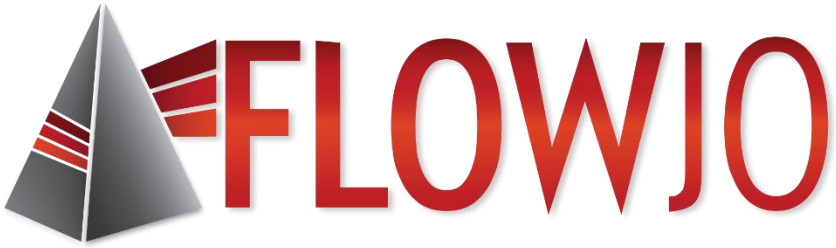
Cytometry Data Analysis in FlowJo V10



產品專員 蔣明涵 Michelle
techsupport@gtbiotech.com.tw

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Outline-Part I

- What is FlowJo?
- Navigating the V10 Workspace
- Graphs, Gating and Ancestry
- The Table Editor
- The Layout Editor
- Batching and Exporting Graphics

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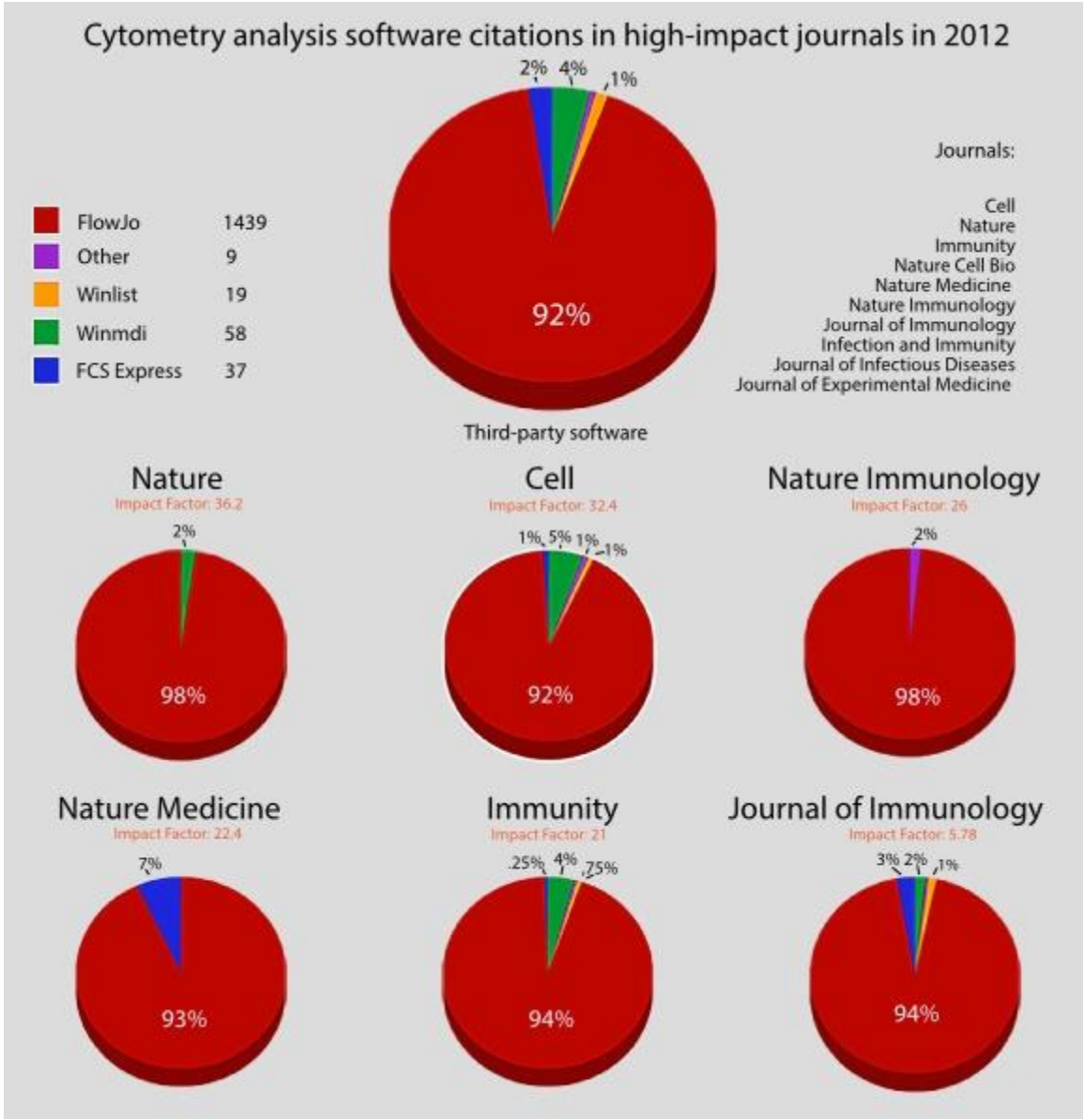
What is FlowJo?

- 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.



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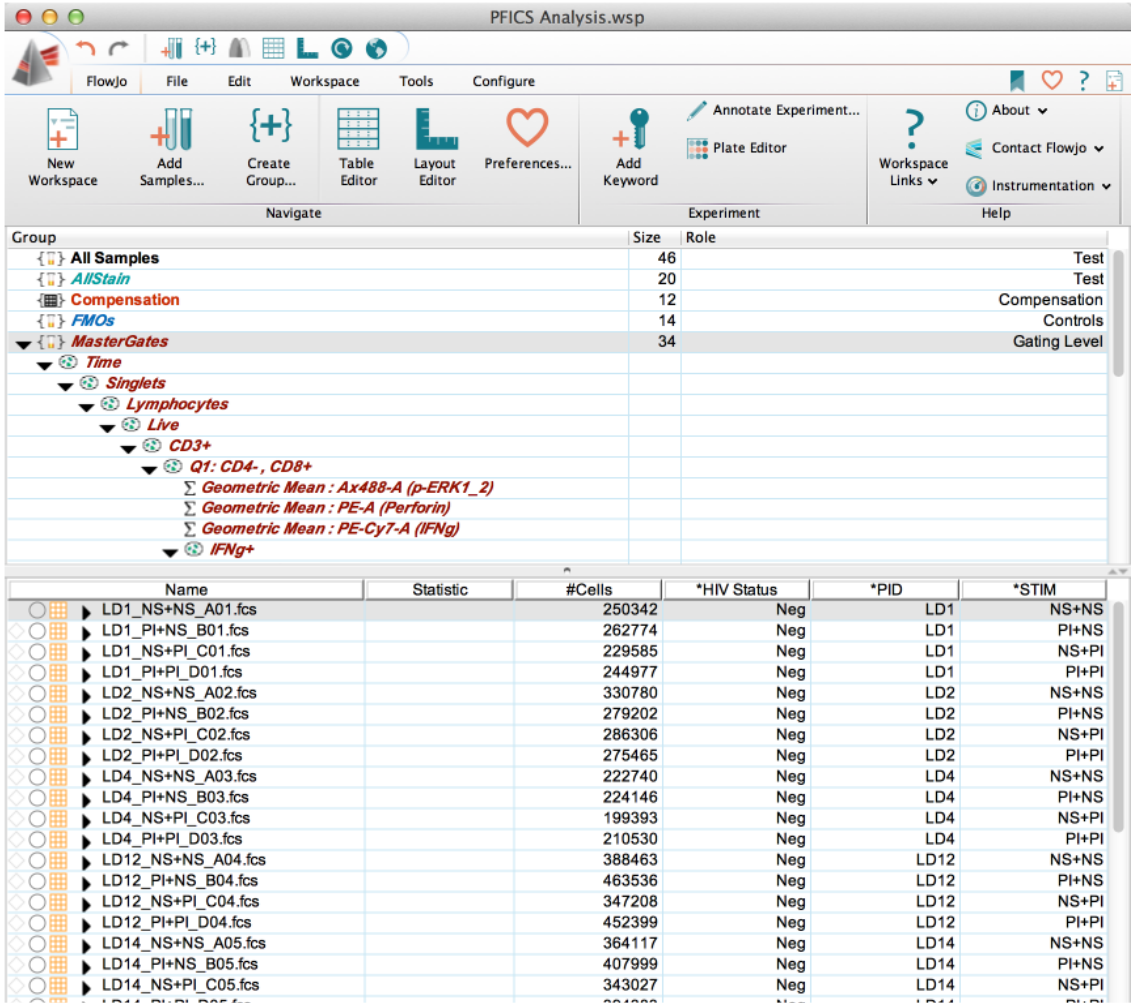
The Leading Analysis Software



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The FlowJo V10 Workspace

- A graphical interface to organize your data.



Ribbon
Tabs and Bands

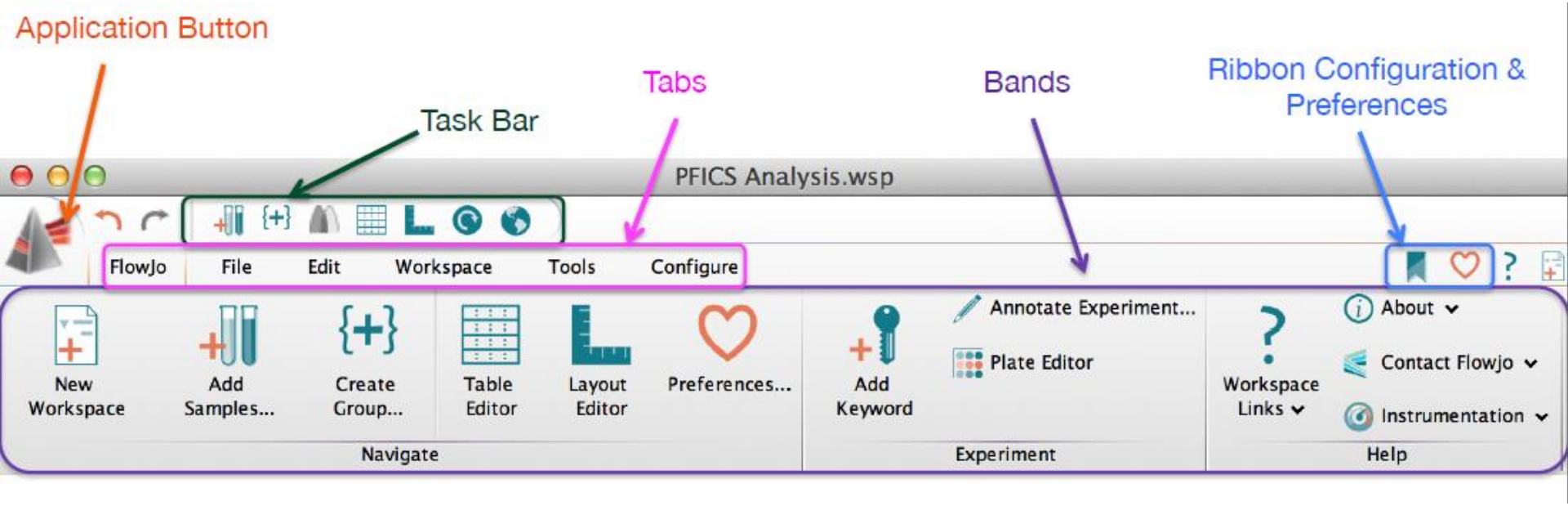
Groups and Group Analysis

Samples and Sample analysis

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Ribbons, Tabs and Bands

- Ribbon organization allows easy visual navigation of workspace functions.

