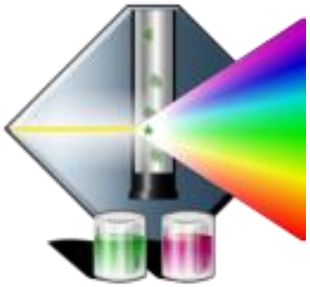


Flow Cytometry Data Analysis

Jack Panopoulos, Ph.D.
Application Scientist

My Goals For Today

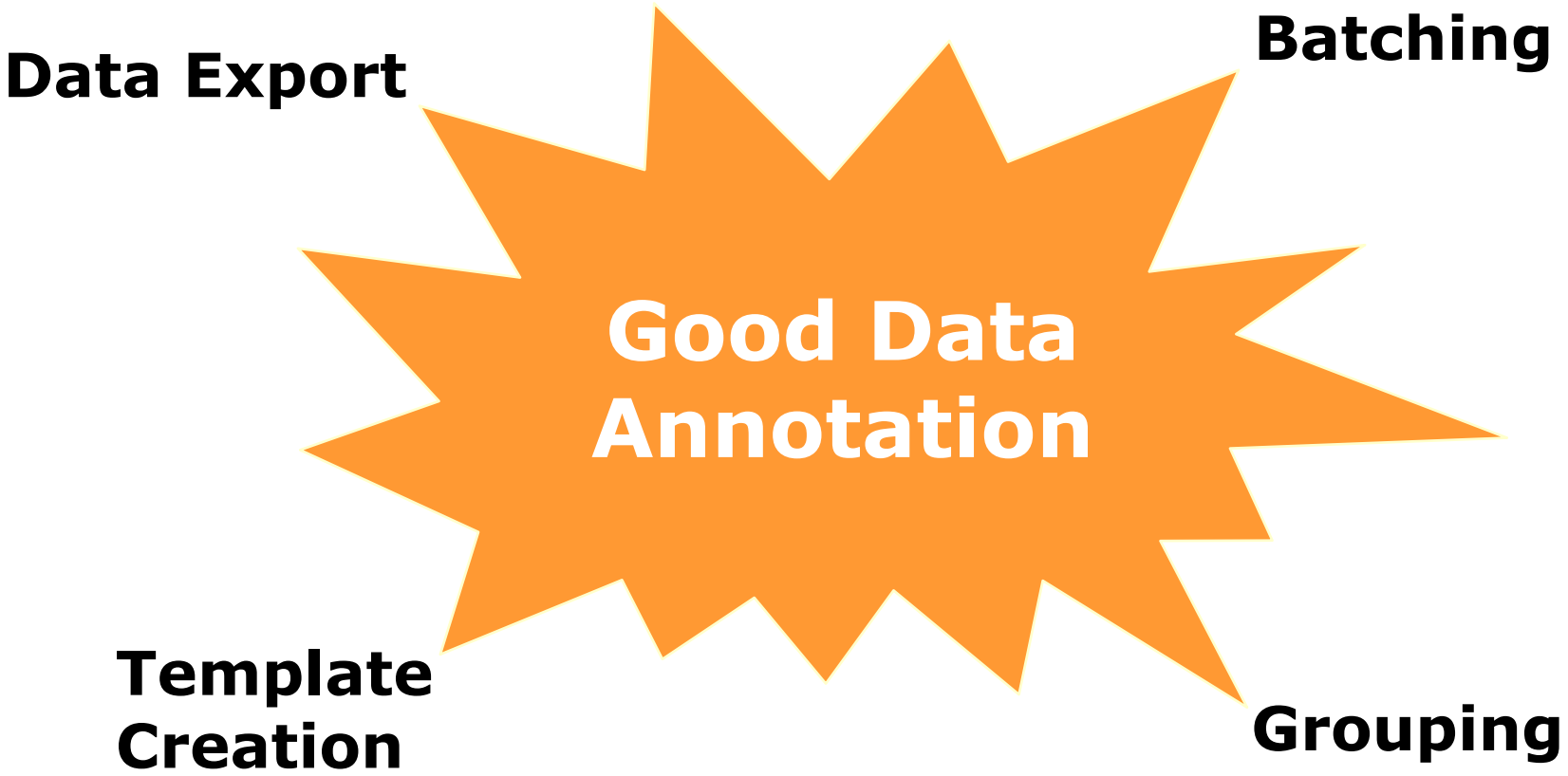
1. Save you time analyzing your data

2. Help you get more out of your data ($$$)

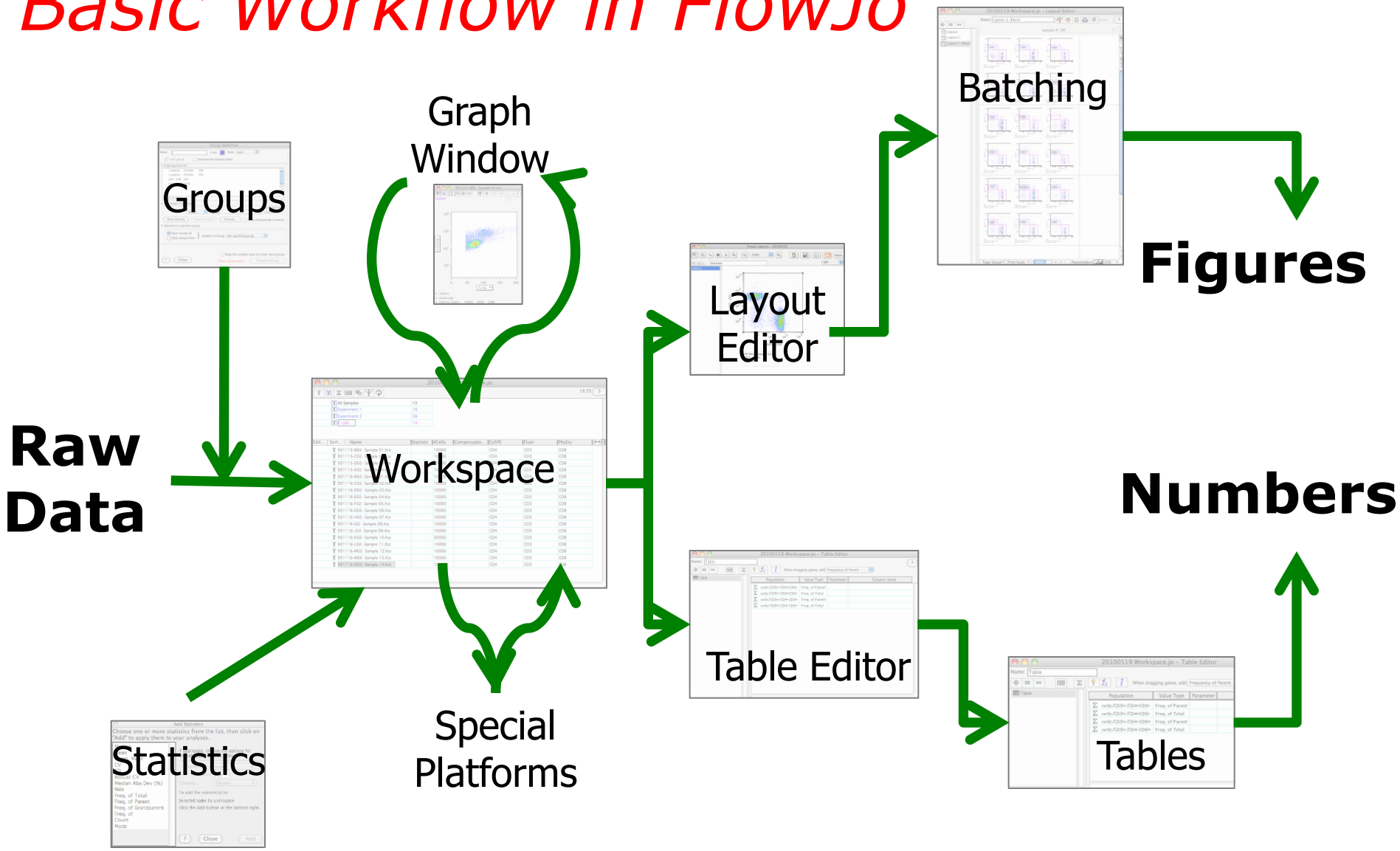
3. Keep you on the cutting-edge

Benefits of Using FlowJo

Keyword-Driven Data Organization

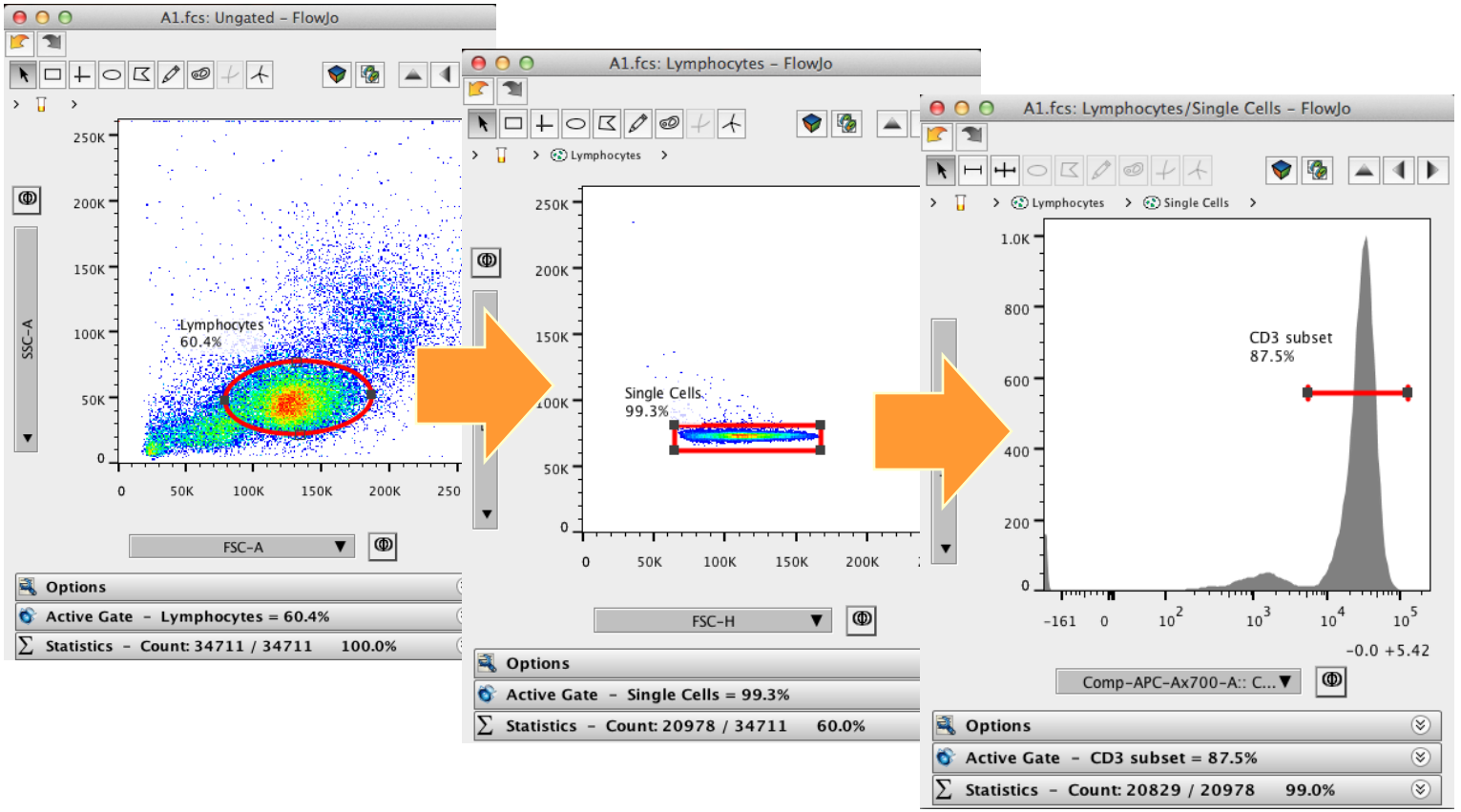


Basic Workflow in FlowJo

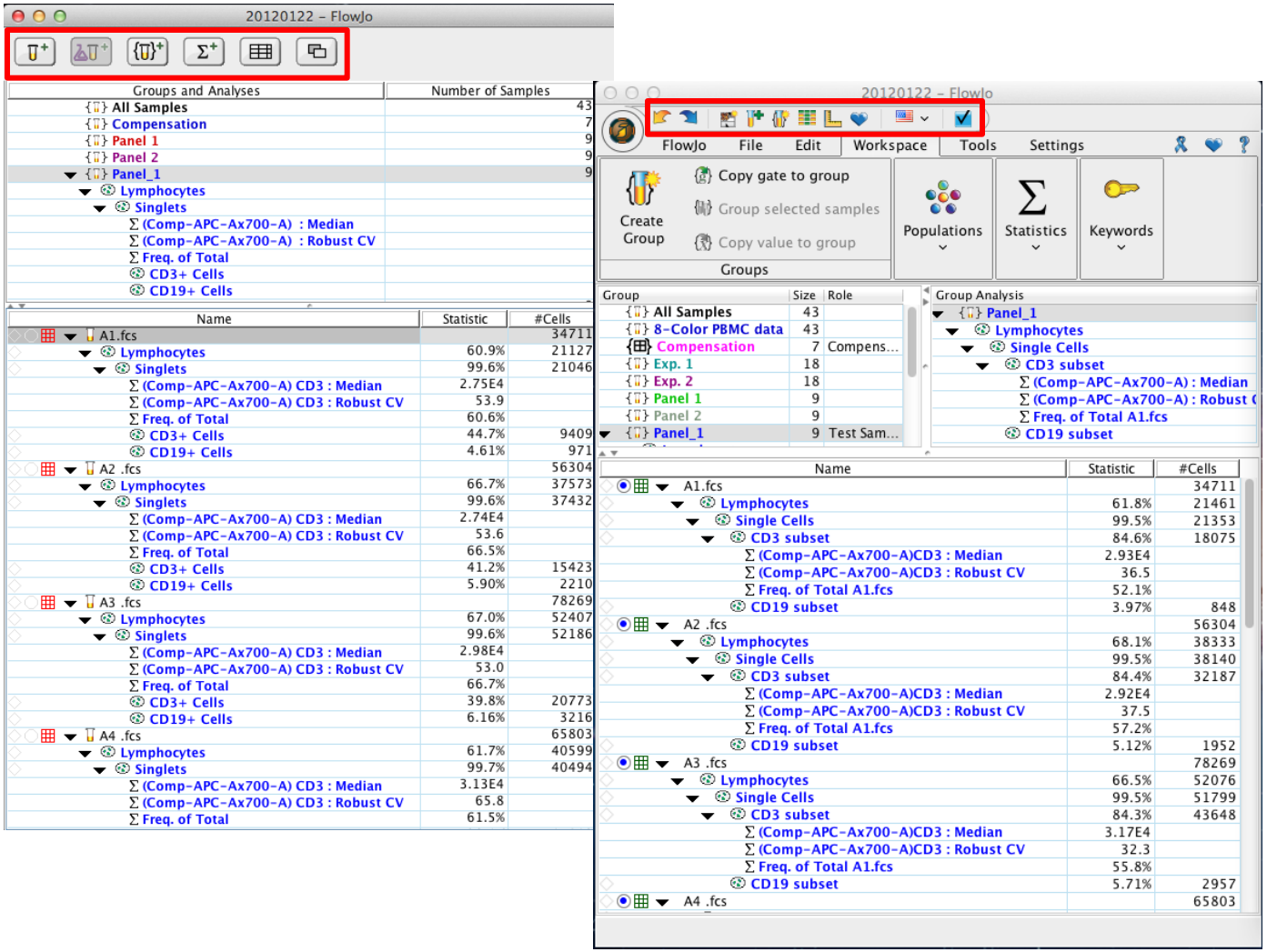


Benefits of Using FlowJo

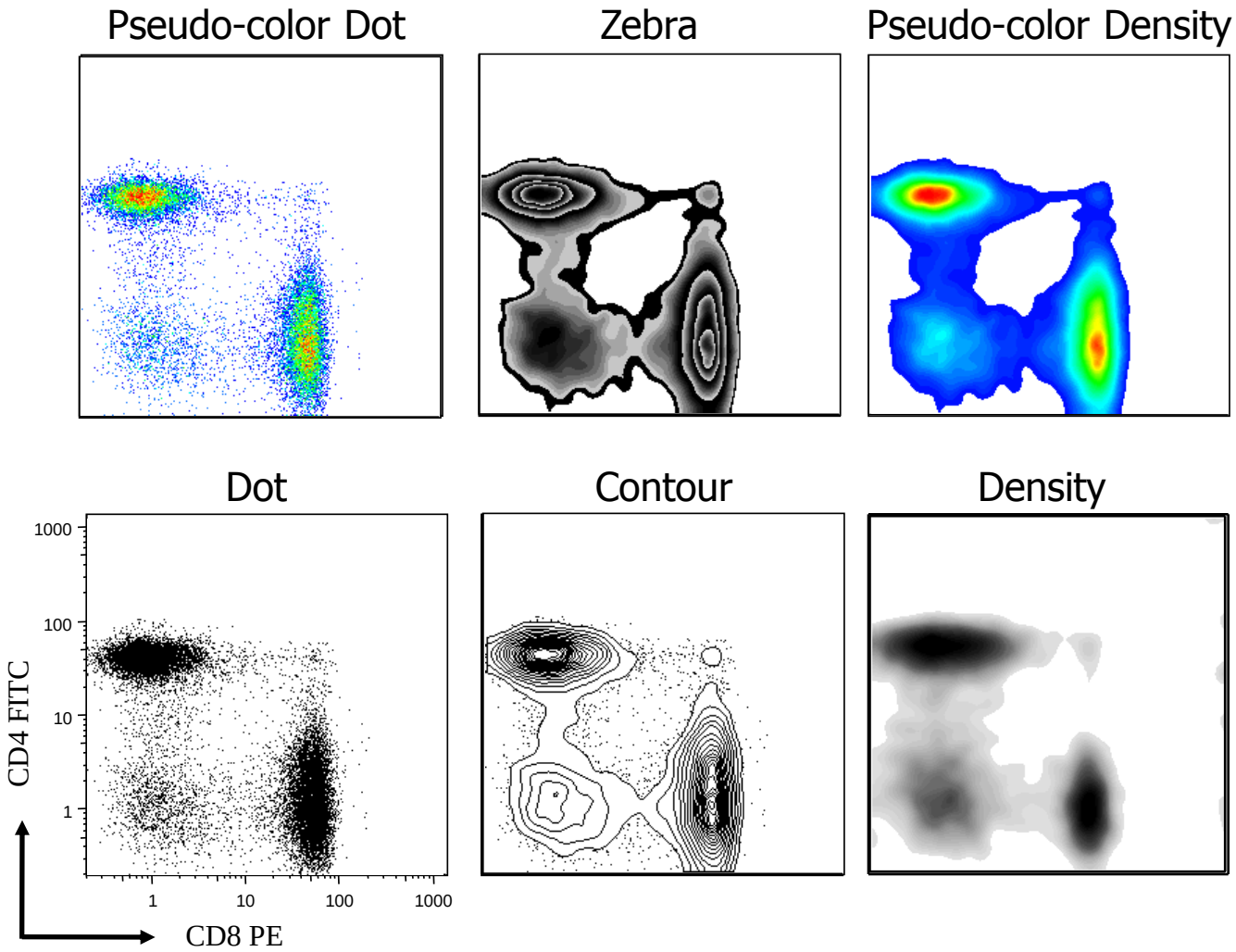
Intuitive Hierarchical Gating



Workspace for Hierarchical Gating

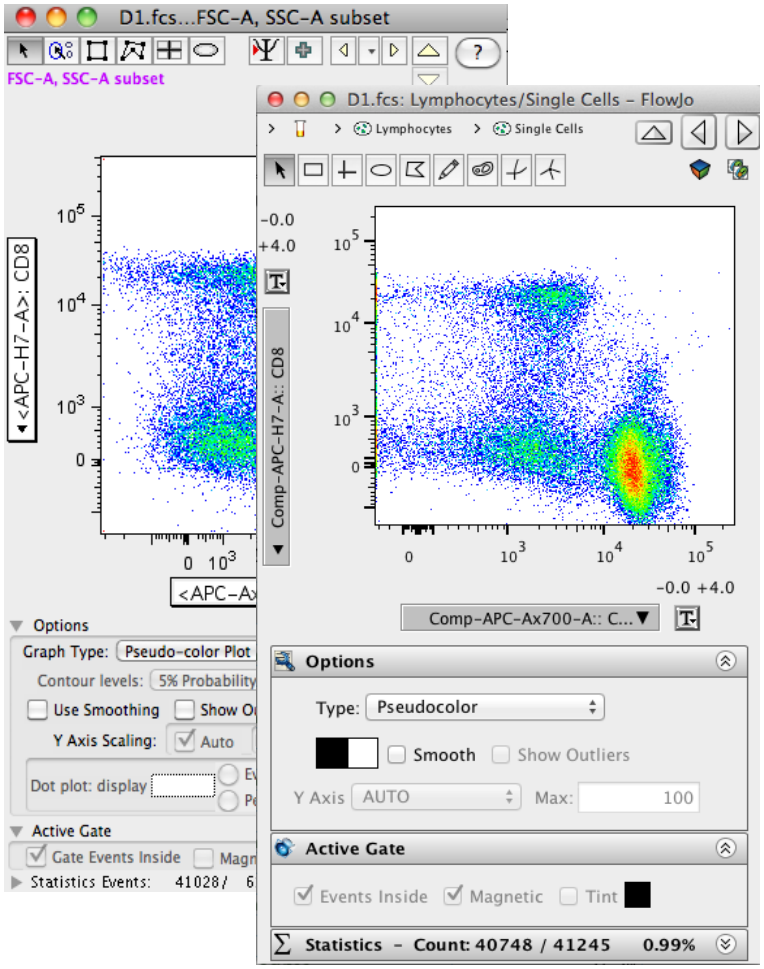


Data Display Types

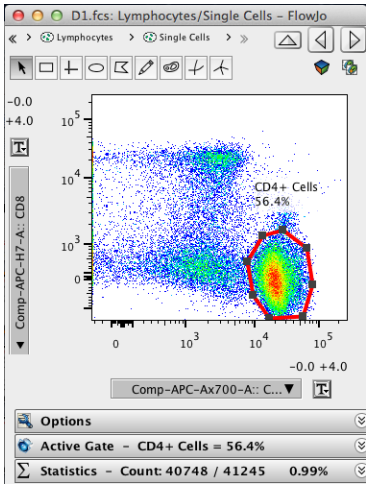


Gating

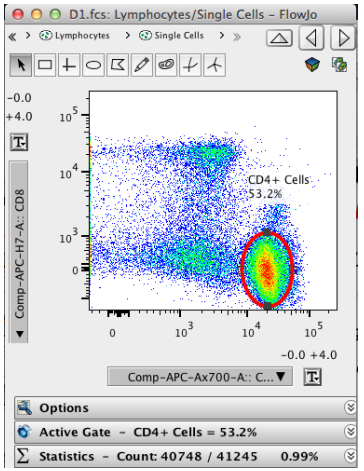
Graph Window



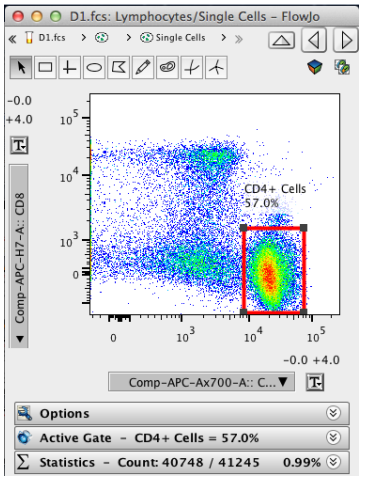
Polygon



Elliptical

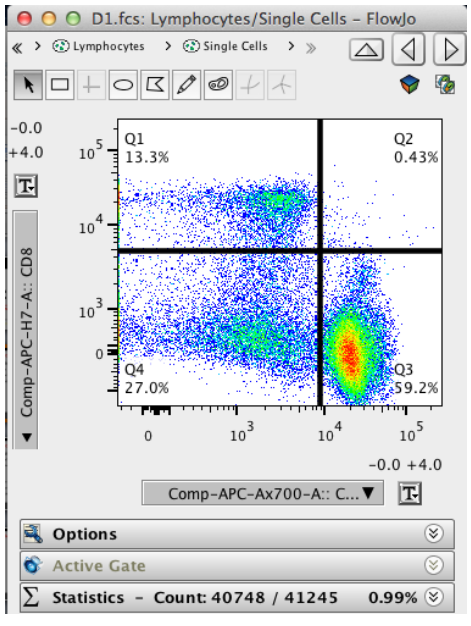


Rectangular

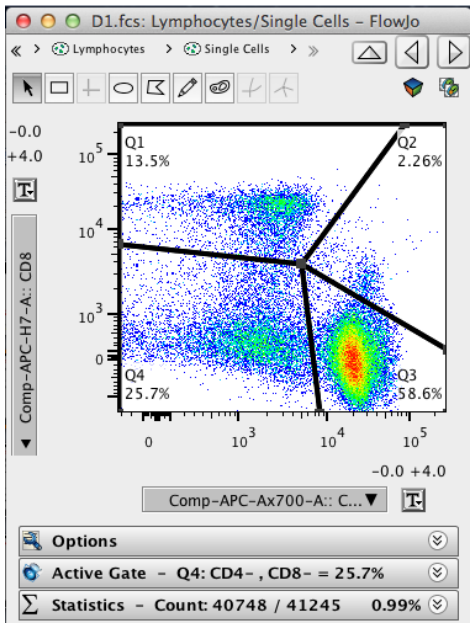


