Aria I/II/III Troubleshooting Guide

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Most common ERRORS:

CST Failed

Problem: Failed due to Blue FSC PMTV

Solution:

- Check neutral density filter is out (on Arial and II), or that 1.0 is in (on AriaIII)
- If the problem is still not fixed, then likely the problem is a dirty flow cell.
  - Put in the closed loop nozzle
  - Select Cytometer-> Cleaning Modes-> Clean Flow Cell with hot 100% contrad on the loader.
  - Set a timer for 5 minutes
  - After 5 minutes is up, put diH2O on the loader and perform a clean flow cell with diH2O.
  - Remove closed loop nozzle and dry where nozzle sits and replace with your nozzle
  - Start stream and try CST again
  - Alternate cleaning with Coulter Clenz.

Problem: Failed due to one or more laser(s) and detector(s) (or one detector on just one laser-not Blue FSC PMTV)

Solution:

- Check the lasers are on in the software (on Arial and II)
- Check main laser button is turned on (side of instrument by power button Arial and II)
- Check the individual laser buttons are turned on (side of instrument by power button AriaIII), then check that the switch for the NearUV/Violet laser is switched to correct configuration setting (CAGT AriaIII)
- Restart stream.
- If this does not fix the problem, remake beads
- If the problem is still not fixed, then likely the problem is a dirty flow cell.
  - Put in the closed loop nozzle
  - Select Cytometer-> Cleaning Modes-> Clean Flow Cell with hot 100% contrad on the loader.
  - Set a timer for 5 minutes
  - After 5 minutes is up, put diH2O on the loader and perform a clean flow cell with diH2O.
  - Remove closed loop nozzle and dry where nozzle sits and replace with your nozzle
  - Start stream and try CST again
  - Alternate cleaning with Coulter Clenz.

Core members:

- *Speak with a BD engineer before performing this step: Take a small head q-tip, break it to just the head of the q-tip, and (with hemostats) very carefully wipe the left- and right-hand sides of the flow cell. Be sure to try and touch all surface area*

CST Setup Failed

Problem: CST did not finish, but instead unloaded and only lists the results of the last time CST ran and finished.

Solution:

- Check cytometer lid is down
- Check that neutral density filter is out for Arial and II, or that the 1.0 is in for the CAGT AriaIII
- Restart stream.
- check that the stream was still in drop form when it was running CST. If it had lost its break-off, stop stream, take out nozzle and dry where it sits, allow nozzle to dry, restart stream, and try CST again.
- If this does not fix the problem:
  - close CST software window
  - In the regular level of software (where you run experiments) open an experiment
  - load the CST beads and look for the beads on the FSC and SSC plot
  - when you find the beads, change the voltage of FSC and SSC, and press record
  - unload the tube and now try CST again
  - If you could not find the beads, load 100% hot contrad and watch for clog clearance (upper lid must be down to see events)- see clog procedure- Sip-line clog for more details
- If you can see beads in an experiment but CST will still not see any events, restart both the computer and the cytometer.

Core members:

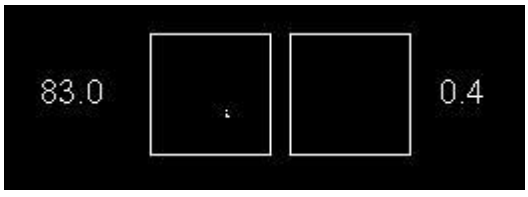
- *Speak with a BD engineer before performing this step: If you can find the beads but they did not look like tight, round populations, take a small head q-tip, break it to just the head of the q-tip, and (with hemostats) very carefully wipe the left- and right-hand sides of the flow cell. Be sure to try and touch all surface area. Now load the beads again and check to see if they are more round. If not, repeat until they are round, using a diH2O dampened q-tip if necessary. After dampened q-tip, wipe outside again with a dry q-tip. Try CST again. Can alternate dampened q-tip with a Zeiss Wipe.*

Accudrop Issues

Problem: Optical Filter values don't add up to 100%

Solution:

- Check brightness has been optimized for the left and waste streams
- Also check that the threshold rate is above 3,000 events/sec (don't go above flowrate 3.0). May need to be way above 3,000 events per second



Problem: Can't get a left (sorted) value higher than 50-80%

Solution:

- Check that precision mode is set to fine tune in sort layout
- Ensure the left stream is falling into the left optical filter box (adjust if necessary)
- Ensure that the % of the gate of population you are sorting is near 100% (FSC vs. SSC plot)
- Use Auto Delay

Other Problems

Problem: Clog

Solution:

- See Clog Sheet (in binder)

Problem: Wrong Merging Pattern for Stream

Solution:

- Check frequency is correct.
- Turn off stream, take out nozzle and key, dry where they sit (and plates and upper area of sort block chamber), and then put key back in, place nozzle back in, and start stream again.
- Sonicate nozzle (try in water 1st with no basket, if that doesn't fix it, try in a tube with coulter clenz, and if it still is not fixed, then in a tube with not heated 100% contrad)

Problem: Software Freeze

Solution:

- Press and hold Ctrl+Alt+Delete, choose Task Manager, Click Applications Tab, Highlight BD FACSDiVa software and choose End Task. Close the black pop-up window (DOS command prompt). Restart software.

Problem: No stream

Solution:

- Check that the air handle on the wall (red behind or to right of instrument) is ON (parallel to floor)
- Check that air and fluid lines are connected to sheath tank.
- Check that the closed loop nozzle is not in the flow cell (ensure the correct nozzle is and matches your configuration).
- If these are not the issue, take out nozzle and dry it and the area where it sits. Put nozzle back in and turn on stream.
- If it still has no stream, see clog sheet for nozzle clogs.

Problem: Unstable stream

Solution:

- With the stream on, click Cytometer-> Sheath Pressure and change the pressure to 70 psi. Wait 5 minutes. During this wait, check the sheath bubble filter for bubbles. Purge if needed. After the 5 minutes, change the pressure back to the original pressure for your nozzle (130um=12psi, 100um=20psi, 85um=45psi).

- If this does not improve stability, stop the stream, take the nozzle out and sonicate (see clog procedure for details of how to sonicate). After sonication, allow nozzle to dry and then place nozzle in the flow cell and restart stream.
- Refill sheath tank (if it was less than ½ full, it could contribute to instability)

Core members:

- o-ring on nozzle may not be able to seal properly (need to replace nozzle)

Problem: Not all lasers show in laser software

Solution:

- Restart the laser software
- If on the LSRFortessa, try unplugging and re-plugging in the black USB on the right side of the instrument (about eye level height), labeled "Laser".

Core members:

- *If still not all lasers are showing, call Dan or Ryan for suggestions*
- *If needed, put in BD Service call.*

Problem: When trying to have >2 side streams, only get 1

Solution:

- Restart DiVa. This is a known bug in DiVa. What happens is, someone before you has sorted a plate, and the software will now only allow the far-left stream to be active until the software has been restarted.

Problem: No signal

Solution:

- Check the upper lid is down
- If on the AriaI: It is possible that the left hinge on upper lid has become partially detached/unscrewed and is preventing the lasers from being un-shuttered
- Remove neutral density filter if one is inserted and check for signal again
- If still no signal, restart the stream
- If still no signal, put in closed loop nozzle
- Perform a cytometer->cleaning modes-> Clean Flow Cell with diH2O. During the clean flow cell, observe if any volume is taken from the diH2O tube
- If volume is taken, look at sample again.
- If volume is not taken, see clog procedure, SIP-line clogs for directions
- If still no signal, restart both cytometer and computer

Core members:

- If still no signal, *call Dan or Ryan for suggestions*
- *If needed, put in BD Service call. Put instrument out of use (shut it down, adjust iLab calendar, and alert users who are affected and help them reschedule)*

Problem: User data fluorescence changed when flowrate was increased

Solution:

There could be many culprits (window extension, software, sample clumping/too concentrated)

- Try changing window extension
    - Try different window extension values in the range of 1.0-25.0, changing it, waiting a moment for it to stabilize, and then changing the flowrate to see if the fluorescence still changes
- To check if it's the DiVa software
    - Restart computer and cytometer
    - Recreate experiment
    - If problem persists, proceed to next step
- Sample clumping/too concentrated
    - Aliquot out a bit of sample and dilute it and filter it.
    - Check to see if the fluorescence is still changing after flowrate adjustment

Problem: Plate errors (x and y)

Solution:

- Re-check Home position
- Adjust if it has changed
- Continue sorting, and report to core after appointment

Core members:

- After appointment is over, *call Dan or Ryan for suggestions*
- *If needed, put in BD Service call.*

