**HCI Imaging Core Standard Operating Procedures**

Live sample imaging is a powerful technique and is commonly performed on many microscopy systems. The guidelines provided below are intended to ensure that researchers are provided with proper protection from potential to biological hazards during live sample imaging. Live sample imaging in the ??? imaging core may potentially involve applications under two Biosafety Levels (BSLs):

 A. BSL-1: Yeast, non-pathogenic *E. coli* (such as K12-derived strains), well established animal (e.g. murine) cells, fixed cells, and other organisms not known to cause disease in humans of normal health (refer to the [BMBL](https://www.cdc.gov/labs/BMBL.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fbiosafety%2Fpublications%2Fbmbl5%2Findex.htm) for further guidance).

 B. BSL-2: All human (including cultured cell lines like HeLa) and non-human primate (e.g., from macaques) which may contain bloodborne or other pathogens (HIV, HCV, HBV, EBV, herpes B, etc.), cell lines which have been transduced with replication deficient viral vectors (which have not been confirmed to be free RCV by PCR or other approved methods), and other specimens containing microorganisms classified as risk group 2. Some materials may require additional protections. This is referred to as BSL-2 enhanced, BSL-2+ or BSL-2 with BSL-3 practices.

 C. Forbidden Applications: Researchers must be aware that specimens requiring BSL-3 or greater containment (e.g. TB, highly pathogenic influenza viruses, coronaviruses, and other potentially airborne pathogens) cannot be live-imaged at HCI. Applications involving agents which are highly resistant to the limited available disinfectants suitable for use with microscopy equipment (e.g. Cryptosporidium) may also not be permitted within the core facility. The Institutional Biosafety Committee (IBC) and Microscopy Core Facility will ultimately determine the feasibility of applications.

General Procedures:

1. Faculty, staff, and students who wish to use the microscope should contact the facility director, ???, to schedule a training session (typically 2 – 3 hours). Each individual user of the microscope must receive instruction in the use and care of the microscope, regardless of whether the user has prior experience with a similar microscope.
2. The microscopes are available to trained, eligible users on an equal basis. Users with less than 15 hours of experience must make an appointment with the director during regular business hours (9am-5pm) for assistance with microscope operation.
3. In order to become an authorized user, the Principal Investigator must submit a risk assessment document (Appendix A), which will be reviewed and approved by the Facility Director, with assistance from the Biosafety Officer, as necessary. The PI must identify:
	1. The types of samples that are being studied.
	2. The Biosafety Level for the work (see Appendix B for guidance).
	3. The Institutional Biosafety Committee (IBC) registration number, if applicable. All work at BSL-2, as well as work with recombinant viral vectors, must be reviewed and approved by the IBC.
4. The main cell culture room, ???, operates as a Biosafety Level 2 (BSL-2) laboratory at all times. All personnel entering this room must follow BSL-2 procedures. The microscope rooms ????? can be increased to BSL-2 level laboratories on a temporary basis. Under these circumstances, a BSL-2 warning sign and a “Do Not Enter” sign must be posted on the door while it is being used and removed once decontamination procedures are complete. Please see the following section on the “BSL-2 Protocol for Safe Use of Imaging Core” for additional information.
5. Scheduling for microscopes and computer workstations is performed online ???????
6. Scheduling procedures for the microscopes:
	1. From 8am to 6pm M-F, each user may sign up for a total of four hours at a time.
	2. Users may sign up for an additional 6 hours of microscope time during off-peak hours (after 6pm M-F, weekends and holidays).
	3. The facility director reserves the right to modify any calendar entry that does not adhere to policy. Any user who violates scheduling policy more than 3 times will lose access to the instrument calendar.
7. Reservations are guaranteed to a user for a period of 30 minutes after the beginning of the time reserved. After 30 minutes, the microscope, if not in use, becomes available to any other user.
8. Users may only sign up for time on the microscope using their own name. The user who reserved time on the microscope must be present for the entire length of the confocal appointment. Under no circumstances may a user sign up for time using another person's name.
9. Cancellations made less than 24 hours in advance without prior approval will be billed at the regular rate.
10. The director reserves the right to bill for unused time at the regular rate.
11. Users who have reserved time on the microscope are responsible for reporting for their scheduled session.
	1. First time no-shows will be billed at the full rate and receive a warning.
	2. Repeat no-shows will lose their microscope privileges.
12. Users are responsible for ensuring the microscope is shut down properly. If the microscope is not scheduled to be used again for at least 1 hour following a session, all components of the microscope should be shut down according to the posted shutdown procedure.
	1. If the microscope is not shut down properly and any component of the microscope (i.e. lasers, computer or mercury bulb) is left on for an extended period of time, users will be charged for this time at the regular rate.
	2. On any subsequent offenses, users will be subject to further penalties (potentially including permanent loss of access to the microscope) at the discretion of the facility director.
13. Users are responsible for ensuring the microscope and surrounding area are kept clean and organized. Microscope objective lenses should be handled with care and cleaned after each use with the provided lens paper and lens cleaning solution. The microscope stage and work area must be decontaminated after use by wiping down with an appropriate disinfectant (70% ethanol or…….). Tissues used to decontaminate surfaces must be placed in the Biohazard waste container.
14. No food or drink is permitted in the microscope room.
15. All sample/slide preparations must be performed within a certified biological safety cabinet (BSC), either in the User’s laboratory or in room ????. This BSC can be reserved with ?????.
16. Personal Protective equipment must be donned upon entering the microscope room and removed prior to leaving. The facility will provide disposable lab coats and nitrile gloves within the rooms. Disposable PPE must be placed in Biohazard waste containers and reusable PPE (such as safety glasses) must be decontaminated prior to leaving the lab. Once the slides/flasks have been placed on the microscope stage and there is no chance of spill, the safety glasses may be removed for visualization of the samples. They must be replaced once complete and prior to moving the samples off the microscope stage.
17. Access to the Imaging Core, is controlled with a card lock that grants access by reading the barcode of an identification card. The lock allows entry at any time of the day, and maintains a record of all entries into the facility. The outer door of the facility should never be left open for any reason. Anyone requiring entry into this area should contact the facility director for access. Users are responsible for the activities of anyone they provide with access to the facility.
18. The microscope computer may be used for temporary data storage only. Each individual user is responsible for his or her own data. No files should be left on the computer any longer than necessary for data acquisition or analysis.
19. Users are expected to pay the current Imaging Core rate for the use of the confocal microscope.
20. Failure to follow training and standard procedures will be subject to penalty at the discretion of the facility director. In general, users will be expected to cover the expenses incurred by any misuse of the microscope. However, if mistakes are made in good faith and reported promptly, these will be considered as mitigating factors.
21. Appeals to this policy are to be submitted in writing to the faculty supervisor of the facility.

