Aria Fusion Guide

Instrument Set-up

1. Check fluidics (refill as needed)
    a. Empty waste down sink & fill bottom with bleach
    b. Refill sheath tank with appropriate sheath fluid
2. Turn on Aria, BioSafety Cabinet, water chiller, and air compressor
3. Login to DiVa software
4. Under Cytometer perform fluidics start-up, if necessary (takes 5 minutes, follow instructions on screen)
5. Open Sorting Chamber and check voltage plates for buildup and clean with di water if necessary
6. Connect cooling lines to appropriate shorting blocks
7. Check current configuration for nozzle and change if necessary.
    a. Cytometer>Configuration
    b. A new window will open behind Diva
    c. Highlight correct configuration
    d. Hit Apply, then Okay
8. Turn on stream
    a. View stream in Sorting Chamber to make sure it's aligned with waste catcher. Adjust by loosening the screws if necessary
    b. Allow stream to settle (about 5 minutes, run CST during this time)
        i. If stream is unstable or potentially clogged, turn it on and off a couple times. If still unstable – sonicate nozzle
    c. Adjust side stream camera (knob to the left of sort block) for brightest waste stream image
9. Run CST
    a. Cytometer>CST
    b. After connecting, select bead lot to match current beads
    c. Vortex beads and hit Run CST.
        i. Beads are in the box in fridge (3 drops per 1mL di water)

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, 80um: ~7-9, 100um: ~9-12)
    i. Correct Gap results in 4 distinct side streams when voltage and test sort are turned on
2. Turn on sweet spot
3. Check if adequate side streams can be created
    a. Turn on voltage (green means off, red means on) and test sort
    b. Ensure side stream laser is focused on the side streams (silver knob next to Sorting Chamber)
    c. Check for distinct side streams in side stream window
    d. Adjust Gap and 2nd, 3rd, 4th drop values if the center stream is fanning (should not need to change these regularly)
    e. Turn off voltage

Accudrop (drop delay) Set-up

1. Accudrop/Sperotech beads are kept in box in fridge (2 drops per 1mL di water)
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. Ensure Flow Rate is set to 1
4. Load beads and run at a threshold rate 3000-6000/sec
5. Click **sort**, and then **cancel** in the pop-up box
6. Turn on voltage and optical filter
    a. Be sure left side stream is within box (may need to adjust side stream sliders)
7. Adjust drop delay 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 a higher number
8. Unload beads

Check Side Stream Aim

1. Tubes
    a. Place tubes in collection block with caps on
    b. Turn on voltage and open waste drawer
    c. Quickly turn test sort on and off to deposit a small droplet on the cap of each test tube

d. Check the location of the droplet and ensure it landed in the center
    i. Adjust sliders for the four individual side streams while re-checking droplet location with each adjustment
e. Close waste drawer and turn off voltage

2. Plates
    a. Insert plate sorting block adaptor at the bottom of the sort chamber
    b. Open sort layout and select your plate type - can be found by expanding the global worksheet folder and then expanding the worksheet itself
    c. Hit the blue triangle button to move plate arm forward
    d. Place plate in plate holder with the lid on
    e. Go to Sort>Home Device and a small window will open
    f. In the Home Device window select your plate type and select Go Home
    g. Turn on voltage and open waste drawer
    h. Set far left sorting stream slider to ~30 and close all other side streams
    i. Open the Sort Chamber and look down through the bottom to ensure the sorting stream does not clip any edges of the open hole of the sorting adaptor
    j. Quickly turn test sort on and off to deposit a small droplet on the lid of well A1
    k. Check the location of the droplet and ensure it landed in the center of the well
        i. For small adjustments, move slider for far left side streams while re-checking droplet location with each adjustment
        ii. For large adjustments, use arrow on Home Device window to adjust arm of the plate holder
            1. If any adjustments were made this way, hit set new home and note the coordinates in the window
    l. Close waste drawer and turn off voltage

Setting up an Experiment

1. Select parameters needed in cytometer window
2. Set voltages based on positive and unstained control that are the same cells as experimental/sample tubes
3. Set appropriate number of events to save from a specific gate
4. Run and record comp controls (and any other controls needed, i.e. negative control, FMO)
5. Set up gates
6. Setup sort layout

7. Load collection tubes/plate
8. **Start sorting! Click acquire and then click on sort; click okay when the pop-up box comes up**
    a. The aria can run 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 the tubes and right click and batch process, save as pdf

Shutdown

1. Run tube of 10% bleach for 5 minutes (15 minutes for BSL-2 work) at a flow rate of 11
2. Run diH_20 for 5 minutes at a flow rate of 11
3. Turn stream off
4. Run fluidics shutdown (follow instructions)
    a. "Cleaning" tube is diH_20
5. Run Cytometer>Clean Flow Cell twice, loading a tube of diH_2O both times

Troubleshooting

- If CST fails
    - If you don't see any events -> turn the Aria off/on and try again
    - You see events but the event rate is too low -> check the volume of CST beads, vortex them and try again
    - Many fields have red warnings -> check the sheath filter for air bubbles
        - If you see bubbles get a paper towel and open the valve on the filter to bleed the air off
        - If you don't see bubbles turn the stream off/on a few times to blast any bubbles in the lines through the systems
        - If you don't see bubble, the flow cell could be dirty -> turn off the stream and do 2 flow cell cleans (Cytometer>cleaning>Flow cell clean) with 10% bleach and wait 5 mins. Afterwards, do 1 flow cell clean with di water and try CST again.
- If there is a clog, close waste catcher if DiVa doesn't - remember, you are quicker than Sweet Spot if you are paying attention! And try:
    - Turn off/on stream
    - Open Sorting Chamber and wipe off voltage 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
    - If the clog will not clear:
        - Remove nozzle
        - Check the o-ring!
        - Sonicate the nozzle for 5 mins
        - Replace nozzle and turn on stream
    - Decontaminate surfaces and run cleaning
        - Run tube of 10% bleach for 5 minutes at a flow rate of 11
        - Run diH20 for 5 minutes at a flow rate of 11

Common Bugs

- No side streams other than the far left plate sorting stream even thoug all sliders are open – turn off stream, change configuration to anything, close configuration window. Change configuration back to what you need. Turn on stream and all side streams should now be visible again
- Plate Sorting – when ACDU is first moved (from 'Go to Home' on the Home Device screen), it will give a movement error. This can be cleared and 'Go to Home' can be clicked again.
- Cameras – both camera windows will show gray fields when a pop up or dialog box is open. This is normal.
- Text fields – occasionally you will be unable to change any text or numbers in a field. To remedy this, follow this procedure:
    - Click **Edit** in the menu bar and select **User Preferences**
    - On the **FCS** tab, check the box for **Export FCS after recording** and click the **Browse** button
    - Ensure you are able to type in the **Folder name:** field
    - Click cancel on this window, uncheck the box for **Export FCS after recording** and cancel out of the **User Preferences** window
    - You should now be able to type in text fields again