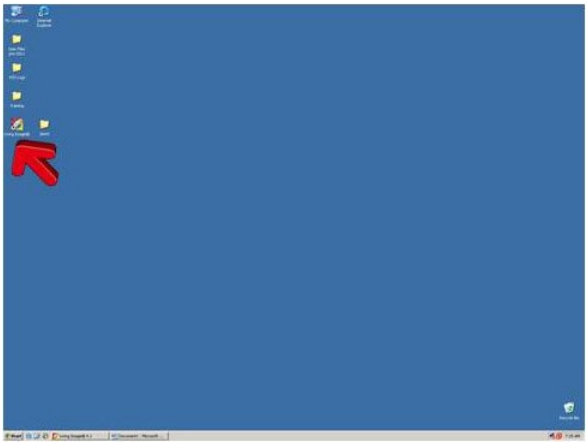
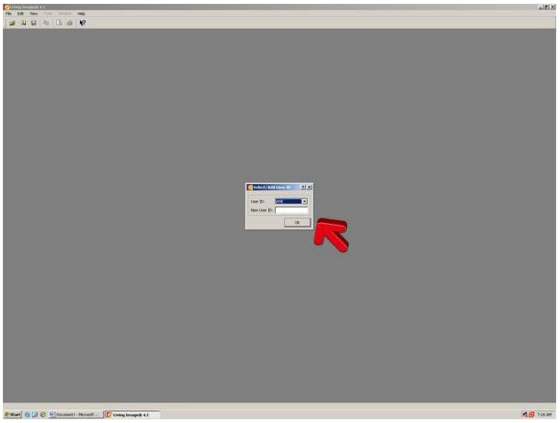
IVIS: Living Imaging System

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).

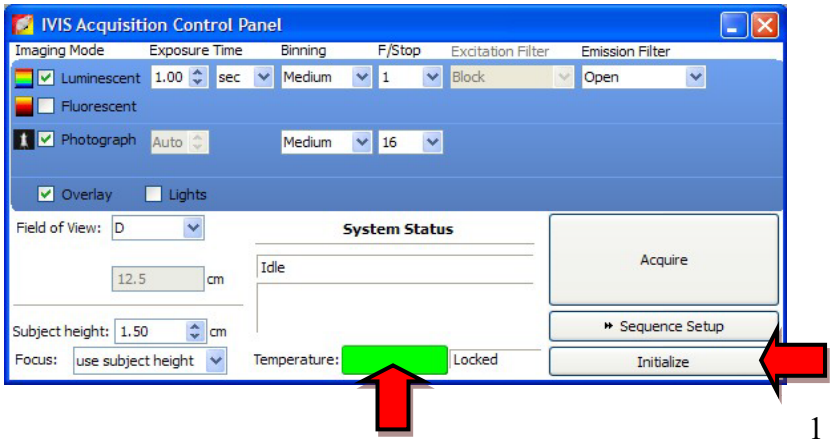


3. Log into the software (choose your initials or add new user by adding your initials to the box).

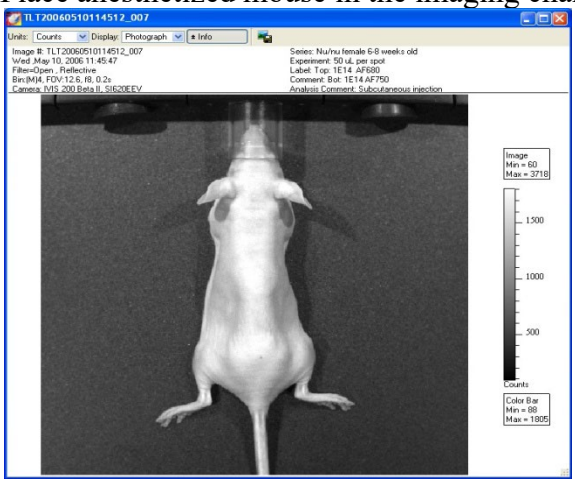


4. Initialize the machine. The window will automatically open when you log in. Click on *Initialize* and wait until the camera is cooled to -90C. The stage is pre-warmed and should be warm all the time.

