





### **Recording Data to Apply compensation:**

1. Record events for the Indo only tube.
2. Record events for the FITC/PE sample tube.
3. Draw a gate around the FITC+, FITC-, and PE+ populations on the FITC vs PE plot.
4. Create a Statistics View to display the FITC and PE mean values.
5. Adjust the compensation manually until the PE median value for the FITC+ and FITC-match.
6. Adjust the compensation manually until the FITC median value for the PE+ and PE-match.

### **Recording Experimental Data:**

1. For the calcium flux experiment set events to record to 1,000,000 and events to display 50,000.
2. Click the Next button on the Acquisition Controls frame to create a new Tube and Label.
3. As positive control for calcium flux, install a sample and adjust flow rate to 200 events/second.
4. Click Record.
5. When 10,000 events have been recorded remove the tube and add 10<sup>-6</sup> Ionomycin to the tube and mix thoroughly. ***DO NOT PUT THE INSTRUMENT ON STANDBY.***
6. Re install the tube and stop recording when cells are no longer reacting to Ionomycin.
7. Install a sample of interest and repeat step 4, 5, and 6, using your own stimulus.