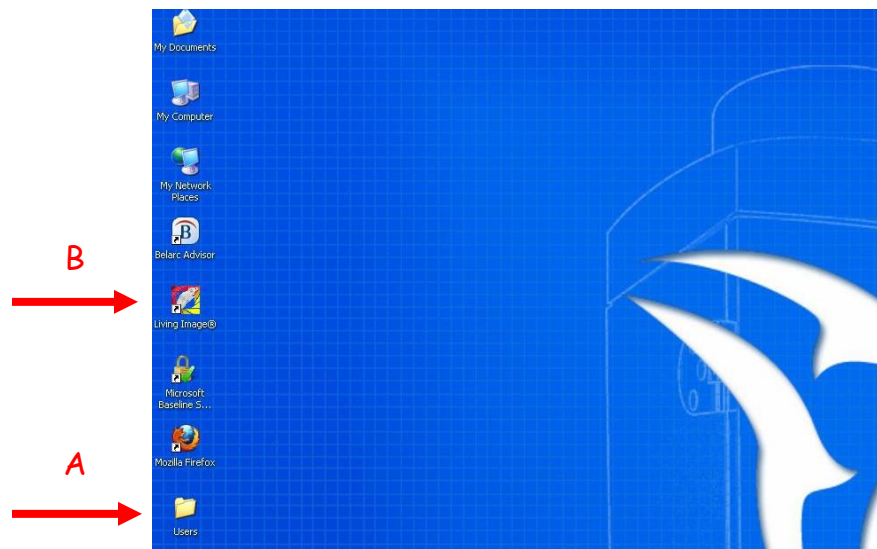


# The Center for Molecular Imaging IVIS Spectrum **Fluorescence** Protocol

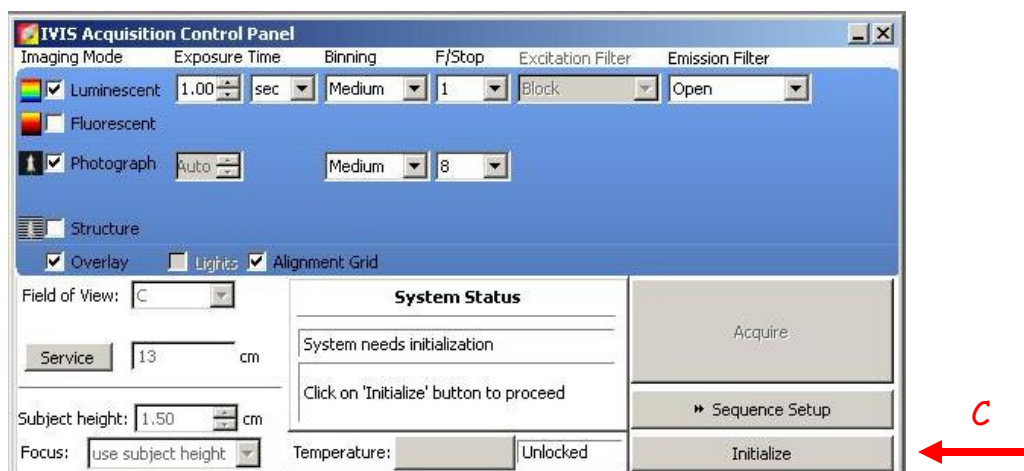


- Open the "Users" folder on the desktop and find your PI's folder. **[A]** If your PI does not have a folder create one with their last name and first initial. You are free to create new folders within your PI's folder but **ALL DATA MUST BE SAVED WITHIN YOUR PI'S FOLDER, NOT ON THE DESKTOP OR IN A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

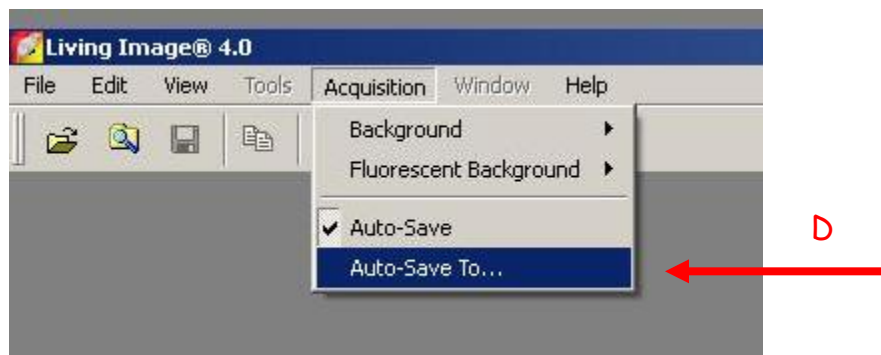


# The Center for Molecular Imaging IVIS Spectrum **Fluorescence** Protocol

- 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.

