LSRII/LSRFortessa Troubleshooting Guide

Most common ERRORS:

No signal/suspected clog

Users:

a. Is the RUN button green when tube is on and arm is under? (as opposed to orangish-green)
   - If yes, put tube of water on SIP and press PRIME, observe if there are bubbles. If bubbles are seen, try sample again
   - If no, try next step

b. Has the o-ring that goes around the tank lid fallen into the tank?
   - If yes, use a gloved hand to retrieve it, replace it and then do fluidics startup again
   - If no, try next step

c. Is there a crack in the tube?
   - If yes, transfer sample to a tube with no crack
   - If no, try next step

d. When you pull up on the pressure release ring (with the lid closed), does it hiss air?
   - If no, re-seat the lid until it is able to pressurize the tank (you will know if it can pressurize by pulling the pressure release ring again- but it does need ~30 seconds to re-pressurize after being closed).
   - If yes, try next step

e. Did you already see cells/signal on a different tube?
   - If yes, try that sample again. If you can see the previous sample's cells, it is your sample that is faulty.
   - If you cannot see the previous sample's data now, try next step

f. There may be too much air in bubble filter/lines.
   - Purge bubble filter again while turning the bubble filter to have the bleed line and connector at the highest point of the filter (see start up procedure on front of instrument). Try to read sample again.
   - If you still cannot see cells, try next step

g. Is there sheath in the sheath tank (no higher than the weld line)?

- If no, fill to weld-line, and complete fluidics startup again, focusing on the bubble filter and purging all bubbles
- If yes, complete fluidics startup again and then try next step

h. Are the lasers turned on in the software?
   - If no, turn on laser software and ensure all lasers are ON and pointing at the appropriate values
   - If yes, try next step

i. If on the LSRFortessa, when you press Acquire Data, do you only see "9 evts" in the Acquisition Dashboard under Processed Events?
   - If yes, you must restart both the computer and the cytometer.
   - If no, try next step

j. If the above are confirmed to be ok, **run clog procedure (below)**, then try sample again.
   - Prime (3x)
   - Place no more than 3 mL of 10% Bleach on the SIP
   - Leave arm over to right for 1 minute
   - Place arm under tube and set to RUN and HI for 5 minutes
   - Place no more than 3 mL of 100% Contrad on the SIP
   - Leave arm over to right for 1 minute
   - Place arm under tube and set to RUN and HI for 5 minutes
   - Prime (2x)
   - Pslace no more than 3 mL of DI Water on the SIP
   - Leave arm over to right for 1 minute
   - Place arm under tube and set to RUN and HI for 5 minutes
   - Try sample again.

   - If still no events, continue to next step

k. Restart both instrument and computer and try sample again.

l. Insert a stylus inside the SIP
   - Get a stylus from the emergency kit (should be located next to the instrument)
   - Unscrew outer SIP and pull down to remove and reveal skinny inner SIP
   - Carefully push stylus up into the center of the SIP (this can be difficult to find the opening, and the stylus will bend, so go slowly until you don't feel resistance and the stylus starts to go in). DON'T LET GO OF THE STYLUS- it can get sucked up into the SIP. Also, it is normal for the SIP to be dripping.
   - Once you have pushed it up as far as you can while still holding it, pull back out
   - Replace outer SIP, tighten
   - Try sample again.

   - If still no events, call/email the CCSC (or after hours #).

Core members:

- *This is likely the hydrophobic filter (MF6) is wet preventing sample from being run. Replace this filter.*
- *If still no events, get a syringe with a female quick connect end*
  - *Fill the syringe with Coulter Clenz*

- *On the right side of the LSRFortessa toward the back, find the waste line and the HTS waste line (not the waste line attached to the waste tank)*
- *Unplug each waste line, plug syringe quick connect into line, and try to push syringe to push any potential clogs clear. Do this for both waste lines*
- *Re-plug the 2 waste lines back into side of instrument*

- *If still no events, call Dan or Ryan to see if repair is possible*
- *If not possible, 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*

