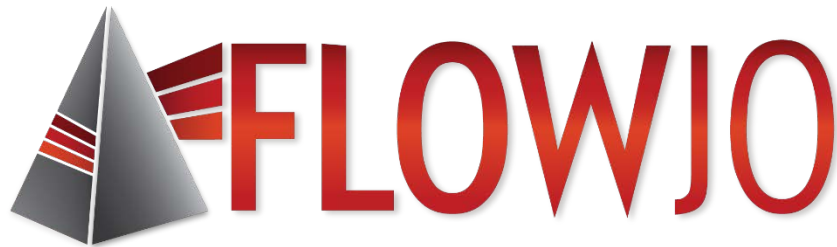


Cytometry Data Analysis in FlowJo V10



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

Outline-Part I

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



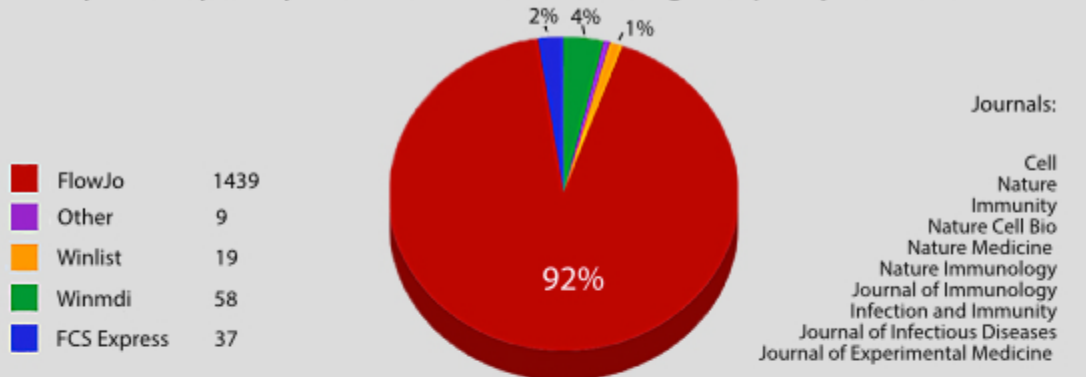
What is FlowJo?

- 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 Leading Analysis Software

Cytometry analysis software citations in high-impact journals in 2012



Third-party software

Nature

Impact Factor: 36.2



Cell

Impact Factor: 32.4



Nature Immunology

Impact Factor: 26



Nature Medicine

Impact Factor: 22.4



Immunity

Impact Factor: 21



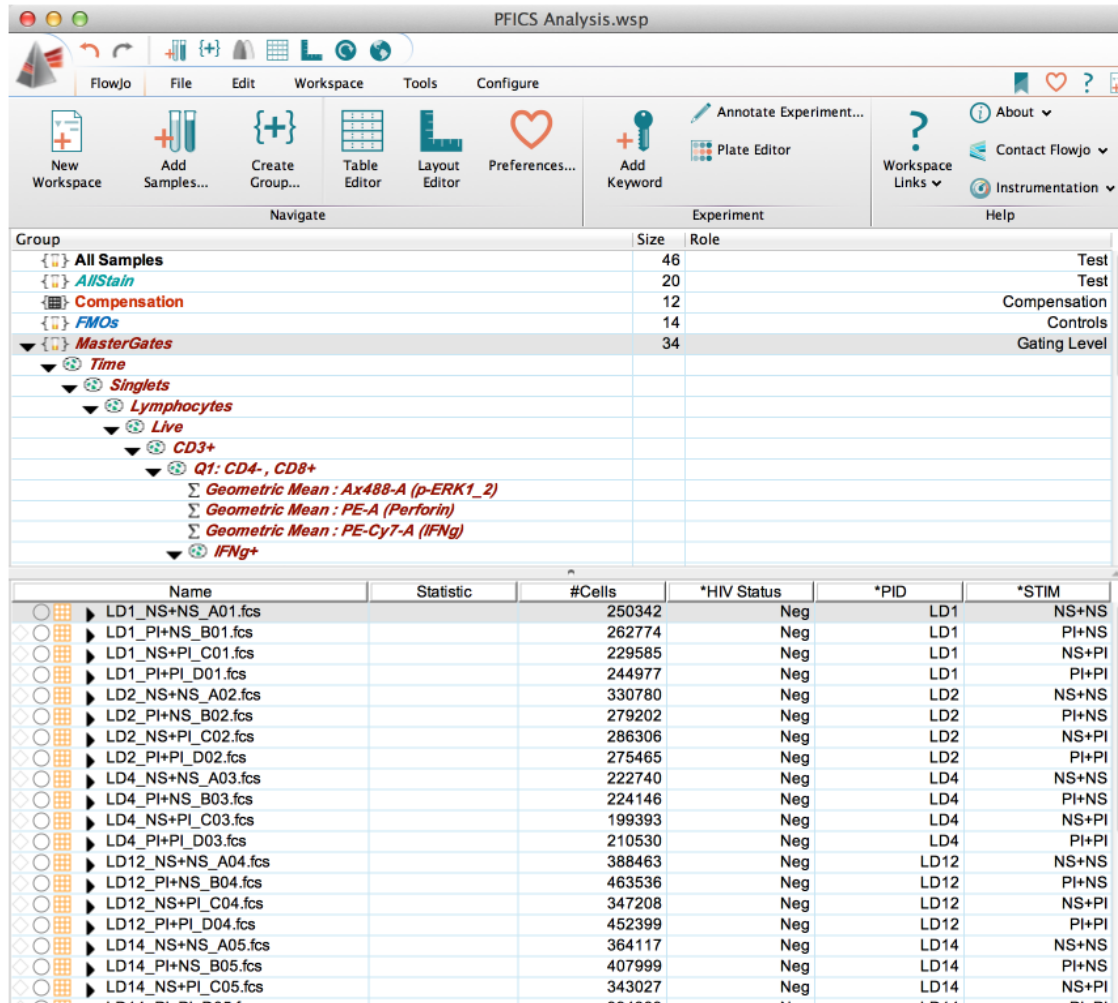
Journal of Immunology

Impact Factor: 5.78



The FlowJo V10 Workspace

- A graphical interface to organize your data.



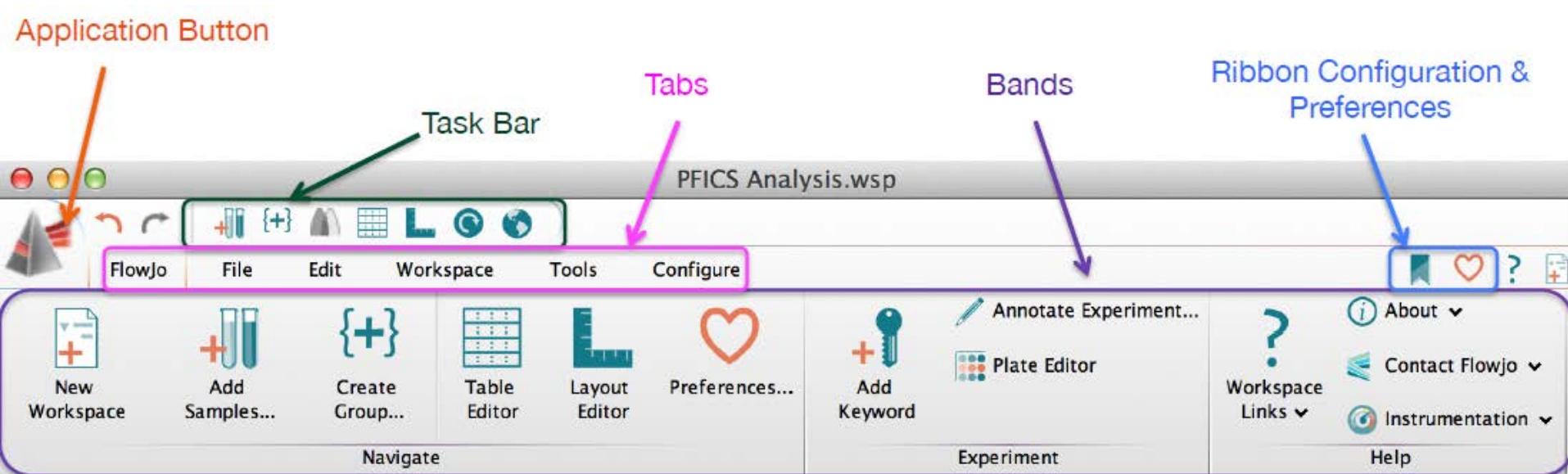
Ribbon
Tabs and Bands

Groups and Group
Analysis

Samples and
Sample analysis

Ribbons, Tabs and Bands

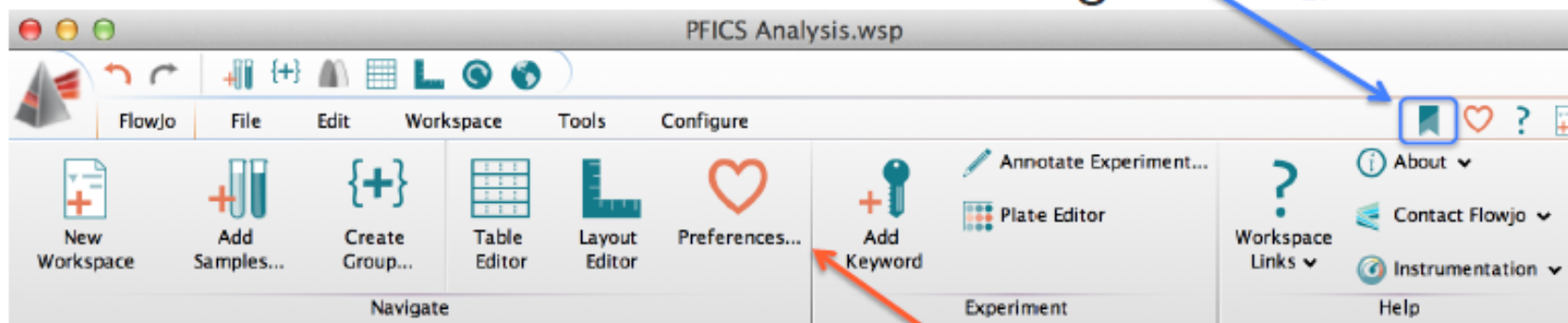
- Ribbon organization allows easy visual navigation of workspace functions.



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

Customizing Ribbons

- Click on the Ribbon icon to configure 1.



