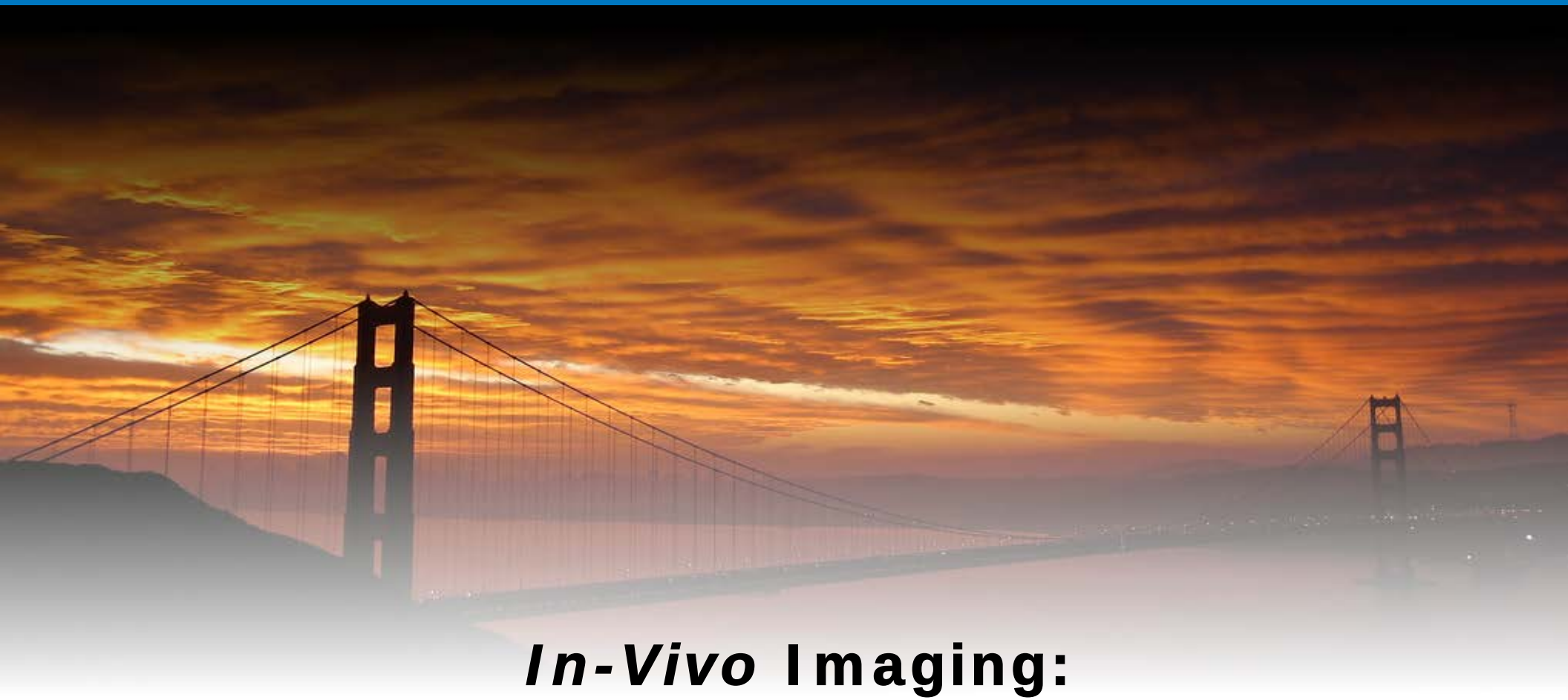


In-Vivo Imaging:
IVIS Spectrum

Brad Taylor, Ph.D.
Advanced Imaging Training Manager

Caliper LifeSciences

XENOGEN
now part of Caliper Life Sciences

What will be covered?

Introduction

- Principles of Optical ‘In Vivo’ Imaging
- Key IVIS® Hardware components
- Overview of Living Image® Software
- Fluorescence Options

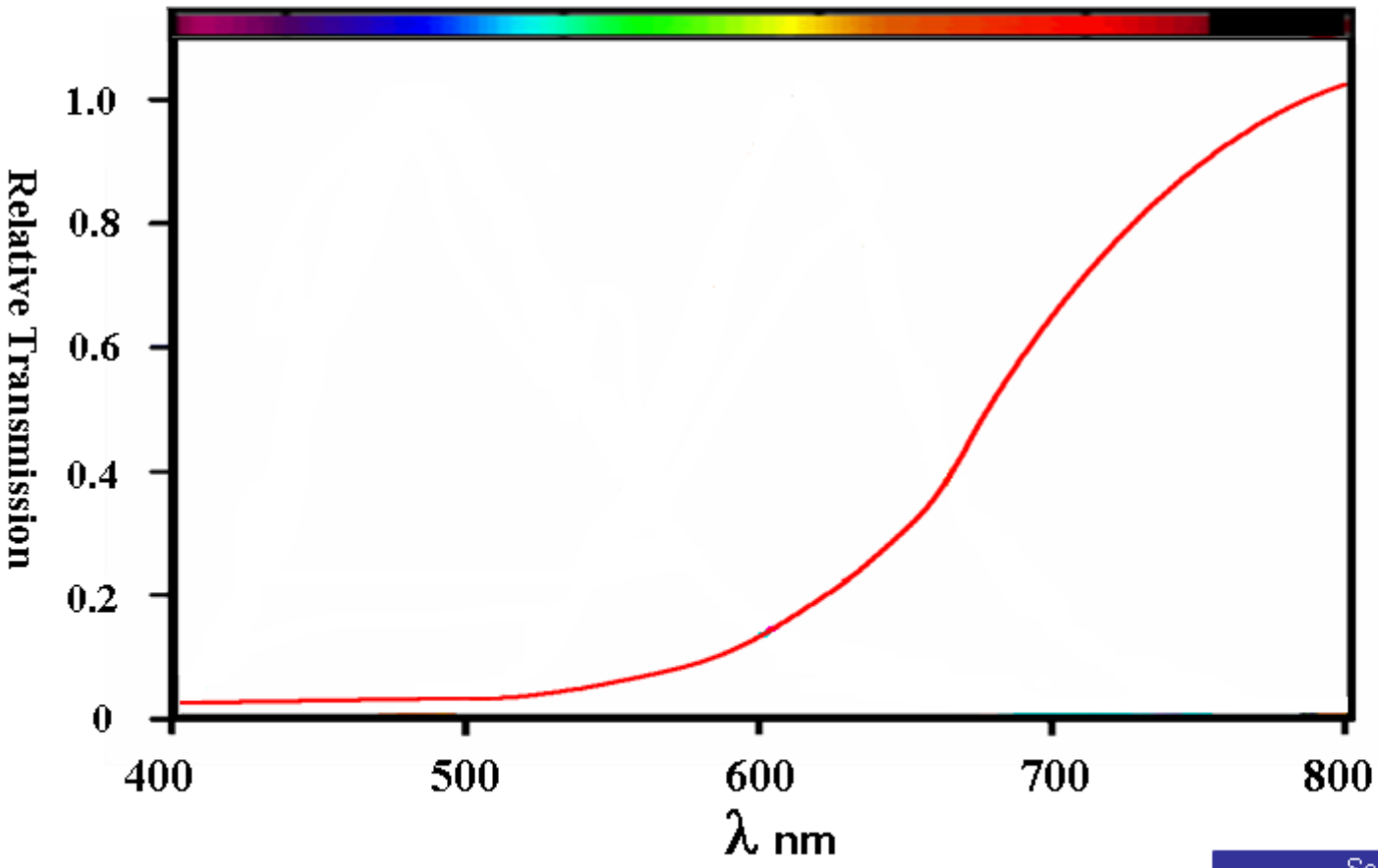
Training

- Hands on Training

Why Optical *In Vivo* Imaging?

- Powerful labeling technique - gene expression results in production of luciferase
  - Amount of light is proportional to number of active live cells
  - Typical applications range from oncology studies, infectious disease (tracer) to imaging transgenic animals (functional)
- Non-invasive – does not require subject to be euthanized
- Relatively simple instrumentation.

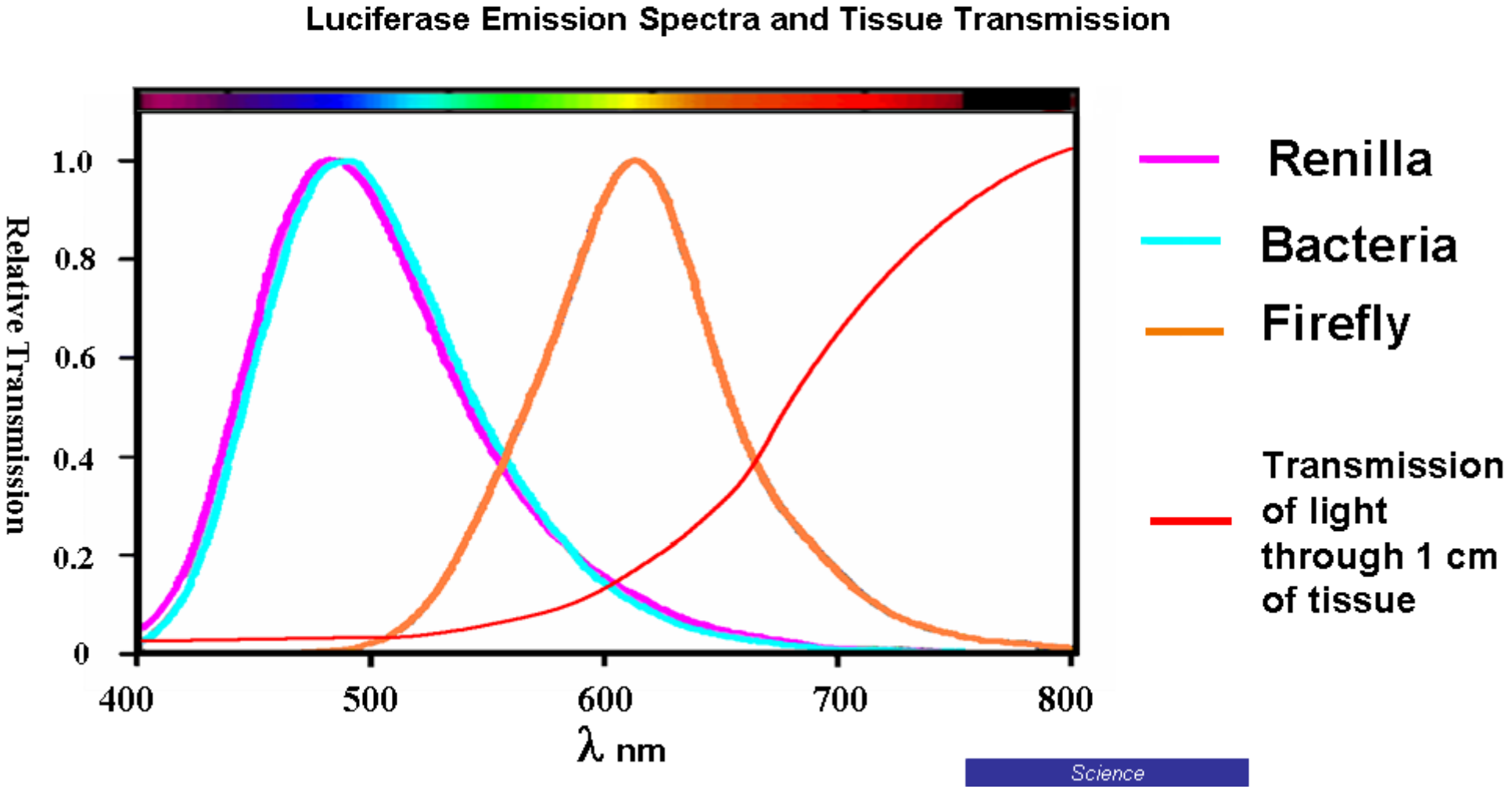
Tissue is not Transparent - Light Absorbance Depends on Wavelength



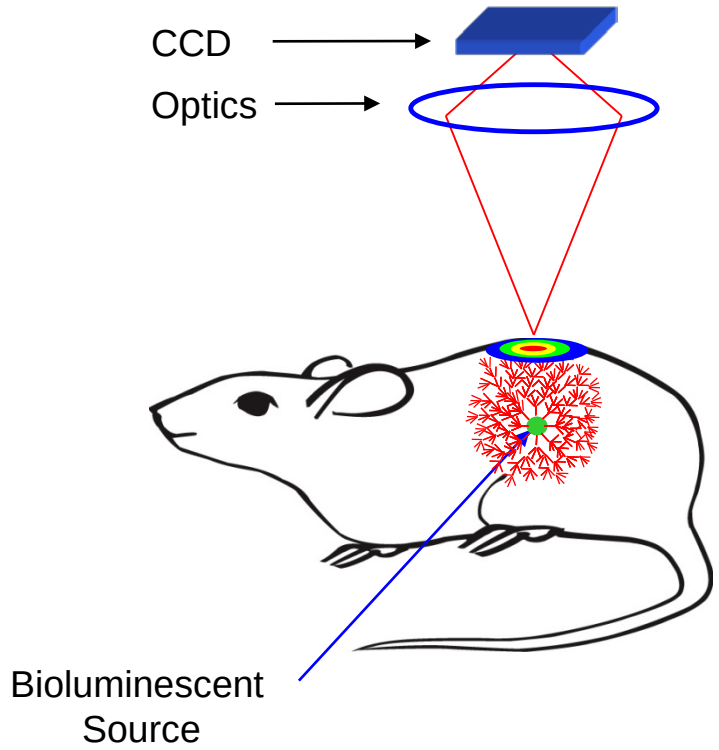
Transmission of light through 1 cm of tissue

Science

Tissue is not Transparent - Light Absorbance Depends on Wavelength

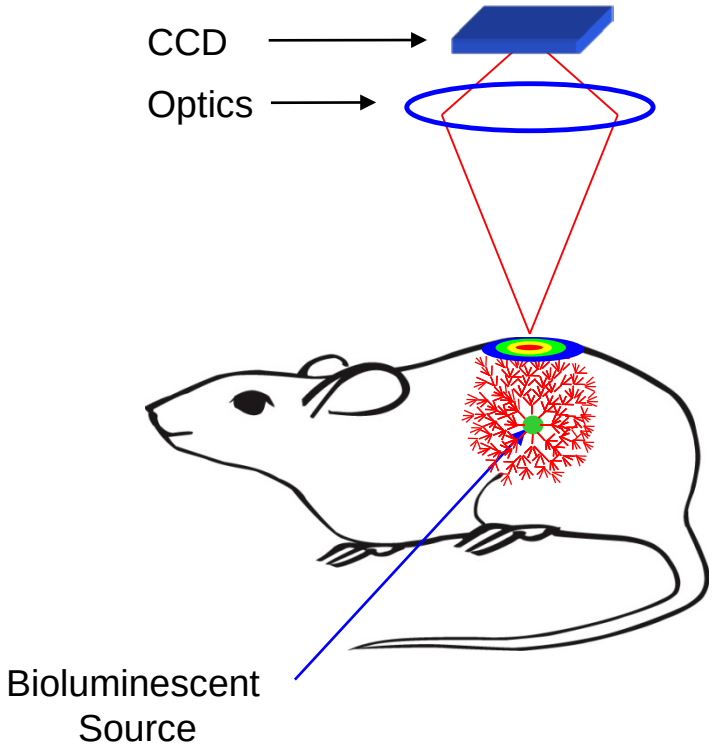


Photons Diffuse Through Tissue and the IVIS® Views this Signal on the Surface of the Subject



- Light traveling through tissue scatters many times creating a "fuzzy" image at the surface of the animal
- The IVIS® views the diffuse image on the surface of the subject

Photons Diffuse Through Tissue and the IVIS® Views this Signal on the Surface of the Subject



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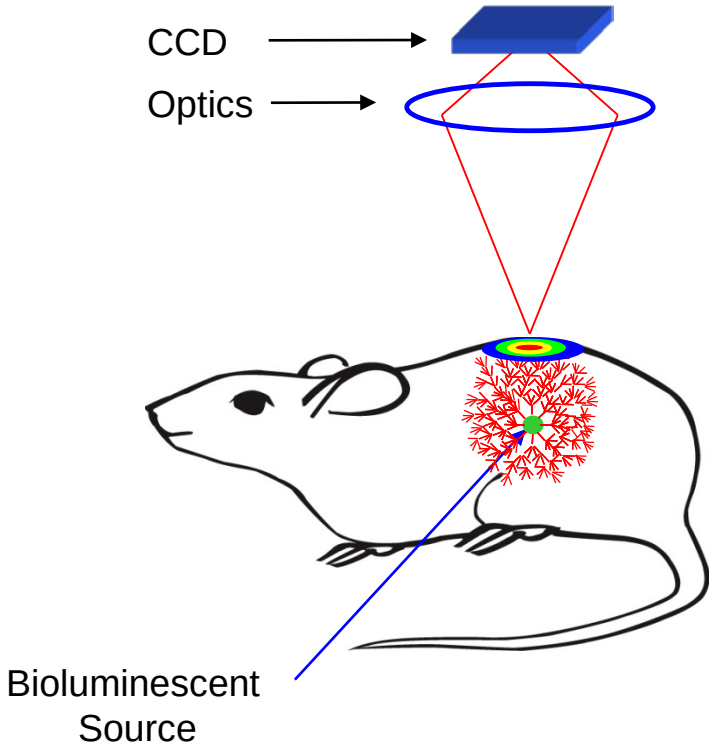
Science

Photons Diffuse Through Tissue and the IVIS® Views this Signal on the Surface of the Subject



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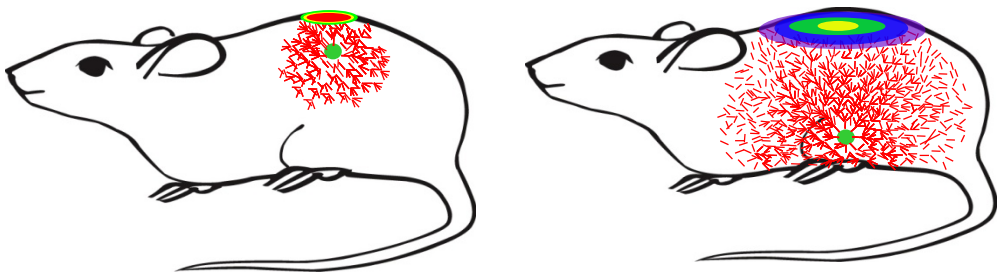


