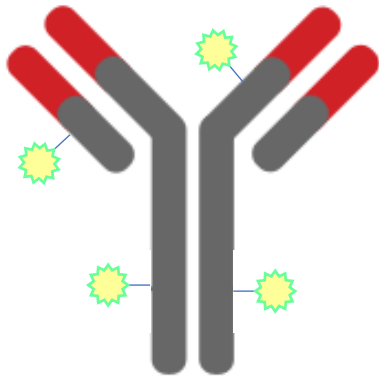
Fluorescent Reagents

What type of applications are you using in flow cytometry?

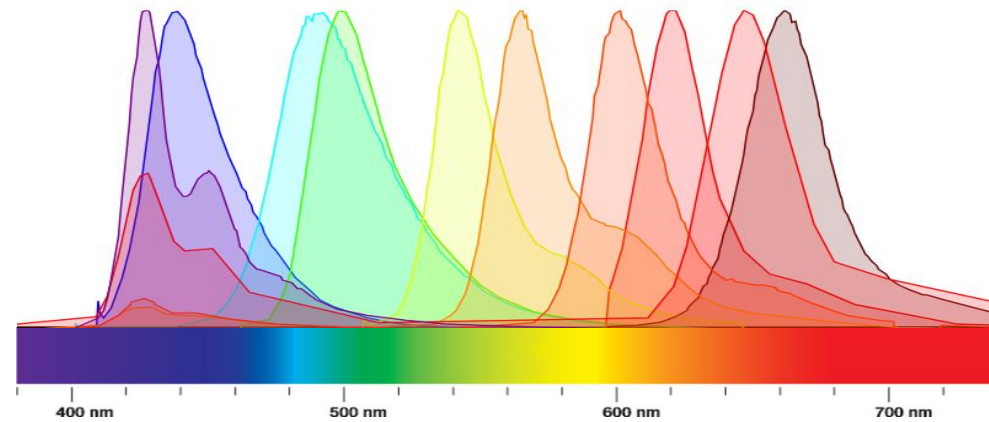


Fluorescent Antibody

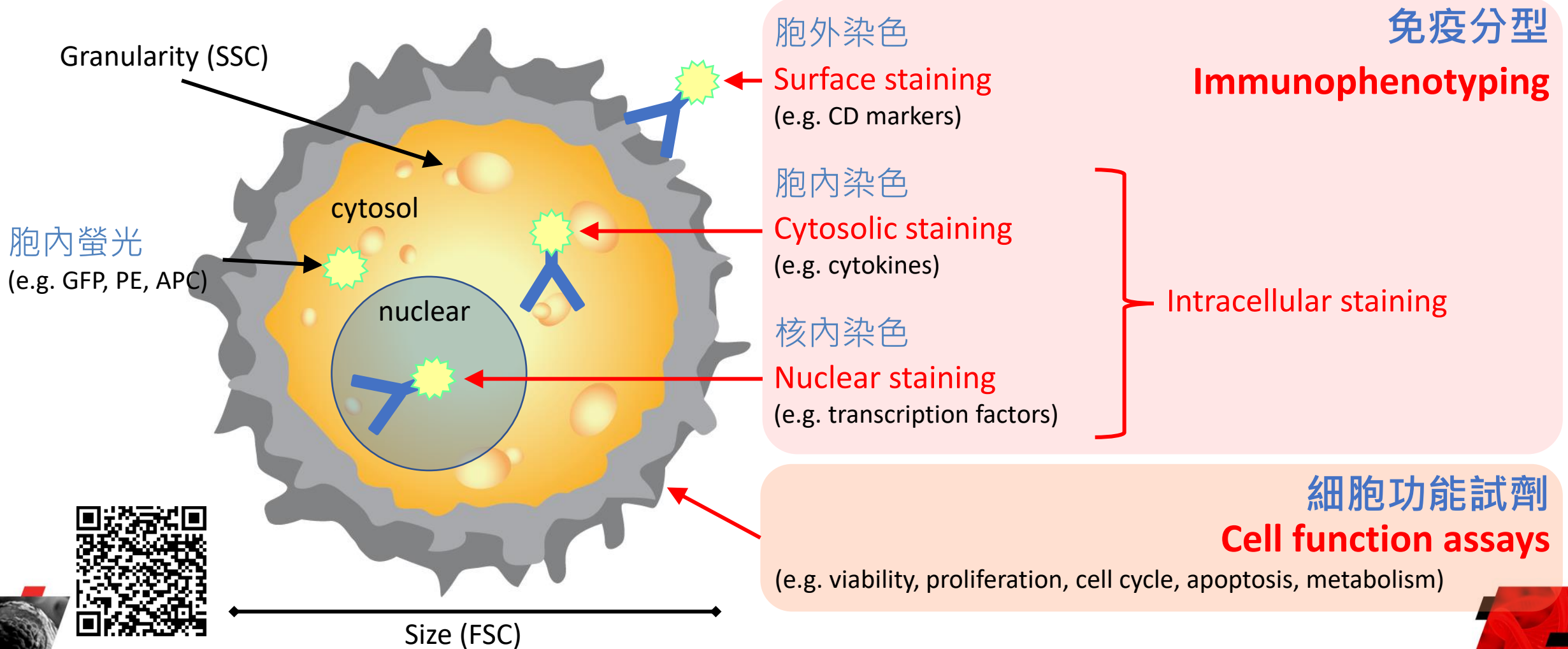
Antibody: Specificity 專一性



Fluorescence: Identity 辨識度



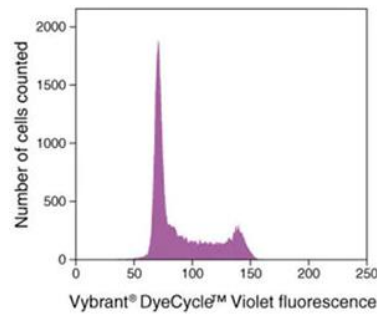
Cell Characteristics by Flow Cytometry



Applications of Flow Cytometry

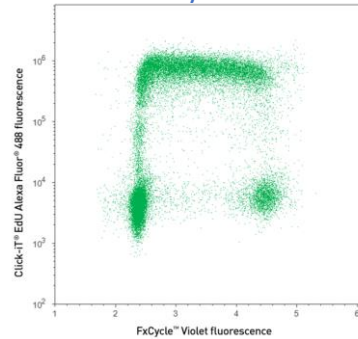
細胞週期

Cell cycle



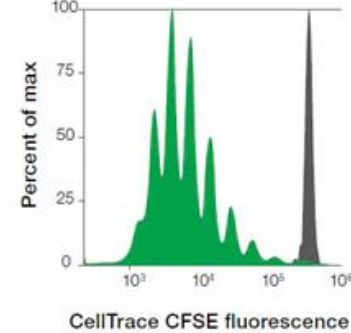
DNA合成

DNA synthesis



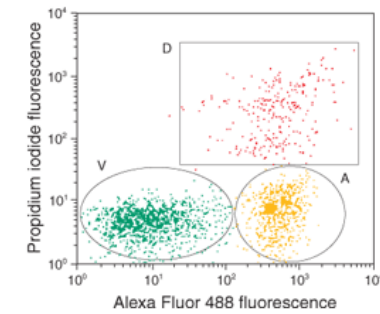
細胞増長

Proliferation



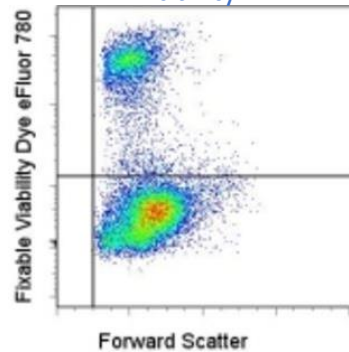
細胞凋亡

Apoptosis



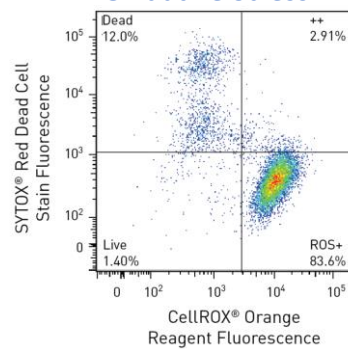
細胞存活

Viability



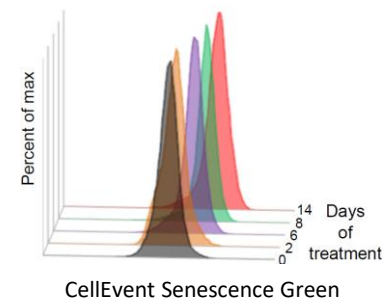
氧化壓力

Oxidative Stress



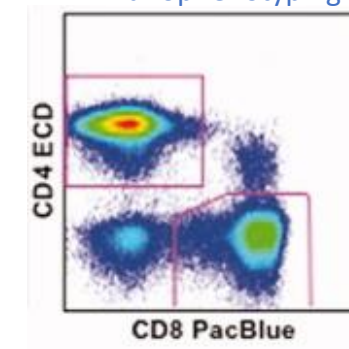
細胞老化

Senescence



免疫分型

Immunophenotyping



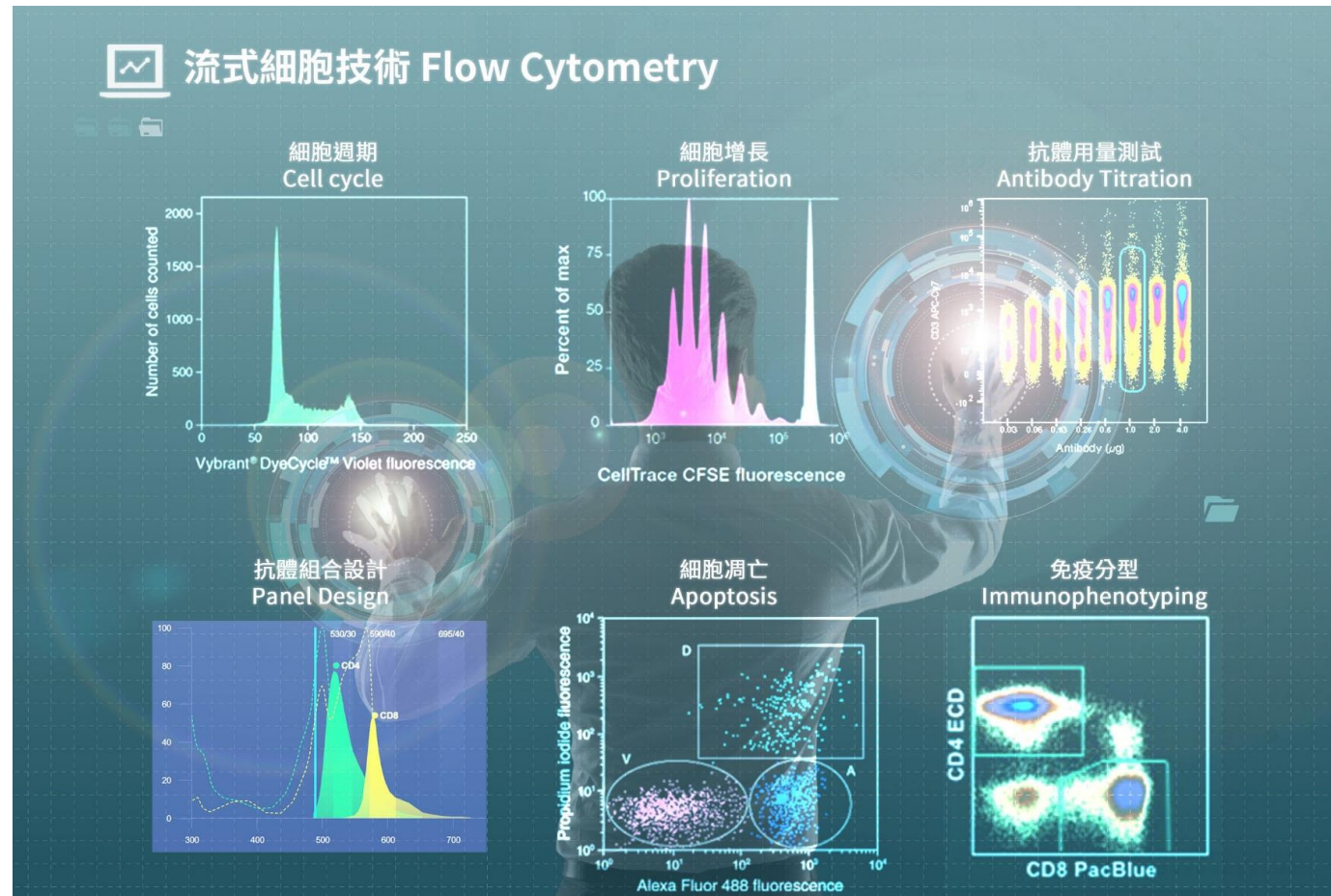
Flow Cytometry 產品諮詢與技術服務

Thermo Fisher Products
Invitrogen
eBioscience
Molecular Probes
Custom Service

歡迎與當區業務

巧盈

聯絡取得相關資訊~



Attune Flow Cytometer

Attune Family



Attune NxT



Attune CytPix

Attune NxT Acoustic Focusing Flow Cytometer

Small in size, big in performance

Flat-Top Laser

平頂雷射均勻激發細胞，
提供穩定且高解析度的分析結果



Syringe Pump

針筒幫浦定量上樣體積，
可絕對計數細胞濃度

Acoustic Focusing

專利聲波輔助流體動力聚焦技術，
大幅提升最高分析流速，同時依舊
維持高解析度

Autosampler

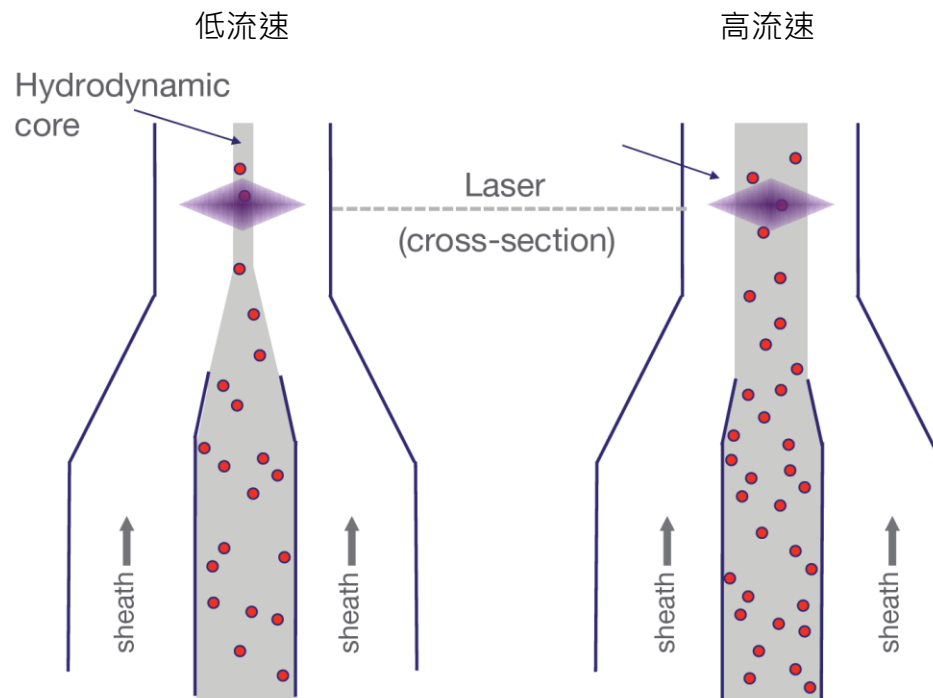
可選配自動上樣機 **CytKick (MAX)**，
盤式上樣更省時方便



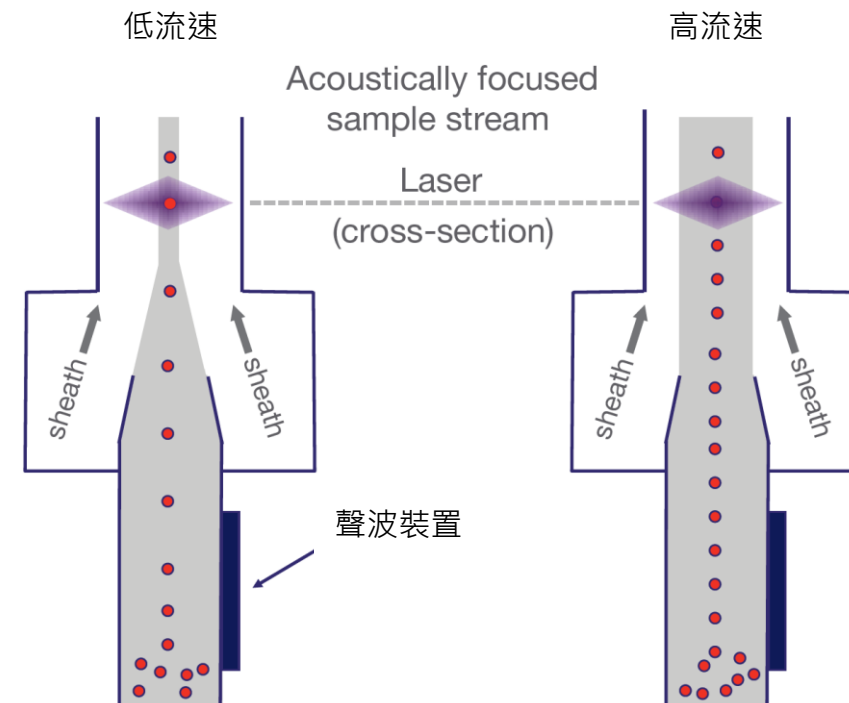
Acoustic Focusing

High sample input flow rates allow for more sample flexibility

傳統流體動力聚焦

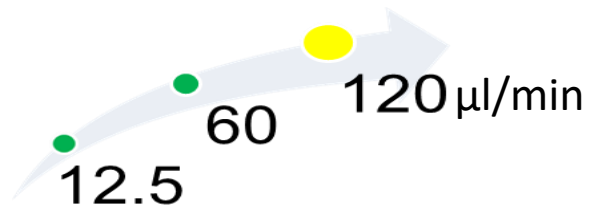


聲波輔助聚焦

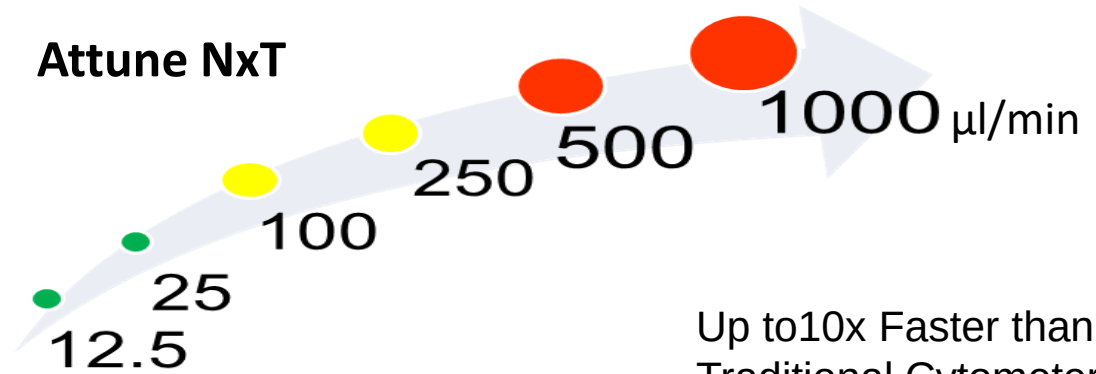


Comparable Results at All Flow Rates

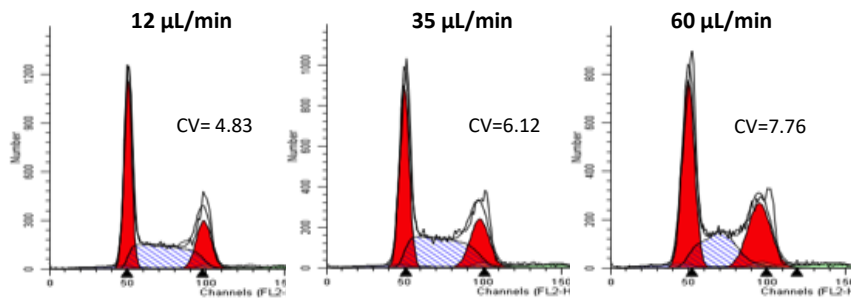
Traditional Cytometers



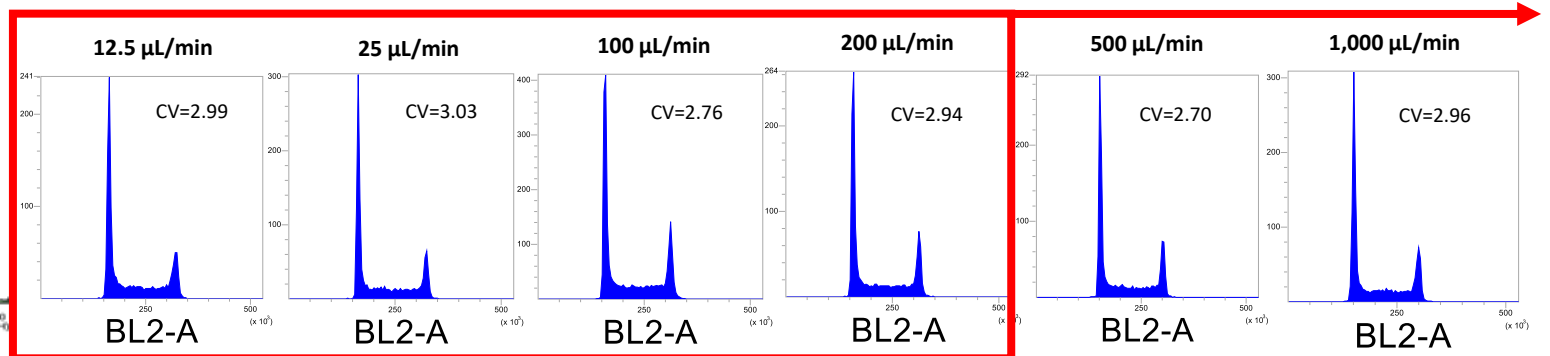
Attune NxT



Up to 10x Faster than Traditional Cytometers



Hydrodynamic Focusing Only



Acoustically Enhanced Hydrodynamic Focusing