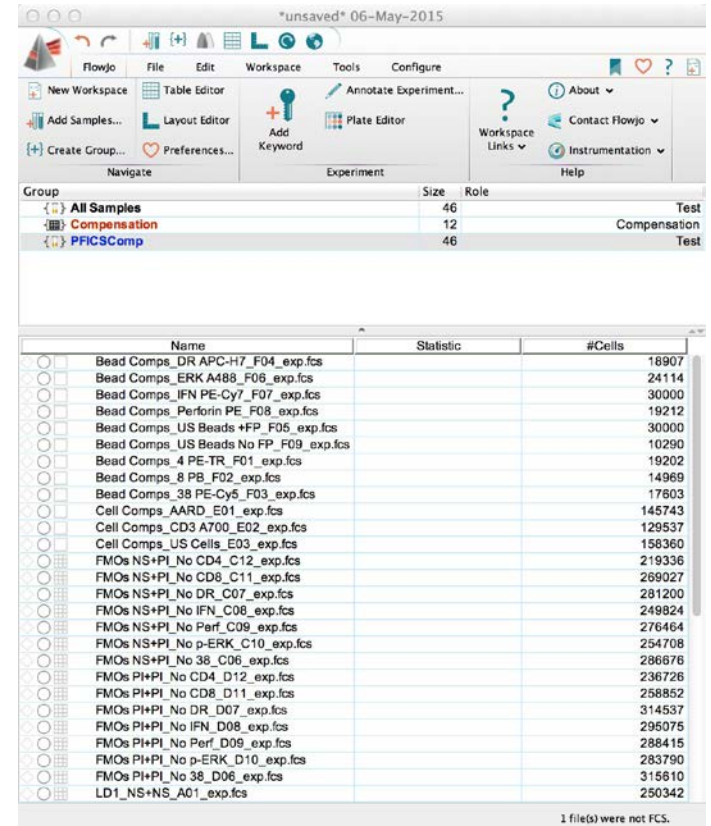
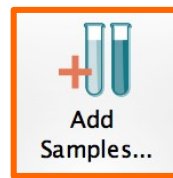
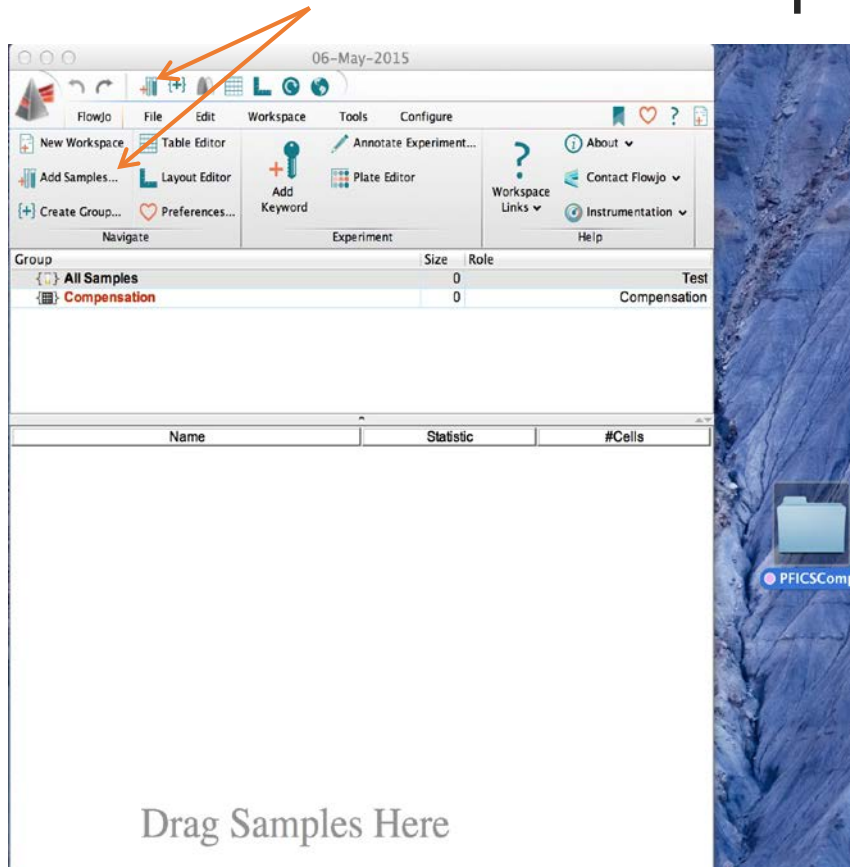
- Drag the icon for any Band into the ribbon → set of Actions added to your selected Tab. 2.



Importing Data

Two possible methods:

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



Samples and Sample Analysis

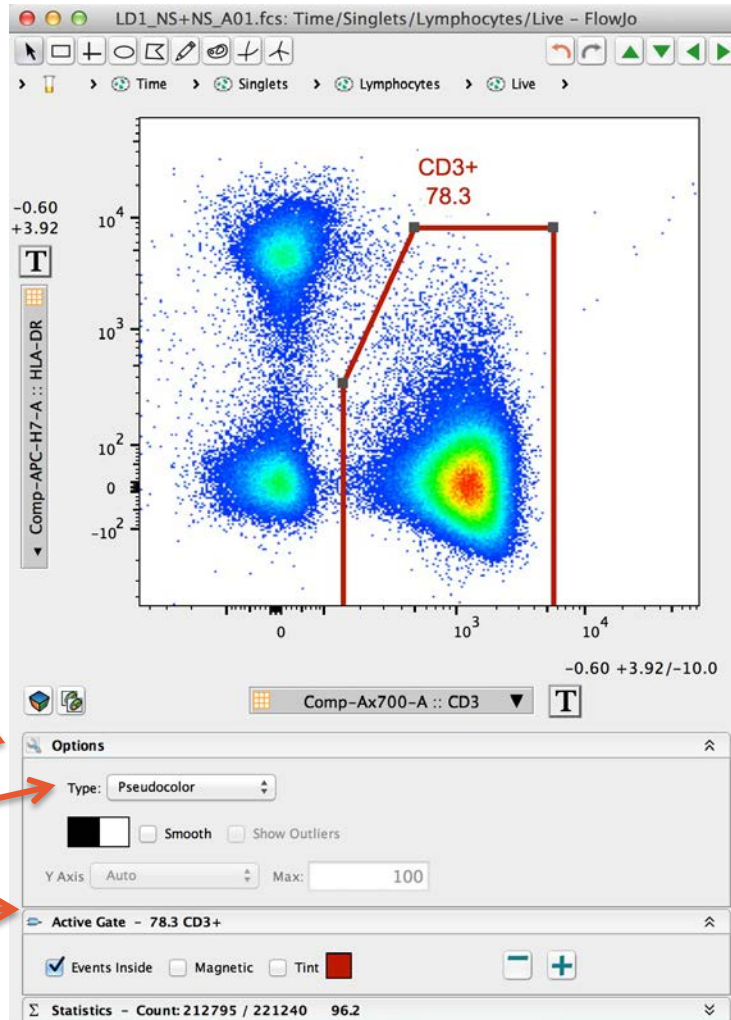
- Displays the sample list and associated analysis of the currently selected group.
- Statistic and #Cells columns are displayed by default. Additional information can be displayed as columns. (Workspace Tab →→ Add Keywords or Configure Tab →→ Edit Columns)

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.

The Graph Window

- Facilitates data visualization and gating.



