

Access Rules

These rules regulate access to the High-resolution electron cryo microscopy facility at the Julius-Maximilian Universität Würzburg (JMU). The Facility is located in the Rudolf-Virchow Centre (Josef-Schneider Straße 2, Building D15, 97080 Würzburg). The facility can be contacted via the function e-mail account cryo-em@uni-wuerzburg.de

What is included?

The facility provides:

- equipment for sample preparation
 - o carbon coater
 - o glow discharge unit
 - o Vitrobot.
- Electron microscope for the analysis of stained samples at room temperature
 - o FEI G2-Spirit (120 kV, LaB6-filament, Eagle CCD-camera)
- High-resolution electron cryo microscope for structure determination of vitrified samples
 - o FEI Krios (300 kV, Schottky-emitter, Falcon III direct detector, EPU-acquisition software)
 - o File-server (64 TB) for short term storage (14 days) and redistribution of data

Who supports the facility?

The facility is supported by Christian Kraft (CK) from Mo-Fri from 9-16:00. CK maintains the equipment, acquires data for users, supports and trains users in using it.

Bettina Böttcher (BB) manages the facility, which includes implementation of new techniques, planning of extensions and overseeing access. In exceptional cases, she can prioritize certain projects in consultation with users.

Who can use the Krios-electron microscope?

There are different types of users, with different access rights and different user fees.

User Group I

Experienced users, who can use the Krios independently and with confidence. Use includes: transfer of samples, basic alignment, set-up of automatic data acquisition and minor trouble shooting. In general these users do not require any support and can support others in using the instrument (act as mentor). The membership in user group I is acknowledged by a “license” that is awarded by CK based on assessment of the general skills and the independent

operation of at least three sessions at the Krios. The license can be revoked in case of reoccurring user problems.

User Group II

These users require help with using the instrument (e.g. sample transfer, alignment and/or setting up sessions). They cannot use the microscope without support. Their access is either facilitated by CK or by a mentor (user of group I). Support can include elements of training to enable users to become independent users of group I.

User Group III

These users request data acquisition on their behalf ("customers"). They hand-over a vitrified sample ready for electron microscopy. Users of group III receive data acquired by CK or another member of User group I according to standardized protocols. This route of access is recommended for all external users and internal users, who only require data.

User Group IV

These users are students of the JMU, who require electron cryo microscopy for their Bachelor or Master projects or students, who participate in structured training courses in electron microscopy as part of the curriculum. Students always require support by a mentor or CK.

Internal Users

Members of the JMU

External, academic Users I

Users from other universities in Germany, who do not have a commercial interest in the data

External, academic Users II

Users from other universities outside Germany.

External users are always supported and cannot be in User Group I. Access for external, academic Users of group II is at the discretion of the facility and always requires signing of individual user contracts before access can be granted.

What is regulated?

The access rules regulate 60% of the available time at the Krios electron microscope. They are valid for all users of groups I-III. The expected available time per year is 200 days, the unavailable time is accounted for by break-downs, repairs (scheduled and unscheduled), FEI user demos (up to 10 per year) and maintenance tasks (e.g. regular warm-up cycles, expert alignments, software updates).

The microscope is operated in daily slots (24 hours, typically from 9:00 am until 9:00 of the following day).

Access of user group IV is independent of the access rules and accounts for 15% of the available core time (30 days). The remaining 25% (ca. 50 days) is administered by BB and is mainly reserved for methods development, pilot studies and implementation of new techniques, which will benefit the facility in longer terms.

What are the rules for booking?

Users of groups I-III can book 1 slot in advance. A regular slot is 24 or 48 hours from Monday to Friday and can be extended to 72 hours when started on Friday and including the weekend. Users book via CK (or BB; function email: cryo-em@uni-wuerzburg.de) in order to co-ordinate the required support and the best use of the instrument. In exceptional cases longer slots can be agreed but will typically require evidence of the feasibility of the study.

If a scheduled slot is cancelled by the facility due to unplanned disruptions, it is rescheduled with highest priority at the next available slot as soon as the instrument becomes operational again.

How should the instruments be used by users?

Users should use all instrumentation in the facility with care and respect. The instruments should be left in a clean and pre-agreed state. If this is not possible, the user should notify the next user about the state of the instrument (e.g. note in log book). The use of the electron microscopes and the Vitrobot should be logged in the log-book.

If problems are encountered with any equipment the users should notify CK (or BB). In case of gross-misuse by the user, costs for repairs can be charged to the user.

New users should not use any of the equipment without supervision by a mentor or CK. CK provides training for all instrumentation in the facility. In times of high demand for training this will be organized in small training groups.

What is the recommended way to obtain data via facilitated access?

