Aria Cell Sorter Start Up SOP

Startup is performed by the FACS Core director between 7:30AM and 9AM, Monday through Friday. In his/her absence (weekends, PTO or holidays), this SOP becomes your responsibility.

1. Empty waste into the lab sink: Unscrew the waste container lid and release the waste probe sensor (set in the large 2L beaker found at each instrument). Take the container to the sink and pour its contents down the drain. Add 1L 100% bleach into the empty waste container.
2. Depressurize the sheath tank and remove the lid. Fill sheath tank with the boxed Blood Bank Saline (found at the left side of the backwall window) to the welding line along the top ¼ circumference of the sheath tank. **DO NOT** use the NERL Diluent 2 Sheath Fluid as this is used for the LSRs.
3. Turn computer on, login into the Window's Admin account: password BDIS#1.
4. Launch the TeraTerm program. This is a terminal window app found on the desktop with a **"T"** icon. Power on the Flow Cytometer: Press the big green power button on the Flow Cytometer. TeraTerm will become active after 2-3 minutes, generating an IP address for the flow cytometer.
5. Ensure the flow cell access door is raised and the sort chamber door is open. You will want to check the plates to make sure they are dry and clean before you turn on the stream.
6. Turn on the air compressor in the corner for the Aria 2u (Captain America) and Aria 3.1 (Falcon). The Aria 3.2 (Captain Marvel) and 3.3 (Spider-Man) do not require this step.
7. Wait 30 minutes for lasers to warm up before running samples (You can proceed with steps 8 through 15 while the lasers warm up).
8. Turn on aerosol management system if you are sorting a BSL-2 sample (Available for Aria 2u, 3.1 and 3.2).
9. Optional: Turn on water bath for cooled sample collection
10. Launch the LabUsage App on the desktop to access the FACSDiva Software (Same user email and password as for the BookMyLabs Calender). At Diva Login select the Administrator account using password "pass." The cytometer will connect to the computer after about 2 minute
11. When the software connects to the cell sorter, select "Use CST settings**"** in the CST dialog box. Most samples are run on a 70u nozzle. If you use another size nozzle follow the instructions for Switching Nozzles on the **laminated instruction form for CST**.
12. Perform Fluidics Start Up (Top dropdown menu: Cytometer>Fluidics Start-up) and follow the instructional prompts. This process takes about 10 minutes.
13. Once complete, as instructed by the Fluidics StartUp prompts, install the nozzle to be used and turn on the stream. Wait 1-2 minutes for stream to stabilize. Apply the SweetSpot to activate the previous optimized drop settings used for sorting.
14. Check the stream comparing today's drop formations to those featured on the laminated images of drops for each nozzle size found at each instrument.

15. Load and run for 5 min each sample tubes of 10% Bleach; 70% EtOH; and MilliQ water with the Flow Rate set to 11 on the Acquisition Dashboard. **Use the FACSDiva experiment found in the Admin Folder for performing Accudrop for the nozzle size you plan to use.**
16. Perform CST performance check if first user of the day (**See CST SOP laminated instructions for details**). (You'll need to turn off SweetSpot)
17. If the CST protocol results in a Pass, proceed to AccuDrop Sort optimization.
18. Optimizing sort settings: Frequencies may vary slightly (+/- 0.2 kHz) from one transducer to another, the stream images above each instrument will reflect the correct setting for that machine. ****DO NOT CHANGE THE FREQUENCY UNLESS ADVICED by the FACS Director****
19. Set the drop delay using the Auto Delay Feature: Note that this will require Accudrops beads. These can be found in the refrigerator by the door on the top shelf either pre-allocated (1drop of concentrated beads per 0.5 mL of MilliQ water) in a labeled 5mL FACS tube or as a concentrate in a black dropper bottle with blue lid).
    - Open a prebuilt Experiment with for the proper nozzle size (Ex. AccuDrop 70μm).
    - In the Acquisition Dashboard, load your Accudrop beads and adjust your sample rate to reflect the correct event/sec rate (for 70uM nozzle, event/sec rate is between 1500-3000 and for 100uM it is between 500-1500.)
    - Within the Experiment, expand the global sheet until it reveals a premade Sort Layout. Sort Precision should be set to **Fine Tune** and population to sort at "**NOT P1**".
    - As needed adjust the brightness of the steam in the Sort Window using the fine focus knob adjacent to the sort chamber door
    - In the Sort Layout Window Press **SORT** but press **CANCEL** when asked to open the Waste Drawer and to charge the sort plates.
    - In the Sort window, manually apply voltage to the sort plates and apply the optical filter using the appropriate icons. Adjust the side stream so that the near left sort stream is in the middle of the optical filter sort box as visible in the Sort Window.
    - Run the **AutoDelay** App found in the top right of the Sort window.
    - Once complete, as necessary, adjust Drop Delay value to get the percentage of events being sorted as close to 100% in the left box of the sort block window as possible.
    - Note that pre-allocated AccuDrop tubes are good for 2-3 days.
20. In the Sort window, set the side streams to the number populations and types of tubes with which you plan to work. Check to make sure each stream drops in the center of the positioned tube by applying Voltage to the Sort Plates and performing a Test Sort. Adjust the side streams as needed.
21. You are ready to sort! Log out of the Administrator Account and Log into your personal account. You needn't turnoff the stream when toggling between FACSDiva Accounts.

