

CytoFlex流式分析仪及应用介绍

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——流式原理简介及应用

——仪器特色介绍



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流式分析原理简介

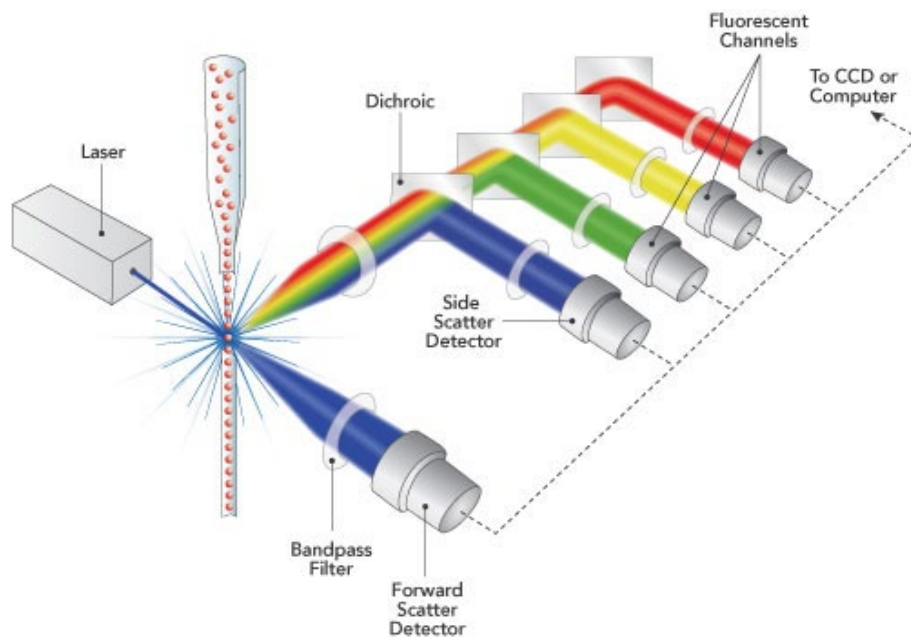
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流式细胞仪的组成

- 鞘流系统;
- 光学系统;
- 电子系统;
- 分选系统 (分选型流式)



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鞘流系统

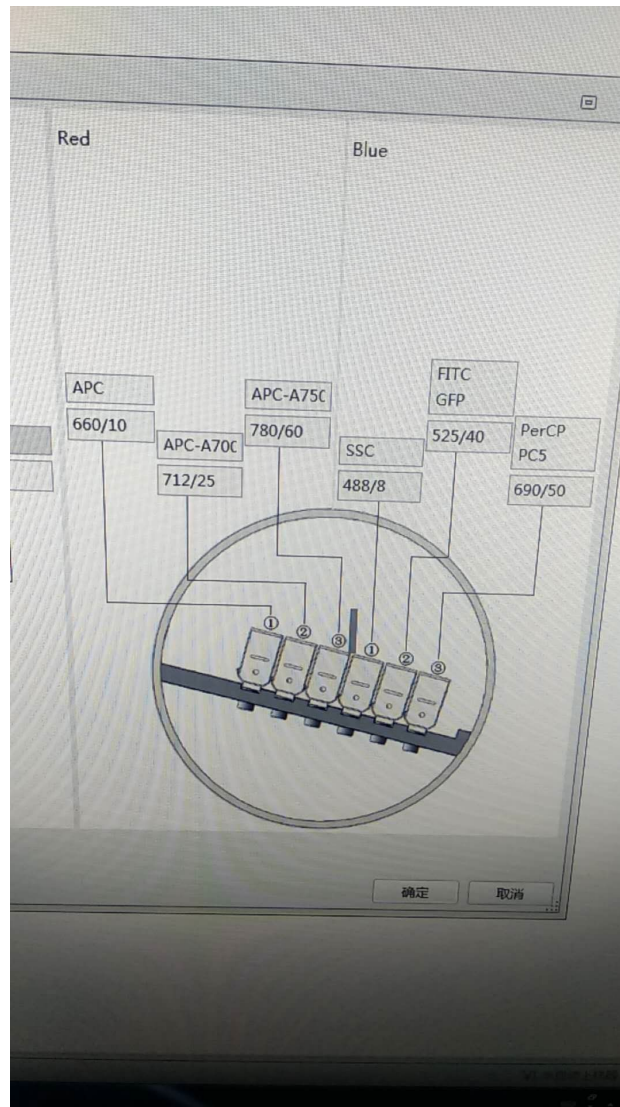
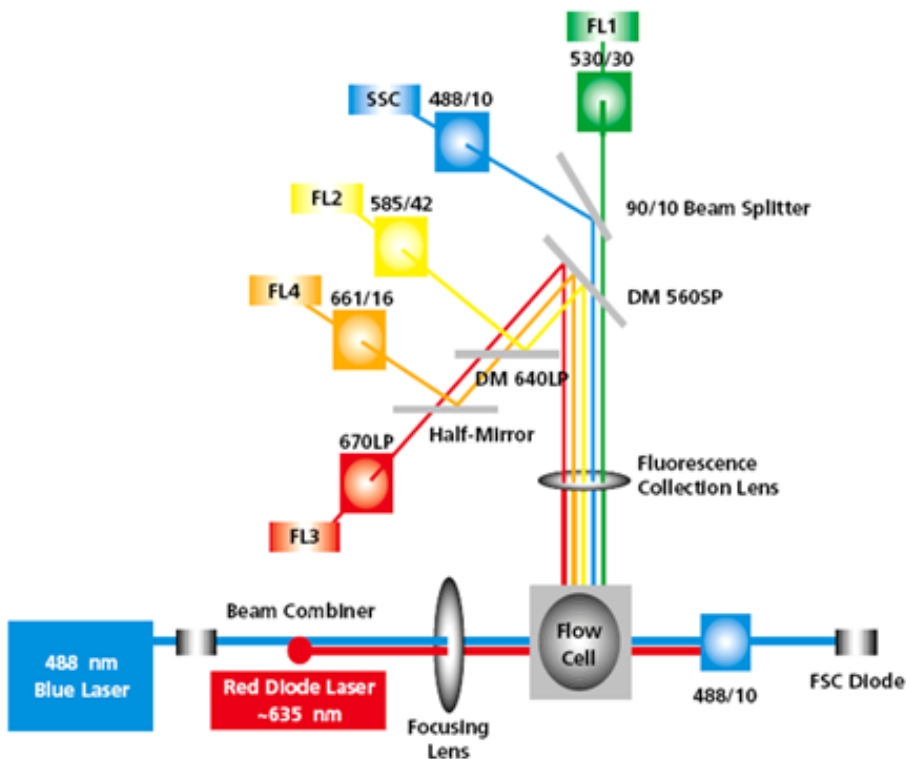
层流原理：

- 稳定的同轴流动状态

鞘液作用：

- 形成单细胞流
- 聚焦
- 防止堵塞

光学系统



在仪器配置信息，我们可以看到仪器所配备的激光器及相应的滤光片信息

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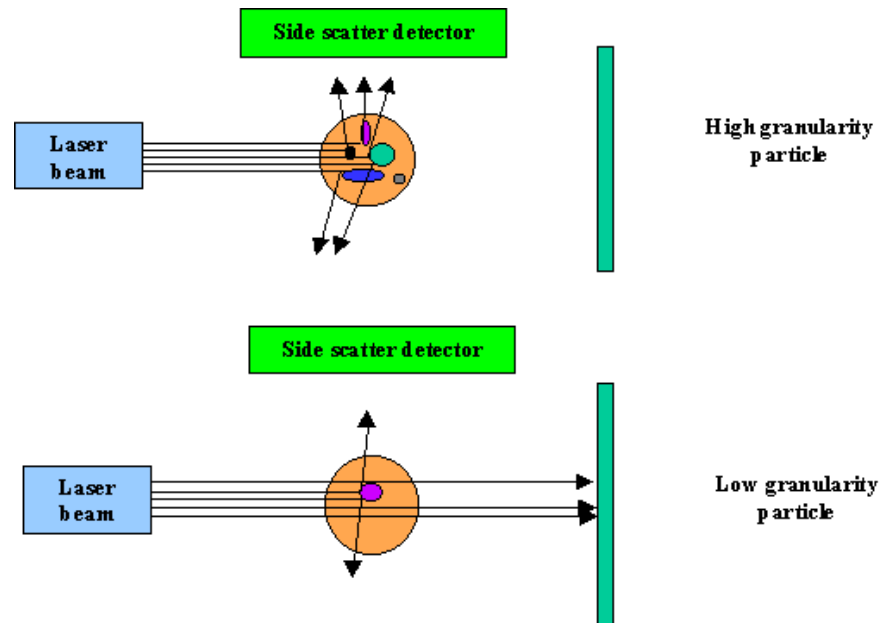
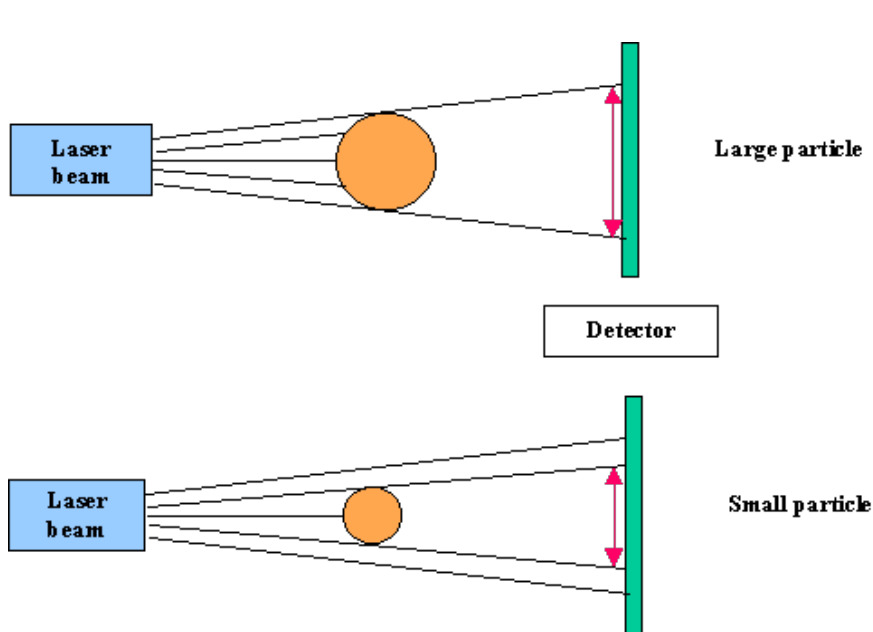
流式细胞术基本原理

