INSTRUMENT TRAINING

Analyzer Training

- 1 on 1 ~1.5hrs
- Actual samples and controls
- Seminars highly recommended prior to training

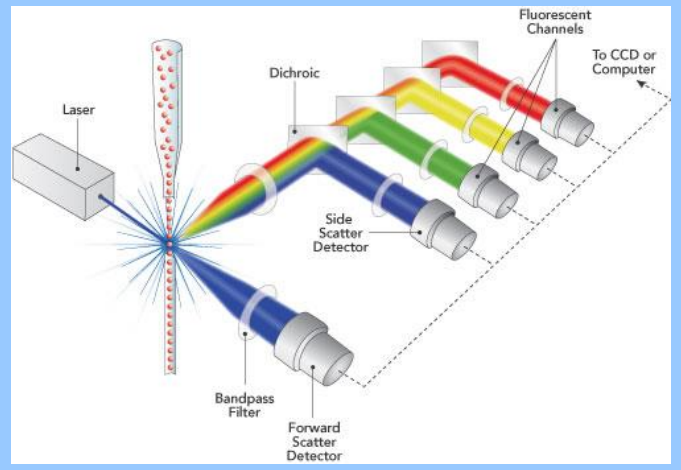
Step 1- contact the lab and request training (flowcytometry@cores.utah.edu)
Step 2- Fill out “request for analyzer Training” form (link in FAQ tab)
Step 3- Fill Out Work Authorization Form
Step 4- Get Trained

Cell Sorter Training

- Contact Lab for details.
- Open to anybody but we try and limit it to After hours needs or High Frequency sorts

SEMINARS

VIDEO RECORDING AVAILABLE ON WEBSITE



Flow Basics

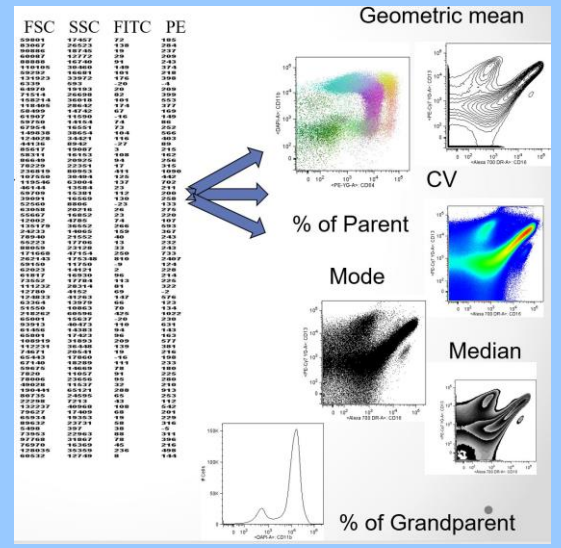
Internal

Step 1: Invitrogen Video

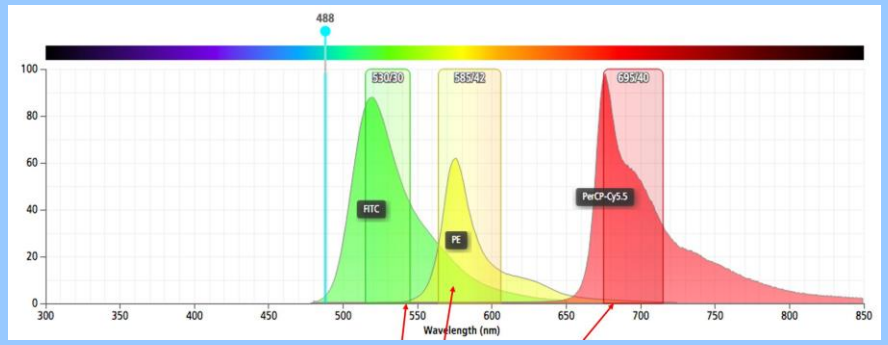
Step 2: 2021 Flow Cytometry **Basics** Short Version *Audio/Video Recording*

Step 3: Flow Cytometry Data Analysis Audio/Video Recording

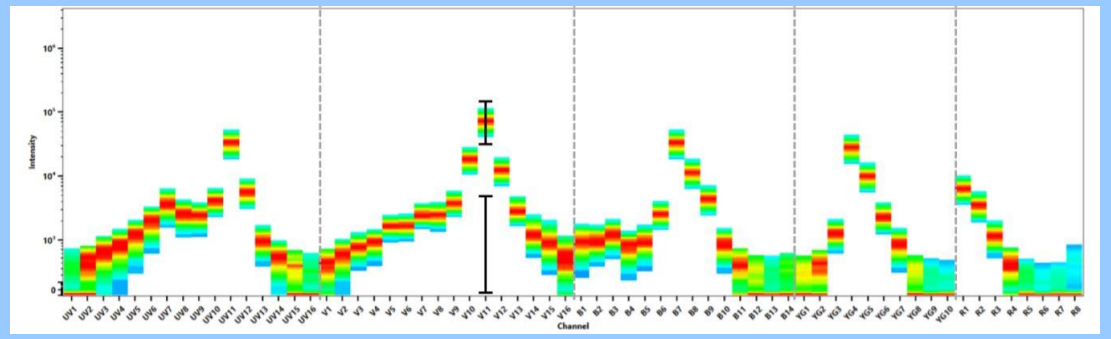
Step 4: Sensitivity *Audio/Video Recording*



