CytoFlex SRT QuickStart Guide

Start Up

1. Check sheath fluids the day before to avoid bubbles in the lines
2. Log into software, turn on cabinet, turn on chiller, and check Wi-Fi connection
3. Go to "*Cytometer*" in menu at the top and select "*Turn On*"
    a. Check the status of the machine in bottom left corner
4. In the bottom right corner select "*Sorter Status*", then select "M*ore Details*" in the pop-up window
5. In the "*Acquisitions*" panel or under the "*Cytometer*" menu, select "*System Start Up*"
6. Insert nozzle into machine and hit "*Next*" in the dialog box
    a. This will take about 15 minutes
    b. Monitor startup to ensure there are no leaks
7. After startup a dialog box will ask if you want to do a QC standardization, select "Yes"
    a. Prepare beads
        i. Shake the bottle of beads vigorously to resuspend them
        ii. Put 3 drops of beads solution into a facs tube
        iii. Check the lot number of the beads on bottle and match the number on the screen
        iv. Select "*Start Standardization*"
    b. If QC Fails:
        i. If DD1 fails, check the stream break off: the break off point should be visible in the top half of the status window.
            1. Run Sort Calibration just long enough for the machine to automatically reposition the stream, then cancel Sort Cal.
            2. If that doesn't work, *gently* wiggle the nozzle to find good break-off height (usually in the second quarter from the top of the stream, but this can vary) and satellite formation and reformation.
        ii. Check for air in the system.
            1. The stream droplets should be visibly sharp and not fuzzy.
            2. Check if there is a bubble present in the filter; tap or vortex the filter to cause non-visible bubbles to rise to the surface.
            3. If there are bubbles, under the "*Cytometer*" menu, run the following in this order:
                a. Sheath filter de-bubble > Run 3 times in a row (skip if there are no bubbles in the filter).
                b. Flow cell de-bubble > Run 3 times in a row.
                c. Backflush > Run 3 times in a row.
        iii. If there is no bead peak, you may not have remembered to vortex the beads. If this is not the case, remake the bead tube and try again.

8. After standardization is complete, you will be prompted to start a “Sort Calibration”. You can select yes and do it now or you can wait and unload the QC beads
    a. If you select “*No*” you can find this again by going to “*Sorting*” in the menu at the top, and selecting “*Sort Calibration*”
    b. If Sort Calibration fails:
        i. *Gently* wiggle the nozzle or debubble and try again.
        ii. Put the machine in standby and remove the nozzle from the machine and reinsert it again.
            1. Select “*Initialization*” in the “*Acquisitions*” panel and wait to re-stabilize the stream. Run “*Sort Calibration*” again
            2. If the previous steps fail to resolve the issue, sonicate the nozzle, found below.
    c. Once “*Sort Calibration*” passes, move the red guideline on the stream to first drop after the satellite drops reform
    d. On rare occasions, if the stream becomes unstable, the machine will stop sorting. You will need to re-run “*Sort Calibration*” during your experiment

Shut Down

1. Remember to make PDF’s of your experiment and export your data
2. Under “Cytometer” select “Daily Clean”
    a. A dialog box will open. Make sure the cleaning time is set to 5 minutes for 10% bleach and for rinsing with Di water
3. Under “Cytometer” select “System shutdown"
    a. A dialog box will open. Follow the prompts on screen
    b. Make sure to dry off the nozzle when you remove it
    c. One of the final steps will ask you remove and clean a bunch of components. Do so with 70% ethanol, Kimwipes, and cotton swabs. Dry and reassemble them.
4. Under “Cytometer” select “Turn off” to power off the machine
5. Spray the sort chamber and bench with 70% Ethanol and wipe clean.
6. Close the sash on the hood and turn off the blower
7. Turn off chiller
8. Log out of the software