# Introduction to FlowJo v10

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## The FlowJo v10 Workspace

An interface to organize your data and initiate actions

Action Toolbar

 Groups and Group Analysis

 Samples and Sample Analysis

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## The Action Toolbar

#### Allows visual navigation of workspace functions



- Tabs group similar Bands together
- Bands group similar Actions together
- Mouse-over an action button  $\rightarrow$  tooltip + hotkey

#### **Importing Data**

Drag-and-drop files into Samples Pane, or click Add Samples



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#### **Group Pane**

#### Groups act as folders to organize samples and initiate actions

Group	Size	Role
{ ]] } All Samples	46	Test
$\{ ] \} *STIM = NS+NS$	5	Test
{ ]] } *STIM = NS+PI	12	Test
$\{ ] \} *STIM = PI+NS$	5	Test
$\{\Pi\}$ *STIM = PI+PI	12	Test
{ T} All Stain	20	Test
Empensation	12	Compensation
{ T} FMOs	14	Controls
▼ { □} Master Gates	46	None
🚽 🚯 Singlets		
🚽 🕲 Live		

- The Group area lists all groups in the workspace, # of samples in each Group (Size) and the Role of that group
- Groups allow for master gating of multiple samples → Group-applied analysis gains the group color
- Groups can be used for batch reporting multiple samples

# Creating and Editing Groups

Click the Create Group icon

**{++**} Create Group...

- Type a Name
- Set options
- Click Create Group
- Drag-and-drop adds samples to the group
- Double click on an existing group to edit its properties



### Sample Inclusion Criteria

Specifies which samples are automatically included in a group

- A "Live group" automatically includes samples based on the user-defined sample inclusion criteria
- These criteria can include characters in the \$FIL field (File Name) or any other combination of keyword attributes

	Modify Group						
Appearance Name *STIM = PI+NS	Color Style Bold						
Role: Test	Parameter Key:						
Sample Inclusion Criter	ria						
Dead, HLA-DR, p-ERK1_2,	, Blank, CD3, Perforin, CD38, IFNg, CD4, CD8						
*STIM - More Choices Few	er Choices Show all keywords in menus						
With reference to samples in another group: Only choose from samples in Group (No specified group)							
Assignments Add Keyword : Add Keyword :	With Value : With Value :						
Help with Groups App	Close Create Group						

### Samples Pane

#### Lists all samples within the selected group

Name	Statistic	#Cells	*PID 🔺	*STIM	WELL ID
LD1_NS+NS_A01_exp.fcs		250342	LD1	NS+NS	A01
UD1_NS+PI_C01_exp.fcs		229585	LD1	NS+PI	C01
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Use State	93.3	198508			
🔷 🛛 🚽 🐼 Live	97.9	194256			
	79.8	155099			
Q1: CD4- , CD8+	18.9	29343			
Q2: CD4+ , CD8+	0.64	993			
Q3: CD4+ , CD8-	78.4	121644			
Q4: CD4- , CD8-	2.01	3119			
LD1_PI+NS_B01_exp.fcs		262774	LD1	PI+NS	B01
LD1_PI+PI_D01_exp.fcs		244977	LD1	PI+PI	D01

- Displays sample-level gating analysis hierarchy
- Statistic and #Cells columns are displayed by default
- Additional Keyword attribute columns can be added

## Keywords

#### Sample-level descriptive metadata

- Within the workspace, keywords can be:
  - Added
  - Displayed
- Keywords are used to:
  - Organize and sort samples
  - Create groups
  - Generate batch reports

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	LD12	_PI+NS_B0	4_exp.fcs		4635	36	LD12	PI+NS	B	04 Neg	
	LD12	_PI+PI_D04	_exp.fcs		4523	99	LD12	PI+PI	D	04 Neg	
	LD14	_NS+NS_A	05_exp.fc	s	3641	17	LD14	NS+NS	A	05 Neg	
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#### Practice time



# The Graph Window

- Facilitates data visualization and gating
- Click on an X or Y axis label to choose and view different measured parameters



# Gating Tools

Subset events into populations based on marker expression

- Draw a Gate →
  Frequency of parent statistic
- Gates can always be modified or removed
- Double-click within a gate to focus on that population in a new graph window



## **Gating Hierarchies**

Display parent/child/sibling relationships between populations

- Viewed in the workspace samples pane
- Can be:
  - Collapsed or expanded
  - Drag-and-dropped to other gates, samples, groups, or areas of the program

Name	Statistic	#Cells
LD1_NS+NS_A01_exp.fcs		250342
LD1_NS+PI_C01_exp.fcs		229585
LD1_PI+NS_B01_exp.fcs		262774
🔷 🚽 🕲 Singlets	95.3	250347
Lymphocytes	89.6	224202
🔷 🚽 🕲 Live	97.6	218763
	82.9	181325
Q1: CD4- , CD8+	24.0	43469
$ \sum Median : Comp-Ax488-A (p-ERK1_2) $	407	
Median : Comp-PE-A (Perforin)	62.0	
IFNg+	44.1	19160
Perf+	32.7	14221
	95.3	41446
🔷 🕑 IFNg-	55.9	24309
🔷 💣 Perf-	67.3	29248
	4.65	2023
IFNg+Perf+pERK+	27.7	12056
IFNg+Perf+pERK-	1.47	637
IFNg+Perf-pERK+	14.6	6360
IFNg+Perf-pERK-	0.25	107
IFNg-Perf+pERK+	2.36	1028
IFNg-Perf+pERK-	1.15	500
IFNg-Perf-pERK+	50.6	22002
IFNg-Perf-pERK-	1.79	779
Q2: CD4+ , CD8+	0.56	1023
	72.9	132235
	422	
	26.2	
Q4: CD4- , CD8-	2.54	4598

### Graph Window Display Options

Customize your data visualization



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#### Practice time



### **Transforming Data**

#### Click the Transformation [T] button $\rightarrow$ Customize Axis...





## Transforming Data

Only affects visual display & scaling of data, not the raw values

- +/- Buttons adjust range
- Sliders adjust transform
  - Extra Neg Decades
  - Width Basis
  - Positive Decades
- Apply button → applies selected settings to chosen parameters



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## Transformation



- Gets rid of the "squishing" of cells
- Ensures the visual population center better correlates with the statistical center (median)
- Makes high resolution compensated digital cytometry data more appealing to the eye

