Advanced tool FlowJo introduction

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Senior Product Specialist
BD Biosciences

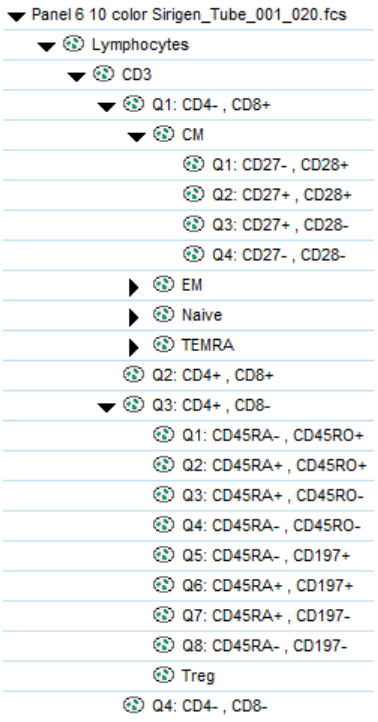


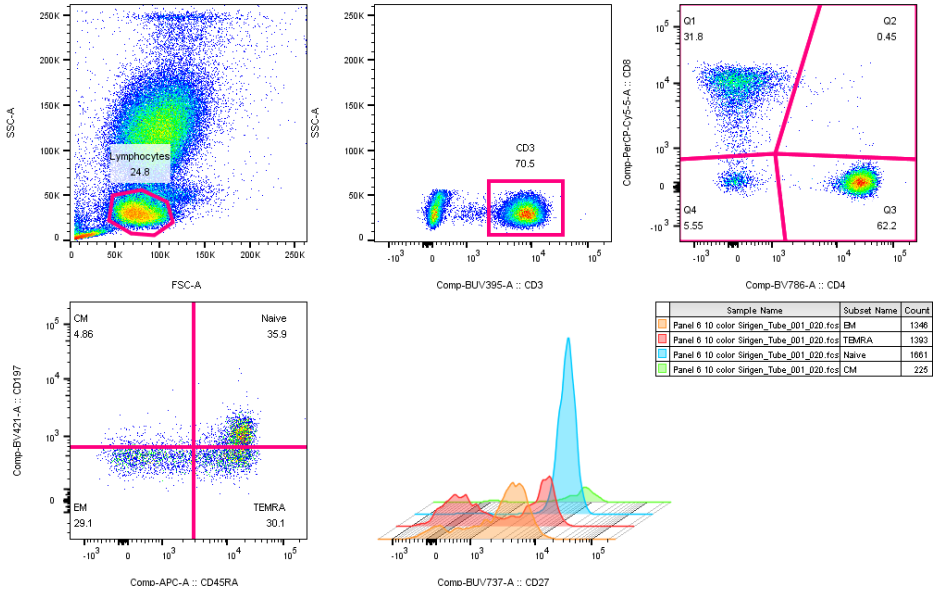


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Flow Data Analysis

- **分析圈選階層**
  (Population Hierarchy)

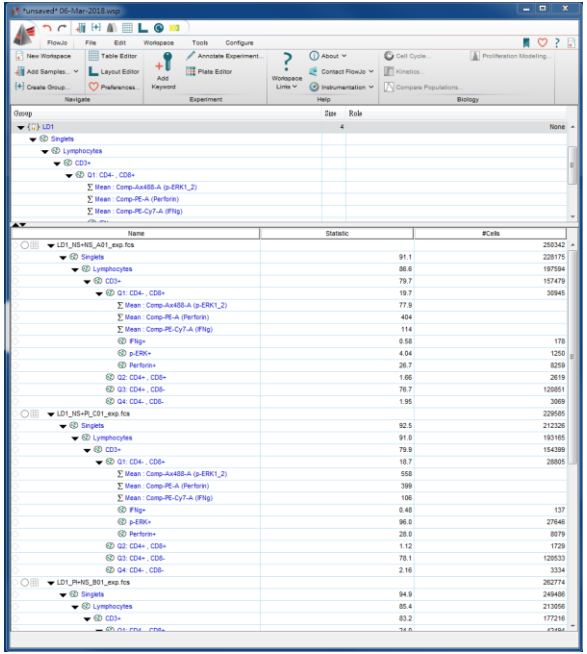




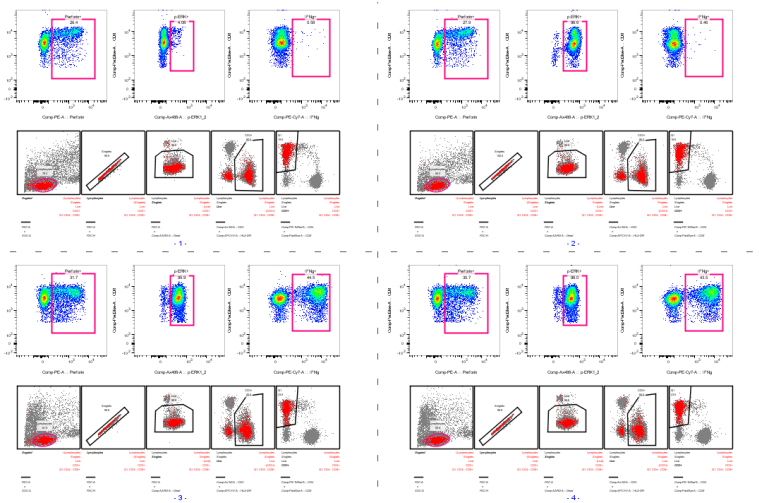
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Flow Data Analysis

- 儲存分析模板
  (Workspace Template)



- 批次分析報告輸出 **(Batch Report)**



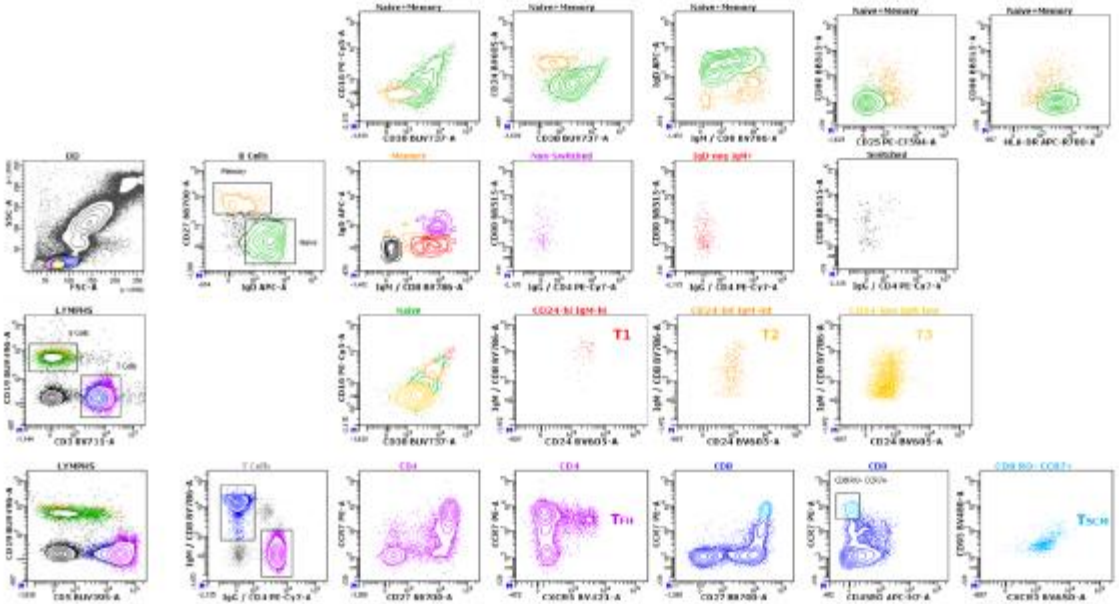
- 統計輸出/Heat Map 統計

Ancestry Subset Statistic For	p-ERK1/2 Mean	Perforin Mean	IFN-R Mean	CD8 %	CD4 %
LD1_NS+NS_A01_exp.fcs	78.0	402	114	19.6	77.0
LD1_NS+PI_C01_exp.fcs	562	397	106	18.6	78.3
LD1_PI+NS_B01_exp.fcs	414	463	3020	23.9	72.6
LD1_PI+PI_D01_exp.fcs	411	463	3018	23.5	73.0
Mean	*366*	*431*	*1564*	*21.4*	*75.2*
SD	*205*	*36.7*	*1680*	*2.69*	*2.85*

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Manual methods waste too much

- 2^{18}=262144 Plots
- 23 plots is 0.88% of 262144 plots



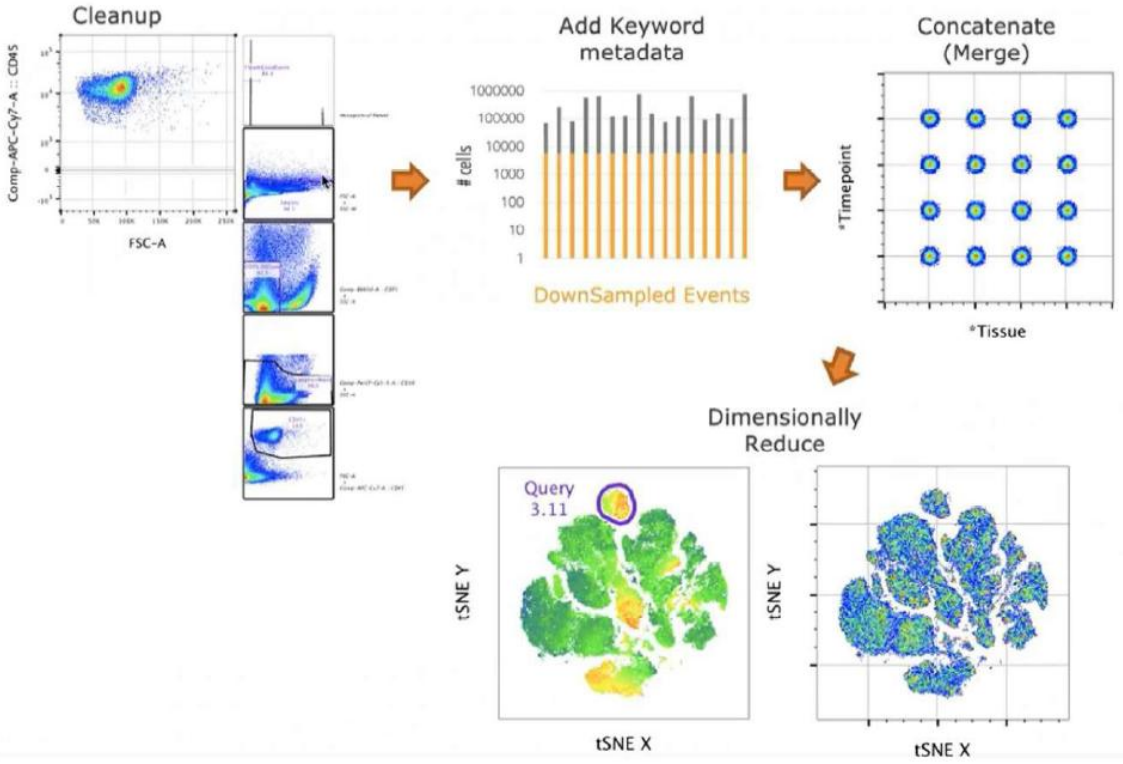
18 Colors

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High Parameter Analysis Workflow

for Discovery

- cleanup
- concatenate
- dimensionally-reduce
- cluster
- gate
- comparisons
- dig deeper

