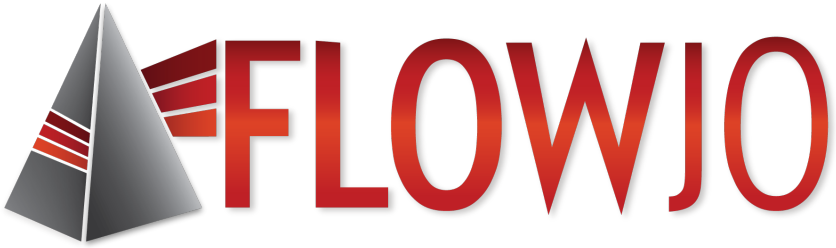
Cytometry Data Analysis in FlowJo V10



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Outline – Part I

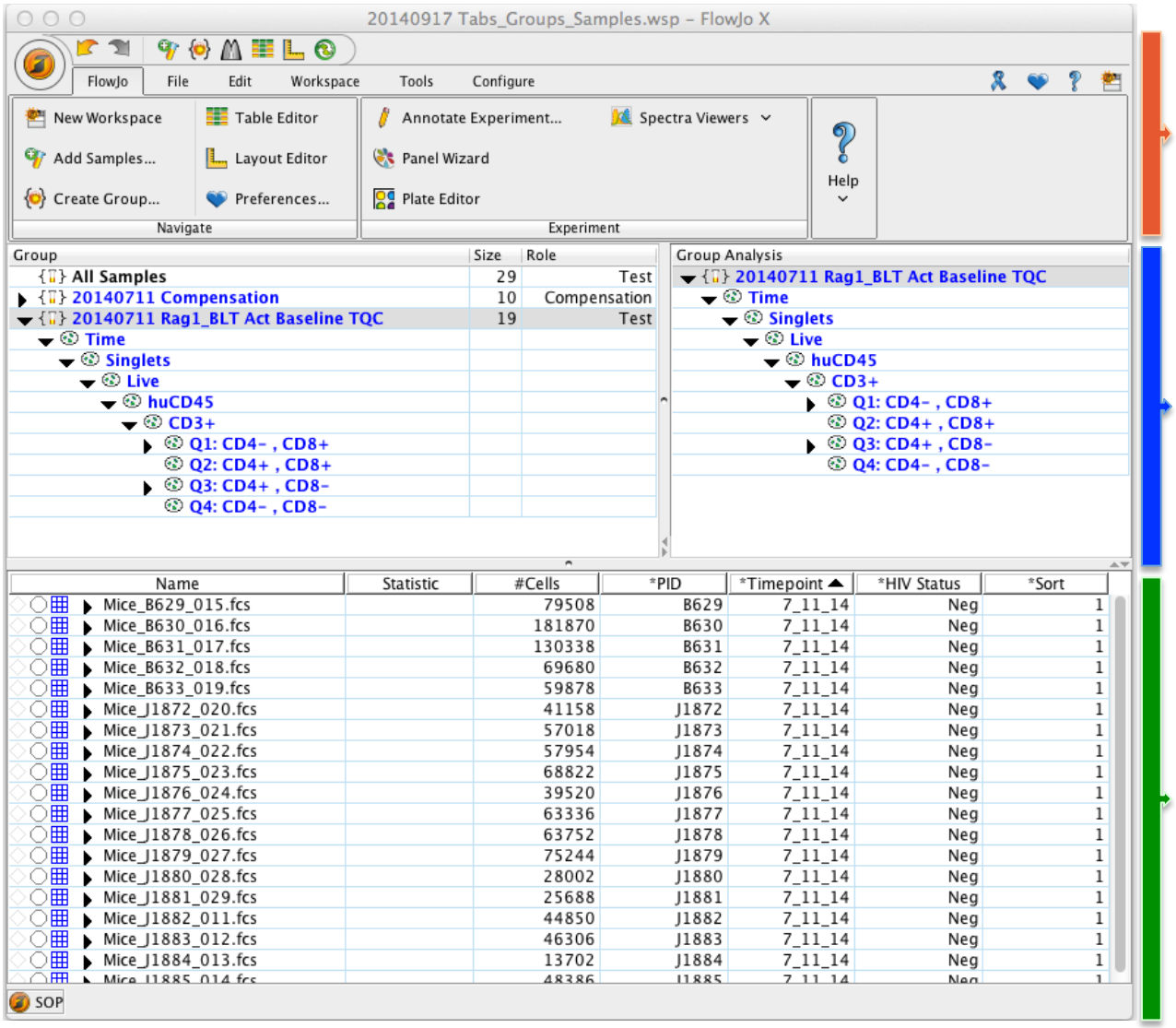
- What is FlowJo?
- Navigating the V10 Workspace
- Customizing Ribbons
- Demo Data Background
- Creating and Editing Groups
- Graphs, Gating and Ancestry
- The Layout Editor
- Batching and Exporting Graphics
- The Table Editor

What is FlowJo?

- An integrated environment for viewing and analyzing flow cytometry data.
- Uniformly analyze whole experiments encompassing many related samples.
- Sophisticated tools allow generation of graphs and statistical reports, driving discovery of biological mechanisms.

The FlowJo v10 Workspace

- A graphical interface to organize your data.



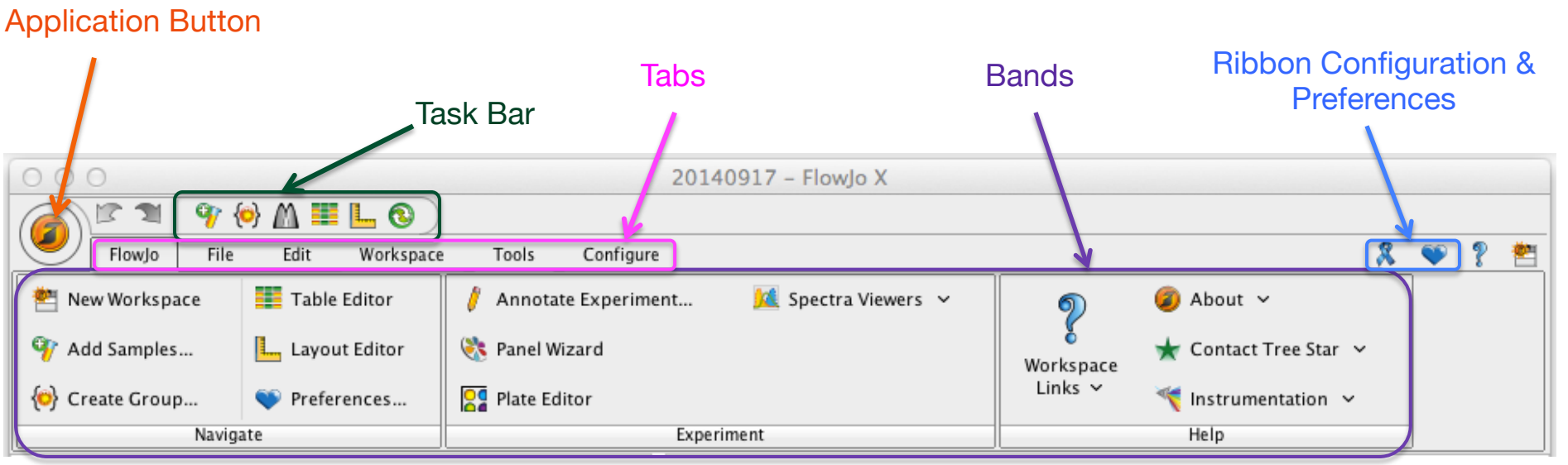
Ribbon
Tabs and Bands

Groups and
group analysis

Samples and
sample analysis

Ribbons, Tabs and Bands

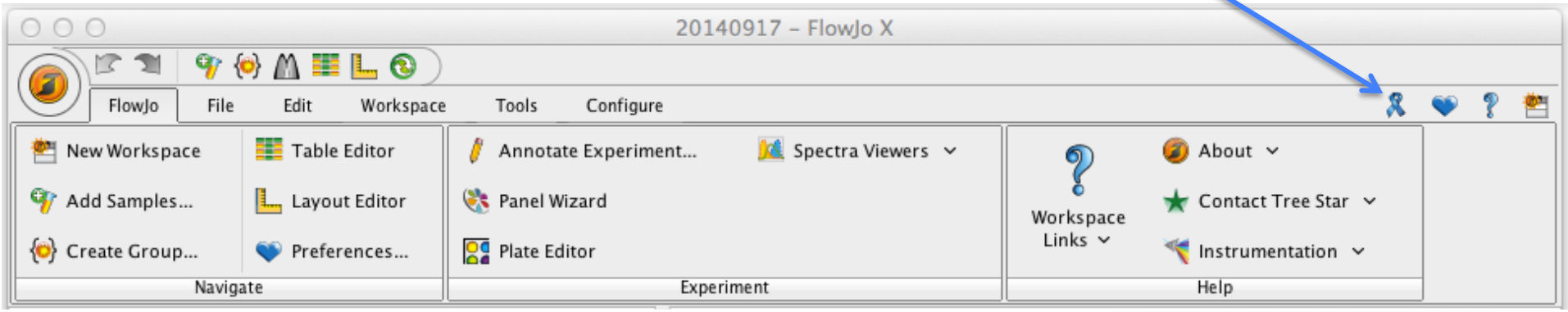
- Ribbon organization allows easy visual navigation of workspace functions.



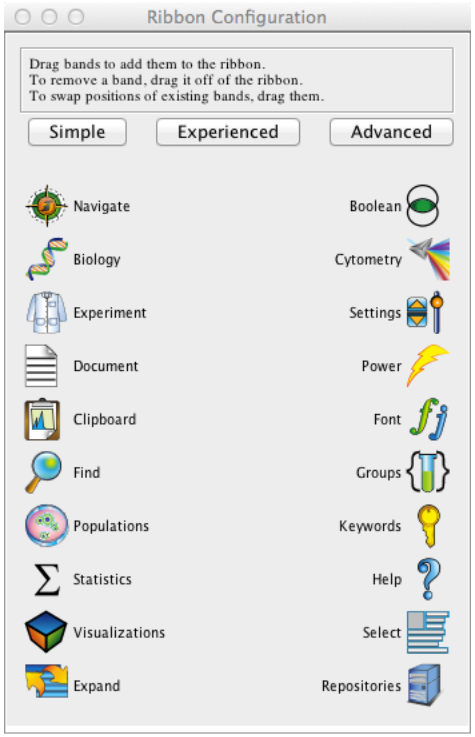
- Tabs group similar Bands together.
- Bands group similar Actions together.

Customizing Ribbons

- Click on the Ribbon icon to configure



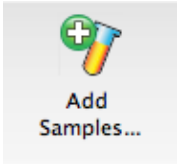
- Click on Simple, Experienced or Advanced to change the number of Tabs displayed.
- Drag the icon for any Band into the ribbon to add that set of Actions to your selected Tab.

