Introduction to FlowJo v10

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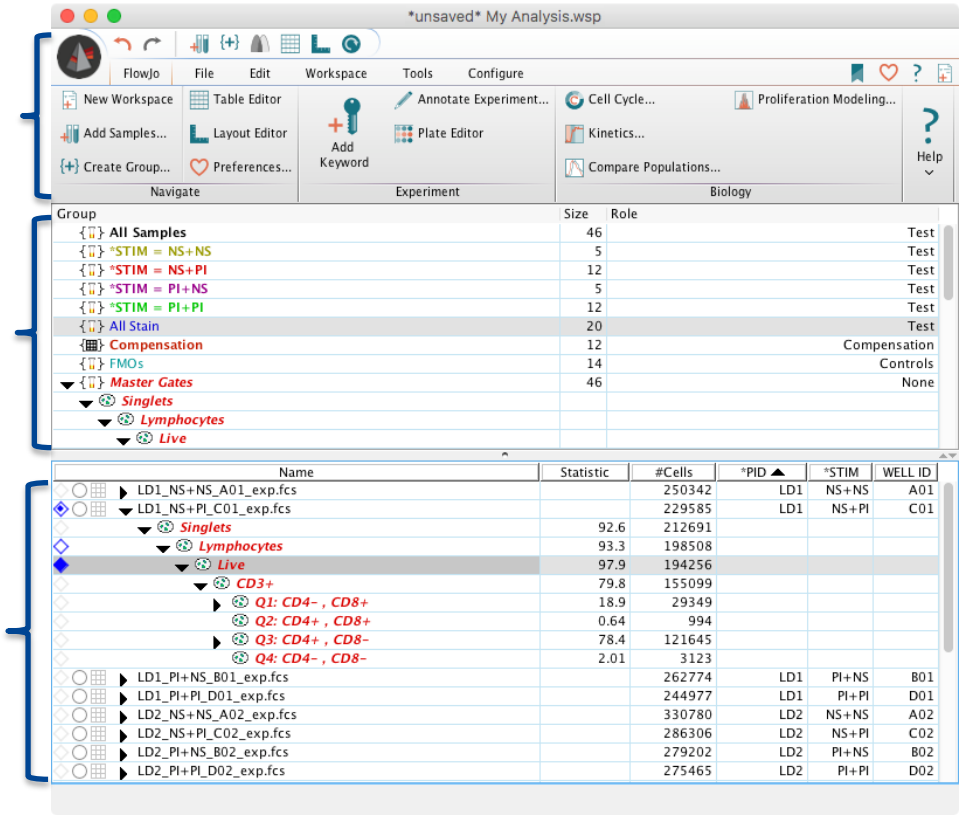
The FlowJo v10 Workspace

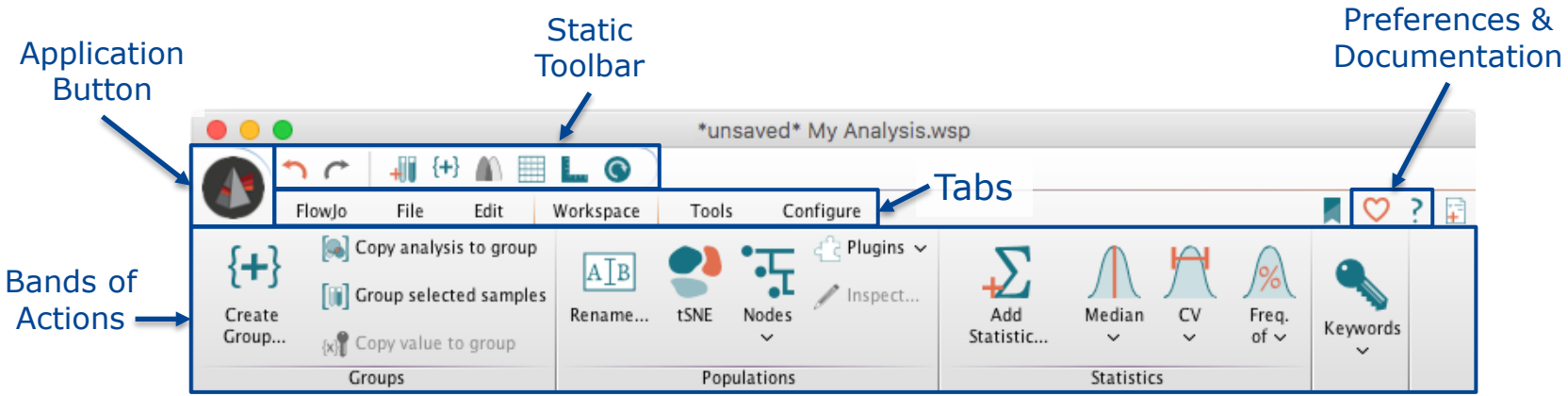
An interface to organize your data and initiate actions

- Action Toolbar
- Groups and Group Analysis
- Samples and Sample Analysis



The Action Toolbar

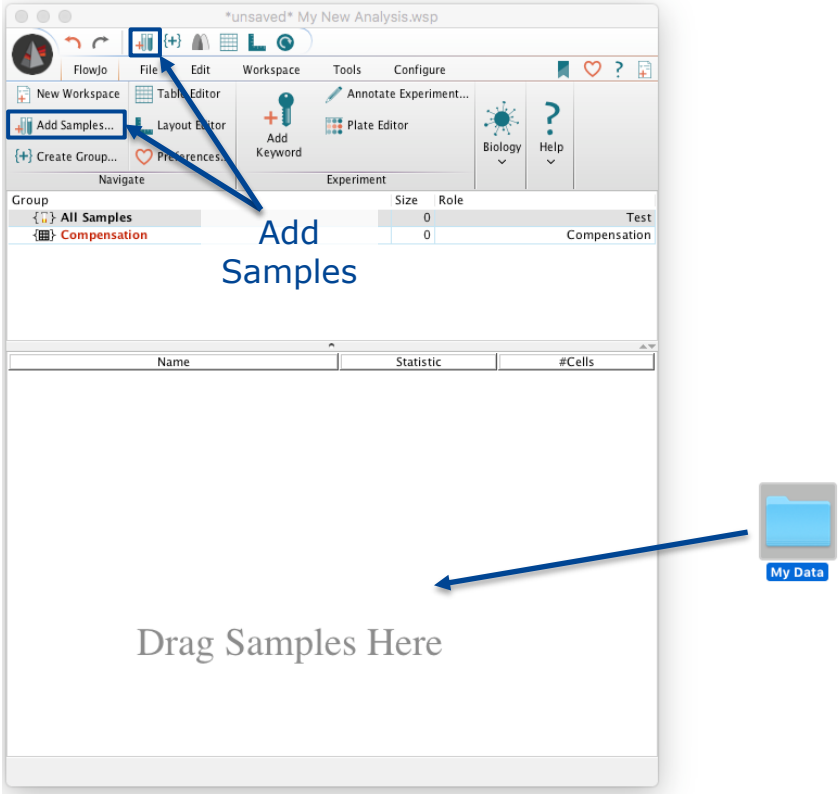
Allows visual navigation of workspace functions

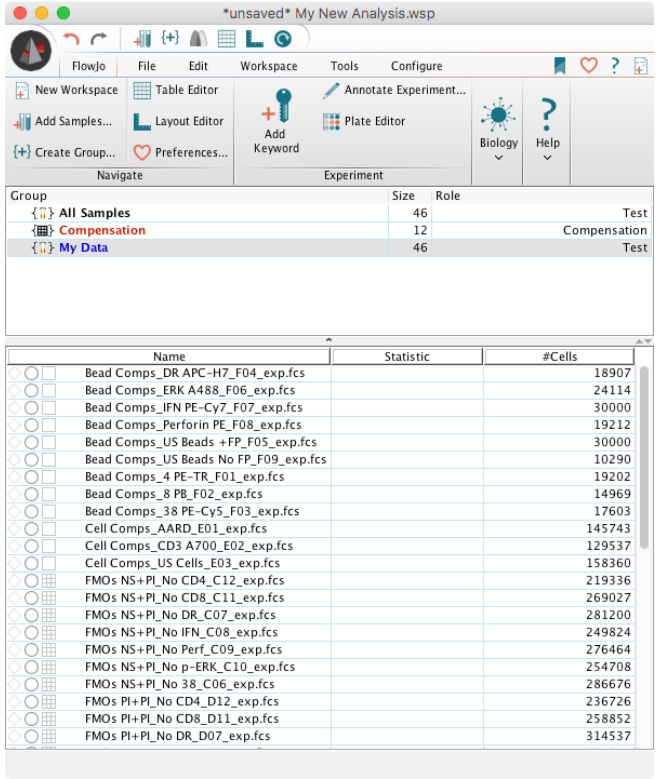


- Tabs group similar Bands together
- Bands group similar Actions together
- Mouse-over an action button → tooltip + hotkey

Importing Data

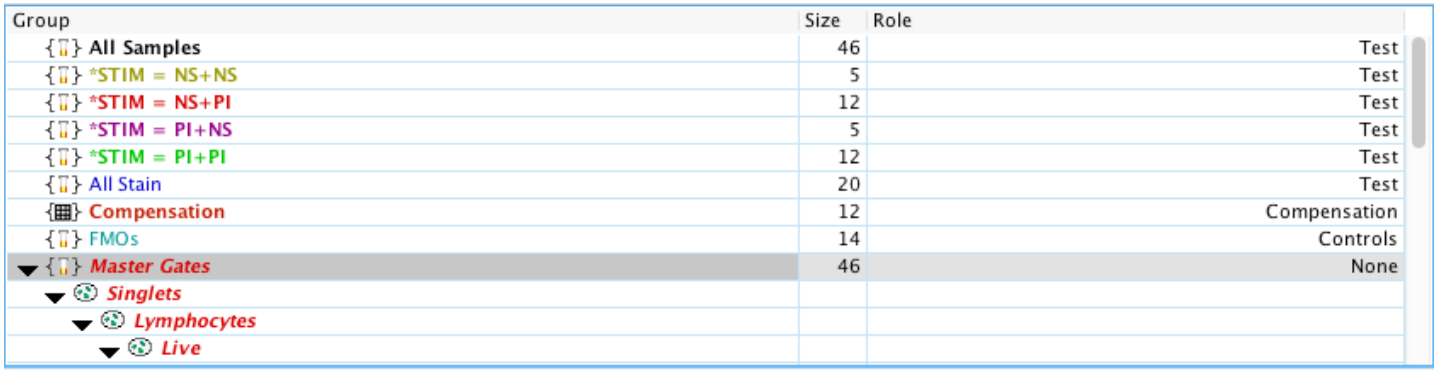
Drag-and-drop files into Samples Pane, or click Add Samples





Group Pane

Groups act as folders to organize samples and initiate actions

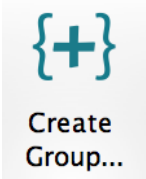


Group	Size	Role
All Samples	46	Test
*STIM = NS+NS	5	Test
*STIM = NS+PI	12	Test
*STIM = PI+NS	5	Test
*STIM = PI+PI	12	Test
All Stain	20	Test
Compensation	12	Compensation
FMOs	14	Controls
Master Gates	46	None
Singlets		
Lymphocytes		
Live		

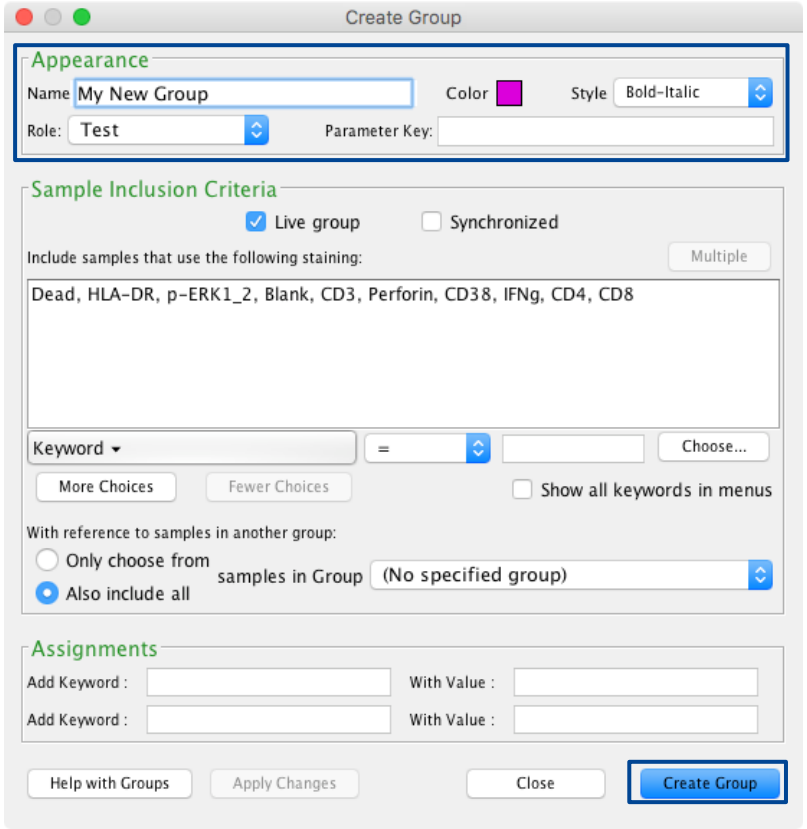
- The Group area lists all groups in the workspace, # of samples in each Group (Size) and the Role of that group
- Groups allow for master gating of multiple samples → Group-applied analysis gains the group color
- Groups can be used for batch reporting multiple samples

Creating and Editing Groups

Click the Create Group icon



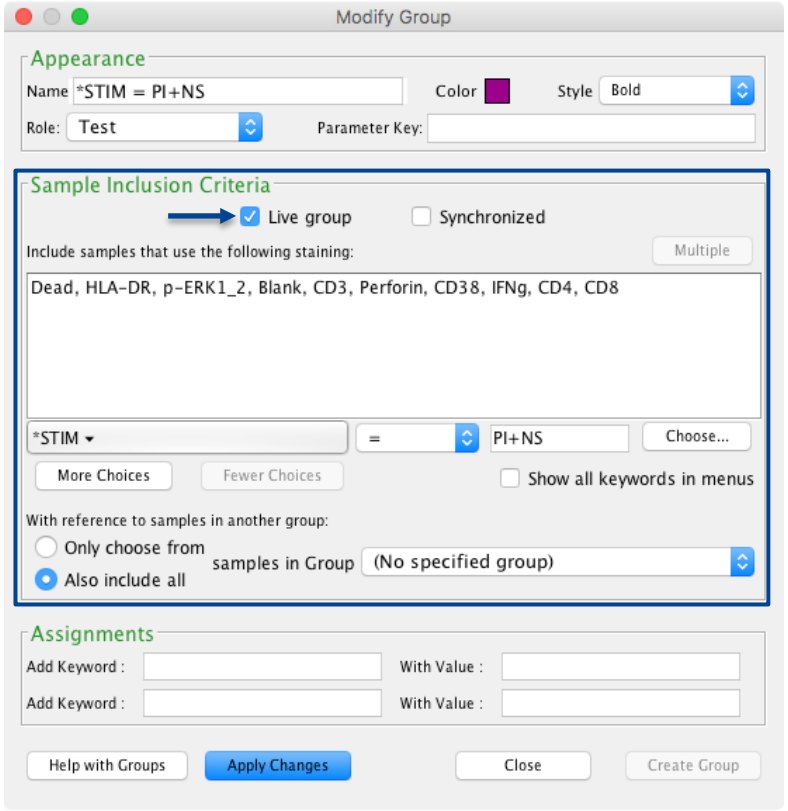
- Type a Name
- Set options
- Click Create Group
- Drag-and-drop adds samples to the group
- Double click on an existing group to edit its properties



Sample Inclusion Criteria

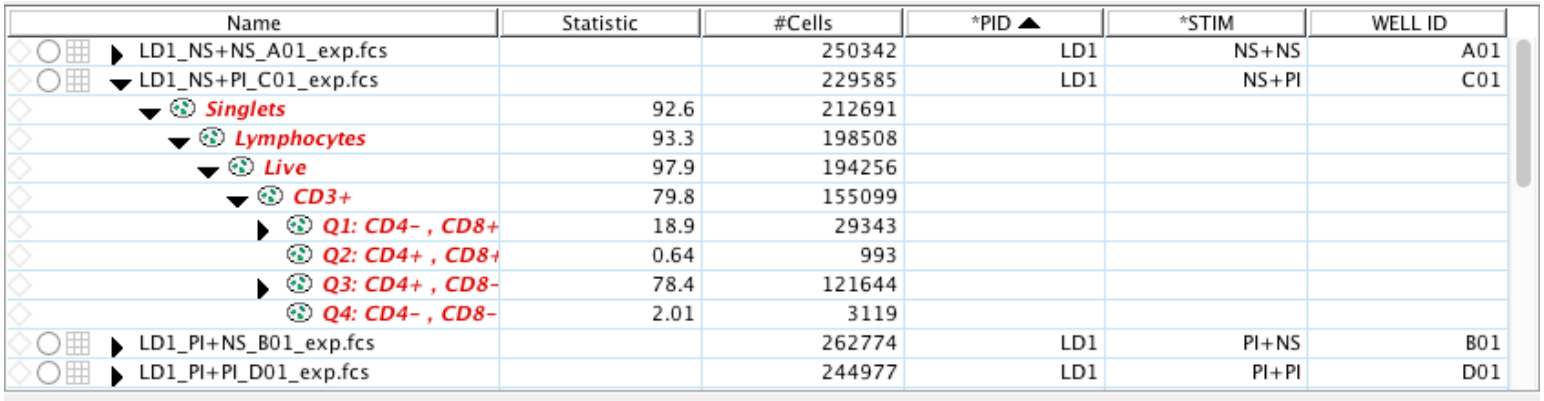
Specifies which samples are automatically included in a group

- 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



Samples Pane

Lists all samples within the selected group



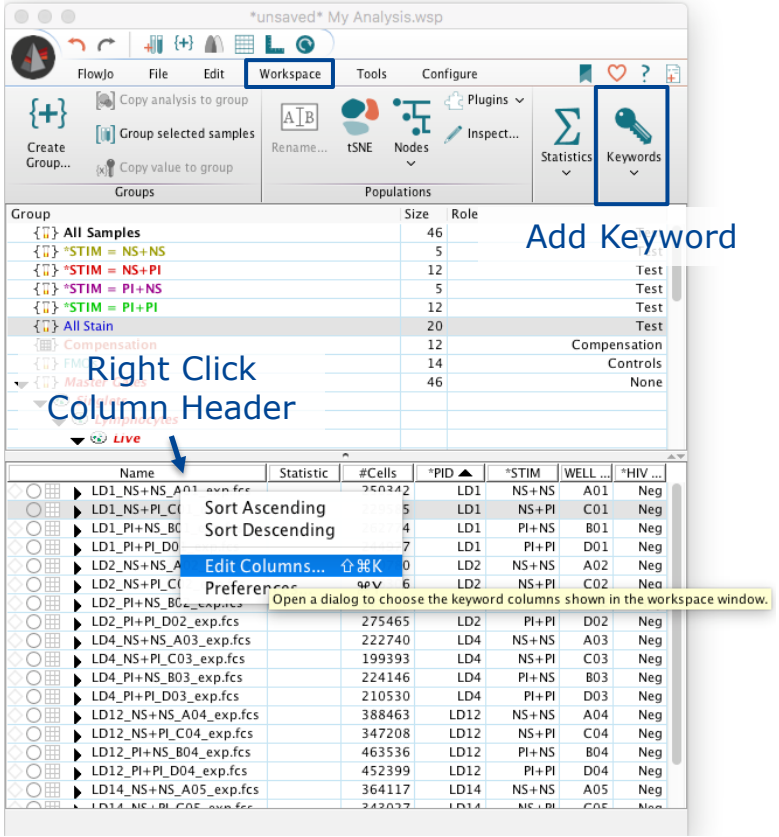
Name	Statistic	#Cells	*PID	*STIM	WELL ID
LD1_NS+NS_A01_exp.fcs		250342	LD1	NS+NS	A01
LD1_NS+PI_C01_exp.fcs		229585	LD1	NS+PI	C01
Singlets	92.6	212691			
Lymphocytes	93.3	198508			
Live	97.9	194256			
CD3+	79.8	155099			
Q1: CD4- , CD8+	18.9	29343			
Q2: CD4+ , CD8+	0.64	993			
Q3: CD4+ , CD8-	78.4	121644			
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

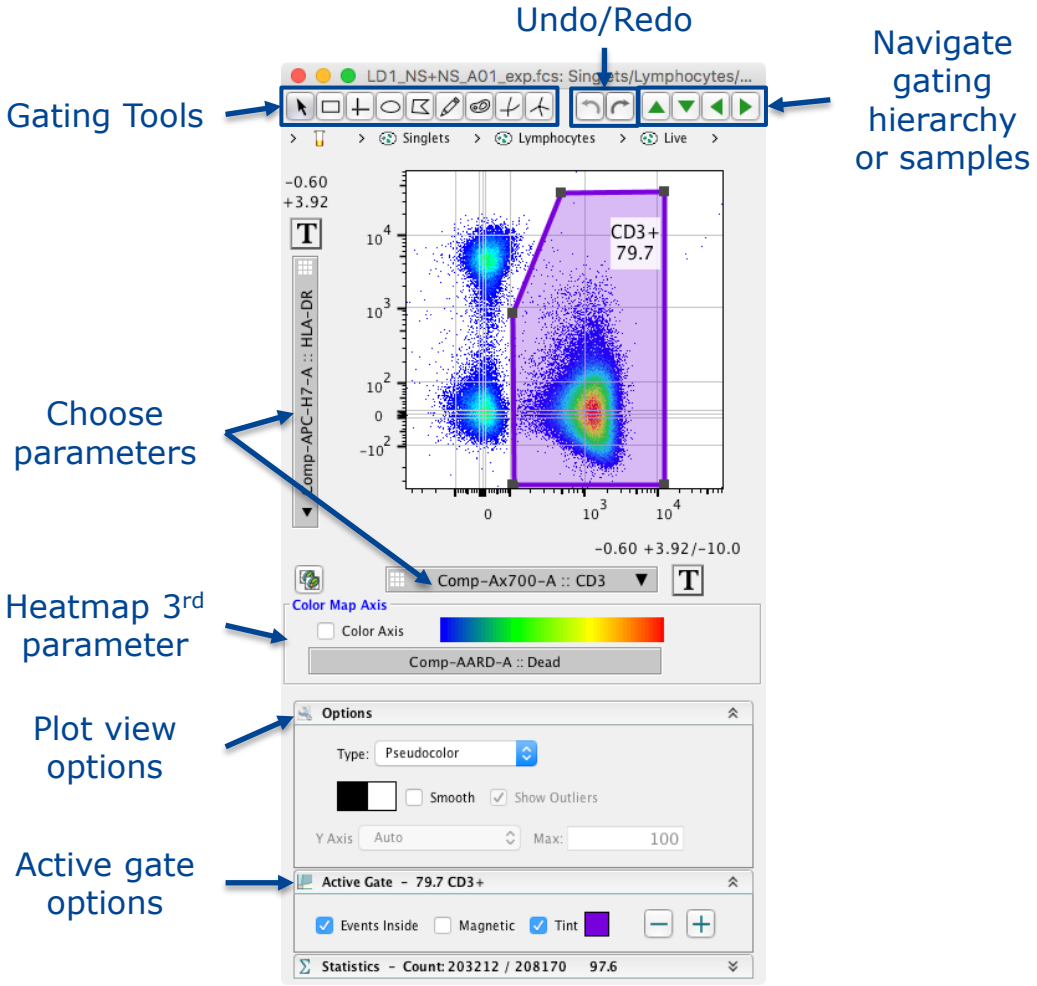
- Within the workspace, keywords can be:
  - Added
  - Displayed
- Keywords are used to:
  - Organize and sort samples
  - Create groups
  - Generate batch reports