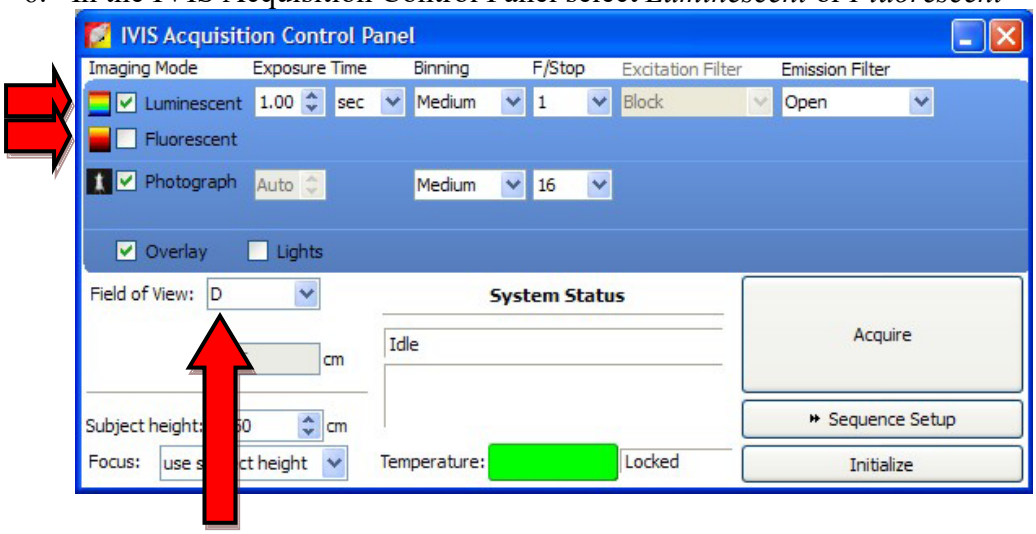
   When the system is ready the temperature indicator will turn green and there will be a green light on the bottom left corner of the imaging chamber.



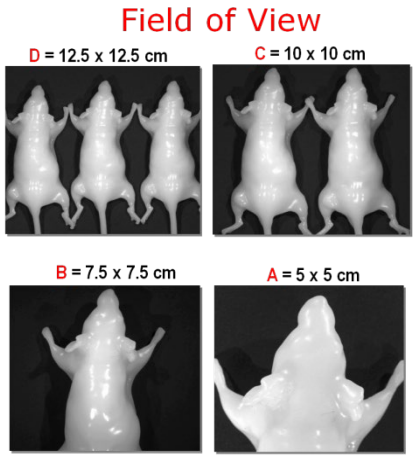
5. Place anesthetized mouse in the imaging chamber.



6. In the IVIS Acquisition Control Panel select *Luminescent* or *Fluorescent*



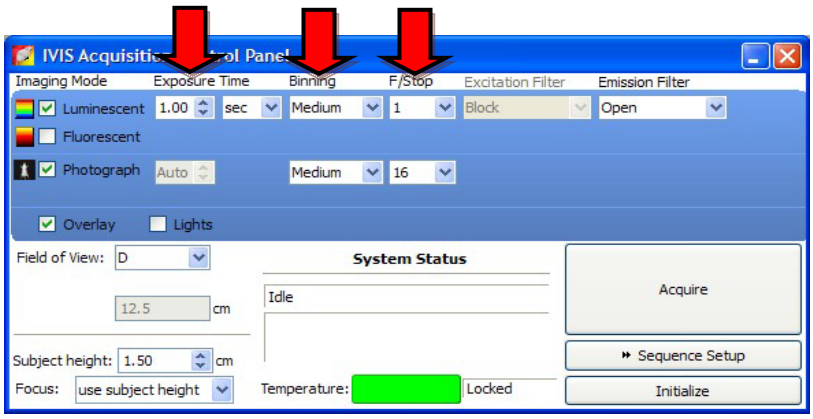
7. Chose field of view (D, C, B or A)



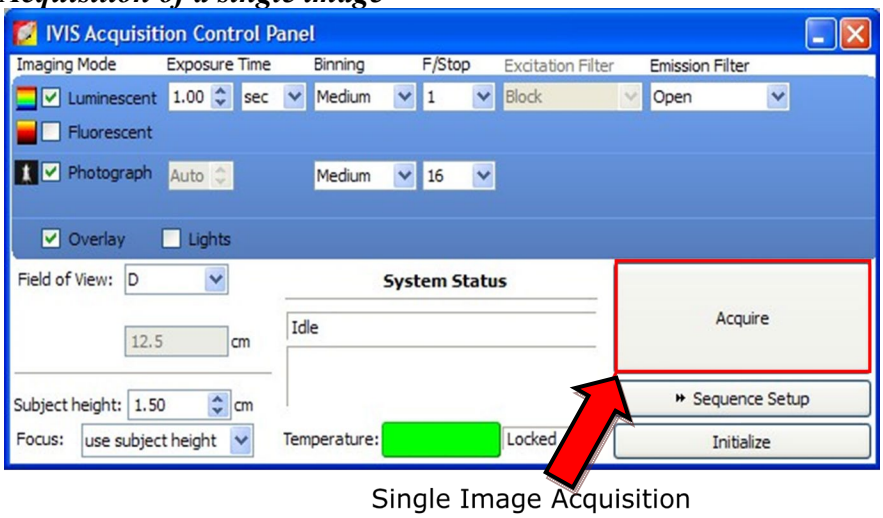
Setting Sensitivity – Luminescent Signal Level:

**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)

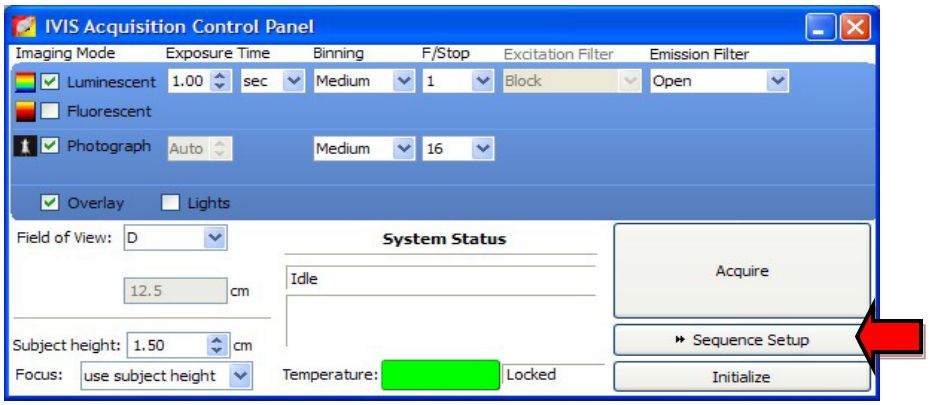


2. ***Acquisition of a single image***

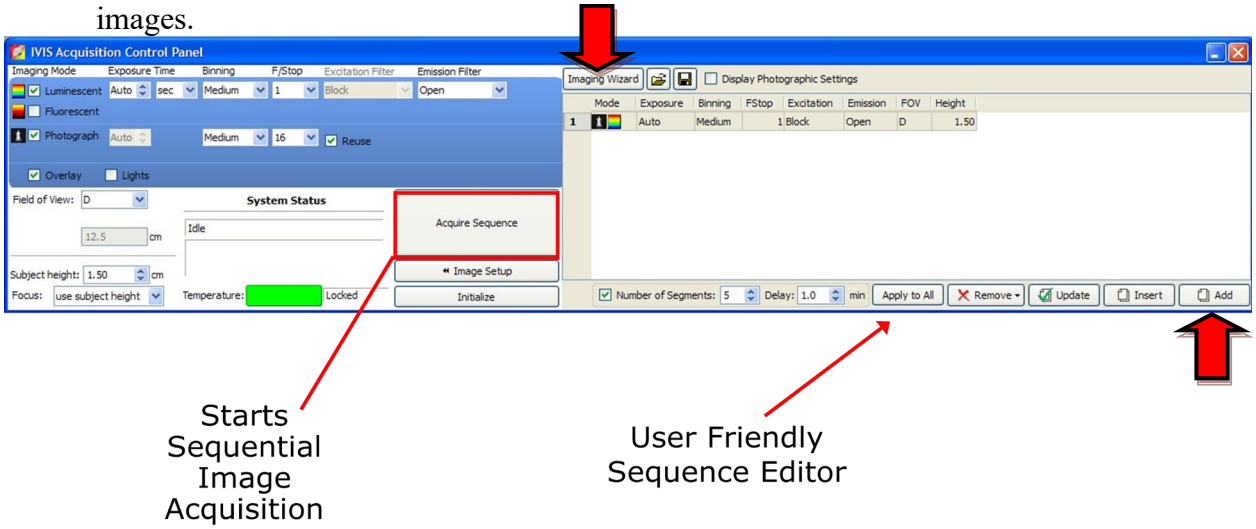


Single Image Acquisition

Or ***Acquisition of an image sequence***



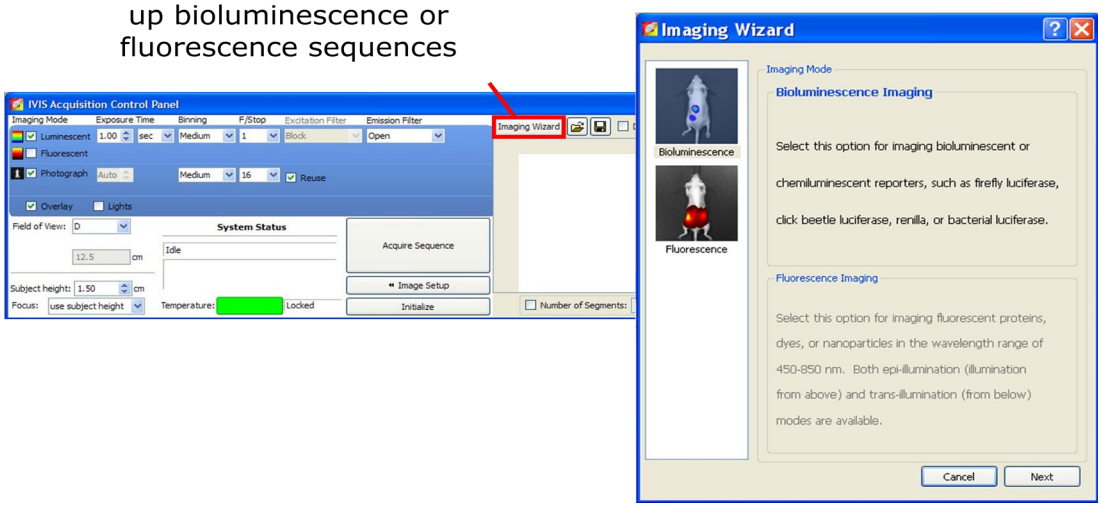
Enter values for exposure time, binning and f/stop and add to imaging sequence