ARIA FUSION START UP GUIDE (version 1)

1. **Put on PPE and disinfect work areas**: lab coat and gloves required in the sort room.
2. In this order: (a) **Computer**: Log in (Username: Admin, Password: BDIS#1), (b) **Aria Fusion:** ON at green button, (b) **Water-bath:** Power on and hit "enter" button, (c) **Compressor:** ON at back left (d) **Tera Term:** icon on desktop (e) **AMS Controller:** ON (BSC button set to Low; *use High for clog*), and (f) **BSC Fan:** ON (flashing LED = off, solid LED = on).
3. **Coherent Connection** (CC) desktop icon: START buttons for lasers (UV: Slide voltage for UV laser to max > start UV laser)
4. **Diva Software:** Open desktop icon and log in> username: administrator / no password
5. Check (a) **Sheath Tank** and then (b) **Connect Sheath/Air fittings** to Sheath Filter/Sheath Tank (pull out the fluidics drawer)
    a. Sheath Tank: *Use autoclaved, unopened carboy only*. If there is ethanol in the Sheath Tank, decant into Ethanol Metal Reserve tank on floor across from Aria Fusion (under ShadowFACS sheath bench). **Use aseptic technique, do not touch anything to the tank opening**. Rinse tank: add 1-2L of sterile buffer. Close lid, swish liquid in tank then empty into sink. Fill with remaining buffer.
    b. Tank fittings: Check and Reconnect all proper tubing/fittings
        i. Bottom of Sheath Filter (1) Should be connected to Sheath Line (1-S)
        ii. Sheath Tank should have an Air Line (2) connected to the Air Port (2-S)
        iii. The 2 Waste Fittings from Waste Tank should be connected to the 2 fluidics cart waste ports (A) and Sheath Sensor and Waste Sensor are connected to the Sheath/Waste sensor ports on the Fluidics Cart
6. **Use CST Settings**: Select this once Cytometer is Connected in Diva Software and window pops up with message
7. Run **Fluidics Start Up** (Cytometer Menu> Fluidics Start Up) to flush ethanol from sorter and fill with sheath
8. **Sonicate nozzle 5min** as Start Up is proceeding. **In Diva, select correct nozzle settings if needed** (100um)

    Any other nozzle besides 100um ask staff first: note also that you are responsible for setting back to 100um and checking sort chamber and AccuDrop laser alignment before leaving the sorter

    a. **Sort Set up for nozzle**: Sort Menu> Sort Set Up > *your nozzle of choice* (100um)
    b. **Set Configuration** if needed (Cytometer Menu > View Configurations > Click on configuration needed > Select Set Configuration > Select OK (e.g. "**100 CST Salk Inst 2B-2R-4V-3YG-2UV**").
9. **Clean Flow Cell:** (Cytometer Menu > Cleaning Modes) Use new tube of filtered MQ H20 **See that water fluid level in tube goes down, if not, sample line may be clogged**
10. **Flush Flow Cell and sample line with bleach and water** (*Note: when turning on/off stream, take care to let the action fully complete before proceeding (e.g. installing/removing a nozzle). Status is indicated in the **Cytometer WIndow***).
    a. **With Closed loop nozzle (CLN) installed and tube of 10% bleach** (at least 3ml), run Clean Flow Cell.
    b. **Remove CLN and Start Stream**, RUN bleach at Flow rate 11 at least 5min. Stop, turn off stream
    c. **Install CLN and tube of MQ H2O** (at least 3ml), run Clean Flow Cell.
    d. **Remove CLN and Start Stream**, RUN H2O at Flow rate 11 at least 5min. Stop, turn off stream.
    e. **Dry with kimwipe**: Sort Chamber and the high voltage plates.
    f. **Install nozzle** (dry any liquid around nozzle outside the Flow Cell if present), then start Stream
    g. **Ensure that droplets are fast merging (satellites disappear after a few drops)**: refer to pictures taped to BSC BSC for that nozzle size (sonicate nozzle again if drops look misshapen or there are many satellites).
11. **Check CS&T bead profiles using FCCF's QC template followed by running CS&T in Diva**
    a. **Prepare beads**: Add 2 drop CS&T beads to FACS tube (mini fridge door; blue label CS&T beads NOT ORANGE ones; label tube w Blue CST, Bead lot# and date). Dilute in approx. 500ul filtered MQ H2O from syringe.
    b. **Record CS&T bead file and assess (make a PDF and email to fccf@salk.edu):** Open the "CST Beads" experiment in the Browser. Find the appropriate Month/Bead lot# specimen (e.g "03Mar-91193") and create an empty tube ready to record a file for today. Set Flow rate 1, load beads and record. Compare to examples taped to BSC. If instrument is dirty (scatter looks weird e.g. odd shape, stretched out profile), lots of debris, or bright bead peak is looking wide for some channels) repeat Step 10 to flush. *Cleaning steps described in Shutdown can also be carried out (e.g. Steps 3-7).*

