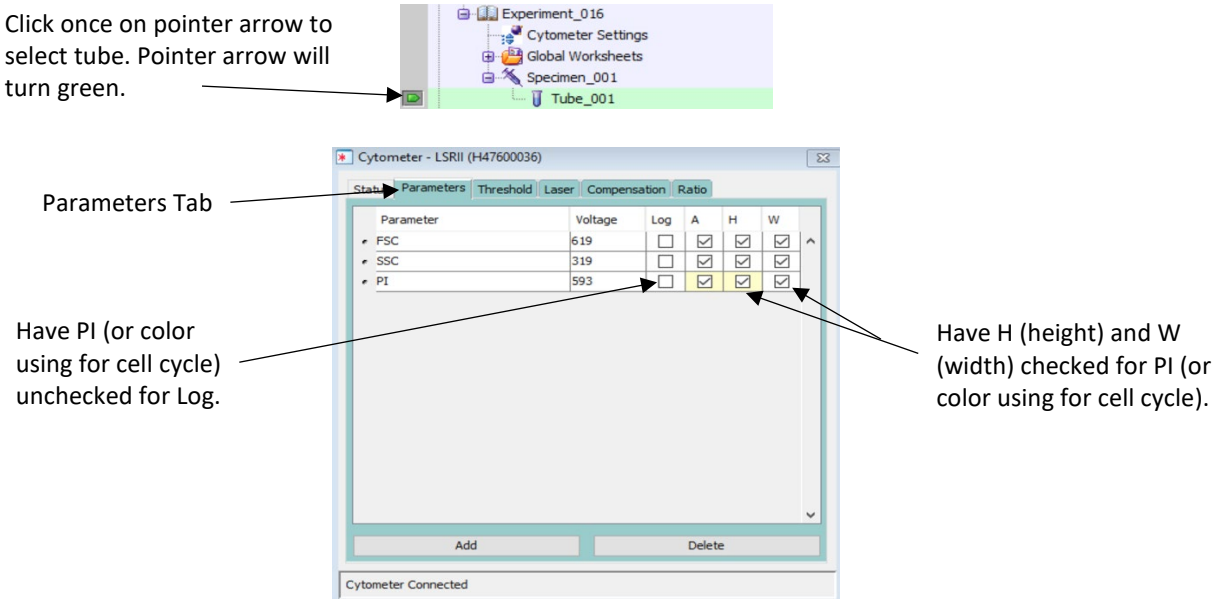
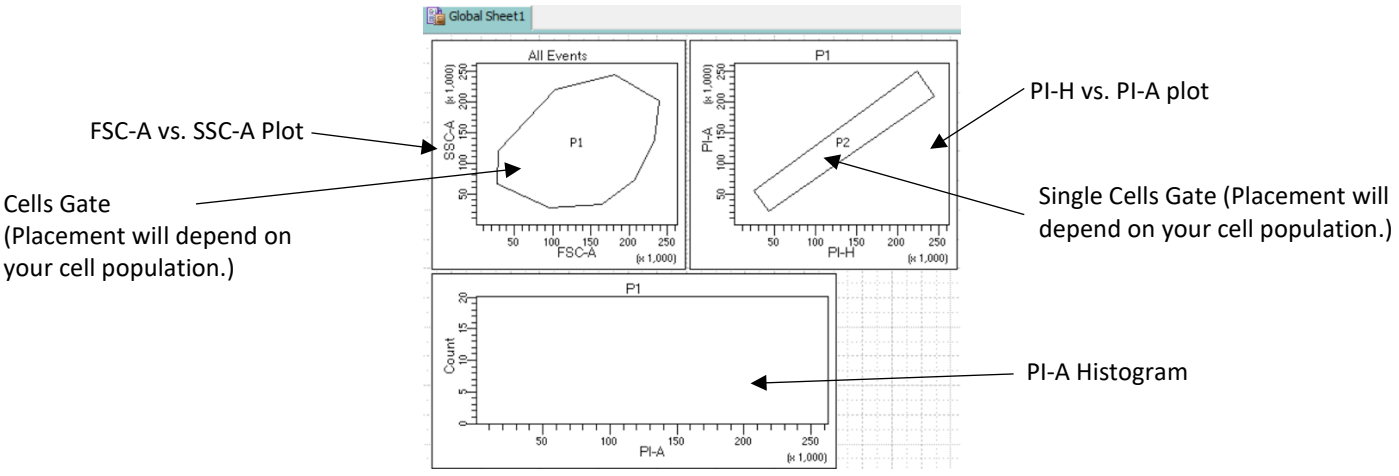
Cell Cycle on Flow Cytometer

