I. LSRII startup and Shutdown

A. LSRII Startup

1. Check the sheath fluid by lifting the 2 tanks (sheath fluid on the left and waste on the right). If the clean sheath fluid card box container is empty, replace it with a new card box sheath fluid container. Keep the empty card box, add 2 liters of pure bleach (under the sink) and replace the waste container. Please write WASTE on it and indicate the date.
2. Turn ON the fluidic pump.
3. Turn on the LSRII (big green button).
4. If you are the first to turn on the cytometer, shut down the computer and restart it. **Login: Administrator Password: BDIS.** If you are after a user, you can skip to 6.
5. Turn on the Green Laser. Turning on the LSRII doesn't automatically turn on the green laser. Double-click on the shortcut for the green laser. In the terminal window, type in **On** and hit enter. If answer is 'syntax error' try again. If the answer is 'OK', then write **SetPower 150** and enter (both are case and space sensitive) to turn the green laser on. After you do this, let the LSRII warm up for 15 min before doing the laser time delay or running samples. To check whether the laser was turned on, write qPower and enter. The value should slowly rise to 149-150.
6. Flush the fluid system of the LSRII out 1-2 times by hitting the **PRIME** button. (Note: Pushing the **PRIME** button will make it will turn red. Wait until the instrument goes back into **STANDBY** mode before priming again.)
7. After the machine has warmed up, you should set the laser time delay and the area scaling.

B. LSRII Shutdown

1. Run 10% bleach on **HIGH for 5 minutes**, then deionized water on **HIGH for 3 minutes**.
2. Leave the deionized water on the LSRII and turn off the machine.
3. Be sure to log out of the DiVa software.
4. Place the instrument in stand by (if the following user talks to you directly) or switch off the LSRII if needed (i.e. if you did not talk to the next user).

II. LSRII Time Delay and Scaling

1. Open and login to the Diva Software. Login: your first initial followed by your last name. Password: first name (If you do not have one, contact Marielle Cavrois at: mcavrois@gladstone.ucsf.edu).
2. Open up an existing Rainbow Bead experiment. Select the latest bead tube and duplicate it without data and rename with today's date. Note: If you do not have this experiment in your Browser window, please notify Marielle.
3. Take the tube of rainbow beads ($$$) from the fridge (if empty, refill with ~3-5 drops from the rainbow 5 ml bottle in the black tray in the fridge), vortex, place on the sample injection port and start acquiring at 200-400 events/sec. You will generally run on LOW and use the fine tune knob to adjust the flow rate. Start with FSC ~550 and SSC ~320.
4. Click on the Threshold tab of the Instrument panel and set the FSC threshold level to ~5,000.
5. Click on the Laser tab on your Instrument panel and change the Window Extension from 6.0 to 0.0.
6. Move the beads gate on FSC and SSC plot to accommodate the changes on the bead population.
7. Adjust the time delay for each laser (except the Blue) in increments of 0.1 (up or down) until the signal for the channels running on that particular laser are maxed out (the peak at the most right position). It is easiest to monitor one channel for each laser; a suggestion is Pacific Blue for the violet laser, APC for the red laser, and PE for the green laser.
8. After adjusting the time delays, change the Window Extension back to 6.0. Remember to adjust the beads gate.

10. Adjust area scaling for each laser until the geometric mean for the respective channels are as close to 2620 - 2680. The channels used for area scaling are FITC for the blue laser, Pacific Blue for the violet laser, APC for the red laser and PE for the green laser.
11. Adjust the FSC Scaling factor so the bead peak on the FSC-A plot is the same brightness as on the FSC-H plot.
12. Record the beads' ~5000 events.

III. LSRII High Throughput Sampler (HTS)

Before installing the HTS unit, make sure the LSRII is on and the time delay and area scaling have been set

A. Installation

1. Put the LSRII in “Standby” mode. Make sure there is enough sheath fluid in the card box container. Remove any tubes and the drip tray from the LSRII sample injection area, including the tube of water on the SIT.
2. Carry the HTS over from the counter to the LSRII. Align the metal rod on the back of the HTS with the hole on the LSRII and slide the HTS unit into the LSRII to have at least 1 inch of the rod is in the hole (the HTS will be slid all the way in after step 6).
3. Take off the HTS cover for easier access to tubing and cables. Connect the waste line (orange) to the orange connector and sheath (clear) to the white connector.
4. Connect the sample coupler tubing to the SIT by sliding the coupler onto the SIT until you have reached a hard stop. Hold the coupler with one hand and tighten the nut with the other hand by turning it clockwise (looking from the top).
5. Connect the serial cable and the power cable (remove the tube that protects it) to the rear right side of the HTS unit. When plugging in the power and serial cable, be careful not to touch the power connector to any metal as this can cause short-circuiting.
6. Tuck extra cables into the LSRII and push the HTS unit into the LSRII.
7. Turn on the HTS unit with the power button located on the right side. Flip the rocker switch (located 5 in. above the LSR green power button) to the plate mode.
8. Place your plate in the HTS and put the HTS cover on.
9. Put the LSRII in “RUN”.
10. From the HTS menu in the DiVa software, select “Reinitialize”. Wait 2–3 min for the prompt.
11. From the HTS menu in the DiVa software, select “Prime”. Wait 2–3 min for the prompt.
11. Run your plate providing your plate template using the command in the DiVa software.
12. Clean the HTS station by following the instruction given after selecting ‘clean’ from the HTS menu of the DiVa software.

