Flow Cytometry Core Facility, Camelia Botnar Laboratories, Room P3.016
UCL Institute of Child Health. 30 Guilford Street, London WC1N 1EH

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 l 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 Inonomycin.
7. Install a sample of interest and repeat step 4, 5, and 6, using your own stimulus.

Flow Cytometry Core Facility, Camelia Botnar Laboratories, Room P3.016
UCL Institute of Child Health. 30 Guilford Street, London WC1N 1EH
Tel 020 7405 9200 ext 0198