HD-cryoNet: Terms of Use

1.1 Introduction

Cryo-EM network of Heidelberg University (HD-cryoNet) is an equipment center located on the campus of Heidelberg University. HD-cryoNet operates as a network in the framework of which users work independently on the devices and are participating in maintaining the network, sharing duties or providing limited assistance to other users. The goal of HD-cryoNet is to foster collaborative research that relies on cryo-EM methods within Heidelberg University and to teach cryo-EM methods to students and new groups interested in this technology of Heidelberg University. The majority of the full costs of use (basic operation and administration, service and maintenance contracts, ongoing expenses for buildings and maintenance) is funded by a scientific research institution (Heidelberg University). User fees are implemented to cover the part of the above-mentioned costs.

1.2 Contact

Administrative, technical and scientific contact persons are listed in the Annex.

1.3 Equipment

The equipment center provides access to the following equipment:

Instrument name	**Instrument purpose**	**Location**	**Biosafety level**	**Instrument Class** (according to 55_04)
Carbon Coater	Sample preparation	INF 267	0	n. a.
Glow discharger	Sample preparation	INF 267	0	n. a.
Vitrobot I	Sample preparation	INF 267	0	n. a.
Vitrobot II	Sample preparation	INF 328	1	n. a.
Aquilos	cryo-FIB-SEM	INF 229	2	Class II
Talos 120	Sample preparation	INF 328	1	Class II
Glacios-Falcon3EC	Sample screening/ Data acquisition for SPA	INF 328	1	Class III

Krios-K3 (energy-filtered)	Data acquisition: high-resolution SPA and cryo-electron tomography	INF 229	2	Class III

1.4 Access Rules

Each individual user is required to read and sign the Terms of Use document, as well as to follow the HD-cryoNet rules and regulations described therein. Group leaders will be held responsible and liable in cases of gross misconduct (negligence, damage, littering, and safety misconduct) of their group members with regard to the HD-cryoNet infrastructure. Access to an instrument within HD-cryoNet is restricted to registered and trained users exclusively. Instruments can be accessed only after booking in the PPMS booking system. Every user is expected to attend HD-cryoNet meetings. Any equipment, as well as the equipment rooms themselves, have to be handled with great care. Before use, the instrument needs to be visually inspected. Damage, problems or incidents must be reported to the HD-cryoNet microscope-responsible operator(s) immediately and recorded in a log-book and the PPMS booking system. Relevant biosafety, work safety, and radiation safety instructions must be followed. The responsibility for compliance with biosafety and ethics regulations is solely within the responsibility of the user and HD-cryoNet cannot be held accountable in the case of user misconduct against these regulations. Group leaders are responsible to pay user fees indicated in the Annex.

1.5 Application process

Group leaders requesting access to the HD-cryoNet infrastructure are required to contact HD-cryoNet. The application form (obtainable from the HD-cryoNet microscope-responsible operators) must be sent to Dirk Flemming, who will circulate the application among the steering committee members for review. Votes received within two weeks after application count towards a majority decision. The applicant can either apply for an *independent* or *collaborative project* with an established HD-cryoNet group leader if sufficient expertise is present. Preliminary data might be required as a prerequisite: for single particle analysis projects, e.g. sample purity (gel filtration) and negative stain EM data can be included. For independent projects, the acceptance depends not only on the feasibility of the project and available time on the instruments but also on training and assisting capacity.

1.6 Users

There are 3 types of users based on affiliation:

- **Internal users** are either members of the University Heidelberg.
- **Heidelberg Molecular Life Sciences (HMLS) users** are members affiliated with an institute that is a member of HMLS other than Heidelberg University. The user time for all HMLS users together is limited to a maximum of 10% of available uptime per equipment per year.
- **External users** are users outside of the University of Heidelberg and HMLS institutions without a commercial interest in the data. The user time for external users is limited to a maximum of 5% of available uptime per equipment per year.

There are different types of users based on cryo-EM proficiency:

User group I. User who can operate an instrument independently and with confidence. User must be able to transfer of samples, perform basic alignment, set-up of automatic data acquisition, set up a cryo-cycle, camera heating cycle and to use Vitrobot. Such users are defined as *Autonomous users* in the PPMS booking system. Autonomous users will have after-hours access that allows HD-cryoNet infrastructure usage without standard working hour restriction (24/7). The status of an autonomous user 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 acquisition sessions). They cannot use the microscope without support and access for all user group II is restricted to standard working hours (8:00 – 17:00, weekdays only). Such users are defined as *Novice users* in the PPMS booking system. Novice users are automatically requested to have assistance with loading samples to a specific microscope or operating Vitrobots. Novice users are assisted either by an autonomous user from their research group or can request assistance from Götz Hofhaus or Dirk Flemming.

1.7 Training and assisted sessions

All Users that want to operate HD-cryoNet instruments have to be trained as described in the Annex. Training must be performed only by an HD-cryoNet microscope-responsible operator or specific members of HD-cryoNet group leaders. Assistance can be provided by any other independent user (User group I).

