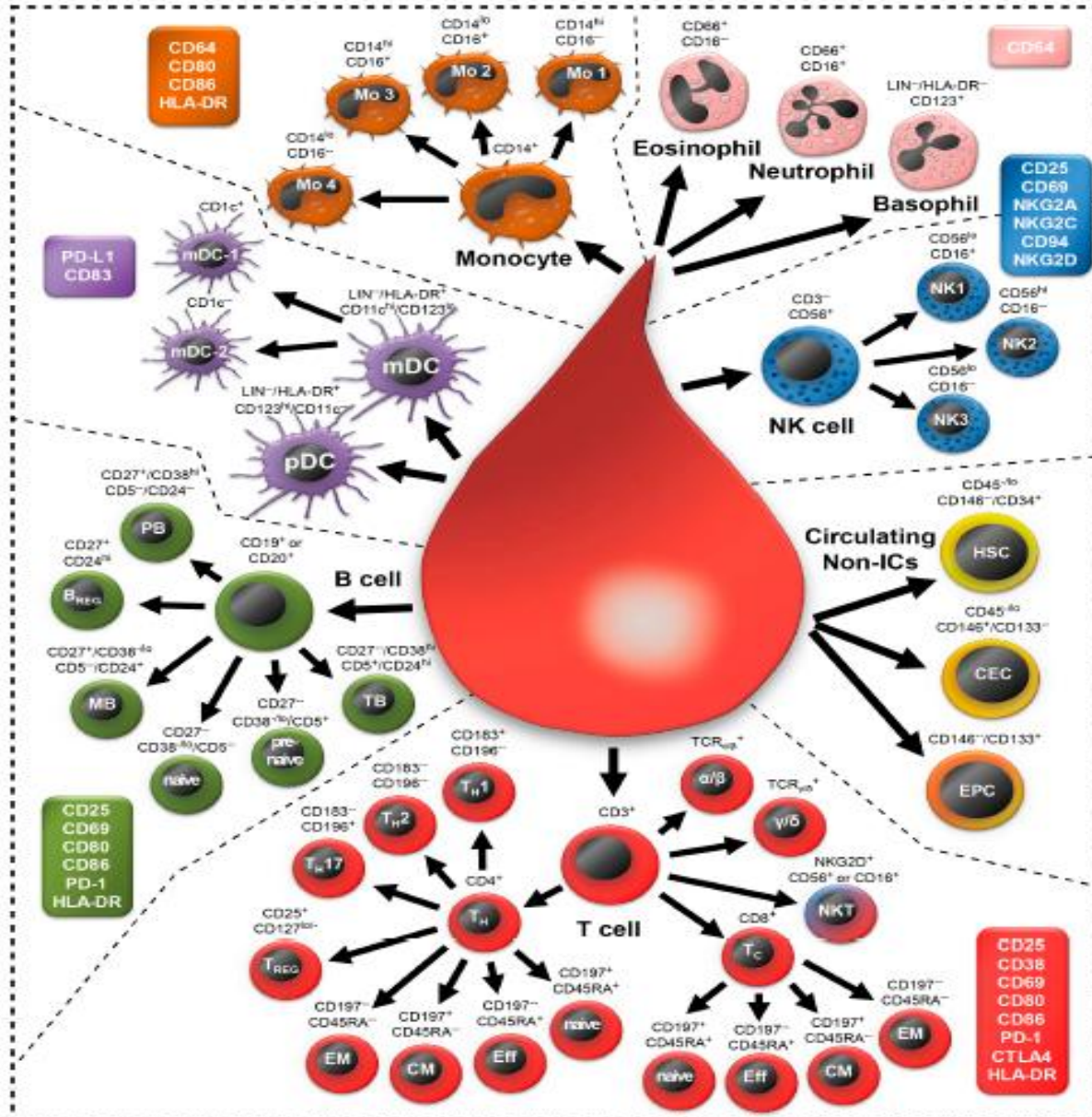


多色流式 ——抗体组合设计

贝克曼库尔特生命科学

多色实验助力细胞研究!



如何建立一个多色实验???

- 仪器的配置(选择染料)
- 荧光素的特性(荧光强度和串色能力)
- 多色组合规则(抗原的表达)

