Protocol for LSRII

Cytometry & Cell Sorting
NYULMC Divison of Advanced Research Technologies

1. Turn on the fluidics first, then analyzer
2. *Check fluidics – This is the most important step!*
    a. Make sure sheath fluid is full (if it is empty, the machine was run dry which is very bad)
    b. Make sure waste is empty
    c. Check sheath filter for bubbles and check plenum for cracks
3. Run tube of sheath fluid and hit PRIME (gets out bubbles)
4. Check filters! Lift up lid of machine and refer to diagrams for correct wavelengths
5. When machine is in RUN the sample is being aspirated
6. Samples:
    a. At least 1 million cells/ml and at least 250,000 cells per sample
    b. Minimum volume 250 ul
    c. Use 5 ml polystyrene tubes
    d. **CONTROLS**: positive control; negative control; isotype control; and single fluorochromes (for compensation)
7. Run samples using FACSDiVa (*refer to FACSDiVa user guide*)
    a. Always vortex
    b. Be sure RUN light is green – orange could mean tube is cracked
    c. RUN in LOW (12ul/min) MED (30ul/min) HI (60ul/min)
    d. Dial on left further tunes pressure (it can turn 10 times, usually leave in middle)
8. *Clean up!*
    a. RUN 10% bleach for 5 minutes on HI
    b. RUN detergent for 5 minutes on HI
    c. RUN sheath for 5 minutes on HI
9. **Leave machine in LO and STANDBY**
10. If you are last user turn off analyzer (lasers have a limited lifetime!)

Troubleshooting

- If you are not seeing anything in FACSDiVa:
    - 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)
    - Restart program
- If your data looks weird
    - Check filters in analyzer (refer to maps on lid of machine)
    - Be sure compensation was set correctly
- 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