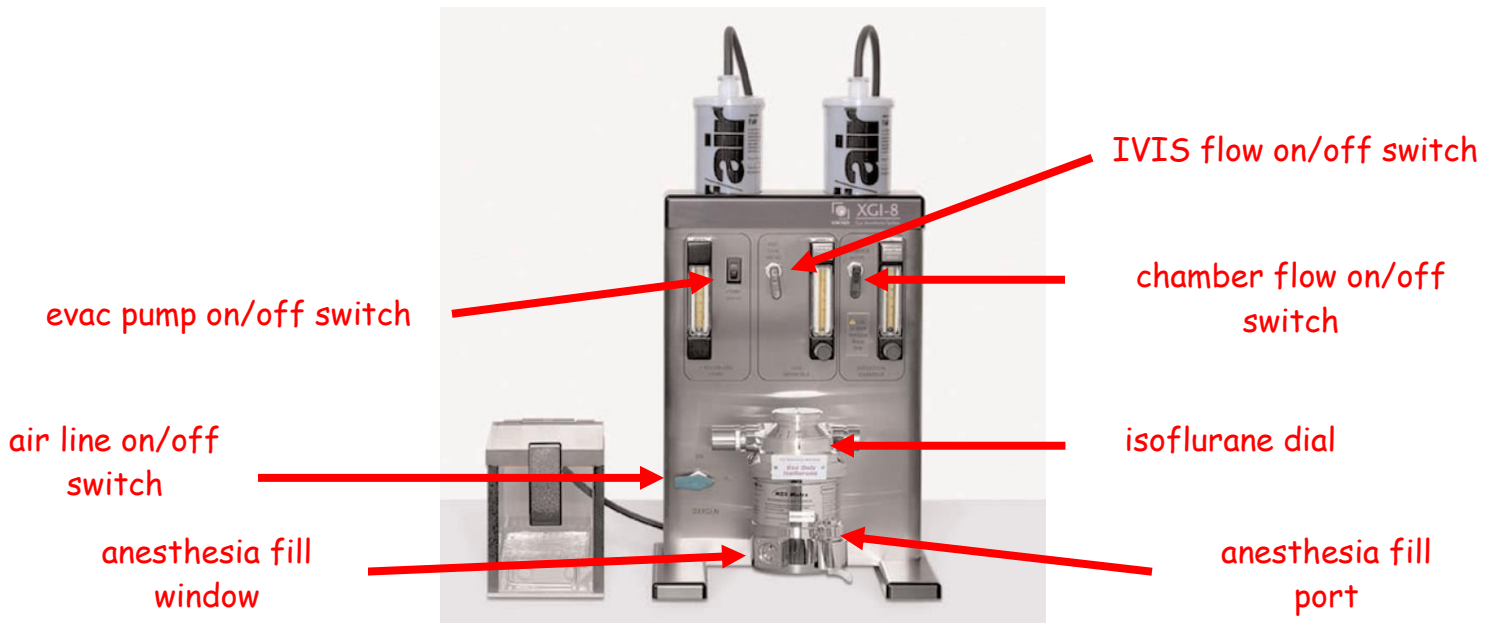


- Set the program to autosave to the folder you created in the beginning. Click on "Acquisition" and then "Auto-Save To..." [D] Find your data file within your PI's folder in the User folder and select it for autosaving.



## The Center for Molecular Imaging IVIS Spectrum **Fluorescence** Protocol

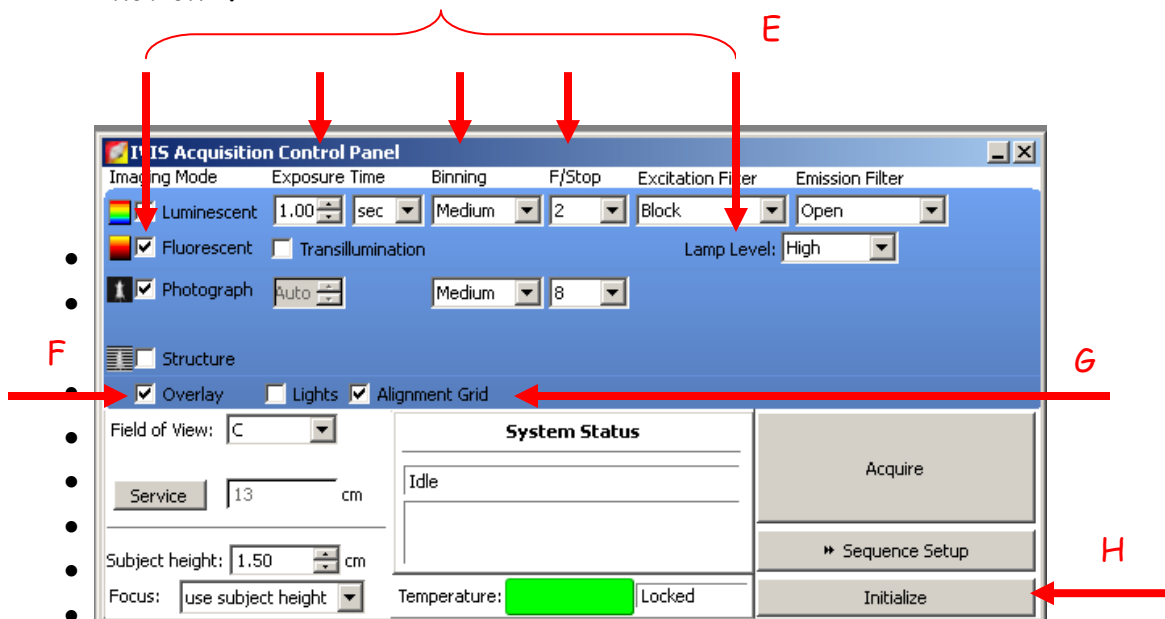
- Make sure the isoflurane chamber is full. When you are ready to anesthetize the animals, make sure the pump (left) and chamber (right) switches are on. Turn the isoflurane dial to 2% for induction and 1.5% for maintenance.



