UCMERCED
Stem Cell Instrumentation Foundry

BD FACS Aria Start-up Guide and SOP

START-UP GUIDE AND SOP

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 samples, use the auxiliary Air Management Option (AMO) at all times. Keep the sort block door closed during sorting. If you need to access the sort block and you are working with highly infectious samples, consider turning off the stream before opening the sort block door.

**This document is a modified version of the SOP found on the Garvan Flow Cytometry Facility website:
http://flow.garvan.org.au/flowcytometryinformation/bd-facs-aria-start-guide-and-sop

PREFACE

This guide is intended for users who have prior Flow Cytometry experience. You should have attended the compulsory sorter theory training sessions, and well as have practical experience operating an analyzing cytometer.

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.

Page 4 of this document offers a general step-by-step overview of the start-up process. These 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.

The BD FACSAria may be autonomously run by appropriately trained users. If in this instance you are the first user of the day, the stream should already be turned on with the appropriate nozzle size, QC completed and the frequency node set. All other aspects of operation are intended to be run by users. With this independence comes an 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 x4508 (office), (559) 240-6492 (cell phone) or Aron Phong at x4039 (office), (916) 388-4712 (cell phone).

CONTENTS

START UP

Note. If you are the first user of the day, steps 1-9 will already be completed for you. If you need to change nozzles or experience a major blockage, 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˚C, Aria III only).

4) Log in to windows (password *BDIS*). Open the program *BD FACSDiva*, log in using your username and password[1]. 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. Turn on the stream (red *X* on the stream window) (E). Allow 3-5 minutes for the stream to settle. Adjust the Amplitude so that the break-off point (S) is on screen (Drop 1 should be ~100-300), and the *Gap* is stable: 70um ~6-10;   100um ~10-18

The frequency node should be appropriate at the previously set frequency. 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.

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.

FINAL CLEAN

1) Run 10% bleach for 5 minutes
2) Run DI H_2O for 5 minutes
3) Log out of your username in *BD FACSDiva*

SHUT DOWN

1) Run 10% bleach for 5 minutes
2) Run DI H_2O for 5 minutes
3) Turn the stream off (green *tick* on the Stream Window) (E)
4) Remove the nozzle and place it into its allocated 5ml tube containing fresh DI H_2O. Install the closed loop nozzle. With ~4ml of DI H_2O on the sample loading station (T), select*Cytometer>Cleaning Modes>Flow Cell Clean* from the drop down menu. Repeat.

5) Export your data.
6) Quit *BD FACSDiva*, turn off the cytometer (C), computer and AMO. De-pressurise the sheath tank (B).

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:

-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 piezo 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 waterbath sonicator for 1-5 minutes. View the orifice of the nozzle under 10x zoom 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[2].
3) Integrated O-ring nozzle: -Insert the nozzle.
Non-Integrated O-ring nozzle: -Place O-ring on the nozzle (used O-rings may be OK to use) before inserting
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.
4) Bleed sheath line filter (D) of air. Turn on the stream (red *X* on the stream window) (E). Allow 3-5 minutes for the stream to settle. Adjust the Amplitude so that the break-off point (S) is on screen (Drop 1 should be ~100-300), and the *Gap* is stable: 70um ~6-10; 100um ~10-18
The frequency node should be appropriate at the previously set frequency. 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).**

4) Enter the *Gap* and *Drop 1* values on the right side (live values) (I) into the corresponding text box on the left side (set values) (J). Turn the *Sweet Spot* (G) on.[3]

5) CST should be re-run with each nozzle change.

FINDING THE FREQUENCY NODE

Droplet creation is controlled by vibrating the stream using a piezo crystal. 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 of the overall system may be subtly different from day to day. Finding the frequency node is notoriously difficult to understand and accomplish.

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.

1) Adjust the frequency up in increments of 0.1 MHz[4]. 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[5].

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 median 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.

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[6]. 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 ~30 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 2 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).

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 2 drops/0.5ml PBS (existing bead solution may be OK to use if generated in past week).

2) Open the 70um or 100um Accudrop delay experiment, 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[7].

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*[8].

7) Select *fine tune* sort precision. Adjust the drop delay until the left side stream is at its highest.

8) Select the *sort precision* you are planning to use for your sort[9]. Adjust the drop delay until the left side stream is at its highest, then

a) Adjust the drop delay up one whole integer, and note the number the left side stream reads.

b) Adjust the drop delay down 2 whole integers (now 1 integer below the central number). Note the number the left side stream reads.

c) Adjust the drop delay such that when toggling both 1 integer up and down around the central number, the left side stream reads the same number.

d) Repeat a) – c), this time using a smaller increment (eg. 0.5)[10].

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

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 (NB. 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 housing accordingly. You can sort fewer populations than a housing can accommodate if desired.

2) Fill the housing 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.

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[1]Your UCM net ID is usually designated as your Diva username. Password is your choice.

[2]If the Closed Loop Nozzle is in place, store it in the nozzle holder (H) to the right of the sort chamber.

[3]The values on the right side are the live values of the G*ap/Drop 1*.

The sweet spot function will automatically make adjustments to the amplitude, endeavoring to match the live values to the set values that you entered on the left side. By entering the live values observed when the stream is optimal, sweet spot will therefore endeavor to keep the stream optimal. Further, any settings made that are dependant on break-off position (eg. Drop delay) depend on break-off position consistency, thus it is important that sweet spot be on, set to a point where the stream behaves well.

[4]The up/down arrows of the *frequency* field will adjust the frequency by 0.1 MHz

[5]One node of potentially several

[6]The bead lot is displayed on the bead vial

[7]When the *optical filter* is applied, you will not be able to see the side stream. Turn the filter on and off again to approximate the position of the side stream within it.

[8]If you cannot attain 100, try adjusting the *Accudrop laser.*

[9]For most 1 or 2-way applications, this will be *purity*. For most 3 or 4-way applications, this will be *4-way.*For a plate sort, this will be *Single Cell.*

[10]The goal here is to accurately find the point where the most beads (highest left side stream number) are deflected. This implies that the drop delay reflects the time is takes for a segment of stream to move from the laser interrogation point to the droplet break-off (S).

APPENDIX - IMAGES

On/off
(C)

Sample
loading
station
(T)

Emergency
Shutdown

Closed Loop
Nozzle Holder (H)

Flow Cell

Deflection Plates
NB: HIGH VOLTAGE!

Accudrop
Laser
Adjust
(L)

Waste
Drawer

Waste Tank (A)

Sheath filter (D)

Sheath tank (B)

Ethanol tank
(Fluidic
Shutdown only)

Stream
on/off
(E)

Sweet spot
on/off
(G)

Stream

Sweet Spot

Breakoff drop (S)

Satellite
Drop

length

Freq

Drop 1: 155   162

Gap: 6   7

Live values (I)

Set values (J)

Deflection
plate voltage
On/off
(O)

Test sort
On/off
(P)

Optical filter
On/off (K)

Waste drawer
Open/Close
(Q)

Drop delay
indicator
(N)

Automatic
drop delay
Calculator
(M)

70 micron

Voltage   Test Sort   Optical Filter   Attenuation   Waste Drawer   Drop Delay: 42.83   Auto Delay

Side
Stream
(F)

Centre
Stream

0   20   19   0

Voltage Center: Plate Voltage: 6,000

2nd Drop: 19   3rd Drop: 10   4th Drop: 0   Phase:

Side Stream
Charge sliders
(R)