TAQKEY SCIENCE

21st ANNIVERSARY

Basics of Flow Cytometry & Attune NxT

20230829

Taqkey Science

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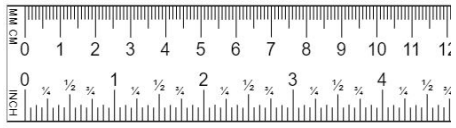
thermo scientific

Authorized Distributor

Basics of Flow Cytometry

What is Flow Cytometry?



Cyto	Metry
Cell	Measurement

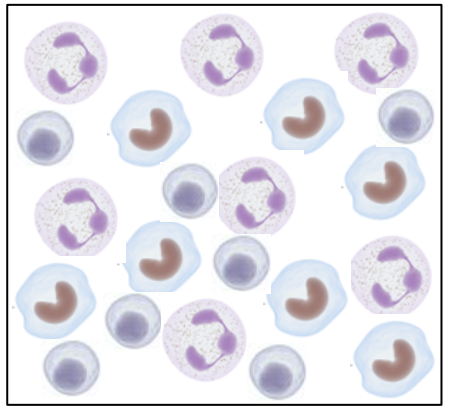
Measurement of cell properties

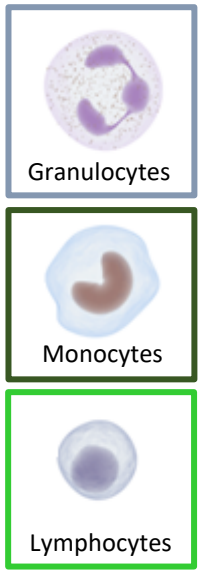
- Performed using **single cell** suspensions in fluidics
- Cells can be measured based on **size, shape, granularity, and light scattering** properties
- **Fluorochromes** are used to label the cell's physical properties to help provide measurements

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

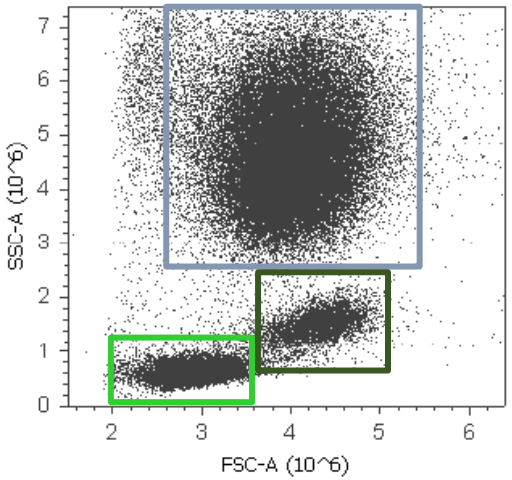
Microscope

Lysed human whole blood





Flow Cytometer



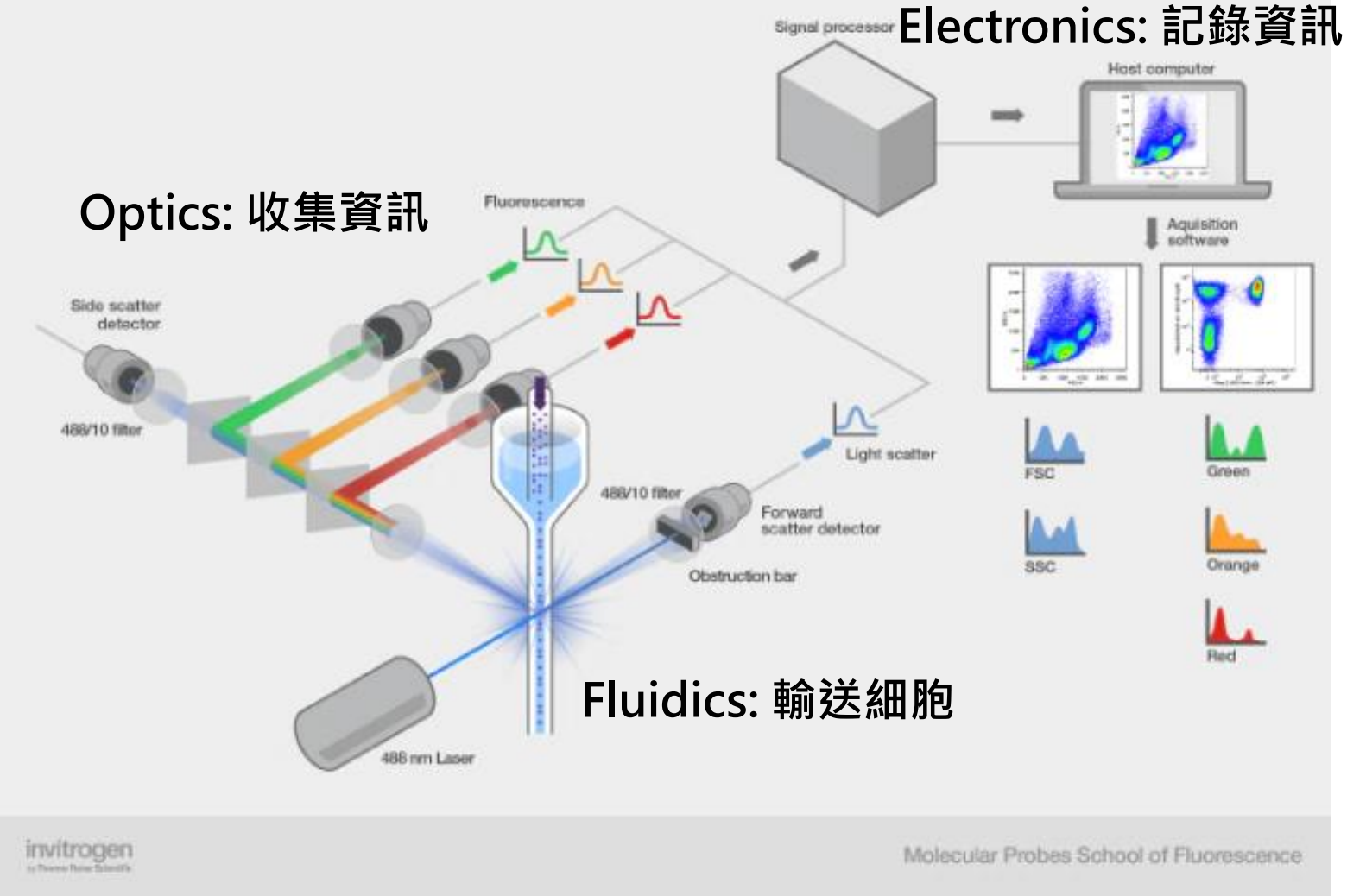
Advantage

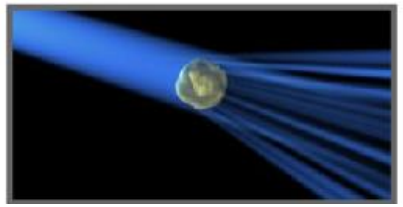
- Quantitative
- Rare population
- Multiple phenotype

Disadvantage

- Lost structure information of tissue/cell

Flow Cytometer Components



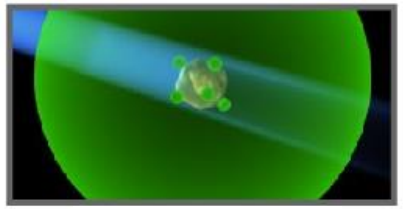


Size

Forward scatter (FSC)

Complexity

Side scatter (SSC)



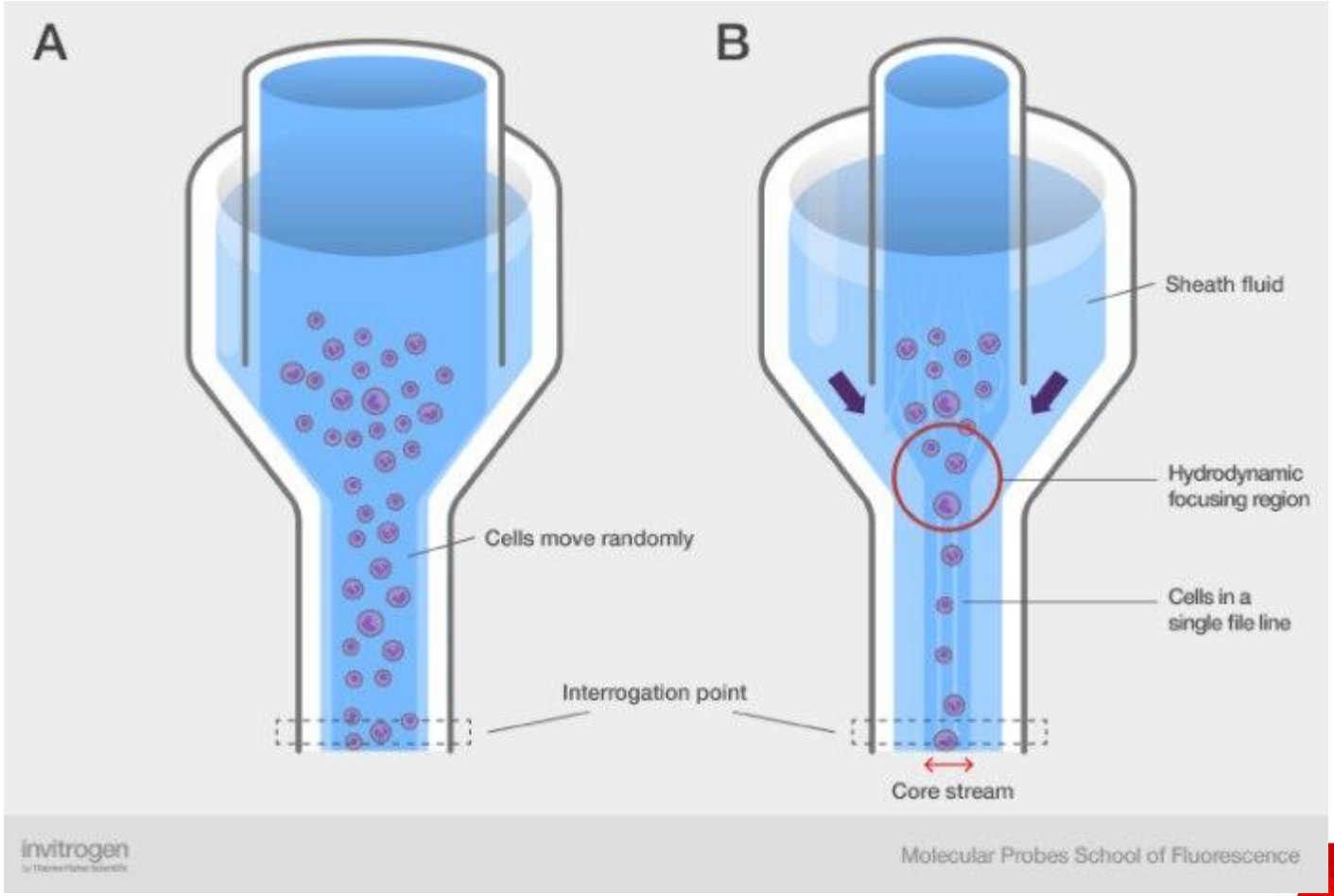
Phenotype

Fluorescence

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/how-flow-cytometer-works.html