Configurations

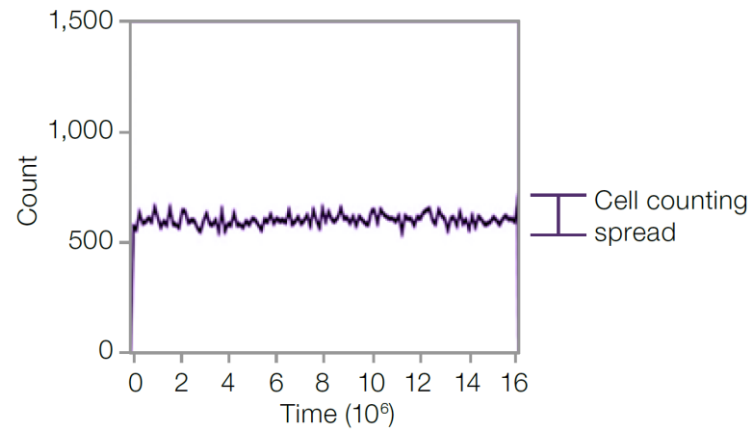


	4			7				11							14
Attune NxT Cat.#	A24864	A28995	A24861	A24863	A24862	A29002	A28997	A24860	A28999	A28993	A24859	A29003	A29004	A29001	A24858
Detectors	4	7	7	7	8	9	10	11	11	10	11	12	14	14	14
Channel	Emission filter (nm)														
BL1	530/30	525/50	530/30	530/30	530/30	530/30	525/50	530/30	525/50	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							
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	
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
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
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		

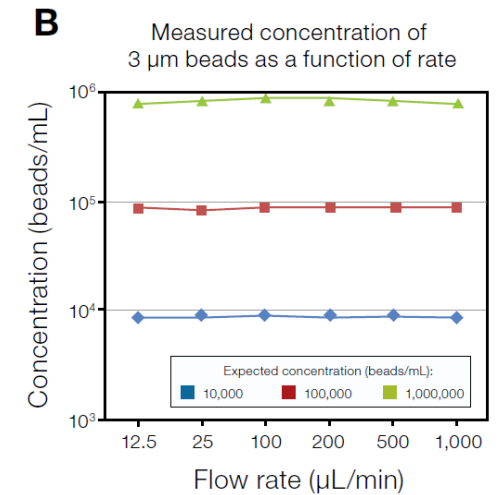
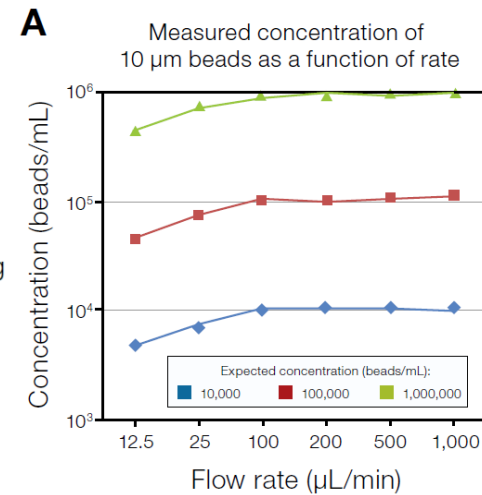
Syringe Pump



Time vs. count plots showing a moving average of the number of events



- Smooth delivery of samples
- Consistent concentration results
- Resist to clog



Attune CytPix

2023 Edison Awards, MedTech

*"With a high-speed brightfield camera that records images of individual events as they pass through the flow cell, **Attune CytPix** helps to ensure the events are single cells. This is useful in almost any flow cytometry experiment to help researchers understand the morphology of each cell population and crucial in cell and gene therapy research."*

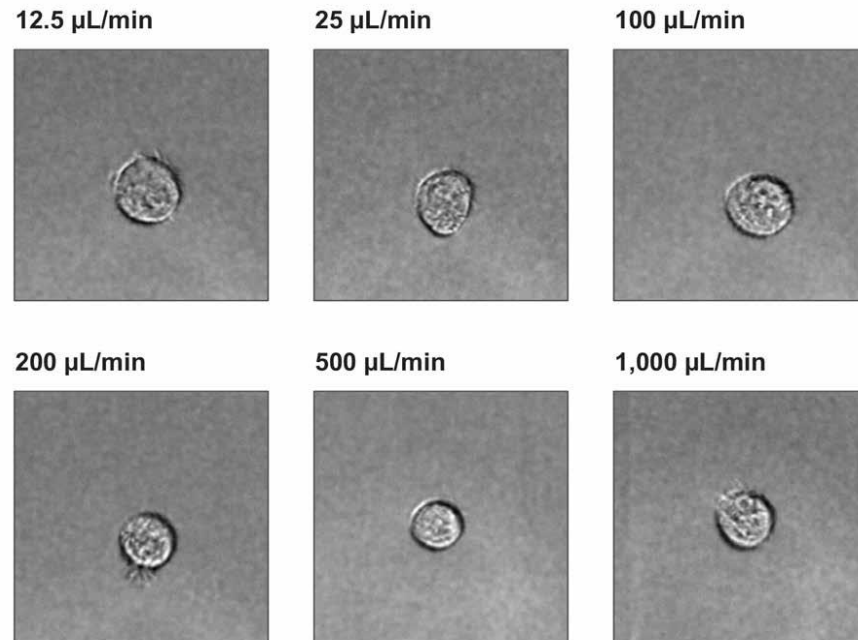


Plus

Bright-field imaging; capture up to 6,000 images per second

- Correlate event to image
- **0.3 μm per pixel, 0.8 μm particle detection (20X Objective)**
- **25 parameters** from image analysis

Bright-Field Image and Image-Derived Parameters



Consistent image quality even at high flow rates

Image-derived parameters

Intensity features

Maximum intensity	Intensity skewness
Minimum intensity	Intensity kurtosis
Total intensity	Intensity entropy
Average intensity	Average normalized intensity
Intensity standard deviation (SD)	Normalized intensity SD
Intensity %CV	Normalized intensity %CV

