Protocol for FACScan and FACSCalibur

Cytometry & Cell Sorting
NYULMC Divison of Advanced Research Technologies

1. Turn on the analyzer first, then computer (if computer is already on, turn it off)
2. *Check fluidics – This is the most important step!*
    a. Flip pressure switch to depressurize
    b. Fill sheath fluid supply (if it is empty, the machine was run dry which is very bad)
    c. Empty waste, make sure tubes are not twisted
    d. Flip pressure switch back to pressurize
3. PRIME (gets out bubbles) or DRAIN for 10 sec then FILL for 10 sec
4. When machine is in RUN the sample is being aspirated
5. Samples:
    a. At least 1 million cells/ml
    b. At least 250,000 cells per sample
    c. Minimum volume 250 ul
    d. Use 5 ml polystyrene tubes
    e. **CONTROLS**: positive control; negative control; isotype control; and single fluorochromes (for compensation)
6. Run samples using CellQuest (*refer to CellQuest user guide*)
    a. Always vortex
    b. Be sure RUN light is green (on Scan, machine says RUN)– If orange, refer to troubleshooting
    c. RUN in LO = 15ul/min, MED = 30ul/min (only on Calibur), or HI = 60ul/min
7. Clean up!
    a. RUN 10% bleach for 3 minutes on HI
    b. RUN detergent for 3 minutes
    c. RUN sheath for 3 minutes
8. **Leave machine in LO and STANDBY**
9. If you are the last user, turn off the analyzer AND computer (lasers have a limited lifetime!)

Troubleshooting

- If event rate is low:
    - Vortex sample again (it is fine to vortex while acquiring, it will continue when you put tube back on)
    - Check for clumps, you may need to filter your sample
- If you are not seeing anything or data looks funny in Cell Quest:
    - Make sure machine is dripping every 4 seconds when in RUN
    - Make sure tube isn't cracked
    - Hit PRIME (with or without tube of sheath on)
    - Check fluidics (check for bubbles)
    - Turn off both computer and analyzer and start over
- If you suspect a clog: come find a core tech!
- If you see a big bubble in filter: come find a core tech!