BIOLOGICAL IMAGING DEVELOPMENT COLAB

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IVIS Spectrum
Whole Animal Imager

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Contents

1   Introduction

The IVIS Spectrum is a whole animal imaging system used for imaging fluorescent and bioluminescent markers in mice. Please remember:

1. All users are responsible for supplying their own isoflurane and refilling whatever they use so that the next user begins their experiment with the isofluane reservoir full. See Figure:1
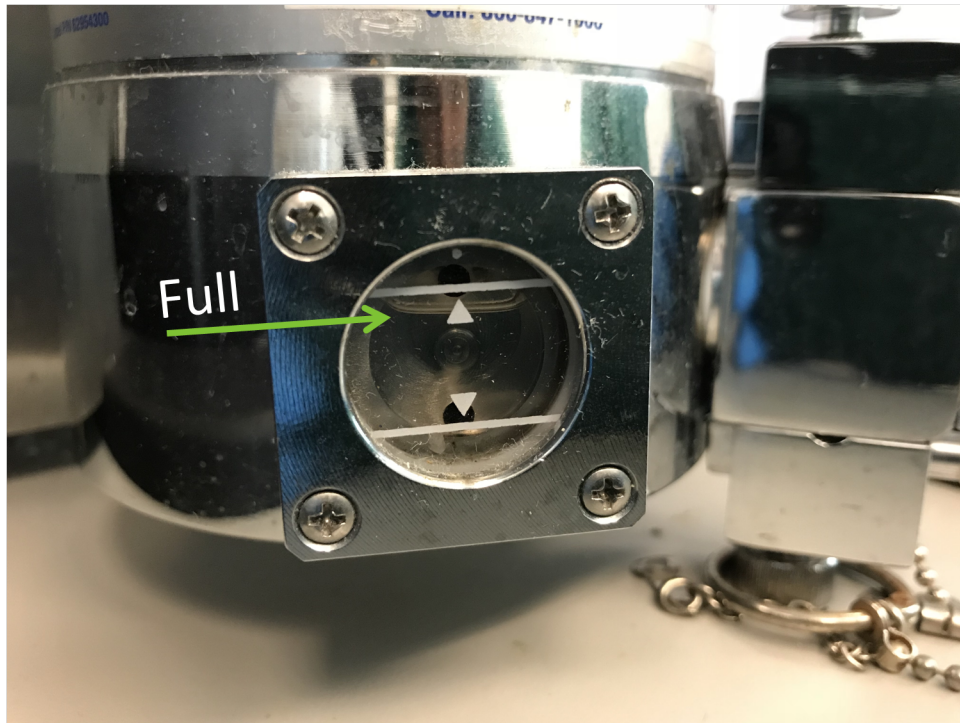

Figure 1: The isoflurane chamber pictured above is indicated as full. Notice that the meniscus is midway through the upper triangle. If the isoflurane level is lower than this at the end of an experiment, users are required to fill it.

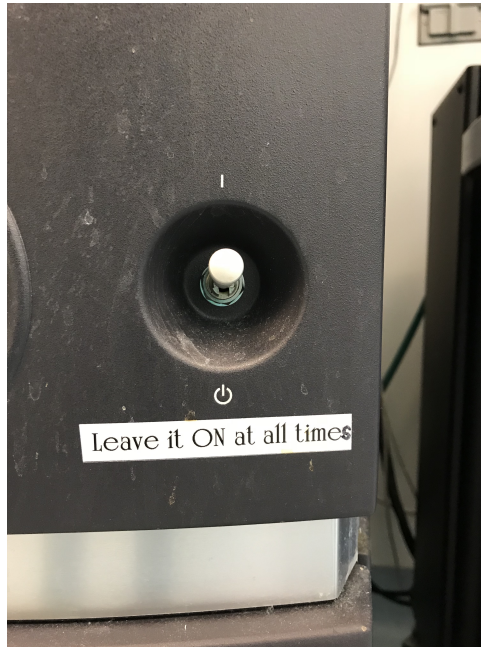2. IVIS Spectrum machine should always be on. See Figure:2

