流式細胞儀 --- FACSCanto
之基本原理與應用

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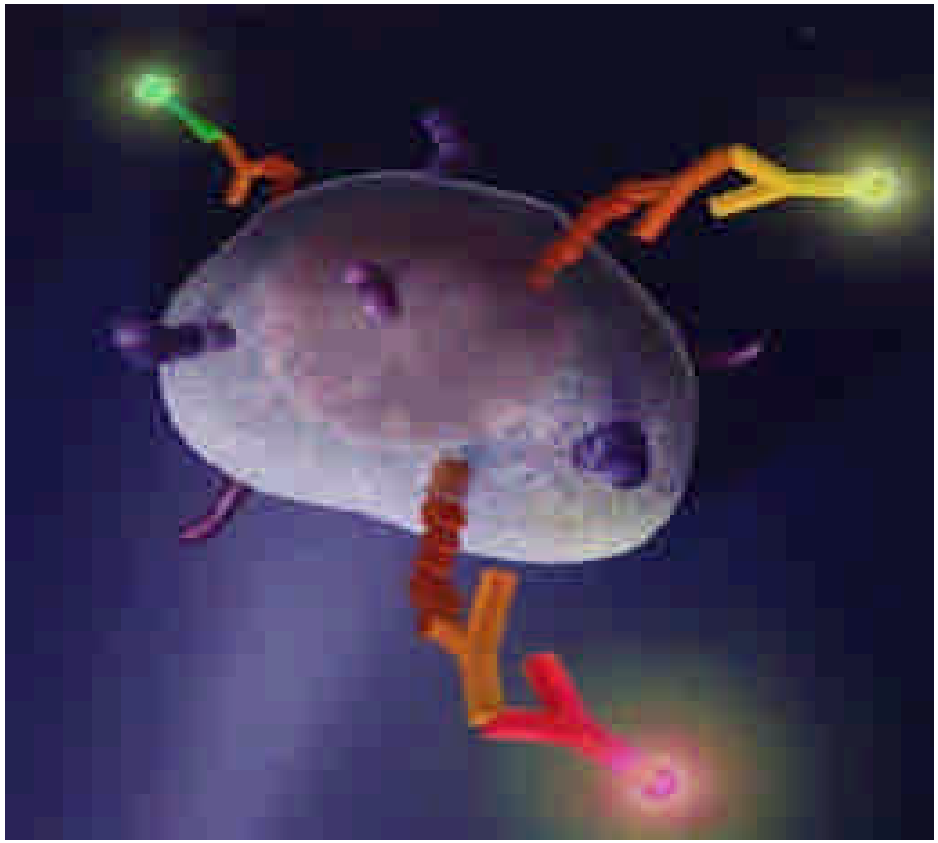
大綱

- 實驗設計原理
- 流式細胞儀運作原理

實驗設計原理

- 藉由螢光抗體標識細胞之抗原特性
- 藉由螢光化合物標識細胞特性
- 藉由螢光染劑標識細胞特性
- Cytometry Beads Array



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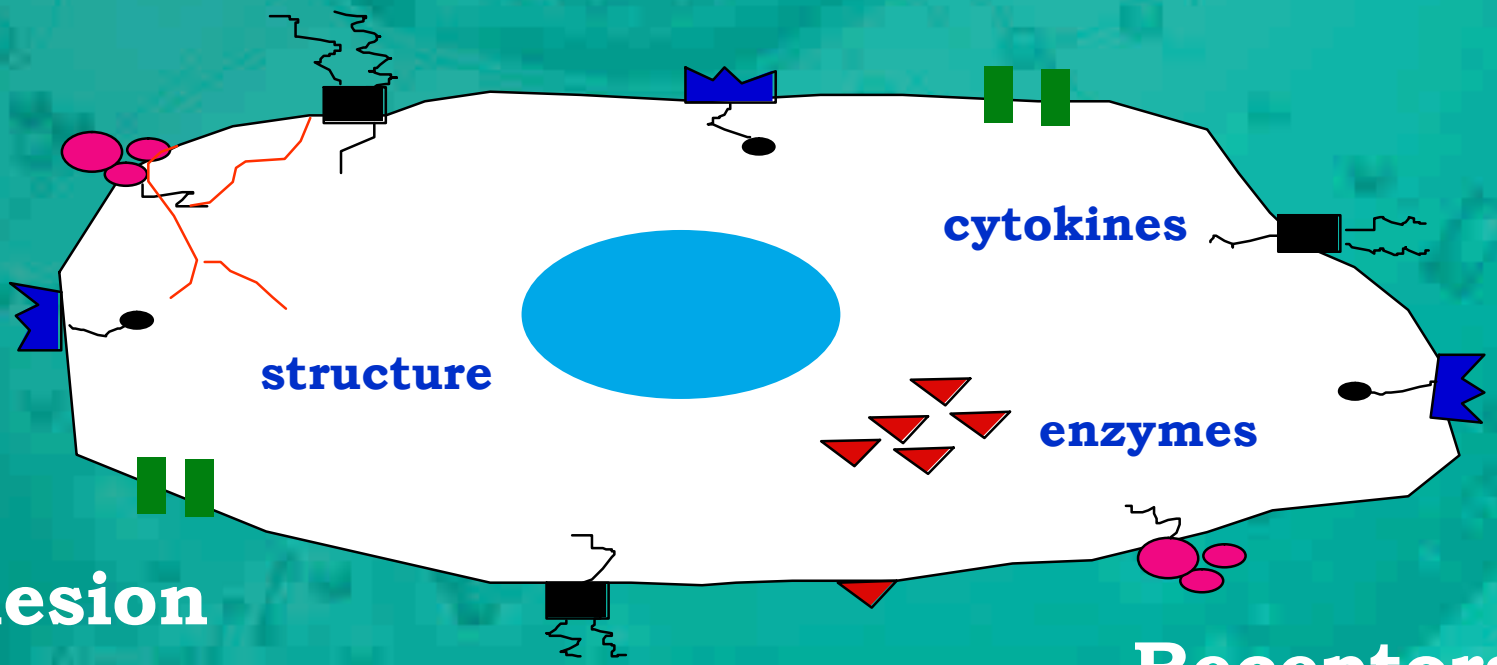
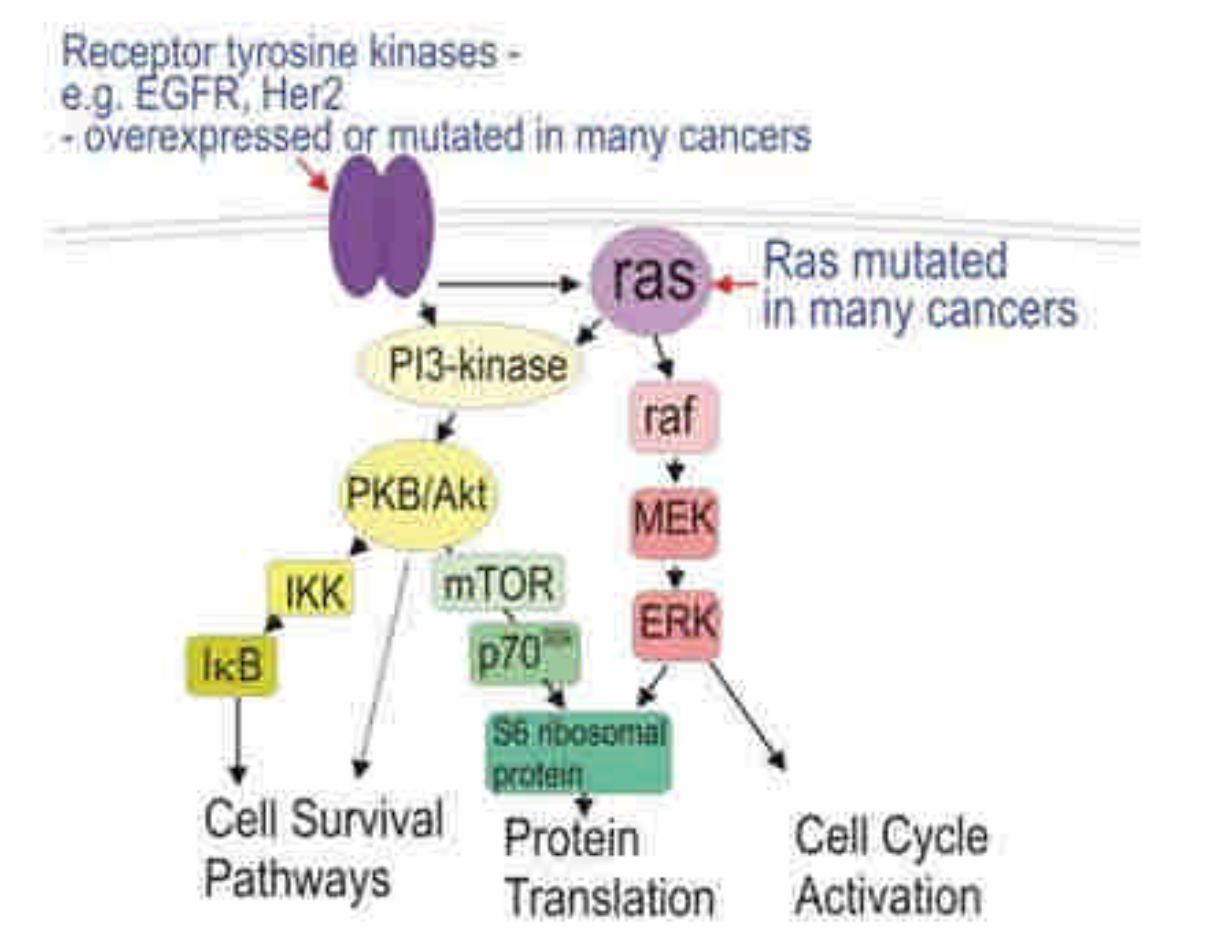


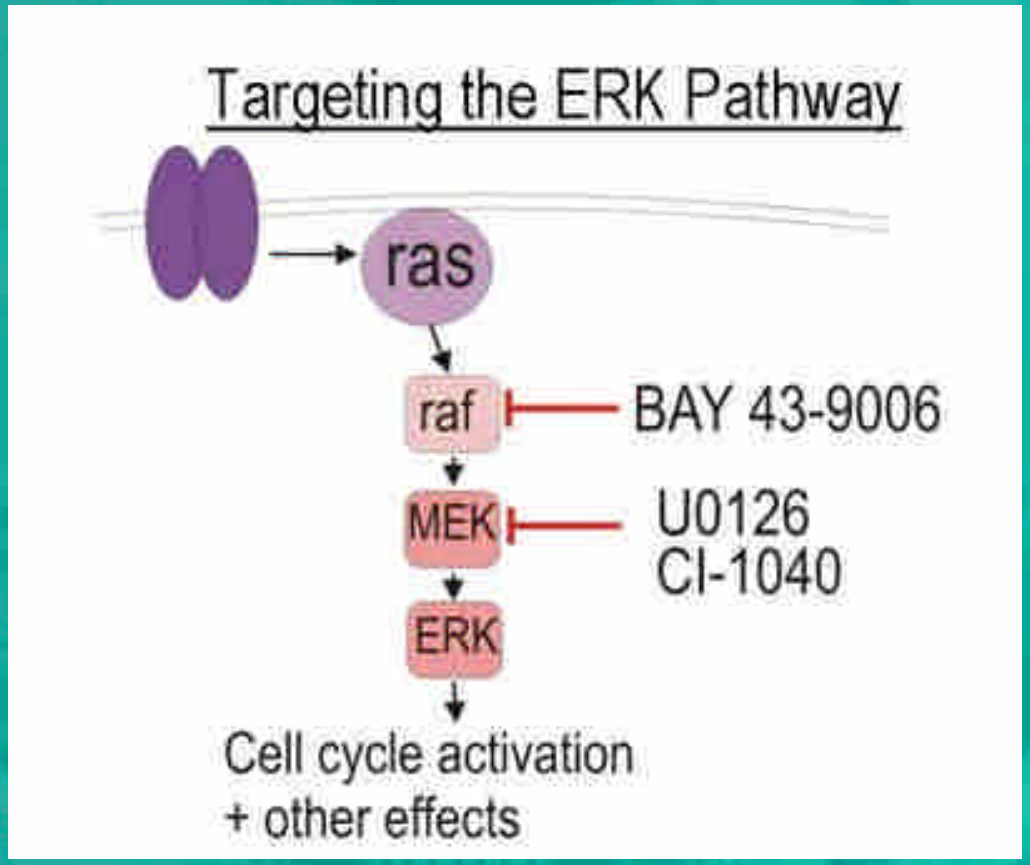
淋巴球免疫分型

FITC	PE	使用原理
CD45	CD14	儀器可自動依據 CD45 與 CD14 之免疫螢光染色，測量三群白血球的比例，淋巴球 (CD45 Bright /CD14-/Low FSC/ Low SSC) ，單核球 (CD14+ /CD45 Intermediate/High FSC/Intermediate SSC) ，顆粒性球 (CD14 dim/ CD45 dim/High FSC/High SSC) ，細胞碎片與紅血球應是 (CD14-/CD45- /Low FSC/Low SSC) 。
IgG1	IgG1	根據陰性對照組之染色程度來劃分陽性/陰性之界線，依此界線在陰性對照的樣品中，假陽性(Quardrant1+2+4)不得超過百分之二。
CD3	CD19	T 細胞必須表達有 CD3+ 抗原， B 細胞表達有 CD19+ 抗原。此兩群細胞與 NK 細胞之總和應約略等於淋巴球的總數，可作爲品管的指標。
CD3	CD4	CD4+ T 細胞必須同時表達有 CD3+ 與 CD4+ 抗原。
CD3	CD8	CD8+ T 細胞必須同時表達有 CD3+ 與 CD8+ 抗原。
CD3	CD16+ or CD56+	NK 細胞必須不表達有 CD3 抗原，同時表達有 CD16+ 或 CD56+ 抗原。 NK 細胞與 T & B 兩群細胞之總和應約略等於淋巴球的總數，可作爲品管的指標。

訊息傳導



治癌藥物



實驗設計原理

- 藉由螢光抗體標識細胞之抗原特性
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- 藉由螢光染劑標識細胞特性
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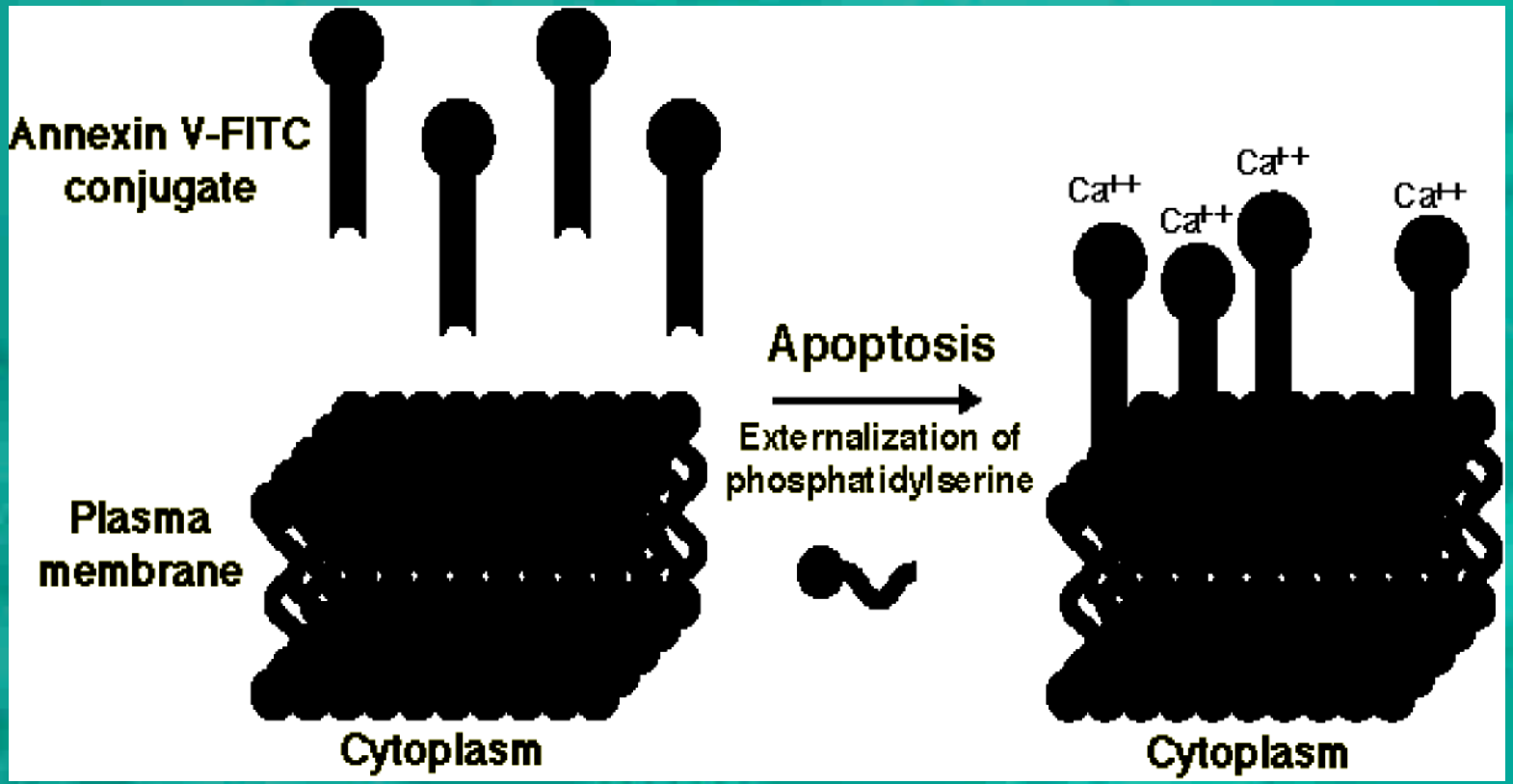
Annexin V Assay



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實驗設計原理

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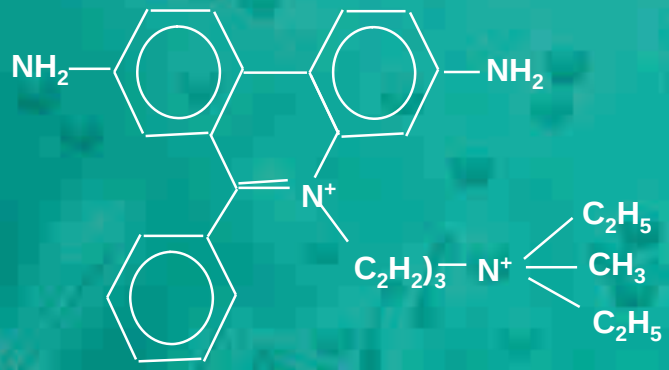
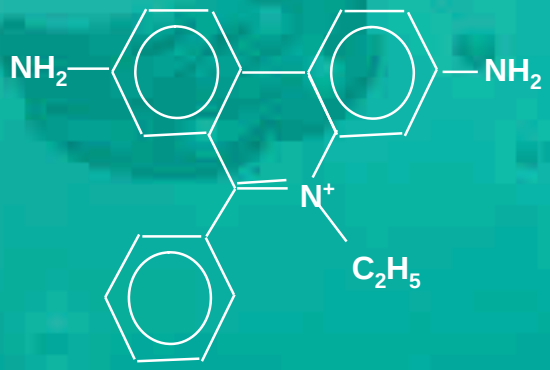
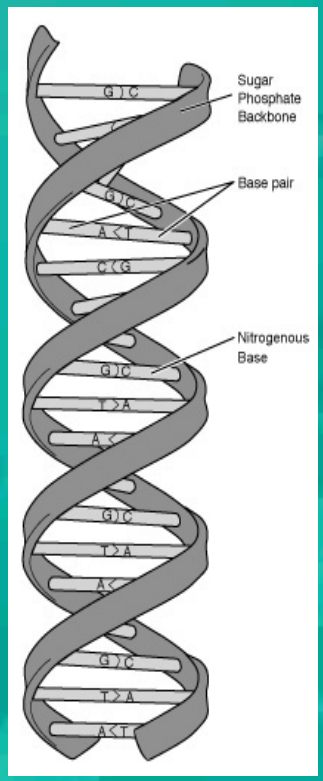
DNA 特異性染劑