Practice time

The Graph Window

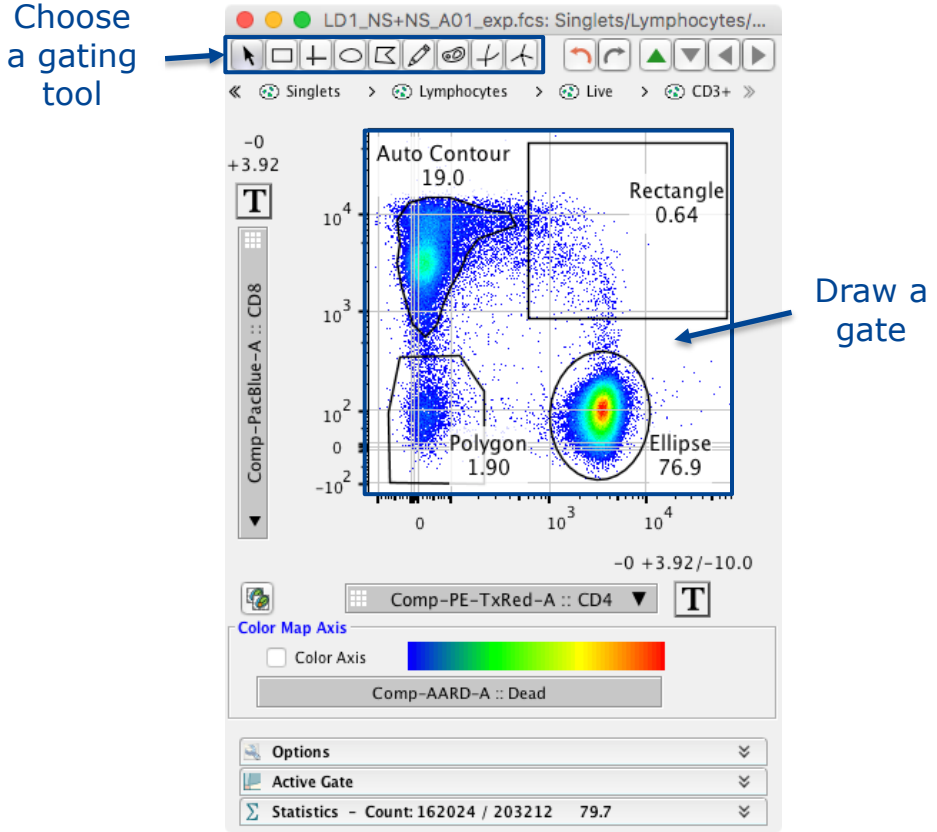
- Facilitates data visualization and gating
- Click on an X or Y axis label to choose and view different measured parameters



Gating Tools

Subset events into populations based on marker expression

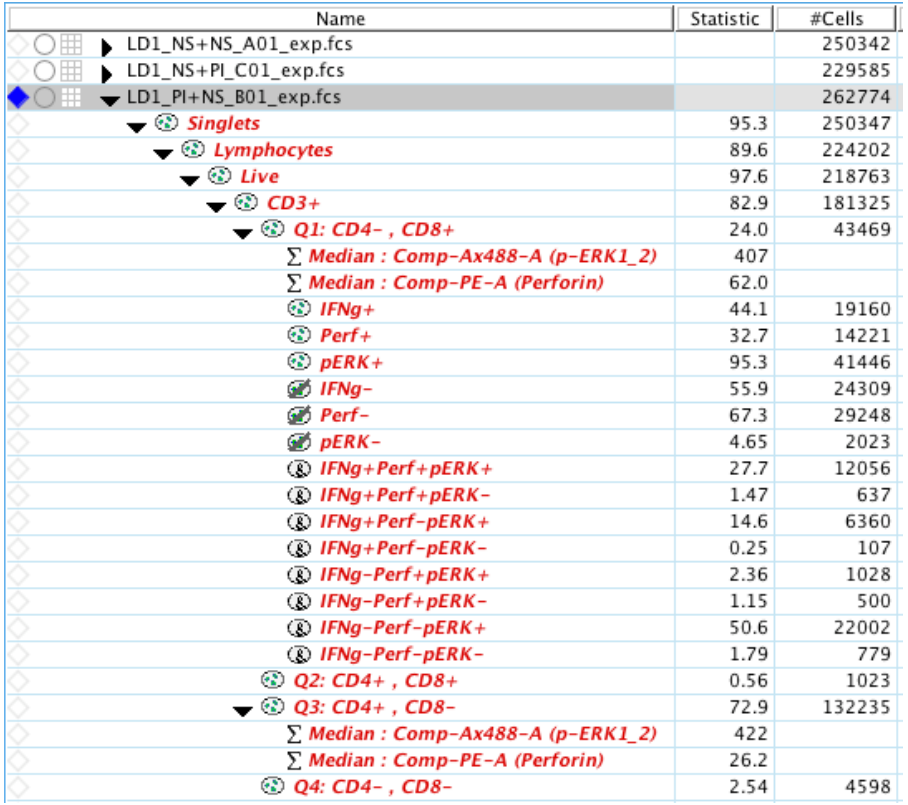
- Draw a Gate → Frequency of parent statistic
- Gates can always be modified or removed
- Double-click within a gate to focus on that population in a new graph window



Gating Hierarchies

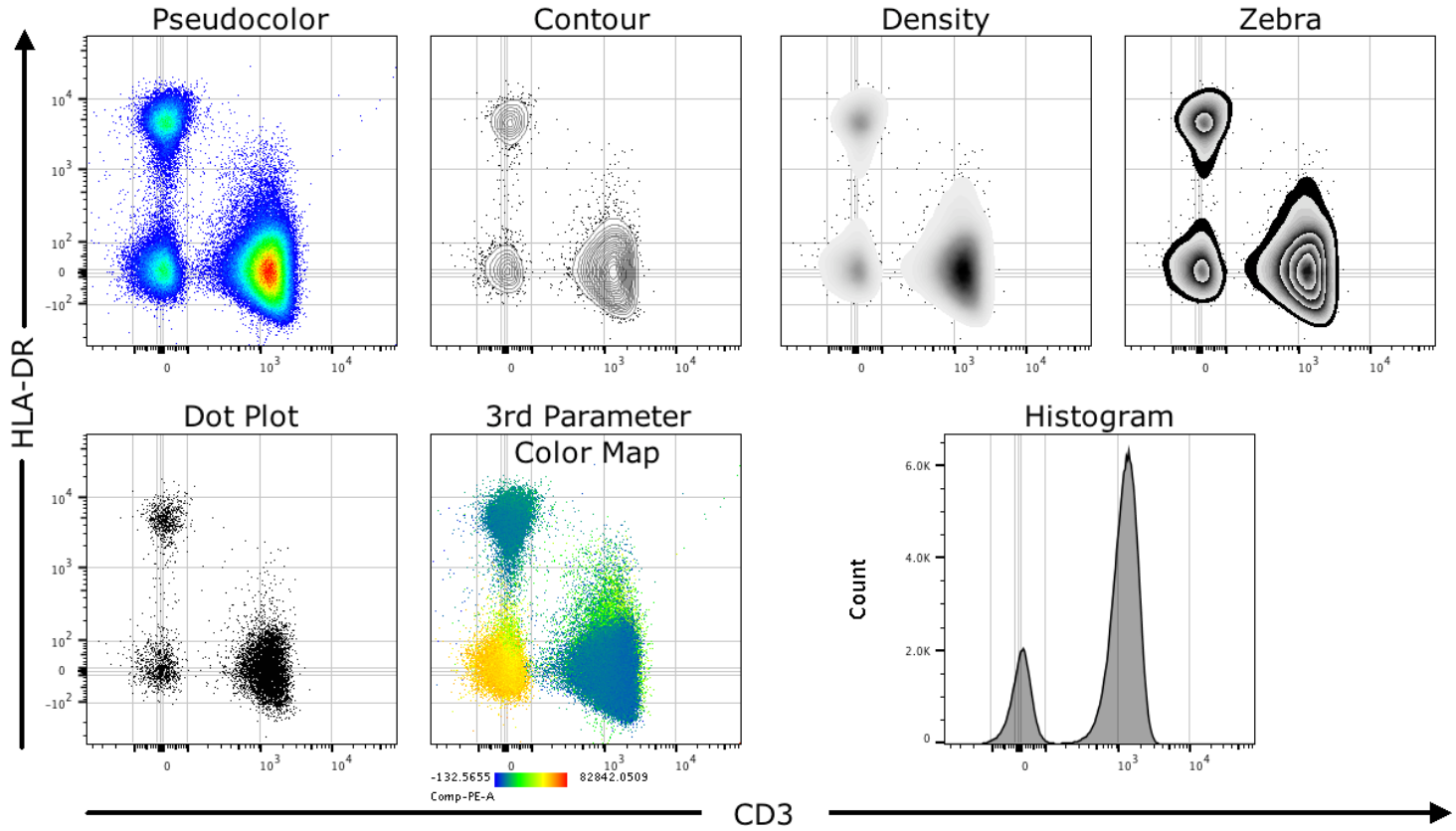
Display parent/child/sibling relationships between populations

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



Graph Window Display Options

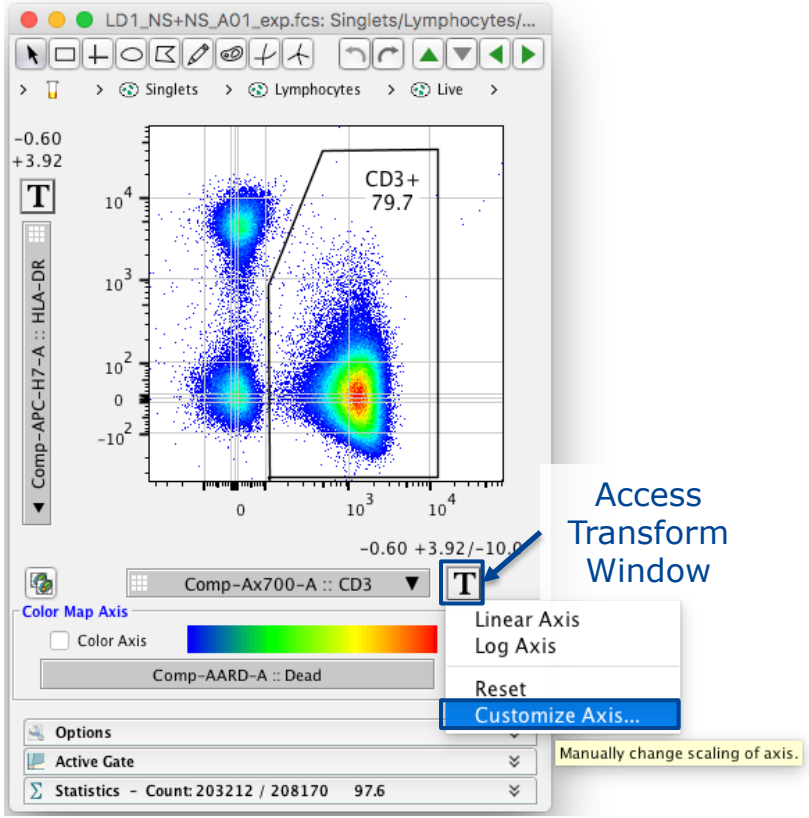
Customize your data visualization

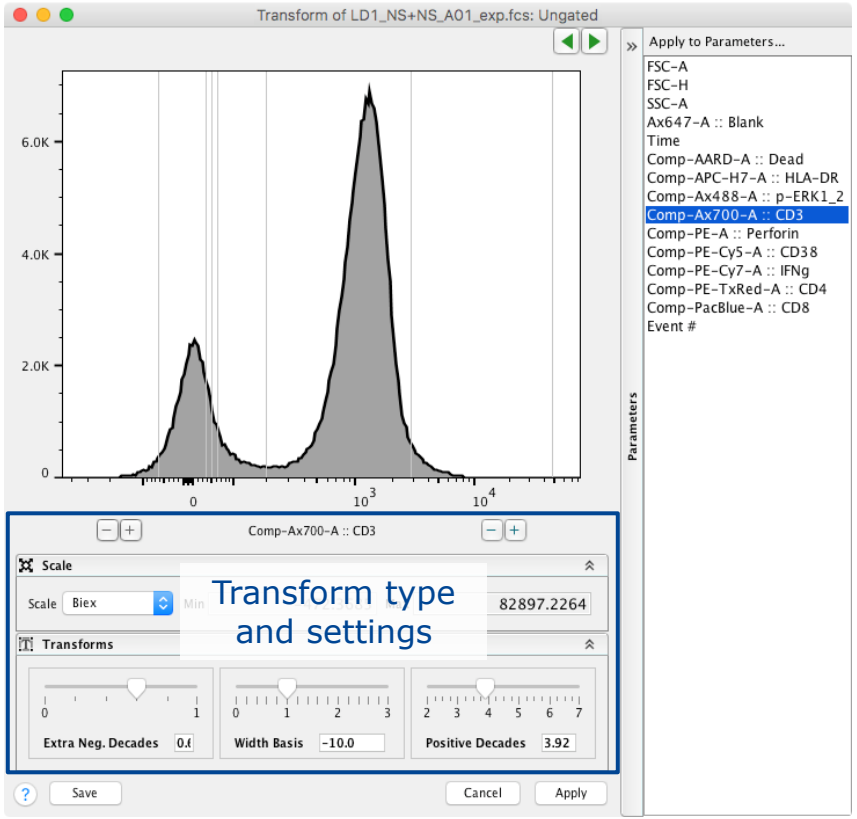


Practice time

Transforming Data

Click the Transformation [T] button → Customize Axis…

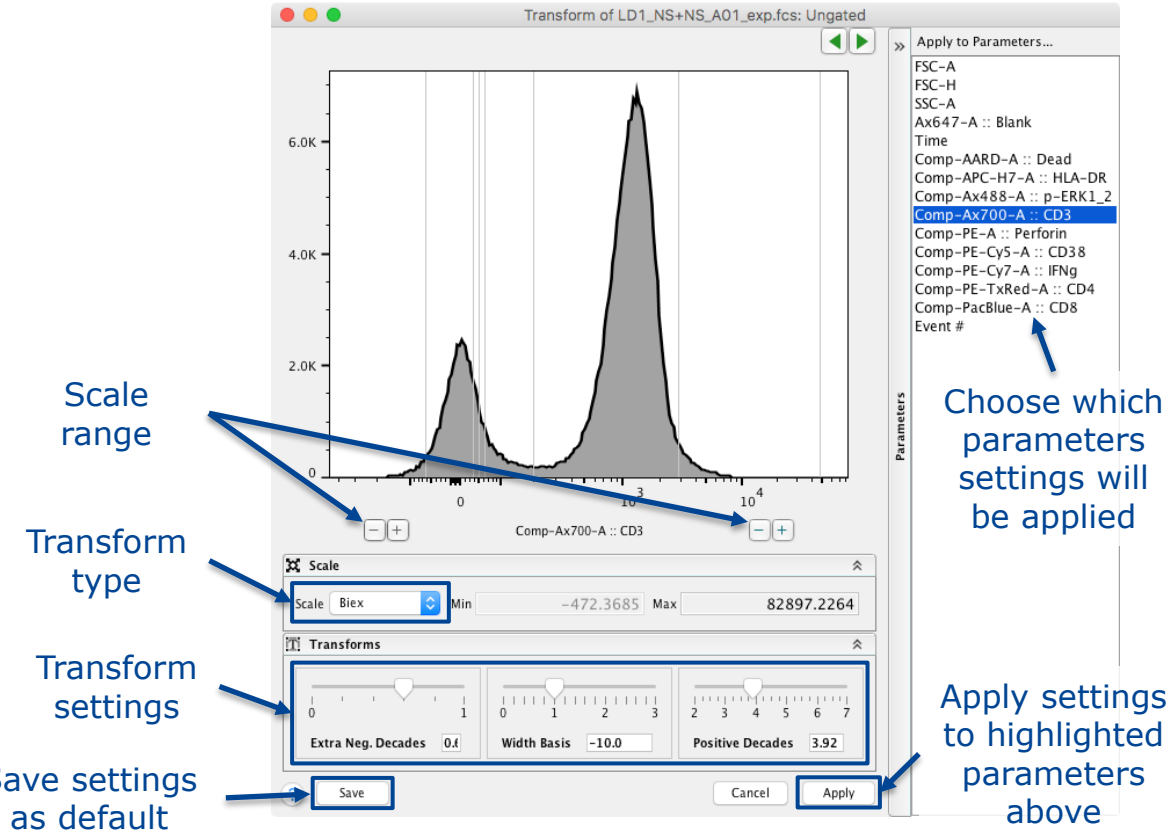




Transforming Data

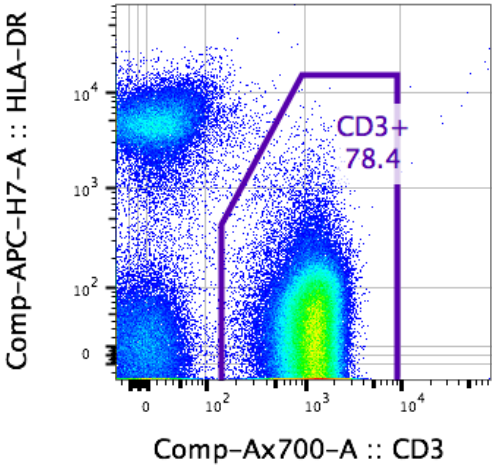
Only affects visual display & scaling of data, not the raw values

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

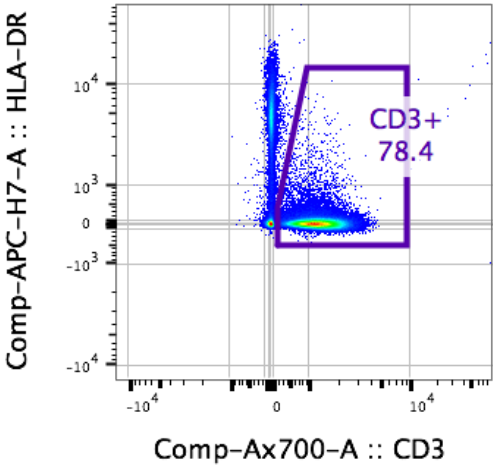


Transformation

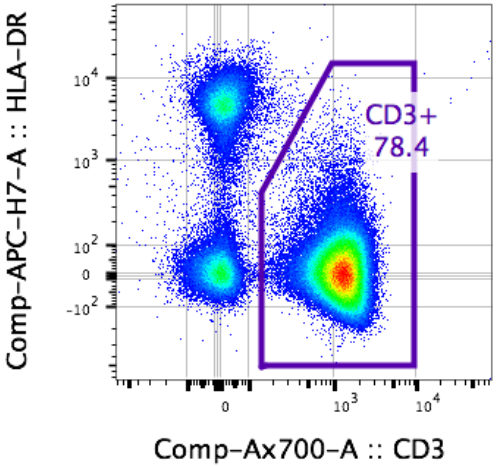
Extra Neg Decades = 0
Width Basis = -1
Extra Pos Decades = 3.92



Extra Neg Decades = 1
Width Basis = -200
Extra Pos Decades = 3.92



Extra Neg Decades = 0.6
Width Basis = -10
Extra Pos Decades = 3.92

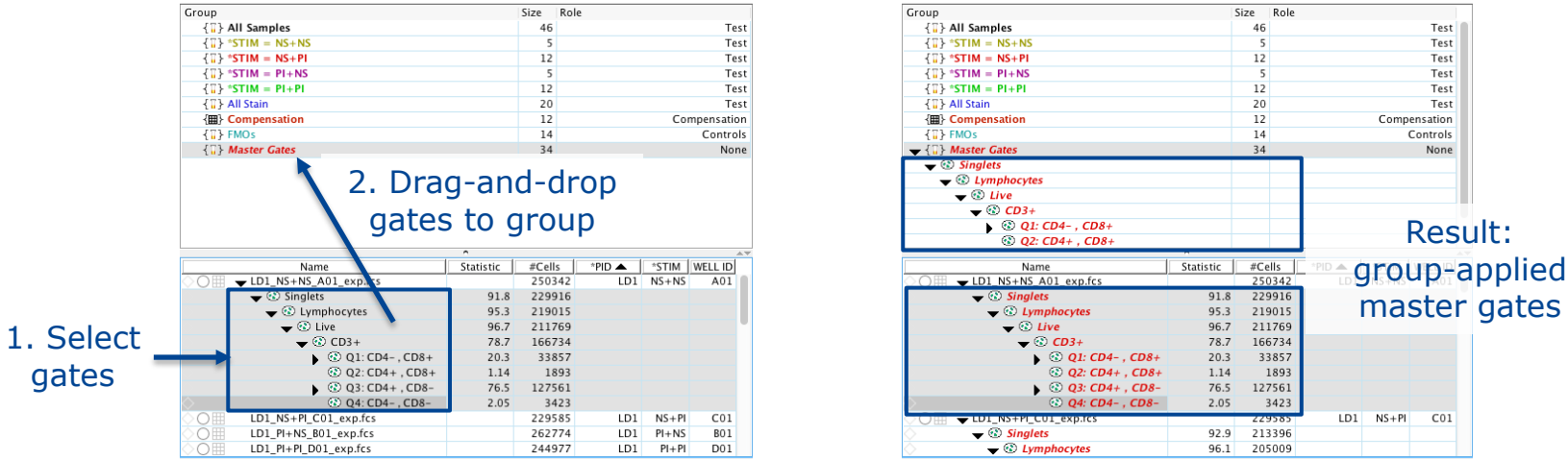


- Gets rid of the "squishing" of cells
- Ensures the visual population center better correlates with the statistical center (median)
- Makes high resolution compensated digital cytometry data more appealing to the eye

Practice time

Group Application of Gates

Allows for master gating of multiple samples

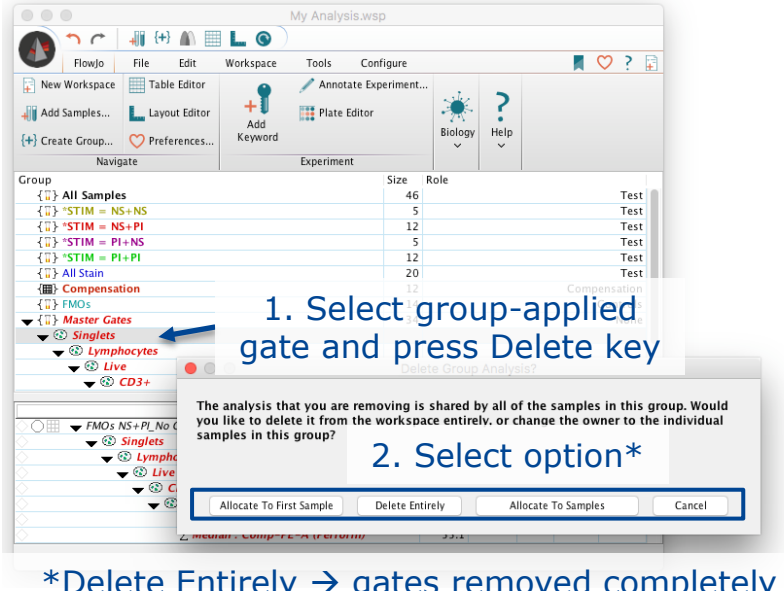


- Select gray gates on a single sample and:
  - drag-and-drop gates from a single sample to a group, or
  - right click on gates and choose ”Copy analysis to group”
- Group-applied gates turn the group color to denote that they are identical

