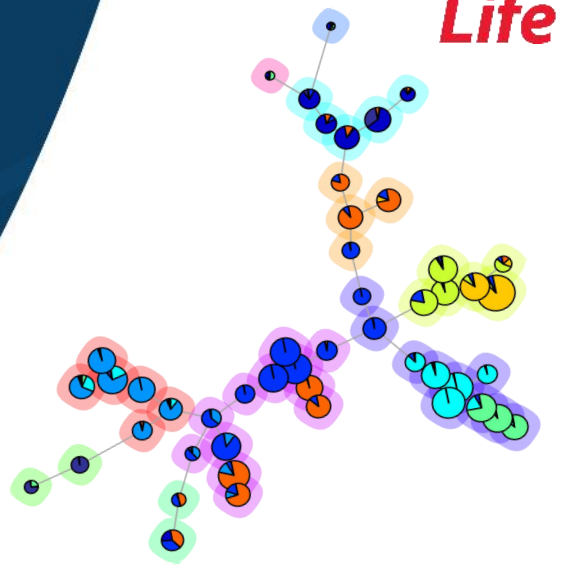
高维数据的艺术：用Cytobank实现降维分析的最佳实践

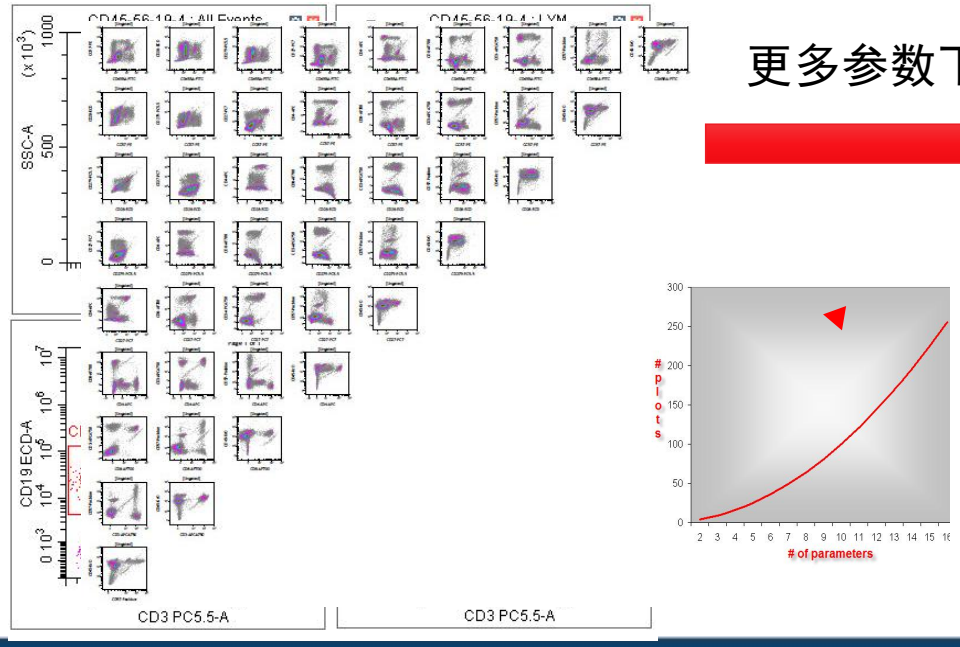
贝克曼库尔特生命科学



流式分析中遇到的问题？

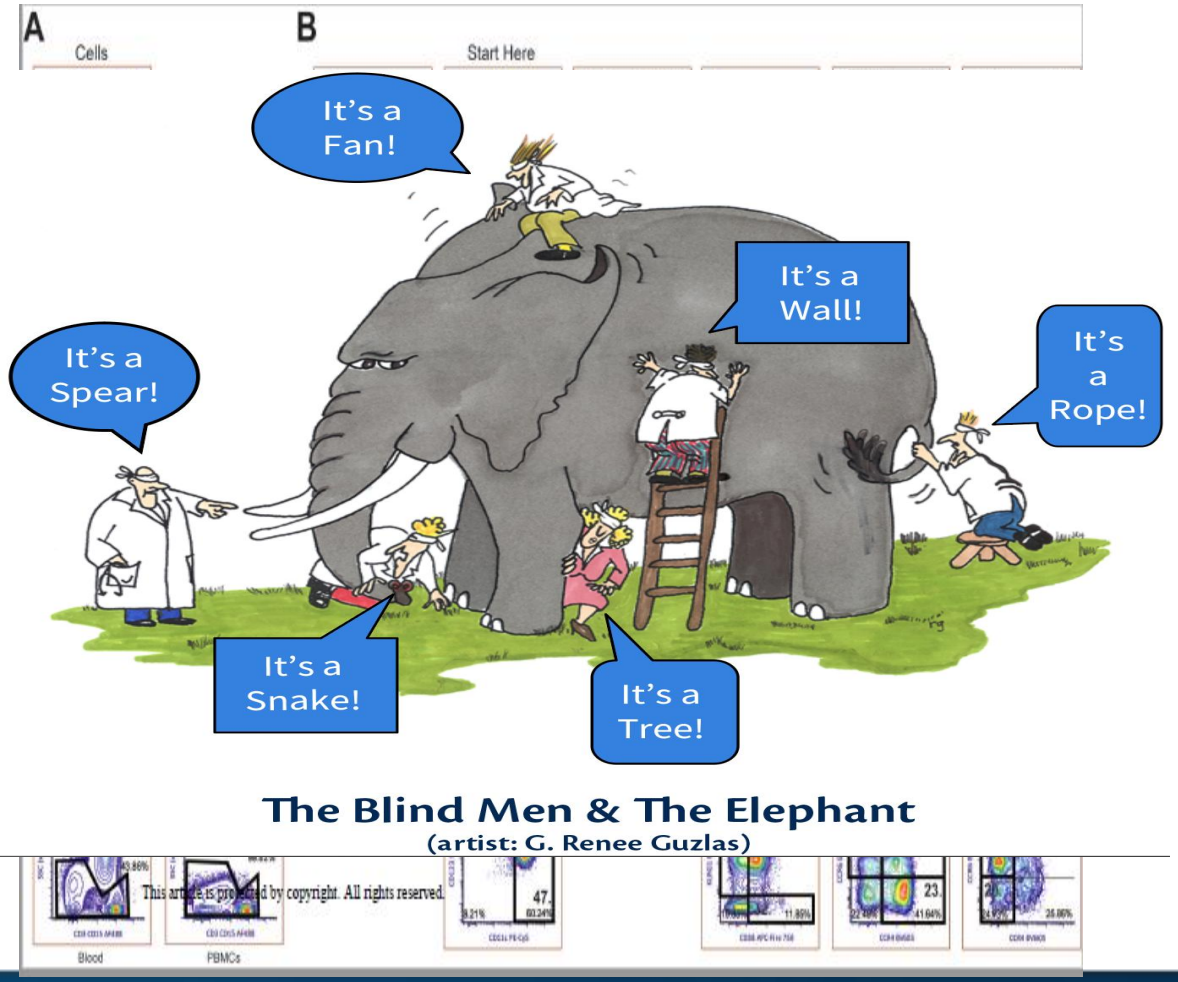
- 多参数下数据分析难度加大
- 按现有免疫学知识，错失可能的群体
- 不同样本分群差异，不能按原有SOP圈门
- 人为圈门差异或逻辑差异导致重复性差

