Flow Cytometry

Basic Introduction and Principle- FACSCanto II

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Topic

- **Introduction of Flow Cytometry**
- **Instrument setup**
- **Application of Flow Cytometry**

What is Flow Cytometry?

Flow = Fluid

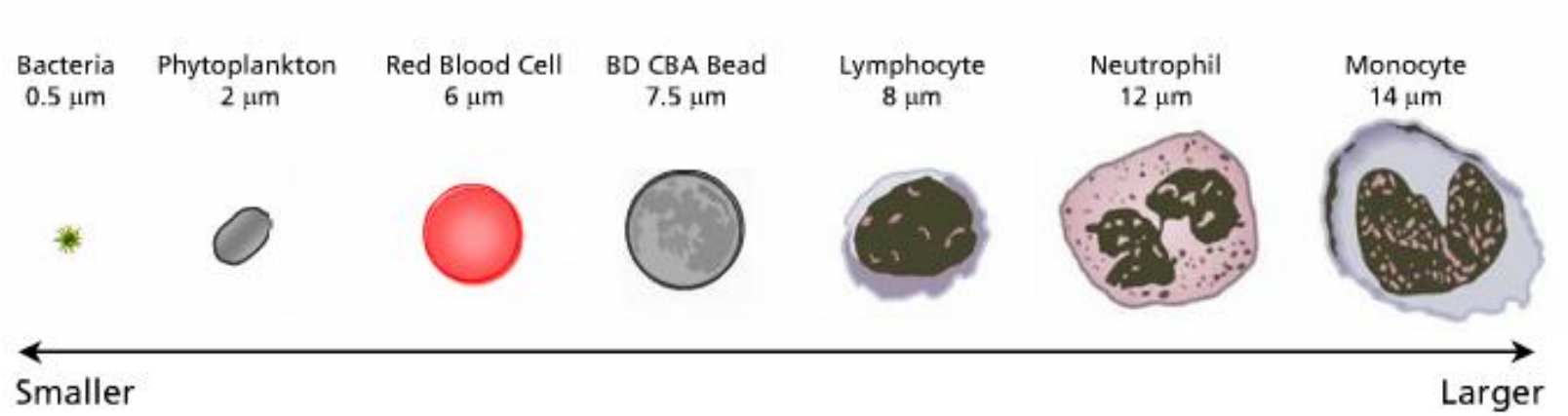
Cyto = Cell

Metry = Measurement

A variety of measurements are made on cells, cell organelles, and other objects suspended in a liquid and flowing at rates of several thousands per second through a flow chamber.

Particle Size

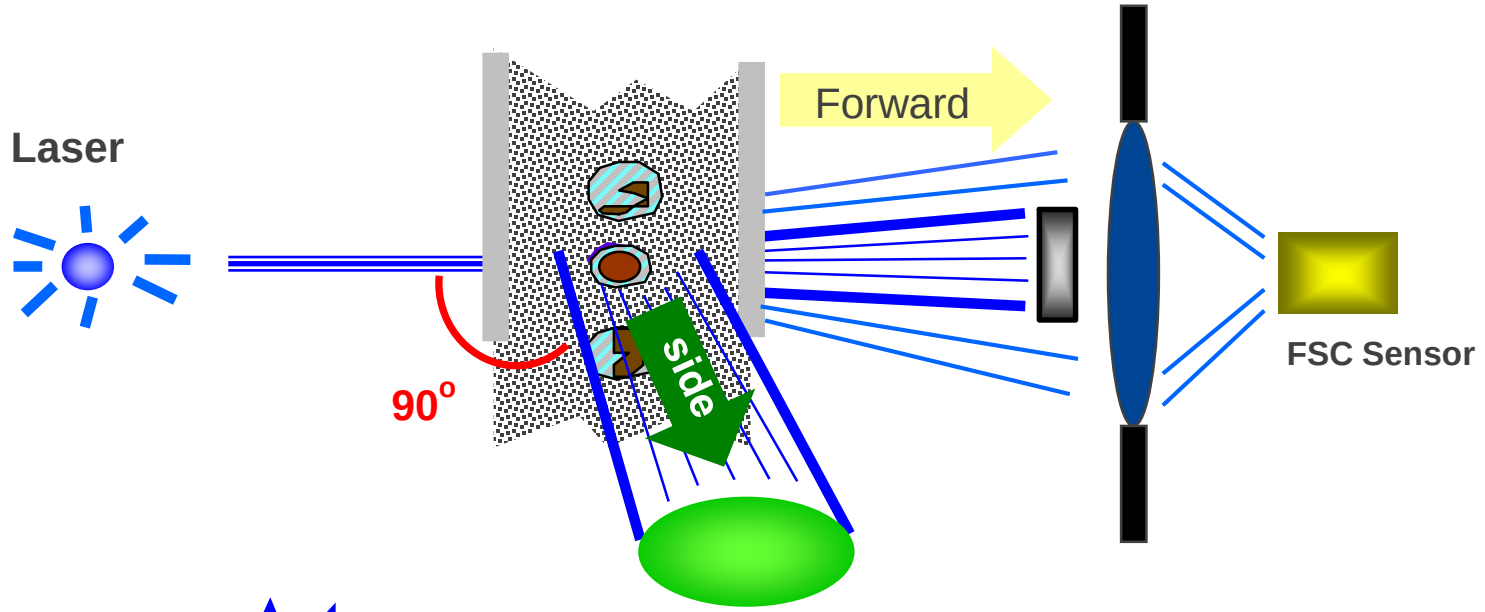
- Detection range: 0.5~50μm

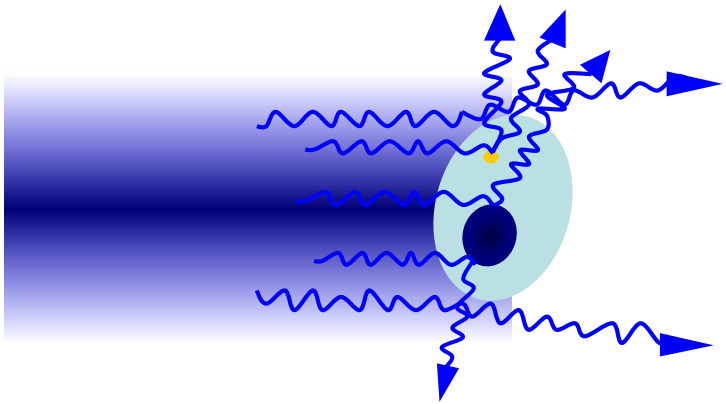


What Can a Flow Cytometer Tell Us About a Cell?

- Its relative size (Forward Scatter—**FSC**；前向散射光)
- Its relative granularity or internal complexity (Side Scatter—**SSC**；側向散射光)
- Its relative **fluorescence intensity**

Scatter Light





Forward Scatter—diffracted light

- **Related to cell surface area**
- **Detected along axis of incident light in the forward direction**

Side Scatter—reflected and refracted light

- **Related to cell granularity and complexity**
- **Detected at 90° to the laser beam**

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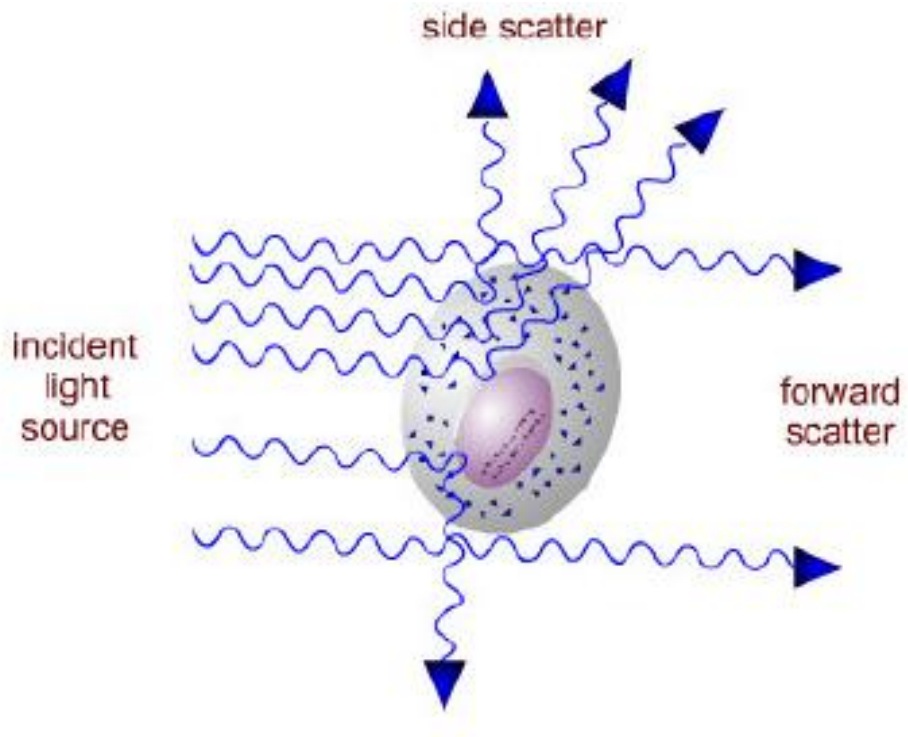
Scatter Light

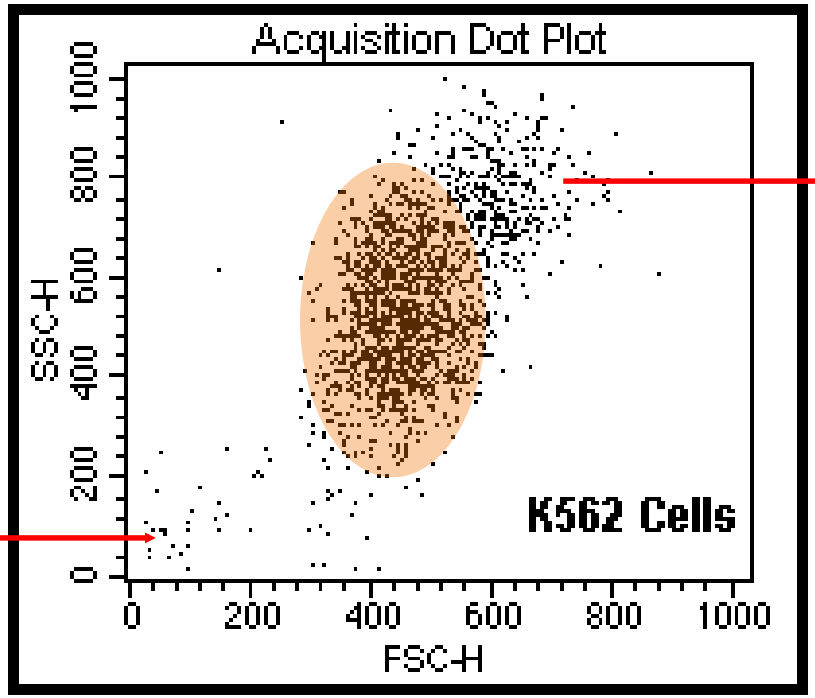


SSC ***Internal Complexity Granularity***

FSC ***Size Shape Surface Refractive index***

Scatter Light indicating physical properties of cell

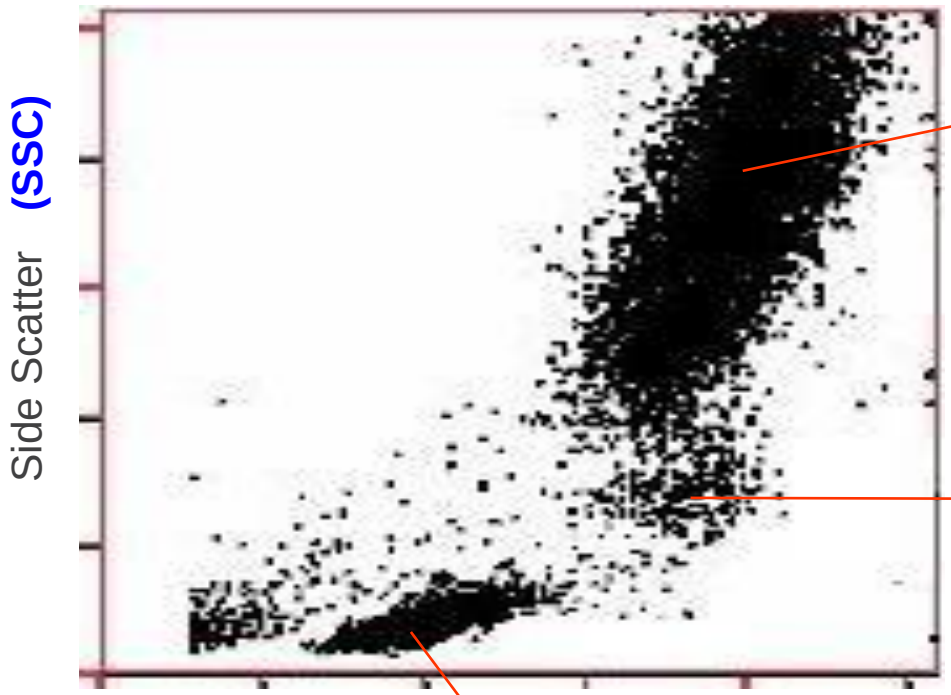
Cell Line



Aggregation

Cell debris

Ex. Lysed Whole Blood



Neutrophils

Monocytes

Lymphocytes

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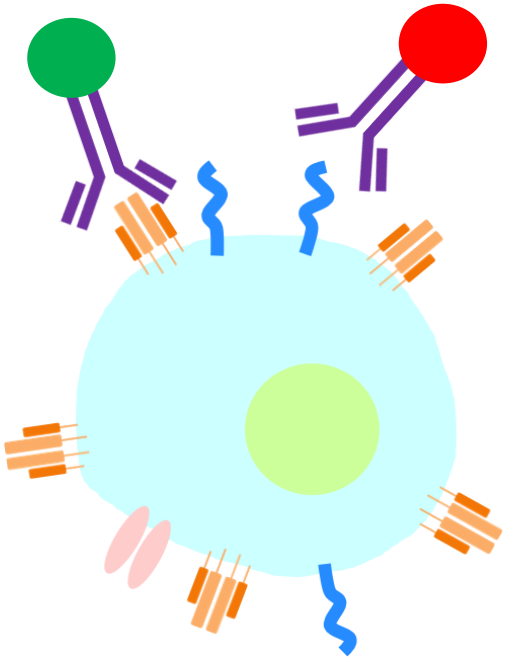
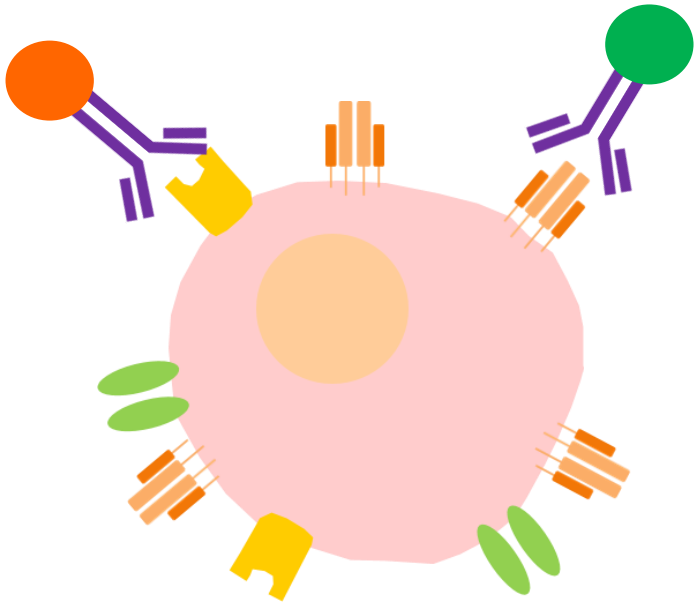
Flow Cytometry Detection Principle



Helper T



Cytotoxic T

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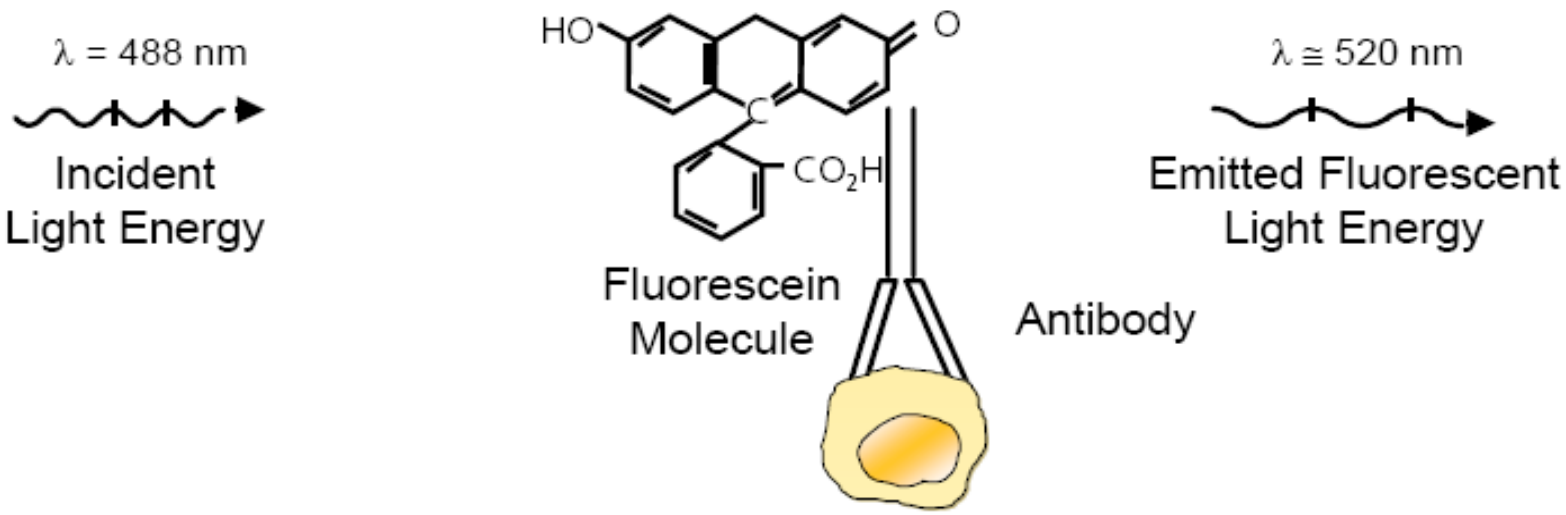
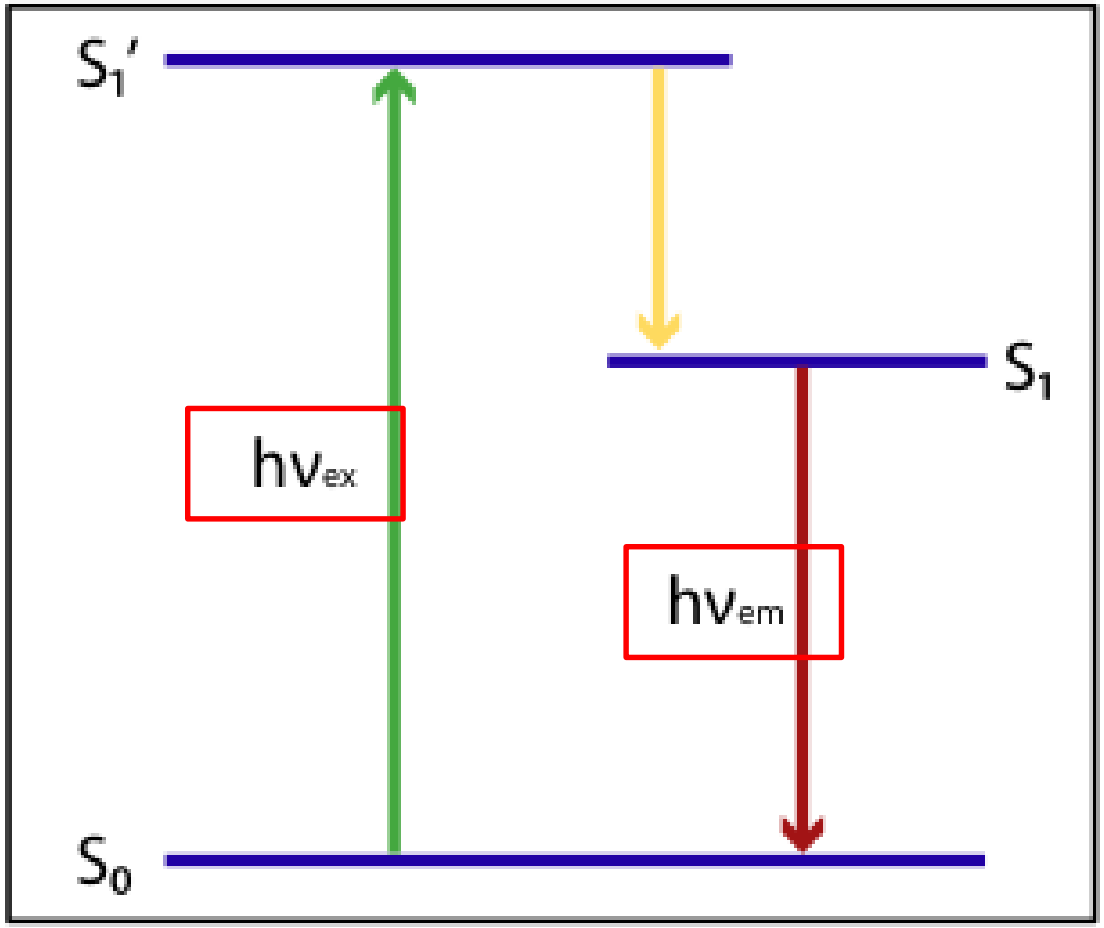
Fluorescence Light

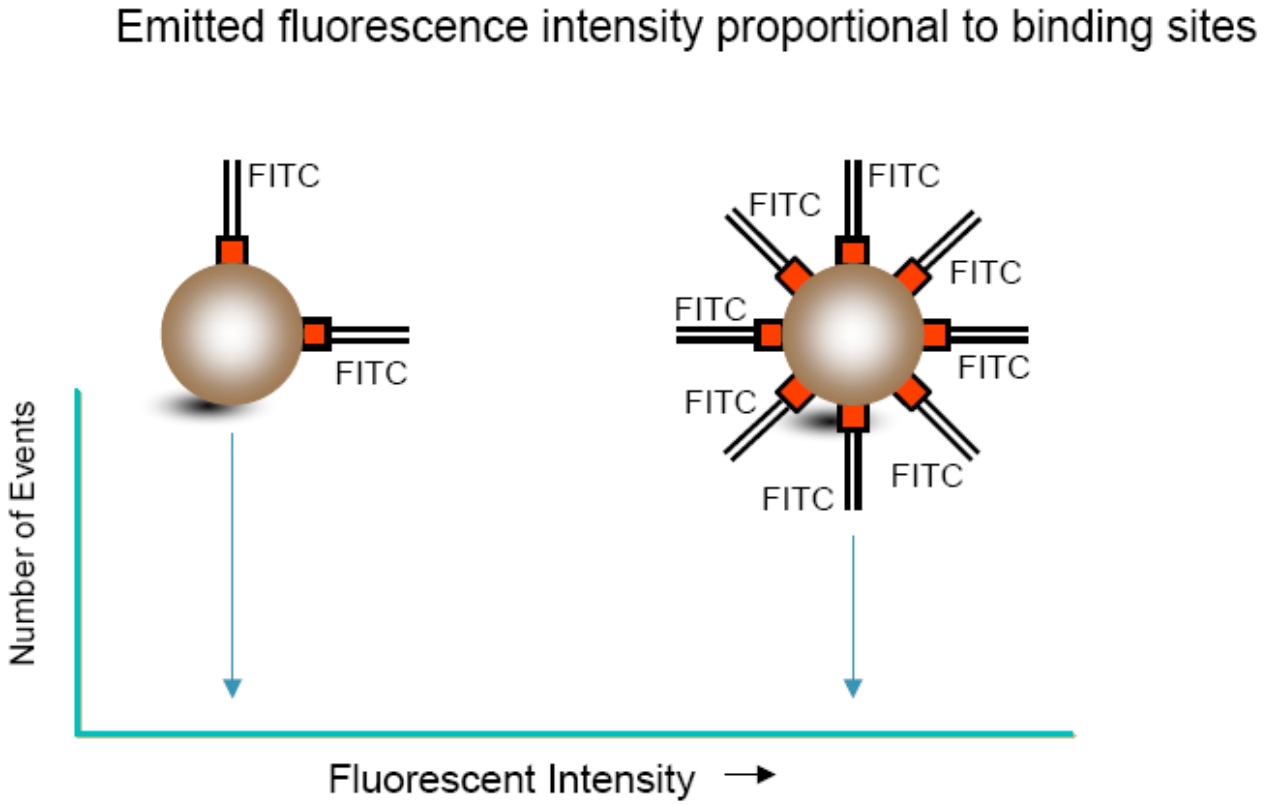


- The fluorochrome absorbs energy from the laser.
- The fluorochrome releases the absorbed energy by:
  - vibration and heat dissipation.
  - emission of photons of a longer wavelength.