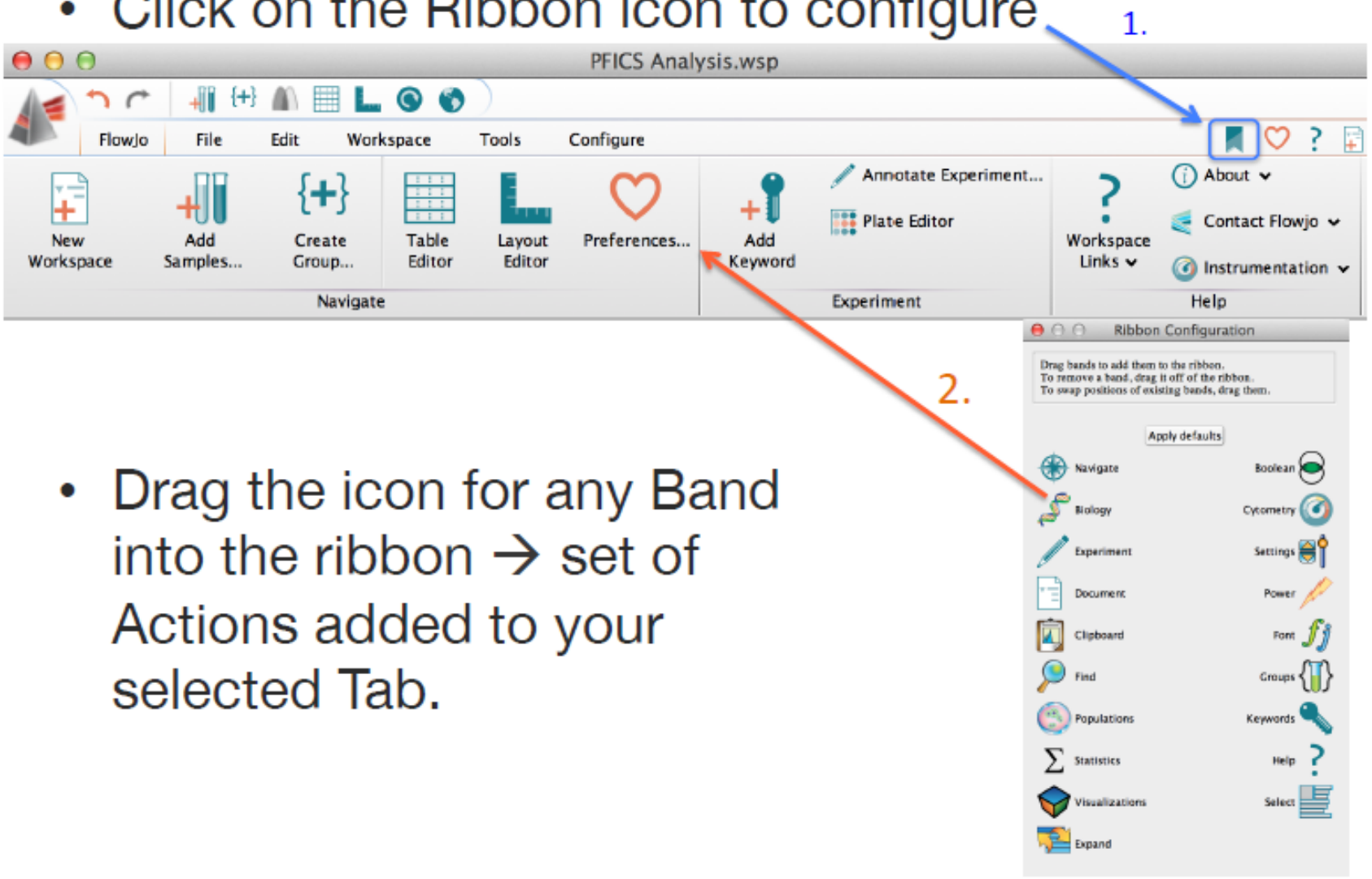


- Tabs group similar Bands together.
- Bands group similar Actions together.

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Customizing Ribbons

- Click on the Ribbon icon to configure



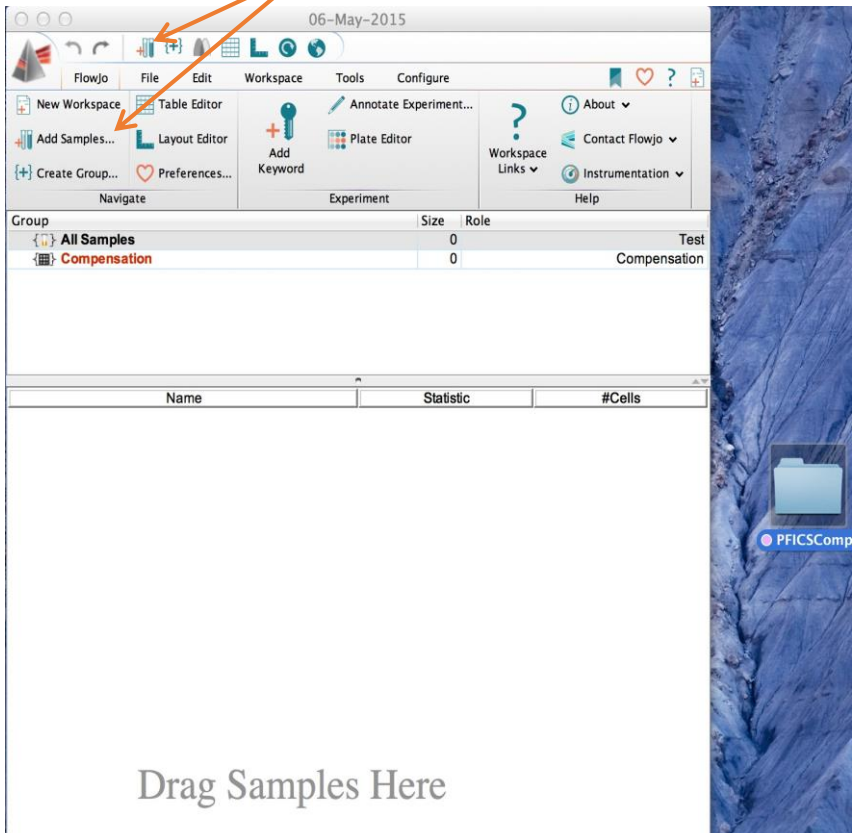
- Drag the icon for any Band into the ribbon → set of Actions added to your selected Tab.

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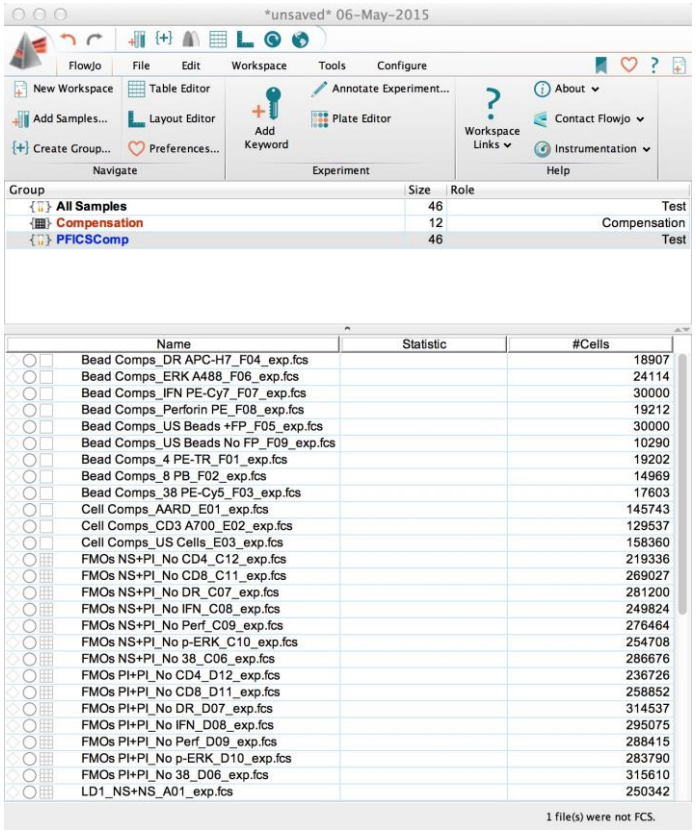
Importing Data

Two possible methods:

1. Drag and drop into samples pane
2. Click Add Samples button







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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)

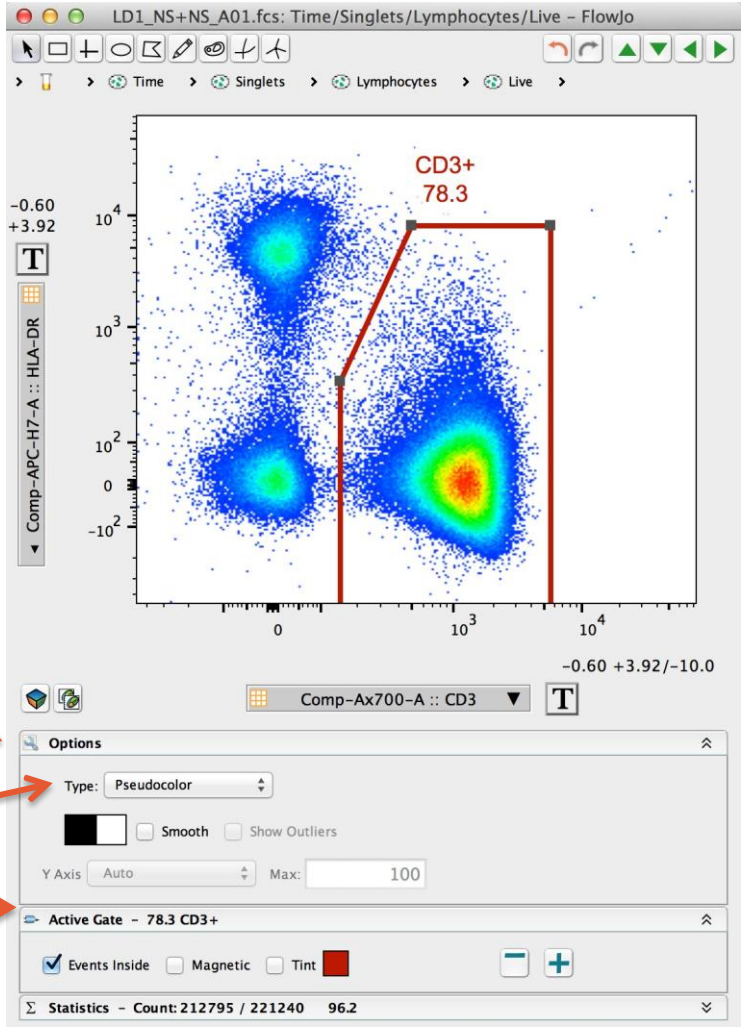
Name	Statistic	#Cells	*HIV Status	*PID	*STIM
LD1_NS+NS_A01.fcs		250342	Neg	LD1	NS+NS
LD1_NS+PI_C01.fcs		229585	Neg	LD1	NS+PI
LD1_PI+NS_B01.fcs		262774	Neg	LD1	PI+NS
Time	99.7	261964			
Singlets	96.2	252097			
Lymphocytes	93.7	236200			
Live	96.2	227167			
CD3+	81.4	184893			
Q1: CD4- , CD8+	24.0	44355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

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The Graph Window

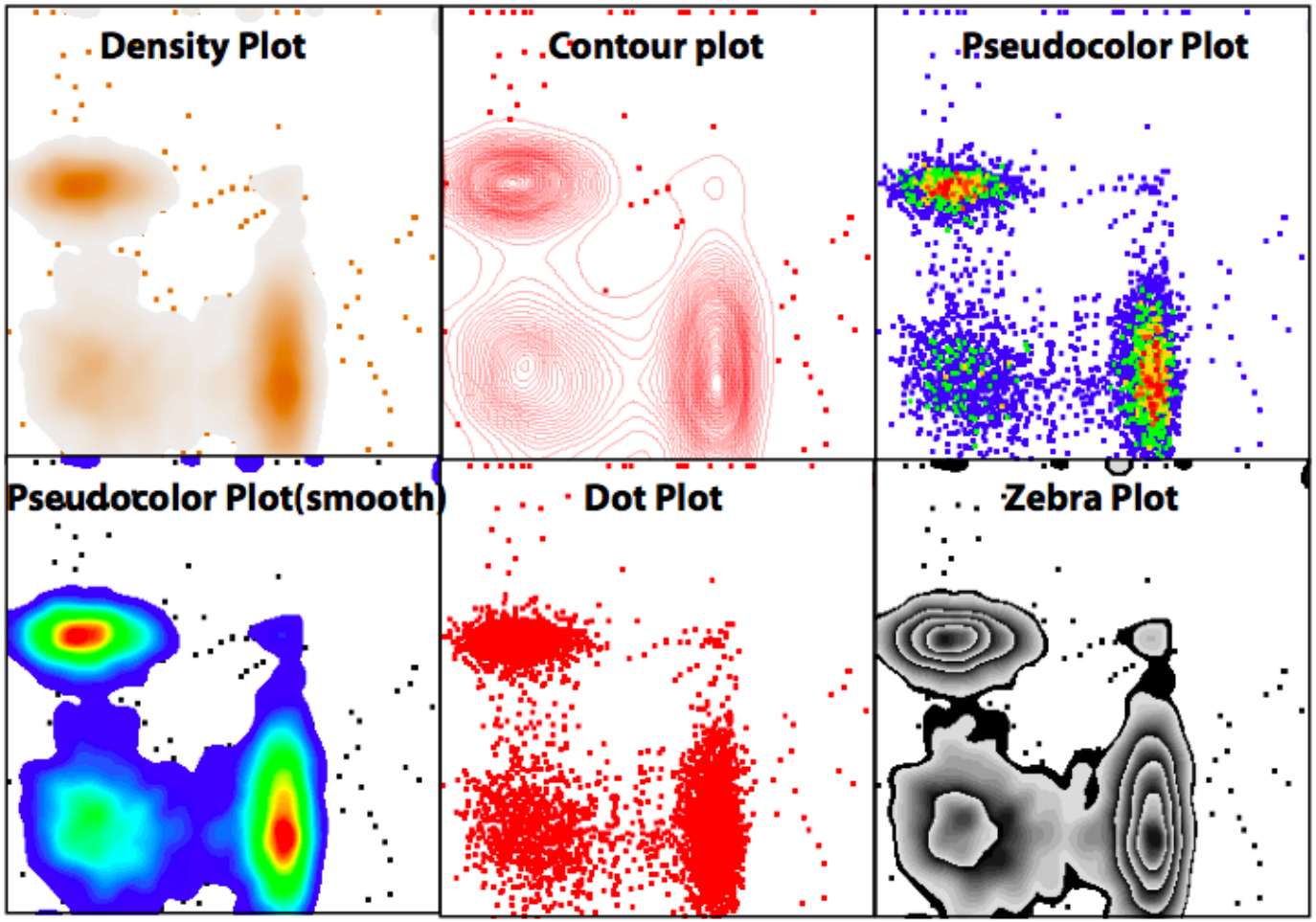
- Facilitates data visualization and gating.



