

Troubleshooting the Stream

Observation

Recommended Solutions

Possible Causes

Stream not in center of aspirator

Difference in keyed stream position between nozzles

If you have just changed the nozzle, use an Allen wrench to loosen the screws on either side of the sort block. Adjust the angle of the sort block until the stream flows into the center of the waste aspirator, and then tighten the screws. See

Figure 1-11 on page 32.

Nozzle inserted improperly

Turn off the stream. Remove the nozzle and ensure that the O-ring is in place. Reinsert the nozzle at a slight downward angle to prevent loss or movement of the O-ring. Make sure the nozzle is completely seated against the back wall of the cuvette flow cell.

Clogged or damaged nozzle

Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope.

- If debris is visible, clean the nozzle. See [Cleaning a Nozzle on page 188](#).
- If the nozzle appears damaged, replace it. See [Changing the Nozzle on page 140](#).

Troubleshooting the Stream (continued)

Observation

Possible Causes

Recommended Solutions

No stream or dripping stream

Grounding plate inserted improperly

Make sure the grounding plate is inserted with the notched end toward you. See [Cleaning the Nozzle and Grounding Plate on page 165](#).

Clogged or damaged nozzle

Stream control disabled Plenum not full or no stream when Stream

control clicked Air lock in filter

Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope.

- If debris is visible, clean the nozzle. See [Cleaning a Nozzle page 188](#).
- If the nozzle appears damaged replace it. See [Changing the Nozzle on page 140](#).

Wait for the plenum to fill.

Prime the system with the corresponding fluid.

If the control is still disabled, remove the filter, install bypass tubing, and repeat the priming procedure until you see fluid in the line.

When fluid is running through the line, remove the bypass tubing, install the filter, and repeat the

Troubleshooting the Stream (continued)

Observation

Recommended Solutions

Possible Causes

No stream when Stream control clicked

Sheath container low or empty Refill the sheath tank. See [Refilling Containers on page 103](#).

Note that when the empty tank warning message is not dismissed after 15 minutes, the stream shuts off automatically.

Air in sheath line

Prime the sheath tank. See [Prime After Tank Refill on page 172](#).

Air in filter

Purge the filter. See [Purging Filters on page 182](#).

Dry filter

1 Install bypass tubing in place of the filter for the affected fluid, and run Prime After Tank Refill.

2 Reinstall the filter and open the bleeder valve to purge the filter. See [Purging Filters on page 182](#).

Fanning around center stream

Nozzle inserted improperly

Reinsert the nozzle. Push it gently all the way forward without rocking it from side to side.

Unstable stream

Debris in flow cell or nozzle

Remove the nozzle and run the stream with no nozzle in place for approximately 1 second. (Click the Stream control on, and then off.) Sonicate the nozzle and reinstall it.

Troubleshooting the Stream (continued)

Observation

Possible Causes

Recommended Solutions

Leaking or spraying around nozzle

Nozzle inserted improperly

Turn off the stream. Remove the

nozzle and the grounding plate at thoroughly clean and dry the plate and the area around the plate. See Cleaning the Nozzle and Grounding Plate on page 165 for instruction:

Extra a-ring blocking nozzle

Remove the nozzle and use a cotton swab to clear out the cuvette.

Grounding plate inserted improperly

Make sure the grounding plate is inserted with the notched end toward you. See Cleaning the Nozzle and Grounding Plate on page 165.

Drop breakoff too long

Bubbles in flow cell

Open the flow cell access door and check for bubbles in the flow cell. they are visible, turn off the stream wait a few seconds, and turn on the stream again.

Attenuation on

Turn off attenuation.

Amplitude too low

Increase the amplitude until you can see drops. If you need a very high amplitude (>70 volts) to see drop you might have air bubbles in the flow cell.

Acquisition Troubleshooting

Observation

Possible Causes

Recommended Solutions

No events in plots after clicking Load or Acquire

Acquisition pointer not set to current Tube

Click to move the Acquisition pointer in front of the appropriate Tube.

Laser shutter engaged

Make sure the flow cell access door is completely closed.

Laser power off

Viewing plots for a different

Tube

Turn on the laser power.

Double-click the current Tube in Browser to display the plots for that Tube.

Acquisition Troubleshooting (continued)

Observation

Recommended Solutions

Possible Causes

No events in plots after clicking Acquire (continued)

Uncolored events in plot

- Format the plot to display all events.
- Assign a color to the population displayed in the plot.
- Verify the population drawing order.

Current Instrument Configuration different from optical setup

No sample in tube

Verify that the instrument optics setup matches the current Instrument Configuration. See [Application Options on page 220](#).

Add sample to tube or install new sample tube.

Sample not mixed properly

Threshold not set to correct parameter (usually FSC)

Increase the Sample Agitation rate. See [Sample Agitation on page 81](#).

Set the threshold to the correct parameter for your application.

Multiple Threshold parameters not set correctly Verify that the correct Boolean logic (And/Or) was used for the Threshold parameters.

Threshold channel too low or too high

Adjust the Threshold channel. See [Adjusting the Voltages and Threshold on page 124](#).

Optical filter(s) not completely seated. Make sure the filters are pushed all the way in.

Acquisition Troubleshooting (continued)

Observation

Possible Causes

Recommended Solutions

No fluorescent signal

Current Instrument Configuration different from optical setup

Verify that the instrument optics setup matches the current Instrument Configuration.

Wrong filter installed or filter not completely seated

Make sure the appropriate filter is installed for each fluorochrome; see [Application Options on page 220](#) for suggestions. Make sure the filters are pushed all the way in.

