Cytek Spectral Analyzers [Northern Lights / Aurora]

- Troubleshooting and FAQs –

These troubleshooting guidelines are intended to help resolve minor issues with the equipment. If the problems cannot be resolved right away, please always notify the facility. Please do not attempt to repair the equipment on your own in ways that exceed the scope of these troubleshooting guidelines. Even minor issues must be recorded in the logbook.

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1. Device won't start / Software won't connect

Symptoms: SpectroFlo won't connect to the cytometer.

- ➔ Check if the cytometer is turned on.
- ➔ Close SpectroFlo and reopen it; this is often enough to resolve the issue.
- ➔ If the machine still is not connecting: Restart the computer and the software; if that doesn't work, restart the instrument as well.

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2. Daily QC / Performance check fails

Symptoms:
QC fails; the beads are not detected properly. Although the bead population is visible at the start of QC, the population has a diffuse shape in the FSC/SSC channels and disappears from the dot plot as QC progresses.
Possible problems:

- Insufficient QC bead concentration in the working stock solution
  - ➔ Vortex the QC beads before use if you haven't done this already. Otherwise, prepare a QC working stock with a sufficient number of beads.
- The prepared working stock QC bead solution is too old [H2O-sheath older than 2 days; FACS flow-sheath older than 5 days].
  - ➔ If necessary, prepare a new QC bead solution [1 drop of QC beads + 300 µL of the sheath fluid used in the flow cytometer]. Make sure the QC beads are thoroughly mixed before use (vortex briefly).

Symptoms:
QC failed; the QC values do not match the reference values. Many Parameters fail and are "red"
Possible problems:

- The QC beads from another than the designated QC reference lot were used.
  - ➔ Verify that the correct lot of QC beads is being used.

Symptoms: QC has failed; one or more channels (often the ultraviolet or violet channel) have a CV (coefficient of variation) above the reference values.
Possible problems:

- The instrument has not yet reached operating temperature
  - ➔ Make sure you have waited for the 30-minute warm-up period before performing QC.
- Dirty flow cell
  - ➔ Perform the Clean Flow Cell Routine.

- Laser beam path slightly misaligned. If the troubleshooting above has not resolved the issue, the lasers are likely to be misaligned.
  ➔ Notify the Core Facility Team.

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3. No signals are detectable after starting the measurement

Symptoms: Normal flow rate, but no or almost no events appear in the dot plot.
Possible problem:

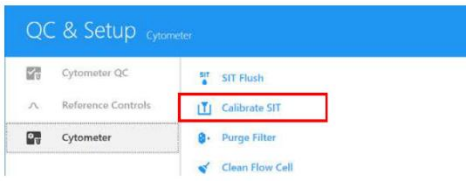
- The threshold is set too high, or the FSC/SSC settings are incorrect
  ➔ Check the threshold settings – Is it set too high, or is the wrong channel (default FSC) selected?
  ➔ Check if any events are detected when the FSC/SSC parameters are changed (+/-).

Possible problem:

- The sample has completely sedimented.
  ➔ Make sure the sample is mixed thoroughly before starting.

Symptoms: Low flow rate and only very few to no events.
Possible problems:

- The SIT is resting on the bottom of the sample vessel, thereby blocking sample uptake.
  ➔ Check whether the SIT is set to the correct height; The tip should not touch the bottom of the tube, but should not be too far from the bottom of the sample vessel.
  ➔ If necessary, recalibrate the SIT Lift Distance and adjust it so that the sample line does not touch the bottom of the tube.
  Automated SIT calibration is performed when the machine is turned on and when you switch to autoloader measurement. SIT Lift Distance can be changed for plate measurements, but is only available in the admin settings for normal tube measurement.



- The SIT is blocked and therefore cannot take up the sample.
  ➔ If the flow speed remains lower than expected, perform a SIT flush and then measure the sample again.
  ➔ If the SIT flush didn´t resolve the problem: Perform the Clean Flow Cell routine [Rinse the flow cell with 10% bleach and then with DI water. 10% bleach can be incubated in the flow cell for up to 10 minutes to remove contaminants from the flow cell].

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4. Signals disappear, or populations move during the measurement

Symptoms: The flow rate (volume uptake per minute) gets unstable or lower than expected [Standard values: Low ~15 µL/min; Mid ~30 µL/min; ~60 µL/min] Events appear only irregularly or sporadically

Possible problem:

- The SIT is blocked by debris in the sample and therefore cannot take up a sample.

Plot the events (SSC or FSC) against the time parameter to determine whether the sample uptake is indeed irregular.

➔ Perform a SIT flush and measure the sample again.

➔ If the problem recurs: Filter the samples to remove bigger aggregates. Sample material prepared from tissues should always be filtered!

➔ If the problem persists: Perform the Clean Flow Cell routine [Rinse the flow cell with 10% bleach and subsequently with DI water. 10% bleach can be incubated in the flow cell for up to 10 minutes to remove contaminants from the flow cell].

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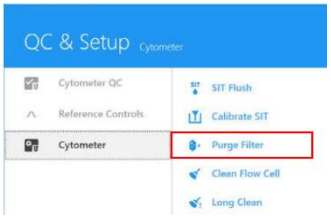
5. Poor separation / distorted scatter data

Symptoms:
The populations are not located in the expected positions in the FSC/SSC.
Possible problems:

- The sample quality is poor. Low cell viability or hypertonic buffers can increase scatter
- An air bubble might be trapped in the flow cell (e.g., if air was drawn in at the end of the last sample)
  ➔ Perform a SIT flush.
- Air in the sheath filter
- ➔ Perform a filter purge and check whether the sheath tank is empty.
- Flow cell contaminated with debris
  ➔ Perform the flow cell cleaning procedure.



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6. No automatic backflush / carry-over from the previous sample

Symptoms:
Backflush is not triggered after the sample tube is removed.
Possible problems:

- The Tube-clip sensor might be stuck due to salt crusts
  ➔ Carefully remove the salt crust from the sensor with a cotton swab soaked in dest. H2O.
    - Carefully open the door that contains the sample line and clean the point marked in the picture with the arrow. Take care that the electronic parts don´t come into contact with water!