Please note:

If images acquired on IMCES instruments are used in publications we would be grateful if you could mention the facility in the Acknowledgements

1.  Turn on the electric socket
2.  Turn on the main power switch and the camera on the backside of the system
3.  Turn on the computer (Windows password is "password")
4.  If you want to do Fluorescent images turn on the Schott DCRIII (besides the monitor)
5.  Start the "Living Image" software
6.  Select a user ID from the drop-down list or enter a new User ID (up to three letters), and click "OK"
7.  Initialize the system , wait for the camera to cool down till the temperature button turns green.
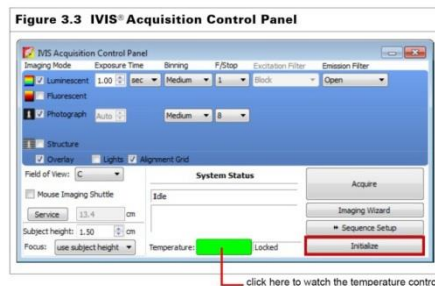


## Operating the XGI-8 Anesthesia System



Before turning on the XGI-8 anesthesia machine it is important to check/do the following

Weigh the F-Air canisters. The F-Air canister MUST BE REPLACED if the canister weight has **increased by 50 grams** from its original weight.
The original weight of the new canister is noted on the outside of the canister

8. Check the level of Isoflurane in the vaporizer. (the filler is located in the drawer)
9. Position the nose cones and light baffles on the 5 station manifold.
   ➢ It is very IMPORTANT to use the stopper on the manifold ports that are not going to be used.
10. Turn on the "EVACUATION PUMP" switch [1] (located on the front upper left of the XGI-8). Confirm that the flow is greater than 6 liters per minute (Lpm) i.e. check where the silver ball is located in the flow meter.
11. Open the oxygen regulator at the wall. It is a 50 psi PRESET regulator.
12. Turn the GREEN OXYGEN HANDLE [2] located on the front (lower left corner) of the XGI-8 to the ON position.
13. Make sure the turn dial on the vaporizer [3] is set to 0% i.e. move the dial from the OFF setting to 0% by pushing down on the black release tab.
14. Turn ON the IVIS Manifold, "IVIS FLOW on/off" [4] toggle switch (i.e. raise the toggle switch). Adjust the flow using the manifold rotameter to 0.25 Lpm.
15. Turn ON the Induction Chamber, "CHAMBER on/off" [5] toggle switch i.e. raise the toggle switch located in the front upper right area of the XGI-8. Adjust the flow using the manifold rotameter to 2 Lpm.
16. Turn OFF the toggle switch to BOTH [4 AND 5] THE IVIS MANIFOLD AND THE INDUCTION CHAMBER.
17. Rotate the dial on the vaporizer [3] to the appropriate setting for animal induction, typically 2.5%
18. Take care the 3 way valve is set to the correct position.
   ➢ If you want to work with infected mice use the "infected"-induction chamber and the "infected"-mannifold in the IVIS chamber. Swap over the GAS-OUT tubing in the chamber if necessary.
20. Place the animals in the induction chamber [6]. Close the chamber and ensure the lid is tightly closed.
21. Turn ON the Induction Chamber, "CHAMBER on/off" [5] toggle switch. The first time you do this it will take some time to induce the animals because the induction chamber and tubing need to fill with the agent.
22. Turn ON the IVIS Manifold, "IVIS FLOW on/off" [4] toggle switch. Allow time for the anesthesia gas to reach the manifold nose cones prior to removing the mice from the induction chamber. This can take from 2 minutes.
23. Turn OFF the Induction Chamber, "CHAMBER on/off" [5] toggle switch before removing the mice from the induction chamber.
24. Quickly place mice to the nose cones in the IVIS. Make sure that the manifold ports you do not need are sealed with the black rubber stoppers.
25. To minimize anesthesia exposure, keep the induction chamber lid closed and latched when not moving or replacing mice.

26. Choose a black paper as a base. (located in the drawer)
28. Chose the correct field of view to get all your animals at once.