Shape features

Area (μm^2)	Major axis (μm)
Perimeter area (μm)	Minor axis (μm)
Circularity (%)	Minor to major axis ratio (%)
Pseudo diameter (μm)	Eccentricity (%)

Object feature

Particle count

Pixel feature

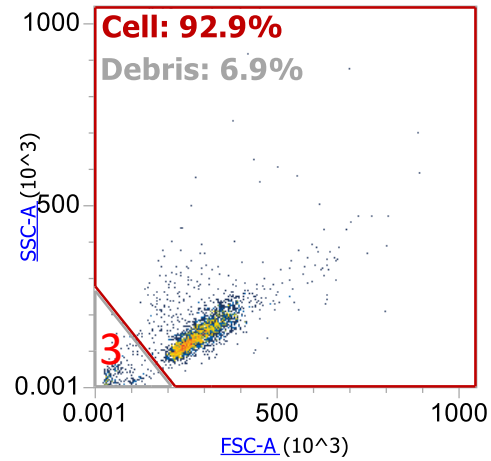
Pixel count

System features

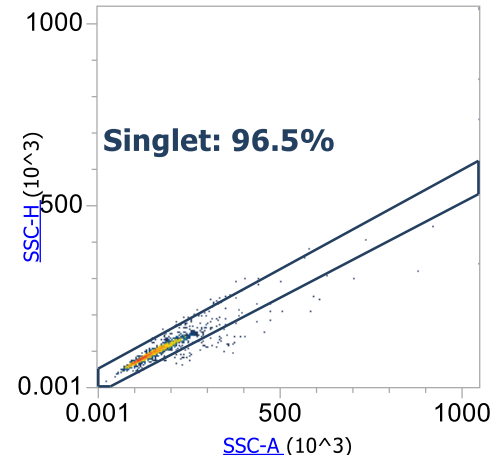
On border	Processed
Confidence score	Processable

Human AML Cell Line

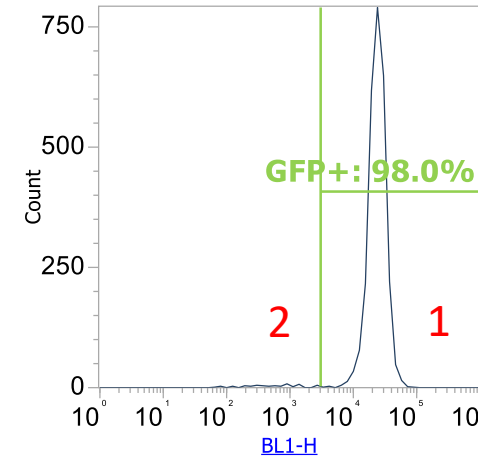
All Events - THP1_500



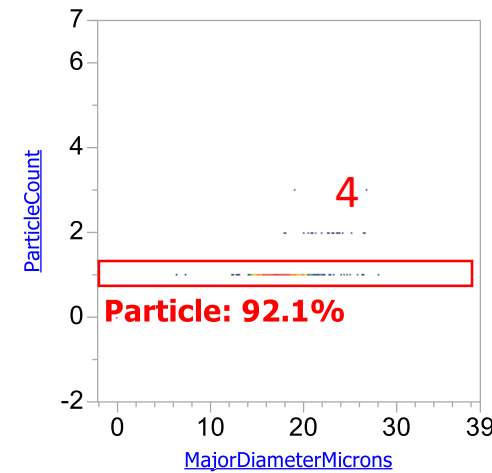
Cell - THP1_500



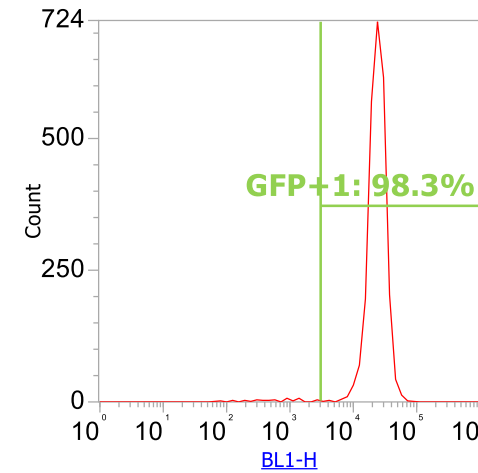
Singlet - THP1_500



Singlet - THP1_500



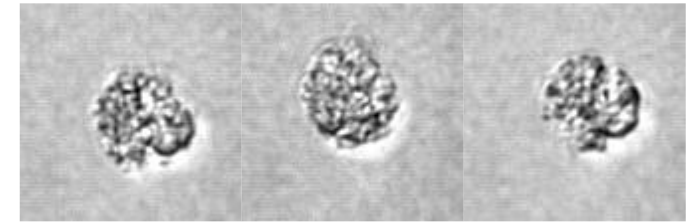
Particle - THP1_500



1. THP-1, GFP+



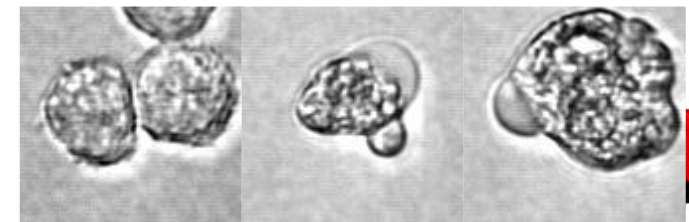
2. THP-1, GFP-

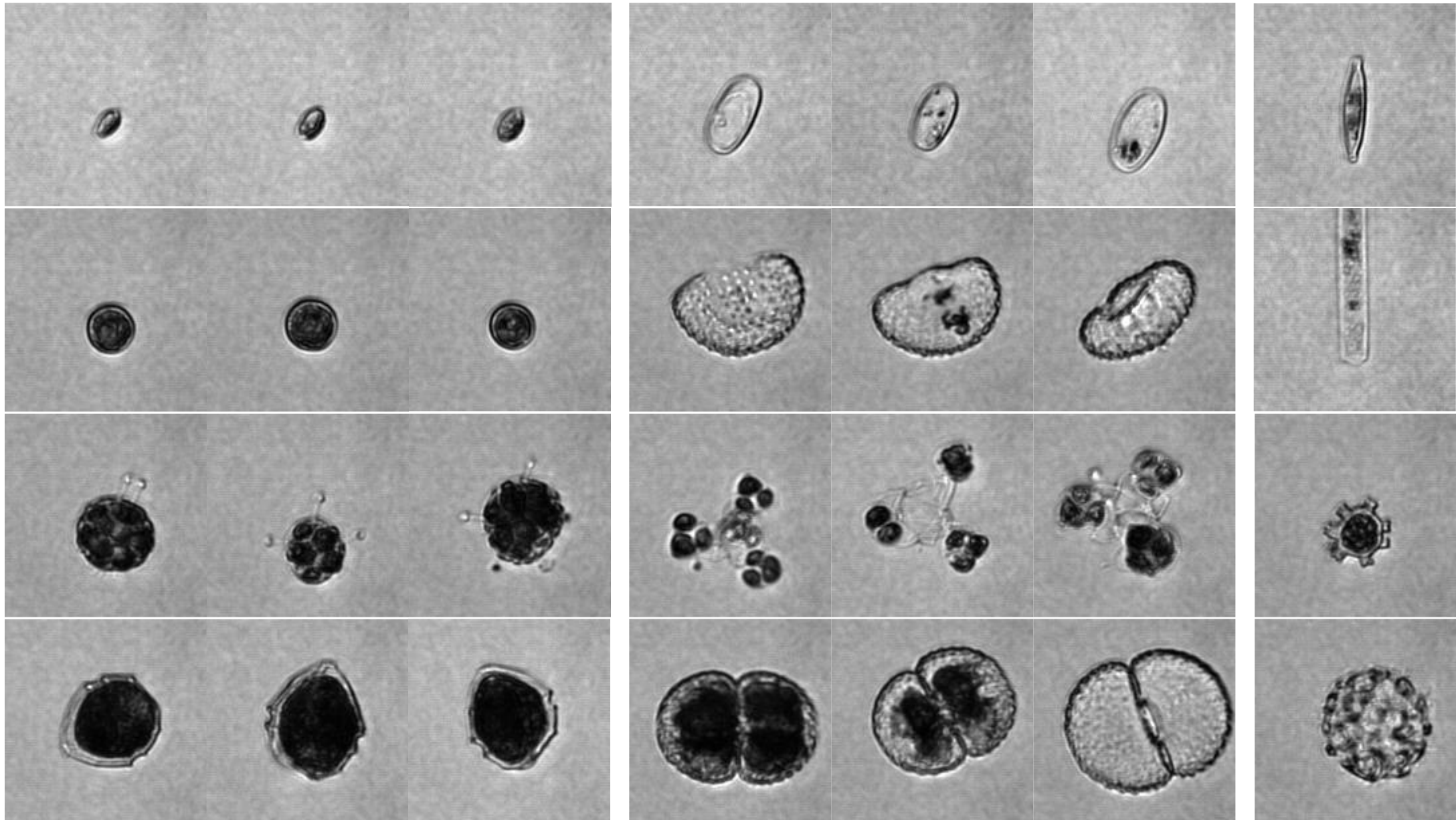


3. Debris



4. Particle Count > 1

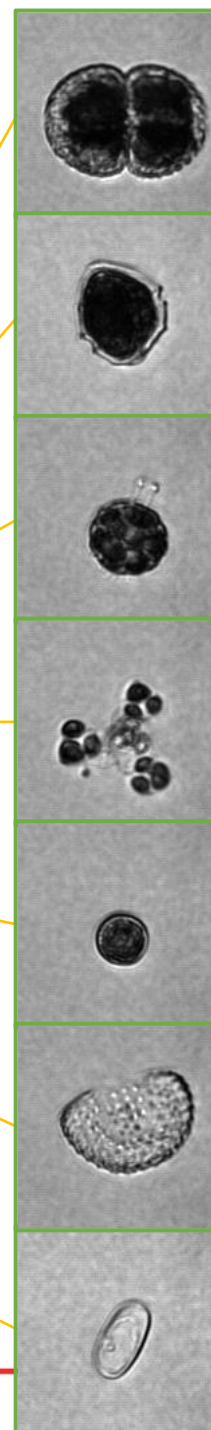
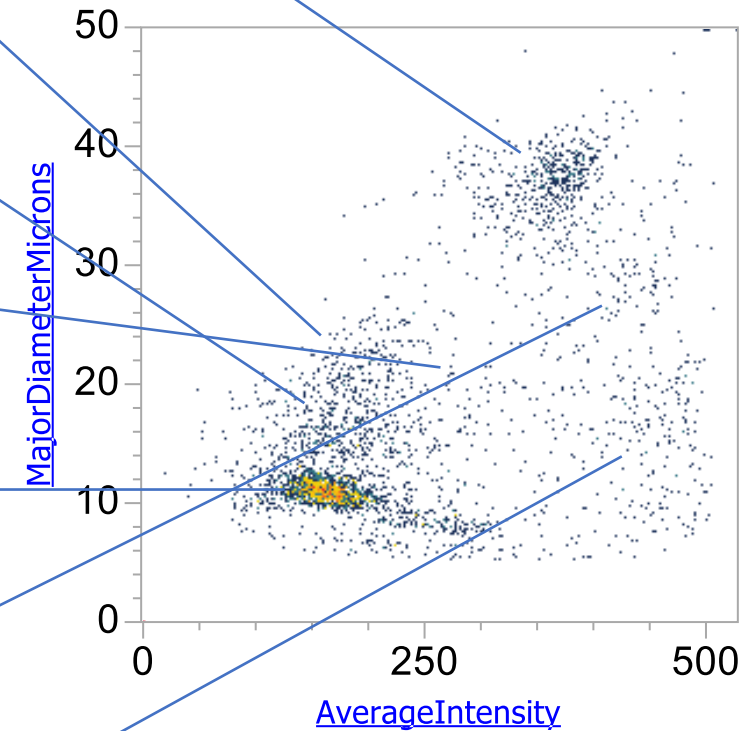
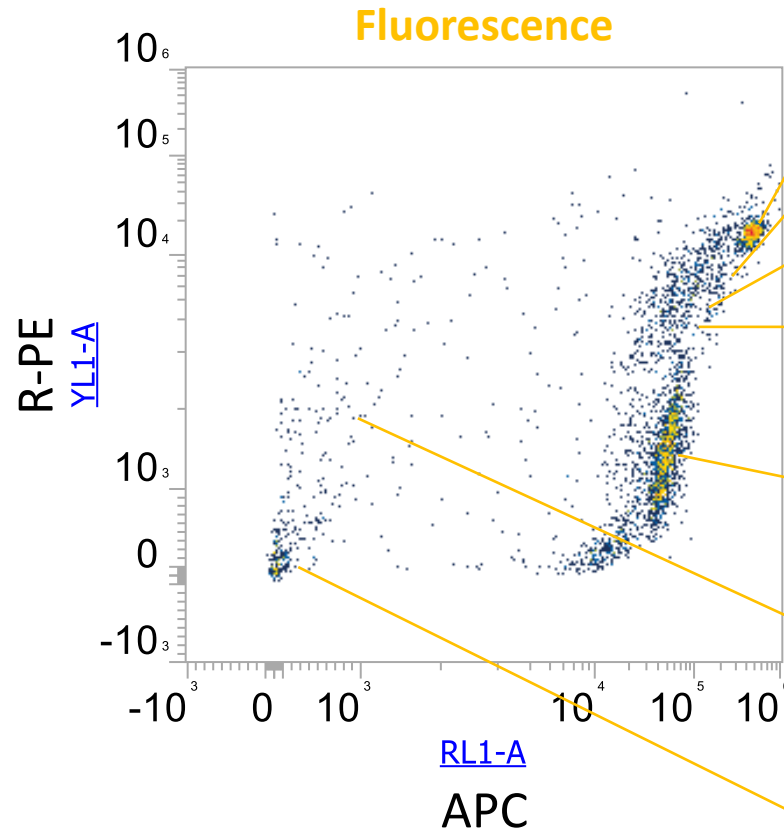




Pond Water

Bright-Field Image

Image-Derived Parameters



Workflow of Flow Cytometry with Attune NxT

Flow Cytometry Workflows

- Panel Design – Flow Cytometry Panel Builder
- Antibody Titration
- Controls

實驗規劃

- Sample preparation
- Cell staining
- Flow Cytometer Start Up
- Select **Channels**
- Setup **Workspace** (**Cell > Singlet**, gating strategies, controls for threshold setup)
- Setup **Collection Panel**
- Setup **PMT** (signal min. from unstained control, signal max. from positive sample)
- Setup **Compensation** (single stained control for all fluorophore)
- Analyze Samples
- Data Export (FCS 3.0 or higher)
- Flow Cytometer Shutdown

樣本製備與染色

上樣分析流程

- Data Analysis

數據分析

Principles of Panel Design

1. Know your flow cytometer (***channels***).
2. Identify ***markers*** of interest (literatures for gating strategy; Immune cell guide).
3. Know the ***spectrum of fluorophores*** and minimize spillover.
4. Brighter fluorophores for lower-expressed markers, and vice versa.
5. Use spectrally similar fluorophores for different cell subpopulations.