Flow Cytometry

流式细胞仪工作原理

前向散射光 FSC 反应细胞的大小

侧向散射光 SSC, 反应细胞的复杂程度



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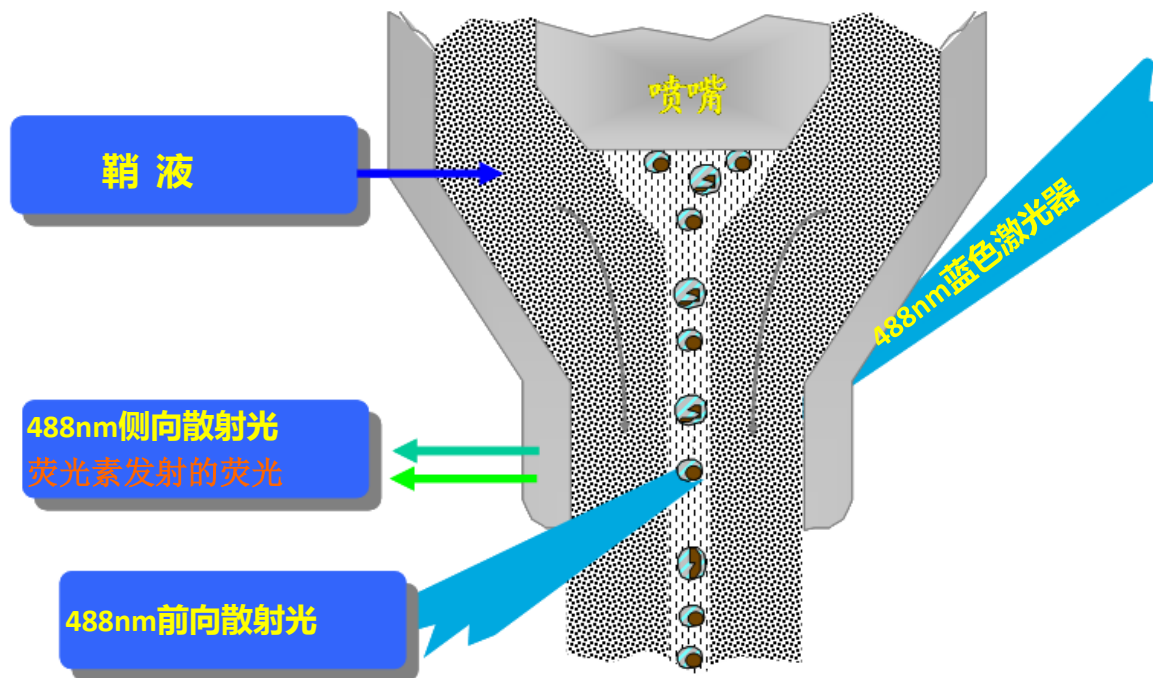
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光学系统

Flow Cytometry

荧光标记的单细胞液
流经过激光的照射



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• 流式试剂相关知识

CD3- FITC
CD4- PE
CD8- PC5
CD14- APC
Annexin V- FITC

抗体分子

荧光素

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荧光素相关知识

★ 任何发荧光的物质分子都具有这两个特征光谱 (nm)

区别

★ 激发光谱 (Excitation, Ex) : -选择激光器

★ 是指能特异性地激发某种荧光素的一定波长范围内的光线, 也称为吸收光谱。

★ 激发波峰 (最大吸收波长) : Ex-Max

★ 发射光谱 (Emission) : -流式滤光片接收的光

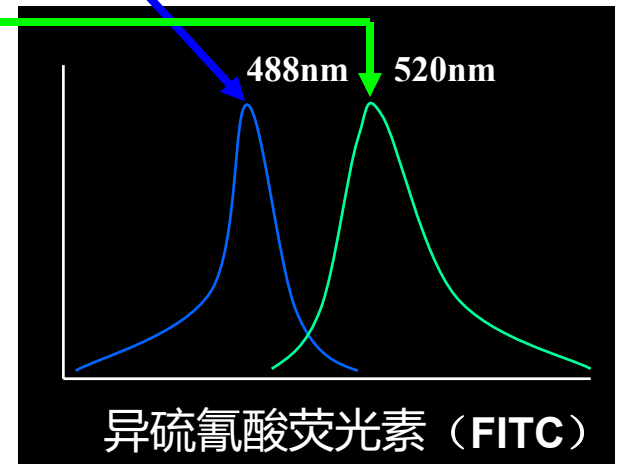
★ 是指某一波长激发光引起荧光素发射的一定波长范围内的荧光

★ 发射波峰 (最大发射波长) : Em-Max

★ 荧光素的使用:

★ 选择正确的激光器

★ 确定所需荧光探测器 (PMT+滤光片)



常见荧光素或荧光染料

488nm激发的荧光素

- FITC
- PE
- ECD
- PerCP
- PC5
- PerCP-CY5.5
- PC7
-

633nm激发的荧光素

- APC
- Alexafluor700
- Alexafluor750
-

405nm激发的荧光素

- Pacific blue
- Krome Orange
-

荧光染料:

- PI
- GFP
- TO
- DAPI
-



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何为荧光补偿？

为何要进行荧光补偿？

如何进行荧光补偿

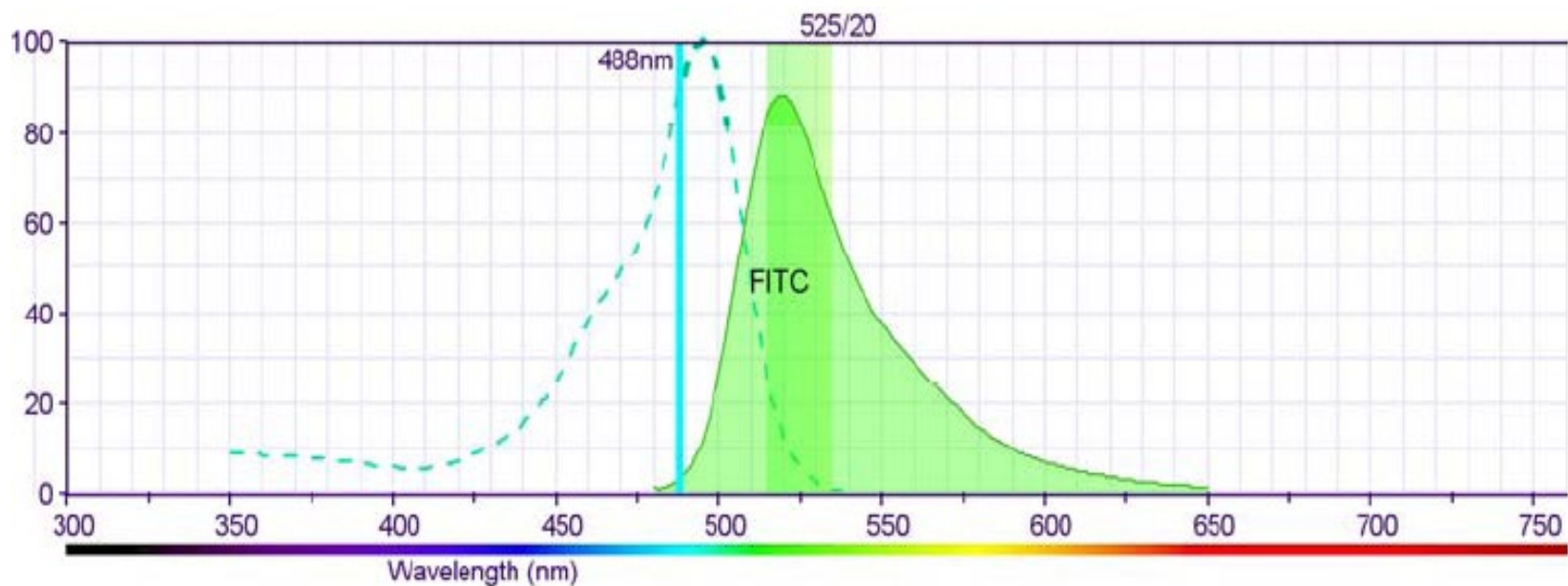
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Fluorescence Spectrum Viewer

FITC



Fluorochrome

Ex% Ex Em

Filters

Filter Performance Estimates (for FWH)

FITC	88	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	525/20	<input checked="" type="checkbox"/>	FITC
						--

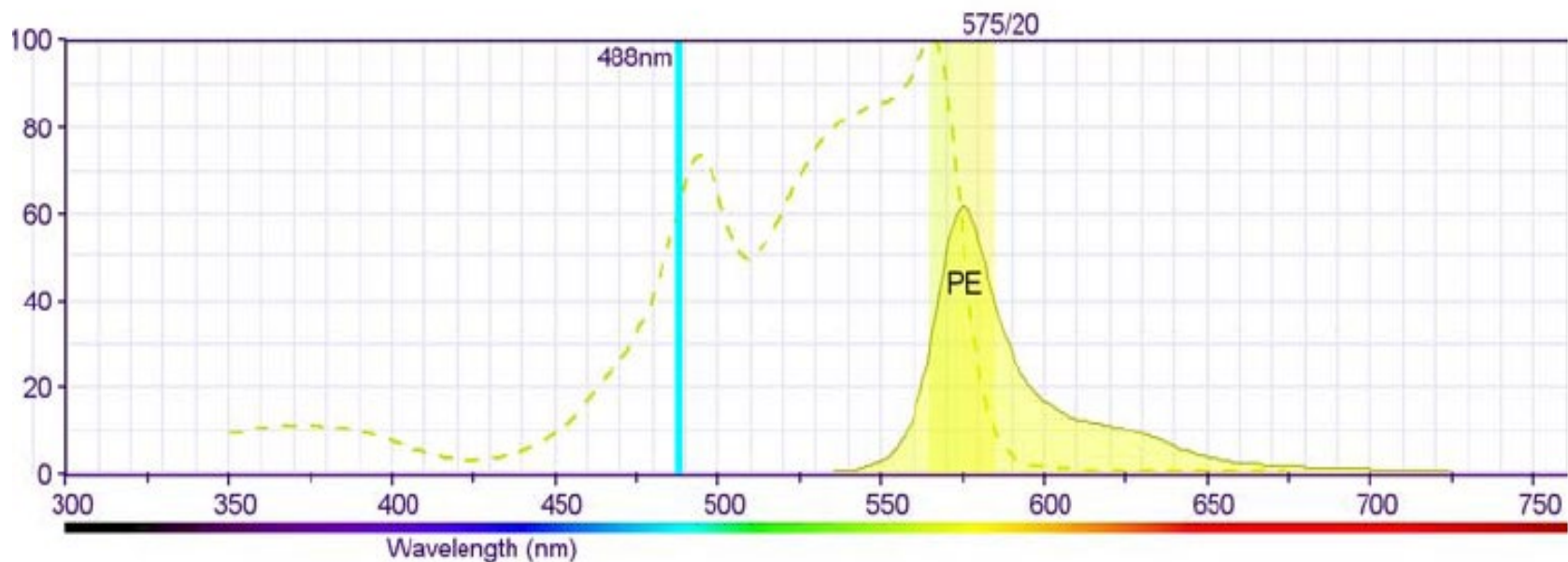
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Fluorescence Spectrum Viewer