29. For the FOV E [(24x24cm) 4-5 animals] you have to insert the XFOV-24 magnetic lens out of the black suitcase **and** tick the XFOV-24 in the IVIS Acquisition control panel.
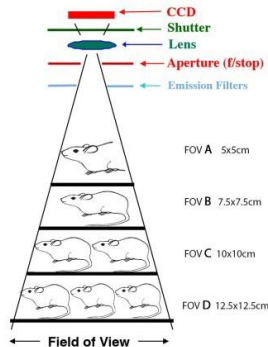


30. Set the correct "subject height" or focus manually.

### Summary of Basic Camera Settings
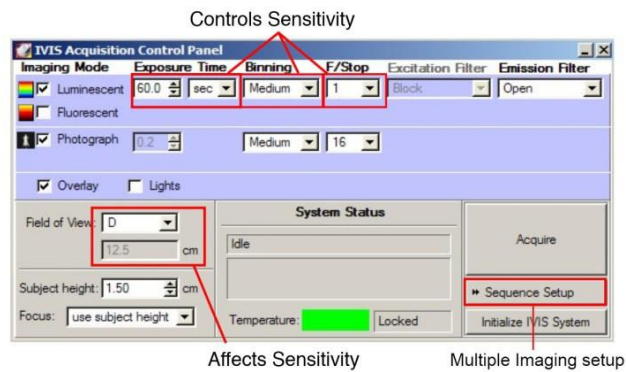


27. Acquire a Photograph only to confirm your sample is in the right focal plane.

When you are acquiring the first image of the session the system asks you if you want to autosave every taken image. Decide and adjust to the desired folder.

If you want you can change the directory in the task bar "Acquisition" "autosave to".

31. Choose if you want to acquire Luminescence, or Fluorescence images.
    - If you aquire "Luminescent" images, choose the "open" Emission filter.
    - If you want to take "Fluorescent" images choose the excitation and emission filter according to your sample (for some examples see the table below)
32. Choose "auto" at the exposure, or set up your own exposure time.
33. Think about the right binning and F/Stop (take F/Stop 16 for Photograph and 1, or 2 for Luminescent or Fluorescent images)

### Pixel Binning (CCD Resolution)



- Binning refers to the grouping of pixels into a larger super-pixel
- Large Binning = High Sensitivity/ Lower resolution
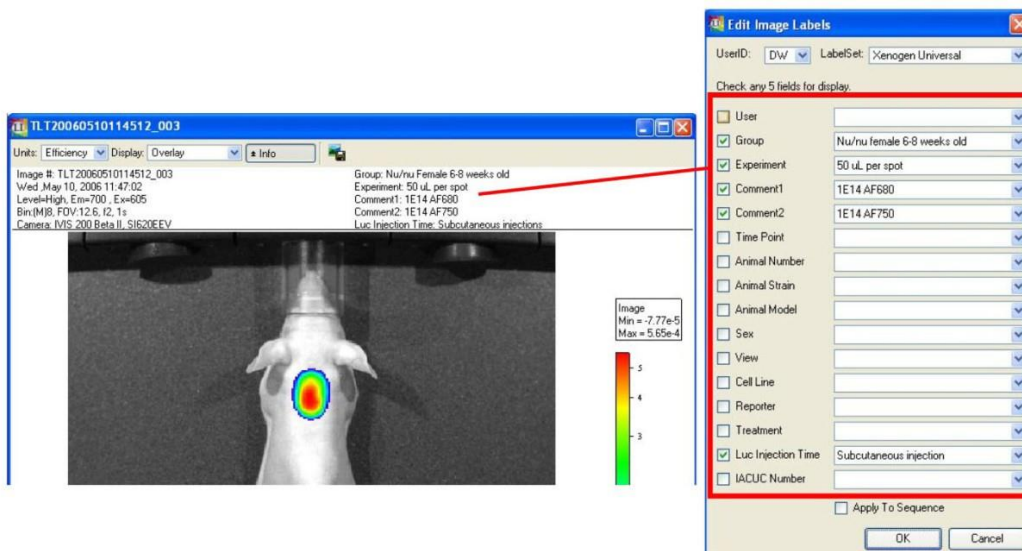- Small Binning = High Resolution/ Lower Sensitivity
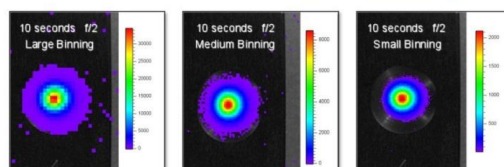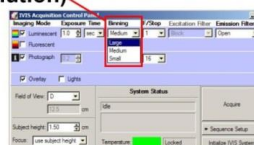
34. Either "Acquire" you image or set up a time series with the "sequence setup" & follow the "imaging wizard"
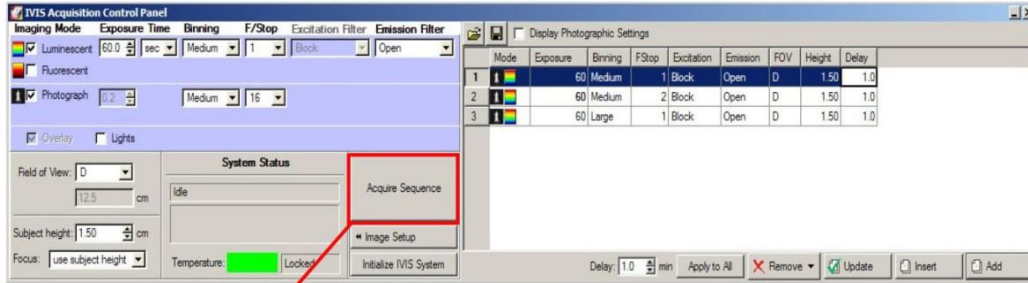


