LAB_035 General Use of Intravital Microscopy

I. OBJECTIVE

To describe the general procedural expectation for performing safe and humane non-recovery intravital microscopy in rodents.

II. DEFINITIONS

Competent - "the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments." (as per, Australian code for the care and use of animals for scientific purposes, 2013)

Intravital microscopy - an optical microscopy technique applied to living animals (or tissue) that enables real-time imaging of cellular events (e.g. multiphoton, or second/third harmonic generation microscopy)

Non-recovery - a procedure from which the animal is not recovered to consciousness prior to death. In the context of this SOP the animal is anaesthetised and then humanely kill while still under general anaesthesia.

III. COMMENTS / RECOMMENDATIONS

- This procedure has been written in specific context to non-recovery imaging. Relative to animal ethics applications, when using this SOP, the following must be described in the individual ethics application: organ(s) to be imaged and how the relevant organ(s) will be exposed, expected duration of imaging, method of general anaesthesia, method of euthanasia, and any variation to this SOP.
- As this is a non-recovery procedure consideration must be taken as to the potential for stressful stimuli that may be perceived by other animals (auditory, olfactory, and visual). Efforts must be made to isolate these potential stressors:
    - Conscious animals awaiting imaging procedure should be held in isolation of animals undergoing imaging (considering animals undergoing imaging may have internal organs surgically exposed),
    - The procedural space should be well ventilated,
    - The area must be cleaned prior to use, and between animals,
    - Different species (i.e. rats and mice) should not be euthanised in the same area at the same time
- Dander and other dust particles will affect microscope operations and imaging quality. For this reason, fur should not be removed in the imaging room. Instead, as outlined in this procedure, this should be done on the day prior to imaging, within the animal facility.
- Further to this, dander is a common allergen. Surfaces must be draped to keep the area clean and minimise risk of disseminating lab animal allergens, e.g. work stations beside the microscope, the transportation trolley, other any other area containing rodents.
- This procedure has been written with specific reference to the Institute for Molecular Biology's (IMB) Microscopy room (6.034). If using a different facility to perform intravital microscopy this novel location, and any variations of equipment and infrastructure that have the potential impact to animal wellbeing must be described in the individual animal ethics application, if using this SOP.
- Generally, imaging will take 2-3 hours per animal. Realistically, research groups should only plan to perform 2-3 animal imaging sessions in one working day.

IV. EQUIPMENT

- Personal Protective Equipment (PPE)

Requirements vary dependent on facility and the type of work being conducted. Generally, requirements include lab gown, gloves, hair bonnet, face mask (ideally P2 standard), eye protection, closed in shoes.

- General anaesthetic agents,
  - If using isoflurane, as per: LAB_060 Rodent Anaesthesia – Isoflurane
  - If using injectable agents, as per: LAB_025 Rodent Anaesthesia – Injectable Agents
- Electric clippers and depilatory cream (e.g. Nair™ Hair Removal Cream)
- Protective eye lubrication (sterile and aqueous e.g. Lacrilube®)
- Microscopy room key (6.034) – for the IMB facility
- Heat mats (and any other items for thermal support, e.g. bubble wrap)
- Surgical patient drapes (or other barrier sheeting e.g. Glad® Press'n Seal)
- Surgical table drapes (or other table barrier sheeting e.g. absorbent blue pads)
  Drapes should be clean, but do not need to be sterile, given non-recovery nature of the surgery
- PhysioSuite® – rodent anaesthetic monitoring equipment
- Surgical instruments
- Gauze and normal saline (for tissue irrigation)
- Intravital microscope and associated infrastructure
- Cadaver bags (and storage)

V. PREPARATION

- Before commencing, the researcher must ensure they have appropriate support to conduct the procedures e.g. at IMB this includes ensuring you know where the list of phone numbers of relevant microscopy facility staff is located within the imaging room (in the event microscope difficulties occur)
- The researcher should ensure the microscopy room (6.034) door is locked before opening the secondary containment area – this is for the safety of people that may try to incidentally enter the room while you are operating lasers.