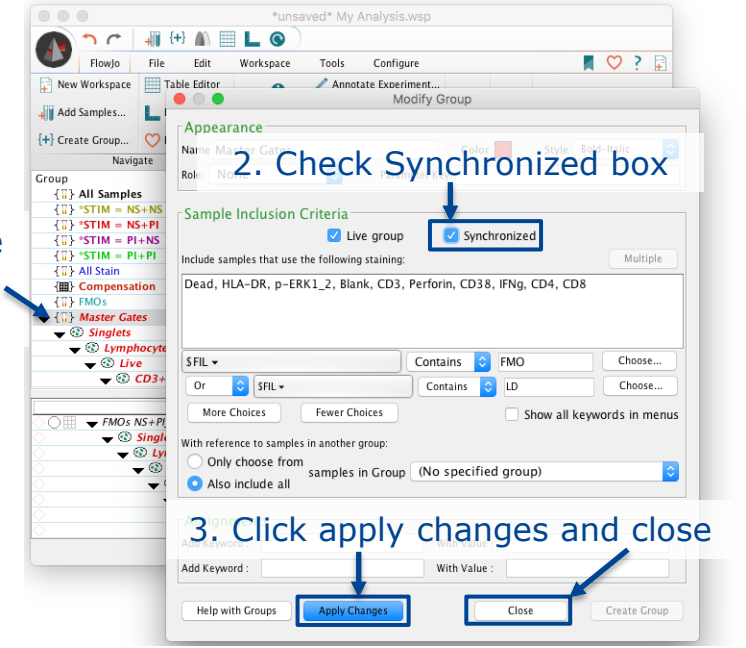
Group-Applied Gates

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

(A)



(B)



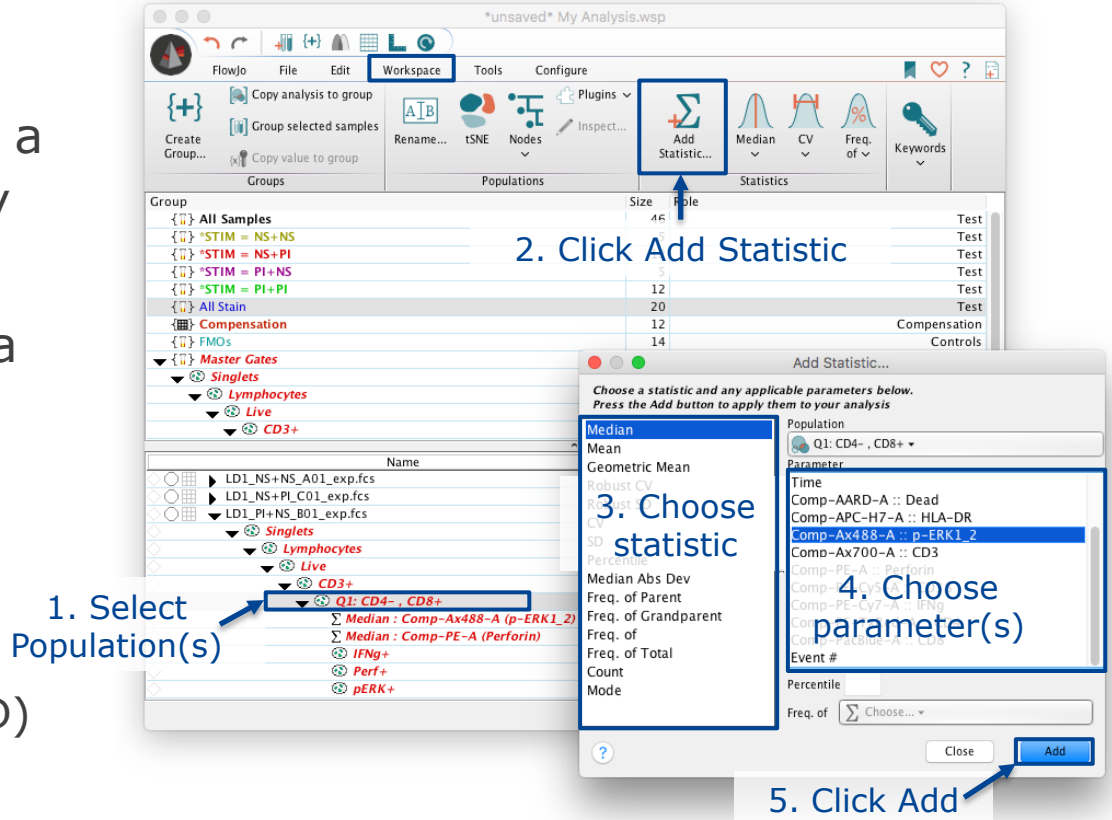
*Delete Entirely → gates removed completely

Allocate → removes group gating, deeps gates on sample(s)

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)

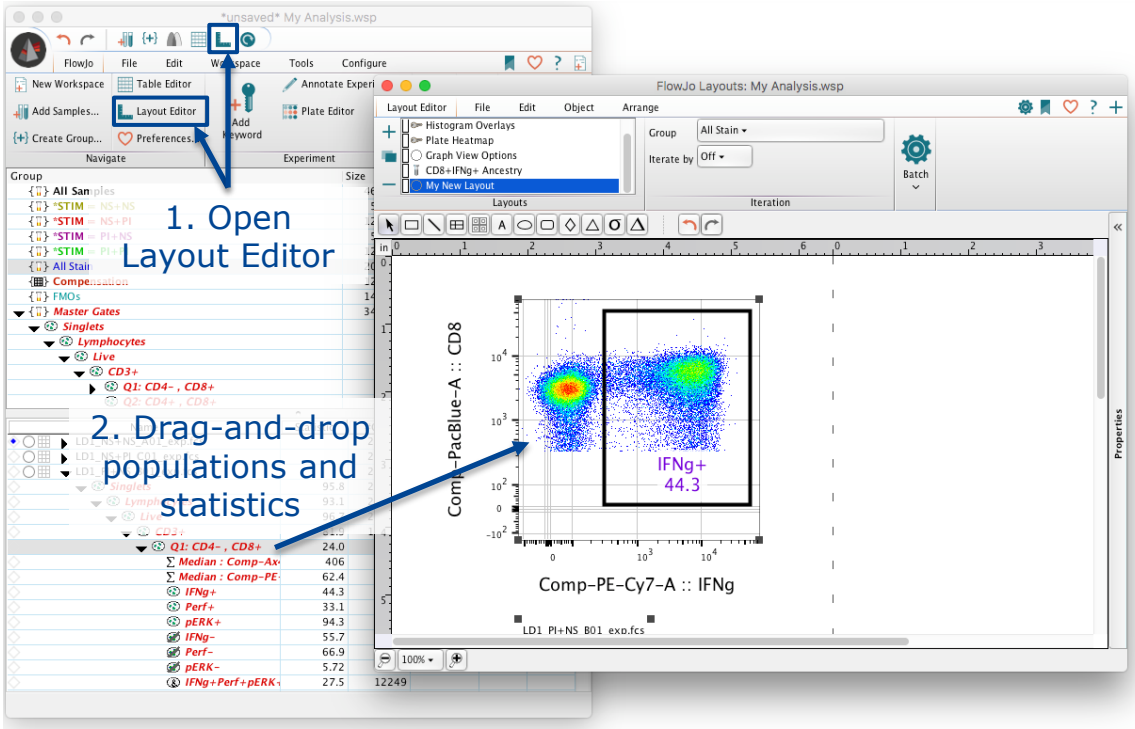


Practice time

The Layout Editor

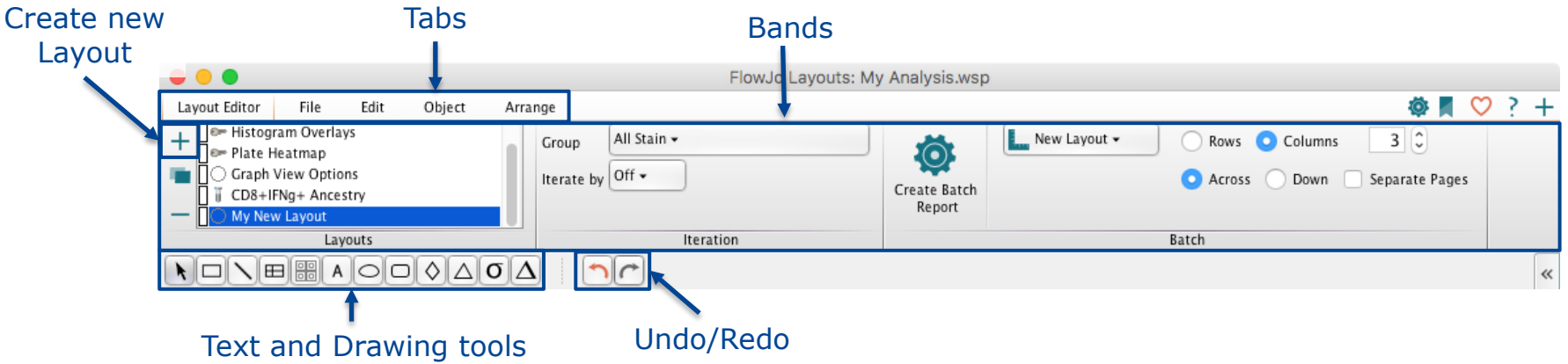
A tool for creating graphical reports

- 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

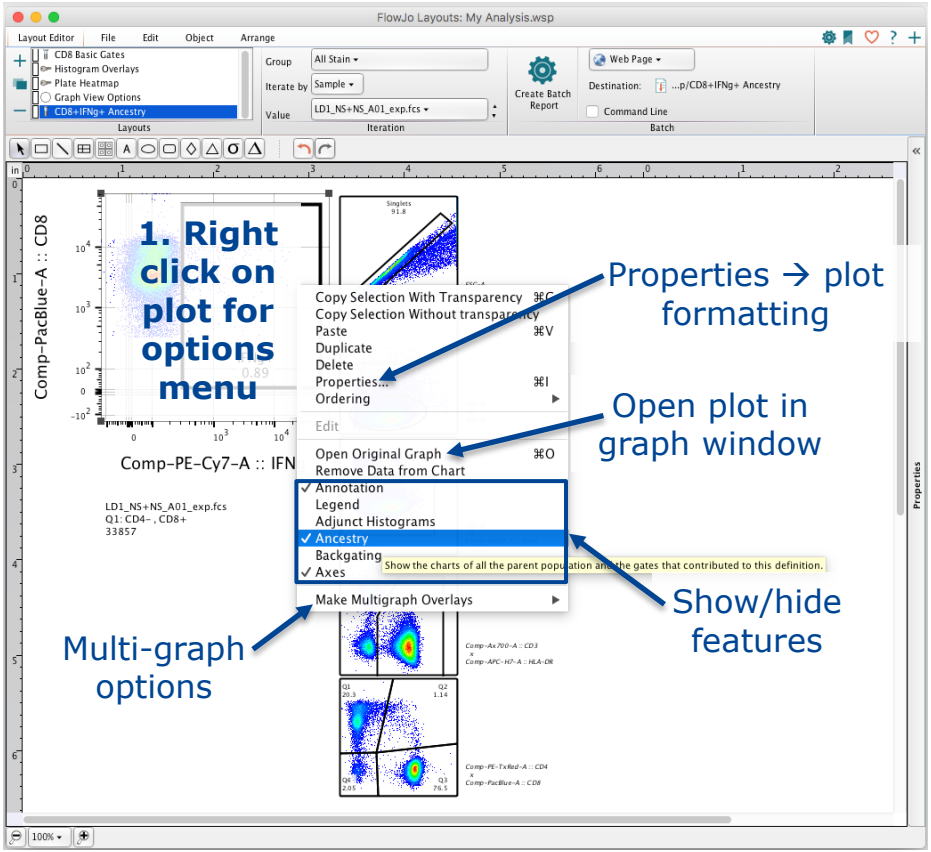
- Similar to the Workspace, the Layout Editor has its own action taskbar with tabs and bands to organize actions



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

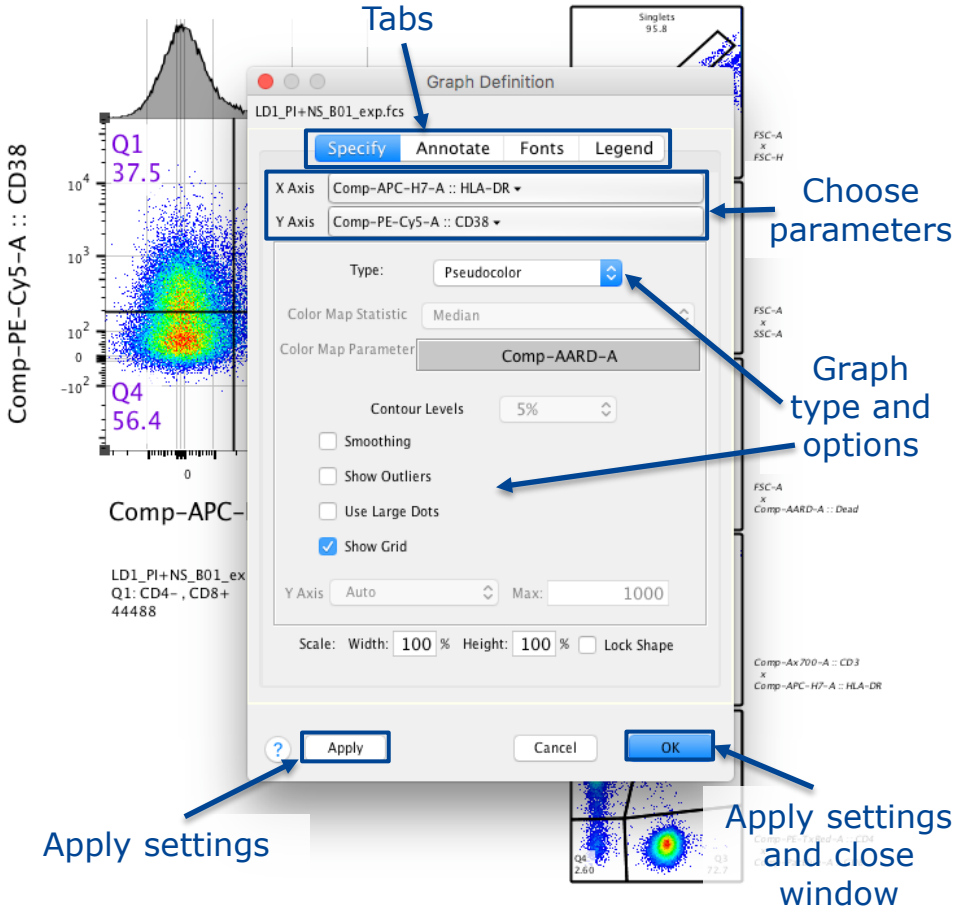
Within the Layout Editor

- 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

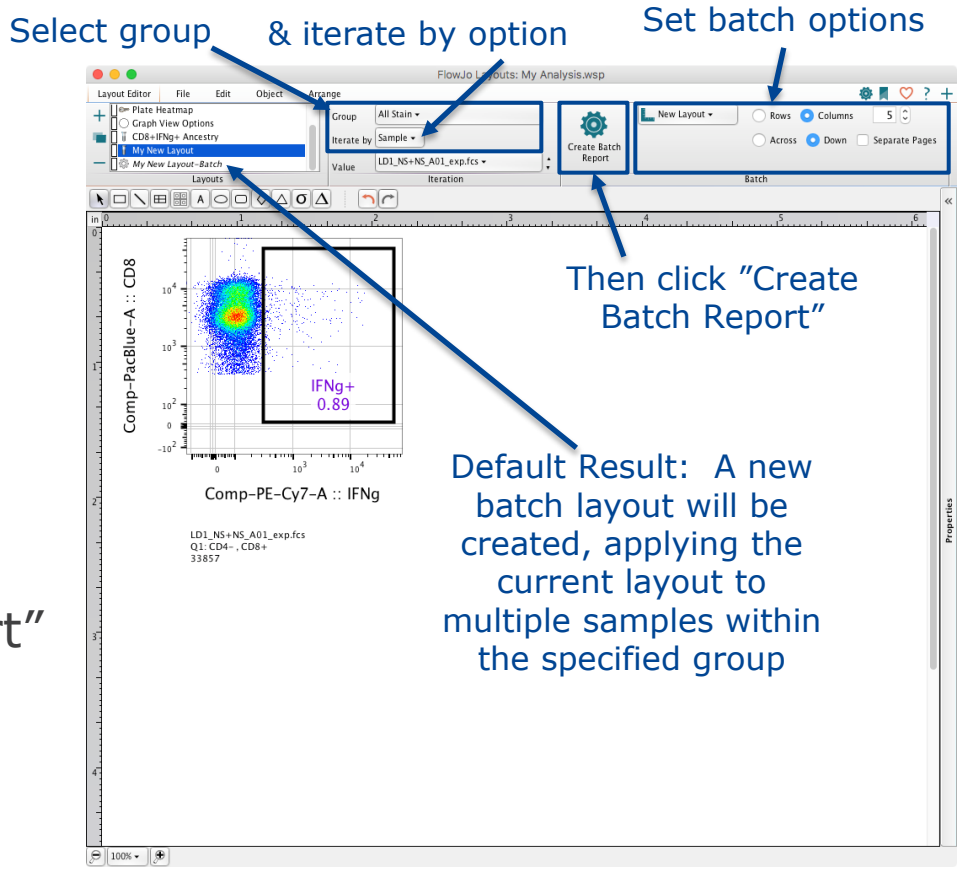
- Right click on plot and select Properties → Graph Definition window
- 4 tabs with formatting options for:
  - Specify graph style
  - Annotation
  - Fonts
  - Legend



Batch Analysis of Layout Graphics

Applies the layout across multiple samples in a group

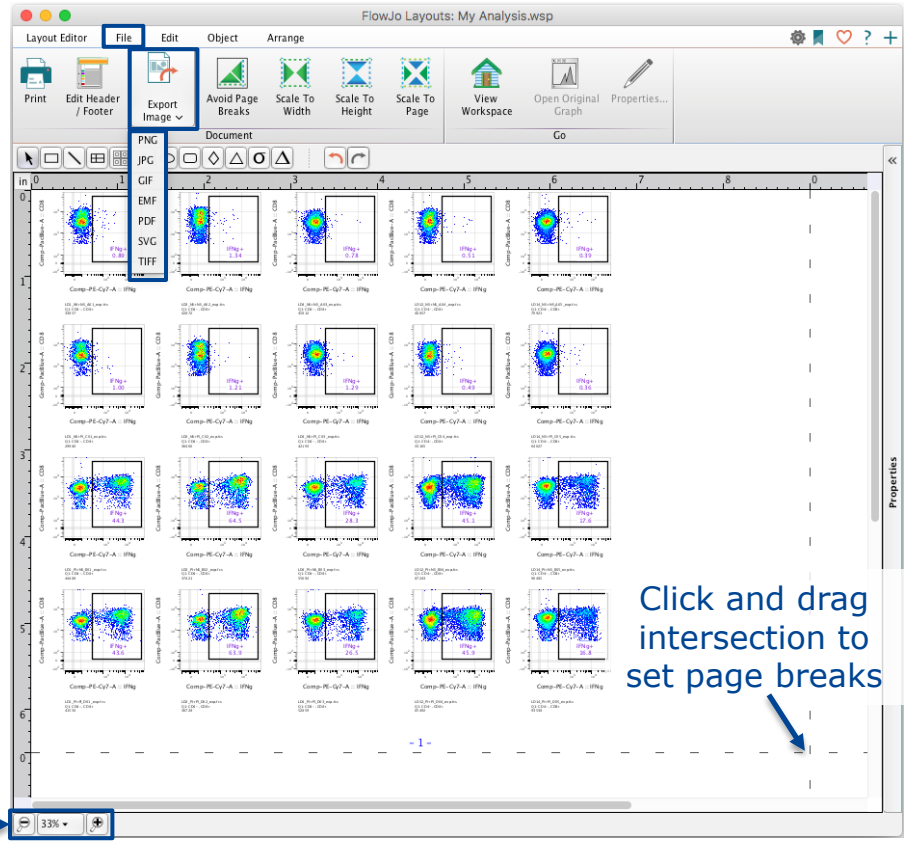
- To batch a layout:
  - Specify a group
  - Choose iterate by option
    - Sample
    - Panel
    - Keyword
  - Set batch options
  - Click “Create Batch Report”



Export Image Options

Are available under the File tab

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



Iteration

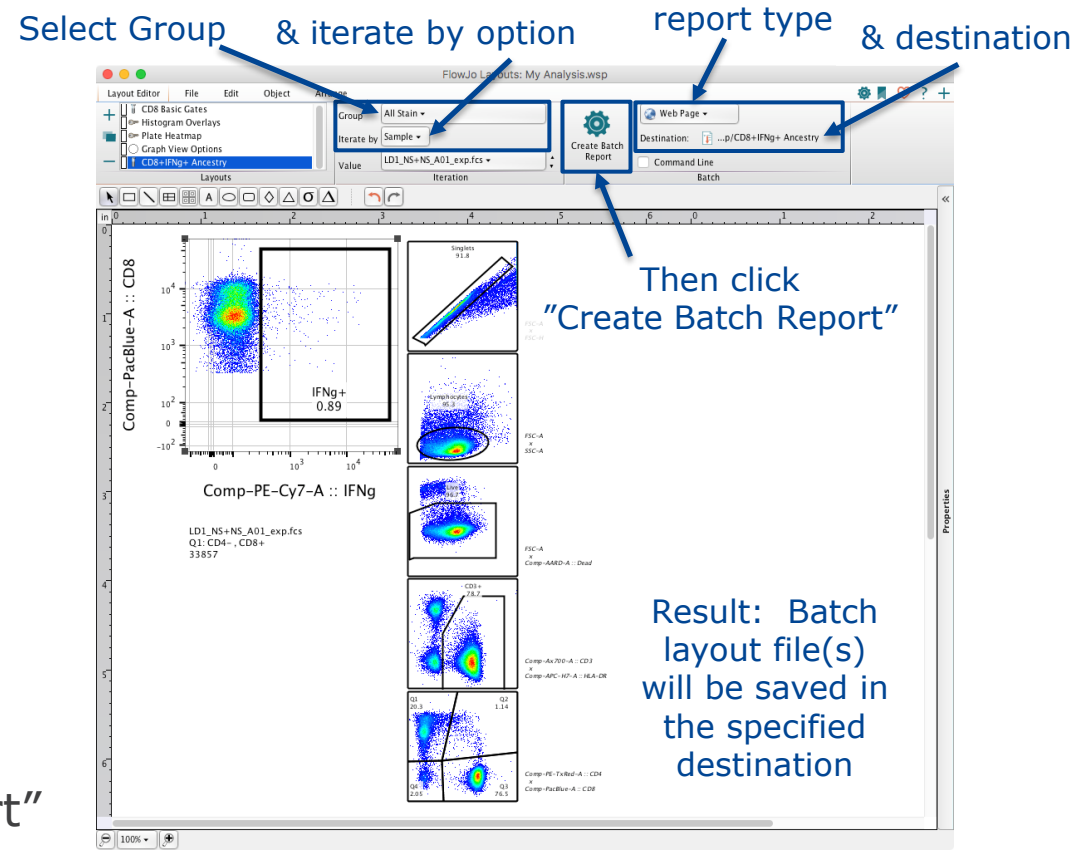
Enables scrolling through samples in a group