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#### Practice time



## **Group Application of Gates**

#### Allows for master gating of multiple samples





- Select gray gates on a single sample and:
  - drag-and-drop gates from a single sample to a group, or
  - right click on gates and choose "Copy analysis to group"
- Group-applied gates turn the group color to denote that they are identical

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## **Group-Applied Gates**

- Can be
  - Modified on a single sample
  - Removed or deleted from the group gating tree (A)
  - Synchronized through the group properties menu (B)



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(A)

### **Additional Statistics**

To enumerate properties of gated populations

- Add a statistic to any gated population within a sample gating hierarchy
- Statistic Nodes can be group-applied just like a gate
- Example statistics:
  - Count
  - Median
  - Standard Deviation (SD)



#### Practice time



## The Layout Editor

A tool for creating graphical reports

- To create a layout:
  - Click on the Layout
    Editor icon in the
    workspace ribbon
  - Drag-and-drop populations from a sample gating hierarchy in the workspace to the Layout Editor window



## Working in the Layout Editor

• Similar to the Workspace, the Layout Editor has its own action taskbar with tabs and bands to organize actions



- Try clicking on the different tabs to see what types of actions are available
- Click the + button to create new layout reports

## Within the Layout Editor

- Graphs can be organized and reformatted
- Statistics, keywords, text and shapes or objects can be added to illustrate your analysis
- Right click on a graph plot for a list of options including Ancestry, Backgating and Properties/Formatting
- Right click on plot axis label → Parameter selection (also available w/in graph properties



## Formatting Graph Plots in the Layout Editor

- Right click on plot and select Properties → Graph Definition window
- 4 tabs with formatting options for:
  - Specify graph style
  - Annotation
  - Fonts
  - Legend



## Batch Analysis of Layout Graphics

Applies the layout across multiple samples in a group

- To batch a layout:
  - Specify a group
  - Choose iterate by option
    - Sample
    - Panel
    - Keyword
  - Set batch options
  - Click "Create Batch Report"



## **Export Image Options**

Are available under the File tab

Zoom

options

- Options include:
  - PNG
  - JPG
  - GIF
  - EMF
  - PDF
  - SVG
  - TIFF



### Iteration

#### Enables scrolling through samples in a group

- With iteration by set to Sample:
  - Click on the Value menu to select and view a specific sample in the specified group
  - Click the up/down arrows next to the value menu to scroll through the samples in a group one by one



# **Batch Options**

#### Enable reports to be exported directly out of FlowJo

- To save batch report to disc:
  - Specify a group
  - Choose iterate by option
  - Choose report type
    - Printer
    - Web Page
    - PPT
    - PDF

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- Choose destination (location to save)
- Click "Create Batch Report"



#### Practice time



### The Table Editor

A tool for creating statistical reports

- To create a Table:
  - Click on the Table
    Editor icon in the
    workspace ribbon
  - Drag-and drop
    Populations and
    Statistics to the Table
    Editor window
  - Click "Create Table"



## Within the Table Editor

• Again, the Table Editor has its own taskbar ribbon with tabs and bands to organize actions



- Click the + button to create new tables
- Enumerating and displaying table statistics is similar to batching layouts
  - Specify the group you wish to report, then click "Create Table"
  - Tables can also be sent to a layout report, or saved in various standard file formats (click the Display menu for options)

## Table Editor Visualize Tools

Add visual formatting to your displayed

- Options include:
  - Heat Map
  - Standard Deviation
  - Expected Range
- To add a visualization
  - Highlight rows(s)
  - Select a visualization option to apply
  - Click "Create Table"

	)	FlowJo Tables: My Analysis.wsp		
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11 E	Singlets/Lymphocytes/Live/CD	03+/Q1: CD4- , CD8+/Q1: HLA-DR- , CD38+	Freq. of Parent	
12 E	Singlets/Lymphocytes/Live/CD	3+/Q1: CD4- , CD8+/Q2: HLA-DR+ , CD38+ 🕌	Freq. of Parent	
13 E	Singlets/Lymphocytes/Live/CD	3+/Q1: CD4- , CD8+/Q3: HLA-DR+ , CD38-	Freq. of Parent	
14 E	Singlets/Lymphocytes/Live/CD	3+/Q1: CD4- , CD8+/Q4: HLA-DR- , CD38-	Freq. of Parent	

## **Table Editor Output**

- Visual formatting is maintained if table is created for Display, saved as HTML or sent to a layout
- Values in a displayed table can be copied and pasted outside of FlowJo

				Table - My	New Table			
	*PID	*STIM	Q1: CD4- , CD8+ p-ERK1_2 Median	pERK+ Freq. of Parent	IFNg+ Freq. of Parent	Perf+ Freq. of Parent	IFNg+Perf+pERK+ Freq. of Parent	CD4/CD8 Ratio
LD1_NS	LD1	NS+NS	68.2	4.62	0.89	30.2	0.13	▲ 3.77
LD1_NS	LD1	NS+PI	550	95.0	1.00	30.0	0.42	▲ 4.09
LD1_PI+	LD1	PI+NS	406	94.3	44.3	33.1	27.5	▲ 3.03
LD1_PI+	LD1	PI+PI	401	94.4	43.6	32.3	26.6	▲ 3.05
LD2_NS	LD2	NS+NS	75.5	0.33	1.34	55.5	0.033	2.79
LD2_NS	LD2	NS+PI	590	92.3	1.21	52.8	0.59	▲ 3.02
LD2_PI+	LD2	PI+NS	472	93.5	64.5	51.4	47.0	▲ 2.87
LD2_PI+	LD2	PI+PI	454	93.5	63.9	50.7	46.4	▲ 2.91
LD4_NS	LD4	NS+NS	77.8	7.58	0.78	20.9	0.044	1.51
LD4_NS	LD4	NS+PI	641	97.2	1.29	23.5	0.30	1.51
LD4_PI+	LD4	PI+NS	489	96.8	28.3	23.6	19.8	▼ 1.20
LD4_PI+	LD4	PI+PI	484	96.3	26.5	22.4	18.3	▼ 1.22
LD12_N	LD12	NS+NS	62.0	3.86	0.51	36.7	0.056	▲ 3.62
LD12_N	LD12	NS+PI	481	89.6	0.49	34.7	0.26	▲ 4.26
LD12_PI	LD12	PI+NS	390	84.6	45.1	39.7	21.1	1.93
LD12_PI	LD12	PI+PI	381	83.5	45.9	40.2	21.3	1.93
LD14_N	LD14	NS+NS	67.4	3.86	0.39	14.2	0.054	2.10
LD14_N	LD14	NS+PI	530	95.5	0.36	13.7	0.15	2.29
LD14_PI	LD14	PI+NS	396	94.6	17.6	18.1	12.7	1.65
LD14_PI	LD14	PI+PI	384	93.0	16.8	18.1	11.5	1.66
Mean			370	70.7	20.2	32.1	12.7	2.52
SD			191	39.7	23.1	13.1	15.4	0.95