10CD45-4-8-3抗体管 combinations



更多参数下分析

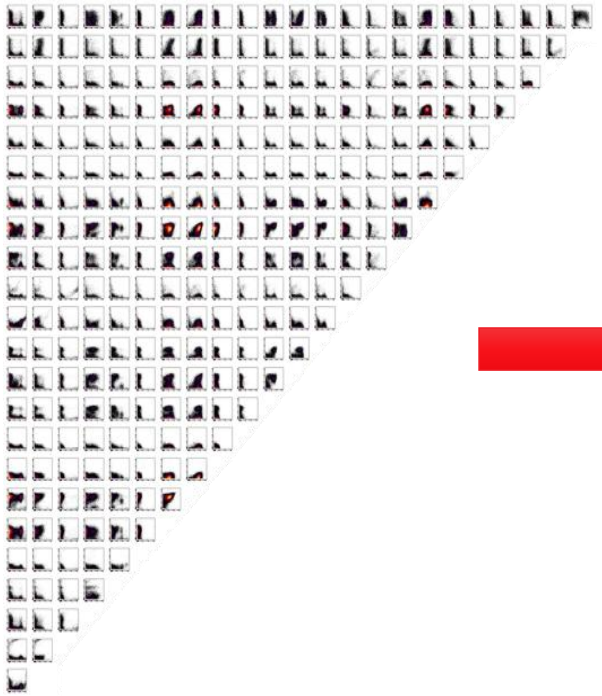
25色人外周血免疫监测

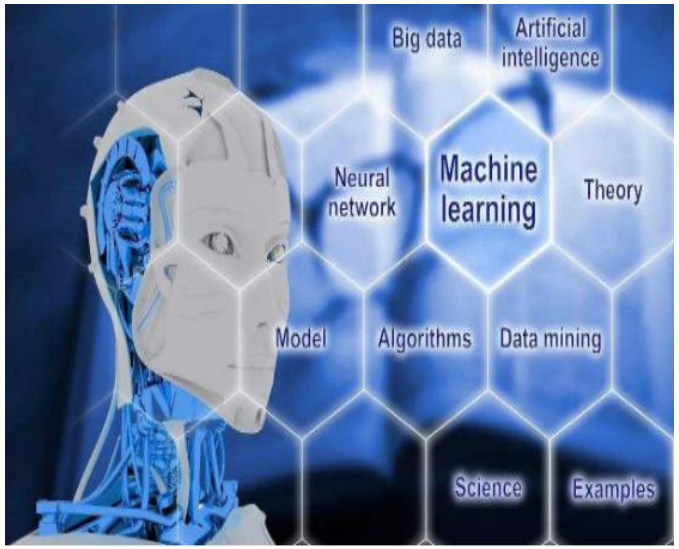


如何提高效率的同时减少误差？

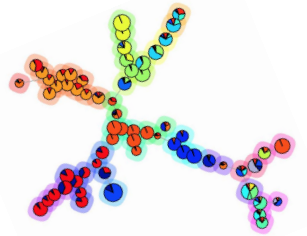
机器学习助力流式数据分析

传统的数据分析

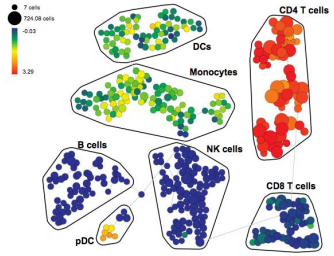




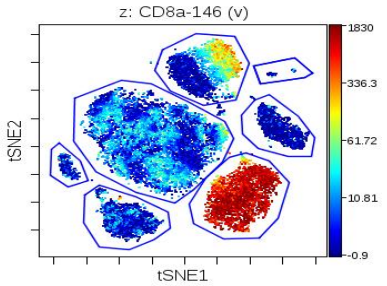
聚类&降维分析



FlowSOM

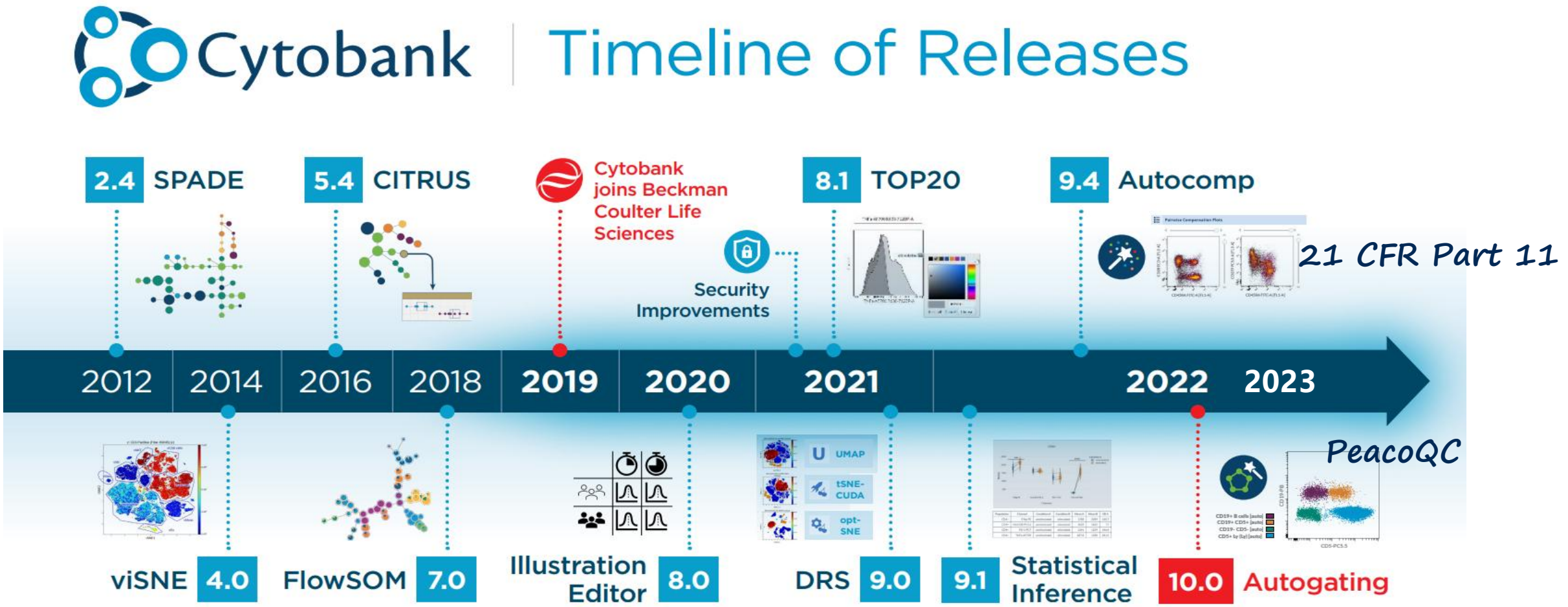


SPADE

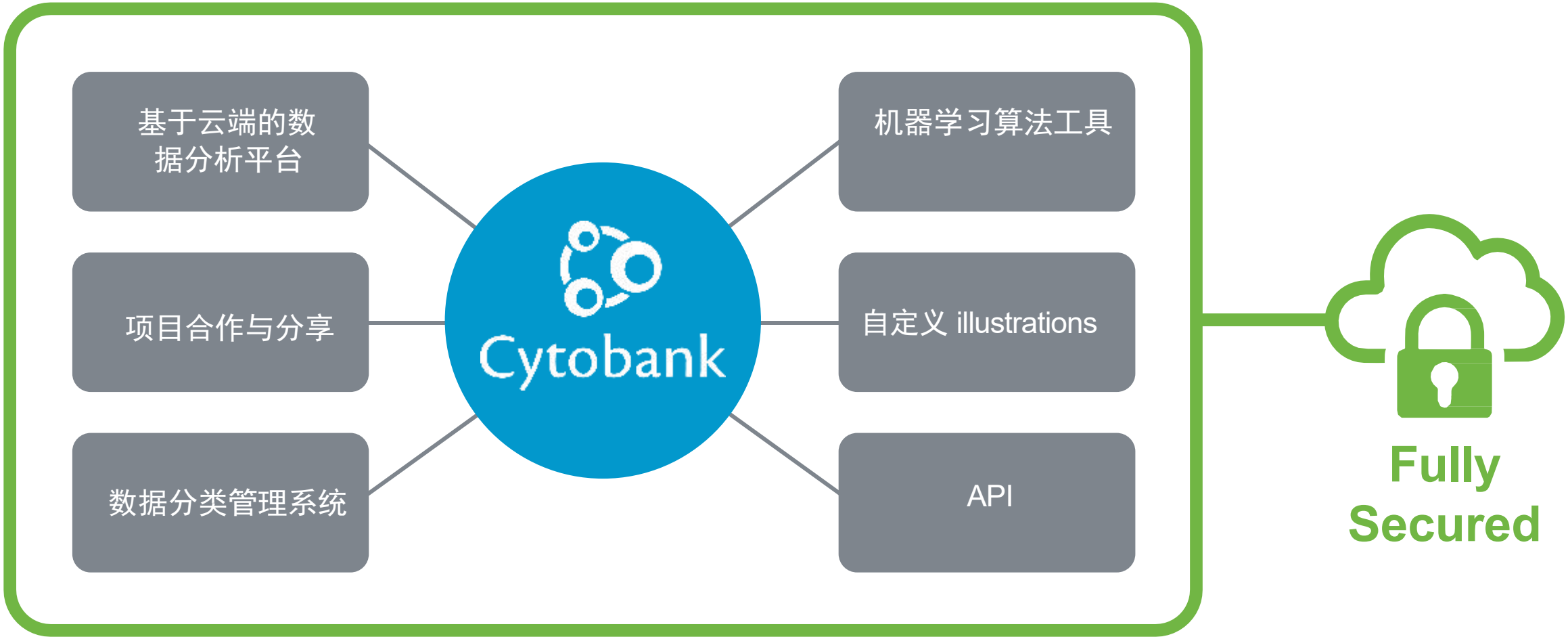


viSNE

更新的脚步从未停止...

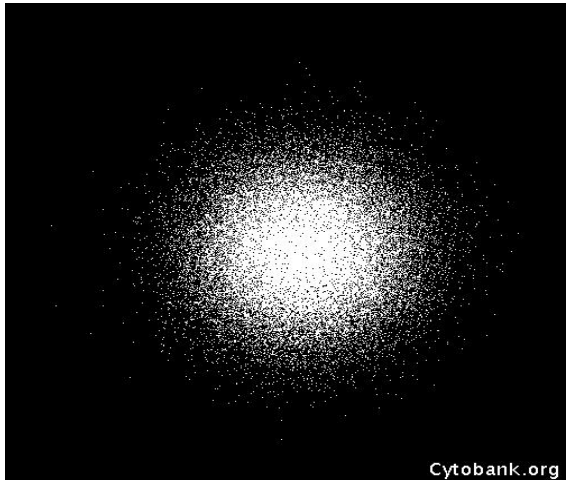


何为Cytobank platform?



t-SNE (viSNE): t-分布随机邻接嵌入算法
t-Distributed Stochastic Neighbor Embedding

一种探索高维数据的降维算法，常用于可视化数据的整体结构。

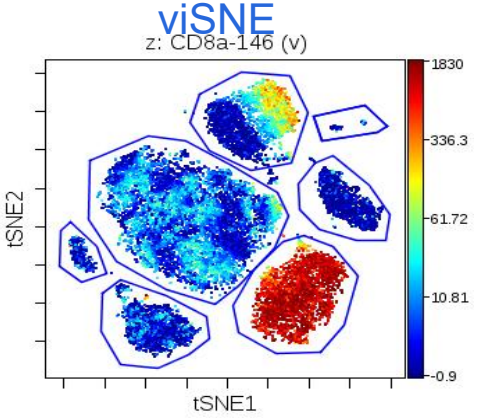


- 多维数据通过迭代过程简化为二维“地图”
- 每个点表示一个细胞/事件
- 在viSNE“图上相邻的细胞彼此更相似，每个“小岛”为相似的细胞群
- 可通过细胞颜色判断Marker表达强弱

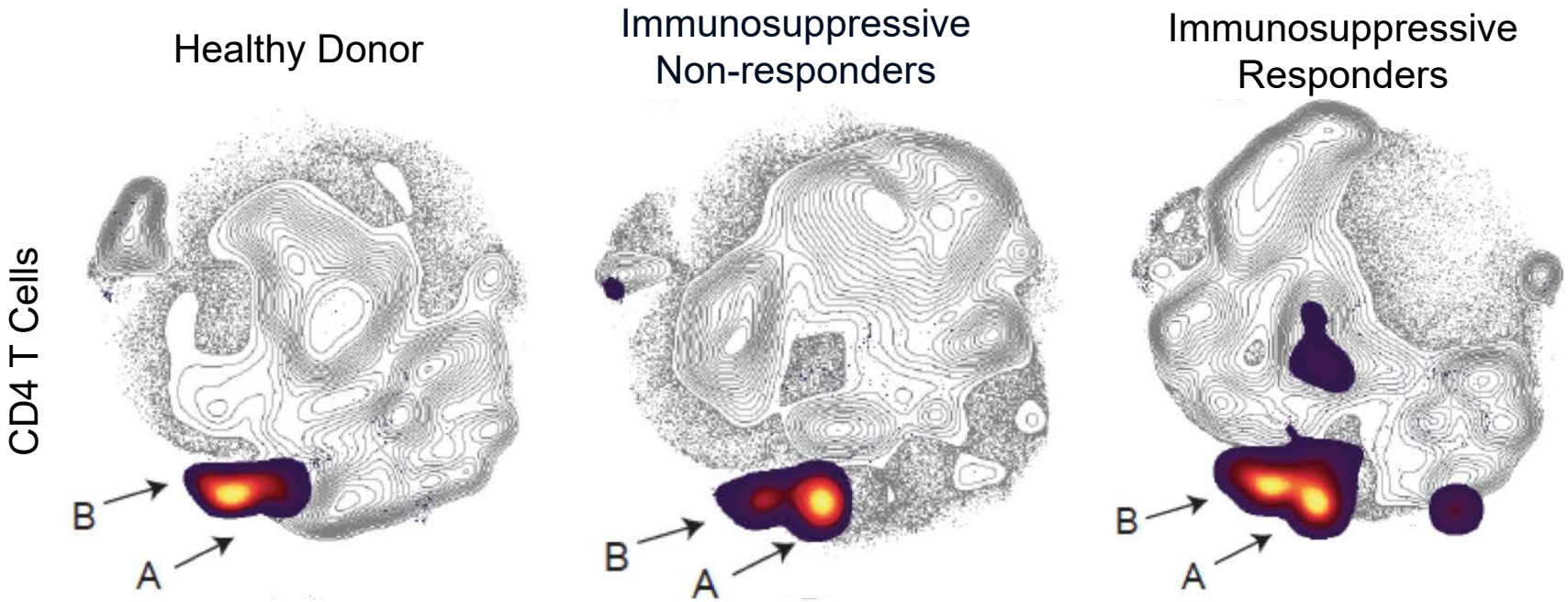
viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia

El-ad David Amir[1], Kara L Davis[2,3], Michelle D Tadmor[1,3], Erin F Simonds[2,3], Jacob H Levine[1,3], Sean C Bendall[2,3], Daniel K Shenfeld[1,3], Smita Krishnaswamy[1], Garry P Nolan[2,4] & Dana Pe'er[1,4]

NATURE BIOTECHNOLOGY VOLUME 31 NUMBER 6 JUNE 2013



客观地观察所有细胞有助于新发现



Highlighted Treg: $CD25^{+}Foxp3^{+}CD127^{-}$

- viSNE density plots revealed two Treg subpopulations
- Non-responders have more A subpopulation than responders/healthy
- B subpopulation has more memory/activation phenotype

降维算法在传统流式中多色分析流程

检测试剂：DuraClone

数据采集：CytoFLEX

数据分析:手动和降维

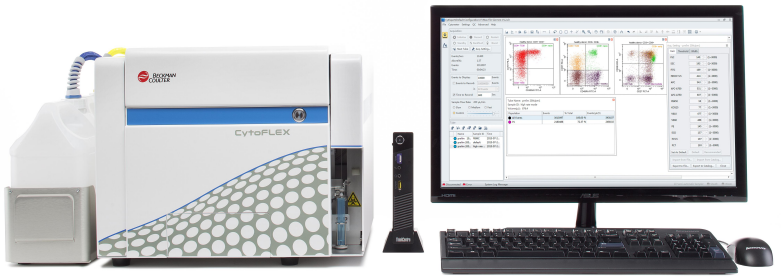
	405 nm		488 nm					633 nm				
Product	PB	KrO	FITC	PE	ECD	PC5.5	PC7	APC	AF647	AF700	APC-AF700	APC-AF750
IM Phenotyping Basic Tube B53309 (25 tests RUO)	-	CD45	CD16	CD56	CD19	-	CD14	CD4	-	CD8	-	CD3
IM B Cell Tube B53318 (25 tests RUO)	IgM	CD45	IgD	CD21	CD19	-	CD27	CD24	-	-	-	CD38
IM T Cell Subsets Tube B53328 (25 tests RUO)	CD57	CD45	CD45RA	CCR7	CD28	PD1	CD27	CD4	-	CD8	-	CD3
IM Dendritic Cells Tube B53351 (25 tests RUO)	HLA-DR	CD45	CD16	Lineage**	-	CD1c	CD11c	Clec9A	-	-	CD123	-
IM TCRs Tube B53340 (25 tests RUO)	TCRVδ2	CD45	TCRγδ	TCRαβ	HLA-DR	-	TCRVδ1	CD4	-	CD8	-	CD3
IM Treg Tube B53346 (25 tests RUO)	Helios	CD45	CD45RA	CD25	-	CD39	CD4	-	FoxP3	-	-	CD3
IM Granulocytes Tube B88651 (25 tests RUO)	CD15	CD45	CD294	-	CD16	CD33	CD11b	PD-L1	-	-	Lineage**	CD62L
IM Count Tube C00162 (25 tests RUO)	-	-	CD45	Counting Beads		7-AAD	-	-	-	-	-	-

Quality Standard: ISO 13001:2008

Designed by expert labs from ONE STUDY

* : CD3 / CD19 / CD20 / CD14 / CD56

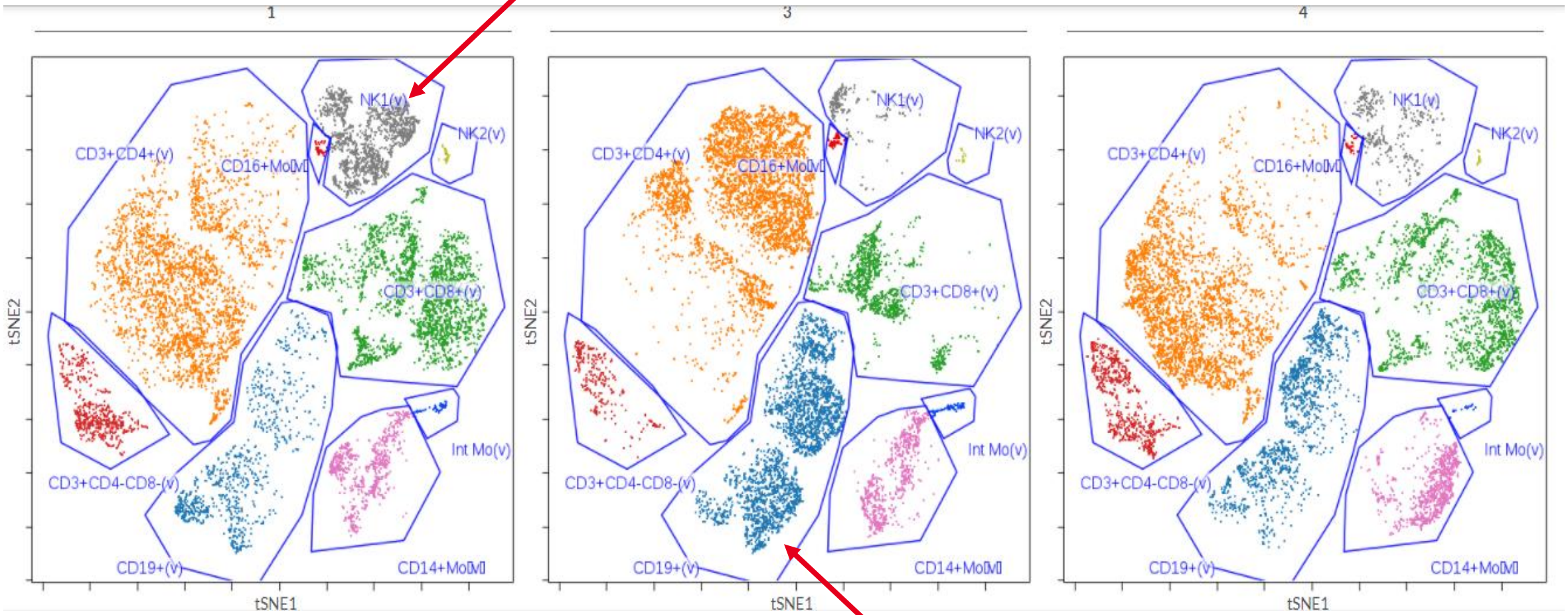
** : CD3 / CD14 / CD19 / CD56



Cytobank

FITC	PE	ECD	PC7	APC	APC-A700	APC-A750	BV421	KO
CD16	CD56	CD19	CD14	CD4	CD8	CD3	HLA-DR	CD45

降维图中识别所有细胞群的变化

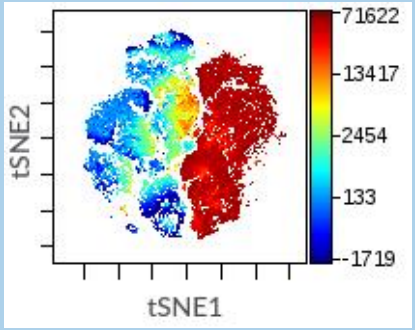


Data from BCLS China

Cytobank 的多种降维算法

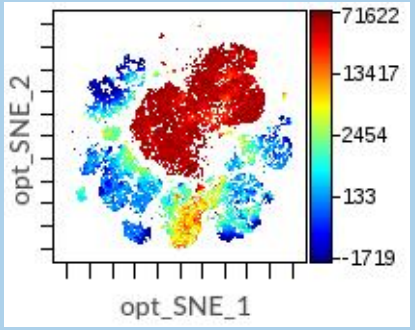
- 相比viSNE/ tSNE，更新更快功能更强大的降维工具，助力降维中的参数自动优化以及缩短分析时间