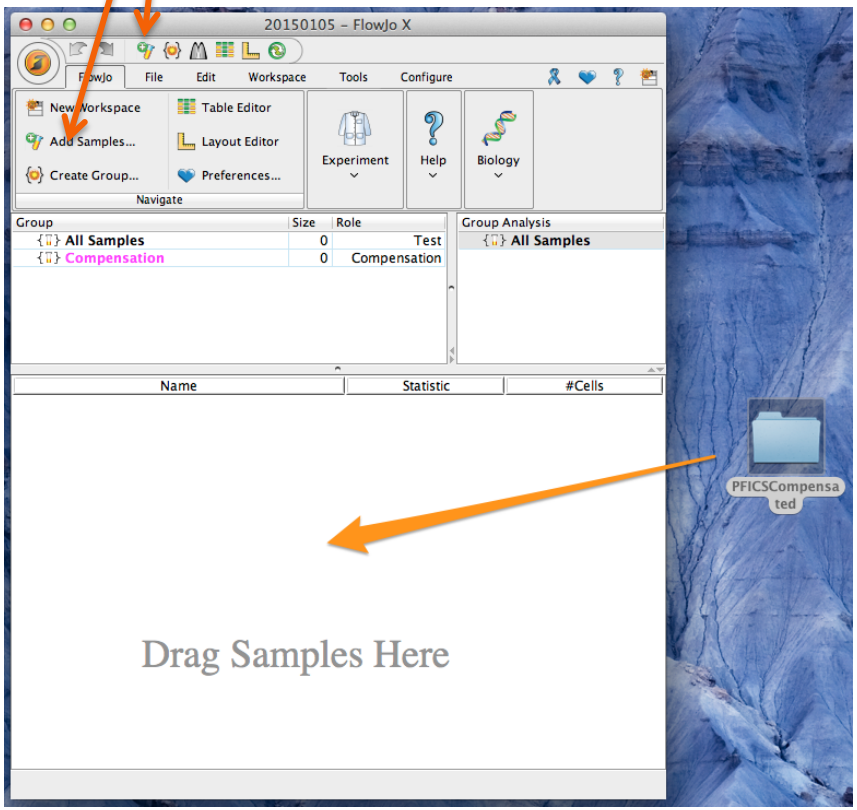


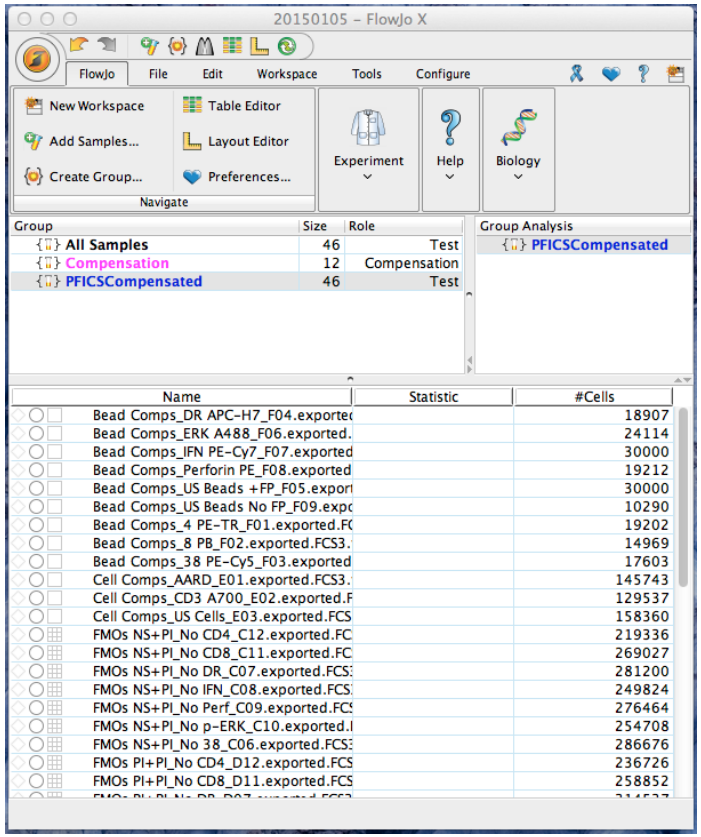
Importing Data Into FlowJo

Three possible methods:

1. Drag and drop into samples pane
2. Click Add Samples button
3. Press ⌘ ;







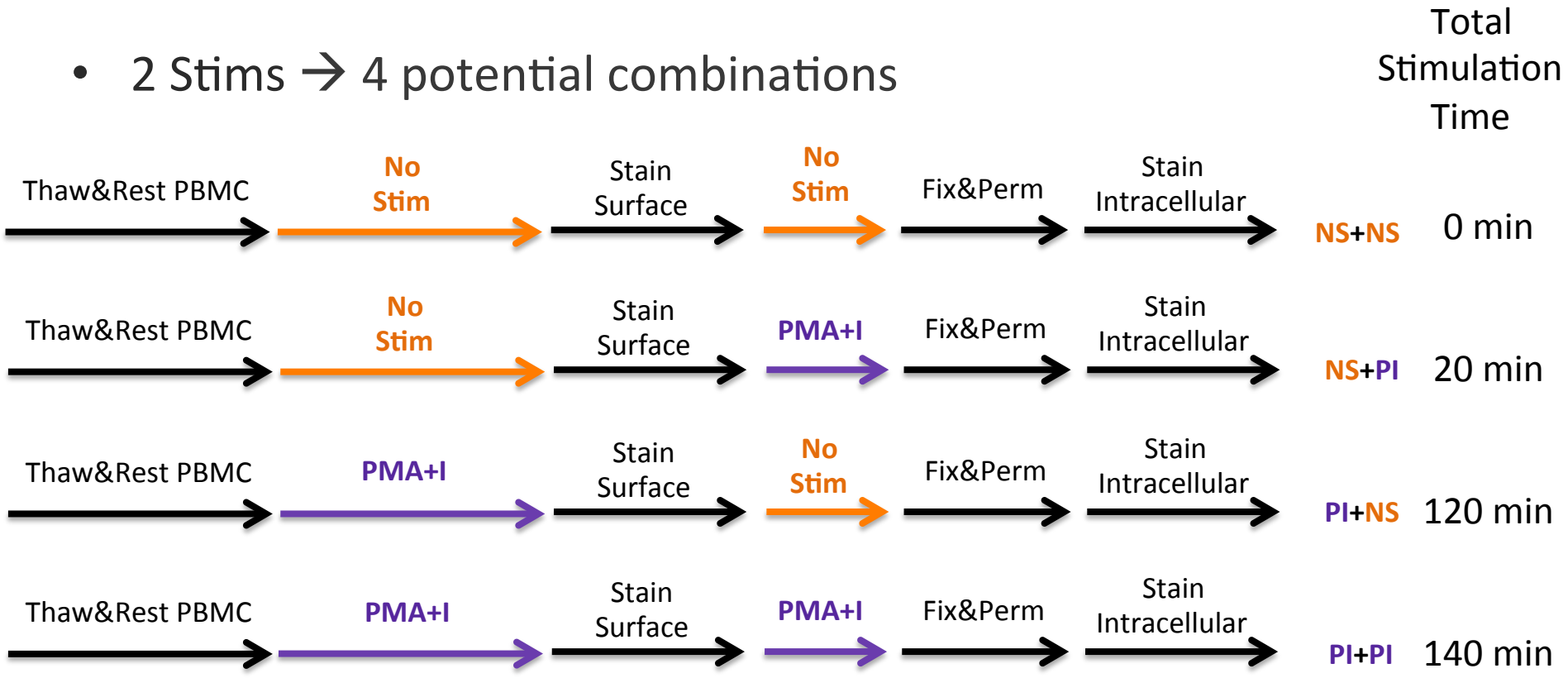
Todays Demo Data Set: Phospho-Flow + Intracellular Cytokine Staining (PFICS)

Polyclonal PFICS Assay:

- Thaw and rest cryopreserved human PBMC overnight
- No Stim (NS) or stimulate with PMA+Ionomycin (PMA+I or PI) for 2 hours
- Stain for viability (AARD) and surface antigens (CD3, CD4, CD8, CD38 and HLA-DR)
- No Stim or stimulate PMA+I for 20 minutes
- Fix, permeabilize and stain for intracellular antigens (phopho-ERK1/2, IFN-γ and Perforin)

PFICS Stim Conditions

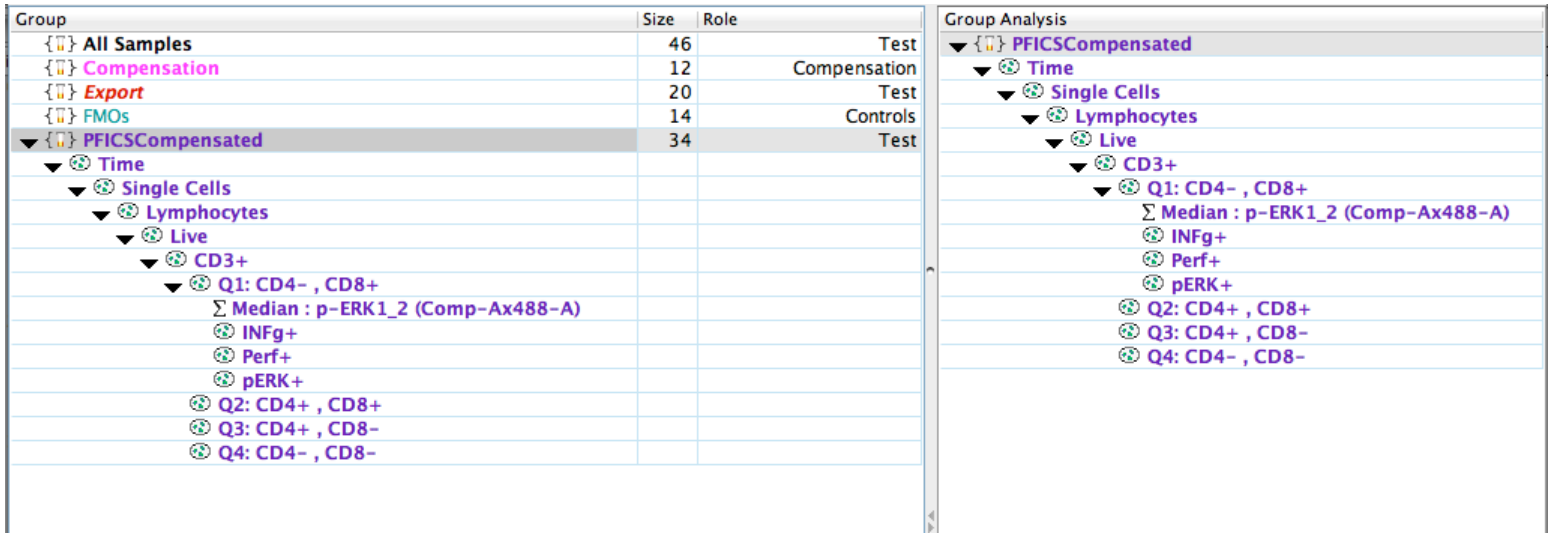
- 2 Stims → 4 potential combinations



- 5 donors X 4 conditions = 20 experimental samples
- 1 donor with 7 FMOs X 2 stim conditions = 14 FMO controls
- 12 Compensation Controls

