BioSorter Quick Start Guide

Startup

1. Check Fluidics: Ensure appropriate sheath type and amount is in the tank. Empty waste and add enough Bleach to result in 10% final concentration when tank is full.
2. Turn on Jun-Air Compressor: On/Off switch is on the back right side
    a. Ensure pressurization via the regulator on the left side of fluidics cart, should be in the 50-65 PSI range (should not be changed!)
3. Ensure the appropriate FOCA is in place:
    a. The FOCA 1000 is larger, and the tubing goes through the pinch valve
    b. The FOCA 500 is smaller, and the tubing runs through the lower rotary valve
4. Turn on Bio Sorter: On/Off switch is on the right-side, by the monitor
5. Open software: FlowPilot III (located in the center of the desktop)
6. Warm up the lasers: Select “enable lasers” option in bottom right panel
7. Prime Flow cell twice: “Maintenance” > “Prime Flow Cell” or manual prime (see troubleshooting section below)
8. Prime Sample Cup twice: “Maintenance” > “Prime Sample Cup” or manual prime (see troubleshooting section below)
9. Perform fluid QC: “Maintenance” > “Flow rate”
    a. Ensure 50cc tube is empty and in place on stage
    b. In the bottom panel, set stage, select 50mL position and click Move
    c. Watch the machine as it dispenses liquid. Look for an uninterrupted, laminar flow and the correct amount of liquid dispensed
        i. Expect 45 mL for the FOCA 1000 and 25 mL for the FOCA 500
    d. If the stream looks bad or an incorrect amount of liquid was dispensed see full guide for troubleshooting
10. Run experiment

11. Sample line Cleaning (done manually):
    a. Place 50cc tube on sample port
    b. Ensure Sheath is “off”, and Turn Sample “on”
        i. Run 40mL of 50% bleach
        ii. Run 40mL pink cleaning solution
        iii. Run **TWO** 40mL water washes
12. If sheath was not water do Fluidic Line Cleaning: “Cleaning” > “Water Flush”
13. Close software, turn off BioSorter and air compressor

File Export

File Types:

1. .expr = Experiment file
    a. Contains settings and parameters
2. .samp = Sample file
3. .bxr4 = Data file
4. .lmd = FCS file <---- File compatible with flow cytometry analysis software
5. .txt = Tab/excel file

Copy (at least, all can be backed up) .lmd files and screenshots to Sharepoint (https://caltech.sharepoint.com/sites/flowcytometry/SitePages/Flow-Cytometry-and-Cell-Sorting-Facility-Sharepoint.aspx) - place in your lab’s folder within the Documents section.

Troubleshooting

Manual Prime of sample line – use when air is in sample line and automatic Prime function is not moving it:

1. Remove sample tube and place beaker under the sample line.
2. Turn Sheath ON and Turn Sample ON.
3. Watch for bubbles clearing the sample line and for drips to begin coming out of sample line.
    d. If air is not moving, increase Sheath Flow Rate to 80% or 100%.

    e. Be careful to turn this down quickly once the air is removed. Always return to the original setting.
4. Recommended to run 2x Flow Cell prime followed by 2x Sample Cup Prime

Misaligned waste tray – do when stream is spraying to one side or the sample flow rate test fails. A misaligned tray causes some fluid to be blown off course, resulting in a lower-than-expected volume during the test.

1. Realign by manually moving waste tray via turning the micrometer on the left of the tray.
    a. Dial in the tray by turning the micrometer until the stream is fully disrupted, then turn until stream is seen, plus a half a rotation further than needed, to ensure stream is passing fully through the tray.