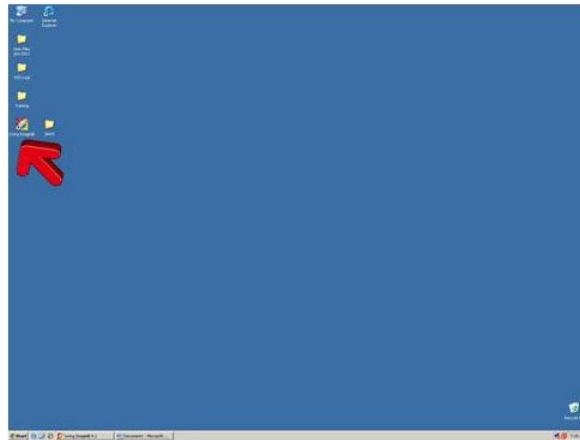


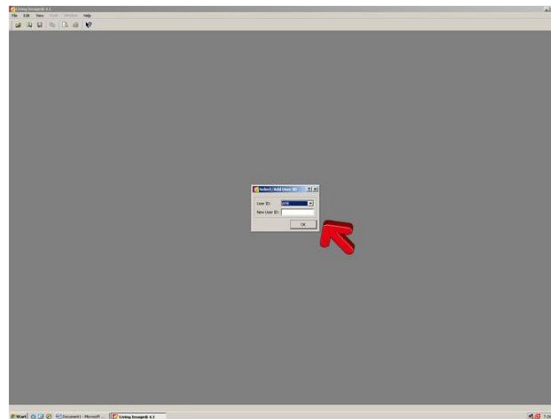
IVIS: Living Imaging System

Start up

1. Log on to the computer. The computer username is *administrator*, the password is *password*.
2. Start up the Living Image software (double click to open).

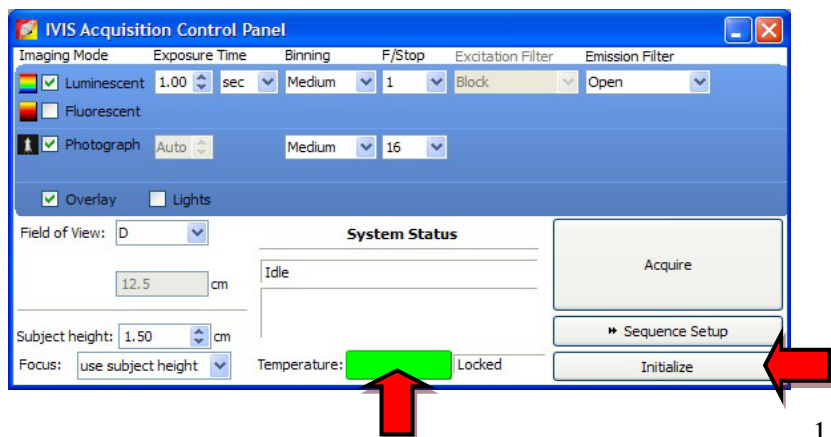


3. Log into the software (choose your initials or add new user by adding your initials to the box).

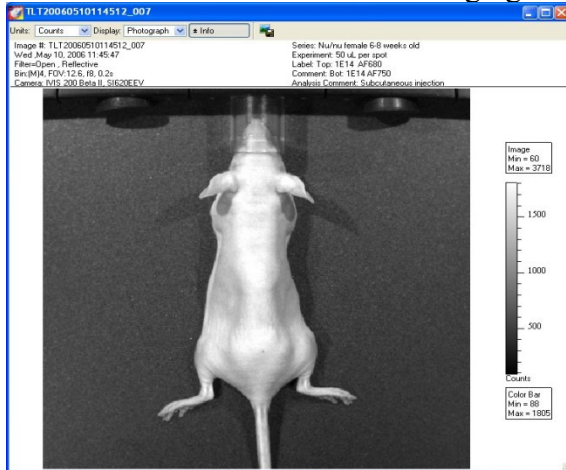


4. Initialize the machine. The window will automatically open when you log in. Click on *Initialize* and wait until the camera is cooled to -90C. The stage is pre-warmed and should be warm all the time.