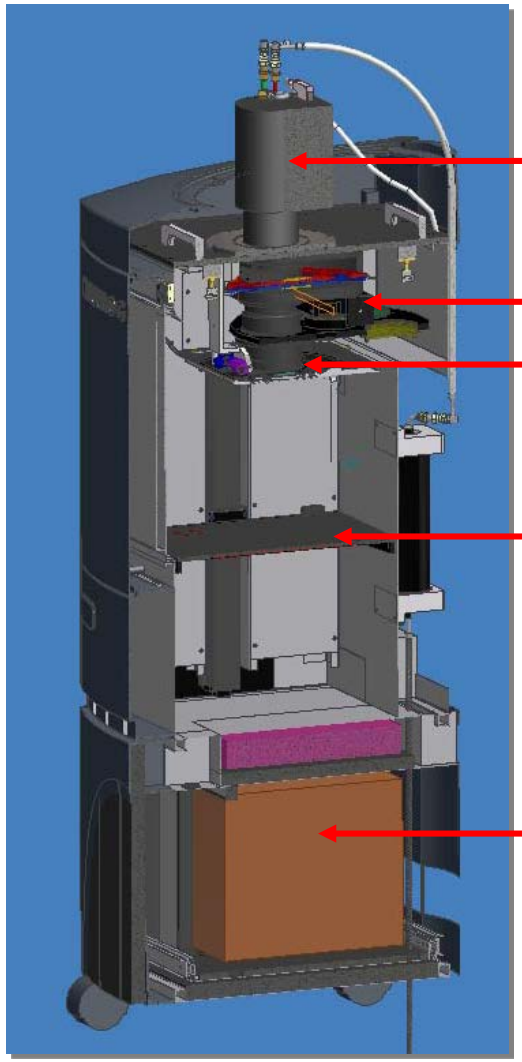
Science

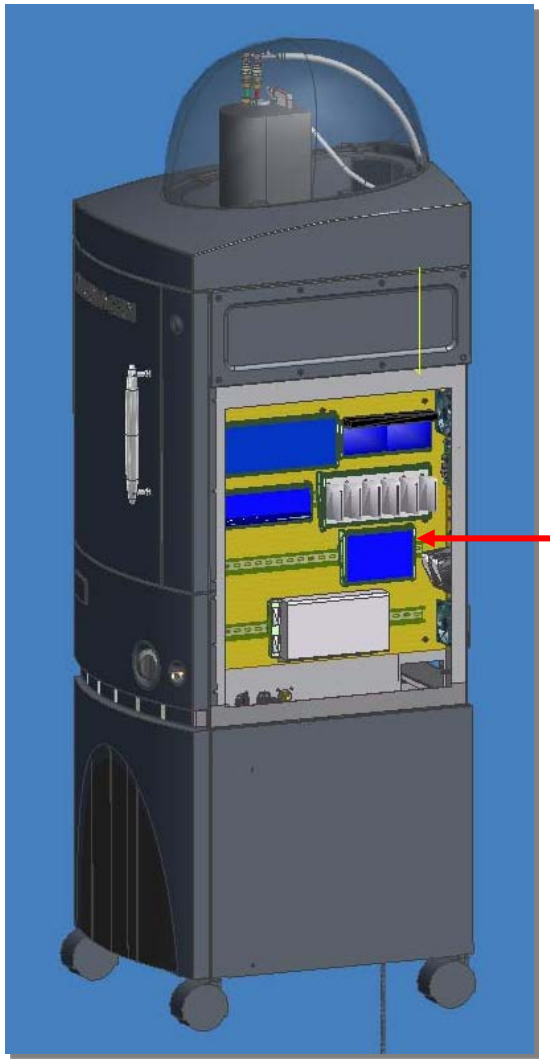
IVIS® Spectrum Imaging System - Hardware

- Customized for *in vivo* imaging
- Highly sensitive camera with a large dynamic range

Hardware

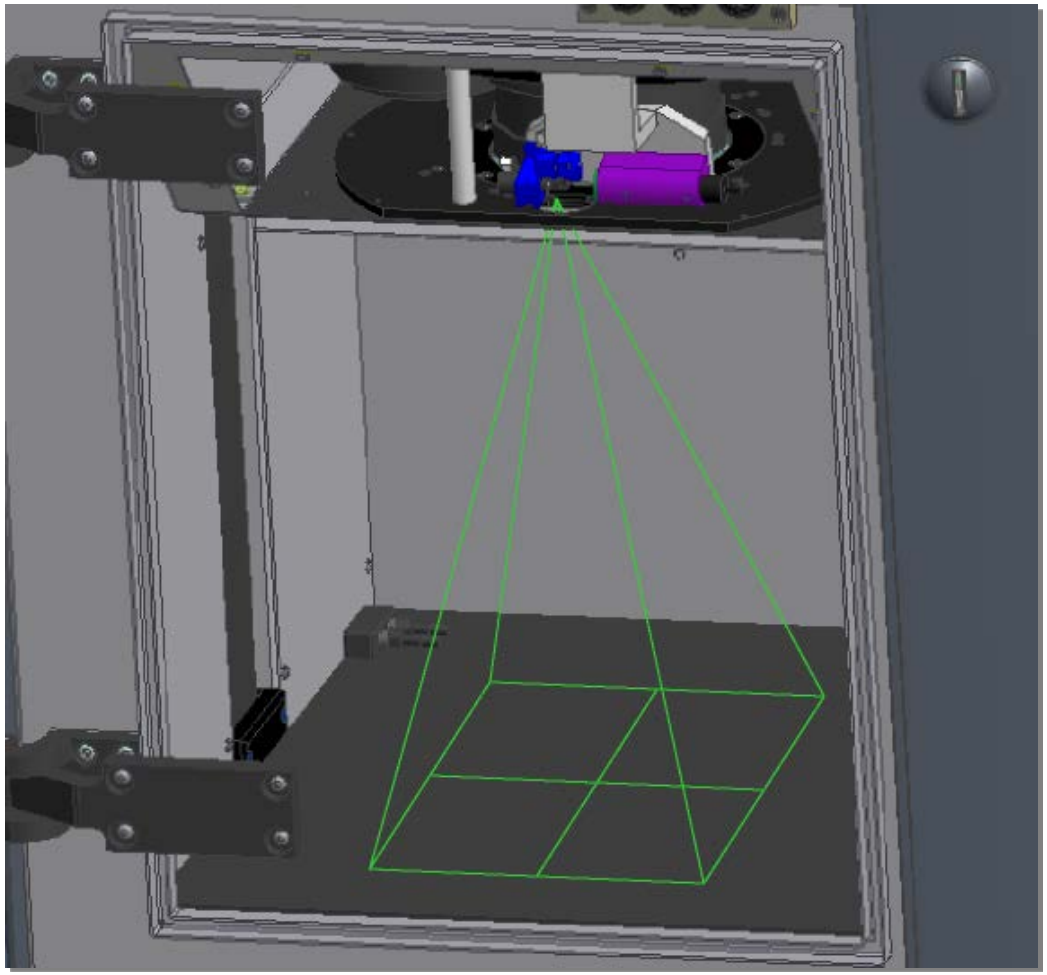
IVIS® Spectrum Imaging System – Hardware



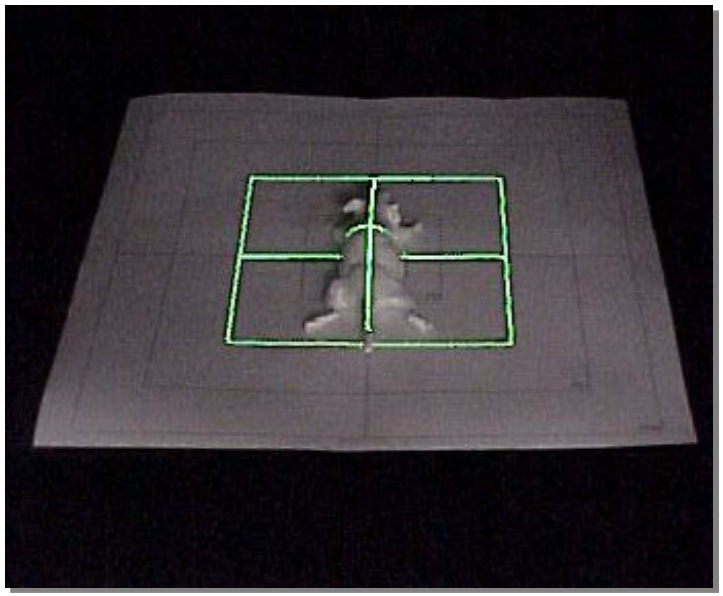


Hardware

Alignment Light Projector



- Allows rapid and reproducible positioning of subjects.
- Size changes with Field of View setting

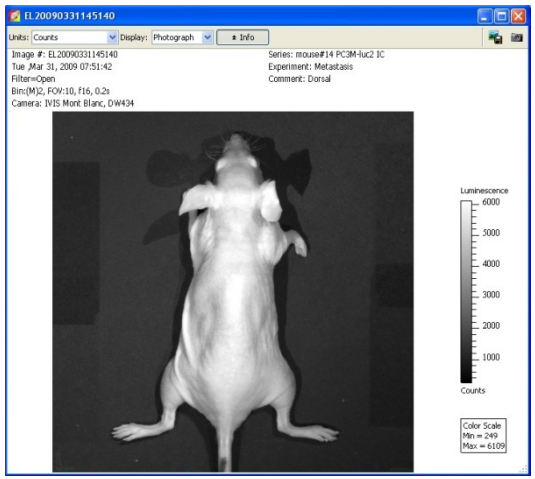


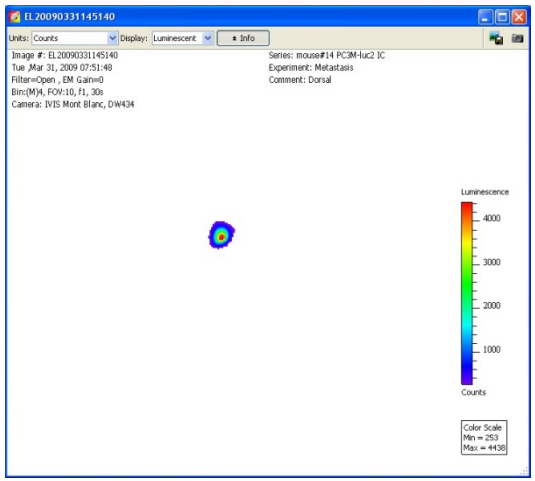
Hardware

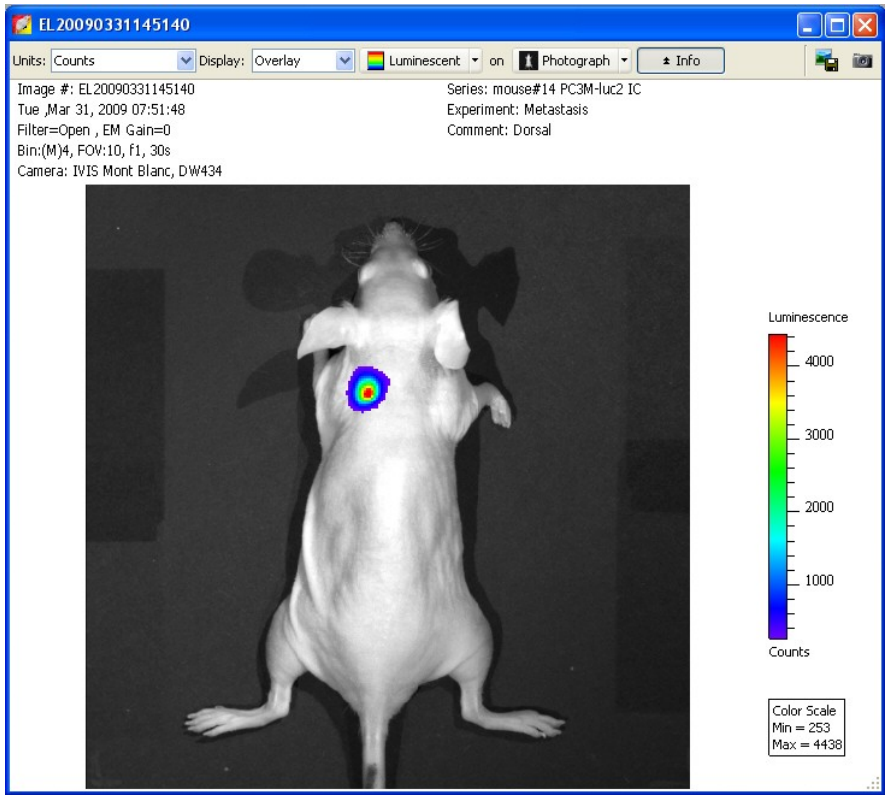
Living Image® Software

- Controls all settings in the IVIS® system (fully computer controlled)
- Provides advanced cataloging and browsing tools
- Provides analysis tools for quantification
- Instrument settings are analogous to photography
- Images are acquired in a two step process

Standard Images are Composed of Two Images Photographic + Luminescent = Overlay



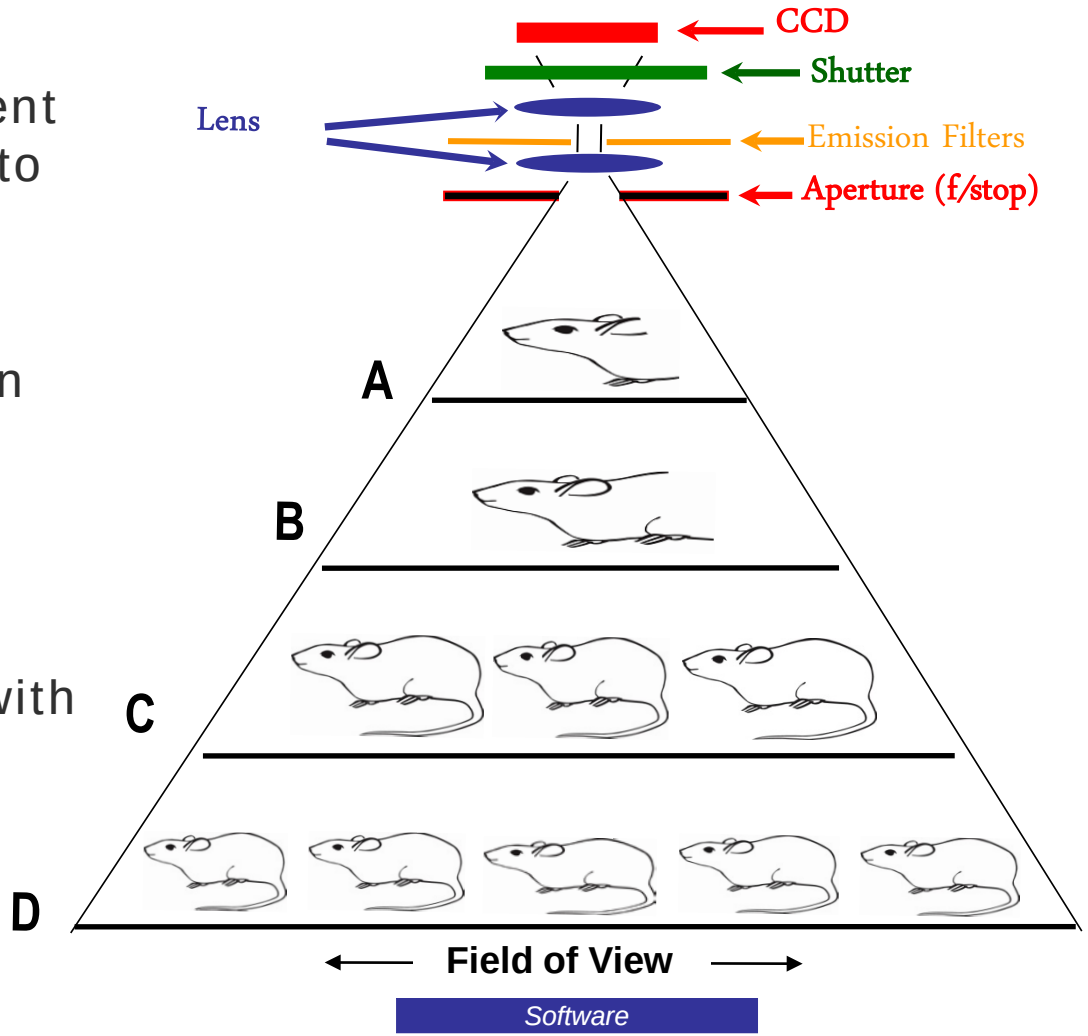




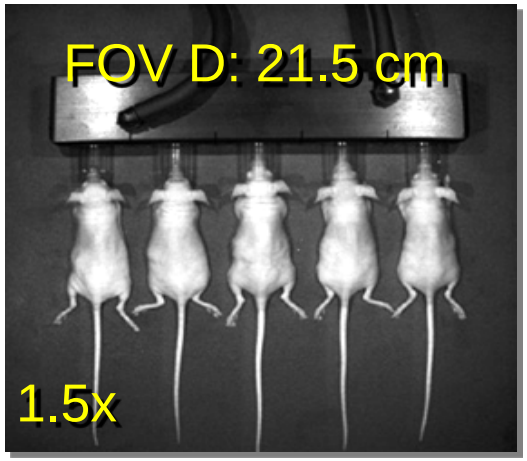
Software – Acquisition

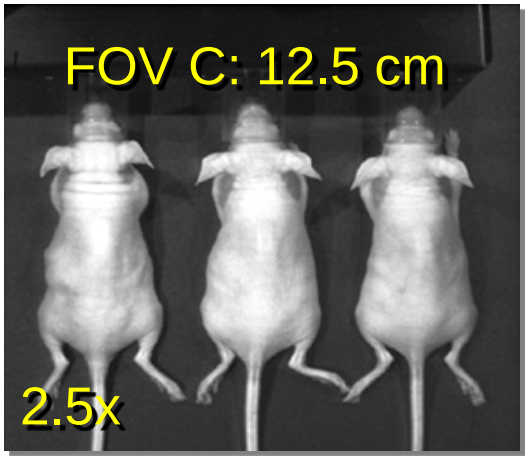
Camera and Lens Settings are Analogous to Those Used in Standard Photography

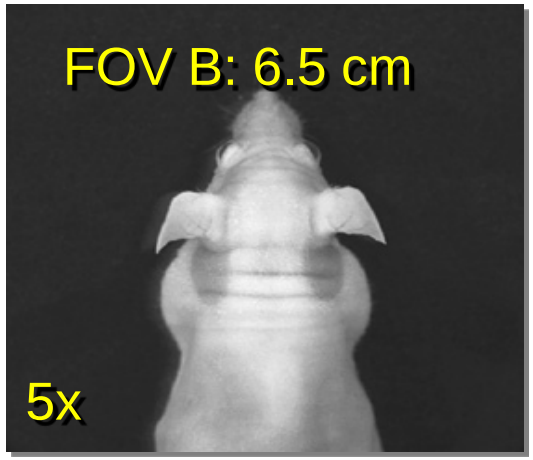
- Field of View (FOV) is dependent on the distance from the lens to the sample
- Light collected is proportional to how long the shutter is open (exposure time)
- Aperture (f/stop) controls the amount of light collected
- Digital pixel binning possible with CCD - for further increase in sensitivity

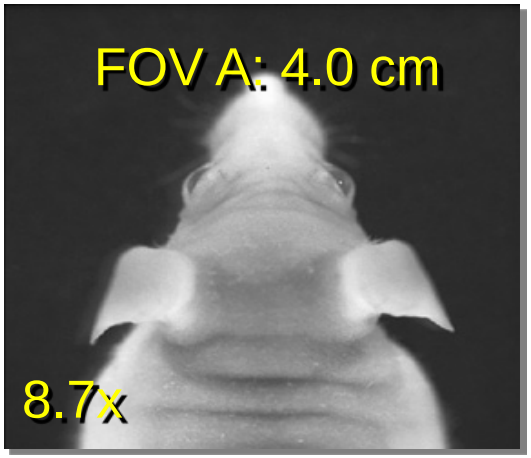


Field-of-View (typical)