1-Color Cell Cycle (PI)

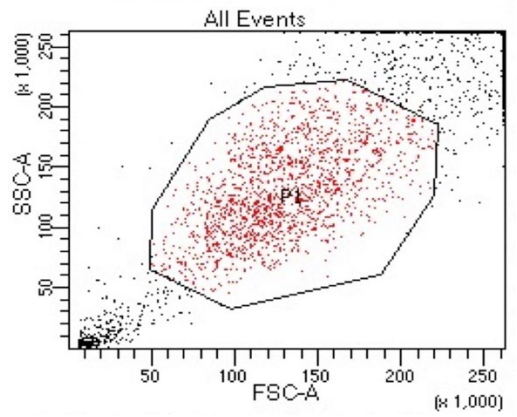
1. For the purpose of this SOP, we're going to use PI as the color being used for cell cycle. Color being used for cell cycle (PI) should be in linear mode. Should also have height and width as options for PI. In FACSDiva, with your tube selected, options for linear versus log mode and options for height and width measurements for the pulse can be found in the Parameters tab in the Cytometer window.



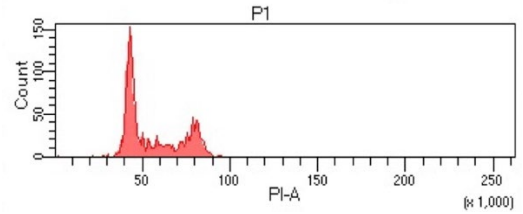
2. Setup the worksheet. Create a 2-parameter FSC-A versus SSC-A plot to discriminate your cells versus debris. Create a 2-parameter PI-H versus PI-A plot to discriminate your singlets versus doublets. Also, create a 1-parameter PI-A histogram for viewing your cell cycle signal.



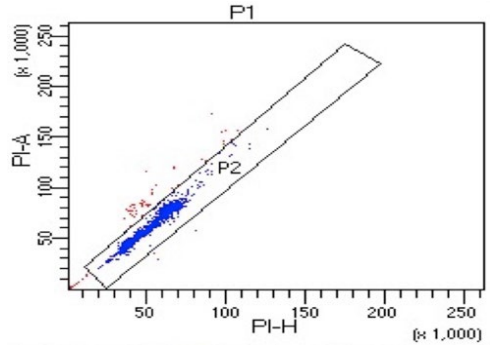
3. Run sample in LOW flow rate.
4. Adjust your voltage settings using a sample that is positive for PI. While running sample in LOW flow rate, adjust FSC and SSC voltages to view cells on the FSC versus SSC plot. Once cells are in view on the FSC versus SSC plot, create a gate on your cells.



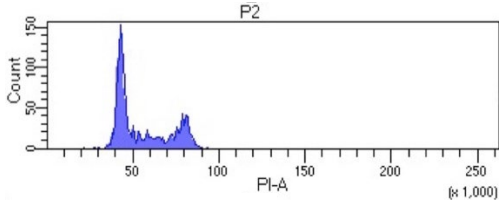
5. Choose to only show events from the cell gate on the PI-A histogram. In FACSDiva, right click on the PI-A histogram and choose Show Population and select your cell gate. Adjust PI voltage so that the first cell cycle peak shows up at about the 50 mark on the PI-A histogram and the second peak falls at about the 100 mark.



6. Choose to only show events from the cell gate on the PI-H versus PI-A plot. In FACSDiva, right click on the PI-H versus PI-A plot and choose Show Population and select your cell gate. Gate on the events that are well correlated, that form a straight line between PI-H and PI-A, to gate on your single events. This step is crucial as otherwise you will overestimate your G2/M phase.



7. Choose to only show your single events on the PI-A histogram.

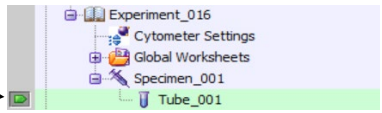


8. Record samples in the LOW flow rate.

2-Color Cell Cycle (PI and BrdU)

1. BrdU is an analog of thymidine and is readily incorporated into DNA during DNA synthesis, so it can be used as a marker for S phase. Other S phase markers can be used, but for the purpose of this SOP, we're going to use BrdU in addition to PI.
2. Color being used for cell cycle (PI) should be in linear mode and have height and width checked as options for PI. BrdU should be in log mode. In FACSDiva, with your tube selected, options for linear versus log mode and options for height and width measurements for the pulse can be found in the Parameters tab in the Cytometer window.

Click once on pointer arrow to select tube. Pointer arrow will turn green.

