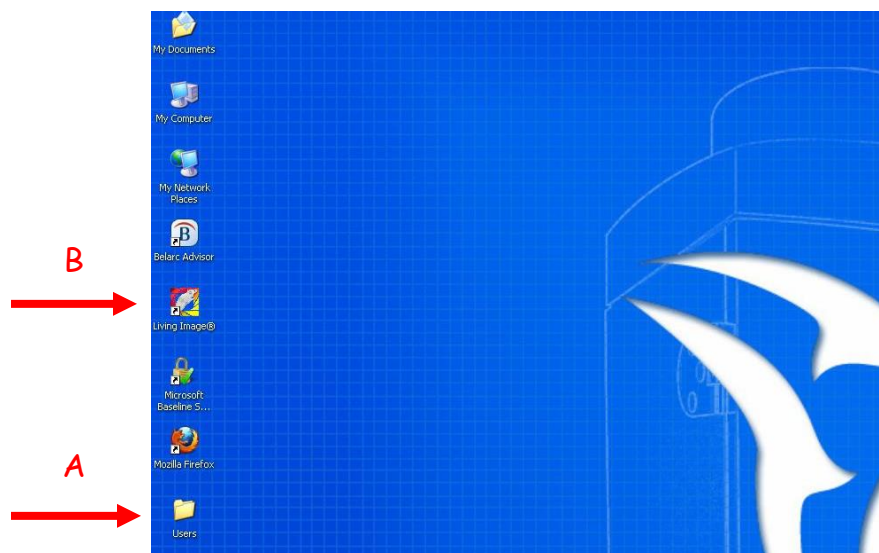


The Center for Molecular Imaging IVIS Spectrum **Bioluminescence** Protocol

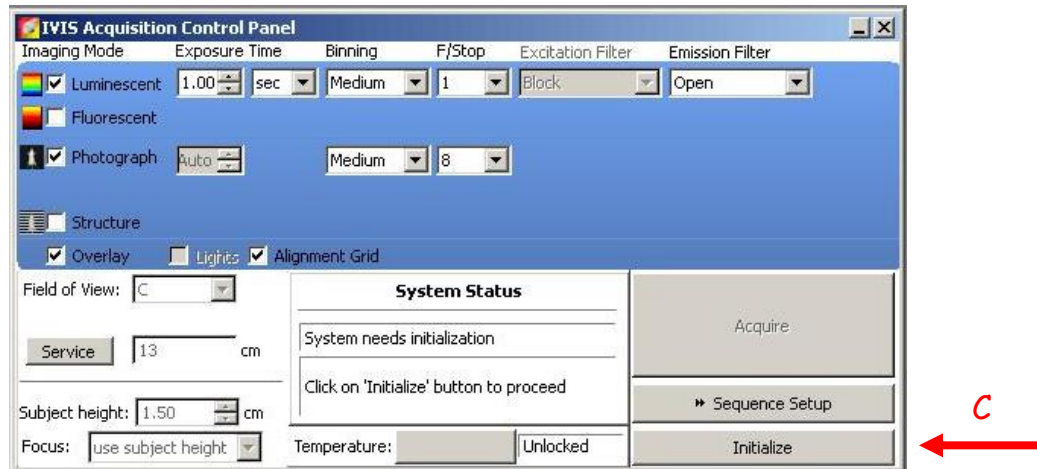


- Open the "Users" folder on the desktop and find your PI's folder. [A] If your PI does not have a folder create one with their last name and first initial. You are free to create new folders within your PI's folder but **ALL DATA MUST BE SAVED WITHIN YOUR PI'S FOLDER, NOT ON THE DESKTOP OR IN A PERSONAL FOLDER.**
- Open the Living Image program (v.4.0). [B] When prompted, enter your initials or select from the list then click "done".

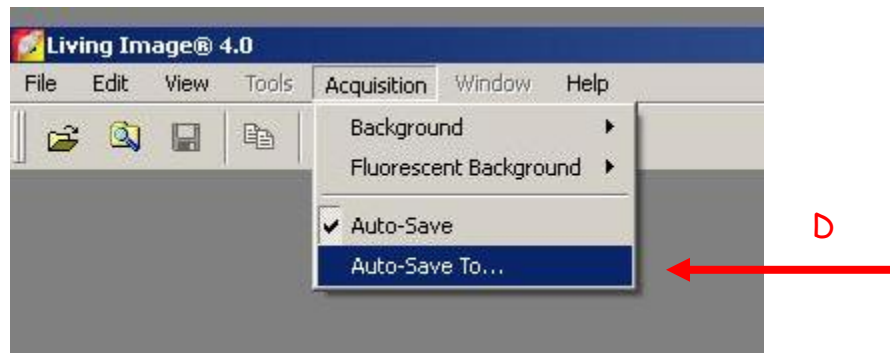


The Center for Molecular Imaging IVIS Spectrum **Bioluminescence** Protocol

- When the program opens click "initialize" on the lower, right corner of the control panel. [C] When initialized, the temperature box will be green and system will be idle.

