

SPECIALIZED MICROSCOPY MODULE POLICIES AND PROCEDURES

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Each principal investigator (PI) is responsible for the conduct of his or her personnel in the Core laboratory. Everyone's cooperation is needed to maintain a facility where safe, high-quality research can be conducted. Your help in observing the laboratory guidelines is appreciated by all users of our Specialized Microscopy Core facility. Work supported by NEI R01 grants always has first priority.

ORIENTATION AND TRAINING

All new personnel (investigators, technicians, fellows, residents, and students) who want to use the major equipment in the Specialized Microscopy Core facility (e.g., transmission and scanning electron microscopes, Zeiss photomicroscope, confocal or fluorescence microscopes, ultramicrotomes, cryostat, and darkroom facilities) must be trained or certified by the laboratory staff (Ernesto Barron or Anthony Rodriguez). All PIs who want their personnel trained to use the laboratory equipment must submit a brief summary of the user's qualifications and goals to the laboratory supervisor. Users are responsible for procuring any additional needed help and/or training deemed necessary from their supervisor or PI. During the orientation period laboratory personnel will explain safety precautions and cleanup procedures and discuss which supplies will be provided by the Core facility and which supplies the PI must provide. Each PI is responsible for ensuring that his or her personnel conform to the policies and procedures in the Specialized Microscopy Core facility as set forth by the laboratory's director, co-director, and supervisor.

USE OF EQUIPMENT

Most of the equipment in the Specialized Microscopy Core facility is available on a sign-up basis, but work supported by NEI R01 grants will be given priority. Sign-up sheets are available in the laboratory at least two weeks in advance. If two people want to sign up for the same piece of equipment, research work to meet a grant or meeting deadline takes priority over other research. The laboratory supervisor arbitrates all problems concerning the use of equipment. If there are disputes over the use of equipment, the PI should discuss the matter with the facility co-director. The laboratory supervisor is to be notified immediately whenever problems occur with the equipment. Every effort will be made to correct problems in a timely manner.

AFTER-HOURS USE OF SPECIALIZED MICROSCOPY CORE FACILITY

Only qualified personnel will be allowed to use the equipment after 6:00 pm and on weekends. Those who wish to use the darkroom, electron microscopes, photoscopes, confocal microscopes, etc., will need to obtain a key from the laboratory supervisor. The laboratory supervisor must be notified in advance, and the equipment to be used must be specified. The laboratory supervisor must be contacted if an emergency occurs after hours. Emergency numbers are posted on the front of the main door to the laboratory.

PILOT PROJECTS

Principal investigators must complete and submit to the laboratory supervisor a standard form termed "Tentative Projects Protocol Sheet" for every pilot project requiring the use of the Specialized Microscopy Core facility or technical assistance from the laboratory supervisor or

technician. A "Tentative Projects Protocol Sheet " can be obtained from the laboratory supervisor and completed after consultation. Before any work begins, the core director or co-director will discuss the project with the PI and decide how much of the laboratory supervisor's or laboratory technician's time will be required, as well as the amount of supplies to be used. A pilot project is defined as an unfunded project expected to lead to a R01 grant and lasting no longer than six months.

PROBLEMS AND GRIEVANCES

If a user has concerns about how the laboratory is being used or how it is being run, the user should contact the laboratory supervisor. If the supervisor is unable or unwilling to rectify the problem, the issue should be taken to the laboratory director.

SPECIFIC LAB PROCEDURES

1. Each user must leave his/her work space in the Core facility clean and orderly. All chemicals must be returned to their proper places. Glassware must be cleaned, dried, and put back in the cabinets. The laboratory supervisor must be notified before the last of any supply item is used.
2. Osmium tetroxide, electron microscopy film, and darkroom paper must be supplied by each PI. Other films and chemicals will be provided.
3. No papers or pictures are to be taped or pinned to the walls or doors.
4. The phones in the Specialized Microscopy laboratory are not for personal use. Others should be discouraged from calling users working in the Specialized Microscopy laboratory unless necessary.
5. To ensure that the laboratory supervisor has time to complete his own work, all projects that require the supervisor's assistance should be scheduled before 2:00 p.m.

