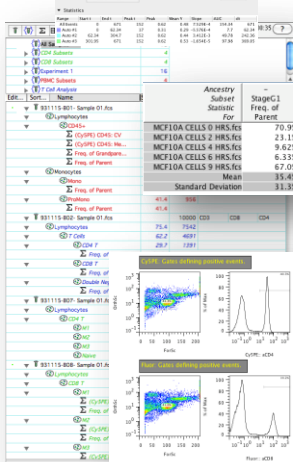
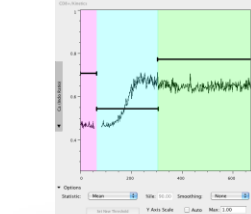
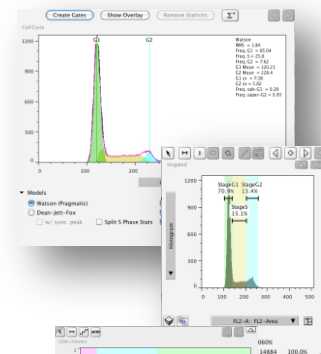


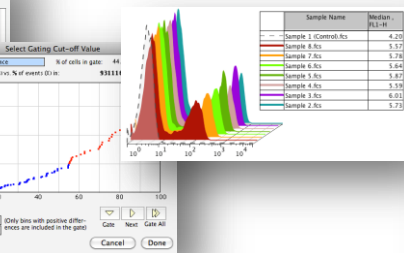
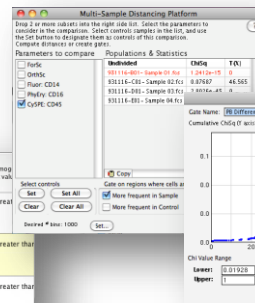
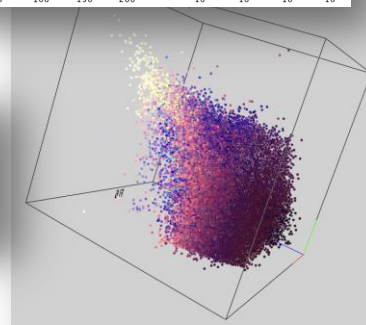
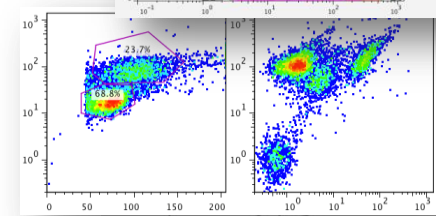
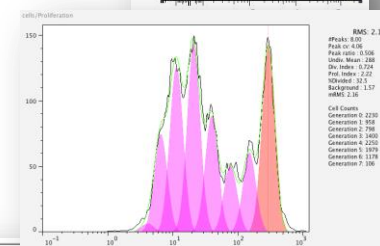
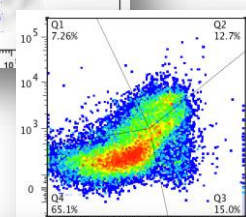
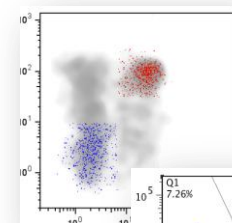


Flow Cytometry Data Analysis

Jack Panopoulos, Ph.D.
Application Scientist



Sample	StageG1 Freq. of Parent	StageG2 Freq. of Parent	StageG5 Freq. of Parent
MCF10A CELLS 0 HRS-IG	70.9%	13.4%	15.1%
MCF10A CELLS 2 HRS-IG	23.1%	13.4%	62.1%
MCF10A CELLS 4 HRS-IG	9.62%	8.32%	78.2%
MCF10A CELLS 6 HRS-IG	6.33%	6.64%	75.5%
MCF10A CELLS 9 HRS-IG	67.0%	17.5%	11.7%
Mean	35.4%	11.9%	48.3%
Standard Deviation	11.3%	4.37%	32.8%



My Goals For Today

1. Save you time analyzing your data
2. Help you get more out of your data (\$\$\$)
3. Keep you on the cutting-edge



Benefits of Using FlowJo

Keyword-Driven Data Organization

Data Export

Batching



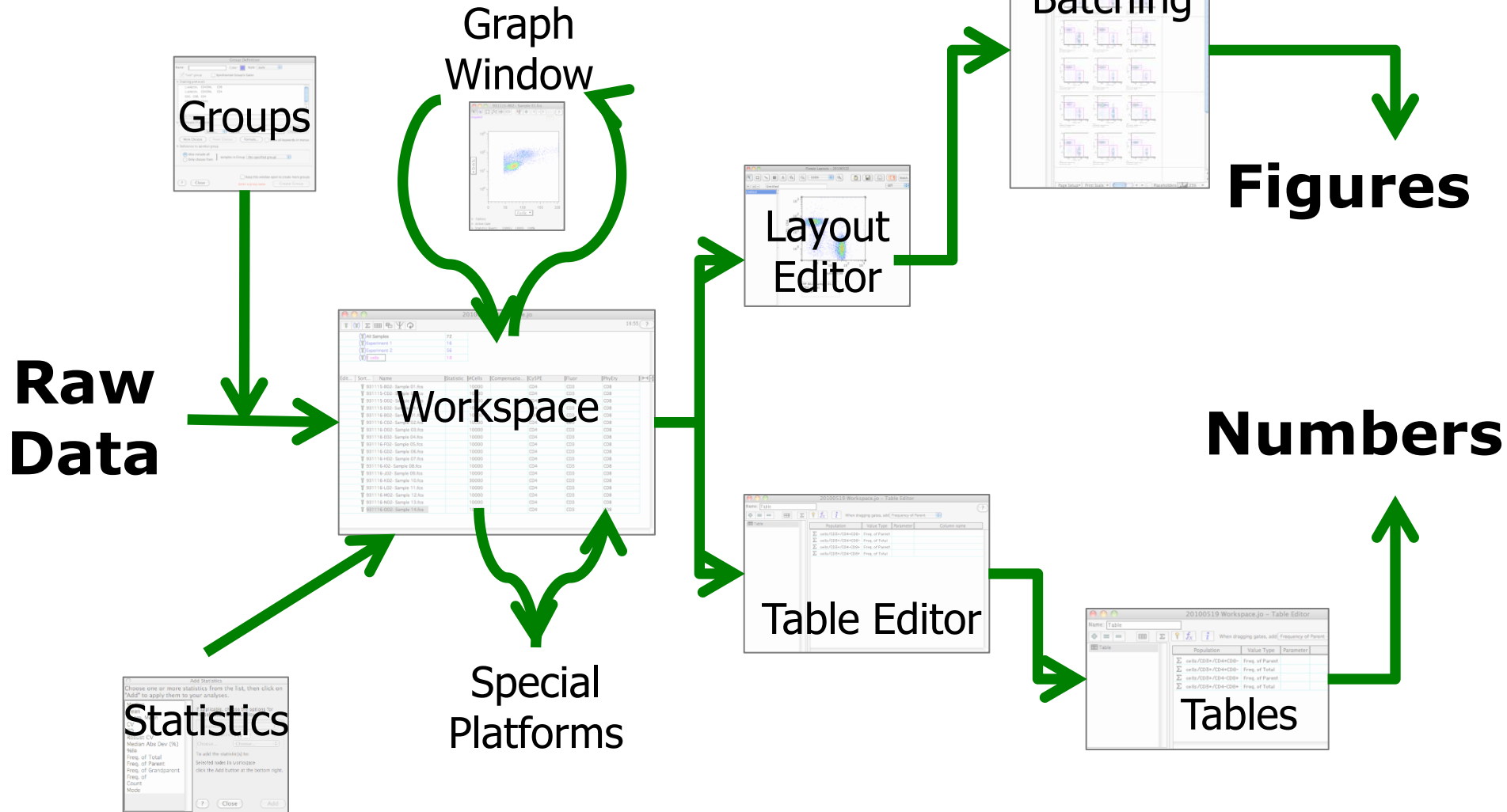
**Good Data
Annotation**

**Template
Creation**

Grouping

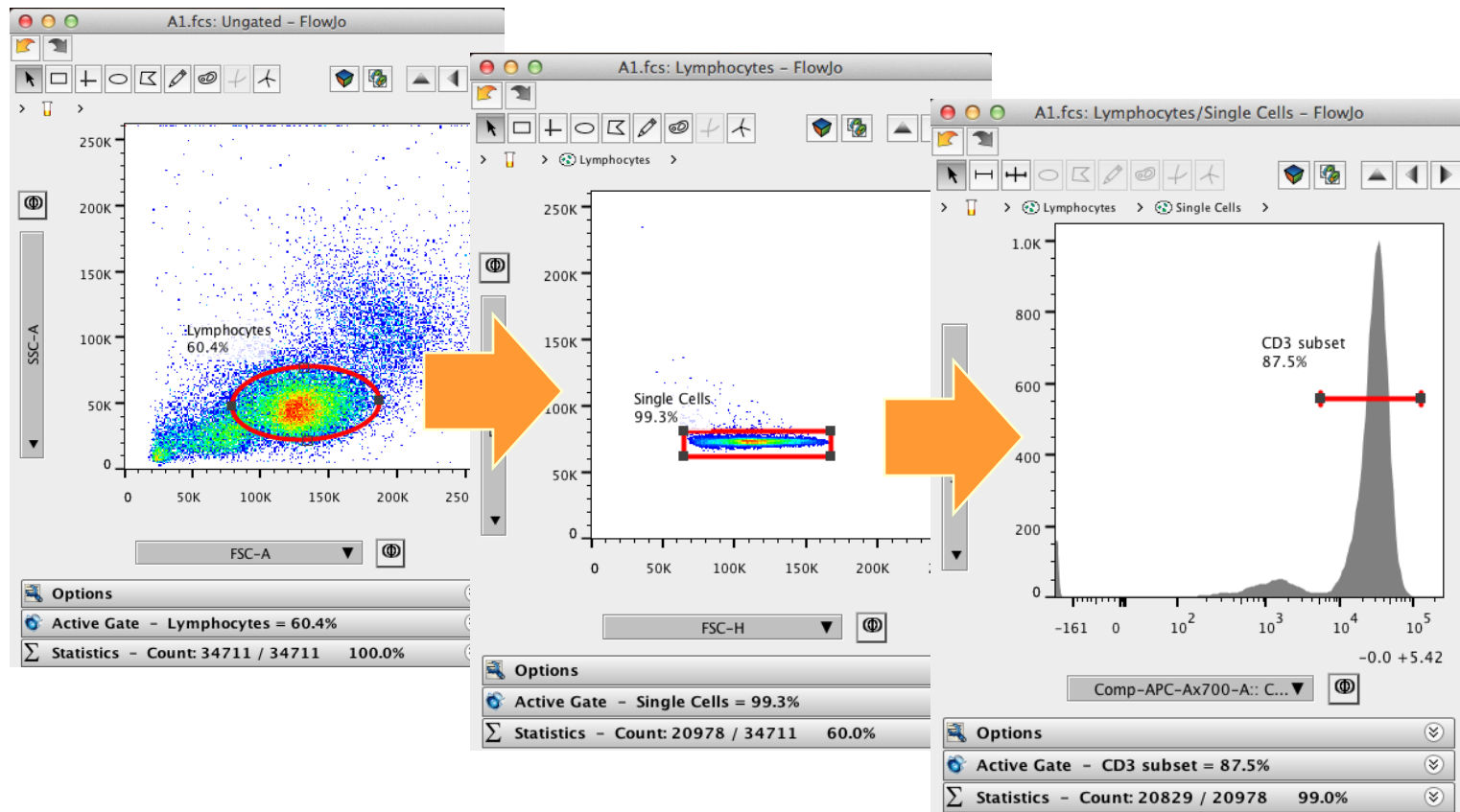


Basic Workflow in FlowJo



Benefits of Using FlowJo

Intuitive Hierarchical Gating



Workspace for Hierarchical Gating

The image displays two screenshots of the FlowJo software interface, illustrating the workspace for hierarchical gating analysis.

Left Screenshot: Groups and Analyses

The top toolbar shows icons for creating groups and analyses. A red box highlights the 'U' icon (Create Group).

The 'Groups and Analyses' table lists the hierarchy of samples and gates:

Groups and Analyses	Number of Samples
All Samples	43
Compensation	7
Panel 1	9
Panel 2	9
Panel_1	9
Lymphocytes	
Singlets	
Σ (Comp-APC-Ax700-A) : Median	
Σ (Comp-APC-Ax700-A) : Robust CV	
Σ Freq. of Total	
CD3+ Cells	
CD19+ Cells	

The bottom table shows the statistical results for each gate:

Name	Statistic	#Cells
A1.fcs		34711
Lymphocytes	60.9%	21127
Singlets	99.6%	21046
Σ (Comp-APC-Ax700-A) CD3 : Median	2.75E4	
Σ (Comp-APC-Ax700-A) CD3 : Robust CV	53.9	
Σ Freq. of Total	60.6%	
CD3+ Cells	44.7%	9409
CD19+ Cells	4.61%	971
A2.fcs		56304
Lymphocytes	66.7%	37573
Singlets	99.6%	37432
Σ (Comp-APC-Ax700-A) CD3 : Median	2.74E4	
Σ (Comp-APC-Ax700-A) CD3 : Robust CV	53.6	
Σ Freq. of Total	66.5%	
CD3+ Cells	41.2%	15423
CD19+ Cells	5.90%	2210
A3.fcs		78269
Lymphocytes	67.0%	52407
Singlets	99.6%	52186
Σ (Comp-APC-Ax700-A) CD3 : Median	2.98E4	
Σ (Comp-APC-Ax700-A) CD3 : Robust CV	53.0	
Σ Freq. of Total	66.7%	
CD3+ Cells	39.8%	20773
CD19+ Cells	6.16%	3216
A4.fcs		65803
Lymphocytes	61.7%	40599
Singlets	99.7%	40494
Σ (Comp-APC-Ax700-A) CD3 : Median	3.13E4	
Σ (Comp-APC-Ax700-A) CD3 : Robust CV	65.8	
Σ Freq. of Total	61.5%	

Right Screenshot: Group Analysis

The top toolbar shows icons for creating groups and analyses. A red box highlights the 'U' icon (Create Group).

