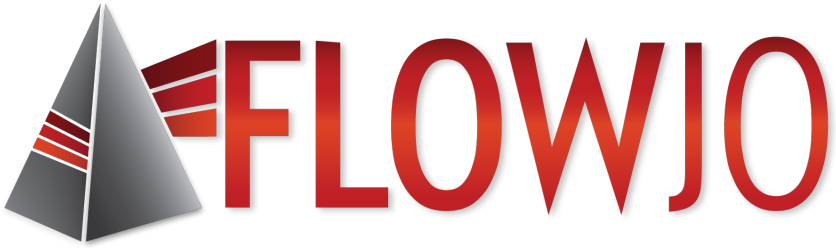
Cytometry Data Analysis in FlowJo V10



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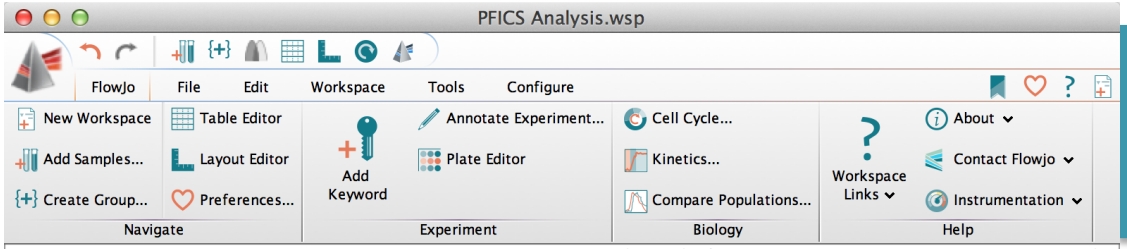
Outline

- Navigating the V10 Workspace
- Demo Data background
- Creating and editing Groups
- Keyword attributes and their uses
- The Graph Window, gating and ancestry
- The Layout Editor – exporting graphics
- The Table Editor – exporting statistics
- Workspace Templates
- Compensation

The FlowJo v10 Workspace

- A graphical interface to organize your data.



Ribbon
Tabs and Bands

Group	Size	Role
All Samples	46	Test
All Stain	20	Test
Compensation	12	Compensation
Compensation		
FMOs	14	Controls
Master Gates	34	None
Time		
Singlets		
Lymphocytes		
Live		
CD3+		
Q1: CD4- , CD8+		
Σ Geometric Mean : Comp-Ax488-A (p-ERK1_2)		
IFNg+		
Σ Freq. of Parent		
Perf+		
pERK+		

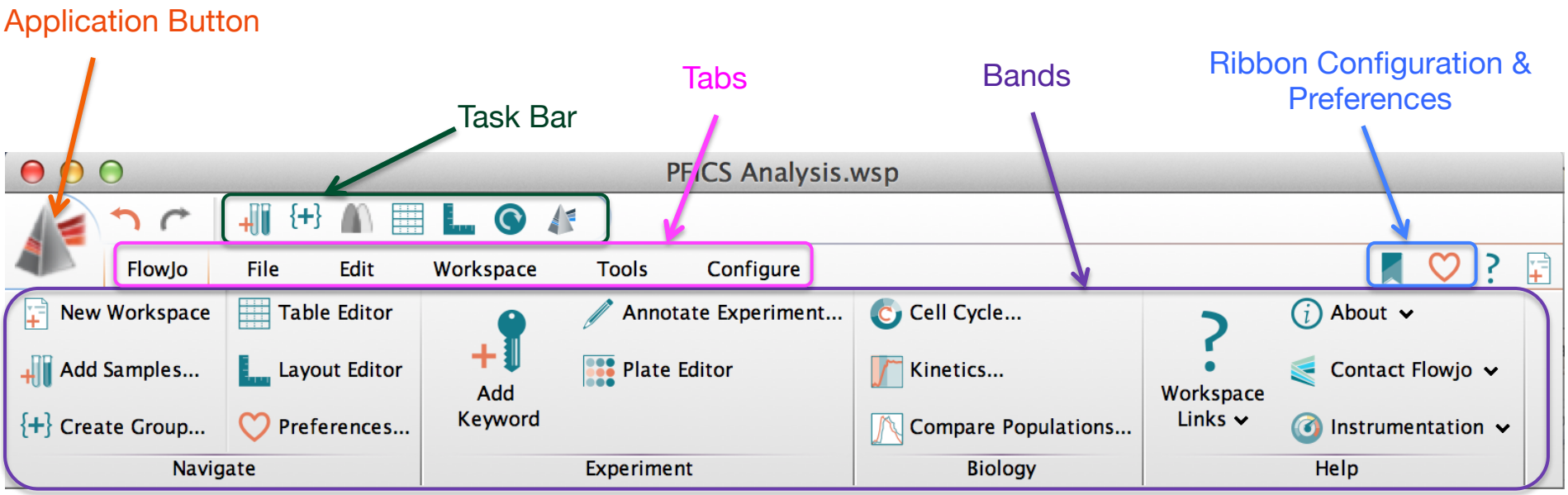
Groups and Group
Analysis

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
LD1_PI+NS_B01_exp.fcs		262774	LD1	PI+NS	B01
LD1_PI+PI_D01_exp.fcs		244977	LD1	PI+PI	D01
LD2_NS+NS_A02_exp.fcs		330780	LD2	NS+NS	A02
LD2_NS+PI_C02_exp.fcs		286306	LD2	NS+PI	C02
LD2_PI+NS_B02_exp.fcs		279202	LD2	PI+NS	B02
Time	100.0	279199			
Singlets	96.3	268967			
Lymphocytes	91.3	245663			
Live	73.6	180798			
CD3+	81.7	147761			
Q1: CD4- , CD8+	25.1	37017			
Σ Geometric Mean : Comp-Ax488-A (p-ERK1_2)	424				
IFNg+	64.1	23716			
Σ Freq. of Parent	64.1				
Perf+	52.9	19580			
pERK+	93.2	34514			

Samples and
sample analysis

Ribbons, Tabs and Bands

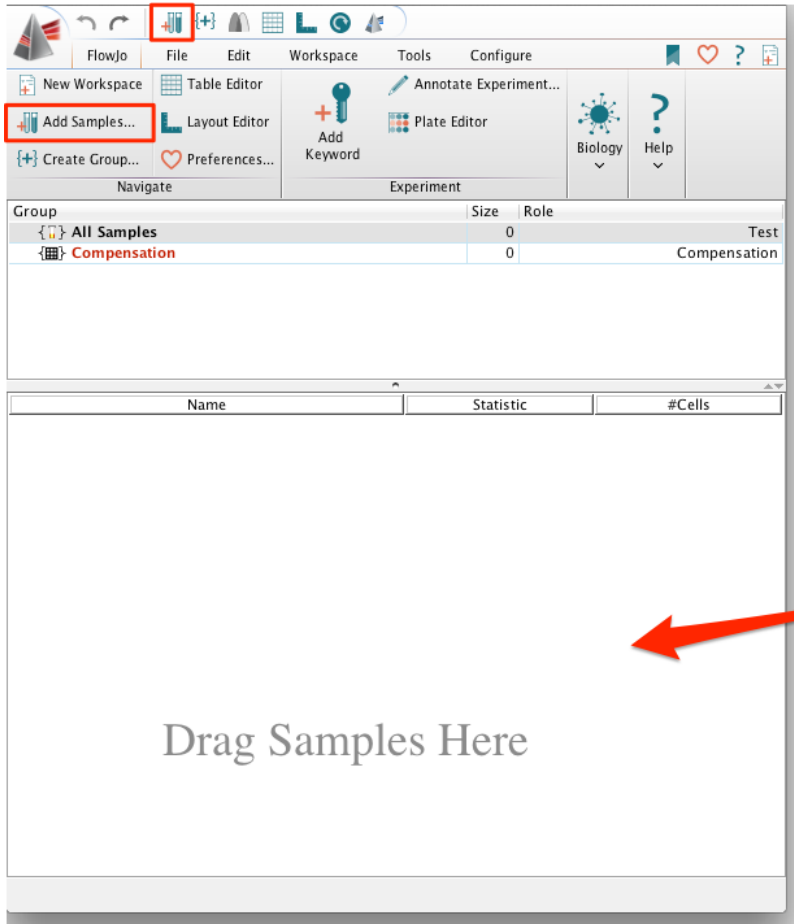
- Ribbon organization allows easy visual navigation of workspace functions.

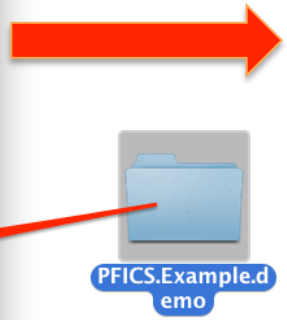


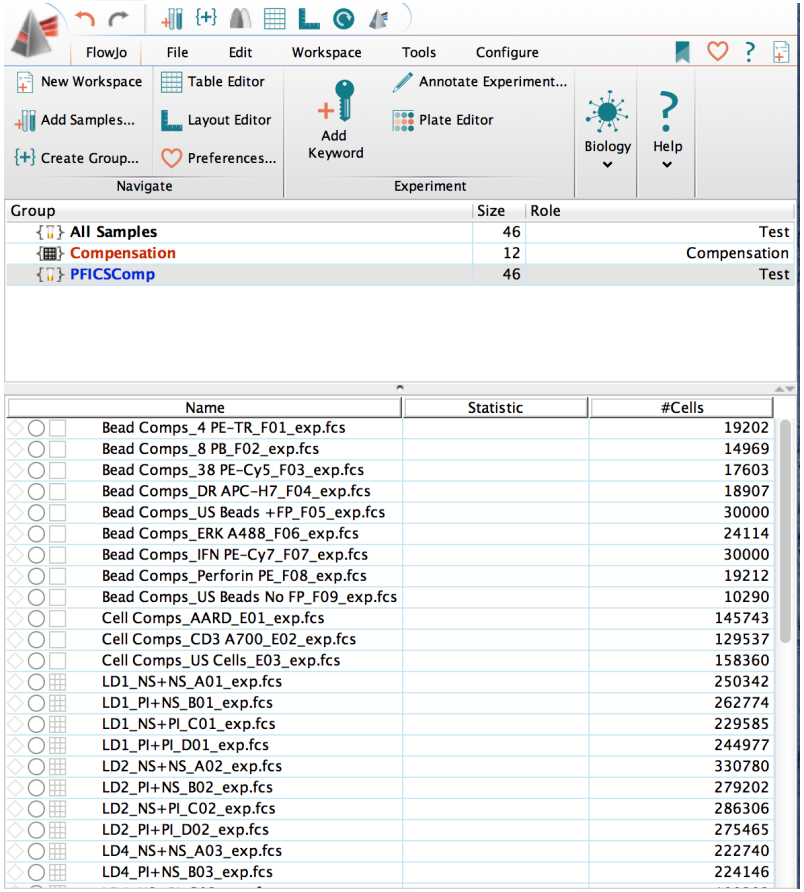
- Tabs group similar Bands together.
- Bands group similar Actions together.

Importing Data

Drag and drop into Samples Pane or click the Add Samples button







Todays Demo Data Set: Phospho-Flow + Intracellular Cytokine Staining (PFICS)

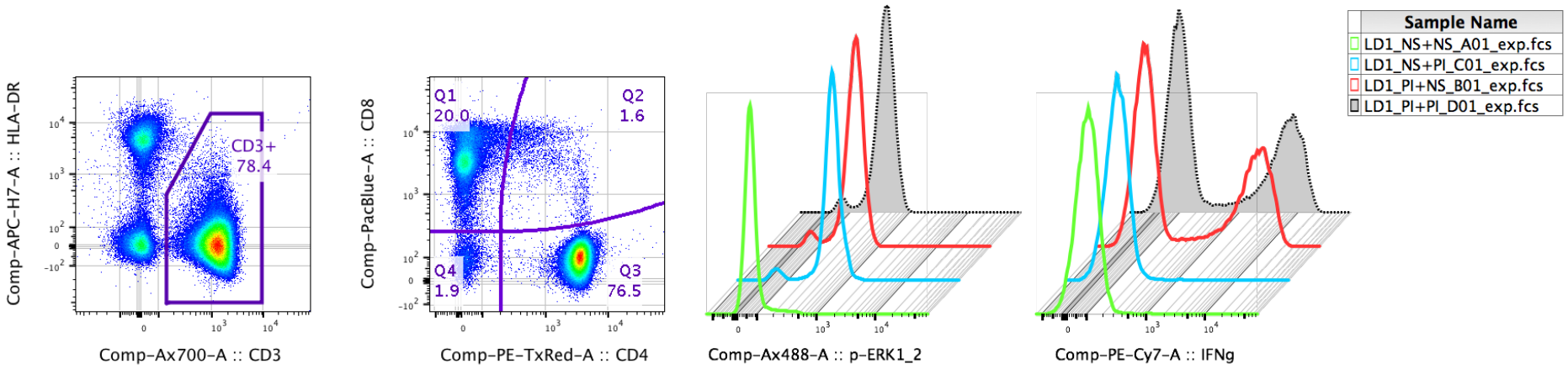
Polyclonal PFICS Assay:

- Thaw and rest cryopreserved human PBMC overnight
- Stimulate with PMA+Ionomycin (PI) for 2 hours or rest (NS) while blocking protein secretion → signaling and cytokines
- Stain for viability (AARD) and surface antigens (CD3, CD4, CD8, CD38 and HLA-DR)
- Stimulate PI for 20 minutes or NS rest
- Fix, perm and stain for intracellular antigens (phospho-ERK1/2, IFN-γ and Perforin)

PFICS Stim Conditions

- 2 Stimulations → 4 potential *STIM combinations/conditions

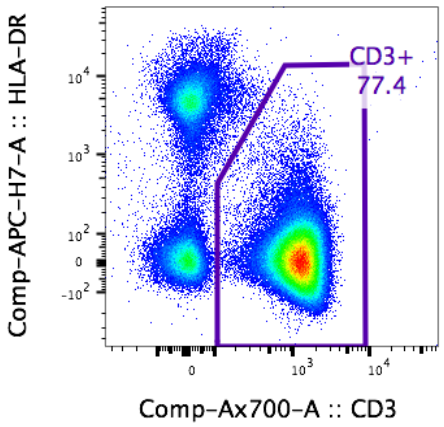
*STIM Condition	Total *STIM Time	phospho-ERK Response	IFN-γ Response
NS+NS	0 min	-	-
NS+PI	20 min	++++	-
PI+NS	120 min	+++	+++
PI+PI	140 min	+++	+++



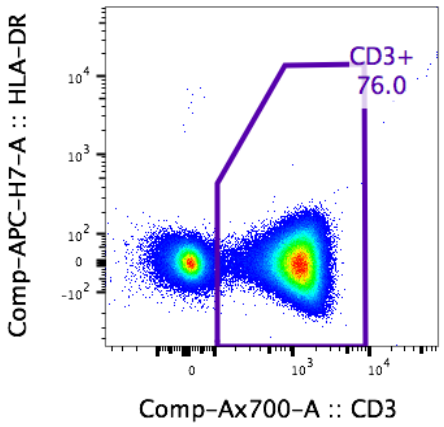
PFICS Samples

- 46 Total Samples
  - 20 experimental ***All Stain*** samples – Stained with all reagents in the panel → Real Experiment
  - *14* ***Fluorescence Minus One (FMO)*** controls – Leave one reagent out of panel → Gating Control
  - 12 ***Compensation*** controls – Stained with single reagent to isolate the fluorochrome emission spectrum and determine spillover into detectors.

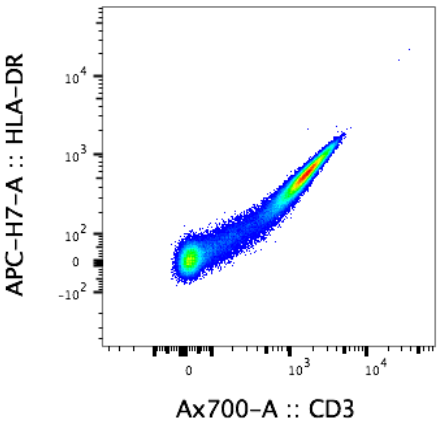
All Stain



No HLA-DR FMO

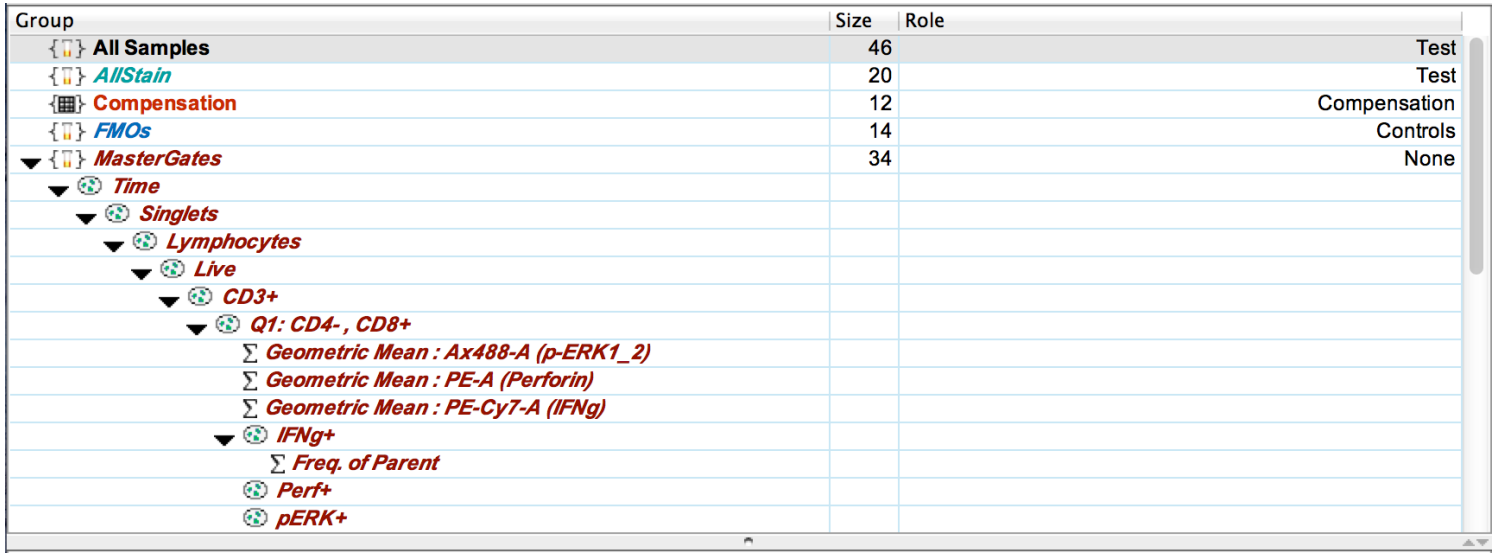


CD3 Ax700 Comp



Group Pane

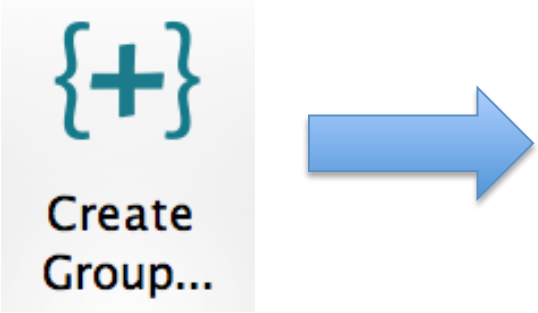
- The Group area lists all groups in the Workspace, # of samples in each group (Size), and the Role of that group (ex. Test, Compensation, Controls) .
- Groups act like folders to organize your samples, allows master gating and unique report generation.

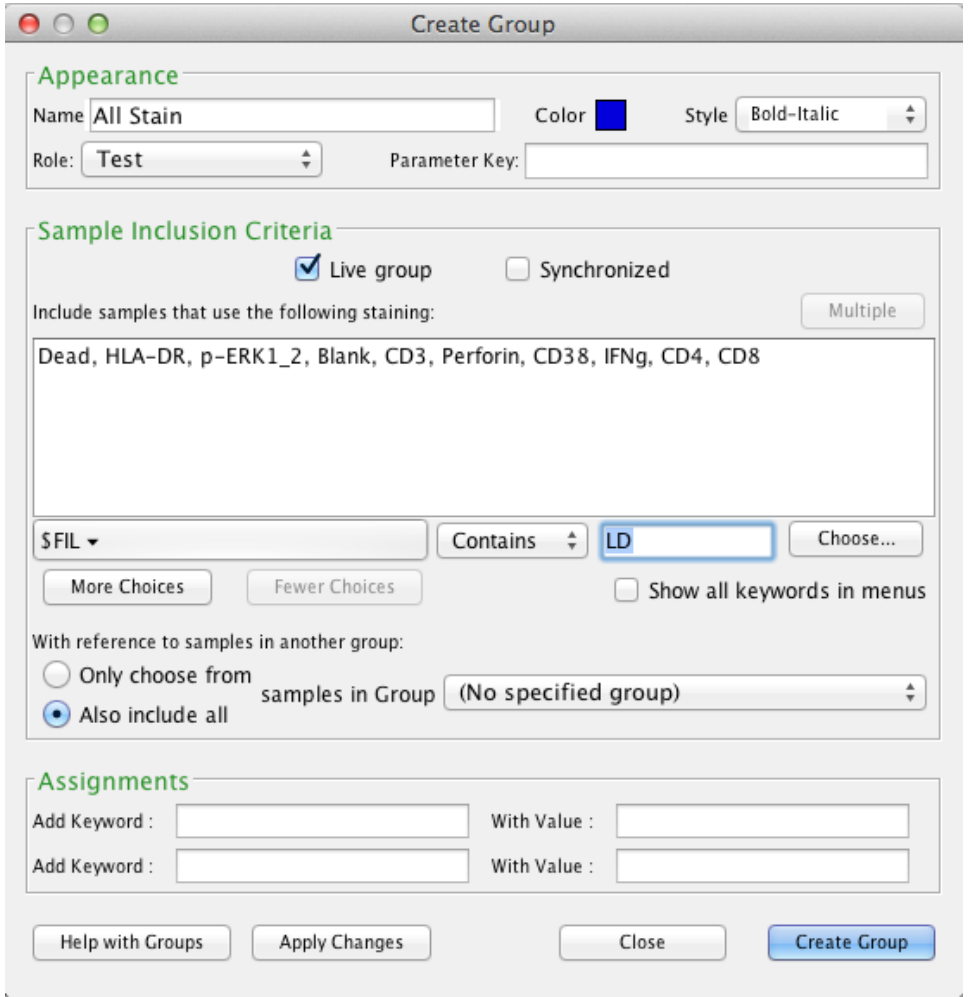


- Group owned analysis gains the group color.

Creating and Editing Groups

- Click the Create Group Icon located in either the task bar at the top of the workspace, or within the Navigate band.



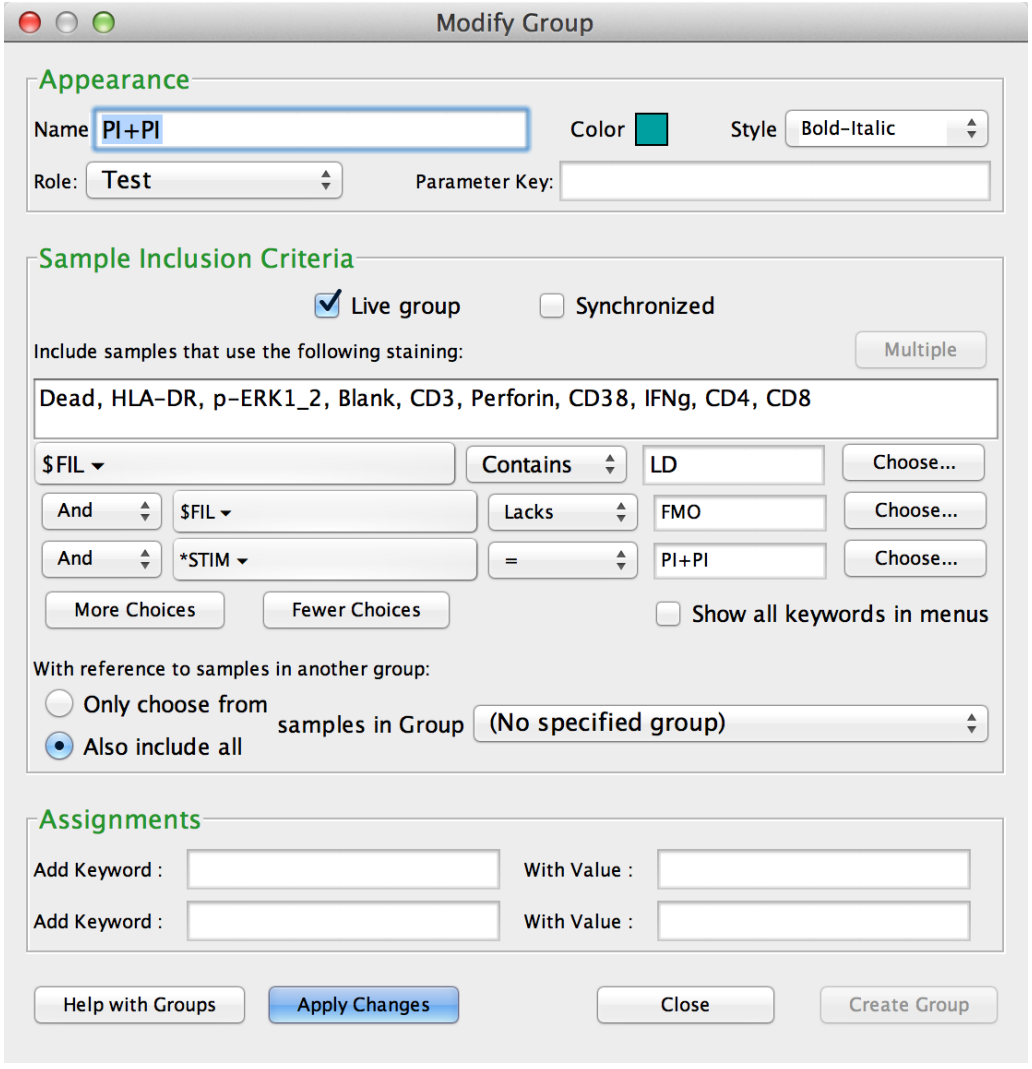


- Double click on an existing group to edit its properties.

Sample Inclusion Criteria

- **Live groups** automatically include samples based on user-defined **Sample Inclusion Criteria.**

- Sample Inclusion Criteria could include the staining panel, characters in the $FIL (file name), a user defined Keyword attribute, or a combination of features.



