

General Policies

The ATC Core Facility is dedicated to the development of the scientific community with a focus in the TMC region. To ensure that these resources are available to investigators at the optimum performance level, the following policies have been established:

- The ATC Core Facility at Baylor College of Medicine is BSL-1. This type of lab has the lowest level of biosafety, the agents worked with in this lab do not ordinarily cause human disease. If you have a need to work with BSL-2, please contact the core directors. Violating this policy could result in a permanent ban from the facility, as well as BCM disciplinary actions.
- If you experience any problems while operating the microscope, stop what you are doing, close the beam valve, and notify a core staff member immediately. Do not try to fix the issue on your own. Attempting to do so may result in damage to the microscope and cause the equipment to be offline for several days or possibly even weeks.
- If a more serious maintenance problem arises and service engineers are required to address the issue, core staff will handle all communication with the engineers and will report to all users when the microscope comes back online. Users must refrain from interrupting the engineers as they are working.
- If a user error or failure to follow policy interrupts a session, the time scheduled for that session will be fully billed. If this results in extended downtime, at our discretion we may bill for additional time the instrument is unavailable.
- If any equipment is undergoing maintenance, affected reservations will be cancelled and will not be billed. Once the equipment is back online, the schedule will continue as normal. Reservations that were canceled, may be rescheduled. Users are responsible for rescheduling their reservations on a first-come first-serve basis.
- Instruments/Tools supplied by the core are for your use and may not be taken out of the lab. Please treat all tools and instruments with care, and report any missing or damaged instrument to core staff before the start of your session. Community tools (tweezers, storage boxes, blue transfer dewar) are relied

upon by most users in this facility. Abusing this policy will result in removal of these items and users will be required to bring their own tools.

- Liquid nitrogen and other specialty gases may only be used in the facility and for any cryo-electron microscopy related procedures. When you empty a liquid nitrogen tank, please put the empty sign on the tank. The empty sign is located on the wall close to the door. This will help core staff monitor the amount of liquid nitrogen available for user use.
- All acquired microscope images and data are required to be uploaded to the CryoEM Database (cryoemdb.bcm.edu) during or after your scheduled session. Once done, you may back up your data and remove it from the microscope computer. The fast data and archive hard disks are for temporary storage, and these drives will be cleaned to make space for new projects periodically. No advance warning is given prior to removal of old files. You are responsible for ensuring that your data has been transferred immediately after your session.
- Users may not, by any means, operate the equipment if their reservation is still pending staff approval. Any individual that is caught using the microscope or any other equipment when they are not supposed to will have their access to the facility restricted. This includes untrained individuals operating any equipment alone at any time, trained individuals without off peak access operating the equipment during off peak hours or trained individuals using equipment without a scheduled reservation.
- You are responsible for leaving each workspace as you found it for the next user. All equipment rooms and benchtops should be kept clean. Trash should be thrown in the appropriate waste bin. Microscope rooms will be checked after every session to ensure the room is cleaned up properly. If a user does not leave the room as they found it, core staff will clean up after the user and the user will be billed for staff time.
 - Things we will be looking for:
 - Consolidating nitrogen into as few dewars as possible
 - Disposing gloves, kimwipes, papers etc.
 - Putting tools back where they were found
 - Blue dewars should be returned to their proper area by the sink

- We are required by BCM to account for staff time, and bill for any staff related assistance. These fees may be added to your account. The only exclusion is for assisting with spontaneous equipment failures.

External User Policies

- An external user is defined as any individual who is not employed by Baylor College of Medicine but participates in the usage and handling of equipment and instruments at the Baylor CryoEM Core Facility.
- External users are required to have a specific permission level per BCM's new external user policy. This policy states that external users can operate the CryoEM Core Facility equipment during peak hours only. Peak hours are from 8:00 am to 6:00 pm Monday through Friday, excluding holidays.
- Per BCM's external user policy, a core staff member must periodically check on external users throughout their session. Because of this, an hour of staff assistance will be automatically added to the microscopy session.
- If an external user wishes to reserve a microscopy session during off-peak hours, an email must be sent to the core staff at least 2 days prior to the desired session date. This request must be approved by core staff, however, if it is not approved, the session must end before off-peak hours begin or it can be rescheduled during peak hours.
- Any time an external user uses the microscope during off-peak hours, a core staff member must be present, meaning a staff assistance charge will be added to the microscopy session.

