

**INSTITUTO DE MEDICINA MOLECULAR JOÃO LOBO ANTUNES
FLOW CYTOMETRY FACILITY
USER GUIDELINES**

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Cell Analyzers

BD Accuri C6 Plus

BD LSRFortessa

BD LSRFortessa X-20

BD FACSymphony A5 SE

CYTEK Aurora

CYTEK Amnis ImageStreamX MKII

How to get training and permission to use the analyzers:

- All new users, regardless of their previous expertise, must undergo two sessions of training: a theoretical session, and a practical session. You can reach out to us in person (**room P2A-32**), by phone (ext. **47224**), email (imm-flowcytometry@medicina.ulisboa.pt), or use the Agendo booking system (imm.agendoscience.com) to request access to a cell analyser. To use the Agendo booking system, you must create an Agendo account.
- The theoretical training session happens regularly, and you can book it on the Flow Cytometry Wiki (imm.medicina.ulisboa.pt/facility/flowcytometry) (New user > Principles of Flow Cytometry - all analyzers - and/or Principles of Spectral Flow Cytometry- spectral instruments). Afterwards, the facility staff will schedule the practical training session on the cell analyser, depending on the equipment availability and the planned date of your first experiment. Ideally, you should be trained a few days before your first usage. The training duration will depend on your know-how and previous expertise. The training can be individual, or performed with a small group of users.
- After the training, you will be kept as an “in training” user on the Agendo booking system for the next few sessions. This means that when you book a slot, the facility staff will receive a notification that you will need assistance, and one of the members will be present to answer your questions as they arise and guide you through the acquisition protocol. Therefore, you will only be able to book during facility hours (9h to 18h on weekdays).
- You will be given full permission as soon as you are autonomously using the equipment.

Rules for analyzer usage

- Your bookings must be under your own name.
- You must have a valid reservation in the booking system before using an analyser.
- The analyzers can be booked up to 1 month in advance.
- You must include 15 minutes in your booking for the cleaning protocol.
- On weekdays, if there is a slot available at 9h, the UCF staff will switch the machines ON, perform the Start-up Cleaning Procedure, and perform the Quality Control check.
- On Weekends, Holidays and outside weekly working hours (9h to 18h), 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 (Page 4-6).

- If you need help in your acquisition, you can ask for assistance as you book your slot on the Agendo booking system. Assistance requests for the analysers work as pre-reservations and require confirmation from the UCF staff according to staff availability. Therefore, make your reservations in advance to ensure that one of the staff members is available.
- We provide assistance on weekdays from 10h to 17h00.
- Do not request assistance during lunchtime (12h to 14h).
- Users can book unlimited slots on the analysers.
- Bookings can only be cancelled up to 3 hours before the slot starting time.
- Last minute cancelations (within 3h before the booking start time) must be requested to the UCF staff, and may result in penalization. The penalty charge corresponds to the running cost of the system, if the session is not booked by another user.
- Always record start and finishing times on the log sheet (cleaning time included). Use the log sheet to report any problems, or if the user before you did not leave the cytometer in appropriate conditions.
- If you swapped optical filters, you must replace them at the end, leaving the system with the default configuration.
- You must exit the analyzer software and log off from Windows at the end of your session.
- On *BD LSRFortessa*, *BD LSRFortessa X20* and *Cytek Amnis ImageStreamX*: If you are the last user booked or if the analyzer will not be used for at least 3h, you must switch OFF the system.
On *BD Accuri C6 Plus*, *BD FACSymphony A5 SE* and *CYTEK Aurora*: Weekly, UCF Staff will turn OFF the system at 17h, if there is no one booked afterwards. Thus, during weekdays from 9h to 17h, you do not need to turn OFF these equipment. However, weekly after 17h, or on weekends, if you are the last user booked, you must switch OFF the system.
If the systems are not switched OFF at night, the last user booked will be charged the number of hours the system was left ON. The facility staff will change your booking time to comprise the full time the system was ON.
- Users are fully responsible for exporting their own data when they finish their acquisition.
- After your acquisition, it is mandatory to perform and record the cleaning protocol (Page 6-8).
- On *BD LSRFortessa*, *BD FACSymphony* and *CYTEK Aurora*: when you finish, refill the sheath tank with PBS+Azide. If during your acquisition, the waste container gets full, a high pitch sound will go off. You should disconnect the black sensor to stop the noise. As soon as possible, put the cytometer on Standby, and disconnect the orange connector. Replace the lid of the new container, and re-attach the connectors.
On *BD Accuri C6*: when you finish, check if the waste reservoir is full (empty it if necessary), refill the sheath tank with H₂O at the end of your session.
On *Amnis ImageStreamX*: when you start, check if the waste reservoir is full (empty it if necessary), refill the sheath tank with PBS and check the beads and the buffer container levels.



