

## Services, Rules, Policies, & Rates at the Beckman Center for Cryo-Electron Microscopy at the University of Pennsylvania

The instruments in the Beckman Center for Cryo-EM represent a substantial investment and resource for the University of Pennsylvania research community. To ensure that these resources are available at the optimum performance level and a minimum of downtime, the Internal Cryo-Electron Microscopy Advisory Committee (CEMAC) has established the following services, rules and policies. Please note that the services, policies, rules and rates will be reviewed periodically by the committee and are subject to change.

### Internal Cryo-Electron Microscopy Advisory Committee (CEMAC):

- Dr. Vera Moiseenkova-Bell (Faculty Director)
- Dr. Sudheer Molugu (Core Director)
- Dr. Darrah Johnson-McDaniel (Manager)
- Dr. Kristen Lynch (Chair, Department of Biochemistry and Biophysics)
- Lisa Ward (Financial and Administrative Officer, Department of Biochemistry and Biophysics).

**Beckman Center for Cryo-EM** is separated into two facilities:

- **Cryo-EM Screening Facility** located in the basement of the Anatomy Chemistry Building, Room B33.
- **Titan Krios Imaging Facility** located in the basement of the Singh Center for Nanotechnology, Rooms 011 and 013.

**The Beckman Center for Cryo-EM (Cryo-EM Screening Facility and Titan Krios Imaging Facility) is designated as a BSL-1 facility. If your project requires BSL-2, please contact the CEMAC committee for further discussion.**

### SERVICES OFFERED

#### Cryo-EM Screening Facility:

- The **Cryo-EM Screening Facility** houses **two FEI microscopes (T12 and TF20)**, Vitrobot and two Leica cryo plungers, as well as all the accessories needed to perform sample screening using negative stain imaging and cryo-EM.
- All users will first be trained to self-sufficiency and proficiency in microscope operation and sample preparation. The ultimate goal of user training is to create independent users who require minimal assistance from center staff.
- The **Cryo-EM Screening Facility does not** provide cryo-EM data processing and reconstruction services.
- The use of the facility and contributions of the staff must be acknowledged in the “Materials and Methods” and “Acknowledgment” sections respectively.
- **For T12 microscope, the first user for the day can start using the microscope 30 min in advance and cool the ACD, ramp up HT and filament.**

#### Titan Krios Imaging Facility:

- The Beckman Center for Cryo-EM also provides high resolution data acquisition on the **Titan Krios microscope** located in the Basement of the Singh Center for Nanotechnology.
- The facility houses a **Titan Krios** microscope, phase plate, Vitrobot cryo plunger, and all accessories needed to perform sample preparation for cryo-EM.
- The facility is currently restricted to the “center staff only” members for operation and only prescreened and **Krios-qualified samples** are allowed for data acquisition.
- The Cryo-EM data are encrypted and returned to the user.
- The facility **does not** provide cryo-EM data processing and reconstruction services.

- The use of the facility and contributions of the staff must be acknowledged in the “Materials and Methods” and “Acknowledgment” sections respectively.

#### **Pay Per Service Negative Stain Imaging at the Cryo-EM Screening Facility:**

- The Beckman Center for Cryo-EM Screening Facility only provides pay-per-service for negative stain imaging. These short (no more than 3 hours per sample) imaging sessions can be requested after a detailed discussion with CEMAC members.
- This service is only provided for **internal** users (UPenn and CHOP).
- The price for a staff assisted negative stain imaging session is \$350 per sample. This includes negative stain TEM grids (up to 3 grids per sample), negative stain, and 3 hours on the T12 microscope operated by a staff member.
- If further sample and imaging optimizations are required by the user, a new staff assisted negative stain imaging session will need to be requested.
- The use of the facility and contributions of the staff must be acknowledged in the “Materials and Methods” and “Acknowledgment” sections respectively.

#### **Collaborations:**

- The Beckman Center for Cryo-EM is also interested in direct **Collaborations** with labs performing cryo-EM experiments in their research.
- A thorough discussion of the theme and scope of the project will need to be discussed in detail with the CEMAC before agreement to the collaboration.
- Upon successful agreement, the participating staff members and their significant intellectual contribution to the project should be honored and considered for co-authorship on all resulting publications.

### **TRAINING POLICIES**

Hands on training is provided at our Screening Facility only, and the training is absolutely critical to learning how to operate the microscopes. Center staff members will provide training on all of the equipment as needed.