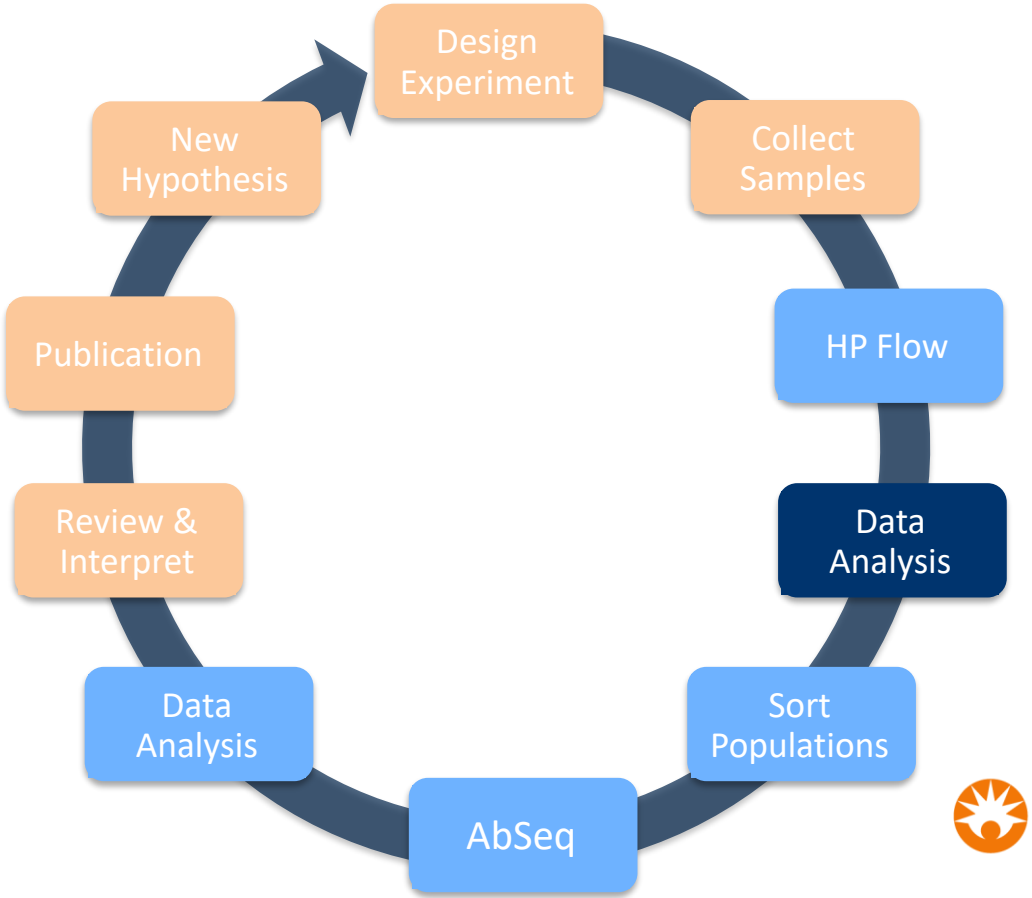


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High Parameter Analysis Workflow

for Discovery

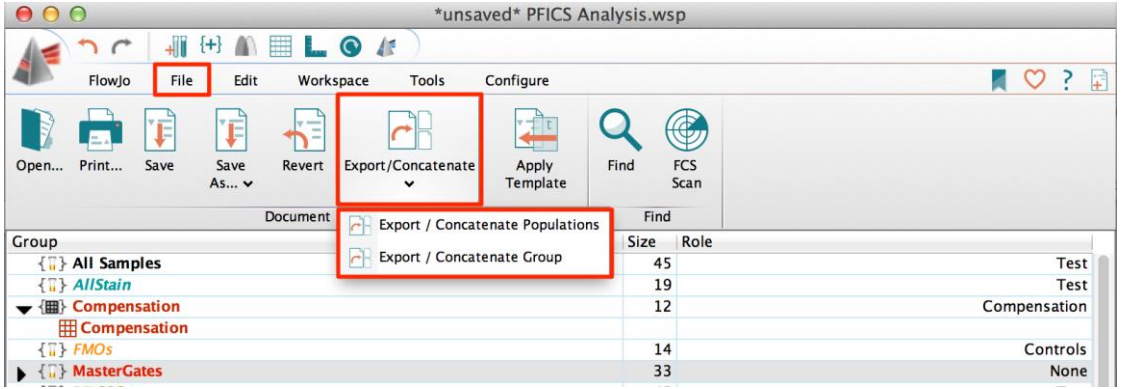
- cleanup
- concatenate
- dimensionally-reduce
- cluster
- gate
- comparisons
- dig deeper



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輸出/連鎖 Export/Concatenate

- Initiation from the Workspace

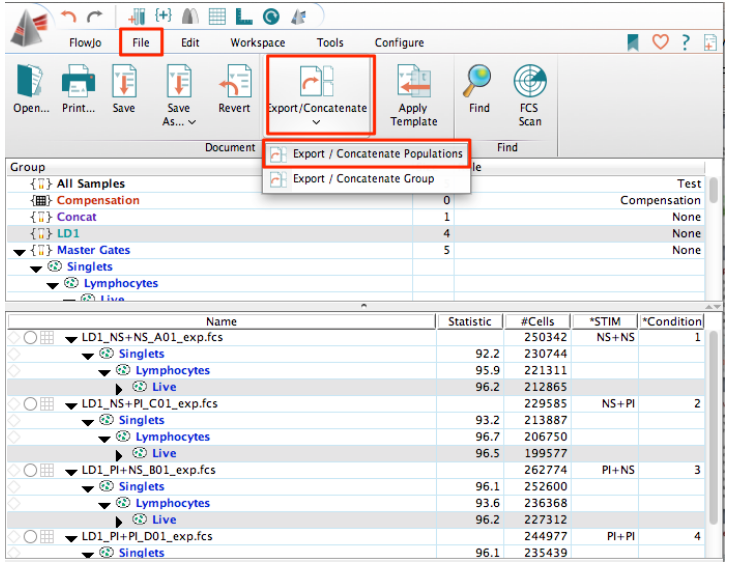


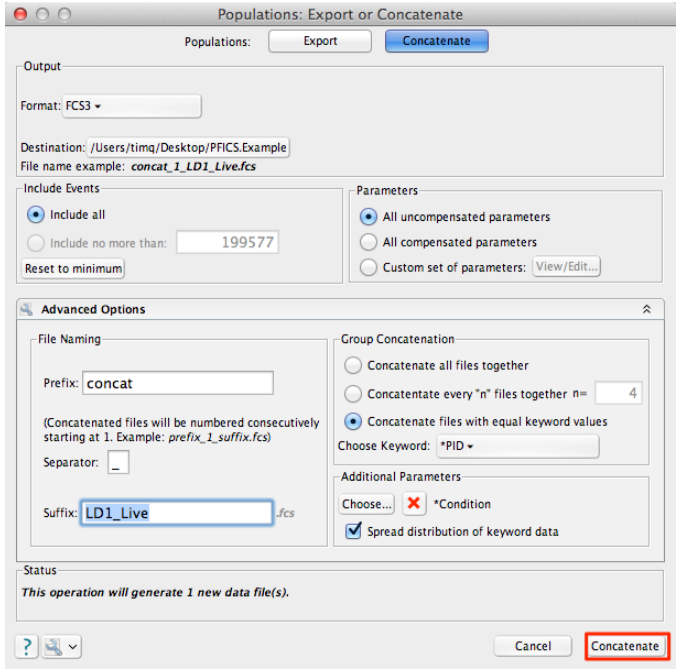
  - Two options:
    - Export/Concatenate Populations → select gated populations on sample gating hierarchy
    - Export/Concatenate Group → select group or group owned gate in the groups pane

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連鎖特定細胞群 Concatenating Populations

- Highlight the equivalent population nodes within the gating tree of samples you wish to merge.
- Choose Export/Concatenate Populations.

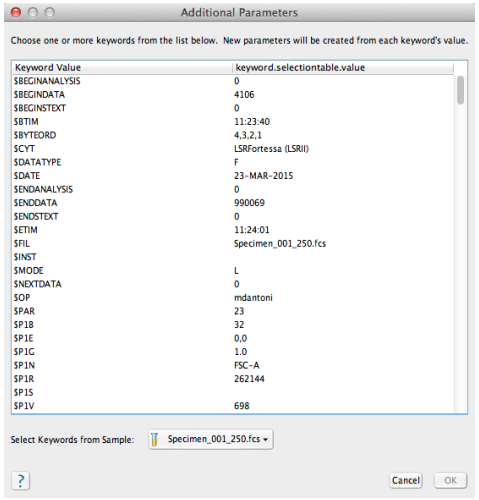


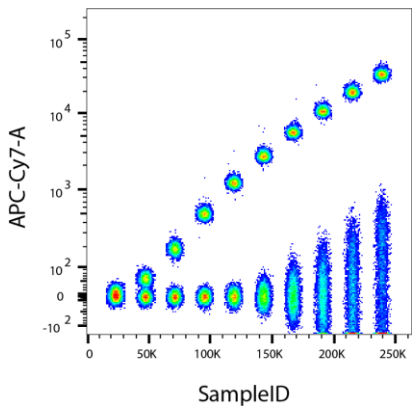


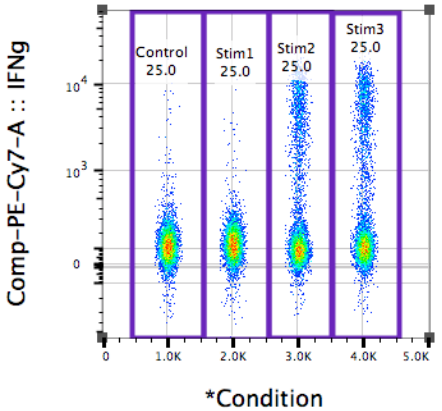
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Additional Parameters

- You can select one or more keywords to create new parameters in the concatenated output file.
- Note that you will always get a new parameter called Sample ID in the concatenated file. Selecting Sample ID allows you to see the different samples that were merged.



