

EVERY
event matters.



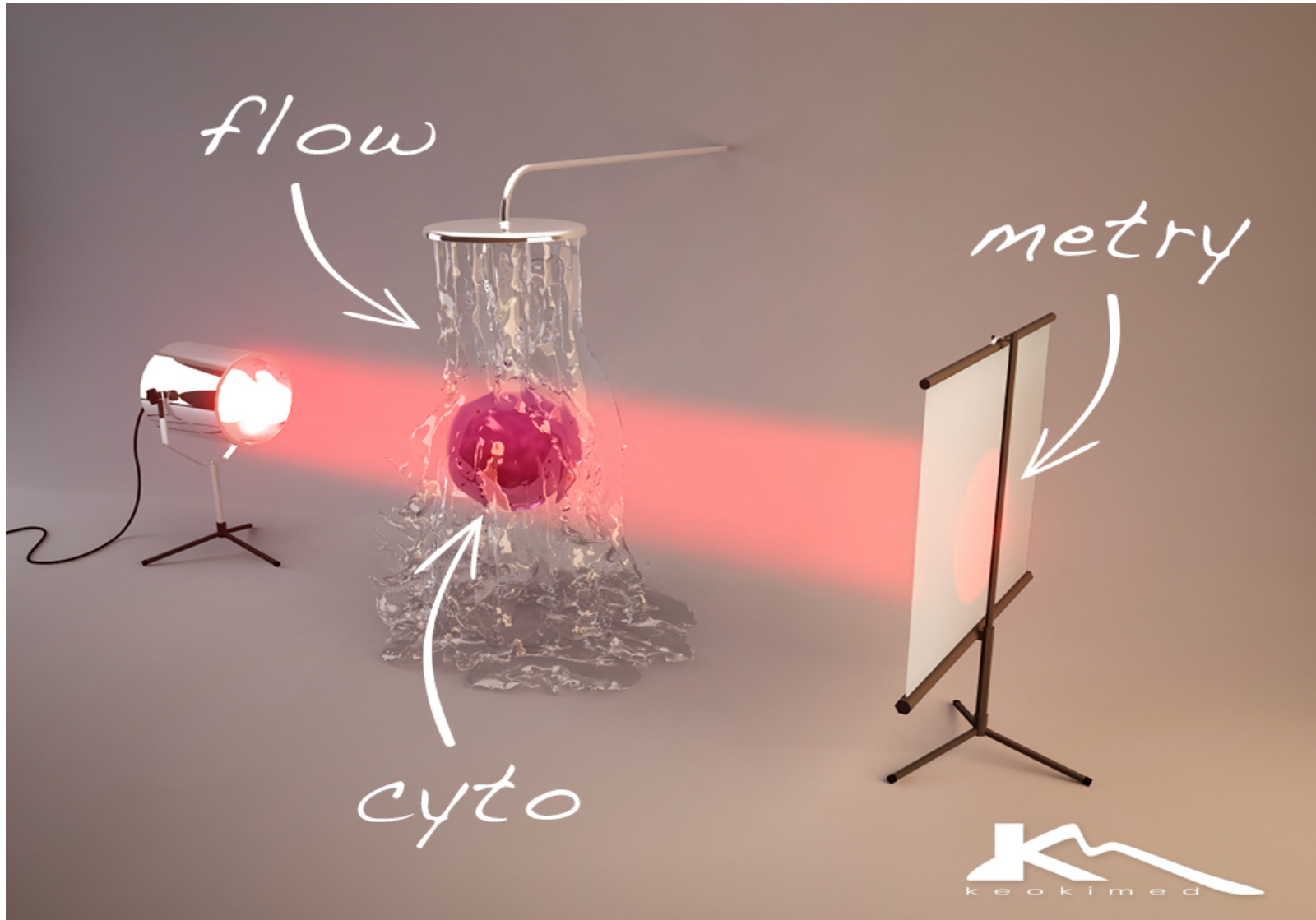
Flow cytometer Principles and Concepts

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Flow Cytometer



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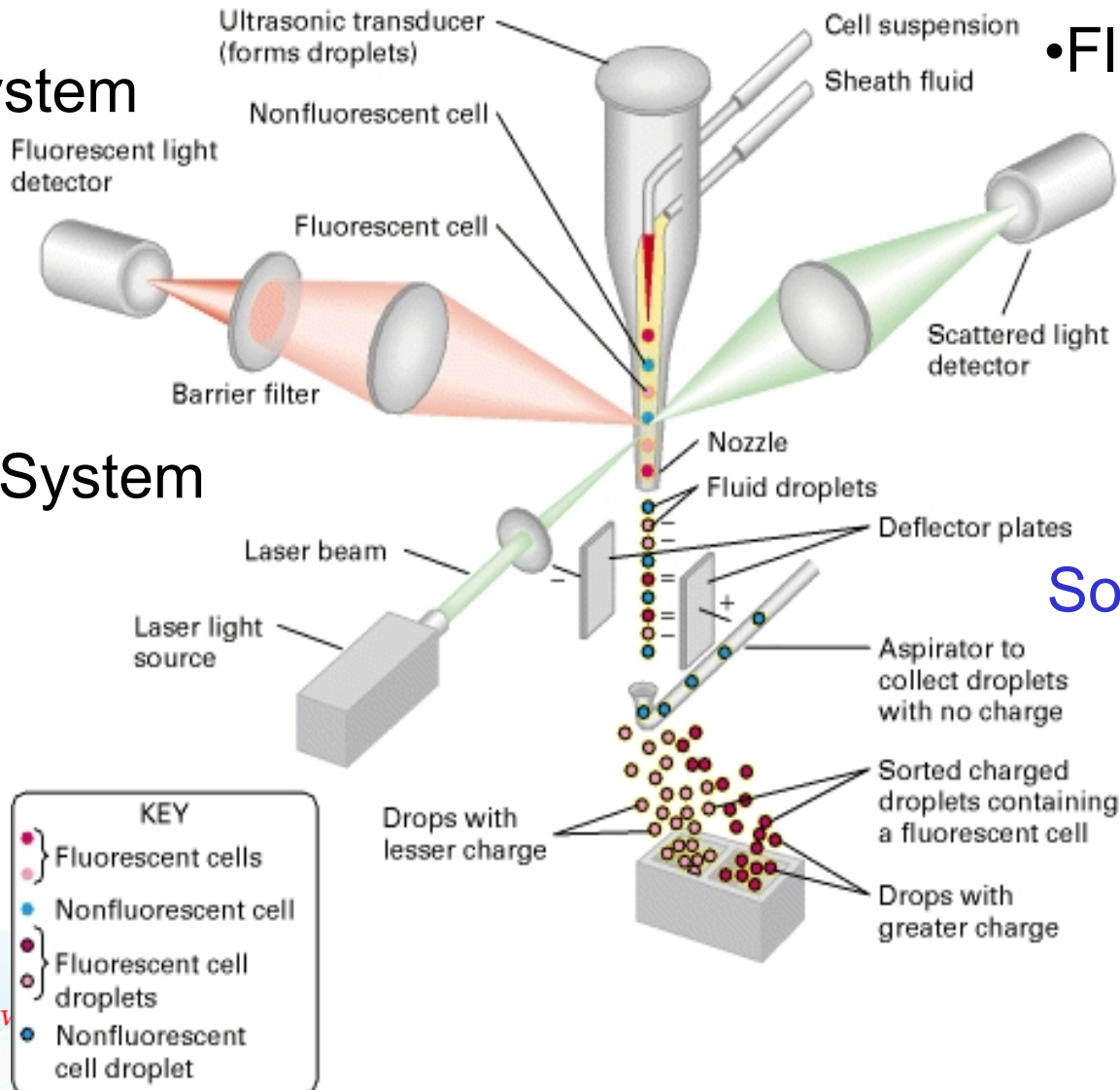
Flow Cytometry Is Made Of

• Fluid System

Electronic System

• Optical System

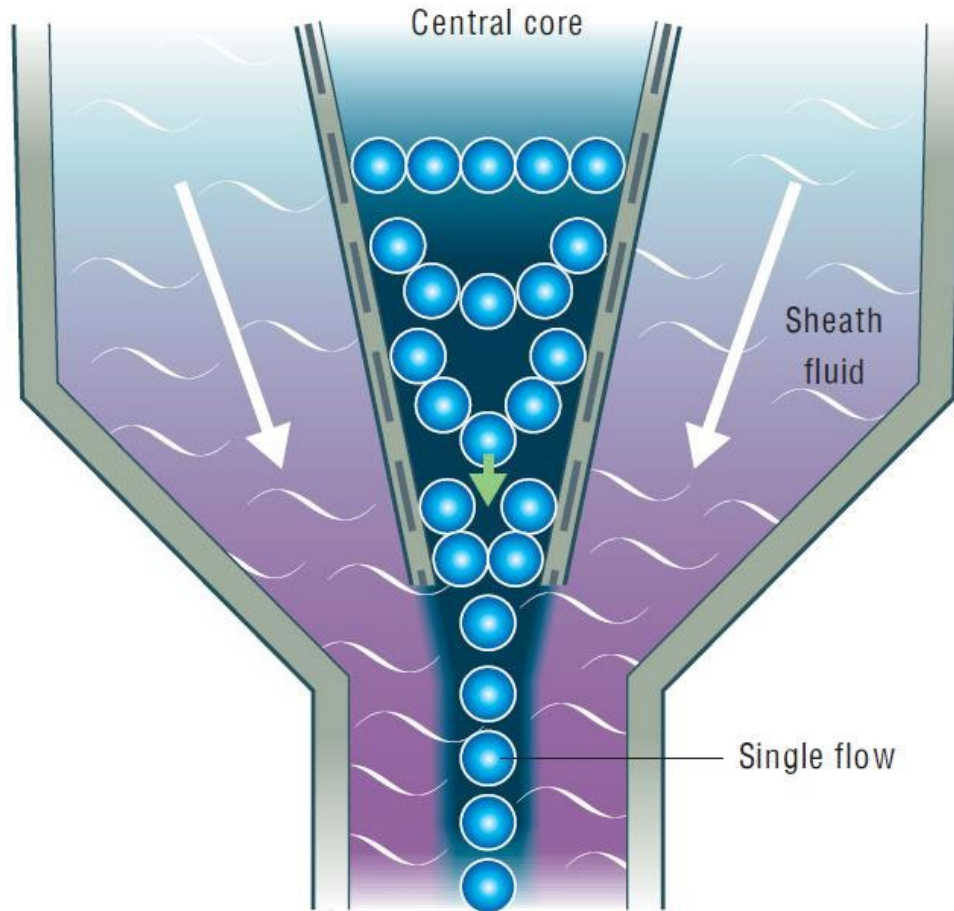
Sorting System



Flow Cytometry Is Made Of

- **Fluid System**
- Optical System
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- Computer System
- **Sorting System**

1. Fluid System: **Hydrodynamic Focusing** (流體動力聚焦)



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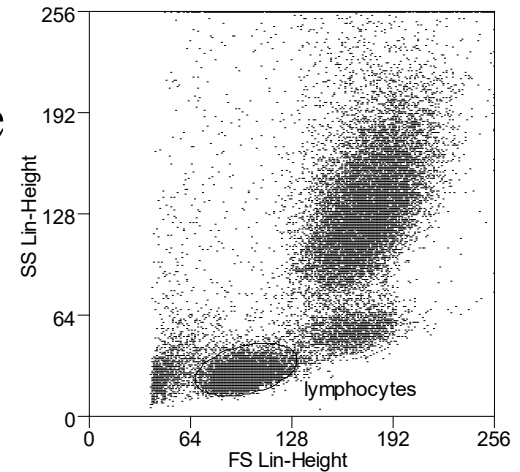
What Flow Cytometry Give You

- Light Scatter for cell morphology
 - Forward Angle Light Scatter (FS) for cell size
 - Side Scatter (SS) for cell complexity

- Fluorescences (FL) for specific labeling

- FL 1 : (530/40 nm BP) for FITC
- FL 2 : (580/30 nm BP) for PE
- FL 3 : (613/20 nm BP) for PE-*TexRed*
- FL 4 : (670/30 nm BP) for PE-Cy5
- FL 5 : (457/50 nm BP) for DAPI or Hoechst blue
- FL 6 : (628/32 nm BP) for Hoechst Red

FL 7 : (670/30nm BP) for APC



488nm Laser

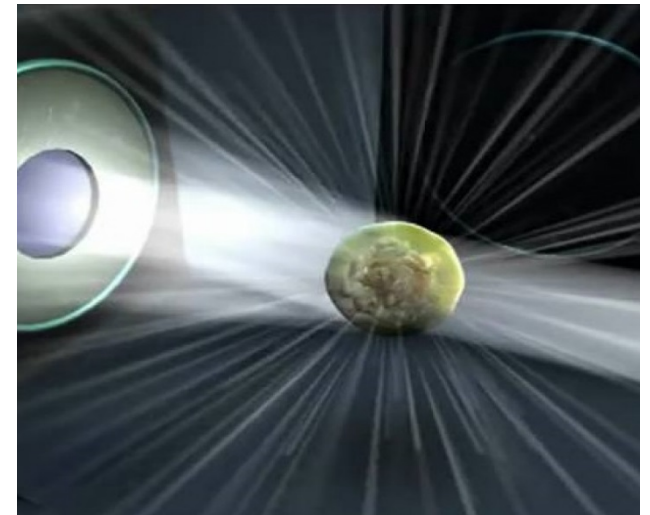
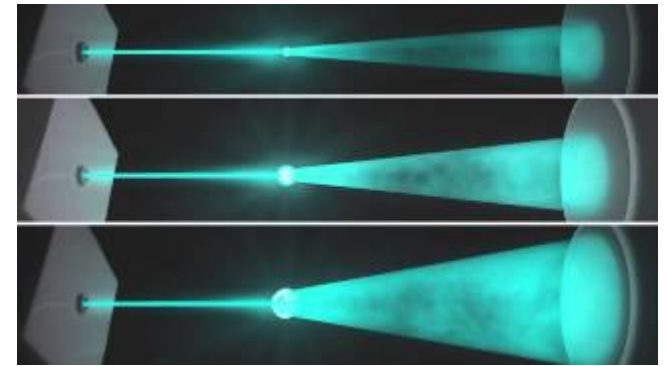
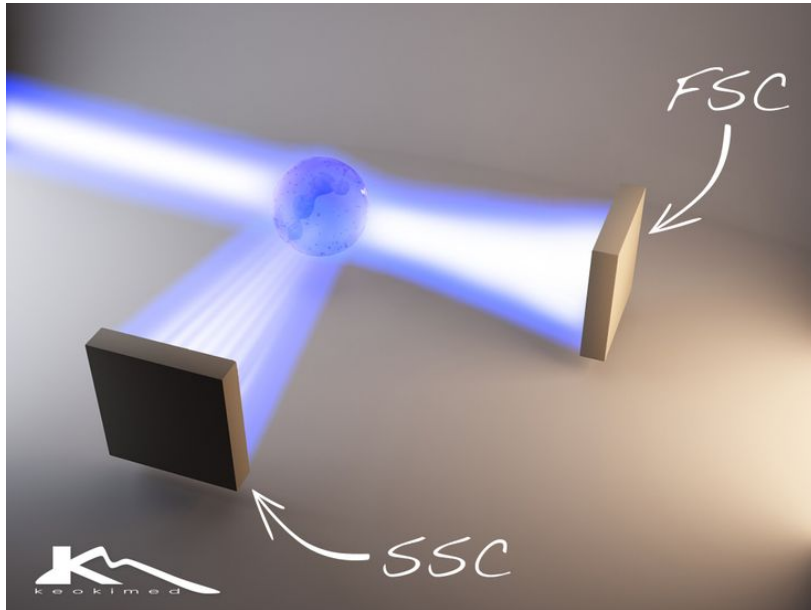
UVnm Laser

