



揚州大學
YANGZHOU UNIVERSITY

实用流式细胞技术及其样品处理

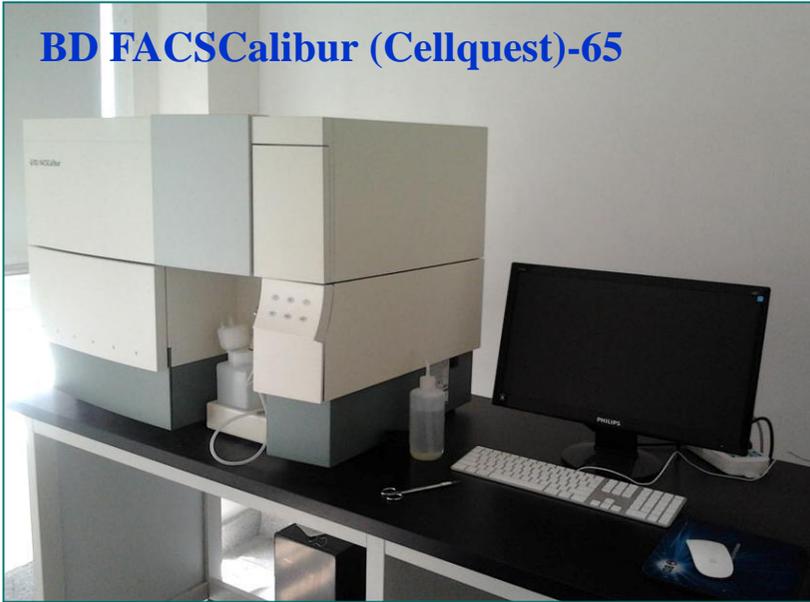
Practical flow cytometry and sample preparation

胡茂志

扬州大学测试中心



BD FACSCalibur (Cellquest)-65



Beckman Coulter: Cyan ADP7 (Summit) -87



BD Accuri C6 (CFlow)