VI. PROCEDURE

1 day prior to imaging, within the animal facility:

1. Within the animal facility, ensure mice are appropriately identifiable.
2. Anaesthetise the appropriate mouse, as per *LAB_060 Rodent Anaesthesia - Isoflurane*
   This includes use of a thermal support (e.g. heat mat) and protective eye lubrication.
3. Using a depilatory cream (e.g. Nair™ Hair Removal Cream) remove fur from the area of interest, as required to facilitate imaging. As per *UQBR Guideline 3 Rodent Hair Removal*;
   a) Fur may be clipped first,
   b) Depilatory cream is applied directly to the area using a cotton tip or swab,
   c) Depilatory cream is left in place for a maximum of 45 seconds,
   d) The cream is then removed using a damp cotton tip or swab (ensuring all traces of cream and fur are removed – to avoid skin irritation in the mouse)
4. Continuously monitor the mouse until recovered from anaesthesia (normal reflexes, normally responsive to external stimuli, able to ambulate, eat, and drink and toilet normally), as per *LAB_060 Rodent Anaesthesia - Isoflurane*

On the day of imaging:

1. Transport the rodent(s), in their home cage or a clean cage with bedding and nest material, from the relevant animal facility to the intravital imaging room (at IMB, that is 6.034)

As per LAB_003 Transportation of Laboratory Rodents

2. Set up heat mat(s) and surgical drapes.
   Mice awaiting imaging, regardless of anaesthetic state, should be provided thermal support – this is because advanced imaging rooms are often kept at cooler temperatures for (equipment) operational reasons.
3. Collect a rodent for imaging and induce general anaesthesia, as per LAB_025 Rodent Anaesthesia – Injectable Agents. Options for alternative injectable anaesthetics may be explored through discussions with a UQBR Veterinarian (see Reference information, below).
   Precision vaporiser units (e.g. isoflurane) are not often available in advanced microscopy imaging rooms. Injectable anaesthetics are often the only option. Given this procedure is non-recovery, the relative anaesthetic safety issues associated with injectable anaesthetics are of less importance (relative to animal welfare considerations).
4. Connect the Physiosuite® anaesthetic monitoring probes to the rodent and begin recording vital signs: pulse oximetry (heart rate and oxygenation), respiratory rate, blood pressure, rectal temperature probe.
5. Ensure the rodent is under a deep plain of anaesthesia (i.e. surgical plain) before proceeding: check for the absence of ocular and toe-pinch reflexes.
   If the mouse is not adequately anaesthetised, you may need to wait longer (e.g. 15min post initial anaesthetic dose), or provide an anaesthetic "top up" (see reference information below).
6. Once the mouse is under a deep plain of anaesthesia move the mouse to the surgical workspace, if appropriate surgically drape the animal, then commence surgery to expose the organ(s) of interest.
7. Carefully place the mouse, with exposed organs of interest into the imaging platform of the microscope.
8. Commence imaging of the organ(s) of interest
9. Administer anaesthetic "top-ups" intermittently (as required), throughout the imaging procedure (see reference information, below).
   It is important that the mouse remains deeply anaesthetised. "Top-ups" are required to keep the mouse appropriately anaesthetised for a 2-3hr imaging sessions (see reference information below).
10. Exposed internal organs will need to be periodically irrigated throughout imaging to maintain their normal cellular architecture (i.e. prevent them drying out and shrivelling up). Details should be discussed with the imaging facility manager.
11. Once imaging is complete, humanely kill the rodent via one of the established laboratory animal methods of euthanasia.
12. Place the carcass into a plastic cadaver bag. Seal the bag and place this within a secondary container.
    At IMB, this must be done before opening the external door to 6.034
13. Ensure the area and equipment is cleaned between animals and at completion.

VII. REFERENCE INFORMATION

Anaesthetic "top ups" for injectable anaesthetics:

Common injectable anaesthetics, such as ketamine/xylazine (100mg/kg, 10mg/kg) and Zoletil®/xylazine (40mg/kg, 10mg/kg) will provide ~30 min of "deep" surgical anaesthesia (and 1-2hrs "sleep time"). Given the imaging may take up to 3 hours, repeat administration of anaesthetics is required. This can be done either as "top-ups" every ~30-60 minutes or via constant rate infusion. Alternatively, longer acting non-recovery anaesthetics do exist (e.g. urethane). The exact details of your anaesthetic protocol should be established with a UQBR veterinarian.

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approved Until
1	MBS	16/12/2021	16/12/2024

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).