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HUMAN HEALTH | ENVIRONMENTAL HEALTH

# In Vivo Imaging
# IVIS Spectrum


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# What Will Be Covered?

**Introduction**

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

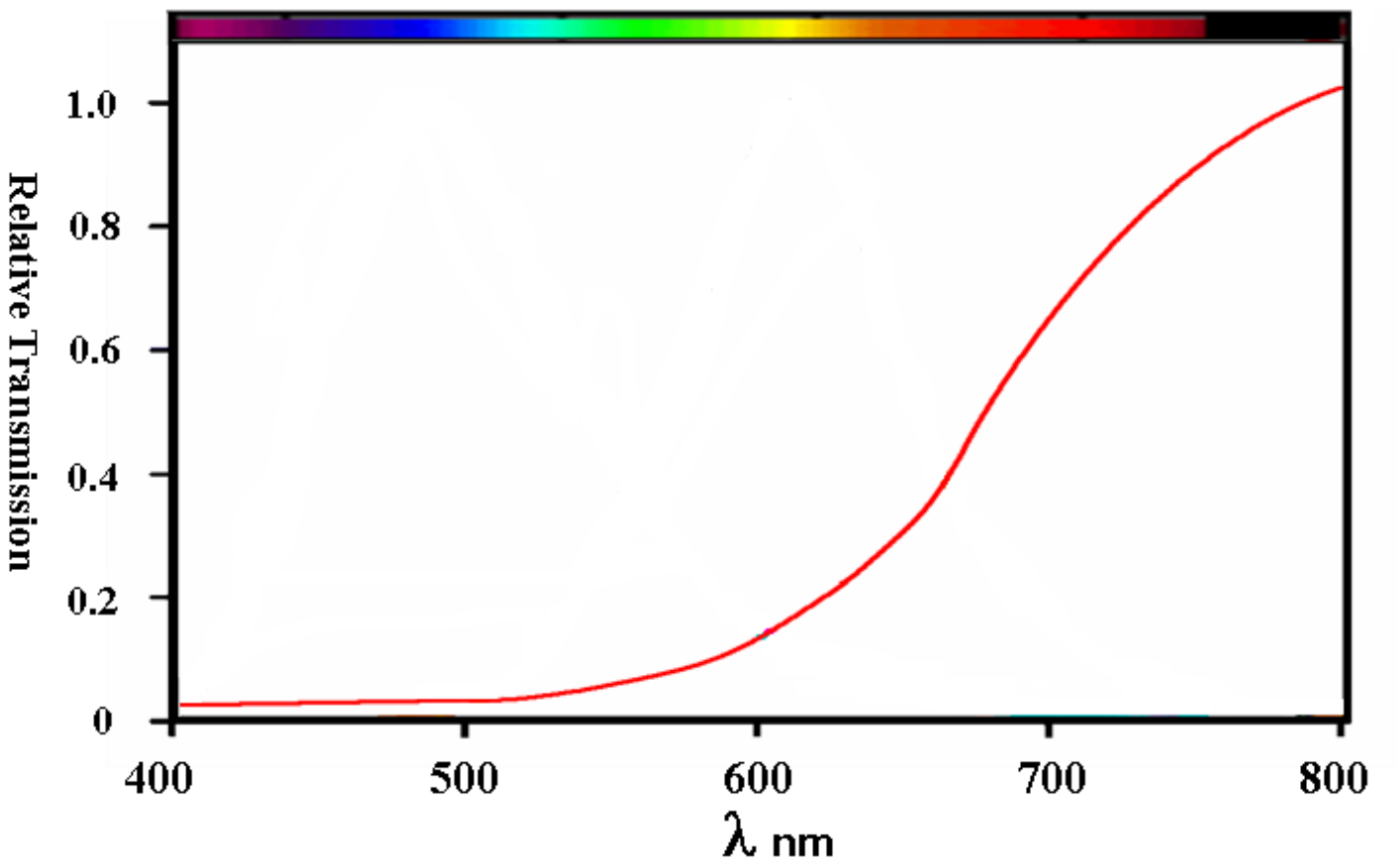
**Training**

- Hands-on Training

# Why Optical In Vivo Imaging?

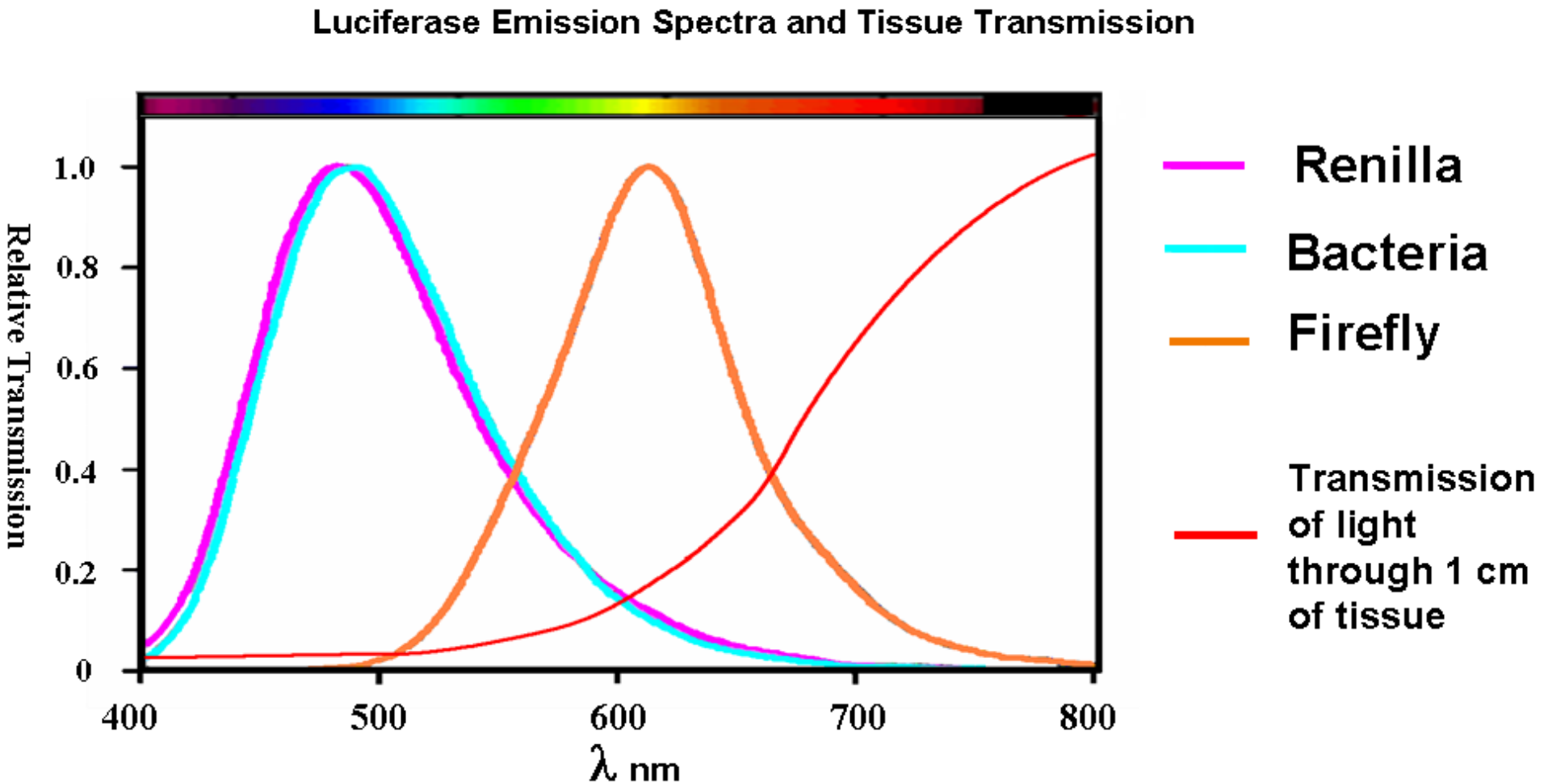
- Powerful labeling technique – gene expression results in production of luciferase
  - Amount of light is proportional to number of live active cells
  - Typical applications range from oncology studies, infectious diseases, imaging transgenic animals, stem cell development
- Non-invasive
  - Does not require subject to be euthanized
- Relatively simple instrumentation