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

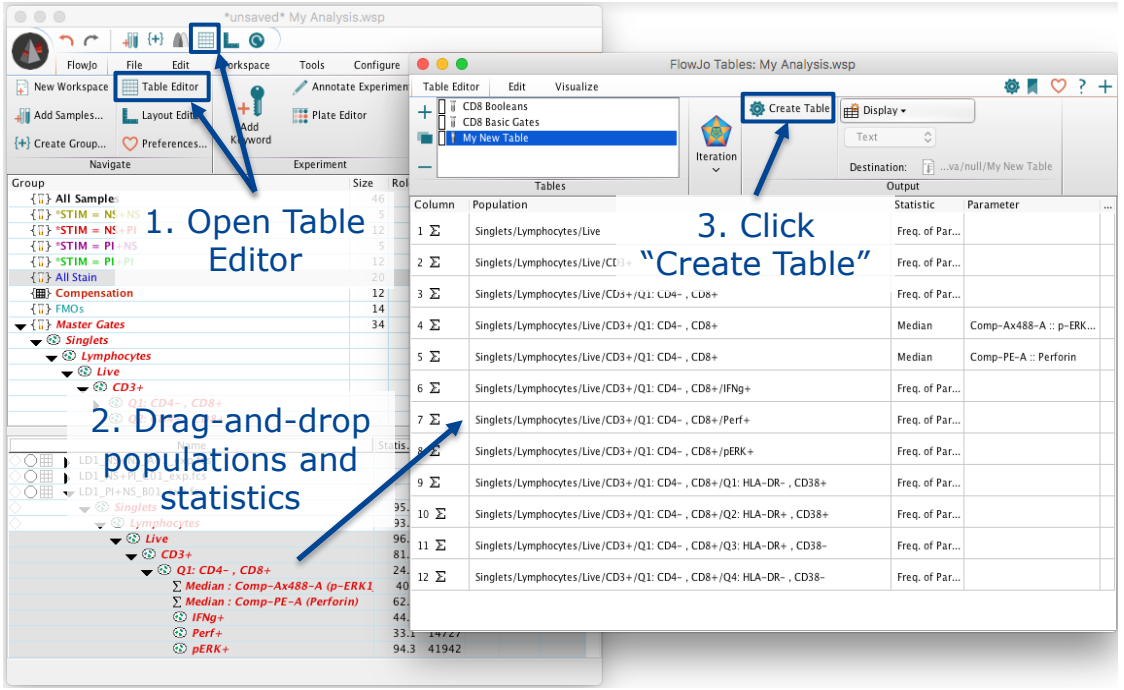


Practice time

The Table Editor

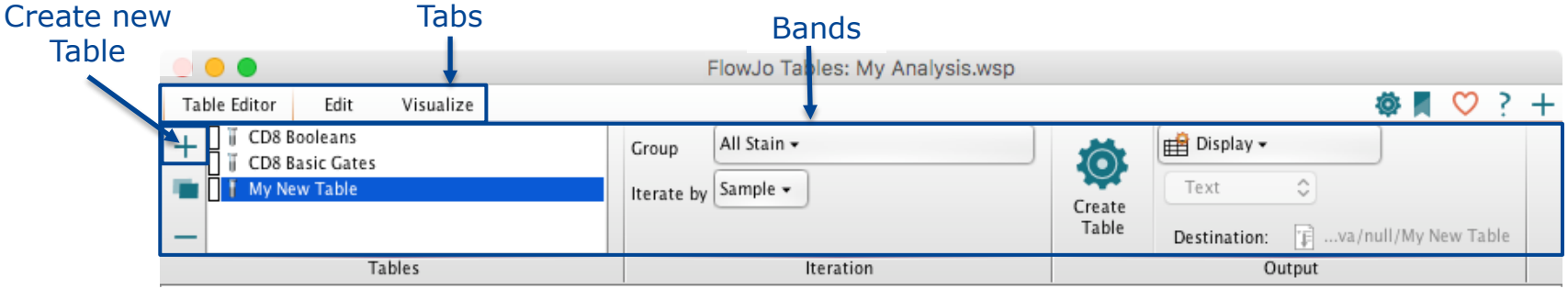
A tool for creating statistical reports

- 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”

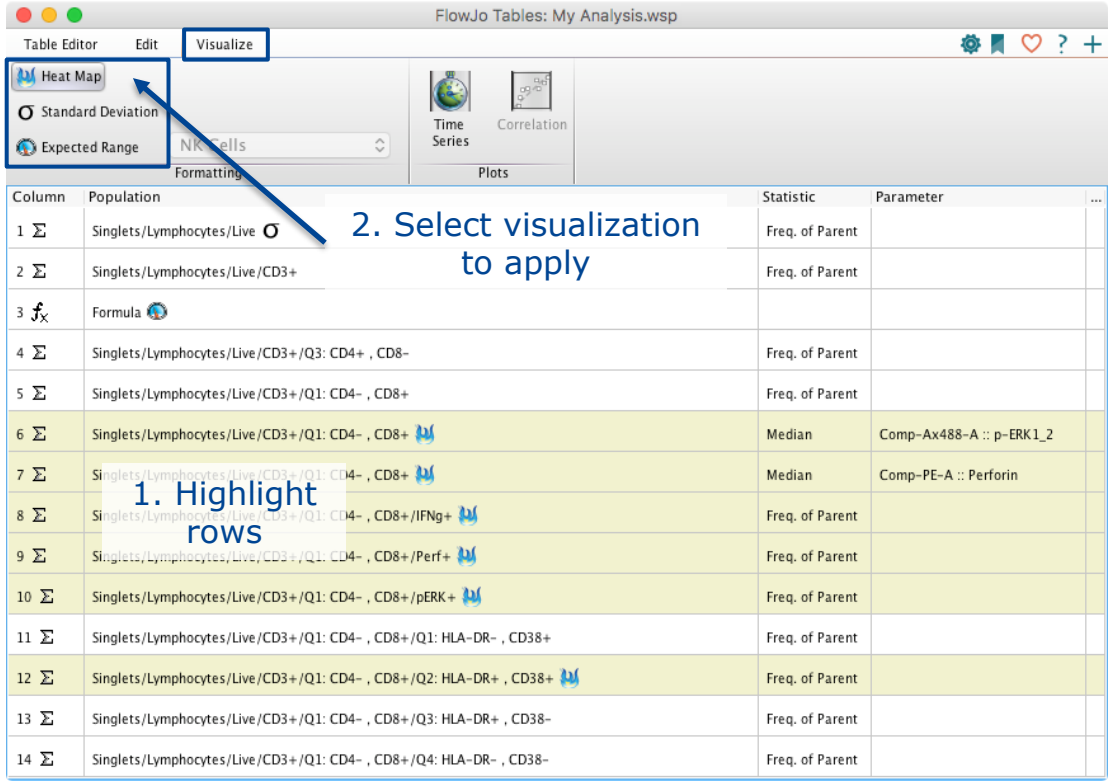


Table Editor Output

- 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

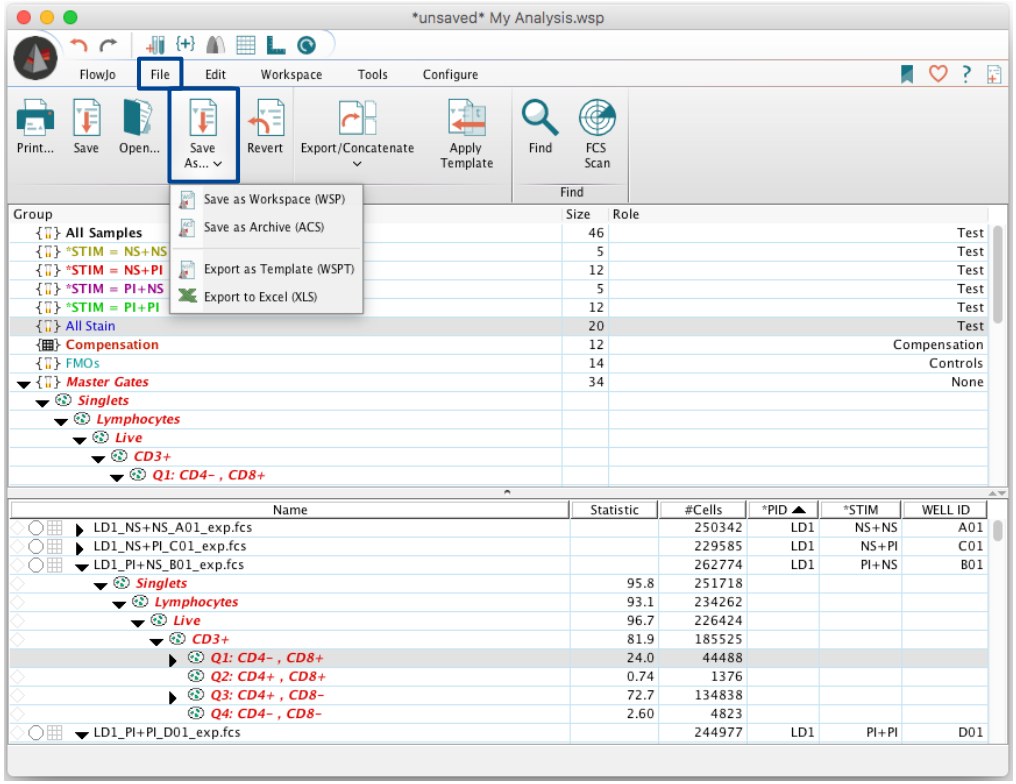
	*PID	*STIM	Q1: CD4-, CD8+ p-ERK1_2 Median	pERK+ Freq. of Parent	IFNg+ Freq. of Parent	Perf+ Freq. of Parent	IFNg+Perf+pERK+ Freq. of Parent	CD4/CD8 Ratio
LD1_NS...	LD1	NS+NS	68.2	4.62	0.89	30.2	0.13	▲ 3.77
LD1_NS...	LD1	NS+PI	550	95.0	1.00	30.0	0.42	▲ 4.09
LD1_PI+...	LD1	PI+NS	406	94.3	44.3	33.1	27.5	▲ 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

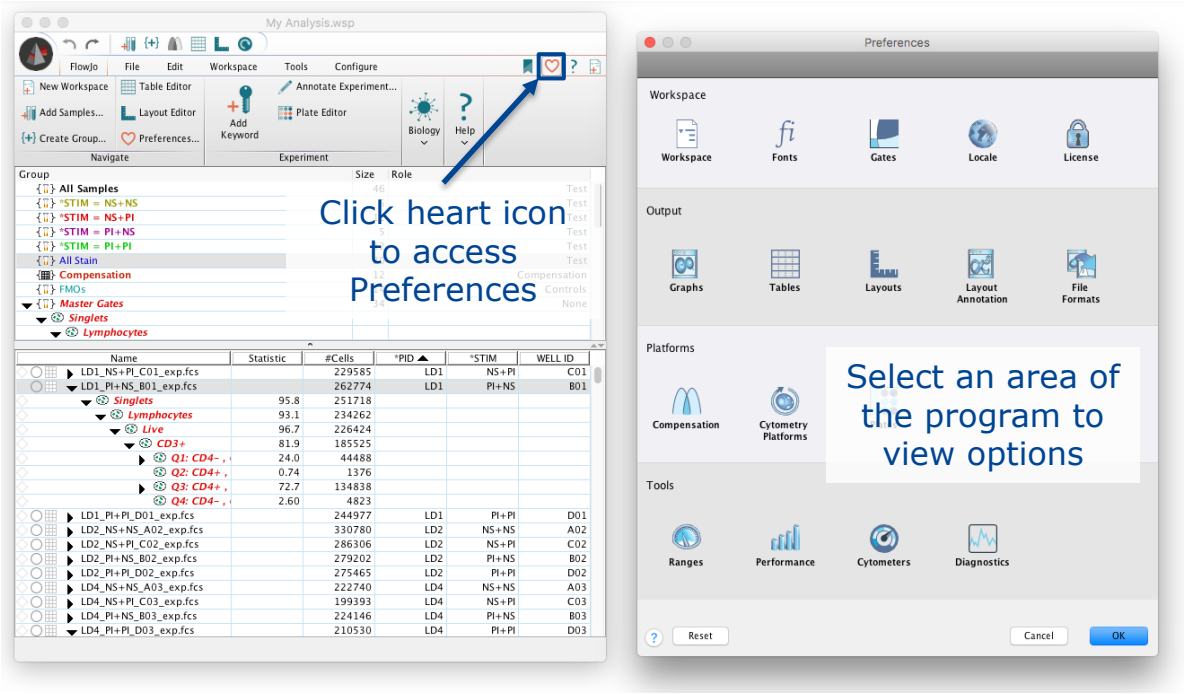
- Save as options are located within the File tab
  - WSP (Normal Save)
  - ACS (Zip file, incl. data)
  - WSPT (Template)
- Templates streamline repetitive analysis of multiple runs using the same staining panel(s)



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



Thank You!

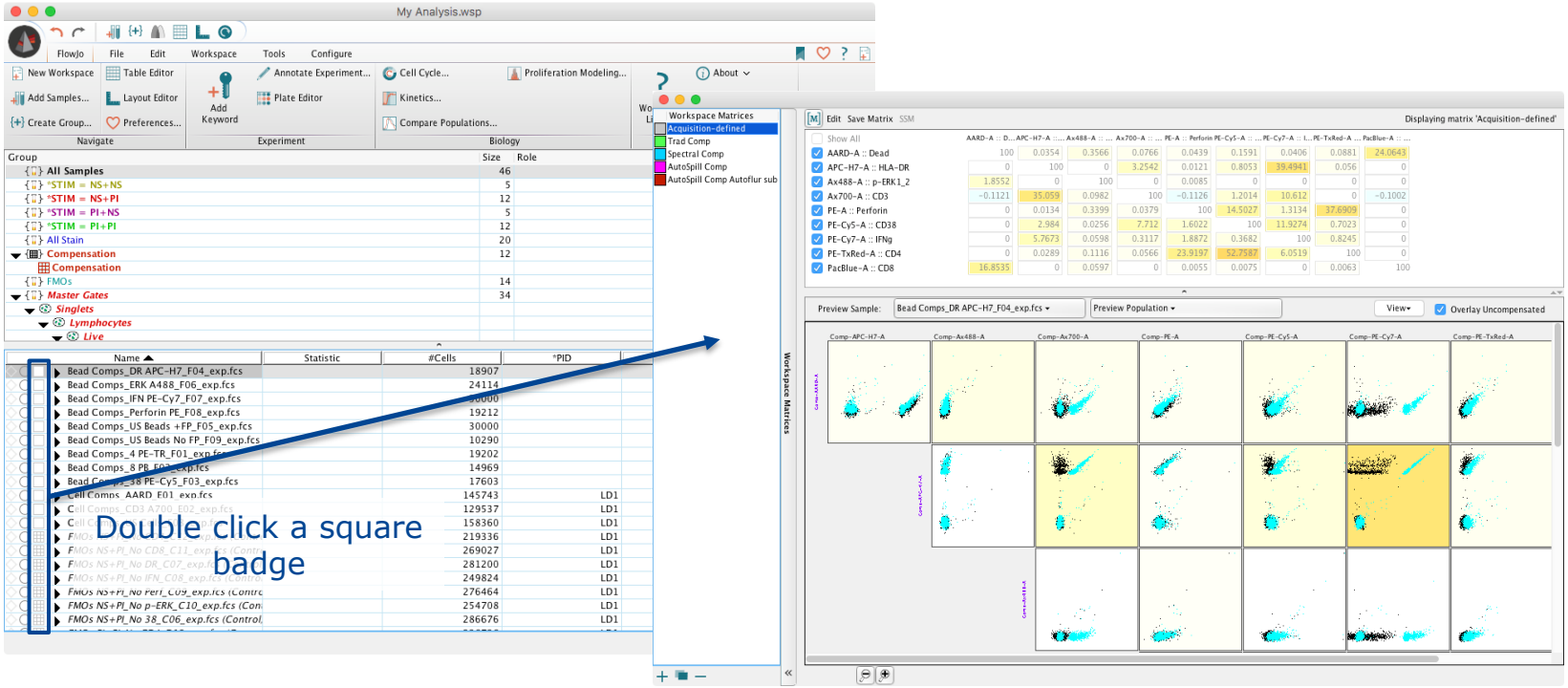
Questions?

Compensation in FlowJo v10

Previewing Compensation

FlowJo™ v10

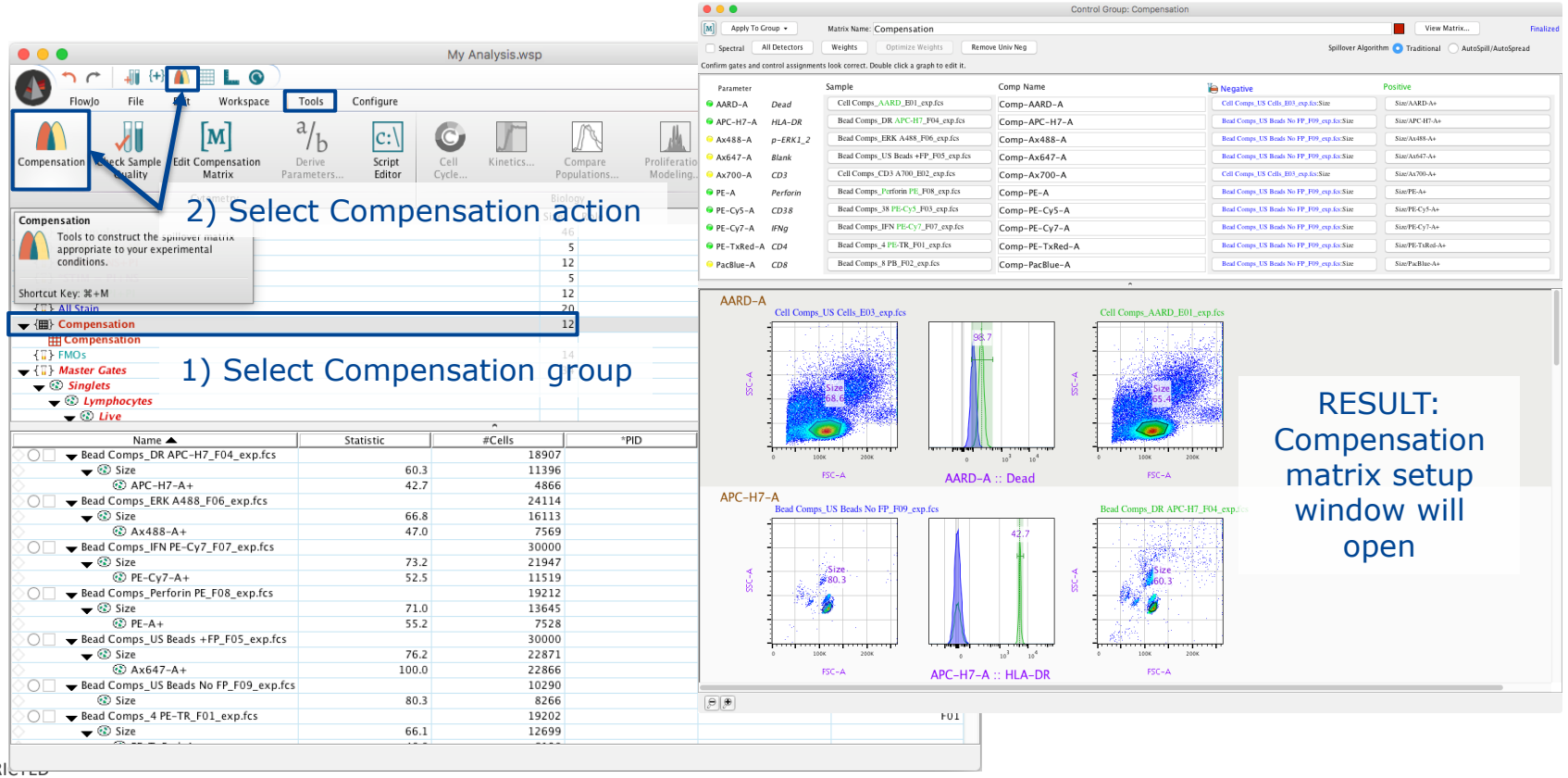
Double click on the square matrix badge next to a sample name



Initiating Compensation

FlowJo™ v10

Place single color controls into the Compensation group and

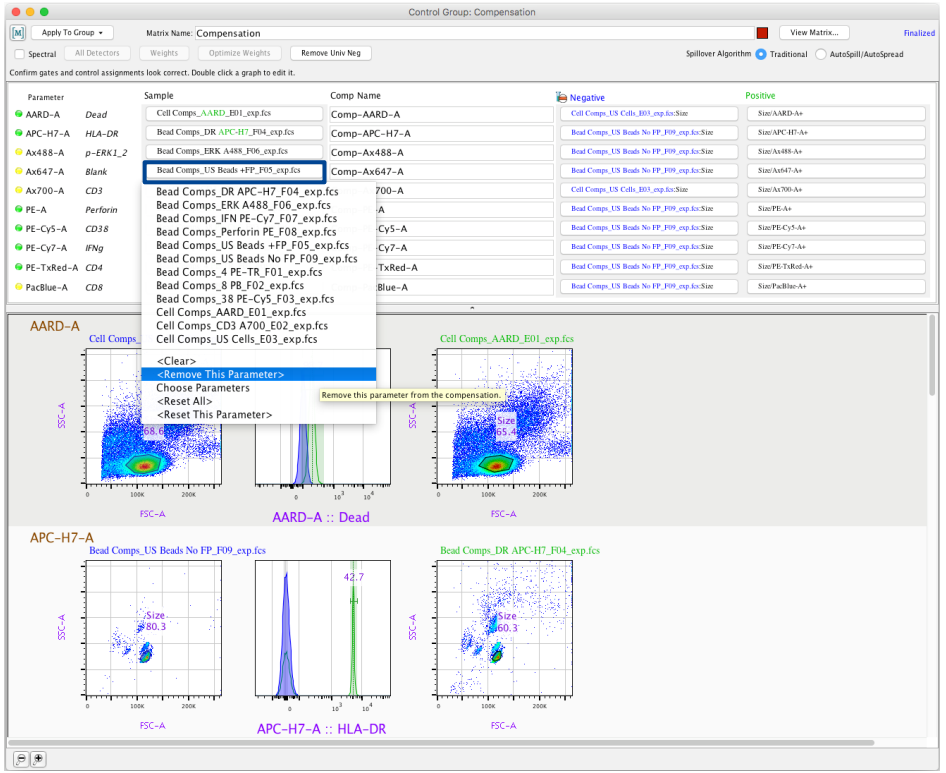


Choose or Remove Parameters

FlowJo™ v10

Click on a Sample column field → drop-down options, including:

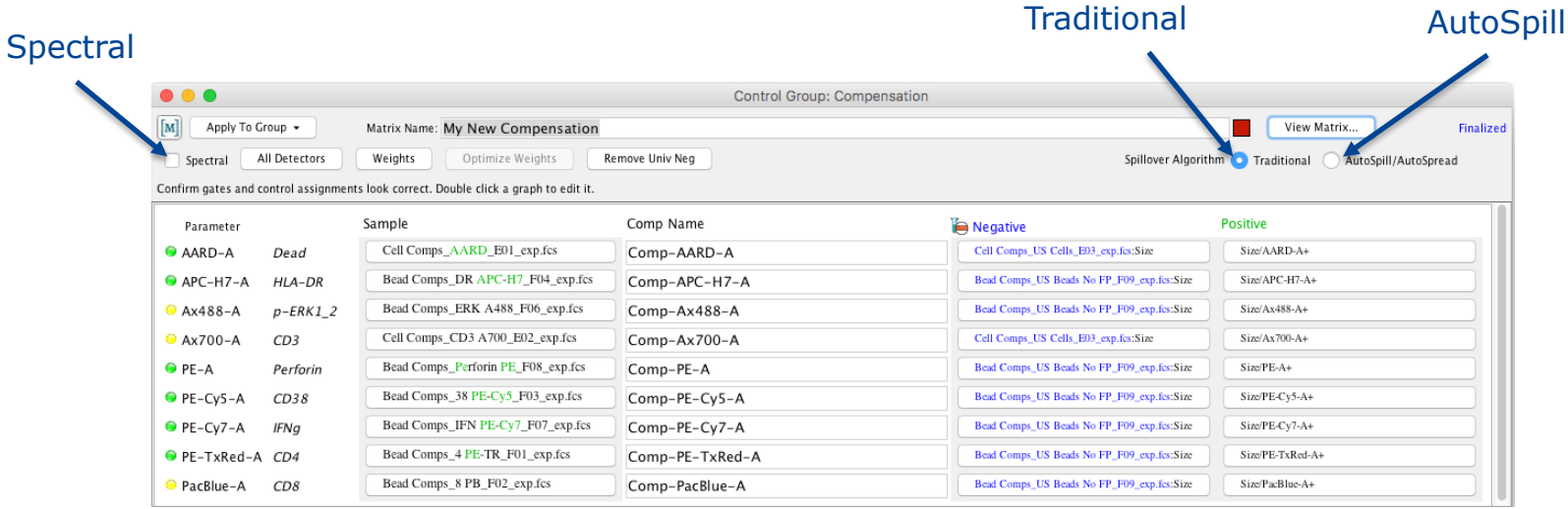
- Single color control selection
- Remove Parameter
- Choose Parameters
- Reset All or single parameter



