

The Gene Center cryo-electron microscopy facility (GC-CEM)

Guidelines

The Gene Center cryo-electron microscopy core facility (GC-CEM) of the Ludwig-Maximilians-Universität München provides access to and advice on cryo-electron microscopy equipment and advice on sample preparation as well as data collection. Usage of the hardware is subject to usage fees. The core facility is located in the Biozentrum, Großhaderner Str. 2 in Martinsried.

The three main rooms are located in the basement of the B wing of the Biozentrum, room numbers B.U 1.061, 1.065, and 1.059.

Contact: For current contact information please see the web site of the core facility at: <http://www.beckmann.genzentrum.lmu.de/the-cryo-electron-microscopy-facility/>

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 “Elektronenmikroskopie”).

§1 Access

The GC-CEM provides opportunities to all scientists of the Gene Center (GC). Capacities not used by GC researchers are made available to applicants from other institutions: 1. LMU with Klinikum. 2. Other academic institutions.

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

1. For new imaging projects, a consultation meeting of user, group leader and GC-CEM staff should be held to determine the optimally suited approach concerning sample preparation by the user and microscope hardware 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. The user signs up for the online booking system. This can be done in parallel to the previous step, but the user will not be approved until the signed forms arrive.
4. Users from outside the GC will submit a 1-2 page project summary. Access for

outside projects may be prioritized or denied on reasons of capacity of the facility or scientific merit of the project.

5. For hands-on training see §4
6. When training is completed the user can book via the booking system, which is supervised by GE-CEM staff. Please be aware that using the core facility equipment is subject to usage fees. Booking and not using it (no-show) is also subject to fees. See below for details.
7. New users have access to the machines during office hours of the facility staff so that we are available if help is required.
8. Experienced users who have a transponder for the Biozentrum can have their transponder activated for the respective facility room and will have access 24/7. For booking see §5.

§2 Equipment

The current list of available equipment can be found on the facility web site.

§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 Introduction to instruments

Training consists of two parts: theoretical and practical. Each user should have a basic theoretical understanding of the underlying imaging concept. To this end facility staff will regularly give lectures on basic topics. In addition, self-study is required. “Learning & Teaching” on our website lists books and websites for this purpose.

For practical, hands-on training, facility staff and the user will together schedule a training session on the respective instrument. Prerequisite is sufficient expertise on the entry-level TEM *Morgagni*, which can be gained under tuition of staff from AG Haszprunar (users from Biozentrum/LMU) or AG Beckmann (users from Gene

Center). Practical training for CEM may consist of several sessions, depending on the complexity of the machine and previous knowledge.

Three successive levels of training are offered by the facility in a strict order: (i) in the first phase grid making, negative stain procedures and the use of the simplest electron microscope, the Morgagni, are taught; (ii) during the second phase users are made familiar with the production of vitrified samples using the Vitrobots and with the handling of the screening cryo-microscope, the 120 KeV Spirit; (iii) lastly, in the third phase handling of the high-end microscopes will be taught.

For example, a first practical introduction to cryo-electron microscopy will take about 3 hours. For an introductory session on these microscopes, the normal usage fee for 3 hours will be charged. Depending on user experience more sessions may be necessary.

§5 Booking, logging and billing of usage fees

Online booking of instruments is usually possible two weeks in advance. Normally, one session (i.e.: maximum half-days for the screening microscopes, 2 days for the high-end EMs) can be booked by a single user and needs to be used before booking the next one.

In general GC-CEM staff will perform the online booking for the user according to the demand and availability. Advanced CEM will be performed by staff of the GC-CEM, unless the user has proven a sufficient degree of expertise.

If earlier reservation is required, e.g. for student courses or long term experimental planning, please contact the GC-CEM staff. The facility staff may prioritize certain projects, such as images for manuscripts under revision, if necessary.

