Policies for the Yale CryoEM Resource

The instruments in the Yale CryoEM Resource (YCR) are housed at three sites: West Campus, Sterling Hall of Medicine (SHM), and Science Hill. They represent a substantial investment and resource for the Yale research community. To ensure that these resources are available to all interested investigators and are performing at their optimum level with a minimum of down-time, the following policies have been established. These policies were developed by the Yale CryoEM Resource staff and Faculty Advisory Committee (https://cryoem.yale.edu/contact/advisory-committee) and are subject to periodic review and change.

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1. Prerequisites for new users
   a. YCR is managed through a web-based tool called Facility Online Manager (FOM). Each on-site user needs to setup a FOM account, through which they can apply for instrument access, reserve instruments, request services and manage their accounts. Please follow the steps at this link (https://cryoem.yale.edu/announcements/yale-fom-registration – note VPN required for FOM).

   b. Users must have completed the Laboratory Chemical Safety training (https://ehs.yale.edu/trainings/laboratory-chemical-training) and Biosafety training (https://ehs.yale.edu/trainings/biological-safety-training-part-1 and https://ehs.yale.edu/trainings/biological-safety-training-part-II). Users who perform negative staining must also have completed the trainings of Radiation Safety for Unsealed Sources (both part I and II, https://ehs.yale.edu/trainings/radiation-safety-basic-concepts). In addition, users must submit the certificates of the completion to FOM.

2. User levels
   a. For all EM except Krios, three categories of users are authorized and defined based on their level of training and expertise: Novice user, Certified user, and Superuser. Training is handled separately at each of the three YCR sites and staff at each site are responsible for certification (see Policy 7).
1) **A Novice user** is one that has begun training, but has not yet passed certification for that instrument. The Novice user can use the instrument, but requires support from YCR staff, Certified user, or Superuser. A Novice user is only allowed to operate the microscope during regular hours when YCR staff are present.

If a Novice user would like to use the microscope after hours and has established a collaboration with a qualified user (i.e., **Certified user** or **Superuser**), the qualified user may reserve the microscope on behalf of the collaboration and gain access to the instrument after hours. The qualified user will be fully responsible for the EM experiment, while the usage fee may be charged to the Novice user.

2) **A Certified user** has completed basic training, passed certification, and is able to work independently on particular instruments on which they are certified. The Certified user can use the microscope independently at any time when the YCR is open, including after hours, with the exception of docking and undocking cryo samples. If docking and/or undocking cryo samples is needed during afterhours, weekends, or holidays, the Certified user must get a Superuser to perform these procedures.

3) **A Superuser** is one that has passed certification and has received additional special training from YCR staff on loading grid cartridges into a cassette and transferring the cassette into the TEM with an autoloader (e.g., Glacios and Krios) or using a cryo side-entry holder to transfer a grid into the TEM without an autoloader (e.g., Tecnai T12 and Talos L120C). These processes are technically difficult and highly prone to errors that can potentially result in microscope down time. A Superuser may use the instruments for which they are certified at any time when the YCR is open.

b. **For Krios**, the access is determined by the sample quality, not the user’s experience (see policy 3a). However, only Certified users and Superusers are allowed to operate the Krios unsupervised. Other prospective users who want to become certified to operate the Krios should first contact the SHM or the Science Hill sites to receive the appropriate training. Alternatively, if a user has established a collaboration with a qualified user, the qualified user may submit a request on behalf of the collaboration and gain access to the instrument. In this case, the qualified user will be responsible for the EM experiment, while the user fee may be charged to the collaborator. Special requests for access to the Krios will be evaluated in collaboration with the director on a case-by-case basis.

c. **For all accessory equipment** (including the Chameleon, Vitrobot, UVOCS, Plasma cleaner, grid cartridge assembly station, cassette transfer station, cryo holder dry pumping station, etc.), the user must have received prior training from YCR staff.

d. For users experienced with a given technique, a complete training on this technique by YCR staff may be waived at the YCR staff discretion. Certification (for EM users) or a refresher training (for accessory equipment users) from YCR staff remains mandatory but free of charge.

