BD LSRIIs and LSR-Fortessa Maintenance Guidelines for Autonomous Users

Note: *During the After-Hours, autonomous users can book and operate BD LSRIIs / LSR-Fortessa when the instruments are unavailable to regular users. Please report any and all problems / concerns to the FCRC Staff as soon as possible by filling up* ***FCRC Problem Report Form*** *on FCRC website; and FCRC staff member will get back to you as soon as possible*

1. **Log into Windows using your RUNet Login and password**
2. On the on-screen user interface **click only the upper button once** to switch "**ON**" both the **BD LSRII** (or **BD LSR-Fortessa**) and **FACSFlow system**
3. Please make sure that the **green lights** on both - Instrument (BD LSRII or BD LSR-Fortessa) and FACSFlow system are actually "**ON**"
4. If the fluorochromes / dyes you use in the experiment require the **UV** or **445 nm laser** to be "**ON**" please make sure to follow the appropriate steps:
    a. If you use the **UV-excited fluorochromes / dyes** (Indo, Hoechst, AF350, CellTrace Calcein Blue AM, LIVE/DEAD Fixable Blue, etc.) please make sure that the UV laser on the BD LSRII-1 is "**ON**" by following the steps in "**BD LSRII-1 UV Laser Operation**"
    b. If you use the **445nm-excited fluorochromes / dyes** (CFP, mCerulean, etc.) please make sure that the 445 nm laser on the BD LSRII-2 is "**ON**" by following the steps in "**BD LSRII-2 445 nm Laser Operation**"
5. If you need to change the filter(s) for particular channel(s) for the first time, please make sure to inform the FCRC Staff in advance and get proper training
6. Before you start running samples please:
    a. Check if the **Instrument**'s fluidic system is functioning properly:
        i. Remove the tube with MQ water from the SIP (Sample Injection Port)
        ii. Push "RUN" and "High" fluidic control buttons
        iii. Check if the buffer starts dripping from the SIP
            - If "yes" - please proceed to the Step [6b]
            - If "not" - please push the "Standby" button immediately and report the problem to FCRC Staff (*filling up* ***FCRC Problem Report Form***)
    b. **Check if the trap filter** attached to the pressurized plastic tank is **free of air bubbles** by bleeding sheath fluid from two ports:
        i. Open the roller clamp on the top of the trap/bubble filter for 3-5 seconds

ii. Sequentially open the second roller clamp (on the right side of the instrument) for 3-5 seconds

iii. Check if you are getting consistent stream release from both ports

- If "yes" - please proceed to the Step [6c]
- If "not" — you should suspect pressure problems or presence of bubbles, please immediately push the "Standby" button and report the problem to FCRC Staff (*filling up **FCRC Problem Report Form***)

c. **Wash the instrument** before you run samples:

i. "RUN" on "High" 2 ml of 10% bleach for 5 minutes

ii. Take a **new tube** with blue sticker from FCRC drawer next to the sink. Refill it with MQ water to the bottom line of sticker

iii. "RUN" at "High" 2 ml of MQ Water for 5 minutes

iv. Push "Standby" button and leave tube with 1 ml of MQ Water on the SIP

***Note:** Please be sure to never exceed the **maximum allowed volume of 2 ml** in the sample tube*

7. **Log in to DiVa. Wait until instrument get connected. Always choose to "Use CS&T Settings"**

8. **In absence of the FCRC Staff on-site it is essential for researcher to RUN "QC_Checkup" EXPERIMENT:**

a. From the "**File**" menu click on "**Import**" > "**Experiments**". In the folder "**T:\Application_Settings\QC_Checkup**", find the most recent QC Experiment named by "**CST LX YYYYMMDD_operator's initial**" (make sure to use the **latest** file)

b. Open the imported QC experiment. Right click on the name and choose "**Duplicate Without Data**"

c. Open the duplicated experiment. In the DiVa "Inspector" window **rename the experiment** by changing the date to the current date and operator's initial to user's first name. Also **change the date** on the **Specimen**

d. In the fridge locate Box #1 to find the **CS&T Research Beads** (**CST**) **(**Lot #33629**) with red label**. Take the one which is in use (has less volume or without parafilm):

e. Unload the water tube from the SIP. Change the flow rate from "**High**" to "**Low**". Make sure the instrument is on "RUN"

f. Vortex the CST tube with bead suspension. In DiVa software, select "CST" tube and load the CST tube on the SIP

g. Click on "**Acquire Data**" and as soon as picture stabilizes, click "**Restart**", then "**Record Data**", and record 5,000 events (events rate ~50-250 events/sec). Immediately unload the CST tube from the SIP

h. Close the tube with the lid and put it away into Box #1 in the fridge for storage and possible future use

i. On the Worksheet for "**CST**" check the appearance of the bead populations and make sure that:

   i. On **FSC/SSC** scatter plot you see two populations within "P1" gate

   ii. For **each channel,** the brightest peak is relatively sharp and located close to the interval gate (+/- 20,000 channel range)

   Note 1: *On LSRII-1 if you didn't turn on UV laser, you're not supposed to see the highest peaks for all UV (355) channels*

   Note 2: *On LSRII-2 if you didn't turn on 445 laser, you're not supposed to see the highest peaks for all 445 channels*

   Note 3: *If some populations are not in the allowed range, please report to FCRC Staff by filling up* ***FCRC Problem Report Form****; and proceed to the "General Notes" at the end of this SOP*

j. **After "QC_Checkup" is completed,** Click "**File**" menu > "**Export**" > "**Experiments**". **Export** the completed experiment to folder "**T:\Application_Settings\QC_Checkup**"

k. Change the flow rate from "**Low**" to "**High**". Wash the instrument with 10% bleach for 5 min, followed by water for 5 min

9. **PROCEED WITH RUNNING YOUR EXPERIMENT: Create a new experiment or import the old one and duplicate without data. Run your experiment. When you finish your experiment, export data to the "T:\ folder". Copy them from "T" to the CFS. Delete your experiment from DiVa Database. Log off DiVa to avoid unnecessary charges**
10. When you are done with the experiment, and if you have used the **UV** or **445 nm laser, be sure to switch it "OFF".** Refer to Step [7] of "**BD LSRII-1 UV Laser Operation**" or Step [3] of "**BD LSRII-2 445 nm Laser Operation**", respectively
11. If in Step [5] you have changed the filter(s) for particular channel(s), please make sure to **switch back to default settings** and put the spare filter(s) back into the storage place
12. When you are done with the experiment **be sure to wash the Instrument (BD LSRII** or **BD LSR-Fortessa**) **properly:**
    a. "RUN" on "High" 2 ml of 10% bleach for 10-15 minutes
    b. "RUN" at "High" 2 ml of MQ Water for 10-15 minutes
    c. Push "Standby" button and leave tube with 1 ml of MQ Water on the SIP
13. Before you leave FCRC please be sure to switch "**OFF**" the BD LSRII (or BD LSR-Fortessa) and FACSFlow system by **clicking only the upper button once** on the on-screen user interface
14. Please **be sure to log off Windows** before you leave FCRC. Otherwise in 10 minutes Windows will automatically lock the session under your name and will prevent other researchers from using the Instrument

General Notes:

Note 1: *Please report all problems / concerns to FCRC Staff as soon as possible by filling up* ***FCRC Problem Report Form****; and FCRC staff will get back to you as soon as possible*

Note 2*: Please leave the note of "****Instrument Out of Order****" on the instrument's keyboard; proceed on step 12-14 from the above list and turn off the instrument*

Note 3*: FCRC Staff will update PPMS to notify users about the instrument issues and block the future instrument booking until issues are solved*