Failure to comply with these rules will result in penalty charges and/or user banning

Usage Log on BD LSRFortessas, BD FACSymphony A5 SE, CYTEK Aurora and BD Accuri C6 Plus software

Each user is given an individual login and password for FACSDiva and SpectroFlo. For Accuri C6 Plus software, there is a general password and user account that will be provided. This will enable us to track instrument usage per user and adjust billing charges. It is mandatory to **logout from FACSDiva, SpectroFlo and Accuri C6 Plus** software at the end of your session. It is also mandatory to **logout from Windows (BD LSR Fortessas, BD FACSymphony A5 SE and BD Accuri C6 Plus)** at the end of your session. We will use the Agendo login to calculate the monthly billing, and you may incur in extra charges if you do not log out from the software. Users will be billed according to the actual starting time or booked starting time (whichever occurs first), and the actual ending time or booked ending time (whichever occurs last).

Data management on analyzers

The Flow Cytometry staff is not responsible for data handling and storage

- Users **must always export** their data **and delete** it from the database. Do not book the system for data transfer or data analysis, otherwise you will be charged. If you only use the computer to export data, check with an X the “Diva only”, “Spectroflo only” or “PC only” (depending on which equipment you are using) box in the log sheet. The use of Diva and SpectroFlo to analyse data will be charged. To analyze your data, you can also use the MacFlow workstation (free of charge).
- Keeping data on the acquisition software may result in database problems, which can only be solved by restoring the database with an empty one, **resulting in ALL DATA being deleted**.
- Data transfer using USB connected devices is **prohibited** due to the high risk of virus or malware contamination.

Cleaning Protocols

On weekdays, the UCF staff arrives at 9h, and performs the following tasks:

- Turning on all machines
- Cleaning procedure
- Quality control check

This means that at around 9h30, the machines are ready to be used. Each instrument also has a weekly “Long Clean” that takes up a full morning.

On Weekends, Holidays, and outside weekly working hours (9h to 18h), if **you are the first user of the day**, you must turn ON the machine 30 minutes prior acquisition, and perform the Start-up Cleaning Procedure.

For BD Accuri C6 Plus:

Are you starting up?

(First user of the day on Weekends, Holidays and outside weekly working hours)

Turn on the Cytometer and the Computer
Login on Windows and Accuri C6 Plus software
Follow the **startup cleaning procedure**:



WATER+AZIDE
RUN + FAST
wait 15 min

You are ready to run your samples!

Thank you!

For BD LSRFortessa 2

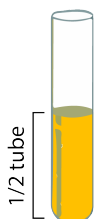
For BD LSRFortessa X20:

Are you starting up?

(First user of the day on Weekends, Holidays and outside weekly working hours)

⚠ Please don't prime

Turn on the Computer, the Cytometer and the FFSS
Login on Windows and FACSDiva
Follow the **startup cleaning procedure**:



CLEAN
RUN + HIGH
arm to the center
wait 10 min



WATER+AZIDE
RUN + HIGH
arm to the center
wait 10 min

You are ready to run your samples!

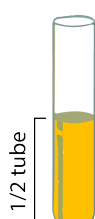
Thank you!

Are you starting up?

(First user of the day on Weekends, Holidays and outside weekly working hours)

⚠ Please don't prime

Turn on the Computer and the Cytometer
Login on Windows and FACSDiva
Follow the **startup cleaning procedure**:



CLEAN
RUN + HIGH
arm to the center
wait 10 min



WATER+AZIDE
RUN + HIGH
arm to the center
wait 10 min

You are ready to run your samples!

Thank you!

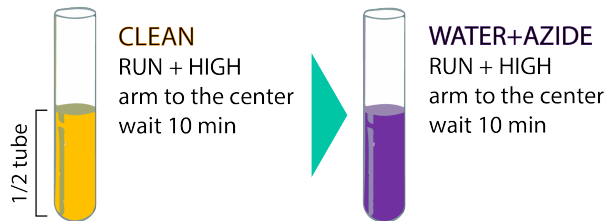
For BD FACSymphony A5 SE

For CYTEK Aurora:

Are you starting up?

(First user of the day on Weekends, Holidays and outside weekly working hours)

Turn on the Computer, the Cytometer and the FFSS.
Login on Windows and turn ON all the lasers on the BD Coherent Connection 4.
Login on BD FACSDiva.
Follow the startup **cleaning procedure**:



Perform the CS&T Check.
Run the Quality Control with CS&T beads.