Training Policies

- EM training packages include hands-on training on the 2100 microscope and grid preparation. The grid preparation includes training on the Vitrobot Mark IV, Leica, the glow discharge machines, negative stain preparation, and applying various films on grids.
- A trainee will be considered a trained user on the 2100 microscope if they meet the two requirements below.
 - The core staff feels comfortable leaving the user alone to operate the microscope

- The user feels fully comfortable operating the microscope by themselves
- The 2200 microscope is not included in the EM Training package. Once an individual has operated the 2100 microscope on their own for a minimum of 5 sessions without significant errors, they may submit two EM refresher courses requesting 2200 training. Access to this training is at the discretion of the core staff who will approve your request for training and coordinate training days. Appeals may be made to the core directors.

Collaborative policies

- If a drop-off time is agreed upon by both parties, everyone is responsible for arriving at the appropriate time and location. If the collaborator is running late, a core staff member must be notified as soon as possible. If core staff is not notified, the collaborator will be fully charged for the scheduled sessions, as well as the sessions scheduled after the sample arrives. All normal session cancellation policies apply.
- If a screening session exceeds the four hours scheduled, microscope usage and staff assistance will be added to the screening request. This addition will be for every hour following the end of the initial session.

Sample, Grid, and Personal Equipment Storage

- We have a 4°C refrigerator with plenty of room for sample and buffer storage. There are racks on each shelf that contain individual compartments. These are perfect for small, white storage boxes. Please ask a core staff member for assistance if needed.
- When adding samples to the refrigerator please email a core staff member with the name of those samples. In addition, please label your specimen. We keep an inventory of samples that are stored in this refrigerator.
- If you need to store cryo grids, there are storage dewars in the back walkway that leads to the nitrogens tanks. Contact core staff before using these. We maintain the nitrogen weekly. There is a small dewar closest to the door that leads out to the hallway which may be used for temporary/emergency storage.
- Numbered spaces in storage dewars are assigned by core staff. You will be required to use your spot, and only your spot to store your samples. If your samples are found in an unassigned spot or another users spot, your sample will be moved to the smaller dewar by the nitrogen tanks, but we do not

guarantee their integrity. If your assigned spot exceeds six falcon tubes, contact a core staff member to acquire another storage spot.

- There are three storage dewars that belong to other labs. These dewars are off limits to users not belonging in those labs. Other specimens found in these dewars will be disposed of.
- When storing your samples, please fold a piece of tape on the end of the string attached to your falcon tube so you may label it. Please include your name and the name of your sample.
- Please be considerate to users sharing a storage dewar with you. Keep your strings and storage spot organized. It is really easy to get the strings tangled.
- As a trained user, you will have some kind of supplies that you may want to store in the lab. We have drawers downstairs that may be used for storage. Space is limited, so please buddy up with another individual and share a drawer together. Make sure to label your drawer with your name on a piece of tape. This helps us keep track of empty drawers.
- If you have a box that contains all your supplies and do not wish to use a drawer, there are shelves right above the drawers that may be used. The labelled drawers are NOT community property, and "borrowing" items without the owner's permission may result in a permanent ban from the facility. If you have questions, or believe that someone is no longer using the facility, please contact core staff.

Pumping Station and Holders

- The last user of the day is responsible for putting their cryo-holder in the pumping station, turning the pumping station on, and decontaminating the tip by heating it to 90°C.
- Please fill out the log by the pumping stations. Information needed is the date, holder type, whether you pumped the zeolite or the tip, what time it started, your name, and the name of your lab.
- Cryo-holders should always be placed in a pumping station for overnight storage, unless otherwise directed. Cryo-holders left overnight in a microscope room without core staff approval will result in a warning.
- Never, under any circumstance, may a user clean or replace the O-rings on any of the holders. A user should report to core staff of any visible debris or extremely slow pumping of the microscope. Never insert a dirty holder into the column. Core staff will address and fix the situation as soon as they can.

Vitrification Room and Equipment

- Cooling the stage and preparing to freeze is part of your scheduled session, and may not be done before your reservation starts.
- Please help us keep the vitrification room clean. Please dispose of used pipette tips, kimwipes, filter paper and other trash. Return freezing tools back to their designated spot.
- Once you are finished freezing please remove used filter paper and close the main ethane tank valve. Shut down the equipment properly when you are finished using it and dispose of your used liquid nitrogen and ethane under the fume hood.
- If an individual is scheduled before you on the Leica, please stop by the vitrification room a little before your reserved time to remind that individual not to bake it out. You will be responsible for your own time.
- Nitrogen dewars with nitrogen in them should not be left in the Vitrification room. Please give the dewar to a core staff member or offer the nitrogen to other users in the facility.

Glow discharge scheduling policy

- The glow discharge machine (old or new) should be reserved for every use. This means, for every freezing session scheduled, whether it is the Leica or the Vitrobot, a glow discharge reservation must be made. If a reservation is not scheduled for the glow discharge, and the machine was used by the user, core staff will add a one hour glow discharge session for the user.