The 'Group Analysis' table shows the statistical results for each gate:

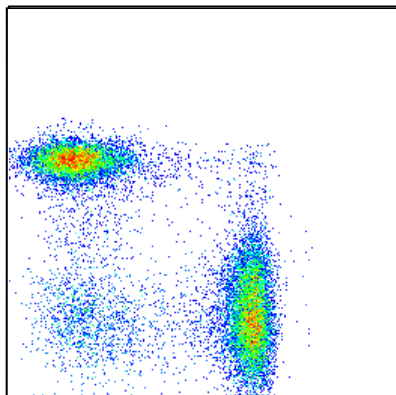
Name	Statistic	#Cells
All Samples		43
8-Color PBMC data		43
Compensation		7
Exp. 1		18
Exp. 2		18
Panel 1		9
Panel 2		9
Panel_1		9
Lymphocytes		9
Singlets		9
Σ (Comp-APC-Ax700-A) : Median		
Σ (Comp-APC-Ax700-A) : Robust CV		
Σ Freq. of Total A1.fcs		
CD19 subset		

The bottom table shows the statistical results for each gate:

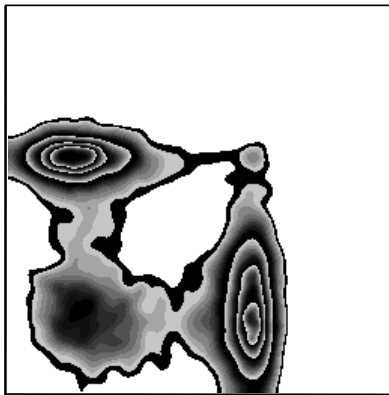
Name	Statistic	#Cells
A1.fcs		34711
Lymphocytes	61.8%	21461
Single Cells	99.5%	21353
CD3 subset	84.6%	18075
Σ (Comp-APC-Ax700-A)CD3 : Median	2.93E4	
Σ (Comp-APC-Ax700-A)CD3 : Robust CV	36.5	
Σ Freq. of Total A1.fcs	52.1%	
CD19 subset	3.97%	848
A2.fcs		56304
Lymphocytes	68.1%	38333
Single Cells	99.5%	38140
CD3 subset	84.4%	32187
Σ (Comp-APC-Ax700-A)CD3 : Median	2.92E4	
Σ (Comp-APC-Ax700-A)CD3 : Robust CV	37.5	
Σ Freq. of Total A1.fcs	57.2%	
CD19 subset	5.12%	1952
A3.fcs		78269
Lymphocytes	66.5%	52076
Single Cells	99.5%	51799
CD3 subset	84.3%	43648
Σ (Comp-APC-Ax700-A)CD3 : Median	3.17E4	
Σ (Comp-APC-Ax700-A)CD3 : Robust CV	32.3	
Σ Freq. of Total A1.fcs	55.8%	
CD19 subset	5.71%	2957
A4.fcs		65803

Data Display Types

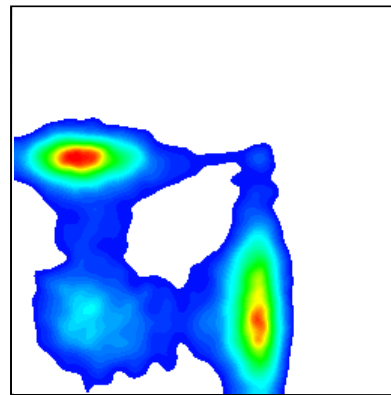
Pseudo-color Dot



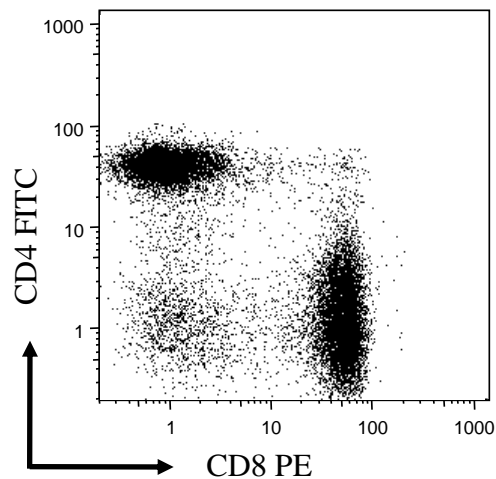
Zebra



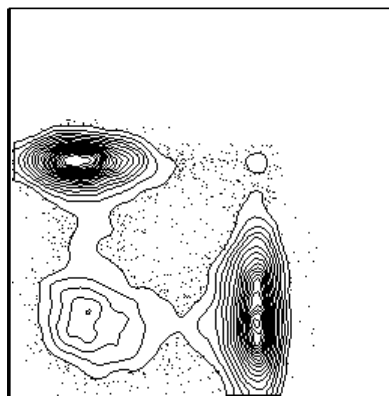
Pseudo-color Density



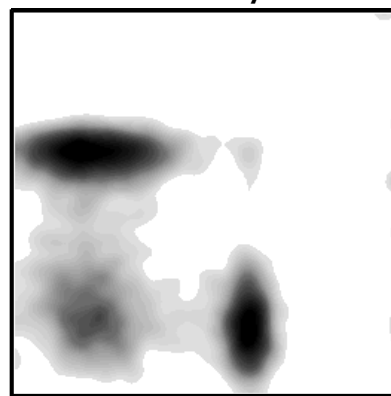
Dot



Contour

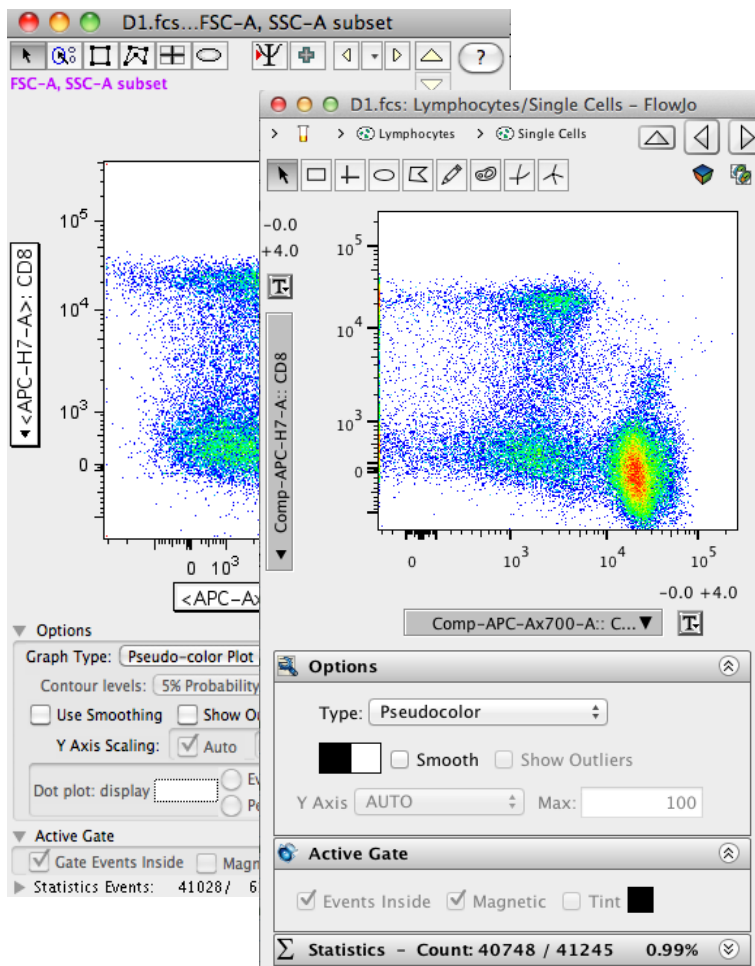


Density

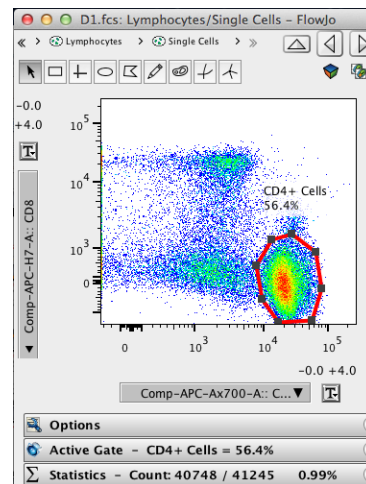


Gating

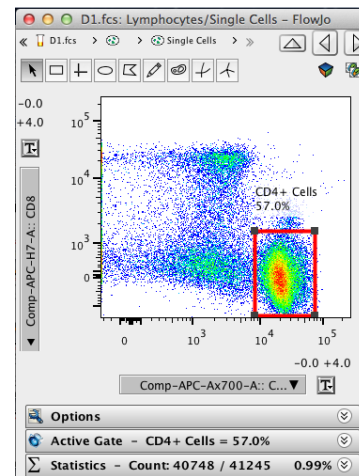
Graph Window



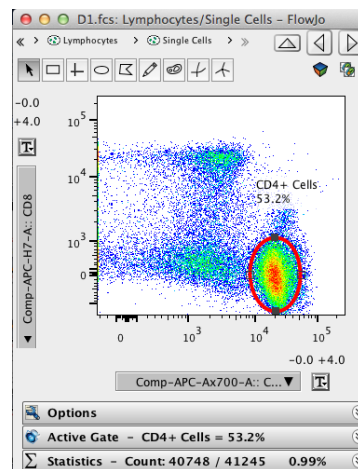
Polygon



Rectangular

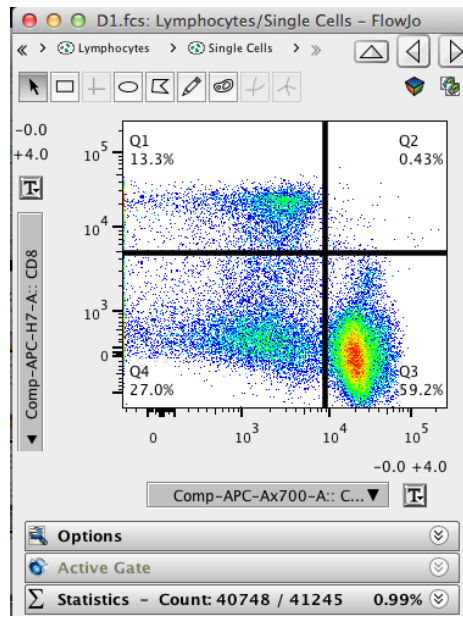


Elliptical

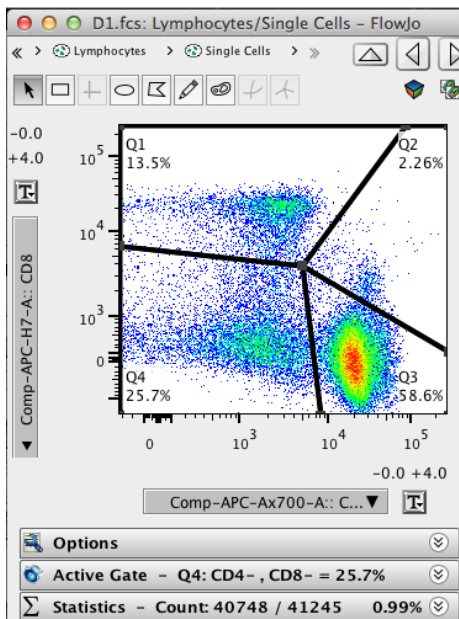


Advanced Gating

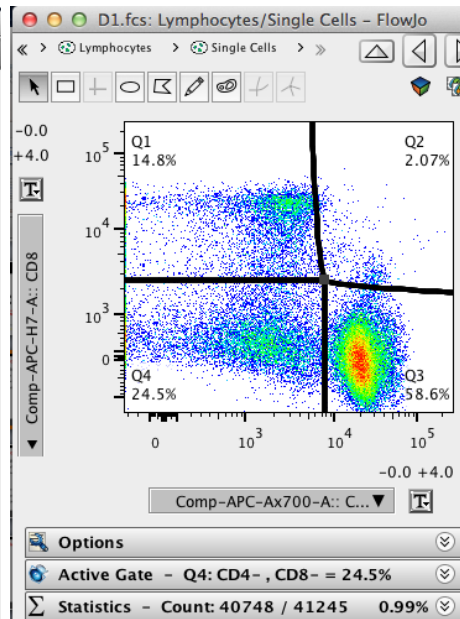
Quad



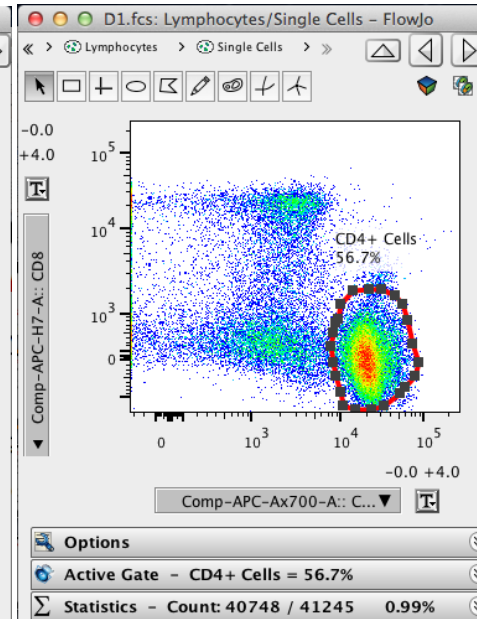
Spider



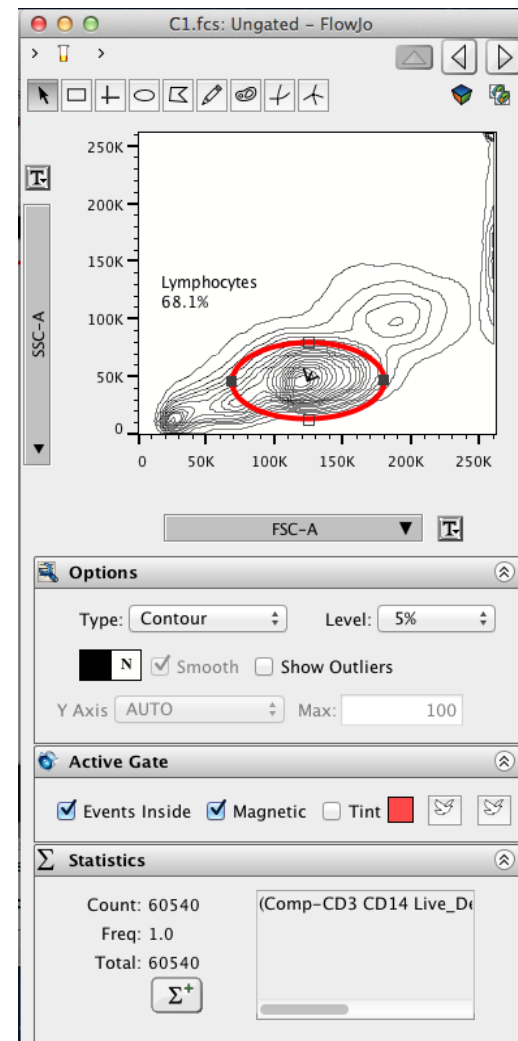
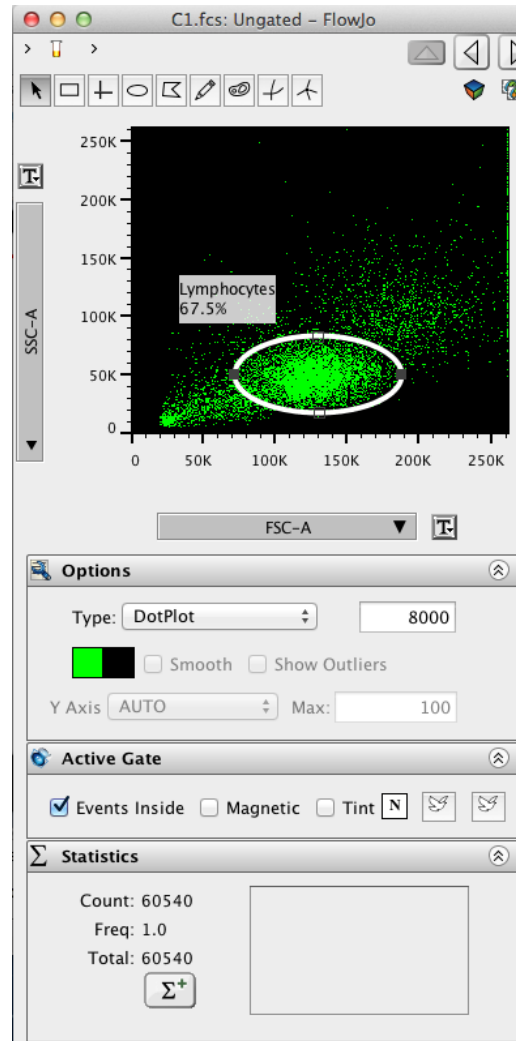
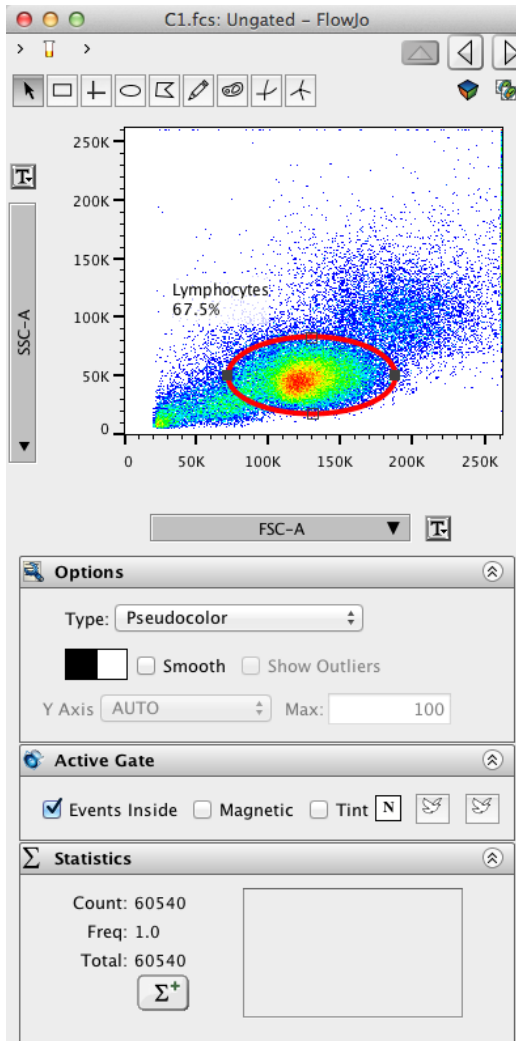
Curly



Auto



Advanced Plotting Tools



Grouping Samples

Samples quickly organized using keywords

The image displays two screenshots of the FlowJo software interface, illustrating sample grouping and analysis.