Default is Traditional Compensation

FlowJo™ v10

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



Review and Adjust Gates

FlowJo™ v10

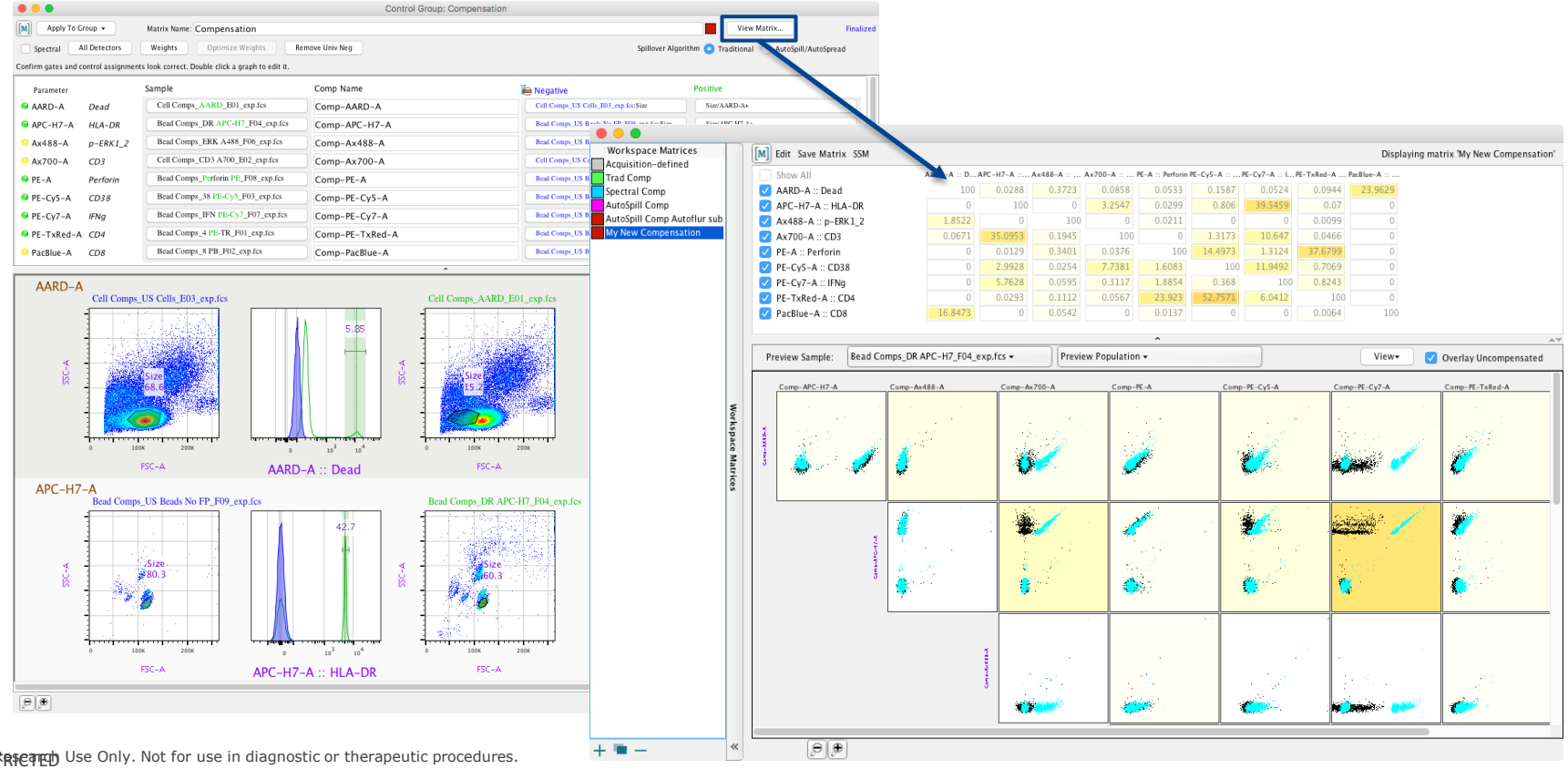
Parameters used and auto-gating for populations

- 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

View Matrix

FlowJo™ v10

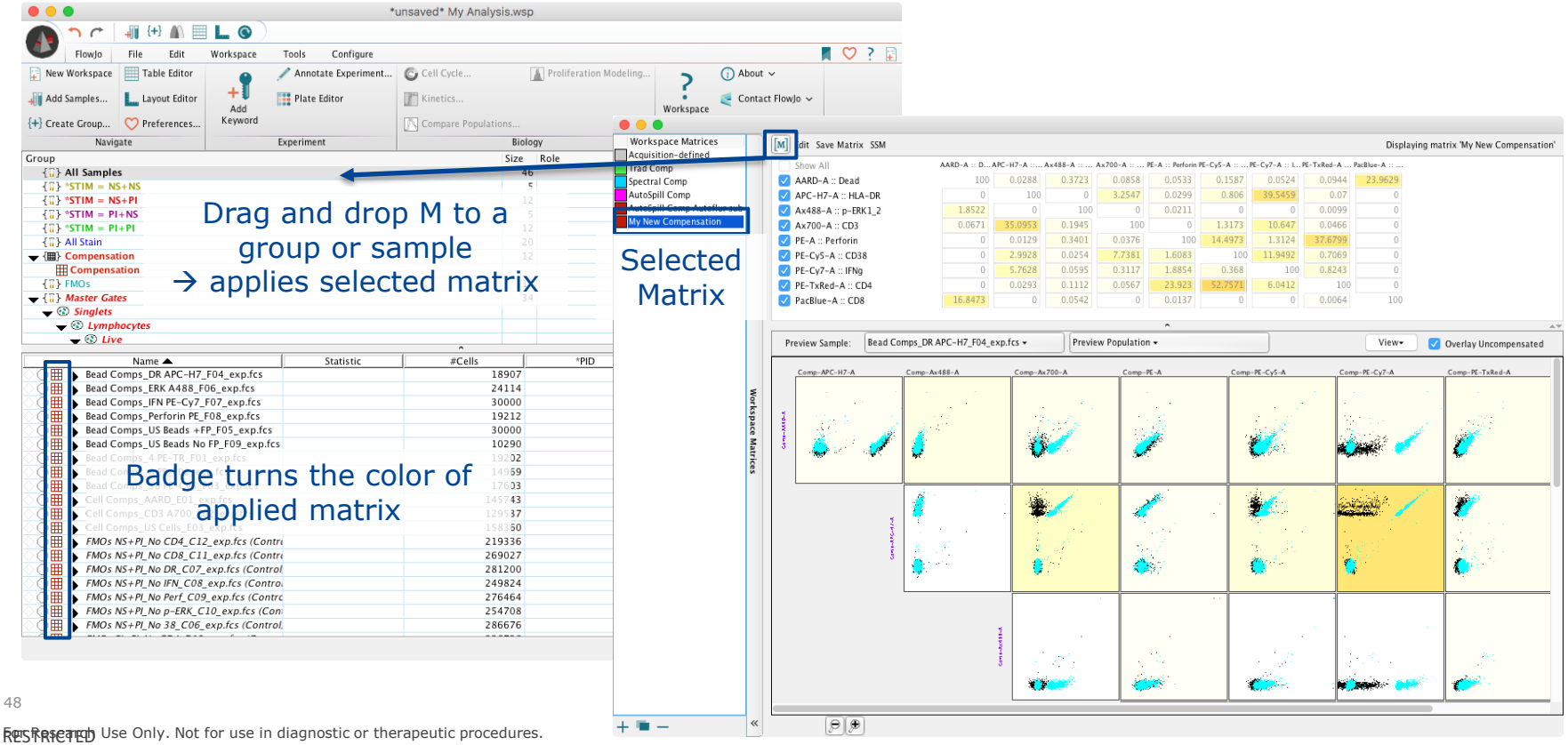
Once choices are finalized, click to view and apply



Apply Compensation

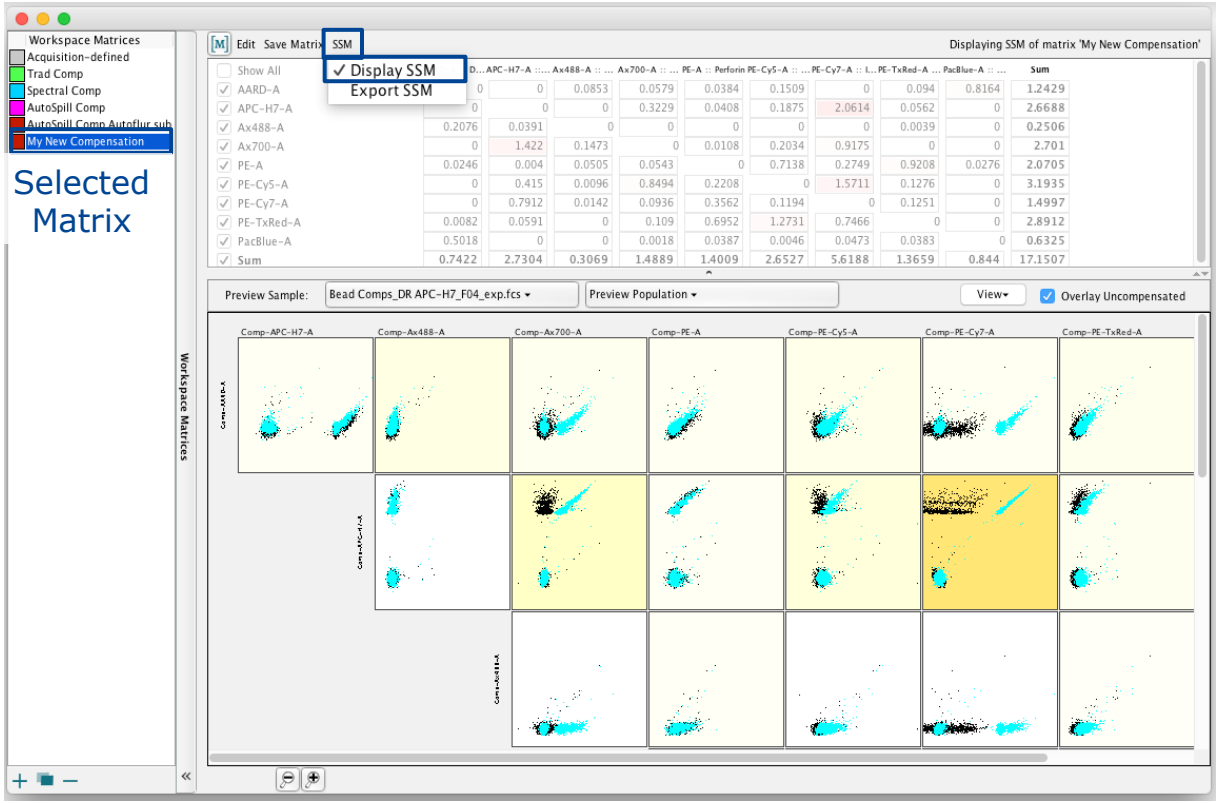
FlowJo™ v10

Select matrix, then drag-and-drop



Accessing the Spillover Spreading Matrix

Select matrix, then click SSM to Display or Export



Using a Spillover Spreading Matrix

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

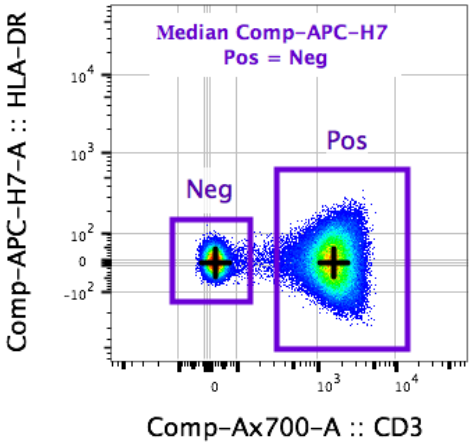
→ Loss of sensitivity for low expressing populations in that secondary channel *if* that primary fluor antigen is expressed on the same population.

Show All	B515-A :: G...	B610-A :: IL...	B660-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 :: IL...	R730-A :: C...	R780-A :: C...	U390-A :: C...	U450-A :: Li...	U500-A :: C...	U570-A :: C...	U660-A :: C...	U740-A :: C...	U785-A :: C...	V450-A :: IL...	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
B610-A	0.1127	0	2.6497	1.7932	0.8328	1.1919	[illegible]	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	[illegible]	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	0	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	[illegible]	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	[illegible]	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	9.8021	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	[illegible]	0.9366	0.5497	0.1097	0.3004	0	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	0	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	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
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
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

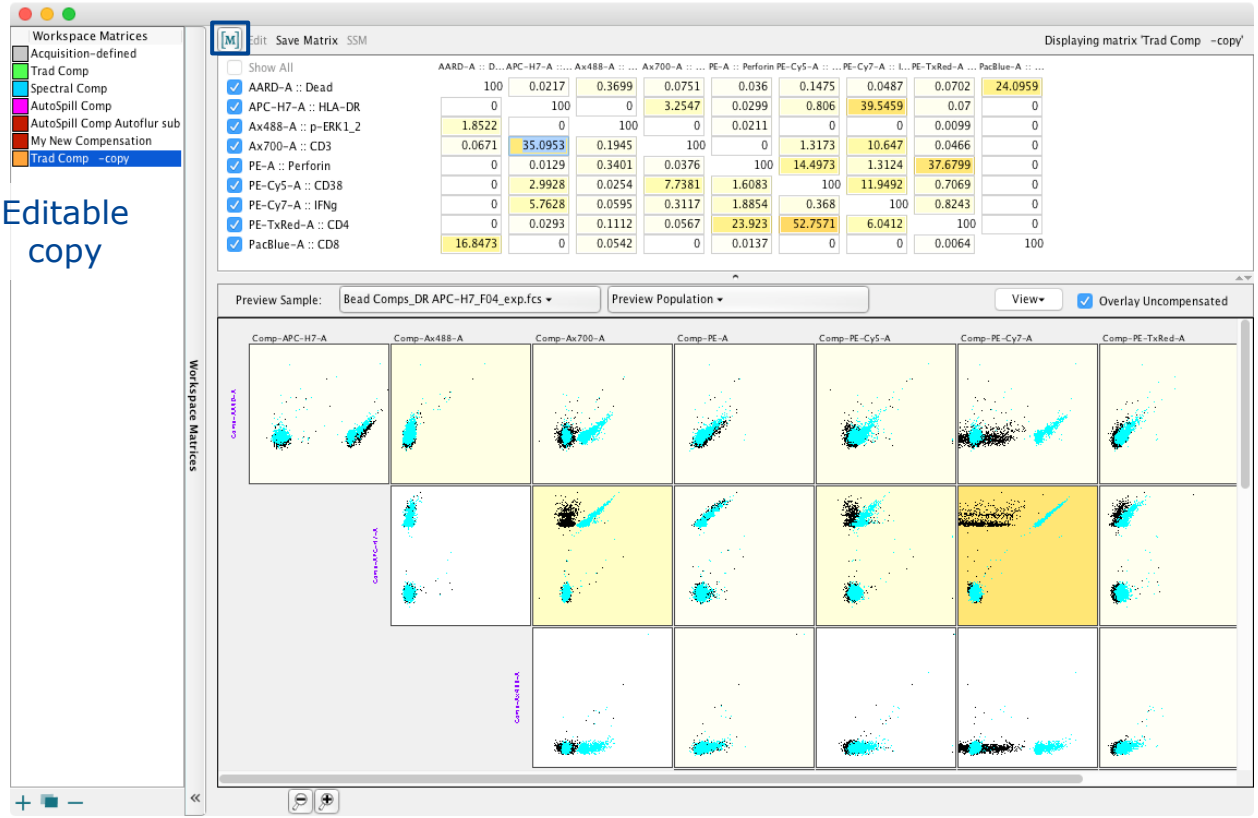
Editing Compensation

FlowJo™ v10

Click Edit → Copy where you can change values



Editable copy

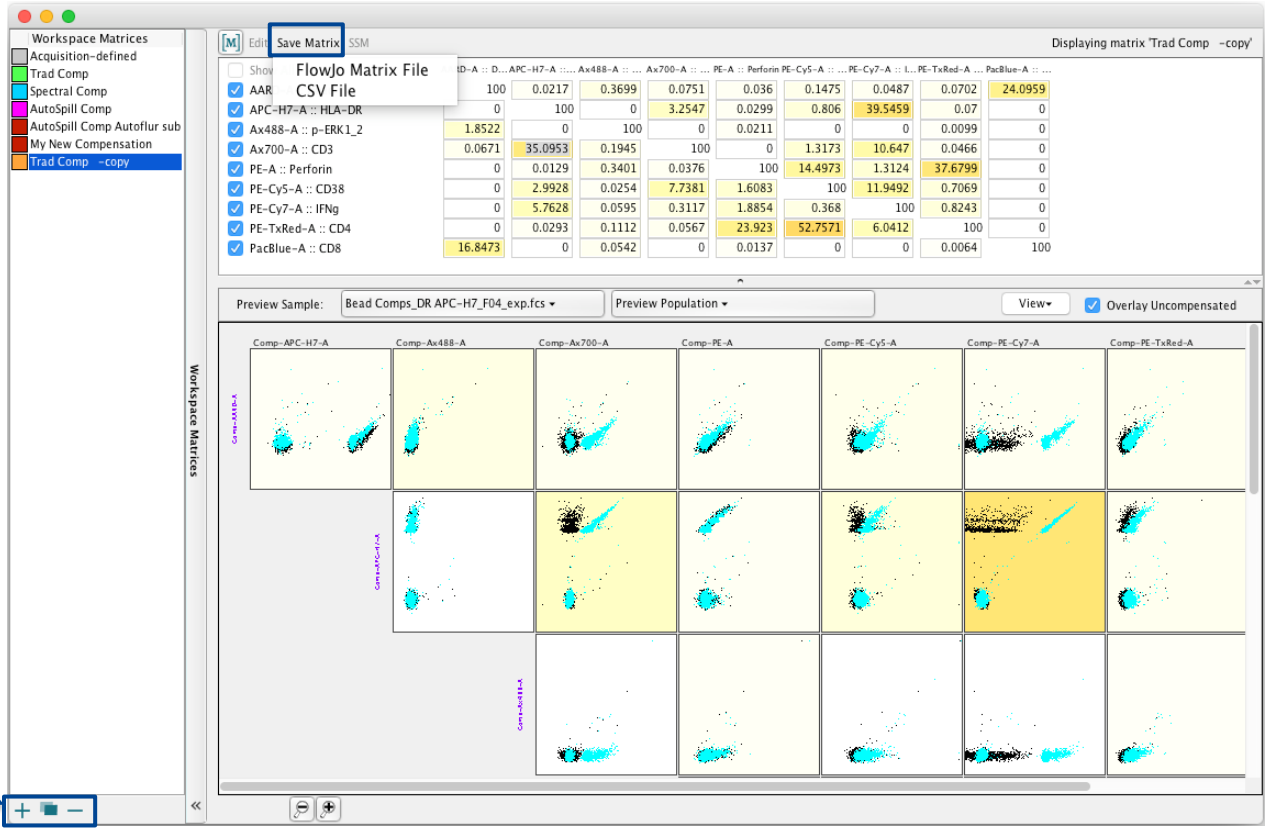


Saving Compensation

FlowJo™ v10

Click Save Matrix

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



Plugin setup

Plugins

Apps that run some external algorithm or function

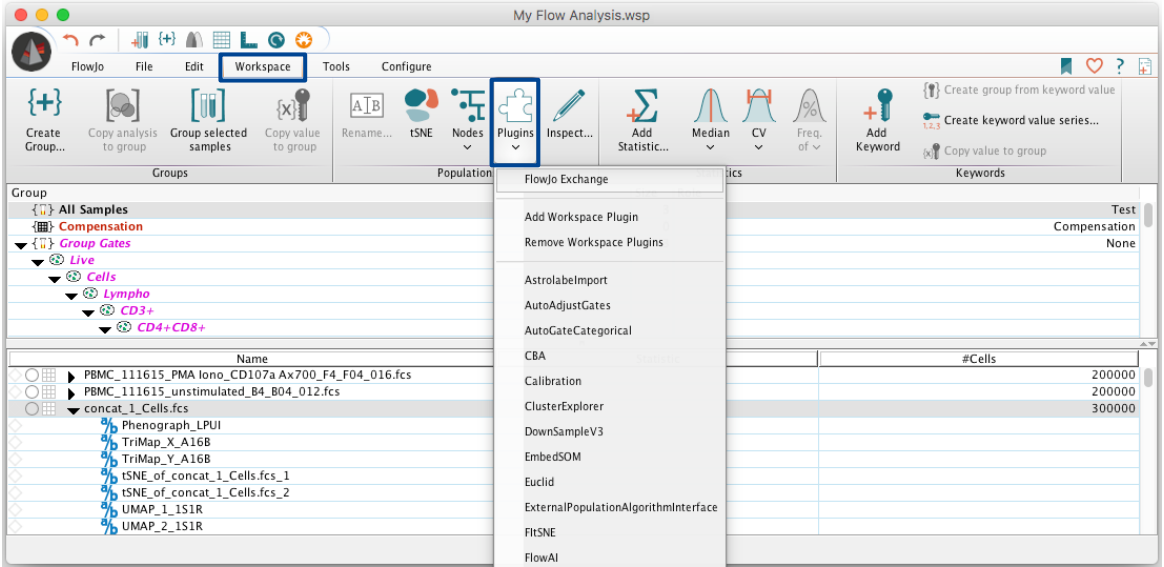
- Can be used to:
  - Cleanup samples
  - Embed data in fewer dimensions (dimensionality reduction)
  - Classify events into populations based on similarity (clustering)
  - Visualize population relationships (comparisons)

- Must be downloaded and installed prior to use

The Plugin Menu

Is located in the Workspace Tab → Populations Band

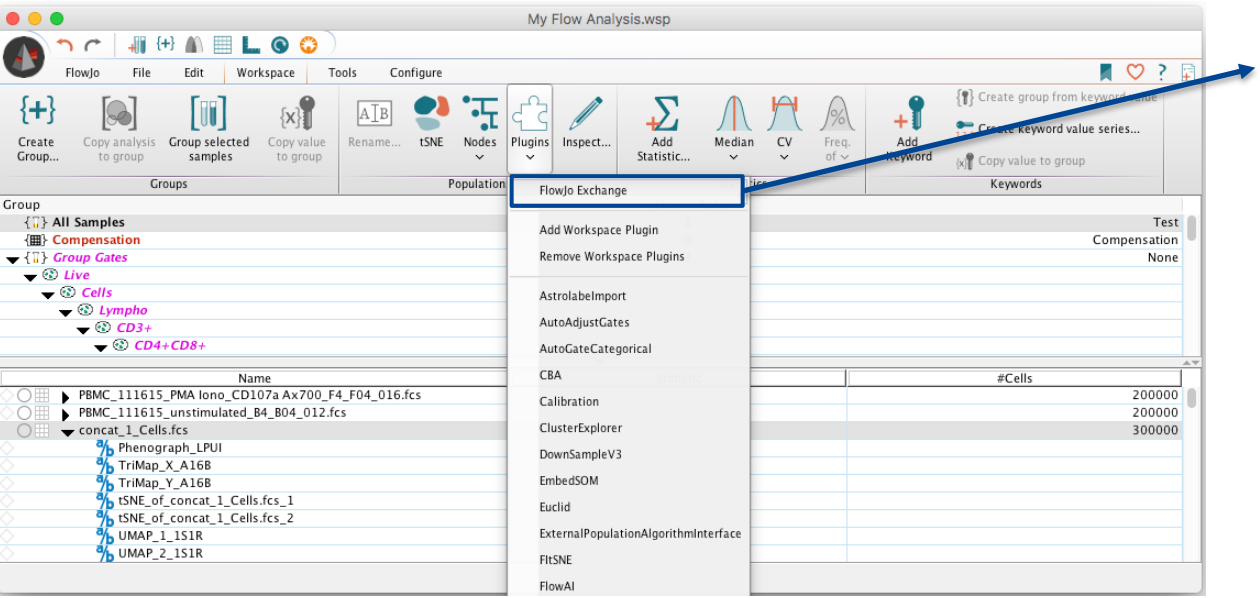
- Once plugins are downloaded and installed, they will be:
  - listed in the drop-down Plugins menu
  - used like an action button to initiate a plugin process