 To save a table in text, CSV, Excel or SQL format, choose that option using the Display button next to "Create Table"
## Workspace Templates

Save all analysis without referencing data

- Save as options are located within the File tab
  - WSP (Normal Save)
  - ACS (Zip file, incl. data)
  - WSPT (Template)
- Templates streamline repetitive analysis of multiple runs using the same staining panel(s)

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							244977	LD1	PI+PI	D01

### Preferences

#### To specify your own personalized defaults

- Click the heart icon at the top left corner of a window to access Preferences
- Allows each user to modify default functionality and appearance



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License

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File

Formats

OK

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#### Thank You!

Questions?

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## Compensation in FlowJo v10



## **Previewing Compensation**



Double click on the square matrix badge next to a sample name

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#### **Initiating Compensation**



Place single color controls into the Compensation group and

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Bead Comps_Perforin PE_F08_exp.fcs		19212			1 22			- 1 🖌 🖌 👘		
Size	71.0	13645			1 1			- 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1		
PE-A+	55.2	7528		_	1.10	<u>M</u>		and the second		
Bead Comps_US Beads +FP_F05_exp.fcs	76.3	30000		_	4. day		t	4.83		
	/6.2	22871			5 10	on 2000 0	10' 10"	- 100K 200K		
Part Comps US Parts No ED E00 over fra	100.0	22866		-		PSC-A APC-H	7-A :: HLA-DR	FSC-A		
Size	80.3	8266								
- Read Comps 4 PE-TR E01 exp for	80.3	19202		r.		601	1			
- @ Size	66.1	12699				101				
V V Jac		12055					_			

## **Choose or Remove Parameters**



#### Click on a Sample column field $\rightarrow$ drop-down options, including:

- Single color control selection
- Remove Parameter
- Choose Parameters
- Reset All or single
   parameter

				Control C	roup: Compensation		
M Apply To Gr	oup •	Matrix Name: Compensation					View Matrix Fit
Spectral Al	I Detectors	Weights Optimize Weights Re	nove Univ Neg			Spillover Alg	orithm 💽 Traditional 🗌 AutoSpill/AutoSpread
onfirm gates and co	ntrol assignment	ts look correct. Double click a graph to edit it.					
Parameter		Sample	Comp Na	me		le Negative	Positive
AARD-A	Dead	Cell Comps_AARD_E01_exp.fcs	Comp-A	ARD-A		Cell Comps_US Cells_E03_exp.fcs:Size	Size/AARD-A+
APC-H7-A	HLA-DR	Bead Comps_DR APC-H7_F04_exp.fcs	Comp-A	PC-H7-A		Bead Comps_US Beads No FP_F09_exp.fcs:Size	Size/APC-H7-A+
Ax488-A	p-ERK1_2	Bead Comps_ERK A488_F06_exp.fcs	Comp-A	x488-A		Bead Comps_US Beads No FP_F09_exp.fcs:Size	Size/Ax488-A+
Ax647-A	Blank	Bead Comps_US Beads +FP_F05_exp.fcs	Comp-A	x647-A		Bead Comps_US Beads No FP_F09_exp.fcs:Size	Size/Ax647-A+
Ax700-A	CD3	Bead Comps DR APC-H7 F04 e	cp.fcs	700-A		Cell Comps_US Cells_E03_exp.fcs:Size	Size/Ax700-A+
PE-A	Perforin	Bead Comps_ERK A488_F06_ex	o.fcs	A		Bead Comps_US Beads No FP_F09_exp.fcs:Size	Size/PE-A+
PE-CV5-A	CD38	Bead Comps_IFN PE-Cy7_F07_e:	cp.fcs	Cv5-A		Bead Comps_US Beads No FP_F09_exp.fccSize	Size/PE-Cy5-A+
PE-CV7-A	IENa	Bead Comps_Periorin PE_F08_e	exp.fcs	Cy7-A		Bead Comps US Beads No FP F09 exp.fccSize	Size/PE-Cv7-A+
PE-TyPad-A	CD4	Bead Comps_US Beads No FP_FC	9_exp.fcs	TyPod_A		Bead Comps US Beads No FP. FDP. exp for Size	Size/PE-TaRed-A+
Pro-TARed-A	CD-4	Bead Comps_4 PE-TR_F01_exp. Read Comps_8 PR_E02_exp_fcs	cs	Dive A		Read Comp. 178 Reads No. 578 (200) are for Size	Similar to
PacBlue-A	CD8	Bead Comps_38 PE-Cy5 F03 ex	p.fcs	BIUE-A		INNE COMPS_US BERR NO PP_PU7_exp.ks:Size	oter/%BIR-A+
		Cell Comps_AARD_E01_exp.fcs			^		
AARD-A	Call Comm	Cell Comps_CD3 A700_E02_exp	.fcs		I Comme AARD FOL a	we for	
SSC-A	0 10	<pre>cReset Al&gt; cReset This Parameter&gt; 68.000000000000000000000000000000000000</pre>	Remove this	A O	ompensation. (4.1.4) Size (5.4.4) IOSK 2004		
		FSC-A AARD-	A :: Dead		FSC-A		
4	Bead Comps	LUS Beads No FP, F09_exp.fs	42.7 +4	B V-52S	ad Comps_DR APC-H7.	POL expfcs	
		APC-H7-	A :: HLA-	UK	PDC-A		