Groups and Group Analysis

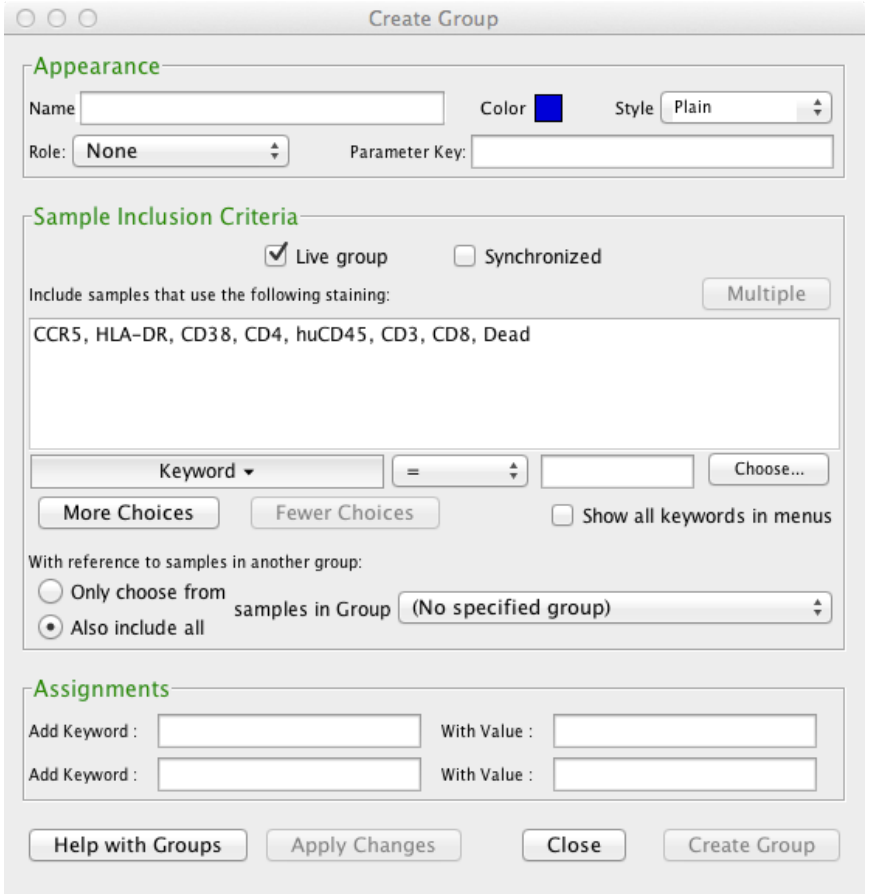
- The Group area lists all groups in the Workspace, # of samples in each group (Size), and the Role of that group (ex. Test, Compensation, Controls) .
- Groups act like folders to organize your sample files and allow unified master gating and analysis.



- Group Analysis displays all analysis within a group.

Creating and Editing Groups

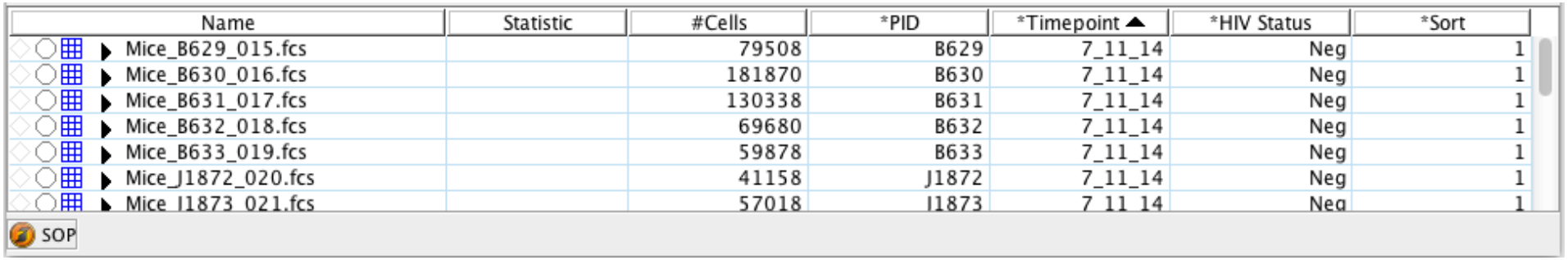
- To create a new group type ⌘ G, or click the Create Group Icon located in either the task bar at the top of the workspace, or within the Navigate band.



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

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 information can be displayed as columns. (Workspace Tab → Add Keywords or Configure Tab → Edit Columns)



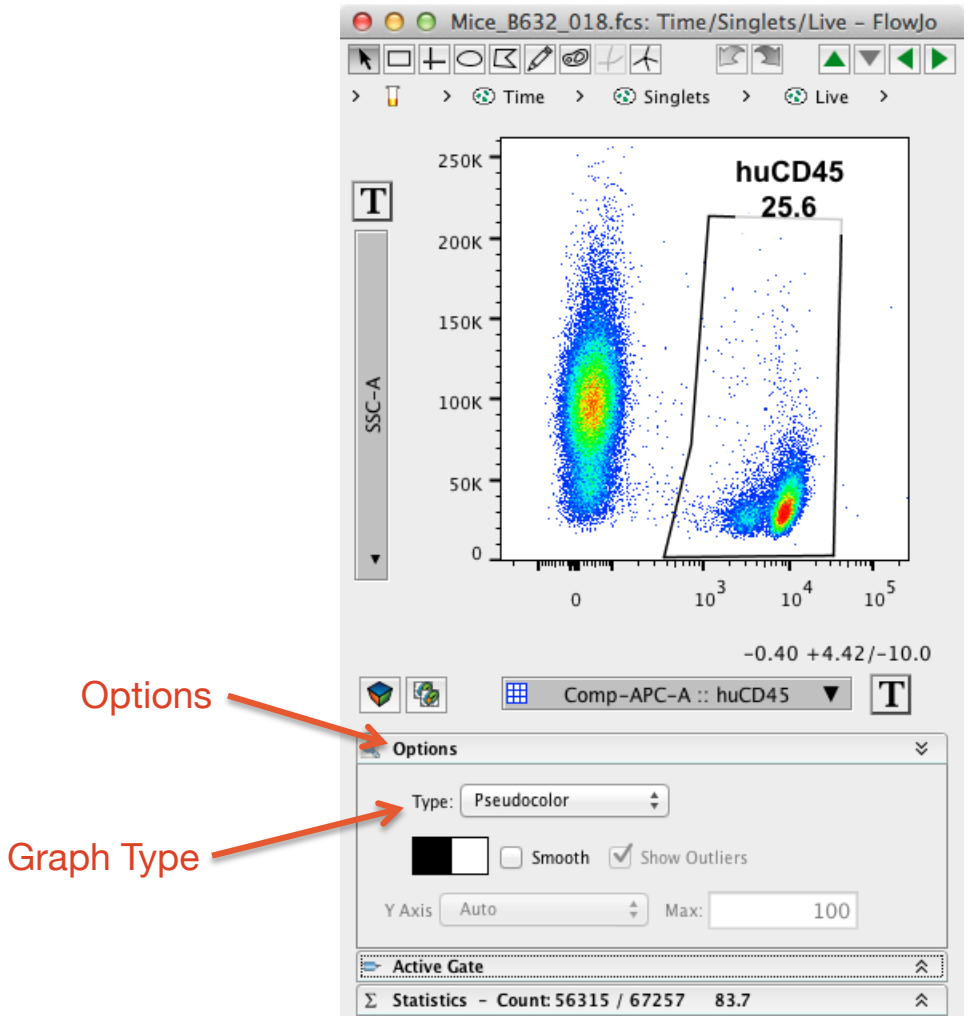
Name	Statistic	#Cells	*PID	*Timepoint	*HIV Status	*Sort
Mice_B629_015.fcs		79508	B629	7_11_14	Neg	1
Mice_B630_016.fcs		181870	B630	7_11_14	Neg	1
Mice_B631_017.fcs		130338	B631	7_11_14	Neg	1
Mice_B632_018.fcs		69680	B632	7_11_14	Neg	1
Mice_B633_019.fcs		59878	B633	7_11_14	Neg	1
Mice_J1872_020.fcs		41158	J1872	7_11_14	Neg	1
Mice_J1873_021.fcs		57018	J1873	7_11_14	Neg	1

SOP

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

Graphs

- The Graph Window facilitates data visualization and gating.

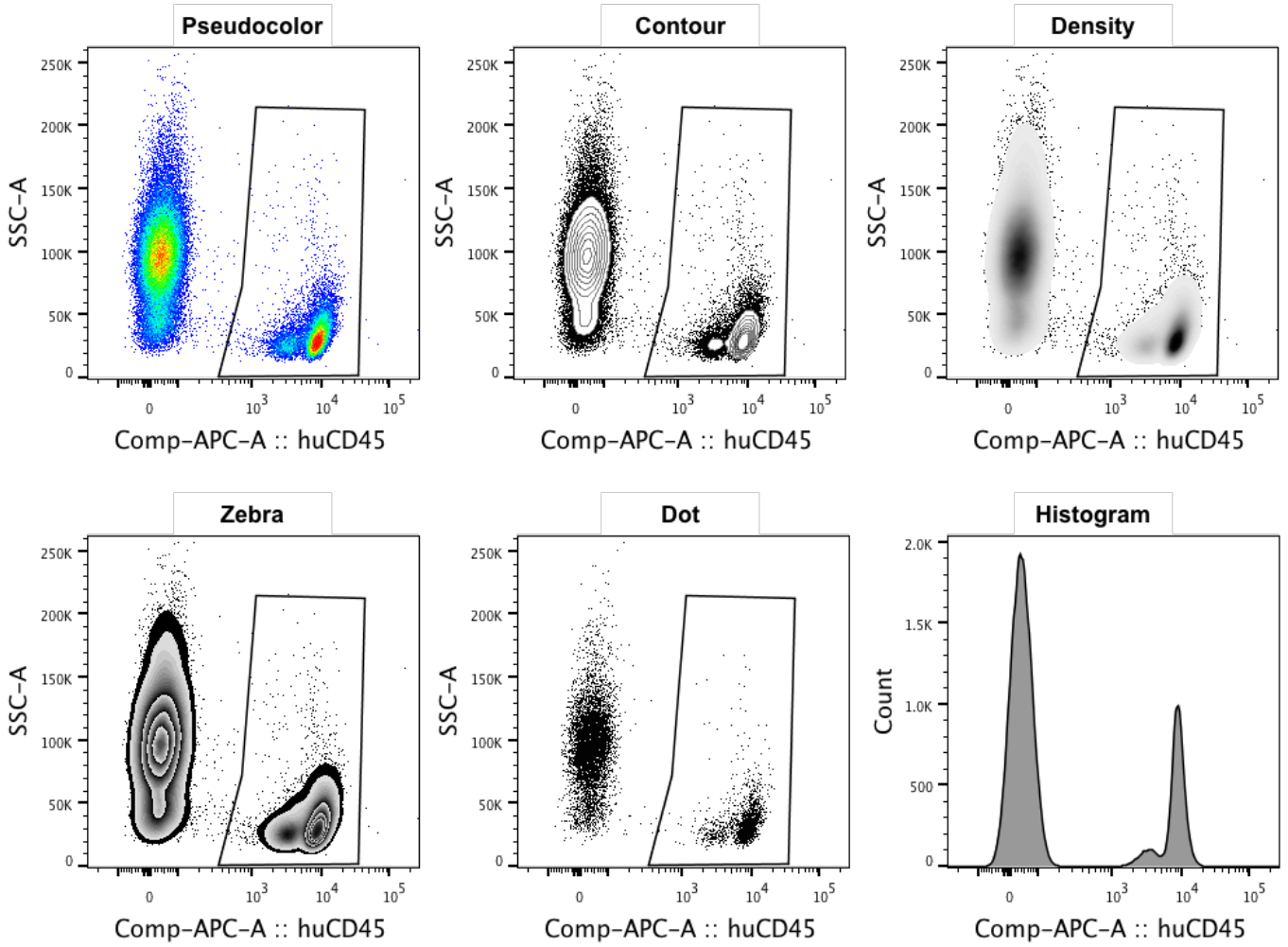


- Several different kind of plots are available to display flow data.