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, eYFP, * β-carotene
	574/26	BL2	Alexa Fluor 546, PE(phycoerythrin), 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	
Red-637 nm	670/14	RL1	APC, Alexa Fluor 647	phycocyanin
	720/30	RL2	Alexa Fluor 680, Alexa Fluor 700, APC-Alexa Fluor 700	
	780/60	RL3	APC-Alexa Fluor 750, APC-Cy7	

Flow Cytometry Panel Builder

Step 1: Cytometer 機器規格

STEP 1

Your cytometer
Attune NxT

Violet 405nm Blue 488nm Yellow 561nm Red 637nm

530/40	530/40	595/16	670/14
625/50	695/40	620/15	720/30
610/20		760/60	760/60
660/20			
710/50			
780/60			

[Edit cytometer settings](#) [Load an existing panel](#) [Clear current panel](#)

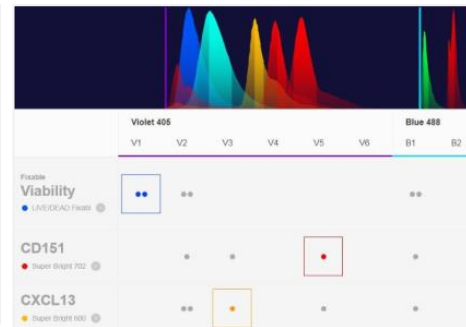
Step 2: Antigens 設定抗原

Target species
Human

Antigens

Antigen name CD4	Target species Human	Open advanced options
Antigen name CD8	Target species Human	Open advanced options
Antigen name CD3	Target species Human	Open advanced options
Antigen name CD103 (Integrin alpha E)	Target species Human	Open advanced options

Step 3: Fluorochromes 配置螢光



Step 4: Products 選擇產品

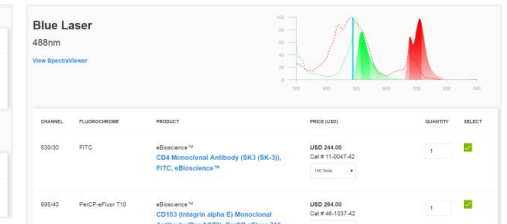
CD4, FITC

PRODUCT ID	CLONE	TARGET SPECIES	PRICE (USD)	
eBioscience™ CD4 Monoclonal Antibody (SK3) (SK-3), FITC, eBioscience™	SK3 (SK-3)	Human	USD 244.00 Cat # 11-0047-42	Selected

CD8, PE

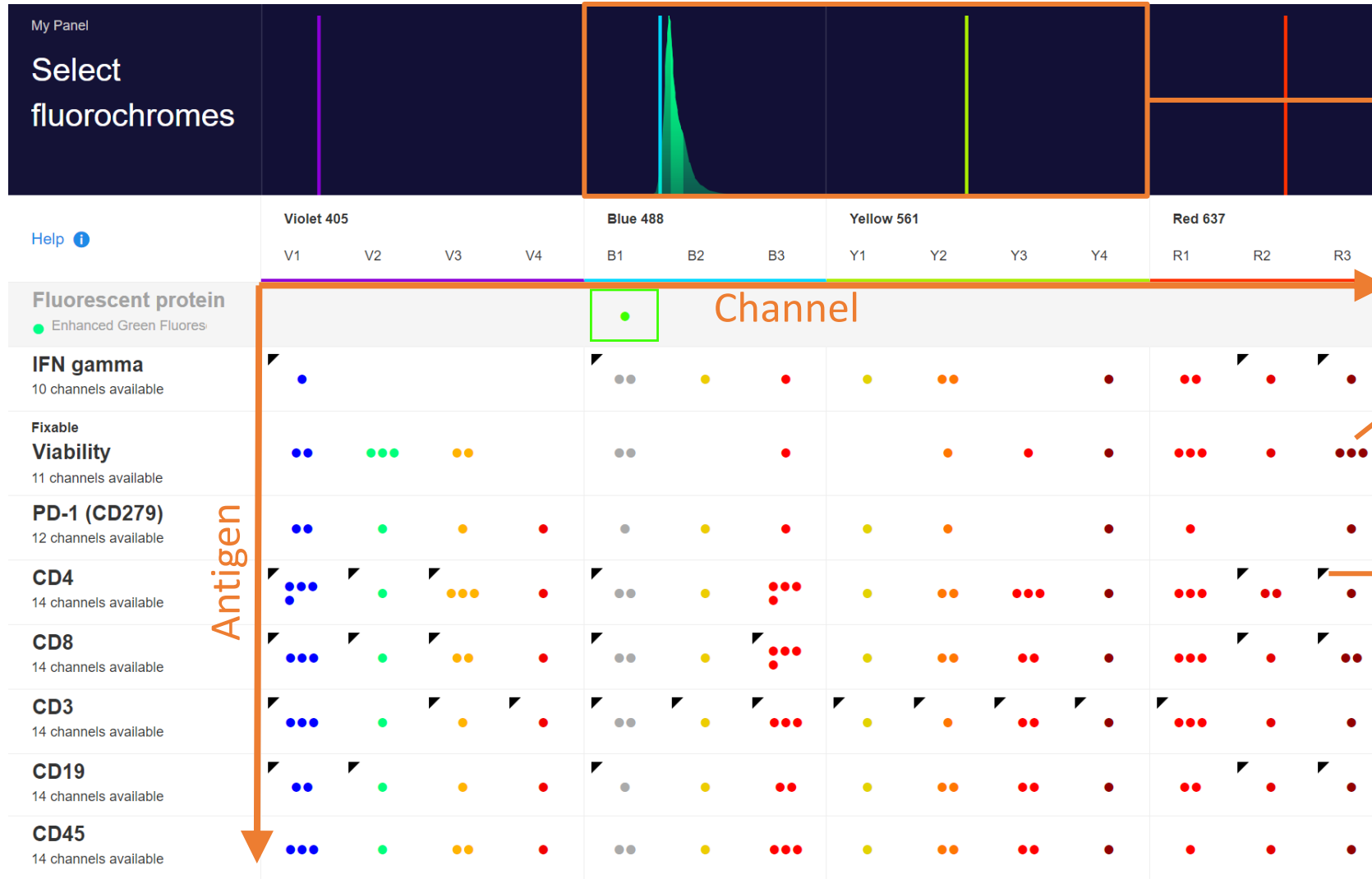
PRODUCT ID	CLONE	TARGET SPECIES	PRICE (USD)	
eBioscience™ CD8 Monoclonal Antibody (CB8), PE	CB8	Human	USD 271.00 Cat # 11-0047-42	Selected

Step 5: Summary 輸出規劃



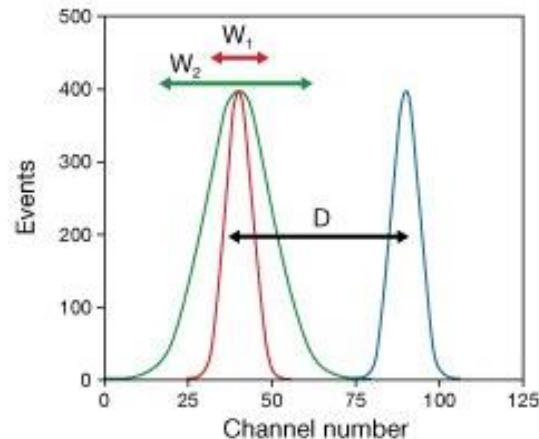
<https://www.thermofisher.com/order/panel-builder/#/>

Flow Cytometry Panel Builder



Antibody Titration

- Use antibodies at the **right concentration**
 - Antibody **batch dependent**
 - **Reduce background** and increase signal to noise ratio
 - **Reduce cost** of antibodies
1. Setup target cell type, protocol, and cytometer configurations
 2. Label cells with serial dilution of antibodies
 3. Examine **Stain Index** to find optimized antibody concentration



$$\text{Stain Index} = D/W$$

Where:

D is the difference between positive and negative peak medians.

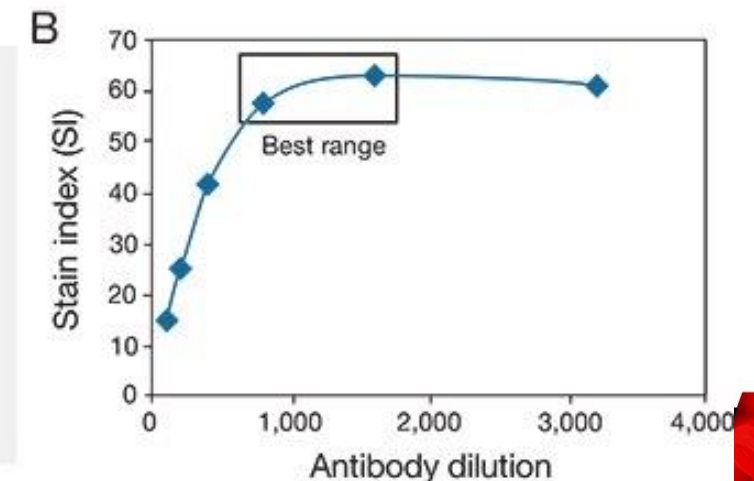
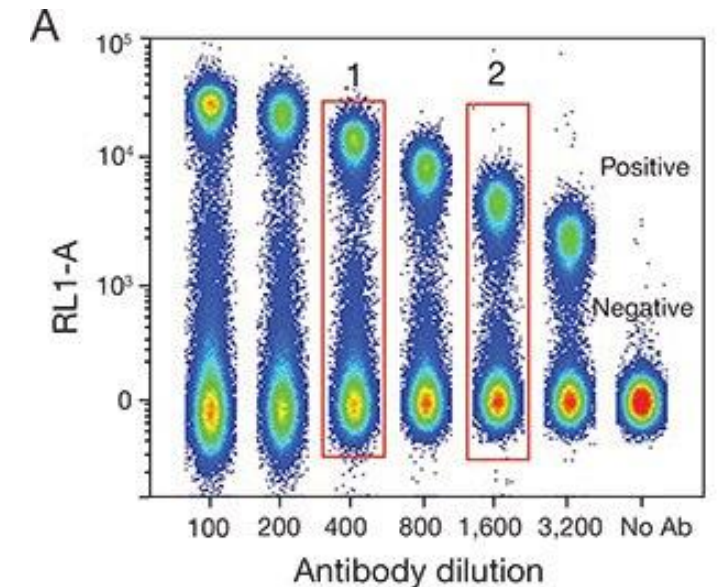
W is the spread of the negative peak and is equal to $2 \times \text{rSD}$.

rSD is the robust standard deviation.

$$\text{Signal-to-noise ratio} = \text{MFI (positive cells)} / \text{MFI (negative cells)}$$

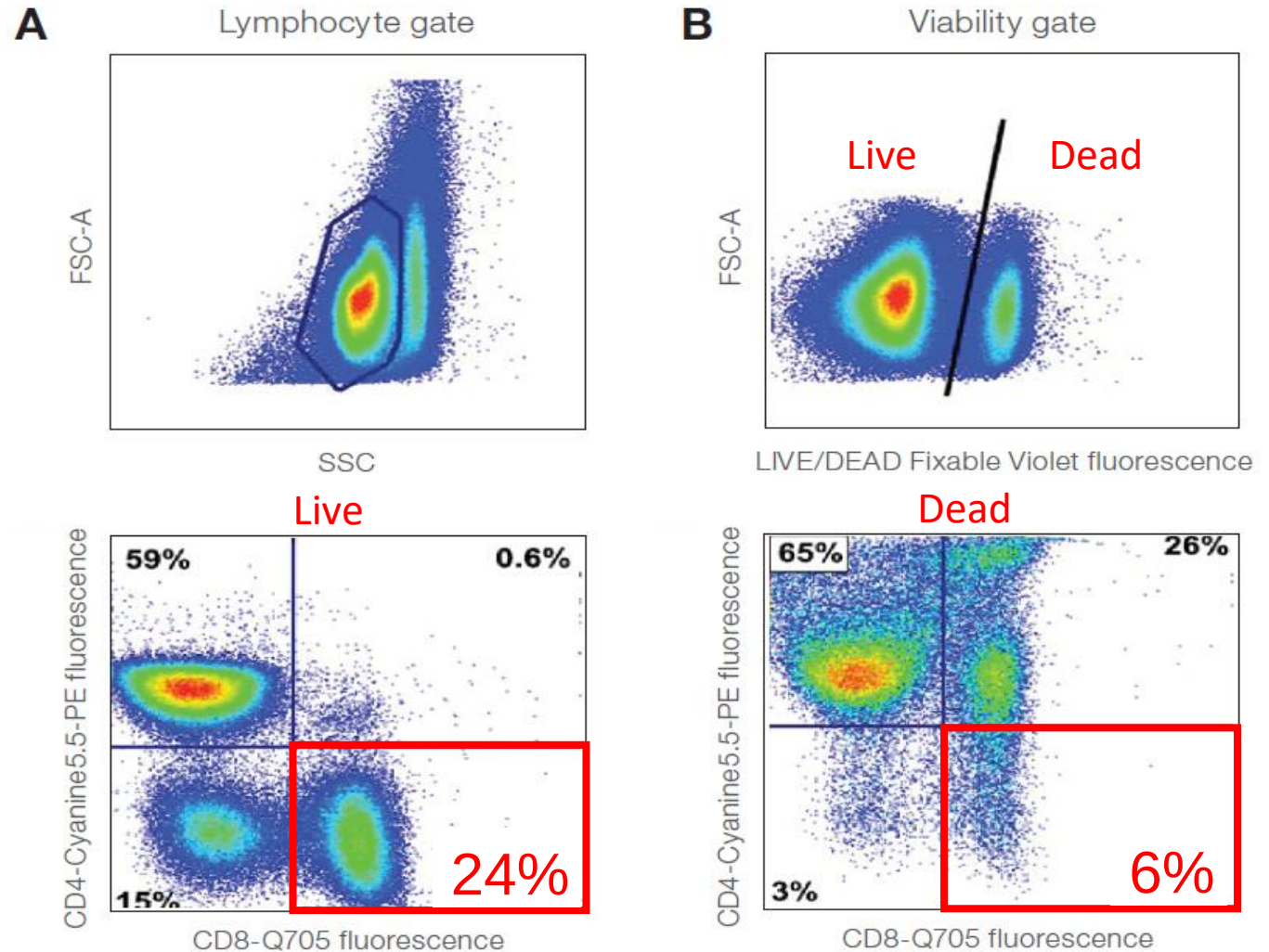
Where:

MFI is the median fluorescence intensity.