633nm Laser

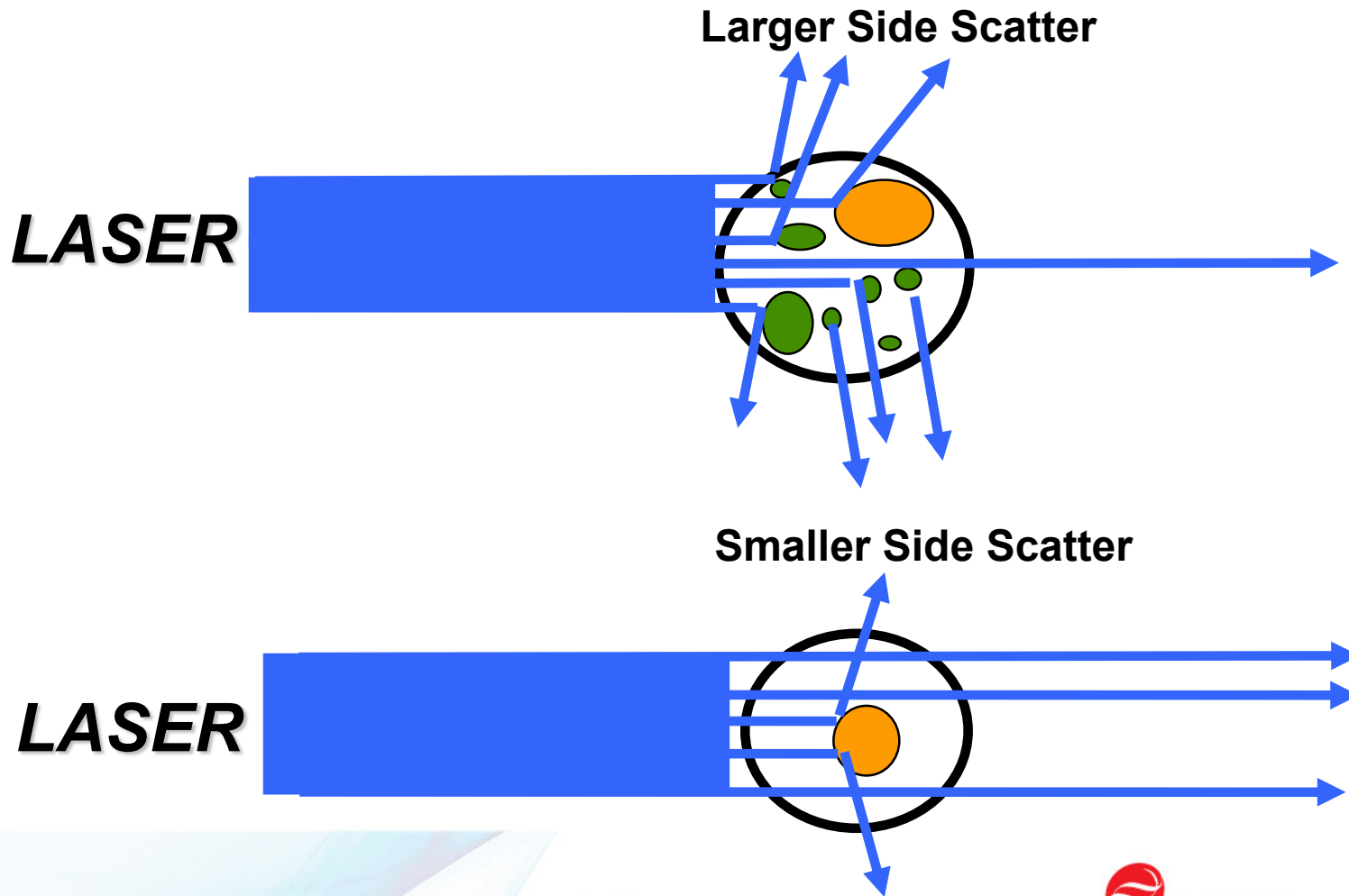
MoFlo XDP

	FL1, 529+28nm	FL2, 575+25nm	FL3, 620+29nm	FL4, 670+30nm	FL5 457+50nm	FL6 628+32nm	F7 670+30nm
488nm Blue Laser	FITC, Alexa Flour488	PE	PE-Texas Red, PerCP	PE-Cy5, PE-Cy5.5 PerCP-Cy5.5			
	GFP	YFP	PI (DNA)	7-AAD (DNA)			
633 nm Red Laser					APC APC- eFluor® 647 Cy5		
355nm Violet Laser						Hoechst Blue DAPI	Hoechst Red

Forward Scatter (FS) vs. Side Scatter (SS)



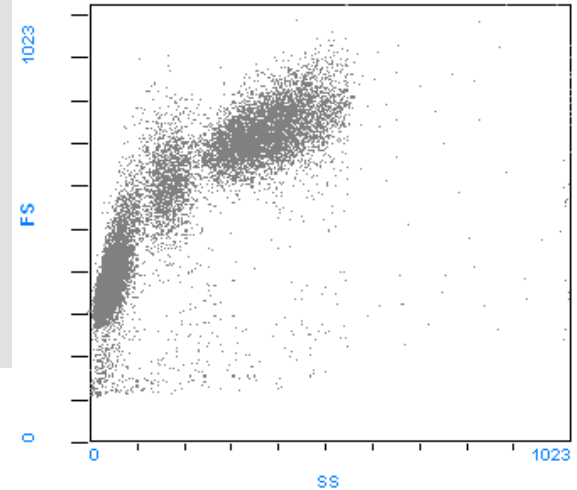
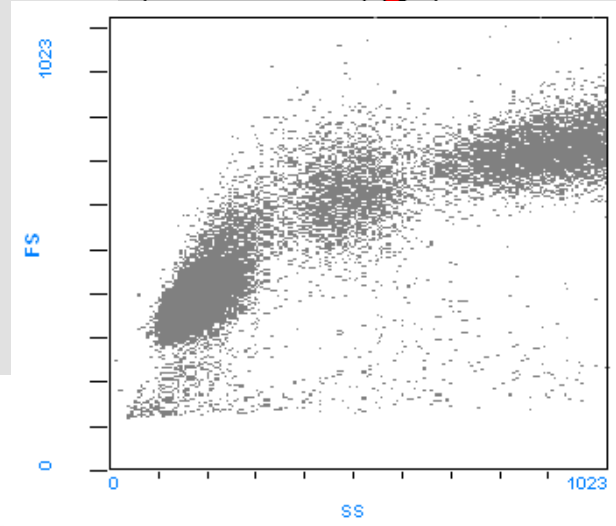
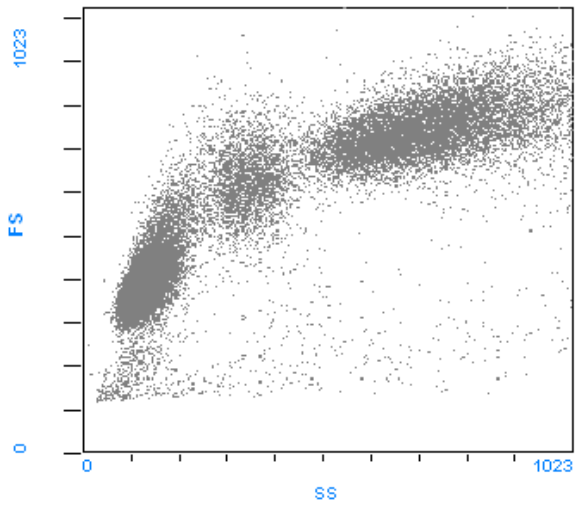
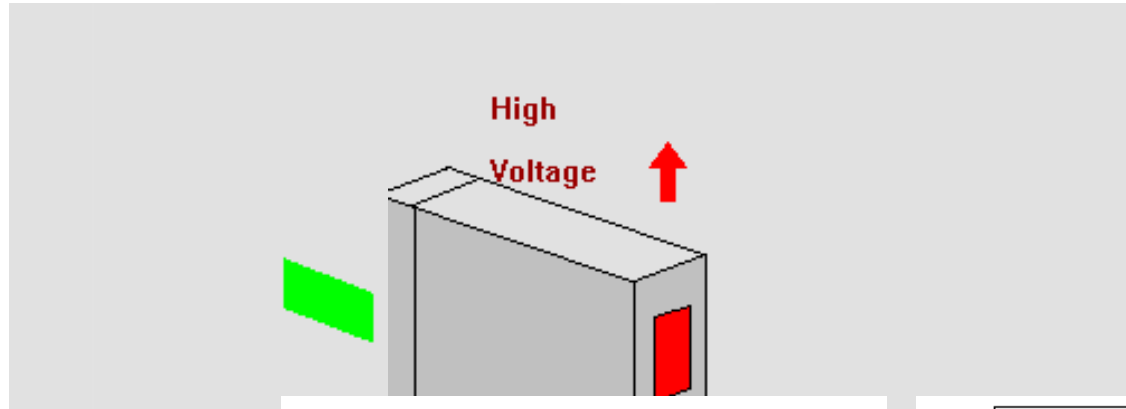
Light Scatter : How Granular is that Cell?



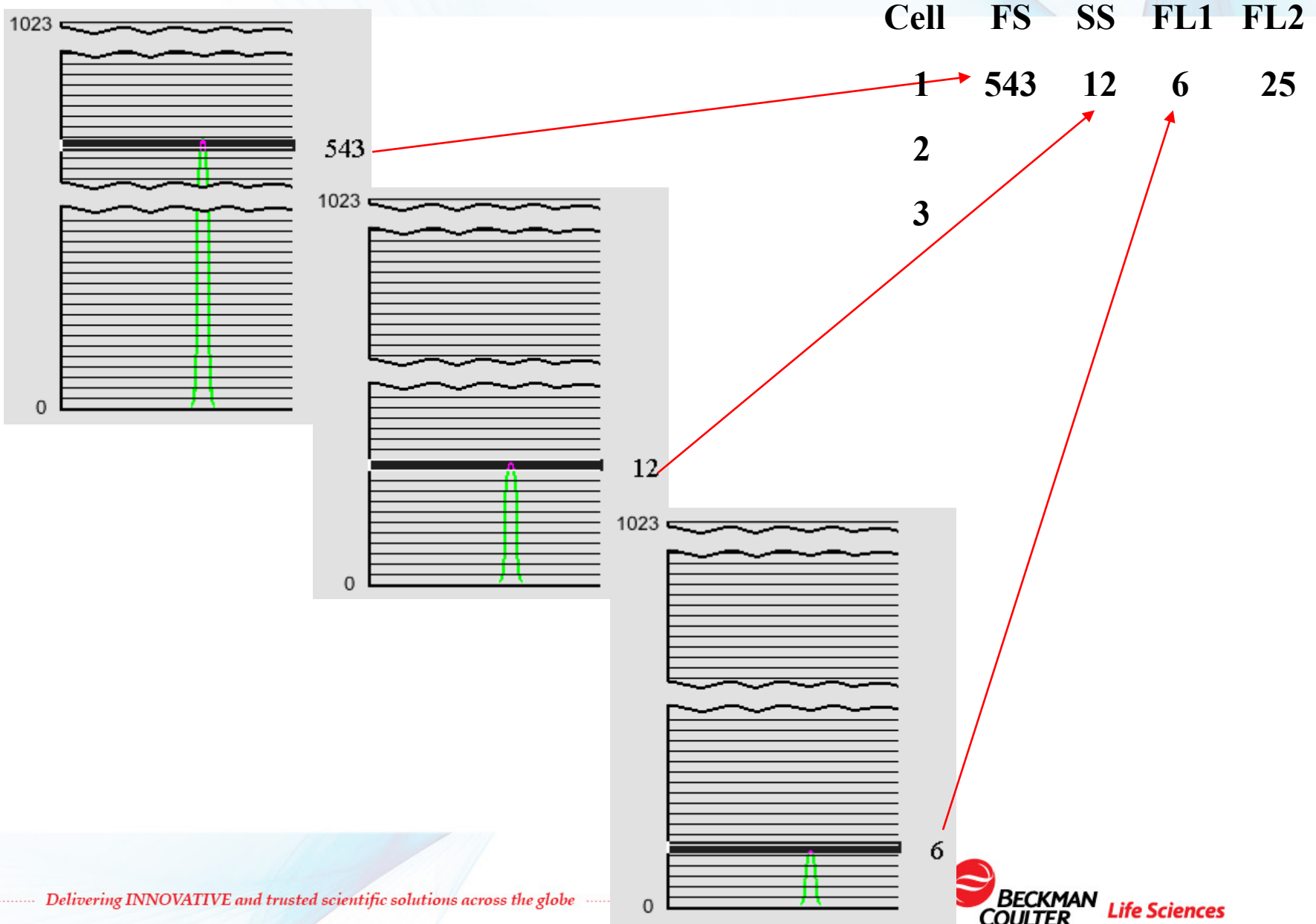
Flow Cytometry Is Made Of

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Photo-electric Multiplier Tube, 光電倍增管



Event: Analog to Digital

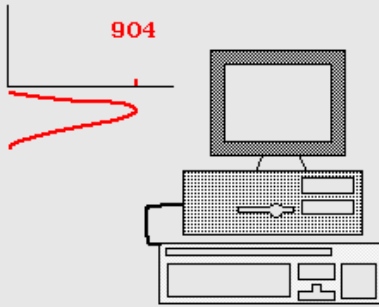


System: Linear Scale Convert to Log Scale

If there is a wide range of pulse heights, the operator may elect to have the channels converted to a log scale. The computer software converts and stores the data.

Channels

904

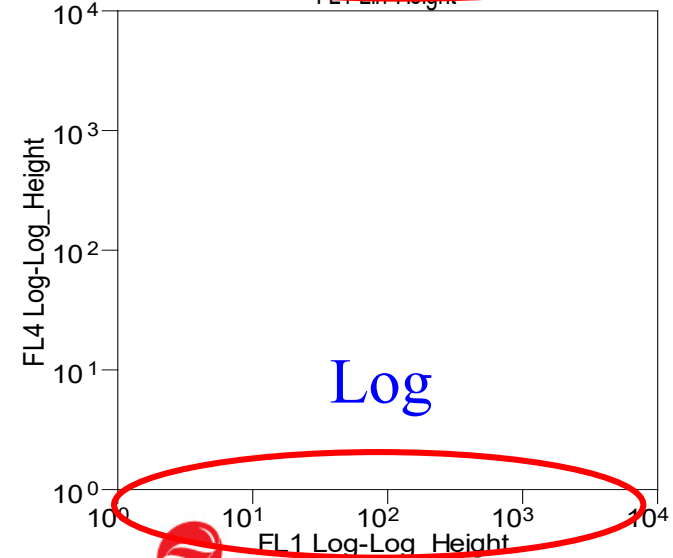
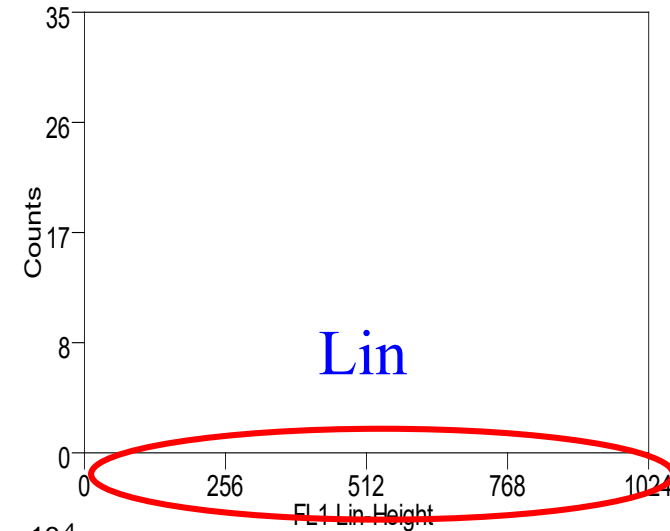


Computer

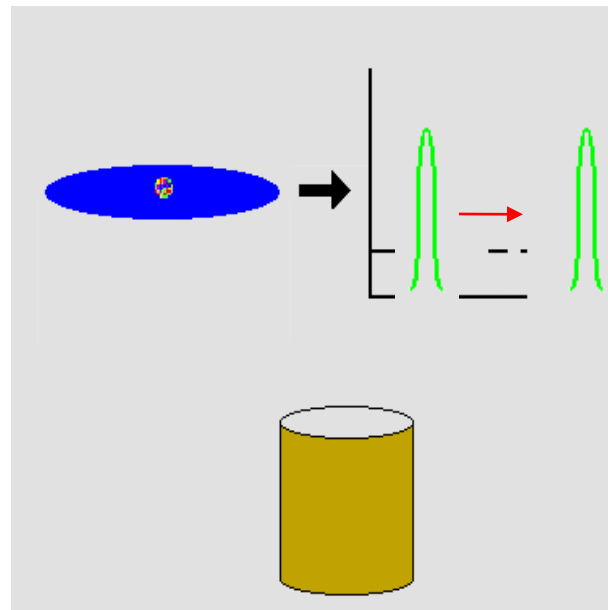
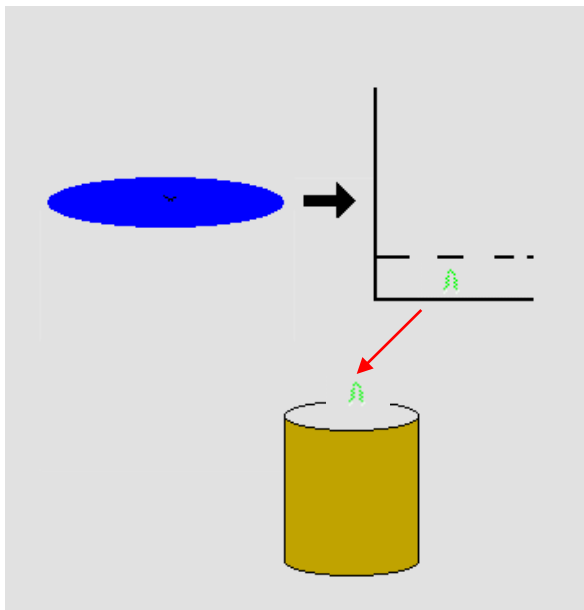
0 -> .102	100 -> .252	900 -> 335.7
1 -> .103	101 -> .254	901 -> 338.7
2 -> .105	102 -> .256	902 -> 341.8
3 -> .106	103 -> .259	903 -> 344.9
4 -> .107	104 -> .261	904 -> 348.0
5 -> .108	105 -> .263	905 -> 351.1
6 -> .109	106 -> .266	906 -> 354.3
7 -> .110	107 -> .268	907 -> 357.5
8 -> .111	108 -> .271	908 -> 360.7
9 -> .112	109 -> .273	909 -> 364.0