Left Screenshot: Groups and Analyses

This panel shows a hierarchical tree structure of sample groups. The top-level group is "All Samples", which contains "Compensation", "Panel 1", and "Panel 2". "Panel 1" is expanded, showing "Lymphocytes" and "Singlets". "Lymphocytes" is further expanded, showing "Singlets", "Σ (Comp-APC-Ax700-A) : Median", "Σ (Comp-APC-Ax700-A) : Robust CV", "Σ Freq. of Total", "CD3+ Cells", and "CD19+ Cells". "Panel 2" is also expanded, showing "Singlets", "Σ (Comp-APC-Ax700-A) : Median", "Σ (Comp-APC-Ax700-A) : Robust CV", "Σ Freq. of Total", "CD3+ Cells", and "CD19+ Cells".

Right Screenshot: Main FlowJo Interface

This panel shows the main FlowJo interface with a menu bar (FlowJo, File, Edit, Workspace, Tools, Settings) and a toolbar. The "Groups" panel on the left lists the following groups:

Group	Size	Role
{ } All Samples	43	
{ } 8-Color PBMC data	43	
{ } Compensation	7	Compensa...
{ } Exp. 1	18	
{ } Exp. 2	18	
{ } Panel 1	9	
{ } Panel 2	9	
{ } Lymphocytes		
{ } Single Cells		
{ } Panel 1	9	Test Sample
{ } Lymphocytes		
{ } Single Cells		
{ } CD3 subset		
Σ (Comp-APC-A		
Σ (Comp-APC-A		
Σ Freq. of Total /		
{ } CD19 subset		
{ } Panel 2	9	

The "Group Analysis" panel on the right shows a list of groups for analysis, including "Exp. 1".

Grouping Samples

Samples quickly organized using keywords

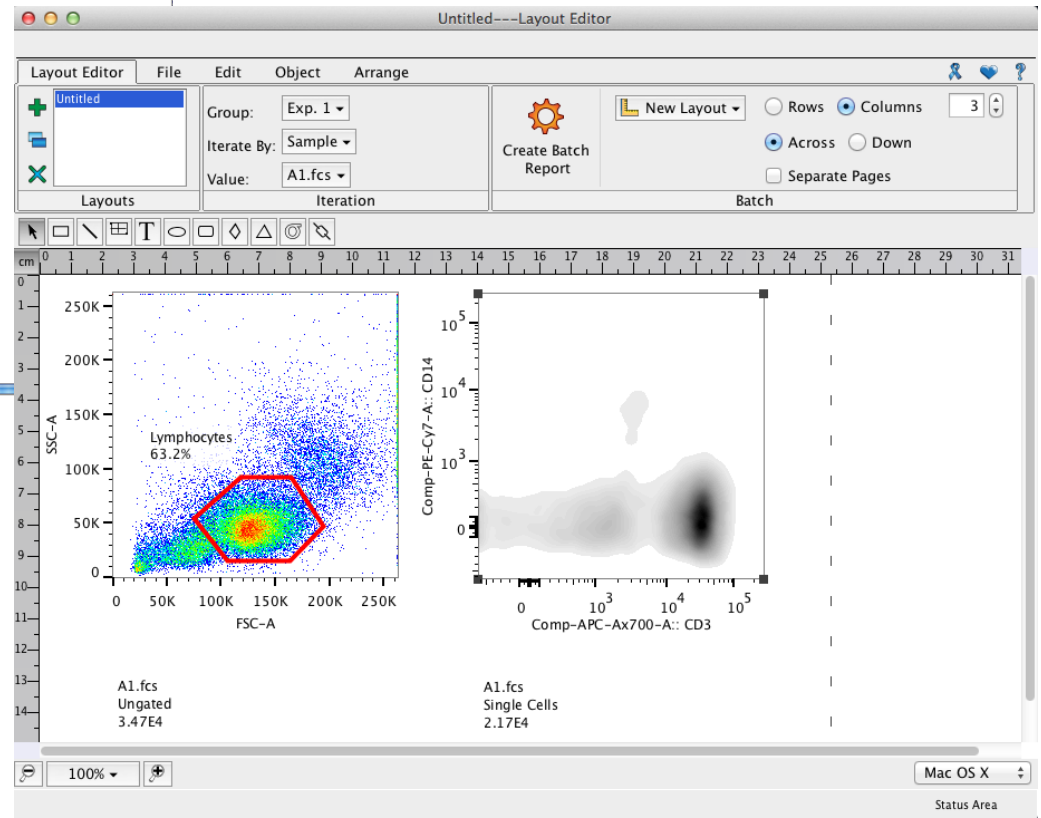
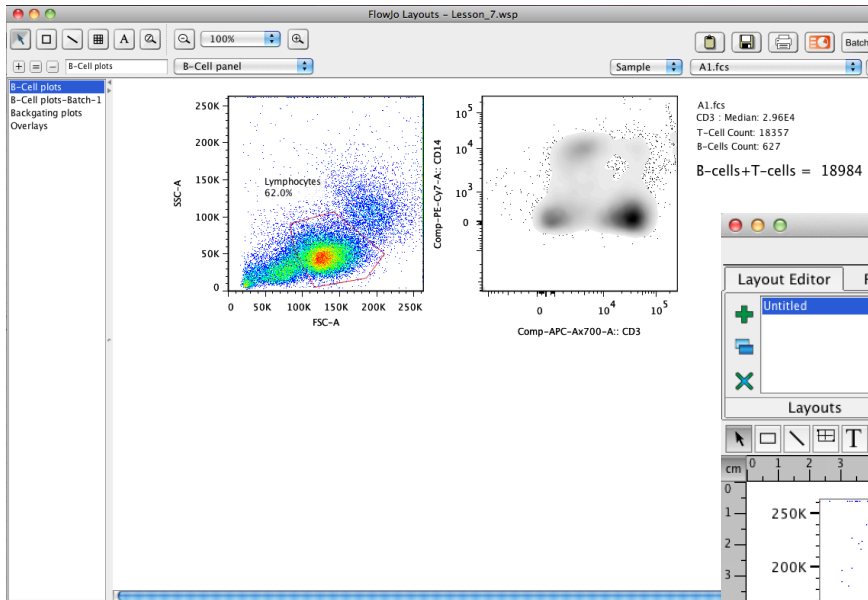
The screenshot displays the 'Create Group' dialog box with the following components:

- Appearance Section:**
 - Name:** New Group
 - Color:** Blue
 - Style:** Plain (dropdown menu with options: Plain, Bold, Italic, Bold-Italic)
 - Role:** Test Sample
 - Parameter Key:** (empty field)
- Sample Inclusion Criteria Section:**
 - Live group:** ☒ **Synchronized:** ☐
 - Include samples that use the following staining:** (Multiple) CD32, CD86, CD209, CD14, 7AAD, 2H2, CD3, CD19, CD56, CD11c, CD3, CD45RO, 7AAD, 2H2, CD4, CD8
 - Operator:** = (dropdown menu with options: =, #, >, >=, <, <=, Contains, Lacks, Exists)
 - Keyword:** *Any Keyword* (dropdown menu with options: *Any Keyword*, \$B1M, \$CYT, \$DATE, \$ENDSTEXT, \$ETIM, \$FIL, \$INST, \$MODE, \$OP, \$P1N, \$P1S, \$P2N, \$P2S, \$P3N, \$P3S, \$P4N, \$P4S, \$P5N)
 - Buttons:** More Choices, Fewer Choices, Show all keywords in menus (checkbox)
- With reference to samples in another group:**
 - Only choose from:** ☐ **Also include:** ☒ samples in Group (No specified group) (dropdown menu)
- Buttons:** Apply Changes, Close, Create Group

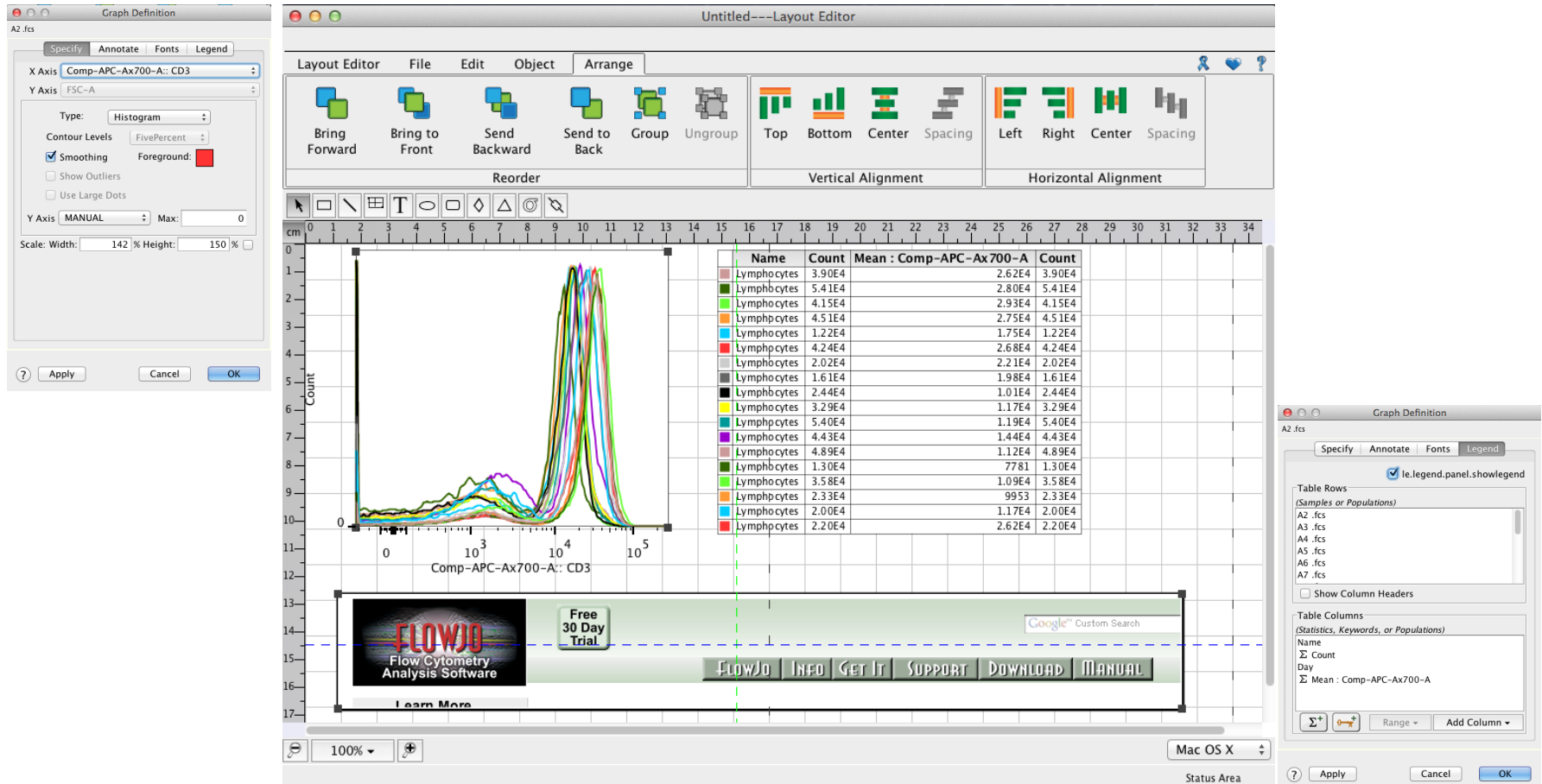
Red arrows indicate the following interactions:

- Arrow pointing to the keyword list on the left.
- Arrow pointing to the 'Style' dropdown menu.
- Arrow pointing to the 'Contains' operator in the criteria section.
- Arrow pointing to the 'Also include' radio button.

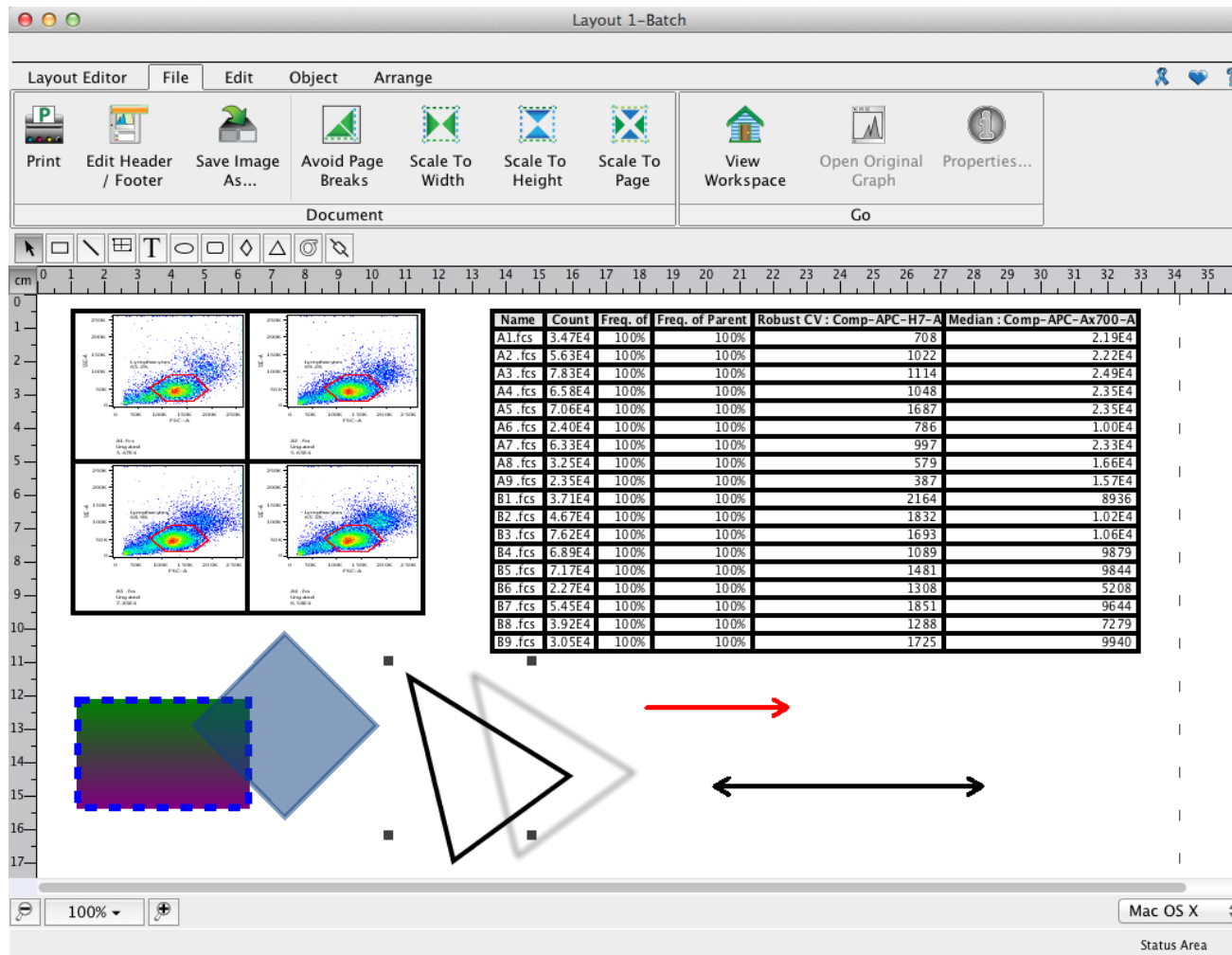
Layout Editor: Creating Figures



Layout Editor: Creating Figures



Layout Editor: Creating Figures



Iterative Analysis Through “Batching”

FlowJo does the work for you!

