WVU IACUC - APPROVED SOP:
Animal Models and Imaging Facility (AMIF)

I. Introduction

The WVU Animal Models and Imaging Facility (AMIF) is a fee-for-service shared resource facility that provides equipment and services to support preclinical research using small animal models. This facility currently has equipment for optical, microCT, ultrasound, body composition analysis, and multi-photon imaging, as well as for image-guided irradiation and metabolic monitoring. The AMIF also has spaces designated for performing procedures and surgeries. The AMIF can provide full service to investigators or can train researchers for unassisted use of the equipment. In addition, the core provides colony management services and performs other general procedures. The AMIF staff is readily available for consultation and assistance with experimental design and data interpretation. For more details on the facility's resources and services, please see our website at https://www.hsc.wvu.edu/resoff/research/shared-research-resources/animal-models-and-imaging-facility-amif/.

This document describes the general operations of the AMIF and is not intended to supersede any existing policies from the Office of Laboratory Animal Resources (OLAR). Each PI that will be working with the facility should reference this SOP in their IACUC protocol. In addition, each investigator ***must*** provide the details that are specific to their project, including any procedures that vary from this SOP. Templates of appendixes (included at the end of this document) should be modified to add experimental details and added to investigator protocols.

II. Operations

A. IACUC & IBC Protocols

All animals remain on the individual PI's IACUC protocol; all appropriate AMIF staff members that will be working with these animals ***must*** be included in each PI's animal protocol. In addition, the AMIF staff ***must*** be included on relevant IBC protocols. A copy of all approved protocols should be maintained in the AMIF.

B. Training & Access

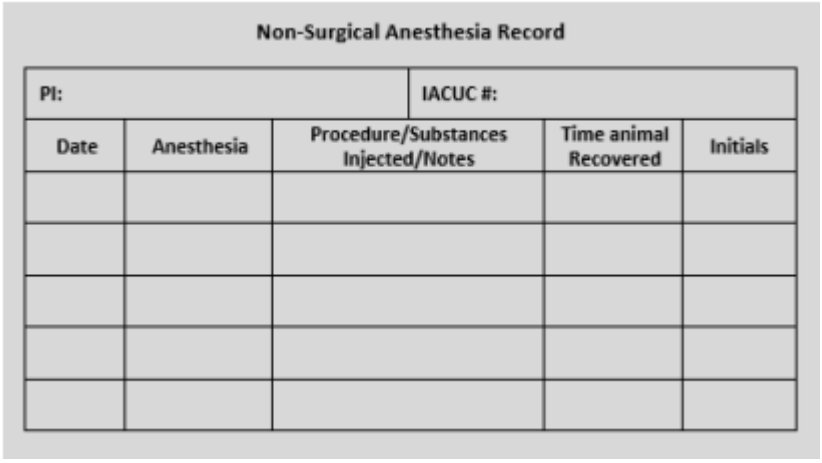
Entrance into the AMIF is controlled by card access. Users ***must*** complete OLAR and AMIF training before they will be granted access to the core. Each user ***must*** be trained by AMIF staff and demonstrate technical mastery before they will be allowed to have independent use of the equipment.

C. Animal Housing

Animals are in the AMIF for less than 24 hours; housing is provided within the OLAR vivarium. The AMIF staff or trained researchers are responsible for transporting animals between the designated holding rooms and rooms in the AMIF. The exception to this is the CLAMS metabolic monitoring cage system which is located within the main OLAR vivarium.

D. Anesthesia Record

Gray "Non-Surgical Anesthesia Record" cards should be used when animals have been anesthetized for imaging procedures. The cards document anesthesia, procedure, any substances injected, and when the animal was recovered.



Non-Surgical Anesthesia Record

PI:		IACUC #:		
Date	Anesthesia	Procedure/Substances Injected/Notes	Time animal Recovered	Initials

III. Cleaning & Biosafety

A. Biosafety

Cages containing animals that are designated as ABSL-2 (due to the use of human tissues, cell lines, pathogens, etc.) will be transported to the AMIF with secondary containment. When these animals enter an imaging/procedures room, this space will be designated at ABSL-2; a sign will be posted on the door listing the agent and any entry requirements. All waste will be autoclaved out of the rooms.