Common issues/Problems with BD Cell Sorters:

1. Fluidics Start-Up/Shut-down Fails: The most common reason is either the waste is full or the sheath tank is not properly sealed. Empty the waste tank and add 1L of bleach as described above. For the sheath tank, make sure you hear no hissing from the sheath tank while fluidics is starting. If you do, unscrew the top of the tank and pull up until you cannot hear hissing. Retighten the tank and you should feel the lid push up tightly against the tank.

2. Clogged nozzle: Place the nozzle in a capped 5ml tube containing 70% Ethanol. Place this tube in our water bath sonicator for 1 to 2 minutes. View the o-ring orifice of the nozzle with the microscopes under 10x zoom to visually verify there are no obstructions to the orifice. Also, inspect to ensure that the red o-ring looks OK (no cracks).

3. Stream Break off and Droplet Formation is Unstable:

A. Check CST configuration in place matches the configuration you need for your current sort and ensure that the proper nozzle is in place.

B. Check the pressure gauge on the left side of the fluidics cart below the sorter. If it is reading below 90 psi, BD Service will have to be called. Note that one can likely still use a 100μM nozzle as it requires a much lower pressure setting (20 psi) than the 70 μm nozzle (70 psi).

C. Dense samples can also affect stream stability. Dilute your sample so that your sample rate doesn't exceed 10% of the sort droplet frequency. For instance, a 70μm nozzle is set to a frequency that generate 88,000 droplets per second. As a result, your event rate should never exceed 8,800 events per second.

D. Dirty nozzle/flow cells: You can clean the nozzle as described above and performing a flow cell cleaning. This is done by installing the Closed Loop Nozzle, inserting a sample tube with 70% bleach (heated as able) and navigating the top drop down Menu in FACS Diva: Cytometer>cleaning>clean flow cell. This step can be repeated 3-4 times for a full flush.

E. Over time, the designated numerical value for the Drop 1 breakoff can drift. To correct, you will have to manually enter the correct breakoff point in the Drop 1 dialogue box. Check periodically for the best sort efficiencies.

F. If you are not able to get satellite drops to join parent drop before 5 drops, remove and reinsert the nozzle. On a rare occasion, you may have to readjust the frequency slightly. Frequencies are typically best between 87 and 89 kHz. **Consult the director if the frequency appears to be an issue**.

4. Stream camera is obstructed from stream clog or spraying: Dry the camera lens and general area under the flow cell with a cotton swab until you can no longer see the obstruction on the camera.

5. Stream fanning: Typically due to an instable stream or an enlarged drop gap. Make sure your stream is stable, your drop formation as desired and that the correct gap is set between the last drop still connected to the mainstream and the first independent drop. Reference settings are listed on the **laminated Drop Image sheet** on each instrument. Check that the 2nd, 3rd and 4th drop are set at 20-17, 6-10, and 0-3 respectively.

6. High voltage error: There is excessive liquid in the sort block, under the flow cell, or on the defection plates. Turn off the cell sorter (do not vent the sheath tank) and clean the sort block, defection plates, and chamber with ethanol and ensure no liquid or accumulated slats are present. Press in the emergency eject button to reset the system. Restart the machine and reboot the computer returning to the point before your error occurred.

7. Can't see side streams, waste stream does not hit middle waste chamber: This is due to either the laser being out of synch with the stream, the camera that see the drops has shifted, or the sort block was moved from a previous sort adjustment. Ensure that the stream hits the middle waste chamber by adjusting the sort chamber using the Allen wrench provided. Adjust the fine focus for the stream using the knob to the left of the sort chamber door.

8. No signal is seen when samples are running: Make sure the front lid covering the fluidics portion of the cell sorter is closed completely. If this does not fix the problem, turn the stream off, log out of FACS Diva and restart the sorter manually.

If you are still having problems with the sorters and none of these suggestions have helped, please call Brandon Carter at +1 (775) 722-4672 or email Brandon at bcarter@pavir.org.

Alternatively, you can call BD directly at 1-877-232-8995, prompt 2. You will need to provide the serial number of the sorter listed below

Aria Serial Numbers:

Aria 2u (Captain America):	P99900023
Aria 3.1 (Falcon):	P28200111
Aria 3.2 (Captain Marvel):	P64828200017
Aria 3.3 (Spider-Man):	P64828200406