Core members ONLY:

Problem: CST sets UV or Violet laser delay to 0

Solution:

Easy way

- Run baseline
- Run CST performance check
- Turn off computer and cytometer
- Turn on
- Turn on OPSL/Coherent
- Turn on UV
- Close OPSL/Coherent
- Turn on DiVa
- Run CST performance check

Hard Way

- Delete the config
- Create new config
- Baseline

Stubborn Way

- Find in the CST results the last time the CST passed and write down the UV laser delay from that successful CST run

- Load CST beads in an experiment that has the UV detectors open and enter the UV laser delay you wrote down
- Adjust delay manually until you can see signal in the UV plots
- Take a small head q-tip, break it to just the head of the q-tip, and (with hemostats) very carefully wipe the left- and right-hand sides of the flow cell. Be sure to try and touch all surface area. Now load the beads again and check to see if they are round and tight and if you can see signal in the UV channel. If not, repeat until they are round, using a diH2O dampened q-tip if necessary. After dampened q-tip, wipe outside again with a dry q-tip. Can alternate dampened q-tip with a Zeiss Wipe.
- Try CST again. If CST checks the first 2 of the 4 checkmarks and there is no UV signal on the UV CST plot, abort CST and repeat steps 2-4 until CST recognizes UV signal. This may take several iterations, so be prepared.

Problem: CST fails with a negative PMTV value

Solution:

- Check to see whether the instrument has recently had service. Contact the engineer and report results to ask if we should re-baseline

Problem: Closed Loop Nozzle Leaking

Solution:

- If the closed loop is spraying liquid, see "**Closed Loop Nozzle sprays liquid upon removal from flow cell"**
- If the closed loop is not spraying liquid, but is wet, either the closed loop is clogged, a line of the closed loop is clogged, or the o-ring on the closed loop is damaged.
    - Remove closed loop nozzle from the line it is attached to by unscrewing the nut peek fitting (tan piece). A white ferrule should be in the closed loop in the spot where the fitting unscrewed from. It seems to not be able to be removed like other ferrules, so leave it in place. Be careful to not lose the nut peek fitting.
    - Sonicate closed loop nozzle in sonicator water with no basket (o-ring side up)
    - Dry the area where the nozzles go in the flow cell, as well as the key and where it sits and the plates and sort chamber
    - Dry nozzle without touching the o-ring
    - Reconnect the line to the closed loop nozzle by pushing the line to have tension against the spot where it goes and at the same time tightening the nut peek fitting back in place. When this is done properly, the line should stay connected to the closed loop nozzle.
    - Re-insert the closed loop nozzle and do a clean flow cell or fluidics startup.
    - Remove closed loop nozzle and determine if it is still leaking (is the closed loop nozzle and the area where it sits wet?)
    - If no, you have solved this issue. If yes, proceed to next step.
- If the closed loop is still wet, try replacing the sip line part of the closed loop.
    - Remove closed loop nozzle from the line it is attached to by unscrewing the nut peek fitting (tan piece). A white ferrule should be in the closed loop in the spot where the fitting unscrewed from. It seems to not be able to be removed like other ferrules, so leave it in place.
    - Unscrew nut peek fitting on other side of tan sip-line (a ferrule may come out; it can be discarded). If the ferrule does not come out, you will need to remove it

with the ferrule removal tool. The tan sip-line piece can also be discarded (or you can try sonicating it).
    - Obtain new sip-line of approximately the same length and re-connect it by pushing the line to have tension against the spot where it goes and at the same time tightening the nut peek fitting back in place. Do this for both ends of the sip-line (you will need a new ferrule for the non-closed loop side of the sip-line). When this is done properly, the line should stay connected to the closed loop nozzle and the other connector.
    - Dry the area where the nozzles go in the flow cell, as well as the key and where it sits and the plates and sort chamber
    - Dry nozzle without touching the o-ring
    - Re-insert the closed loop nozzle and do a clean flow cell or fluidics startup.
    - Remove closed loop nozzle and determine if it is still leaking (is the closed loop nozzle and the area where it sits wet?)
    - If no, you have solved this issue. If yes, proceed to next step.
- If the closed loop is still wet, order a new closed loop nozzle (the o-ring is probably damaged).

