Introducing BD FACSLyric™ and FACSMelody

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Topic

- **Basic Concept of Flow Cytometer**
- Introduction to Instrument
- Compensation Theory
- Daily Workflow - FACSuite Software

BD

What is Flow Cytometry?

Flow = Fluid

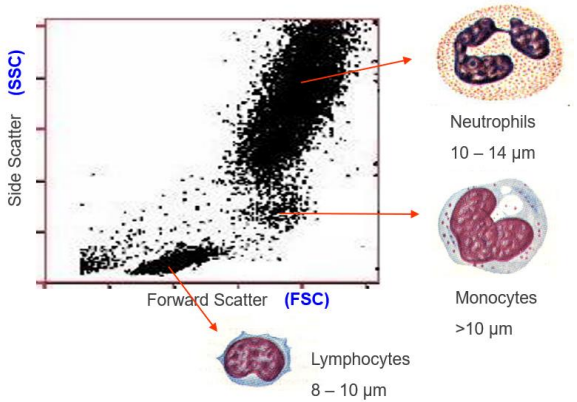
Cyto = Cell

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.

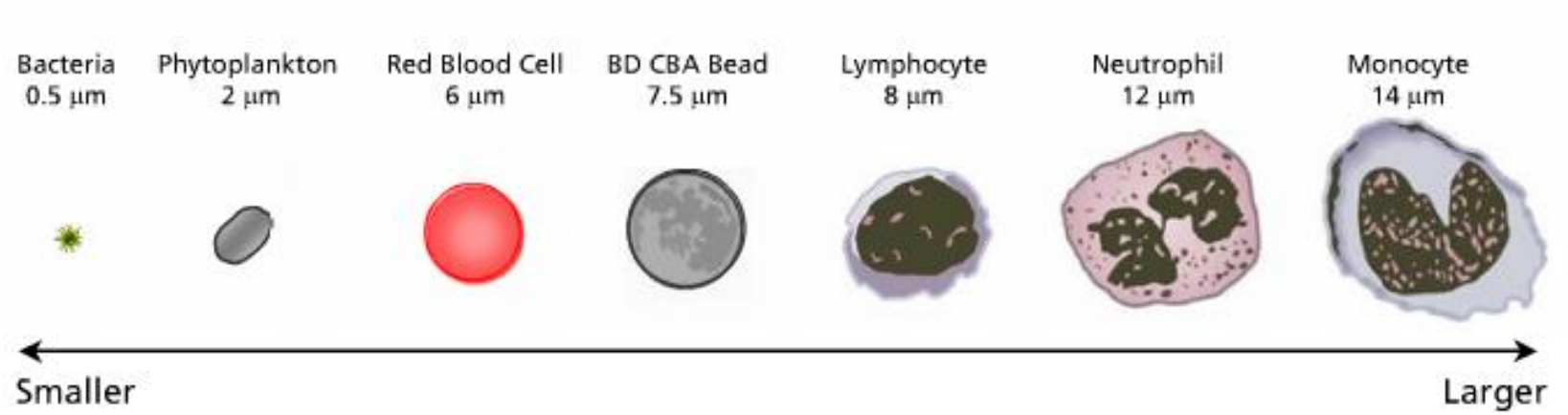
What Can a Flow Cytometer Tell Us About a Cell?

- 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**

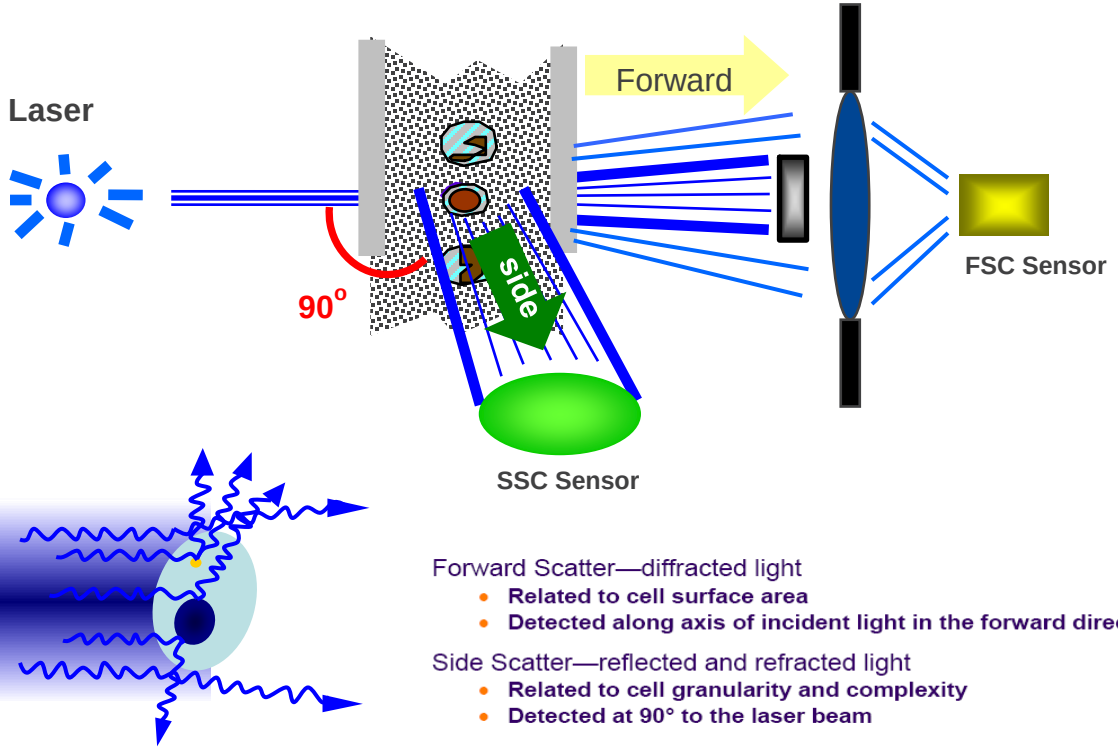


Particle Size

- Detection range: 0.2~50μm



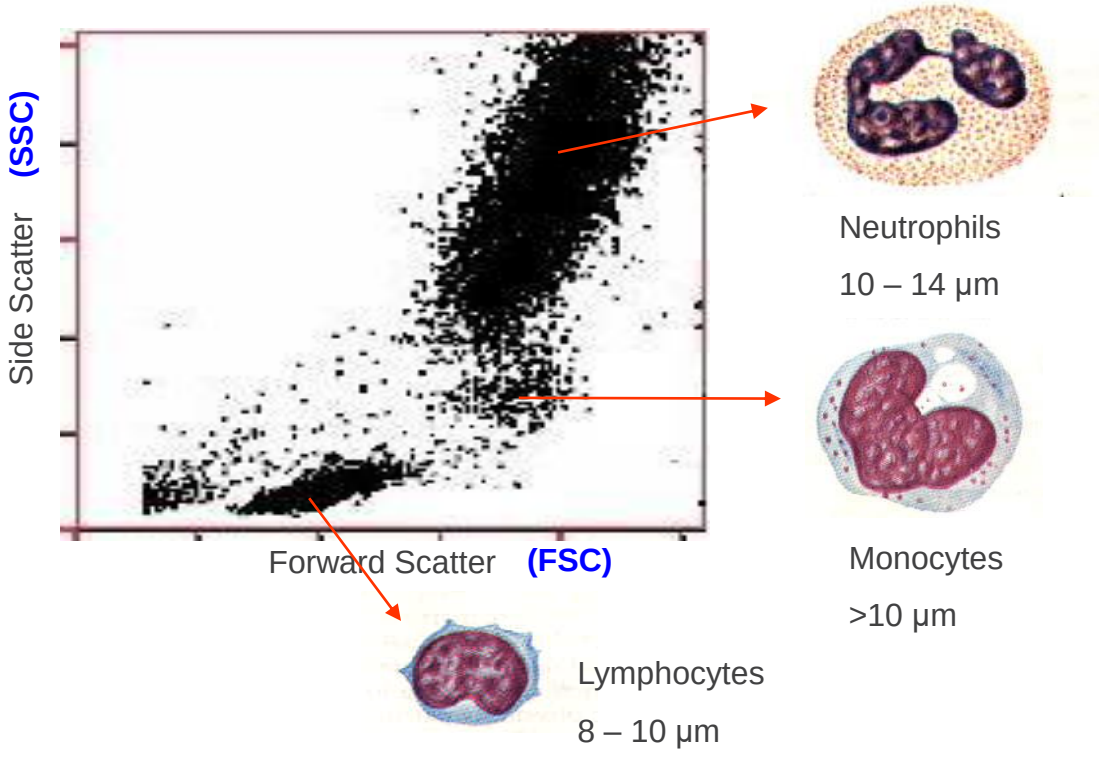
Scatter Light



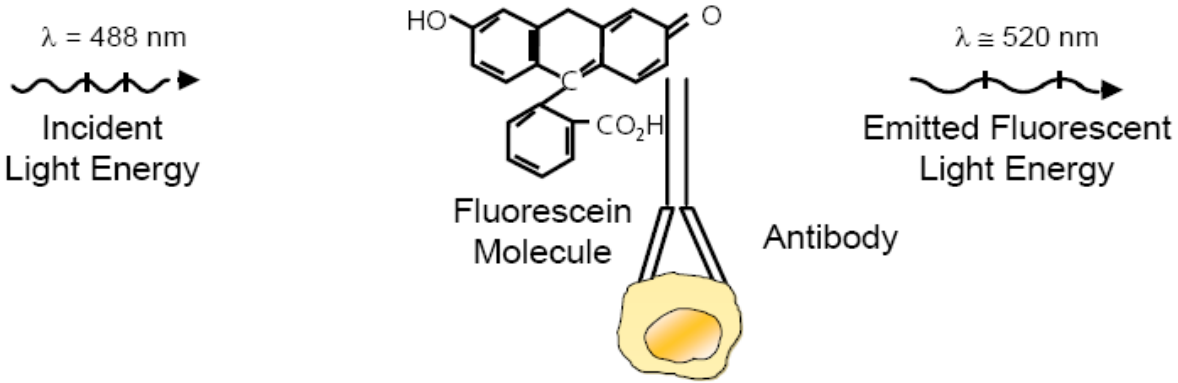
Forward Scatter—diffracted light
- **Related to cell surface area**
- **Detected along axis of incident light in the forward direction**

Side Scatter—reflected and refracted light
- **Related to cell granularity and complexity**
- **Detected at 90° to the laser beam**

Ex. Lysed Human Whole Blood

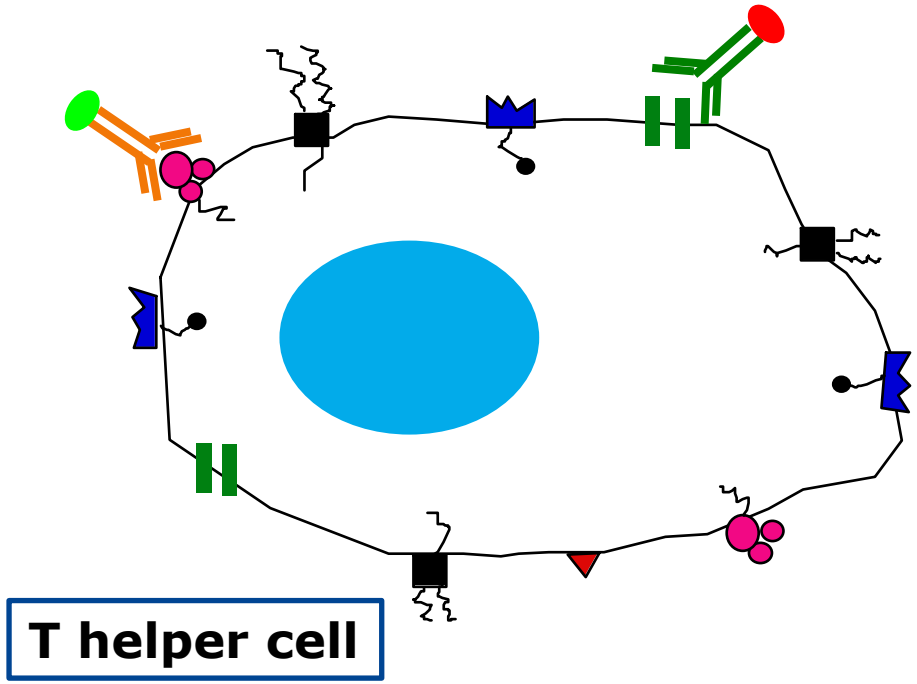


Fluorescence Light



- 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.