Data Analysis



Advanced Compensation



Others- Spectral Flow Cytometry, Basics of Imaging Cytometry, more to come

Rules for Compensation Control

1)First and foremost, there must be a single-stained control for every parameter in the experiment!

In addition, there are a handful of additional rules for “good” compensation controls:

2) Background fluorescence should be the same for the positive and negative control

3) Compensation controls must match the exact experimental fluorochrome

4) Compensation controls must be treated (fix, perm etc) in the same manner as experimental samples.

5) Compensation controls need to be at least as bright or brighter than any sample the compensation will be applied to

6) Collect enough data while running controls to have a significant cell number in positive gate.

7) Never blindly trust Autocomp

Rule #1-There must be a single-stained control for every dye in the experiment!

-If you have an 8 color panel, you should have 8 single color controls

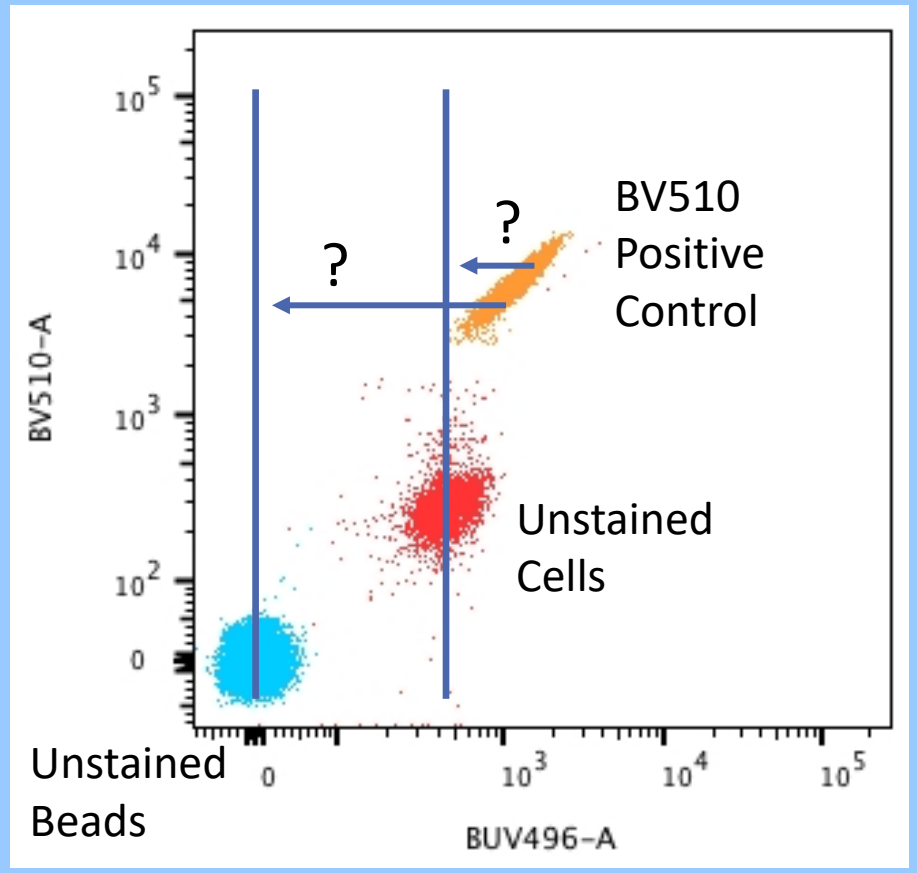
-If you have 3 panels with 3 different PECY7 dyes, you should probably be running 3 different controls for PECY7