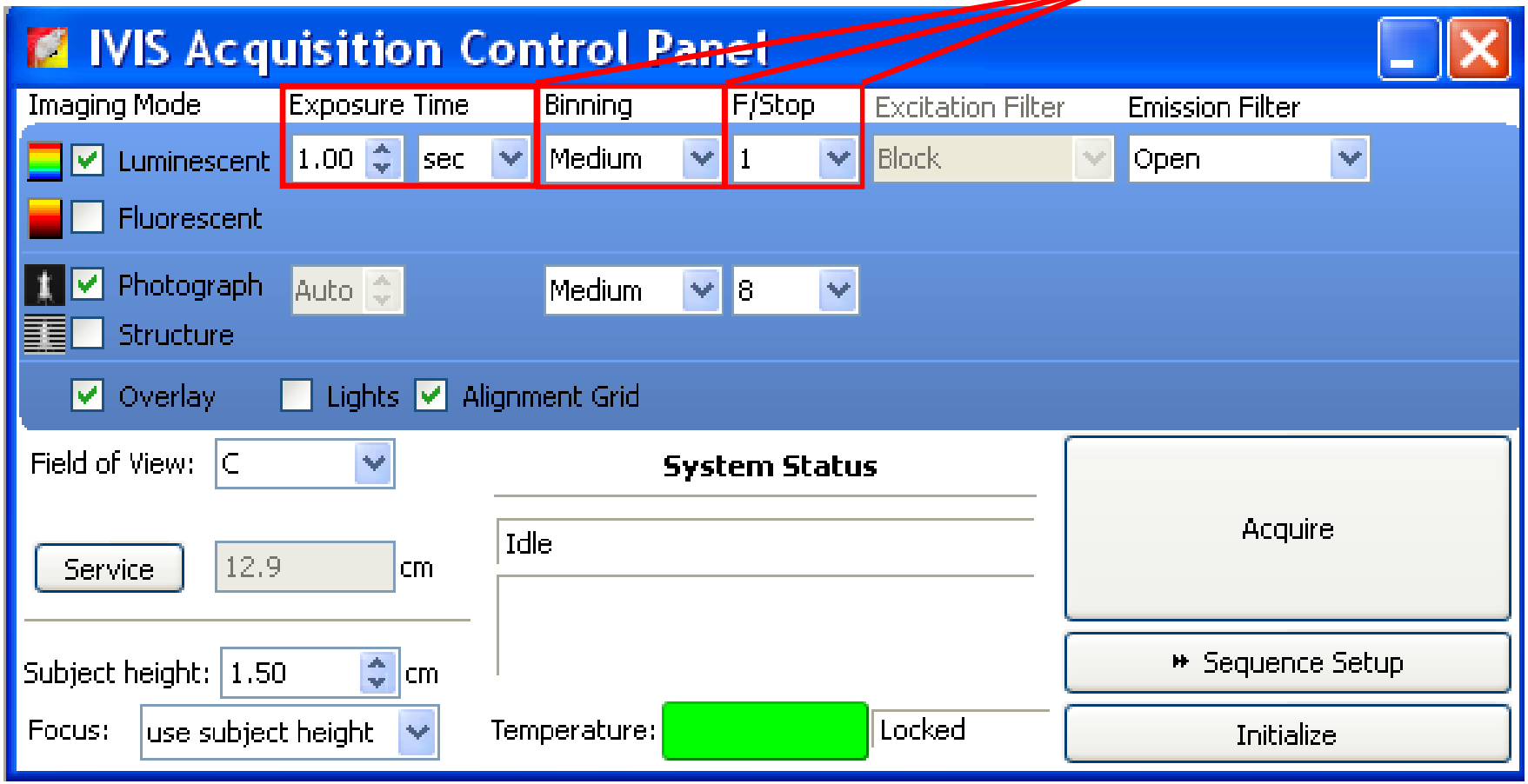
Software

Setting Sensitivity – Luminescent Signal Level

- The IVIS® CCD camera has a raw signal range of 0 to 65535 Analog to Digital Counts (2^{16}).
- Adjust camera settings to obtain a signal level of 600 to 60,000 counts.
- Settings that control signal level are:
  - Exposure time
  - Binning (CCD Resolution)
  - f/stop (Aperture)
- Instrument is calibrated to automatically compensate for changes in sensitivity settings

Living Image Control Panel

Controls Sensitivity



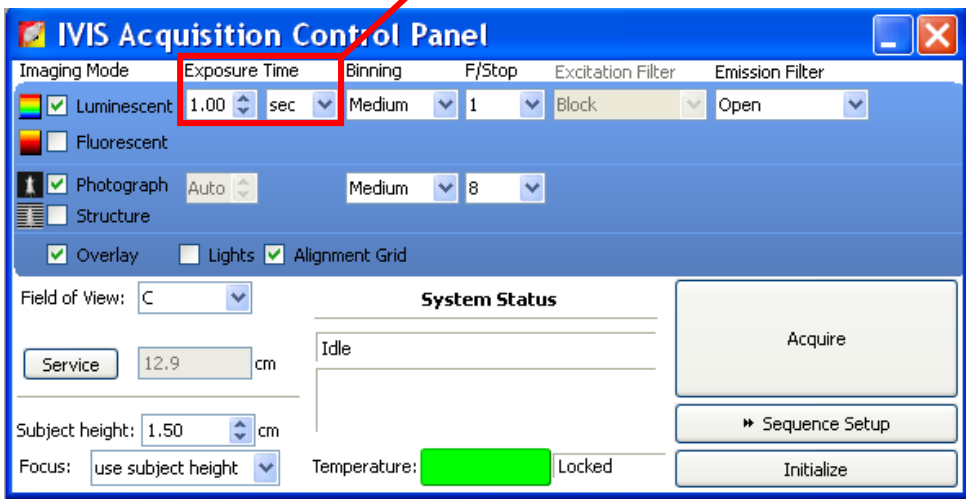
Exposure Time

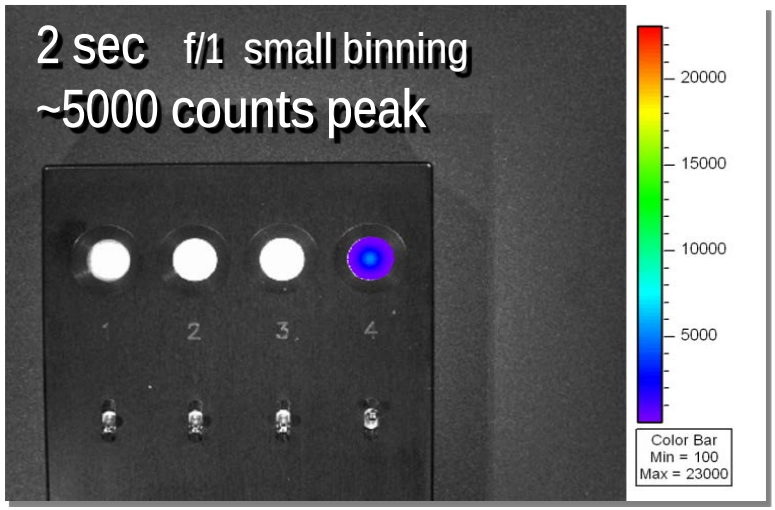
- Signal level is directly proportional to exposure time
- Shorter exposure time improves throughput

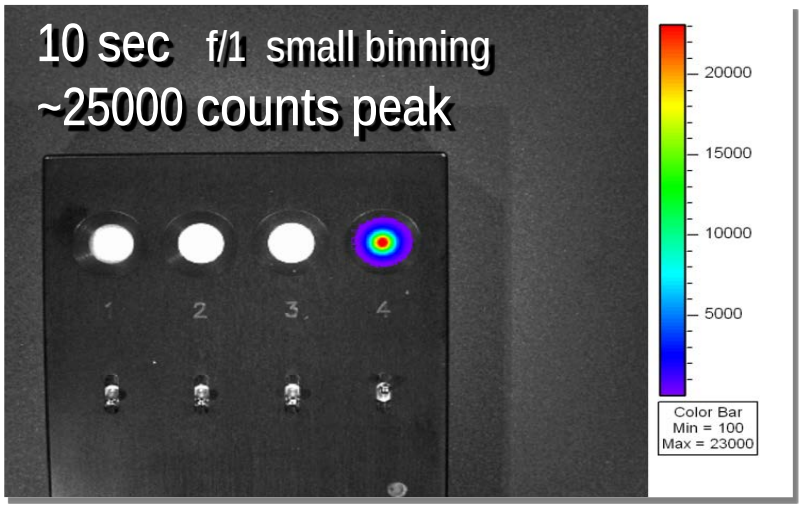
{Recommended min exposure time > 0.5 secs}

- Longer exposure time increases signal

{Recommended max exposure time < 5 mins}



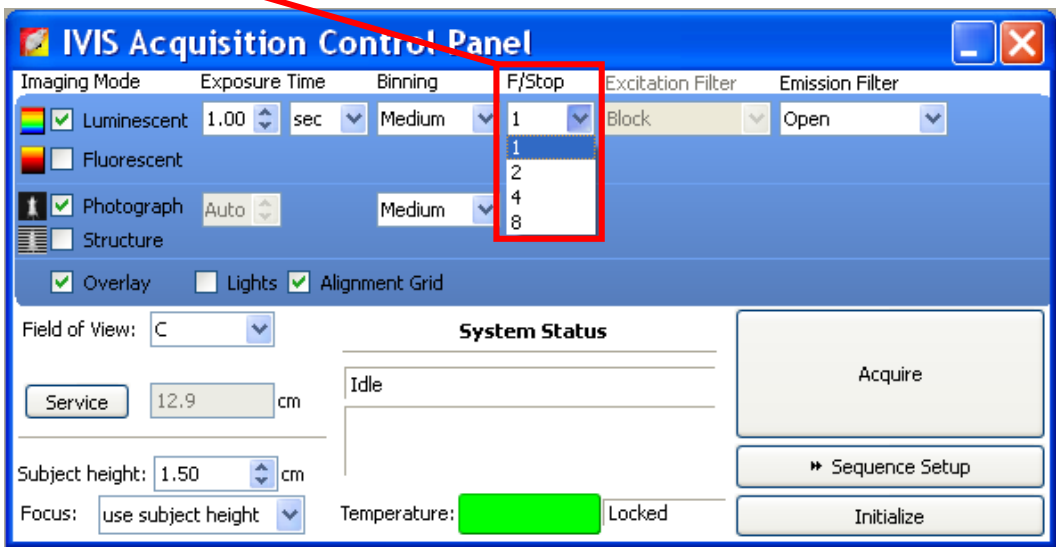




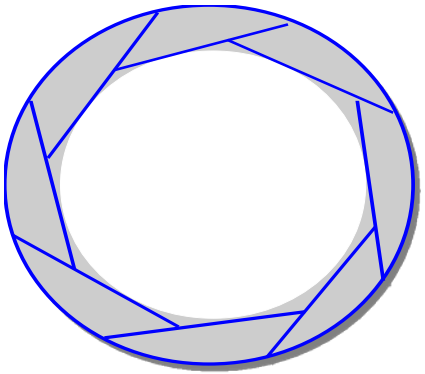
Software – Acquisition

f/stop (lens aperture)

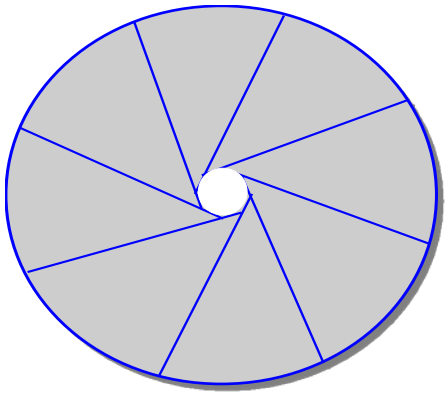
- f/stop controls the amount of light received by the CCD
- f/1 is wide open, maximum light collection - default for luminescent
- f/8 is smallest aperture, best resolution - default for photo



f/1



f/8

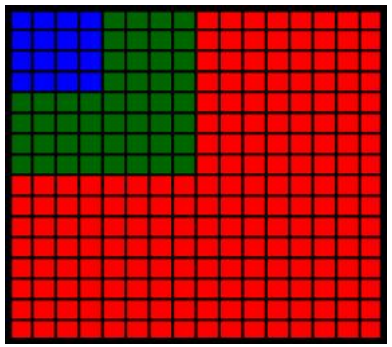


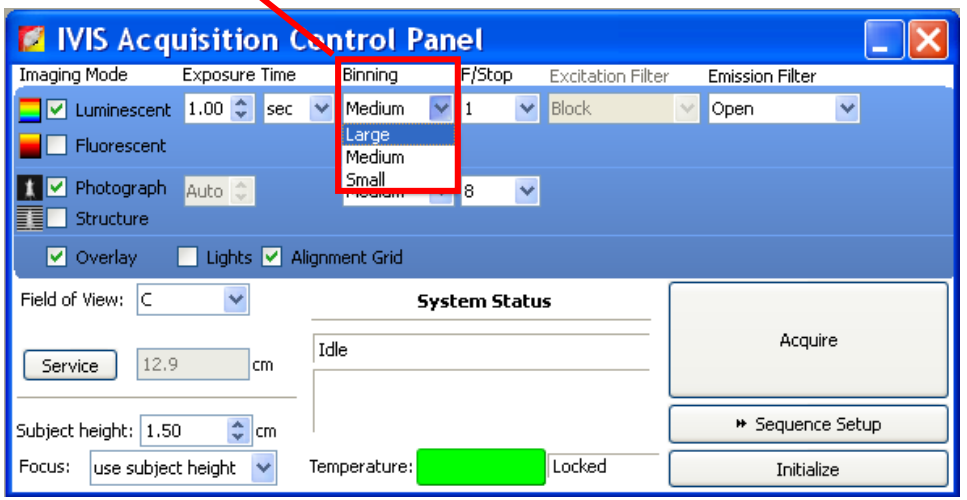
Software – Acquisition

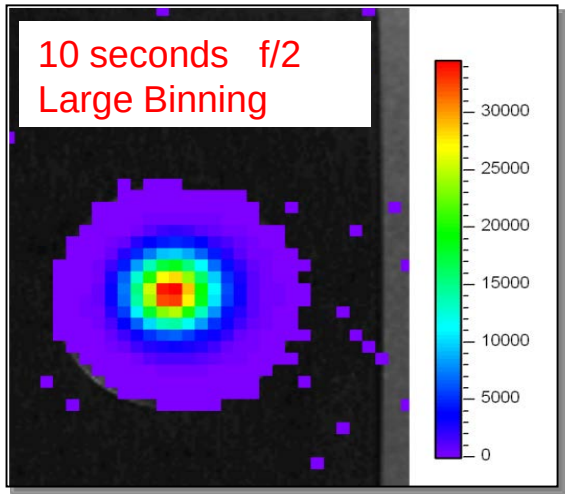
Pixel Binning (CCD Resolution)

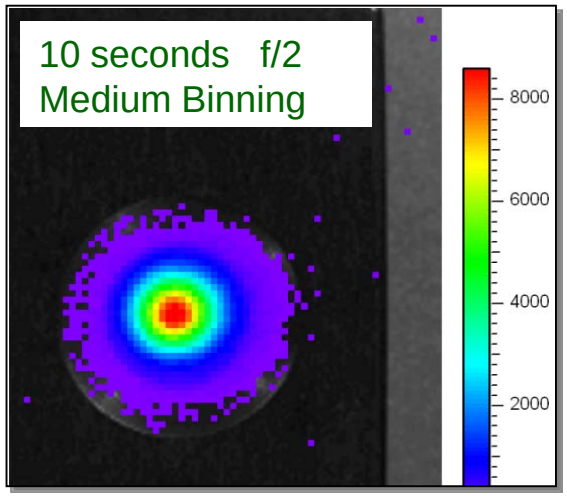
Binning refers to the grouping of pixels into a larger super-pixel

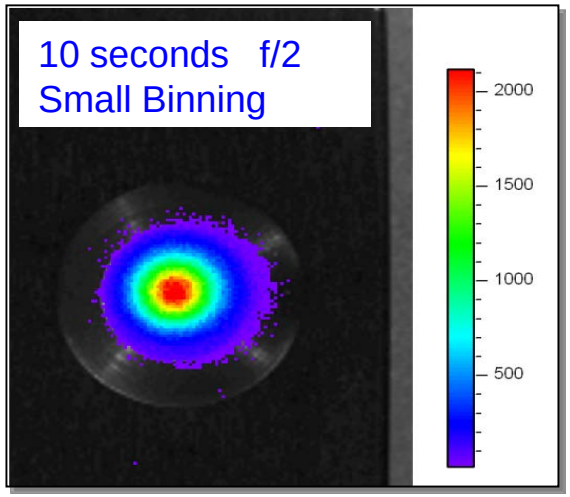
- **Large Binning (16) Higher Sensitivity / Lower Resolution**
- **Medium Binning (8)**
- **Small Binning (4) Higher Resolution / Lower Sensitivity**







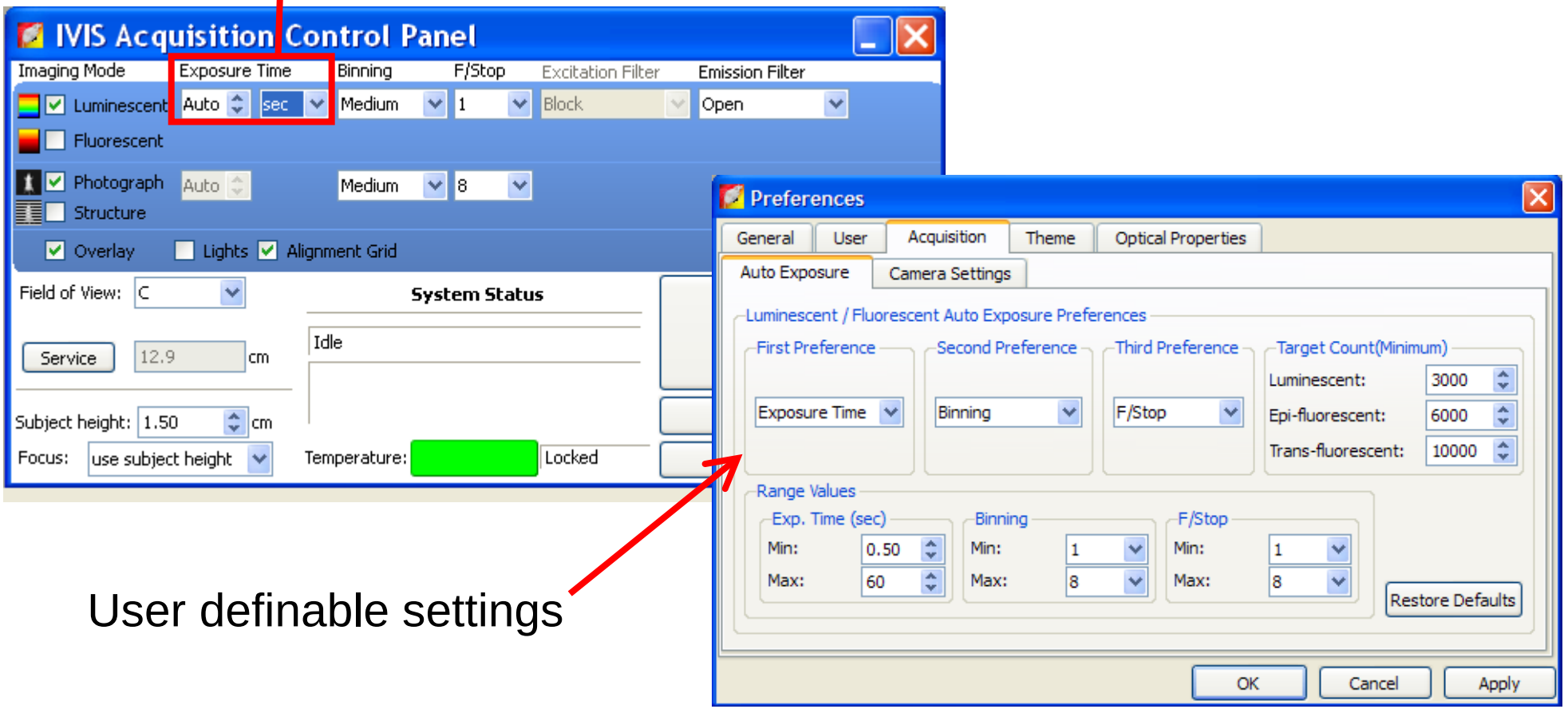




Software – Acquisition

Auto-exposure

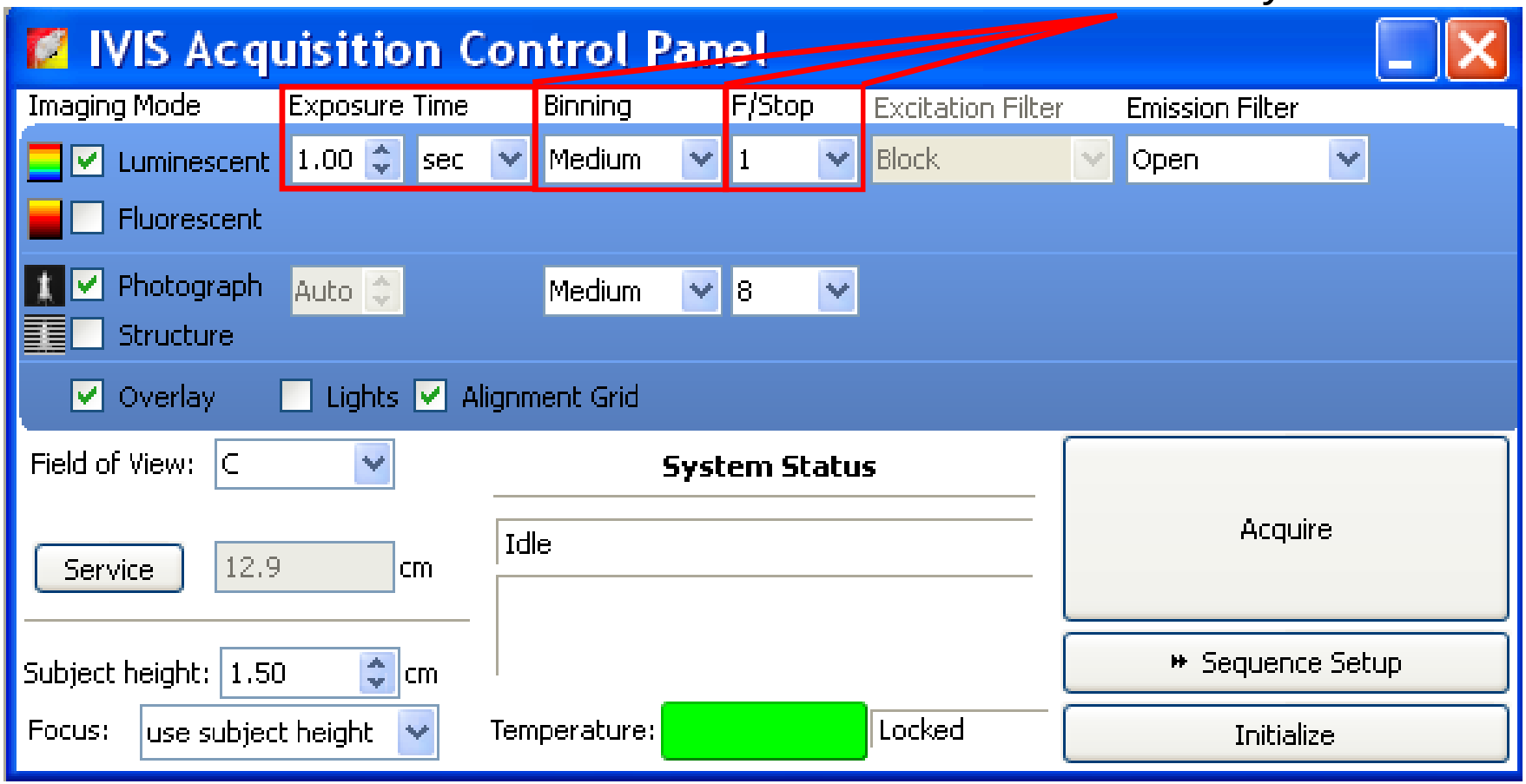
Auto-exposure feature available for bioluminescence and fluorescence