or select *Imaging Wizard* for assistance, set delay between images for a sequence with multiple images.



Starts Sequential Image Acquisition

User Friendly Sequence Editor

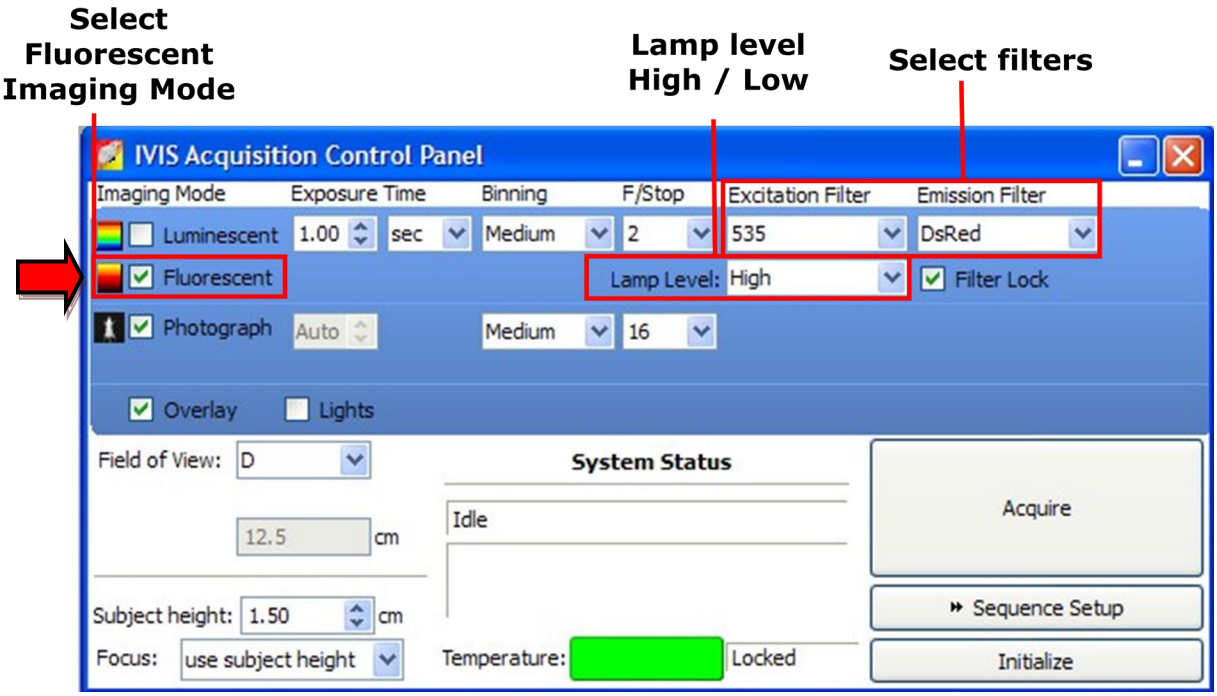
Select for assistance in setting up bioluminescence or fluorescence sequences



Fluorescent imaging:

Signal should be above 3000 counts to avoid background.