Propidium

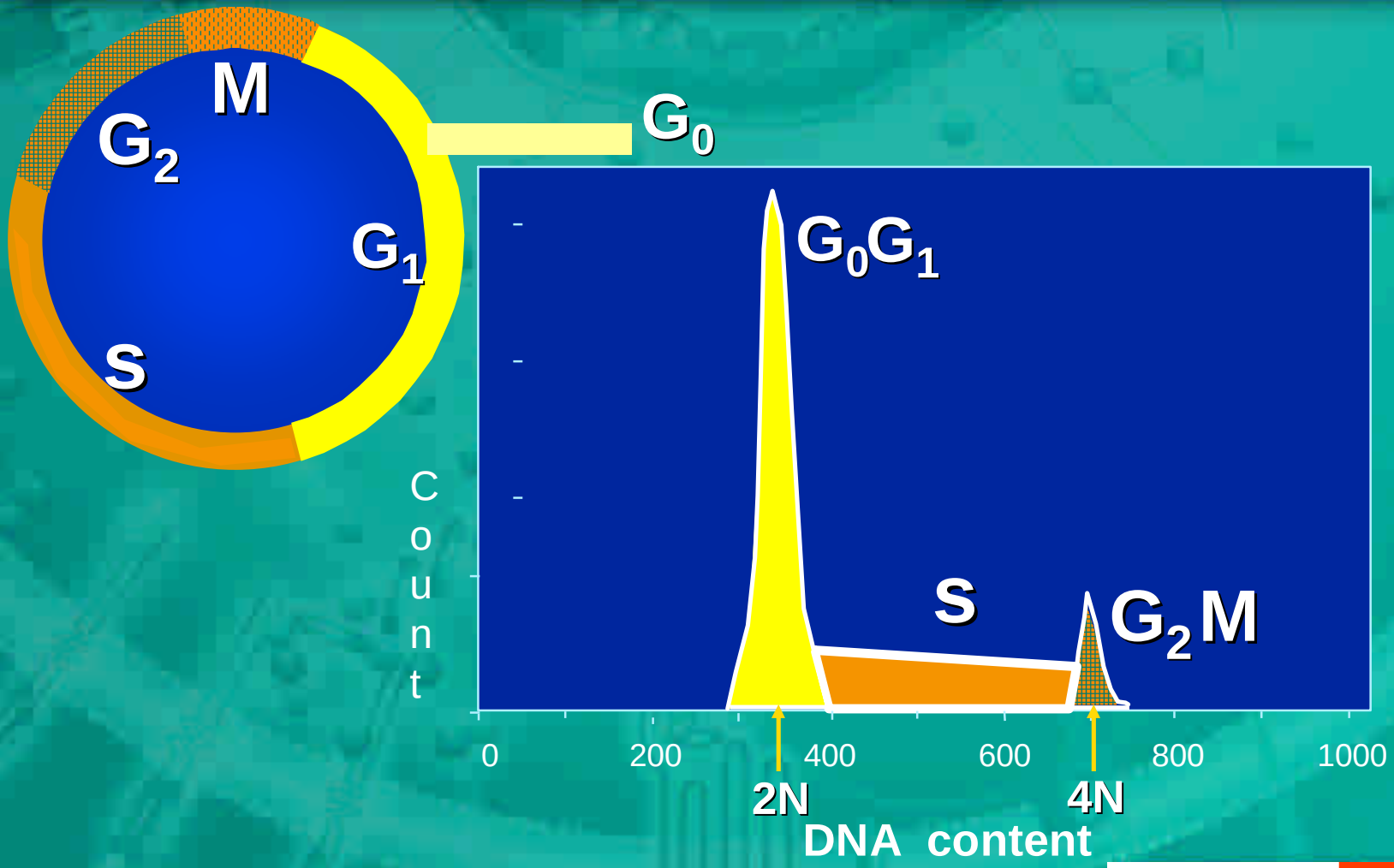


Ethidium





細胞周期位相的決定



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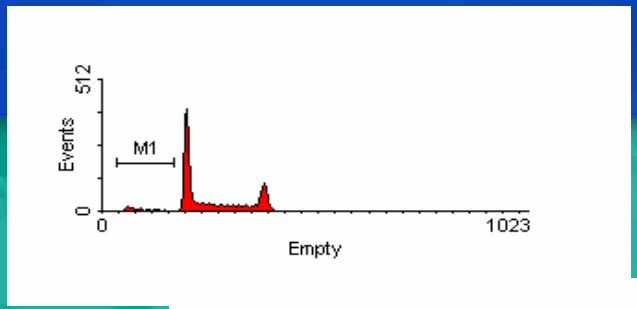
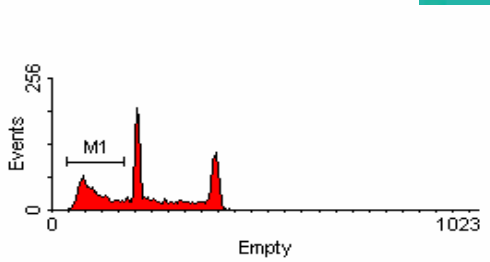
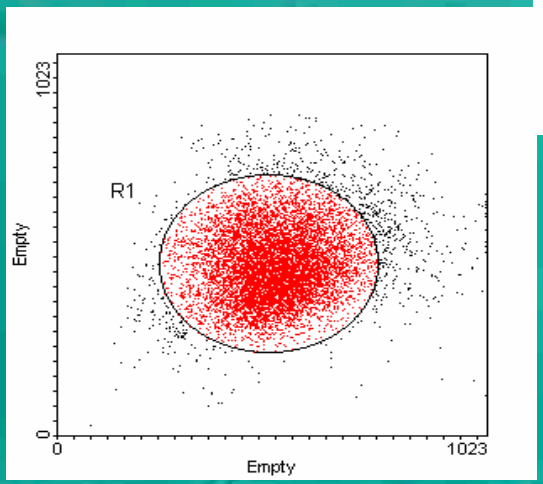
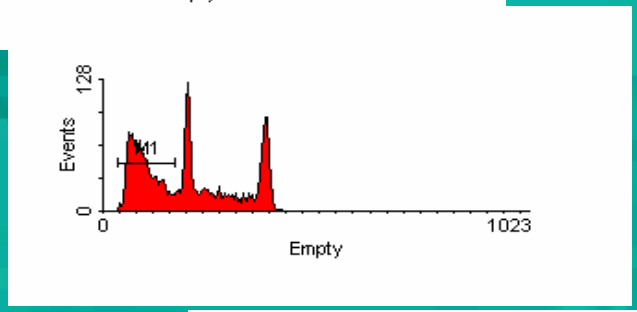
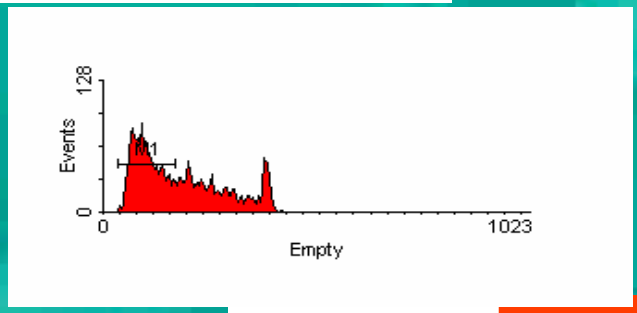
Sub G1











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Mitochondria Membrane Potential

細胞增殖反應

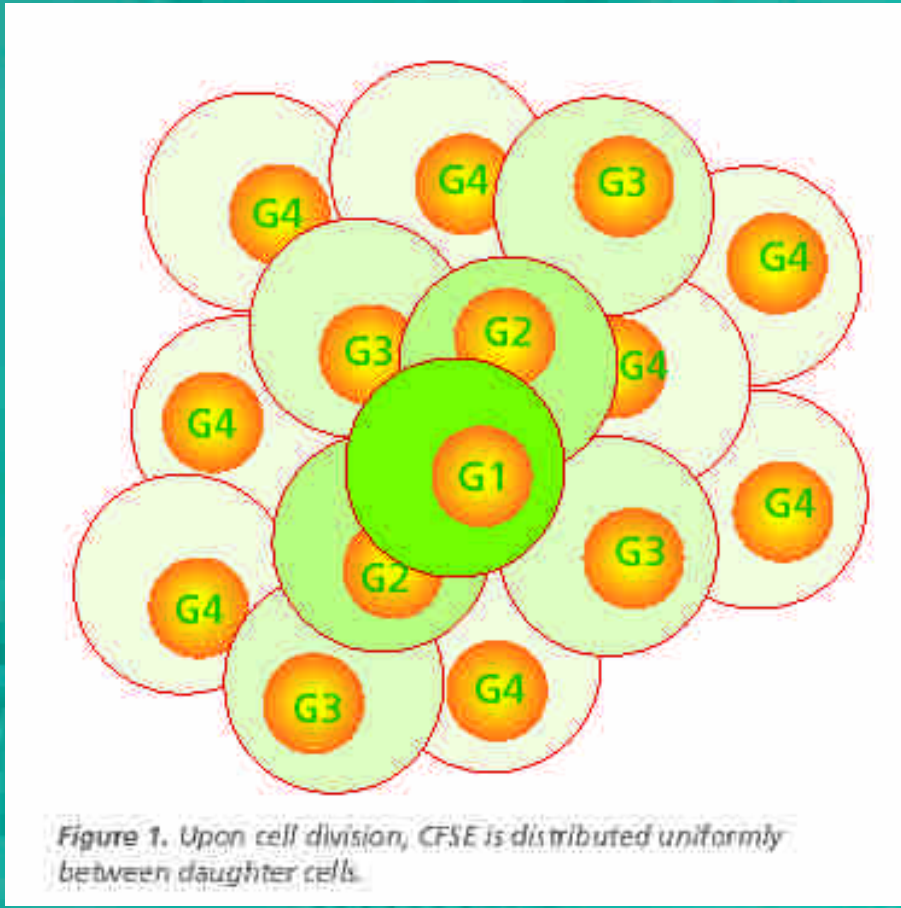

Figure 1. Upon cell division, CFSE is distributed uniformly between daughter cells.

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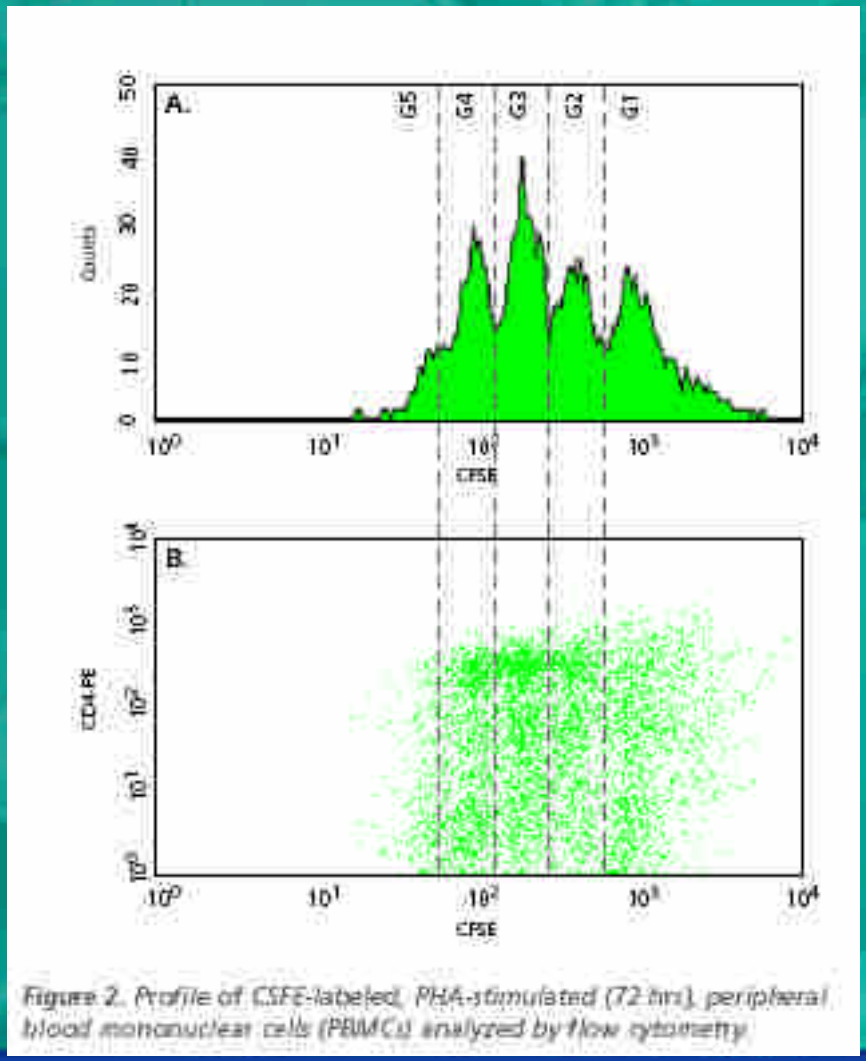
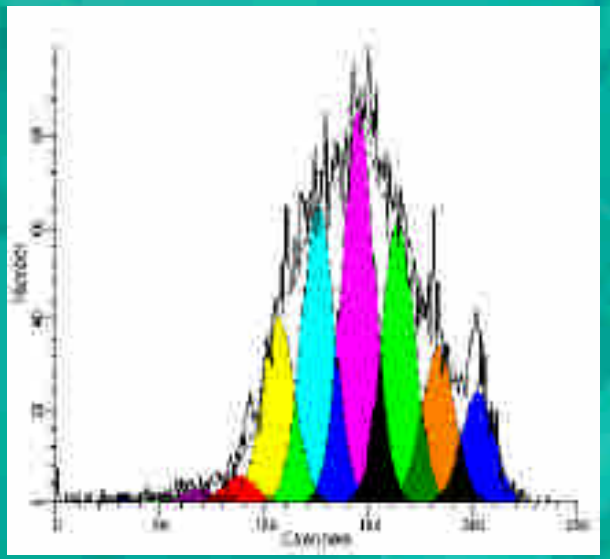


Figure 2. Profile of CSFE-labeled, PHA-stimulated (72 hrs) peripheral blood mononuclear cells (PBMCs) analyzed by flow cytometry



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實驗設計原理

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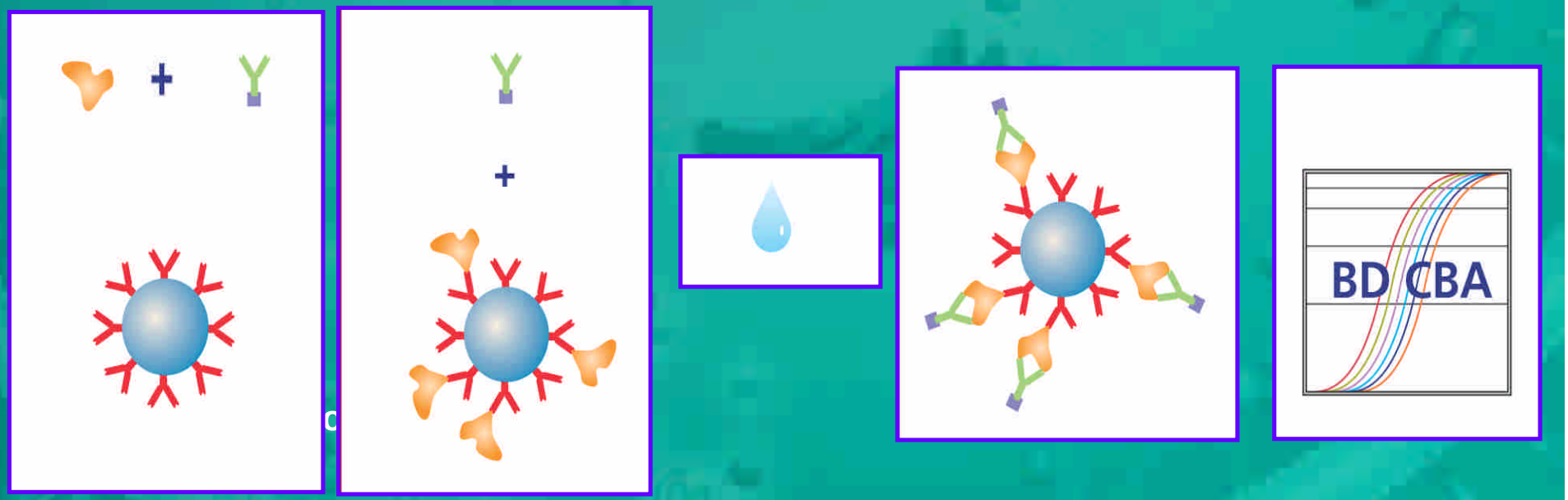
- Cytometry Beads Array

Cytometric Beads Array (CBA)



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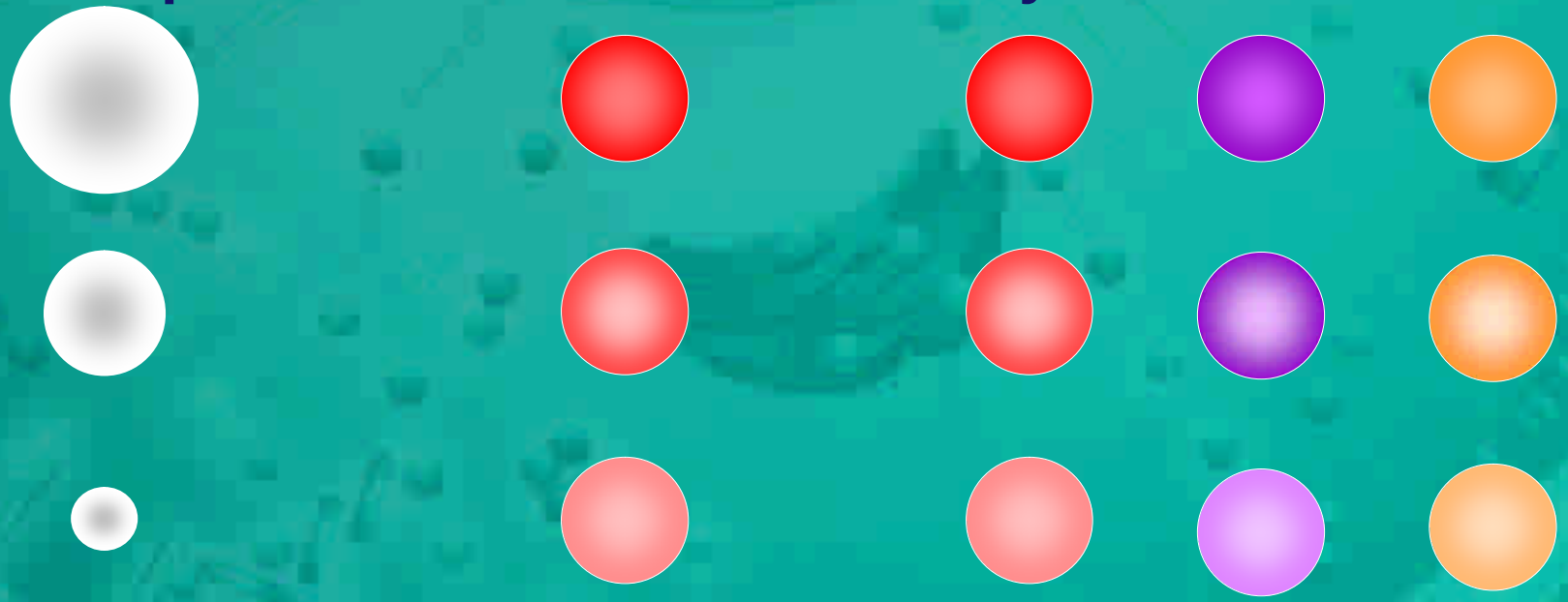
Beads Provide a Flexible Platform

Beads provide an expandable assay platform for use with a flow cytometer



Multiple sizes

Different intensities*

Different colors with different intensities

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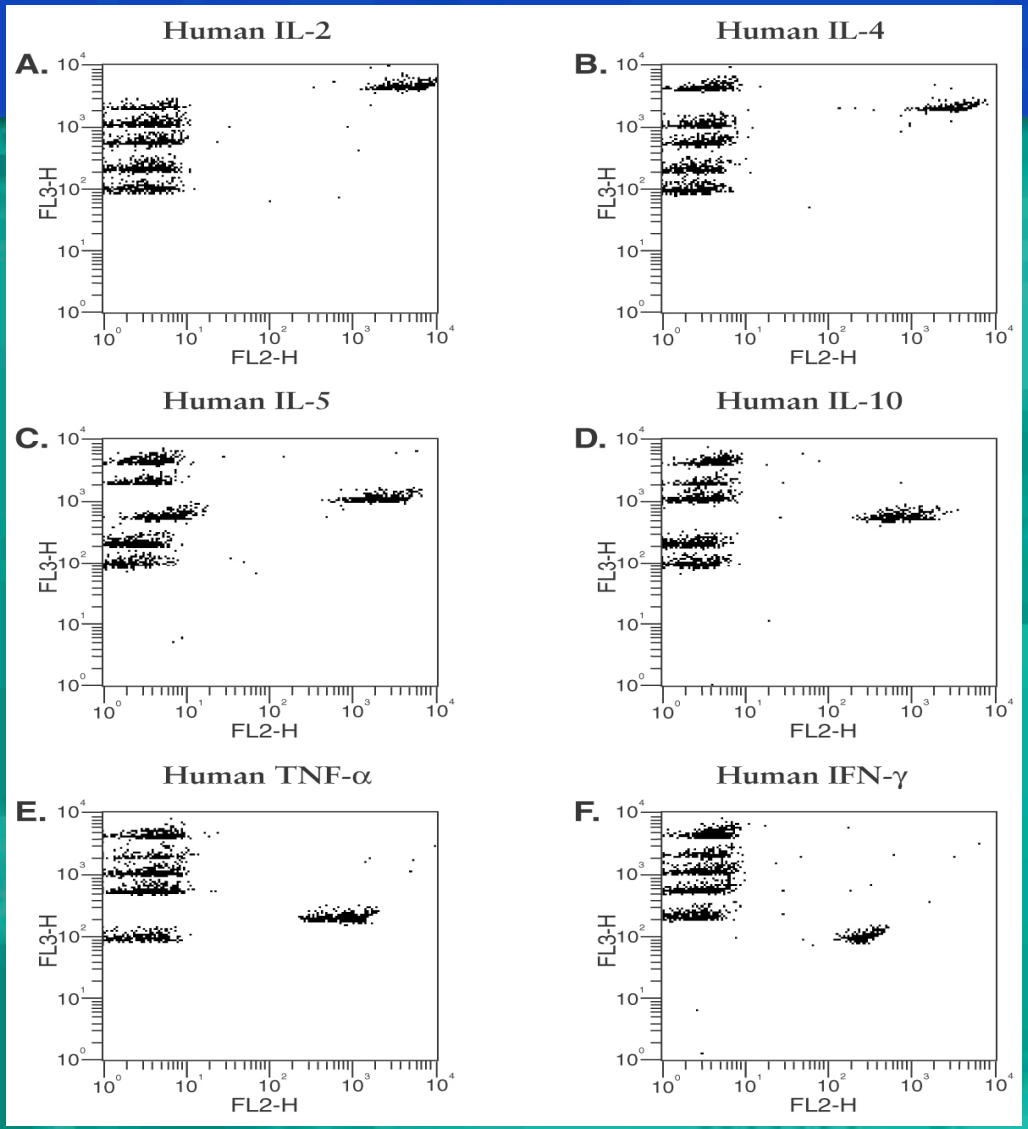
Advantages of Bead-Based Immunoassays

