



Standard Operating Procedures – CryoEM Lab

The Cryo-EM Laboratory is a shared resource of the Sealy Center for Structural Biology and Molecular Biophysics (SCSB). All users share responsibility for careful, error-free usage of the microscopes and other equipment in the lab. Usage of the facility can be obtained as an SCSB certified microscopist or via the staff microscopists. All microscopists must be either trained SCSB microscopy personnel or certified by the manager. All usage must be logged and authorized.

STEPS TO OBTAIN AUTHORIZED USAGE:

1. Complete the SCSB Memorandum of Understanding – User of Center Instrumentation Resources form and submit for approval.
2. Register each active project in the EMEN2 database, listing the appropriate investigator(s)/PI(s) and microscopist.
3. Reserve microscope usage utilizing the web-based sign-up software (currently <http://cryoem.utmb.edu/cgi-bin/emcal/index.cgi> or email emcal@cryoem.utmb.edu).
4. Users who wish the EM Lab staff to run their samples, should so indicate on the MOU under “user personnel” and arrange times according to staff work schedules.
5. To receive notification of user cancellation on a particular microscope, subscribe to the email list for the corresponding microscope. (Slots are filled on a first come/first serve response basis).

SUPPLIES:

SCSB Cryo-EM Laboratory will provide:

1. Basic chemicals necessary for EM including typical negative stains.
2. Bare EM grids.

User will provide:

1. Specialty chemicals required for the project.
2. More expensive EM grids (“C-flat” and/or “Quantifoil”).
3. Tools such as EM tweezers, cryo-buttons (cryo-storage grid boxes).

SCSB CRYO-EM LABORATORY USER RESPONSIBILITIES:

1. User sessions must start on time. *Failure to show up with an hour of the start time will result in forfeiture of the slot if others are ready.*
2. All sessions must end on time, with provisions made for staff or certified microscopist scheduling.
3. No food or drink allowed in microscope rooms or chemical/specimen preparation labs.
4. Immediately report equipment malfunctions to M. Sherman or M. Woodson. Users are not authorized to attempt to repair equipment.
5. Notify EM Lab staff if liquid nitrogen tank runs dry or if Lab-supplied chemicals/grids/gases run out.
6. Tools must remain in the microscope rooms.
7. After each use, replace tools in their designated storage.
8. Wear rubber gloves when working with vacuum coater, plasma cleaner and film scanner to prevent equipment contamination.
9. Clean specimen carriers before inserting them into the coater or plasma cleaner.

10. Specimen carriers must not be touched with bare hands until they are removed from vacuum.
11. At the end of a microscope session, empty the specimen dewar (cryo-EM) and turn it upside down to allow it to warm up and dry out.
12. Clearly label all specimen containers if stored in the refrigerator/freezer in EM Lab. *Unlabeled containers will be flooded with Cavicide and autoclaved before being discarded.*
13. As a shared use facility, it is important that each user cleanup at the end of his/her session.

USER GUIDELINES FOR LAB EQUIPMENT USAGE AS MICROSCOPIST:

1. Align microscope.
2. Start "EM-DASH" program on a GATAN PC. *(The program will upload images to EMEN2 database for permanent storage. Users may retrieve images from EMEN2 using the supplied web interface).*
3. Cool down the microscope.
4. Transfer specimen to the microscope.
5. Maintain liquid nitrogen level in specimen holder dewar and in ACD dewar.
6. Close session with centering specimen stage.
7. Take out specimen holder.
8. Warm up specimen in a dry-pumping station.
9. Activate zeolite cycle.
10. Activate ACD bakeout cycle in the microscope by inserting ACD heater into ACD dewar and activating "ACD bake" in the microscope control software (either TEMCON, or TEM Center). Plug specimen airlock with blanking plug after specimen holder is removed.
11. Use of JEM 2200FS when BSL-3 containment is locked requires operation of the microscope remotely from 1.206. (Only BSL-3 certified staff members are allowed in the containment.) Under these circumstances, the microscope will be cooled down and specimens transferred to the microscope, and the session will be closed by the Lab staff.
12. Use of JEM 2200FS when BSL-2 containment is enabled allows operation of the microscope remotely from 1.206 or by BSL-2 certified microscopists.

THE FOLLOWING SPECIAL CIRCUMSTANCES REQUIRE PRIOR AUTHORIZATION OF THE DIRECTOR, SCSB:

1. Override of reservations for emergencies, EM failures, urgent maintenance or repair of equipment.
2. Extraordinary/continuous use by a group to complete a project, or additional time to accommodate a collaborator visit.