PE



Fluorochrome	Ex%	Ex	Em	Filters	Filter Performance Estimates (for FWH)	
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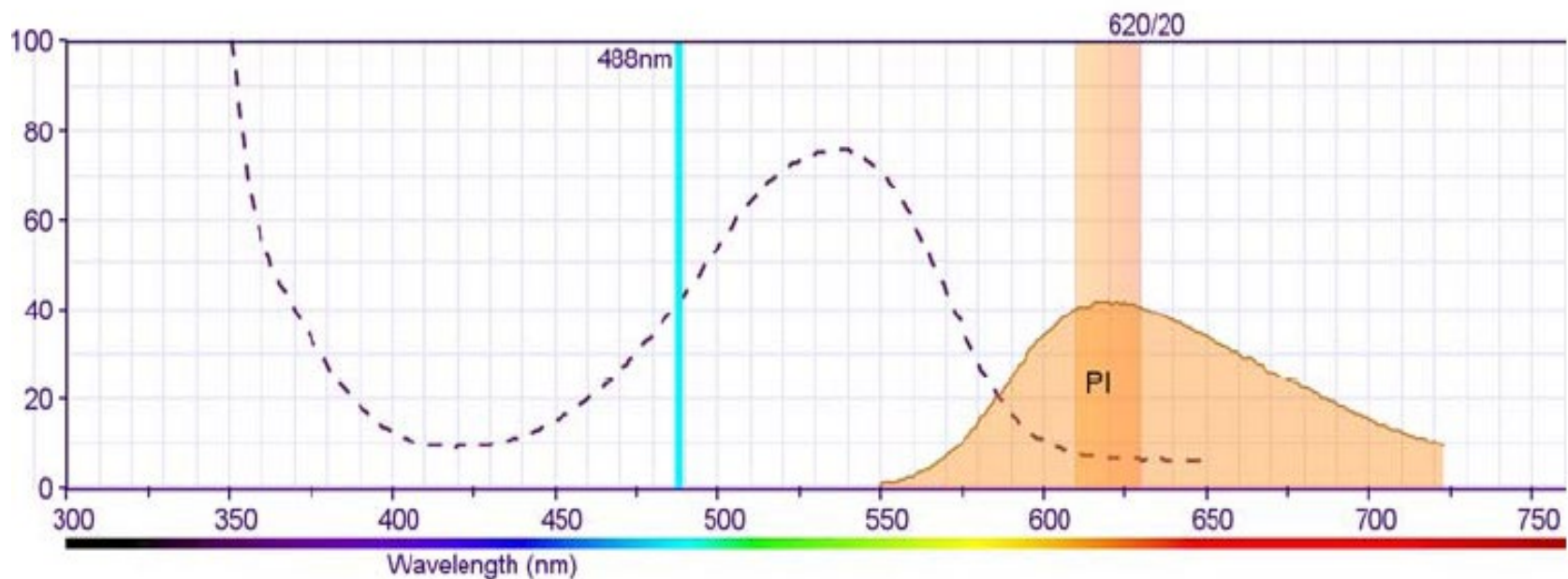
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Fluorescence Spectrum Viewer

PI



Fluorochrome	Ex%	Ex	Em	Filters	Filter Performance Estimates (for FWH)	
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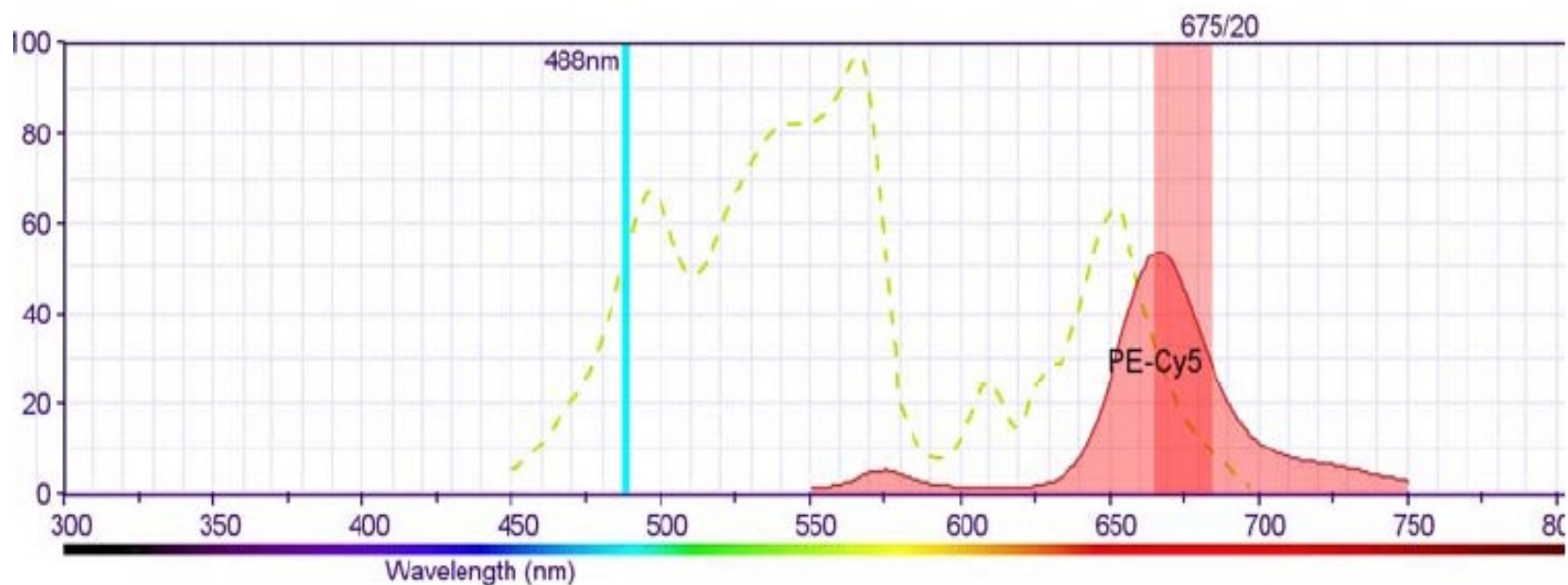
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Fluorescence Spectrum Viewer

PE-Cy5



Fluorochrome Ex% Ex Em Filters Filter Performance Estimates (for FWH)

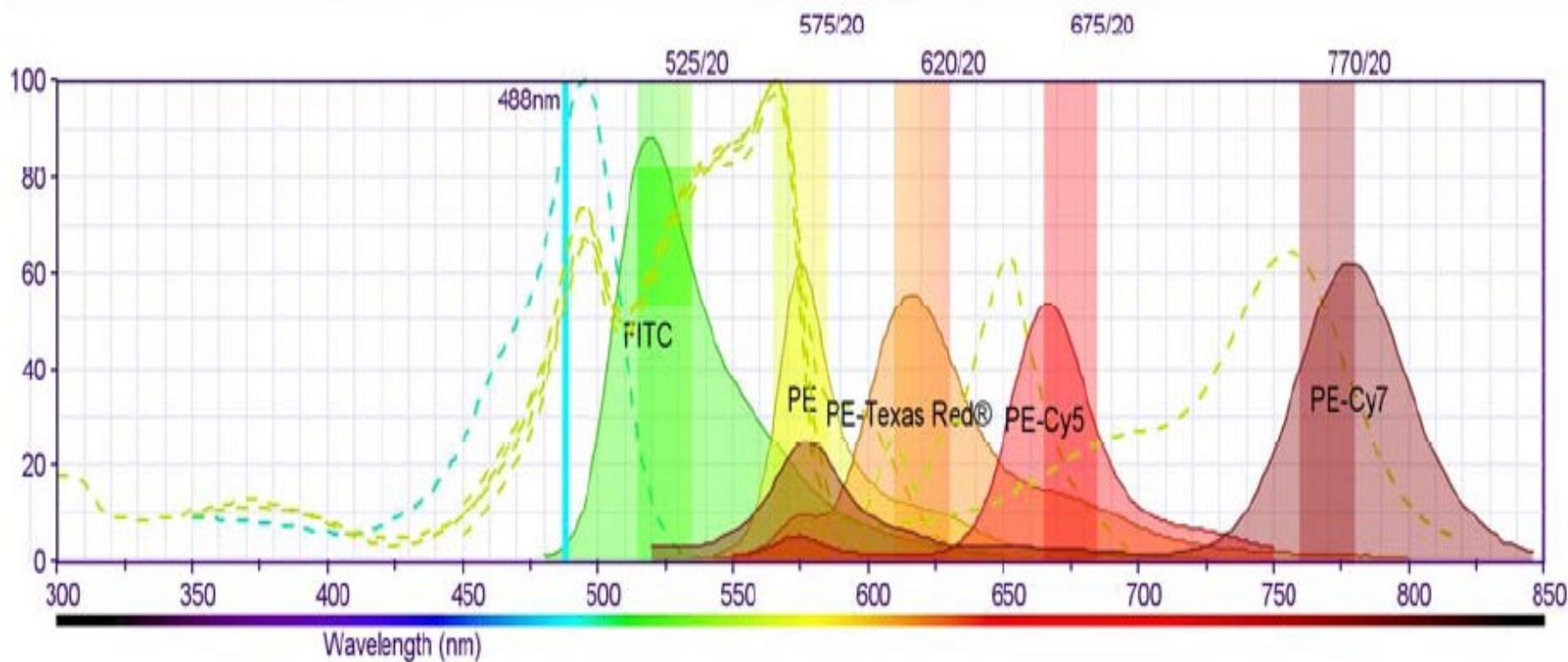
PE-Cy5 54 675/20

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荧光发射光谱重叠 spectral overlap



荧光补偿 (compensation) 是指在流式细胞多色分析中, 纠正荧光素发射光谱重叠的过程, 即从一个被检测的荧光信号中去除任何其他的干扰荧光信号. 将单种荧光素标记的单克隆抗体分别进行单色荧光染色.