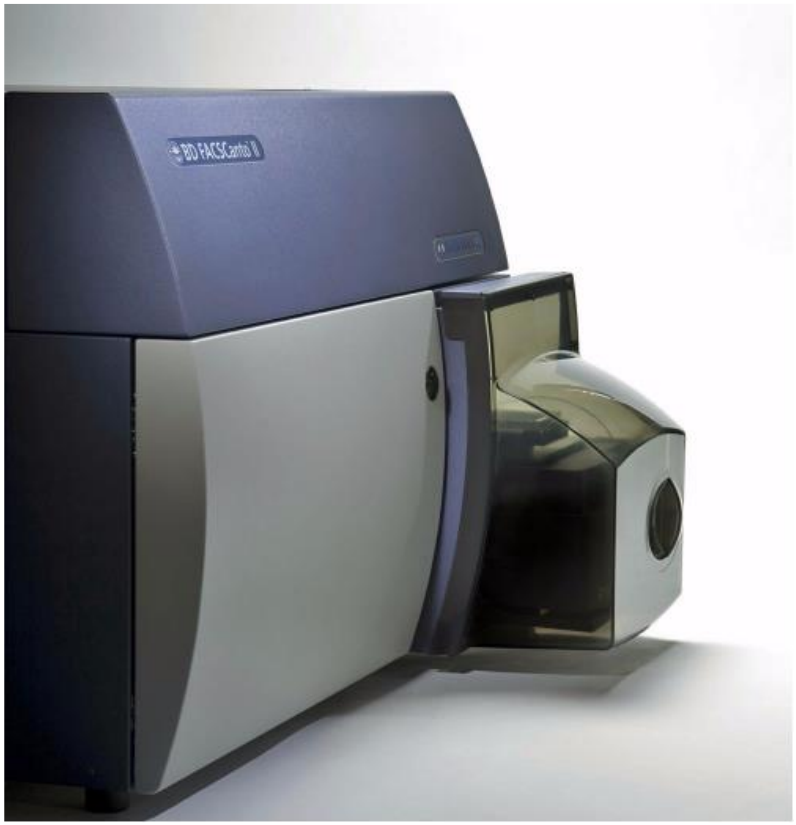
Fluorescence Light



Fluorescence intensity



BD FACSCanto II™



Main Component

Fluidics 液流系統

To introduce and focus the cells for interrogation.

Optics 光學系統

To generate and collect the light signals.

Electronics 電子系統

To convert the optical signals to proportional digital signals, process the signals, and communicate with the computer.

Fluidics - BD FACSCanto II™

Housekeeping Solution Capacities

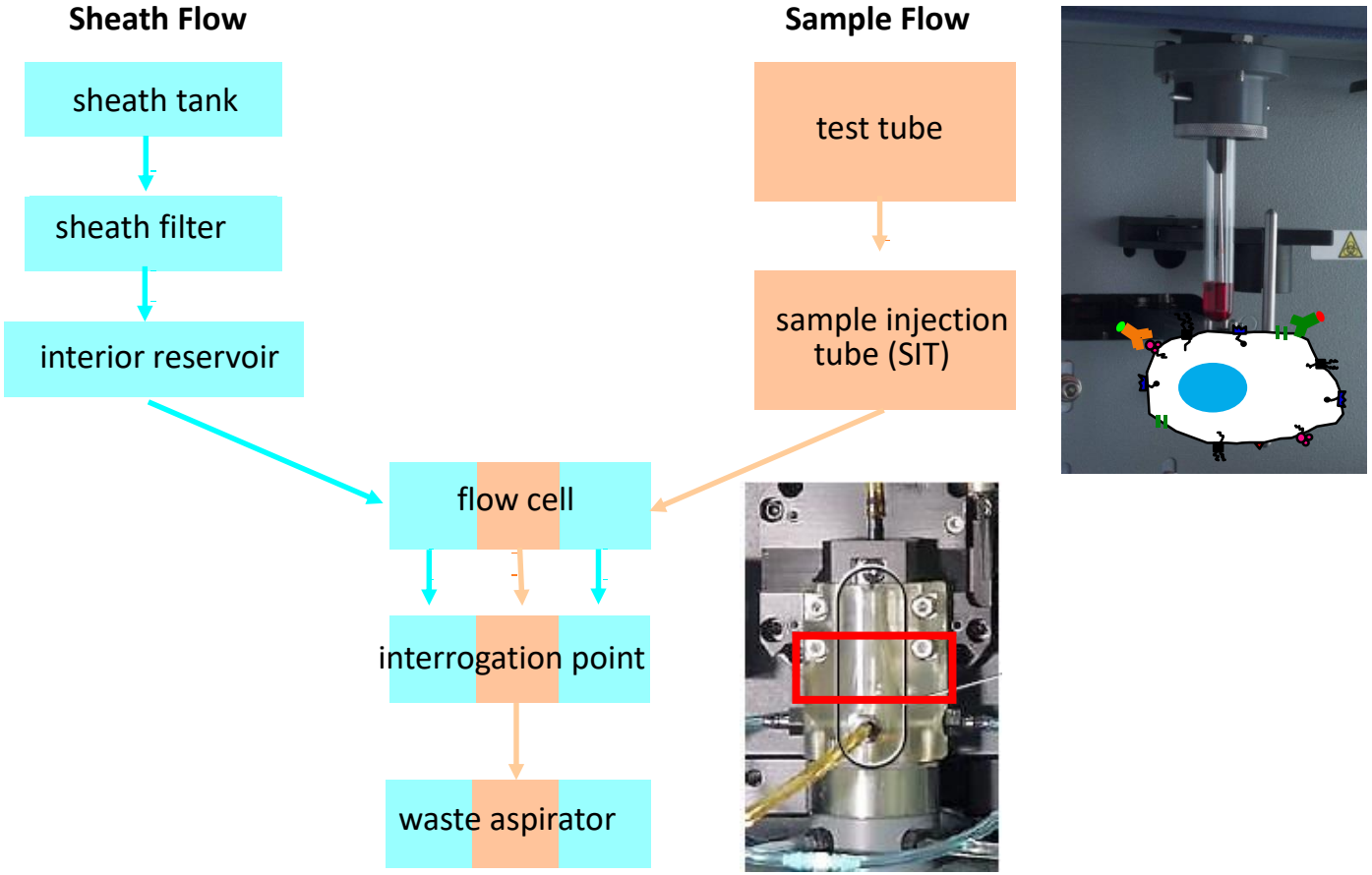
BD FACSFlow™ sheath solution 20 L

BD™ FACSClean solution 5 L

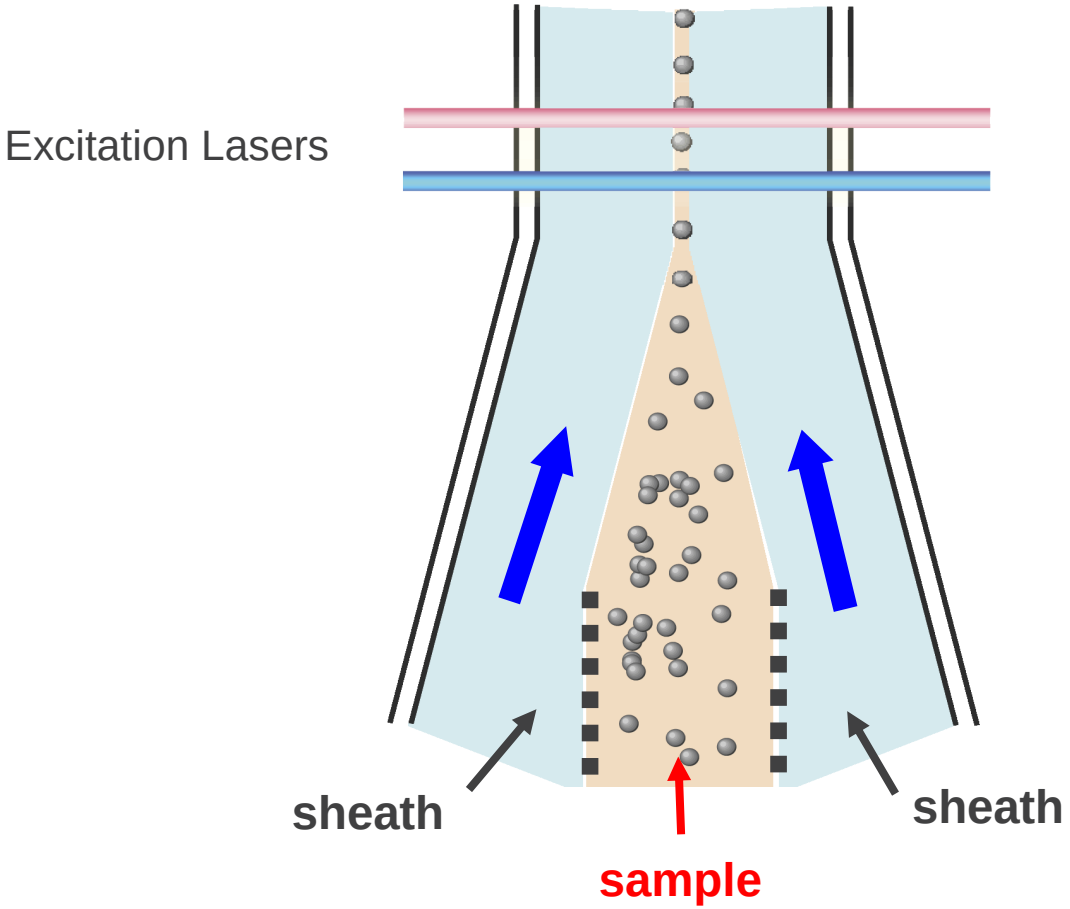
BD FACS™ shutdown solution 5 L

Waste tank 10 L

Fluidics - BD FACSCanto II™



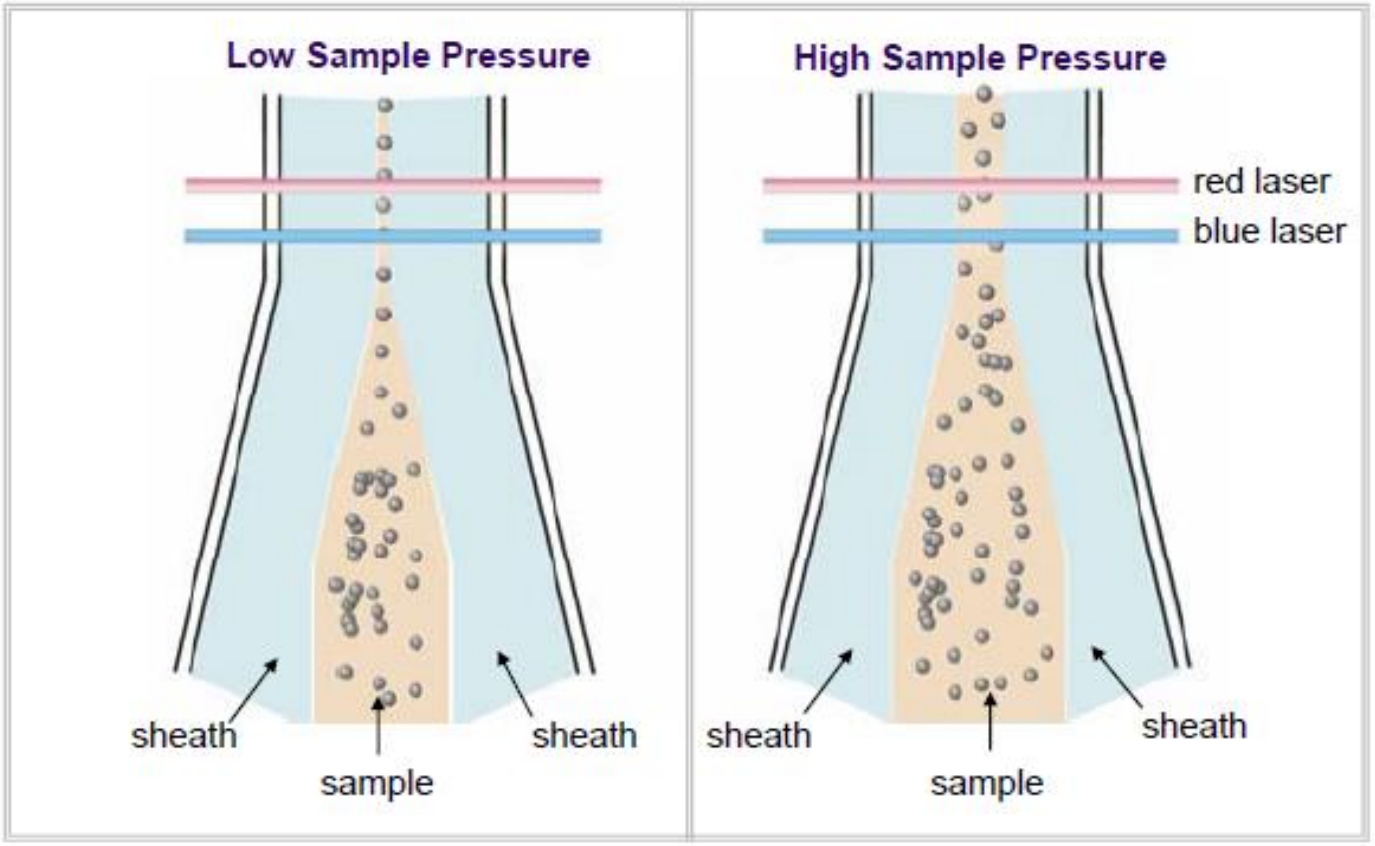
Sample Flow



Hydrodynamic Focusing

Flow Cell

Sample Differential



FACSCantoII flow rate

Low = 10 μL/min, Medium = 60 μL/min, High = 120 μL/min

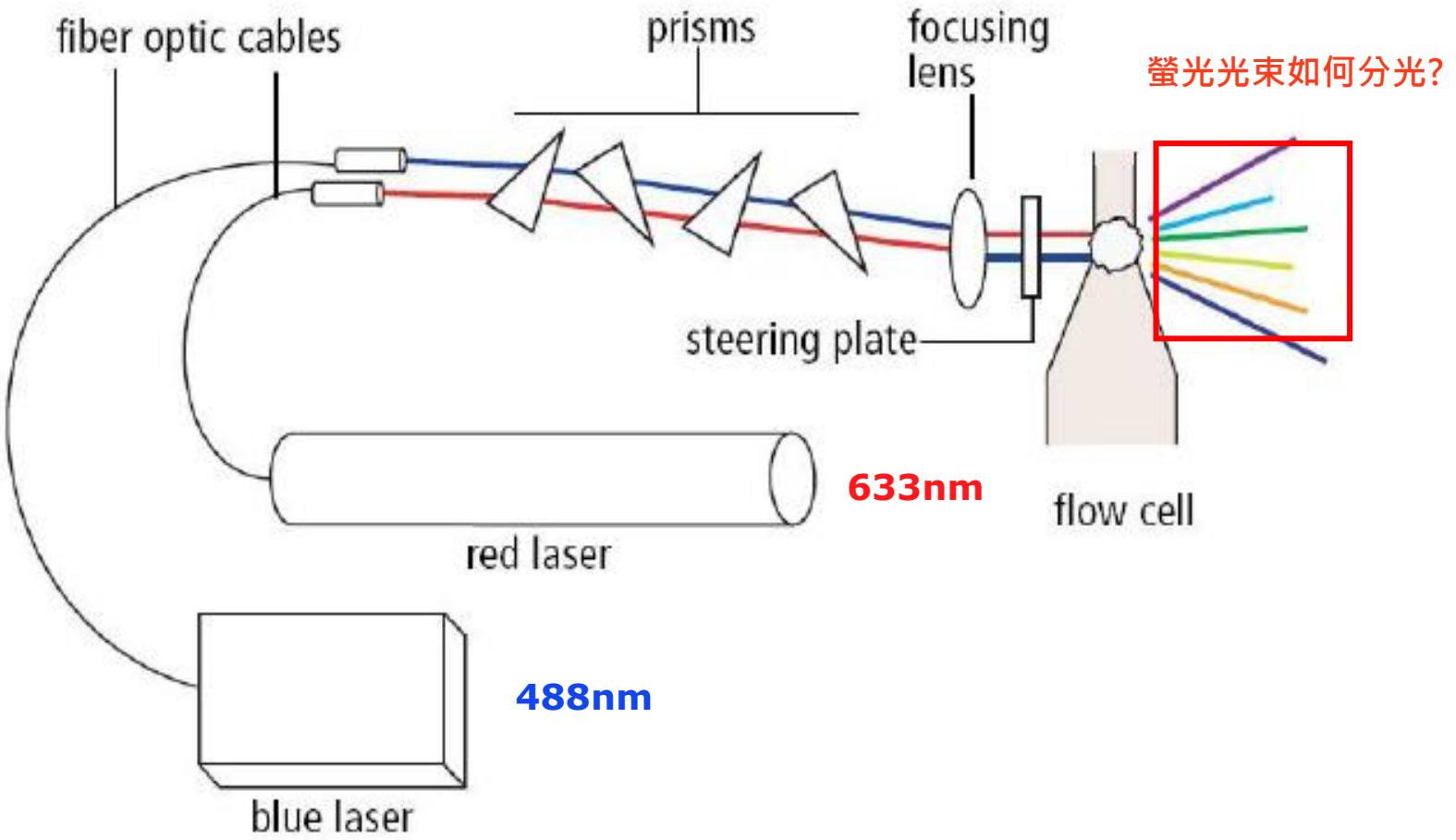
＊細胞週期實驗請選用低流速上機

Optical

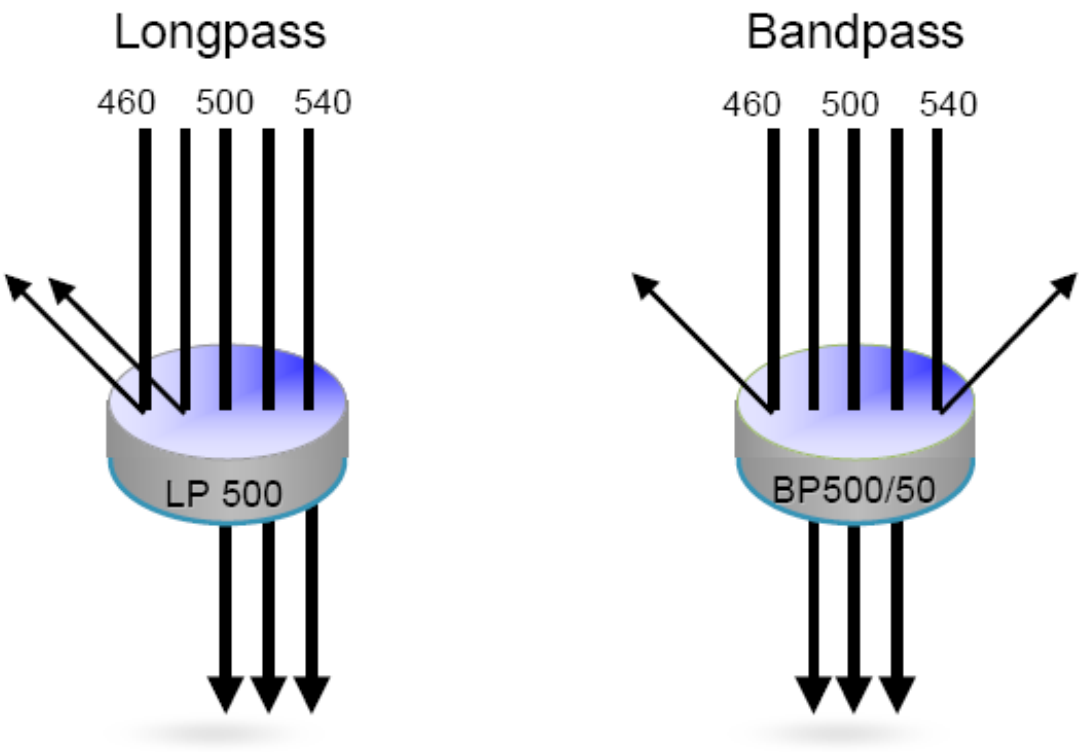
- Excitation optics:
  - Lasers
  - Filters and mirrors that route the laser light to the fluid stream