- 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 Center for Molecular Imaging IVIS Spectrum **Fluorescence** Protocol

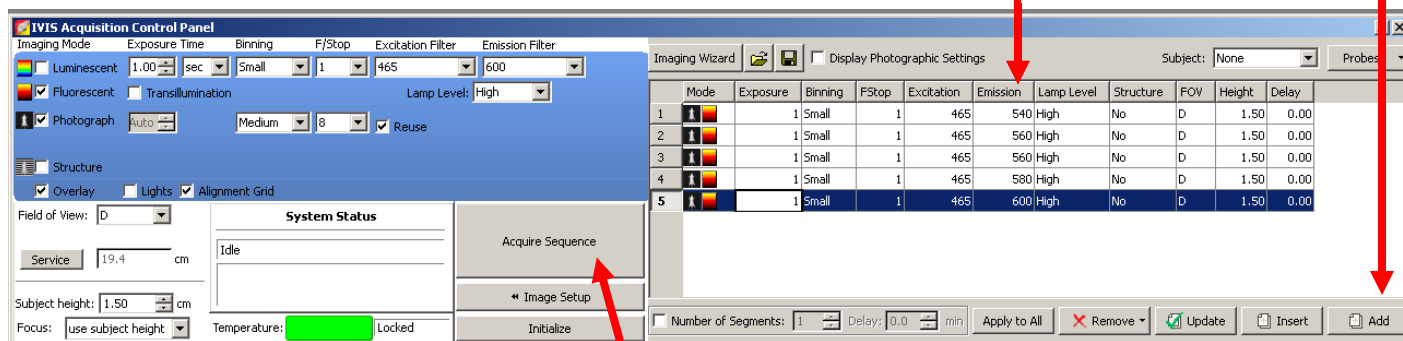
- Set fluorescent imaging mode, exposure time, binning, f/stop and lamp level. [E] If you do not know what setting to use try setting a 1-2 second exposure time, medium binning, f/stop 2 or 4 and lamp level to high. For a hot signal, decrease time first, if necessary lower binning, last increase the f/stop. 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 fluorescent 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 check, make sure "enable alignment grid" is checked [G] and a green grid will appear on the Xenogen platform.
- Click "Sequence Setup" to set the parameters for a sequence of image acquisitions. [H]

# The Center for Molecular Imaging IVIS Spectrum **Fluorescence** Protocol

- In the sequence editor click "Add" [I] and a scan consisting of the acquisition parameters you set on the left will appear. Each row in the table specifies the acquisition parameters for one image in the sequence. Select an excitation wavelength between the min and max for your dye and maintain that excitation wavelength for all images. Select a range of emission wavelengths (starting below the min for your dye) by adding multiple rows to the acquisition sequence. Once added you can double click on the "Emission" for each row and change it. [J]



- Turn on the IVIS anesthesia flow and place the animals in the chamber, arranging them as necessary.
- To acquire the sequence click "Acquire Sequence" [K] in the control panel.
- When all the images are acquired and displayed in the image window confirm that the signal of interest is above the noise level and below the CCD saturation. Double clicking on any one of the scans will open it in a new window that will have a scale indicating the signal intensity. 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.

## The Center for Molecular Imaging IVIS Spectrum **Fluorescence** Protocol

- Repeat for all animals. 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.
- **You must analyze your data on the analysis computer located in G005.** Use of this system is free while analysis done on the acquisition station will incur the imaging hourly rate. You should remove your data from the acquisition hard drive after each imaging session.
- 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.