FACScan Protocol

Startup

1. Turn on FACScan
2. Turn on computer
3. Open lower compartment
   1. Verify the vent switch is up (this closes the vent and pressurizes the sheath fluid tank)
   2. Check all tubes for crimps and correct if required
   3. Check sheath fluid and waste levels
      1. Sheath fluid should be filled above the line (~80%)
      2. Waste should be below the line (~10%, fluid is bleach)
   4. Clear any bubbles from filter by removing cap and purging fluid, return cap when finished
4. Open middle compartment and flush flow cell 3x
   1. Toggle switch to “drain” and watch fluid level fall
   2. Toggle switch to “fill” and watch fluid level rise
   3. After 2 flushes, no bubbles should be seen during fill
5. Leave switch at “fill”
6. Make sure there are no bubbles in tube leading to waste container- wait for all bubbles to leave tube
7. Toggle switch to standby
8. Ensure fluid control is set to high
9. Launch CellQuest program

Setting up a New Document

1. Create new plots
   1. X: FSC, Y:SSC
   2. X:FL1 (GFP), Y:FL2 (RFP)

Opening Previous Settings and Preparing to Run a Sample

1. Open acquisition template
   1. File 🡪Open
2. Connect Computer to cytometer
   1. Acquire 🡪 Connect to Cytometer
3. Open Cytometer Controls
   1. Cytometer 🡪 Detector/Amps
4. Load Historical detector/amp settins
   1. Cytometer 🡪 Instrument Settings 🡪 Open File, Set, Done
5. Open Sample Descriptions
   1. Acquire 🡪 Parameter Descriptions
      1. Set Folder
      2. Reset Sample ID count
      3. Name Sample
6. Open Counter
   1. Acquire 🡪

Running a Sample and Acquiring Data

1. Sample should be single suspension of cells in ~1mL media, try no tto exceed 1MM cells per mL
2. Transfer sample to appropriate tube
3. Enter appropriate sample ID
4. Ensure selection of “setup” is appropriate
   1. If “setup” is checked, data is not acquired and saved; use this to make adjustments to amps or gates
   2. If “setup” is unchecked, data will be acquired- 10,000 counts total, only 200 displayed
5. Remove storage tube- move support to right, and pull tube down
6. Set to Run
7. Load sample tube- push sample up until seated, move support in place
8. Once settings are appropriate, uncheck setup and click Acquire
   1. System will run until all 10,000 counts are made
   2. Data is automatically saved
   3. If you want, print the display or record any preliminary data
9. Repeat this procedure for all of your samples
   1. `Do NOT leave a sample on the machine under STANDBY, this will drain sample
   2. In general unless you’re setting up parameters or acquiring data, your sample should be off
   3. Always replace a tube with sheath fluid when cytometer not in use

Shutdown

1. Acquire 🡪 Disconnect from cytometer
2. Set to Run
3. High flow bleach
   1. 1 min with arm right (run)
   2. 5 min with arm center (run)
      1. NOTE: this is a good time to do analysis of data
4. Repeat 3 with DI H2O
5. Leave 2 cm DI H2O on
6. Turn to Standby
7. Fill Sheath Fluid Tank
8. Empty Waste and add bleach
9. Turn off computer
10. Turn off FACScan

Data Analysis

1. Make new template (File 🡪New)
2. Make new graph
3. Using negative control, draw in gates
4. Add Chart with Gate/Area stats
5. Record % gated for the region of interest