Problem: Closed Loop Nozzle sprays liquid upon removal from flow cell

Solution:

- Replace the bulkhead male quick disconnect on waste cart
    - Inspect male bulkhead for damage. It is located on the waste tank side of the fluidics cart, where the waste tank plugs in to the cart through the male port.
    - Unscrew purple handles of fluidics cart and remove; set aside
    - Unscrew remaining screws on grey/tan housing of fluidics cart and pull away from cart
    - Unscrew nut around male bulkhead connector on inside side of fluidics cart
    - Remove damaged bulkhead connector and replace with new one (you can use old nut)
    - Ensure connector is fully connected to the waste line and is tightened.
    - Replace grey/tan housing
    - Replace purple handles
    - Plug in waste tank female line to new male bulkhead connector.
    - You can now either run a fluidics startup and after it's completed, check if closed loop still sprays when removed
- If replacing the bulkhead male quick disconnect on waste cart did not stop the closed loop from spraying, *call Dan or Ryan for suggestions*
    - *If needed, put in BD Service call.*

Problem: Stream Reflection from Violet laser

Solution:

- Heat up 100% contrad to hot hot hot
- Take out nozzle and key
- Clean flow cell 4 times (make sure you have enough contrad)
- Let sit at least 20 minutes
- Clean flow cell with air (no tube)
- Turn on stream with no nozzle and no key (10 seconds)

- dry everything
- insert nozzle and check for reflection
- If reflection remains, leave flow cell soaking in coulter clenz overnight.
- *If reflection remains, call Dan or Ryan for suggestions*
- *If needed, put in BD Service call.*

Problem: Amplitude is consistently too low/high

Solution:

- Click attenuation and change amplitude to the opposite end of the amplitude range (example, if the amplitude is too low, click attenuation and change amplitude to 80, if too high, click attenuation and change amplitude to 10)
- Evaluate whether this setting is in a better amplitude range than the previous setting
    - If yes, then keep these settings
    - If no, change back and go to next step
- Try changing the frequency
    - Not all frequencies are created equal, so here are some know ones that you can try first:
        - 70um= 87.8, 88.7, 89.2
        - 85um= 45, 44.2
        - 100um= 34, 34.7, 36.9, 37.9, 42.5
        - 130um= 15.4, 16.4, 16.8
    - After choosing a frequency, evaluate whether it:
        - Has a break off in the range of amplitude of 10-70
        - Has the correct merging pattern
        - Can make 4 distinct side streams (desired gap may need to be adjusted/reset)
        - Can achieve acceptable values for accudrop

Problem: Can't get accudrop lower than 4%

Solution:

- Perform an accudrop and find the lowest value in the right optical filter box possible
- Log out of DiVa administrator account and in to BDService, password superman
- Open accudrop experiment and again get the lowest value showing in the optical filter boxes
- In cytometer window, click the Image tab and find the Camera Controls section, click the "Stream" radio button
- Adjust contrast and brightness as little as possible to allow for a 0.1% low value in the right optical filter box

Problem: Backflushing into sample tube during unloading sample

Solution:

- Ensure no tube is loaded. If loaded, unload tube.
- Lift upper lid
- Locate clear part of SIP-line that is threaded through the pinch valve (caution: pinch valve can be hot)
- Grasp clear part of SIP-line on each side and pull toward yourself until SIP-line comes out of pinch valve

- Massage SIP-line
- Push SIP-line back into pinch valve
- Check to see if still backflushing into tube upon unloading
- If still backflushing*, call Dan or Ryan for suggestions*
- *If needed, put in BD Service call.*

Problem: Loss of pressure/Slow uptake on flow rates 1 & 2

Solution:

- With sample unloaded, lubricate the black circular part of the bulk injection loader (tube loader)
- Try sample again
- If problem is still noted, *call Dan or Ryan for suggestions*
- *If needed, put in BD Service call.*

Problem: Stream camera off-center

Solution:

- Stream camera needs to be adjusted to make stream more centered (small physical adjustment). This is the small camera on the left side of the sorting area under the upper lid. It tends to adjust in notches, so push or pull while stream is on to get image centered better. Try to make the smallest adjustment possible as this will affect all other nozzles/streams, but you are not able to evaluate how much they are affected until you change nozzles.

Problem: BISH Switch Timeout

Solution:

- *Call Dan or Ryan for suggestions*
- *If needed, put in BD Service call. Put instrument out of use (shut it down, adjust iLab calendar, and alert users who are affected and help them reschedule*