Log conversion chart

Output



System: Discrimination or Threshold (排除雜訊)

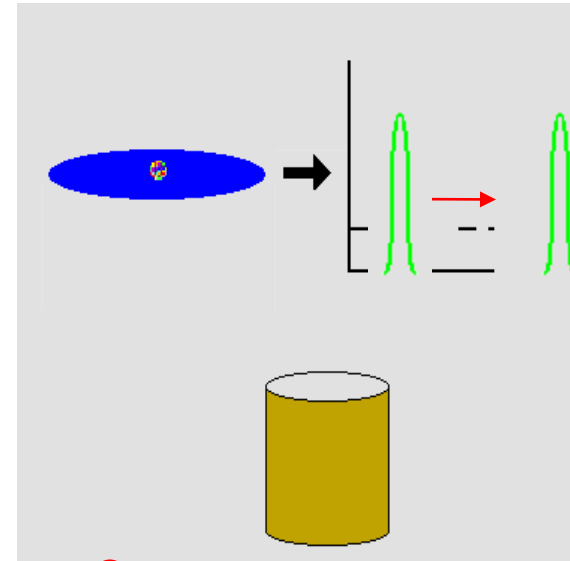
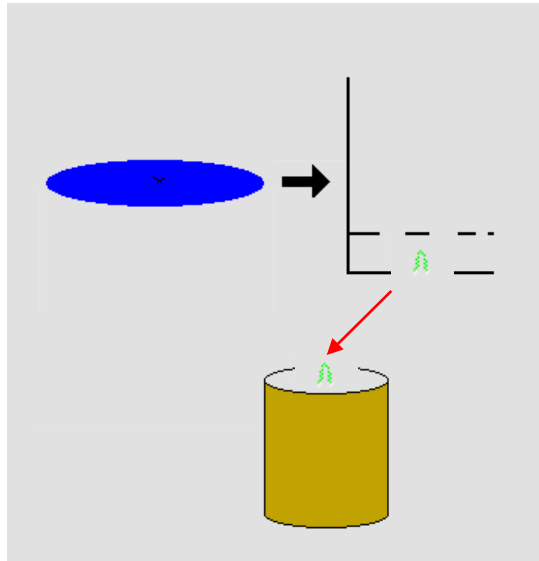


Example:

Discriminator: FS=5%

Cell	FS	SS	FL1	FL2		
1	101	50	125	200	→	OK
2	80	30	700	90	→	OK
3	20	70	33	234	→	×
4	420	26	77	894	→	OK

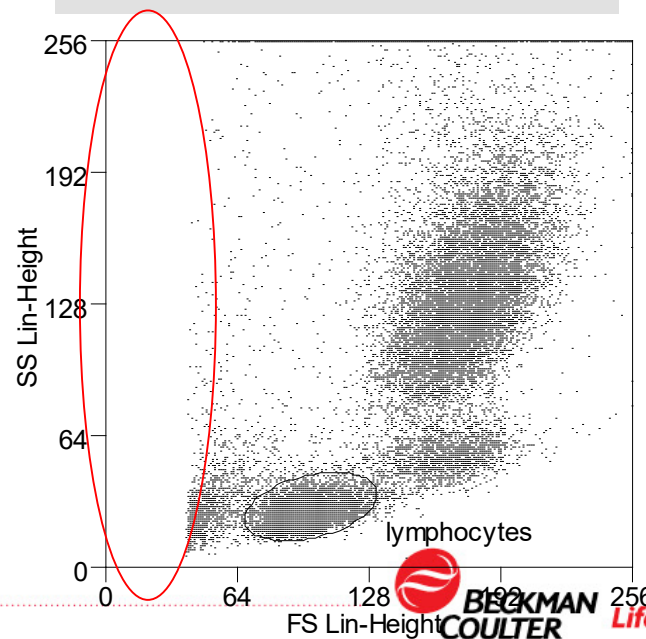
System: Discrimination or Threshold (排除雜訊)



Example:

Discriminator: FS=5%

Cell	FS	SS	FL1	FL2	
1	101	50	125	200	→ OK
2	80	30	700	90	→ OK
3	20	70	33	234	→ ✗
4	420	26	77	894	→ OK



Flow Cytometry Is Made Of

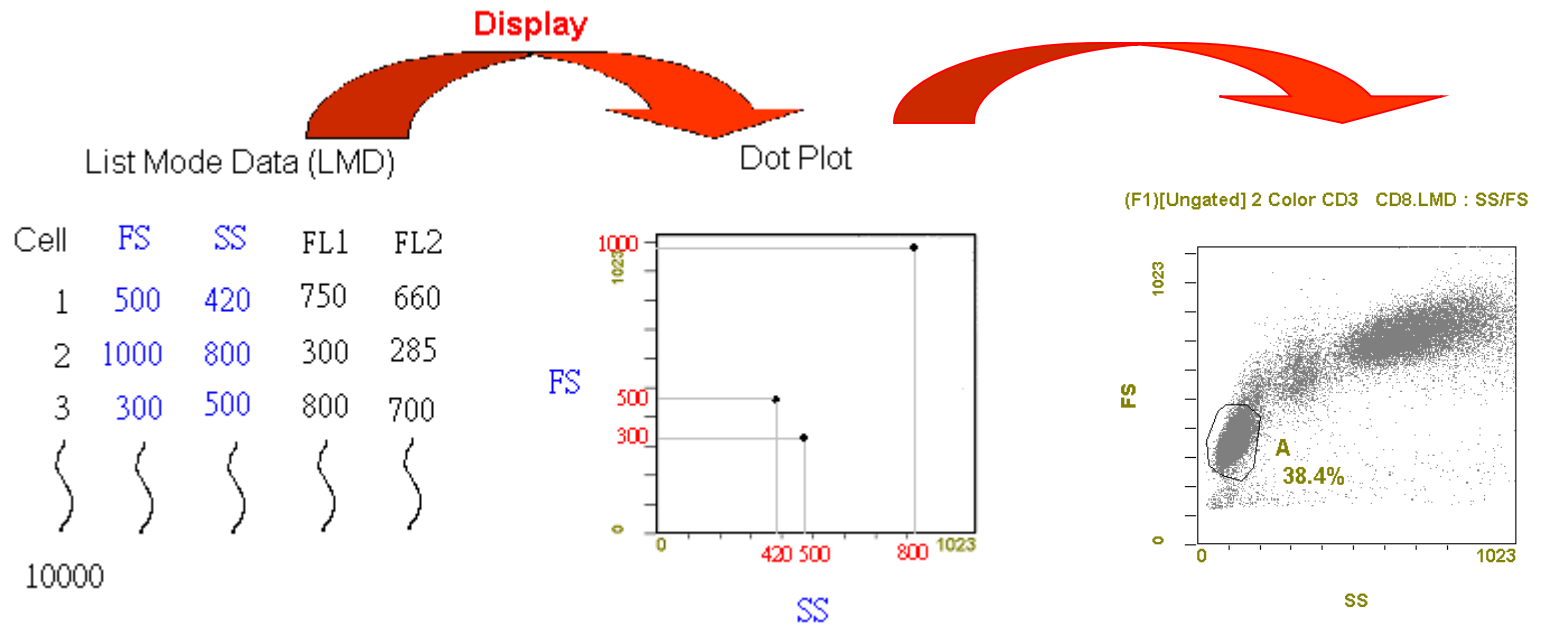
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Flow Raw Data: List Mode Data

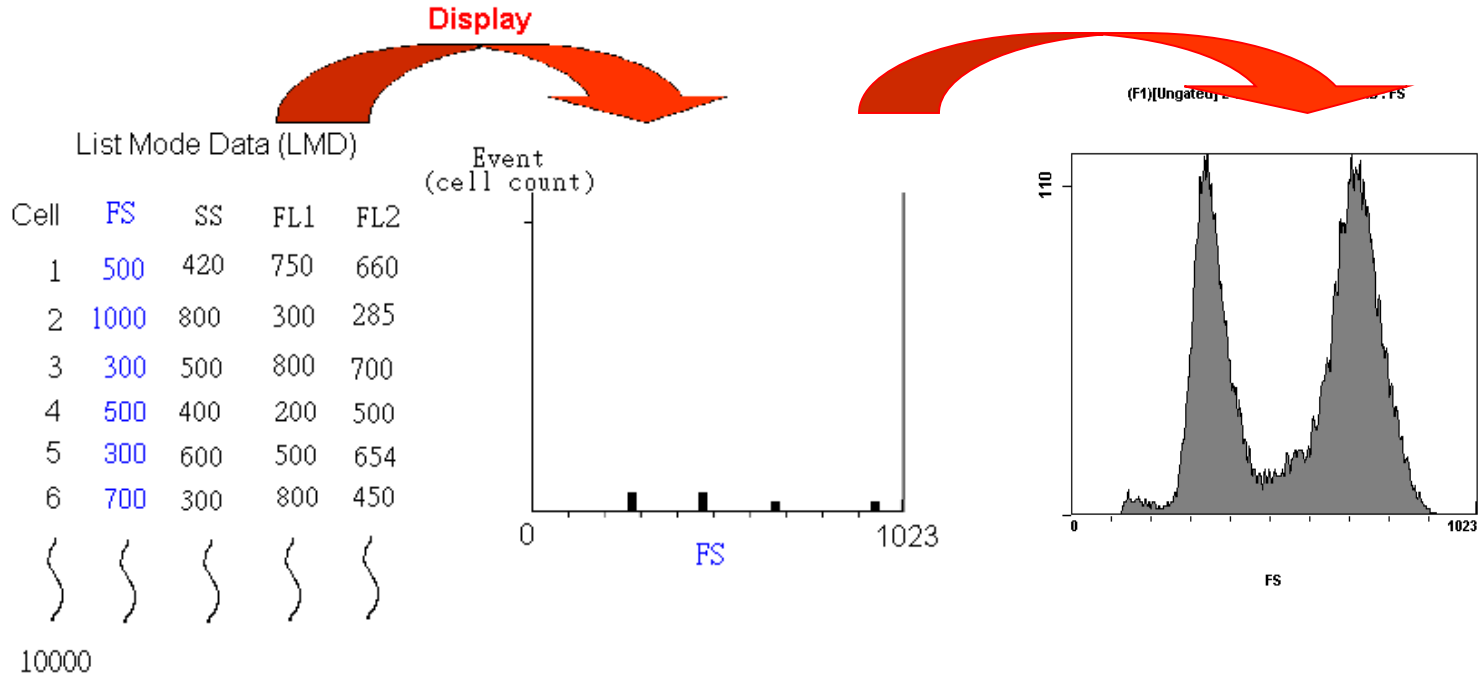
List Mode Data (LMD)

Cell	FS	SS	FL1	FL2
1	500	420	750	660
2	1000	800	300	285
3	300	500	800	700
⋮	⋮	⋮	⋮	⋮
10000				

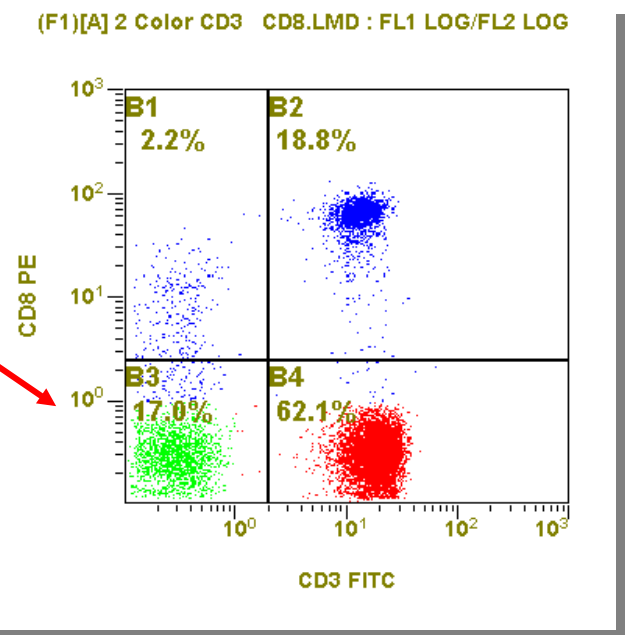
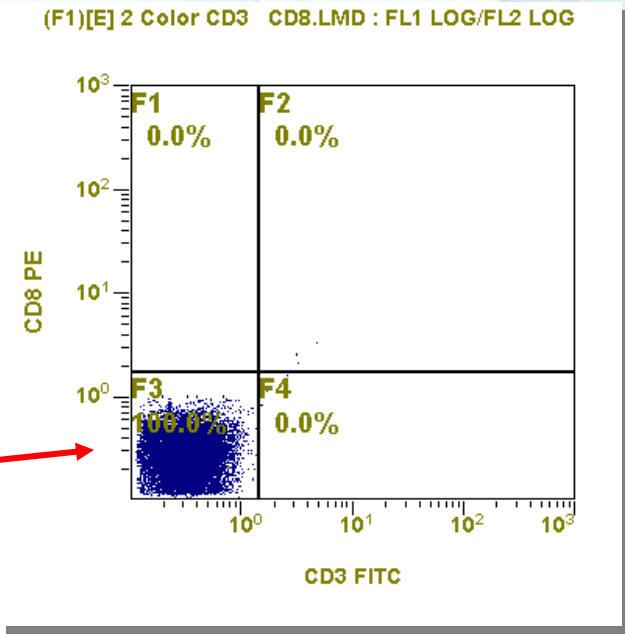
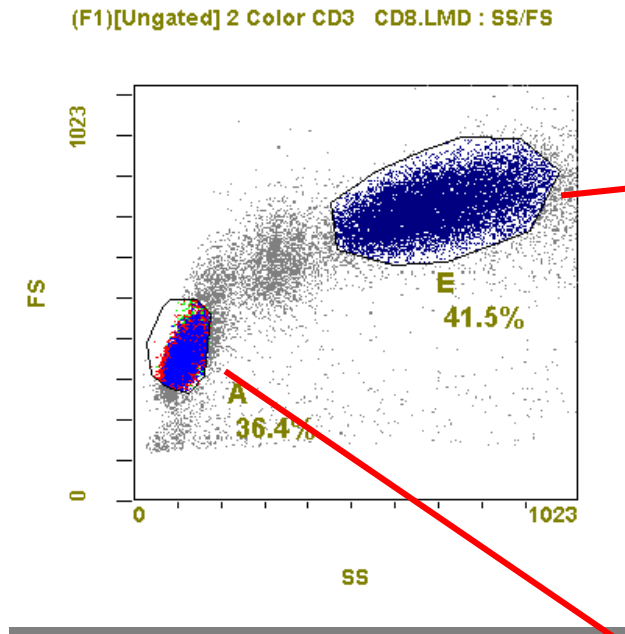
• Computer System: Data Display