- Analyze multiple analytes simultaneously
- Reduced sample volume requirements
- Reduced hands-on time by parallel analysis of samples
- Wide dynamic range of fluorescence detection (requires fewer sample dilutions)



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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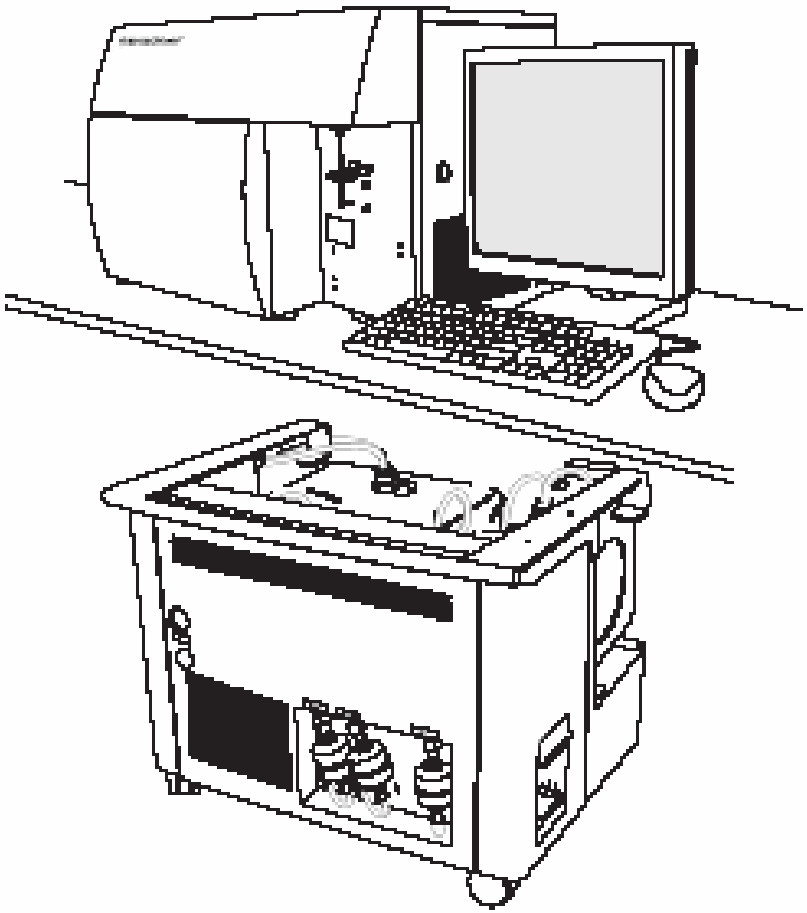
BD FACSCanto™

Figure 1-1 BD FACSCanto system



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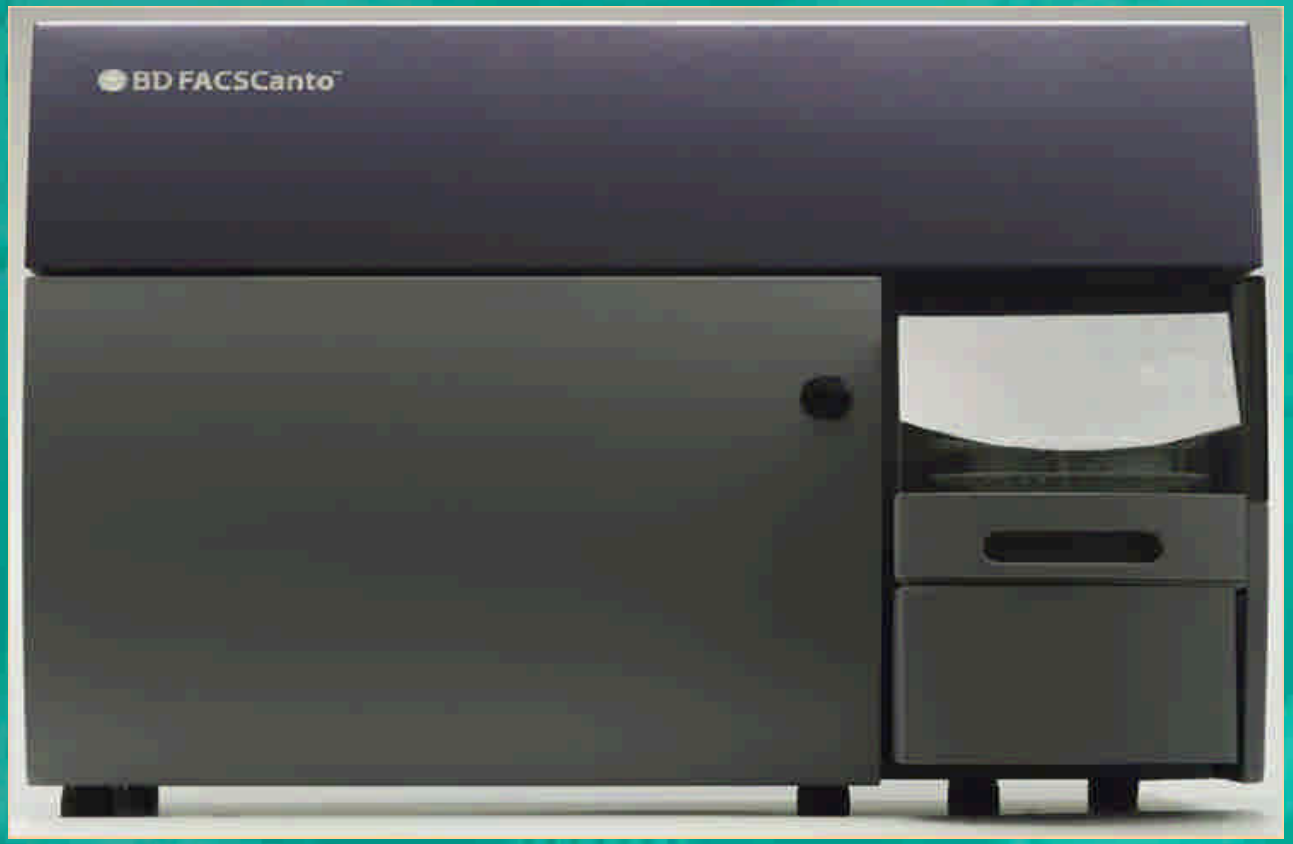

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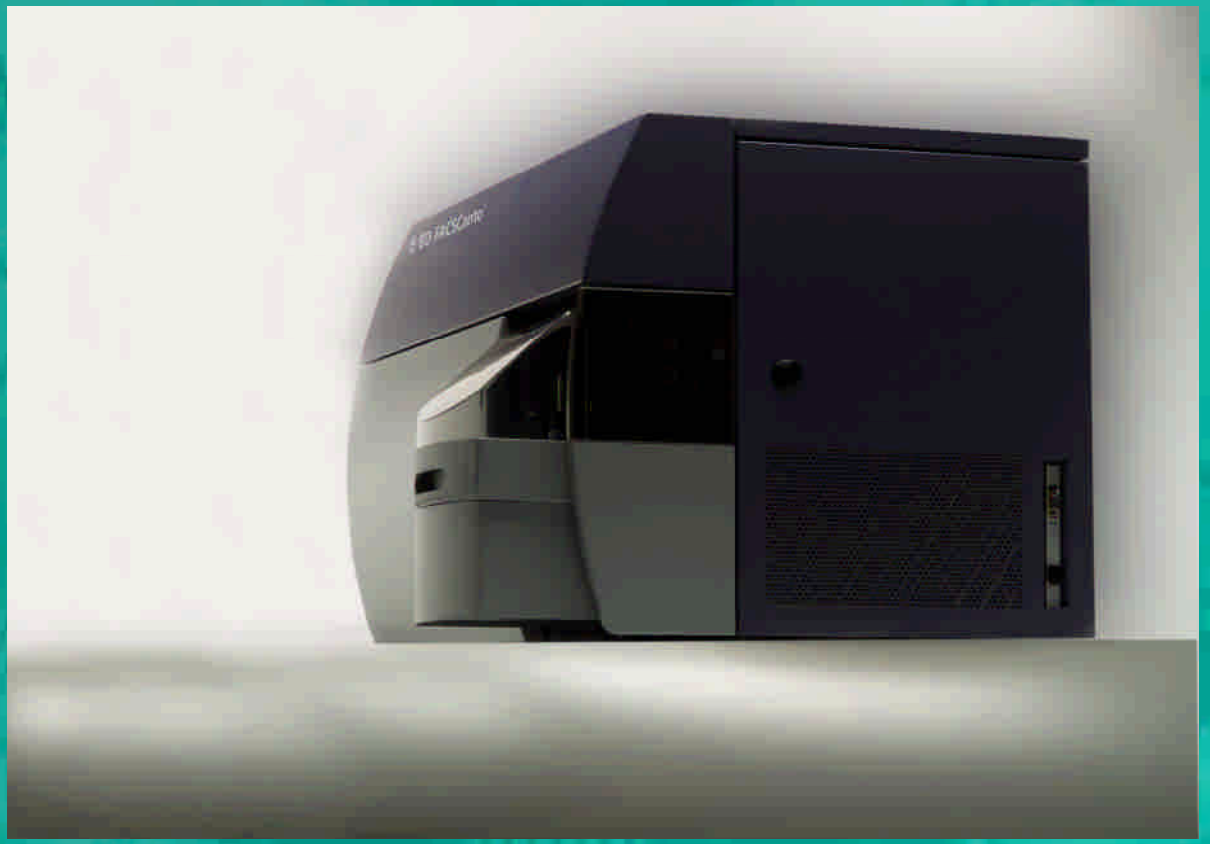

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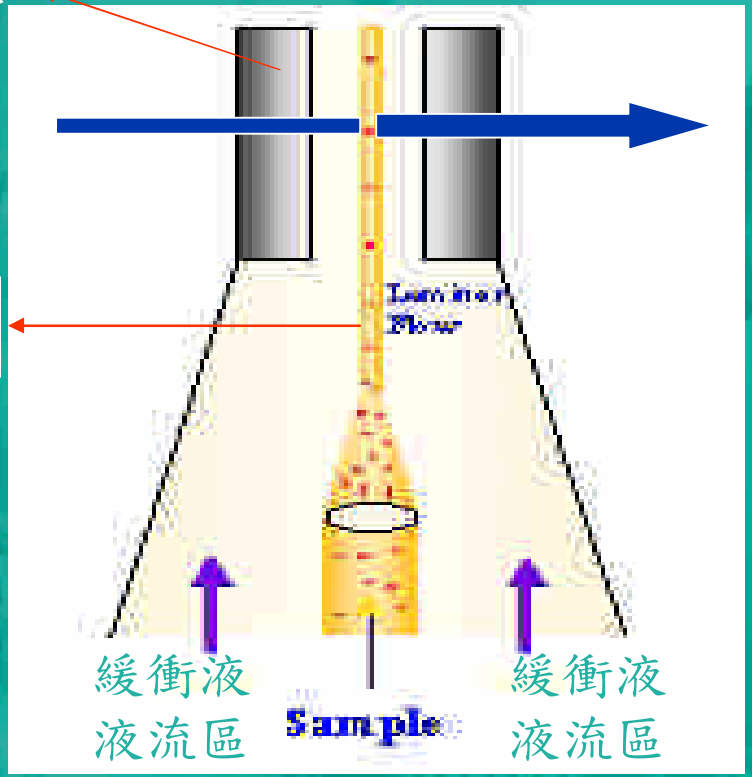
流式細胞儀的工作原理

偵測區

激發光源

樣品流動區



-螢光
-散射光

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流式細胞儀能測量：

- 散射光
  - 細胞大小 (前方散射光)
  - 細胞折射率 (側方散射光)
- 各色螢光

綜合三個系統的功能:

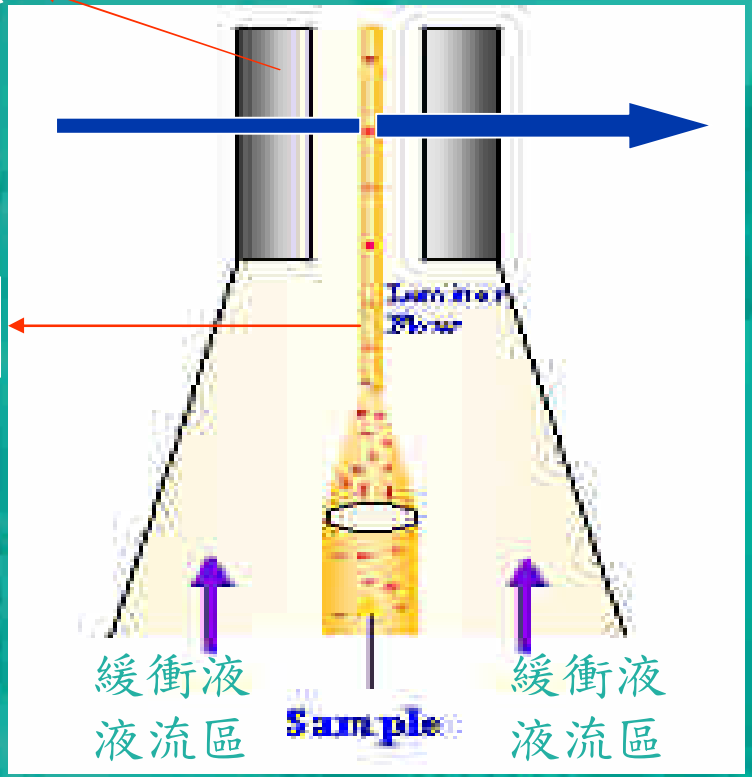
- 液流系統:將細胞依序送到測量區受檢。
- 光學系統:產生並收集螢光、光散射等信號。
- 電子系統:
  - 將光學訊號轉換成電子訊號。
  - 分析所輸出的電流訊號，以脈衝高度、寬度、積分面積顯示。
  - 量化訊號並傳至電腦。

液流系統

偵測區

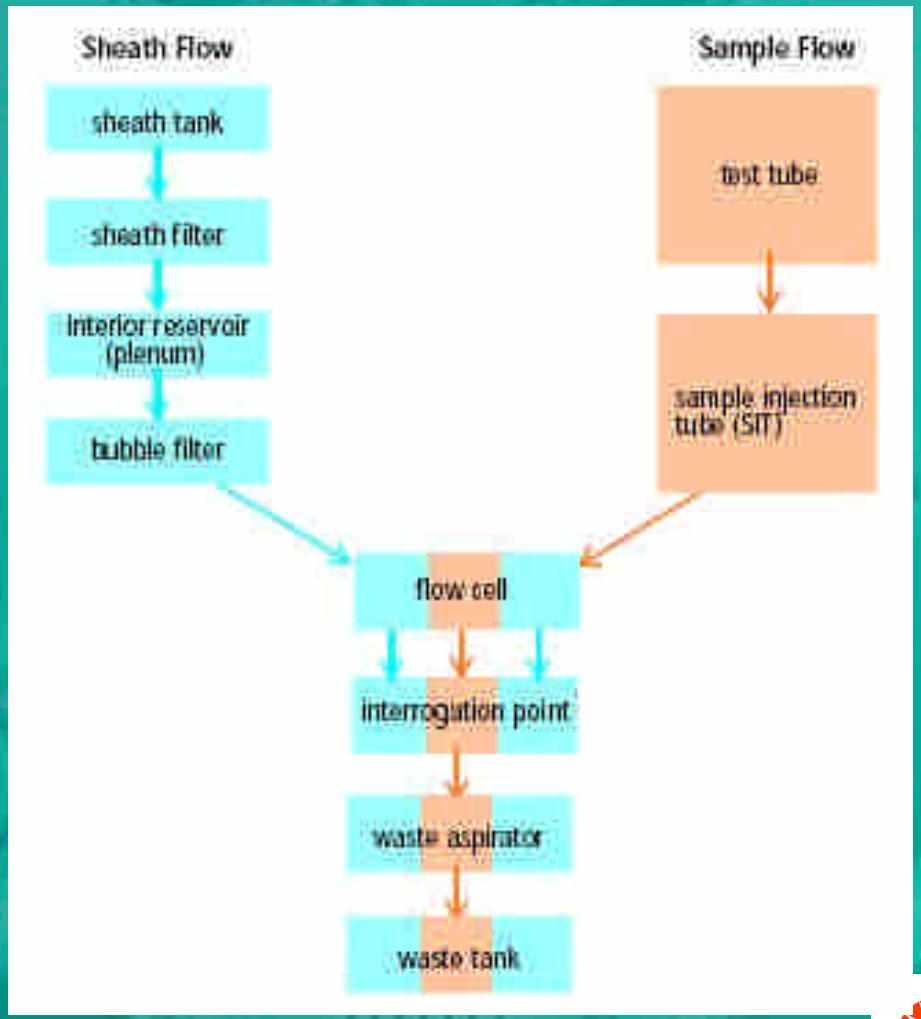
激發光源

樣品流動區



-螢光
-散射光

FACSCanto 的液流系統



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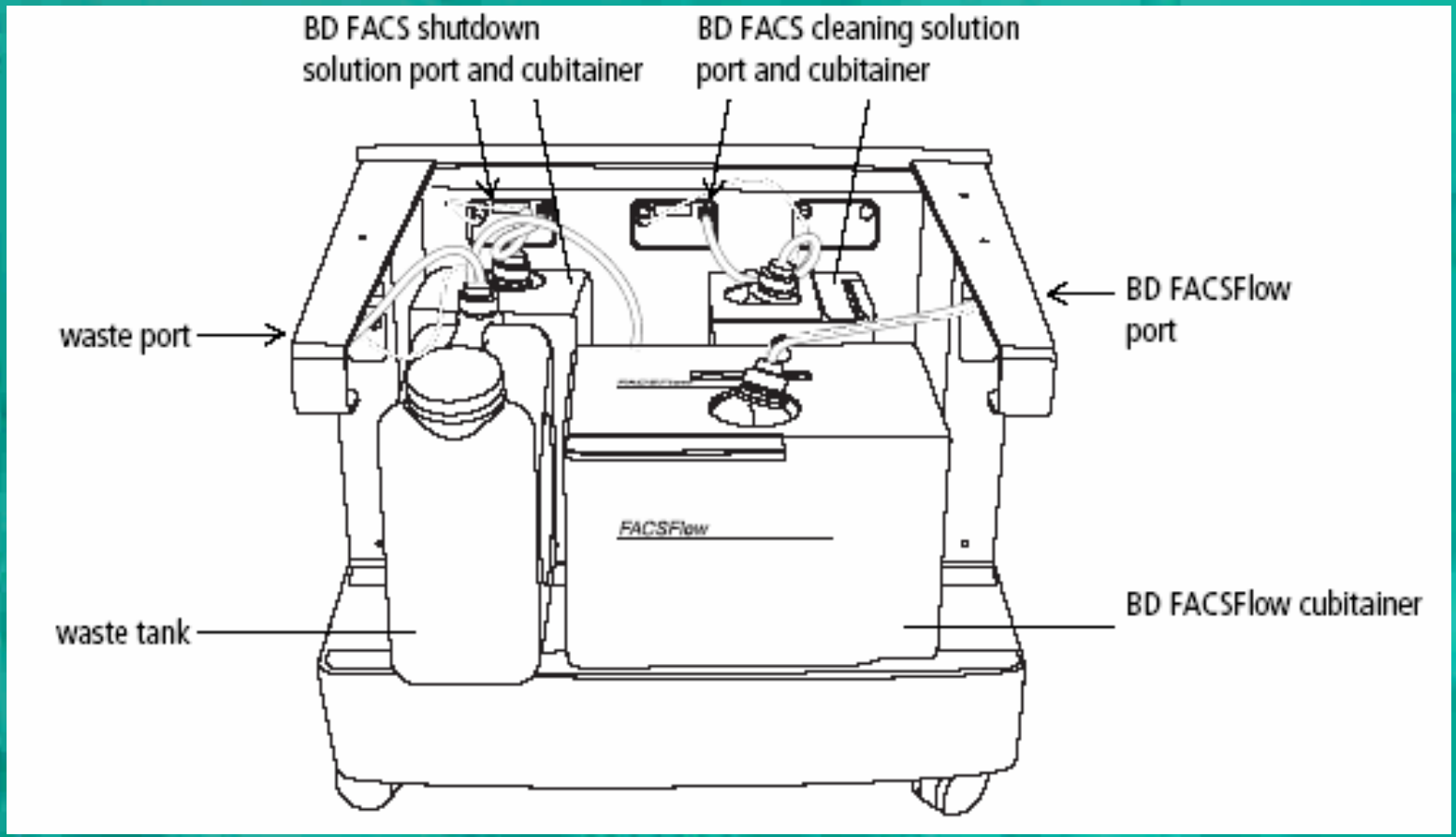

- Compressor
- 10 or 20 L sheath reservoir
- 5 L rinsing solution
- 5 L shutdown solution

