

Flow Cytometry

流式細胞儀原理介紹

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大綱

- 流式細胞儀原理介紹
- 流式細胞儀應用介紹



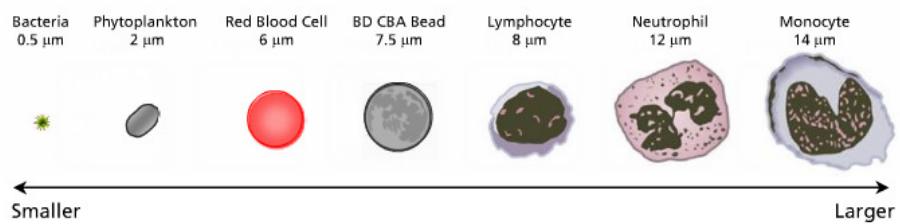
何謂流式細胞儀 Flow Cytometry

- Flow = Fluid 液流
- Cyto = Cell 細胞
- Metry = Measurement 量測
- 流式細胞術:為一種使用液流將懸浮的樣品(細胞, 胞器, 或其他粒子)以每秒數千到上萬顆的速度送入流式細胞儀偵測的技術。



目標粒子尺寸(Target Size)

- 可偵測粒子大小範圍: 0.5~50 μm

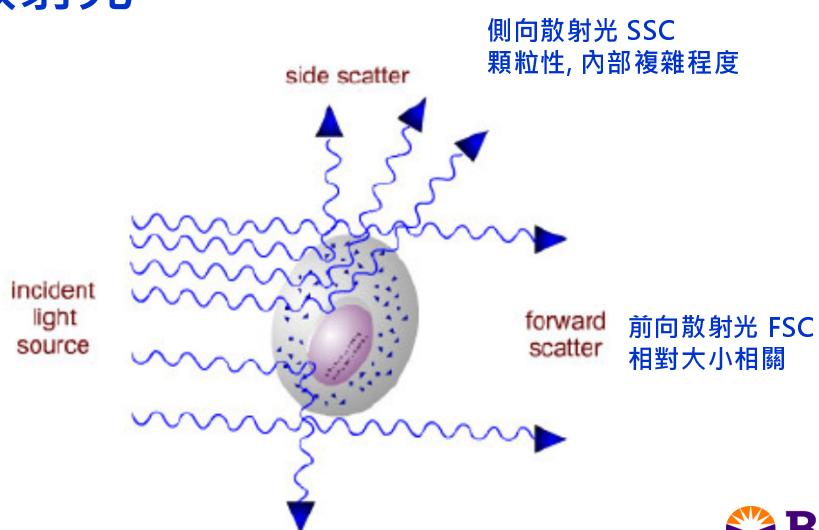


流式細胞儀可偵測之細胞特性

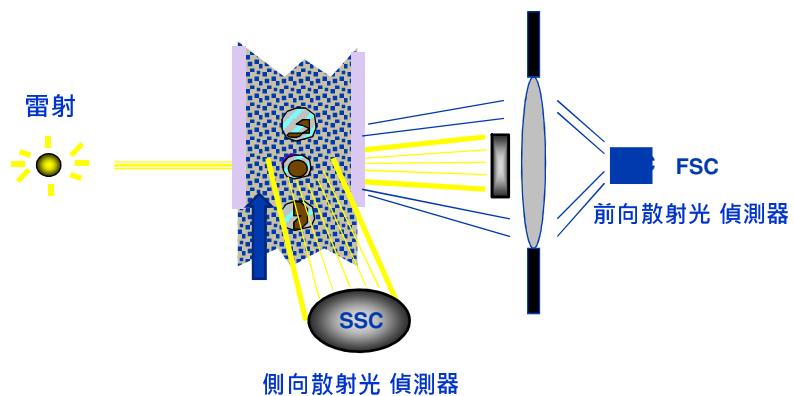
- 細胞相對大小—前向散射光
(Forward Scatter—FSC)
- 細胞相對的顆粒性與內部構造的複雜程度-
側向散射光(Side Scatter—SSC)
- 細胞相對螢光強度



散射光

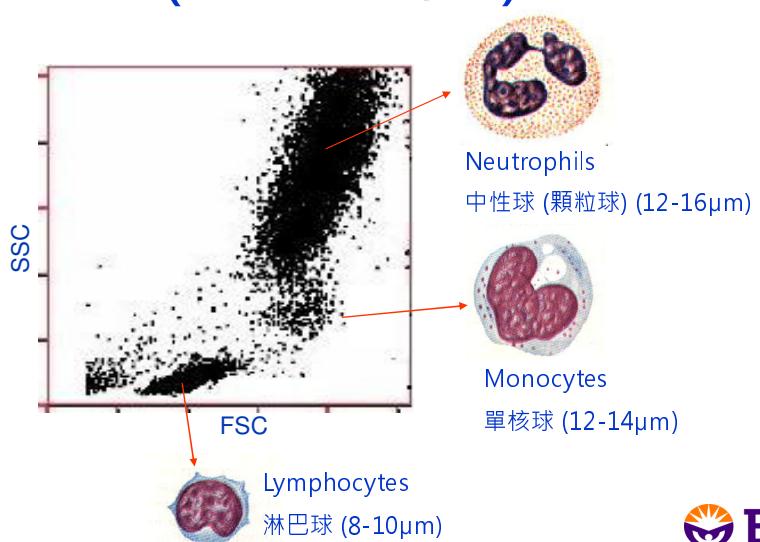


散射光



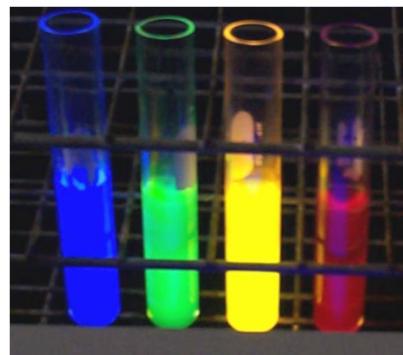
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溶血白血球(混合細胞樣品)



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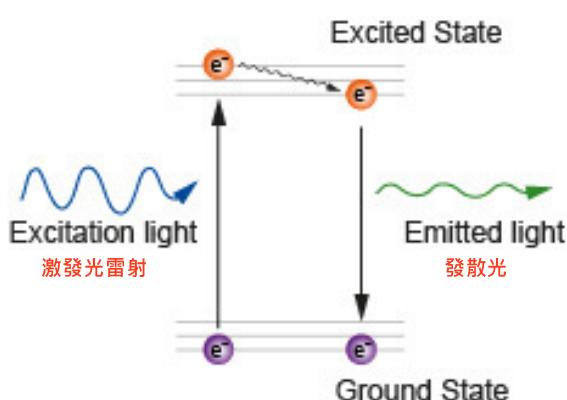
螢光



- 螢光分子可拮抗於抗體上, 用以偵測細胞表面或內部的蛋白質或生物標記, 使細胞發螢光
- 亦有試劑是可以直接偵測某種細胞功能或化學現象, 同樣使細胞發螢光



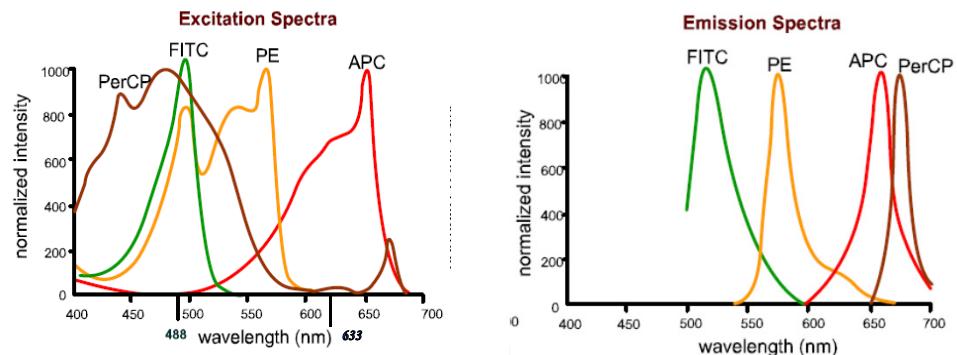
螢光-原理



- 螢光分子接收固定波長激發雷射光(Excitation)之能量後, 跃遷到激發態, 損失掉部分能量後, 剩餘的能量再度以光的形式發散出(Emitted light)即成為使用者觀察的螢光
- 因此一個螢光分子最重要的兩個資訊為最大激發波長 Ex , 與最大發散波長 Em