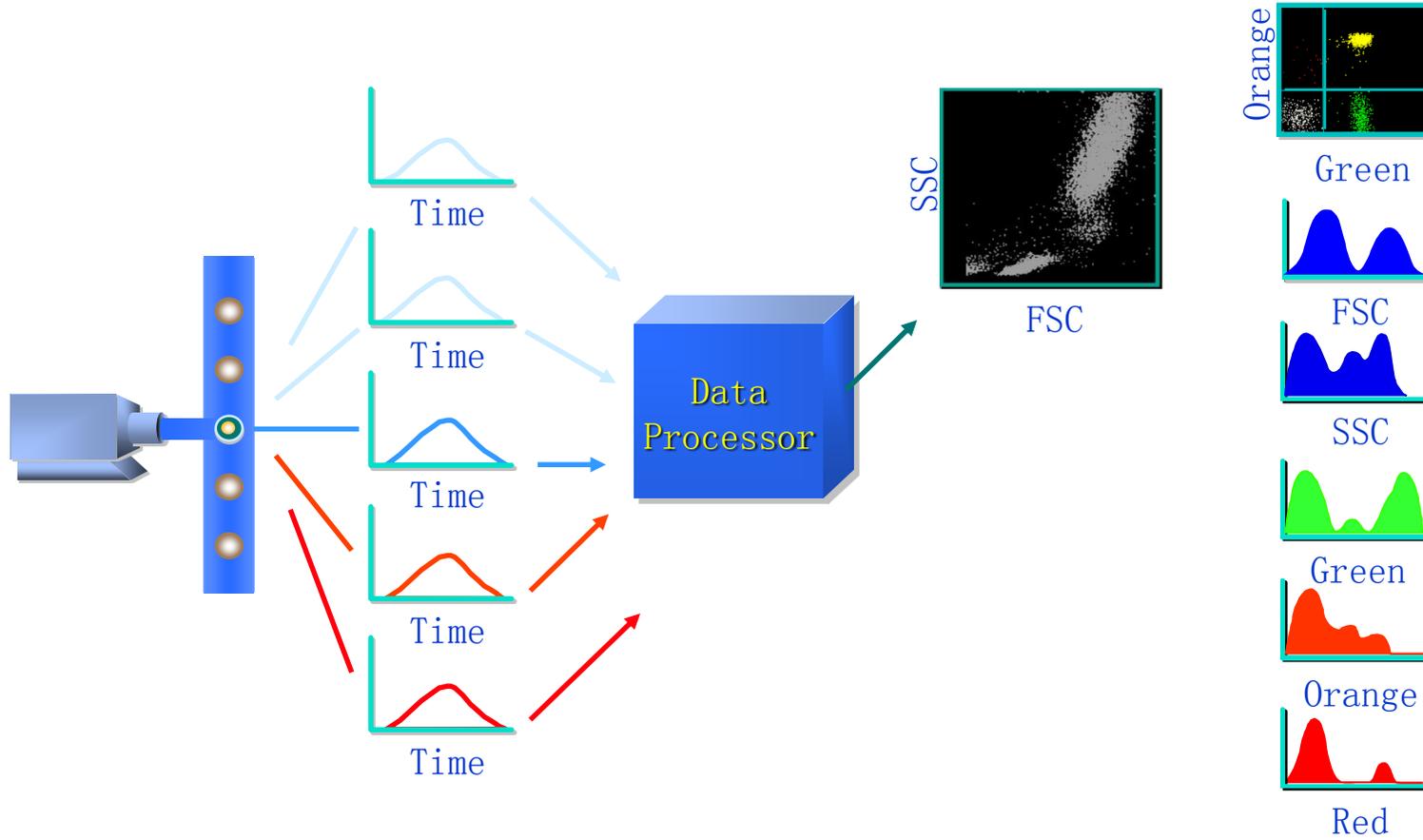
BD LSRFortessa

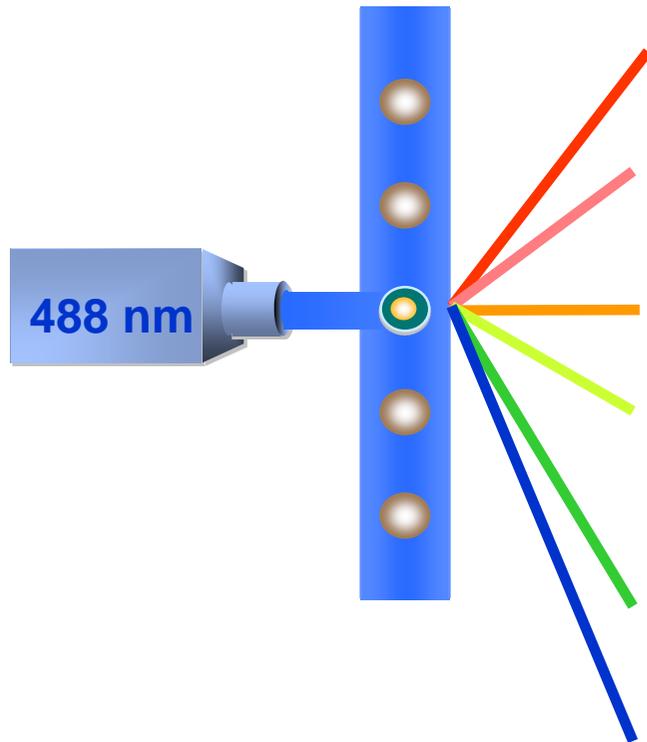




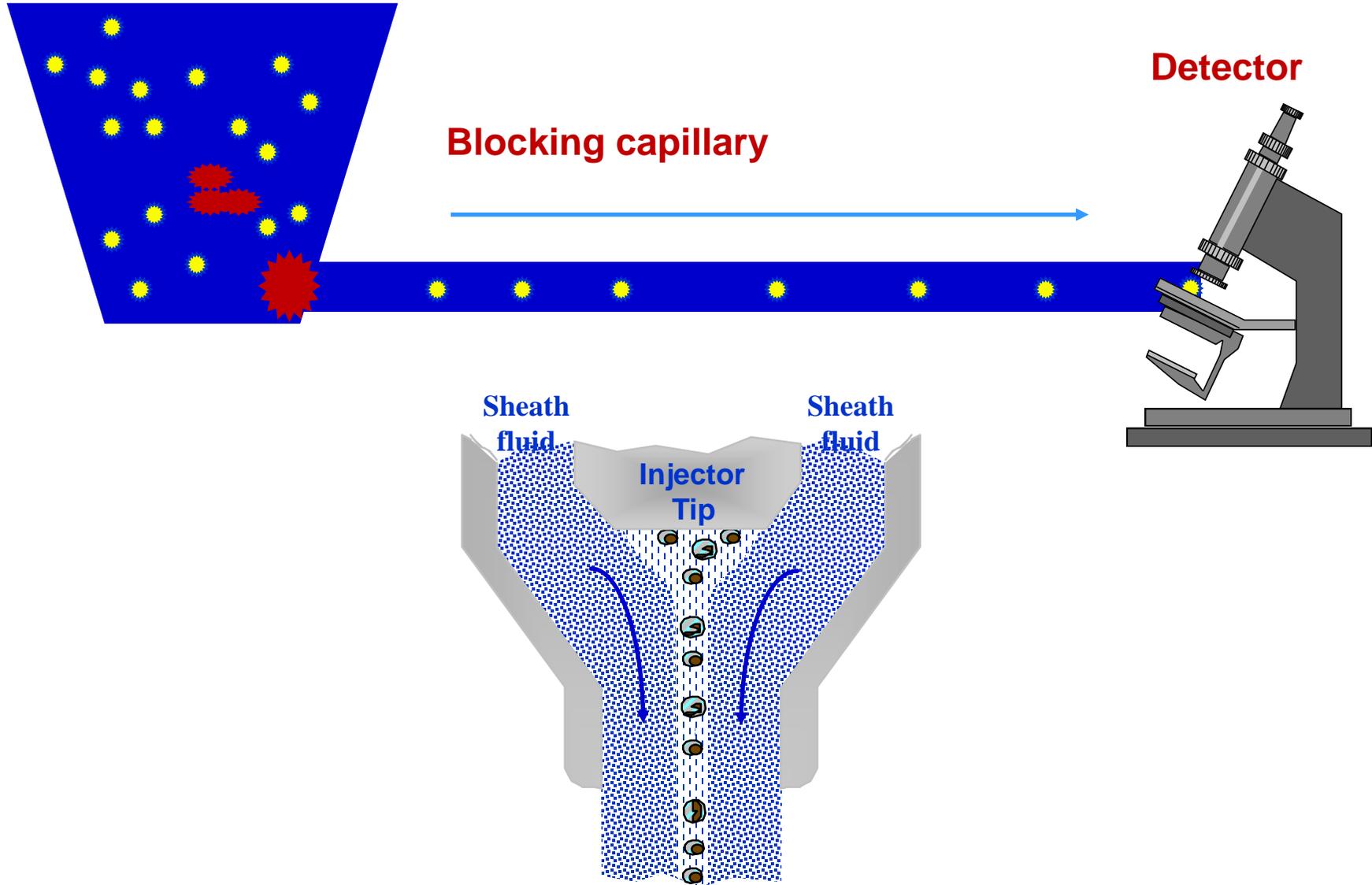
- **基本原理(Basic principle)**
- **应用(Application)**
- **样品处理(Sample preparation)**

一、基本原理





LP Mirror	BP Filter	Intended Dye
735	780/60	PE-Cy7
655	695/40 675/20	PerCP-Cy5.5 or PI PerCP
595	610/20	PE-Texas Red
556	575/26 585/42	PE or PI Alternative for PE/PI when not using PE-Texas Red
502	530/30	FITC
—	488/10	Side scatter (SSC)





利用流式细胞术可以获得哪些信息

- 细胞的相对计数、绝对计数
- 细胞成分的相对定量、绝对定量
- 单参数或多参数
- 细胞表面、细胞内成分
- 可溶性成分
- 流式分子表型

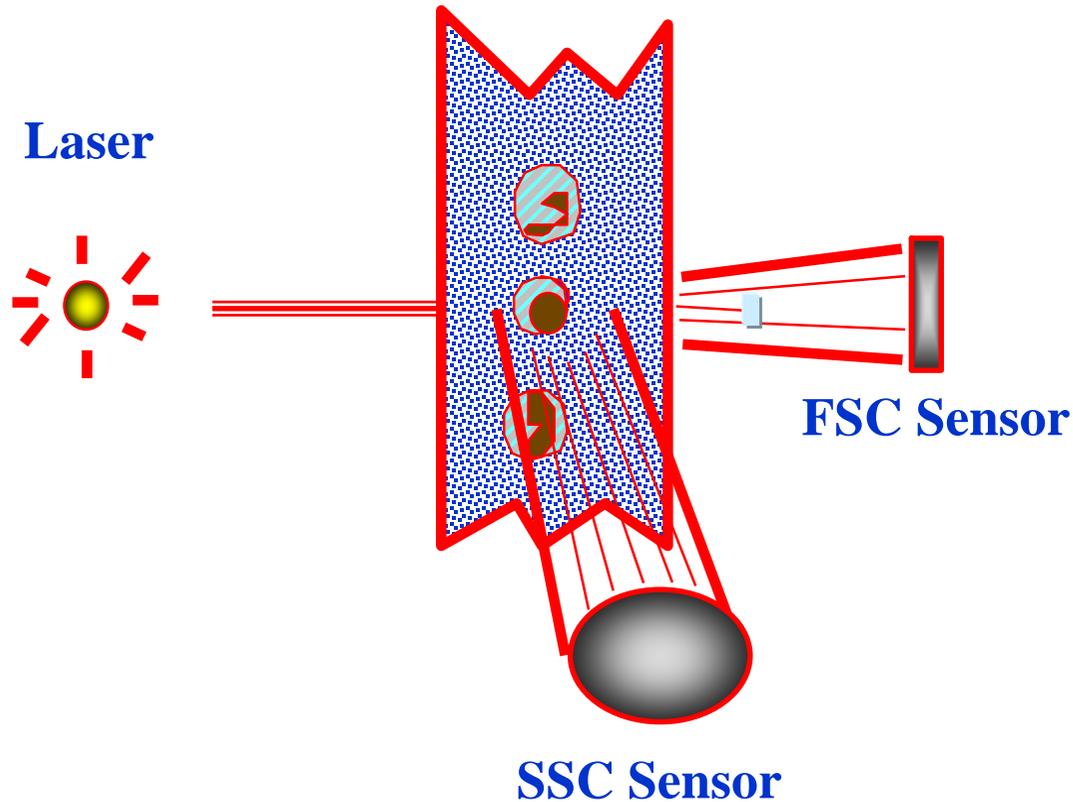


流式细胞术可以产生哪些信号

细胞的物理参数： 散射光(Scatter light)