Figure 2: Always leave the Ivis power switch set to ON

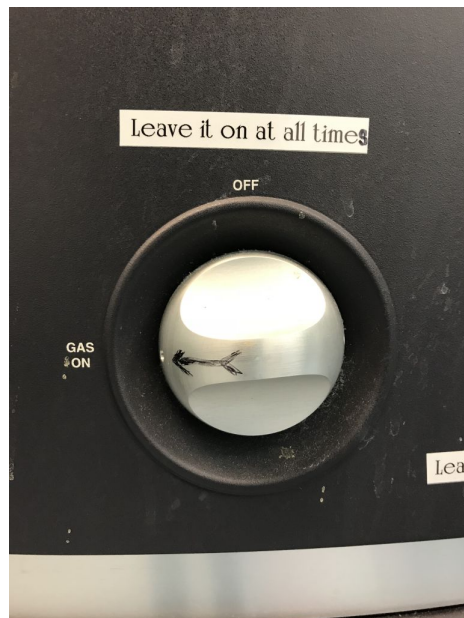3. Gas exchange knob should always be on. See Figure:3

Figure 3: Always leave the Ivis gas switch set to ON

4. Do NOT use ethanol or bleach on the IVIS instrument. Use only the Nolvosan detergent (blue liquid) provided to clean the surfaces of the IVIS.

2   Hardware - Startup

1. Begin filling out the IVIS Spectrum check-in sheet when you get to the instrument. Please note the level of isoflurane in the vaporizer. Remember, After your experiment you are responsible for refilling the vaporizer to 100% before you leave the instrument. Make sure there is enough isoflurane in the unit to anesthetize your animals.

2. Check the O_2 tank by opening the valve on the regulator, it should be above the empty marker on the regulator as seen in figure 4. If it is not, take the regulator off and change it to a new O_2 tank. Remove the plastic seal and screw the regulator into place. See Figure:4

Figure 4: A full O_2 tank is depicted above.

3. Weigh the filter canisters used for scavenging exhaled gasses before each use, using the provided scale. If canister is above the permissible weight, replace the canister with a fresh one. If you have any questions please contact the BIDC or ask a LARC employee for a new canister.

4. Turn the Oxygen valve to the ON position, located at the bottom left of the anesthesia system unit.

5. Turn on the Evacuation Pump using the switch located on the front left side of the anesthesia system unit. See Figure:5
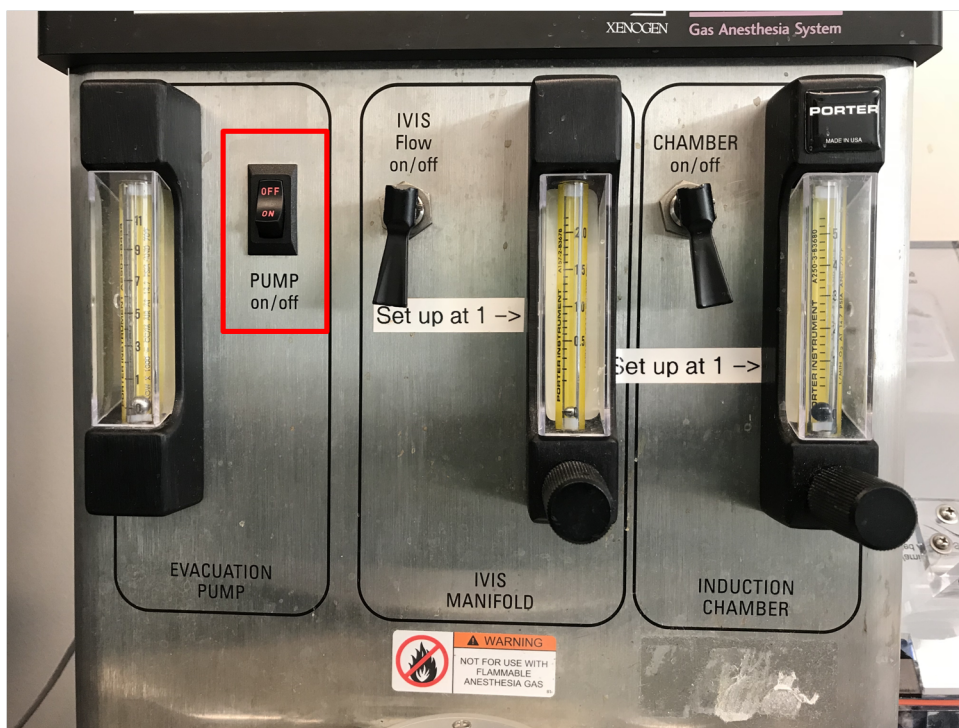
Figure 5: The figure above shows the Gas Anesthesia System. The evacuation pump switch is highlighted in red.

