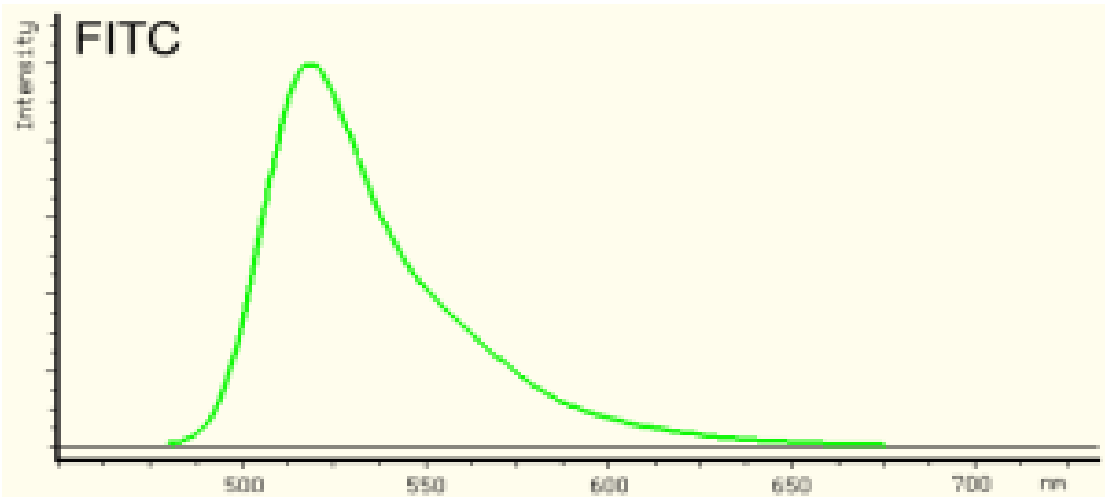
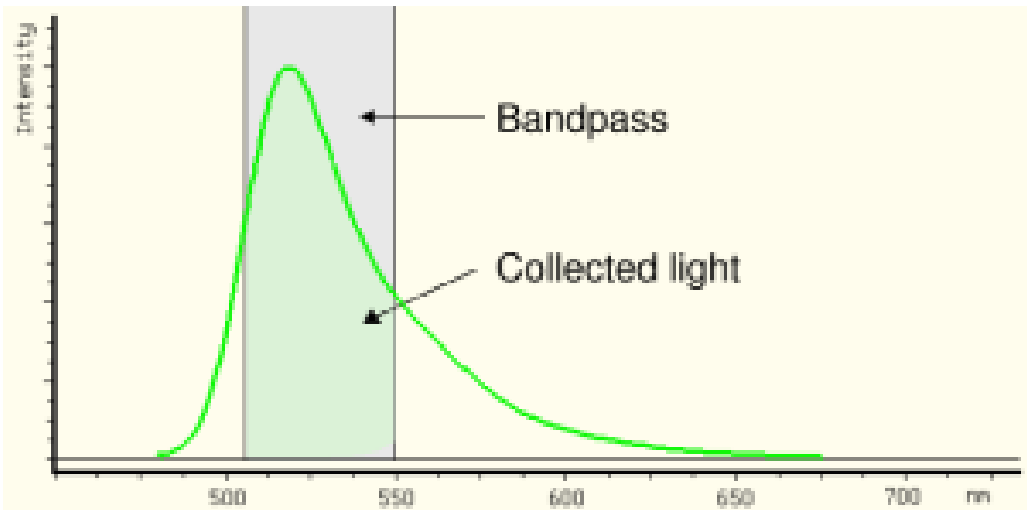
Compensation
by Ronald Mathieu

- Why do we need compensation?
  - 1) Because of long emission spectrum of a dye like FITC.