## Default is Traditional Compensation (M) Flow Jo<sup>™</sup> v10

# Options for utilizing Spectral unmixing math and AutoSpill robust linear regression can be selected in the header

				Traditional	Auto
				N	/
• • •			Control Group: Co	mpensation	
M Apply To G	roup 👻	Matrix Name: My New Compensation			View Matrix Finalize
Spectral A	II Detectors	Weights Optimize Weights R	emove Univ Neg	Spillover Alg	orithm Traditional AutoSpill/AutoSpread
Confirm gates and co	ontrol assignmen	ts look correct. Double click a graph to edit it.			
Parameter		Sample	Comp Name	ie Negative	Positive
😑 AARD-A	Dead	Cell Comps_AARD_E01_exp.fcs	Comp-AARD-A	Cell Comps_US Cells_E03_exp.fcs:Size	Size/AARD-A+
APC-H7-A	HLA-DR	Bead Comps_DR APC-H7_F04_exp.fcs	Comp-APC-H7-A	Bead Comps_US Beads No FP_F09_exp.fcs:Size	Size/APC-H7-A+
⊖ Ax488-A	p-ERK1_2	Bead Comps_ERK A488_F06_exp.fcs	Comp-Ax488-A	Bead Comps_US Beads No FP_F09_exp.fcs:Size	: Size/Ax488-A+
⊖ Ax700-A	CD3	Cell Comps_CD3 A700_E02_exp.fcs	Comp-Ax700-A	Cell Comps_US Cells_E03_exp.fcs:Size	Size/Ax700-A+
😑 PE-A	Perforin	Bead Comps_Perforin PE_F08_exp.fcs	Comp-PE-A	Bead Comps_US Beads No FP_F09_exp.fcs:Size	: Size/PE-A+
PE-Cy5-A	CD38	Bead Comps_38 PE-Cy5_F03_exp.fcs	Comp-PE-Cy5-A	Bead Comps_US Beads No FP_F09_exp.fcs:Size	: Size/PE-Cy5-A+
PE-Cy7-A	IFNg	Bead Comps_IFN PE-Cy7_F07_exp.fcs	Comp-PE-Cy7-A	Bead Comps_US Beads No FP_F09_exp.fcs:Size	: Size/PE-Cy7-A+
● PE-TxRed-A	CD4	Bead Comps_4 PE-TR_F01_exp.fcs	Comp-PE-TxRed-A	Bead Comps_US Beads No FP_F09_exp.fcs:Size	: Size/PE-TxRed-A+
PacRlue_A	CDS	Bead Comps 8 PB F02 exp.fcs	Comp-PacBlue-A	Bead Comps_US Beads No FP_F09_exp.fcs:Size	: Size/PacBluc-A+

## **Review and Adjust Gates**



#### Parameters used and auto-gating for populations

- Define positive and negative
- Choose from dropdowns lists or drag-and drop populations from workspace gating tree
- Double click graph plot preview to modify gates



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#### View Matrix



#### Once choices are finalized, click to view and apply



## **Apply Compensation**



#### Select matrix, then drag-and-drop



# Accessing the Spillover Spreading Matrix

# Select matrix, then click SSM to Display or Export

✓ Display SSM APC-H7-A Av488-A Show All Ax700-A PE-A ·· Perforin PE-CvS-A PE-Cv7-A ··· I PE-TyRed-A Pac Blue-A Sum Trad Comp 1.2429 Spectral Comp AARD-A Export SSM AutoSpill Comp ✓ APC-H7-A 2.6688 utoSpill Comp Autoflur 0.2506 ✓ Ax488-A ✓ Ax700-A V PE-A 0.7138 Selected ✓ PE-Cv5-A ✓ PE-Cy7-A 0.1194 1.4997 Matrix 2.8912 ✓ PE-TxRed-A 0.7466 0.6325 ✓ PacBlue-A √ Sum 0.7422 2.7304 0.3069 1.4889 1.4009 2.6527 5.6188 1.3659 0.844 17.1507 Preview Sample: Bead Comps DR APC-H7 F04 exp.fcs -Preview Population -View-Overlay Uncompensated Comp-APC-H7-A Comp-PE-TxRed-A Comp-Ax488-A Comp-Ax700-A Comp-PE-A Comp-PE-Cv5-A Comp-PE-Cy7-A Workspace **#** Mati <u>.</u> **\*** ٢. C.A. ÐÐ << 

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## Using a Spillover Spreading Matrix

Large numbers with red shading  $\rightarrow$  primary detector's fluorochrome (columns) will spread in secondary detector's channel (Rows)

 $\rightarrow$  Loss of sensitivity for low expressing populations in that secondary channel *if* that primary fluor antigen is expressed on the same population.