3. **Samples allowed on the EMs in the YCR**

a. Only non-hazardous samples are allowed to be imaged in YCR. For all **new samples** that will be imaged in the YCR, a Core Facility Registration Form (Biosafety Form) has to be completed in FOM and approved by EHS as non-hazardous samples prior to bringing the sample to the YCR. Upon approval, the sample will immediately be allowed to be imaged on corresponding EMs with the exception of the Krios.

The **Krios** should be primarily used for large-scale data collection on optimized samples. Therefore, **for each new project on Krios, an additional scientific review is required**. The user must submit a Krios Access Request Form (available at [https://cryoem.yale.edu/policies-and-privacy](https://cryoem.yale.edu/policies-and-privacy))...
rates). Information provided through the form is intended to help identify potential experimental problems and maximize the likelihood of successful data acquisition. In the form, single particle users are required to provide representative cryo-EM micrographs and basic information about their sample (molecular weight, stoichiometry, symmetry, etc.), and are encouraged to show 2D averages and other results if available. Tomography users are asked to provide a brief description of their sample and target of interest, and have frozen grids prepared for imaging. The YCR staff may provide suggestions and feedback based on the information provided through the access request form. Decisions concerning access to the Krios will be made within three business days. Permission to access the Krios will be based on the quality of the preliminary data and the readiness of the sample, as not all projects may be suitable for data acquisition with the Krios.

b. Users may ship their samples to the YCR with the address information provided here: https://cryoem.yale.edu/contact. Yale users can use Intra-Campus Shipping service provided by eShipGlobal: https://your.yale.edu/yale-link/eshipglobal-express-shipping. Prior to shipping, each new sample shipped to the YCR must have an approved Yale University Core Facility Use Questionnaire on file.

c. Both cryo and negatively stained samples can be imaged on Tecnai T12 and L120C, whereas only cryo samples are allowed on the Krios and Glacios.

d. The achieved resolution is largely dependent on the quality of samples. Therefore, checking the quality of a new sample by negative staining in Tecnai T12 or L120C is recommended.

e. The Glacios can be used for both cryo-sample screening and cryo-EM data collection. Better than 2.5Å resolution of apoferritin has been achieved from our benchmarking experiments on the Glacios.

4. Access to the YCR

a. The YCR is open 24/7 unless otherwise informed by a YCR manager or director. The YCR is secured by an electronic card access control system. Access to the YCR is granted by a YCR manager or director following proper training. Visitors or unauthorized users must be accompanied by an authorized user when present in the YCR (unless otherwise approved by a YCR staff).

b. Only users of the YCR as defined in Policy 2 are allowed to operate the instruments (unless otherwise approved by YCR manager or director).

c. During regular hours (9am-5pm, Monday through Friday, excluding holidays), YCR staff are available to assist users as necessary. During off-hours, YCR staff should only be contacted in case of an emergency (such as issues in YCR infrastructure or hardware and software issues that could potentially result in damage to the microscopes).

5. Sign up

a. All instrument access in YCR must be reserved through FOM (http://fom.yale.edu).

b. Rolling sign-up – Instruments are available on a first-come basis for all users with the following limitations: each user can reserve a maximum of one (2-days or 3-days) slot on Krios, one (4-48 hr, or 72 hr if includes one weekend day) slot on Glacios, two (1-8 hr) slots on the Talos L120C or Tecnai T12, and/or two (1-3 hr) slots on Vitrobot. The only exception is the “Grab rule” (please see Policy 5g below). Once the user has finished a session, the user can then sign up for a new open slot.

c. Two type of sessions are available for signing up for each EM: attended and unattended sessions. An attended session only applies to weekdays and YCR staff will provide partial or full service
as requested; An unattended session applies to any days and the user will conduct experiments independently. In an unattended session, the user is fully responsible for monitoring progress of their experiment, although YCR staff will help and engineers may provide technical support for sessions during regular hours on Monday-Friday. Only qualified users (i.e., Certified user or Superuser; see Policy 2), are eligible to reserve unattended sessions.

d. For Krios or Glacios: because attended sessions rely on the availability of YCR staff and are currently in high demand, users should contact YCR staff directly by email to request time. Their email request should also include an Access Request Form (see policy 3, Forms can be downloaded at https://cryoem.yale.edu/policies-and-rates) containing session-specific information to facilitate the reservation. The time stamp of a valid request email determines the position in the queue.

