

Cytometry Data Analysis in FlowJo V10



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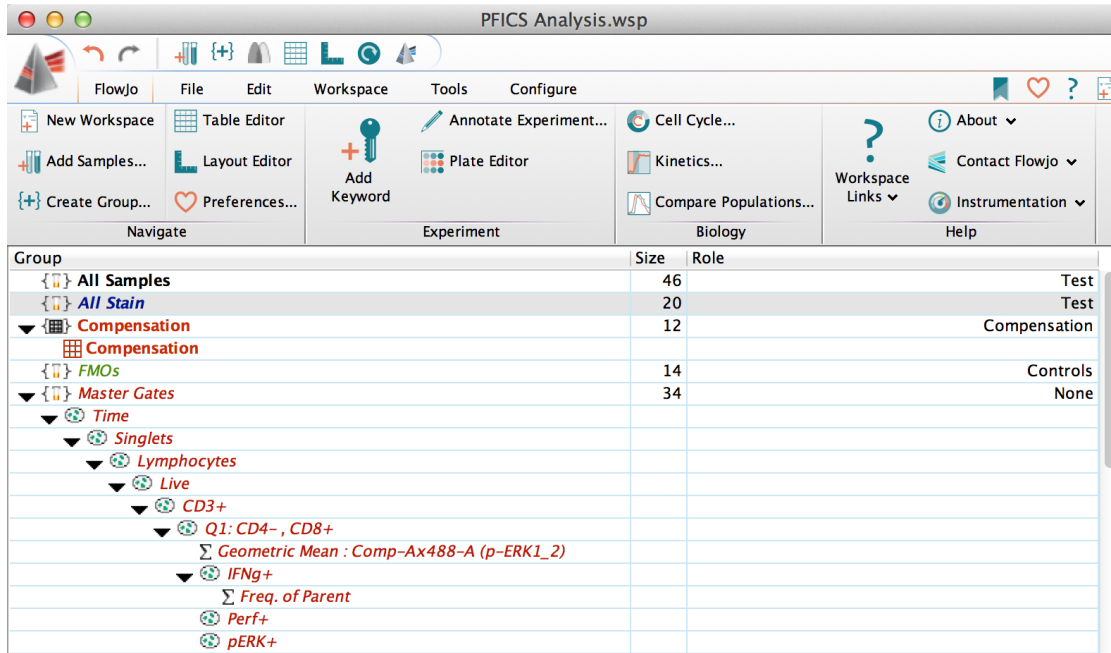
Outline – Part I

Intro to 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

The FlowJo v10 Workspace

- A graphical interface to organize your data.



Ribbon
Tabs and Bands

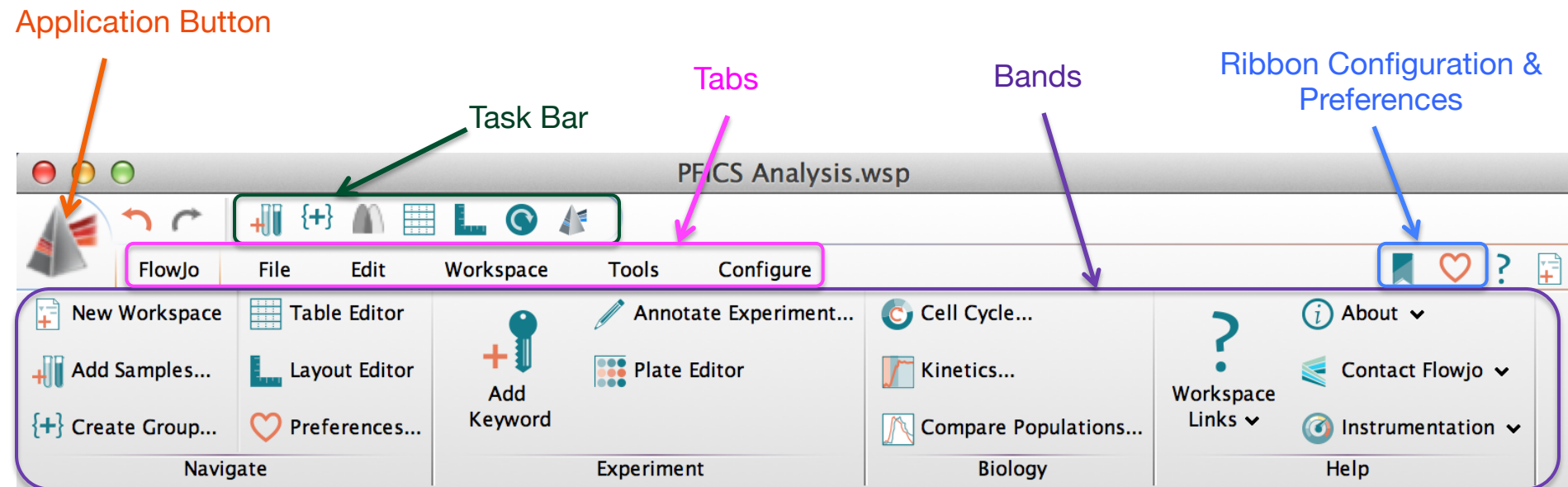
Groups and Group
Analysis

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			
Geometric Mean : Comp-Ax488-A (p-ERK1_2)	424				
IFNg+	64.1	23716			
Freq. of Parent	64.1				
Perf+	52.9	19580			
pERK+	93.2	34514			

Samples and
sample analysis

Ribbons, Tabs and Bands

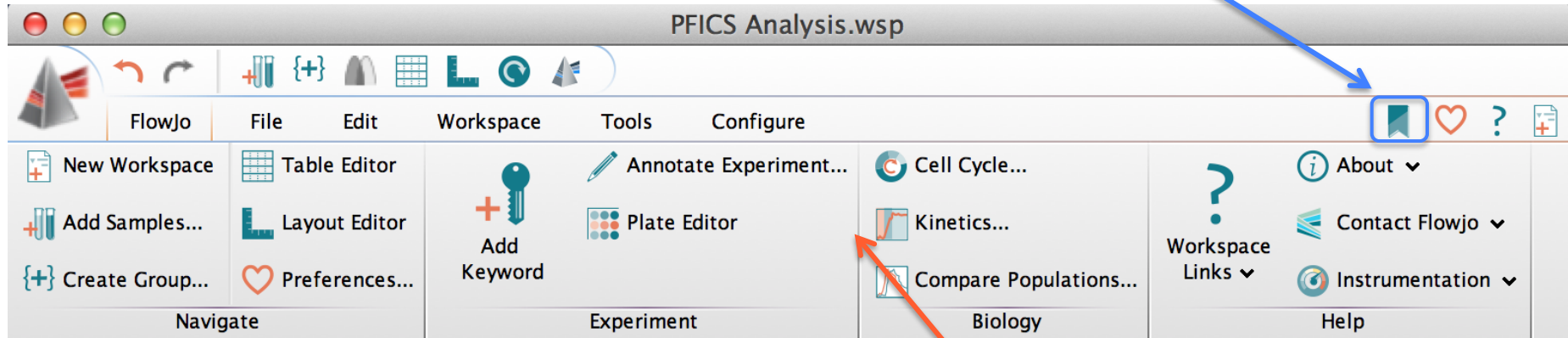
- Ribbon organization allows easy visual navigation of workspace functions.



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

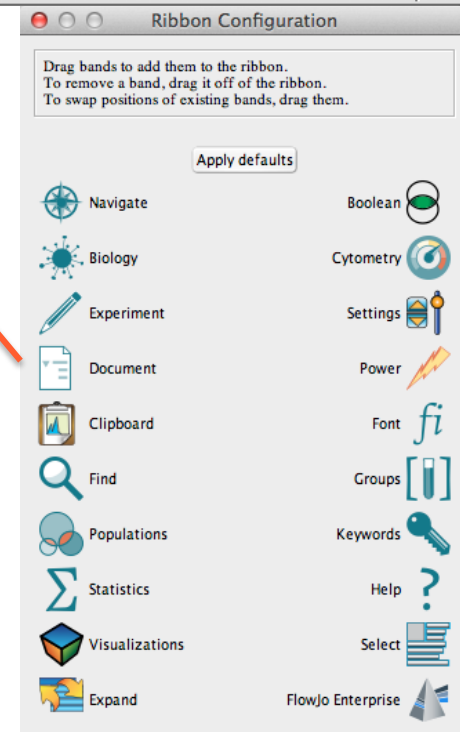
Customizing Ribbons

- Click on the Ribbon icon to configure



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

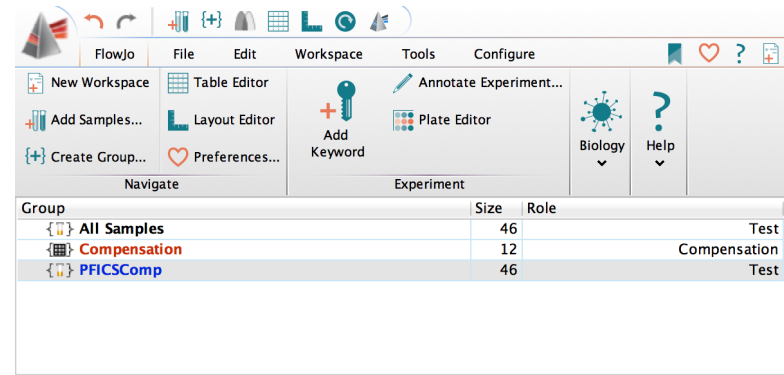
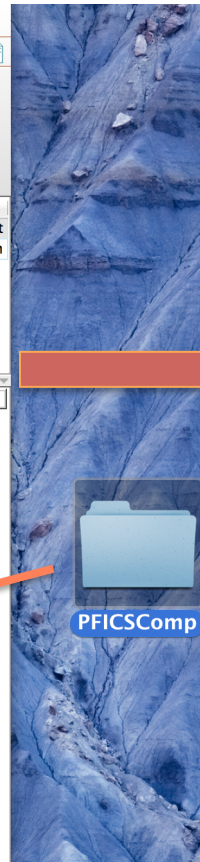
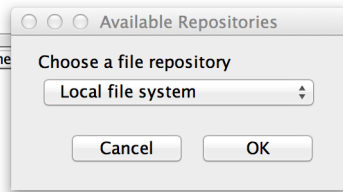
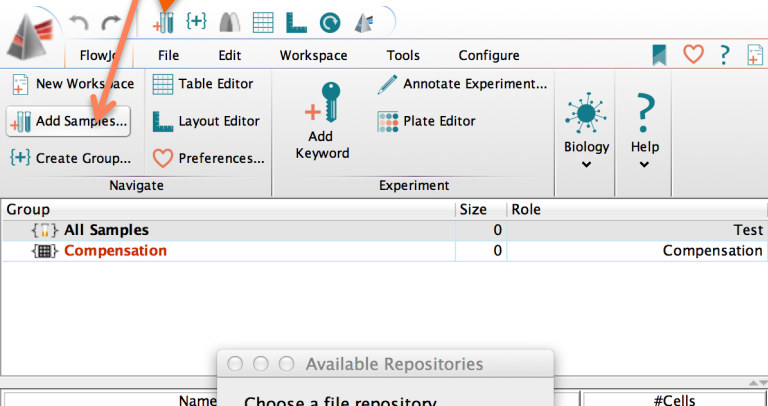
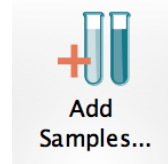
2.



Importing Data

Three possible methods:

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



Group			Size	Role
{ } All Samples			46	Test
{ } Compensation			12	Compensation
{ } PFICSComp			46	Test

Name			Statistic	#Cells
<input type="checkbox"/>	Bead Comps_4 PE-TR_F01_exp.fcs			19202
<input type="checkbox"/>	Bead Comps_8 PB_F02_exp.fcs			14969
<input type="checkbox"/>	Bead Comps_38 PE-Cy5_F03_exp.fcs			17603
<input type="checkbox"/>	Bead Comps_DR APC-H7_F04_exp.fcs			18907
<input type="checkbox"/>	Bead Comps_US Beads +FP_F05_exp.fcs			30000
<input type="checkbox"/>	Bead Comps_ERK A488_F06_exp.fcs			24114
<input type="checkbox"/>	Bead Comps_IFN PE-Cy7_F07_exp.fcs			30000
<input type="checkbox"/>	Bead Comps_Perforin PE_F08_exp.fcs			19212
<input type="checkbox"/>	Bead Comps_US Beads No FP_F09_exp.fcs			10290
<input type="checkbox"/>	Cell Comps_AARD_E01_exp.fcs			145743
<input type="checkbox"/>	Cell Comps_CD3 A700_E02_exp.fcs			129537
<input type="checkbox"/>	Cell Comps_US Cells_E03_exp.fcs			158360
<input type="checkbox"/>	LD1_NS+NS_A01_exp.fcs			250342
<input type="checkbox"/>	LD1_PI+NS_B01_exp.fcs			262774
<input type="checkbox"/>	LD1_NS+PI_C01_exp.fcs			229585
<input type="checkbox"/>	LD1_PI+PI_D01_exp.fcs			244977
<input type="checkbox"/>	LD2_NS+NS_A02_exp.fcs			330780
<input type="checkbox"/>	LD2_PI+NS_B02_exp.fcs			279202
<input type="checkbox"/>	LD2_NS+PI_C02_exp.fcs			286306
<input type="checkbox"/>	LD2_PI+PI_D02_exp.fcs			275465
<input type="checkbox"/>	LD4_NS+NS_A03_exp.fcs			222740
<input type="checkbox"/>	LD4_PI+NS_B03_exp.fcs			224146

Drag Samples Here

Today's 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 Stims → 4 potential combinations

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	+++	+++

- 5 donors X 4 stim conditions = 20 experimental ***All Stain*** samples
- 1 donor with Fluorescence Minus One (FMO) controls
7 x 2 stim conditions = 14 ***FMOs***
- 12 ***Compensation controls***


Group Pane

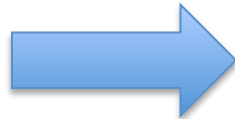
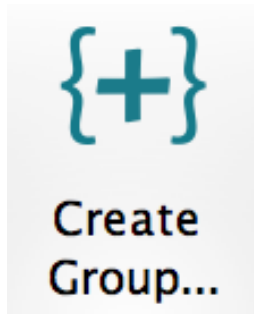
- The Group area lists all groups in the Workspace, # of samples in each group (Size), and the Role of that group (ex. Test, Compensation, Controls) .
- Groups act like folders to organize your samples, allows master gating and unique report generation.