- **Computer System: Data Display**



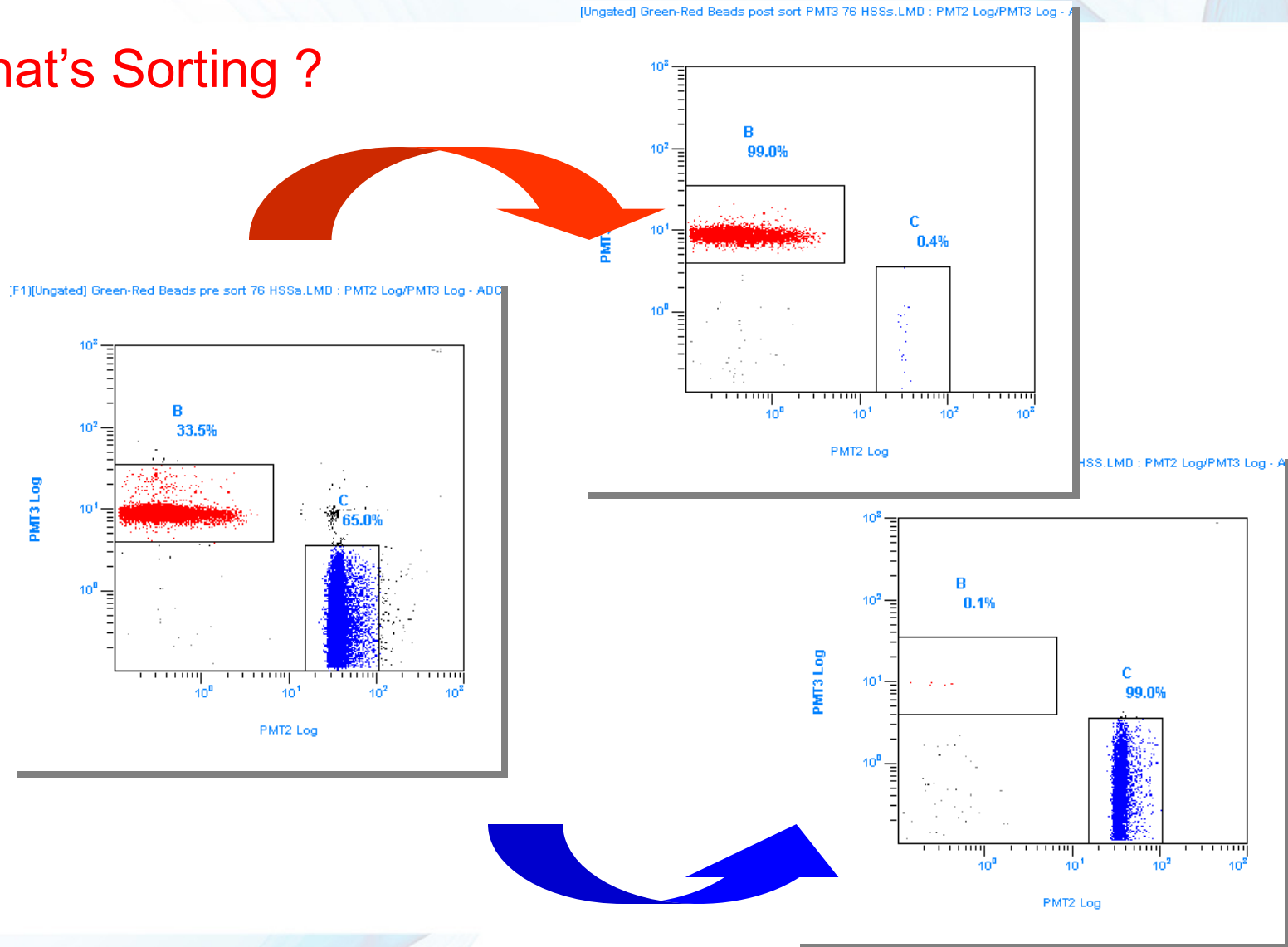
- Computer System: Gate & Analysis

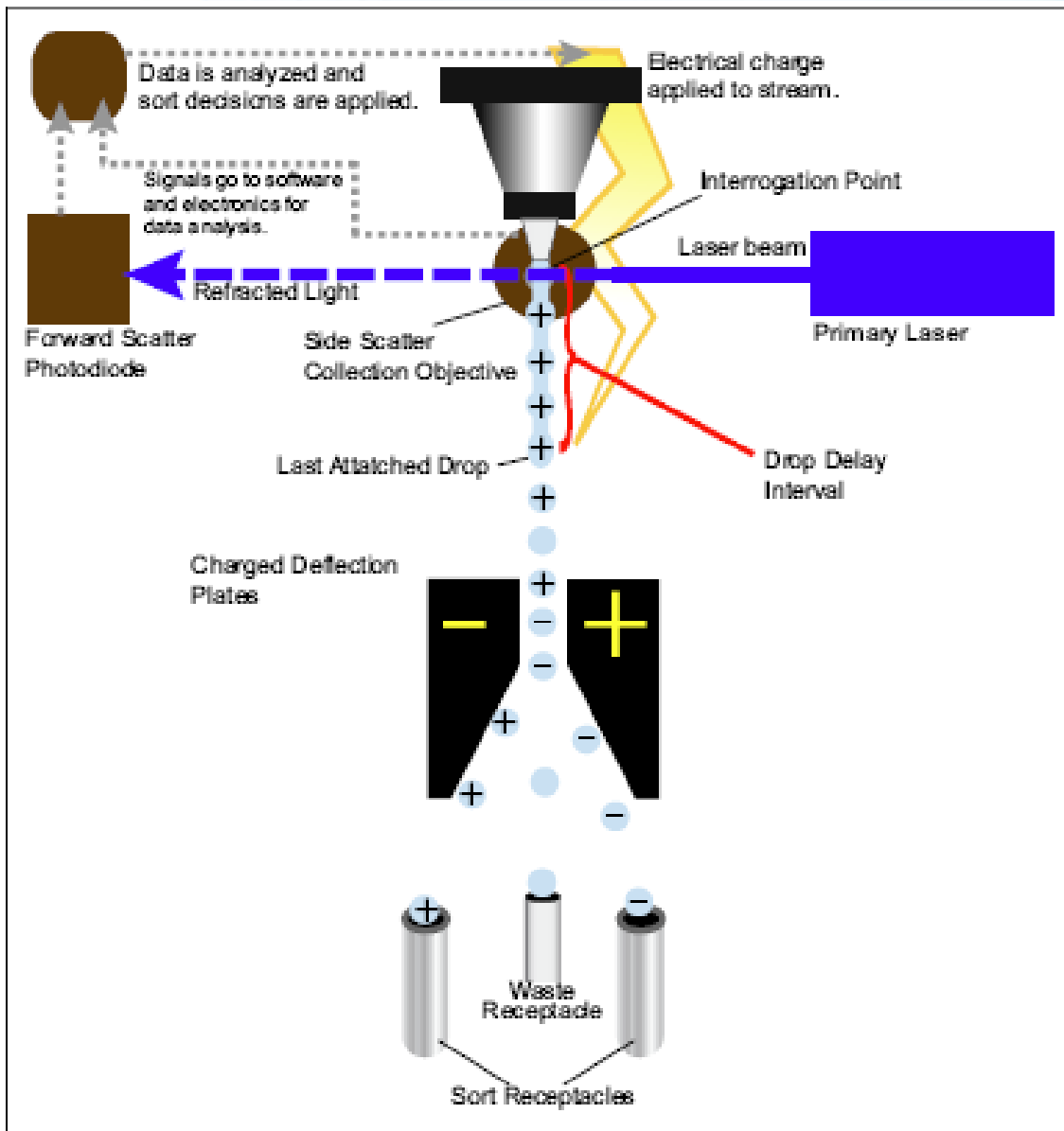


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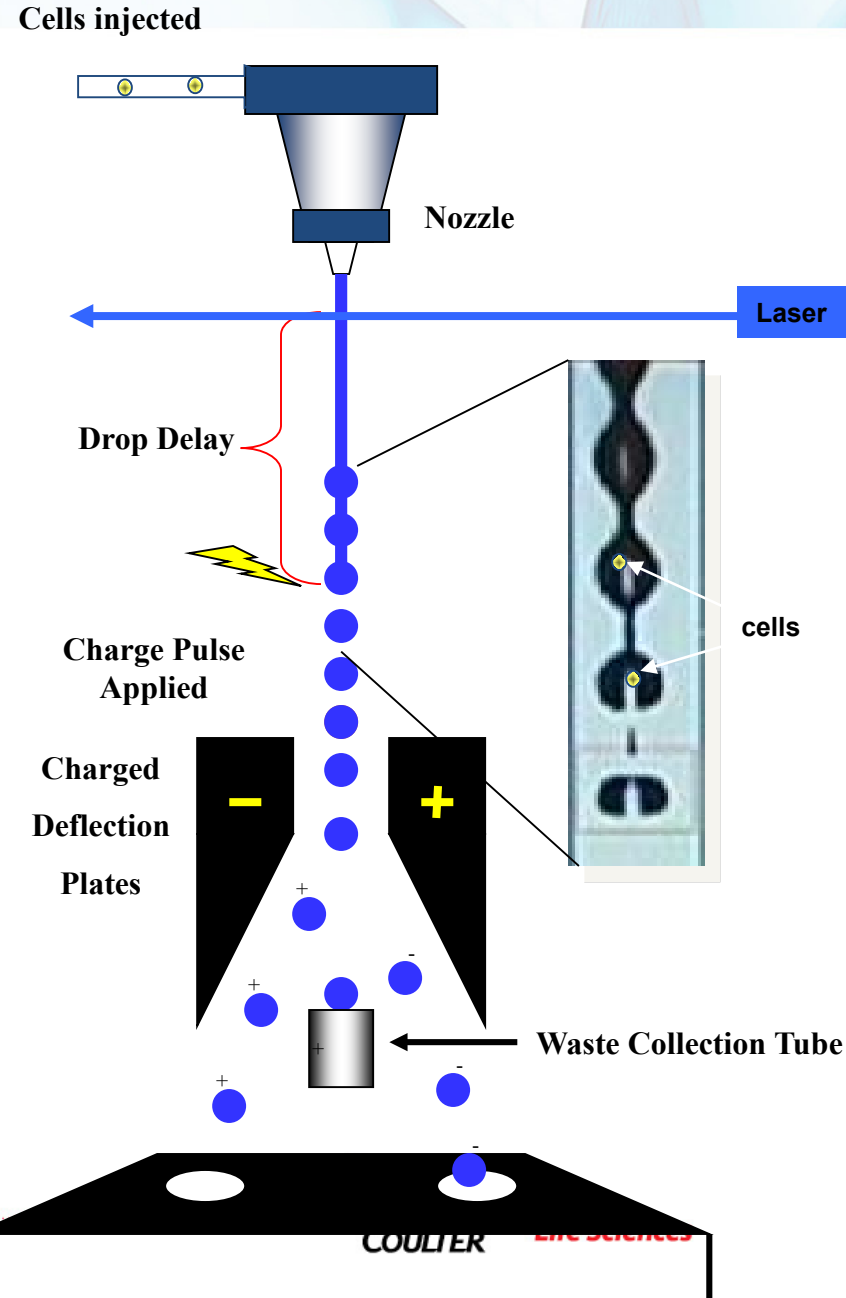
What's Sorting ?



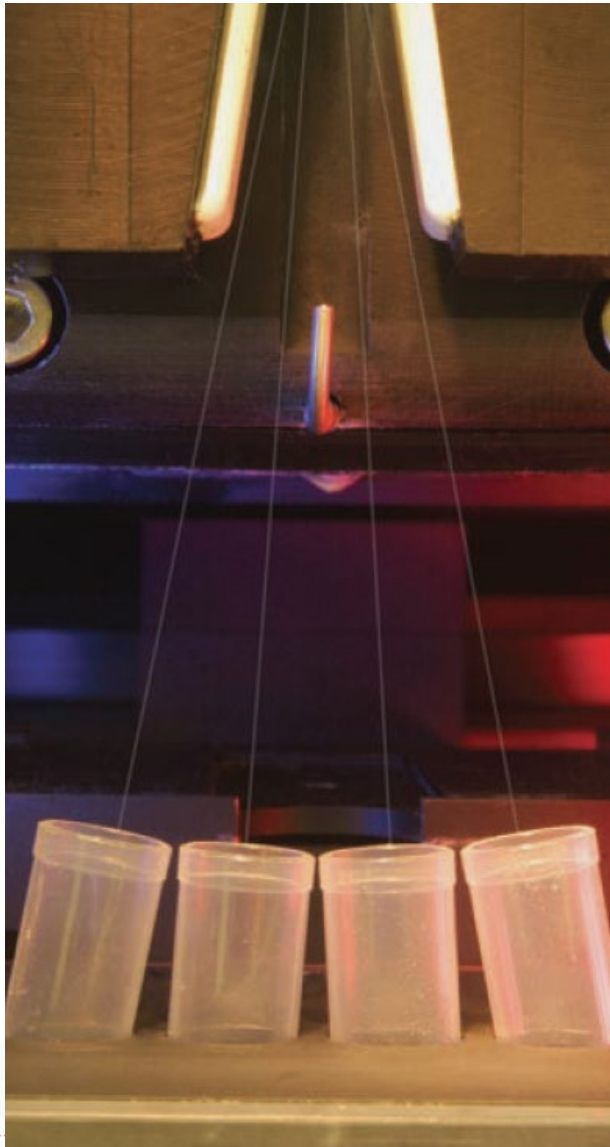


MoFlo XDP's sorting system

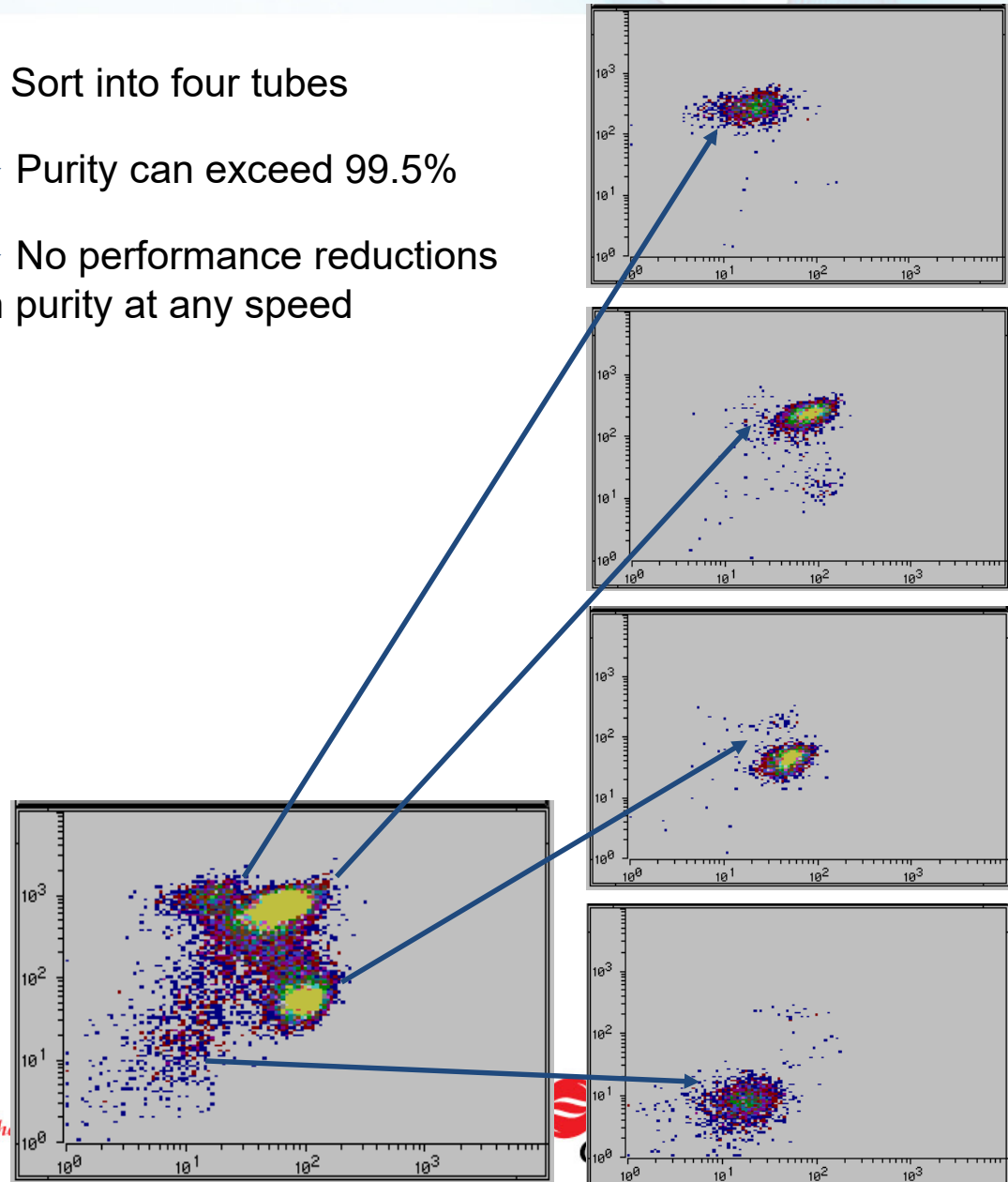
- **Drop formation frequency: 200 kHz**
 - 每秒可震出 200,000 顆水珠
 - 為維持分選純度，平均每 3 顆水滴含有 1 顆細胞為最佳狀態
- **Sort speed up to 70,000 eps**
 - Purity > 99% ; Yield > 90%
- **IntelliSort: 自動化協助設定分選條件，並監控分選過程**
- **4Way sorting**
- **3 different sort modes and mixed mode**
 - Enrich mode
 - Purity mode
 - Single mode
- **CyClone deposition system**



4WAY™ Sorting



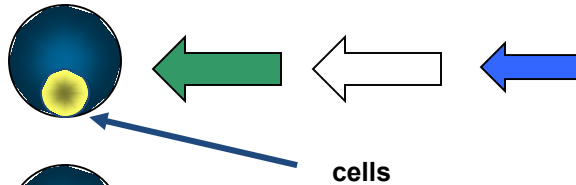
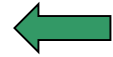
- Sort into four tubes
- Purity can exceed 99.5%
- No performance reductions in purity at any speed



Sort Modes



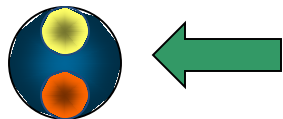
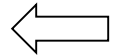
Enrich mode



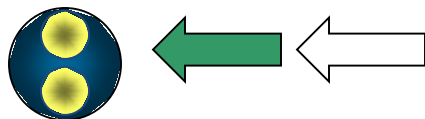
All positive cells are sorted except hard coincidences (cells too close together to discriminate).