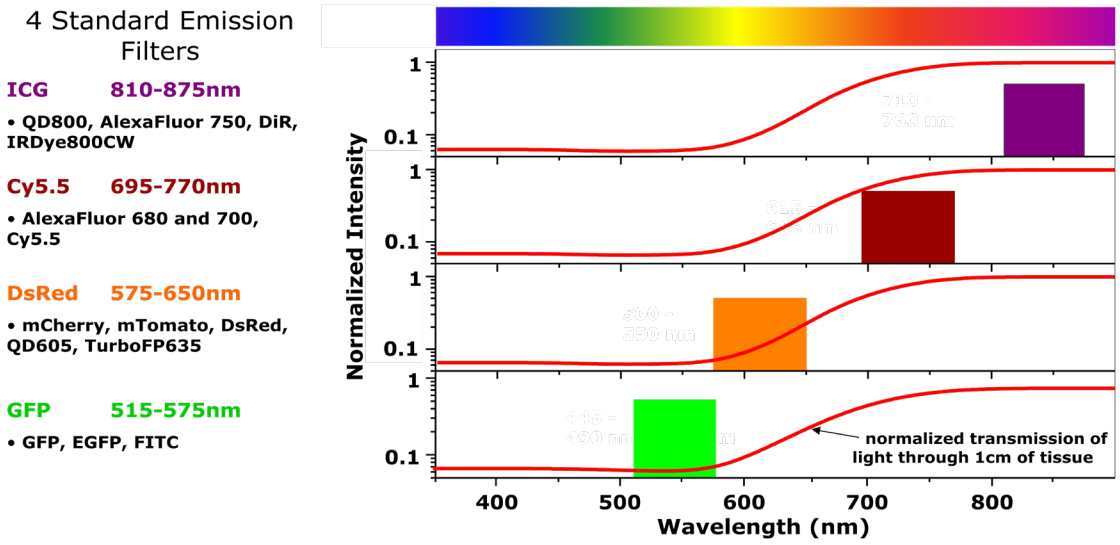
1. Fluorescent data acquisition, check the fluorescent box and select lamp level and appropriate filters.



Reference for excitation and emission filter selection:

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

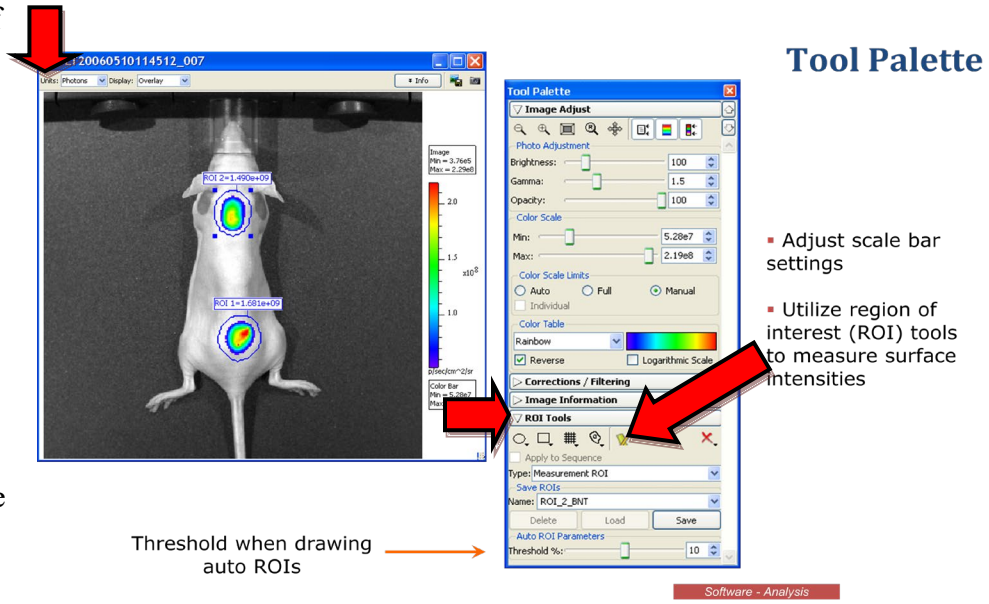
Standard Filter Set For Imaging Multiple Reporters