B. Personal Protective Equipment

PPE requirements for room entry are the same as your animal housing room. With animal use this typically includes gown, face mask, and gloves. Closed toe shoes are required at all times in the animal facility; Use a set of shoe covers if working with ABSL-2 animals.

C. AMIF Cleaning Procedures

All surfaces that come into contact with the animal ***must*** be cleaned thoroughly. Use the provided cleaner (T-Spray or CaviWipes) on the ultrasound transducers, keyboards, imaging stages and inside any imaging system. Use OLAR approved disinfectant (Virkon, Rescue, etc.) on non-sensitive surfaces such as the induction box, hood and counters. Surfaces should be cleaned prior to beginning and ***must*** be cleaned after the completion of room use. CHARM testing is being performed biannually by OLAR to confirm cleaning efficacy.

IV. Imaging Facility Equipment

Preclinical imaging is a critical tool for small animal models of human disease. Longitudinal studies using imaging to monitor developmental or disease progression will increase the accuracy of the study with fewer animals. The imaging offered in the AMIF is non-invasive and animals can be imaged multiple times without any adverse effects.

A. IVIS Spectrum CT - Optical and micro–CT Imaging

Fluorescence light imaging is rapid, painless and harmless to the animal. The typical experiment involves shining excitation light of the desired wavelength on the animal at a fluence rate of approximately 4-20 mW/cm^2. This fluence rate is about the same as a brightly lit room. The emission light reflected from the animal is then imaged with a camera. A typical image takes 1 second to 1 minute to acquire.

Bioluminescence imaging is a high-sensitivity, low-noise, non-invasive technique used for visualizing, tracking, and monitoring specific cells and genetic activity in an animal. This specificity comes from the 'tagging' of the cells with a gene expressing the luciferase enzyme. When the substrate is processed by the luciferase enzyme, the cells emit light which the system can record. For those experiments that require the luciferin substrate, a typical dosage is 150 mg luciferin/kg body weight injected intraperitoneally, with a maximum volume of 1 ml. A typical image can take 1 to 5 minutes to acquire.

The IVIS SpectrumCT uses micro-CT imaging to generate a 3D image of a mouse. The optical images (fluorescence or bioluminescence) can then be overlaid onto the 3D model, increasing accuracy of localization of the optical signal as well as increasing accuracy of the quantitation of signal. CT uses x-rays to create images. In the IVIS system, the mouse is rotated (while the x-ray source and detector remain stationary), to collect a series of x-rays from different angles that are then computed to create a 3D image.

The IVIS SpectrumCT has 4 modes for live animal CT imaging which affect the image resolution and the dose of radiation. The manufacturer cites an immuno-compromising dose as 1,000-2,000 mGy and an LD 50/30 dose as 5,000-7500 mGy.

Mode Name	Resolution (mm)	FOV L x W x H (cm)	Dose (mGy)	Total Time (s)	Typical Use
Fast	850	12.5 x 12.5 x 3	13	90	Needed for FLIT/DLIT, longitudinal CT
Standard (1 Mouse)	425	12.5 x 12.5 x 3	53	140	Anatomical reference, largest FOV
Standard (2 Mice)	425	12.5 x 12.5 x 3	23	150	Anatomical reference, largest FOV
Medium Res	225	6 x 6 x 3	132	210	Best for soft tissue, organ contrast

B. VevoF2 - Ultrasound Imaging

Ultrasound uses high-frequency sound waves to generate in vivo images of organs and tissues. This preclinical imaging system is non-invasive and can be used for physiological measurements such as heart function, blood flow, tumor volume and embryonic development.