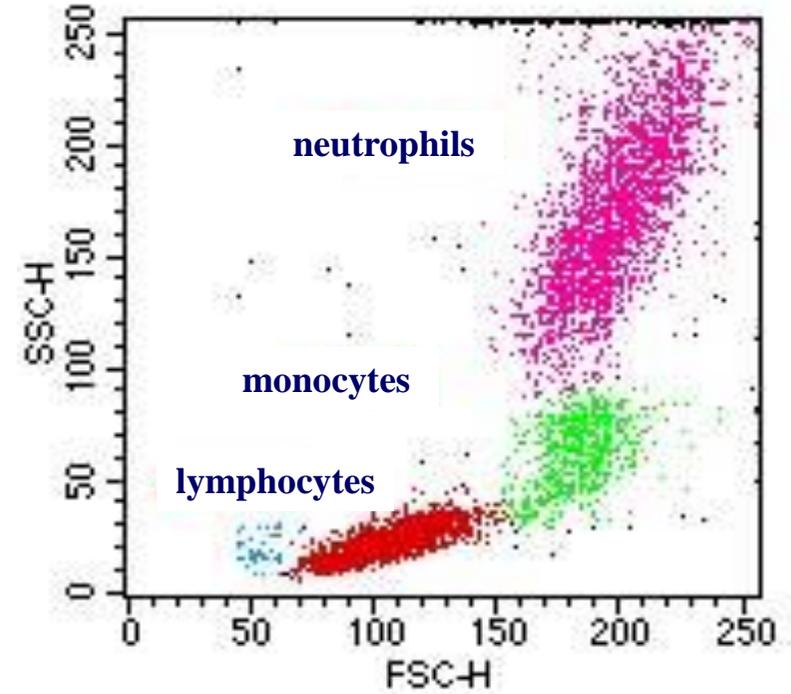
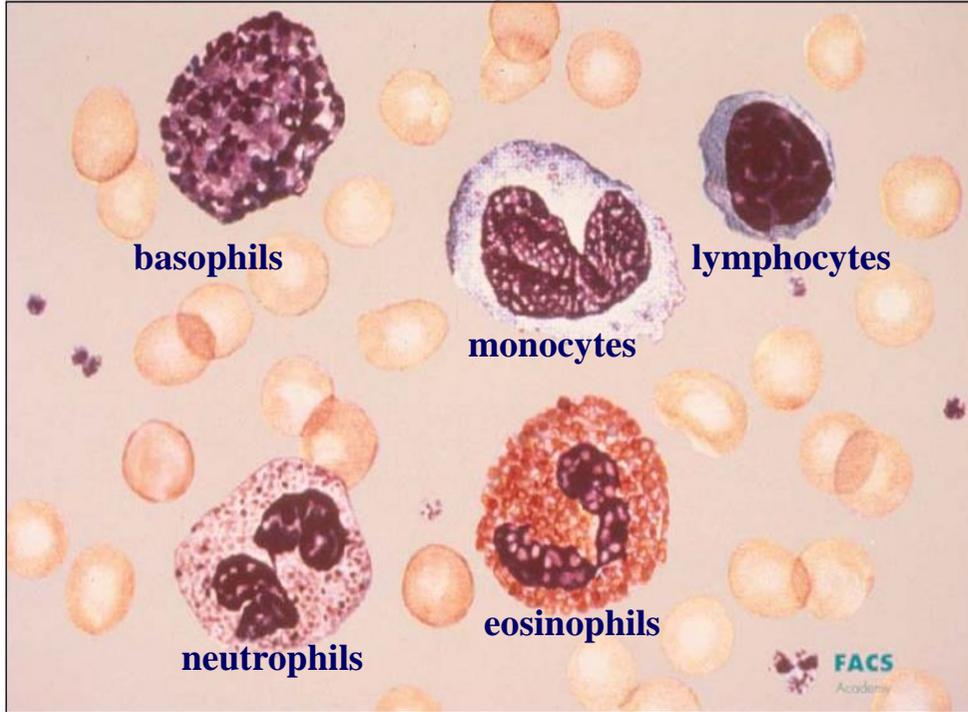
细胞的理化参数： 荧光(Fluorescence)