Perform an operation once and instruct the software to repeat it.

Data can be exported in a variety of ways:

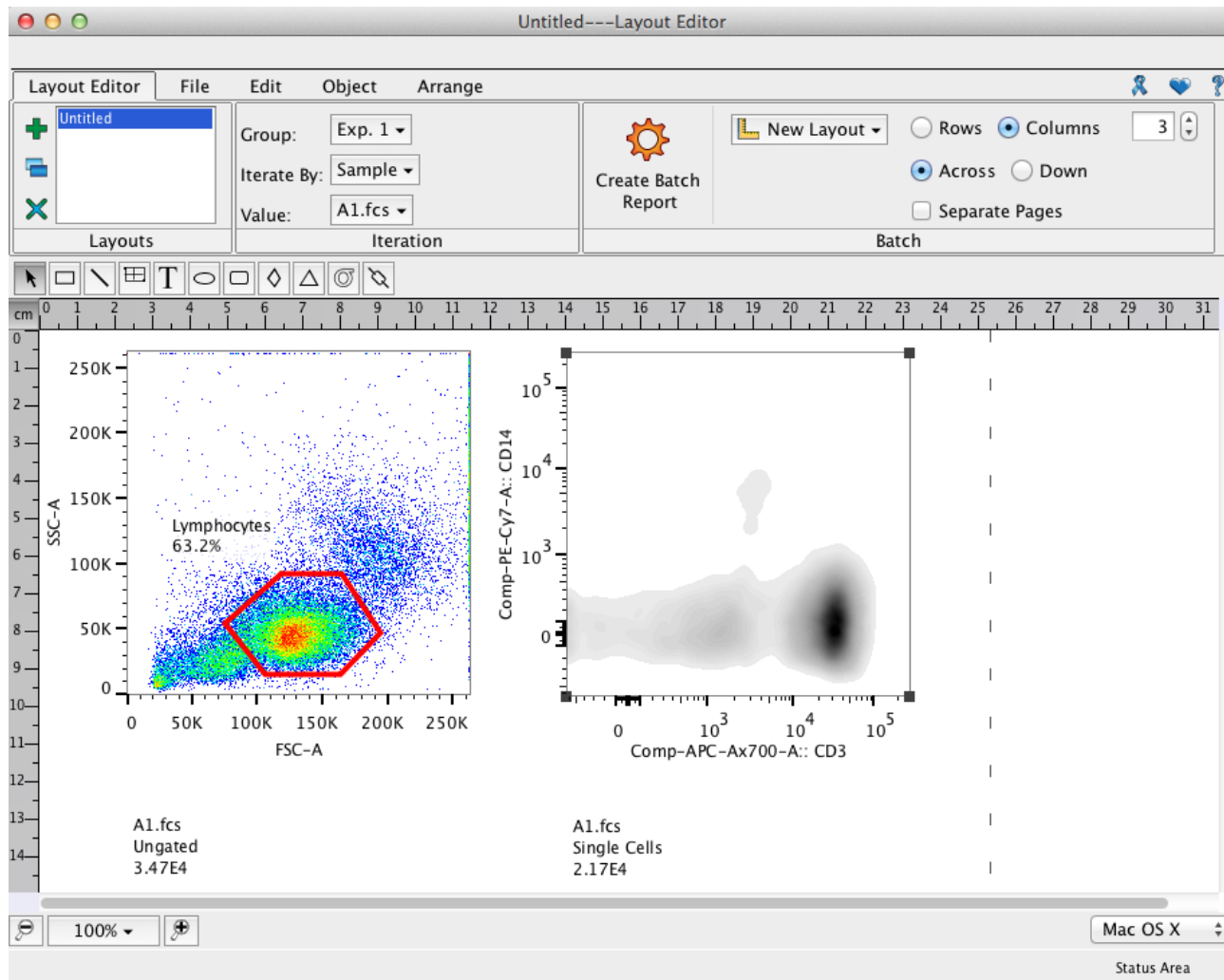
PC



Mac



Iterative Analysis Through “Batching”



Iterative Analysis Through “Batching”

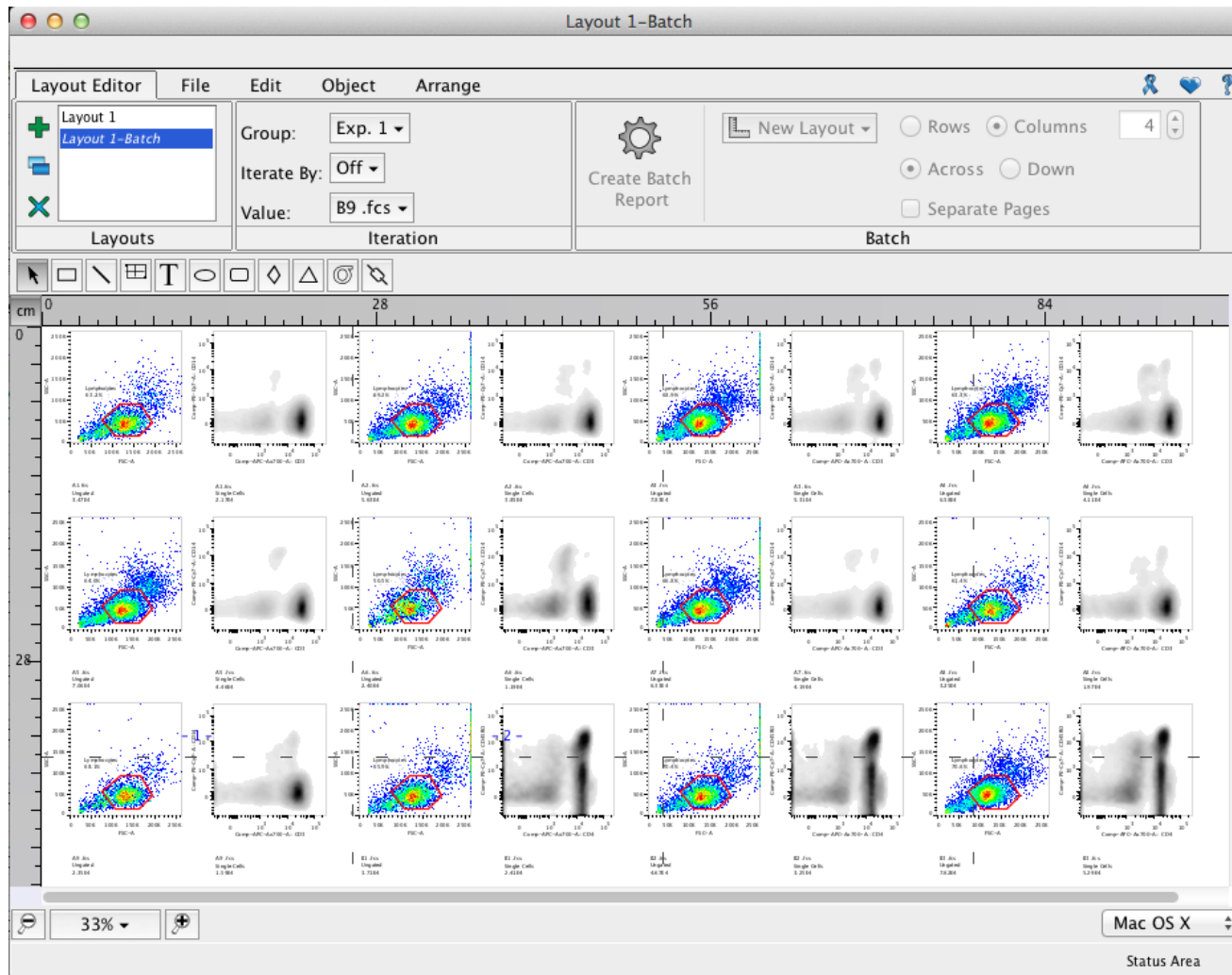


Table Editor: Creating Statistics

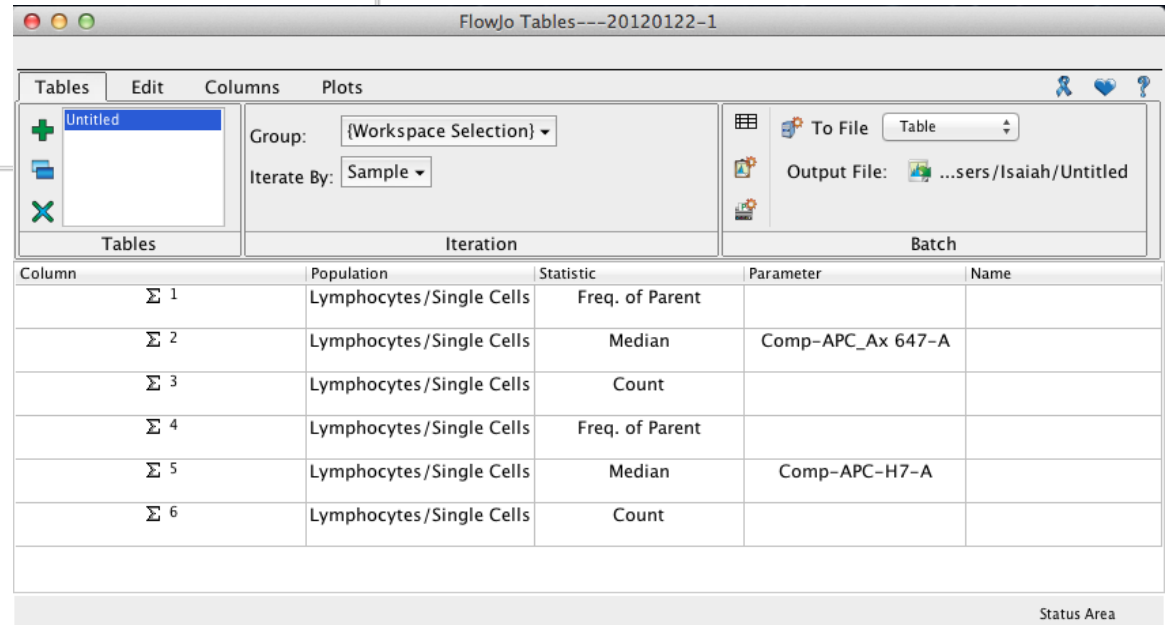
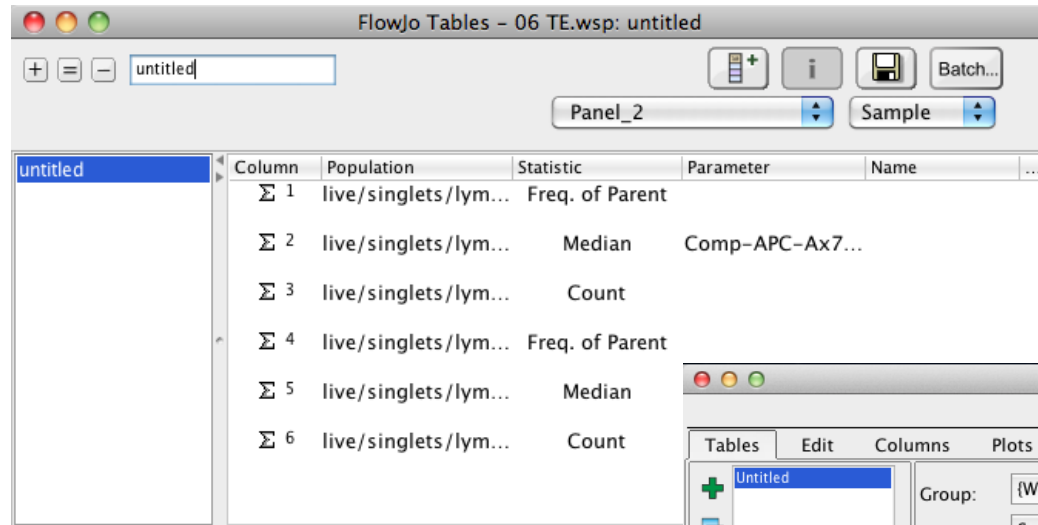


Table Editor: Creating Statistics

The screenshot displays the FlowJo Table Editor window, titled "FlowJo Tables---20120122-1". The interface includes a left sidebar with a list of parameters, a top menu bar with "Tables", "Edit", "Columns", and "Plots", and a main workspace. A red arrow points to the "Add Column" button in the "Columns" tab. Another red arrow points to the "Heat Map" button in the "Formatting" section. A third red arrow points to the "References..." button in the "Insert Reference" field of the "Formula" tab.

The "Columns" tab is active, showing a table with the following data:

Column	Population	Statistic	Parameter	Name
Σ 1	Lymphocytes/Single...	Freq. of Parent		
Σ 2	Lymphocytes/Single...	Median	Comp-APC_Ax 647-A	
Σ 3	Lymphocytes/Single...	Count		
4	Day			

The "Formula" tab is selected, showing the formula: `<Column "Lymphocytes/Single Cells|Lymphocytes/Single Cells|Freq. of Parent"> / 2`. The "Insert Reference" field is set to "References...", and the "Insert Function" field is set to "Functions...".

References...

Lymphocytes/Single Cells|Lymphocytes/Single Cells|Freq. of Parent

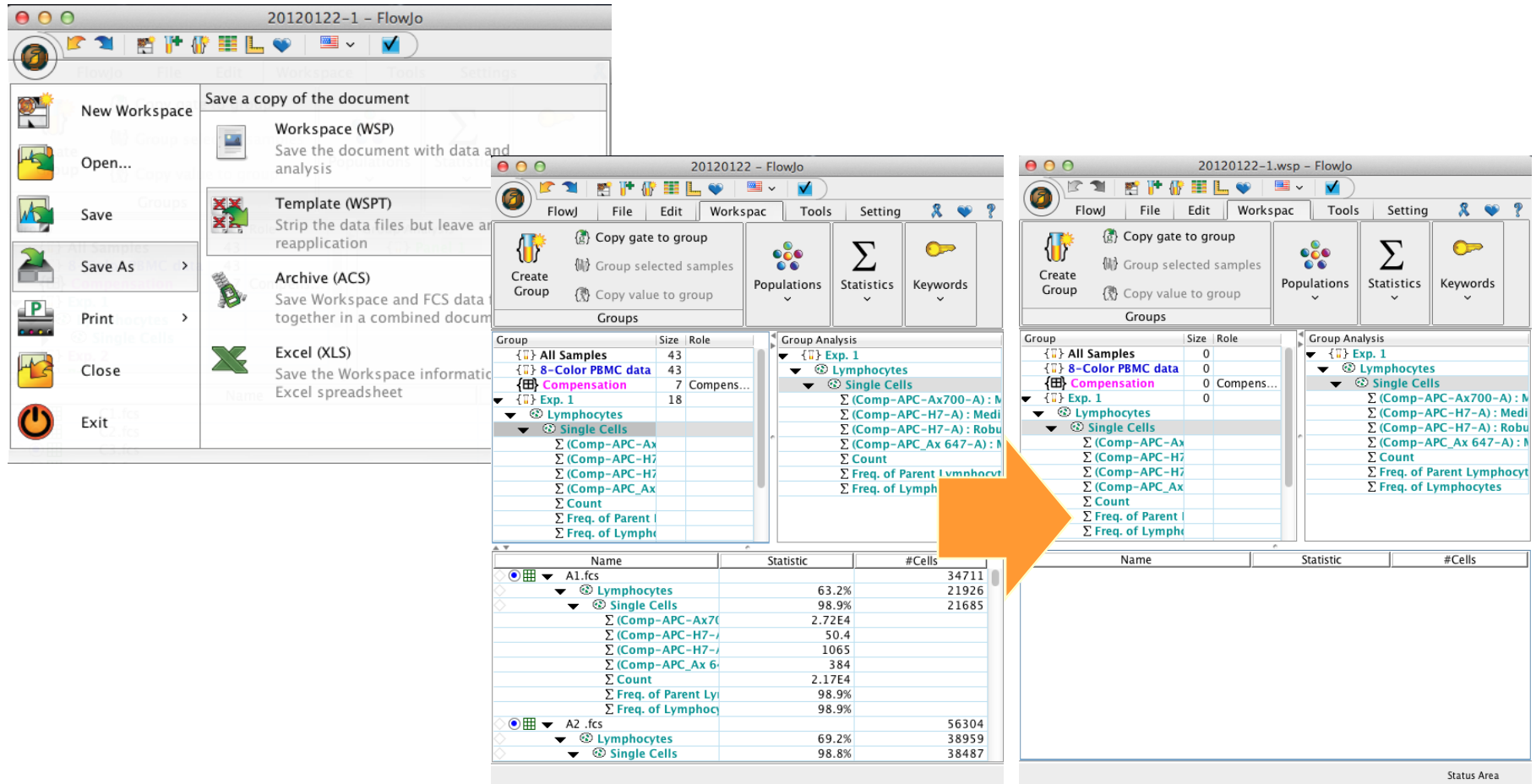
Lymphocytes/Single Cells|Lymphocytes/Single Cells|Median (Comp-APC_Ax 647-A)