1.8 Booking Rules

Booking of the equipment is always obligatory without exception. Even if the equipment is momentarily free it needs to be booked before usage. Booking is performed exclusively via the

online HD-cryoNet booking system PPMS (https://ppms.eu/cryoem-hd) using the personal account. To ensure compliance and prevent overbooking, these booking rules are implemented into the HD-cryoNet booking software and therefore it is not possible to violate them. Each registered user can book an instrument once at a time. **Double booking is not allowed except for I) Krios and Glacios 2-day weekend slots (Weekend slot 1 = Friday and Saturday; Weekend slot 2 = Sunday and Monday) and II) slots that are booked less than 24h in advance (users must leave a comment during the booking process: 'short-term booking').** If longer time slots are required or more long-term planning of experiments is required, a microscope-responsible operator(s) must be contacted. A deviation from these rules is possible if a particular experimental setup demands it. Any deviation needs to be discussed and approved by the user group. Potential conflicts due to overbooking of instruments will be resolved between the microscope-responsible operators and group leaders. For teaching purposes (officially organized teaching courses), booking should be done well in advance. Microscope booking for teaching courses has priority over booking for research usage. Booking can be deleted or edited only up to a certain number of days (see table) before the booked date without consequence. If the booking is deleted or edited less than the given day limit before the booked time, user fee charges may apply (see user fee section for more details). Any permanent changes of these rules need to be approved by the HD-cryoNet steering committee. 8:00 – 17:00 are considered as peak hours. 17:00-8.00, weekend and holidays are considered as off-peak hours. Sundays might be reserved for a cryo-cycle on some instruments. Every session has to be recorded in an instrument's log-book.

Instrument	Maximum time	Minimum booking slot	Free cancelation up to:
Krios	24 hours per week	4 hours	3 days
Glacios	24 hours per week	2 hours	2 days
Talos 120	6 hours per day	1 hour	1 day
Aquilos	2 days per week	6 hours	1 day
Vitrobots	2x 2 hours per day	1 hour	N.A.

1.9 Consumables

Consumables such as C-rings, autogrids, grid boxes, and grids are provided by each group. However, if needed HD-cryoNet will provide consumables for a fee (see table). Any damage to tools such as bent forceps and broken loading tools will be charged on the group if the damage is caused by mishandling the tools. LN_2 and ethane consumption is included in user fees. A calendar has been established according to which groups are rotating on a weakly basis to refill

the Krios Dewar. Nevertheless, the User having a session on the Krios is responsible for assuring a sufficient level of LN2 in the Krios Dewar. The Krios Dewar can be only refilled from 8:00-12:00 on working days.

2. Data policy

Management of the acquired data is at the sole responsibility of the user. Data are allowed to remain stored on the system's local hard drive for up to 3 days, after which they will be automatically deleted. There is no backup or protection of the locally saved data in HD-cryoNet and HD-cryoNet takes no responsibility in case of potential data loss accidents. The users are encouraged to transfer the acquired data right upon completion of data acquisition within the timeframe of their booked session. Large data sets can also be transferred directly to the Large Scale Data Facility (LSDF) of Heidelberg University using the high-speed IT infra-structure (10Gb/s optical fibers), which connects the INF229 and INF328 building with the University computing center (URZ). It is recommended to archive the original raw data in the file format used by the microscope software.

3. Publication and acknowledgment policy

If results are published that were acquired making use of the HD-cryoNet infrastructure and/or consultation with an HD-cryoNet user, a statement: **"We would like to acknowledge access to the infrastructure and support provided by the Cryo-EM Network at the Heidelberg University (HD-cryoNet)"** must be included in the acknowledgment section of the publication and a pdf of the published paper needs to be sent to the administrative manager of the HD-cryoNet for internal quality management and reporting to granting agencies. An HD-cryoNet user that is actively involved in research projects in a collaborative manner beyond providing basic HD-cryoNet infrastructure and consultation is regarded as co-author in the respective publication in addition to the acknowledgment statement.

4. Safety policy

Proper safety policy implementation is crucial to minimize the risk of injury associated with the usage of HD-cryoNet infrastructure. Users are briefed about the potential hazards and safety rules during the introductory training session by their group leader and must attend a basic safety training offered by their institute. Compliance with radiation safety and bio-safety necessitates the participation (mandatory for all users) in annual user training seminar organized by dedicated microscope-responsible operators or HD-cryoNet members. These seminars are organized annually and the presence of the HD-cryoNet users is required. HD-cryoNet

infrastructure complies with the University of Heidelberg's general laboratory safety rules. The rooms containing liquid nitrogen, are equipped with O_2 sensors. In the case of a visual and audible warning, the users must vacate the room immediately and notify the microscope-responsible operator(s). HD-cryoNet maintains all equipment and performs experiments in certified BSL-2 according to the respective regulations of the University of Heidelberg. HD-cryoNet cannot be responsible for any injury or property damages resulting from the improper use of the equipment or violation of HD-cryoNet Terms of Use and Safety Policy. HD-cryoNet infrastructure-specific safety aspects are discussed in an annex.