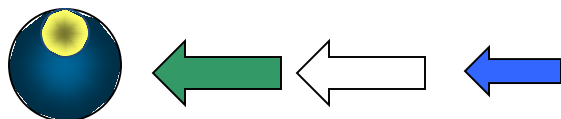
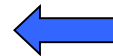
Purify mode



All negative cells are aborted and all positive cells are sorted.



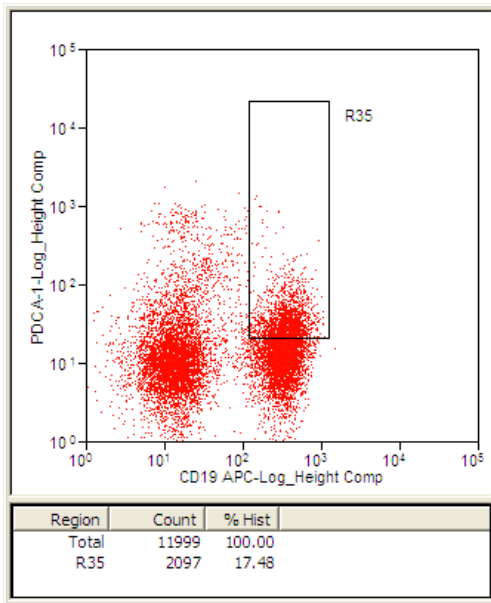
Single mode



All negative cells are aborted and there must be only one positive cell in the drop. This very important mode is used when sorting into 96 well plates.

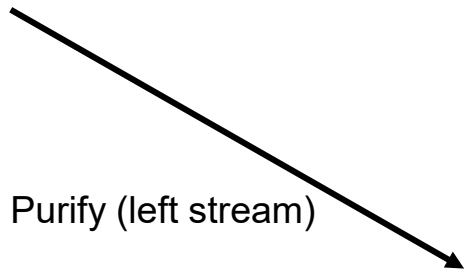
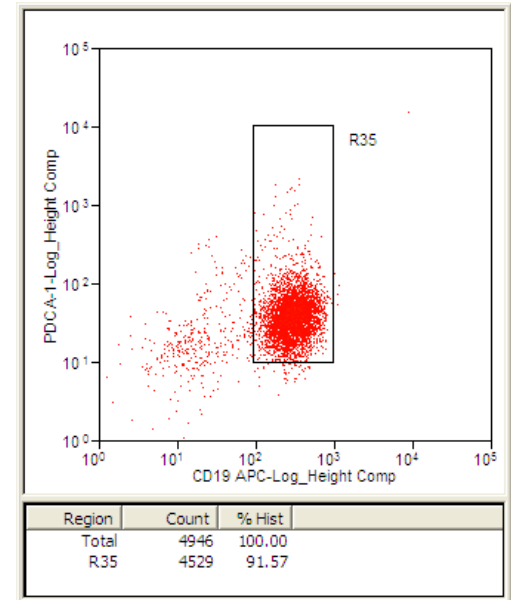


'Mixed Mode' Sorting

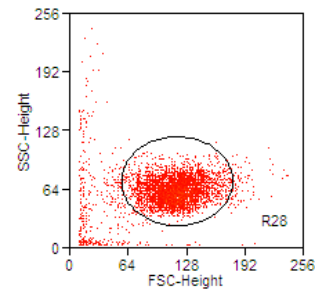
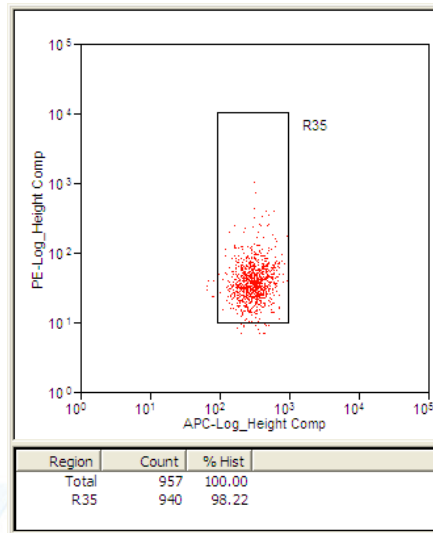


Enrich (right stream)

Sorted at >70,000eps for >2hrs



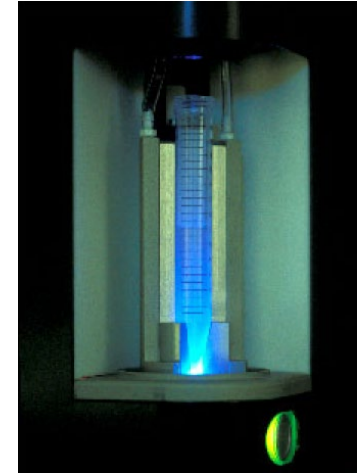
Purify (left stream)



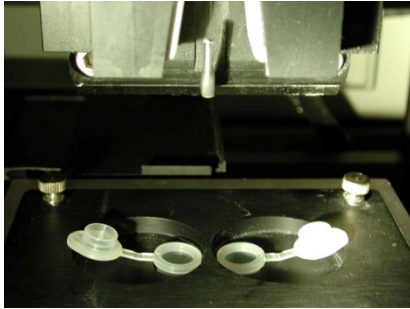
Good viability !

SmartSampler Features

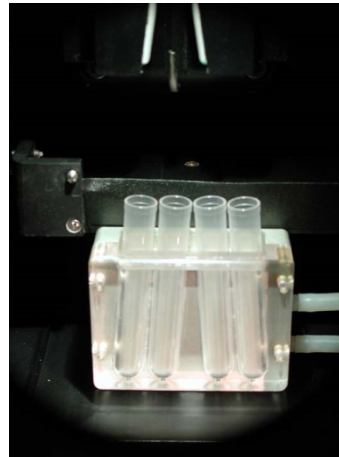
- Wide variety of tube formats: 0.5, 1.5, 5, 14, 15 and 50 mL
- Automatic agitation settings and automatic backflush.
- An air detection feature alerts the user if air enters the sample line, allowing full aspiration of the sample, & preventing air from entering the nozzle.
- Samples can be maintained at temperatures from -4 to 40°C throughout the run with the water bath option.
- Automated cleaning and shutdown
- Environmental safety has been considered by directing depressurized air through a dual HEPA filtration system prior to return to the laboratory



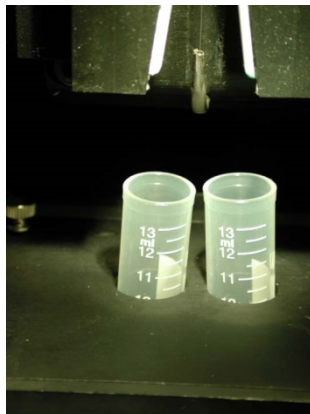
Collection Tubes at MoFlo XDP



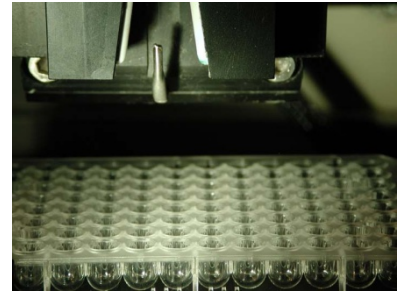
Eppendorf tubes



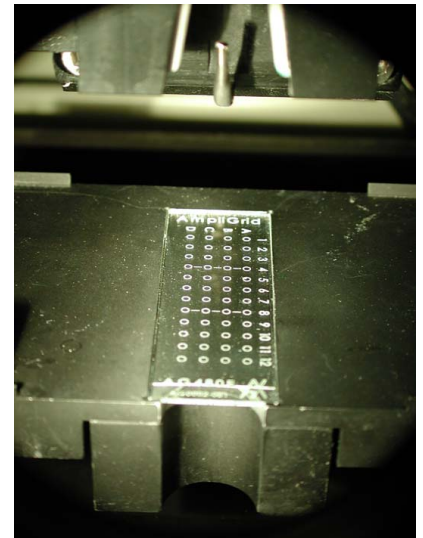
one to four tubes
/populations



5 ml to 50 ml Falcon tubes



multiwell plates
6 – 1536 wells



onto slides or directly
onto filters
or nitrocellulose membranes.

- The volume of one sorted drop is 1.4 nl (70 μ m, 100kHz, 60psi).
10⁶ cells result in 1.4 ml.
- Give 1-2 ml serum into the collection tube or whatever
your cells will be happiest in !

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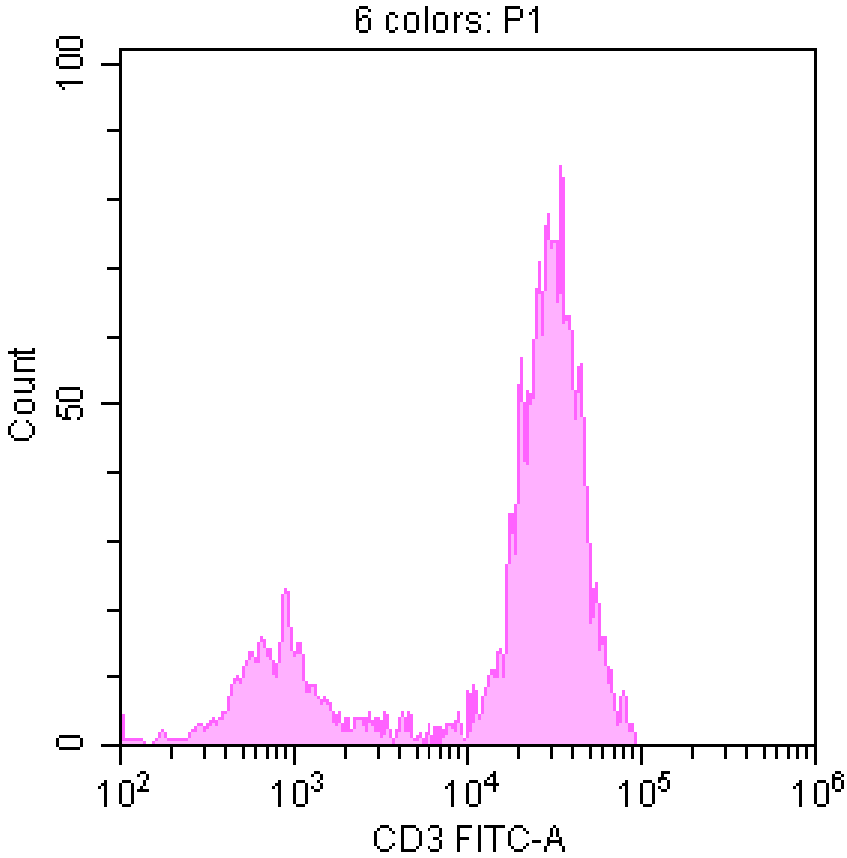


DATA ANALYSIS :PLOT &STATISTICS

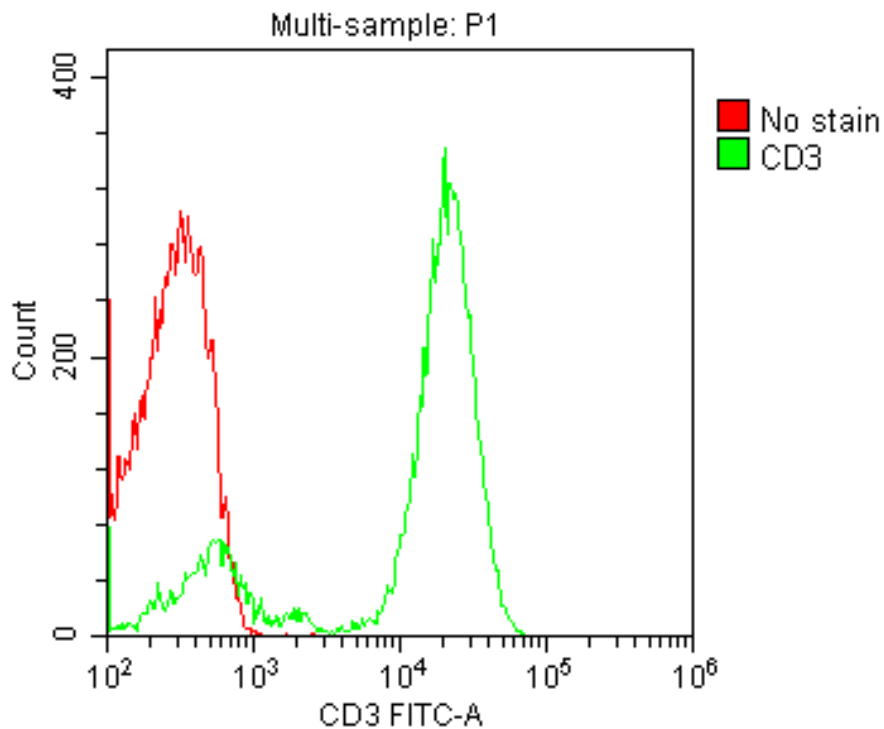
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Plots

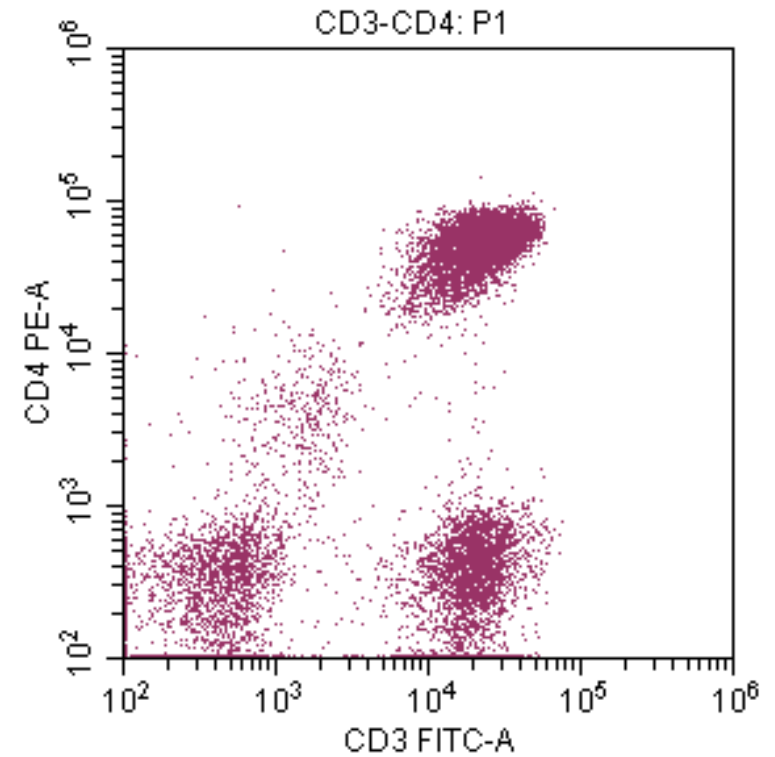
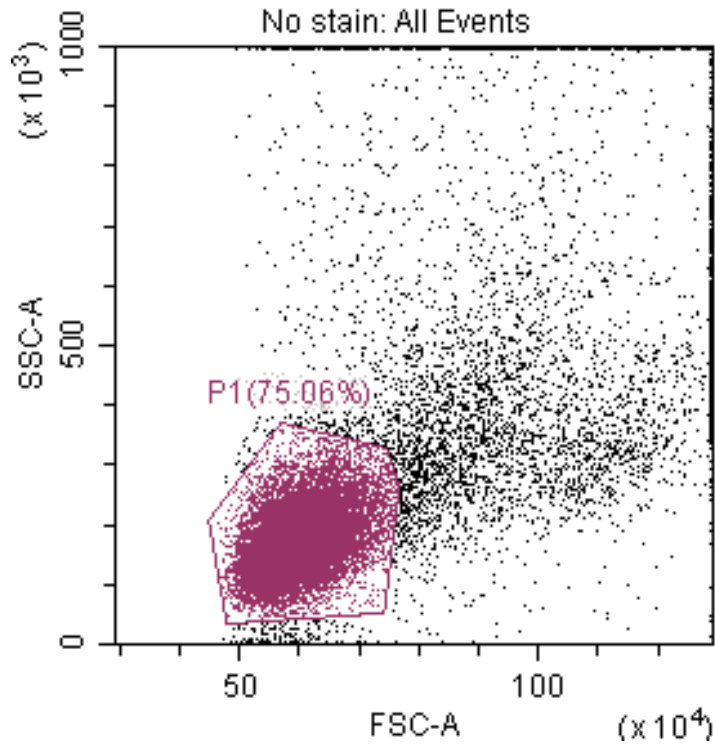
1. Single parameter histogram