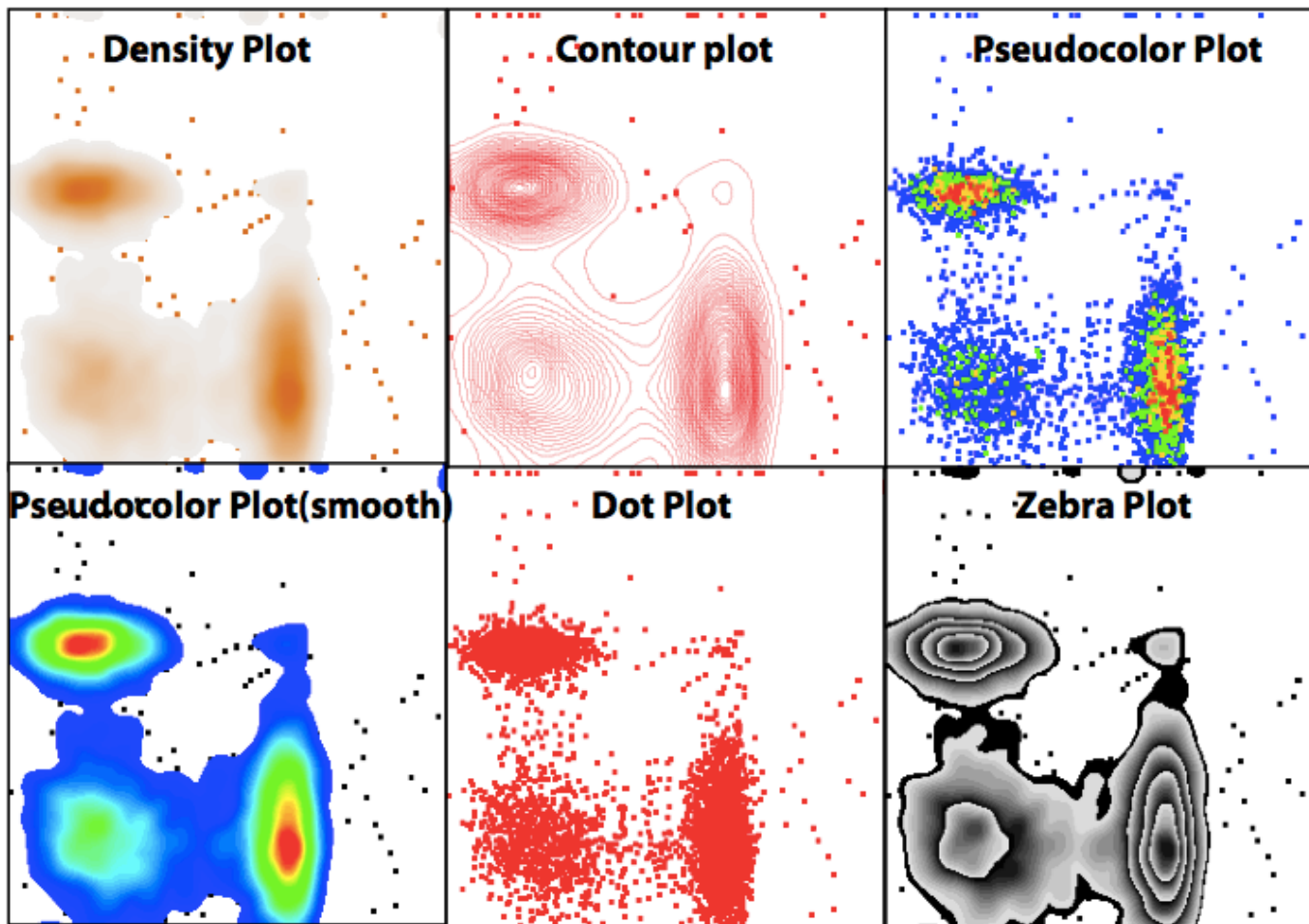
Plot View
Options

Graph Type

Active Gate
Options

- 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

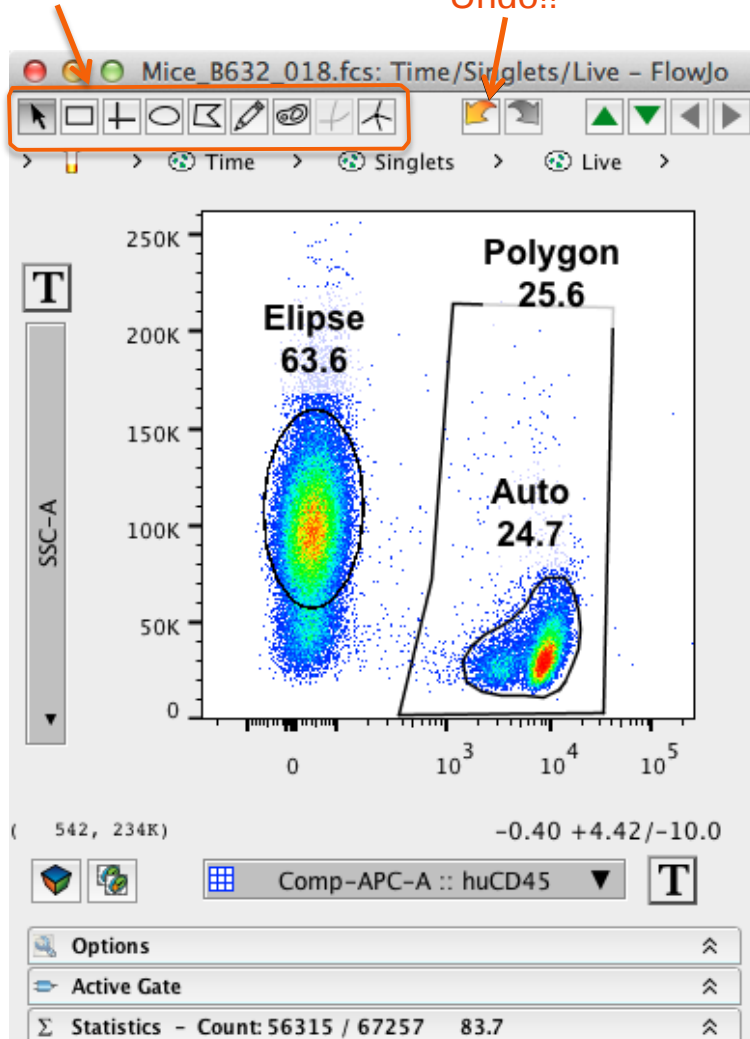


Gating tools

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

Gating Tools

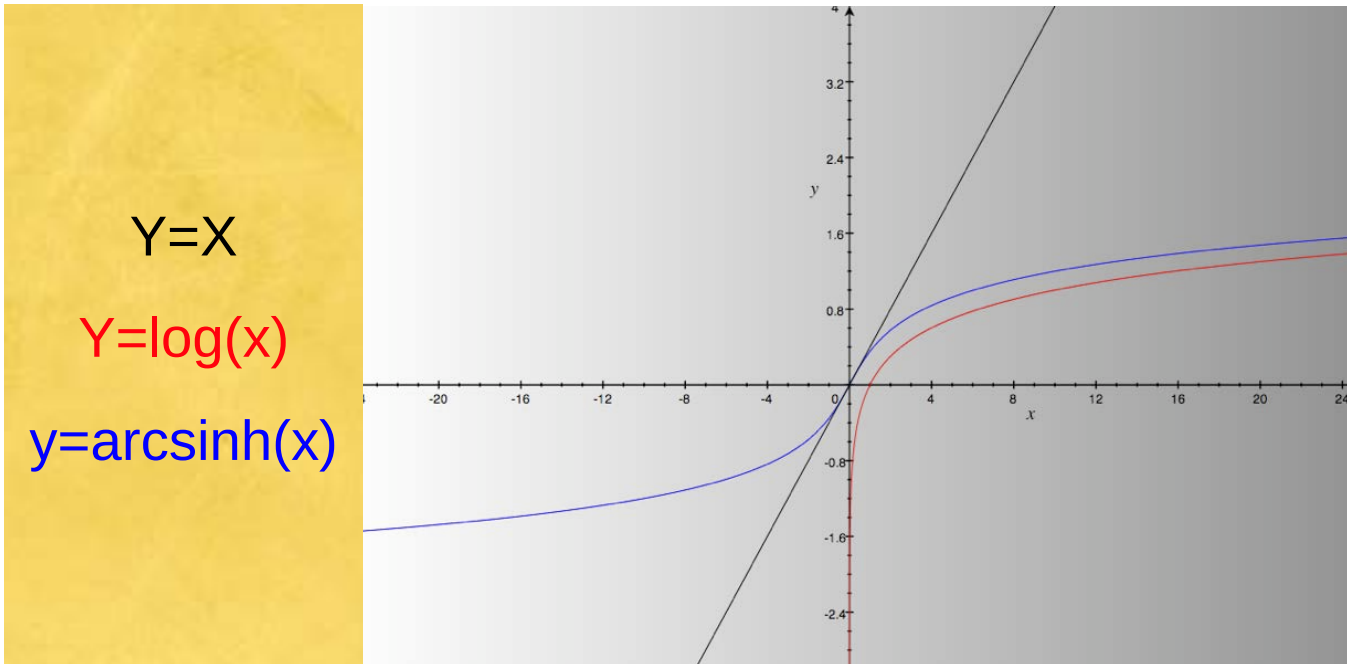
Undo!!



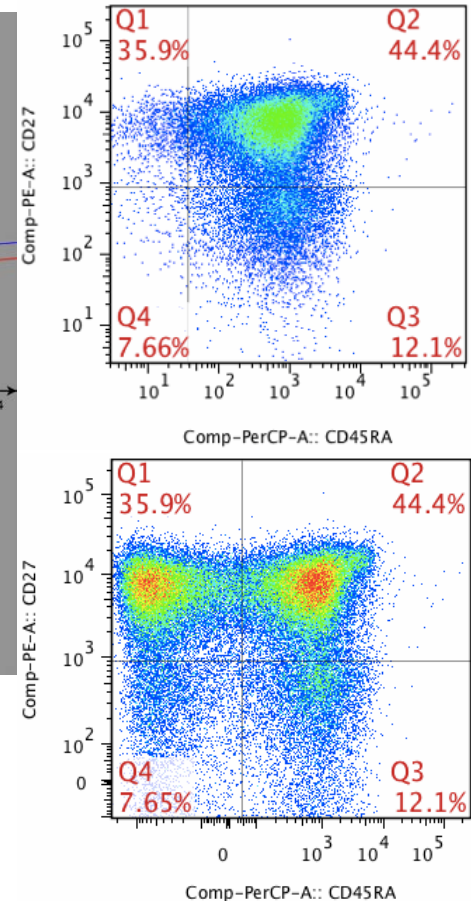
- Gates can always be modified or removed, so don't be shy.
- Explore the gating options and pick what works best for you.

Biexponential Transformation

- to Better Visualize Data After Compensation

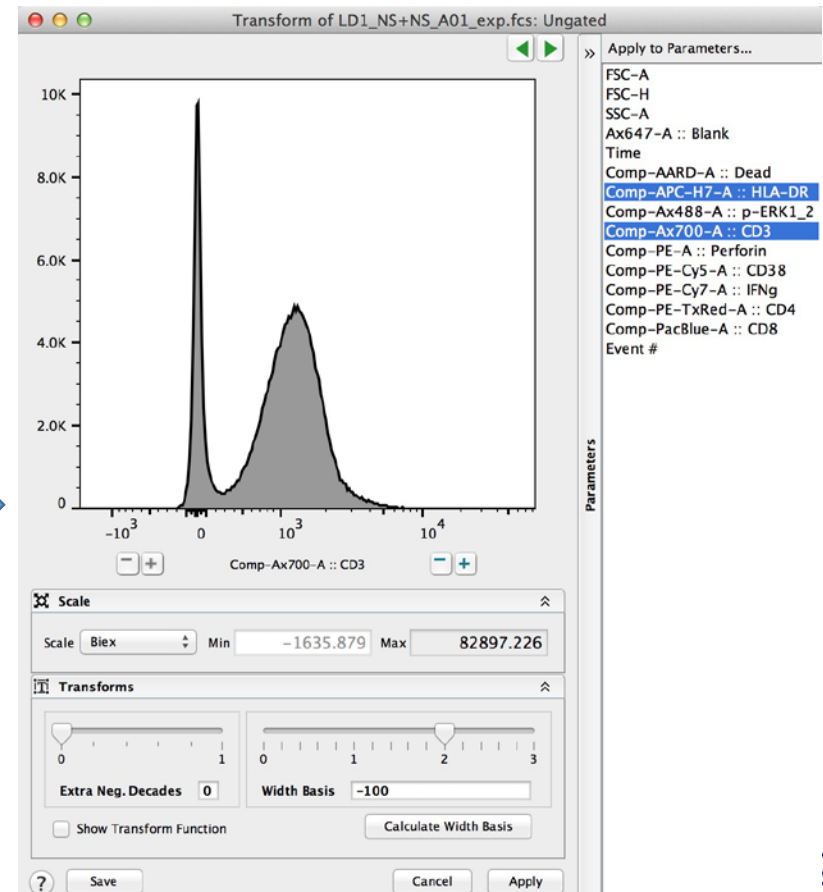
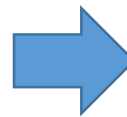
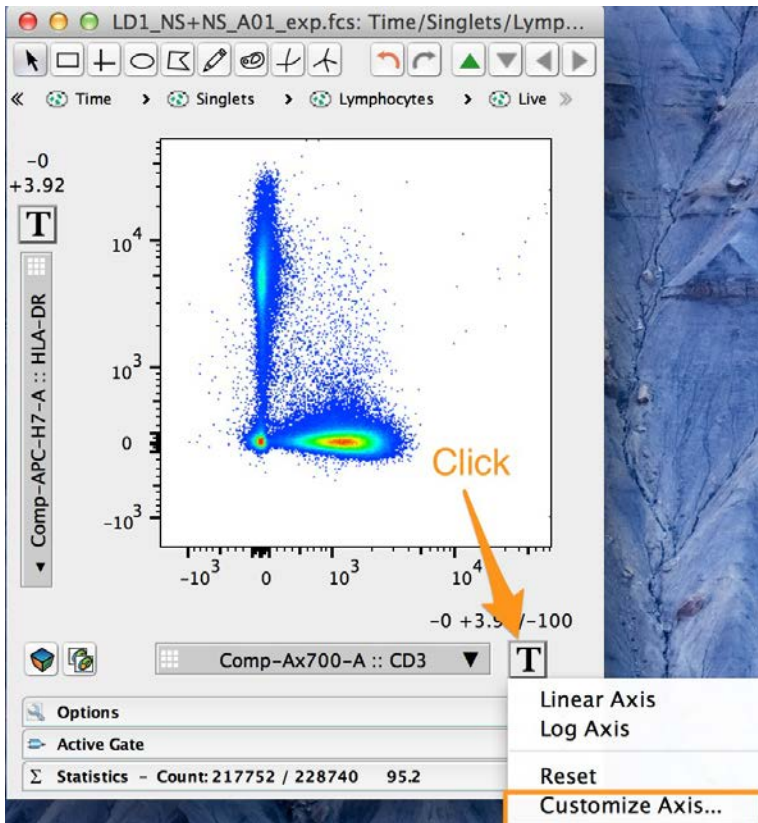


- Transformation is a bi-exponential display tool by introducing linear scales into your axes to better visualize your data.
- It does not change your data!