- Click on the Options Menu below the graph image and select graph Type from the dropdown menu.

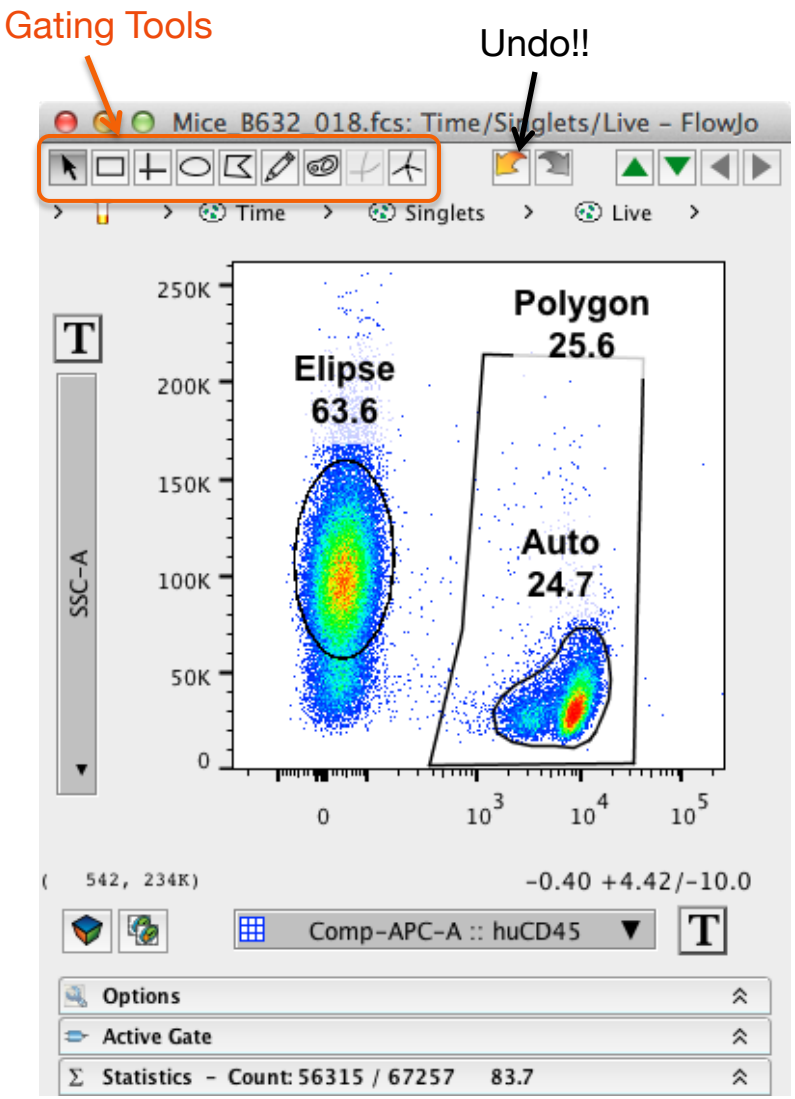
Graph Display Options

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



Gating tools

- Are located at the top of a Graph Window.

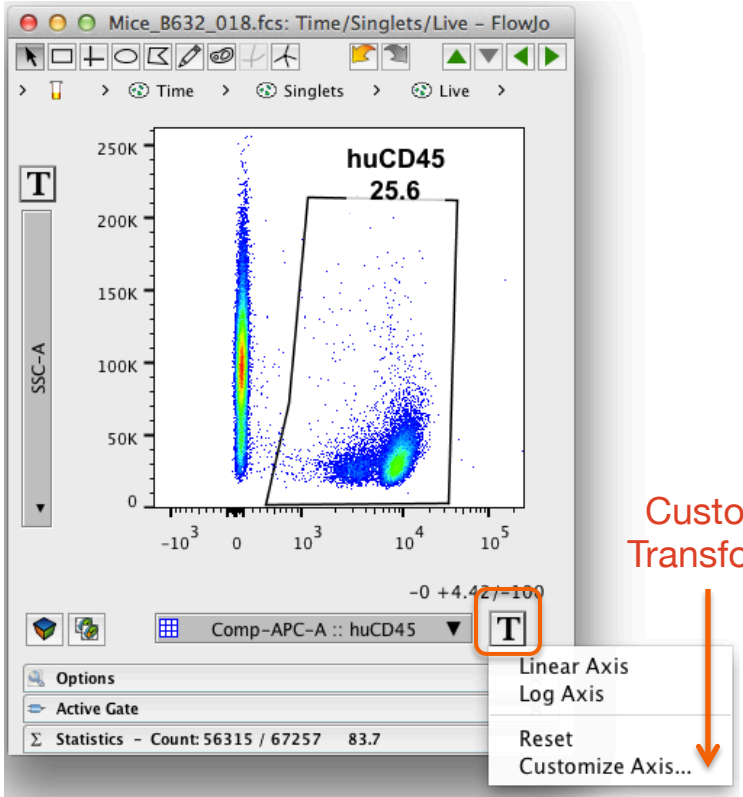


- Gates can always be modified or removed, so don't be shy.

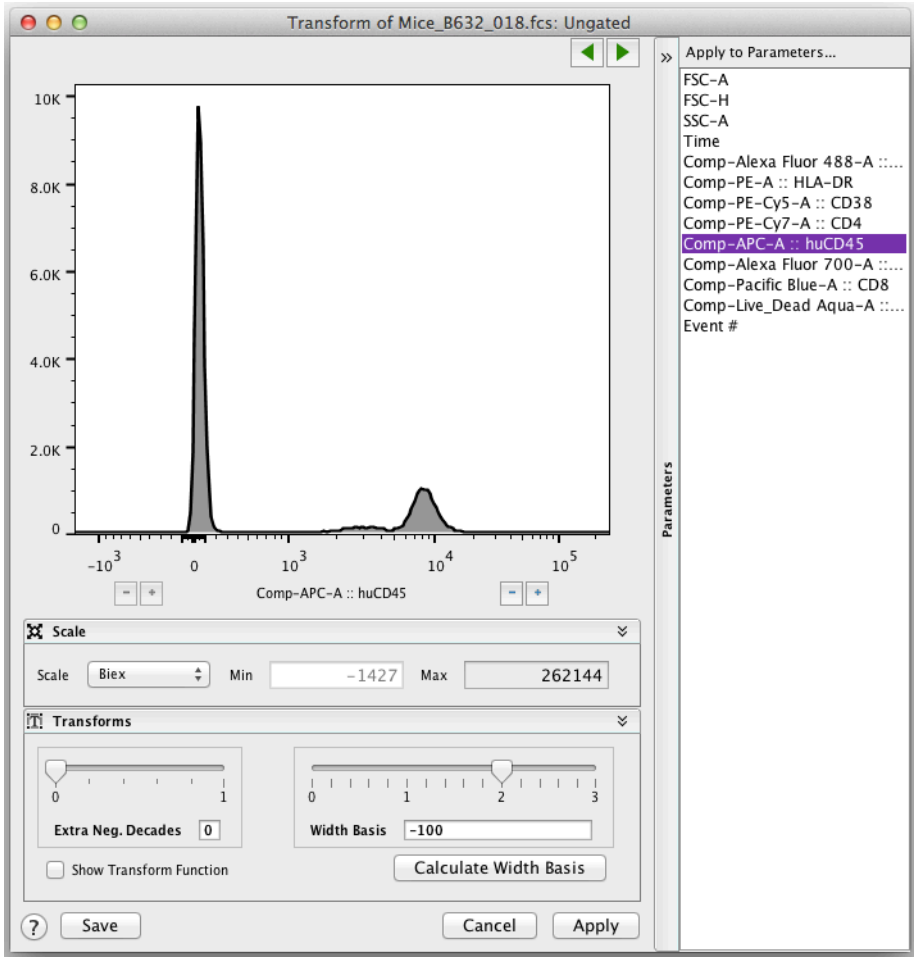
- Explore the gating options and pick what works best for you.

Transforming Data

- Your data may initially look ‘squished’.
- Click the Transformation icon to change the visual display.