Advanced Gating

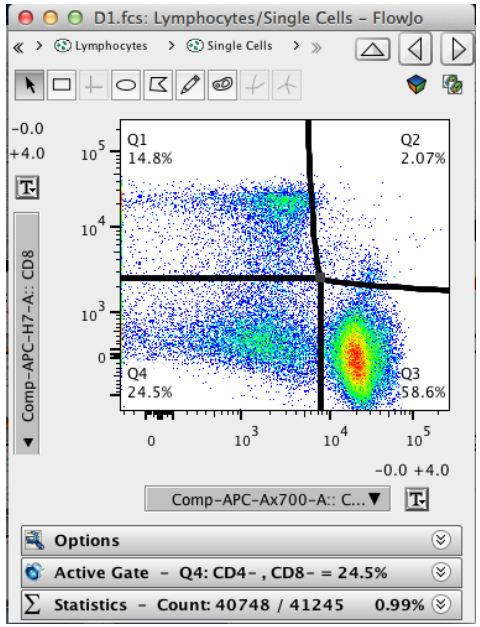
Quad



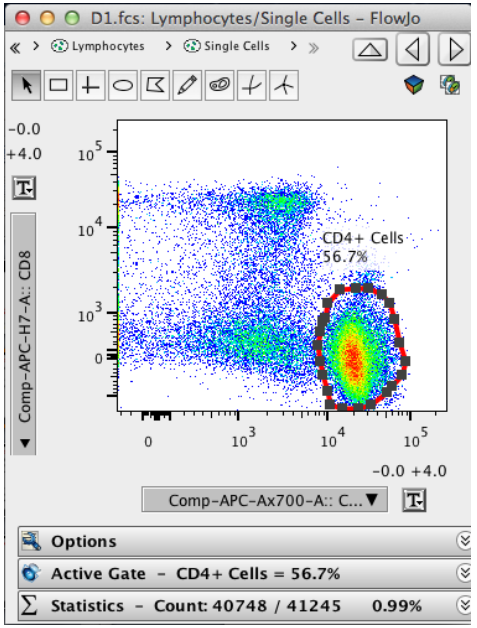
Spider



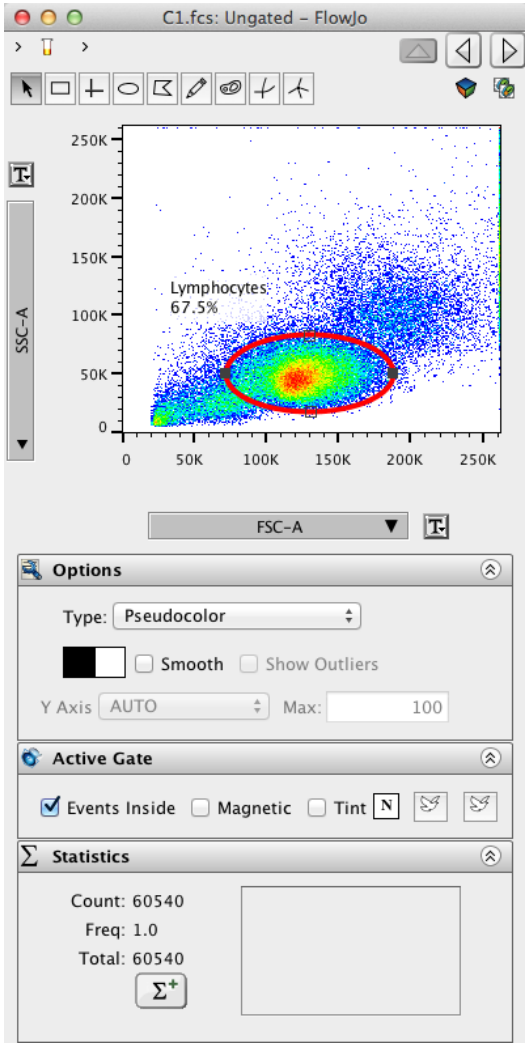
Curly

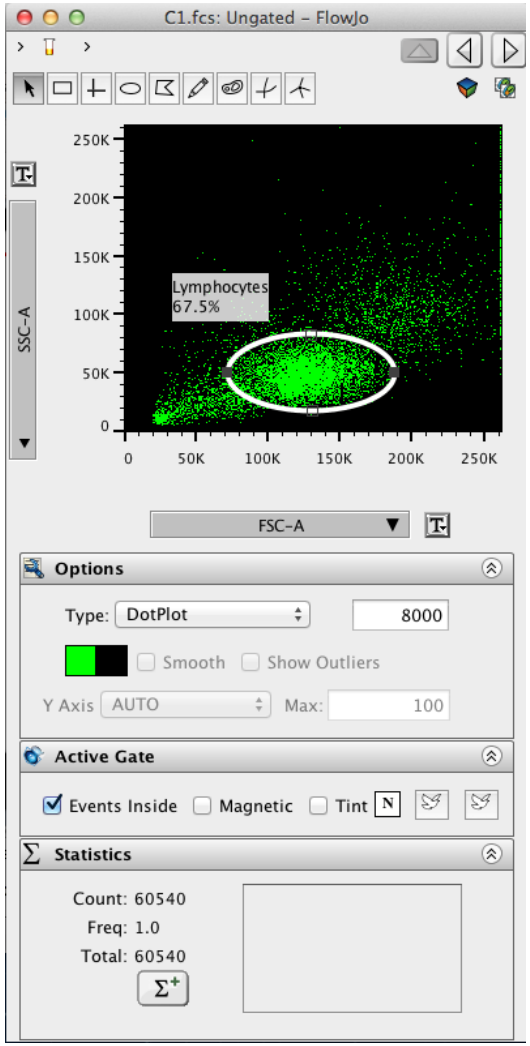


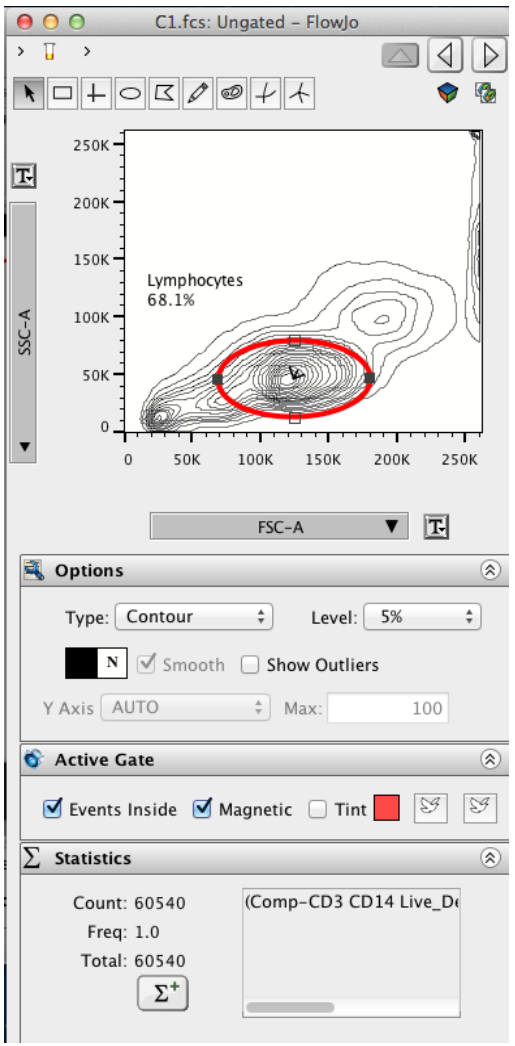
Auto



Advanced Plotting Tools

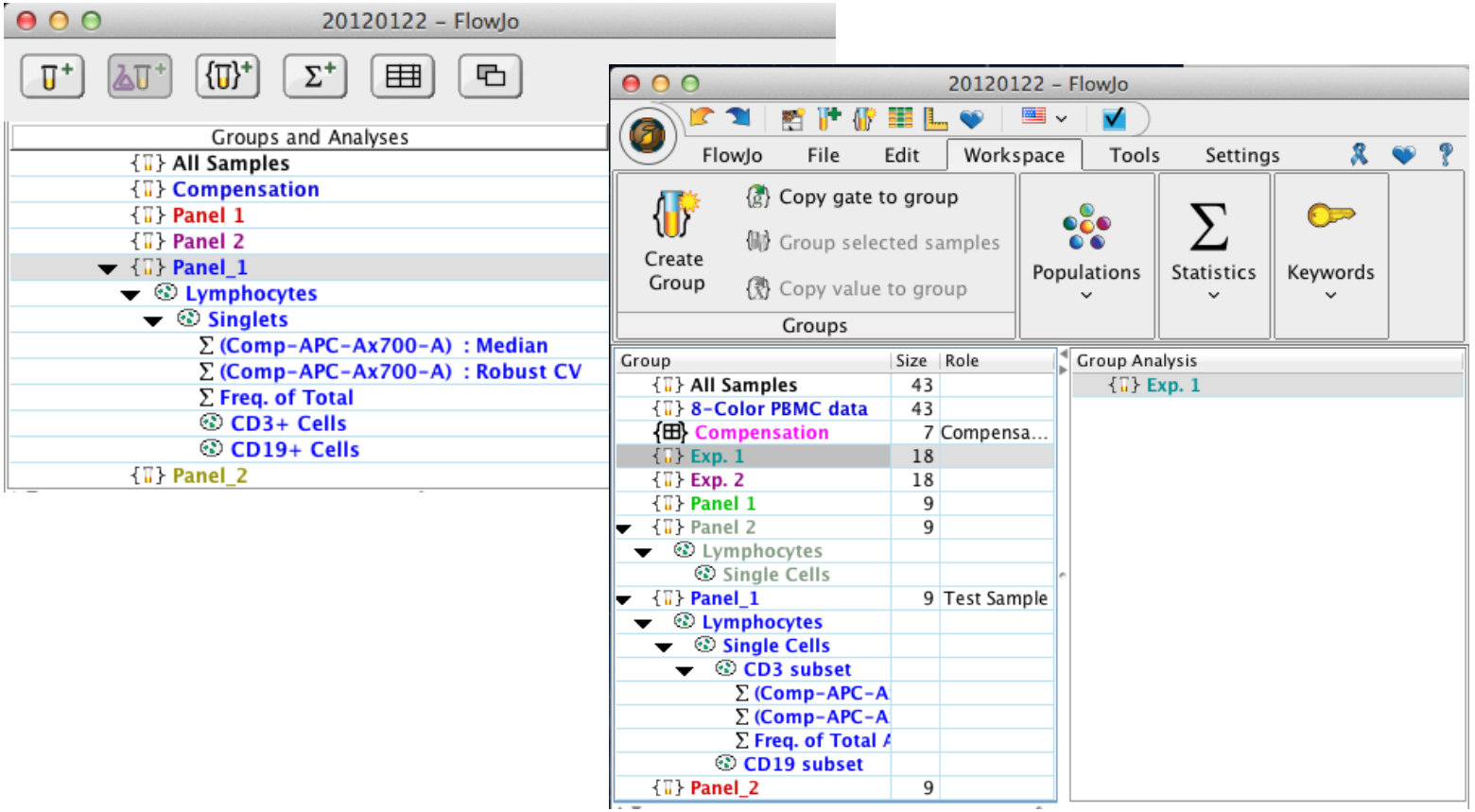






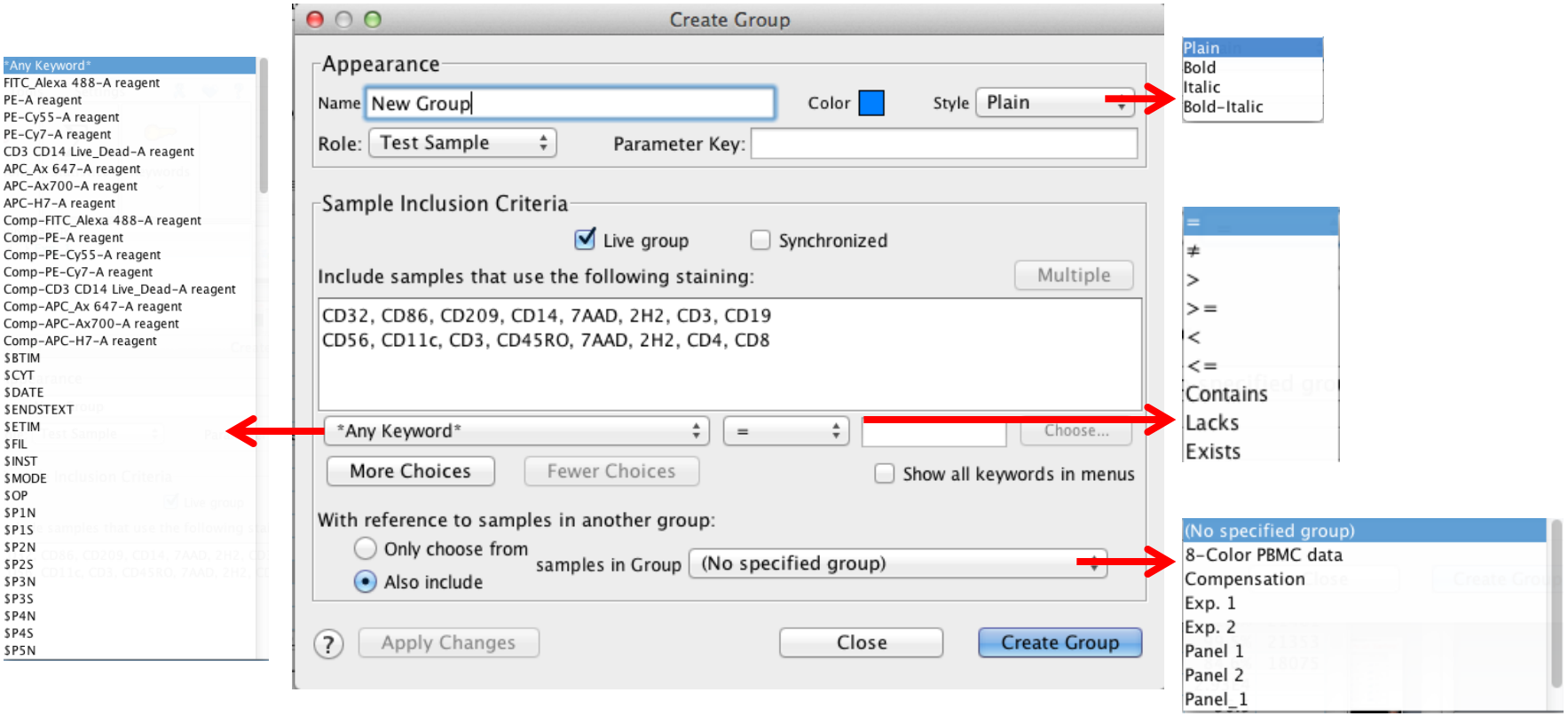
Grouping Samples

Samples quickly organized using keywords

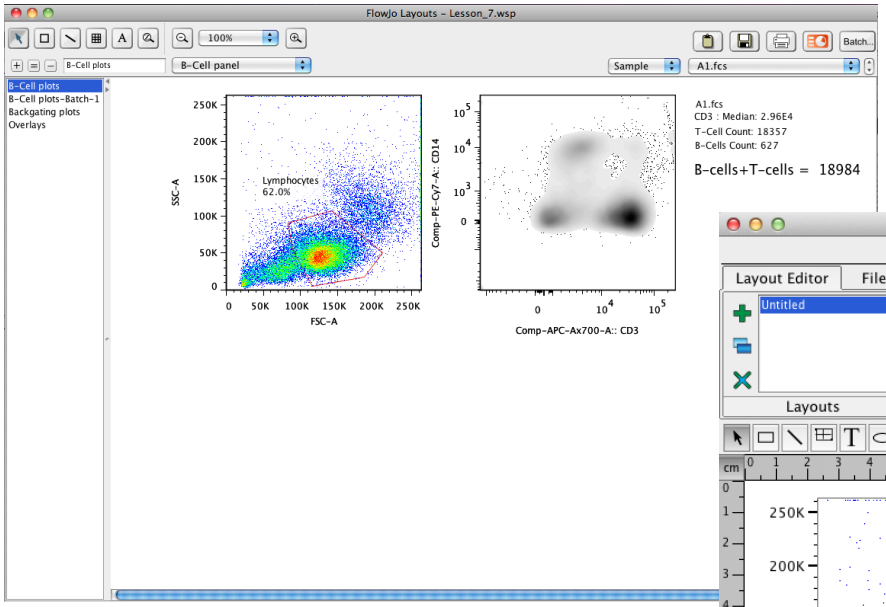


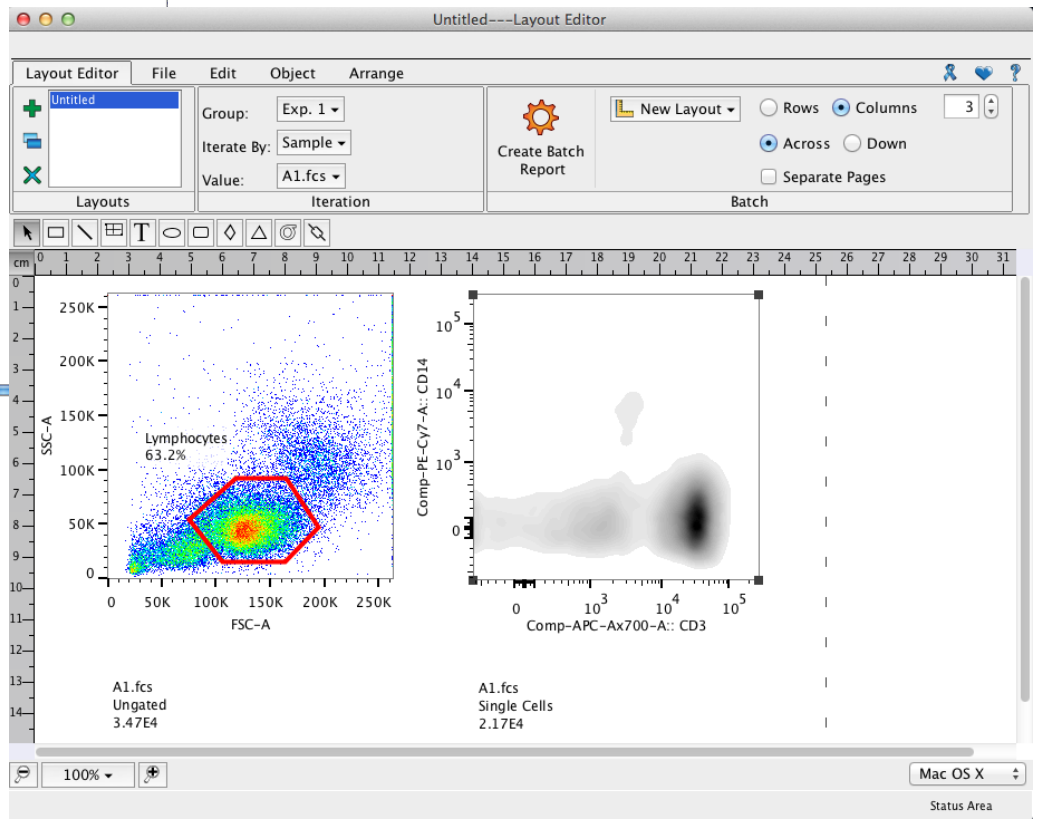
Grouping Samples

Samples quickly organized using keywords

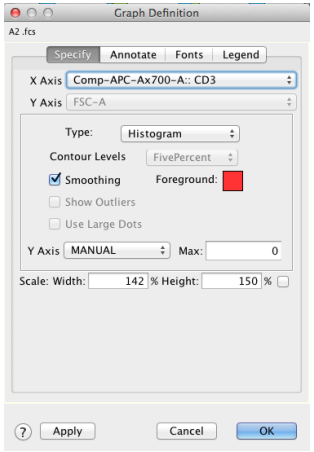


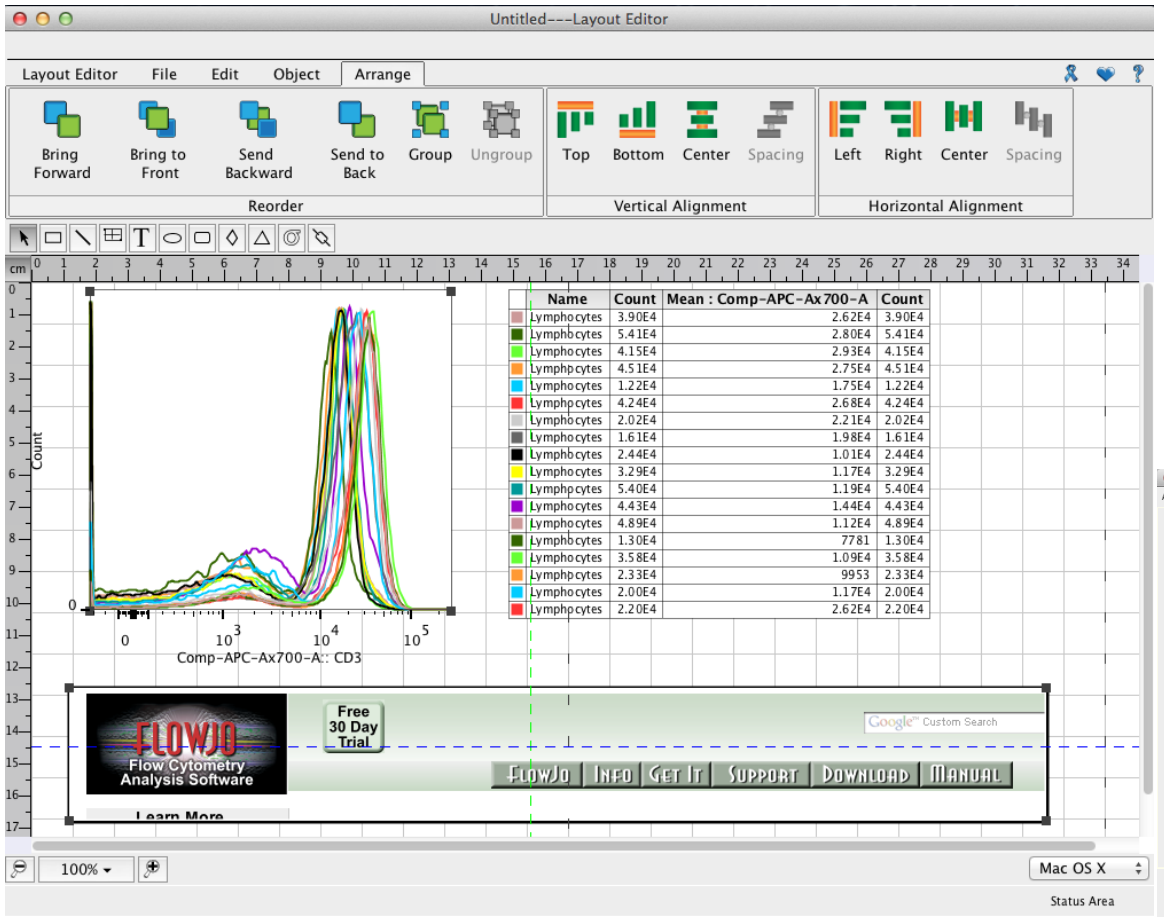
Layout Editor: Creating Figures

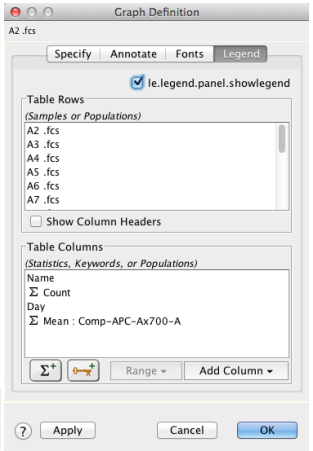




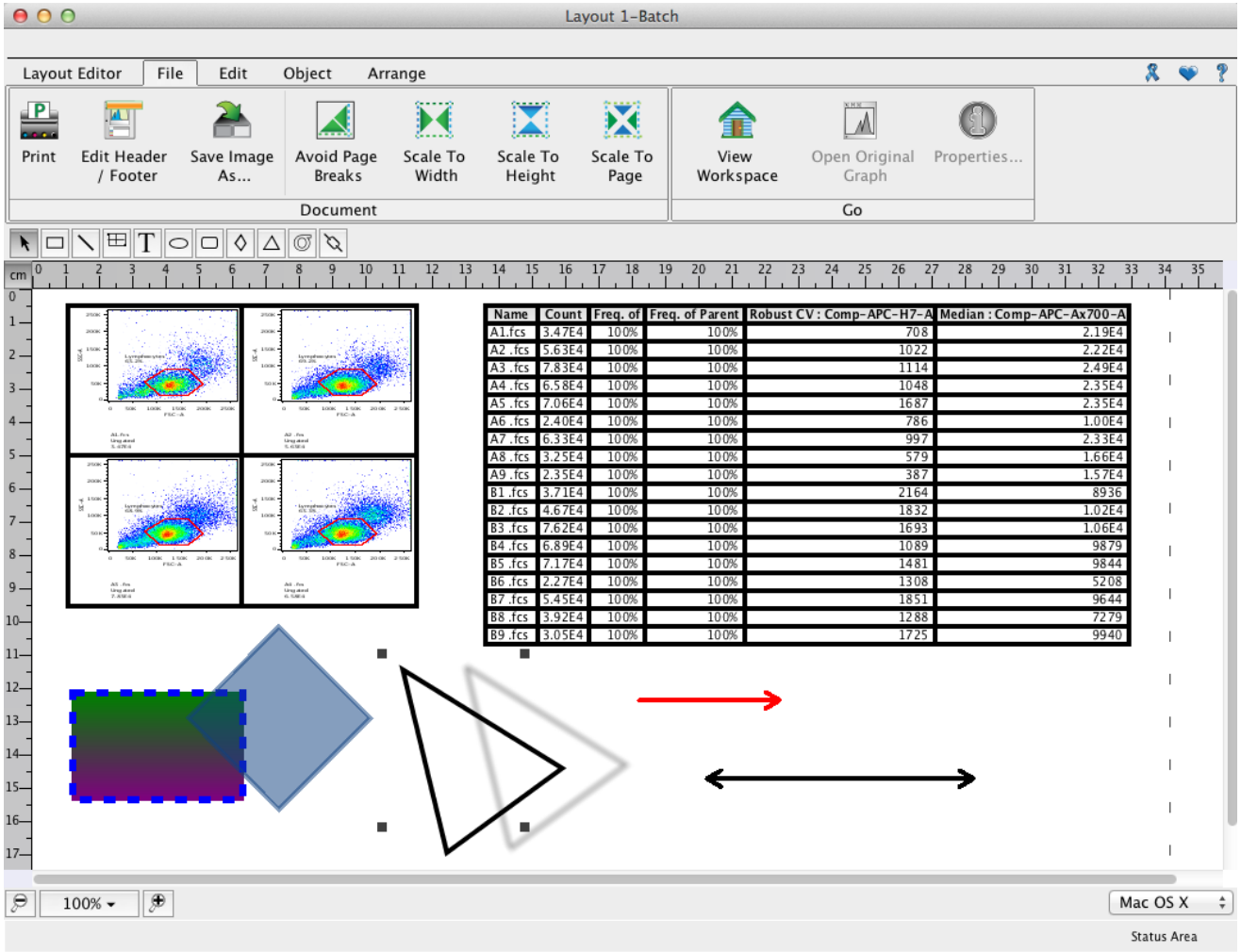
Layout Editor: Creating Figures







Layout Editor: Creating Figures



Name	Count	Freq. of	Freq. of Parent	Robust CV : Comp-APC-H7-A	Median : Comp-APC-Ax700-A
A1.fcs	3.47E4	100%	100%	708	2.19E4
A2 .fcs	5.63E4	100%	100%	1022	2.22E4