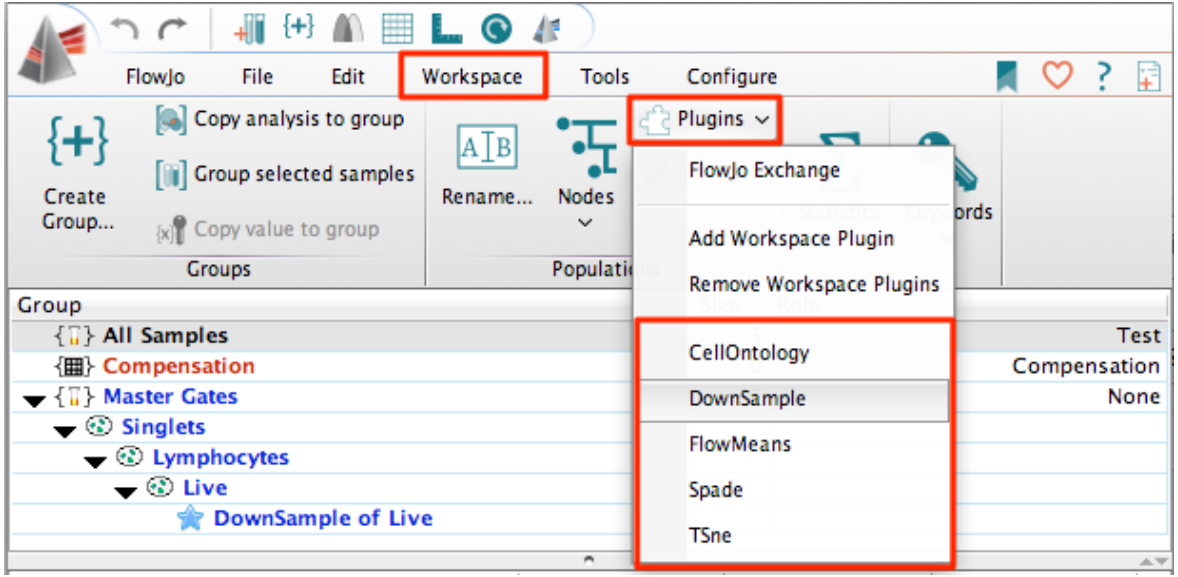




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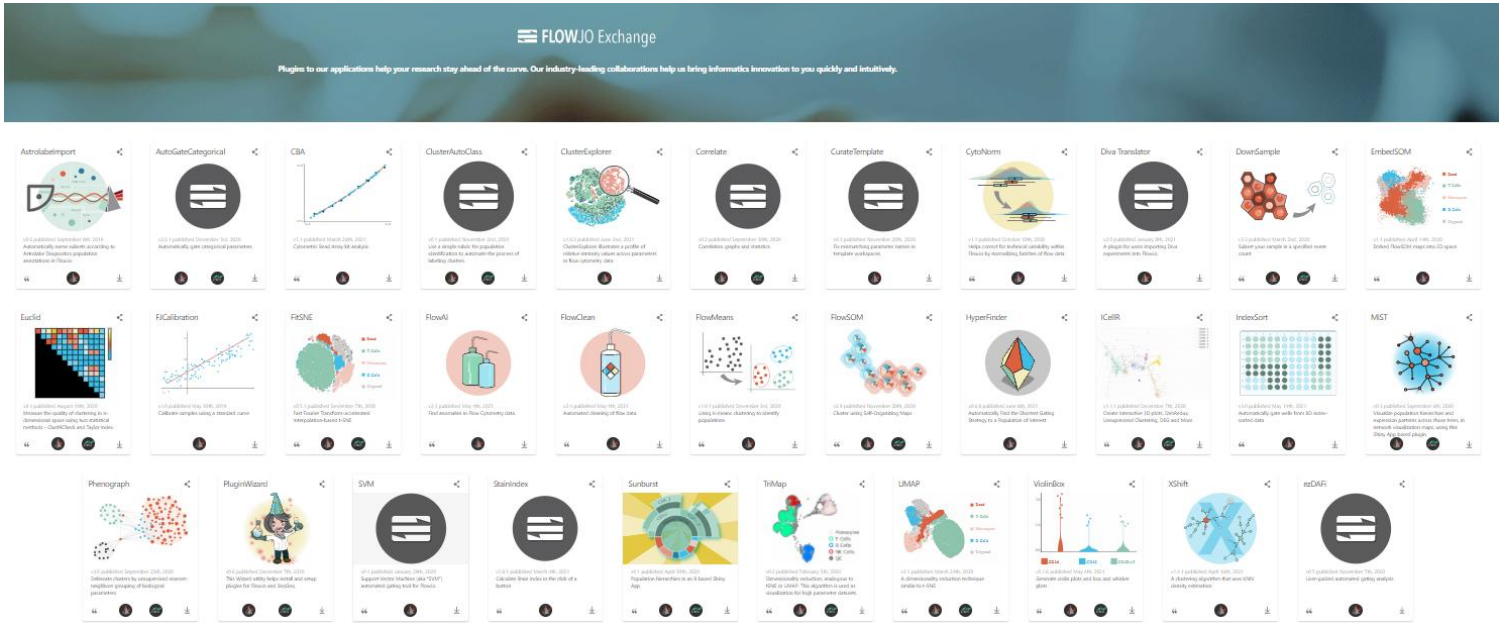
Plugins

- Java programs that extend the functionality of FlowJo.
- Accessed from the Plugins menu
  - Workspace tab → Populations band → Plugins menu



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Currently Available Plugins



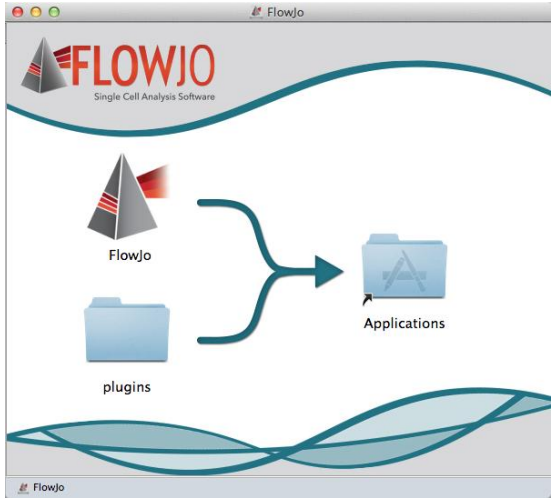
*Requires the R Statistical Computing Environment

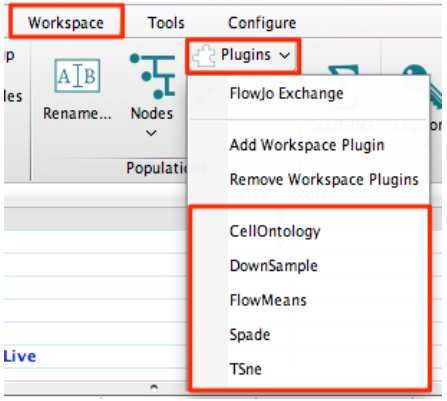
Available at: https://cran.r-project.org/

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Installing Plugins

- 5 plugins are included with the FlowJo v10.2 release
- Download the installation package for your OS and follow the instructions.
- Open FlowJo and look under the Workspace tab → Populations band → Plugins menu.





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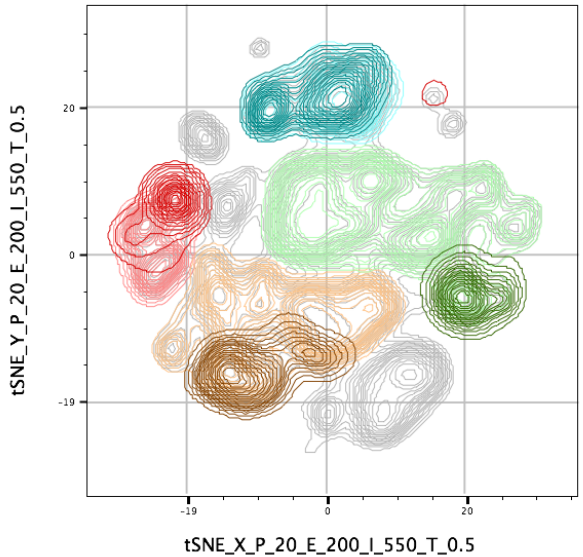
DownSample

- Selects a limited number of data points/events from a sample or gated population
  - Events are evenly distributed across parent sample or gated population → random
  - Creates a gate containing selected events
  - Purposes:
    - Reduce number of events for algorithm calculation
    - Normalize cell number to compare distribution of populations across samples

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tSNE

- T-Distributed Stochastic Neighbor Embedding (tSNE)
  - An algorithm for performing dimensionality reduction
  - Allows visualization of complex multi-dimensional data in fewer dimensions while still maintaining the structure of the data



Full Gating Path
Lymphocytes/DownsampleDP.Pop/Q3: CD3+ , CD19–/Q3: CD4+ , CD8–/CD127–CD25+
Lymphocytes/DownsampleDP.Pop/Q3: CD3+ , CD19–/Q3: CD4+ , CD8–
Lymphocytes/DownsampleDP.Pop/Q3: CD3+ , CD19–/Q1: CD4– , CD8+/CD127–CD27+
Lymphocytes/DownsampleDP.Pop/Q3: CD3+ , CD19–/Q1: CD4– , CD8+
Lymphocytes/DownsampleDP.Pop/Q1: CD3– , CD19+/IgD+
Lymphocytes/DownsampleDP.Pop/Q1: CD3– , CD19+
Lymphocytes/DownsampleDP.Pop/Q4: CD3– , CD19–/CD56+/CD197+
Lymphocytes/DownsampleDP.Pop/Q4: CD3– , CD19–/CD56+
Lymphocytes/DownsampleDP.Pop

Maaten and Hinton (2008). "Visualizing data using t-SNE." Journal of Machine Learning Research, 9: 2579–2605.

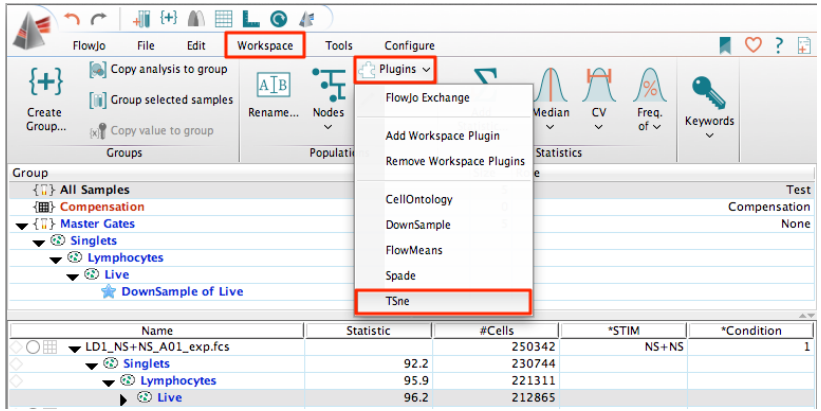
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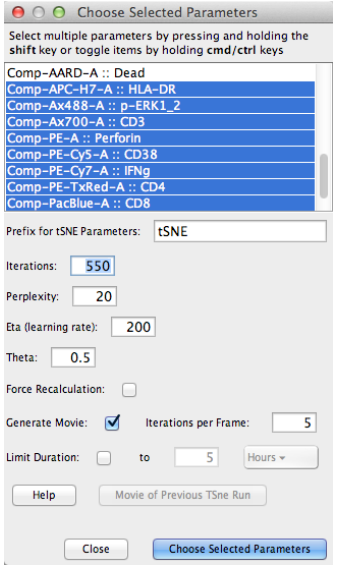
tSNE