※几色分析就需要制备几个补偿对照管

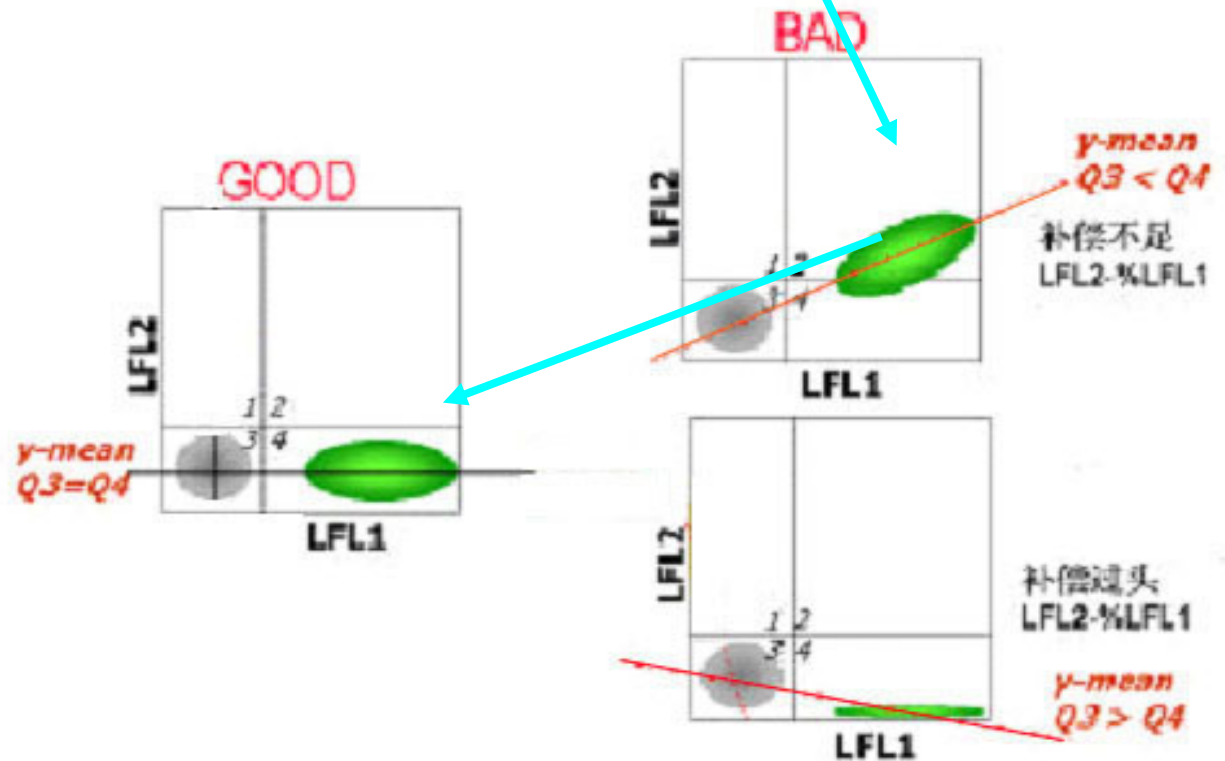
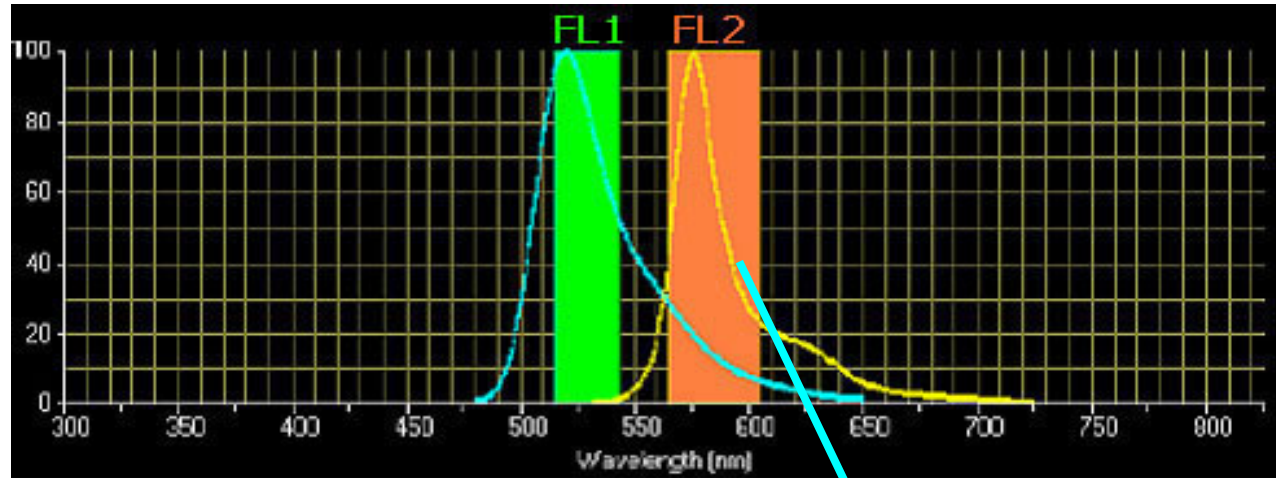
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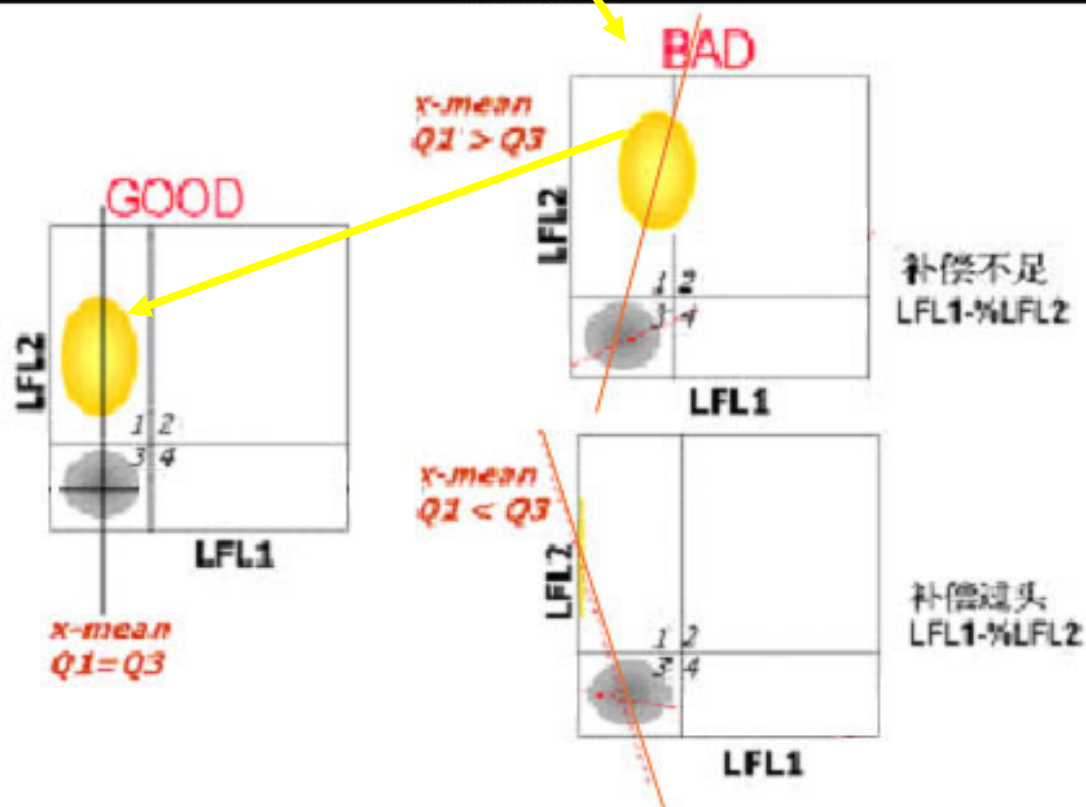
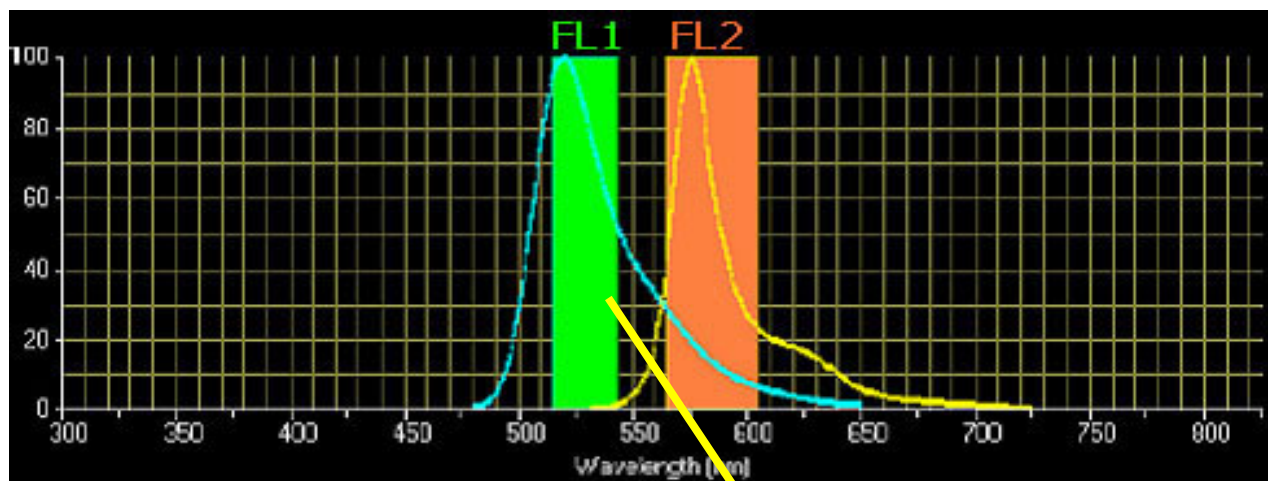
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如何进行荧光补偿

