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Stem Cell Instrumentation Foundry

SOP for FACS Aria Instrument Setup

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A 12,000-volt potential exists between the deflection plates when they are on. Contact with the charged plates results in serious electrical shock. Do not touch the deflection plates when the voltage warning light is illuminated, or when the software indicates that the plate voltage is on. The plates remain energized even when the sort block door is open.

⚠ ☢

Lasers emit intense, coherent electromagnetic radiation that can cause irreparable damage to skin and eyes. To prevent retinal burns and possible blindness, do not remove laser shielding, adjust laser controls, or attempt to service the cytometer any place where laser warning labels are attached.

⚠ ☣

Cell sorters can produce aerosols around the sample stream. When acquiring biohazardous samples, use the auxiliary Air Management Option (AMO) at all times and refer to our BSL-2 sorting protocol.

**This document is a modified version of the SOP from the Garvan Flow Cytometry Facility:

PREFACE

This guide is intended for advanced users who have prior Flow Cytometry experience. You should be proficient in using a flow analyzer, have attended the mandatory sorter theory training session, the hands-on FACS Aria training, and have written authorization from the SCIF that you are eligible to use FACS Aria sorters outside of business hours. This procedure is also demonstrated in a video on our YouTube channel:

https://www.youtube.com/channel/UCDeleBdiFFJ7DQ1TZw9z8eA

This guide will take you through turning on, setting up and shutting down a cytometer. This guide does not describe how to run an experiment. The startup steps make reference to other processes (in **bold** type) that are further expanded upon in subsequent pages. *Italicized* words indicate a button or specific action in FACSDiva software. Red letters refer to the images at the end of this document.

During business hours the SCIF staff will perform the entire setup process for users. Users choosing to operate the instruments outside of business hours are subject to inherent risk to your sample and

experiment. While the UC Merced SCIF will endeavor to help you should you need it, this may not always be possible.

If you need help running your experiment, or experience technical difficulties with the BD FACSAria, contact David Gravano at (209) 228-4039 (office) or (209) 285-5726 (cell phone). Text is preferred outside of business hours.

CONTENTS

1. START UP

Note. If you are the first user of the day during business hours, all steps will already be completed for you. If you are using the instrument outside of business hours If you need to change nozzles or experience a major blockage, contact staff during business hours or start from step 1.

1) Empty waste (A) down the lab sink if full. Decant 1L 100% bleach into the empty waste container.

2) Refill sheath tank (B) with 1x PBS to the top 'fill line' (Note. Use the autoclaved 1x PBS prepared especially for this instrument. DO NOT use the NERL Diluent Sheath Fluid that is used for the LSR2). Ensure air and fluid lines are connected to tank.

3) Switch on the computer and log in to Windows using the Admin account and password "BDIS". Wait for Windows to load until the network icon appears in the lower right system taskbar. Turn on the compressed air line (orange valve on the wall). Then turn on the instrument (C), AMO (Aria III only) and circulating water bath.

4) Open the program *BD FACSDiva* and log in using your username and password. Wait for the cytometer to connect.

5) From the dropdown menu, select *Cytometer>Fluidics startup,* follow the prompts.

6) Note the nozzle size needed. Select the appropriate nozzle, and follow the steps in **Installing the Nozzle** to ensure the configuration is correct.

7) Bleed sheath line filter (D) of air if any bubbles are seen in the top of the filter (this is usually not necessary). Turn on the stream (red *X* on the stream window) (E). Allow 3-5 minutes for the stream to stabilize. Adjust the Amplitude so that the break-off point (S) is on screen (Drop 1 should be ~150-300), and the *Gap* is stable: 70um ~6-8; 100um ~10-14. It is recommended to adjust the amplitude to get the actual Drop 1 value as close as possible to the previous Drop 1 set value.

The standard, pre-optimized frequency value for each nozzle is indicated on the monitor. It is not recommended to deviate from this. If you want to inspect/change the frequency node, see **Finding**

the Frequency Node. A good check for stream stability is that side streams (F) are clean and stable (See **Setting Side Stream Trajectory).**

8) Run *CST* (see **Running CST**).

9) Turn the *Sweet Spot* (G) on. Sweet spot adjust the amplitude automatically to maintain a stable gap.

10) Set the drop delay (see **Setting the Drop Delay**).

11) Set the side stream (F) trajectory (See **Setting Side Stream Trajectory**).