-Single Color means Single Color

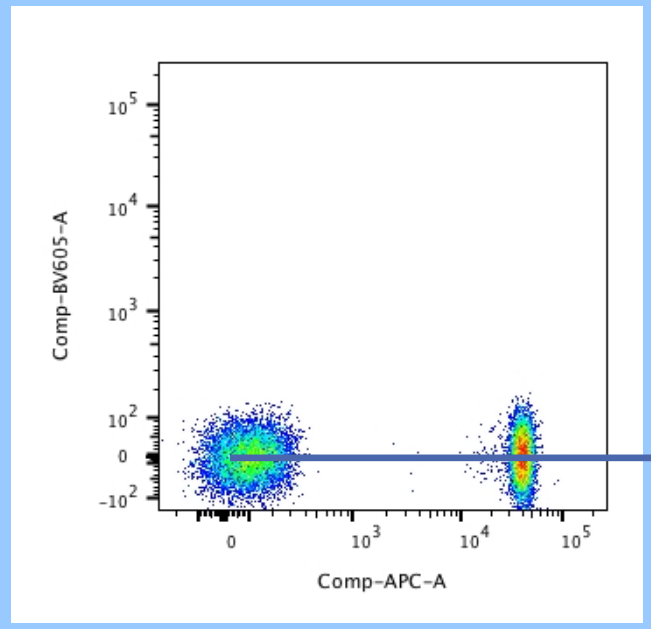
- ~~“I added DAPI to all my controls”~~
- ~~“All of my controls have GFP”~~

Rule #2 Background fluorescence should be the same for the positive and negative control

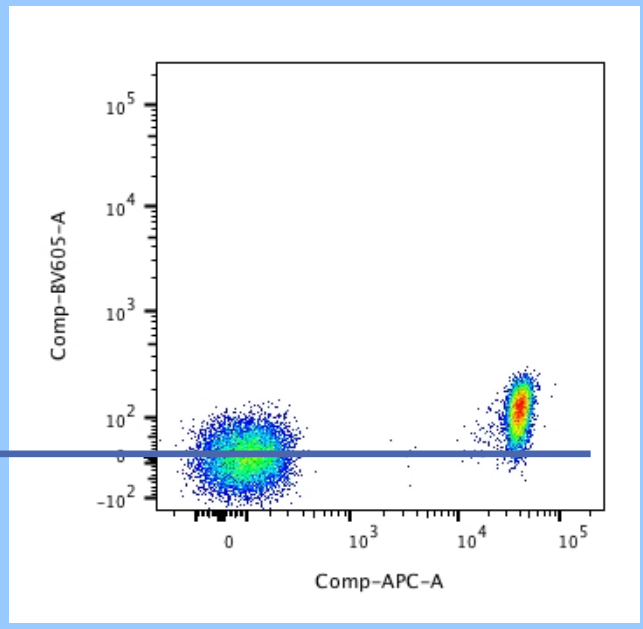
Example 1



Example 2



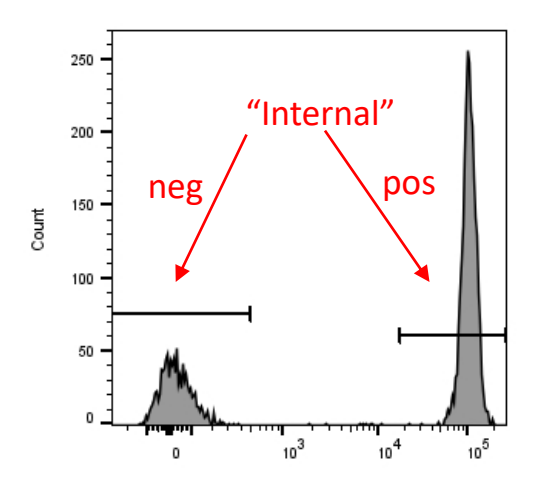
Used beads to set up comp matrix



Used Monocytes as universal negative and beads as the positive

Rule #2- Background fluorescence should be the same for the positive and negative control

Universal negative
VS
Internal negative



Edit Reference Group — Spectroflow

Define Unstained Control Matching Sample Autofluorescence

Name	Control Type	View Stored Unstained Controls
Unstained	Cells	3 Controls

1-Unstained Cells control

Define Universal Negative Control(s)

Name	Control Type
Unstained Beads	Beads

2-Additional Unstained controls
Cells or beads

Add Remove

Fluorescent Tags

Fluorescent Tag	Control Type	Label	Universal Negative	View Stored Reference Controls
BUV805	Cells		Unstained	1 Control
Super Bright 436	Cells		Unstained	N/A
BV480	Cells		Unstained	1 Control
BV510	Cells		Unstained	1 Control
FITC	Cells		Unstained	1 Control
PerCP-eFluor 710	Cells			N/A
PE-Cy5	Cells			1 Control
APC	Cells		Unstained	1 Control
Alexa Fluor 700	Cells		Unstained	1 Control
APC-Fire 810	Cells		Unstained	N/A
BUV395	Beads		Unstained Beads	1 Control
BV605	Beads		Unstained Beads	1 Control
PE	Beads		Unstained Beads	1 Control