双色荧光补偿



双色荧光补偿



光学系统

Flow Cytometry

流式细胞仪工作原理

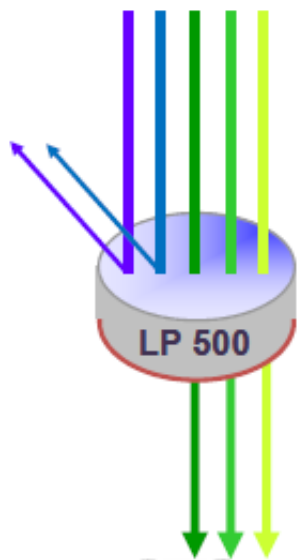
长通滤光片

短通滤光片

带通滤光片

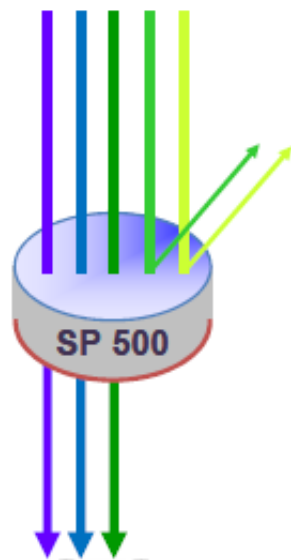
Long pass (LP)

460 500 540



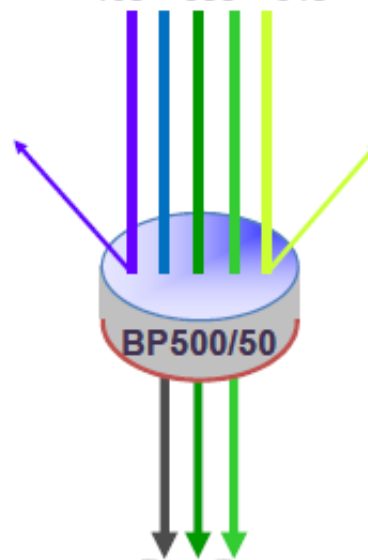
Short pass (SP)

460 500 540



Band pass (BP)

460 500 540



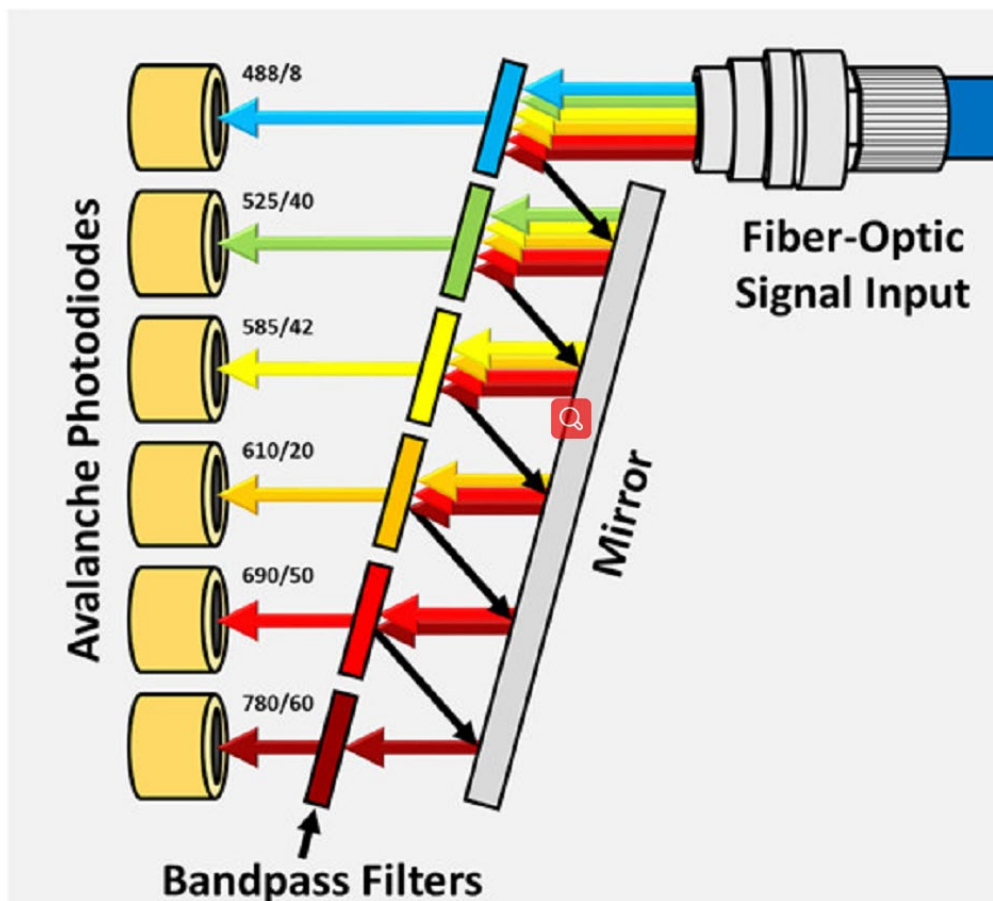
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光学系统

A. Wavelength-Division Multiplexing



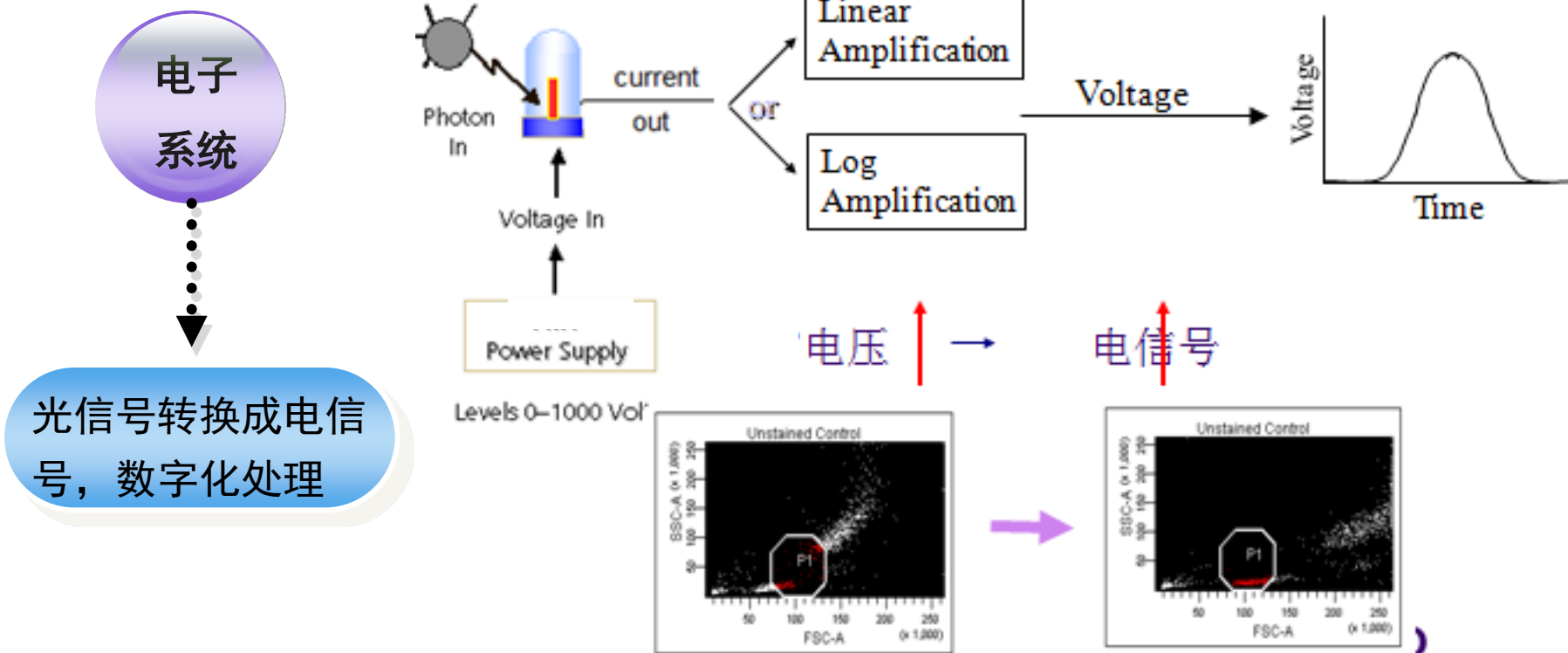
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电子系统

光电管和检测系统：放大器



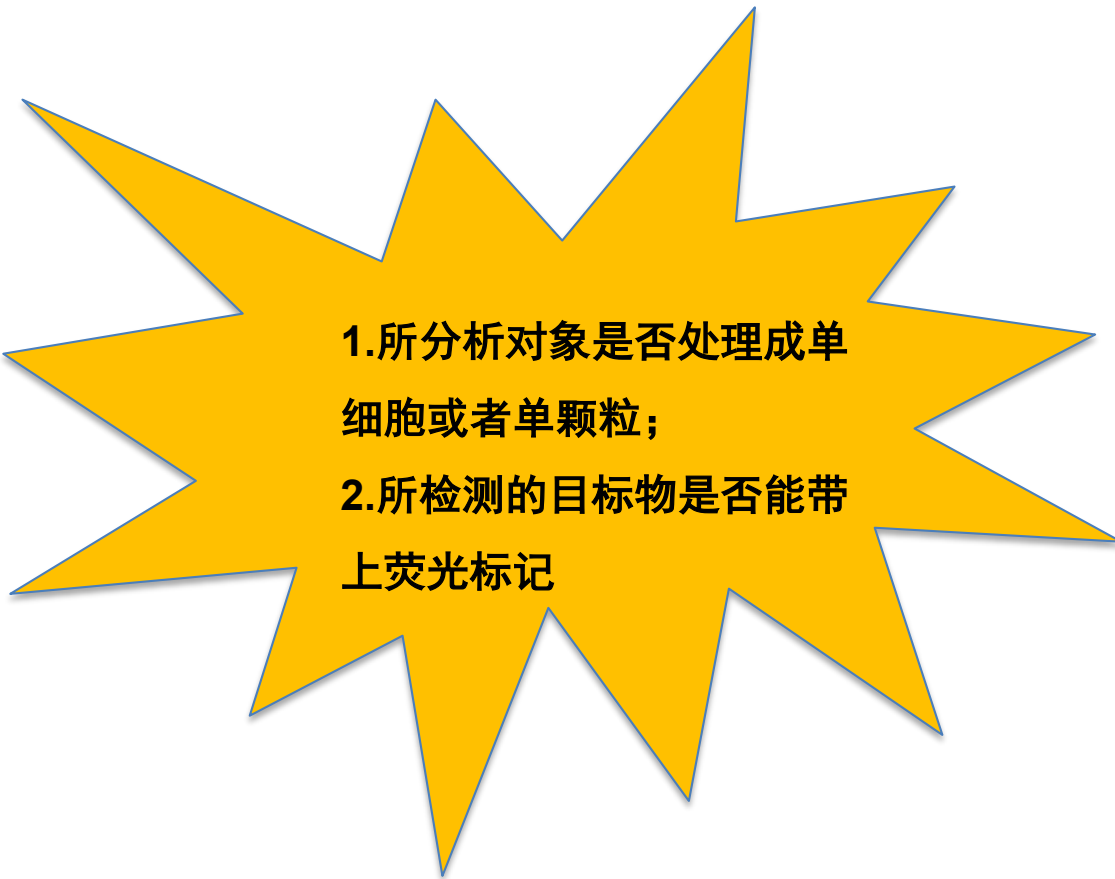
光信号转换成电信号，数字化处理

★ 基本原则

- 信号强弱差 < 10 倍 or 需线性关系：使用 **线性** 放大器 (如散射光, DNA 分析)
- 信号强弱差 > 10 倍 or 需分析弱荧光：使用 **对数** 放大器 (如荧光)