*If you are able to use it but it is still out of spec, please contact us as it may need an extended FC soak to get it clean **before** doing an ethanol shutdown.*

c. **Execute CS&T in Diva, assess results (Export PDF, send to fccf@salk.edu):** Cytometer Menu > CS&T > wait for it to connect > Select Performance Check from the drop down menu (Bead lot ID# should be highlighted already in drop down menu) > Press Run. Once it is done running CS&T, check results for anything highlighted in red. Export PDF (select report from left); exit to return to Diva.

12. **Optimize breakoff at Stream and Start Sweet Spot**
    a. Adjust the amplitude until Drop 1 current value (on grey background) matches typable text box
    b. Turn on Sweet Spot (within stream window)
13. **Calculate Drop Delay using Auto Delay** button (calibrates drop delay to charge drops for sorting)
    a. Open **Drop Delay experiment** > Expand Specimen > Expand tube, double click to open **Sort Layout**
    b. In Sort Stream window, optimize AccuDrop laser brightness (silver knob; left of sort chamber door)
    c. Click **Optical Filter** button. Turn on plates (**Voltage** button) and use Test Sort button: **adjust left stream voltage** (position stream to fall within optical filter window).
    d. **Starting sorting:** Load beads (vial in mini fridge door: check for already made up beads as they are stable and can be kept diluted at 4C. Use 1 drop/ml if making fresh). In Sort Layout, start sorting the NOT gate (to sort ALL of the beads). At the window that pops up, click **Cancel** (no need to open the waste drawer at this time) but you must manually **turn on the plates (click Voltage icon).**
    e. Click **Auto Delay** button > select RUN and follow the prompts. Once completed, exit window (When finished, the final status within Auto Delay window should read "drop delay set" and show an upside down "U" shape. If drop delay cannot be set, take a picture of Auto Delay window and set to fccf@salk.edu).
14. **Sterilize and then backflush:** RUN 10% bleach 5min then backflush 2min (Cytometer > Cleaning Mode)
15. **During and sorting:**
    a. **Biosafety aerosol door**: must be closed to contain aerosols from collection chamber when sorting.
    b. **Sample Temperature:** check to make sure appropriate temp is selected (Cytometer Menu).
    c. **Flow Cell Clean (take photo of Diva showing timestamps):** Record a *5min FCS file every 30min of use (filtered DiH20 after Flow Cell Clean with 30% contrad, then water).*
    d. **Leaving sorter for next user:** Follow step (1) and (2) of Shutdown Guide, leave stream ON with 100um nozzle.
    e. **Send PDFs (CS&T profiles and report) and photos (cleaning and shutdown if relevant)** to fccf@salk.edu

ARIA FUSION SHUTDOWN GUIDE (version 1)

Procedure for cleaning up after sorting, changing back to 100um nozzle (if using other nozzle size), verifying instrument is clean before shutting down the system.

1. **Backflush sample line and RUN 10% bleach**: Once you have unloaded your last sample, backflush for 1 minute to clear all sample from sample line. (Cytometer Menu > Cleaning modes > Sample Line backflush > window will appear, Click **Start**. **Cancel** to exit). Load a tube of 10% Bleach and RUN at Flow Rate 11 for 5min.
2. **Switch back to 100um sort setting and configuration if other nozzle was used** (install freshly sonicated 100um nozzle and check sort chamber/waste stream alignment, and adjust AccuDrop laser to brightest on the waste stream
3. **Stop the stream, install Closed-Loop Nozzle (CLN)**
    a. Turn off the Stream (wait for fluidics action to complete)
    b. Stow nozzle: Remove nozzle, place it the labelled tube and add MQ DiH20 to cover before capping
    c. Install Closed-Loop Nozzle
4. **Clean Flow Cell and Sample Line with 10% Bleach (perform 2x)**
    a. Clean Flow Cell (Cytometer > Cleaning modes) with 3ml of 10% Bleach→ Let sit for 3min (back up data, make PDFs, export templates and DELETE OLD DATA during this time)
    b. Take out the CLN; start the stream (with no nozzle, this is to flush out debris from the flow cell)
    c. RUN tube of 10% bleach at Flow Rate 11 for 2 minutes
    d. Unload the tube; turn off Stream (wait for fluidics action to complete), install CLN
    e. Repeat steps a-d.
5. **Clean Flow Cell and Sample Line with MQ H20**

a. Clean Flow Cell (Cytometer > Cleaning modes) with 3ml of MQ H2O → Let sit for 3min (delete data!)
b. Take out the CLN; start the stream
c. RUN tube of MQ H2O at Flow Rate 11 for 2 minutes
d. Unload the tube; turn off Stream (wait for fluidics action to complete), install CLN