You are ready to run your samples!

Thank you!

Are you starting up?

(First user of the day on Weekends, Holidays and outside weekly working hours)

Turn on the Computer and the Cytometer.
Login on Windows and Login on SpectroFlo.
Follow the startup **cleaning procedure**:



Run the Quality Control with SpectroFlo beads.

You are ready to run your samples!

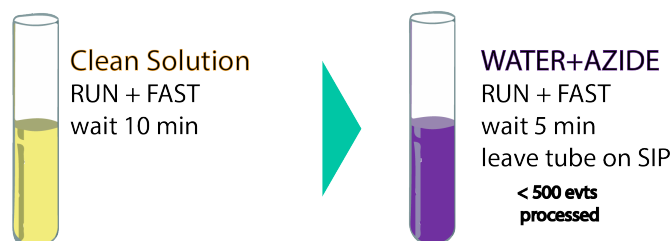
Thank you!

After your acquisition, it is mandatory to perform and record the following cleaning procedures:

For BD Accuri C6 Plus:

Are you Done?

After acquiring your data, please **export it** to the server and **delete it** from the computer
Start the **cleaning procedure** and **record the cleaning steps** in Accuri C6 Plus:



Quit from Accuri C6 Plus and Log off from Windows
Refill the sheat tank with diH₂O
Empty the waste reservoir, in case it is halfway full

Thank you!

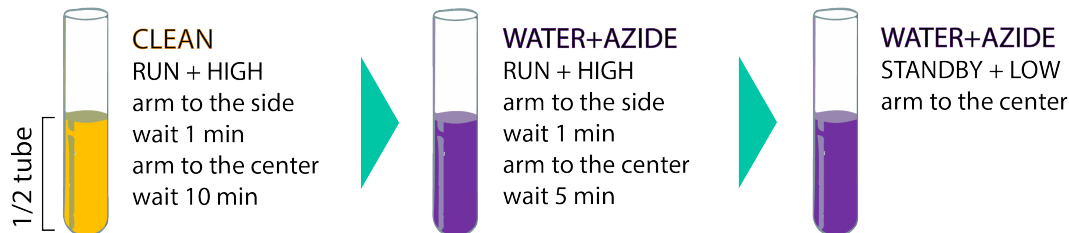
For BD LSRFortessa and LSRFortessa X-20:

Are you Done?

After acquiring your data, please **export it** to the server and **delete it** from the computer

Prepare new **CLEAN** and **WATER+AZIDE** tubes

Start the **cleaning procedure** and **record the cleaning steps** in FACSDiva:



Log off from FACSDiva and Windows

Refill the sheat tank with PBS + Azide

Replace the waste reservoir, in case it gets full (sound bip)

record the cleaning steps



Thank you!

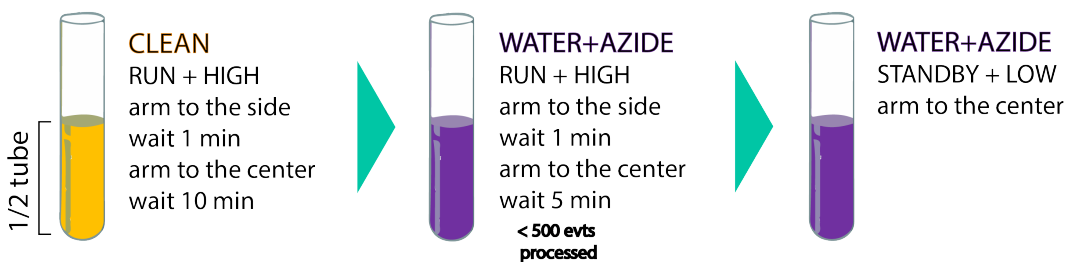
For BD FACSymphony A5 SE:

Are you Done?

After acquiring your data, please **export it** to the server and **delete it** from the computer

Prepare new **CLEAN** and **WATER+AZIDE** tubes

Start the **cleaning procedure** and **record the cleaning steps** in FACSDiva:



record the cleaning steps



Turn off all the lasers on the BD Coherent Connection 4.

Log off from FACSDiva and Windows

Refill the sheat tank with PBS + Azide

Replace the waste reservoir, in case it gets full.

Thank you!

