Instrumentation, Policies, Services, and Rates
at UTHealth Houston Cryo-EM Core Facility

Electron Cryo-Microscopy (Cryo-EM) Core Facility at McGovern Medical School provides researchers access to the state-of-the-art cryo-EM resources for 3D analysis of both molecular and cellular structures. The Cryo-EM Core has high-end cryo-EM instrumentation and expertise for near-atomic resolution single particle analysis of molecular structures (SPA cryo-EM) and for cellular structure determination by electron cryo-tomography (cryo-ET).

These policies are intended to maximize performance and minimize downtime for the electron cryo-microscopes housed at the Cryo-EM Core Facility. Please note that the policies, rules and rates will be reviewed periodically and are subject to change.

Available instruments at the Cryo-EM Core Facility

1. **Polara F30**: This is a 300 keV instrument equipped with a K2 Summit direct electron detector and 6-slot multiple specimen holder and single axis stage tilt (±70°). Automated data collection can be acquired through SerialEM. This microscope can be used for screening cryo-specimen, preliminary cryo-EM data collection and electron cryo-Tomography (cryo-ET).
2. **Titan Krios G3**: This is a 300 keV instrument with an X-FEG electron source, equipped with a post-column Gatan BioQuantum energy filter, a K2 Summit direct electron detector, Volta Phase Plates, 12-position Autoloader. And dual axis stage tilt (±70°). Automated data collection can be acquired through EPU, Tomo and SerialEM.
3. **Ancillary equipment**
   a. Vitrobot Mark IV
   b. Manual cryo-plungers
   c. PELCO easiGlow Glow Discharge system
   d. Cressington 108C Auto Carbon Coater
   e. Denton Vacuum Carbon evaporator

Location:
The Cryo-EM Core facility is located in the McGovern Medical School Building (MSB):

**6431 Fannin Street, Houston, TX 77030**

* Tecnai G2 Polara Microscope – G.606
* Titan Krios G3 – G.601
* Cryo-grid preparation – 6.630 and G.606
SERVICES

The Cryo-EM Core facility will offer services that are adaptable to the user’s level of competency regarding electron cryo-microscopes and peripheral instruments. Staff time and availability will be reflected in cost and scheduling.

Polara
a. Only certified users are allowed to operate the microscope independently
b. Assisted users – Cryo-EM Core staff will provide all services related with cryo-EM operation (grids transfer, data acquisition). Cryo-specimen preparation services can be added at an additional cost. Because this option requires more Core staff time, requests for this service will need to be scheduled with Core staff based on their availability (for details see below “Sign-up and microscope usage rules”).
c. Training on the Polara microscope is no longer permitted.

Titan Krios

To maintain optimal Titan Krios performance, we do NOT accept pre-clipped grids. Only pre-clipped grids that have been previously imaged using a Krios, are permitted. However, we reserve the right to inspect these grids and reject them for loading based on their condition. Cryo-EM Core staff will always load clipped cartridges into the cassette and load the cassette into the Autoloader.

The Core will provide free of charge 2 cartridges/2 C-clips per session. Any additional supplies for clipping cryo-grids and for storage of clipped grids will be provided per change:

1x C-Clip & 1x Cartridges ($40)*
1x Box for storage of the clipped grids ($15)**
   *Used Cartridges are NOT permitted
   **Users can bring their own storage boxes

a. Experienced/Certified Cryo-EM users are allowed to clip grids themselves and will hand clipped grids to Core staff, who will load them into the Titan Krios for data collection. Users will be given an overview on how to use the data collection software EPU during their initial session in the microscope, if needed. These users will be allowed to perform some steps of direct alignment.
b. Staff-Assisted Data collection: users will hand off pre-screened grids to the Cryo-EM Core staff, who will clip the grids and perform grid transfer. Based on consultation with the
PI/lab the data collection will be set up by Cryo-EM Core staff. Cryo-EM Core staff will perform all alignments. Because this option requires more Core staff time, requests for this service will be scheduled with Core staff based on their availability.

**Data Storage**

Users will be required to provide a path to a data storage target or bring adequate hard drives to accommodate the data set. The storage location provided should have enough capacity to accommodate the anticipated data.

The cryo-EM facility will have the capacity to store data locally for 1 week following the data collection session. Data remaining on the Core Facility computers after 1 week may be deleted and will not be recoverable at a later date. Microscope usage fees will still apply regardless of whether the data is downloaded by the end user.

**Training**

Hands-on user training is available for peripheral cryo-EM equipment (glow discharger/cryo-plunger/evaporation). Training on the Polara microscope is no longer permitted.

**POLICIES**

**New Project Application**

All Cryo-EM Users are required to fill out one preliminary application form per project on the iLab page. Within 48 hours, a Core Facility member will schedule a time for a detailed discussion about the project feasibility, pricing, and goals. The proposal should contain the following sections:

1. **Project.** This should be a brief, one-paragraph description of the project accessible to a broader audience. This is only required for new projects. Subsequent proposals requesting additional time on the same project do not need to include this summary.
2. **Preliminary data.** This is the section where any data regarding the sample quality (e.g. negative stain images, SEC profiles, gels) should be presented.
3. **Timeline.** This section gives users a chance to indicate any circumstances that should be taken into account when assigning instrument time, such as grant deadlines, competitive publication situations, etc.
4. **Intended means of data storage.** Please specify where you will transfer the data collected at the Cryo-EM Core.