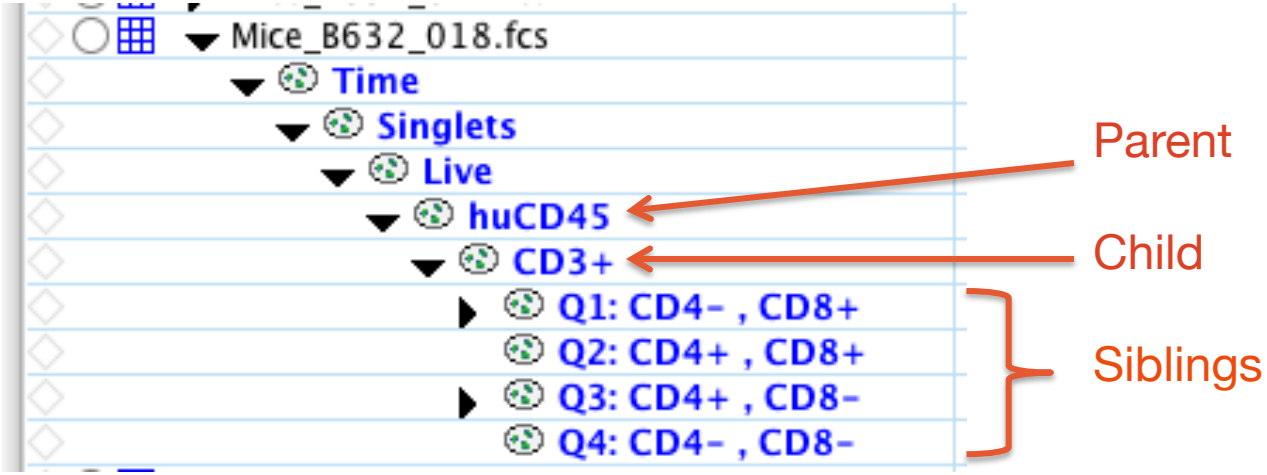


Custom Transform



Gating Hierarchy

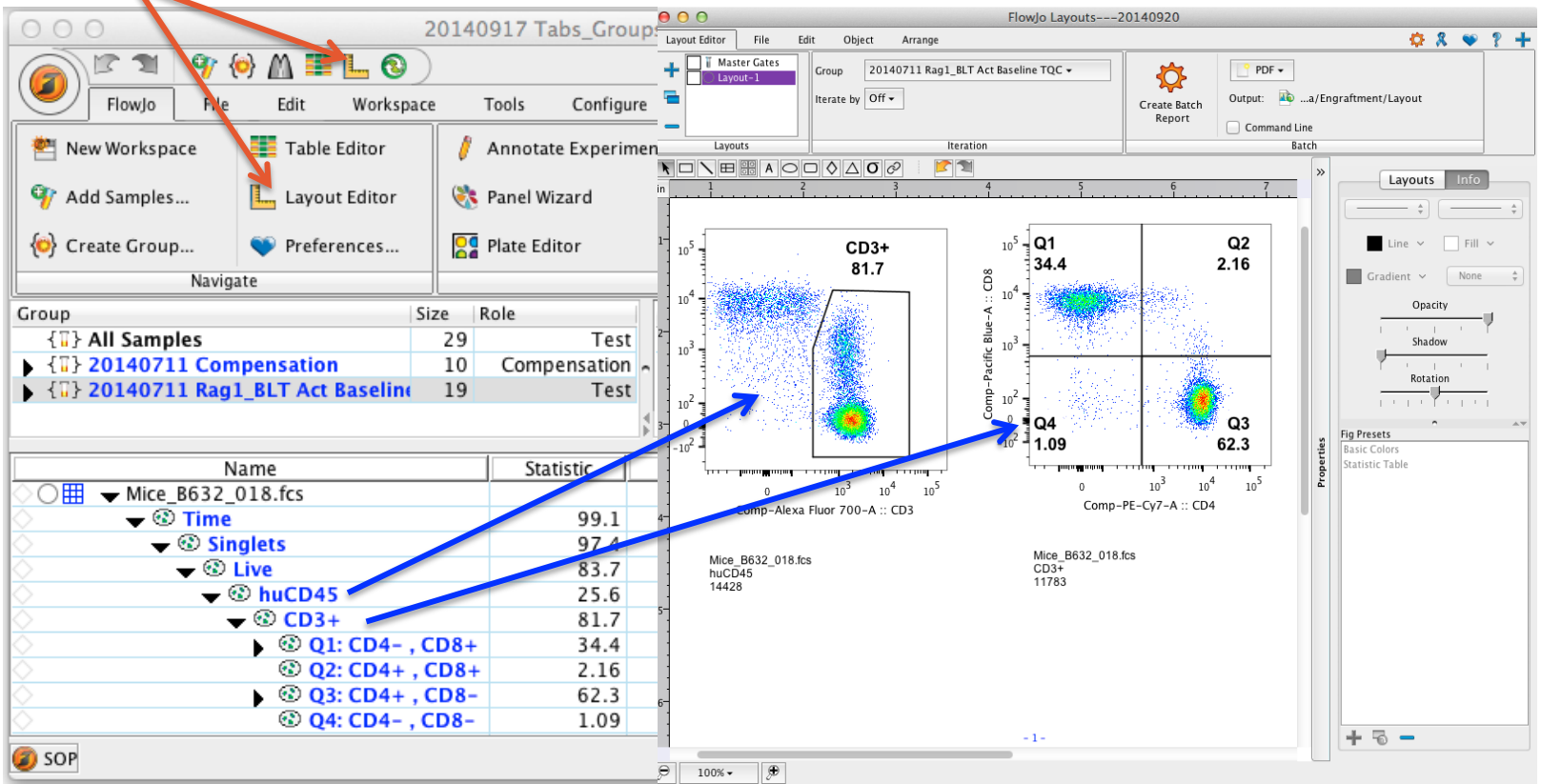
- When you create a gate on a sample, FlowJo shows you this gate (subset population) as a genealogical tree.
- The subset population is a child of the parent sample.



The Layout Editor

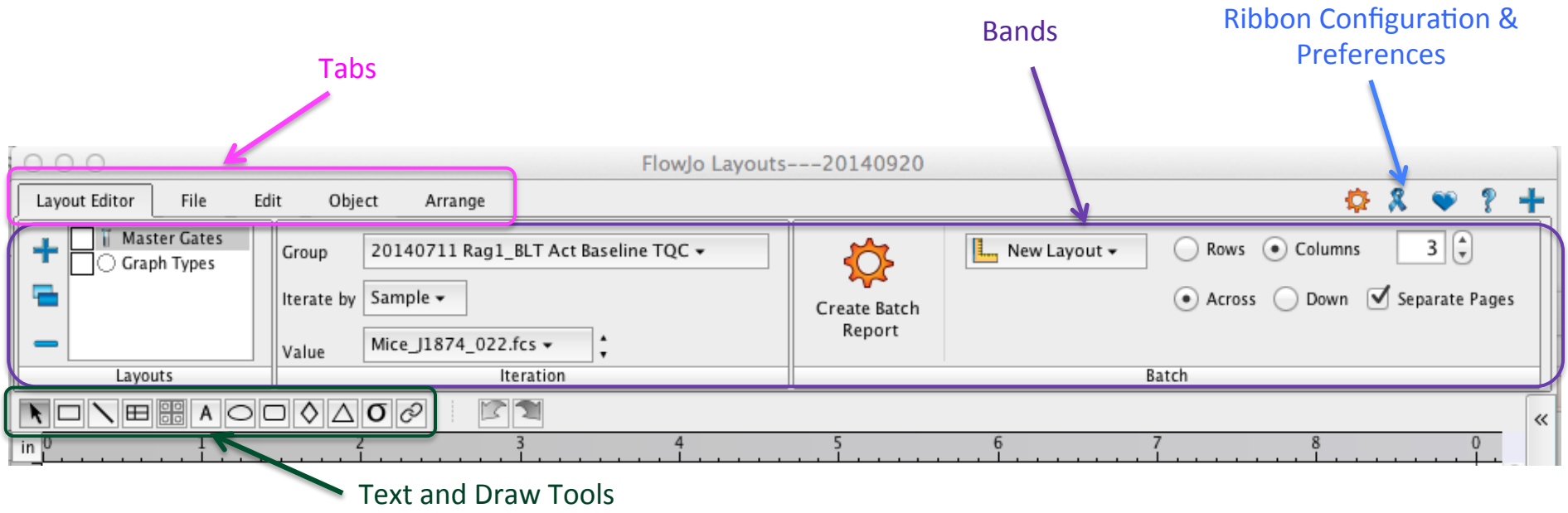
- A tool for creating graphical reports.
- Type ⌘ L, or click on the Layout Editor icon.
- Drag populations from a sample to Layout Editor.

Layout Editor



Working in Layout Editor

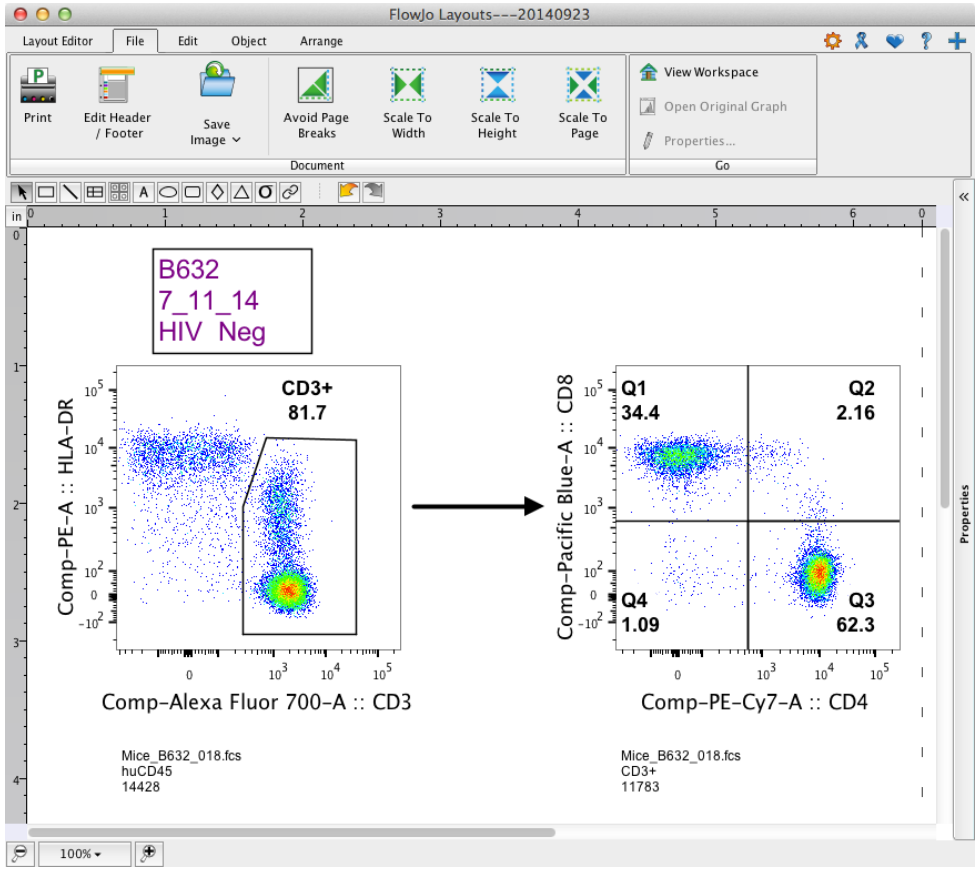
- Similar to the Workspace, the Layout Editor has its own customizable Ribbon with Tabs and Bands to organize actions.



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

Within Layout Editor

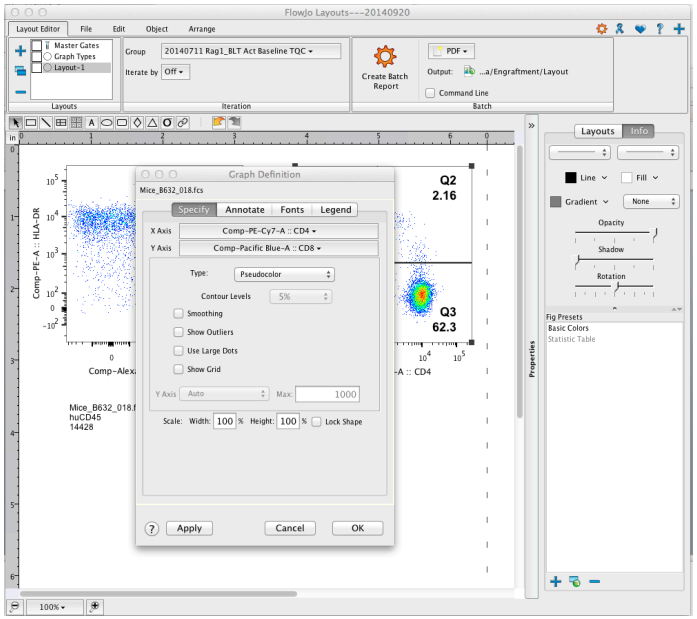
- Graphs can be organized and re-formatted.
- Statistics, keywords, text and even shapes or objects can be added to illustrate your analysis.