Actual (known) Clog

Users:

a. Get a tube of beads (CST or Rainbow) from the fridge (there is a fridge in room T105 with the AriaII sorter and in room M902)
   - If you can see the beads, it is most likely not an instrument issue. *You may need to change the FSC and SSC to see the beads.
   - If still no events, try next step

b. Did you already see cells/signal on a different tube?
   - If yes, try that sample again. If you can see the previous sample's cells, it is your sample that is faulty.
   - If you cannot see the previous sample's data now, try next step

c. Put a tube of water on SIP and press PRIME, observe if there are bubbles.
   - If bubbles are seen, try sample again
   - If no bubbles, try next step

d. **Run clog procedure (below)**, then try sample again.
   - Prime (3x)
   - Place no more than 3 mL of 10% Bleach on the SIP
   - Leave arm over to right for 1 minute
   - Place arm under tube and set to RUN and HI for 5 minutes
   - Place no more than 3 mL of 100% Contrad on the SIP
   - Leave arm over to right for 1 minute
   - Place arm under tube and set to RUN and HI for 5 minutes
   - Prime (2x)
   - Place no more than 3 mL of DI Water on the SIP
   - Leave arm over to right for 1 minute
   - Place arm under tube and set to RUN and HI for 5 minutes
   - Try sample again.

   - If still no events, continue to next step

e. Insert a stylus inside the SIP
   - Get a stylus from the emergency kit (should be located next to the instrument)
   - Unscrew outer SIP and pull down to remove and reveal skinny inner SIP
   - Carefully push stylus up into the center of the SIP (this can be difficult to find the opening, and the stylus will bend, so go slowly until you don't feel resistance and the stylus starts to go in). DON'T LET GO OF THE STYLUS- it can get sucked up into the SIP. Also, it is normal for the SIP to be dripping.

- Once you have pushed it up as far as you can while still holding it, pull back out
- Replace outer SIP, tighten
- Try sample again.

- If still no events, call/email the CCSC (or after hours #).

Core members:

- *Try to insert a stylus again*
- *If still no events, this is likely the hydrophobic filter (MF6) is wet preventing sample from being run. Replace this filter.*
- *If still no events, call Dan or Ryan to see if repair is possible*
- *If not possible, 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*

SIP dripping when arm to the side/No liquid being taken up when arm to the side

Users:

a. Check that the switch on the side (LSRFortessa) or front (LSRII) of the instrument is pushed up to "tube"
   - If it is not, push the switch up to tube. Dripping should stop.
   - If it is on tube already, inform the core, but you can continue analyzing cells, with 2 additional precautions.
     1) In between samples, put a tube of DiH20 on the SIP (arm under) in RUN for at least 5 seconds.
     2) When performing the shutdown cleaning procedure, you can skip the "1 minute with the arm to the side" part of the protocol

Core members:

- *The problem is likely a clogged DCM pump waste line. See "Unclogging DCM Pump on LSRII" in labtest1.*
- *If still dripping, Call Dan or Ryan to see if repair is possible*
- *If not possible, put in BD Service call.*

Tube gets forcefully blown off SIP when arm to the side

Users:

a. Are you using the correct tubes?
   - BD has patented the size of tubes that fit best on their analyzers. For convenience, the core sells the below tubes:

     - 352052 (5 mL tubes with no lid). $12.50/pack of 125 tubes
     - 352054 (5 mL tubes with lids). $15/pack of 125 tubes
     - 352235 (5 mL filter-cap tubes). $25/pack of 25 tubes

   - If you have ensured that you are using the correct tubes for the cytometer and still experience issues, please call/email CCSC.