1) Additional rules for Krios:

i. Only one active request per lab (i.e., PI group) is allowed in the queue. Additional requests will be accepted upon completion of the existing reservation. If a request is submitted on behalf of a collaborator’s lab, the active request will be counted toward that collaborator's lab, i.e., the lab that the project belongs to and typically pays for the usage, while a member from a different lab may handle the practical aspects of running the experiment.

ii. Reservations will be made on behalf of the user based on the availability of the user, the Krios, and the staff. Unattended slots apply to holidays, weekends, and days that the staff is out of office. The scheduling window is three weeks. Slots within three weeks are assigned to specific users. See access request form for more details.

2) Additional rules for Glacios:

i. A 4-hour morning session may only take place from 9am-1pm to enable session time for additional users on the same day. The only exception is the “48-hours grab rule” (please see policy 5g).

ii. A full-day(s) (1-3 days) session must end at no later than 9 am of the last day to ensure next user’s time. The only exception is the “48-hours grab rule” (please see policy 5g).

e. For Tecnai T12 or Talos L120C: users may sign up for unattended sessions without an Access Request Form, whereas attended sessions need to be requested by contacting YCR staff and including a Tecnai T12 or Talos L120C Access Request Form (Available at https://cryoem.yale.edu/policies-and-rates) from users.

f. Each user is responsible for finishing on time if there is another user right after you. If no one is signed up to use the instruments after your session, you can extend your time. The rate for extended time will be the lower one if the rates for your signed time and extended time are different. However, you need to record the actual end time in the logbook accordingly.

g. Grab rules: to ensure high usage efficiency of an instrument, users are encouraged to grab instrument time on a first-come basis if it has not been signed up within a certain period (see below). The grabbed time does not count towards the overall allowed sign up for each user/lab. But it must also be entered in the reservation calendar in FOM and should be marked as “Grabbed” in the “notes” of the calendar to avoid possible confusion.

1) A “One-week grab rule” will apply to Krios as it is highly used. If there are open slots in the scheduler, up to one week prior to the start of the session time, these slots may be booked by qualified users in consultation with the director without submitting review materials, including for the purpose of screening cryo samples.
2) A “48-hours grab rule” will apply to Glacios, whereas a “24-hours grab rule” will applied to all the other instruments.

6. Cancellation, Tardiness, Early logoff, and Loss of session

a. For multi-user facilities, cancellation, tardiness, and early logoff affect other users’ experiments. Therefore, cancellation, tardiness, and early logoff caused by non-emergency circumstances are discouraged, and may be subject to charge (see below).

b. Cancellation: active slots can be cancelled. For Krios and Glacios, if the cancellation occurs more than 48 hours (two business days) before the beginning of the reserved slot, it is free of charge; if it occurs less than 48 hours in advance and no user fills the reserved time, the sign-up user will be billed for one quarter of the reserved time.

c. Tardiness: if a user does not show up after ONE hour from the starting time, the reservation may be lost, the slot is then open to other users to sign-up. If no user uses the session, the sign-up user will be billed for one quarter of the reserved time.

d. Early logoff: if a reservation is ended early due to an instrument issue, the user will not be billed for the remainder of the booked time. Otherwise, any early logoff is discouraged.

e. Instrument Down: as customary for multi-user facilities, sessions lost due to unforeseen instrument down time and service will not be made up or cause schedule shifts (and will not be charged). However, once the regular schedule resumes after the microscope becomes available again, you will be placed at the top of the request queue and will be assigned a new session by the director as soon as new slots open.