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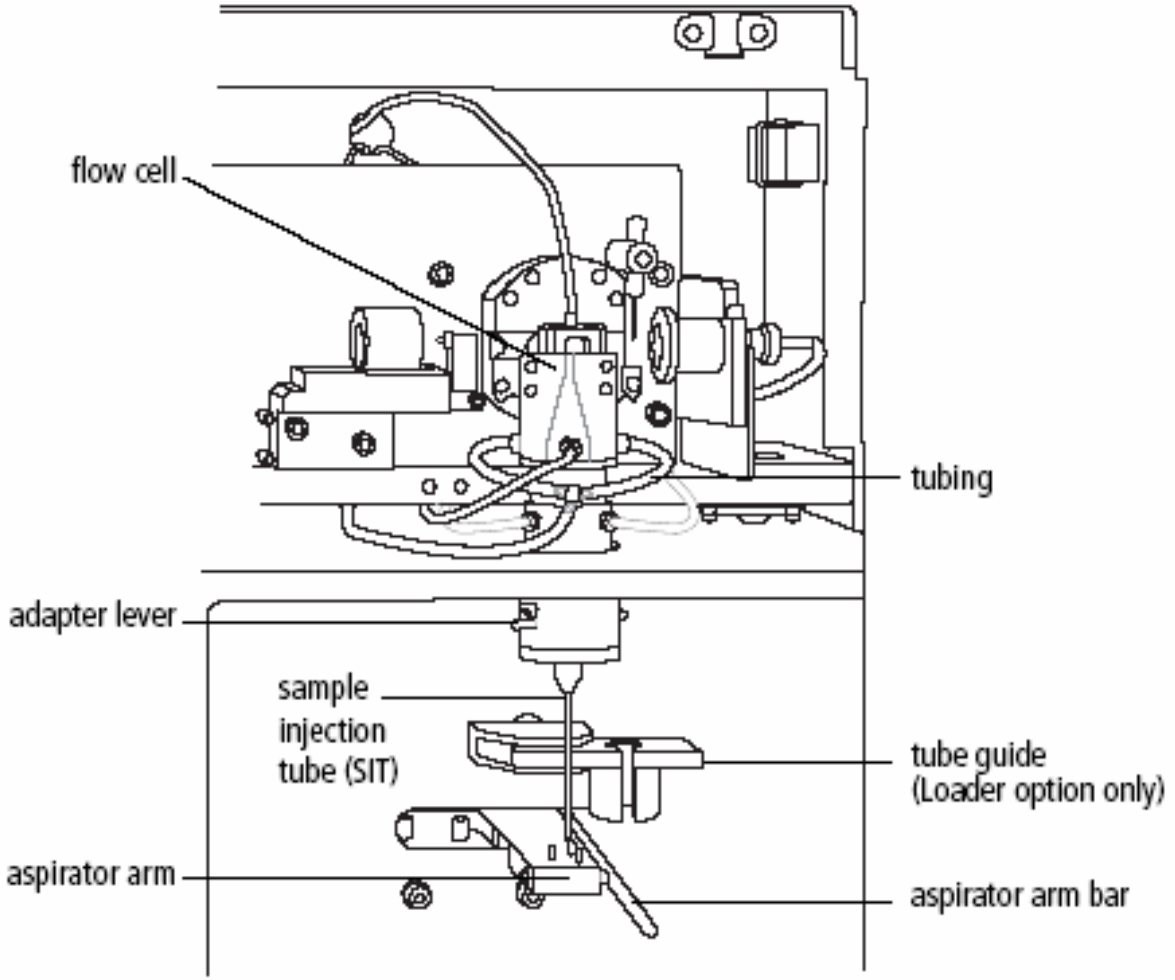
Figure 1-2 Sample injection tube



10,000 events/sec

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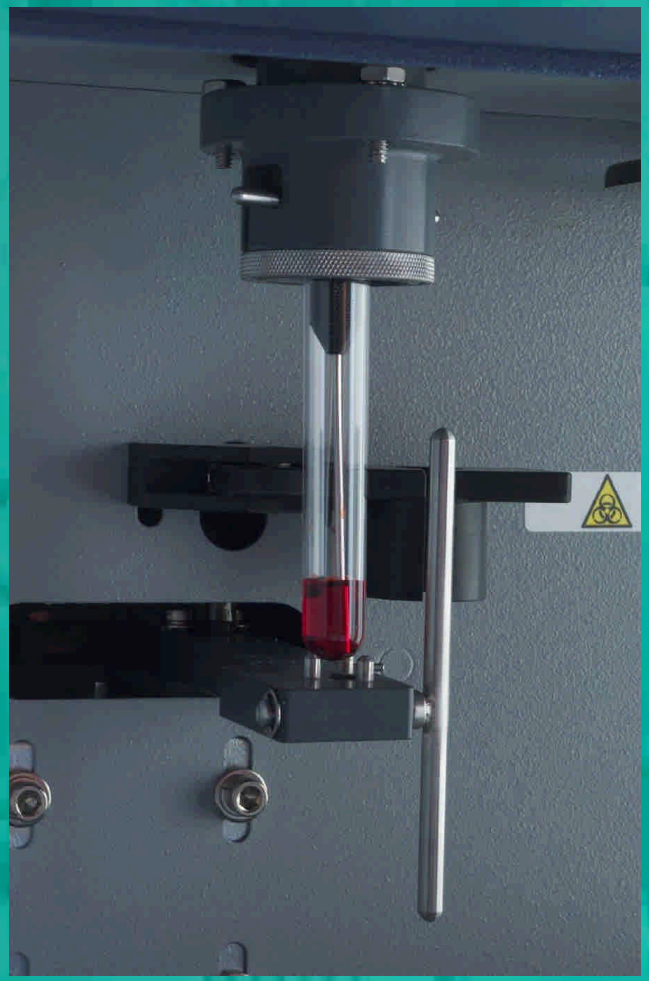

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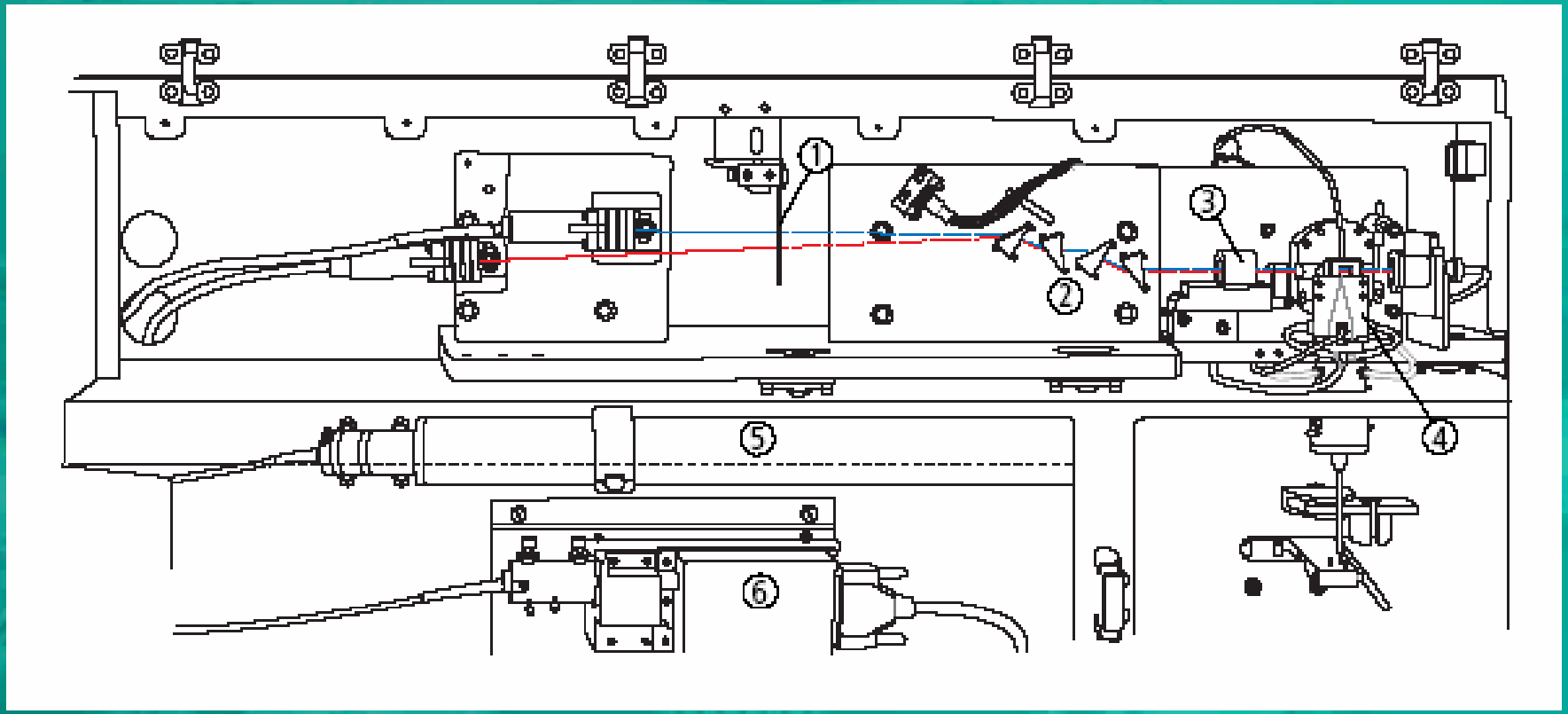
光學系統

Sensitivity : 100 FITC MESF, 50 PE MESF



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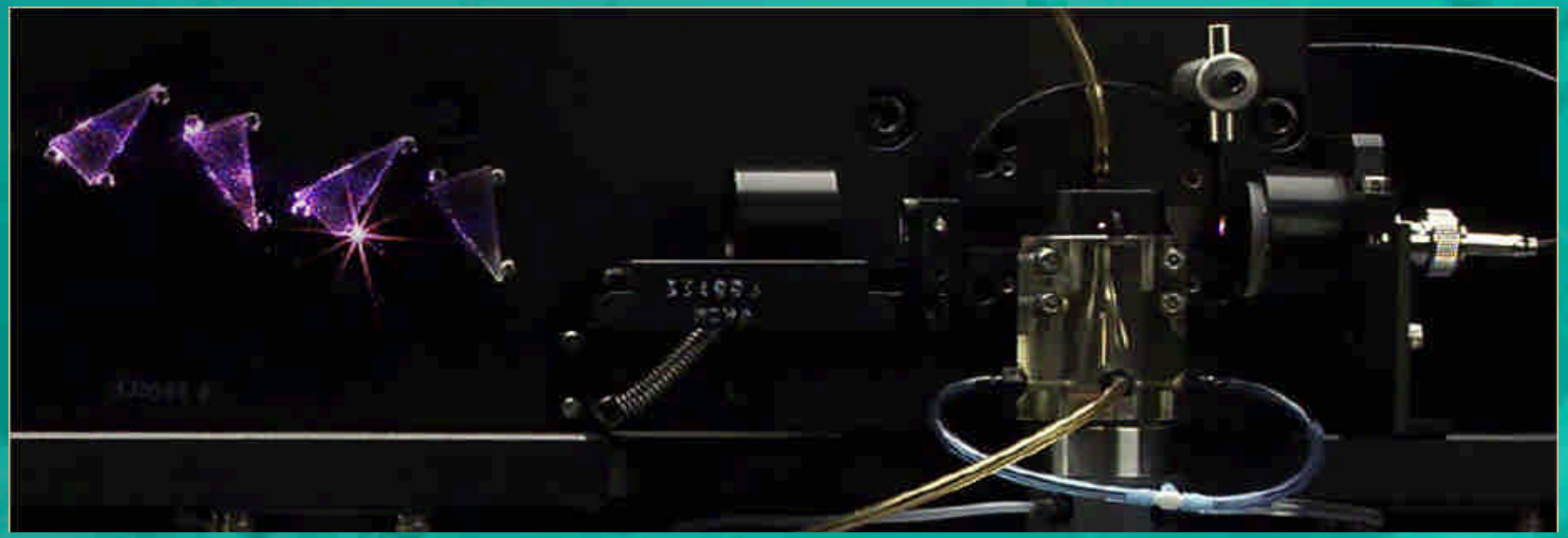

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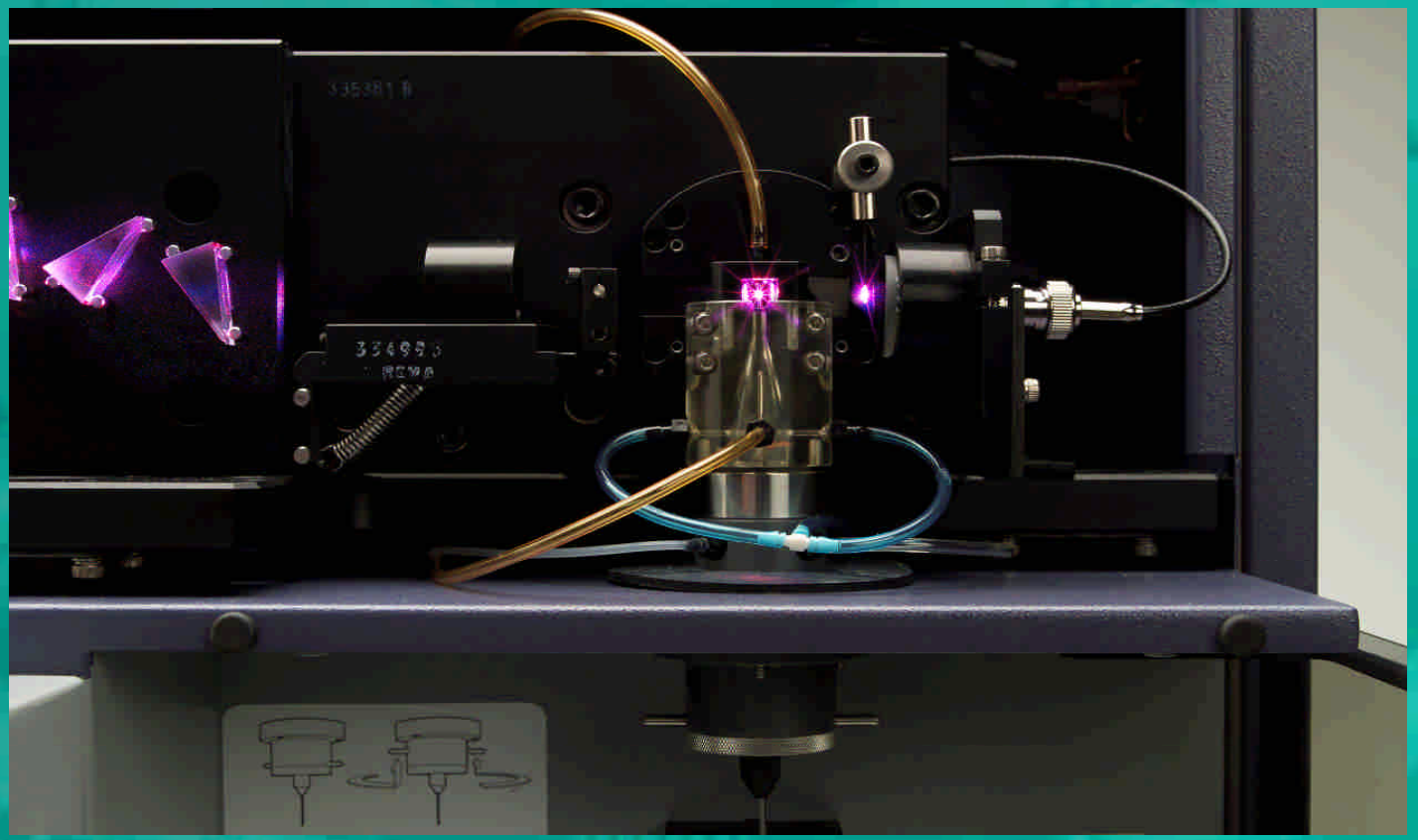

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Cuvette Flow Cell & Fiber Optics

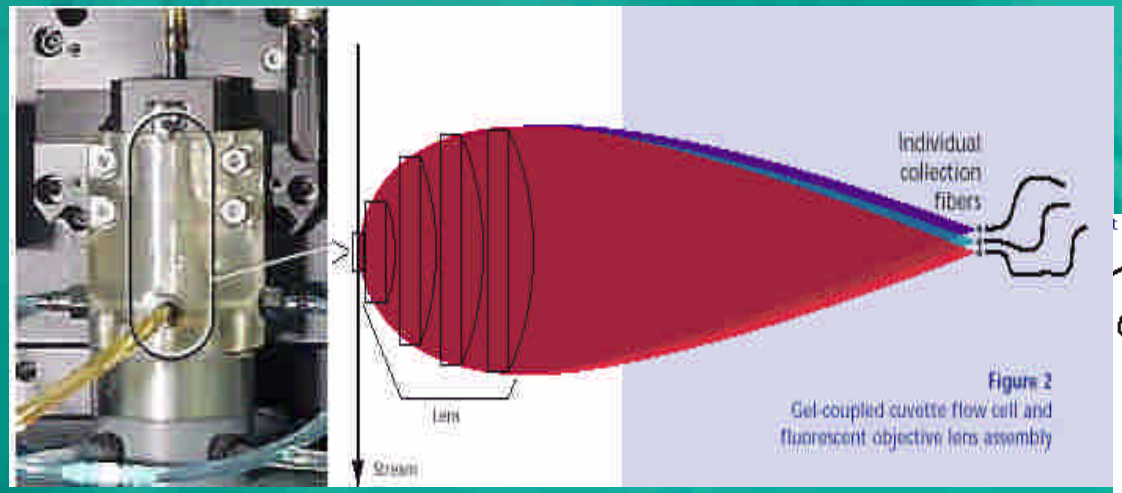

Figure 2
Gel-coupled cuvette flow cell and fluorescent objective lens assembly

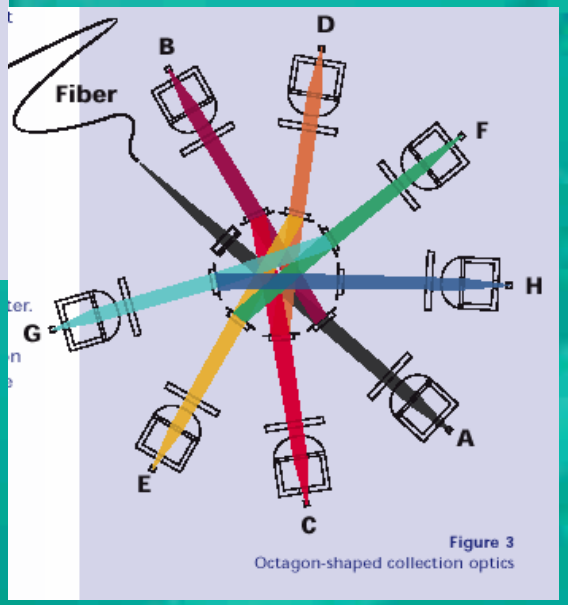

Figure 3
Octagon-shaped collection optics

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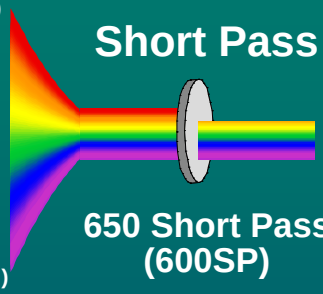
螢光濾片

Wavelengths

LONG (700nm)

SHORT (500nm)

Pass Through Filters

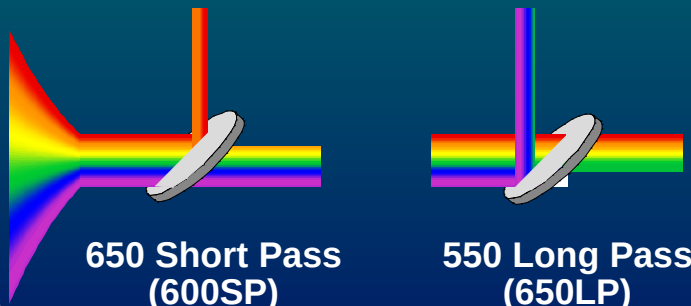


	Short Pass	Long Pass	Band Pass
	650 Short Pass (600SP)	550 Long Pass (650LP)	600/100 Band Pass (600/100)
Transmitted	<650 nm	>550 nm	550 - 650 nm (600?0)
Blocked	>650 nm	<550 nm	<550 nm & >650 nm

Dichroic Filters

LONG (700nm)

SHORT (500nm)



	650 Short Pass (600SP)	550 Long Pass (650LP)
Transmitted	<650 nm	>550 nm
Diflected 90	>650 nm	<550 nm

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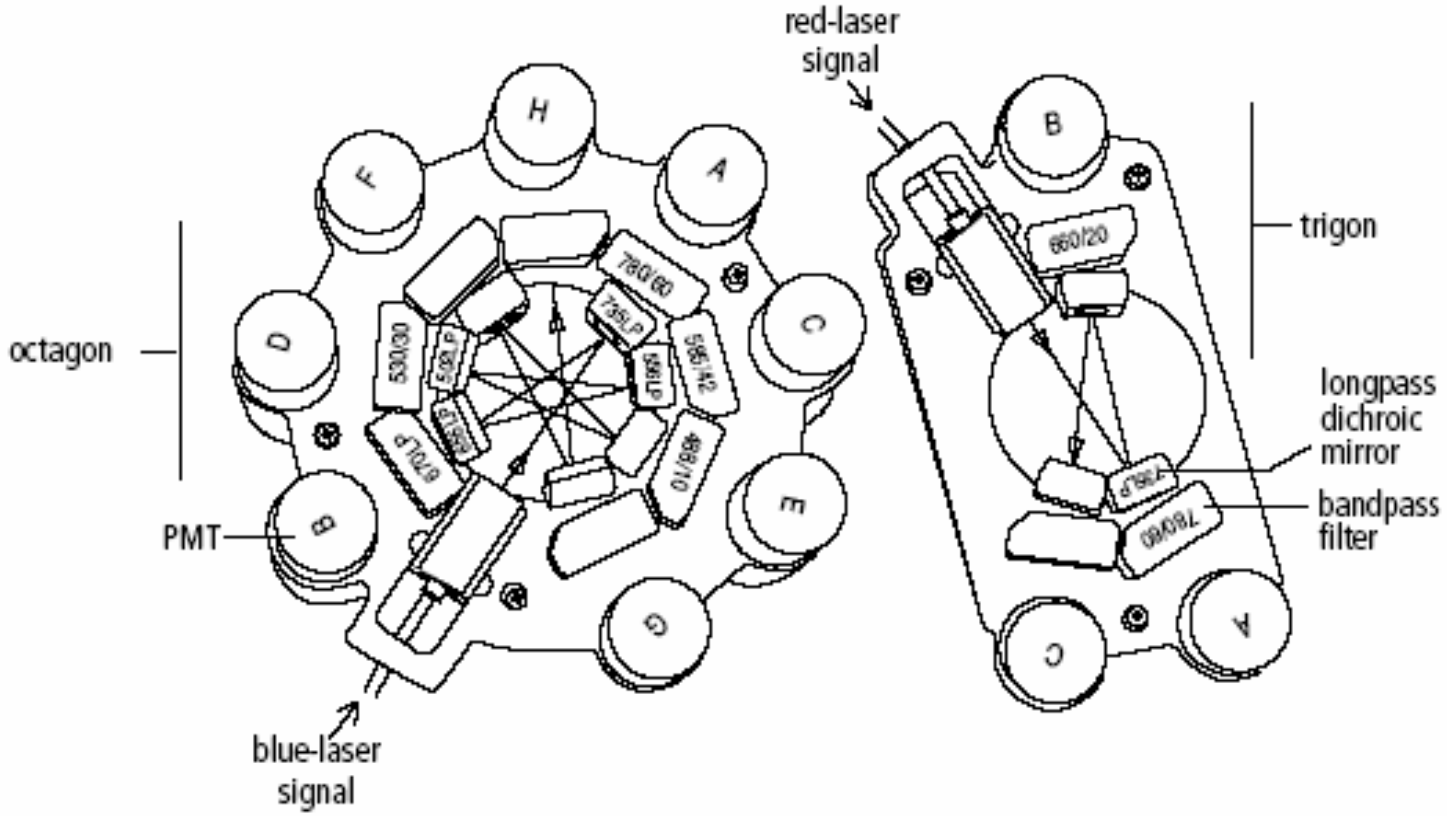
Figure 1-5 Octagon and trigon detector arrays



