Cytek Aurora Maintenance Guidelines for **Regular Users**

General Notes:

Note 1: *SIT flush is an important step to keep the instrument clean. By default, 2x SIT flushes are performed whenever a tube is removed from the SIP. If additional cleaning is needed, in "Acquisition Control" click "SIT Flush". Always wait until 2nd SIT flush is completed, then move to the next step*

Note 2: *It is very important to keep the level of 10% Bleach and 30% Contrad in the tube to be lower than the tip of the SIP (~2.6ml), and level of water tube should be higher than the tip of the SIP (~3.3ml)*

Note 3: *Please report all the problems / concerns to FCRC Staff:*

During business hours *— Go to FCRC office and tell the FCRC staff member*
After hours *— Leave the note on the instrument's keyboard and turn off the instrument if possible. Fill up* ***FCRC Problem Report Form*** *on FCRC website and FCRC staff member will get back to you as soon as possible*

Note 4: *Before you leave FCRC, please double check and make sure that the SIT is submerged in the MQ water (it will lift up if you open SpectroFlo software again after fluidics shutdown procedure is done). If not please repeat IV. Instrument Shutdown again*

I. Instrument Startup

1. **Log in** to Windows using your RUNet Login and password
2. Check if the Power control panel on screen shows that instrument is "**ON**" and verify that the instrument is actually "ON" (blue power button on instrument is lit and fan is running)

   Note*: If instrument is "OFF", please email to FCRC_Staff@rockefeller.edu and we will help*
3. Open the lid of the loader. Ensure that the tube of MQ water is loaded on the SIP before launching SpectroFlo software. Make sure the SIT is down to the bottom of the tube

   Note: *A tube is required for the SIT depth and flow rate calibration*
4. Open the **SpectroFlo** software from desktop. Sign in to SpectroFlo with "**wash**" and password "**wash**"
5. Maximize the SpectroFlo window to fit the screen
6. Select **"Acquisition"** from the "Get started" menu
7. Make sure that the system calibrates the SIT depth, and after the 2nd measurement SIT is not curved

***Note**: If SIT is curved, exit the SpectroFlo software by clicking the "X" in the upper-right corner of the application window. After one minute, proceed to Step [I.4] and follow the consequent steps of this protocol*

8. Wait for the software to connect with the cytometer and check if fluidics status is OK

   ***Note:** When instrument is connected the green checkmarks for **Sheath**, **Waste** and **Cytometer** will be seen in the lower-right hand corner of the screen (loader stays red if it is off)*

9. In the Acquisition Experiment menu, choose and open experiment "**AUX_Clean_Checkup_YYYYMM_User_WASH**" from "**My Experiments**"
10. Expand the group "**AUX_Water_Med_10min_Checkup**" and duplicate the last tube from this group. Change the name for the newly made tube with your name and current date. Move the pointer to the new tube
11. Remove the water tube from the SIP
12. **Dump** the remaining water and **refill water** tube to bottom line of sticker and load onto SIP
13. In the Acquisition control window click on **"Start"** to see the events and check the flow rate in **"Low"** (10-30 µL/min), **"High"** (60-100 µL/min), and **"Medium"** (25-55 µL/min)

    ***Note 1:** The event rate during wash is slightly higher than when running the samples due to the high volume in the wash tube*

    ***Note 2:** If you see differences in actual vs. listed flow rates (in µL/min) in any mode ("Low", "Medium" or "High"), unload the tube, wait until 2x SIT flushes are completed, then in "Acquisition Control" click "SIT Flush" for additional cleaning*

    ***Note 3:** If on "High" you observe significant fluctuations of the flow rates (in µL/min, with more than 10% variations), unload the tube, wait until 2x SIT flushes are completed, then in "Acquisition Control" click "SIT Flush" for additional cleaning*

14. **Record** the washing process by running MQ water for 10 minutes on flow rate "**Medium**"
15. When done, remove the tube with MQ water and wait until 2x SIT flushes are completed
16. **Review** the recorded data, and if satisfactory move to the next step
17. Expand the group "**AUX_SF_Low_5000_Checkup**" and duplicate the last tube from this group. Change name for the newly made tube with your name and current date. Move the pointer to the new tube
18. Pick up the tube with the **SF beads** from the fridge (with the green label premade by FCRC Staff)
19. Change Flow Rate to "**Low**". Vortex SF beads, remove lid and load the SF beads tube onto the SIP
20. **Record** SF beads for 5,000 events on flow rate "**Low**"
21. When done, remove the tube with **SF beads**, close the lid, and put it **back into the fridge**
22. DO NOT move any gate. **Review** the recorded data, and if satisfactory move to the next step
23. Close the experiment and **save changes**
24. **Sign Out** from SpectroFlo software but do not close the software