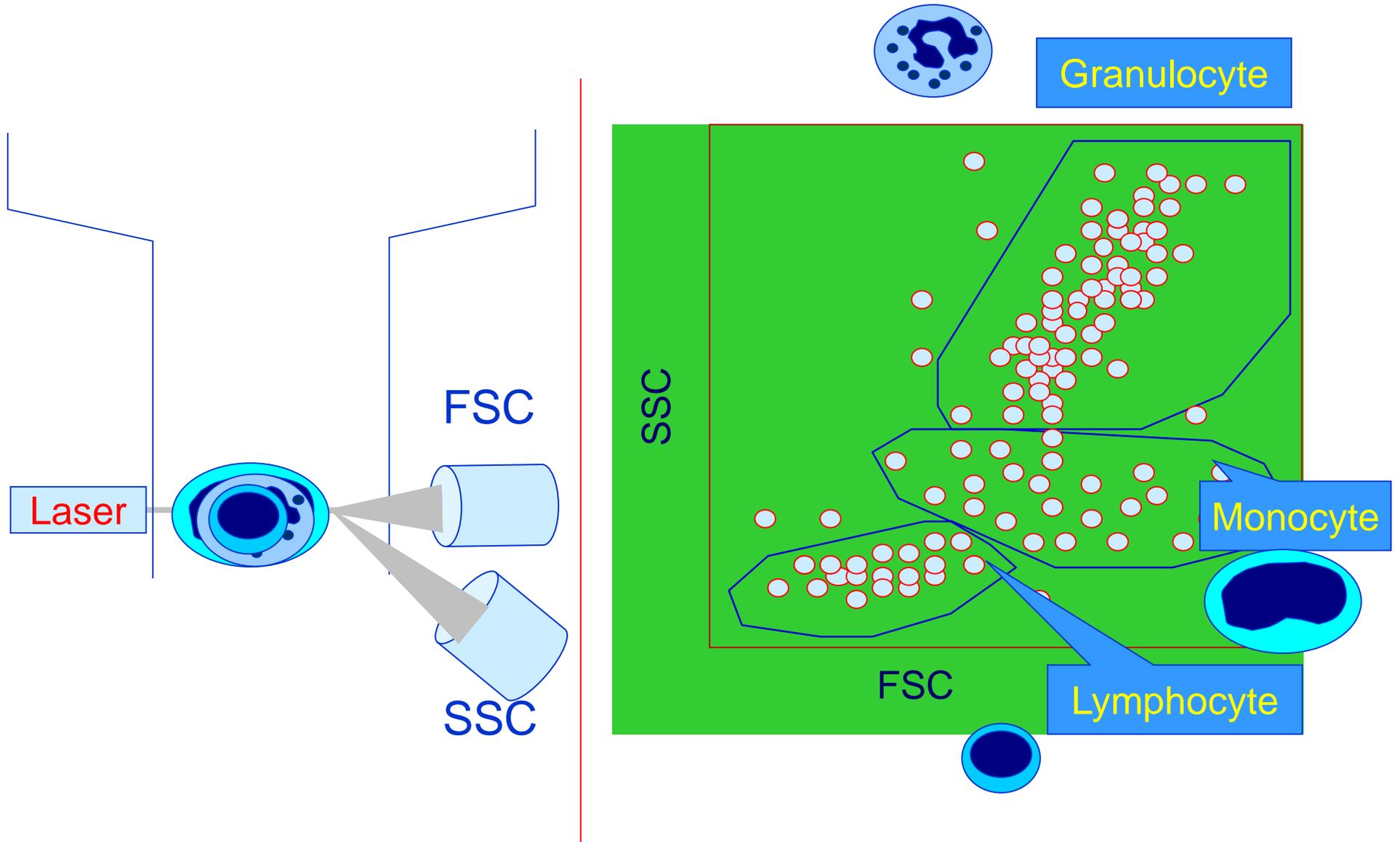
Scatter light signal



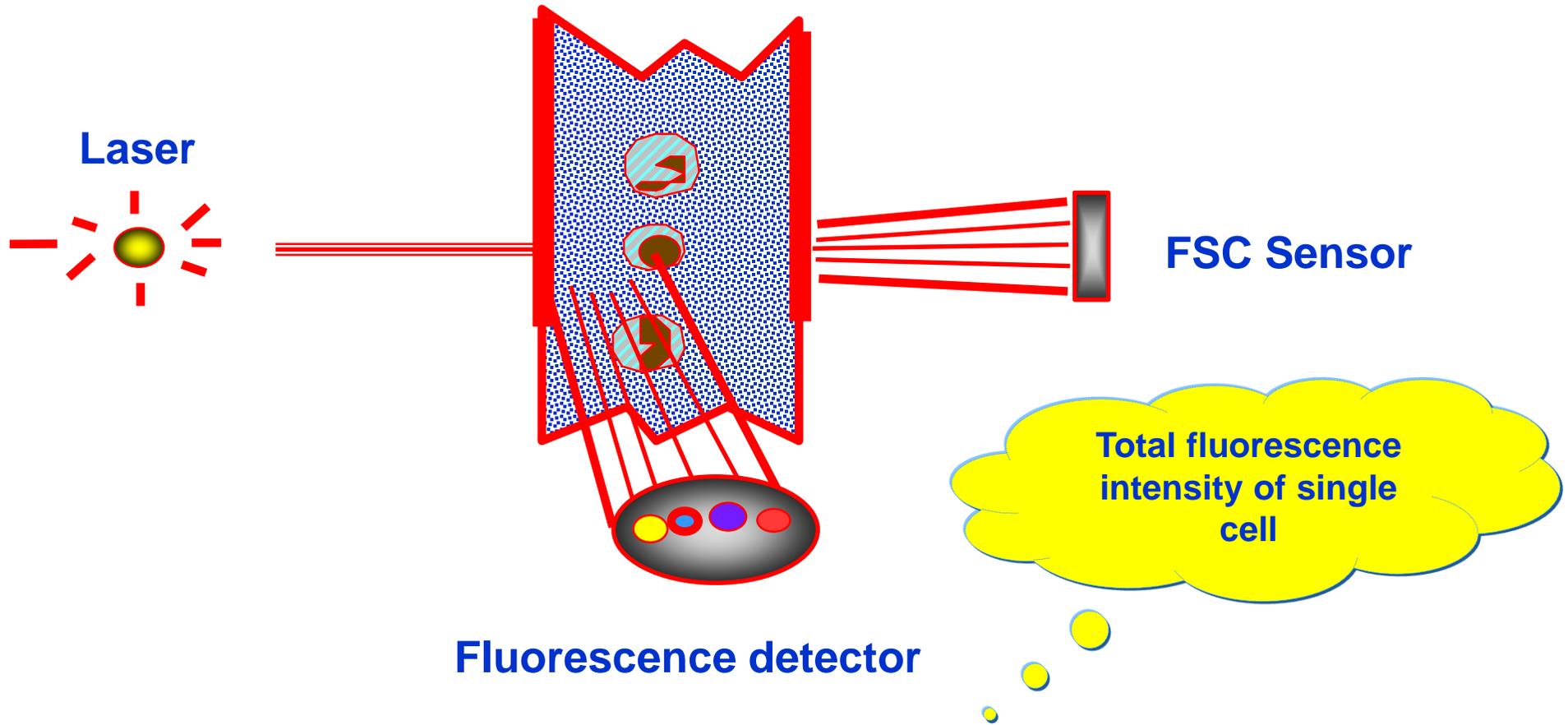
✚ FSC: 与球形细胞直径的平方密切相关。

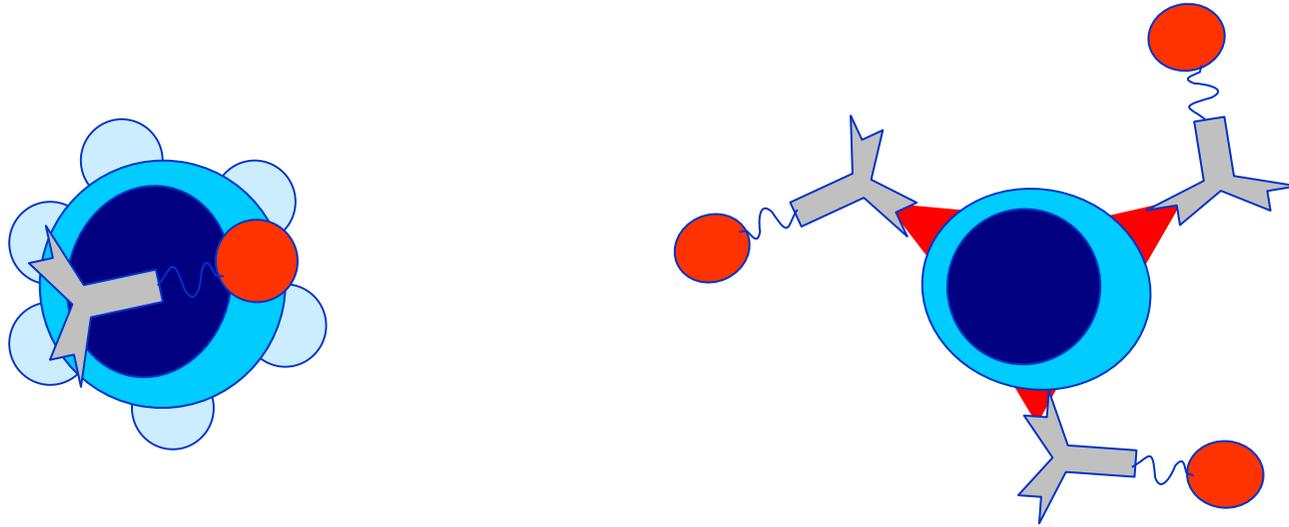