7. Training, Practice, and Certification

a. Training on the use of the instruments in the YRC will be provided by YRC staff. A trainee will need intense practice to gain sufficient knowledge and proficiency to receive certification, which will be assessed by the YCR manager or director. Any disputes regarding certification will be adjudicated by the YRC director in collaboration with relevant faculty and administration.

b. Four types of training based on the trainee’s skill level can be provided:

1) **Negative staining EM training on Talos L120C or Tecnai T12** is designed for users who aim to independently perform negative staining EM using the Talos L120C or Tecnai T12.
   - It consists of five 2-hours training sessions and prepaid 6 hours of EM time. The training must be completed in 10 weeks after the initial training session. The prepaid EM time will allow the trainees to practice and improve their skills after the training is completed. The unused prepaid hours expire in 3 months after successful completion of the training.
   - Each trainee will be charged with a Training flat fee without additional instrument fee.
   - One user will be trained by one YCR staff only.
   - Trainees will be required to pass a certification (practical test) during the final training session, which will approve the trainees as Certified users of Talos L120C or Tecnai T12, and grant independent usage of corresponding EM and scheduling of future independent EM sessions using the FOM system. The certification of Talos L120C will be valid for Tecnai T12 with a refresher training (free of charge; see Policy 7b4)) and vice versa.

2) **Cryo EM basic training on Glacios** is designed for users who aim to independently prepare cryo samples and screen them using Glacios with SerialEM.
   - It consists of three full-day training sessions, which must be completed within 10 weeks after the initial training session. Although topics for each training session are well designed, they can be customized based on the trainee’s experience and imaging needs.
• The training is charged hourly for staff time plus the instrument usage fee.
• Training can be provided either in a group of two (if the trainees prefer to avoid excessively long waiting time and/or share the cost) or individually, with a cost of $1275/trainee and $2550/trainee, respectively.
• Trainees will be required to pass a certification during the final training session or a post-training user’s session, which will approve the trainees as Certified users of Glacios, and grant independent usage of corresponding Glacios. The certification of YSB Glacios will be valid for the SHM Glacios with a brief refresher training (free of charge) and vice versa.

3) **Advanced training** is provided for specific techniques and applications. For example, advanced training on SeraiEM enables trainees to collect high resolution (either SPA or Tomography) data using SerialEM and Glacios, or advanced training for cryo side-entry holder enables Talos L120C users to screen cryo samples on Talos L120C.
• The training is charged hourly for staff time plus the instrument usage fee, but can be conducted during user’s data collection session to reduce cost.
• Up to 2 trainees can be trained in a group if the trainees prefer to avoid excessively long waiting time and/or share the cost.

4) **Refresher training** enables users with previous experience to get instrument-specific and lab-specific knowledge. Up to one hour of refresher training is provided free of charge.

c. To receive training, users need to request training via email to YCR staff. Their email request should also include an EM Training Request Form (Available at [https://sciencehill-cryoem.yale.edu/training](https://sciencehill-cryoem.yale.edu/training) and [https://cryoem.yale.edu/policies-and-rates](https://cryoem.yale.edu/policies-and-rates)). YCR staff will follow up with the user to assess the project and associated training requirements. Applications are further assessed and approved by the YCR manager/director. Upon approval, the order of training will be set by the sequence of received applications and YCR staff will schedule training based on the availabilities of trainee, trainer, and instrument.

d. The Negative staining EM training, Cryo EM basic training, and Advanced training that does not result in trainees’ data collection does not count towards the overall allowed sign up for each user/lab.

e. For scheduled training, users who anticipate being tardy for more than 20 minutes should notify the YCR manager in advance. Tardiness for >60 minutes caused by non-emergency circumstances will result in cancellation of the training though the training session fee will be charged.

f. **Certification** is required for independent operation of each microscope. It can be requested by trainees once they feel confident about their skills and will be supervised by YCR staff. During the certification, individual users must demonstrate proficiency in performing the full workflow introduced in the training manual. The trainees who can independently (i.e., without referring to manual or notes) complete the test and demonstrate good judgement on problems that require immediate notification of the YCR staff will be approved by YCR manager or director as a Certified user.

