LSR/Symphony Operation

URMC Flow Cytometry Shared Resource Lab

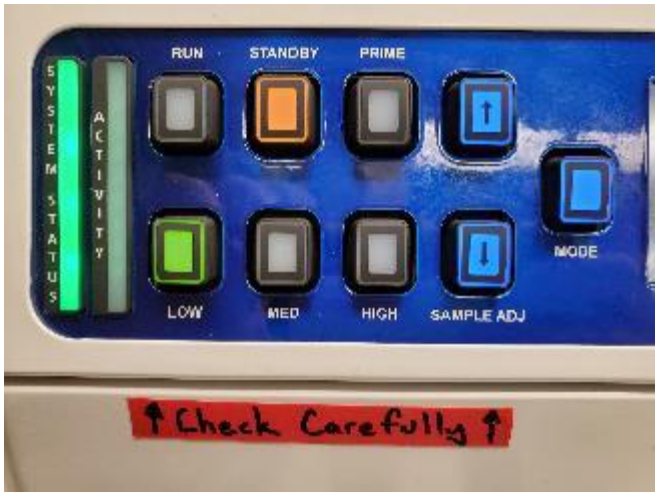
January 2022

The purpose of this document is to familiarize the user with the fluidics and operational components of the BD LSR and Symphony instruments. This is meant to be a basic operational guide and does not cover troubleshooting or the FACSDiva software.

Fluidics Control Panel

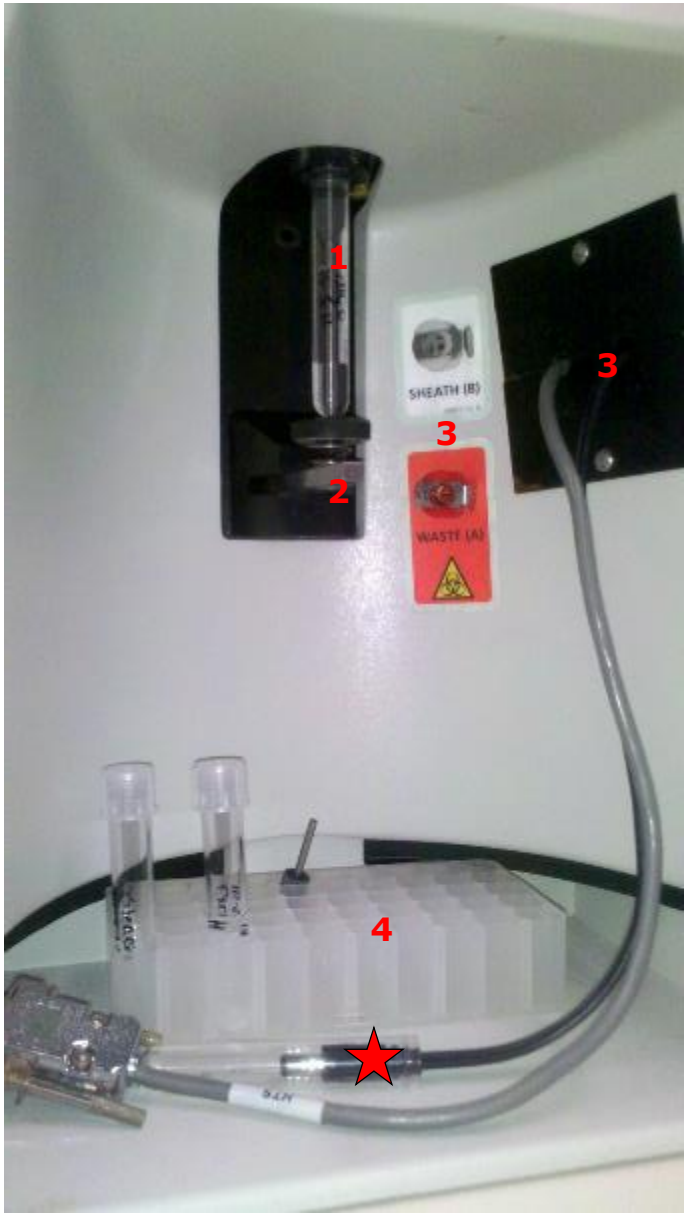
- Lo, Med and Hi:
  - Controls the sample flow rate
  - Lo is always recommended
- Sample Fine Adj:
  - Fine control of the sample flow rate
- Standby:
  - Depressurizes the system
  - Any time the instrument is not in use
- Run:
  - Sheath tank and SIP are pressurized and fluid is flowing through the LSRII
  - Running samples/cleaning
- Prime:
  - Empties and refills the flow cell
  - Clears air bubbles/minor clogs from the flow cell
  - Before every run