FITC

KrO

PE

PacBlue

ECD

APC-A750

PC5.5

APC-A700

PC7

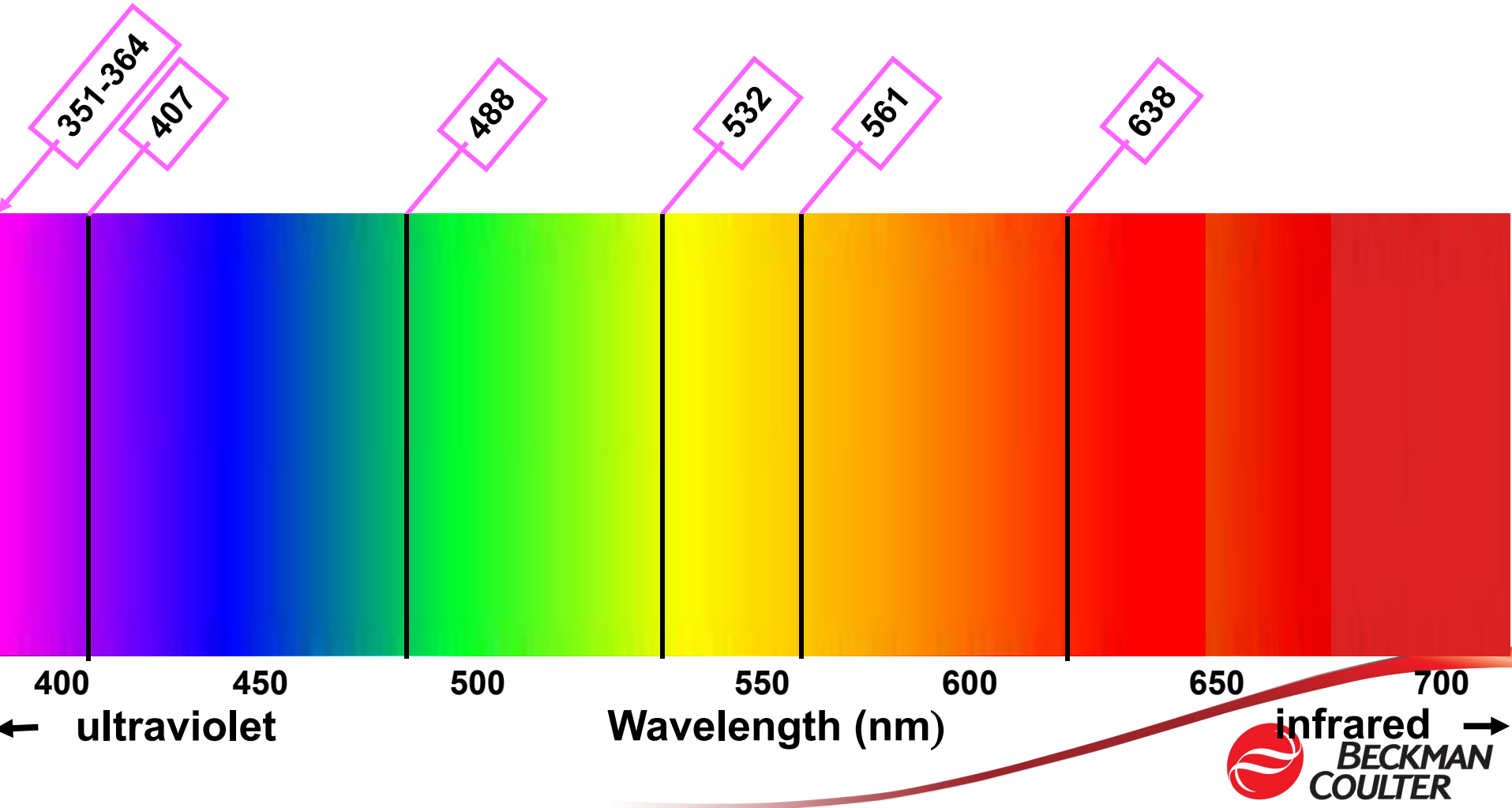
APC




















1. 仪器的配置

激光器: Lasers

确定仪器有哪些激光器, 目前常用的有355, 375, 405, 488, 561, 638, 808nm的激光



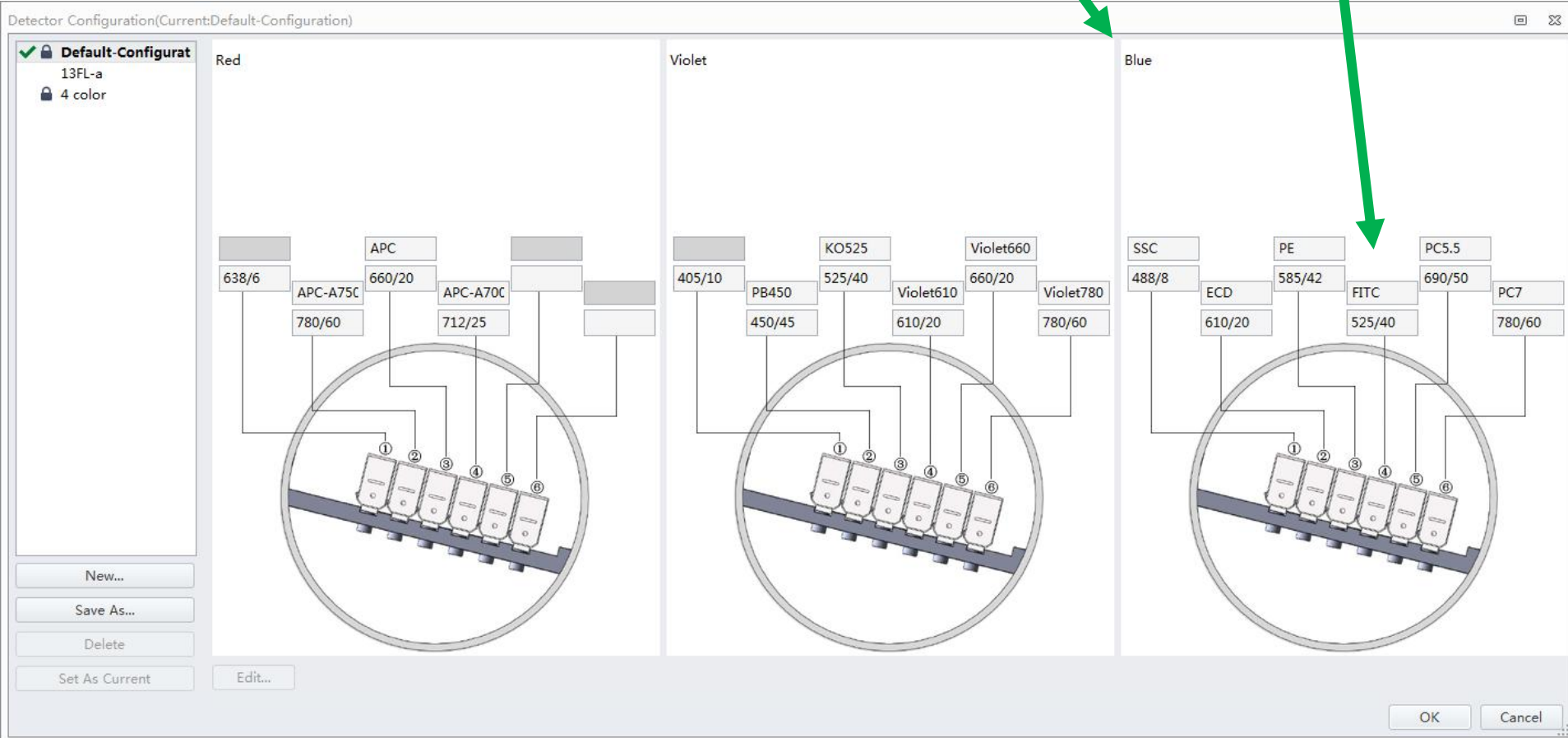
CytoFLEX s

激光器	荧光通道	CytoFLEX 通道名称	通用的荧光染料
 405 nm	 450/45 BP	V450	Pacific Blue 染料、V450、eFluor 450、BV421
	 525/40 BP	V525	Krome Orange、AmCyan、V500、BV510
	 610/20 BP	V610	BV605、Qdot 605
	 660/10 BP	V660	BV650、Qdot 655
 488 nm	 525/40 BP	B525	FITC、Alexa Fluor 488、CFSE、Fluo-3
	 690/50 BP	B690	PC5.5、PC5、PerCP、PerCP-Cy5.5、PI、DRAQ7
 561 nm	 610/20 BP	Y610	ECD、mCherry、PE-CF594
	 585/42 BP	Y585	PE、DsRed
	 690/50 BP	Y690	PC5.5、PC5、PI、DRAQ7
	 780/60 BP	Y780	PC7、DRAQ7
 638 nm	 660/10 BP	R660	APC、Alexa Fluor 647、eFluor 660、Cy5
	 712/25 BP	R712	APC-A700、Alexa Fluor 700、Cy5.5、DRAQ7
	 780/60 BP	R780	APC-A750、APC-Cy7、APC-H7、APC- eFluor 780、DRAQ7

确保该仪器可以做哪些荧光素

细胞仪→检测器配置

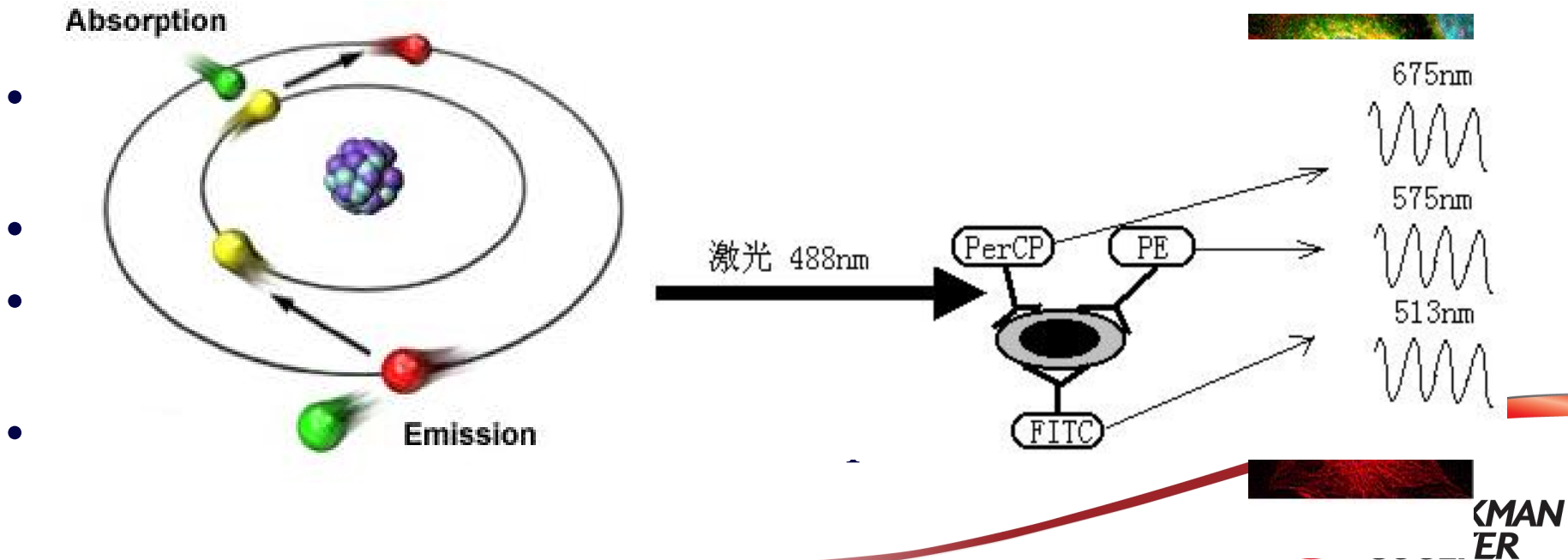
FITC—Ex:488nm Em:525



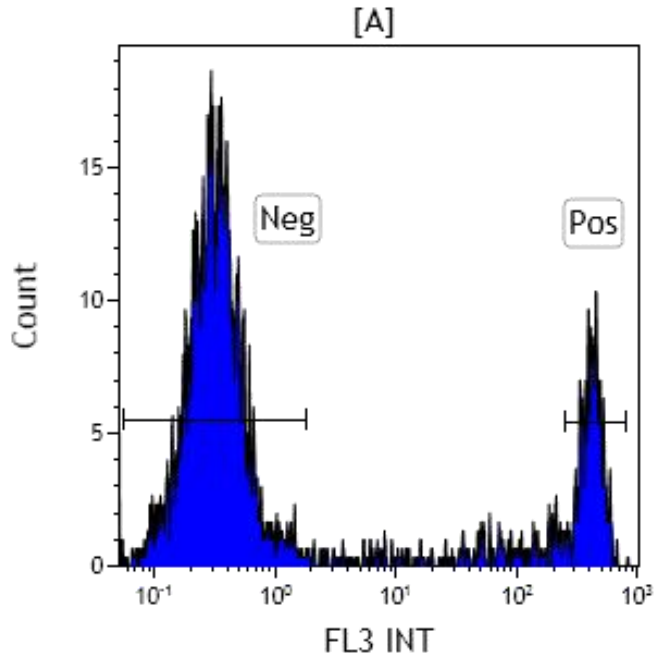
2. 荧光素特性

What is fluorescence ?