Fluidics: 輸送細胞

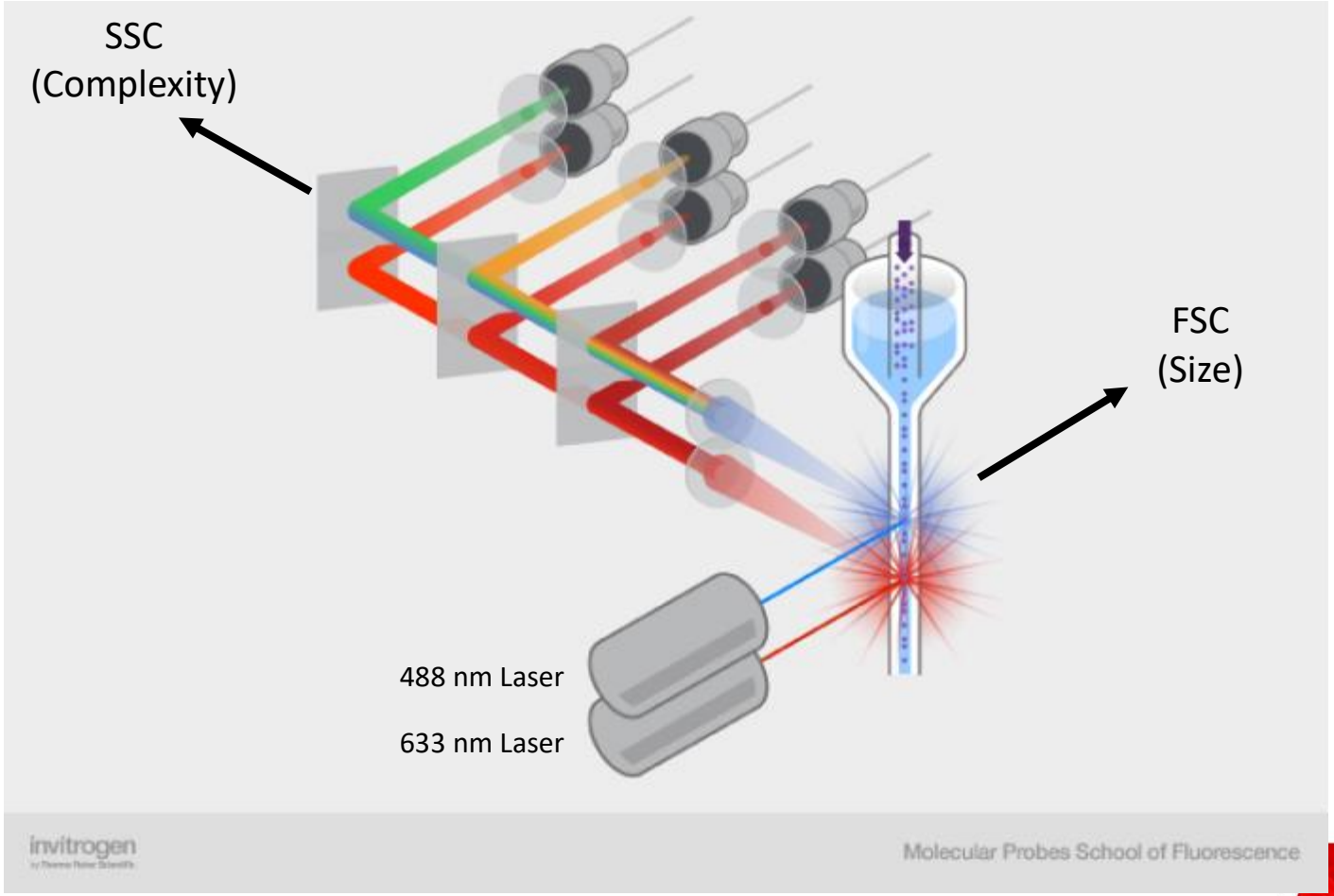
The **fluidics system** of a flow cytometer is responsible for transporting sample from the sample tube to the flow cell.



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

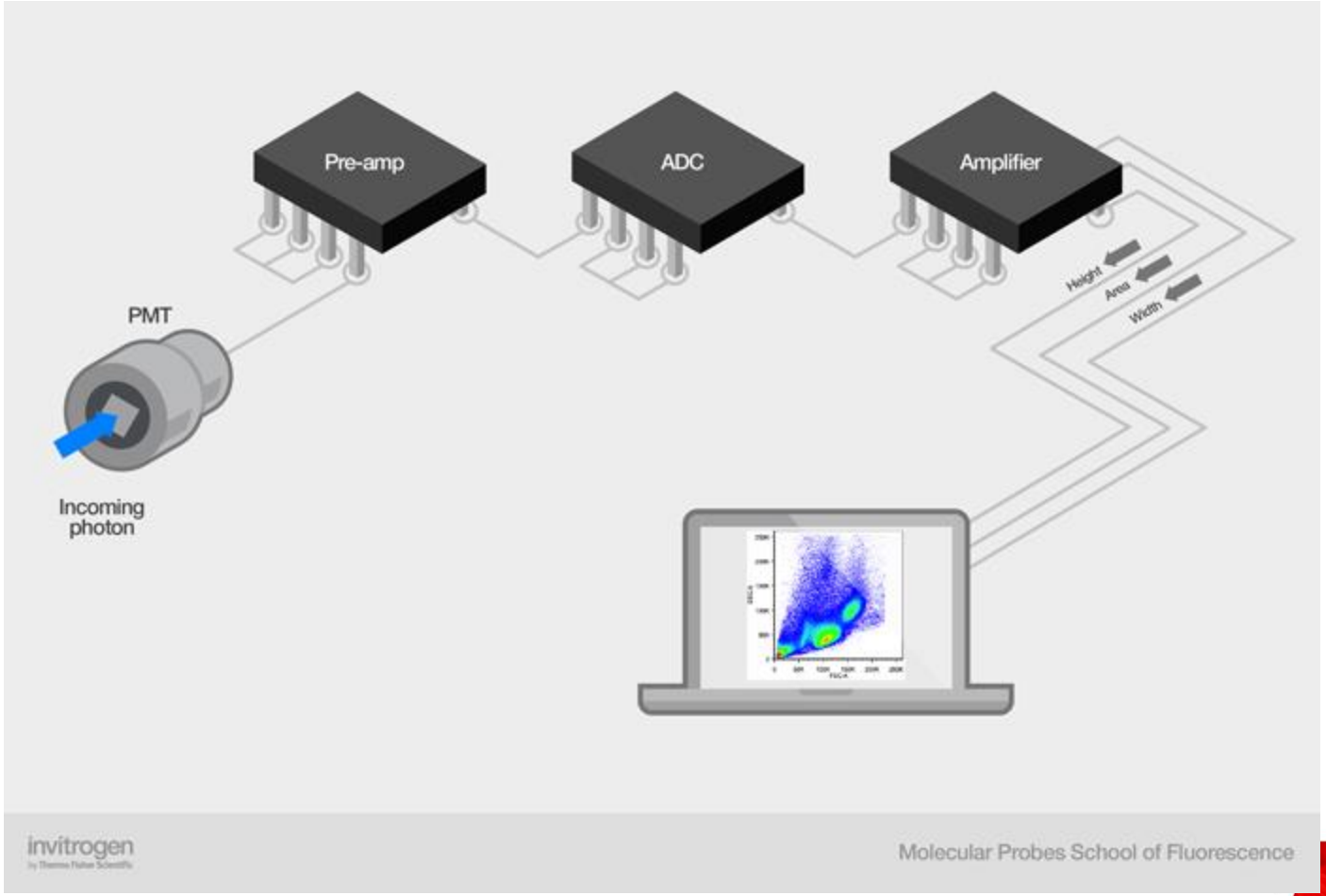
The components of the **optical system** include excitation light sources, lenses, and filters used to collect and move light around the instrument and the detection system that generates the photocurrent.



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.



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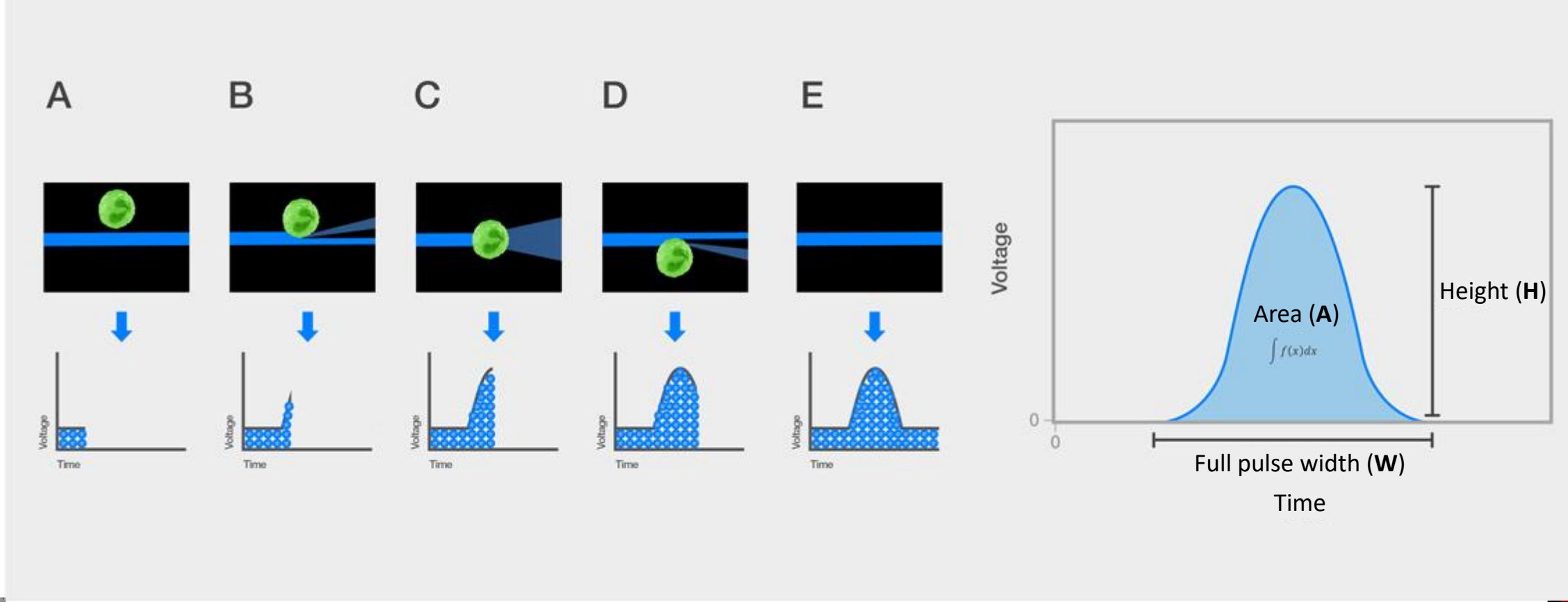
Signal Pulse



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/electronics-flow-cytometer.html