Samples and Sample Analysis

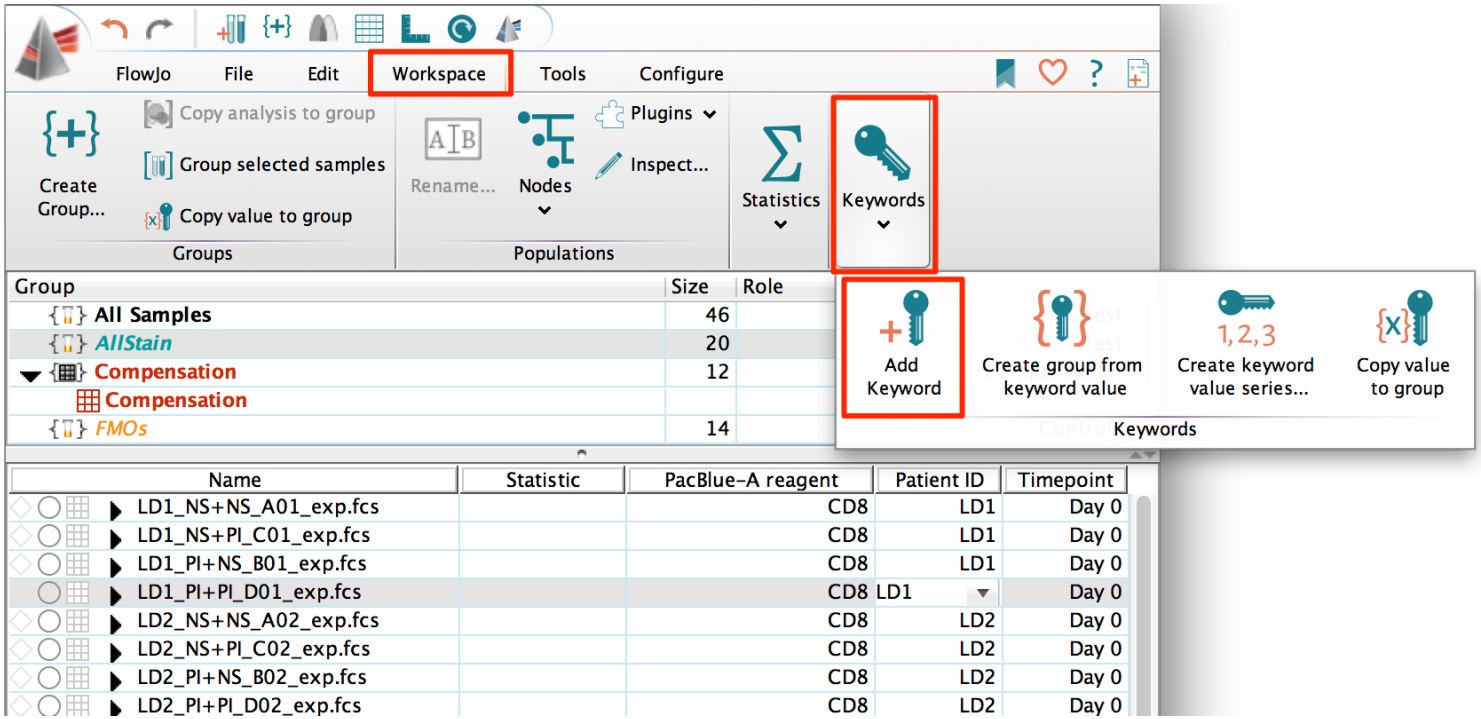
- Displays the sample list and associated analysis of the currently selected group.
- Statistic and #Cells columns are displayed by default. Additional Keyword attributes can be displayed as columns.

Name	Statistic	#Cells	*HIV Status	*PID	*STIM
LD1_NS+NS_A01.fcs		250342	Neg	LD1	NS+NS
LD1_NS+PI_C01.fcs		229585	Neg	LD1	NS+PI
LD1_PI+NS_B01.fcs		262774	Neg	LD1	PI+NS
Time	99.7	261964			
Singlets	96.2	252097			
Lymphocytes	93.7	236200			
Live	96.2	227167			
CD3+	81.4	184893			
Q1: CD4- , CD8+	24.0	44355			
Q2: CD4+ , CD8+	1.13	2090			
Q3: CD4+ , CD8-	72.7	134352			
Q4: CD4- , CD8-	2.22	4096			
LD1_PI+PI_D01.fcs		244977	Neg	LD1	PI+PI

- Double click on a sample to open a Graph Window and add gates.

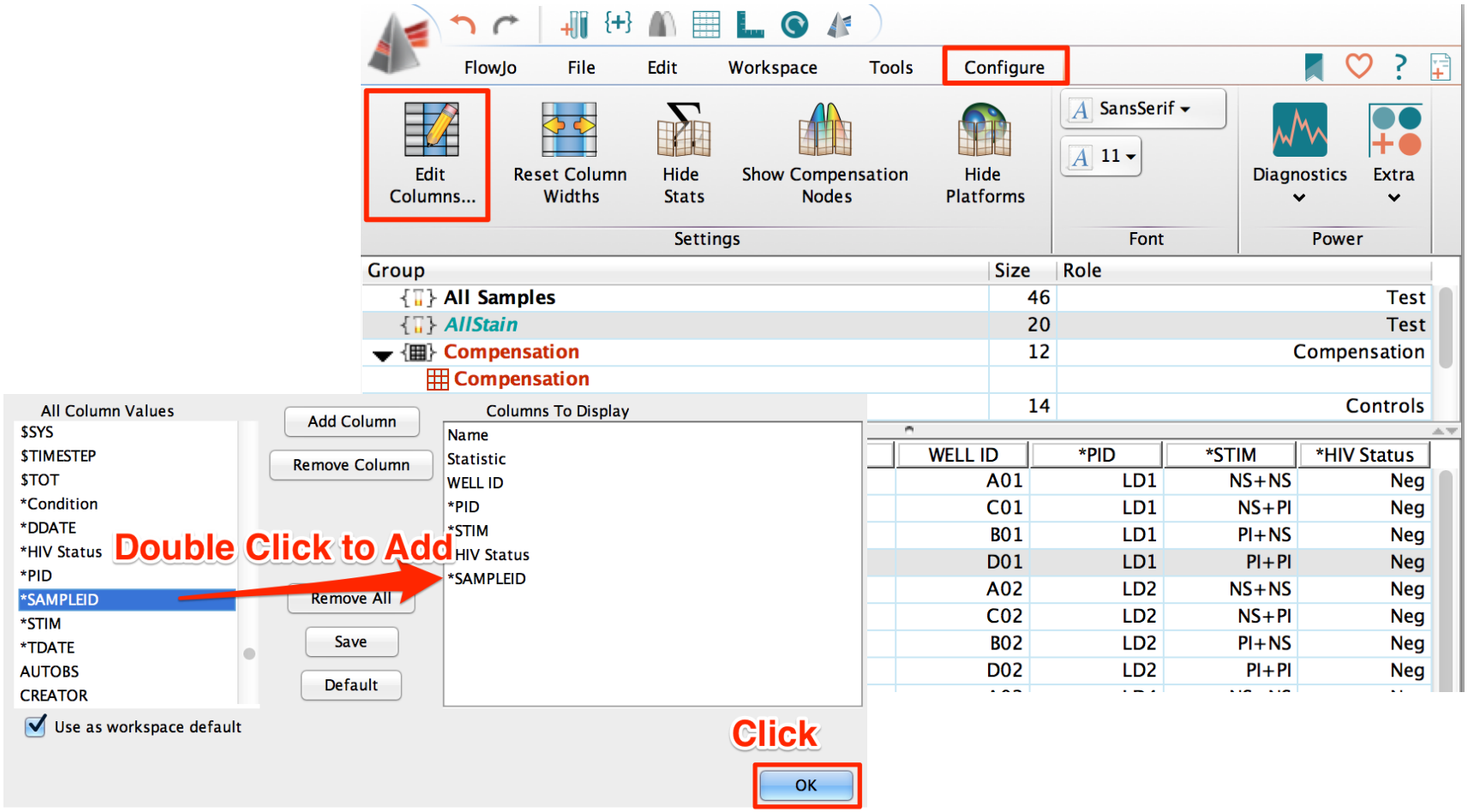
Add New Keywords

- Keywords attach descriptive metadata to samples Examples:
  - FCS file standard required keywords (ex. $FIL, $PnS)
  - User defined descriptive Keywords (ex. Patient ID, Timepoint)
- Workspace Tab → Keywords Band→ **Add Keyword** allows user to define new Keywords and add metadata



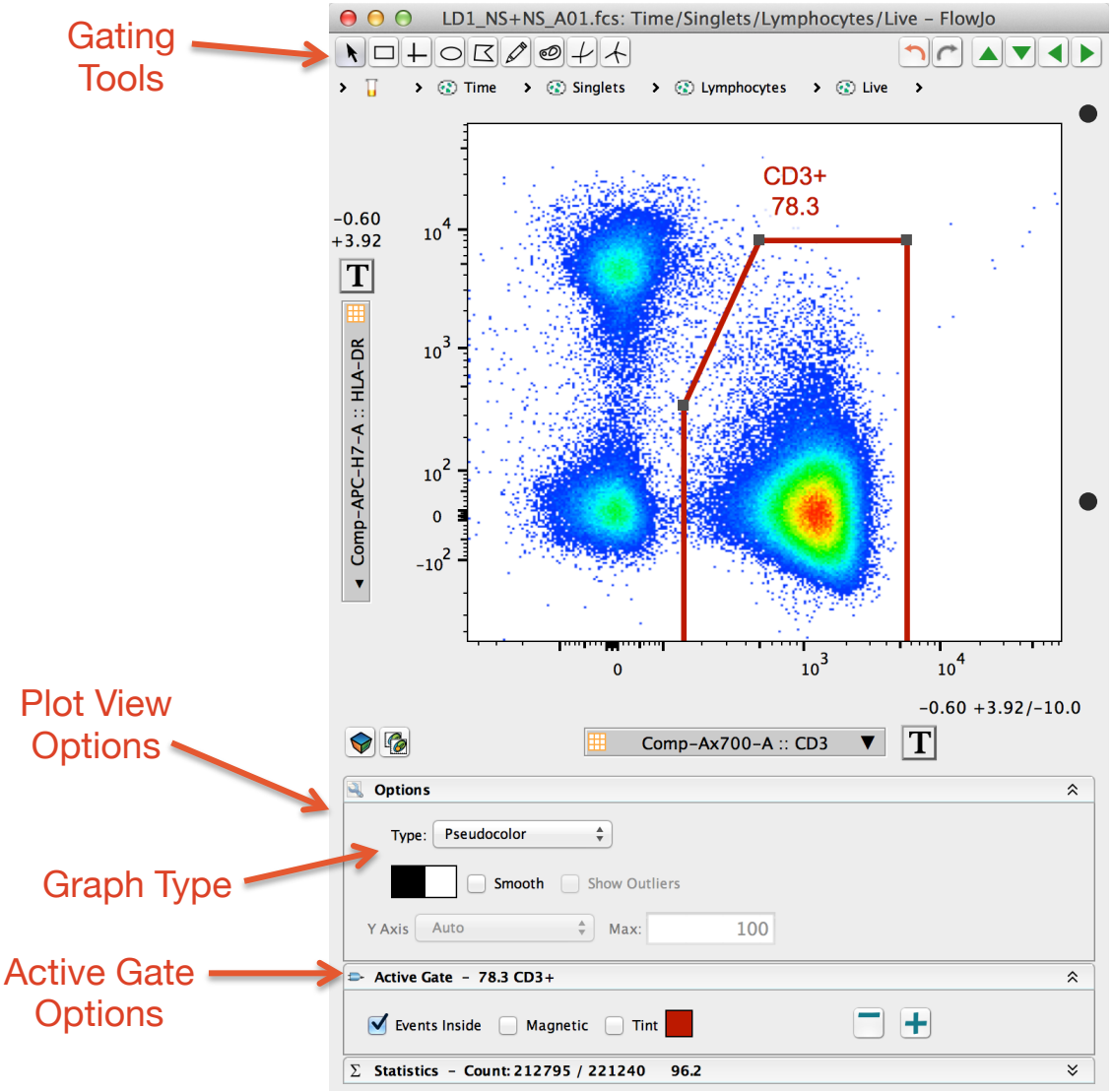
Display Existing Keywords

- Configure Tab → Settings Band → **Edit Columns** allows user to display Keywords as columns in the workspace samples pane



The Graph Window

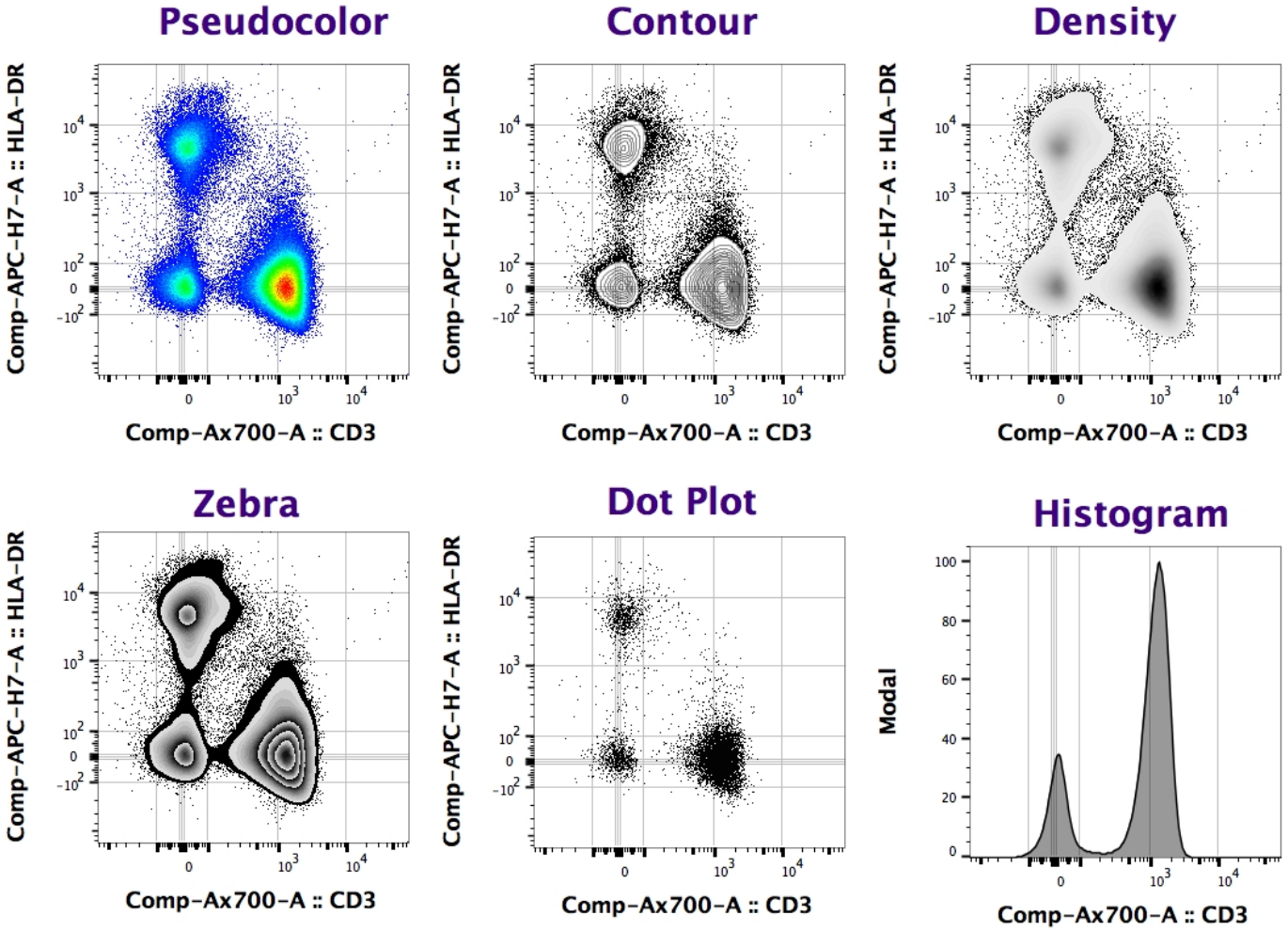
- Facilitates data visualization and gating.



- Several different plot types are available to display flow data.
- Click on the Options Menu below the graph image and select Graph Type from the dropdown menu.

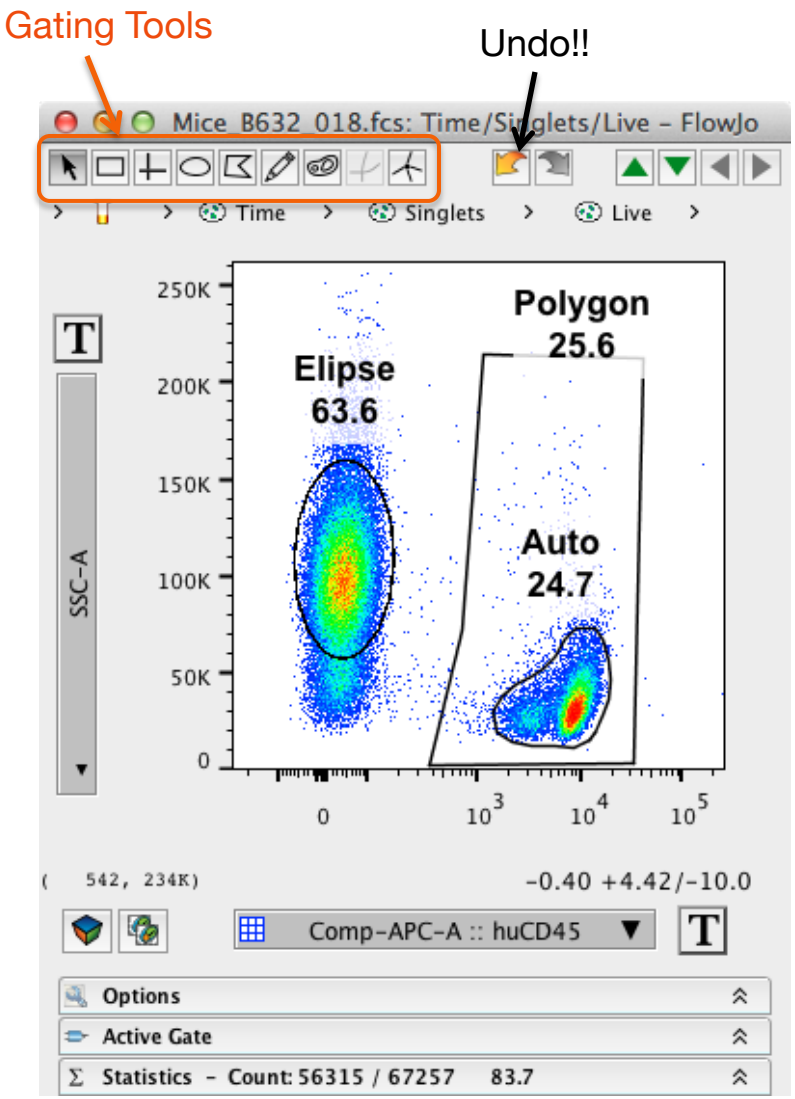
Graph Display Options

- Try them all and pick what pleases you, or best represents your data.



Gating tools

- Are located at the top left in a Graph Window.

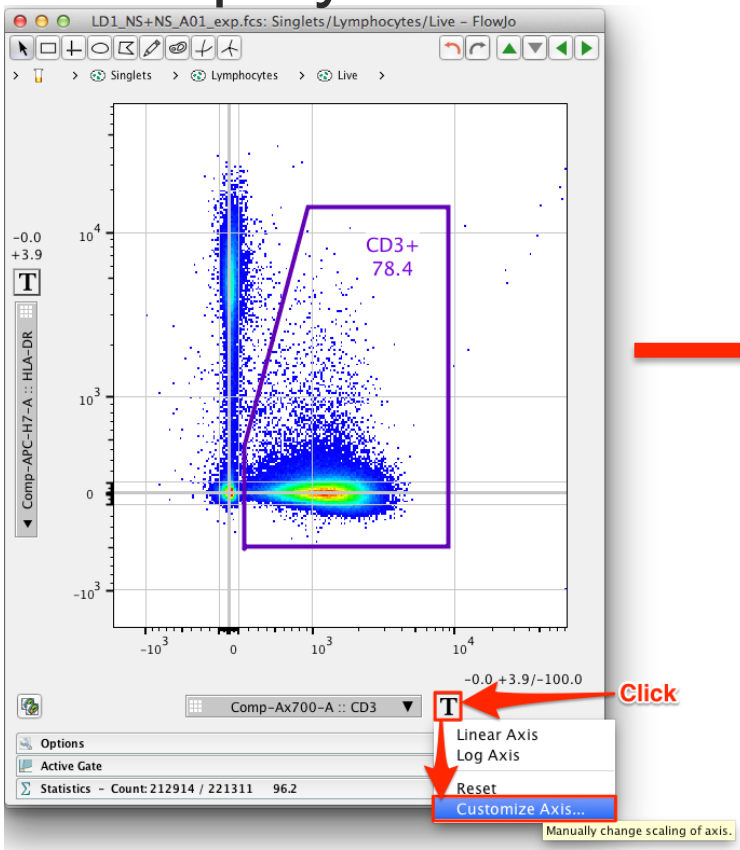


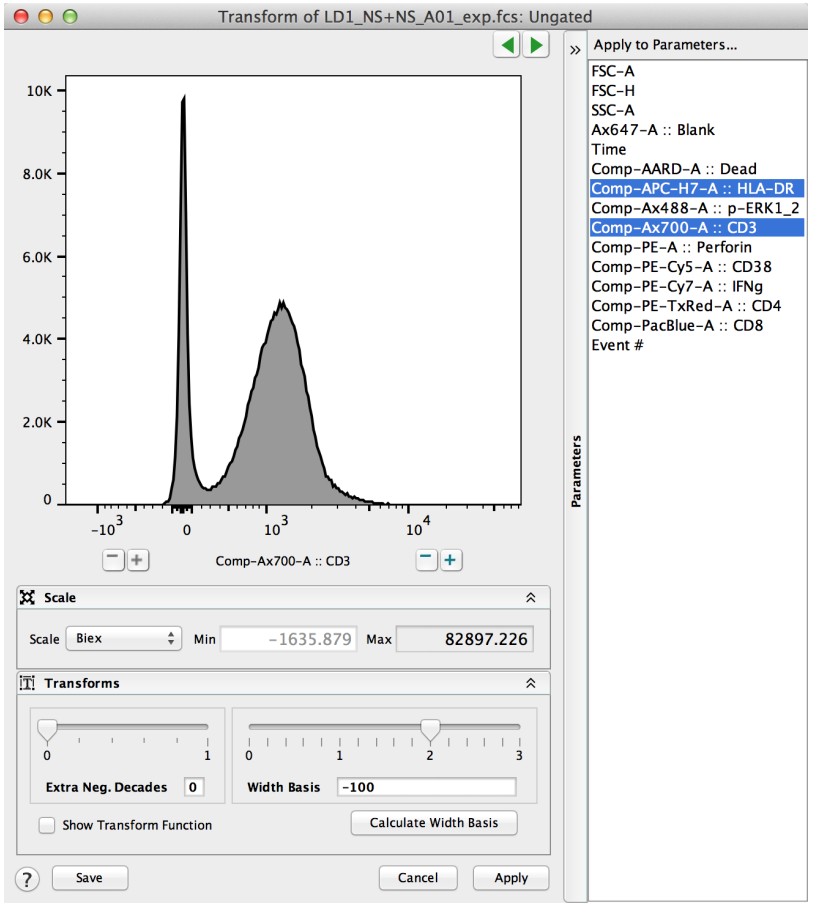
- Gates can always be modified or removed, so don't be shy.

- Explore the gating options and pick what works best for you.

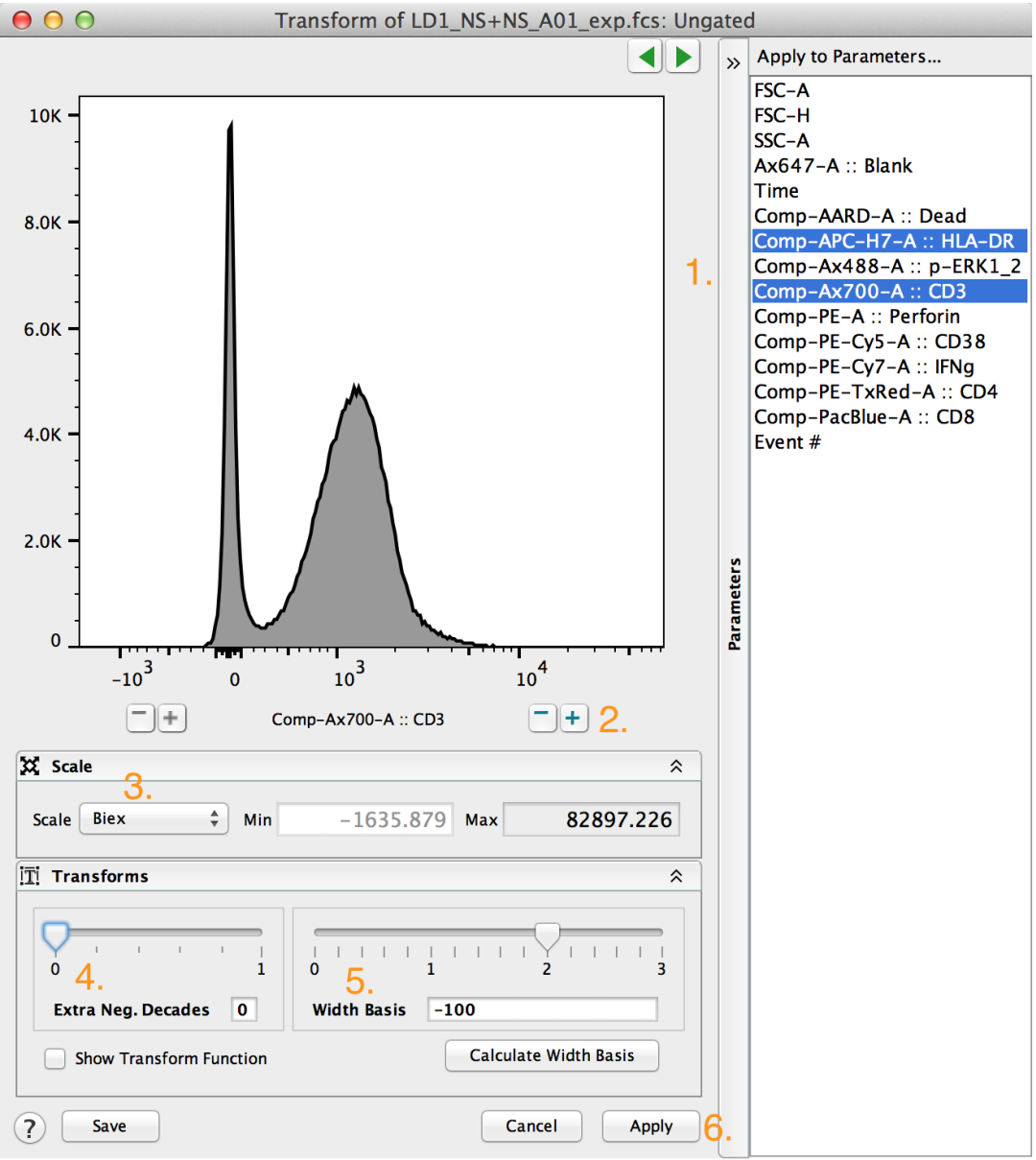
Transforming Data

- Your data may initially look ‘squished’.
- Click the Transformation [T] button and Select Customize Axis… to change the visual display.