5. Annex

5.1 Administrative, technical and scientific contact persons

1. HD-cryoNet steering committee: Petr Chlanda, Stefan Pfeffer, Rasmus Schröder, Irmgard Sinning, user group
2. User group: Dirk Flemming, Jan Rheinberger, Götz Hofhaus
3. The biological safety project leader: Petr Chlanda
4. Radiation safety instructor: Dirk Flemming, Lisa Veith
5. Preliminary administration support for HD-cryoNet: Catarina Vill-Härtlein
6. 'User Group': all users as registered in PPMS
7. Persons responsible for specific equipment and instruments:

Equipment	**Location**	**Responsible Operator(s)**
Carbon Coater	INF 267	Setsuko Fujita-Becker
Glow discharger	INF 267	Setsuko Fujita-Becker
Vitrobot I	INF 267	Götz Hofhaus
Vitrobot II	INF 328	Dirk Flemming
Krios-K3	INF 229	Götz Hofhaus, Stefan Pfeffer
Aquilos cryo-FIB-SEM	INF 229	Petr Chlanda
Glacios	INF 328	Dirk Flemming, Jan Rheinberger
Talos L120	INF 328	Dirk Flemming, Jan Rheinberger

5.2 User Fees

The user fees for the use of instruments are currently waived for internal users. Fees may apply for external users.

Krios

User	Weekdays (Mo-Fr)	Weekends (Sa-Su)
Internal users group I	10 €/ hour	10 €/ hour
External HMLS users	59.5 (including tax 19%) * €/ hour	n. a.
External (no HMLS)	95.2 (including tax 19%) * €/ hour	n.a.

Glacios

User	Weekdays (Mo-Fr)	Weekends (Sa-Su)
Internal users group I	10 €/ hour	10 €/ hour
External HMLS users	59.5 (including tax 19%) * €/ hour	n. a.
External (no HMLS)	95.2 (including tax 19%) * €/ hour	n.a.

Aquilos

User	Weekdays (Mo-Fr)	Weekends (Sa-Su)
Internal users group I	10 €/ hour	10 €/ hour
External HMLS users	n.a	n. a.
External (no HMLS)	n.a	n.a.

Talos 120

User	Weekdays (Mo-Fr)	Weekends (Sa-Su)
Internal users group I	10 €/ hour	10 €/ hour
External HMLS users	35.7 €/ hour	n. a.
External (no HMLS)	n.a	n.a.

Costs originating from the use of peripheral instrumentation (Vitrobots, glow discharger, and carbon coater) are included in the user fees of the Krios, Glacios, Talos 120 and Aquilos. (*)The user fees for external users are subjected to taxation 19%.

5.3 User training

Training capacity is limited and will be offered when time on the instruments and time of the microscope-responsible operator allows. User fees for the training will be charged with the same rate as listed for assisted sessions (internal user group II). Training is offered only to internal users and it is mandatory for everybody in order to become a user. If the user does not use the instrument for more than 6 months, training must be repeated. After a novice user has undergone the initial training, she/he must request assistance in any future session. It is upon the instructor's judgment to decide whether the novice user (User group II) can be work autonomously and become an autonomous user (User group I).

HD-cryoNet offers the following training:

- Training on Vitrobot and sample handling
- Training on Krios, Glacios, and Talos
    - Sample loading
    - Grid mapping
    - Image acquisition using EPU
- Training on Aquilos

5.4 Biosafety

The general safety rules that apply to any area exposed to genetically modified organisms (GMOs) also apply to HD-cryoNet microscopy rooms. GMO related documentation is solely the user's and the group leader's responsibility. HD-cryoNet cannot be held responsible if such documentation is missing or incomplete. In addition, the HD-cryoNet infrastructure located in INF229 is designed to allow research on living organisms and pathogens. For this reason, the entire infrastructure is placed under BSL-2 safety containment according to respective regulations of the University of Heidelberg. All users working with known or potential human pathogens or other potentially biohazardous agents must comply with Heidelberg University Biosafety Guidelines. The biological safety project leader of the HD-cryoNet holds a database of biohazard agents on which research has been granted, together with the biological agent-specific risk assessment as well as a description of proper work practices, decontamination methods, and/or specialized personal protective equipment. HD-cryoNet is recording what types of infectious organisms are examined using the HD-cryoNet infrastructure. Documentation thereof is implemented in the booking procedure where users need to specify the type of infectious agent they are working with. In the case research needs to be conducted on a living organism (BSL-1) or pathogen (BSL-2) which is not registered within the HD-cryoNet infrastructure (and therefore not in the HD-cryoNet database) or on a new pathogen, the user (group leader) needs to contact the HD-cryoNet Biosafety project leader to pursue the necessary clearances. BSL-2 project can be started only after the required clearance has been obtained and forwarded to the administrative manager of HD-cryoNet. HD-cryoNet microscopy rooms in INF229 are labeled with biohazard signals indicating which infectious agent is in use. Instructions for cleaning and decontamination procedures in the case of spill accidents are located in each room. HD-cryoNet is recording what types of biological samples and pathogens are examined using its infrastructure. When material needs to be transported from the BSL-2 laboratories into the HD-cryoNet area it must be carried in the vitrified state in LN_2 Dewar.