12) Run 10% bleach for 2 minutes, followed by DI H20 for 1 minute.

Once you have finished your sort, you should check the calendar booking system to see if you are the last user of the day. If there is someone after you, complete the **Final Clean.** If you are the last user of the day, complete the **Shut Down** procedure.

2. FINAL CLEAN

1) Run 10% bleach for 5 minutes on a flow rate of 11.

2) Run DI H_2O for 5 minutes and turn off the stream.

3) Log out of *BD FACSDiva* and notify the next user that they are responsible for shutdown. If you are the last user of the day, complete Shut Down.

3. SHUT DOWN

1) With the stream off, remove the nozzle and place it in the storage tube with sterile diH2O. Install the closed loop nozzle.

2) Load a tube with at least 4ml of 10% bleach. Select *Cytometer>Cleaning Modes>Clean Flow Cell* from the menu. Repeat one time.

3) Load a tube with at least 4ml of FACS Rinse. Select *Cytometer>Cleaning Modes>Clean Flow Cell* from the menu. Repeat one time.

4) Load a tube with at least 4ml of sterile diH2O. Select *Cytometer>Cleaning Modes>Clean Flow Cell* from the menu. Repeat one time.

5) Export your data.

6) Quit *BD FACSDiva*, turn off the cytometer (C), computer and AMO. De-pressurize the sheath tank (B). Turn off the air valve on the wall.

4. INSTALLING THE NOZZLE

The BD FACSAria offers 4 interchangeable nozzle sizes: 70um, 85um, 100um and 130um. There is also a closed loop nozzle used for cleaning. We only commonly use the 70um and 100um nozzles.

Choice of nozzle size will vary depending on:

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-The size of the particle (cell) being sorted: Particles must be no bigger than 1/5 the diameter of the nozzle. Keep in mind that you may be passing very large particles through the cytometer, even though you are not interested in them.

-The type of sort: A plate sort should be performed using a 100um nozzle. A bulk sort can use any nozzle size.

-The amount of sample being sorted: Larger nozzles operate using lower frequencies, which means less droplets/sec, thus less sample throughput over time.

Note. Sample size and concentration is usually prepared/adjusted to accommodate time restrictions associated with a nozzle selection based on the above parameters.

Note. It is important that the stream be set up correctly so that the sweet spot function can act effectively (ie Appropriate installation of a clean nozzle, appropriate frequency and amplitude settings).

Process:
1) Place the nozzle in a capped 5ml tube containing DI H_2O. Place this tube in a water bath sonicator for 1-5 minutes. If necessary, view the orifice of the nozzle under 10x zoom on the dissecting microscope to visually verify there are no obstructions to the orifice. Also ensure that the orange o-ring looks OK (eg. no cracks).
2) Remove the previous nozzle from the instrument and store it in the appropriate 5ml FACS tube containing about 2ml of DI H_2O.
3) Before turning the stream on (in *BD FACSDiva*), open from the dropdown menu *Cytometer>View Configurations*, and select the configuration that corresponds to the nozzle you have prepared. Click *Set configuration*, *OK* in the dialogue box that appears (assuming the PMT configuration has not changed), and then *OK* in the CST module. Exit the module.
5) CST must be re-run with each nozzle change. Each nozzle also runs at a different pressure and frequency.

5. FINDING THE FREQUENCY NODE

Droplet creation is controlled by vibrating the stream using a piezo electric transducer. The frequency of this vibration can be changed, and there is an optimal frequency (or frequency node) giving maximum stability for each different nozzle size, sheath pressure etc (overall system). The frequency node for each nozzle/pressure is quite stable and the frequency value has been previously optimized. It is not recommended to change this.

The frequency node is defined as a point in which the direction of the break-off (S) movement changes when changes in frequency are applied in a constant direction. At this frequency, any slight changes to the overall system (thus optimal frequency node) will hopefully minimally affect the break-off position thus optimal drop-delay.

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1) Adjust the frequency up in increments of 0.1 MHz. The break-off (S) should soon move to the drop above. Continue adjusting the frequency up until the break-off moves to the drop below (you may need to adjust amplitude to keep the break-off on-screen). This is roughly the position of a node.

2) Adjust the frequency back down in increments of 0.1 MHz until the break-off returns the drop just before it changed direction. Note this as frequency *A*. Adjust the frequency down in increments of 0.1 MHz until the break-off point moves to the drop below it. Note this as frequency *B*.