II. Running Experiment

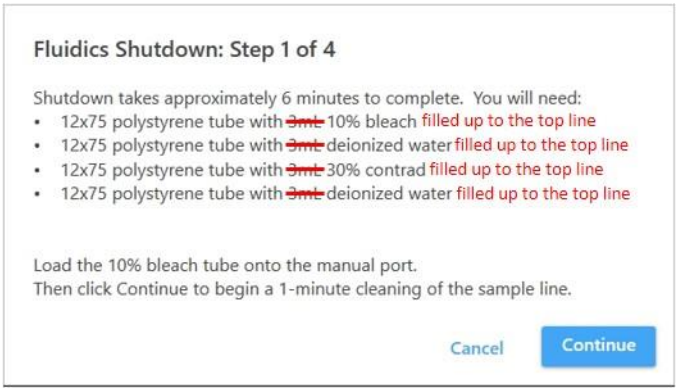
1. Sign in SpectroFlo with **your username** and **password**
2. Click the "**Experiment**" icon in the far left panel. Using the wizard, open a template, the default, or new experiment. If needed import, open, and continue an acquisition on the previously recorded experiment
3. Run your experiment
4. When you finish your experiment, **export data to the "T:\" folder**. **Copy** it from "T:\" to the **CFS**
5. Delete your experiment from "**My Experiments**"
6. **Sign Out** from SpectroFlo software but do not close the software
7. When experiment is completed please be sure to perform **"Instrument Wash"**

III. Instrument Wash

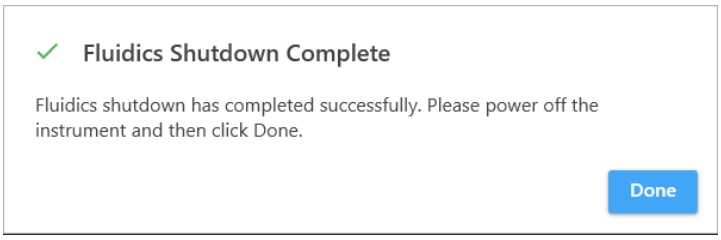
1. Log in to SpectroFlo with "**wash**" and password "**wash**"
2. Select **"Acquisition"** from the "Get started" menu
3. In the Acquisition Experiment menu, choose and open experiment "**AUX_Clean_Checkup_YYYYMM_User_WASH**" from "**My Experiments**"
4. Expand the group "**AUX_Water_Med_10min_Checkup**" and duplicate the last tube from this group. Change name for the newly made tube with addition of EOE (short for "end of experiment"). Move the pointer to the new tube
5. **Dump** the remaining water and **refill water** tube to bottom line of sticker and load onto SIP
6. **Record** washing process by running MQ water for 10 minutes on flow rate "**Medium**"
7. When done, remove the tube with MQ water and wait until 2x SIT flushes are completed
8. Expand the group "**AUX_SF_Low_5000_Checkup**" and duplicate the last tube from this group. Change name for the newly made tube with addition of EOE
9. Pick up the tube with the **SF beads** from the fridge (with the green label premade by FCRC Staff)
10. Change Flow Rate to "**Low**". Vortex SF beads, remove lid and load the SF beads tube onto the SIP
11. **Record** SF beads for 5,000 events on flow rate "**Low**"
12. When done, remove the tube with **SF beads**, close the lid, and put it **back into the fridge**
13. Close the experiment and save changes
14. From "My Experiments" export the completed experiment and overwrite it to the "**T:\QC_Checkup**"
15. Before you leave FCRC please be sure to perform "**Instrument Shutdown**"

IV. Instrument Shutdown

1. "De-maximize" the SpectroFlo window to fit the screen to make the Power control panel visible
2. Click on the "Cytometer" icon in the far-left panel menu and select **"Fluidics Shutdown"**. Follow the wizard



3. When "Fluidics Shutdown" is completed the message pops-up:



4. Leave the tube of MQ water on the SIP. Make sure the SIT is submerged in the MQ water at the end of procedure
5. Click on "**Done**" from the message. Check the time and PPMS schedule to decide if you need to switch instrument "**OFF**":
    a. If it is **BEFORE 5pm**, or someone is signed up after you, **DO NOT SWITCH OFF** the instrument
    b. If it is **AFTER 5pm**, and you are the last user of the day, **SWITCH OFF** the instrument by clicking the "ON" upper button on the Power control panel from computer desktop to turn off the instrument
6. **Exit the SpectroFlo** software by clicking the "X" in the upper-right corner of the application window
7. **Sign out from Windows** by using "Ctrl+Alt+Del" keyboard command
8. **Close the lid** of the loader
9. **Clean-up** the work area