# MICROSCOPY IMAGING CORE

**Rules & Regulations**

The instruments in this Light Microscopy Imaging Division are very sensitive and enormously expensive. These instruments must be treated with care by ALL users, as improper or careless use could render these systems unusable for extended periods of time, hampering multiple research programs. Even under service contract, many things will take weeks to get it fixed, and service contracts ***do not*** cover negligence on the part of the users. Thus, to keep all of the equipment functioning properly for *everyone*, the following rules apply to all jobs done in this shared microscope core lab.

1.**GENERAL**

1. No foods are allowed in the microscope rooms at any time.
2. No sitting/standing on the anti-vibration table.
3. No installing software onto the computer.
4. No using the computers for purposes unrelated to microscopy [*for instance, checking email, surfing internet, etc.]*
5. The microscope room is equipped with Class IIIb and Class IV lasers. Therefore,

### unauthorized entry into the facility is prohibited all the time. 2.TRAINING

1. Before training, please arrange a 15-minute appointment to determine the applicability of the facility equipment to your research goals. Please send an email to Dr. Thomas Abraham, Director of Microscopy Imaging at tabraham1@pennstatehealth.psu.edu for appointments.
2. Fill out a *User Registration Form* and return it to tabraham1@pennstatehealth.psu.edu
3. Users need to attend 3 hours of training before being allowed to sign up to use the equipment independently. Additional collaborative opportunities, including image processing, image analysis and data interpretation, etc. are available for those who need it.
4. ***Refresher training*** may be required for anyone who has not used the facility equipment for ***6 months***.

### (e) Users can schedule initial training only with Dr. Abraham and subsequent instrument scheduling with Wade Edris. Users will not train other users under any circumstances, i.e., EACH user has to receive training and be approved to use the instruments. Any violation of this rule will result in retraction of facility privileges.

3.**SIGN-UP**

1. Users may sign up for use of the microscopy equipment between the hours of 8 am – 6pm, Monday through Friday only. After-hour and weekend appointments may be made only after consultation with Wade Edris.
2. You may schedule equipment time online. Sign-up is available online at: <http://microscopyimaginghmc.calendarhost.com/cgi-bin/calweb/calweb.cgi>

User ID: MicroUsers

Password: 3dlivecell

1. Cancellation must be given at least 24 hours in advance of your time slot, preferably sooner. Failure to cancel in advance will result in the following penalties. First offense, a warning. Second offense, time slot missed billed in full. Note that if you can find someone to fill in your cancelled time, there will be no penalty. If you finish early you must contact the next person signed up.
2. Sign-up slots may be cancelled at any time to schedule needed maintenance or repairs.
3. Users must include their phone number (and email) during online sign-up.

4.**USE**

1. Use of the facility is a privilege. Access to confocal computer system will be activated only after successful completion of all training sessions. You will use your Penn State Hershey ID and password for log-in, once those have been activated for you following successful completion of training.
2. Users must follow the standard operating procedures (SOP) when operating the instruments. If you are unsure of any procedures, please contact Dr. Abraham or Wade Edris for further information.
3. For the user/ service fee, please contact Kathy stauffer at kstauffer2@pennstatehealth.psu.edu

### MIS-USE: Any misuse of the facility will result in immediate prohibition.

5.**SHUT-DOWN AND CLEAN-UP**

1. Check the schedule to see if someone else is signed up after you. Please follow the SHUT-DOWN PROCEDURE included in the standard operating procedures (SOP). Make sure all equipment are cooled down and turned off properly. ***You are responsible for logging off properly to avoid extra time charges, and for turning off ALL of the equipment.***
2. Users are responsible for cleaning up the microscope tables upon completion of imaging work.

 (c) Slides, coverslips, specimens, and other tools must be removed from the microscopes and the air table should be cleaned up. Users must wipe oil/ water with *lens tissue or Q-tips* from all oil/ water objectives used.



**6. CITATION**

When you publish images generated from our latest **Leica SP8 Confocal or Leica SP8 STED or Nikon Multiphoton Systems**, please include relevant SIG grant number(s) in the “Acknowledgements” section.

**For Leica SP8 Confocal:** *1S10OD010756-01A1 (CB)*

**For Leica SP8 STED:** *PA Tobacco Settlement Fund*

**For Nikon Multiphoton:** *1S10OD018124-01A1 (TA)*

**7. Safety**

Safety for all users and Core personnel is a top priority. Since the Core facility is a shared laboratory and is designated as a Biosafety Level II facility, there are certain guidelines that all users are responsible to be aware of and follow. This policy is summarized in the Safety Guidelines. They include guidelines for the safe use of lasers as well as procedures for imaging any biohazards and proper clean-up procedures. Any imaging of cells or materials classified with Biosafety Levels 2 or 2+ (including but not limited to e.g. live primary cells, cells containing pathogenic viruses) must be approved by both the Institutional Biological Safety and Recombinant DNA Committee and independently by the Microscopy Imaging operator Wade Edris and the Microscopy Imaging Safety Committee before scheduling or using the instruments. Not all BL2+ work will be approved; NO Biosafety classification work above BL2+ is ever accepted.

**8. EMERGENCY**

### In the event of an emergency (e.g. fire, water leaks), please act CALM and ENSURE your own personal safety first. If possible, shut down all running equipment, cover equipment if possible, and exit the building.