Flow Cytometry Detection Principle



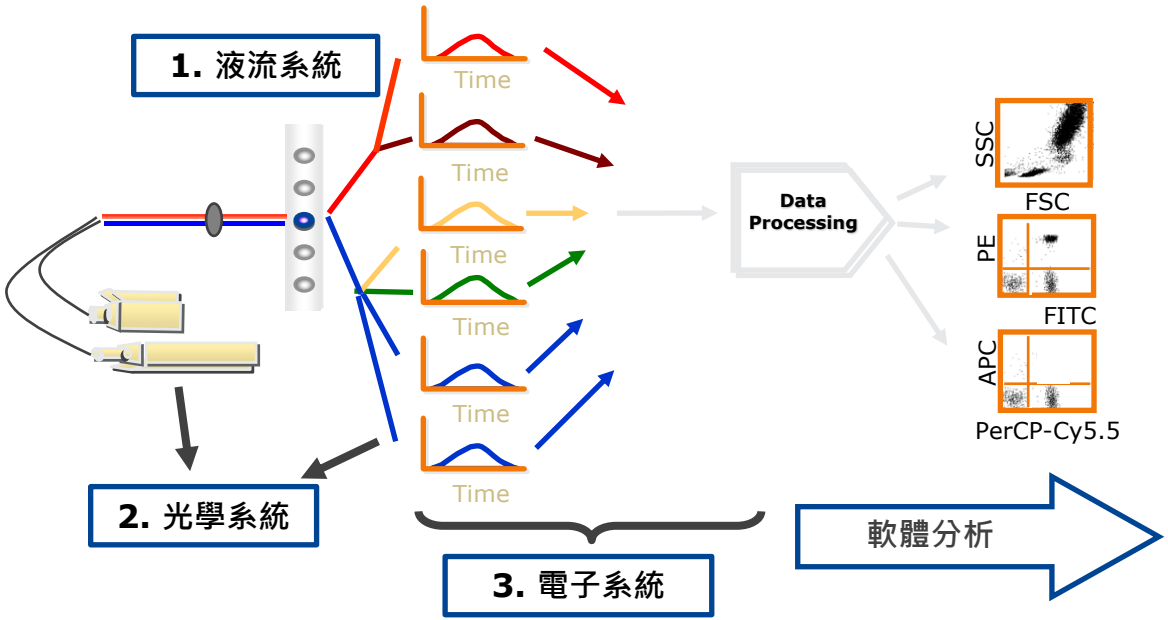
:Antigen CD4

:Antigen CD3

:Anti-CD3

:Anti-CD4

Flow Cytometer Overview



Main Component

Fluidics 液流系統

To introduce and focus the cells for interrogation.

Optics 光學系統

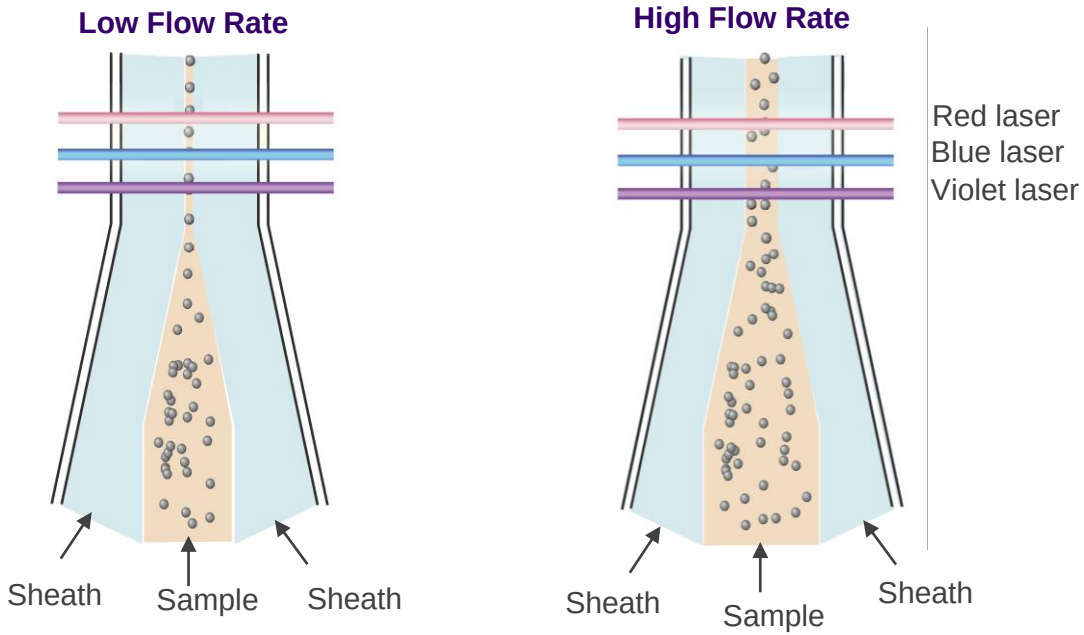
To generate and collect the light signals.

Electronics 電子系統

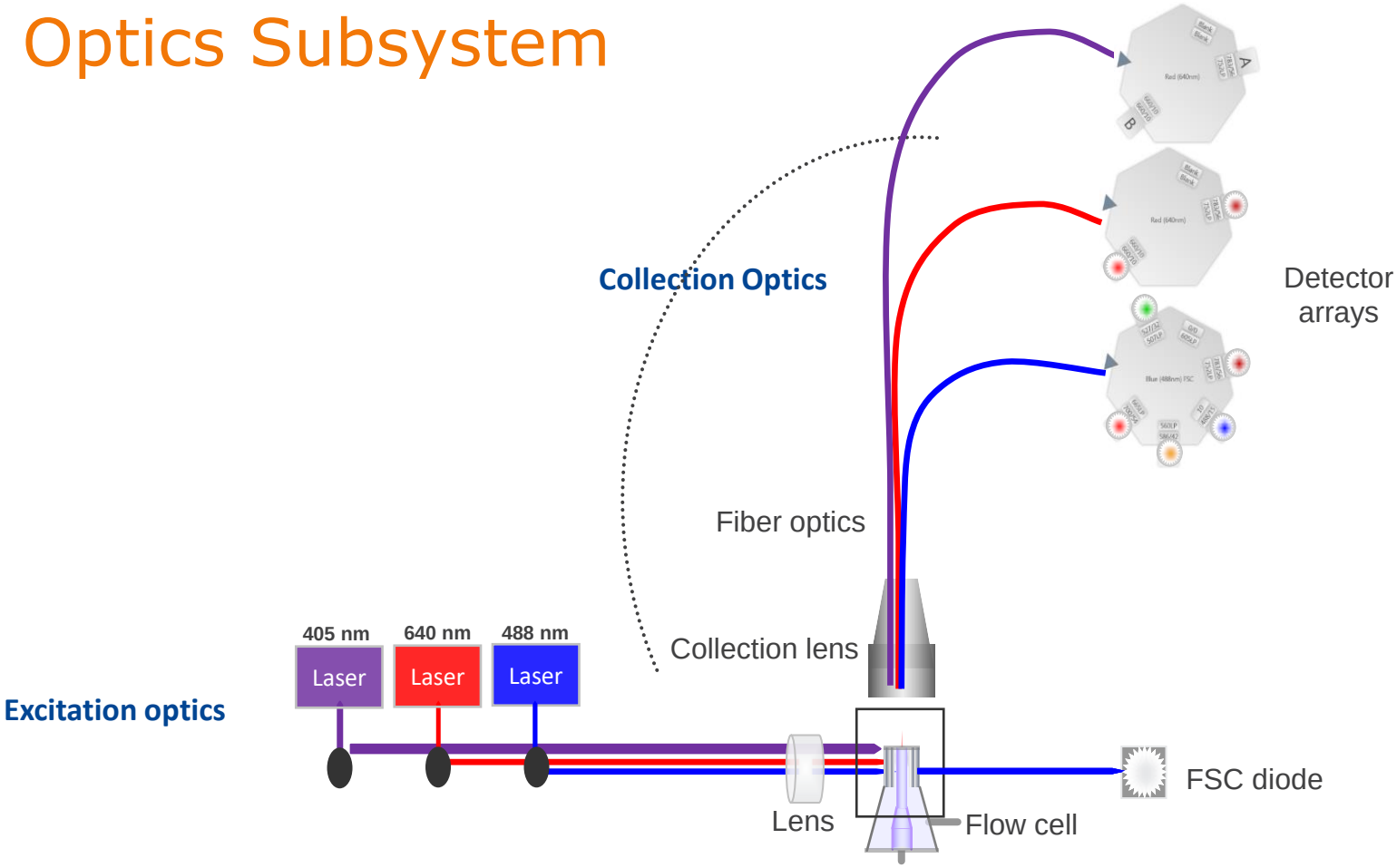
To convert the optical signals to proportional digital signals, process the signals, and communicate with the computer.

Sample Flow

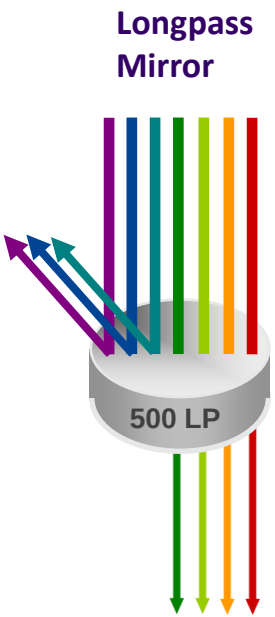
Low: 12 µL/min
Medium: 60 µL/min
High: 120 µL/min
High sensitivity: 50 µL/min