Compensation

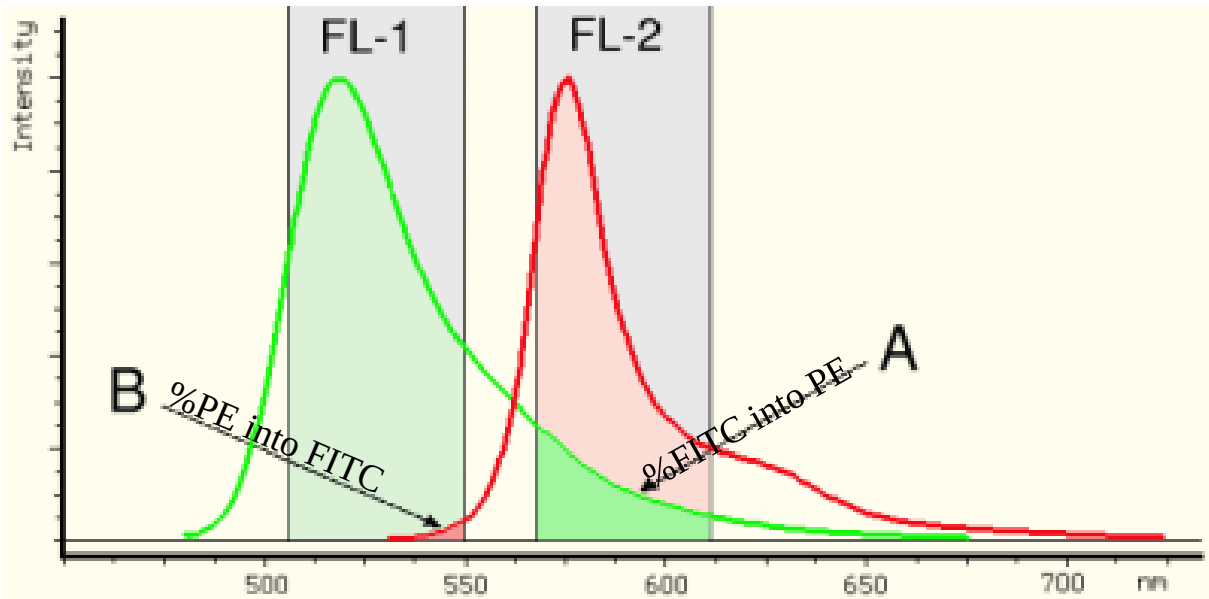
– 2) Because of the use of bandpass and dichroic filters in the instrument in order to separate fluorescence emission from the excitation light source .



Compensation

THE END RESULT IS SPECTRUM OVERLAP

WHERE FITC BLEEDS INTO THE PE CHANNEL AND PE BLEEDS BACK INTO FITC.



Compensation

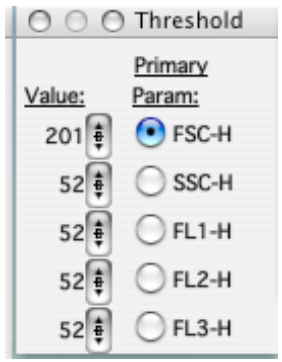
To correct for spectrum overlap during multicolor flow experiments **color compensation** must be performed.

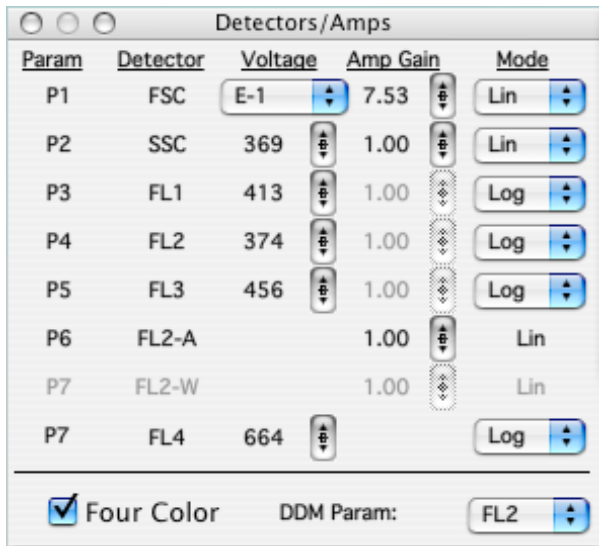
The goal of color compensation is to correctly quantify each dye with which a particular cell is labeled. This is done by subtracting a portion of one detector's signal from another, leaving only the desired signal.