A3 .fcs	7.83E4	100%	100%	1114	2.49E4
A4 .fcs	6.58E4	100%	100%	1048	2.35E4
A5 .fcs	7.06E4	100%	100%	1687	2.35E4
A6 .fcs	2.40E4	100%	100%	786	1.00E4
A7 .fcs	6.33E4	100%	100%	997	2.33E4
A8 .fcs	3.25E4	100%	100%	579	1.66E4
A9 .fcs	2.35E4	100%	100%	387	1.57E4
B1 .fcs	3.71E4	100%	100%	2164	8936
B2 .fcs	4.67E4	100%	100%	1832	1.02E4
B3 .fcs	7.62E4	100%	100%	1693	1.06E4
B4 .fcs	6.89E4	100%	100%	1089	9879
B5 .fcs	7.17E4	100%	100%	1481	9844
B6 .fcs	2.27E4	100%	100%	1308	5208
B7 .fcs	5.45E4	100%	100%	1851	9644
B8 .fcs	3.92E4	100%	100%	1288	7279
B9 .fcs	3.05E4	100%	100%	1725	9940

Iterative Analysis Through "Batching"

FlowJo does the work for you!

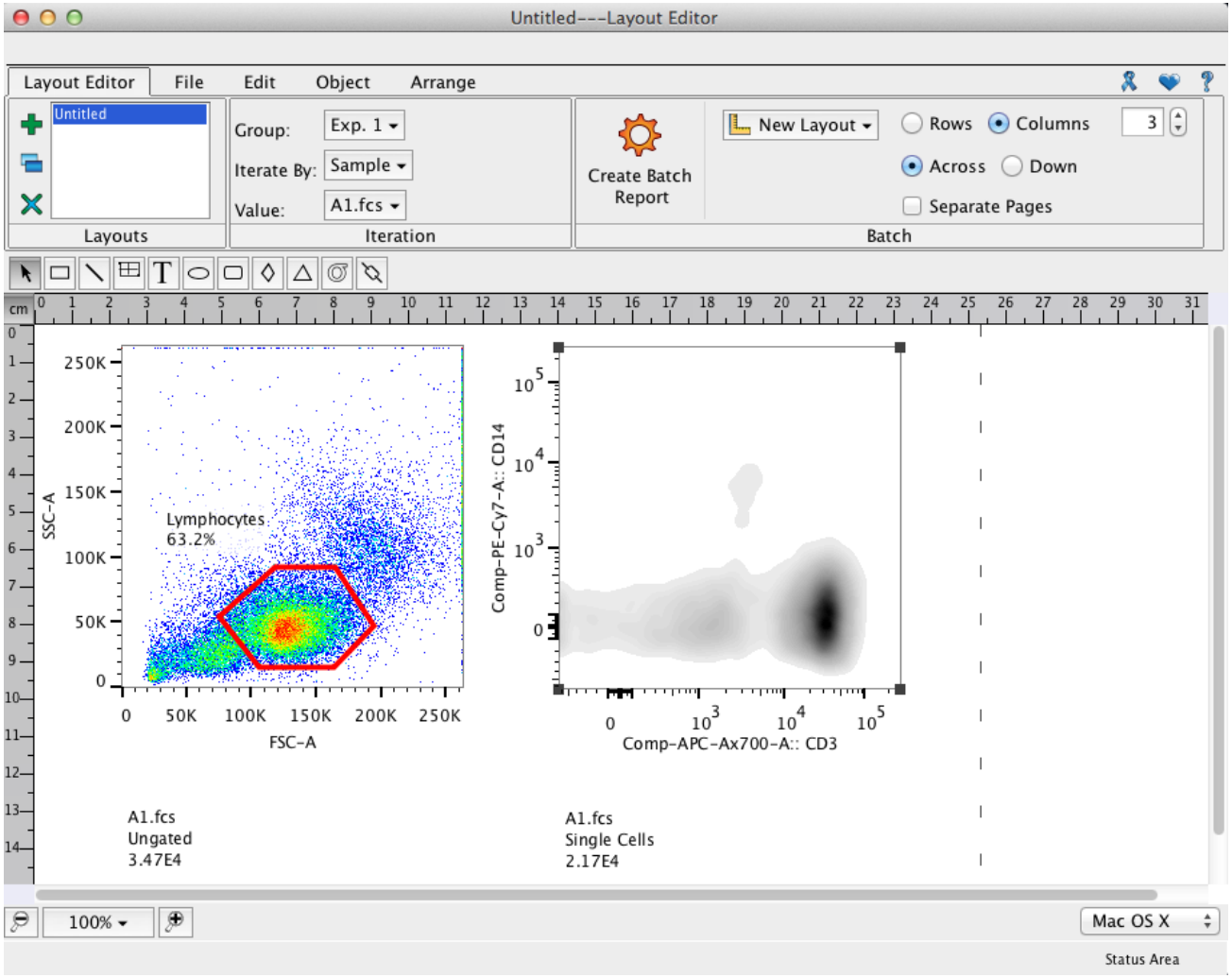
Perform an operation once and instruct the software to repeat it.

Data can be exported in a variety of ways:

PC

Mac

Iterative Analysis Through "Batching"



Iterative Analysis Through "Batching"

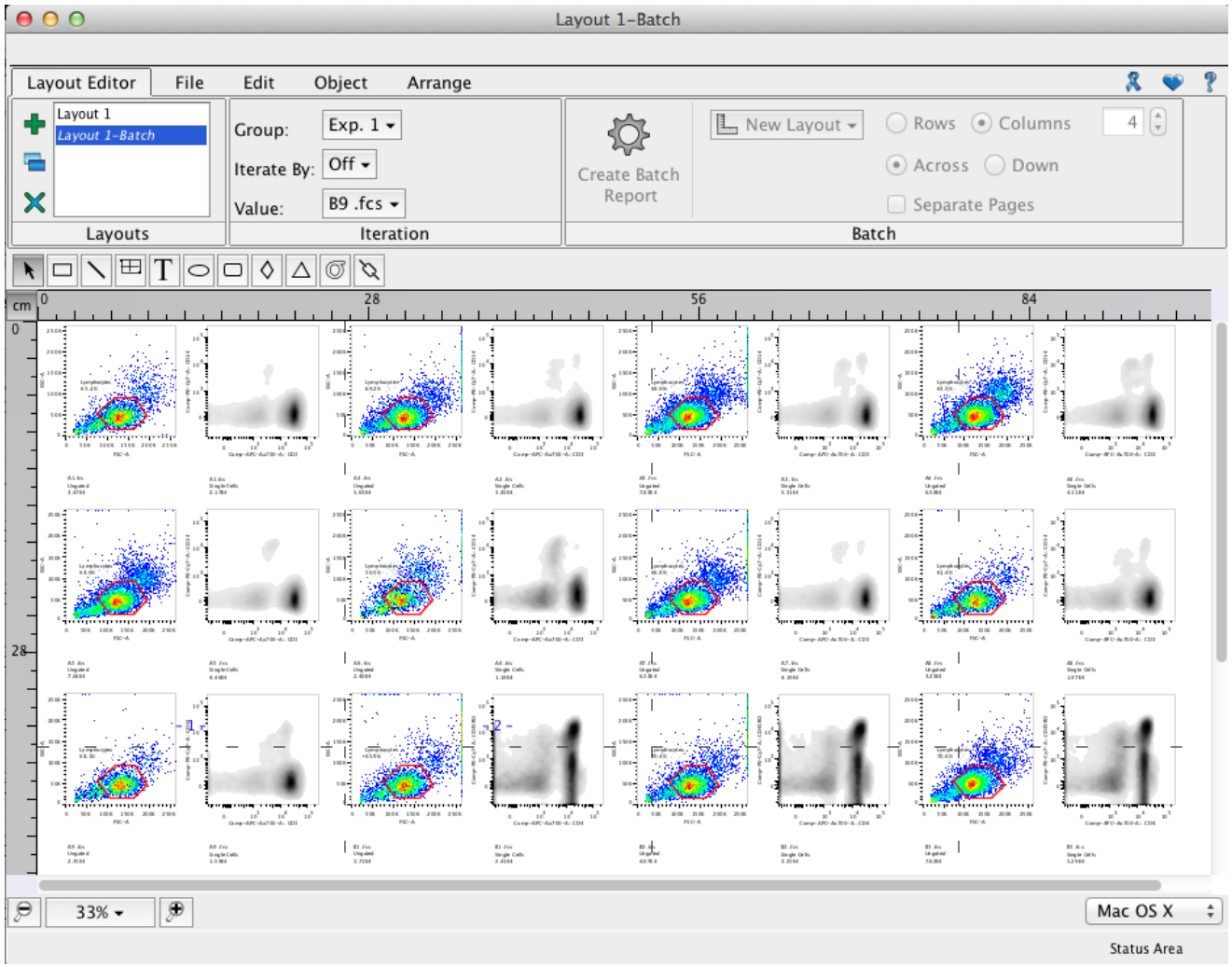
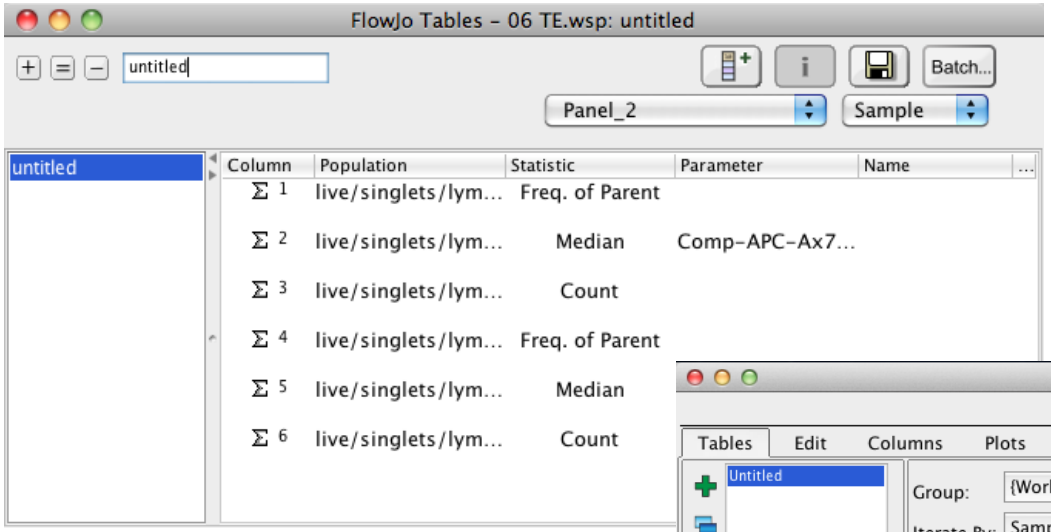