- Collection optics:
  - Fiber optic cables that direct the emitted light to the appropriate emission block
  - Filters that direct the signals in the emission block to the appropriate photomultiplier tube (PMT)

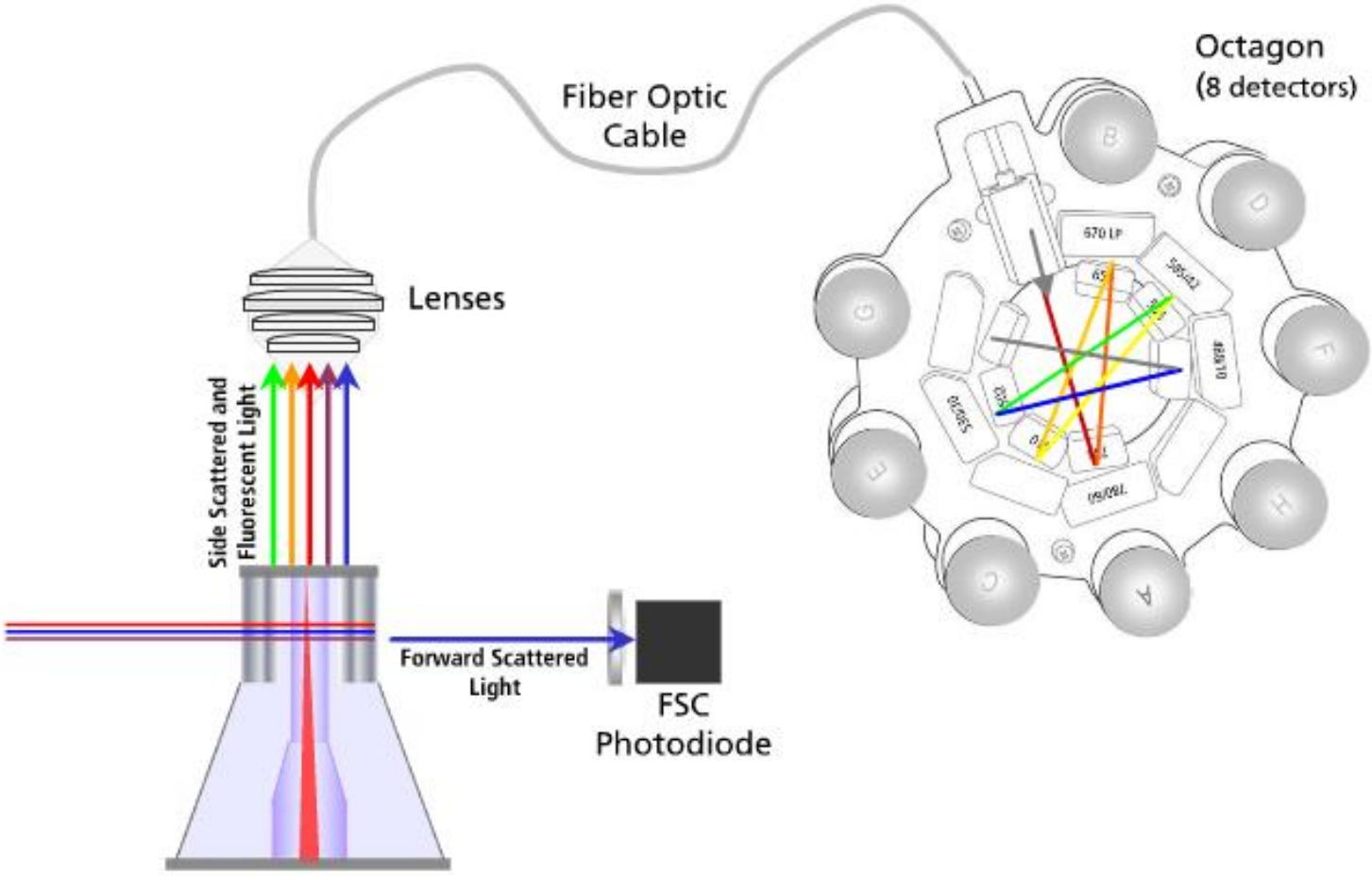
Excitation Optics



Optical Filters



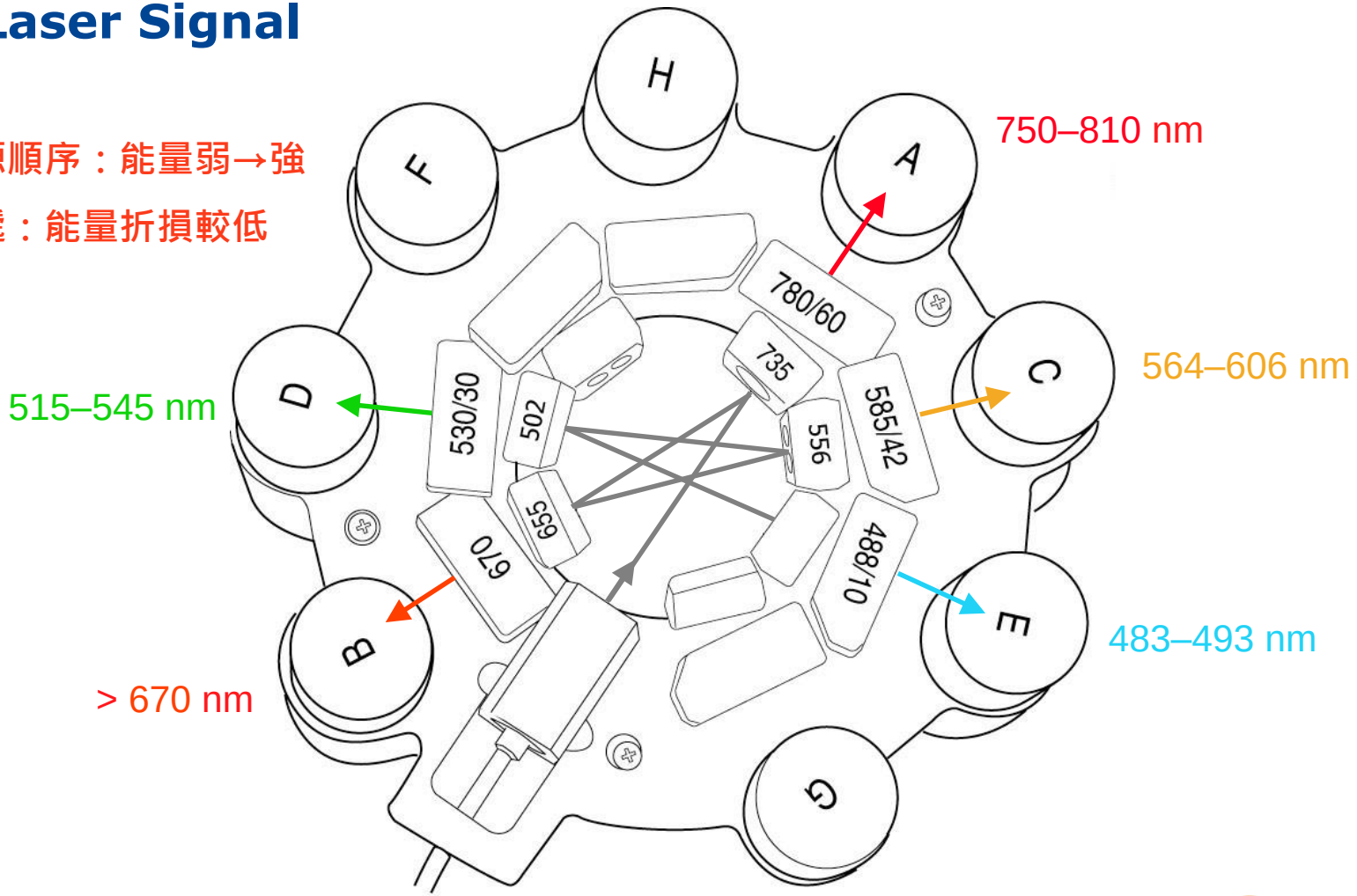
FACSCanto II™ - Collection Optics



Collection Optics—Octagon

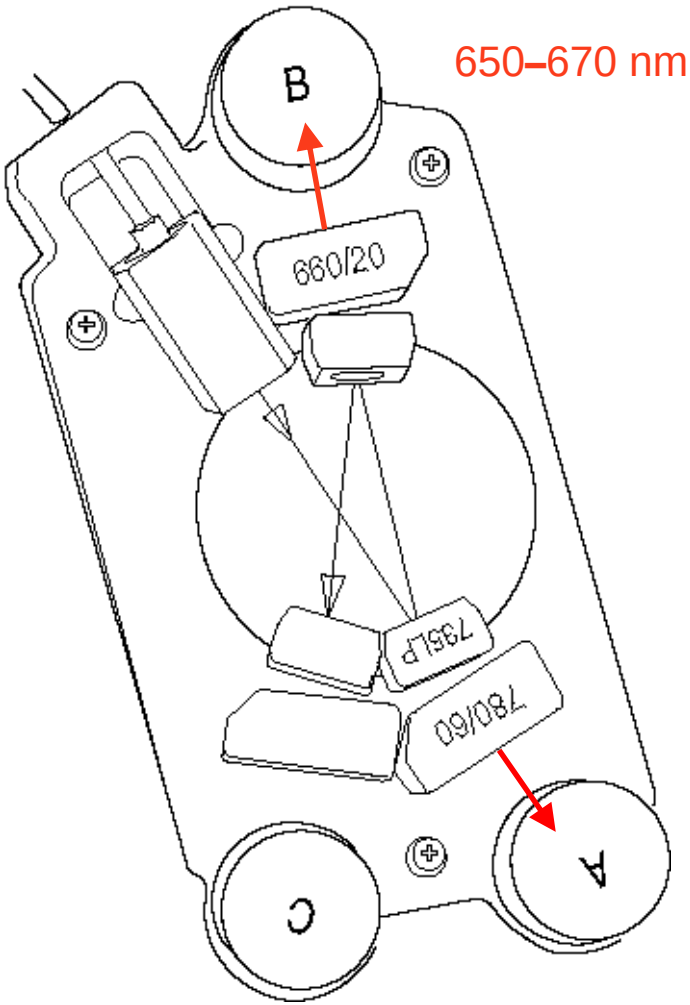
Blue Laser Signal

- 接收光源順序：能量弱→強
- 反射傳遞：能量折損較低



Collection Optics—Trigon

Red Laser Signal



650–670 nm

750–810 nm

FACSCanto II™—Octagon and Trigons

(BD專利設計)

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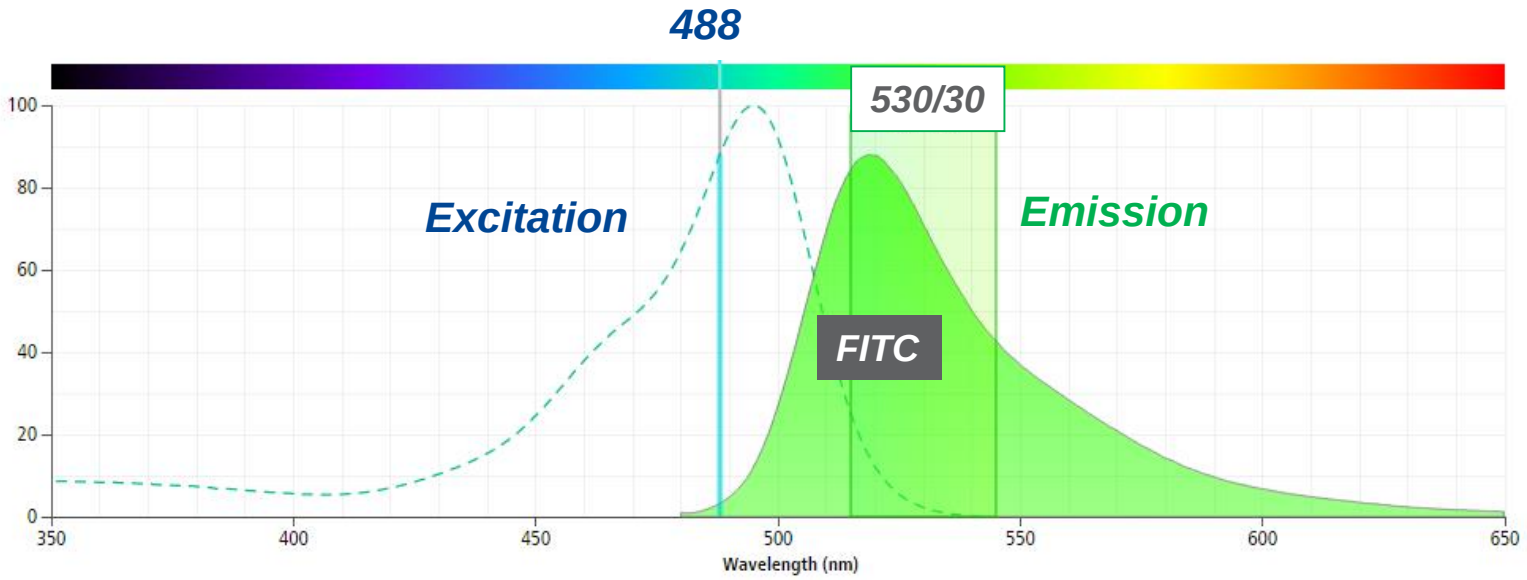
Excitation and Emission

- Use the maximum excitation wavelengths to **determine lasers** that can be used to excite the fluorochrome.

- Use the maximum emission wavelengths to **determine filters and PMTs** that can be used to measure the signal.

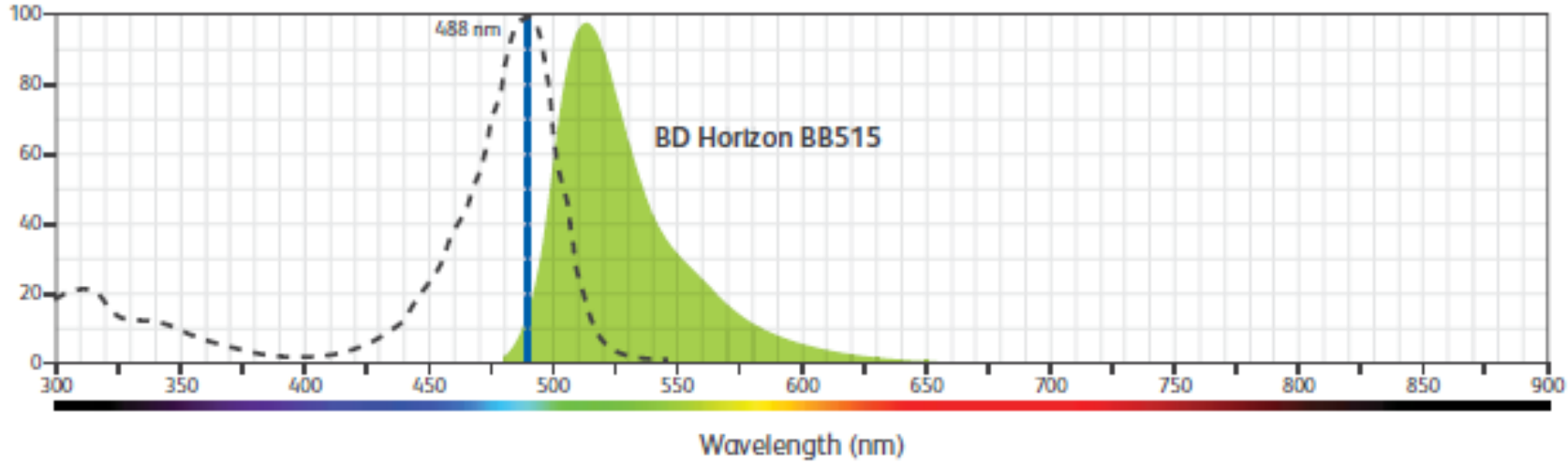


BD FACSCanto Configuration

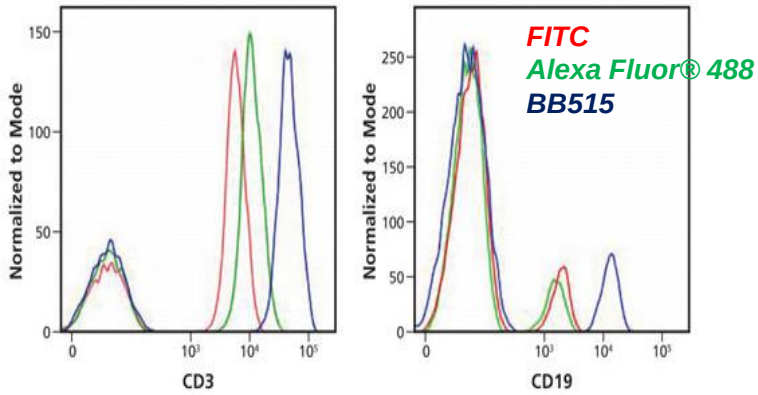
2 lasers, 4 – 2 Configuration

488nm Blue Laser		
Alexa Fluor 488, FITC, BB515	530/30	502LP
PE	585/42	556LP
	NA	610LP
PI, PerCP, PerCP-Cy5.5, BB700	670LP	655LP
PE-Cy7	780/60	735LP
640nm Red Laser		
APC, Alexa Fluor 647	660/20	N/A
	NA	685LP
APC-Cy7, APC-H7	780/60	735LP

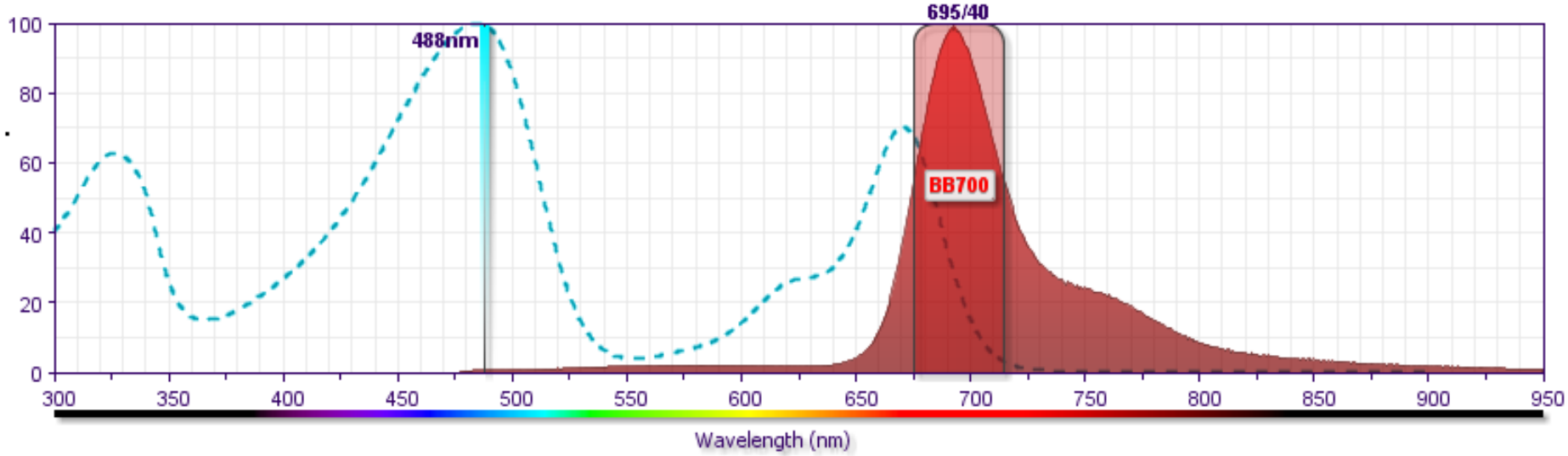
BD Horizon Brilliant Blue dyes -BB515



- BB515 offers a significantly brighter alternative to FITC
- BB515 has less spillover into the PE channel compared to FITC

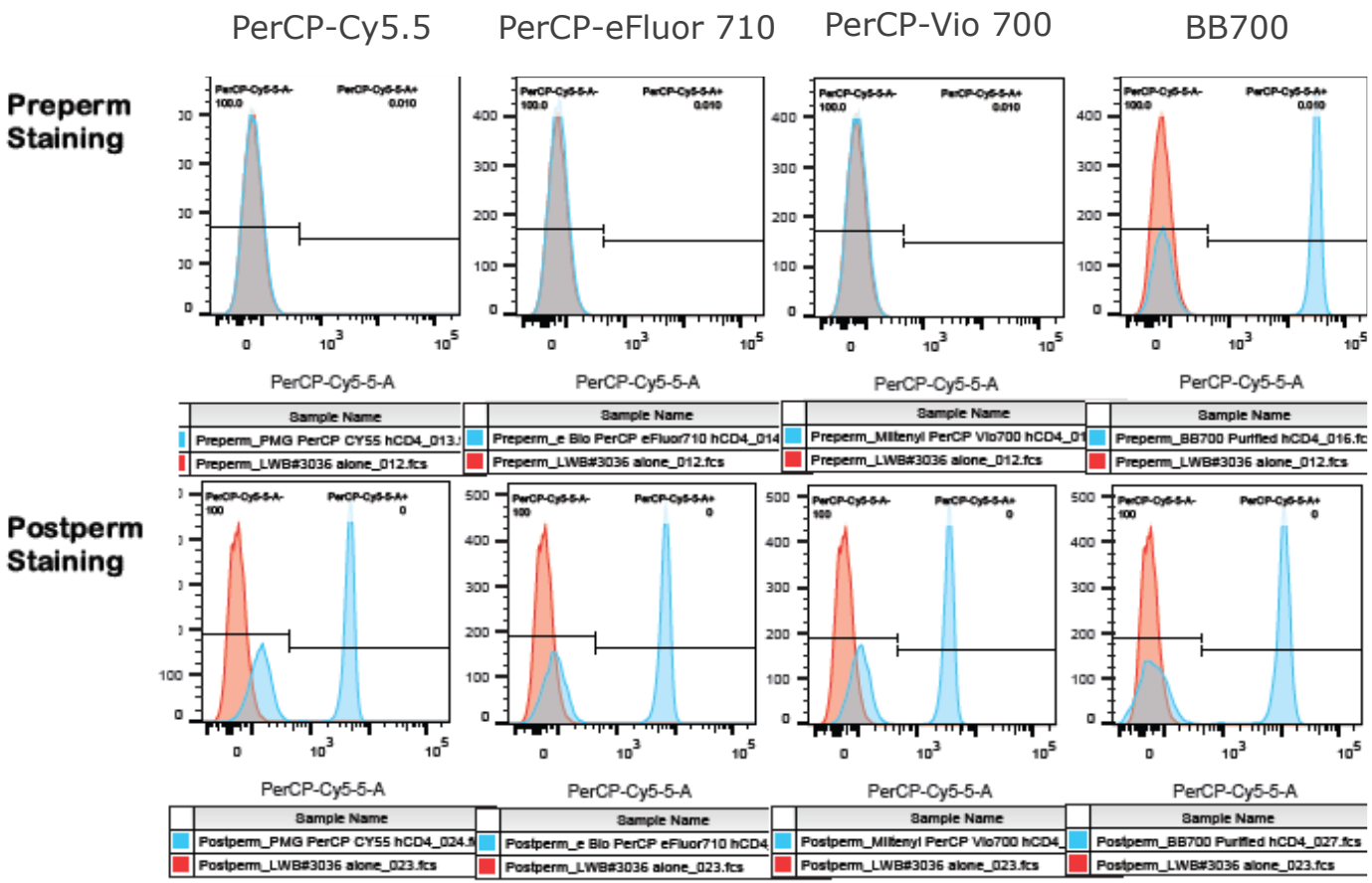


BB700 is detected in the same channel as PerCp-Cy5.5



Tandem Dye: BB core + Cy5.5 like acceptor dye
Brightness: Very Bright
Ex Max: 485 nm
Em Max: 693 nm
Filter: same as PerCP-Cy5.5 (e.g. 695/40)

BB700 is more compatible with Perm Buffer III



Electronics

- PMTs and preamps convert photons to voltage pulses.
- Analog-to-digital converters translate analog signals to proportional digital signals.
- Compute area and height for each pulse.
- Perform compensation and calculate ratios and width.
- An embedded computer interfaces with the computer workstation for data transfer.