3   Start-Up Software

1. Log into the computer with your UCSF User Name and Password (your MyAccess credentials).

2. Double click the Living Image application on the desktop. Select your user ID, or create a User ID by clicking the + icon located on the pop-up window. You are not required to enter a password. See Figure:6
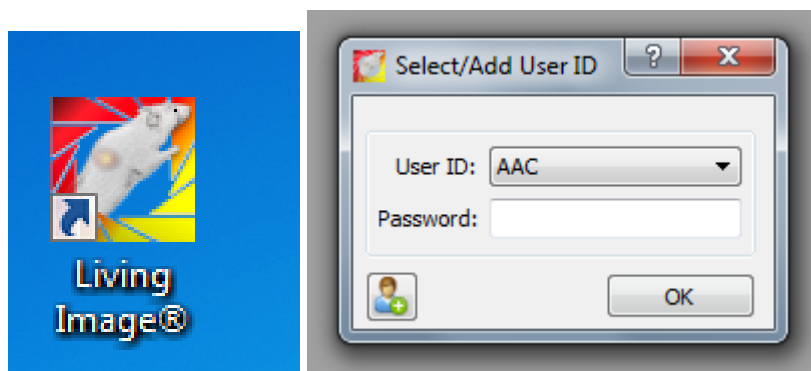

Figure 6: The figure above shows the Living Image desktop icon and the Select/Add User window.

3. Click the 'Initialize' button within the software pop up window. This will initialize the hardware by homing the stage and cooling the camera The status of the camera is denoted by the red/green bar at the bottom of the window. See Figure:7
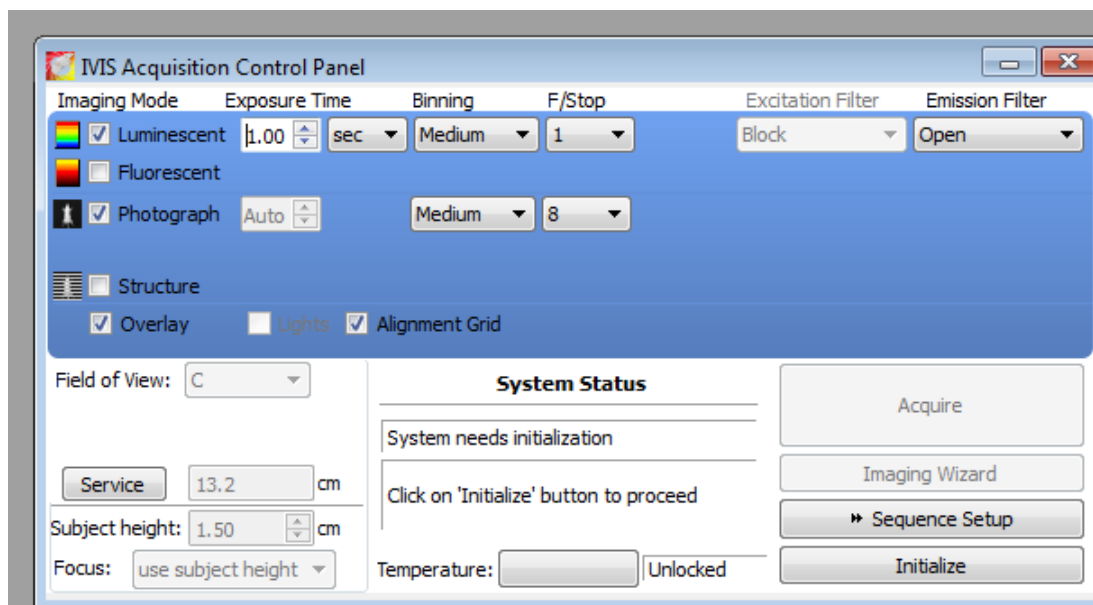
Figure 7: The figure above shows the Ivis initialization window.