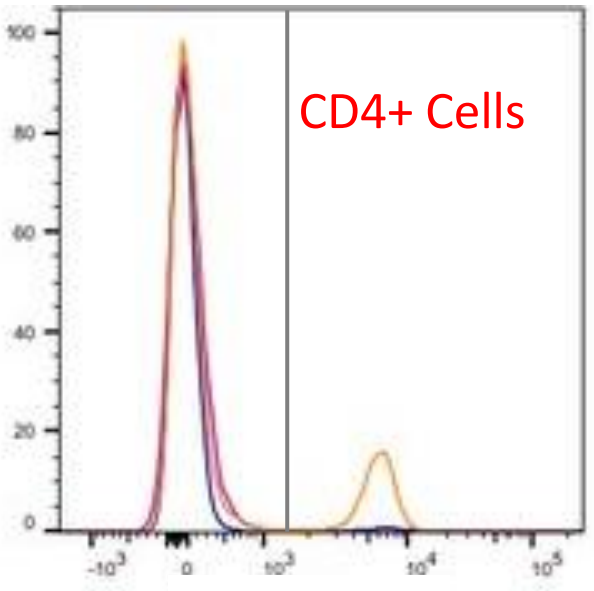
Data of Flow Cytometry

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

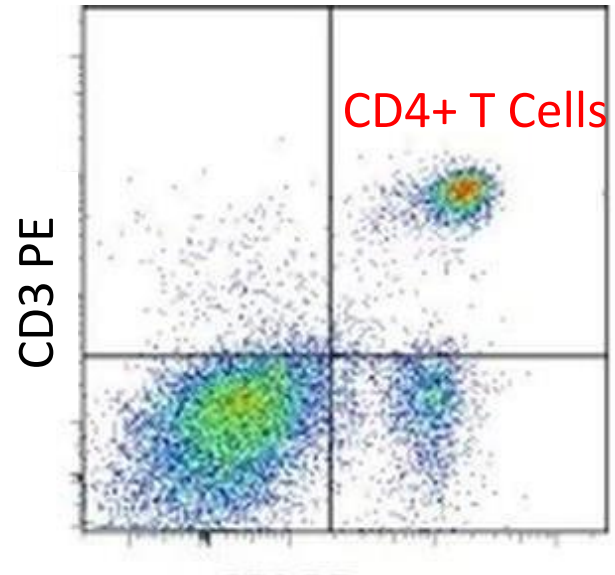
1 marker



CD4 FITC

Histogram

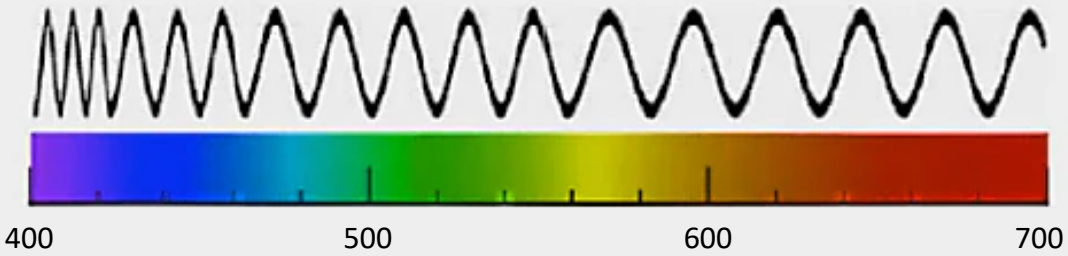
2 markers



CD4 FITC

Dot plot

The Visible Light Spectrum



Short wavelength

Higher frequency

Higher energy

Longer wavelength

Lower frequency

Lower energy

$$E = h\nu = \frac{hc}{\lambda}$$

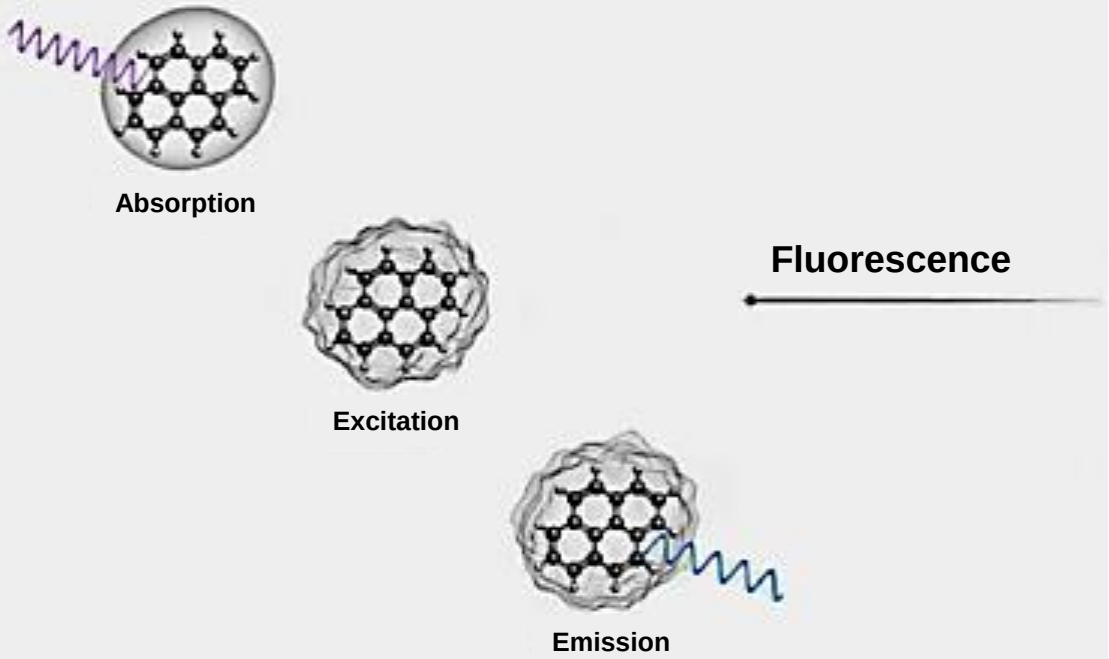
E: energy
h: Planck constant
v: frequency
c: speed of light
λ: wavelength

invitrogen
by Thermo Fisher Scientific

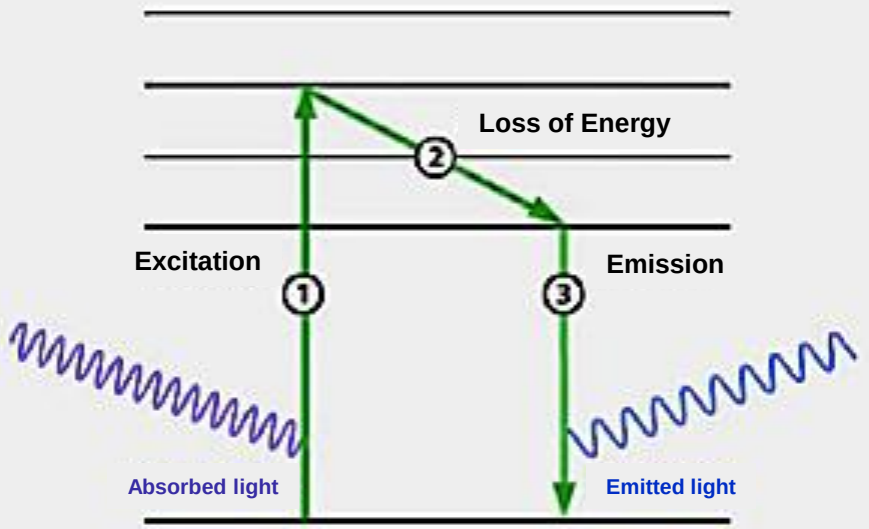
Molecular Probes School of Fluorescence

Fluorescence

Definition of Fluorescence



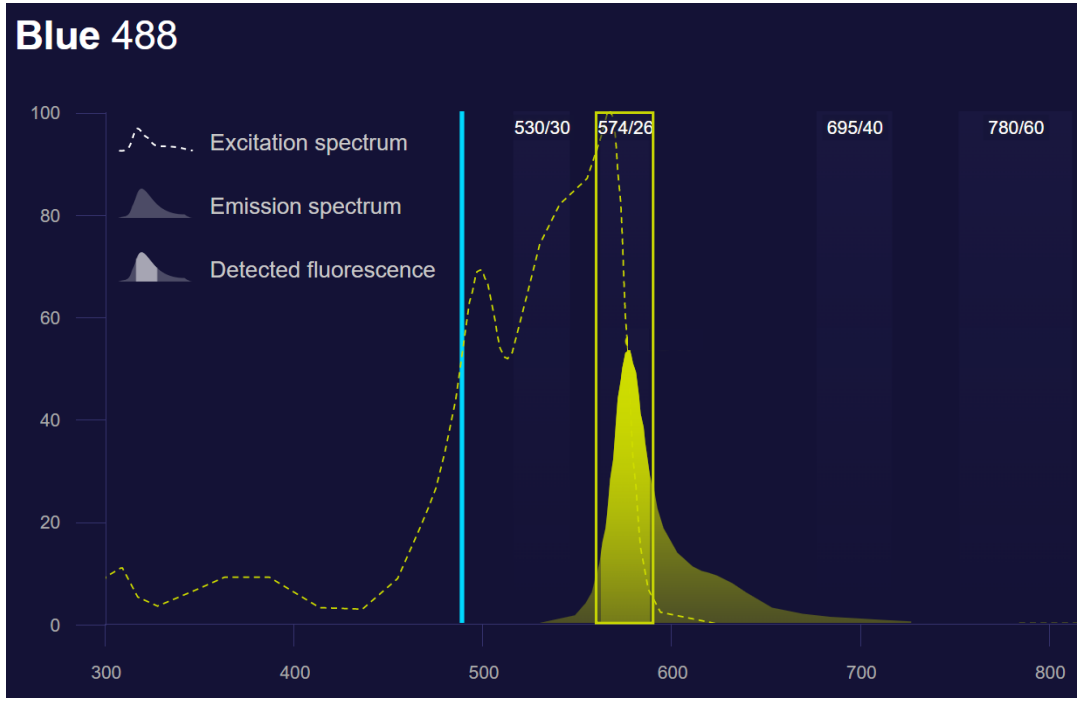
Jablonski Diagram Summary



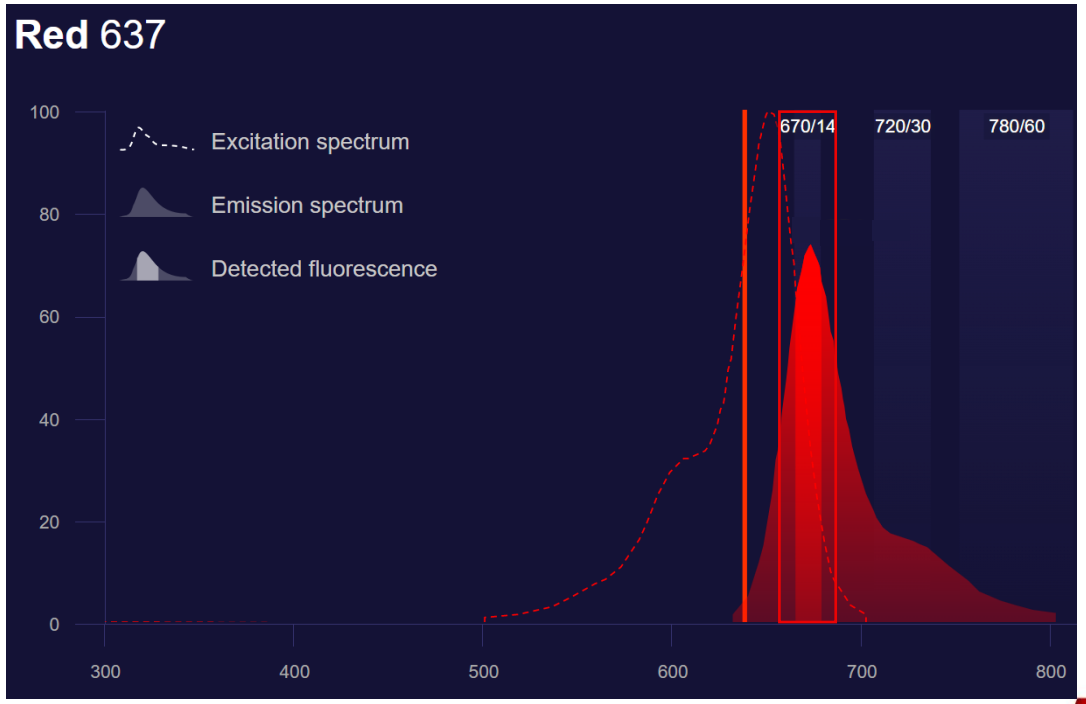
https://www.thermofisher.com/tw/en/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/fluorescence-basics/fluorescence-fundamentals/process-fluorescence.html