螢光-原理



- 激發光譜:
使用何種波長的雷射光才能激發該螢光分子

- 發散光譜:
了解使用何種波長範圍的的螢光偵測器才能
偵測到該螢光分子的訊號



BD Flow Cytometer

FACSCantoll

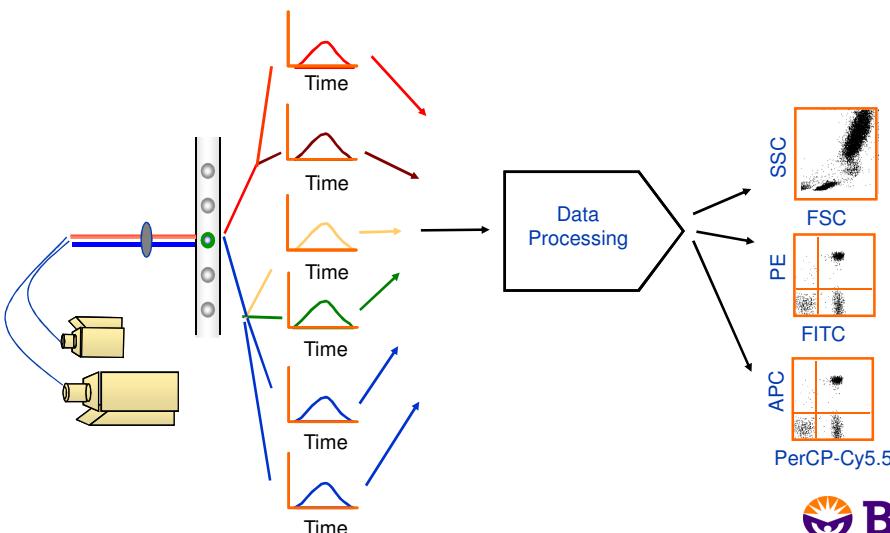


- 細胞分析儀主體
- 系統液流車
- BD FACStation電腦工作站

2 Lasers, 6 Colors



Overview



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儀器子系統介紹

Fluidics 液流系統

將細胞引導入儀器內

Optics 光學系統

產生並收集光學訊號

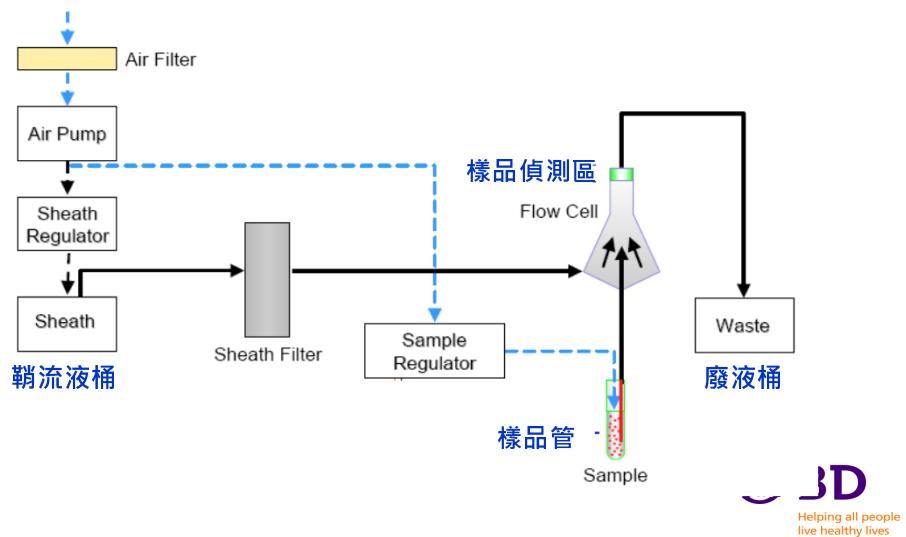
Electronics 電子系統

將光學訊號轉換為相對應的數位電子訊號，量化處理後送入電腦系統

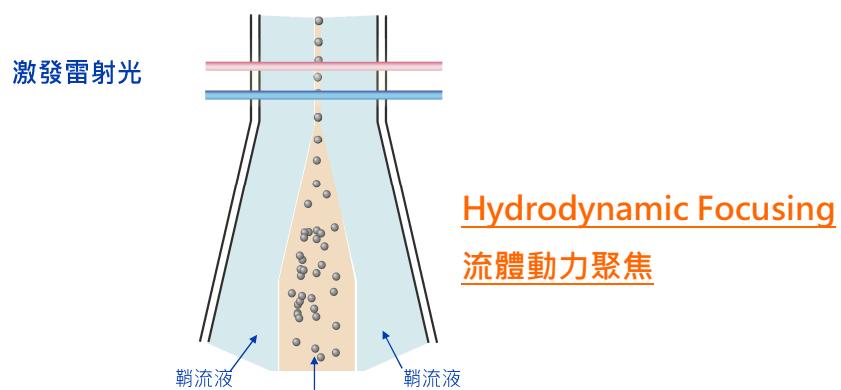


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Fluidics 液流系統



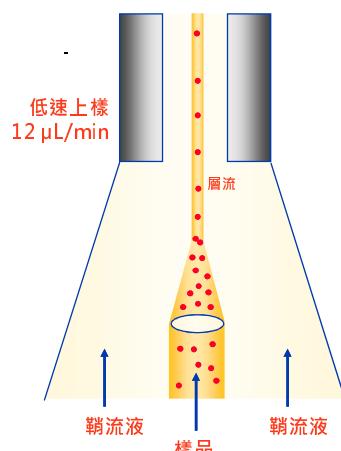
樣品偵測區



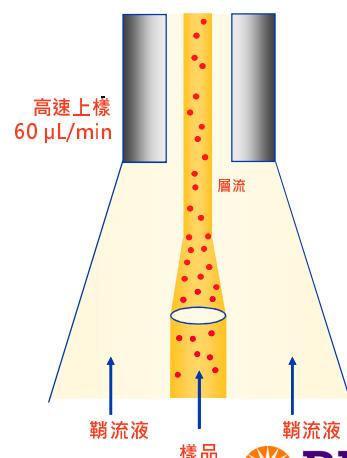
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不同的上樣速度

低樣品-鞘流液壓力差



高樣品-鞘流液壓力差



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螢光物質光譜表

Fluorochrome	Fluorescence Emission Color	Ex-Max (nm)	Excitation Laser Line (nm)*	Em-Max (nm)
Hoechst 33342	Blue	350	355, 375	461
BD Horizon™ V450	Blue	404	405	448
Pacific Blue™	Blue	401	405	452
BD Horizon™ V500	Green	415	405	500
AmCyan	Green	457	405	491
Alexa Fluor® 488	Green	495	488	519
FITC	Green	494	488	519
PE	Yellow	496, 564	488, 532, 561	578
BD Horizon™ PE-CF594	Orange	496, 564	488, 532, 561	612
PI	Orange	351	488, 532, 561	617
7-AAD	Red	543	488, 532, 561	647
APC'	Red	650	633, 635, 640	660
Alexa Fluor® 647	Red	650	633, 635, 640	668
PE-Cy™5'	Red	496, 564	488, 532, 561	667
PerCP	Red	482	488, 532	678
PerCP-Cy™5.5	Far Red	482	488, 532	695
Alexa Fluor® 700	Far Red	696	633, 635, 640	719
PE-Cy™7	Infrared	496, 564	488, 532, 561	785
APC-Cy7	Infrared	650	633, 635, 640	785
BD APC-H7	Infrared	650	633, 635, 640	785

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Optics 光學系統

- 激發光源系統
 - 雷射
 - 光學菱鏡(調整雷射光束用)
- 收集光學系統
 - 光學反射鏡折射鏡等將光訊號引導至偵測器
 - 光學透鏡與光學濾片限縮偵測器的偵測波長範圍

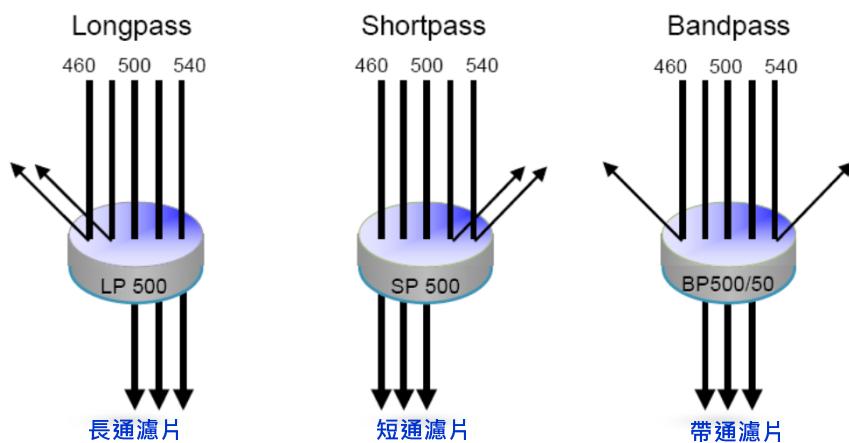


激發光學

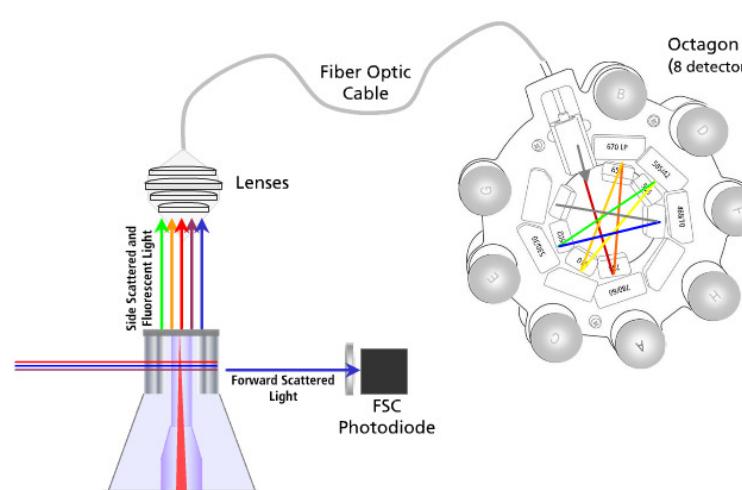
- *FACSCantoII*
 - 488nm 藍光雷射
 - 633nm 紅光雷射