Table Editor: Creating Statistics



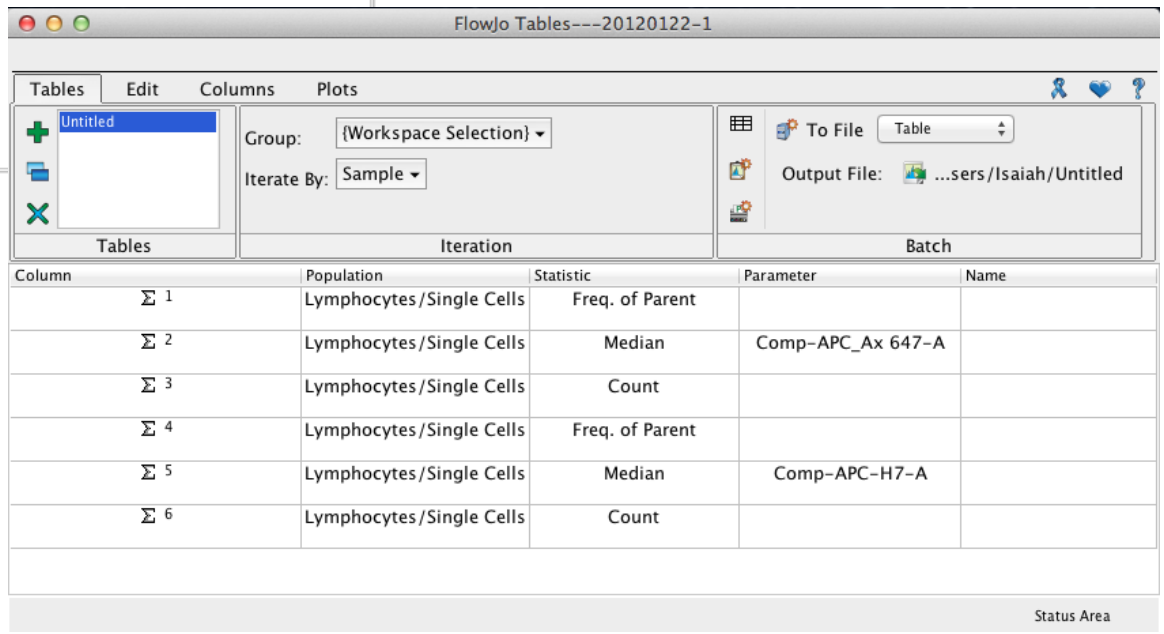


Table Editor: Creating Statistics

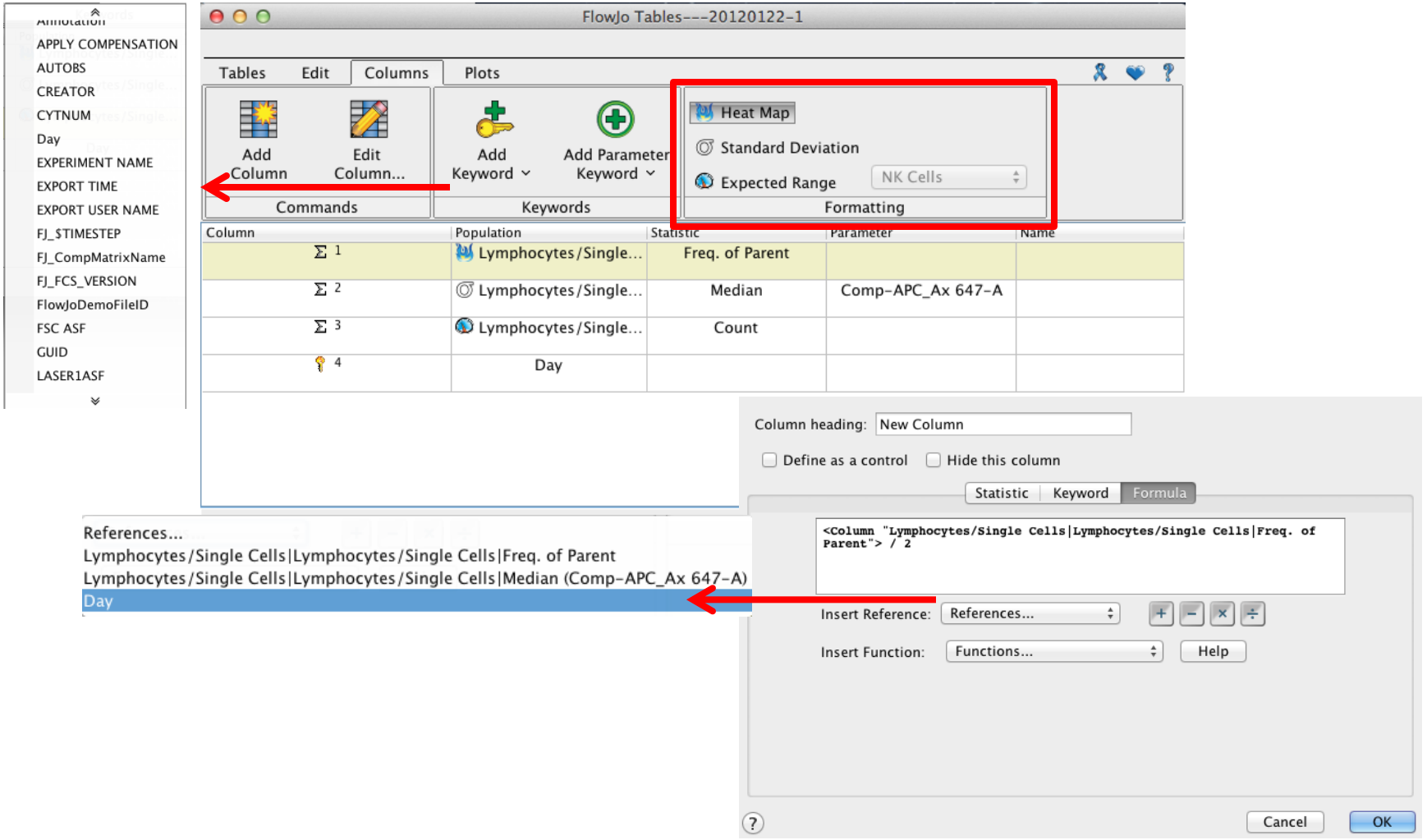
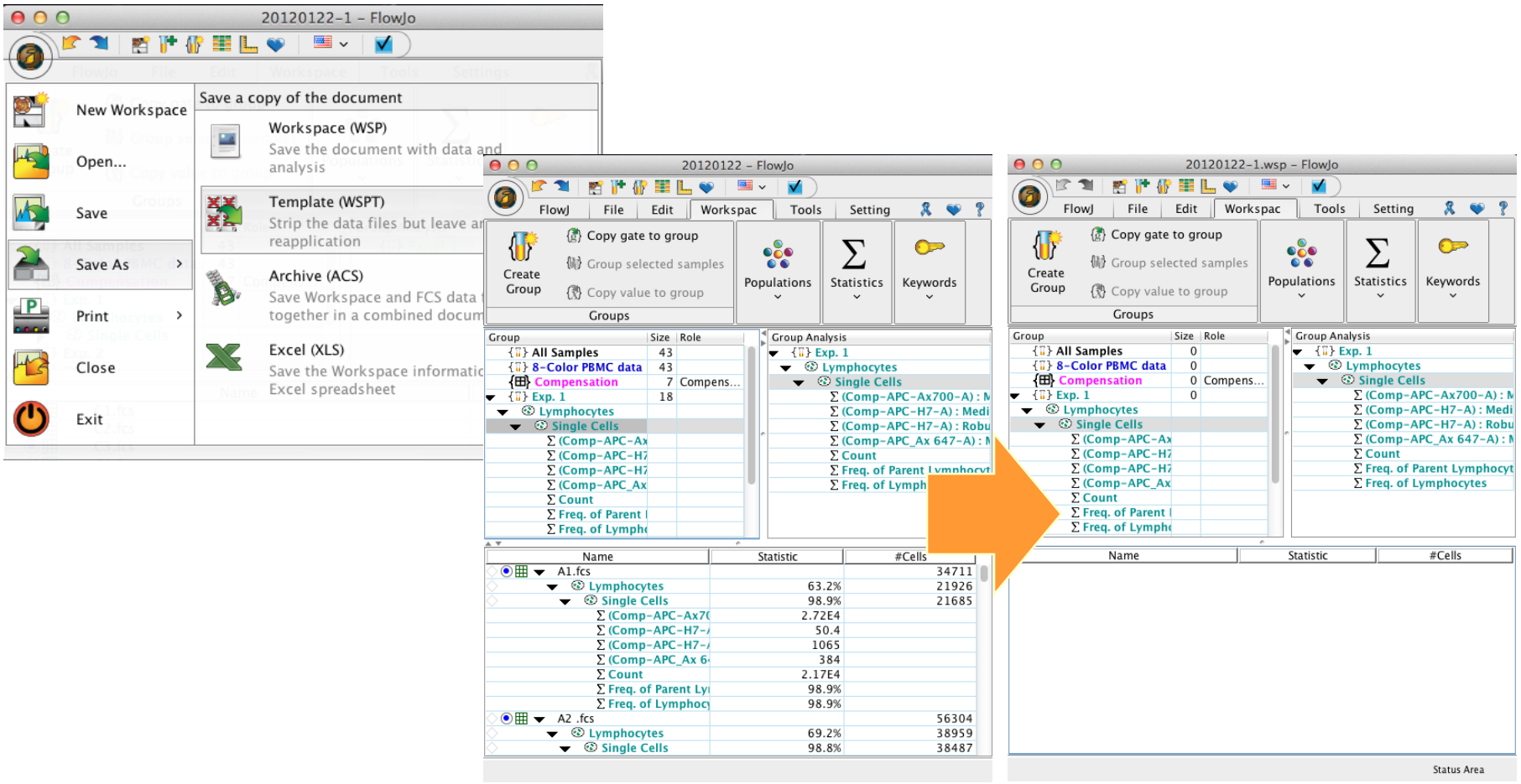


Table Editor: Exporting the Table

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%	*150*	▲ 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