It is a functional group in a molecule which will absorb energy of a specific wavelength and re-emit energy at a different (but equally specific) wavelength.



荧光强度的评价



Gate	X-Mean	X-Stdev
All	78,37	161,02
Neg	0,37	0,23
Pos	420,15	82,32

Signal-to-Noise Ratio:

$MFI(pos) / MFI(neg),$

$$420.15/0.37 = \underline{1136}$$

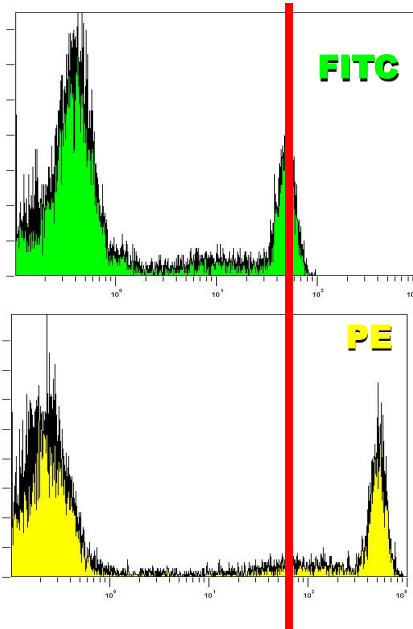
Staining Index:

$(MFI(pos) -$
 $MFI(neg))/2 * SD(neg),$

$$(420.15 - 0.37) / 2 * 0.23 = 913$$

- 荧光强度 (Brightness)

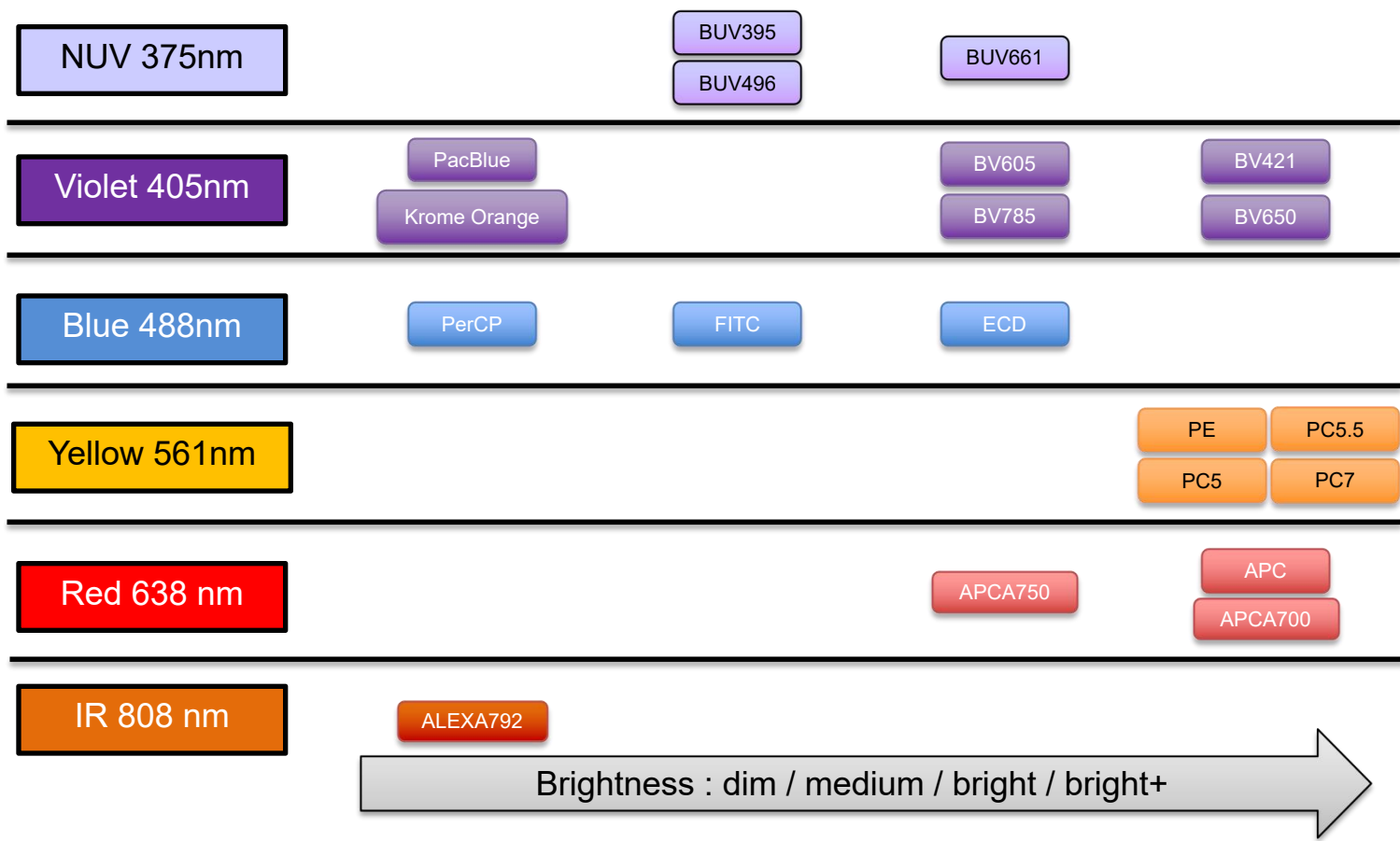
CD8 conjugates



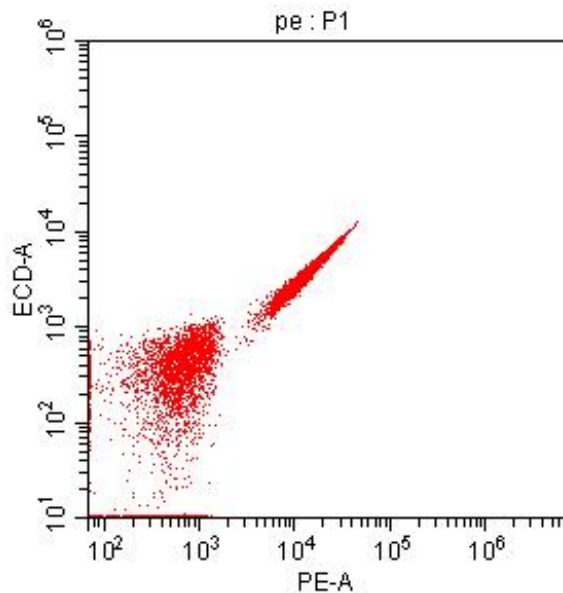
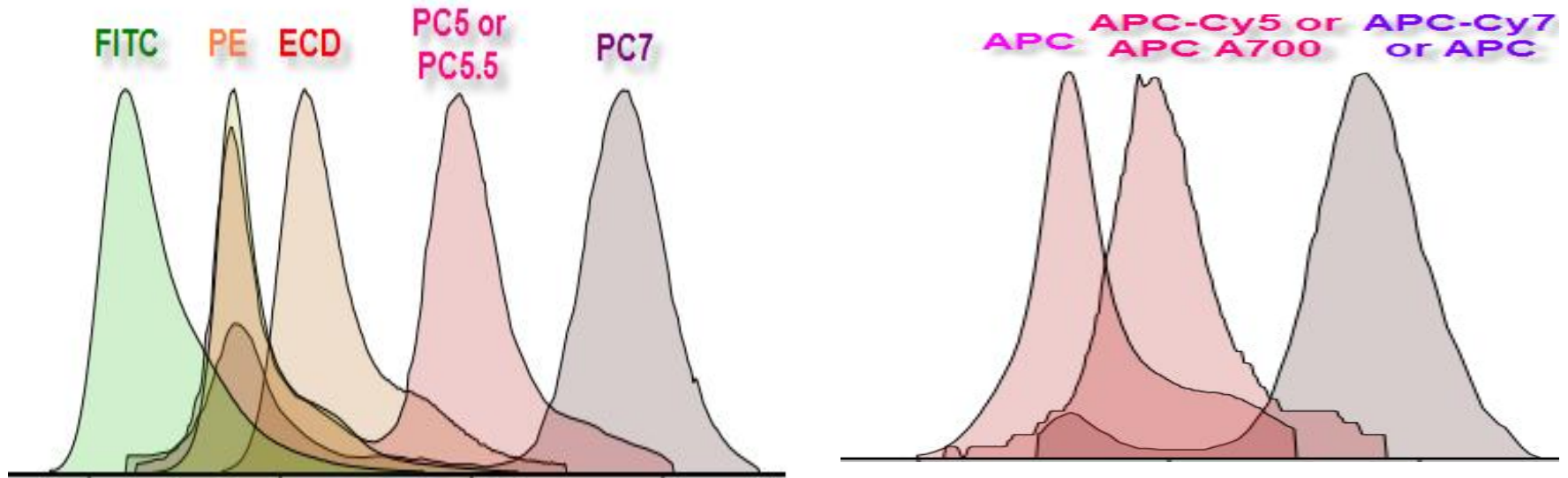
Fluorochrome	Stain Index
PE	2906.5
APC	450.98
ECD	1287.3
PB450	335.69
Violet660	173.86
PerCP-Cy5.5	62.72
Violet610	103.29
APC-A750	90.1
AF700	89.5
FITC	318.6
KO525	79.55
PerCP	62.72