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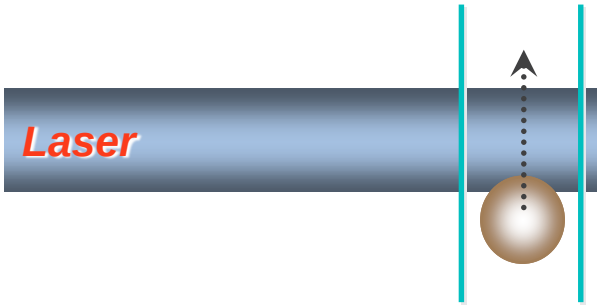
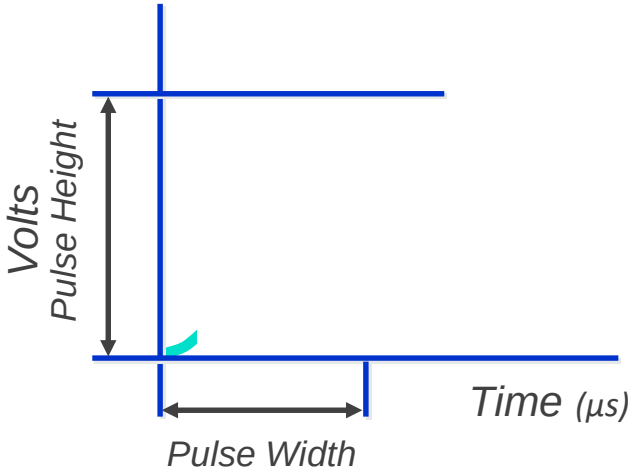
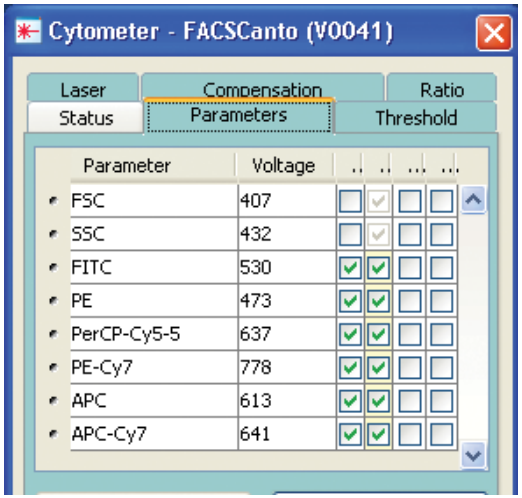
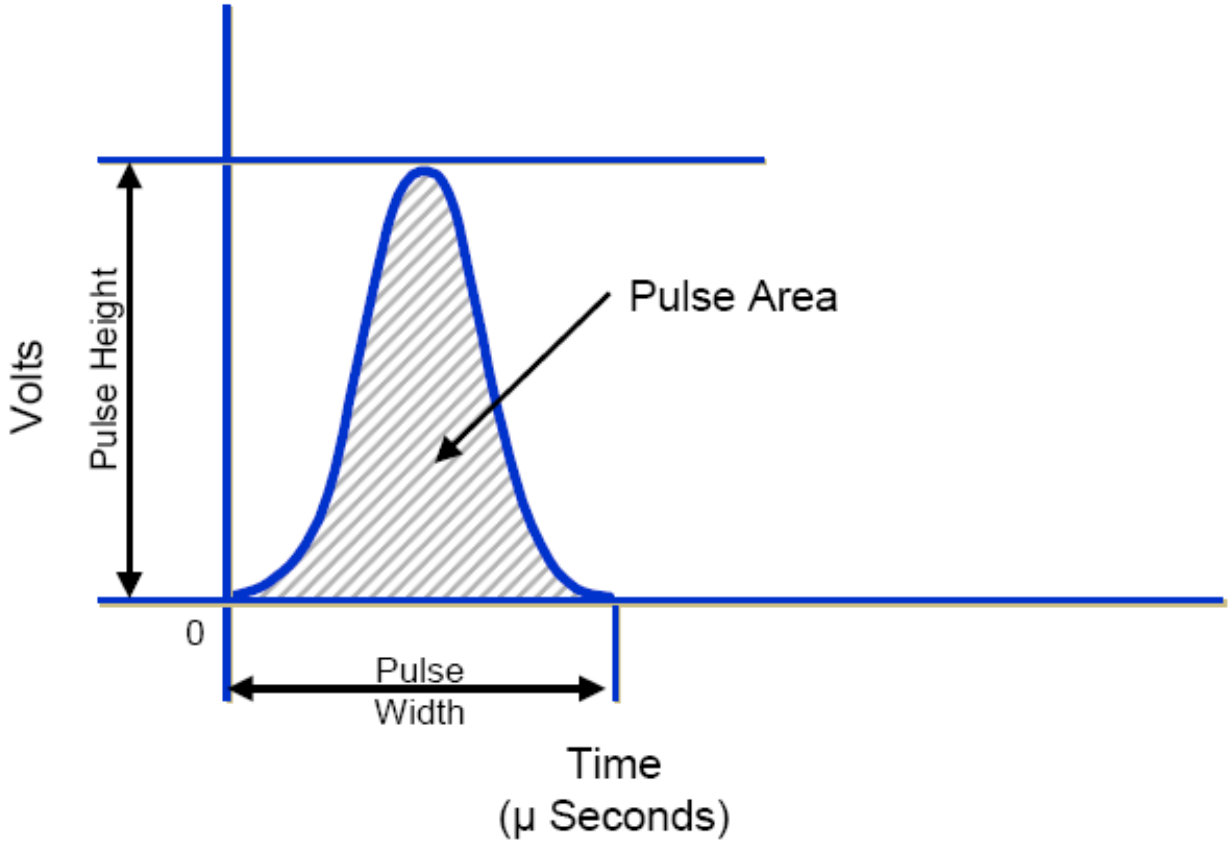
Creation of a Voltage Pulse







Quantification of a Voltage Pulse

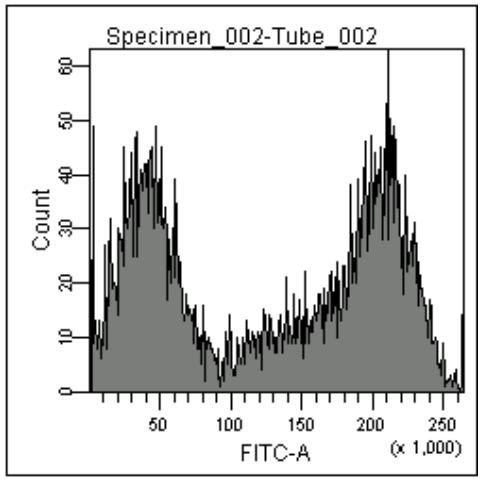


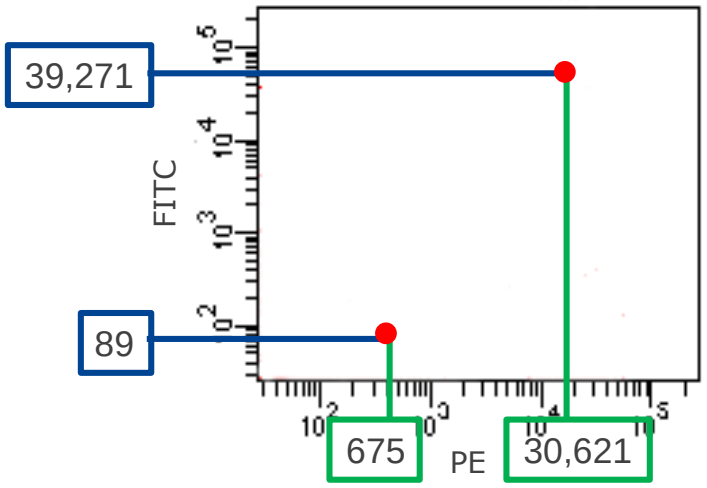
Data Storage

List-Mode Data

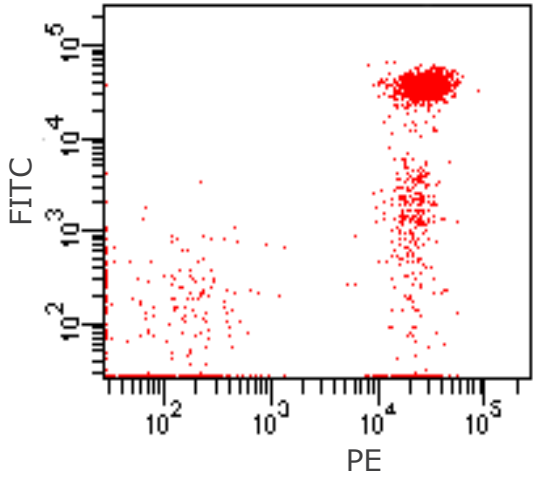
	Time	FSC	SSC	FITC	PE
Event 1	0	60	120	89	675
Event 2	10	160	65	39,271	30,621
Event 3	30	650	160	22,688	6,189

Histogram



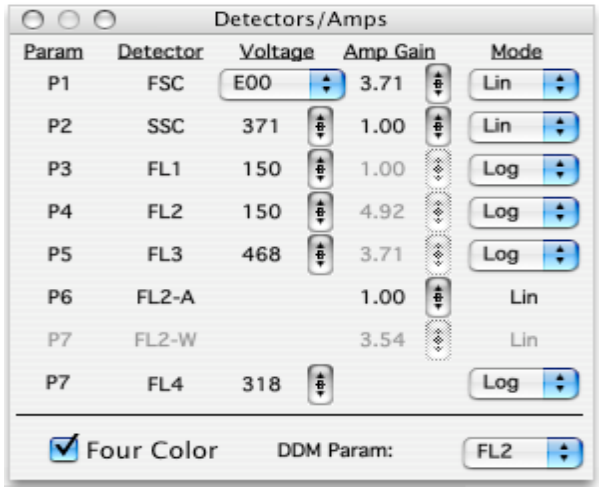


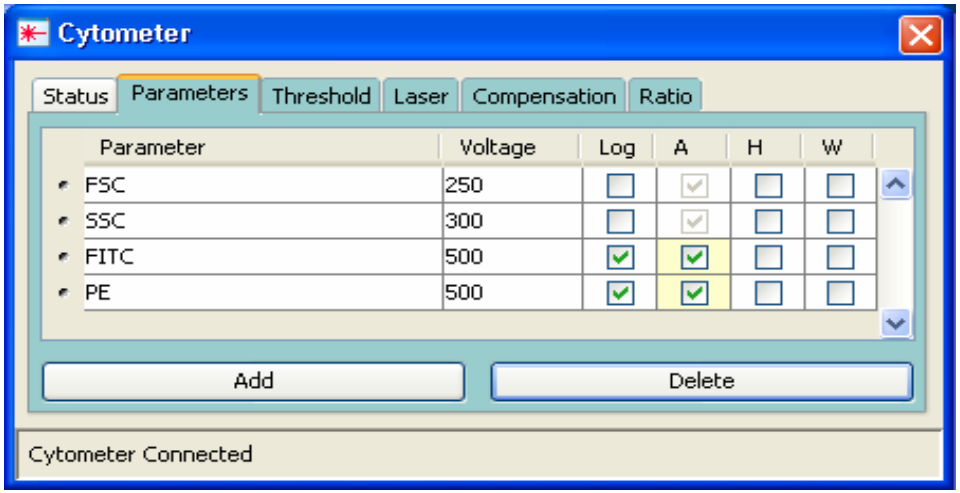
Dot Plot



Data Display

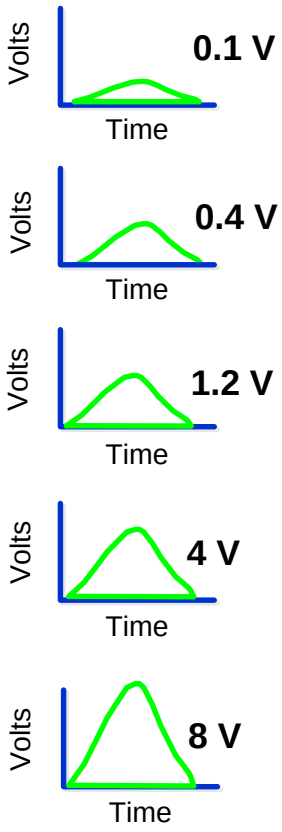
- Linear Scaling
- Log Scaling



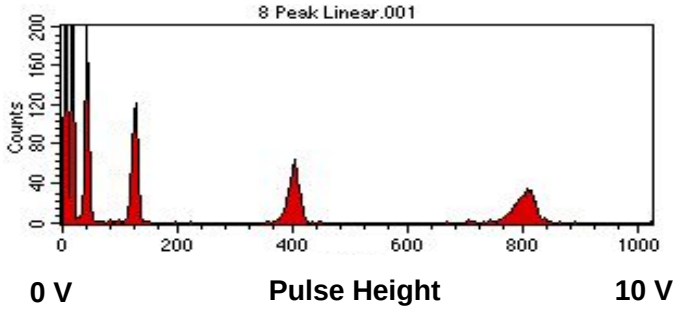


Linear vs. Log

FL1 Voltage Pulses

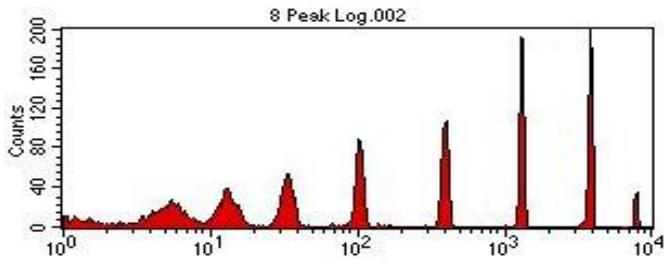


Linear mode



0 V **Pulse Height** **10 V**

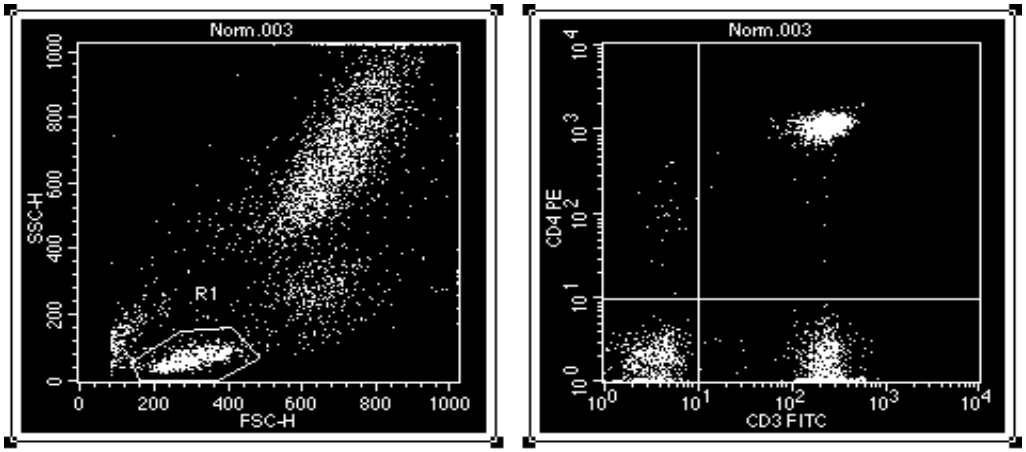
Log mode



0.001 V **0.01 V** **0.1 V** **1 V** **10 V**

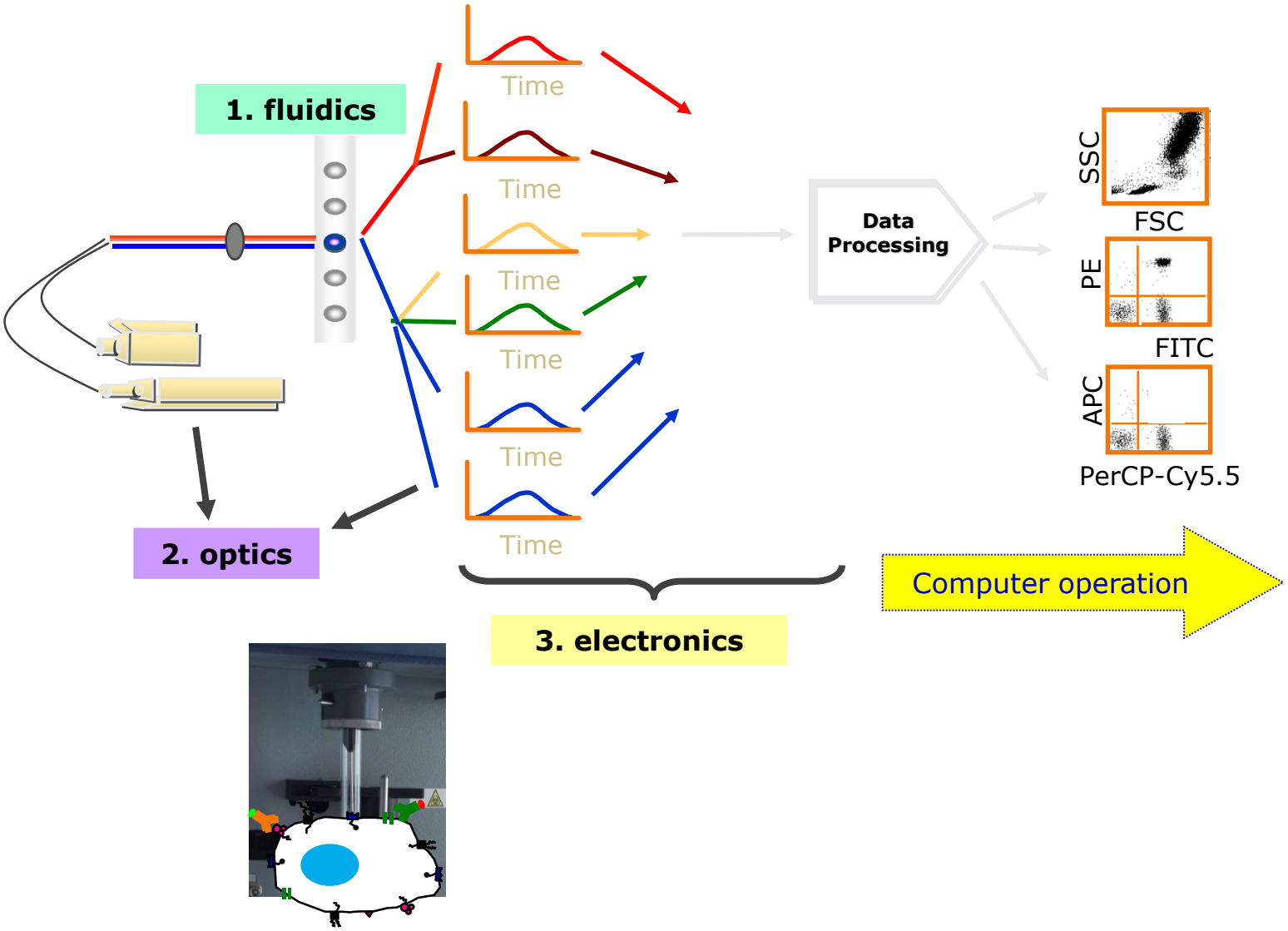
Pulse Height

Linear v. Log Amplification



- **Linear** amplification is usually used for light scatter parameters and DNA analysis.
- **Log** amplification is used for fluorescence signals with a large dynamic range.