# Tissue Is Not Transparent – Light Absorbance Depends on Wavelength

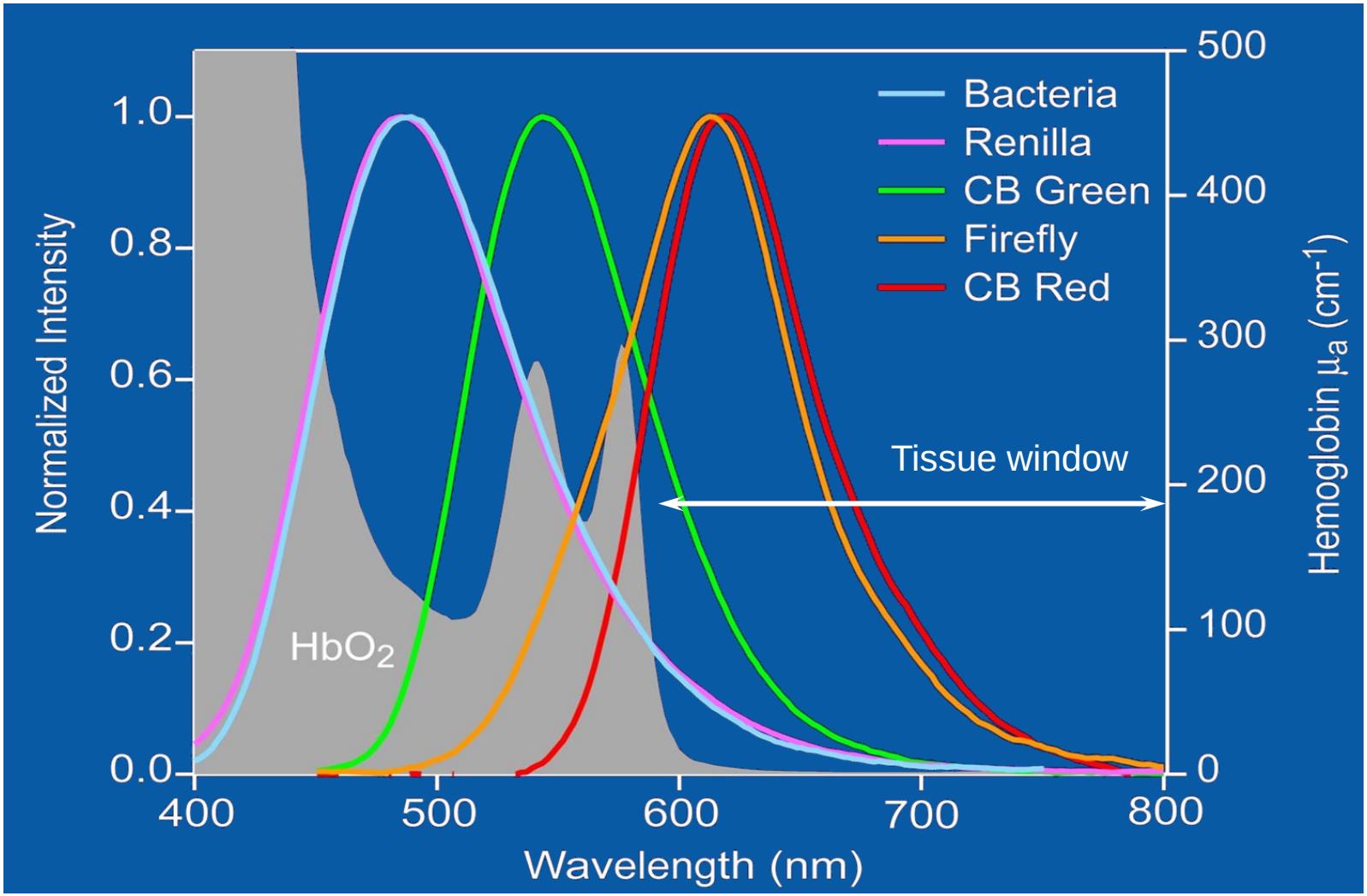



Transmission of light through 1 cm of tissue

# Tissue Is Not Transparent – Light Absorbance Depends on Wavelength



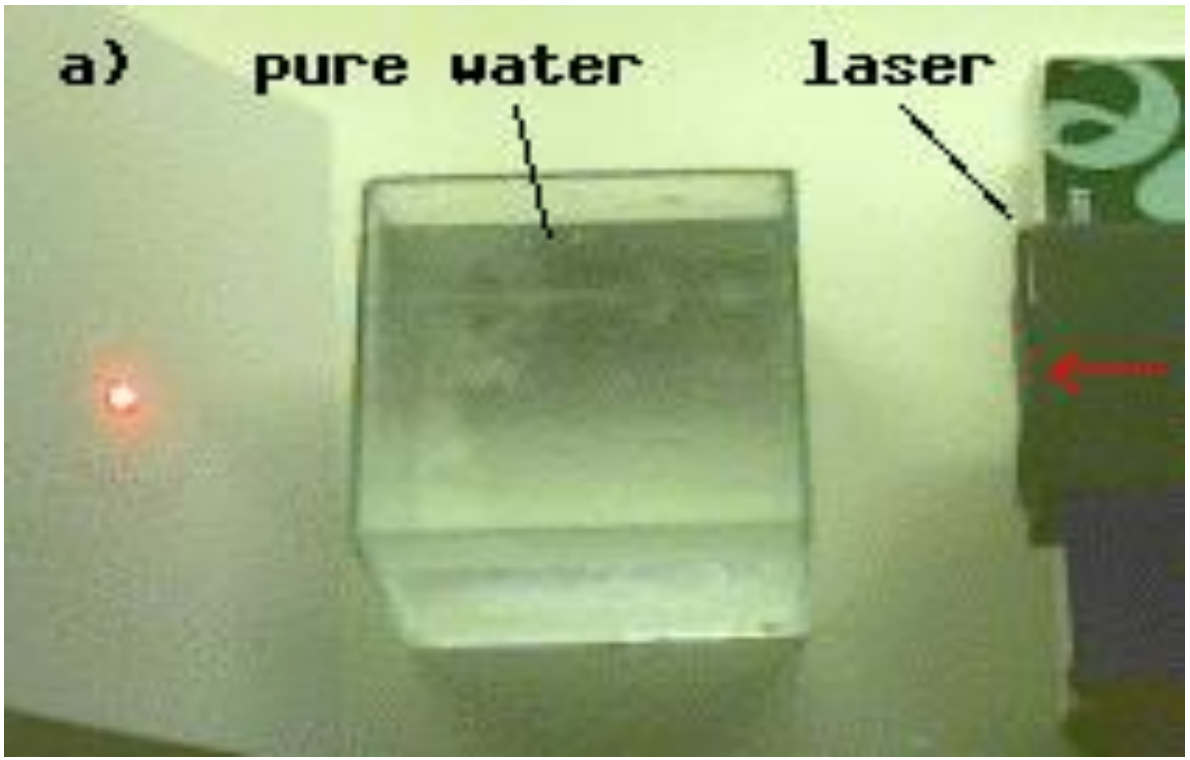
# Tissue Absorption





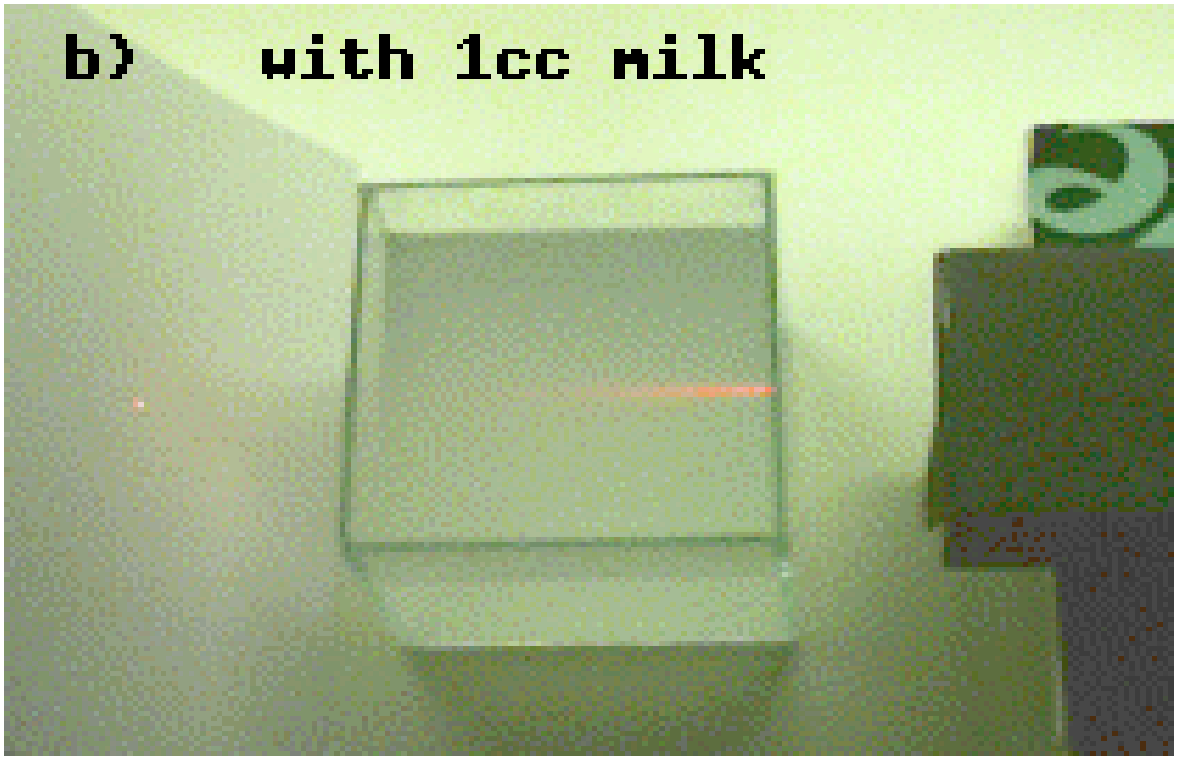

Science

# Light Transmission Through Clear Media




- Light will not scatter or diffuse traveling through pure water.

# Light Transmission Through Turbid Media

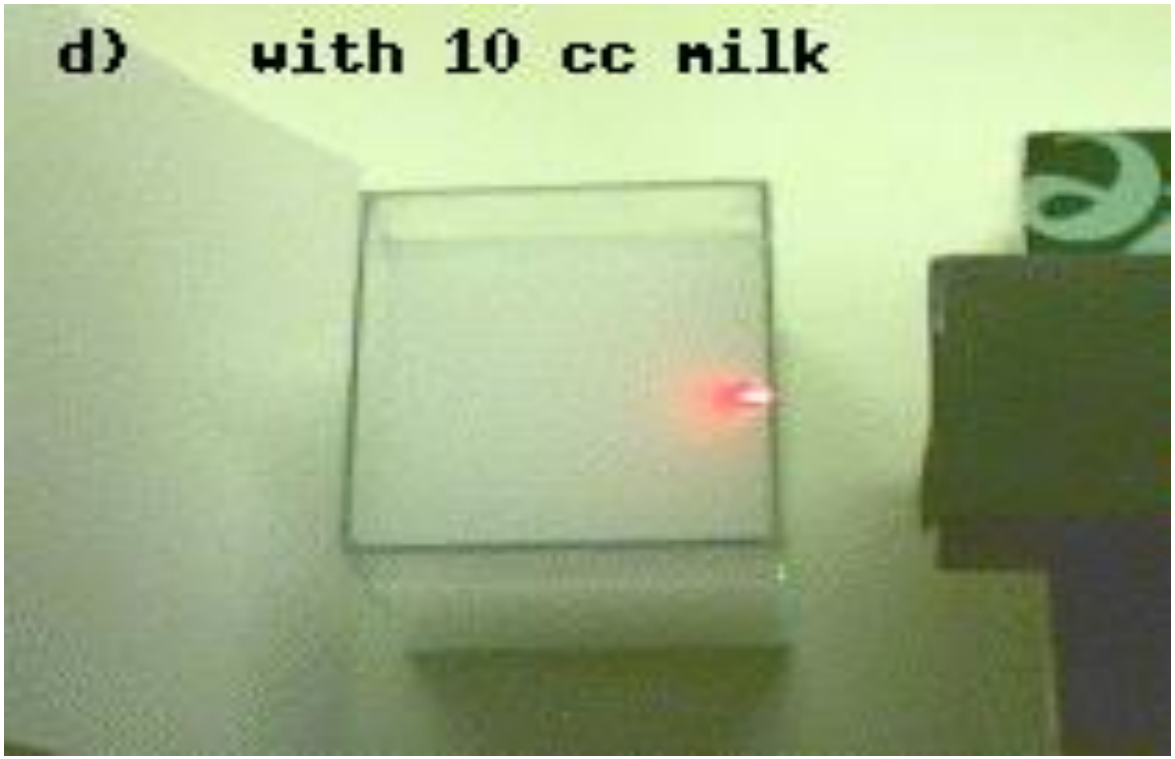



- Light scatters and diffuses traveling through water with added milk.