Channels for Fluorochrome

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

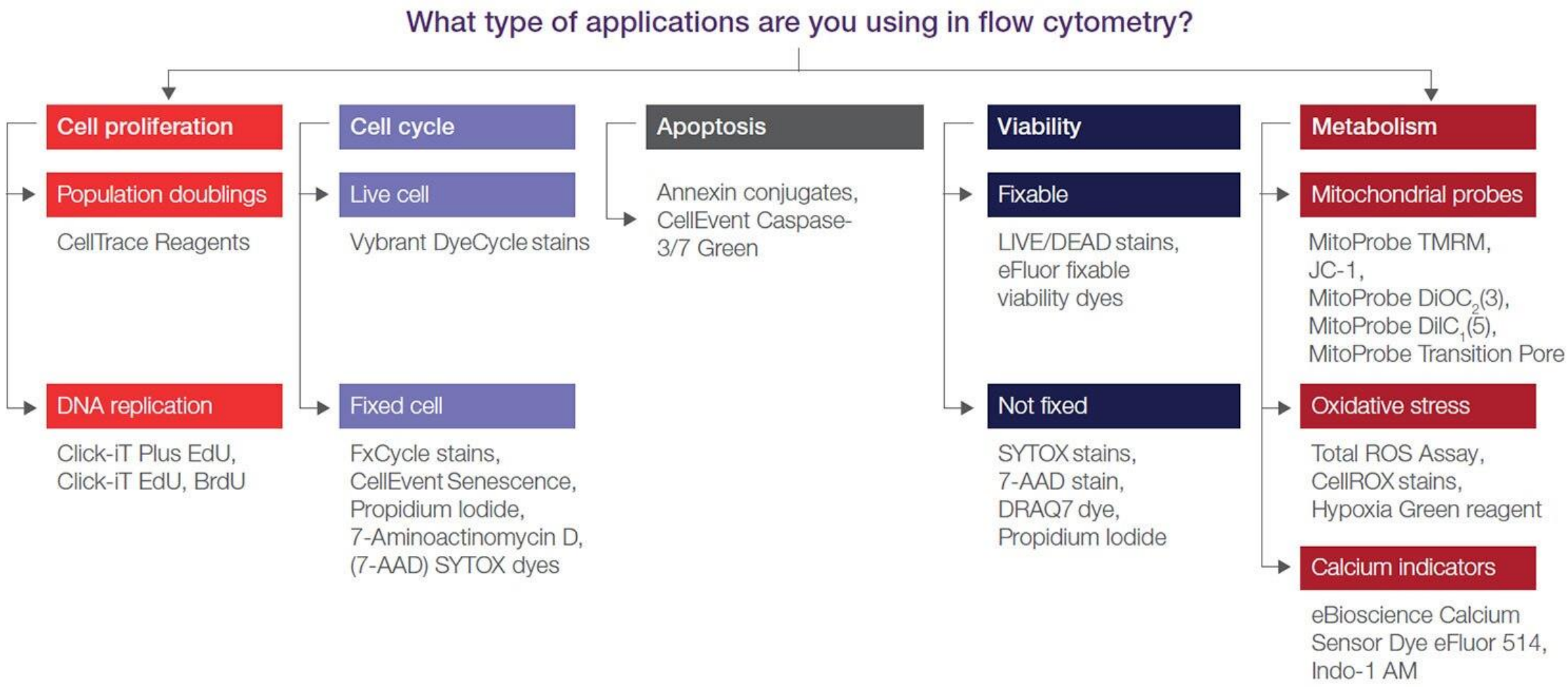


Fluorochrome: APC
Channel: Ex 637, Em 670/14



https://www.thermofisher.com/order/fluorescence-spectraviewer#!/

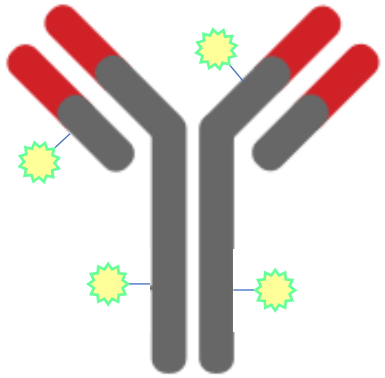
Fluorescent Reagents



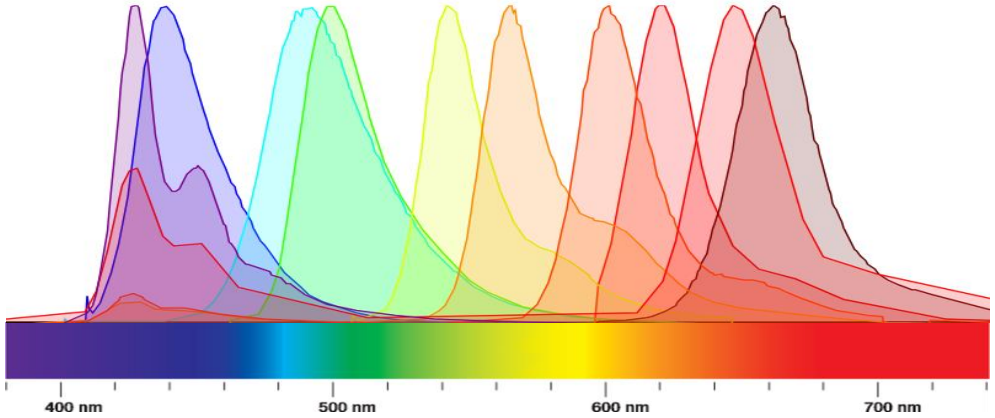
https://www.thermofisher.com/tw/en/home/life-science/cell-analysis/flow-cytometry/flow-cytometry-assays-reagents.html

Fluorescent Antibody

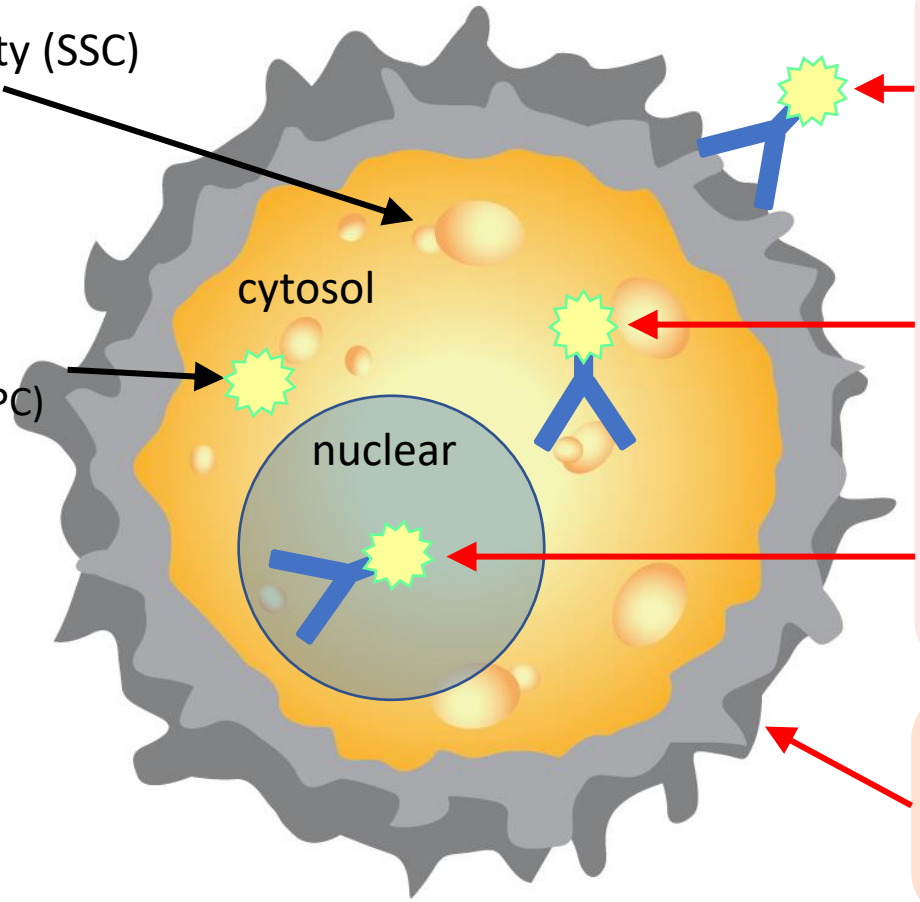
Antibody: Specificity 專一性



Fluorescence: Identity 辨識度



Cell Characteristics by Flow Cytometry



胞内螢光
(e.g. GFP, PE, APC)

胞外染色
Surface staining
(e.g. CD markers)

免疫分型
Immunophenotyping

胞内染色
Cytosolic staining
(e.g. cytokines)

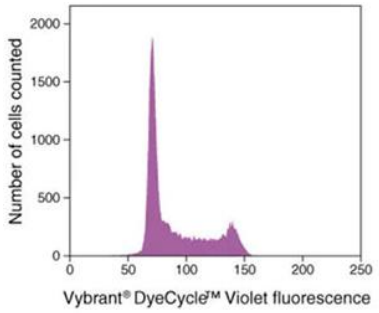
核内染色
Nuclear staining
(e.g. transcription factors)

Intracellular staining

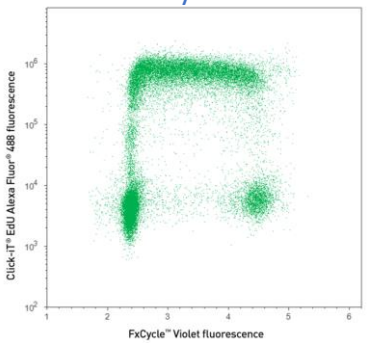
細胞功能試劑
Cell function assays
(e.g. viability, proliferation, cell cycle, apoptosis, metabolism)

