Advanced tool FlowJo introduction

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BD Biosciences

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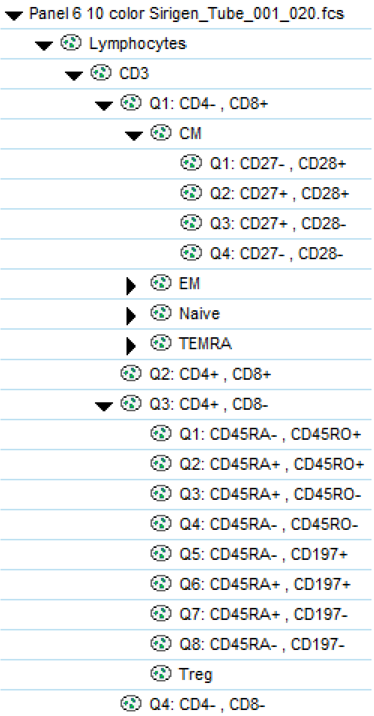
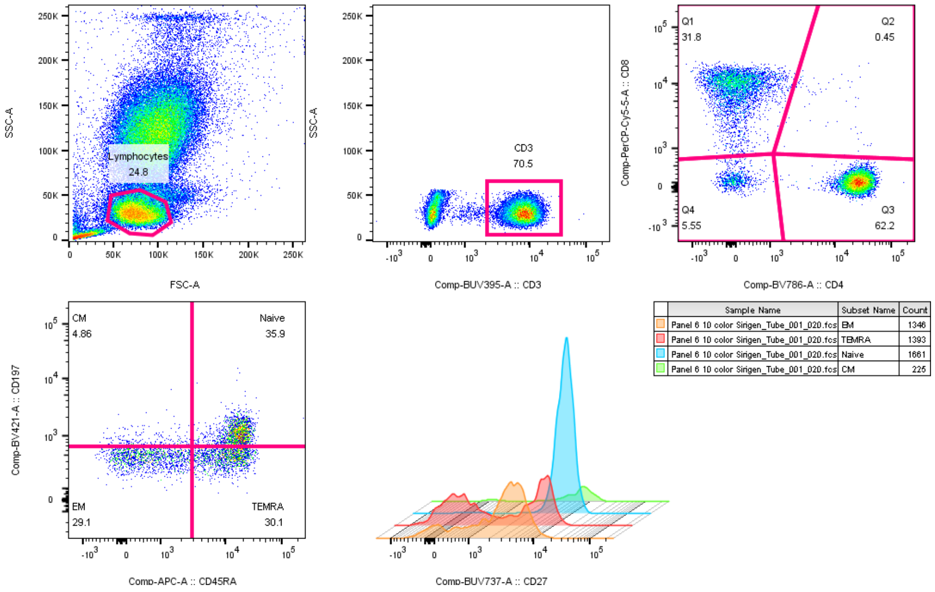
Flow Data Analysis

- **分析圈選階層**
  (Population Hierarchy)



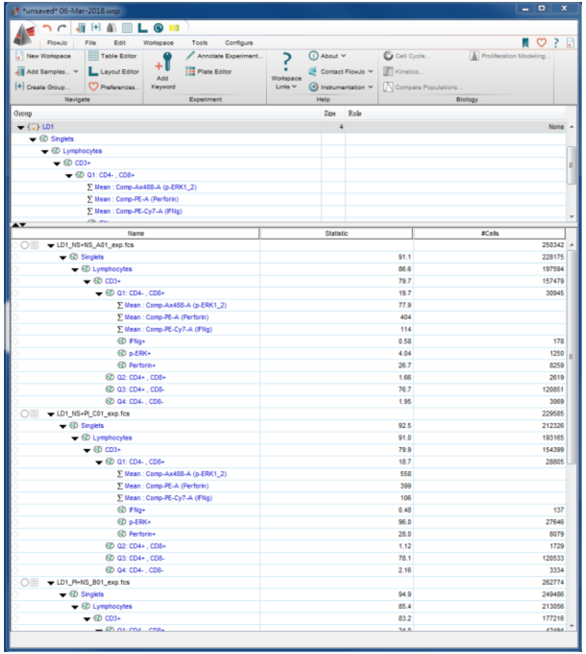
- Panel 6 10 color Sirigen_Tube_001_020.fcs
  - Lymphocytes
    - CD3
      - Q1: CD4- , CD8+
        - CM
          - Q1: CD27- , CD28+
          - Q2: CD27+ , CD28+
          - Q3: CD27+ , CD28-
          - Q4: CD27- , CD28-
        - EM
        - Naive
        - TEMRA
      - Q2: CD4+ , CD8+
      - Q3: CD4+ , CD8-
        - Q1: CD45RA- , CD45RO+
        - Q2: CD45RA+ , CD45RO+
        - Q3: CD45RA+ , CD45RO-
        - Q4: CD45RA- , CD45RO-
        - Q5: CD45RA- , CD197+
        - Q6: CD45RA+ , CD197+
        - Q7: CD45RA+ , CD197-
        - Q8: CD45RA- , CD197-
        - Treg
      - Q4: CD4- , CD8-



	Sample Name	Subset Name	Count
🟧	Panel 6 10 color Sirigen_Tube_001_020.fcs	EM	1346
🟥	Panel 6 10 color Sirigen_Tube_001_020.fcs	TEMRA	1393
🟦	Panel 6 10 color Sirigen_Tube_001_020.fcs	Naive	1661
🟩	Panel 6 10 color Sirigen_Tube_001_020.fcs	CM	225

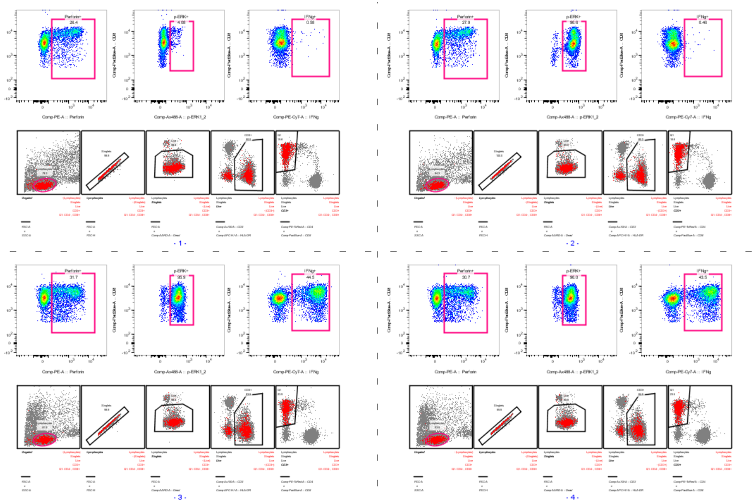
Flow Data Analysis

- 儲存分析模板 (Workspace Template)

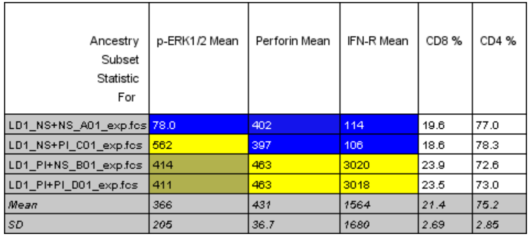


Name	Statistic	#Cells
LD1_NS+NS_A01_exp.fcs		250342
Singlets	91.1	228175
Lymphocytes	86.6	197594
CD3+	79.7	157479
Q1: CD4- , CD8+	19.7	30945
Σ Mean : Comp-Ax488-A (p-ERK1_2)	77.9	
Σ Mean : Comp-PE-A (Perforin)	404	
Σ Mean : Comp-PE-Cy7-A (IFNg)	114	
IFNg+	0.58	178
p-ERK+	4.04	1250
Perforin+	26.7	8259
Q2: CD4+ , CD8+	1.66	2619
Q3: CD4+ , CD8-	76.7	120851
Q4: CD4- , CD8-	1.95	3069
LD1_NS+PI_C01_exp.fcs		229585
Singlets	92.5	212326
Lymphocytes	91.0	193165
CD3+	79.9	154399
Q1: CD4- , CD8+	18.7	28805
Σ Mean : Comp-Ax488-A (p-ERK1_2)	558	
Σ Mean : Comp-PE-A (Perforin)	399	
Σ Mean : Comp-PE-Cy7-A (IFNg)	106	
IFNg+	0.48	137
p-ERK+	96.0	27646
Perforin+	28.0	8079
Q2: CD4+ , CD8+	1.12	1729
Q3: CD4+ , CD8-	78.1	120533
Q4: CD4- , CD8-	2.16	3334
LD1_PI+NS_B01_exp.fcs		262774
Singlets	94.9	249486
Lymphocytes	85.4	213056
CD3+	83.2	177216

- 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*

試用版申請方式

*一台電腦僅能申請一次

1. **安裝FlowJo軟體**

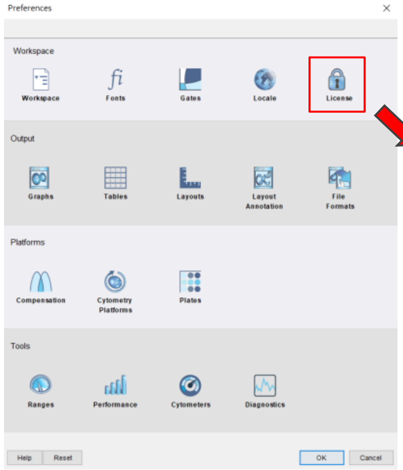
a. 進入FlowJo官網: https://www.flowjo.com/

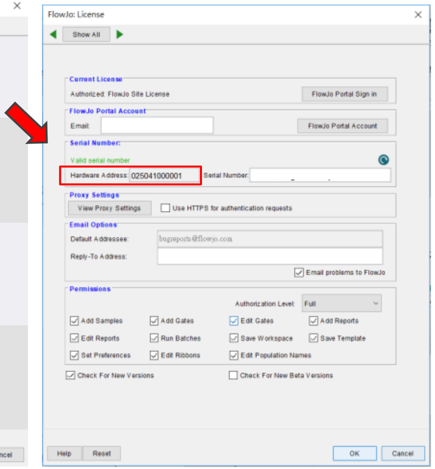
b. Download>Download FlowJo

c. 依照電腦系統選擇安裝檔.

試用版申請方式

2. 找到電腦序號





Current License
Authorized: FlowJo Site License

FlowJo Portal Account
Email:

Serial Number:
Valid serial number
Hardware Address: 025041000001
Serial Number:

Proxy Settings
☐ Use HTTPS for authentication requests

Email Options
Default Addressee: bugreports@flowjo.com
Reply-To Address:
☑ Email problems to FlowJo

Permissions
Authorization Level: Full
☑ Add Samples
☑ Add Gates
☑ Edit Gates
☑ Add Reports
☑ Edit Reports
☑ Run Batches
☑ Save Workspace
☑ Save Template
☑ Set Preferences
☑ Edit Ribbons
☑ Edit Population Names

☑ Check For New Versions
☐ Check For New Beta Versions

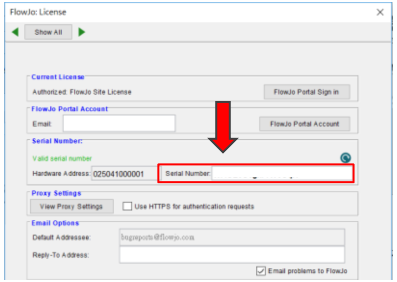
試用版申請方式

3. 回到官網, 申請試用金鑰

a. Download> Free Trial

b. 填妥表單並送出, 即會收到FlowJo提供30天試用序號

c. 開啟FlowJo, 輸入試用序號



Authorized: FlowJo Site License

Email:

Valid serial number

Hardware Address: 025041000001

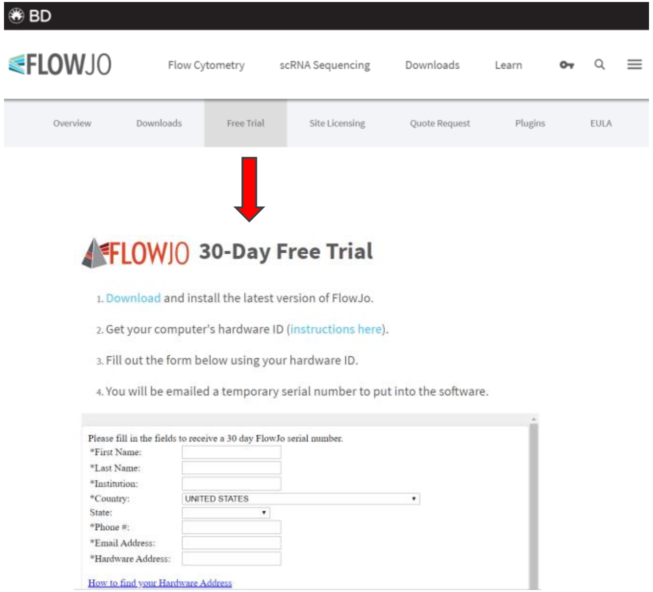
Serial Number:

☐ Use HTTPS for authentication requests

Default Addressee: bugreports@flowjo.com

Reply-To Address:

☑ Email problems to FlowJo



30-Day Free Trial

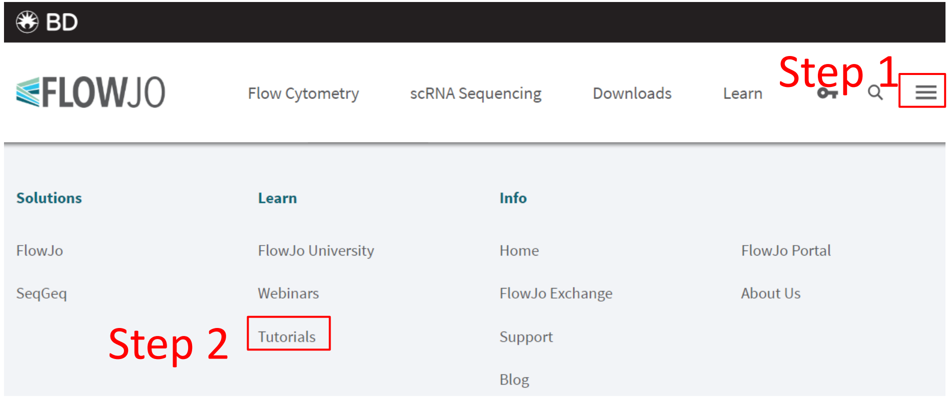
1. Download and install the latest version of FlowJo.
2. Get your computer's hardware ID (instructions here).
3. Fill out the form below using your hardware ID.
4. You will be emailed a temporary serial number to put into the software.