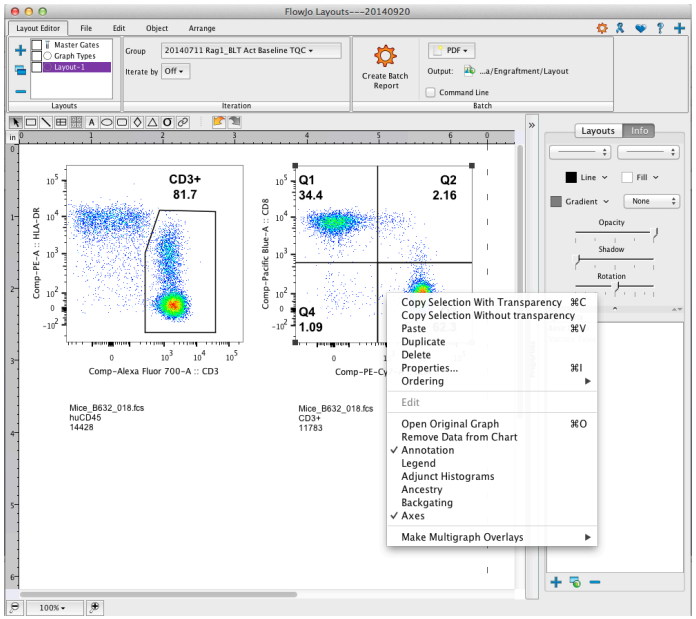


- We encourage you to explore the tools and display features available to improve the visualization of your data.

Working in Layout Editor

- Click once on a graph or object to select it.
- Double Click a graph to change its properties.

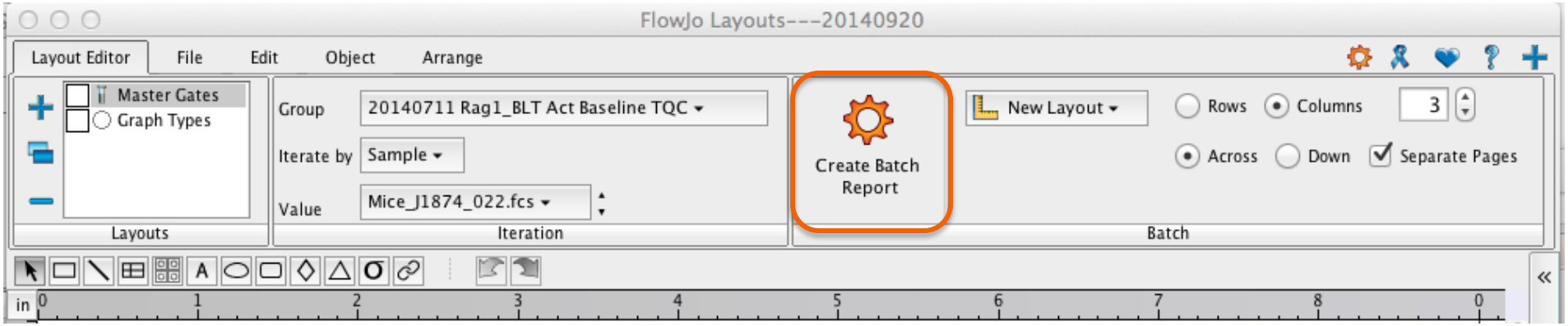




- Right click the graph for even more options.
- Hold down shift and click on multiple graphs to select and edit their properties simultaneously.

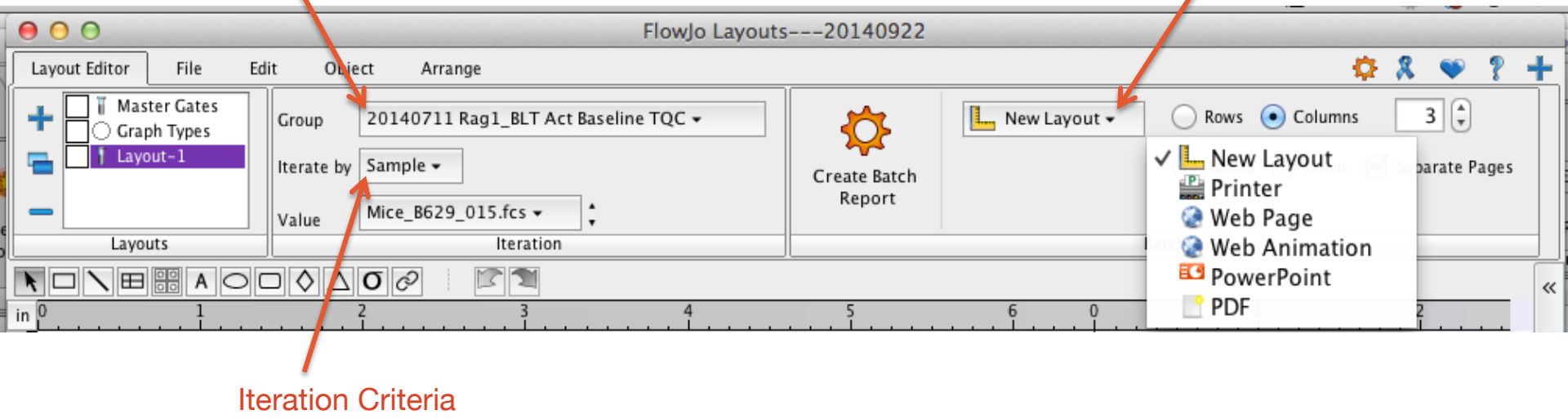
Batch Analysis of Layout Editor Graphics

- Batch operations perform repetitive analysis on multiple samples, applying the layout to an entire set of samples.
- Within the Layout Editor Tab, Look for the Create Batch Report icon.



Batch Analysis of Layout Editor Graphics

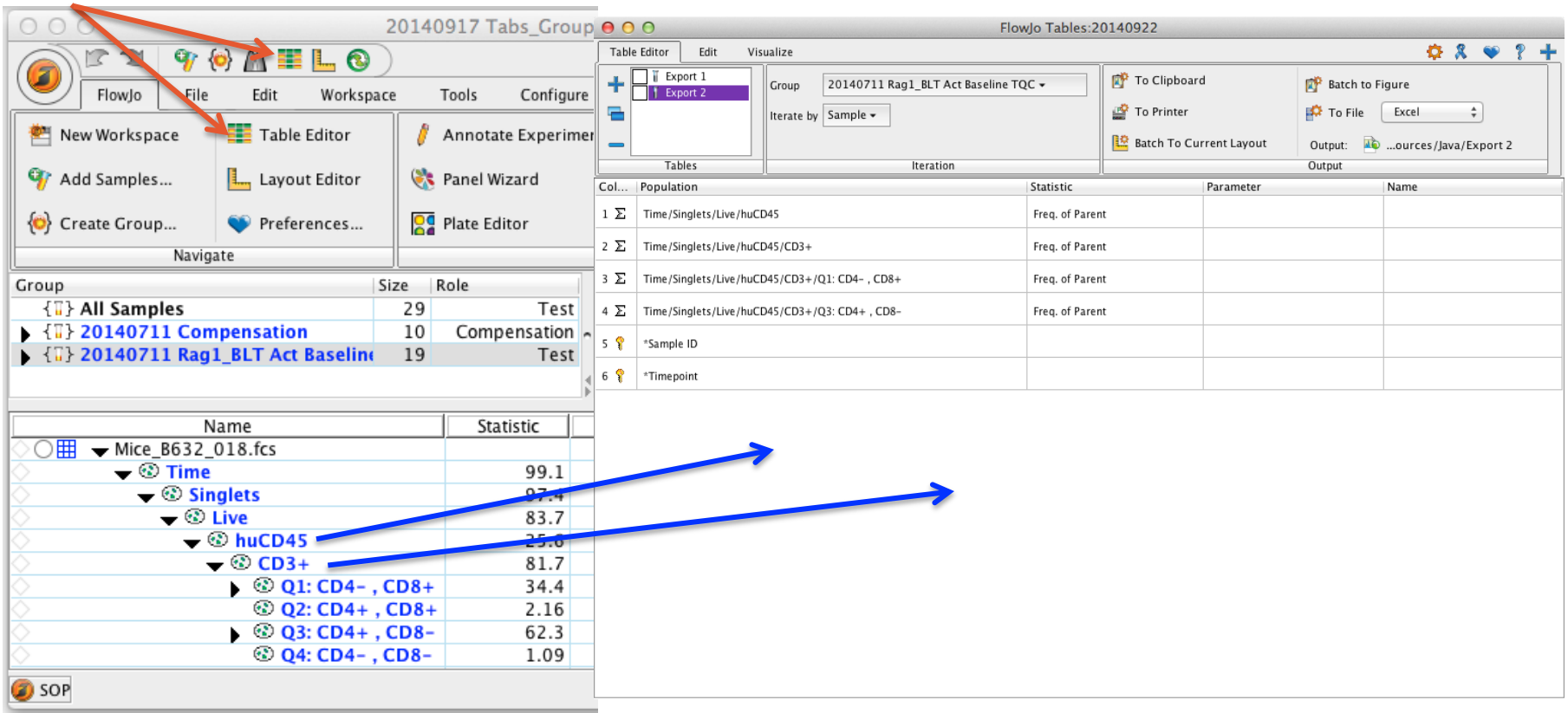
- Specify the group you wish to batch, and how to iterate the batch process (ex. by sample or keyword), then specify where you want the batch output to go. Finally, click on



The Table Editor

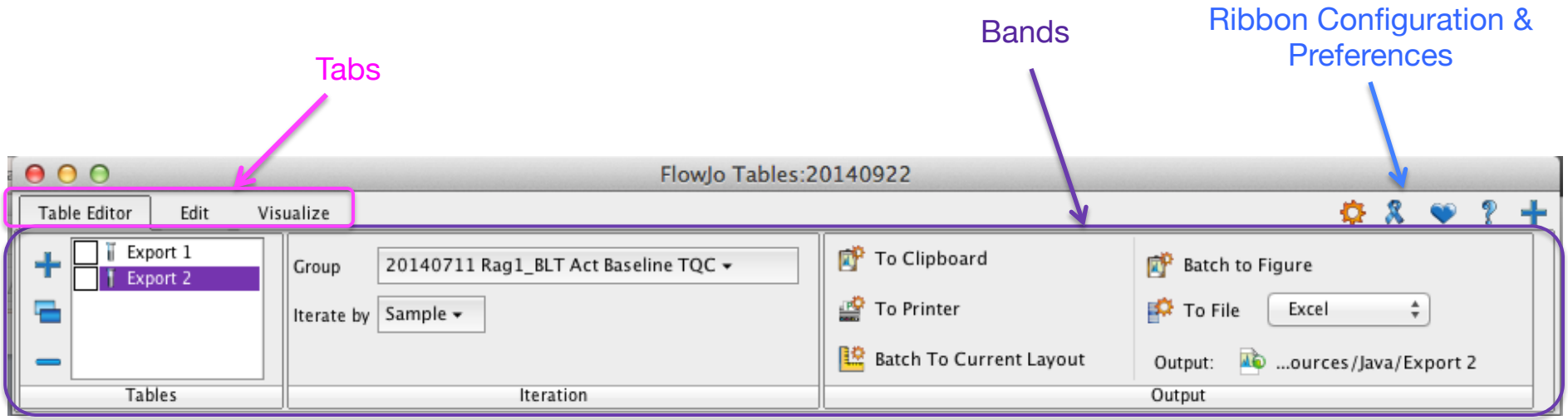
- A tool for creating statistical reports.
- Type ⌘ T, or click on the Table Editor icon.
- Drag populations from sample to Table Editor.