6. **Clean Flow Cell and Sample Line with 1.5% Citranox**
   a. Clean Flow Cell (Cytometer > Cleaning modes) with 3ml of 1.5% Citranox→ Let sit 3min (delete data!)
   b. Take out the CLN; start the stream
   c. RUN tube of 1.5% Citranox at Flow Rate 11 for 2 minutes
   d. Unload the tube; turn off Stream (wait for fluidics action to complete), install CLN
7. **Clean Flow Cell and Sample Line with MQ DiH20**
   a. Clean Flow Cell (Cytometer > Cleaning modes) with 3ml of MQ H2O (no need to let sit)
   b. Take out the CLN; start the stream
   c. RUN tube of MQ H2O at Flow Rate 11 for 5 minutes
8. **Check CS&T bead profiles using FCCF's QC template followed by running CS&T in Diva:** See Fusion Start Up (Step 11). Export PDF of beads and report to send fccf@salk.edu.

If the instrument is dirty and fails CS&T, repeat cleaning steps 4-8 while you contact core staff as we may coordinate with you to perform an extended Flow Cell soak.

9. **For Regular shutdown (only if directed; default is FULL ETHANOL SHUTDOWN), if CS&T passed and Fusion looks clean:**
   a. Unload beads and backflush sample line (Cytometer > Cleaning modes) 2min.
   b. Repeat (6) to fill Flow Cell with 1.5% Citranox for storage.
   c. Continue at Step 12
10. **For Full Ethanol Shutdown through the Sheath Tank (default procedure), if CS&T passed and Fusion looks clean:**
    a. Unload beads and backflush sample line (Cytometer > Cleaning modes) 2 min.
    b. Turn off stream (wait for fluidics action to be complete), install CLN
    c. **Remove Sheath Tank and empty:** Release (i) Sheath sensor: plastic connector pulls straight out from drawer wall panel, (ii) Sheath tubing: Metal connector/blue tubing, (ii) Air Line: Metal connector/clear tubing.
    d. **Add ~6-inch depth 70% Ethanol** (for level sensor to read): from labelled metal tank on floor by ShadowFACS
    e. **Install and Re-connect tank:** Sheath tubing, Air Line, and Sheath sensor
    f. **Perform Fluidics Shutdown** (Cytometer Menu> Fluidics Shutdown: follow prompts within window)
       i. Load a tube of 70% Ethanol when it prompted to load cleaning solution
       ii. Leave a "Shutdown in Ethanol" sticky note on the top of the sheath tank
    g. Continue at Step 12
11. **For Ethanol Shutdown with Small Metal Tank (only if directed by staff), if CS&T passed and Fusion looks clean:**
    a. Unload beads and backflush sample line (Cytometer > Cleaning modes) 2 min.
    b. Turn off stream (wait for fluidics action to be complete), install CLN
    c. **Fill Small Metal Ethanol Tank** (located in Fluidics Drawer) to at least 4-inch depth 70% Ethanol
    d. **Move connections from Sheath tank to Small Metal Tank:** (i) Sheath tubing, (ii) Air Line
    e. Perform Fluidics Shutdown (Cytometer Menu> Fluidics Shutdown: follow prompts within window)
       i. Load a tube of 70% Ethanol when it prompted to load cleaning solution
    f. Continue at Step 12
12. **Transfer your data to your folder on filer** and delete data from DIVA software
13. **Turn off/Close:** Aria Fusion, water bath, compressor, BSC, AMS, BSC fan, DIVA, Tera Term, Coherent Connection
14. **Empty Waste** into sink > Add 1L bleach to waste container and reinstall
15. **Send us pictures/files to** fccf@salk.edu
    a. **Photo of Diva browser** with cleaning files (5min cleaning every 30min of use)
    b. **CS&T PDFs and reports** before and after using
    c. **BSC closed with light OFF**, showing **AMS OFF** (blue LED) and **BSC Fan OFF** (FAN LED light: FLASHING is off!)
    d. **Water bath OFF**

e. **Compressor OFF**
f. **Sorter OFF** (Green power button OFF)