HDcryoNet: Terms of Use

1. General information

1.1. Introduction

The Cryo-EM network of Heidelberg University (HDcryoNet) is a technology platform located on the campus of Heidelberg University. HDcryoNet operates as a network of expert users who independently use the equipment and share duties. Its overarching goal is to foster collaborative research that relies on cryo-EM methods within the Heidelberg University and to teach cryo-EM methods to students and groups of Heidelberg University interested in the technology. The available equipment allows users to perform a variety of methods from sample preparation, sample screening, to high-resolution data collection, *e.g.*, for single particle analysis or (in-situ) cryo-electron tomography.

1.2. Available equipment

The platform provides access to the following equipment:

Instrument name	**Instrument purpose**	**Location**	**Biosafety level**	**Instrument Class** (according to DFG 55_04)
Glow discharger	Sample preparation	INF 267	1	n. a.
Vitrobot I	Sample preparation	INF 267	1	n. a.
Vitrobot II	Sample preparation	INF 328	1	n. a.
Aquilos 2	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-Bioquantum-K3 (energy-filtered)	Data acquisition: high-resolution SPA and cryo-electron tomography	INF 229	2	Class III

1.3. Administration, technical and scientific contact persons

HDcryoNet is formally managed by the BZH and headed by Prof. Dr. Cristina Paulino (BZH). She is the spokesperson of the steering committee that meets about two to three times per year and is composed of:

- Prof. Dr. Cristina Paulino (BZH)
- Dr. Petr Chlanda (BioQuant)
- Dr. Stefan Pfeffer (ZMBH)
- Prof. Dr. Rasmus Schröder (BioQuant)
- Prof. Dr. Irmgard Sinning (BZH)
- User Group* representatives (Dr. Dirk Flemming, Dr. Götz Hofhaus and Dr. Jan Rheinberger)

Safety instructors are:

- Biological safety instructor: Dr. Petr Chlanda
- Radiation safety instructor: Dr. Götz Hofhaus

Additional entry-level technical support is provided by Rebecca Déchaud (BZH). Administration support for HDcryoNet is provided by the BZH and includes: Raquel Jungmann (orders), Annette Retzbach (invoices), Catarina Vill-Härtlein and Mandy Roscher (accounting).

Persons responsible for specific equipment and instruments:

Equipment	**Location**	**Responsible Operator(s)**
Glow discharger	INF 267	Götz Hofhaus
Vitrobot I	INF 267	Götz Hofhaus
Vitrobot II	INF 328	Dirk Flemming, Jan Rheinberger
Aquilos 2	INF 229	Petr Chlanda
Talos 120	INF 328	Dirk Flemming, Jan Rheinberger
Glacios	INF 328	Dirk Flemming, Jan Rheinberger
Krios	INF 229	Götz Hofhaus, Jan Rheinberger

* The term ‘User Group’ refers to all users as registered in PPMS.

2. Access and Users

2.1. Application process

Group leaders or users requesting access to the HDcryoNet infrastructure are **required to get in contact with Dirk Flemming and/or Jan Rheinberger** (dirk.flemming@bzh.uni-heidelberg.de, jan.rheinberger@bzh.uni-heidelberg.de). If a user is requesting access, their supervisor (group leader) needs to be included in the initial communication. For newly submitted projects a discussion meeting will be scheduled, which requires the presence of the group leader. Applicants can either apply for an *independent* project*,* if sufficient expertise is present, or a *collaborative project* with an established HDcryoNet group leader. Preliminary data is required as a prerequisite: for single particle analysis projects, *e.g.*, sample purity (gel filtration) and negative stain EM data should be included. Acceptance depends not only on the feasibility of the project and available time on the instruments, but also on training and assisting capacity. We do not offer a full project service from sample preparation, data acquisition, image processing and interpretation. However, if capacity allows, we might be able to offer a 1-2 day 'feasibility check' by our experts at additional cost.

2.2. Access Rules

Each individual user is required to read and agree to the Terms of Use document (in PPMS, https://ppms.eu/cryoem-hd), and agrees to follow the HDcryoNet rules and regulations described therein. In addition, **group leaders need to sign the Terms of Use** for their laboratory and both will be held responsible and liable in cases of gross misconduct of the user with regard to the HDcryoNet infrastructure (negligence, damage, littering, and safety misconduct). Access to an instrument within HDcryoNet is restricted to registered users exclusively. In addition, users need to attend and follow the annual biological and radiation safety instruction and confirm their participation (see safety section).

