BD LSRIIs and BD LSR-Fortessa Maintenance Guidelines

1. Login into Windows using your RUNet Login and password

2. Check if the on-screen user interface shows **BD LSRII** (or **BD LSR-Fortessa**) and **FACSFlow** system "ON". If it is "OFF" be sure to click only upper button once to switch "ON" both **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 lasers** 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 filter(s) on particular channel(s), for the **first time** please make sure to inform FCRC staff in advance and get proper **training**

6. Before you start running samples please:

   a. **Check if the BD LSRII** (or **BD LSR-Fortessa**) **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 (see the notes on the end of the document)
   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 (at 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 (see the notes on the end of the document)
   c. **Wash the instrument** before you run samples
      i. "Run" at "High" 2 ml of 10% Bleach for 5 minutes

    ii.   "Run" at "High" 2 ml of MQ Water for 5 minutes

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

    *Note: Please be sure in all the cases not to exceed the **maximum allowed volume of 2 ml** in the sample tube*

7. Log in DiVa. Always choose to "use CS&T settings". Create new experiment, or import the old one and duplicate without data. Run your experiment. Export data. Copy them to the CFS. Delete your experiment from the DiVa Database. Log off DiVa to avoid unnecessary charges

8. When you are done with the experiment if you have used the **UV** or **445 nm laser be sure to switch it off.** Refer to the step 7 of **"BD LSRII-1 UV Laser Operation"** or step 3 of **"BD LSRII-2 445 nm Laser Operation"**, respectively

9. If you change filter(s) on particular channel(s) for your experiment, please make sure to **switch back to default settings** and put the spare filter(s) back to the storage place

10. When you are done with the experiment **be sure to wash BD LSRII** (or **BD LSR-Fortessa**) **properly**

    a.   "Run" at "High" 2 ml of 10% Bleach for 10 minutes
    b.   "Run" at "High" 2 ml of MQ Water for 10 minutes
    c.   Push "Standby" button and leave tube with 1 ml of MQ Water on the sip

11. Before you leave FCRC please be sure to switch instrument off if required. **Policy of keeping instruments "ON" or "OFF" depends on the time of the day**

    a.   **During the extended daytime (10:00 am – 10:00 pm)**, we keep the analyzers "**ON**" all the time
    b.   If you finish your BD LSRII (or BD LSR-Fortessa) experiment **after 9:00 pm and you are the last user of the day,** we request to switch "**OFF**" the **BD LSRII** (or **BD LSR-Fortessa**) and **FACSFlow** system by using the on-screen user interface. Please click only upper button once to switch "**OFF**" both **BD LSRII** (or **BD LSR-Fortessa**) and **FACSFlow** system

12. 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 usage of the instrument

Note 1: Please report all the problems / concerns to FCRC Staff:

During business hours *— Go to FCRC office and tell the FCRC staff member*
After hours *— Leave the note on the instrument's keyboard and turn off the instrument if possible. Fill up FCRC Problem Report Form on FCRC website and FCRC staff member will get back to you as soon as possible*

✉ FCRC Problem Report Form
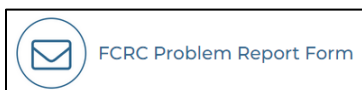
Note 2: FCRC Staff shut down the instruments remotely at 10 pm during weekdays. If you plan to use instrument after regular hours during the weekdays, please notify FCRC in advance by email to FCRC_Staff@rockefeller.edu, FCRC will keep the instrument "ON" and you are responsible to switch instrument "OFF" after your usage