User definable settings

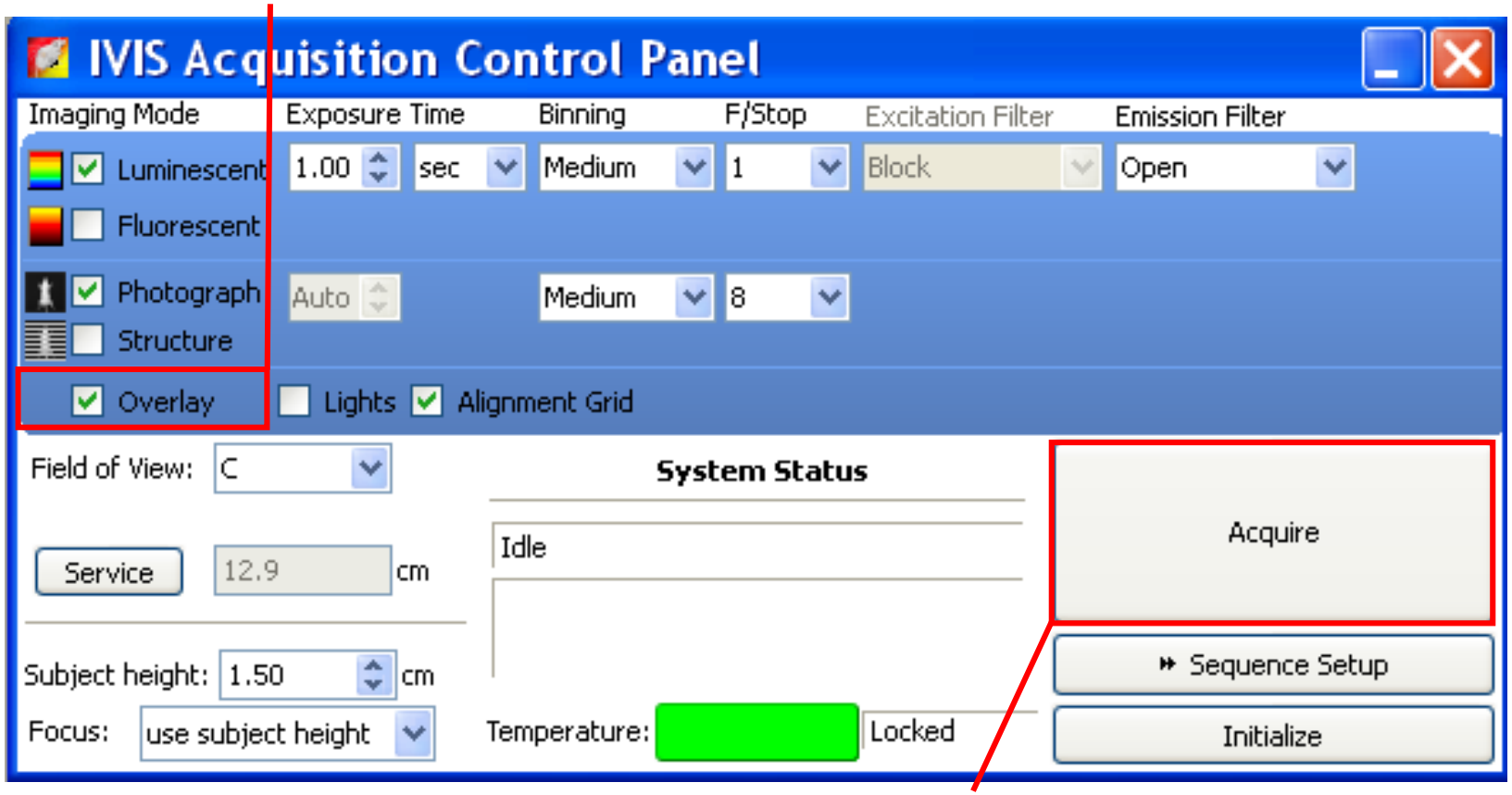
Summary of Basic Camera Settings

Controls Sensitivity



Acquisition – Single Image

Overlay will automatically take Photo + Luminescent

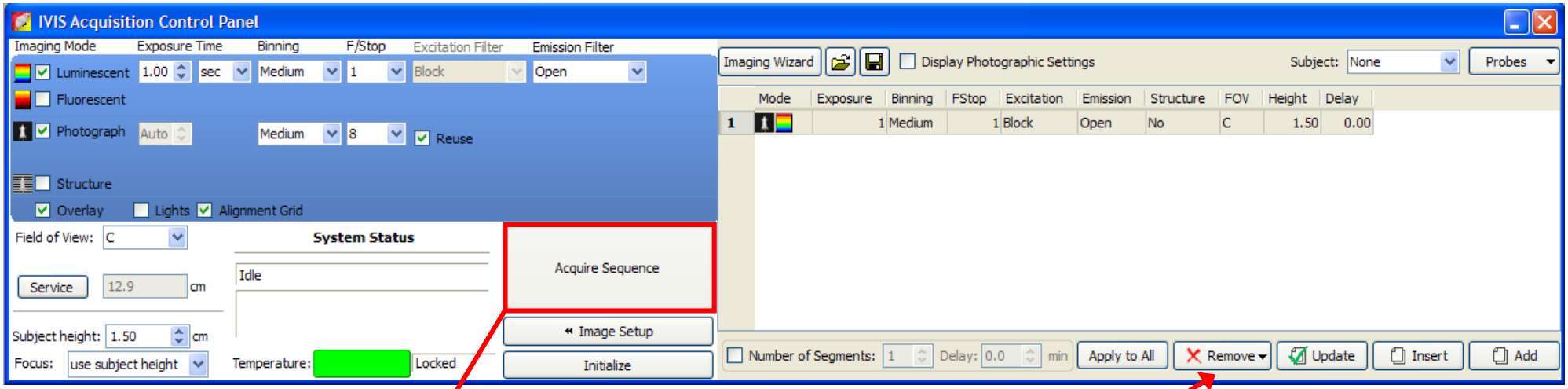


Single Image Acquisition

Acquisition – Sequential mode

Allows automatic acquisition of a series
of images separated by fixed time points.

(useful option for kinetic studies and DLIT 3D reconstruction)



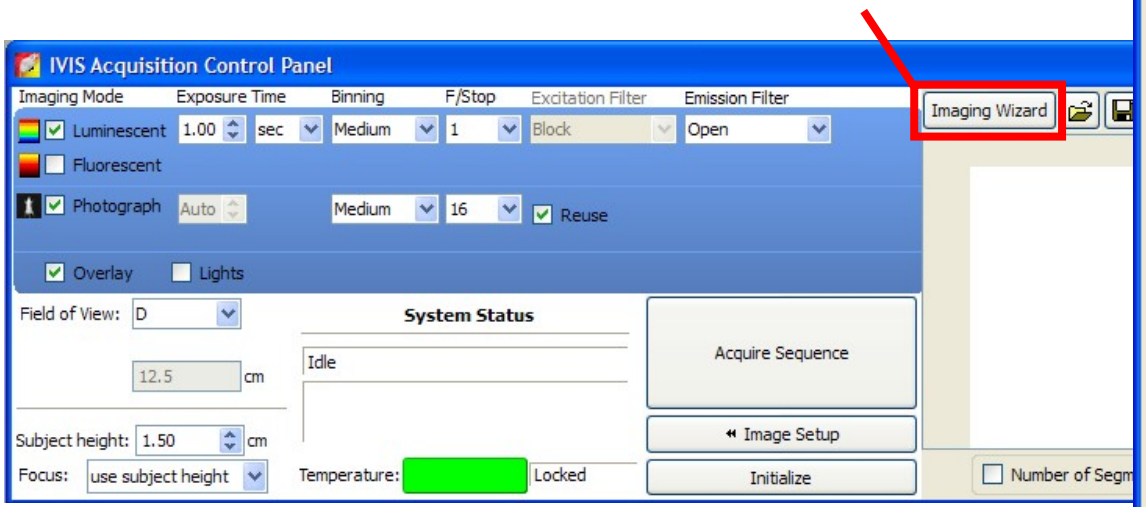
Starts Sequential Image Acquisition

User Friendly Sequence Editor

Software – Acquisition

Imaging Wizard

Select for assistance in setting up bioluminescence or fluorescence sequences



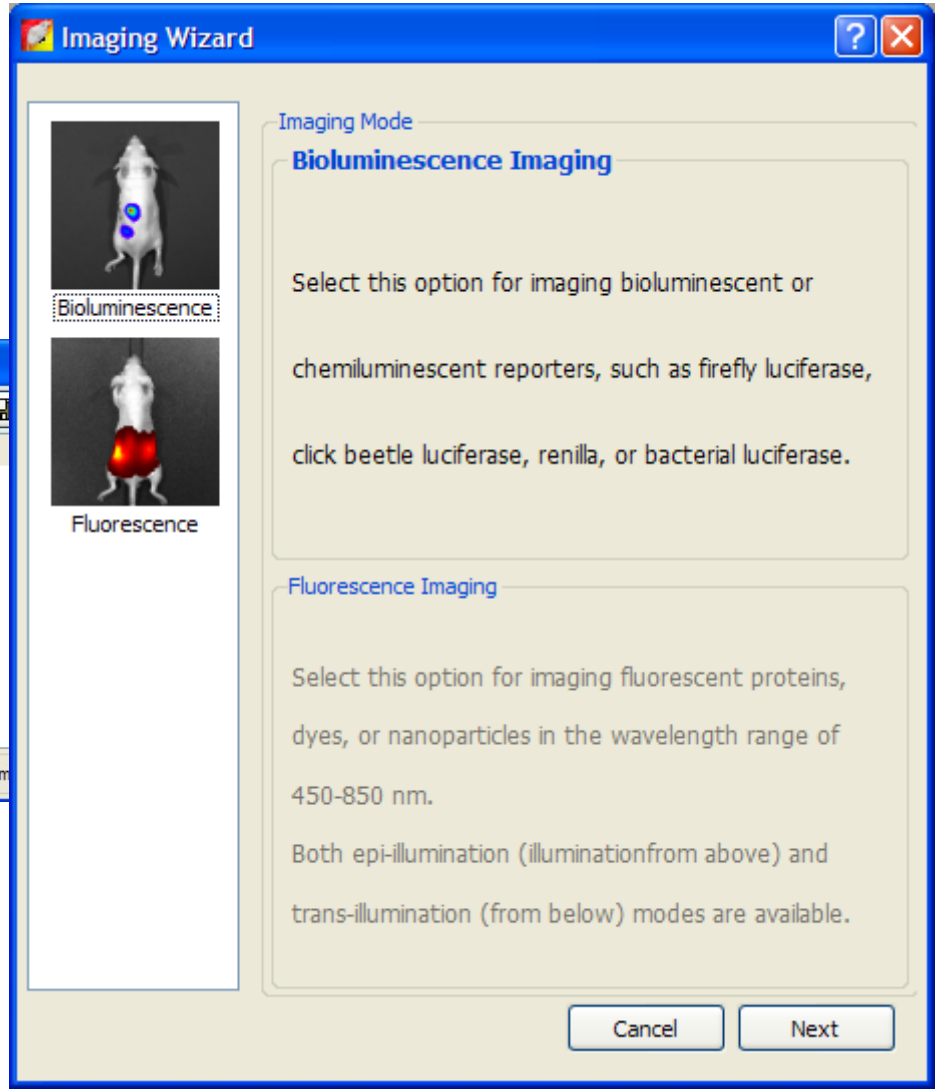
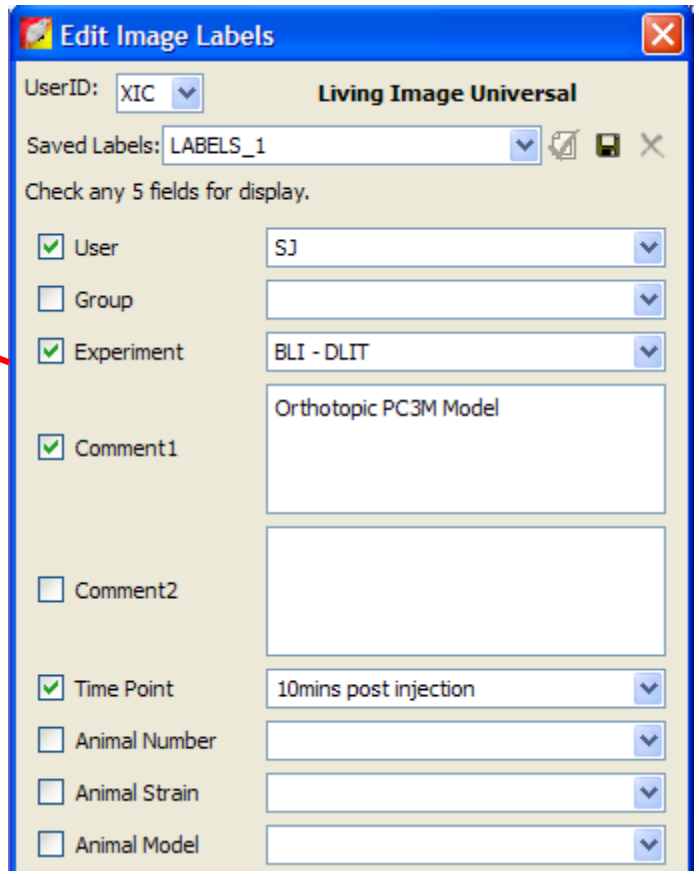