3) Find the midpoint of frequencies *A* and *B* and apply this number to the frequency of the stream.

4) Repeat 1-4 for multiple frequency nodes. Find the drop where the incremental change between the drop moving up and the drop moving down is the largest. Set the frequency to this node.

6. RUNNING CST

CST (Cytometer Setup and Tracking) is a module that runs within *BD FACSDiva*. As the name suggests, it is used to set-up certain components of the system (eg. determine laser delays), and to track these parameters over time. The module must be run using *CST beads*, from the correct bead lot. With every new bead lot, a *baseline* is first established, and a *performance check* is subsequently run (at least) daily.

1) Vortex and invert *CST* vial several times for ~10 seconds. Make up bead solution as 2 drops in 0.5 ml 1x PBS (existing bead solutions may be OK to use in made in past 5 days).
2) Open *Cytometer>CST* from the drop down menu. Select appropriate bead lot. If you cannot find the correct bead lot, contact SCIF staff.
3) Load beads on to the loading port and select *Check Performance*, press *Run* and follow the prompts thereafter. The performance check process takes around 5 minutes.
4) Assuming the performance check finishes (either passed or failed), it is important to review the report provided. By reviewing the *Performance Tracking*, we can both monitor the trends of the cytometer settings over time, and also check that the laser delays have been set. If the CST program does not finish, try the following troubleshooting steps:
a) Re-run the *CST* module using a fresh bead solution.
b) Restart the cytometer and computer.
c) Re-sonicate and reseat the nozzle.
d) Contact SCIF Flow staff.

5) If *CST* does not pass, judge whether or not to use the cytometer (SCIF may have insight into this). Restarting the whole system may help to pass *CST*.
6) Exit the *CST* module. *Use CST settings* in the dialogue box that appears. Remember to re-apply *Sweet Spot* (G).

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7. SETTING THE DROP DELAY

The Cytometer knows the precise moment to charge the stream, thus deflecting a specific droplet containing a particle of interest, based on the drop delay. The drop delay is the period of time it takes for a segment of stream to move from the laser interrogation point to the droplet break-off (S).

1) Make up *Accudrop* bead solution at around 1 drop/0.5ml PBS (existing bead solution may be OK to use if generated in past 5 days).

2) Open the 70um or 100um Accudrop delay experiment template, depending on which nozzle you're using. Set the inner left side stream (F) trajectory (see **Setting Side Stream Trajectory**) so that, when the *optical filter* (K) is applied, the inner left side stream is in the center of the box that appears.

3) Adjust the *Accudrop laser* (small silver dial to the left of the sort chamber) (L) so that the side stream is at its brightest (do this when the *optical filter* is not applied).

4) Run *Accudrop beads* at a threshold of:

<1500 events/sec for 100um nozzle

<3000 events/sec for 70um nozzle

On a FSC/SSC plot, place a small gate away from the bead population. It should have no events in it. Right click the gate and make an inverse gate called "Not P1".

5) Create a sort layout where the inner left sort population is the "Not P1" population. *Sort.*

At this point, you may continue either by using the automatic drop delay calculator, or calculating the drop delay manually (the automatic function is much easier). If you wish to use the automatic function, simply press *Auto Delay* (M) and follow the prompts thereafter.

The automatic drop delay calculator is often more time consuming and uses more beads then setting the drop delay manually. If you wish to set the drop delay manually, continue from step 6.

6) Select *initial* sort precision. Adjust the drop delay (N) until the left side stream reads *100.* Note, the drop delay value is quite stable if the same frequency, pressure, and nozzle combination is used. If you are finding that the drop delay is substantially off from past use (whole number differences), this is a red flag and staff should be contacted.

7) Select *fine tune* sort precision. Adjust the drop delay by increments of .03 using the arrows until the left side stream is at its highest. This should consistently be greater than 97%. It is helpful to overshoot and undershoot the drop delay value so you can find the exact midpoint where the drop delay is deflecting the highest percentage of beads. Around the optimal point, find values where only 90% of the beads are correctly sorted and take the midpoint of those for the optimal value.

8) Take note of the drop delay (this is handy for quick reference/peace of mind).

8. SETTING THE SIDE STREAM TRAJECTORY

Collection apparatus will vary depending on the type of sort (ie. bulk vs. plate), the number of populations to be collected, and also the desired collection tube type. 4x 5ml collection tubes can be housed simultaneously, as can 2x 15ml tubes, and 1x plate (Set up for a plate sort requires additional training).