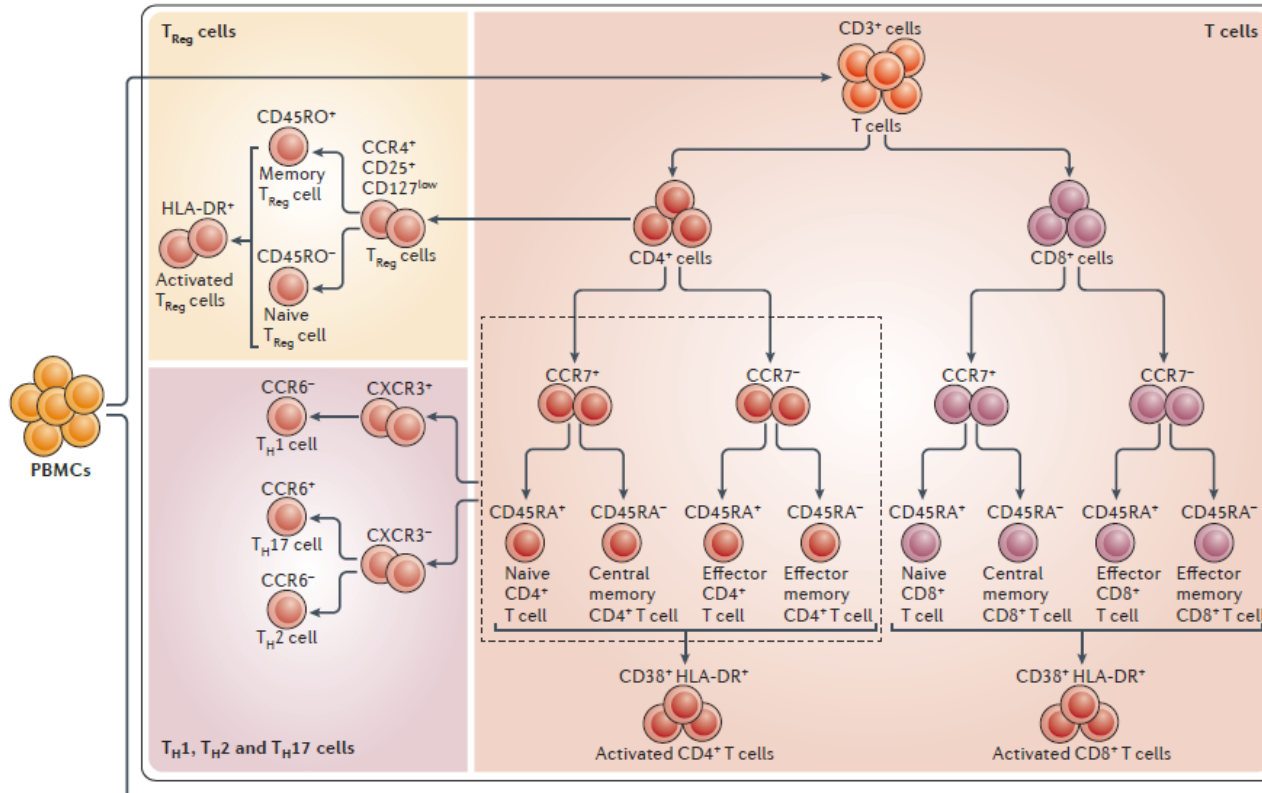
流式能做什么？

- ❖ 细胞表型鉴定
- ❖ 细胞胞内及核内因子检测
- ❖ 胞外分泌型蛋白检测
- ❖ 在细胞周期检测中的应用
- ❖ 在细胞增殖检测中的应用
- ❖ 在细胞凋亡检测中的应用
- ❖ 在细胞外囊泡中的检测应用
- ❖



1. 所分析对象是否处理成单细胞或者单颗粒；
2. 所检测的目标物是否能带上荧光标记

流式细胞术的应用举例-细胞表型鉴定



Standardizing immunophenotyping for the Human Immunology Project. Holden T ect. Nature Rev Immunol . ; 12(3): 191–200.

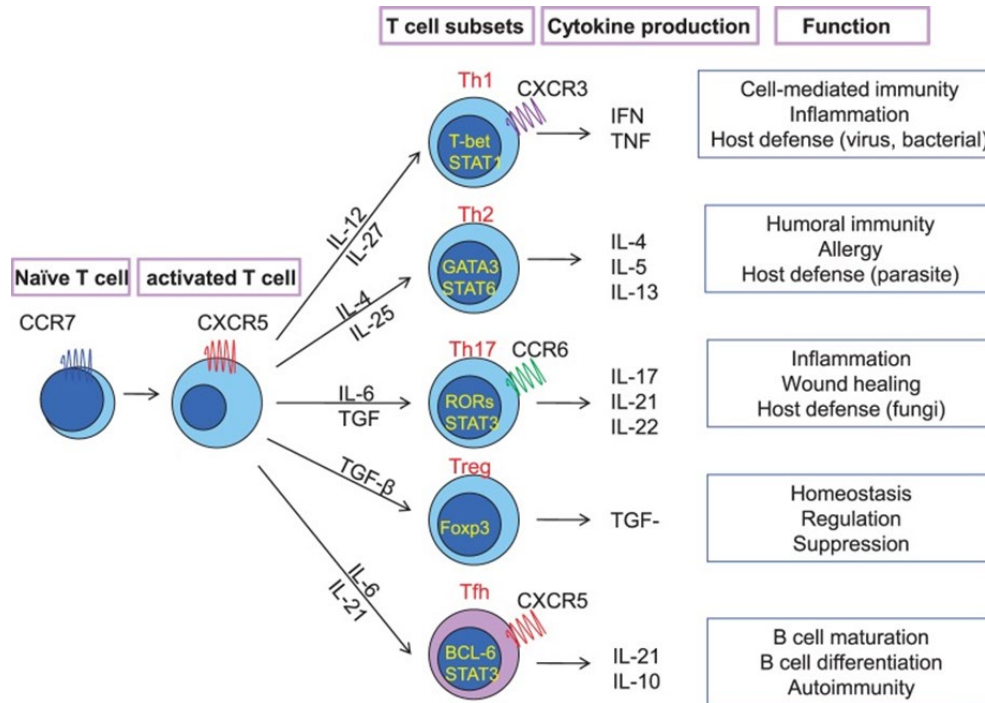
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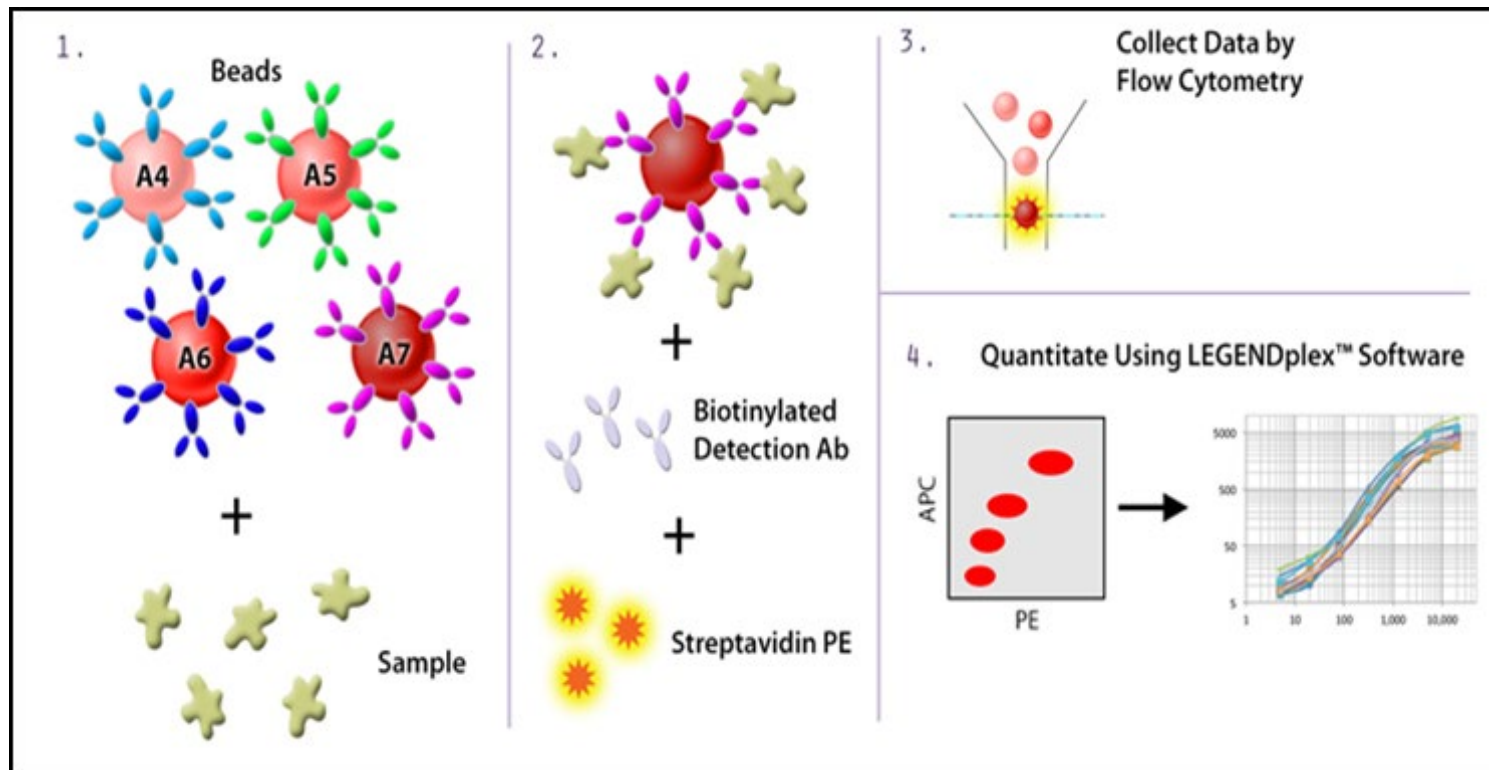
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流式细胞术的应用举例-细胞胞内及核内因子检测