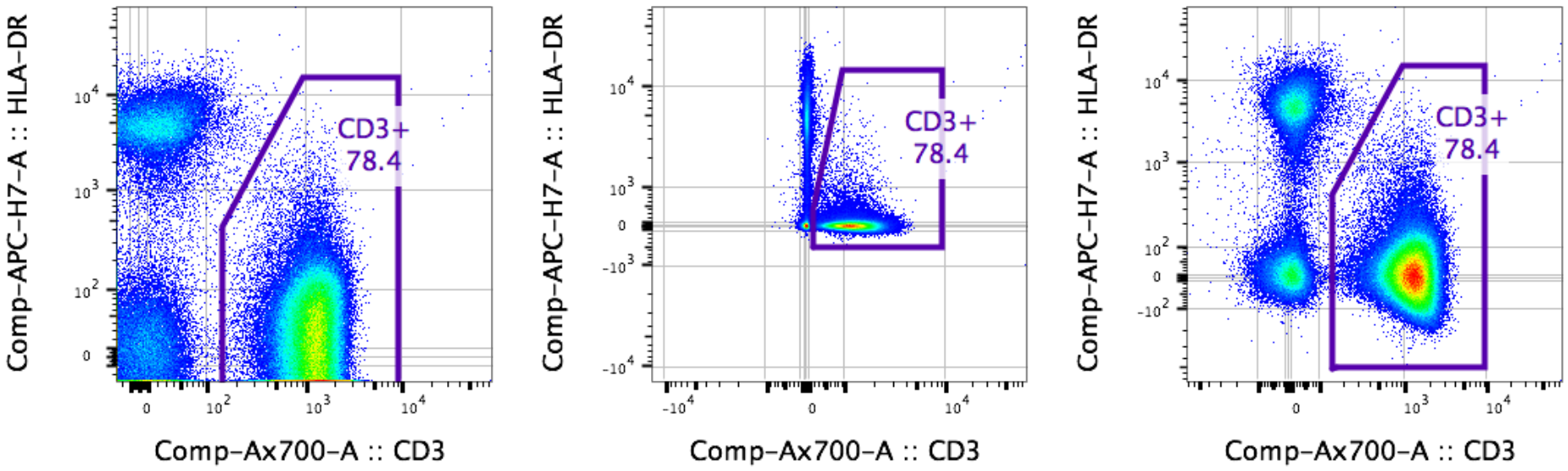


Transform Options



1. Select parameter(s)
2. Add or remove extra Pos. decades/range on top end
3. Select scale (Biex displays linear around zero and log further out)
4. Add or remove extra Neg. decades/range on bottom end
5. Width Basis scales how much visual display is given to linear vs. log range of the Biex scale
6. Applies the transformation settings to all selected parameters

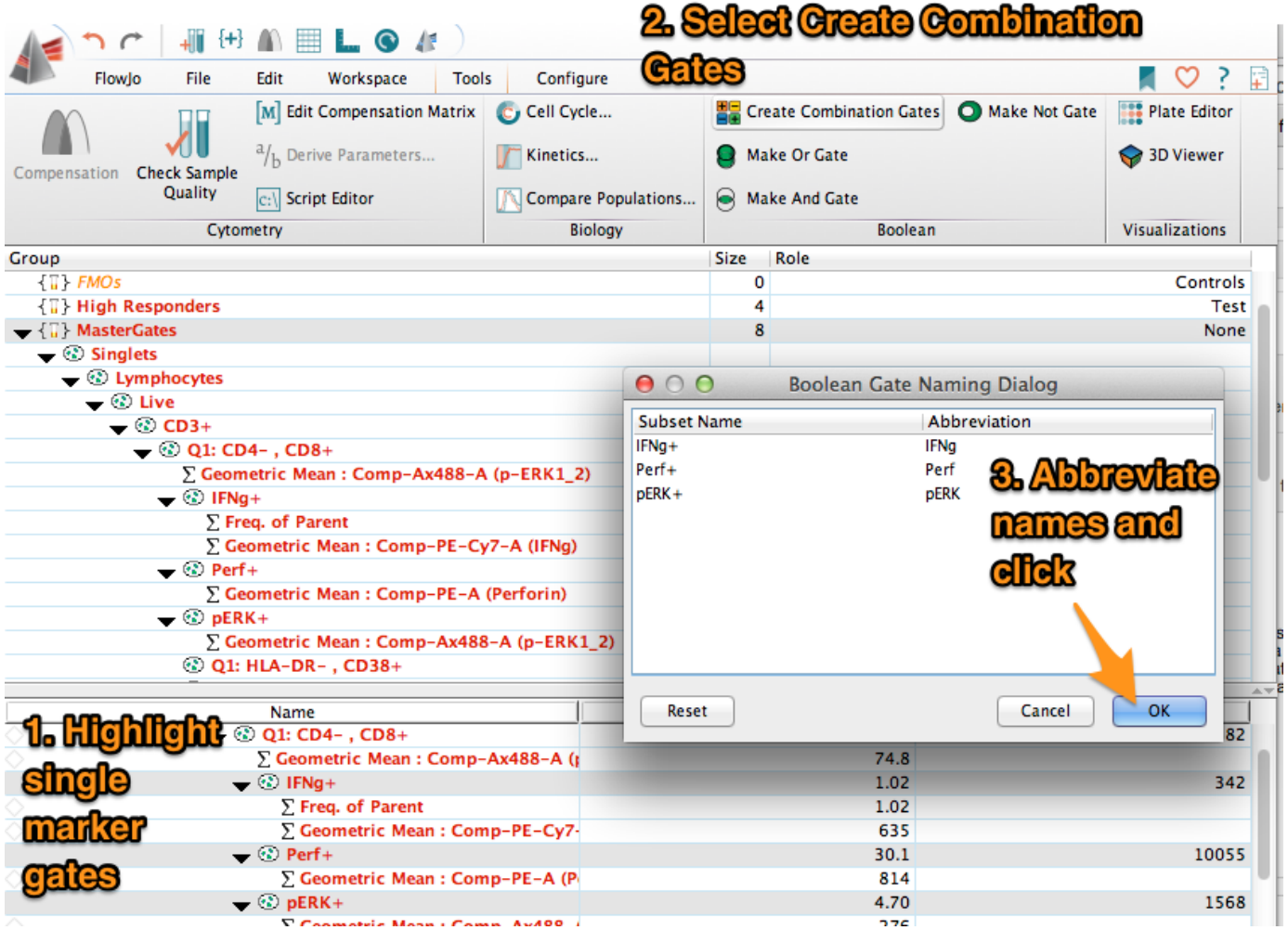
Effects of Transformation



1. Gets rid of the “squishing” of cells.
2. Ensures the visual population center better correlates with the statistical center (median).
3. Makes high resolution compensated digital cytometry data more appealing to the eye.

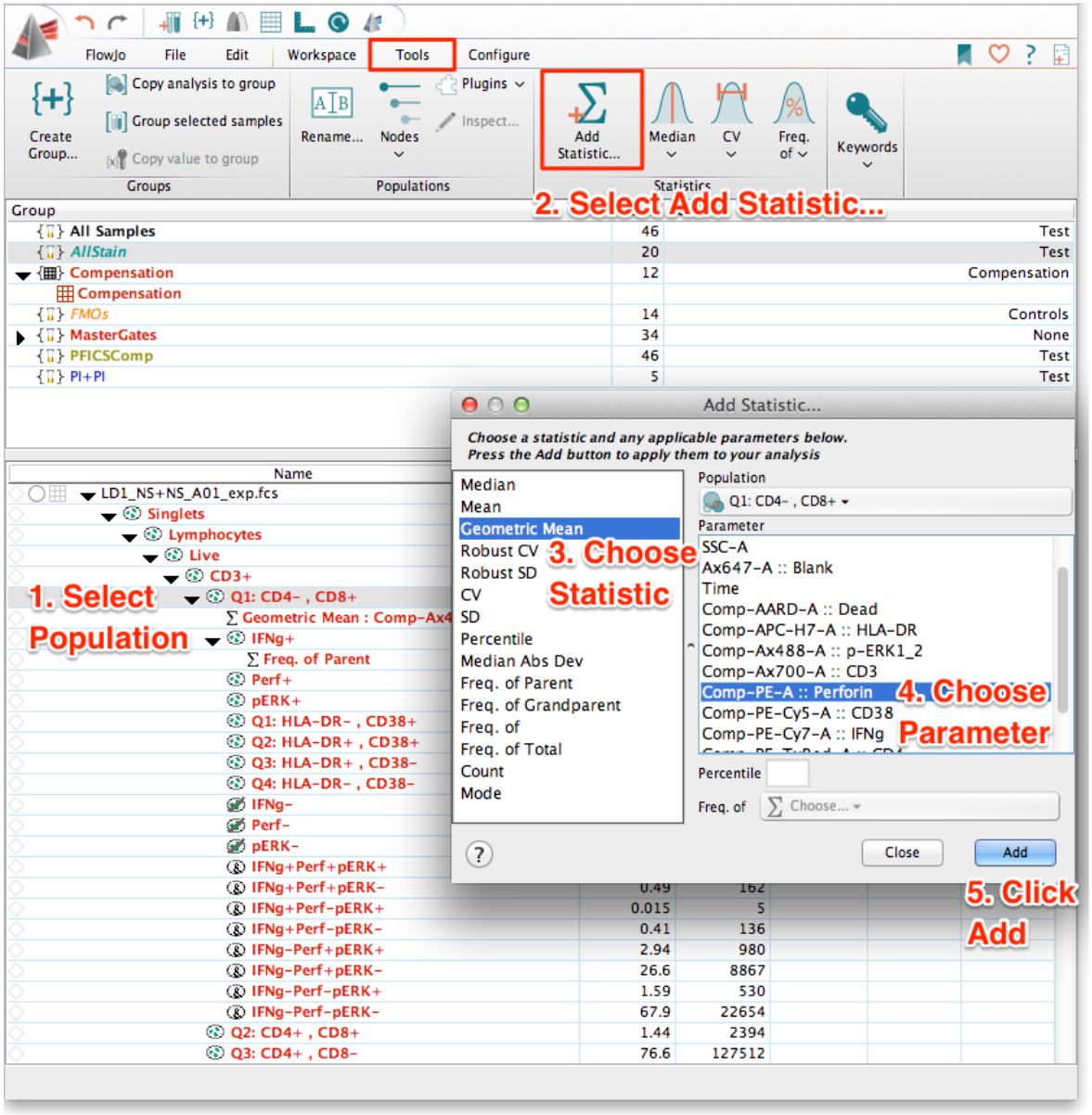
Boolean Combination Gates

- Calculate all possible combinations based on single marker gates (#combinations = $2^{\#gates}$).



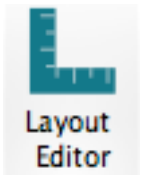
Adding Statistics

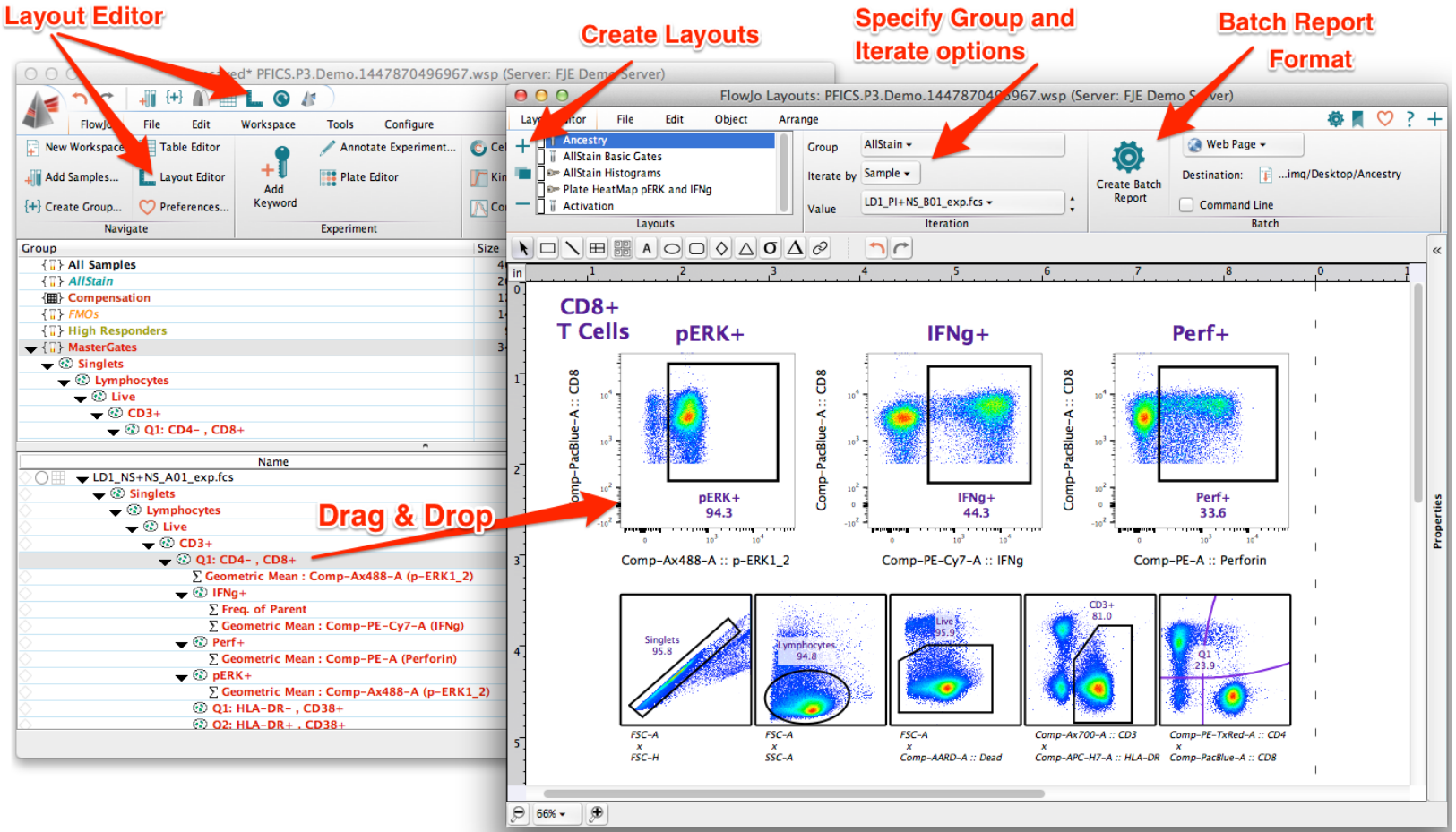
- Add a statistic to any gated population selected within a sample gating hierarchy.
- Statistic nodes can be group-applied just like a gate.



The Layout Editor

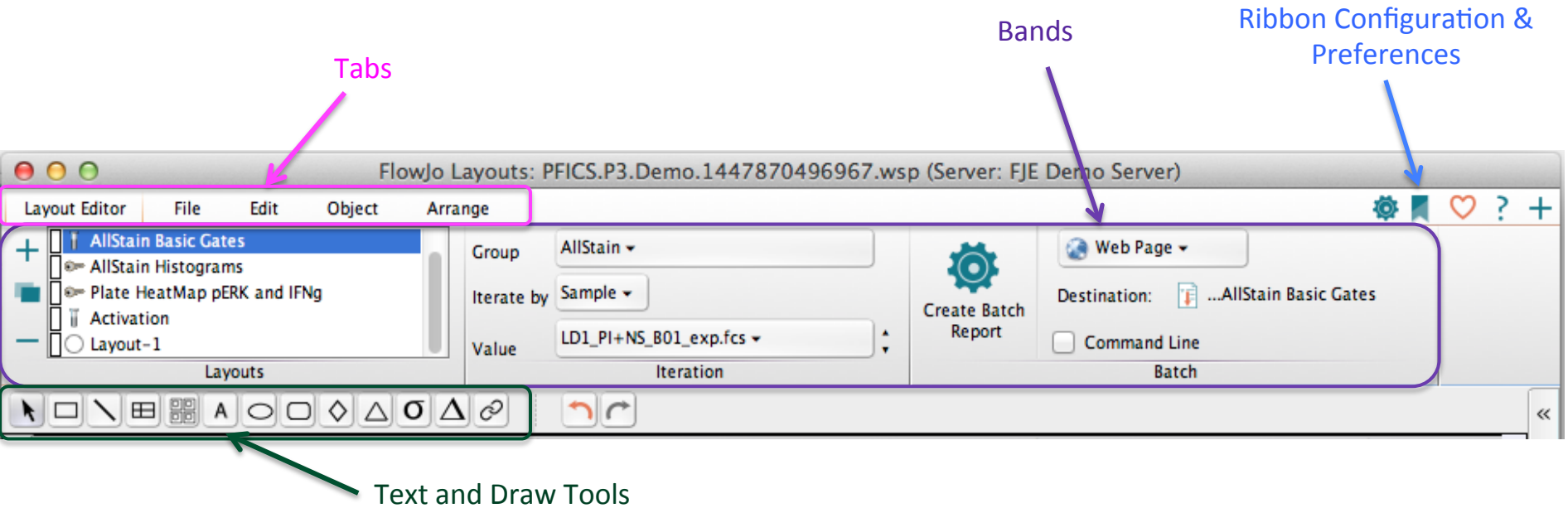
- A tool for creating graphical reports.
- Click on the Layout Editor icon.
- Drag populations from a sample to Layout Editor.





Working in Layout Editor

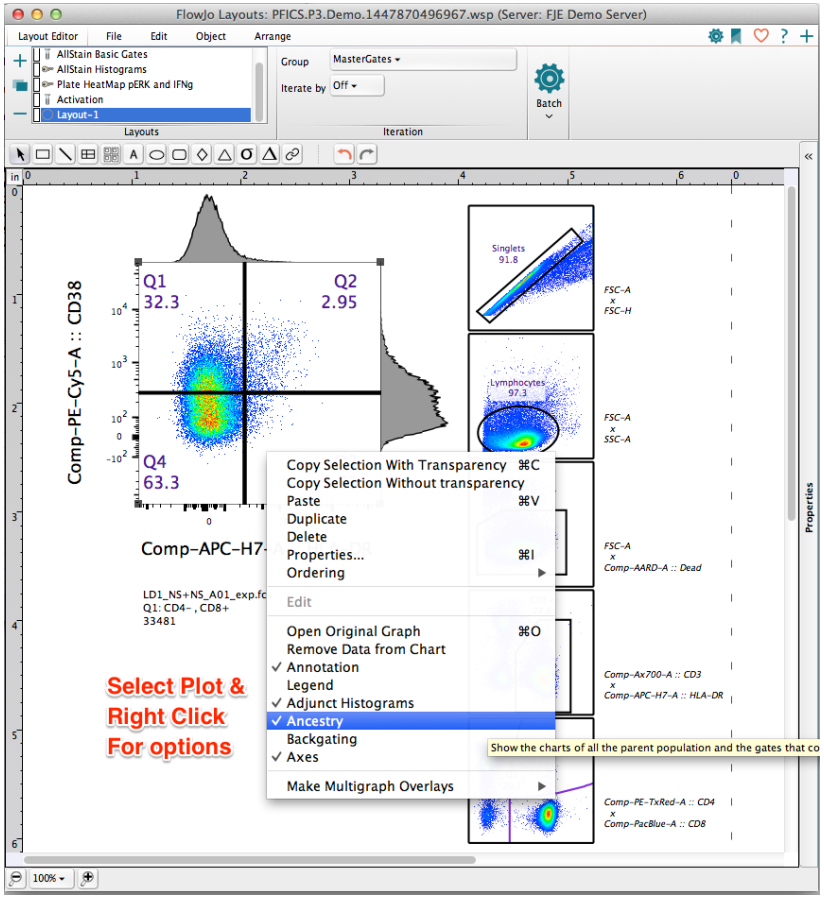
- Similar to the Workspace. Layout Editor has its own customizable Ribbon with Tabs and Bands to organize actions.



- Try clicking on the different tabs to see what types of actions are available.

Within Layout Editor

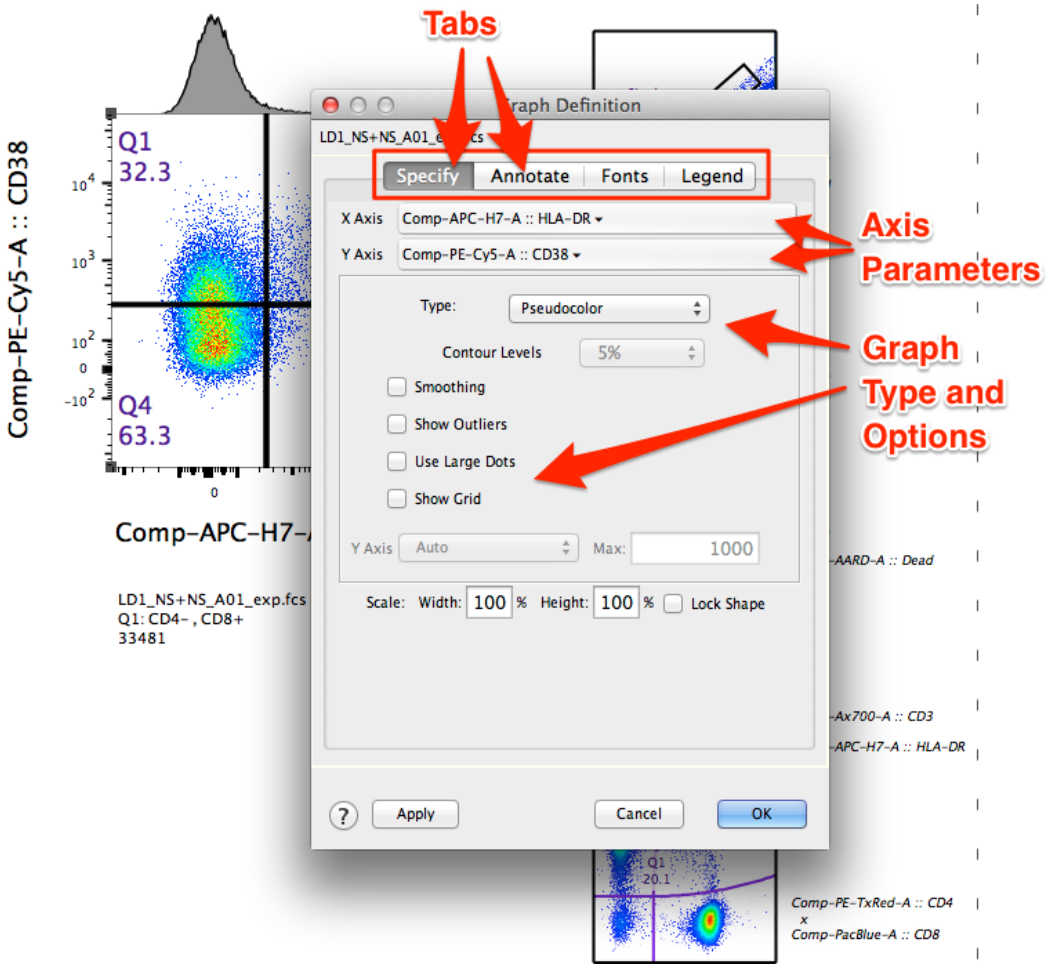
- Graphs can be organized and re-formatted.
- Statistics, keywords, text and even shapes or objects can be added to illustrate your analysis.

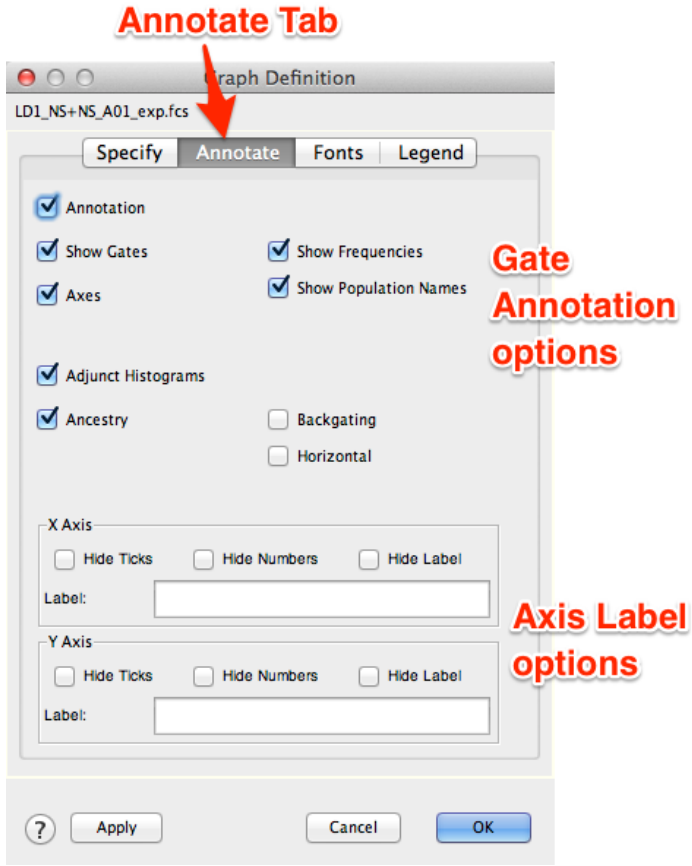


- Right Click on a graph plot for Ancestry and Backgating options
- Right click and select Properties for additional graph formatting

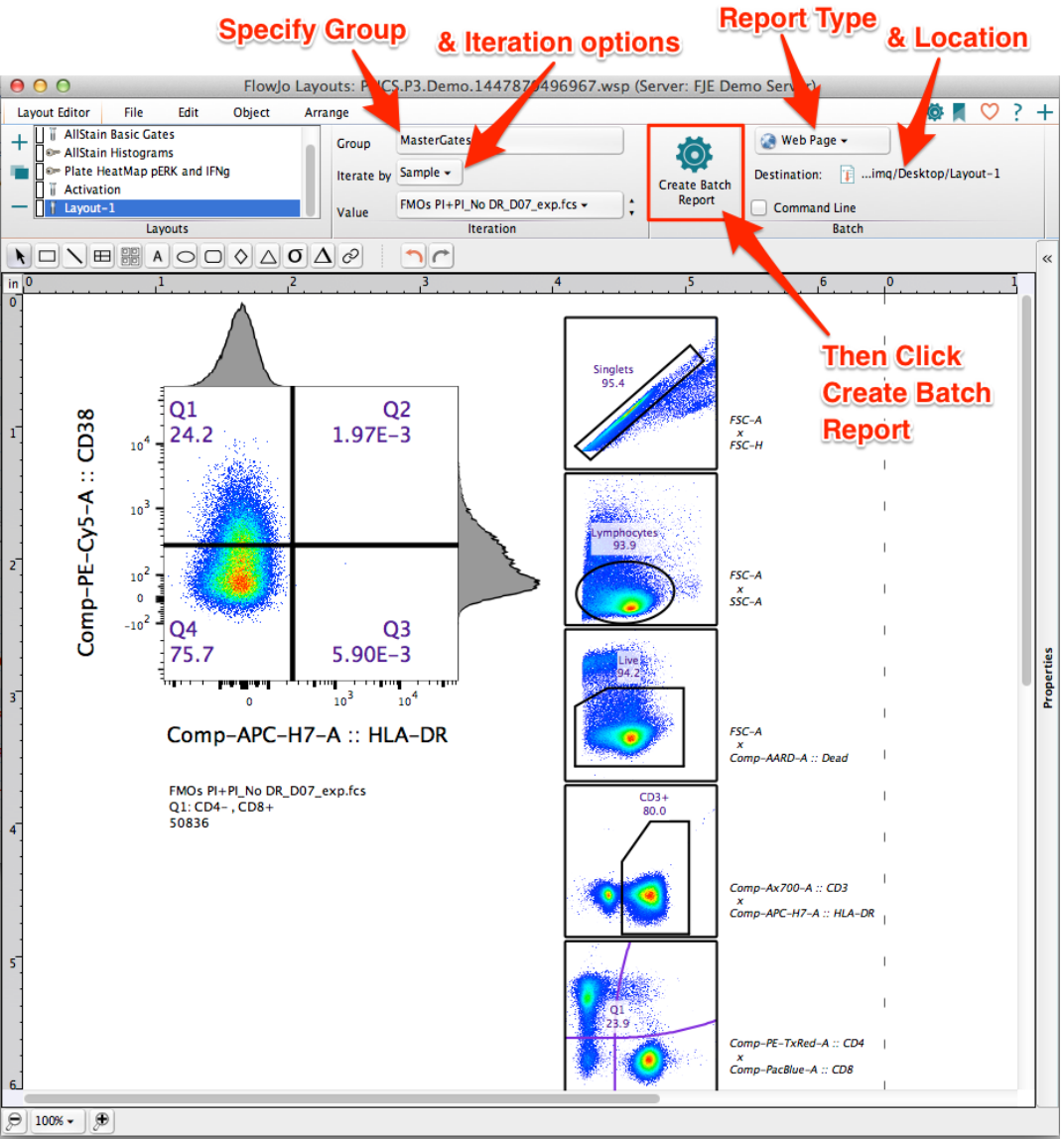
Working in Layout Editor

- Double Click a graph to change its properties/ formatting with 4 tabs of Graph Definition options

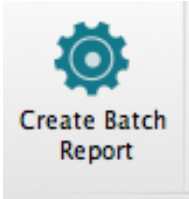




Batch Analysis of Layout Editor Graphics

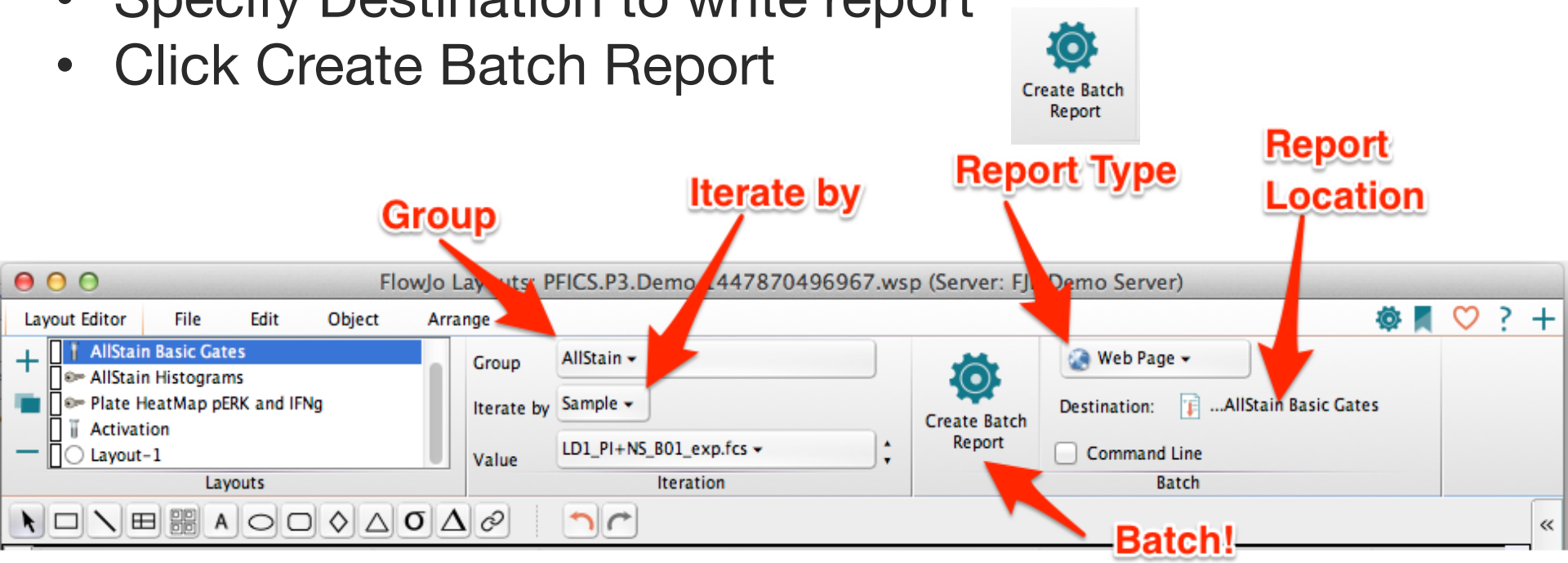


- Batch operations perform repetitive analysis on multiple samples, applying the layout to an entire set of samples.
- Specify Group, Iterate by, Report type and Location, then Click Create Batch Report .