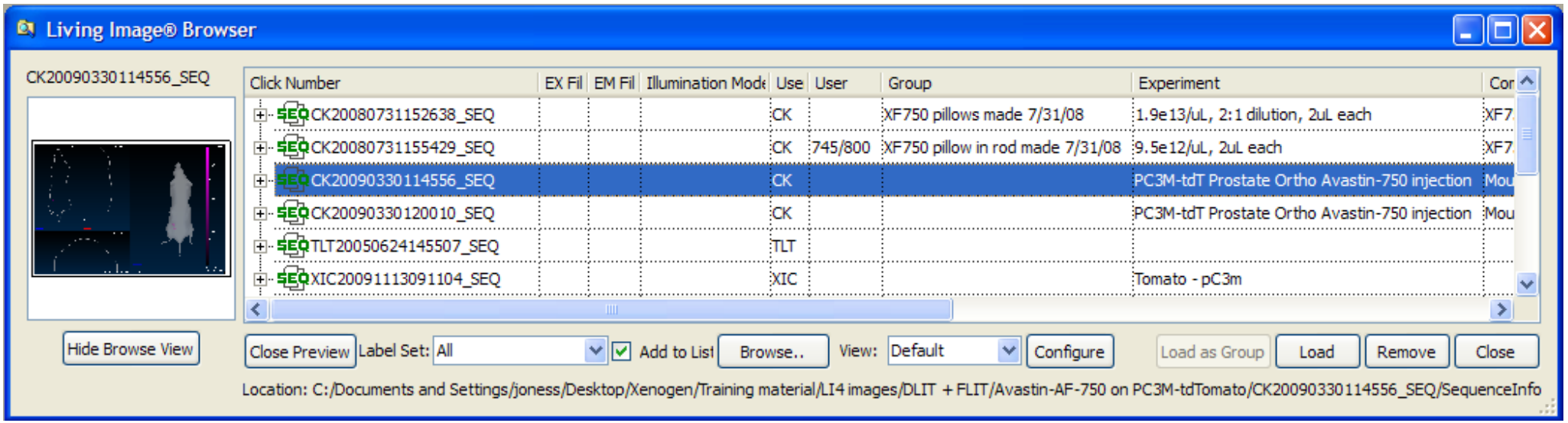
Image Labeling

- Good labeling practices are necessary for effective data browsing



Software – Cataloging

Image Cataloging & Browsing Tools

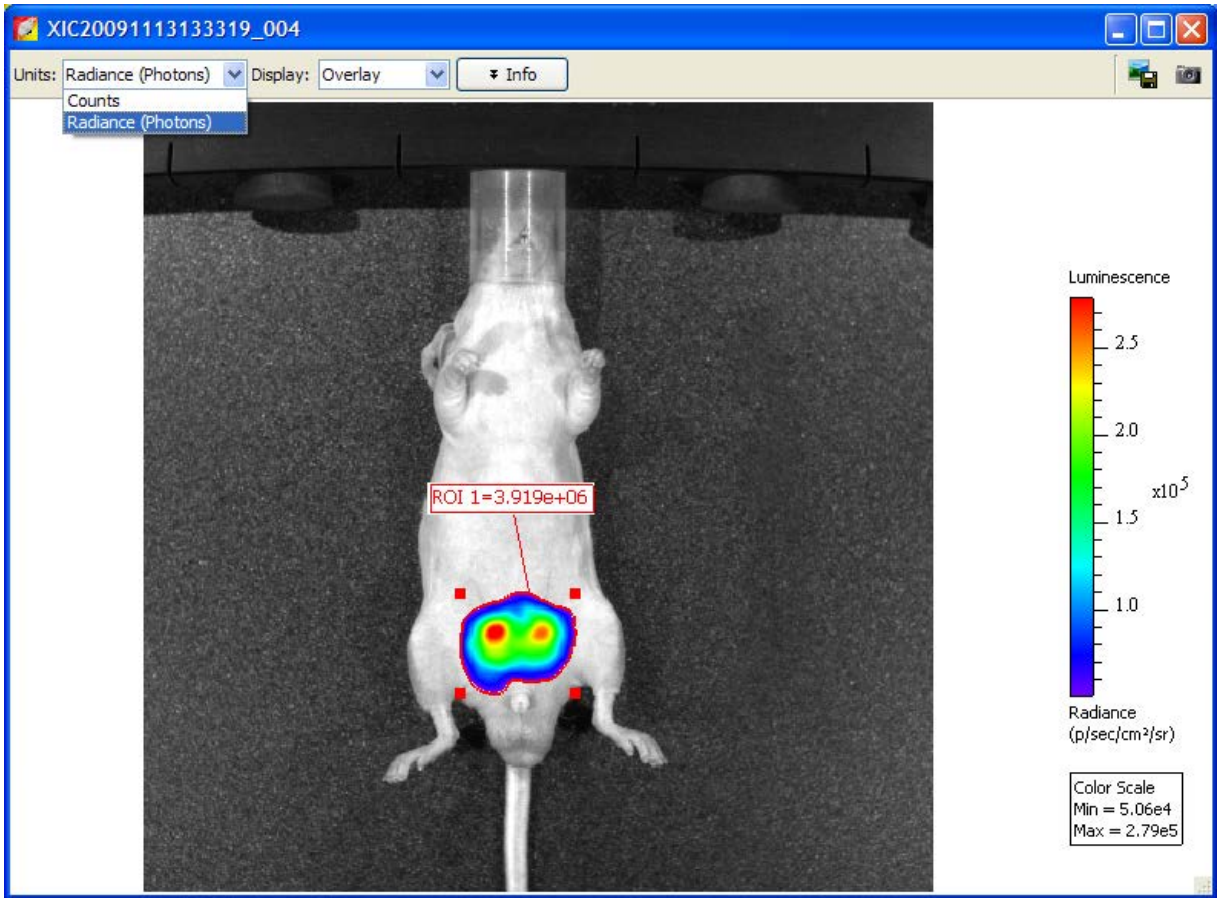


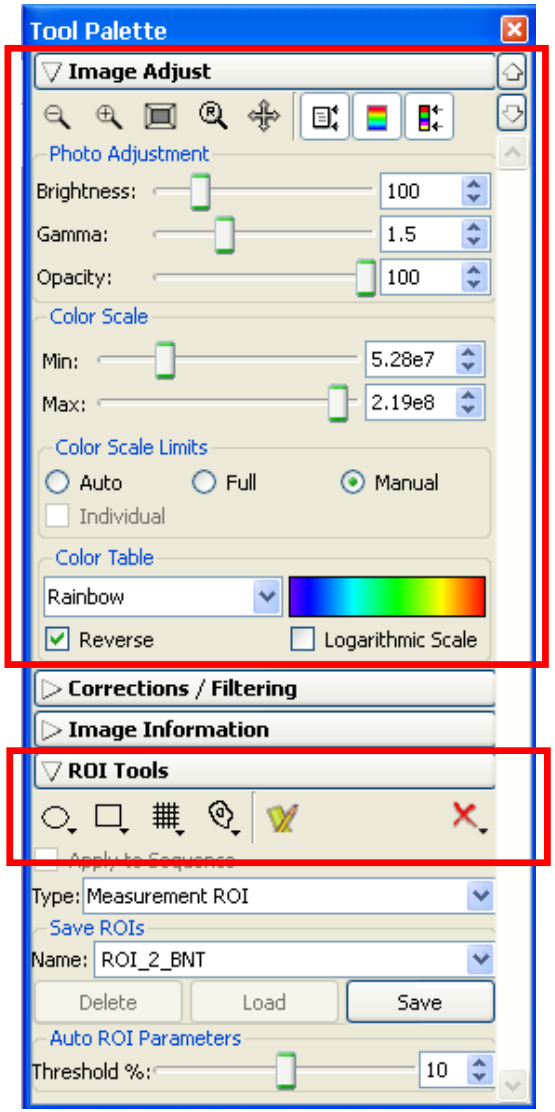
User defined information

Software – Cataloging

Quantification

- Tool palette for adjusting scale/opacity etc.
- Region of interest (ROI) tools to measure surface intensities





Software - Analysis

Regions of Interest Tools

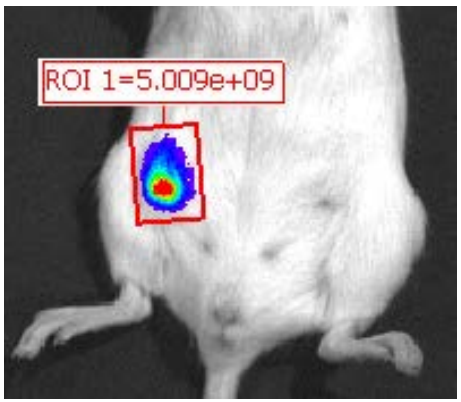
ROI shapes available:

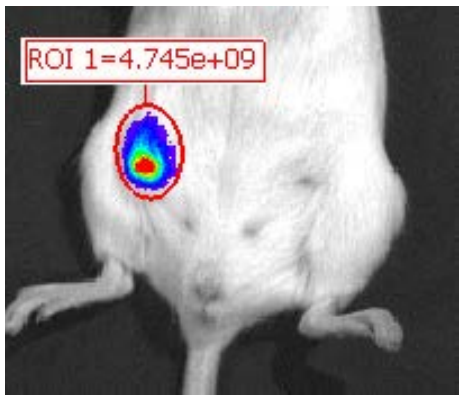
- Square
- Circle
- Contour
- Grid

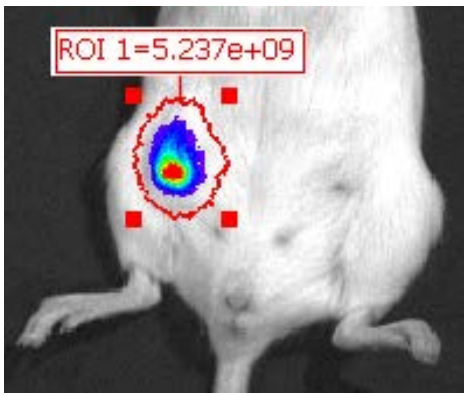
ROI's can be created:

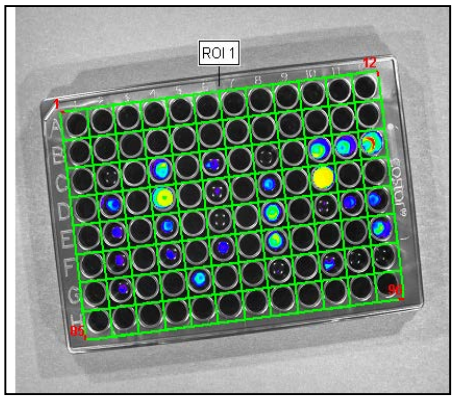
- Manually
- Automatically
- Free Draw

Important to be consistent with ROI selections

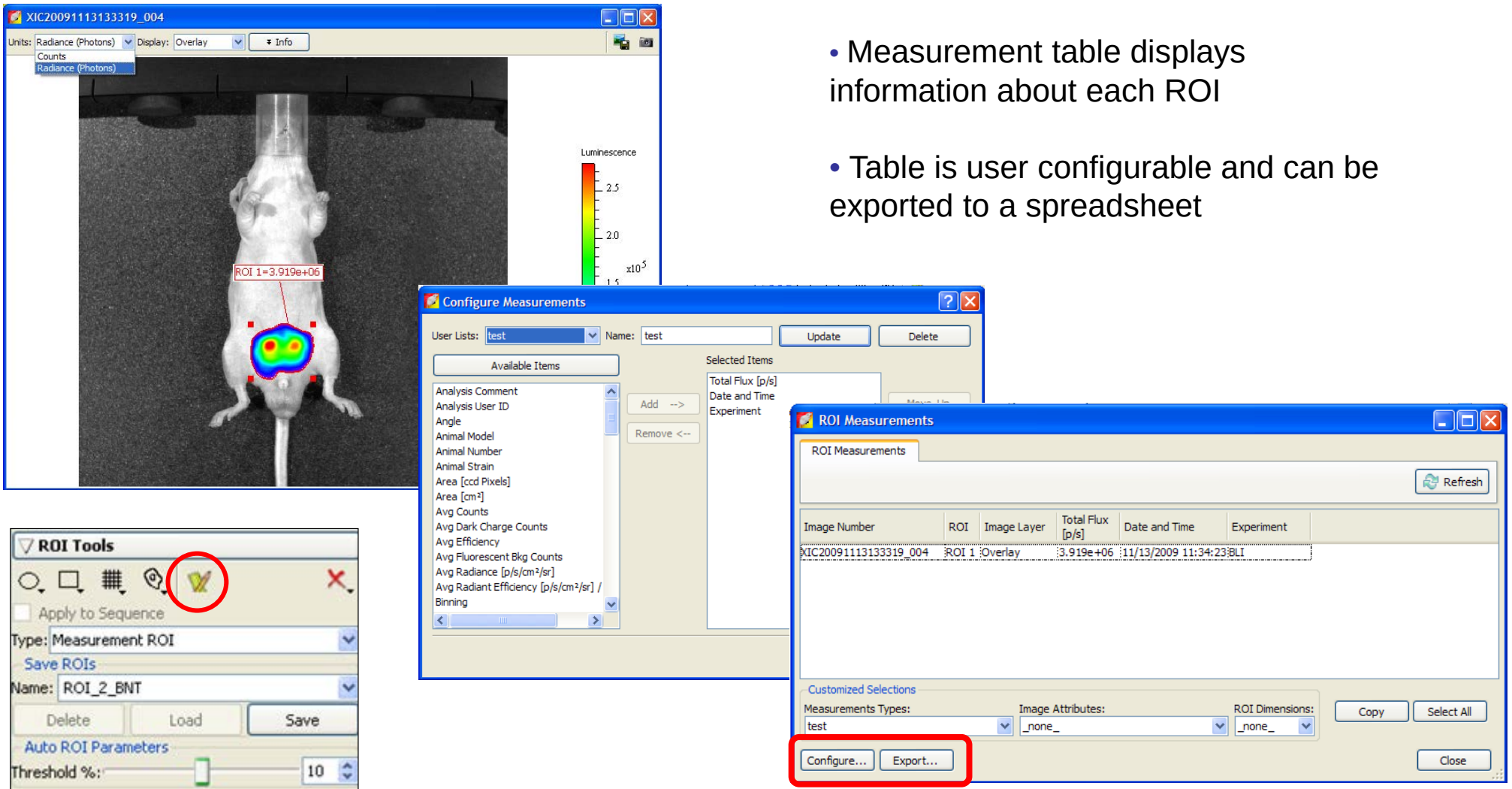








Measurement Table

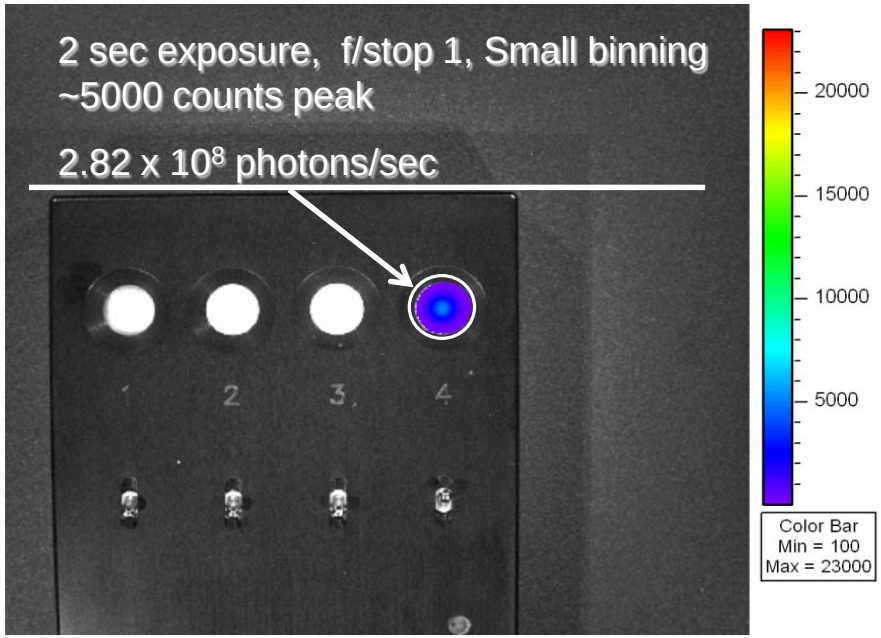


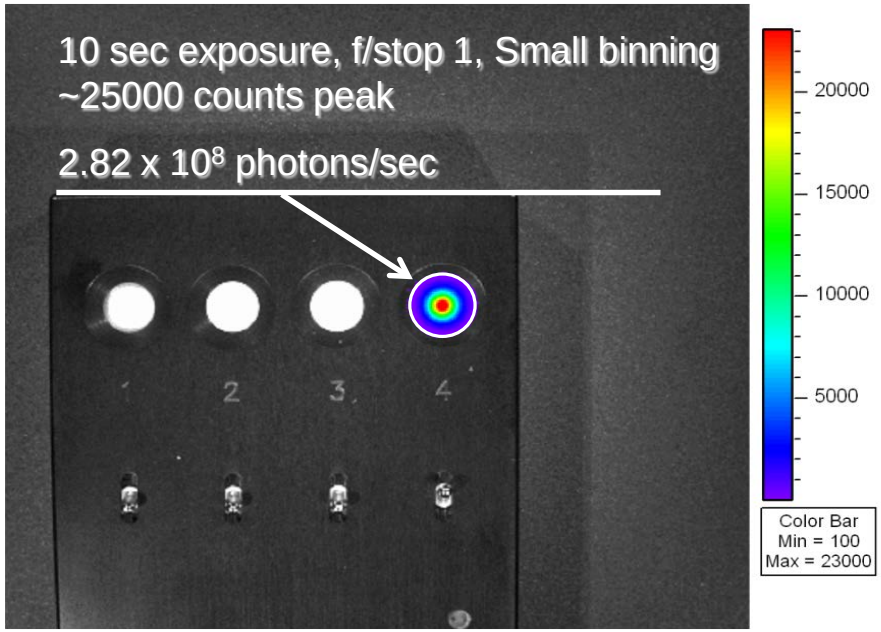
- Measurement table displays information about each ROI
- Table is user configurable and can be exported to a spreadsheet

Software - Analysis

Calibrated Physical Units

- Living Image® automatically compensates for device settings: Exposure time, f/stop, Binning, and Field of View.
- Calibrated units are Photons per Second, representing the flux radiating omni-directionally from a user defined region.

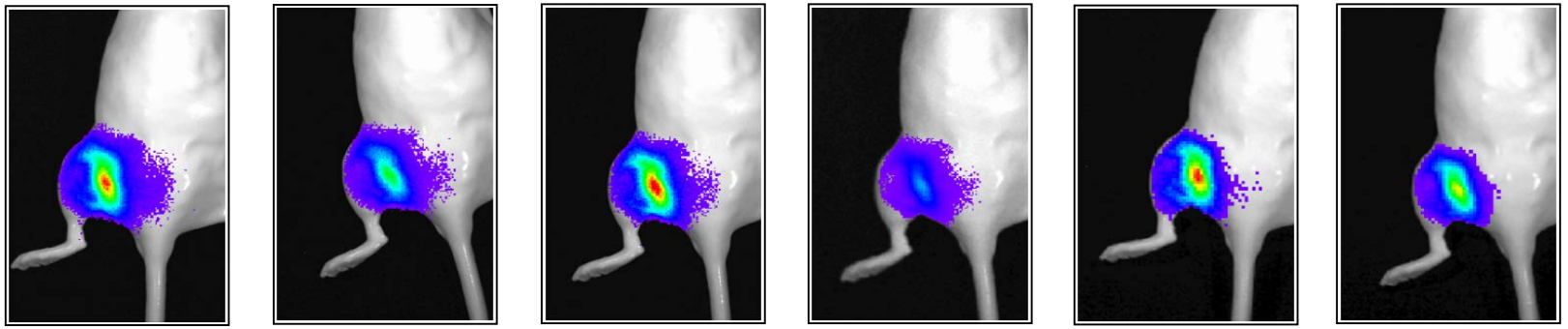




Software - Analysis

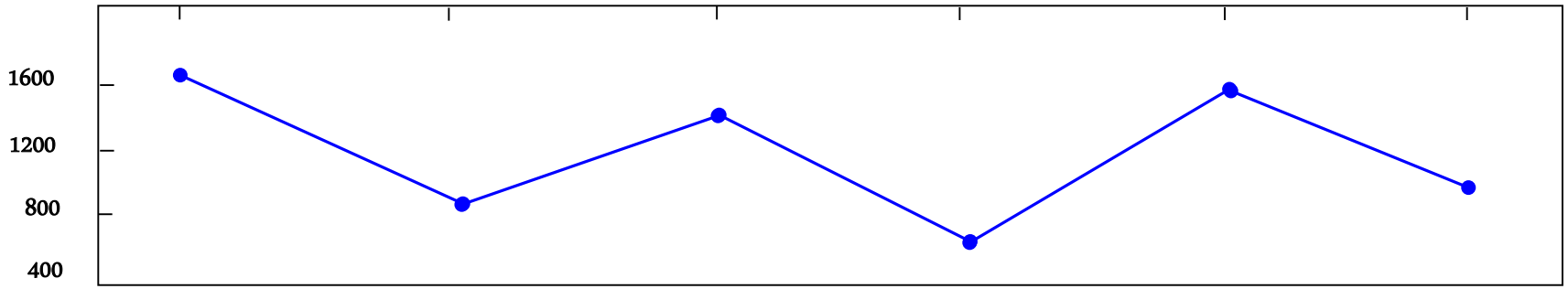
Calibrated Physical Units *vs* Raw Signal - Example

Raw Signal
(*Counts*)



Exp time:	30 sec	30 sec	60 sec	60 sec	60 sec	60 sec
Binning:	small	small	small	small	medium	medium
Day:	1	2	3	4	5	6

Peak Counts

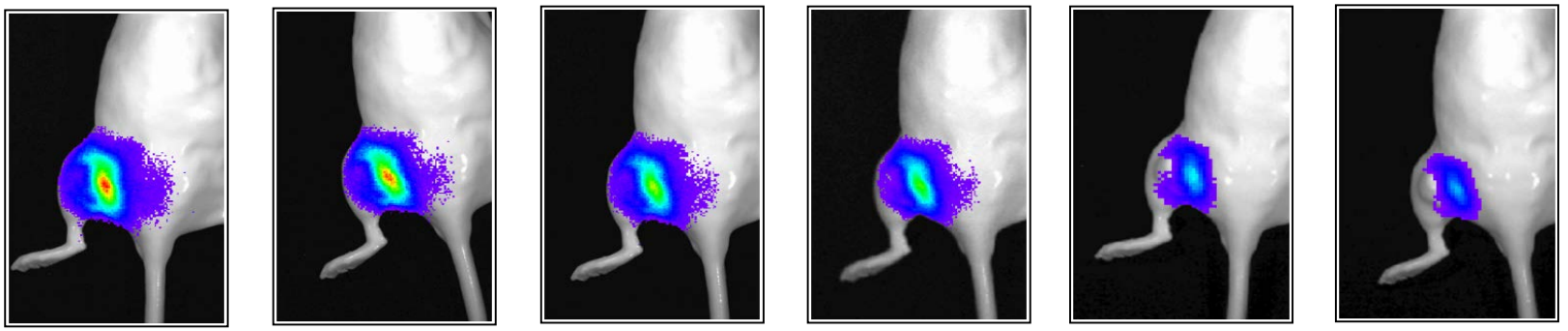


Software - Analysis

Calibrated Physical Units *vs* Raw Signal- Example

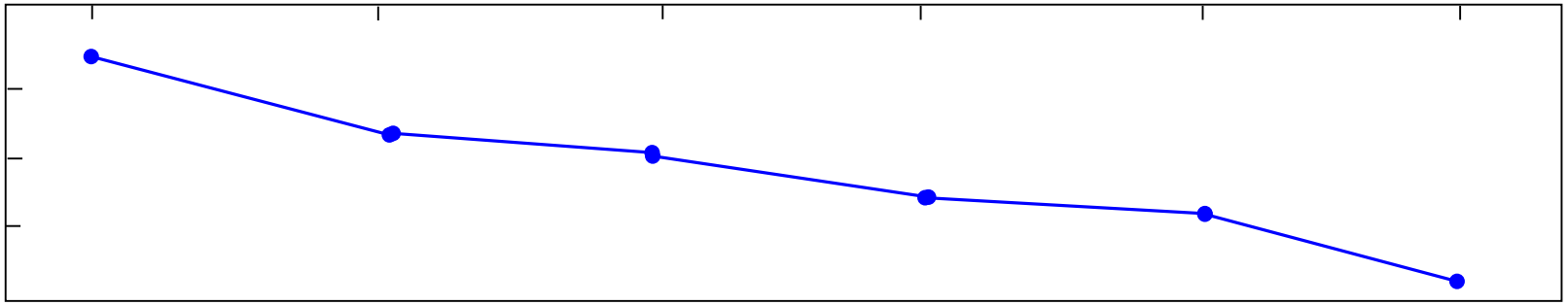
Calibrated Signal

(*Photons per second*)