Flow Cytometry Detection Principle

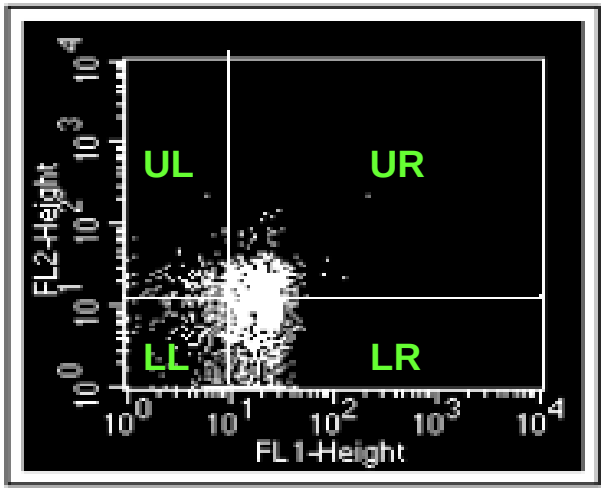


Topic

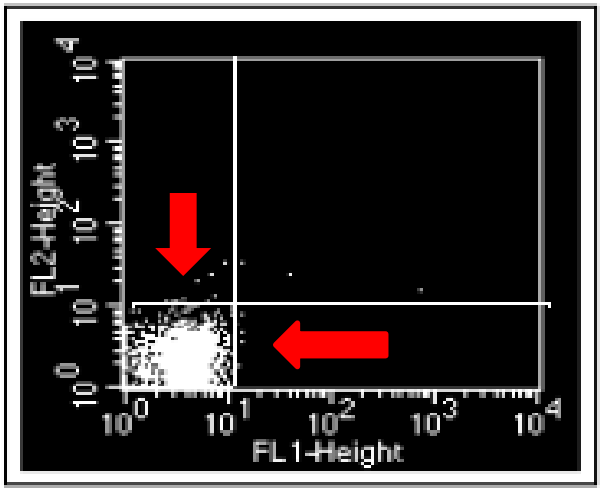
- **Introduction of Flow Cytometry**
- **Instrument setup**
- **Application of Flow Cytometry**

Auto-fluorescence

Non-stain sample



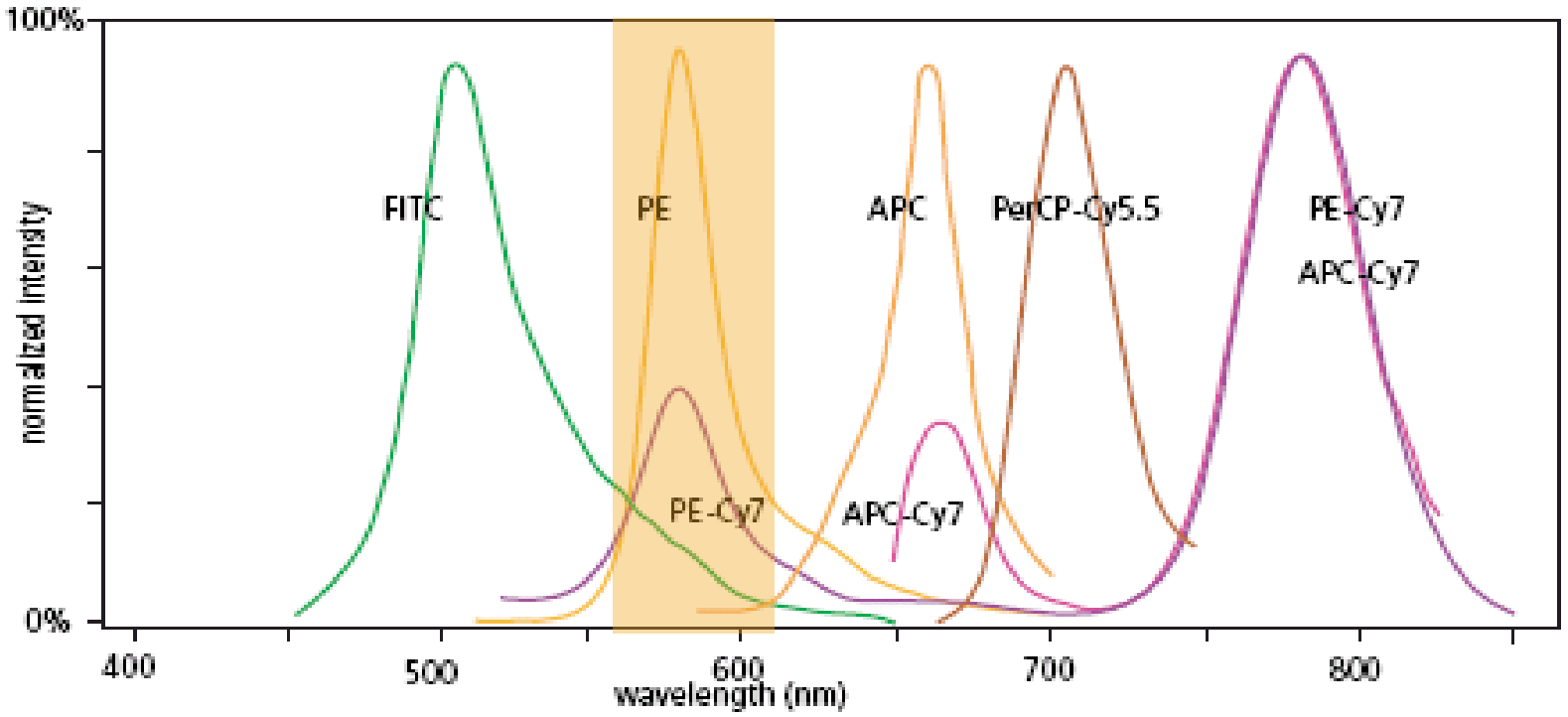
Auto-fluorescence



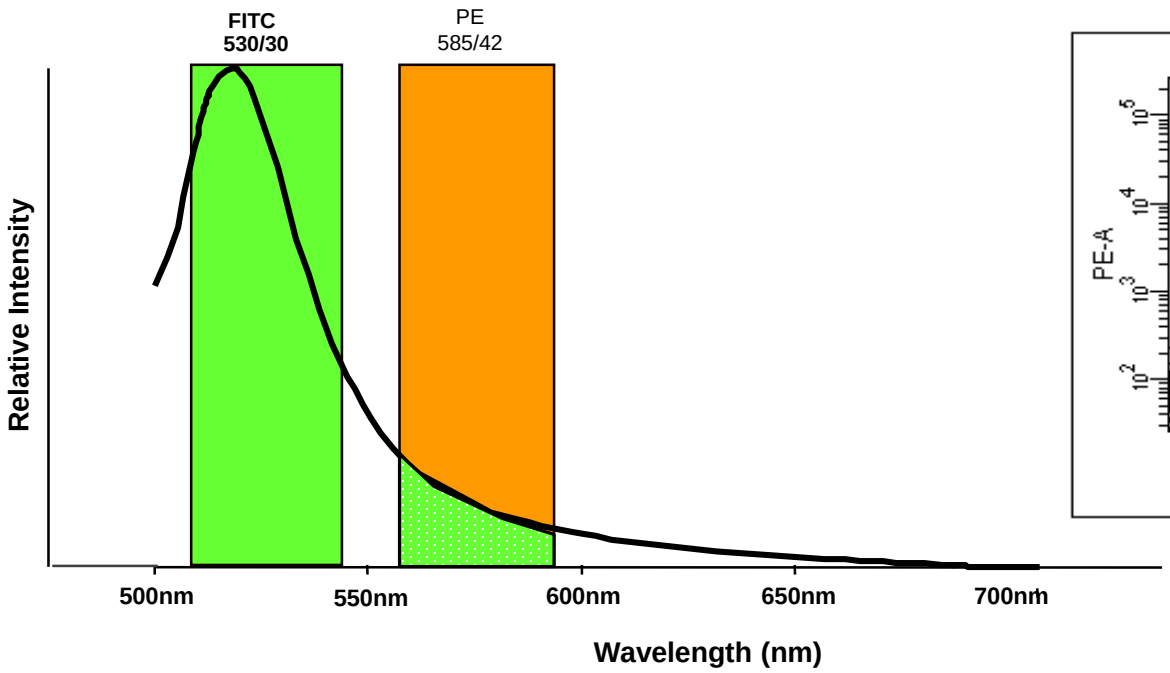
After voltage adjustment

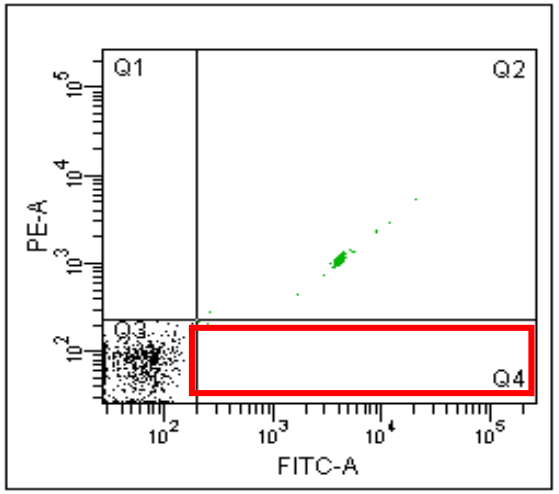
Compensation theory

Emission Optics

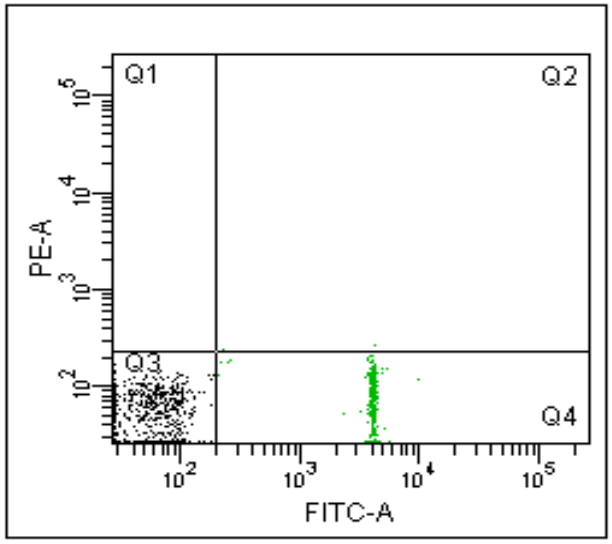


FITC Spillover





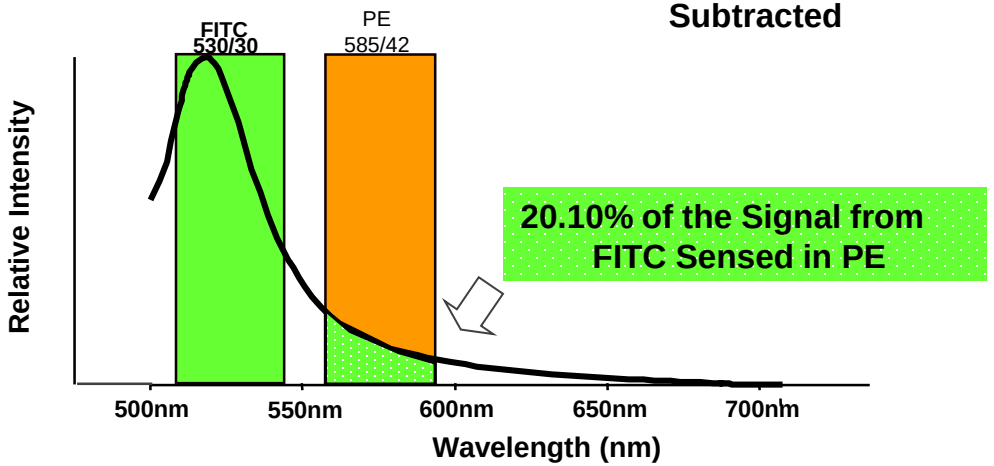
FITC Compensation



To Lower Cluster

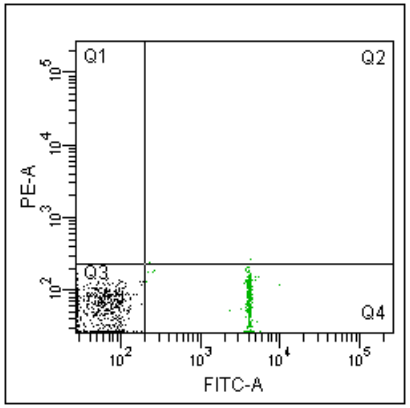
	Fluorochrome	- % Fluorochrome	Spectral Overlap
•	PE	FITC	20.10

Increase % Subtracted

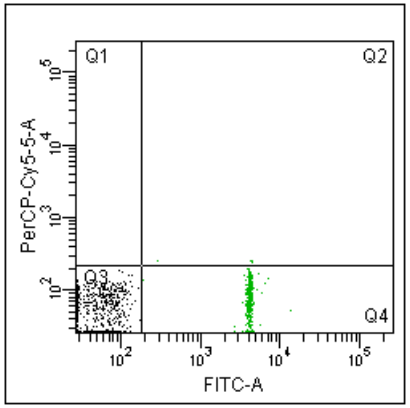


20.10% of the Signal from FITC Sensed in PE

FITC Compensation



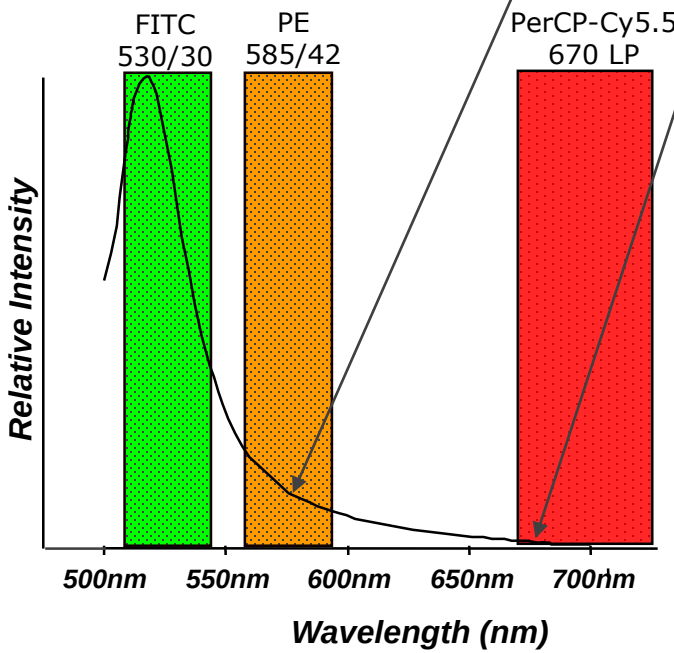
Population	PE-A Mean
Q3	61
Q4	60



Population	PerCP-Cy5-5-A Mean
Q3	69
Q4	66

To lower cluster, increase value.

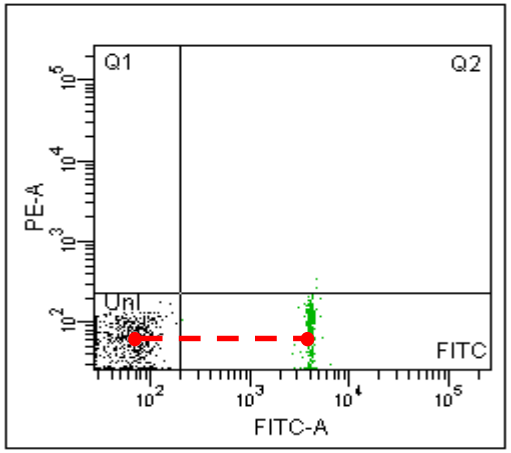
	Fluorochrome	- % Fluorochrome	Spectral Overlap
•	PE	FITC	20.10
•	PerCP-Cy5-5	FITC	0.90



Compensation Examples

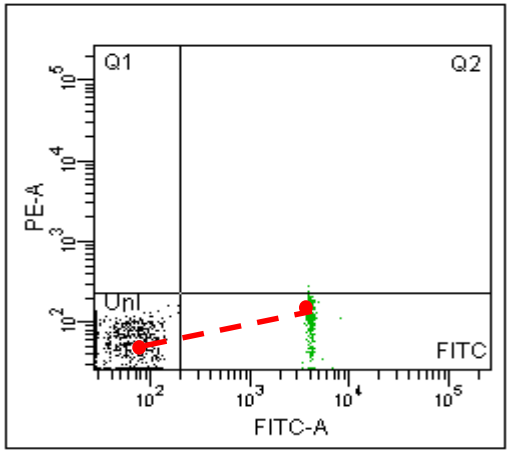
Incorrect Compensation

Correct Compensation



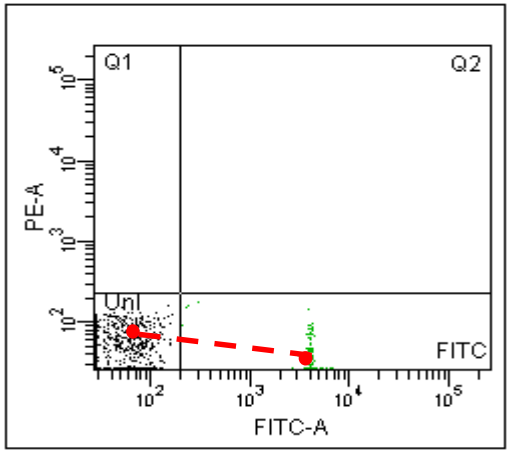
Population	PE-A Mean
Unl	64
FITC	69

Undercompensation



Population	PE-A Mean
Unl	61
FITC	96

Overcompensation



Population	PE-A Mean
Unl	62
FITC	-1

Topic

- **Introduction of Flow Cytometry**
- **Instrument setup**
- **Application of Flow Cytometry**

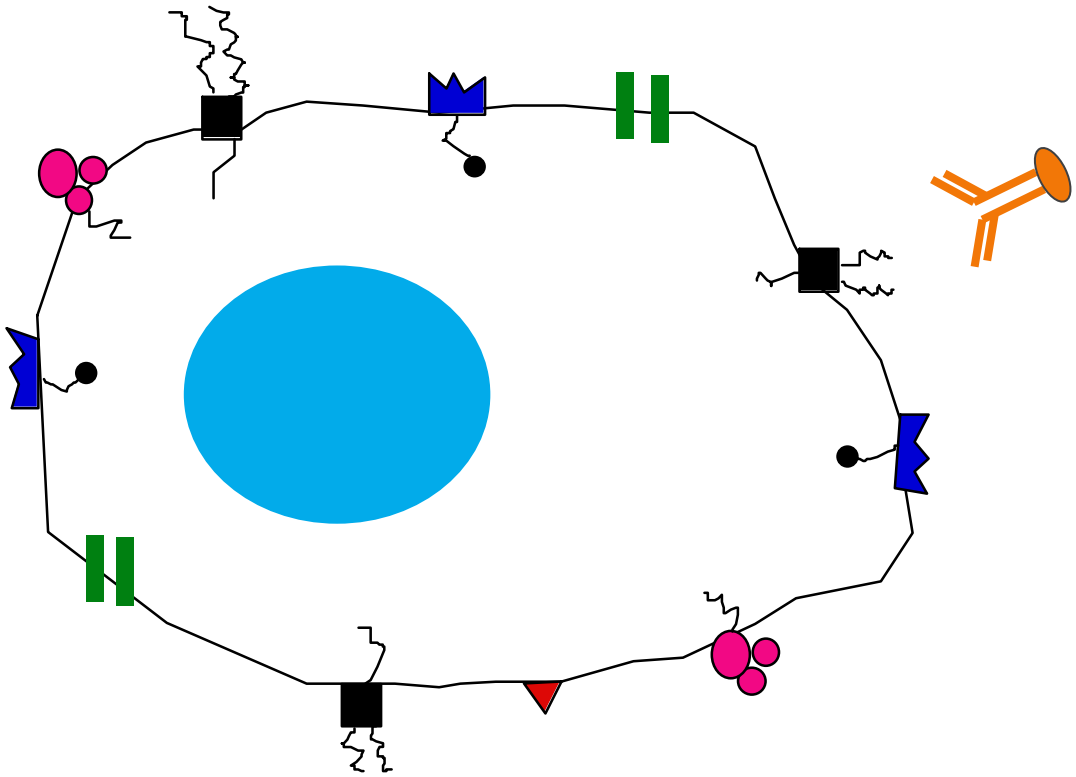
Applications

- **Phenotype Analysis**
  (Cell Surface Antigens/Markers)
- **Intracellular Analysis**
  -- Eg. Cytokines, Signal Transduction molecules…etc.
- **DNA Analysis**
  -- Eg. Viability, Cell cycle, Apoptosis…etc.
- **Cell Fuction Analysis**
  -- Eg. Free radicals, Ca^{2+}, Reporter genes…etc.
- **CBA (Cytometric Bead Array)**