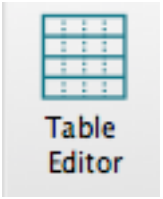
Batch Report Layouts

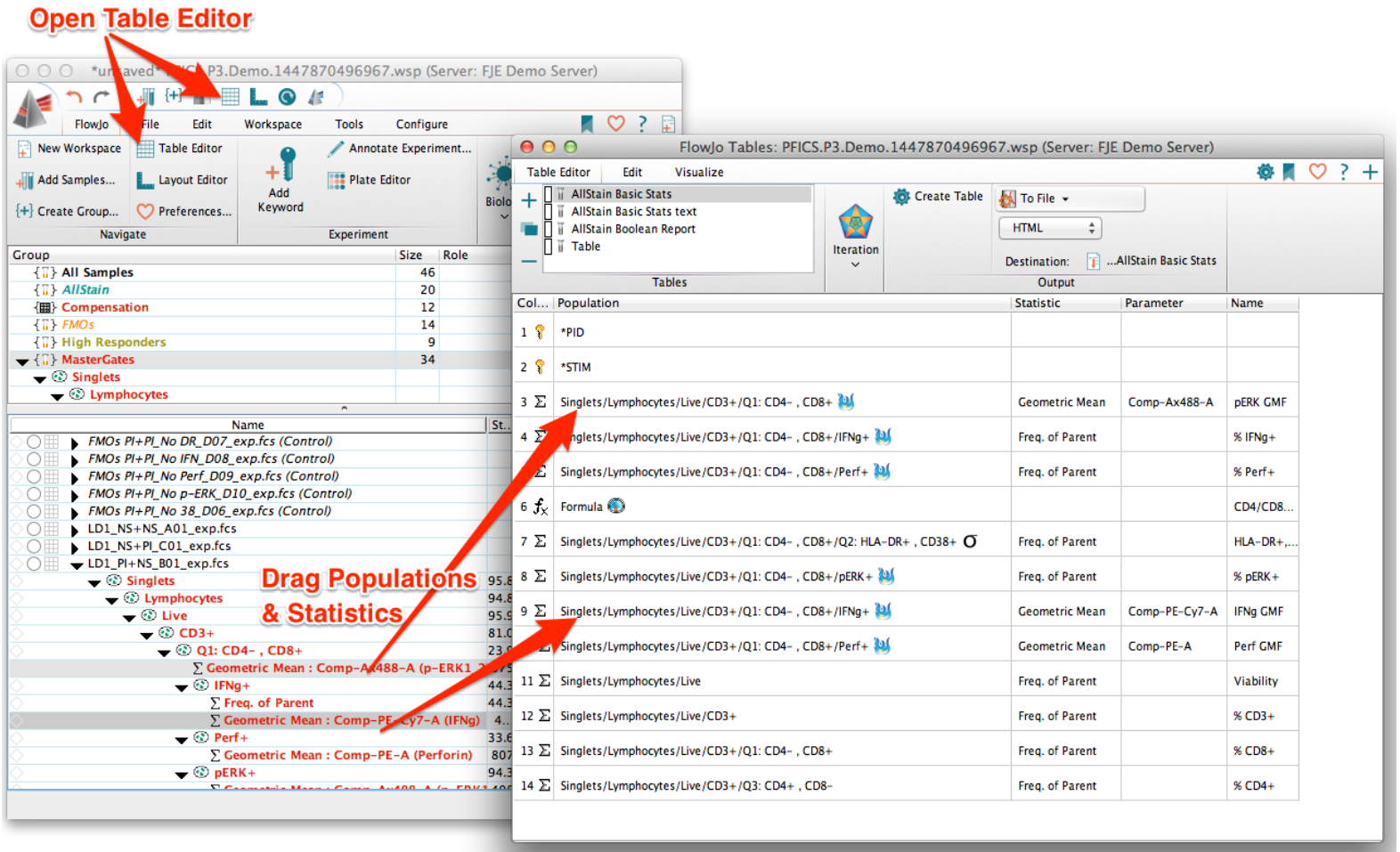
- Specify Group
- Choose Iterate by option
  - Sample
  - Panel
  - Keyword
    - Iterate By (must be Same for all samples displayed in layout)
    - Discriminator (must be Different for all samples displayed in layout)
- Specify type of Report
- Specify Destination to write report
- Click Create Batch Report



The Table Editor

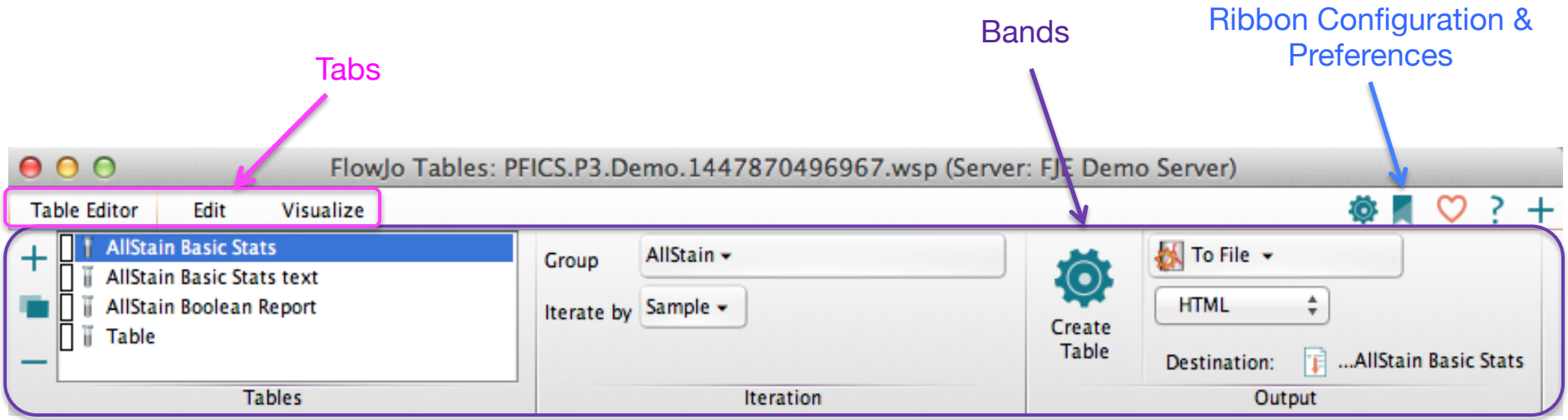
- A tool for creating statistical reports.
- Type ⌘ T, or click on the Table Editor icon.
- Drag Populations & Statistics to Table Editor.





Within Table Editor

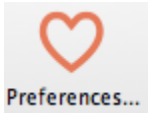
- Again, the Table Editor has its own customizable Ribbon with Tabs and Bands to organize actions.



- Specify the group you wish to batch, and how to iterate the batch process, then in the Output band, specify where you want the batch output to go.

Table Editor Visualize Tools

- Table formatting/visualization options such as heat mapping are contained within the Visualize Tab.
- Highlight row(s), then select the visualization.
- Expected Ranges can be set within Preferences



→ Ranges

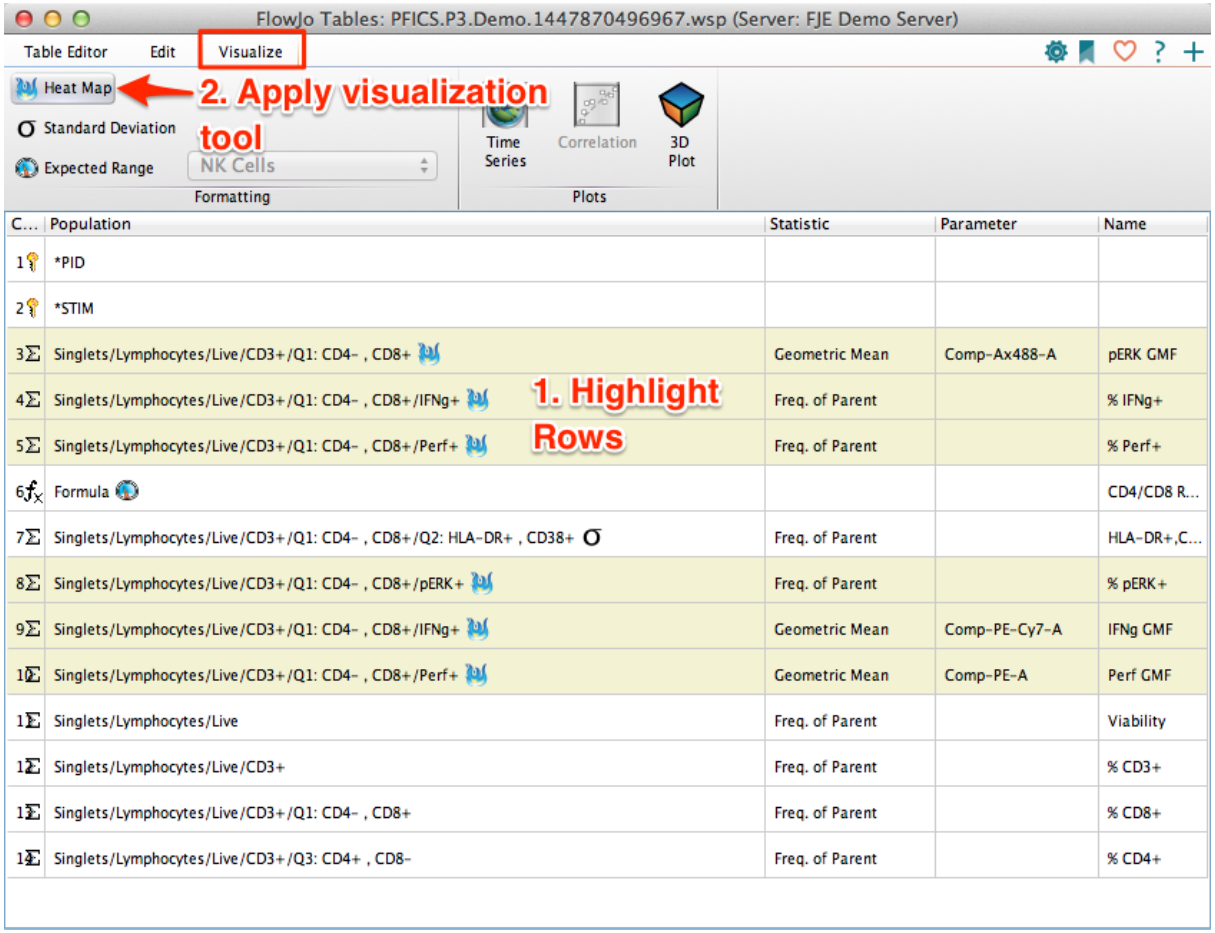


Table Editor Output

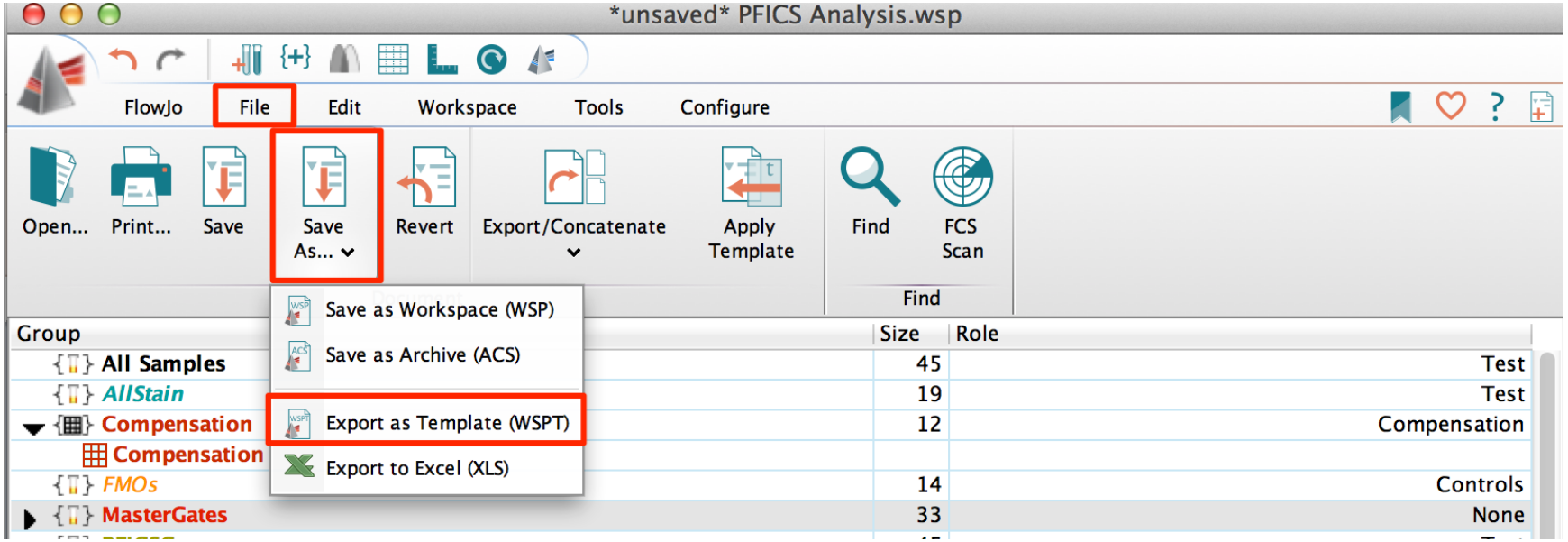
- Formatting/visualization options are maintained when a table is batched to either Display or HTML formats.
- Other file types (ex. Text, CSV, Excel) produce statistics tables lacking visualization formatting.

Table – AllStain Basic Stats

Ancestry Subset Statistic For	*PID	*STIM	pERK GMF	% IFNg+	% Perf+	CD4/CD8 Ratio	HLA-DR+,(	% pERK+	IFNg GMF	Perf GMF
LD1_NS...	LD1	NS+NS	74.1	1.09	30.2	▲ 3.81	*2.95*	4.70	642	812
LD1_NS...	LD1	NS+PI	503	0.96	30.0	▲ 4.13	*2.72*	94.9	504	809
LD1_PI+...	LD1	PI+NS	375	44.3	33.6	▲ 3.04	2.26	94.3	4917	807
LD1_PI+...	LD1	PI+PI	373	43.8	32.7	▲ 3.06	1.94	94.5	4907	816
LD2_NS...	LD2	NS+NS	75.6	1.83	55.9	2.80	2.07	0.45	509	818
LD2_NS...	LD2	NS+PI	496	1.91	53.4	▲ 3.01	1.87	91.0	425	752
LD2_PI+...	LD2	PI+NS	420	64.0	52.1	▲ 2.86	*1.27*	92.6	5894	739
LD2_PI+...	LD2	PI+PI	407	63.7	51.4	▲ 2.91	*1.46*	92.7	5768	734
LD4_NS...	LD4	NS+NS	86.6	1.05	21.1	1.52	*2.71*	8.08	494	740
LD4_NS...	LD4	NS+PI	596	1.74	23.6	1.52	*2.80*	97.1	403	775
LD4_PI+...	LD4	PI+NS	456	28.2	23.8	▼ 1.21	1.74	96.8	5298	577
LD4_PI+...	LD4	PI+PI	449	26.5	22.6	▼ 1.22	*1.48*	96.4	5035	566
LD12_N...	LD12	NS+NS	67.5	0.74	37.5	▲ 3.64	*2.93*	4.14	755	440
LD12_N...	LD12	NS+PI	414	0.50	35.3	▲ 4.28	*3.19*	89.3	683	444
LD12_PI...	LD12	PI+NS	327	45.3	40.8	1.94	*1.50*	84.8	4632	408
LD12_PI...	LD12	PI+PI	319	46.1	41.4	1.94	*1.64*	83.7	4793	403
LD14_N...	LD14	NS+NS	72.4	0.50	14.3	2.11	1.90	4.11	689	811
LD14_N...	LD14	NS+PI	483	0.45	13.8	2.30	2.19	95.5	595	829
LD14_PI...	LD14	PI+NS	366	17.7	18.2	1.66	*1.21*	94.8	3708	650
LD14_PI...	LD14	PI+PI	351	17.0	18.3	1.67	*1.10*	93.2	3565	644
Mean			336	20.4	32.5	2.53	2.05	70.7	2711	679
SD			167	23.0	13.4	0.96	0.65	39.5	2259	152

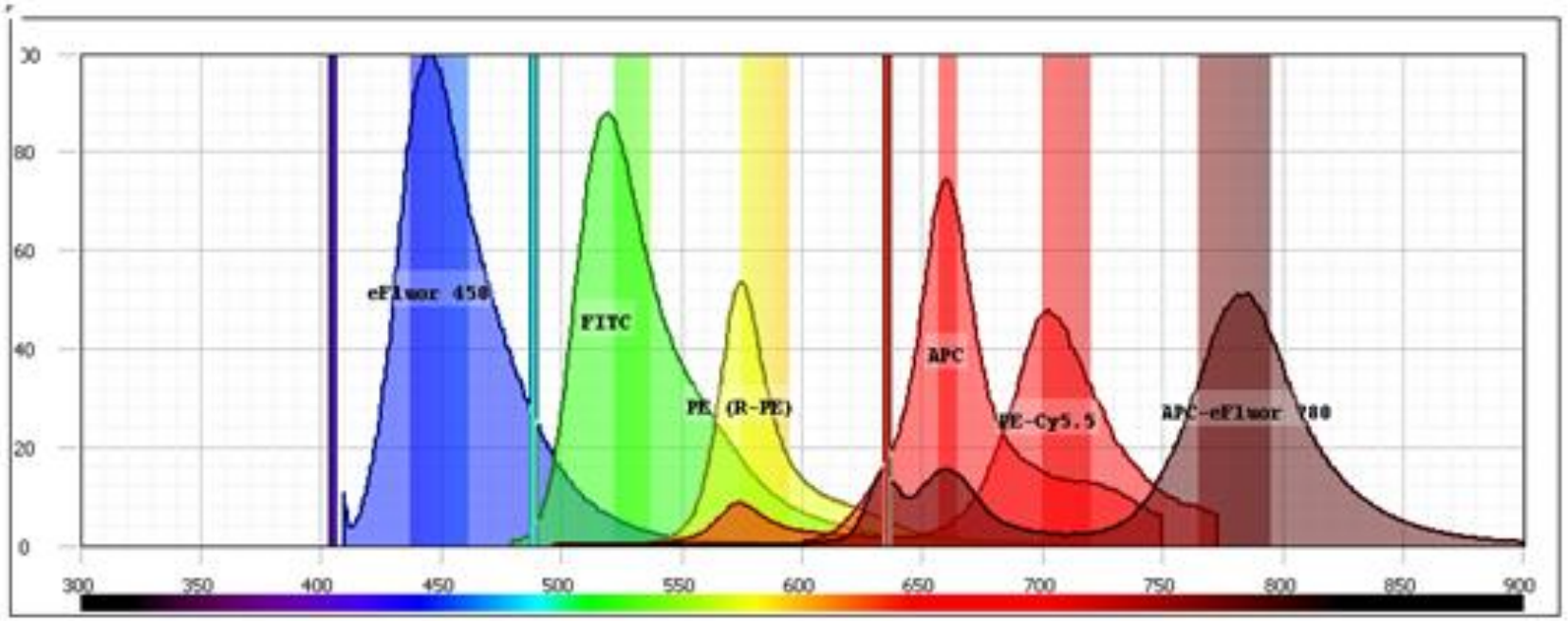
Workspace Templates

- Allows saving all analysis reports in your workspace without data.
- Streamlines repetitive analysis of multiple runs using the same staining panel(s).
- File Tab → Document Band → Save As…→ **Export as a Template (WSPT)**



Compensation

- Compensation corrects for spillover between fluorochrome emission spectra.



- Compensation is essential for multicolor panels.

Three Rules of Compensation

- First, there must be a single stained control for every parameter in the experiment!

- In Addition, there are three *rules* for 'good' compensation controls.

1. Controls need to be at least as bright or brighter than any sample the compensation will be applied to.
2. Background fluorescence should be the same for the positive and negative control.
3. Compensation controls MUST match the exact experimental fluorochrome.

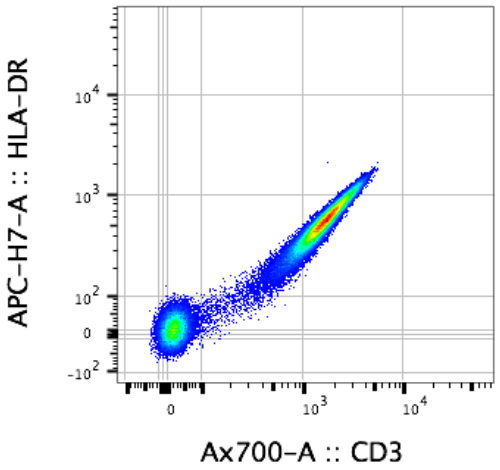
http://flowjo.typepad.com/the_daily_dongle/2011/09/three-rules-for-compensation-controls.html

PFICS Compensation Controls

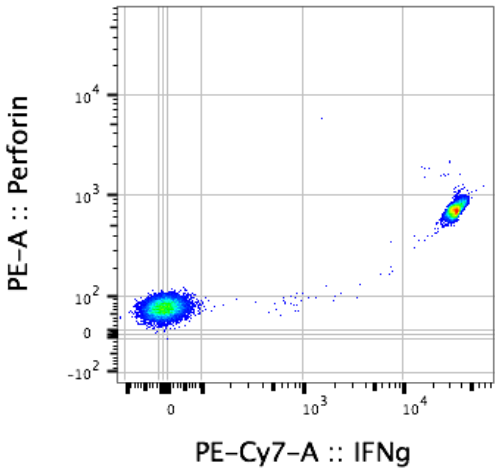
- **PBMC Cells**
  1. Unstained Cells
  2. AARD
  3. CD3 Alexa700

- **Compensation Beads**
  1. Unstained Beads with Fix and Perm
  2. CD4 PE-TexasRed
  3. CD8 Pacific Blue
  4. CD38 PE-Cy5
  5. HLA-DR APC-H7
  6. *Unstained Beads without Fix and Perm*
  7. *p-ERK1/2 Alexa 488*
  8. *IFNg PE-Cy7*
  9. *Perforin PE*

CD3 Ax700 Cell Comp



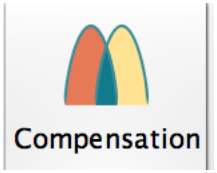
IFNg PE-Cy7 Bead Comp



Tim's Additional Rule (#4)

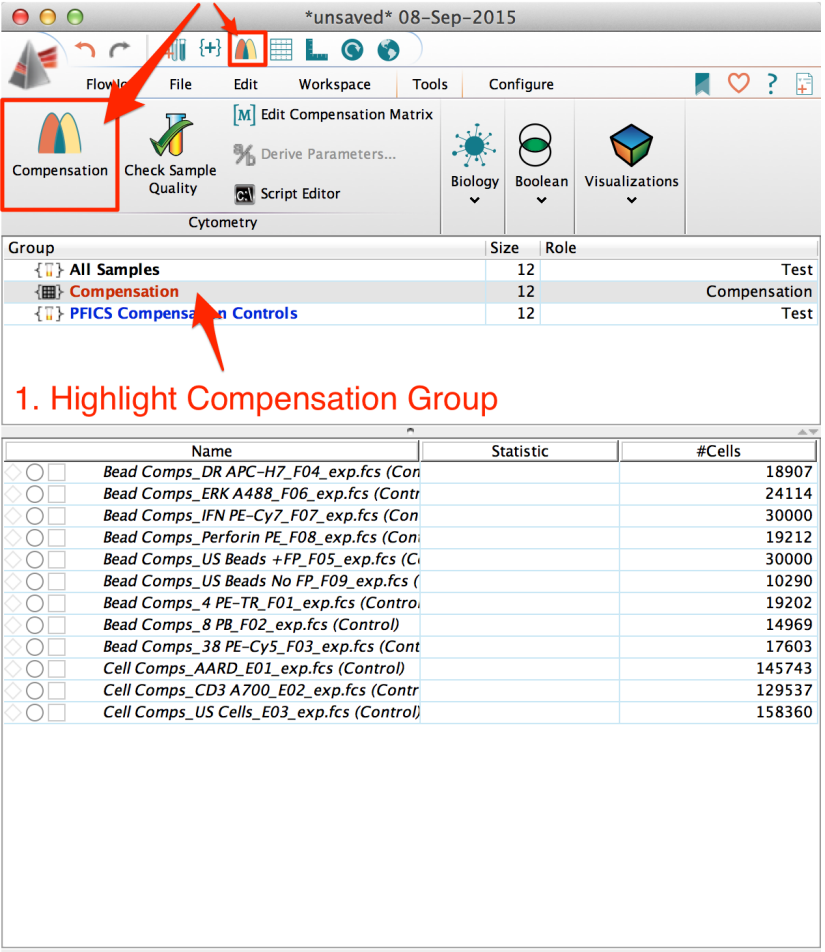
-Treat your comps like you treat your cells.