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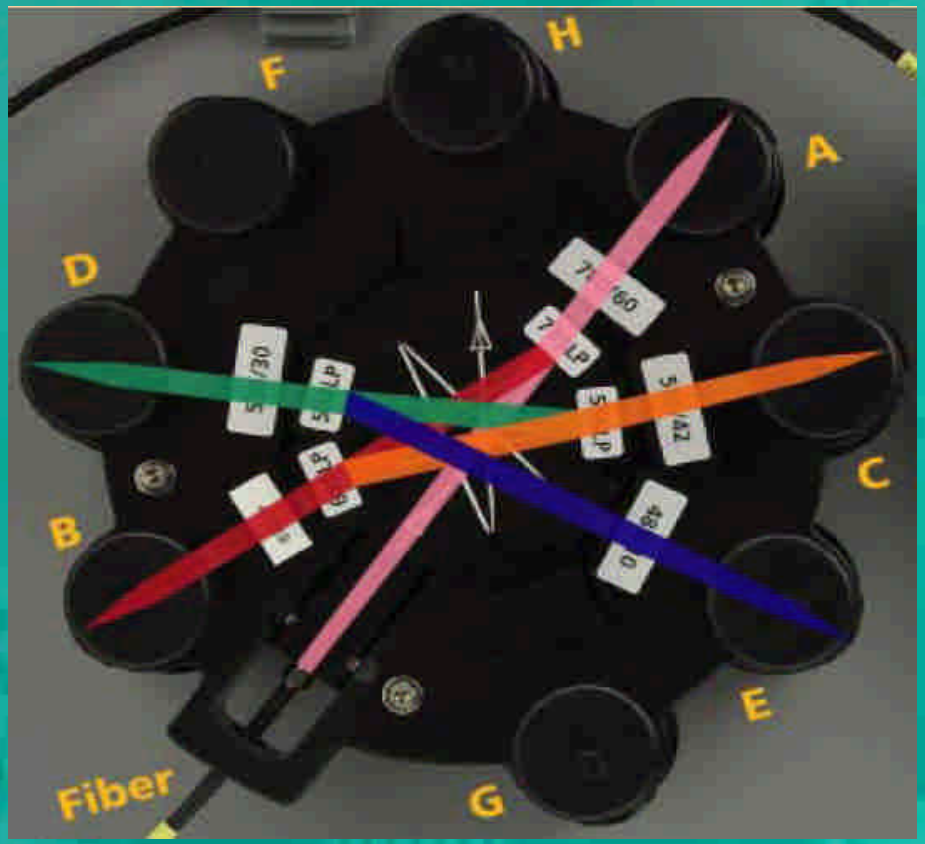

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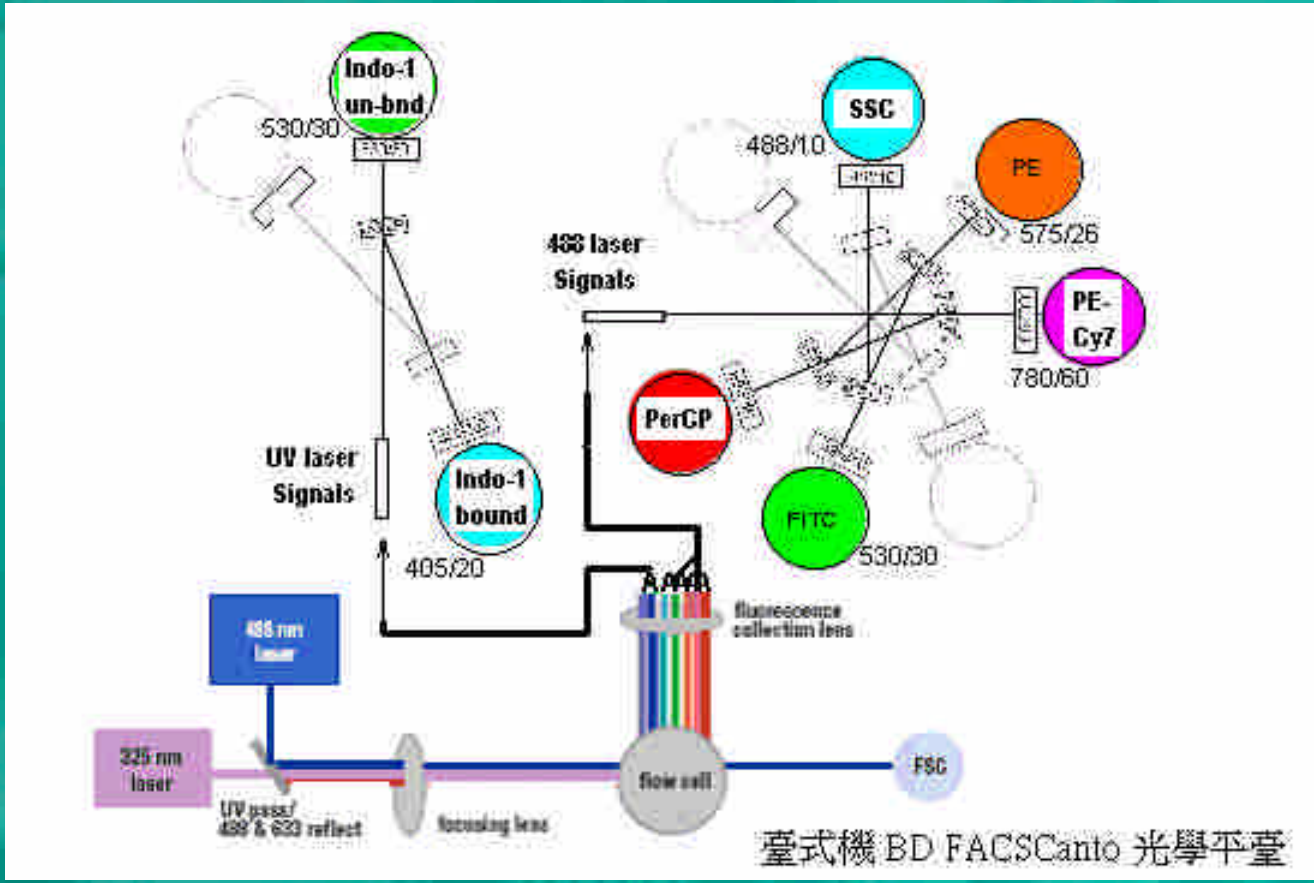
FACSCanto 的光學系統



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Laser	Wavelength (nm)	Min. Power[a] (mW)	Commonly Used Fluorochromes
Coherent® Sapphire™ Solid State	488 (blue)	20	FITC, PE[b], PE-Texas Red®, PerCP, PerCP-Cy5.5, PE-Cy7, PI
JDS Uniphase™ HeNe Air Cooled	633 (red)	17	APC, APC-Cy7

BD FACSCanto – Default dyes

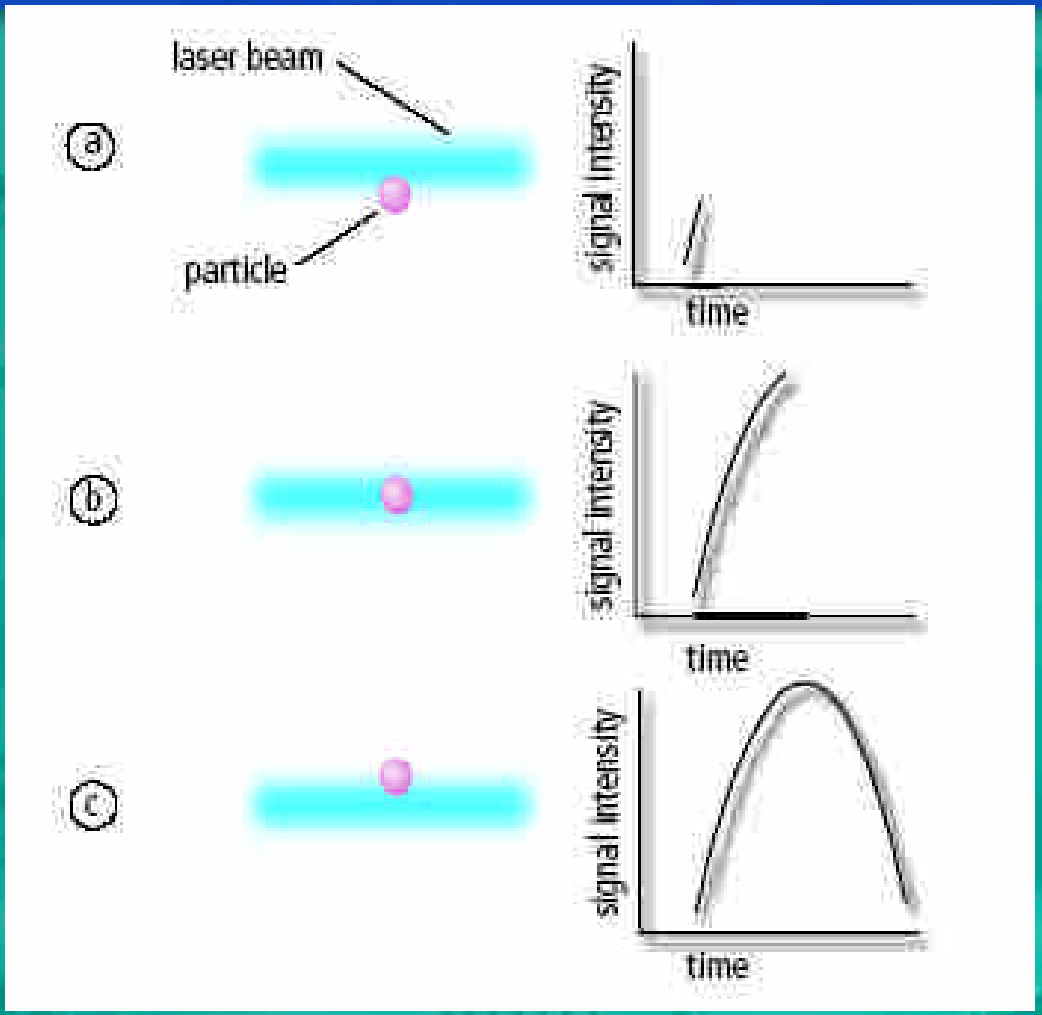
Instrument	Laser	Excitation Laser Line (nm)	Fluorescence Channel	Fluorochromes from BD Biosciences	
BD FACSCanto Flow Cytometer	Blue	488	Green	FITC	
			Yellow	PE	
			Red	PerCP	PerCP-Cy5.5
			Infra Red	PE-Cy7	
	Red	633	Red	APC	
			Infra Red	APC-Cy7	

Table 1
Validated fluorochrome combinations with the BD FACSCanto flow cytometer.

Table 1-1 Octagon and trigon optical filters

Detector Array (Laser)	PMT Position	LP Mirror	BP Filter or LP Mirror	Intended Dye
Octagon (488-nm blue laser)	A	735	780/60	PE-Cy7
	B	655	670 LP	PerCP-Cy5.5, PerCP
	C	556	585/42	PE
	D	502	530/30	FITC
	E	blank optical holder	488/10	Side scatter (SSC)
	F	blank optical holder	blank optical holder	—
	G	blank optical holder	blank optical holder	—
	H	blank optical holder	blank optical holder	—
Trigon (633-nm red laser)	A	735	780/60	APC-Cy7
	B	blank	660/20	APC

電子系統



BD
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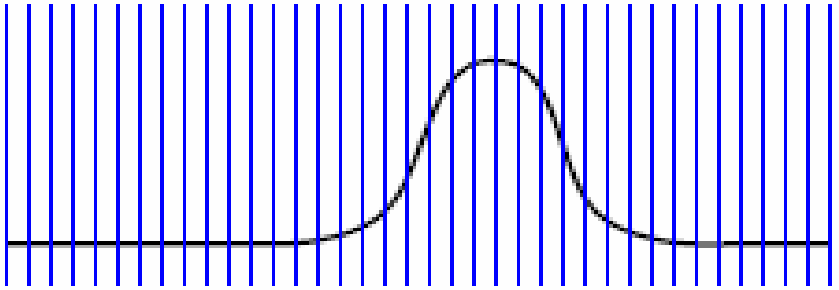
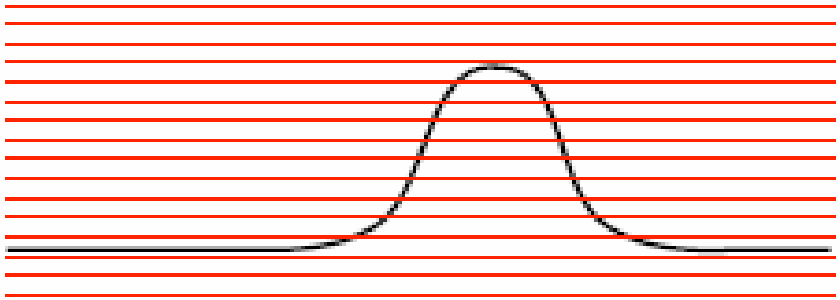
measured 10,000,000 times every second



digitized into 16,384 levels



BD

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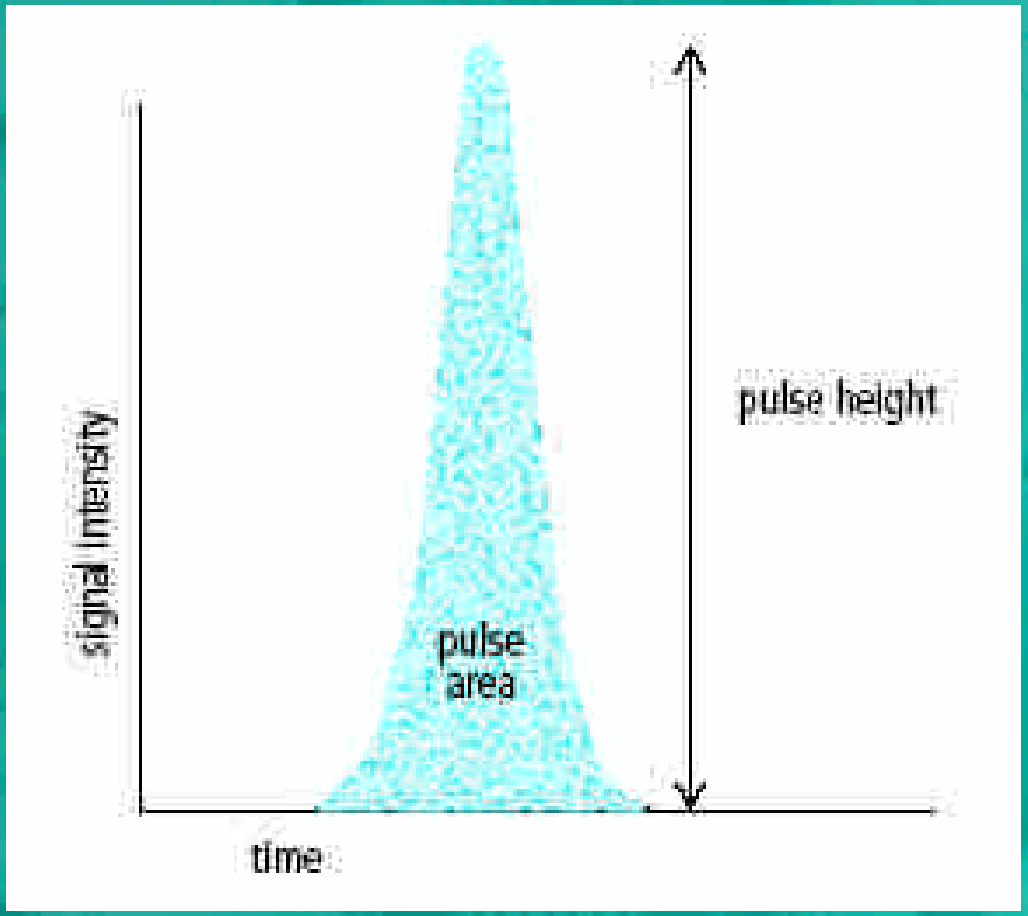
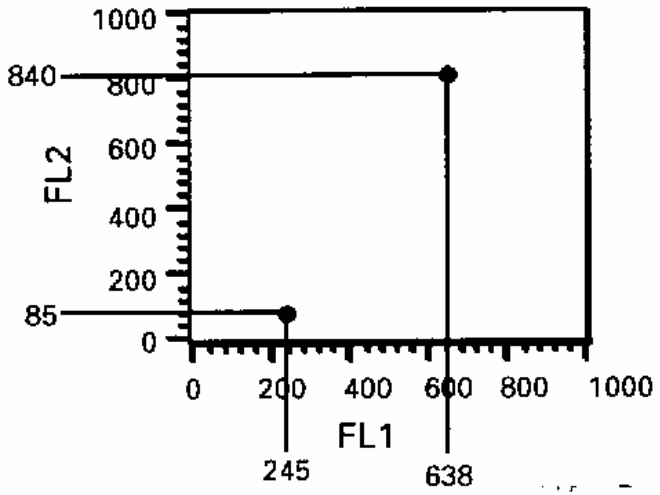
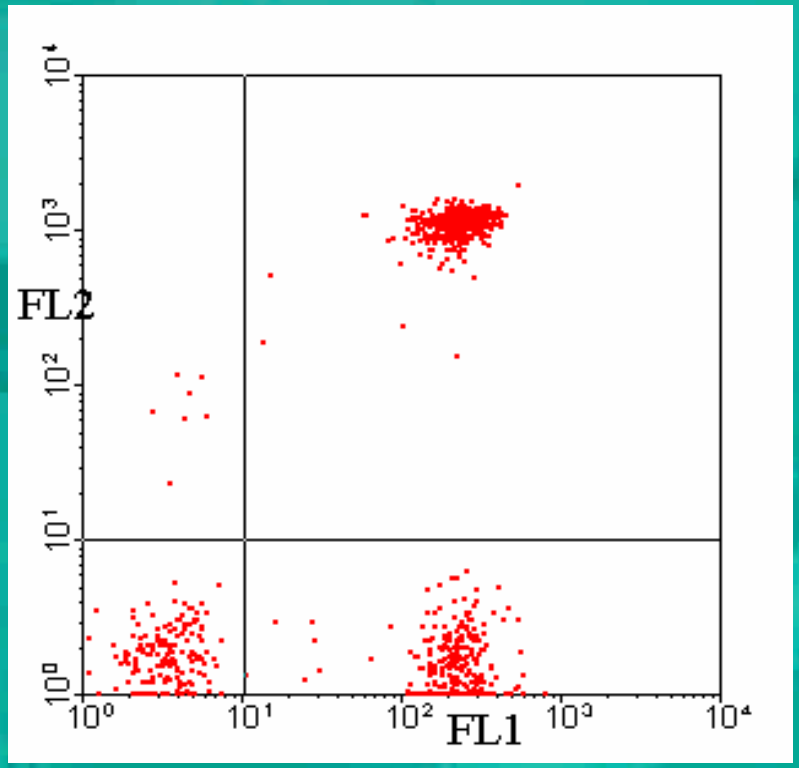


實驗數據的呈現

List-Mode Data

	FSC	SSC	FL1	FL2
Event 1	30	60	638	840
Event 2	100	160	245	85
Event 3	300	650	160	720





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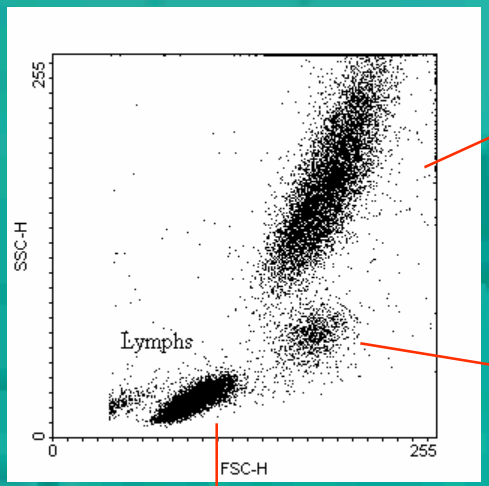
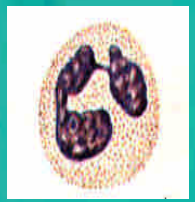
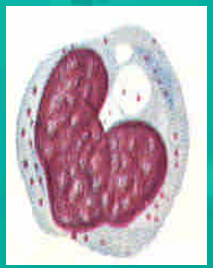
常見的數據呈現