Please fill in the fields to receive a 30 day FlowJo serial number.

*First Name:
*Last Name:
*Institution:
*Country: UNITED STATES
State:
*Phone #:
*Email Address:
*Hardware Address:

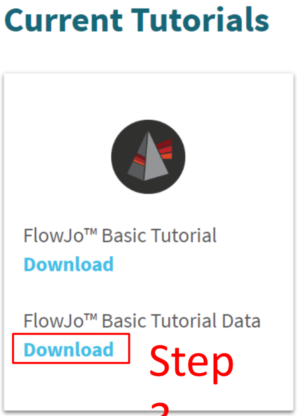
How to find your Hardware Address

練習檔案下載



Step 1

Step 2


Current Tutorials

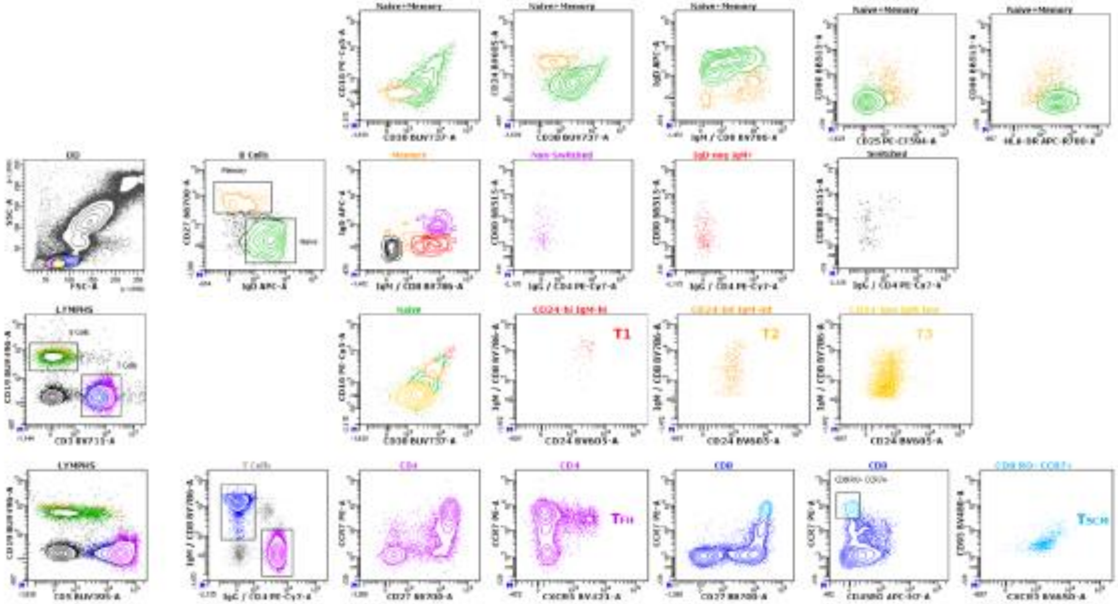
FlowJo™ Basic Tutorial
Download

FlowJo™ Basic Tutorial Data
Download

Step 3

Manual methods waste too much

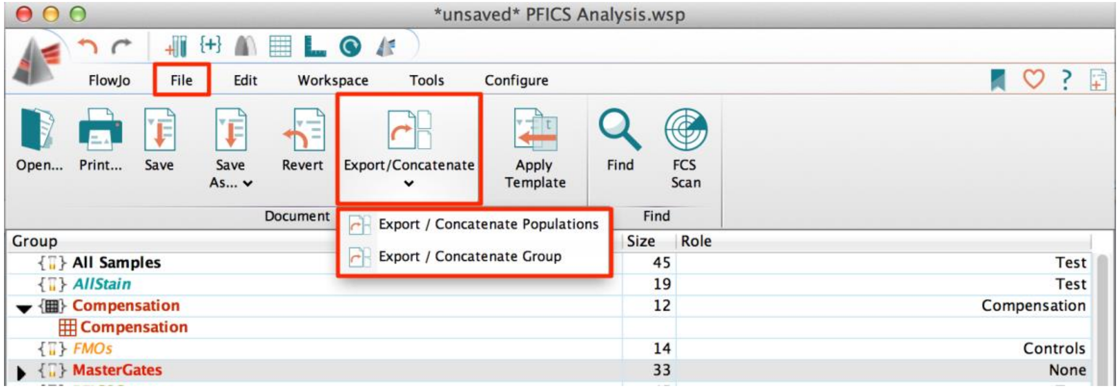
- 2^{18}=262144 Plots
- 23 plots is 0.88% of 262144 plots



18 Colors

輸出/連鎖 Export/Concatenate

- Initiation from the Workspace



Group	Size	Role
All Samples	45	Test
AllStain	19	Test
Compensation	12	Compensation
Compensation		
FMOs	14	Controls
MasterGates	33	None

- 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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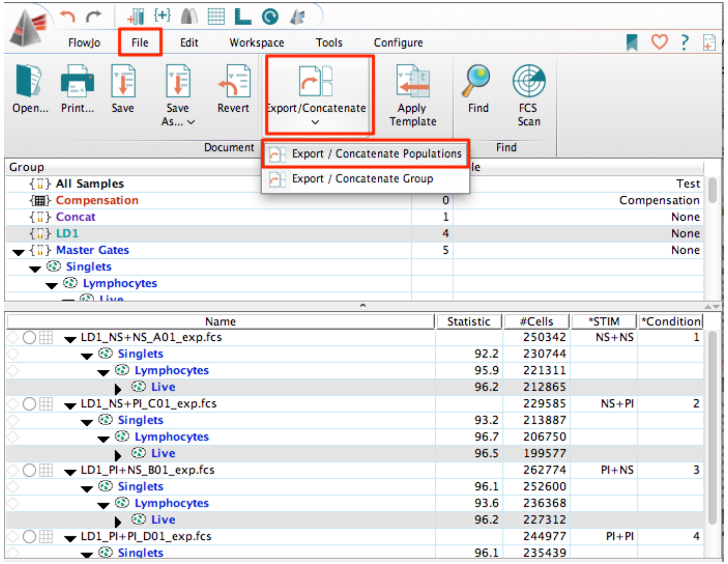
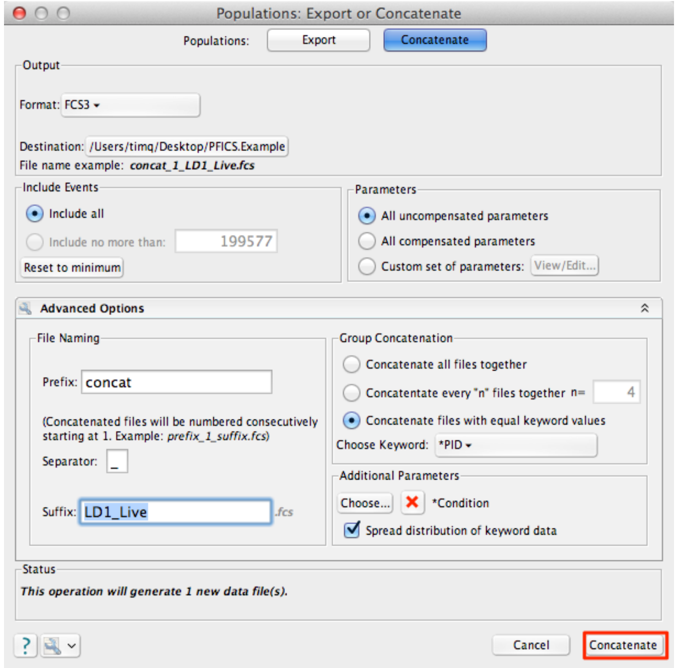
連鎖特定細胞群 Concatenating Populations

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



Group		
All Samples		Test
Compensation	0	Compensation
Concat	1	None
LD1	4	None
Master Gates	5	None
Singlets		
Lymphocytes		
Live		

Name	Statistic	#Cells	*STIM	*Condition
LD1_NS+NS_A01_exp.fcs		250342	NS+NS	1
Singlets	92.2	230744		
Lymphocytes	95.9	221311		
Live	96.2	212865		
LD1_NS+PI_C01_exp.fcs		229585	NS+PI	2
Singlets	93.2	213887		
Lymphocytes	96.7	206750		
Live	96.5	199577		
LD1_PI+NS_B01_exp.fcs		262774	PI+NS	3
Singlets	96.1	252600		
Lymphocytes	93.6	236368		
Live	96.2	227312		
LD1_PI+PI_D01_exp.fcs		244977	PI+PI	4
Singlets	96.1	235439		



Populations: Export or Concatenate

Populations:

Output

Format: FCS3

Destination: /Users/timq/Desktop/PFICS.Example

File name example: *concat_1_LD1_Live.fcs*

Include Events

● Include all

○ Include no more than: 199577

Parameters

● All uncompensated parameters

○ All compensated parameters

○ Custom set of parameters:

Advanced Options

File Naming

Prefix: concat

(Concatenated files will be numbered consecutively starting at 1. Example: *prefix_1_suffix.fcs*)

Separator: _

Suffix: LD1_Live .fcs

Group Concatenation

○ Concatenate all files together

○ Concatentate every "n" files together n= 4

● Concatenate files with equal keyword values

Choose Keyword: *PID

Additional Parameters

*Condition

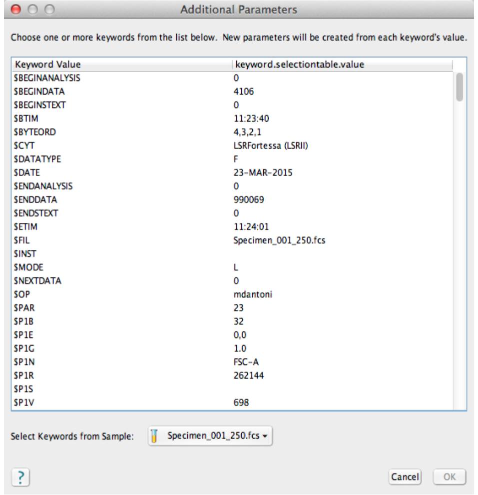
☑ Spread distribution of keyword data

Status

This operation will generate 1 new data file(s).

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.

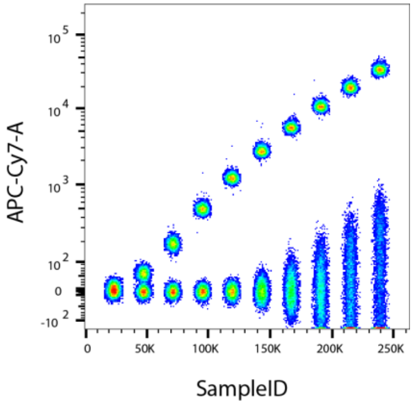


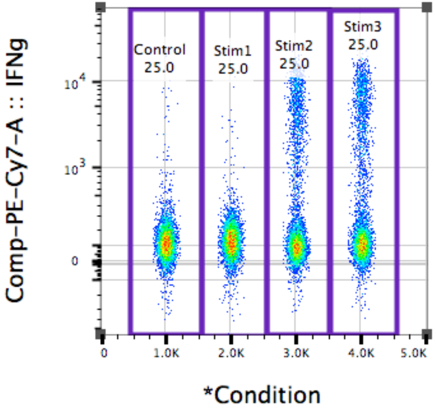
Additional Parameters

Choose one or more keywords from the list below. New parameters will be created from each keyword's value.

Keyword Value	keyword.selectiontable.value
$BEGINANALYSIS	0
$BEGINDATA	4106
$BEGINSTEXT	0
$BTIM	11:23:40
$BYTEORD	4,3,2,1
$CYT	LSRFortessa (LSRII)
$DATATYPE	F
$DATE	23-MAR-2015
$ENDANALYSIS	0
$ENDDATA	990069
$ENDSTEXT	0
$ETIM	11:24:01
$FIL	Specimen_001_250.fcs
$INST	
$MODE	L
$NEXTDATA	0
$OP	mdantoni
$PAR	23
$P1B	32
$P1E	0,0
$P1G	1.0
$P1N	FSC-A
$P1R	262144
$P1S	
$P1V	698

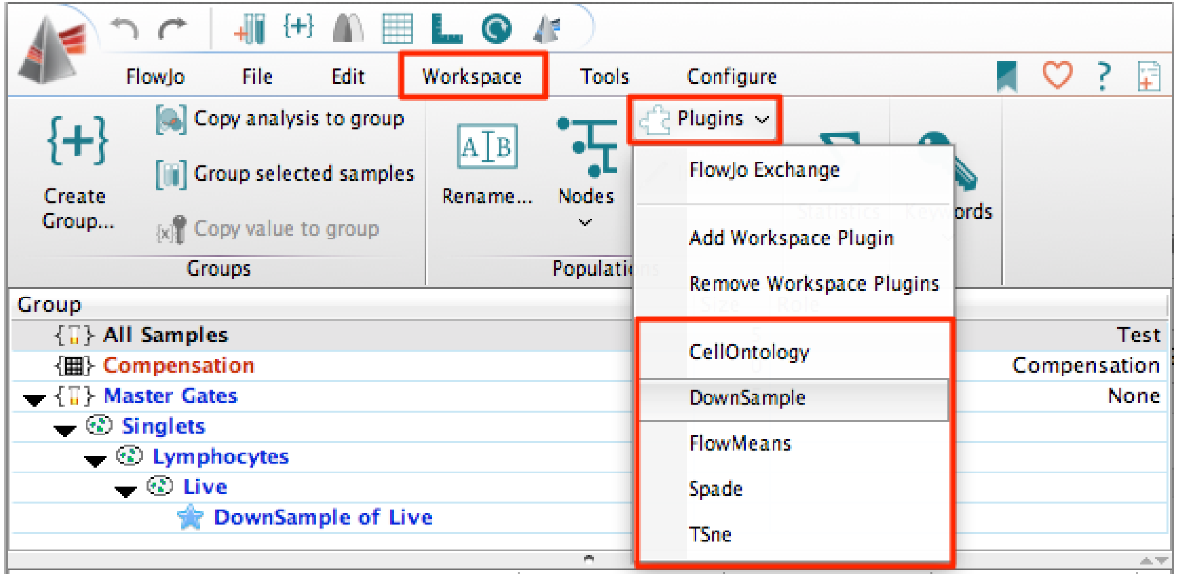
Select Keywords from Sample: Specimen_001_250.fcs