Compensation

This example will show you how to compensate a FITC and PE experiment using a facscalibur

Open the Detectors/Amps and threshold windows by selecting them from the **Cytometer** drop down menu**.**



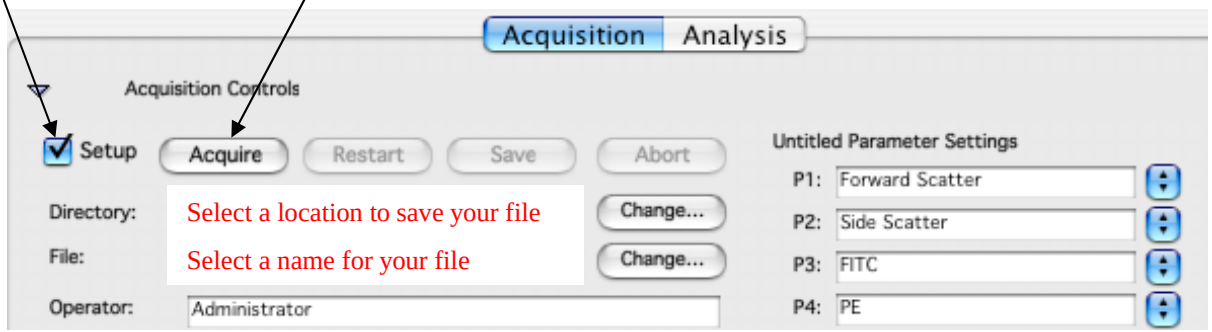


Compensation

Loading your negative control

Put the machine on run mode and put your negative control on the SIP

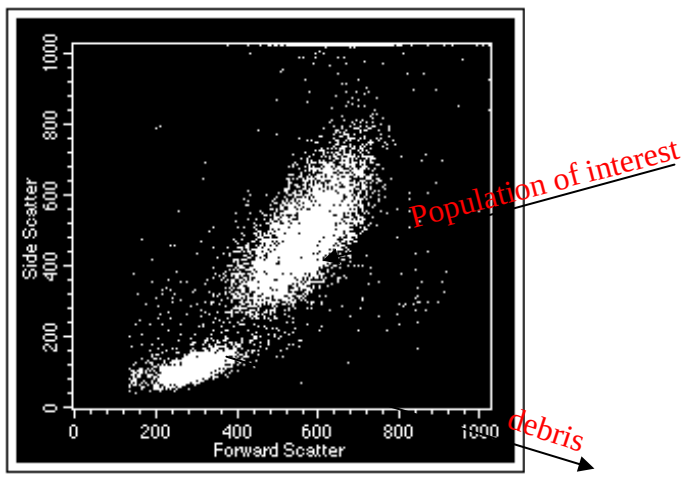
While the negative control is running under setup mode press acquire and make the following changes

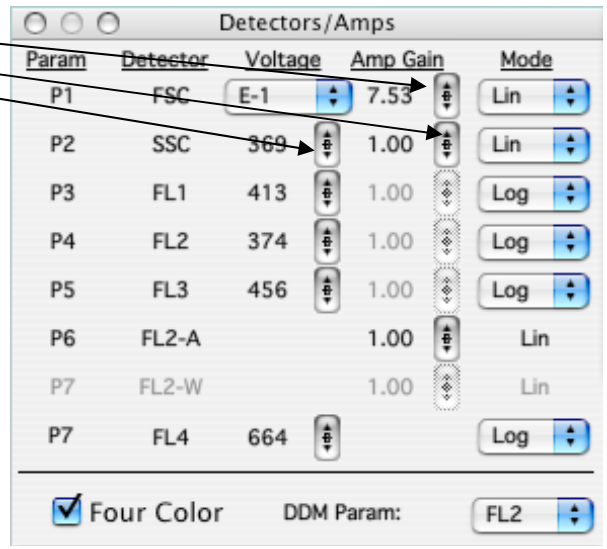


Compensation

Make sure the population of interest is clear visible by making adjustment to the FSC/ SSC.

Adjust the forward and side scatter by sliding the bar to bring the population of interest toward the center of the plot

