General

Registration with the Cytometry Facility, as well as attending the technical introduction by the facility staff is a prerequisite to use the Symphony**.**

Questions / reporting technical problems:

Feedback form

Emergencies:

079 252 27 65 (Priya)
079 831 55 96 (Philipp)

I. Instrument startup

1. Press the green button on the right side of the Symphony (before starting the computer!).
2. Turn on the instrument PC and log in using your core domain user account.
   For instance John Doe: user name = j.doe
3. Wait for the BD Coherent Connection software to start up and connect to all 5 lasers. If any laser fails to connect, shutdown the computer and Symphony and repeat the startup. See BD Coherent Connection SOP on how to start the UV laser if needed.
4. Close the BD Coherent Connection software after confirming all lasers are connected.
5. Start FACSDiva Software and log in with **Diva** and **no password** (simply press enter).
6. Wait for the software to connect with the cytometer.
7. Accept “Use CS&T Settings” if prompted.
8. Perform “Prime” on the instrument without a water tube attached. Repeat one more time.

II. Acquisition

- Create a new experiment or open a previous experiment template.
- Make sure you have filtered and vortexed your samples well.
  Filter your samples using a filter mesh size appropriate for your cell size to remove larger aggregates (generally ca. 35 – 70 µm).
- Place your sample tube on the SIT arm and press “Run” on the instrument. Keep the system on “Run” when switching samples to flush the needle.
- Press “Acquire” after activating the correct tube in FACSDiva (green arrow).
- Adjust sample flow rate if needed. We recommend lower flow rates for better data quality.
- Adjust the voltage for FSC & SSC to observe your cells on scale.
- Optimize the voltages for the fluorescence channels according to your panel (use at least 500V, if possible, for best signal-to-noise ratio).
- For compensation go to Experiment -> Compensation Setup and record your single stains.
- Set your gates and the event number you would like to measure and press “Record”.

III. Wet Cart Handling

- See Wet Cart SOP on how to change FACSFlow or waste on the wet cart.

IV. Data Export

Note: Delete all data from the Diva database before you log off!

- Export your data as FCS files v3.1 (**never as 2.0!**) to the “data” folder on our server (data storage for 3 months) or to your institute server (if available). **Important: Check that the size of each exported FCS file is > 0 kb in destination folder.** If files have 0 kb, repeat the export procedure!

- To protect yourself from data loss due to incorrectly exported FCS files you may additionally export your data as "*Experiment"* to your "data" folder on the server.
- If you wish to keep your experiment settings as a template, right click the experiment and choose "Duplicate without data". Save the empty duplicate in the "Home" folder on the server for later use. You can reimport a template using File → Import.
- For easy reimport of your experiment with the data, export as "Experiment". However, the FCS files created that way must not be used for analysis in third party software!
- **Before closing Diva, remove all experiments and templates from the database!**
- For safety reasons, the use of USB sticks is blocked.

V. Cleaning

- **The instrument must be cleaned between every user**. If you are running problematic samples, you might have to clean in between samples to avoid clogging.
- Set the sample flow rate to "High" and run
  a) 3 min **FACS Clean** (bleach)
  b) 3 min **FACS Rinse** (detergent)
  c) 3 min **DI H_2O**
- Confirm that the instrument is clean during the water run by setting FSC to 350 V.
  Clean DI H_2O should show a threshold rate of **0-4 events/sec** on average.
- If you are the **last user of the day**, record the water run and save it as PDF under "D:/Last User Clean" with your name and date.

VI. Handover / Shutdown

- Check the instrument booking calendar to see if somebody is booked after you.
- **Between different users of the day:**
  a) Clean the instrument (see Cleaning).
  b) Delete your experiments in FACSDiva and close the software.
  c) Log out of the computer. Please note that your log out time may be used for billing.
  d) Switch the instrument to "Standby" and "Low" flow rate and leave the H_2O tube on the instrument. **Make sure that there is enough room in the tube for 1 – 2 ml backflow!**
  e) Leave instrument and computer on for the next user & clean the work area
- **Last user of the day:**
  a) Clean the instrument (see Cleaning) and save the water run PDF.
  b) Delete your experiments in FACSDiva and close the software.
  c) Switch the instrument to "Standby" and "Low" flow rate and leave the H_2O tube on the instrument. **Make sure that there is enough room in the tube for 1 – 2 ml backflow!**
  d) Turn off the computer & clean the work area.
  e) Turn off the instrument by pressing the green button on the right side of the Symphony.

Note: Any violation of these rules will result in penalty points (see SOP "Penalty points")!