

The BioSysM FACS Core Facility (FCCF) Guidelines

The BioSysM FACS core facility (FCCF) of the Ludwig-Maximilians-Universität München provides access and advice on flow cytometry equipment. Usage of the instruments is subject to usage fees. The core facility is located in the BioSysM, Butenandtstr.1 81377 Munich. The main room is located on the third floor in room K 03.041. of the BioSysM building.

Contact Info: For current contact information please see the web site of the core facility at: <http://www.fccf.genzentrum.lmu.de/>

It is possible to apply for usage fees to be spent at the core facility in DFG grant applications according to the “Hinweise zu Gerätenutzungskosten und zu Gerätezentren” (http://www.dfg.de/formulare/55_04/index.jsp, Introduction and chapter on “flow cytometry“).

§1 Access

The FCCF provides opportunities to all scientists of the Gene Center, Faculty of Chemistry and Pharmacy (CUP) and all interested academic groups of the LMU as far as capacity allows. We offer professional and dedicated services for all our users, including training and helping with analyzing and fully operated sorts. We aim to stay in touch with the work of each individual user. We offer guidance and help, not only on technical aspects, but also on scientific questions whenever possible. We would like to encourage all users to acknowledge the facility on any papers that were generated with the help of instrumentation of the core facility or credit authorship to members of staff that have significantly helped the science published.

To obtain access to any instruments, the following steps are made:

1. For new flow cytometry projects, a consultation meeting of user and FCCF staff should be held to determine the optimally suited approach concerning sample preparation by the user and instrument to be used. Such a meeting is mandatory for all users. All such discussions will be treated as confidential by facility staff.
2. The user and the respective group leader sign agreements in which they both accept the guidelines of the facility (see appendices).
3. For hands-on training see §4
4. When training is completed the user can send a request to tafrishi@genzentrum.lmu.de or facsgenzentrum@gmail.com which will be received and proceed by FCCF staff. Please be aware that booking and not using it (no-show) is also subject to fees. See below for details.
5. New users have access to the machines during office hours of the facility staff (8:00-17:30) so that we are available if help is required.
6. Experienced users who have a transponder for the BioSysM can have their transponder activated for the respective facility room and will have access 8:00-20:00. For booking see §5.

§2 Equipment

The current list of available equipment is:

- Instrument 1: FACS Analyzer, BD LSRFortessa
- Instrument 2: S1 FACS Sorter, BD FACSAria Fusion
- Instrument 3: S2 FACS Sorter, BD FACSMelody

§3 Responsibilities of users and group leaders

Responsibilities of the user and the respective group leader are regulated in the respective agreements (see Appendices). Failure to follow these rules may result in temporary or permanent loss of usage privileges for the user or the respective group, at the discretion of the facility staff.

§4 Training

Training consists of two parts: theoretical and practical. Each user should have a basic theoretical understanding of flow cytometry. To this end facility staff will regularly give lectures on basic topics. In addition, self-study is required.

For practical, hands-on training, facility staff and the user will together schedule a training session on the respective instrument. Practical training for flow cytometry may consist of several sessions, depending on the complexity of the machine and previous knowledge.

Service	Definition/Description
Training of MSc/BSc students	<p>As most MSc/BSc students are usually repeating existing protocols and rarely have to create new panels, their training will be simplified and reduced to:</p> <ul style="list-style-type: none">• Starting the instrument/switching the instrument off + cleaning the instrument• Waste disposal• Running analyses from pre-existing templates including compensation calculation• Exporting and deleting data• Data safety and integrity <p>Training comes at no extra cost other than the run time of the analyzer.</p>

<p>Training of PhD students/PostDocs/Technicians</p>	<p>Training for research technicians / PhD students / Postdocs will be 1:1 sessions and require a simple stained sample supplied by the user. This will most optimally be a 4-5 color panel if the user is thinking of performing multi-color FACS analysis. The training will include all necessary steps to establish and perform FACS analyses.</p> <p>The training covers:</p> <ul style="list-style-type: none"> • Starting the instrument/switching the instrument off + cleaning the instrument, waste disposal • Performing CST and validating CST reports • Creating new experiments and templates • Compensation theory and PMT optimization • Doublet exclusion and sensible gating strategies • Exporting and deleting data • Possible data analysis strategies <p>Training comes at no extra cost other than the runtime of the analyzer.</p>
<p>Cell sorting</p>	<p>Cell sorting is fully serviced and performed by facility staff only. We offer regular and sterile cell sorting using our Aria Fusion and Melody sorter.</p>

§4-a Working with the cytometers:

Only researchers or students that have been introduced into the facility by a member of facility staff may operate the instrument. Introductions to the instruments not done by a member of the facilities staff do not entitle anybody to work with facility equipment.

Users are asked to leave the analyzer and the surrounding equipment (pipets, buffers, filters, etc.) in the same clean state as they have found it. If users find the analysis station untidy, please approach the facility staff to ensure the previous user can be contacted and informed about his or her failure to clean.