Transforming Data

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



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

*Gating basic

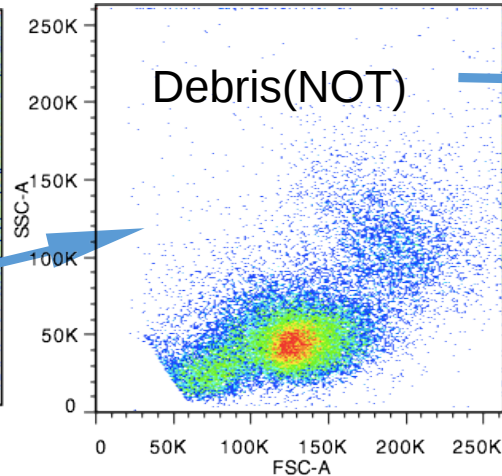
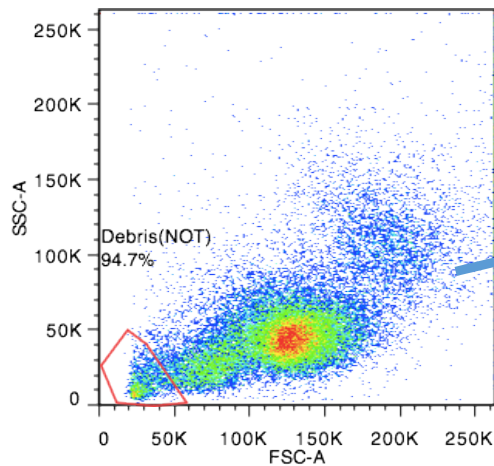
- Gate on population of interest and gate out population not of interest
- Gate out debris, dead cells, doublets/cell aggregates, Focus on POI
- Is both objective and subjective

*Gating tools

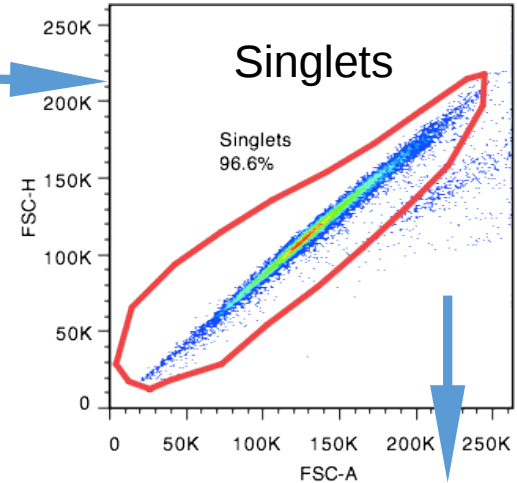
- Hierarchical gating

Gating Strategy

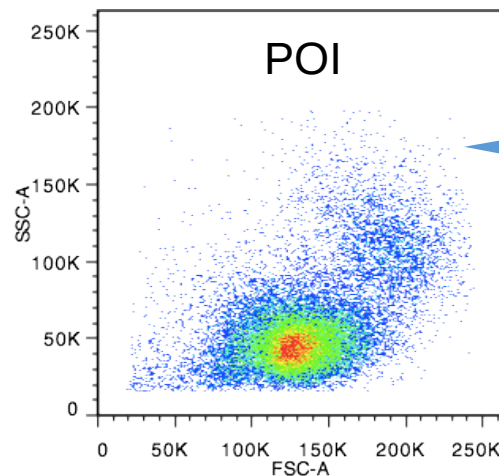
Gate out Debris



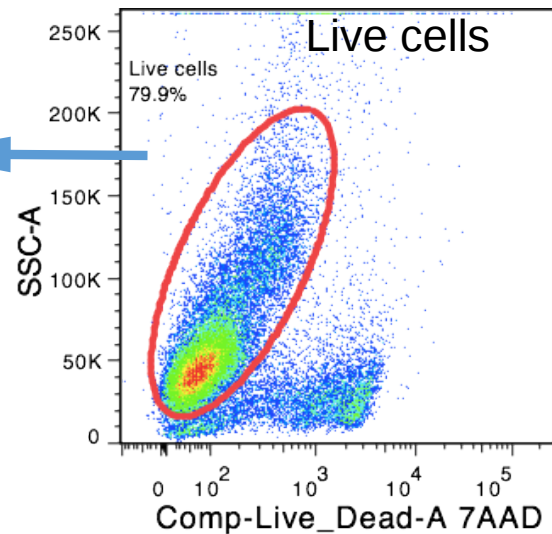
Gate out doublets



Plot on FSC&SSC



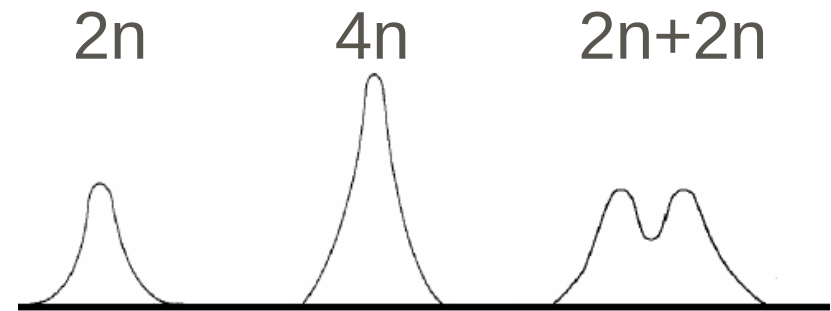
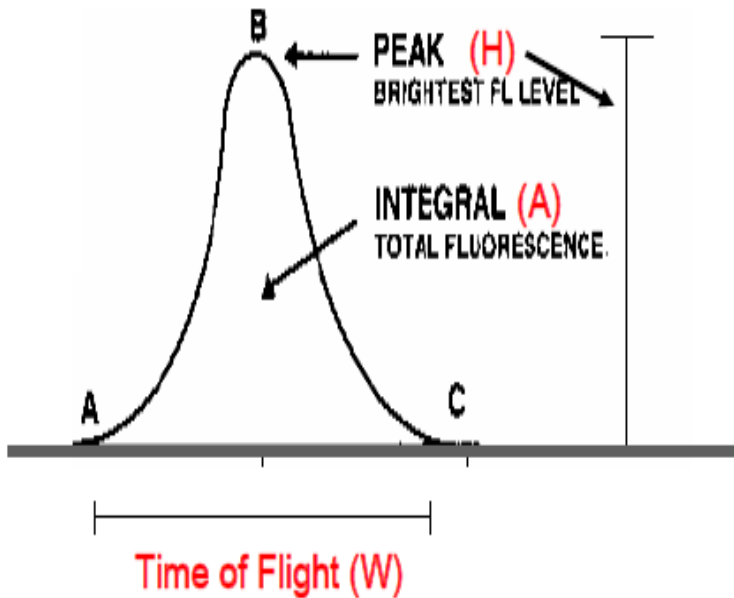
Gate out dead cells



Doublets Discrimination

What are doublets and why are they harmful?

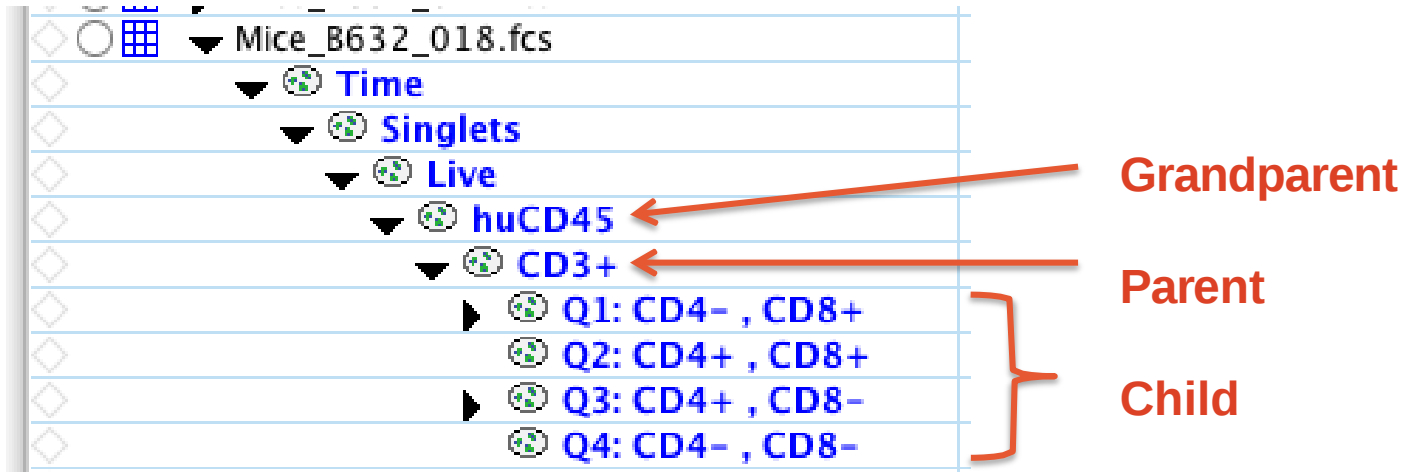
- Doublets are two cells stick together
- Doublets can lead to higher background and false positive and especially harmful for cell cycle analysis.



H	1	2	1
A	1	2	2
W	1	1	2

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.



The Table Editor

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



Table Editor

The screenshot displays the FlowJo software interface, specifically the 'Table Editor' and 'Visualize' tabs. The 'Table Editor' tab shows a list of samples with columns for Group, Size, and Role. The 'Visualize' tab shows a table with columns for Col..., Population, Statistic, Parameter, and Name. Red arrows point from the 'Table Editor' to the 'Visualize' tab, and blue arrows point from the 'Table Editor' to the 'Table Editor' tab.

Table Editor (Left Panel):

Group	Size	Role
{ } All Samples	29	Test
{ } 20140711 Compensation	10	Compensation
{ } 20140711 Rag1_BLT Act Baseline	19	Test

Table Editor (Right Panel):

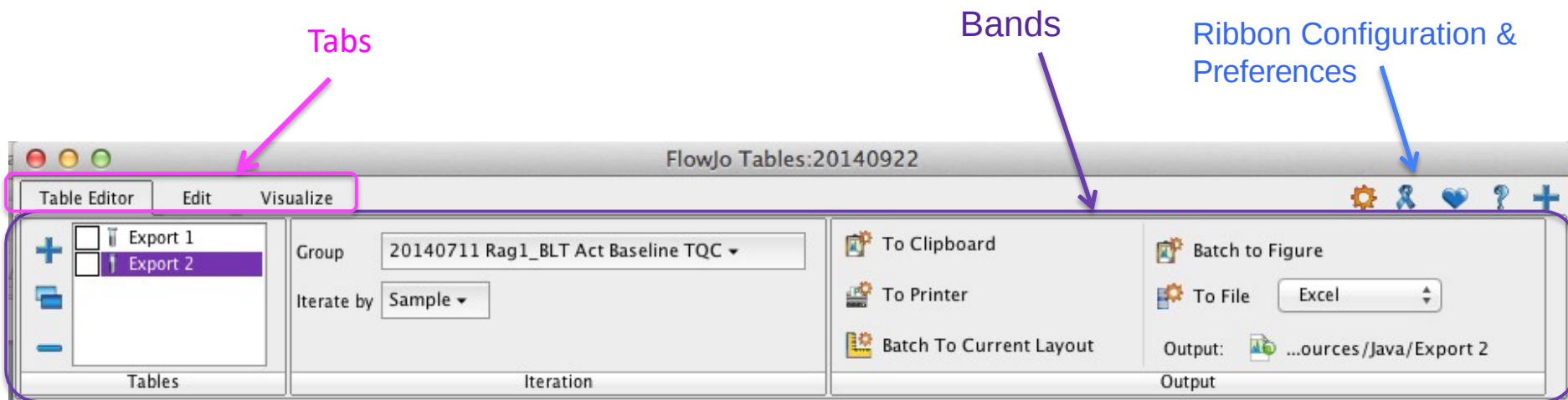
Col...	Population	Statistic	Parameter	Name
1	Σ Time/Singlets/Live/huCD45	Freq. of Parent		
2	Σ Time/Singlets/Live/huCD45/CD3+	Freq. of Parent		
3	Σ Time/Singlets/Live/huCD45/CD3+/Q1: CD4- , CD8+	Freq. of Parent		
4	Σ Time/Singlets/Live/huCD45/CD3+/Q3: CD4+ , CD8-	Freq. of Parent		
5	*Sample ID			
6	*Timepoint			

Table Editor (Bottom Panel):

Name	Statistic
▼ Mice_B632_018.fcs	
▼ Time	99.1
▼ Singlets	97.4
▼ Live	83.7
▼ huCD45	25.6
▼ CD3+	81.7
▶ Q1: CD4- , CD8+	34.4
▶ Q2: CD4+ , CD8+	2.16
▶ Q3: CD4+ , CD8-	62.3
▶ Q4: CD4- , CD8-	1.09

With in Table Editor

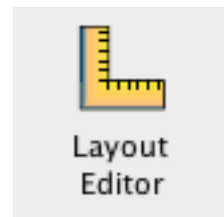
- Again, the Table Editor has its own customizable Ribbon with Tabs and Bands to organize actions.



