General remarks

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

Questions / reporting technical problems:	Emergencies:
Feedback form	**+41 79 152 20 69 (Mario)** **+41 79 559 65 58 (Claudia)**

I. Instrument startup

1. Start the instrument and support computers, set the switch board to "support" and login with your core domain login at the support computer. The connection to the instrument computer will be established automatically (in case of failure, establish the connection via the shortcut "Connect to Instrument Computer" on the desktop of the support computer or switch the switch board to "instrument" and use the "Admin" account on the local computer, password "BDIS#1").
2. Start FACSDiva Software and log in with **Diva** and **no password**.
3. Press the green button on the left side of the instrument.
4. Wait for the software to connect with the cytometer.
   For troubleshooting see SOP "Reconnecting Cytometer & Computer"
5. Accept "Use CS&T Settings" if prompted.
6. **Check the sheath filter at the wet cart for air bubbles**. If necessary, release the upper white cap to bleed the filter. Close cap shortly before liquids runs out of the filter.
7. Go to "Cytometer" and perform a "Fluidics Startup".

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 the tube with your cells on the SIT arm.
- Activate the tube in the Diva software and press "Acquire".
- Adjust the flow rate and 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.
- Set your gates and the cell number you would like to measure and press "Record".

III. Changing empty FACSFlow container

- In case the FACSFlow is "empty" (shown in the DIVA Software as red sheath fluid container), replace the empty 20 L box with a new one. The container will still contain about a third of liquid. Label it "from Canto" and place it next to the FACSymphony.
- The instrument will recognize that the FACSFlow is full again. In the DIVA software the red container will switch to being green. Prime the tank line to remove air bubbles: Cytometer → Cleaning modes → Prime after tank refill.

IV. Data Export

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

- Export your data as *FCS* files (**use the newest format FCS 3.1**) to the "data" folder on our server (data storage for 3 months) or to your institute server (if available). **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.
- To protect yourself from data loss due to incorrectly exported FCS files we strongly recommend to 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 (1GB size, will stay on the server as long you are registered with the facility) for later use (Right click → Export → *Experiments*). You can reimport a template using the import function (File → Import.).
- **Before closing DIVA software, you have to remove all experiments and templates from the database**
- For safety reasons the use of USB sticks is blocked. Check "Data Management & Access" under "IT Infrastructure" on our website for more information.

V. Cleaning

- The instrument must be cleaned between every user. If you are running problematic samples, you might have to clean in between your samples to avoid clogging.
- Set the 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 400 V.
  Clean DI H_2O should show a threshold rate of 0-4 events/sec.

VI. Handover / Shutdown

- Check in the instrument booking calendar if somebody is booked after you.
- **Between different users of the day:**
  a) Close the FACSDiva Software <u>without performing a fluidics shutdown</u>.
  b) Log out of the computer. Please note that your log out time may be used for billing.
  c) Leave instrument and computer running for the next user.
  d) Clean the work area.
- **Last user of the day:**
  a) Check shutdown solution level. If required refill with ddH_2O from tank opposite the device next to the Symphony or tank on the sink in Y44G3h and perform fluidics shutdown "Cytometer" → "Fluidics Shutdown".
  b) After fluidics shutdown is complete, close FACSDiva and turn off the computers.
  c) Turn off the instrument by pressing the green button on the left side of the Canto.
  d) Exchange the waste tank. Attach an empty tank to the device and decontaminate the full waste container.
  e) Clean the work area – don't leave used tubes, gloves, etc. behind.