Group	Size	Role
{ } All Samples	46	Test
{ } AllStain	20	Test
{ } Compensation	12	Compensation
{ } FMOs	14	Controls
▼ { } MasterGates	34	None
▼ { } Time		
▼ { } Singlets		
▼ { } Lymphocytes		
▼ { } Live		
▼ { } CD3+		
▼ { } Q1: CD4-, CD8+		
Σ Geometric Mean : Ax488-A (p-ERK1_2)		
Σ Geometric Mean : PE-A (Perforin)		
Σ Geometric Mean : PE-Cy7-A (IFNg)		
▼ { } IFNg+		
Σ Freq. of Parent		
{ } Perf+		
{ } pERK+		

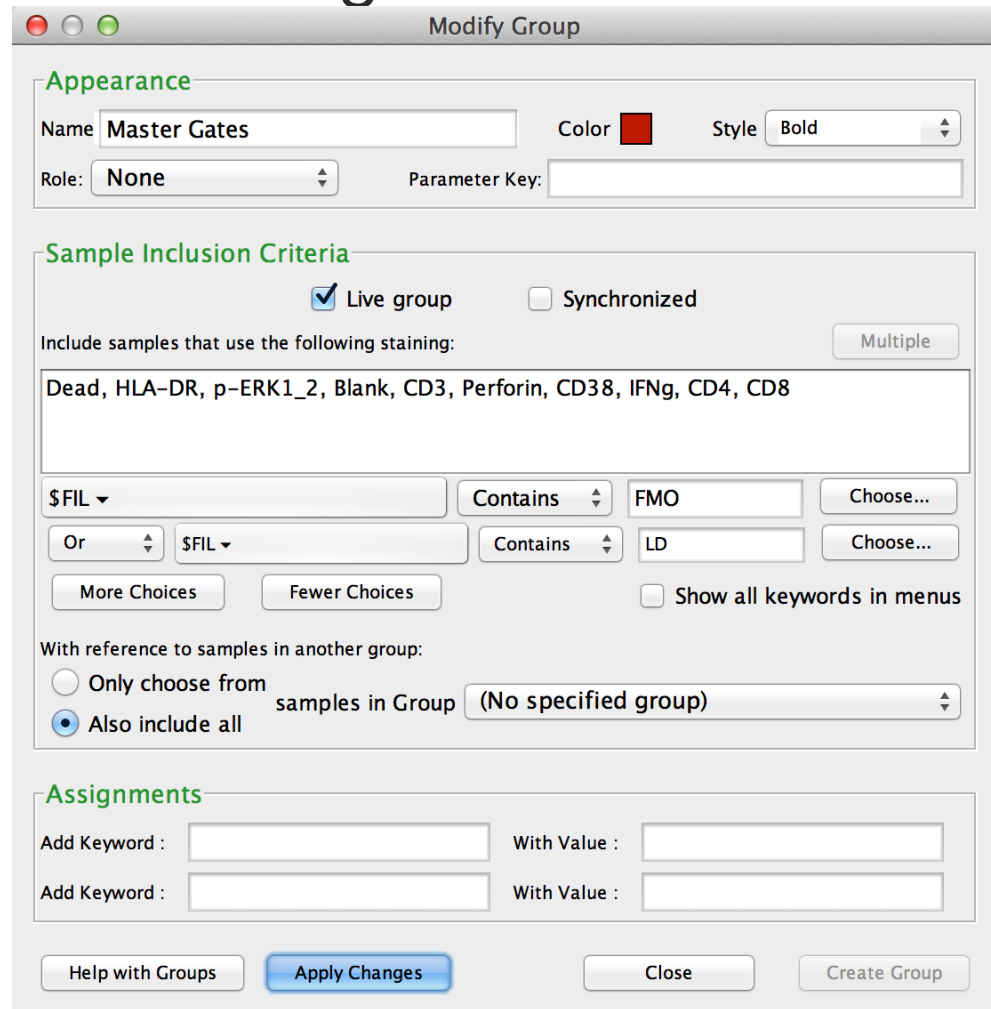
- Group owned analysis gains the group color.

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.



Modify Group

Appearance

Name: Color: Style:

Role: Parameter Key:

Sample Inclusion Criteria

☒ Live group ☐ Synchronized

Include samples that use the following staining:

\$FIL Contains

Or \$FIL Contains

☐ Show all keywords in menus

With reference to samples in another group:

☐ Only choose from ☐ Also include all samples in Group

Assignments

Add Keyword : With Value :

Add Keyword : With Value :

Sample Inclusion Criteria

- Live groups automatically include samples based on user-defined inclusion criteria.
- Criteria could include the staining panel, a keyword, characters in the file name, or any combination of these features.

The screenshot shows a 'Modify Group' dialog box with the following sections:

- Appearance**
 - Name:
 - Color:
 - Style:
 - Role:
 - Parameter Key:
- Sample Inclusion Criteria**
 - ☒ Live group ☐ Synchronized
 - Include samples that use the following staining:
 - Complex criteria builder:
 - Row 1: - Row 2: - Row 3:
 - ☐ Show all keywords in menus
 - With reference to samples in another group:
 - ☐ Only choose from
 - ☒ Also include all
 - samples in Group:
- Assignments**
 - Add Keyword : With Value :
 - Add Keyword : With Value :

Buttons at the bottom:

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)

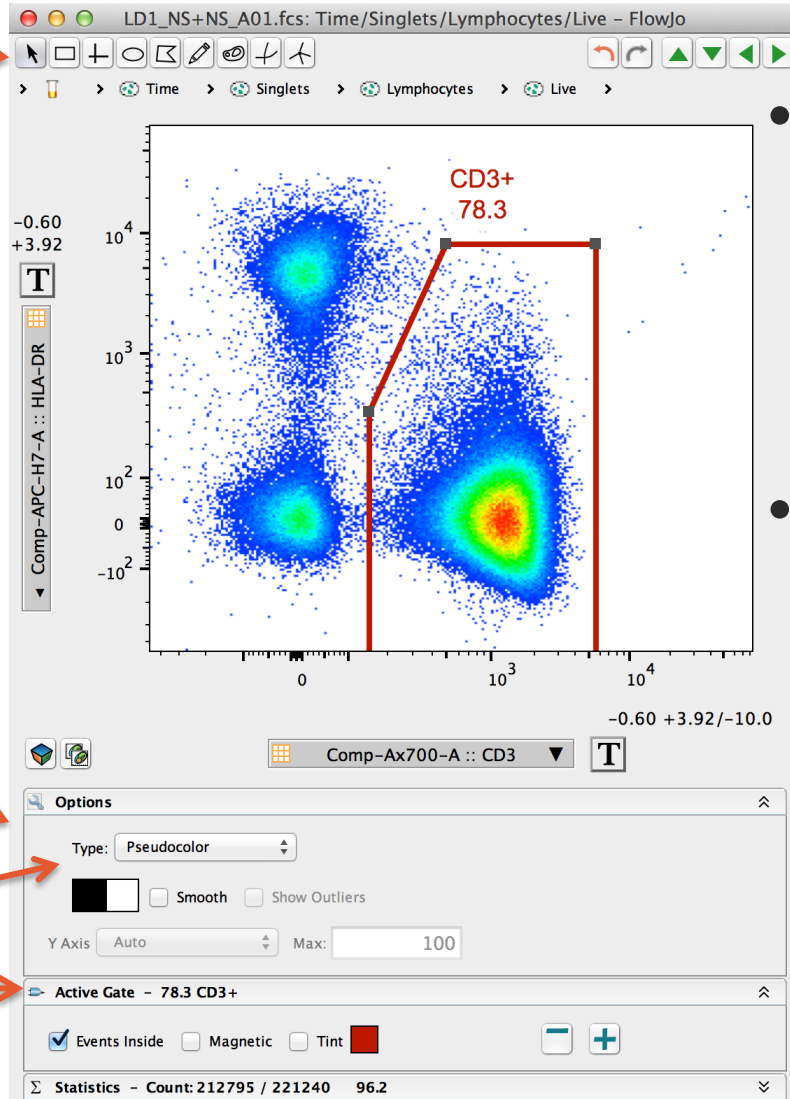
Name	Statistic	#Cells	*HIV Status	*PID	*STIM
LD1_NS+NS_A01.fcs		250342	Neg	LD1	NS+NS
LD1_NS+PI_C01.fcs		229585	Neg	LD1	NS+PI
LD1_PI+NS_B01.fcs		262774	Neg	LD1	PI+NS
Time	99.7	261964			
Singlets	96.2	252097			
Lymphocytes	93.7	236200			
Live	96.2	227167			
CD3+	81.4	184893			
Q1: CD4-, CD8+	24.0	44355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

Gating Tools



Plot View Options

Graph Type

Active Gate Options

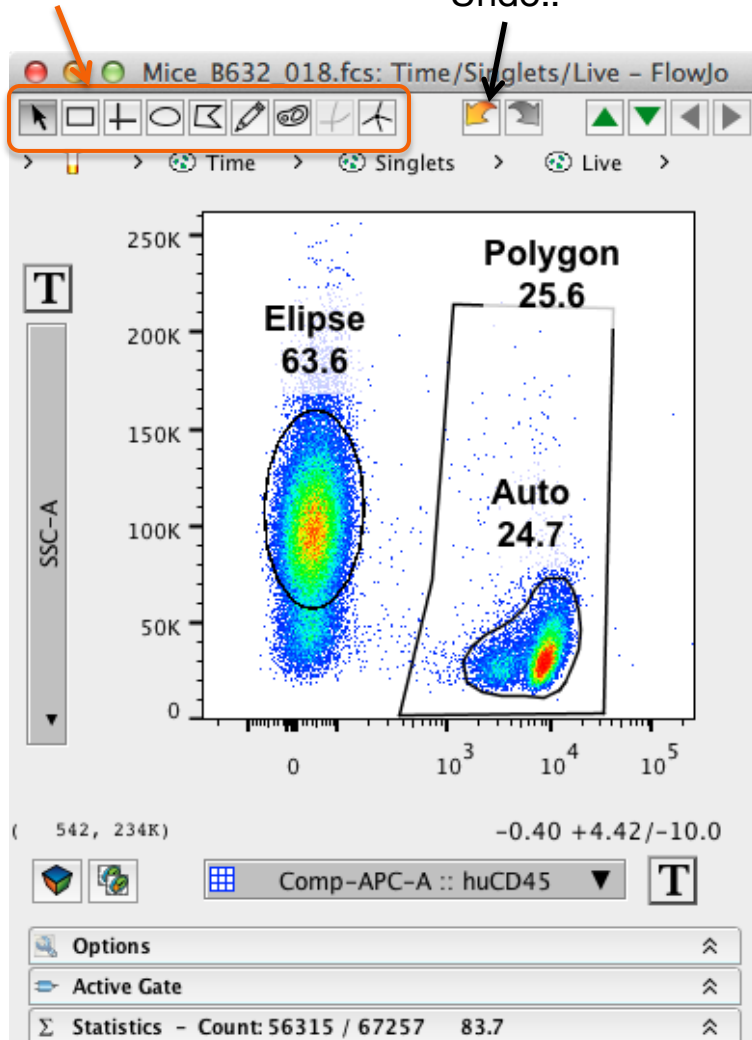
- Several different plot types are available to display flow data.
- Click on the Options Menu below the graph image and select Graph Type from the dropdown menu.

Gating tools

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

Gating Tools

Undo!!

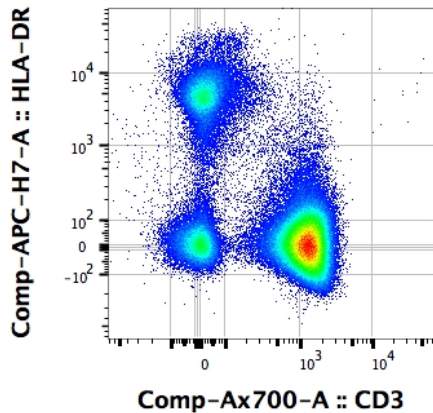


- Gates can always be modified or removed, so don't be shy.
- Explore the gating options and pick what works best for you.

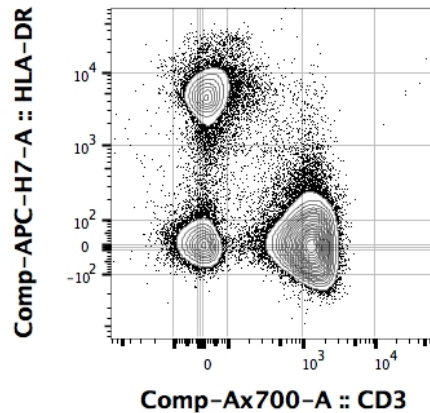
Graph Display Options

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

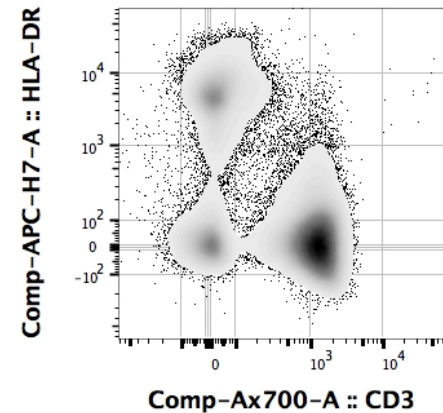
Pseudocolor



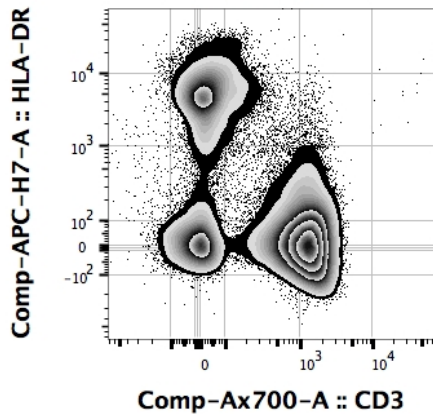
Contour



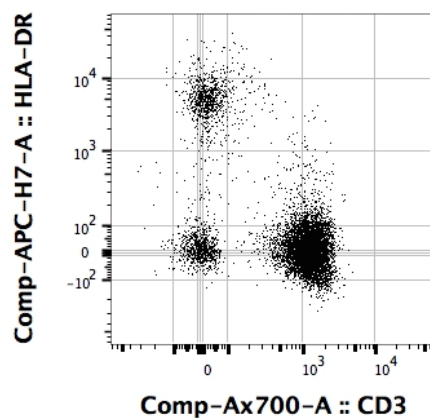
Density



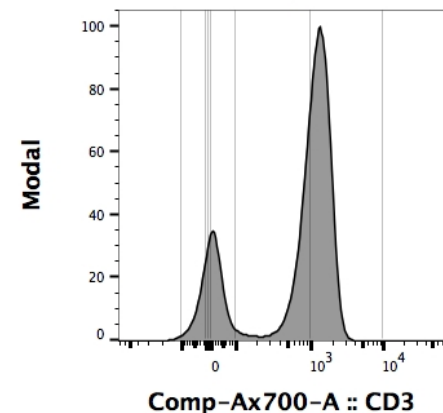
Zebra



Dot Plot

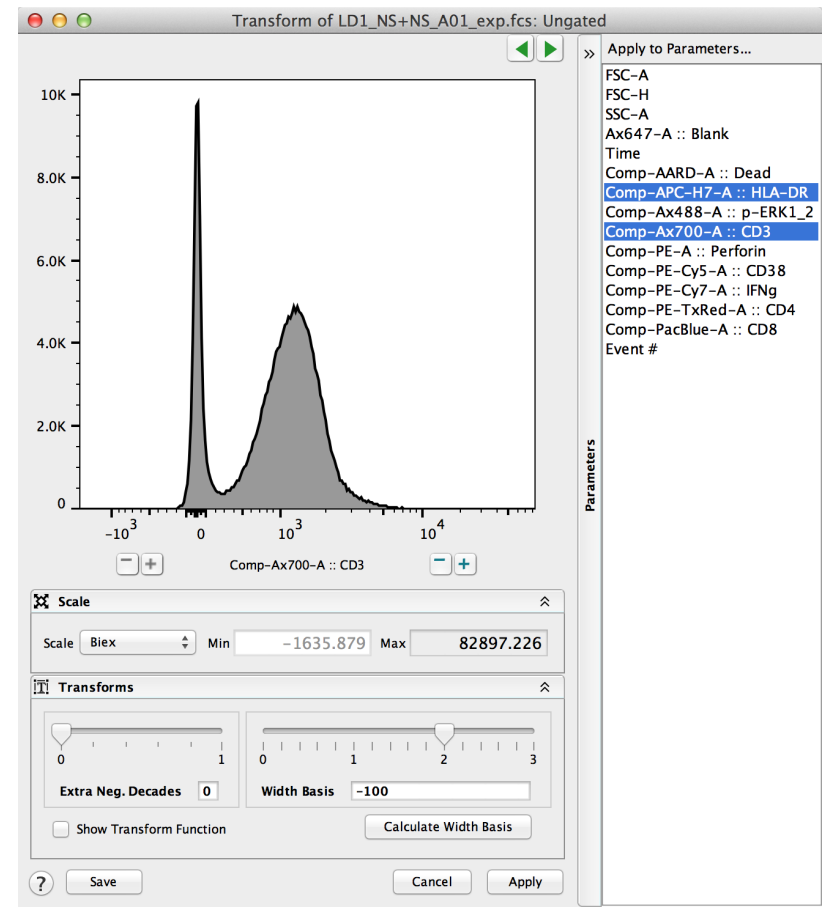
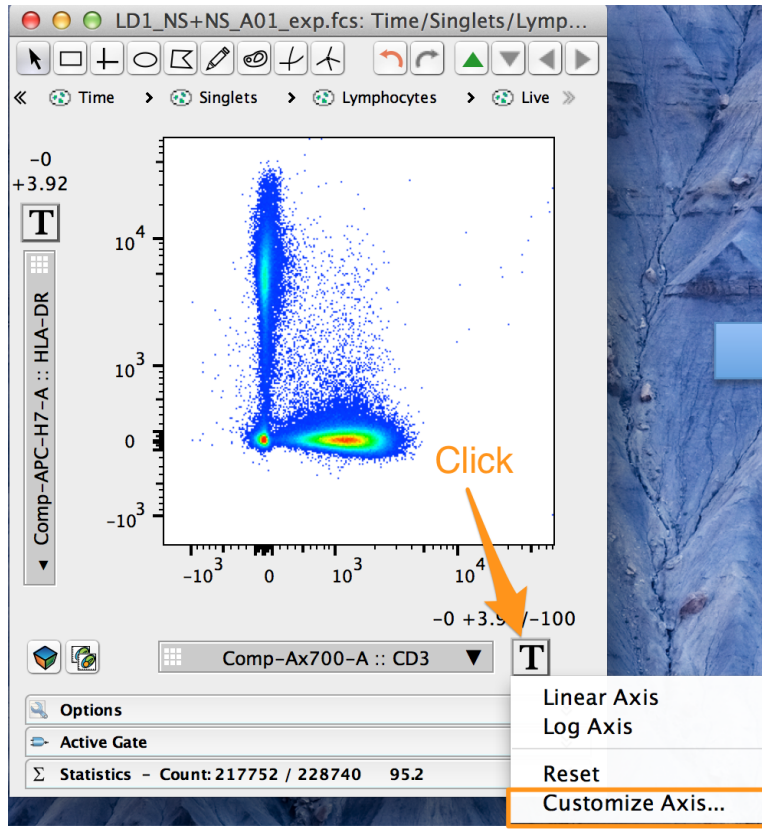