Add Remove

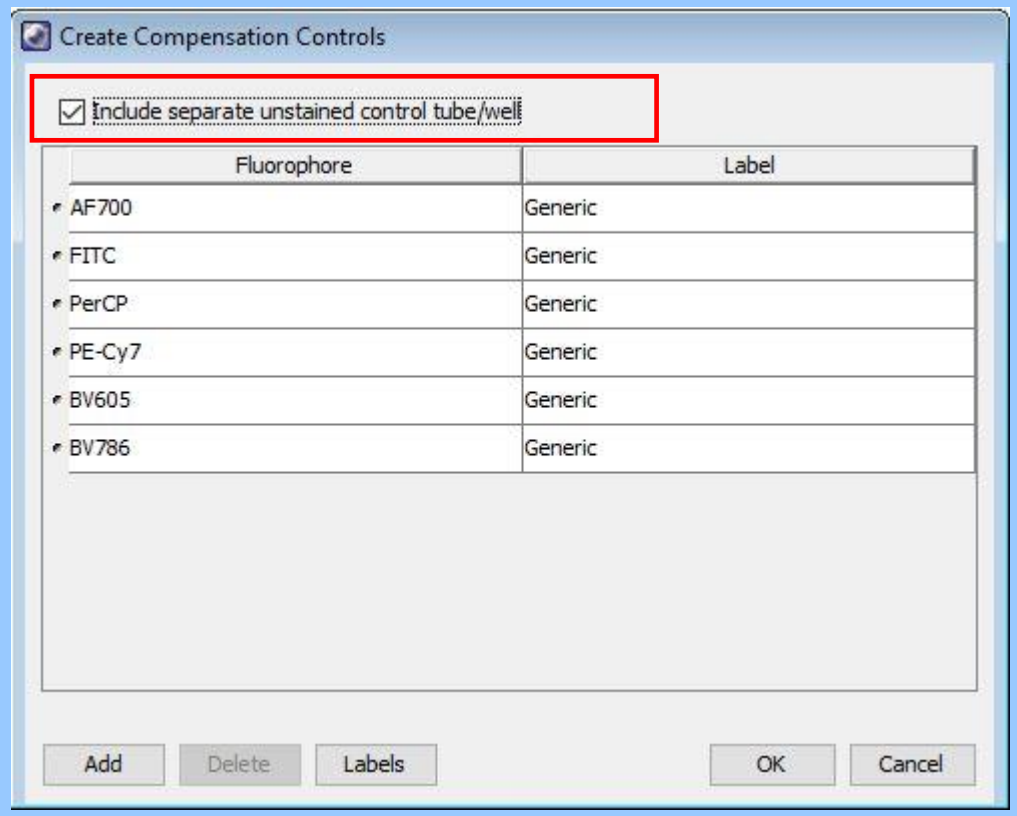
Cancel Save

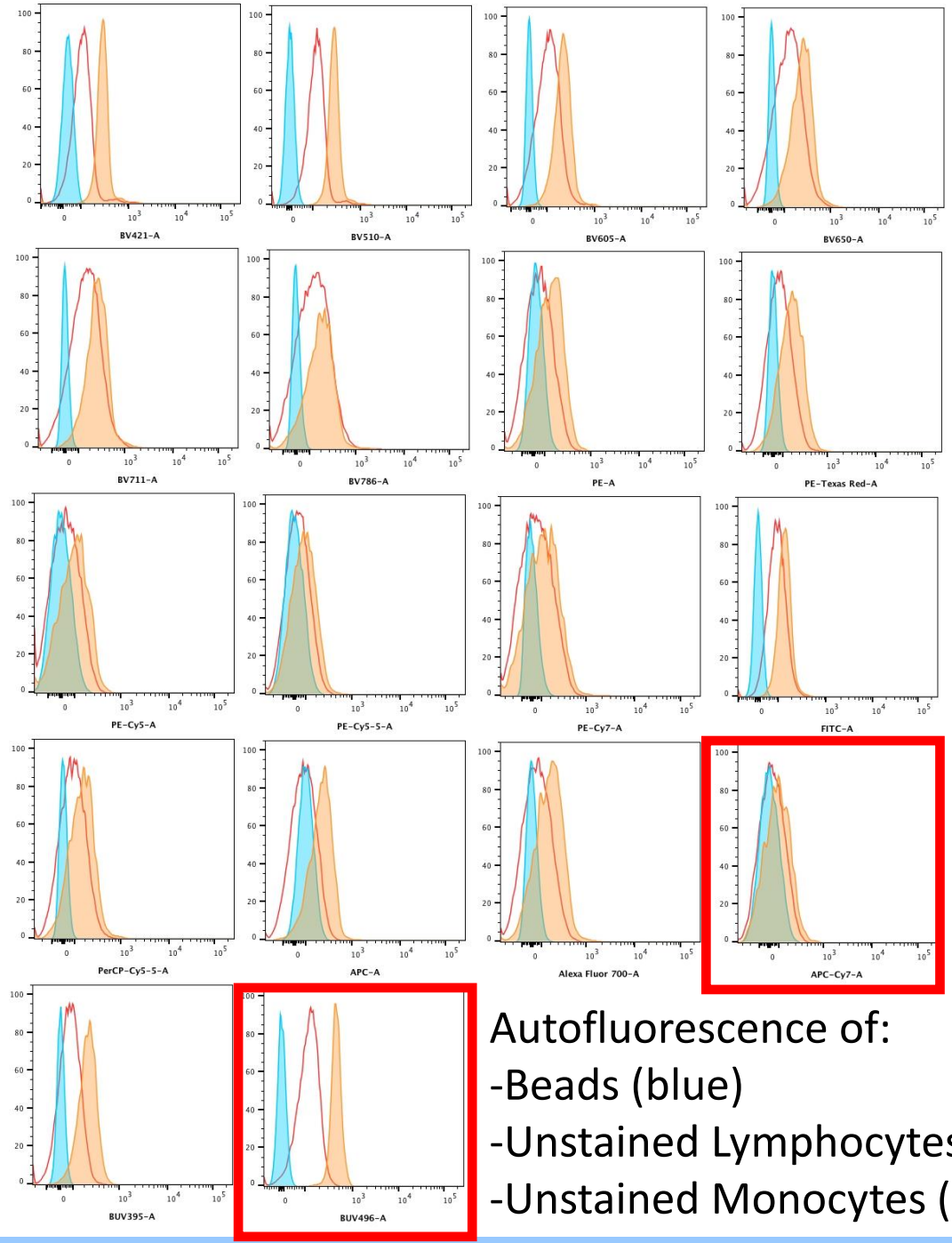
3-Define Positive controls
Cells or beads