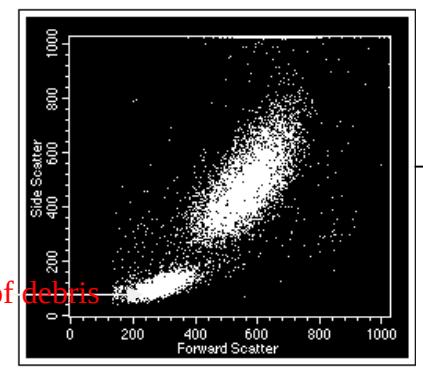




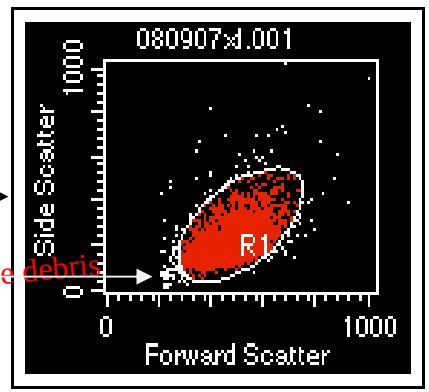
Compensation

Separates your population from debris by using the threshold menu

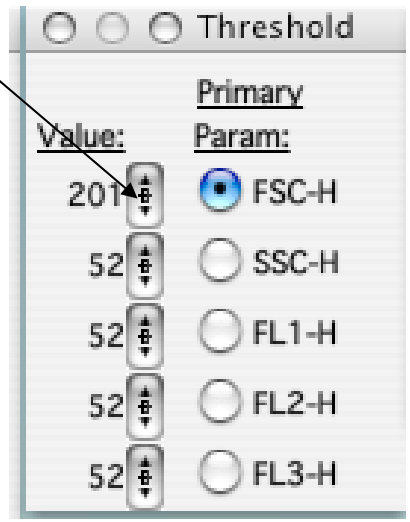
Adjust the threshold value by sliding the bar on the threshold window enough to remove most of the debris but not all of them. It is a good idea to have some debris on scale to make sure you are not threshoulding out your population.



Lots of debris



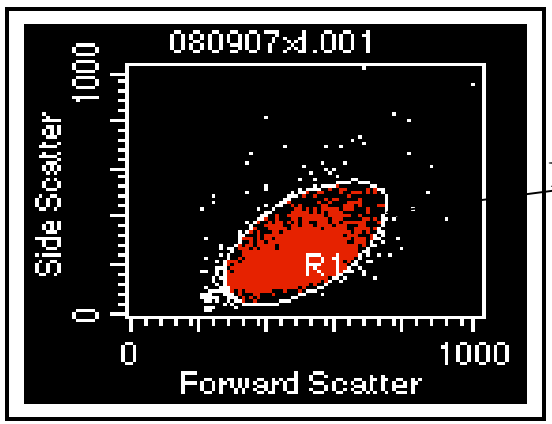
Very little debris



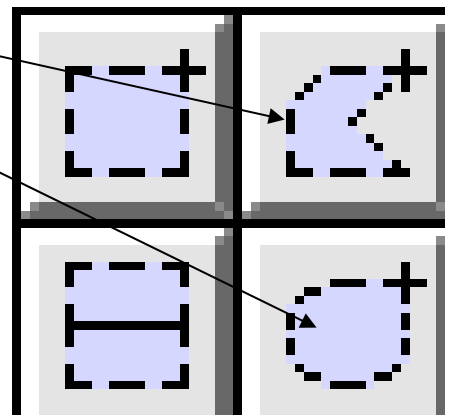
Compensation

Select your population of interesting by creating a gate around that population

draw a gate, which will allow you to remove unwanted debris and aggregates, around the population of interest by selecting a circle or a polygon from the tool template and applies it to all subsequent plot.