Compensation I

- Select a Compensation Group in the groups window, then click 

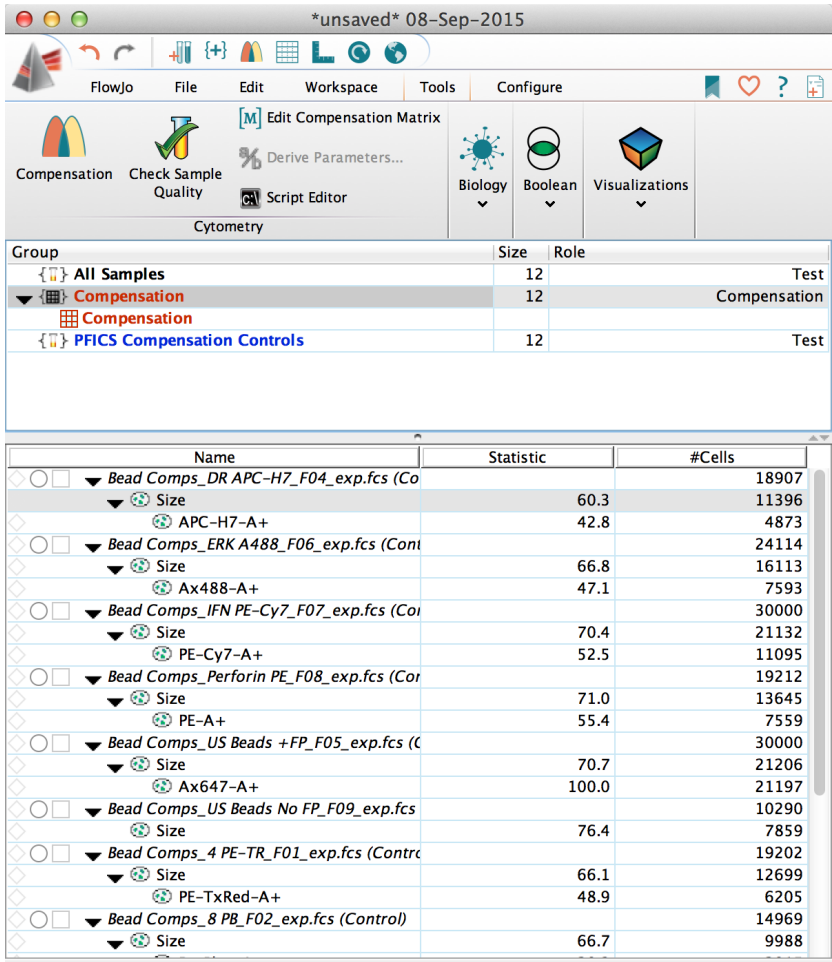
in the task bar.

2. Click the Compensation Tool



1. Highlight Compensation Group

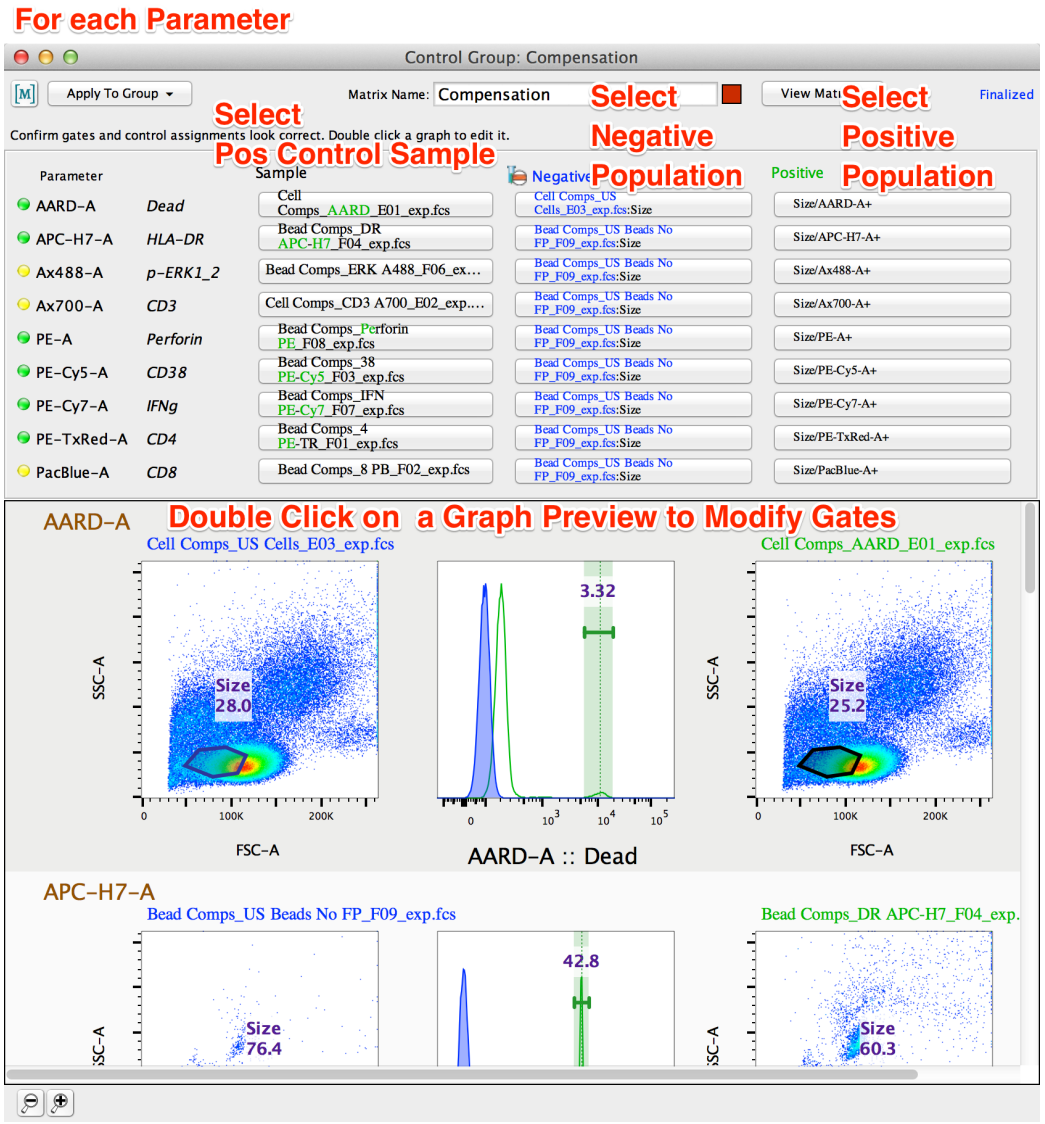
The wizard auto gates samples

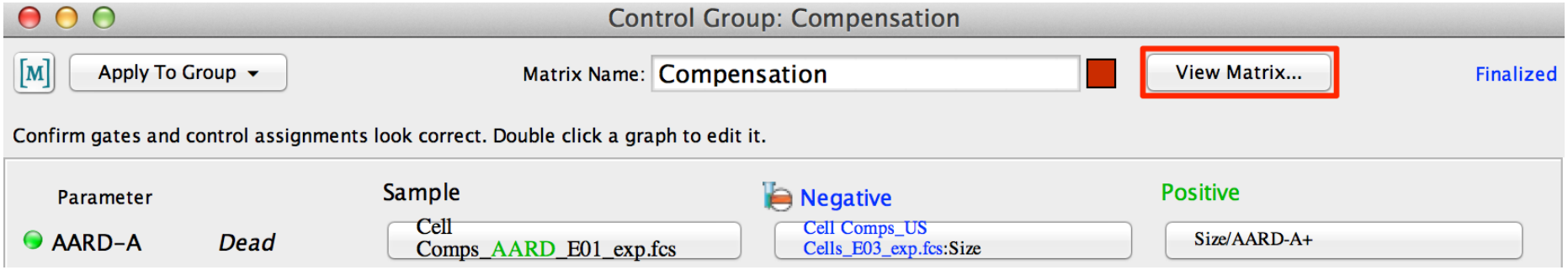


Compensation II

- Define positive and negative sample populations.

- Choose from the dropdown lists for each parameter, or drag and drop to populate fields.
- Double click graph preview to modify gates.

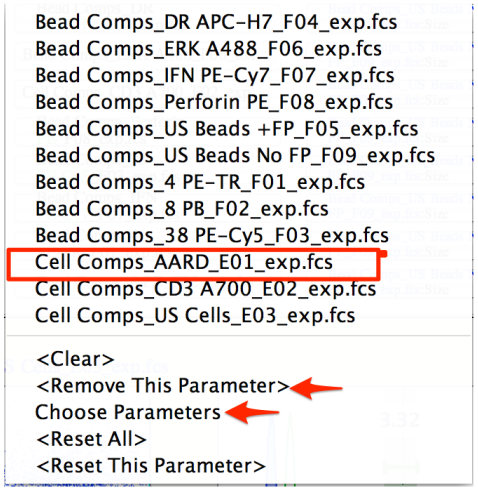




Use Sample drop down list

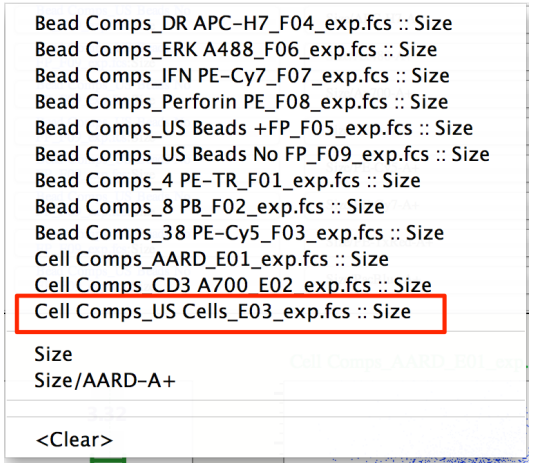
to select Pos Control Sample and

Choose or Remove Parameters



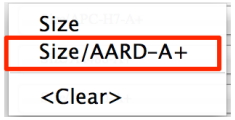
Use Negative drop down list

to Select Negative Sample or Population



Use Positive drop down list

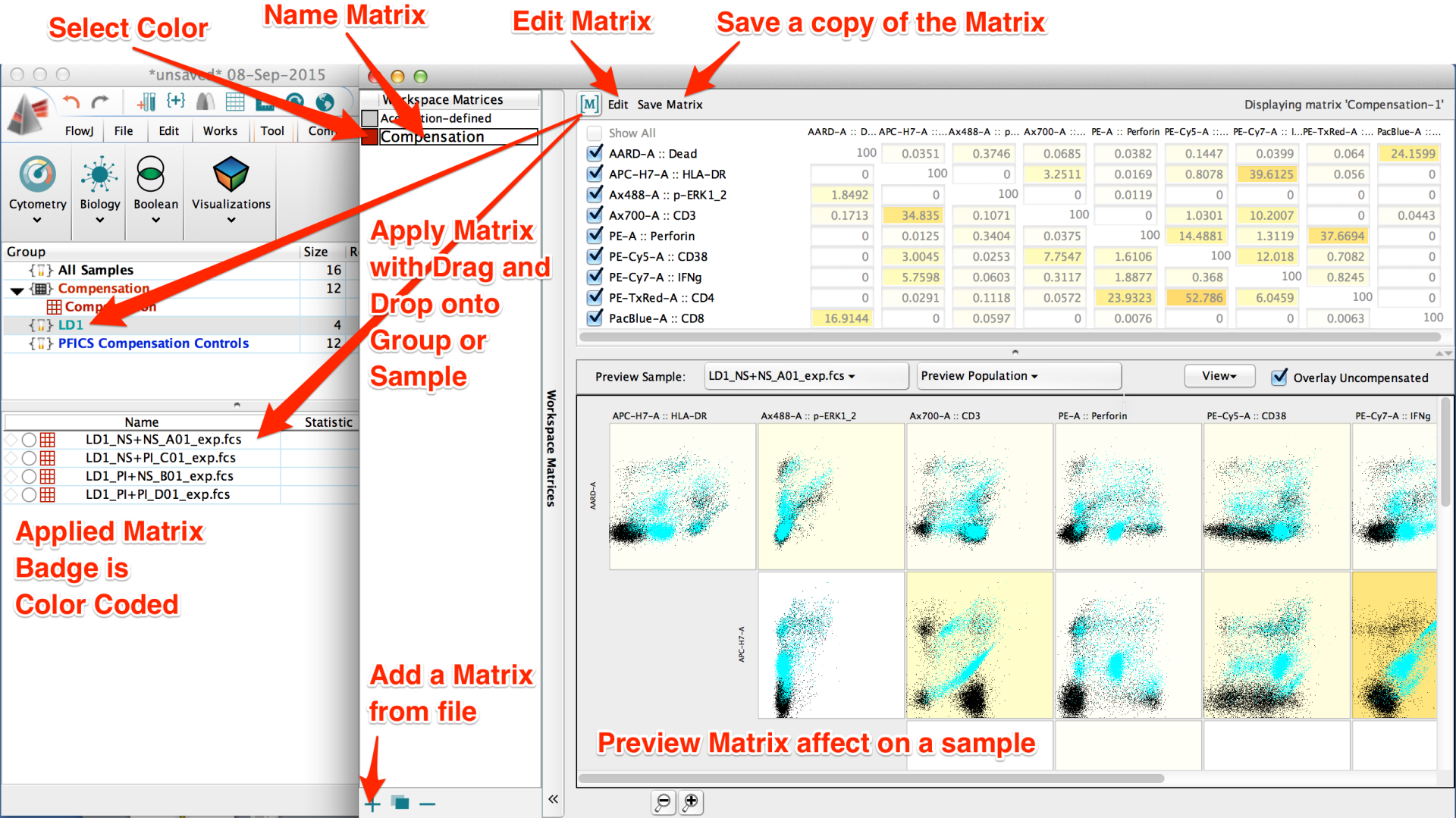
to Choose Positive population



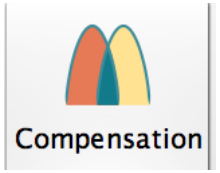
- Note that you can always create your own gates on a sample and then choose those from the drop down menus.
- When set up is complete, select View Matrix (top right) to Modify, Apply, Save or Preview the matrix you've created.

Compensation III

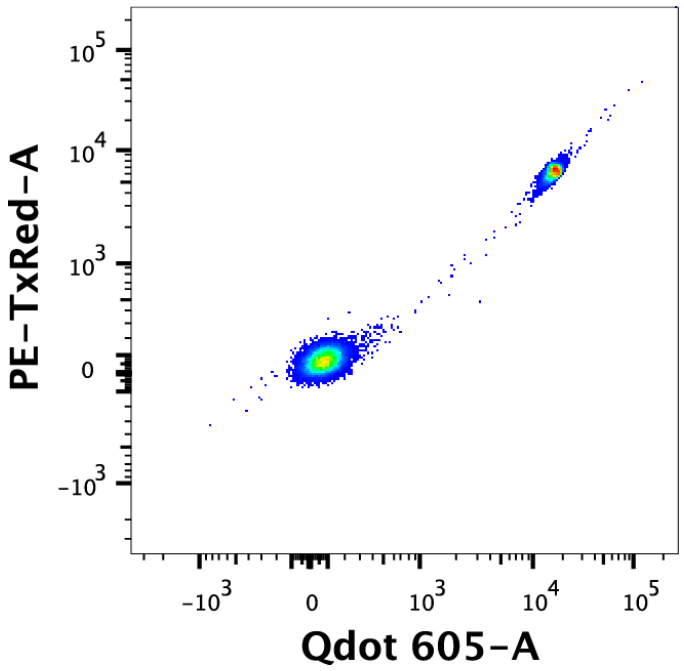
- Preview and apply the calculated matrix



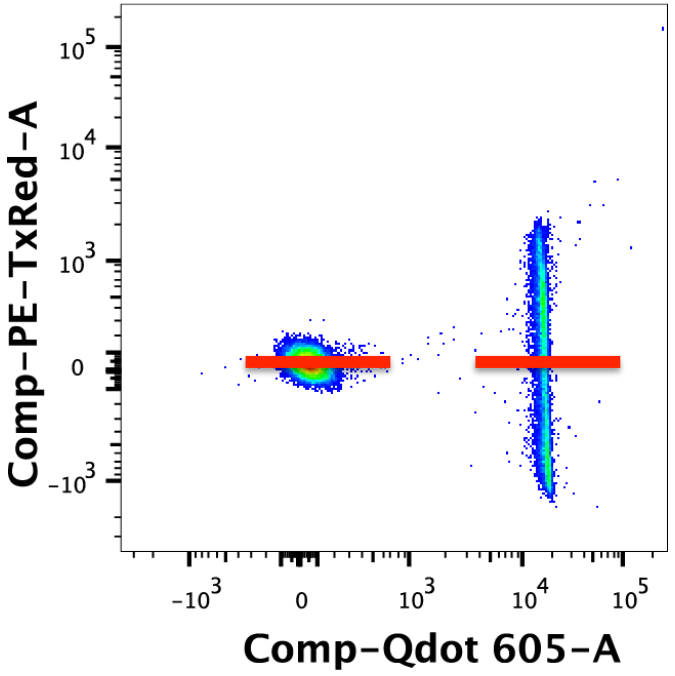
Effect of Compensation



Uncompensated



Compensated



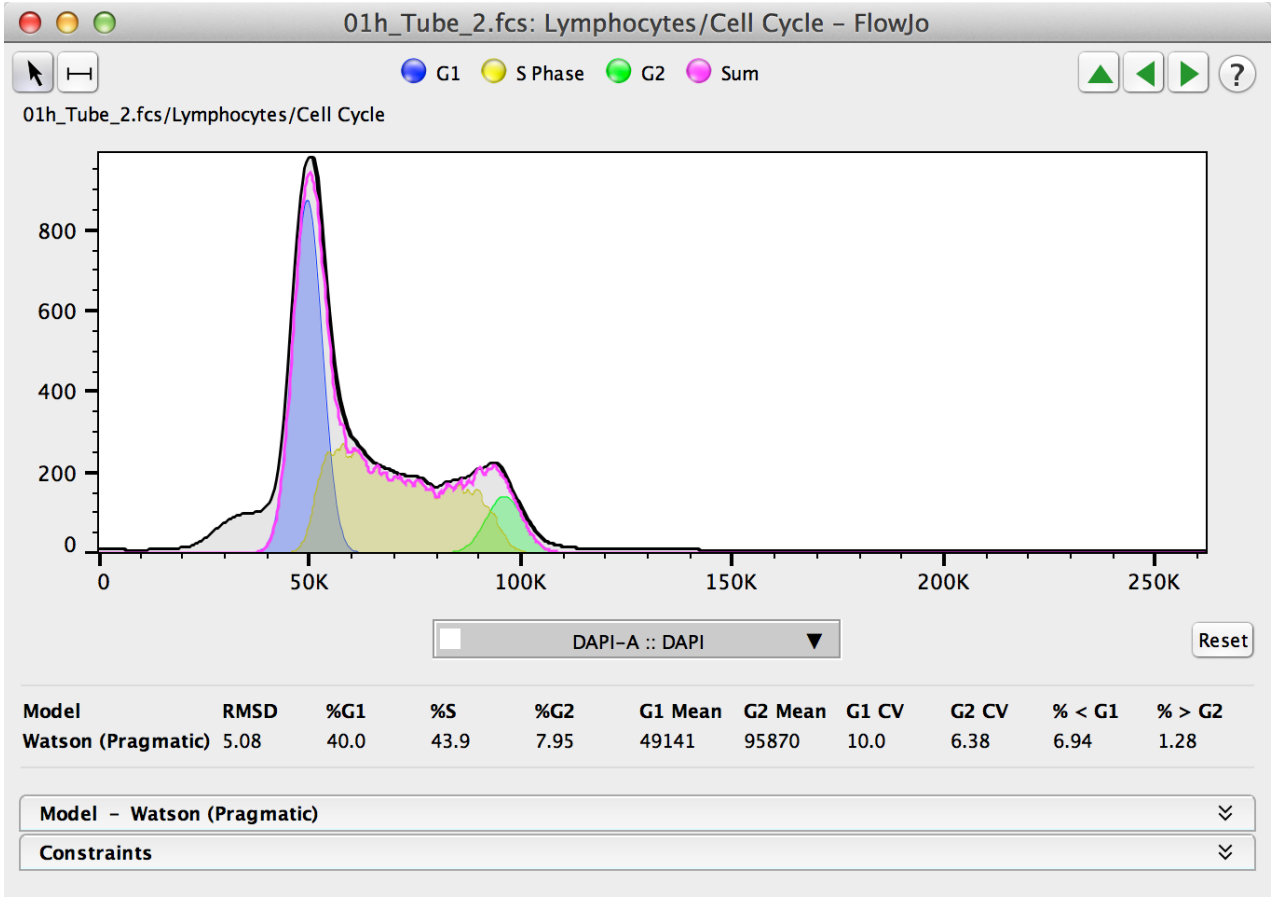
Outline – Part II
Advanced Tools and Platforms

- Cell Cycle Analysis
- Plate Tools
- Export/Concatenate
- Plugins
- Additional Resources
- Q&A

Cell Cycle Analysis

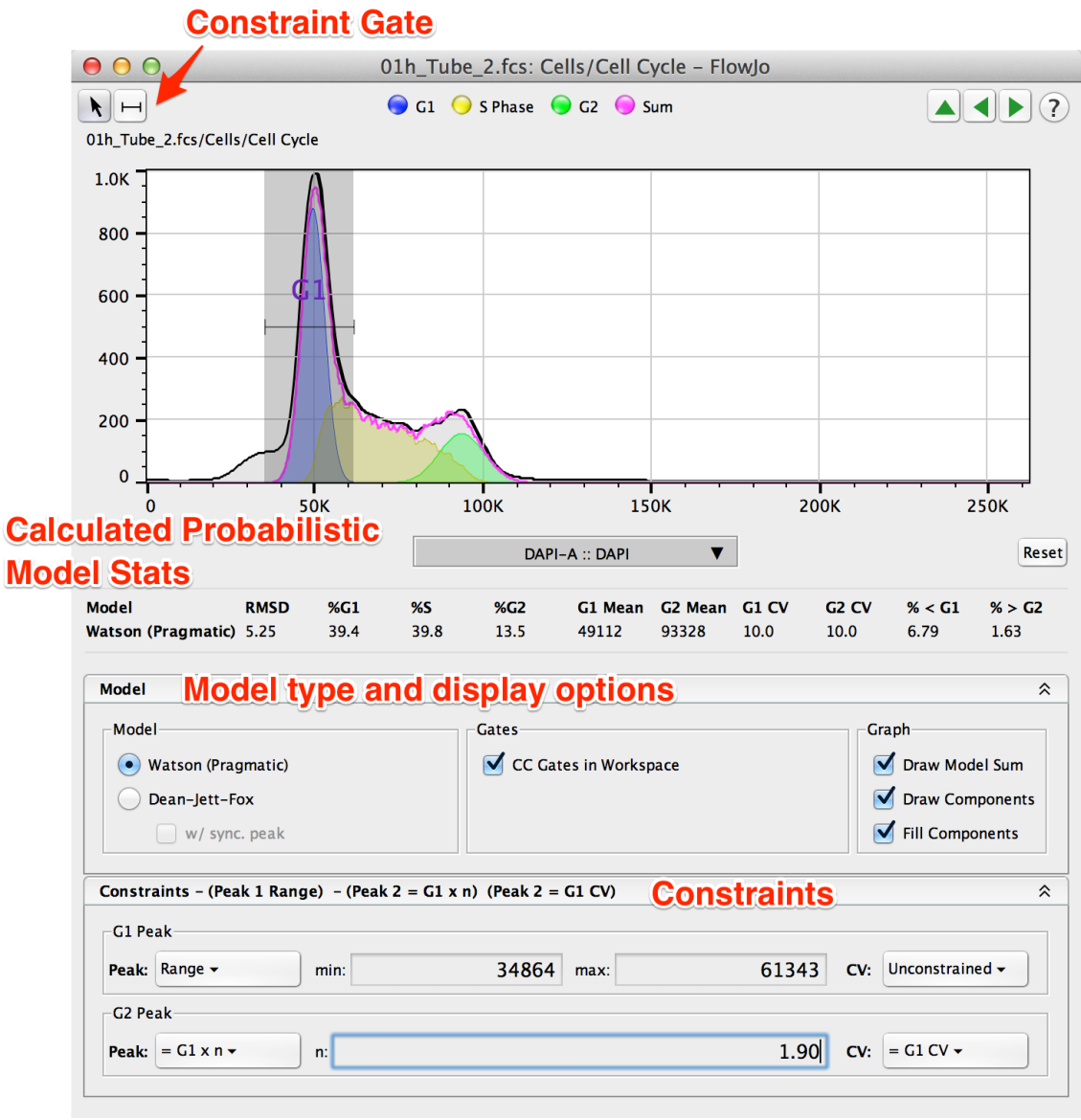
- The Cell Cycle platform allows 1D modeling of cell cycle phases based on DNA content.

- V10 has 1D Watson pragmatic (polynomial S-phase fit) and Dean-Jett-Fox (Gaussian fit) models.

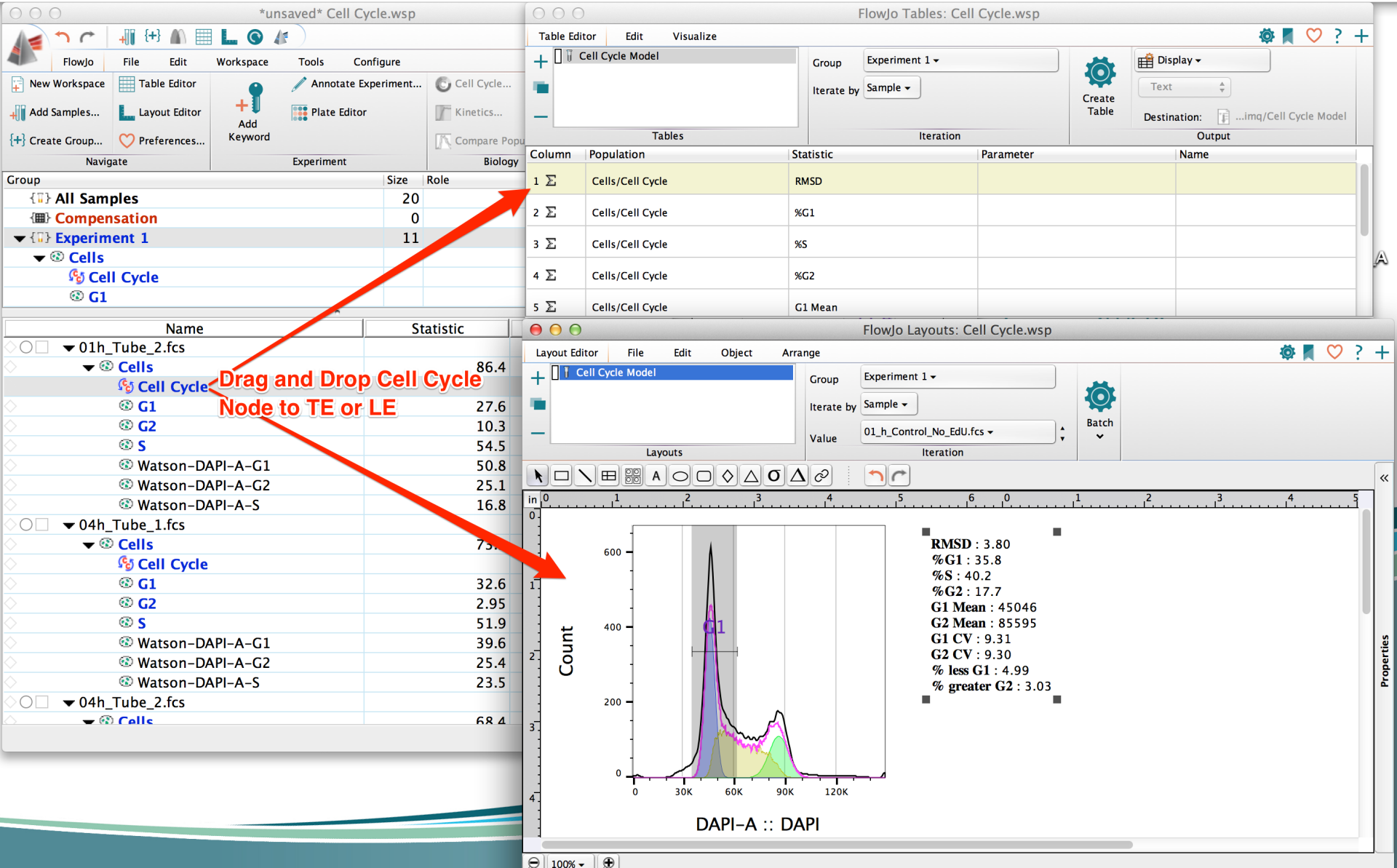


Cell Cycle Analysis Workflow

- Initiate CC modeling from the Biology Band
- Select model type
- Set constraints on G1/G2 peaks and CVs.
- Set model individually for each sample, or group-apply a model to all samples in a group.

