# BioSorter

**Overview**

- Currently have 500 FOCA and 1000 FOCA flow cells
- Sample Injection Cup can be swapped for 50ml Falcon Tube or 750mL Sample Cup
    - Minimum sample volume =2mL
- Instruments have low pressure, under 3-5PSI
- Waste is blown into waste tray by an air diverter, turns off to allow desired sample to drip down into collection cup
- Unsorted material can be re-collected in the sample recovery cup
- Sample can dispense into 2 plates, 1 plate, 5mL CC, 15mL CC, 50mL CC, or 425mL CC conical tubes
- Features three sample modes:
    - Pure = only sorts desired sample, will sort multiple instances of desired particles at once
    - Pure No Doubles = only sorts ONE desired sample, will NOT sort multiple desired and undesired particles
    - Enrich = will sort desired particles, as well as any undesired particles that come along for the ride
- Bleach must be diluted 1:1
- Bring at minimum 3L of sheath, if not more for large volume samples
    - Running new sheath for 15min prior to loading sample should suffice to flush sheath filter
    - Not applicable if bypassing sheath filter, run 3 min to clear old sheath if bypassing
- Parameters:
    - Extinction = SSC, ie. How much of a shadow is being cast by particle
    - Time of Flight (TOF) = FSC, ie. How long the particle takes to clear the light, beginning to end
    - 2 lasers: 488nm, 561nm
        - 3 filters
    - PMT voltage must be at 200 minimum, or else you will need to titrate

**Guide:**

1. Check Fluidics
    a. Ensure sheath is appropriate/full, use bypass filter if using non-standard sheath
        i. When swapping between two samples/sheaths, either swap filter (if needed), run new sheath for 15 minutes (to flush and remove traces of previous sheath) or simply start the sort if diluting previous sheath will be non-harmful to the sample.
    b. To remove a tank, disconnect quick connects on lid of tank, not the one on the fluidics cart
2. Turn on Jun-Air compressor
    a. On/Off switch is on the back right side
    b. Ensure pressurization via the regulator on left side of fluidics cart, should be in the 50-65 PSI range
3. Check which 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 valve
4. Turn on Bio Sorter
    a. On/Off switch is on the right-side, by the monitor
5. Open software: FlowPilot III located in the center of the desktop
    a. The last experiment will automatically open
    b. File Types:
        i. .expr = Experiment file
            1. Contains settings and parameters
        ii. .samp = Sample file
        iii. .bxr4 = Data file
        iv. .lmd = FCS file
        v. .txt = Tab/excel file
6. Warm up the lasers
    a. Select “enable lasers” option in bottom right panel
7. Prime the Machine
    a. Prime Flow Cell twice: “Maintenance” > “Prime Flow Cell”
        i. FOCA size is automatically detected, visible in the bottom left panel
        ii. Watch the sample lines, to ensure all bubbles move through
    b. Prime Sample Cup twice: “Maintenance” > “Prime Sample Cup”
    c. Trouble shooting:
        i. Remove sample cup and place beaker under sample line.

ii. Turn Sheath ON and Turn Sample ON.
iii. Watch for bubbles clearing the sample line and for drips to begin coming out of sample line.
iv. If air is not moving, increase Sheath Flow Rate to 80% or 100%.
v. Be careful to turn this down quickly once the air is removed. Always return to the original setting.

8. Perform QC
    a. In the bottom panel, set stage, select 50mL position and load tube on stage
    b. “Maintenance” > “Flow rate”
    c. Watch the machine as it dispenses liquid
        i. Look for an uninterrupted, laminar flow and the correct amount of liquid dispensed
        ii. 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:
        i. Check Sheath flow rate is at 50%
        ii. Misaligned: If the waste tray is opened/ misaligned, this is indicated by the stream spraying to one side. This causes some fluid to be blown off course, resulting in a lower-than-expected volume
            1. Realign by manually moving waste tray via turning the setting screws. Dial in the tray by turning the setting screw half a rotation further than needed, to ensure tray locks into place
        iii. Clogged:
            1. Unscrew the sample cup and Prime
            2. To unclog, “maintenance” > “Unclog” (This will backflush air from the sample line)
        iv. Too much air in flow cell
            1. Re-do Primes noted above
9. To save data:
    a. “Acquire” > save data to folder
        i. This automatically occurs outside of QC/setup mode
        ii. Data and experiment files should be stored in different folders
    b. Screenshot: “File” > “Save Screenshot”
10. To set Labels:
    a. “View” > “Settings”
11. To make a dot plot:
    a. “Layout" > “dot plot”
    b. Defaults scale:

        i. Right click > “Rescale” > Apply” > “Ok”
        ii. Change axis with a right click
    c. Right click on the center of the plot > “Gate”, right click around plot to add vertices around desired population
    d. Right click > “gate” > Reassign gate hierarchy
        i. Profile graph is set as R1 by default
    e. Ungate everything at once using the “Ungate” icon
12. Manual compensation: “Setup” > “Parameter Math”
13. Triggering/Threshold: “Advanced Settings” > “Trigger and Threshold tab”
    a. Triggering requires a minimum input value to register an event
    b. Pre-event and Trailing Thresholds = an event outside of the trigger threshold that is still recorded
14. Profiler setup: “Setup” > “Profiling Setup”
    a. Allows for orientation, partial profiling, number/location of peaks, and peak definition to determine the front/back ends of an event based off profile/size/fluorescence criteria
15. Dispensing: (Requires gates to be drawn)
    a. Bulk sort: Select “move to Home”
        i. “Setup” > “Coincidence” > “Choose Mode” > “[enrich, purity, or pure, no doubles]” > [Move to desired tube home] > [select desired Gate] > “Sort”
            1. “Enrich” = dispenses so long as there is sample of interest, includes non-desired sample in order to not miss desired sample; best for initial bulk sorts, sorts where having some non-desired sample is okay or for sorts where speed is essential
            2. “Purity” = will only dispense sample of interest, excludes sample of interest that is too close to sample of noninterest; best for refining a bulk sort or for sorts where a pure population is essential
            3. “Pure no doubles” = will only dispense a SINGLE particle of interest; best to know exact number of samples dispensed, and concentration of fluid added
    b. Plate sort: “Move Stage” = load A or B (left and right plate holders, respectively)
        i. To make a new plate template: “Setup” > “Plate” > “Calibrated Plate”

1. For 384 well plates, you will need to make a template for *each **brand**,* due to the low margin of error allowed for this plate type. All other plates are okay with a default template

ii. Gate each well or select sort gate

1. If the sort gate is greyed out and not selectable, this is because the "Gate each well" option has been selected in the plate builder

16. Plate calibration: (or add a new configuration)
    a. Put plate with a cover into plate holder A, ensuring that A1's position correlates to the marked A1 position on the holder
    b. "Setup" > "Plates" > [Select your plate] > "Close"
    c. "Setup" > "Plates" > "Recalibrate" > "Start (left)"
    d. A dialog box will open. (**DO NOT** hit enter at any time while the dialog box is open. This will cause the box to close without saving your changes)
        i. X-step/Y-step/Z-step values = how far the plate moves in the X/Y/Z direction
        ii. "Move to Position" = moves stage
        iii. Drop Test = Number of Droplets > "Test"
        iv. Custom Position = creates a position point where the stage currently is
        v. "Go To" = moves the stage to selected position point
        vi. "Test" = dispense one drop, may need to wipe the plate off and try again if the droplet does not dispense or misfires
        vii. "Set Position" = tells the robot arm where the position is set
    e. Set the positions for each "Go To" in order of the prompts, using the Step and Test functions to position the stage so a droplet is dispensed centered of each well
    f. Once you have set each position, name your plate (include brand for 384 well plates) and select "Ok"
    g. "Setup" > "Plates" > [Select your plate] > "Close" to load your template
17. Sort Delay Setup (For new samples only):
    a. Setup plate template, 1 sample per well
    b. "Setup" > "Coincidence" > "Pure no doubles"
    c. "Advanced" > "Delay Setup" > select a different delay every 6 or 12 wells, increasing the delay by increments of 0.5
        i. Set the initial delay, starting below the desired sort delay, to allow room to work up to and past the desired delay

ii. Set “Number of Repeats” = 6

d. Fill your plate and then check the sample per well. Your ideal drop delay will have 1 sample/well
    i. If your accuracy is bad, then reduce your drop width and increase “Number of Repeats” = 12, then run again
    ii. Make sure to clean/use a different plate in between runs

18. To compare/overlay datasets:
    a. “Data” > “Compare Datasets”
        i. Select desired dataset and color of desired dataset
        ii. Graphs the old/new dataset together
        iii. NOTE: you can change the names of dataset on the display without changing the names of the actual saved datafiles
19. Adjust Mixer speed: “advanced”>View dropdown > Mixer velocity
    a. Can also pause/oscillate
        i. Oscillate must be checked and then stop/start the mixer to apply oscillation
20. Cleaning after experiment
    a. Run 1:1 bleach (45mL total volume)
    b. Run non-diluted cleaning solution (45mL total volume)
    c. Run TWO water washes (45mL each)
    d. If using anything other than Di water as sheath, flush sheath lines: “Cleaning” > “Water Flush”