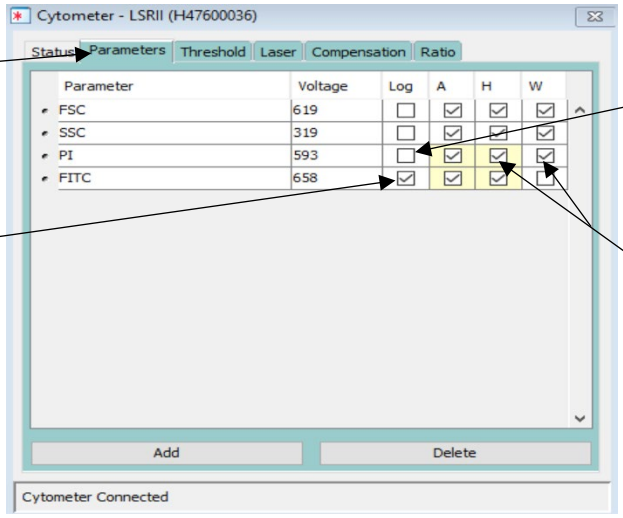


Parameters Tab

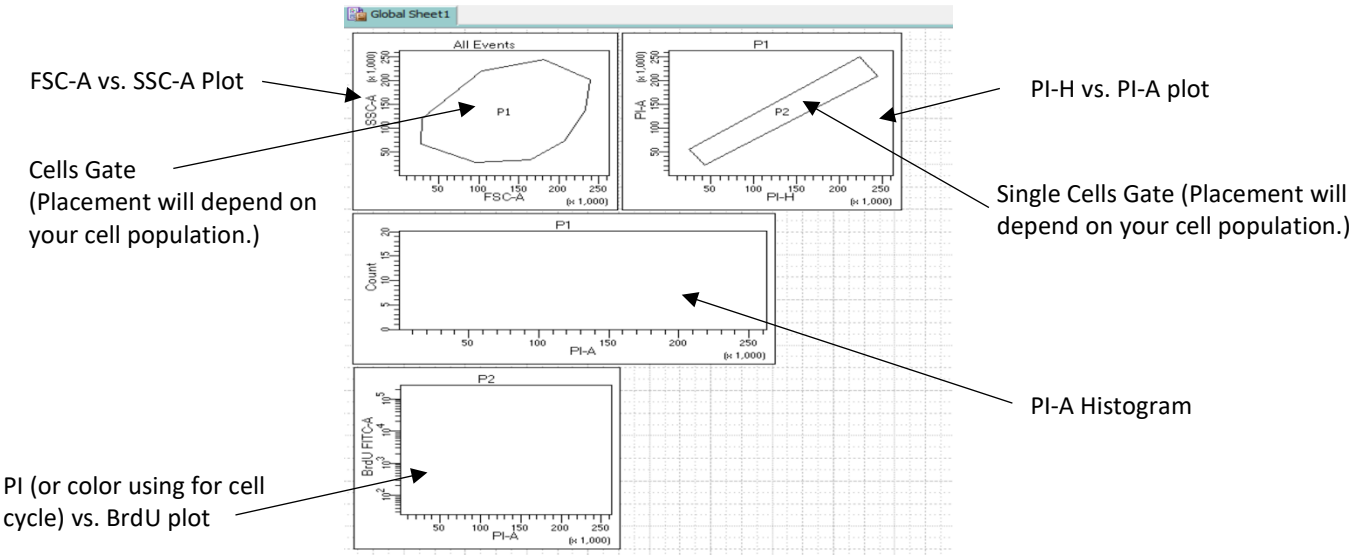
Have PI (or color using for cell cycle) unchecked for Log.

Have fluorochrome attached to BrdU checked for Log.

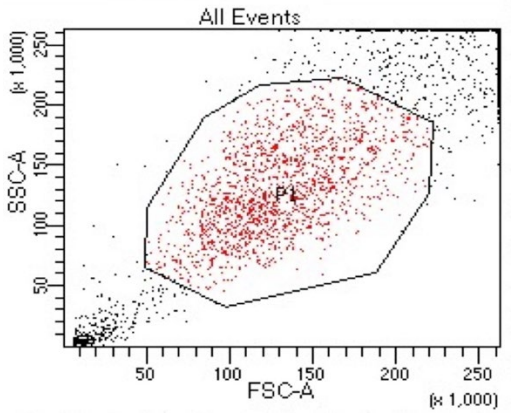
Have H (height) and W (width) checked for PI (or color using for cell cycle).