Put Viability Dye into Consideration - Dead Cell Exclusion

Dead cells adds significant staining *artifacts* to analysis.



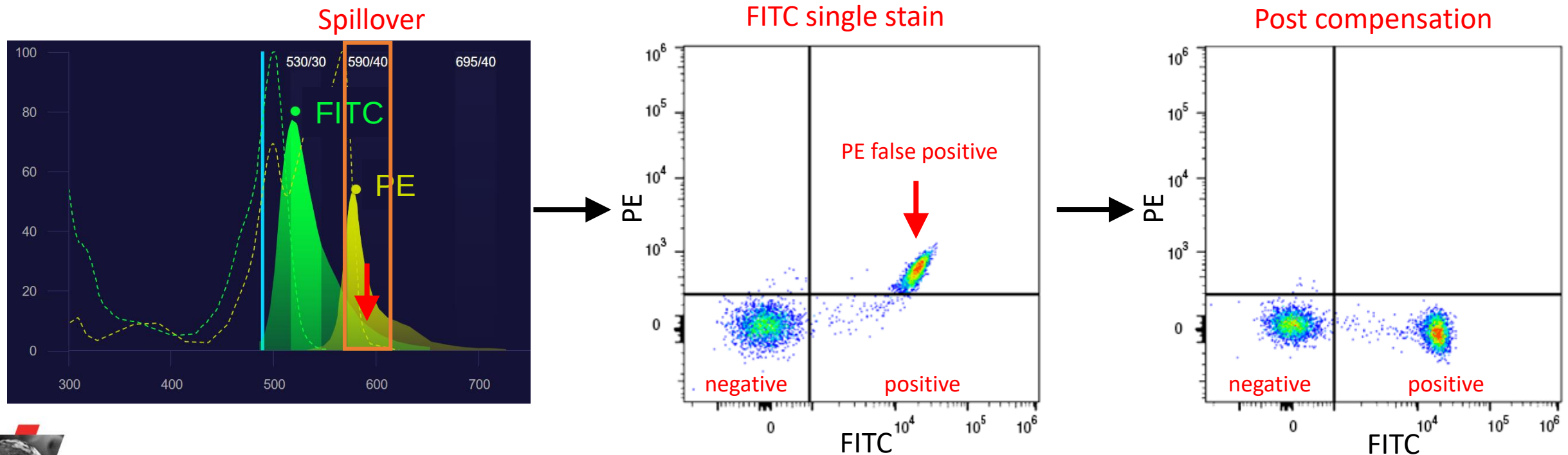
Perfetto et al. (2006) J Immunol Methods 313:199

Flow Cytometry Controls

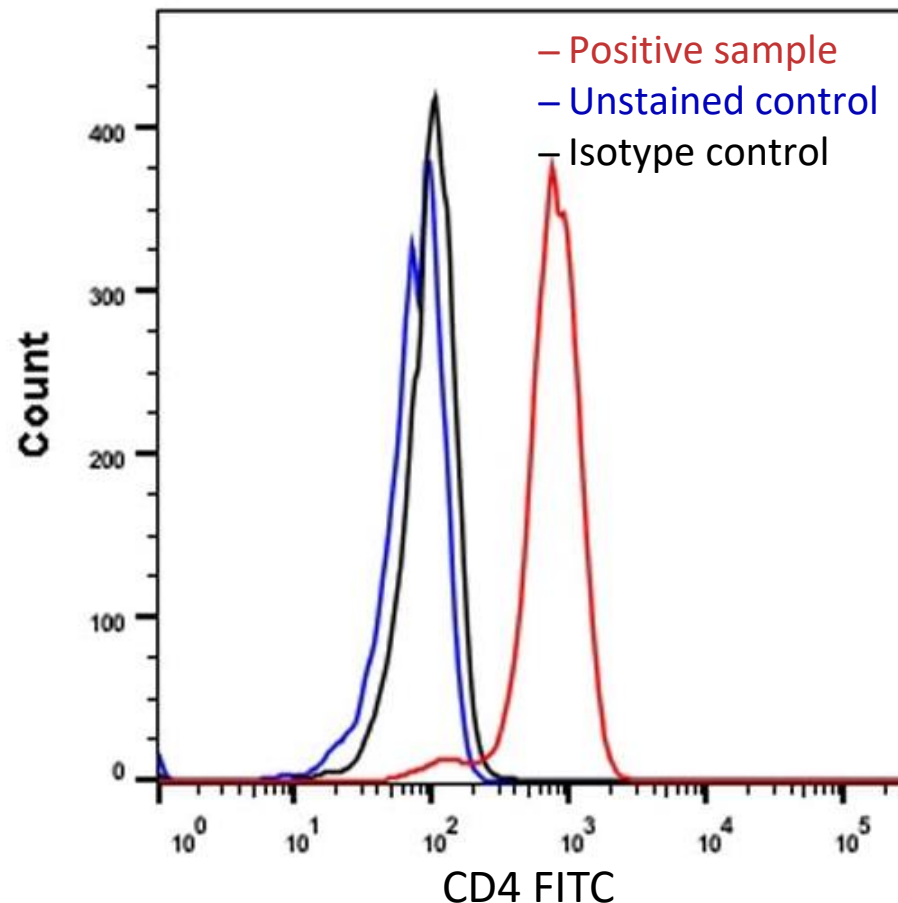
- **Single stained control** for compensation
- **Negative control:** 判斷訊號背景值
 1. Unstained control
 2. Isotype control
 3. Fluorescence minus one (FMO) control for multicolor panel
 4. FMO + isotype control
- **Positive control:** 確認實驗流程正確，可以得到預期訊號

Flow Cytometry Controls – Single Stained for Compensation

- **Compensation** is the mathematical method used to correct the emission overlap from one **fluorophore** into the emission channel of another **fluorophore**.



Flow Cytometry Controls – Unstained and Isotype Control

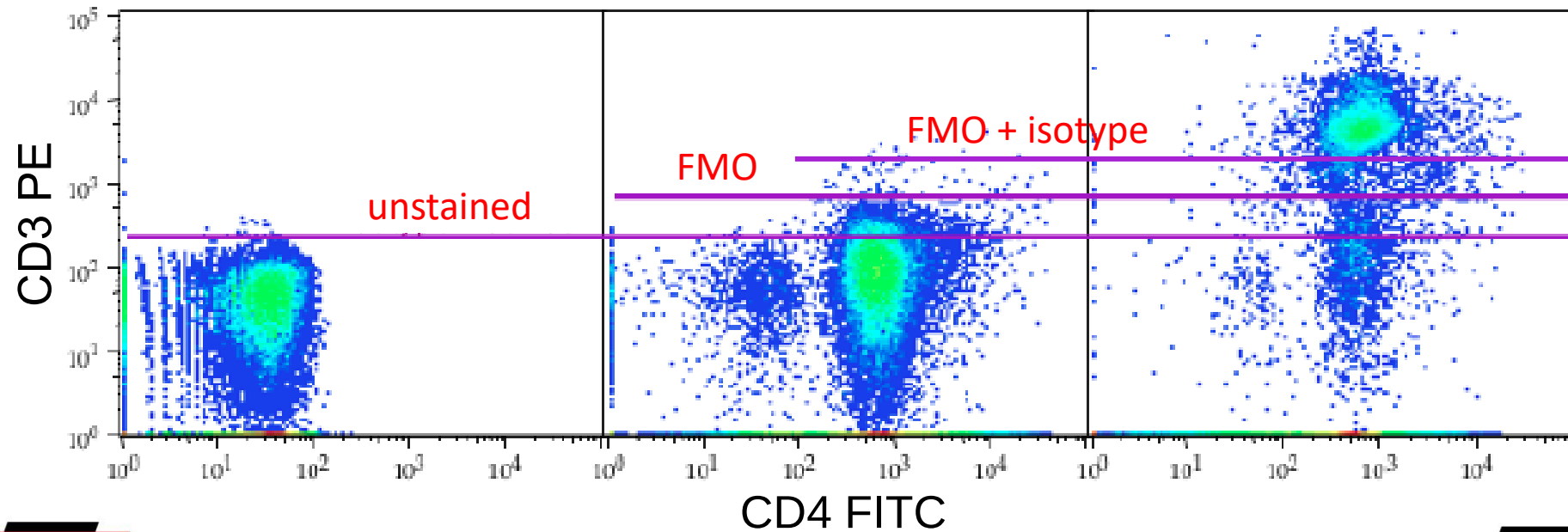


- CD4 Monoclonal Antibody (RM4-5), FITC
Expression System: Rat IgG2a kappa
- Recommended Isotype Control:
Rat IgG2a kappa Isotype Control (eBR2a), FITC

Isotype control for non-specific binding background

Flow Cytometry Controls – FMO control

	Unstained Control	FMO control	Fully Stained
FITC	-	CD4	CD4
PE	-	- + isotype Ab	CD3
PerCP	-	CD8	CD8
APC	-	CD45	CD45



Flow Cytometry Workflows

- Panel Design – Flow Cytometry Panel Builder
- Antibody Titration
- Controls

實驗規劃

- Sample preparation
- Cell staining

樣本製備與染色

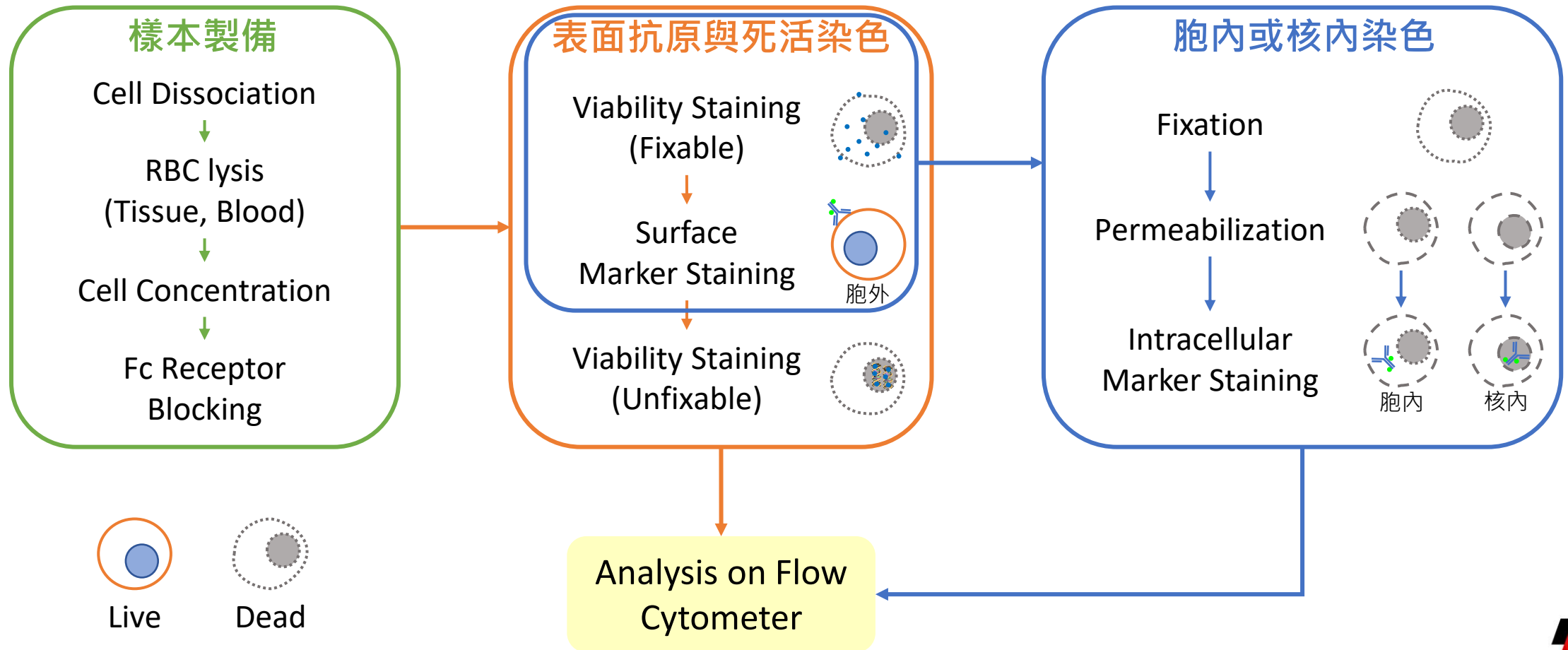
- Flow Cytometer Start Up
- Select **Channels**
- Setup **Workspace** (**Cell > Singlet**, gating strategies, controls for threshold setup)
- Setup **Collection Panel**
- Setup **PMT** (signal min. from unstained control, signal max. from positive sample)
- Setup **Compensation** (single stained control for all fluorophore)
- Analyze Samples
- Data Export (FCS 3.0 or higher)
- Flow Cytometer Shutdown

上樣分析流程

- Data Analysis

數據分析

Immunophenotyping with Flow Cytometry



Cell Preparation for Flow Cytometry Protocols

- Cell preparation for flow cytometry protocols
 - Protocol A: Tissue Culture Cells
 - Protocol B: Lymphoid Tissue
 - Protocol C: Non-lymphoid Tissue
 - Protocol D: Isolation of PBMC from Whole blood
- [Worthington Tissue Dissociation Guide](#)
The Worthington Tissue Dissociation Guide provides a useful summary and guide of the various methods that can be used for tissue dissociation.