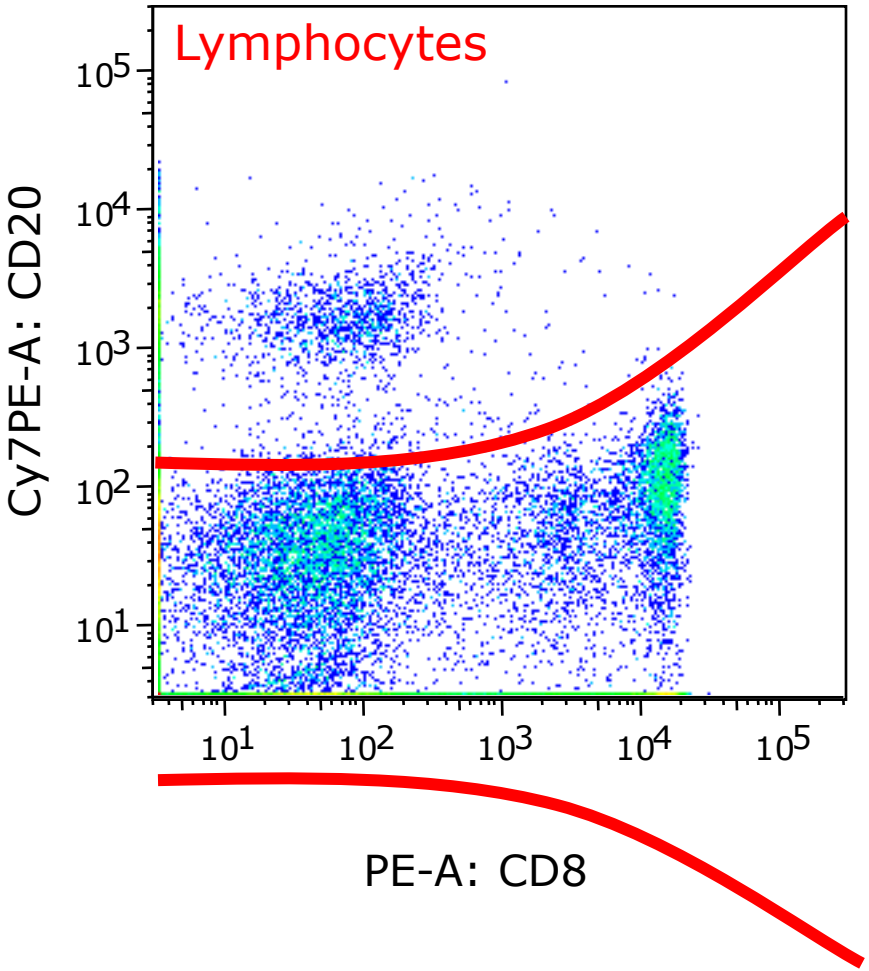


FlowJo Version 10 - Chimera

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

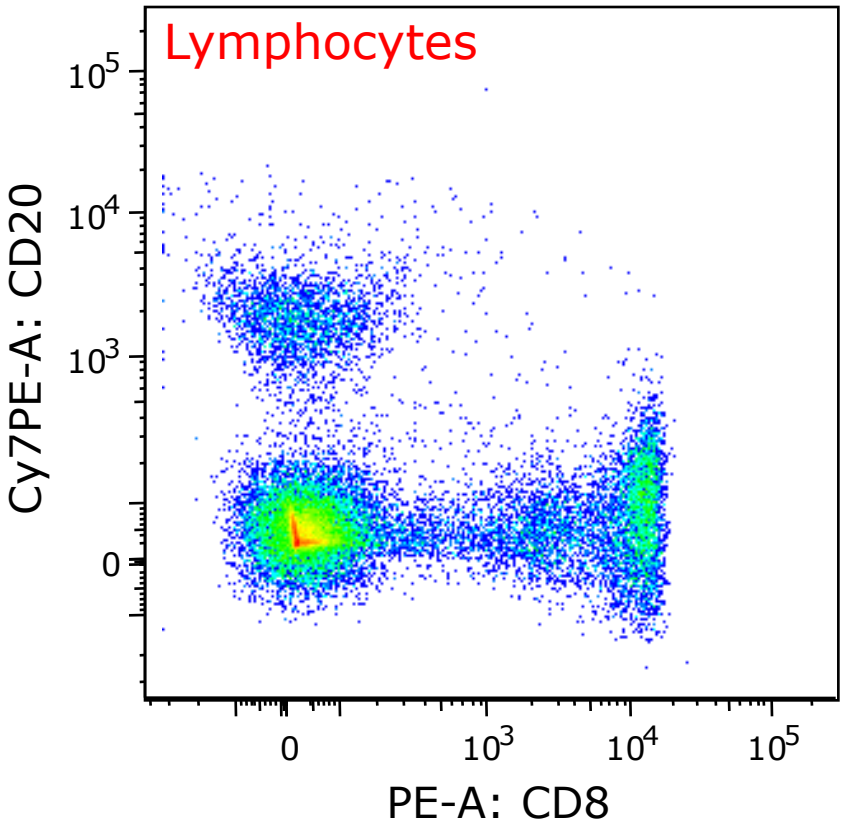
Biexponential Transformation

The Actual Spread

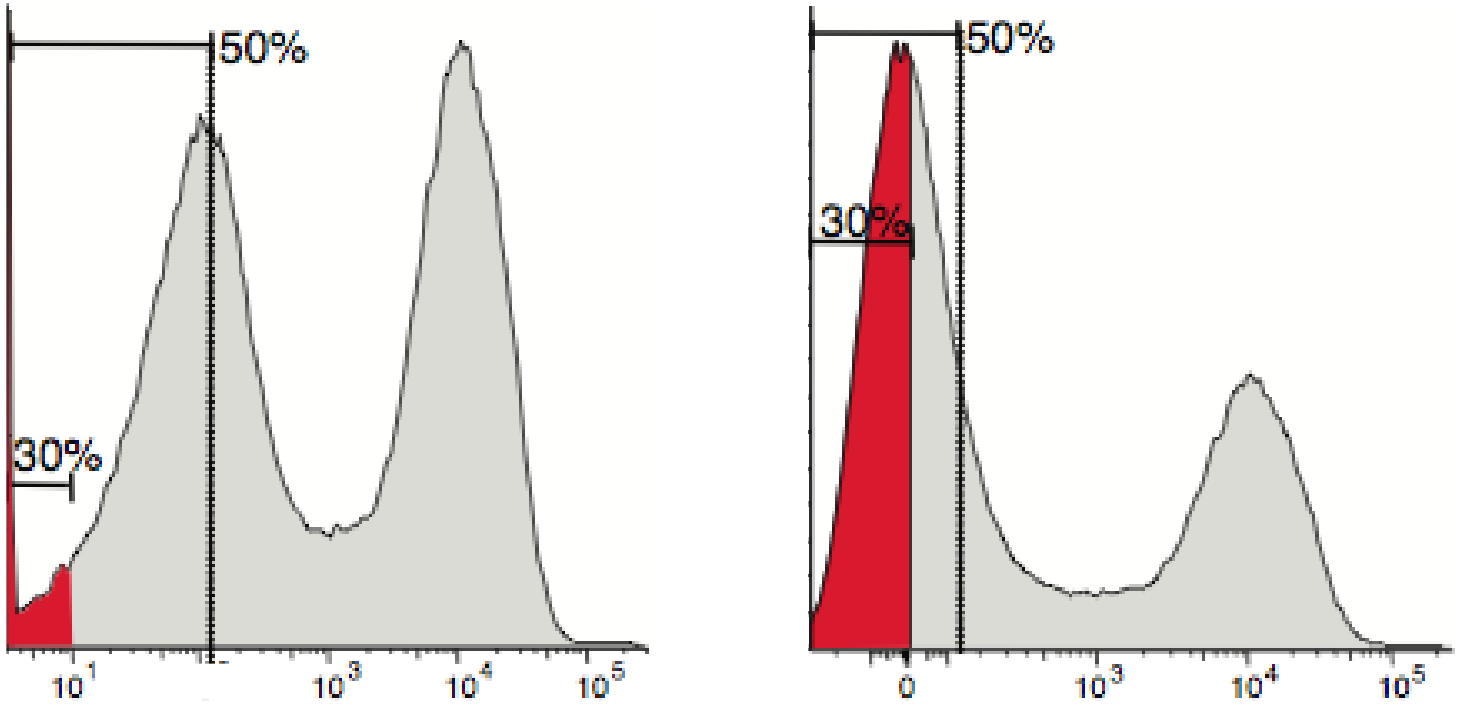


Biexponential Transformation

New Display



Biexponential Transformation

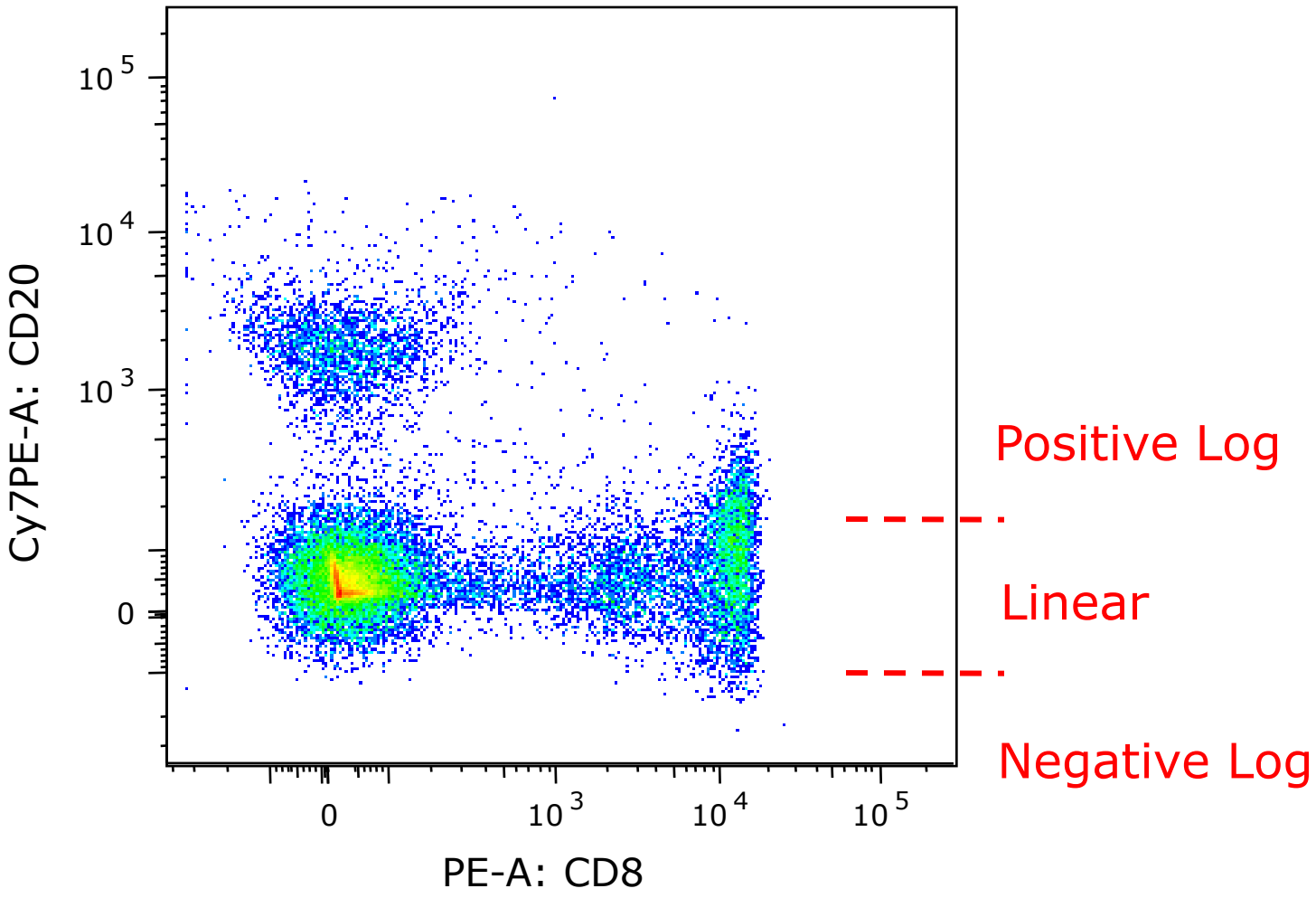


Baseline subtractions or compensation result in NEGATIVE values

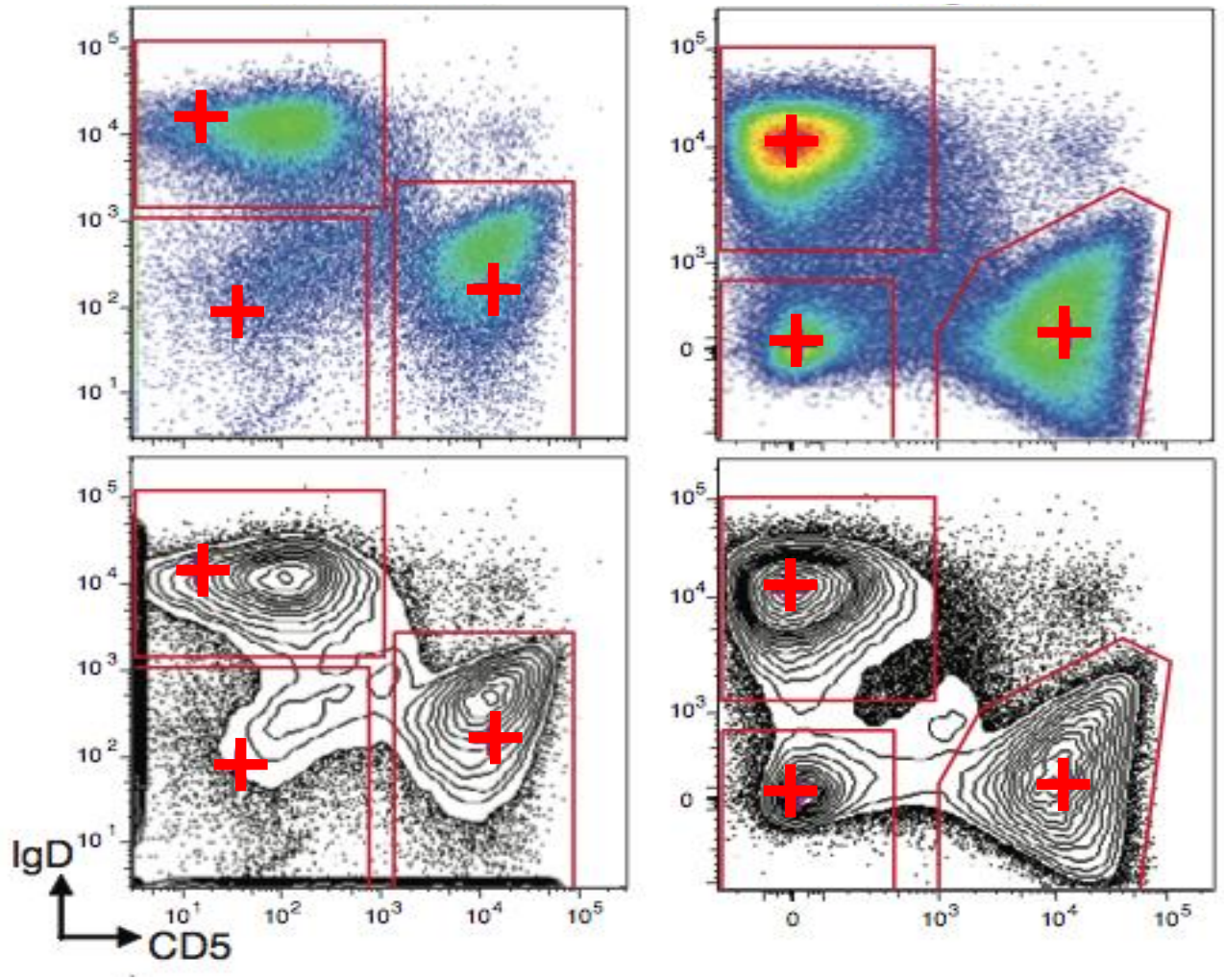
Transformation “EXPANDS” log scale for lows and negatives

Data values are NOT altered – only the scale is altered

Biexponential Transformation



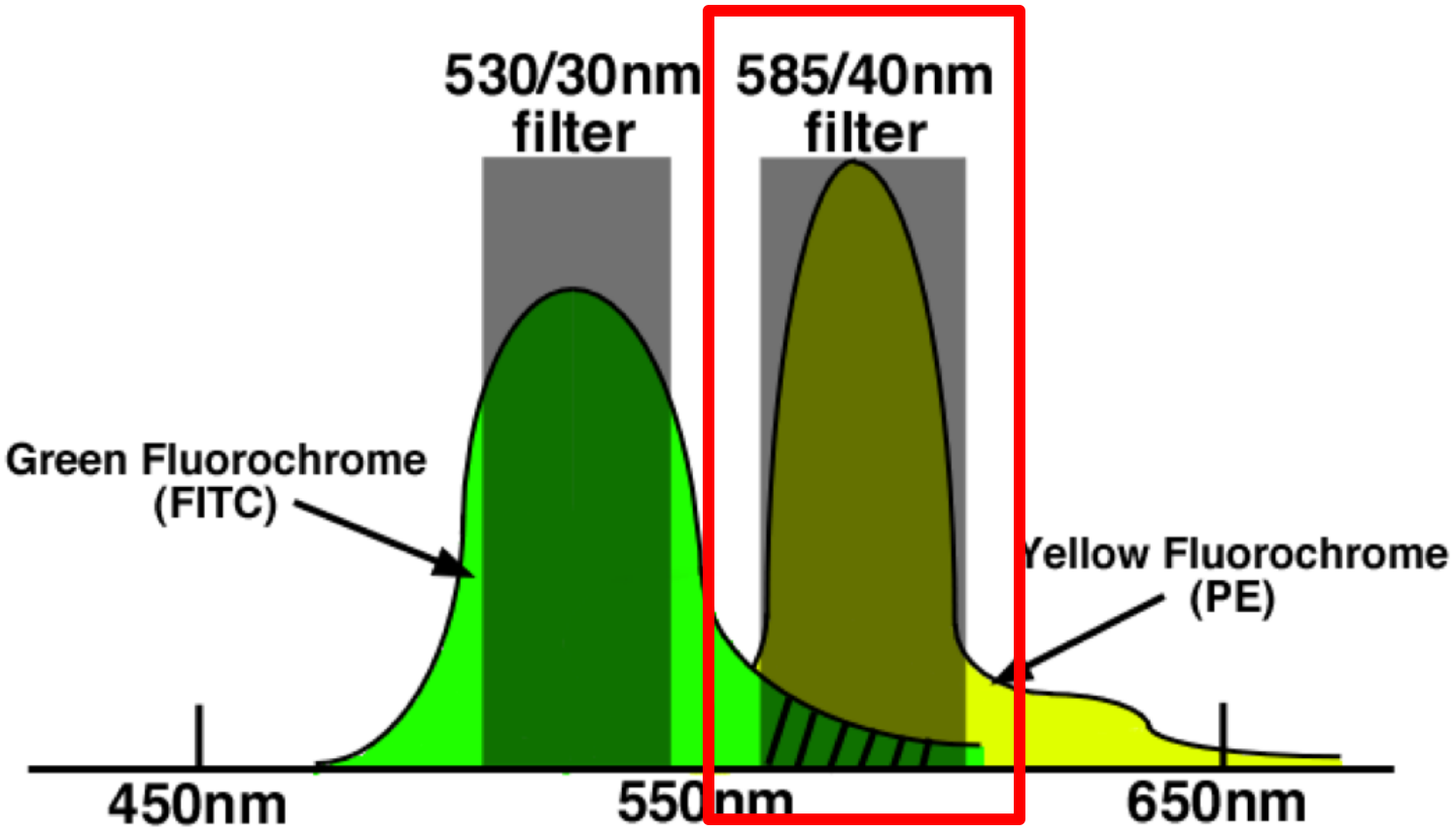