Core members:

- *Change the BAL seal o-ring at the top of the SIP*

- *If the tubes keep popping off the SIP, try unclogging the DCM pump waste line. See "Unclogging DCM Pump on LSRII" in labtest1.*
- *If the tubes keep popping off the SIP, most likely the problem is a stuck valve on the back side of the DCM pump. Call Dan or Ryan to see if repair is possible*
- *If not possible, put in BD Service call.*

Backflushing into tube when arm is under/over

Users:

a. Inform the core. You can continue analyzing cells, with 2 additional precautions.
   1) In between samples, put a tube of DiH20 on the SIP (arm under) in RUN for at least 5 seconds.
   2) When performing the shutdown cleaning procedure, you can skip the "1 minute with the arm to the side" part of the protocol

Core members:

- *The problem is likely a clogged DCM pump waste line. See "Unclogging DCM Pump on LSRII" in labtest1.*
- *If still backflushing, call Dan or Ryan to see if repair is possible*
- *If not possible, put in BD Service call.*

Not all lasers show in laser software (LSRFortessa only)

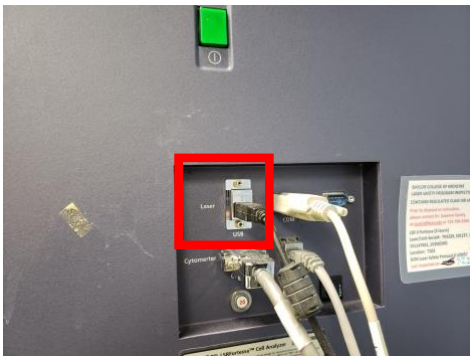
Users:

a. Restart the laser software:
   - Close the laser software.
   - Double-click the laser software icon to open.
   - Maximize the window and click "tile windows."
   - If <5 laser found or errors appear, restart the laser software until all 5 lasers are found. (This may take several restarts.)
   - If there are still error lights, click Stop Lasers, then Start Lasers.
   - Once all lasers are working properly, close the Coherent software by clicking the **red X** at top right of window.
   - If restarting the laser software does not cause all 5 lasers to show, try next step

b. Restart both the computer and cytometer.
   - If you still don't see all 5 lasers, call/email the CCSC (or after hours #).

Core members:

- *Try unplugging and re-plugging the laser USB cord*
- *If all 5 lasers still will not show up, call Dan or Ryan to see if repair is possible*
- *If not possible, put in BD Service call. Confirm signal is still able to be seen in all fluorescent channels before allowing users back on the instrument.*



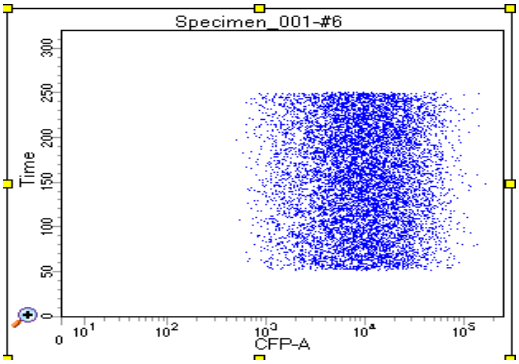
Liquid leaking from sheath tank at quick connect

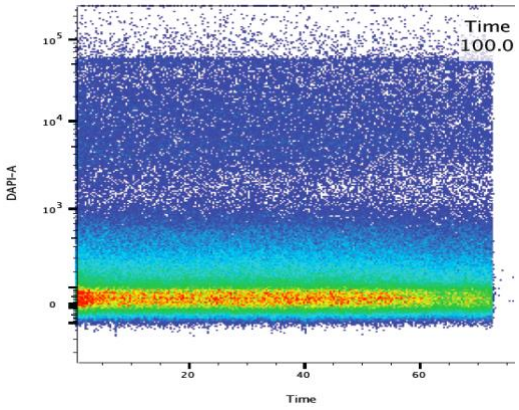
Users:

Inform CCSC as soon as possible.