**BSL-2 Protocol for Safe Use of Imaging Core**

**Imaging Core guidelines**: **Protocol for Safe Imaging of BSL-2 Live Samples**

# Prior to training, each user must provide an appropriate protocol and IBC approval letter for their BSL-2 agent (Appendix A). The protocol must include safe containment and PPE requirements for the agent (Contact the Biosafety Office at Biosafety@ehs.utah.edu if you need assistance determining this information.)

1. All BSL-2 agents must be clearly marked, declared, and approved in advance by the director of the facility. Admission to the BSL-2 areas is restricted to authorized personnel when work with BSL-2 samples is in progress. The provided sign on each door must indicate the current biosafety level of the room at all times.
2. BSL-2 samples must be prepared in the investigator’s lab or in Room 1410, using appropriate protocols and safety procedures.
3. BSL-2 samples must be transported in a closed, secondary container, labeled with the Universal Biohazard sign and with absorbent materials in the bottom of the container if there is a risk of spill. The outside of the secondary container must be decontaminated with an effective disinfectant. Remove PPE, including gloves, when transporting the samples between rooms.
4. Imaging dishes must be tight-fitting and or designed to be fully closable (for example, ibidi µ-Slides or Corning Microplates), with parafilm utilized to further secure lids and minimize risk of spills. The outer surfaces of the dishes must be wiped with an appropriate disinfectant prior to transfer to the microscope room.
5. Disposable lab coats and nitrile gloves will be provided by the facility. Each user must supply additional personal protective equipment, including safety glasses and respirators (if needed). See Appendices B and C for PPE requirements.
6. Use appropriate personal protective equipment (including lab coat, safety glasses, and gloves) when moving the dish onto the stage. Remove and discard gloves and clean hands with an alcohol-based sanitizer before touching the microscope or computer.
7. After imaging BSL-2 samples, the microscope stage and the surrounding work area must be wiped down with a tissue soaked in 70% ethanol.
8. Minor spills resulting in contamination of outer surface areas microscopy equipment: wipe down the area thoroughly with tissue damp with disinfectant (…..). Waste materials generated during spill response will be disposed of in the biohazard waste container.
9. Major spills resulting in potential contamination of inner/inaccessible areas of microscope must be cleaned up after advice from the Core Director or staff. Research staff will remove PPE and leave room immediately, and notify the Microscopy Core staff of the incident. Due to presence of electrical shock hazard and potential for damage to sensitive components of the microscope, research staff will not attempt to disinfect or otherwise access internal surfaces of microscope. Major spills on work surfaces or the floor will be cleaned up as described in Appendix D.
10. All PPE must be removed prior to leaving the laboratory.
11. Hands must be disinfected with appropriate alcohol-based hand sanitizer (e.g., Purell) before leaving the microscope room and then washed with soap and water as soon as possible.
12. All solid waste (including microscope slides) must be placed in the 20 gallon biohazard waste containers provided.
13. Plates containing less than 1ml of liquid AND completely sealed to prevent leakage must be placed in the 20 gallon biohazard waste containers provided
14. Plates containing more than 1ml of liquid or cannot be completely sealed must be transported back to the Cell Culture room in a sealed secondary container (see #4). In the Cell Culture room, the plate can be opened in the BSC and the media can be either aspirated into a flask containing bleach or have bleach added (at least 1/10th of the volume of media) can be added to the plate. After 20 minutes, the treated media may be disposed down the sink with running water.
15. No eating or drinking is permitted inside the Imaging Core at any time.