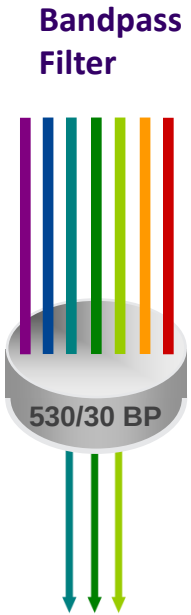


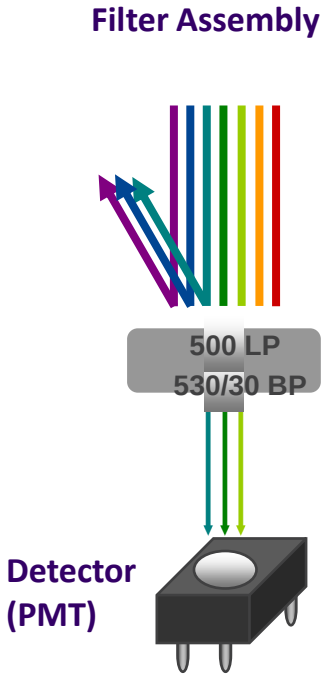
Optics Subsystem



Collection Optics: Filters



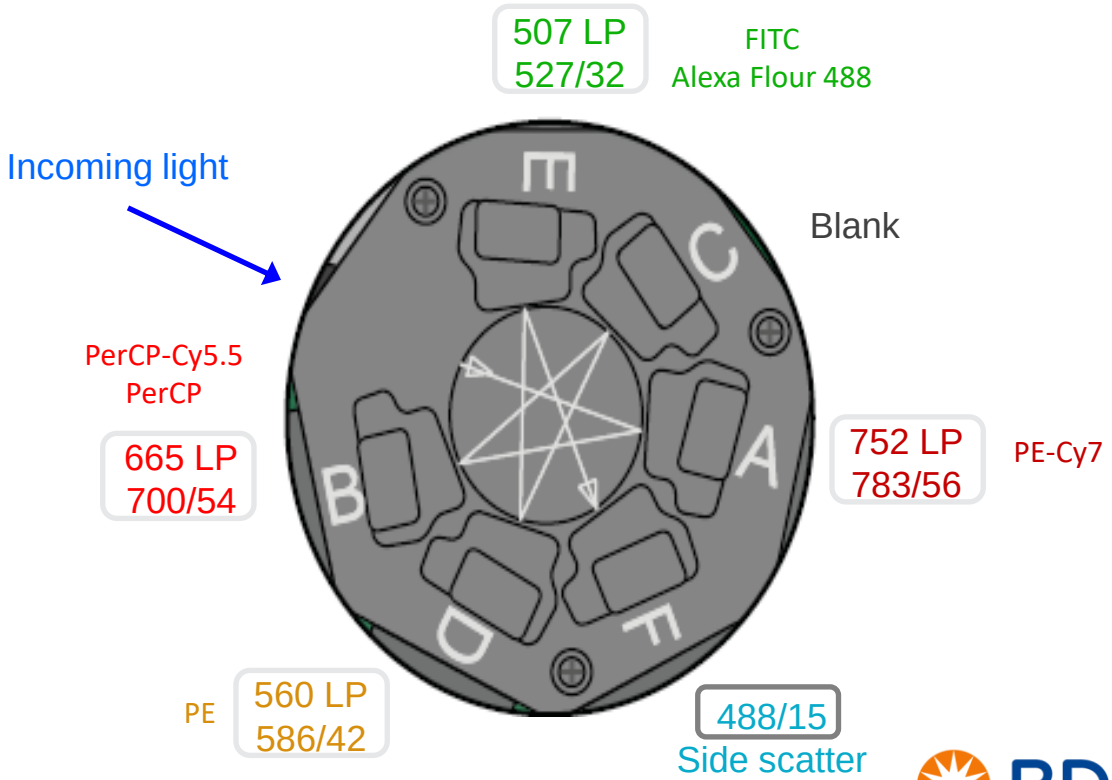




- Miniaturized collection blocks
- All filters installed for simple change
- Chip ID for each filter cassette
- Automatic detection of configuration

Collection Optics: Heptagons

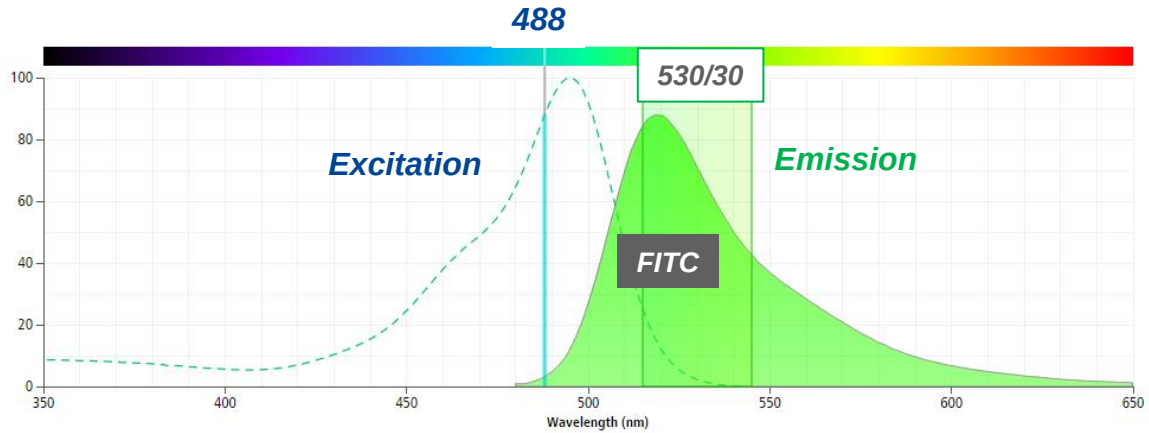
- 全球第一以”轉折”代替多重穿透設計：保留螢光能量
- 接收光源順序：能量弱→強
  BD專利設計
- FACSLyric進一步改良，將分光鏡及濾鏡整合，使光學訊號以最少路徑讓接收，以達成最強光學訊號呈現



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.

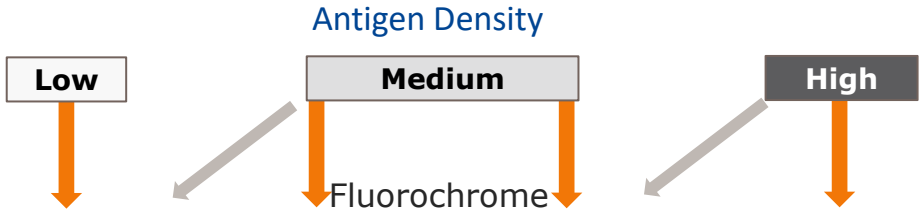


FACSLyric Configurations

Dyes	Filters	Mirrors
Blue Laser (488 nm)		
SSC	488/15	none
BD Horizon™ BB 515, FITC	527/32	507 LP
PE, PI	586/42	560 LP
PerCP, PerCP-Cy™5.5 , BB 700	700/54	665 LP
PE-Cy™7, RB780	783/56	752 LP
Red Laser (640 nm)		
APC	660/10	660/10
Red 718, APC-R700, AF700	720/30	705 LP
APC-Cy™7, APC-H7	783/56	752 LP
Violet Laser (405 nm)		
BD Horizon™ V450, BD Horizon Brilliant™ Violet 421	448/45	448/45
BD Horizon™ V500-C, BD Horizon Brilliant™ Violet 510	528/45	500 LP
BD Horizon Brilliant™ Violet 605	606/36	606/36
BD Horizon Brilliant™ Violet 711	715/50	715/50
BD Horizon Brilliant™ Violet 786	755 LP	755 LP

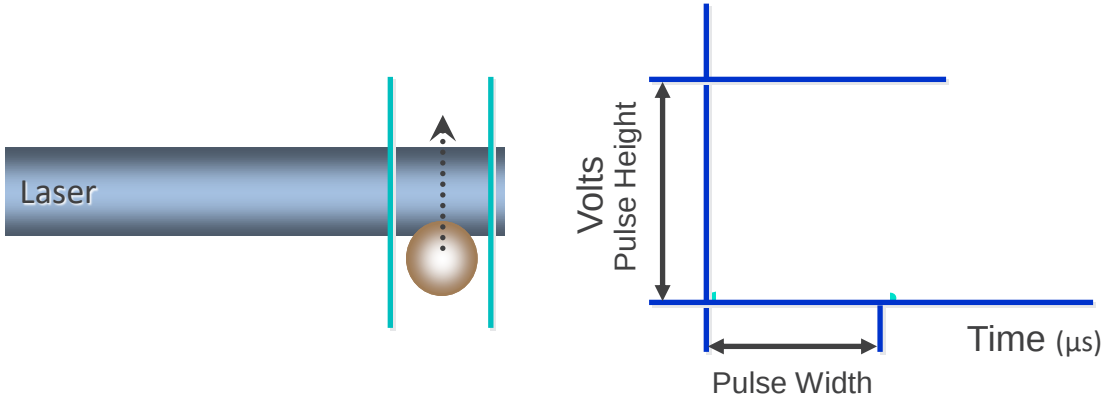
4B-3R-5V

Fluorochrome/Antigen Combination

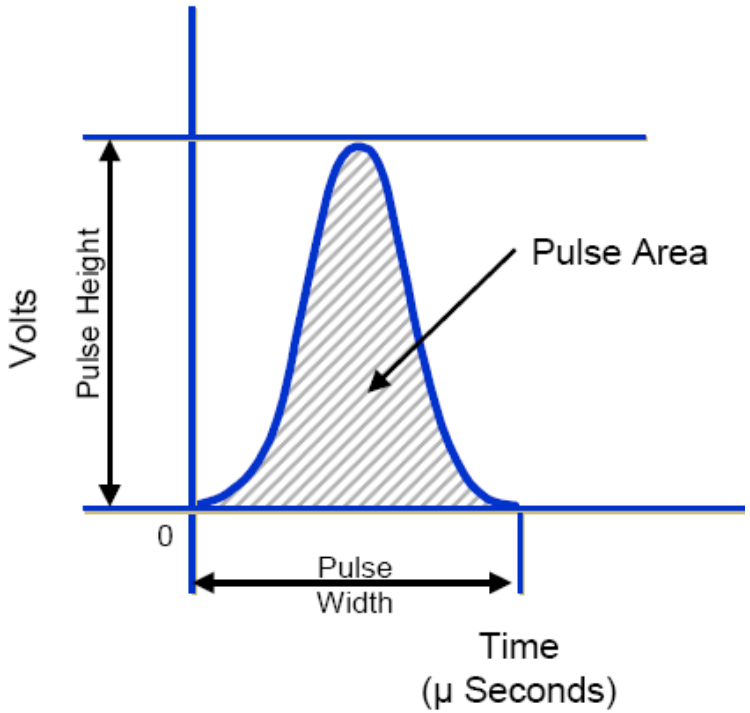


Laser	Very Bright	Bright	Moderate	Dim
Ultraviolet (355 nm)		BD Horizon BUV661 BD Horizon BUV737 BD Horizon BUV563	BD Horizon BUV395 BD Horizon BUV496	BD Horizon BUV805
Violet (405 nm)	BD Horizon BV421 BD Horizon BV650 BD Horizon BV711	BD Horizon BV480 BD Horizon BV786	BD Horizon BV510 BD Horizon BV605	BD Horizon V450 BD Horizon V500
Blue (488 nm)	BD Horizon BB515 BD Horizon BB700 BD Horizon PE-CF594 PE-Cy™5	PE PE-Cy™7	FITC Alexa Fluor® 488 PerCP-Cy™5.5	PerCP
Yellow/Green (561 nm)	PE BD Horizon PE-CF594 PE-Cy5 PE-Cy7			
Red (640 nm)		APC Alexa Fluor® 647 BD Horizon APC-R700		Alexa Fluor® 700 APC-H7 APC-Cy7

光學-電子訊號轉換過程



電子訊號的量化

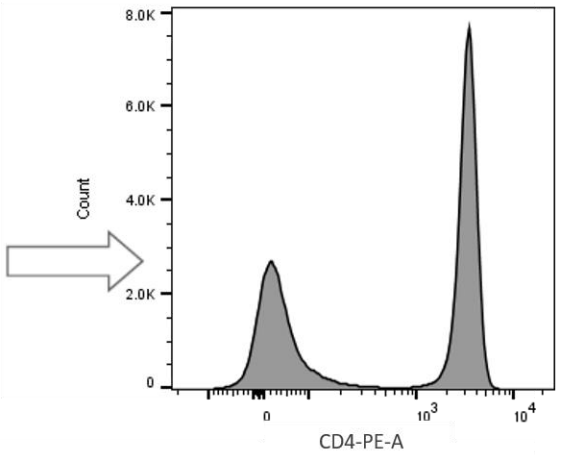


- **H**
  Height粒子通過雷射激發的訊號/螢光瞬間最大量
- **W**
  Width粒子通過雷射的時間
- **A**
  Area粒子通過雷射激發的訊號/螢光總量