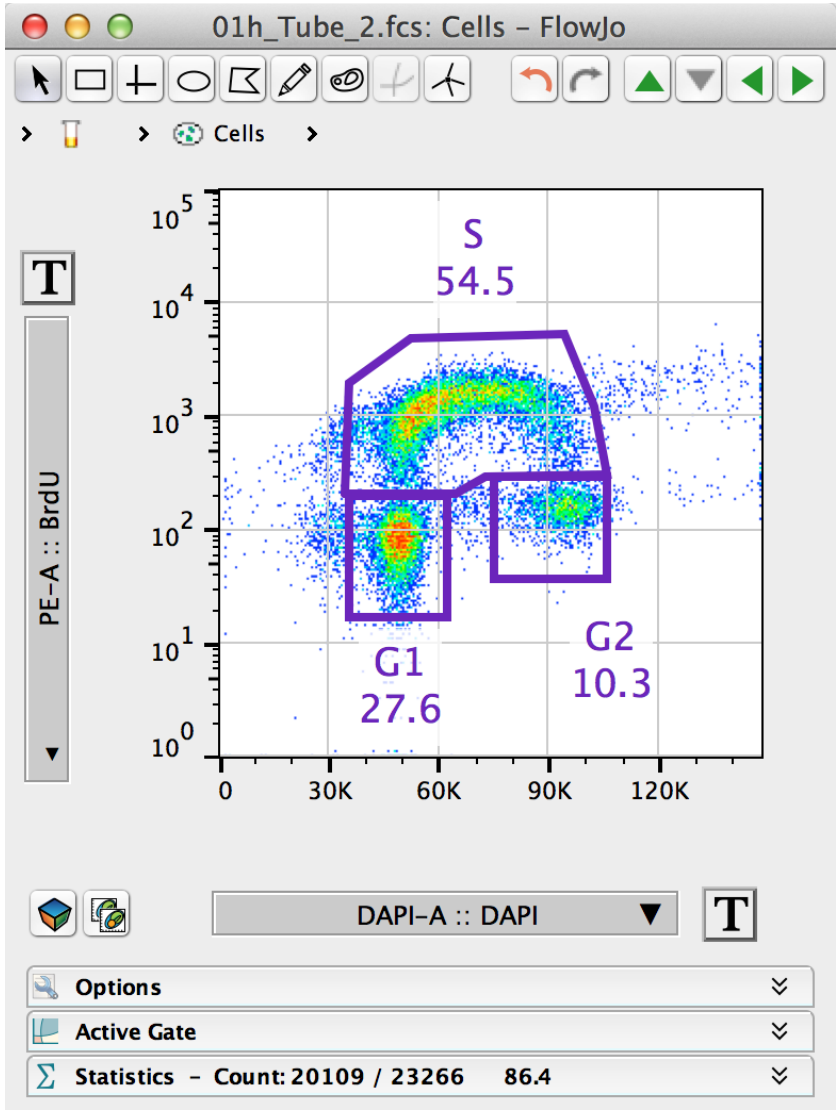


Cell Cycle Analysis Reporting



Cell Cycle Analysis in 2D

- Note that if using an S-phase specific marker in your panel, you can also use standard 2D gating to define G1, S, and G2.
- This may produce results that are more accurate than the 1D probabilistic models.



The Plate Editor

- Viewer to add keywords in a plate format
- Located in the visualizations Band within the Tools Tab
- Add new keyword/value pairs to the right. Drag and drop on selected wells.

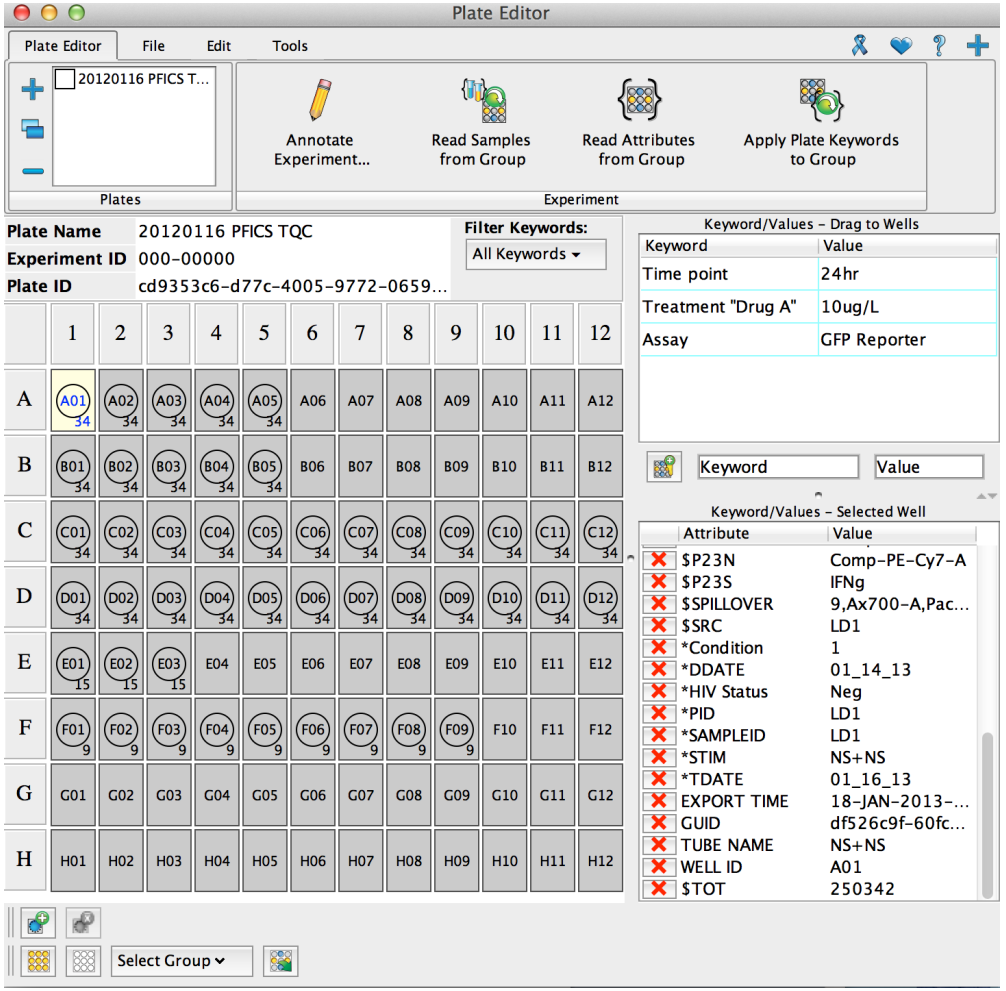
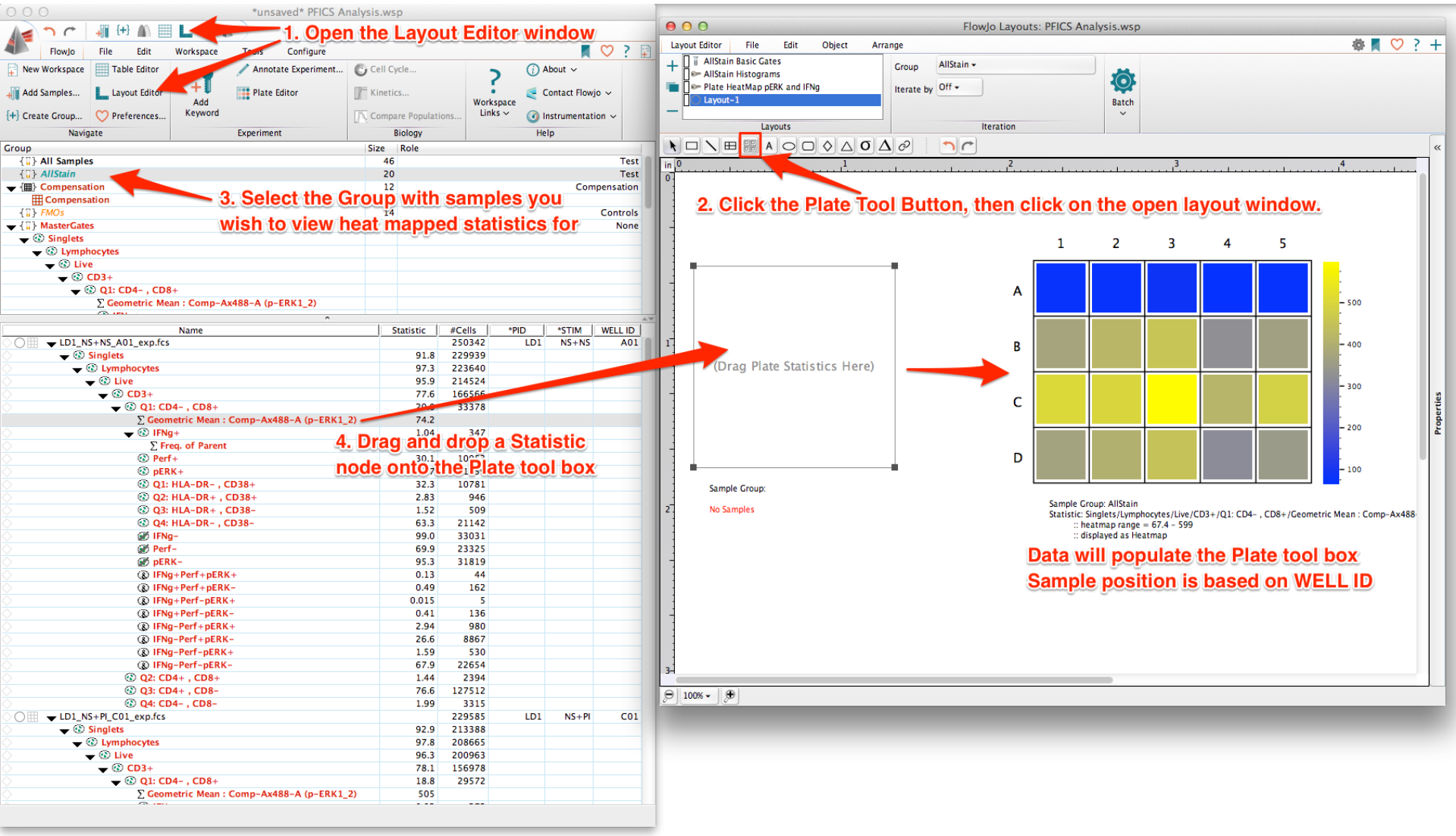
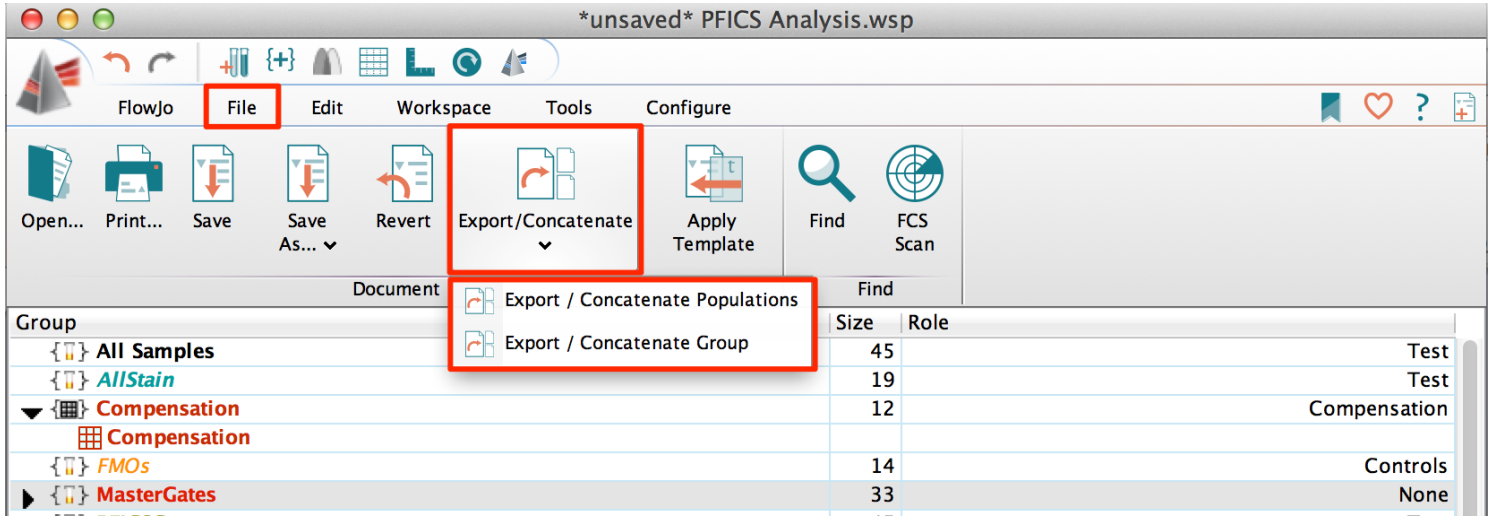


Plate Statistics Heatmap



Export/Concatenate

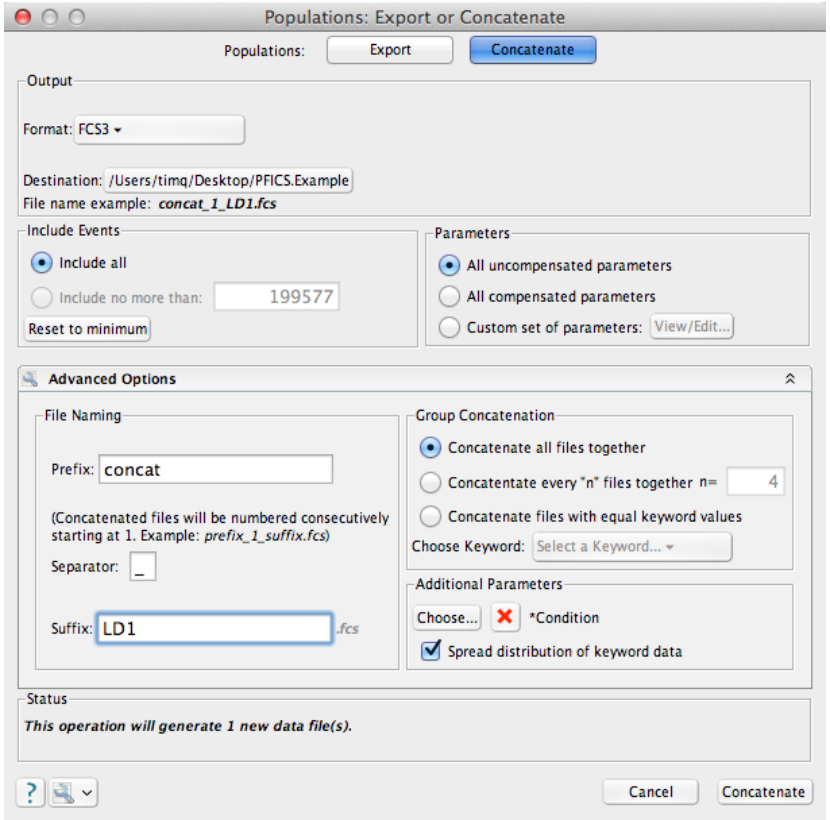
- Initiation from the Workspace



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

Concatenate Options

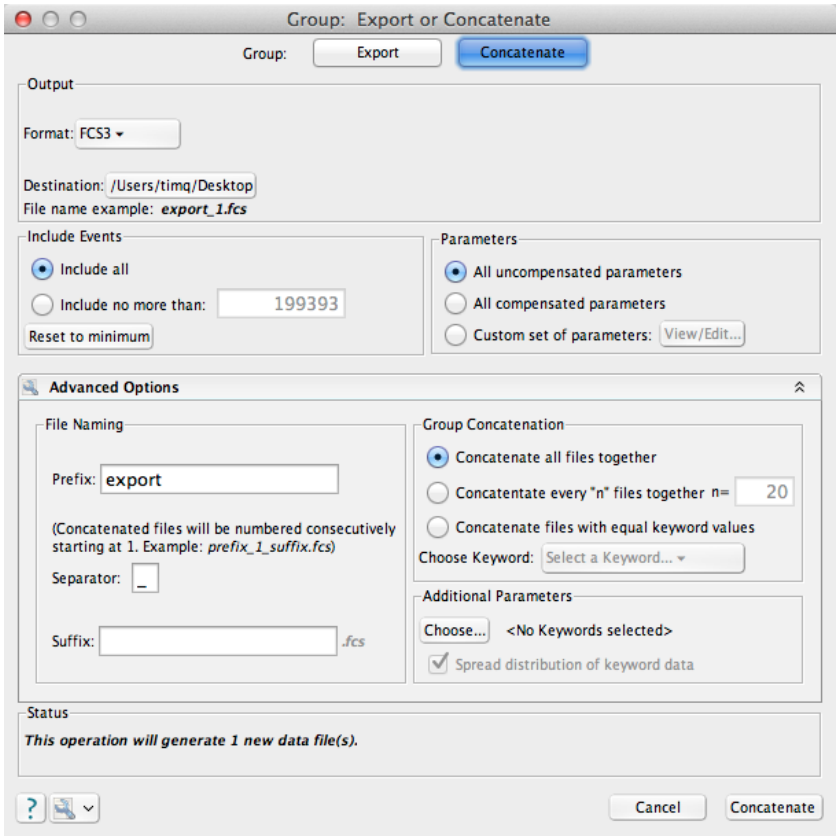
- **Output**
  - Format: select file format (FCS3 or CSV)
  - Destination: specify save location
  - File name example: displays example of naming scheme as specified in Advanced Options → File Naming
- **Include Events**
  - Include all events is the only option when concatenating
- **Parameters**
  - Suggestion: Always leave Choose All uncompensated parameters selected. If the sample is compensated, compensated parameters will be written to the concatenated file.
- **File naming**
  - Prefix: specify a common prefix
  - Suffix: specify a suffix
- **Status**
  - Number of files that will be generated



Concatenating Groups

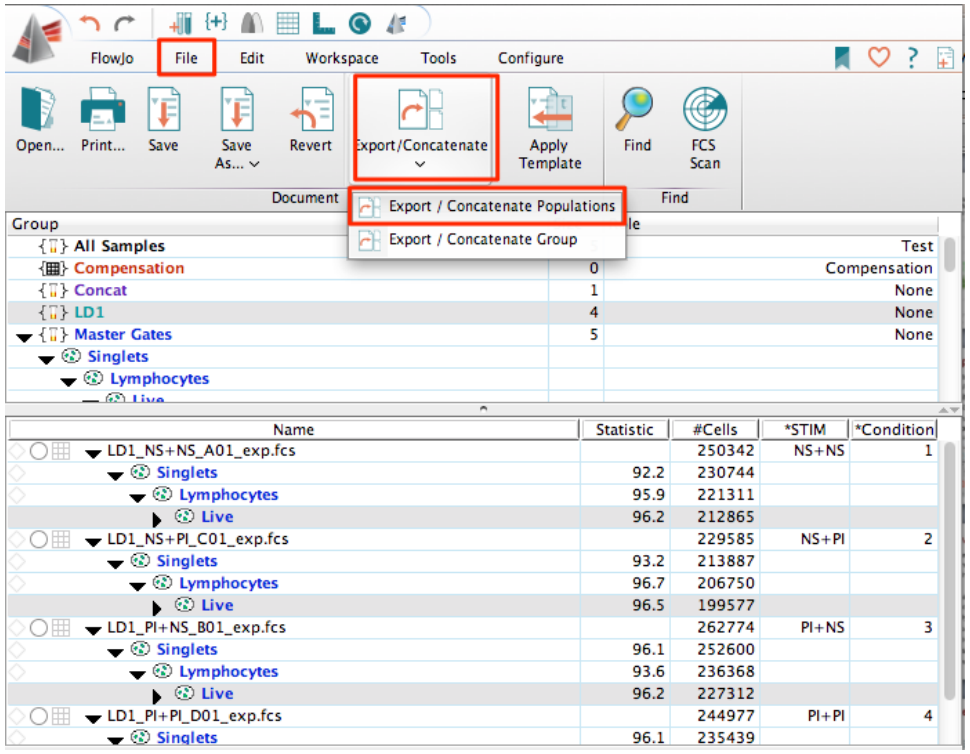
- Highlight a group or group owned population. The group should contain all the samples you wish to export.
- Choose Export/Concatenate Group and click the Concatenate button at the top of the UI

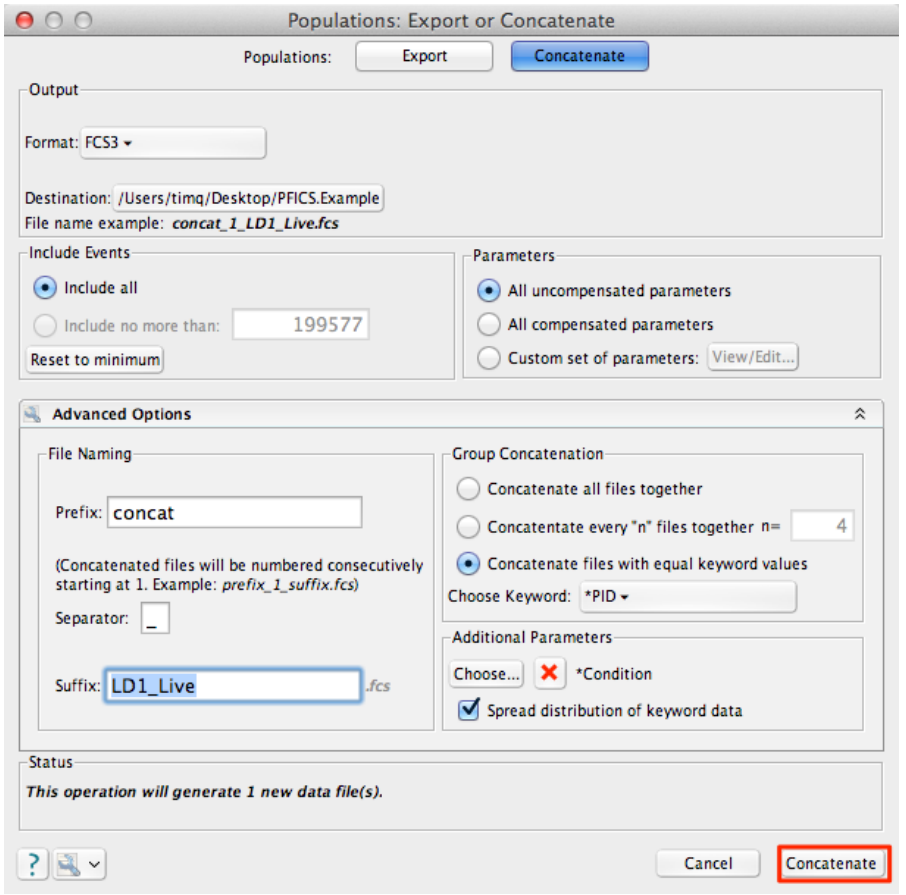
- **Group Concatenation**
  - Concatenate all files together
  - Concatenate every "n" files together
  - Concatenate files with equal keyword values
- **Additional Parameters**
  - Creates new derived parameter(s) from selected Keyword attribute(s) → Order files or group together based on common feature (example: Timepoint or Sick vs Healthy



Concatenating Populations

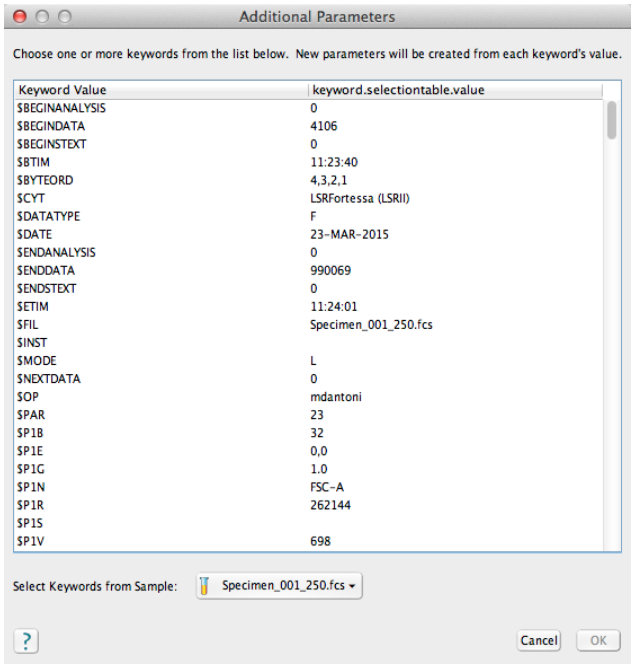
- Highlight the equivalent population nodes within the gating tree of samples you wish to merge.
- Choose Export/Concatenate Populations.