✚ SSC: 对细胞膜、细胞质的折射率敏感。对细胞质内较大的颗粒也有反映。用来获取有关细胞内部精细结构和颗粒性质的有关信息。



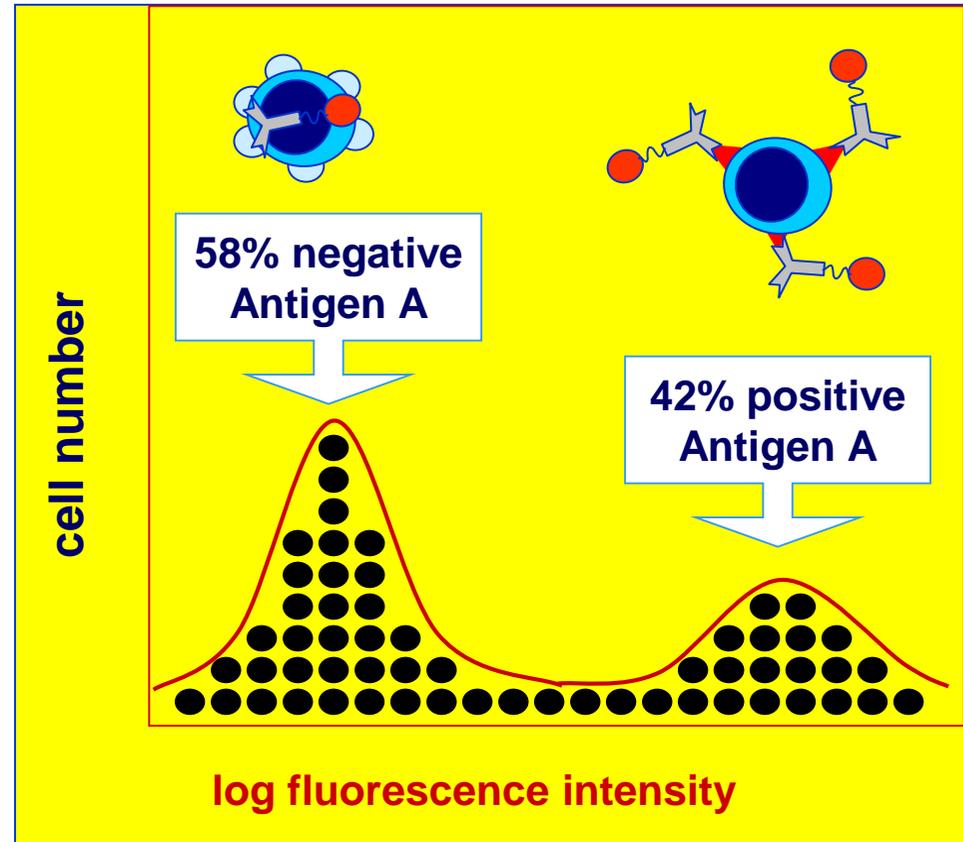
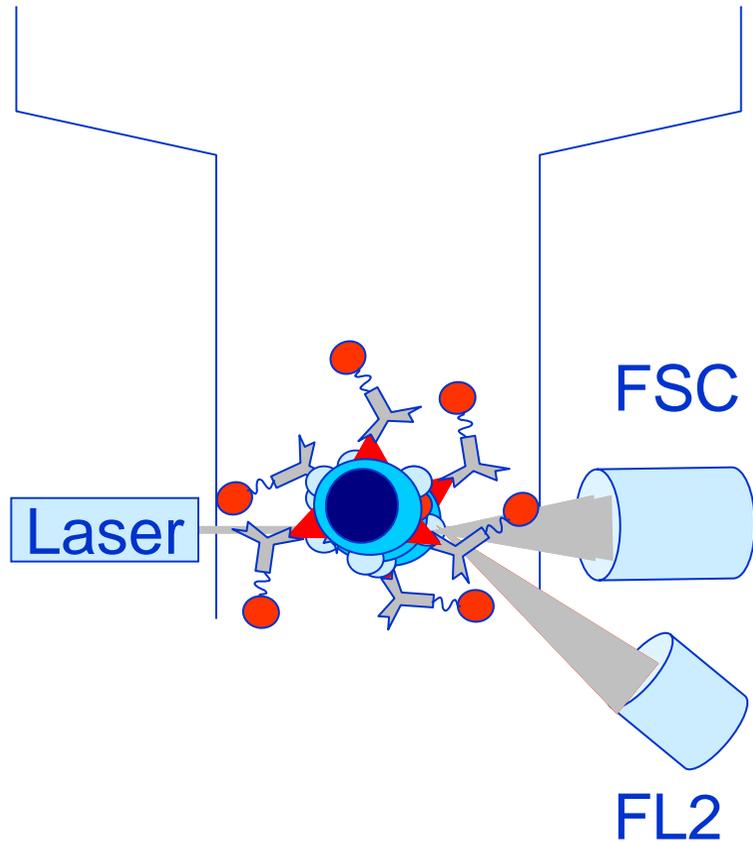