Optical Filters 光學濾片



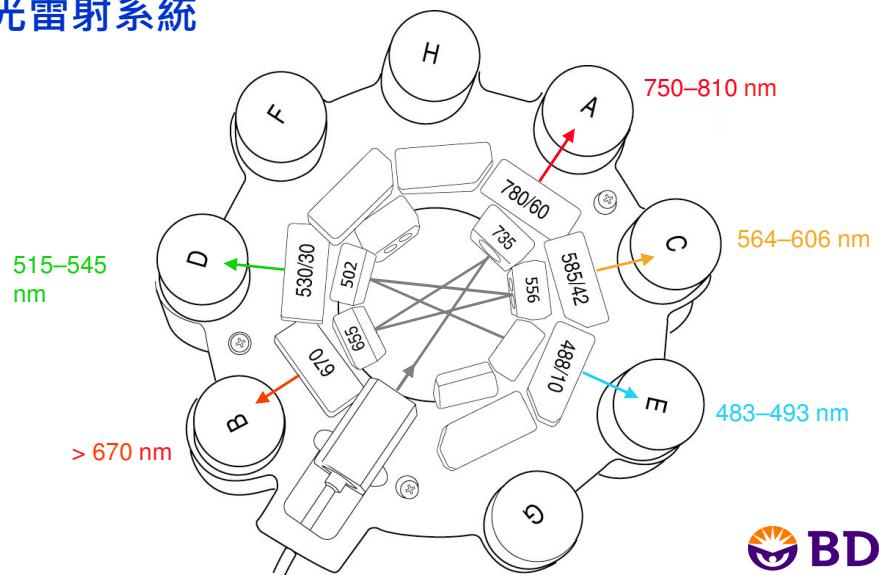
FACSCanto 收集光學系統



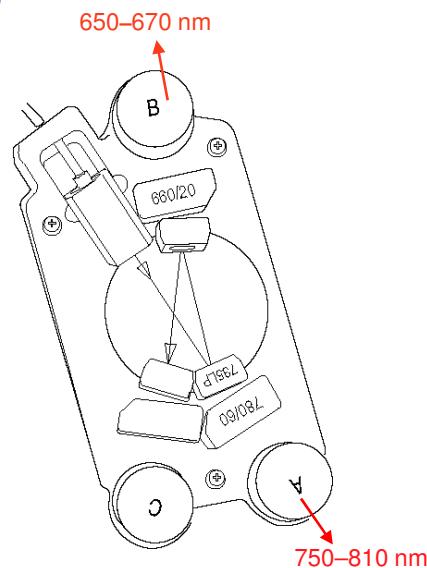
FACSCanto—Octagon and Trigons



收集光學系統—Octagon 藍光雷射系統



收集光學系統—Trigon 紅色雷射訊號系統



FACS CantoII configuration

	激發波長	偵測器發散波長	常見螢光物質名稱	其他螢光
	Excitation	PMT Position(nm)	Intended Dyes	Other Dyes
藍光 雷射 488	488 (blue)	A (750-810) B (>670) C (564-606) D (515-545) E	PE-Cy7 PerCP, PerCP-Cy5.5 PE FITC Side Scatter (SSC)	PI, PE-Cy5.5, DSRed PI GFP
	633(red)	A (650-670) B (750-810)	APC-Cy7 APC	Alexa Fluor® 633

設計實驗購買試劑前，請先確認所用螢光物質
激發波長Ex及發散波長Em是否跟儀器規格符合

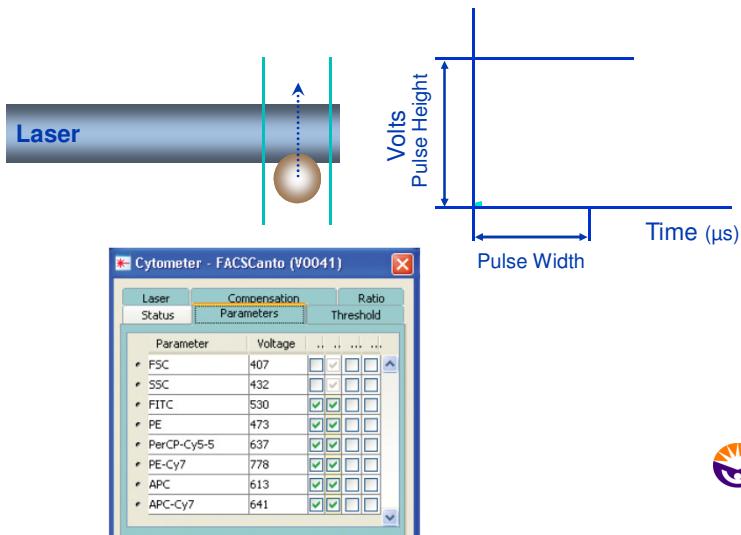


電子系統

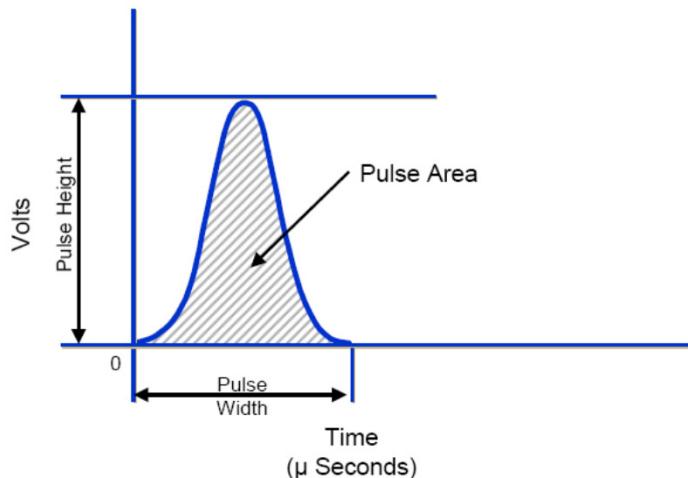
- Convert analog signals to proportional digital signals.
將類比訊號轉換成數位訊號
- Compute area, height and width for each pulse.
計算電子脈衝的面積, 高度及寬度
- Interface with the computer for data transfer
作為數據與電腦轉換的中介



Creation of a Voltage Pulse 產生電壓脈衝

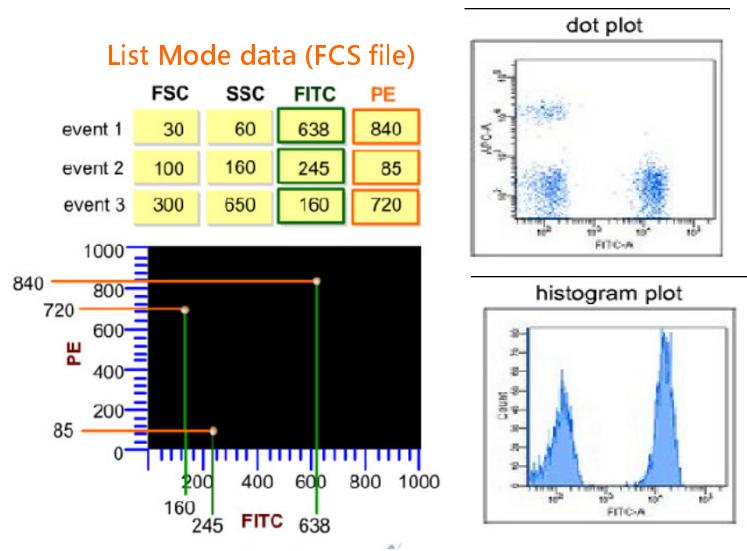


Quantification of a Voltage Pulse 量化電子脈衝



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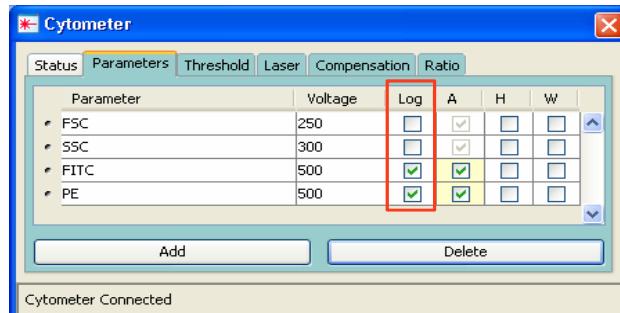
Data Storage 數據儲存