Instruments can be accessed only after booking in the PPMS booking system. Any equipment, as well as the equipment rooms themselves, have to be handled with great care. Written notices in the respective rooms need to be followed. Before use, the instruments need to be visually inspected. Damage, problems or incidents must be reported to the HDcryoNet microscope-responsible operator(s) (see 1.3) immediately and recorded in a log-book. While LN_2 dewars are filled regularly, users are requested to check the levels before and during their session and inform the responsible operator.

The responsibility for compliance with biosafety and ethics regulations and the report of this is solely within the responsibility of the user and the respective supervisor and HDcryoNet cannot be held accountable in the case of user misconduct against these regulations.

To ensure that everyone is kept up to date, follows standardized operation, and to foster an exchange of knowledge, users are expected to regularly check the PPMS system for new indications (new terms, workflows, notes, etc.) and to attend and participate in the HDcryoNet meetings (Fridays 1:15 pm).

Group leaders are responsible to pay the costs for the use of HDcryoNet instrumentation, services and/or consumables according to the user fee rates indicated in the Annex.

2.3. Users

With respect to booking, support allocation and pricing (see annex) we differentiate several user types. We define three types of users based on their affiliation:

- HDcryoNet affiliated users are either members of the founding institutes (BZH, BioQuant) or members of institutes that financially contribute to the network (ZMBH). These users are generally given priority access over others.
- Heidelberg University users are members affiliated with the Heidelberg University (including CIID and the Medical Faculty) which do not belong to the HDcryoNet affiliated user group.
- External users are users outside of the Heidelberg University without a commercial interest, this includes the Heidelberg University Hospital, DKFZ, MPI, and EMBL. The user time for external users is generally limited to 5% of available uptime per equipment per year, unless capacity allows otherwise.

We further differentiate two types of booking users based on cryo-EM proficiency. This classification is system specific:

- User group proficiency I are users that can operate the booked instrument independently and with confidence. This includes, among others, using the Vitrobot, transferring samples, performing basic microscope alignment, setting up automatic data acquisition, setting up a cryo-cycle, and starting a camera heating cycle. Such users are defined as *autonomous users* in the PPMS booking system. *Autonomous users* will have after-hours access that allows HDcryoNet infrastructure usage beyond standard working hour restrictions (24 hours a day, 7 days a week). The **status of an autonomous user is given after passing an independence test defined by the *Responsible Operator* and assessed by Dirk Flemming and/or Jan Rheinberger**, with a subsequent use of the microscope within 3 months. The status of an autonomous user can be revoked in case of reoccurring user problems or inactivity for more than 6 months.
- User group proficiency II are users that cannot operate the instruments independently and require assistance, e.g. during training or due to lack of expertise and experience (e.g. sample transfer, alignment and/or setting up acquisition sessions). They **are not**

allowed to use the microscope without support and access for all group II users is restricted to standard working hours (9:00 – 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 or microscopes. Assistance can be provided by an autonomous user from their research group or by the *Responsible Operator* (1.3) of the equipment, which have to be present during the entire session. The assisting person needs to be indicated during booking in PPMS. A change to user group I can be made after showing the ability of independent operation of the equipment, and passing the 'independence test' defined by the *Responsible Operator* and assessed by Dirk Flemming and/or Jan Rheinberger.

Before leaving the university, users are kindly asked to inform Dirk Flemming and/or Jan Rheinberger about their departure.

2.4. 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 booking system PPMS using a personal account. To ensure compliance and prevent overbooking, these booking rules are implemented into the booking software and therefore cannot be violated. Each registered user can book an instrument once at a time. Double booking is not allowed except for I) Krios, Glacios and Aquilos 2-day weekend slots (Weekend slot 1 = Friday and Saturday; Weekend slot 2 = Sunday and Monday) and II) slots that are booked less than 24 hours in advance (users must leave a comment during the booking process: 'short-term booking'). Booking can also only be done 4 weeks in advance. If longer time slots are required or more long-term scheduling 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. Cancelation or changes of booking can only be done up to a certain number of days (see table) before the booked date without consequence. For bookings canceled or edited after the given time limit, charges may apply (see user fee section for more details). Any permanent changes to these rules need to be approved by the HDcryoNet steering committee. 9:00 – 17:00 are considered peak hours. 17:00-9.00, 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 the respective instrument's log-book.

Billing is performed on the basis of booking. If during a session technical issues arise that are not related to misuse of the equipment or poor-quality samples and which result in an early termination or long delay (> 3 hours) of the session, a reduction or cancelation of the user fees (see Annex) will be applied.