  -- cytokine detection

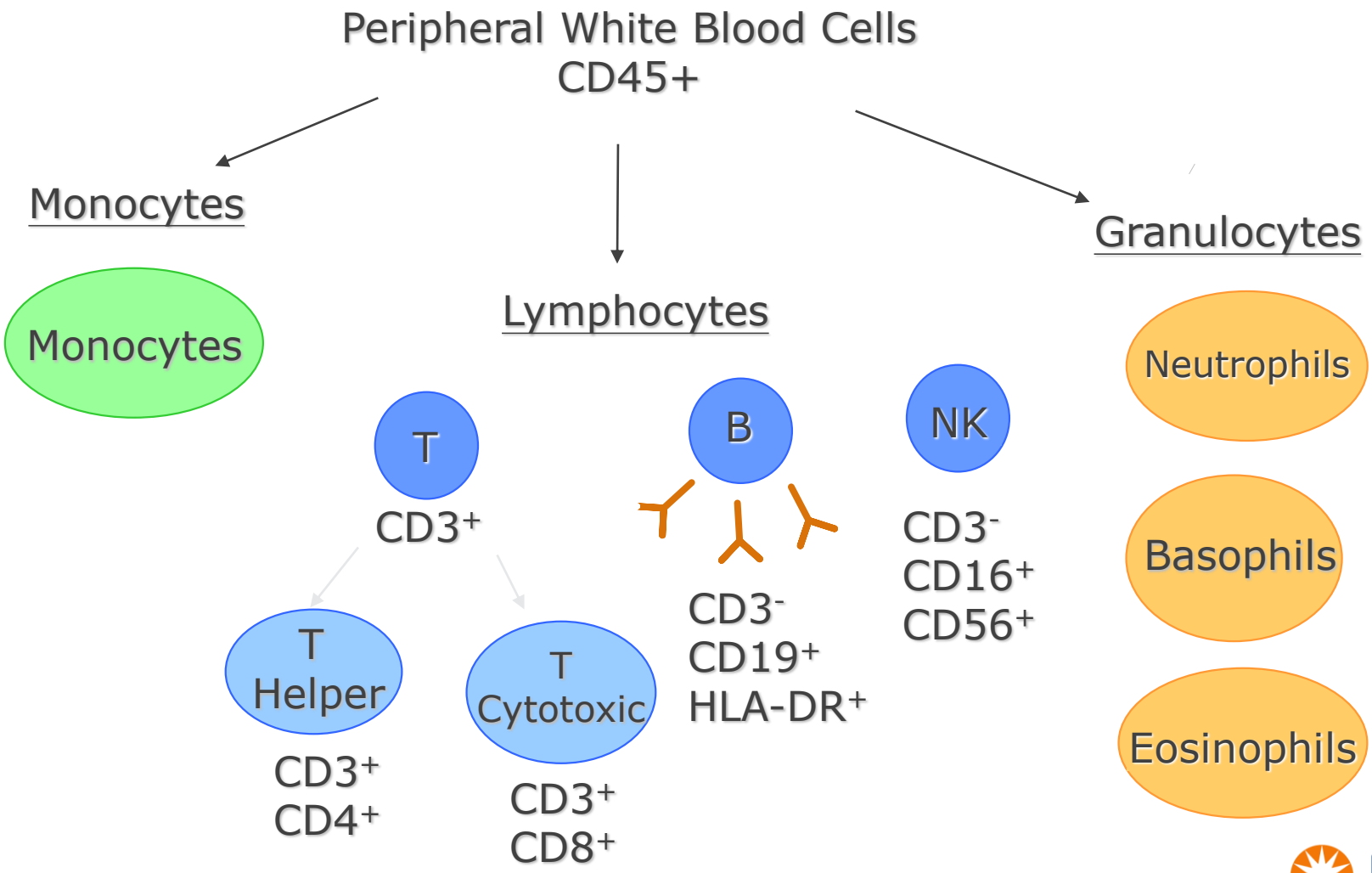
BD

Phenotype Analysis

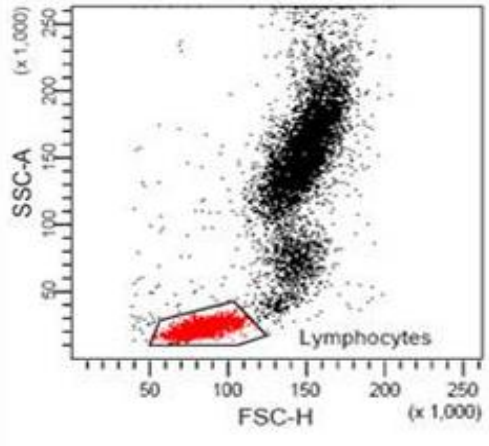


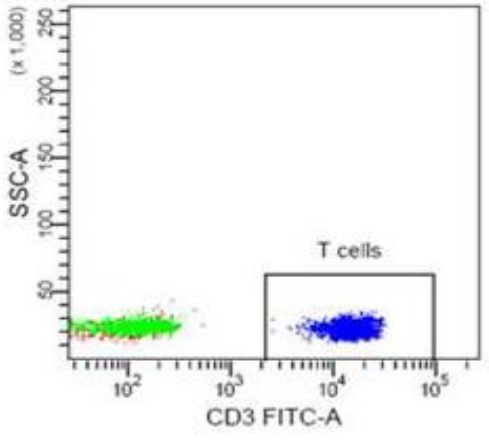
- Ligand
- Receptor
- Adhesion molecule
- …etc

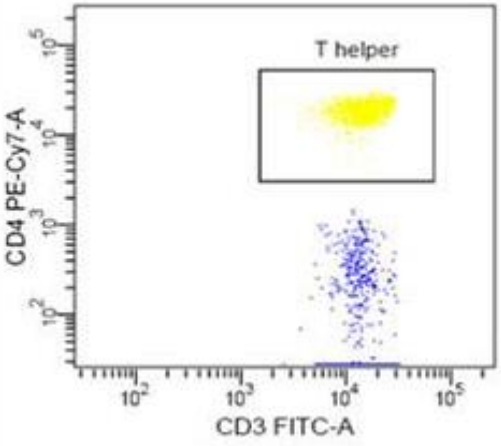
Lysed Whole Blood Components

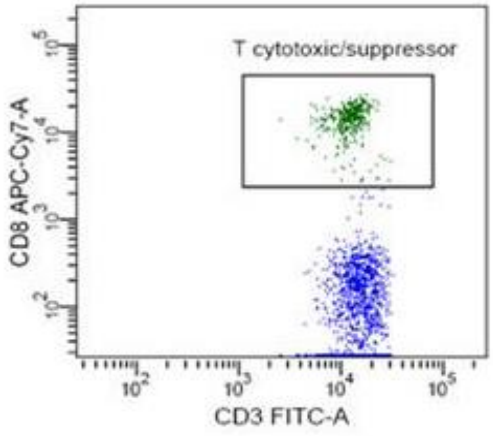


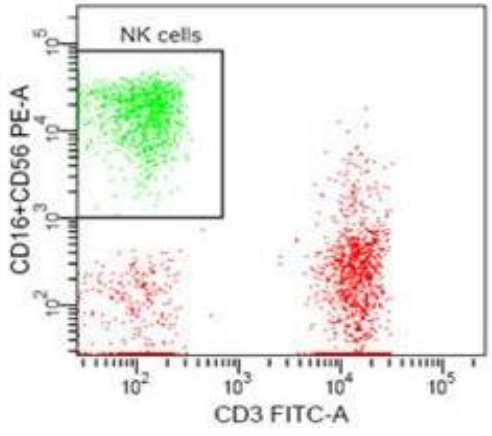
Lymphocyte Subset

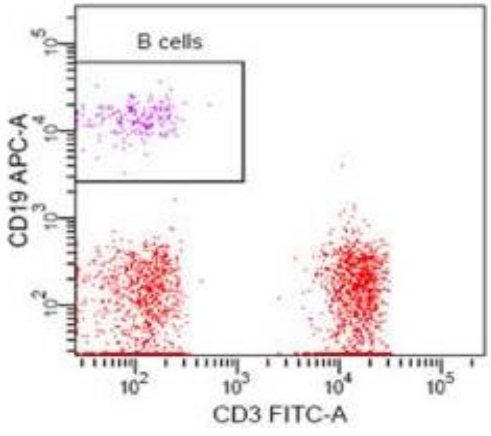




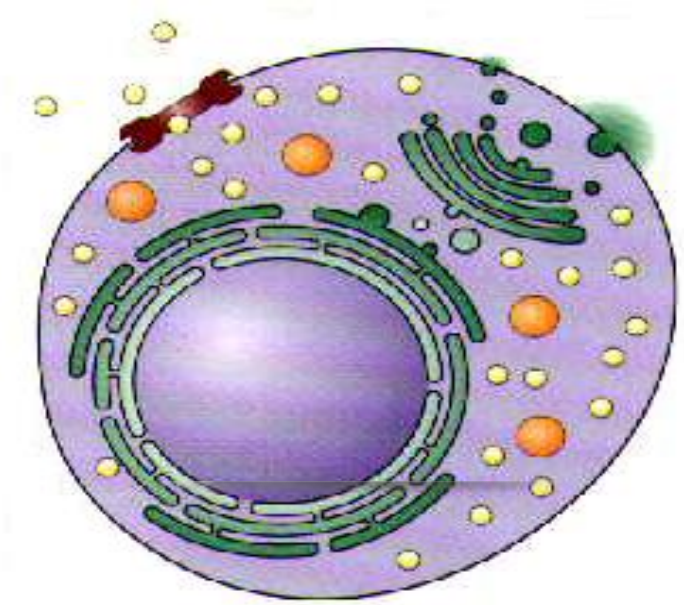






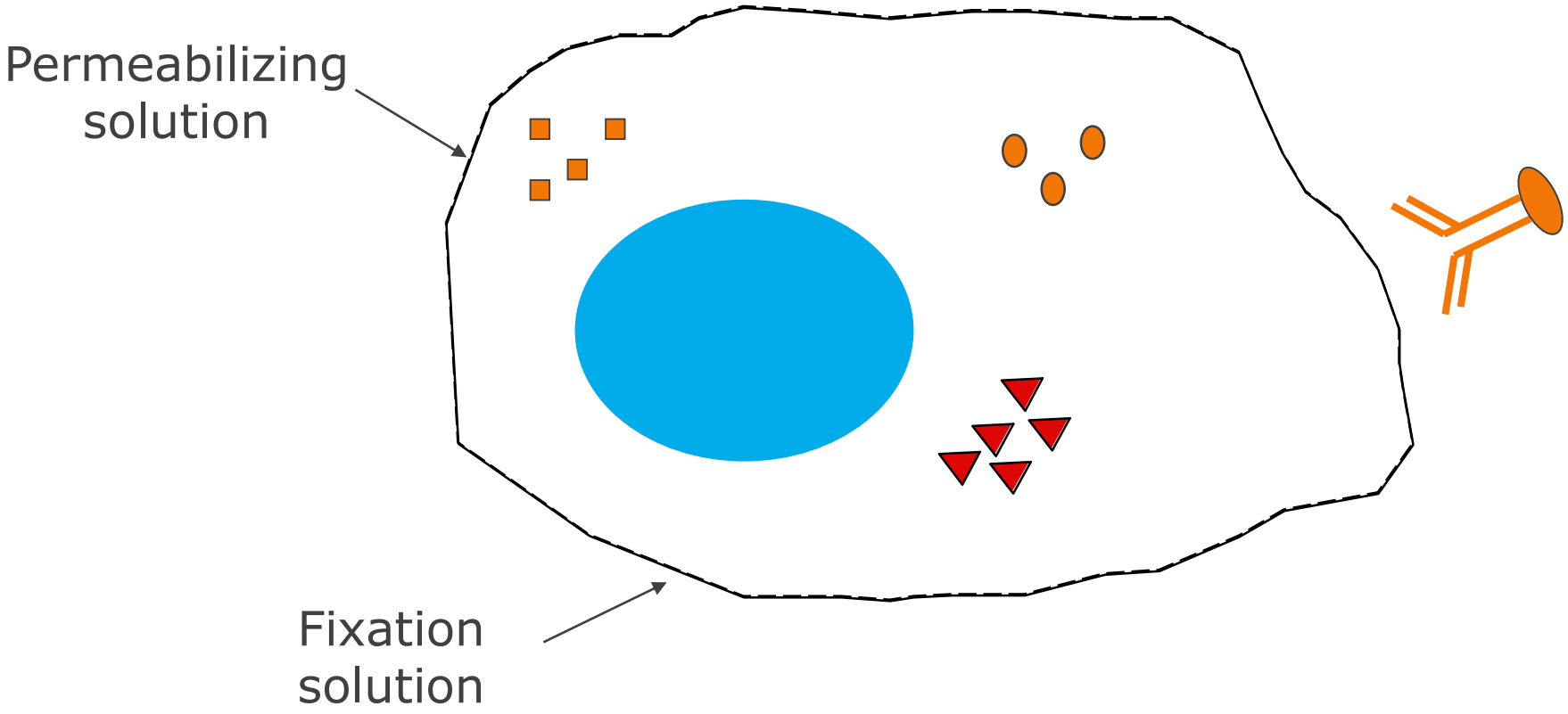


Intracellular Analysis

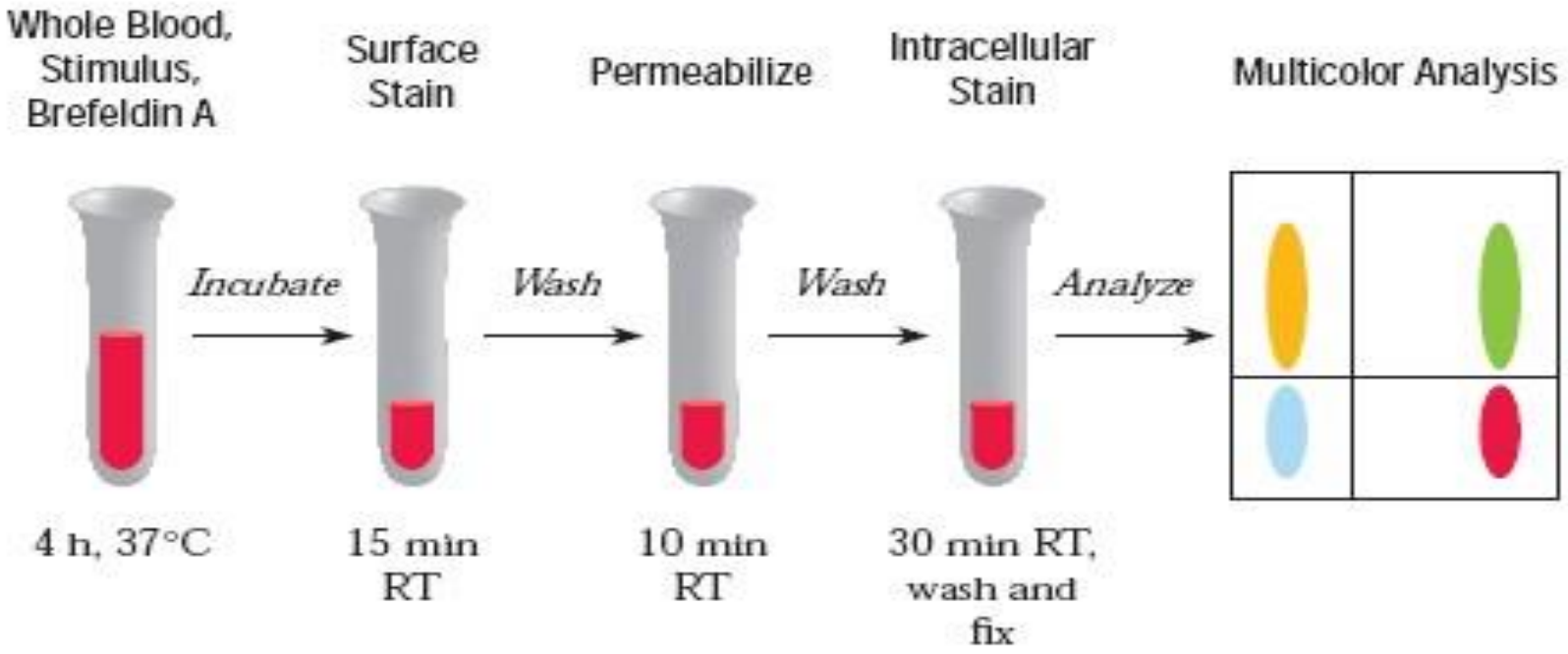


- Cytokine
- Enzymes
- Structure Proteins
- Intracellular Signaling

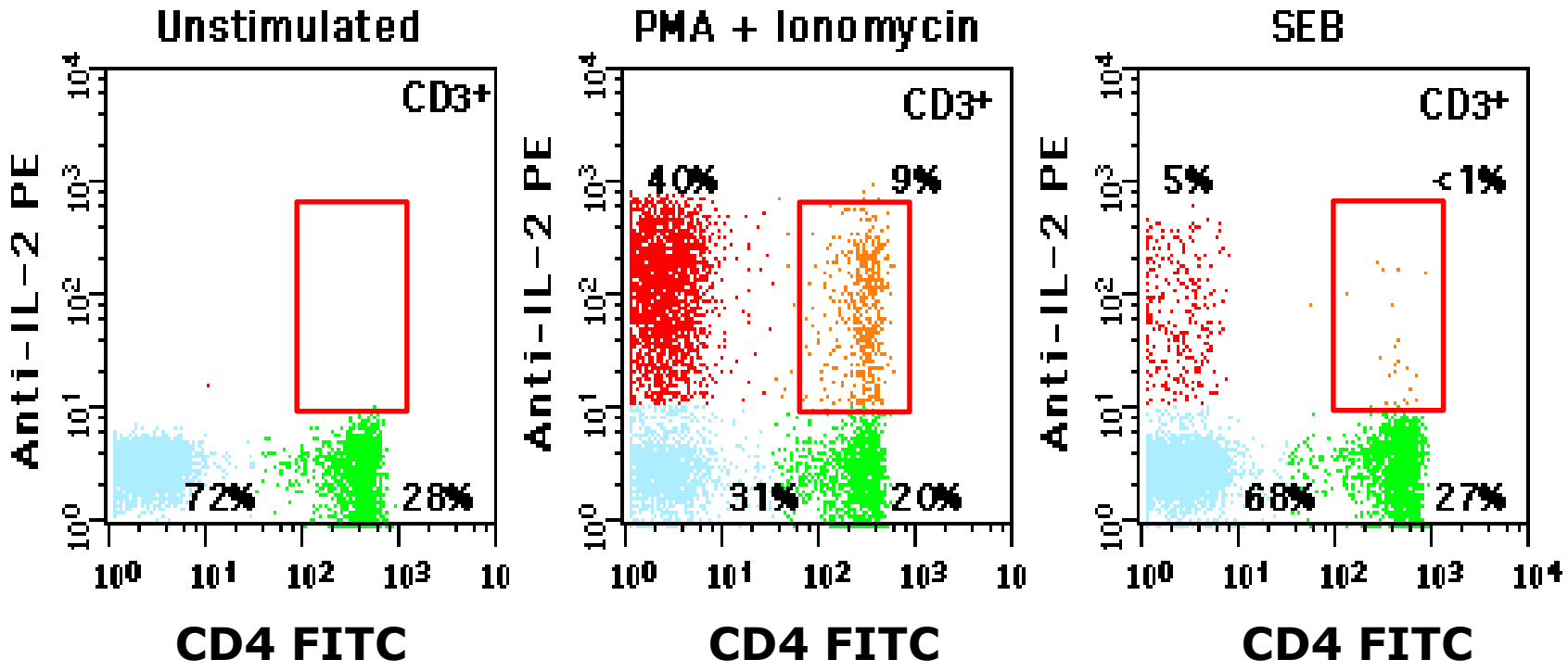
Intracellular Analysis



Cell Surface and Cytoplasmic Stain protocol

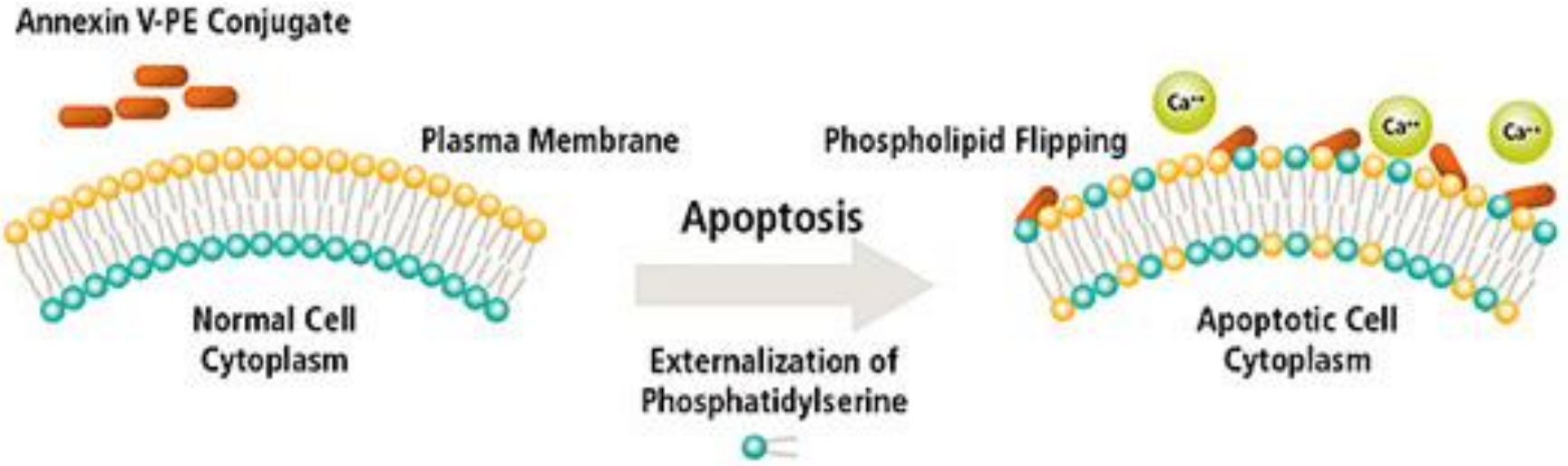


Detection of Cell Surface and IC Cytokine

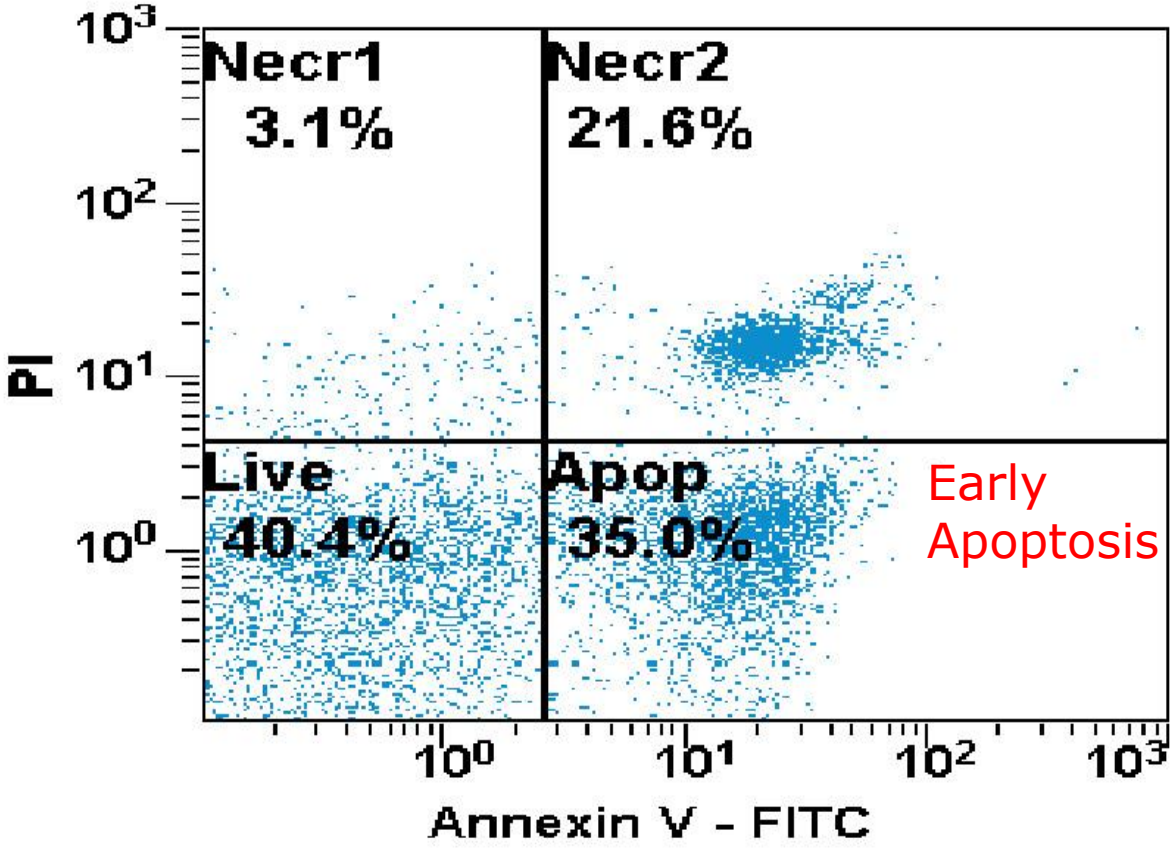


Cell Apoptosis 細胞凋亡

- Annexin V Apoptosis Assay



Annexin V/PI Double Staining



Cell Apoptosis 細胞凋亡

- Mitochondria potential change-JC-1 (BD Mitoscreen)

