IVIS/Spectrum Standard Operating Procedure

General remarks and tips before starting:

- IVIS imaging and anesthesia procedures must be approved on your IACUC protocol and may only be performed by approved personnel.
- Bring a newly formatted thumb drive to copy your files after you're done.
- Bring isoflurane to refill the anesthesia machine.
- You will need to have your own nose cones (mouse-VWR #921609, rat-Vet Equip #921614).
- Make sure nose cones are sterilized after each use by soaking for 20 minutes in Clidox.
- 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 and demonstrated proper use of the entire equipment protocol.
- 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.
- Cleaning of the induction chamber and imager will be handled by Comparative Medicine after your scheduled imaging session.
- Notify the Lewis Lab and CM before imaging any animals that have been exposed to biological, chemical, or radiation hazards. Special procedures will need to be followed.
- **NEVER spray anything in the machine!**
- **Never** turn the IVIS off, or the camera will be damaged. If there is ever a problem, contact James Askew (Lewis Lab 402-559-8271) immediately!
- This room is a shared research space. Please dispose of waste properly and sweep up after use.

I. LOGGING ON

1. **Sign the isoflurane notebook, then log-in to the computer:**
   Username: administrator
   Password: password
2. **Start the Living Image software** (icon on Desktop).
   The "User ID" = users initials (limited to three).
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. Check the isoflurane level. As each user must fill after they're finished, it should be full.
   b. Turn ON the oxygen supply: "oxygen ON/OFF".
   c. Turn ON the pump: "Pump ON/OFF".
   d. Close the switches to the induction chamber "Chamber ON/OFF" and imaging box "IVIS Flow ON/OFF" by flipping the switches down.
   e. Place your mice for anesthesia in the induction chamber. Close it tightly.

f. Turn the isoflurane knob to 5.
g. Open the switch to the induction chamber: “Chamber ON/OFF”
i. Once the mice are anesthetized (4 to 5 minutes, unresponsive to pinch), reduce isoflurane to 1.5+, open the isoflurane switch to the imaging box “IVIS Flow ON/OFF” and transfer the mice to the nose pieces in the IVIS.

II. IMAGE YOUR MICE

- The "field of view" is determined by how many animals you wish to image in the same picture.
- 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.
- You can use the image wizard or choose your own settings: These are recommended:

IVIS system				
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				

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, the better the data
Measurements= can export as a text file for prism, or cvs for excel

Fluorescence: 10 excitation filters/8 emission filters
Exposure time = set to auto
Radiant efficiency = emission light / excitation
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 alfalfa-free diet due to 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 and hides in the machine, contact James Askew (402-559-8271, james.askew@unmc.edu)

III. LOGGING OFF

1. Turn the isoflurane dial to zero.
2. Switch the oxygen supply to OFF.
3. Turn off vacuum pump.
4. Open both switches and let the oxygen out of the anesthesia machine.
5. Turn the pump OFF and the anesthesia machine OFF.
8. Refill the isoflurane with the black/violet connector located in the drawer.
9. Exit Living Image software and log off the computer (leave the computer ON at all times).
10. Weigh each charcoal canister noting the weight on the side.
11. Download data: always download your data to a jump drive. There should be ***no*** analysis performed at the IVIS computer. There are three computers around campus that also have the Living Image software, and further analysis can be completed at one of these. These labs are the Lewis lab in BCC (Rm# 9.12.375, extension 98271), Oupicky lab in DRC1 (Rm# 1027s, extension 95256), and Mahato lab (Virender.kumar@unmc.edu, Amit.chaudhary@unmc.edu) in DRC2.
12. Remove your jump drive.

Happy Imaging!

Lewis Lab (402-559-8271)
James Askew – james.askew@unmc.edu