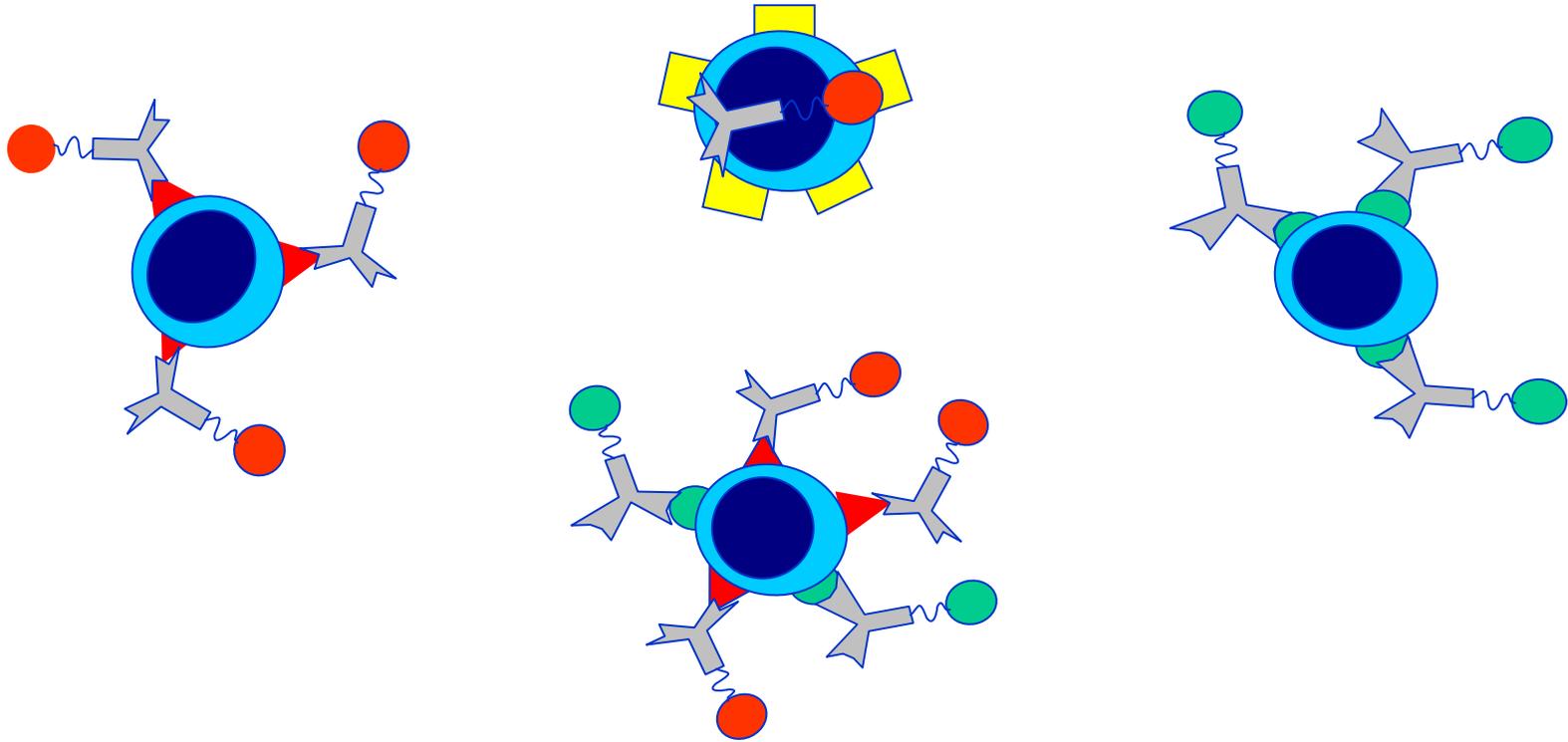
Fluorescence signal



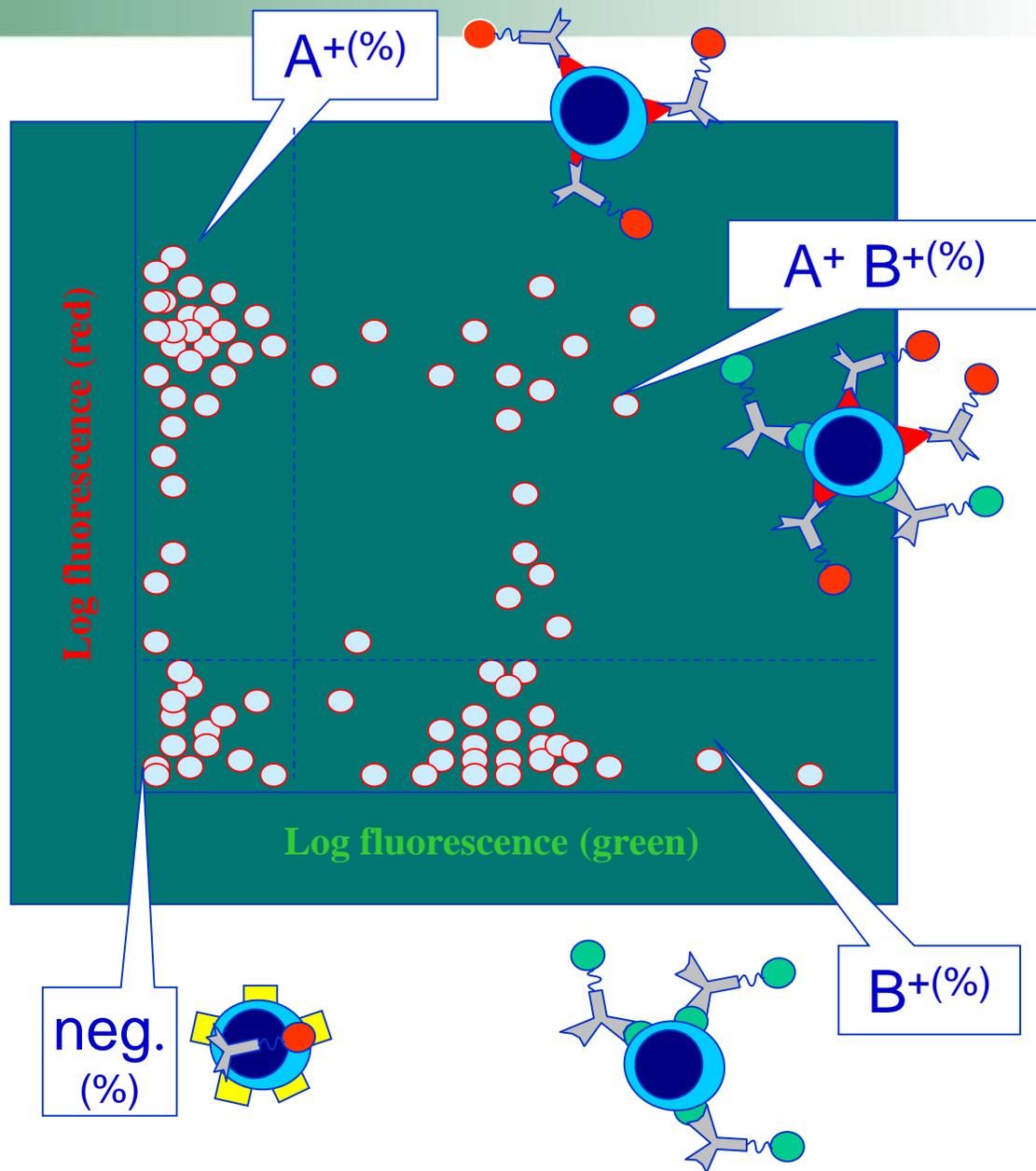
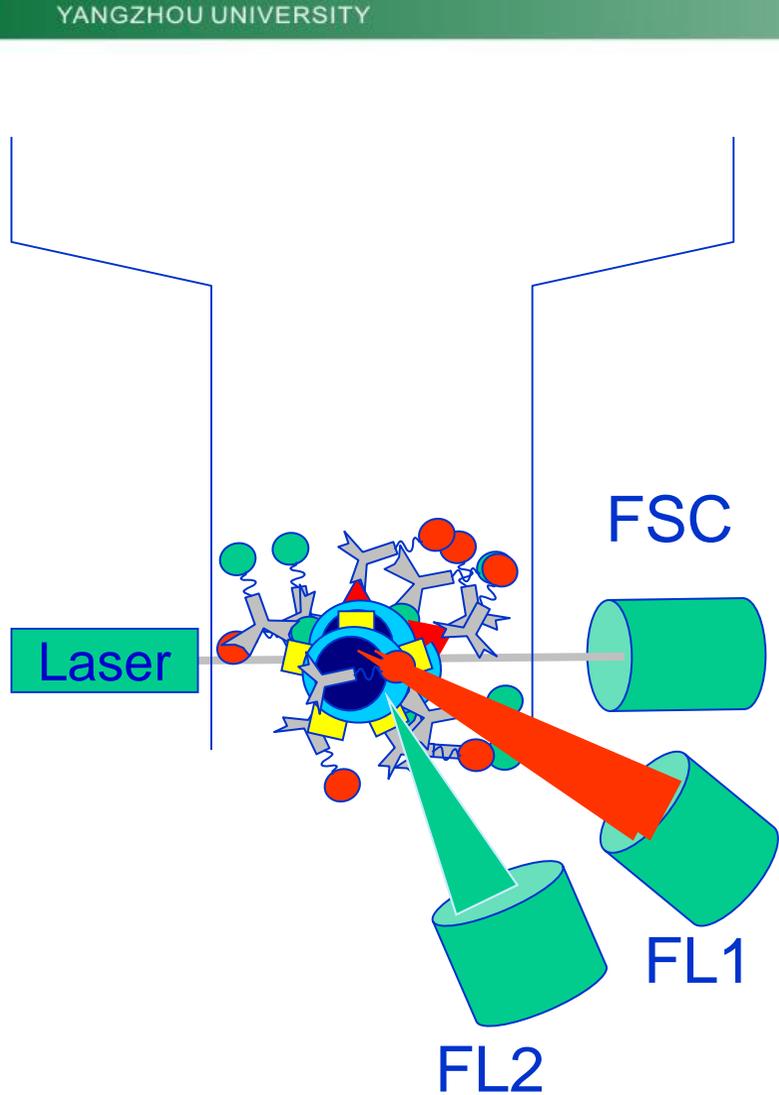