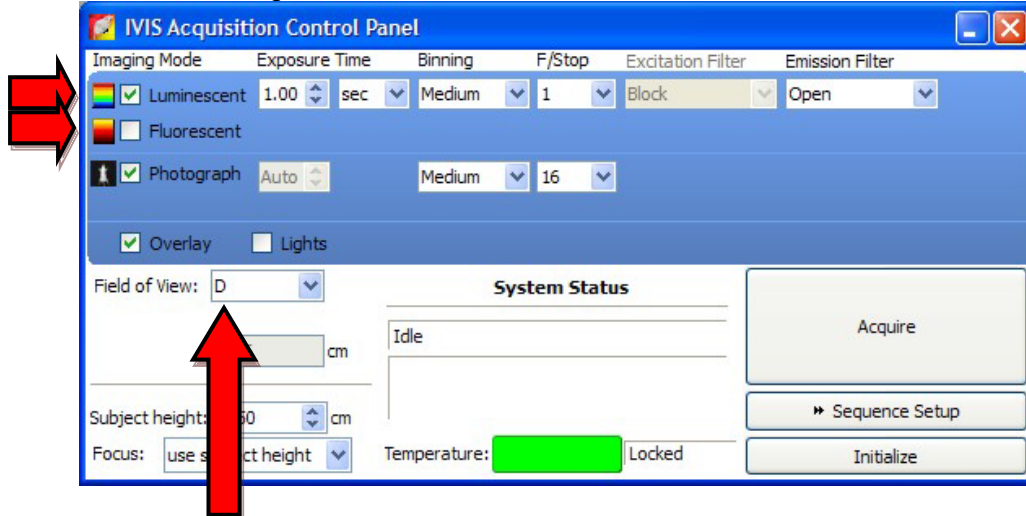
When the system is ready the temperature indicator will turn green and there will be a green light on the bottom left corner of the imaging chamber.



- Place anesthetized mouse in the imaging chamber.

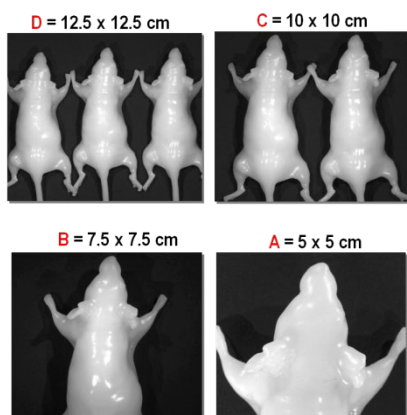


- In the IVIS Acquisition Control Panel select *Luminescent* or *Fluorescent*



- Chose field of view (D, C, B or A)