CytoFlex 常用荧光素的 Brightness

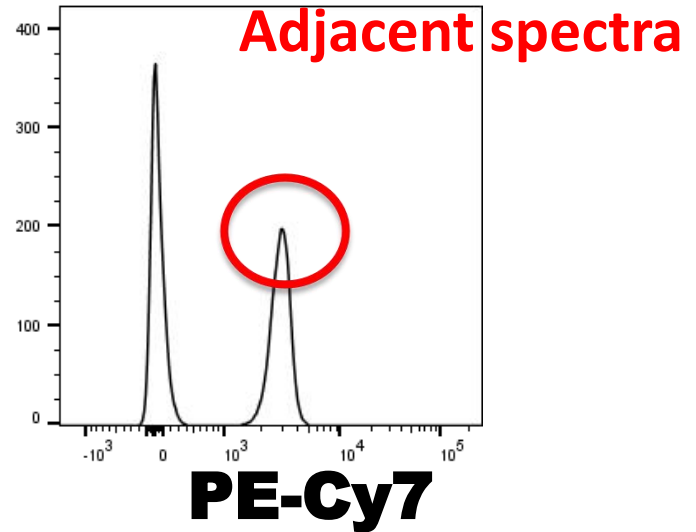
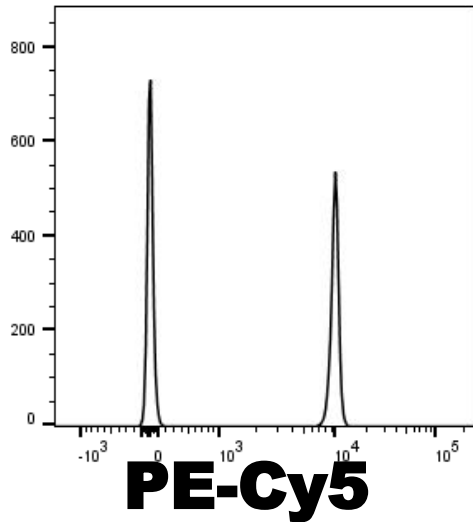


荧光溢出 (Spill Over)

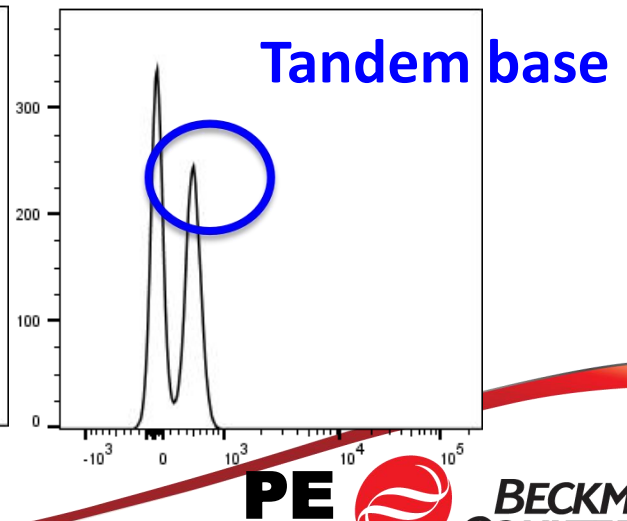
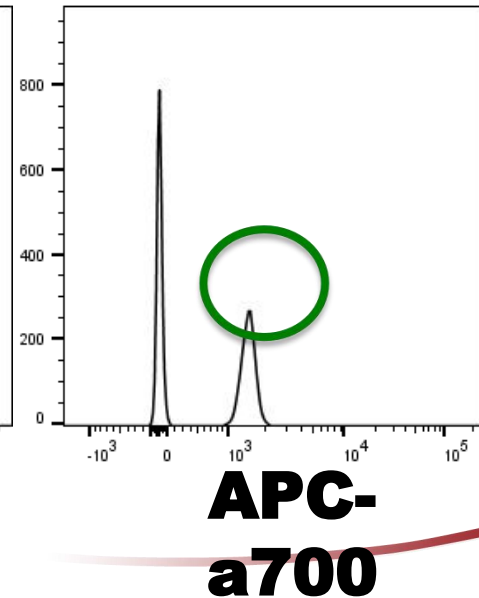
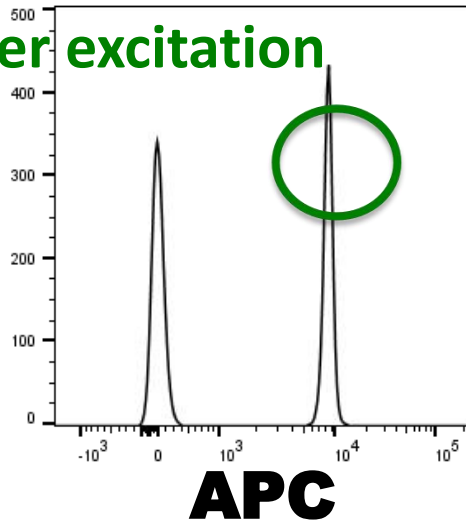


- 1. Adjacent spectra (FITC/PE)**
- 2. Tandems and their bases (PE/PE-CY5)**
- 3. Cross laser excitation (APC/PC5)**