***Note that the buttons are reversed on the Symphony instruments. The Run, Standby and Prime buttons are on the top.



The SIP

1. The SIP is where tubes are attached for running.
   - Tubes must create an air tight seal with the o-ring at the top.
   - When the tube is pressurized the fluid is pushed toward the flow cell.
2. The sample arm has two functions:
   - Helps prevent the tube from falling if seal is lost.
   - When pushed to the side the system will automatically flush itself.
     a. We recommend ~8-10 seconds of rinsing between each tube. Longer rinsing has diminishing returns.
     b. If a tube is on the SIP it will be flushed into the waste. Samples can be lost if they are in place with the arm open.
3. Two quick connects and two cables are for HTS use: Not present on all instruments
   - The HTS involves a separate training.
   - There is a black switch on the front panel that controls tube mode vs HTS (plate) mode.
   - This is a live power cable and should always be kept covered.
4. Tube rack at each instrument
   - Tubes of bleach, ethanol and water are provided.
   - Extra fluids can be found on the center table.



The Sheath Tank

1. The fluid line brings sheath fluid into the LSRII and to the flow cell. The sheath filter is ~18 inches up the tube from the tank.

2. The pressure line automatically pressurizes the sheath tank when its plugged in.

3. The pressure release valve allows the operator to release the pressure from the tank for refilling.

4. The lid seals the tank closed using the air pressure within the tank and a rubber o-ring.

The Waste Tank

1. The waste tank is a reservoir to capture the liquid waste. There should be no pressure in the tank.

2. The waste connection. Note the orange color of the male and female connectors

3. The empty line is usually marked with lab tape. The bleach should be added such that it covers the bottom of the tank, but does not need to be measured.

At the Start of Your Run

1. Check the waste tank to make sure the level is below the line.
2. Briefly check the sheath tank to be sure there is fluid (you do not have to remove the lid)
3. Remove the water tube from the SIP and leave the arm to the side.
4. Press Prime (from the control panel) and wait 23 seconds. The prime button will turn red during the prime.
5. The instrument will default back to Standby when the Prime is completed.
6. Place the water tube back on the SIP and close the arm.
7. Press Run (on the control panel) and Lo.
8. There is no need to go back to Standby until the experiment is completed.
    - Between samples the arm can stay open allowing the SIP to flush itself
9. The water should remain running while you get your experiment set up in the FACSDiva software.

At the End of the Run: Cleaning Process

Cleaning can be done while exporting and transferring files

1. Run the cleaning fluids.
    1. Run 2 minutes of Bleach on the SIP on High.
    2. Run 2 minutes of Ethanol on the SIP on High.
    3. Run 2 minutes of Water on the SIP on High.
2. Switch the instrument into Standby.
3. Check the waste tank. **If the waste is above the "Empty Now" line the tank must be emptied.**
    1. Unhook the quick connect (Push the metal tab on the side to release).
    2. Unscrew the cap and dump the waste into the sink with running water.
    3. Pour bleach (under the sinks) back into the tank to the "Bleach" line.
    4. Reconnect the orange quick connects (the connectors should audibly snap into place when connected)
4. If the waste tank had to be emptied then the sheath tank must be filled. **The tanks should always be filled/emptied in unison.**
    1. Unhook both of the quick connects. The order for releasing does not matter.
    2. Unscrew the lid and release the pressure by pulling straight up on the pressure release valve.
    3. Fill the tank up to the weld seam using the sheath fluid located at each LSRII.
    4. Place the lid back into position and screw the cap until its snug. O-ring must be in place.
    5. Reconnect the quick connects. The order for reconnecting does not matter.
5. Verify the instrument is in Standby and all components are in place prior to leaving

Important Tips

1. Remember that the fluidics control is separate from the software control allowing flexibility when running.
   - You can find your own rhythm for switching samples and clicking buttons in the software.
   - You can clean the instrument while exporting data.
2. Do not leave the sample arm open when your sample tube is on the SIP. The flush pump will suck up your sample and send it to waste.
   - Leave the arm open with nothing on the SIP if its going to be a minute.
   - Put the water on and close the arm if its going to take longer.
3. There is a troubleshooting guide posted on each instrument to help with fluidics issues and software issues.
   - **You can always contact the staff via the Slack channel, but the guide is your first resource when trying to fix instrument issues on your own.**