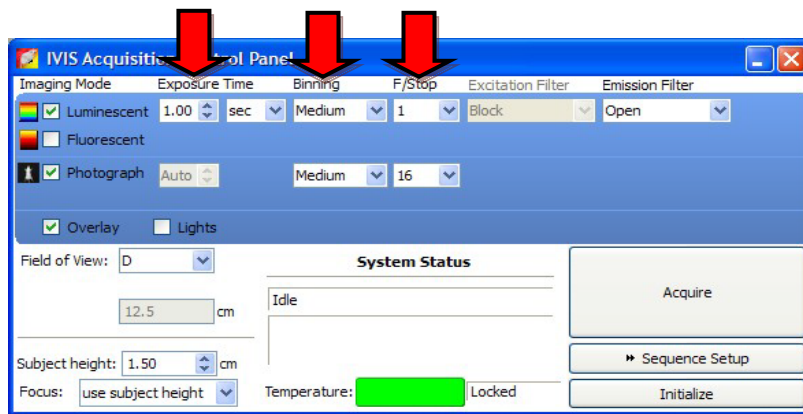
Field of View



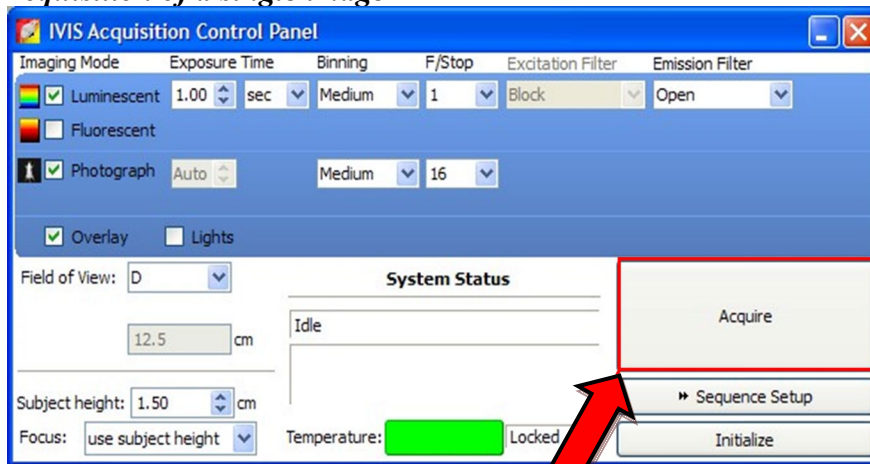
Setting Sensitivity – Luminescent Signal Level:

**Adjust camera settings to obtain a signal level of 600 to 60,000 counts.

1. Controls that control the signal level are:
 - a) Exposure time (AUTO)
 - b) Binning (CCD Resolution)
 - c) f/stop (Aperture)