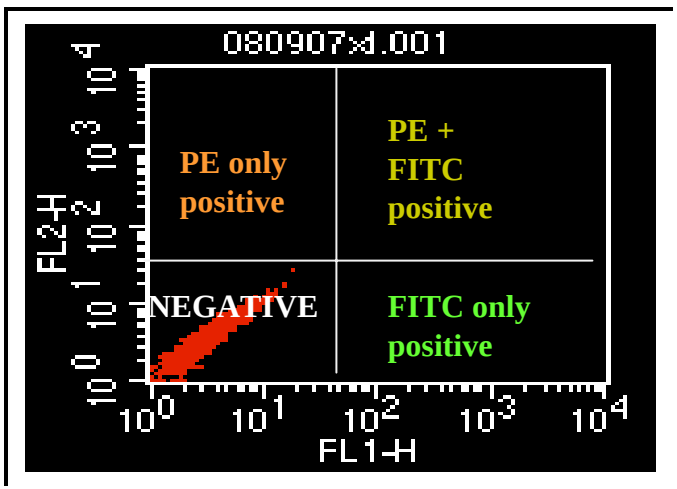
REGION R1

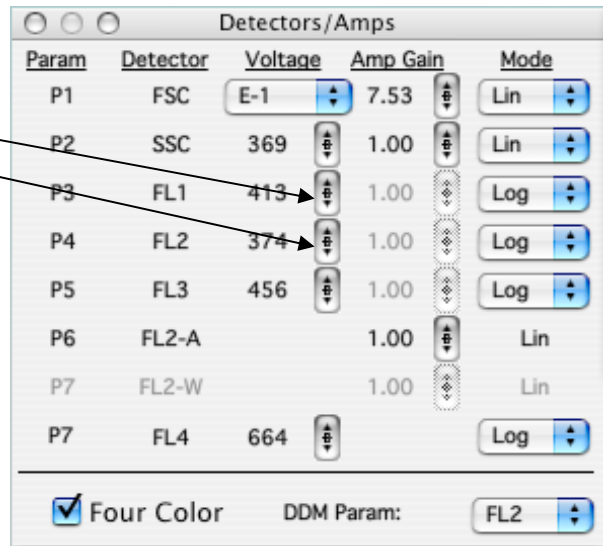


Compensation

Adjusts the voltage on the FITC and PE channel to put the cells in the center of the first log.

Adjust the FL1 and FL2 voltage by sliding the bar up or down to bring the population in the middle of the first log.

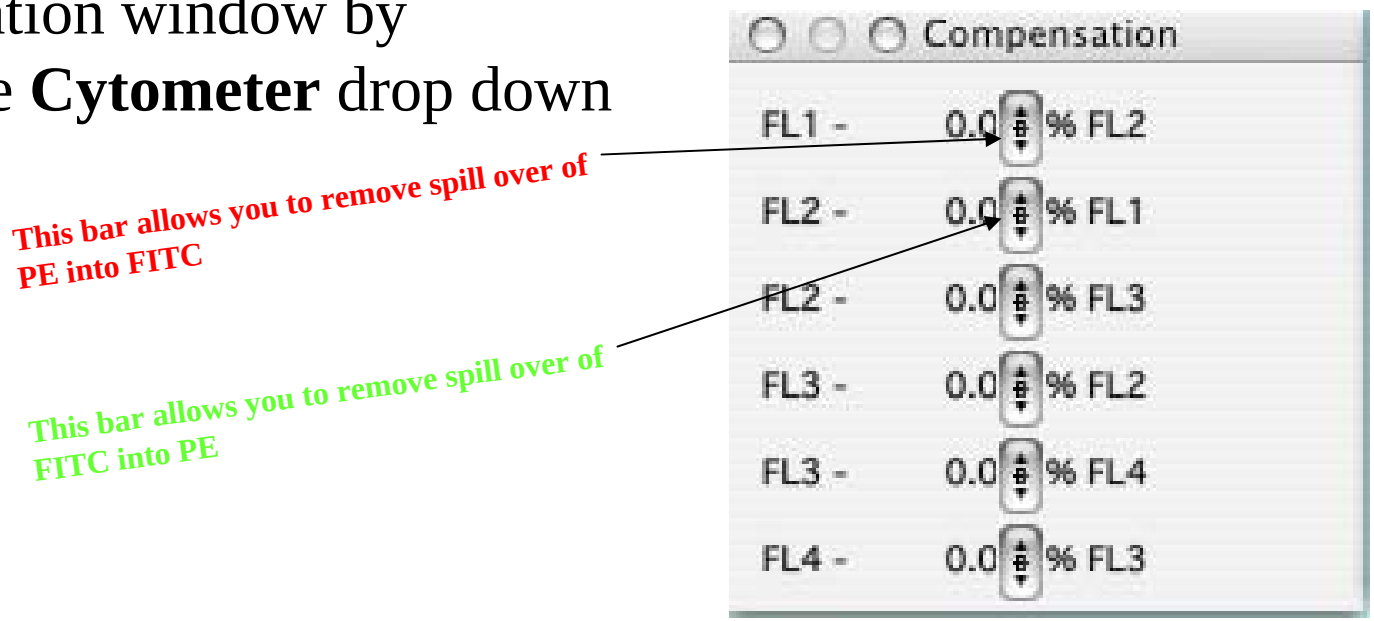




Compensation

You are now done with the negative control and ready to move on to the first single stain control

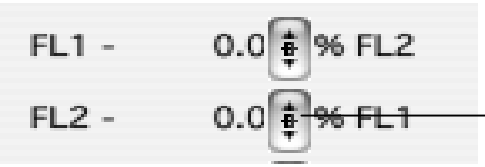
Open the compensation window by selecting it from the **Cytometer** drop down menu**.**