- 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.

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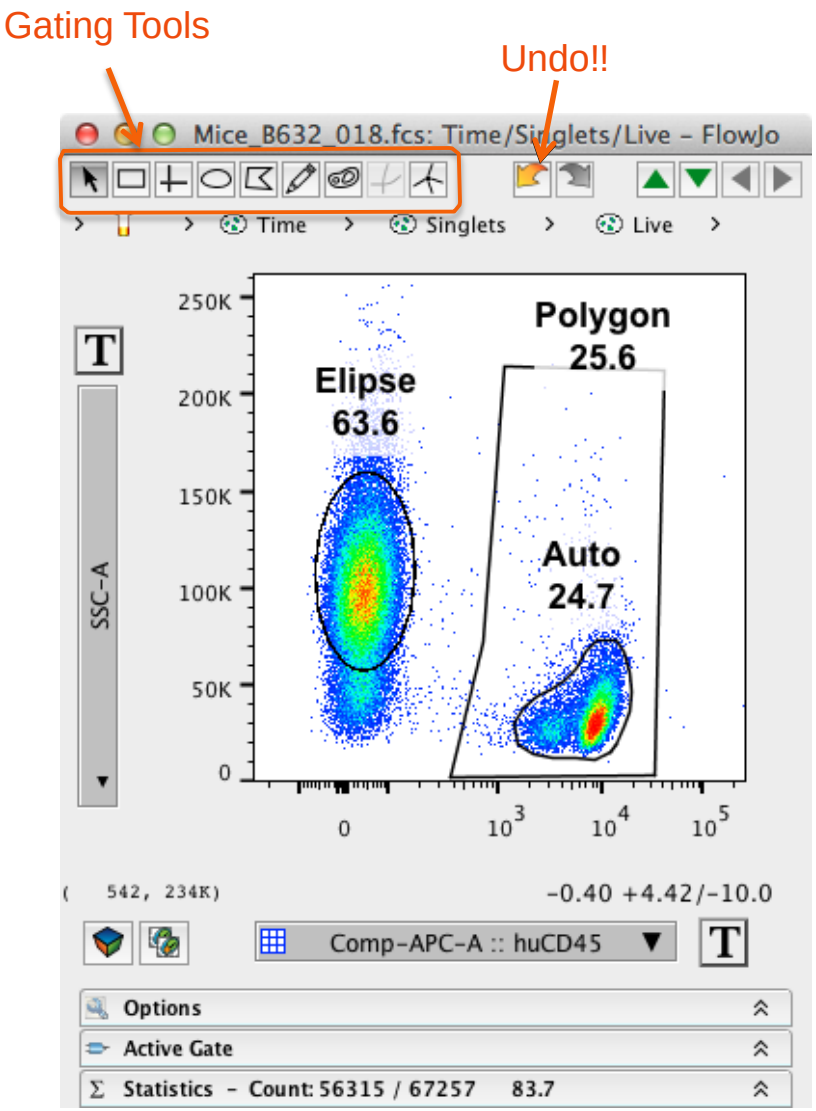
Graph Display Options



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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.

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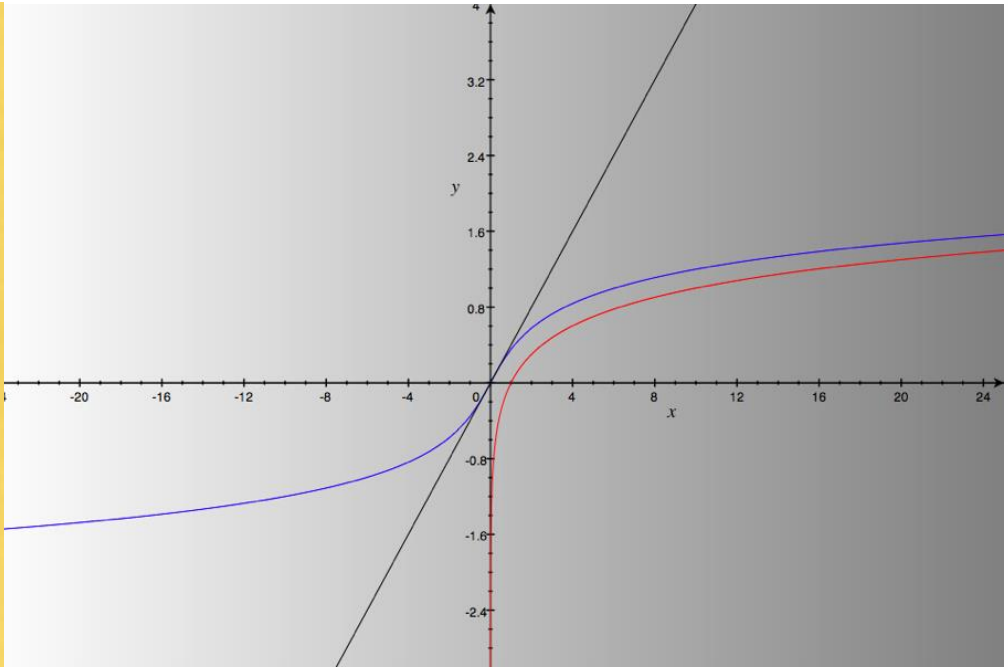
Biexponential Transformation

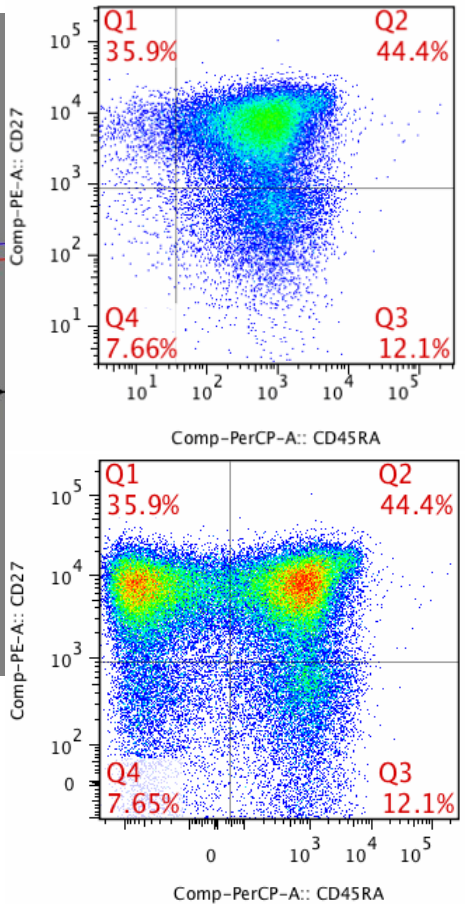
- to Better Visualize Data After Compensation

Y=X

Y=log(x)

y=arcsinh(x)





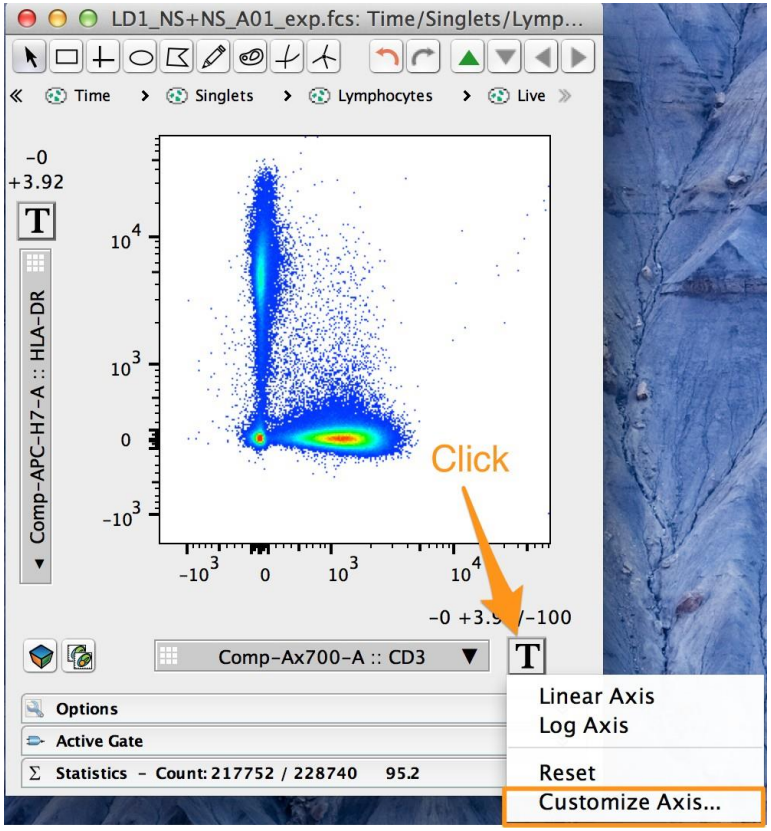
- Transformation is a bi-exponential display tool by introducing linear scales into your axes to better visualize your data.
- It does not change your data!

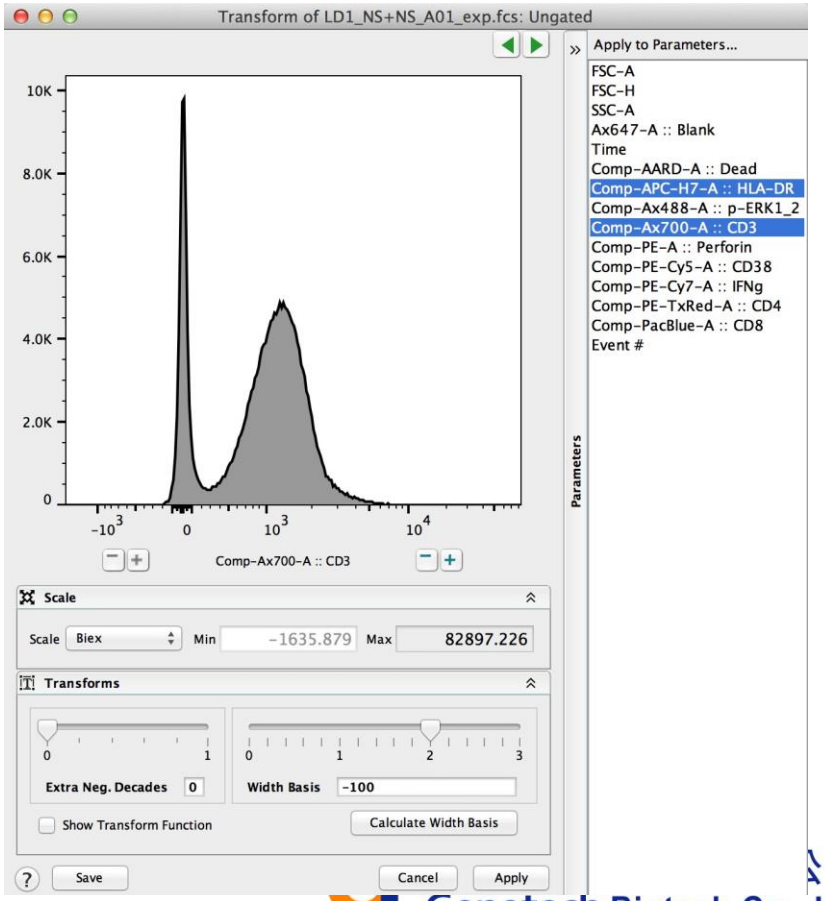
Herzenberg et. al. Nature Immunology 2006