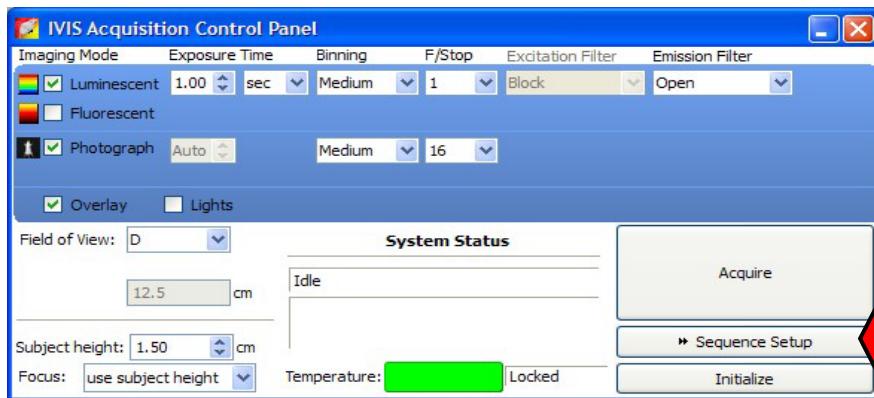


2. Acquisition of a single image

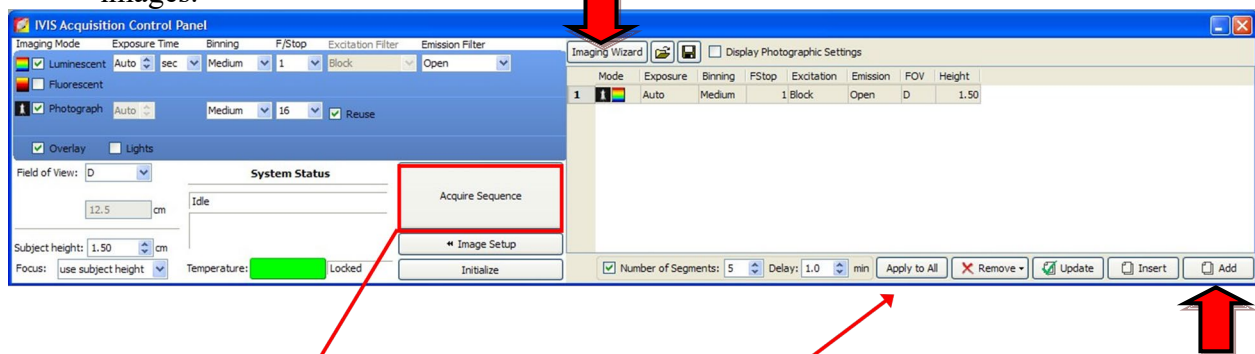


Single Image Acquisition

Or Acquisition of an image sequence



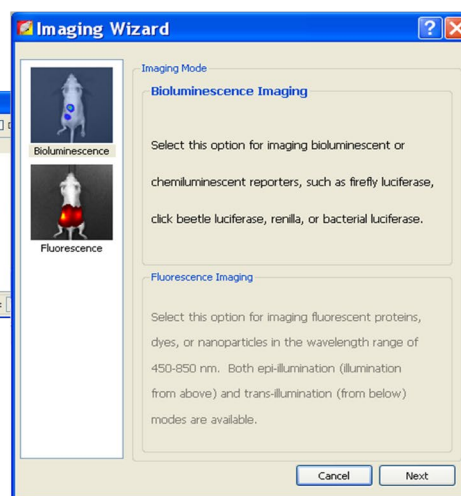
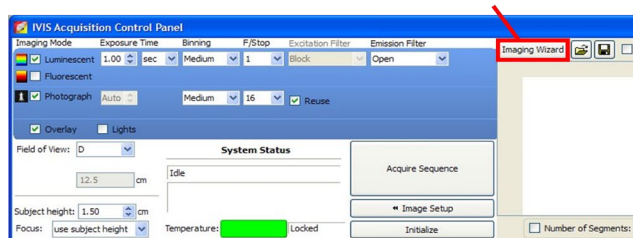
Enter values for exposure time, binning and f/stop and add to imaging sequence or select *Imaging Wizard* for assistance, set delay between images for a sequence with multiple images.



Starts
Sequential
Image
Acquisition

User Friendly
Sequence Editor

Select for assistance in setting
up bioluminescence or
fluorescence sequences



Fluorescent imaging:

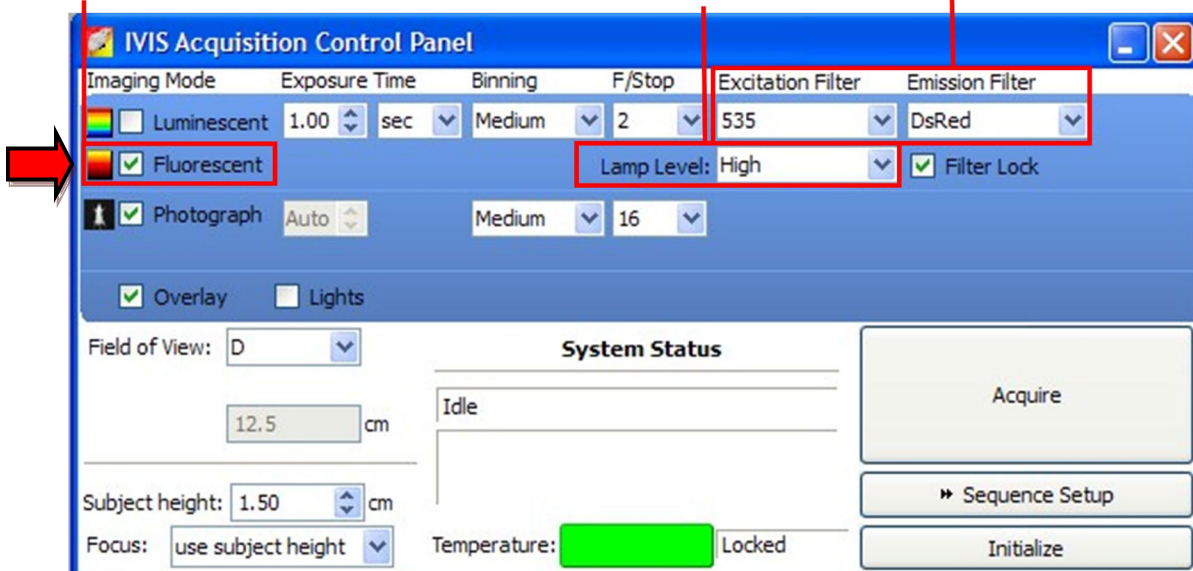
Signal should be above 3000 counts to avoid background.

