## General remarks

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

Questions / reporting technical problems:           Emergencies:

⌨ **Feedback form**

☎ **044 635 02 17**
☎ **044 635 02 07**

## I. Task for the 1ˢᵗ user of the day

- Check that a spare waste tank is available next to the instrument. If you cannot find any, empty the decontaminated waste tank in the cell culture (Y44G3h) and bring it back to the instrument.

## II. Instrument startup

1. Turn on the computer and log in using your core domain user account.
   For instance John Doe → user name = j.doe
2. Start FACSDiva Software and log in with **Diva** and **no password**.
3. Press the green button on the right 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. Perform a "Prime" on the instrument without a water tube attached.

## III. Acquisition

- Create a new experiment or import 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.
- Activate the tube in Diva software and press "Acquire".
- Adjust 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".

## IV. Wet Cart Handling

- See "Wet Cart SOP" on how to change FACSFlow and waste on the instrument.

## V. Data Export

Note: Delete all data 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 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, 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 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.

## VI. 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" and run
    a) **3 min FACS Clean** (bleach)
    b) **3 min FACS Rinse** (detergent)
    c) **3 min DI H$_2$O**
- Confirm that the instrument is clean during the water run by setting FSC to 450 V.
  Clean DI H$_2$O should show a threshold rate of 0-4 events/sec.
- **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.**
- Switch the instrument to "Standby" and "Low" flow rate and leave the H$_2$O tube on the SIT arm to avoid salt crystallization. **Make sure that there is enough room in the tube for 1 – 2 ml backflow!**

## VII. Handover / Shutdown

- Check the instrument booking calendar if somebody is booked after you.
- **Between different users of the day:**
    a) Close the FACSDiva Software.
    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) After cleaning the instrument and saving the water run pdf, close the FACSDiva software.
    b) Shut down the computer.
    c) Turn off the instrument by pressing the green button on the right side of the Fortessa.
    d) Clean the work area – don't leave used tubes, gloves, etc. behind.

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