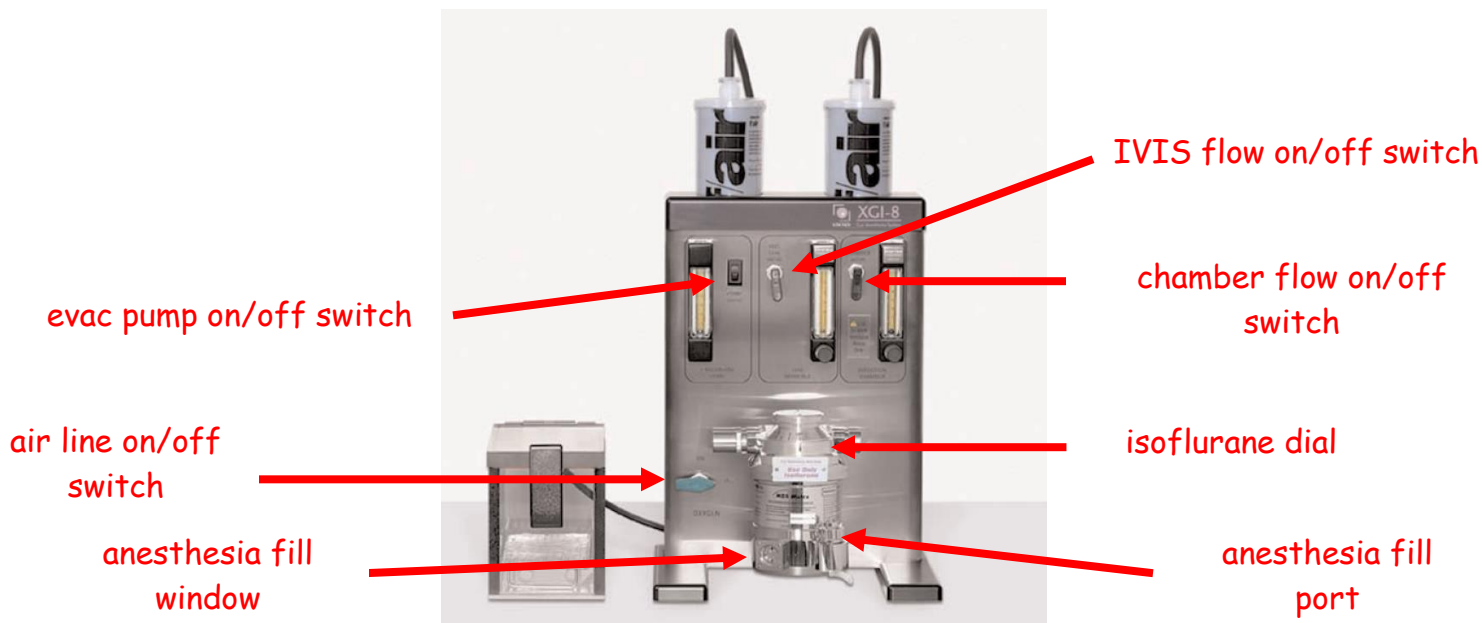


- Set the program to autosave to the folder you created in the beginning. Click on "Acquisition" and then "Auto-Save To..." [D] Find your data file within your PI's folder in the User folder and select it for autosaving.