Show All	B515-A :: G	B610-A :: IL I	8660-A :: IL	B710-A :: IF	B780-A :: C	G575-A :: C	G610-A :: Pe	G660-A :: T	G710-A :: Fo	G780-A :: IL	. R670-A :: Ⅲ	R730-A :: C	R780-A :: C	U390-A :: C	U450-A :: Li	U500-A :: C	U\$70-A :: C	U660-A :: C	U740-A :: C	U785-A :: C.		V510-A :: C	V570-A :: C	V605-A :: IL	V655-A :: Ki	V710-A :: C	V750-A :: T	V785-A :: C	Sum
📝 B515-A	0	0.3386	0.3656	0.2974	0.1239	0.621	0.4031	0.0041	0.2543	0	0.1968	0.0021	0	0.1381	0.0467	0.336	0.3264	0.1082	0.0023	0	0	0.3933	0.2711	0.2993	0.1361	0	0	0	4.6644
🖌 8610-A	0.1127	0	2.6497	1.7932	0.8328	1.1919	11110	0.92	1.0927	0.7799	2.3488	0.5584	0.0615	0.2085	0.0195	0	0.5252	1.2206	0.3809	0.2716	0.2722	0	0.3333	9.9098	1.7473	0.8158	0.9363	1.0448	44.1706
✓ B660-A	0.1364	0.5315	0	2.3386	1.3725	0.5631	0.4942	3.2433	2.3834	1.1011	- Haller	2.0263	0.2674	0.3172	0	0	0.1873	2.0194	0.5647	0.4282	0.6809	0.0722	0.5765	0.7269	8.6741	1.31	1.5452	1.921	47.7461
✓ B710-A	0.1354	0.5441	1.0453	(	2.2489	1.9492	0.5709	0.2963	4.6855	1.6941	2.118	3.2236	0.4014	0.4654	0.0065	0.0511	0.1883	0.4156	1.0155	0.6654	0.7323	0.0938	0.5453	0.3853	1.3669	5.7264	2.737	3.1052	36.4127
📝 B780-A	0.2341	0.6986	0.5431	0.7403	0	0.8329	0.6307	0.1459	0.3853	8,5401	0.2949	0.3526	0.4928	1.566	0.0459	0	0.2651	0.1327	0.4942	2.1341	0.9449	0.0057	0.6124	0.4714	0.36	0.377	2.4492	15.1787	39.9286
📝 G575-A	0.0424	1.9982	0.8639	0.6754	0.3003	0	2.9765	0.3844	0.5238	0.342	0.457	0.1293	0.0367	0.2014	0	0	2.7368	0.1877	0.1293	0.0986	0.1759	0	1.6275	1.5294	0.5182	0.2372	0.2317	0.2867	16.6903
✓ G610-A	0	0.347	1.0752	1.0007	0.48	0.5437	0	0.6176	1.2095	0.8448	0.7095	0.3916	0.0942	0.1008	0	0	0.1334	0.3651	0.2312	0.1763	0	0	0.0052	0.8931	0.6319	0.3944	0.4322	0.5483	11.2257
✓ G660-A	0.238	1.0988	21.6117	7.4463	2.391	2.2169	1.0328	0	3.8736	3.0857	14.401	1.3753	0.2239	0.3759	0.0053	0	0.2182	1.9735	0.7133	0.5421	0.84	0.0058	0.6127	1.0494	9,8023	2.1461	1.3706	2.0264	81.0784
📝 G710-A	0.2323	0.6409	3.4267	29.6799	2.2866	4.034	1.0822	0.3501	0	3.1982	1.146	3,7772	0.4419	0.4325	0.0281	0	0.4711	0.2663	1.2556	0.6027	1.0011	0	0.7166	0.3659	0.7419	8,1753	2.4534	2.1129	68.9194
🗹 G780-A	0.1681	0.5254	0.3026	0.371	41,2453	0.9366	0.5497	0.1097	0.3004	C	0.2124	0.1826	0.2638	0.679	0	0	0.1168	0	0.2428	1.0268	0.4652	0	0.2974	0.1936	0.0943	0.0834	0.8785	8.4637	28.7071
✓ R670-A	0	0.0715	1.0139	0.7532	0.4003	0.2028	0	0.7894	0.8355	0.7507	C	1.6043	0.3281	0	0	0	0	0.1893	0.1496	0.1257	0	0	0.0994	0	0.3975	0.2077	0.1932	0.3082	8.4203
✓ R730-A	0	0.1368	0.2564	1.4657	0.6963	0.5515	0.1605	0	1.8614	1.1939	0.1382	(	0.4558	0.2842	0.0843	0	0.128	0	0.7259	0.5524	0	0	0.1326	0	0.136	0.883	1.1473	1.3907	12.3809
📝 R780-A	0	0.2077	0	0.4514	3.0128	0.4101	0.2436	0	0.4743	6.552	0.21	0.7009	0	1.1959	0.1289	0	0	0	0.3985	1.9065	0.2052	0	0.2013	0.3174	0.2938	0	1.3902	5.4008	23.7013
📝 U390-A	0	0.1216	0	0	0	0.4568	0.1426	0	0	0	0	0	0	0	0.4907	0.2984	0.2899	0	0.002	0.1043	0.2781	0	0.1676	0.1306	0	0	0.1305	0	2.6131
✓ U450-A	0.1743	0.2722	0	0.1743	0	1.9562	0.3811	0.1386	0	0	0	0	0	3.0982	0	1.9053	1.113	0.1791	0.105	0.1115	4.3162	0.793	0.3126	0.2351	0	0.1194	0	0.1569	15.542
V U500-A	0.5774	0.422	0	0.2431	0	3.1989	0.7145	0	0.2549	0.0119	0.3459	0	0	1.3282	0.6859	0	1.6785	0.3189	0.2847	0.2392	0.3414	2.0067	0.6367	0.618	0.2763	0	0.2098	0.0146	14.4075
🗹 U570-A	0.0665	3.7848	1.3291	0.6307	0.2852	28.6861	5.8792	0.7111	0.6288	0.4377	0.7776	0.2031	0	0.5972	0.1268	0.0711	0	0.4764	0.3182	0.2725	0.5088	0	2.7078	2.4427	0.5675	0.1576	0.1342	0.1839	51.9846
✓ U660-A	0	0.1707	2.9006	1.2139	0.6039	0.4625	0.306	2.3327	1.4807	1.0328	8.2951	1.8182	0.325	0.9696	0.134	0.113	0.1055	0	1.6182	1.3161	0.0777	0.0063	0.1344	0.2498	2.5206	0.6624	0.7575	0.889	30.4962
✓ U740-A	0	0.2222	0.4975	2.8962	2.4377	0.552	0.2029	0	1.2127	1.4977	0	5.3914	0.6845	1.7906	0.169	0.0985	0.1624	0.1376	0	2.9492	0.1723	0	0.1178	0.0048	0.1208	0.8175	2.2198	2.6786	27.0337
V U785-A	0	0.1545	0	0.3911	0.757	0.3086	0	0	0.2901	1.1045	0.2235	0.2231	0.7381	3.1007	0.3551	0.2563	0.2959	0	0.4701		0 0	0	0.3055	0.2381	0	0	0.6004	2.5838	12.3964
🗹 V450-A	0.0446	0.0598	0	0	0	0.4282	0.1316	0	0.0538	0	0.0028	0	0	1.0718	1.0495	0.3322	0.209	0.0465	0.034	0	0	0.6973	0.3662	0.2574	0.1036	0.0513	0.0629	0.1	5.1025
V510-A	0.7941	0.2297	0	0.1547	0.0793	1.6991	0.7555	0.087	0.1627	0	0.203	0.1256	0	1.7821	1.0184	2.1514	0.7977	0.1951	0.1277	0.0752	0.9707	0	1.4708	1.2915	0.5662	0.2906	0.3788	0.3997	15.8066
V570-A	0	1.0696	0.7577	0.6661	0.3129	7.6936	2.7641	0.3708	0.6984	0.5536	0.6443	0.3454	0	0.3164	0.1472	0	3.3054	0.5143	0.3617	0.2696	0.7871	0.0108	0	2.4184	1.5168	0.7472	0.985	1.1078	28.3642
V605-A	0	0.6501	0.9981	0.8946	0.4701	2.035	4.712	0.6073	0.9499	0.7616	2.0555	0.7046	0.0731	0.3739	0.0795	0.0627	0.9419	1.4062	0.5725	0.4948	0.4507	0.0812	0.4823	0	2.3078	1.2075	1.5683	1.7195	26.6607
V655-A	0	0.0852	0.9766	0.7422	0.3716	0.2956	0.6482	0.7012	0.7338	0.611	2.5225	1.205	0.1616	0.4517	0.0979	0	0.1138	2.5522	0.8206	0.6425	0.5817	0.1265	0.1449	0.7955	0	1.9323	2.2523	2.2755	21.8419
V710-A	0	0.1485	0.5393	3.0619	1.0766	0.5284	0.1851	0.0896	1.6216	0.9927	0.2695	4.7297	0.4512	1.2071	0.1098	0	0.0846	0.2322	2.6149	1.7435	0.7764	0.1344	0.201	0.1698	0.4375	0	5.6643	6.6018	33.6714
V750-A	0	0.1209	0	0.9053	1.4112	0.192	0	0.0029	0.4393	1.1097	0.0047	1.5818	0.35	1.419	0.0604	0	0.0911	0	2.0175	2.0226	0.4913	0.1015	0.1343	0.1044	0.1377	0.9992	0	8,4272	22.124
V785-A	0	0.0542	0	0.2421	0.8591	0	0.1295	0	0.2055	1.4545	0.0037	0.428	0.3196	1.9129	0.062	0	0.1037	0.0614	0.6316	2.6335	0.5631	0.0813	0.1321	0.1187	0.1355	0.3181	3.0446	0	13.4947
Sum	2.9563	14.7051	41.153	59.2293	35.0533	62.5467	39.2397	11.902	26.6119	37.6502	52.2474	31.0801	6.1706	25.3843	4.9514	5.676	14.589	12.9983	16.2825	21.4049	15.6332	4.6098	13.3453	25.2163	33.5904	27.6594	33.7732	69.9257	745.5853