散點圖反映細胞形態





10 to 14 μm
Neutrophil



15 to 20 μm
Monocyte

8 to 10 μm
Lymphocyte

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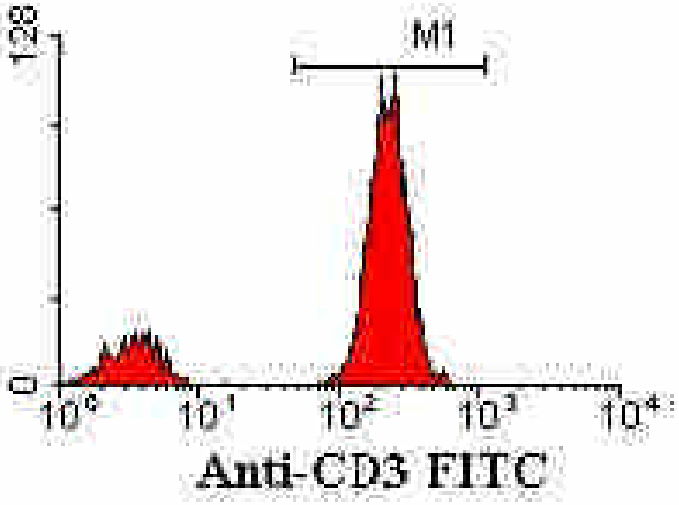
直方圖分析報告

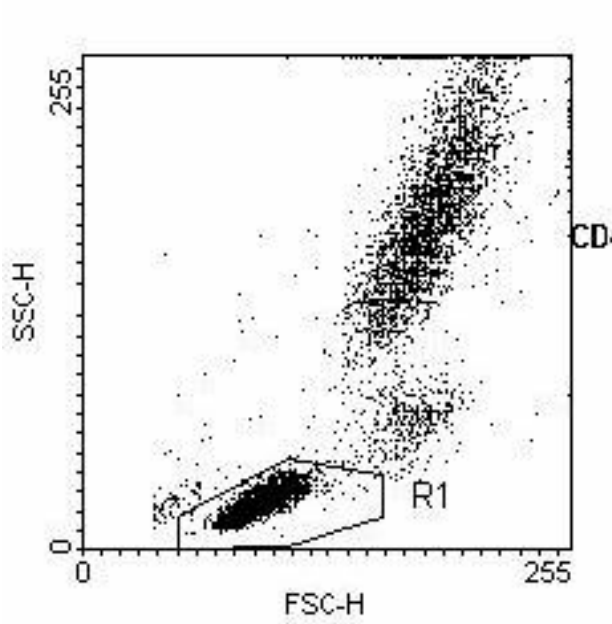


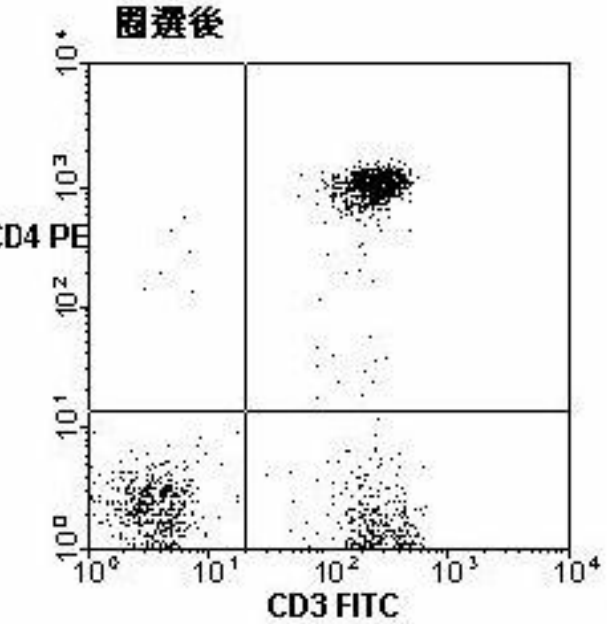
File: IS20924004
Date: 24-Sep-93
Total Events 6000
Gated Events 2785

M	Low,High	Events	%Gated	GMean	CV
0	0, 255	2785	100.00	106.55	35.70
1	108, 196	2270	81.51	231.20	6.06

CV=S.D./Mean

散點圖分析報告



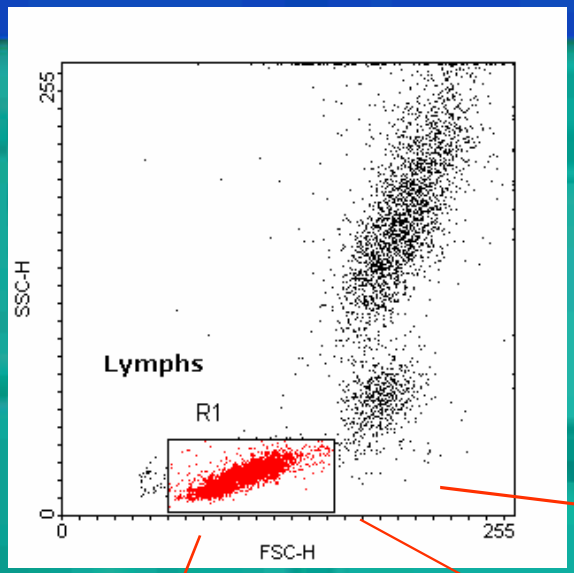


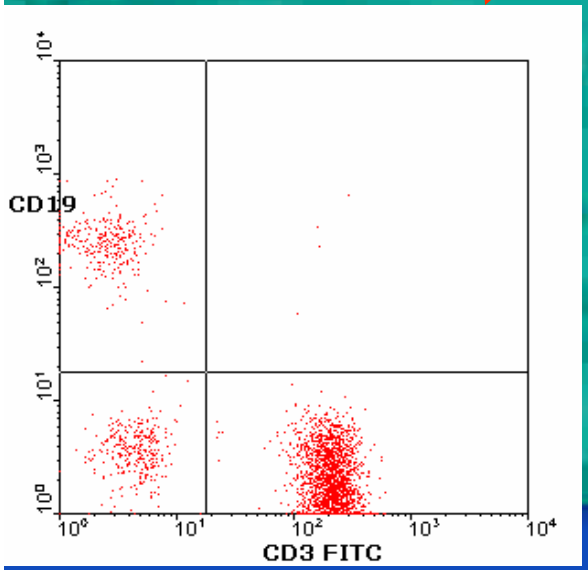
四象限統計 Quadrant Statistics

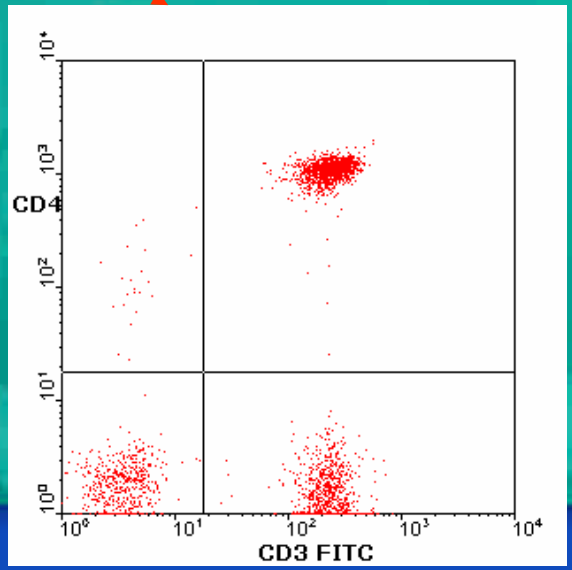
Gates: R1
Gated Events: 2354
Quad Stats
FL1-H(Log) vs FL2-H(Log)
Quadrant x,y: 83,72

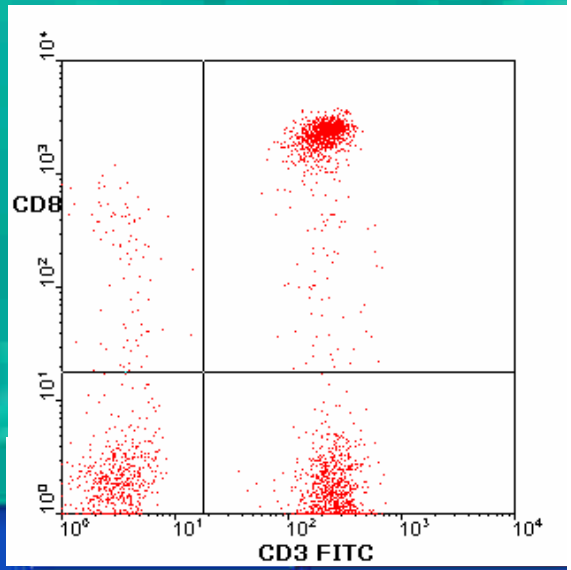
Quad	X-Mean	Y-Mean	Events	%Gated
UL	5.2	262.6	6	0.25
UR	252.9	977.9	1139	48.39
LL	3.4	2.0	508	21.58
LR	288.1	1.3	701	29.78

免疫細胞分型


SSC-H
255
0
Lymphs
R1
0
255
FSC-H


CD19
CD3 FITC


CD4
CD3 FITC


CD8
CD3 FITC

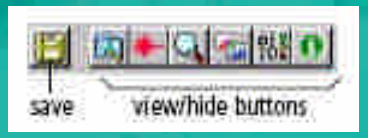
Running Samples with BD FACSDiva Software

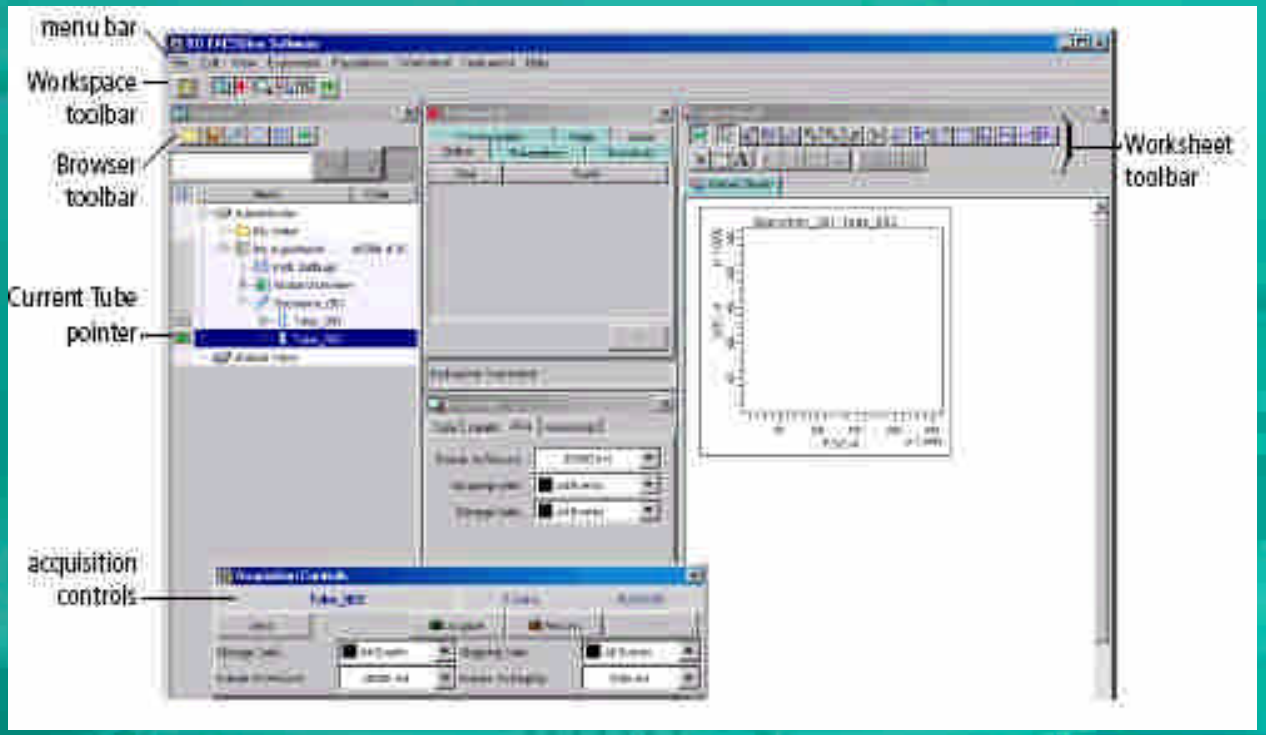
The following topics are covered in this chapter:

儀器之調試

- 設閾參數
- 閾值
- FSC / SSC 散點圖
- 螢光信號接受器 FL 1-4
- 自動化色差補償

User Log In





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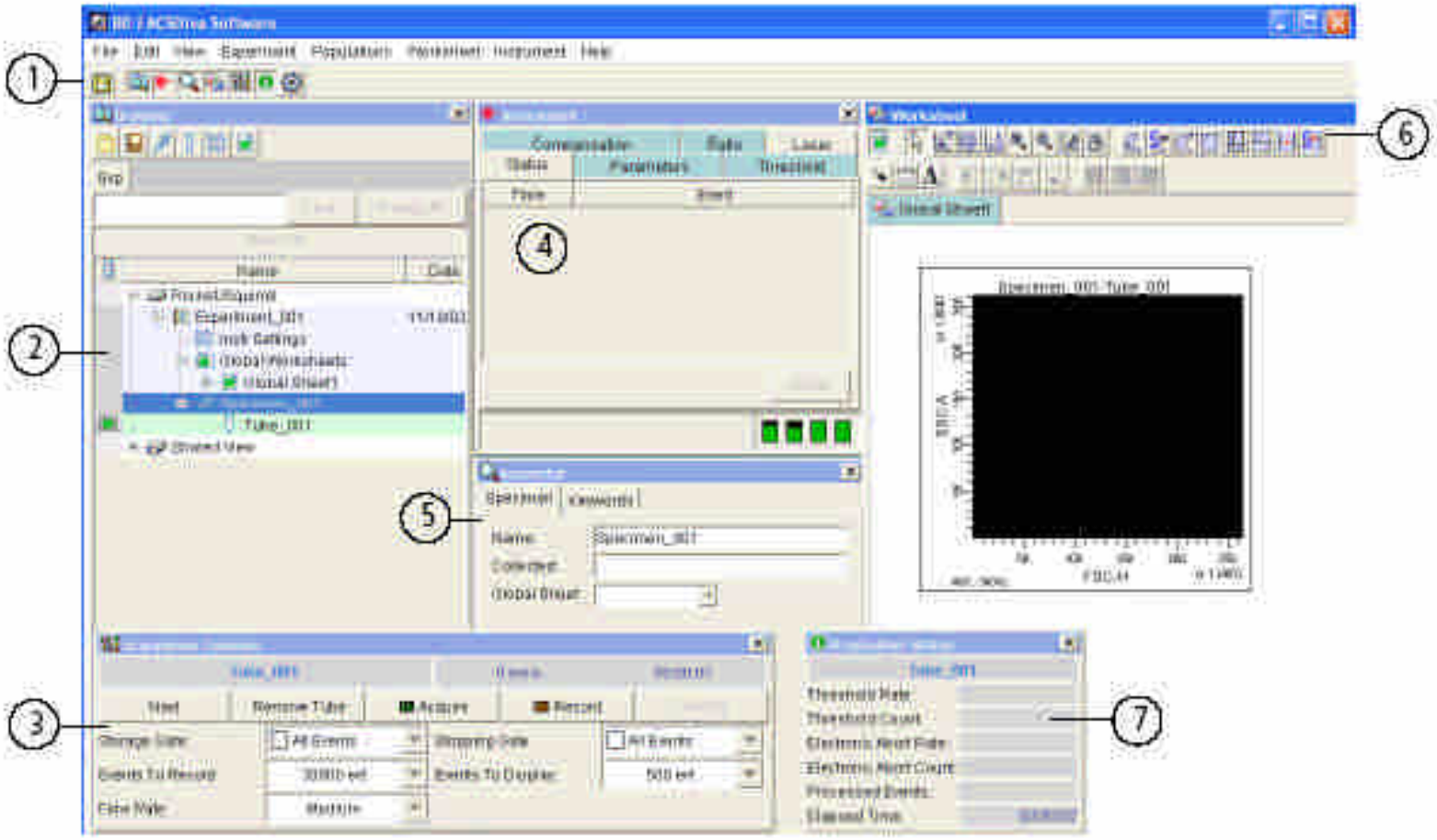
FACSDiva Workspace

Figure 2-1 BD FACSDiva workspace



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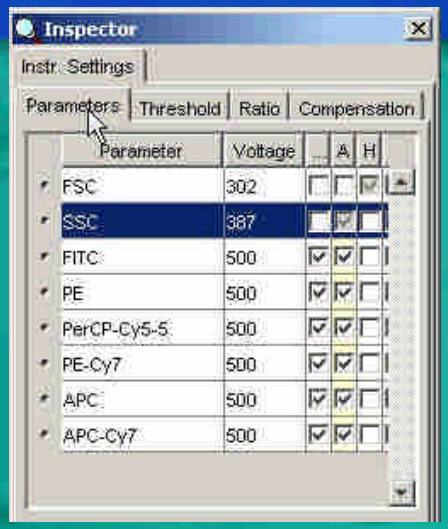
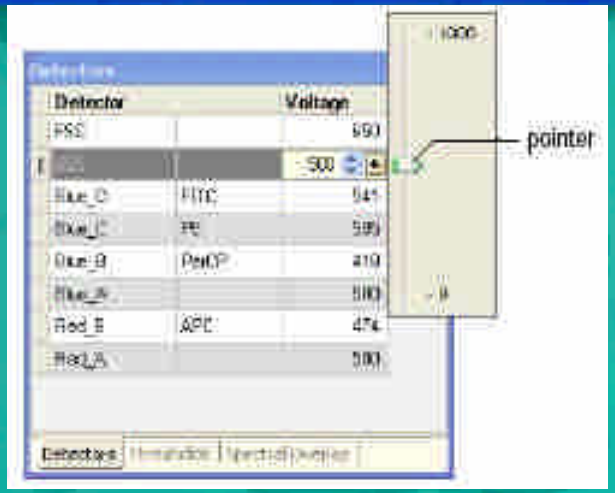
常用螢光染劑

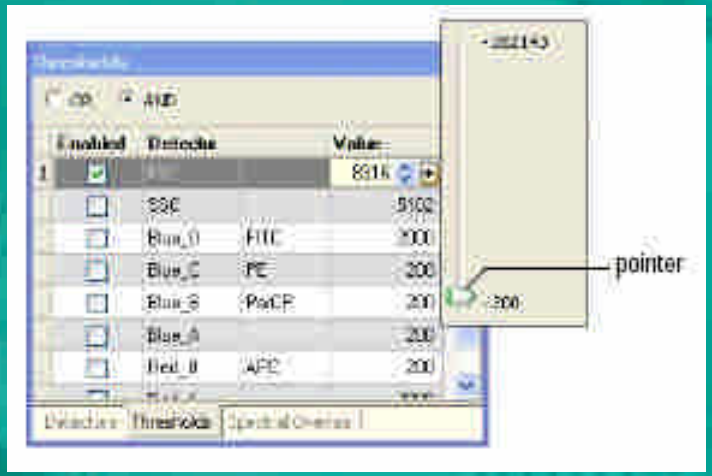
Instrument	Laser	Excitation Laser Line (nm)	Fluorescence Channel	Fluorochromes from BD Biosciences	
BD FACSCanto Flow Cytometer	Blue	488	Green	FITC	
			Yellow	PE	
			Red	PerCP	PerCP-Cy5.5
			Infra Red	PE-Cy7	
	Red	633	Red	APC	
			Infra Red	APC-Cy7	

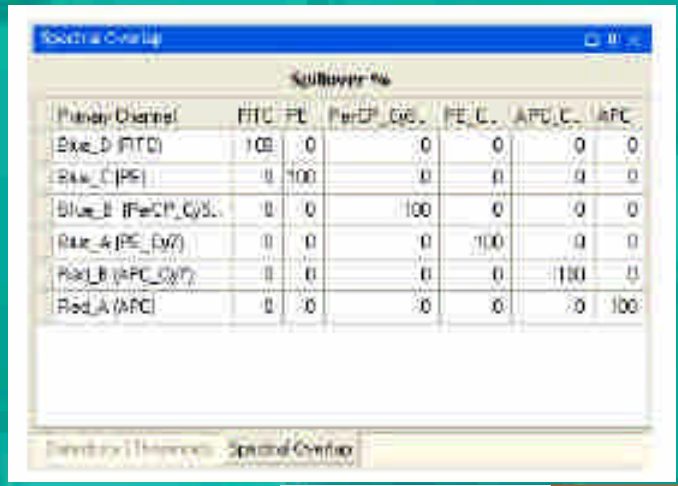
Table 1
Validated fluorochrome combinations with the BD FACSCanto flow cytometer.