Laser delay set incorrectly

Adjust the laser delay settings. See [Instrument Quality Control on page 105](#).

Low Area signal

Area Scaling factor too low

Adjust Area Scaling for the corresponding laser. See [Instrument Quality Control on page 105](#).

Unexpected events in plot Incorrect logic in Population Hierarchy

Verify the gating strategy.

Incorrect population(s) in plot

Right-click the plot and choose Show Populations. Verify that the appropriate populations are displayed.

Incorrect drawing order

Verify that the required population is not hidden by another population Right-click the plot and choose Order Populations by Count.

Acquisition Troubleshooting (continued)

Observation

Recommended Solutions

Possible Causes

Unexpectedly high event rate

Threshold channel too low

Adjust the Threshold channel. See [Adjusting the Voltages and Threshold on page 124](#).

Sample too concentrated

Dilute the sample.

Event rate too high

Decrease the Flow Rate in the Acquisition Controls frame.

Bubbles in flow cell

Turn off the stream, wait a few seconds, and turn on the stream again.

Unexpectedly low event rate

Sample not adequately mixed

Increase the Sample Agitation rate. See [Sample Agitation on page 81](#).

Threshold channel too high

Adjust the Threshold channel. See [Adjusting the Voltages and Threshold on page 124](#).

Sample too dilute
Concentrate the sample.
Sample line clogged
Perform a sample line backflush. See Sample Line Backflush on page 170. If necessary, change the sample line.
Sample line clogged or kinked
Backflush the sample line. See Sample Line Backflush on page 170.
Look for visible kinks in the line. If
kinks are noted, change the sample line.

Acquisition Troubleshooting (continued)

Observation
Possible Causes
Recommended Solutions
Distorted parameters or high CV s
Instrument settings adjusted incorrectly
Optimize the scatter parameters. Adjusting the Voltages and Threshold on page 124.
Flow rate too high
Decrease the Flow Rate in the Acquisition Controls frame.
Window Extension too low
Increase the Window Extension.
Bubbles in flow cell
Turn off the stream, wait a few seconds, and turn on the stream again.
Nozzle clogged or dirty
Flow cell dirty
Clean the nozzle as described in [Cleaning a Nozzle on page 188](#).

Clean the flow cell with a detergent such as Contrad. See Clean Flow Cell on page 171. Let the detergent sit 5 minutes before turning on the stream.

Poor sample preparation
Repeat sample preparation.
Area scaling factor too low
Verify area scaling. See Verifying Area Scaling and Laser Delay on page 112.
Excessive amount of debris Threshold channel too low in plots
Increase the Threshold channel. Adjusting the Voltages and Threshold on page 124.

Acquisition Troubleshooting (continued)

Observation
Possible Causes
Recommended Solutions
High electronic abort rate
rate) [Window Extension too high](#) (>10% of system event rate)
[Threshold channel too low](#)

Event rate too high

Decrease the Window Extension.
Increase the threshold channel.
Sample aggregated
Decrease the Flow Rate in the Acquisition Controls frame.
Sample too concentrated

Filter the sample. Dilute the sample.
Fewer events than expected Window Extension set
in gated population incorrectly
Adjust the Window Extension. Refer to the BD FACSDiVa Software User's Guide, if needed.
Laser delay set incorrectly
Adjust the laser delay settings. See Instrument Quality Control on page 105.
Plot zoomed
Unzoom the plot or make the gate bigger.
Events left out of gate
When drawing a gate, make sure events on the axis are included.
Increasing threshold results Window Extension too low in decreased Area signal
Slightly increase the Window Extension to maximize Area signal.

NOTICE Increasing the Window Extension too much results in more electronic aborts

Fluidics Cart Troubleshooting

Observation

Recommended Solutions

Possible Causes

No fluid in line during system prime

Air lock in filter

Remove the filter for the

corresponding fluid, install bypass tubing, and run Prime After Tank Refill. Repeat the priming procedure until you see fluid in the line.

When fluid is running through the line, remove the bypass tubing, install the filter, and repeat the priming procedure one last time.

Long clean fails

See previous recommendations.

Air lock in filter

Fluid line detached

Verify the fluid line connections on the fluidics cart and on the instrument. Push firmly on each line to ensure it is connected.

Fluidics cart air flow <70 psi

Contact your BD Biosciences service engineer.

Air leak

Fluidics cart air flow >100 psi

Regulator not adjusted properly

Contact your BD Biosciences service engineer.

Fluid leak under fluidics cart or below side door

Condensation from pressure relief valve

This is a normal phenomenon that occurs when water is condensed from room air. Condensation is greater in humid environments. To avoid slipping, check and wipe up the water daily.

Electronics Troubleshooting

Observation

Recommended Solutions

Possible Causes

"Instrument Disconnected" in Instrument frame

Instrument power off

Turn on the instrument main power.

. Quit the software and then restart it.

Communication failure between workstation and instrument

- If restarting does not work, reset the instrument electronics: switch off the main power, wait 10 seconds until the system is fully depressurized, and then switch the power back on.

- Restart the computer and the instrument.

Ethernet cable disconnected between workstation and instrument

Unplug and then plug in the cable connectors and make sure they are secure.

IP address changed

Enter the correct IP address. Call BD Biosciences for assistance.

"Master DAQ Overflow" Event rate too high in Instrument frame

Decrease the event rate or verify the threshold.

"Instrument not responding" in Status tab

Unknown

Perform the suggestions for a communication failure, above.