* 1. ***Notify Wade/ Other Core Staff immediately and phone Campus EMERGENCY at 8888***

***These rules and regulations are subject to change at any time. Thank you very much for your cooperation.***



**Rules for the *Safe* Use of the Confocal Microscopy Facility**

The Confocal Microscopy Facility operates as a **Biosafety Level 2** laboratory. These **Rules** (below) are reviewed and revised as necessary each year and each facility user is responsible to review them on a regular basis to insure they are in compliance. Briefly, the rules for safe use of BSL-2 materials as applied specifically to the Confocal Microscopy Facility are as follows:

* + 1. Admission to the laboratory is restricted to authorized personnel when work with human samples or known infectious agents is in progress.
		2. **All human, primate, biohazardous or genetically modified cells must be assigned appropriate BSL levels for research use by the Biological Safety and Recombinant DNA Committee** (Ralph Keil, Ph.D., Chair) before they are allowed to be used in the Confocal Microscopy Core. See the following link for more information: [**http://infonet.hmc.psu.edu/policy/Safety/HM15SPM.htm**](http://infonet.hmc.psu.edu/policy/Safety/HM15SPM.htm)
		3. **All samples that are intended for imaging and all unfixed biohazardous cells intended for analysis must be clearly identified and approved in advance by the Microscopy Imaging Safety Committee.** Generally this is covered by the above process of evaluation by the Biological Safety and Recombinant DNA Committee.
		4. In general, unfixed primary human and primate cells will not be allowed to be imaged on the Confocal, although some exceptions may be made if the cells have been certified as biohazard/pathogen-free. Any need to image unfixed cells must be justified and pre-approved by the Microscopy Imaging Safety Committee. Some otherwise “forbidden” cells MAY be permissible on the Confocal Microscopes if they are fixed according to an accepted process documented to inactivate HIV and other biohazards. In any case, protocols for safe containment and clean-up must be submitted to and approved by the Microscopy Imaging Safety Committee and the Confocal Specialist Wade Edris before scheduling or performing any imaging of BSL2 or BSL2+ materials. Please discuss requirements for imaging any BSL2 or BSL2+ materials with the Confocal Microscopy Specialist well in advance of the need to image the materials
		5. All procedures must be performed carefully to minimize the creation of aerosols.
		6. All samples must be logged onto the facility in the log books provided. Samples that have been fixed for imaging on the Confocal Microscopes may be monitored and recorded to insure that the fixation is adequate.
		7. Work surfaces are to be decontaminated with 10% household bleach after any spillage of sample or hazardous material and after each user completes his/her work.
		8. Instrument decontamination shall be performed according to protocols posted on each instrument, an addition to any special procedures required for particular samples (as decided by the **Microscopy Imaging Safety Committee**).



* + 1. All spills, however small, must be recorded in the accident book and reported to the Confocal Microscopy Specialist Wade.
		2. All liquid and solid waste and disposal is to be **removed** from the laboratory as biohazardous material by each user.
		3. Gloves must be worn whenever handling or running biohazardous samples on the microscope.
		4. Hands must be washed after handling biohazardous materials and before leaving the laboratory.
		5. Regardless of biosafety level, thorough cleanup of the microscope, including e.g. oil-immersion and other lenses, sample stage, and other surfaces, is required after every imaging session. Failure to clean-up properly will first result in a warning; continued failure to leave the microscope in a completely useable “ready” state for the next researcher will result in loss of privileges on the microscope.
		6. Neither syringes nor hypodermic needles should be used in the facility without the express consent and knowledge of the Confocal Specialist Wade Edris. This consent must be obtained each time syringes or hypodermic needles are used – consent for use today does not imply that consent is given for later dates.
		7. No eating, drinking, smoking or applying cosmetics in the work area.
		8. No mouth pipetting.
		9. No animals are permitted within the laboratory.

In addition to the above guidelines and because this facility uses Class III, the following

**Laser Safety guidelines** shall also be observed:

* + - 1. Access to the laboratory will be limited to only those persons necessary to the running of experiments when any laser is operating.
			2. Only qualified personnel shall operate or adjust any laser.

Any questions regarding these guidelines may be directed to the Confocal Microscopy Specialist (Wade Edris, Ext. 284149), to the Director of the Microscopy Imaging Facility (Thomas Abraham, Ext. 285486).

## In the event of a potential retrovirus exposure

This item refers to exposure of skin or mucous membranes to infectious materials, and is modeled after the NIH program, “3 Emergency Steps to Take in the Event of a Potential HIV Exposure”. Remember that the risk of infection is very dependent upon the titer of virus and the route of exposure. Thus the risk of infection by contact of intact skin with infectious body fluids is probably truly zero, and although the risk of infection by contact of mucous membranes or non-intact skin with infectious body fluids may be extremely small, it is likely to be no-zero.



## The 3 emergency steps are:

* + - * 1. Immediately initiate first aid at the work site.

Contaminated skin should be meticulously cleaned for 10 minutes using a povidone iodine solution (such as Betadine) and copious amounts of water.

Contaminated eyes and mucous membranes should be irrigated for 5 minutes using water.

Notify your supervisor, if he or she is **immediately** available. More importantly, go on to step 3.

Report to the hospital Emergency Room to activate the invasive accident protocol (needlestick protocol), which will include evaluation, counseling, and provision of antiretroviral treatment if deemed appropriate. Do this immediately (i.e. within the half-hour).