

## FACSORT Instrument Startup/Shutdown

1. Turn on the cytometer and flip the vent switch to pressure. Check the fluid levels for the sheath fluid and waste... the sheath should be full and the waste empty.
2. Re-boot the computer to connect the computer to the cytometer.
3. Launch CellQuest 3.1 from the Apple Menu.
4. Under the Acquire Menu... connect to cytometer, Acquisition Control box should appear.
5. Under the Cytometer Menu... call up the Detectors/Amps and Status boxes.

If you already have your instrument settings saved, select instrument settings under the cytometer menu, then click open. Navigate to your individual folder and open the settings needed for your current study. Click on Set, which will change the settings on the Detectors/Amps to your selected settings, then click on Done.

If you do not have settings saved, these can be set while the first or control sample is being run.

6. If you already have your histograms and plots saved, go under the File Menu... select Open that opens the CellQuest 3.1 folder, select Users, then your individual folder to call up your histograms.

If you do not have histograms saved, you can use the cytometer tools to draw the histograms and plots you want or you can go to the menu and pull down plots to select the ones for this experiment.

7. At this point, go under the Acquire Menu and call up Parameter Description. Click on the folder box to choose a destination folder. Make sure the initial folder shown on the top is your individual folder. Click on New to make a new folder for your current studies. Click on Create then click select (at the bottom of this box) so the correct path for you saved data is chosen.

Note: On the Parameter Description Box, click on file then set the file name prefix to Sample ID. This will permit the name of the file to be what ever you label your sample ID. Also, for initial studies set the file count to 1... then click OK.

8. Under the Acquire Menu... call up the Counters, which will show you the number of events (cells) per second that are flowing through the instrument.

9. If you are doing a study for the first time (new histograms and instrument settings) you will need to set the number of and location of events that you want to save to the computer.

Under the Acquire Menu... call up Acquisition and Storage. Under Collection Criteria select the number of events (cells) you want to examine and the area i.e. G1 = R1 or all of which you are interested. This tells the computer to stop acquiring data when a given number of cells are reached.

Note: If you have already saved your histograms and plots from a previous study and have called this file back up, the Collection Criteria will already be set from the previous study. The only reason you may need to call up the Acquisition and Storage box is if you want to change anything from the previous study.

## **YOU ARE NOW READY TO EXAMINE YOUR SAMPLES**

To examine your cells:

1. Slide the arm underneath the tube on the sample device to the side, remove the tube, and replace with your sample.
2. Using the knob on the front of the cytometer, set the instrument to run.

Note: the cytometer can remain in the run position throughout your study, just be sure a sample tube or a tube of water is always on the instrument.

3. In the Acquisition Control box, make sure the Setup box is checked, and then click Acquire. At this point, NO information is being saved to the computer. This is just to check if the correct instrument settings have been selected and that your sample is being processed through the cytometer. Also, if you do not have previously saved instrument settings, which were called up, now is the time to establish the settings for this particular study.
4. To save your data to the computer, click on pause then abort in the Acquisition Control box and remove the check from the setup box. In the Parameter Description box give the sample a name under Sample ID, then click acquire in the Acquisition Control box. When the predetermined number of events has been processed by the cytometer, the computer will automatically quit acquiring data.
5. Remove your sample from the collection device and replace with the next sample. Change the name of the sample in the Sample ID box, then click acquire in the Acquisition Control box.

### **WHEN ALL SAMPLES HAVE BEEN RUN:**

1. Remove your last sample from the collection device and replace with a tube containing 10% bleach. Allow the instrument to run for at least 5 minutes.
2. Remove the tube of 10% bleach from the collection device and replace with a tube containing distilled water. Allow the instrument to run for at least 5 minutes.
3. Remove the tube of distilled water from the collection device and replace with a tube containing a small (about a ml) of water. Turn the instrument on Standby.
4. Exit CellQuest or disconnect from the cytometer under the Acquire Menu if you want to shut off the cytometer and continue to analyze your data.
5. Depressurize the cytometer by switching the pressure valve from Pressure to Vent... empty the waste container in the sink and fill the Sheath fluid with PBS (located above the cytometer) to the black line on the Sheath container. Turn off the cytometer.