viSNE/ tSNE



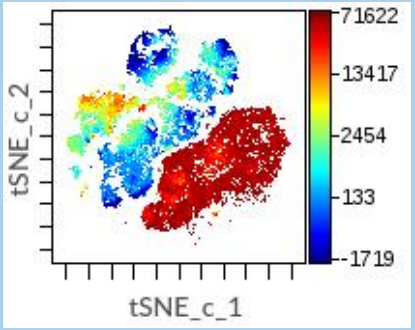
t-分布随机近邻嵌入（t-Distributed Stochastic Neighbor Embedding,t-SNE）是一种非线性降维算法，在亚群细节展示方面会有优势，能够更好的展示亚群之间的细微差异最高可2M events

opt-SNE



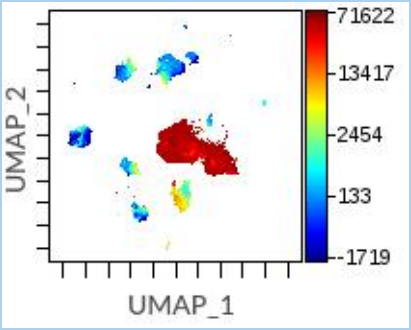
在t-SNE的基础上，针对所选数据集自动优化参数设置，从而得到更好的数据分辨率以及降低数据分析时间，最高可3M events

tSNE-CUDA



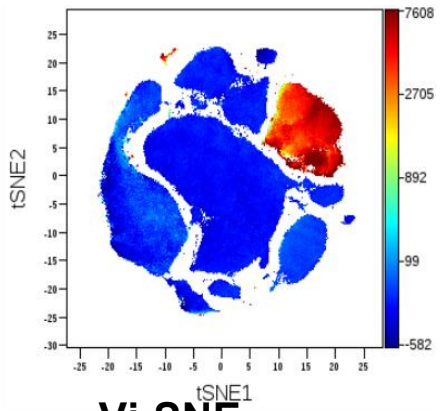
在t-SNE的基础上，基于GPU加速，运算速度更快，节约时间，最高可10M events

UMAP

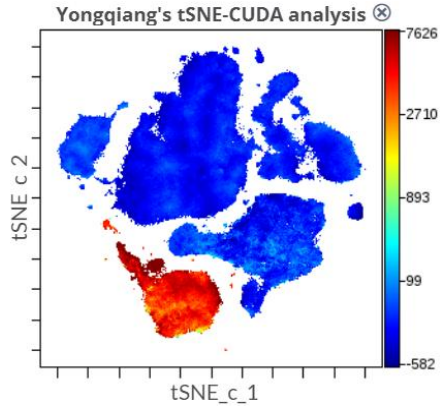


与t-SNE不同，它使用拓扑框架对数据集的底层模式进行建模，相比其他算法，更为保存全局的整体结构，同时基于GPU加速，运算速度更快，节约时间，最高可10M events

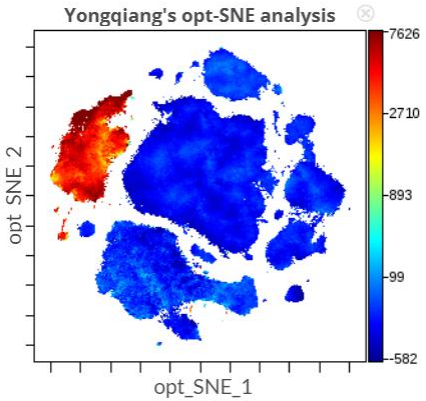
新算法更好解决降维分析中的难点



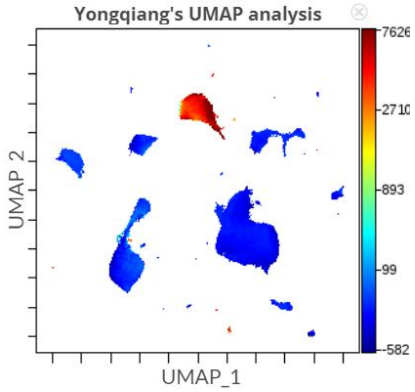
Vi-SNE
5.68h



tSNE-CUDA
5min

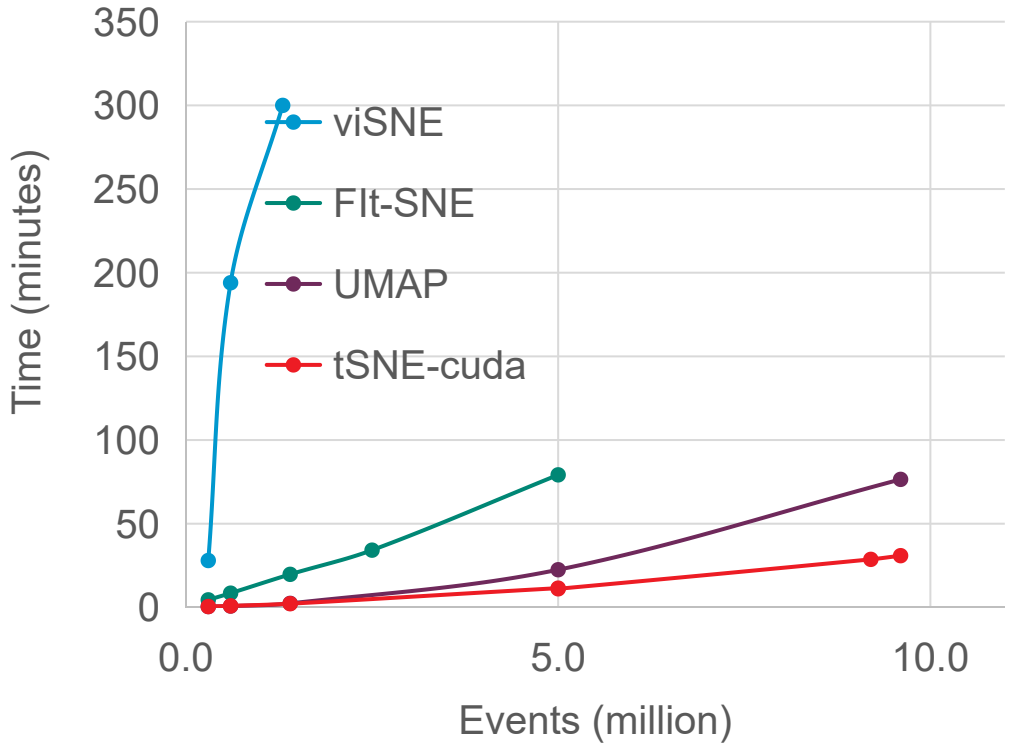


Opt-SNE
33min



UMAP
5.3min

Speed as events increase
(preliminary data)

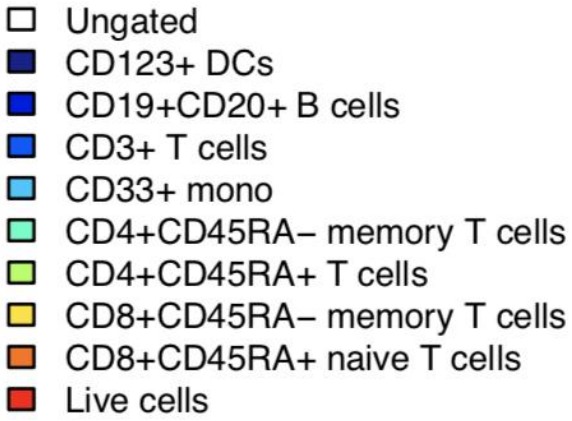


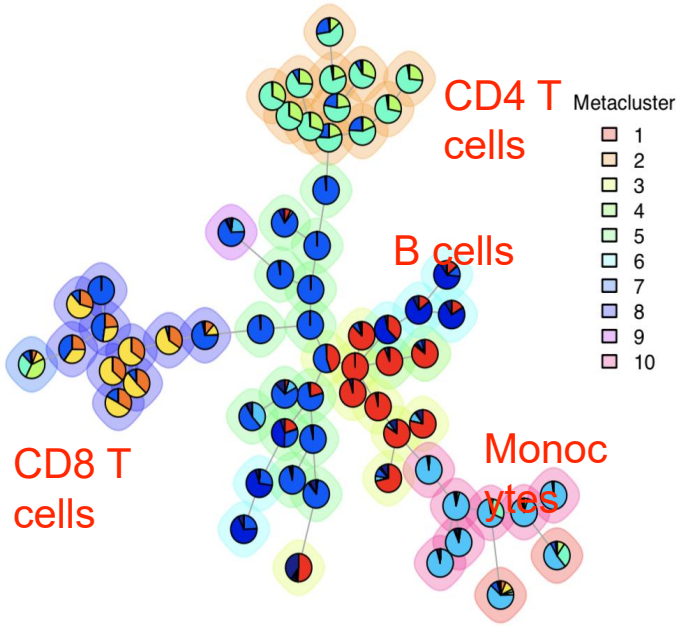
Cytometry Part A . 93A: 402. 405, 2018

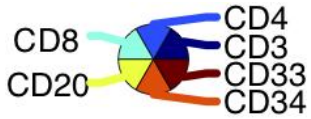
FlowSOM: 一种快速准确的聚类算法

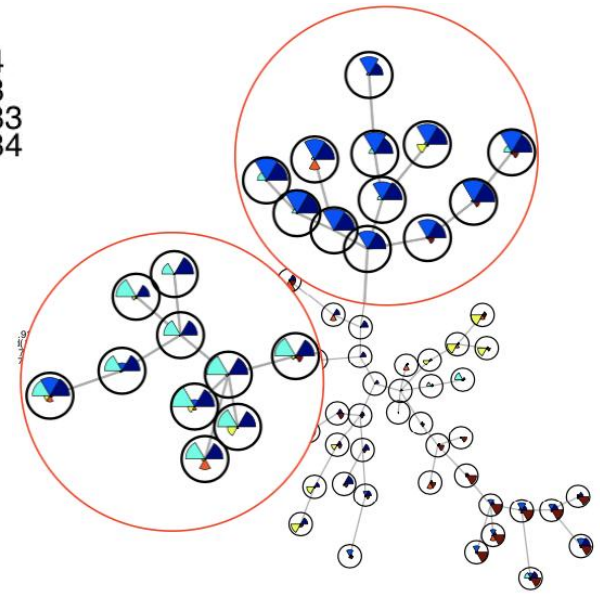
- 使用自组织映射(SOM)来聚类显示所有细胞上的所有标记
- SOM被映射到最小生成树上，并且有星图便于数据可视化分析
- 使用饼图来检测难以定义的子集
- 速度快，可同时运行多个FLOWSOM分析
- 集群自动圈门