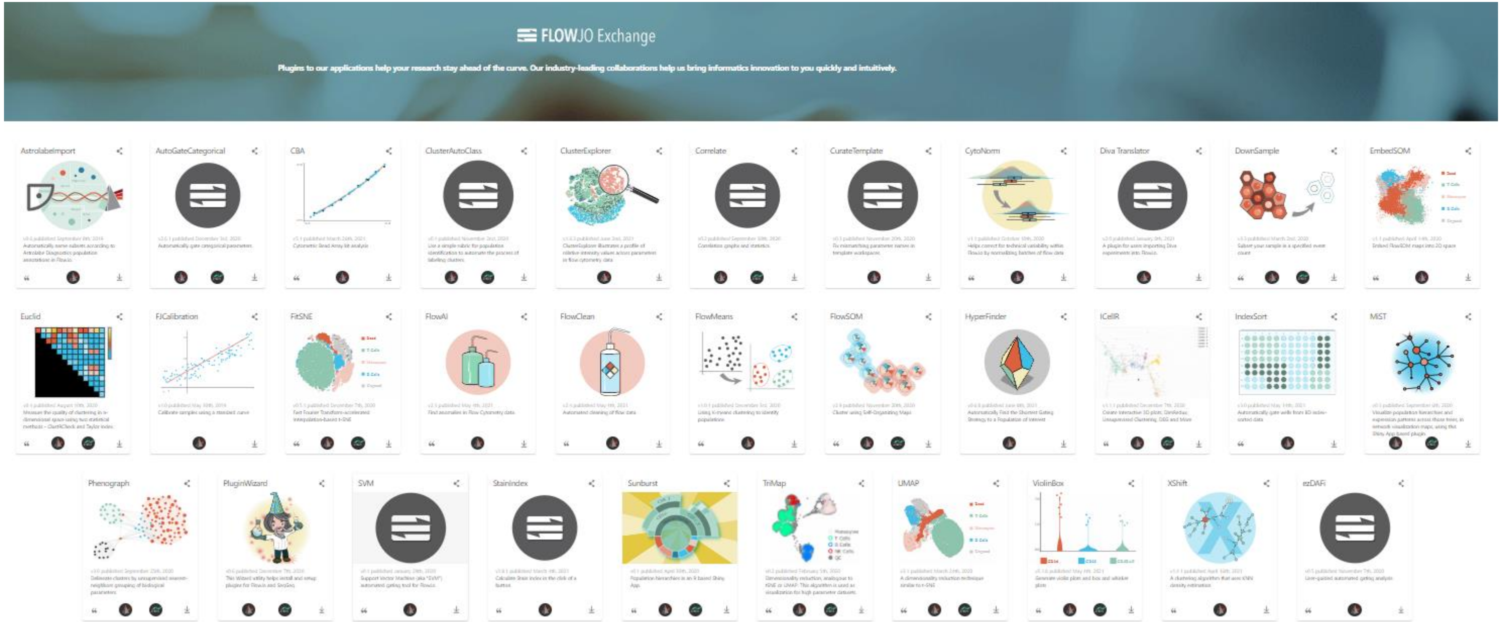


Plugins

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



Currently Available Plugins



FLOWJO Exchange

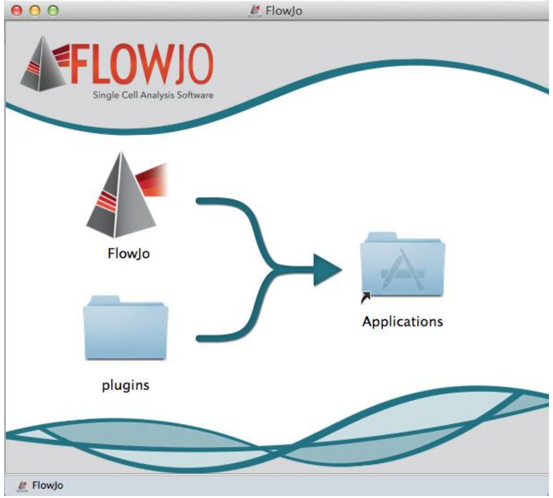
Plugins to our applications help your research stay ahead of the curve. Our industry-leading collaborations help us bring informatics innovation to you quickly and intuitively.

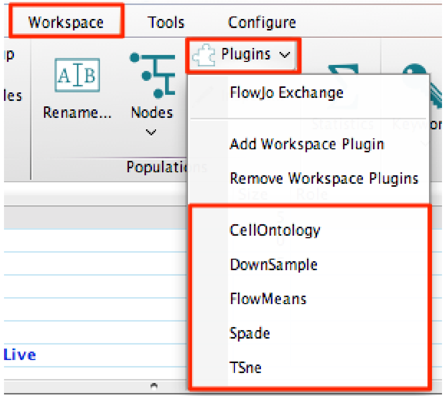
*Requires the R Statistical Computing Environment

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

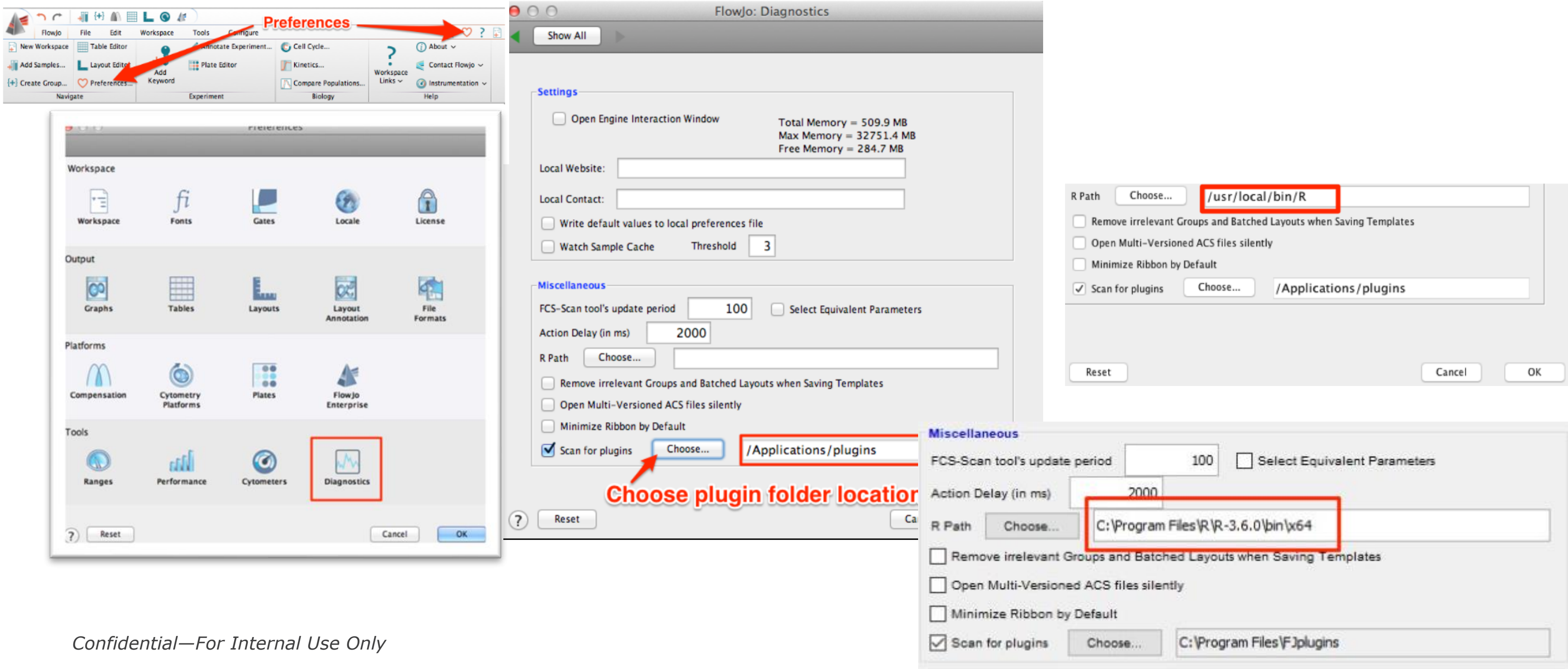
Installing Plugins

- 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.





Choosing R and plugins path



Preferences

Settings

☐ Open Engine Interaction Window

Total Memory = 509.9 MB
Max Memory = 32751.4 MB
Free Memory = 284.7 MB

Local Website:

Local Contact:

☐ Write default values to local preferences file

☐ Watch Sample Cache Threshold 3

Miscellaneous

FCS-Scan tool's update period 100 ☐ Select Equivalent Parameters

Action Delay (in ms) 2000

R Path Choose...

☐ Remove irrelevant Groups and Batched Layouts when Saving Templates

☐ Open Multi-Versioned ACS files silently

☐ Minimize Ribbon by Default

☑ Scan for plugins Choose... /Applications/plugins

Choose plugin folder location

R Path Choose... /usr/local/bin/R

☐ Remove irrelevant Groups and Batched Layouts when Saving Templates

☐ Open Multi-Versioned ACS files silently

☐ Minimize Ribbon by Default

☑ Scan for plugins Choose... /Applications/plugins

Miscellaneous

FCS-Scan tool's update period 100 ☐ Select Equivalent Parameters

Action Delay (in ms) 2000

R Path Choose... C:\Program Files\R\R-3.6.0\bin\x64

☐ Remove irrelevant Groups and Batched Layouts when Saving Templates

☐ Open Multi-Versioned ACS files silently

☐ Minimize Ribbon by Default

☑ Scan for plugins Choose... C:\Program Files\FJplugins

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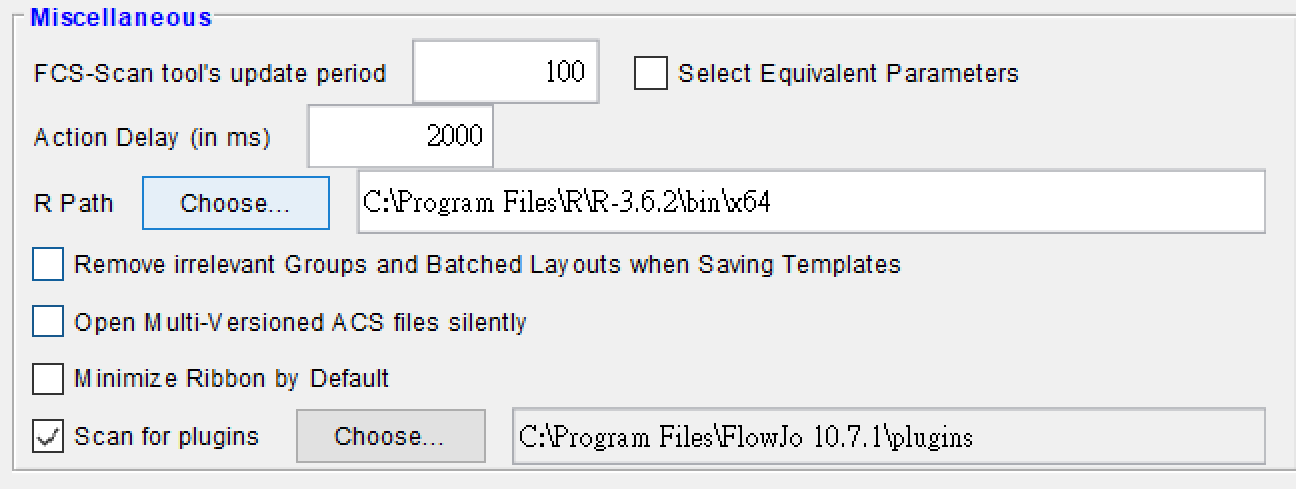

Miscellaneous

FCS-Scan tool's update period: 100

☐ Select Equivalent Parameters

Action Delay (in ms): 2000

R Path: C:\Program Files\R\R-3.6.2\bin\x64

☐ Remove irrelevant Groups and Batched Layouts when Saving Templates

☐ Open Multi-Versioned ACS files silently

☐ Minimize Ribbon by Default

☑ Scan for plugins: C:\Program Files\FlowJo 10.7.1\plugins

```
> if (!requireNamespace("BiocManager", quietly = TRUE))
+ install.packages("BiocManager")
Installing package into ‘C:/Users/10321131/AppData/Local/R/win-library/4.3’
(as ‘lib’ is unspecified)
--- Please select a CRAN mirror for use in this session ---
trying URL 'https://cran.csie.ntu.edu.tw/bin/windows/contrib/4.3/BiocManager_1.$
Content type 'application/zip' length 495555 bytes (483 KB)
downloaded 483 KB

package ‘BiocManager’ successfully unpacked and MD5 sums checked

The downloaded binary packages are in
        C:\Users\10321131\AppData\Local\Temp\RtmpgRcPoU\downloaded_packages
> BiocManager::install()
```

When you run a Plugin

- You must save the FlowJo Workspace first
  - If not, prompted to save
- Many plugins take a gated population from FlowJo, uses it to run some sort of operation or algorithm calculation producing associated derivative files, and returns results to FlowJo.
- The derivatives files are saved in a folder, created the first time a plugin is run in a Workspace.
- The folder is named the same as the Workspace and saved in the same location as the Workspace.
- All subsequent plugins run from that Workspace will be saved to that same derivatives folder.