1. Fluorescent data acquisition, check the fluorescent box and select lamp level and appropriate filters.

**Select
Fluorescent
Imaging Mode**

**Lamp level
High / Low**

Select filters



Reference for excitation and emission filter selection:

*Note—long wavelength fluorphores (810-875 nm) are far superior for this type of imaging. Less tissue autofluorescence.

Standard Filter Set For Imaging Multiple Reporters

4 Standard Emission Filters

ICG 810-875nm

• QD800, AlexaFluor 750, DiR, IRDye800CW

Cy5.5 695-770nm

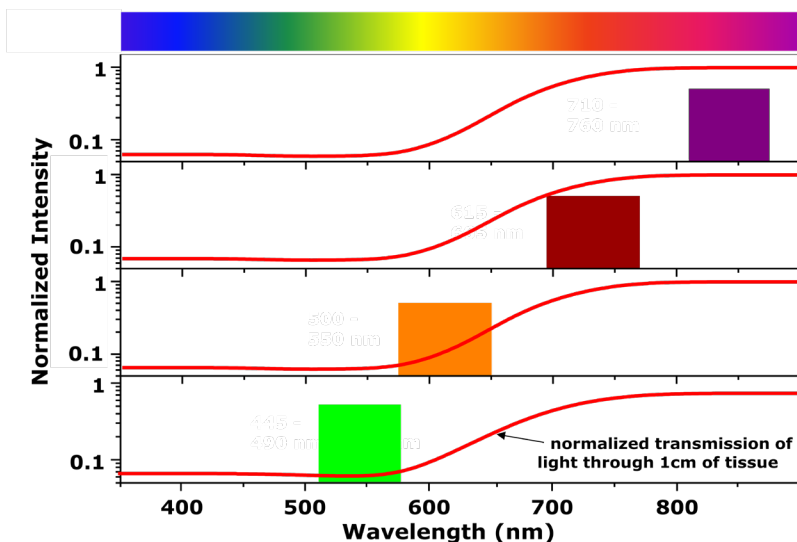
• AlexaFluor 680 and 700, Cy5.5

DsRed 575-650nm

• mCherry, mTomato, DsRed, QD605, TurboFP635

GFP 515-575nm

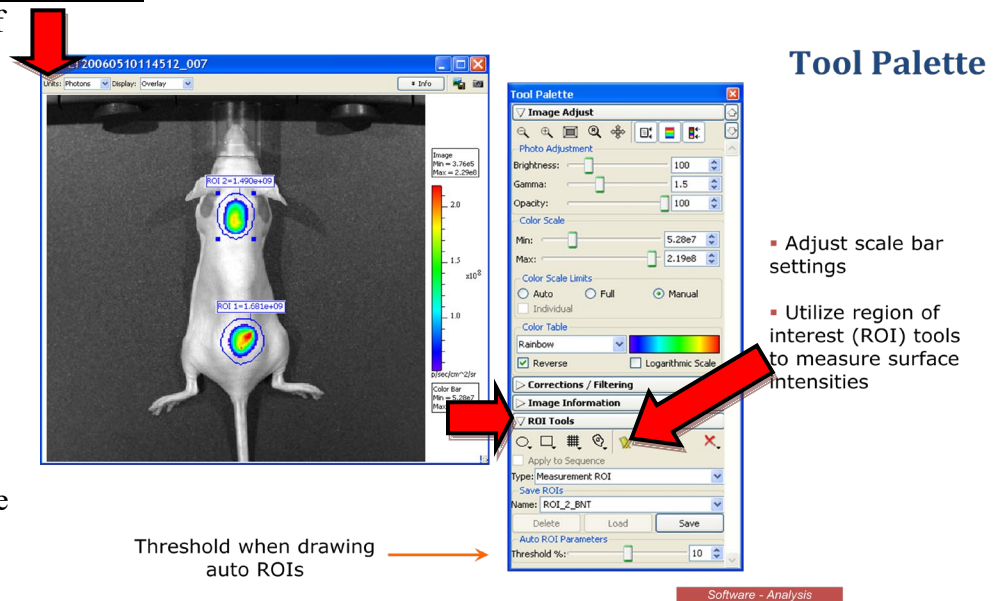
• GFP, EGFP, FITC



2. Acquire single image or set up a sequence of imaging using *Sequence Setup* as shown above.

Region Of Interest (ROI) Selection:

1. Change the units of the image to **Photons** for illuminance and **Efficiency** for fluorescence
2. In *Tool Palette*, ROI Tools draw an ROI around the signal, if there is more than one image in the sequence hit *Apply to Sequence* and the ROI will appear on every image in the sequence.