B. Removal

1. Turn off the HTS. Put LSRII on “Standby”. Remove the cover.
2. Detach the sample coupler from the SIT by loosening the nut (turn counterclockwise looking from the top).
3. Pull the HTS unit out from the LSRII slowly to keep about 1 inch of support rod inserted. Be careful not to strain the sheath tubing and cables.
4. Disconnect the sheath and waste lines from the cytometer.
5. Disconnect the power and communication cables from the rear right side of the HTS unit. Put an empty and dry 5 ml FACS tube on the end of the power cable to prevent short-circuiting.
6. Switch the tube/plate rocker back to tube mode.
7. Slowly pull the HTS unit back out and away from the LSRII. Put the HTS unit back on the counter.
8. Replace the drip tray and deionized water tube on the LSRII. Shutdown LSRII if needed (i.e. if you did not talk to the next user).

C. LSRII HTS Loader Parameters

1. HTS Modes of Operation. The HTS has two modes of operation, Standard mode and High Throughput mode. In Standard mode, one syringe is used to mix the sample, aspirate the running volume, and push the sample through the LSR. In the High Throughput mode, the first syringe mixes and aspirates the sample while the other pushes the sample through the LSR.
2. Sample Volume per Well: To determine the volume to be analyzed, it is important to take into consideration the following volumes:
   - The dead volume of a well (~30 µl) is a volume of sample from each well that cannot be reached by HTS probe (often plate dependent).
   - The excess volume (~20-22 µl) is a volume of sample from each well that will be taken up by the HTS but that will not be run through the LSR (will go to the waste without analysis).
   - ***For example, to run 100 µl of sample per well in standard mode, each well should contain at least 150 ul of sample. To run 10 µl in High Throughput mode, each well should contain at least

62 µl (75 µl recommended to account for pipetting error).

3. Other Parameters
    - **Mixing volume** should be no more than half the well volume.
    - **Number of mixes**: Generally 2 but can be increased if sticky samples
    - **Wash volume:** 2 washes with 400ul is currently used by Marielle Cavrois
    - **Sample Flow Rate** should be set to not exceed 10,000 events/sec. Make sure the threshold is appropriate to remove debris. For example, 500,000 cells per 100 µl, the Flow Rate can be computed as follows: (100 µL/0.5*10^6 cells) * (10^4 cells/sec) = 2 µl/sec.

IV. Data management

1. Export your file to D: /BDExport/FCS Right click on the experiment, select Export, then FCS files and finally D: /BDExport/FCS folder or Desktop/LSR2Users
2. Import your files in the Vishnu server: Login Vishnu by clicking on the short cut on the desktop. If the flowcore data folder opens right away, it means that someone had login into Vishnu before and you might encounter problems regarding privilege access. To disconnect from vishnu, go in the start menu, select my computer, select Tools, select Disconnect Drive and Click on the vishnu server. Then login with your own ID and password, after double click on the short cut to vishnu on the desktop. Finally, drag your folder from D: /BDExport/FCS or Desktop/LSR2Users to NewLSR2User folder or in the Mission Bay LSR2
3. Retrieve your data by Connecting to Vishnu.ucsf.edu from your computer

    From a Mac: In the Finder under the Go menu, choose “Connect to Server…” In the dialog box, type vishnu.ucsf.edu in the Server Address space, then click Connect. A login box should appear. Select FlowCoreDataA and select the appropriate lab folders.

    From a PC: If you are in the GIVIFLOWCORE workgroup, Vishnu will appear in My Network Places. If not, choose Map Network Drive under the Tools menu. For the directory to map, type //vishnu.ucsf.edu/flowdataa/gladstone/NewLSR2Users. To disconnect, select "Disconnect Network Drive" from the Tools menu in My Computer or My Network.

For the benefit of all users, please delete your files from the DiVa software when you are done with the analysis of your sample.

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