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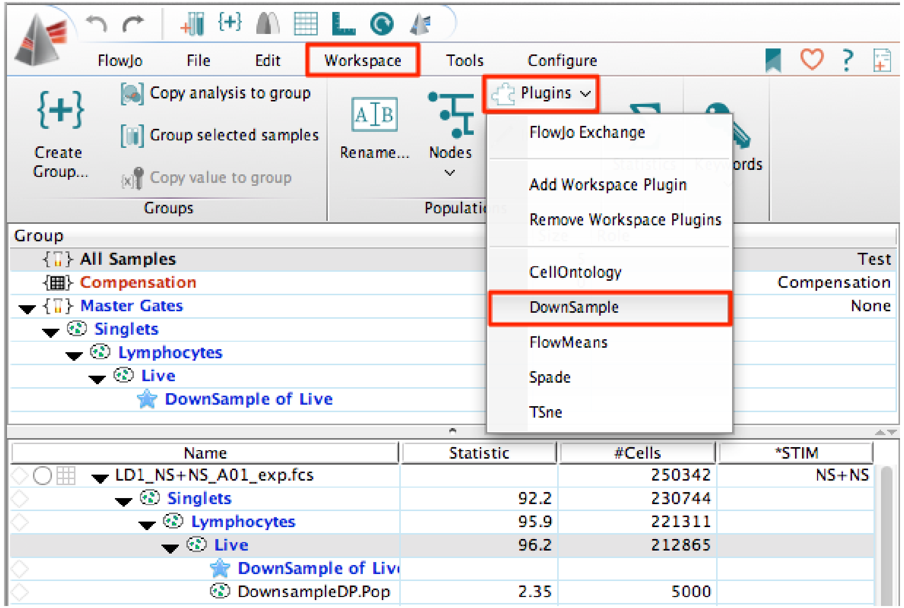
DownSample

- 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

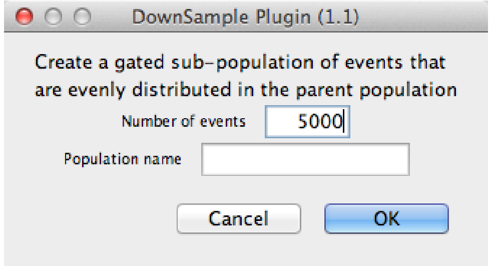
DownSample

- Initiating DownSample from the Workspace
- Workspace>Plugins>DownSample



Group	
All Samples	Test
Compensation	Compensation
Master Gates	None
Singlets	
Lymphocytes	
Live	
DownSample of Live	

Name	Statistic	#Cells	*STIM
LD1_NS+NS_A01_exp.fcs		250342	NS+NS
Singlets	92.2	230744	
Lymphocytes	95.9	221311	
Live	96.2	212865	
DownSample of Liv			
DownsampleDP.Pop	2.35	5000	


DownSample Plugin (1.1)

Create a gated sub-population of events that are evenly distributed in the parent population

Number of events: 5000

Population name:

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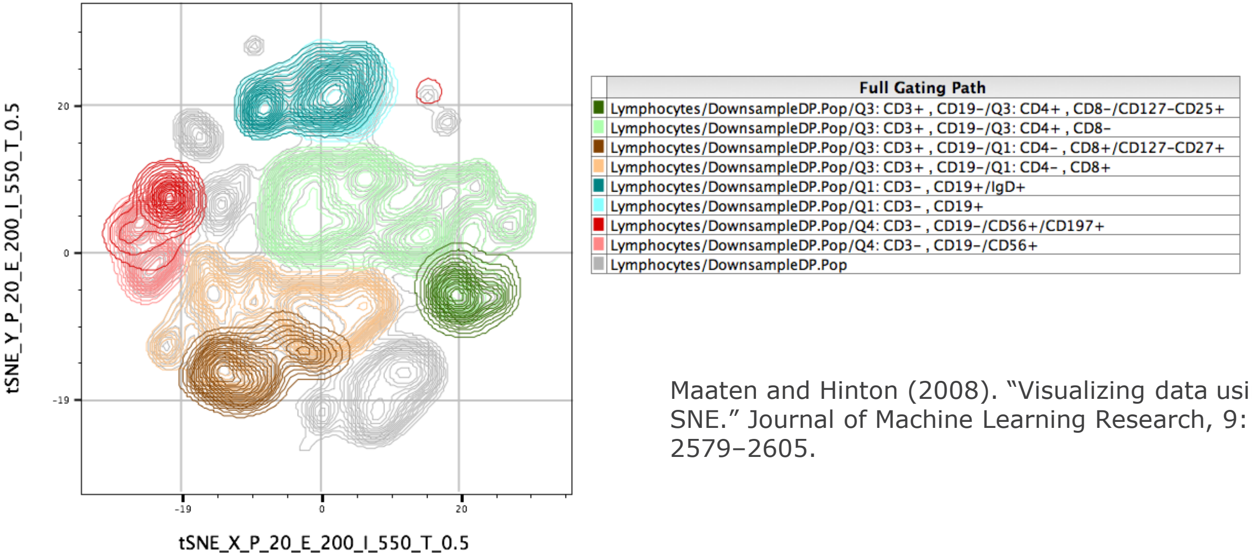
tSNE

- 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

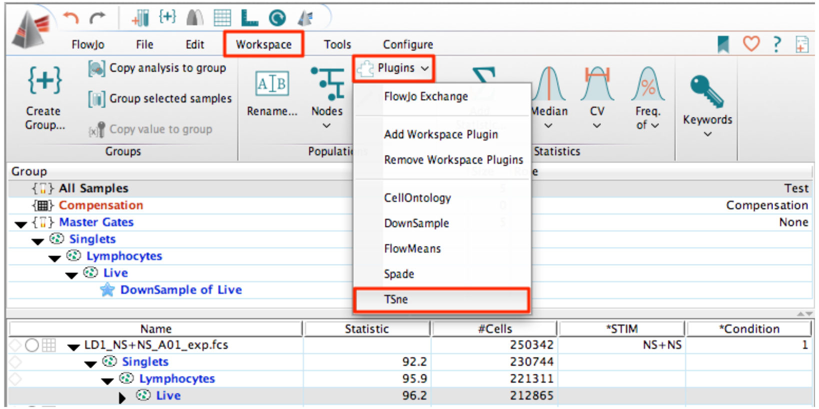


	Full Gating Path
🟩	Lymphocytes/DownsampleDP.Pop/Q3: CD3+ , CD19−/Q3: CD4+ , CD8−/CD127−CD25+
🟩	Lymphocytes/DownsampleDP.Pop/Q3: CD3+ , CD19−/Q3: CD4+ , CD8−
🟫	Lymphocytes/DownsampleDP.Pop/Q3: CD3+ , CD19−/Q1: CD4− , CD8+/CD127−CD27+
🟧	Lymphocytes/DownsampleDP.Pop/Q3: CD3+ , CD19−/Q1: CD4− , CD8+
🟦	Lymphocytes/DownsampleDP.Pop/Q1: 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

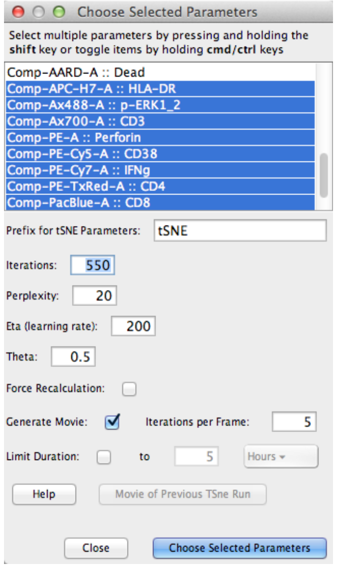
Maaten and Hinton (2008). “Visualizing data using t-SNE.” Journal of Machine Learning Research, 9: 2579–2605.

tSNE

- Workflow
  → Downsample, Concatenate, tSNE, Gate/Cluster, Explore
- Initiating tSNE from the Workspace



Name	Statistic	#Cells	*STIM	*Condition
LD1_NS+NS_A01_exp.fcs		250342	NS+NS	1
Singlets	92.2	230744		
Lymphocytes	95.9	221311		
Live	96.2	212865		



Select multiple parameters by pressing and holding the **shift** key or toggle items by holding **cmd/ctrl** keys

Comp-AARD-A :: Dead
Comp-APC-H7-A :: HLA-DR
Comp-Ax488-A :: p-ERK1_2
Comp-Ax700-A :: CD3
Comp-PE-A :: Perforin
Comp-PE-Cy5-A :: CD38
Comp-PE-Cy7-A :: IFNg
Comp-PE-TxRed-A :: CD4
Comp-PacBlue-A :: CD8

Prefix for tSNE Parameters: tSNE
Iterations: 550
Perplexity: 20
Eta (learning rate): 200
Theta: 0.5
Force Recalculation: ☐
Generate Movie: ☑ Iterations per Frame: 5
Limit Duration: ☐ to 5 Hours

- **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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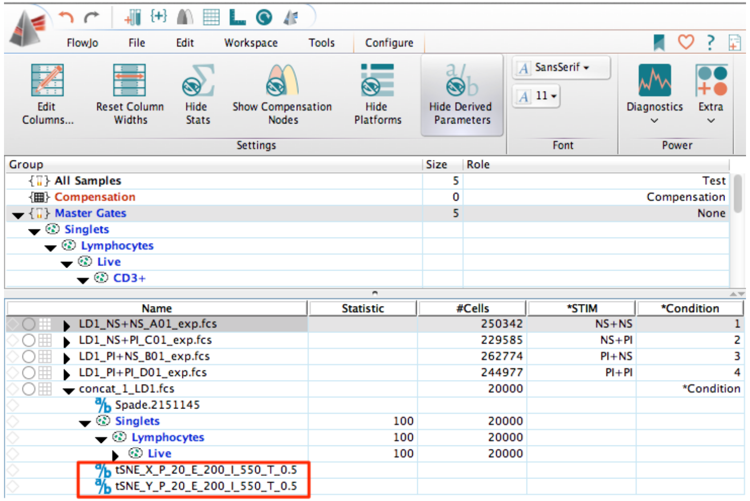
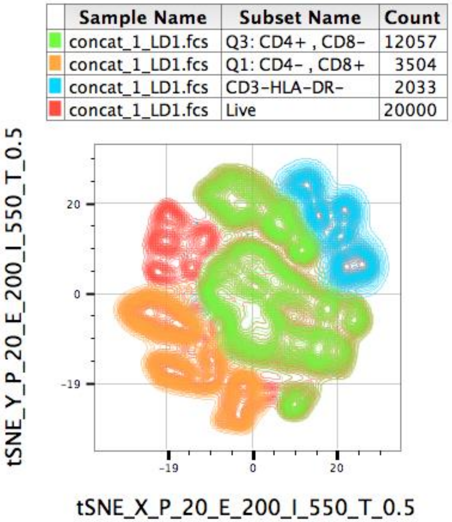
tSNE

- 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.



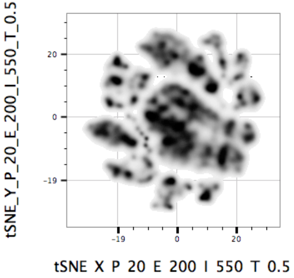
Group	Size	Role
All Samples	5	Test
Compensation	0	Compensation
Master Gates	5	None
Singlets		
Lymphocytes		
Live		
CD3+		

Name	Statistic	#Cells	*STIM	*Condition
LD1_NS+NS_A01_exp.fcs		250342	NS+NS	1
LD1_NS+PI_C01_exp.fcs		229585	NS+PI	2
LD1_PI+NS_B01_exp.fcs		262774	PI+NS	3
LD1_PI+PI_D01_exp.fcs		244977	PI+PI	4
concat_1_LD1.fcs		20000		*Condition
Spade.2151145				
Singlets	100	20000		
Lymphocytes	100	20000		
Live	100	20000		
tSNE_X_P_20_E_200_I_550_T_0.5				
tSNE_Y_P_20_E_200_I_550_T_0.5				

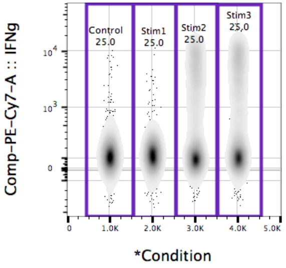


	Sample Name	Subset Name	Count
🟩	concat_1_LD1.fcs	Q3: CD4+ , CD8-	12057
🟧	concat_1_LD1.fcs	Q1: CD4- , CD8+	3504
🟦	concat_1_LD1.fcs	CD3-HLA-DR-	2033
🟥	concat_1_LD1.fcs	Live	20000

