IVIS Training Guide

Introduction

- FOM: Always reserve time & sign up, even on weekends. The computer monitor will not work if you don't log on to your reserved time and will shut off if the reservation time in FOM is exceeded. Remember to log off FOM when finished.
- Instrument is always left on. Please do not sign out of user or turn off computer.
- Located in BE-39 (shares room with BSC and downdraft table) and BE-357 (shares room with MRI)
    - Items stocked by room tech- disinfectant, 70% ETOH, 1% Chlorhexidine, DI H2O, blue pads
    - User provides study-specific materials- syringes, needles, injectate, isoflurane
    - Please clean up after yourself!
    - Report any room or equipment issues to CM, or Imaging staff.

Background

- Animals don't naturally produce light
- Tag cells with tracers→ track position and quantity of cells
- Photons diffuse through tissue and IVIS captures the signal

Things to check before you image

1. Isoflurane
2. Oxygen
3. Living Image Software/IVIS machine

Procedure

- Open Living Image software (set up user account, create lab folder on desktop)
- To Initialize→ Camera needs time to be cooled (~10min) and heated platform warms
    - First, open door to make sure nothing is on the platform (except the manifold)
    - Click initialize platform moves to check focus, stage hydraulics, etc.
    - Wait for camera to cool=> check by clicking progress bar, don't open when red
- Equipment→All equipment that potentially encounters the animal must be disinfected before/after use.
    - Plastic pads for animals
        1. Spray sheet outside of the IVIS and wipe dry with paper towel
        2. Rinse with DI H_2O and wipe dry with paper towel
    - Or use black construction paper provided (not for fluorescence imaging)
        1. Rip out from packet underneath table
        2. Throw away when done
    - Nose cones need to be disinfected outside the machine: spray paper towel and wipe inside and outside the cone
    - Manifold is movable, but limited in how far
- Anesthesia→ User provides own isoflurane and responsible for filling machine after each use.
    - Remove rubber stoppers or pins, insert nose cones, and animal dividers if desired.
    - Open main O_2 tank
    - Turn exhaust pump to "on" (if adjustable flow meter, set to 5 L/min)
    - Turn O_2 lever on

- Open switch to “Induction Chamber” and set to 1L/min
- ***Anesthetize animal (2 to 2.5% isoflurane)***
- Open lever for “IVIS manifold” (takes a few seconds for gas to travel through the tubing to reach the manifold) and set to 1 L/min
- Transfer animal from induction box to IVIS manifold

- Imaging→Exposure Time: set to Auto, this acquires an image to the brightest pixel is approx. = to the specific target max. “Redder is Better” 600-60000 counts in range. If signal is low increase auto expose time default (edit, preferences, acquisition, auto exposure tab). If saturation occurs reduce exposure or cover\take out saturated animal. (check saturation map to determine)
  - FOV: Green laser projects FOV on stage, also determines height of subject
    D=5 animals
    C=3 animals
    B=1 animal
    A=less than one animal (tissue)
  - Binning: Controls the pixel size on the camera. Increasing binning increases the pixel size and the sensitivity but reduces spatial resolution. Binning a luminescent image can significantly improve the SNR
  - F/stop: Sets the size of the camera lens aperture. The aperture size controls the amount of light detected and the depth of field. A larger f/stop number corresponds to a smaller aperture size and results in lower sensitivity because less light is collected for the image. However, a smaller aperture usually results in better image sharpness and depth of field
  - Open filter most common: gives the most sensitivity.
  - Imaging Wizard: Step by step help with setting imaging parameters
- Shut down procedure→
  - Recover animal(s)
  - Turn off isoflurane
  - Open switch to “IVIS manifold” and “Induction Chamber”
  - Close O_2 tank and allow for all gas to flush from the system
  - Close switch to “IVIS manifold” and “Induction Chamber”
  - Turn O_2 lever off
  - Turn exhaust pump to “off”
  - Refill isoflurane machine

- Saving data→ Comp. Med. is not responsible for data. It is the responsibility of the user to copy and store their data in a different location.
  - Map network drive (instructions available in imaging room)
  - Transfer data from the desktop to your user drive
  - When complete, disconnect from the mapped drive
  - Once data transfer is confirmed, please delete data from desktop at next convenience

- FOM→ log off so that you are not charged for further time

- Analyzing data→3 copies of Living Image software on Arnold Library computers #6, #7, and (Mac) Copernicium
- Wanting more material or have questions, please do not hesitate and ask Preclinical Imaging @preclinicalimaging@fredhutch.org or Brianna bwrights@fredhutch.org.