4. You can image up to five mice at a time, using the clear nose cones provided. If you are not imaging the maximum number of mice, use the plugs provided to stop up the spaces not being used. This will limit the amount of isoflurane released into the chamber. See Figure:8

Figure 8: The figure above shows the nosecones and plugs properly placed inside of the Ivis chamber.

5. Use the imaging wizard while the camera is cooling. Choose bioluminescence, fluorescence, or Cherenkov imaging. See Figure:9

6. Choose FOV, as shown by the green grid on the imaging stage. FOV A is for a small FOV on one mouse, B is for imaging one whole mouse, C is for 3 mice, and D is for 5 mice. See Figure:10

7. Focus should be set at "use subject height," which is 1.5 cm. You may set a time series within this window. Click 'Next' to apply these settings to your Acquisition Control Panel.
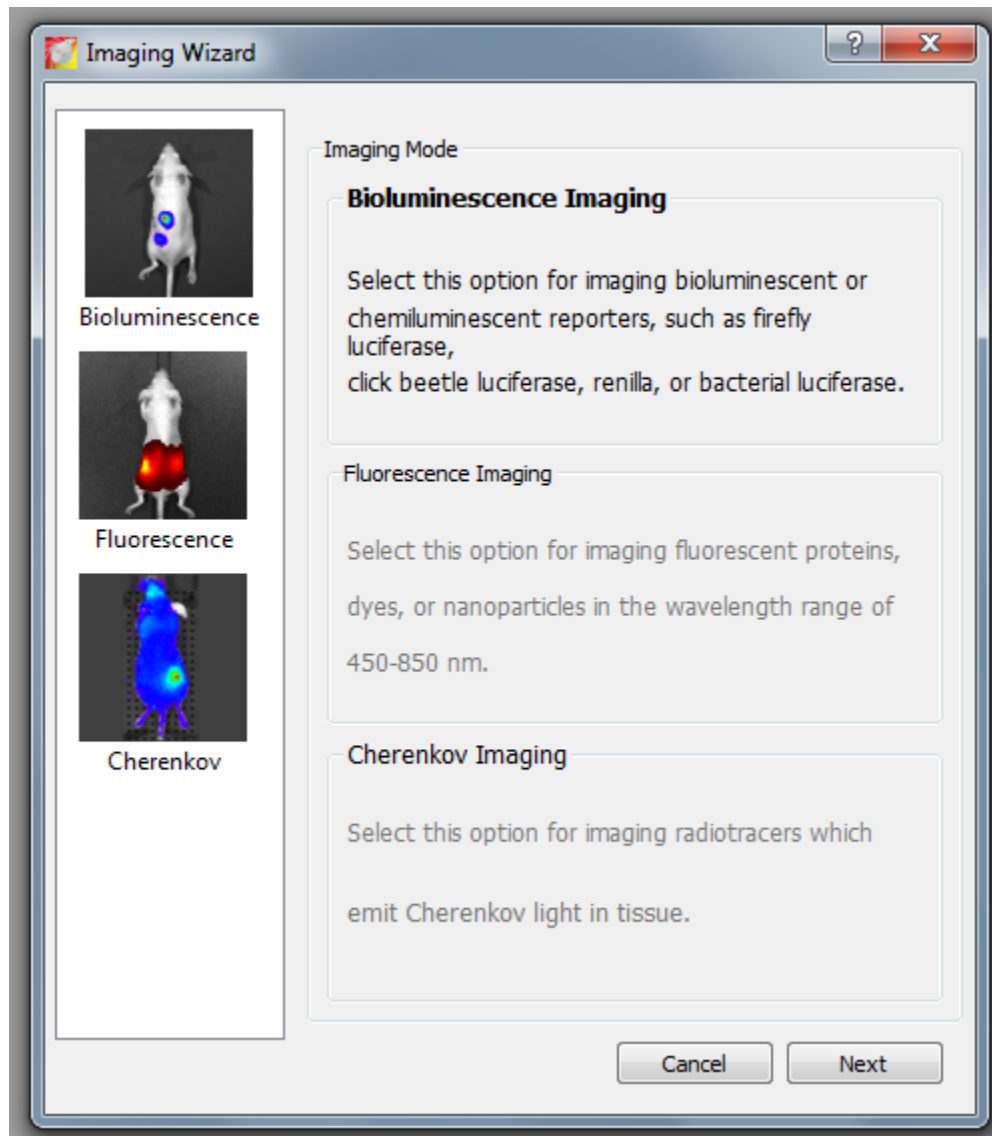