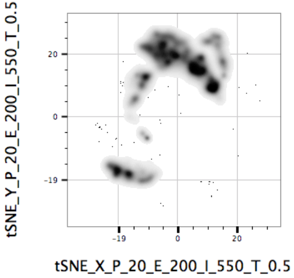
tSNE



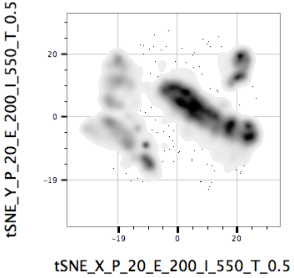
All Concatenated Events



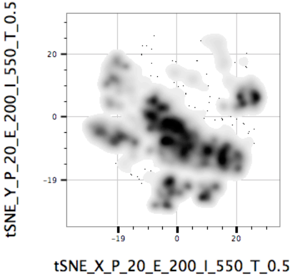
Gating



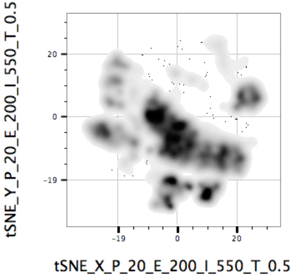
Control



Stim1



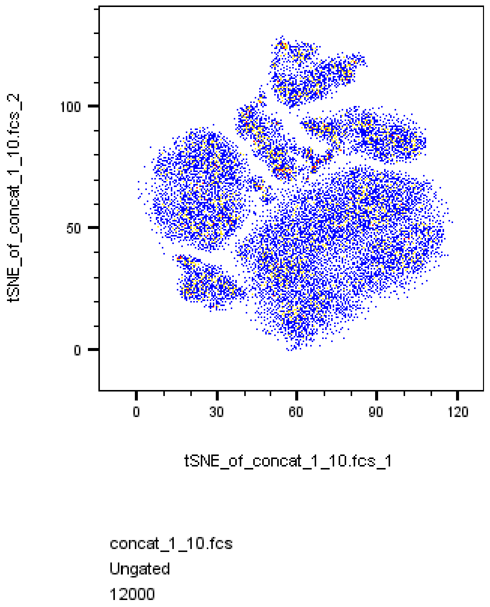
Stim2

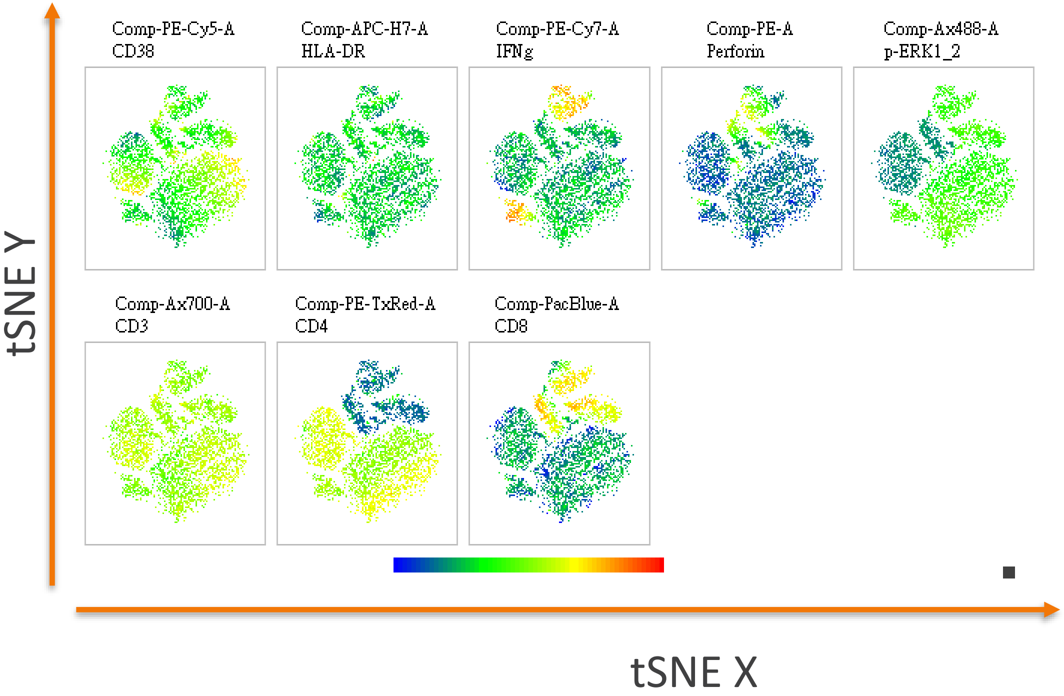


Stim3

An example of analyze data through plugins

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





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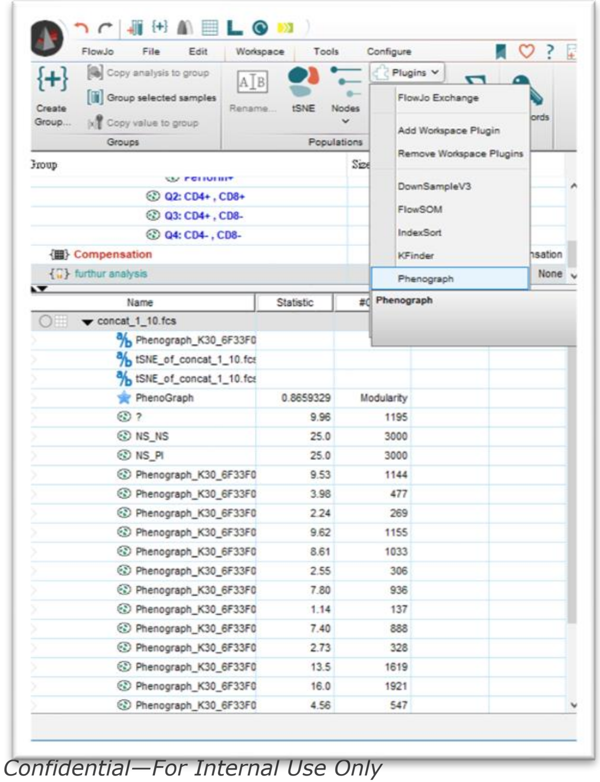
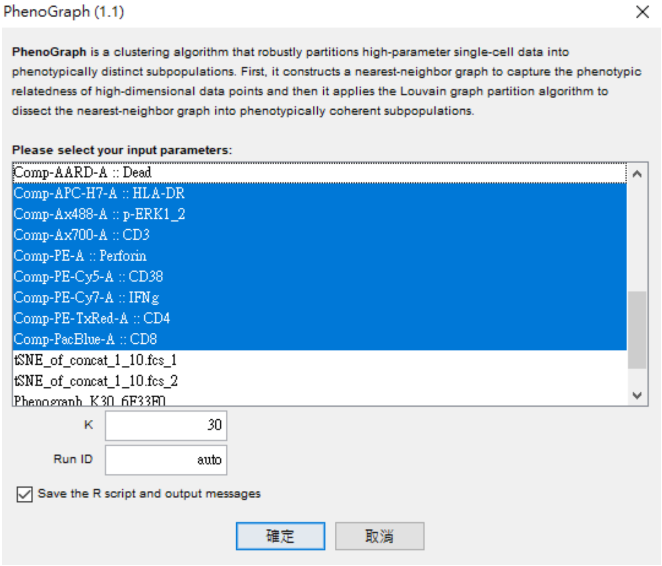
Discovery workflow using tSNE/Phenograph



Name	Statistic	#C
concat_1_10.fcs		
Phenograph_K30_6F33F0		
tSNE_of_concat_1_10.fcs		
tSNE_of_concat_1_10.fcs		
PhenoGraph	0.8659329	Modularity
?	9.96	1195
NS_NS	25.0	3000
NS_PI	25.0	3000
Phenograph_K30_6F33F0	9.53	1144
Phenograph_K30_6F33F0	3.98	477
Phenograph_K30_6F33F0	2.24	269
Phenograph_K30_6F33F0	9.62	1155
Phenograph_K30_6F33F0	8.61	1033
Phenograph_K30_6F33F0	2.55	306
Phenograph_K30_6F33F0	7.80	936
Phenograph_K30_6F33F0	1.14	137
Phenograph_K30_6F33F0	7.40	888
Phenograph_K30_6F33F0	2.73	328
Phenograph_K30_6F33F0	13.5	1619
Phenograph_K30_6F33F0	16.0	1921
Phenograph_K30_6F33F0	4.56	547

PhenoGraph

Identifies subpopulations in high-dimensional single-cell data. PhenoGraph is a computational method that was developed to avoid the disadvantages of manual gating.



PhenoGraph is a clustering algorithm that robustly partitions high-parameter single-cell data into phenotypically distinct subpopulations. First, it constructs a nearest-neighbor graph to capture the phenotypic relatedness of high-dimensional data points and then it applies the Louvain graph partition algorithm to dissect the nearest-neighbor graph into phenotypically coherent subpopulations.

Please select your input parameters:

- Comp-AARD-A :: Dead
- Comp-APC-H7-A :: HLA-DR
- Comp-Ax488-A :: p-ERK1_2
- Comp-Ax700-A :: CD3
- Comp-PE-A :: Perforin
- Comp-PE-Cy5-A :: CD38
- Comp-PE-Cy7-A :: IFNg
- Comp-PE-TxRed-A :: CD4
- Comp-PacBlue-A :: CD8
- tSNE_of_concat_1_10.fcs_1
- tSNE_of_concat_1_10.fcs_2
- Phenograph_K30_6F33F0

K: 30

Run ID: auto

☑ Save the R script and output messages

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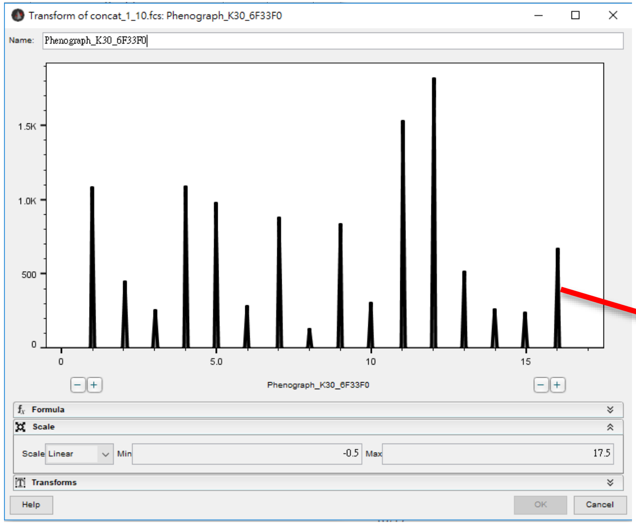
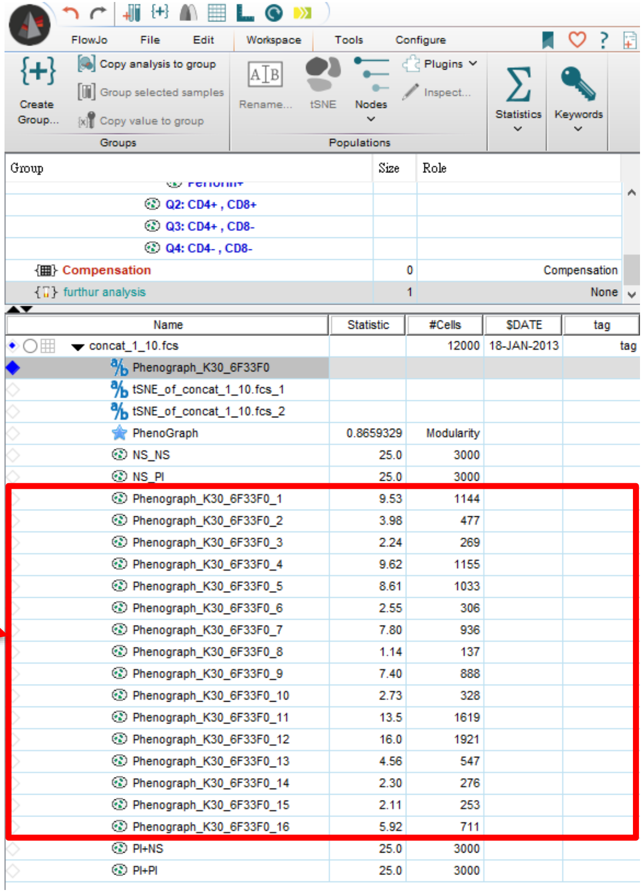
Cell clustering

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



Name: Phenograph_K30_6F33F0

Scale: Linear Min: -0.5 Max: 17.5



Group	Size	Role
Perforin+		
Q2: CD4+ , CD8+		
Q3: CD4+ , CD8-		
Q4: CD4- , CD8-		
Compensation	0	Compensation
furthur analysis	1	None

Name	Statistic	#Cells	$DATE	tag
concat_1_10.fcs		12000	18-JAN-2013	tag
Phenograph_K30_6F33F0				
tSNE_of_concat_1_10.fcs_1				
tSNE_of_concat_1_10.fcs_2				
PhenoGraph	0.8659329	Modularity		
NS_NS	25.0	3000		
NS_PI	25.0	3000		
Phenograph_K30_6F33F0_1	9.53	1144		
Phenograph_K30_6F33F0_2	3.98	477		
Phenograph_K30_6F33F0_3	2.24	269		
Phenograph_K30_6F33F0_4	9.62	1155		
Phenograph_K30_6F33F0_5	8.61	1033		
Phenograph_K30_6F33F0_6	2.55	306		
Phenograph_K30_6F33F0_7	7.80	936		
Phenograph_K30_6F33F0_8	1.14	137		
Phenograph_K30_6F33F0_9	7.40	888		
Phenograph_K30_6F33F0_10	2.73	328		
Phenograph_K30_6F33F0_11	13.5	1619		
Phenograph_K30_6F33F0_12	16.0	1921		
Phenograph_K30_6F33F0_13	4.56	547		
Phenograph_K30_6F33F0_14	2.30	276		
Phenograph_K30_6F33F0_15	2.11	253		
Phenograph_K30_6F33F0_16	5.92	711		
PI+NS	25.0	3000		
PI+PI	25.0	3000		

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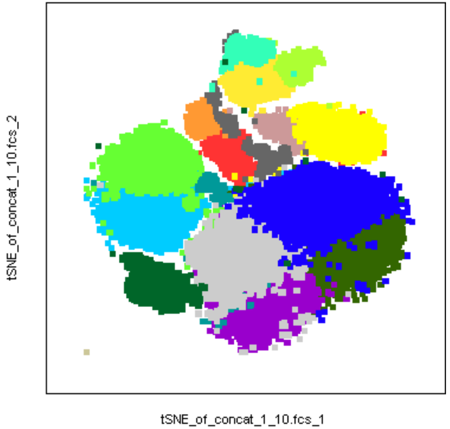
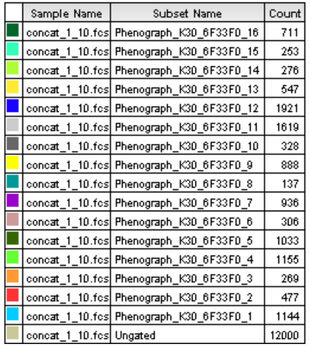
Apply Phenograph clusters to tSNE map

Overall

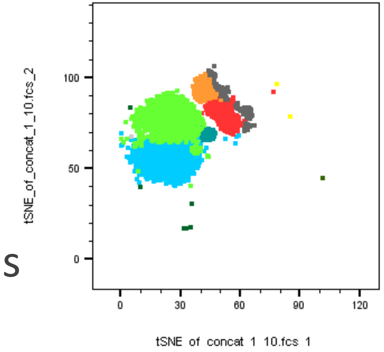




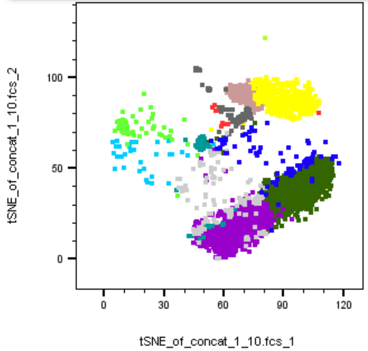
	Sample Name	Subset Name	Count
🟩	concat_1_10.fcs	Phenograph_K30_6F33F0_16	711
🟩	concat_1_10.fcs	Phenograph_K30_6F33F0_15	253
🟩	concat_1_10.fcs	Phenograph_K30_6F33F0_14	276
🟨	concat_1_10.fcs	Phenograph_K30_6F33F0_13	547
🟦	concat_1_10.fcs	Phenograph_K30_6F33F0_12	1921
⬜	concat_1_10.fcs	Phenograph_K30_6F33F0_11	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

