

On Weekdays, if there is a slot available at 9am, the UCF staff will switch the machine ON and perform the Quality Control.

If the cytometer will not be used within 3 hours, UCF staff will turn the cytometer OFF. In this case, if you are the next user you must turn it ON 30 minutes prior acquisition.

If you are not sure if the cytometer is ON or OFF, please stop by the unit to check.

On Weekends, Holidays and outside weekly working hours (9am to 6pm), if you are the 1st user of the day you must turn ON the machine 30 minutes prior acquisition and perform the Start-up Cleaning Procedure.

1. Turn ON the FFSS (on the floor)
2. Turn on the computer and the cytometer
3. Login with your Agendo Credentials:
Username – **Your email** (@*medicina.ulisboa.pt* - for internal users)
Password - **Your Agendo's password**
4. Login on DIVA using your own password
5. Wait for cytometer's connection and click "**Use CST Settings**"
6. **Make sure the sheath tank is full before you start.** If the sheath tank becomes empty during acquisition it will cause major problems in the instrument.

STARTING UP

- **(if you are the first user of the day and the machine is OFF)**
 7. Wait 30 minutes for the lasers to warm up and stabilize while performing the daily cleaning procedure:
 8. Remove the tube with Water.
 9. Run a tube with FACSClean on HIGH for 10 min (support arm closed)
 10. Run a tube with WATER+AZIDE on HIGH for 10 min (support arm closed)
 11. Run a tube with PBS on LOW (support arm closed) for 1 min.
 12. Fortessa 1 has the PerCP filter by default. Fortessa 2 has PerCP-Cy5.5 by default. If you need you can change them. **BUT DO NOT FORGET TO REVERT TO THE DEFAULT CONFIGURATION WHEN YOU FINISH.**
 13. Run your samples.
- **(if the machine is ON)**
 14. Run a tube with PBS on LOW (support arm closed) for 1 min.
 15. Fortessa 1 has the PerCP filter by default. Fortessa 2 has PerCP-Cy5.5 by default. If you need you can change them. **BUT DO NOT FORGET TO REVERT TO THE DEFAULT CONFIGURATION WHEN YOU FINISH.**
 16. Run your samples.

FINISHING ACQUISITION AND DATA HANDLING

17. **REPLACE THE DEFAULT FILTERS ON THE CYTOMETERS IF YOU HAVE MADE ANY CHANGES.**

18. **Always export your data** to your Lab folder on the IMM server (shortcut on the desktop). To access the IMM server you must use your email credentials. After this, right click on the experiment > Export > Experiment > Choose folder.

19. If you want, **export Experiment Template** into your Lab folder in order to keep a template with settings in the computer (right click on the experiment > Export > Experiment Template > On "Type" select your Lab)

20. If you want to analyze your data in one of our Analysis Stations, you can also **export your data directly to them**. To access these stations, right click on the shortcuts on the desktop (MacFlow 1 and 2) and use the following credentials:

User- flowcytometryuser Password- BDIS

21. Delete your data from DIVA as soon as you are certain that your data has been copied to the network and you have your own backup. **You cannot store experiments on DIVA for long periods of time as the software will start to malfunction. You must delete your data from DIVA and to keep backups.**

22. **Clean the cytometer according the protocol on top of the table. DO NOT FORGET TO RECORD IT.**

23. **Refill the Sheath tank**

24. If during your acquisition, **the waste container gets full, a HIGH PITCH SOUND will go off**. You should disconnect the black sensor (the noise will stop). As soon as possible, when convenient, put the cytometer in **Standby**, and **disconnect the orange connector**. Close the container with the red cap that is on top of it and replace the container with a new one.

TURNING OFF THE MACHINE

Switch OFF the system if you are the last user booked or the cytometer will not be used for at least 3 hours. Penalties will incur if you fail to do so.

1. Turn off the cytometer
2. Turn off the computer
3. Turn off the FFSS