Applications of Flow Cytometry

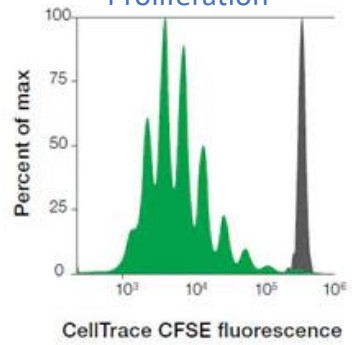
細胞週期
Cell cycle



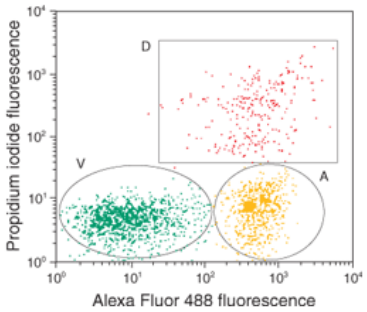
DNA合成
DNA synthesis



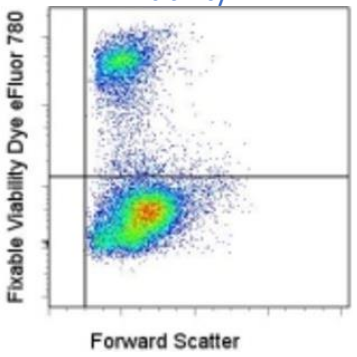
細胞增長
Proliferation



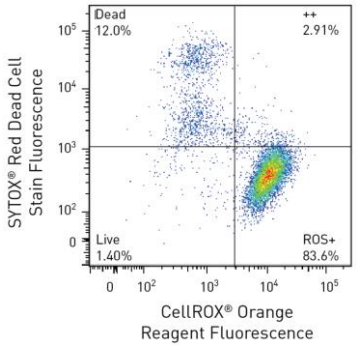
細胞凋亡
Apoptosis



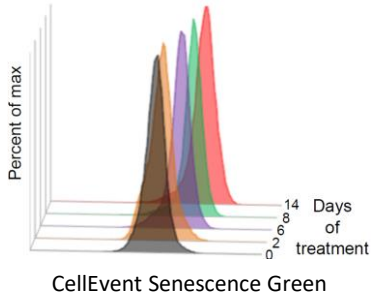
細胞存活
Viability



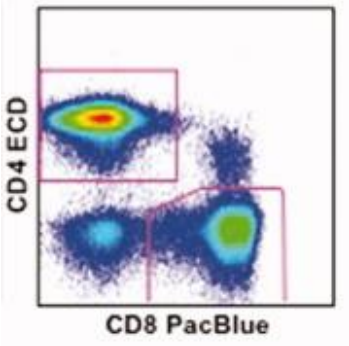
氧化壓力
Oxidative Stress



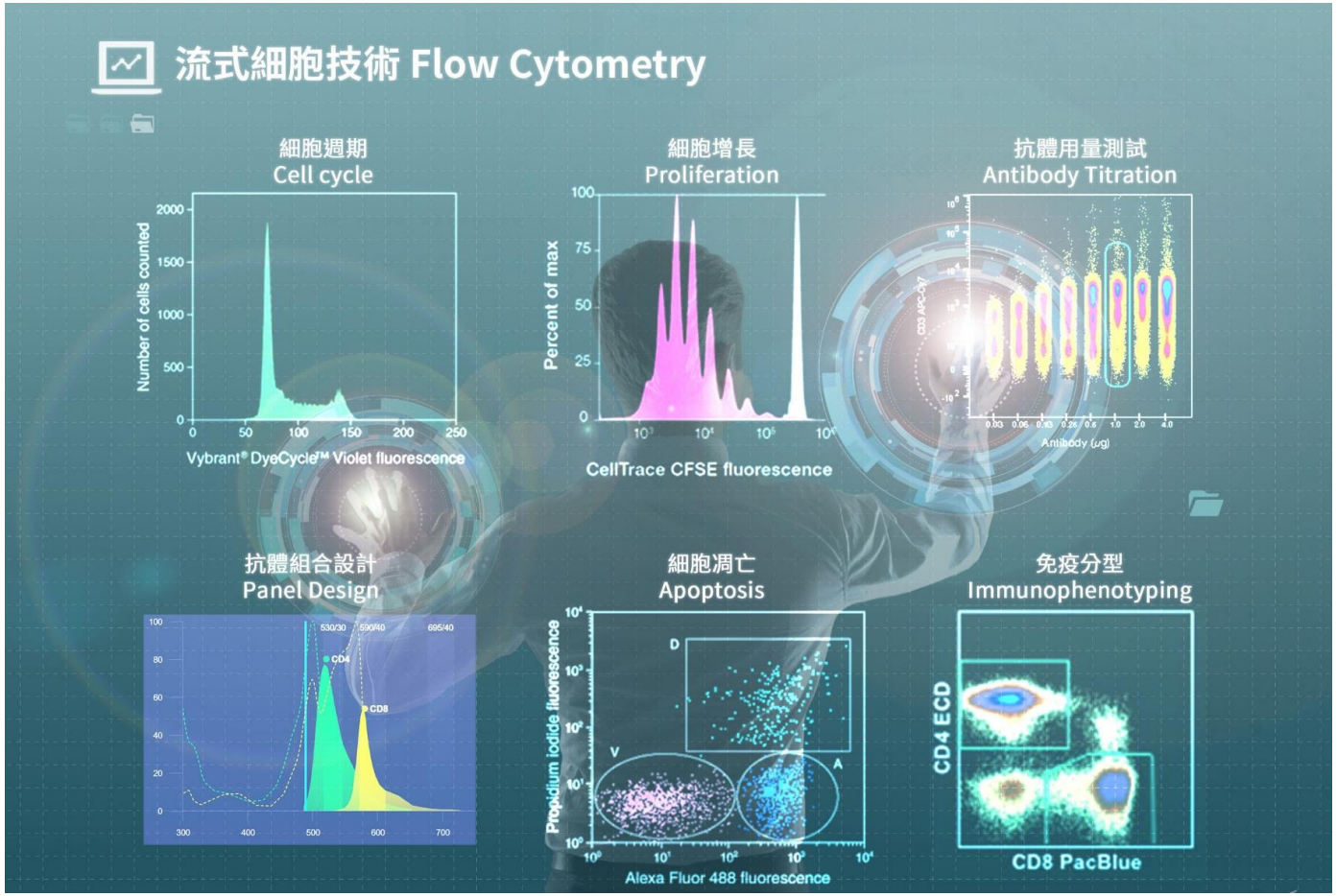
細胞老化
Senescence



免疫分型
Immunophenotyping



Flow Cytometry產品諮詢與技術服務



歡迎與當區業務

巧盈

聯絡取得相關訊息~

Attune NxT Flow Cytometer

Attune NxT Acoustic Focusing Flow Cytometer

Small in size, big in performance

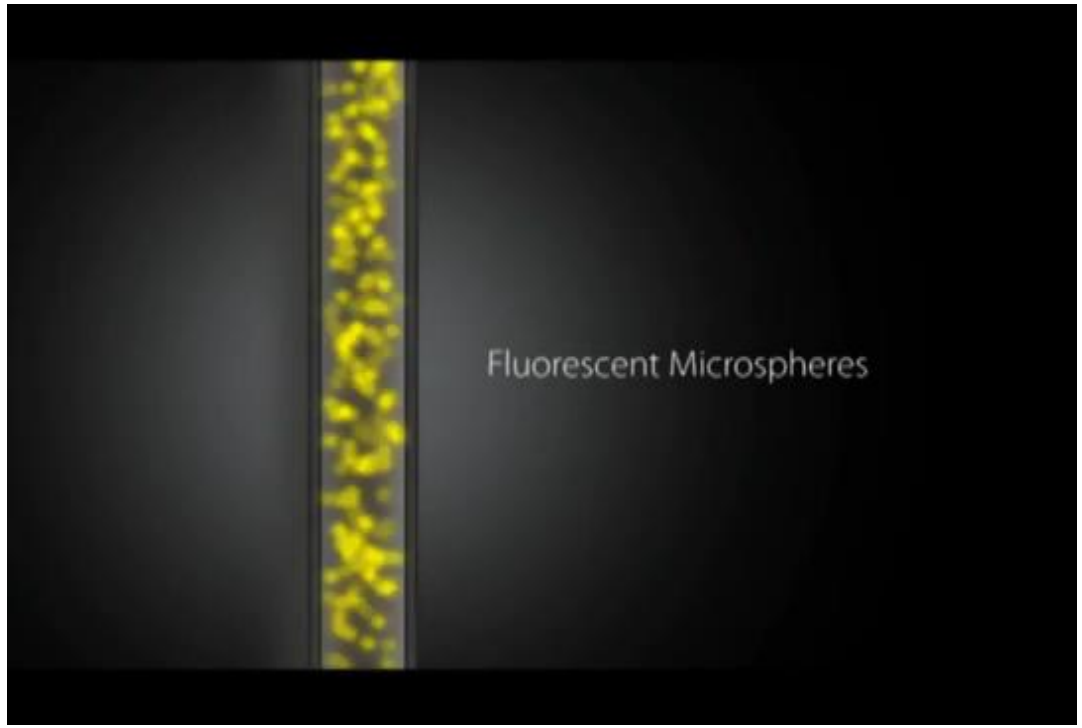
Flat-Top Laser
平頂雷射均勻激發細胞，
提供穩定且高解析度的分析結果

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

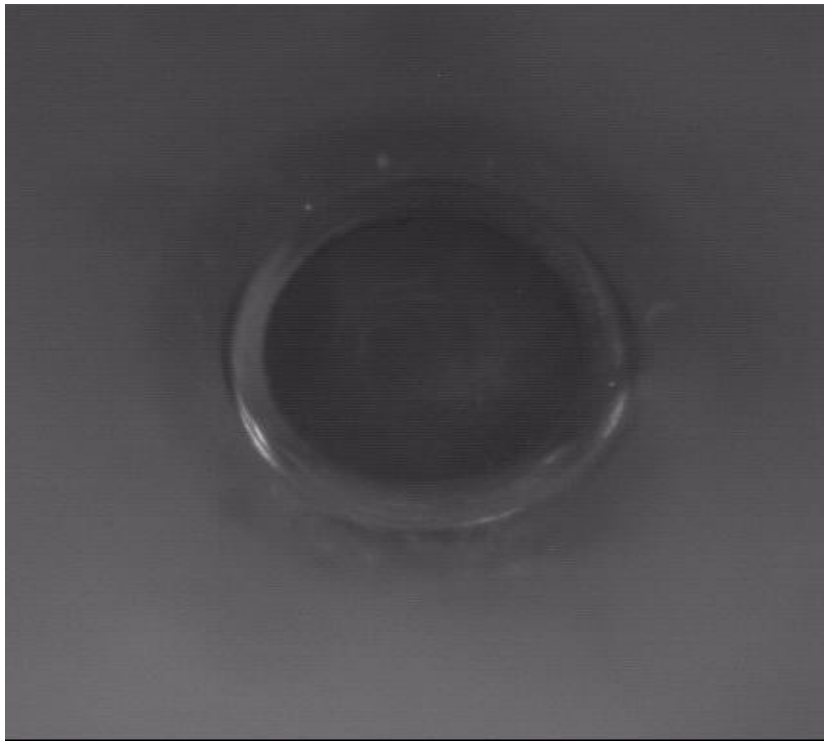
Autosampler
可選配自動上樣機**CytKick (MAX)**，
盤式上樣更省時方便

Syringe Pump
針筒幫浦定量上樣體積，
可絕對計數細胞濃度

Acoustic Focusing



End-on view of capillary

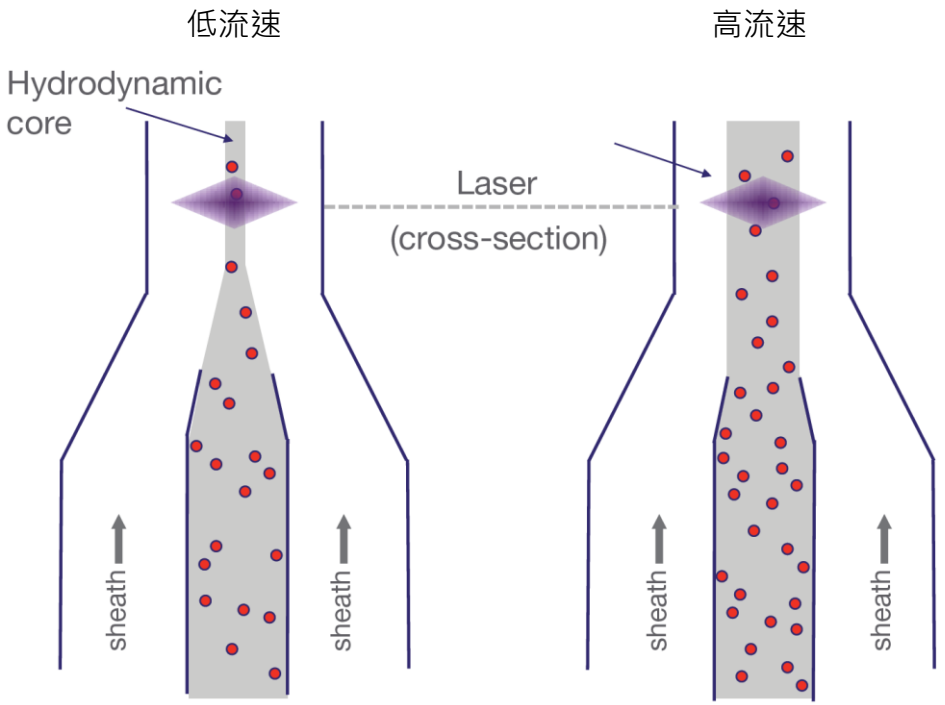


https://www.thermofisher.com/tw/en/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/flow-cytometry-basics/flow-cytometry-fundamentals/fluidics-flow-cytometer.html