18. Observe the following laser safety guidelines while imaging: never look into the laser beam or insert anything reflective into the beam path.

##### **APPENDIX A: Work Authorization Request Form**

##### **Contact Information Date:**

* 1. **Principal Investigator (Laboratory Director):**

Phone number:

Fax number:

E-mail:

Laboratory Location (Building and Room):

##### Authorized Researcher:

Phone Number:

E-mail:

Add Additional Authorized Researchers, as needed.

1. **Project Information**
	1. **Project title:**
	2. **Summary or description of project**: (Provide details related to types of cells to be imaged, such as source of cells, whether they have been transduced with a recombinant viral vector or infected with a pathogen)
	3. **Has the project been approved by the Institutional Biosafety Committee?**
		1. **YES 🞎**
			1. **IBC registration number & expiration date:**
			2. **Approved containment level:**
		2. **NO 🞎**
	4. **Will the cells be fixed prior to imaging:**
		1. **Yes 🞎**

If Yes, describe the fixation protocol in detail (e.g., fixative, fixative concentration and exposure time).

* + 1. **No 🞎**
	1. **What is the approved Biosafety Level for this work:**
		1. **BSL-1 🞎**
		2. **BSL-2 🞎**
		3. **BSL-2+ (BSL-2 enhanced) 🞎**

**Note: NO work requiring BSL3 or higher containment may be performed in this facility**

* 1. **PPE will be worn while imaging the cells (check all that apply):**
		1. **Standard Lab Coat 🞎**
		2. **Rear closing lab coat 🞎**
		3. **Nitrile Gloves**
			1. **1 pair 🞎**
			2. **2 pairs 🞎**
		4. **Latex Gloves**
			1. **1 pair 🞎**
			2. **2 pairs 🞎**
		5. **Safety Glasses 🞎**
		6. **Surgical/medical mask 🞎**
		7. **Face mask 🞎**
		8. **N95 respirator 🞎**

I have read the above questions carefully and certify the information provided to be correct. All authorized personnel have reviewed and understand these procedures, including spill clean up (Appendix E). Any changes in the risk assessment will be communicated by completion of a new Authorization Form.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Signature (Principal Investigator)

**Appendix B: Biosafety Level Determination for Cell Imaging**

|  |  |  |
| --- | --- | --- |
|  | **BSL-1** | **BSL-2** |
| **Risk Assessment Condition** | **Pathogen-free Human /NHP cells****Uninfected non-primate cells** | **Unscreened Human /NHP cells****Infectious but with low risk assessment (e.g. Risk Group 2)**  |
| **Example: Sample type or Agents1** | Normal murine cellsYeast or non-pathogenic *E. coli*3rd generation Lentivirus transduced cells (non-human cells or pathogen-free human cells: >72 hours post transduction)Fixed Cells | Unscreened or partially screened human cell lines**1**2nd generation Lentivirus transduced cells or 3rd generation lentivirus in unscreened human cellsCells infected with human pathogens |
| **Respirator** |  | N-95 or better2 |
| **Eye protection** | Safety Glasses (if risk of splash) | Safety Glasses plus face shield or mask (if risk of splash or BSL2+) |
| **Lab Coat** | Lab coat | Lab coat or wrap around rear closure, cuffed or disposable sleeves |

1Example: Sample type or Agents - the samples and/or agents listed represent only a partial list of agents which may be included in each category. A risk assessment should be conducted for all samples/agents prior to imaging, and the appropriate biosafety level determined in collaboration with Biosafety specialists, subject matter experts and the IBC. Human cell lines purchased from commercial companies may have been screened some adventitious agents but must be handled at BSL-2. For additional information please consult the following web sites: [http://www.phac-](http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php) [aspc.gc.ca/msds-ftss/index-eng.php](http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php); <https://www.cdc.gov/biosafety/publications/bmbl5/index.htm>

2If a respirator is required, according to the risk assessment, they must remain on during all procedures. For human pathogens with a containment recommendation of BSL-2 and are not respiratory hazards, but which may pose a risk if exposed to mucous membranes, only mucous membrane protection is required.

