

FACSCAN OPERATION GUIDELINES

INSTRUMENT START-UP:

1. Check fluid tanks - fill sheath and empty waste if needed (Note: ALWAYS empty the waste tank if ANY sheath fluid has been added). Add about 400 ml of bleach to waste tank before returning it to the cytometer.
2. When re-capping the sheath tank, be sure the cap is tightly closed (it must hold pressure for the system to operate correctly).
3. Turn on the cytometer first (there will be a 4-5 min laser warm-up delay) then the computer.

PREPARING TO RUN SAMPLES:

1. Start *CellQuest* by double clicking on the desktop icon.
2. Connect to the cytometer (under the "Acquire" drop down menu).
3. Open an Acquisition window (from the "Acquisition" folder or your lab's folder).
4. Load Instrument Settings (from the "Settings" folder or your lab's folder).
5. From the "Parameter Description" window, click on the "Folder" button and go to your lab folder to create a folder for storage of the data you are about to collect. Click on the "File" button to create a unique name to be used for each of your data files.

"NOT READY" STATUS APPEARS DURING OPERATION:

1. The sheath fluid is low and/or the waste tank is full; however BOTH must be serviced.
2. Put the Fluidics Control in "Standby" and open the lower door to the fluidics compartment.
3. Vent the sheath tank by pushing down the "Vent Valve" (large toggle switch located between the two tanks).
4. Fill sheath and empty waste (ALWAYS empty the waste tank if ANY sheath fluid has been added). Add about 400 ml of bleach to waste tank before returning it to the instrument.
5. Be sure the cap on the sheath tank is tightly closed (it must hold pressure for the system to operate correctly).
6. Re-pressurize the system by returning the Vent Valve to the "up" position.
7. Put the Fluidics Control in "Run" and continue analyzing samples.

BLOCKAGE OF SAMPLE FLOW:

1. Do not use "Back Flush" on Fluidics Control.
2. Open the sample support arm for 30-60 seconds. This flushes clean, filtered sheath fluid back through the sample uptake tube and should push out any obstructions. To confirm that the sample uptake tube is clear, with the sample support arm open, remove the outer metal tube by unscrewing the knurled connector at the top of the tube. Slide the outer tube off and sheath fluid should drip from the sample uptake tube if it is clear. Replace the outer tube and *GENTLY* re-tighten the knurled connector at the top of the tube.
3. If there is an obstruction in the flow cell it can be cleared by doing a "Drain and Fill". Open the upper front panel on the FACScan and observe the flow cell while putting the fluidics control in "Drain". After a few seconds the fluid level will drop as the flow cell is evacuated. After about 10 seconds put the fluidics control in "Fill" and observe the flow cell refilling slowly with sheath fluid. If any bubbles are observed during the fill process drain the flow cell again and re-start the fill process.
4. If there is still a problem try running undiluted bleach for 2-3 minutes (rinse well afterwards).
5. If you have repeated problems with blockage, or you see "bursts" of data on your acquisition dot plots, it means your specimen has too many cell aggregates and each sample must be

filtered before running it on the instrument.

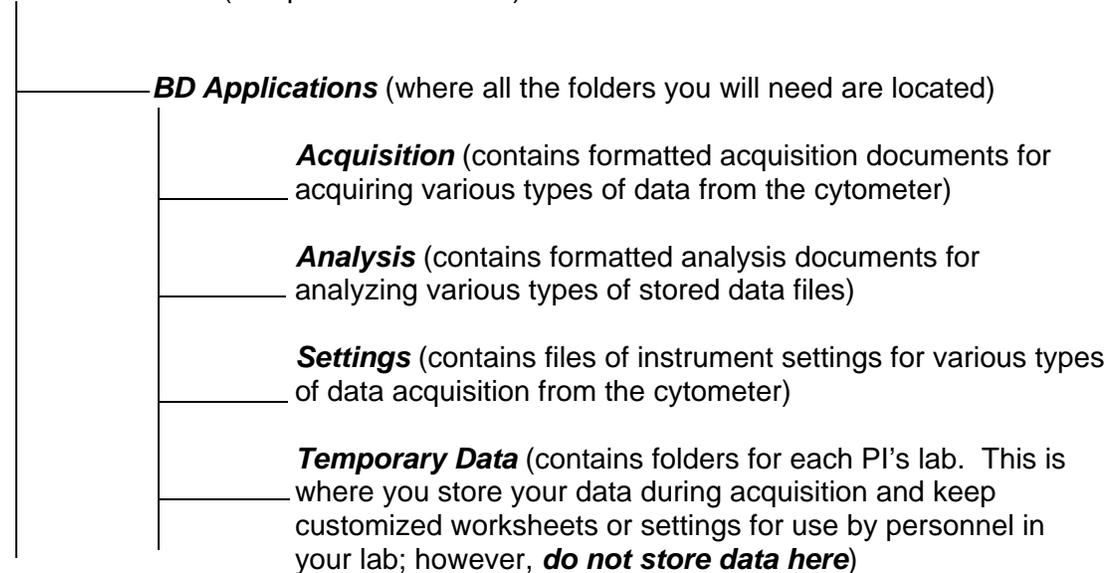
INSTRUMENT SHUT DOWN:

NEVER skip complete cleaning when you finish running your samples!!

1. Fill tube 2/3 full with **10% bleach**, place on sample uptake and with the arm open allow half the volume to run through. Close the arm and allow bleach to run for an additional **4 min**.
2. Fill tube 2/3 full with **distilled water**, place on sample uptake and with the arm open allow half the volume to run through. Close the arm and allow water to run for an additional **4 min**.
3. Place the Fluidics Control in "Standby" always leaving a water tube on sample uptake.
4. After hours, leave the cytometer in "Standby" for about 5 min to allow cooling of the laser, then turn off the power on the cytometer, computer and power supply/surge protector.

HARD DRIVE ORGANIZATION (folders & files):

FACStation G4 (computer's hard drive)



When you finish acquiring your data, transfer the data file to some other storage device (ex. ZIP disk, flash drive, etc.) or to your own computer via AppleTalk and **delete the original file** from the FACStation G4 hard drive. If data files are stored on the hard drive it will rapidly get too full for optimal operation (i.e. it will get real slow).