Instrument	Maximum time	Minimum time	Free cancelation up to:
Krios	24 hours per week	8 hours	72 hours
Glacios	24 hours per week	4 hours	48 hours
Talos 120	6 hours per day	1 hour	24 hours
Aquilos 2	48 hours per week	4 hours	24 hours
Vitrobots	4 hours per day	1 hour	N.A.

2.5. Grid storage

If required, HDcryoNet offers a grid storage solution from SubAngstrom located at the BZH. Users of this storage are required to get an introduction by Dirk Flemming and/or Jan Rheinberger, keep the associated online inventory system up-to-date and clean their storage regularly. Unregistered grid boxes will be removed during the annual cleaning cycle.

3. Safety policy

Proper safety policy implementation is crucial to minimize the risk of injury associated with the usage of HDcryoNet 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 seminars organized by dedicated microscope-responsible operators or HDcryoNet members. These seminars are organized annually and the presence of the HDcryoNet users is required. HDcryoNet 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/or audible warning, the users must vacate the room immediately and notify the microscope-responsible operator(s). HDcryoNet maintains all equipment and performs experiments in certified BSL-2 according to the respective regulations of the University of Heidelberg. HDcryoNet cannot be responsible for any injury or property damages resulting from the improper use of the equipment or violation of HDcryoNet Terms of Use and Safety Policy.

4. Data policy

Management and subsequent processing and analysis 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 or the **preprocessing servers for up to 5 days, after which they will be deleted without further notice**. There is no backup or protection of the locally saved data and HDcryoNet takes no responsibility in case of potential data loss accidents. Large data sets can be transferred directly to the Scientific Data Storage (SDS)[†] and processed on the bwForCluster[‡] of the University computing center (URZ), using the high-speed IT infra-structure (10-40Gb/s optical fibers) that connects the INF229 and INF328 buildings with the URZ.

5. Publication and acknowledgment policy

If results are published that were acquired making use of the HDcryoNet infrastructure and/or strongly benefited from consultation with an HDcryoNet user, the **following statement must be included in the acknowledgment section of the publication**:

"*We would like to acknowledge access to the infrastructure and support provided by the Cryo-EM Network at the Heidelberg University (HDcryoNet), which is funded and supported by the German Research Foundation (DFG), the Federal Ministry of Education and Research (BMBF) and the Ministry of Science Baden-Württemberg, among others, within the framework of the Excellence Strategy of the Federal and State Governments of Germany*".

New publications should be reported to Jan Rheinberger and Dirk Flemming and **entered in the respective section in the PPMS system**. Please not that it is crucial for a platform to keep an up-to-date track record for its own annual reporting.

[†] https://www.urz.uni-heidelberg.de/en/service-catalogue/storage/sdshd-scientific-data-storage
[‡] https://www.urz.uni-heidelberg.de/de/forschung-und-lehre/forschungsnahe-projekte/bwforcluster-helix

ANNEX I – Fees, Consumables and Billing

User Fees

The infrastructure fees are indicated below and vary according to the instrument, user affiliation and user proficiency:

TFS Titan Krios

User	User group I	User group II
Heidelberg University users	20 €/ hour	30 €/ hour
External users	40 €/ hour*	60 €/ hour*

TFS Glacios

User	User group I	User group II
Heidelberg University users	15 €/ hour	22.5 €/ hour
External users	30 €/ hour*	45 €/ hour*

TFS Aquilos 2

User	User group I	User group II
Heidelberg University users	15 €/ hour	22.5 €/ hour.
External users	30 €/ hour*	45 €/ hour*

* The user fees for external users are subjected to 19% VAT.

TFS Talos 120

User	User group I	User group II
Heidelberg University users	10 €/ hour	15 €/ hour
External users	20 €/ hour*	30 €/ hour*

Costs originated from the use of peripheral instrumentation (Vitrobots, glow discharger, and carbon coater) are currently included in the user fees of the Krios, Glacios, Talos 120 and Aquilos.

We note that for special cases an additional staff fee might be charged, for example when extra assistance, full service or consultation by our expert operators were requested or required for.

Consumables

LN_2 and ethane consumption is included in the user fees. Consumables such as grids, C-rings, autogrids and grid boxes are generally purchased and managed by each group. However, if required/preferred HDcryoNet can provide all typical consumables at a fee.

Billing

Invoices are sent out twice a year to the respective PI by Annette Retzbach usually in June and December covering the periods of December - May and June - November, respectively.

* The user fees for external users are subjected to 19% VAT.