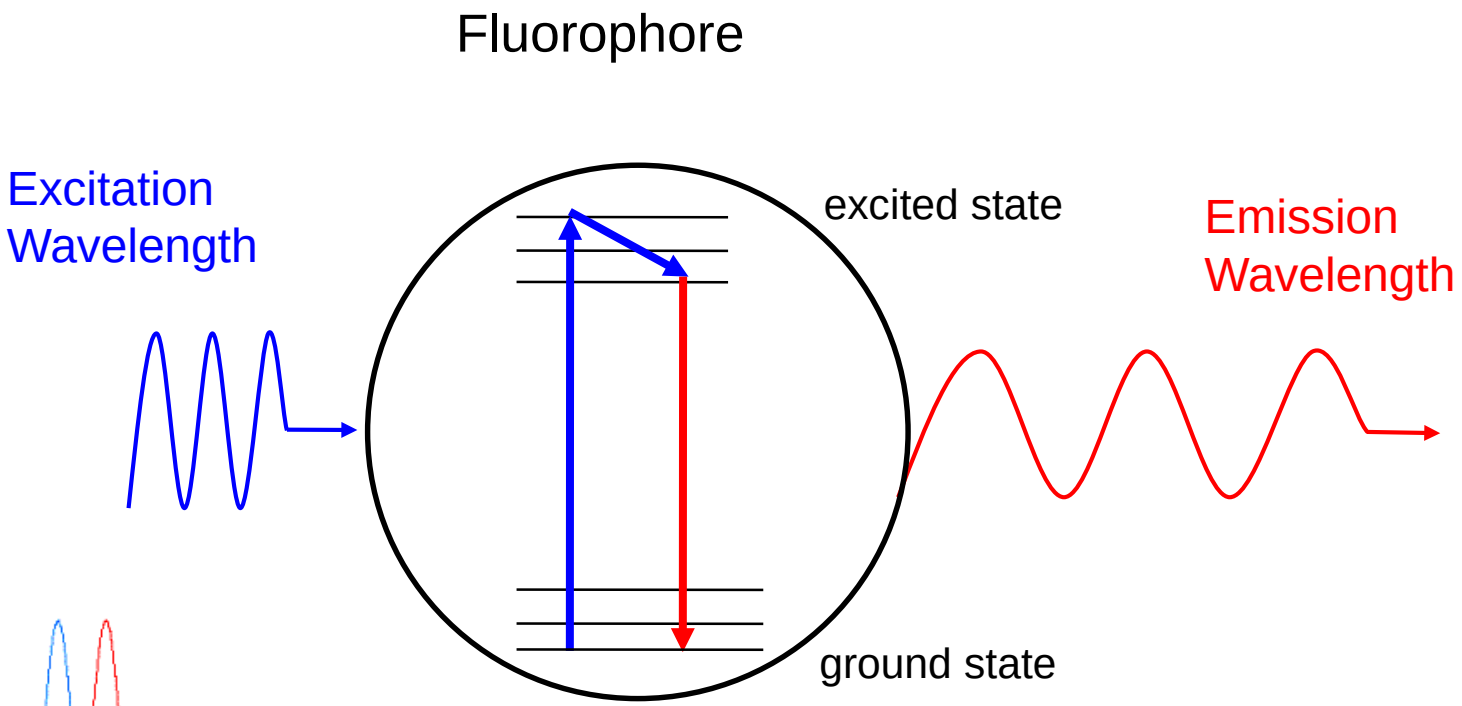
Exp time:	30 sec	30 sec	60 sec	60 sec	60 sec	60 sec
Binning:	small	small	small	small	medium	medium
Day:	1	2	3	4	5	6

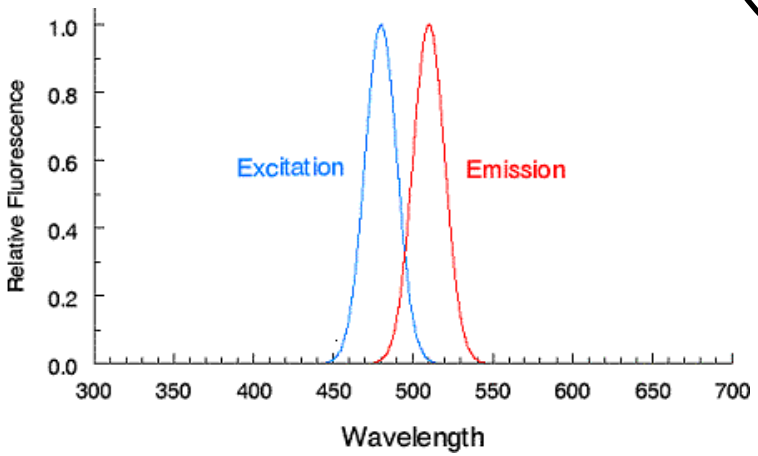
Radiance: *Photons per second*



Software - Analysis

Fluorescence

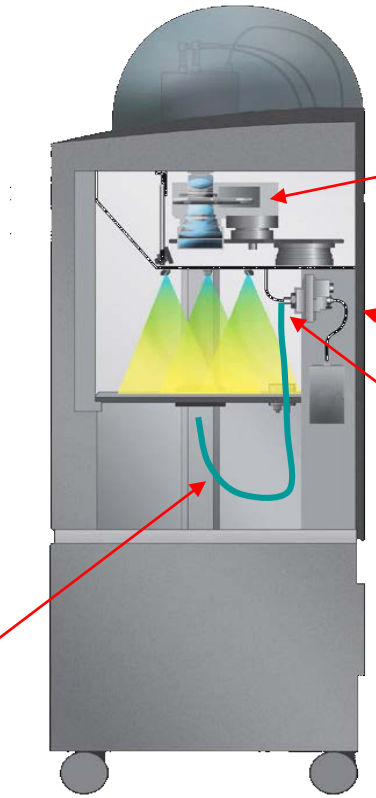




Fluorescence

IVIS® Fluorescence Imaging

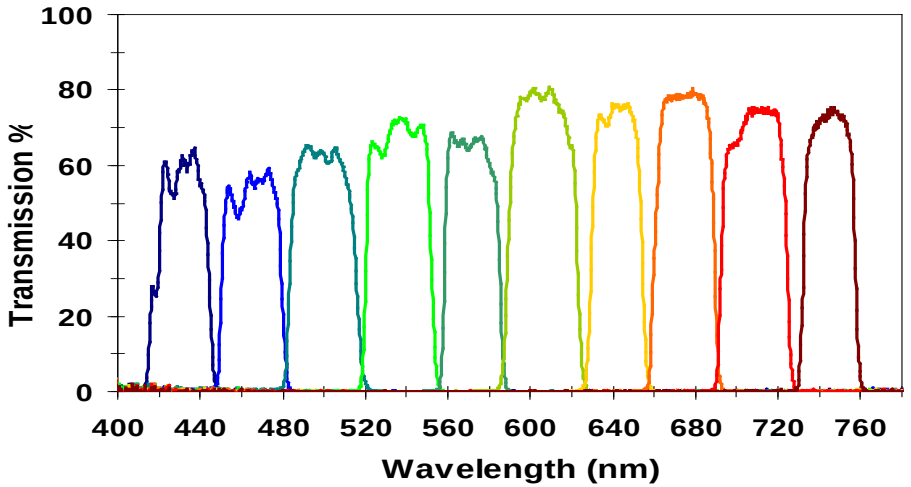
- 18 Emission filters (computer controlled)
- 10 Excitation filters (computer controlled)
- 150 Watt Tungsten/Halogen lamp (computer controlled intensity)
- Low Auto Fluorescence optics and fibers

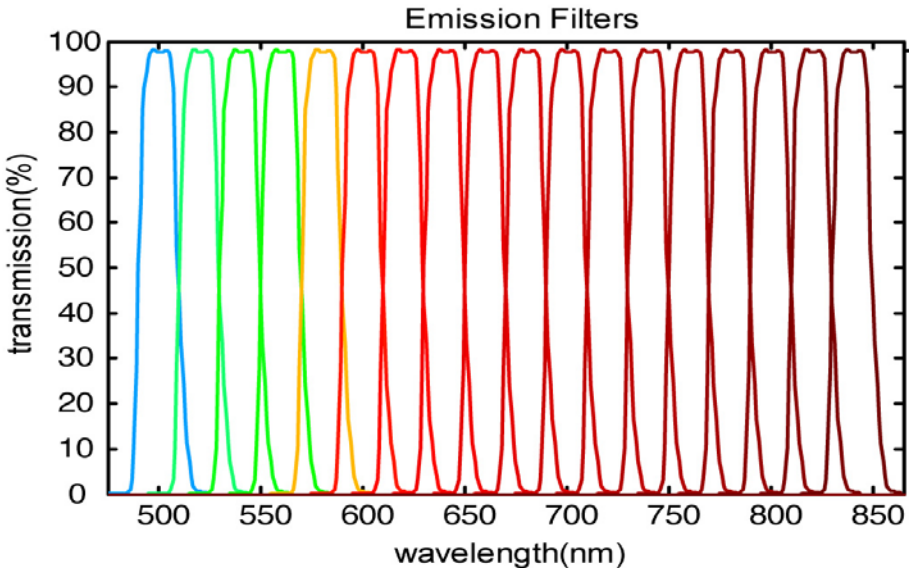


Fluorescence

Excitation and Emission Filters

10 Excitation filters –
35 nm bandwidth





18 Emission filters –
20 nm bandwidth

Fluorescence

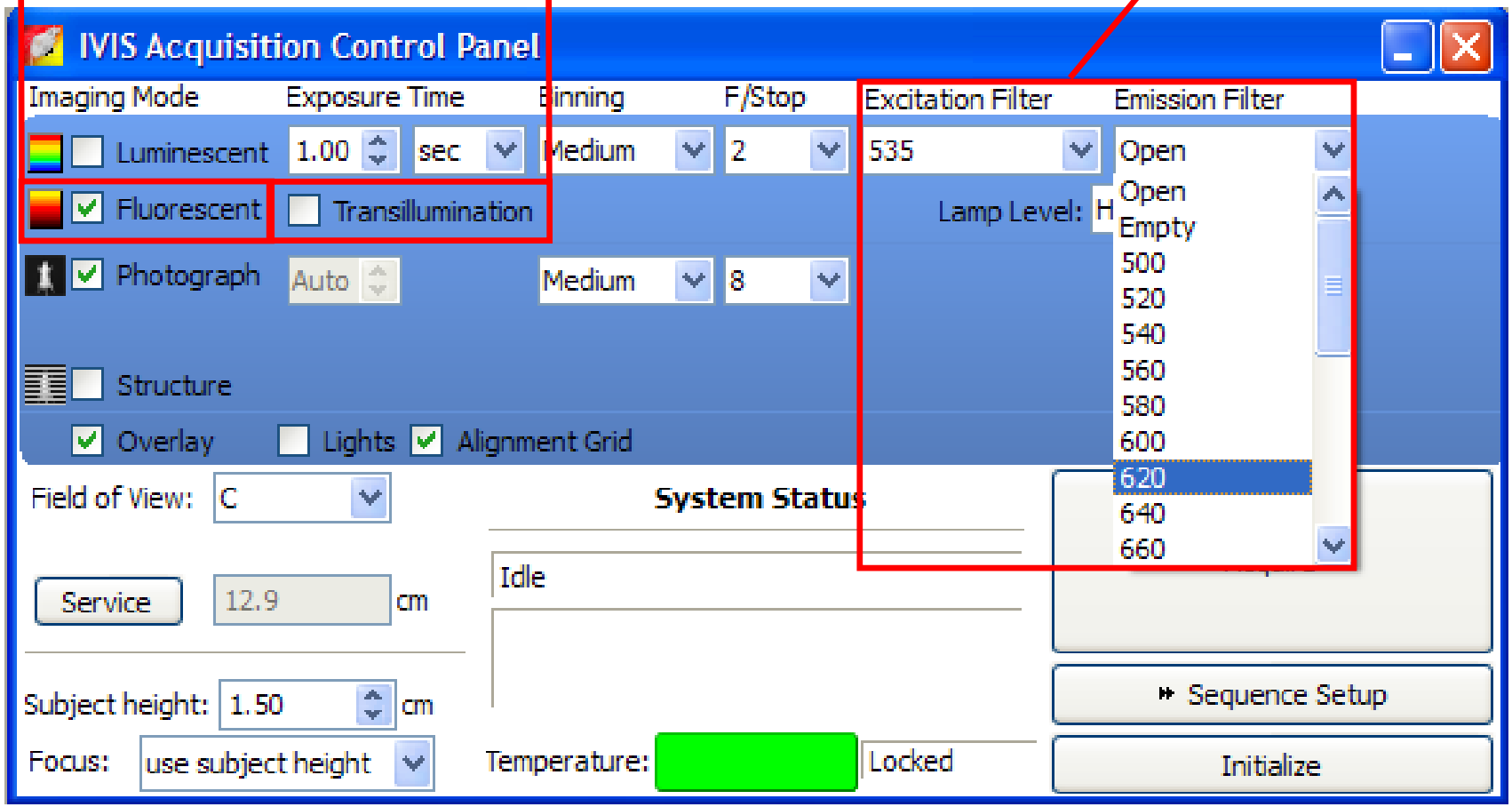
Fluorescence Acquisition

Select Fluorescent Imaging Mode

Trans-illumination

Lamp level High / Low

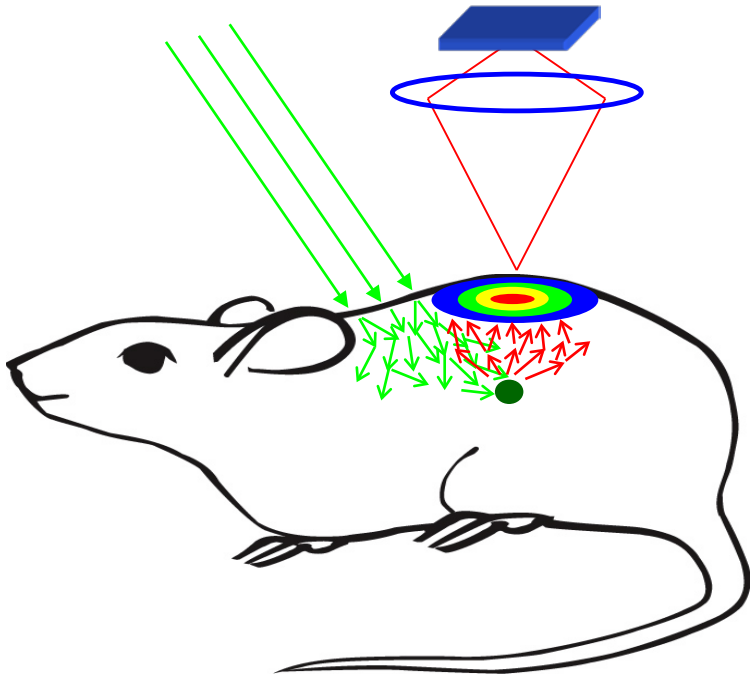
Select filters



Fluorescence

Fluorescent Calibrated Units: Radiant Efficiency

$$\text{Radiant Efficiency} = \frac{\text{Emission Light (photons/sec/cm}^2\text{/str)}}{\text{Excitation Light } (\mu\text{W/cm}^2)}$$



Fluorescence

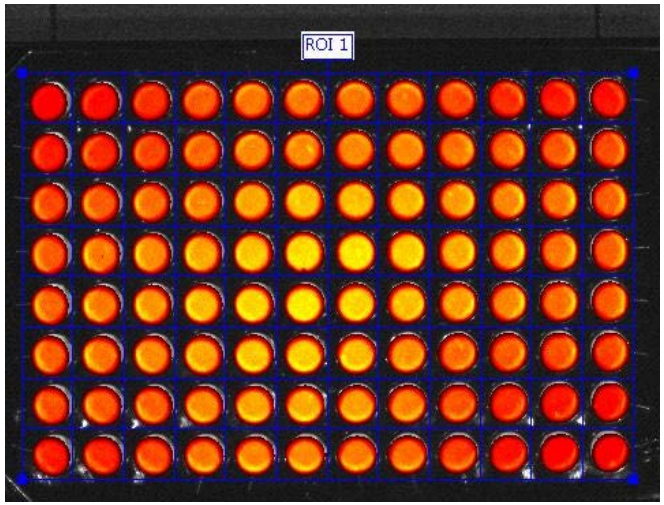
Fluorescent Calibrated Units: Radiant Efficiency

Excitation Light Pattern

Units of 'Radiant Efficiency' compensates for non-uniform excitation light pattern

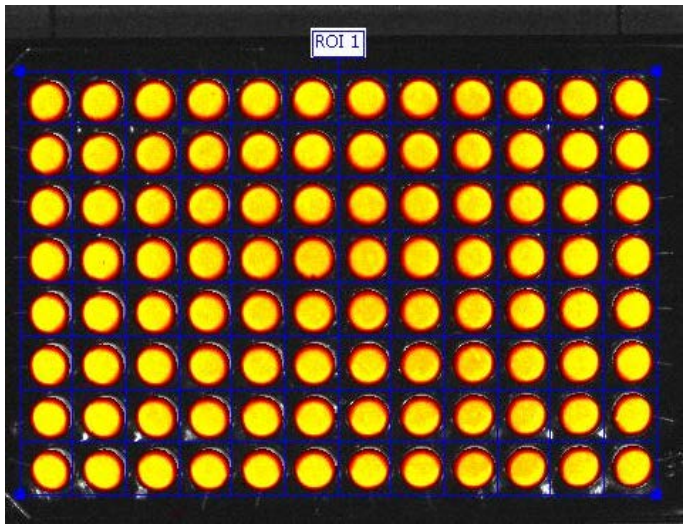
GFP Well Plate Uncorrected

Counts



vs.