Ultrasound may also utilize contrast agents. Ultrasound-based contrast agents are typically small micron sized micro-bubbles that may be air or gas filled. Tissue typically reacts linearly to ultrasound energy while micro-bubbles react in a non-linear fashion to the same energy. As these microbubbles are intravascular, they are easily introduced intravenously and pass through the vascular stream mimicking red blood cell movement. We will utilize two types of contrast agents. The Vevo MicroMarker Non-Targeted Contrast Agent Kit is for tissue enhancement, perfusion and microcirculation applications. The Vevo MicroMarker Target-Ready Contrast Agent Kit is for performing targeted molecular applications to quantify biomarker expression. These microbubbles will be conjugated to an antibody or other targeting reagent related to the primary investigators research goals. Both kits are designed for use in small animals and are non-toxic. These are administered as a tail vein (IV) injection at 20-32 mg/kg.

Because of its high-resolution imaging and the ability to provide real-time visualization, the Vevo ultrasound system can guide injections of cells, drugs, genetic material or metabolic agents into developing small animal embryos as early as embryonic day 5. Also, the system allows visualization and needle guidance into adult small

animal models to include but not be limited to: embryos, cardiovascular system (myocardium or ventricles), liver, kidney, placenta and spleen, and can also be used for extraction procedures (biopsy).

C. Multiphoton Microscopy

The AMIF partners with the Microscope Imaging Facility (MIF) to image live animals with the Nikon AIR-HD multiphoton microscope. This system generates images using fluorescent light which is non-invasive so the animals can be imaged multiple times.
Prior to imaging the animals, it is typically necessary to surgically place a "window" to image through. Common examples include: cranial window to observe brain (vasculature following TBI or stroke or to observe plaques or other markers in blood vessels), skin flap imaging into a subcutaneous tumor, lung window or spinal window. The surgeries to place these windows will be described by each investigator as it pertains to the specific experiments.
The animal will be anesthetized and positioned with the window site of interest under the microscope objective. The animal will typically need to be injected with a fluorescent probe that is targeted to the area of interest. The multiphoton microscope then shines fluorescent light on the animal and collects the signal to generate the image.

D. EchoMRI – Body Composition Analysis

EchoMRI uses nuclear magnetic resonance (NMR) technology to analyze body composition. NMR creates contrast between soft tissues by emitting radio pulses which cause proton spins to process and emit radio signals which are then received and analyzed. The amplitude, duration, and spatial distribution of these signals are related to properties of the material scanned. The high contrast between fat, lean, and free water is further enhanced by application of specially composed radio pulse sequences. Our researchers use body composition analysis to accurately show changes in fat mass, muscle mass, and body fat percentage.

E. XenX Irradiation

Radiation therapy is a staple in treatment of many types of cancer, and the ability to target the radiation beam limits damage to the normal tissues surrounding a tumor. The AMIF has an Xstrahl XenX X-ray irradiator for whole body and targeted irradiation studies on small animals. The XenX system allows researchers to perform radiation therapy in small animals in a manner that is similar to the way patients are treated, providing a more clinically-relevant treatment option for translational research.

F. CLAMS

The Columbus Instruments Comprehensive Lab Animal Monitoring System (CLAMS) is a system of metabolic cages which allows for simultaneous measurement of numerous metabolic parameters. The CLAMS system will simultaneously monitor open circuit calorimetry, activity, feeding, running wheel, all contained within an environmental chamber with optional temperature and lighting modifications. Mice will be individually housed in cages. Enrichment can not be used as it will interfere with the motion tracking. A typical study length is 5 days. This is a completely non invasive procedure as it is simply monitoring the mice within a home cage environment.

G. MRI – Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a noninvasive imaging technique that produces detailed images of structures within the body. Images are generated by using a large magnet and radio waves. The magnetic field

temporarily realigns water molecules within the body. Radio waves cause these aligned atoms to produce faint signals, which are used to generate 3D images of the organ or tissue. MRI is good at telling the difference between types of soft tissues and differentiating between normal and abnormal soft tissues.

V. Animal Procedures

The AMIF supports our users' imaging experiments by providing assistance or training for a variety of animal procedures. The following list includes our most common procedures.