3. Measurement tool will display measurement of surface intensities within the ROI of each image.

ROI measurements display: **Total Flux** is the measurement of concern.

ROI Measurements

ROI Measurements

Image Number	ROI	Image Layer	Total Flux (p/s)	Avg Radiance	Stdev Radiance	Min Radiance	Max Radiance
TLT20060510114512_007	ROI 1	Overlay	1.681e+09	7.363e+07	5.798e+07	1.665e+07	2.295e+08
TLT20060510114512_007	ROI 2	Overlay	1.490e+09	7.497e+07	5.283e+07	1.490e+07	1.901e+08

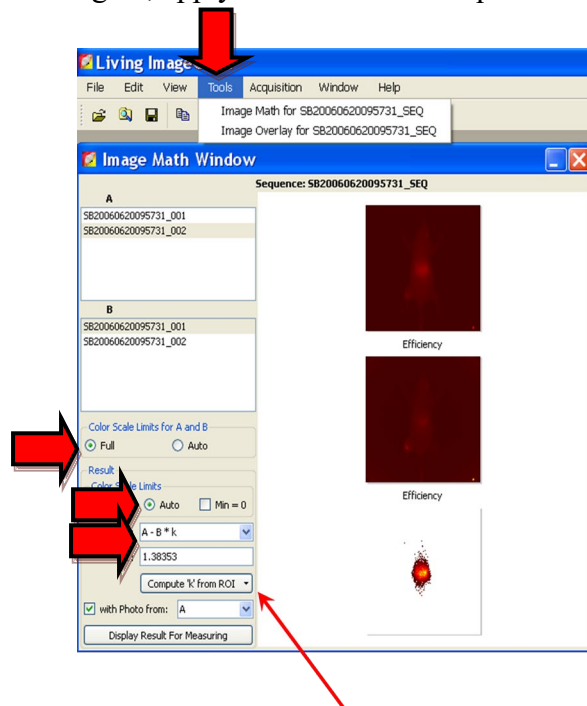
Customized Selections

Measurements Types: Photons Image Attributes: _none_ ROI Dimensions: _none_ Menu Copy Select All

Refresh Configure... Export... Close

Background reduction for fluorescence imaging:

1. Open image of background only, select ROI around signal, apply that ROI to the sequence of images.
2. Open image of signal plus background
3. Go to *Tools*, *Image Math for*
4. Color scale limits for A and B, check FULL
5. Result Color Scale Limits, check AUTO
Result $A-B*k$
6. Push Compute 'k' from ROI



Automatic K value computation
for image math