4-Define matching autofluorescent negatives
-Unstained cells
-Unstained beads
-Blank= internal negative
-Other

Diva

-If this is checked you are using a “universal negative”





Rule #2- Background fluorescence should be the same for the positive and negative control

Compensated=Median of the Positive cells equals median of the negative cells

-If you use a “universal negative” it needs to match what you use for positive controls.

-If you use an internal positive and negative for each tube they need to match.

Autofluorescence of:
-Beads (blue)
-Unstained Lymphocytes (Red)
-Unstained Monocytes (Orange)

Rule #2- Background fluorescence should be the same for the positive and negative populations

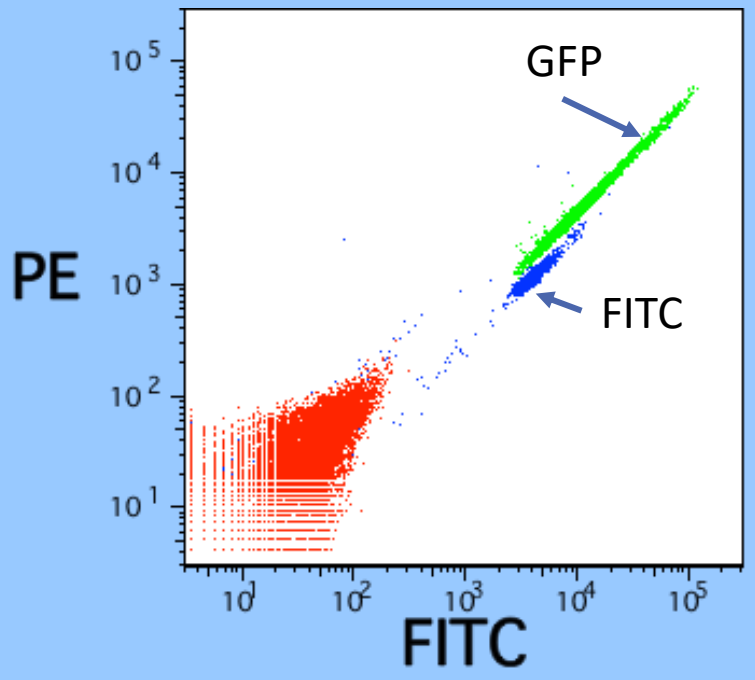
Note: You can run Beads for some colors and cells for other colors in the same experiment.

However – the negative control for each type must match the positive control: do not use a “universal negative” unstained beads (or cells) for all controls!

Autofluorescence of controls does NOT need to match experimental samples.