Users are expected to follow the general start-up, cleaning, and shut-down procedure present at each machine in writing and taught during the training session.

Every user is expected to refresh sheath fluid if it is running empty and to exchange waste tanks either when they are 50% full or when they shut down the instrument as last user. Waste treatment is described during the introduction and all necessary reagents can be found at the sink in the FACS lab.

Users should treat the analyzers with care when operating the system and loading samples. If you are in doubt of your operating procedures, please contact the staff and you will be shown proper use and procedure.

Users are asked to export their experiments from the DiVa database immediately after recording their experiments. Deleting the file has to be done after checking the integrity of their data but no more than **96h** must pass between recording and deletion. The DiVa database will be cleaned **every first Monday of a new month**.

FACS analysis is user operated once the training on the instrument has been attended. The facility will be open for help at any time if we are not busy otherwise. The facility will not run your samples as part of the analyzer booking (unless you mentioned in your request and ask for the operating by core facility staff service). We don't offer staining services. All samples must be stained by the user.

§4-b Cell sorting:

- Cell sorts are done in close cooperation with facility staff. All information on the panel and the sample numbers/conditions must be made available to the facility staff prior to the sort.
- The facility asks every sorter user to contact us prior to the planned sort in order to discuss sample size, volume, nozzle setup, anticipated amount of cells, the required controls, recharge issues, and sample timing.
- The facility expects users to be on time for their sorting appointment (+/- 10min). Charging will start as booked in the calendar. If the sort requires more time and the facility can manage to provide this extension, the booking will be amended accordingly.
- All samples will be filtered at the facility through a 30 µm mesh filter prior to loading on the cytometer
- The facility charges 30 min increments for sorts and adds 30 minutes for startup and setup onto the actual sort time. If a sterile setup is required, the facility will run a long sterilization protocol with an extra charge of 45 min.
- Collection tubes can be offered by the facility, but they are untreated. If the user wishes to treat his tubes, he or she must come before hand to pick up the tubes. We recommend overnight coating with sterile FCS at 4°C on a tumbler or rotator.
- Cell numbers obtained from the sorter depend on the quality of the material handed to the facility. The facility reserves the right to stop a sort when
 - 1) The booked time is over.
 - 2) The facility core working hours (8:00-17:30 GMT) have passed and no late sort has been agreed on.
 - 3) The quality of the sample is too bad to proceed with.
 - 4) If the facility obtains knowledge that the sample is not compliant with the associated risk assessment. The latter will result in action from the facility management in compliance with Health and Safety to prevent such an incident from happening again.
- The facility will not count your sorted cells, but we make very sure that the instrument met all criteria that the number of expected cells sorted is about 80-85% of the software predicted amount (this is an approximation by experience and relatively reliable).
- When you book on the cell sorter, the facility will provide the following services or items as part of the usage fee:
 - 1) Instrument setup and sort procedure
 - 2) Full operator surveillance of your sort
 - 3) Pipetting equipment and cell strainers prior to the sort
 - 4) Data transfer to the Sorting folder
 - 5) Printout of your sort data and an instrument-based assumption of the total number of sorted cells (no guarantee, it is an estimation!)

§5 Booking, logging and billing of usage fees

Checking the availability of instruments is possible in advance. For heavily booked equipment, the facility staff can set limitations to bookings per week or during certain day times for individual users or users from a group or from a chair. Even if no such limitations are in place, FCCF staff may cancel excessive bookings (e.g. several days in a row). If intense booking is experimentally required, please contact the facility staff in advance. On heavily booked equipment, the facility may restrict booking to certain time slots (e.g. 9-12, 12-15, 15-18) to optimize usage time.

A user may use a machine until the next user is actually showing up at the facility (please consider instrument cleaning time in your booking) or is calling in to announce her/his immediate arrival (see web site for phone numbers of the facility rooms). If a machine is not booked by others, a user may extend a session. In both cases, the usual usage fees apply.

Computers at the facility mostly have an automated logging and monitoring of usage times. These data will be used to calculate usage fees, in addition to booked times. Booked times are charged also if a session began late or ended early. For those machines without automatic monitoring, the booking times only will be used for calculation of usage fees. The user is then responsible for extending the booking times to any elongation of the originally booked time.

Billing of the usage fees will in principle follow the suggested fees provided by the DFG. However, dependent on the support through the central maintenance funds of the LMU we will be able to offer access at a reduced fee (which may become subject to further reduction):

	Operated by user	Operated by core facility staff
BD LSRFortessa	25 € per hour	65 € per hour
BD FACS Aria Fusion BD FACS Melody	40 € per hour	80 € per hour

§6 Cancellation of bookings

Bookings of the instruments are not to be changed within a three working day period prior to the actual appointment. Booking durations are set within the three working day period and can only be changed or modified in agreement with the facility members. Short-term cancellations (1-3 work days before the booked day) may be charged with 25-100% of the usage fees, if the slot was not picked up by other users. The height of the cancellation fee is decided by the facility staff, considering arguments brought forward by the user. For all cancellations, the user should alert other frequent users on the same machine to the free time.