Histogram

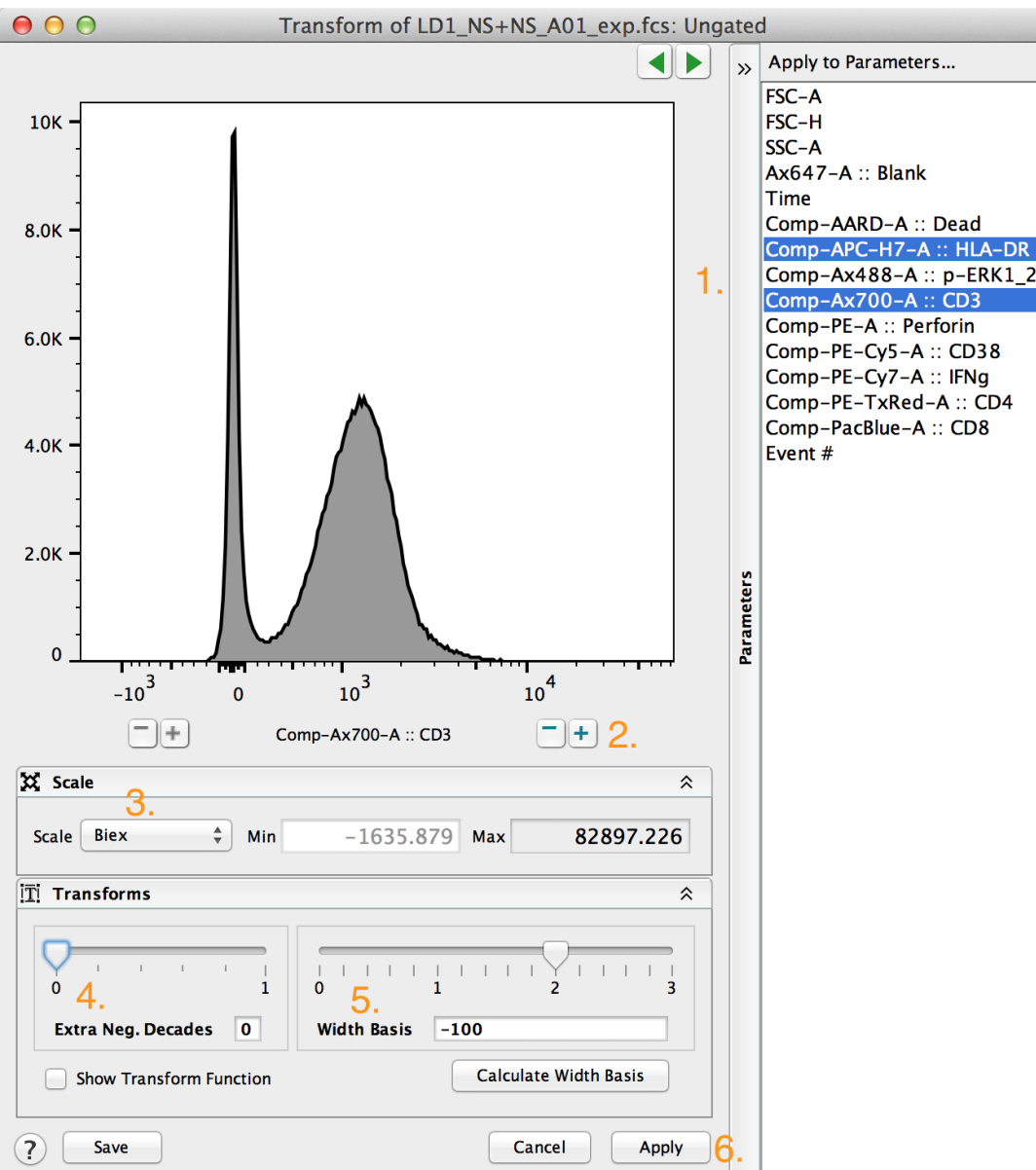


Transforming Data

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

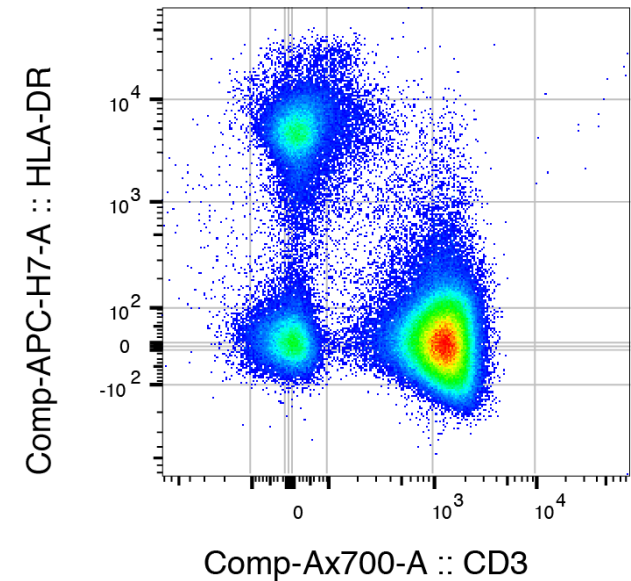
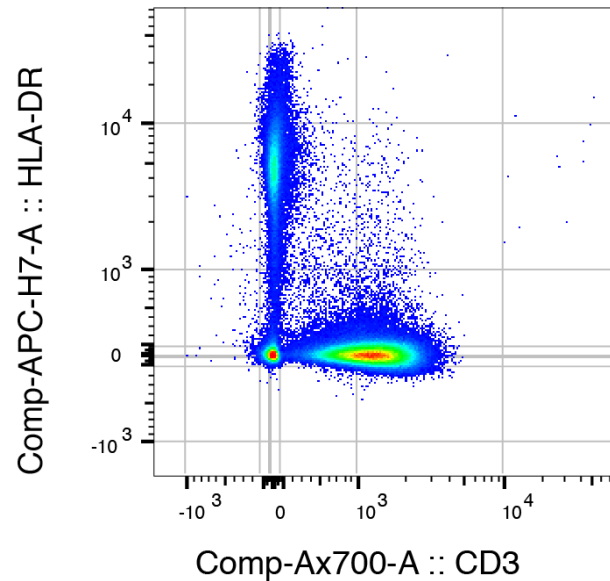
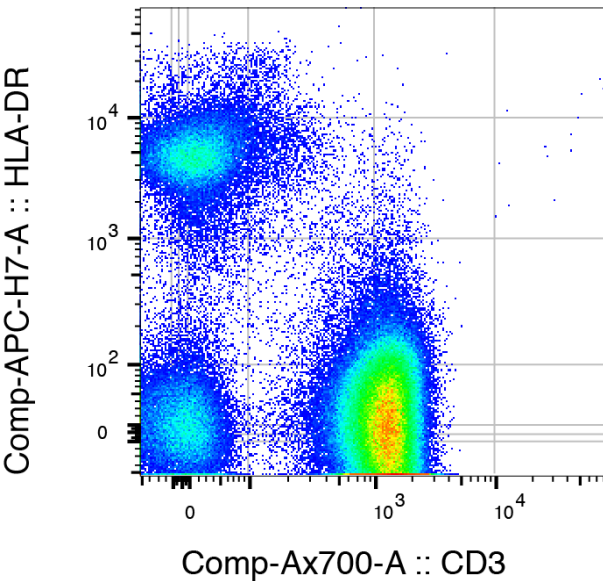


Transform Options



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. Click the Apply button at bottom right to apply the transformation settings to selected parameters

Effects of Transformation



Effects:

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

Boolean Combination Gates

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

2. Select Create Combination Gates


3. Abbreviate names and click

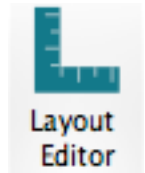
1. Highlight single marker gates

Group	Size	Role
{ } FMOs	0	Controls
{ } High Responders	4	Test
▼ { } MasterGates	8	None
▼ Singlets		
▼ Lymphocytes		
▼ Live		
▼ CD3+		
▼ Q1: CD4-, CD8+		
Σ Geometric Mean : Comp-Ax488-A (p-ERK1_2)		
▼ IFNg+		
Σ Freq. of Parent		
Σ Geometric Mean : Comp-PE-Cy7-A (IFNg)		
▼ Perf+		
Σ Geometric Mean : Comp-PE-A (Perforin)		
▼ pERK+		
Σ Geometric Mean : Comp-Ax488-A (p-ERK1_2)		
Q1: HLA-DR-, CD38+		

Name	74.8	342	10055	1568
Q1: CD4-, CD8+				
Σ Geometric Mean : Comp-Ax488-A (p-ERK1_2)	74.8			
IFNg+	1.02	342		
Σ Freq. of Parent	1.02			
Σ Geometric Mean : Comp-PE-Cy7-A (IFNg)	635			
Perf+	30.1		10055	
Σ Geometric Mean : Comp-PE-A (Perforin)	814			
pERK+	4.70			1568
Σ Geometric Mean : Comp-Ax488-A (p-ERK1_2)	775			

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.