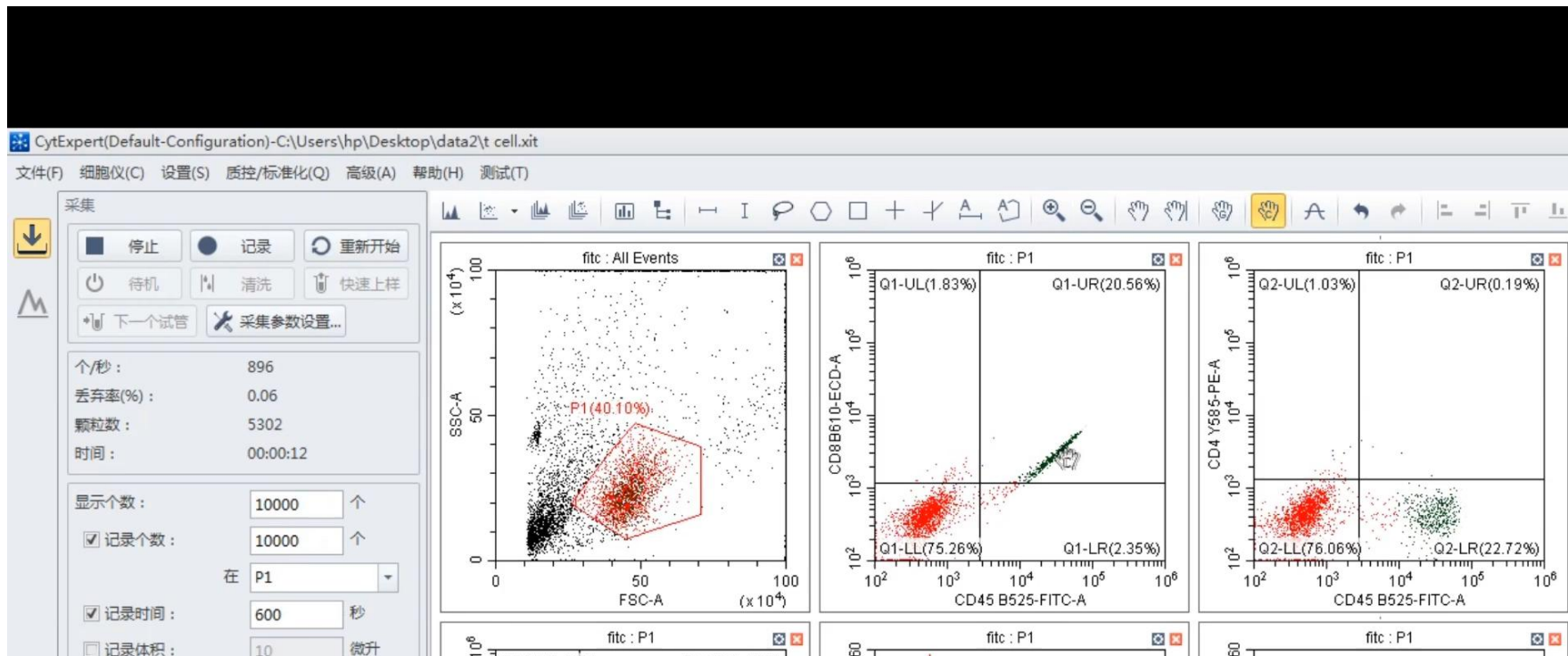
PE-Cy5 Single Color



Cross laser excitation



荧光补偿



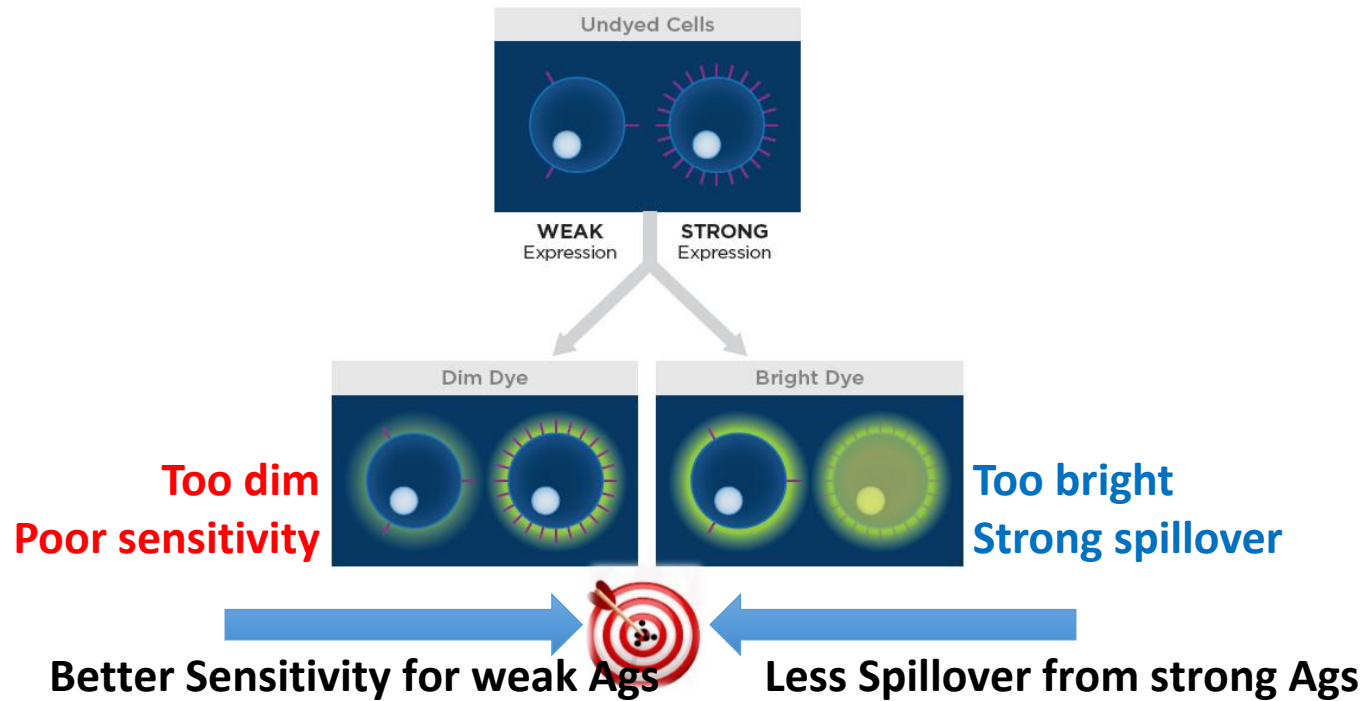
3. 多色组合规则

抗原表达组合达到最佳灵敏度的7大法则

- 弱表达抗原选择强荧光染料，强表达抗原选择弱荧光染料（“老法则”）
- 弱表达抗原放在“untouched”通道，强表达抗原放在“silent”通道
- 互相排斥的抗原允许spillover
- 共表达的抗原避免放在相互干扰的通道
- 上级抗原检测通道避免对下级抗原检测通道造成干扰
- 阳性设门：用阳性细胞群来区分阴阳性/弱阳和阴性间的界限时，允许互相串色
- 使分析的复杂程度降到最低

荧光染料的选择-强弱搭配

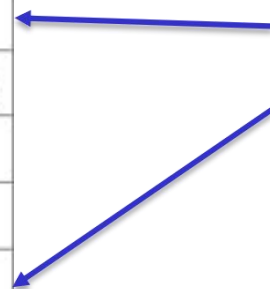
➤ 抗原表达水平



Marker与荧光素匹配

Laser	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
488nm	FITC	FITC, Alexa Fluor 488, CFSE, Fluo-3, GFP, eGFP
	PC5/perCP	PC5, PerCP
561nm	PE	PE, PI
	ECD	ECD, PE-Texas Red, PE-CF594, PI, DsRed, dTomato, mCherry
	PC5.5	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI
	PC7	PC7
638nm	APC	APC, Alexa Fluor 647, eFluor 660
	APC-A700	APC-A700, Alexa Fluor 700
	APC-A750	APC-A750, APC-Cy7, APC-H7, APC-eFluor 780
405nm	PB450	Pacific Blue dye, V450, eFluor 450, BV421
	K0525	Krome Orange, AmCyan, V500, BV510
	Violet610	BV605, Qdot 605
	Violet660	BV650, Qdot 655

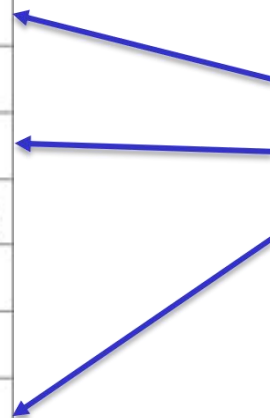
两色实验



Marker与荧光素匹配

Laser	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
488nm	FITC	FITC, Alexa Fluor 488, CFSE, Fluo-3, GFP, eGFP
	PC5/perCP	PC5, PerCP
561nm	PE	PE, PI
	ECD	ECD, PE-Texas Red, PE-CF594, PI, DsRed, dTomato, mCherry
	PC5.5	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI
	PC7	PC7
638nm	APC	APC, Alexa Fluor 647, eFluor 660
	APC-A700	APC-A700, Alexa Fluor 700
	APC-A750	APC-A750, APC-Cy7, APC-H7, APC-eFluor 780
405nm	PB450	Pacific Blue dye, V450, eFluor 450, BV421
	K0525	Krome Orange, AmCyan, V500, BV510
	Violet610	BV605, Qdot 605
	Violet660	BV650, Qdot 655

三色实验



Marker与荧光素匹配

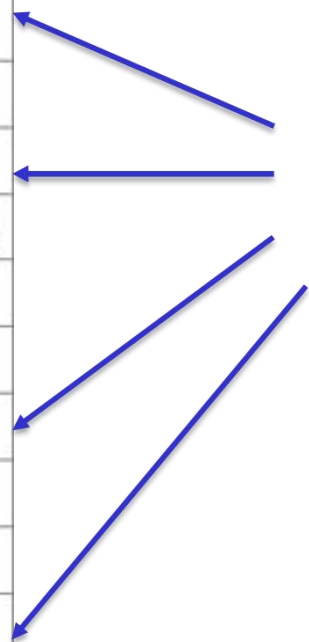
Laser	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
488nm	FITC	FITC, Alexa Fluor 488, CFSE, Fluo-3, GFP, eGFP
	PC5/perCP	PC5, PerCP
561nm	PE	PE, PI
	ECD	ECD, PE-Texas Red, PE-CF594, PI, DsRed, dTomato, mCherry
	PC5.5	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI
	PC7	PC7
638nm	APC	APC, Alexa Fluor 647, eFluor 660
	APC-A700	APC-A700, Alexa Fluor 700
	APC-A750	APC-A750, APC-Cy7, APC-H7, APC-eFluor 780
405nm	PB450	Pacific Blue dye, V450, eFluor 450, BV421
	K0525	Krome Orange, AmCyan, V500, BV510
	Violet610	BV605, Qdot 605
	Violet660	BV650, Qdot 655