The most efficient way for running the microscope is to minimize time for transfer and screening and to maximize time for measurements. For this, we typically load 2 grids per sample from several users/samples (up to 5 samples, 10 grids) at the same time. After loading, all grids from all users are pre-screened (gridmap, 1 high resolution image from thin ice and 1 high resolution image from thick ice). Screening incurs a pre-screening fee per grid (see table). After pre-screening, it will be decided, from which of the two grids data will be acquired or whether the quality is insufficient for further data collection. Subsequently data from the committed grids will be acquired during the next available 24/48 h time-slot (see table for fees). The time-slot includes autoloading of the committed grid (ca. 15 min), direct alignments of the microscope (ca. 45 min), set-up of the EPU-session (ca. 2-3 hours, depending on the number of holes and mode of acquisition and the quality of the grid) and data acquisition (rest of the time).

We expect that samples arrive on the day before scheduled loading until 12:00. This enables us to pre-mount grids into the autogrids. To minimize contaminations, a cryo cycle of the microscope will be run over night before loading auto grids into the microscope. For fast and efficient set-up, users are mildly discouraged to attend data acquisition or pre-screening in person. Users should provide external hard discs together with their grids for data storage.

What happens with the acquired data?

The data that is automatically acquired by EPU at the Krios is transferred from the acquisition computer via a private network to a fileserver. After the transfer is completed the data will be deleted from the acquisition computer. The fileserver is accessible to users independent of the microscope and can be used for the redistribution of the data via the network (only RVZ internal, slow) or to external USB-3 hard-discs (provided by users, recommended). The

fileserver provides short-term storage for a maximum of 14 days and is not backed up. It is the responsibility of the users to recover their data within 14 days. Data will be deleted from the fileserver after 14 days without further notice. The facility does not take any responsibility for data loss.

What are the costs of using the facility?

Krios

User Group	24 h Mo-Fr [#]	48 h Sa-Su
Group I (internal)	840 €	840 €
Group II,III (internal, collaborative basis)	840 €	840 €
Group II,III (internal, noncollaborative basis)	1200 €	1200 €*
Group II,III (external academic I)	1360 €	1360€*
Group II,III (external academic II)	2200 €	2200 €
All user groups	20 € per loaded auto grid for the autogrid	
All academic Users	100 €/Grid for pre-screening (Grid-map, 1 high res image in thin ice, 1 high res image in thick ice) as part of a shared loading session	

*) Saturday slots for groups II and III are only in combination with a Friday slot: The support is only on Friday for setting up the session. The session will run unattended over the weekend. If the session crashes over the unattended weekend-slot time will not be compensated.

#) If a session crashes during the unattended EPU data collection, the 24h-slot shall count as completed if more than 16 hours were completed. Whenever possible, a failed unattended measurement of less than 16 hours will be resumed to complete the slot (at least 16 hours).

Time for loading, alignment set-up of the session is part of the booked slot. Time for sample preparation for user group III is also part of the booked slot.

Slots are charged according to the booked time. Cancellations 48 hours in advance of the start of the slot or the agreed loading date are free of charge. Cancellations at shorter notice are only free of charge, if another user takes the slot.

The access to the Krios is sponsored by the university. This is taken into account for internal users and external academic users I but not for external academic users II.

The use of the peripheral instrumentation (Vitrobot, glow discharge and carbon coater) is included in the use of the Krios (as required by the DFG-rules for the use of periphery).

Many consumables have delivery times of several weeks. The facility provides a limited stock of these consumables at costs. However, users are strongly advised to make their own arrangements to benefit from the lower costs by directly buying from the suppliers.

Consumable	cost per piece €	rent for 10 days €	max number of items per booked session
FEI-Autogrid	20	Cannot be reused	12
*Cryo grid box for 4 grids	20	Included in access	4
*Grid box for 4 autogrids	20	Included in access	4
*Quantifoil 1.2/1.3 400 mesh copper/carbon	10	-	20
*Quantifoil Ultragold 1.2/1.3. 300 mesh	15	-	12
*Quantifoil 1.2/1.3 with 2 nm carbon, 400 mesh copper carbon	15	-	12
*External USB-hard disc 3TB	200	Included in access	2

*) subject to availability and not always in stock

Some of the items can be reused. If these items are returned clean and undamaged within 10 days after the end of the session the use was included in the access fees (see rent for 10 days) otherwise it will be charged at the quoted “costs per piece”.

How should the facility be acknowledged?

Users should acknowledge the facility in all research publications that made use of the facility.

“Electron Cryo Microscopy was carried out in the cryo EM-facility of the Julius-Maximilians University Würzburg (INST 93/903-1).

In addition, users are requested to inform the facility management (BB) about any publications that made use of the facility (pdf, PubMed entry).

Future users are encouraged to discuss planned projects with the facility management. The facility management will help with costing of projects and planning.