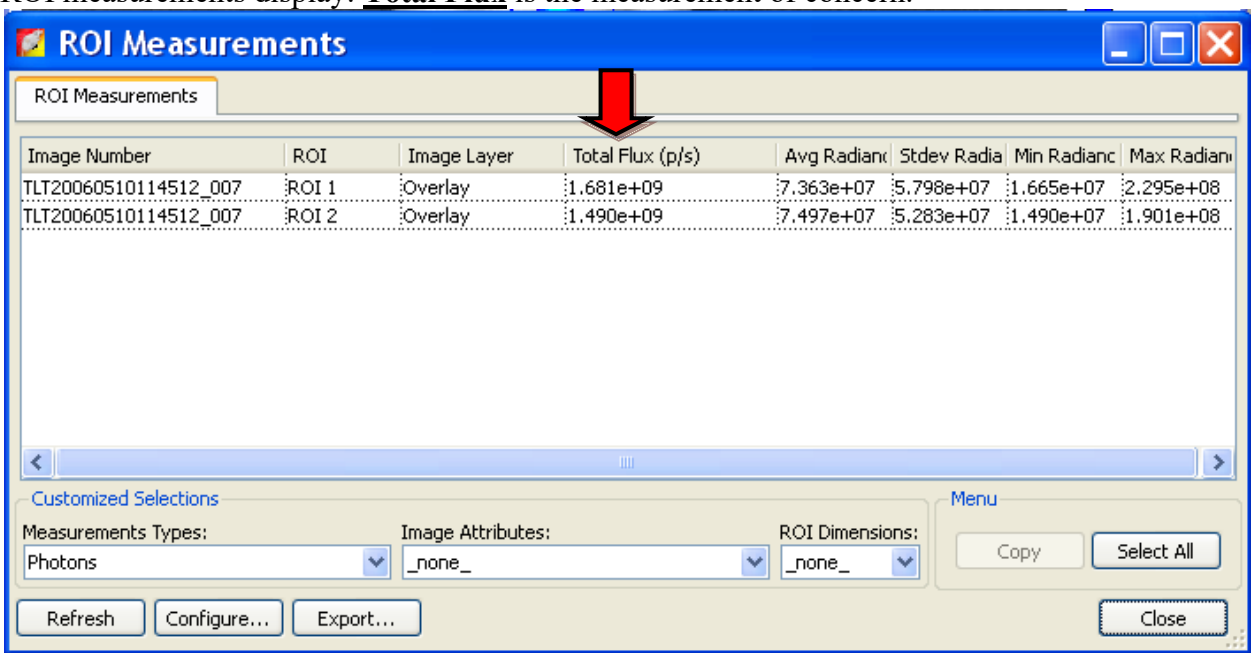
2. Acquire single image or set up a sequence of imaging using *Sequence Setup* as shown above.

Region Of Interest (ROI) Selection:

1. Change the units of the image to **Photons** for illuminescence 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.



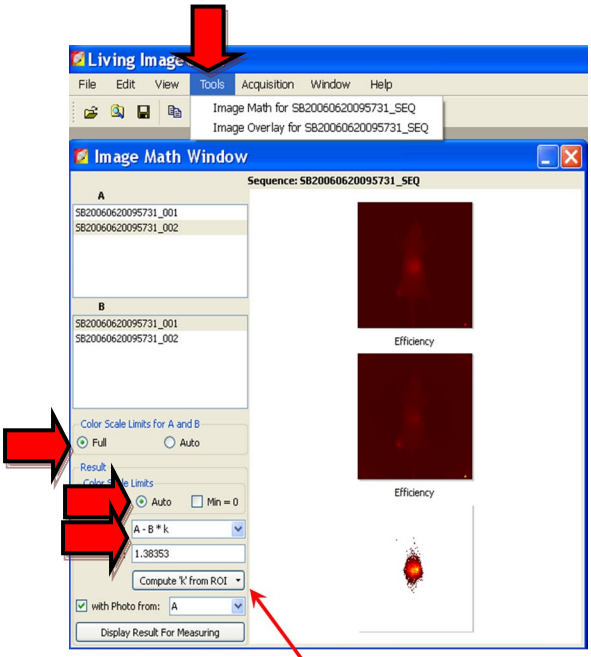
3. Measurement tool will display measurement of surface intensities within the ROI of each image.
   ROI measurements display: **Total Flux** is the measurement of concern.



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