Apoptosis通常與mitochondria的膜電位(Δψ)去極化有關性

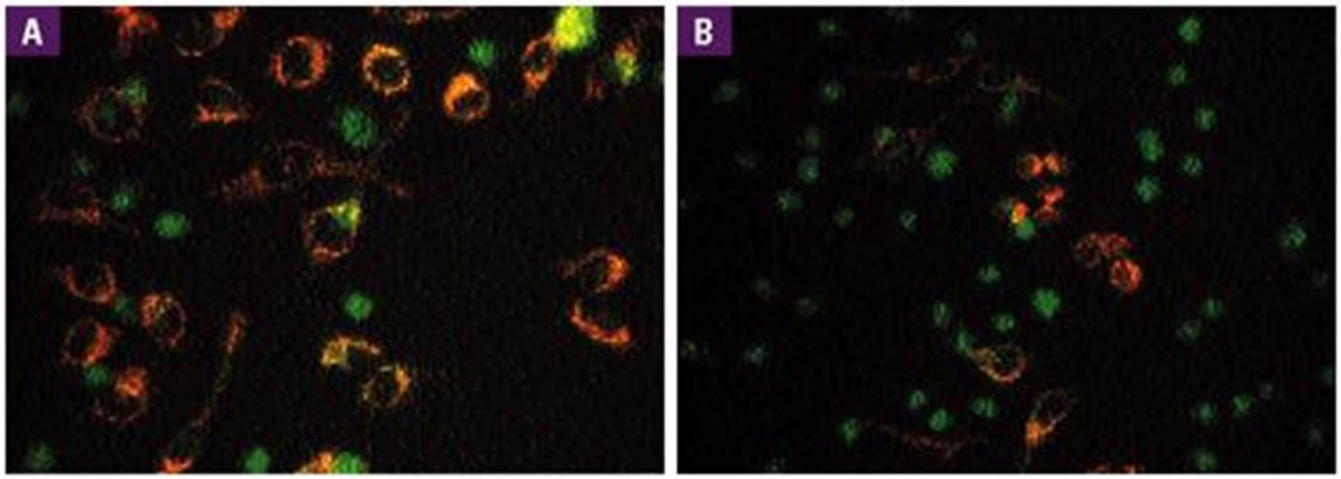
- JC-1 = J-aggregate-forming Cationic Δψ sensitive dye

  J-aggregates:膜電位Δψ 極化(polarized)時為此型式

  JC-1 monomers: 膜電位Δψ (depolarized)去極化時存在此型式

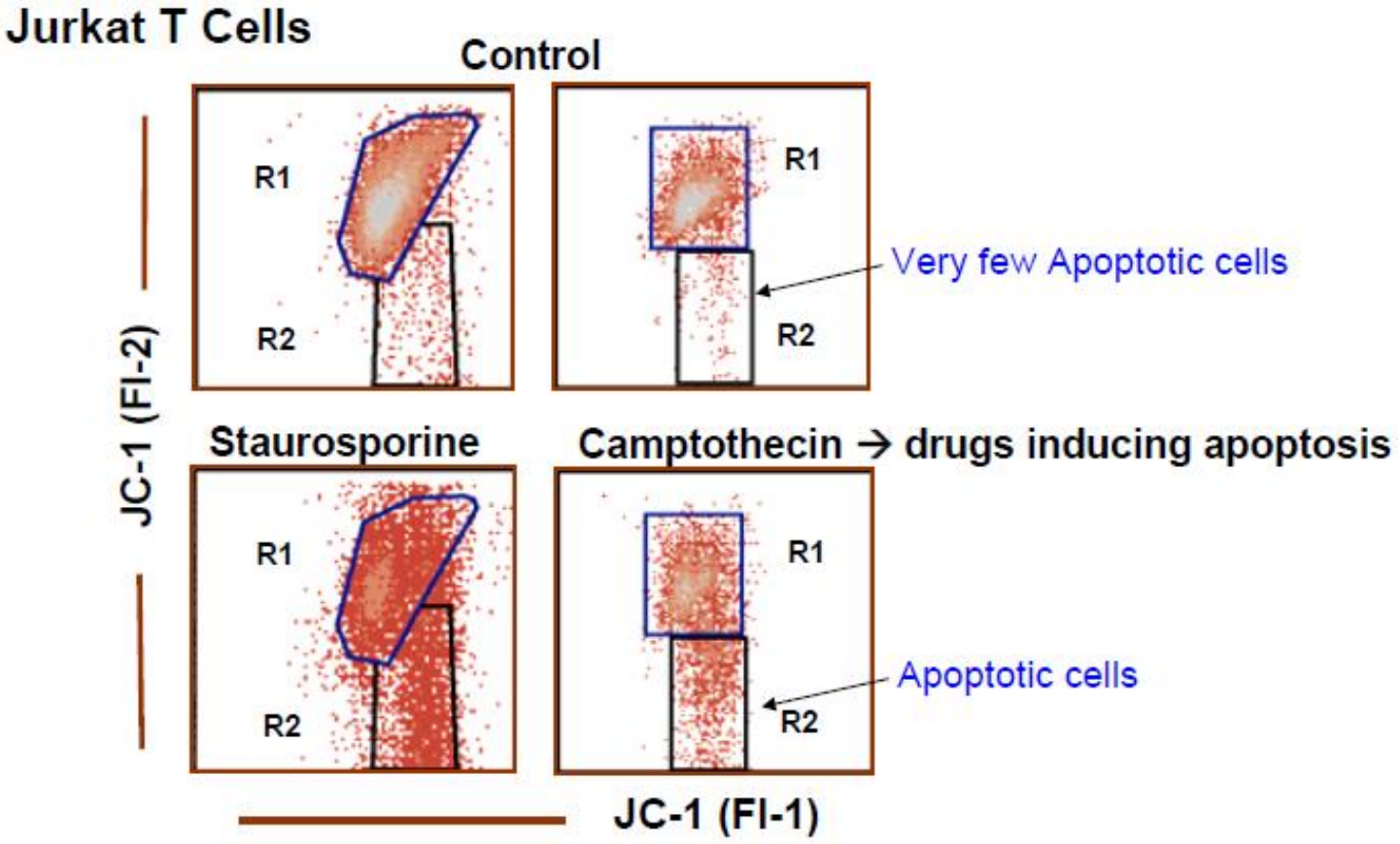
J-aggregates 存於健康細胞，且螢光為FL1及FL2 channels (綠色及橘紅色螢光)

JC-1 monomers通常但非絕對存於Δψ 去極化的apoptosis細胞，且只剩下FL1 (綠色螢光)



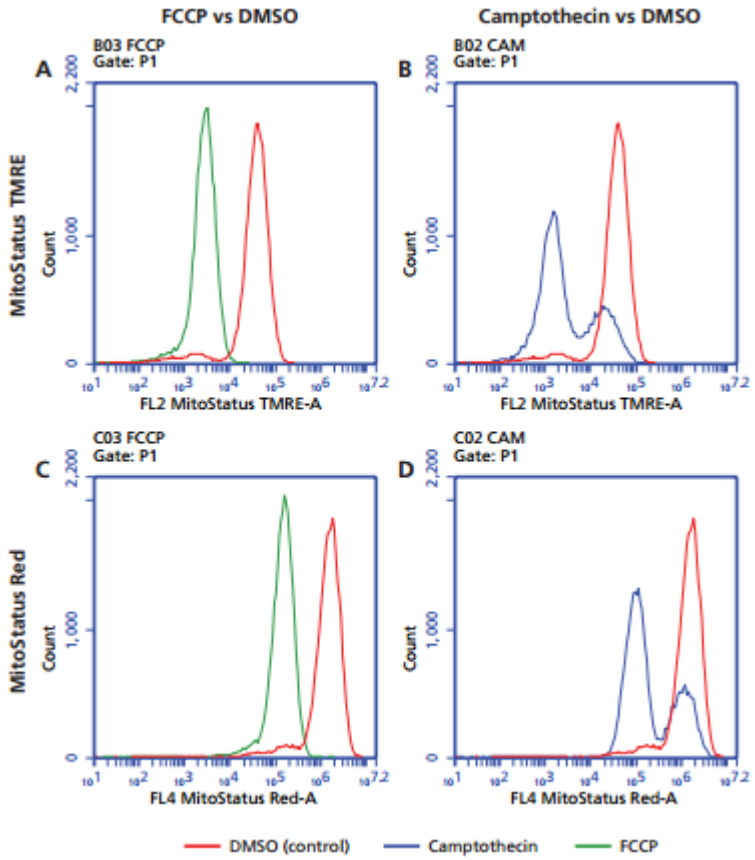
Cell Apoptosis 細胞凋亡

- JC-1(BD Mitoscreen) Jurkat T cell data



Cell Apoptosis 細胞凋亡

- MitoStatus TMRE/Red



Characteristic	MitoStatus TMRE	MitoStatus Red
Excitation peak	549 nm	622 nm
Emission peak	574 nm	648 nm
Laser	488 nm (blue)	640 nm (red)
Detector	FL2	FL4
Equivalent fluorochromes*	PE	APC Alexa Fluor® 647

*Do not use these fluorochromes in the same tube with the corresponding MitoStatus dye.

Table 1. Fluorescence characteristics of MitoStatus dyes on the BD Accuri C6.

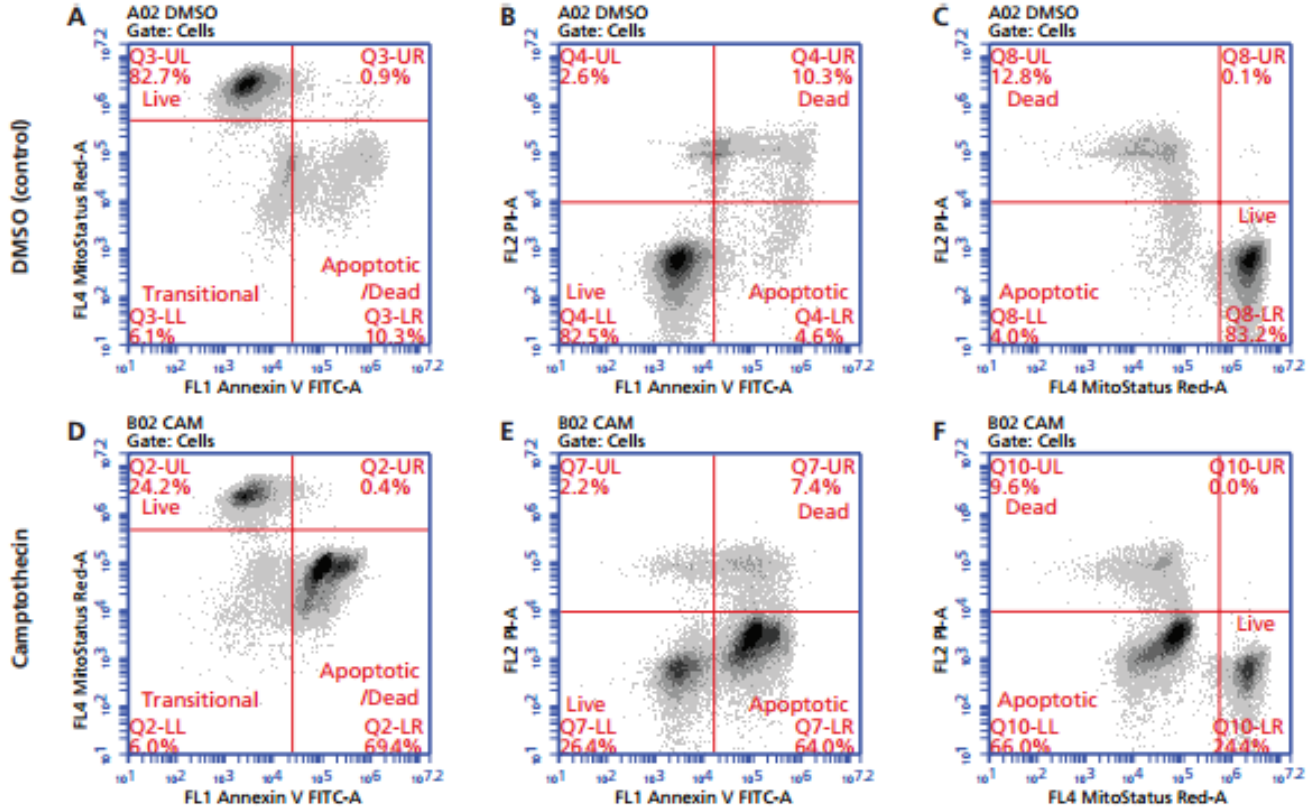


Figure 2. Detecting apoptosis with MitoStatus Red and Annexin V

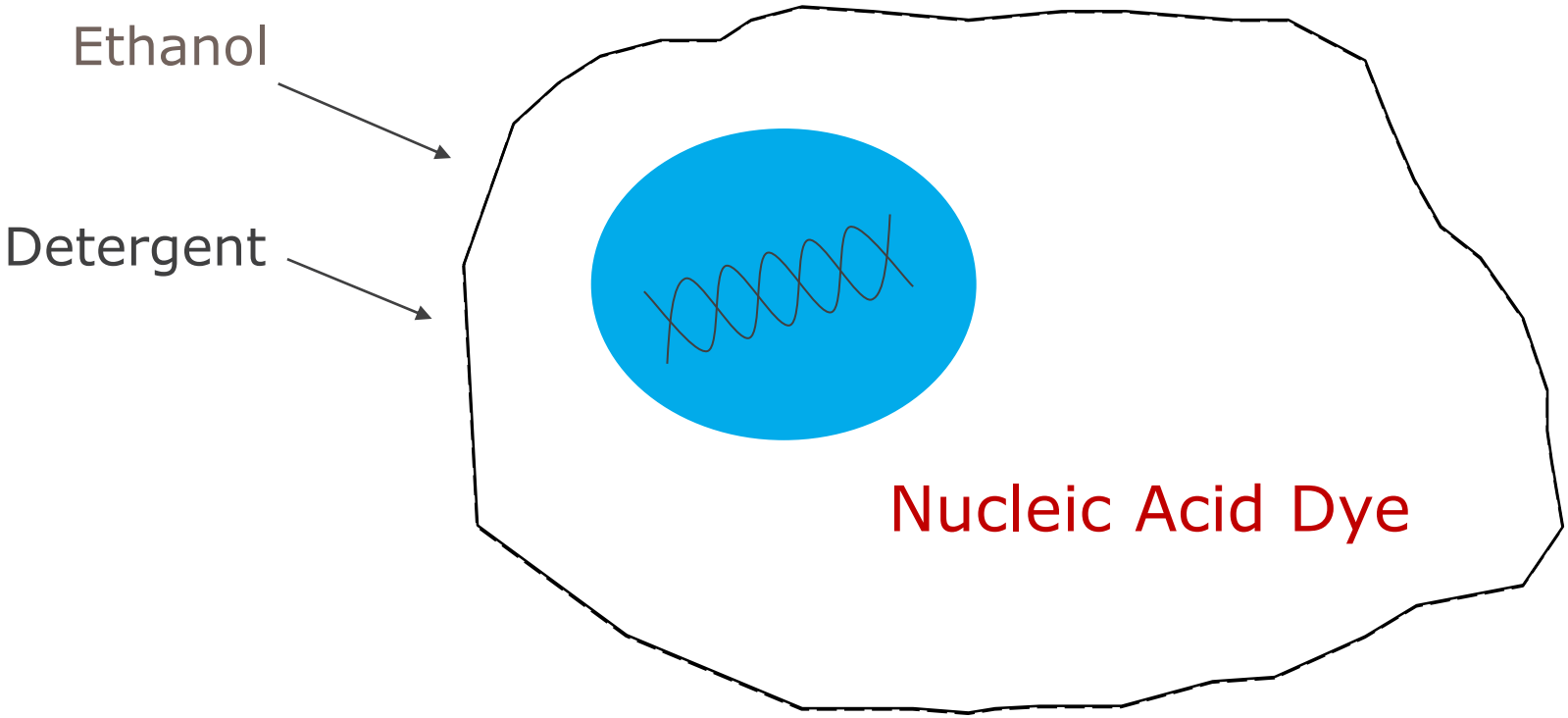
Related Products

Description	Cat.No.
BD™ MitoScreen (JC-1) Kit	551302
BD Pharmingen™ Annexin V Apoptosis Detection Kit	556570 (FITC) 559763 (PE)
BD Pharmingen™ Stain Buffer (FBS)	554656

Ordering Information

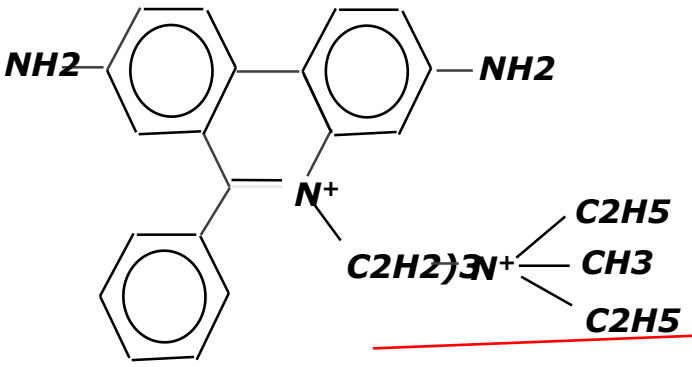
Description	Quantity	Cat.No.
BD Pharmingen™ MitoStatus TMRE	25 mg	564696
BD Pharmingen™ MitoStatus Red	100 µg	564697