Table Editor



Within Table Editor

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



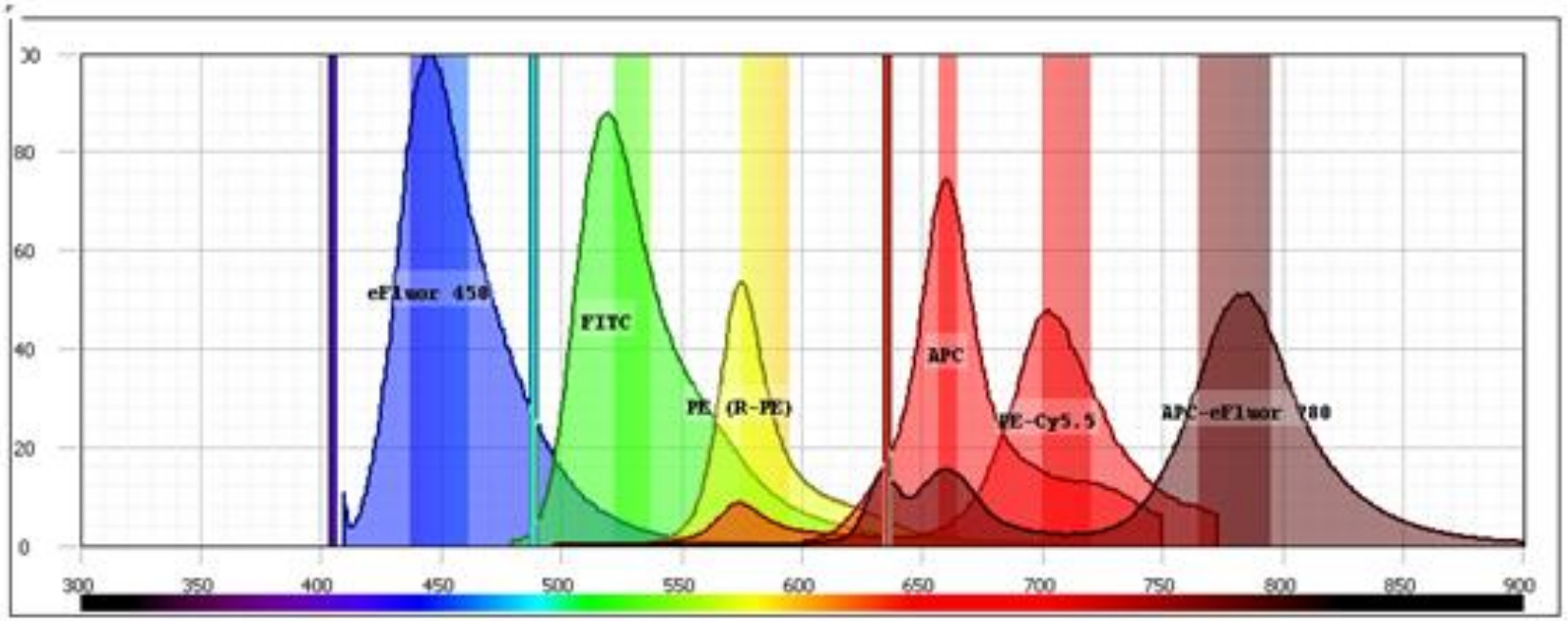
- Specify the group you wish to batch, and how to iterate the batch process, then in the Output band, specify where you want the batch output to go.

Outline – Part II

- Compensation
- Templates
- Command Line FlowJo

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.

PFICS Compensation Controls

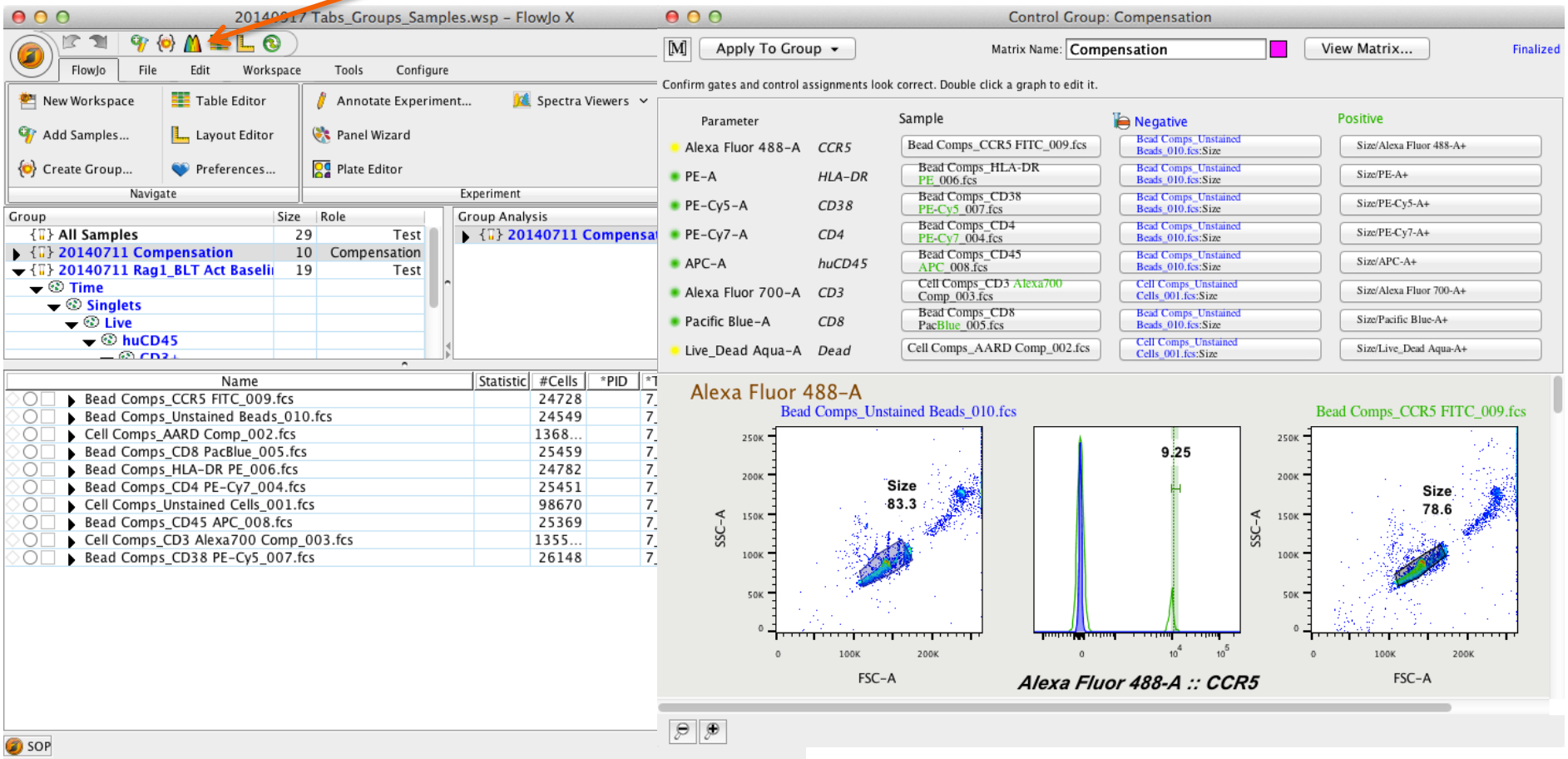
- PBMC Cells
  1. Unstained Cells
  2. AARD
  3. CD3 Alexa700

- 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. IFN-g PE-Cy7
  9. Perforin PE

Compensation Wizard

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

in the task bar.

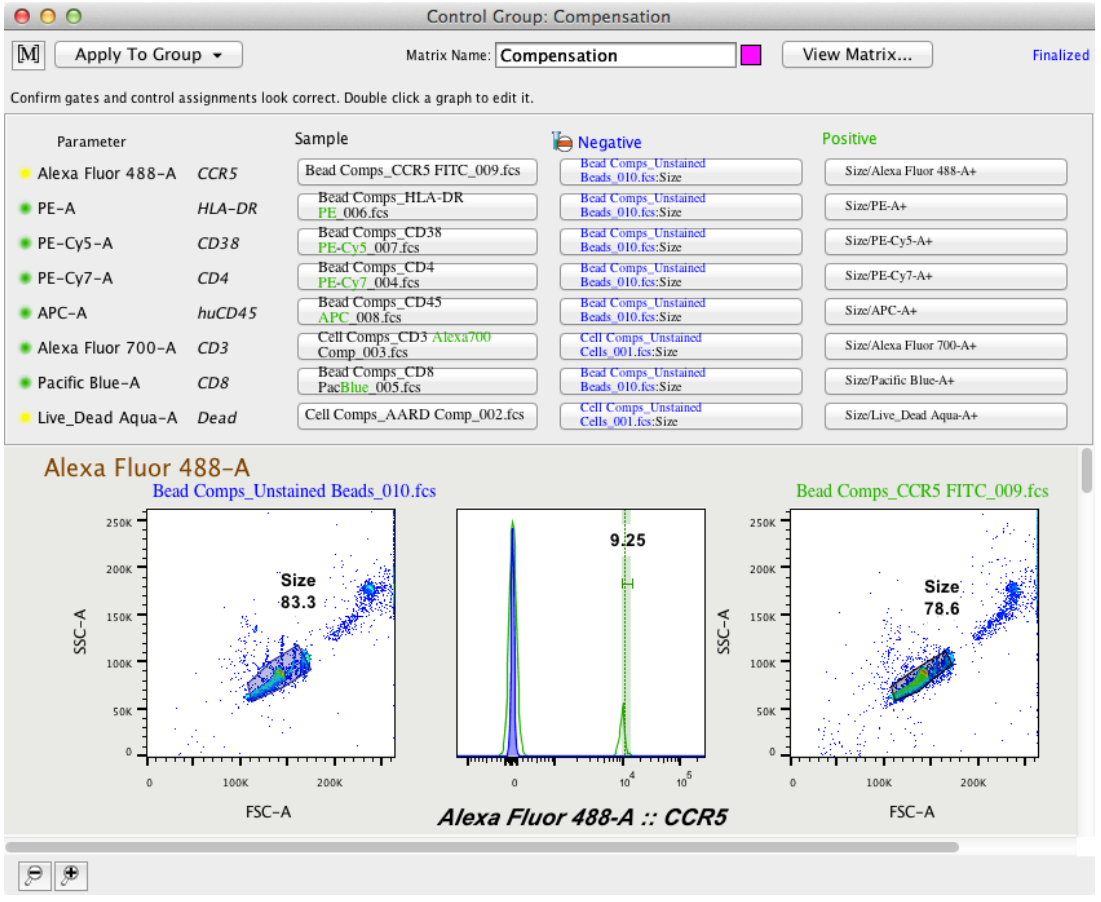


Compensation Wizard

- The wizard auto gates your compensation samples and fills in the positive and negative.