## **Editing Compensation**



#### Click Edit $\rightarrow$ Copy where you can change values



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Comp-APC-H7-A :: HLA-DR

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## Saving Compensation



#### **Click Save Matrix**

- CSV → use outside of FlowJo
- FlowJo Matrix (.mtx) → import back into FlowJo workspace

Add, duplicate or delete a matrix



# Plugin setup



**BD RESTRICTED** 

## Plugins

Apps that run some external algorithm or function

- Can be used to:
  - Cleanup samples
  - Embed data in fewer dimensions (dimensionality reduction)
  - Classify events into populations based on similarity (clustering)
  - Visualize population relationships (comparisons)
- Must be downloaded and installed prior to use

## The Plugin Menu

Is located in the Workspace Tab  $\rightarrow$  Populations Band

- Once plugins are downloaded and installed, they will be:
  - listed in the drop-down Plugins menu
  - used like an action button to initiate a plugin process

			My Flow Anal	ysis.wsp								
- 🖍 🥆 r 🗐 👭 🕀 🛄 上 💿 😳 🔵												
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Image: Create Group         Copy analysis         Image: Create Group selected to group         Copy value to group	Plugins	Add Statistic	Median	cv cv	Add Keyword	<ul> <li>Create group from keyword value</li> <li>Create keyword value series</li> <li>Copy value to group</li> <li>Kewordf</li> </ul>						
Group		ropulation	FlowJo Exchan	ge					Reywords			
{□} All Samples {m} Compensation ▼{□} Group Gates ▼ \$0 Live	Add Workspac Remove Works	e Plugin ;pace Plugins					Test Compensation None					
	<ul> <li>◆ ③ Cells</li> <li>◆ ③ Lympho</li> </ul>					Astrolabelmport AutoAdjustCates						
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Name PBMC_111615_PMA lono_CD107a Ax700_F PBMC_111615_unstimulated_84_B04_012.fc concat_1_Cells.fcs Phenograph_LPUI TriMap_Y_A168 TriMap_Y_A168 TSNE_of_concat_1_Cells.fcs_1 StSNE_of_concat_1_Cells.fcs_2 UMAP_1_ISIR UMAP_1_SIR		CBA Calibration ClusterExplore DownSampleV EmbedSOM Euclid ExternalPopula FItSNE	r 3 ationAlgorithmIni	terface				*Cells 200000 200000 300000				
			FlowAl									

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#### **Download Plugins**

From the FlowJo Exchange

• 1<sup>st</sup> plugin menu item is a link to the Exchange

• • •	My Flow Analysis.wsp		11 A Martin Contraction of the
n n c   41 (+) 🗈 📰 L 💿 😳 )			ELOW IO Exchange
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Gloup to group samples to group V	Statistic V	(x) Copy value to group	TriMap <
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Phenograph_LPUI	DownSampleV3		•
TriMap_X_A168	EmbedSOM		v0.2 published February 5th, 2020
SNE_of_concat_1_Cells.fcs_1	Fuelid		Dimensionality reduction, analogous to
tSNE_of_concat_1_Cells.fcs_2	Euclid		Dimensionality reduction, analogous to
○ <u>%</u> UMAP_1_1S1R	ExternalPopulationAlgorithmInterface		tSNE or UMAP. This algorithm is used as
UMAP_2_151R	FItSNE		visualization for high parameter datasets.
	FlowAl		

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## The FlowJo Exchange

Lists all available plugins with download links

 Click on a link to download the plugin and associated documentation



## **Installing Plugins**

Requires a few initial steps

For each new Plugin you want to use:

- Step 1 Download the Plugin package and unzip
- Step 2 Place Plugin.jar in your Plugins folder

If this is your first time installing a Plugin

• Step 3 – Setup/Configure Preferences

If the plugin requires R

- Step 4 Install R (if not already installed)
- Step 5 Run the Plugin package installation script within R

### Steps 1 & 2

Apply to all plugins

#### 1. Download plugin package and unzip



- 2. Put the Plugin.jar file in your plugins folder
- Restart FlowJo → the plugin name will display in your Plugins action menu

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# Step 3 – Configure Diagnostics Preferences

Tells FlowJo the location of your plugins and R installation

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Reset

Cancel

- Choose the location your plugins folder and R installation
- •• Restart FlowJo  $\rightarrow$  applies the change

### Step 4 – Install R

#### Required for certain plugins to run

- Navigate to <a href="https://cran.r-project.org/">https://cran.r-project.org/</a>
- Download and Install the R base package for your operating system

Download and Install R

Precompiled binary distributions of the base system and contributed packages, **Windows and Mac** users most likely want one of these versions of R:

- Download R for Linux
- Download R for (Mac) OS X
- Download R for Windows
- In addition to the base R package, also download and install:
  - X-Quartz If using a Mac. Link: <u>https://www.xquartz.org/</u>
  - R-Tools If using a PC

base	Binaries for base distribution. This is what you want to install R for the first time.
contrib	Binaries of contributed CRAN packages (for $R \ge 2.13.x$ ; managed by Uwe Ligges). There is also information on <u>third party</u> <u>software</u> available for CRAN Windows services and corresponding environment and make variables.
old contrib	Binaries of contributed CRAN packages for outdated versions of R (for R < 2.13.x; managed by Uwe Ligges).
Rtools	Tools to build R and R packages. This is what you want to build your own packages on Windows, or to build R itself.