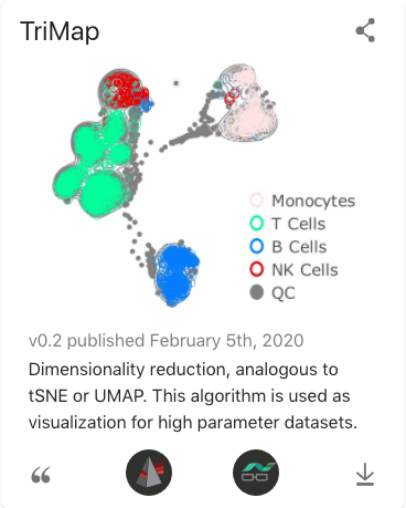


Download Plugins

From the FlowJo Exchange

- 1st plugin menu item is a link to the Exchange

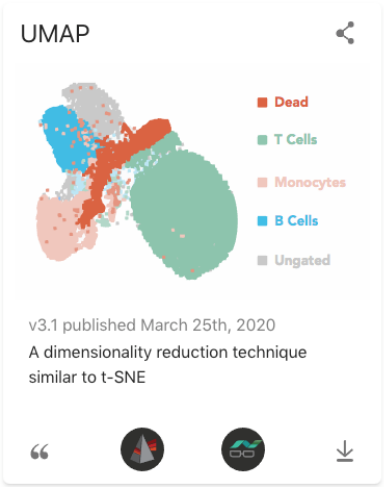


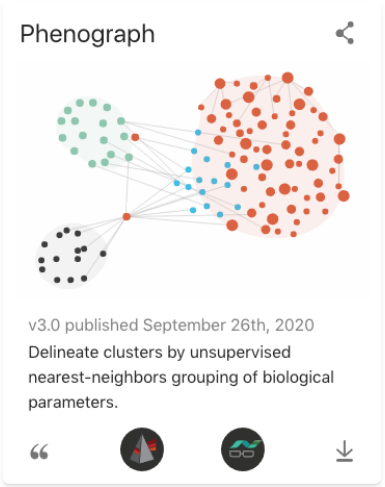


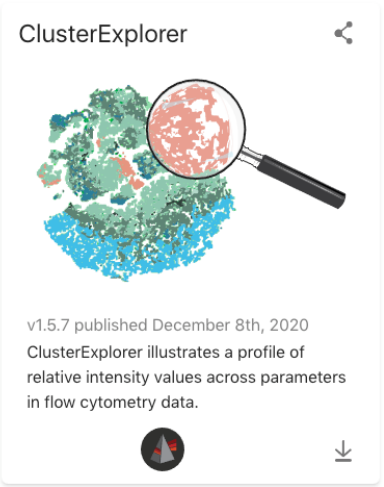
The FlowJo Exchange

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

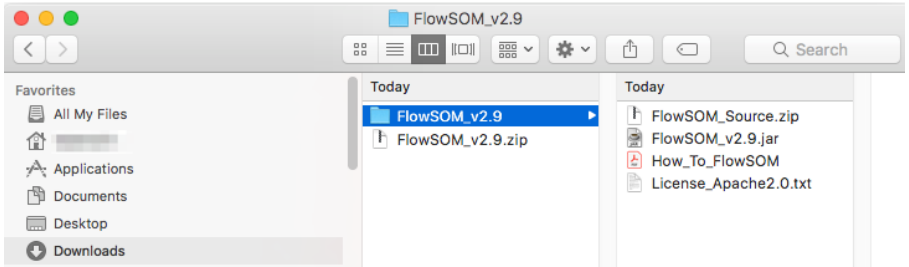
If the plugin requires R

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

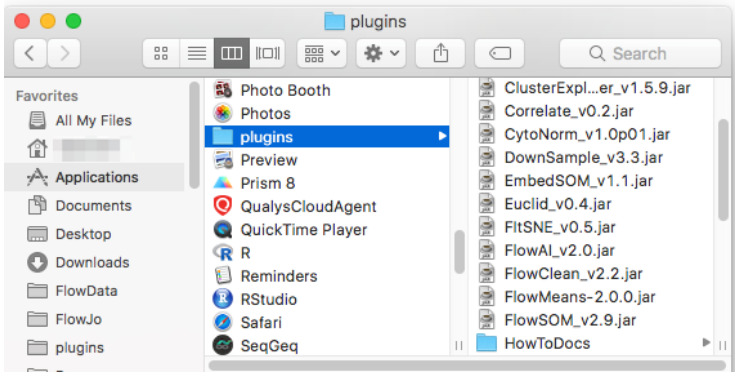
Steps 1 & 2

Apply to all plugins

1. Download plugin package and unzip



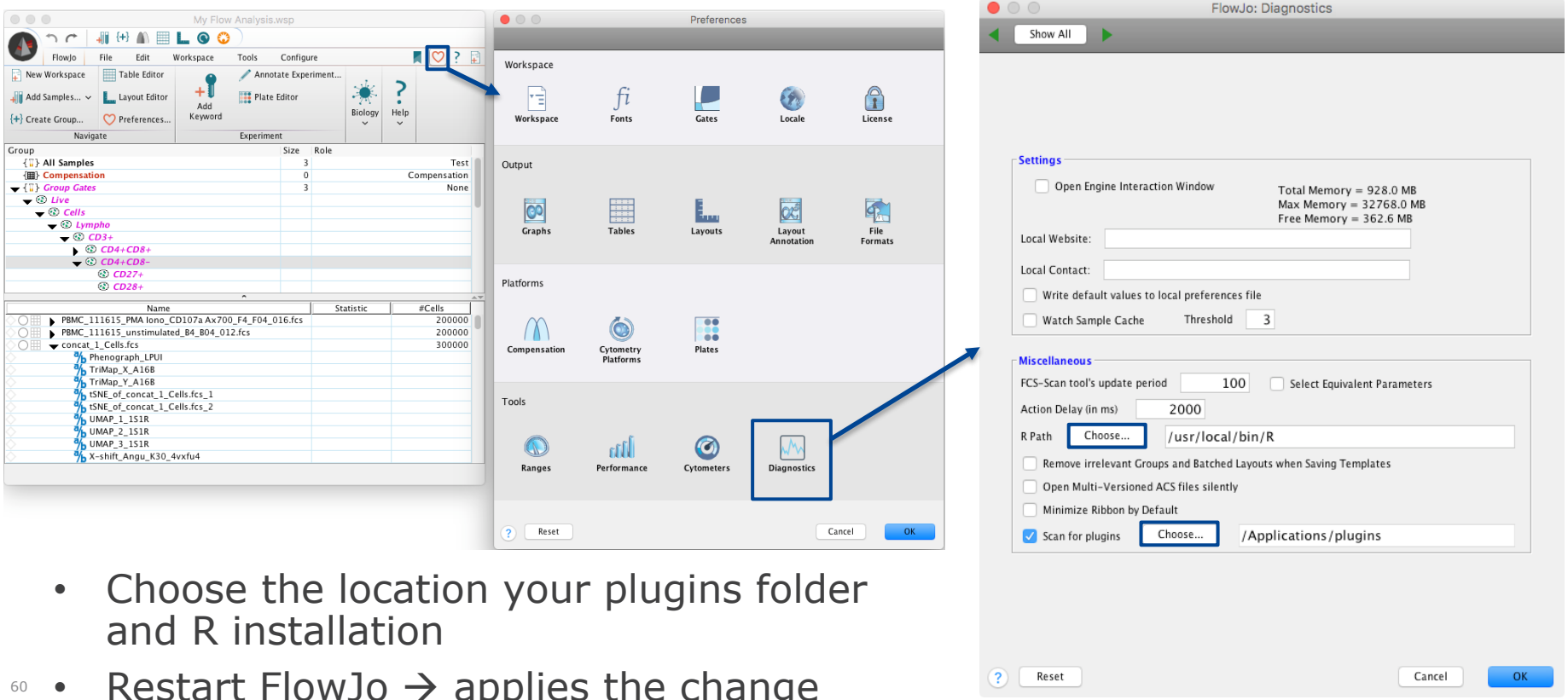
2. Put the Plugin.jar file in your plugins folder



- Restart FlowJo → the plugin name will display in your Plugins action menu

Step 3 – Configure Diagnostics Preferences

Tells FlowJo the location of your plugins and R installation



- Choose the location your plugins folder and R installation
- Restart FlowJo → applies the change

Step 4 – Install R

Required for certain plugins to run

- Navigate to https://cran.r-project.org/
- 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

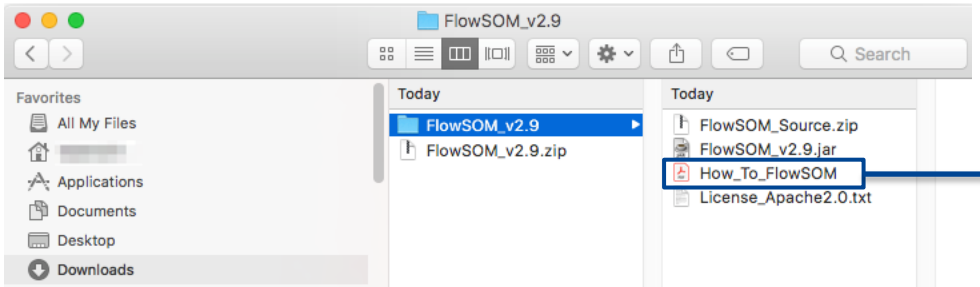
- In addition to the base R package, also download and install:
  - X-Quartz – If using a Mac. Link: https://www.xquartz.org/
  - R-Tools – If using a PC

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 >= 2.13.x; managed by Uwe Ligges). There is also information on third party software 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.

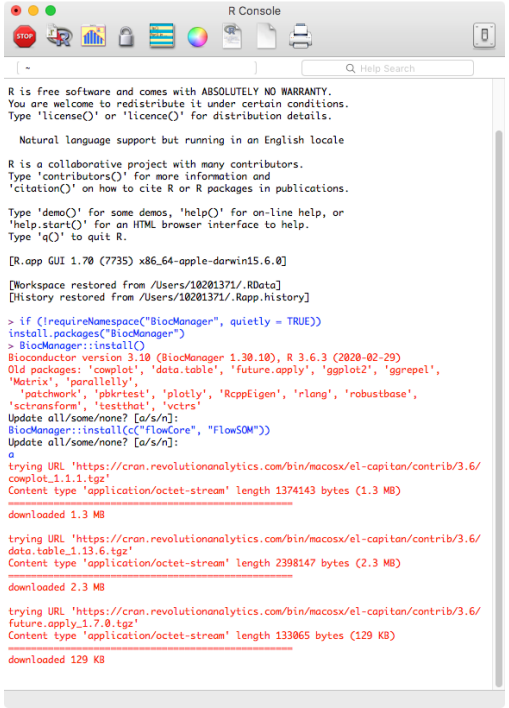
- Each Plugin downloaded from the FlowJo Exchange site will come with a "How To" document, which specifies the packaging scripts for any R dependencies.



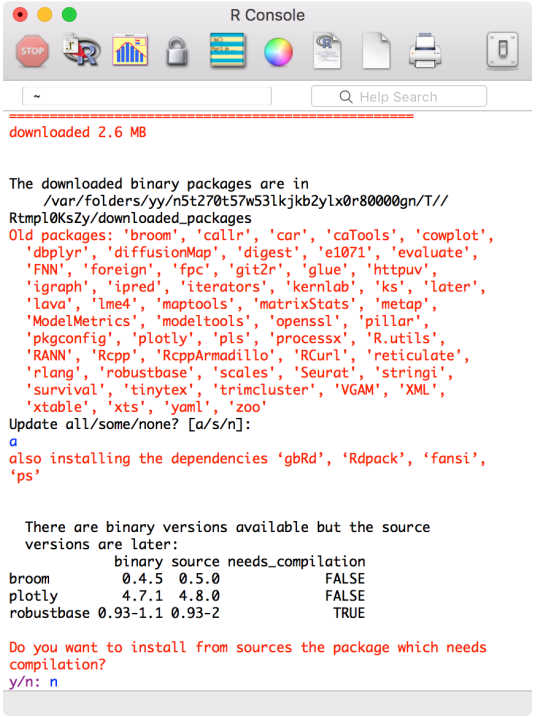

```
if (!requireNamespace("BiocManager", quietly = TRUE))
install.packages("BiocManager")
BiocManager::install()

BiocManager::install(c("flowCore", "FlowSOM"))

install.packages("pheatmap")
```

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)



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
FlowAI	TriMAP	Phenograph	***Euclid***
FlowClean	***EmbedSOM***	***flowMeans***	
IndexSort			

Visualization	Utility
ViolinBox	CBA
MiST	StainIndex
Sunburst	***iCellR***
Correlate	PluginWizard

Bold-Italic → Requires R

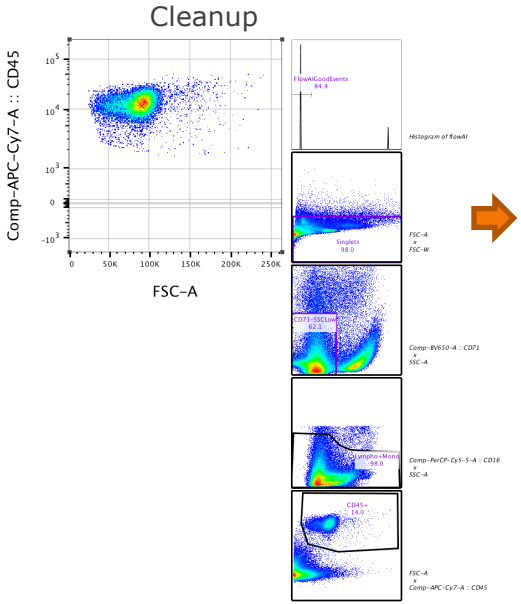
Why Pugins?

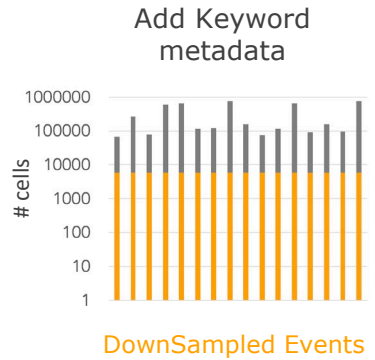
- Plugins Enable:
  - Quick access to algorithmic processes
  - Point and click interface for non-programmers
- Allowing for:
  - Data clean up
  - Population discovery
  - Exploration
  - Visualization
  - Computational Sorting

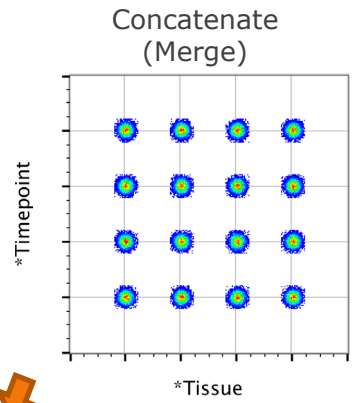
Employing Plugins

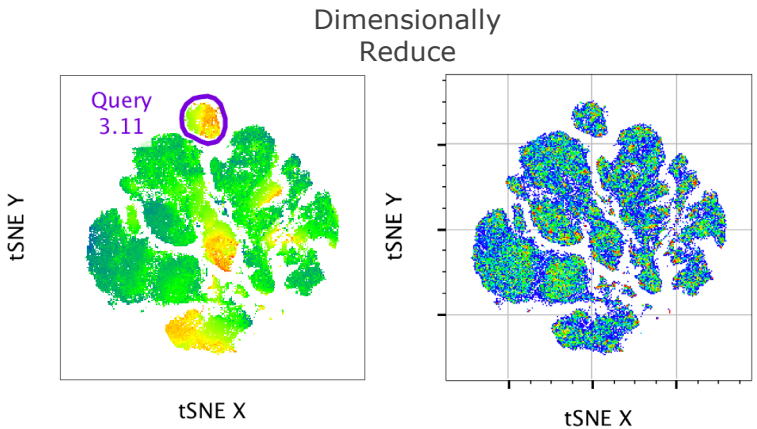
As part of a discovery workflow

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





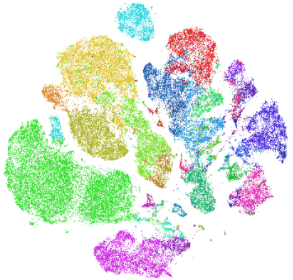




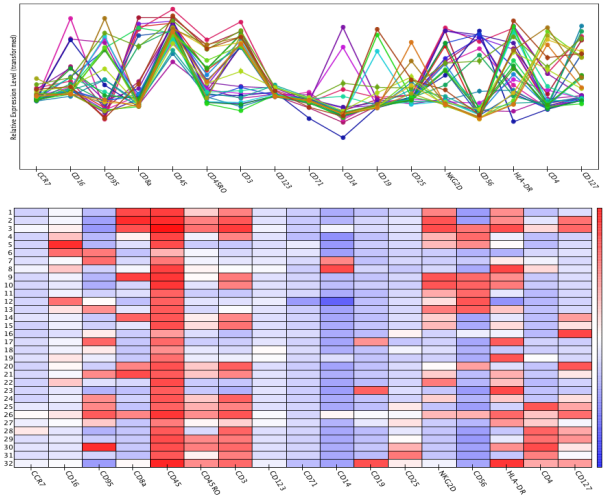
Employing Plugins

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

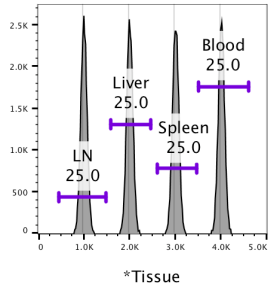
Cluster



Explore



Gate



Identify Differentially Expressed Populations

Compare Conditions

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

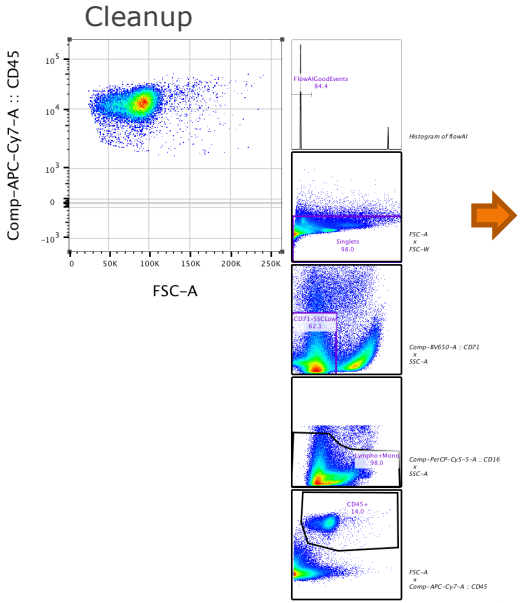
Dimensionality Reduction and Clustering

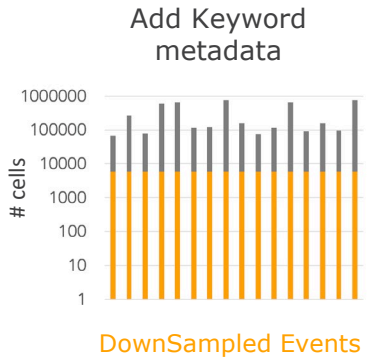
Analysis Workflow

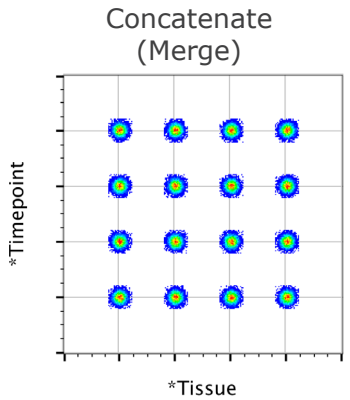
FlowJo™ v10

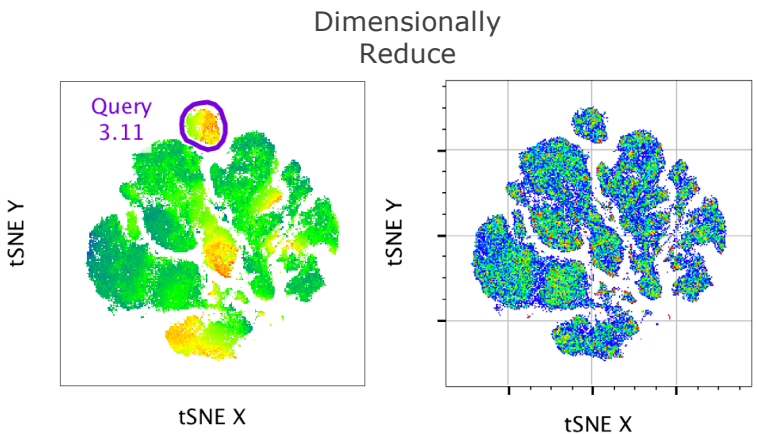
for Discovery

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







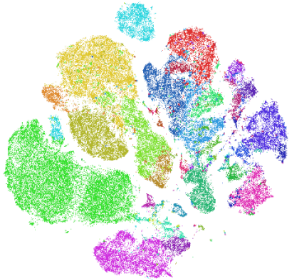


Analysis Workflow

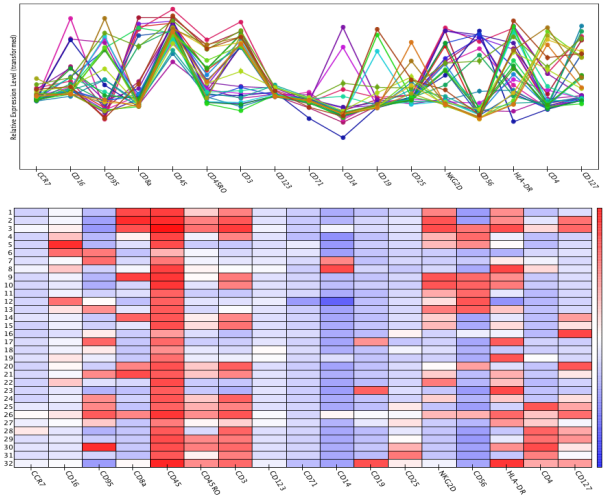
for Discovery

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

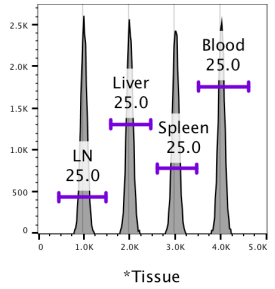
Cluster



Explore



Gate



Identify
Differentially Expressed
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Compare Conditions

FlowJo™ v10

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IndexSort			

Visualization	Utility
ViolinBox	CBA
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Correlate	PluginWizard