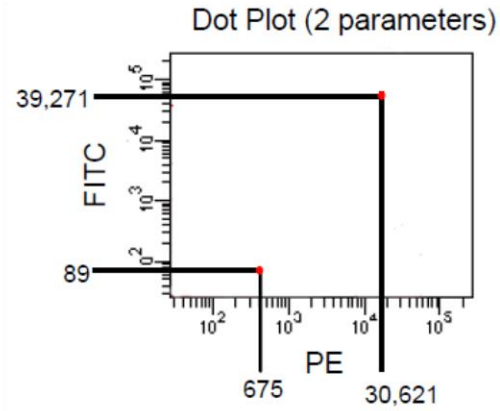
數據的儲存

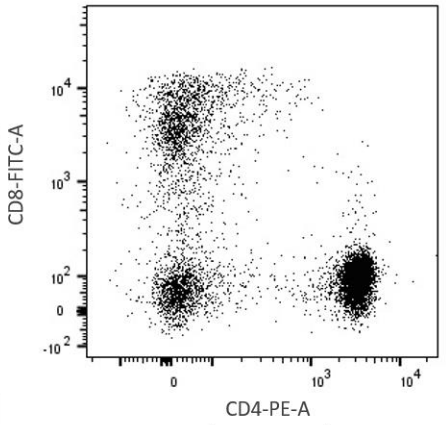
List-Mode Data

	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



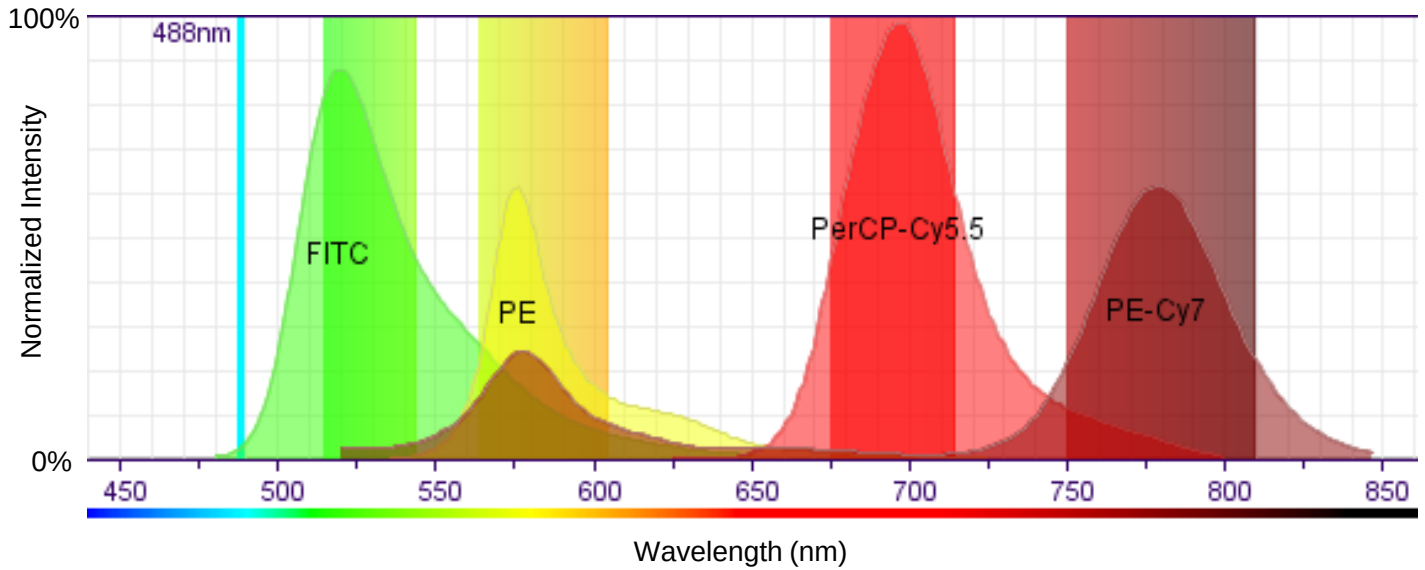
直方圖



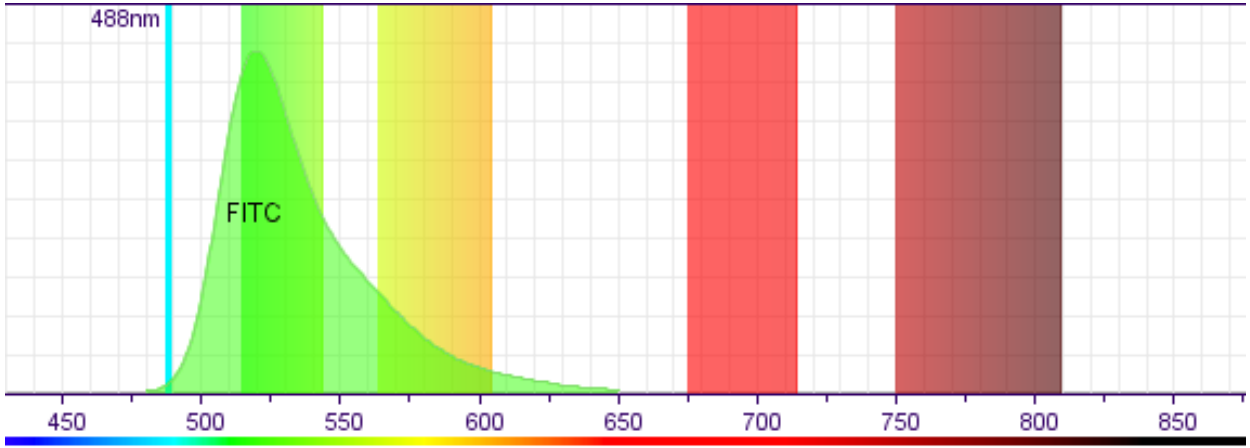


散點圖

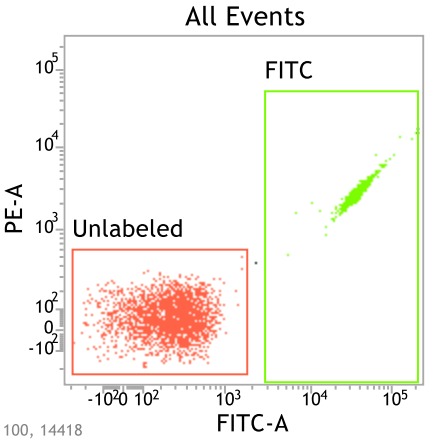
Spectral Overlap



FITC Spillover

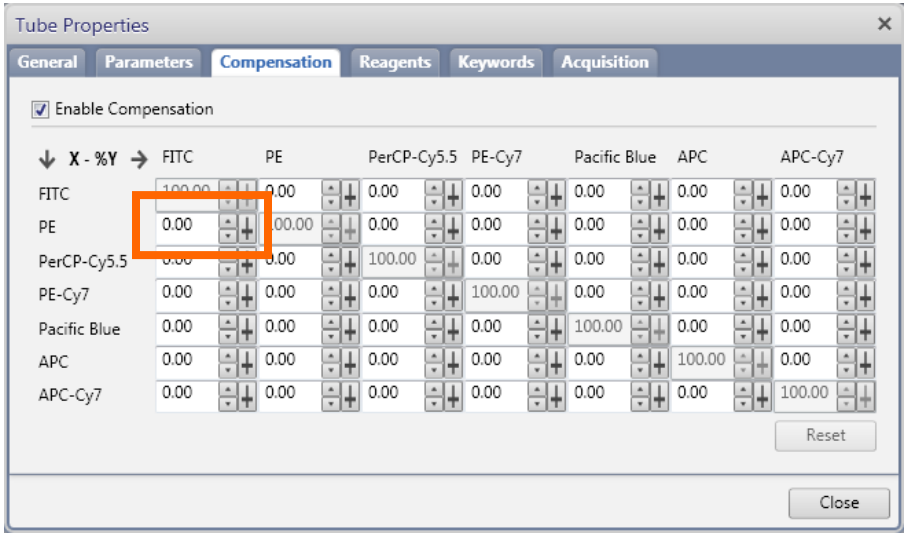


Correcting Spillover



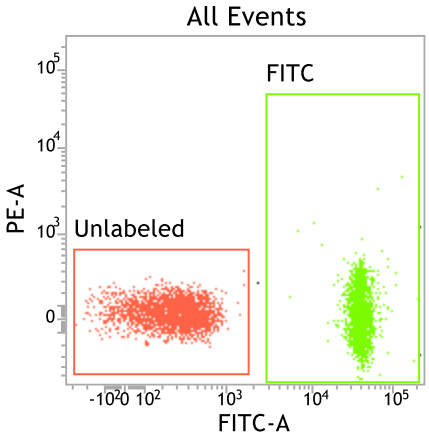
Statistics

Name	PE-A Median
FITC Stained Control:Unlabeled	55
FITC Stained Control:FITC	2,691



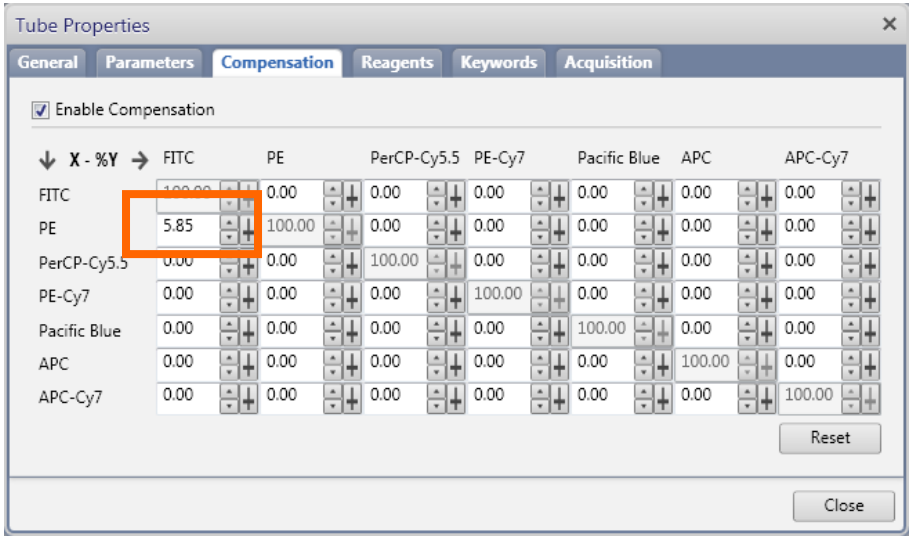
Which control do you adjust?

Correct Compensation



Statistics

Name	PE-A Median
FITC Stained Control:Unlabeled	34
FITC Stained Control:FITC	35

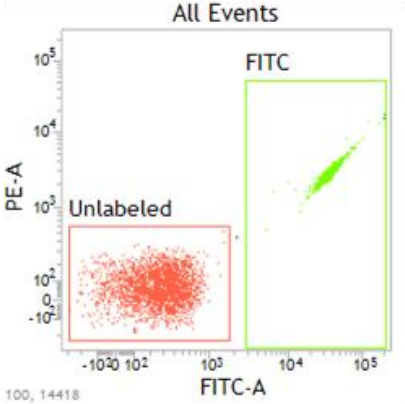


Which control do you adjust?