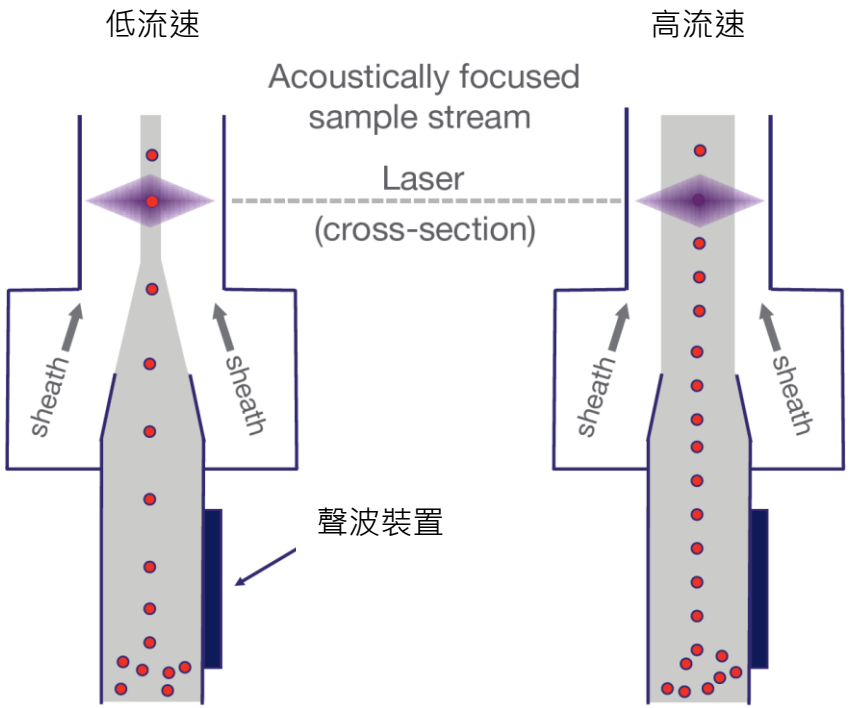
Acoustic Focusing

High sample input flow rates allow for more sample flexibility

傳統流體動力聚焦

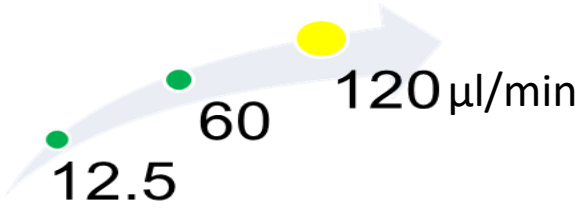


聲波輔助聚焦

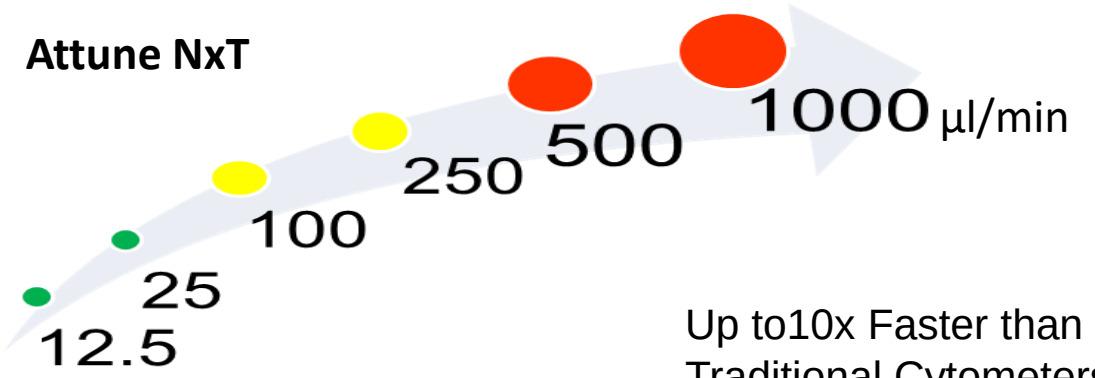


Comparable Results at All Flow Rates

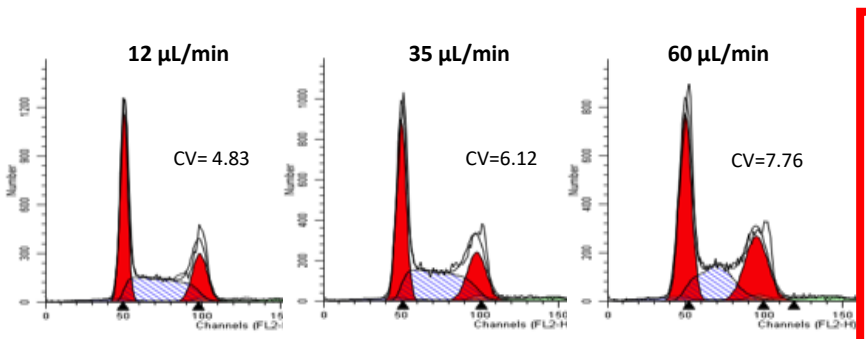
Traditional Cytometers



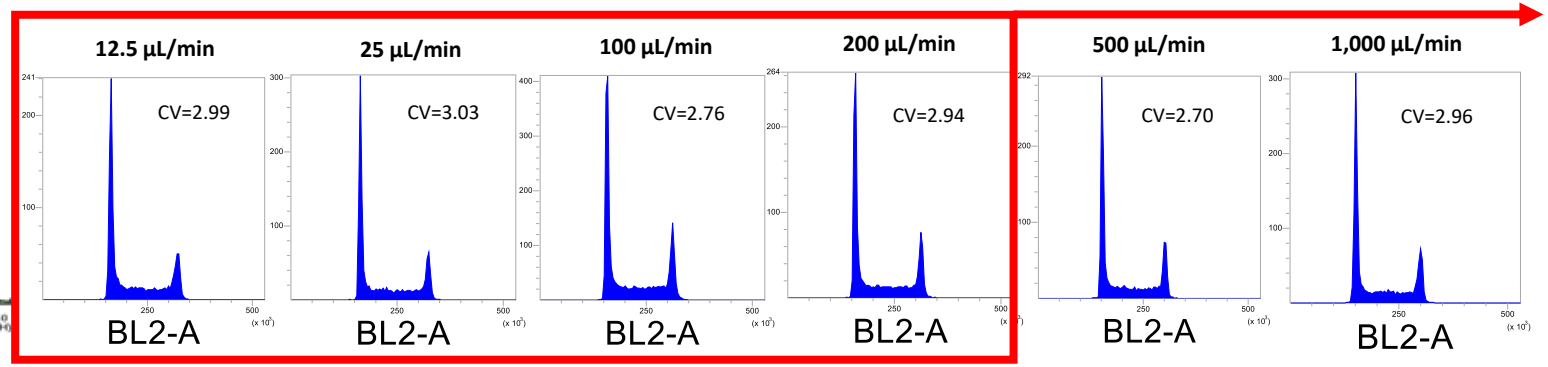
Attune NxT



Up to10x Faster than Traditional Cytometers



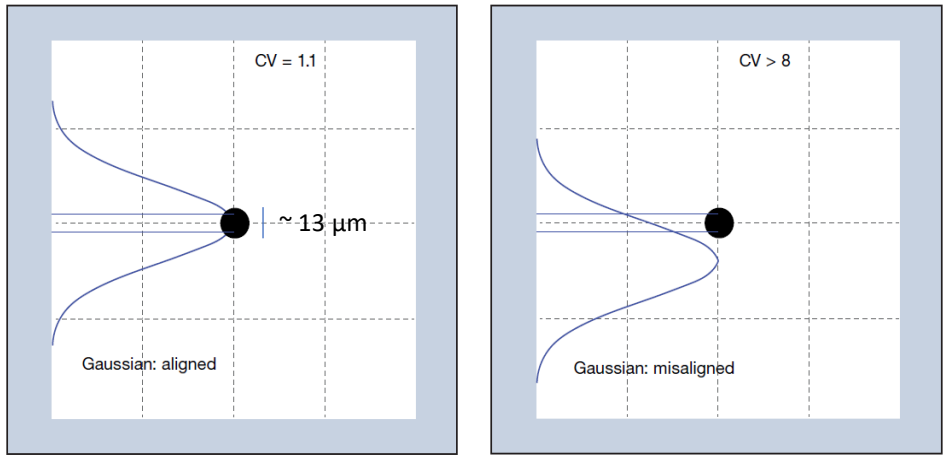
Hydrodynamic Focusing Only



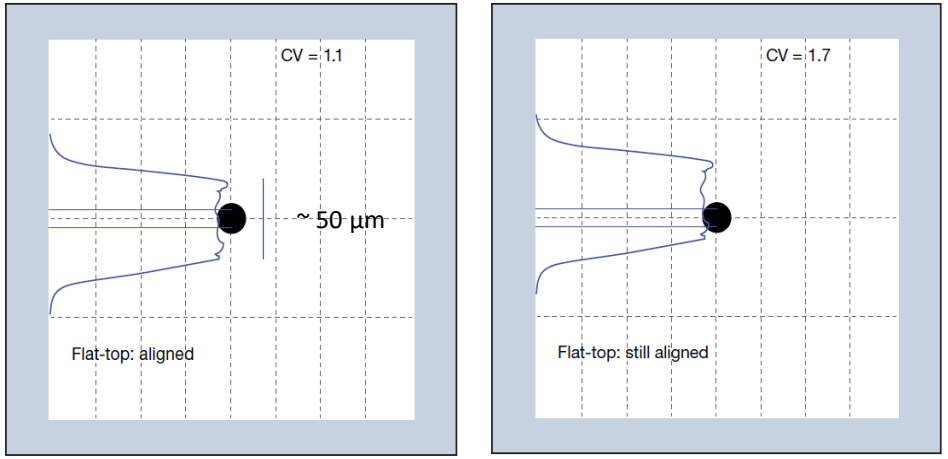
Acoustically Enhanced Hydrodynamic Focusing