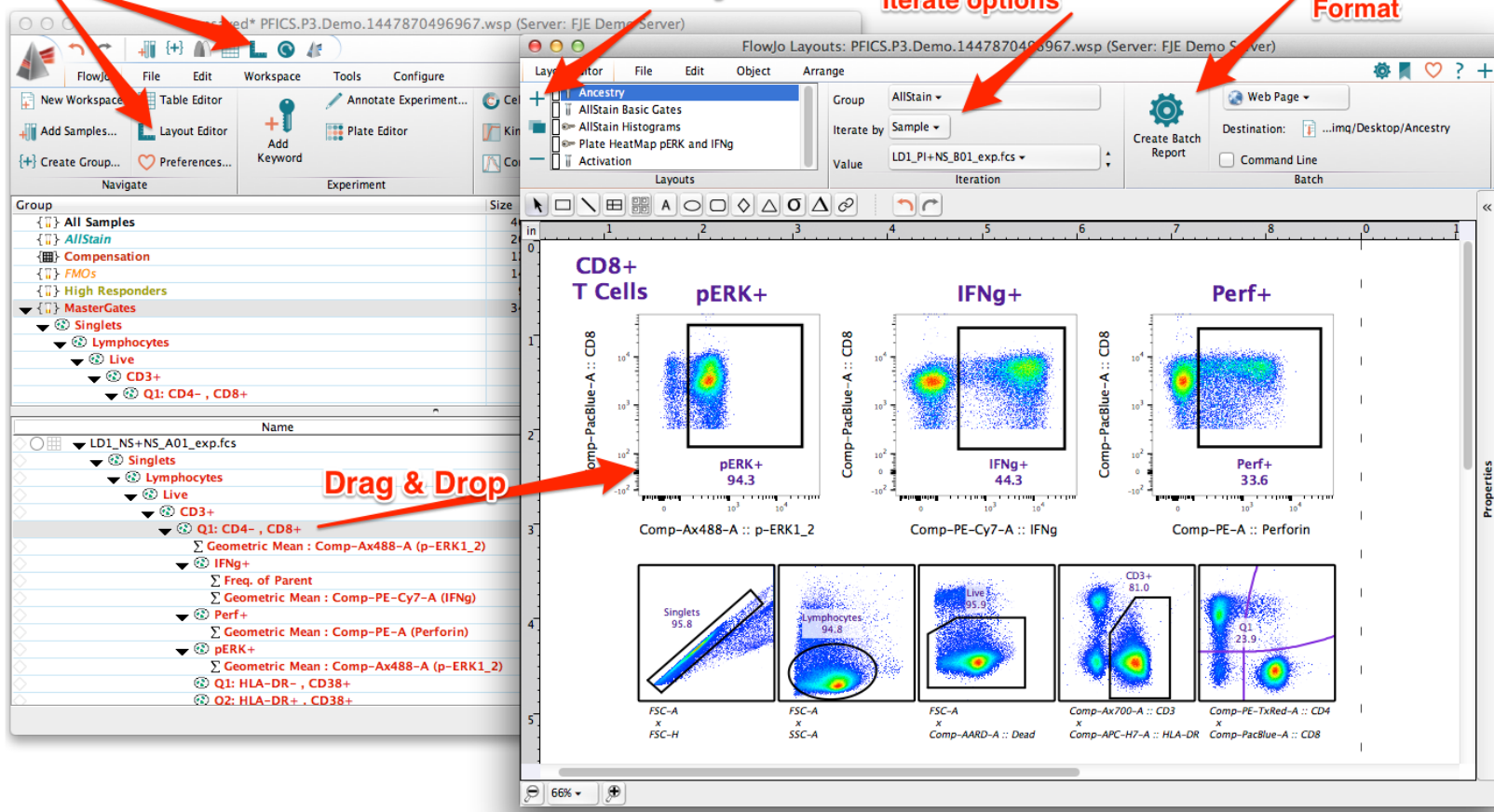


Layout Editor

Create Layouts

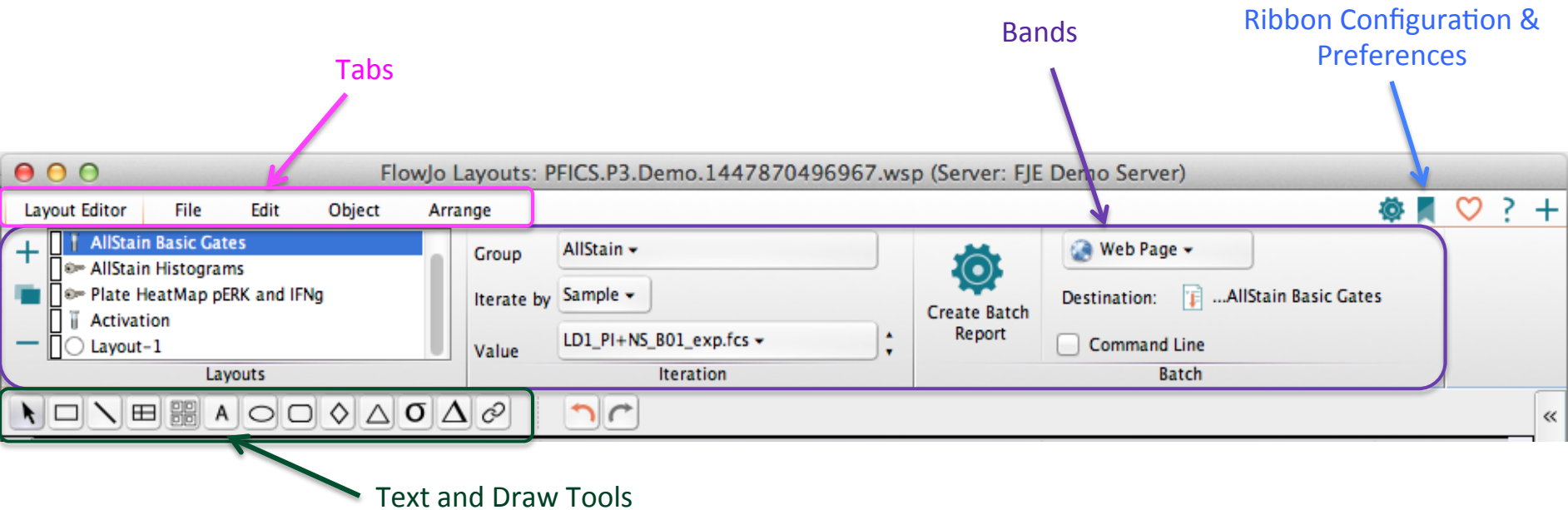
Specify Group and Iterate options

Batch Report Format



Working in Layout Editor

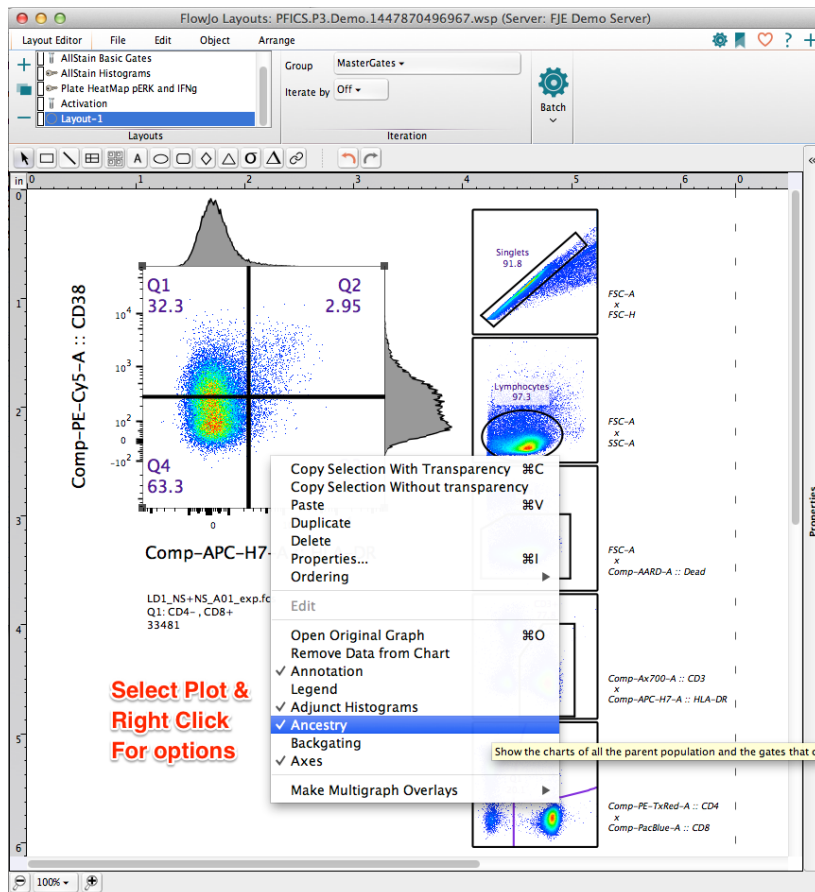
- Similar to the Workspace. Layout Editor has its own customizable Ribbon with Tabs and Bands to organize actions.



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

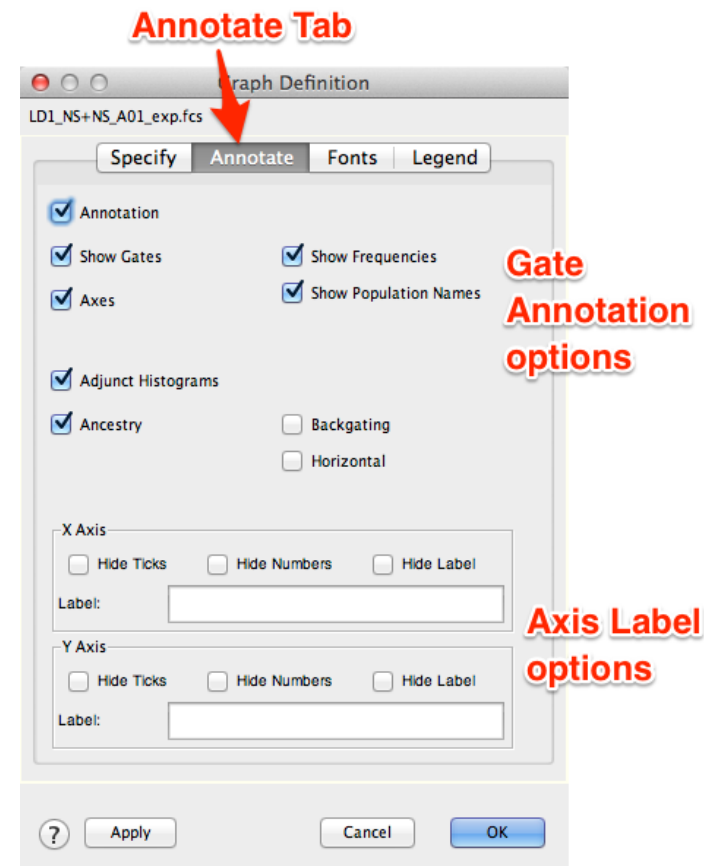
Within Layout Editor

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

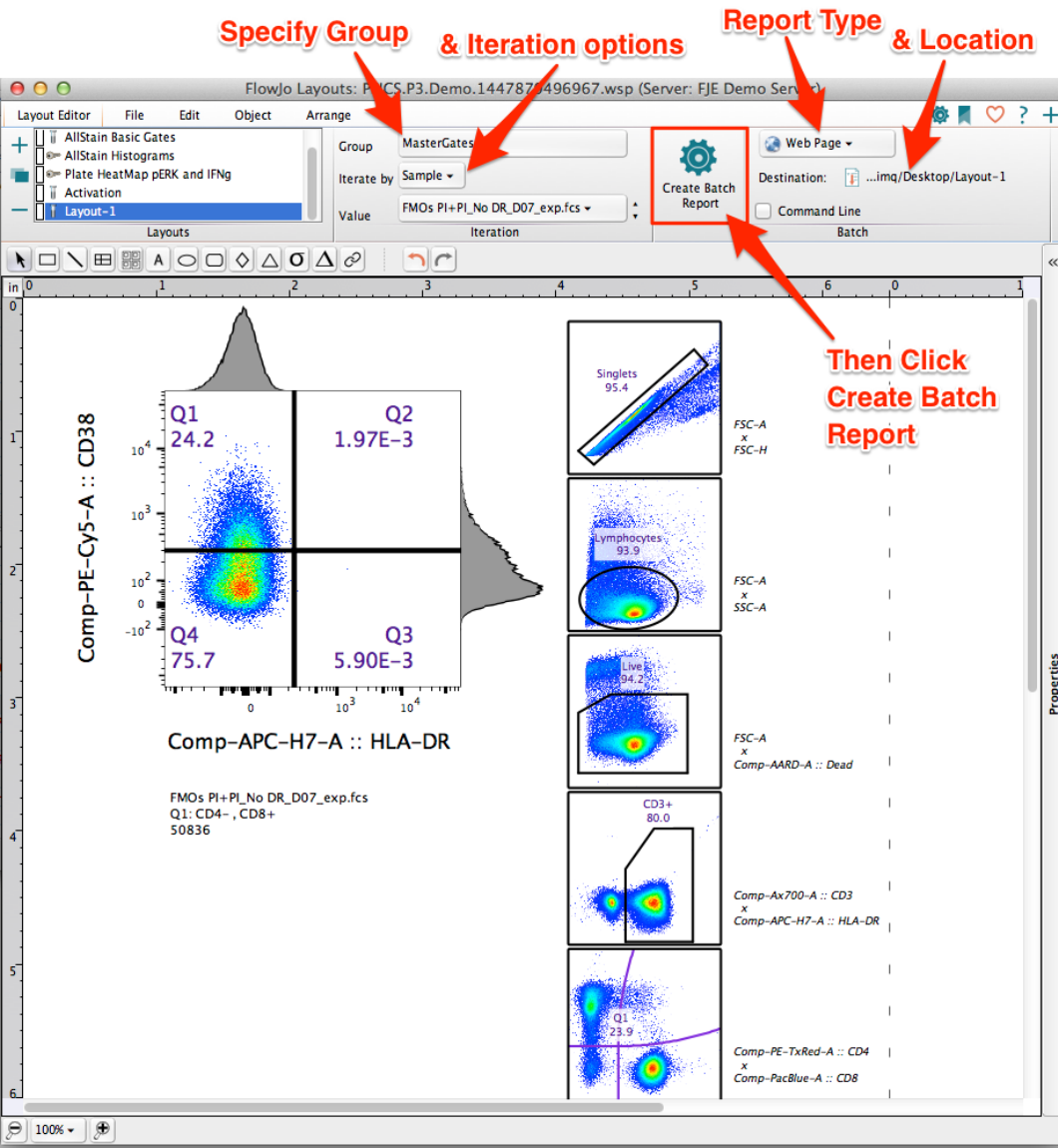


- Right Click on a graph plot for Ancestry and Backgating options
- Right click and select Properties for additional graph formatting

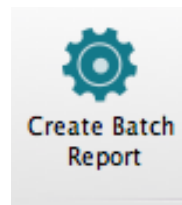
- Double Click a graph to change its properties/ formatting with 4 tabs of Graph Definition options



Batch Analysis of Layout Editor Graphics

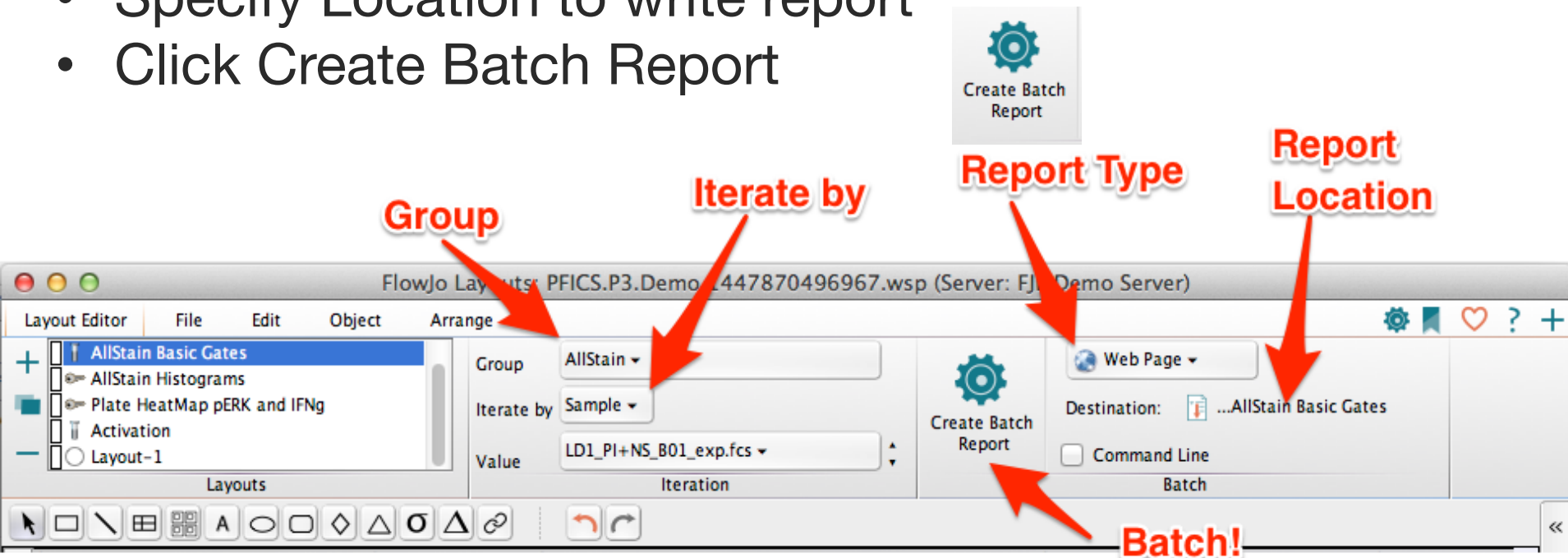


- Batch operations perform repetitive analysis on multiple samples, applying the layout to an entire set of samples.
- Specify Group, Iterate by, Report type and Location, then Click Create Batch Report .



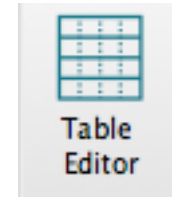
Batch Report Layouts

- Specify Group
 - 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 Location to write report
- Click Create Batch Report



The Table Editor

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



Open Table Editor

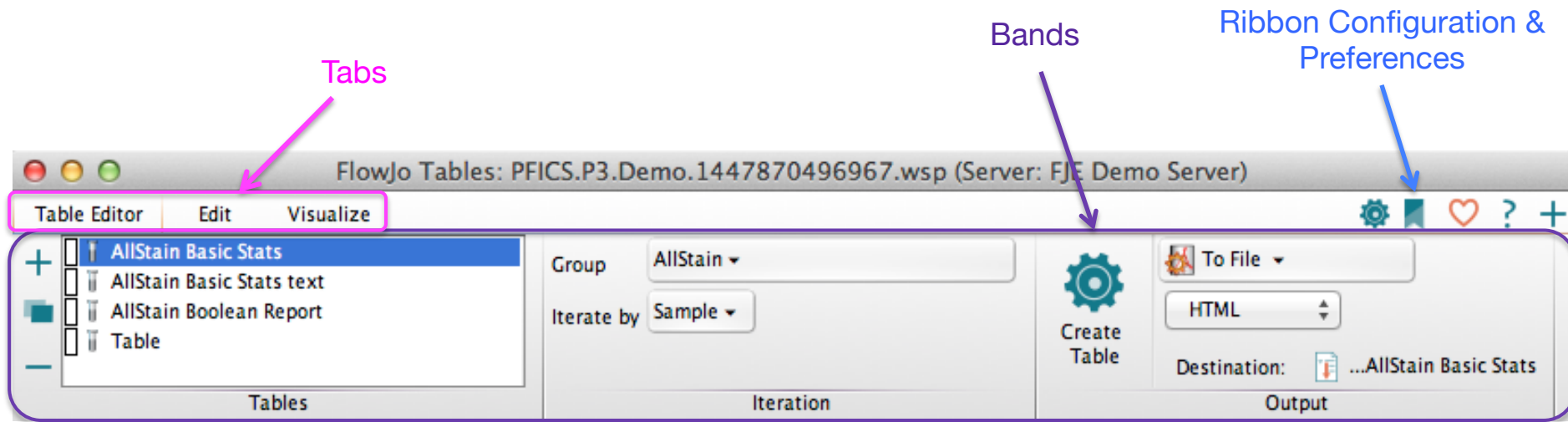
Drag Populations & Statistics

FlowJo Tables: PFICS.P3.Demo.1447870496967.wsp (Server: FJE Demo Server)

Col...	Population	Statistic	Parameter	Name
1	*PID			
2	*STIM			
3	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+	Geometric Mean	Comp-Ax488-A	pERK GMF
4	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+/IFNg+	Freq. of Parent		% IFNg+
5	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+/Perf+	Freq. of Parent		% Perf+
6	Formula			CD4/CD8...
7	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+/Q2: HLA-DR+, CD38+	Freq. of Parent		HLA-DR+, ...
8	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+/pERK+	Freq. of Parent		% pERK+
9	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+/IFNg+	Geometric Mean	Comp-PE-Cy7-A	IFNg GMF
10	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+/Perf+	Geometric Mean	Comp-PE-A	Perf GMF
11	Singlets/Lymphocytes/Live	Freq. of Parent		Viability
12	Singlets/Lymphocytes/Live/CD3+	Freq. of Parent		% CD3+
13	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+	Freq. of Parent		% CD8+
14	Singlets/Lymphocytes/Live/CD3+/Q3: CD4+, CD8-	Freq. of Parent		% CD4+

Within Table Editor

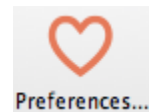
- Again, the Table Editor has its own customizable Ribbon with Tabs and Bands to organize actions.



