SOP FOR USE OF IVIS TO IMAGE BSL-1 OR BSL-2 SPECIMENS IN NLS 26B

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1) **Purpose**

The Perkin Elmer In Vivo Imaging System (IVIS) Spectrum is used to expand the capabilities to understand critical cellular processes in specimens using fluorescent or bioluminescent cells, proteins or probes. The IVIS images whole specimens, living or not, under anesthesia if needed, and can detect fluorescent or bioluminescent signals within the specimens, including but not limited to rodents, c. ellegans, sea urchins, drosophila, plants, etc. The system can view and capture images from up to 5 specimens in the main IVIS imaging chamber or up to 3 specimens at one time using the isolation imaging chamber. The IVIS can image deep fluorescent and luminescent signals and perform tomographic reconstructions of the whole animal. Fluorescent signals are captured using matched excitation and emission filters. Bioluminescence signals are measured in cells or probes expressing luciferase following introduction of luciferin.

Currently, in order to study changes in specimen models like mice, it is necessary to sacrifice and harvest tissues. The use of the IVIS to perform biophotonic imaging (BPI) would allow sequential imaging of the same specimen to follow changes over time for longitudinal comparisons, thereby reducing the number of animals needed for studies. Imaging of live anesthetized specimens will allow for the use of fewer animals by obtaining quantitative data from an individual animal at multiple time points throughout the study. This will eliminate the need to repeatedly sacrifice groups of specimens for data collection at each time point.

2) **Responsible Individuals**

- Animal Technicians
- Veterinarians
- Principal Investigators (PI)
- Research Technicians
- Office of Laboratory Animal Care (OLAC)

3) **Materials**

- The IVIS Spectrum integrated imaging system:
  - CCD camera in a light-tight imaging chamber
  - Thermoelectric cooling unit
  - Fluorescence light source
  - Windows-based computer system for data acquisition and analysis.
- If imaging with isoflurane anesthesia:
  - Portable oxygen tanks (E-tanks)
  - Isoflurane
  - Waste gas scavenger system (charcoal canister(s))
  - Dual diverter and evacuation tubing
  - ABSL1 Anesthesia machine and induction box
  - ABSL2 Anesthesia machine and induction box
- If imaging with BSL-2:
  - Class II type A2 biosafety cabinet
  - ABSL2 XIC-3 Animal Isolation Chamber
- Luciferin for bioluminescence studies

4) **References**

These protocols reference standard operating procedures (SOPs) that are part of the IACUC Policies and Guidelines, including:

1. SOP on Animal Movements and Transfers - Shipping and Receiving
2. SOP on Routes of Administration for Research and Teaching Animals
3. SOP on Anesthesia - Isoflurane Gas Anesthesia Machine Operation
4. SOP on Biocontainment Husbandry - Animal Biosafety Level 2

5) **Procedures**

Considerations prior to use:

Approval requirements for IVIS imaging:
Complete details of IVIS imaging methods must be described on an approved BUA and, when applicable, APF, before work takes place. Interested users must reach out to the IVIS manager for orientation and training, including IVIS imaging use and IVIS isoflurane anesthesia machine use. Authorized users who wish to use the IVIS Spectrum for imaging studies must reserve time in the facility.

Preparing the specimens for imaging:
Animal hair is highly effective at blocking, absorbing, and scattering light during optical imaging. Even light within the NIR spectrum, which typically shows minimal scattering and absorbance in tissue, is significantly absorbed and scattered by hair. For example, nude mice, or immunocompetent hairless mice, do not require depilation; however conventional strains of haired mice, like BALB/c or C57BL/6, may require depilation. Note: Black mice will have a ~10X reduction in the bioluminescent signal and ~20X reduction in fluorescence as compared to white, shaved or nude mice. If required, fur will be removed on and around the areas of the animal that are to be imaged with small electric hair clippers or with Nair. Animals may be kept under isoflurane anesthesia for this procedure. Hair will be removed using an electric animal shaver and/or depilatory cream (e.g., Nair). After 30-90 seconds, the depilatory cream will be wiped off and any remaining agent will be thoroughly removed using wet tissue to prevent burns.

Furthermore, regular rodent chow contains chlorophyll that auto-fluoresces around 700 nm and can interfere with signals collected from this agent. Consider switching to low fluorescence chow around two weeks before the imaging study.

Transportation from animal housing to NLS 26B:
Transfer animals into clean cage set ups, complete with filter top. Primary enclosures should be decontaminated with Cidex plus (3.4% glutaraldehyde), Cavicide or other approved disinfectant before being placed in a single layer into a secondary container that will be provided by the IVIS facility. Secondary containers containing animals must be transported on a cart to NLS 26B. The secondary container will be large and opaque, have a secure lid, and have air holes in the top and sides (example Figure 1). Do not stack primary enclosures or secondary containers.

Figure 1: Example of transport containers and cart

Maintaining BSL-1 and BSL-2 spaces:
In order to maintain biosafety, BSL-1 and BSL-2 will have separate work spaces (described below and in Figure 2). All work with specimens at BSL-1 will be completed on the BSL-1 work table, using the BSL-1 induction station (Figure 2A-B). BSL-1 specimens will be imaged in the standard IVIS imaging window using nose cones.

