

Can't log onto Windows computer

- Username: MyAurora
- Password: BDIS#1

PPMS is not running in order to log in

- Launch PPMS using the shortcut on the desktop

Can't log into PPMS (incorrect username/password combination)

- Check if Caps Lock is on
- Make sure there are no spaces before your username and/or password

Can't log into SpectroFlo

- Start typing last name of PI in the username field and select the appropriate group
- Password: facs123

Cytometer disconnected (Cytometer indicator icon in bottom right-hand corner of SpectroFlo software is yellow instead of green)

- Check cytometer is powered on
- Close SpectroFlo software and reopen

Plate loader is disconnected (if using plate loader, does not affect using tubes)

- Check that plate loader power is on (switch on back side of plate loader)
- Close SpectroFlo software and reopen

Cytometer/Plate loader still disconnected

- Power down cytometer (small silver button on left hand side of cytometer) and wait 2 minutes
- Shut down computer and wait 2 minutes
- Power on cytometer
- Power on Windows PC (see login info above)
- Open SpectroFlo and cytometer should connect automatically

Cytometer says 'Tube is Present' when it isn't (won't perform SIT Flush)

- Please see attached 'Cytek Aurora SIT Flush Troubleshooting Guide'

Unmix button is grayed out/unavailable

- All reference control tubes must contain recorded data in order to unmix – this includes group-specific unstained controls. Record all reference controls and proceed to unmixing.

Low event rate or Flow rate is less than 5 uL/min

- The instrument may be clogged:
 - o Filter your samples just prior to acquisition on the instrument (after staining)
 - o Perform several SIT flushes (Acquisition control pane -> SIT Flush)
 - o If flow rate is still low, perform a Clean Flow Cell: Go to the Cytometer -> Clean Flow Cell and follow the software prompts

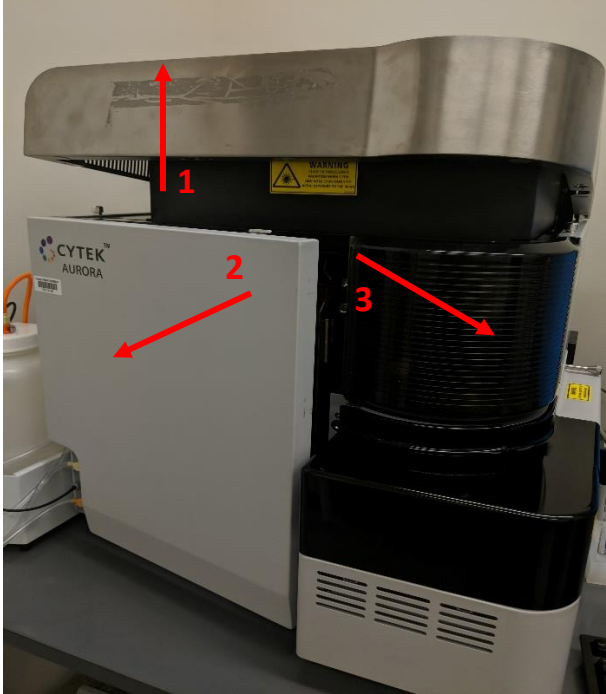
- If using microtiter/bullet tubes inside a regular FACS tube, the SIT lift distance may need adjusting:
 - o Install a FACS tube with bullet tube inside on the SIP
 - o Go to Cytometer -> Calibrate SIT
 - o Increase the number in the SIT Lift Distance field by 1 millimeter (For example, if SIT lift distance is set at 0.75, increase the number to 1.75)
 - o Hit the 'Calibrate SIT' button and check that the sample line depth is satisfactory when the probe extends during calibration. The probe should not be flush with the bottom of the tube – a small space is required to avoid clogging. Click Ok if distance is satisfactory or enter a new number in the SIT Lift Distance field to try a new distance.
- If using the plate loader, the SIT lift distance may need adjusting:
 - o Install plate on loader (it will calibrate on well A1 – it is okay if there are cells present in the well, as performing a calibration does not take any sample)
 - o Go to Cytometer -> Calibrate SIT
 - o The recommended starting point for U bottom plates is a lift distance of 1.5, this number may need to be increased for flat bottom plates
 - o Hit the 'Calibrate SIT' button and check that the sample line depth is satisfactory when the probe extends during calibration. The probe should not be flush with the bottom of the well – a small space is required to avoid clogging. Click Ok if satisfactory or enter a new number in the SIT Lift Distance field to try a new distance.

Problems with transferring files

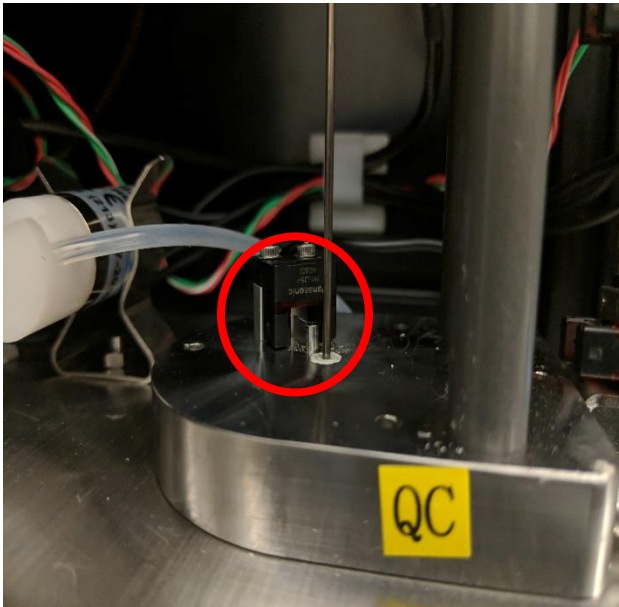
- Make sure the experiment in SpectroFlo is not open
- Close SpectroFlo and try the file transfer again

Cytek Aurora SIT Flush Troubleshooting Guide

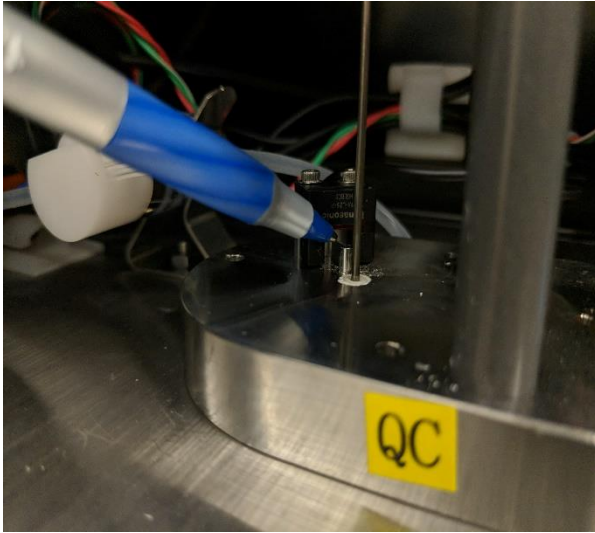
1. Without a tube loaded, open the ribbed black plastic part (#3) of the machine above where the tubes are loaded (follow the arrows in numerical order)



2. You are looking for the following part (circled)



3. Use a small, thin object to push the small metal part down. **!!DO NOT TOUCH ANY OTHER PARTS IN THIS AREA OR INSIDE THE MACHINE!!**



4. Close the machine before running your next tube and notify core staff by email that the part is sticking. We will clean it at the earliest available time.