## Step 5 – Installing R Packages

- For each Plugin that utilizes the R environment, the algorithm package installation script must first be run within R.
- Each Plugin downloaded from the FlowJo Exchange site will come with a "How To" document, which specifies the packaging scripts for any R dependencies.

Q Search	
ce.zip i.jar OM ie2.0.txt	

# Step 5 – Installing R packages

• Open R and copy/paste, or type the script into the R console



- You will notice the package components being downloaded
- If prompted to update a previously installed package, select all (a), unless the component requires compiling, then select no (n)



The downloaded binary packages are in /var/folders/yy/n5t270t57w53lkjkb2ylx0r80000gn/T// Rtmpl0KsZv/downloaded\_packages Old packages: 'broom', 'callr', 'car', 'caTools', 'cowplot', 'dbplyr', 'diffusionMap', 'digest', 'e1071', 'evaluate', 'FNN', 'foreign', 'fpc', 'git2r', 'glue', 'httpuv', 'igraph', 'ipred', 'iterators', 'kernlab', 'ks', 'later', 'lava'. 'lme4'. 'maptools'. 'matrixStats'. 'metap'. 'ModelMetrics', 'modeltools', 'openssl', 'pillar', 'pkgconfig', 'plotly', 'pls', 'processx', 'R.utils', 'RANN', 'Rcpp', 'RcppArmadillo', 'RCurl', 'reticulate', 'rlang', 'robustbase', 'scales', 'Seurat', 'stringi', 'survival', 'tinytex', 'trimcluster', 'VGAM', 'XML', 'xtable', 'xts', 'yaml', 'zoo' Update all/some/none? [a/s/n]: a

also installing the dependencies 'gbRd', 'Rdpack', 'fansi', 'ps'

There are binary versions available but the source versions are later:

	binary	source	needs_compilation
broom	0.4.5	0.5.0	FALSE
plotly	4.7.1	4.8.0	FALSE
robustbase	0.93-1.1	0.93-2	TRUE

Do you want to install from sources the package which needs compilation?  $y/n\colon n$ 

#### **Available Plugins**

#### Can be classified into several groups

Pre-processing	Dimensionality Reduction	Clustering	Interpretation +Dig Deeper
Downsample	tSNE	X-Shift	ClusterExplorer
CytoNorm	UMAP	FlowSOM	HyperFinder
FlowAl	TriMAP	Phenograph	Euclid
FlowClean	EmbedSOM	flowMeans	
IndexSort		Visualization	Utility
IndexSort		Visualization	Utility
IndexSort <b>Bold-Italic →</b>	Requires R	Visualization ViolinBox	Utility CBA
IndexSort Bold-Italic →	Requires R	Visualization ViolinBox MiST	Utility CBA StainIndex
IndexSort Bold-Italic → E FLOWJO Ex	<b>Requires R</b> change	Visualization ViolinBox MiST Sunburst	Utility CBA StainIndex <i>iCellR</i>

# Why Pugins?

- Plugins Enable:
  - Quick access to algorithmic processes
  - Point and click interface for non-programmers
- Allowing for:
  - Data clean up
  - Population discovery
  - Exploration
  - Visualization
  - Computational Sorting

# **Employing Plugins**

#### As part of a discovery workflow



# **Employing Plugins**

Explore



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## **Additional Resources**

To empower your discovery

• Download Plugins:

https://www.flowjo.com/exchange/

Searchable Documentation

https://docs.flowjo.com/flowjo

• FlowJo University

https://www.flowjo.com/learn/flowjo-university/flowjo

• Technical Support

flowjo@bd.com

# Dimensionality Reduction and Clustering



## Analysis Workflow





## Analysis Workflow

FlowJo<sup>™</sup> v10





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#### **Available Plugins**

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IndexSort <b>Bold-Italic →</b>	Requires R	Visualization ViolinBox	Utility CBA
IndexSort Bold-Italic →	Requires R	Visualization ViolinBox MiST	Utility CBA StainIndex
IndexSort Bold-Italic → E FLOWJO Ex	<b>Requires R</b> change	Visualization ViolinBox MiST Sunburst	Utility CBA StainIndex <i>iCellR</i>

## **Dimensionality Reduction**

#### creates new derived parameters



• Goal: low-dimensional representation of a high-dimensional dataset that preserves the overall structure of the data as much as possible

•



Events with a similar multidimensional expression pattern group together within the dimensionally reduced data space

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# **Dimensionality Reduction Options**

- t-SNE = t-Distributed Stochastic Neighbor Embedding
- UMAP = Uniform Manifold Approximation and Projection
- TriMAP = Triplet Manifold Approximation and Projection
- EmbedSOM = 2D visualization of FlowSOM clustering output



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# **DR Method Comparison**

- Gate on CD8+ Phenograph Clusters  $\rightarrow$  7 populations
- Populations are • fragmented with t-SNE but group together in UMAP or TriMap







## t-SNE



#### the GUI details

tSNE\_of\_concat\_1\_Cells.fcs

Run Name: tSNE\_of\_concat\_1\_Cells.fcs

Population: concat\_1\_Cells.fcs

Comp-B515-A :: GzmB FITC
Comp-B610-A :: IL-8 BB630
Comp-B660-A :: IL-13 BB660
Comp-B710-A :: IFNg BB700
Comp-B780-A :: CD137 BB790
Comp-G575-A :: CD28 PE
Comp-G610-A :: Perforin Ax594
Comp-G660-A :: TCR GD PE-Cy5
Comp-G710-A :: FoxP3 PE-Cy55
Comp-G780-A :: IL-22 PE-Cy7
Comp-R670-A :: IL-21 Ax647
Comp-R730-A :: CD107a Ax700 or APC-R700
Comp-R780-A :: CD3 APC-H7
Comp-U390-A :: CCR7 BUV395
Comp-U450-A :: Live Dead UV Blue
Comp-U500-A :: CD4 BUV496
Comp-U570-A :: CD25 BUV563
Comp-U660-A :: CD39 BUV661
Comp-U740-A :: CD95 BUV737
Comp-U785-A :: CD8 BUV805