- Workflow

  → Downsample, Concatenate, tSNE, Gate/Cluster, Explore

- Initiating tSNE from the Workspace



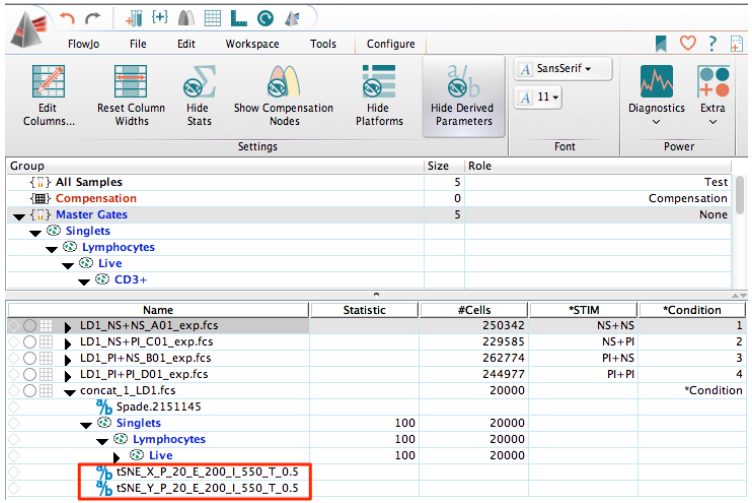


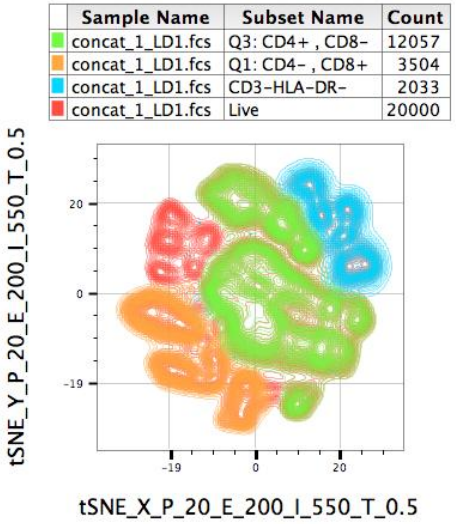
- **Iterations –** Maximum number of iterations the algorithm will run.
- **Perplexity –** Perplexity is related to the number of nearest neighbors that is used in learning algorithms. In tSNE, the perplexity may be viewed as a knob that sets the number of effective nearest neighbors. The most appropriate value depends on the density of your data. Generally a larger / denser dataset requires a larger perplexity.

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tSNE

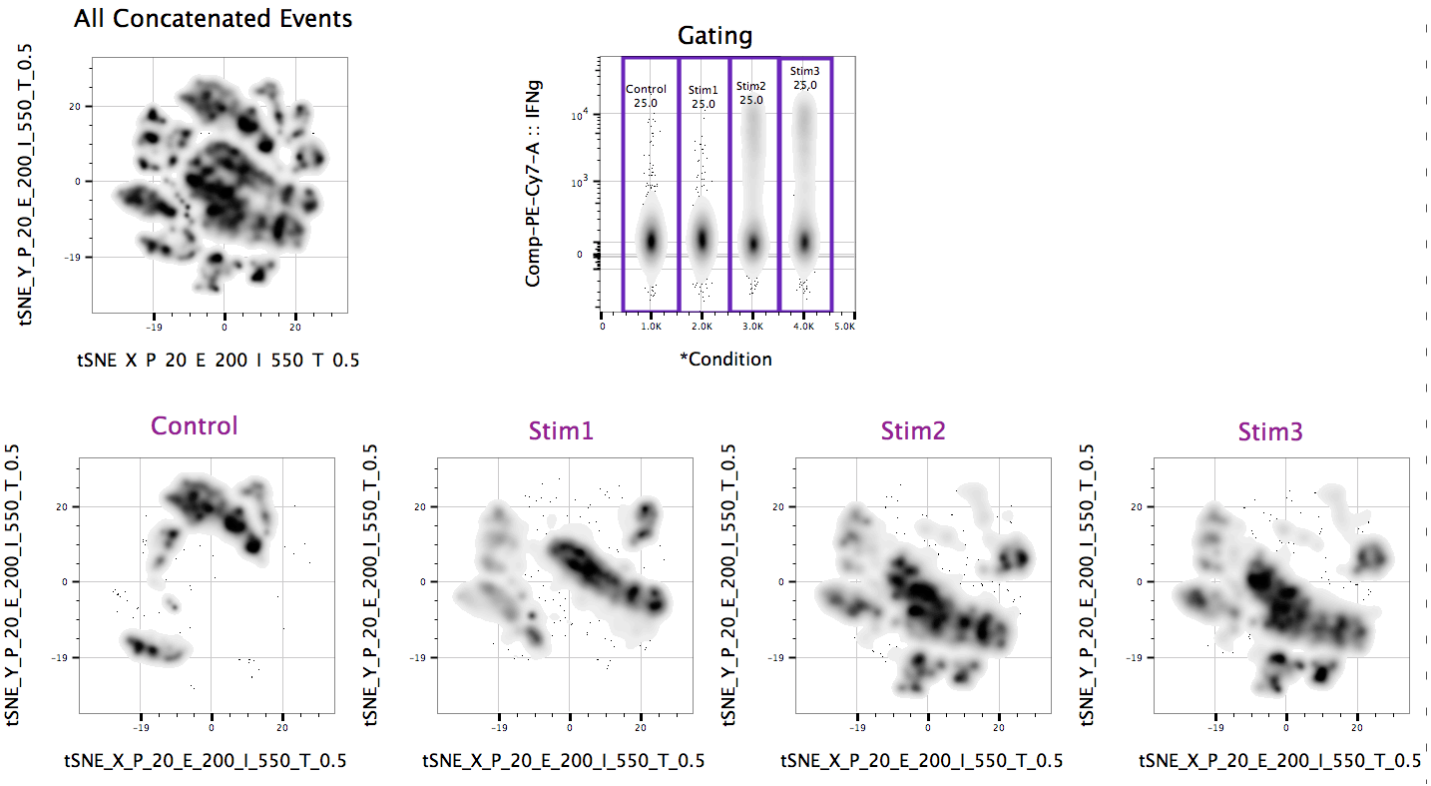
- Creates two new derived parameters from user selection, optimized in such a way that observations/data points which were close to one another in the raw high dimensional data are close in the reduced data space.





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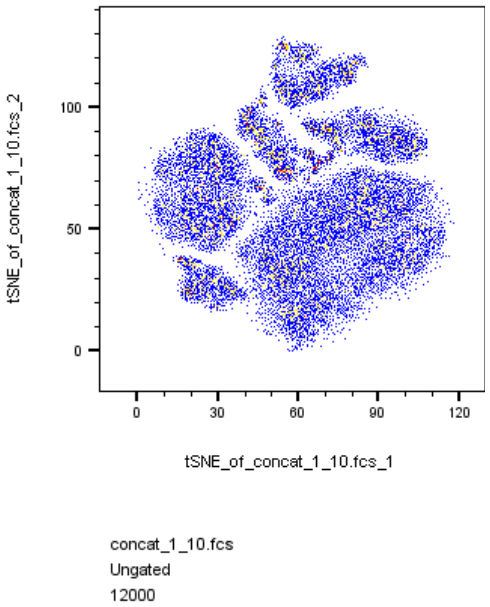
tSNE

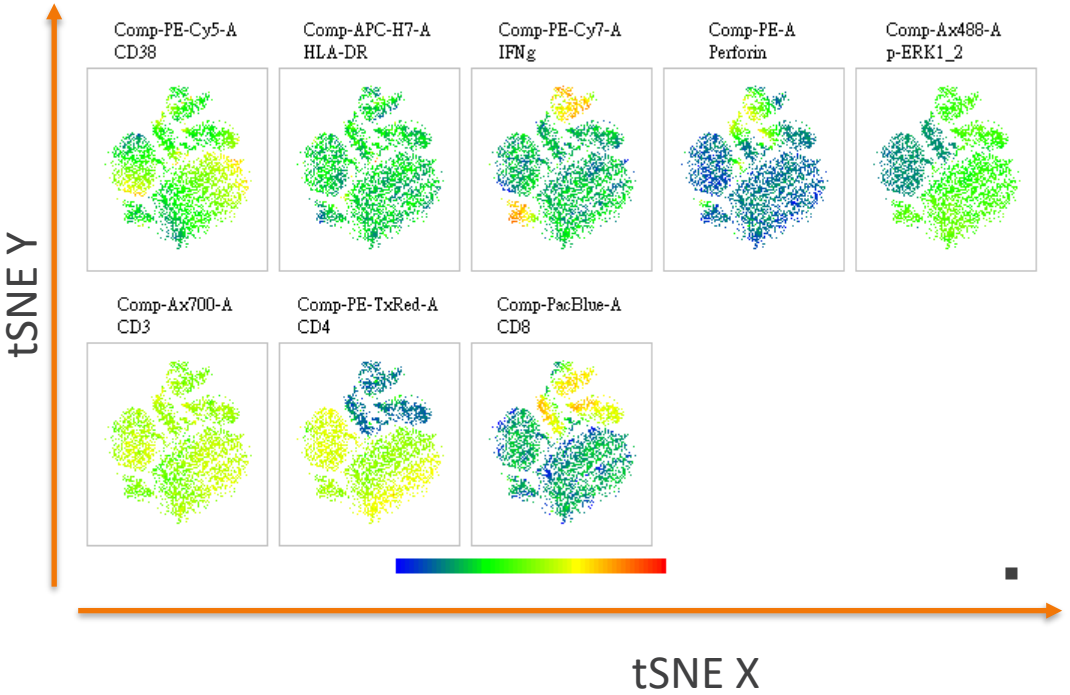


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An example of analyze data through plugins

Clean up → down sample (optional) → concatenation (optional) → Visualization (ex. tSNE) → Clustering (ex. Phenograph)