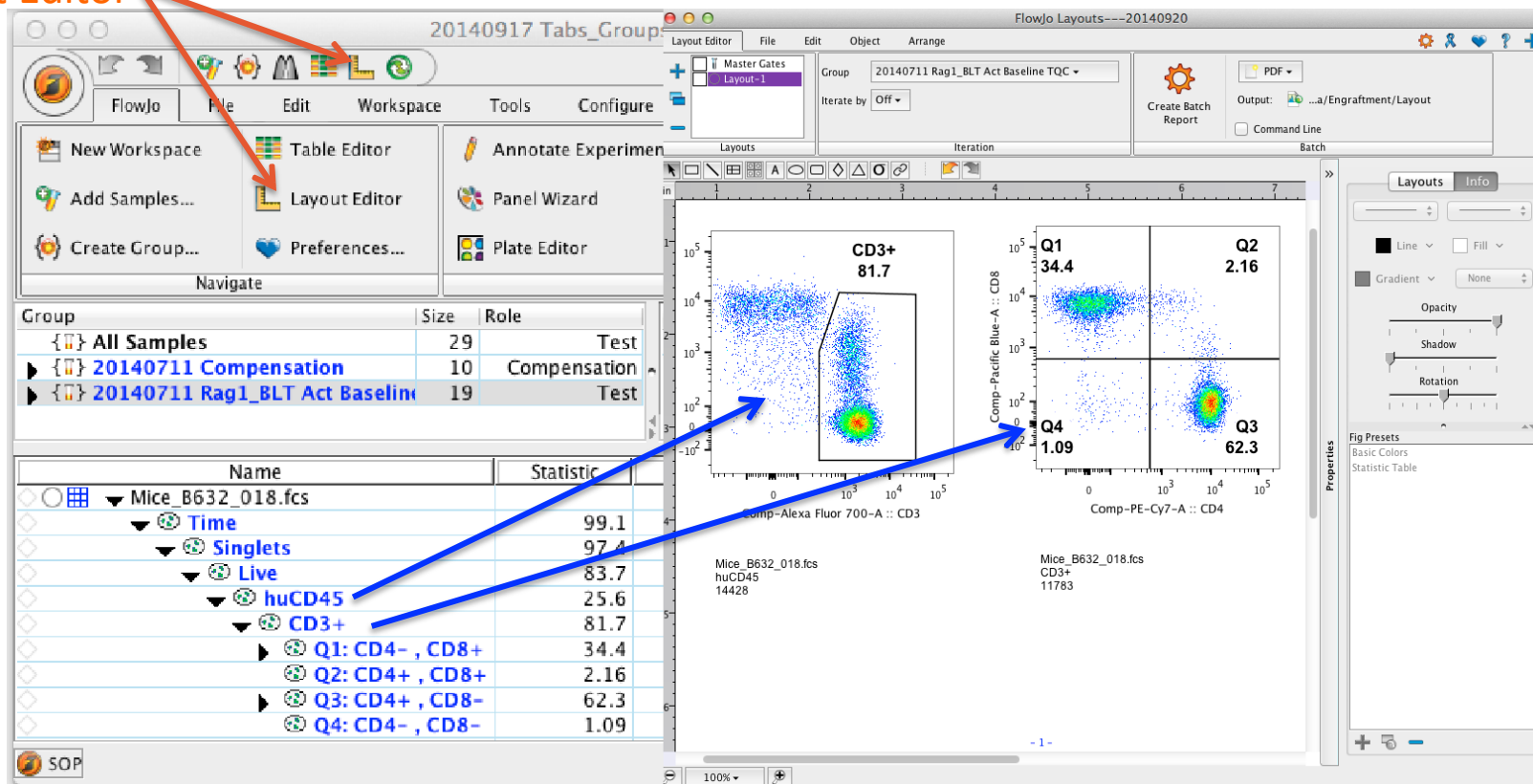
- Specify the group you wish to batch, and how to iterate the batch process, then in the Output band, specify where you want the batch output to go.

The Layout Editor

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

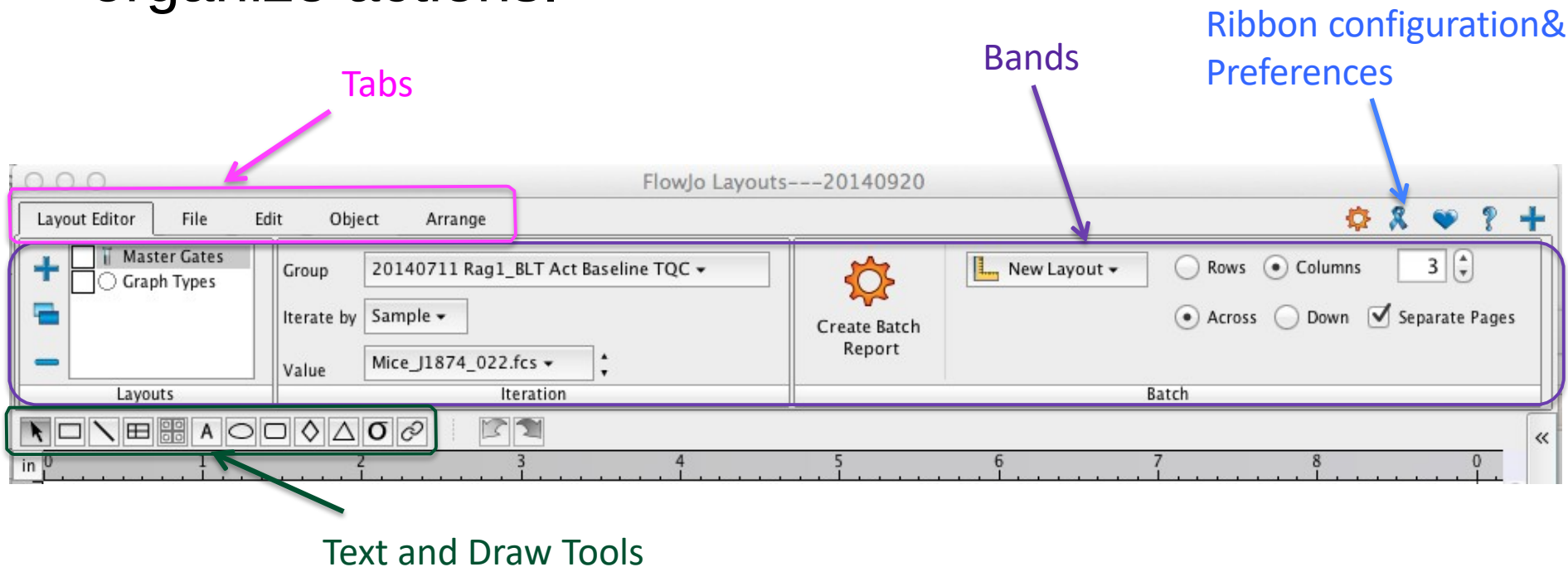


Layout Editor



Working in Layout Editor

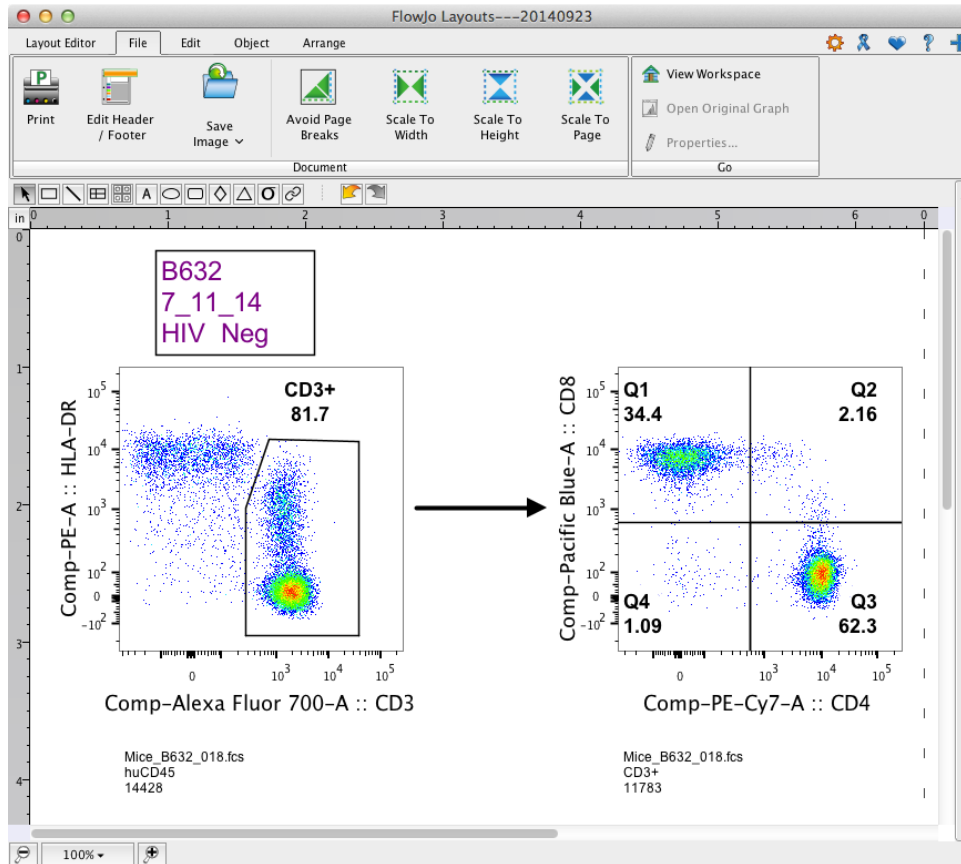
- Similar to the Workspace, the Layout Editor has its own customizable Ribbon with Tabs and Bands to organize actions.



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

Within Layout Editor

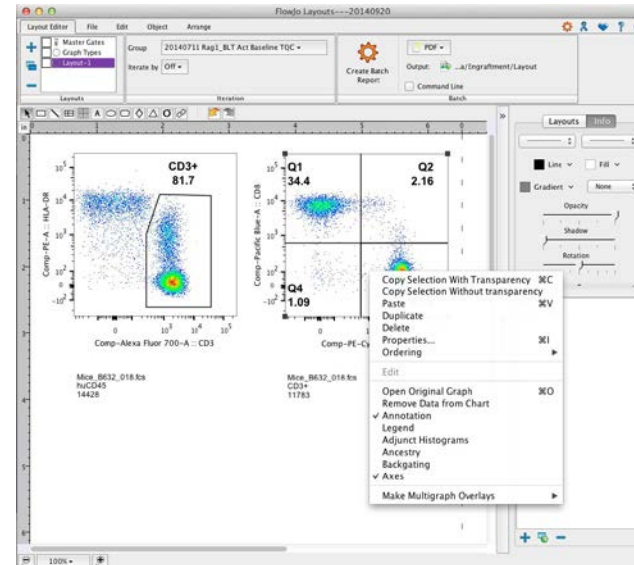
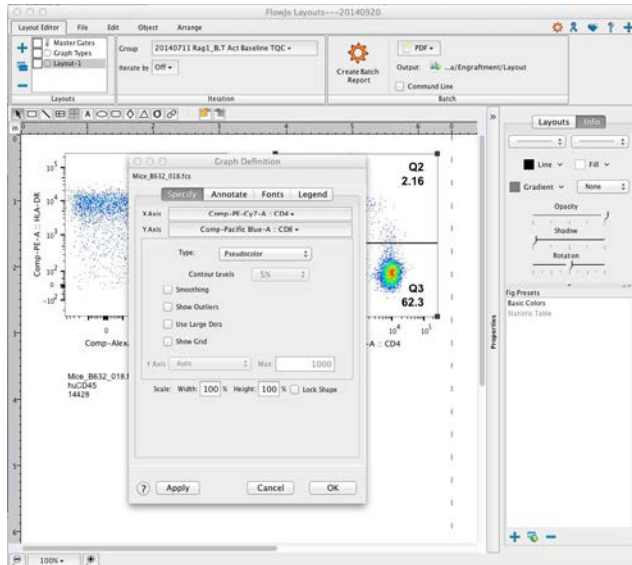
- Graphs can be organized and re-formatted.
- Statistics, keywords, text and even shapes or objects can be added to illustrate your analysis.