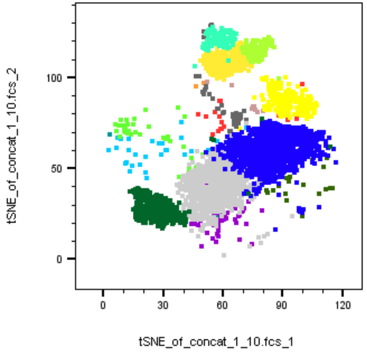
NS+NS



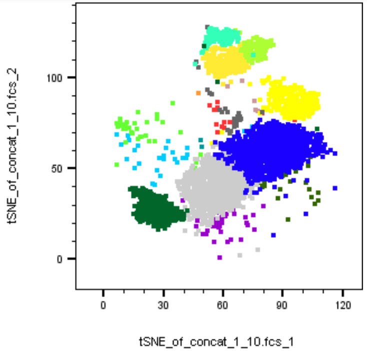
PI+NS



NS+PI



PI+PI



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Additional Plugin Resources

The FlowJo Exchange

http://exchange.flowjo.com/

Future plugin releases

Featured plugins

Updates

Developer documentation

Scripts

Documentation

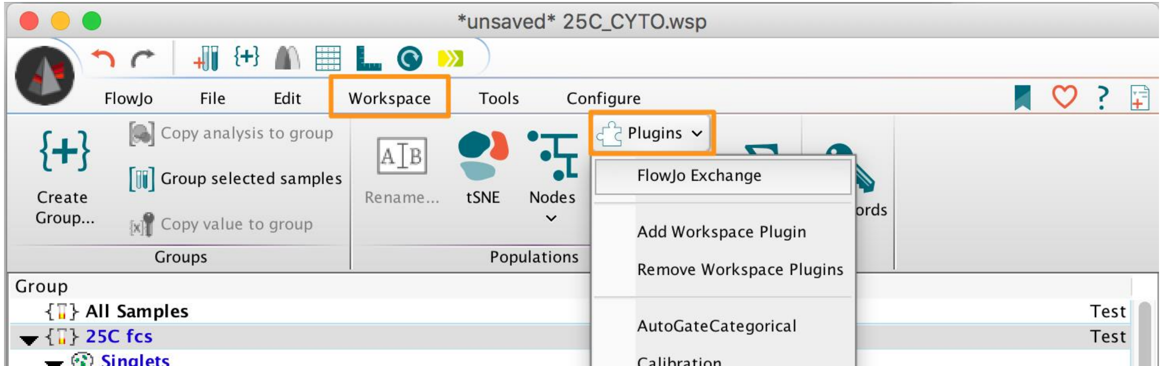
http://docs.flowjo.com

- Search for Plugins → pages describing plugin setup and functionality

FlowJo Exchange Plugins

Apps that extend the functionality of FlowJo

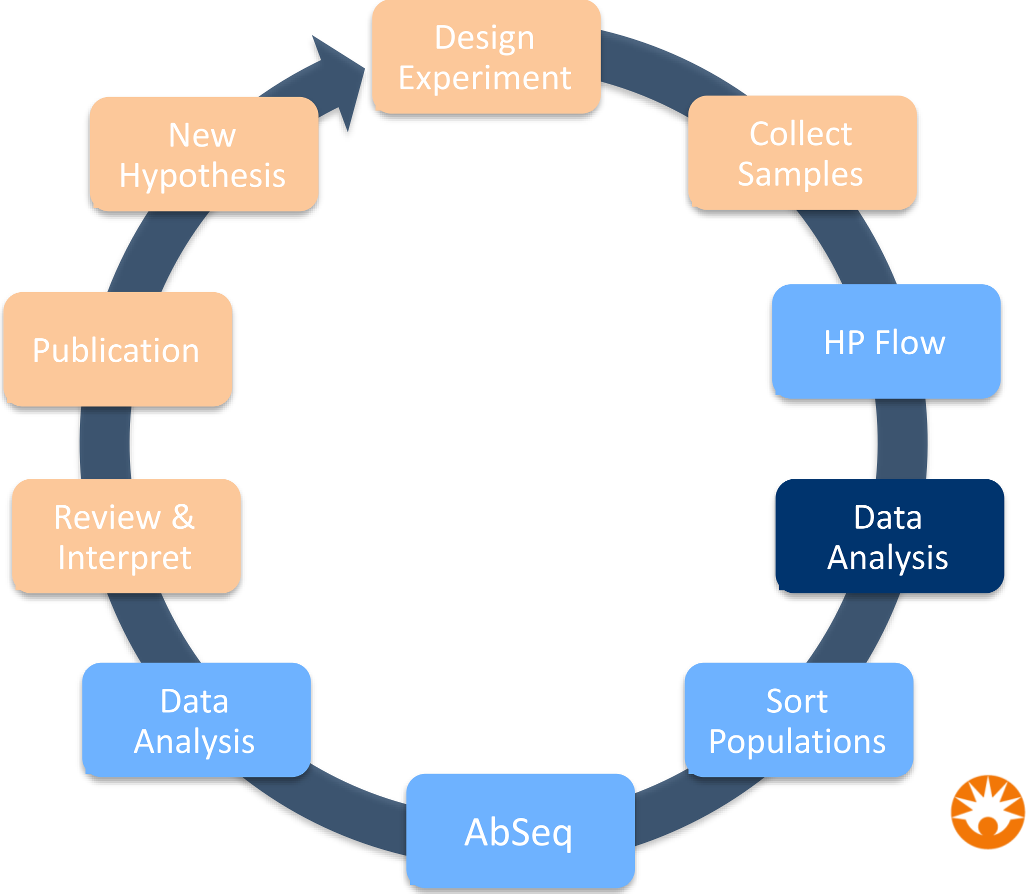
Pre-processing	Visualization	Clustering	Interpretation +Dig Deeper
Downsample	tSNE	FlowSOM	ClusterExplorer
IndexSort	FItSE	Phenograph	HyperFinder
FlowAI	UMAP	flowMeans	
FlowClean	EmbedSOM	X-Shift	
AutoGateCategorical	ViolinBox		



High Parameter Analysis Workflow

for Discovery

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



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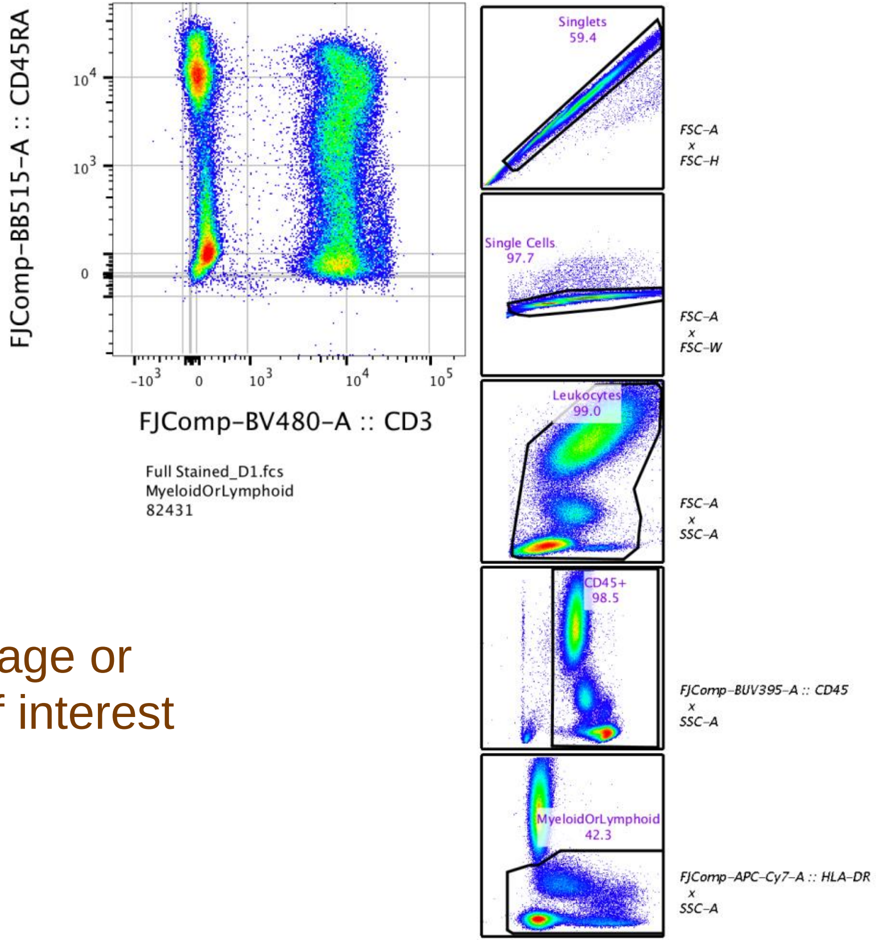
Cleanup Gate

remove doublets, dead cells and debris

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



- Focus on a lineage or compartment of interest

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DownSample

normalize the number of events contributed

cleanup

concatenate

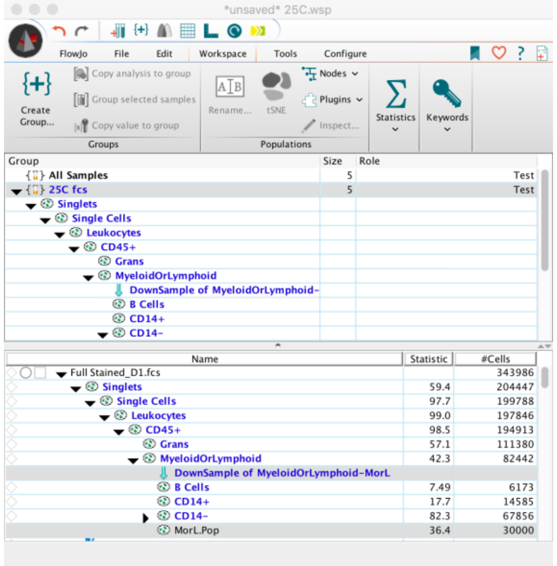
dimensionally-reduce

cluster

gate

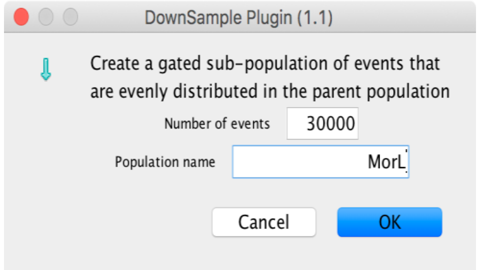
comparisons

dig deeper



Group	Size	Role
All Samples	5	Test
25C fcs	5	Test
Singlets		
Single Cells		
Leukocytes		
CD45+		
Grans		
MyeloidOrLymphoid		
DownSample of MyeloidOrLymphoid-		
B Cells		
CD14+		
CD14-		

Name	Statistic	#Cells
Full Stained_D1.fcs		343986
Singlets	59.4	204447
Single Cells	97.7	199788
Leukocytes	99.0	197846
CD45+	98.5	194913
Grans	57.1	111380
MyeloidOrLymphoid	42.3	82442
DownSample of MyeloidOrLymphoid-MorL		
B Cells	7.49	6173
CD14+	17.7	14585
CD14-	82.3	67856
MorL.Pop	36.4	30000



Create a gated sub-population of events that are evenly distributed in the parent population