Compensation

Compensating the FITC Tube

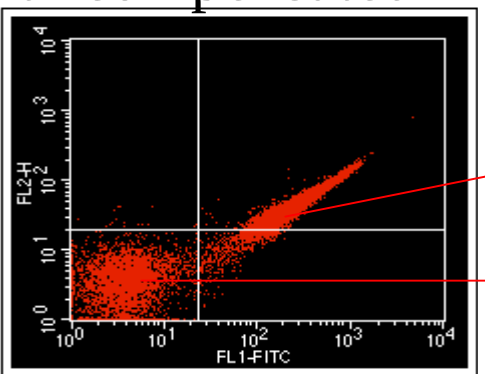
Still under the setup Mode, load the Single FITC control in the machine then press acquire. Your sample will look like the data on the left. Adjust the slide on the FL2- %FL1 to remove the % of FITC spill over in the PE channel. Try not to over compensate, pay attention to the mean of the negative and the FITC positive, they should be about the same.



We went from 0.0% to 26.0%

uncompensated

compensated

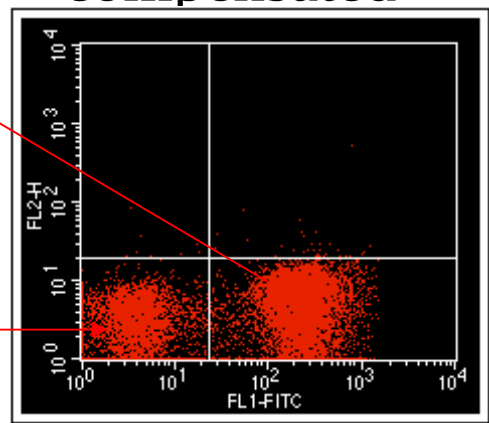


Y mean = 65

Y mean = 5

Y mean = 6

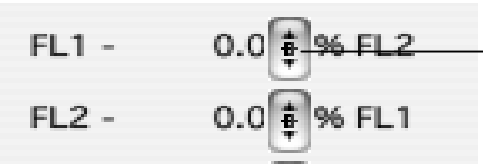
Y mean = 5



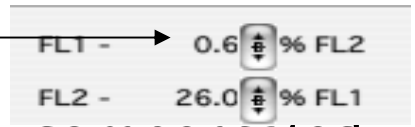
Compensation

You are now done with the FITC tube and ready to compensate your PE control

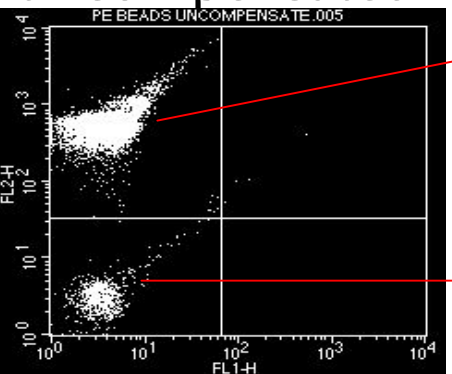
Still under the setup Mode, load the PE single control in the machine then press acquire. Your sample will look like the data on the left. Adjust the slide on the FL1- %FL2 to remove the % of PE spill over in the FITC channel. Try not to over compensate, pay attention to the mean of the negative and the PE positive, they should be about the same.



We went from 0.0% to 0.6%



uncompensated



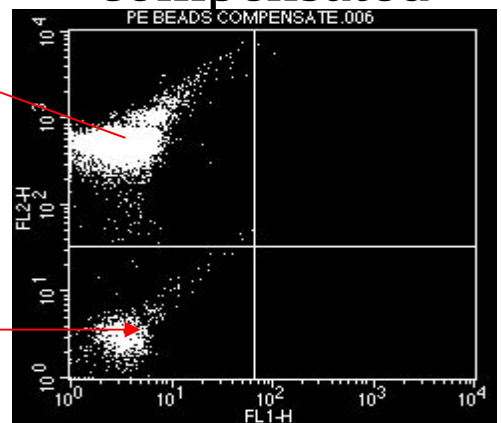
X mean = 9

X mean = 5

compensated

X mean = 6

X mean = 5



Compensation

THE FITC AND PE COMPENSATION IS DONE.

YOU ARE NOW READY TO RECORD DATA AND SAVE YOUR INSTRUMENT SETTING.

TO SAVE YOUR INSTRUMENT SETTING:

GO TO CYTOMETER MENU THEN SELECT INSTRUMENT SETTING. LOCATE YOUR DIRECTORY AND PRESS SAVE THEN PRESS DONE.