Observations

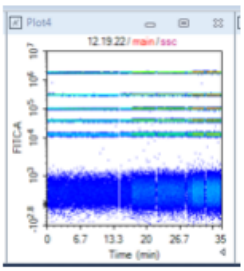
Observation	Possible Causes	Recommended Solutions
Daily QC does not complete	Wrong QC bead sample	Ensure you are running SpectroFlo QC beads.
	Bead sample not properly mixed	Mix the bead sample.
	Bead sample too dilute	Concentrate the bead sample or prepare a fresh bead sample.
	Air bubble is sample line	Run a SIT Flush.
Daily QC failed	Dirty flow cell	Run a Clean Flow Cell.

Hellmanex (let sit 15min) and MiliQ water
-then run MiliQ water for 30min on "High"
**if it still does not pass, CONTACT SRL STAFF and wait until next business day

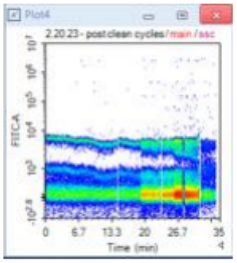
Observation	Possible Causes	Recommended Solutions
Data in scatter parameters appear distorted	Air bubble in flow cell	Run a SIT Flush.
	Air in sheath filter	Run a Purge Filter.
	Dirty flow cell	Run a Clean Flow Cell.
	Poor sample health	Check the viability of the cells.
High CVs	Air bubble in fluidics	Run a SIT Flush and a Purge Filter.
	Sample flow rate set to High	Set the sample flow rate to Low or Medium.
	Dirty flow cell	Run a Clean Flow Cell. If the problem persists, run a Clean Flow Cell using 25%–50% Contrad 70, followed by DI water.
SIT hitting bottom of well/tube	SIT Lift Distance set too low	Set the SIT Lift Distance to at least 1.5. See "Calibrating the SIT" on page 103.

Unstable signal/drift in scatter signal	Dirty flow cell, debris in fluid line, bubbles in fluid line	Clean Flow Cell: Hellmanex (let sit 15min) and MiliQ water -then run MiliQ water for 30min on "High" **if it still does not pass, CONTACT SRL STAFF and wait until next business day

To check stability of signal: set x-axis to Time and the y-axis to which channel you had a drift in signal. The signal should remain steady with no dips or peaks



Stable



Unstable

Observations

Observation	Possible Causes	Recommended Solutions
No events displayed (flow rate lower than expected)	No sample in tube	Add sample or install a new sample tube.
	Sample not properly mixed	Mix the sample to suspend cells/particles.
	Clogged SIT	Run a SIT Flush. Then run a Clean Flow Cell with 10% bleach, followed by a Clean Flow Cell with DI water. If the clog persists, replace the sample line. ★
	For loaders, the SIT Lift Distance set too low (touching bottom of tube)	Increase the SIT Lift Distance. See "Calibrating the SIT" on page 103.
No events displayed (flow rate normal)	Insufficient gain for threshold parameter	Increase the gain for the threshold parameter.
	Threshold too high	Lower the threshold.
	Laser delay not correct	Ensure the laser delay values match those from the latest Daily QC run. See "Instrument Control" on page 44 for the laser delay location. If the values do not match, rerun Daily QC.
	Threshold set to incorrect parameter	Set the threshold to the appropriate parameter for the application (usually FSC).
	Gated plot with no data in gate	Delete or move the gate.
Low sample event rate	Threshold too high	Lower the threshold.
	Insufficient gain for threshold	Increase the gain for the threshold parameter.
	Sample not properly mixed	Mix the sample to suspend cells/particles.
	Sample too dilute	Concentrate the sample. Set the flow rate to Medium or High.
	Clogged SIT	Run a SIT Flush. Then run a Clean Flow Cell with 10% bleach, followed by a Clean Flow Cell with DI water. If the clog persists, replace the sample line. ★
Erratic event rate	Partially blocked SIT	Run a SIT Flush. Then run a Clean Flow Cell with 10% bleach, followed by a Clean Flow Cell with DI water.
	Clumpy sample	Vortex, filter, or disaggregate the sample.