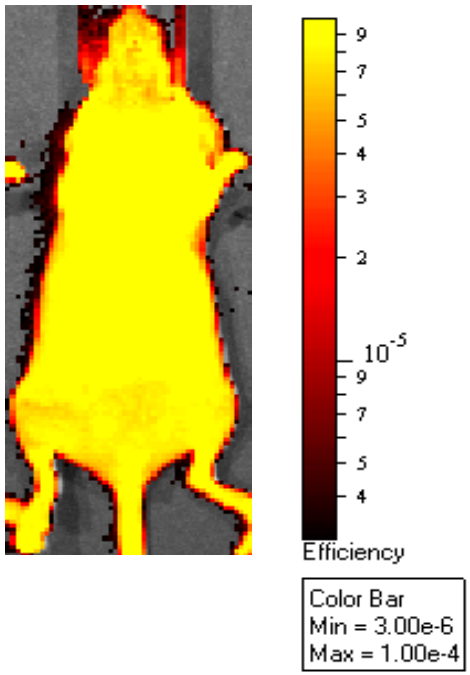
GFP Well Plate Corrected



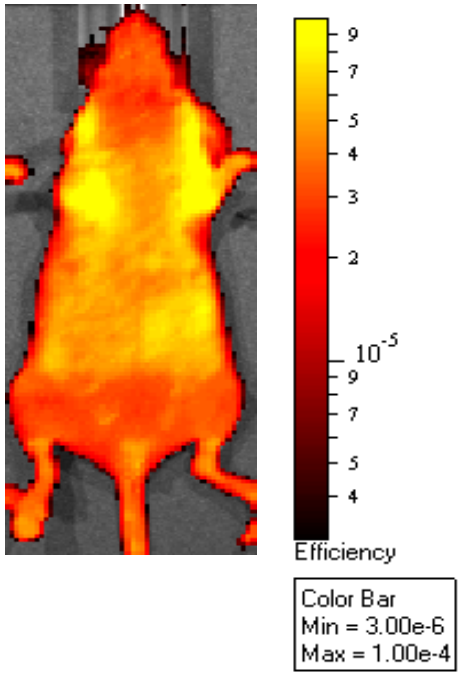
Radiant Efficiency

Auto-fluorescence of Control Mice

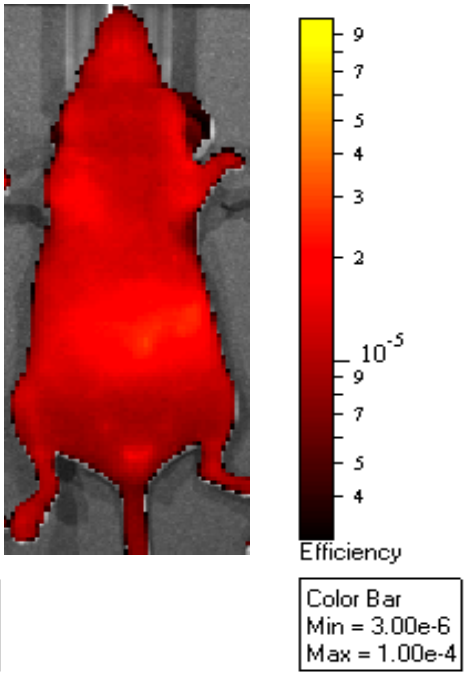
λ = 550nm



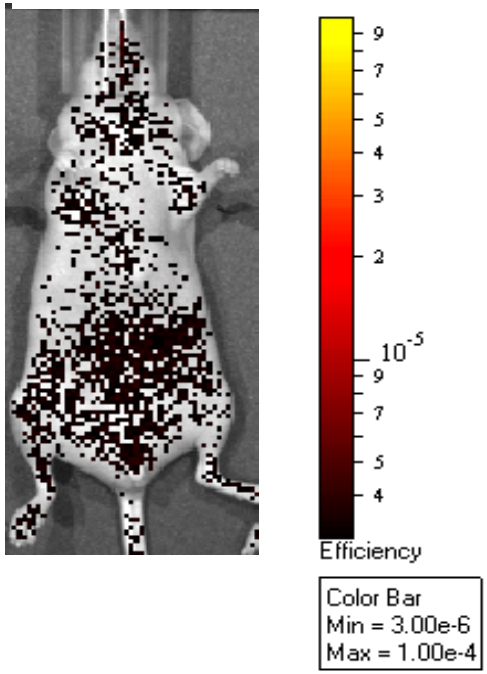
λ = 610nm



λ = 740nm



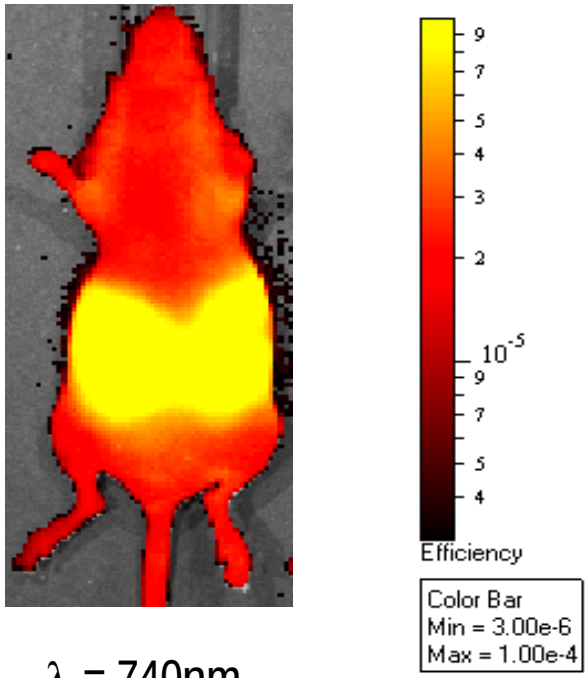
λ = 840nm



Fluorescence

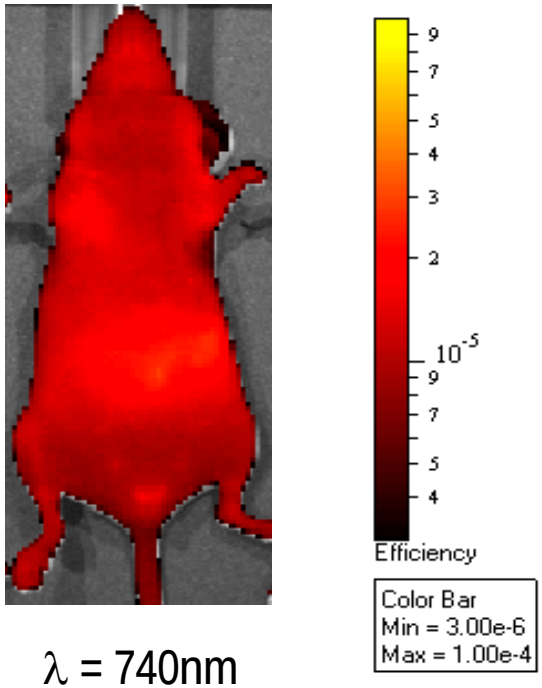
Animal Diet Auto-fluorescence in Control Mice

Regular Rodent Food



λ = 740nm

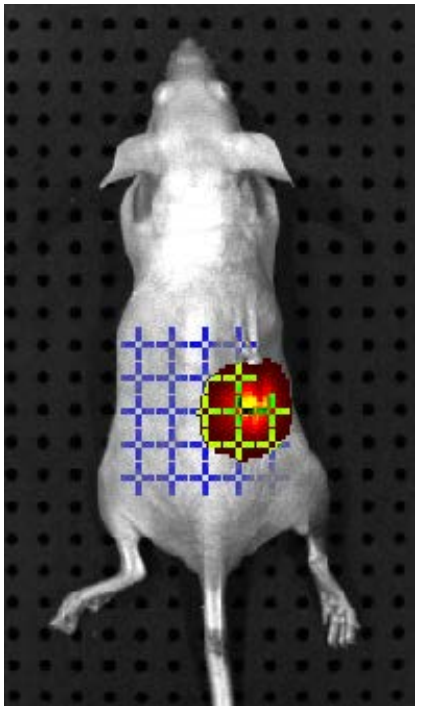
Alfalfa Free Rodent Food



λ = 740nm

Fluorescence

Transmission Fluorescence

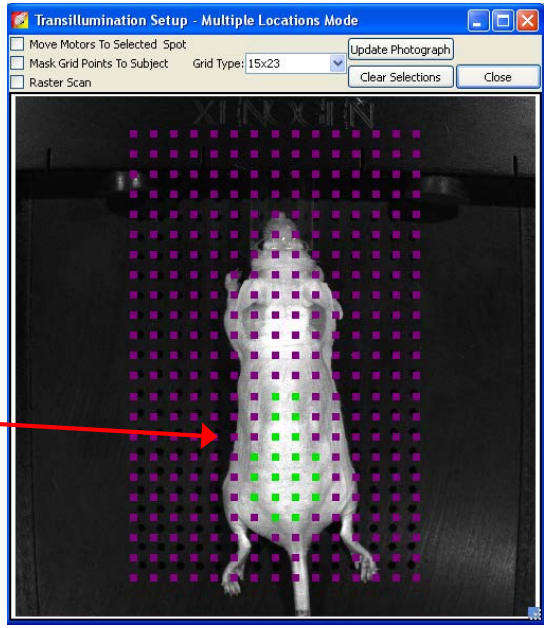


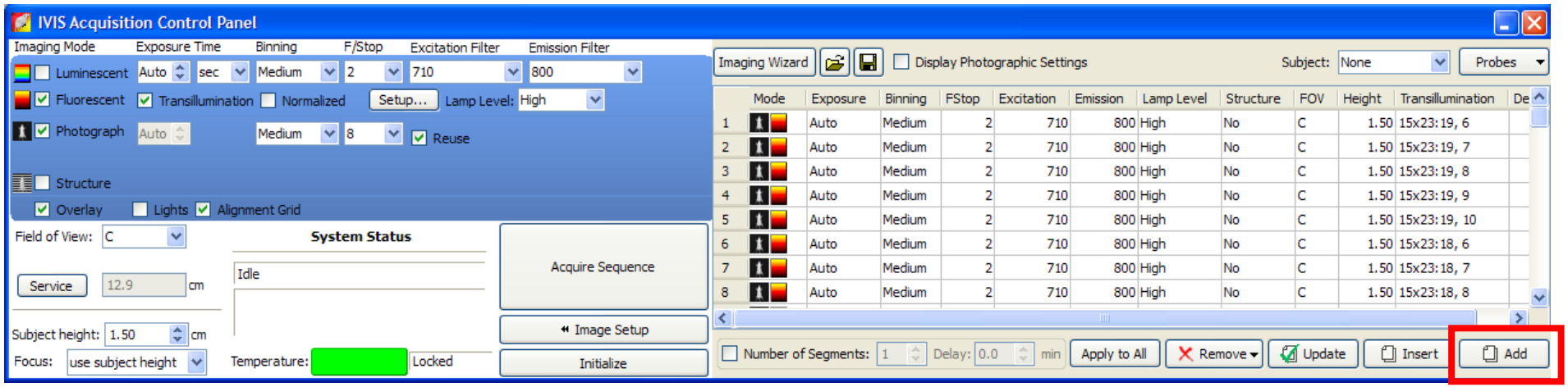
- Optimized for imaging deep signals, improves signal/noise

Trans-illumination Sequence

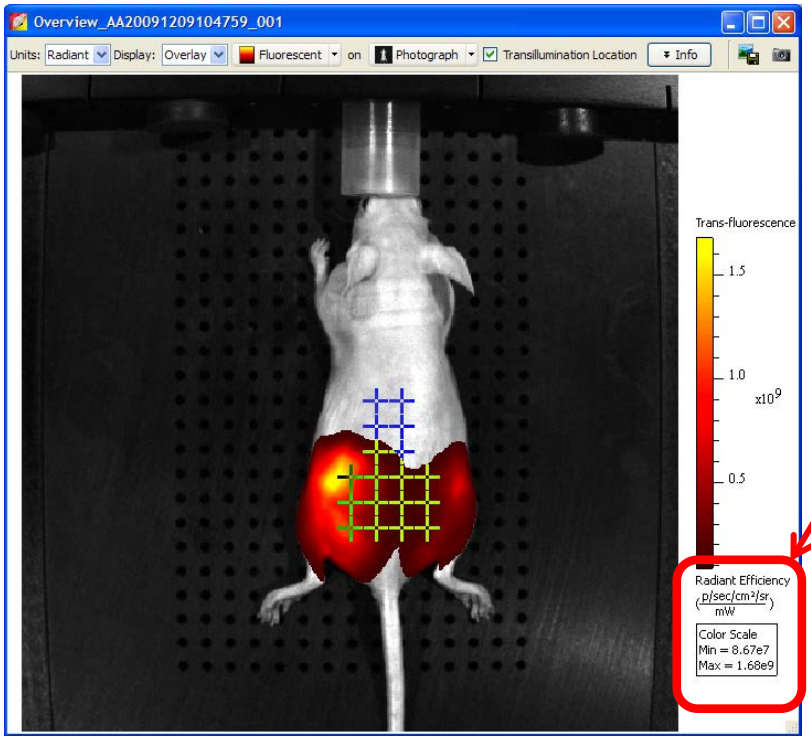
Highlight multiple locations of interest (or entire subject) and add to the image sequence

Select trans-illumination points





Trans-illumination units



Imaging units are defined as **Radiant Efficiency**;

Emission Radiance ÷ Excitation light power

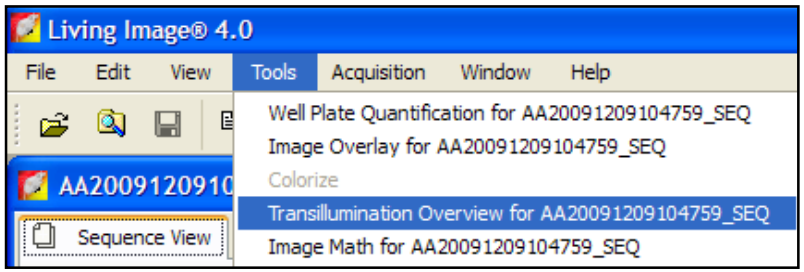
Radiant Efficiency $\left(\frac{p/sec/cm^2/sr}{mW}\right)$

Color Scale
Min = 6.27e6
Max = 3.98e7

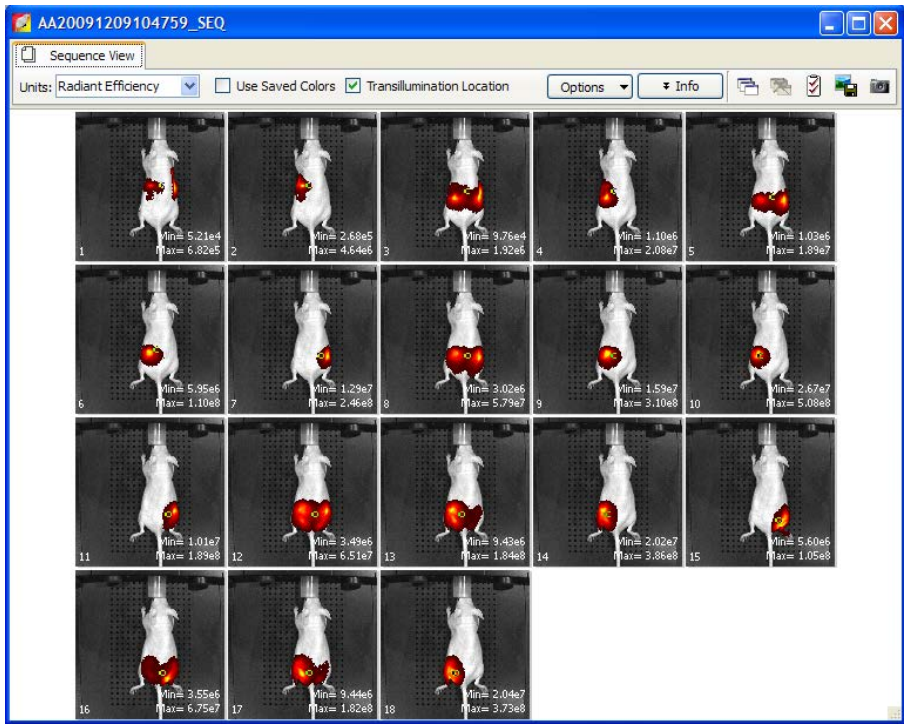
Note:
Quantification not comparable
to Epi-Fluorescence images;

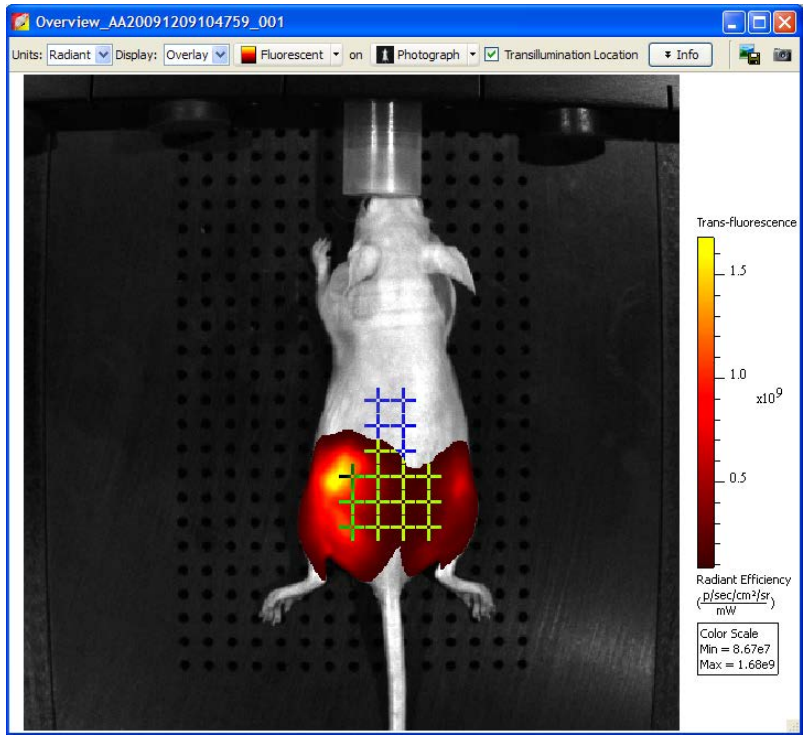
Emission Radiance ÷ Excitation power density (per area)

Trans-illumination Sequence Overview



Combined image overview of multiple trans-illumination images

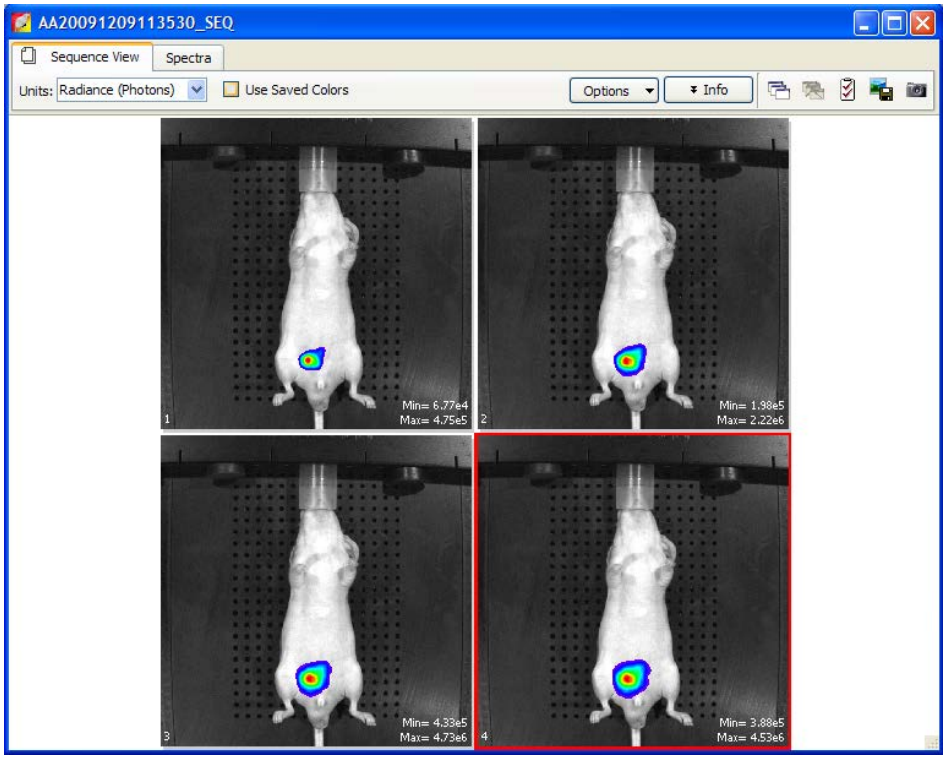


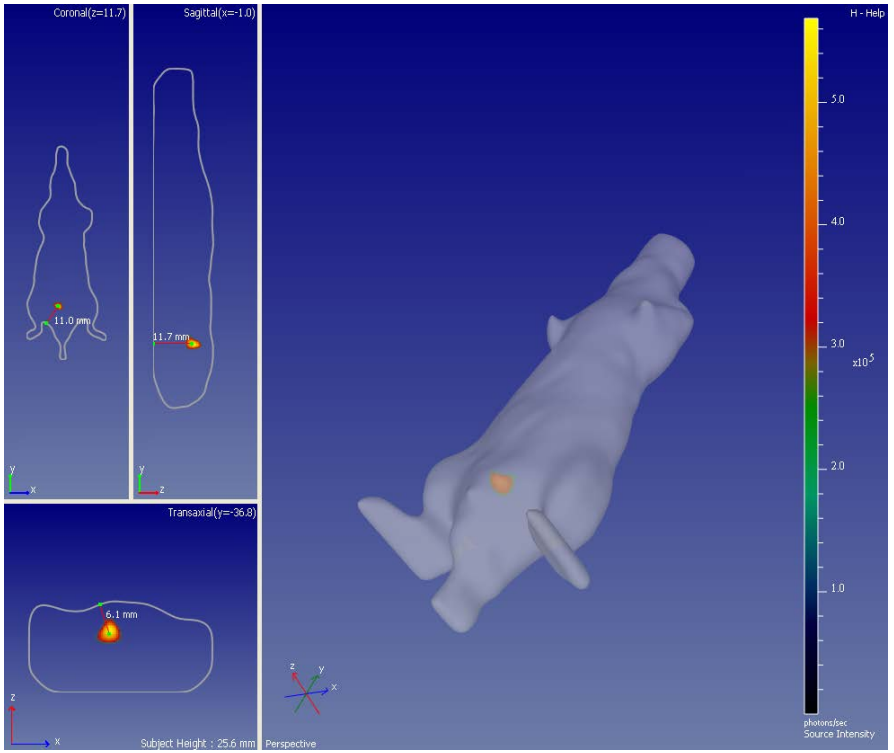


Advanced topics (preview)