三色实验

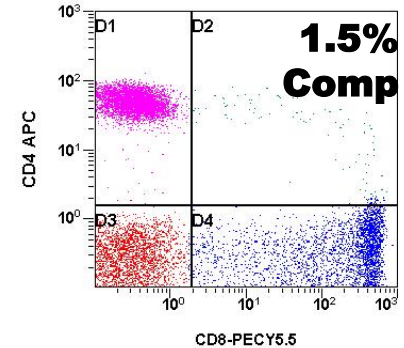
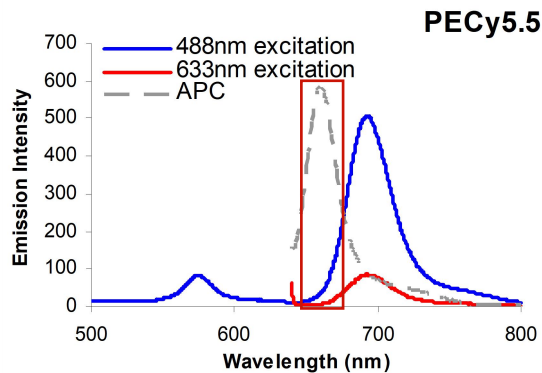
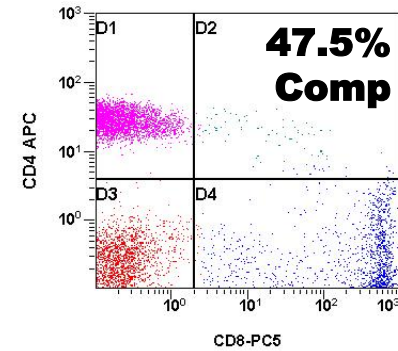
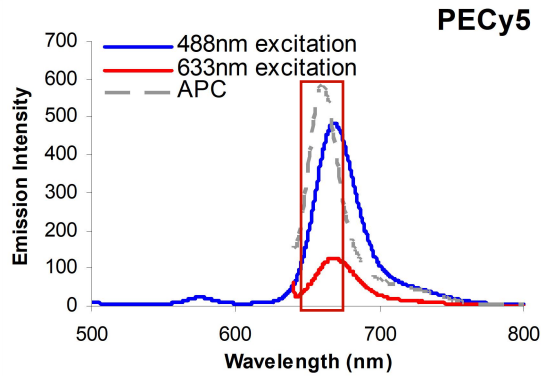
Marker与荧光素匹配

Laser	CytoFLEX Channel Names	Commonly used Fluorescent Dyes
488nm	FITC	FITC, Alexa Fluor 488, CFSE, Fluo-3, GFP, eGFP
	PC5/perCP	PC5, PerCP
561nm	PE	PE, PI
	ECD	ECD, PE-Texas Red, PE-CF594, PI, DsRed, dTomato, mCherry
	PC5.5	PC5.5, PC5, PerCP, PerCP-Cy5.5, PI
	PC7	PC7
638nm	APC	APC, Alexa Fluor 647, eFluor 660
	APC-A700	APC-A700, Alexa Fluor 700
	APC-A750	APC-A750, APC-Cy7, APC-H7, APC-eFluor 780
405nm	PB450	Pacific Blue dye, V450, eFluor 450, BV421
	K0525	Krome Orange, AmCyan, V500, BV510
	Violet610	BV605, Qdot 605
	Violet660	BV650, Qdot 655

四色实验



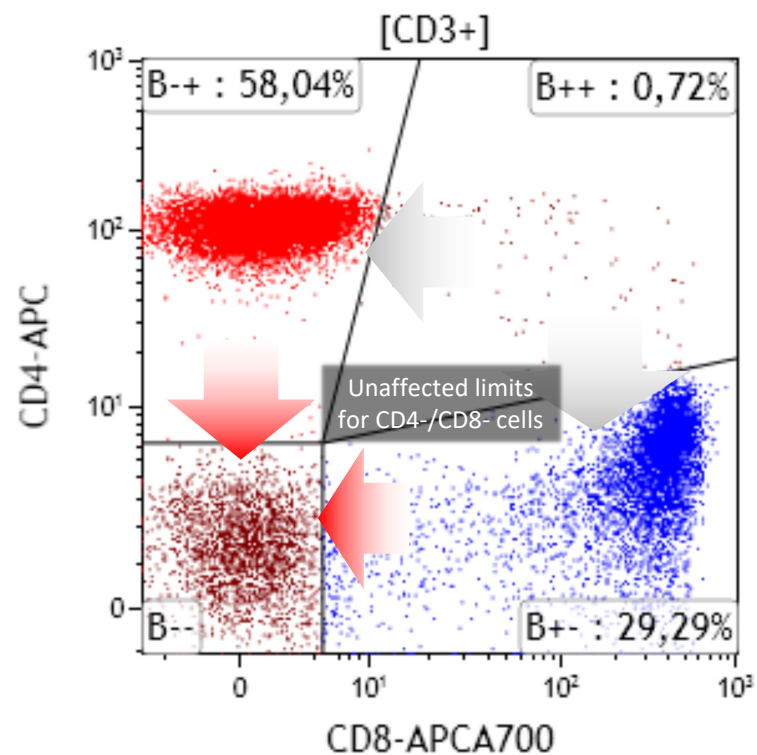
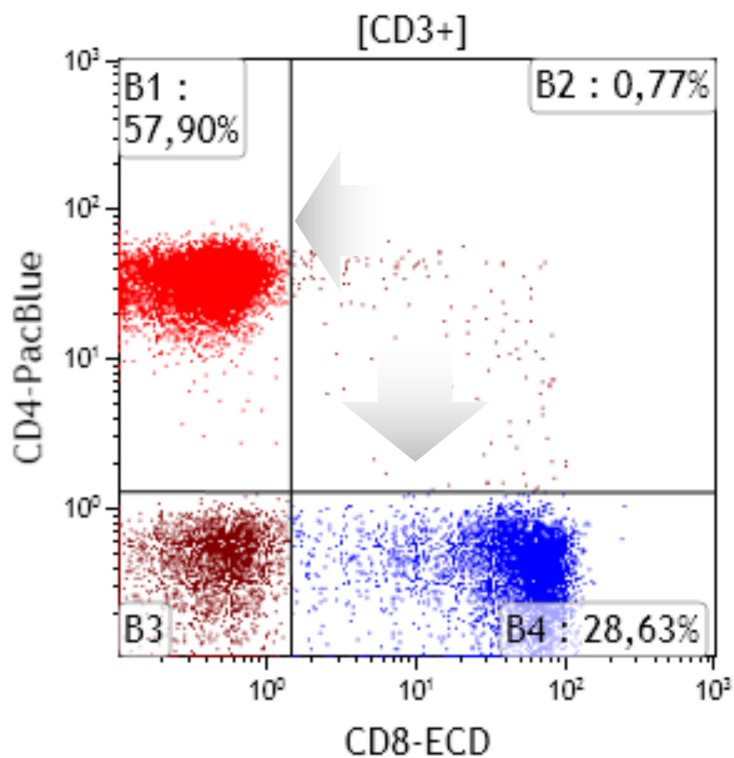
荧光抗体的搭配