Frequent cancellations by a user or a group may result in increasing cancellation fees and/or in revoking of user privileges.

§7 Biosafety

All FACS core facility (FCCF) equipment resides in biosafety level 1 laboratory space in room K 03.041. Therefore, all users must be up to date with biosafety level 1 training (contact Claudia Buchen buchen@genzentrum.lmu.de beforehand). Radioactive samples are not allowed in our facility under any circumstances.

Correct personnel protection equipment must be worn and proper containment procedures must be practiced at all times in this location. Specifically:

- Only biosafety level 2 samples, for which approval by the local authorities have been granted can be analyzed or sorted at the facility (for more information visit <http://www.fccf.genzentrum.lmu.de/> or contact Dr. Neda Tafrishi tafrishi@genzentrum.lmu.de beforehand).
- All biosafety level 2 samples must be transported to this location in a secondary, waterproof container with a secure lid (such as a tupperware, styroform is not acceptable).
- Users must wear lab coat, eye protection goggles, gloves located by the front entrance and remove this equipment before leaving the facility.
- Closed toe shoes are required in this location at all times.
- The FACS analyzer (BD LSRFortessa) and the S1 sorter (BD FACSAria Fusion) are strictly biosafety level 1. No unfixed or untested (HIV, HBV, HCV) human samples (or other biosafety level 2 material) are allowed to be analyzed on those instruments at any time.
- Please do not use lab supplies, sinks, and trash bins that belong to other labs that share the FCCF floor space.

§8 Acknowledgement of the core facility in publications

The FCCF will be evaluated by funding bodies. Thus, it is of high importance for the future of the facility that it is visible in the published record. By using the facility, users and their group leaders commit to explicitly acknowledge the FCCF in any publication that contains data recorded or processed on facility equipment and/or where significant advice of the facility staff was obtained. Such publications include bachelor, master and doctoral theses. A digital copy of each publication will be made available to the facility staff for archiving. The facility may list such publications on the web site.

Appendix 1: Agreement between user, group leader & FCCF

The signees declare that they accept the guidelines of the core facility and will adhere to the following rules:

- All experiments must comply with local regulations (Nutzerordnungen), in particular concerning cleaning and disinfection after the experiment.
- All experiments with human material must comply with local ethics and biosafety regulations.
- For damages caused by improper usage, the user and his group and institution are responsible and have to cover cost for repairs.
- Data storage on facility devices is for short term periods only. The user will delete all files as soon as they are safely available on other systems. Facility staff may delete data left on facility devices after a certain period week, whereas the users will be informed ahead of time.
- The user and the respective group leader are responsible for the scientific integrity of the data and for long term storage of the original data. It is noted that the DFG rules on Safeguarding Good Scientific Practice (http://www.dfg.de/download/pdf/dfg_im_profil/reden_stellungnahmen/download/empfehlung_wiss_praxis_1310.pdf) request a storage time of ten years in recommendation 7: “Primary data as the basis for publications shall be securely stored for ten years in a durable form in the institution of their origin.” This is also reflected by the “Richtlinien der LMU München zur Selbstkontrolle in der Wissenschaft“,
- Specifically, §7 of the guidelines is accepted concerning acknowledgement of the FCCF and its staff in publications.
- The group leader agrees to cover for the usage fees caused by the user.

Project name (or work group):

City, Date:,

Signature Group leader:

Signature User

User (in block letters):

Appendix 2: Additional agreement between user and core facility

The signee declares to accept the guidelines of the FCCF and to adhere to the following rules:

- The user has to handle all equipment with utmost care and according to instructions to avoid damage and expensive repairs. Cleaning of the instruments after each session is performed according to guidelines explained during the introduction. When in doubt, the user will ask the FCCF staff for advice.
- The user is responsible to check the equipment for cleanness and obvious damage before starting to work. Any problems inherited from a previous user have to be reported to the facility staff or they will become the responsibility of the current user. At the end of the work, the equipment has to be left in a state as taught by the facility staff. Users have to sign into a user book. No left-overs will be left at the workplace. Left samples may be discarded by the facility staff.
- No food or drinks are allowed in the microscope rooms (see also §7 for specific rules regarding biosafety).
- If problems or unusual or improper behavior of the hardware or software are noticed, the user will inform the facility staff.
- Users have to use and to stay within their dedicated user-accounts.
- No dangerous chemicals or instrumentation will be brought into the facility without explicit consent of facility staff.
- In case of any accidents, the facility staff will be immediately notified.
- It is expected that users refill the sheath tanks and empty the waste tanks.
- The user will not 'lend her/his login credentials to others. I am aware that usage of the instruments is logged via the respective computers. For each login, the user name and on/off-times are recorded.

City, Date:

Signature User:

User (in block letters):