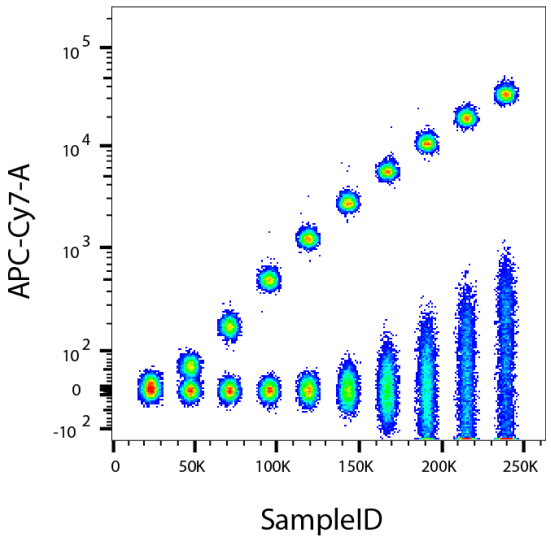


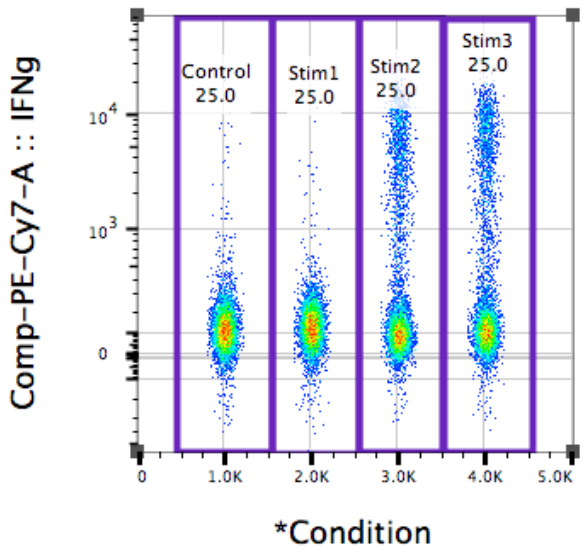


Additional Parameters

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

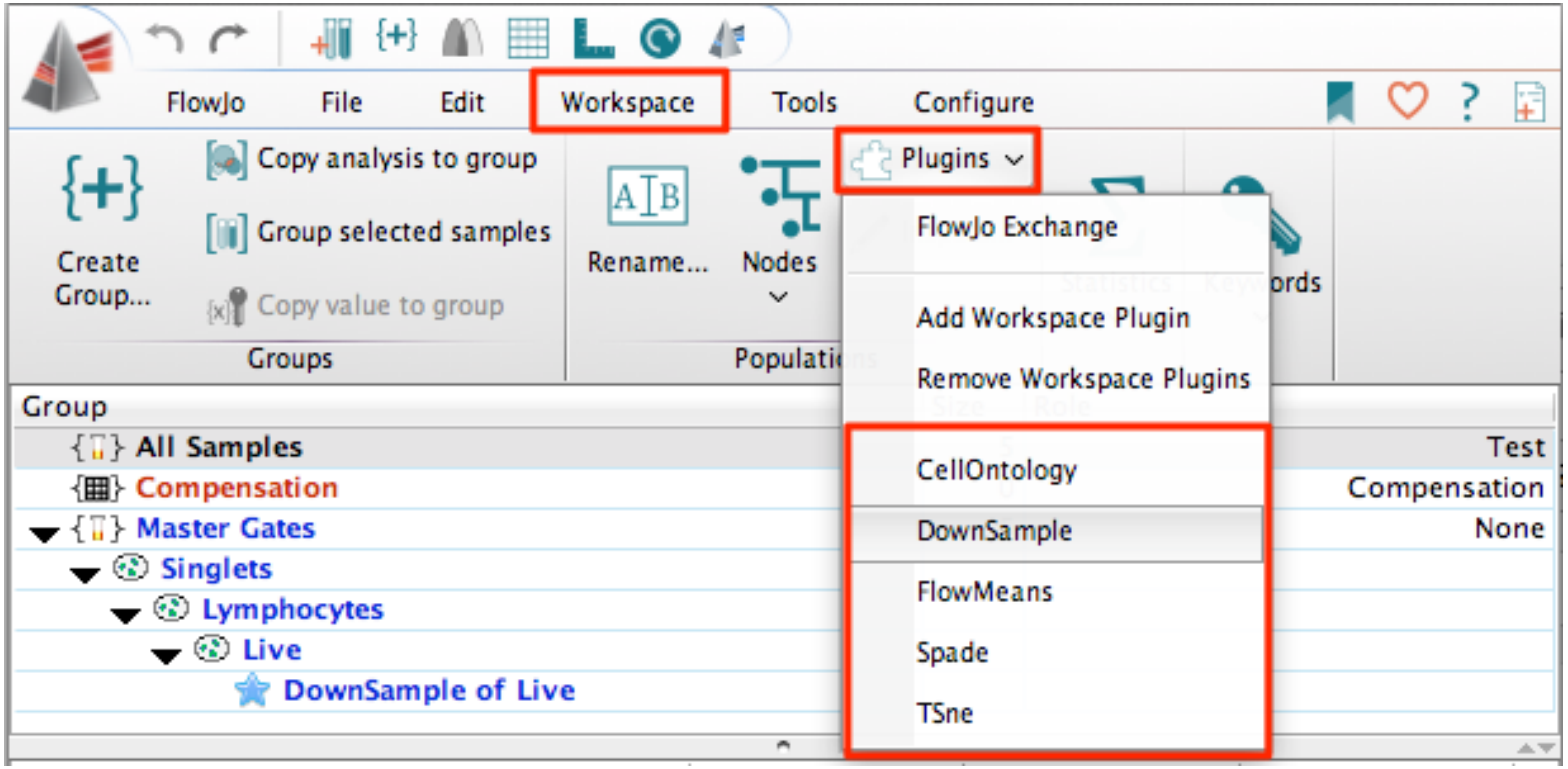






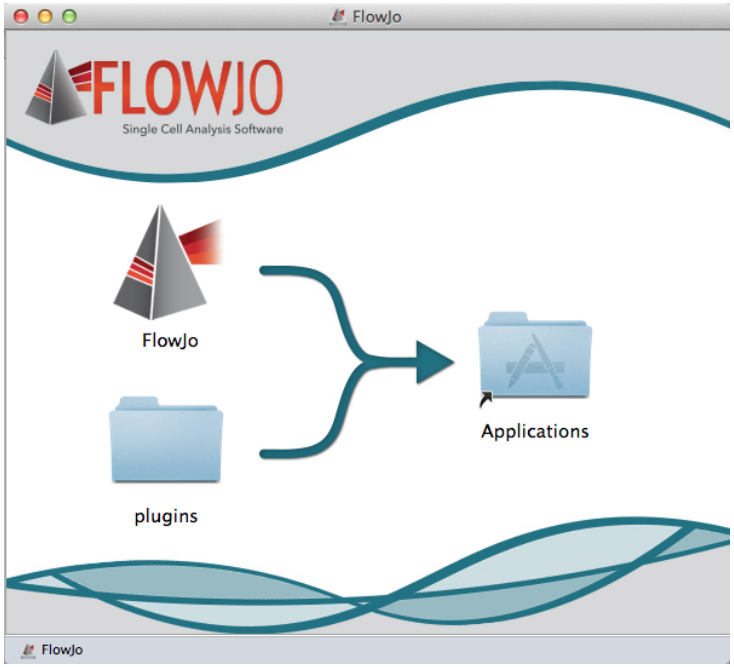
Plugins

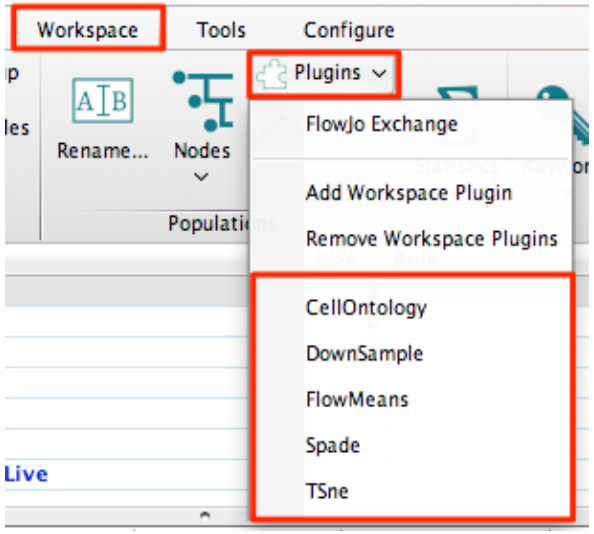
- Java programs that extend the functionality of FlowJo.
- Accessed from the Plugins menu
  - Workspace tab → Populations band → Plugins menu



Installing Plugins

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





Currently Available Plugins

- ***DownSample*** - Create a child gate containing a limited number of events, selected randomly from the parent population
- ***tSNE*** – Dimensionally reduce high parameter data into 2D
- ***SPADE**** - Automated clustering with minimal spanning tree
- ***FlowMeans**** - Automated clustering with K-means
- ***CellOntology**** - Query the GO Database to identify unknown populations.

*Requires the R Statistical Computing Environment

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

When you run a Plugin

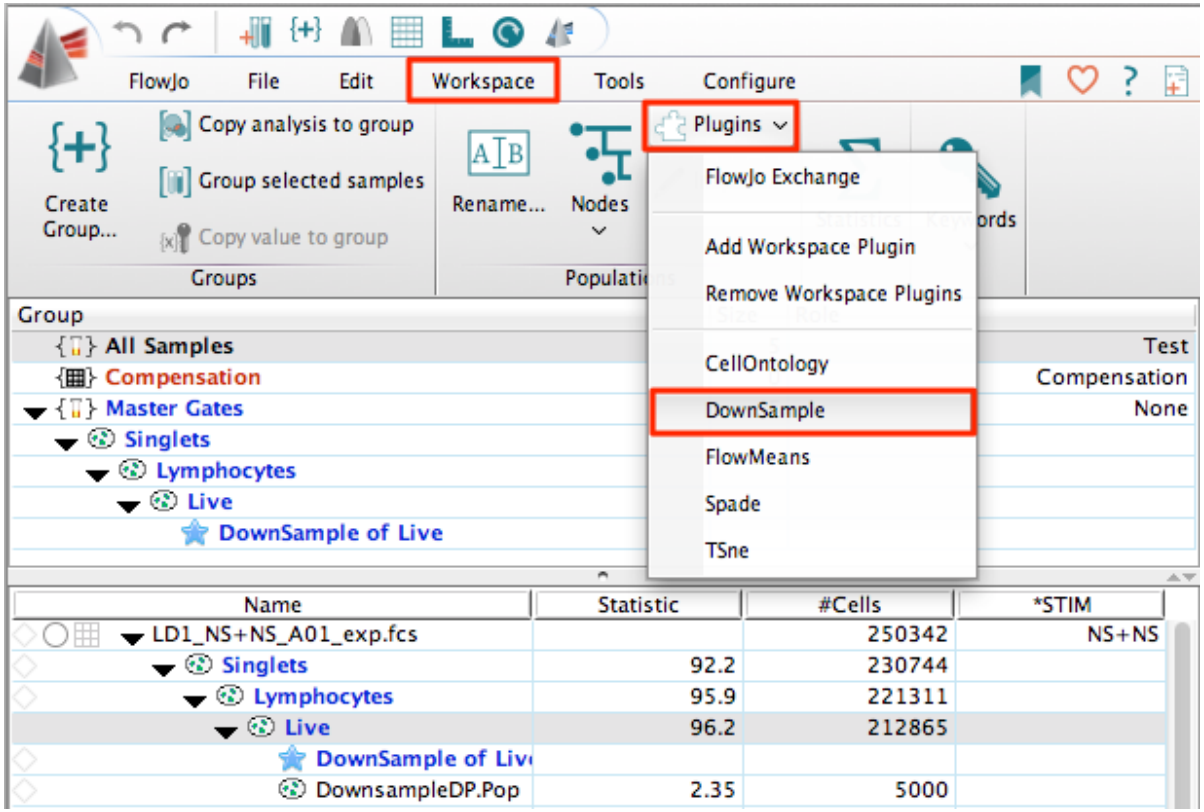
- You must save the FlowJo Workspace first
  - If not, prompted to save
- Many plugins take a gated population from FlowJo, uses it to run some sort of operation or algorithm calculation producing associated derivative files, and returns results to FlowJo.
- The derivatives files are saved in a folder,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

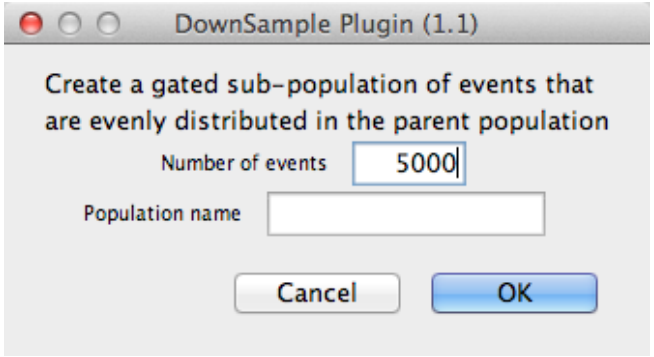
DownSample

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

DownSample

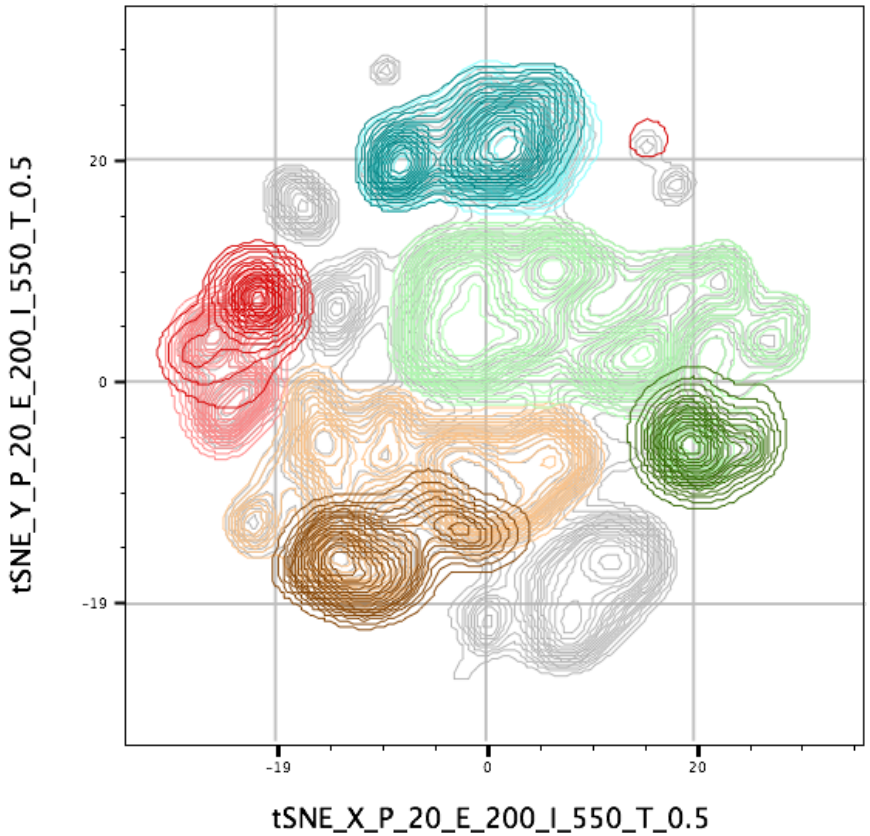
- Initiating DownSample from the Workspace





tSNE

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

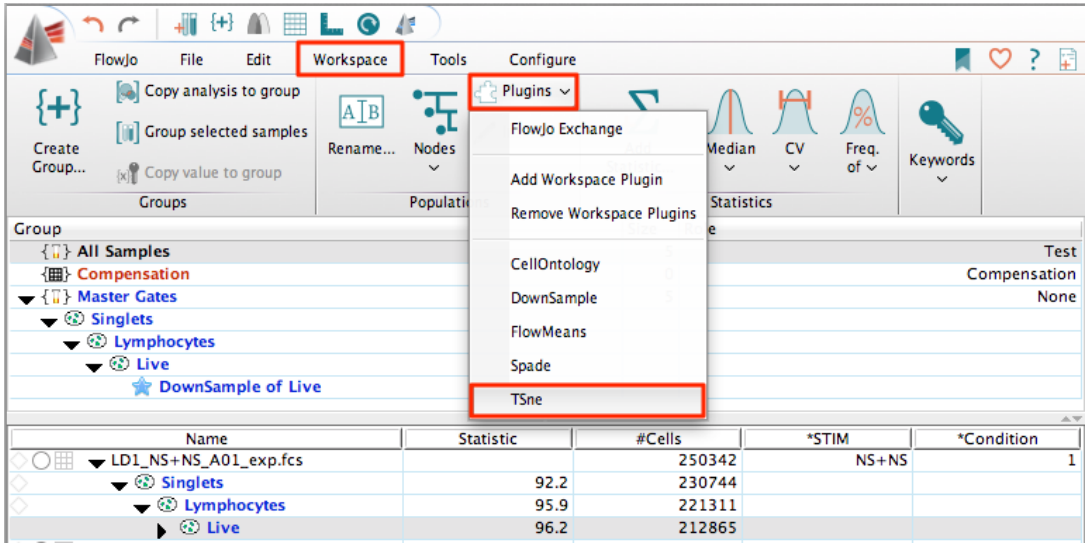


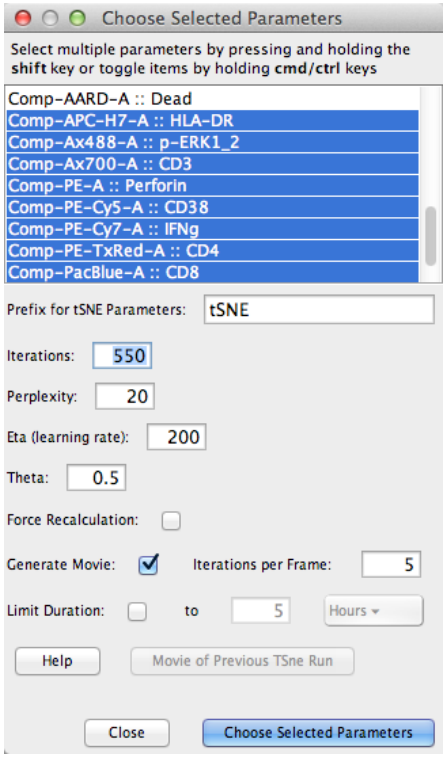
Full Gating Path
Lymphocytes/DownsampleDP.Pop/Q3: CD3+ , CD19-/Q3: CD4+ , CD8-/CD127-CD25+
Lymphocytes/DownsampleDP.Pop/Q3: CD3+ , CD19-/Q3: CD4+ , CD8-
Lymphocytes/DownsampleDP.Pop/Q3: CD3+ , CD19-/Q1: CD4- , CD8+/CD127-CD27+
Lymphocytes/DownsampleDP.Pop/Q3: CD3+ , CD19-/Q1: CD4- , CD8+
Lymphocytes/DownsampleDP.Pop/Q1: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

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

tSNE

- Initiating tSNE from the Workspace

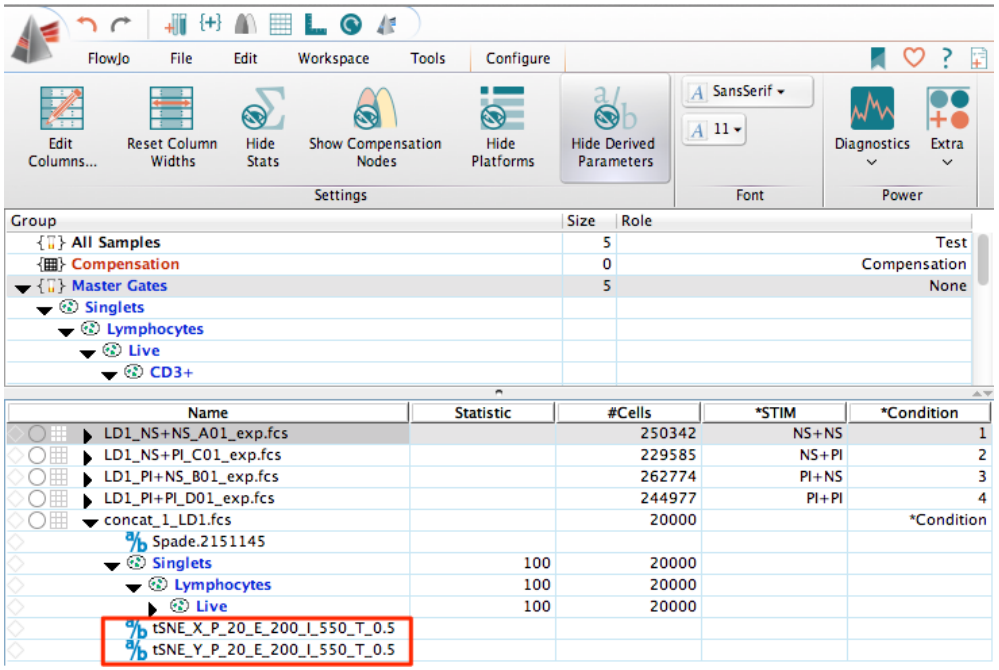




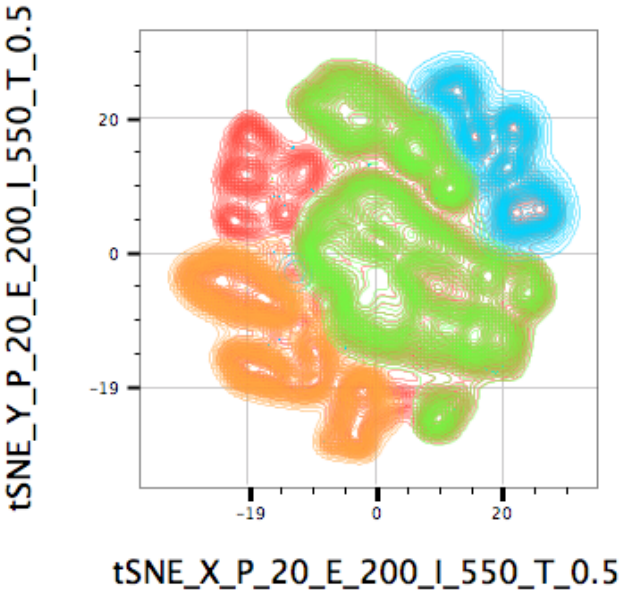
- **Iterations –** Maximum number of iterations the algorithm will run.
- **Perplexity –** Perplexity is related to the number of nearest neighbors that is used in learning algorithms. In tSNE, the perplexity may be viewed as a knob that sets the number of effective nearest neighbors. The most appropriate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

tSNE

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

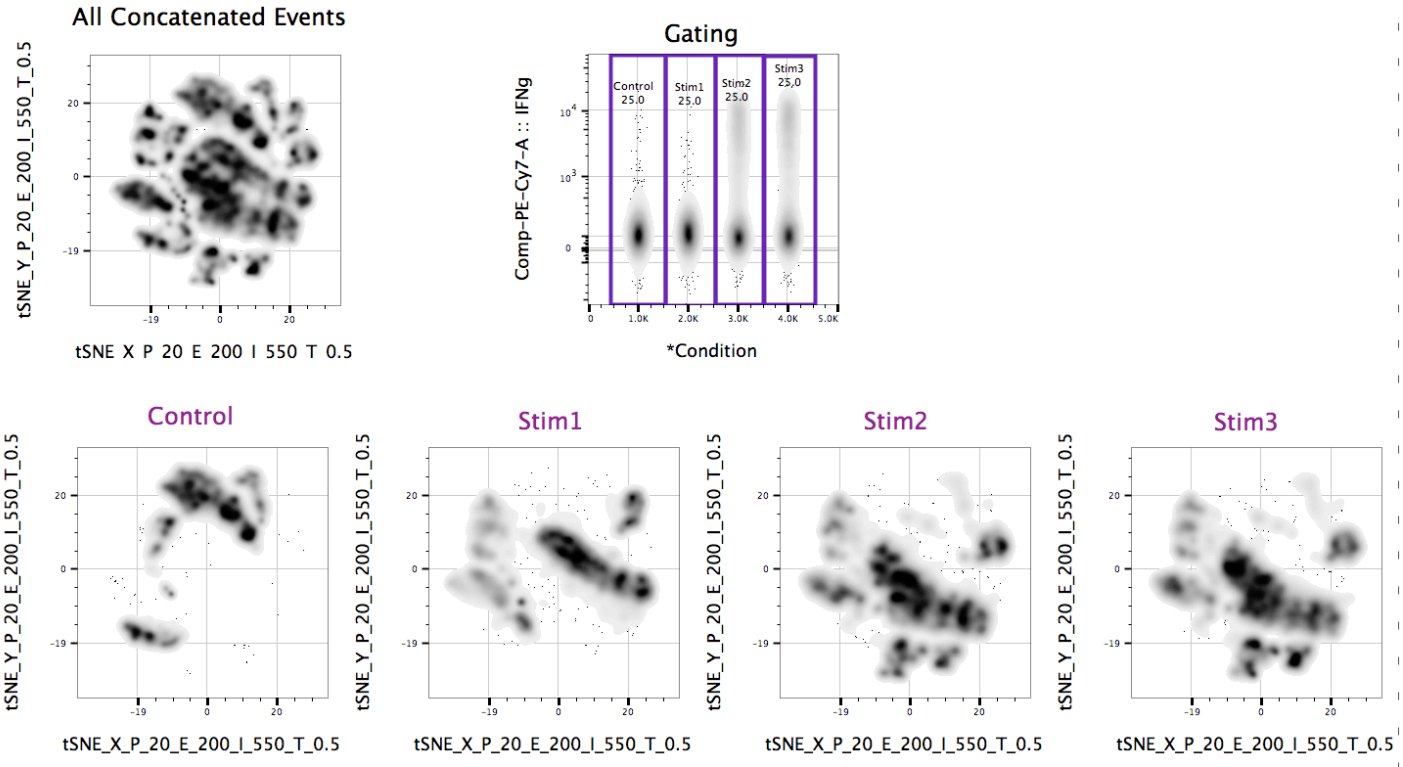


Sample Name	Subset Name	Count
concat_1_LD1.fcs	Q3: CD4+ , CD8-	12057
concat_1_LD1.fcs	Q1: CD4- , CD8+	3504
concat_1_LD1.fcs	CD3-HLA-DR-	2033
concat_1_LD1.fcs	Live	20000



tSNE

- Practical Considerations
  - Cleaning up the data
  - DownSample
  - Parameter Selection
  - Workflow



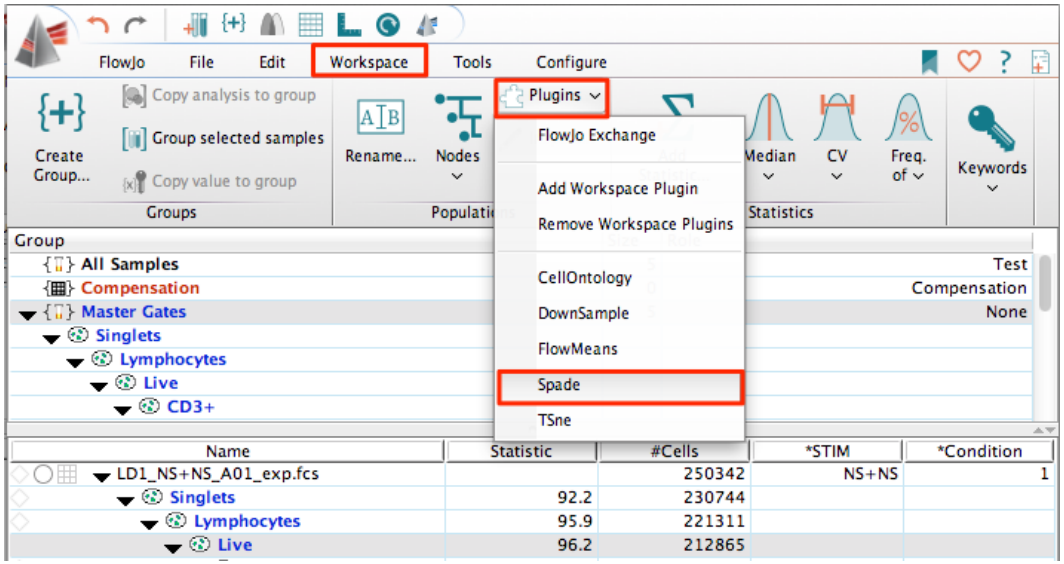
SPADE

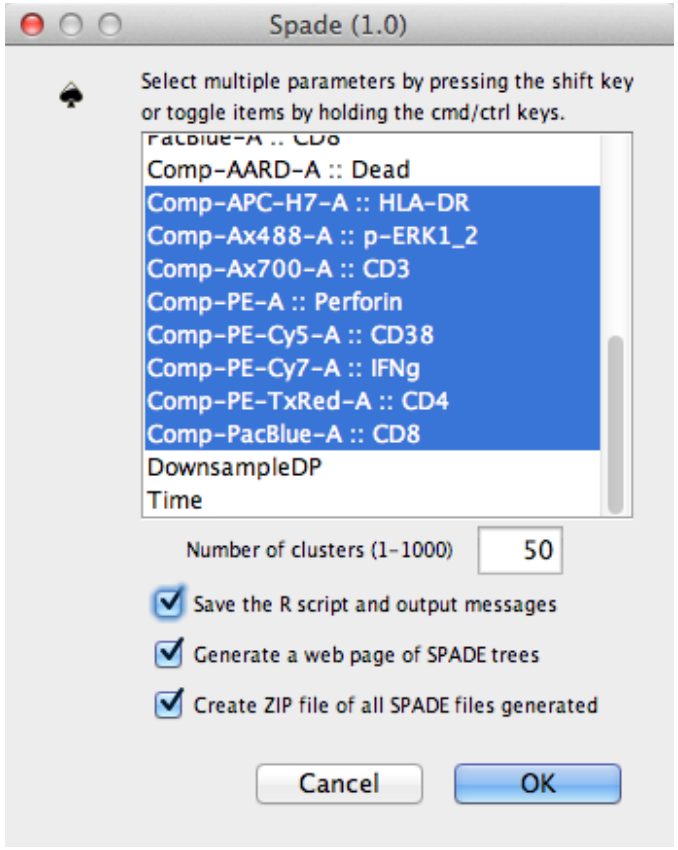
- Spanning tree Progression of Density normalized Events (SPADE) is an algorithmic visualization tool for high dimensional flow and mass cytometry data.
- Requires R and the R package SPADE
  - install.packages("devtools")
  - library(devtools)
  - devtools::install_github("nolanlab/Rclusterpp")
  - source("http://bioconductor.org/biocLite.R")
  - devtools::install_github("nolanlab/spade")
- Produces
  - Gated cluster populations as children of the parent reference population in the FlowJo workspace
  - Zip file containing a network GML file, PDFs of the graphs, tables, and FCS files with the "cluster " column appended.

