Set Up Calcium Flux Experiment  
(From BD LSRII Instrument User’s Guide)

To set up Calcium flux experiment

1) Verify configuration of instrument. Make sure that the configuration lists the Indo-1 (Violet) and Indo-1 (Blue) parameters.

2) Click the Parameters tab in the Inspector and make sure the following changes:
   - Delete all parameters except FSC, SSC, Indo-1 (Blue), and Indo-1 (Blue).
   - Verify that the Log checkbox is deselected for all parameters.

3) Click the Ratio tab and click the Add button; choose Indo-1 (Violet)-A for the Numerator and Indo-1 (Blue)-A for the Denominator.

4) Create the following dot plots for the Ca 1 Tube.
   - FSC-A vs SSC-A
   - Indo-1 (Violet) vs Indo-1 (Blue)
   - Time vs Ratio: Indo-1 (Violet)/Indo 1 (Blue)

5) Create a Statistics view and display the mean for the UV parameters and the ratio.

6) In the Acquisition Controls frame, set the Events To Record to 1,000,000 evts and the Events to Display to 500 evts.

Optimizing the Calcium sample

1) While viewing the FSC vs SSC plot, make the following adjustments:
   - Adjust the FSC and SSC voltages to place the sample on scale in the FCS vs SSC dot plot
   - Adjust the FSC threshold to remove debris without cutting into the population of interest
   - Draw a gate around the population of interest

2) Format the remaining two dot plots to show the population of interest.

3) Adjust the Indo-1 (Violet)-A and the Indo-1 (Blue)-A voltages to optimize the signal. The signal should extend along the Indo-1 (Blue) axis and should be slightly off the baseline for both axes.
4) Adjust the Ratio Scaling to set the baseline between 0-50,000.

Select the Tube in the Browser and click the Instr.Settings>Ratio tab in the Inspector

To adjust the setting, select the value in the Scaling field, enter a new value, and press Enter. Repeat as needed to achieve the required results.

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**Measuring Calcium Flux**

Do the following to record data for a calcium flux experiment

1) Change the Events to Display to 50,000 events.

2) Verify that the unstimulated sample is still running: adjust the event rate to approximately 200 events/second

3) Click Record

4) When approximately 10,000 events have been recorded, remove the unstimulated sample tube from the cytometer (Keep the instrument in RUN mode as you do this. Do not stop recording or acquisition or the data display will reset to zero)

5) Add the stimulus to the tube and mix thoroughly

6) Reinstall the tube on SIP. After a few seconds, the Ca^{++} concentration begins to increase on the Time vs Ratio plot.
7) Click the Acquisition pointer when the cells are no longer reacting to the stimulus.

8) Remove the tube from the cytometer.

9) Clean the fluidics system with 10% bleach for 5 minutes, and then with DI water for 5 minutes (Make sure to remove any remaining stimulus that would activate cells in subsequent samples).

10) For subsequent samples repeat steps 3 through 10.