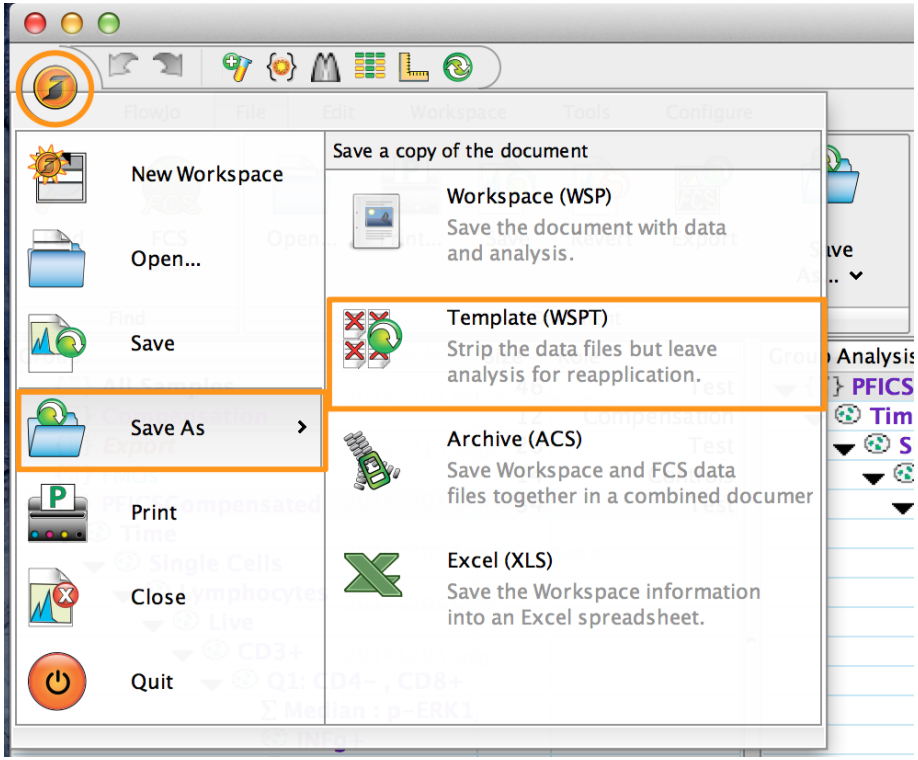
- To select a different sample, or gate choose from the dropdown lists.

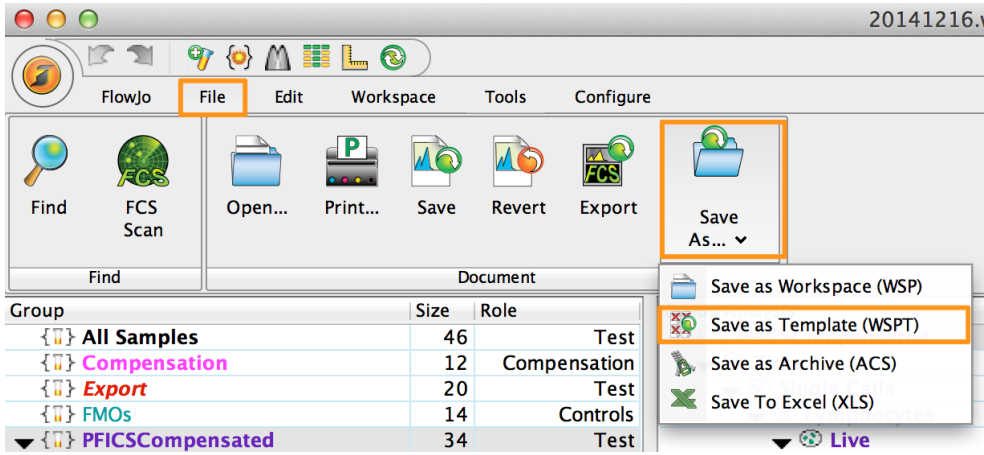
- Double click on a graph in the wizard to modify the gate



Workspace Templates

- Allow saving all analysis reports in your workspace without data
- Streamlines repetitive analysis of multiple runs using the same staining panel(s).





Command Line FlowJo (Ninja Skills)

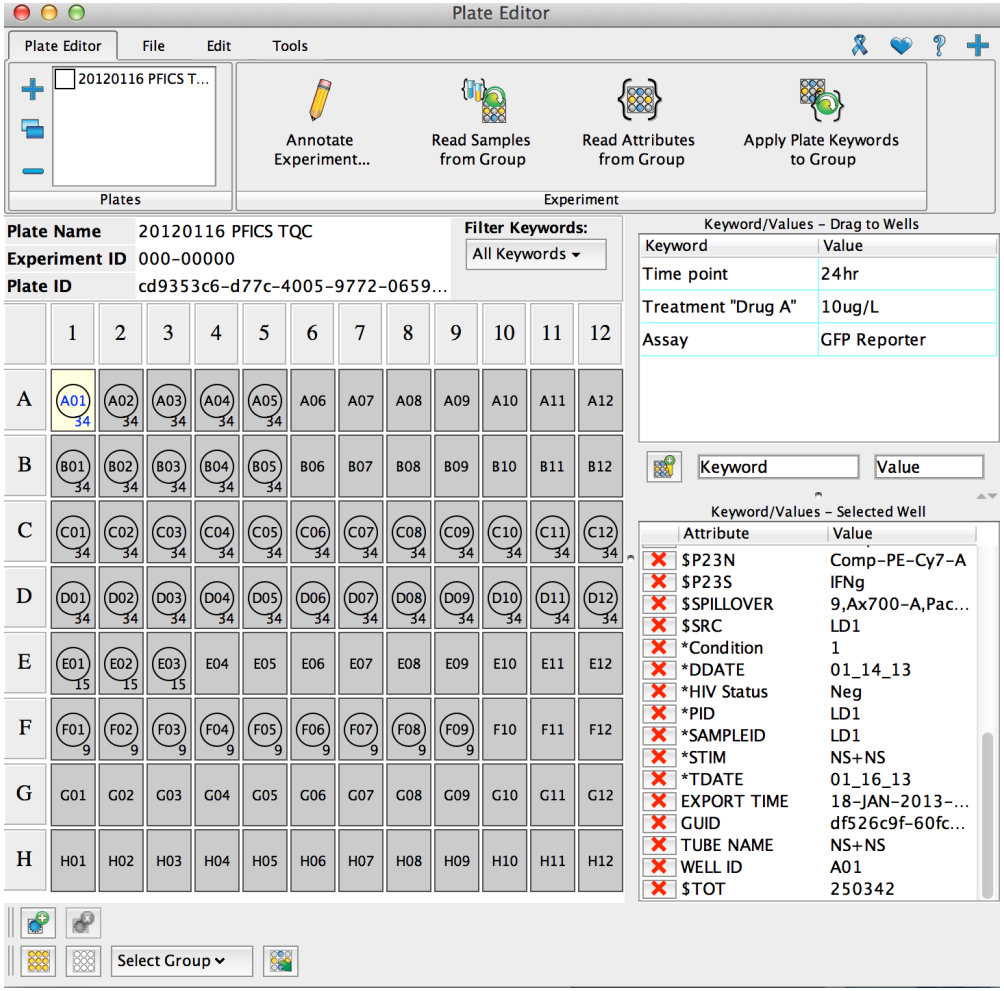
- FlowJo can be run “headless” through command line interface, applying templates and generating automated reports.

- FlowJo Enterprise is a server based version of FlowJo 10 that spawns command line tasks through a web browser interface.

- Designed to assist with data management, analysis and report generation for high dimensional, high throughput flow or mass cytometry.

The Plate Editor

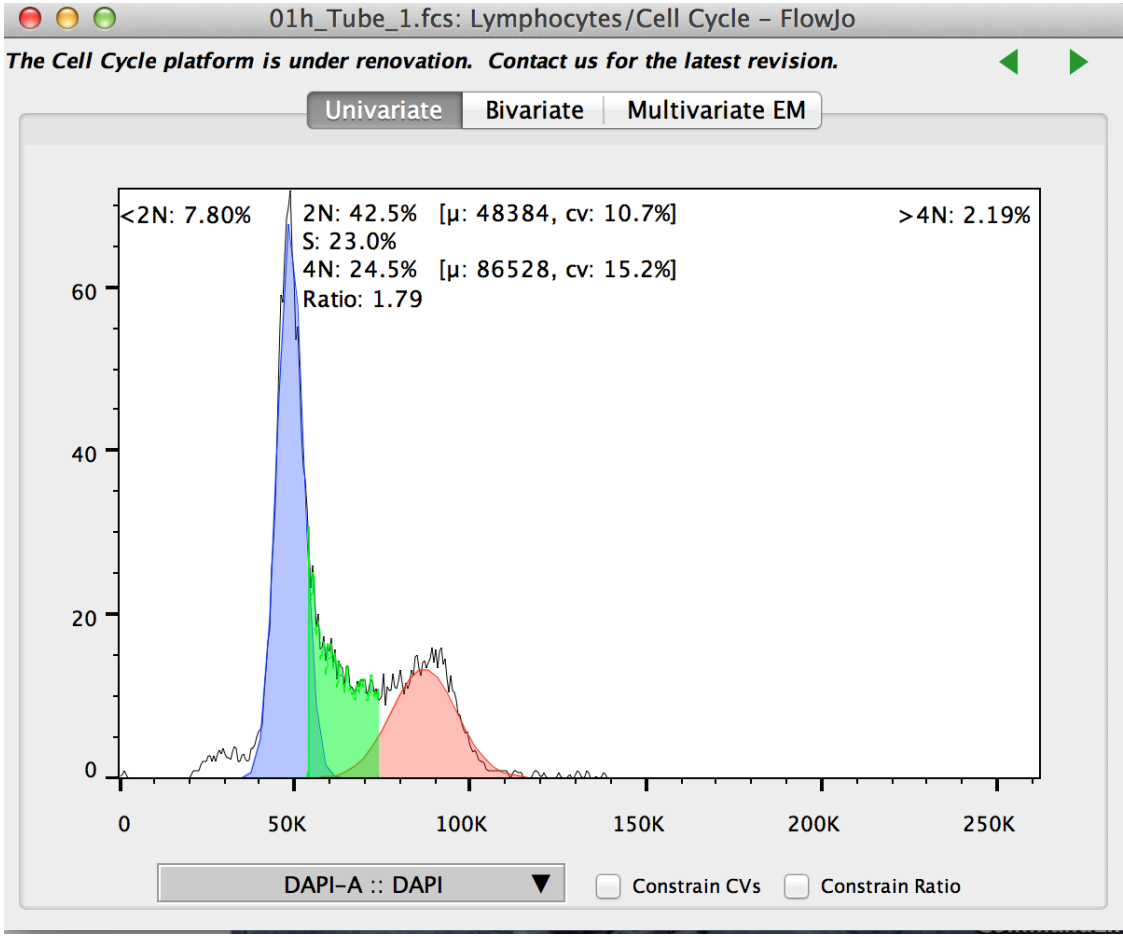
- Viewer to add keywords in a plate format
- Can export layout for use in FACS Diva acquisition software.
- Located in the visualizations Band within the Tools Tab
- Add new keywords/Value pairs to the right. Drag and drop on selected wells.



Cell Cycle Analysis

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

- Currently under development, the upcoming point release of v10.0.8 will have 1D Watson and Dean-Jett-Fox models, similar to FlowJo v9 for Mac



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/

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 and information on FlowJo Enterprise.

Thank You!