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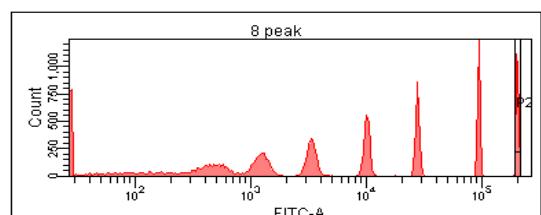
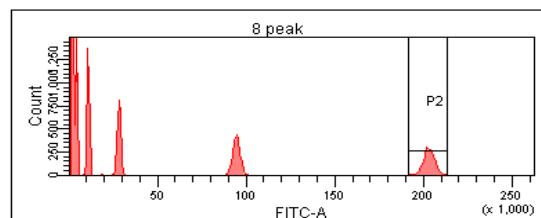
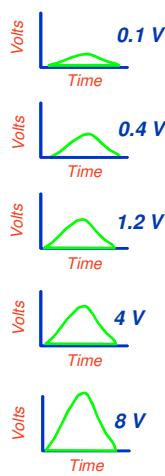
Data Display 數據顯示

- Linear Scaling 線性尺度
- Log Scaling 對數尺度

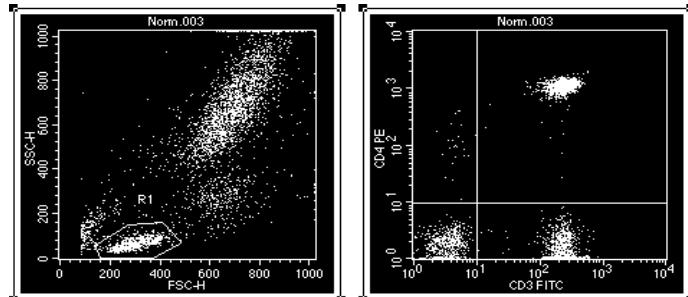


Linear v.s Log

Voltage Pulses



Linear v. Log Amplification

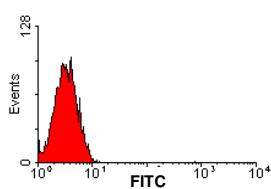


- **Linear** amplification is usually used for light scatter parameters and DNA analysis. 散射光與DNA分析使用線性
- **Log** amplification is used for fluorescence signals with a large dynamic range. 螢光訊號通常使用對數表示

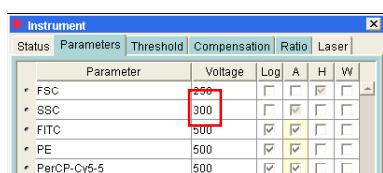
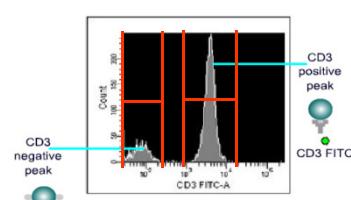


Instrument setting (Single color)

Unstained control



Single stained test sample

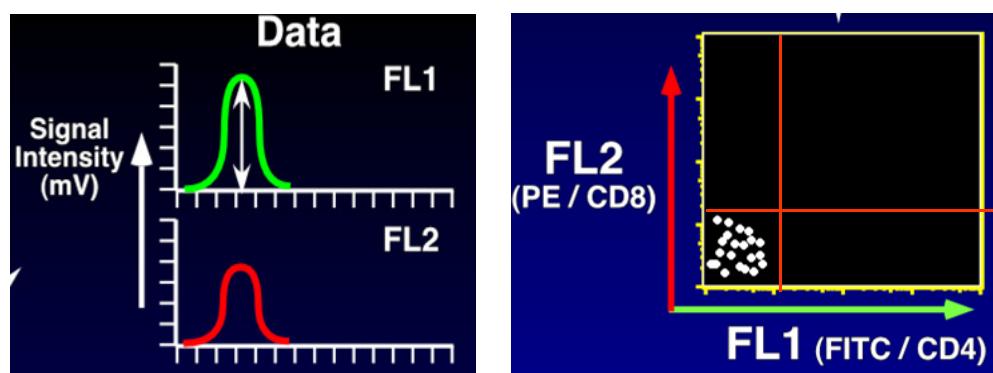


Tube: 13d-1			
Population	#Events	%Parent	%Total
All Events	12,789	####	100.0
Cell	10,240	80.1	80.1
CD3-	1,068	10.4	8.4
CD3+	9,177	89.6	71.8



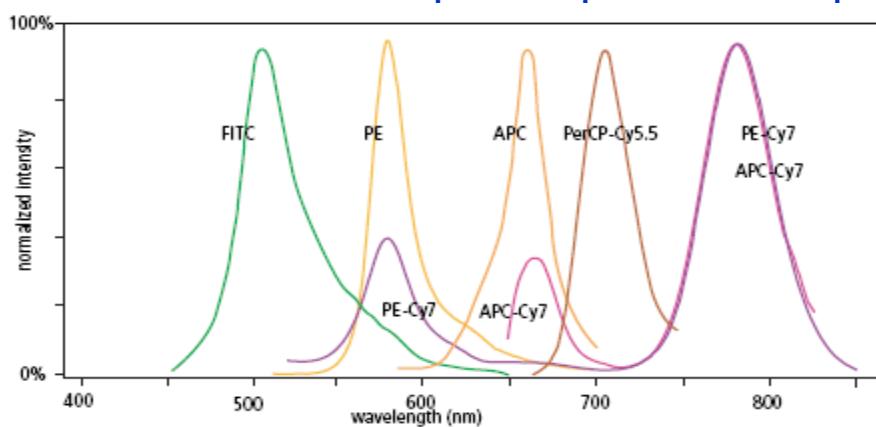
Instrument setting (Multi-color)