Starts Sequential Image Acquisition

Easy to use Sequence Editor

36. Don't forget to do a proper labeling to be able to find back your analysis later.



37. Please check your pixel values. In your region of interest you should obtain values (Counts) from at least 6000 to 60000! If your pixel values are much lower either choose a higher binning, or raise the exposure time.
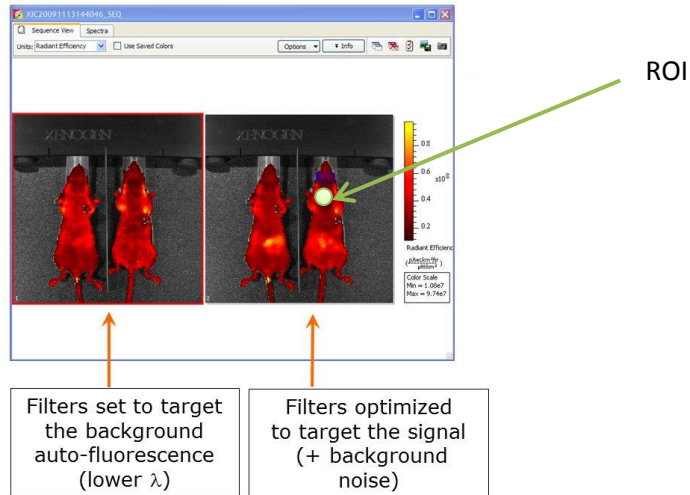
For quantification of your fluorescence signal chose the units drop down menu: "radiance efficiency".

38. Perform a background subtraction for getting rid of autofluorescence signal.

> ➢ Use the Image Wizard and choose your desired imaging setting. Press "add".
> ➢ Add another measurement with the same emission filter but the corresponding background excitation-filter (see table below)

| Filter Set | Label | Background Passband | Excitation Passband | Emission Passband | Fluorescent Dyes & Proteins |
|---|---|---|---|---|---|
| 1 | GFP | 410-440 | 445-490 | 515-575 | GFP, EGFP, FITC |
| 2 | DsRed | 460-490 | 500-550 | 575-650 | DsRed2-1, PKH26, CellTracker™ Orange, 605 Qdot® Bioconjugate, TdTomato |
| 3 | Cy5.5 | 580-610 | 615-665 | 695-770 | Cy5.5, Alexa Fluor® 660, Alexa Fluor® 680, 705 Qdot® Bioconjugate |
| 4 | ICG | 665-695 | 710-760 | 810-875 | Indocyanine green (ICG), 805 Qdot Bioconjugate |

| Imaging Center Essen | Institut für Experimentelle Immunologie und Bildgebung | Universitätsklinikum Essen |
|---|---|---|

**S**tandard **O**perating **P**rocedure
**Ivis Luminall**

Page **5** von **6**

Last change: 02.08.2018

39. Create a ROI on a Position where you don´t expect a specific signal in your animal.



ROI

Filters set to target the background auto-fluorescence (lower $\lambda$)

Filters optimized to target the signal (+ background noise)

40. Go to the task bar, choose: "tools" and "Image math"

- ➢ Use your image with the desired wavelength as "A"
- ➢ And the Background image as "B"
- ➢ Use "A-B*K" in the Result drop down menu
- ➢ Press "Compute k from ROI" (image A, ROI from A)
- ➢ And "display result for measuring"

