

## 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:



**Feedback form**

Emergencies:

☎ 079 152 20 69 (Mario)

☎ 079 684 56 81 (Tatiane)

## I. Task for the 1<sup>st</sup> user of the day

- Check that a spare waste tank is available next to the instrument otherwise check the sink and empty the inactivated waste (tank should have been labelled with “bleach added”).

## 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 the 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 your 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 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”. Next, save the empty duplicate in the “home” folder on the server for later use (Right click → Export → *Experiments*; *note that* the files will stay on the server as long your account is active). You can reimport a template using the import function (File → Import.).
- **Before closing Diva you need to remove all experiments and templates from the database!**
- For IT safety reasons the use of USB sticks is blocked. Check our website “IT Infrastructure” → ”Data Access & Saving Policies” for more information.

## VI. Cleaning

**Note: The Fortessa has no shutdown procedure, therefore the cleaning process is 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 need 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<sub>2</sub>O**
- Confirm that the instrument is clean during the water run by setting FSC to 450 V. Clean DI H<sub>2</sub>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<sub>2</sub>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 to see 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 both computers.
  - 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”)!