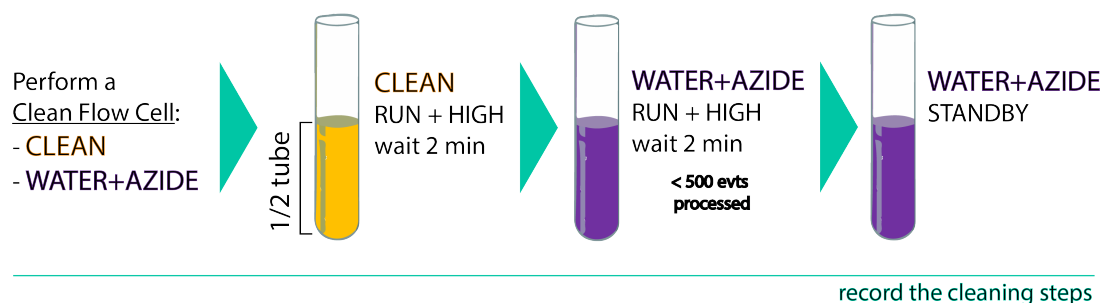
For CYTEK Aurora:

Are you Done?

After acquiring your data, please **export it** to the server and **delete it** from the computer

Prepare new **CLEAN** and **WATER+AZIDE** tubes

Start the following **cleaning procedures** and **record the cleaning steps** in SpectroFlo:



Sign Out from SpectroFlo and leave the software open

Refill the sheath tank with PBS + Azide

Replace the waste reservoir, in case it gets full.

Thank you!

Performing the cleanings as indicated ensures that the performance of the instruments remains stable and optimal throughout the day. Please be mindful of others, make sure you refill the sheath tank, leave the workspace clean and ready for the next user, and contact them if you finish early. Remove anything that does not belong to the core facility, including used gloves, pipettes, tip boxes or FACS tubes. Do not damage facility equipment and resources.

Material that is left behind can be found on the “lost and found” area of the facility.

In case of technical problems or necessary maintenance, resources will be blocked on the booking calendar by UCF staff as quickly as possible and users with conflicting reservations will be notified.

Cell Sorters

BD FACSAria III

BD FACSAria Fusion

BD FACSsymphony S6 SE

Rules for sorter usage

- The sorters can be operated by **Flow Cytometry Facility Staff** and by **Power Users** (for more information on this topic, please talk to the UCF staff).
- You can reach out to us in person (**room P2A-32**), by phone (ext. **47224**), email (**imm-flowcytometry@medicina.ulisboa.pt**), or use the Agendo booking system (**imm.agendoscience.com**) to request access to the cell sorter.
- Your bookings must be under your own name.
- Reservations for the cell sorters work as pre-reservations and require confirmation by the UCF staff according to staff availability. Therefore, make your reservations in advance to ensure that one of the staff members is available.
- Users must have a valid reservation on the booking system for sorting. If you agree to swap your sort with a colleague, this must be updated in the booking system, or communicated to the UCF staff.
- The cell sorters can be booked **up to 1 month in advance**, between 10h and 18h.
- If you exceptionally need to book a sorter outside the regular working hours, please contact the UCF staff beforehand to check for staff availability.
- When booking, **include time for performing new settings, purity checks and cleaning** (minimum 30 min).
- Please **contact us if you make a last-minute reservation on the booking system**.
- Sorter cancellations can be made in the Agendo software **up to 7 days in advance**. Cancellations within 6 days in advance must be communicated to the UCF staff, and may result in a penalization. The **penalty charge** corresponds to the running cost of the system if the session is not booked by another user.
- **No shows** will be charged the running cost of the system for the duration of the booked slot.
- If you are late for a sort, contact the UCF staff as soon as possible. If the sorter is already running when you notify us of the delay, you will be charged according to the booking start time.

Acknowledgements

If publications or any other formal presentations were supported by data generated on our equipment, or by the services provided by the Flow Cytometry Facility of IMM, this should be acknowledged, representing the effort of the staff and funding. You are welcome to use the following examples for reference:

In Materials and Methods:

“All Flow Cytometry experiments were performed at the Flow Cytometry Facility of Instituto de Medicina Molecular João Lobo Antunes, Lisboa, Portugal”.

In Acknowledgements:

“We would like to thank the Flow Cytometry Facility of Instituto de Medicina Molecular João Lobo Antunes for their services and assistance”.

Important Information and Communication with the Flow Cytometry staff

If you want to know **more about our Facility**;

If you want to use our cytometers and you want to know **How to Prepare your samples**;

If you want to know **what to expect when sorting**;

If you want training and some tips and tricks on **How to analyse your data**;

Come by the Unit (P2A49) and visit our Wiki:

imm.medicina.ulisboa.pt/facility/flowcytometry

- Talk to us: before, during and after. We are here to help!
- Use the email imm-flowcytometry@medicina.ulisboa.pt for cancellations, questions, suggestions, complaints, etc.