Cell Staining Protocols

- Viability Dye Staining
 - Protocol A: Staining Dead Cells with Propidium Iodide or 7-amino-actinomycin D (7-AAD)
 - Protocol B: Staining Live Cells with Calcein Dyes
 - Protocol C: Staining Dead Cells with Fixable Viability Dyes (FVD)
- Staining cell surface targets protocols
 - Protocol A: Cell Suspensions
 - Protocol B: Human Lysed Whole Blood
- Staining Intracellular Antigens protocols
 - Protocol A: Two-step protocol: intracellular (cytoplasmic) proteins
 - Protocol B: One-step protocol: intracellular (nuclear) proteins
 - Protocol C: Two-step protocol for Fixation/Methanol

Flow Cytometry Workflows

- Panel Design – Flow Cytometry Panel Builder
- Antibody Titration
- Controls

實驗規劃

- Sample preparation
- Cell staining

樣本製備與染色

- Flow Cytometer Start Up
- Select **Channels**
- Setup **Workspace** (**Cell > Singlet**, gating strategies, controls for threshold setup)
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- Setup **Compensation** (single stained control for all fluorophore)
- Analyze Samples
- Data Export (FCS 3.0 or higher)
- Flow Cytometer Shutdown

上樣分析流程

- Data Analysis

數據分析

Attune NxT上樣分析流程

1. 檢查機器外觀(Fluid bottles and connections, Syringe, SIP) , 緩衝液是否充足 , 廢液是否過多。
2. 開啟Attune NxT與電腦電源。
3. 啟動Attune NxT分析程式 , 登入使用者帳號 (operator: 執行Performance Test)。
4. 執行**Startup** (約5分鐘)。
5. 設定**Experiment**
6. 勾選**Channels** , 以及欲觀察的A , H , W數值。
7. 設定**Workspace**: *Cell (FSC-A, SSC-A) > Singlet (SSC-A, SSC-H) > Chart for markers*。
8. 設定**Collection Panel**: 吸取樣本體積 , 分析流速 , 數據蒐集目標
9. 調整**PMT voltage**: 以unstained樣本觀察各channel背景值 , 以正式染色樣本觀察各channel最大值 , 調整各channel PMT voltage。
- 10.調整**Compensation**: 使用大於一種螢光顏色時 , 上樣單染樣本以利軟體進行自動Compensation。
- 11.依序上樣: 其他controls以及正式染色樣本。
- 12.輸出實驗結果: atx原始數據檔案 , FCS3.1檔案 , excel檔案 , 與PDF報告。
- 13.執行**Shutdown** (約40分鐘)。
- 14.關閉Attune NxT程式 , 關閉電腦與Attune NxT電源。
- 15.清空廢液桶。

Main Menu



Performance Test



Cat.#4449754

Attune™ Performance Tracking Beads



Performance Test Results

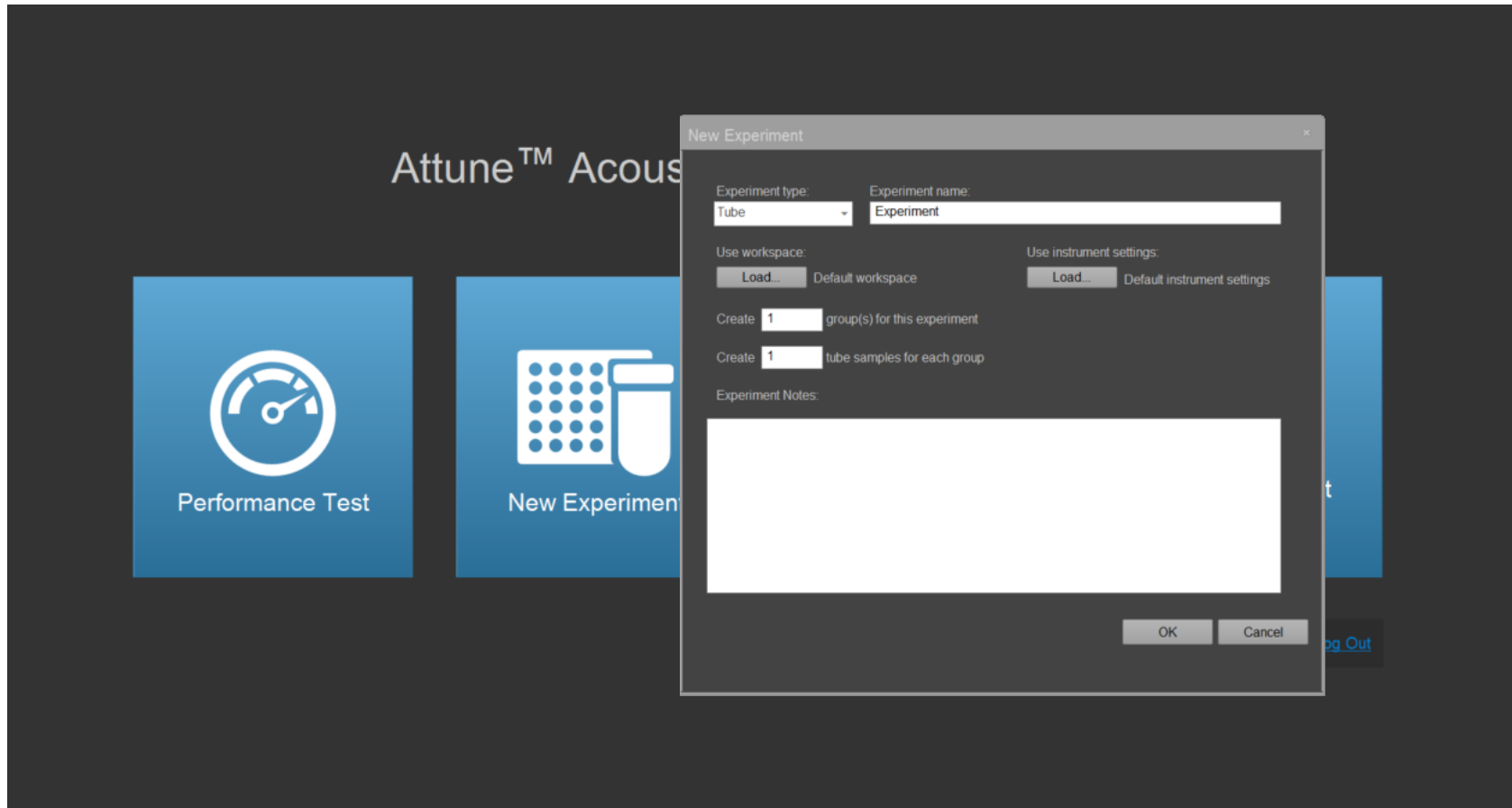
✓ Performance test successful

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
Blue	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	✓
Green	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	✓
Red	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	✓
Purple	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	✓
Yellow	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	✓

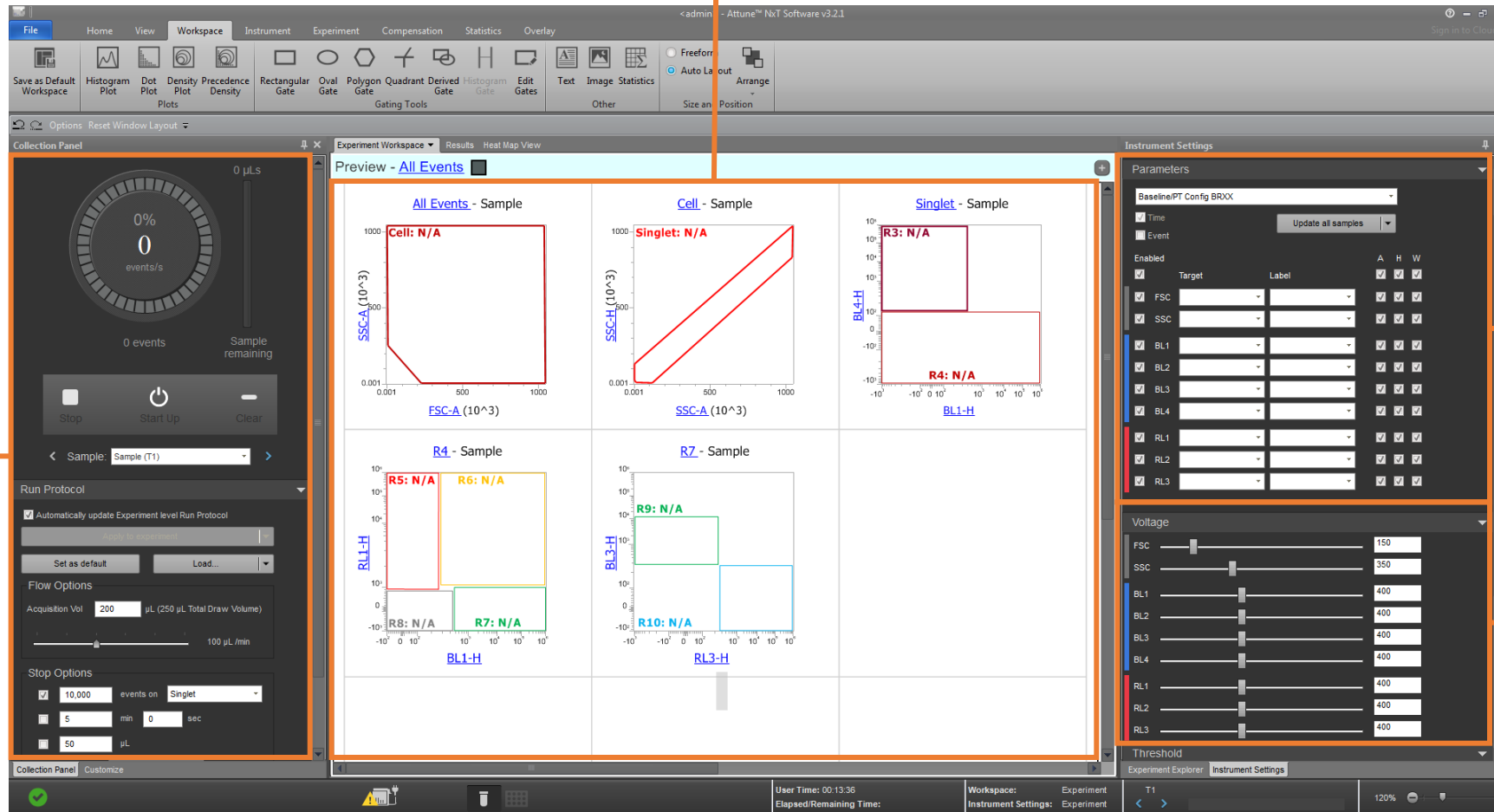
Main Menu – New Experiment



設定儀器參數

2. Workspace

3. Collection Panel



1. Channel

4. PMT V.

設定儀器參數 – 1. Channels

勾選預計觀察的Channels

Parameters

Baseline/PT Config BRXX

☒ Time ☐ Event Update all samples

Enabled	Target	Label	A	H	W
☒			☒	☒	☒
☒ FSC			☒	☒	☒
☒ SSC			☒	☒	☒
☒ BL1	CD4	FITC	☒	☒	☒
☒ BL2	CD8	R-PE	☒	☒	☒
☒ BL3			☒	☒	☒
☒ BL4			☒	☒	☒
☒ RL1			☒	☒	☒
☒ RL2			☒	☒	☒
☒ RL3			☒	☒	☒

