Aria II Quick Guide

Instrument Set-up

1. Check fluidics (refill as needed)
    a. Empty waste down sink & fill bottom with 10% bleach or 1:3 wescodyne
    b. Refill sheath with BD FACSFlow
2. Turn on Aria
3. Login to DiVa using your Kerberos ID
    a. Always click “Use CST settings”
4. Open Aria hood
    a. Clean off plates with H20
    b. Spray and clean collection block and sample tube area with EtOH
5. Perform fluidics start-up if necessary (takes 5 minutes, follow instructions)
6. Turn on stream ***DO NOT TOUCH FLUIDICS CART WHILE STREAM IS ON***
    a. Allow stream to settle (about 5 minutes)
        i. If stream is unstable or potentially clogged, turn it on and off a couple times
    b. Adjust sort block (two screws on sides) to ensure stream is centered in waste catcher
    c. Adjust side stream camera (knob to the left of sort block) for brightest waste stream image
7. Run CS&T
    a. Cytometer>CST
    b. After connecting, select bead lot to match current beads
    c. Run CST.
        i. Beads are in the “Aria Box” in room fridge (3 drops per 1mL sheath)

Stream Set-up

1. Adjust amplitude to correct stream settings
    a. ‘Drop1’ actual value should be as close to the ‘Drop1’ set value as possible
        i. If ‘Drop1’ changes, note which direction (up or down)
    b. Ensure ‘Gap’ is correct for specific nozzle type (70um: ~5-7, 100um: ~9-12)
        i. Correct Gap results in 4 distinct side streams when voltage and test sort are turned on

c. The 'Drop1' and 'Gap' set values (along with the plate voltage, drop delay, and other stream settings) should load in with the configuration for each nozzle

2. Turn on sweet spot
3. Check side stream aim (can be done now or after accudrop!)
    a. Place "test" tubes in collection block
    b. Ensure side stream laser is focused on the side streams (silver knob)
    c. Turn on voltage (green means off, red means on) and 'test sort', open waste drawer
    d. Look inside and check streams
        i. Adjust sliders for the four individual side streams while checking that the stream is aimed at the bottom center of the tube (must look in from above)
    e. Adjust 'Voltage Center' if waste stream moves in waste catcher with voltage on
    f. Adjust 2^{nd}, 3^{rd}, 4^{th} drop values if the center stream is fanning (should not need to change these regularly)
    g. Close waste drawer and turn off voltage

Accudrop (drop delay) Set-up

1. Accudrop beads are kept in "aria box" in fridge (2 drops per 1mL sheath)
2. Open accudrop experiment (can be imported from desktop),
    a. Open sort layout - can be found by expanding the global worksheet folder and then expanding the worksheet itself
3. Load beads and run at a threshold rate 3000-6000/sec
4. Click **sort**, and then **cancel**
5. Turn on voltage and optical filter
    a. Be sure left side stream is within box (may need to adjust side stream sliders)
6. Adjust drop delay so that 0-1% in right box, 99- 100% in left box (>95% is acceptable)
    a. Drop delay adjustment direction depends on where your 'Drop1' moved compared to previous settings
        i. If 'Drop1' increases, the breakoff got longer, which means the drop delay will be higher

Setting up an Experiment

1. Select parameters needed in cytometer window
2. Set voltages based on unstained control that are the same cells as experimental/sample tubes (good idea to check voltages with positive sample as well)
3. Run comp controls (and any other controls needed, FMOs?!)
4. Save sample data (set appropriate amount of events to save)
5. Set up gates

6. Setup sort layout - **<u>For all sorts, select 4 tube and 4-way purity</u>**
7. Load collection tubes
8. **Start sorting! Click acquire and then click on sort; click ok when the prompt comes up**
    a. The aria can sort max 20,000 cells/sec (70um nozzle) or 10,000 cells/sec (100um nozzle), and don't go higher than 7 with the flow rate
9. To create PDFs: batch process – highlight all of the tubes and right click and batch process, save as pdf

Shutdown

1. Run tube of 70% Ethanol for 5 minutes at a flow rate of 11
2. Run H_20 for 5 minutes at a flow rate of 11
3. Turn stream off
4. If last user on a friday night, run fluidics shutdown (follow instructions)
    a. "Cleaning" tube is H20
5. If not last user, run Cytometer>Clean Flow Cell twice, mounting a tube of H2O both times

Troubleshooting

- If there is a nozzle clog (or tube runs empty):
    - Close waste catcher if DiVa doesn't - remember, you are quicker than Sweet Spot if you are paying attention!
        - pay attention to event rate - it will slow down and then suddenly speed up at the end
    - Open aria and wipe off plates
        - Use q tip and wedge up where drop delay camera is
        - You can right click on the stream window to toggle between raw image and processed image
    - Turn stream back on
    - If the clog will not clear:
        - Remove nozzle
        - Check for o-ring!
        - Sonicate nozzle in H20 in a tube
        - After sonicating (at least 5 min)
        - Replace nozzle and turn on stream
- If the sample line is clogged:
    - Can check with a sample line backflush: Cytometer>Cleaning modes>Sample line backflush. Click start and note that sheath drips from the smaple probe.
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- DiVa will freeze if you play around with stuff while you are sorting. Don't add new plots or gates.
    - If it does not unfreeze, use red panic button
- When sorting, be sure side streams are all on (make sure voltage sliders are at correct number) - may not see anything at low sort rates
- O-rings are integrated to Aria II nozzles but can eventually degrade and fall off. Please let Core staff know if you discover any issues with the integrated o-rings.