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Transforming Data

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





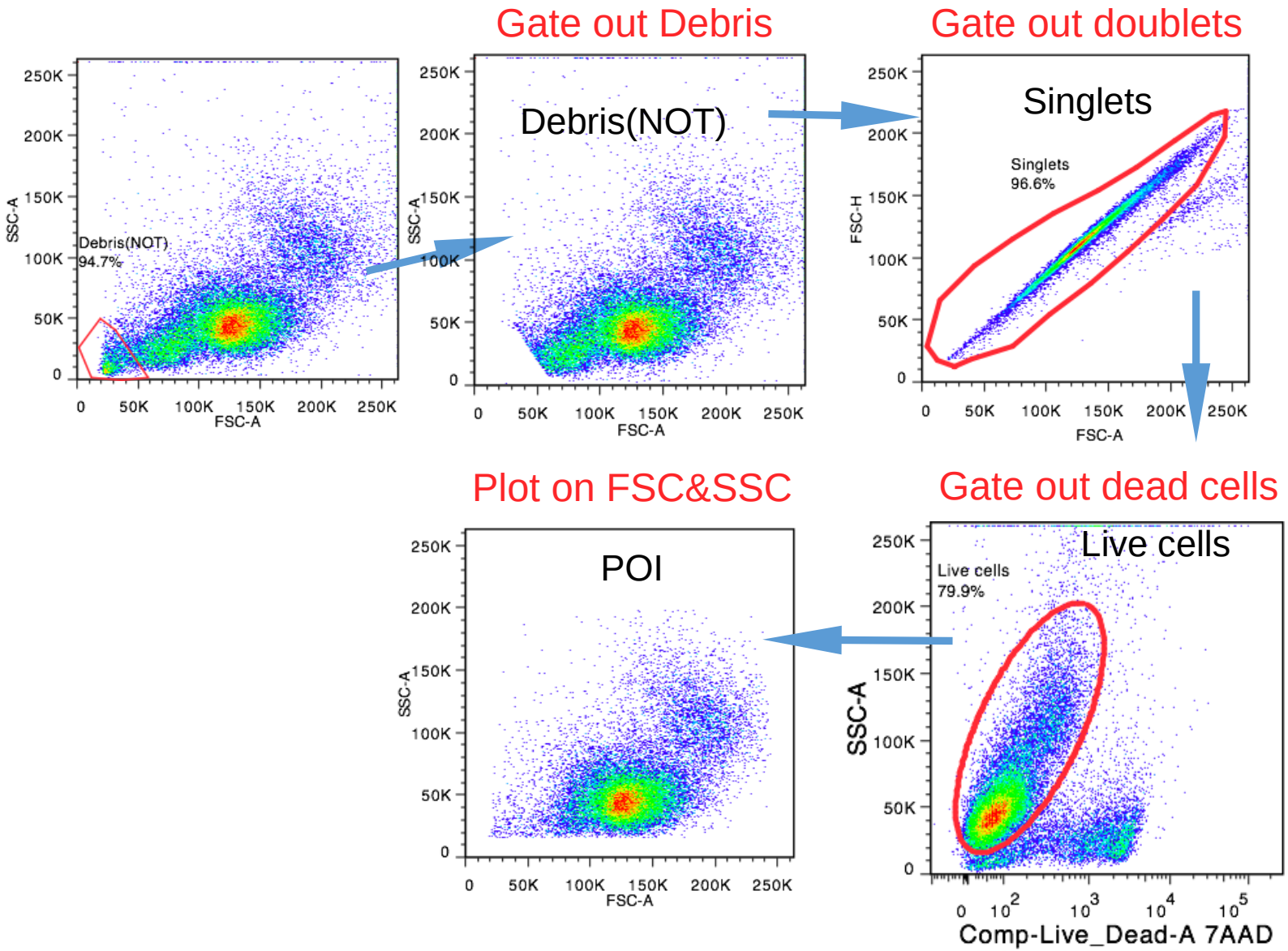
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Gating: Define Population(s) of Interest (POI)

- **Gating basic**
  - Gate on population of interest and gate out population not of interest
  - Gate out debris, dead cells, doublets/cell aggregates, Focus on POI
  - Is both objective and subjective
- **Gating tools**
  - Hierarchical gating

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Gating Strategy

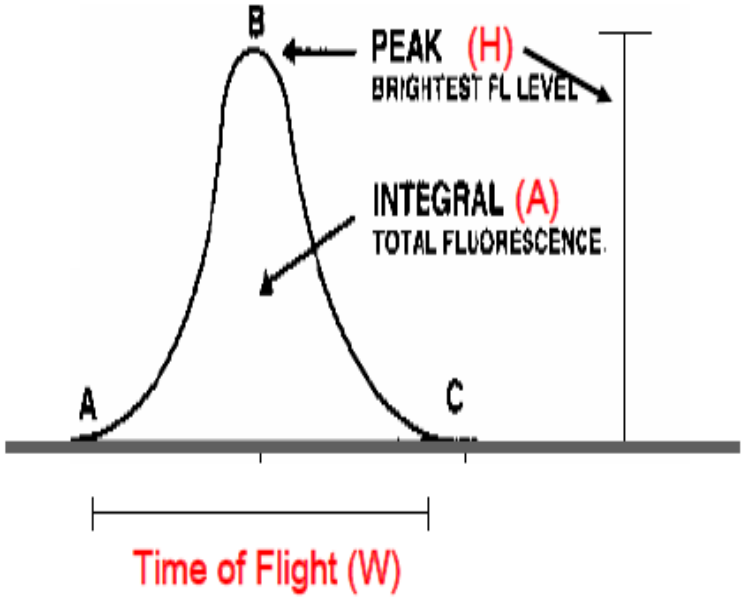


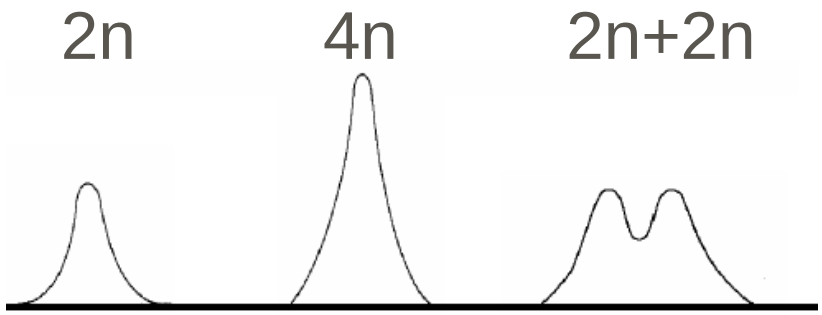
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Doublets Discrimination

What are doublets and why are they harmful?

- Doublets are two cells stick together
- Doublets can lead to higher background and false positive and especially harmful for cell cycle analysis.



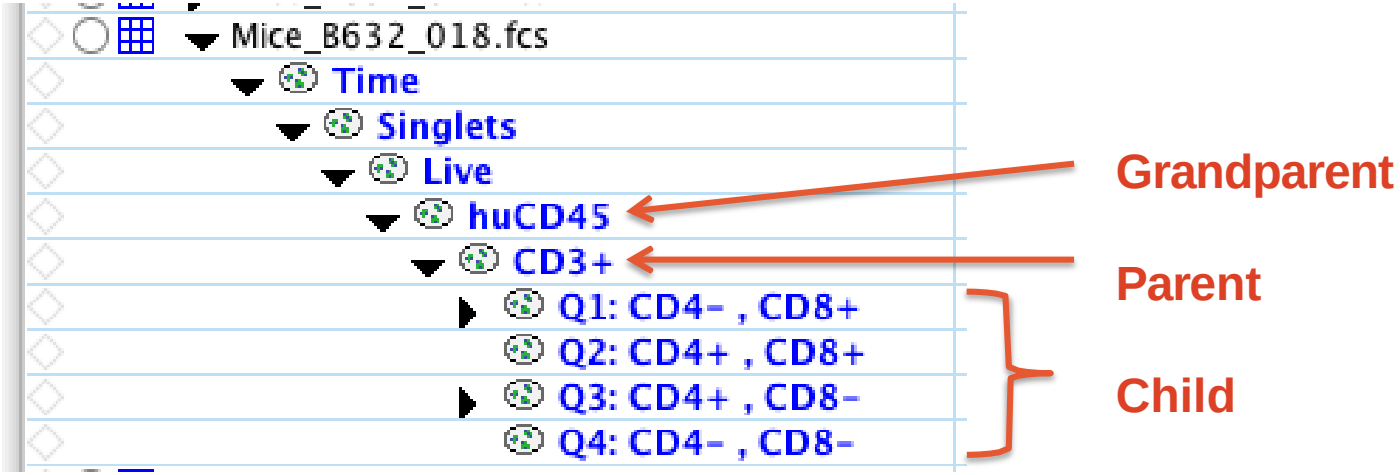


	2n	4n	2n+2n
H	1	2	1
A	1	2	2
W	1	1	2

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Hierarchical Gating

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



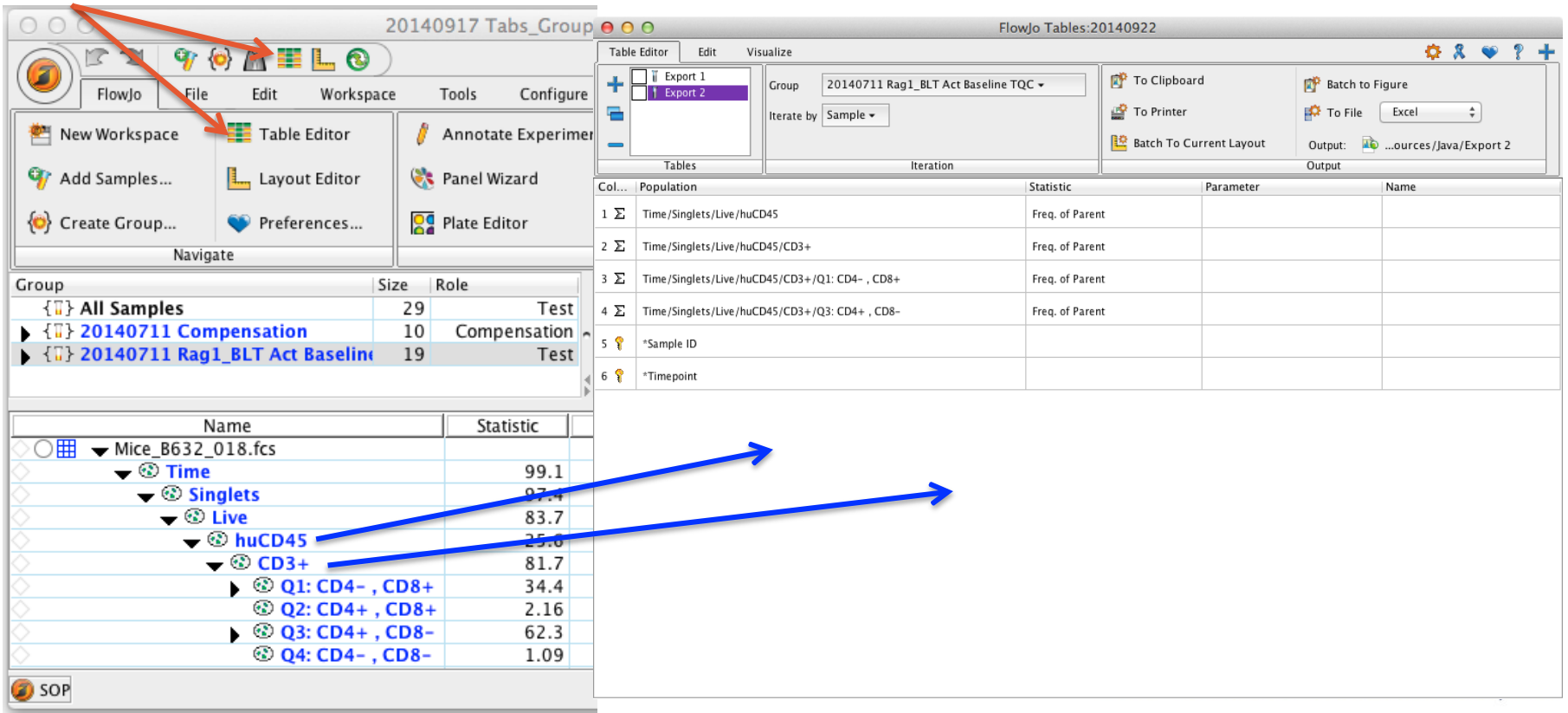
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The Table Editor

- A tool for creating statistical reports.
- Click on the Table Editor icon.
- Drag populations from sample to Table Editor.

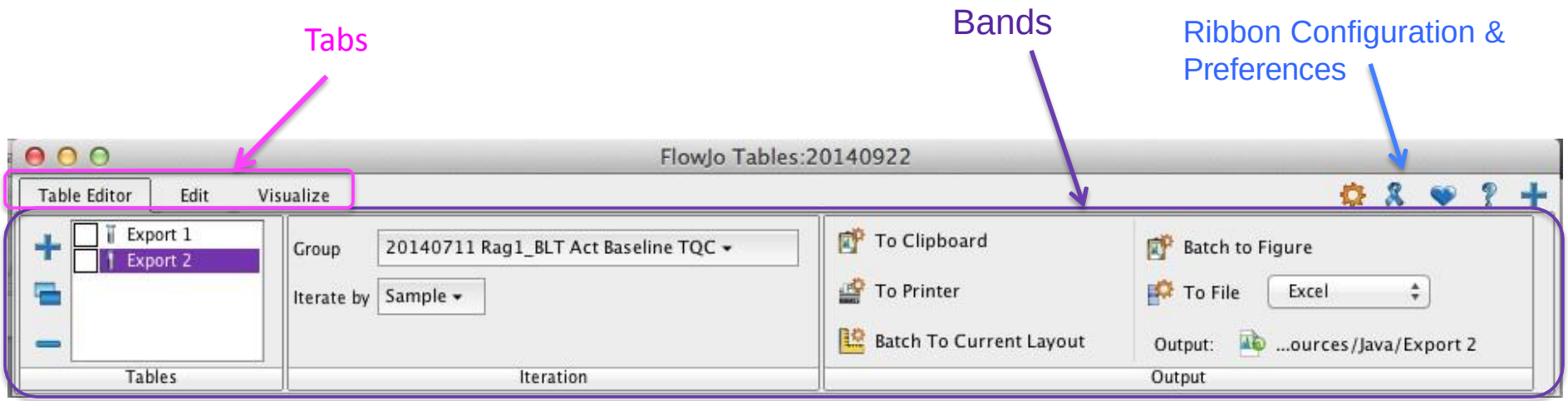


Table Editor



With in Table Editor

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

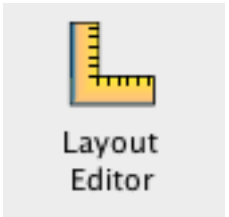


- Specify the group you wish to batch, and how to iterate the batch process, then in the Output band, specify where you want the batch output to go.