Instrument Set Up









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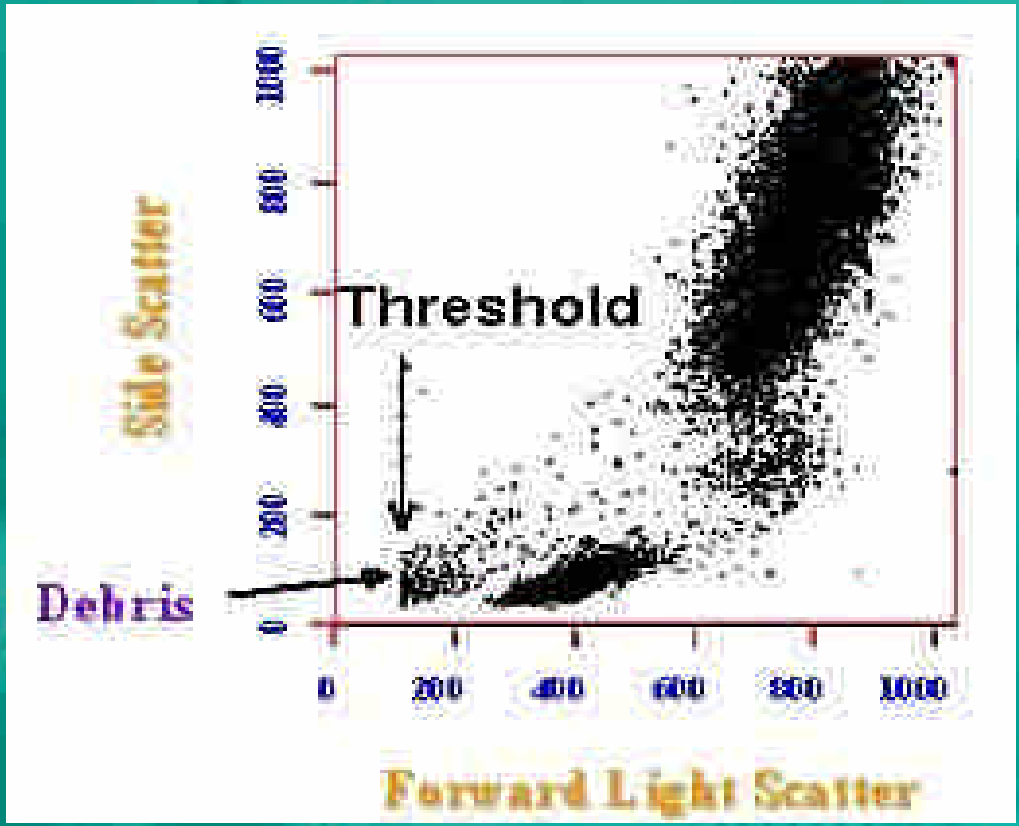
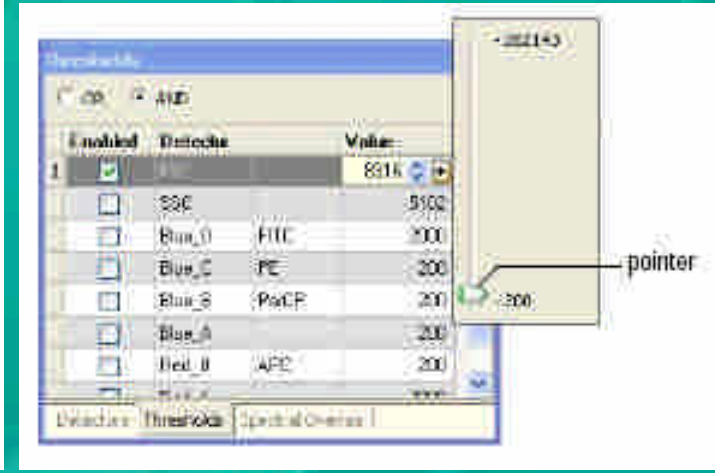
閥值的調整





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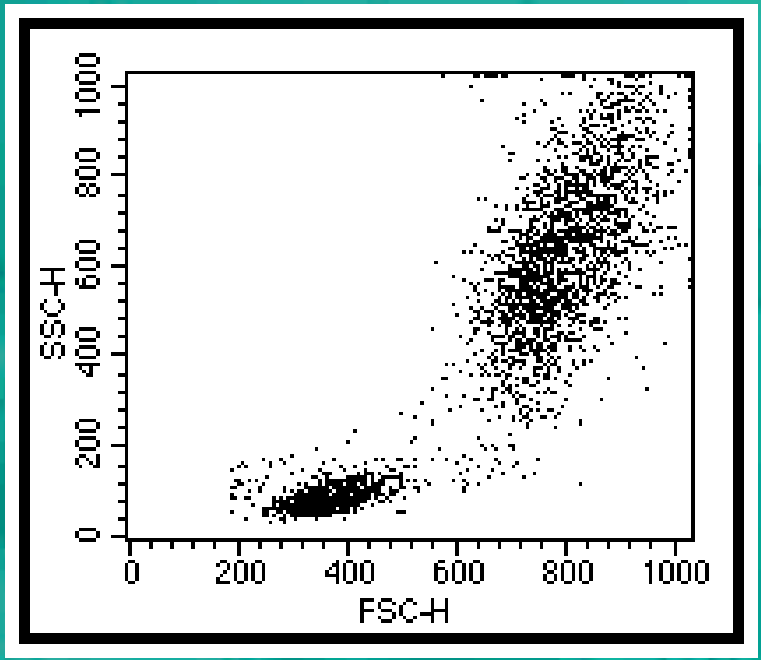
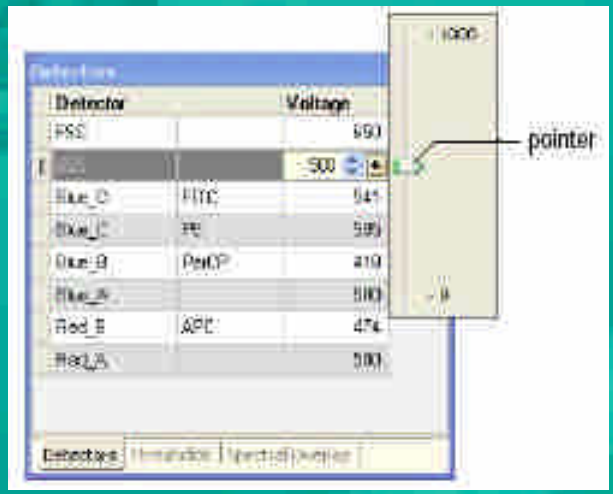
散射光信號的調整





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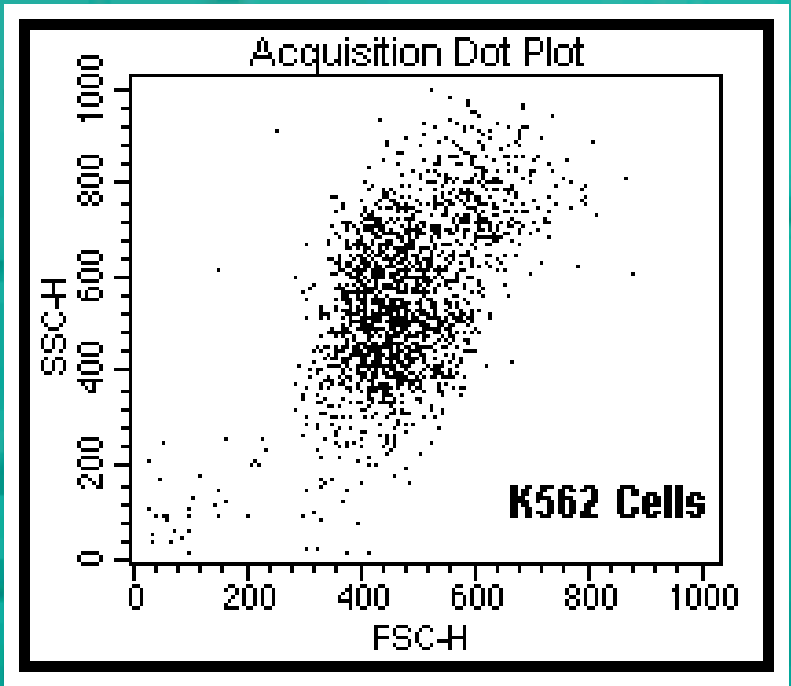
散射光信號的調整
Cell Line



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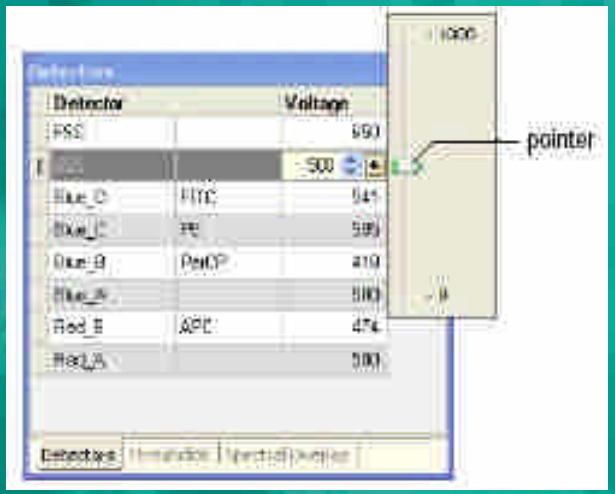
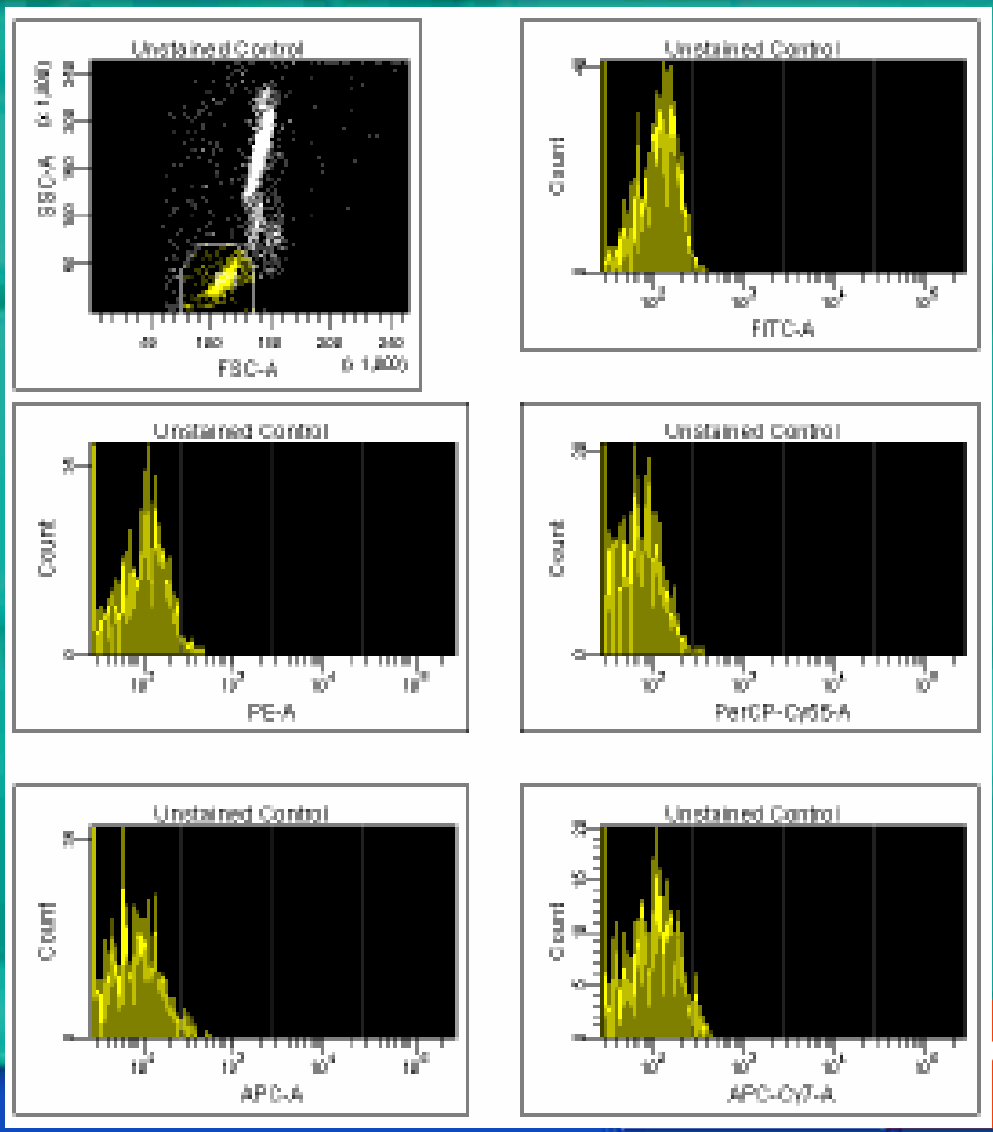
自體螢光信號的調整





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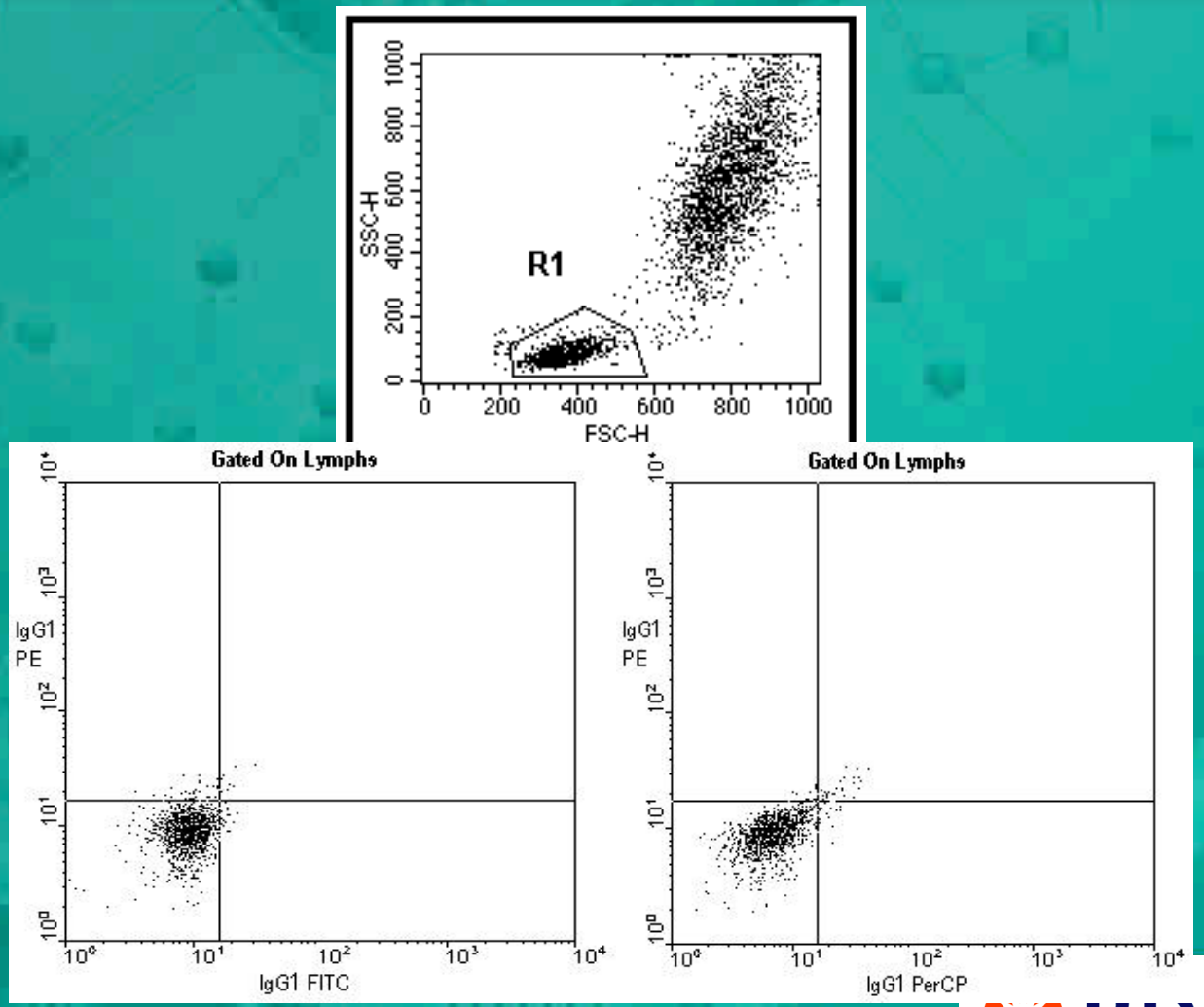
自體螢光信號的調整 2-3C



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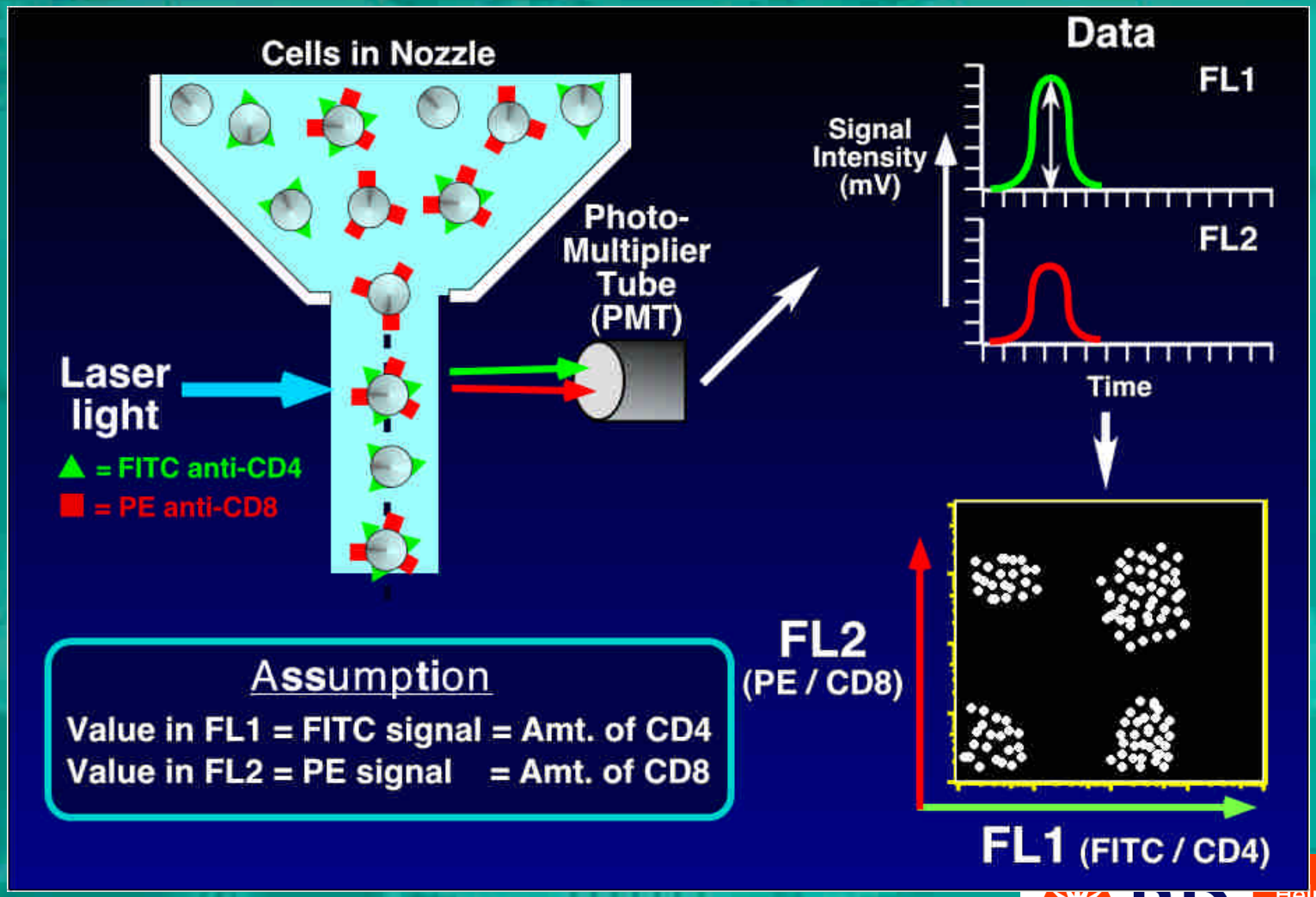
螢光補償調整 (The Problem)



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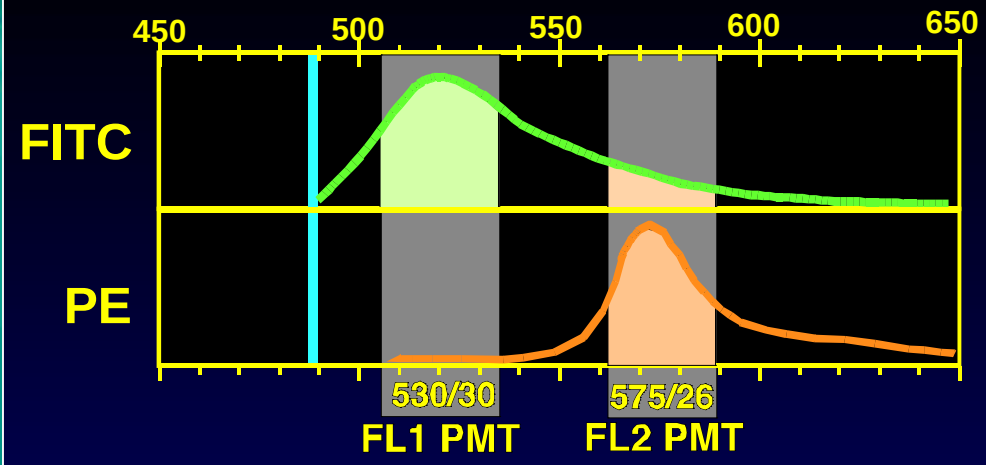
螢光補償調整 (The Problem)



FL1 = FITC + x% PE

FL2 = PE + y% FITC

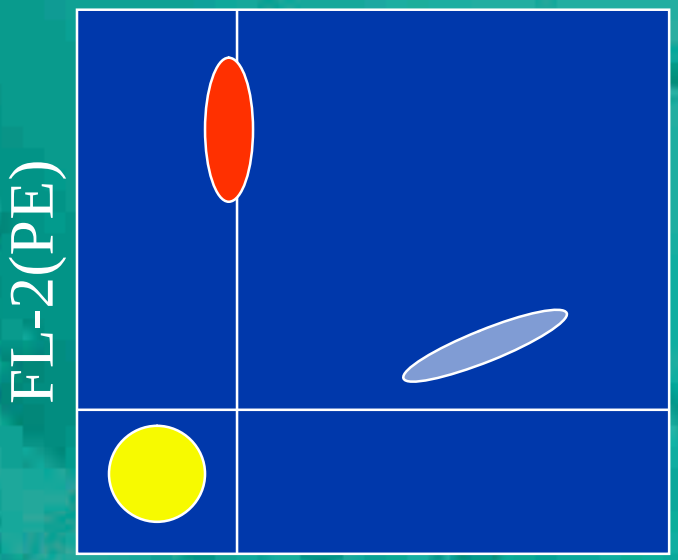
Remember the basic assumption of flow analysis:

The signal in FL1= the signal from FITC and only FITC and
the signal in FL2= the signal from PE and only PE.

