

## Cytek Aurora Maintenance Guidelines for **Autonomous Users**

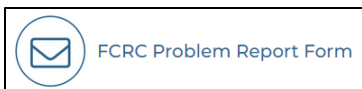
### **General Notes:**

**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 the 2<sup>nd</sup> 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:** If you see any abnormality in instrument performance:

- a. Report any and all problems / concerns to FCRC Staff by filling out [FCRC Problem Report Form](#) on FCRC website; and FCRC staff member will get back to you as soon as possible



- b. Take screenshot(s) (by pressing "Alt + Print Screen") and paste as picture(s) into the PowerPoint. Save the file as "AUX\_YYYYMMDD\_YourFirstName.pptx" to "T:\\" for further troubleshooting by FCRC Staff
- c. Proceed on part IV to shut down the instrument
- d. Please leave a note "**Instrument Out of Order**" on the instrument's keyboard
- e. FCRC Staff will update PPMS to notify users about instrument issues and block future instrument booking until issues are solved

**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 into** Windows using your RUNet Login and password
2. On the computer desktop click on "**OFF**" upper button on the Power control panel to turn "**ON**" the instrument

**Note:** The power control panel will turn on the Power Conditioner (you will hear a click), which will be followed by turning on the instrument

3. Open the lid of the loader. Ensure that a 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. Log into 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 system calibrates the SIT depth, and after the 2<sup>nd</sup> 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. Close the SpectroFlo software and log off Windows. **Wait for 30 min for the instrument to be warmed up**
10. After instrument is warmed up, proceed the instrument startup by following Steps [I.1, 4-8]
11. In the Acquisition Experiment menu, choose and open experiment "**AUX\_Clean\_Checkup\_YYYYMM\_User\_WASH**" from "**My Experiments**"
12. 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
13. Remove the water tube from the SIP
14. **Dump** the remaining water and **refill water** tube to bottom line of sticker and load onto SIP
15. In the Acquisition control window click on "**Start**" to see the events and check the flow rate in "**Low**" (10-30  $\mu\text{L}/\text{min}$ ), "**High**" (60-100  $\mu\text{L}/\text{min}$ ), and "**Medium**" (25-55  $\mu\text{L}/\text{min}$ )  
***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  $\mu\text{L}/\text{min}$ ) 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  $\mu\text{L}/\text{min}$ , 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*
16. **Record** the washing process by running MQ water for 10 minutes on flow rate "**Medium**"
17. When done, remove the tube with MQ water and wait until 2x SIT flushes are completed
18. **Review** the recorded data, and if satisfactory move to the next step
19. Expand the group "**AUX\_SF\_Low\_5000\_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
20. Pick up the tube with the **SF beads** from the fridge (with the green label premade by FCRC Staff)
21. Change Flow Rate to "**Low**". Vortex SF beads, remove lid and load the SF beads tube onto the SIP
22. **Record** SF beads for 5,000 events on flow rate "**Low**"
23. When done, remove the tube with **SF beads**, close the lid, and put it **back into the fridge**

24. DO NOT move any gate. **Review** the recorded data, and if satisfactory move to the next step
25. Close the experiment and **save changes**
26. **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**"

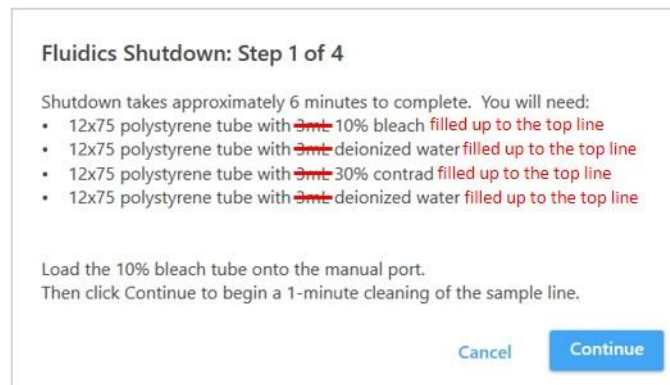
## III. Instrument Wash

1. Log into 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 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**

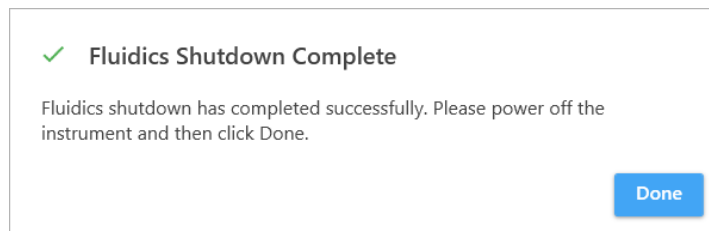
13. Close the experiment and save changes
14. From "My Experiments" export the completed experiment and overwrite it to the "T:\QC\_Checkup"
15. Before you leave FCRC please be sure to perform "Instrument Shutdown"

#### 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. **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