Single-stained sample

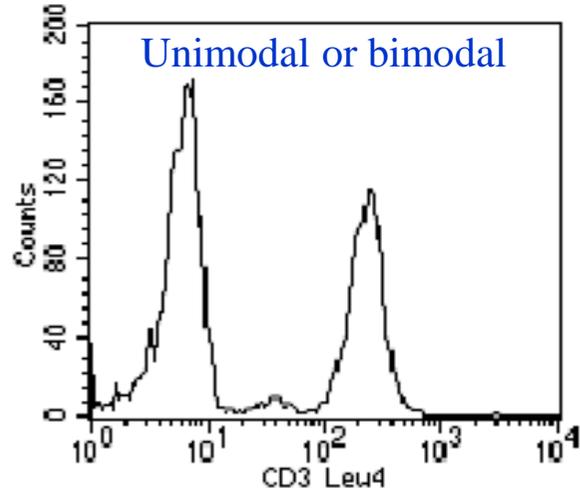




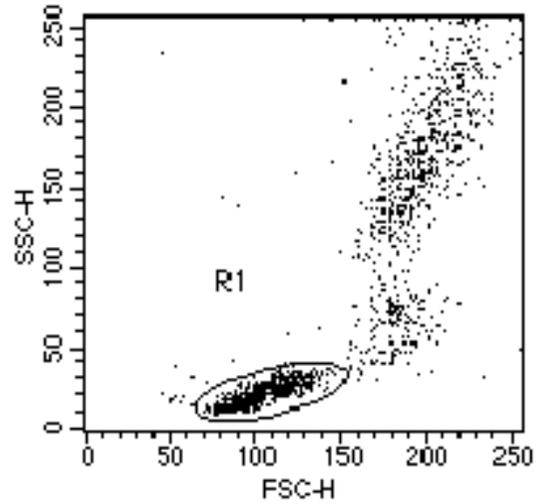
Double-stained sample



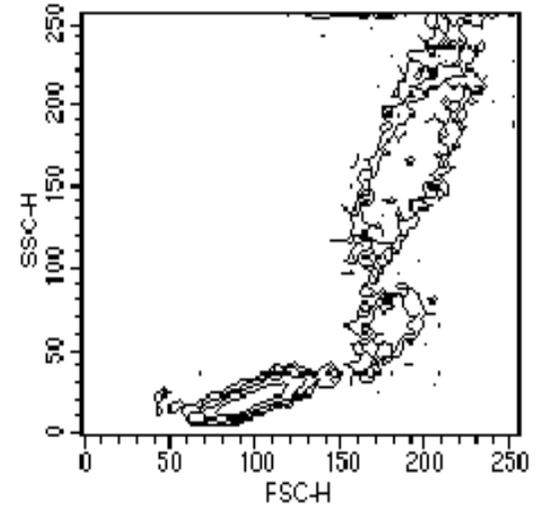
Histogram



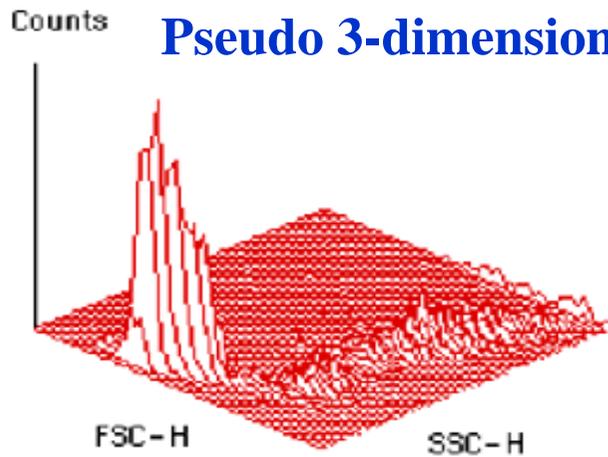
Dot Plot



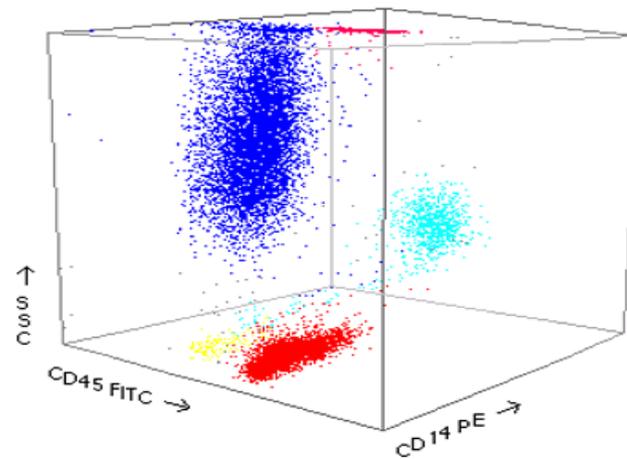
Contour Plot



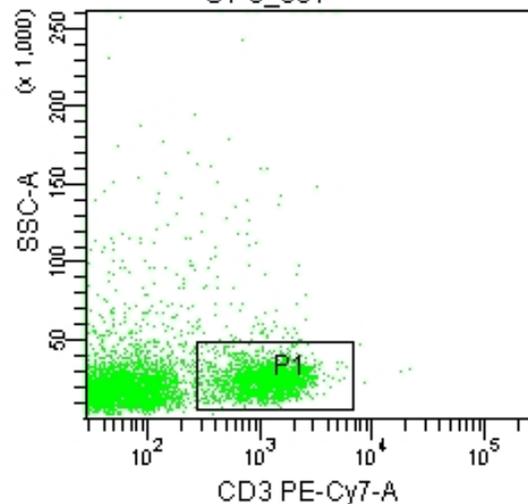
Pseudo 3-dimension Plot



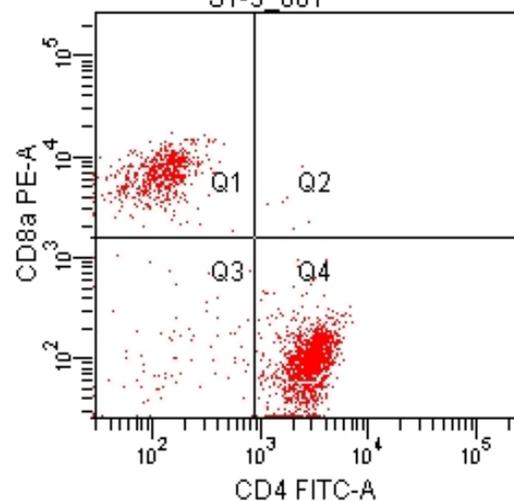
3-dimension Plot



S1-5_001



S1-5_001



Tube: 5_001

Population	#Events	%Parent	%Total
All Events	10,000		100.0
P1	1,910	19.1	19.1
Q1	576	30.2	5.8
Q2	7	0.4	0.1
Q3	84	4.4	0.8
Q4	1,243	65.1	12.4

Experiment Name: T lymphocyte subsets

Specimen Name: S1

Tube Name: 5_001

Record Date: May 31, 2007 8:10:32 PM

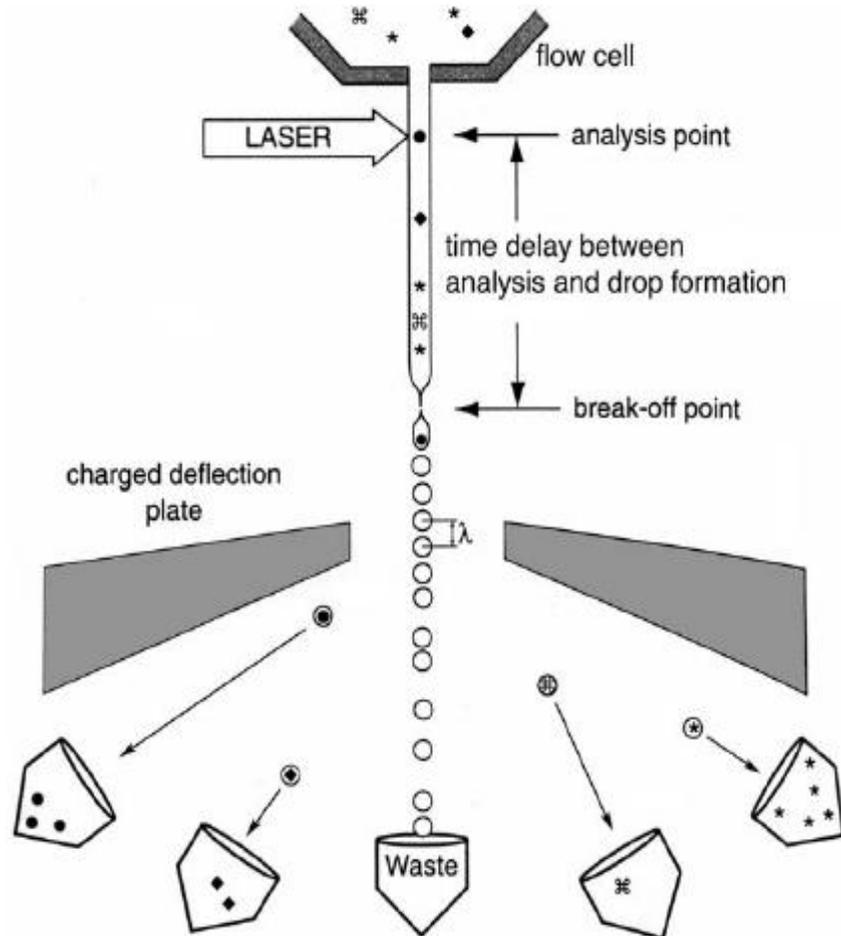
\$OP: Biotechnology

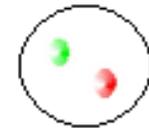
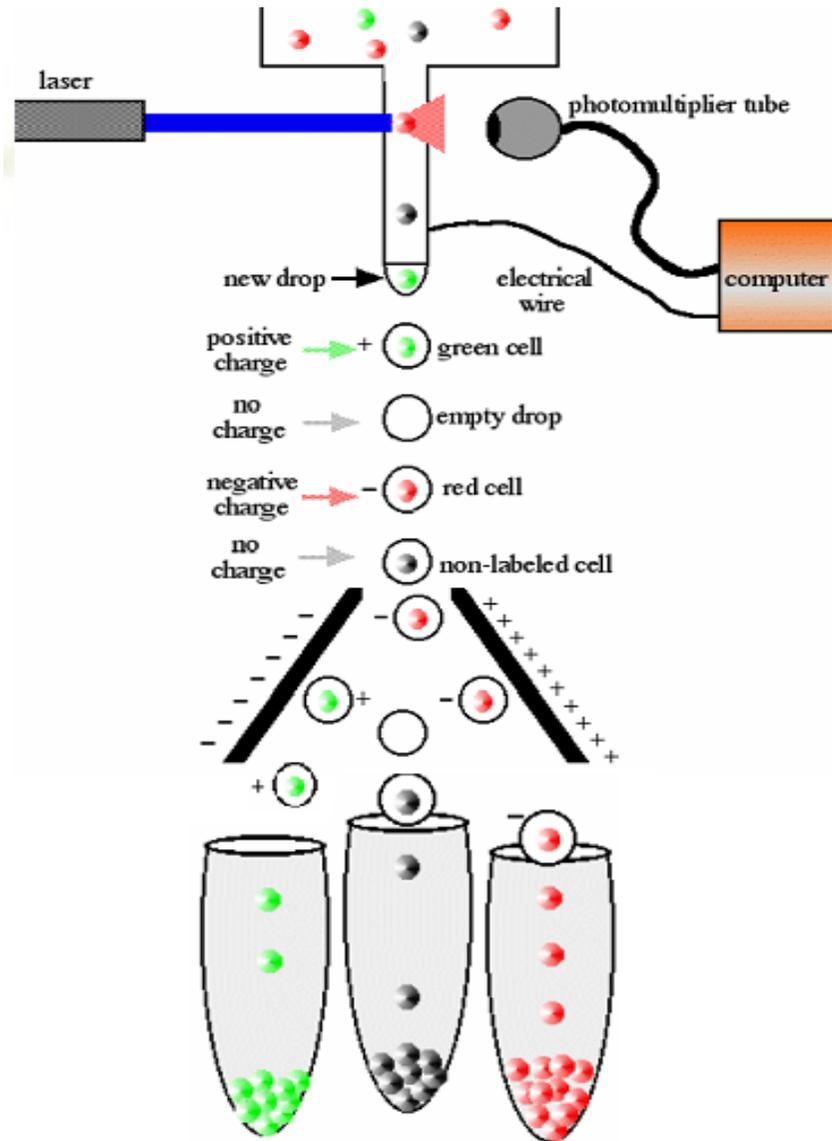
Population	#Events	%Parent	SSC-A Mean	CD4 FITC-A Mean	CD4 FITC-A CV	CD8a PE-A Mean	CD8a PE-A CV	CD3 PE-Cy7-A Mean	CD3 PE-Cy7-A CV
All Events	10,000	###	21,205	442	233.7	467	393.8	276	236.9
P1	1,910	19.1	23,747	1,927	78.1	2,229	158.3	1,276	51.7
Q1	576	30.2	25,121	124	51.7	7,089	37.8	1,038	44.9
Q2	7	0.4	23,864	1,999	28.7	4,309	55.4	933	48.4
Q3	84	4.4	25,365	274	93.6	175	123.2	989	110.9
Q4	1,243	65.1	23,001	2,873	32.7	104	74.6	1,408	47.0