# Light Transmission Through Turbid Media

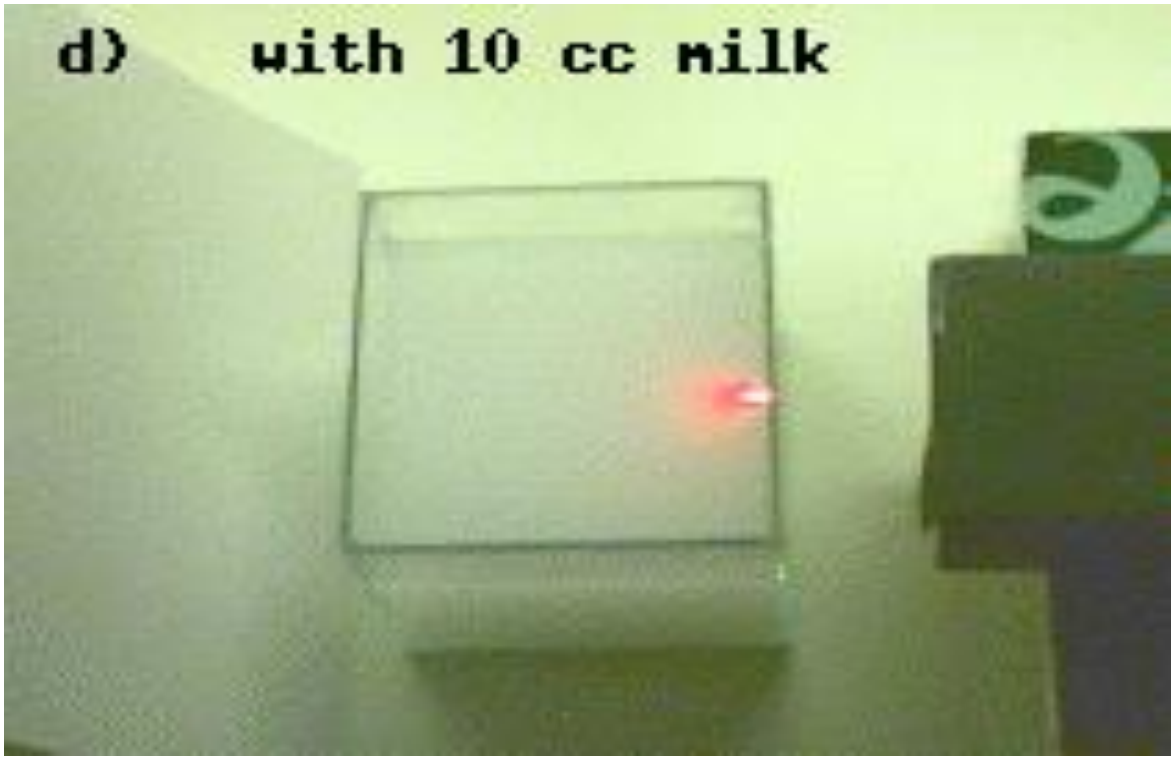

- Light scatters and diffuses traveling through water with added milk.

# Light Transmission Through Turbid Media



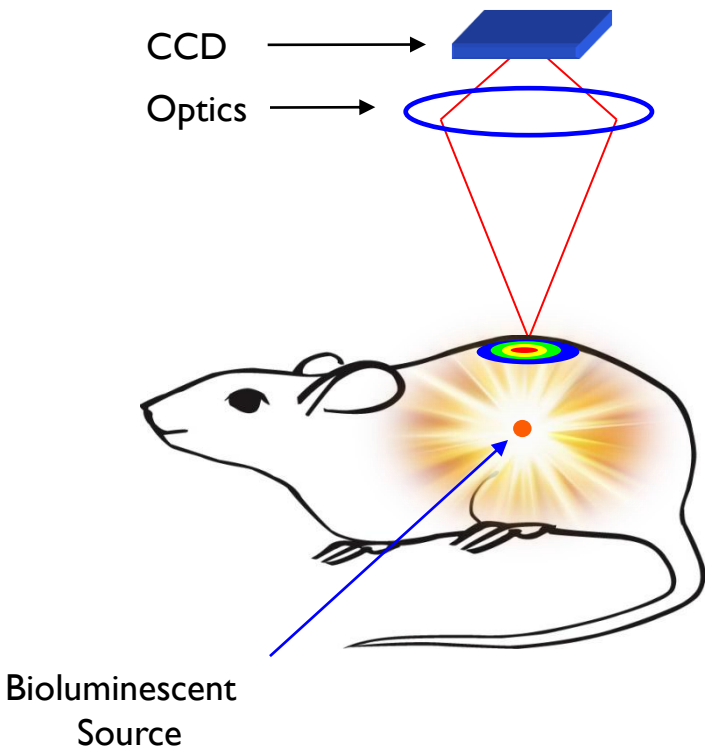

- Light scatters and diffuses traveling through water with added milk.

# Tissue Scatters Light Like Turbid Media




- Light scatters and diffuses traveling through water with added milk.

# Photons Diffuse Through Tissue – Surface Light Pattern is Recorded

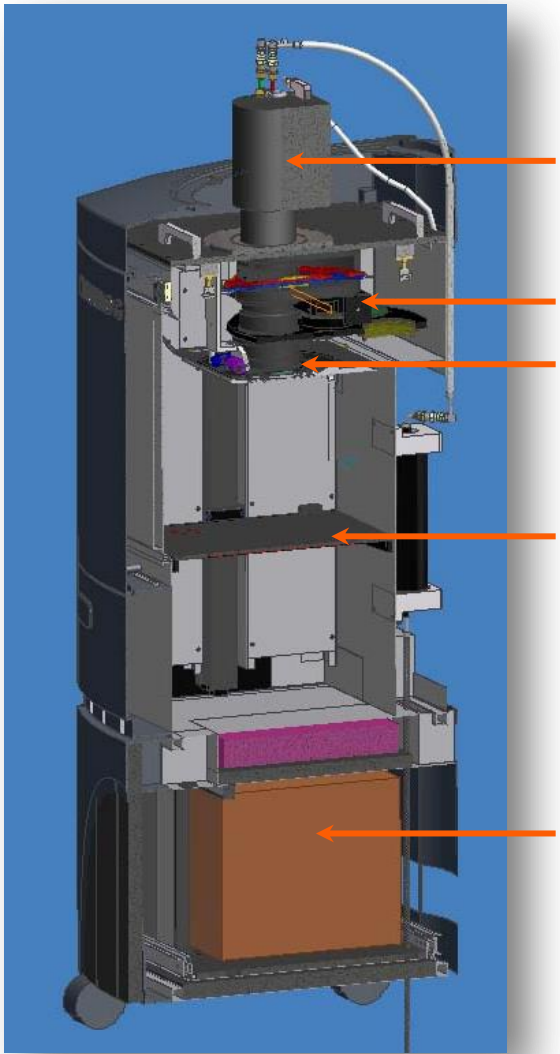


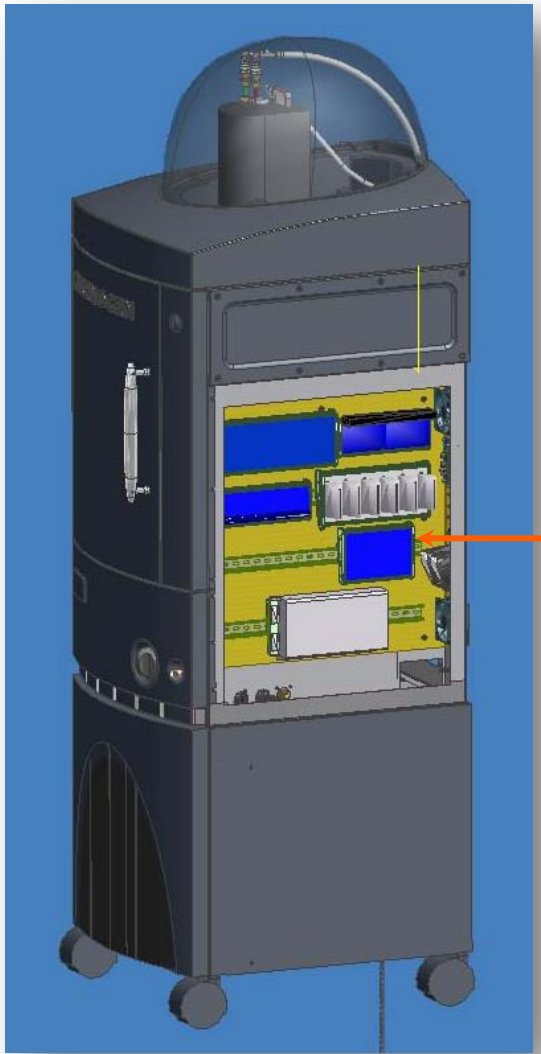

- Light traveling through tissue scatters many times creating a “fuzzy” light diffusion pattern on the surface of the animal
- The IVIS® views the diffuse light on the camera-facing (top) surface of the subject
- Not all light from the source will make it to the camera – light absorption will occur as signal exits the animal