#### **T12 Training:**

1. A new user starts with Training on the T12 microscope. Training is one-on-one and consists of three, 2-hour sessions.
  - a. During the 1<sup>st</sup> session, the EMRL core staff will walk you through the entire process of the instrument's SOPs, rules and regulations, go over negative staining, glow discharge operation, sample holder insertion and removal, basic alignments and troubleshooting
  - b. During the 2<sup>nd</sup> and 3<sup>rd</sup> sessions, you are allowed to practice under the supervision/guidance of the staff. If additional training is required for a particular case, the user shall consult the staff and coordinate with their calendar.
2. A successful candidate is tested for his/her proficiency and are then allowed to sign up for TF20 training.

#### **TF20 training:**

1. Training is one-on-one and consists of four, 4-hour sessions.
  - a. During the 1<sup>st</sup> session, the EMRL core staff will walk you through the instrument's SOPs, rules and regulations and go over Vitrobot usage, cryo-holder and pumping station operation and loading cryo samples.
  - b. During the 2<sup>nd</sup> session, EMRL core staff will walk you through TF20 usage, low dose setup, alignments, image collection and Falcon III usage, and troubleshooting.

- c. During the 3<sup>rd</sup> and 4<sup>th</sup> sessions, you are allowed to practice under the supervision/guidance of the staff. If additional training is required for a particular case, the user shall consult the staff and coordinate with their calendar.
2. The ultimate goal of any user is to be an **independent user** in the **Cryo-EM Screening Facility**.

### **Definition of Independent User:**

We define an **independent user** as one certified to operate T12 and TF20 microscopes in the **Cryo-EM Screening Facility** during normal operational hours, as well as weekends, holidays and overnight without the supervision of staff. To be independent, individual users must demonstrate proficiency in: 1) handling holders, 2) microscope alignments, 3) low dose imaging and data collection, 4) ending of session, and 5) basic troubleshooting (and being able to assess what does/does not constitute “basic troubleshooting” vs problems that require immediate notification of the staff). Users should be able to perform all necessary steps safely and without having to refer to their notes.

## **USAGE AND BILLING**

All New Users are required to first fill out the consultation form on the Beckman Center for Cryo-EM web page. Once you submit the form, a staff member will schedule a time for a detailed discussion about the project feasibility, pricing, and goals.

New internal laboratories to the Beckman Center for Cryo-EM, with center approved projects, are required to first gain access to Electron Microscopy Resource Center (EMRL) and Beckman Center for Cryo-EM with appropriate account assignments in CAMS. In addition, you are required to set up an iLab account for accessing services and billing once your CAMS account(s) are activated and approved by your department business office.

For new external labs, you will need to create a PO and then submit it to our Core Facilities Coordinator. After your PO is approved, you are required to set up an iLab account for accessing services and billing.

### **Cryo-EM Screening Facility:**

- All users must sign-up for instruments in the **Cryo-EM Screening Facility** on the iLab website. You can reserve up to 14 days in advance. You can modify or cancel your reservation up to 24-hours prior to your reserved time slot. Users will be charged for the entire reserved slot, even if the user finishes early. If your sample is not viable and the session needs to be cancelled on the same day in the **Cryo-EM Screening Facility**, please contact a staff member as soon as possible. Failure to notify a **Cryo-EM Screening Facility** staff member in the first hour of the session will result in full financial responsibility for the session.
- New users will coordinate their training and sign up with the staff members of the Beckman Center for Cryo-EM.
- The minimum length for a negative stain sample screening session for an independent user is 1 hour on the T12 in the **Cryo-EM Screening Facility**. Sign up accordingly.
- The minimum length for a cryo-EM sample screening session on the TF20 microscope is 4 hours in the **Cryo-EM Screening Facility**. 4-hour sessions can be reserved on iLab in the following time slots: 9 am-1 pm and 1 pm-5 pm. Additionally, an 8-hour screening session on the TF20 is allowed (9 am-5 pm). Sessions can be extended provided proper session extension is reflected on iLab. If you only require a 2-hour cryo-EM screening session, you can use the T12 microscope. **Failure to obey these rules will result in a revocation of privileges to the Cryo-EM Screening Facility.** Sign up accordingly.

- All users must clean up their work areas, return all used items to their proper storage location and leave all rooms, microscopes and accessory instruments in the **Beckman Center for Cryo-EM** in a condition such that they are ready for the next user by the end of their session.