Bold-Italic → Requires R

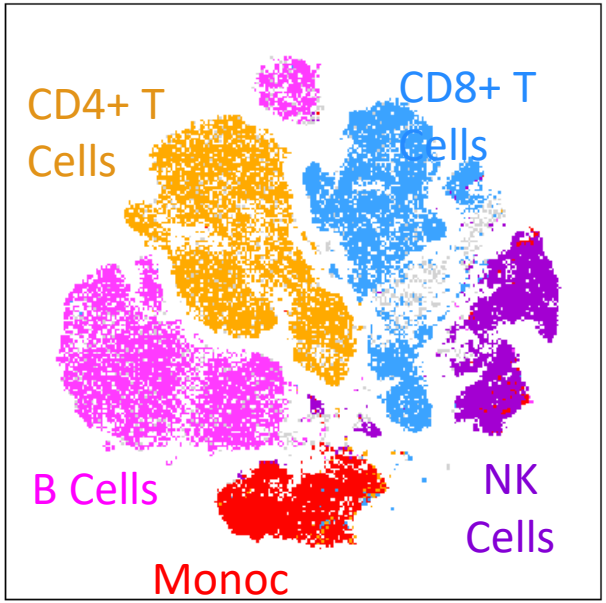
Dimensionality Reduction

FlowJo™ v10

creates new derived parameters

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

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

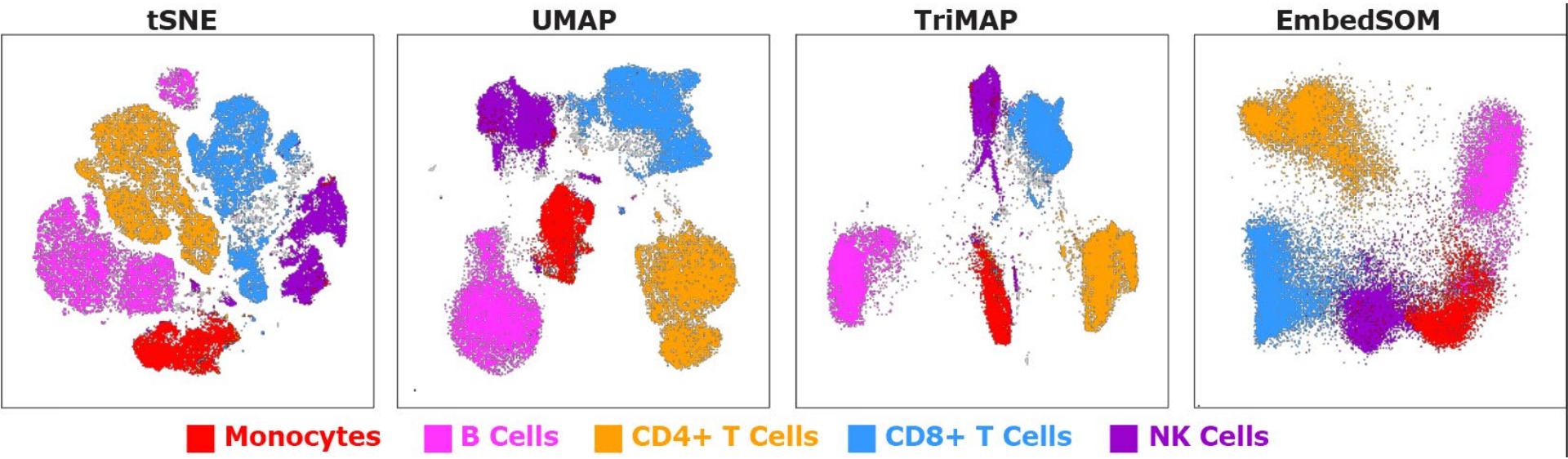


- Events with a similar multi-dimensional expression pattern group together within the dimensionally reduced data space

Dimensionality Reduction Options

FlowJo™ v10

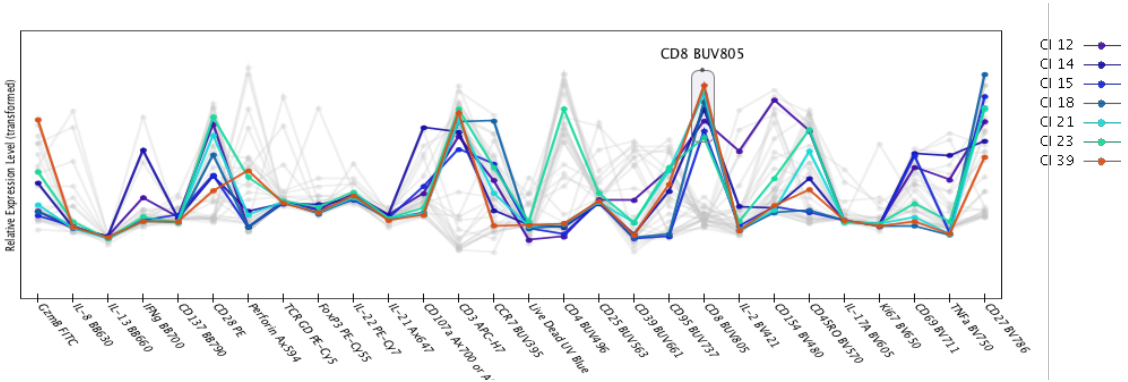
- t-SNE = t-Distributed Stochastic Neighbor Embedding
- UMAP = Uniform Manifold Approximation and Projection
- TriMAP = Triplet Manifold Approximation and Projection
- EmbedSOM = 2D visualization of FlowSOM clustering output

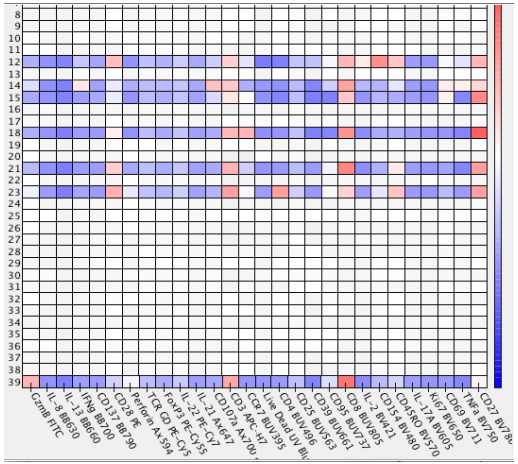


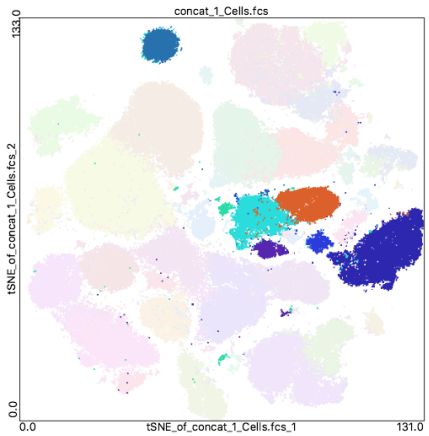
DR Method Comparison

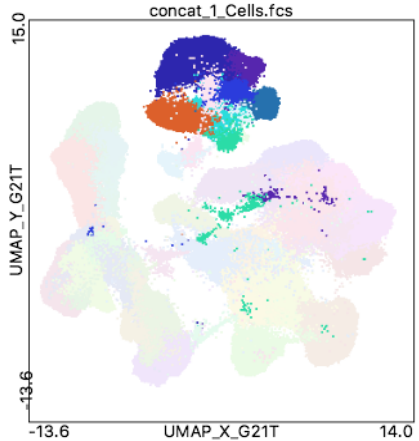
FlowJo™ v10

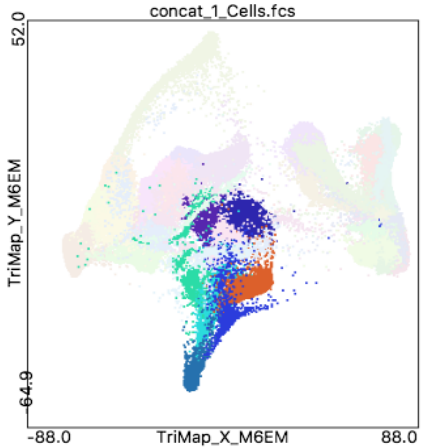
- Gate on CD8+ Phenograph Clusters → 7 populations
- Populations are fragmented with t-SNE but group together in UMAP or TriMap





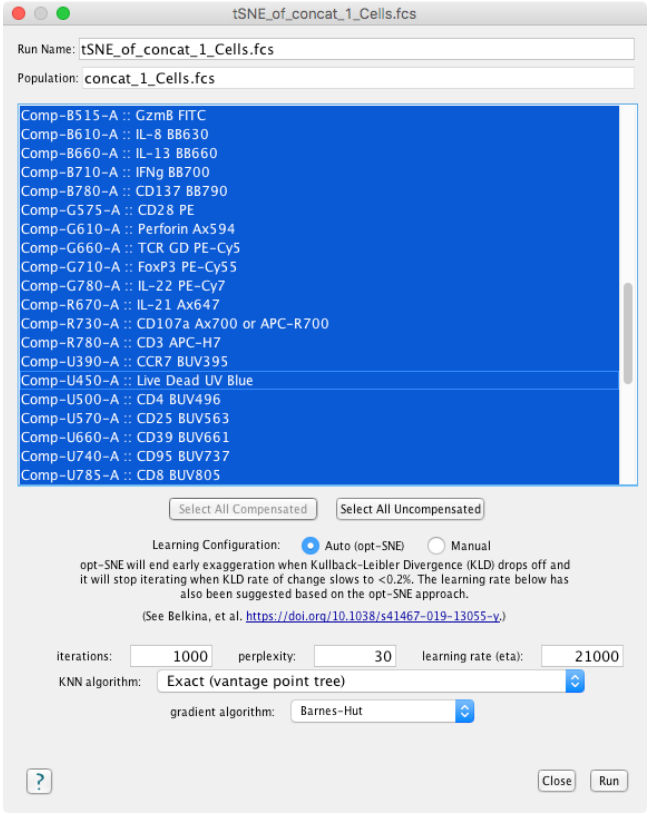


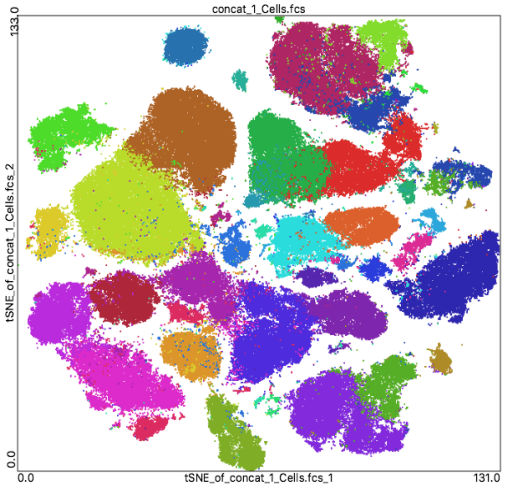




t-SNE

the GUI details





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

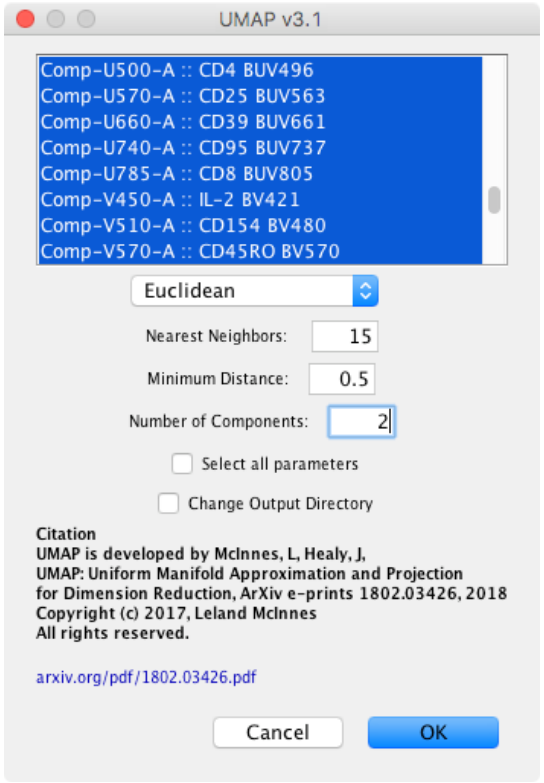
Learning Rate (eta) – How fast you get to a solution. Small → gradual changes per iteration, slower. Large → larger changes, faster.

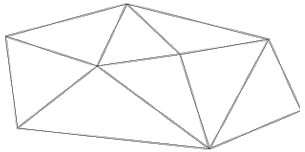
KNN – Exact (vantage point tree) vs Annoy (Random Forest Projection)

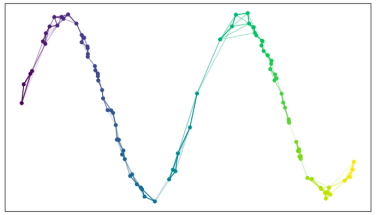
Gradient Algorithm – Barns-Hut vs Fit-SNE

UMAP

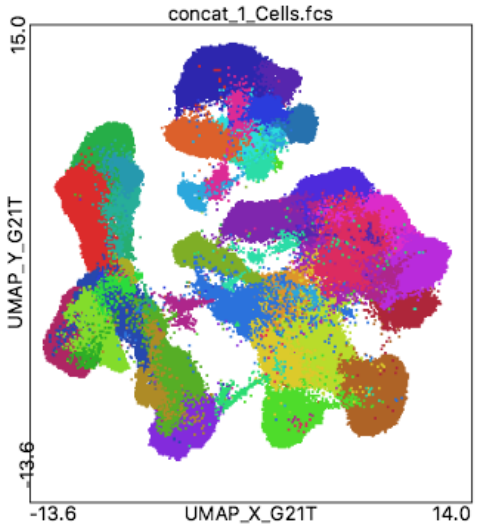
FlowJo™ v10




A simplicial complex

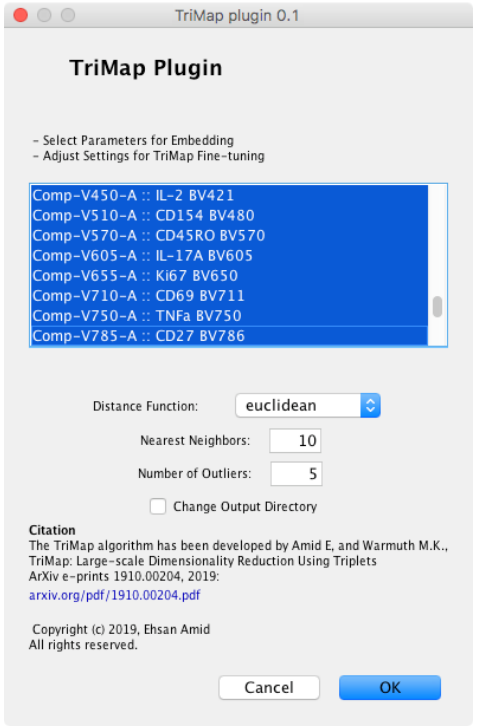


- **How does it work?**
  - Neighbor graph style approach that uses simplices to build a manifold approximation of the data structure, making an effort to preserve the topological global structure of that data.
- **How well does it work?**
  - Equally meaningful representations compared with t-SNE
  - 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

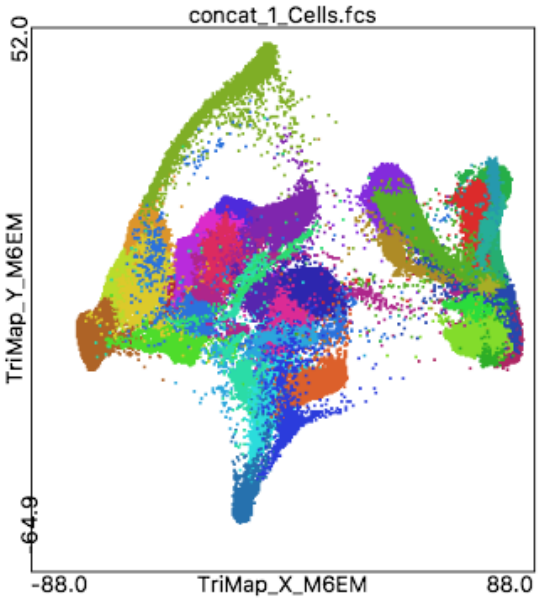


TriMAP

FlowJo™ v10



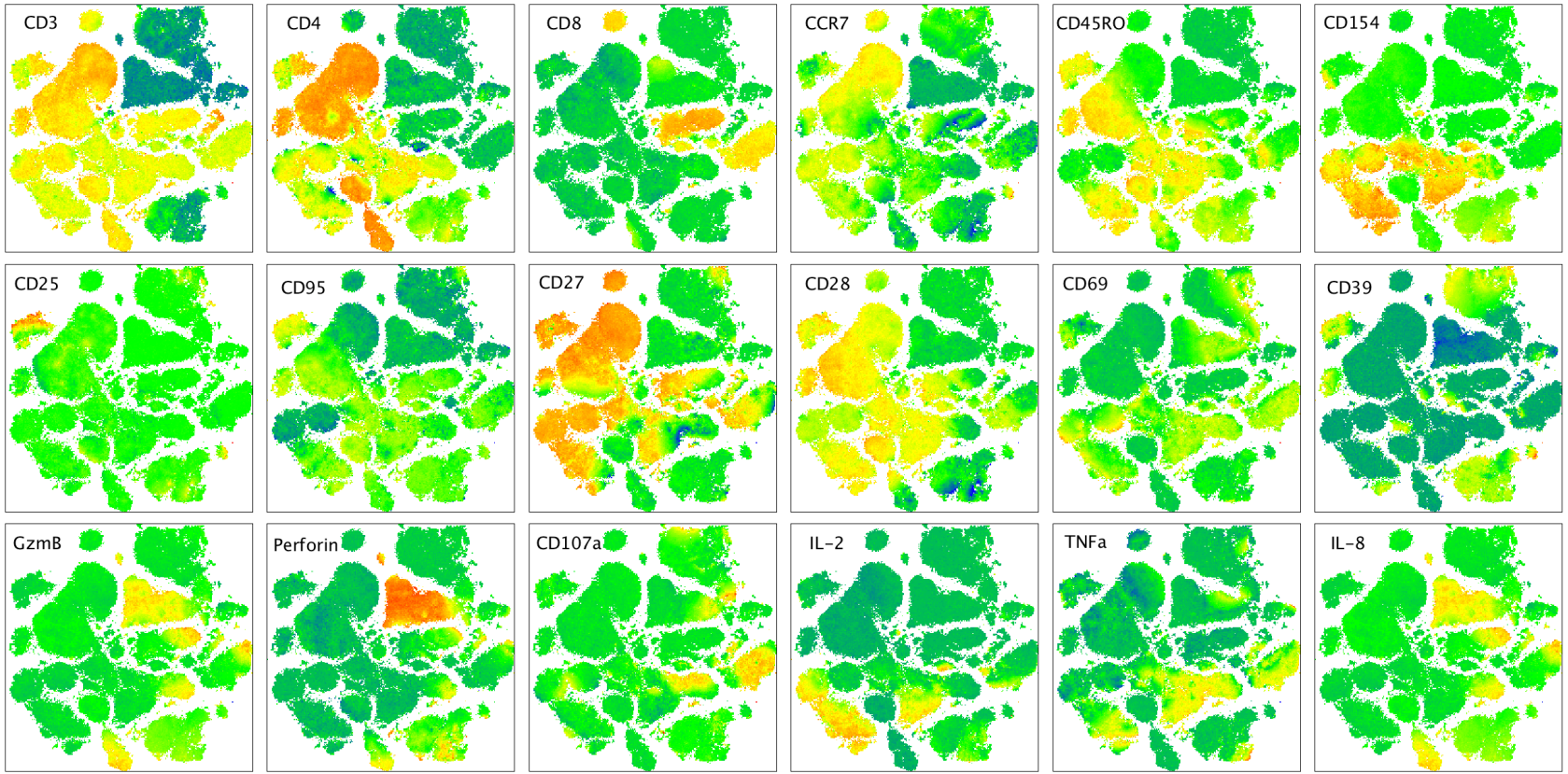
- **How does it work?**
  - Semi-supervised metric learning
  - Initialized with low dimensional PCA embedding, and this embedding is then modified using a set of selected triplets from the high-dimensional representation
- **How well does it work?**
  - Meaningful representations
  - Faster than tSNE
  - Scales well (comparable to UMAP)
  - Preserves *Global Structure*



3rd Parameter Heat Map

FlowJo™ v10

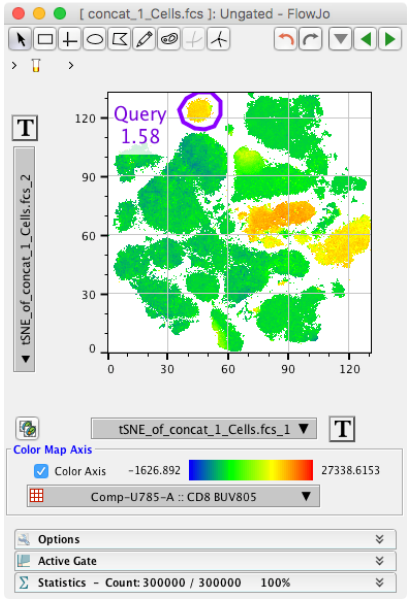
orient and identify

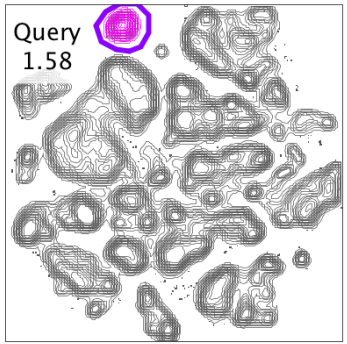


Query Gate

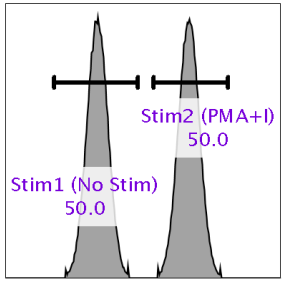
and explore

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

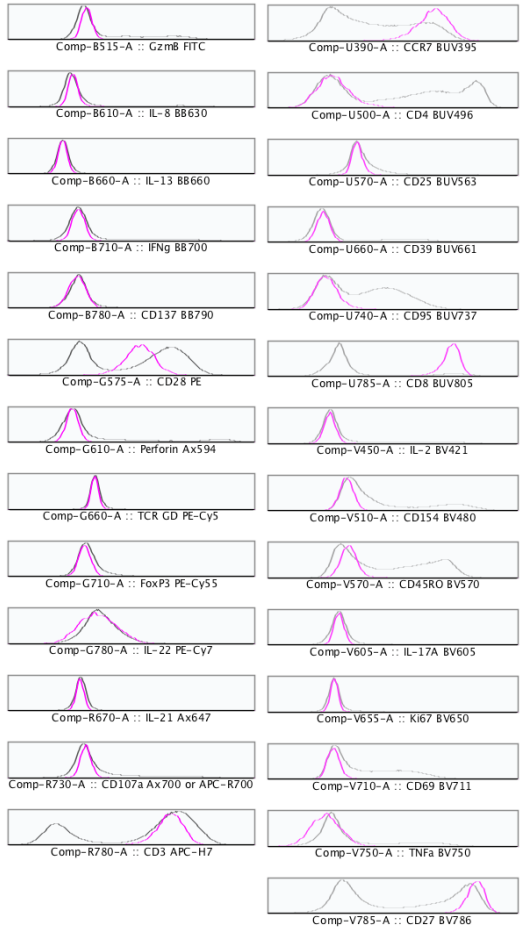




Sample Name	Subset Name	Count
concat_1_Cells.fcs	Query	4748
concat_1_Cells.fcs	Ungated	300000



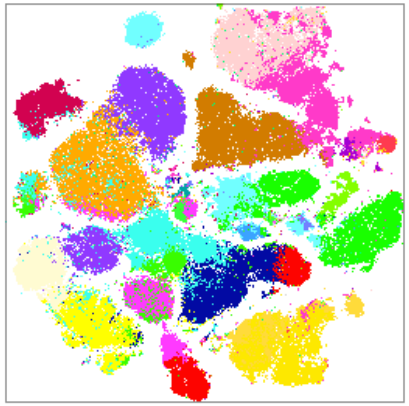
Name	Statistic
Query	1.58
Stim1 (No Stim)	98.3
Stim2 (PMA+I)	1.66