Select All Uncompensated Learning Configuration: O Auto (opt-SNE) Manual opt-SNE will end early exaggeration when Kullback-Leibler Divergence (KLD) drops off and it will stop iterating when KLD rate of change slows to <0.2%. The learning rate below has also been suggested based on the opt-SNE approach. (See Belkina, et al. https://doi.org/10.1038/s41467-019-13055-y.) perplexity: learning rate (eta): 21000 iterations: 1000 30 KNN algorithm: Exact (vantage point tree) gradient algorithm: Barnes-Hut ? Close Run **Select Parameters** – Only highlighted parameters will be considered

Learning Configuration – Defaults to Opt-SNE

**Iterations** – Maximum number of iterations



**Perplexity** – Related to the number of nearest neighbors that is used in learning algorithms. May be viewed as a knob that sets the number of effective nearest neighbors. & Learning Rate

**Learning Rate (eta)** – How fast you get to a solution. Small  $\rightarrow$  gradual changes per iteration, slower. Large  $\rightarrow$  larger changes, faster.

**KNN** – Exact (vantage point tree) vs Annoy (Random Forest Projection)

Gradient Algorithm – Barns-Hut vs Fit-SNE

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UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction, ArXiv e-prints 1802.03426, 2018 Copyright (c) 2017, Leland McInnes All rights reserved.

#### arxiv.org/pdf/1802.03426.pdf



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A simplicial complex

#### How does it work?

Neighbor graph style approach that uses simplices to build a manifold approximation of the data structure, making an effort to preserve the topological global structure of that data.

#### How well does it work?

- Equally meaningful representations compared with t-SNE
- Better representation of multi-branched continuous trajectories (ex. hematopoietic development, lineage relationships)
- Faster than Barnes-Hut tSNE
  Scales better with high #s of parameters
- > Improved Global Structure representation
- Can produce multiple components





# **TriMAP**





#### • How does it work?

- > Semi-supervised metric learning
- Initialized with low dimensional PCA embedding, and this embedding is then modified using a set of selected triplets from the high-dimensional representation

#### How well does it work?

- Meaningful representations
- Faster than tSNE
- Scales well (comparable to UMAP)
- Preserves Global Structure





# 3<sup>rd</sup> Parameter Heat Map

#### orient and identify



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# **Query Gate**



### and explore



cleanup



concatenate

dimensionallyreduce

cluster

#### gate

comparisons

dig deeper

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Stim1 (No Stim) 50.0



# Clustering



### creates populations



cleanup



concatenate



dimensionallyreduce

cluster





dig deeper



tSNE X

Overlay cluster populations in the LE

٠

Right click and choose Make Multigraph Overlays → Histograms



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# **Clustering Options**

- X-Shift Fast KNN Density Estimation
- PhenoGraph Nearest Neighbors Graph  $\rightarrow$  Communities
- FlowSOM Self Organizing Map





# **FlowSOM**



 Analyzes using a self-organizing map and K nearest neighbors to generate cluster populations

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X-Shift is a clustering algorithm that uses k-nearest neighbor density estimate to find cell populations in multiparametric single-cell data. First, it constructs a nearest-neighbor graph that captures the phenotypic similarity of high-dimensional data points. Then, it uses distances to K nearest neighbors to compute local density estimate for each data point. Finally, it searches for local density maxima in the KNN graph and applies density gradient ascent to create distinct subpopulations.

Citation: Samusik N et al, Nat Methods. 2016 Jun;13(6):493-6. doi: 10.1038/nmeth.3863

X-Shift v1.3 clustering parameters

Select parameters for clustering:		
Comp-U500-A :: CD4 BUV496		
Comp-U570-A :: CD25 BUV563		
Comp-U660-A :: CD39 BUV661		
Comp-U740-A :: CD95 BUV737		
Comp-U785-A :: CD8 BUV805		
Comp-V450-A :: IL-2 BV421		
Comp-V510-A :: CD154 BV480		
Comp-V570-A :: CD45RO BV570		
Comp-V605-A :: IL-17A BV605		
Comp-V655-A :: Ki67 BV650		
Comp-V710-A :: CD69 BV711		
Comp-V750-A ·· TNE2 PV750		
Num. nearest neighbors (K):	32	
Distance metric:	Angular	0
Subsampling limit:		100000
Run ID:	auto	
	Cancel	OK

K-nearest neighbor density estimation



## PhenoGraph



PhenoGraph v2.4

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

#### Please select your input parameters:

	Comp-U570-	-A :: CD25 BUV5	63					
	Comp-U660-	-A :: CD39 BUV6	61					
	Comp-U740-	-A :: CD95 BUV7	37					
	Comp-U785	-A :: CD8 BUV80	5					
	Comp-V450-	-A :: IL-2 BV421						
	Comp-V510-A :: CD154 BV480							
	Comp-V570-A :: CD45R0 BV570							
	Comp-V605	-A :: IL-17A BV6	05					
	Comp-V655-	-A :: Ki67 BV650						
	Comp-V710-	-A :: CD69 BV71	1					
	Comp-V750-	-A :: TNFa BV75						
	Comp_1/785	A CD27 PV/78	G					
	К	30						
	Run ID	auto						
Save the R script and output messages								
To learn more, check out the algorithm author's citation: www.cell.com/cell/fulltext/S0092-8674(15)00637-6								
				Cancel	OK			

• Constructs a nearest neighbor graph and partitions the graph into communities





# ClusterExplorer

### gating and comparison of cluster populations





# Now What?

- You've found one or more interesting populations by high parameter clustering
- You would like to isolate this population for additional study, but don't have a hierarchical gating tree...

Question becomes: How do you sort a population identified with a clustering algorithm?





# HyperFinder

### generate an optimized gating strategy for any population



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# **Computational Sorting Workflow**





# Thank You!

Questions?

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# Thank You!

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