Number of events: 30000

Population name: MorL

- 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

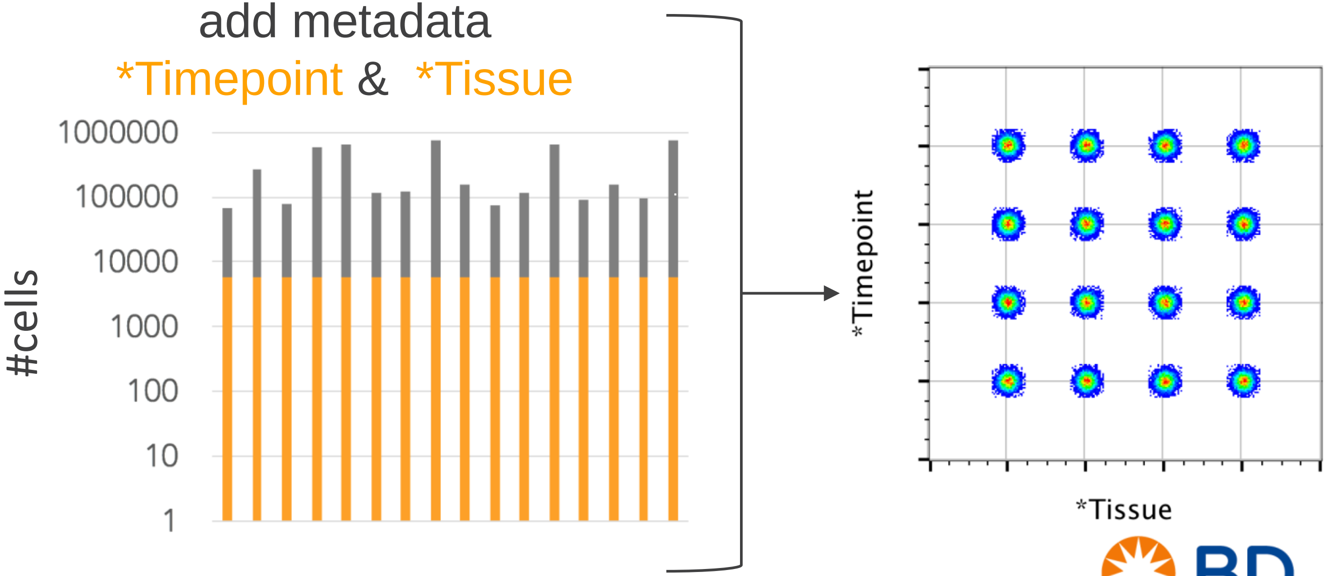
comparisons

dig deeper

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

add metadata

*Timepoint & *Tissue



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Dimensionality Reduction

creates new derived parameters

cleanup

concatenate

dimensionally-reduce

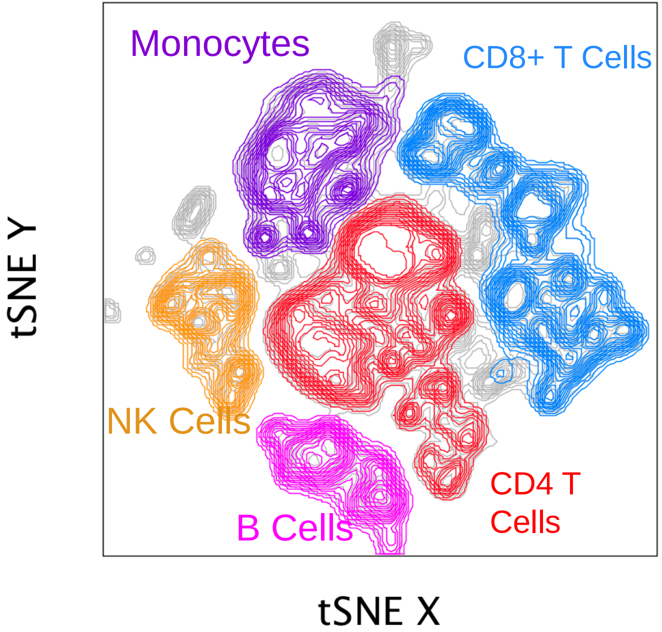
cluster

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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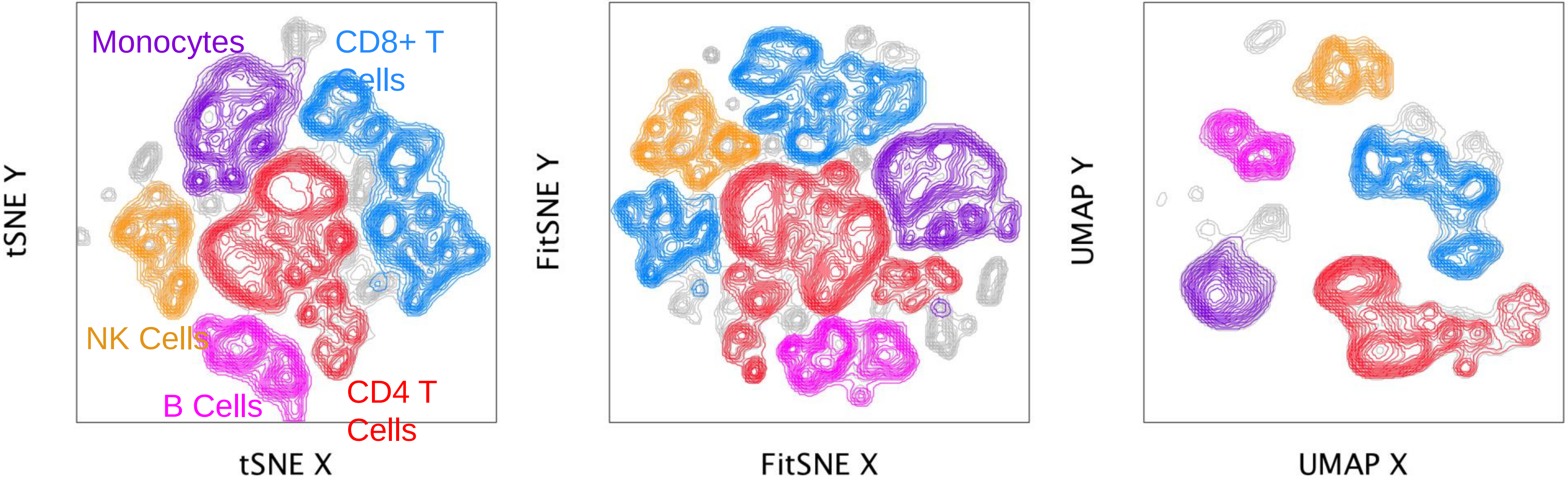
Dimensionality Reduction Options

- 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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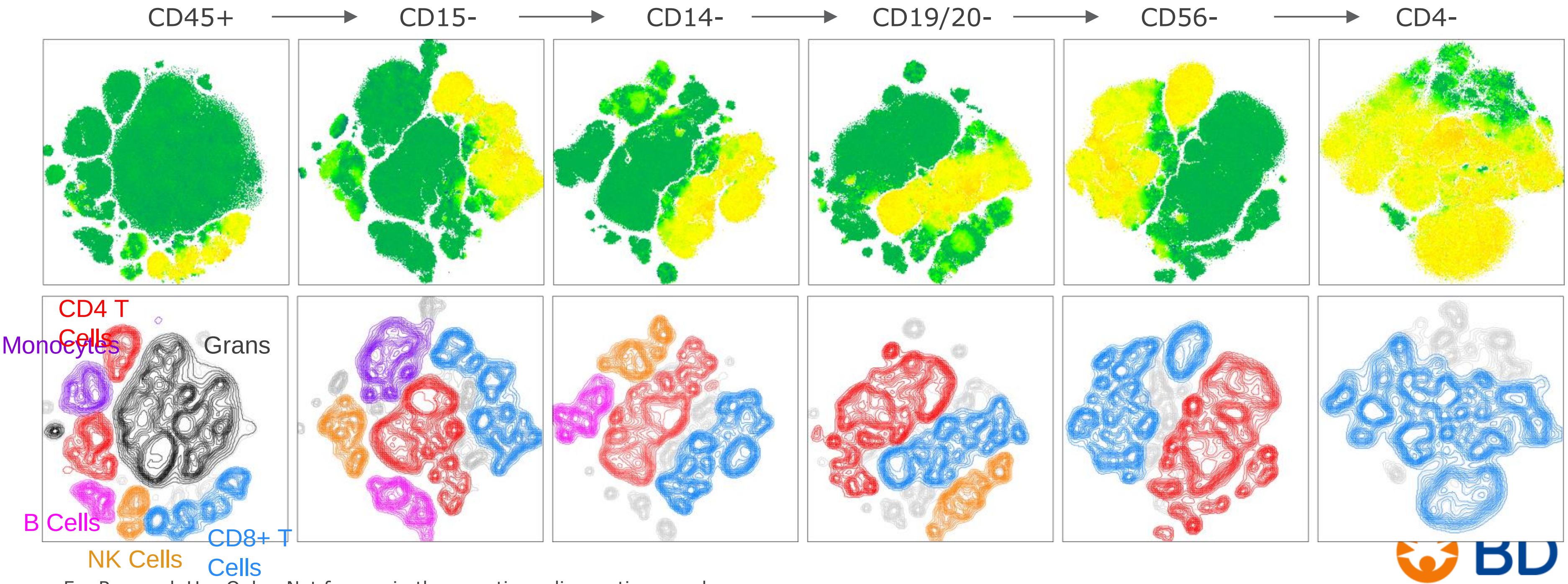
What to Dimensionally Reduce?

depends on the panel and experiment

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



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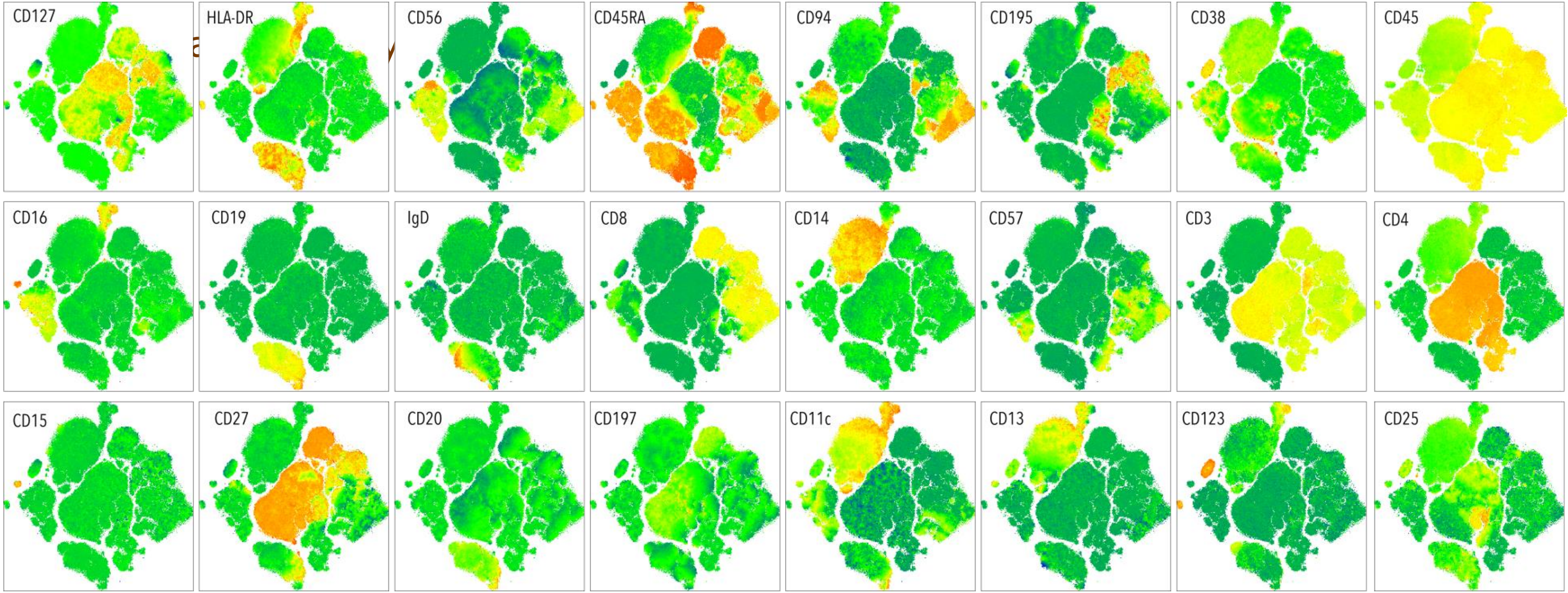
3rd Parameter Color Maps



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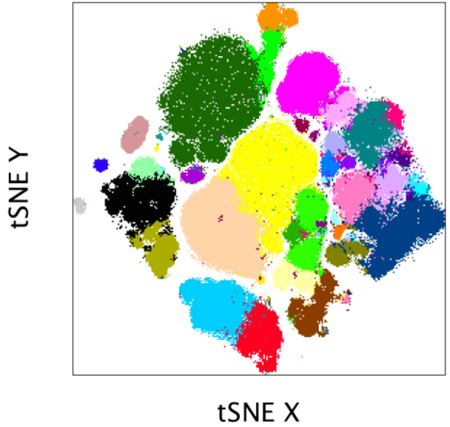
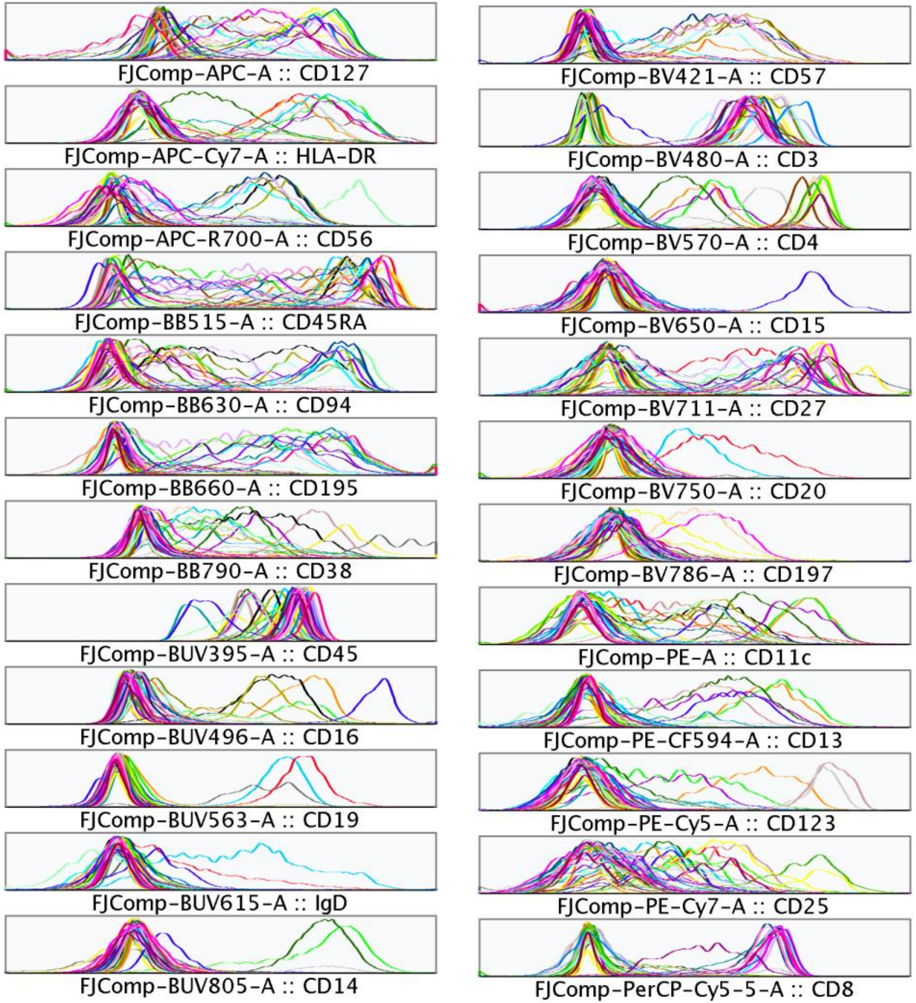
Clustering

creates populations

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





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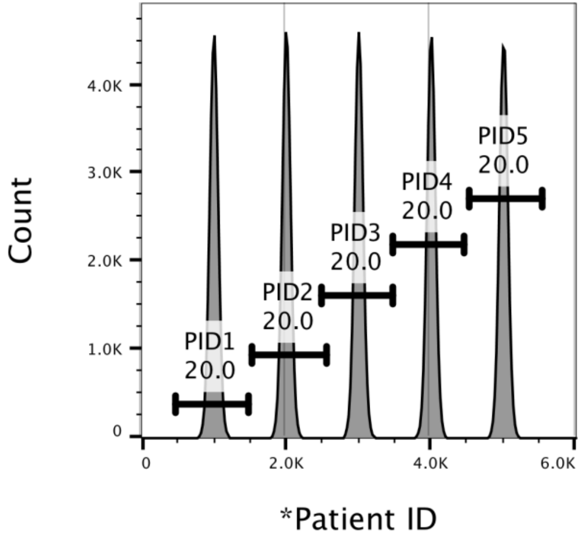
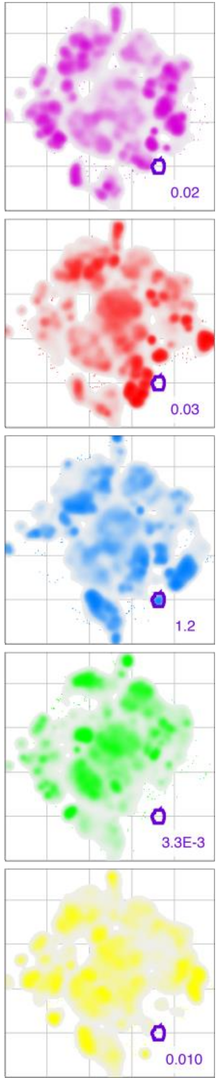
Gate on Keyword Parameter