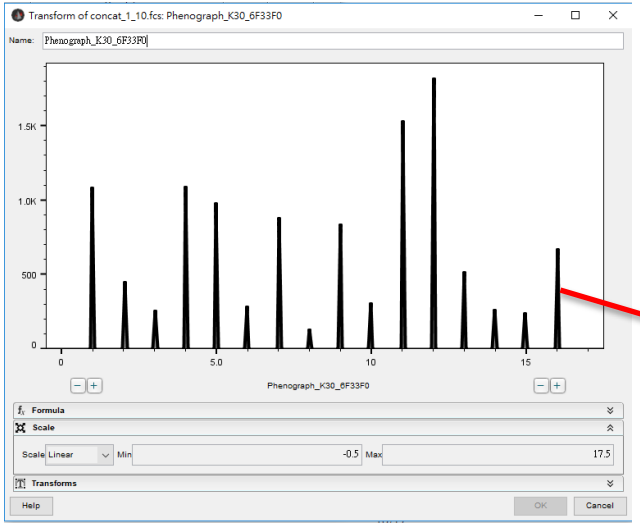


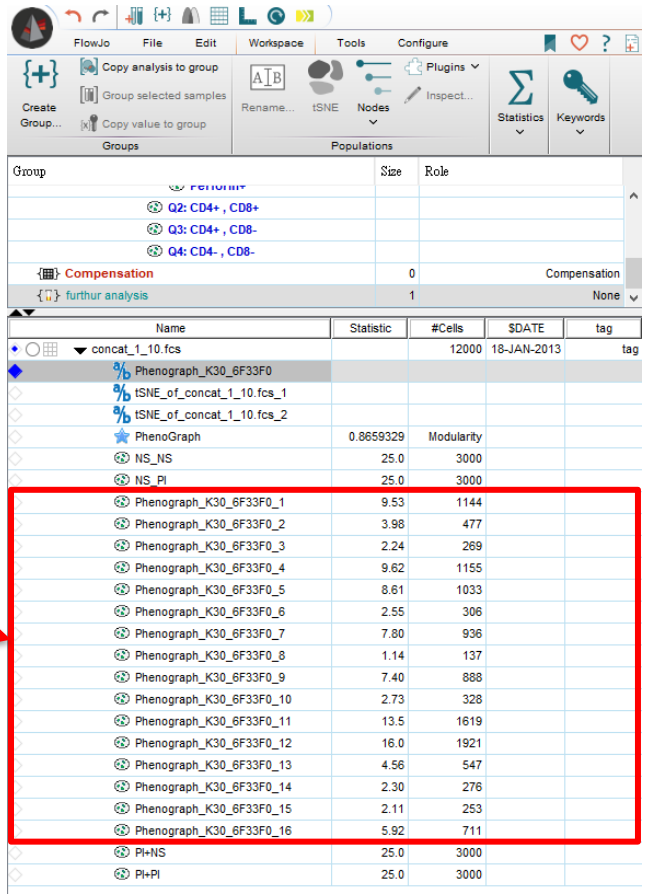
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Cell clustering

- In this case, 16 clusters were identified based on Phenograph algorithm among comparative parameters



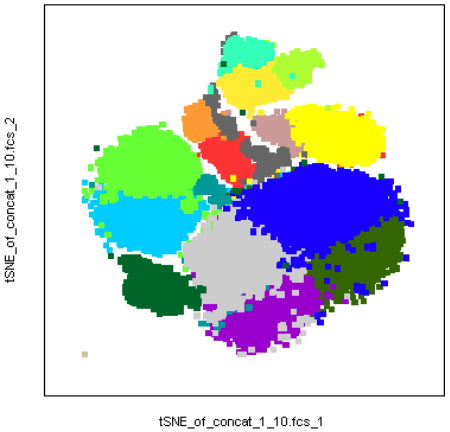


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Apply Phenograph clusters to tSNE map

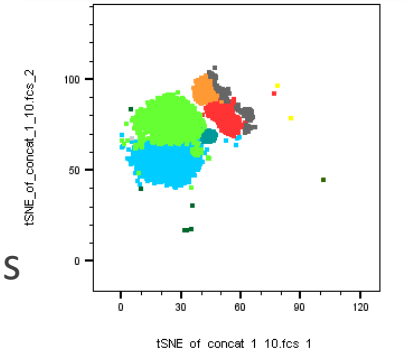
Overall



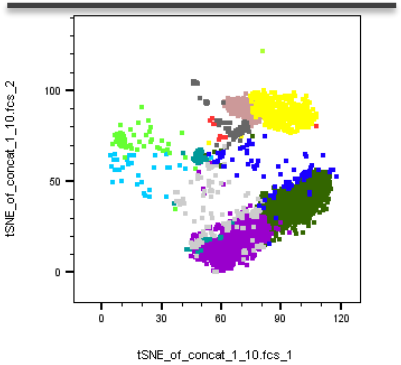
	Sample Name	Subset Name	Count
	concat_1_10.fcs	Phenograph_K30_6F33F0_16	711
	concat_1_10.fcs	Phenograph_K30_6F33F0_15	253
	concat_1_10.fcs	Phenograph_K30_6F33F0_14	276
	concat_1_10.fcs	Phenograph_K30_6F33F0_13	547
	concat_1_10.fcs	Phenograph_K30_6F33F0_12	1921
	concat_1_10.fcs	Phenograph_K30_6F33F0_11	1619
	concat_1_10.fcs	Phenograph_K30_6F33F0_10	328
	concat_1_10.fcs	Phenograph_K30_6F33F0_9	888
	concat_1_10.fcs	Phenograph_K30_6F33F0_8	137
	concat_1_10.fcs	Phenograph_K30_6F33F0_7	936
	concat_1_10.fcs	Phenograph_K30_6F33F0_6	306
	concat_1_10.fcs	Phenograph_K30_6F33F0_5	1033
	concat_1_10.fcs	Phenograph_K30_6F33F0_4	1155
	concat_1_10.fcs	Phenograph_K30_6F33F0_3	269
	concat_1_10.fcs	Phenograph_K30_6F33F0_2	477
	concat_1_10.fcs	Phenograph_K30_6F33F0_1	1144
	concat_1_10.fcs	Ungated	12000

Under different conditions

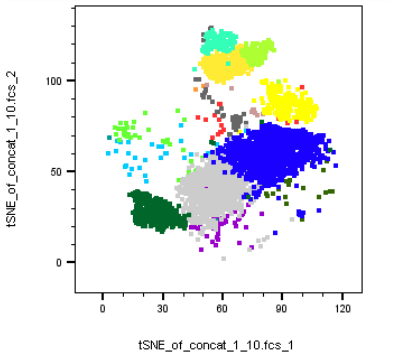
NS+NS



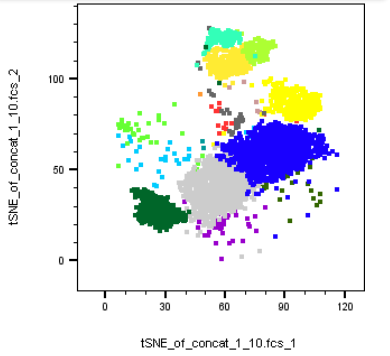
PI+NS



NS+PI



PI+PI



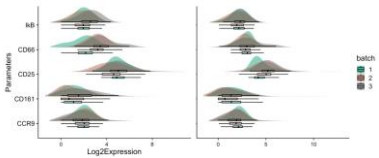
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FlowJo Exchange Plugins

Processing

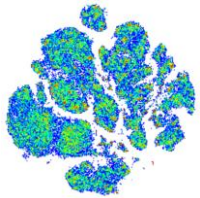
FlowAI
FlowClean
PeacoQC
FlowCut

CytoNorm
CyCombine
MNN



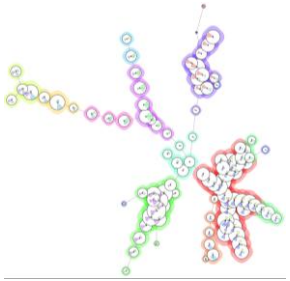
Dimensionality reduction

tSNE
UMAP
TriMAP
PacMAP
PHATE



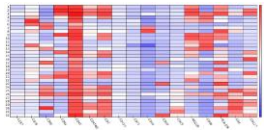
Clustering

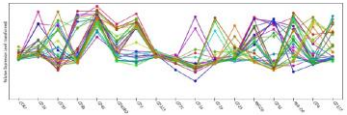
FlowSOM
Phenograph
X-Shift



Interpretation

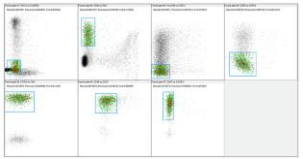
ClusterExplorer
MEM
T-REX





Validation

HyperFinder
Embed



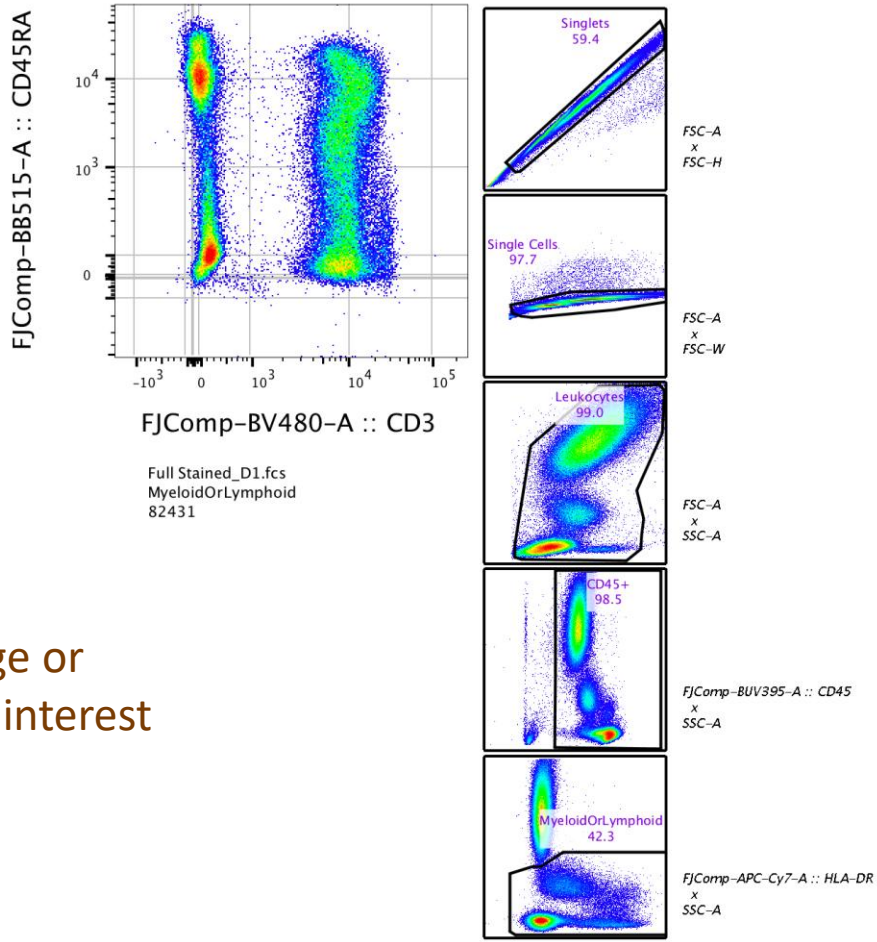
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Cleanup Gate

remove doublets, dead cells and debris

- cleanup
- concatenate
- dimensionally-reduce
- cluster
- gate
- comparisons
- dig deeper

