Standard Operating Procedures (SOPs): BD LSRFortessa

General remarks

Following the introduction course and signing the Cell Analytics Facility policies are mandatory prerequisite to use the BD LSRFortessa.

Report any technical problems or questions to:

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I.   Instrument Start-Up

- Check the level of FACSFlow and replace with a new box if needed (lift to check).
- Empty the waste in the red containers if needed.

  If the instrument is running while you need to change a solution, switch off the supply system chariot! When done press "restart" on the Supply System chariot.

- Press the green button on the right side of the instrument.
- Allow the laser to warm-up for 30min
- Turn on the computer, log into windows. **Password = BDIS**
- Start FACSDiva Software and login within the **USERS session, no password**.
- Wait for the software to connect with the cytometer.
- Accept "Use CS&T Settings" if prompted.
- Perform 3x "Prime" on the instrument with a DI water tube attached.

  Change DI water tube after priming! It will be contaminated.

  It's a good idea to pass some clean-rinse-water 5-10min each during warm-up.

II.   Acquisition

- Create a folder with your name.
- Create a new experiment or import a previous experiment template.
- Delete all non-needed parameters of the cytometer settings.
- Draw all needed graphs and prepare the worksheet.
- Make sure you have filtered and vortexed your samples well.

  When analyzing large cells, filter sample with 0.45µm filter directly before analysis.

- Place your sample tube on the SIT arm and press "Run" on the instrument.

  Do not switch in between samples on "Standby" because this will clog the sample needle! Instead remove the tube from the instrument and leave the sample arm open (backflush dripping to avoid carry over and clogs between samples).

  NEVER put the instrument in STANDBY without cleaning it.

  NEVER put on STANDBY while running a sample.

- Activate the tube in Diva software and press "Acquire".
- Adjust the voltage for FSC & SSC to observe your cells on scale.

  Lower the voltage for fluorescence channels only if off scale due to high fluorescence intensity. CS&T PMT voltage settings are minimum voltages for optimal resolution.

- For compensation go to "Experiment → Compensation Setup" and record your single stains. Then go to "Experiment → Compensation Setup → Calculate compensation"

- Set your gates and the cell number you would like to measure and press "Record". Use the "low" speed to avoid big CVs.

III.   Data Export

Note: All data has to be deleted from the Diva database before you log off!

- Export your data as FCS files (3.0 or 3.1; never as 2.0!) to the "data" folder on the user folder. Important: Open the destination folder and check that the size of each FCS file is > 0 kb. If files have 0 kb this may indicate a corrupted data export and you will need to repeat the export procedure!
- If you wish to keep your experiment settings as a template, right click the experiment and choose "Duplicate without data".
- Before closing FACSDiva you have to remove all experiments from the database! Only keep templates.

IV.   Cleaning

Note: The Fortessa has no shutdown procedure; therefore, the cleaning process is very essential to avoid clogging or contamination of the instrument.

- The instrument must be cleaned between every user. If you are running problematic samples, you might have to clean in between your experiments to avoid clogging.

- **Set the flow rate to "High", open the arm of the sample holder**
- **Run FACSClean with the open arm for 1min**
- **Close the arm under the sample and run FACSClean for 5min**
- **Repeat the procedure with FACSRinse**
- **Repeat the procedure with a new DI Water tube**

- Clean DI H2O should show a threshold rate of 0-10 events/sec. If not, repeat the cleaning procedure.
- Switch the instrument to "Standby" and "Low" flow rate and leave the water tube on the SIT arm to avoid salt crystallization instrument.

V.   Shutdown

- Check on Open IRIS if somebody is booked after you: http://iris.science-it.ch

- *Between different users of the day:*
  - o   Close the FACSDiva Software.
  - o   Leave instrument and computer running for the next user.
  - o   Clean the work area - don't leave used tubes, gloves, and etc. behind.

- *Last user of the day:*
  - o   Close the FACSDiva Software.
  - o   Shut down the computer.
  - o   Turn off the instrument by pressing the green button on the right side of the Fortessa.
  - o   Clean the work area – don't leave used tubes, gloves, and etc. behind.