FITC single stain control : PE-%FITC

Compensation

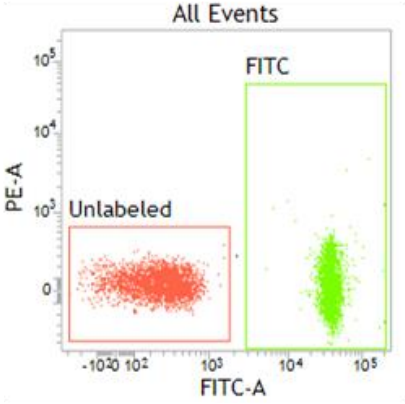
Uncompensated



Statistics

Name	PE-A Median
FITC Stained Control:Unlabeled	55
FITC Stained Control:FITC	2,691

Compensated

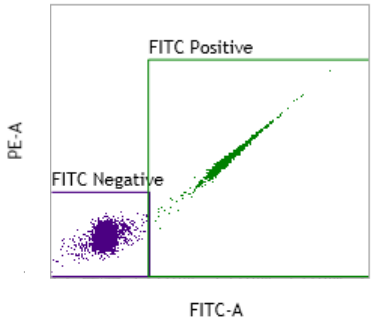


Statistics

Name	PE-A Median
FITC Stained Control:Unlabeled	34
FITC Stained Control:FITC	35

Compensation Troubleshooting

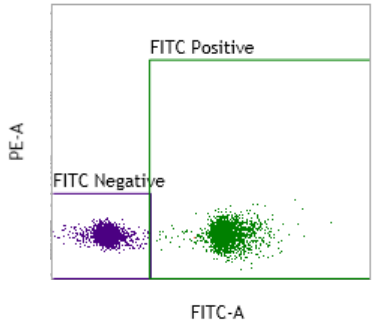
Undercompensated



FITC:FITC RunPointerStatistics

Name	PE-A Median
FITC Negative	13
FITC Positive	3,119

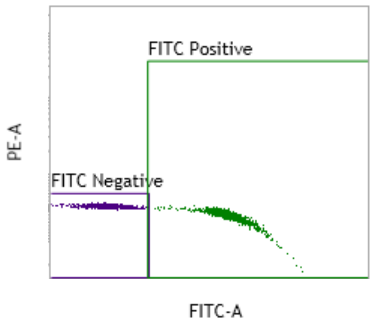
Correctly compensated



FITC:FITC RunPointerStatistics

Name	PE-A Median
FITC Negative	9
FITC Positive	9

Overcompensated



FITC:FITC RunPointerStatistics

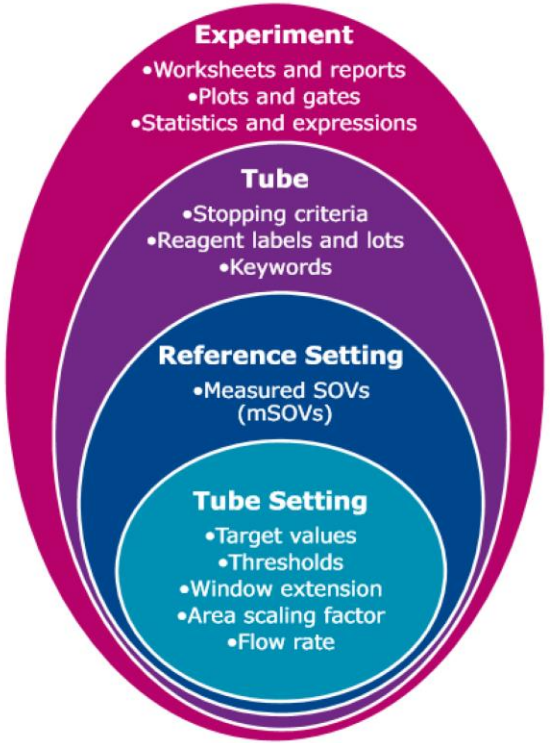
Name	PE-A Median
FITC Negative	8
FITC Positive	-250

名詞解釋

Tube setting =
傳統PMT電壓在特定MFI設定值

Reference setting =
螢光補償

- Tube Setting
  - Target Values
  - Thresholds
  - Window extension
  - Area scaling factor
  - Flow rate
- Reference Setting
  - Measured SOVs
- Tube
  - Stopping criteria
  - Reagent labels and lots
  - Keywords
- Experiment
  - Worksheets and reports
  - Plots and gates
  - Statistics and expressions

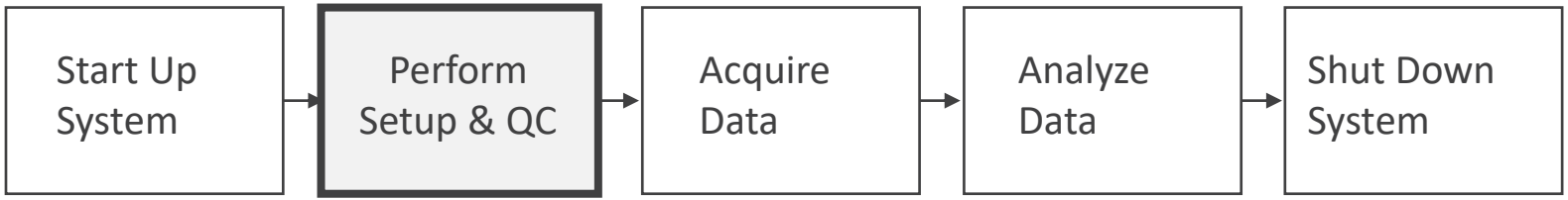


Daily Workflow

1. Turn on power to the instrument
2. Turn on computer and log into BD FACSuite software
3. Verify software connection to the instrument 

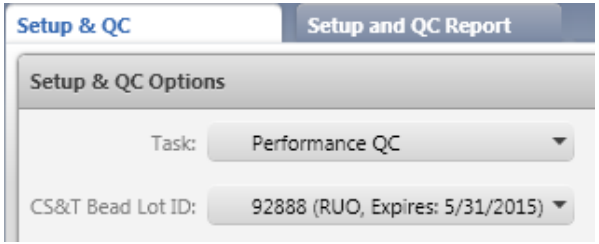
4. Check fluid levels 

Daily Workflow



Running Performance QC

1. Prepare the CS&T research beads.
2. Verify the bead lot ID and select the **Performance QC** task.
3. Run performance QC.
4. View the performance QC reports (normal and high-sensitivity fluidic modes).
5. View Levey-Jennings charts in the **QC Tracking** tab.



Viewing the Performance QC Reports

Cytometer Performance QC Report
4-Blue 2-Red 2-Violet (RUO)

Performed: 3/24/2011 2:46 PM

Cytometer:	Liberty	User:	Admin User
Cytometer Name:		Institution:	None
Serial Number:	12345		
Fluidics Mode:	Normal		
Last Characterization QC:	3/24/2011 2:33:21 PM		

Configuration Name: 4-Blue 2-Red 2-Violet (RUO) — Last Modified: 3/24/2011 2:20 PM

SUMMARY: PASSED

%rCV, linearity, sensitivity

DETECTOR SETTINGS

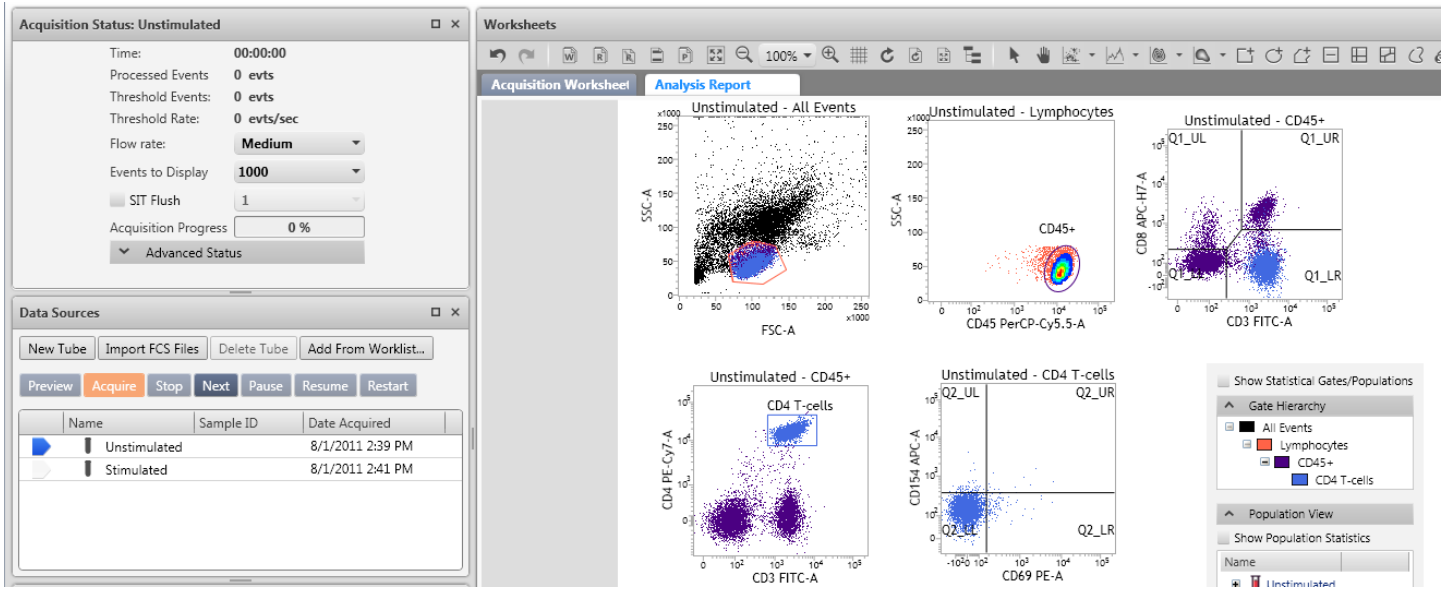
Lot Info	Detector				PMTV		Bright Bead		Linearity (±2%)		Resolution			
	Name	Mirror	Filter	Position	Actual	Δ	Median	% rCV	Min Channel	Max Channel	Sensitivity Actual	Sensitivity % Diff	Qr (x10³)	Br
LASER: Blue (Wavelength = 488nm)														
X	FSC	-	-	FSC	398.6	0.0	124980	1.5	N/A	N/A	300	0	N/A	N/A
X	SSC	10	488/15	F	351.1	0.0	125090	3.1	N/A	N/A	300	0	N/A	N/A
X	FITC	507LP	527/32	E	659.5	0.1	100061	1.5	5	219688	300	0	86.9	393
X	PE	560LP	586/42	D	580.7	-0.1	99893	1.2	5	220051	300	0	584.6	382
X	PerCP-Cy5.5	665LP	700/54	B	708.9	-0.1	99960	2.7	5	220425	300	0	30.3	277
X	PE-Cy7	752LP	783/56	A	790.8	-0.2	99930	5.2	26	219957	300	0	30.9	14
LASER: Red (Wavelength = 640nm)														
X	APC	660	660/10	B	634.9	-0.1	100018	1.7	45	219887	300	0	49.8	476
LASER: Red (Wavelength = 640nm)														
X	APC-Cy7	752LP	783/56	A	690.1	-0.1	99951	3.7	35	220029	300	0	12.0	1856
LASER: Violet (Wavelength = 405nm)														
X	V450	448	448/45	B	628.5	0.0	99892	1.9	27	219724	300	0	56.8	11356
X	V500	500LP	528/45	A	545.1	0.0	99892	1.8	27	219621	300	0	41.0	6162

Daily Workflow: Experiment

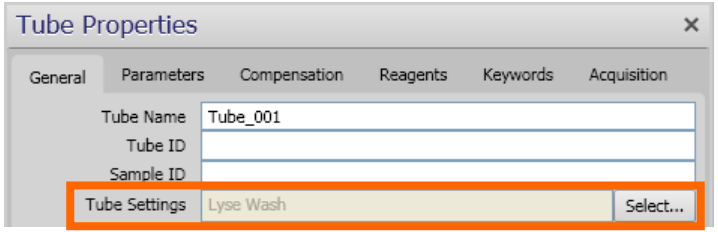
- Create or open an experiment
- Add tubes and create worksheet elements (plots, gates, stats)
- Adjust instrument settings and create tube settings, as needed
- Customize tube properties
- Acquire data

Experiment

- A customized group of elements used to acquire and analyze data.
- An experiment is flexible and can be saved as an assay for use in a worklist.