可视化展示：饼图与星图

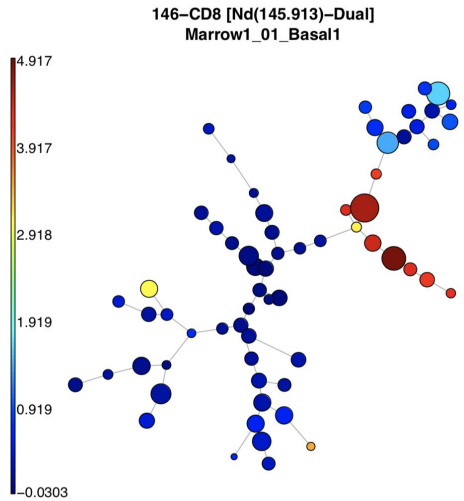






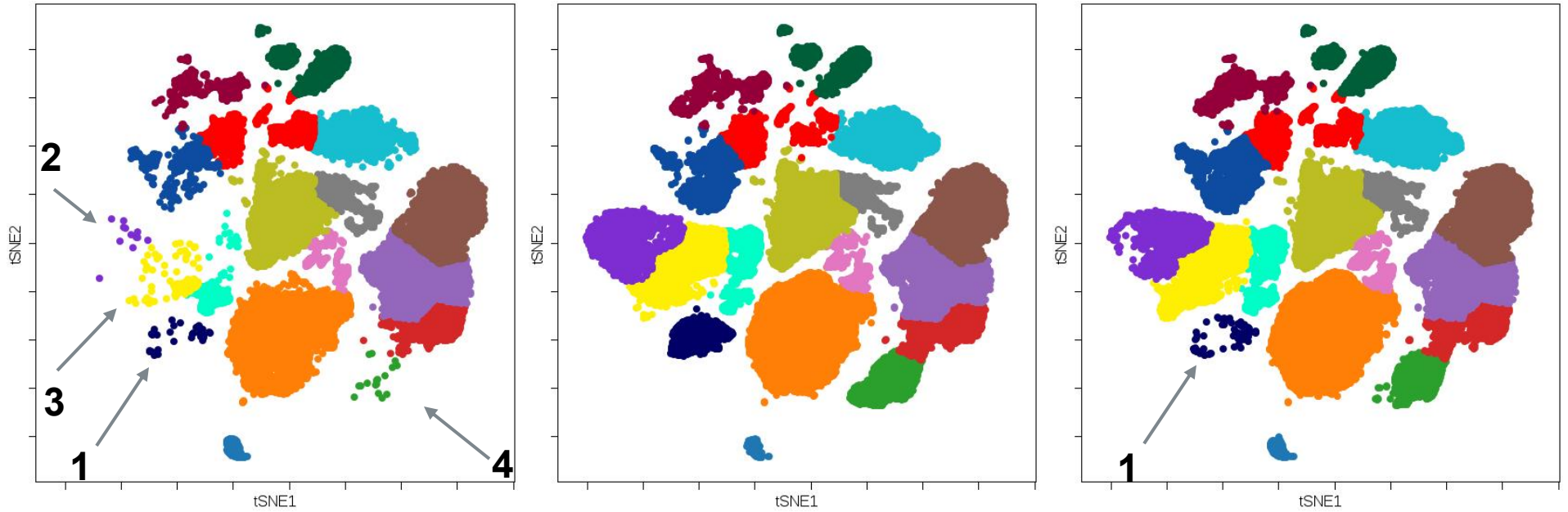


星图

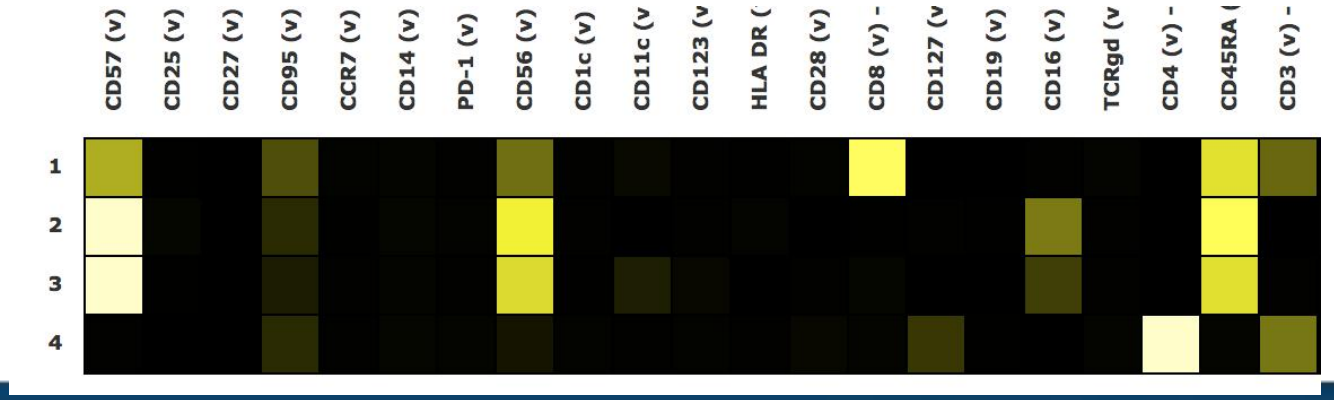


细胞簇抗原表达情况

Automatic Clustering with FlowSOM on viSNE



Donor 1 Donor 2 Donor 3



14

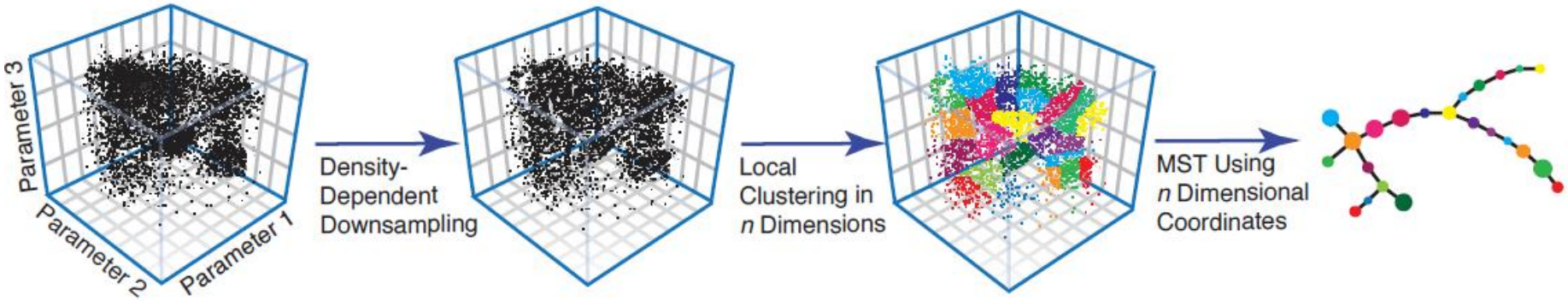
SPADE:密度标准化事件的生成树连续分析

Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE

Peng Qiu[1,2], Erin F Simonds[3], Sean C Bendall[3], Kenneth D Gibbs Jr[3], Robert V Bruggner[3], Michael D Linderman[4], Karen Sachs[3], Garry P Nolan[3] & Sylvia K Plevritis[1]

VOLUME 29 NUMBER 10 OCTOBER 2011 NATURE BIOTECHNOLOGY

SPADE method:



1) 获取数据

2)降低取样
(降低含量高细胞群比例，保留稀有细胞群)

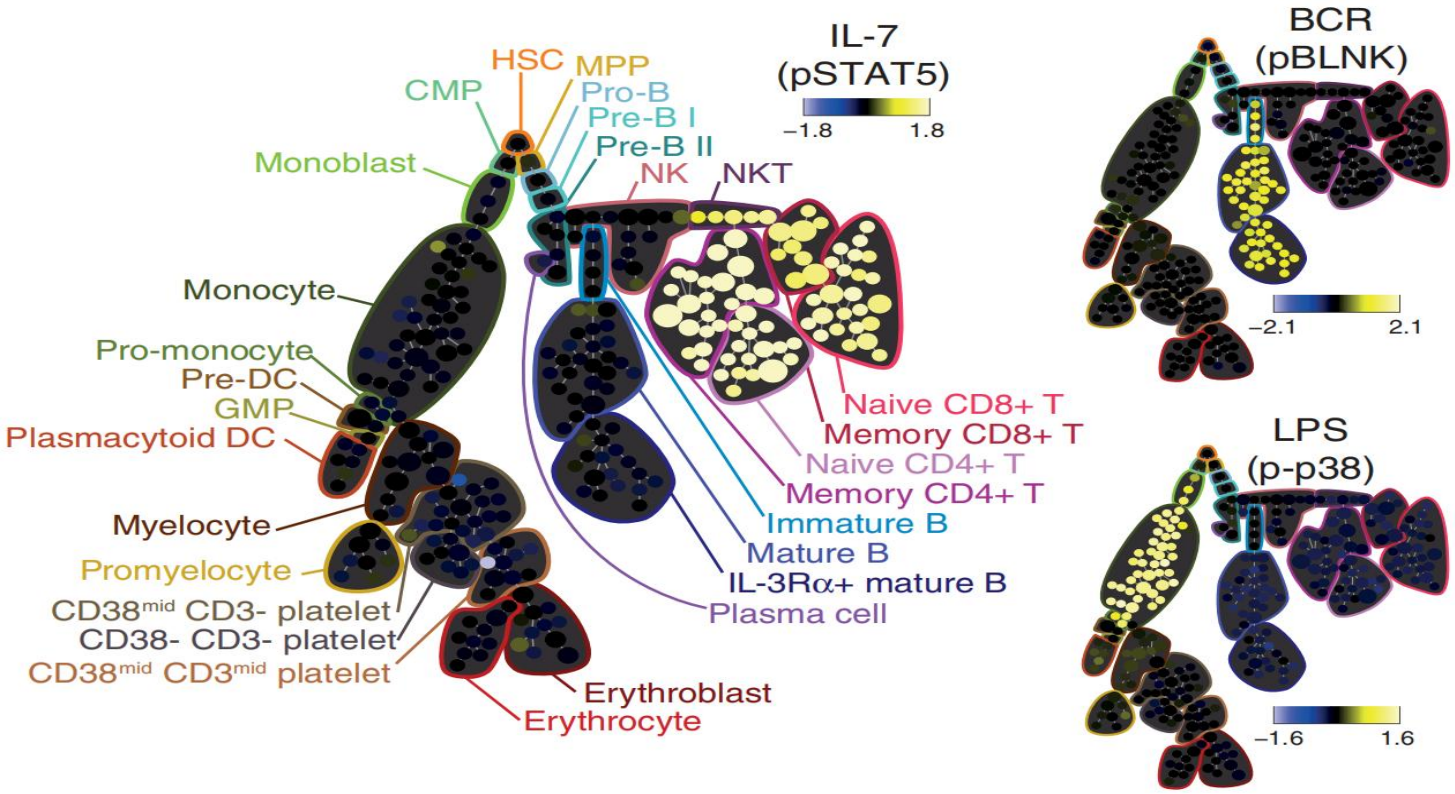
3) 聚类
(按相似性分类)

4) 2D投影
(最小化生成树)

15

免疫系统中单细胞信号分子表达

- 骨髓细胞采用IL-7, BCR和LPS 刺激，检测pSTAT5
- IL-7, BCR和LPS刺激后pSTAT5 水平变化分别发生在T cells, B cells, 和 monocytes



Bendall et al. Science. (2011)