### **Titan Krios Imaging Facility:**

- To qualify a sample/project for data collection on the **Titan Krios**, users must first submit an application for Krios usage that will be reviewed by the qualification committee on a weekly basis.
  - **Application for single particle cryo-EM:** users must include, at least, cryo-EM pre-screening images from the same grids made from the same plunge freezing session (particles should be clearly visible) and 2D classification of their sample. If the application is successful, a Titan Krios time slot is granted. If necessary, the user can apply for an extension.
  - **Application for cryo-ET:** users must include, at least, cryo-ET pre-screening images.
- **Titan Krios Qualification Committee:**
  - Dr. Vera Moiseenkova-Bell (Faculty Director)
  - Dr. Sudheer Molugu (Core Director)
  - Dr. Darrah Johnson-McDaniel (Manager)
- The minimum length for **Titan Krios** data collection is 1 day in the **Cryo-EM Imaging Facility**. Each 1-day session is from 9 am to 9 am of the following day.
- The maximum session length on the **Titan Krios** is 3 days. Under extenuating circumstances (very heterogeneous sample, etc.), longer sessions can be accommodated.
- Due to the complexities of the instruments, user error, or unforeseen circumstances, unplanned outages do occur potentially interrupting user's session. When this occurs, the billed usage will be rounded up to the nearest half-day. Furthermore, due to the complexities of trying to rearrange scheduling when outages occur, any user with an interrupted session would need to sign up for a new session at the next available time slot. We aim to minimize any downtime within our control.

### **DATA STORAGE AND ACCESS**

- All University of Pennsylvania **Cryo-EM Screening Facility** users will get an account created on the data computer located in the corridor of B33, Anatomy Building. An account is setup for you by the core director during the initial consultation. Your account is connected to your Penn+Box, and the images collected on T12 or TF20 will automatically sync to the data computer and are stored on your Box account. Be sure to download the images to the local drive on your personal computer and backup your data. If you would like to use the Falcon III camera in counting mode, bring an external hard drive with you and copy the data onto the external drive.
- All External **Cryo-EM Screening Facility** users will get an account created on the data computer located in the corridor of B33, Anatomy Building. Your account is setup for either Box sync or Google drive sync. The images that are collected on the microscopes will be synced to the cloud. Be sure to download the images to the local drive on your personal computer and backup your data.

### **TREATMENT OF INSTRUMENTS/TOOLS/WORK SPACE**

- The **Beckman Center for Cryo-EM** provides shared instruments 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 and cannot remove instruments/tools/chemicals from

the facility at any time. If any of the tools are missing or damaged, it is the user's responsibility to report this to the facility manager at the beginning of the user session.

- All users must follow the **Beckman Center for Cryo-EM** Standard Operating Procedures.
- All users must clean up their work areas after they have finished their session, return all used items to their proper storage location and leave all rooms, microscopes and accessory instruments in the **Beckman Center for Cryo-EM** in a condition such that they are ready for the next user.
- Due to limited space, users should not store their samples, solutions, or other materials (grids, tweezers, etc.) in the **Beckman Center for Cryo-EM** unless authorized by the staff members or in the "grid motel" Dewar for short-term storage.
- **VERY IMPORTANT:** 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 manager). On the microscopes: stop operations, close the column valves and contact the facility manager immediately (24-hour contact is posted). Attempting to correct a malfunction and inadvertently damaging the microscopes can void our service agreement with FEI. If an error causes termination of the session the user will not be billed for the remainder of the booked time.

#### **CURRENT RATES AT BECKMAN CENTER FOR CRYO-EM**

- **Vitrobot/Leica cryo plungers:**

Internal Independent Use (UPenn and CHOP only): **\$25.00/hr**

Academic Independent Use (Temple, Drexel, Jefferson, Swarthmore and etc.): **\$30.00/hr**

Corporate Independent Use (External): **\$50.00/hr**

- **T12 and TF20 microscopes at Cryo-EM Screening Facility:**

Internal Independent Use (UPenn and CHOP only): **\$50.00/hr**

Academic Independent Use (Temple, Drexel, Jefferson, Swarthmore and etc.): **\$70.00/hr**

Corporate Independent Use (External): **\$100.00/hr**

- **Staff assisted negative stain imaging session:**

Internal Use (UPenn and CHOP only): **\$350.00/sample**

- **Training on Vitrobot, T12 and TF20 microscopes at Cryo-EM Screening Facility:**

Internal Independent Use (UPenn and CHOP only): **\$50.00/hr**

Academic Independent Use (Temple, Drexel, Jefferson, Swarthmore and etc.): **\$70.00/hr**

Corporate Independent Use (External): **\$100.00/hr**

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- **Titan Krios Imaging Facility Rates:** to be determined soon.