

UCF IMM USER GUIDELINES

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ANALYZERS

How to get permissions to use the facility and get training:

- All users, regardless of their previous expertise, must talk to UCF staff before using the analyzers. This is to make everyone aware on how the unit operates, get access to the room and the booking system, etc. Get in touch in person or by email:

imm-flowcytometry@medicina.ulisboa.pt

- The “inductions” can be quick when users already know the instruments and are familiar with flow cytometry or can include one-to-one tutorials on flow cytometry.
- We are available to help users at any stage and will be ready to guide you through the acquisition protocol until you are confident to do it on your own. This should be scheduled by email.
- After inductions, you will be given permission to use the booking system (after IMM Booking system registration has been given by IMM lab management) and you should send us an email with **your full name, unit and phone extension** in order to get **permission to access the room**.

Guidelines for analyzer usage

- Book for yourself and not for others.
- At the end of your acquisition it is mandatory to contact the next person on the list.
- Always leave your contact (extension number) on the booking so you can be reached.
- Always record start and finishing times on the log sheet (cleaning time included). Also record if you had any sort of problems or if the user before you did not leave the cytometer in appropriate conditions.
- Users can book unlimited slots on the analyzers, but can only cancel up to 3 hours before their slots. Last minute cancelations (within the 3 hour gap) must be made by email to imm-flowcytometry@medicina.ulisboa.pt. The email request must mention the reason for the cancelation. These last-minute cancellations will incur in the following **penalty charges**:
 - Cancelation 3-1 hours in advance: booking time will be charged 2x
 - Cancelation <1 hour in advance or don't turn up (No Show): booking time will be charged 3x

Note: For clinical samples or mouse samples requiring "same-day" genotyping penalties are not applied. Booking time will be charged in 1x.

Filter replacement:

Users who swap filters and then fail to replace the original filter configuration will be banned from using the analyzer for a week on a first instance, two on the third and three weeks if this occurs for a third time.

Switching instruments off at night:

The analyzers must not be left on during the night as this result in severe loss of laser lifetime and is damaging to the instruments.

Users are free to use the analyzers at any time of day and night. However, the last user booked is responsible for switching the instrument off. Failing to do so will result, on a first occurrence, on a warning to the user and the payment of the number of hours during which the machine was left on. On a second occurrence the user will further be banned from using the analyzers and making bookings for a week. Banning from using the analyzer for a whole month will occur if this occurs for a third time.

During the night, users are encouraged to try to communicate as much as possible with each other to ensure that this type of situation does not take place. They should therefore leave their contact details on their bookings so that they can be reached by other colleagues.

Login System on LSRFortessa and Accuri:

Each user is given an individual login and password for Diva and Accuri C6 software. This will enable us to track instrument usage per user, making it easier to identify users who don't delete data, problems with the databases, and identify users who have been acquiring without booking or recording their usage on the logsheet.

It's mandatory to logout from DIVA at the end! We will use those records to calculate the monthly billing. Users are requested to always record in the spreadsheet all the acquisition and analysis time and any problems taking place during instrument usage.

Data management on the analyzers

UCF staff are not responsible for data storage

- Users MUST always export their data and delete it from the database. Don't book for data transfer, otherwise you will be charged. Always record the data transfer into the log sheet.
- Failing to do so may result in UCF having to restore the database with an empty one resulting in ALL DATA being deleted.

We do not have enough resources to have someone deleting user's data: THIS IS THE USER'S RESPONSABILITY.

Cleaning the analyzers tidying the workspace:

YOU MUST ALWAYS CLEAN THE MACHINES, empty the waste reservoir and refill the sheath tank with PBS1X+Azide.

We clean the machines every morning and perform weekly cleans, but this will not be enough if you don't clean after using the instruments! Also, make sure you do not leave the sheath empty and the waste full for the next user. Do not leave gloves, pipettes, tip boxes or FACS tubes lying around.

Analysing data (adjusting compensations, etc) on the Fortessa:

If you book the Fortessa to adjust compensation without using the machine you will be charged. To do your analysis/compensation you should use the PC located in room P2A32 (free of charge).

Golden Rules for using the analyzers at UCF IMM

- Inductions before using the instruments are compulsory.
- Book for yourself and not for others.
- Always leave your contact (extension number) on the booking so you can be reached.
- Always record start and finishing times on the log sheet (cleaning time included). Also register if you had any problems while acquiring.
- Clean the instruments after usage. Tidy up the workspace and take care of the reservoirs.
- Create a backup of your data to a safe location and delete your experiments from DIVA database.
- Communicate with your colleagues and never leave instruments on during the night.
- Re-install the correct filters at the end of your acquisition.
- When in doubt, ask for help!

SORTERS

1. The FACSArias can be booked up to 2 months in advance by the users between 10am and 10pm. When booking, allow enough time for purity checks and cleaning between sorts (15minutes).

UCF Staff kindly requests you to let us know if you decide to make a last minute reservation

2. Always mention which nozzle you will use (70um, 85 um or 100um) on booking description.
3. The sorter cancelations can be made up to 7 days in advance free of charge. Within this time period cancelations must be made by email (imm-flowcytometry@medicina.ul.pt). The latter will incur in the following **penalty charge**:

- 7-5 days in advance: booking time will be charged 2x
- 4-3 days in advance: booking time will be charged 3x
- 2 days in advance: booking time will be charged 3,5x
- 1 day in advance, on the day or don't turn up for a sort (No Show): booking time will be charged 4x

Note: For clinical samples or mouse samples requiring "same-day" genotyping penalties are not applied. Booking time will be charged in 1x.

4. When users are late for a sort, this will be charged according to the schedule, unless UCF staff is warned of the delay before the instrument is switched on. In this situation be aware that you may have to wait until a UCF staff member is available to start your sort.
5. We have been having problems when users swap sorting slots and misunderstandings have occurred:
 - a. When you agree to swap a sort (or give your slot to colleague) this must be updated on the booking system. In situations when nothing is written and there are disputes for sorting times, our staff will follow what is on the booking system.

6. Sample preparation:

If you are new to sorting, speak to us beforehand so that we can help you plan your experiment.

Come to UCF fully prepared with controls, collection tubes and media (for dilution and collection).

Always filter your cells with the correct size filter immediately before sorting (or acquiring in either of the cell sorters)! (For most users this would be a 70 μ m filter).

Users sorting big cells must let us know in advance so that we can prepare the instrument with a bigger nozzle (100 μm).

7. Acquisition on the FACS Aria: When acquiring on the Aria, the normal rules and charges are applied, but you will be taught how to acquire and we will be around in case problems occur. **We do not acquire samples for you.**

Golden Rules for sorting at IMM

- Don't forget your bookings.
- Avoid last minute cancelations.
- Plan your experiment.
- Avoid delays.
- We are flexible enough to accommodate unpredictable events, but when you need us to make extra hours make sure we are warned about it.
- Come prepared: **correct controls, filtered cells, collection tubes, sample buffer, extra cell strainers.**
- Talk to us: before, during and after. We are here to help and good communication is crucial.
- Use the email address: **imm-flowcytometry@medicina.ulisboa.pt** for cancelations, suggestions, complaints, etc.