Flat-Top Lasers

Gaussian laser

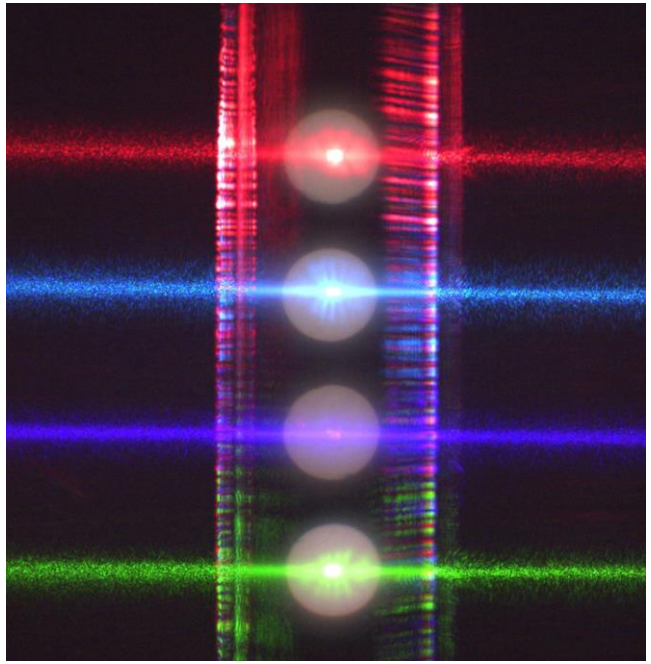


Flat-Top laser



- 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!**

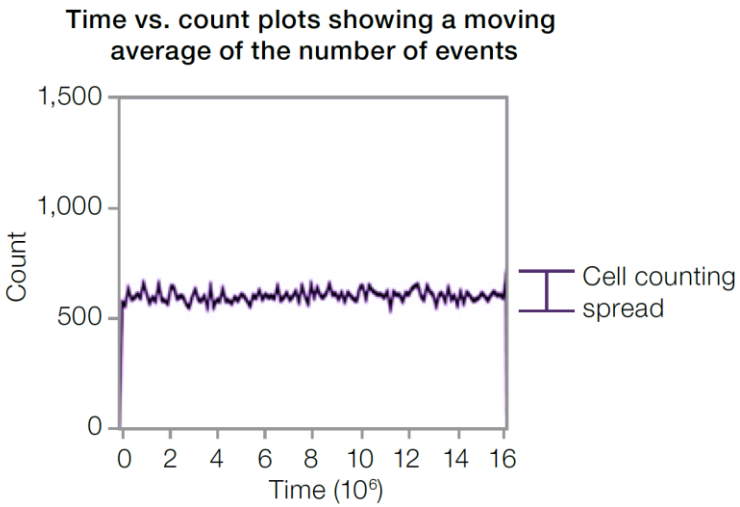
Attune NxT Configurations

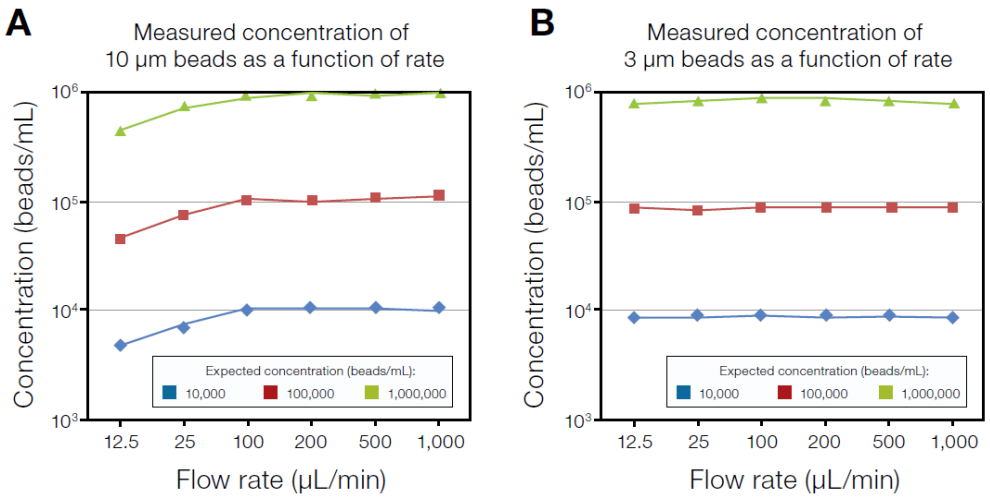
Upgrade Violet Laser

7 → 11

Cat. No.	A24864	A28995	A24861	A24863	A24862	A29002	A28997	A24860	A28999	A28993	A24859	A29003	A29004	A29001	A24858
Detectors	4	7	7	7	8	9	10	10	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





- Smooth delivery of samples
- Consistent concentration results
- Resist to clog
- Buffer consumption ~ 1.8L/Day

CyKick (MAX) Autosampler

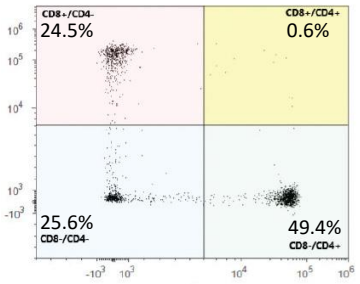
CytKick 與 CytKick MAX自動上樣機

- 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

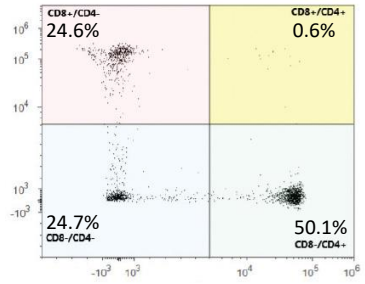
CytKick MAX自動上樣機

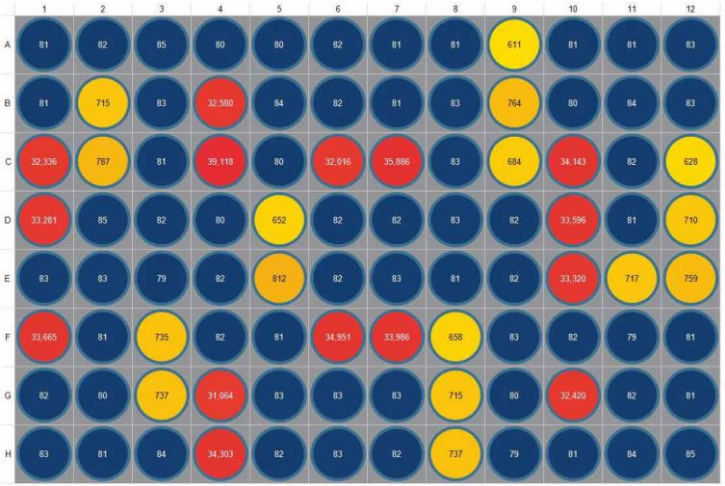
- 可選用24孔離心管(1.5-2 mL)上樣架
- 96孔盤式上樣總時間 < 42 mins
- 96孔盤與24孔離心管上樣架可被動降溫

Tube

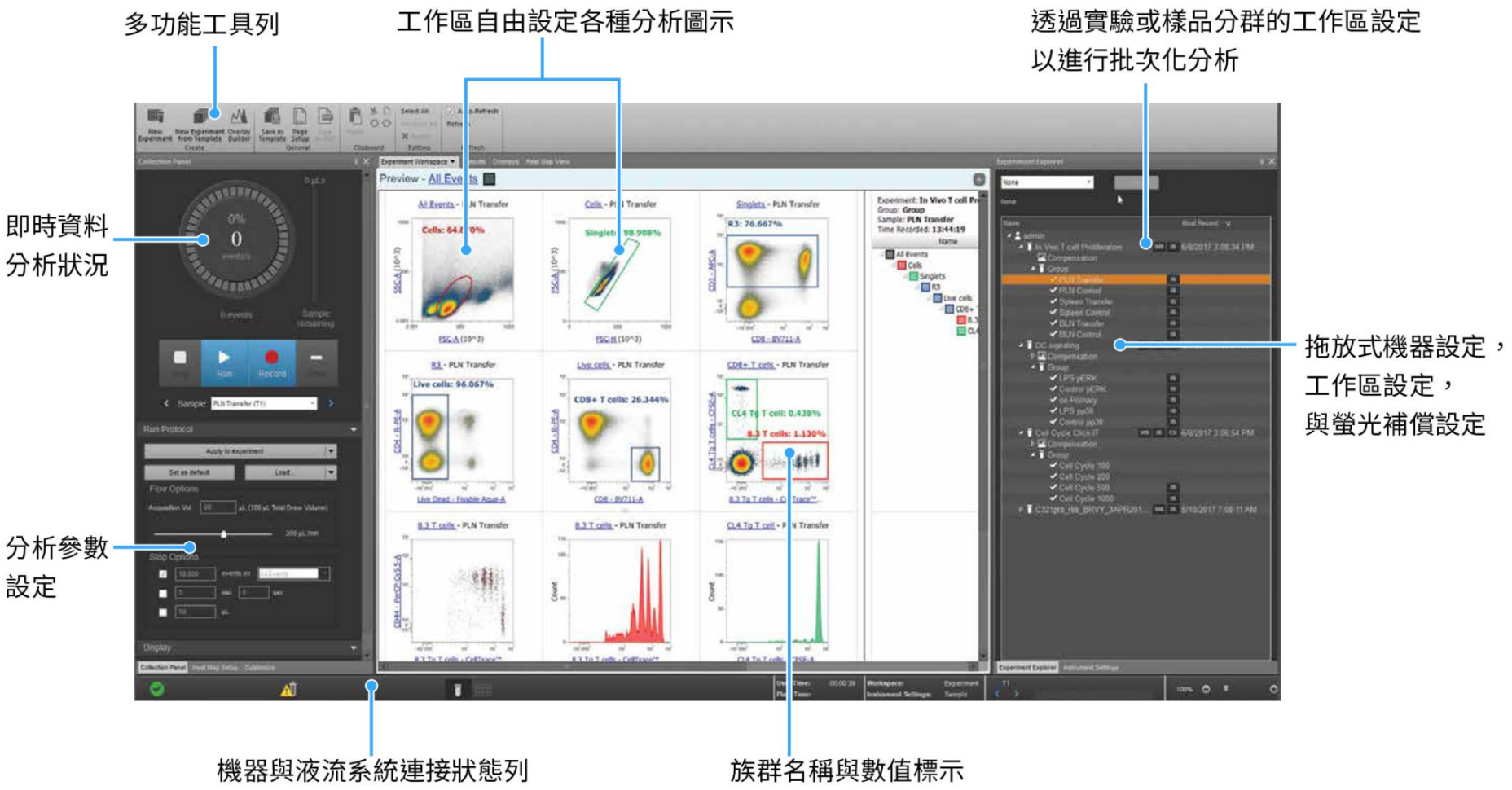


Autosampler





Attune NxT Software



Attune CytPix

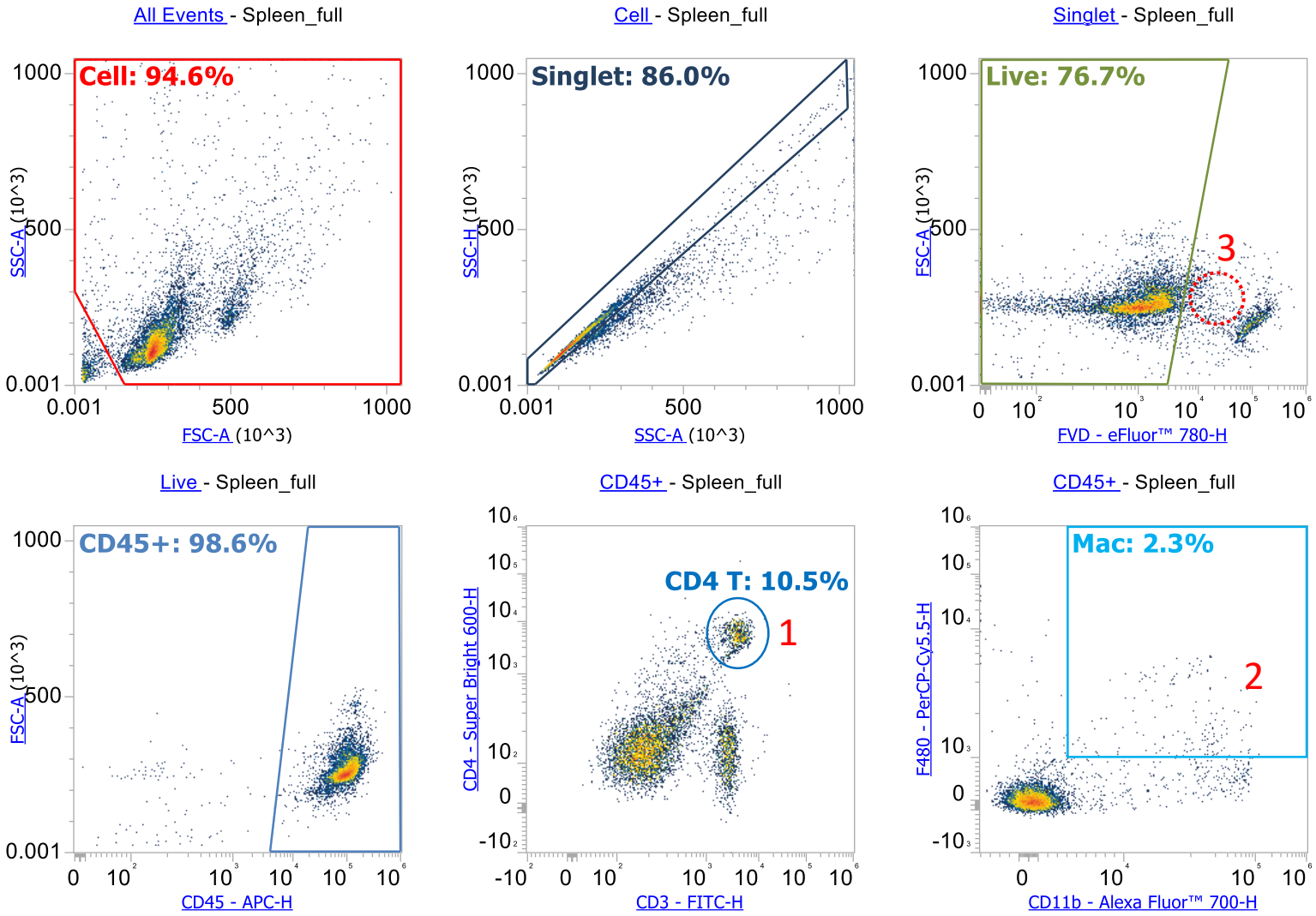
Plus

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

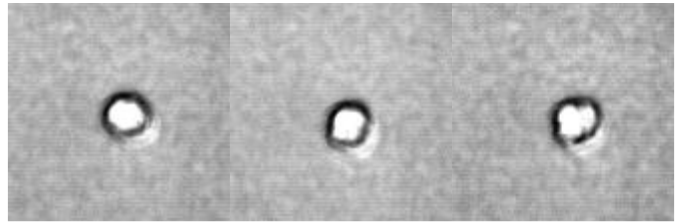
- Correlate event to image
- **0.3 µm** per pixel, 0.8 µm particle detection (20X)
- **25 parameters** from image analysis

https://www.thermofisher.com/tw/en/home/life-science/cell-analysis/flow-cytometry/flow-cytometers/attune-nxt-flow-cytometer/models/cytpix.html