Rule #3 Compensation controls MUST match the exact experimental fluorochrome

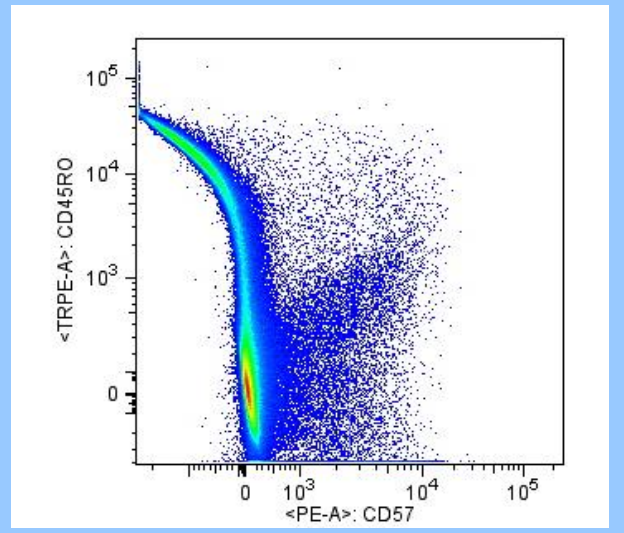
Example 1



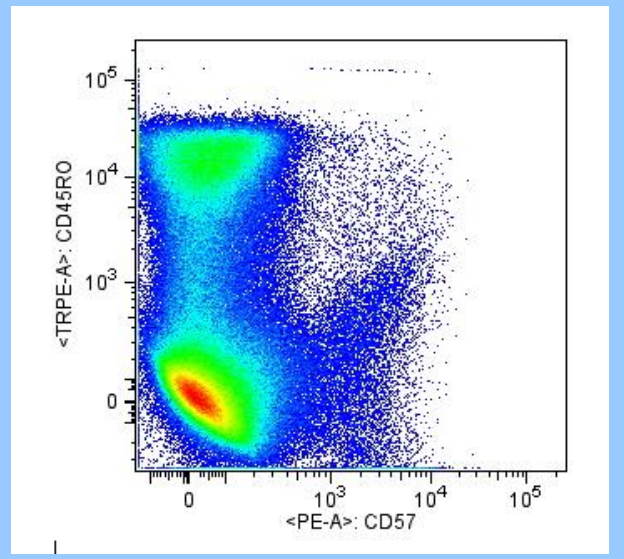
FITC ≠ GFP

Example 2

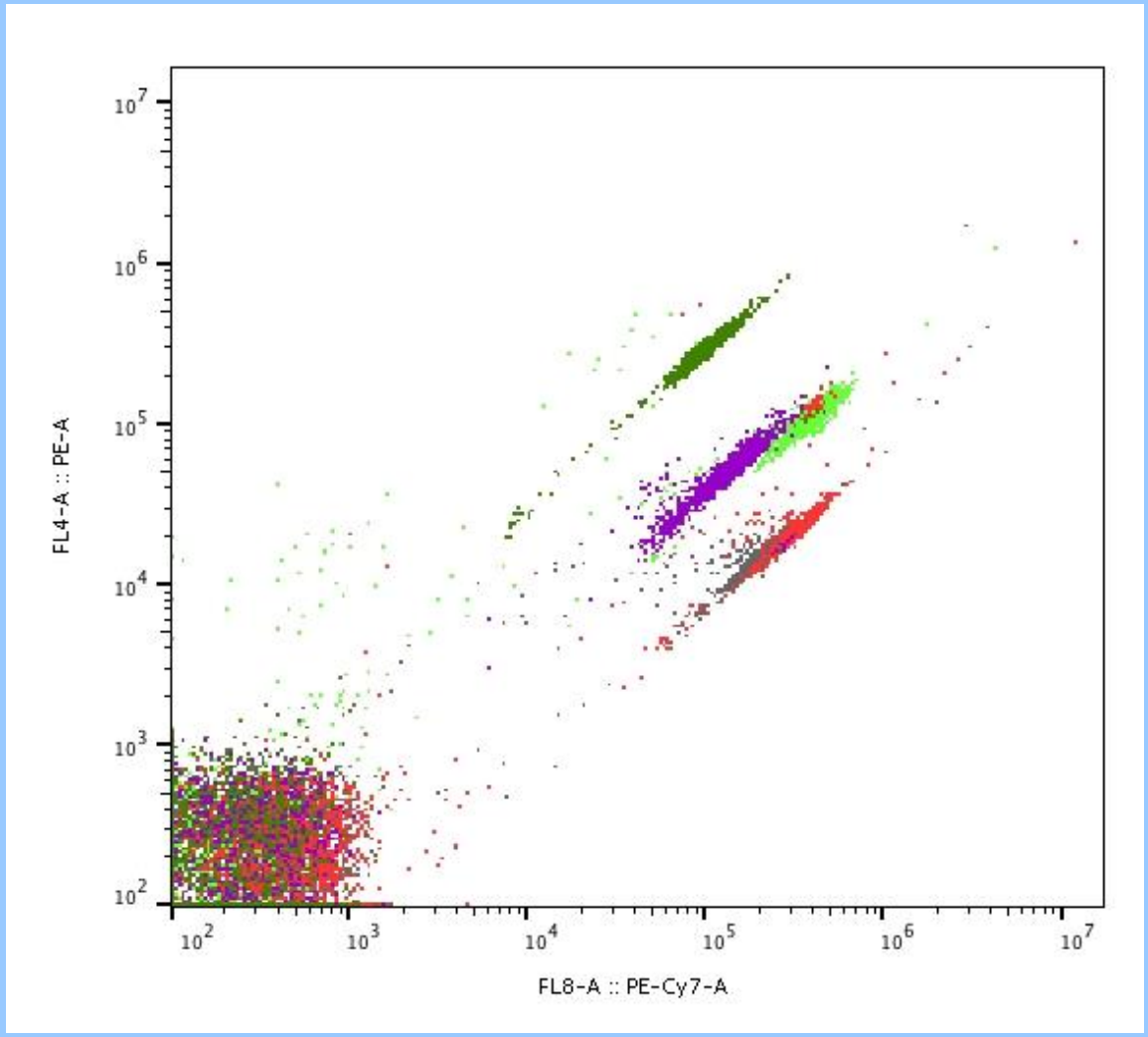
Wrong PE-TxRed comp control used



Correct PE-TxRed comp control used