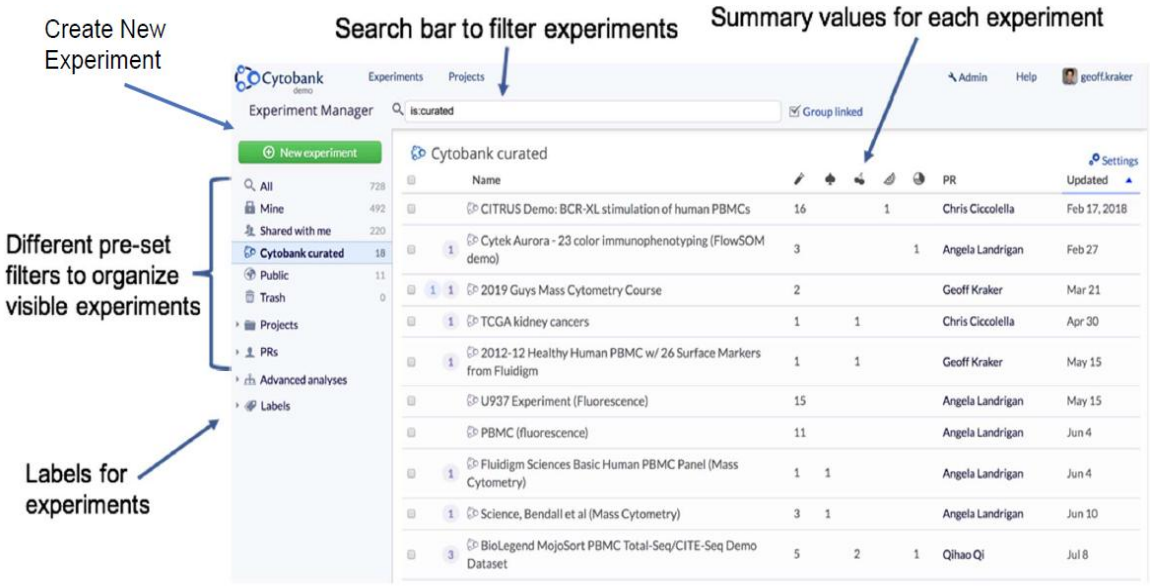
降维分析操作流程

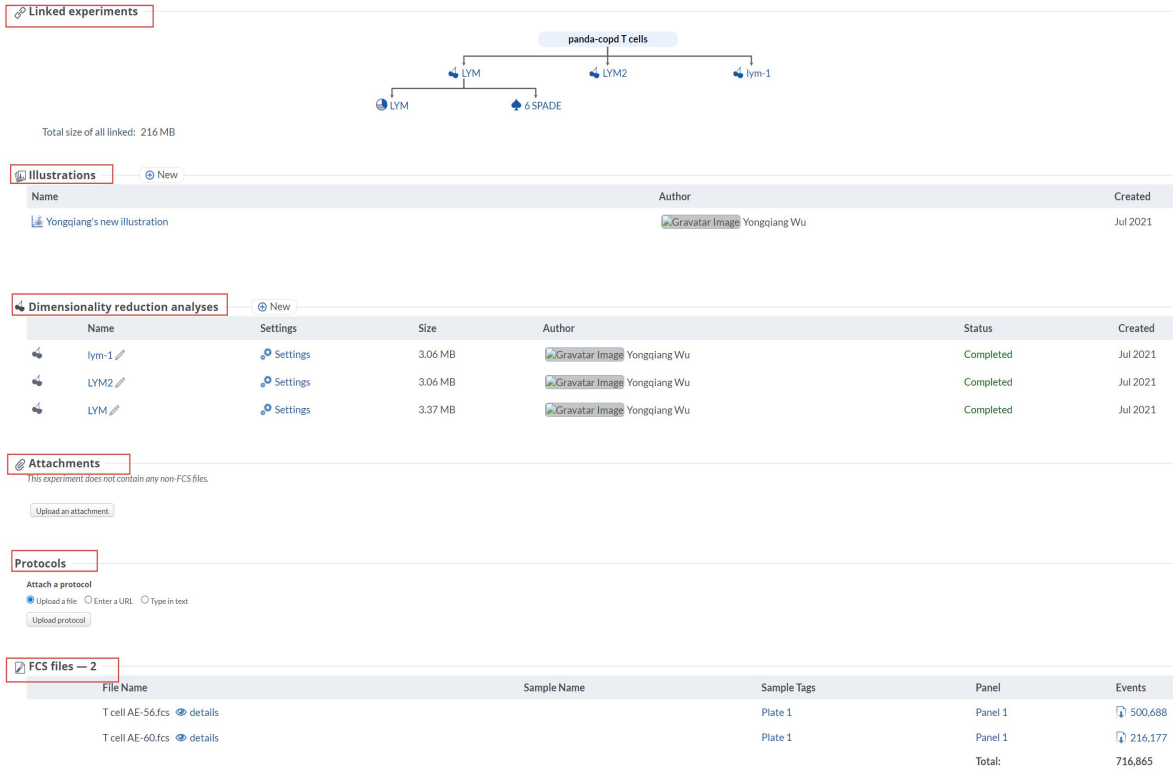
Cytobank 账号登录

网址：https://www.cytobank.cn

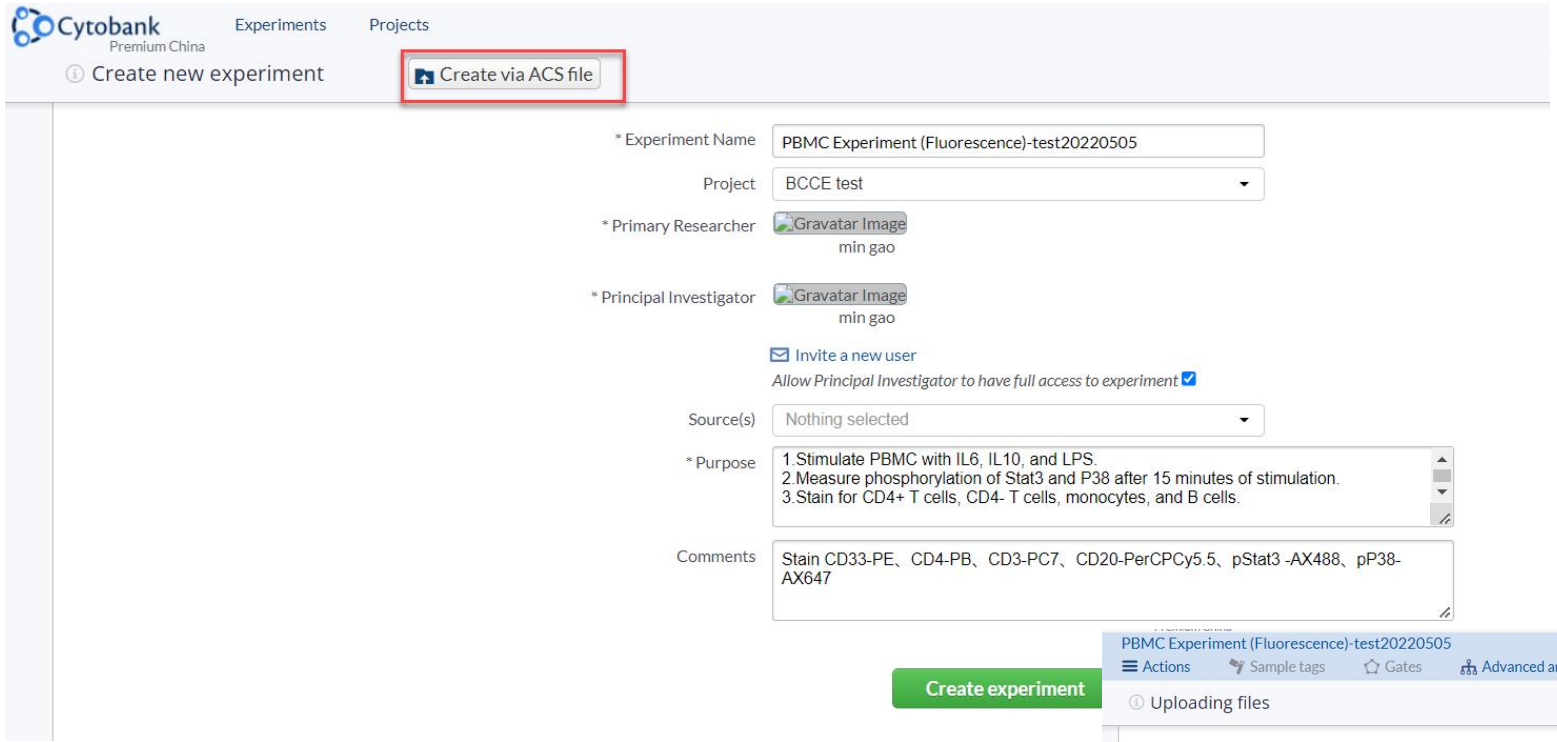
便捷的数据分类管理--软件主界面

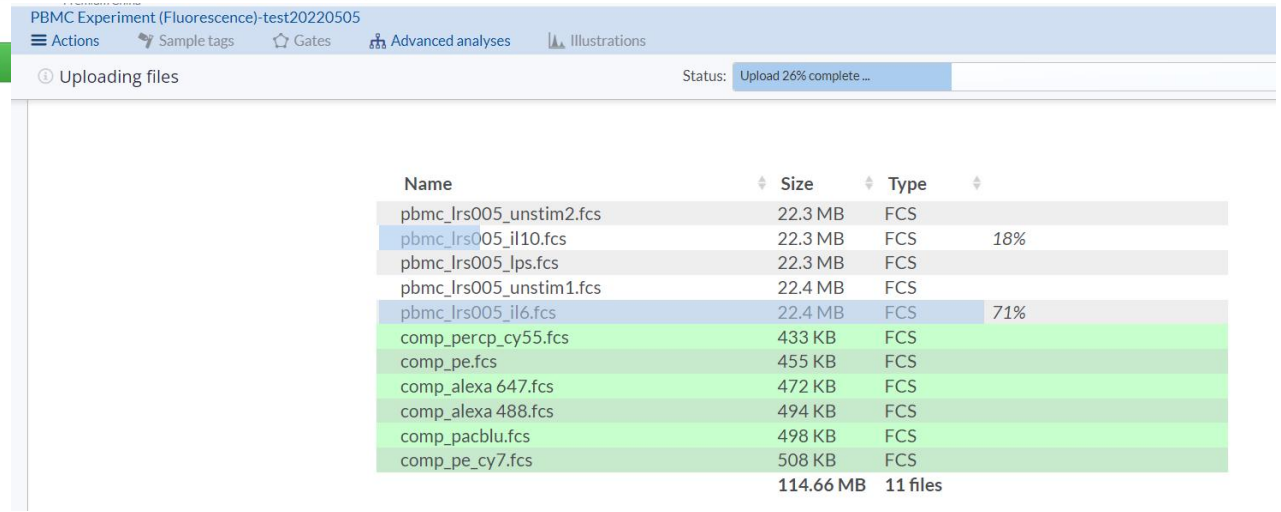
- 所有数据基于标签分类和关键词查找
- 单个实验以总表形式呈现，一键溯源分析
- 数据分析，即刻自动保存，避免丢失信息



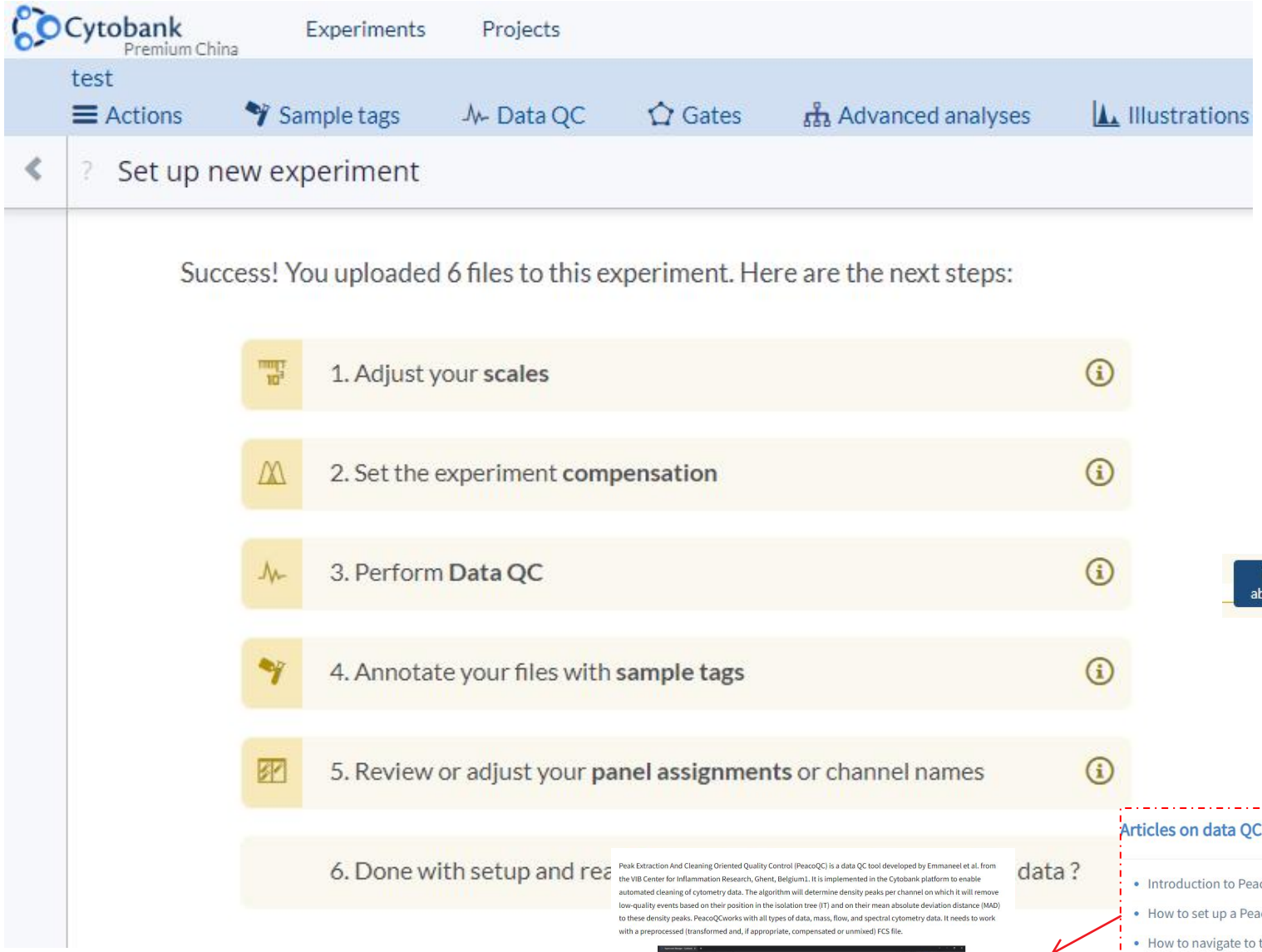


数据上传--Cytobank





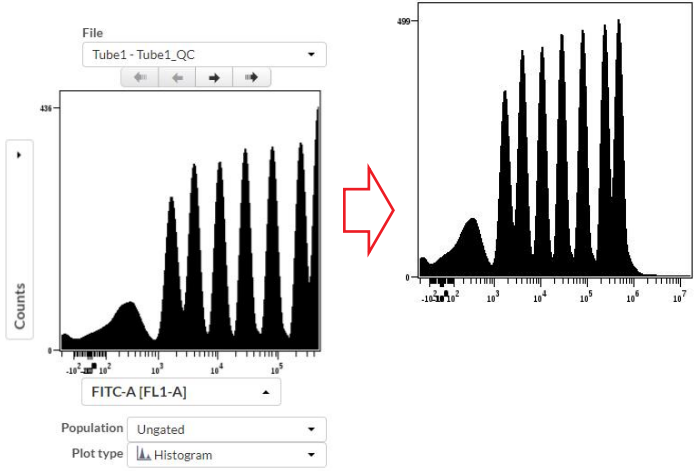
创建实验

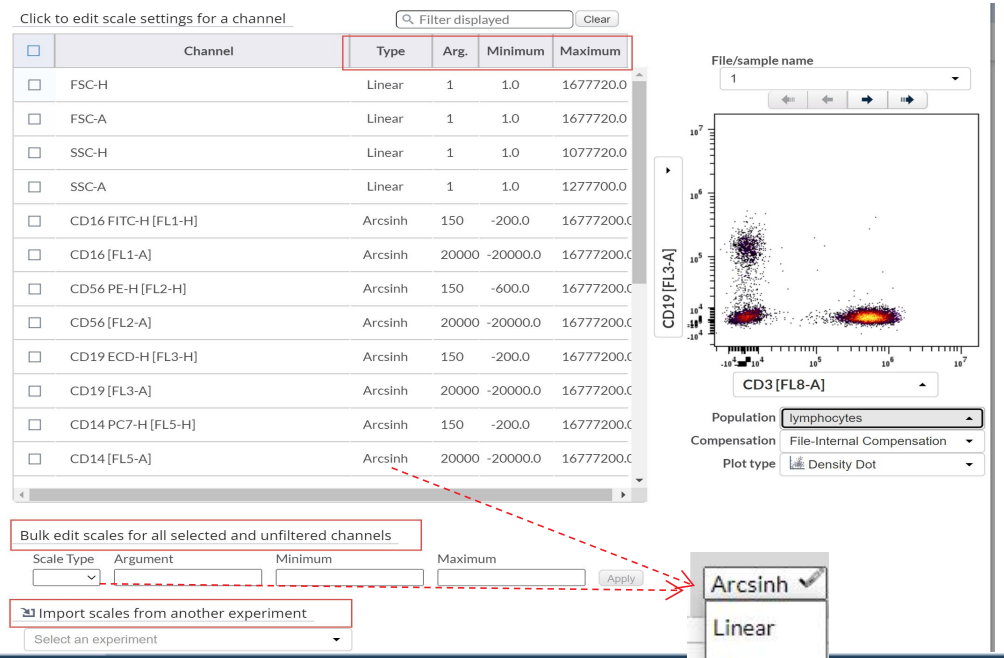


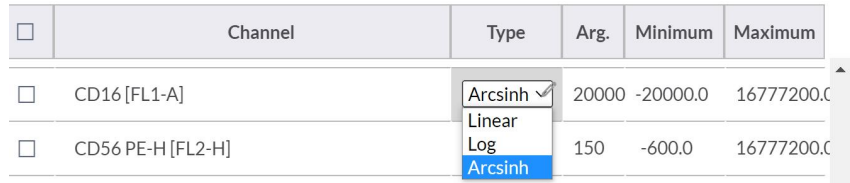
1.调整坐标轴--Adjust your scales

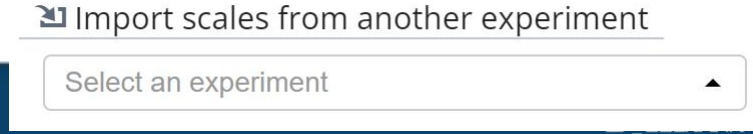
Cytobank支持便捷的坐标轴调整

- 支持荧光通道坐标轴显示方式切换，以及动态范围自定义展示
- 批量修改坐标轴
- 相似方案坐标轴设置参数一键导入







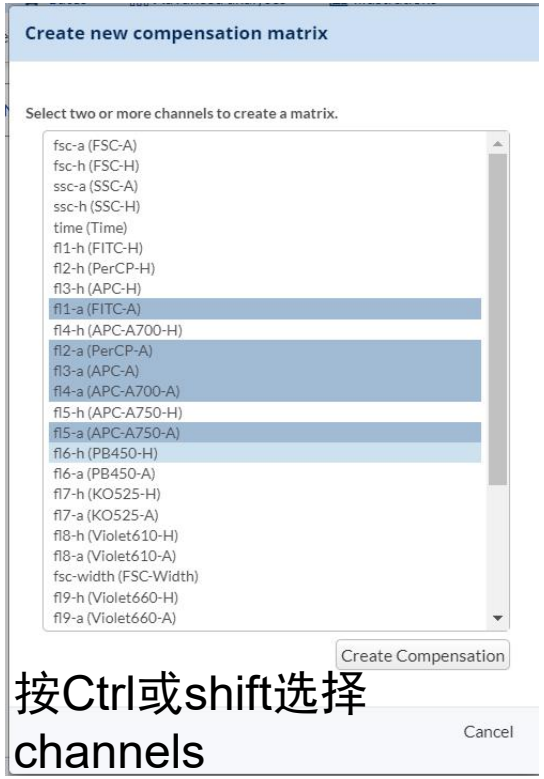


2.设置补偿-- Set the experiment compensation

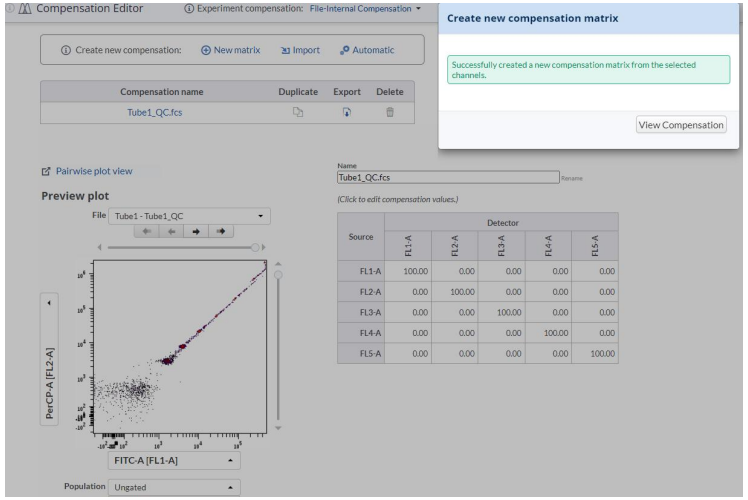
Tips:

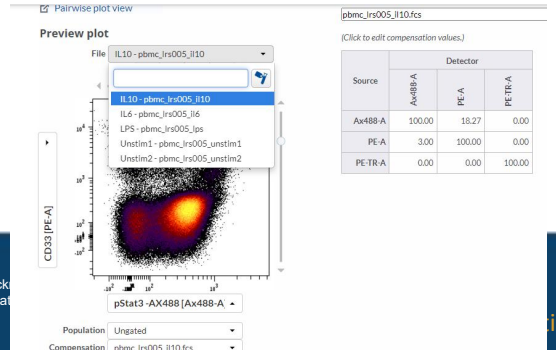
1.需把补偿管设置到compensation files（界面Sample tags→Assign panels）中

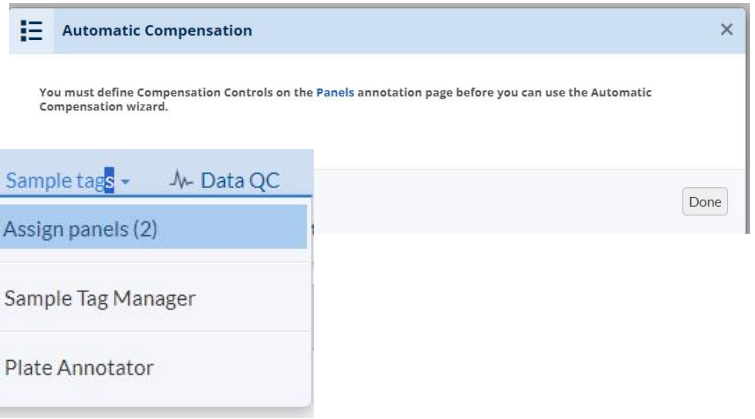
2.手动补偿时选择不了 设置在compensation files中的文件（管）

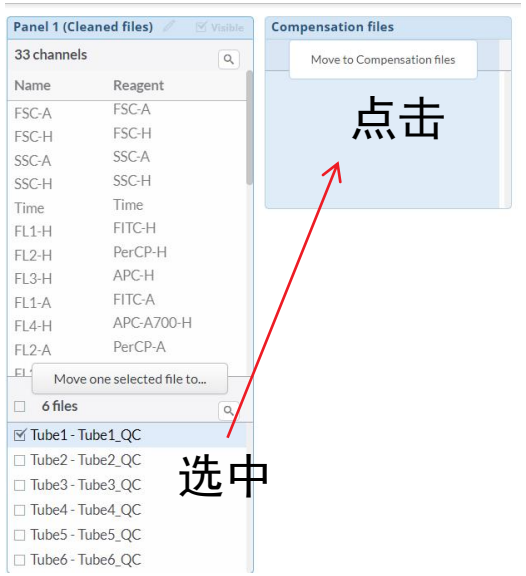


按Ctrl或shift选择channels

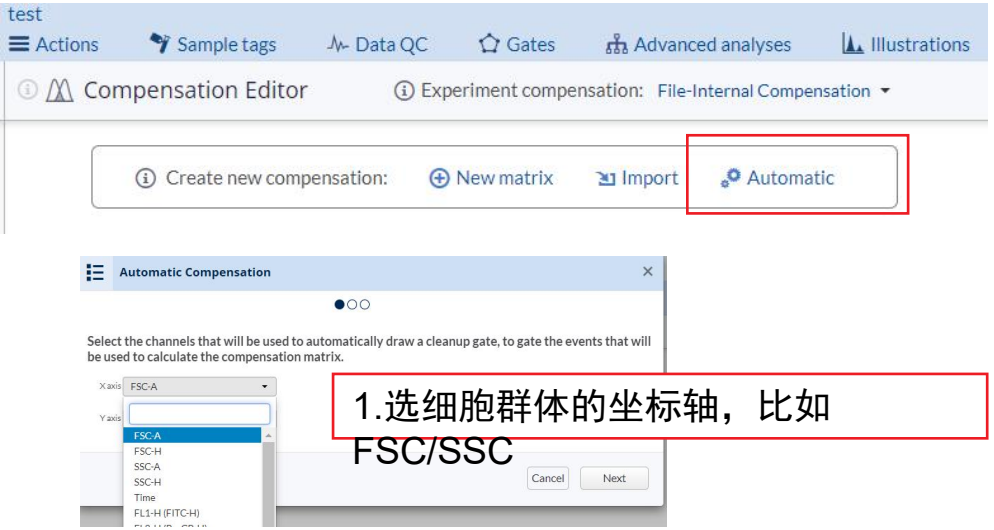




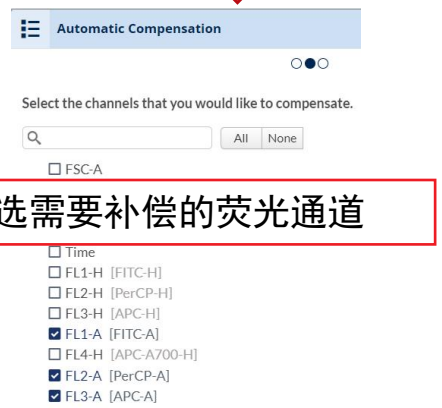




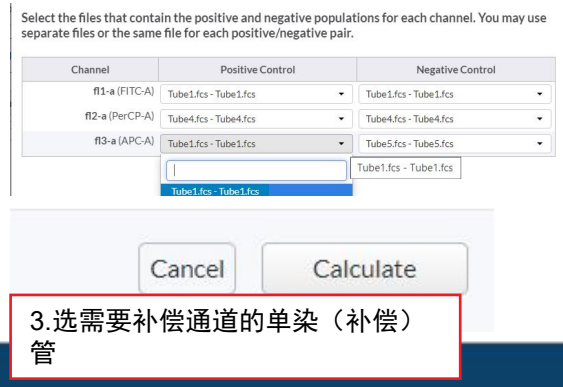
2.设置补偿-- Set the experiment compensation