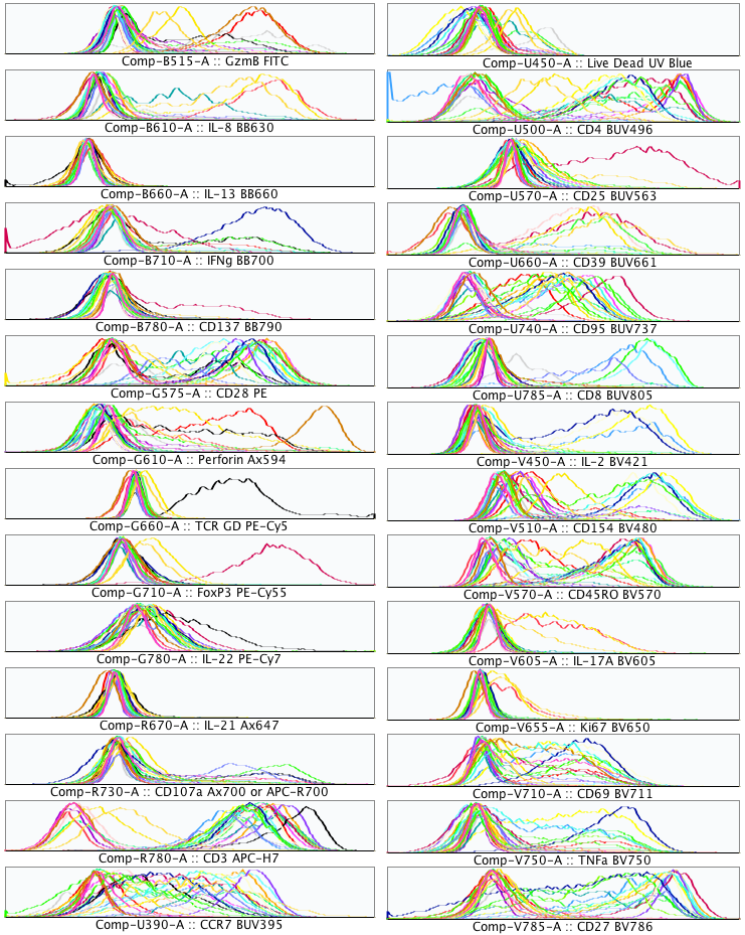
Clustering

creates populations

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



- Overlay cluster populations in the LE
- Right click and choose Make Multigraph Overlays → Histograms

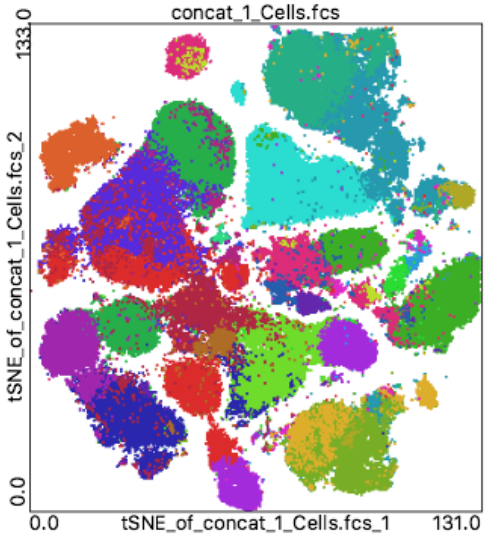


Clustering Options

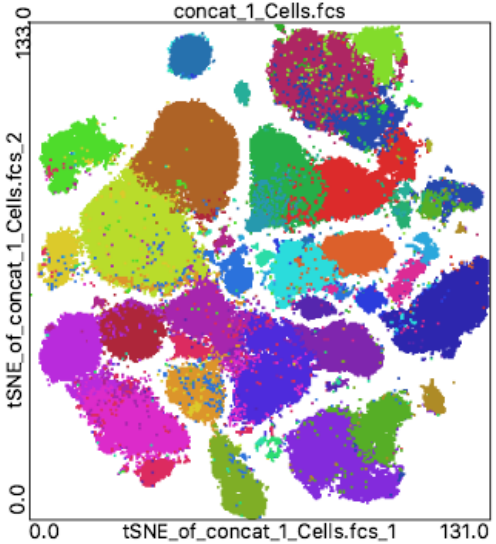
FlowJo™ v10

- X-Shift – Fast KNN Density Estimation
- PhenoGraph – Nearest Neighbors Graph → Communities
- FlowSOM – Self Organizing Map

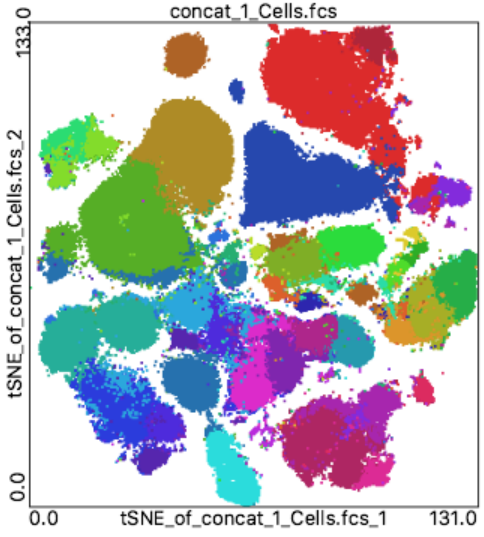
X-Shift



PhenoGraph

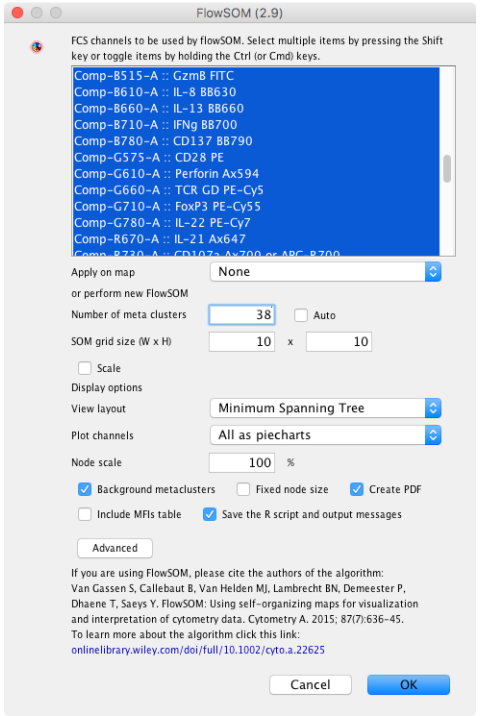


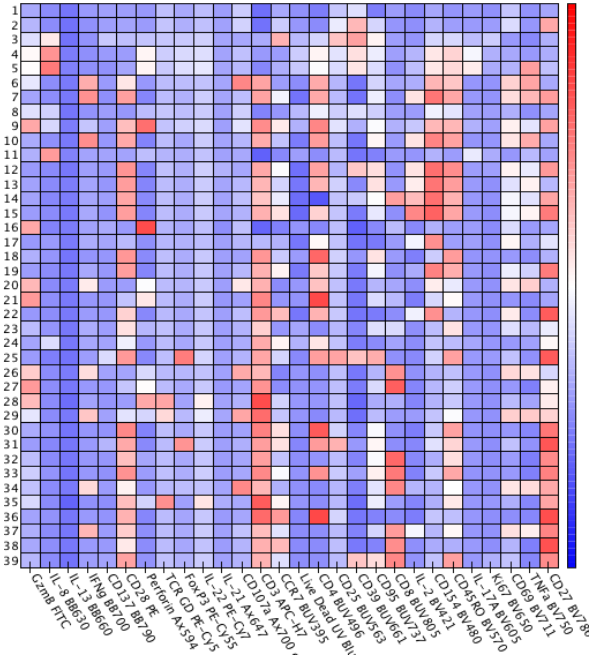
FlowSOM

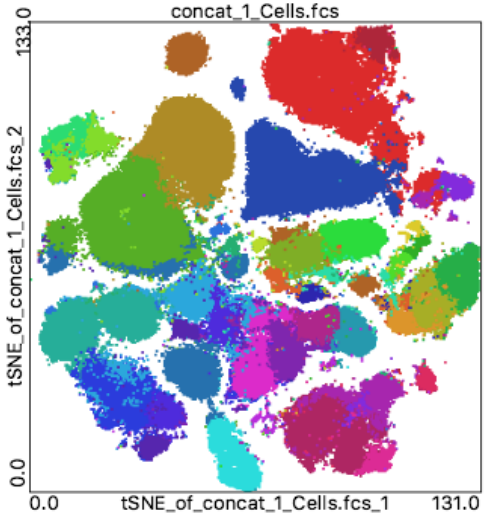


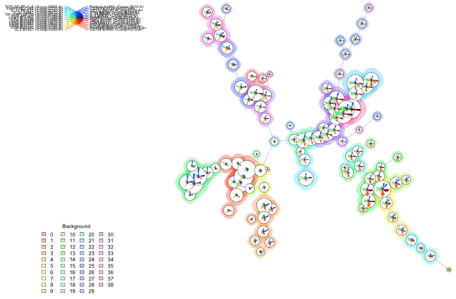
FlowSOM

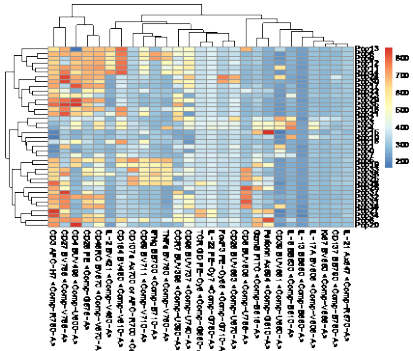
FlowJo™ v10







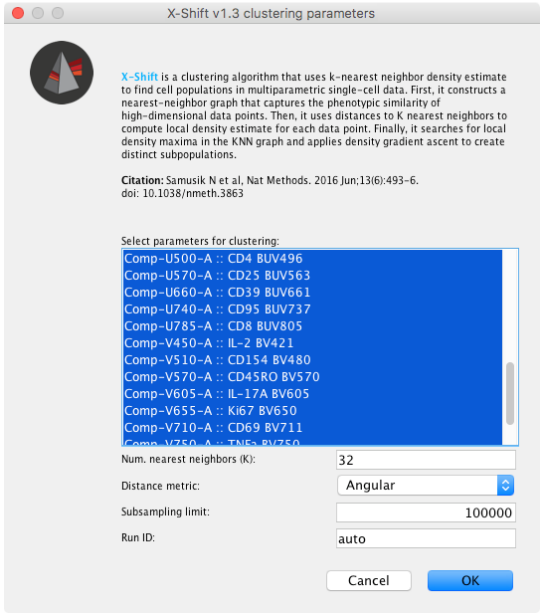


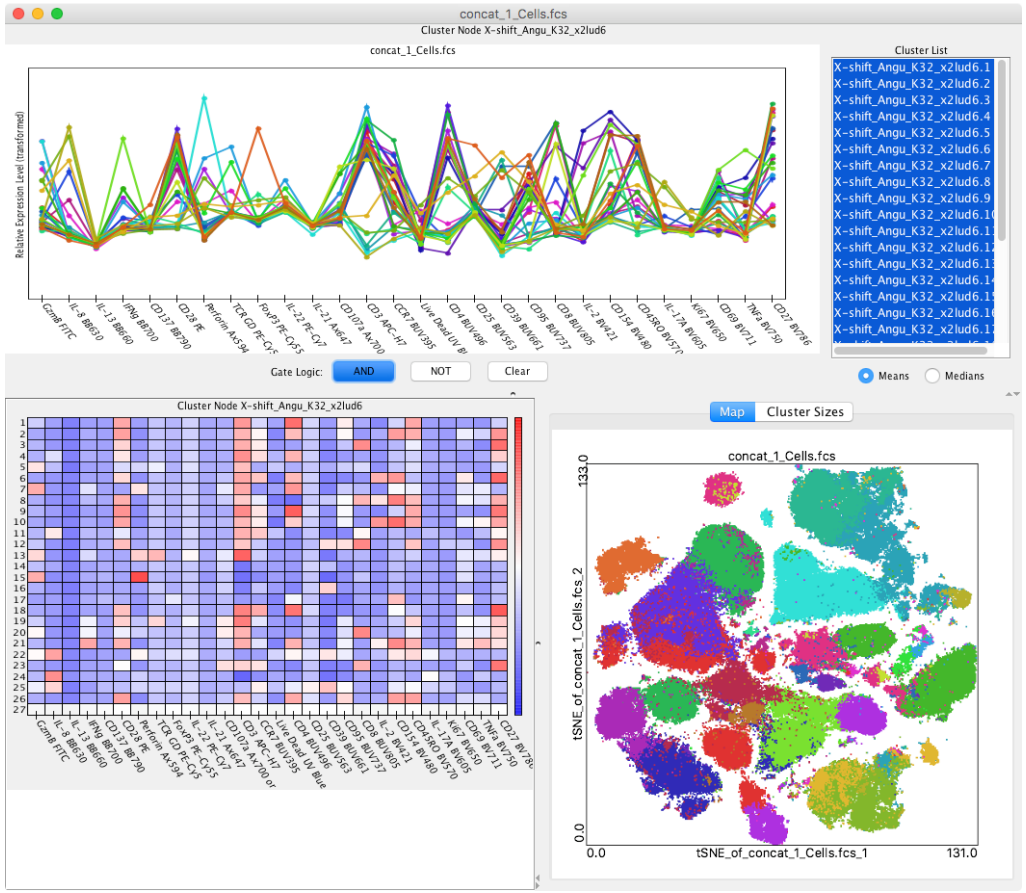


- Analyzes using a self-organizing map and K nearest neighbors to generate cluster populations

X-Shift

FlowJo™ v10

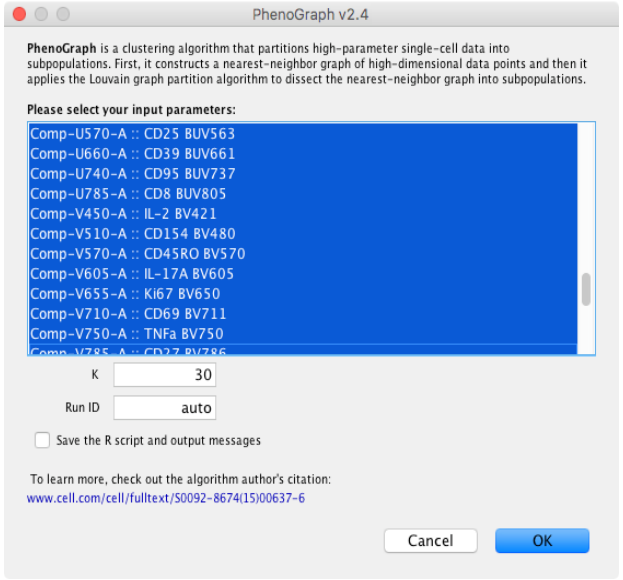




- K-nearest neighbor density estimation

PhenoGraph

FlowJo™ v10



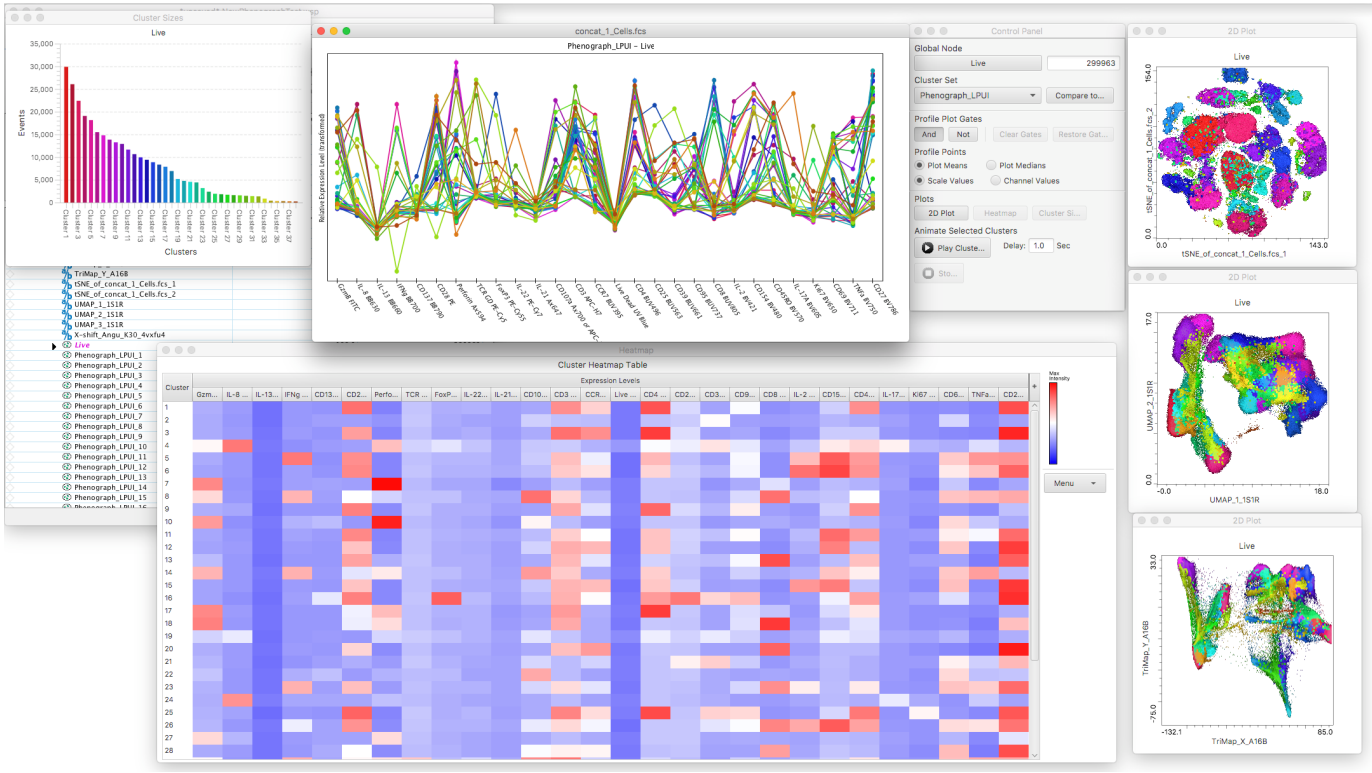
- Constructs a nearest neighbor graph and partitions the graph into communities

ClusterExplorer

FlowJo™ v10

gating and comparison of cluster populations

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



Now What?

FlowJo™ v10

- 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…

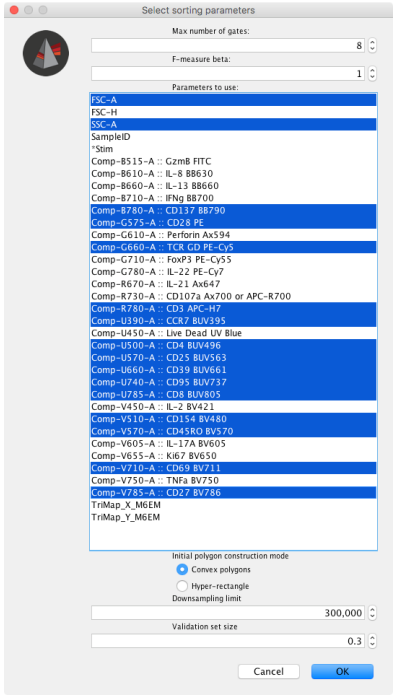
Question becomes: How do you sort a population identified with a clustering algorithm?

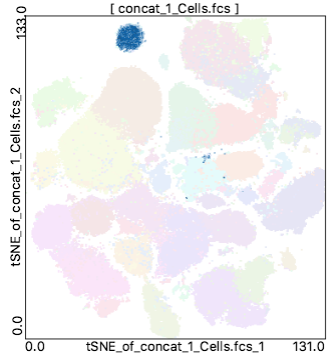
HyperFinder

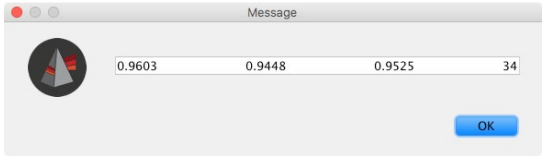
FlowJo™ v10

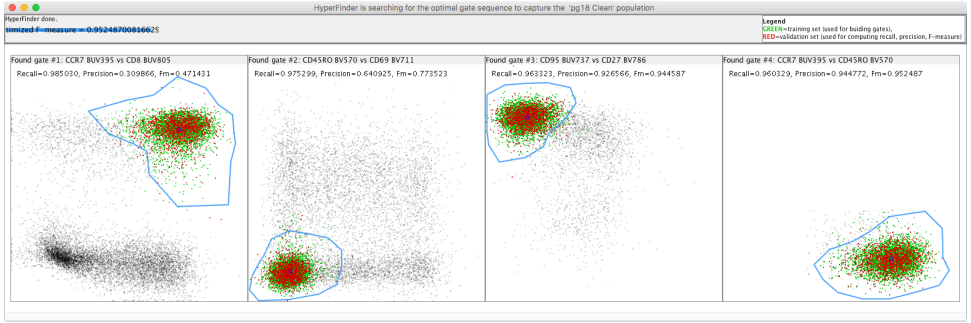
generate an optimized gating strategy for any population

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







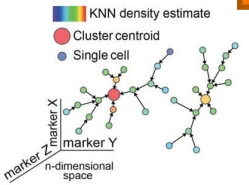


Computational Sorting Workflow

FlowJo™ v10

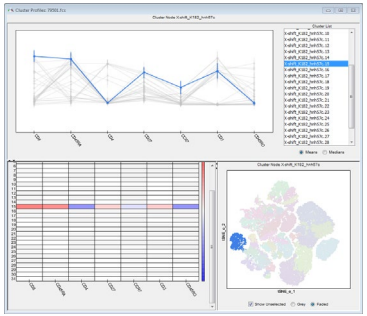
1) Clustering

FlowJo plugins
X-shift
PhenoGraph
FlowSOM



Finding the cluster of interest

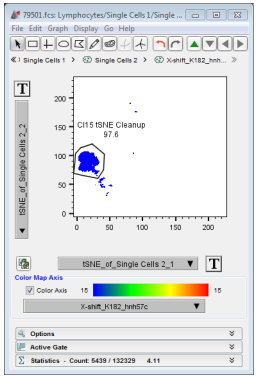
ClusterExplorer FlowJo plug-in



10-15 min on 100K dataset

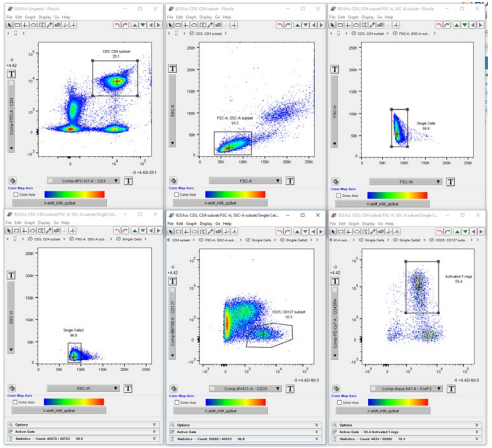
t-SNE Cleanup

Removing the outliers by t-SNE gating on the clustered population



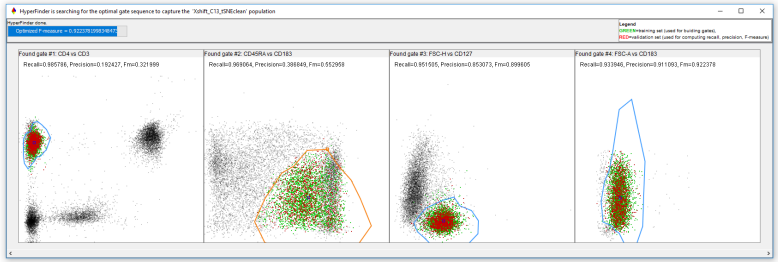
Use case:
Objectively define a gating strategy for a multidimensional clustered population

2) Conventional gating

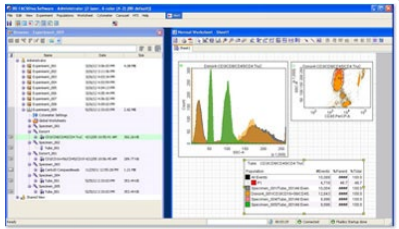


Use cases:
1) Shorten the gating strategy
2) Try and capture the same population while leaving some markers out

HyperFinder

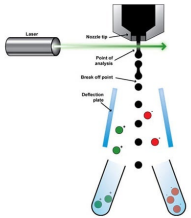


BD FACSDiva™



BD FACSDiva™ Export function available in FlowJo v10.6+

Sorting



Thank You!

Questions?

Thank You!