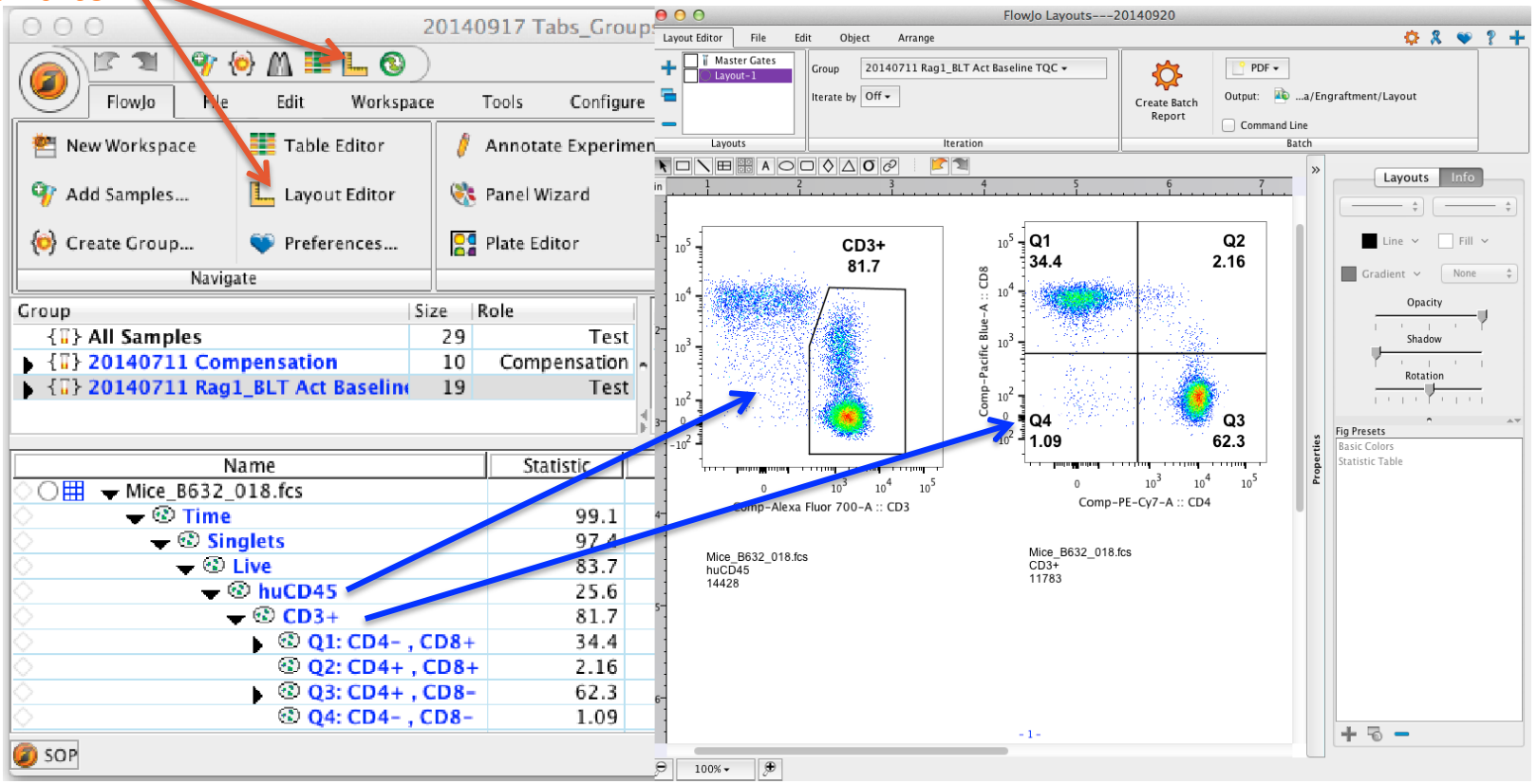
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The Layout Editor

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



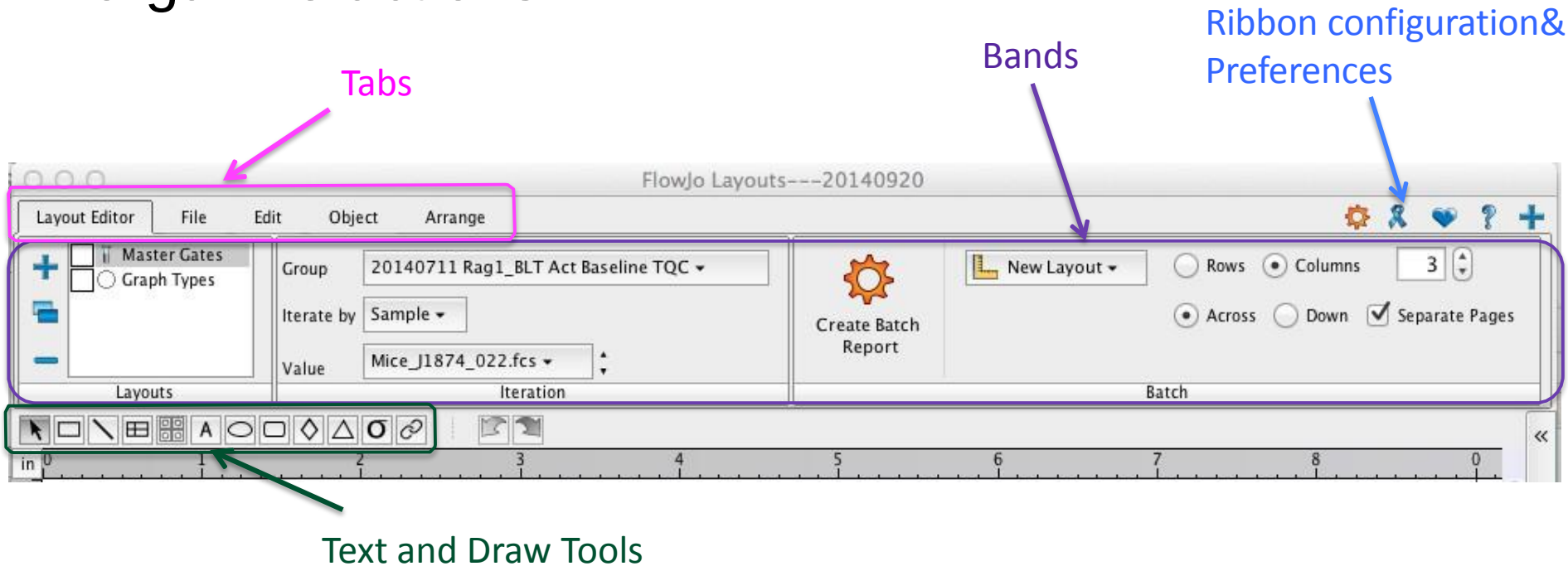
Layout Editor



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Working in Layout Editor

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

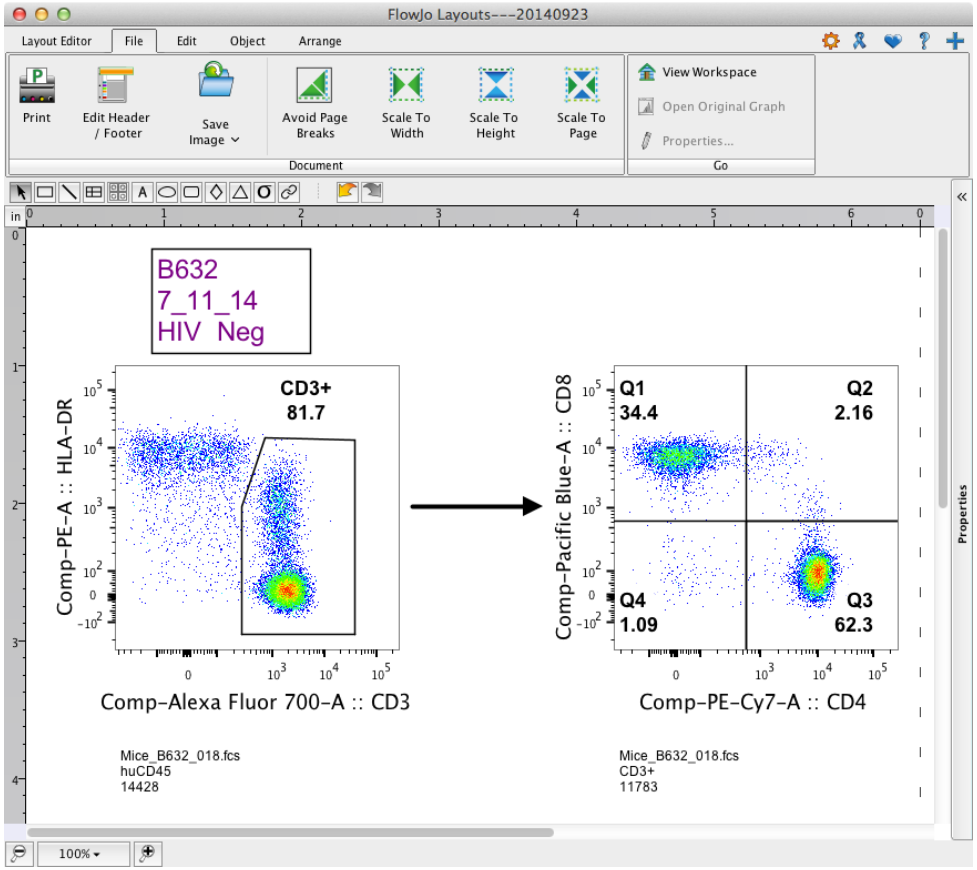


- Try clicking on the different tabs to see what types of actions are available.

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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.

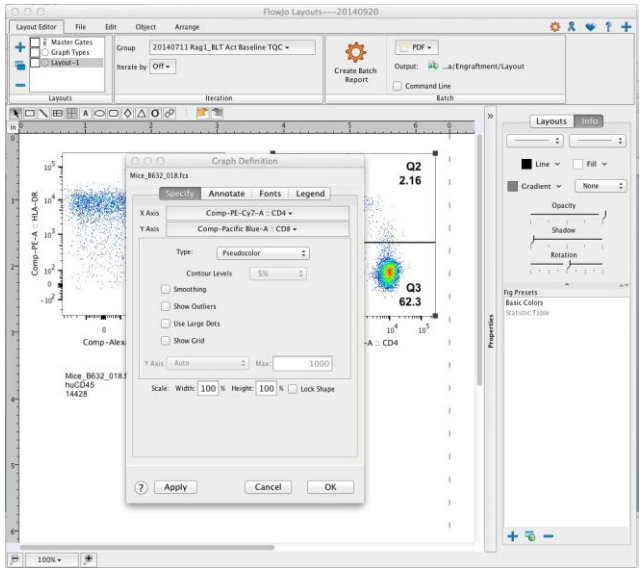


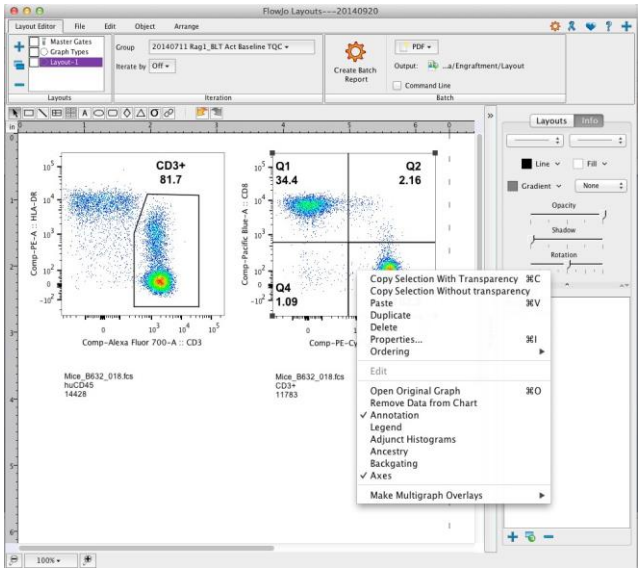
- We encourage you to explore the tools and display features available to improve the visualization of your data.