All work with specimens at BSL-2 must be completed inside the biosafety cabinet or the XIC-3 isolation chamber (Figure 2C), following protocols established for cage changes in the ABSL-2 animal husbandry SOP. Briefly, to ensure limited exposure to the outside environment, BSL-2 specimens should be transported into NLS26B in primary containment and sterilely transported into the biosafety cabinet. Specimens can be introduced to the BSL-2 induction chamber and/or the XIC-3 isolation chamber while in the biosafety cabinet. Specimens will be transported to the IVIS and imaged inside of the XIC-3 isolation chamber (Figure 2D). The XIC-3 isolation chamber allows analysis of specimens (up to 3 anesthetized mice) in a biologically sealed system, consisting of charcoal filters for isoflurane scavenging and bacteria/virus filters, while imaging using the IVIS system. Specimens will be removed from the XIC-3 isolation chamber and return to their primary containment only in the biosafety cabinet.

If the fluorescent or luminescent signal is not strong enough to allow use of the XIC-3 isolation chamber, it may be necessary to image specimens outside the isolation chamber due to weak signal, or other complications of using the isolation chamber. The reproducible penetration and emission of light and signal capture with the IVIS can be vary dependent on specimen body position and distance from the camera. The isolation chamber increases the field of view, limiting how close the camera can come to the specimens. Prior approval must be obtained before imaging outside the isolation chamber.

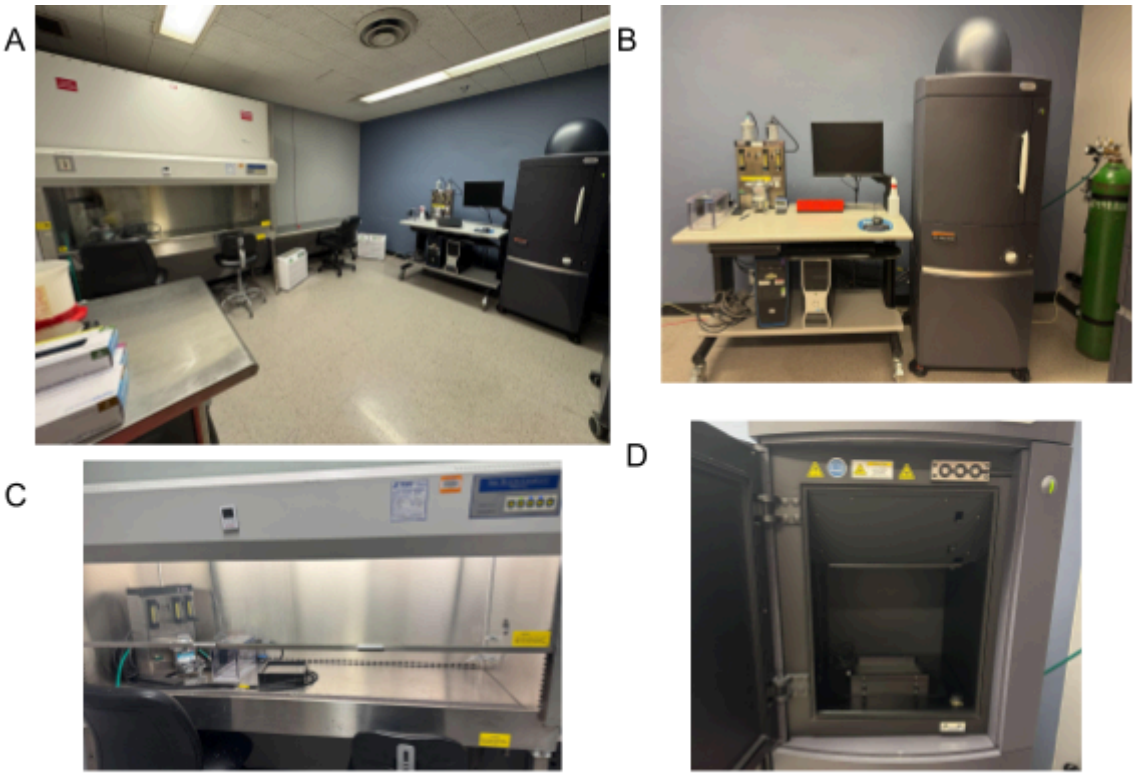

Figure 2: A) Arrangement of room NLS 26B including (from left to right) biosafety cabinet and BSL-2 anesthesia machine and induction chamber, BSL-1 table, BSL-1 anesthesia machine and induction chamber, IVIS computer station and imaging compartment. B) BSL-1 induction station and IVIS computer station and imaging compartment. C) biosafety cabinet, BSL-2 anesthesia machine and induction chamber, and BSL-2 XIC isolation chamber. D) BSL-2 XIC isolation chamber moved to IVIS imaging compartment for BSL-2 imaging.

Returning animals to housing:
Prior to imaging, arrangements must be made with the OLAC manager who will determine the appropriate housing area to return animals to ensure biosecurity in the animal facility.