# IVIS Spectrum Imaging System - Hardware

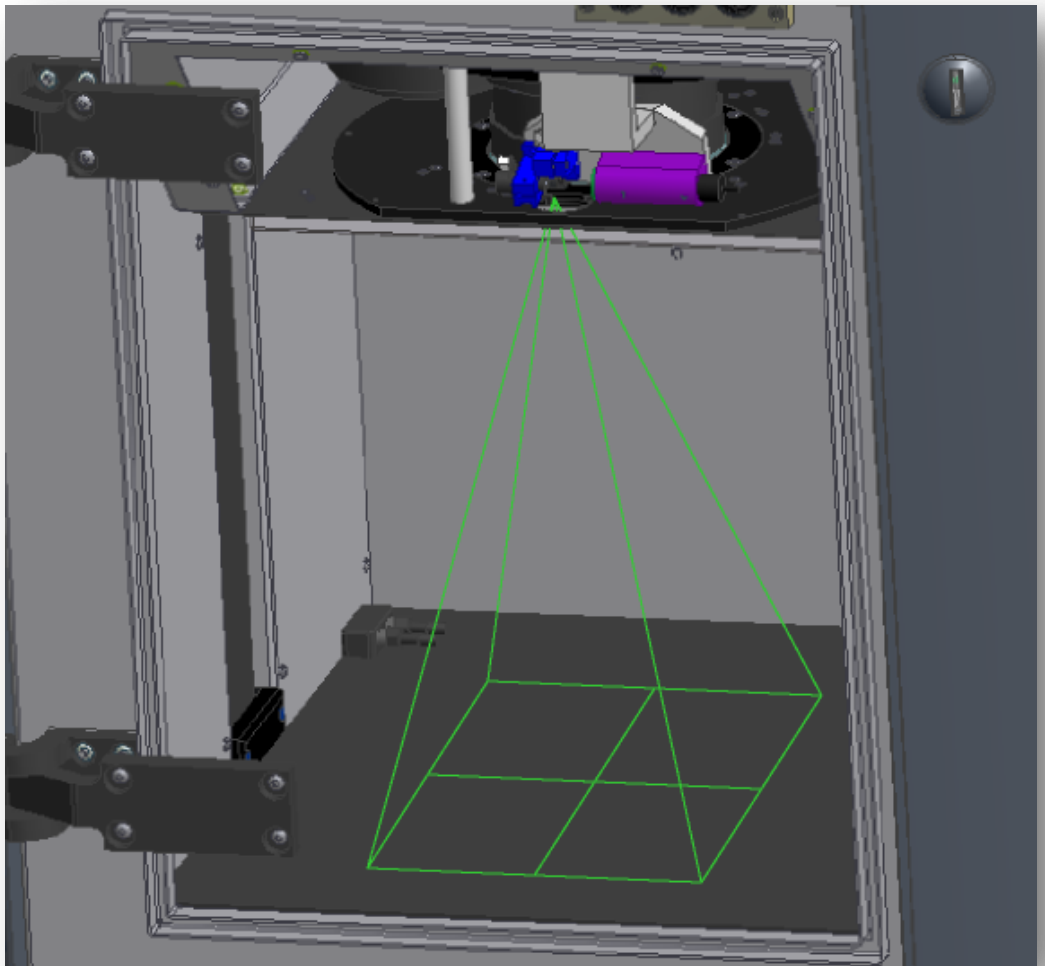

- Customized for *in vivo* imaging
- Highly sensitive camera with a large dynamic range

# IVIS Spectrum Imaging System - Hardware

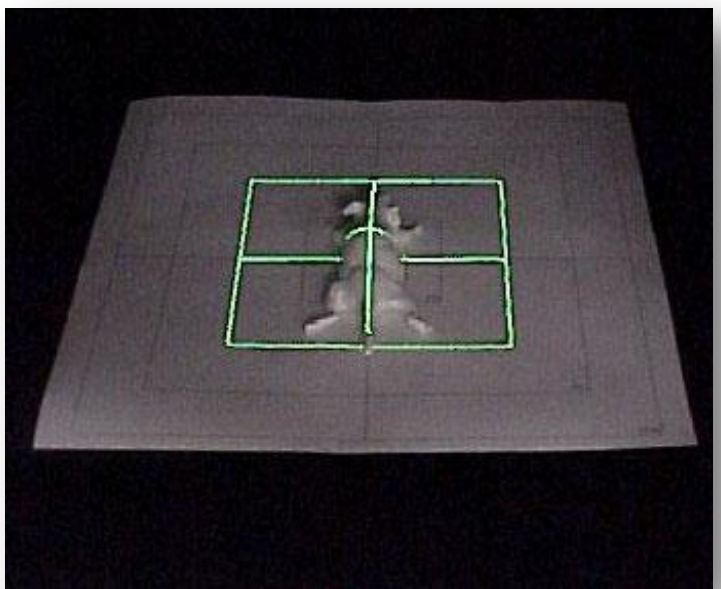


# Alignment Light Projector



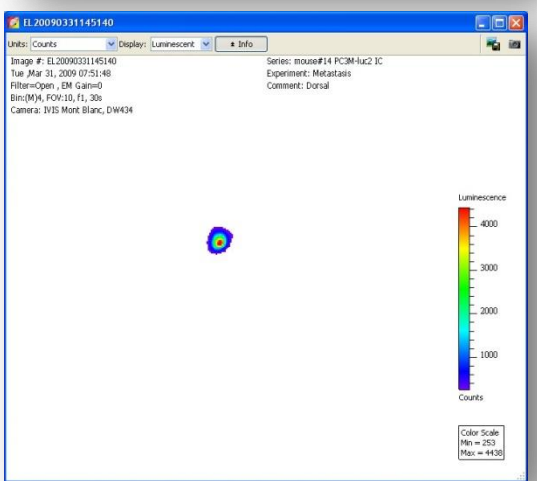
- Allows rapid and reproducible positioning of subjects
- Size change with Field of View setting



# Living Image® Software

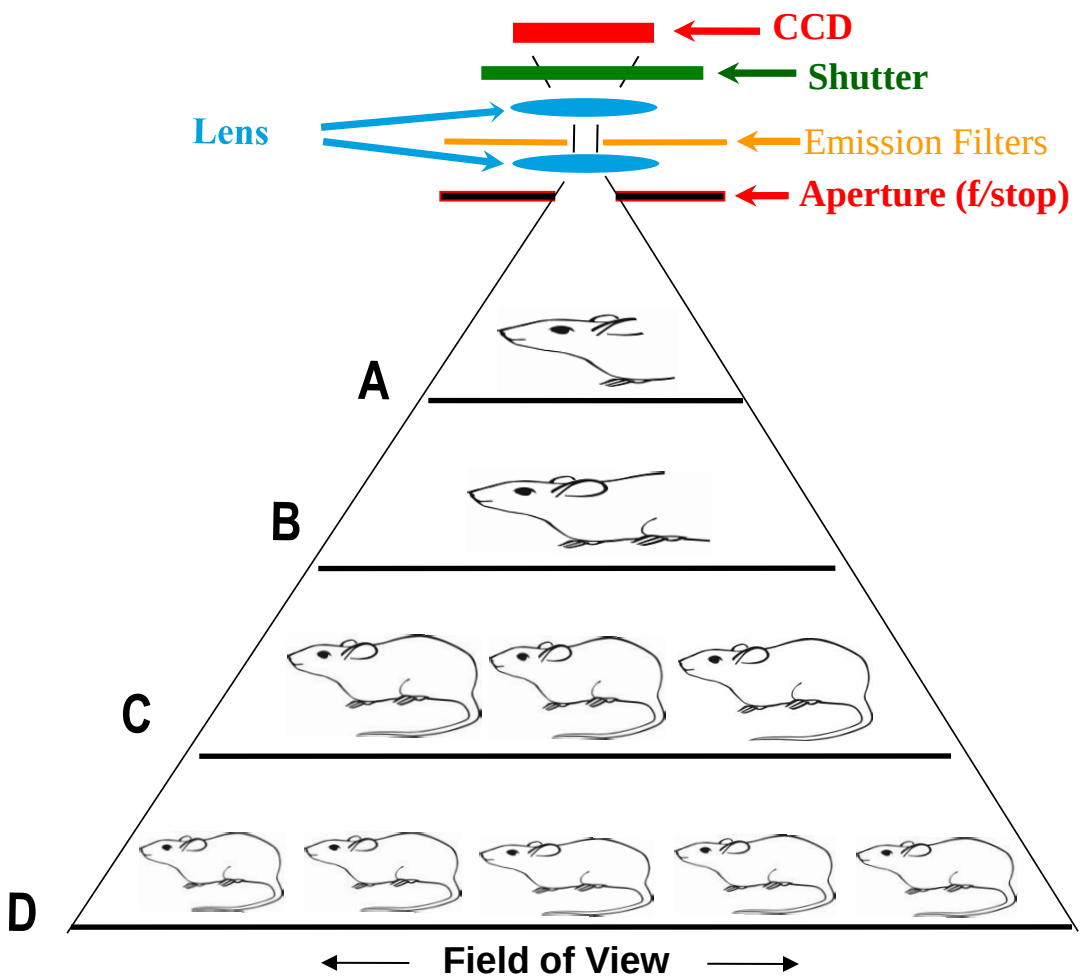
- Controls all settings in the IVIS® system (fully computer controlled)
- Provides advanced cataloging and browsing tools
- Provides analysis tools for quantification
- Instrument settings are analogous to photography
- Images are acquired in a two-step process