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Working in Layout Editor

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



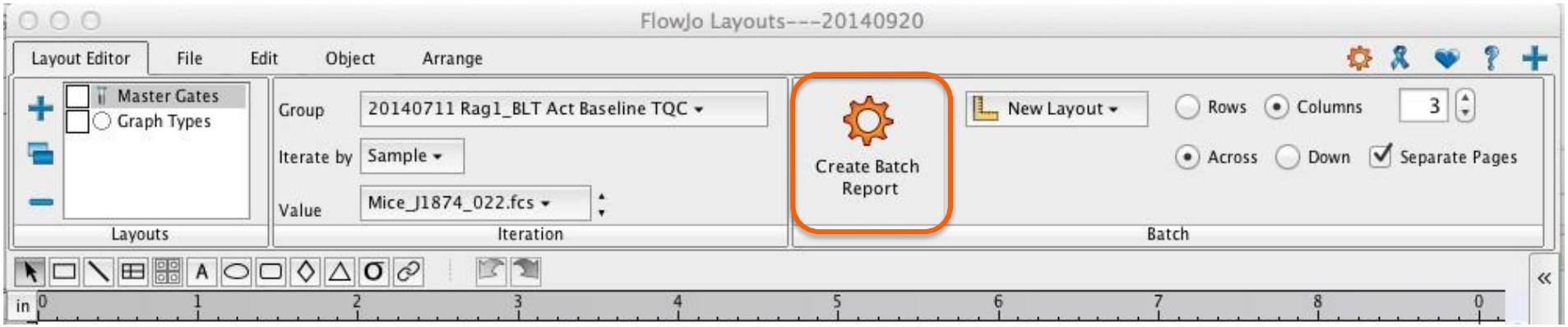


- Right click the graph for even more options.
- Hold down shift and click on multiple graphs to select and edit their properties simultaneously.

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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.

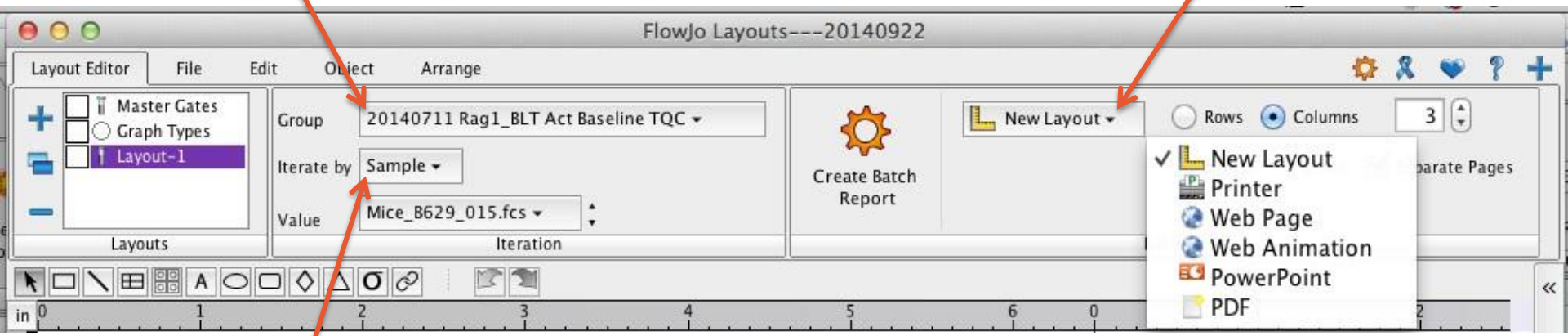


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Batch Analysis of Layout Editor Graphics

- 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





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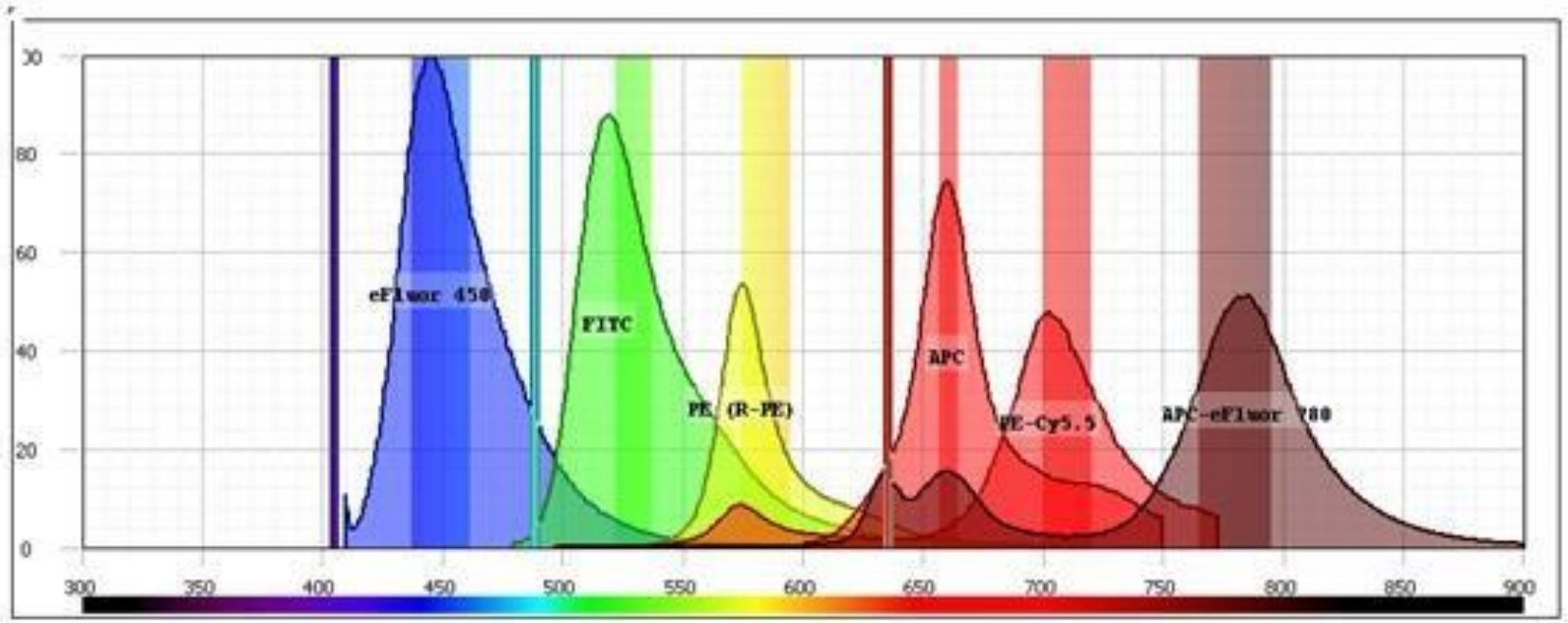
Outline – Part II

- Compensation
- Templates
- Special Analysis Platform:
  - Cell cycle
  - Proliferation (CFSE)
  - Cell Kinetics (Calcium flux)

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Compensation

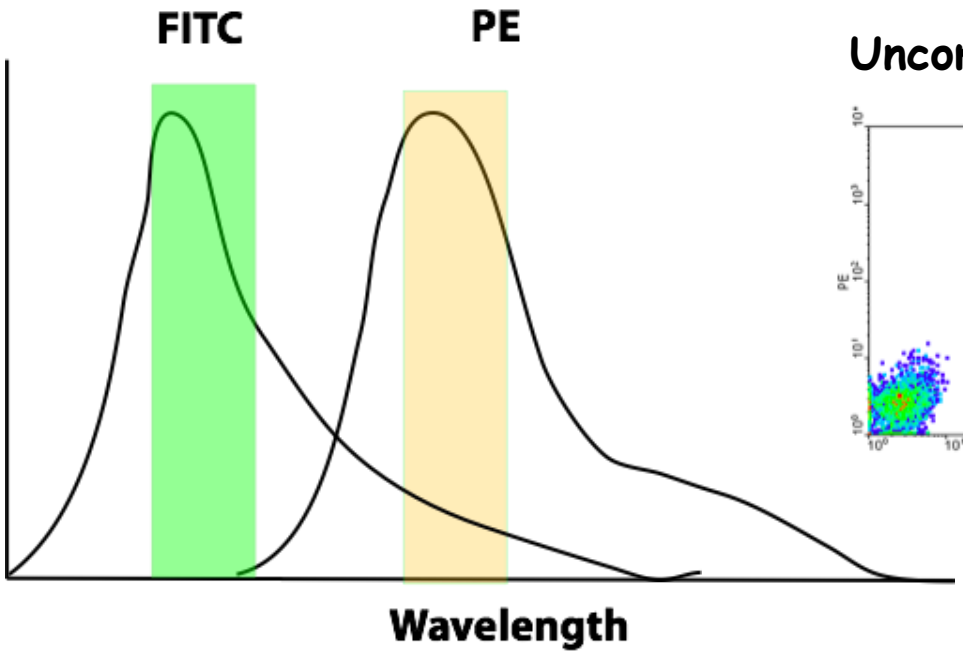
- Compensation corrects for spillover between fluorochrome emission spectra.

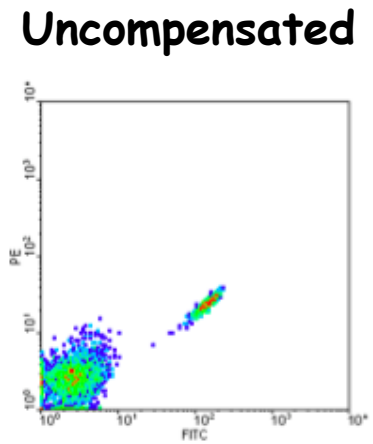


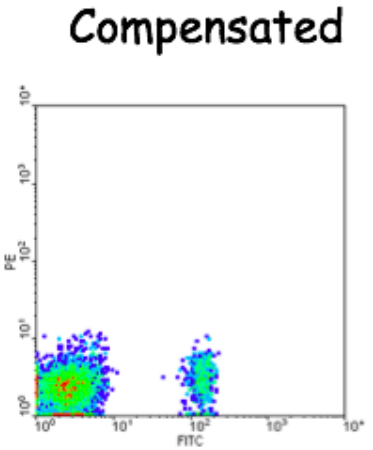
- Compensation is essential for multicolor panels

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Compensation Controls-To Correct Spectra Spillover







True PE =total FL2 -FITC spill over

	FITC median fluorescence		PE median fluorescence	
	Negative	Positive	Negative	Positive
Uncompensated	125	3,540	185	1,650
Compensated	125	3,560	135	135

$$\% \text{ Spillover} = \frac{1{,}650 - 185}{3{,}540 - 125} \times 100$$

Slide credit to Dr. Holden Maecker Stanford University

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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.

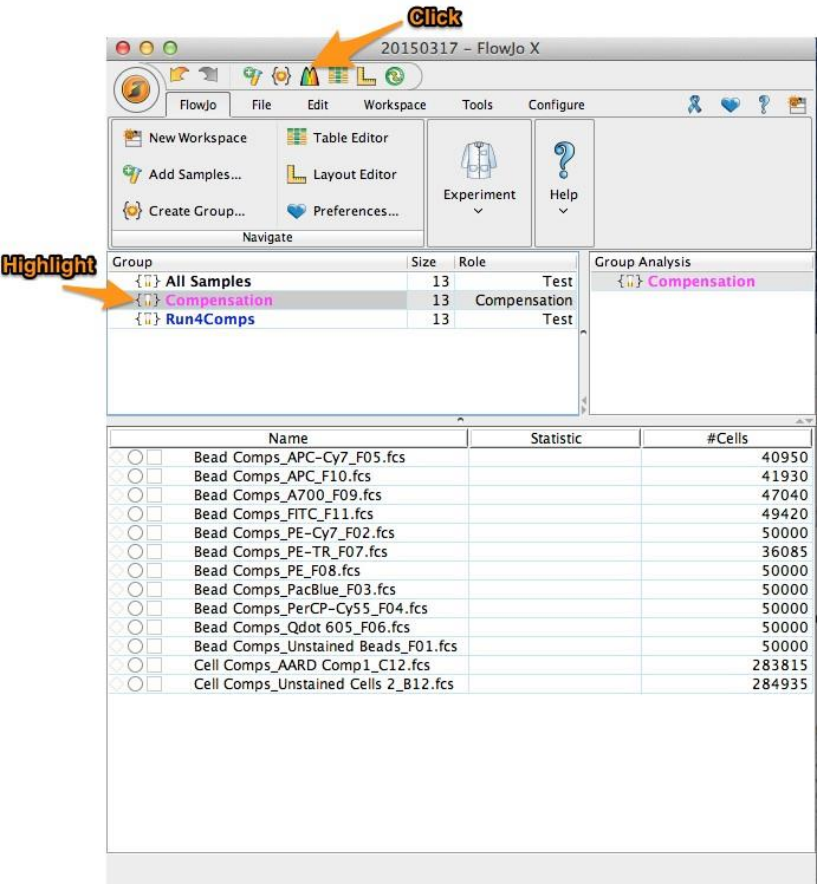
1. Controls need to be at least as bright or brighter than any sample the compensation will be applied to.
2. Background fluorescence should be the same for the positive and negative control.
3. Compensation controls MUST match the exact experimental fluorochrome.