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

Working in Layout Editor

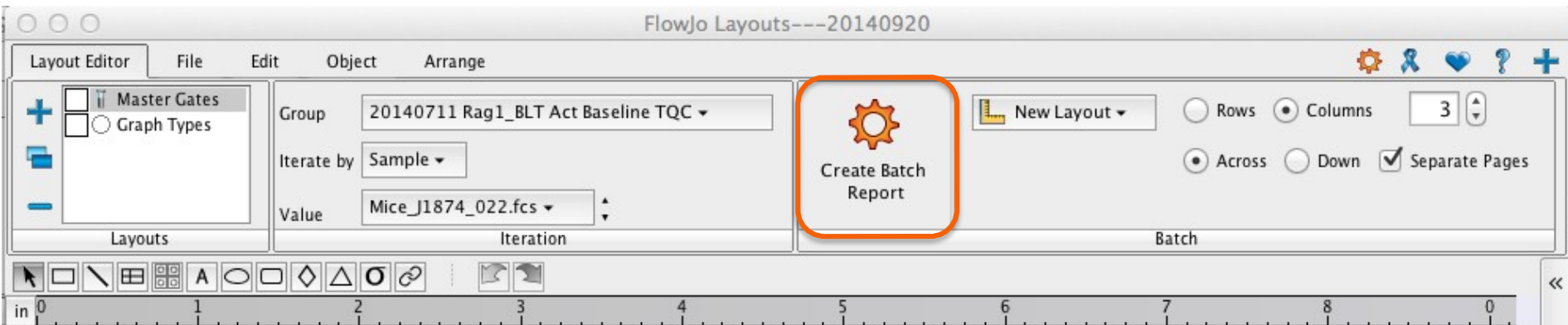
- Click once on a graph or object to select it.
- Double Click a graph to change its properties.



- 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.



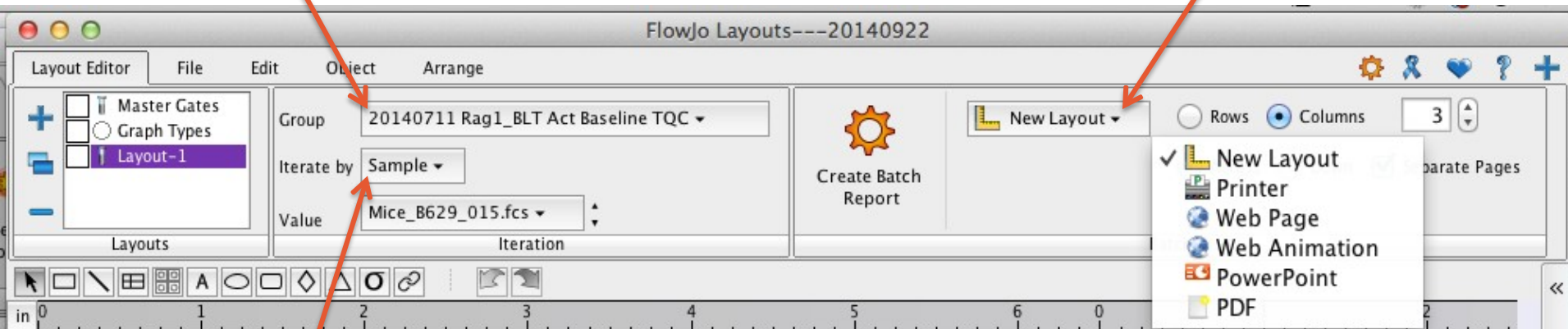
Batch Analysis of Layout Editor Graphics

- 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



Batch Output

Group



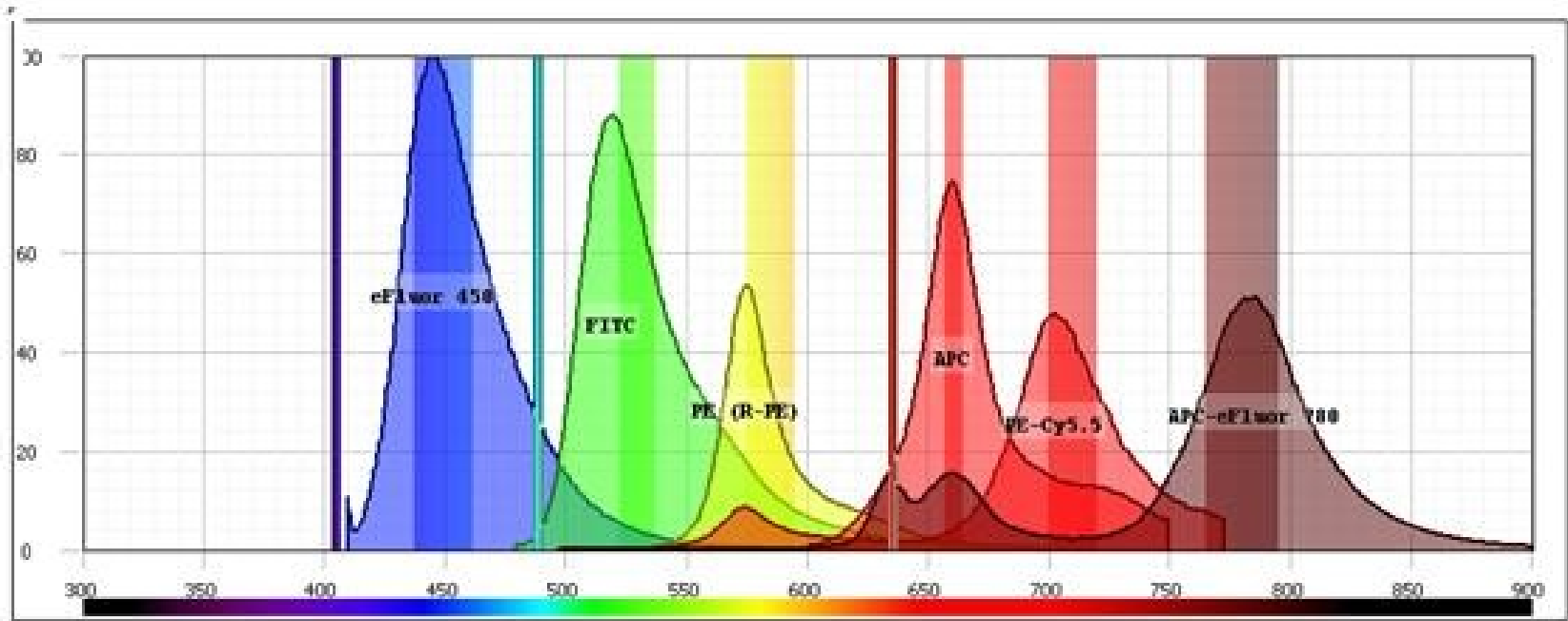
Iteration Criteria

Outline – Part II

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

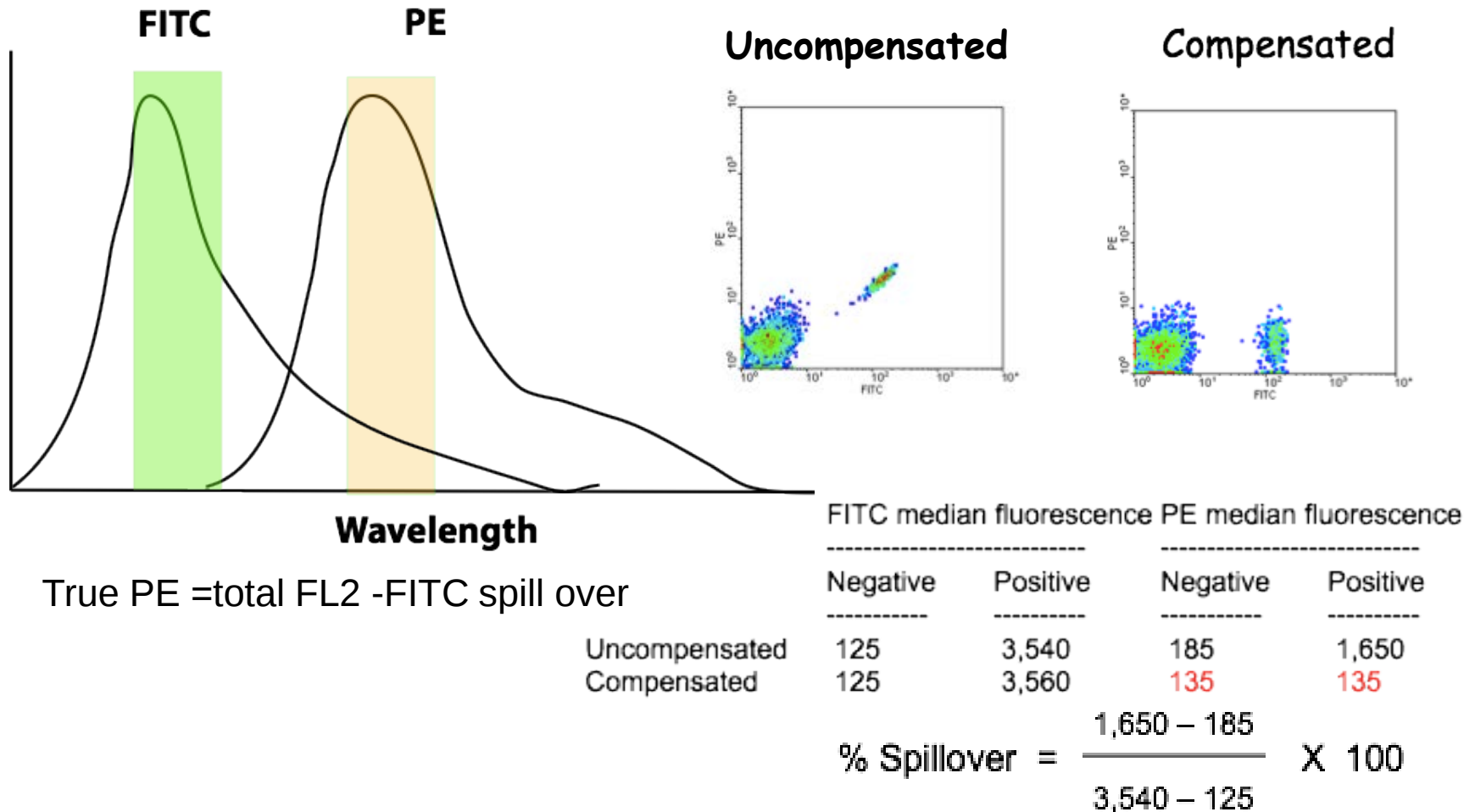
Compensation

- Compensation corrects for spillover between fluorochrome emission spectra.



- Compensation is essential for multicolor panels

Compensation Controls-To Correct Spectra Spillover



Slide credit to Dr. Holden Maecker Stanford University

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.