The Center for Molecular Imaging IVIS Spectrum **Bioluminescence** Protocol

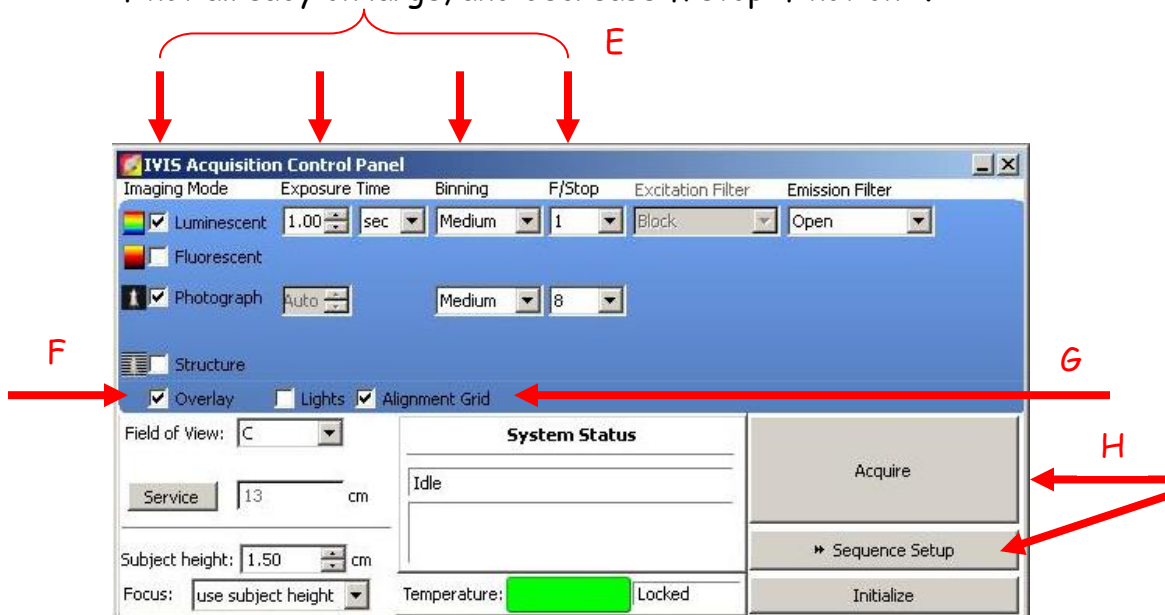
- Make sure the isoflurane chamber is full. When you are ready to anesthetize the animals, make sure the pump (left) and chamber (right) switches are on. Turn the isoflurane dial to 2% for induction and 1.5% for maintenance.



- Prepare the IVIS imaging chamber by lining it with a piece of black cardstock, which you will discard when you are done imaging. You should also place the nose cones that you will need and get the black dividers for in between your animals.
- The standard in vivo procedure is to inject animals with a substrate, such as luciferin, and wait about 10 minutes before imaging. If your experiment requires such an injection proceed with it now. For mouse studies the standard injection is 100 μ L of a 40 mg/mL stock. Set a timer for 10 minutes and anesthetize animals with roughly 5-6 minutes remaining so that they are in the IVIS chamber and ready for imaging by the 10 minute mark.

The Center for Molecular Imaging IVIS Spectrum **Bioluminescence** Protocol

- While you wait the 10 minutes set luminescent imaging mode, exposure time, binning, and f/stop to the right level. [E] If you do not know what setting to use try 30-60 second exposure time, large binning and f/stop 1. For a hot signal, decrease time first, if necessary lower binning, last increase the f/stop. Do not lower time below 5 seconds. For a low signal increase time, raise binning if not already on large, and decrease f/stop if not on 1.



- Make sure Overlay is checked (for both luminescent and photographic images). [F] If you just want to take a picture, check only photographic.
- Set the proper field of view and make sure the animals are completely within the grid. To see, make sure "enable alignment grid" is checked [G] and a green grid will appear on the platform.
- Place animals in the chamber and arrange them as necessary so that you can begin imaging at roughly 10 minutes post injection.
- Click "Acquire" to start image acquisition or select "Sequence Setup" [H] and run multiple acquisitions in order to obtain a curve of signal intensity over time. Acquiring a sequence will allow you to record the peak signal intensity for each individual animal.

The Center for Molecular Imaging IVIS Spectrum **Bioluminescence** Protocol

- When the scan is complete you will see a screen like the one below (without the red circles). If you get a message saying the image is saturated or if there is too little signal, adjust the settings as explained above and immediately run the scan again. It is recommended that the signal of interest be greater than 600 counts and less than ~ 60,000. If you get a message saying the image is saturated or if there is too little signal, adjust the settings as explained above and run the sequence again.



- **You must analyze your data on the analysis computer located within 6005.** Use of this system is free while analysis done on the acquisition station will incur the hourly imaging rate. You should remove your data from the acquisition hard drive after each imaging session.
- Please maintain MI technique as much as possible. Spray gloves and clean equipment with provided Virex solution in between animal cages to reduce the possibility of cross contamination.
- When you are done imaging you can check within the folder you created to ensure your files have saved. Then exit out of Living Image and proceed with cleaning up the area.
- Discard the black paper. Spray some paper toweling and wipe off the platform, NEVER spray directly into the imaging chamber. Clean the nose cones and dividers that you used and return them to the blue bins. Clean out the induction box and the bench top and ensure that vaporizer is shut down properly. Refer to the posted instructions if you have questions about how this should be done.