ELECTRON MICROSCOPES

- a) TEM users will be trained to use the Zeiss EM10 transmission electron microscope and allowed to use the scope independently once proficiency is demonstrated.
- b) SEM users will be trained to use the Hitachi scanning electron microscope and work station and allowed to use the system independently once proficiency is demonstrated.
- c) The laboratory supervisor will align the scopes and change filaments as required.

CONFOCAL MICROSCOPE

- a) Users will be trained on the Zeiss 510 Confocal/Multiphoton microscope by the laboratory supervisor or technician. Only when proficiency is demonstrated will users be allowed to use the scope independently.
- b) Each user will be expected to know how to:
 1. Turn the instrument (and lasers) on and off.

2. Align the brightfield illumination.
3. Clean lenses
4. Acquire, save, and print images.
5. Transfer files to work station.
6. Delete files.

c) The laboratory supervisor will also offer additional training in digital image handling.

MICRODISSECTOR / MICROCAPTURE / MICROINJECTOR MICROSCOPE

- a) Investigators intending to use the instrument should meet with the laboratory supervisor to discuss the optimal method of slide preparation for the microdissector.
- b) Users will be trained by the laboratory supervisor or technician and will be allowed to use the PALM-Zeiss microscope independently once they can show proficiency on the instrument.

DARKROOM

- a) Each user is expected to know how to use the darkroom equipment.
- b) All technicians using the darkroom are expected to participate on a rotating schedule to clean the print processing equipment.
- c) The darkroom is to be left clean and tidy and free of splattered rapid-fix. Negative carriers are to be returned to the cabinets, and the covers are to be put back on all processing equipment.
- d) It is the responsibility of each user to be aware of the condition of the developing solutions and to change them if necessary. Each user must make up new solutions if he/she uses the last solution from a bottle or tank.

ULTRAMICROTOMES

- a) Although many people prefer to use a certain ultramicrotome, each user is strongly encouraged to learn how to operate all the ultramicrotomes.

COMPUTER

- a) The laboratory supervisor will provide initial instruction on the various computer applications in the Specialized Microscopy laboratory, as well as training on the various workstations.
- b) Since the computers are multi-user machines, all users should store their work files on CD or zip disks. The hard drive should be reserved for large program files.

GENERAL

- a) Each user is requested to use the sign-up sheets at all times, even if there is not a high demand for the instrument/area. Please remember that work supported by NEI R01 grants will be given priority
- b) If someone has already reserved an instrument, another user may not use it until it becomes available. A user may not sign up for the entire day without prior consent of the laboratory supervisor or technician.
- c) Users who are unable to use an instrument during their reserved time should call to advise the laboratory supervisor or technician or cross their names off the sign-up sheet so that others will know the equipment is available. If a user has not arrived within 20 minutes of the reserved time, another user may assume the time slot.
- d) Please advise the laboratory supervisor when supplies are getting low. He will make an effort to order as soon as possible; however, if a user wants something *immediately*, or *needs a special item*, the user will have to place the order. The Specialized Microscopy laboratory will stock only general use supplies.
- e) In the laboratory supervisor's absence, a user should take note if he/she is the last person to use an instrument for the day. If so, the instrument is to be turned off when the user is finished. The last person to leave the laboratory is to make sure that everything is turned off and the door is locked. A checklist is posted on the bulletin board near the entrance to the laboratory.

INSTRUMENTS AVAILABLE

Zeiss 510 confocal/multiphoton microscope with workstation and printer/cell culture prep room

Zeiss EM 10 transmission electron microscope, MT 6000 and MT2B microtomes, 2 LKB knife breakers

Hitachi S-570 scanning electron microscope with digital acquisition software, and sputter coater

PALM laser microdissector, microcapture and microinjector fluorescence microscope

Olympus inverted fluorescence microscope with Spot II digital camera and computer workstation

Olympus microscope (phase and Nomarski optics available) with Spot II digital camera and computer workstation

Zeiss fluorescence dissecting microscope with Spot II digital camera

Reichert Jung cryostat

NT work station with NEC laser color printer, Umax print/slide/neg scanner Nikon 950 digital camera

Leica upright fluorescence microscope with Spot II digital camera and computer workstation

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