- 3D Tomography (Luminescence and Fluorescence)
- Spectral Un-mixing

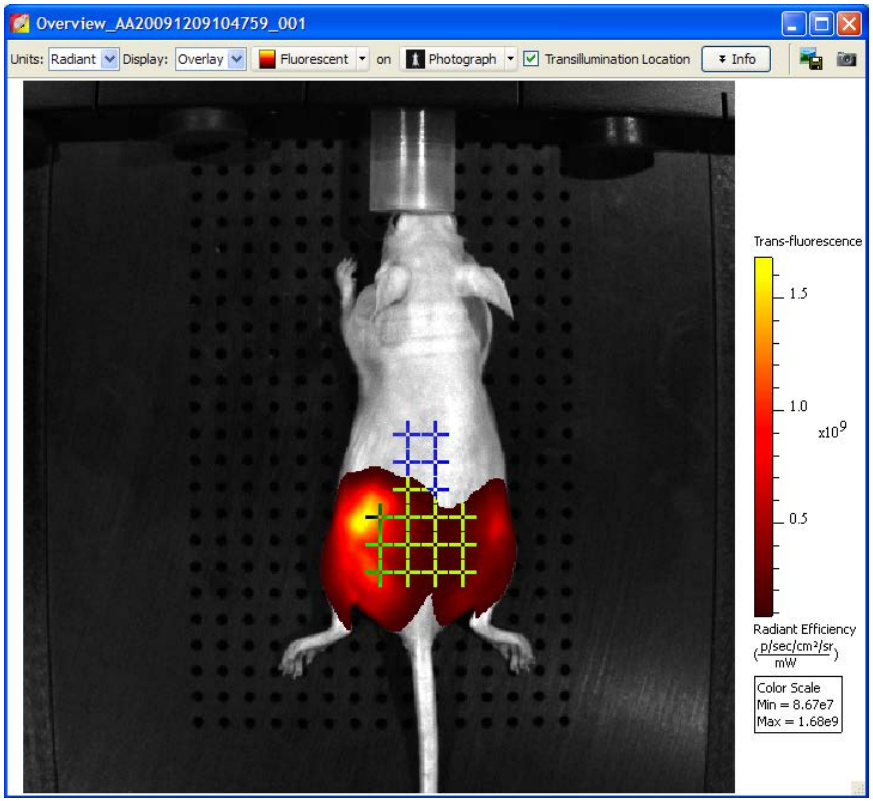
3D Tomography (Luminescence)

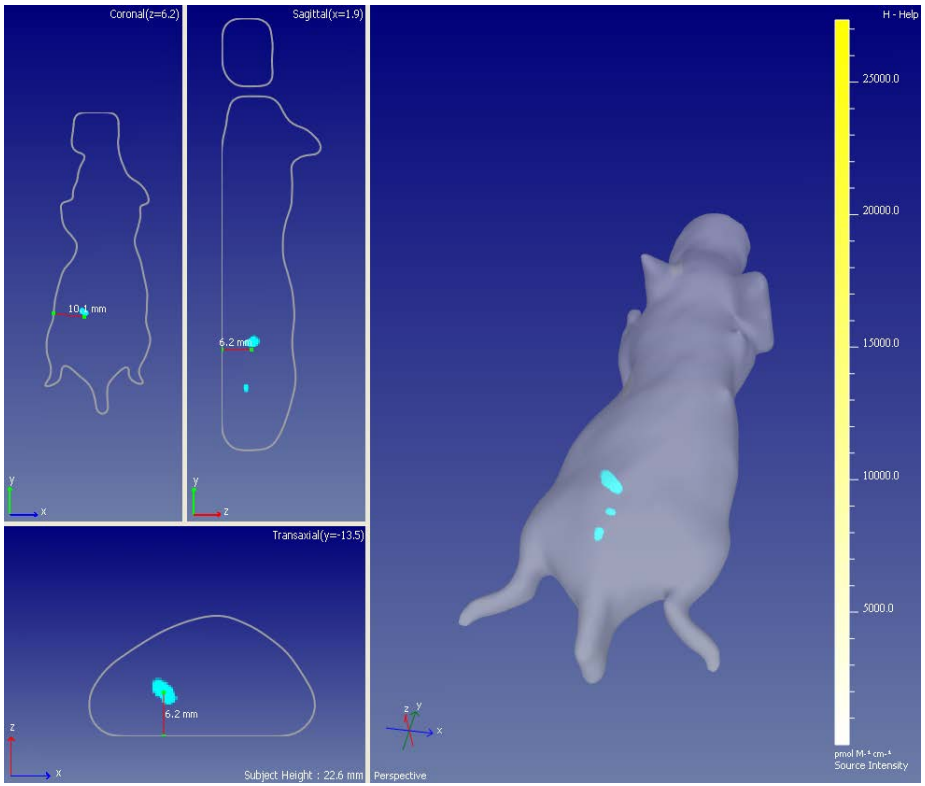




Spectral scans with Emission filters allows for absolute quantification and source localization

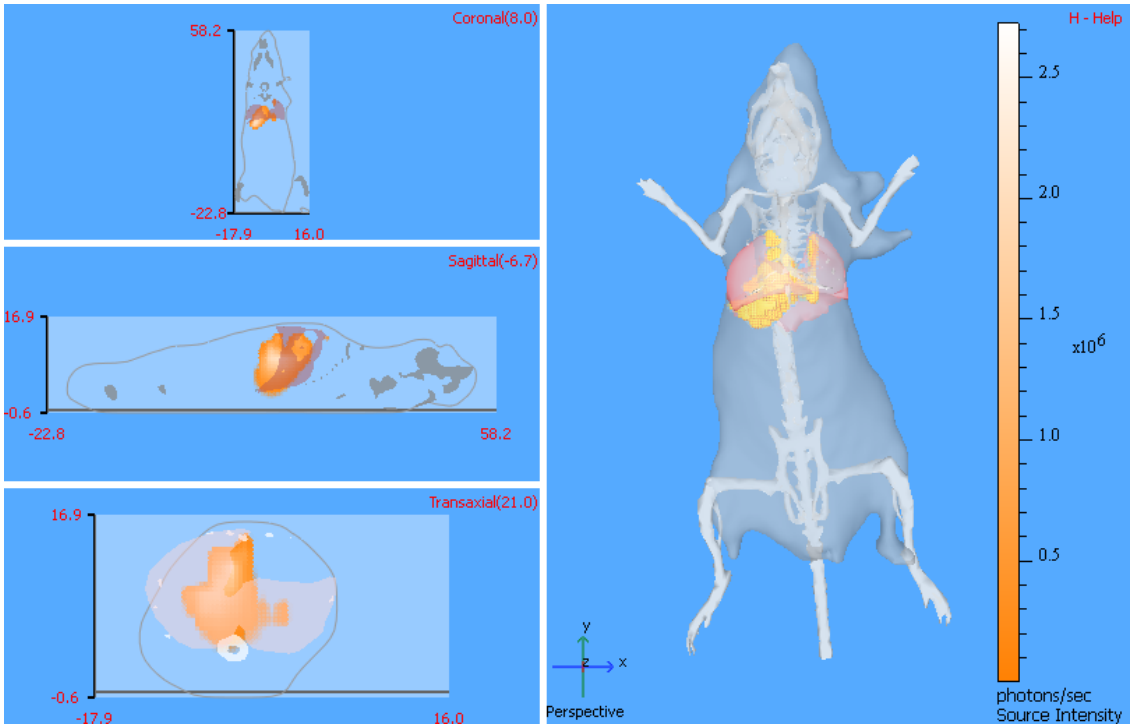
3D Tomography (Fluorescence)

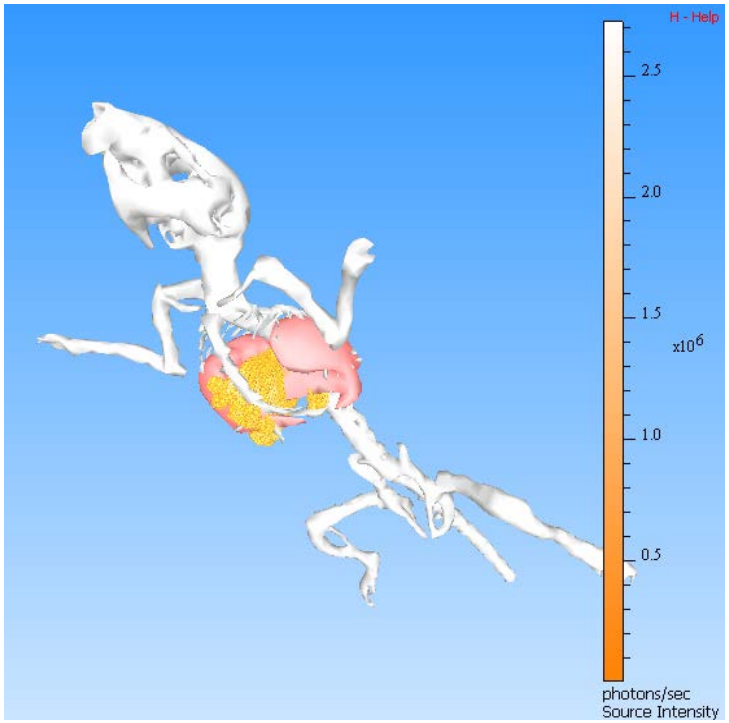




Spatial scans with trans-illumination light source allows for absolute quantification and source localization

3D Tomography with co-registration - μCT and organ atlas database

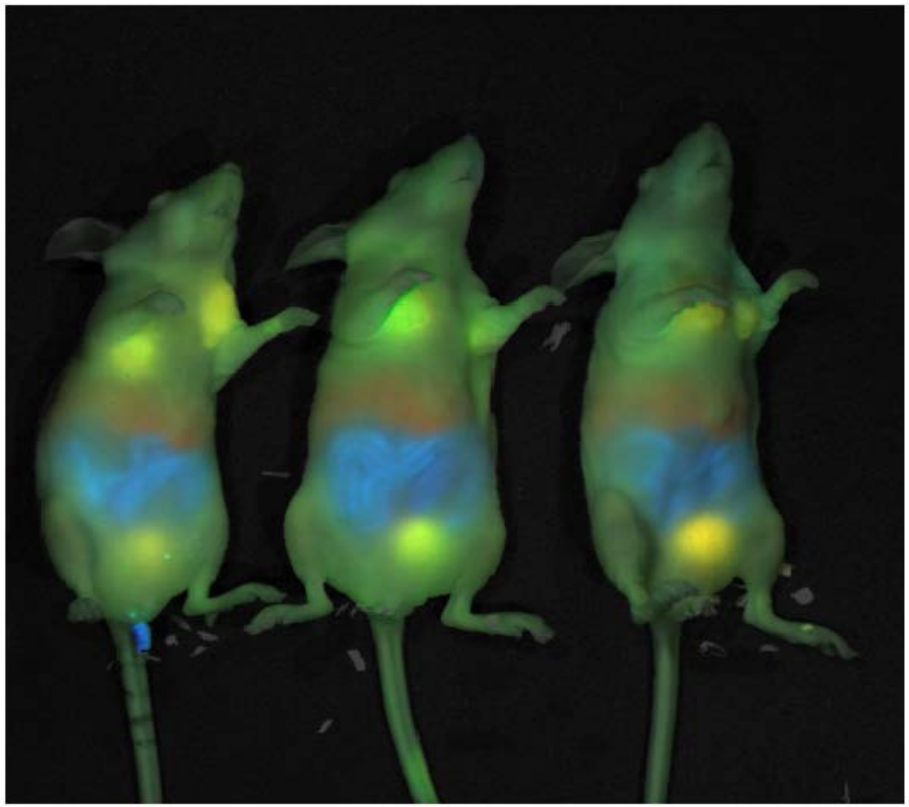




Ability to add and co-register images from a library of μCT / atlas data plus import/export DICOM formatted files

Spectral Un-mixing

- Ability to calculate contributions from different fluorescent components (inc. auto-Fluorescence)



Composite image of 4T1 murine mammary tumor cells implanted in mammary fat pads:

- ProSense 680 (Yellow) activated by cathepsins in tumor cells and accumulates in bladder
- MMPSense750 (Red) activated by metalloproteinases in tumor cells and liver accumulates in bladder
- Auto-fluorescence from chlorophyll in food (blue) and animal tissue background (green)

Summary

Imaging principles

- Light is scattered and absorbed by tissue - dependant on wavelength and depth
- **Calibrated physical units compensate for device settings**

Hardware

- Custom designed for *in-vivo* bioluminescent & fluorescent imaging
- 28 filters make IVIS Spectrum ideal for imaging multiple probes
- Settings are analogous to photography

Software

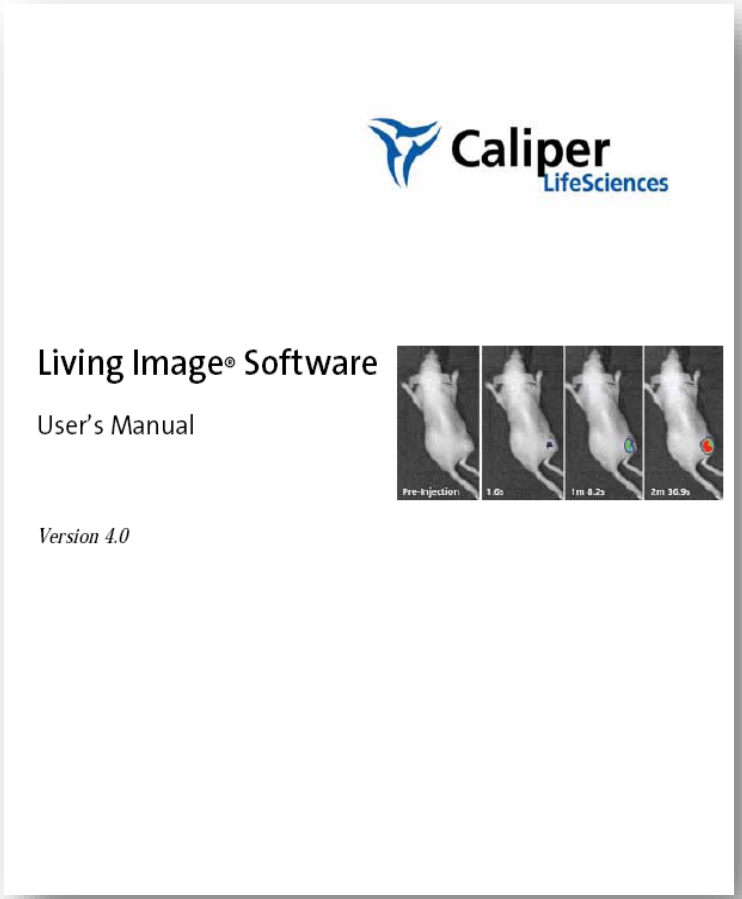
- Living Image® used for acquisition and analysis
- Images are acquired in a two step process
- **Sensitivity is controlled by Exposure time, f/stop and binning**

Fluorescence

- **Two modes of illumination: Reflection (epi) or Transmission**
- Tissue and Instrument Auto-fluorescence can be subtracted

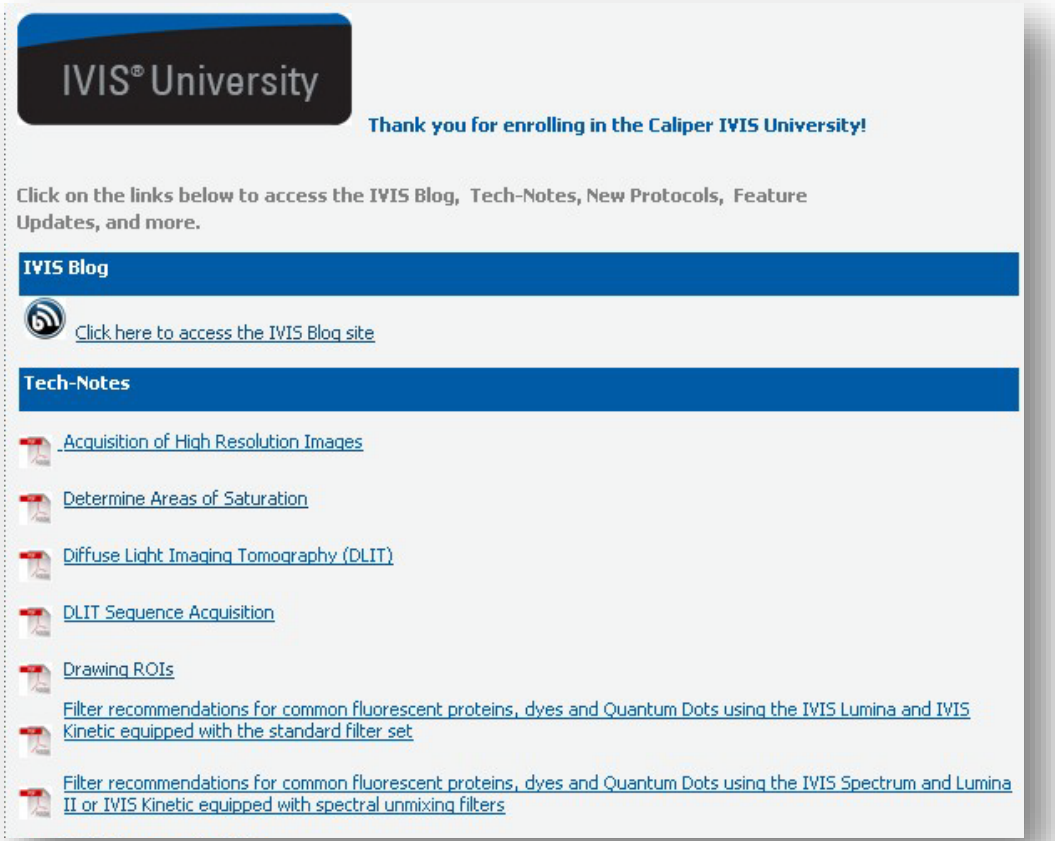
For an In Depth Study

IVIS Software Manual



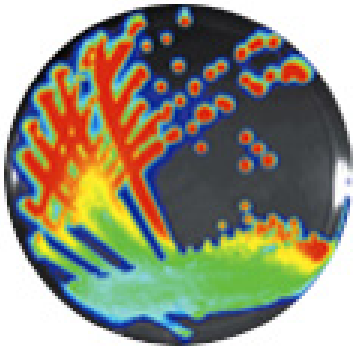
IVIS University Web page

www.caliperls.com/products/optical-imaging/ivis-university.php



Software

IVIS | Bioware and Reagents



- ✓ Bioware
- ✓ Bioware Ultra
- ✓ Bioware Ultra Red

IVIS | XenoLight

Suzen O'Coin
(508) 497-6489
suzen.ocoin@caliperls.com

- ✓ D-Luciferin Substrate

- ✓ NIR Fluorescent Reagents 680, 750, 770nm Protein Labeling Kits

- ✓ DiR

- ✓ RediJect D-Luciferin
- ✓ RediJect D-Luciferin Ultra