Compensation I

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



Click

20150317 - FlowJo X

FlowJo File Edit Workspace Tools Configure

New Workspace Table Editor
Add Samples... Layout Editor
Create Group... Preferences...

Navigate

Group	Size	Role
All Samples	13	Test
Compensation	13	Compensation
Run4Comps	13	Test

Highlight

Name	Statistic	#Cells
Bead Comps_APC-Cy7_F05.fcs		40950
Bead Comps_APC_F10.fcs		41930
Bead Comps_A700_F09.fcs		47040
Bead Comps_FITC_F11.fcs		49420
Bead Comps_PE-Cy7_F02.fcs		50000
Bead Comps_PE-TR_F07.fcs		36085
Bead Comps_PE_F08.fcs		50000
Bead Comps_PacBlue_F03.fcs		50000
Bead Comps_PerCP-Cy55_F04.fcs		50000
Bead Comps_Qdot 605_F06.fcs		50000
Bead Comps_Unstained Beads_F01.fcs		50000
Cell Comps_AARD Comp1_C12.fcs		283815
Cell Comps_Unstained Cells 2_B12.fcs		284935

- The wizard auto gates samples

20150317 - FlowJo X

FlowJo File Edit Workspace Tools Configure

New Workspace Table Editor
Add Samples... Layout Editor
Create Group... Preferences...

Navigate

Group	Size	Role
All Samples	13	Test
Compensation	13	Compensation
Run4Comps	13	Test

Group Analysis

Name	Statistic	#Cells
Bead Comps_APC-Cy7_F05.fcs		40950
Size	74.7	30579
APC-Cy7-A+	35.6	10871
Bead Comps_APC_F10.fcs		41930
Size	74.1	31057
APC-A+	34.0	10549
Bead Comps_A700_F09.fcs		47040
Size	74.9	35227
Ax700-A+	32.8	11548
Bead Comps_FITC_F11.fcs		49420
Size	73.7	36437
FITC-A+	31.3	11407
Bead Comps_PE-Cy7_F02.fcs		50000
Size	64.7	32365
PE-Cy7-A+	31.9	10328
Bead Comps_PE-TR_F07.fcs		36085
Size	66.5	24006
PE-TxRed-A+	34.9	8389
Bead Comps_PE_F08.fcs		50000
Size	45.4	22681
PE-A+	36.2	8221
Bead Comps_PacBlue_F03.fcs		50000
Size	68.8	34393
PacBlue-A+	35.1	12056
Bead Comps_PerCP-Cy55_F04.fcs		50000
Size	76.7	38349
PerCP-Cy5-5-A+	33.3	12754
Bead Comps_Qdot 605_F06.fcs		50000
Size	70.5	35265
Qdot 605-A+	35.5	12510
Bead Comps_Unstained Beads_F01.fcs		50000
Size	88.2	44080
Cell Comps_AARD Comp1_C12.fcs		283815
Size	63.9	181464
AARD-A+	66.2	120140
Cell Comps_Unstained Cells 2_B12.fcs		284935

Compensation II

- Then fills in the positive and negative.

For each parameter

- Choose options from the dropdown lists for each parameter.

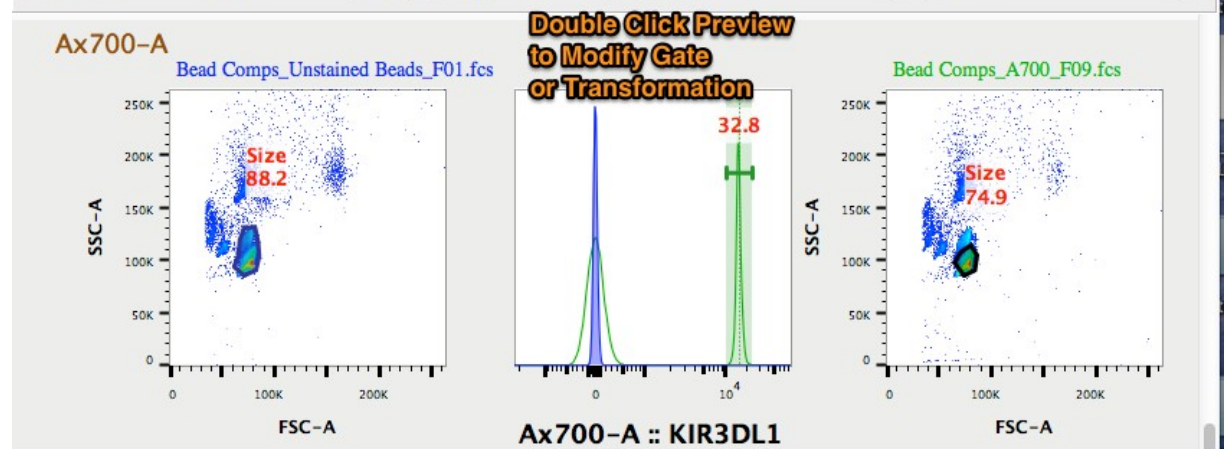
Control Group: Compensation

[M] Apply To Group Matrix Name: Compensation View Matrix... Finalized

Confirm gates and control assignments look correct. Double click a graph to edit it.

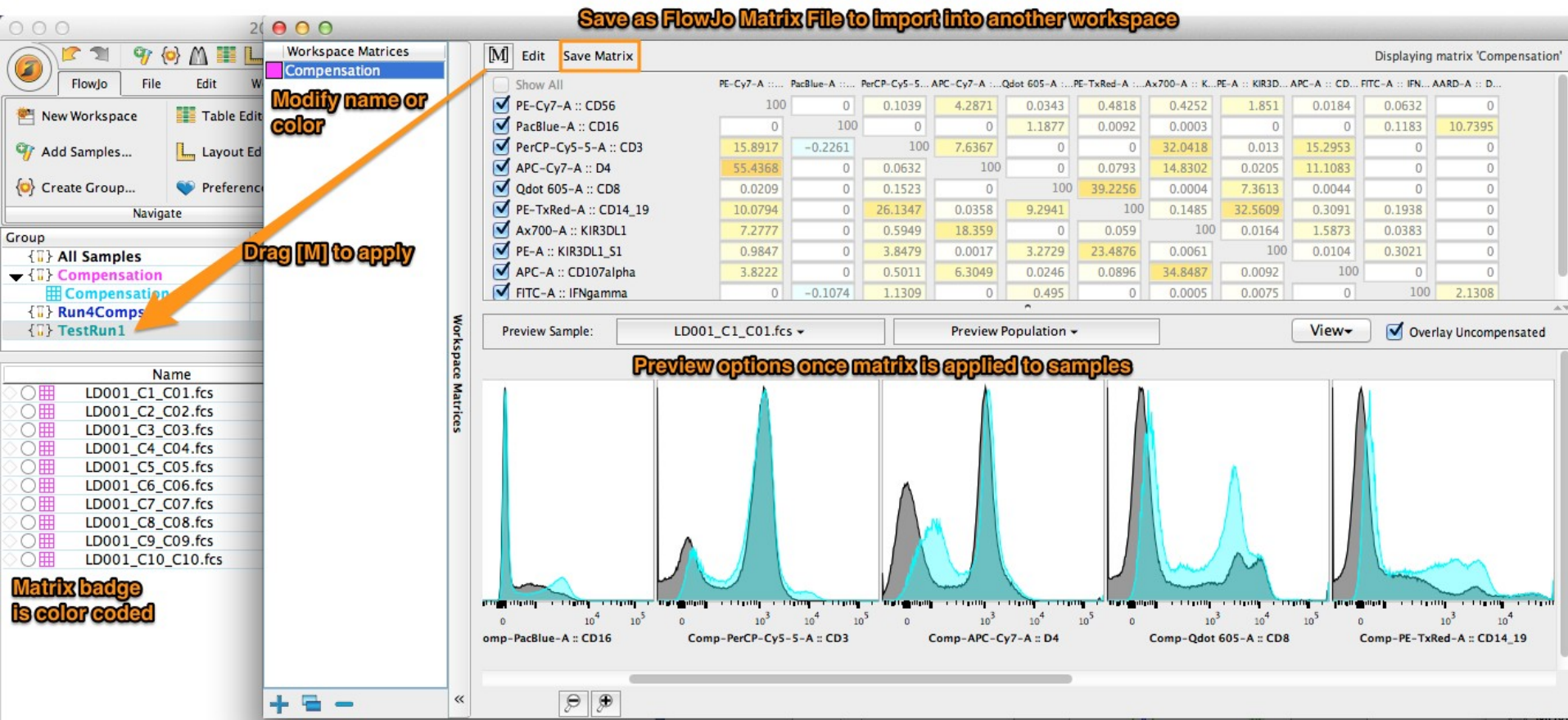
Parameter	Sample	Select Parameters or sample	Pick the Negative	Pick the Positive
PE-Cy7-A	CD56	Bead Comps_PE-Cy7_F02.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/PE-Cy7-A+
PacBlue-A	CD16	Bead Comps_PacBlue_F03.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/PacBlue-A+
PerCP-Cy5-5-A	CD3	Bead Comps_PerCP-Cy55_F04.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/PerCP-Cy5-5-A+
APC-Cy7-A	D4	Bead Comps_APC-Cy7_F05.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/APC-Cy7-A+
Qdot 605-A	CD8	Bead Comps_Qdot 605_F06.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/Qdot 605-A+
PE-TxRed-A	CD14_19	Bead Comps_PE-TR_F07.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/PE-TxRed-A+
Ax700-A	KIR3DL1	Bead Comps_A700_F09.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/Ax700-A+
PE-A	KIR3DL1_S1	Bead Comps_PE_F08.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/PE-A+
APC-A	CD107alpha	Bead Comps_APC_F10.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/APC-A+
FITC-A	IFNgamma	Bead Comps_FITC_F11.fcs	Bead Comps_Unstained Beads_F01.fcs:Size	Size/FITC-A+
AARD-A	Dead	Cell Comps_AARD Comp1_C12.fcs	Cell Comps_Unstained Cells 2_B12.fcs:Size	Size/AARD-A+

- Double click preview graphs to modify properties.