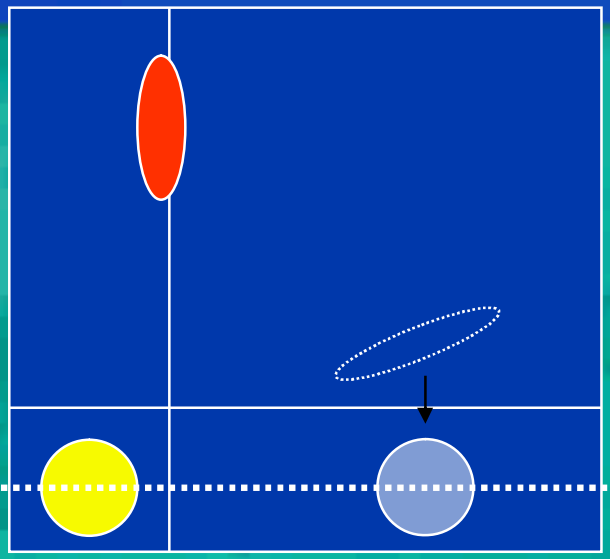
This is NOT TRUE for the raw data!

The process by which each fluorescence channel is "corrected" for this spectral overlap is termed *Fluorescence Compensation*

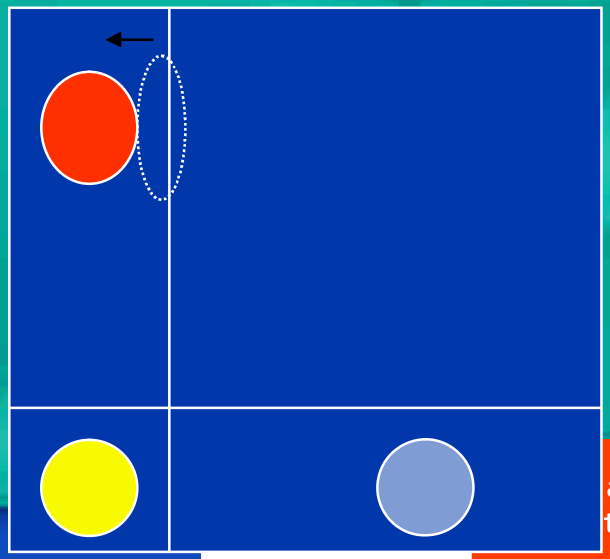
螢光補償調節



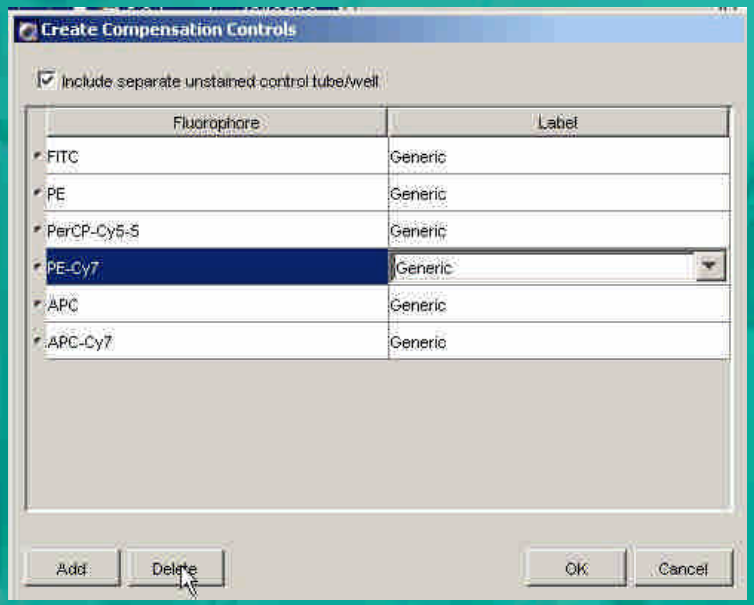
FL2 - %FL1
(18 –30%)

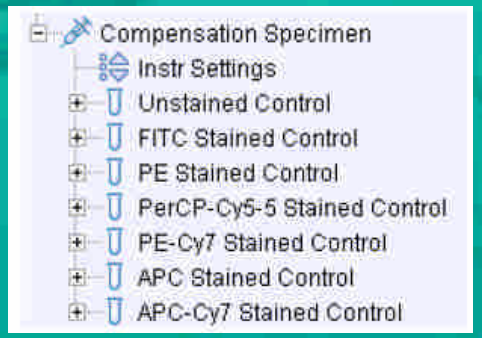


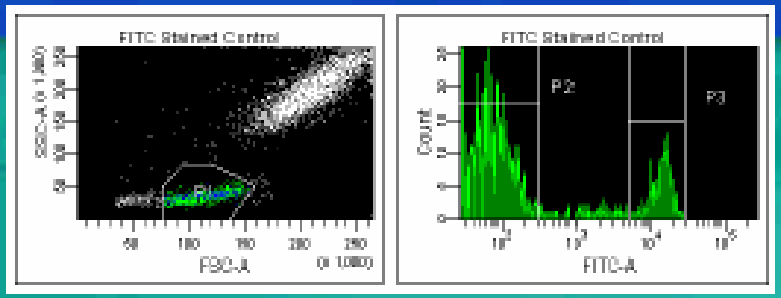
FL1 - %FL2
(0 – 1%)

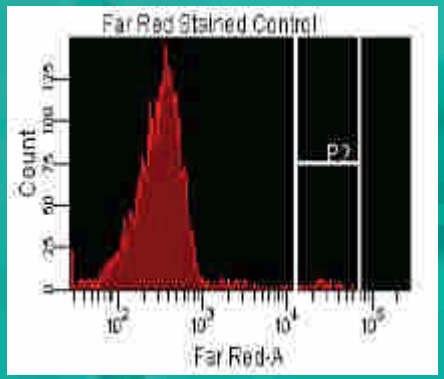


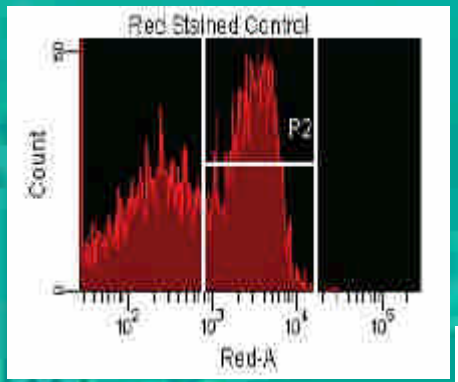
Create Compensation Controls











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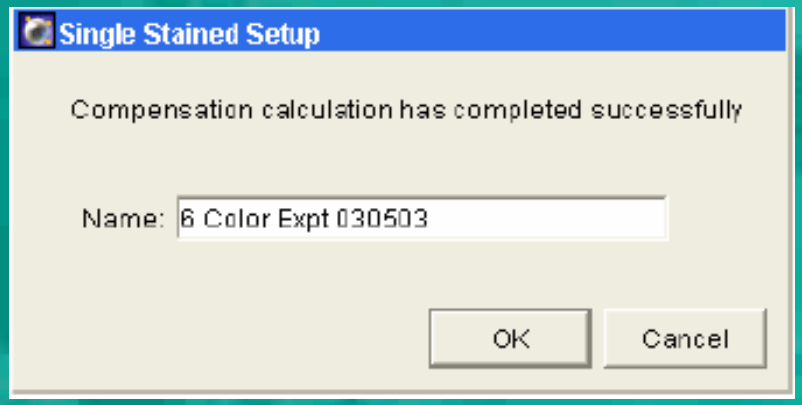
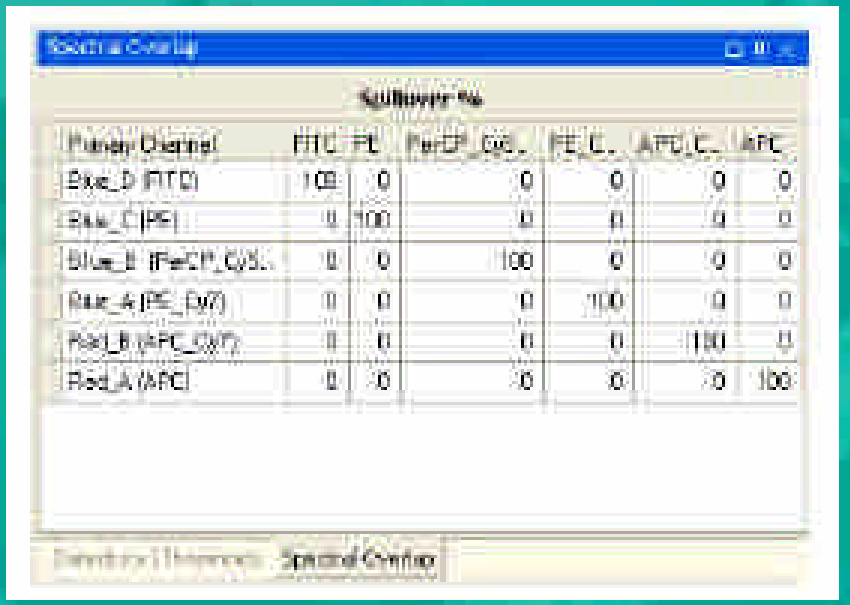
選擇“Instrument>Instrument Setup>Calculate Compensation”命令。





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FACSCanto 各部構造

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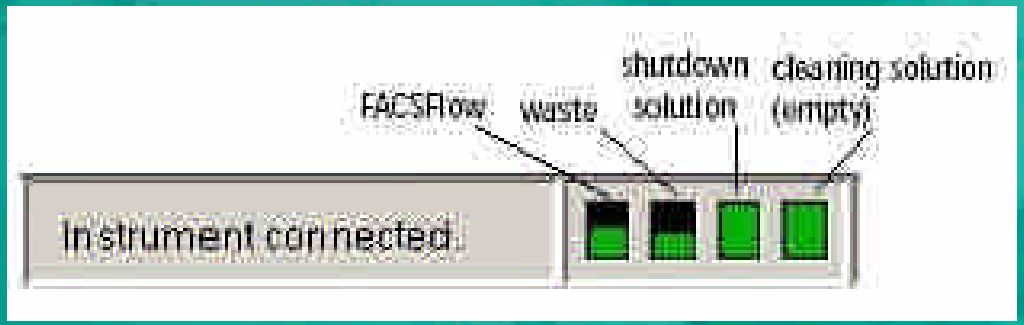
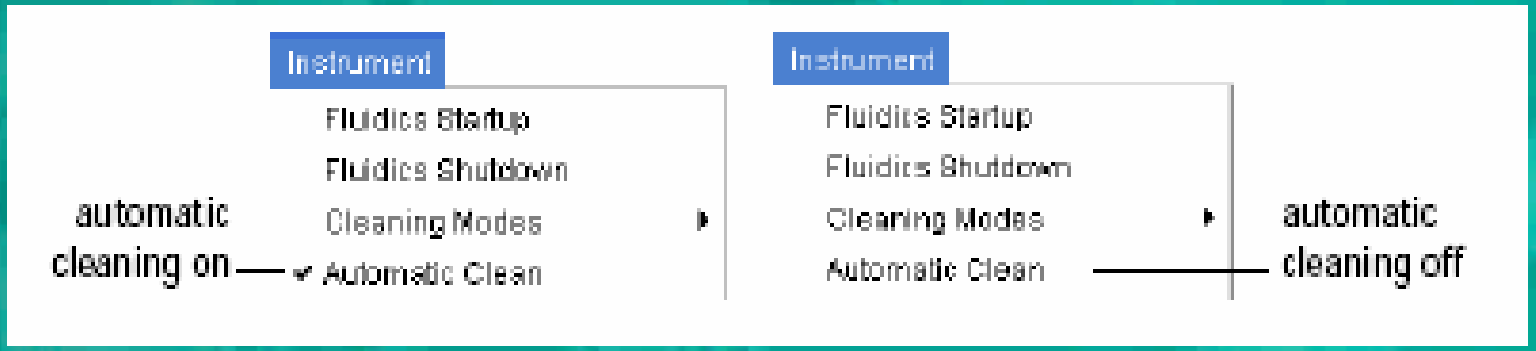
正確開機程序

1. 開啟主電源。
2. 開啟電腦。在密碼登錄框中，輸入BDIS，按Enter。
3. 開啟 BD FACSDiva 軟體。
4. 確定軟體與細胞儀完成連線。
5. 完成連線後，檢視各項試劑液面是否正常。
6. 等待細胞儀自動完成液流啟動程式。
7. 當液流啟動程式完成後，請點選OK。
8. 檢視雷射是否暖機完成。

整個程序約需5~10分鐘。





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上機檢查程序

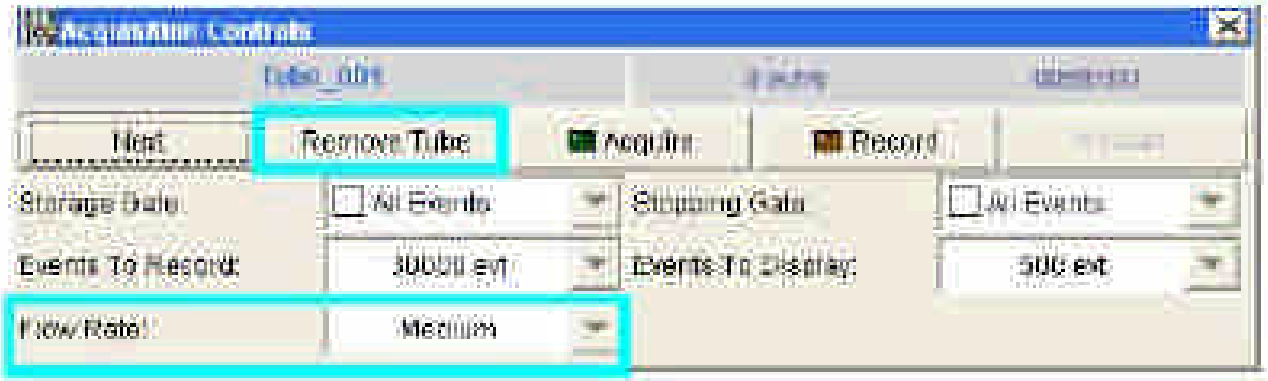
1. 檢品濃度調至1X10^6 cells/ml？一般只需0.5 ml。
2. 是否已小心地去除檢品中之細胞團塊。
3. 是否已將檢品放至FALCON 2052 試管中？試管是否有裂痕？
4. 是否有足量專用鞘液？ 是否已將廢液倒掉？
5. 是否已執行Auto Clean ？
6. 系統氣壓讀數55~65 Psi ？
7. 是否已將液體過濾器中之氣泡排空？
8. 請填寫使用登記表。

建議使用之鞘液液體

1. FACSFlow (BDIS)
2. 經0.22um過濾之自備含0.05%NaN_3之 PBS

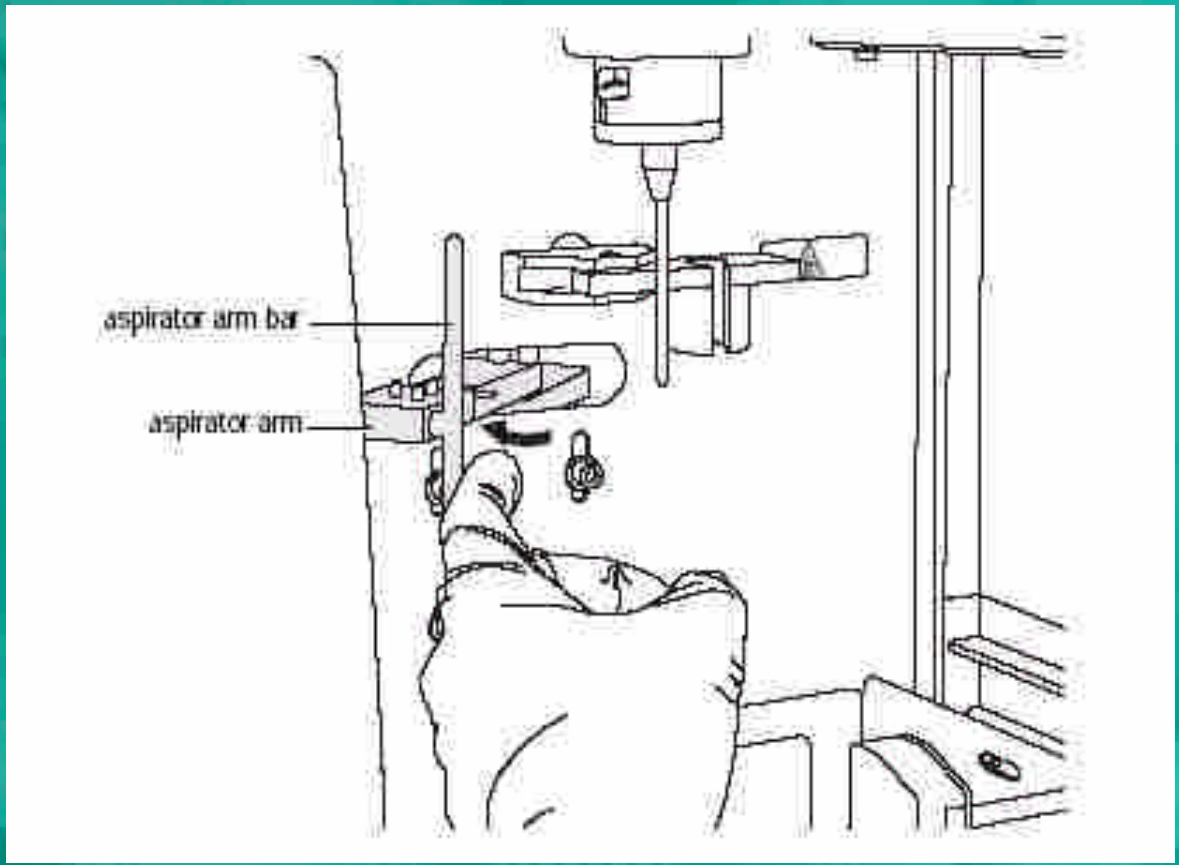
不鼓勵使用下列溶液作為鞘液液體之用：
- Fisher Hematology Diluent
- Isoton III
- Isolac D
- DI water

Figure 2-7 Acquisition controls unique to the BD FACSCanto



Low： 樣品流速= 10 µl /min
Medium： 樣品流速= 60 µl /min
High: 樣品流速=120 µl /min

檢體吸取區(SIT)



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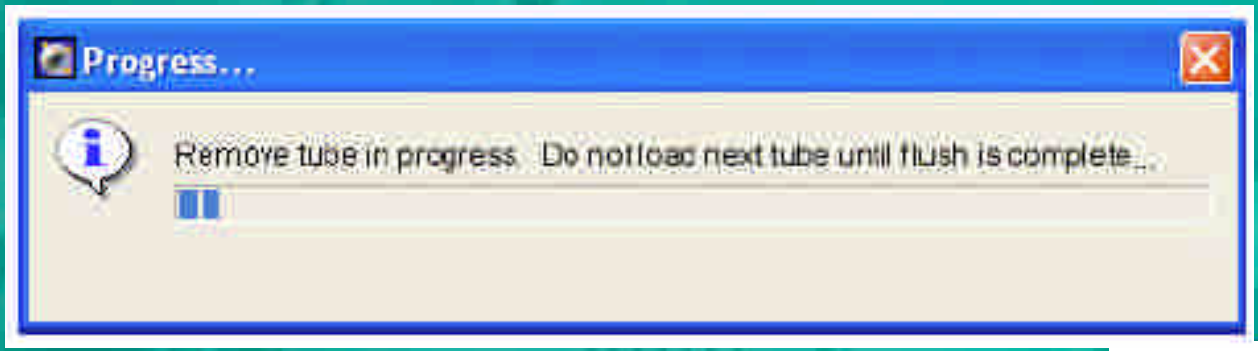
Remove Tube正確步驟

步驟要確實：〔to minimize carry over between samples〕

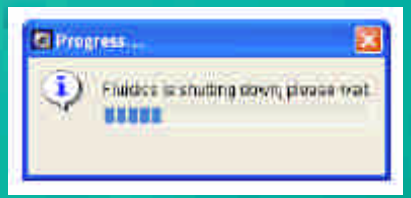
1. 在Acquisition Controls視窗中，點Remove tube；此時，會出現一個進程顯示對話框。
2. 右手握著小管，左手將Aspirator arm向左搬到底。
3. 取下樣品管。
4. 放開左手，使Aspirator arm回到中央；此時，SIT會自動清洗；清洗完成後，進程顯示對話框會自動消失，在清洗動作完成前，請勿放下一管。



儀器清洗與關機正確步驟

清洗步驟要確實：〔尤其是使用PI之後〕

1. 取 3 ml FACSClean（10%Bleach）上樣品。
2. 在Acquisition control上，按Acquire。
3. 讓儀器在Medium Flow Rate模式下，Acquire 5 分鐘，再按Acquire以中止收取。取下樣品管。
4. 換上 3 ml dH2O上樣品。
5. 在Acquisition control上，按Acquire。
6. 讓儀器在Medium Flow Rate模式下，Acquire 5 分鐘，再按Acquire以中止收取。**取下樣品管**。
7. 點選 Instrument > Fluidics Shutdown。液流系統關閉中，會出現下方進度報告。
8. 在允許關閉系統的對話框中，點選OK。
9. 關閉電腦，關閉細胞儀。



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公共儀器的維護

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