# Photographic + Luminescent = Overlay

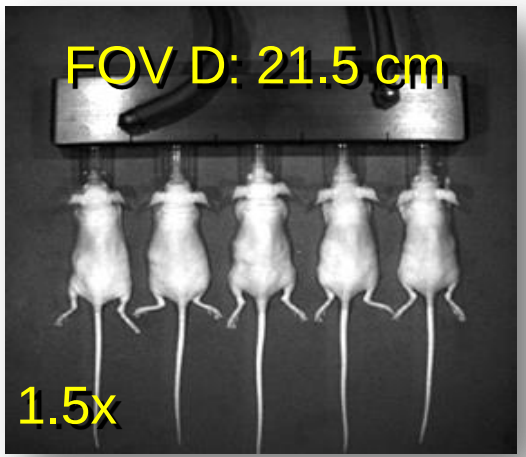



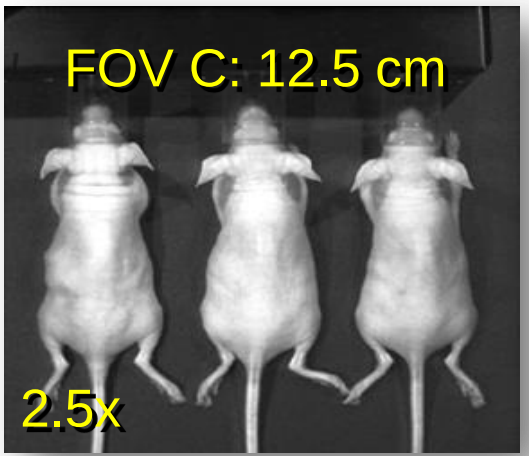

# Camera and Lens Settings are Analogous to Photography

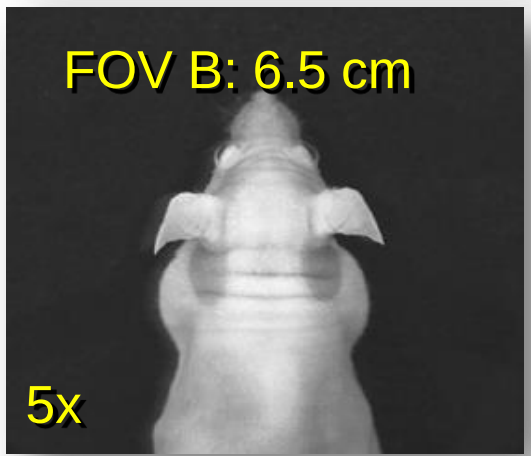
- Field of View (FOV) is dependent on the distance from the lens to the sample
- Light collected is proportional to how long the shutter is open (exposure time)
- Aperture (*f*/stop) controls the amount of light collected
- Digital pixel binning is possible on the CCD – alters sensitivity/resolution

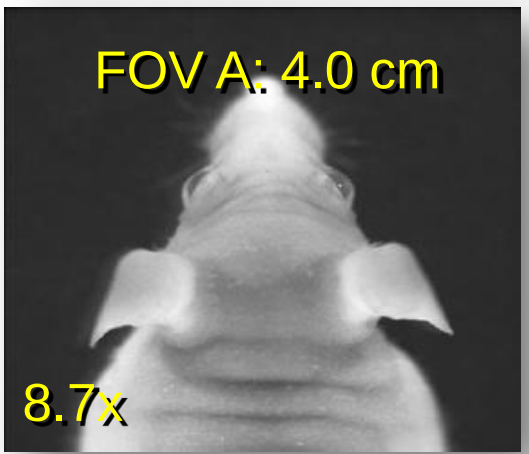


# Field of View Options



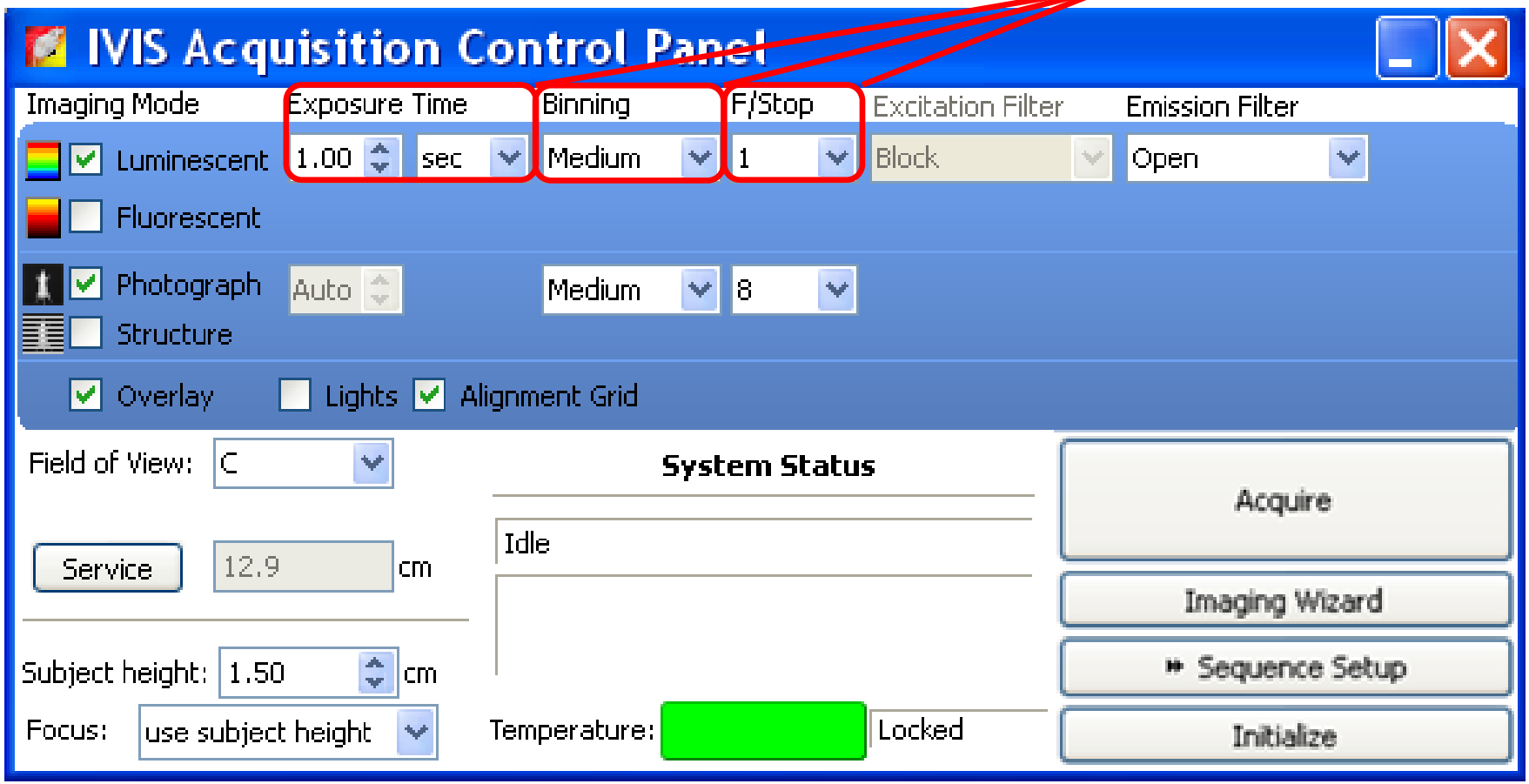



# Setting Sensitivity – Signal Level

- The IVIS® CCD camera has a raw signal range of 0 to 65,535 Analog to Digital counts ($2^{16}$ or 16-bit)
- Adjust camera settings to obtain a signal level of **600 to 60,000 counts** to be within the linear range of the detector
- Settings that control signal level are:
  - Exposure time
  - Pixel binning (CCD resolution)
  - *f*/stop (aperture)
- Instrument is calibrated to automatically compensate for changes in sensitivity settings when count levels are within the linear range

