To set up cell cycle experiment
1) In the “Inspector” select the Height (H) and Width (W) for DAPI (or PI), FSC and SSC.
2) Most of the time the “Threshold” tab for DAPI (or PI) is set at 5,000.
3) Using a “Worksheet”, create the following plots for the DNA tube.
   - FSC-A vs. SSC-A dot plot
   - DAPI-A vs. DAPI-W dot plot
   - DAPI-A histogram
4) Create a Statistics view (select on DAPI-A vs. DAPI-W dot plot → right click “Create Statistics View”).
5) Right click and choose “Edit Statistics View”.
6) On the Statistics tab, select the Mean and CV for DAPI-A and DAPI-W. Set decimal places to 1 for CVs.
7) When running the sample, adjust the event ratio to approximately 200 events/second (LOW, if it is necessary with the Sample Fine Adjust knob….black knob on the left of the Control Panel on LSRII).
8) Drawn and Interval gate around the first two peaks on the DAPI-A histogram; name populations Singlets and Doublets.
   - If the CV is ≤ 6%, continue with next step.
• If the CV is >6%, restart the acquisition. Decrease the flow rate with the Sample Fine Adjust knob until the CS is ≤ 6%, and then re-records data. If the CV does not improve, check air bubble in system, air leak at sheath container, sample not diluted in saline solution/PBS, or poor sample preparation.

10) Save data. Check the linearity:

  • Note the means of the Singlet and Doublet populations. Divide the mean of the Doublets by the mean of the Singlets. The Doublets/Singlets ratio should be 2.00±0.05.

Singlets can be distinguished from aggregates based on size. With BD FACSDiVa software, aggregates can be resolved from singlets on an Area (A) vs. Width (W) plot. On the Area vs Width plot, singlets are distinguished from doublets by the Width measurement singlets have a smaller Width measurement. Discriminating singlets from aggregates enhances the accuracy of cell-cycle analysis.

11) Adjust the DAPI voltage to place the first peak at approximately channel 50 × 10^3 on the DAPI-A axes.