https://github.com/nolanlab/spade

SPADE

- Initiation from the Workspace

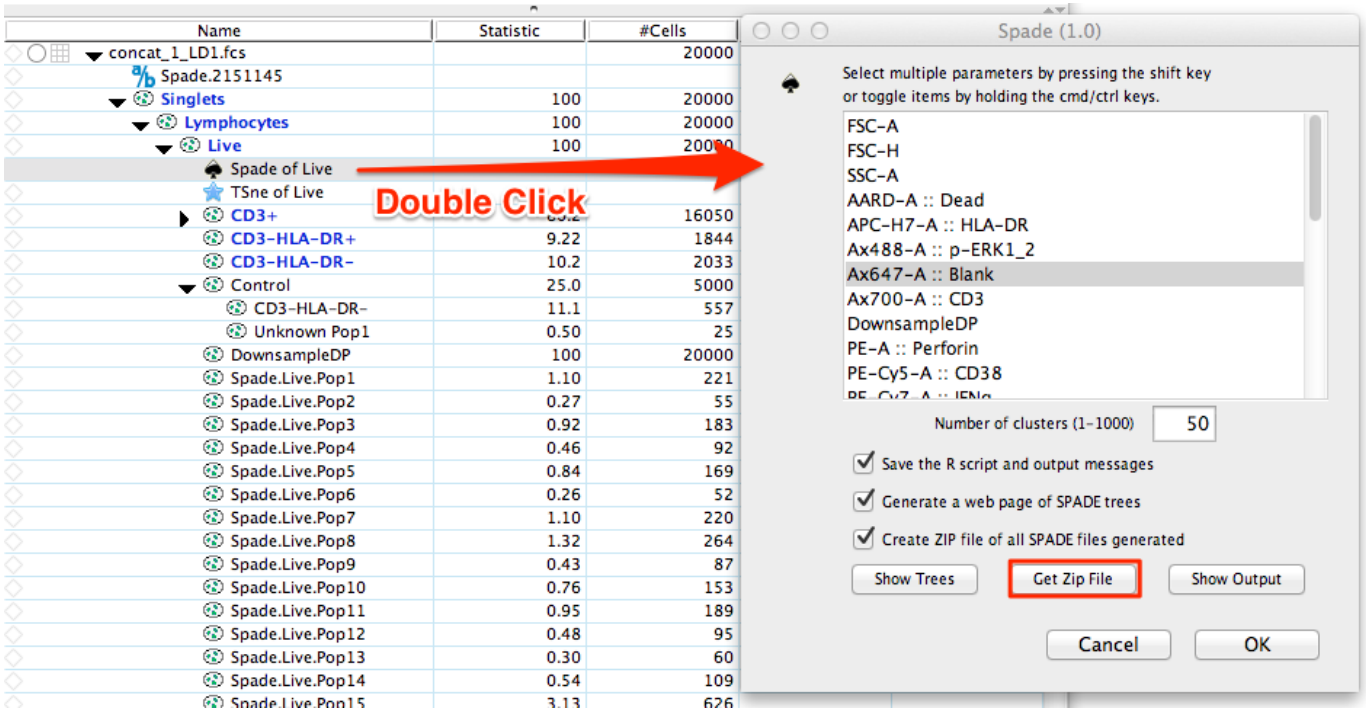




- Enter the desired Number of Clusters and click OK

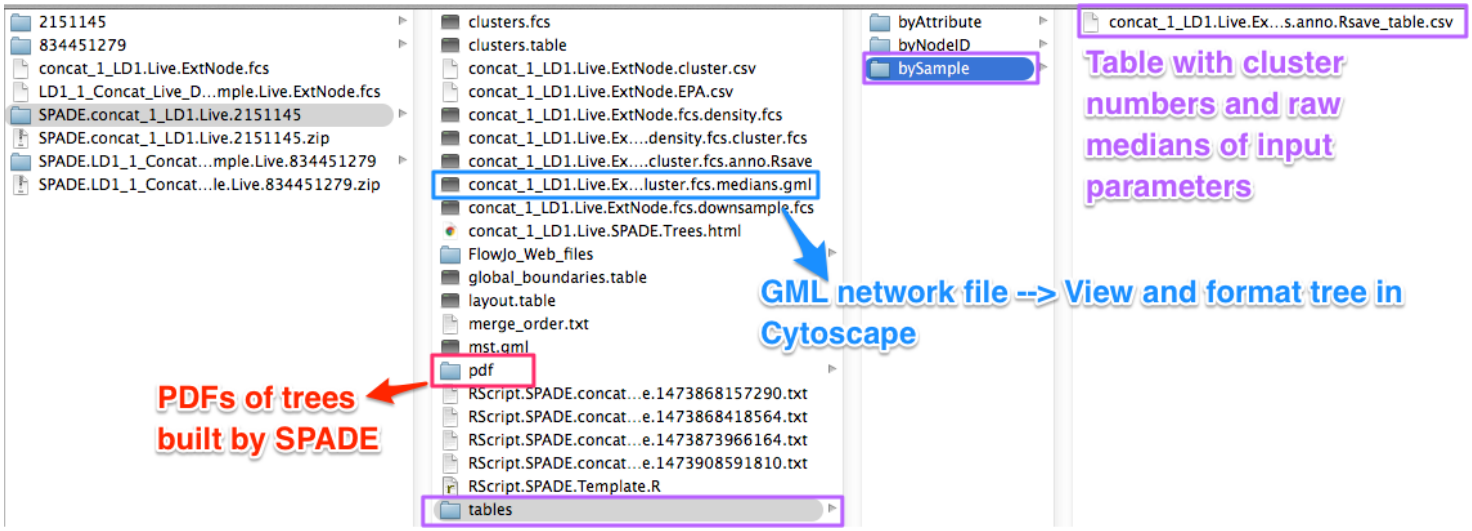
SPADE

- Populations in the workspace represent events within clusters of the spade tree
- Double Click on the Spade of 'Population' node, then select Get Zip File



Within the SPADE .zip

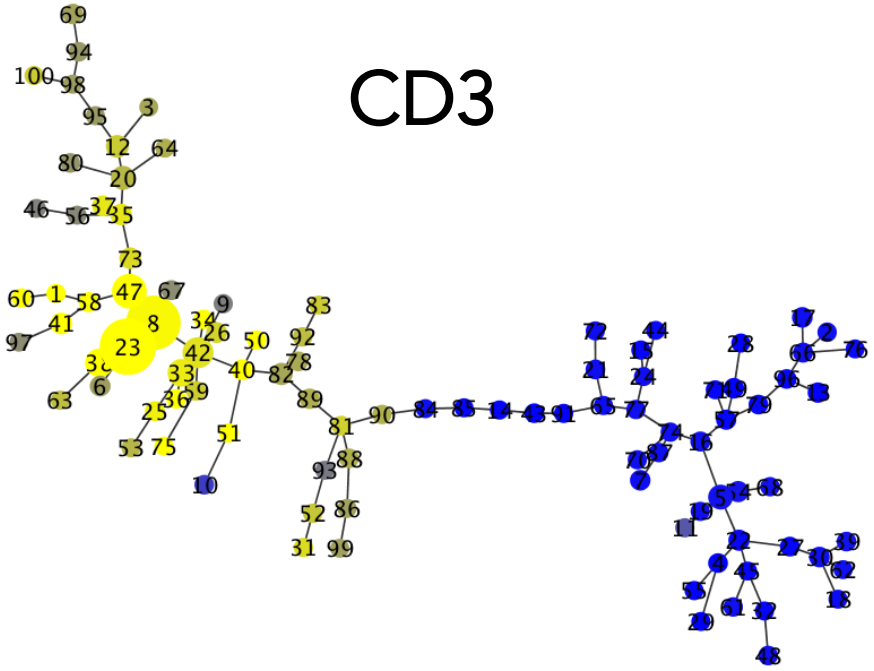
- GML file – Tree network can be visualized using Cytoscape.
- pdf folder - contains PDFs of all the trees built by SPADE
- Tables → By Sample → .csv – spreadsheet with raw medians for every parameter used in the SPADE calculation

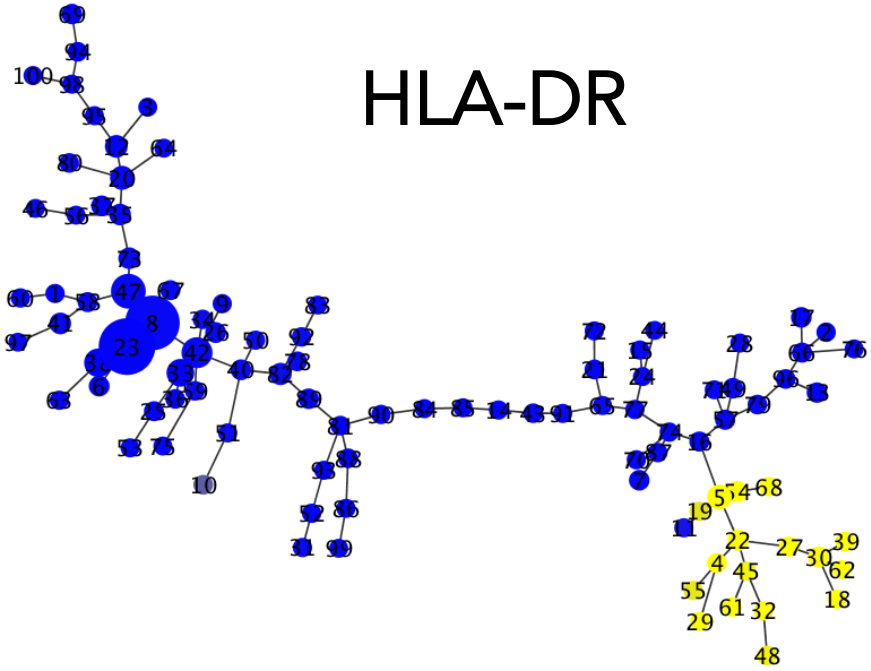


http://www.cytoscape.org/

SPADE

- GML file can be visualized in Cytoscape.

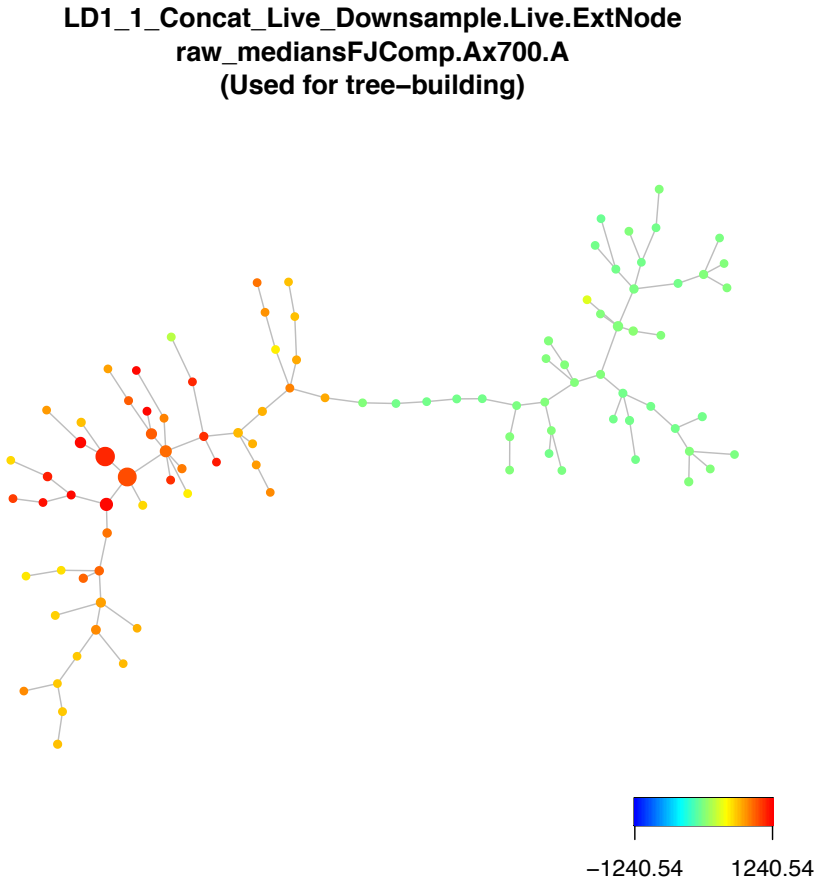




http://www.cytoscape.org/

SPADE

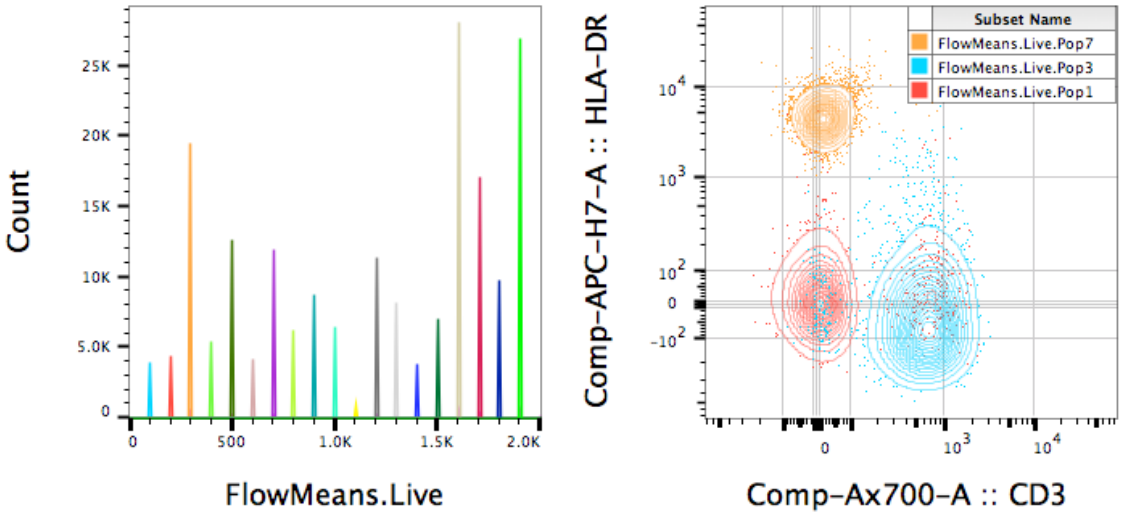
- PDFs aren't as useful, but the By Sample CSV table may be helpful for calling expression.



		CD150	FcgR	IL7R
ID	count	raw_mediansFJComp.PE.A_clust	raw_mediansFJComp.PerCP.Cy5.5.A_clust	raw_mediansFJComp.PE.Cy7.A_clust
1	117	-91.29728699	-187.1949005	-228.2537689
2	75	-114.8877792	1425.162476	-256.3802795
3	607	-131.198349	942.6101685	485.6322632
4	144	45.06650162	-231.4508133	383.0471802
5	176	540.3177338	638.2731018	396.6481018
6	2213	-133.9013367	3068.497314	667.8973389
7	9016	413.6738434	1986.239563	619.8262329
8	126	671.3719482	1045.242371	-225.64505
9	1157	559.7160034	1440.166504	547.5114746
10	111	2026.342407	847.7639771	65.27305603
11	120	2658.671143	-195.1321869	198.4194412
12	405	1883.511353	752.9630127	-291.4768982
13	94	391.3848114	5319.604004	832.4526672
14	118	482.8102112	531.129425	-293.3732147
15	253	-140.9054565	2870.712402	754.9061279
16	54	-137.2172623	450.6687164	-31.0025835
17	66	322.586792	1074.81543	218.1777191
18	632	343.6929016	3988.859375	819.3094177
19	358	1934.900391	-187.7943344	-235.9351273
20	84	-87.44278717	820.6205444	-287.3853455

FlowMeans

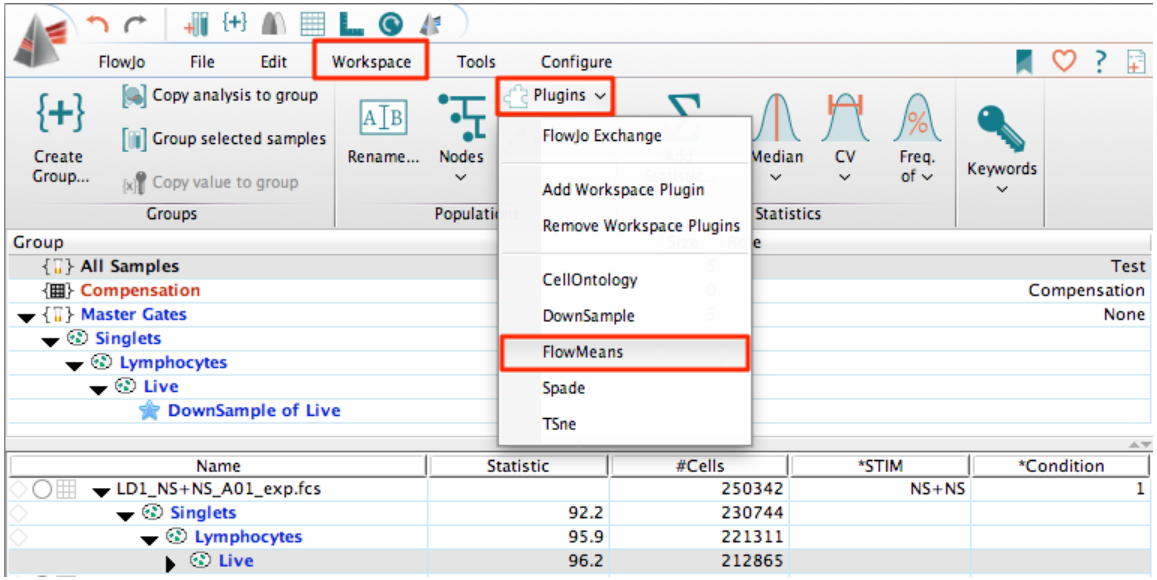
- FlowMeans is a method for automated identification of cell populations based on K-means clustering.
- Requires R and the R package flowMeans
  - source("https://bioconductor.org/biocLite.R")
  - biocLite("flowMeans")
- Produces gated cluster populations as children of the parent reference population.

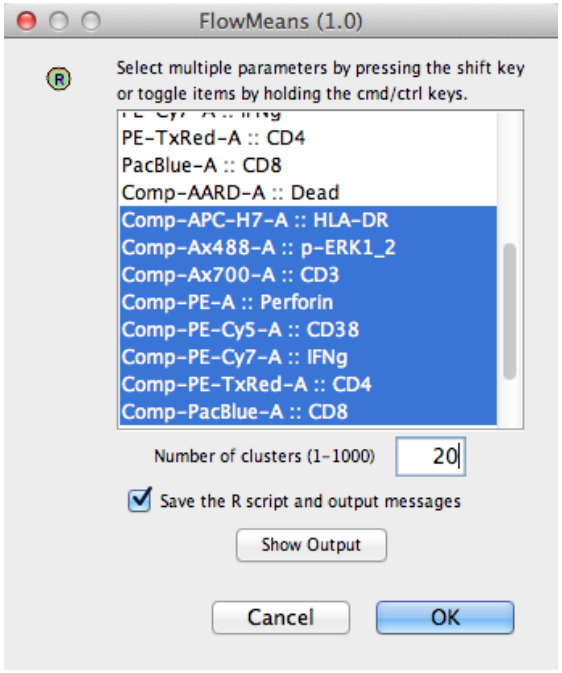


Aghaeepour, N. et. al. (2011) Rapid cell population identification in flow cytometry data. Cytometry A, DOI: 10.1002/cyto.a.21007

FlowMeans

- Initiating flowMeans from the Workspace





- Enter the desired Number of Clusters and click OK

Additional Plugin Resources

The FlowJo Exchange http://exchange.flowjo.com/

- Future plugin releases
- Featured plugins
- Updates
- Developer documentation
- Scripts

Documentation http://docs.flowjo.com

- Search for Plugins → pages describing plugin setup and functionality

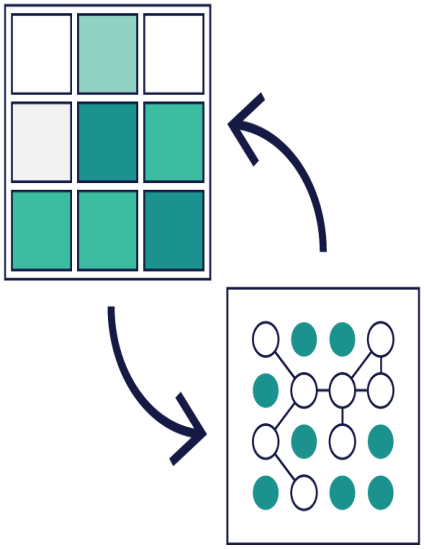
Additional Training Resources

- Webinars on basic and advanced features of FlowJo, held on the 1st and 3rd Thursday of each month.
- Webinar Schedule can be found at http://www.flowjo.com/webinars/
- Technical Documentation for V10 can be found at http://docs.flowjo.com/
- The Daily Dongle provides tips, tricks and answers to common questions.

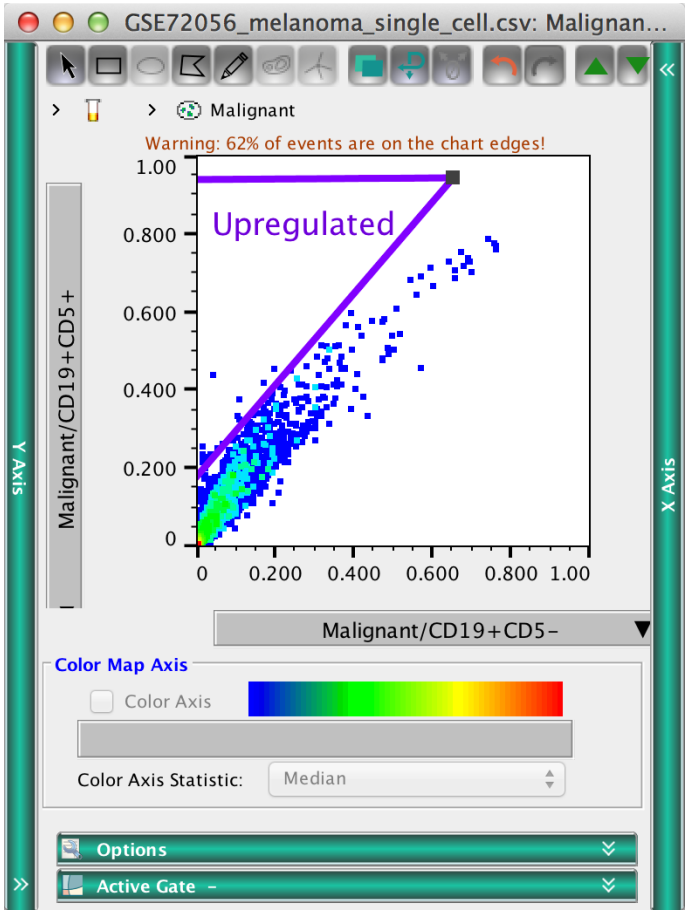
  http://flowjo.typepad.com/

SeqGeq™

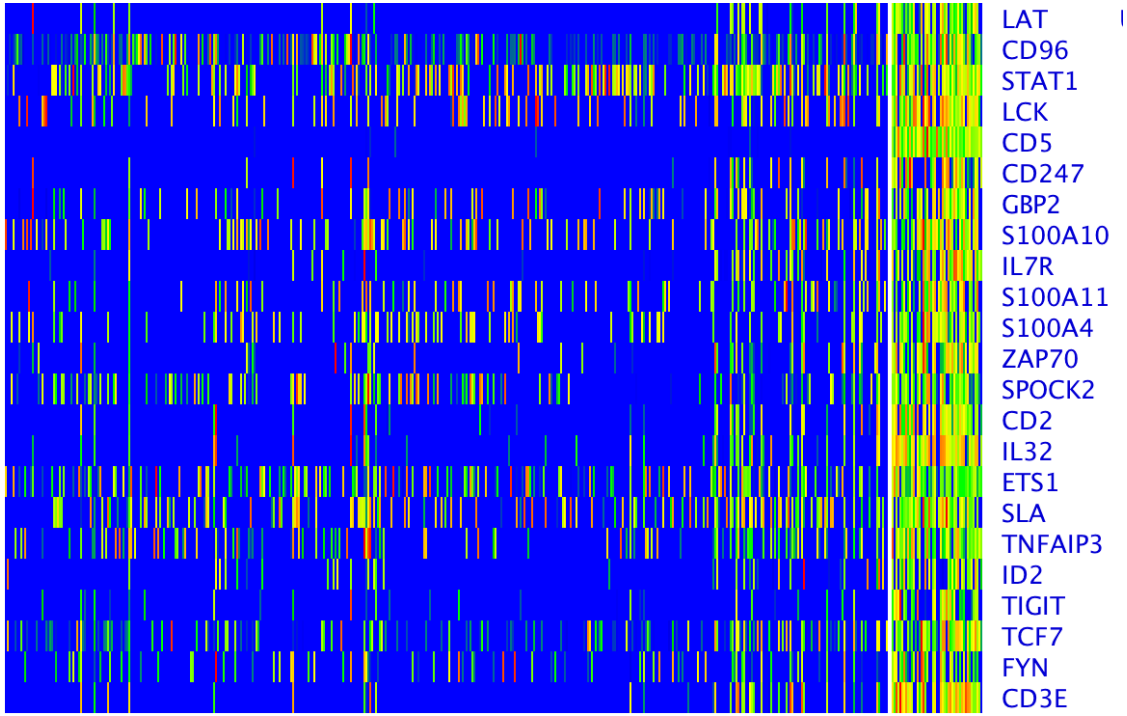
- Single cell gene expression analysis software.
  - Gate on individual genes, gene sets or synthetic parameters to define populations.
  - Pivot the graph to compare populations, define differentially expressed genes and identify new novel populations.
  - Visualize differences.



SeqGeq™



CD19+CD5- CD19+CD5+



www.flowjo.com/solutions/seqgeq

SeqGeq™

Questions?

- FlowJo is here to help with all your cytometry analysis needs.

- Contact techsupport@flowjo.com for general questions and support.

- Contact timc@flowjo.com for science questions, additional training resources.

Thank You!