1.选细胞群体的坐标轴，比如FSC/SSC

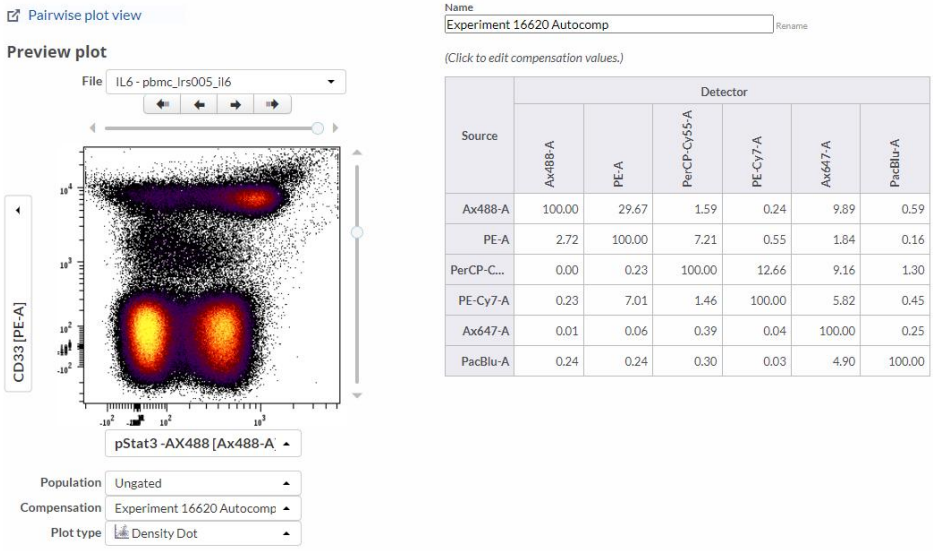


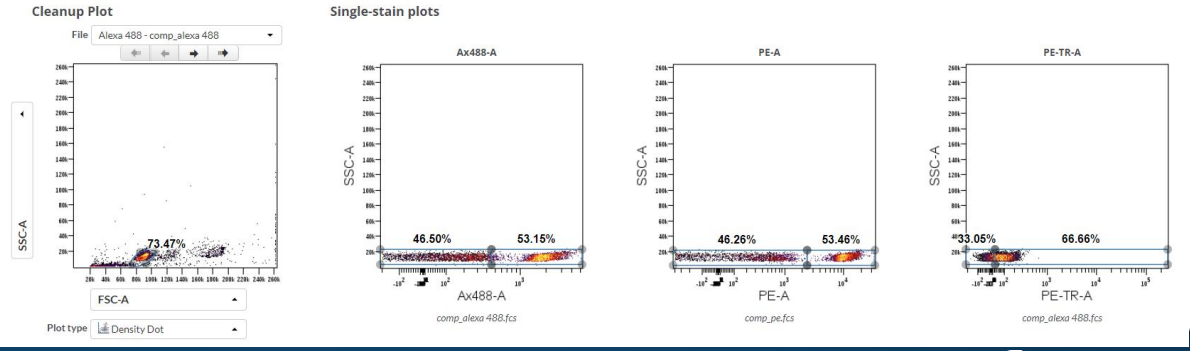
2.选需要补偿的荧光通道



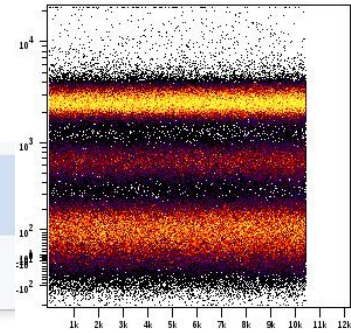
3.选需要补偿通道的单染（补偿）管

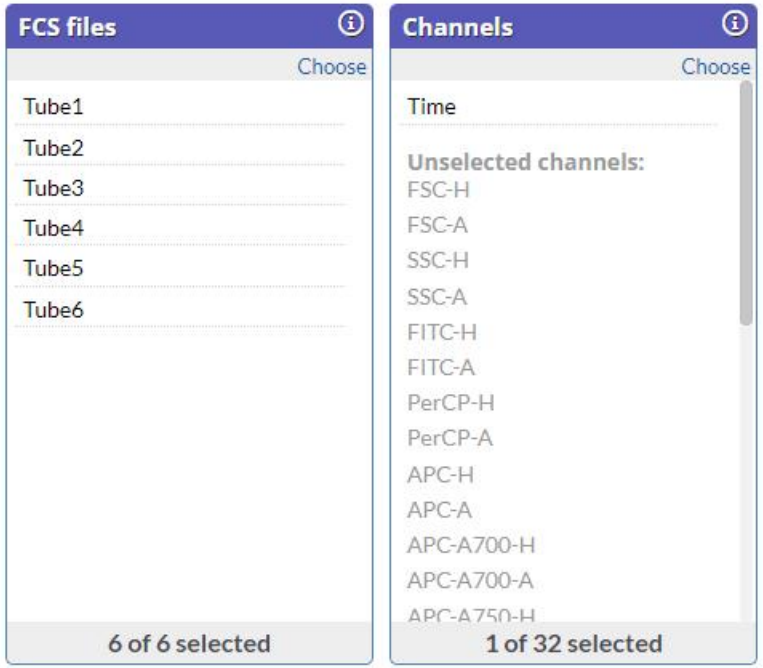
4.用全染管验证补偿，如需，可再微调

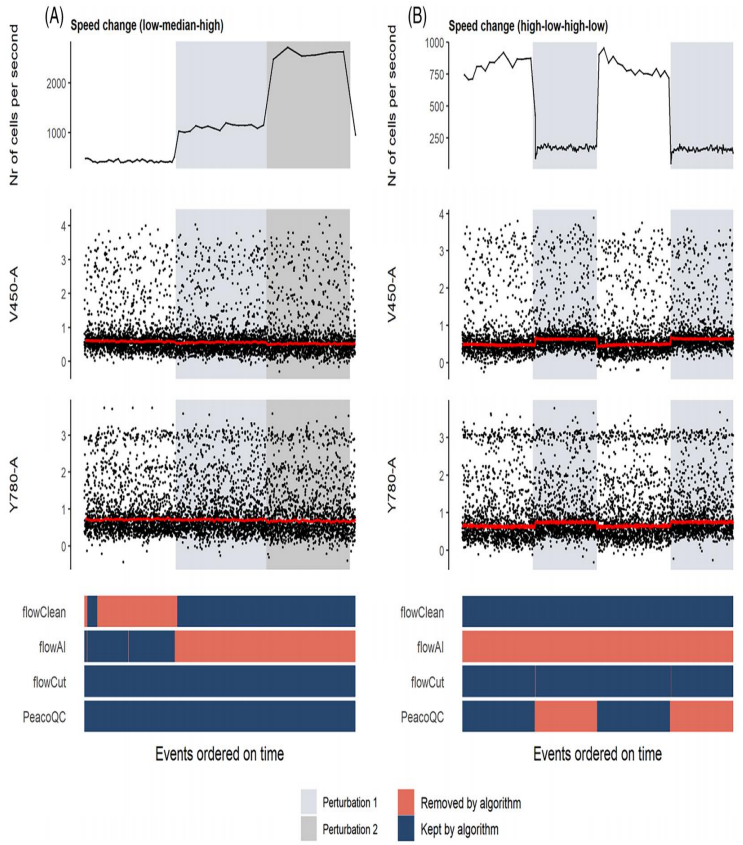


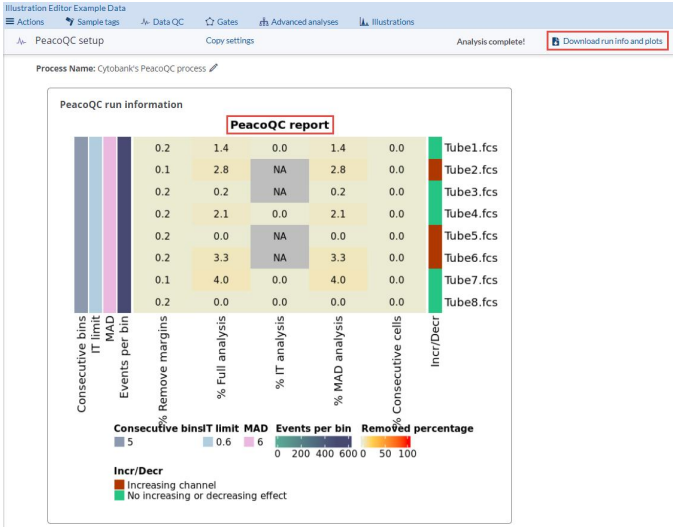


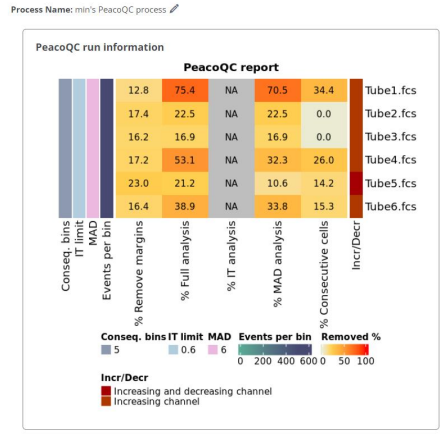
3. 数据质控--Perform Data QC



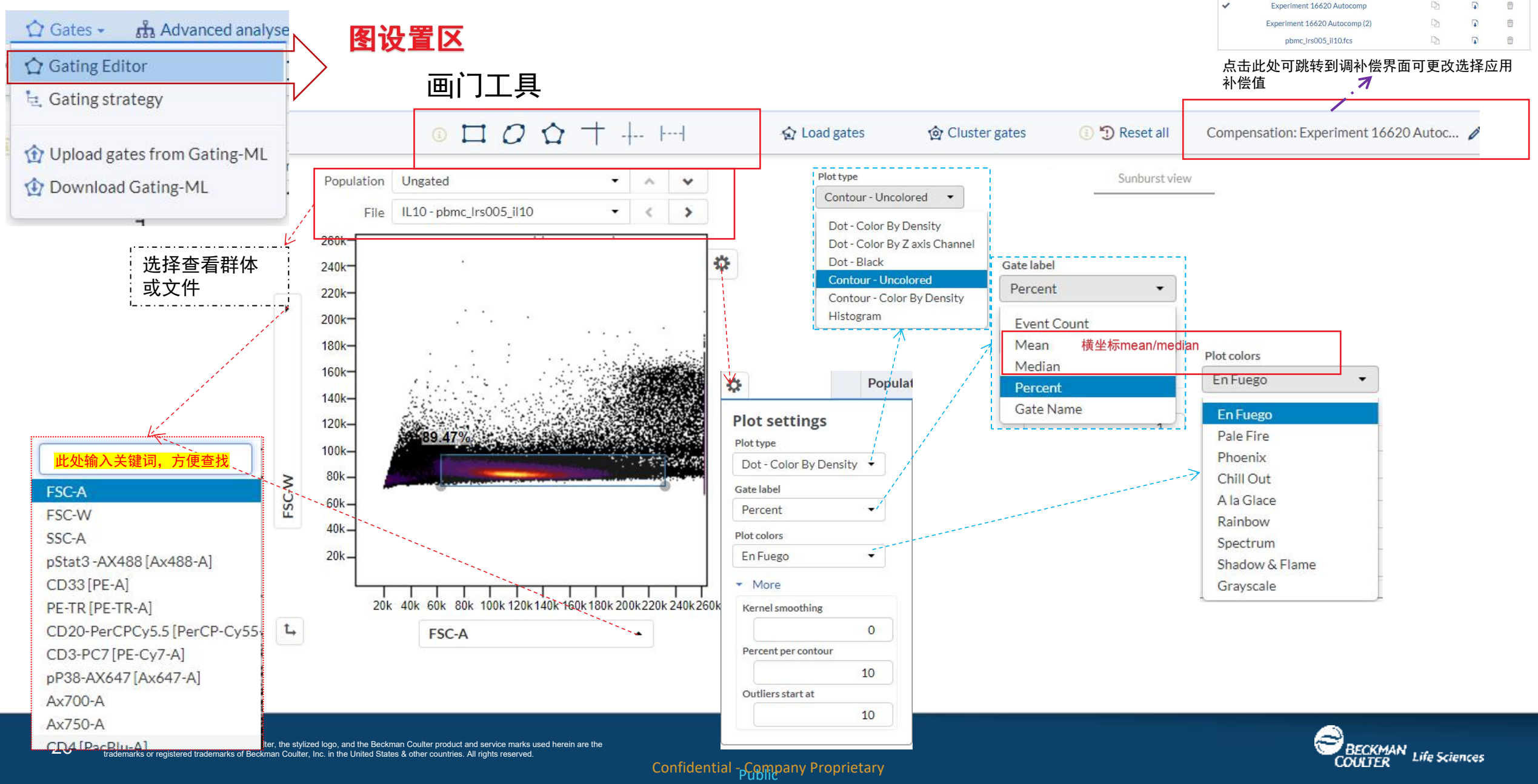




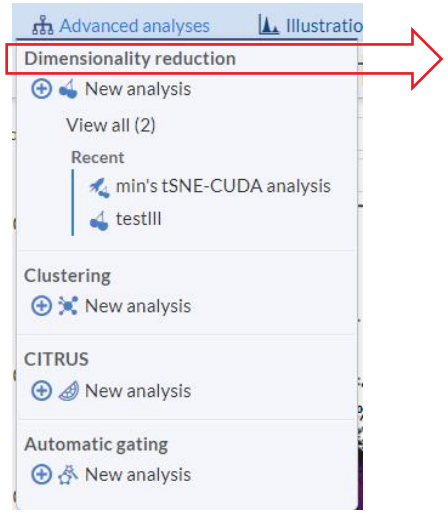


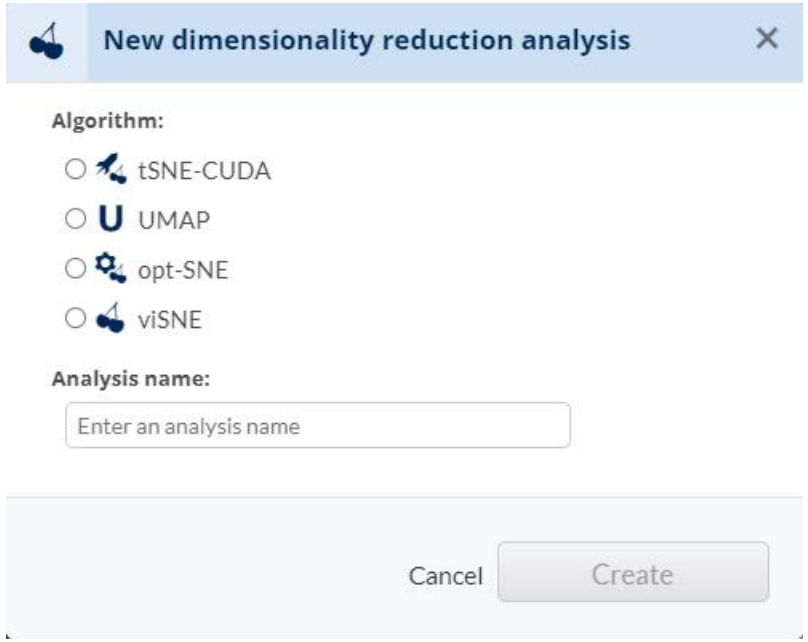


4.Gating Editor

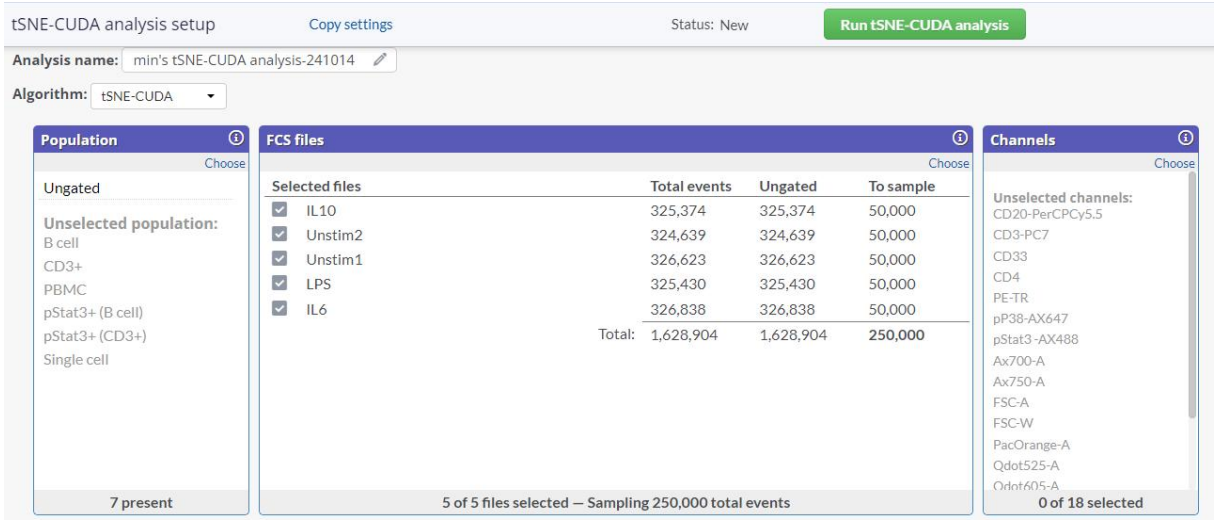


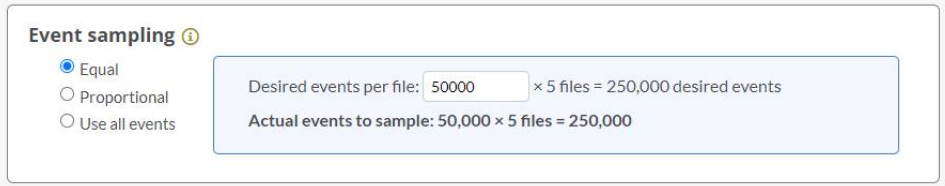
5. 降维ViSNE分析

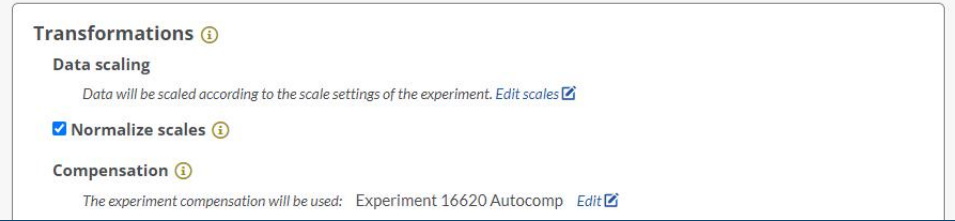


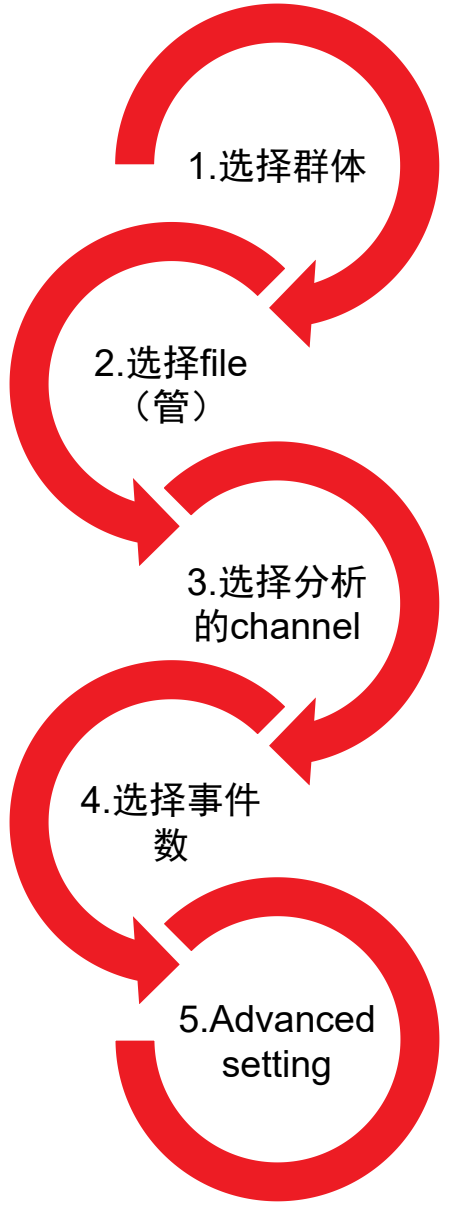


• tSNE-CUDA analysis setup









Equal（均等）
Proportional（按比例）
Use all events（所有）

Iterations
Perplexity
Learning rate
Early exaggeration

6. 分析结束

1.邮件提醒

[Premium China Cytobank] Your tSNE-CUDA analysis is complete

Cytobank_Admin@beckman.com on b
To Gao, Min (周一) 23:02

If there are problems with how this message is displayed, click here to view it in a web browser.
Some of the content in this message couldn't be downloaded because you're working offline or aren't connected to a network.

Hi min,

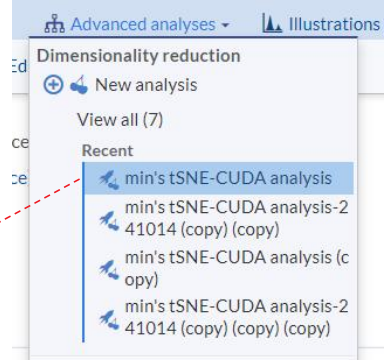
The tSNE-CUDA analysis 'min's tSNE-CUDA analysis-241014 (copy)' for experiment PBMC Experiment (Fluorescence)-test20220505 (Clone) is now complete.