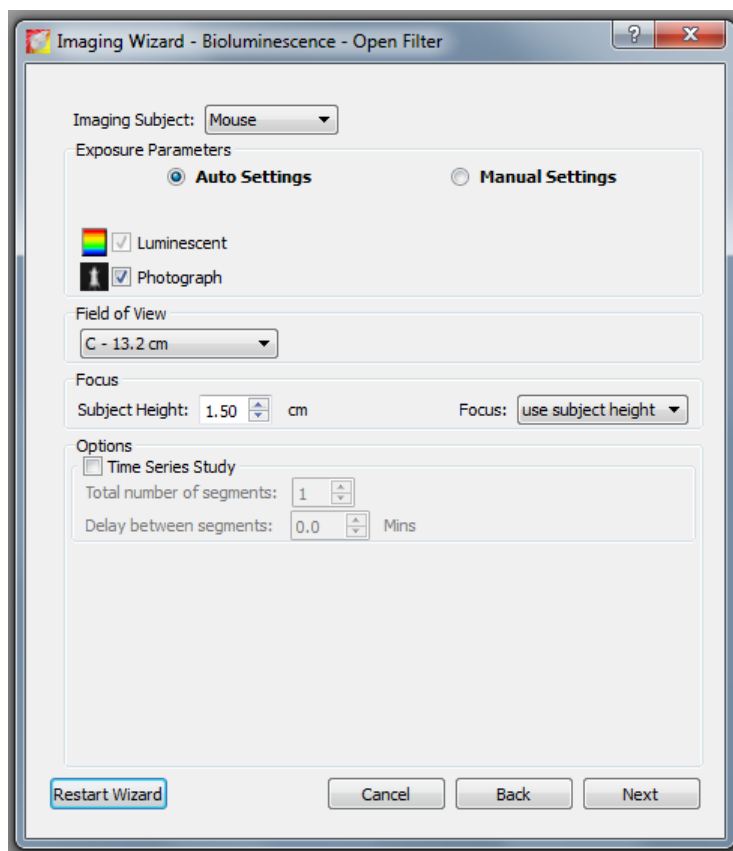Figure 9: The figure above shows the Ivis Imaging Wizard window.

Figure 10: The figure above shows Imaging Wizard:Bio-luminescence Open Filter window.

