

During weekdays and regular working hours (Mon-Fri 9am-6pm), the Flow Cytometry staff will switch on the machines before first booking and perform the Quality Control every day.

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. If the cytometer is turned off:
 - Turn on the cytometer and the computer. During start up, the cytometer automatically flushes the fluidic lines with sheath (dH₂O). This process takes approximately 5 minutes.
 - Login with your Agendo Credentials:
Username - **Your email** (@*medicina.ulisboa.pt* - for internal users)
Password - **Your Agendo's password**
 - Open the software. Place an empty tube on the SIP and click backflush.
 - Place a fresh tube with dH₂O, set time limit for 15 min and fluidics speed to fast. Click RUN. Once time is reached, click DELETE SAMPLE DATA.
2. If the cytometer is turned on:
 - Login with your Agendo Credentials:
Username- **Your email** Password- **Your Agendo's password**
 - Open the software

CHECK FLUID AND WASTE LEVELS

3. **Make sure the sheath bottle has enough dH₂O before you start.** If the sheath bottle is empty refill it with dH₂O (5L reservoir on the floor).
4. Check the Waste bottle and make sure it is empty.

STARTING ACQUISITION

5. Remove the tube with dH₂O that is on the SIP: **while holding the tube, gently push the sample stage back.**
6. Place an empty tube on the SIP and click backflush.
7. Remove the backflush tube and clean the needle using a paper towel.
8. Place your sample on the SIP and **pull the stage forward to support the tube.** The stage clicks into place.
9. Run your samples. Clean the needle using paper towel between samples and use backflush function if necessary.
10. If your samples are too sticky and/or if no cells are going through, perform a "SIP Clean" procedure to solve the clogging issue.

FINISHING ACQUISITION AND DATA HANDLING

11. **REPLACE THE DEFAULT FILTERS ON THE CYTOMETER IF YOU HAVE MADE ANY CHANGES.**

12. **Save your experiment (.ci)** (File > Save workspace) and **always export the FCS files** (File > Export all samples as FCS). FCS Files are automatically exported to the 'FCS Exports' folder on the Desktop. Move your fcs files to the iMM server (shortcut on the desktop). To access the iMM server you must use your email credentials.

13. If you want to analyze your data in one of our Analysis Stations, you can also **export your fcs files 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

14. If you want, **save a template (.cit)** into your Lab folder on the Accuri Experiments folder (on the desktop) in order to keep your settings (File > Save template)

15. **Clean the cytometer:**

- a. Place a tube with **FACSClean solution**, select an empty well and run for **10 minutes on FAST speed**; Repeat the same procedure with **dH₂O** for **5 minutes on FAST speed**.
- b. Leave the dH₂O tube on the cytometer

16. **If necessary, refill the sheath bottle with dH₂O and empty the waste bottle.**

TURNING OFF THE MACHINE

After 5pm, during weekends and holidays, if you are the last user it is your responsibility to switch off the equipment. Penalties will incur if you fail to do so.

1. Turn off the cytometer. The shutdown cycle runs for approximately 15 minutes and then the cytometer automatically powers off.
2. Turn off the computer. (you don't have to wait until the end of cytometer shutdown)