**SOP FOR LIVE IMAGING OF POTENTIALLY BIOHAZARDOUS LIVE SAMPLES**

**Last revised: 03/22/18**

*List Biohazardous/rDNA materials to be live imaged:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

**I. Purpose:** Live sample imaging is a powerful technique and is commonly performed on many confocal 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 VCU research facilities may potentially involve applications under two Biosafety Levels (BSLs):

 A. BSL1: Yeast, *E coli*, well established animal (nonhuman primate) cells, and other organisms not known to cause disease in humans of normal health (refer to the [BMBL](http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm) for further guidance).

 B. BSL2: All human cells (including cultured cell lines like HeLa) which may contain bloodborne pathogens (HIV, HCV, HBV, EBV, etc.), cell lines which have been transformed by 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 under BSL-2 (refer to the [BMBL](http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm) for further guidance).

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

**II. Required Standard Operating Procedure (SOP) for BSL2 Samples** (adherence to this SOP is also strongly recommended imaging applications involving BSL-1 materials):

 A. All research involving agents classified at BSL-2 or greater and/or recombinant DNA materials must be approved and registered through submission of a [Bioraft biological registration](vcu.bioraft.com) prior to utilization of live imaging equipment. If the research involves use of core facility equipment a copy of the Bioraft registration will be provided to core facility director by the researcher prior to initiating imaging.

 B. Completion of adequate biosafety training must be verified on Bioraft prior to permitting any research staff to perform applications involving live-imaging of materials classified at BSL-2 of greater: minimal requirements include direct task-specific training from principal investigator (PI) and completion of EHS “Biosafety Training CDC/NIH Module”.

 C. All sample/slide preparation must be performed within a certified biological safety cabinet (BSC) under the conditions established under the Bioraft registration.

 D. Research staff will don proper PPE during preparation and transport of samples/slides to include: lab coat, examination gloves, proper laboratory attire, and eye protection where necessary.

 E. Imaging dishes must be tight-fitting and or designed to be fully closable (for example, ibidi USA “microslides,” in Vitro Scientific “Multiwell” glass bottom plates), 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 (refer to biological registration) prior to packaging for transfer to core facility.

 F. Transfer of Potentially Biohazardous Materials: If transportation from laboratory to microscopy facility (or vice versa) is required, samples must be transported within a closed secondary container displaying universal biohazard signage on the exterior surface, with sufficient absorbent materials in the bottom of the container to absorb contents in the case of leakage.

 G. Signage must be posted on entry door of room housing microscope by research staff while conducting procedures involving potentially biohazardous agent(s).

 H. Examination gloves will be worn when removing dishes from secondary container and placing onto the stage, the gloves will be removed and hands washed thoroughly prior to contacting the microscope, computer, or other equipment.

 I. After completing imaging, research staff will remove samples from the microscope and wipe down the microscope stage with tissue saturated with disinfectant. Unless otherwise specified (by the conditions of the biological registration and with written verification from the Microscopy Core), 70% ethanol (EtOH) solution will be utilized for disinfection of microscope surfaces and components. Researchers will be required to provide their own disinfectant: stocks of 70% EtOH solution expire 60 days after preparation.

 J. Spills: In the event of any spill, notify core staff immediately. Researchers are responsible for providing a “spill kit” which includes all materials required for conducting response in the event of a spill. The spill kit should at a minimum include disinfectant (70% EtOH), paper towels, red bags, and personal protective equipment (PPE). If the event of a spill, research personnel will leave room immediately to limit exposure to aerosols and avoid reentry into the laboratory for at least 20 minutes to allow for settling of aerosols. Research staff will don appropriate PPE (refer to Bioraft registration) prior to entering space to conduct cleanup.

 1. Work surface (benchtop, floor, etc.) spills: spills must be cleaned thoroughly with an appropriate disinfectant (70% EtOH, unless otherwise specified). Waste materials generated during spill response will be managed/disposed of by research staff as regulated medical waste via university red bag or orange bag procedures as specified on Bioraft registration.

 2. Minor spills resulting in contamination of outer surface areas microscopy equipment: If spill is minor and only affects outer/accessible surface of scope, research staff will wipe down area thoroughly with tissue damp with disinfectant (70% EtOH). Waste materials generated during spill response will be managed/disposed of by research staff as regulated medical waste via university red bag or orange bag procedures as specified on Bioraft registration.

 3. Major spills resulting in potential contamination of inner/inaccessible areas of microscope: research staff will leave room immediately and notify 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.

 K. Hands will be thoroughly washed with soap and water immediately upon completing above tasks.

 L. Following completion of imaging tasks, all samples must be transferred (per line III.E) back to the host laboratory for disposal per conditions of Bioraft registration.

**III. Additional Training Requirements:** Research staff will be trained per the conditions of this SOP and the corresponding Bioraft registration prior to conducting live imaging procedures involving BSL-1 and BSL-2 specimens. Research staff will also be required to complete any training as required by the Microscopy Core prior to use of the facility.

Questions regarding this SOP should be directed to the [Biosafety Office](http://srm.vcu.edu/research-clinical-safety/biological-safety/).