- Focus on a lineage or compartment of interest

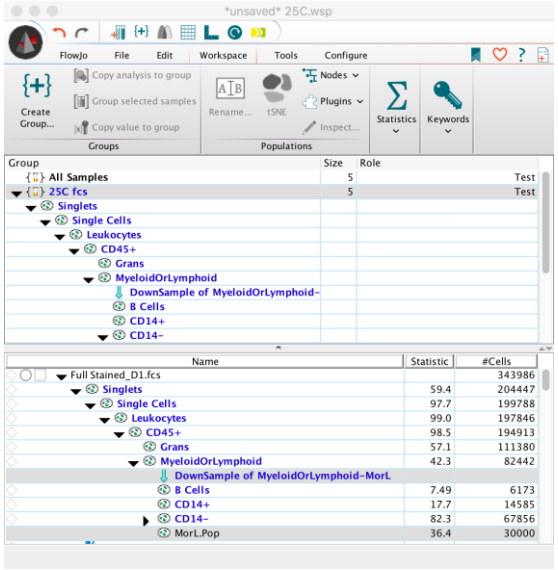


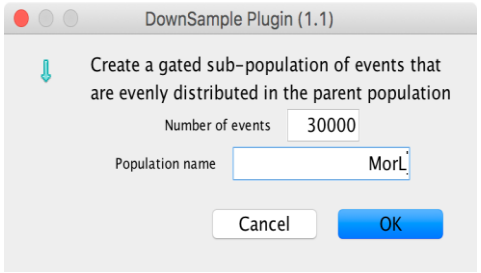
For Research Use Only. Not for use in therapeutic or diagnostic procedures.
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DownSample

normalize the number of events contributed

- cleanup
- concatenate
- dimensionally-reduce
- cluster
- gate
- comparisons
- dig deeper





- Selects equidistant events across acquisition time parameter

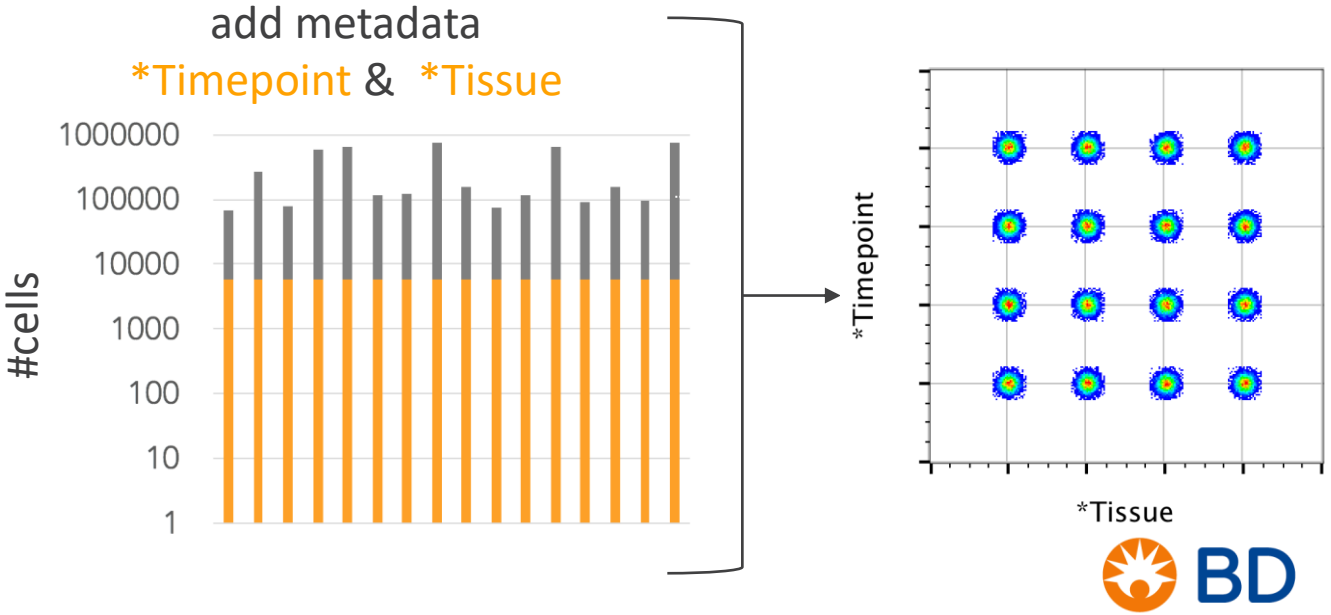
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Concatenate

merge events from all samples into a single file

- cleanup
- concatenate
- dimensionally-reduce
- cluster
- gate
- comparisons
- dig deeper

- New keyword-based parameters can be created when concatenating, allowing the separation of samples and/or experimental conditions



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Dimensionality Reduction

creates new derived parameters

cleanup

concatenate

dimensionally-reduce

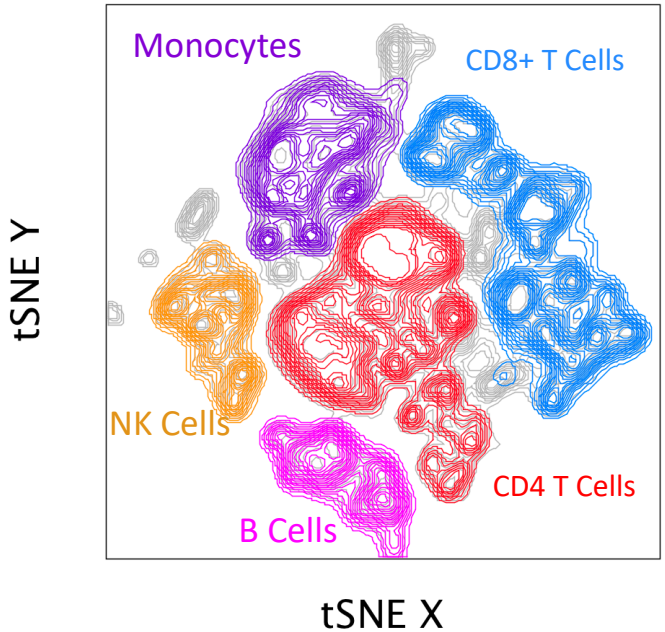
cluster

gate

comparisons

dig deeper

- Allows visualization of complex multi-dimensional data in fewer dimensions while still maintaining its higher order structure

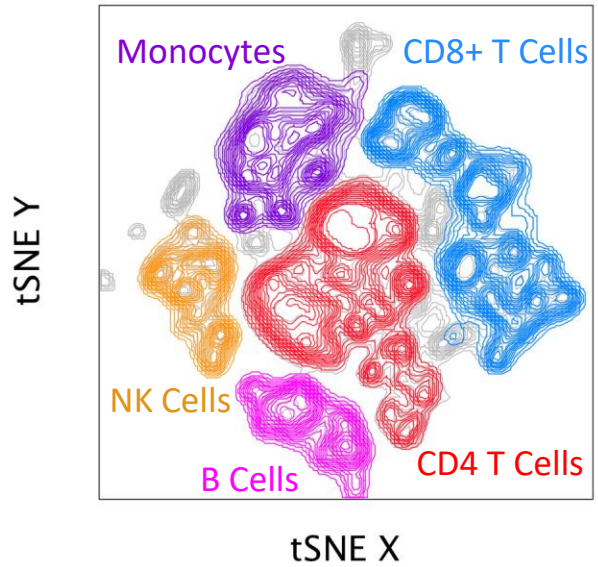


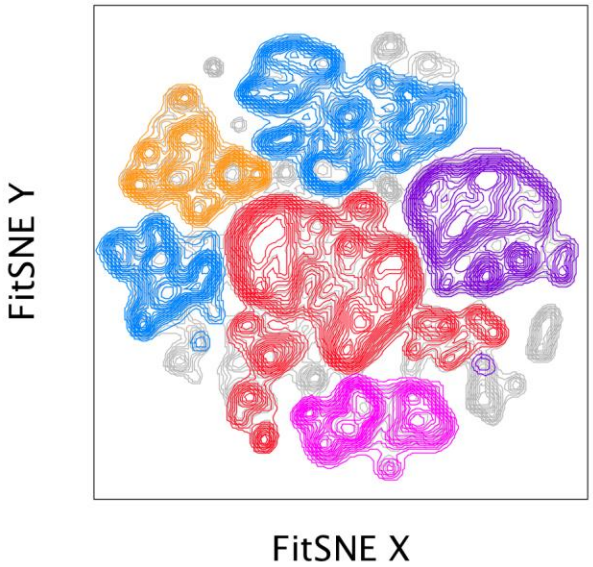
- Events with a similar multi-dimensional expression pattern group together within the dimensionally reduced data space

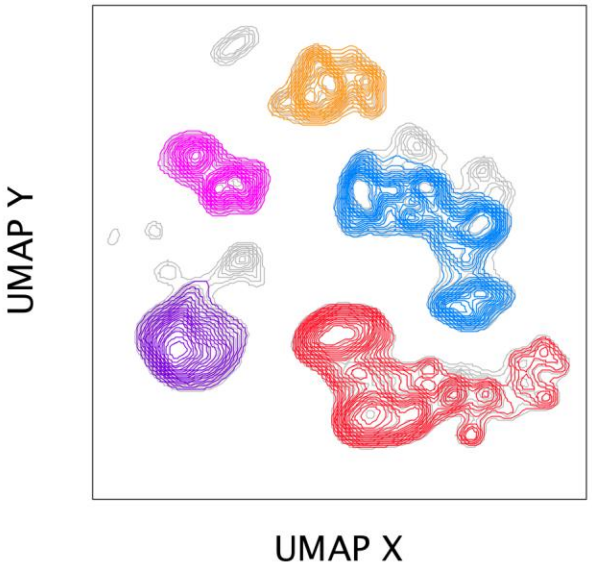
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Dimensionality Reduction Options

- t-Distributed Stochastic Neighbor Embedding (tSNE)
- Fast Fourier Transform-Accelerated Interpolation-based t-SNE (FitSNE)
- Uniform Manifold Approximation and Projection (UMAP)





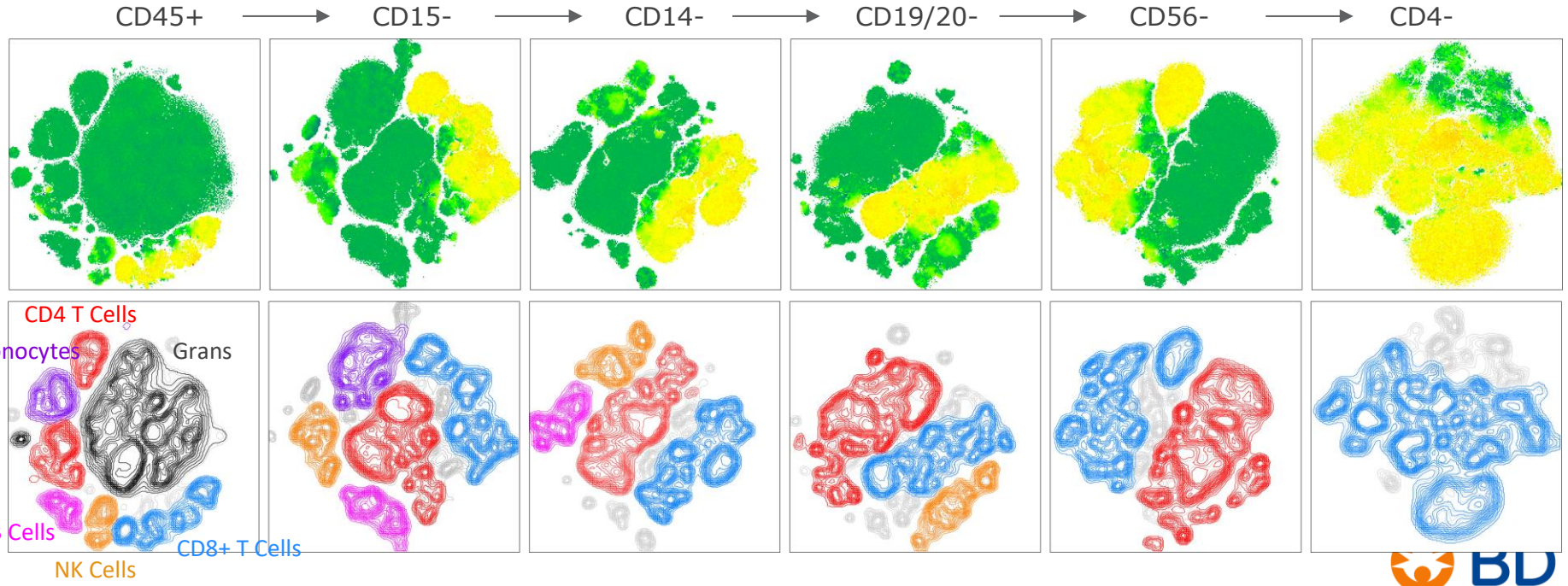


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What to Dimensionally Reduce?