Biexponential Transformation



Parks DR, Roederer M, Moore WA. Cytometry A. 2006 Jun;69(6):541-51.

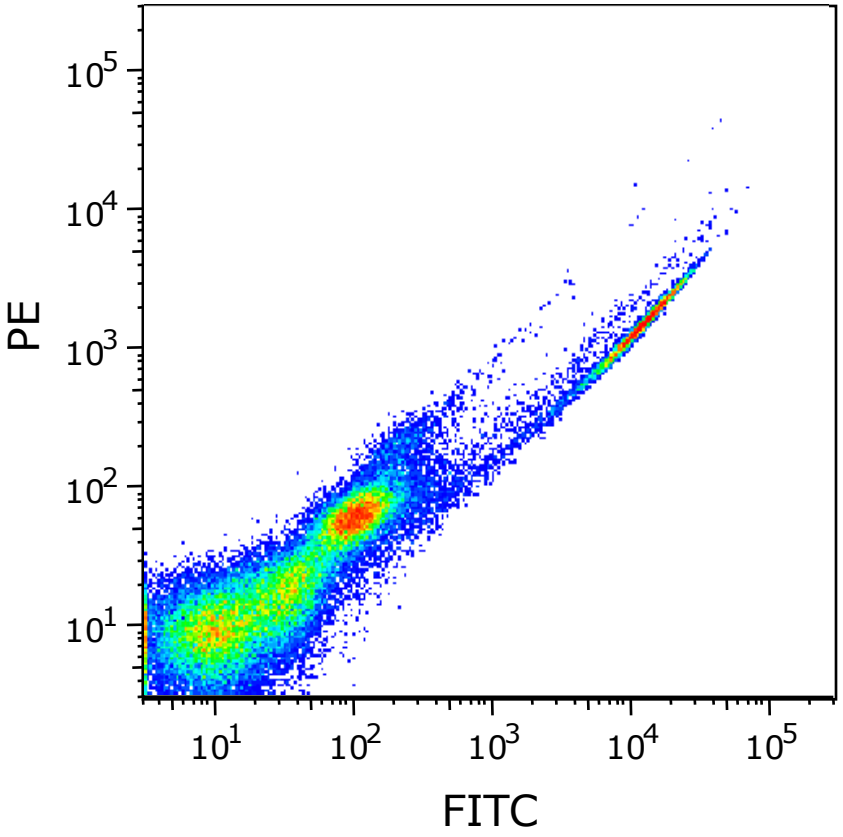
Compensation – What is it?

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

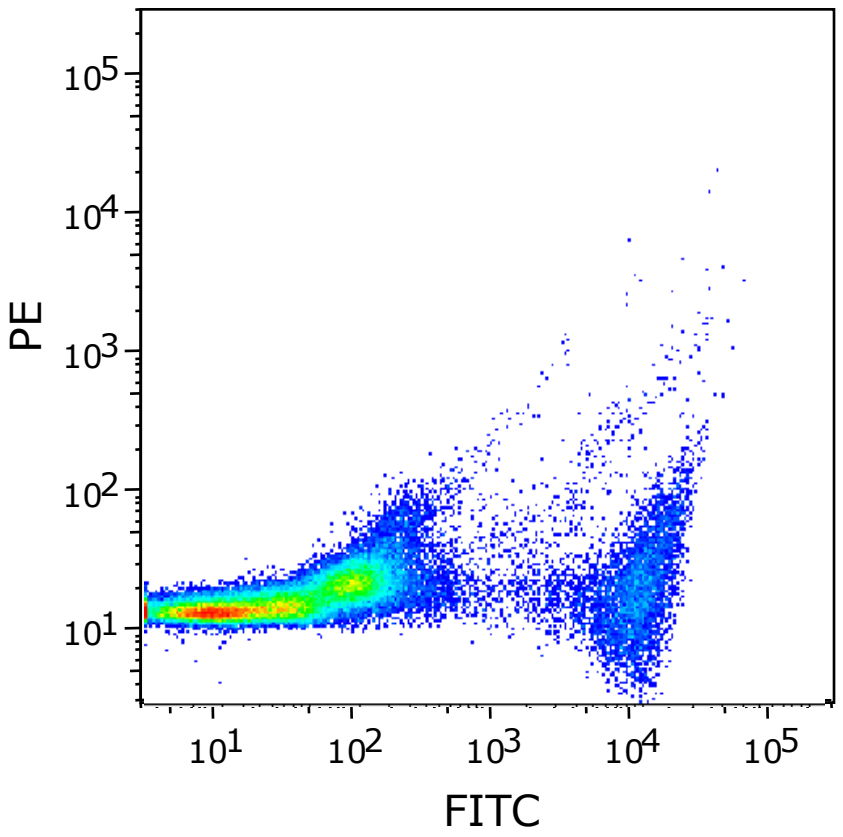


Compensation – Why is it important?

Uncompensated



Compensated



Compensation – How to do it?

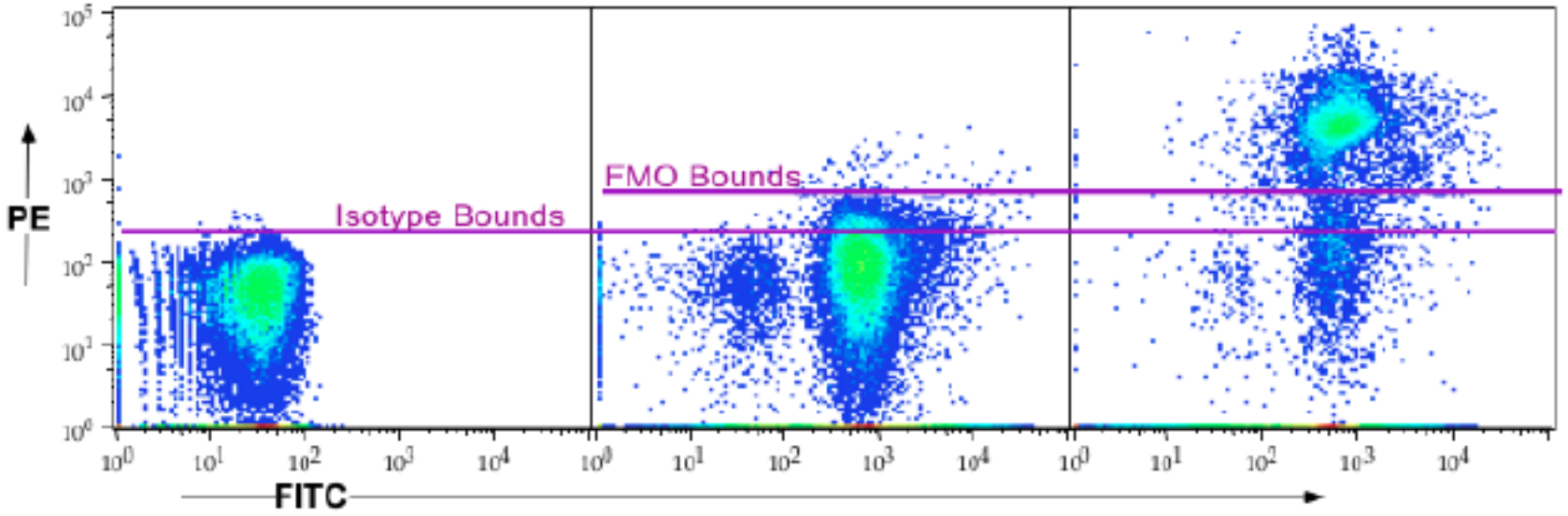
Setting Up Compensation Controls

- 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)

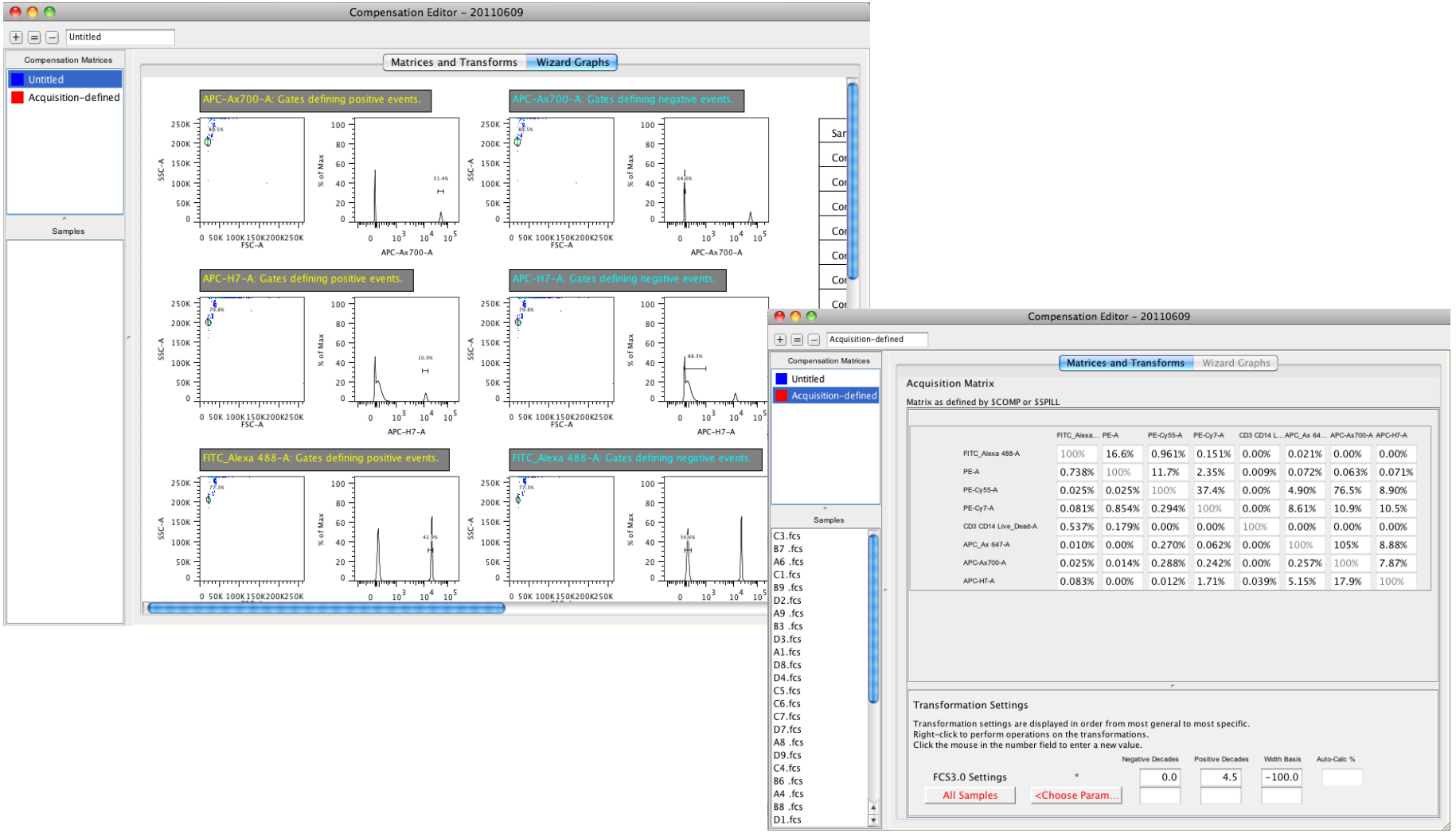
	Unstained Control	FMO Control	Fully Stained
FITC	–	CD3	CD3
PE	–	–	CD4
Cy5PE	–	CD8	CD8
Cy7PE	–	CD45RO	CD45RO