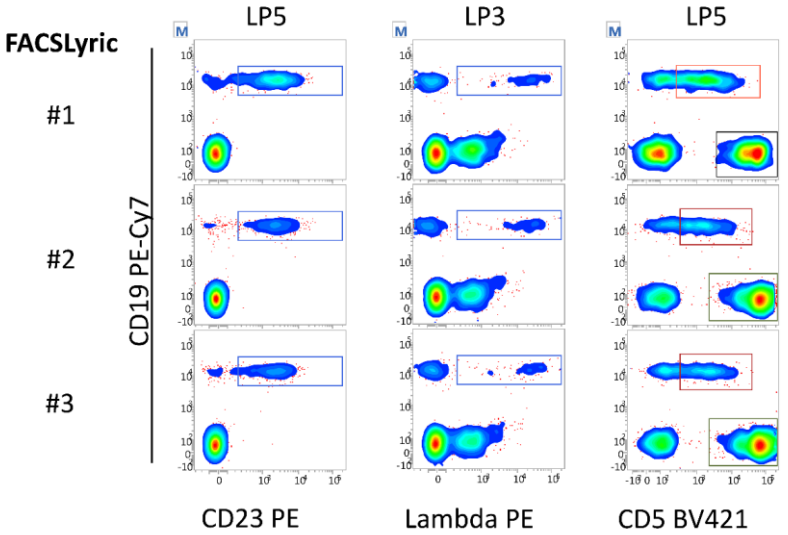
Setting Up the Experiment

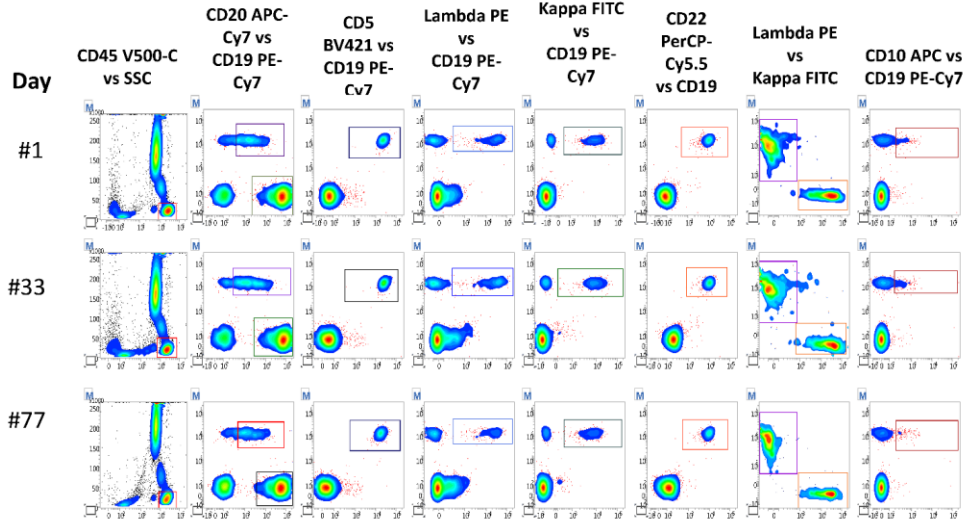


Default Lyse/Wash Reference Settings

- Contain tube settings for normal human lymphocytes
- Contain spillover values
- Values are re-calculated daily based on performance QC

Assay Portability: Unchanged Signal Intensity





Enhanced Reproducibility of Multi-color B-Cell Assays Using the Automated Universal Assay Setup Features of the BD FACSLyric™ and Dry-Format Reagent Panels

Marybeth Sharkey[1], Alan Stall[1], Dina Huckaby[1], Marsha Griffin[1]; BD Life Sciences, San Jose, CA
Jeffrey Jorgensen[2], Sa Wang[2], Russell Higgins[3], Kenneth Holder[3], Chandra Krishnan[4], Cathy Spadaccini[5], Jean Coviello[6], Franklin Fuda[7], Weina Chen[7], Andrea Sheehan[8], Martha Luna[9], April Ewton[10], Kimberly Monnin[11], Laura Sulak[12], Christina Montalvo[12], Nawir Hakim[13], Alireza Torabi[13], Harry Wilson[13]
[2]University of Texas MD Anderson Cancer Center, Houston; [3]UT Health Science Center, San Antonio, [4]Dell Children's, Austin, [5]Christus Santa Rosa, San Antonio, [6]Brooke Army Medical Center, San Antonio, [7]University of Texas Southwestern, Dallas, [8]Texas Children's Hospital, Houston, [9]Renaissance Hospital, Edinburgh, [10]The Methodist Hospital, Houston, [11]Clinical Pathology Laboratories, Austin, [12]St. Lukes Episcopal Hospital, Houston, [13]University Medical Center, El Paso

Fluidics Commands

SIT Flush

Backflushes sheath fluid through the SIT and removes bubbles from the flow cell.

Clean Cuvette

Runs cleaning solution through the flow cell.

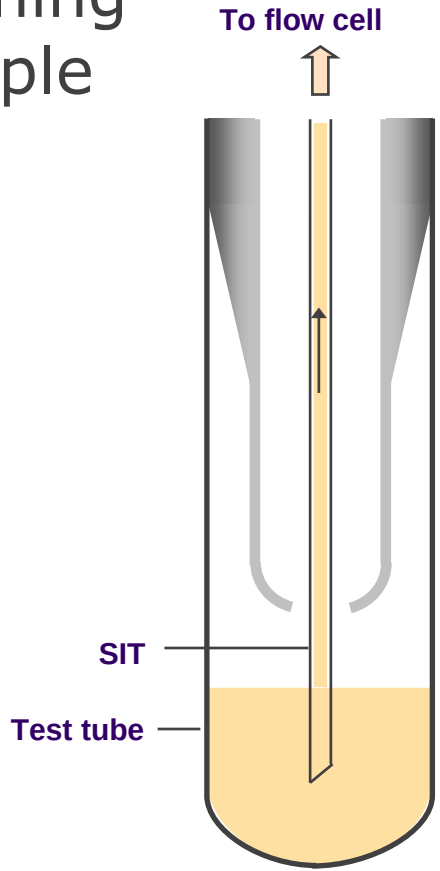
Drain and Fill Flow Cell

Removes persistent bubbles from the flow cell.

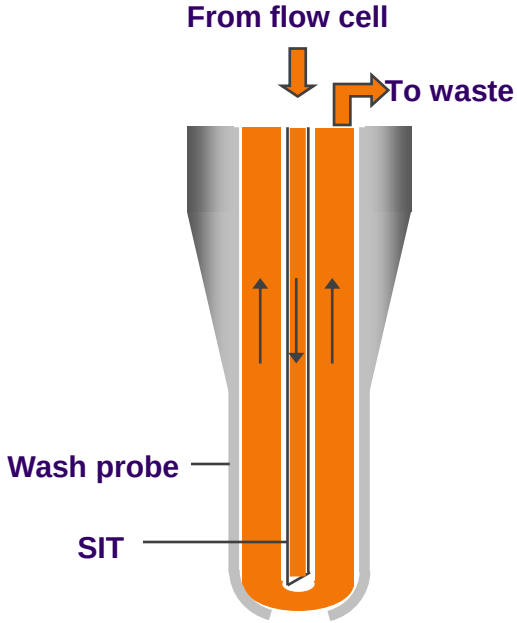
Purge Sheath Filter

Removes air from the sheath filter.

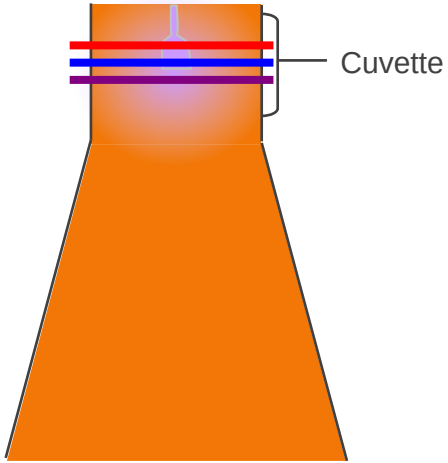
Running sample



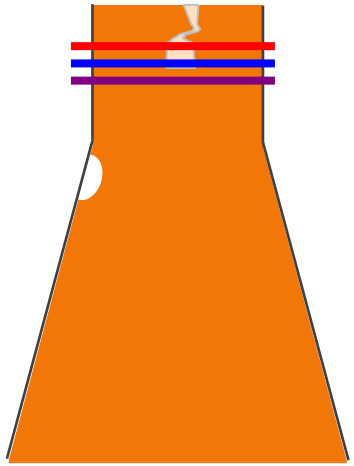
SIT Flush



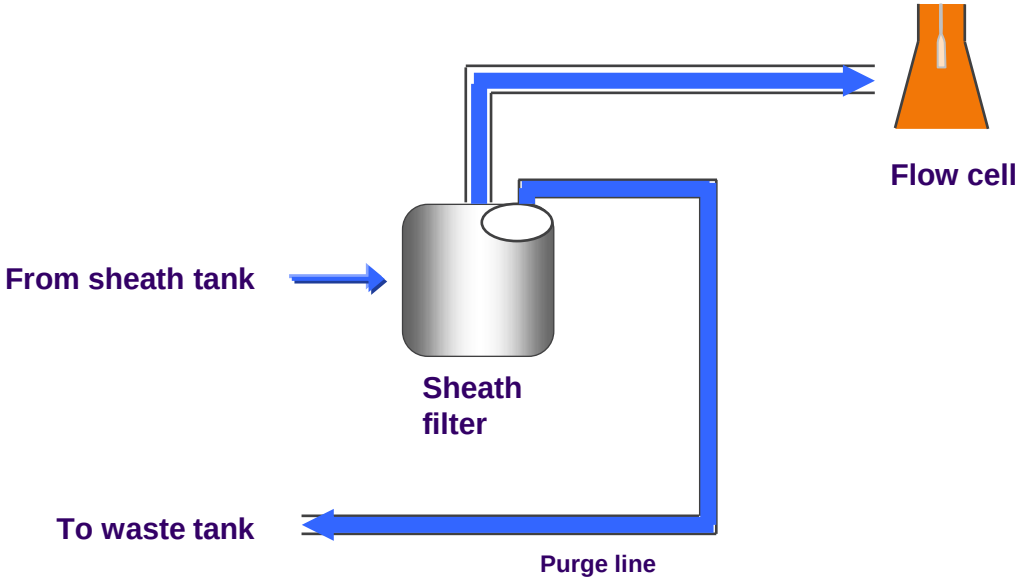
Clean Cuvette



Drain and Fill Flow Cell

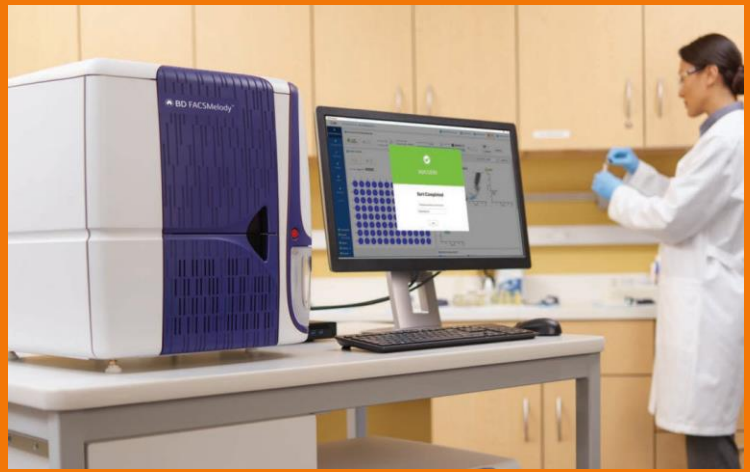


Purge Sheath Filter



BD FACSMelody™ Cell Sorter

The simple solution
for consistent, quality
results



Class 1 Laser Product.
For Research Use Only. Not for use in diagnostic or therapeutic procedures.