Day

Table Editor: Exporting the Table

Table - Untitled				
Ancestry Subset Statistic For	Lymphocytes/Single Cells Freq. of Parent	Lymphocytes/Single Cells Median Comp-APC_Ax 647-A	Lymphocytes/Single Cells Count [0.1-0.24]	Day
A1.fcs	98.9%	384	▲ 2.17E4	0
A2.fcs	98.8%	385	▲ 3.85E4	2
A3.fcs	98.4%	385	▲ 5.31E4	4
A4.fcs	98.7%	392	▲ 4.11E4	6
A5.fcs	98.7%	365	▲ 4.46E4	8
A6.fcs	98.6%	413	▲ 1.19E4	10
A7.fcs	98.9%	452	▲ 4.19E4	12
A8.fcs	98.5%	499	▲ 1.97E4	14
A9.fcs	99.3%	541	▲ 1.59E4	16
B1.fcs	98.6%	108	▲ 2.41E4	0
B2.fcs	98.8%	133	▲ 3.25E4	2
B3.fcs	98.3%	105	▲ 5.29E4	4
B4.fcs	98.9%	144	▲ 4.39E4	6
B5.fcs	98.9%	156	▲ 4.83E4	8
B6.fcs	98.9%	261	▲ 1.28E4	10
B7.fcs	98.6%	99.1	▲ 3.53E4	12
B8.fcs	97.9%	189	▲ 2.29E4	14
B9.fcs	98.9%	362	▲ 1.97E4	16
Mean	98.7%	298	3.23E4	800%
SD	0.30%	149	1.39E4	531%

Rapid-Fire Template Creation



20120122-1 - FlowJo

Save a copy of the document

Workspace (WSP)
Save the document with data and analysis

Template (WSPT)
Strip the data files but leave an application

Archive (ACS)
Save Workspace and FCS data together in a combined document

Excel (XLS)
Save the Workspace information in an Excel spreadsheet

20120122 - FlowJo

FlowJ File Edit Workspac Tools Setting

Create Group Copy gate to group Group selected samples Copy value to group

Populations Statistics Keywords

Groups

Group	Size	Role
All Samples	43	
8-Color PBMC data	43	
Compensation	7	Compens...
Exp. 1	18	
Lymphocytes		
Single Cells		
Σ (Comp-APC-Ax700-A) : M		
Σ (Comp-APC-H7-A) : Medi		
Σ (Comp-APC-H7-A) : Robu		
Σ (Comp-APC_Ax 647-A) : M		
Σ Count		
Σ Freq. of Parent Lymphocyt		
Σ Freq. of Lymphocytes		

Group Analysis

Exp. 1

Lymphocytes

Single Cells

Σ (Comp-APC-Ax700-A) : M

Σ (Comp-APC-H7-A) : Medi

Σ (Comp-APC-H7-A) : Robu

Σ (Comp-APC_Ax 647-A) : M

Σ Count

Σ Freq. of Parent Lymphocyt

Σ Freq. of Lymphocytes

Name	Statistic	#Cells
A1.fcs		34711
Lymphocytes	63.2%	21926
Single Cells	98.9%	21685
Σ (Comp-APC-Ax700-A) : M	2.72E4	
Σ (Comp-APC-H7-A) : Medi	50.4	
Σ (Comp-APC-H7-A) : Robu	1065	
Σ (Comp-APC_Ax 647-A) : M	384	
Σ Count	2.17E4	
Σ Freq. of Parent Lymphocyt	98.9%	
Σ Freq. of Lymphocytes	98.9%	
A2.fcs		56304
Lymphocytes	69.2%	38959
Single Cells	98.8%	38487

20120122-1.wsp - FlowJo

FlowJ File Edit Workspac Tools Setting

Create Group Copy gate to group Group selected samples Copy value to group

Populations Statistics Keywords

Groups

Group	Size	Role
All Samples	0	
8-Color PBMC data	0	
Compensation	0	Compens...
Exp. 1	0	
Lymphocytes		
Single Cells		
Σ (Comp-APC-Ax700-A) : M		
Σ (Comp-APC-H7-A) : Medi		
Σ (Comp-APC-H7-A) : Robu		
Σ (Comp-APC_Ax 647-A) : M		
Σ Count		
Σ Freq. of Parent Lymphocyt		
Σ Freq. of Lymphocytes		

Group Analysis

Exp. 1

Lymphocytes

Single Cells

Σ (Comp-APC-Ax700-A) : M

Σ (Comp-APC-H7-A) : Medi

Σ (Comp-APC-H7-A) : Robu

Σ (Comp-APC_Ax 647-A) : M

Σ Count

Σ Freq. of Parent Lymphocyt

Σ Freq. of Lymphocytes

Name	Statistic	#Cells
------	-----------	--------

Status Area

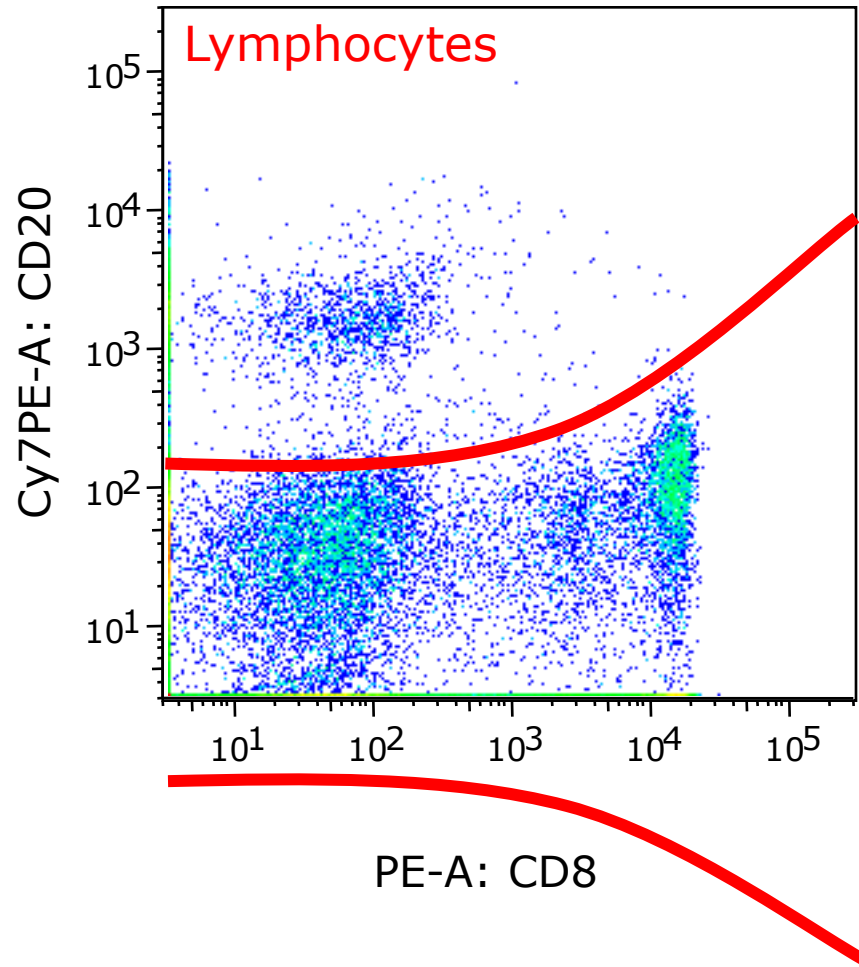
FlowJo Version 10 - Chimera

- New Ribbon Interface
- Translations
- Undo Buttons
- Breadcrumb Bar
- New Compensation Wizard
- Optimized Preference Settings