- 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



FlowJo Tables: PFICS.P3.Demo.1447870496967.wsp (Server: FJE Demo Server)

Table Editor Edit **Visualize**

Heat Map **2. Apply visualization tool**

Standard Deviation

Expected Range NK Cells

Time Series Correlation 3D Plot

Formatting Plots

C...	Population	Statistic	Parameter	Name
1	*PID			
2	*STIM			
3	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+	Geometric Mean	Comp-Ax488-A	pERK GMF
4	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+ /IFNg+ 1. Highlight Rows	Freq. of Parent		% IFNg+
5	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+ /Perf+	Freq. of Parent		% Perf+
6	Formula			CD4/CD8 R...
7	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+ /Q2: HLA-DR+ , CD38+	Freq. of Parent		HLA-DR+ ,C...
8	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+ /pERK+	Freq. of Parent		% pERK+
9	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+ /IFNg+	Geometric Mean	Comp-PE-Cy7-A	IFNg GMF
10	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+ /Perf+	Geometric Mean	Comp-PE-A	Perf GMF
11	Singlets/Lymphocytes/Live	Freq. of Parent		Viability
12	Singlets/Lymphocytes/Live/CD3+	Freq. of Parent		% CD3+
13	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+	Freq. of Parent		% CD8+
14	Singlets/Lymphocytes/Live/CD3+/Q3: CD4+ , CD8-	Freq. of Parent		% CD4+

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.

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_PL...	LD12	PI+NS	327	45.3	40.8	1.94	1.50	84.8	4632	408
LD12_PL...	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_PL...	LD14	PI+NS	366	17.7	18.2	1.66	1.21	94.8	3708	650
LD14_PL...	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

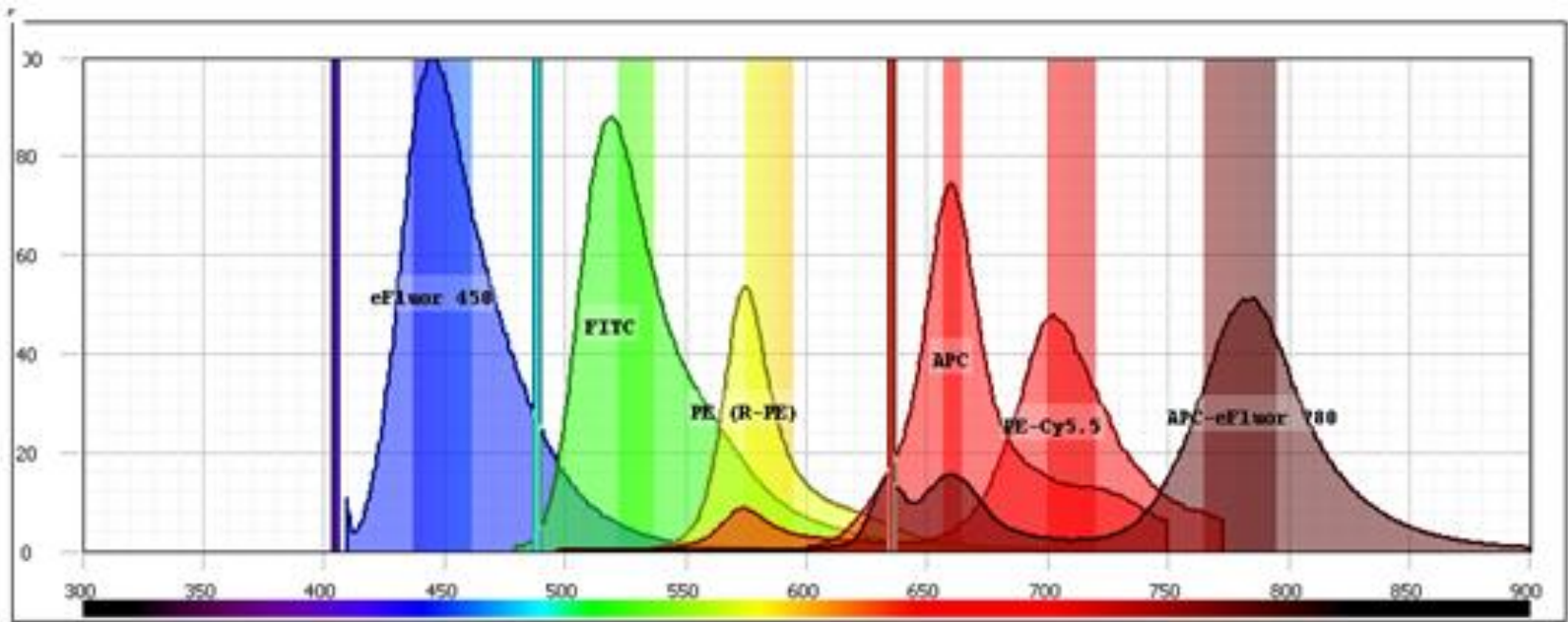
Outline – Part II

Advanced Tools and Platforms

- Compensation
- Export/Concatenate
- Cell Cycle Analysis
- The Plate Editor
- Plugins – Downsample & tSNE
- Templates
- Additional Training Resources

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

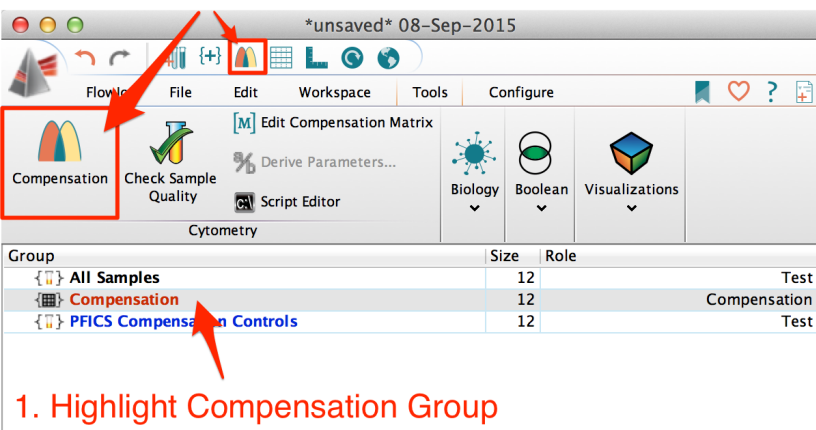
Compensation

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



in the task bar.

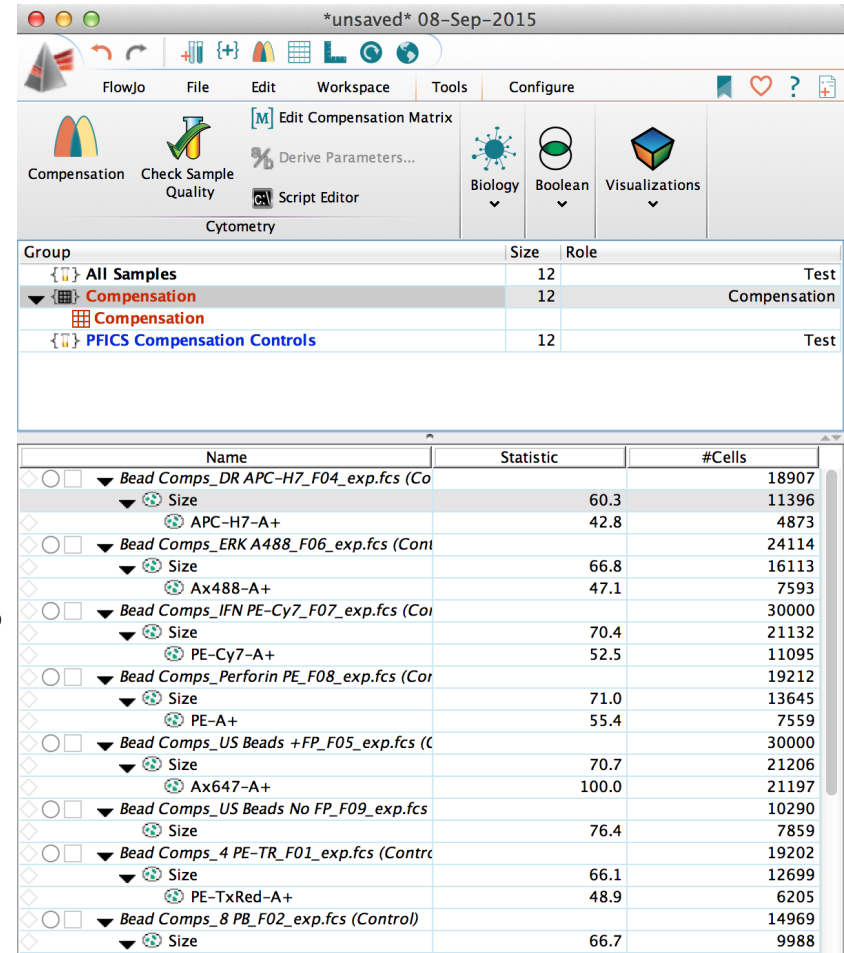
2. Click the Compensation Tool



1. Highlight Compensation Group

	Name	Statistic	#Cells
<input type="checkbox"/>	Bead Comps_DR APC-H7_F04_exp.fcs (Control)		18907
<input type="checkbox"/>	Bead Comps_ERK A488_F06_exp.fcs (Control)		24114
<input type="checkbox"/>	Bead Comps_IFN PE-Cy7_F07_exp.fcs (Control)		30000
<input type="checkbox"/>	Bead Comps_Perforin PE_F08_exp.fcs (Control)		19212
<input type="checkbox"/>	Bead Comps_US Beads +FP_F05_exp.fcs (Control)		30000
<input type="checkbox"/>	Bead Comps_US Beads No FP_F09_exp.fcs (Control)		10290
<input type="checkbox"/>	Bead Comps_4 PE-TR_F01_exp.fcs (Control)		19202
<input type="checkbox"/>	Bead Comps_8 PB_F02_exp.fcs (Control)		14969
<input type="checkbox"/>	Bead Comps_38 PE-Cy5_F03_exp.fcs (Control)		17603
<input type="checkbox"/>	Cell Comps_AARD_E01_exp.fcs (Control)		145743
<input type="checkbox"/>	Cell Comps_CD3 A700_E02_exp.fcs (Control)		129537
<input type="checkbox"/>	Cell Comps_US Cells_E03_exp.fcs (Control)		158360

The wizard auto gates samples



Group	Size	Role
{ } All Samples	12	Test
{ } Compensation	12	Compensation
{ } PFICS Compensation Controls	12	Test

	Name	Statistic	#Cells
<input type="checkbox"/>	Bead Comps_DR APC-H7_F04_exp.fcs (Control)		18907
<input type="checkbox"/>	Size	60.3	11396
<input type="checkbox"/>	APC-H7-A+	42.8	4873
<input type="checkbox"/>	Bead Comps_ERK A488_F06_exp.fcs (Control)		24114
<input type="checkbox"/>	Size	66.8	16113
<input type="checkbox"/>	Ax488-A+	47.1	7593
<input type="checkbox"/>	Bead Comps_IFN PE-Cy7_F07_exp.fcs (Control)		30000
<input type="checkbox"/>	Size	70.4	21132
<input type="checkbox"/>	PE-Cy7-A+	52.5	11095
<input type="checkbox"/>	Bead Comps_Perforin PE_F08_exp.fcs (Control)		19212
<input type="checkbox"/>	Size	71.0	13645
<input type="checkbox"/>	PE-A+	55.4	7559
<input type="checkbox"/>	Bead Comps_US Beads +FP_F05_exp.fcs (Control)		30000
<input type="checkbox"/>	Size	70.7	21206
<input type="checkbox"/>	Ax647-A+	100.0	21197
<input type="checkbox"/>	Bead Comps_US Beads No FP_F09_exp.fcs (Control)		10290
<input type="checkbox"/>	Size	76.4	7859
<input type="checkbox"/>	Bead Comps_4 PE-TR_F01_exp.fcs (Control)		19202
<input type="checkbox"/>	Size	66.1	12699
<input type="checkbox"/>	PE-TxRed-A+	48.9	6205
<input type="checkbox"/>	Bead Comps_8 PB_F02_exp.fcs (Control)		14969
<input type="checkbox"/>	Size	66.7	9988

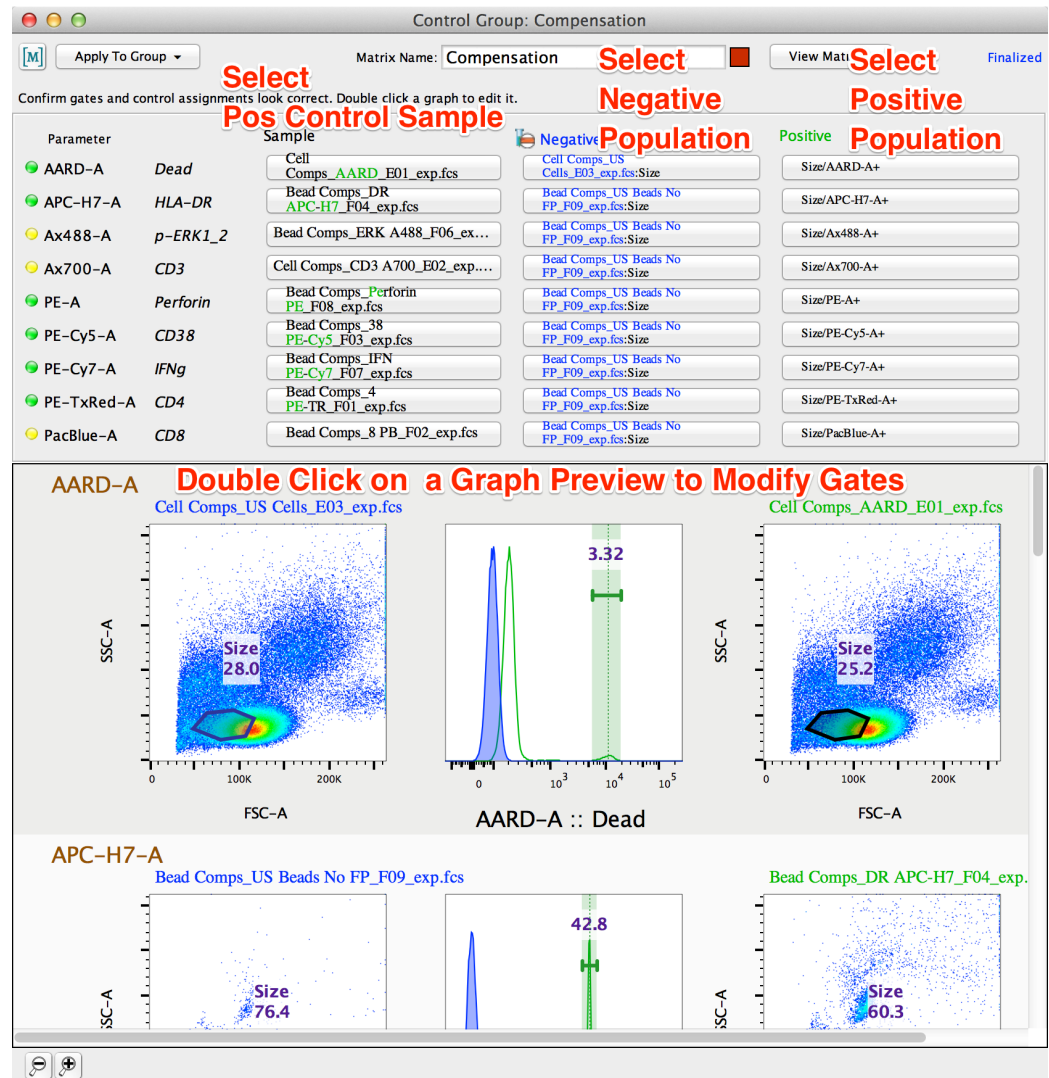
Compensation

- Then fills in the positive and negative.

For each Parameter

- Choose from the dropdown lists for each parameter.

- Double click preview graphs to modify gates.