All labs will be required to set up an iLab account for services and billing prior to being granted access to the scheduler. Only after the project is approved and a PO is set up in iLabs can a time slot on the appropriate instrument(s) be booked.
Sign-up and Microscope Usage Rules

1. The Polara can be used for single particle cryo-specimen screening/data collection, negative stain screening/data collection and cryo-ET data collection. This microscope has been used to collect data resulting in structures solved to ~3.5 Å resolution, however, sample dependent variables (e.g. sample heterogeneity, flexibility) can limit the achievable resolution.

2. Only cryo-samples are allowed on the Titan Krios microscope.

3. The Titan Krios will primarily be used for cryo-data collection. Technically, the Krios can result in structures that are solved to better than 3 Å resolution, however, sample dependent variables (e.g. sample heterogeneity, flexibility) can limit the achievable resolution. As needed, 1-2 ‘screening days’ will be scheduled per month.

4. Krios cryo-cycle and K2-annealing will be performed once a week by the cryo-EM Core staff.

Booking EM slots

a. All users must sign-up for microscopes by submitting a service request to the Cryo-EM Core via iLabs.

b. EM time can be booked to 14 days in advance for Polara and 1 month in advance for Titan Krios. Sign-up for instruments will occur on a first-come basis for all users.

c. Polara time can be booked as screening 4-8-hr day or data collection 12-96 hours. Time slots start at 9 am. Certified users can book the Polara slot directly on the iLabs calendar but still have to submit a service request. All other users will be signed-up for the instrument by Cryo-EM Core staff once they submit a service request for the Polara.

d. Titan Krios time will be assigned by Cryo-EM Core staff after communications with the requesting PI/Lab. Request for the Krios time can be placed on iLabs. Scheduling is aimed to maximize Krios data collection time. Cryo-EM data collection will be available in 24-, 48- or 72-hour slots. Time slots start at 9 am and the session will conclude at 9 am at the end of the booked time slot (see below rules about sign-up cancellations). There is a booking limit of 72 hours per month of total Krios time per lab. Booking over 72 hours per month may be scheduled based on microscope availability.

e. 24 hour vacancy: Any slot still open 24 hours ahead of time becomes available to all certified users on a first-come basis.

f. Peripherals (glow dischargers/cryo-plungers/evaporator) can be booked by certified trained users in the iLabs calendar. All other users must to submit a service request to arrange an assistance of Cryo-EM Core staff.
**Charging and Cancellation**

a. All users must register in *iLabs* to get an access to the Cryo-EM Core Resources.
b. Any slots that are reserved will be charged, even if the user finishes early, unless another lab takes the released time. (In this case, charges will be divided between the labs according to hourly usage).
c. Cancellations: Slots can be cancelled without fee up to 48 hours in advance of their reserved slot. The signed-up lab is responsible for the cost of the slot, unless another lab takes the released hours. Cancellations should be announced to the Core personnel via the email to allow for efficient usage of available microscope time.
d. Down-time: Regularly scheduled maintenance will be pre-booked into the calendar. Should the microscope become unavailable to use during your time slot (*i.e.* due to immediate need for maintenance/repairs) you will not be charged for the unused time. However, your slot will not be made-up or cause schedule shifts, instead the regular schedule resumes after the microscope becomes available again.

**RATES**
Rates are available via *iLabs*:
https://uthealth.corefacilities.org/service_center/show_external/5042/

**USER RESPONSIBILITIES AND EQUIPMENT**

a. The Cryo-EM Core Facility provides key equipment and tools to the research community. The users and the quality of their research rely heavily on them and their pristine condition. Therefore, users must treat the cryo-EM equipment with care (from tweezers to the Krios) and cannot remove instruments/tools from the Core facility at any time. If any of the tools are missing or damaged, it is the user’s responsibility to report this to the Cryo-EM Core staff at the beginning of the user session.
b. Laboratory Supplies: You will need a variety of supplies and consumables. Users are responsible for covering the cost of expendable supplies (grids, grid storage boxes, mica, tweezers for grid preparation, filter paper, scissors, face masks).
c. Logbooks: Logbooks near equipment should be filled out with the user’s name, PI, equipment used and status of the equipment (*e.g.* report anything working ‘differently’, any errors, any broken components). This list will be monitored, however, report any major issues immediately to the Cryo-EM Core staff.
d. Data: It is the users’ responsibility to copy their data to their own storage space/devices within one (1) week from the end of their session.

e. Environment: All users must clean up the work areas after finishing their session, return all used items to their proper storage location and leave all rooms, microscopes and accessory instruments in the cryo-EM facility in a condition such that they are ready for the next user.

f. If users experience any problems while operating the instruments (hardware issues or software/computer errors), the user should not attempt to resolve the problem (unless specifically instructed by the facility staff). On the microscopes: stop operations, close the column valves and contact the facility staff immediately (24-hour contact is posted). Attempting to correct a malfunction and inadvertently damaging the microscopes can void our service agreement with ThermoFisher.

g. The Cryo-EM Core is not liable for any samples lost during any part of the cryo-EM process.

h. Any publication resulting from the use of the UTHealth Houston Cryo-EM Core Facility and any contributions of the staff should be acknowledged in the “Materials and Methods” and “Acknowledgment” sections.