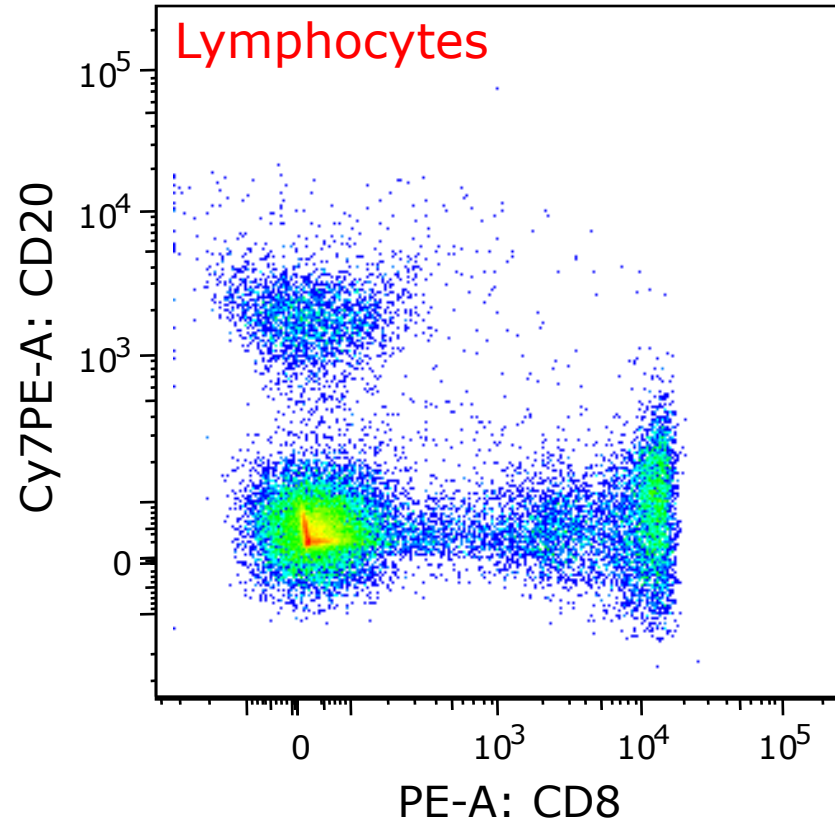
Biexponential Transformation

The Actual Spread

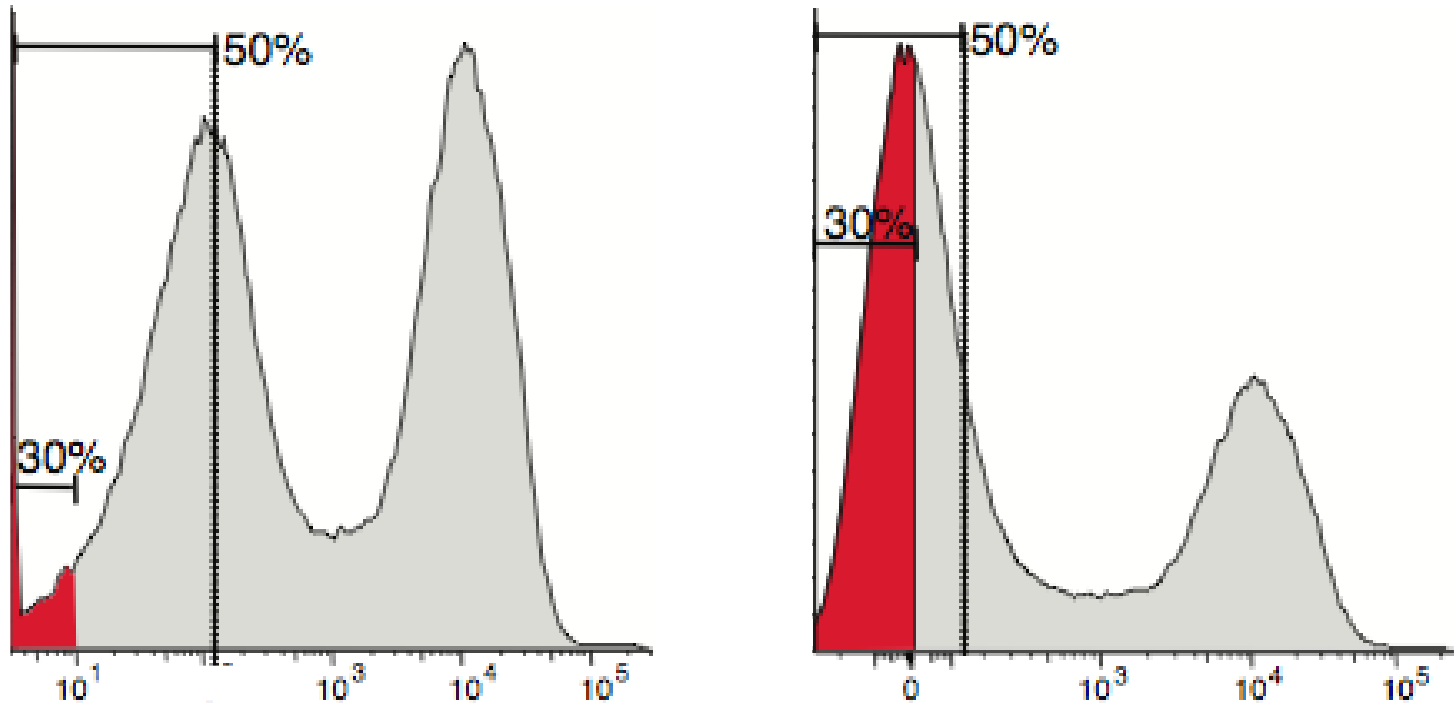


Biexponential Transformation

New Display

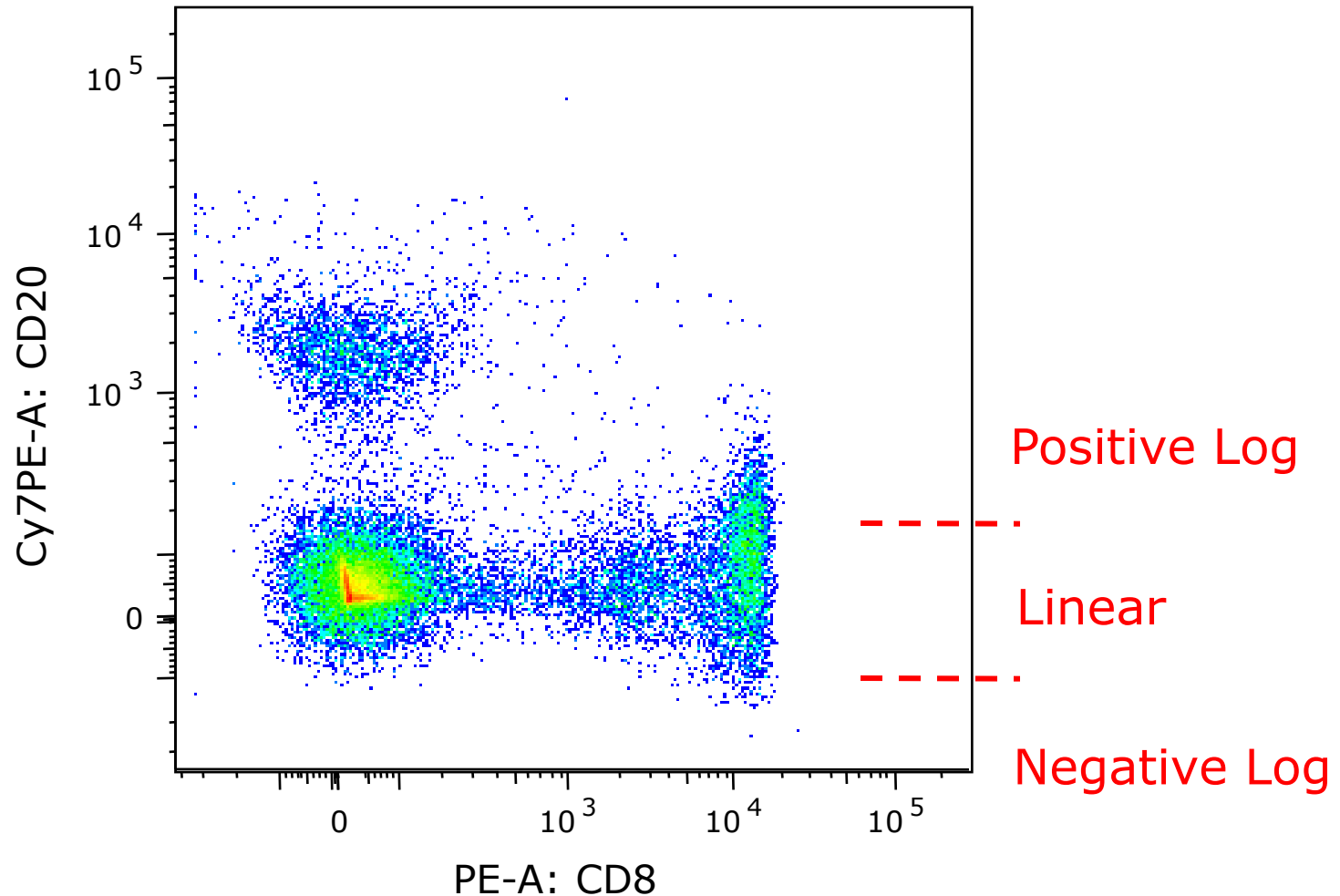


Biexponential Transformation

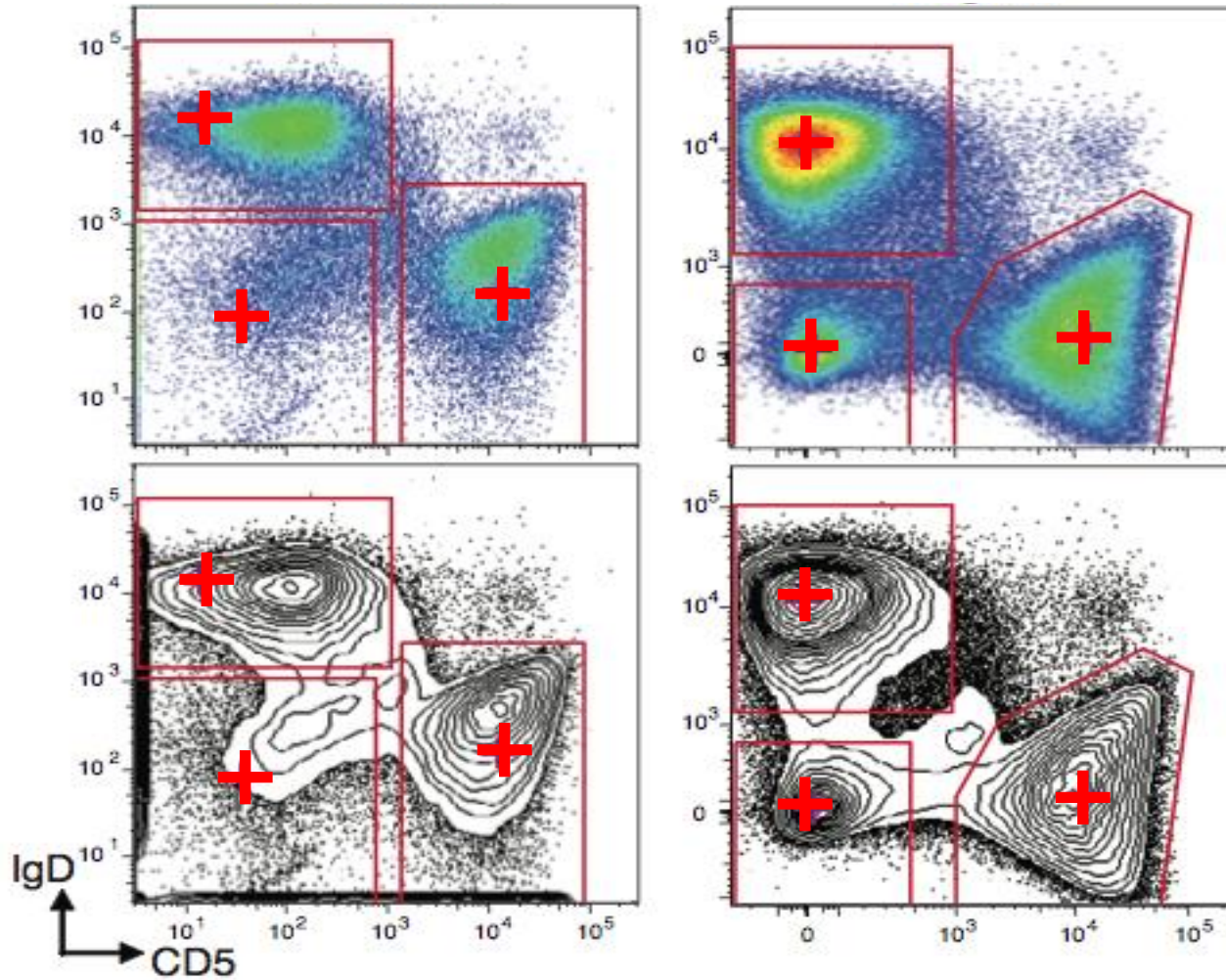


Baseline subtractions or compensation result in NEGATIVE values
Transformation “EXPANDS” log scale for lows and negatives
Data values are NOT altered – only the scale is altered

Biexponential Transformation

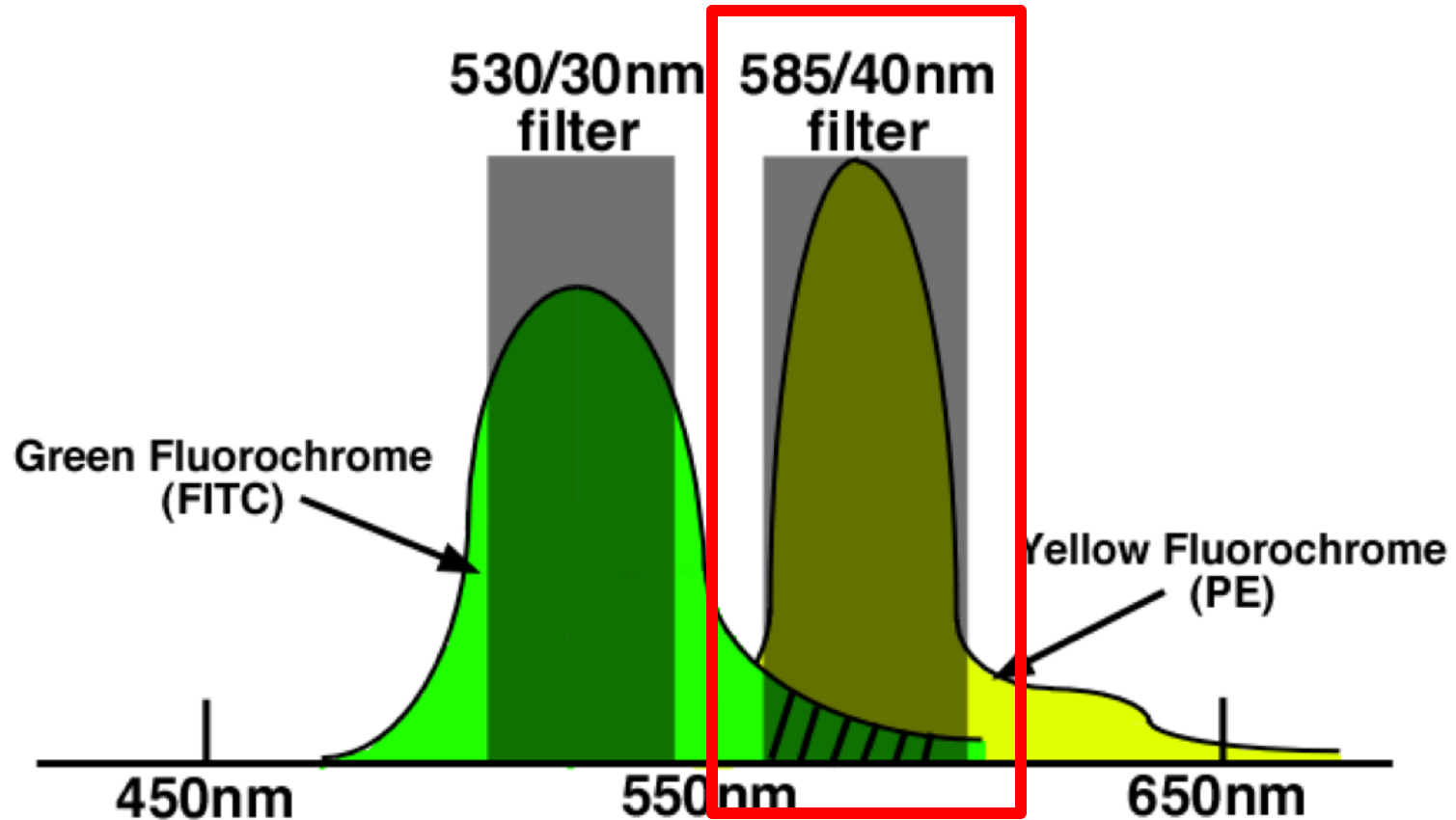


Biexponential Transformation



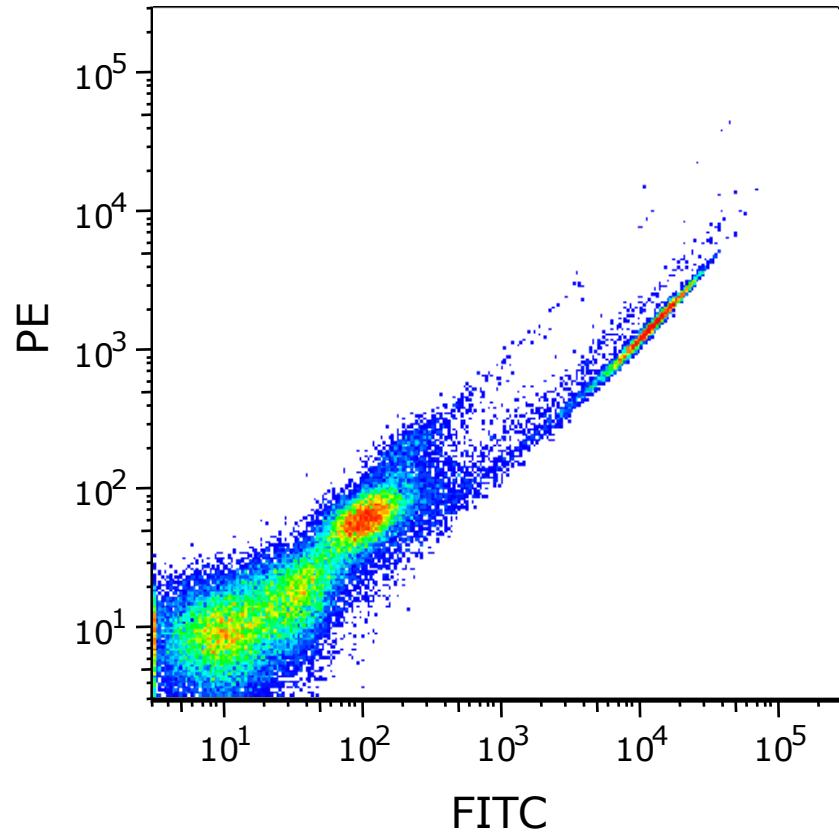
Compensation – What is it?

Removing the spillover fluorescence of a particular probe from the "wrong" channel.

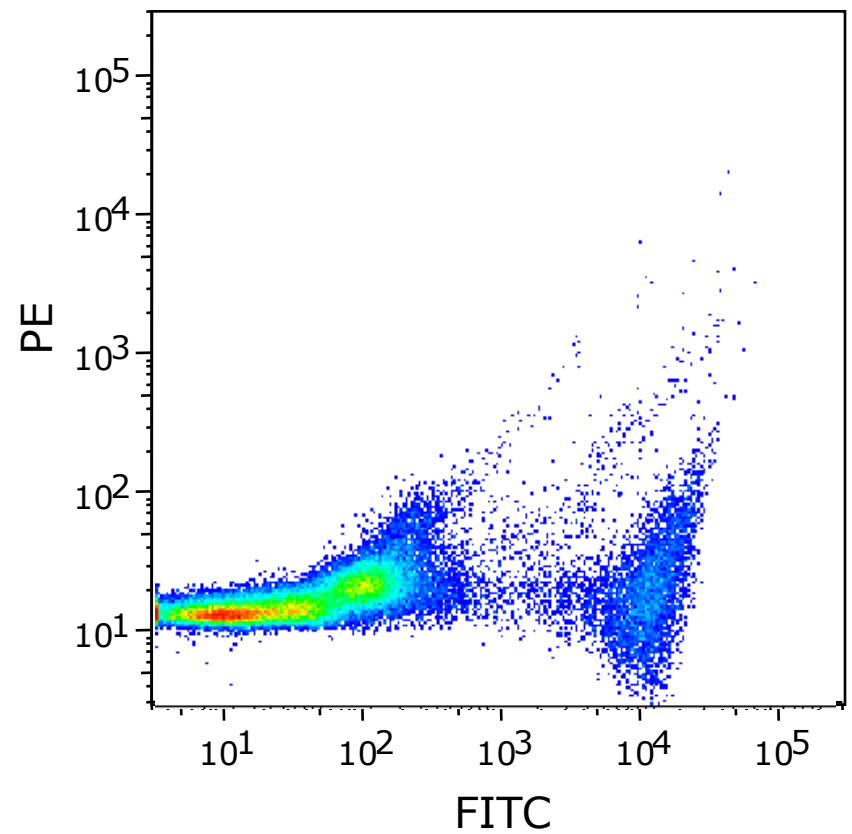


Compensation – Why is it important?

Uncompensated



Compensated



Compensation – How to do it?