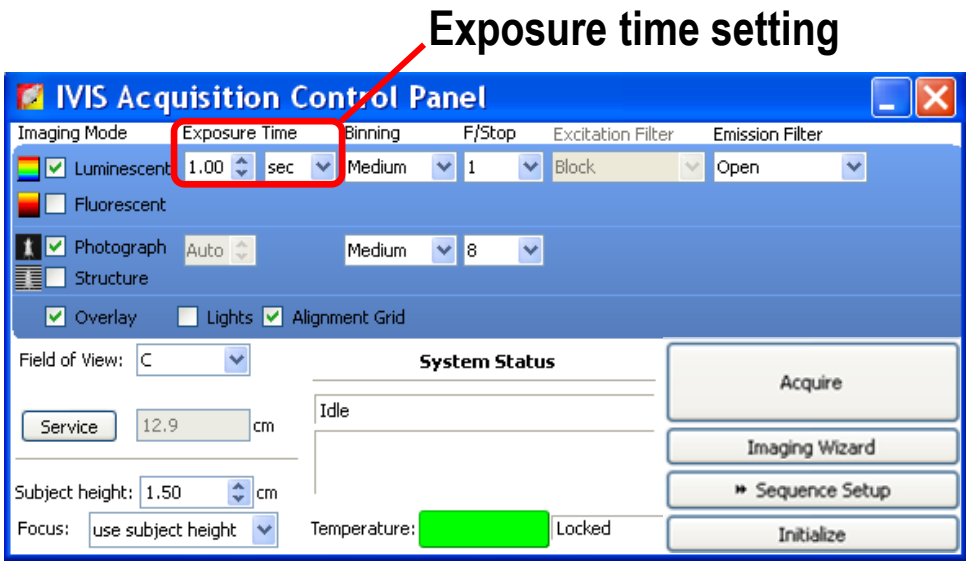
# Living Image® Control Panel

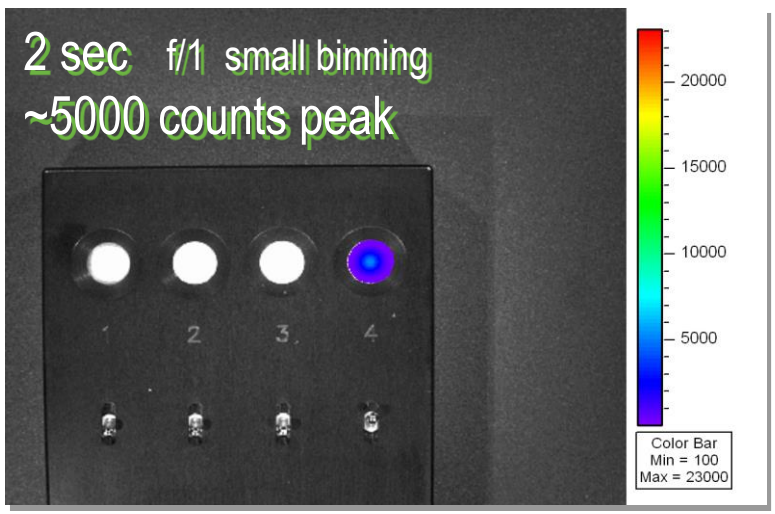
**Controls Sensitivity**

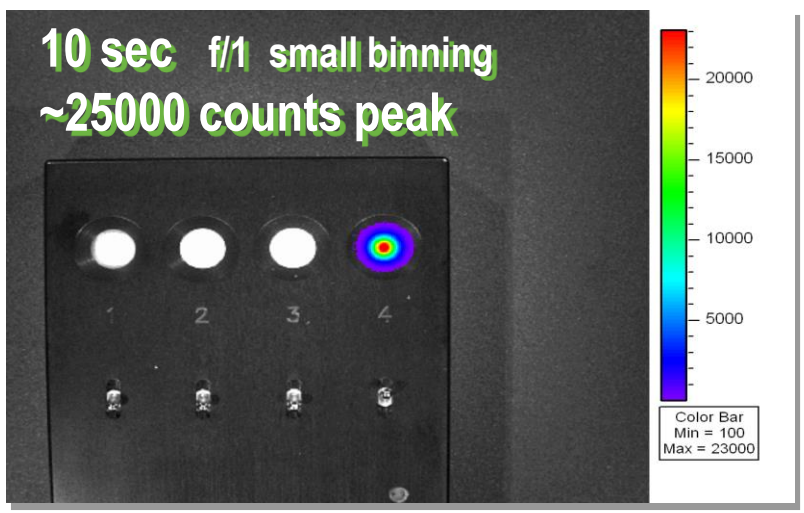


# Exposure Time

- Signal level is directly proportional to exposure time (1:1)
- Shorter exposure time improves throughput
- Recommended minimum exposure time > 0.5 seconds
- Longer exposure times increase signal intensity
- Recommended maximum exposure time < 5 minutes

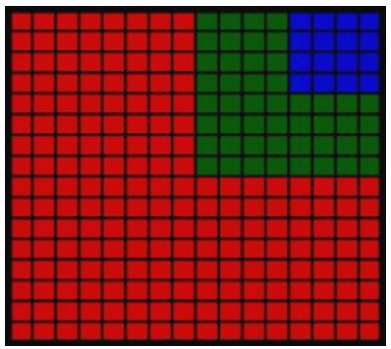


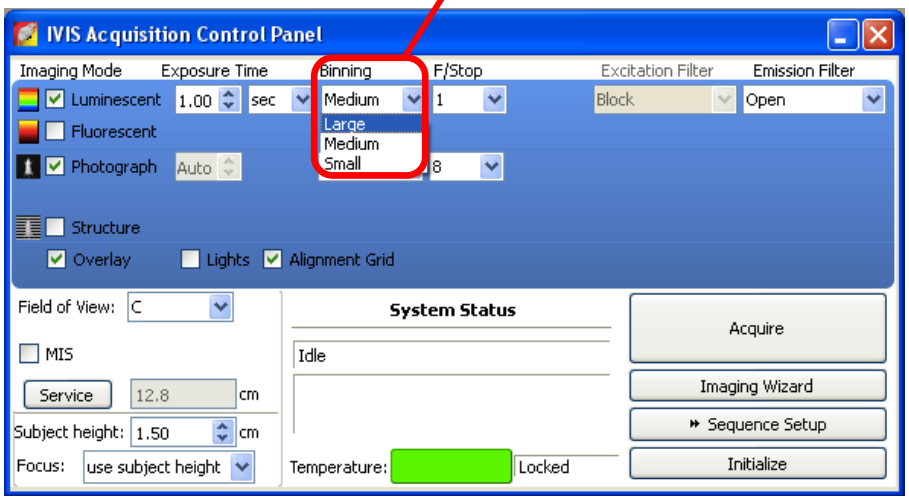



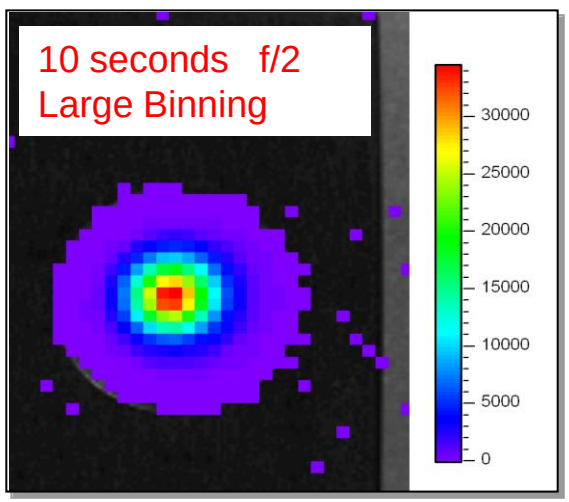
# Pixel Binning (CCD Resolution)

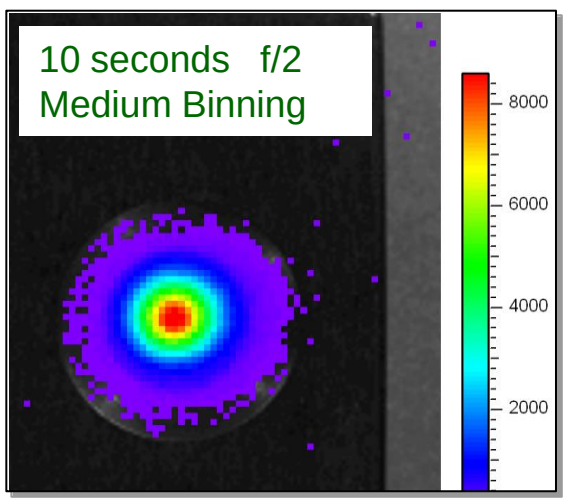
- Binning refers to the grouping of pixels into a larger super-pixel
- Changing binning settings changes counts by a factor of 4
- Large Binning (16)
  Higher Sensitivity/Lower Resolution
- Medium Binning (8)
- Small Binning (4)
  Lower Sensitivity/Higher Resolution

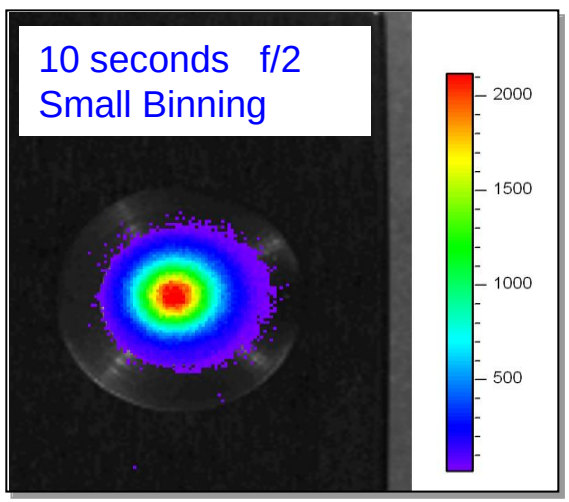


**Pixel binning setting**



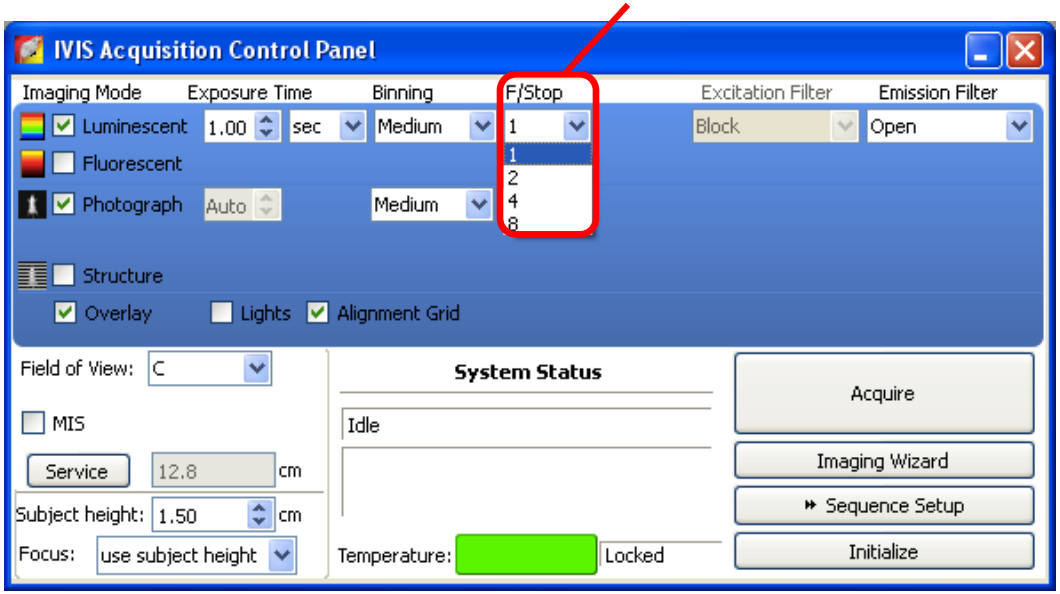



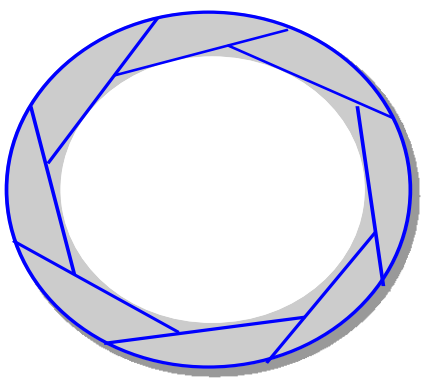
# *f*/stop (Lens Aperture)

- *f*/stop controls the amount of light received by the CCD detector
- *f*/1 is wide open, maximum light collection – default for luminescent
- *f*/8 is smallest aperture, best resolution – default for photo
- Changing f/stop changes counts by a factor of 4