A. Hair Removal

Hair removal will be necessary for many procedures including injections, fluorescent or bioluminescent imaging and ultrasound imaging. For complete fur removal depilatory cream, such as Nair, will be applied. Nair will be applied to the animal with a cotton-tipped swab or cloth and will be wiped off after 15-60 seconds. Some strains may require a second application. The animal will then be wiped with a wet cloth to remove any excess Nair. Alternatively, we may shave them with clippers.

B. Injections

Qualified personnel may inject medications, anesthetics, and/or experimental substances via one or more of the following routes as dictated by study needs.

- Intraperitoneal
- Intravenous via tail vein
- Intravenous via retro-orbital sinus (under general anesthesia only)
- Orthotopic
- Subcutaneous
- Intracardiac (under general anesthesia only)
- Ultrasound image-guided injections

C. Identification

AMIF will identify animals with ear punches or ear tags. This method of identification involves only momentary restraint as well as momentary pain. No anesthesia is required with skilled application of these methods of identification. Tissue from ear punch will be collected to use for genotyping whenever possible.

D. Anesthesia

Animals will be anesthetized by inhalation of isoflurane at 1-5% with oxygen and titrated to effect during the procedure. Animals will first be anesthetized in an induction box and moved for imaging or other procedures once it has been determined that they are in an acceptable depth of anesthesia. Gas anesthesia ports allow inhalant anesthesia to be maintained during imaging sessions while a heated stage/platform/warm pad is used to maintain optimal body temperature, as needed. Eye lubricant will be applied to eyes of all animals maintained under isoflurane via nose cone.
Animals are monitored throughout the experiment and until they fully recover from anesthesia per the WVU IACUC Policies: *Anesthesia and Analgesia in Mice and Rats.* No side effects are anticipated at the dosage used for both acute and longitudinal studies.

The anesthetic vaporizers in the AMIF are calibrated and maintained as described in the WVU IACUC Policy: *Calibration of Vaporizers for Inhalational Anesthesia in Animals*.

VI. Disposition of Animals Following Study

Timing of euthanasia with regard to imaging will be dependent on the length of the study and, therefore, will be dependent on the Principal Investigator's protocol and the health status of the animals.
For animals involved in tumor studies, the WVU IACUC Policy: Tumor Development Endpoints for Euthanasia in Rodents will be strictly followed.
Animals will be euthanized according to the WVU IACUC Policy: Pain and Distress Recognition – Humane Endpoints after consultation with veterinary staff.

Primary euthanasia methods include:

- Anesthesia (isoflurane or injectable) overdose
- Cervical dislocation under anesthesia
- Inhalation of CO_2
- Cervical dislocation on awake animal (***must*** be scientifically justified and performed by appropriately trained and proficient personnel only)

Secondary euthanasia (confirmation) methods include:

- Cervical dislocation
- Bilateral thoracotomy
- Dissection and removal of a vital organ

VII. AMIF Contacts

If you have any questions or comments, please contact:

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Karen Martin, PhD
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Email: kamartin@hsc.wvu.edu

VIII. ACUC Appendix Templates

The following appendixes are available upon request to be used as templates for investigators to include in ACUC protocols. They should be modified to describe the research plans for each investigator. Some of the details that need to be addressed by the investigator are indicated in **blue text**. **Please contact AMIF personnel for copies**.

- Personnel Addition Form
  - All protocols using the AMIF ***must*** add Sarah McLaughlin and Amanda Stewart for the purpose of training or imaging assistance.
- Procedure Rooms
  - List of the AMIF equipment/procedure locations to be included, as needed, in section 6 of the Main Protocol Form
- Appendix A:
  - Cranial Window Surgery
- Appendix B:
  - IVIS Spectrum CT Imaging
  - Vevo Imaging
  - Multi-Photon Imaging
  - EchoMRI Imaging
  - XenX Irradiation
  - CLAMS – metabolic monitoring
  - MRI Imaging
- Appendix C:
  - Common AMIF Drugs
- Appendix O
  - Single Housing – required if using CLAMS