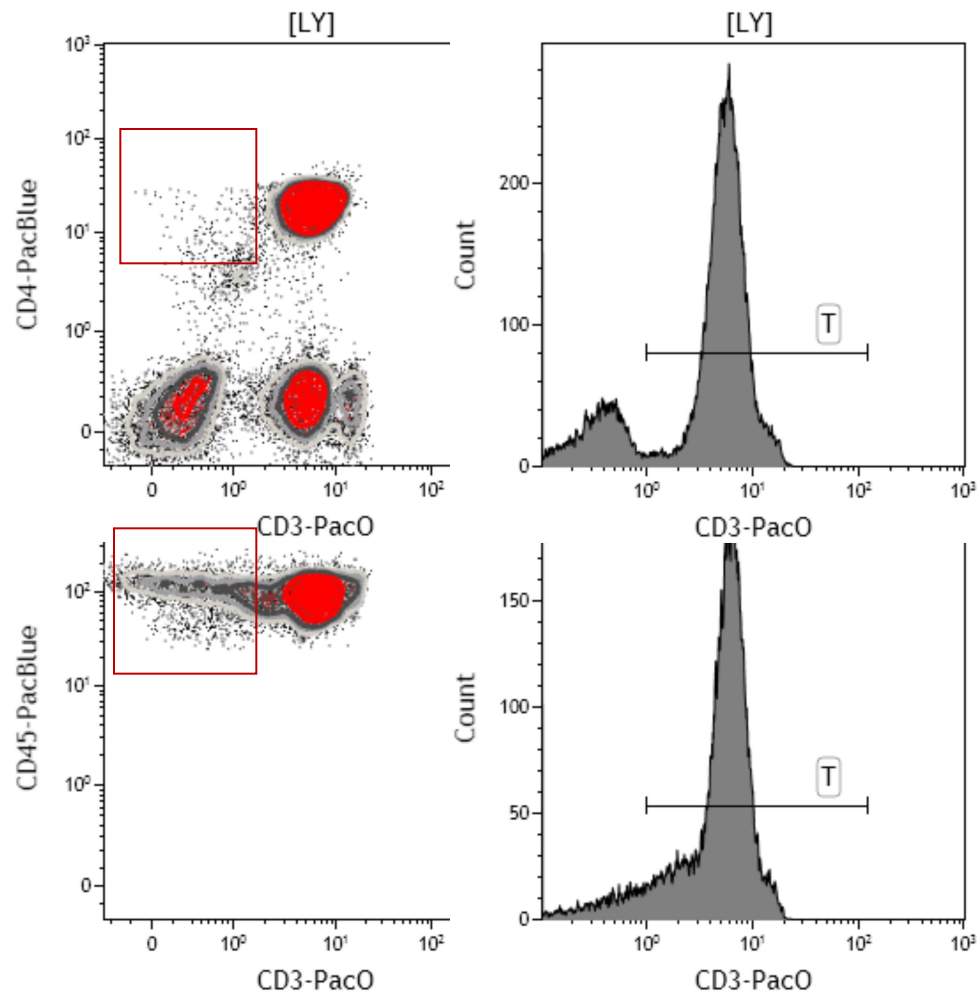
尽量选择“溢出”小的组合



抗原间的关系 - 互相排斥



抗原间的关系 - 亲代和子代

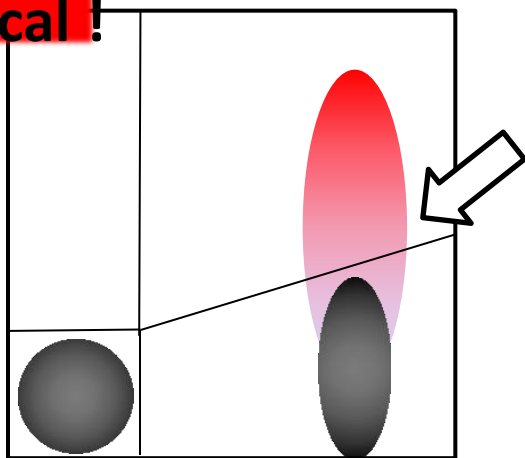


子代可以串亲代，亲代避免串子代

抗原间的关系 - 共表达

连续性共表达

Critical!

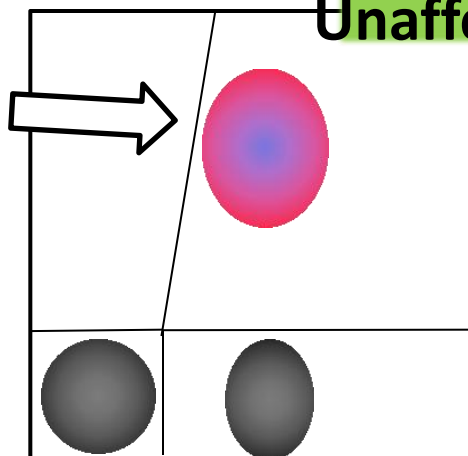


Co-expression of modulated antigens

不连续性共表达

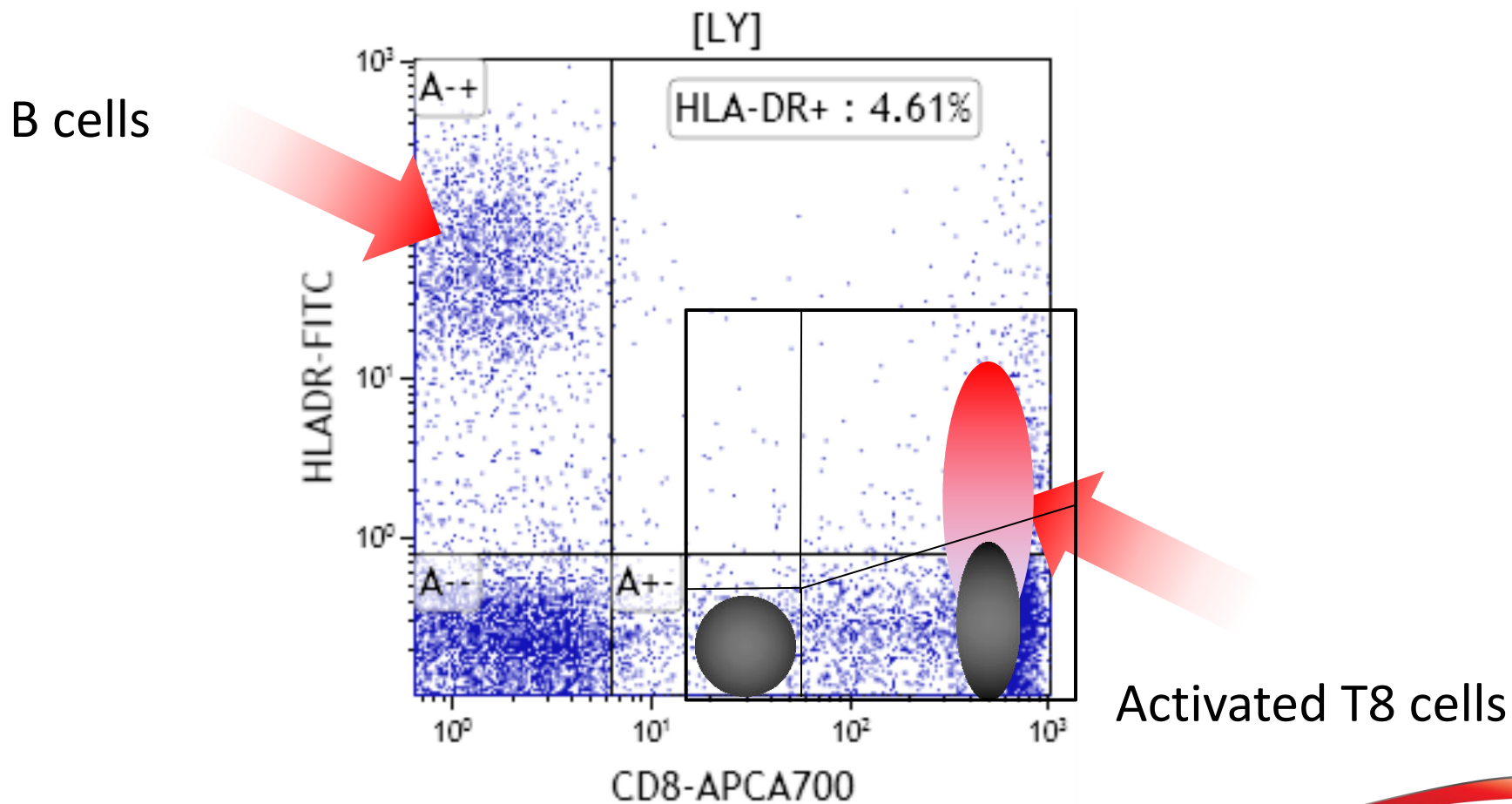
Unaffected!

Hinged quadrants represent data spreading resulting from spillover

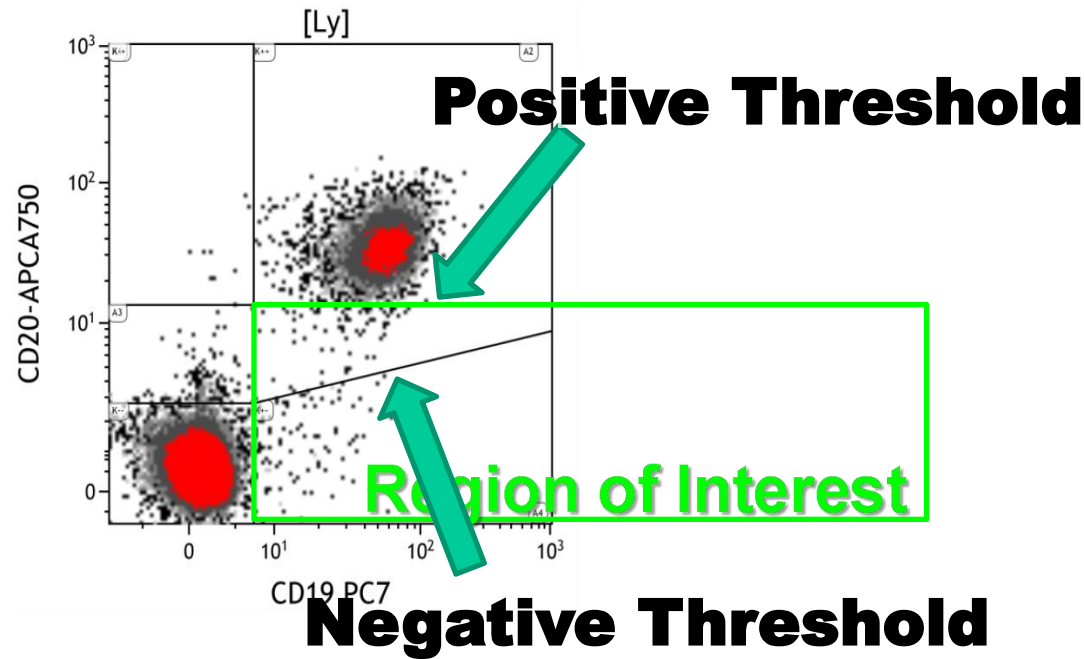
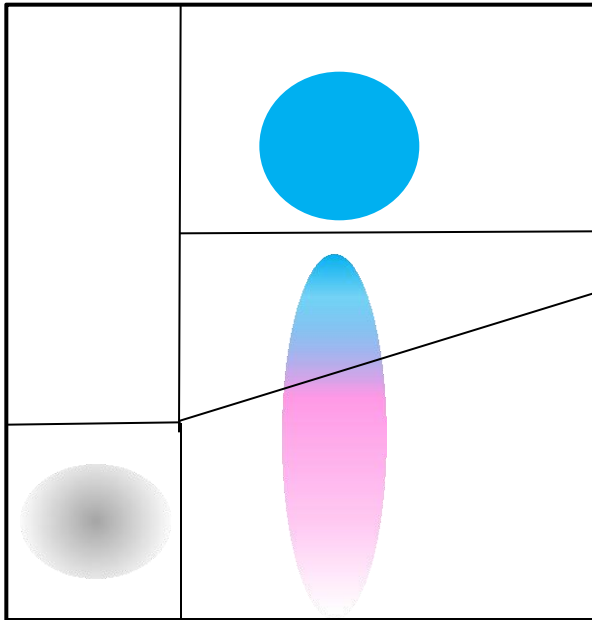