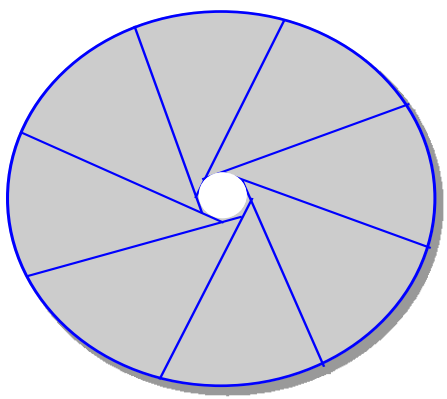
***f*/stop (aperture) setting**




***f*/1**

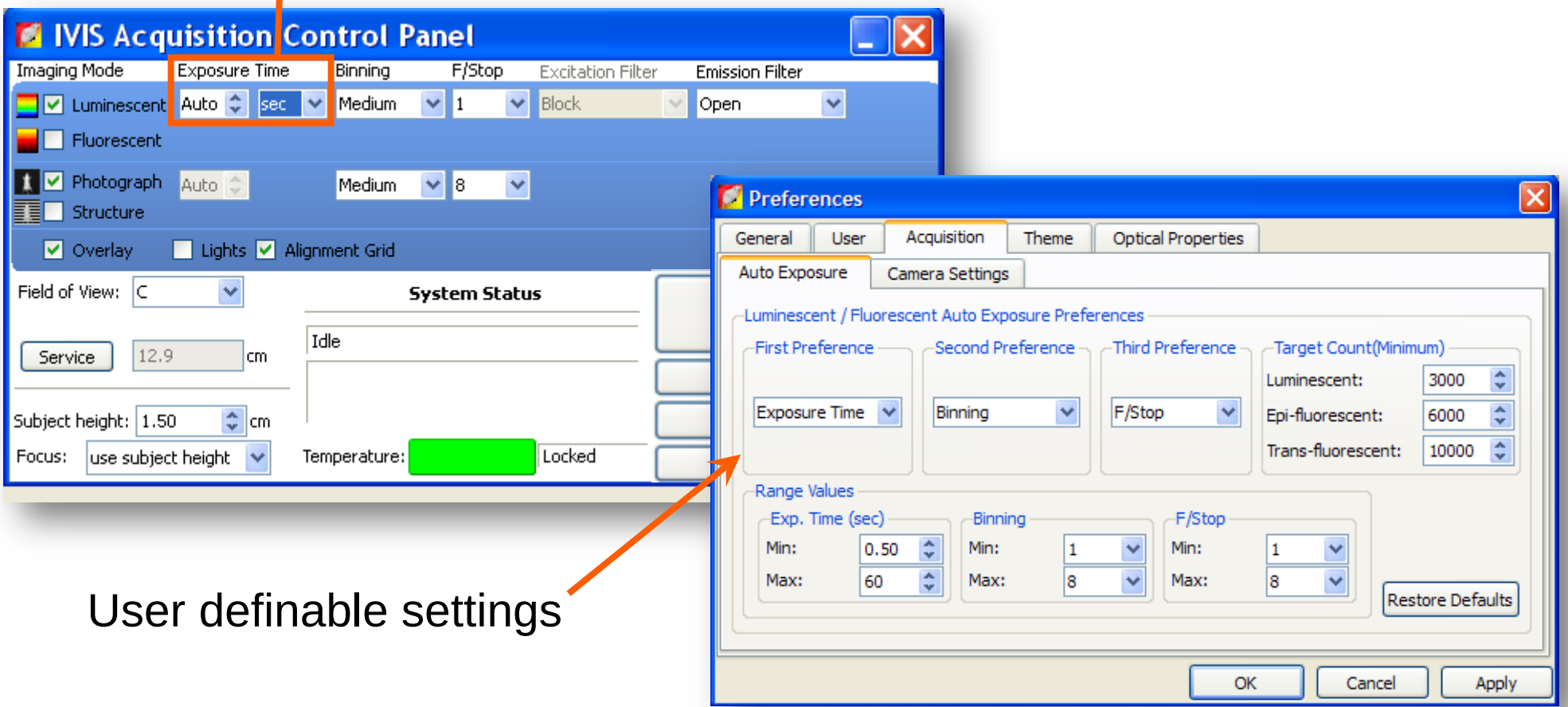


***f*/8**



# Auto-Exposure

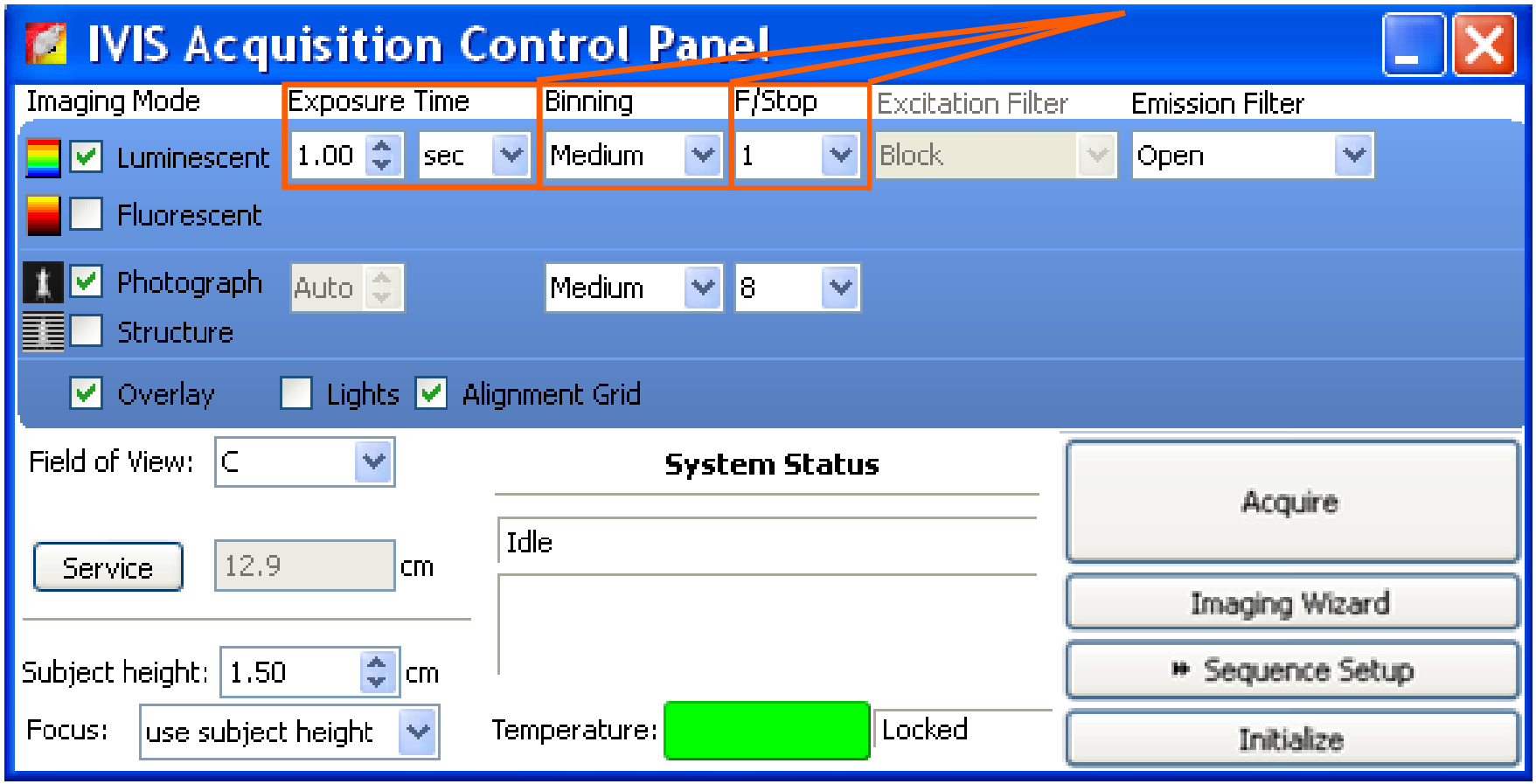
Auto-exposure feature available for bioluminescence and fluorescence