勾選各Channel預計收集的數值

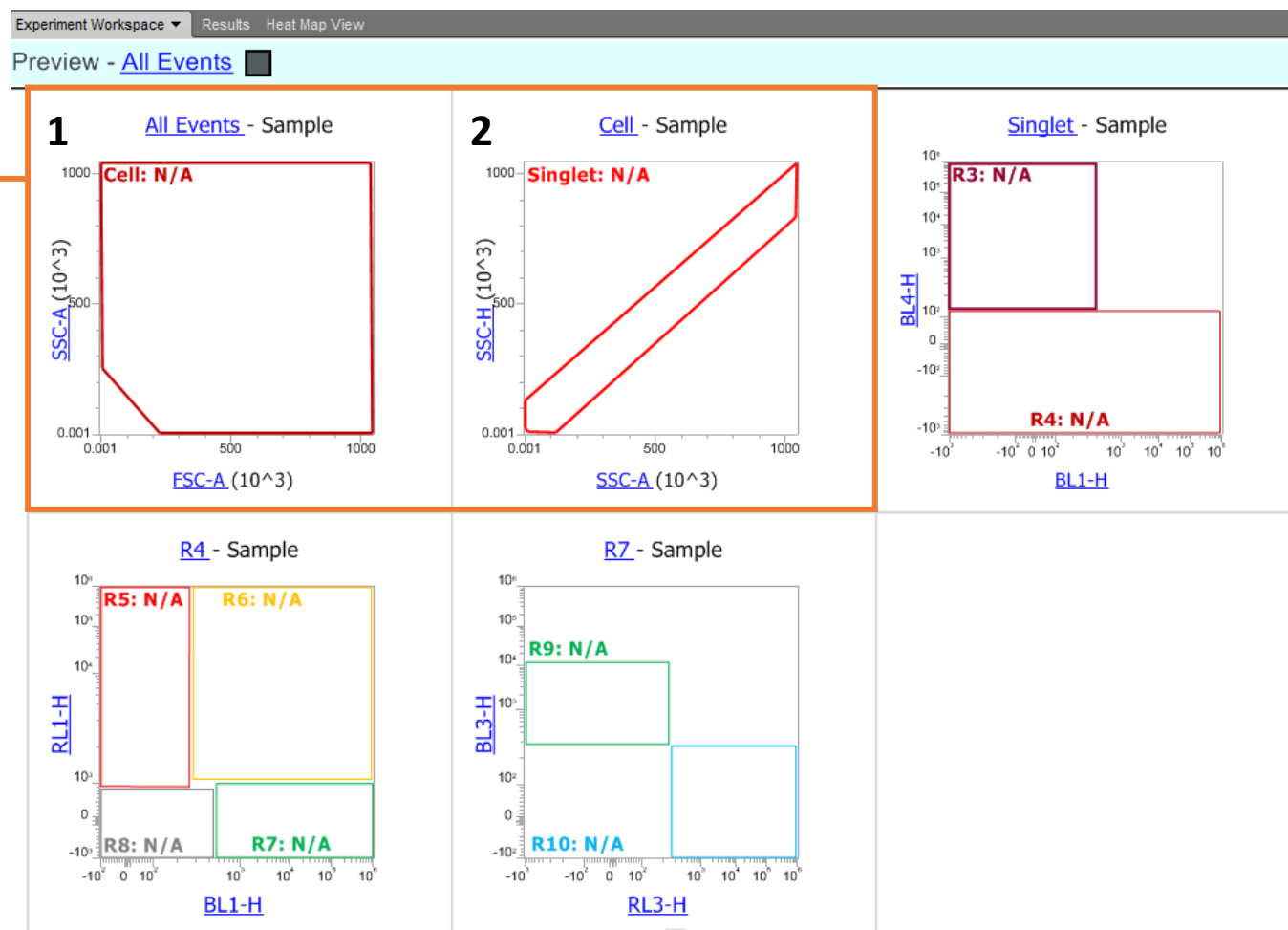
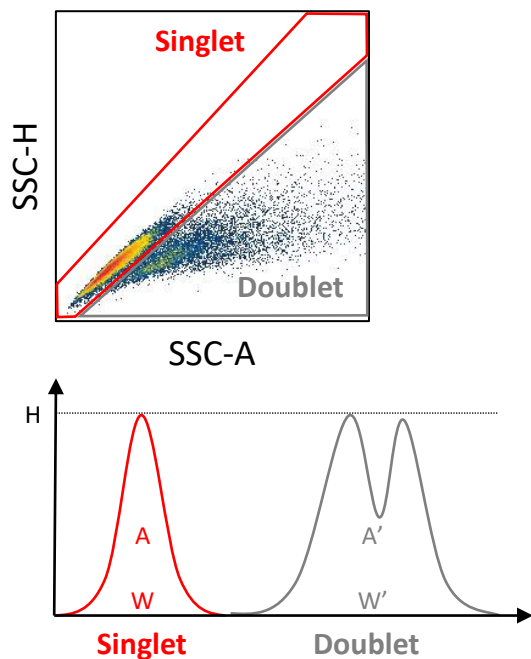
輸入Marker名稱

選擇/輸入螢光名稱

設定儀器參數 – 2. Workspace

最基本的兩個圖：

1. 圈選細胞位置: FSC-A vs SSC-A
2. 圈選單顆細胞: SSC-A vs SSC-H



設定儀器參數 – 3. Collection Panel

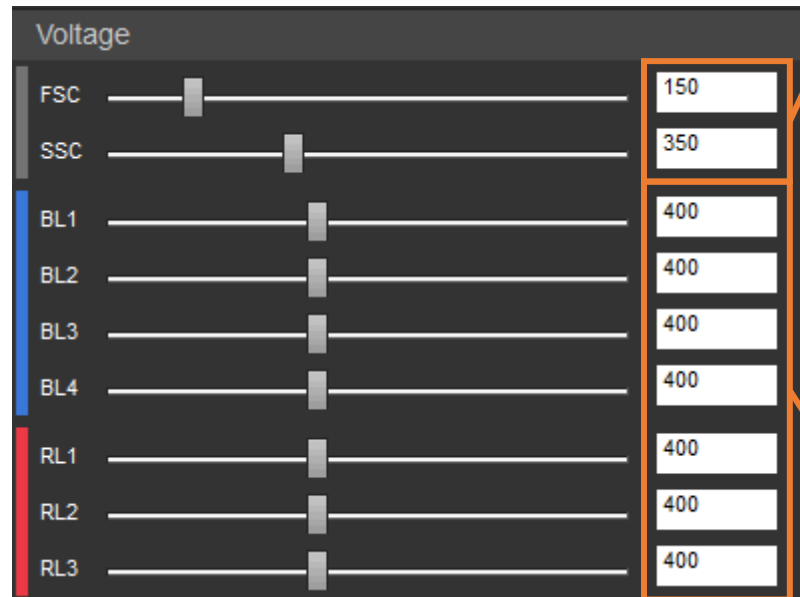
吸取樣本體積
(確認細胞足夠達到蒐集目標)

分析流速

數據蒐集目標

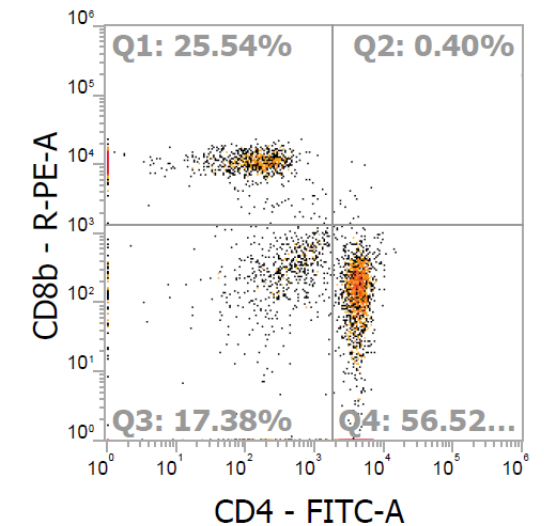
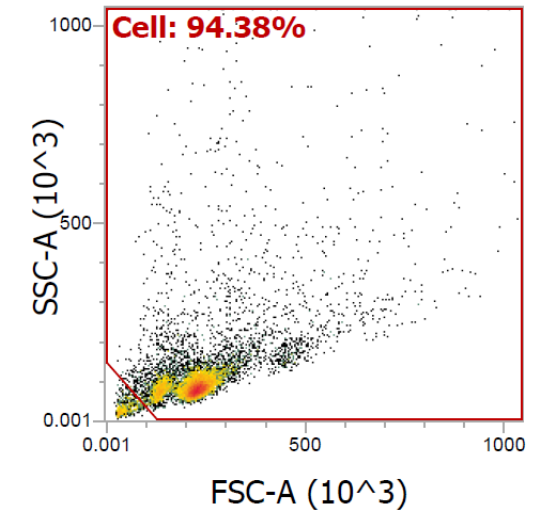
The screenshot shows the 'Collection Panel' of a laboratory instrument. It features a circular progress indicator at the top center showing '0%' and '0 events/s'. To its right is a vertical scale for 'Sample remaining' at '0 µLs'. Below these are three buttons: 'Stop', 'Start Up', and 'Clear'. A dropdown menu shows 'Sample: Sample (T1)'. The 'Run Protocol' section has a checked box for 'Automatically update Experiment level Run Protocol' and buttons for 'Apply to experiment', 'Set as default', and 'Load...'. The 'Flow Options' section has a text input for 'Acquisition Vol' set to '200 µL (250 µL Total Draw Volume)' and a slider for flow rate set to '100 µL /min'. The 'Stop Options' section has three rows of controls: the first row has a checked box, '10,000' events, and 'Singlet' on a dropdown; the second row has an unchecked box, '5' min, and '0' sec; the third row has an unchecked box and '50' µL. Three orange lines with dots point from Chinese text on the left to the 'Acquisition Vol' input, the flow rate slider, and the '10,000' event target respectively.

設定儀器參數 – 4. PMT Voltage



一般哺乳類動物細胞($\sim 10 \mu\text{m}$)建議從 FSC (150)以及SSC (350)開始測試，再根據結果調整以利觀察主要群體

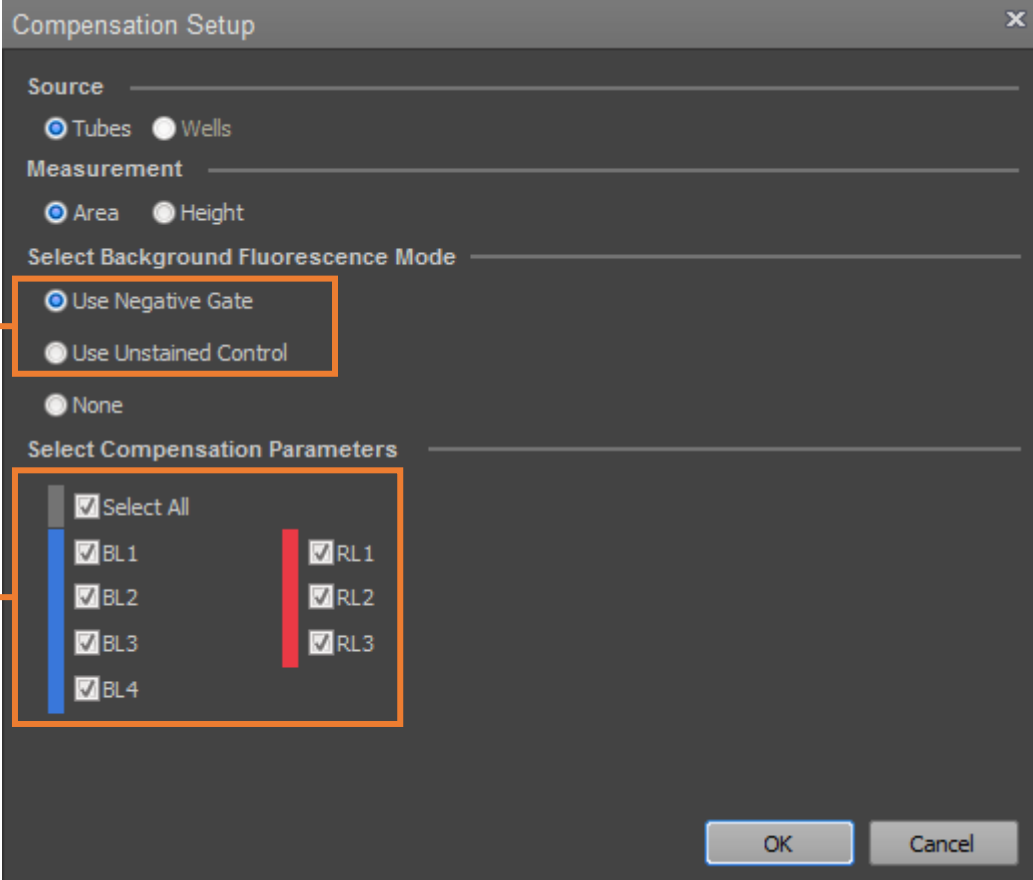
以unstained樣本調整訊號最小值
以正式染色樣本調整訊號最大值



設定儀器參數 – Compensation

選擇螢光背景值的判斷模式

勾選需要進行compensation的
channels



The screenshot shows the 'Compensation Setup' dialog box. It has a dark grey background with white text. The 'Source' section has 'Tubes' selected. The 'Measurement' section has 'Area' selected. The 'Select Background Fluorescence Mode' section has 'Use Negative Gate' selected, which is highlighted by an orange box. The 'Select Compensation Parameters' section has a list of channels: 'Select All', 'BL 1', 'BL 2', 'BL 3', 'BL 4' on the left (highlighted by a blue bar) and 'RL 1', 'RL 2', 'RL 3' on the right (highlighted by a red bar). All these channels are checked. At the bottom right are 'OK' and 'Cancel' buttons. Two orange lines with dots at the end point from the Chinese text on the left to the 'Use Negative Gate' option and the compensation parameters list respectively.

Compensation Setup

Source ☒ Tubes ☐ Wells

Measurement ☒ Area ☐ Height

Select Background Fluorescence Mode

☒ Use Negative Gate

☐ Use Unstained Control

☐ None

Select Compensation Parameters

☒ Select All

☒ BL 1

☒ BL 2

☒ BL 3

☒ BL 4

☒ RL 1

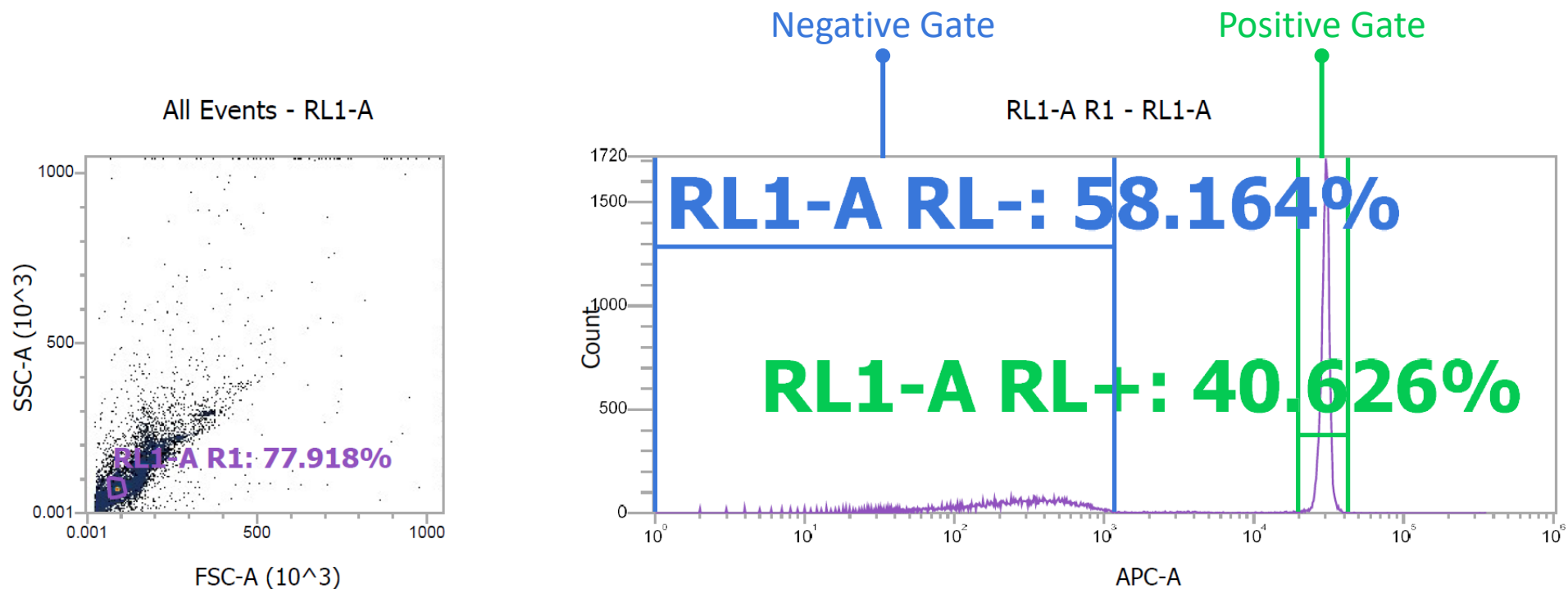
☒ RL 2

☒ RL 3

OK Cancel

Note: Cells for negative and positive signal must have the same level of background fluorescence.