Unstained control

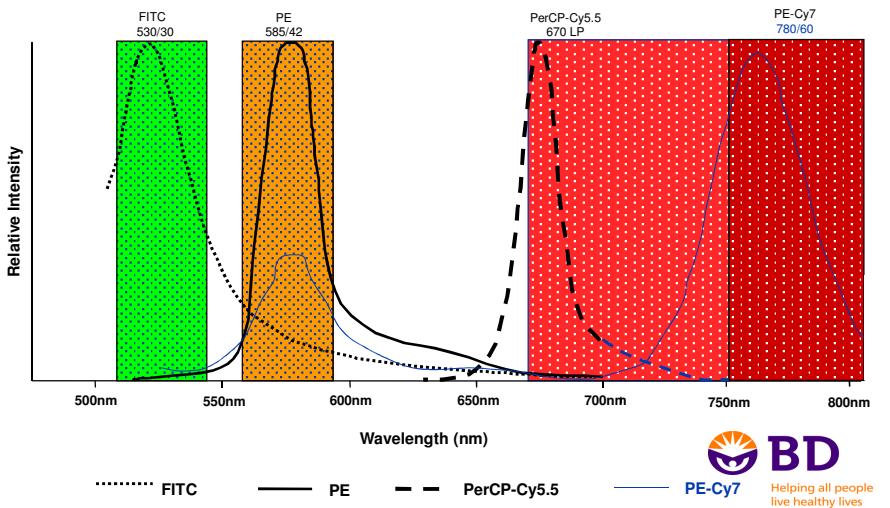


Compensation Theory 螢光補償

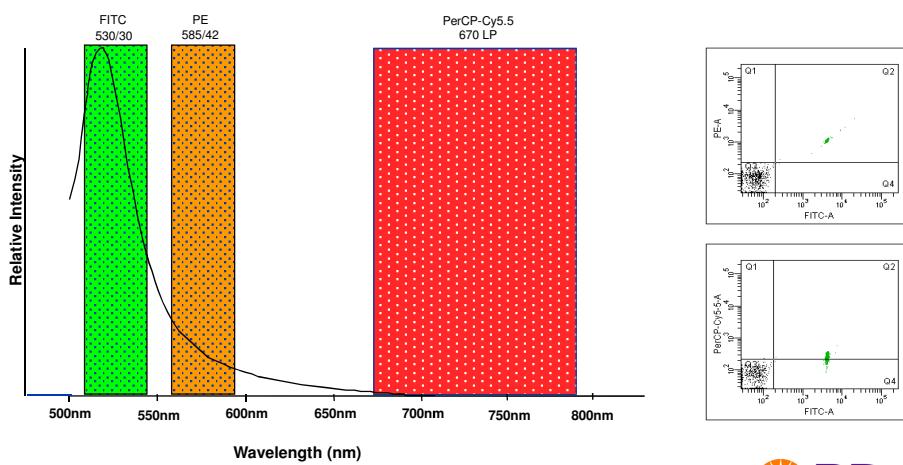
Emission Optics—Spectral Overlap



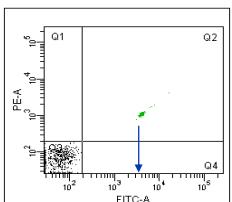
Spillover 融光溢光



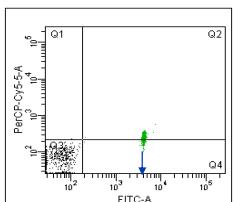
FITC Spillover



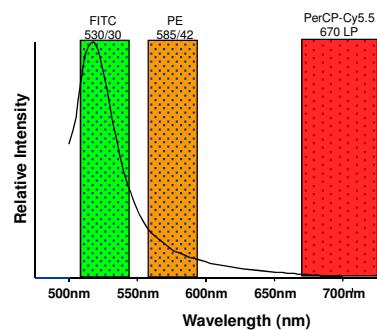
FITC Compensation



Population		PE-A
		Mean
■	Q3	64
■	Q4	179 ***



Population		PerCP-Cy5-5-A
		Mean
■	Q3	69
■	Q4	179

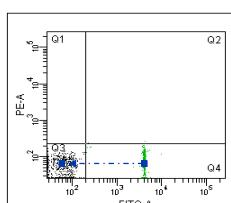


	Fluorochrome	- % Fluorochrome	Spectral Overlap
•	PE	FITC	0.00
•	PerCP-Cy5.5	FITC	0.00

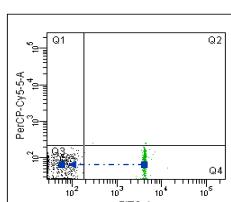
增加數值



FITC Compensation



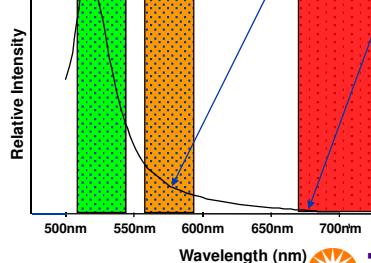
Population		PE-A
		Mean
■	Q3	61
■	Q4	60



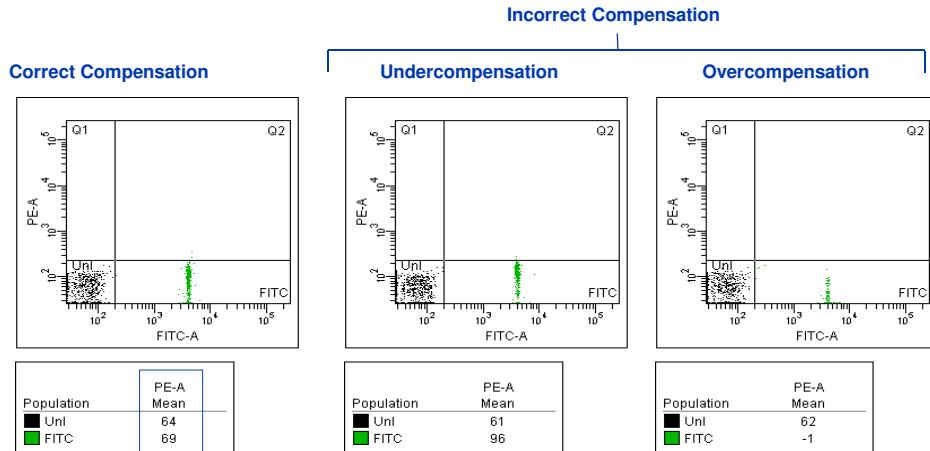
Population		PerCP-Cy5-5-A
		Mean
■	Q3	69
■	Q4	66

To lower cluster, increase value.

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



Compensation Examples 螢光補償範例

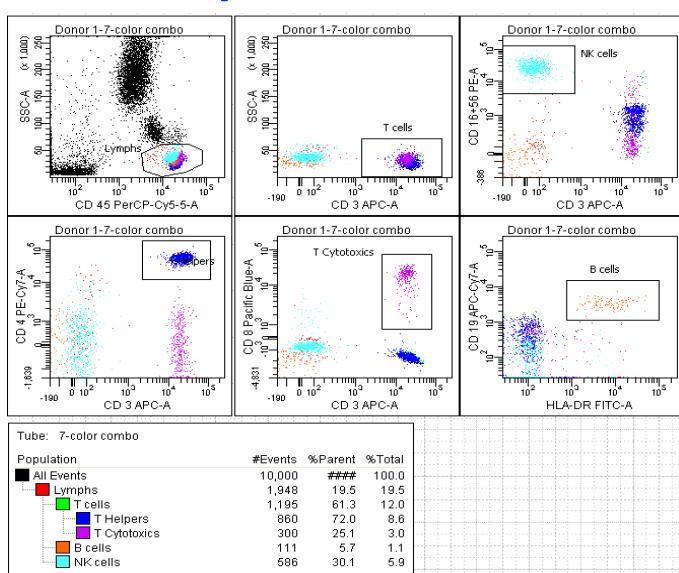


未表現螢光細胞與FITC+細胞的PE
平均螢光接近=兩群細胞均沒有表
現紅色螢光



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Multicolor Analysis 多色分析範例



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Application Examples

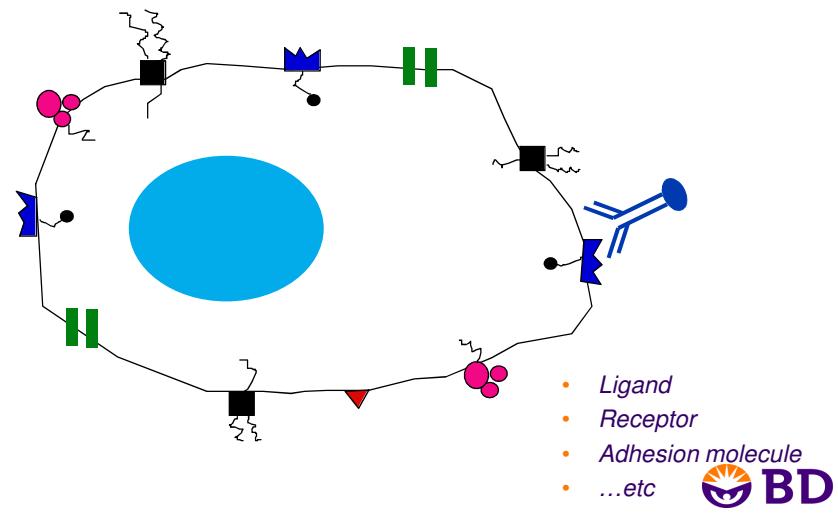


Applications 應用

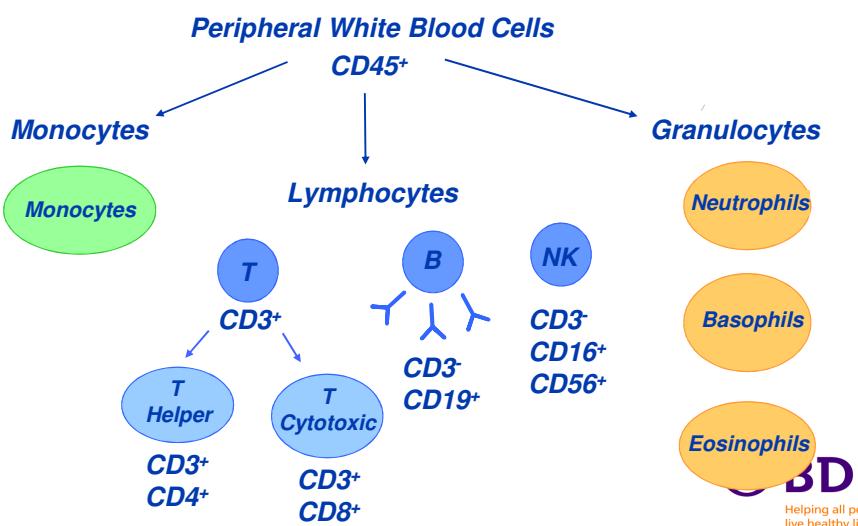
- **Phenotype Analysis (Cell Surface Antigens/Markers)**
- **Intracellular Analysis**
 - Eg. Cytokines, Signal Transduction molecules...etc.
- **DNA Analysis**
 - Eg. Viability, Cell cycle, Apoptosis...etc.
- **Cell Function Analysis**
 - Eg. Free radicals, Ca^{2+} , Reporter genes...etc.
- **CBA (Cytometric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

