IVIS/Spectrum SOP

IVIS can generate images in 3 different modes: epi-illumination, trans-illumination or 3D imaging. Epi-illumination is the standard 2D image that is covered in basic training. Trans-illumination and 3D imaging will require an advanced training session, at the users request.

General remarks and tips before starting:
  You need to be on an active IACUC protocol before being trained

− Bring a thumb drive to copy your files after you're done

- Bring your own isoflurane to refill the anesthesia machine when you´re done
- You will need to have your own nose cones (VWR #921609, $13.40 ea, rat cone #921614 Vet Equip)
- Bring black construction paper: Strathmore Artagain black paper (Amazon)
- Each users time on the machine is logged by the Living image software. This software gives a monthly report of the users which will be used for billing. Thus, please make sure you log out of the system when you are done.

⁻ Access to IVIS machine will be granted after users have been trained by Comparative Medicine or James Askew after they have demonstrated proper use of the equipment.   1-2 training sessions per person is necessary.

⁻ IVIS imaging and anesthesia procedures must be approved on your IACUC protocol and may only be performed by approved personnel.

- Do not allow anyone to use the machine under your login, because if the IVIS/computer is damaged the last user will be billed for the repairs and access will be denied.

⁻ Please clean the machine and induction chamber before and after each use.

⁻ Notify the Lewis Lab and CM before imaging any animals that have been exposed to biological, chemical or radiation hazards. Special procedures must be followed.

Repeat with 70% ETOH to avoid Clidox residue build up, which may show up on scans.

NEVER spray anything in the machine!!

- **Never** turn the IVIS off, or the camera will be damaged. *The IVIS must be turned on and off in a certain order and you are not trained to do that.*

- This room is a shared research space. Please wipe down surfaces, dispose of waste properly and sweep up after use.

Starting April 1, 2017, there will be a charge to use the IVIS machine of $20/hour. There will be an additional charge if you do not refill the isoflurane, and do not schedule appointments on RSS.

I. ON
1. **Sign in to the isoflurane notebook, then log-in to the computer**
        Username: administrator
        Password: password
2. **Start the software Living Image** (icon on Desktop)
- The "User ID" = users initials.

3. **Initialize the system**: It will take several minutes for the check up of the system and for the camera to cool down to -90C (You can check the current temperature by clicking on the red bar at the bottom of the control window). Once the system is initialized and camera is at temperature, the red bar will turn green.

4. Start the anesthesia/isoflurane system

 a. There is no longer a tank of O2 connected to the IVIS. O2 is being pumped into the BCC facility, and the IVIS is receiving O2 from that.   Proceed to the next step.

 b. Check the level of isoflurane, It should be full.

 c. Turn ON the oxygen supply: ″oxygen ON/OFF″

 d. Turn ON the pump: ″Pump ON/OFF″

 e. Close the switches to the induction chamber ″Chamber ON/OFF″ and imaging box ″IVIS Flow

ON/OFF″ by putting the switches vertical.

f. Place your mice for anesthesia in the induction chamber. Close it tightly.

 g. Turn the isoflurane knob to 2.

 h. Open the switch to the induction chamber: ″Chamber ON/OFF″

(the switch is ON when horizontal and OFF when vertical)

 i. Once the mice are anesthetized (4 to 5 minutes), open the isoflurane switch to the imaging box ″IVIS

Flow ON/OFF″ and transfer the mice to the nose piece.

(the switch is ON when horizontal and OFF when vertical)

II. IMAGE YOUR MICE

- The "field view" should be set to "D" for 5 mice. This determines the size of the field that the camera will scan.

- If you image less than 5 mice, close the nose pieces you won′t be using with the black plugs.

- For luminescence/fluorescence: place the black mat on the bottom of the imaging box, matte side up. Then place black construction paper on the mat

- For trans-illumination: remove the black mat

- You can use the image wizard or choose your own settings: These are recommended

IVIS system ctl				
Image Mode	Exposure time	Binning	f/stop	Excitation
Luminescence	1-60sec	Medium	1	block
Photographic:	Don′t change			
FOV: choose A-D depending on how may mice you are imaging	Subject height: 1.5cm is the default			

Image mode	Exposure time	Binning	f/stop	Excitation	Emission
Fluorescence	Varies	Medium	8		

Photographic:	Don't change				
FOV: choose A-d depending on how many mice you are imaging	Subject height: 1.5cm is default				

For Fluorescence: You will need to input the filters used for the fluorescent marker of your choice. The IVIS has several proteins to choose from which can be accessed using the WIZARD.

Acquisition: FOV/exposure time/ F-Stop/ binning

Counts are ″photons per unit of time″ and must be within **600-60,000*****

(quality)   Total flux/ radiance: photons/second (quantification)

Exposure − 0.5sec-3min

Binning: grouping of pixels, default is medium, large has a 4 fold sensitivity (less time), however it is not a good resolution, small is 4 fold less sensitive yet gives high resolution.
 F-Stop: 1=widest position (most light), best for luminescence
        8= sharpest position (least light), best for fluorescence

Photographic= auto, don't ever change

Autoexposure= good for high throughput experiments
Edit image label= add information for the image
ROI= the bigger the region will give the better data
Measurements= can export as a text file for prism, or cvs for excel

Fluorescence: 10 excitation/8 emission
 Exposure time= no more than 1 min
 Radiant efficiency = emission light / excitation
 Counts: there are no counts in fluorescence
 Good: 600-875, using a red signal is better especially for deep tissue/metastatic

 Bad: low GFP signal=poor, auto fluorescence is at GFP, it's suggested to use 740 alpha free diet because of auto-fluorescence

Autosave: create or choose an existing folder on the drive: D
        Edit image labels- you can see that information by clicking information of picture
Overexposure: counts are beyond 60,000. Decrease exposure time first then decrease binning

*If a mouse wakes up during the acquisition process, click on the service button. This will allow the stage to rise, so mouse can be rescued. Then click load and resume imaging.

III. OFF
1. Turn the isoflurane dial to zero
2. Open both switches and let the oxygen out of the anesthesia machine.
3. Turn the pump OFF and the anesthesia machine OFF
4. <u>Clean</u> the induction chamber with 70% ethanol
5. <u>Clean</u> the bottom of the imaging box with 70% ethanol.
6. <u>Refill</u> the isoflurane with the black/violet connector located in the drawer; You will be charged if the isoflurane is not refilled.

7. Quit Living Image and log off the computer (leave the computer ON at all times)
8. Down load data: always down load your data to a jump drive. There will be __*no*__ analysis performed at the IVIS computer.
9. Remove your jump drive.
10**. No analysis of data should be performed on the IVIS computer.** The following labs have a copy of the software on a computer in their lab for public use. Contact them to use their software.
    Lewis lab, Oupicky lab, and Mahato lab

Lewis Lab (9-8271)