設定儀器參數 – Compensation: Use Negative Gate



記錄Compensation controls之前確認:

1. 已調整好各channel的PMT voltage
2. 已設定好R1，Negative，以及Positive Gates

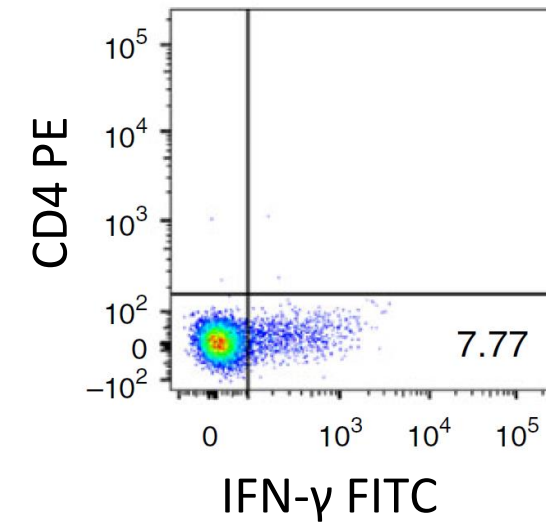
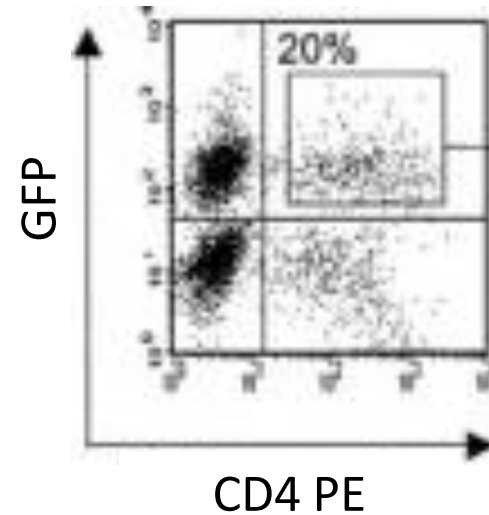
When to Use Compensation Beads



Cat.#01-3333-41

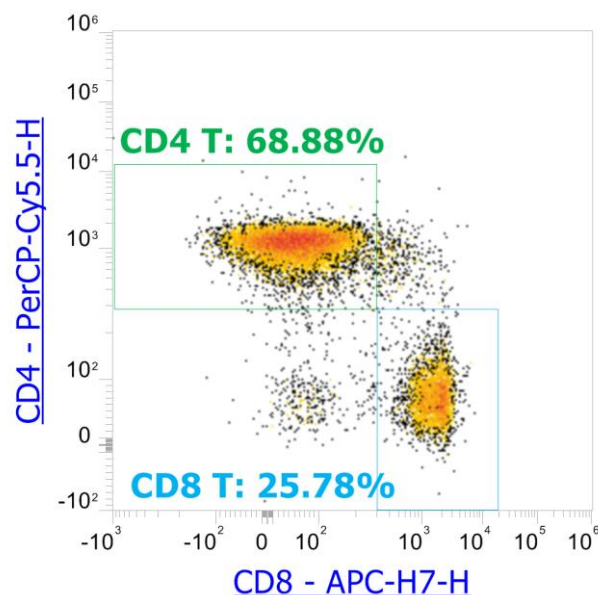
UltraComp eBeads™ Plus Compensation Beads

- Intracellular fluorescence
- Poorly expressed markers
- Limited amount of sample
- Large multicolor panel
- Standardization



設定儀器參數 – Customize

多色螢光實驗進行compensation後，
數值軸建議使用**HyperLog**，以正確呈現過小的數值



Customize

General

Plot Type: ☐ Histogram ☐ Dot ☒ Density ☐ Precedence Density

Resolution: 256 x 256

Mode: Log

Color: [Color bar]

% of Events: 100%

X axis

Parameter: BL1-H

Scale: ☐ Linear ☐ Logarithmic ☒ HyperLog™

Range: ☐ Automatic ☒ Manual

Min: -1000

Max: 1048576

HyperLog™ Transitional Value: 1000

Y axis

Parameter: BL4-H

Scale: ☐ Linear ☐ Logarithmic ☒ HyperLog™

Range: ☐ Automatic ☒ Manual

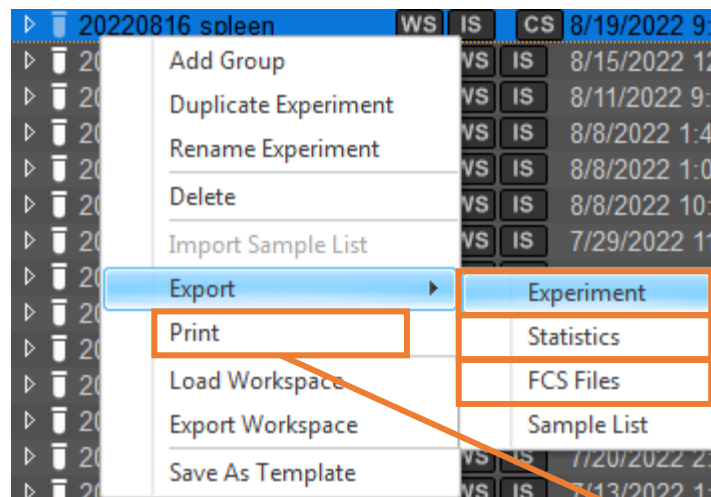
Min: -1000

Max: 1048576

HyperLog™ Transitional Value: 1000

Workspace Chart類型選擇
與參數調整

輸出實驗結果



Experiment (*.atx): 完整原始實驗數據檔案

Statistics (*.csv): 可使用excel開啟的數據檔案

FSC Files (*.fcs): Flow Cytometry通用數據檔案，可使用第三方分析軟體開啟

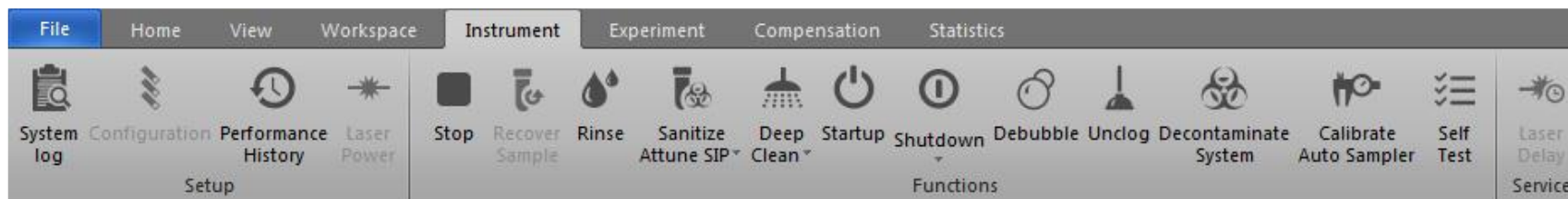
Print (*.pdf): Compensation與Workspace圖檔與統計表格

Data Analysis

- 調整分析流程，留下高品質數據 (單顆細胞，排除死細胞)
- 規劃良好的controls以協助分析結果
- 決定統計數值的呈現方式 (影響數據蒐集目標的設定)
 - % Total
 - % Gated
 - Events/ μ L
 - MFI (mean fluorescence intensity)

File	Home	View	Workspace	Instrument	Experiment	Compensation	Statistics	Overlay
☐ Select All	☐ Plate	☐ Experiment	☒ X parameter	☒ Y parameter	☐ Count	☐ Events/ μ L	☐ X Mean	☐ Y Mean
	☐ Sample	☐ Workspace	☐ Group	☒ Autogate Status	☐ % Total	☒ % Gated	☐ X Median	☐ Y Median
	☒ Gate	☐ Comp Source	☐ Plot Title		☐ Volume (μ L)		☐ X Peak	☐ Y Peak
Tools	General				Event Statistics		Intensity	
							Variation	
							☐ X SD	☐ Y SD
							☐ X %CV	☐ Y %CV
							☐ X rSD	☐ Y rSD
							☐ X %rCV	☐ Y %rCV

清洗功能與錯誤排除



Function	狀況
Rinse	清洗樣本管路
Sanitize SIP	清洗樣本管路與上樣針SIP 不同使用者之間避免樣本互相干擾 使用易沾黏管路的樣本
Deep Clean	清洗樣本管路與flow cell
Debubble	系統偵測到氣泡，清除樣本管路與flow cell氣泡
Unclog	無訊號，樣本管路可能阻塞管路時
Decontamination	儀器管理進行定期保養

狀況無法排除時，問題回傳:

1. System log
2. Print screen

Attune NxT手冊

invitrogen

Attune™ Cytometric Software USER GUIDE

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

Publication Number MAN0026553

Revision B.0

https://downloads.thermofisher.com/Attune_v6.0.1/MAN0026553-RevB-AttuneCytometricSW-UG-EN-27Apr2023.pdf

USER GUIDE

invitrogen

Attune™ NxT Acoustic Focusing Cytometer

Catalog Numbers A24858, A24859, A24860, A24861, A24862, A24863, A24864, A28993

Publication Number 100024235

Revision C.0

https://assets.thermofisher.com/TFS-Assets/LSG/manuals/100024235_AttuneNxT_HW_UG.pdf

其他工具



Human and mouse antigens

ThermoFisher
SCIENTIFIC

其他工具

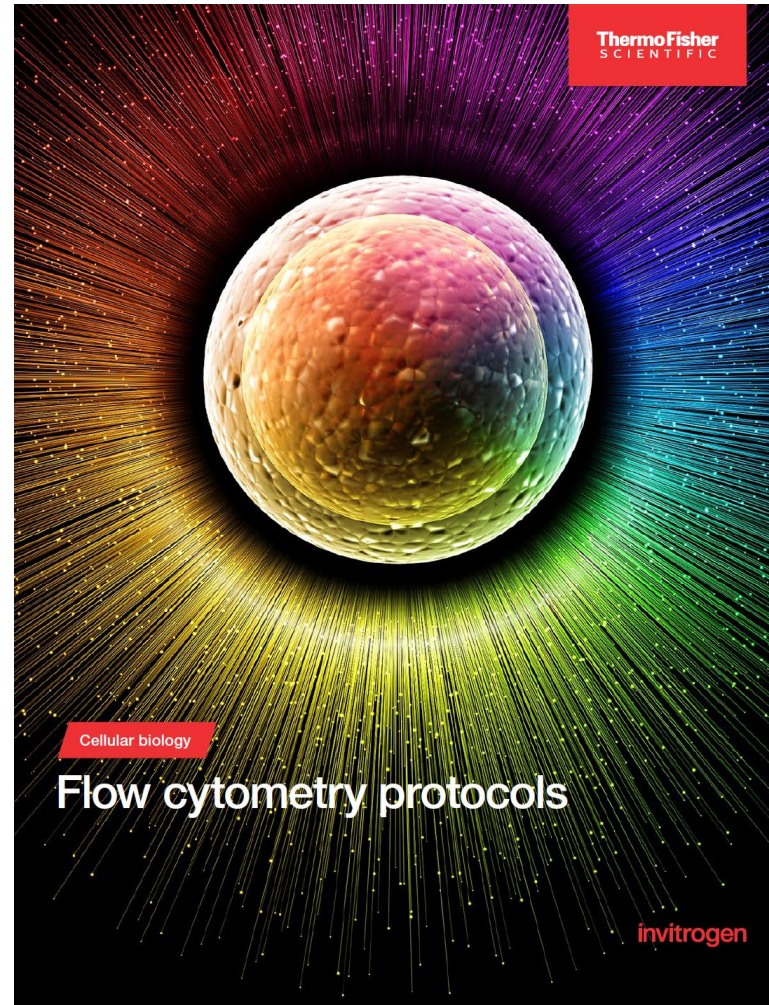


Flow cytometry capabilities guide

Sample preparation | Fluorophore selection | Flow cytometry antibodies and assays |
Attune flow cytometers | PrimeFlow RNA Assay | Fluorophore and reagents

ThermoFisher
SCIENTIFIC

<http://assets.thermofisher.com/TFS-Assets/BID/brochures/flow-cytometry-capabilities-guide-brochure.pdf>



Cellular biology

Flow cytometry protocols

invitrogen

<https://www.thermofisher.com/tw/zt/home/global/forms/flow-cytometry-protocols-handbook.html>

流式細胞儀應用專刊

thermo
scientific
Authorized Distributor



德怡科技股份有限公司

免費專線: 0800 212228 台北(02)86922116 桃園(03)3975447 苗栗(037)625816 花蓮(03)8570182 www.TAQKEY.com



<https://www.taqkey.com/attunenxt-flow-cytometr/>

Thank you

ts@taqkey.com