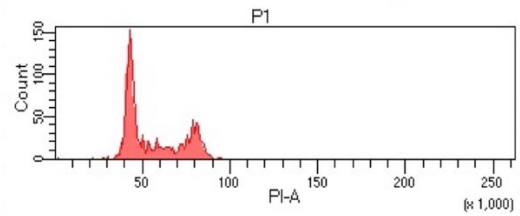
3. Setup the worksheet. Create a 2-parameter FSC versus SSC plot to discriminate your cells versus debris. Create a 2-parameter PI-H versus PI-A plot to discriminate your singlets versus doublets. Create a 1-parameter PI-A histogram for viewing your cell cycle signal. Also, create a 2-parameter PI-A versus BrdU-A plot.



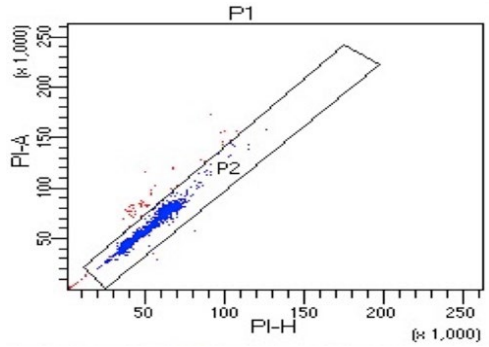
4. Run sample in LOW flow rate.
5. Start adjusting your voltage settings using a sample that is only positive for PI. While running sample in LOW flow rate, adjust FSC and SSC voltages to view cells on the FSC versus SSC plot. Once cells are in view on the FSC versus SSC plot, create a gate on your cells.



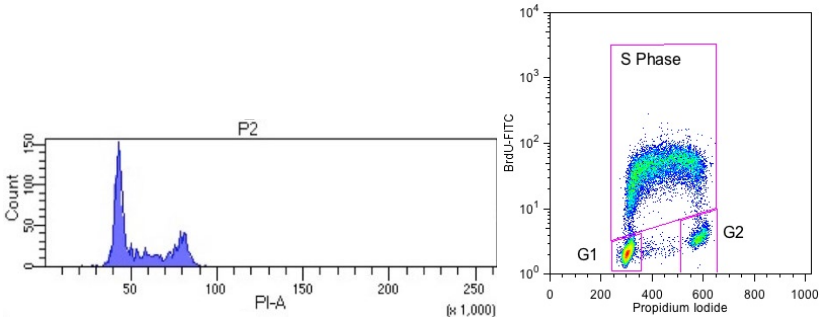
6. Choose to only show events from the cell gate on the PI-A histogram. In FACSDiva, right click on the PI-A histogram and choose Show Population and select your cell gate. Adjust PI voltage so that the first cell cycle peak shows up at about the 50 mark on the PI-A histogram and the second peak falls at about the 100 mark.



7. Choose to only show events from the cell gate on the PI-H versus PI-A plot. In FACSDiva, right click on the PI-H versus PI-A plot and choose Show Population and select your cell gate. Gate on the events that are well correlated, that form a straight line between PI-H and PI-A, to gate on your single events. This step is crucial as otherwise you will overestimate your G2/M phase.



8. Choose to only show your single events on the PI-A histogram and on the PI-A versus BrdU-A plot.



9. Adjust the voltage setting for the fluorochrome attached to BrdU using a sample that is double-positive for PI and BrdU. Looking at the PI-A versus BrdU-A plot, only adjust the voltage for the fluorochrome attached to BrdU down if the signal is too bright. In FACSDiva, make sure that the positive events are below 10^5 on the BrdU-A parameter.
10. The events on the PI-A versus BrdU-A plot should look like a horseshoe or an upside down “U” (classic “hook” profile). If your horseshoe is leaning, you might need to adjust your compensation. If you record negative and positive single-stained controls, you can compensate using computer compensation. If you attempt to compensate manually, single-stained controls do not tend to be as helpful as with typical multicolor flow. If you were to run a BrdU single-stained control with the current setup, you wouldn’t see anything on the PI-A versus BrdU-A plot, since your singlet gate, which are the events showing on the plot, is off of PI. If you change the PI-A versus BrdU-A plot to showing your cell gate instead, you would see events. However, since the events would be negative for PI and PI is in linear mode, the events would appear as a line against the very left edge of the PI-A versus BrdU-A plot. The PI single-stained control would be

more normal for adjusting PI out of BrdU. However, if adjusting BrdU out of PI, you would need to look at the double-stained tube.

* All images except for last PI vs. BrdU plot (with data) was from Indulekha Singaravelu data that I analyzed on PC1.
** Last PI vs. BrdU plot (with data) was from ”Cell Proliferation Round 1: Using Thymidine Analogs With Flow Cytometry” which was published in May 20, 2014 and written by Derek Davies.