depends on the panel and experiment

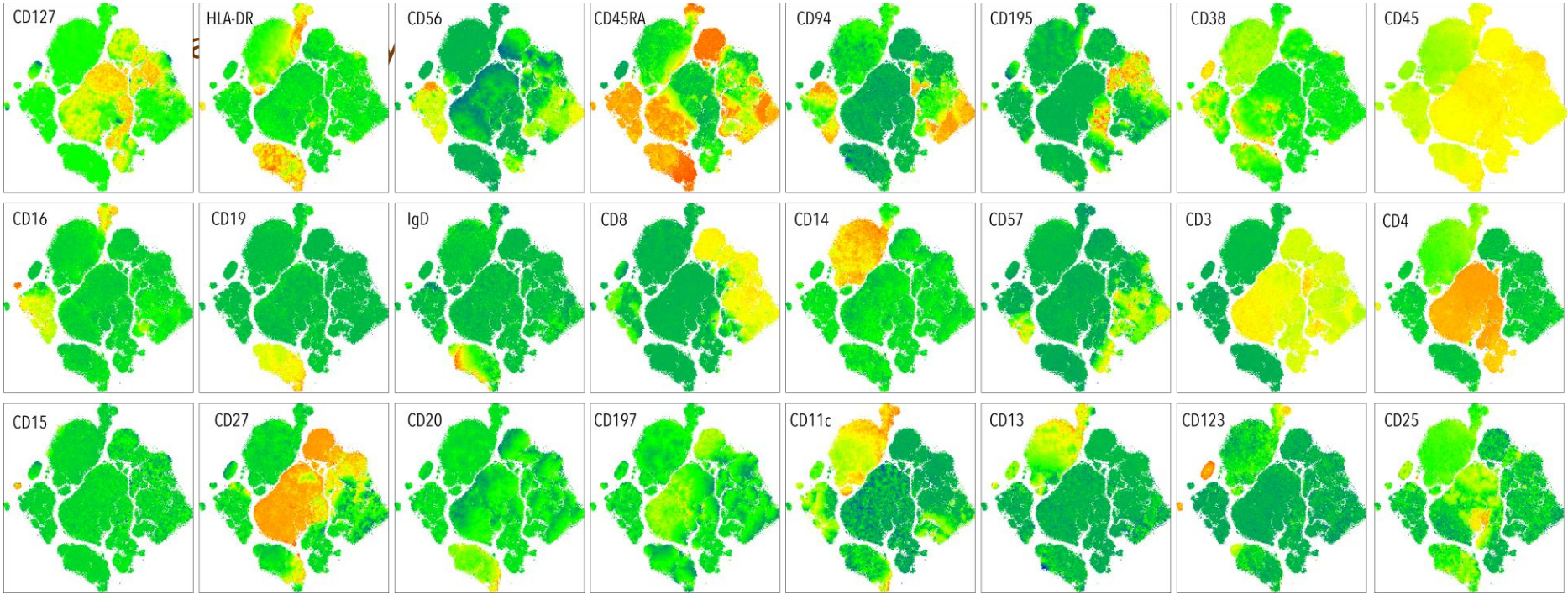
- Focus on a subset to gain detail and resolve changes across conditions



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3rd Parameter Color Maps



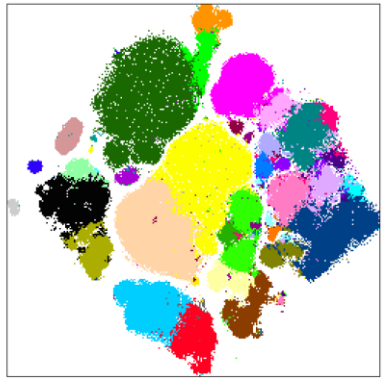
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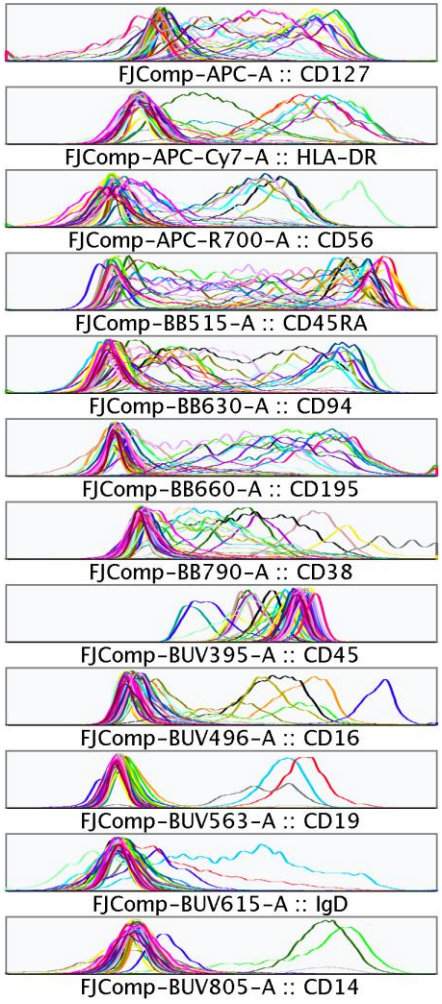
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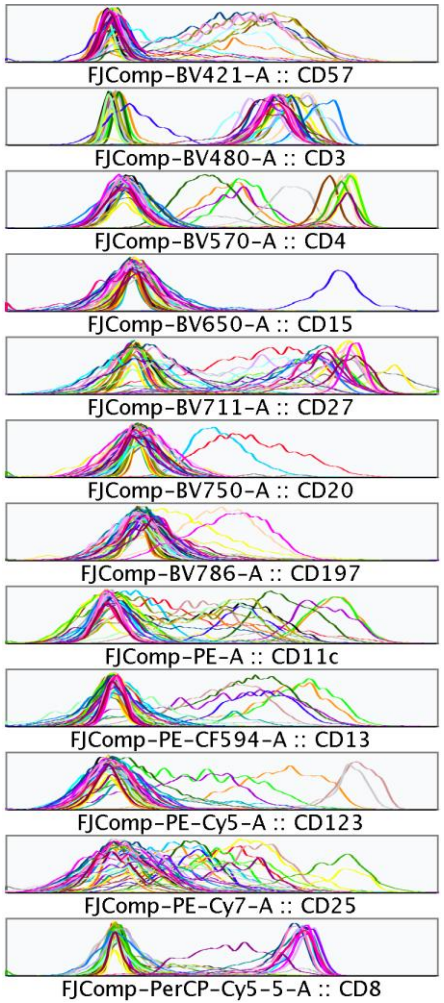
Clustering

creates populations

- cleanup
- concatenate
- dimensionally-reduce
- **cluster**
- gate
- comparisons
- dig deeper





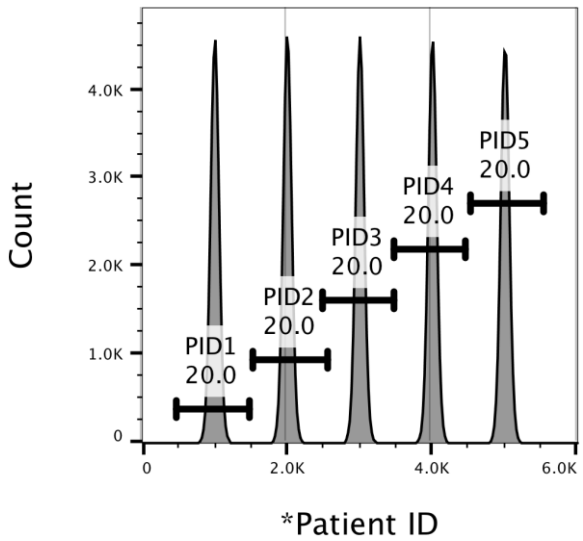


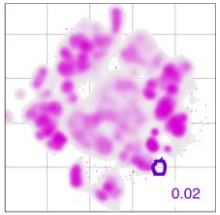
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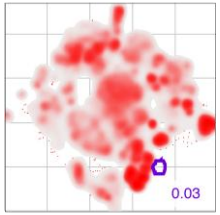
Gate on Keyword Parameter

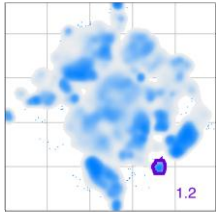
to pull apart samples and/or experimental conditions

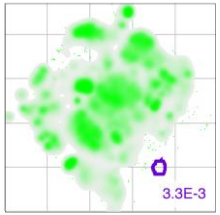
- cleanup
- concatenate
- dimensionally-reduce
- cluster
- gate
- comparisons
- dig deeper

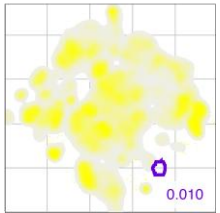










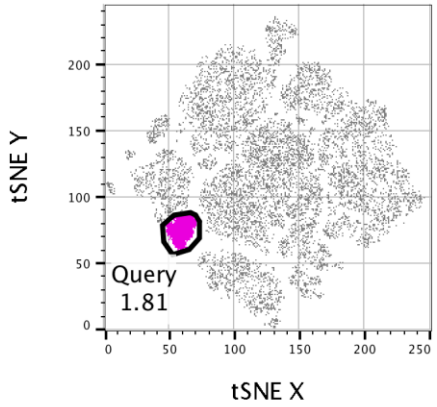


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Query Gate w/in tSNE

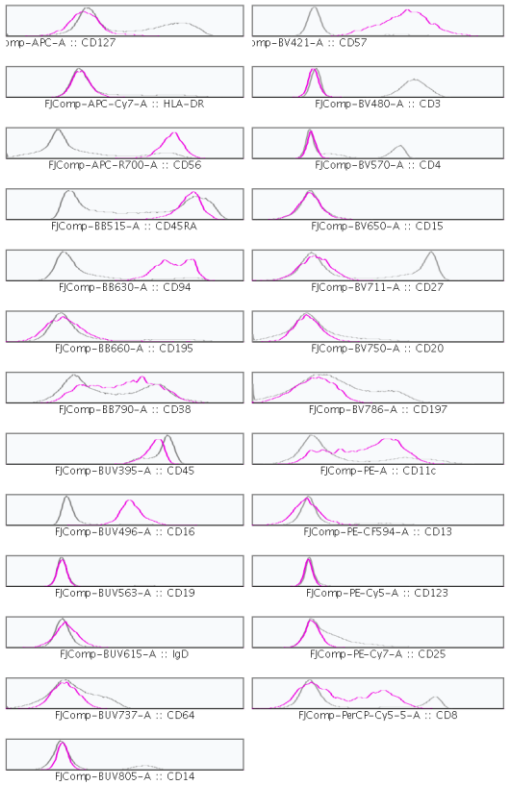
to identify complex phenotype of a node or region