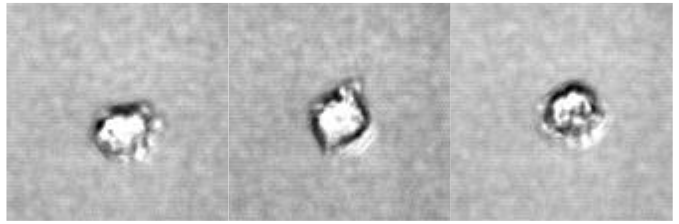
Mouse Splenocyte



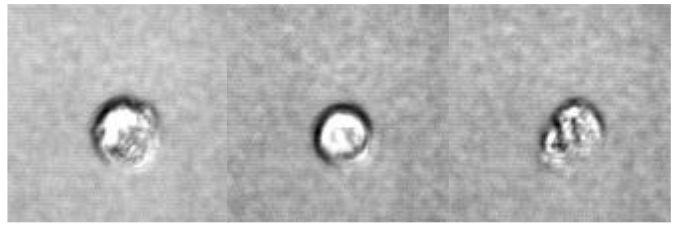
1. CD4 T Cell



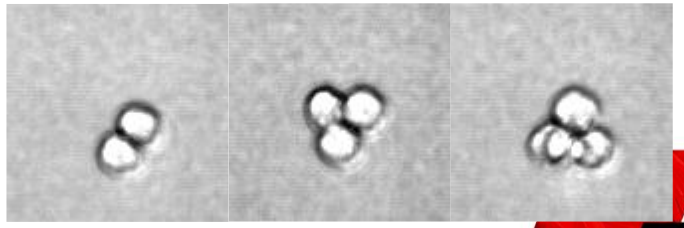
2. Macrophage



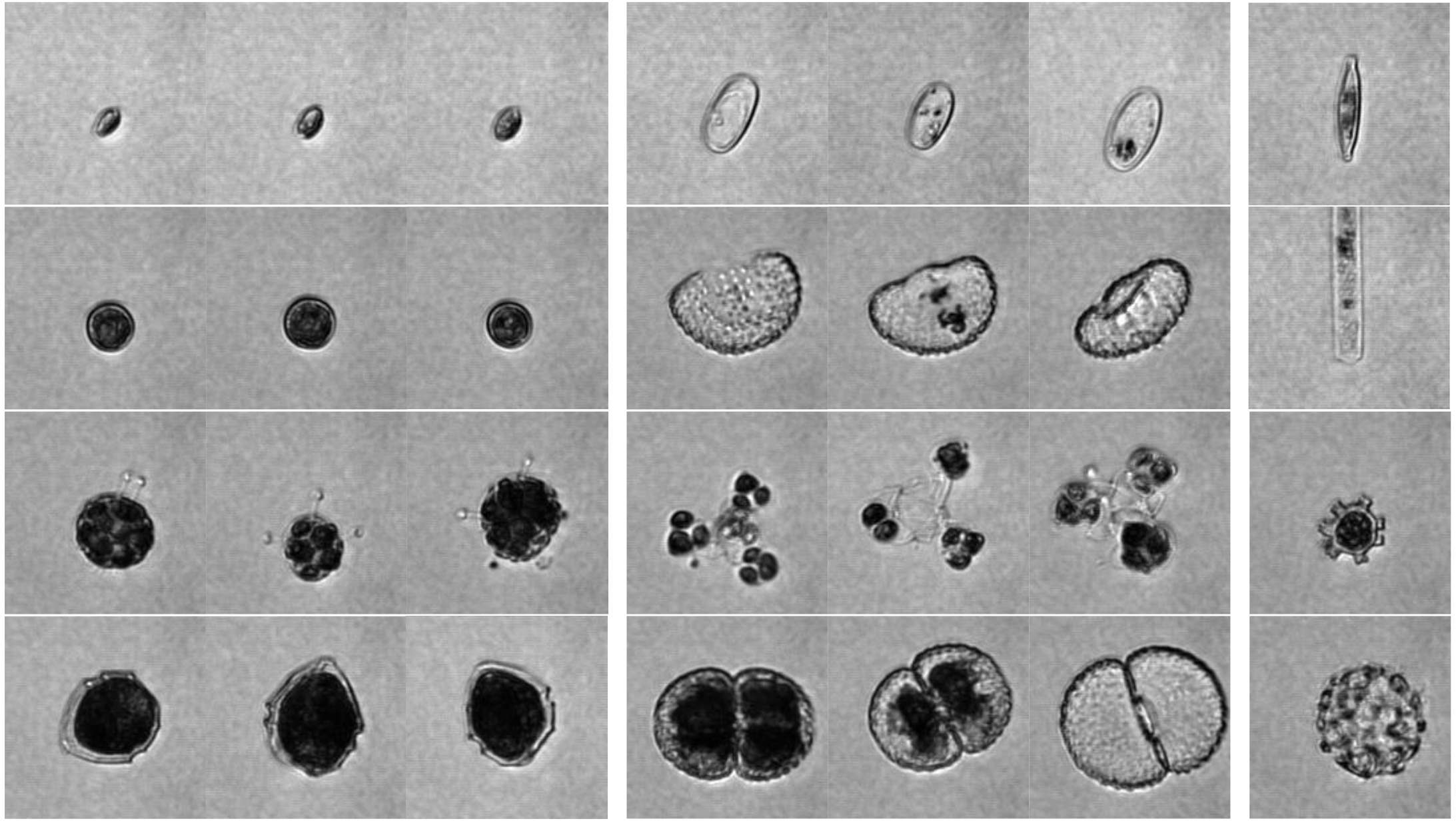
3. Dying Cells?



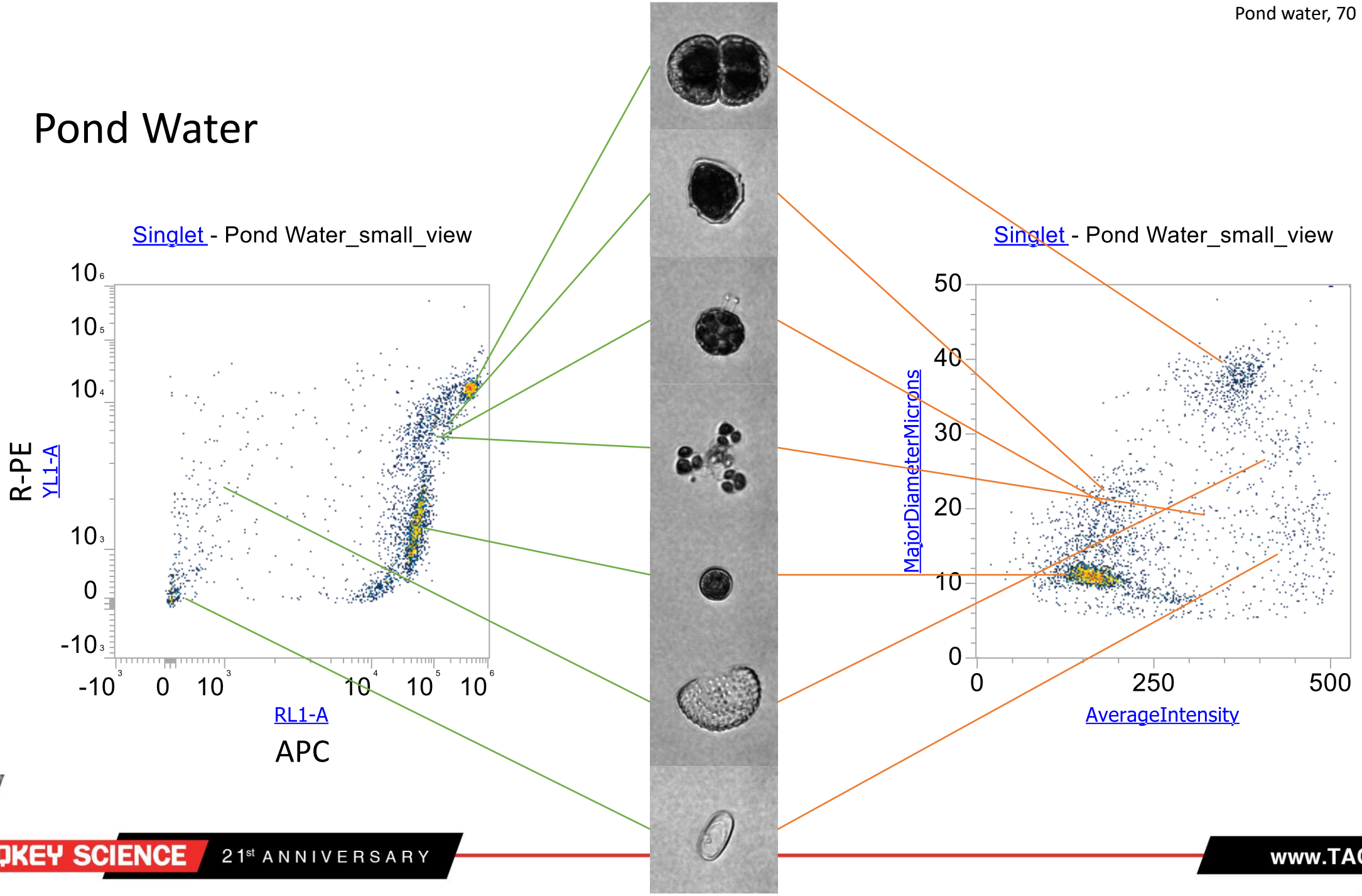
4. Particle Count > 1



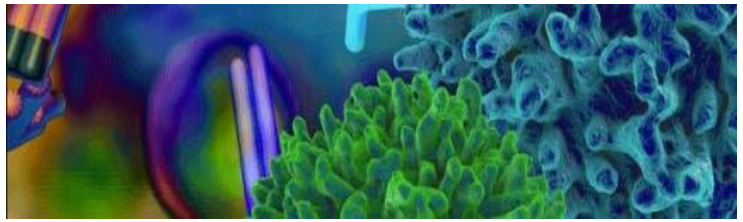
Pond water, 70 µm filtered, fixed



Pond Water


Singlet - Pond Water_small_view
R-PE
YL1-A
RL1-A
APC
Singlet - Pond Water_small_view
MajorDiameterMicrons
AverageIntensity

Flow Cytometry Learning Resource



Flow Cytometry Learning Center

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

Molecular Probes™ 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™ 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.

Thank you

ts@taqkey.com