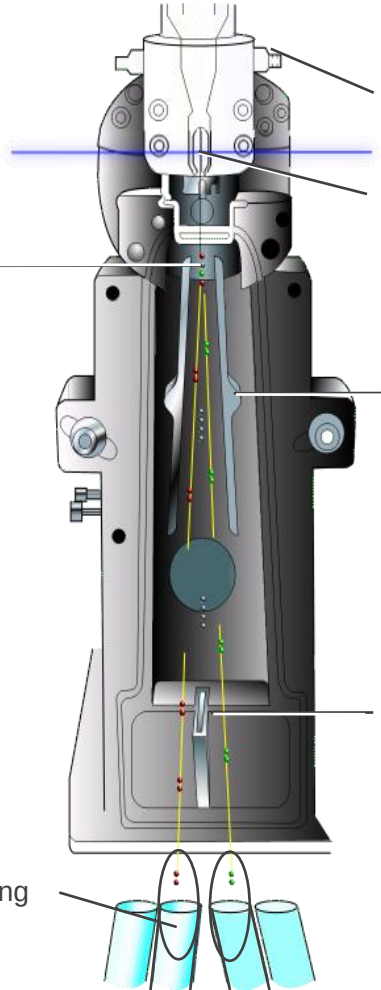
BD FACSMelody™ Cell Sorter

- Available with up to 3 spatially-separated lasers
- Detect up to 9 colors simultaneously
- Purification of up to 4 simultaneous populations into tubes
- Index sorting into plates or slides
- Superior sensitivity for the resolution of low-density antigens
- Easy to learn, use and maintain

BD FACSMelody™ Configuration

488nm	Filter	Mirror
FITC, BB515, AF488, GFP	527/32	507LP
PE, PI	586/42	560LP
PerCP-Cy5.5, PerCP, BB700, PE-Cy5, 7-AAD	700/54	665LP
PE-Cy7, RB780	783/56	752LP
640nm		
APC, AF647	660/10	660/10
APC-Cy7. APC-H7	783/56	752LP
405mm		
BV421, V450, DAPI, Pacific Blue		448/45
BV510, V500	528/45	500LP
BV786		755LP

Sorting Theory



2. Charge is applied via the stream-charging wire.

1. Sample generates light scatter and fluorescent signals; signals are analyzed.

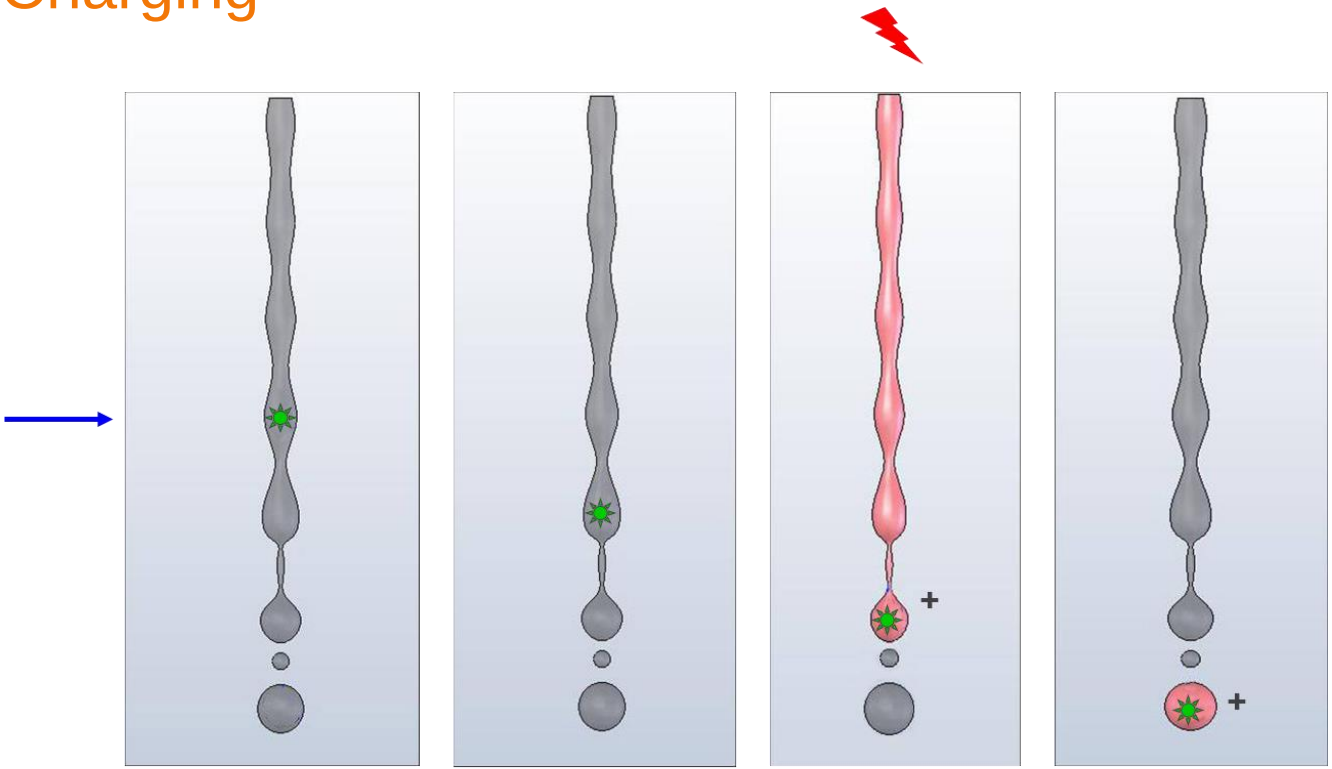
3. Charged droplets break off.

4. Deflection plates attract or repel charged droplet.

5. Uncharged droplets pass to waste.

6. Charged drops containing particles of interest are collected.

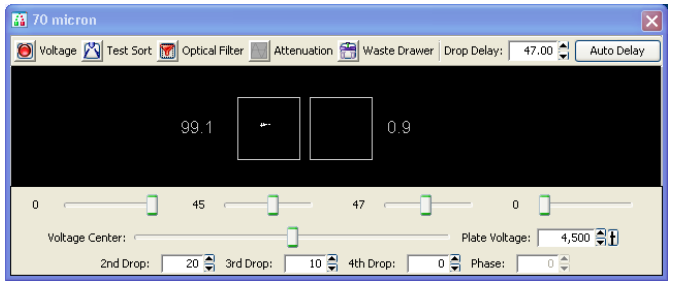
Drop Charging

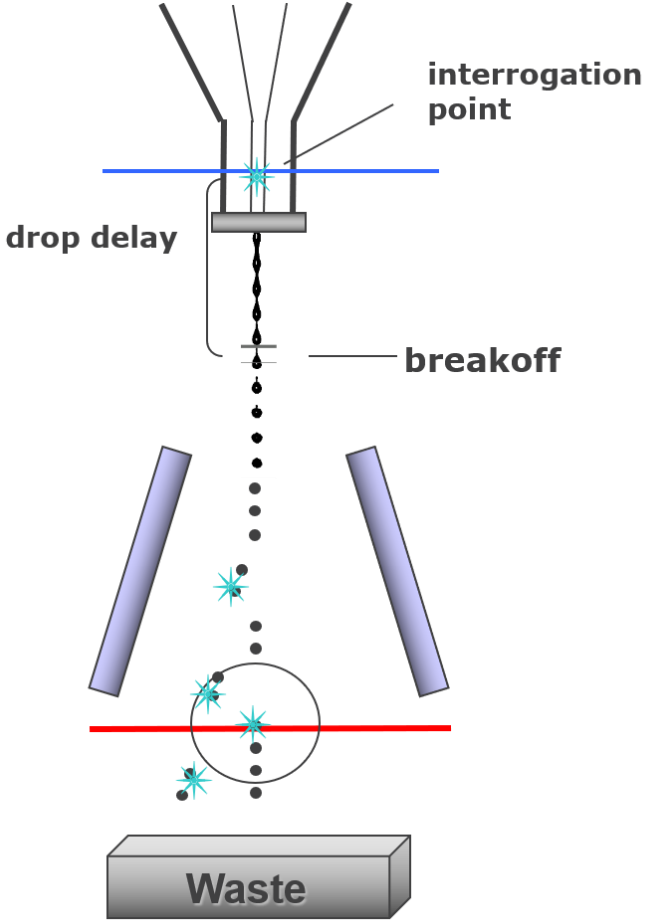


Drop Delay

BD FACS™ Accudrop technology

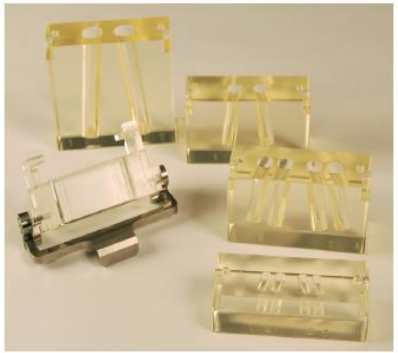
- Accudrop beads
- Diode laser
- Camera
- Optical filter





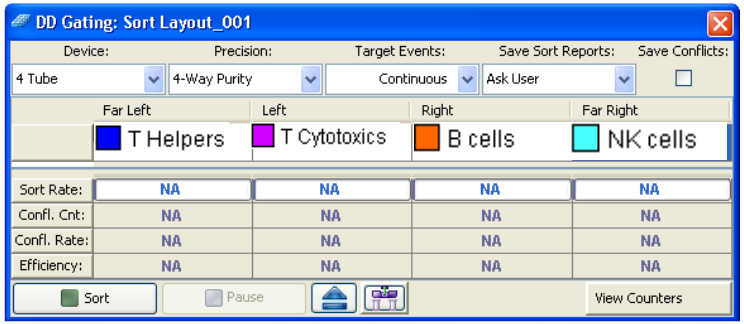
Sort Layout

- Devices
  - 2 or 4 way
  - Multiwell Plates
  - Slides
  - Custom Devices
- Precision mode
  - Yield
  - Purity
  - Single cells
  - 4-Way Purity
  - Custom