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	Negative	Positive
● AARD-A Dead	Cell Comps AARD_E01_exp.fcs	Cell Comps_US Cells_E03_exp.fcs:Size	Size/AARD-A+

Use Sample drop down list

to select Pos Control Sample and

Choose or Remove Parameters

Bead Comps_DR APC-H7_F04_exp.fcs
Bead Comps_ERK A488_F06_exp.fcs
Bead Comps_IFN PE-Cy7_F07_exp.fcs
Bead Comps_Perforin PE_F08_exp.fcs
Bead Comps_US Beads +FP_F05_exp.fcs
Bead Comps_US Beads No FP_F09_exp.fcs
Bead Comps_4 PE-TR_F01_exp.fcs
Bead Comps_8 PB_F02_exp.fcs
Bead Comps_38 PE-Cy5_F03_exp.fcs
Cell Comps_AARD_E01_exp.fcs
Cell Comps_CD3 A700_E02_exp.fcs
Cell Comps_US Cells_E03_exp.fcs

<Clear>
<Remove This Parameter> ↖
Choose Parameters ↖
<Reset All>
<Reset This Parameter>

Use Negative drop down list

**to Select Negative Sample
or Population**

Bead Comps_DR APC-H7_F04_exp.fcs :: Size
Bead Comps_ERK A488_F06_exp.fcs :: Size
Bead Comps_IFN PE-Cy7_F07_exp.fcs :: Size
Bead Comps_Perforin PE_F08_exp.fcs :: Size
Bead Comps_US Beads +FP_F05_exp.fcs :: Size
Bead Comps_US Beads No FP_F09_exp.fcs :: Size
Bead Comps_4 PE-TR_F01_exp.fcs :: Size
Bead Comps_8 PB_F02_exp.fcs :: Size
Bead Comps_38 PE-Cy5_F03_exp.fcs :: Size
Cell Comps_AARD_E01_exp.fcs :: Size
Cell Comps_US Cells_E03_exp.fcs :: Size

Size
Size/AARD-A+
<Clear>

Use Positive drop down list

to Choose Positive population

Size
Size/AARD-A+
<Clear>

- 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

Select Color **Name Matrix** **Edit Matrix** **Save a copy of the Matrix**

Apply Matrix with Drag and Drop onto Group or Sample

Applied Matrix Badge is Color Coded

Add a Matrix from file

Preview Matrix effect on a sample

Workspace Matrices

Compensation

Group

Size

LD1

PFICS Compensation Controls

Name

Statistic

LD1_NS+NS_A01_exp.fcs

LD1_NS+PI_C01_exp.fcs

LD1_PI+NS_B01_exp.fcs

LD1_PI+PI_D01_exp.fcs

Preview Sample: LD1_NS+NS_A01_exp.fcs

Preview Population

View

Overlay Uncompensated

APC-H7-A :: HLA-DR

Ax488-A :: p-ERK1_2

Ax700-A :: CD3

PE-A :: Perforin

PE-Cy5-A :: CD38

PE-Cy7-A :: IFNg

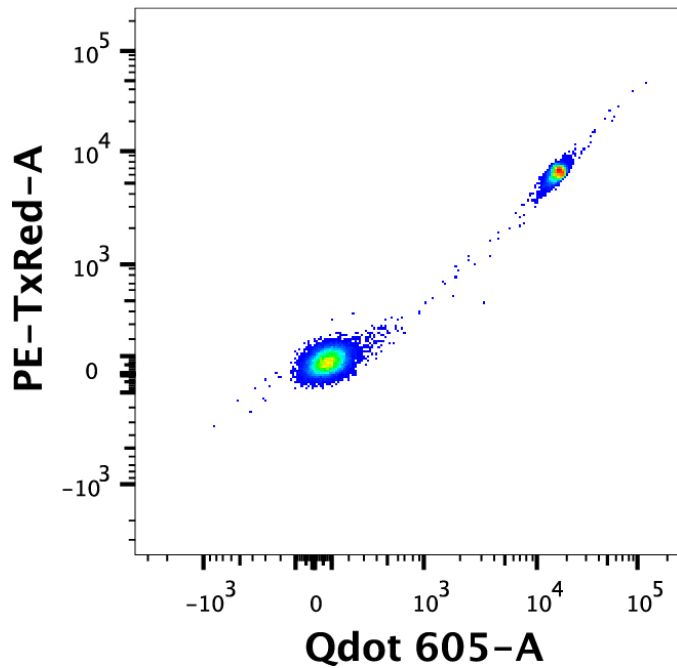
Displaying matrix 'Compensation-1'

	AARD-A :: Dead	APC-H7-A :: HLA-DR	Ax488-A :: p-ERK1_2	Ax700-A :: CD3	PE-A :: Perforin	PE-Cy5-A :: CD38	PE-Cy7-A :: IFNg	LD1_PI+NS_B01_exp.fcs	PacBlue-A :: CD8
AARD-A :: Dead	100	0.0351	0.3746	0.0685	0.0382	0.1447	0.0399	0.064	24.1599
APC-H7-A :: HLA-DR	0	100	0	3.2511	0.0169	0.8078	39.6125	0.056	0
Ax488-A :: p-ERK1_2	1.8492	0	100	0	0.0119	0	0	0	0
Ax700-A :: CD3	0.1713	34.835	0.1071	100	0	1.0301	10.2007	0	0.0443
PE-A :: Perforin	0	0.0125	0.3404	0.0375	100	14.4881	1.3119	37.6694	0
PE-Cy5-A :: CD38	0	3.0045	0.0253	7.7547	1.6106	100	12.018	0.7082	0
PE-Cy7-A :: IFNg	0	5.7598	0.0603	0.3117	1.8877	0.368	100	0.8245	0
PE-TxRed-A :: CD4	0	0.0291	0.1118	0.0572	23.9323	52.786	6.0459	100	0
PacBlue-A :: CD8	16.9144	0	0.0597	0	0.0076	0	0	0.0063	100

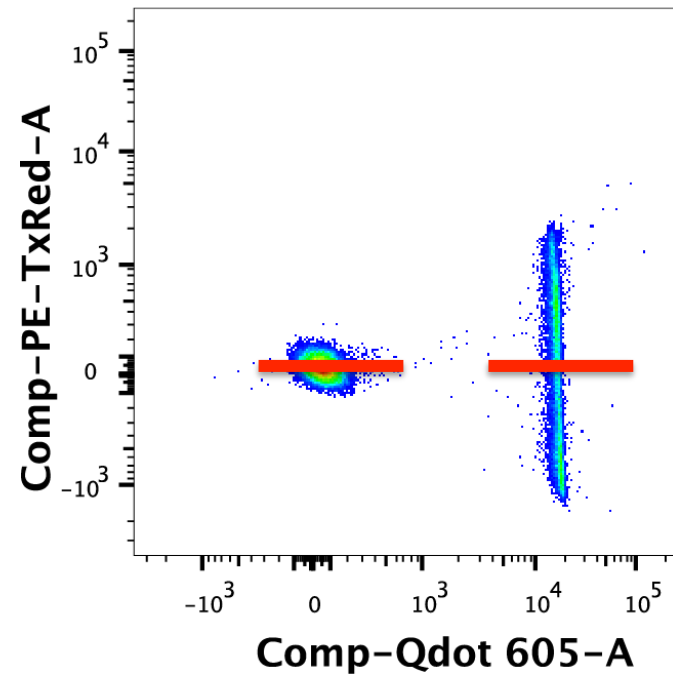
Effect of Compensation



Uncompensated

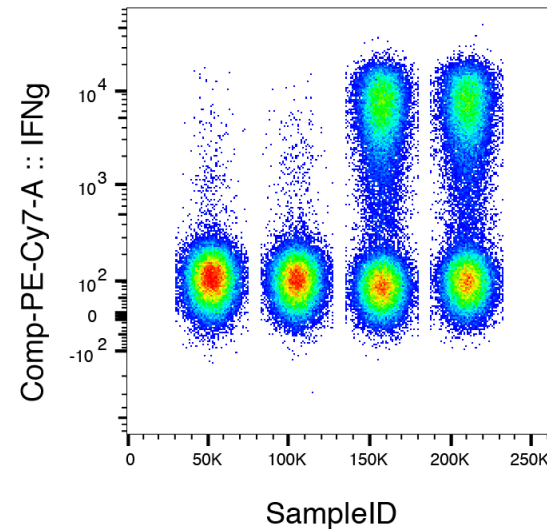
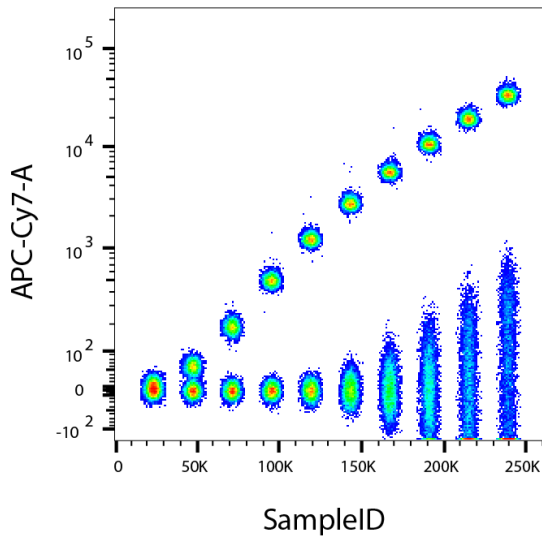


Compensated



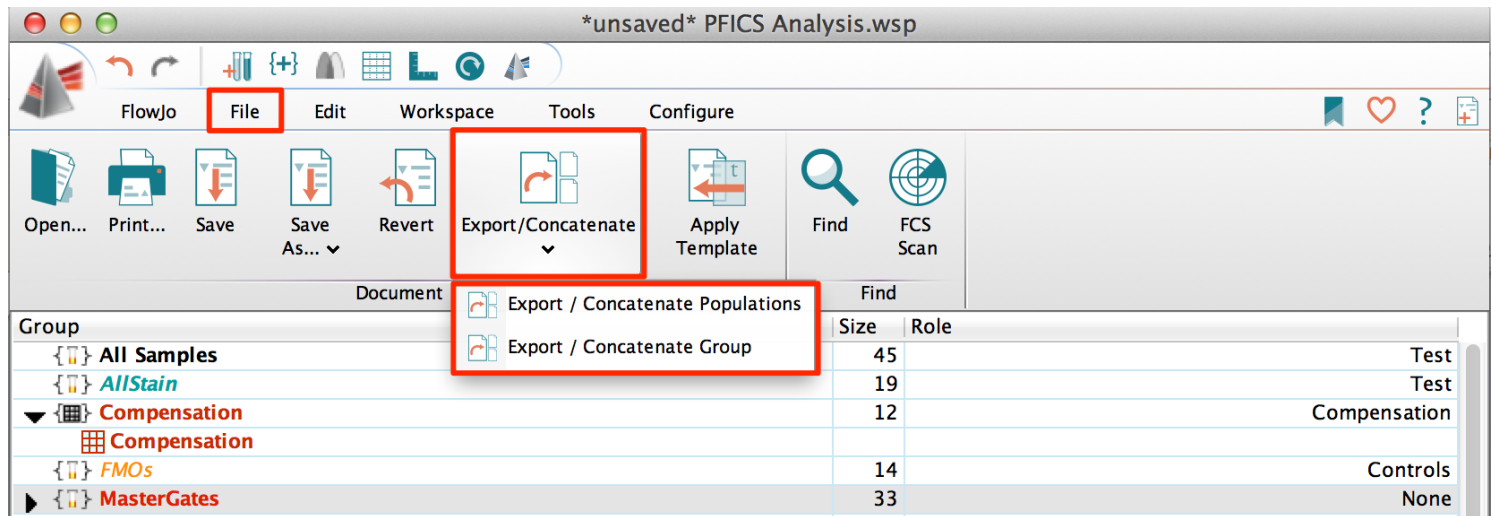
Export and Concatenate

- Add and embed keyword metadata
- Merge data from multiple files
- Identify specific populations
- Isolate events for further computational analysis
- Titrate reagents for optimal staining and stimulation conditions
- Visualize responses



Export or Concatenate Data

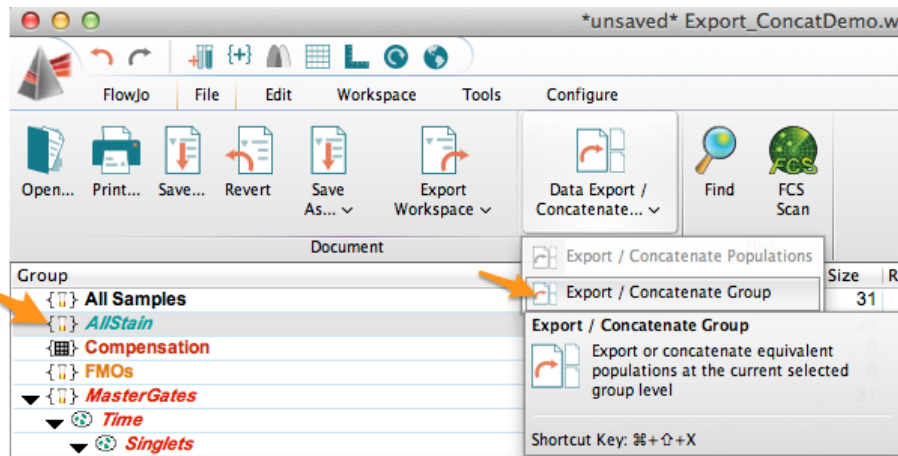
- The Data Export/Concatenate... action button is located by default in the Document band within the workspace File tab.



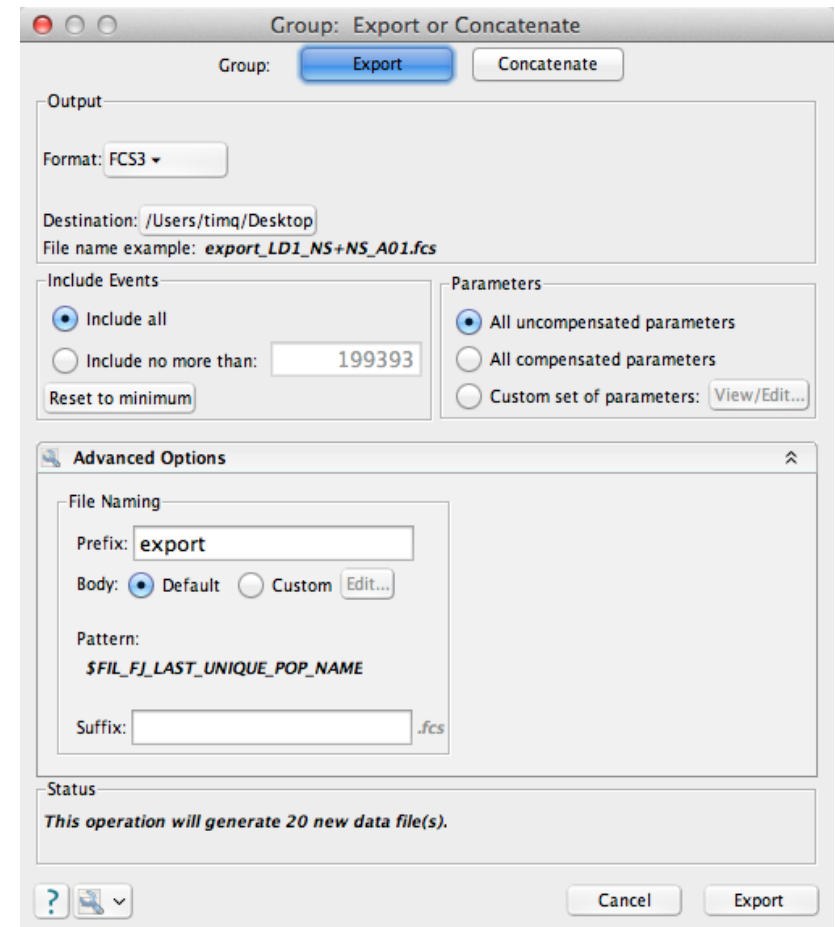
- Choose from two options in the drop down menu:
 - 1) Export/Concatenate Populations
→ subset of events defined by gating hierarchy/phenotype
 - 2) Export/Concatenate Group
→ all or a subset of events independent of phenotype

Exporting Groups

- Highlight a *group* containing the samples you wish to export
- Then, choose Export/Concatenate Group
(hotkey = shift+⌘+X)