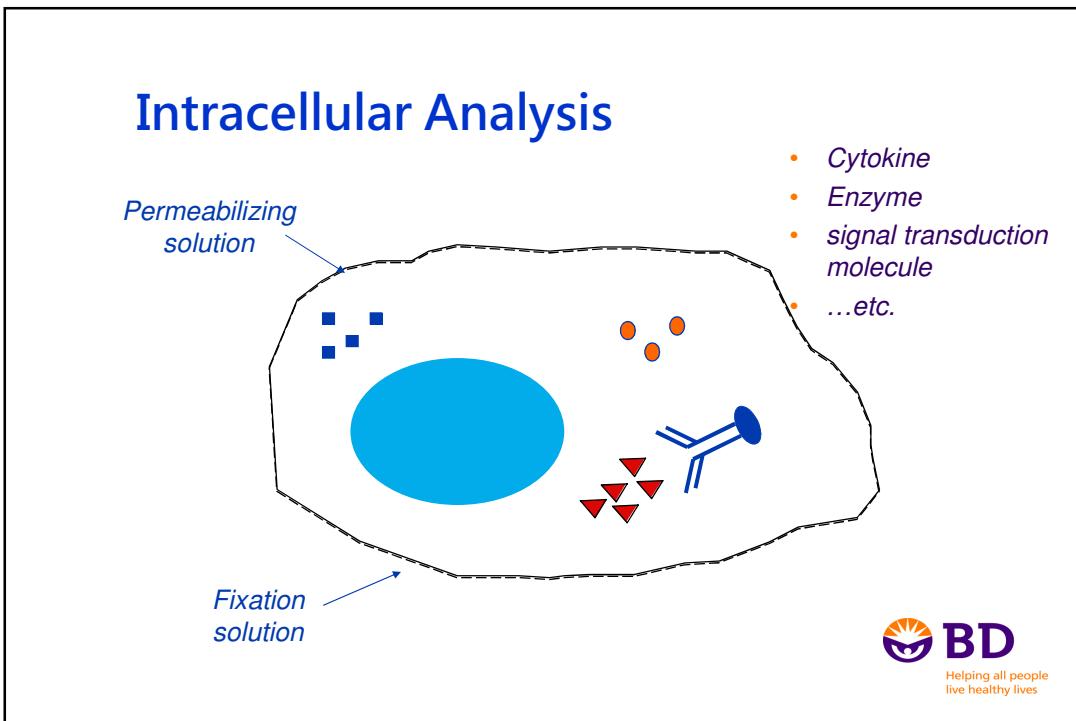
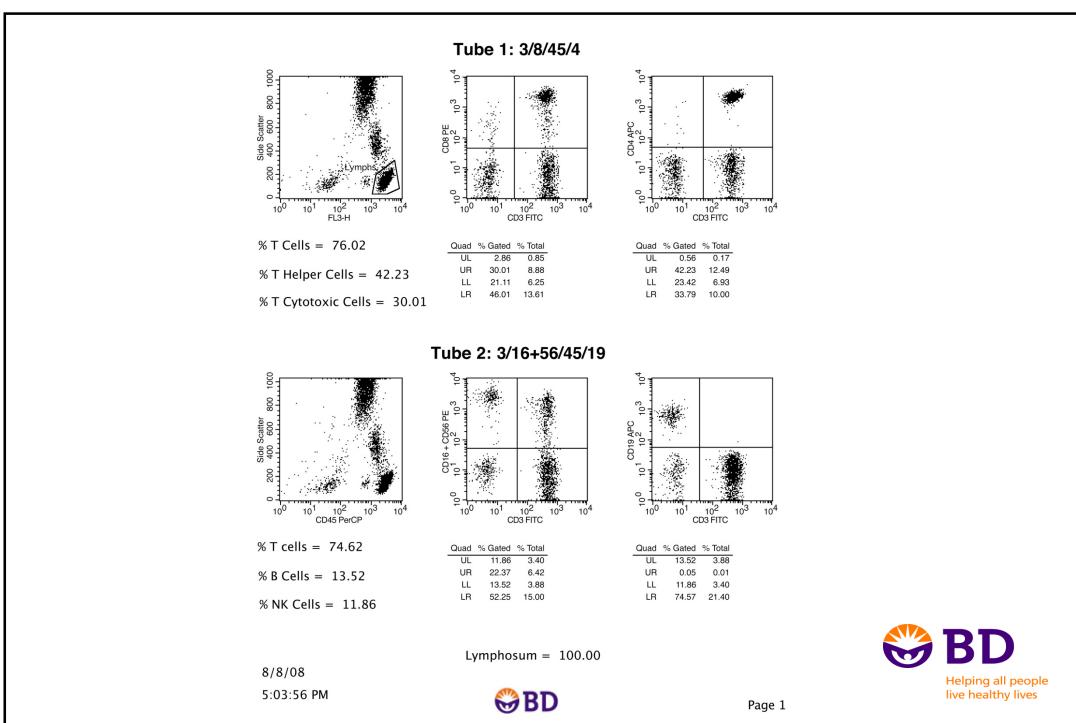