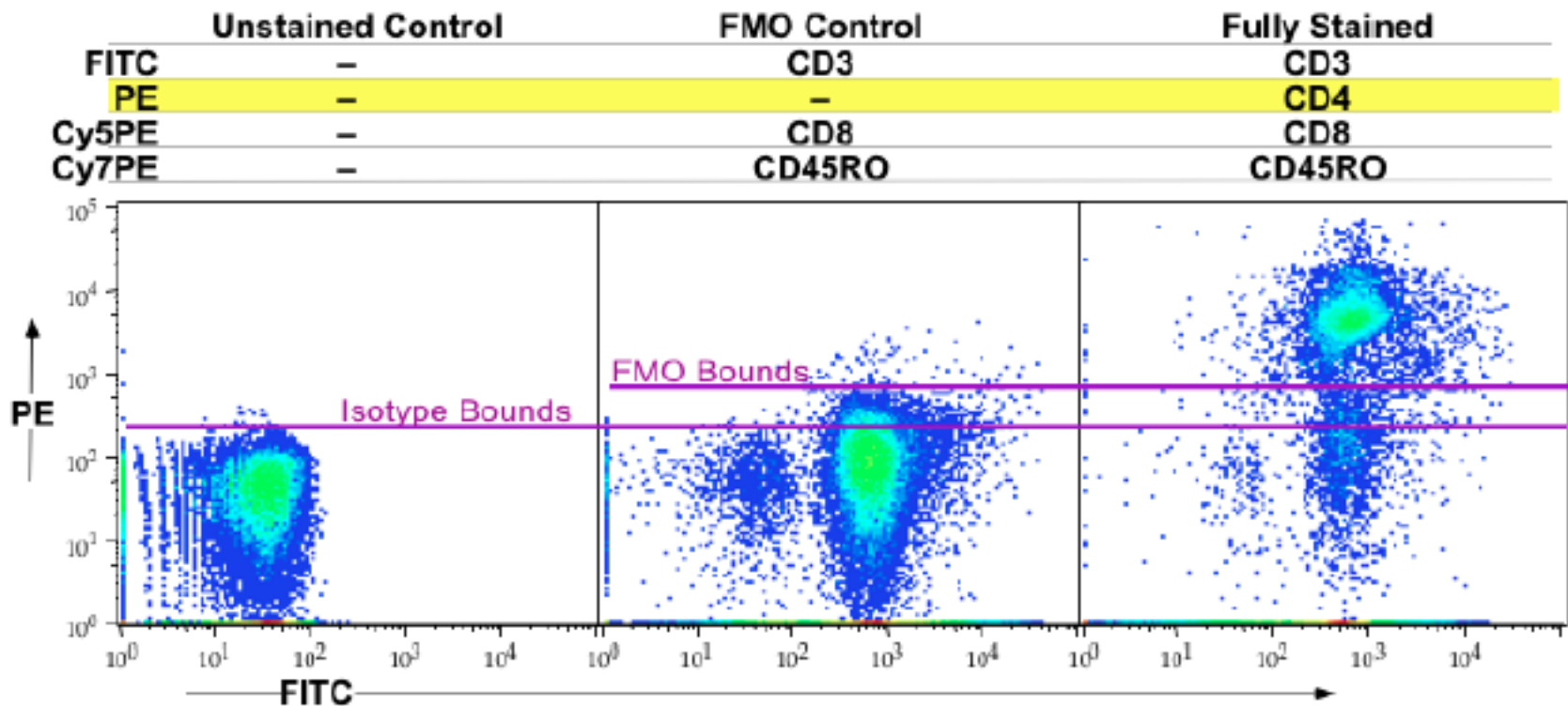
Setting Up Compensation Controls

- Controls should be **brighter** than samples
- Autofluorescence should be the same for positive and negative populations
- Compensation color MUST match experimental color
- Use the same tandem dye from the same manufacturer (and lot#)



Gating Controls

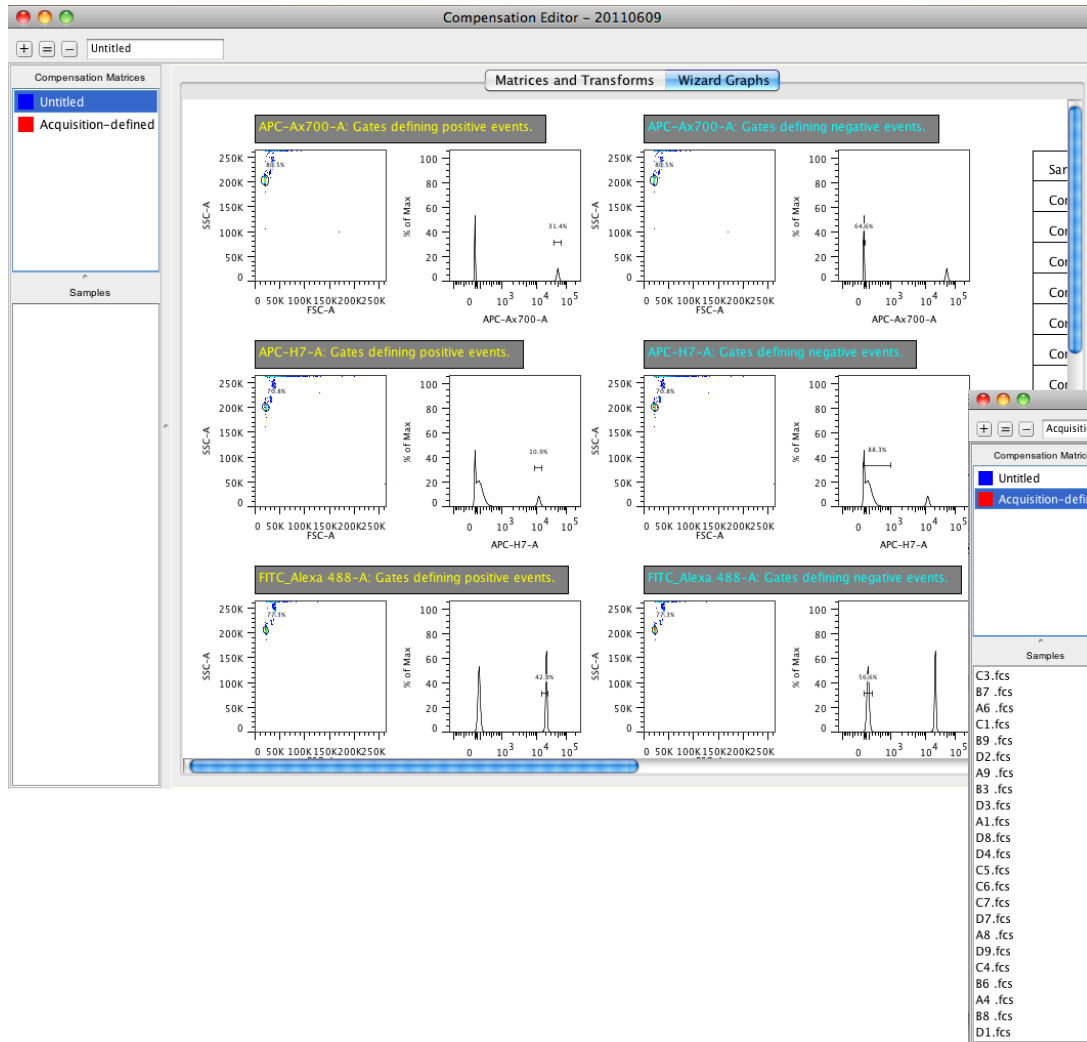
Fluorescence Minus One (FMO) Controls: leave out one reagent at a time (the opposite of single stain controls)



Gating Controls

Tube #	Description	FL1	FL2	FL3
1	<i>Experimental Sample</i>	CD3 FITC	CD4 PE	CD8 Cy5PE
2	<i>Compenstaion Controls</i> (Single stains – one for each fluorochrome used in the experiment)	CD3 FITC	-	-
3		-	CD4 PE	-
4		-	-	CD8 Cy5PE
5	<i>Gating Controls</i> (FMO – leave out one fluorochrome at a time)	-*	CD4 PE	CD8 Cy5PE
6		CD3 FITC	-	CD8 Cy5PE
7		CD3 FITC	CD4 PE	-

Current Compensation Wizard



Compensation Editor - 20110609

Acquisition-defined

Matrices and Transforms Wizard Graphs

Acquisition Matrix

Matrix as defined by \$COMP or \$SPILL

	FITC_Alexa...	PE-A	PE-Cy55-A	PE-Cy7-A	CD3 CD14 L...	APC_Ax 64...	APC-Ax700-A	APC-H7-A
FITC_Alexa 488-A	100%	16.6%	0.961%	0.151%	0.00%	0.021%	0.00%	0.00%
PE-A	0.738%	100%	11.7%	2.35%	0.009%	0.072%	0.063%	0.071%
PE-Cy55-A	0.025%	0.025%	100%	37.4%	0.00%	4.90%	76.5%	8.90%
PE-Cy7-A	0.081%	0.854%	0.294%	100%	0.00%	8.61%	10.9%	10.5%
CD3 CD14 Live_Death-A	0.537%	0.179%	0.00%	0.00%	100%	0.00%	0.00%	0.00%
APC_Ax 647-A	0.010%	0.00%	0.270%	0.062%	0.00%	100%	105%	8.88%
APC-Ax700-A	0.025%	0.014%	0.288%	0.242%	0.00%	0.257%	100%	7.87%
APC-H7-A	0.083%	0.00%	0.012%	1.71%	0.039%	5.15%	17.9%	100%

Transformation Settings

Transformation settings are displayed in order from most general to most specific. Right-click to perform operations on the transformations. Click the mouse in the number field to enter a new value.

FCS3.0 Settings * 0.0 4.5 -100.0

All Samples <Choose Param...



Version 10 Compensation Wizard

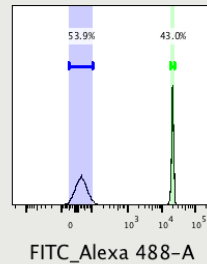
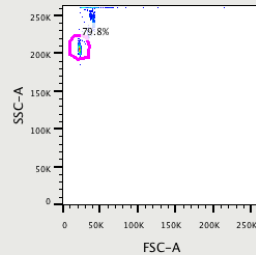
Compensation

[M] Apply To Group ▾ Compensation Matrix Editor Calculate Reset

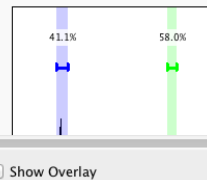
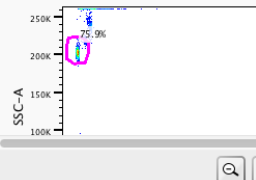
Confirm gates and control assignments look correct. Double click a graph to edit it.

Sample		Negative	Positive
FITC_Alexa 488-A	CompCtrl AF488.fcs	Size/FITC_Alexa 488-A-	Size/FITC_Alexa 488-A+
PE-A	CompCtrl PE.fcs	Size/PE-A-	Size/PE-A+
PE-Cy55-A	CompCtrl PECy55.fcs	Size/PE-Cy55-A-	Size/PE-Cy55-A+
PE-Cy7-A	CompCtrl PECy7.fcs	Size/PE-Cy7-A-	Size/PE-Cy7-A+
APC_Ax 647-A	CompCtrl AF647.fcs	Size/APC_Ax 647-A-	Size/APC_Ax 647-A+
APC-Ax700-A	CompCtrl AF700.fcs	Size/APC-Ax700-A-	Size/APC-Ax700-A+
APC-H7-A	CompCtrl APCH7.fcs	Size/APC-H7-A-	Size/APC-H7-A+

FITC_Alexa 488-A



PE-A



Show Overlay

Compensation

[M] Editing compensation matrix: Compensation Reset Finalized

Show All

	FITC_Alexa...	PE-A	PE-Cy55-A	PE-Cy7-A	APC_Ax 64...	APC-Ax70...	APC-H7-A
FITC_Alexa...	16.35	0.92	0.11	-0.01	0.02	0.00	
PE-A	0.79	11.88	2.31	0.07	0.00	0.00	
PE-Cy55-A	0.04	0.01	37.04	4.59	70.26	8.24	
PE-Cy7-A	0.07	0.86	0.24	0.01	1.90	7.74	
APC_Ax 647-A	0.00	0.00	0.28	0.05	22.53	8.70	
APC-Ax700-A	0.02	0.02	0.33	0.26	0.23	15.04	
APC-H7-A	0.02	0.02	-0.03	1.40	2.51		

Preview Sample: D2.fcs View Overlay Uncompensated Animate

PE-A PE-Cy55-A PE-Cy7-A APC_Ax 647-A APC-Ax700-A APC-H7-A

FITC_Alexa

PE-A

PE-Cy55-A

PE-Cy7-A

APC_Ax 647-A

APC-Ax700-A


APC-H7-A

Status Area Manual editing of the matrix and transform settings override derived values.


FLUORISH! Panel Wizard


- Create an account and join a circle
- Stepwise process for antibody panel design
- Takes into account instrument selection and configuration
- Target-fluorochrome refinement
- Combined product databases from multiple vendors (BD, eBio, BioL, Miltenyi, etc.)

Fluorish




Nicholas Ostrout
Logout
[Account preferences](#)

[Home](#) [Panels](#) [Instruments](#) [Lab Inventory](#) [Cart](#) [Search for Reagents](#) 




Get the Fluorish Panel Wizard
v1.0b25
06/07/2011
[Show System Requirements](#)

We are building this for you...any feedback?




US/Canada 800-366-6045
International 541-201-0022




Core Information

☐ Create a new core account? [?](#)




Cores you manage


 Ultimate Core [Show Users](#)

Cores you have joined

 treecore1 [Remove Me](#)

Cores available to join

 treecore1 [Add Me](#)
 treecore2 [Add Me](#)
 treecore7 [Add Me](#)




Lab Information



☐ Create a new lab account? [?](#)

You do not manage a lab.

Labs you have joined

 treelab1 [Remove Me](#)

Labs available to join

 treelab4 [Add Me](#)
 treelab6 [Add Me](#)

Create An Account

Fill out the fields below to join Fluorish

First name:


Last name:


Email:


Password:


Verify Password:

Institution:

Are you a core facility manager? ☐ 

Are you a lab manager? ☐ 

Are you human? 

 reCAPTCHA™

stop spam.
read books.

Join Fluorish

this is important

Check the box

***up to you**

Instrument Configuration

Calibur 2-laser 4-color

BD Biosciences – FACSCalibur 2 Laser



EPICS 1-laser, 4-color

Beckman Coulter – EPICS XL 3



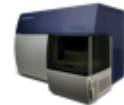
xP5 2-laser, 5-color

Cytek – xP5



Canto II 3-laser, 8-color

BD Biosciences – FACSCanto II



Fortessa 4-laser, 13-color

BD Biosciences – LSRFortessa



Aria #1 3-laser, 9-color

BD Biosciences – FACS Aria II




Aria #2 2-laser, 7-color

BD Biosciences – FACS Aria II



Save Antibody Panels For Life

[Home](#) [Panels](#) [Instruments](#) [Lab Inventory](#) [Cart](#) [Search for Reagents](#)  +72

[Upload a Panel](#)

3 panels exist in your account.

Name: Dual Tetramer

Created: October 17, 2011

Lab:

Investigator: Lisa St John

Description: Determining the frequency of antigen-specific cells (tetramer staining) and the functional capacity, or lack thereof, of those cells (cytokine production after antigen stimulation).
There is no citation information for this panel.

[Add](#) [Options](#)

Name: Maturation

Created: October 17, 2011

Lab:

Investigator: Lisa St John

Description: Determining the specific subsets of T cells which produce cytokine (i.e. are functional) in response to stimulation/activation with antigen.
There is no citation information for this panel.

[Add](#) [Options](#)

Name: Stem Cell Panel

Created: October 11, 2011

Lab:

Investigator: Kathryn Ruisaard

Description:
There is no citation information for this panel.

[Add](#) [Options](#)

1. Welcome
2. Select Cytometer
3. Cytometer Configuration
4. Select Dyes
5. Fluorochromes
6. Targets
7. Conjugations
8. Summary
9. Finalize



version: 1.0b25

Welcome to the Fluorish Panel Wizard

This tool will provide a step-by-step process to aid in building a multicolor antibody panel optimized to your cytometer. The logical progression will assist in efficiently creating a panel that is designed based on your fluorochrome specifications and required targets.

Start New Panel

Edit Saved Panel

Custom Antibody Database

Search Catalogs

Experiment Name

Carol's Panel

Date

October 20, 2011

Investigator

Presentation

Lab

Oxford

Experimental Description

Add an additional field

DISCLAIMER:

Please be aware that the Antibody Selection Tool is designed to aid in the development of antibody panels **for experimental use only**. The user must have a general understanding of the cytometer they will be using including the quantity of the detectors per laser, the detectable wavelengths, and the number of lasers. The contents of the reagent database are supplied by the antibody manufacturers. Product availability is subject to change.

Save

Cancel

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Steps









1. Welcome
2. **Select Cytometer**
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Select Cytometer

Log In

Choose your cytometer from the list below:

 My Cytometers  BD Biosciences  Beckman Coulter  Cytex  iCyt-Sony  Millipore  Partec  Other

Please login or create a [fluorish.com](https://www.fluorish.com) account to populate this list.