Bulk sort:

1) Note how many populations are to be collected and/or collection tube type, and choose collection device accordingly. You can sort fewer populations than a device can accommodate if desired.
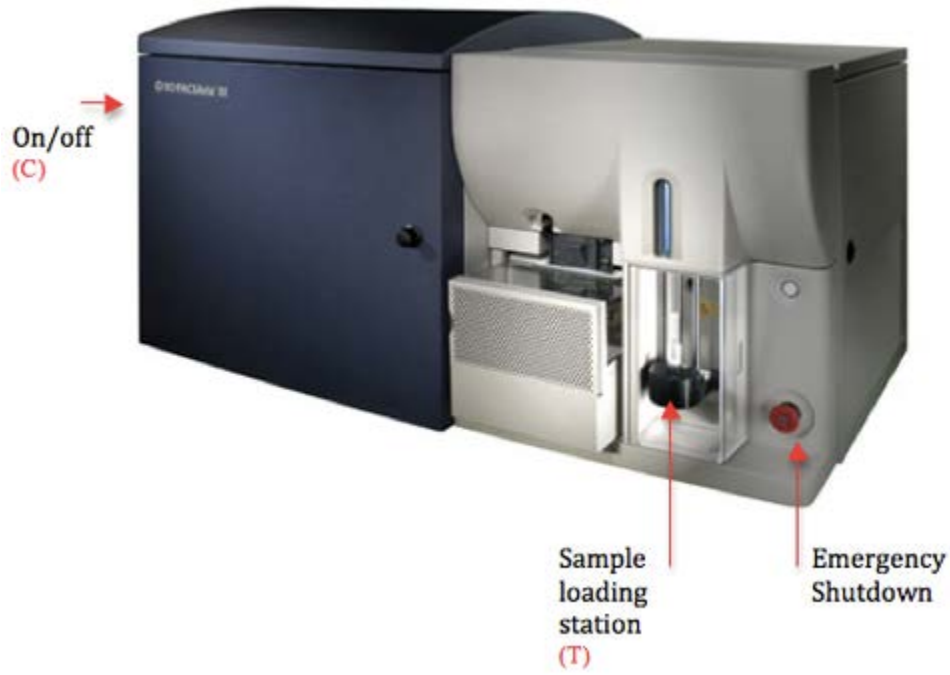
2) Fill the collection device with blank dummy collection tubes and slide into place.

3) Press *voltage* (O) and *test sort* (P). Open the *waste drawer* (Q), and open the front of the sort chamber.