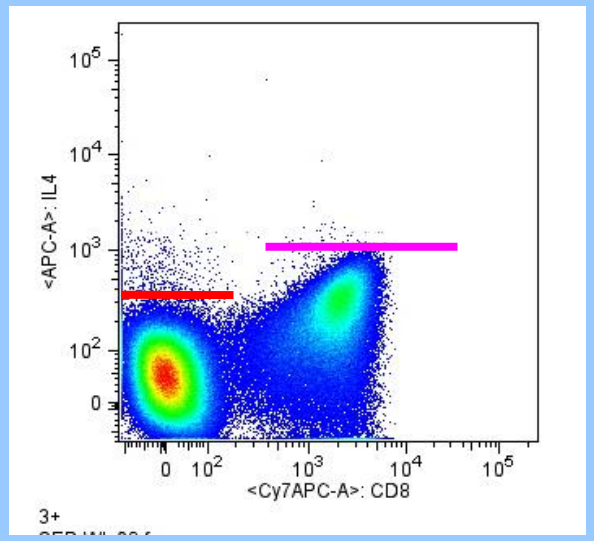
Rule #3 Compensation controls MUST match the exact experimental fluorochrome



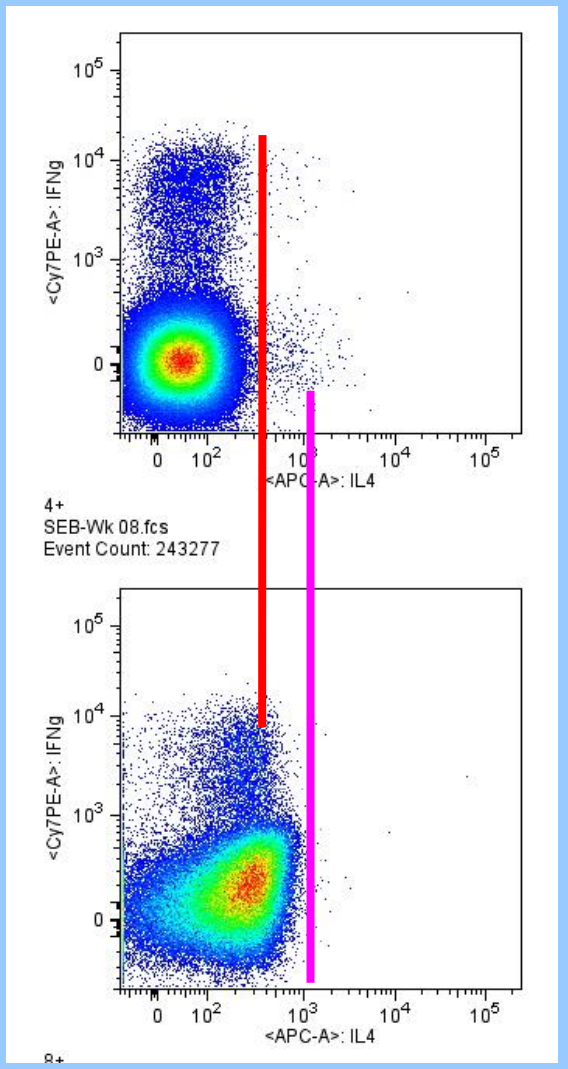
Compensation beads labelled with 5 different PECY7 antibodies

Rule #4 Controls must be treated (fix, perm etc) in the same manner as experimental samples.