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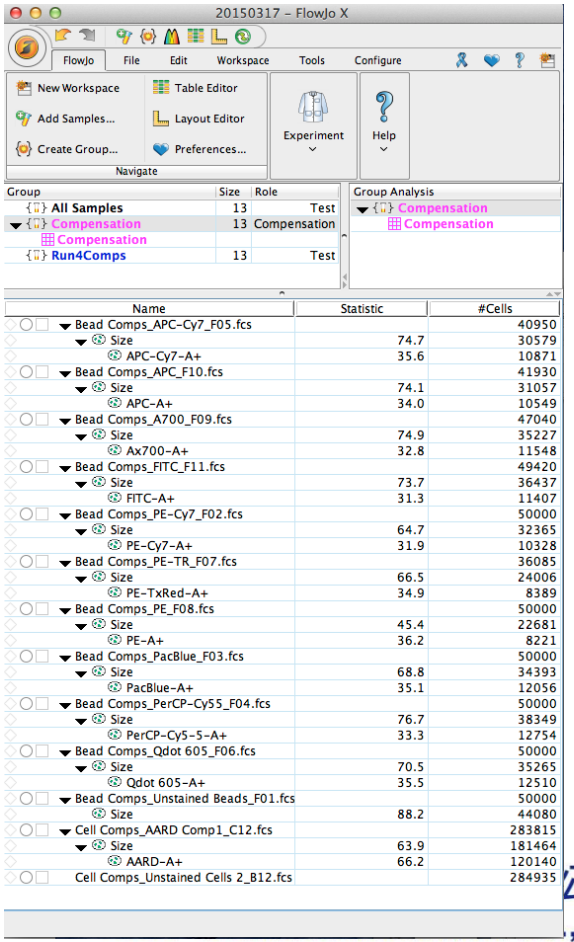
Compensation I

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

in the task bar.

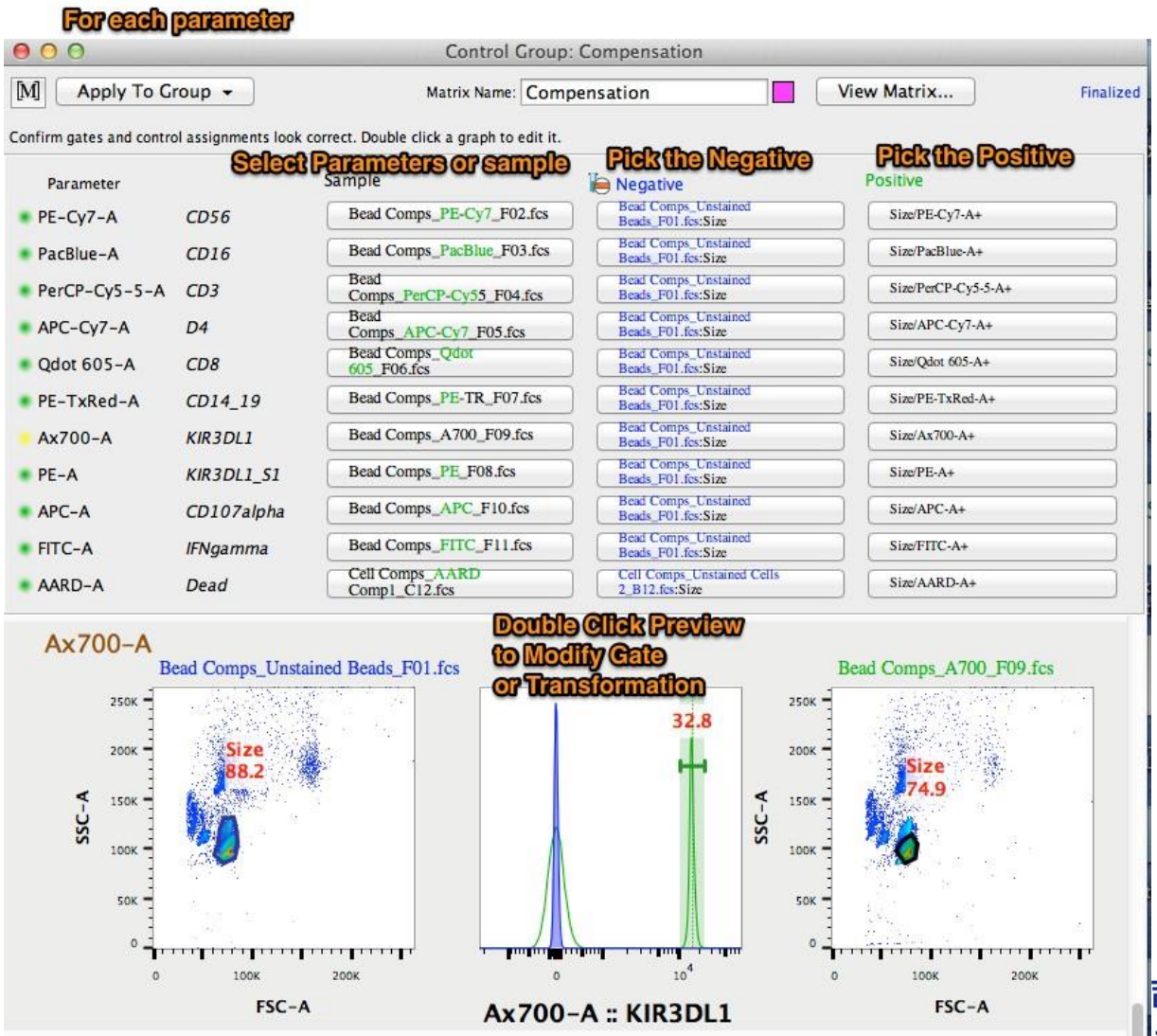


- The wizard auto gates samples



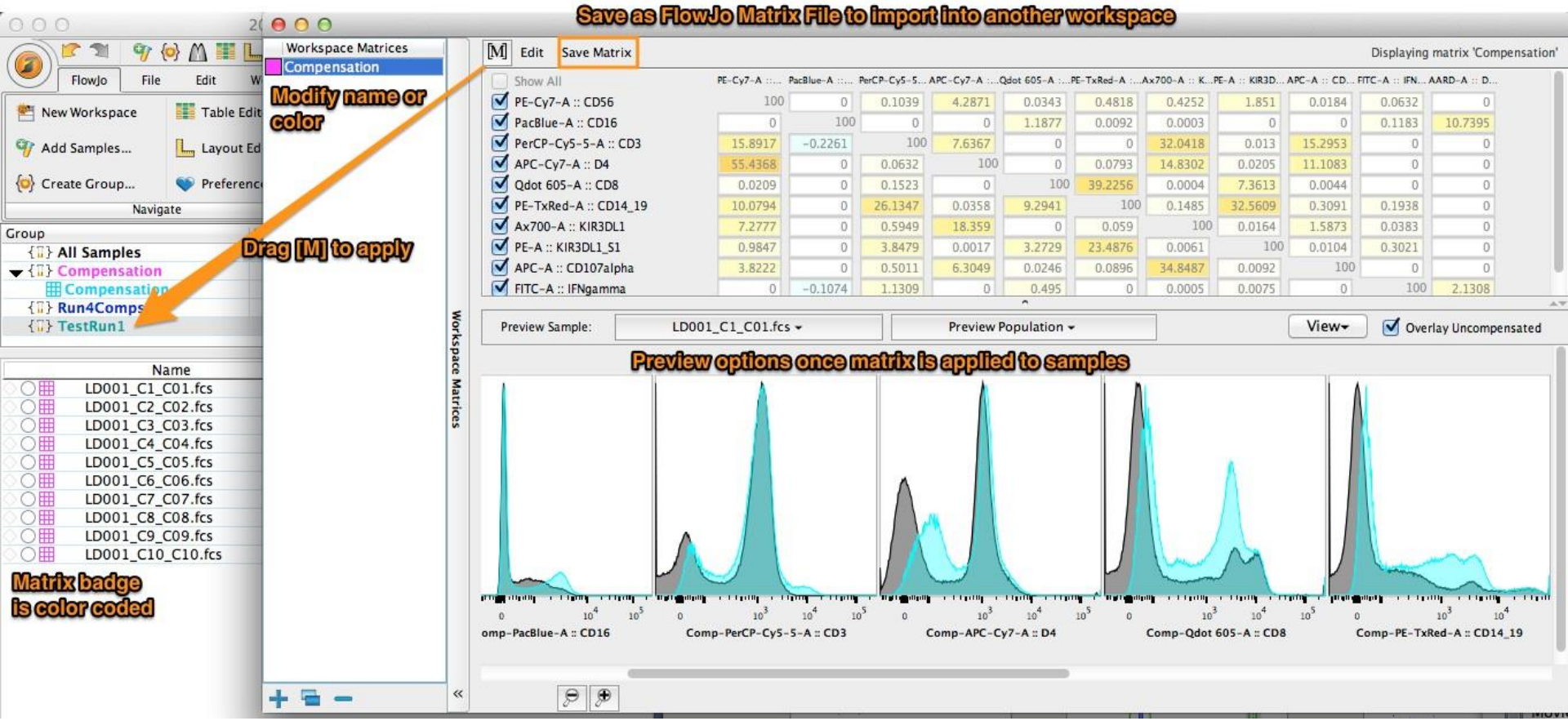
Compensation II

- Then fills in the positive and negative.
- Choose options from the dropdown lists for each parameter.
- Double click preview graphs to modify properties.



Compensation III

- View Matrix… to Modify, Apply, Save or Preview

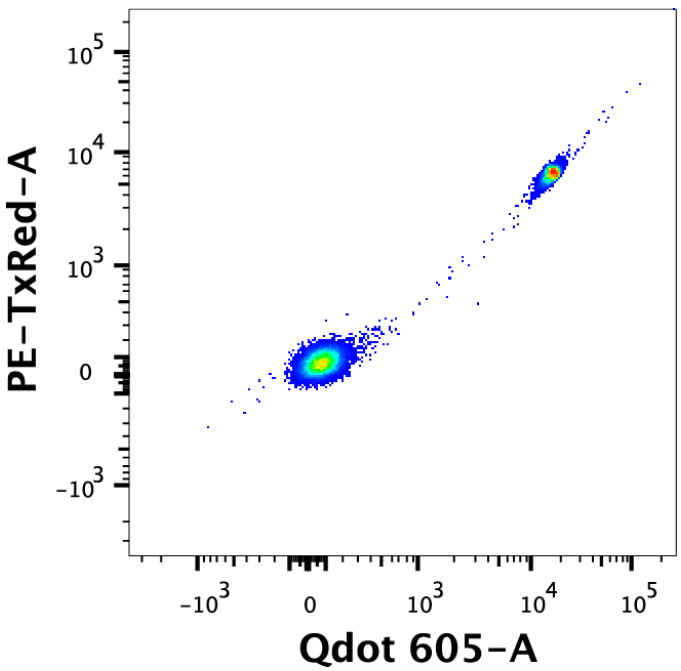


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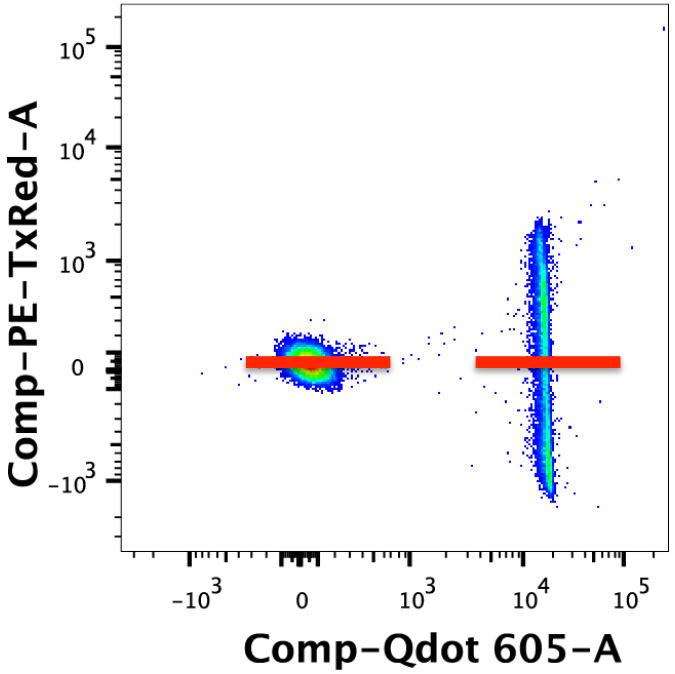
Effect of Compensation