DNA Analysis

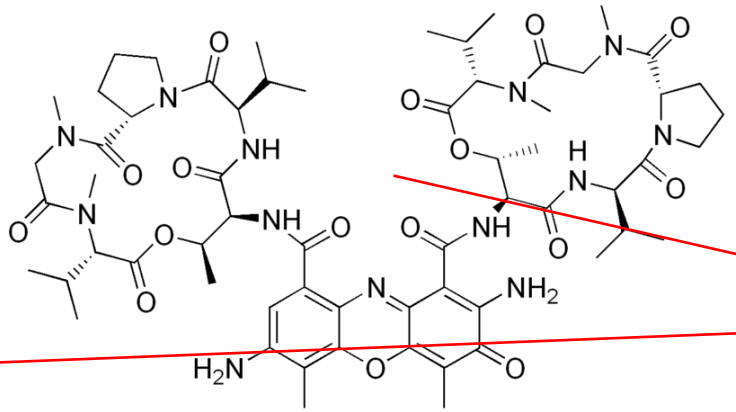


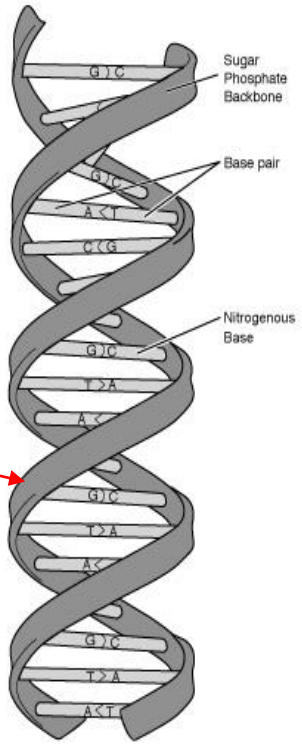
DNA Dye

Propidium

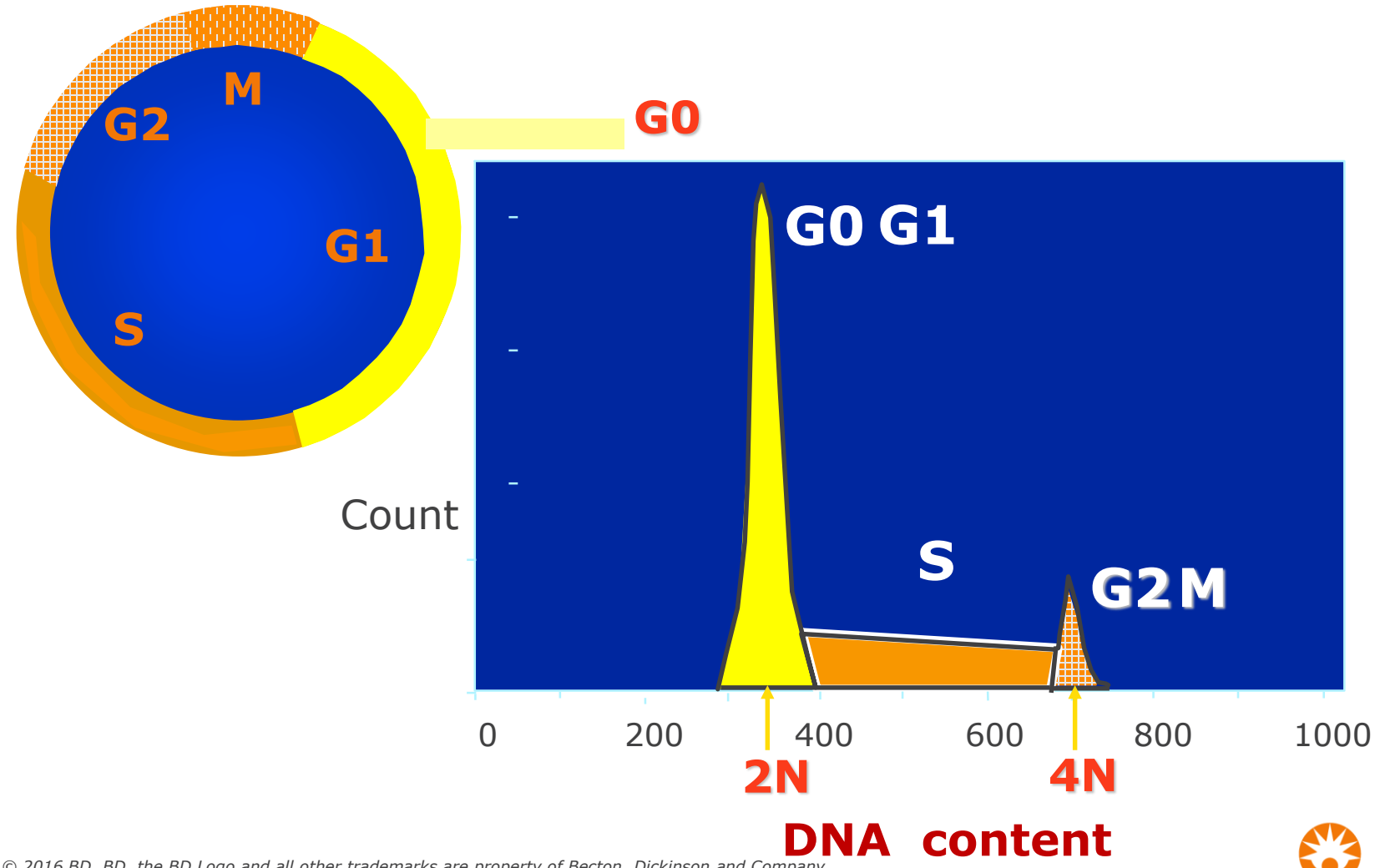


7-AAD

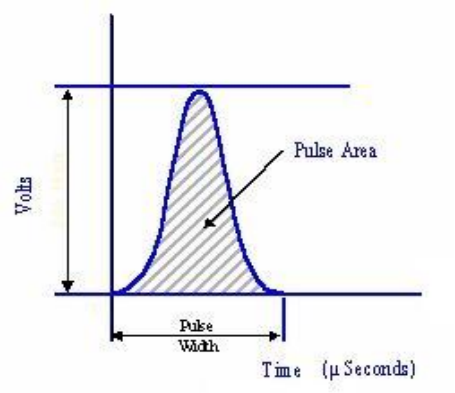




Cell Cycle Analysis

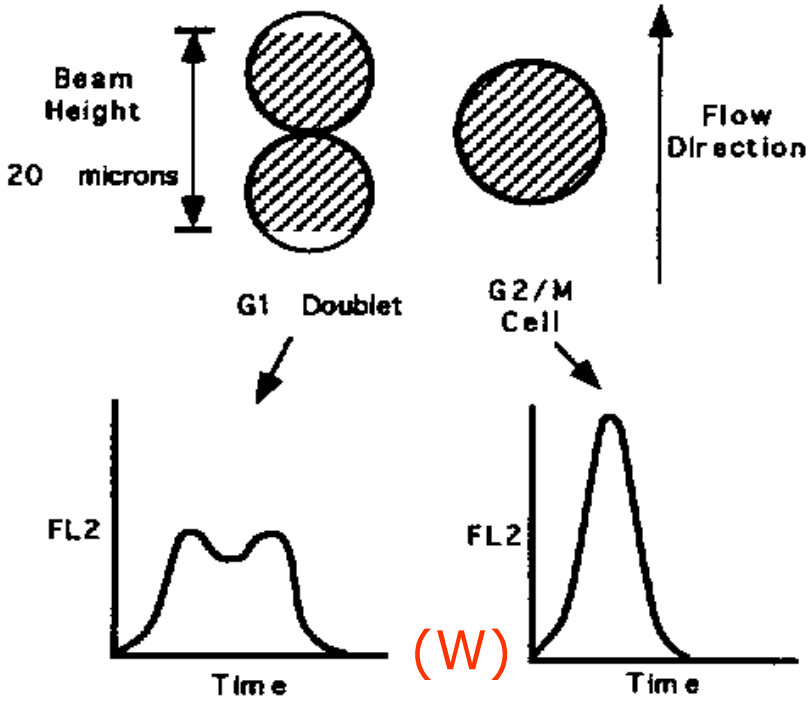


Cell cycle study

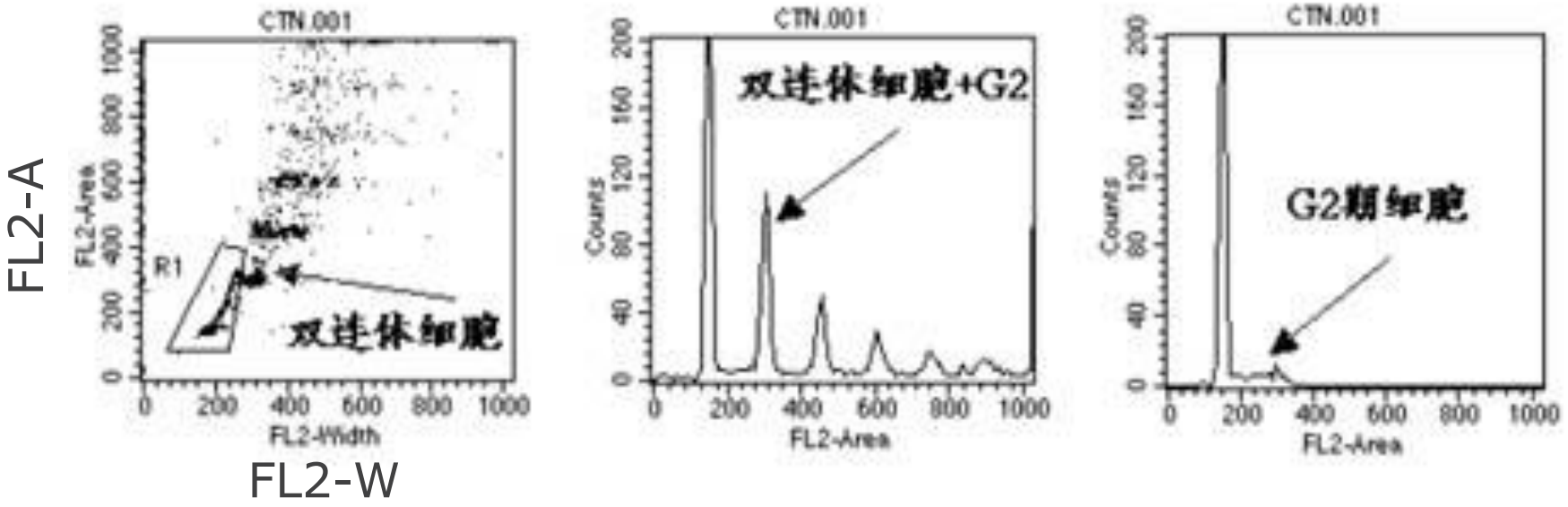


FL2-A

FL2-W

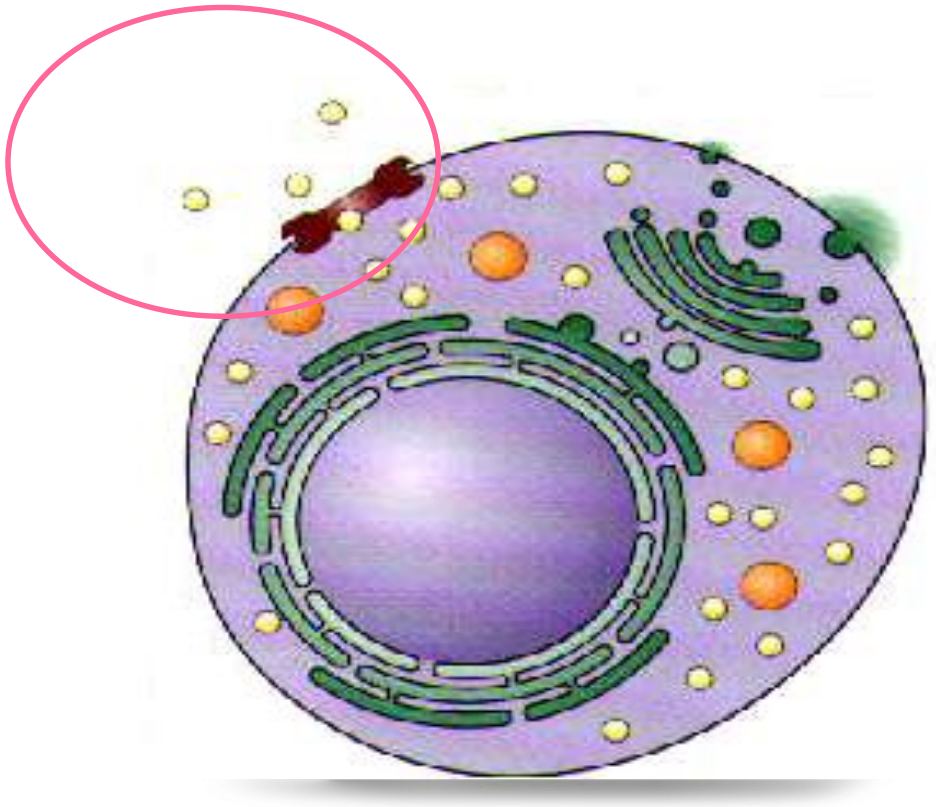


Cell cycle study

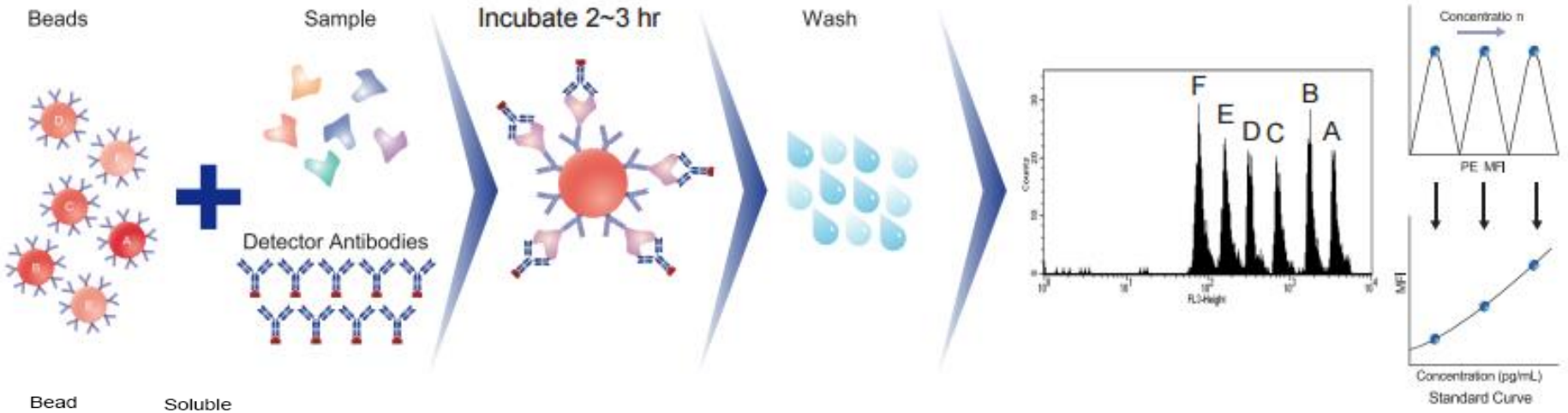


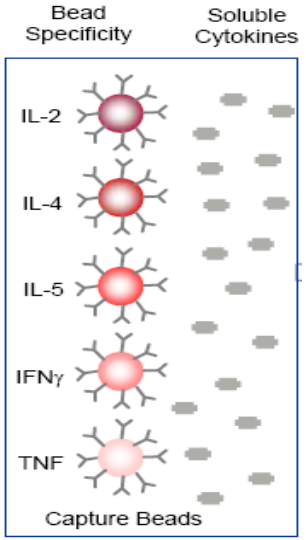
Application of Flow Cytometry

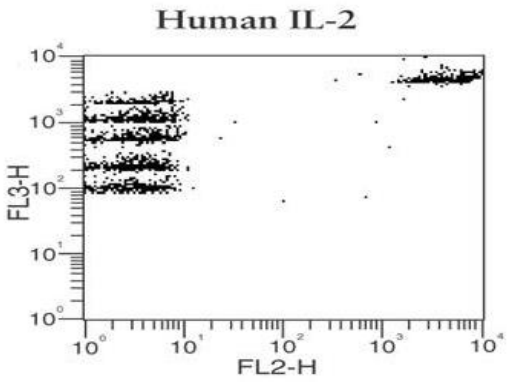
- ***Soluble form protein detection***



Cytometric Beads Array (CBA)

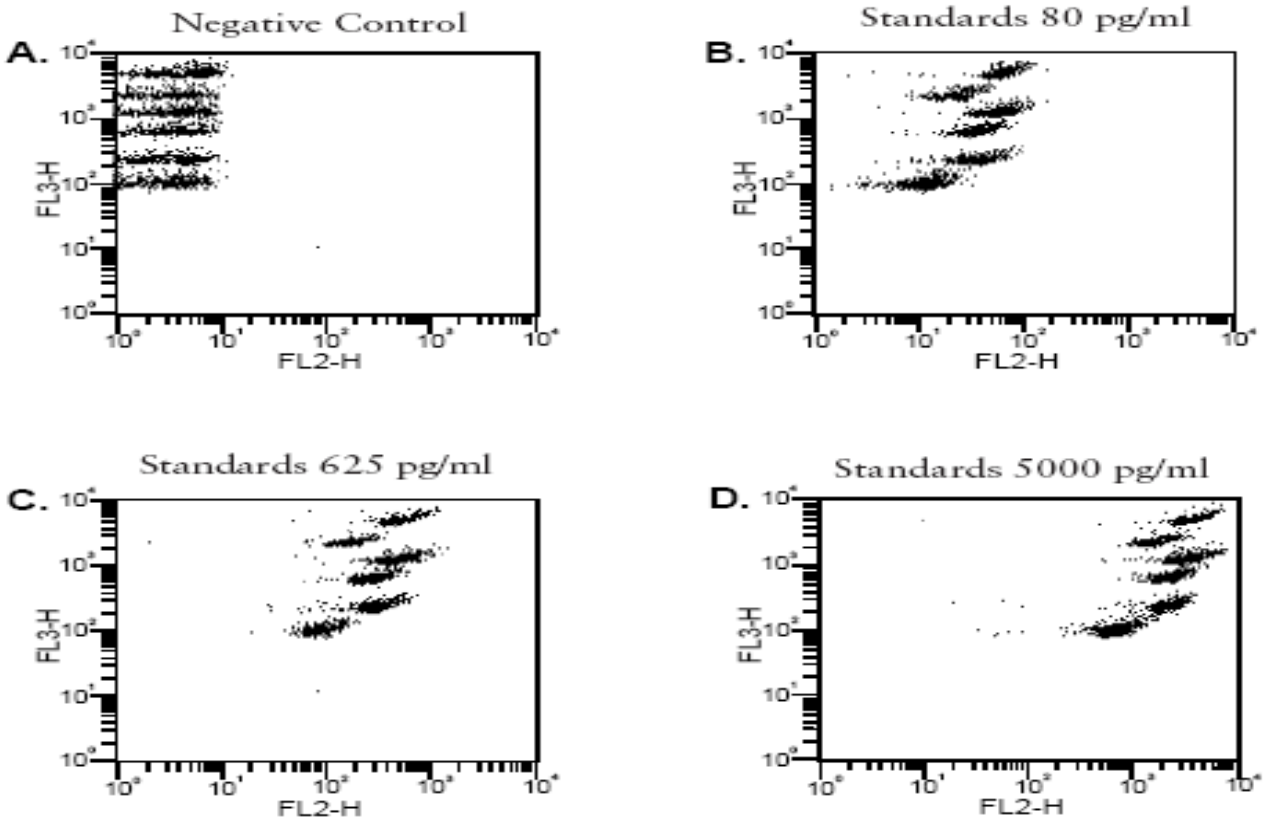




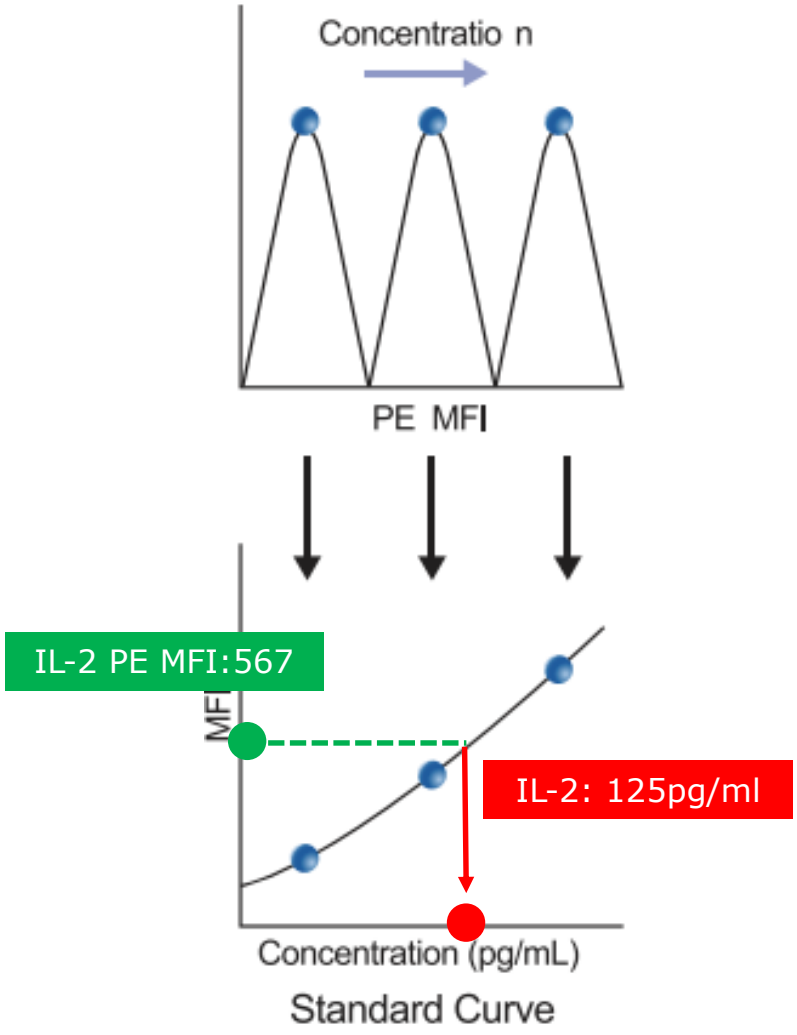


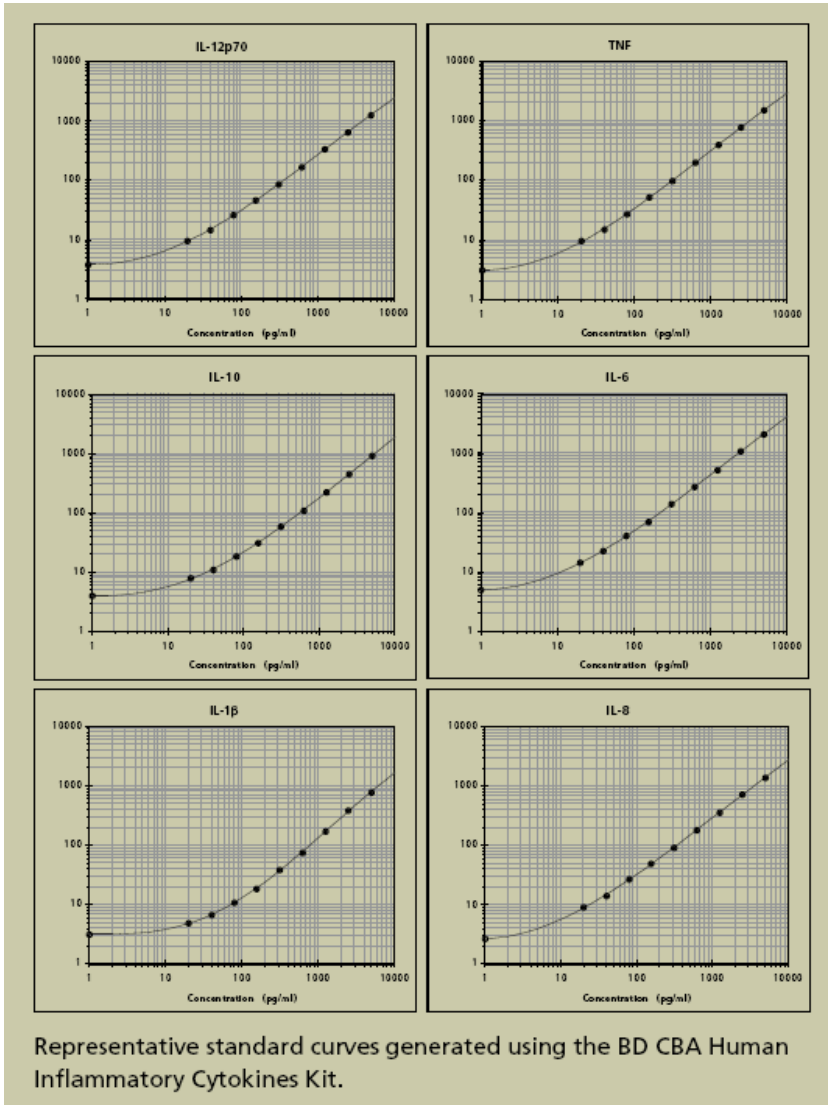
Simultaneous Analysis of Multiple Cytokines

Typical Data



Standard Curves





Representative standard curves generated using the BD CBA Human Inflammatory Cytokines Kit.

BD Pharmingen Product list

Cat#	Name	Content	Size
550285	BD Pharmingen™ PI/RNase Staining Buffer	The reagent is suspended in a phosphate-buffered solution (pH 7.2) with 0.02% (w/v) sodium azide	100ml
556463	BD Pharmingen™ Propidium Iodide Staining Solution	Propidium Iodide Staining Solution (For AnnexinV/PI assay use)	2ml
556419	BD Pharmingen™ Annexin V	Annexin V-FITC	200 test
556547	BD Pharmingen™ Annexin V : FITC Apoptosis Detection Kit I	Annexin V-FITC, Propidium Iodide Staining Solution, Annexin V Binding Buffer	100 test
551302	BD Pharmingen™ BD™ MitoScreen (JC-1)	JC-1 dye and assay buffer	100 test
564696 564697	BD Pharmingen™ MitoStatus TMRE BD Pharmingen™ MitoStatus Red	BD Pharmingen™ MitoStatus TMRE BD Pharmingen™ MitoStatus Red	25mg 100ug

Thank you!

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