8. Sample storage and Data safety

a. Due to limited space, users should not store their materials (samples, etc.) in the YCR, although short term storage (<1 month) of samples in the LN2 Dewar is allowed with prior authorization.

b. Browsing other users’ data without the owner’s permission is prohibited.
c. For maintenance and/or supervision reasons, the YCR staff might need to access a user’s data folder, but will keep the data confidential.

d. **Using YCR computers:** the computers in the lab are for data collection and analysis only. It is prohibited to alter any program on the computers, including installing, uninstalling, upgrading, or downgrading any software without permission from the staff. Users should never use them for web surfing, which increases the risk of exposing the computers to viruses and malware. Users must not insert any external file-storage devices (e.g., portable drive and USB drives) into the computers in the YCR.

e. **Using the Farnam cluster:** due to limited space in facility computers, user must save movie data directly into Farnam as the data collection is in progress or transfer the movie data to Farnam right after the session. Data in Farnam should be organized by date, i.e., each session is given a folder named with the date (e.g., yyyyymmdd-User).

f. Raw data and important files should be transferred immediately after data acquisition to your Farnam archive directory (if your PI has purchased archive space) or to your own personal hard drive. It is users’ responsibility to archive the data on users’ own storage drive to avoid data loss caused by possible failure of the storage space of YCR.

g. The priority is always to have enough space for upcoming sessions. Therefore, the storage space of the YCR (in both YCR computers and Farnam) is only for short term data storage. User must transfer the data to their own storage space. **Data older than two months in the storage space of the YCR will be deleted to release space without prior notification of users.**

9. **Additional user responsibilities**

a. If users experience any hardware related problems while operating the instruments, they must not attempt to resolve the problem by themselves. **Instead, they must first close the column valves, then get support from the YCR staff immediately.** In addition, they need to record the error details in the logbook.

b. **All usages of microscopes and accessory instruments must be recorded in the logbooks with details including name, lab, start time, finish time, and problems during the session.**

c. Users must treat the resources in the YCR with care (from tweezers to the electron microscopes) and cannot remove chemicals, instruments, or tools from the YCR at any time. If any of the tools are found to be missing or damaged, users must report this to the YCR staff **at the beginning of the user’s session.** Although making mistakes is part of the learning process, users are responsible for repairing/replacing damaged devices/instruments when misused or abused.

d. All users must clean up their work areas after finishing their work, return all items to their original locations, and leave the YCR in good condition for the next user.

e. Use of negative staining is confined to the specific bench space. The hazardous and radioactive waste need to be properly disposed in the assigned containers.

f. Spills of chemicals need to be notified and cleaned following Yale’s policy.

g. If users abuse YCR policies and/or act with a lack of respect toward other users or the YCR staff, their access to the YCR may be revoked.

10. **Acknowledgement and Rates**

a. **Acknowledgement of the Yale CryoEM Resource in publications:** considerable and continued funding is essential for the YCR to provide high end support to the researchers with subsidized fees. In order to maintain this level of support it is critical that the YCR and its funding are
acknowledged in publications. Therefore, publications that result from using the Yale CryoEM Resource are required to acknowledge the YCR and the grants, e.g., by adding a text similar to the following to their Acknowledgements: (1) “Cryo-EM data were collected at the Yale CryoEM Resource that is funded in part by the NIH grant 1S10OD023603-01A1.” if the YSB Glacios is used; (2) “Cryo-EM data were collected at the Yale CryoEM Resource.” for the use of all instruments other than YSB Glacios.

b. Information about rates is provided in Table 1.

c. Payment Information – By accessing YCR services, you agree to pay for services rendered by providing a valid COA in advance (internal users). External Users agree to provide payment upon presentation of a YCR invoice. External user payment terms are Net 30 and payable by check, wire transfer or credit card.