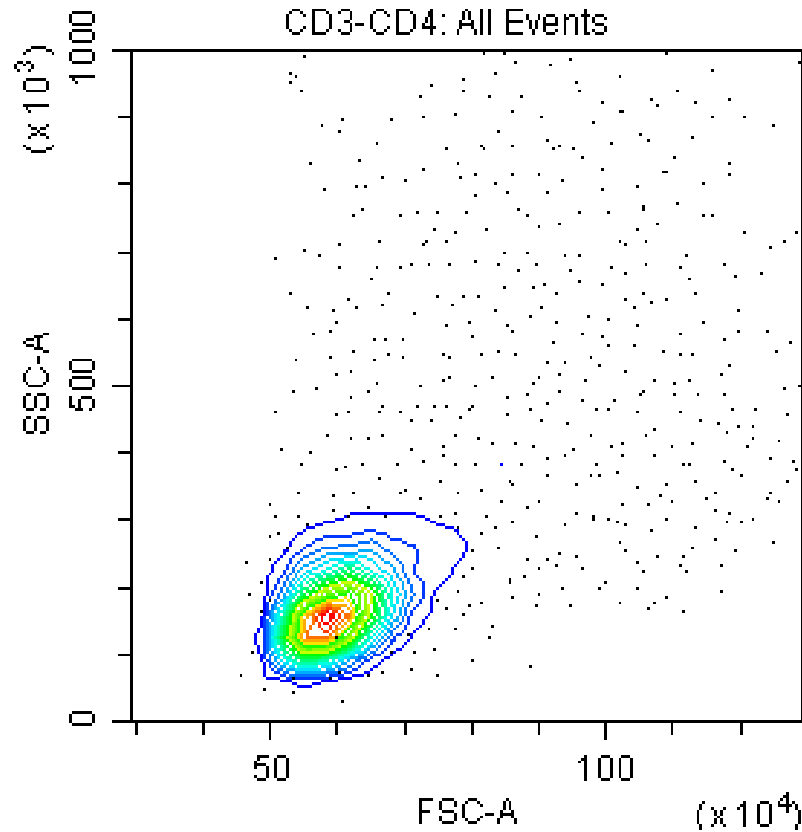
1. Single parameter histogram overlap



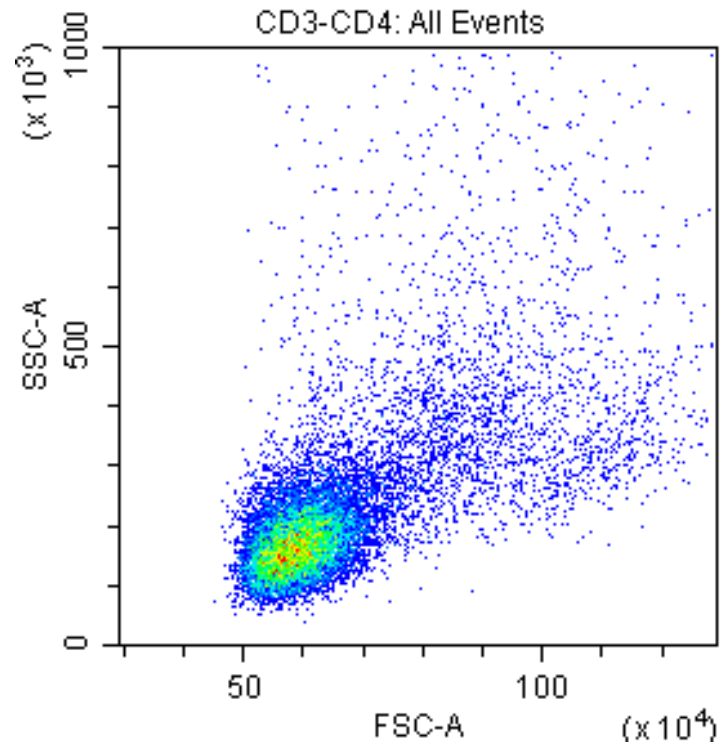
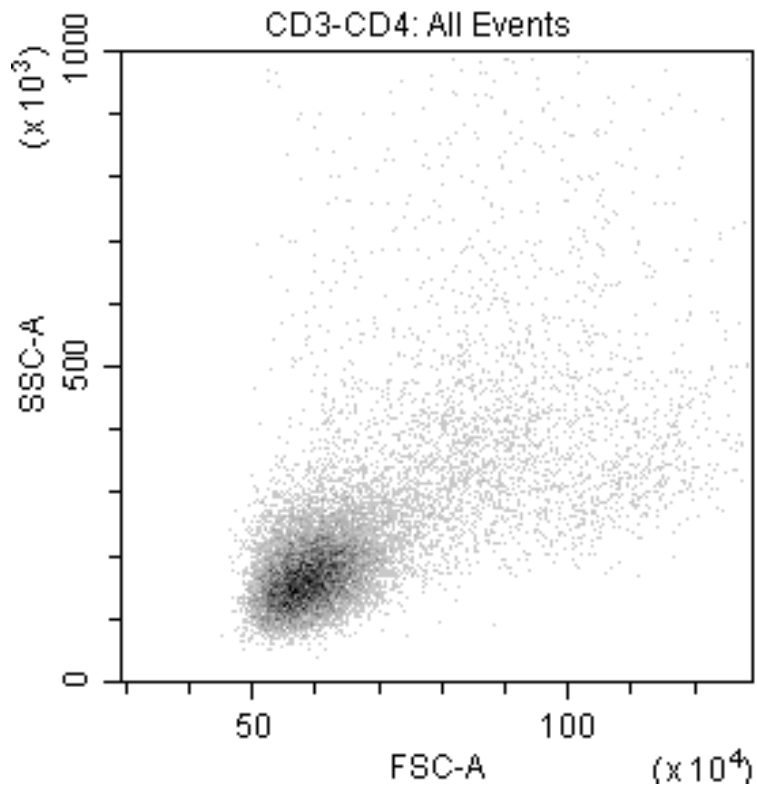
2. Dual parameter histogram – (1) Dot plot



2. Dual parameter histogram – (2) Contour plot (等高線圖)

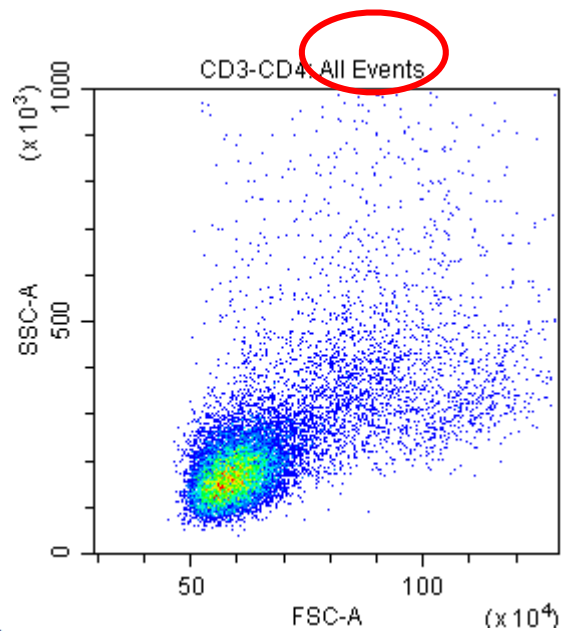


2. Dual parameter histogram – (3) Density plot



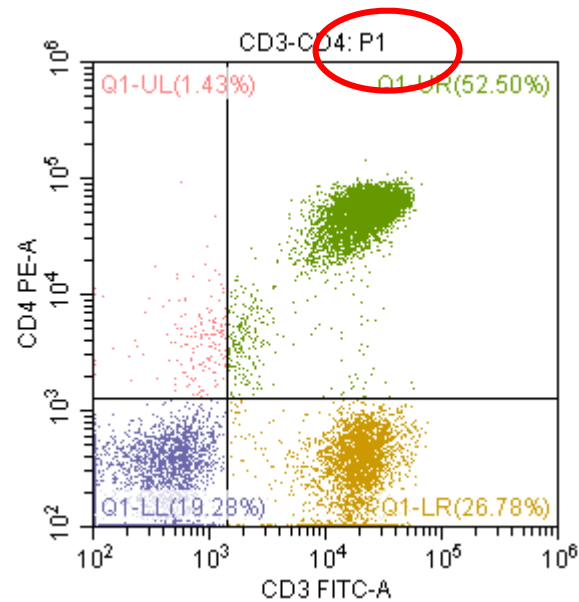
Statistics

1. Percentage : % Total & %Gate



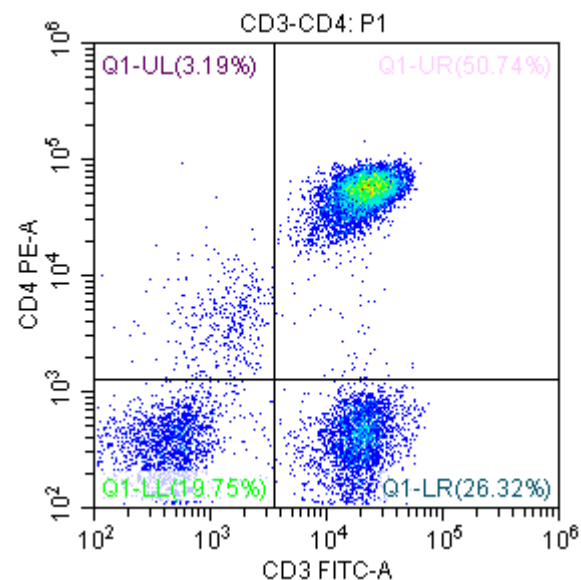
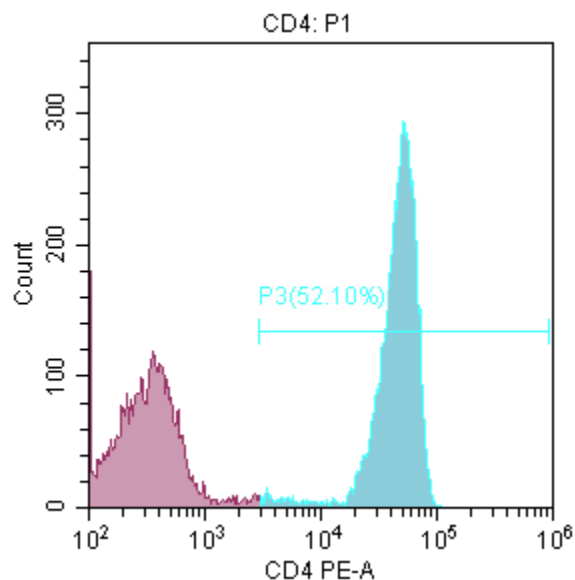
Sample ID:

Population	Events	% Total	% Parent
All Events	14455	100.00%	100.00%
P1	11499	79.55%	79.55%



Population	Events	% Total	% Parent
All Events	14455	100.00%	100.00%
P1	11499	79.55%	79.55%
Q1-UR	6037	41.76%	52.50%
Q1-UL	165	1.14%	1.43%
Q1-LL	2217	15.34%	19.28%
Q1-LR	3080	21.31%	26.78%

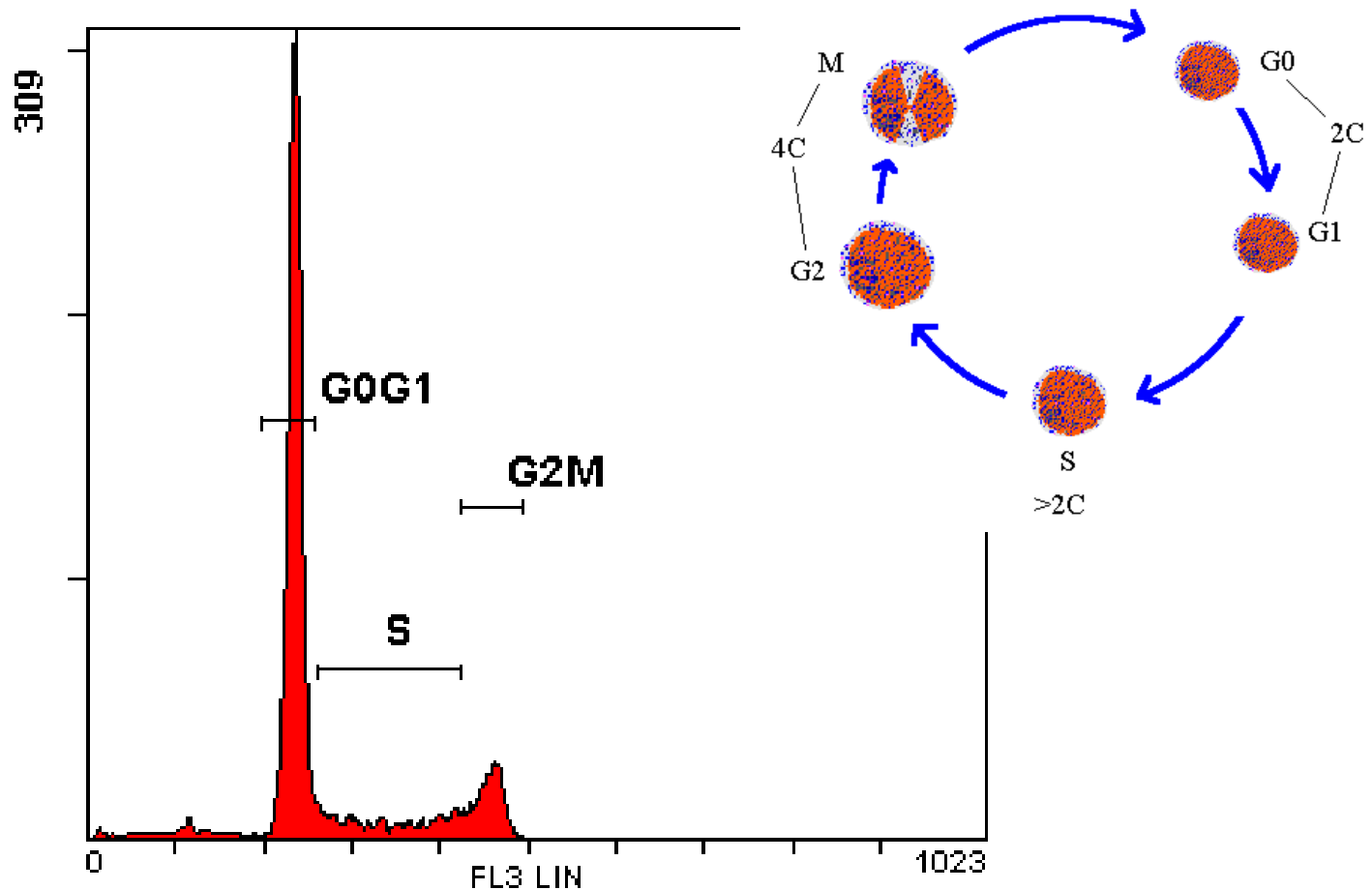
2. Mean : X-mean and Y-mean



Population	Events	% Total	% Parent	Mean CD4 PE-A
All Events	14422	100.00%	100.00%	23427.6
P1	11662	80.86%	80.86%	24870.8
P2	1	0.01%	0.01%	2886.0
P3	6076	42.13%	52.10%	47467.0

Population	Events	% Total	% Parent	Mean CD3 FITC-A	Mean CD4 PE-A
All Events	14455	100.00%	100.00%	16378.6	25265.5
P1	11499	79.55%	79.55%	17241.5	26551.0
P2	8886	61.47%	77.28%	22132.0	34068.2
P3	201	1.39%	1.75%	2728.3	4620.6
Q1-UR	5835	40.37%	50.74%	23003.3	51680.7
Q1-UL	367	2.54%	3.19%	1527.1	5377.8
Q1-LL	2271	15.71%	19.75%	491.2	301.4
Q1-LR	3026	20.93%	26.32%	20607.9	362.0

3. Coefficient of Variant : CV (變異係數)



Region	%Gated	X-CV
G0G1	63.15	3.7
G2M	12.37	3.3
S	15.26	15.0



Current Applications of Flow

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Cell Surface Marker Immunofluorescence analysis