Fix/Perm Changes APC-CY7 Compensation Requirement



The longer APCCY7 is in fixative, the more it “falls apart”, leading to more APC signal



CD4+

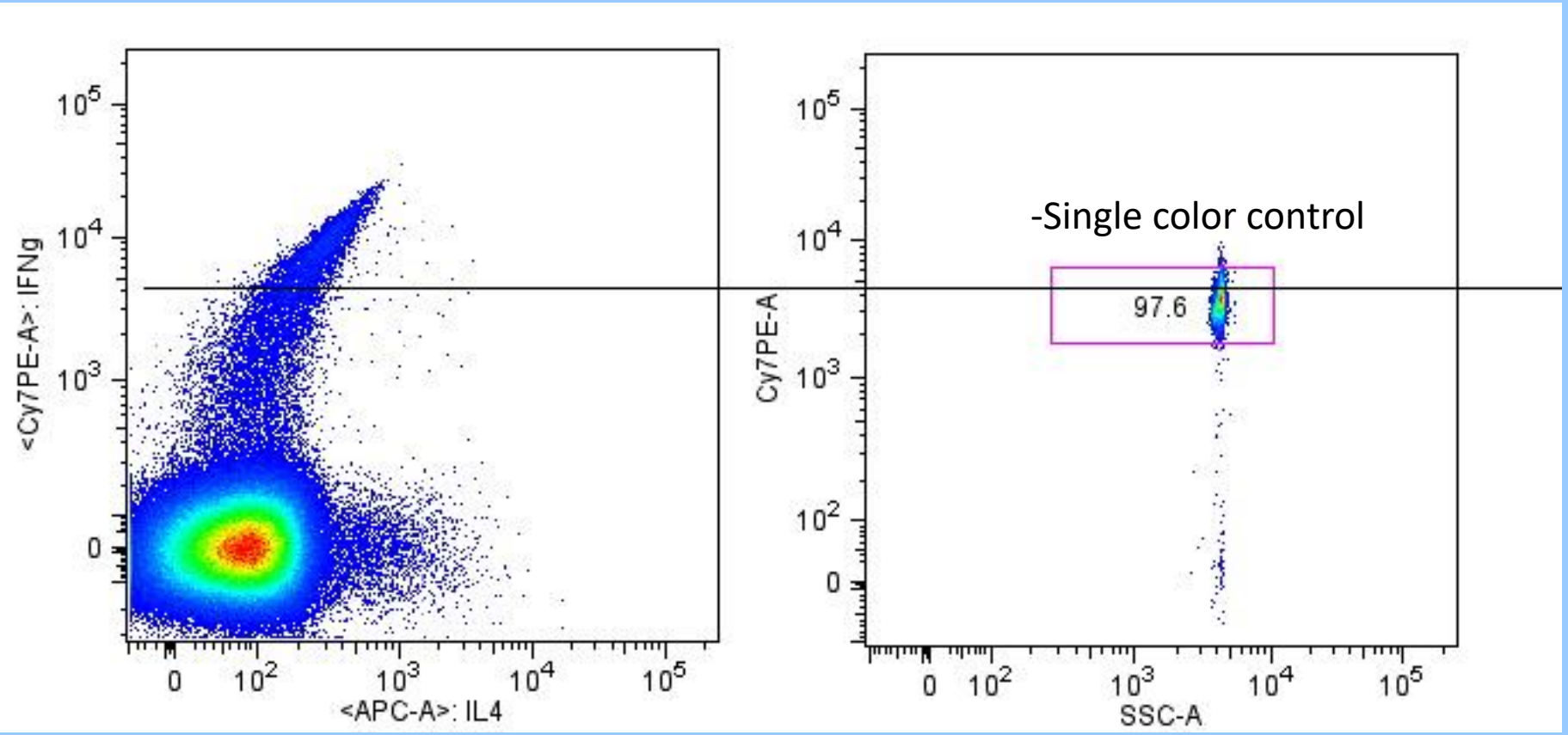
CD8+

Note that this exacerbates the higher “IL4+” gate required for CD8 cells.

The undercompensation would not have been detected except by looking at the APC vs. Cy7APC graphic…

Data generated by Mario Roederer

Rule #5 Controls need to be at least as bright or brighter than actual samples



-Most common when using Cells for Compensation controls
-Activation markers
-GFP
-CD11b, MHCII very high expression levels

Note that either under- *or* over-compensation can result from using comp controls that are too dim!

Data generated by Mario Roederer

Rule #6- Collect sufficient amount of data in your compensation controls

-This is especially important to think about when using cells for compensation controls.

-Aim for 2500

That might mean 5000 total events for some tubes and 500,000 in others

Beads Vs Cells

First of all, it doesn't make any difference that your cells may be nowhere near the same size, granularity, autofluorescence as beads. Follow the rules and you will be ok.

Benefits of using Beads

-Relatively straightforward to accomplish Rules 1-6
-No wasting of precious sample
-Typically provide very bright signal (Rule #5)
-Generally are more precise(Low CV-Low Error). Cells have large variance in background fluorescence leading to more error in compensation values.

Benefits of using Cells

-Must use cells for PI, DAPI, etc
-On rare occasions beads are not bright enough (Rule #5)
-Beads may not bind your specific antibody (Check product details)
-Cheaper