4) Move the side stream charge sliders (R) out to a point where the side stream is targeted to the bottom of its corresponding collection tube (verify this by looking down through the sort chamber to the collection tubes below). Only set up those side streams you are planning to use. If side streams/ center stream are not tight (fanning), ensure the gap value is appropriate for the nozzle chosen. The drop1, drop2, and drop3 values may be adjusted to minimize the degree of fanning, but this is usually not required.
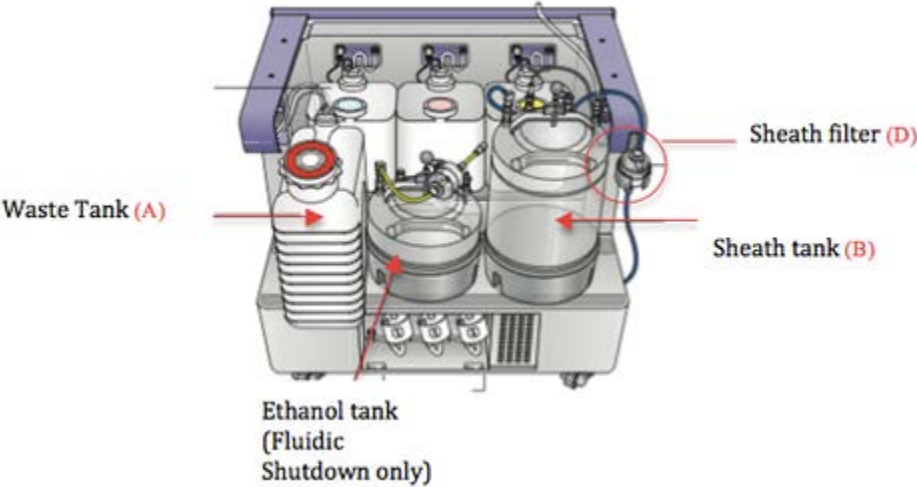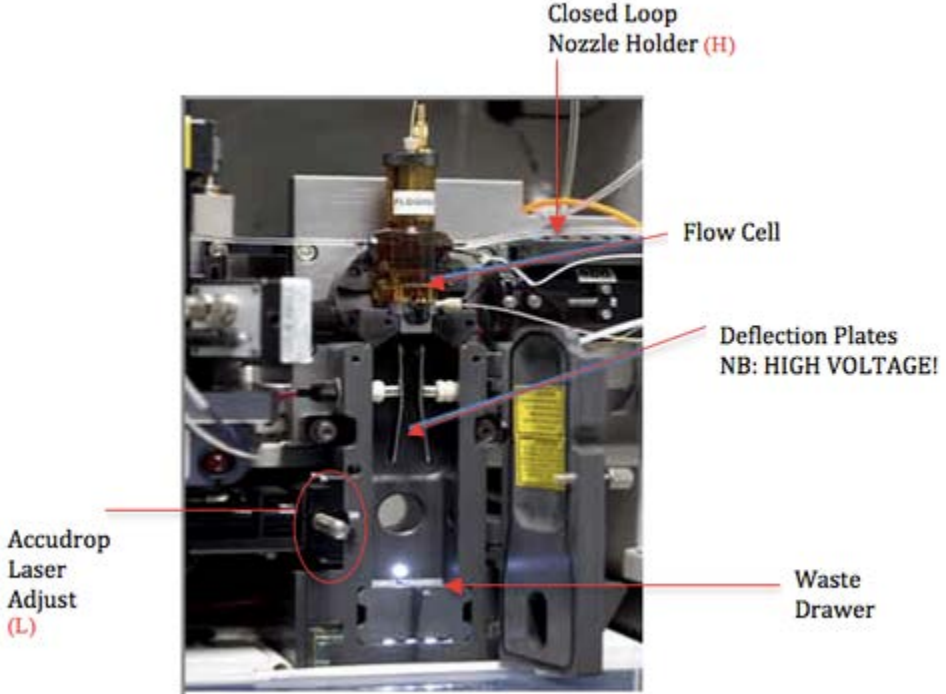
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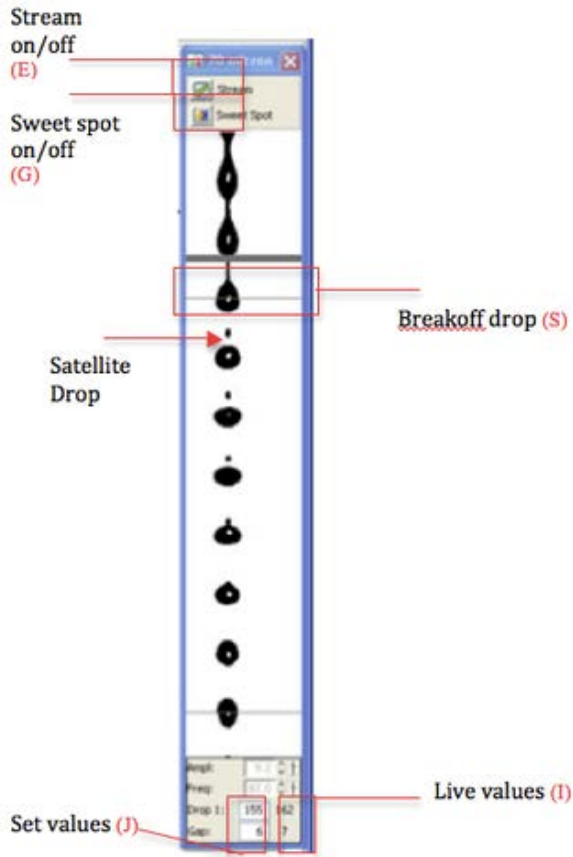
9. APPENDIX - IMAGES

On/off (C)

Sample loading station (T)

Emergency Shutdown

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Closed Loop
Nozzle Holder (H)

Flow Cell

Deflection Plates
NB: HIGH VOLTAGE!

Accudrop
Laser
Adjust
(L)

Waste
Drawer

Sheath filter (D)

Waste Tank (A)

Sheath tank (B)

Ethanol tank
(Fluidic
Shutdown only)

Stream on/off (E)

Sweet spot on/off (G)

Breakoff drop (S)

Satellite Drop

Live values (I)

Set values (J)

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