- cleanup
- concatenate
- dimensionally-reduce
- cluster
- gate
- comparisons
- dig deeper



Sample Name	Subset Name	Count
concat_1_MorL.fcs	Query	2722
concat_1_MorL.fcs	Ungated	150000

Query	1.81
PID1	43.2
PID2	4.59
PID3	0.51
PID4	25.2
PID5	26.6

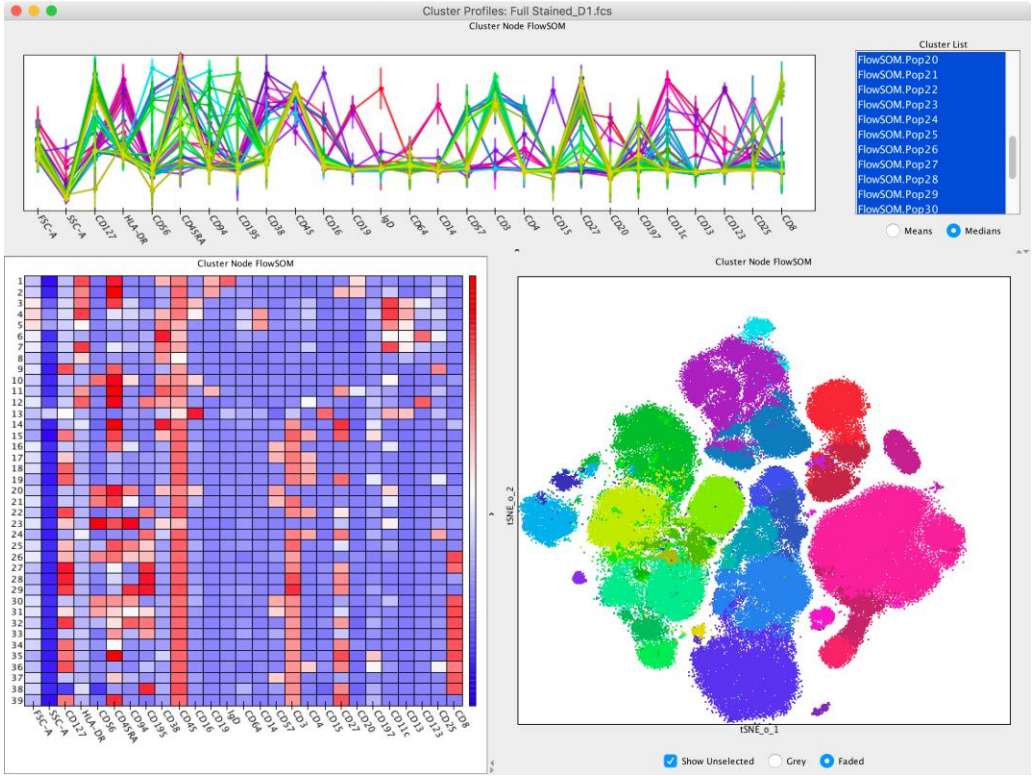


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Cluster Explorer

interactive population phenotype identification

- cleanup
- concatenate
- dimensionally-reduce
- cluster
- gate
- comparisons
- dig deeper



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Cluster Explorer

and comparison

- cleanup
- concatenate
- dimensionally-reduce
- cluster
- gate
- comparisons
- dig deeper

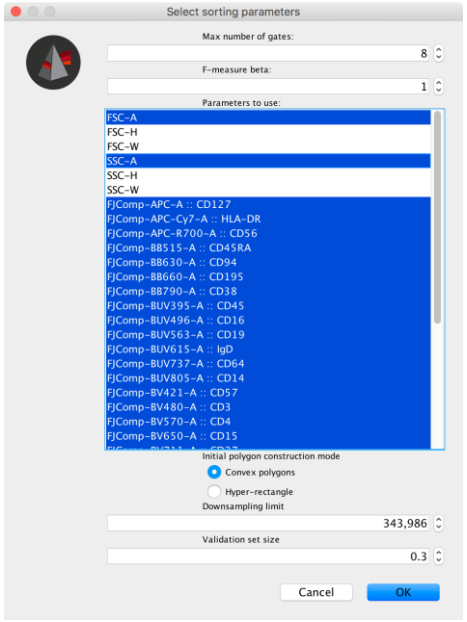


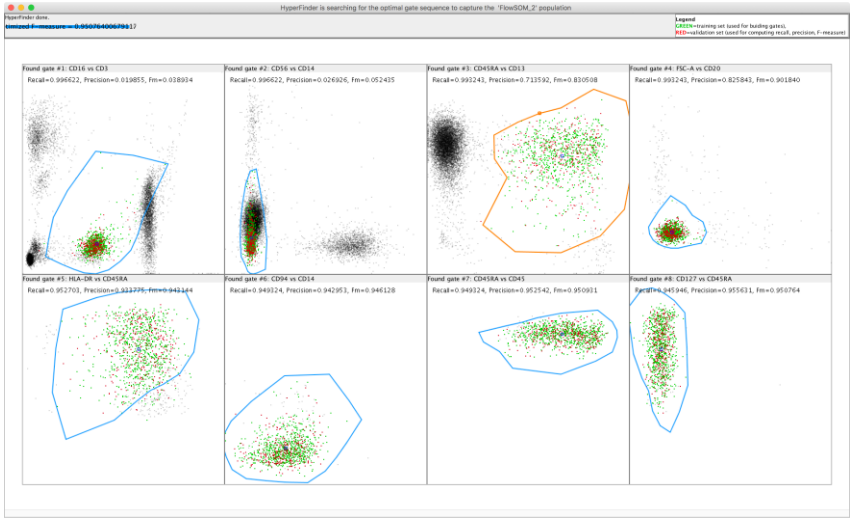
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HyperFinder

generate sort gates for isolating events with a complex phenotype

- cleanup
- concatenate
- dimensionally-reduce
- cluster
- gate
- comparisons
- dig deeper





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Export to FACSDiva

creates a Diva experiment template --> sort the population of interest

cleanup

concatenate

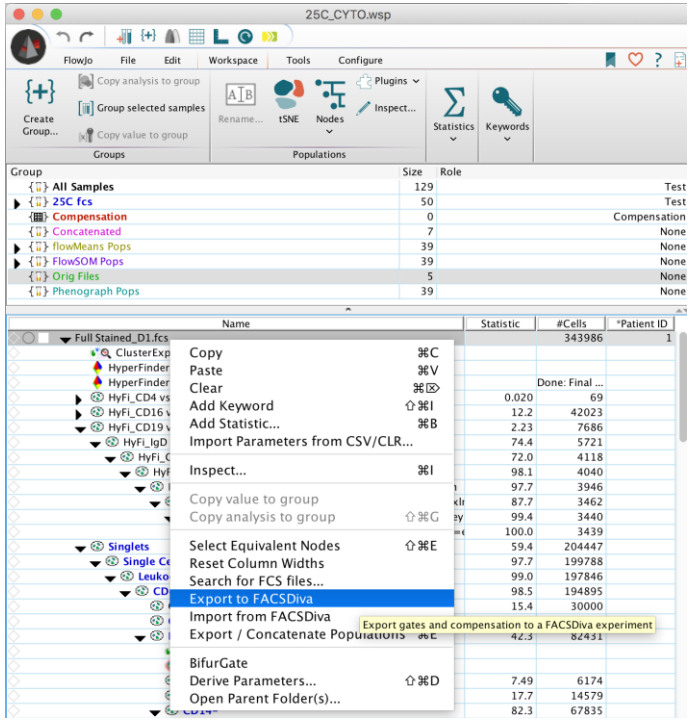
dimensionally-reduce

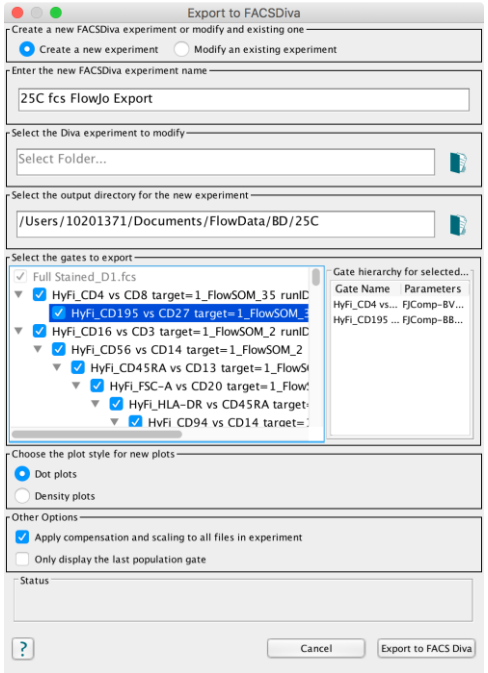
cluster

gate

comparisons

dig deeper





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Summary



FlowJo Plugins facilitate interaction with bioinformatics programming environments, and use of algorithmic tools by bench scientist with limited bioinformatics experience.



Employing plugin tools in the proper workflow allows for discovery of complex, potentially novel populations.

Integration between BD FACSDiva and FlowJo enables transfer of gating information between acquisition and analysis software, and sorting of cluster populations using computationally derived sort gates.



BD Rhapsody with AbSeq & SeqGeq provides a full multiomic solution for single cell analysis.

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Thank you!

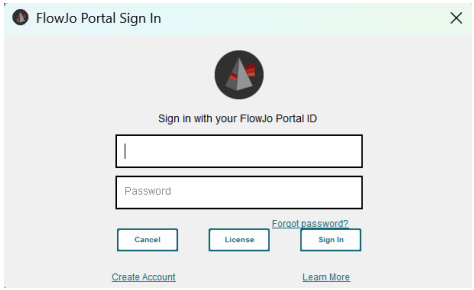
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使用期限	一年期訂閱制
軟體版本更新	永遠可使用最新版本
權限認證	FlowJo 網站帳號(Email)登入



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- 一次購買5個以上的portal帳號就算group license
- 購買的這些帳號會同時開通，同時到期
- 會有一個管理者來統一管理這些帳號(管理者可以不是使用者)
- 管理者有權限去發放及停止這一年內這些帳號要給誰使用
- 限定購買該license的同單位同仁才能申請開通(會限定該單位的email後綴；@bd.com)
- 依照購買帳號數量的級距，價格會有不同

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