to pull apart samples and/or experimental conditions

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





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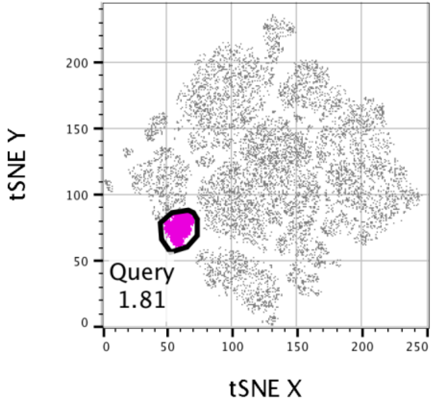
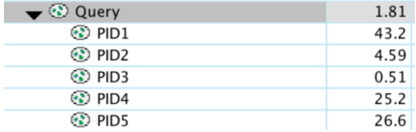
Query Gate w/in tSNE

to identify complex phenotype of a node or region

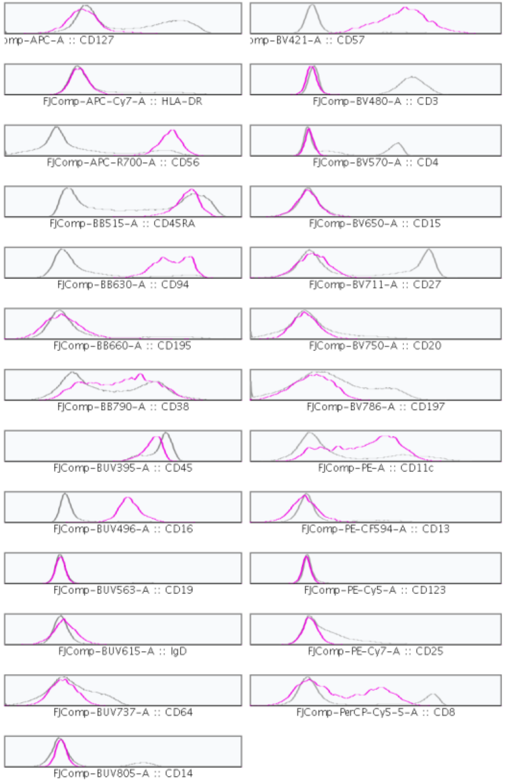
- cleanup
- concatenate
- dimensionally-reduce
- cluster
- **gate**
- comparisons
- dig deeper



	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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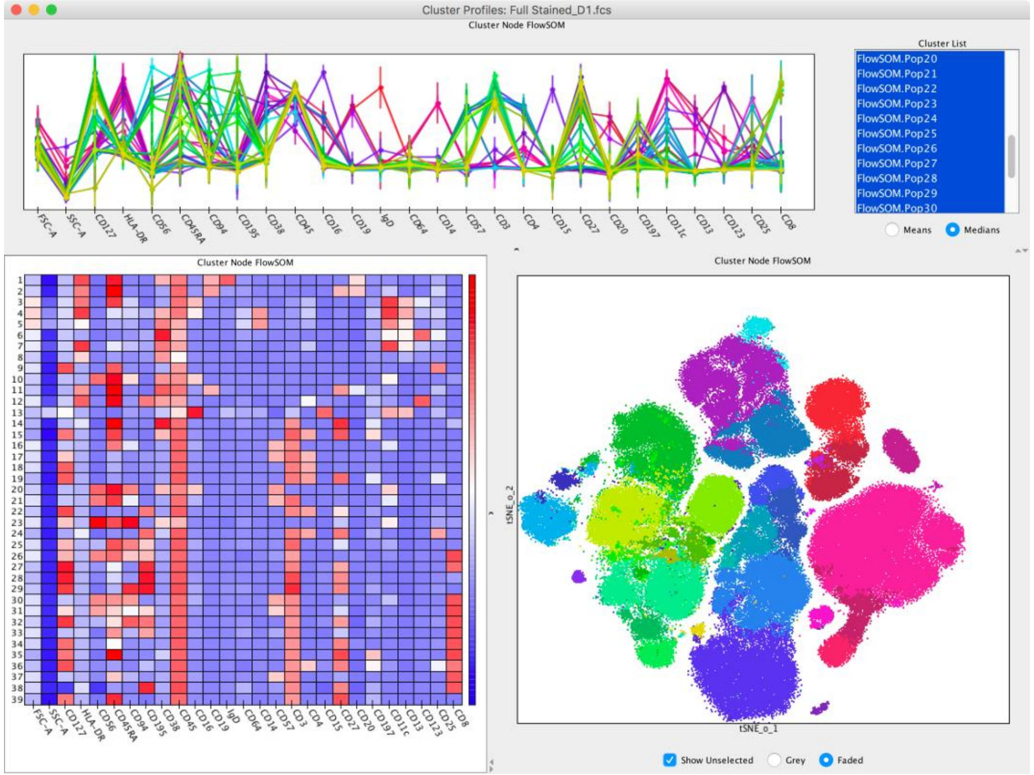
Cluster Explorer

interactive population phenotype identification

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



BD

Cluster Explorer

and comparison

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

○ Means ● Medians

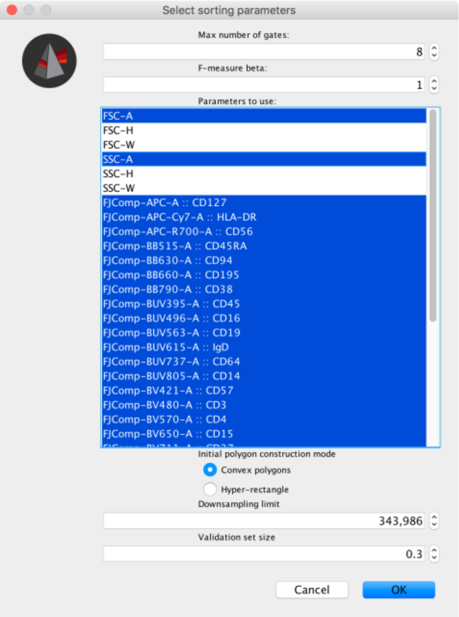
☑ Show Unselected ○ Grey ● Faded

BD RESTRICTED

HyperFinder

generate sort gates for isolating events with a complex phenotype

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



Select sorting parameters

Max number of gates: 8

F-measure beta: 1

Parameters to use:

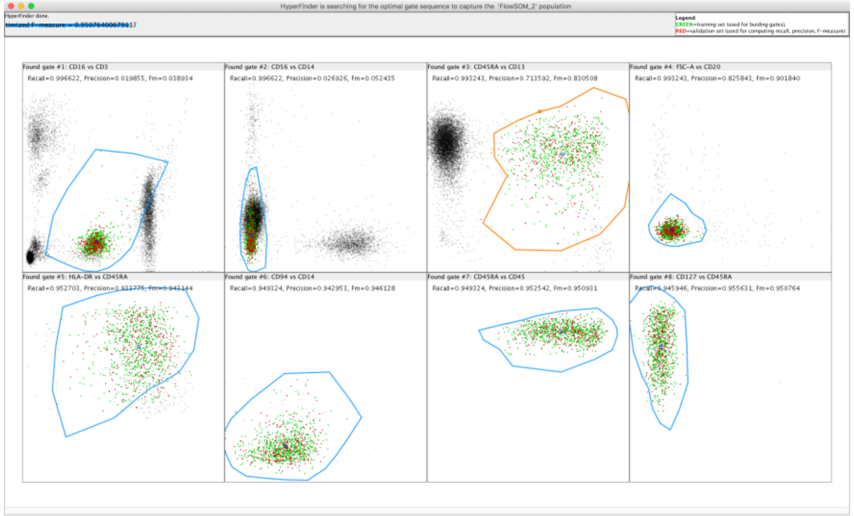
FSC-A
FSC-H
FSC-W
SSC-A
SSC-H
SSC-W
FJComp-APC-A :: CD127
FJComp-APC-Cy7-A :: HLA-DR
FJComp-APC-R700-A :: CD56
FJComp-BB515-A :: CD45RA
FJComp-BB630-A :: CD94
FJComp-BB660-A :: CD195
FJComp-BB790-A :: CD38
FJComp-BUV395-A :: CD45
FJComp-BUV496-A :: CD16
FJComp-BUV563-A :: CD19
FJComp-BUV615-A :: IgD
FJComp-BUV737-A :: CD64
FJComp-BUV805-A :: CD14
FJComp-BV421-A :: CD57
FJComp-BV480-A :: CD3
FJComp-BV570-A :: CD4
FJComp-BV650-A :: CD15

Initial polygon construction mode

● Convex polygons
○ Hyper-rectangle

Downsampling limit: 343,986

Validation set size: 0.3



HyperFinder is searching for the optimal gate sequence to capture the 'FlowSOM_2' population

HyperFinder done.
timized F-measure = 0.95076400679117

Legend
GREEN=training set (used for buiding gates),
RED=validation set (used for computing recall, precision, F-measure)

Found gate #1: CD16 vs CD3
Recall=0.996622, Precision=0.019855, Fm=0.038934

Found gate #2: CD56 vs CD14
Recall=0.996622, Precision=0.026926, Fm=0.052435

Found gate #3: CD45RA vs CD13
Recall=0.993243, Precision=0.713592, Fm=0.830508

Found gate #4: FSC-A vs CD20
Recall=0.993243, Precision=0.825843, Fm=0.901840

Found gate #5: HLA-DR vs CD45RA
Recall=0.952703, Precision=0.933775, Fm=0.943144

Found gate #6: CD94 vs CD14
Recall=0.949324, Precision=0.942953, Fm=0.946128

Found gate #7: CD45RA vs CD45
Recall=0.949324, Precision=0.952542, Fm=0.950931

Found gate #8: CD127 vs CD45RA
Recall=0.945946, Precision=0.955631, Fm=0.950764

Export to FACSDiva

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

cleanup

concatenate

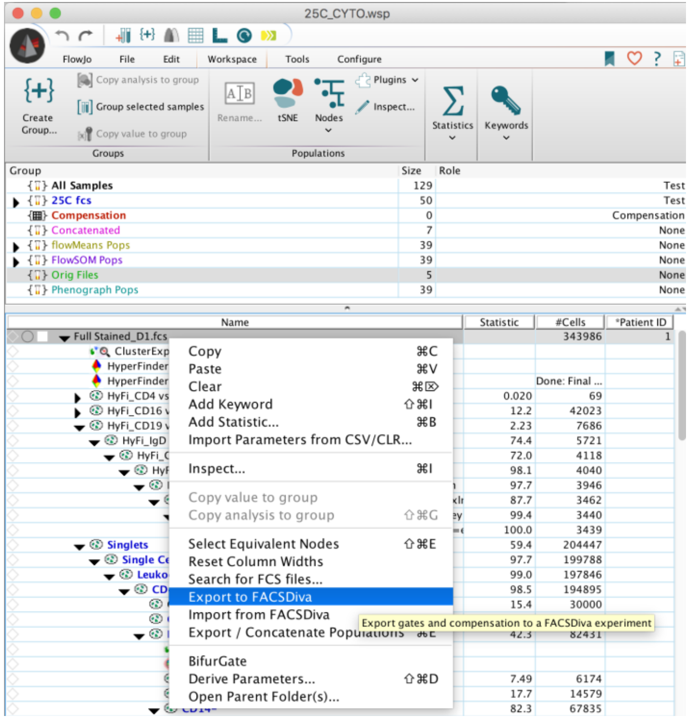
dimensionally-reduce

cluster

gate

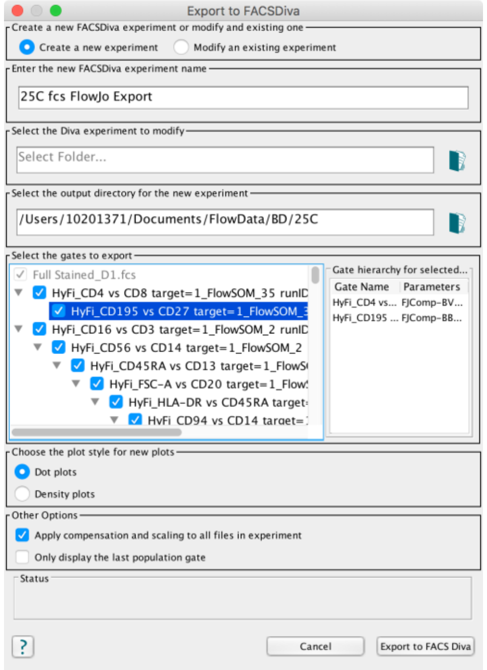
comparisons

dig deeper



Group	Size	Role
All Samples	129	Test
25C fcs	50	Test
Compensation	0	Compensation
Concatenated	7	None
flowMeans Pops	39	None
FlowSOM Pops	39	None
Orig Files	5	None
Phenograph Pops	39	None

Export gates and compensation to a FACSDiva experiment



Export to FACSDiva

Create a new FACSDiva experiment or modify and existing one
● Create a new experiment ○ Modify an existing experiment

Enter the new FACSDiva experiment name: 25C fcs FlowJo Export

Select the Diva experiment to modify: Select Folder...

Select the output directory for the new experiment: /Users/10201371/Documents/FlowData/BD/25C

Select the gates to export
- Full Stained_D1.fcs
  - ☑ HyFi_CD4 vs CD8 target=1_FlowSOM_35 runID
    - ☑ HyFi_CD195 vs CD27 target=1_FlowSOM_3
  - ☑ HyFi_CD16 vs CD3 target=1_FlowSOM_2 runID
    - ☑ HyFi_CD56 vs CD14 target=1_FlowSOM_2
      - ☑ HyFi_CD45RA vs CD13 target=1_FlowS
        - ☑ HyFi_FSC-A vs CD20 target=1_Flow
          - ☑ HyFi_HLA-DR vs CD45RA target
            - ☑ HyFi_CD94 vs CD14 target=

Gate hierarchy for selected...

Gate Name	Parameters
HyFi_CD4 vs...	FJComp-BV...
HyFi_CD195 ...	FJComp-BB...

Choose the plot style for new plots
● Dot plots
○ Density plots

Other Options
☑ Apply compensation and scaling to all files in experiment
☐ Only display the last population gate

Status

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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