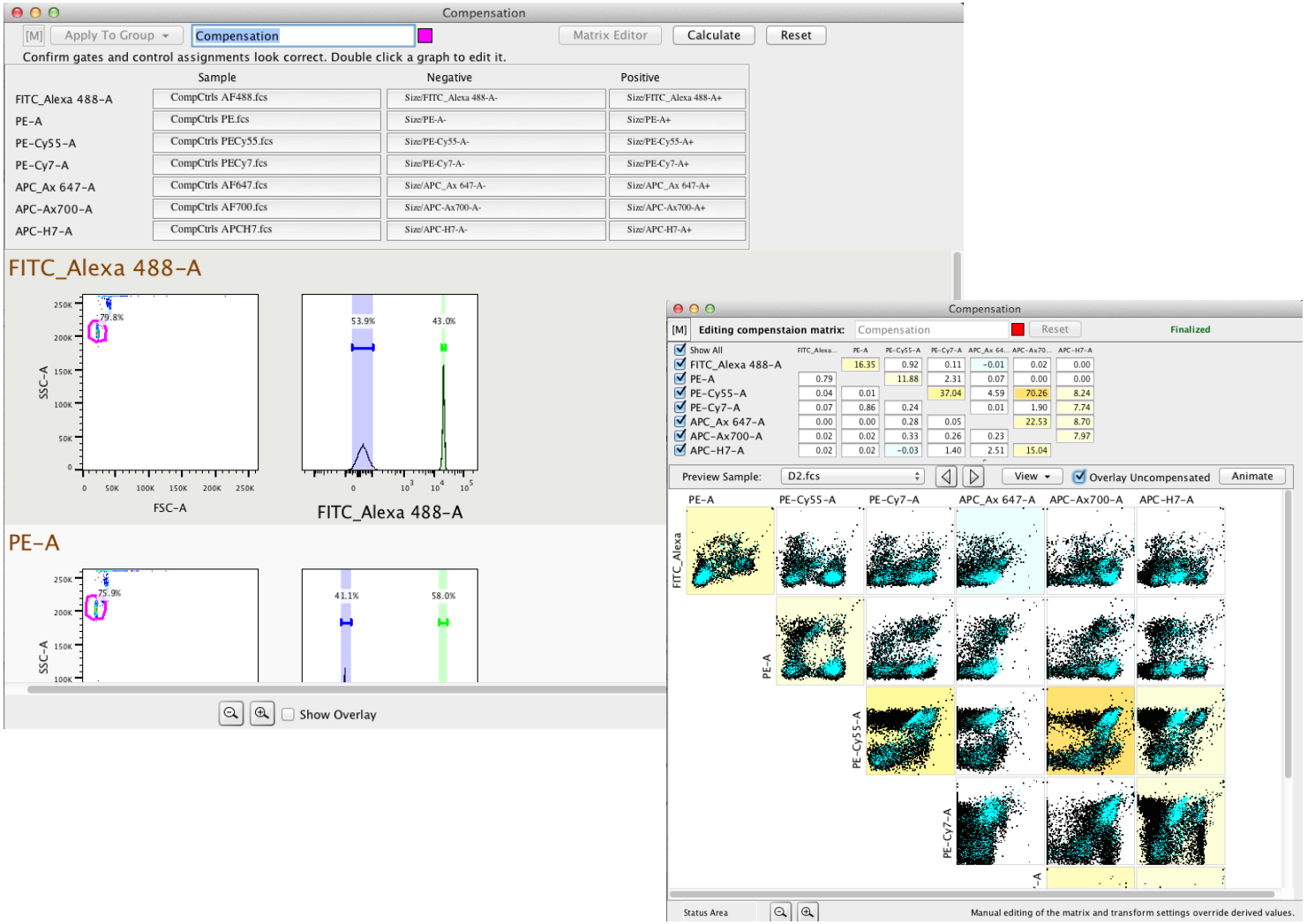
Gating Controls

Tube #	Description	FL1	FL2	FL3
1	*Experimental Sample*	CD3 FITC	CD4 PE	CD8 Cy5PE
2	*Compenstaion Controls* (Single stains – one for each fluorochrome used in the experiment)	CD3 FITC	-	-
3		-	CD4 PE	-
4		-	-	CD8 Cy5PE
5	*Gating Controls* (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



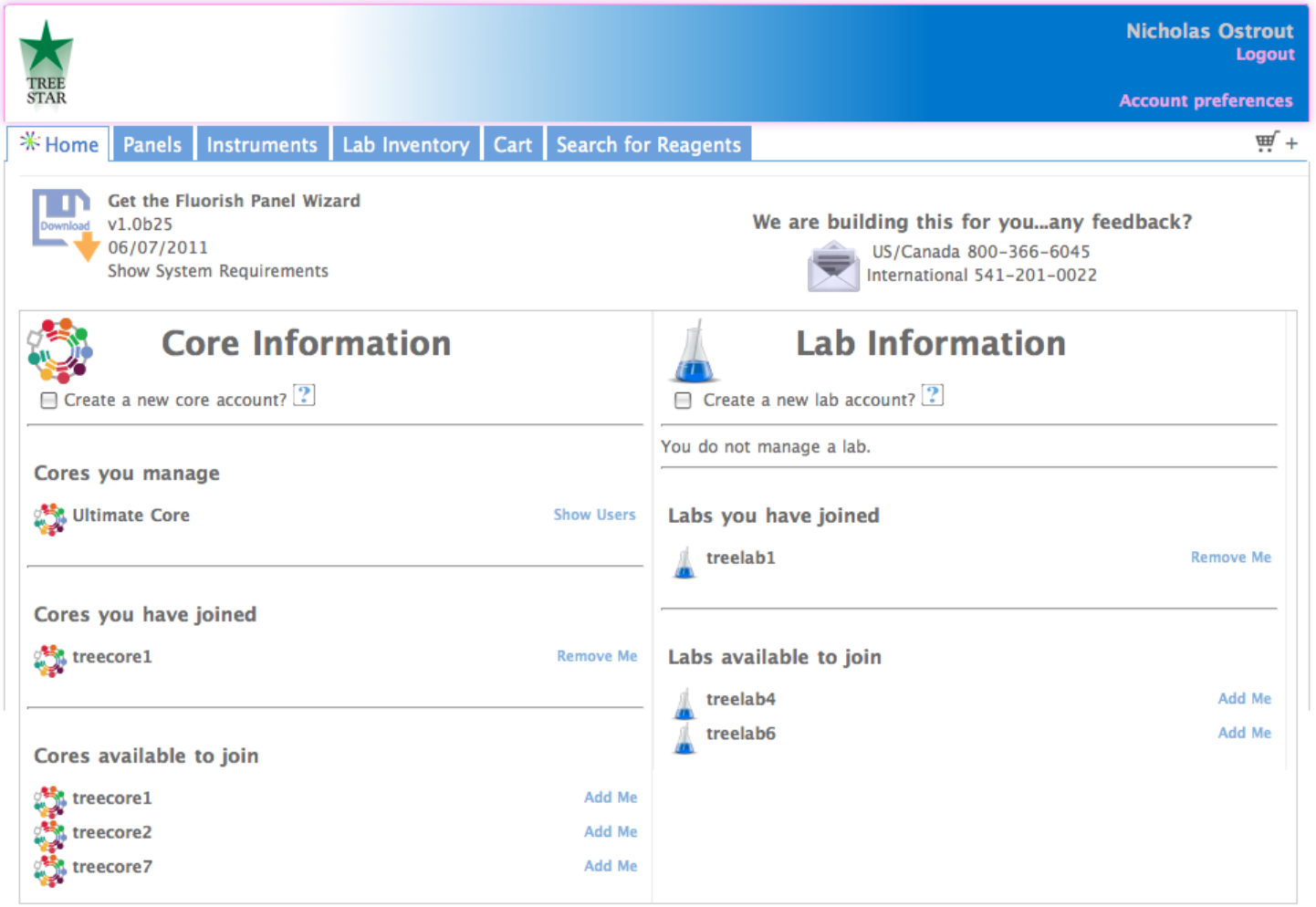
Version 10 Compensation Wizard



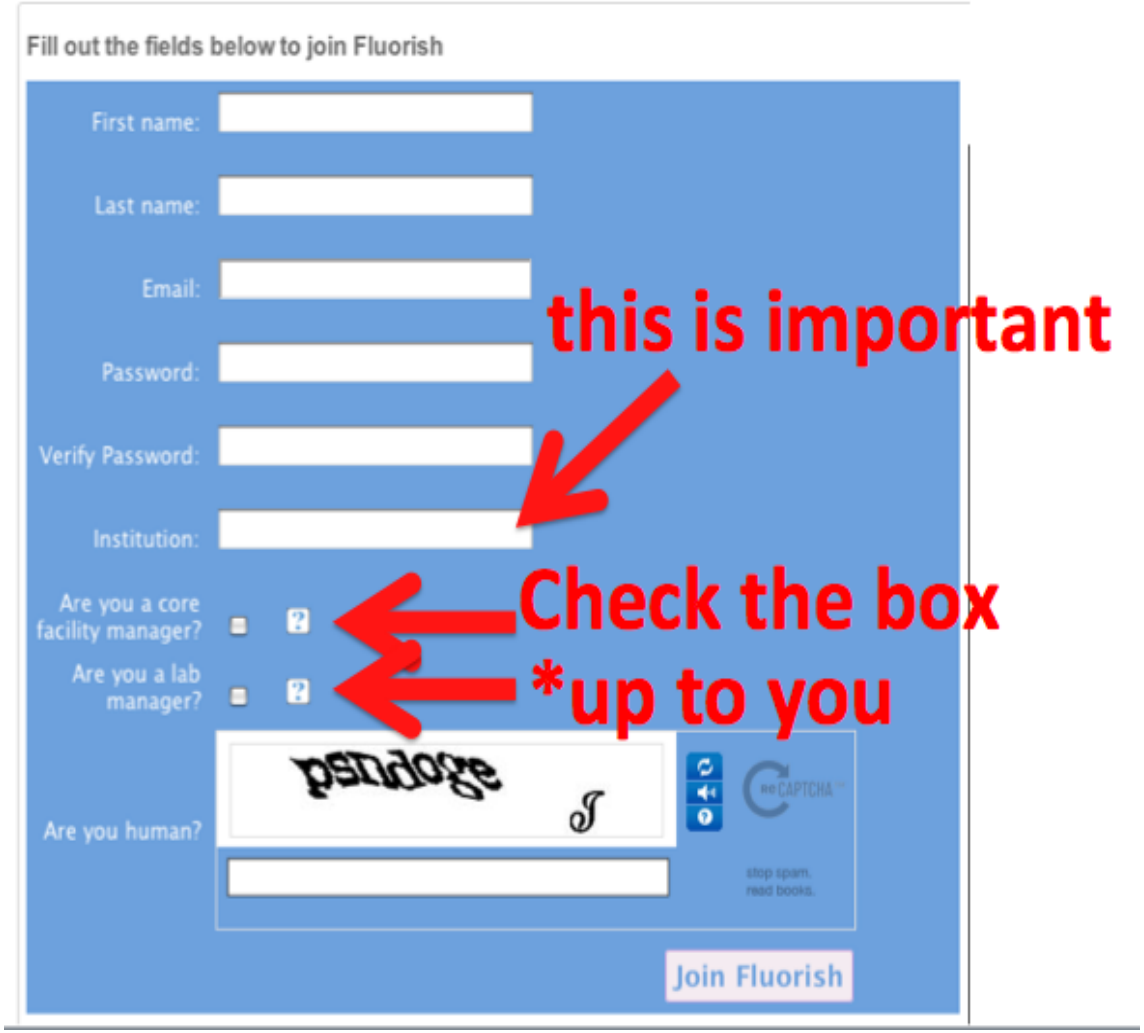
FLUORISH! Panel Wizard

- 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


TREE STAR
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



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

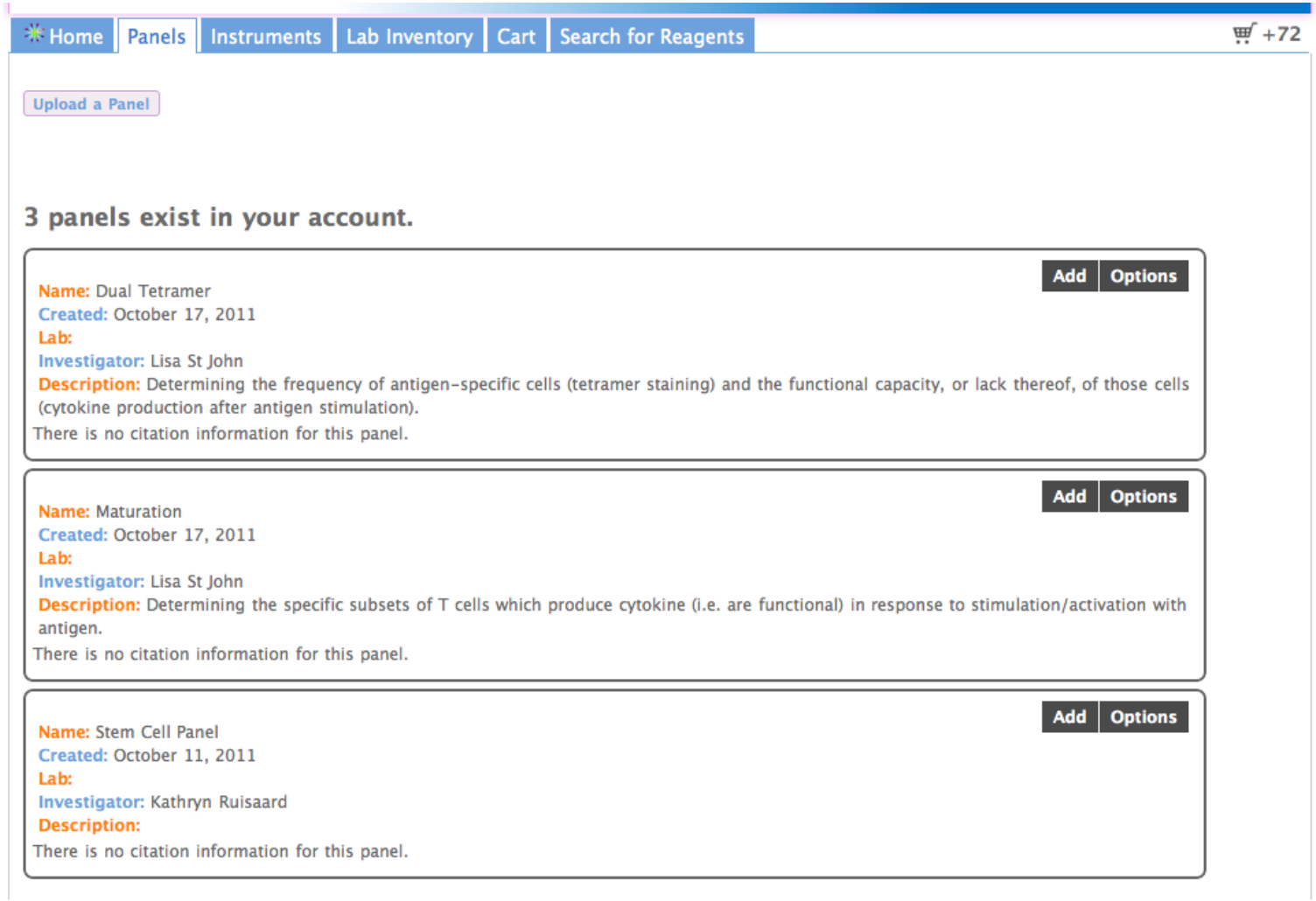
Aria #1 3-laser, 9-color

BD Biosciences - FACSAria II

Aria #2 2-laser, 7-color

BD Biosciences - FACSAria II

Save Antibody Panels For Life



Steps

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

Welcome

Log In

FLUORISH

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 | < Previous | Next > | Upload

Steps

1. Welcome
2. **Select Cytometer**
3. Cytometer Configuration
4. Select Dyes
5. Fluorochromes
6. Targets
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Select Cytometer

Log In

Choose your cytometer from the list below:

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

Please login or create a fluorish.com account to populate this list.