★ CONTACT SRL STAFF

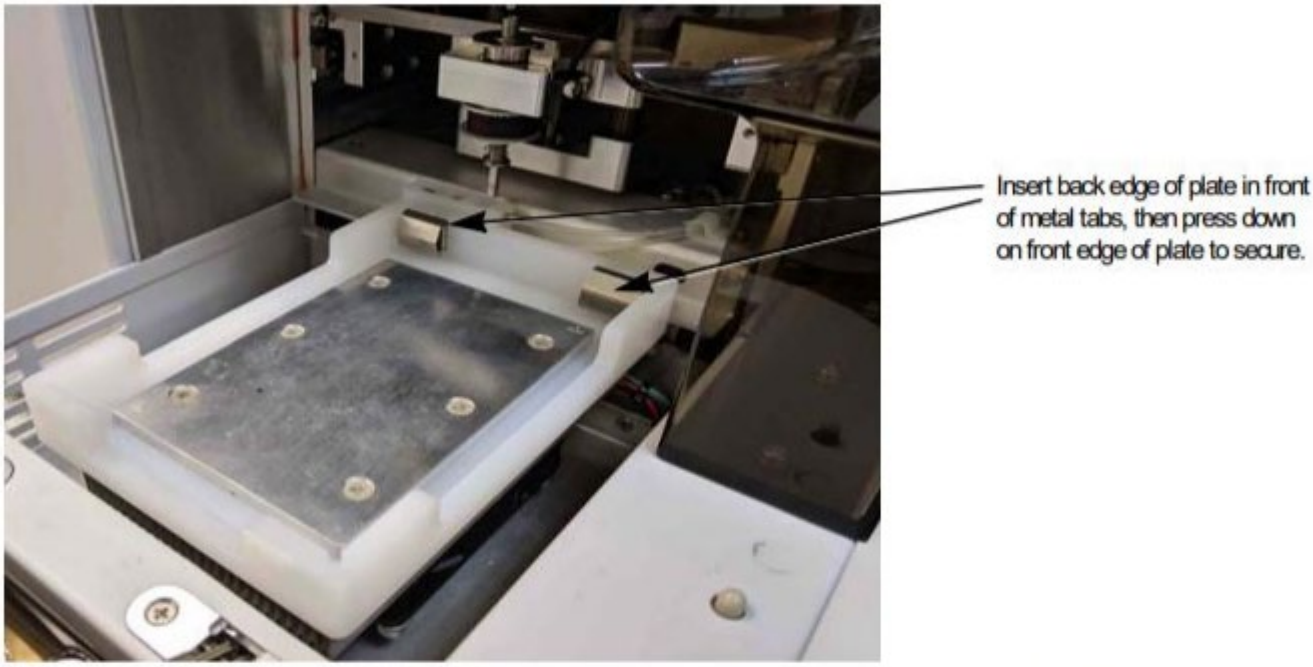
Concentration could also be too high. Dilute, filter and try again

This is also the cause of a DAQ board error

Loading a 96-Well Plate

1 If necessary, press Eject from either the QC & Setup or Acquisition module to eject the stage, bringing it forward.

2 Load a plate on the plate stage so that position A1 is located in the front-left corner. Insert the edge of the plate behind the metal tabs at the front of the plate stage, then press down on the back edge of the plate to secure it in the holder.



3 Press Load from the QC & Setup or Acquisition module to load the plate, followed by Start to begin acquisition.

Or press Start to load the plate and begin acquisition.

4 To switch back to tube mode, set the Sample Delivery Mode in the Acquisition Preferences back to Tube and slide the Loader lever back.

Minimum Well Volume

When acquiring from a well, a boost is applied to deliver the sample to the flow cell as quickly as possible. This process, as well as mixing, results in the loss (consumption) of up to 75 µL of sample, as the sample is neither acquired nor recorded. We recommend a minimum volume of 150 µL of sample per well, keeping in mind that approximately 75 µL will be lost.

■ **NOTE:** With the Loader v1.5, sample consumption is reduced to <35 µL. This can be reduced further by selecting to skip the fluidics boost. For information, see "Acquisition Preferences" on page 81.

You can disable the boost to save sample. For information see "Acquisition Preferences" on page 81.