Phenotype Analysis 細胞表型分析



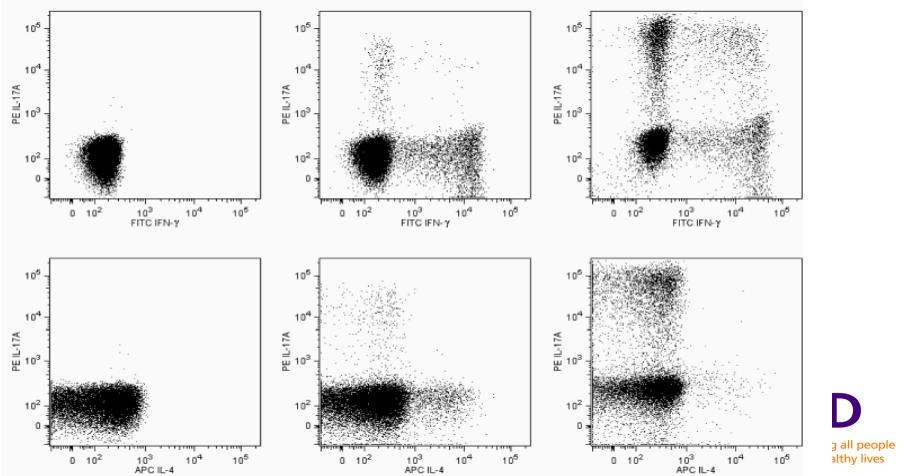
Lymphocyte Immunophenotyping



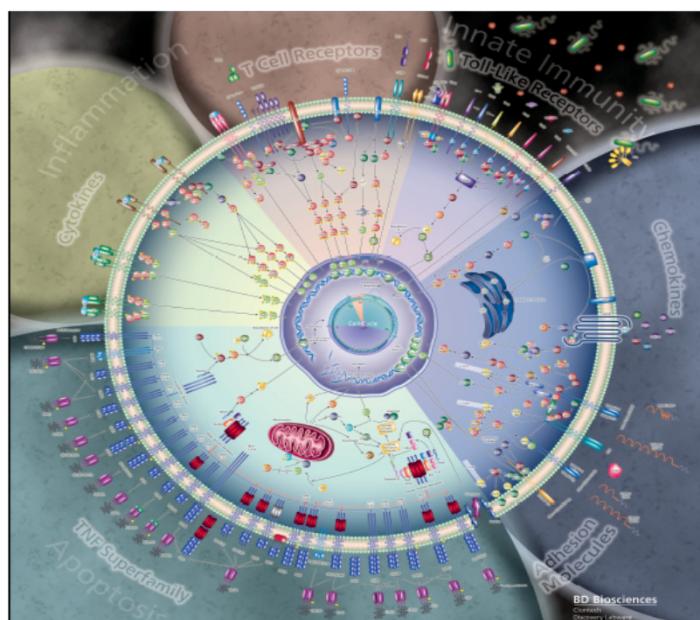


Combination of Cell Surface and Cytoplasmic Staining

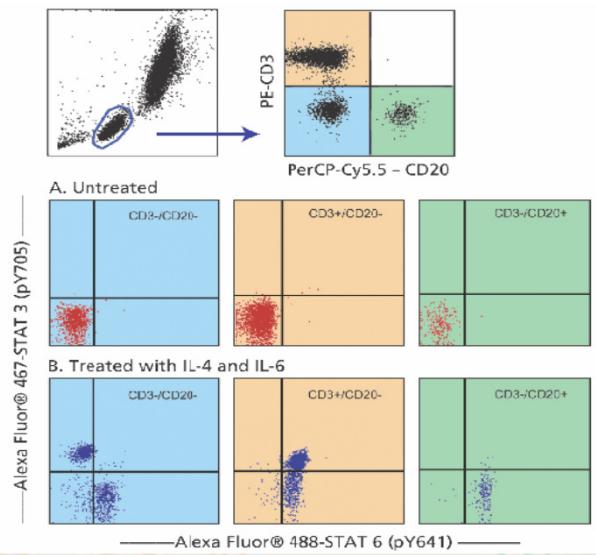
Th1/Th2/Th17 Phenotyping Kit



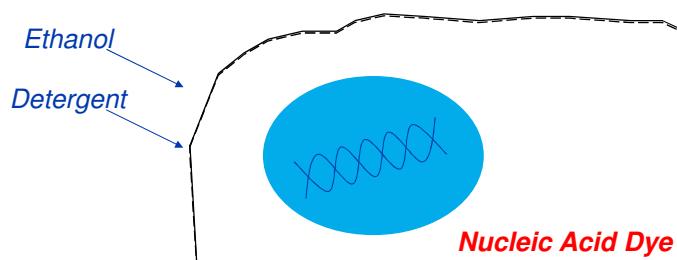
Signal Transduction



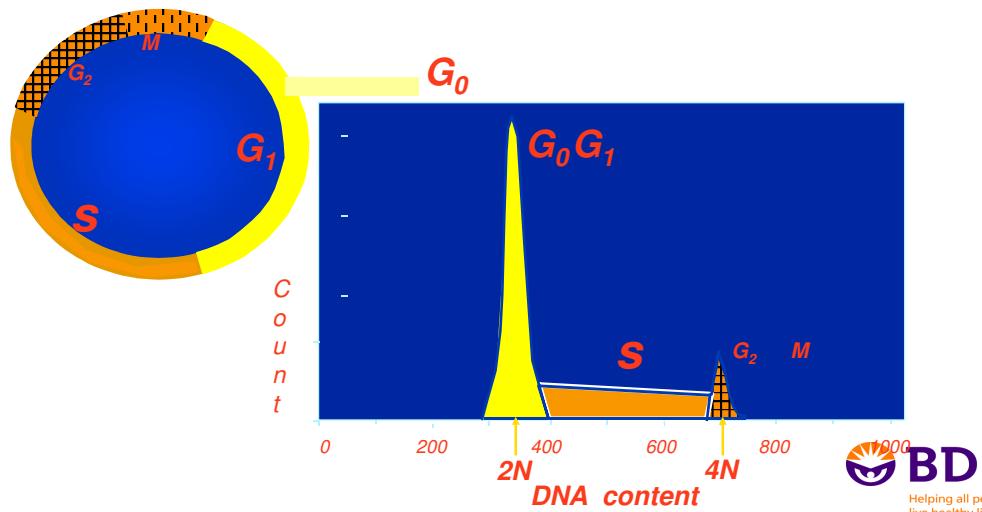
Intracellular Staining in Activated Lysed Whole Blood



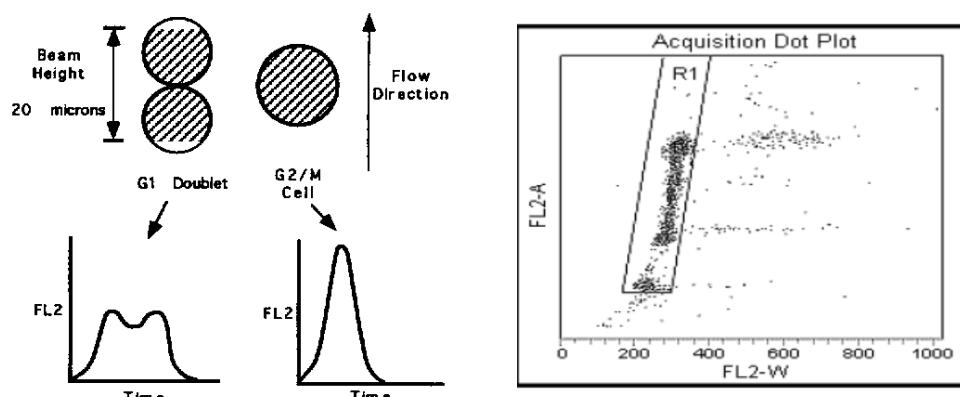
DNA Analysis



Cell Cycle Analysis

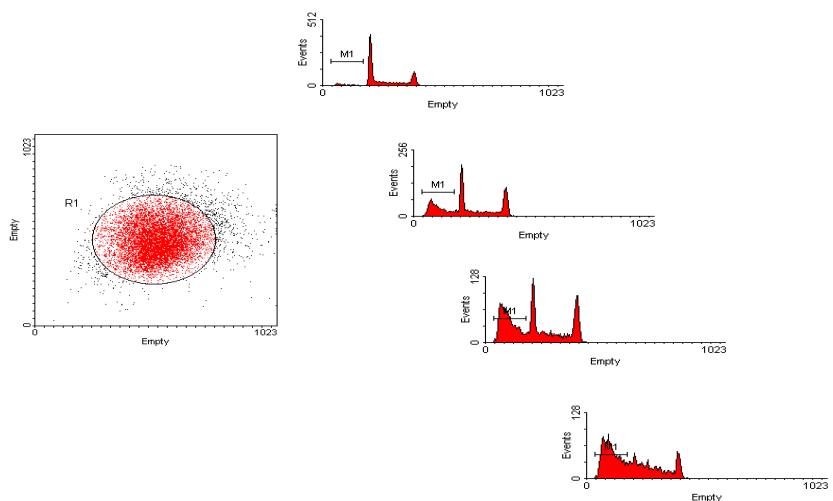


DDM (Doublet Discrimination)



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Apoptosis (Sub G1)

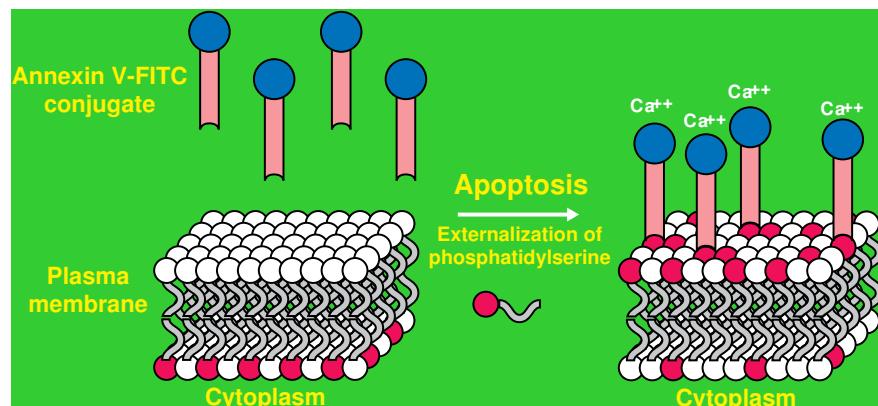


Cell Function Analysis

- Membrane Potential (DiOC₆, JC-1)
- Oxidative Metabolism (Free Radicals)
- Intracellular PH Value (Snarf-1)
- Ca⁺⁺ Influx (Fluo-4/Fura Red, Indo-1)
- Phagocytosis
- Cell Proliferation (PI, BrdU, Intracellular Cyclins)
- Apoptosis (Annexin V, active Caspase-3)

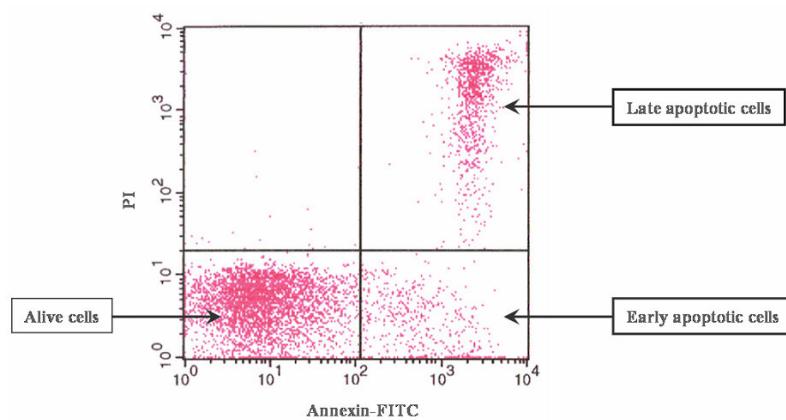


Annexin V Assay



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Annexin V/PI Double Staining