Help | Save | Cancel | < Previous | Next > | Upload

Steps

1. Welcome
2. **Select Cytometer**
3. Cytometer Configuration
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Select Cytometer

Log In

Choose your cytometer from the list below:

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

- ○ Accuri C6
- ○ FACScan
- ○ FACSCalibur 1 Laser
- ○ FACSCalibur 2 Laser
- ○ FACSVantage
- ○ FACSArray
- ○ FACSAria
- ○ FACSAria II
- ○ FACSAria III
- ○ FACSCanto
- ○ FACSCanto II
- ○ FACSVerse
- ○ LSR I
- ○ LSR II
- ◉ LSRFortessa
- ○ Influx
- ○ FACSJazz

Help | Save | Cancel | < Previous | Next > | Upload

Steps

1. Welcome
2. Select Cytometer
3. Cytometer Configuration
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7. Conjugations
8. Summary
9. Finalize

Cytometer Configuration

Log In

Open Recent | Change Cytometer

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.

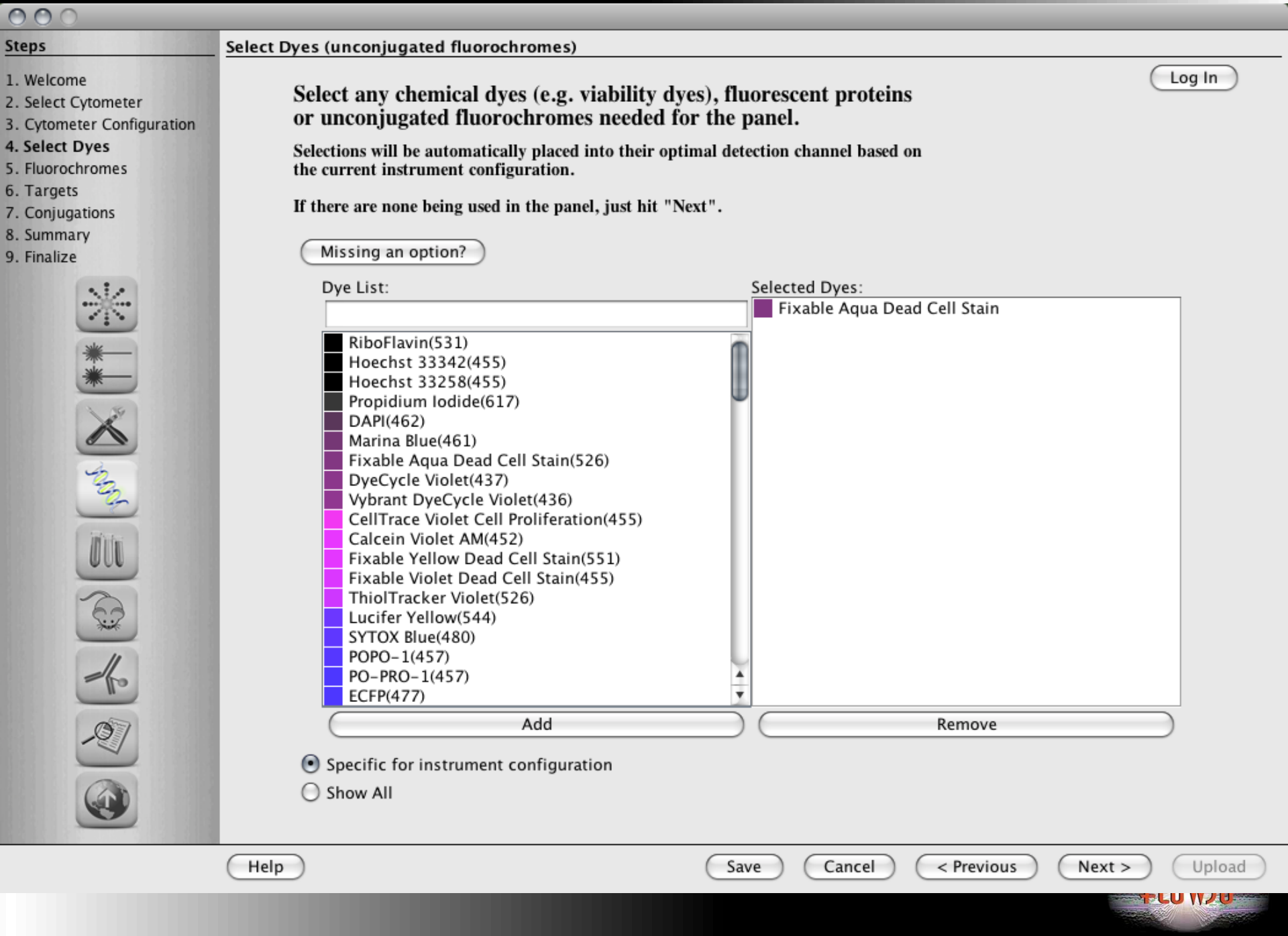
Instrument Configuration

- 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

Help | Save | Cancel | < Previous | Next > | Upload


Steps
1. Welcome
2. Select Cytometer
3. Cytometer Configuration
4. Select Dyes
5. Fluorochromes
6. Targets
7. Conjugations
8. Summary
9. Finalize
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:
Selected Dyes:
Fixable Aqua Dead Cell Stain
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)
Add
Remove
Specific for instrument configuration
Show All
Help
Save
Cancel
< Previous
Next >
Upload

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?

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

Targets

Log In

Select the species and identify the target antigens.

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

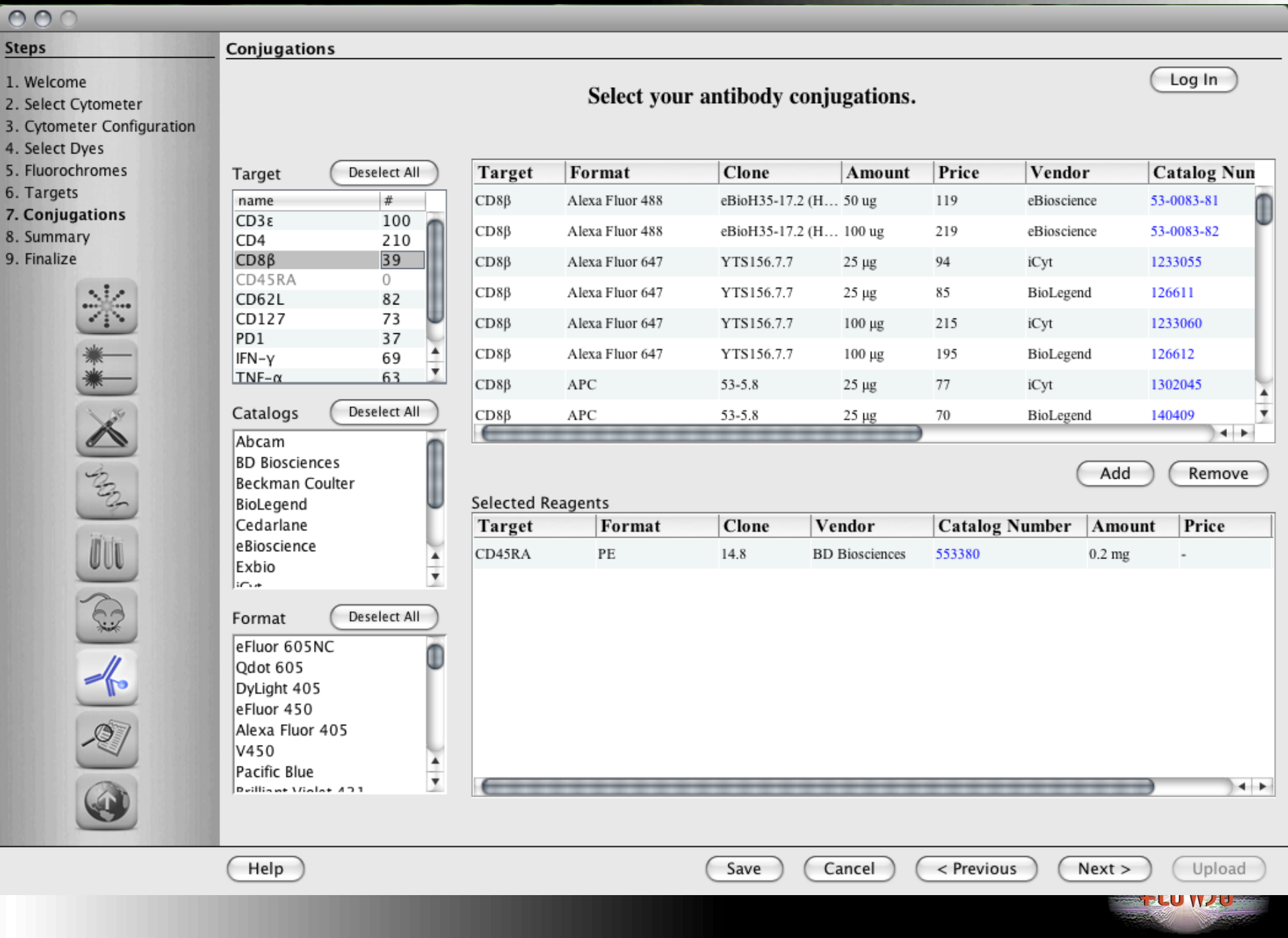
Add >>

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

Help | Save | Cancel | < Previous | Next > | Upload


Steps
1. Welcome
2. Select Cytometer
3. Cytometer Configuration
4. Select Dyes
5. Fluorochromes
6. Targets
7. Conjugations
8. Summary
9. Finalize
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
Format
Deselect All
eFluor 605NC
Qdot 605
DyLight 405
eFluor 450
Alexa Fluor 405
V450
Pacific Blue
Target Format Clone Amount Price Vendor Catalog Nun
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 -
Help
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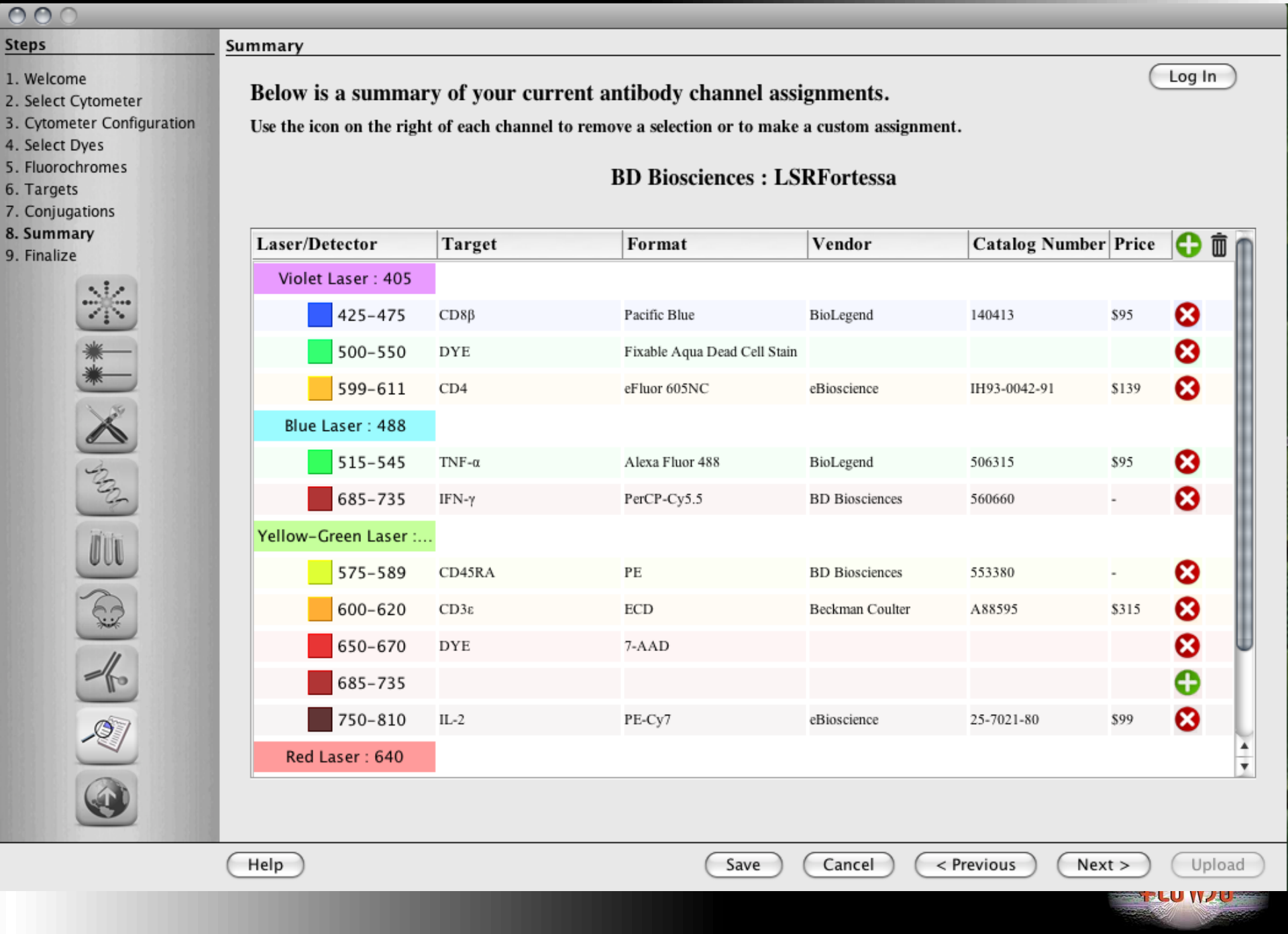
< Previous
Next >
Upload


Steps
1. Welcome
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3. Cytometer Configuration
4. Select Dyes
5. Fluorochromes
6. Targets
7. Conjugations
8. Summary
9. Finalize
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
CD8β
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
CD3ε
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
Help
Save
Cancel
< Previous
Next >
Upload

Steps

1. Welcome
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7. Conjugations
8. Summary
9. **Finalize**

Finalize

Log In

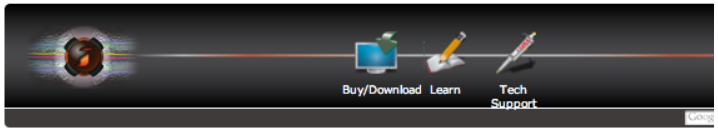
Final Summary

Experimental Description | Save To CSV | Print | Order

Laser/Detector	Target	Format	Vendor	Catalog Number	Price
Violet Laser : 405					
425-475	CD8β	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	CD3ε	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					

Help | Save | Cancel | < Previous | Next > | Upload

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 u demonstrate some of the basic analysis features of FlowJo. You can watch one of our application scientists dem 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 thorough 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

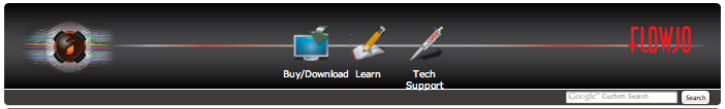
Provides an overview of the proliferation modeling algorithm, suggests model adjustment techniques, and giv explanation of the proliferation statis
Read more...

Kinetics Tutorial

Shows how to analyze time series
Read more...

Batching and Iteration

Grants you access to the most po from the patterns of your dataset(s)
Read more...



FlowJo WebEx

Find this helpful?

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 logon 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 WebExs are listed on our WebEx calendar, here.

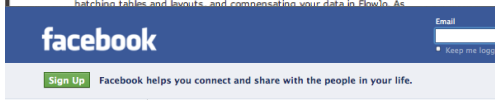
What is covered in a WebEx?

- 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. As

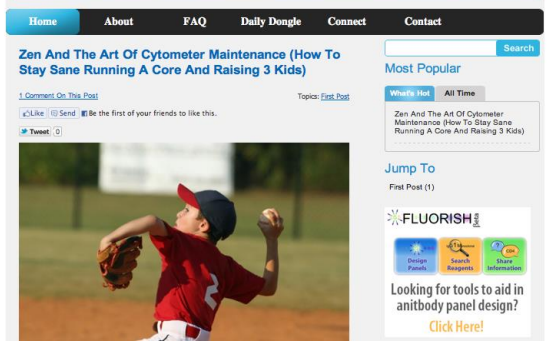
Steps to Joining a WebEx

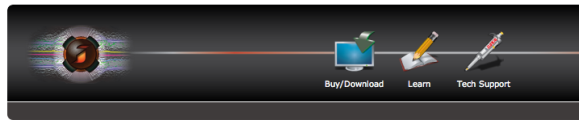
Click on the FLOWJOx icon

Click Join on the calendar page



FLUORISH BLOG





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

Thank You...Questions?

FlowJo Tech Support
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1-800-366-6045

Jack Panopoulos, Ph.D.
Application Scientist