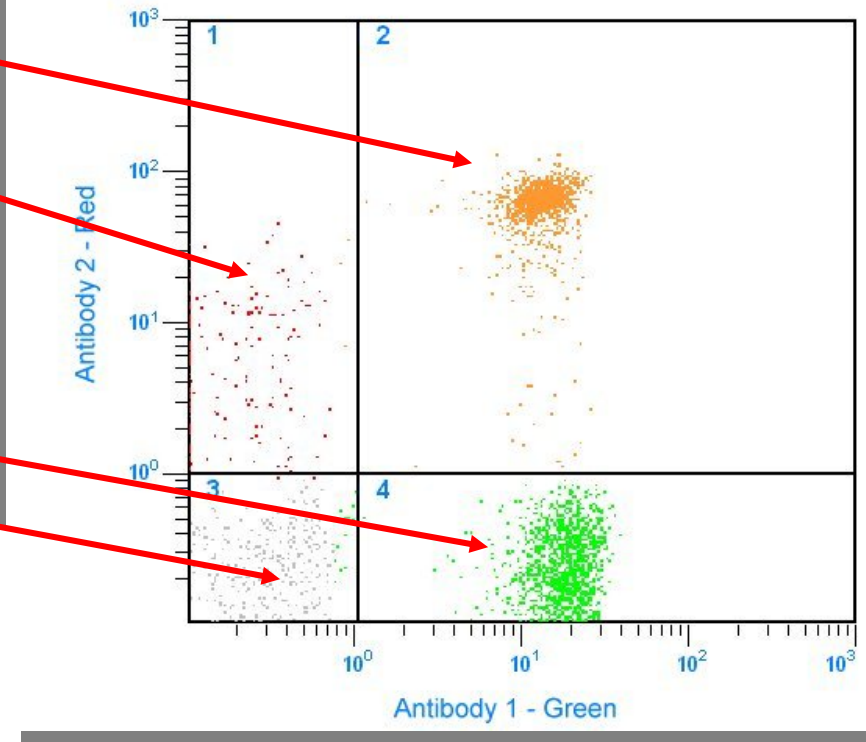
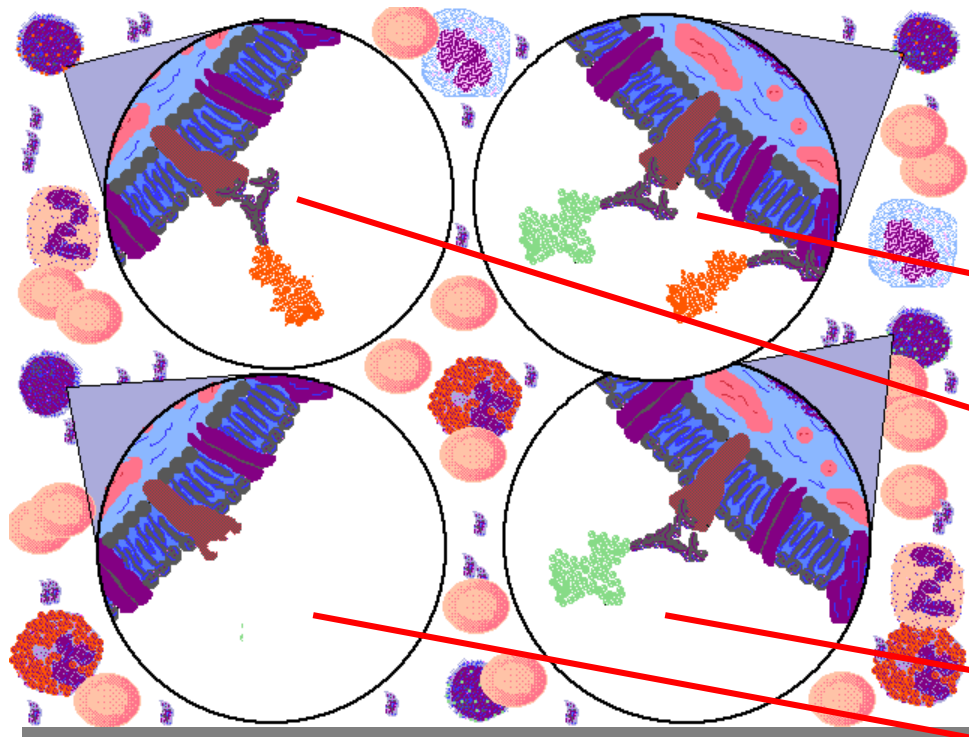
- ✦ Leukocyte Immunophenotyping (淋巴細胞免疫分型)
- ✦ Stem cells tracking and enumeration (幹細胞分析及計量)
- ✦ Analysis of platelets (血小板功能分析)

Cell function assays

- ✦ Cell Cycle and Apoptosis
- ✦ Mitochondrial membrane potential analysis (**DiOC6**、**JC-1**)
- ✦ Calcium kinetic studies (**Fluo-3**)
- ✦ Cellular protein content measurements (**GFP**、**YFP**)
- ✦ NK cell cytotoxicity assay (**CFSE**+ **PI**)
- ✦ Phagocytosis assay (**FITC-E. coli BioParticles** + **PI**)
- ✦ Extracellular cytokines detection (beads array)

Microorganisms analysis

Surface Marker:



Surface Marker: CD chart, cell classification and function

CD Chart

Session	CD	Main antigen expression main activities/functions Alternate Name	Clone
T	CD7	Mature T cells, NK cell activation, cell adhesion gp40, Tp41	
T	CD8 α	Cytotoxic / suppressor with TCR T8, Leu-2	
T	CD8 β	Cytotoxic / suppressor with TCR	
P	CD9	Platelets, activated T cell adhesion and migration p24, MRP-1	
B	CD10	Pre-B cell subset, B cell regulation of B cell growth CALLA, neutral endopeptidase	
Ad	CD11a	Most of lymphoid and myeloid cells LFA-1 α subunit, Integrin	
Ad	CD11b	Myeloid cells and NK cells MAC-1 α subunit, Integrin	
Ad	CD11c	Myeloid cells, NK cells p150,95, Integrin α X s	
Ad	CD11d	Leucocytes Integrin α D subunit	
M	CDw12	Monocytes, granulocytes -	
M	CD13	Monocytes, neutrophils, coronaviruses aminopeptidase N (EC 3.4.11.2)	
M	CD14	Monocytes, macrophages LPS-R	

CD Chart

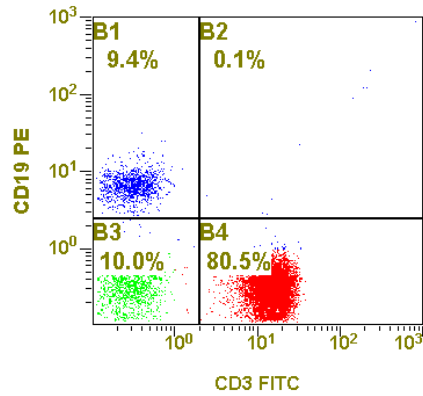
Session	CD	Main antigen Alternate Name
N	CD16a	NK cells, macrophages low-affinity Fc γ RII
N	CD16b	Granulocytes GPI-linked, I
M	CDw17	Neutrophils, signal transduction LacCer
Ad	CD18	Leucocytes I Integrin β_2 s
B	CD19	Precursor B cell B4
B	CD20	Precursor B cell progression B1, Bp55
B	CD21	Mature B cell proliferation, differentiation CR2, EBV-R, CD22
B	CD22	Precursor or mature B cell β form to T1 BL-CAM, Leu-13
B	CD23	B cells, monocytes cytotoxicity I FC γ RIII, BLA
B	CD24	B cells, granulocytes BA-1, HSA (I)
Ok	CD25	Activated T cells IL-2R α , Tac

CD Chart

Session	CD	Main antigen expression main activities/functions Alternate Name	Clone Availability
Q	CD234	Erythroid cells, endothelial cells, some epithelial cells binds various chemokines (IL-8, M-CSF, RANTES, MCP-1) intracellular sink to modulate levels of proinflammatory molecules Fy-glycoprotein, Fy glycoprotein, Duffy antigen, DARC	-
Q	CD235a	Red blood cells, erythroid precursor cells Glycophorin A	+
Q	CD235b	Red blood cells, erythroid precursor cells Glycophorin B	-
Q	CD235ab	Red blood cells, erythroid precursor cells Glycophorin A/B cross reactive mabs	-
Q	CD236	Red blood cells, stem cell subset Glycophorin C/D	-
Q	CD236R	Red blood cells, stem cell subset Glycophorin C	-
	CD237	NA (reserved)	
Q	CD238	Red blood cells, stem cell subset Kell kx	-
Q	CD239	Red blood cells, stem cell subset B-CAM	-
Q	CD240CE	Red blood cells Rh30CE	-
Q	CD240D	Red blood cells Rh30D	-
Q	CD240CE	Red blood cells Rh30CE/CE cross reactive mabs	-