Compensation III

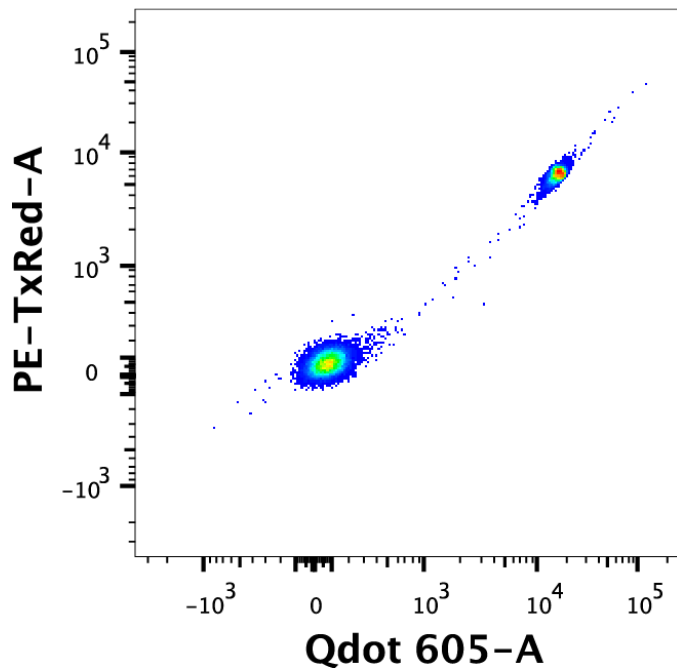
- View Matrix... to Modify, Apply, Save or Preview



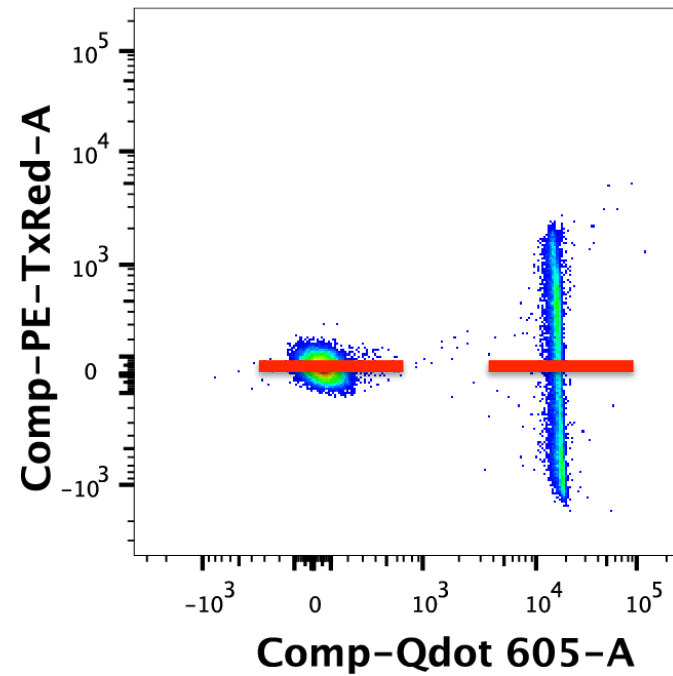
Effect of Compensation



Uncompensated

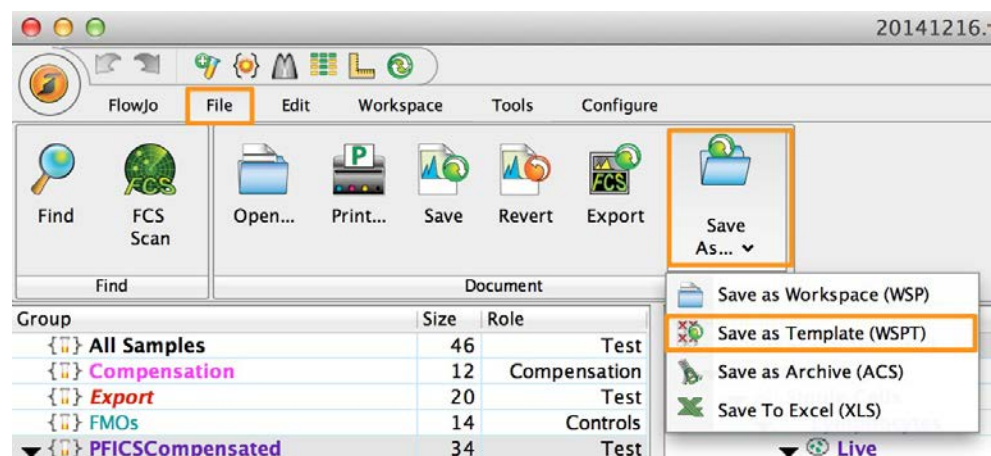
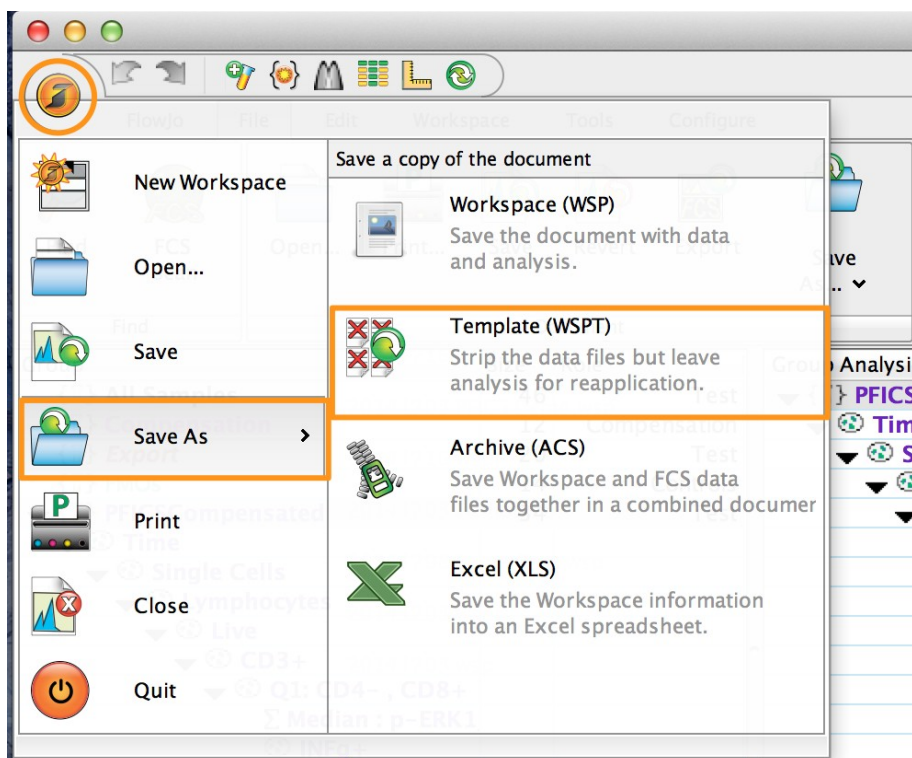


Compensated



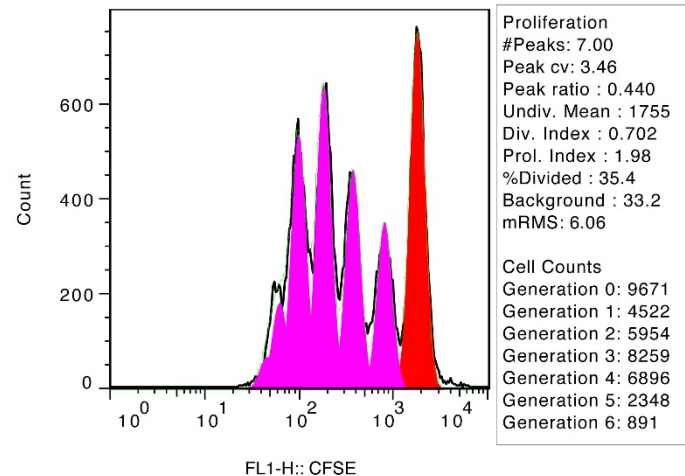
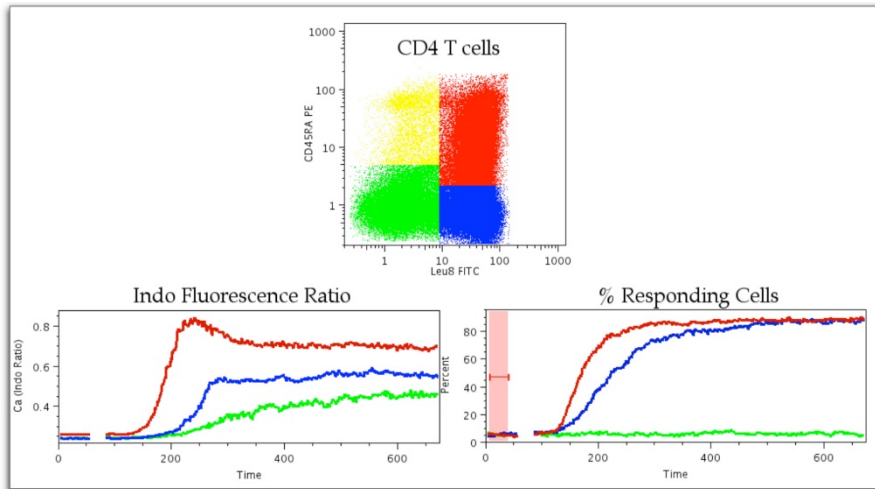
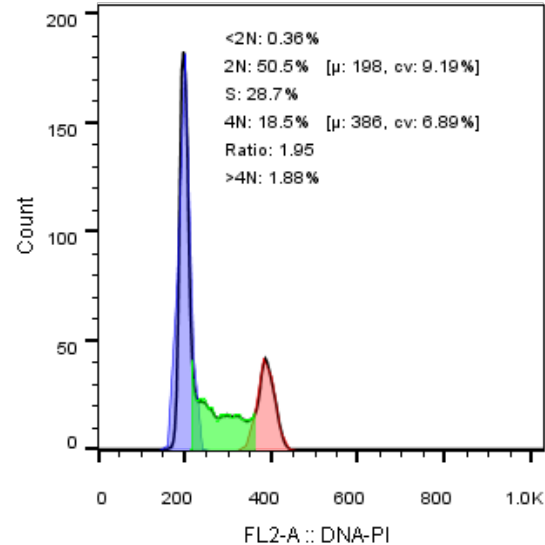
Workspace Templates

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



Special Analysis Platforms

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

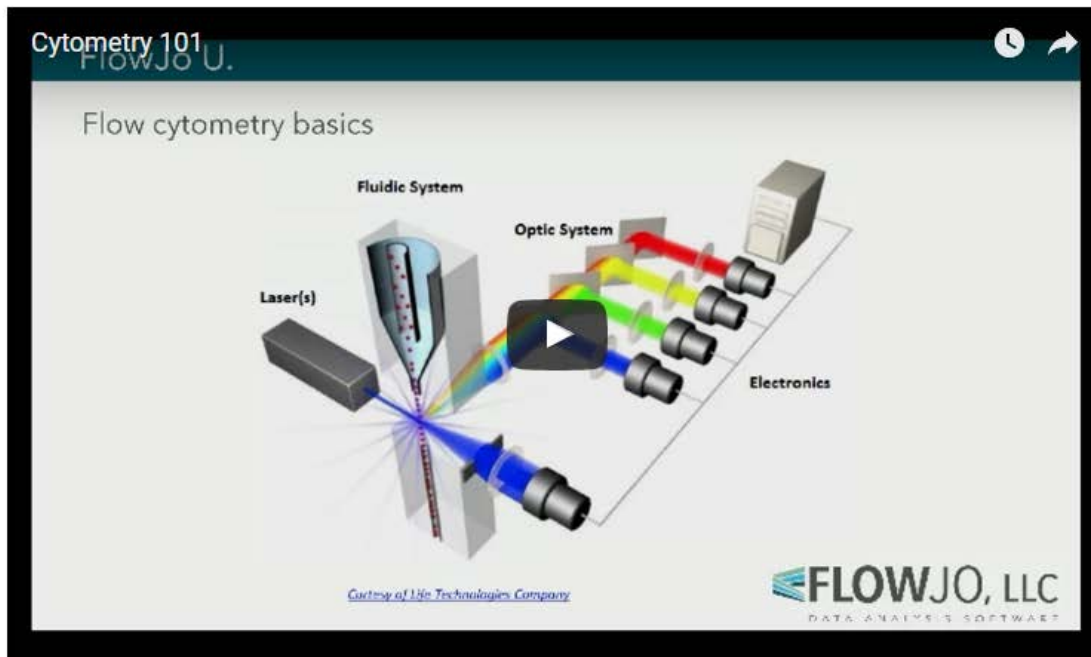




- FlowJo University
- Webinars

 **FLOWJO, LLC**

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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)

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/>



www.flowjo.com

- 30 day free trial
- Video Tutorial
- Webinars
- FlowJo University
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技術支援信箱:
techsupport@gtbiotech.com.tw
02-27965108