Help

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Select Cytometer

Log In

Choose your cytometer from the list below:

My Cytometers BD Biosciences Beckman Coulter Cytex iCyt-Sony Millipore Partec Other

- ☐ Accuri C6
- ☐ FACScan
- ☐ FACSCalibur 1 Laser
- ☐ FACSCalibur 2 Laser
- ☐ FACSVerse
- ☐ FACSArray
- ☐ FACSria
- ☐ FACSria II
- ☐ FACSria III
- ☐ FACSCanto
- ☐ FACSCanto II
- ☐ FACSVerse
- ☐ LSR I
- ☐ LSR II
- ☒ LSRFortessa
- ☐ Influx
- ☐ FACSJazz



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Cytometer Configuration

Open Recent

Change Cytometer

Log In

BD Biosciences : LSRFortessa

The cytometer you selected is typically customized per user specifications.
Please drop your instrument configuration file here.



[Instructions on how to generate your instrument configuration file are here.](#)

Instrument Configuration

▼ 405 nm Laser

425-475

500-550

599-611

▼ 488 nm Laser

515-545

685-735

▼ 561 nm Laser

575-589

600-620

650-670

685-735

750-810

▼ 640 nm Laser

663-677

Violet Laser : 405

Select a laser to view configuration



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Steps

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4. **Select Dyes**
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Select Dyes (unconjugated fluorochromes)

Log In

Select any chemical dyes (e.g. viability dyes), fluorescent proteins or unconjugated fluorochromes needed for the panel.

Selections will be automatically placed into their optimal detection channel based on the current instrument configuration.

If there are none being used in the panel, just hit "Next".

Missing an option?

Dye List:

- RiboFlavin(531)
- Hoechst 33342(455)
- Hoechst 33258(455)
- Propidium Iodide(617)
- DAPI(462)
- Marina Blue(461)
- Fixable Aqua Dead Cell Stain(526)
- DyeCycle Violet(437)
- Vybrant DyeCycle Violet(436)
- CellTrace Violet Cell Proliferation(455)
- Calcein Violet AM(452)
- Fixable Yellow Dead Cell Stain(551)
- Fixable Violet Dead Cell Stain(455)
- ThiolTracker Violet(526)
- Lucifer Yellow(544)
- SYTOX Blue(480)
- POPO-1(457)
- PO-PRO-1(457)
- ECFP(477)

Selected Dyes:

- Fixable Aqua Dead Cell Stain

Add

Remove

- ☒ Specific for instrument configuration
- ☐ Show All

Help

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Fluorochromes

Log In

The fluorochromes matching your cytometer have been selected.

Selecting additional fluorochromes will provide those in the conjugations search results, but they are not optimal for the instrument and will require manual channel assignment.

Missing an option?

<input type="checkbox"/> eFluor 565NC(565)	<input type="checkbox"/> Brilliant Violet 570(571)	<input checked="" type="checkbox"/> Dyomics 547(574)	<input checked="" type="checkbox"/> eFluor 615(622)	<input checked="" type="checkbox"/> APC-A
<input type="checkbox"/> Qdot 565(565)	<input type="checkbox"/> V500(500)	<input checked="" type="checkbox"/> DyLight 550(576)	<input checked="" type="checkbox"/> DyLight 594(618)	<input type="checkbox"/> Cy5.5(617)
<input checked="" type="checkbox"/> eFluor 605NC(605)	<input type="checkbox"/> AmCyan(489)	<input checked="" type="checkbox"/> PE(578)	<input checked="" type="checkbox"/> SureLight P3(662)	<input checked="" type="checkbox"/> Alexa Fluor 647(665)
<input checked="" type="checkbox"/> Qdot 605(605)	<input checked="" type="checkbox"/> PerCP-Cy5.5(695)	<input checked="" type="checkbox"/> RD1(578)	<input checked="" type="checkbox"/> SureLight PBXL-3(662)	
<input type="checkbox"/> eFluor 625NC(625)	<input checked="" type="checkbox"/> PerCP-eFluor 710(710)	<input checked="" type="checkbox"/> PE-Dyomics 590(599)	<input checked="" type="checkbox"/> eFluor 660(658)	
<input type="checkbox"/> eFluor 650NC(650)	<input type="checkbox"/> PerCP(675)	<input checked="" type="checkbox"/> TriColor(613)	<input checked="" type="checkbox"/> Cy5(670)	
<input type="checkbox"/> Qdot 655(655)	<input type="checkbox"/> Cy2(507)	<input checked="" type="checkbox"/> PE-Texas Red(613)	<input checked="" type="checkbox"/> APC(660)	
<input type="checkbox"/> Qdot 800(800)	<input checked="" type="checkbox"/> FAM(518)	<input checked="" type="checkbox"/> ECD(613)	<input checked="" type="checkbox"/> Alexa Fluor 647(665)	
<input type="checkbox"/> Krome Orange(530)	<input checked="" type="checkbox"/> DyLight 488(518)	<input type="checkbox"/> PE-Alexa Fluor 610(628)	<input checked="" type="checkbox"/> Dyomics 647(665)	
<input checked="" type="checkbox"/> DyLight 405(420)	<input checked="" type="checkbox"/> FLMA(520)	<input checked="" type="checkbox"/> PE-Cy5(670)	<input type="checkbox"/> APC-Cy5.5(695)	
<input checked="" type="checkbox"/> eFluor 450(450)	<input checked="" type="checkbox"/> Alexa Fluor 488(519)	<input checked="" type="checkbox"/> PE-Dyomics 647(672)	<input checked="" type="checkbox"/> APC-H7(765)	
<input checked="" type="checkbox"/> Alexa Fluor 405(425)	<input checked="" type="checkbox"/> FITC(520)	<input checked="" type="checkbox"/> PE-Cy5.5(695)	<input checked="" type="checkbox"/> APC-Cy7(767)	
<input type="checkbox"/> Pacific Orange(551)	<input type="checkbox"/> SureLight P1(667)	<input checked="" type="checkbox"/> PE-Alexa Fluor 700(720)	<input checked="" type="checkbox"/> APC-Alexa Fluor 750(775)	
<input checked="" type="checkbox"/> V450(448)	<input checked="" type="checkbox"/> TRITC(572)	<input checked="" type="checkbox"/> PE-Cy7(760)	<input checked="" type="checkbox"/> APC-eFluor 780(780)	
<input checked="" type="checkbox"/> Pacific Blue(455)	<input type="checkbox"/> Cy3(566)	<input checked="" type="checkbox"/> Cy3.5(598)	<input checked="" type="checkbox"/> DyLight 650(672)	
<input checked="" type="checkbox"/> Brilliant Violet 421(423)	<input type="checkbox"/> Alexa Fluor 555(565)	<input checked="" type="checkbox"/> Texas Red(615)	<input checked="" type="checkbox"/> DyLight 649(670)	

*HINT: Don't worry about fluorochromes with nearly identical excitation/emission profiles at this step (e.g. FITC and Alexa Fluor 488). The fluorochromes selected here will be provided in the search results. Specific channel assignments and refinements will be made in the conjugations step to prevent the selection of fluorochromes with identical emission profiles.

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Targets

Log In

Select the species and identify the target antigens.



HUMAN



MOUSE



RAT



PRIMATE



BACTERIA
VIRUS



BOVINE



FELINE



CHICKEN



CANINE



DONKEY



FISH



FROG



GOAT



GUINEA
PIG



HAMSTER



HORSE



LLAMA



RABBIT



SHEEP



SWINE



TURKEY

Target List:

1F-5Ag
2B4
2B4 B6 Alloantigen
2B4.2
2DL4
2H2
2H12
3-FAL
3-FL
3G11 sialoganglioside antigen
4E-BP1
4F2
4F9
5'NT
6C6AG
6XHis Tag
7.1

Add >>

Selected Targets:

Target	Clone
CD3ε	select optional clone
CD4	select optional clone
CD8β	select optional clone
CD45RA	select optional clone
CD62L	select optional clone
CD127	select optional clone
PD1	select optional clone
IFN-γ	select optional clone
TNF-α	select optional clone
IL-2	select optional clone

<< Remove

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FLU W20

Steps

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Conjugations

[Log In](#)

Select your antibody conjugations.

Target

[Deselect All](#)

name	#
CD3ε	100
CD4	210
CD8β	39
CD45RA	0
CD62L	82
CD127	73
PD1	37
IFN-γ	69
TNF-α	63

Catalogs

[Deselect All](#)

Abcam
BD Biosciences
Beckman Coulter
BioLegend
Cedarlane
eBioscience
Exbio
iCyt

Format

[Deselect All](#)

eFluor 605NC
Qdot 605
DyLight 405
eFluor 450
Alexa Fluor 405
V450
Pacific Blue
Brilliant Violet 421

Target	Format	Clone	Amount	Price	Vendor	Catalog Num
CD8β	Alexa Fluor 488	eBioH35-17.2 (H...	50 ug	119	eBioscience	53-0083-81
CD8β	Alexa Fluor 488	eBioH35-17.2 (H...	100 ug	219	eBioscience	53-0083-82
CD8β	Alexa Fluor 647	YTS156.7.7	25 µg	94	iCyt	1233055
CD8β	Alexa Fluor 647	YTS156.7.7	25 µg	85	BioLegend	126611
CD8β	Alexa Fluor 647	YTS156.7.7	100 µg	215	iCyt	1233060
CD8β	Alexa Fluor 647	YTS156.7.7	100 µg	195	BioLegend	126612
CD8β	APC	53-5.8	25 µg	77	iCyt	1302045
CD8β	APC	53-5.8	25 µg	70	BioLegend	140409

[Add](#)[Remove](#)

Selected Reagents

Target	Format	Clone	Vendor	Catalog Number	Amount	Price
CD45RA	PE	14.8	BD Biosciences	553380	0.2 mg	-

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Summary

[Log In](#)

Below is a summary of your current antibody channel assignments.

Use the icon on the right of each channel to remove a selection or to make a custom assignment.

BD Biosciences : LSRFortessa

Laser/Detector	Target	Format	Vendor	Catalog Number	Price		
Violet Laser : 405							
425-475	CD8 β	Pacific Blue	BioLegend	140413	\$95		
500-550	DYE	Fixable Aqua Dead Cell Stain					
599-611	CD4	eFluor 605NC	eBioscience	IH93-0042-91	\$139		
Blue Laser : 488							
515-545	TNF- α	Alexa Fluor 488	BioLegend	506315	\$95		
685-735	IFN- γ	PerCP-Cy5.5	BD Biosciences	560660	-		
Yellow-Green Laser : ...							
575-589	CD45RA	PE	BD Biosciences	553380	-		
600-620	CD3e	ECD	Beckman Coulter	A88595	\$315		
650-670	DYE	7-AAD					
685-735							
750-810	IL-2	PE-Cy7	eBioscience	25-7021-80	\$99		
Red Laser : 640							

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Steps

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9. Finalize



Finalize

Final Summary

Log In

Experimental Description

Save To CSV

Print

Order

Laser/Detector	Target	Format	Vendor	Catalog Number	Price		
Violet Laser : 405							
425-475	CD8 β	Pacific Blue	BioLegend	140413	\$95		
500-550	DYE	Fixable Aqua Dead Cell Stain					
599-611	CD4	eFluor 605NC	eBioscience	IH93-0042-91	\$139		
Blue Laser : 488							
515-545	TNF- α	Alexa Fluor 488	BioLegend	506315	\$95		
685-735	IFN- γ	PerCP-Cy5.5	BD Biosciences	560660	-		
Yellow-Green Laser : ...							
575-589	CD45RA	PE	BD Biosciences	553380	-		
600-620	CD3 ϵ	ECD	Beckman Coulter	A88595	\$315		
650-670	DYE	7-AAD					
685-735							
750-810	IL-2	PE-Cy7	eBioscience	25-7021-80	\$99		
Red Laser : 640							

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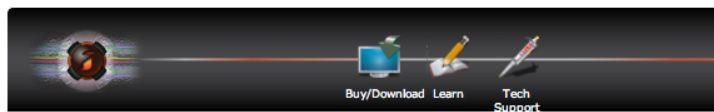
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Online Resources



TUTORIALS, TECH NOTES, AND DEMO DATA

VIDEO TUTORIALS

A collection of FlowJo Videos

STEP-BY-STEP TUTORIALS

All tutorials include a PDF, a demonstration dataset, and completed workspaces.

THE FLOWJO BASIC TUTORIAL

The Basic Tutorial gets you up to speed quickly with key concepts in FlowJo. A common titration experiment is demonstrated some of the basic analysis features of FlowJo. You can [watch](#) one of our application scientists demonstrate Basic Tutorial (about 15 minutes).

8 COLOR PBMC EXPERIMENT

Provides a in-depth overview of the main FlowJo functions in the context of a 'real world' data set. Includes an in to compensation and provides tips on speeding analysis through filtered batch operations.
[Read more...](#)

CELL CYCLE TUTORIAL

Explains the Watson and the Dean, Jett, Fox cell cycle models used by FlowJo and provides stepwise instruction 2-color PBMC experiments..
[Read more...](#)

PROLIFERATION TUTORIAL

Provides an overview of the proliferation modeling algorithm, suggests model adjustment techniques, and gives explanation of the proliferation statistics..
[Read more...](#)

KINETICS TUTORIAL

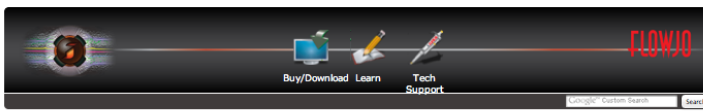
Shows how to analyze time series
[Read more...](#)

BATCHING AND ITERATION

Grants you access to the most powerful patterns of your dataset(s)
[Read more...](#)

FLOWJO PRICING

FlowJo License Option	Commercial Price	Academic Price
Dongle	\$2,995	\$1,995
License Number	\$2,995	\$1,995
1 Year License Number	\$995	\$695
Upgrade v7/8 to v9	\$599	\$599
Upgrade v6 to v9	\$699	\$699
Upgrade v4 to v9	\$799	\$799
Upgrade v3 to v9	\$899	\$899
Site License	Based on Usage	Based on Usage
Trial	Free for 30 Days	Free for 30 Days



FlowJo WebEx

[Click here to be taken to the WebEx calendar!](#)

What is a WebEx?

- A WebEx is a [free](#) online seminar-tutorial and question/answer session. This web based service integrates a conference call, chat and screen sharing, allowing us to share our desktop, and speak or chat, with you in realtime.

When are WebExs?

- Introduction to FlowJo WebEx seminars on the first Friday of every month, at 1:00 PM EST. To participate, on the day of the meeting, five minutes before the start time, click this link [Monthly WebEx](#). You will be directed to a login page, with instructions for calling in toll free. The meetings will last about two hours, and you can logout whenever you need to. Both the Advanced and Basic WebEx are listed on our WebEx calendar, [here](#).

What is covered in a WebEx?

- There are two types of WebExs:

- Basic - A two-hour intro to the main organs and mechanics of FlowJo. This session covers starting FlowJo, adding samples, gates and stats, batching tables and layouts, and compensating your data in FlowJo.

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Software



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Write something...



FlowJo Last week to get FlowJo at it's 2011 Pricing! Buy FlowJo before the price raise. We will adjust prices upward beginning in January 2012. You can view the new price list after the jump.

FlowJo Prices Starting 2012
[www.flowjo.com](#)
FlowJo FlowJo help manual guide lesson help

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Andreiu Thibault does anybody know a FJ version for iPad or iPhone is available? I just see a web phone application on the web!

[Like](#) [Comment](#) - January 12 at 10:18pm · [Like](#)



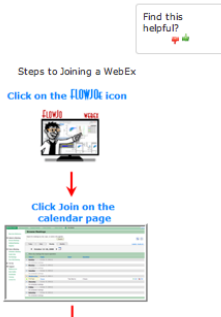
Daniela Mónico Is it possible to convert a .jo file into a .wsp one? I've been working on Mac at Emory University and now I'm trying to do it on my PC in Argentina... I've tried everything I could think of and it's not working... Could you help me?

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FlowJo Daniela, please email the file you are trying to open on your PC to our Tech Support Guru's at [flowjo@brestar.com](#) and they should be able to help get you running again.

[Like](#) [Comment](#) - January 4 at 1:47pm



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Thank You...Questions?

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Jack Panopoulos, Ph.D.
Application Scientist

