

## **FACS Aria Start Up (For Analysis)**

1. Open the Flow Cell Access Door – The flow cell access door is equipped with a shutter mechanism that shuts off the laser light when the door is open. Running the lasers without turning on the stream can degrade the performance of the cuvette flow cell.
2. Turn on the Main Power switch and the power switches for the Blue, Red, and Violet lasers (located on the left side of the FACS Aria). Allow the lasers to warm-up for 30 minutes before running samples.
3. Turn on the HP workstation by pressing the blue button in the center of the HP tower.
4. Press CTRL + Alt + Delete to bypass the Welcome to Windows 2000 Professional prompt.
5. The Administrator User Name does not have a password and can be bypassed by selecting OK.
6. Double-click the BD FACSDiva software icon on the desktop.
7. Click the Instrument Panel. There will be a prompt at the bottom of the Instrument Panel that says “Connecting to Instrument”. When this prompt changes to “Connected to Instrument” select Instrument from the menu bar and then click on Fluidics Startup. A popup message will say, “Fluidics is starting. You will be notified when the system is ready.” If you need your sample to be collected and kept cold, you can now turn on the temperature control unit located to the left of the FACS Aria.
8. At the completion of the Fluidics startup you will be given the prompt, “The system is ready. You can now turn the stream on.”
9. To turn on the stream select the last icon (the Sorting Icon) from the workspace toolbar. The sorting frame will appear. The Stream button will have a red X. Click on the red X to activate the stream.
10. Once the stream has started, wait until at least one drop is visible in the sort frame. You then may click on the Sweetspot button, which will automatically monitor and adjust the GAP and Drop 1 to appropriate values.
11. Now either select or create a folder for yourself from the Browser. To create a new folder in the Browser click on the New Folder Icon in the Browser Toolbar. If you have an existing folder double click on that folder in the Browser to open it.
12. First, select the Instrument Menu key -> Instrument Configuration. Select an appropriate configuration for the left-hand column and click Set Configuration then OK.
13. Next in the instrument panel select the Parameters that you will be using in the experiment. You can add or delete parameters using the Add and Delete buttons at the bottom of the panel.
14. Once you have all the parameters listed you may select the Instrument Menu key -> Instrument Setup -> Create Compensation Tubes.
15. In the browser you will notice a new Compensation Specimen has been created in your folder. It includes compensation tubes for all of the fluorochromes you will be using in the experiment. Select all of the fluorescence histograms while

- holding the shift key (this will put handlebars around all of the fluorescence histograms. Next enter the Inspector dialogue box (lower center of workspace). Select the show grid box and change the histograms background color to a color other than black. Now place a tube of unstained cells on the sample port and select the Load radio button from the Acquisition Controls.
16. Adjust the FSC and SSC voltages to place your desired population on screen. Adjust the fluorochrome voltages to place the unstained peaks in the first decade (the negative decade). Press the Record button in the Acquisition Control bar. Once the tube has been recorded press the Next button and Unload button in the acquisition control bar.
  17. Unload your unstained tube and place a tube of single stained cell on the port. Load the cells and press record. Make sure that the P1 region is around your desired population. Repeat step 17 for however many fluorochromes you are using.
  18. Once you have collect samples of all of your single color controls, place interval gates around the positive and negative peaks. Then from the Instrument Menu at the top of the workspace select -> Instrument Setup -> Calculate Compensation. You will then have to give the compensation for this experiment a name and then click OK.
  19. Now open a worksheet and create the plots needed to analyze your experiment. Load your tube and then press Record once you begin to see events in your plots or histograms.