JEM2100 microscope operation policy

- Core staff members will generally take the responsibility of raising the HT and aligning the microscope for experienced users. Core staff members, ideally, will have the microscope aligned and ready for imaging 30 minutes following the start time of the scheduled session. The exception to this is newly trained microscope users, who will be responsible for aligning the microscope on their own. This will allow new users to develop their skills of handling the microscope further.

- Since core staff will be handling the alignment of the microscope, a minimum of one hour of staff assistance will be added to the microscope session. This will allow core staff to ensure the microscope is in good condition and ready for imaging, and should minimize the impact of previous users.
- Do not use the microscope-attached computers for web browsing, email, or any other purpose! Due to their connection to instrumentation, these machines are not well protected from viruses, so visiting even innocuous websites can potentially disrupt the machine. If you are caught doing this, you may be banned from using the instrument.

JEM2200F microscope operation policy

- Core staff members will generally take the responsibility of raising the HT and aligning the microscope for experienced users. Core staff members, ideally, will have the microscope aligned and ready for imaging 30 minutes following the start time of the scheduled session. The exception to this is newly trained microscope users, who will be responsible for aligning the microscope on their own. This will allow new users to develop their skills of handling the microscope further.
- Since core staff will be handling the alignment of the microscope, a minimum of one hour of staff assistance will be added to the microscope session. This will allow core staff to ensure the microscope is in good condition and ready for imaging.
- The only type of screening allowed on the JEM2200F microscope is cryo-sample screening. There should be **no negative stain imaging** on this microscope.
- Do not use the microscope-attached computers for web browsing, email, or any other purpose! Due to their connection to instrumentation, these machines are not well protected from viruses, so visiting even innocuous websites can potentially disrupt the machine. If you are caught doing this, you may be banned from using the instrument.

JEM3200 microscope operation policy

- The 3200 may be scheduled up to one month in advance depending on the needs of the project and imaging requirements. If you would like to schedule a data collection session please contact any one of our core staff members.
- Collaborative efforts will require core staff to operate the microscope which includes transferring grids, aligning the microscope, and setting up continuous automated data collection. A minimum of two hours of staff assistance will automatically be added to a microscopy session. Any additional assistance performed by core staff will be charged.
- Users and collaborators will have the ability to remotely access the 3200 computer in order to monitor their data collection. However, any adjustments that need to be made, must be done by approved users or core staff. These adjustments will be counted toward the staff assistance time.
- If there are any unexpected errors, please inform core staff immediately so they can assess and address the issue properly.
- 3200 Windows computers are only for microscopy purposes. **No data storage is allowed.**
- Do not use the microscope-attached computers for web browsing, email, or any other purpose! Due to their connection to instrumentation, these machines are not well protected from viruses, so visiting even innocuous websites can potentially disrupt the machine. If you are caught doing this, you may be banned from using the instrument.
- If a cancellation needs to be made, you are responsible for cancelling your reservation up to three days before your start time. If you cancel within the three days, you will be fully billed for the time reserved unless another user reserves the instrument at that time.

Scheduling, Cancellation, and Tardiness

- Microscope sessions may be made only for 4 or 5 hours, arranged such that two sessions/day are possible. If a smaller time block is desired, please discuss splitting a four hour session with another user.

- Newly trained microscope users are required to use the instruments during peak hours until the core approves off peak usage.
- Users with Advanced Trained status may operate the microscope during peak and off peak hours.
- Any trained BCM user may continue collecting data in an existing microscopy session past 6:00 pm, if the reservation began prior to 2:00 pm and core staff is notified before 5:45 pm. No new grids may be transferred after 6:00 pm. This policy does not apply to External users.
- If a user finishes their session early, they will still be charged for the remainder of the reservation unless another user takes over.
- If a cancellation needs to be made, you are responsible for cancelling your reservation 24 hours before your start time. If you cancel within the 24 hours, you will be fully billed for the time reserved unless another user reserves the instrument in your stead.
- If a user begins their session later than originally scheduled, that user will be fully billed for their reservation in addition to the extra time spent on that instrument afterwards.
- If a user does not show up for their microscopy session, they will be fully billed for the time reserved.

Users who abuse any of the policies listed above will be placed on probation and will be restricted from using any core facility equipment. The duration of a users restricted access will depend on the frequency and severity of the abuse. Users who exhibit a lack of respect towards other users, the facility staff, or the laboratory itself may result in access to the facility being revoked permanently. Appeals and disputes may be raised with the core directors.