Tube holders

Plate loader on ACDU



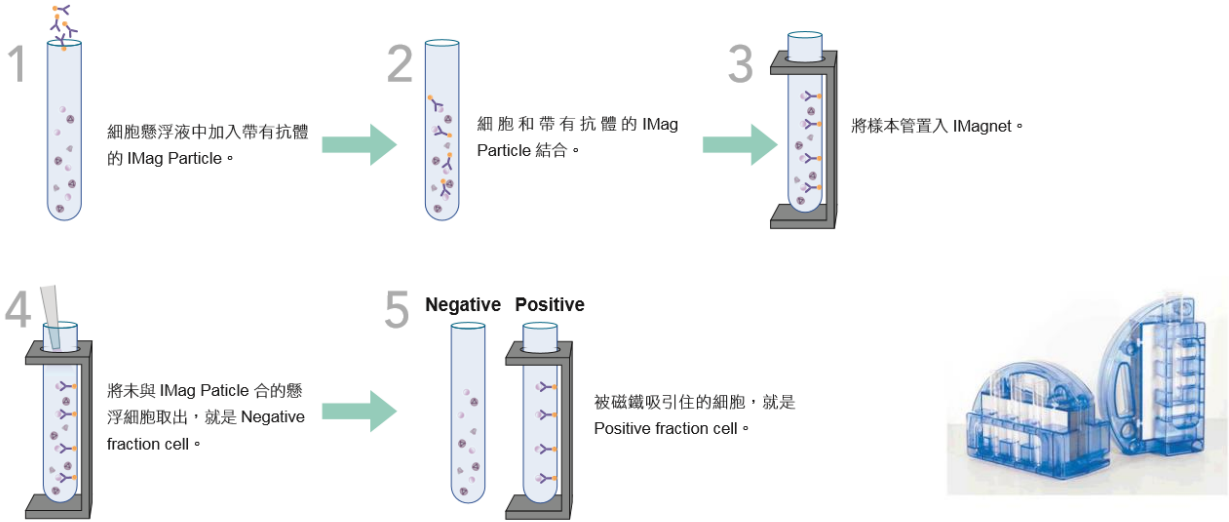
Sort mode

分選模式	功能
Yield	• 回收細胞數>純度的狀況下使用 • 具有目標細胞的液滴即會進行分選, 不考慮純度 • 適用於稀有細胞富集或想盡可能的不損失目標細胞時
Purity	• 純度>回收細胞數 • 可得到高純度的分選結果, 而犧牲部分目標細胞 • 分選速度越高, 分選效率越低, 目標細胞回收較少 • 適用於一般純度優先的分選, 建議準備1.5-2倍的起始理想細胞數量。
Single Cell	**適用於盤式分選**, 希望單一個well中僅分選一顆細胞且純度優先的時候選擇。

- Cell Purity:
  Purity mode=Single Cell mode>Yield mode

- Recovery cell count:
  Yield>Purity>Single Cell

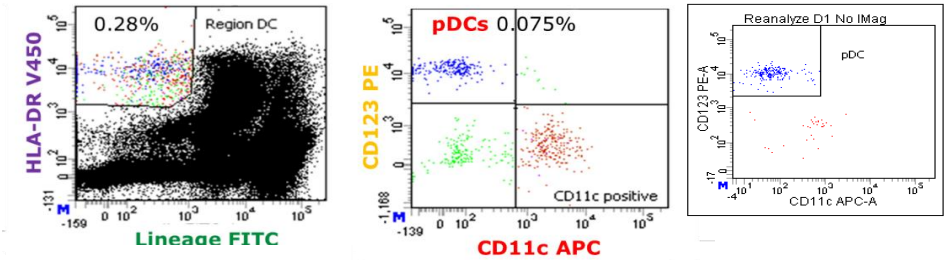
BD IMag 磁珠分選：
Positive or Negative selection or combination



- Positive selection:
  去除上清，將試管移出磁場，分析被磁珠捕獲的細胞，即為目標細胞
- Negative selection:
  分析上清，目標細胞在上清液中

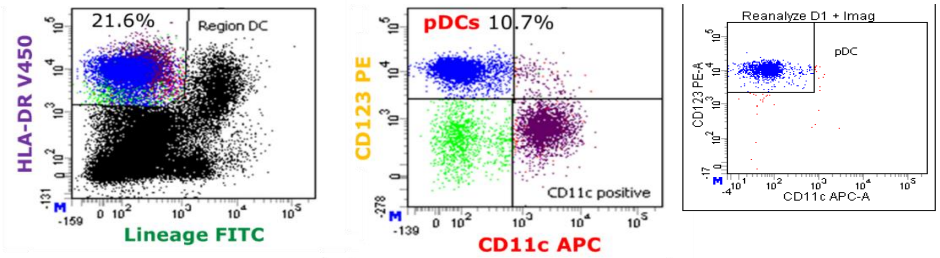
IMag increases purity and reduces time of sorting

w/o IMag enrichment



10^5 DCs
6.5 hours
74.4%

IMag enrichment



10^5 DCs
25 mins
95.3%

套裝試劑組 內含Biotinylated antibody cocktail 及 BD IMag ™ Streptavidin Particles

Human

Cat. No.	Name	Biotinylated Antibody Cocktai Contents	Size
558007	Human B Lymphocyte Enrichment Set - DM	CD3, CD41a, CD43, CD235a	1 x 10^9 cells
557939	Human CD4 T Lymphocyte Enrichment Set-DM	CD8, CD11b/Mac-1, CD16, CD19, CD36, CD41a, CD56, CD123, CD235a, γ δ TCR	1 x 10^9 cells
557987	Human NK Cell Enrichment Set - DM	CD3, CD19, CD36, CD41a, CD66b, CD123, CD235a, IgE	1 x 10^9 cells
557874	Human T Lymphocyte Enrichment Set - DM	CD11b/Mac-1, CD16, CD19,CD36, CD41a, CD56, CD235a	1 x 10^9 cells
557941	Human CD8 T Lymphocyte Enrichment Set - DM	CD4, CD11b/Mac-1, CD16, CD19, CD36, CD41a, CD56, CD123, CD235a, γ δ TCR	1 x 10^9 cells
558521	Human Naïve CD4+ T Cell Enrichment Set - DM	CD8, CD11b, CD16, CD19, CD36, CD41a, CD45RA, CD56, CD123,CD235a, γ δ TCR	1 x 10^9 cells
558569	Human Naïve CD8+ T Cell Enrichment Set - DM	CD4, CD11b, CD16, CD19, CD41a, CD45RO, CD235a, γ δ TCR	1 x 10^9 cells
560030	Human Lineage Cell Depletion Set - DM	CD3, CD14, CD16, CD19, CD41a, CD56, CD235a	1 x 10^9 cells
558520	Human Memory CD4+ T Cell Enrichment Set - DM	CD8, CD11b, CD16, CD19, CD36, CD41a, CD45RA, CD56, CD123, CD235a , γ δ TCR	1 x 10^9 cells
558420	Human Dendritic Cell Enrichment Set - DM	CD3, CD14, CD19, CD41a, CD56, CD66b, CD235a , IgE	1 x 10^9 cells
558454	Human Monocyte Enrichment Set - DM	CD3, CD45RA, CD19, CD56, CD235a, CD41a*	1 x 10^9 cells

＊貨號 558454 的 Human Monocyte Enrichment Set – DM 的 CD41 Bition 抗體是另外獨立小瓶包裝，請研究者依照需求適量使用。

套裝試劑組 內含Biotinylated antibody cocktail 及 BD IMag ™ Streptavidin Particles

Mouse

Cat. No.	Name	Biotinylated Antibody Cocktai Contents	Size
557955	Mouse Dendritic Cell Enrichment Set - DM	CD2, CD3e, CD45R/B220, CD49b, CD147, LY-6G and LY-6C, TER-119/Erythroid cells	1 x 10^9 cells
557954	Mouse NK Cell Enrichment Set - DM	CD4, CD5, CD8a, CD19, CD24, LY-6G and LY-6C, TER-119/Erythroid cells	1 x 10^9 cells
557793	Mouse T Lymphocyte Enrichment Set - DM	CD11b, CD45R/B220, CD49b, TER-119/Erythroid cells	1 x 10^9 cells
557792	Mouse B Lymphocyte Enrichment Set - DM	CD4, CD43, TER-119/Erythroid cells	1 x 10^9 cells
558471	Mouse CD8 T Lymphocyte Enrichment Set - DM	CD4, CD11b, CD45R/B220, CD49b, TER-119/Erythroid cells	1 x 10^9 cells
558131	Mouse CD4 T Lymphocyte Enrichment Set - DM	CD8a, CD11b, CD45R/B220, CD49b, TER-119/Erythroid cells	1 x 10^9 cells
558451	Mouse Hematopoietic Progenitor (Stem) Cell Enrichment Set - DM	CD3e, CD11b, CD45R/B220, LY-6G and LY-6C, TER-119/Erythroid cells	2 x 10^9 cells

成功的分選所需條件

- **了解自己的細胞樣品資訊**

-細胞大小
-貼附或懸浮培養型
-脆弱或生命強韌
-分選前存活率
-是否容易結成團

- **事先溝通實驗流程**

-樣品處理的方式-培養條件-測試組別
-樣品的染色方式(表現螢光或者使用抗體染劑)與條件
-以死活染劑區別分選前已死亡細胞
-樣品液準備
-收集液準備

- **正確的儀器設定與最適化分選條件**

-依照細胞大小與脆弱程度選擇噴嘴
-依照期望的回收細胞數調整細胞濃度與上機速度
-開啟溫控裝置維持細胞生存條件
-回測分選後細胞追蹤成果以為下次實驗改進

實驗樣品準備建議

- Enrich rare cell population if possible
- Avoid cell clumps
  - Always filter your cells before sort!
  - Use Accutase instead of Trypsin
  - Treat cells with DNAse
- Use appropriate sample buffer
  - PBS, HBSS or phenol-red free culture media w/ 25mM HEPES, 5mM EDTA and 1~2% FBS or 0.1~0.2% BSA to maintain cell viability
- Use viability dye to confirm cell viability before sort

實驗樣品準備建議

1.上樣細胞液製備

使用 Falcon, 5mL Tubes with **40 µm filter top cap** P/N: 352235 過濾細胞
細胞濃度調整在$1x10^6$-$2x10^7$/ml 為佳
建議實驗設計時保留 PI or 7-AAD 偵測器作為死活染色分辨死細胞使用

2.收集液製備(收集管)

750 µL - 1 mL for 5 mL tubes
置入高濃度20-50% FBS 培養用medium, 含1X-2X抗生素
分選後細胞需離心後回溶到正常培養濃度medium
(若為單細胞盤式分選可以等細胞貼附在盤底後再抽換medium)

記得多帶兩~三支額外的收集管，如果操作錯誤可替換
對部分較脆弱的細胞類型，可為分選細胞準備1~2 ml小牛血清當底墊 (小牛血清記得請先用0.22um過濾後，再加入收集管內)

Cat. No.	Name	Size
561527	Accutase™ Cell Detachment Solution	100 ml

BD FACS™ Pre-Sort Buffer

- 幫助製備和維持從淋巴組織，骨髓，外周血，培養細胞（包括多能幹細胞）或其他來源製備的單細胞懸液。
- 不含酚紅，是一種透明的緩衝液，有助於最小化背景。
- 減少細胞結塊，從而有助於在分選儀上形成一致的液滴。
- 鈣和鎂含量極低，可最大程度地減少細胞聚集。
- 含FBS
- 不含EDTA
- 適合即將分選及分析的細胞之懸浮，洗滌和儲存。
- 每批 BD FACS Pre-Sort Buffer 都經過內毒素測試，低於 0.96 EU/mL
- 提供理想的 pH 調節，以在細胞製備和分選過程中保持細胞健康

Cat. No.	Name	Size
563503	BD FACS™ Pre-Sort Buffer	250 ml

分選樣品收集管注意事項

- 細胞收集管可以事先加以coating, 提高細胞存活率 (1% BSA或 10% FBS, 4℃, overnight) 。
- 置入高濃度20-50% FBS 培養用medium, 含1X-2X抗生素
  - 使用Polypropylene取代Polystyrene管
- 分選後細胞需離心後回溶到正常培養濃度medium
  (若為單細胞盤式分選可以等細胞貼附在盤底後再抽換medium)
- 分選過程中, 定期更換收集管
- 設定樣品收集區溫度控制, 以提供最佳細胞存活條件
- 樣品流速: 低流速 to minimize CV
- 單一clone細胞之培養較mixed population不易, 主要是適合細胞生長所需環境與營養因子太複雜. 以傳統培養hybridoma clones來說, 通常是需要加入feeder cells或是conditioned media. 可試著使用conditioned media加入culture media(5%, 10%或20%)中來加強培養環境.

Thank you!