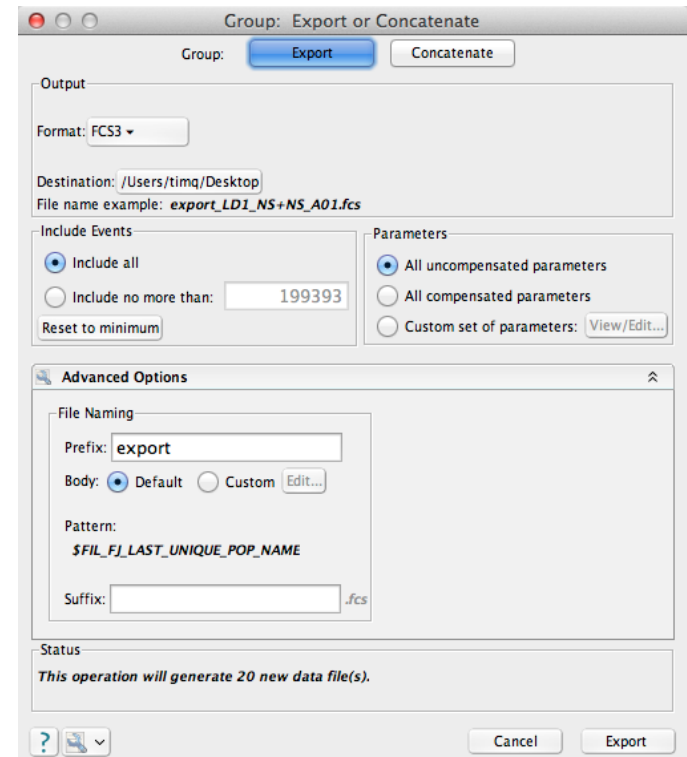


- The Group Export or Concatenate dialog window will appear



Export Options

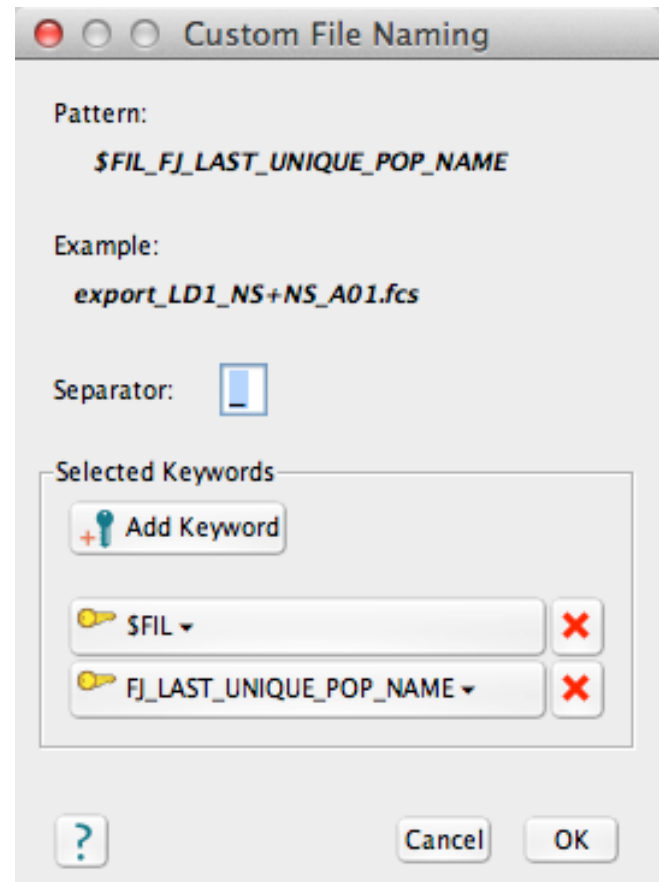
- **Output panel**
 - Format – selects file format (FCS3 or CSV)
 - Destination – specifies directory where output files will be saved
 - File name example – displays example of naming scheme as specified in Advanced Options → File Naming
- **Include Events panel** – Include all events or down-sample randomly with Include no more than #
- **Parameters panel** – Choose All uncompensated, All compensated, or a custom set of parameters for export
- **Advanced Options File naming panel**
 - Prefix – specifies a common prefix to add
 - Body – specifies the keywords to create a unique name for each file
 - Pattern – displays keyword pattern for body naming scheme
 - Suffix – specifies a suffix to add
- **Status panel**
 - Tells how many files will be produced



Custom File Naming

- Specific options for Export function
- Allows unique keyword pattern to be defined as a distinct naming scheme between exported files.

- **Add a Keyword value**
Will add an additional keyword value option
- **To change a Keyword value**
Select from the drop down keyword list
- **To remove a Keyword value**
Click the red X button



Concatenating Groups

- Highlight a *group* containing the samples you wish to export
- Then, choose Export/Concatenate Group and click the Concatenate button at the top of the dialog

- **Group Concatenation panel**

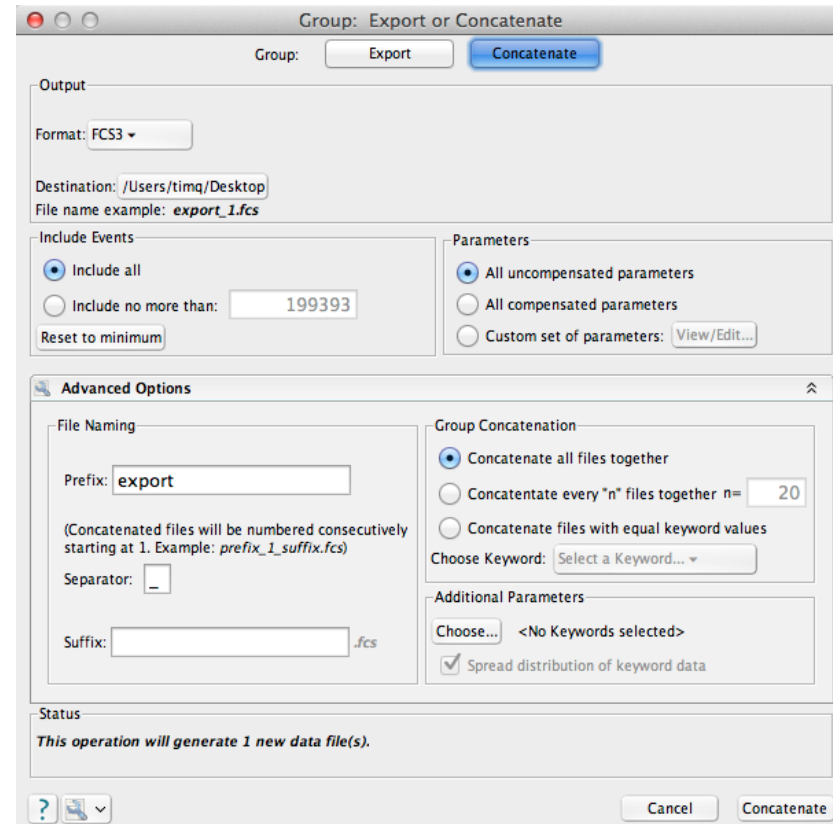
Concatenate all files together

Concatenate every “n” files together

Concatenate files with equal keyword values

- **Additional Parameters panel**

Tells how many files will be produced



Concatenating Populations

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

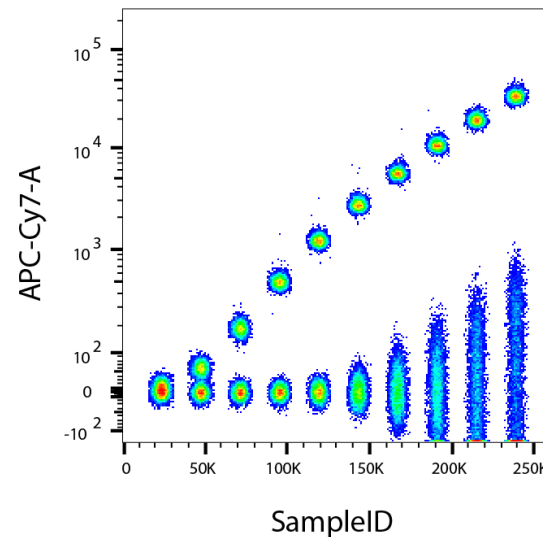
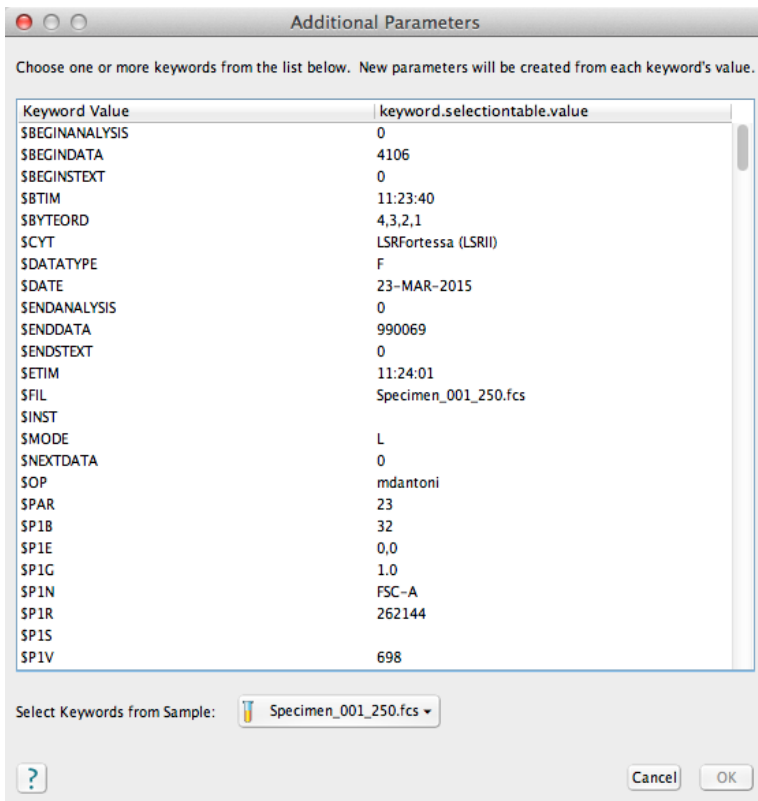
The screenshot shows the FlowJo software interface. The title bar indicates an unsaved workspace named "Export_ConcatDemo.wsp". The menu bar includes FlowJo, File, Edit, Workspace, Tools, and Configure. Below the menu bar is a toolbar with icons for Open, Print, Save, Revert, Save As, Export Workspace, Data Export / Concatenate, Find, and FCS Scan. The main workspace area displays a gating tree on the left under the "Group" pane, showing "All Samples" expanded to "Rainbow Bead Voltage Titration", which contains a "Beads" population. Below the tree is a table with four columns: Name, Statistic, #Cells, and *PMT Voltage. The table lists 10 populations, each with its corresponding statistics.

Name	Statistic	#Cells	*PMT Voltage
Specimen_001_250.fcs		10717	250
Beads	82.2	8811	
Specimen_001_300.fcs		10528	300
Beads	84.7	8916	
Specimen_001_350.fcs		10479	350
Beads	84.6	8866	
Specimen_001_400.fcs		10502	400
Beads	85.0	8923	
Specimen_001_450.fcs		10497	450
Beads	85.1	8935	
Specimen_001_500.fcs		10497	500
Beads	85.1	8934	
Specimen_001_550.fcs		10468	550
Beads	84.6	8861	
Specimen_001_600.fcs		10506	600
Beads	84.6	8886	
Specimen_001_650.fcs		10489	650
Beads	85.4	8958	
Specimen_001_700.fcs		10500	700
Beads	84.8	8900	

The screenshot shows the "Populations: Export or Concatenate" dialog box. The "Populations:" section has "Export" and "Concatenate" buttons, with "Concatenate" selected. The "Output" section shows "Format: FCS3" and "Destination: /Users/timq/Desktop/ExportDemo". The "File name example: export_1.fcs" is shown. The "Include Events" section has "Include all" selected, with "Include no more than: 8811" and a "Reset to minimum" button. The "Parameters" section has "All uncompensated parameters" selected. The "Advanced Options" section has "File Naming" with "Prefix: export" and "Suffix: .fcs", and "Group Concatenation" with "Concatenate all files together" selected. The "Additional Parameters" section has "Choose..." and "<No Keywords selected>". The "Status" section at the bottom says "This operation will generate 1 new data file(s)." and has "Cancel" and "Concatenate" buttons.

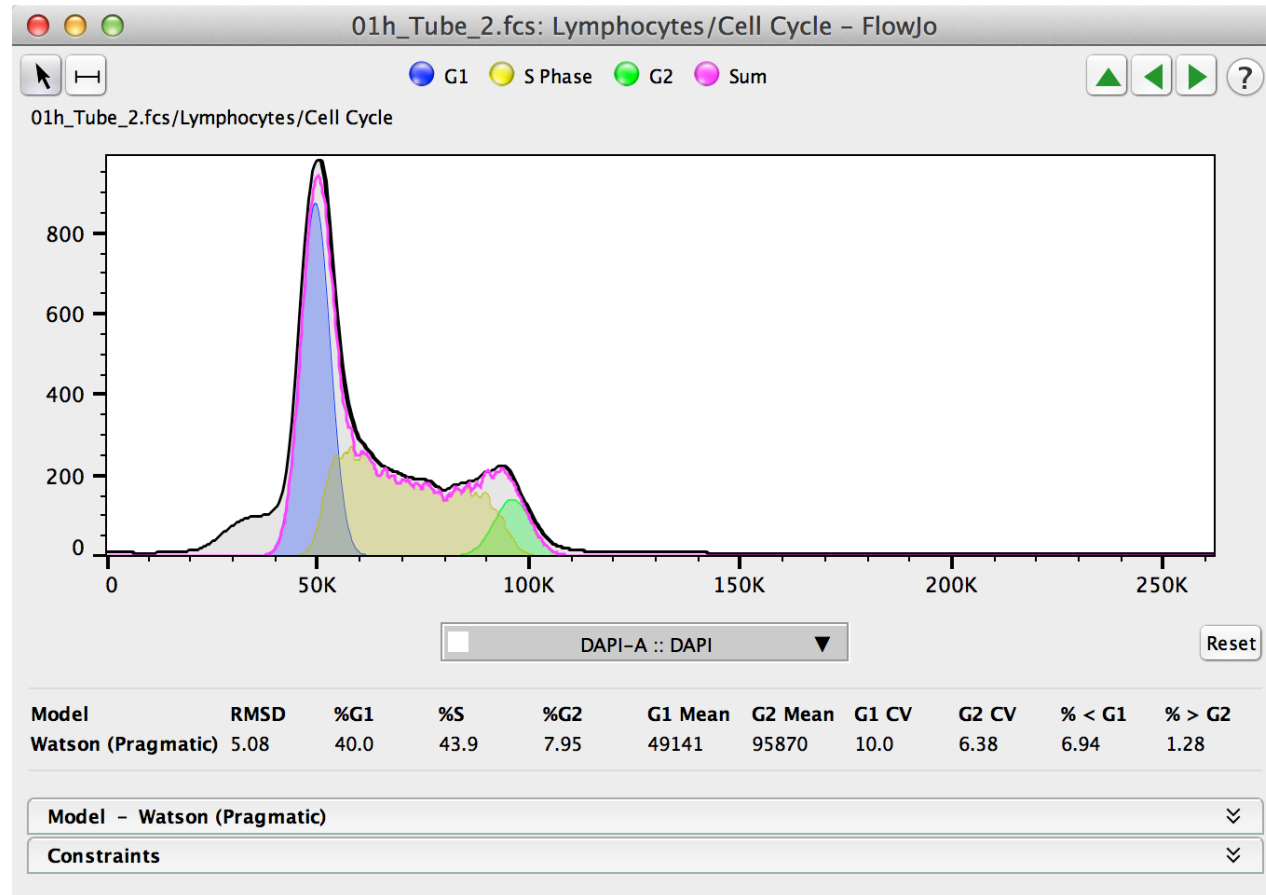
Additional Parameters

- You can select one or more keywords to create new parameters in the concatenated output file.
- Note however, 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 contributing to the concatenated file.



Cell Cycle Analysis

- The Cell Cycle platform allows 1D modeling of cell cycle phases based on DNA content
- V10.1 has 1D Watson and Dean-Jett-Fox 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.