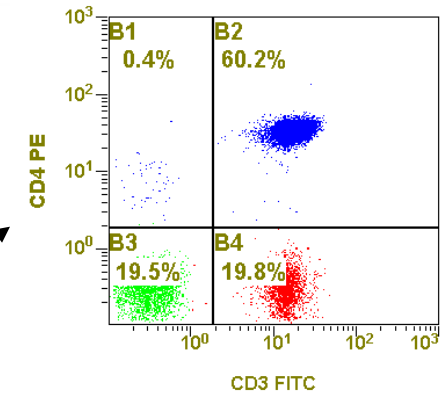
T cell / B cell

F1][A] 2 Color CD3 CD19.LMD : FL1 LOG/FL2 LOG

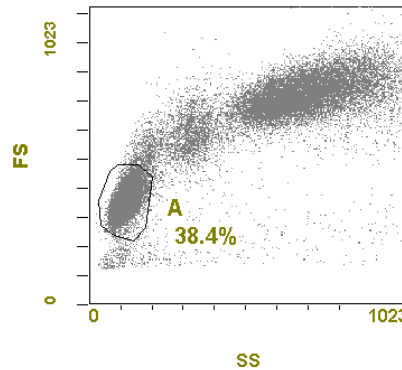


Helper T cell

(F1)[A] 2 Color CD3 CD4.LMD : FL1 LOG/FL2 LOG

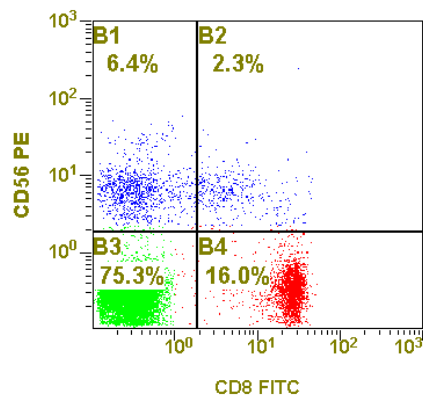


(F1)[Ungated] 2 Color CD3 CD8.LMD : SS/FS



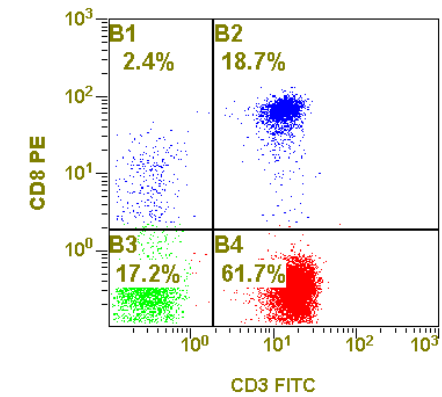
NK cell / Suppressor T

F1][A] 2 Color CD8 CD56.LMD : FL1 LOG/FL2 LOG



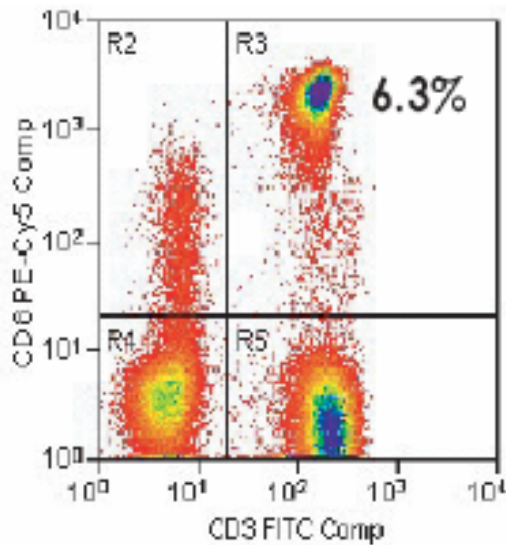
Suppressor T cell

(F1)[A] 2 Color CD3 CD8.LMD : FL1 LOG/FL2 LOG



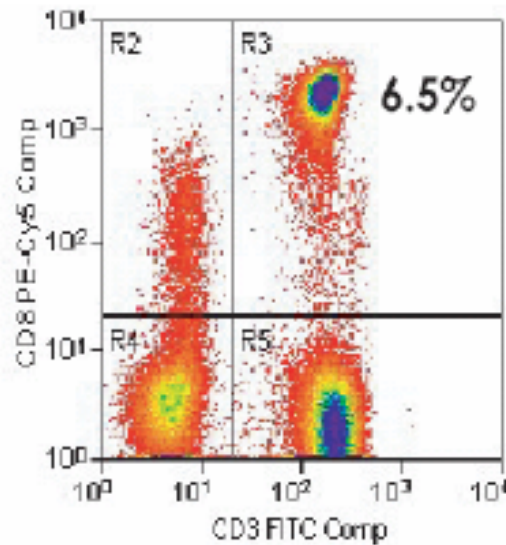
High speed analysis

1 x 10⁶ cells/ml
500,000 events
8 min 18 sec



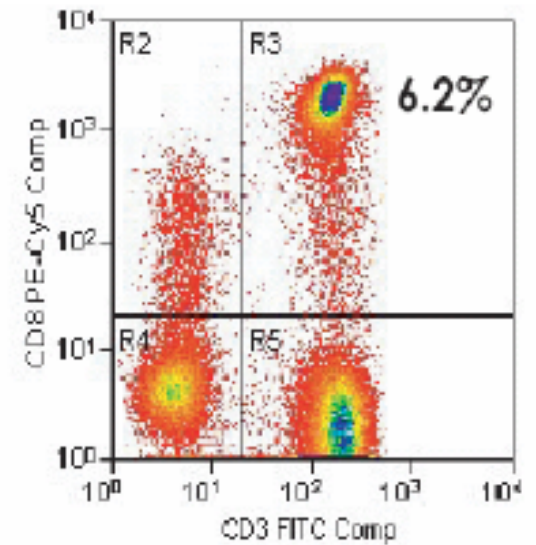
1,000 events per sec

5 x 10⁶ cells/ml
500,000 events
1 min 38 sec



5,000 events per sec

50 x 10⁶ cells/ml
500,000 events
10 seconds

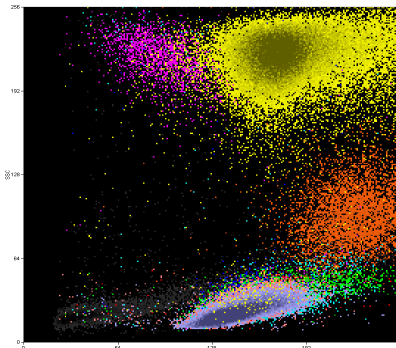


50,000 events per sec

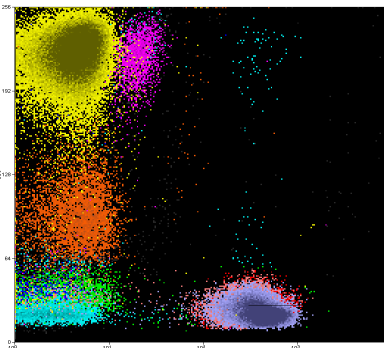
Multi-color immunophenotyping

7 colors: CD45-FITC / CD54-PE / CD3-PE-TR / CD14-PC5 / CD4-PC7
CD19-APC / CD8-APC-Cy7

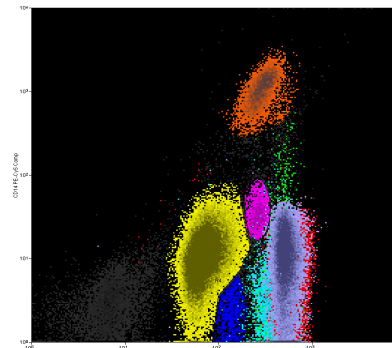
SSC vs. FSC



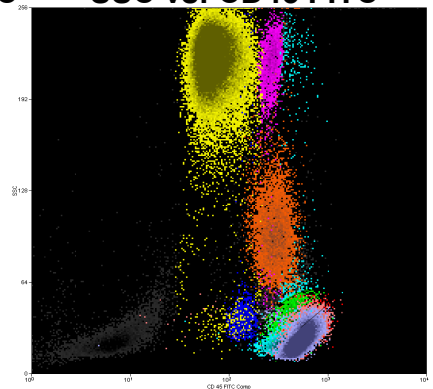
SSC vs. CD3 PE-TR



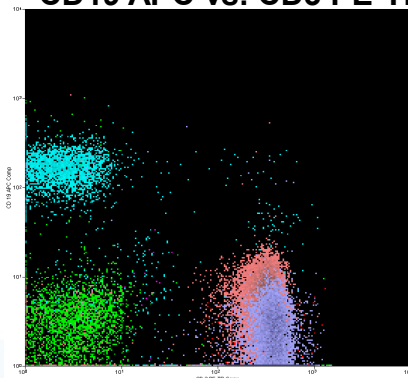
CD14 PE-Cy5 vs. CD45 FITC



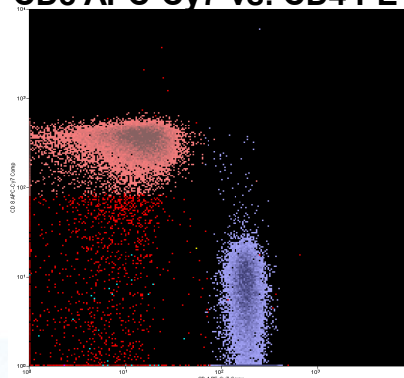
SSC vs. CD45 FITC



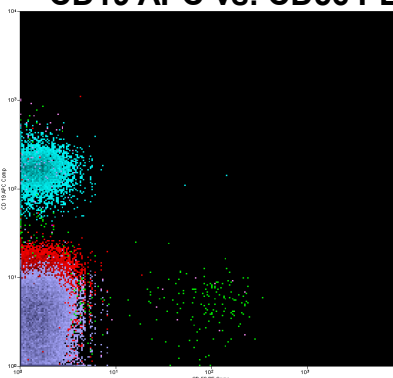
CD19 APC vs. CD3 PE-TR



CD8 APC-Cy7 vs. CD4 PE-Cy7

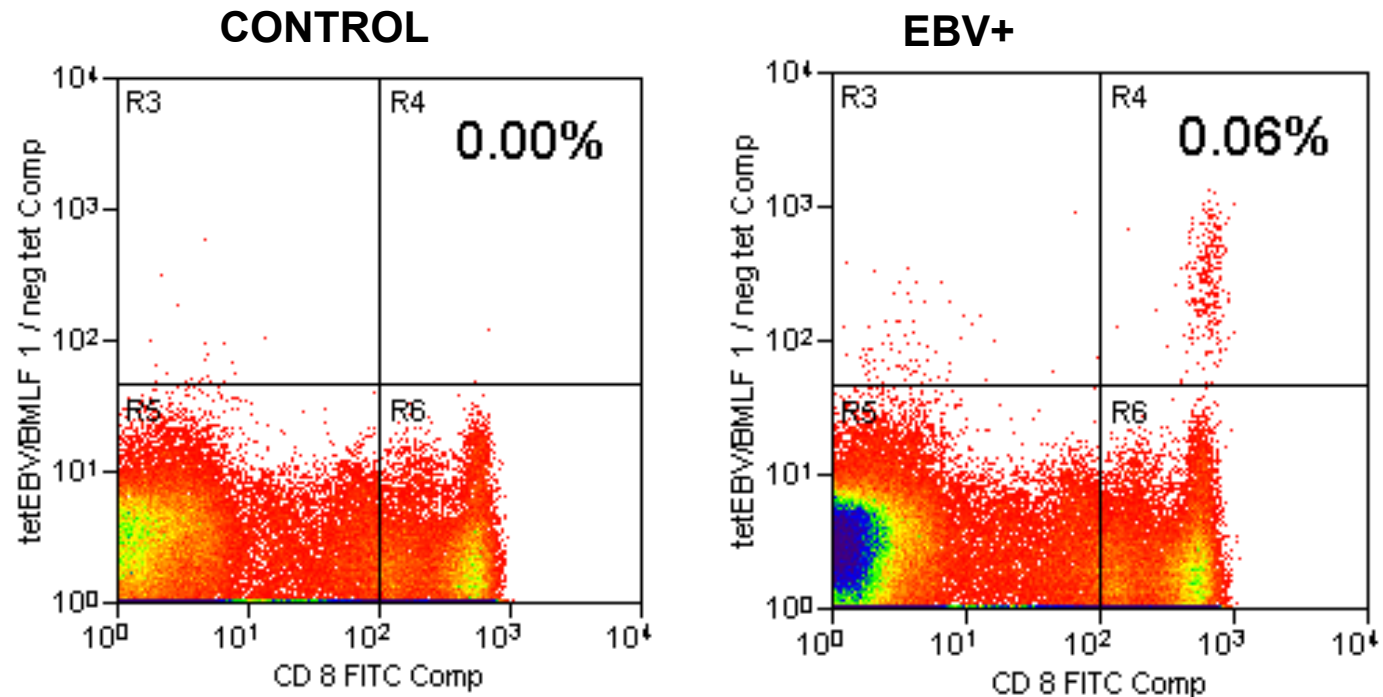


CD19 APC vs. CD56 PE



Rare population: antigen specific T cell

High speed detection of Antigen (Epstein-Barr-Virus) specific tetramers from whole blood (less than 0.1% total blood cells), with very low background.



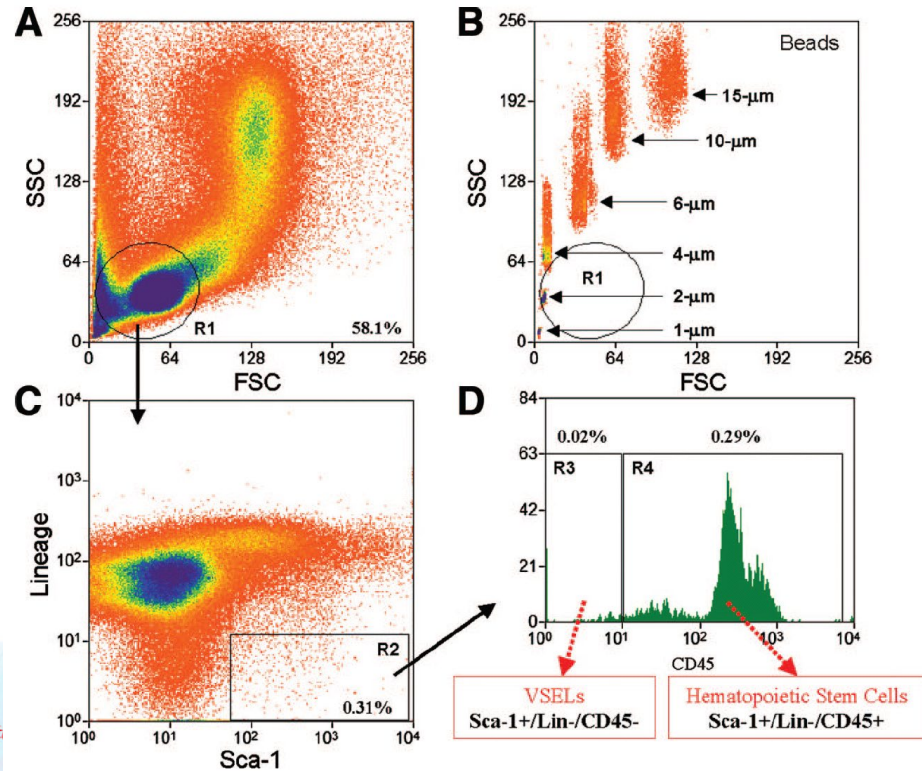
Rare population: very small embryonic-like stem cell

STEM CELLS®

TISSUE-SPECIFIC STEM CELLS

Transplantation of Bone Marrow-Derived Very Small Embryonic-Like Stem Cells Attenuates Left Ventricular Dysfunction and Remodeling After Myocardial Infarction

BUDDHADEB DAWN,^a SUMIT TIWARI,^a MAGDALENA J. KUCIA,^b EWA K. ZUBA-SURMA,^b YIRU GUO,^a SANTOSH K. SANGANALMATH,^a AHMED ABDEL-LATIF,^a GREG HUNT,^a ROBERT J. VINCENT,^a HISHAM TAHER,^a NATHAN J. REED,^a MARIUSZ Z. RATAJCZAK,^b ROBERTO BOLLI^a



CyClone application: single cell PCR

Single cell typing by on-chip Low Volume PCR (LV-PCR)

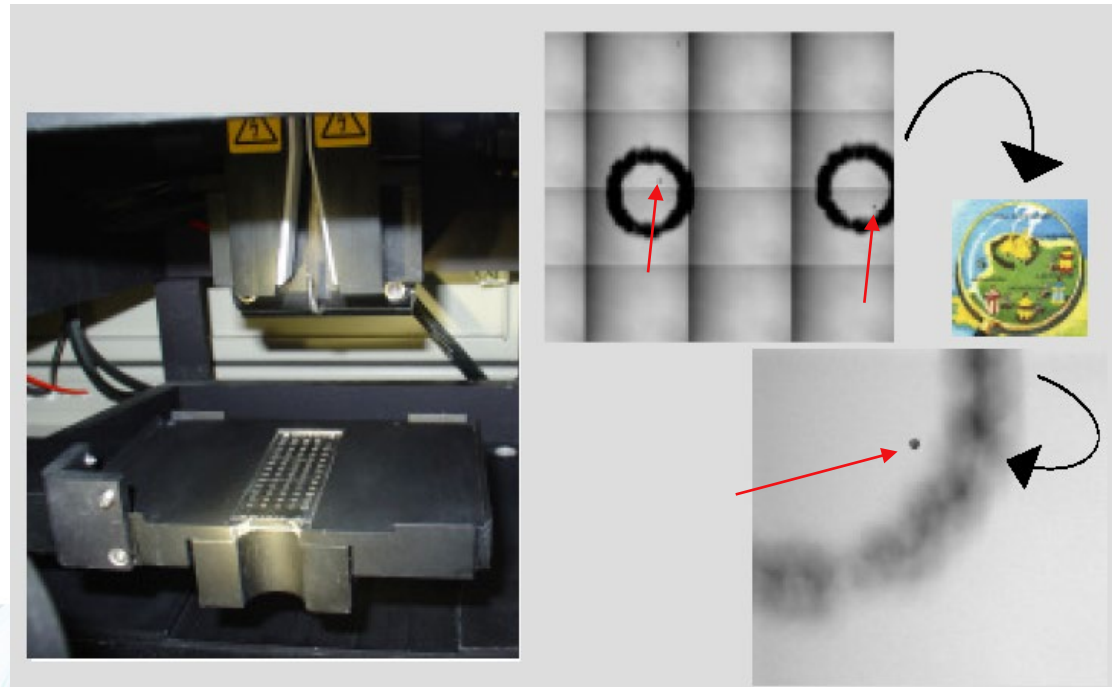
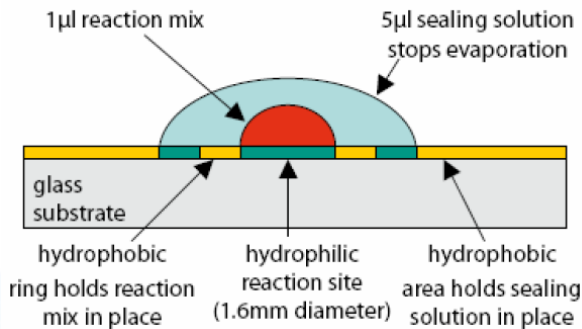
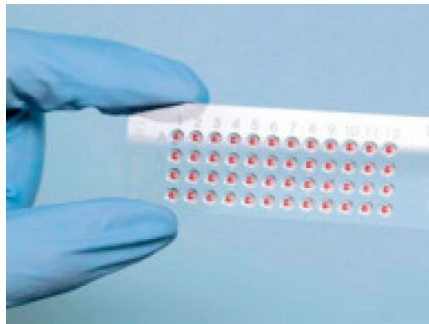
C. Proff[#], W. Mann^b, J.W. Ellwart^c, M.A. Rothschild^a, P.M. Schneider^a

^aInstitute of Legal Medicine, University of Cologne, Germany

^bAdvalytix AG, Brunnthal, Germany

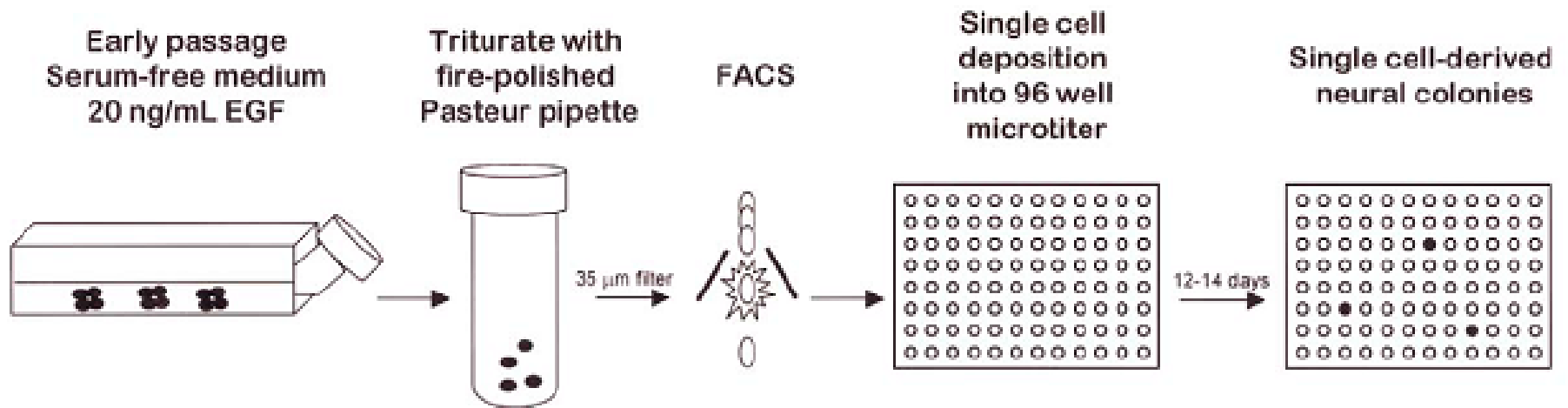
^cGSF-National Research Center for Environment and Health, Munich, Germany

[#] present address: Institut für Blutgruppenforschung LGC GmbH, Cologne, Germany



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Single Cell Sorting



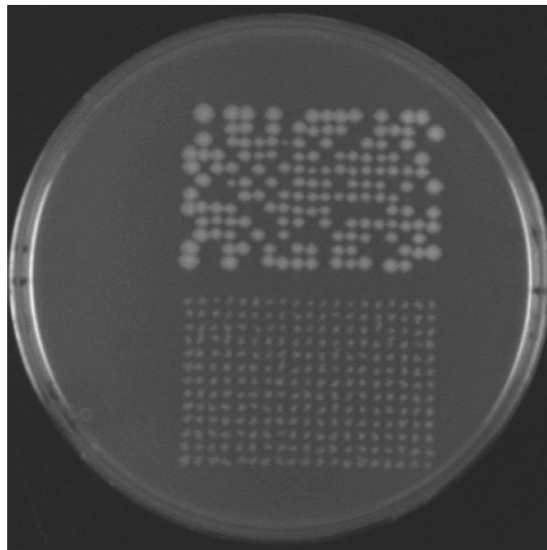
Journal of Neuroscience Methods 117 (2002) 111_/_121

CyClone application: Clone selection and HTS

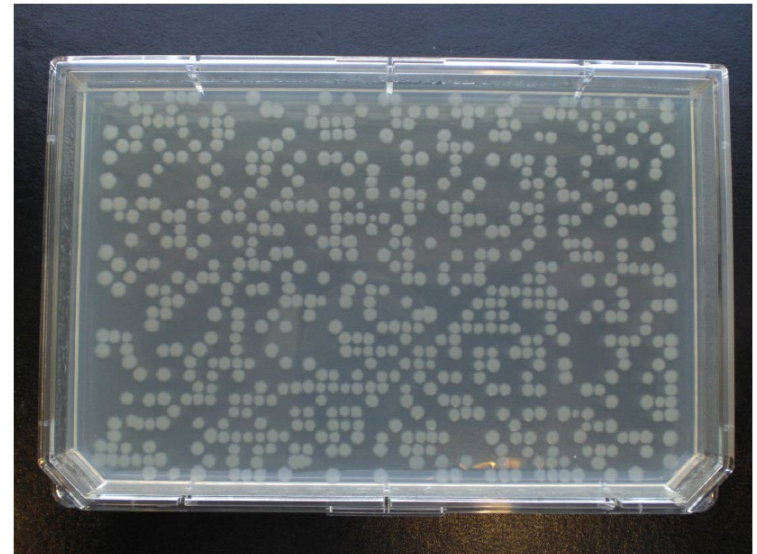


Increasing Well-based High Throughput Screening Efficiency for Protein Engineering by Integration of MoFlo[®] Library Dispensing

E.Coli – GFP (+)
1/well
 $144/228 = 63\%$



E.Coli – GFP (-)
10/well



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