- **“Particle”** can be used as a more general term for any of the objects flowing through a flow cytometer.
- **“Event”** is anything that has been interpreted by the instrument, to be a single particle , correctly or incorrectly.
- Each measurement from each detector is referred to as a **“parameter”**.
- Data are stored in so-called **flow cytometry standard (FCS)** format.
- Data stored on one cytometer may not be analyzable on software from another cytometer.

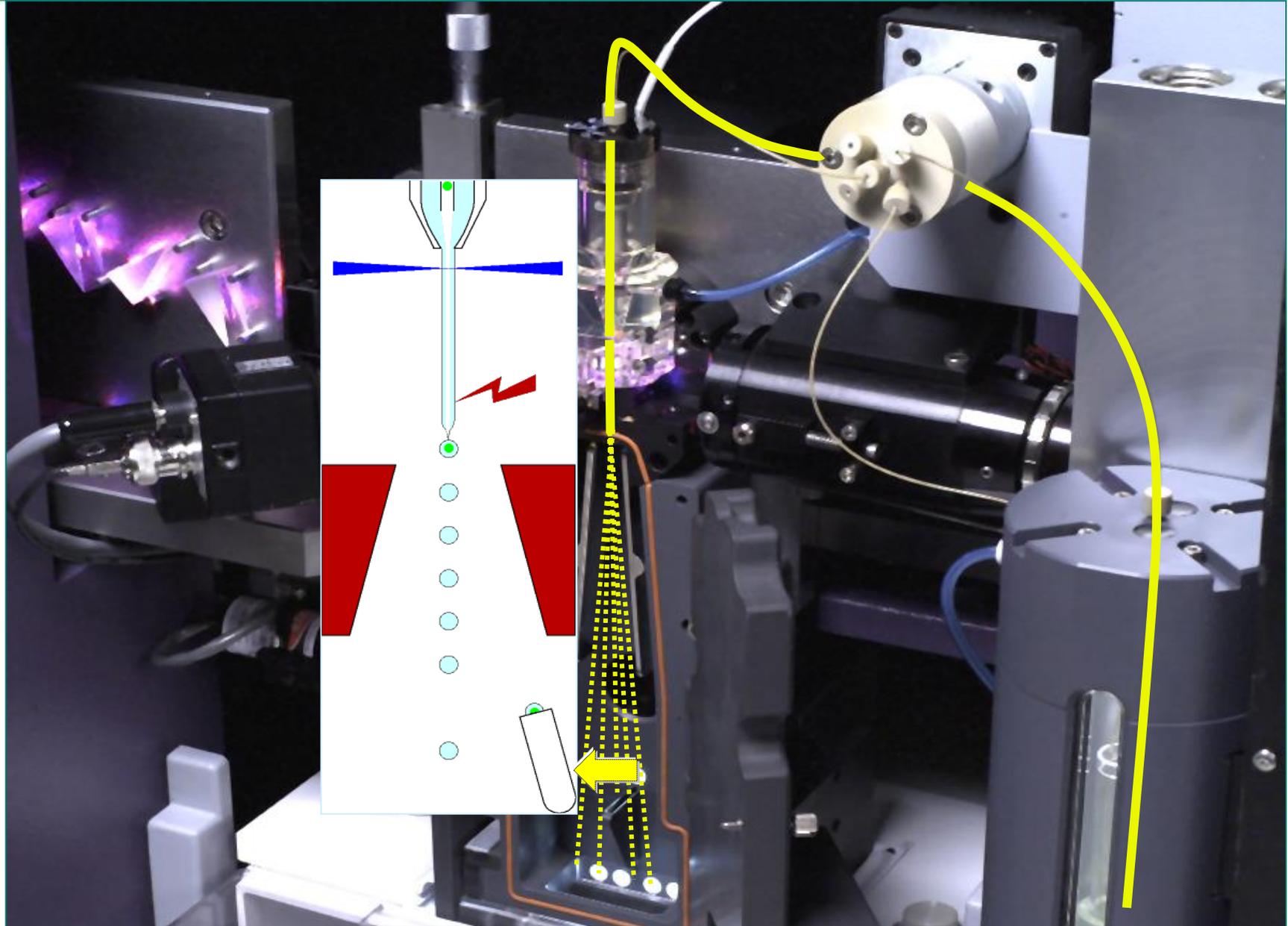
Cell Sort





纯度(purity)

产率(yield)



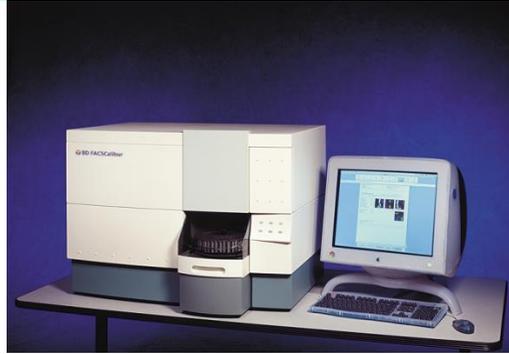


Estab
monoclon

Ernst Bö
B
Institute of A

Cell sorting was performed on a BD FACS Vantage™ flow cytometer (BD Biosciences, San Jose, CA, USA), equipped with a 5-W Argon laser (Coherent, Santa Clara, CA, USA) for 488 nm and a 6-W Argon laser (Spectra Physics, Irvine, CA, USA) for multiline UV excitation, and an automatic cell deposition unit for sorting into 96-well plates. FITC fluorescence was measured in F11 using the standard 530/30BP filter, DAPI fluorescence in F15 with a 424/44BP filter. Data acquisition and analysis were performed with CellQuest™ software.

厂家、
型号、
激光器、
滤光片、
软件
Company,
Pattern number,
Laser,
Light filter,
Software



Becton Dickinson (Maxwell W. Becton and Fairleigh S. Dickinson BD):

FACS

“Fluorescence Activated Cell Sorter”

FACS I,II,III,IV,400,420,440,FACStar,Vantage, DiVa, **Aria** : Sorters. FACS Analyser, can,
Calibur* , LSR, Canto: ‘Benchtop’.





Coulter (Beckman Coulter) : EPICS

“Electronic Programmable Individual Cell Sorter”

Profile, Elite, XL, ALTRA: a mixture of sorters and ‘benchtops’.

Other Manufacturers:

Partec, Ortho (Cytofluorograph, Cytoron),
Skatron, Partec, Dako (Galaxy), Cytomation (MoFlo).

July 2002 : DakoCytomation: Cyan benchtop.



Table 1-1 Default setup for detector arrays

Detector Array (Laser)	PMT	LP Mirror	BP Filter	Intended Dye
Octagon (488-nm blue laser)	A	735	780/60	PE-Cy7
	B	655	695/40 675/20	PerCP-Cy5.5 or PI PerCP
	C	595	610/20	PE-Texas Red
	D	556	575/26 585/42	PE or PI Alternative for PE/PI when not using PE-Texas Red
	E	502	530/30	FITC
	F	—	—	488/10
Trigon (633-nm red laser)	A	735	780/60	APC-Cy7
	B	—	660/20	APC
Trigon (407-nm violet laser)	A	502	530/30	Alexa Fluor 430
	B	—	450/40	Cascade blue, Pacific blue, DAPI, Hoechst, Alexa Fluor 405

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