Table 1. Rates of instruments, Services, and Consumables in Yale CryoEM Resource

<table>
<thead>
<tr>
<th>Instrument, Service, or Consumables</th>
<th>Location¹</th>
<th>Internal Rate</th>
<th>External Academic</th>
<th>External For-Profit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titan Krios Daily (Attended)</td>
<td>WC</td>
<td>$1000/day</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Titan Krios Daily (Unattended)</td>
<td>WC</td>
<td>$660/day</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glacios Daily (Unattended)</td>
<td>SHM, YSB</td>
<td>$600/day</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glacios</td>
<td>SHM, YSB</td>
<td>$60/hr Peak⁴</td>
<td>$45/hr Off-Peak</td>
<td>-</td>
</tr>
<tr>
<td>Talos L120C</td>
<td>YSB²</td>
<td>$50/hr Peak</td>
<td>$106/hr</td>
<td>$225/hr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$37.5/hr Off-Peak</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tecnai T12</td>
<td>SHM</td>
<td>$50/hr Peak</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$37.5/hr Off-Peak</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Cleaning</td>
<td>SHM, YSB</td>
<td>$65/hr</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitrobot</td>
<td>WC, SHM, YSB</td>
<td>$50/hr</td>
<td>$125/hr</td>
<td>$125/hr</td>
</tr>
<tr>
<td>Chameleon</td>
<td>SHM</td>
<td>$75/hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon Coating</td>
<td>SHM, YSB</td>
<td>$60/hr</td>
<td>$136/hr</td>
<td>$150/hr</td>
</tr>
<tr>
<td>Negative staining EM training</td>
<td>SHM, YSB</td>
<td>$1000 flat fee</td>
<td>$1700 flat fee</td>
<td>$2000 flat fee</td>
</tr>
<tr>
<td>Staff Support or Training</td>
<td>SHM, YSB</td>
<td>$70/hr</td>
<td>$112/hr</td>
<td>$125/hr</td>
</tr>
<tr>
<td>Auto-grid ring and C-clip⁴</td>
<td>WC, SHM, YSB</td>
<td>$18.10/set</td>
<td>$27/set</td>
<td>$32/set</td>
</tr>
<tr>
<td>Autogrid box</td>
<td>WC, SHM, YSB</td>
<td>$12.50/each</td>
<td>$18/each</td>
<td>$22/each</td>
</tr>
<tr>
<td>Chameleon grid</td>
<td>SHM</td>
<td>$20/each</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹. WC (West Campus), SHM (Sterling Hall of Medicine), YSB (Yale Science Building).
². Talos L120C is located in Bass.
³. Peak hours are 9am-5pm on weekdays. Off-Peak are 5pm-next 9am, weekends, and Yale holidays.
⁴. Users are responsible for the cost of auto-grid rings and C-clips. Although the YCR provides consumables, users are encouraged to order the consumables directly from Thermo Fisher Scientific.
11. **Feedbacks, Comments, and Concerns**

For techniques and general issues, please direct feedback, comments, and concerns to YCR Staff:

Xinran Liu, [xinran.liu@yale.edu](mailto:xinran.liu@yale.edu) (Interim Director, YCR)
Jianfeng Lin, [jianfeng.lin@yale.edu](mailto:jianfeng.lin@yale.edu) (Associate Director, YCR)
Shenping Wu, [shenping.wu@yale.edu](mailto:shenping.wu@yale.edu) (Research Scientist, West Campus site)
Marc Llaguno, [marc.llaguno@yale.edu](mailto:marc.llaguno@yale.edu) (Cryo EM Manager, SHM site)

If you have additional concerns or inquiries about CryoEM at Yale, please direct them to Ben Myers, [b.myers@yale.edu](mailto:b.myers@yale.edu) (Director of Research Cores).

In addition, the YCR Advisory Committee members are listed below for your reference:
Frederick Sigworth, [fred.sigworth@yale.edu](mailto:fred.sigworth@yale.edu) (Chair of the YCR Advisory Committee; Professor, Cellular & Molecular Physiology)
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