Imaging in NLS 26B:

1. Users will don shoe covers, gowns, eye protection, face masks, and gloves as they cross over the purple tape into NLS 26B. These are provided by the facility.
2. Core manager will have the IVIS machine turned on and software Living Image open. Users will need to login with their username and password. Initialize the software and start the cooling process for the camera which can take ~5-10 min.
3. If using bioluminescence, users will inject luciferin, following their APF protocol and the SOP on Routes of Administration Guidelines. Luciferin will be administered to the animal ~0-15 minutes before induction of anesthesia and the start of imaging.
4. If BSL-1:
    a. Specimens are moved to BSL-1 induction chamber, next to the IVIS computer (Figure 2), and administered isoflurane anesthesia, following the SOP for Anesthesia - Isoflurane Gas Anesthesia Machine Operation. Briefly, specimens are moved to the induction chamber and administered 2 liters of oxygen per minute. Isoflurane dial is turned to 5% for initial induction, and once recumbent, isoflurane is maintained at 1.5-2%. Specimens are continuously monitored for depth of anesthesia.
    b. Once a sufficient plane of anesthesia has been reached, oxygen flow to the IVIS imaging chamber should be turned on to 0.8 liters of oxygen per minute with 1.5-2% isoflurane flow.
    c. Once flow is established, animals will be quickly moved to the imaging chamber and placed directly into the nose cone. The platform can deliver anesthesia to up to 5 animals using a precision vaporizer, providing controlled consistent anesthesia for multiple animals during one imaging session.
    d. Unused nose cones are closed prior to beginning of air\anesthesia flow. Animals will be positioned securely on nonfluorescent black paper on the heated imaging platform (maintained at 37°C) and positioned for imaging. Nose cones and paper are provided by the facility.
    e. If specimens do not need anesthesia, they may be moved directly to the imaging chamber.
    f. IVIS imaging lasts 5-30min. Following imaging, return animals to primary enclosures on the BSL-1 table.
5. If BSL-2:
    a. Cages are sterilely moved into the biosafety cabinet. Inside the biosafety cabinet, specimens are then moved to the BSL-2 induction chamber, which is equipped with bacterial/viral filters between the induction chamber and charcoal gas scavenging system. Specimens are administered isoflurane anesthesia, following the SOP for Anesthesia - Isoflurane Gas Anesthesia Machine Operation, at 2 liters of oxygen per minute. Isoflurane dial is turned to 5% for initial induction, and once recumbent, isoflurane is maintained at 1.5-2%. Specimens are continuously monitored for depth of anesthesia.
    b. Once a sufficient plane of anesthesia has been reached, specimens are quickly transferred to the XIC-3 imaging isolation chamber while still inside the biosafety cabinet. When using the isolation chamber, any animal manipulation will be confined within the biosafety cabinet to adjust the body position of specimens for imaging.

c. If specimens do not need anesthesia, they may be moved directly to the XIC-3 isolation chamber, while still inside the biosafety cabinet.
d. Specimens inside the isolation chamber are quickly moved to the IVIS imaging chamber and re-attached to the isoflurane/oxygen anesthetizing unit within the IVIS imaging chamber. When specimens are imaged using the isolation chamber, tubing connections will be connected to bacterial/viral filters between the imaging chamber and charcoal gas scavenging system and supply lines for isoflurane-oxygen gas anesthesia are connected to the imaging chamber. WARNING: The XIC-3 isolation chamber should not be used in Field of View “A” in an IVIS Spectrum. The Chamber is too tall and will hit the top of the IVIS and possibly damage optical components.
e. IVIS imaging lasts 5-30min. Following imaging, return the XIC-3 isolation chamber to the biosafety cabinet before returning animals to primary enclosures.

6. Monitor animal recovery from anesthesia (walking, active, etc.) while decontaminating all surfaces utilized.
7. Decontaminating all utilized surfaces of IVIS room as follows:
    a. All utilized surfaces will be decontaminated with Cidex plus (3.4% glutaraldehyde), Cavicide or other approved disinfectant.
    b. Biosafety cabinet and isolation chamber will be further decontaminated by water and then 70% ethanol.
    c. If the IVIS imaging chamber is used without the isolation chamber, the surfaces of the imaging chamber, but not the imaging window, will be decontaminated with approved disinfectant applied with a lint free cloth such as Scott Pure, Kaydry EX-L. Cloth shall be saturated with cleaner, wiping external surfaces using a gentle circular motion.
    d. Special care needs to be taken to avoid the imaging window. Imaging window can only be cleaned with 70% Ethanol. WARNING: Reasonable care should be taken to avoid scratching of the surfaces of the isolation chamber and imaging window. Use of paper towels to clean the surface is discouraged. Clean, dry, lint free optical cleaning cloths will prevent scratching the surface of the window.
8. Cleaning cloth and disposable gloves will be discarded as biohazard waste after wiping inside surfaces of IVIS, biosafety cabinet, and isolation chamber.
9. Following imaging, users must shut down the Living Image software, but DO NOT shut down the IVIS or the computer. This will damage the IVIS. Remember to shut down the software. Living Image software gives a monthly report of the users, which will be used for billing.