为了进一步研究细胞功能，我们往往需要检测胞内及核内蛋白水平的变化，如IL-4, IFN- γ , IL-17, 颗粒酶, 穿孔素等等，同时核内转录因子的变化对实验结果也有很重要的影响，如Foxp3, T-bet等等。



流式细胞术的应用举例-胞外分泌型蛋白检测

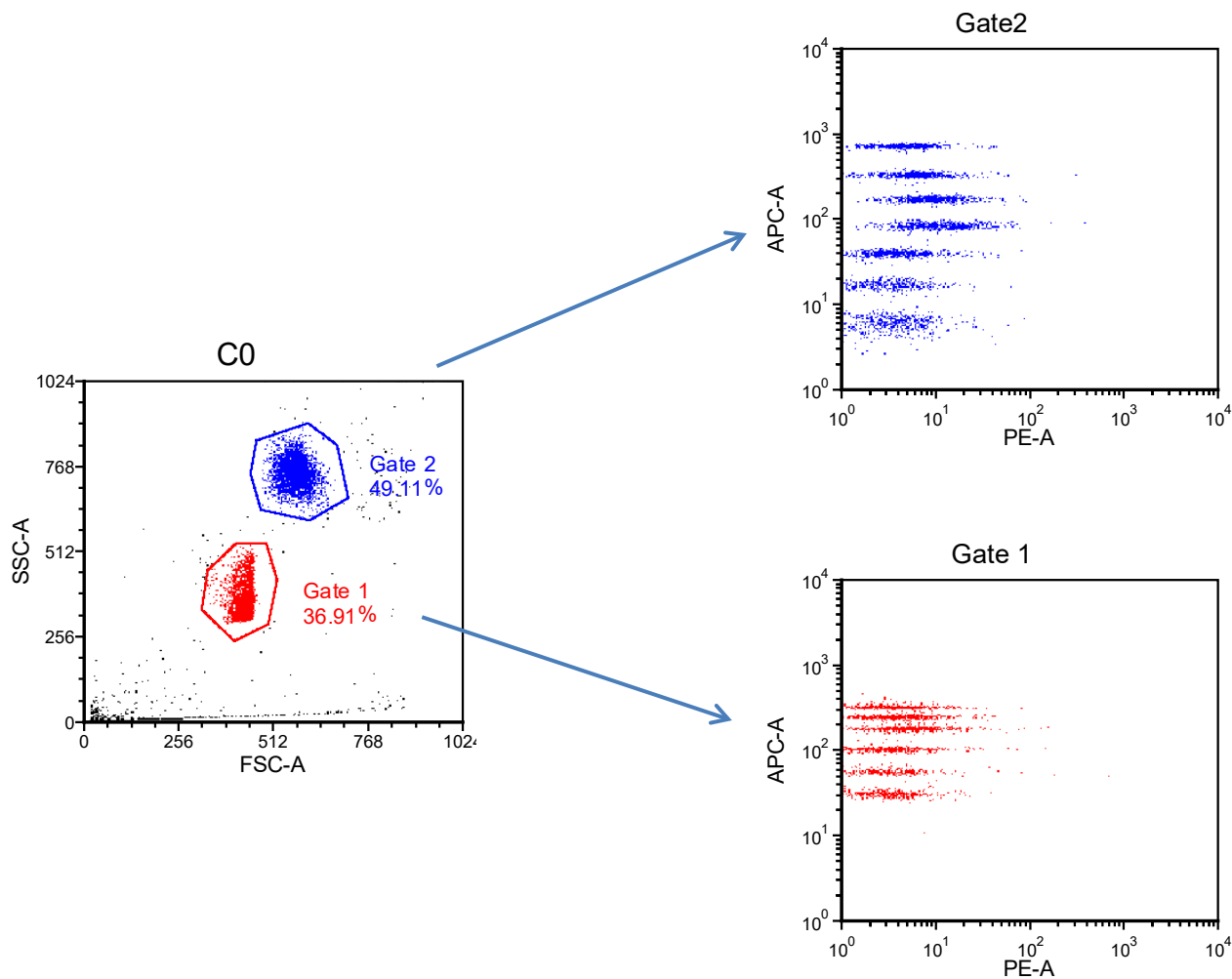


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流式细胞术的应用举例-胞外分泌型蛋白检测



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CytoFlex特色介绍

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高性能/ 技术的颠覆性革新

➤ 革新的流路

创新的流动室设计
专利的无脉冲蠕动泵

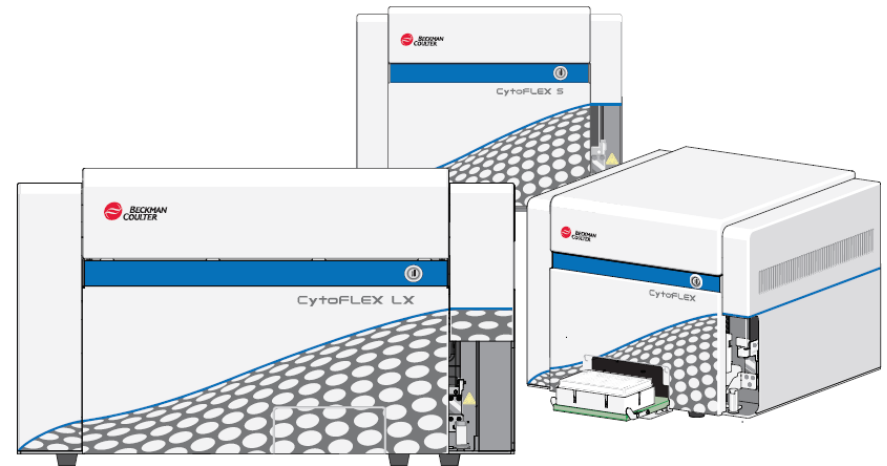
➤ 高效的光路

WDM模块
FAPD高效光电转换器
405nm作为SSC来源，拓展小颗粒检测范围

➤ 强大的电子系统

➤ 便捷的软件

智能补偿库-一次就好
强大的Cytexpert 软件



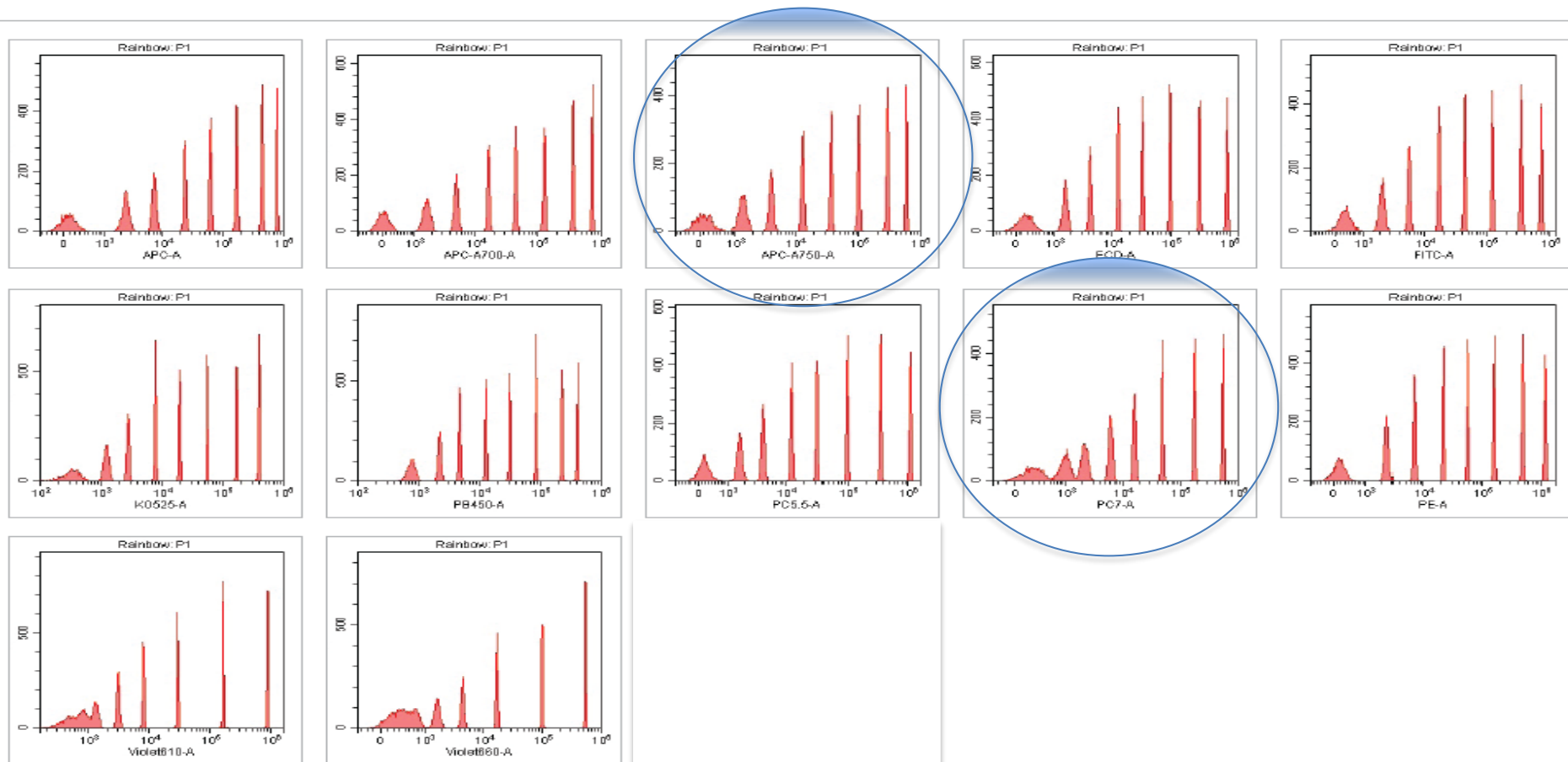
我们的使命：赋能中国每一位生命科学工作者

我们的愿景：持续改进和创新，助力生命科学研究与医药健康产业发展，成为最可信赖的合作伙伴，服务健康中国2030！

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优越的荧光灵敏度和分辨率



8-peak Rainbow Beads

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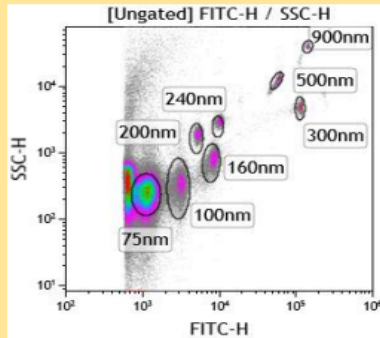
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光路：405nm作为SSC