Uncompensated

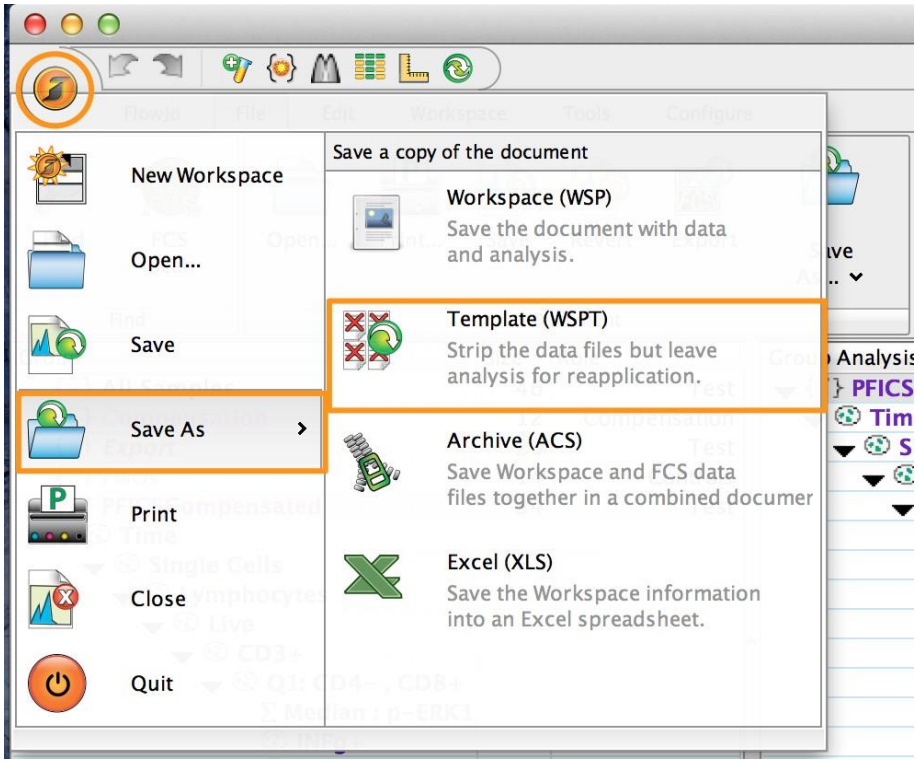


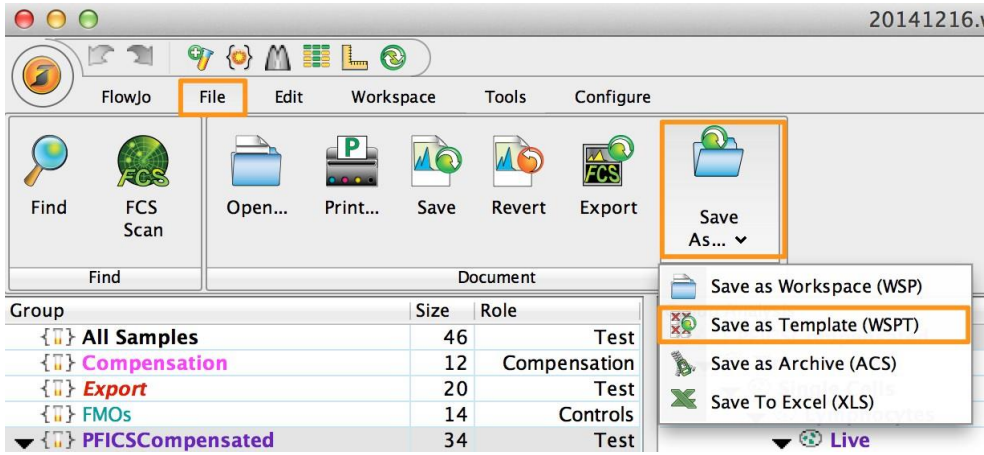
Compensated



Workspace Templates

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

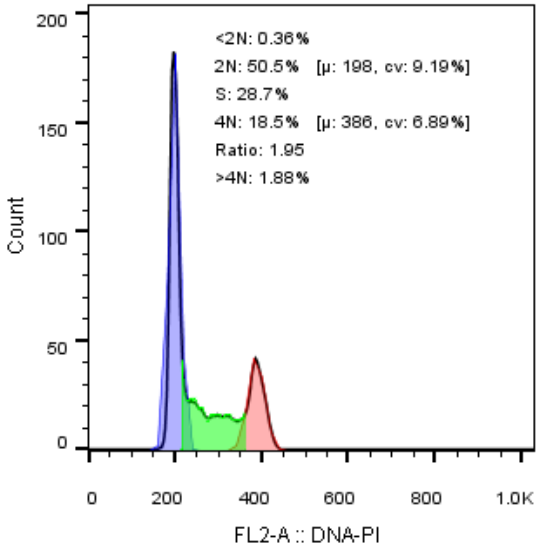


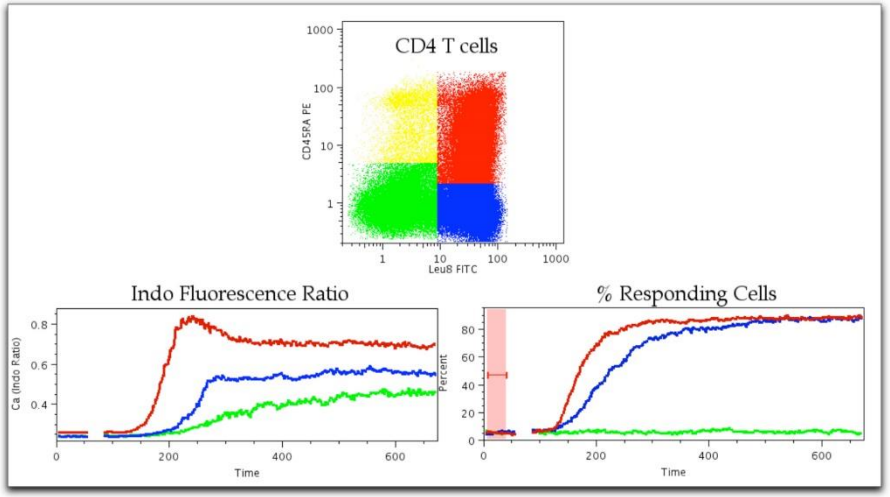


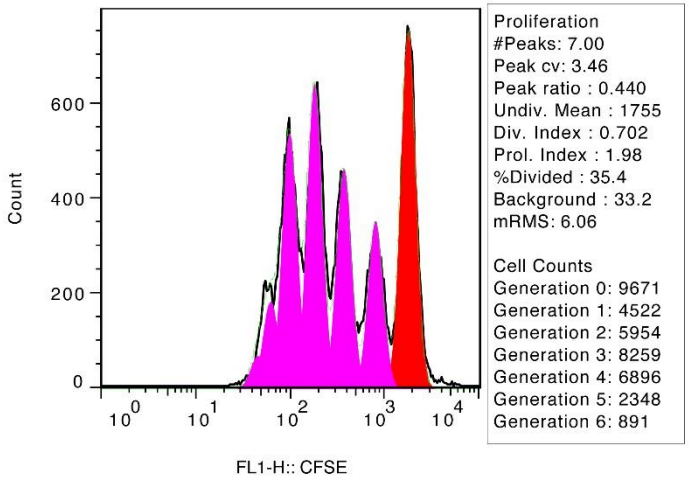
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Special Analysis Platforms

- DNA Cell Cycle
- Proliferation
- Kinetics (Calcium influx)







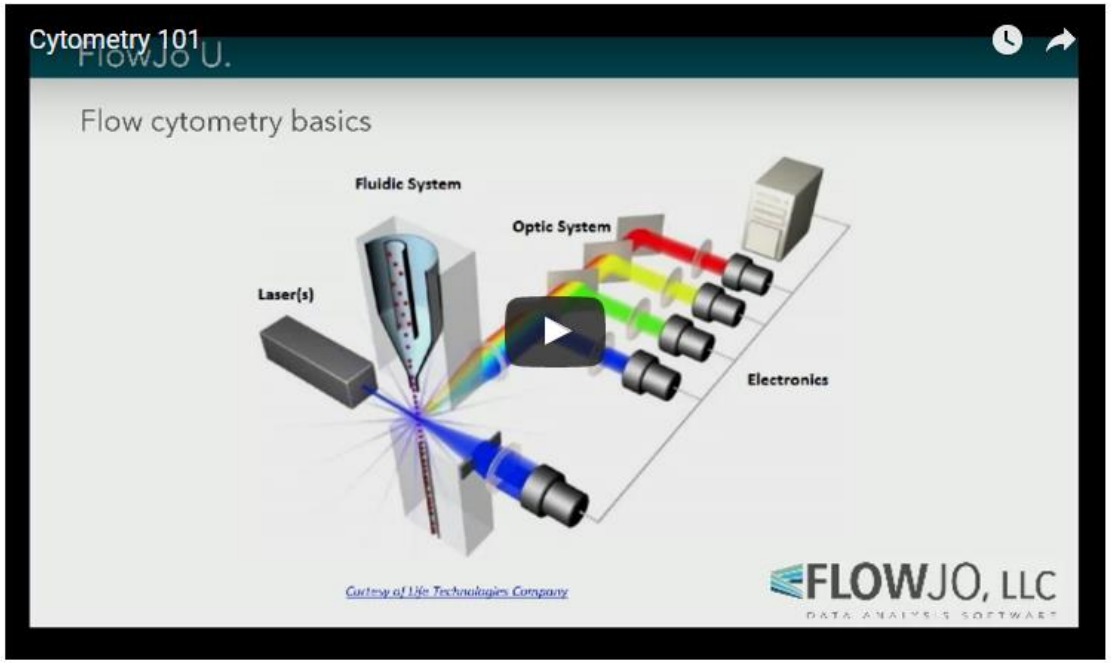
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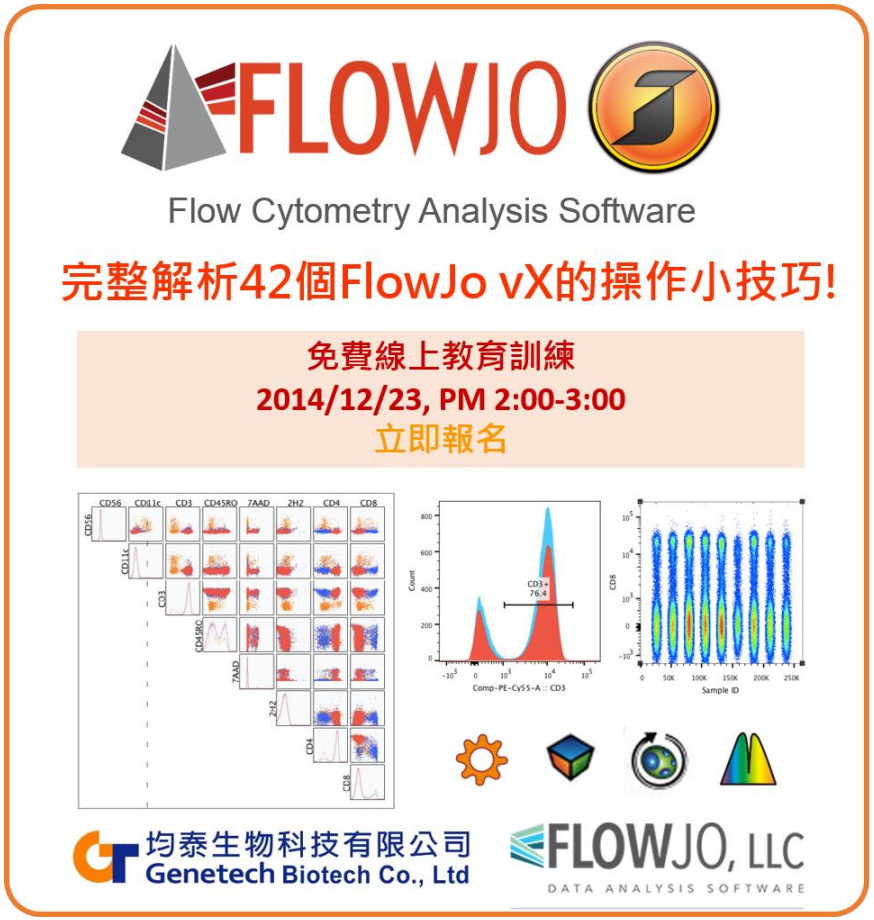


John Quinn, PhD

- Cytometry 101 (1 of 16)
- Intro to the Workspace (2 of 16)
- Introduction (3 of 16)
- Getting Started (4 of 16)
- The Workspace (5 of 16)

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- 均泰生技提供每兩個月一次FlowJo免費線上教育訓練
- 活動訊息請洽均泰官網或均泰FB粉絲團



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/

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