8. In the Acquisition Control Panel, your imaging parameters will appear as set by the setup wizard. See Figure:11
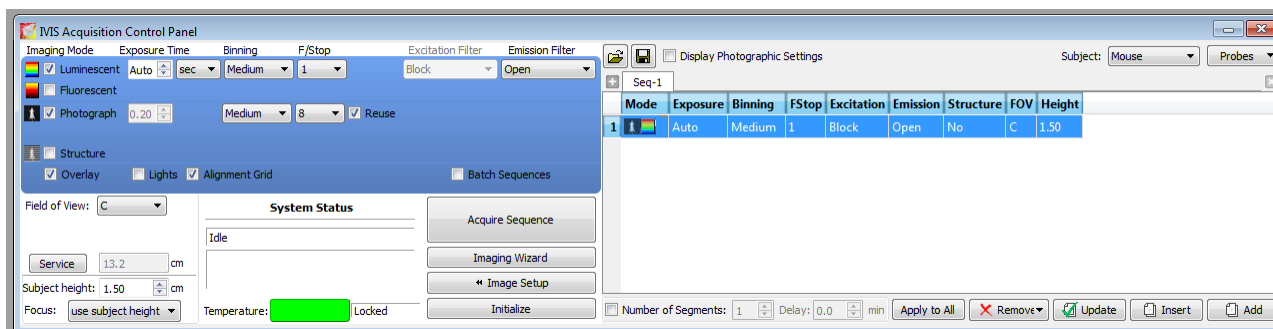

Figure 11: The figure above shows the Ivis Acquisition Control Panel.

9. Exposure will always default to Auto. You may want to select a specific exposure time, in either seconds or minutes. Use the drop down menus to select your settings.

10. Increasing the binning will allow you to amplify your dim signal, at the cost of resolution. Choose your binning signal using the drop down menu. Small binning will result in a higher resolution image than a large binning.

11. FStop refers to the aperture used to image your specimens. A small F number correlates to a wide aperture, resulting in a shallow depth of field, and a large number correlates to a small aperture, resulting in an extended depth of field.

12. Line the bottom of the induction chamber with paper towels to collect mouse droppings. Turn on the valve on the O2 regulator to allow oxygen to flow. Place your mouse or mice into the induction chamber, and lock it down.

13. Open the valve to the Induction Chamber on the anesthesia system unit. Set the valve to approximately 1. This will allow oxygen to flow to the induction chamber. Use the isoflurane vaporizer unit to select an appropriate percentage of isoflurane to anesthetize your animal(s). See Figure:12

14. Lift the switch for the IVIS Manifold, which will allow oxygen and isoflurane to enter the imaging stage.
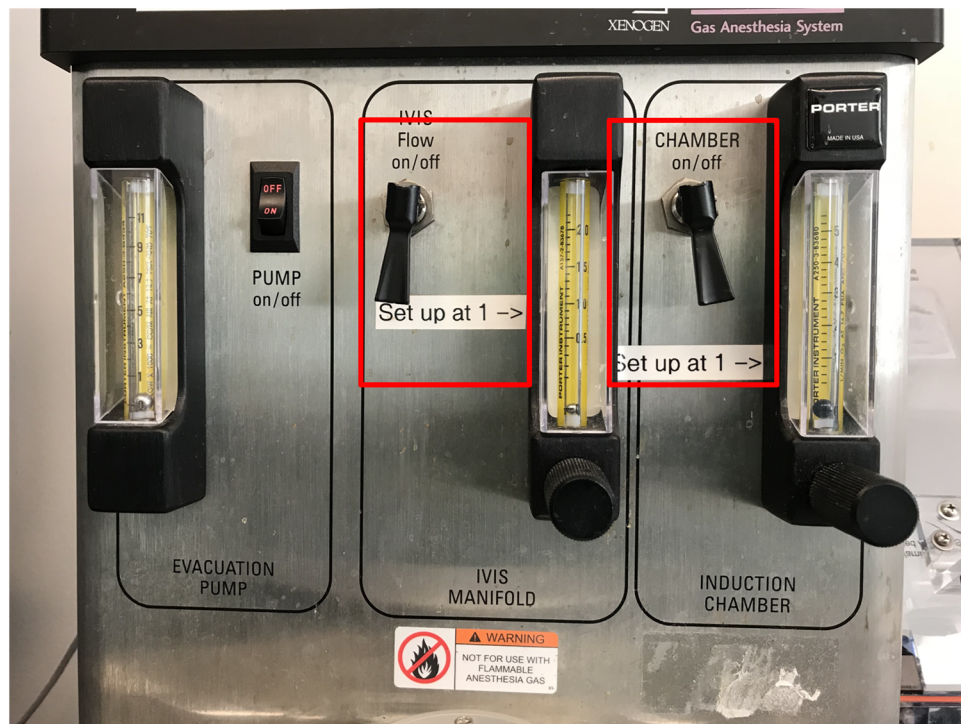