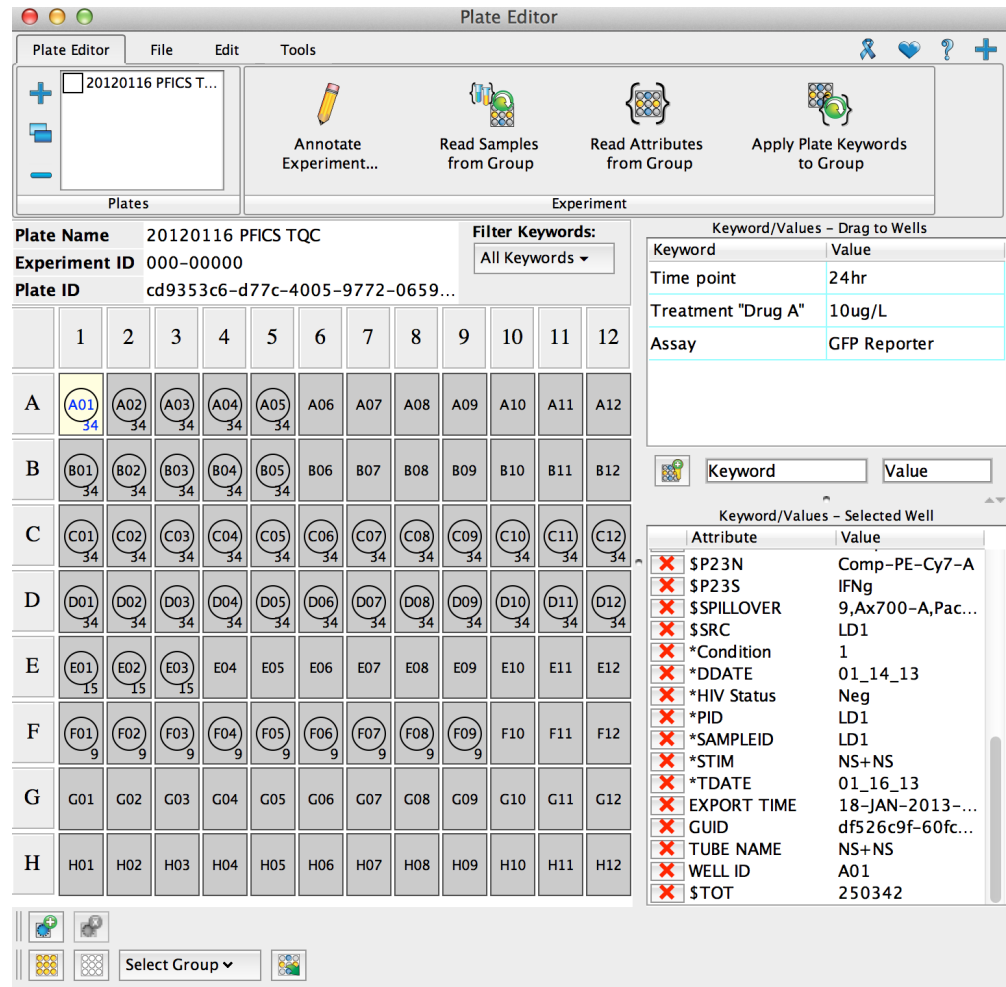
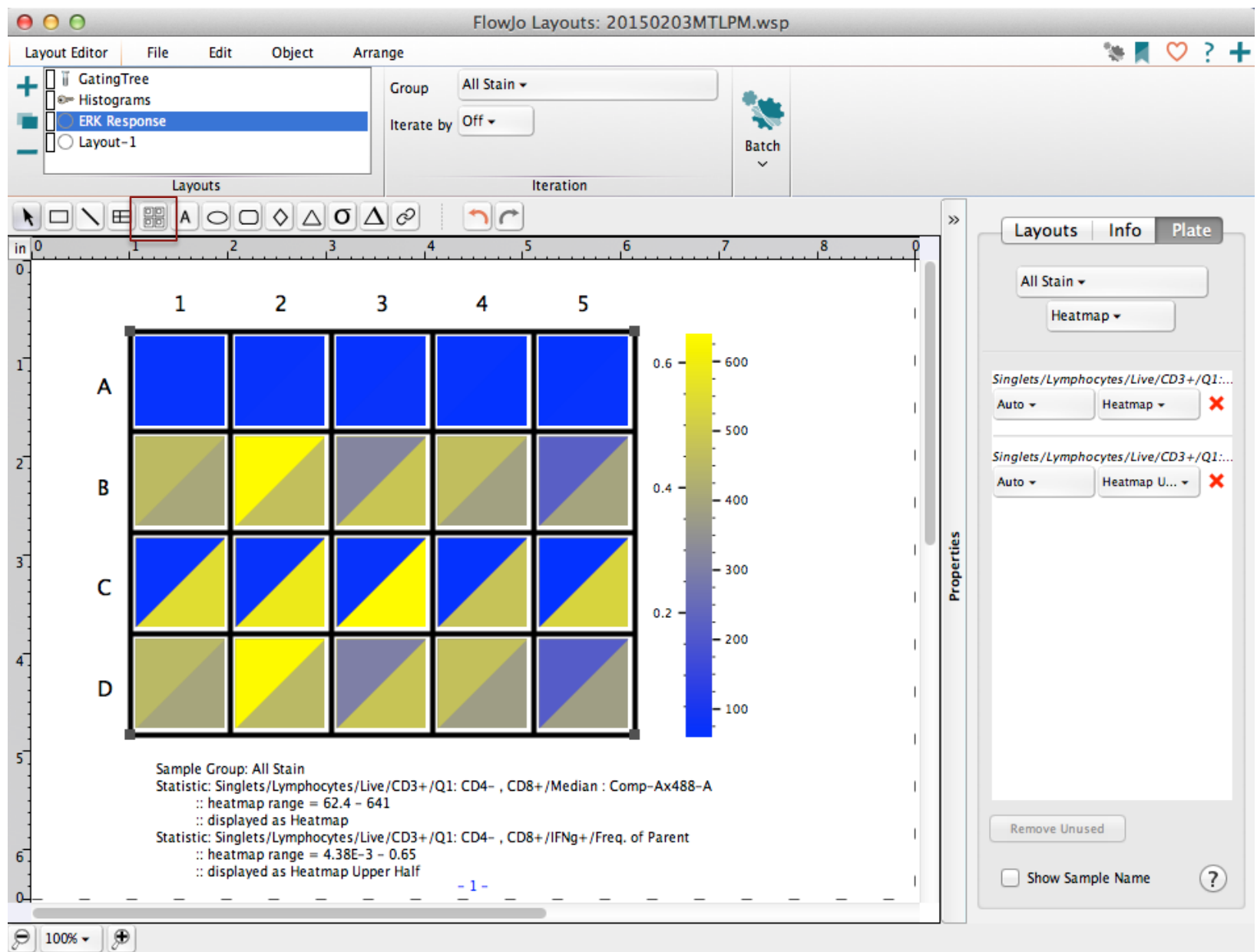
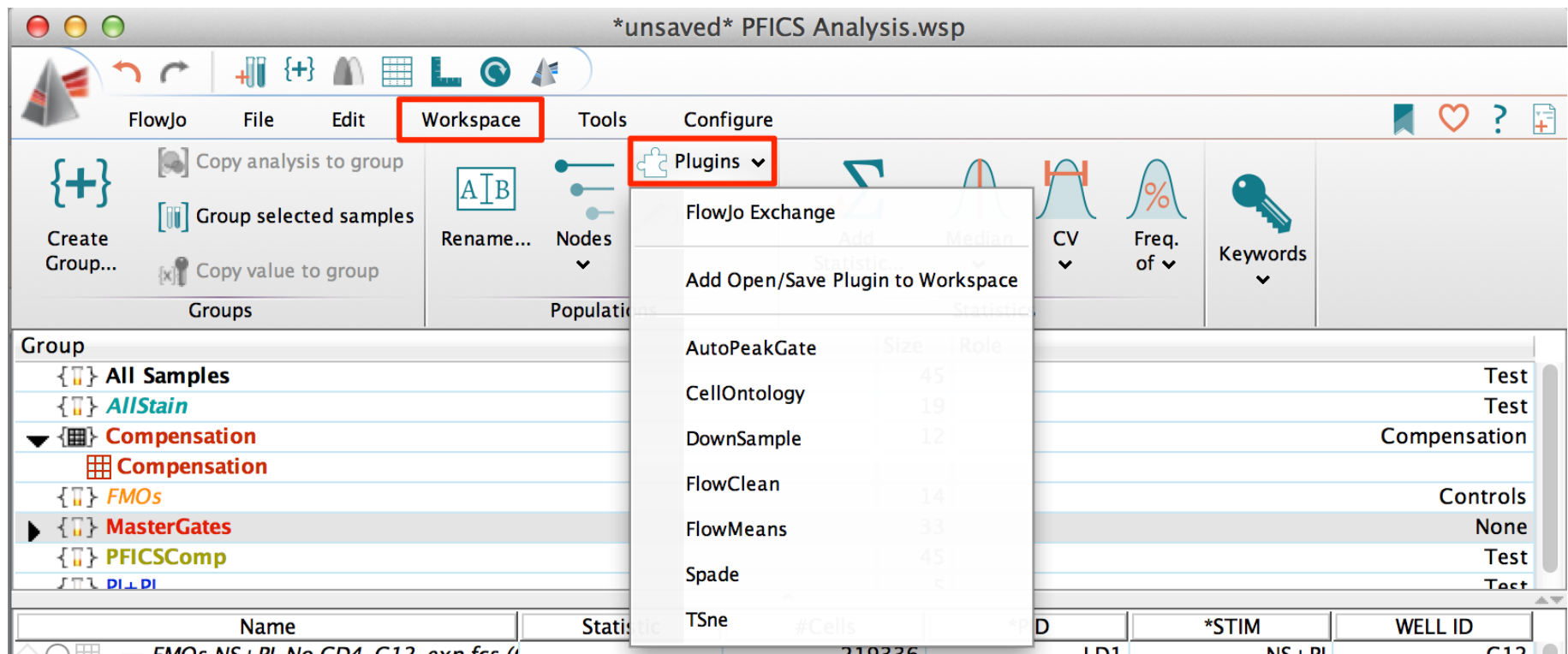


Plate Visualizations



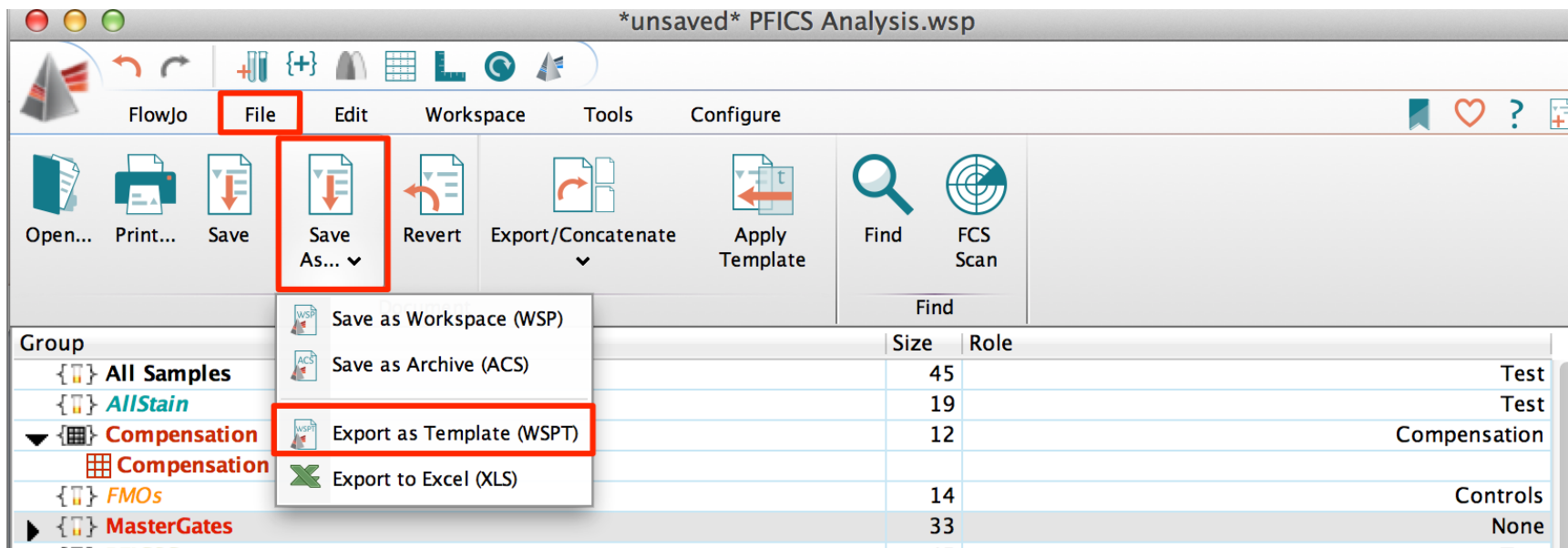
Plugins

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



Workspace Templates

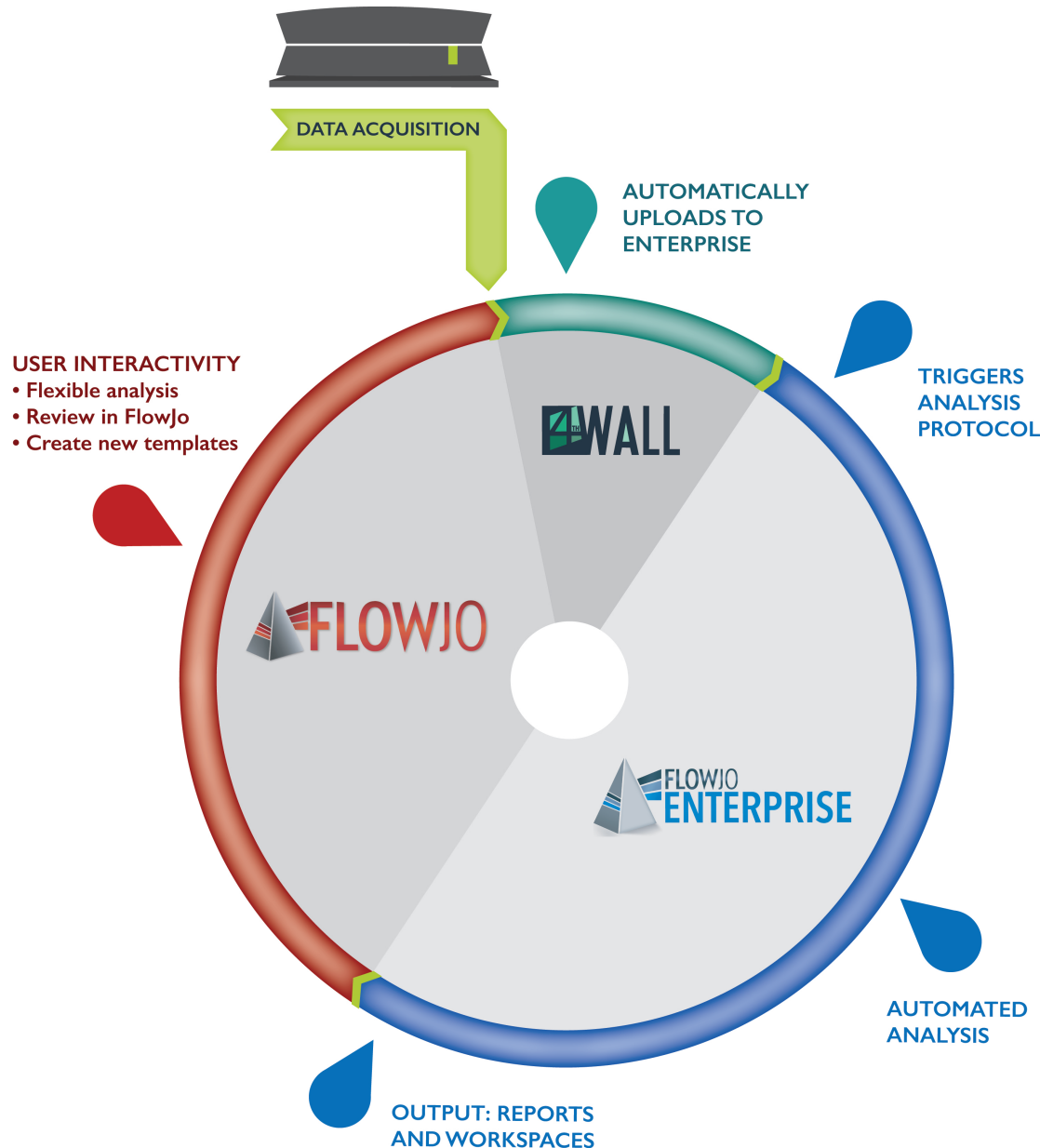
- Allows saving all analysis reports in your workspace without data.
- Streamlines repetitive analysis of multiple runs using the same staining panel(s).
- File Tab → Document Band → Export Workspace As... Save as a Template



FlowJo Enterprise

- Is a server-based version of FlowJo v10, designed to assist with data archiving, analysis, and report generation for high dimension, high throughput flow or mass cytometry data.
- Can handle data upload directly from the cytometer, store it on a secure server, and provide computational power and automated analysis features for scientists.
- Is an optional add-on component of the FlowJo Licensing Server (FLS) institutional site license.
- Is offered as 1 of 4 tiered packages, with each tier introducing additional features and levels of service.

FlowJo Enterprise Components



Email:
enterprise@flowjo.com
for information

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!