

Standard Operating Procedures (SOPs): BD FACSCanto II

General remarks

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

Report any technical problems or questions to:

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Be gentle with the SIT !! Always wait for the backflush to be performed, never force the arm!

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.
- Turn on the computer, log into windows. **Password = BDIS**
- Press the green button on the left side of the instrument.
- 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.
- “Cytometer → Fluidic Startup” (take 10min)
- Check the waste, FACSFlow, Shutdown and cleaning solution level (green square) and change if needed.

If the instrument is running while you need to change a solution, it will beep. Just change the solution, and then go on “Cytometer → Cleaning Modes → Bubble filter purge after tank refill”

- Allow the laser to warm-up for 20min
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.
- Activate the tube in Diva software and press “Acquire”.
Maximum event rate is 10'000 events/sec.
- 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. Cleaning

Note: 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”**
- **Run FACSClean for 5min**
- **Run FACSrinse for 5min**
- **Run DI Water for 5min**

- Clean DI H₂O should show a threshold rate of 0-10 events/sec. If not, repeat the cleaning procedure.
- Do not leave any tube on the instrument !

IV. Shutdown

- Check on Open IRIS if somebody is booked after you: <http://iris.science-it.ch>
- *Between different users of the day:*
 - Logout the FACSDiva Software.
 - Leave instrument and computer running for the next user.
 - Clean the work area - don't leave used tubes, gloves, and etc behind.
- *Last user of the day:*
 - Run the “fluidics shutdown ” procedure on “Cytometer” (take 10min).
 - Close the FACSDiva Software.
 - Shut down the computer.
 - Turn off the instrument by pressing the green button on the left side of the FACSCanto II.
 - Clean the work area – don't leave used tubes, gloves, and etc behind.

V. 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.