Figure 12: The figure above shows the Gas Anesthesia System. The Ivis Manifold switch is highlighted in red on the left and the Induction Chamber switch is highlighted in red on the right.

15. Place mouse on imaging stage, keeping in mind the green grid denoting the imaging area. Make sure your area of interest on the mouse is within the imaging area.

16. Begin image sequence.

17. Manual saving of image: File → Save As → C:/ → Data → Make a folder for yourself

18. Living Image saves images in a unique format. Analysis must be done within Living Image. ImageJ/FIJI will not open these files.

19. To Analyze Images:

   Select image View→Tool Palette→ ROI Tools

   Choose an ROI shape then click and drag handles around the ROI to change size. Click and drag ROI to change position then click 'Measure ROIs' within the Tool Palette to measure ROIs. Export measurements into a text/csv file if needed. See Figure:13
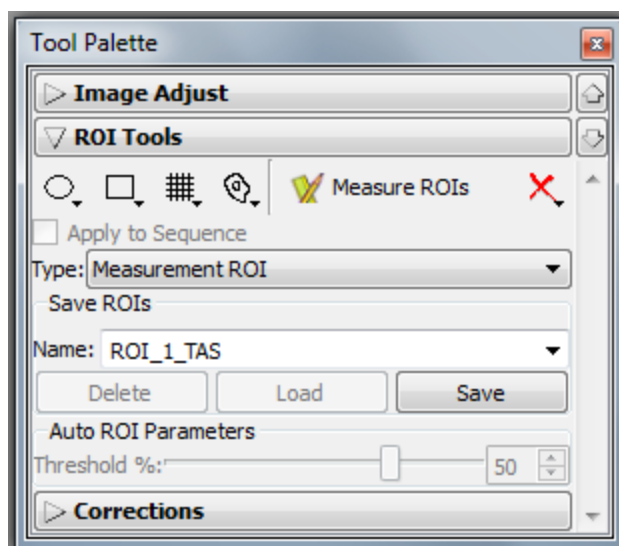
Figure 13: The figure above shows the Ivis Tool Palette window.

4   Shut Down

1. Remove mice from imaging chamber. Users are responsible for proper mouse handling/disposal after imaging is complete.

2. Set isoflurane vaporizer unit to off. Allow oxygen to flow into induction chamber to flush isoflurane before opening the chamber. Clean the induction chamber with bleach after removing mouse debris and paper towels if used.

3. Turn off both pressure manifold switches on anesthesia system. Turn the oxygen valve to OFF on the anesthesia system. Turn Evacuation Pump switch OFF.

4. Close the valve to the O_2 regulator.

5. Clean the IVIS imaging stage with Nolvasan ONLY. Clean any surfaces outside of the IVIS with 70% ethanol or bleach as needed.

6. Refill the isoflurane to 100%. Fill out the IVIS sign in/sign out sheet. The IVIS machine always stays on, and the valve to the gas always stays on as well.

Contact the BIDC

The BIDC office is located in Medical Sciences Building Room S1109.
The BIDC office phone number is 415-476-4550.
If you need immediate assistance and no one is available in the office, or it is after business hours, please call the
BIDC Hotline at 415-745-2432.