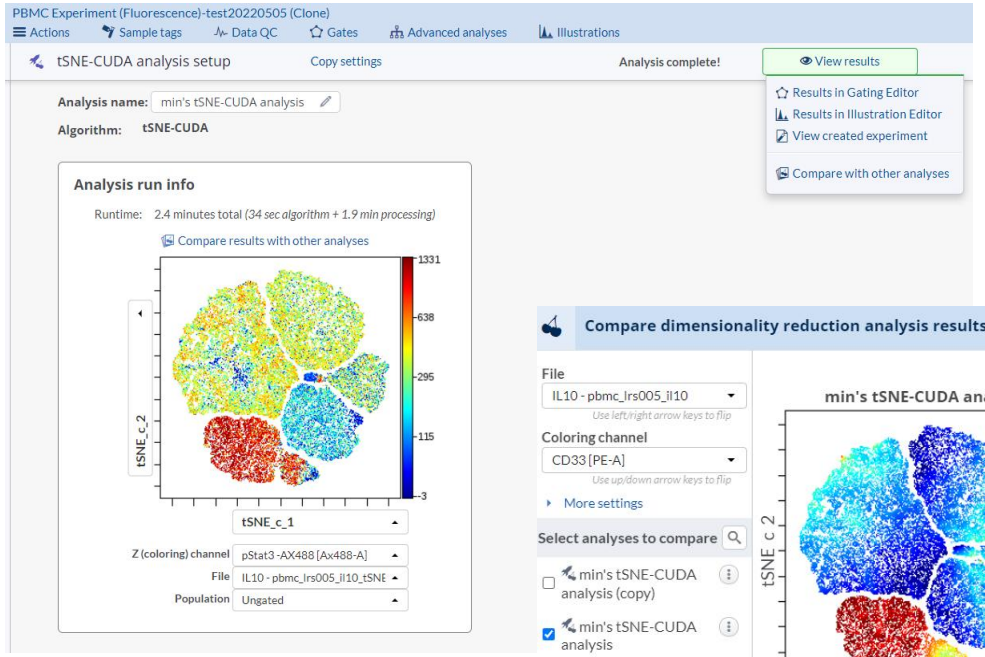
View results

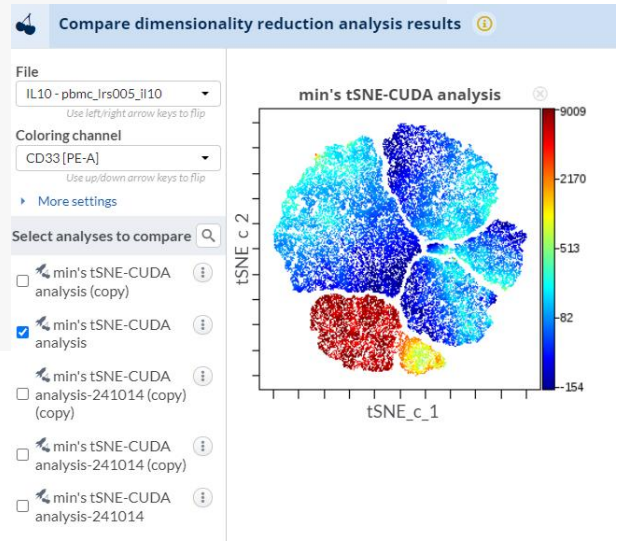
- The Cytobank Team

2.软件界面会出结果

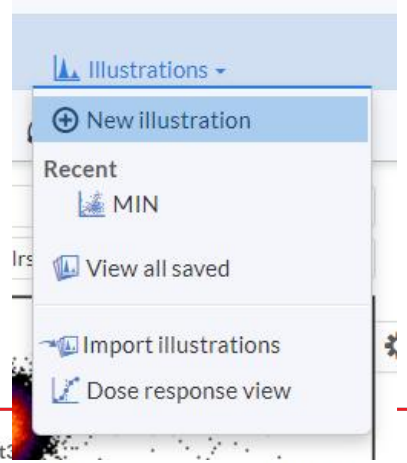
查看

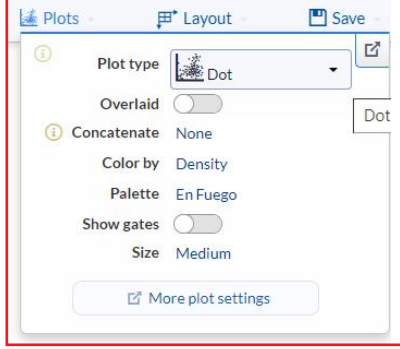




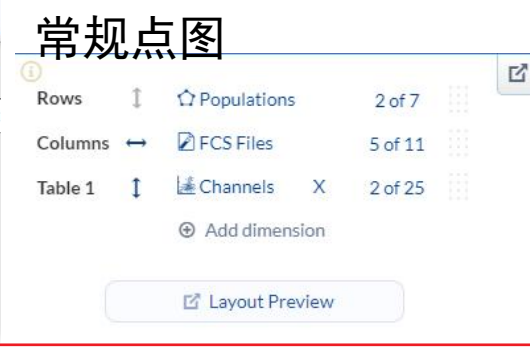


7. 结果展示

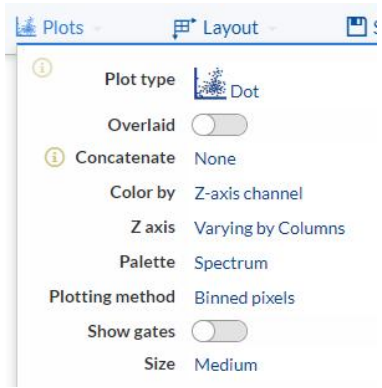


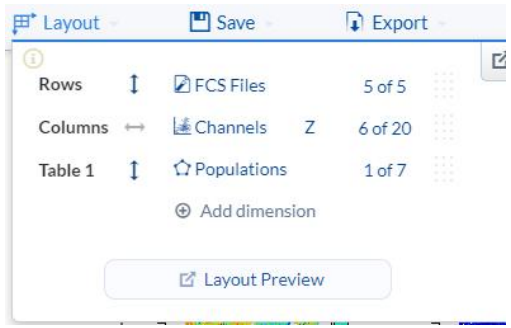


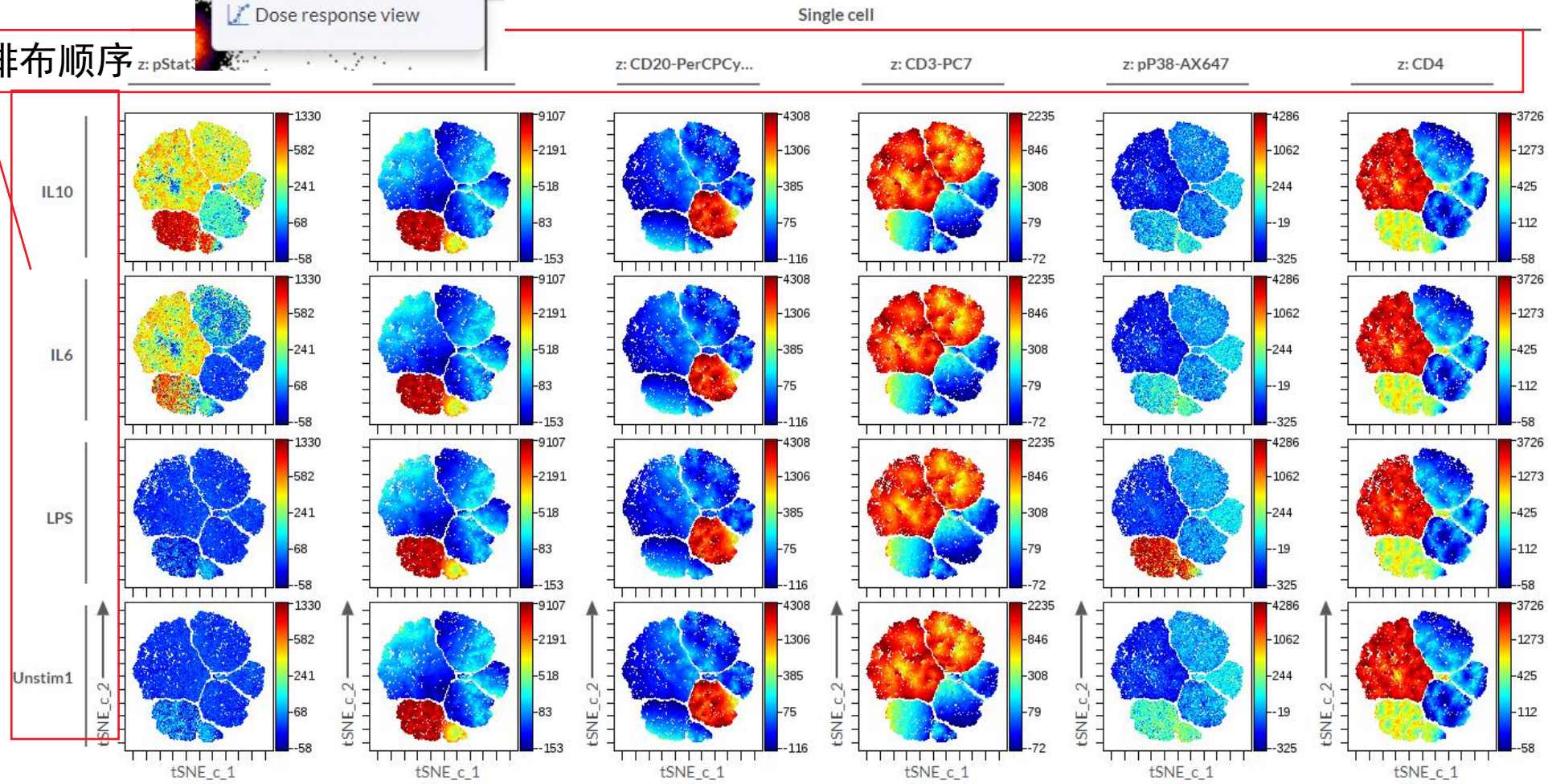
常规点图



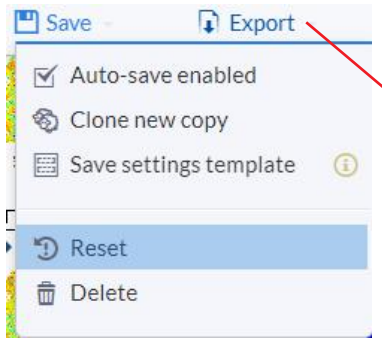
拖动此处可以改排布顺序

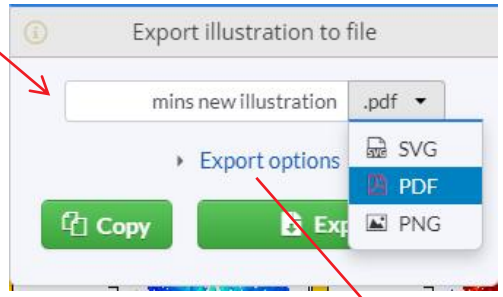


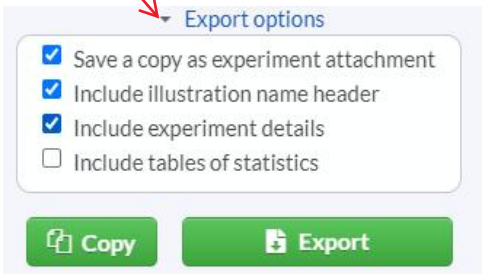


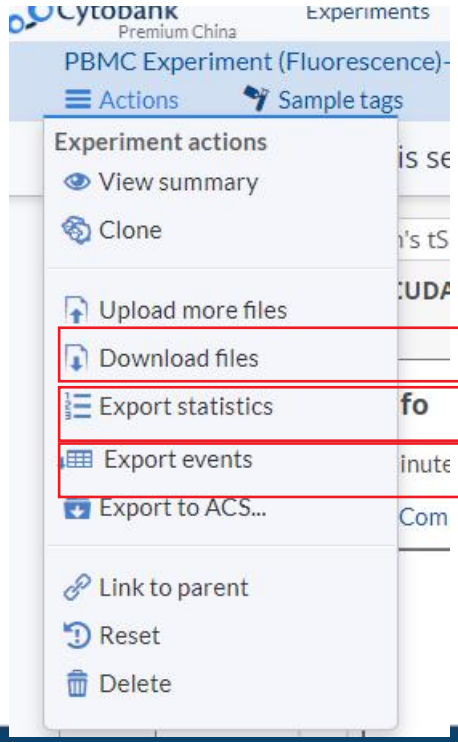


8.数据保存及导出





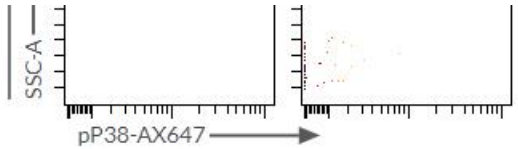


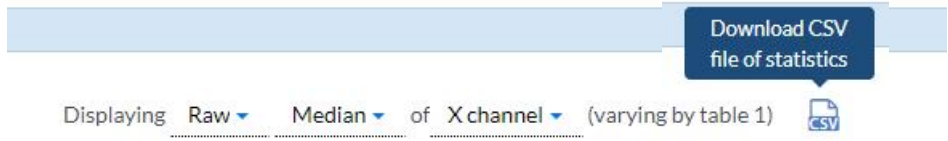


可下载.FCS文件

可导处门内每个事件（event）的信息（光强度）

可导处门控的信息（百分比、MFI，keyword等）





pStat3 -AX488

	pStat3+ (B cell)	pStat3+ (CD3+)
IL10	449.735	423.774
IL6	441.028	423.207
LPS	476.51	313.274
Unstim1	485.396	213.673
Unstim2	2665.882	224.553

pP38-AX647

	pStat3+ (B cell)	pStat3+ (CD3+)
IL10	-35.681	-116.637
IL6	-20.708	-127.047
LPS	224.919	-83.813
Unstim1	1114.999	63.116
Unstim2	8061.213	-53.486

哪里使用Cytobank?

哪里使用 Cytobank?

是否有教程?

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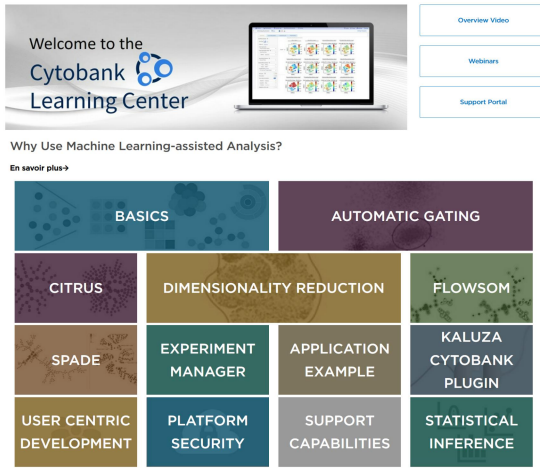
还有问题怎么办?

网址：https://www.cytobank.cn/



网址：https://www.beckman.fr/flow-cytometry/software/cytobank-premium/learning-center

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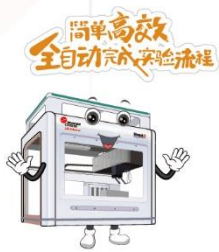

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