Parent and descendant (exclusive coexpression)

抗原分布 - 分群明显 vs 连续表达



抗原间的关系 - 阳性设门



流式实验中对照的设置

1.同型对照

- 同种属来源
- 同抗体类型
- 同荧光素标记
- 等量加入

例如：

FITC标记小鼠抗人CD4单克隆抗体（IgG1）

其同型对照为：

FITC标记 小鼠 IgG1

流式实验中对照的设置

2.空白对照

- 不进行标记的细胞
- 回答细胞自身本底荧光问题，不是画门的阴性对照。

流式实验中对照的设置

3.单染对照

- 根据多色方案确定补偿单标管
- 例
- 多色方案：

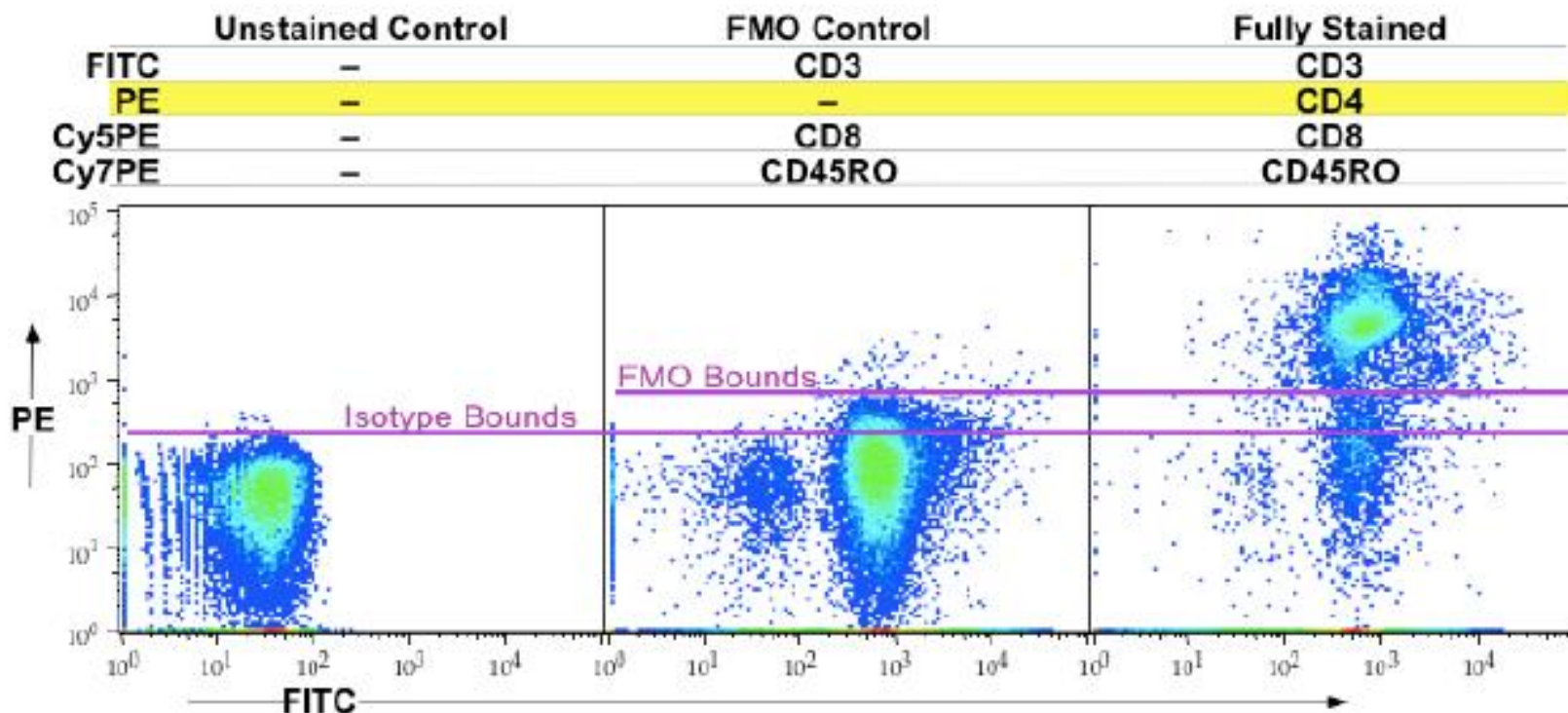
CD45-FITC,CD4-PE,CD8-ECD,CD3-PC5

- 补偿单标管：
- 1.CD45-FITC
- 2.CD4-PE
- 3.CD8-ECD
- 4.CD3-PC5

流式实验中对照的设置

4.单管扣除 (FMO) 对照

FMO : Fluorescence Minus One,即除目标抗体外, 其他抗体都加上。



流式实验中对照的设置

5. 未处理阴性对照

细胞凋亡：未处理对照

GFP检测：未转染对照

细胞因子：不刺激对照

抗体组合设计很大程度上会影响你的结果！

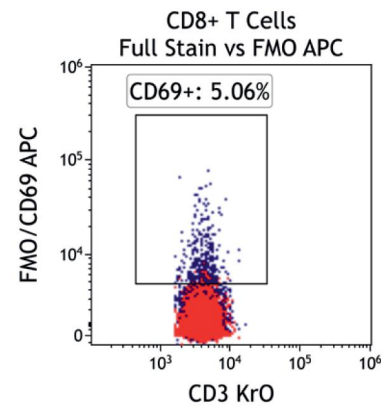
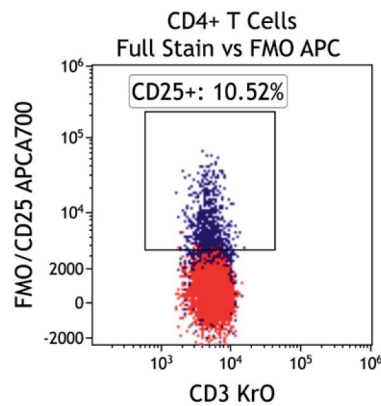
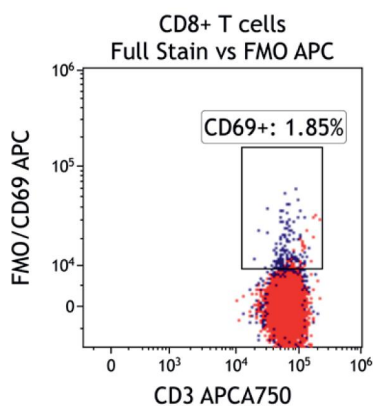
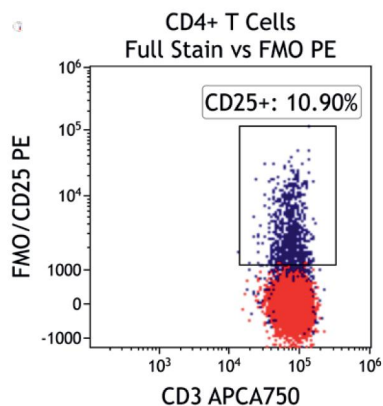
	405 Excitation		488 Excitation				633 Excitation			
	Pacific Blue	Krome Orange	FITC	PE	ECD	PC5.5	PC	APC	APC-A700*	APC-A750*
①	CD4	HLA-DR	CD45RA	CD25	CD45RO	CD117	CD127	CD69	CD8	CD3
②	CD4	CD3	CD8	CD45RA	HLA-DR	CD117	CD45RO	CD69	CD25	CD127

Figure 2.2

Figure 2.3

Figure 2.4

Figure 2.5



Spreading影响阳性率

多色方案设计

选择染料

不同仪器选择不同的荧光染料
复合荧光染料的制作工艺
复合荧光染料的稳定性
荧光染料的强度，性能



抗原表达模式

抗原间的表达关系
七大法则

染料搭配

减少干扰从而减少补偿调节
荧光素之间的搭配

谢谢大家！