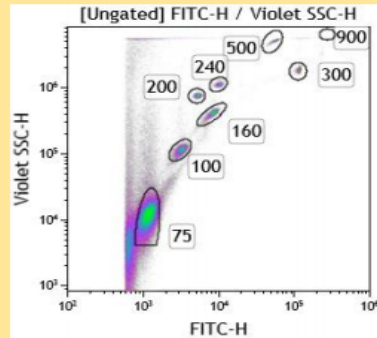
- 将小颗粒研究范围降至75nm左右，研究Microvesicle甚至部分Exosome

E) CytoFLEX vSSC resolves 75 nm beads

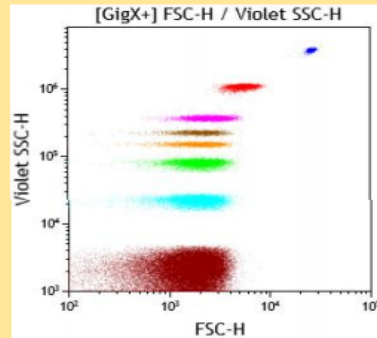
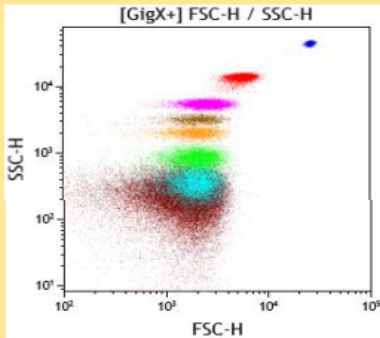
Blue SSC



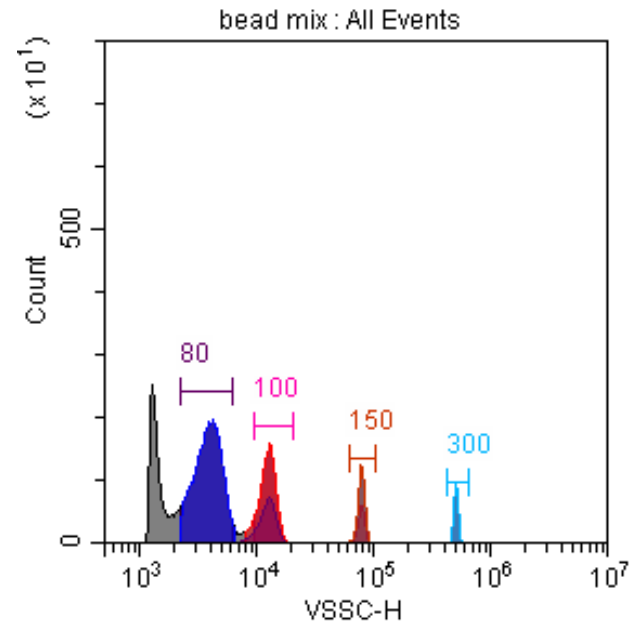
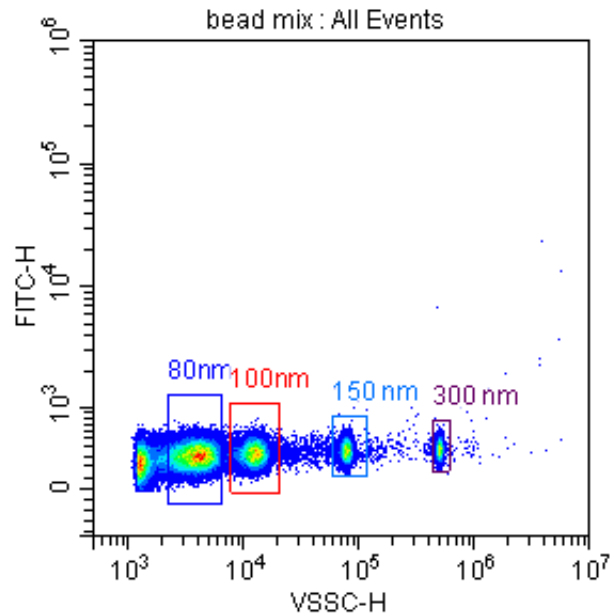
Violet SSC



- FITC trigger & Gigamix**
- Fluorescent 75 nm beads added
- Wide gap between 75 & 100 nm beads**
- # from Blue SSC: 75 nm ~ 100 nm.
- Note 1: Raw data from CytoFLEX retreated using B.C. Kaluza v 1.5 (flexible choice of decades # among 7 in CytoFLEX dynamic range).*
- Note 2: CytoFLEX daily Q.C. is NOT applicable in vSSC setup! → use Gigamix for SMP-oriented Q.C.*



光路：405nm作为SSC



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操作易

- 支持Windows 7, 8, 10
- 支持中英文双语，方便学习和操作
- 提供线上课程，方便随时学习



➤ 软件开放



Online Operator Training

The CytoFLEX system brings you easily upgradeable detection capabilities for up to 3 lasers and 13 color research flow cytometry right on your bench top.

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操作易-电压、补偿和阈值调节

The screenshot displays the CytExpert software interface. The top-left panel contains acquisition controls with buttons for Run, Record, Restart, Standby, Backflush, Boost, Next Tube, and Acq. Setting... Below these are status indicators for Events/Sec (0.0), Abort(%), Events (0), and Time (00:00:00). The middle-left panel shows display settings: Events to Display (1000), Events to Record (10000), and Time to Record (600). The bottom-left panel is labeled 'Tube' and contains a table with one entry: Tube1.

Name	Sample ID	Time
Tube1		

The main workspace features two plots for 'Tube1: All Events'. The left plot shows SSC-A (y-axis, 0 to 100) versus FSC-A (x-axis, 0 to 100). The right plot shows PE-A (y-axis, 10² to 10⁶) versus FITC-A (x-axis, 10² to 10⁶). A status bar at the bottom indicates 'Connected', 'Ready', and '[2015-02-06 16:36:23] Acquisition Completed.' The system tray shows 'Semi-automatic Sampler', 'Sheath', and 'Waste'.

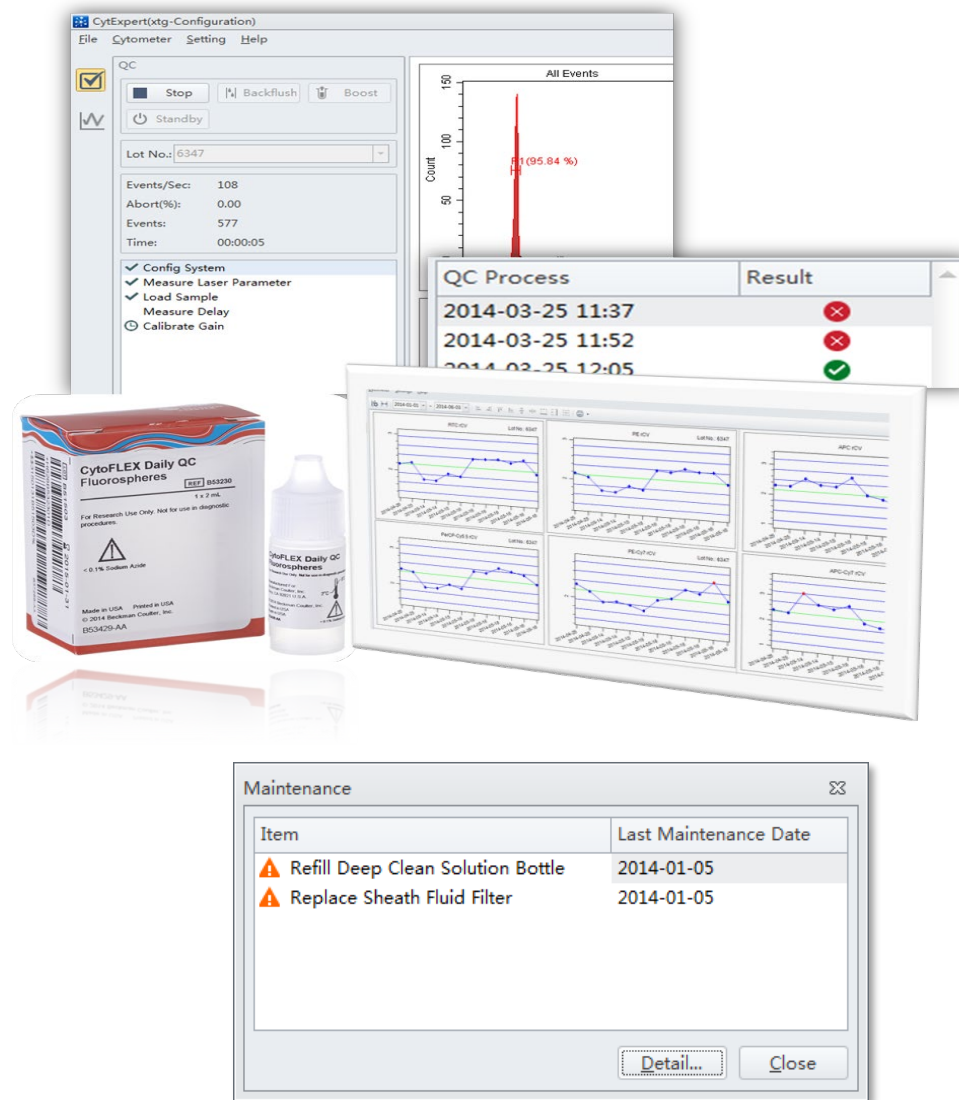
操作易

➤ 自动化QC

➤ 多种仪器控制程序

- 启动
- 清洗
- 灌注
- 反冲
- 深度清洗

➤ 仪器维护提示



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感谢聆听！

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