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, GC-CEM 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 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):

a) 1 hour at a screening cryo-microscope: 50,- Euro

b) 1 hour at a high-end Titan Krios: 100,- Euro

For non LMU users 60,- Euro and 120,- Euro will be charged for a) and b), respectively (taking into account the sales tax/MWSt.).

§6 Cancellation of bookings

Booked times or times canceled less than 24h before the reservation may be charged fully even when the machine was not used by the user (no-show), except if another user was using the equipment.

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 freed time.

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

§7 Acknowledgement of the core facility in publications

The GC-CEM 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 core facility GC-CEM 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.

The facility staff is available to read, comment and help on the imaging part (including methods) of respective manuscripts, to ensure a faithful and reproducible description of the applied techniques.

Collaborations beyond mere introduction to a device, when substantial input has been obtained by facility staff usually will warrant co-authorship. Examples for such input include detailed experimental planning, regular joint microscopy sessions with facility staff or development of experimental approaches and models together with or by facility staff. Procedure for such projects should be discussed at an early stage to avoid misunderstandings. Co-authorship is in no case precluded by paying usage fees.

Appendix 1: Usage fees

Instrument usage fees paid by academic users are required for running costs of the facility. They do not cover costs for instrument acquisition or staff. Usage fees may be different for different user groups. The current table of fees can also be found on the web site under "Instrumentation":

a) 1 hour at a screening cryo-microscope: 50,- Euro

b) 1 hour at a high-end Titan Krios: 100,- Euro

For non LMU users 60,- Euro and 120,- Euro will be charged for a) and b), respectively (taking into account the sales tax/MWSt.).

Please note that it is explicitly accepted by the DFG to apply for usage fees of electron microscopes in grant applications. See "Hinweis Richtwerte für die Beantragung von Nutzungskosten", DFG form 55.04, point „Elektronenmikroskopie“, for details on electron microscopy, http://www.dfg.de/formulare/55_04/55_04_de.pdf.

Usage fees can also be applied for in BMBF or EU grants.

Appendix 2: Agreement between user, group leader & core facility cryo-electron microscopy

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 regulations.
- For damages caused by improper usage, the user and his group and institution are responsible and have to cover cost for repairs. For external users it is strongly recommended to negotiate a professional liability insurance.
- 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. It is strongly advised to keep all original image data at least twice, on independent devices, to avoid data loss in case of hardware failure (hard drive crash; encryption by Trojan...). Facility staff may delete data left on facility devices after one week.
- 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. The facility recommends to store all data in the proprietary file format of the microscope manufacturer, since these file formats often contain information such as exposure times, selected filters, etc. It is, however, the user's responsibility to confirm which information is stored automatically and which has to be noted in the lab journal. 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", https://www.uni-muenchen.de/einrichtungen/zuv/uebersicht/dez_ii/formulare/download/dez_ii_personal/062_f.pdf
- Specifically, §7 of the guidelines is accepted concerning acknowledgement or coauthorship of the cf GC-CEM and cf GC-CEM staff in publications.
- The group leader agrees to cover for the usage fees caused by the user.

Project name (can be specific like SWI/SNF, or generic, like AG XY):

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City, Date:

.....,

Signature Group leader:

.....

Signature User

.....

User (in block letters):

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Appendix 3: Additional agreement between user and core facility

The signee declares to accept the guidelines of the core facility cryo-electron microscopy 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 immersion objectives after each session is performed according to guidelines explained during the introduction. When in doubt, the user will ask the cf GC-CEM 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 and note after the session requested details like filament hours or type of specimen holder. 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.
- 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, e.g. leakage of liquids into the instruments or contamination of the workplace with chemicals, the facility staff will be immediately notified.
- It is expected that users refill the liquid nitrogen tanks and fill up the storage tanks.
- The user is responsible for the complete documentation of applied imaging conditions, microscope settings and image manipulations. The cf GC-CEM staff is available to give advice on this documentation, if requested.
- The user will not 'borrow' his login credentials to others. I am aware that usage of the microscopes 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):.....