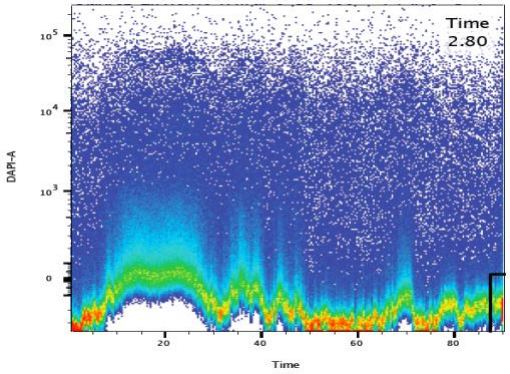
If you choose to continue analyzing samples, add a dot plot to your analysis that has the fluorescence of one of your fluorophores that excite on a laser other than the blue laser (like APC, any Brilliant Violet dye, or Brilliant UV dyes). You are monitoring for fluctuations in the signal over time. This would indicate the data you are acquiring is not consistent, and that you should **not** continue to analyze. Consistent fluorescence over time would indicate it is fine to continue analyzing.

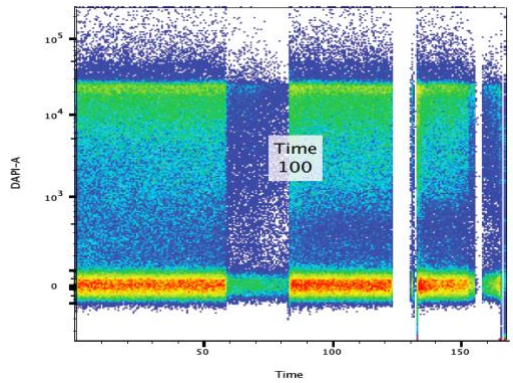
Examples of Stable Sample Runs





Examples of Unstable Sample Runs





Core members:

- *Replace o-ring on male quick connect of the port that is leaking.*
- *If leaking persists, replace quick connect(s).*

Leak from under the instrument

Users:

Inform CCSC as soon as possible. It is not advised to continue analyzing samples.

Core members:

- *To the extent that it is possible, unscrew panels and covers to see if you can determine where the leak is originating from*

- *If possible, repair, or call Dan or Ryan to see if repair is possible*
- *If not possible, 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*

Data shifting

Users:

a. Re-attach all fluidic lines
   - This includes all waste lines to waste tank- the waste line(s) may be pinched & causing back pressure. Re-connect the connectors on waste tank to prevent lines from kinks)
   - Re-purge bubble filter (when the bubble filter line is detached and reattached, it typically creates a bubble. Ensure you see a bubble in the tubing and that it moves). Also ensure the filter is turned to make the purging tube the highest point (so air will go out into the purging tube).
   - Try sample again. If data continues to shift, call/email the CCSC (or after hours #).

Core members:

- *Change window extension to 25 and see if fluctuation stops.*
  - *If yes, it is a fluidics issue.*
    - *Verify bulkhead waste quick connect is secure with no tears in o-ring. If compromised, replace.*
    - *Check for leaks in airline and check the condition of lines on waste tank. Replace lines on waste tank and/or airline if needed.*
    - *Perform "Advanced Clean-LSRII" or "Advanced Clean- LSRFortessa" in labtest1.*
    - *Try replacing the MF6 hydrophobic filter (could be wet preventing sample from being run consistently).*
    - *Check for T-junction leak inside of instrument & replace if needed.*
    - *If you cannot find a leak and none of the above helped, call Dan or Ryan to see if repair is possible*
    - *If not possible, 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*
  - *If fluctuation continues, get a syringe with a female quick connect end*
    - *Fill the syringe with Coulter Clenz*
    - *On the right side of the LSRFortessa toward the back, find the waste line and the HTS waste line (not the waste line attached to the waste tank)*
    - *Unplug each waste line, plug syringe quick connect into line, and try to push syringe to push any potential clogs clear. Do this for both waste lines*
    - *Re-plug the 2 waste lines back into side of instrument*
  - *If fluctuation does not stop, call Dan or Ryan to see if repair is possible*
  - *If not possible, 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*

DiVa states: Hardware Key is not accessible, Application will be terminated! Status Value: 3

Users:

a. Turn off both cytometer and computer
b. Look on the back and front of the computer for the DiVa dongle/USB
c. Unplug and re-plugin the DiVa dongle/USB
d. Turn on cytometer and computer again.