Now a new window, including your calculated image should appear. Here you can change the contrast as usual.
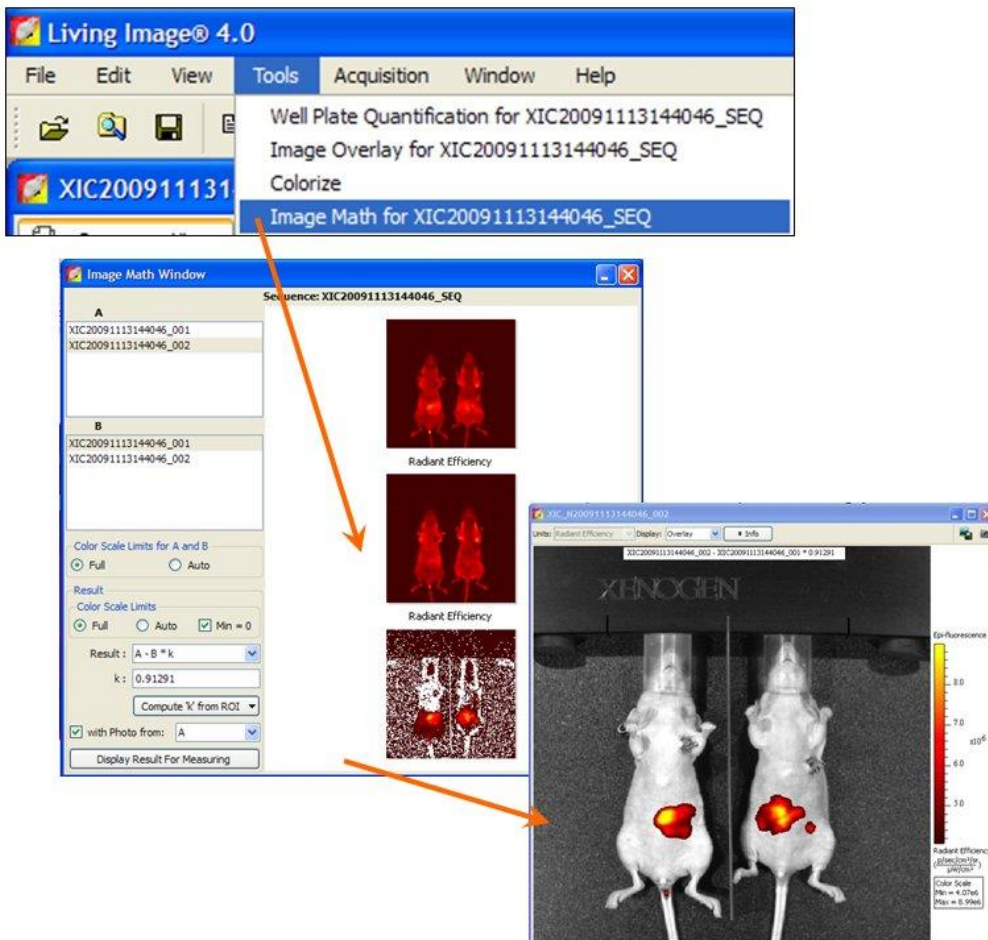
41. When you are done with your imaging, TURN the VAPORIZER [3] to the OFF position.
42. Turn ON the Induction Chamber, "CHAMBER on/off" [5] toggle valve for 5 minutes to allow pure oxygen to flow through the induction chamber.
43. After purging the induction chamber with oxygen, turn OFF the Induction Chamber, "CHAMBER on/off" [5] toggle switch.
44. Turn OFF the oxygen supply at the regulator on the wall.
454. Turn OFF Turn ON all toggle [4 and 5] switches to DEPRESSURIZE the XGI-8 system. This is completed when the silver balls in all the rotometers are at the bottom of their respective tubes.
45. Once the flow had stopped through the rotometers, turn OFF all toggle switches [2,4 AND 5].
46. Turn OFF the EVACUATION PUMP [1].
47. CLEAN the induction chamber and IVIS.

After finishing your experiments CLEAN YOUR WORKSPACE & THE INDUCTION CHAMBERS. You want to have a clean Workspace as well.

Tell the staff if the F-Air Canister needs to be replaced. (an E-Mail is enough if there is no staff available)