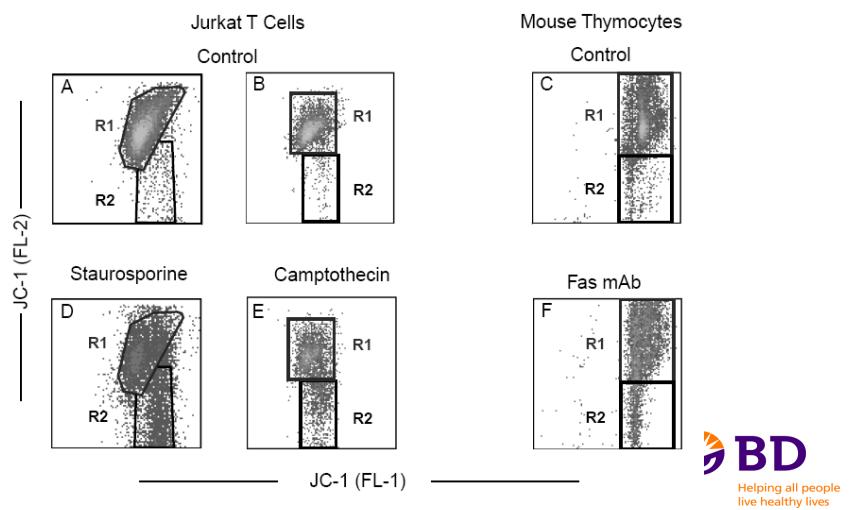


Bordón et al. Radiation Oncology 2009 4:58

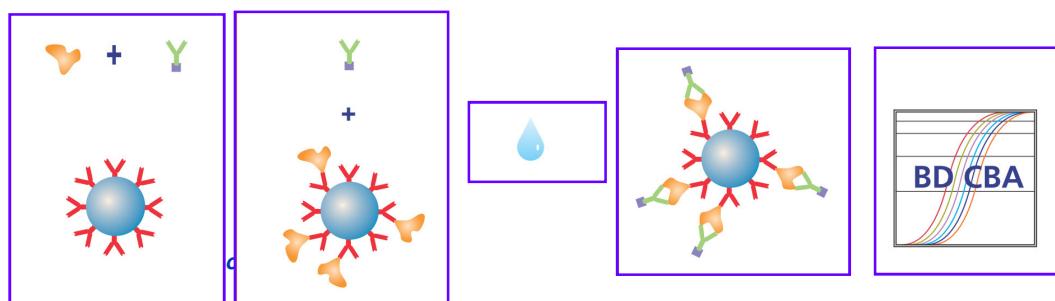


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Mitochondria Membrane Potential (JC-1)

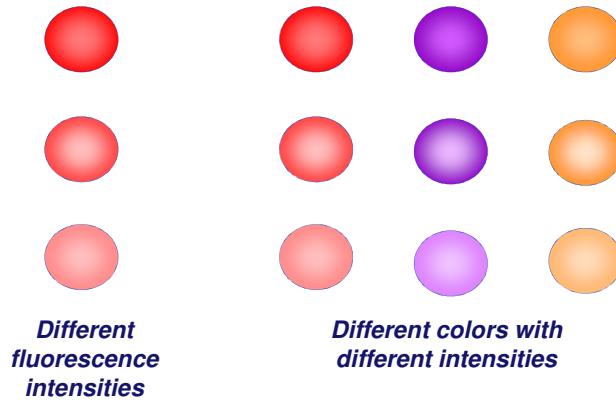


Cytometric Beads Array (CBA)

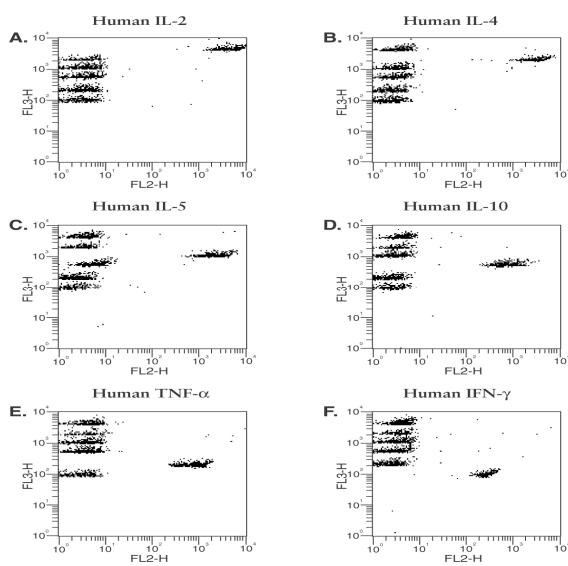


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Beads Provide a Flexible Platform

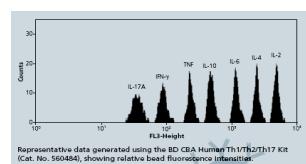


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Proteins Measured

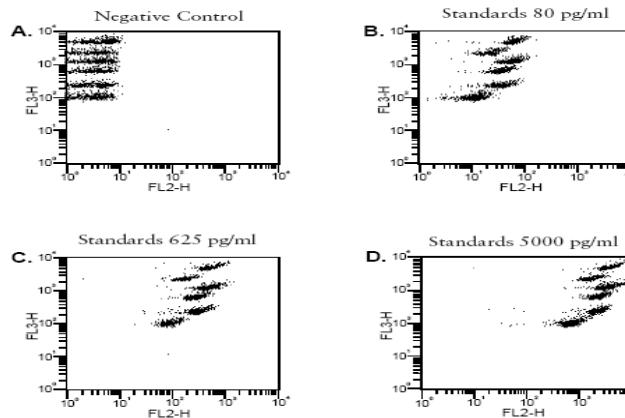
- A. Interleukin (IL)-2
- B. IL-4
- C. IL-5
- D. IL-10
- E. Tumor Necrosis Factor- α
- F. Interferon- γ



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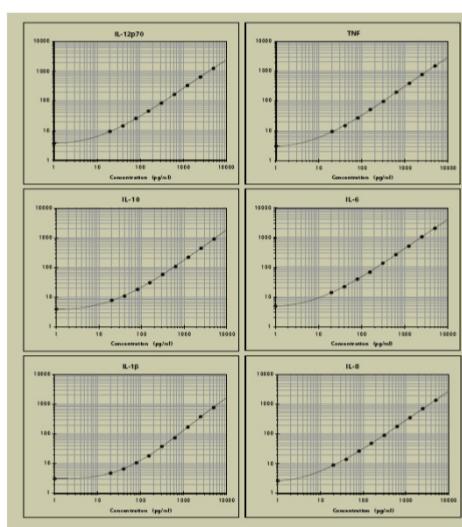
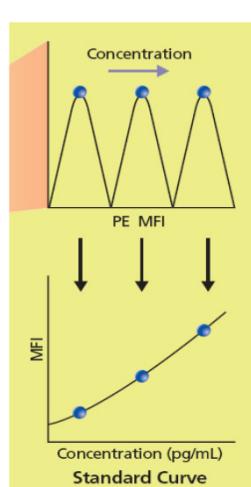
Cytometry Beads Array (CBA)

Typical Data



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Standard Curves

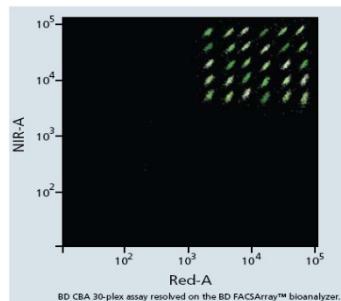


Representative standard curves generated using the BD CBA Human Inflammatory Cytokines Kit.

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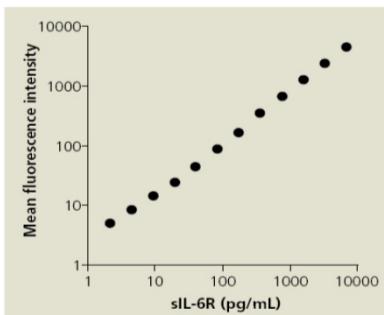
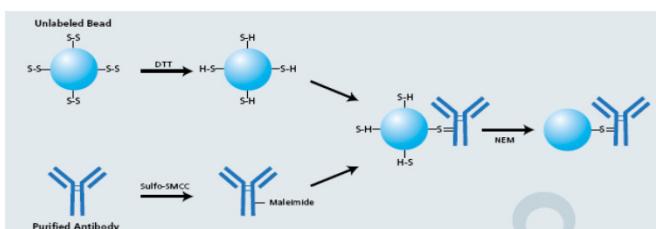
CBA Flex Sets

- Open configuration (Up to 30 plex)
- Clustering based on Red and NIR fluorescence intensity



CBA Functional Beads

- Can be conjugated with any Ab



Standard curve for a soluble IL-6 receptor assay generated using BD CBA Functional Bead E4 following the conjugation procedure in the BD CBA Functional Bead Conjugation Buffer Set manual.

Data courtesy of Joseph Cannon and Gloria Sloan, Medical College of Georgia.

Thank You!