**APPENDIX C**

**User Specific Safety Equipment:**

* 1. **Personal Protective Equipment (PPE) for Biosafety Level 1 (BSL-1) or Biosafety Level 2 (BSL-2) Laboratory:**
		1. **Disposable Lab coat**
		2. **Gloves**
		3. **Eye Protection**: Safety glasses (Impact resistant and side protection): once the slides/flasks have been placed on the microscope stage and there is no chance of spill, the safety glasses may be removed for visualization of the samples. They must be replaced once complete and prior to moving the samples off the microscope stage.
	2. **Personal Protective Equipment (PPE) for Biosafety Level 2 enhanced (BSL-2+):**
		1. **Isolation-style solid-front or wrap-around gown**, with cuffed sleeves or disposable sleeve covers
		2. **Gloves (double pair)**. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Gloves and protective clothing must not be worn outside the laboratory and must be disposed of with other contaminated waste.
		3. **Eye protection:** Safety goggles, face shield, splatter guard, or integral respirator/face shield that provide mucous membrane protection as required for anticipated splashes or sprays of infectious agents or other hazardous materials.
			+ **Respirator:** National Institute for Occupational Safety and Health (NIOSH)- approved respirators may be required, based on the risk assessment. Approved respirators include N-95, N-99, or N-100 filtering face-piece respirators or powered air-purifying respirators (PAPR) with integral face shield. Respirators must remain on during all procedures associated with sample manipulation.

All individuals using respirators must be enrolled in the University of Utah Respiratory Protection Program. Questions should be directed to EHS at 801-581-6590

**APPENDIX D**

**Disinfection:**

The choice of disinfectant is dependent upon a variety of factors including the agent in use, the chemical resistance of the cell sorter components, and potential of exposure of lab personnel to the chemical disinfectant. Broad-spectrum disinfectants are desirable in a facility in which agent use is varied. For work involving human or non-human primate cell lines it must be an [EPA-Registered disinfectant](https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants).

Sodium hypochlorite solutions (1:10 dilution of household bleach in H2O; final concentration of 5,250-6,150 ppm of chlorine) offer several advantages over alcohols and other disinfectants; bleach has broad-spectrum antimicrobial activity, does not leave toxic residues, is unaffected by water hardness and is inexpensive and fast acting. However, because of the corrosive nature to metals, exposure to instrumentation should be limited to times determined to be maximally efficacious to microbial killing. In addition, bleach solutions must be prepared fresh due to loss of free available chlorine. However, there are commercially available sprayers that mix the bleach and water when sprayed, eliminating the need to make fresh solutions daily.

Quaternary Ammonium Chloride-based disinfectants (QUATs) are typically the best choice for cleaning surfaces that may be sensitive to harsh chemicals. However, ensure that in cases where infectious agents are known to be present that the agent is susceptible to the disinfectant chosen. Examples of appropriate general purpose disinfectants include Simple Green dPro5, Lysol I.C,, Microban, Cavicide, and Conflikt.

**APPENDIX E**

**Spill Clean-up**

All personnel must be trained on this procedure before beginning work

* 1. Stop work. Remove PPE and wash hands (with sanitizer)
	2. Ensure that any other people in the room are notified that a spill has occurred and that the room should be evacuated. Post the “Do Not Enter” notice, contained in the Spill Kit, on the door.
	3. Notify the Core Director or staff.
	4. If you need assistance with the spill clean-up, call EHS (1-6590).
	5. Wait 60 minutes before re-entering the room to allow aerosols to settle.
	6. A spill kit is available in the room. Don PPE, including lab coat, eye protection and face shield or mask, 2 pair of gloves, shoe covers.
	7. Contain the spill by covering with paper towels (to avoid splashes or aerosols)
	8. Saturate spill with the disinfectant (XXXXXXX)*.* Let sit for 20 minute exposure time.
	9. Wipe areas around the spill that may have splatter and any reusable equipment disinfectant*.*
	10. Wipe up spill, disposing of towels in the 20 gallon Biohazard waste container: if sharps (such as microscope slides) may be present use tongs or a brush and pan to pick up waste.
	11. Work concentrically to clean up the absorbent material. Always work from the outer edge of the spill toward the center.
	12. Spray spill area with disinfectant*.* Allow to air dry.
	13. Remove PPE: discard disposable PPE as biohazardous waste and wash hands. Clean reusable PPE with a disinfectant or soap and water.
	14. Remove the “Do Not Enter” sign and inform others that it is safe to re-enter the room.

**Sources and References**

University of Maryland Imaging Core SOP

Virginia Commonwealth University Live Imaging SOP