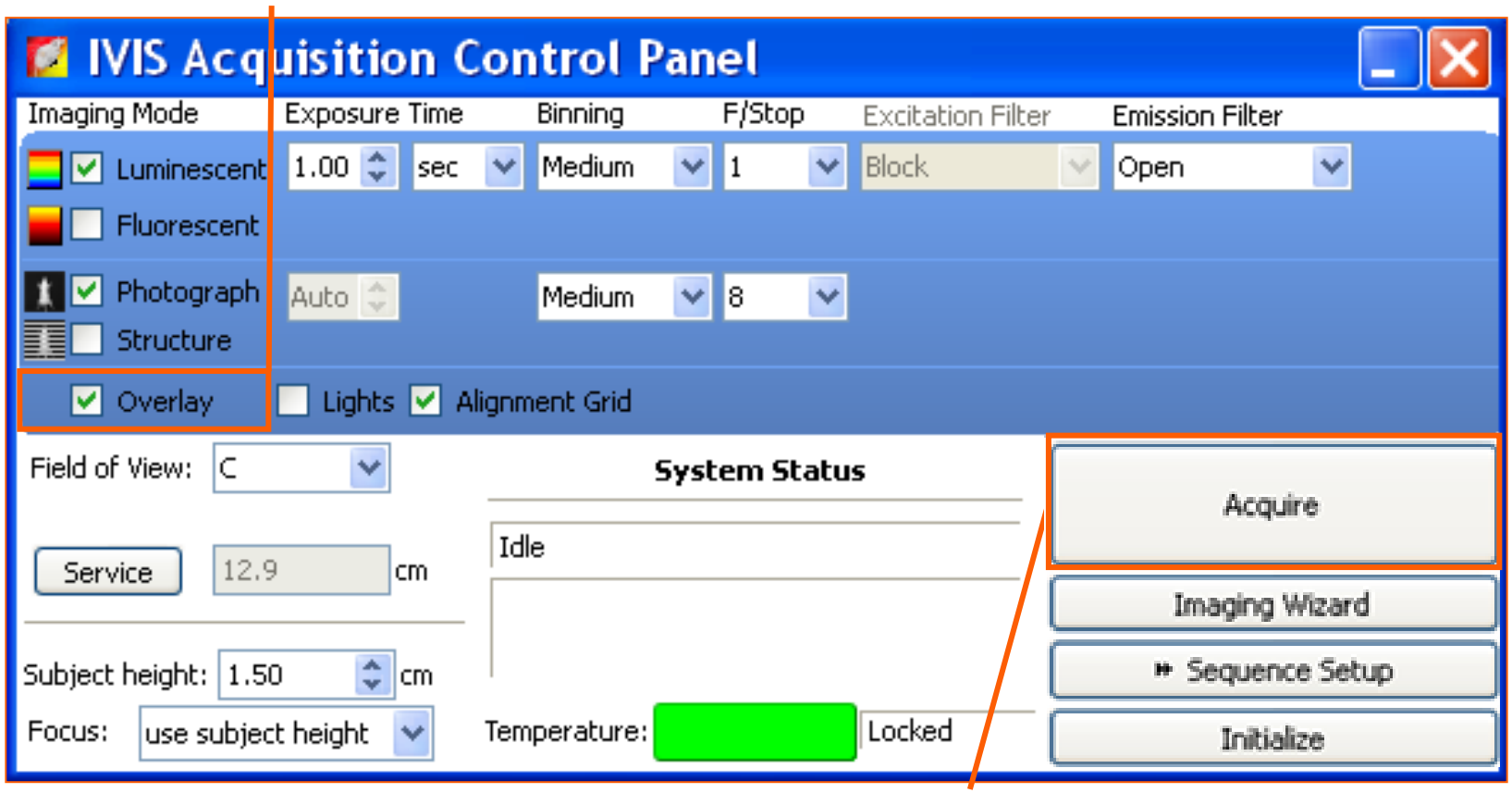

User definable settings

# Summary – Basic Camera Settings

Controls Sensitivity



Overlay will automatically take Photo + Luminescent

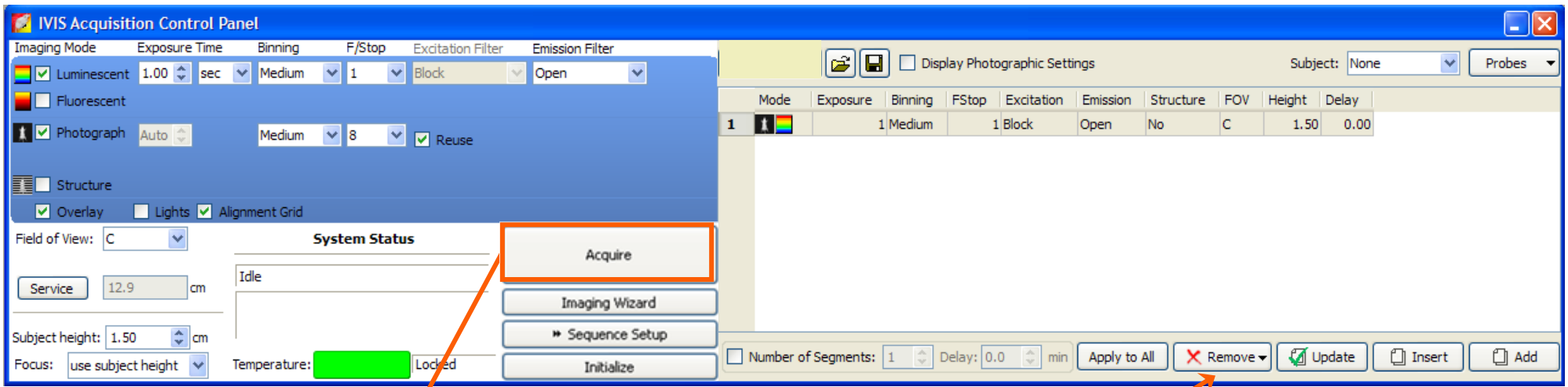



Single Image Acquisition

# Sequence (or Imaging Wizard) Acquisition

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

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

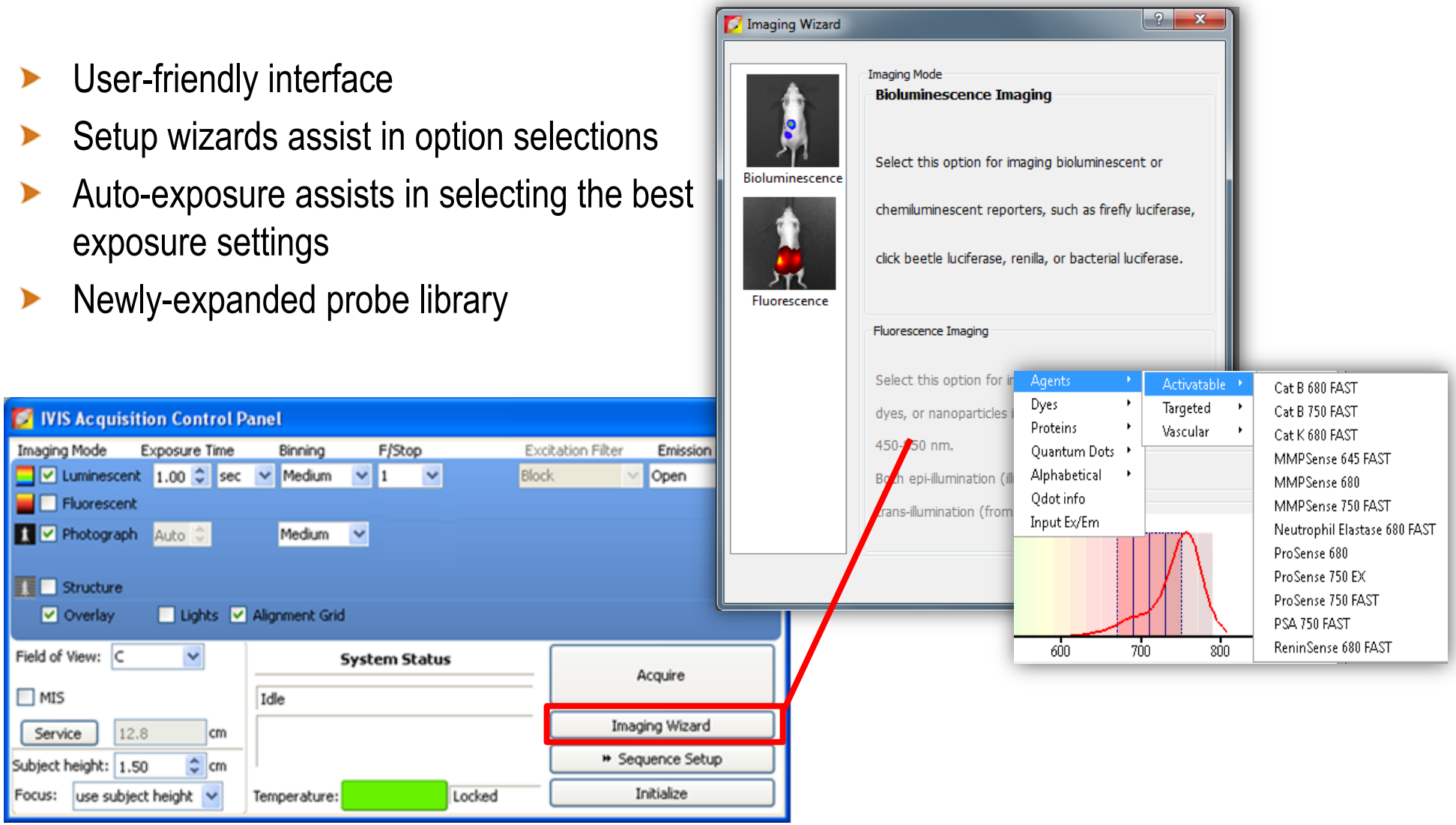



Starts Sequential Image Acquisition

User Friendly Sequence Editor

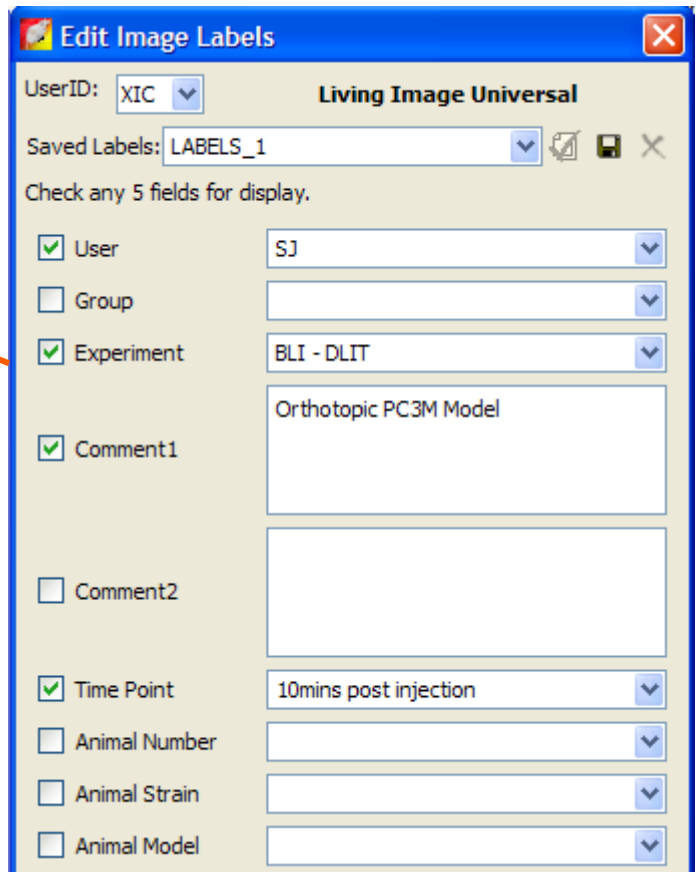
# Living Image

- User-friendly interface
- Setup wizards assist in option selections
- Auto-exposure assists in selecting the best exposure settings
- Newly-expanded probe library

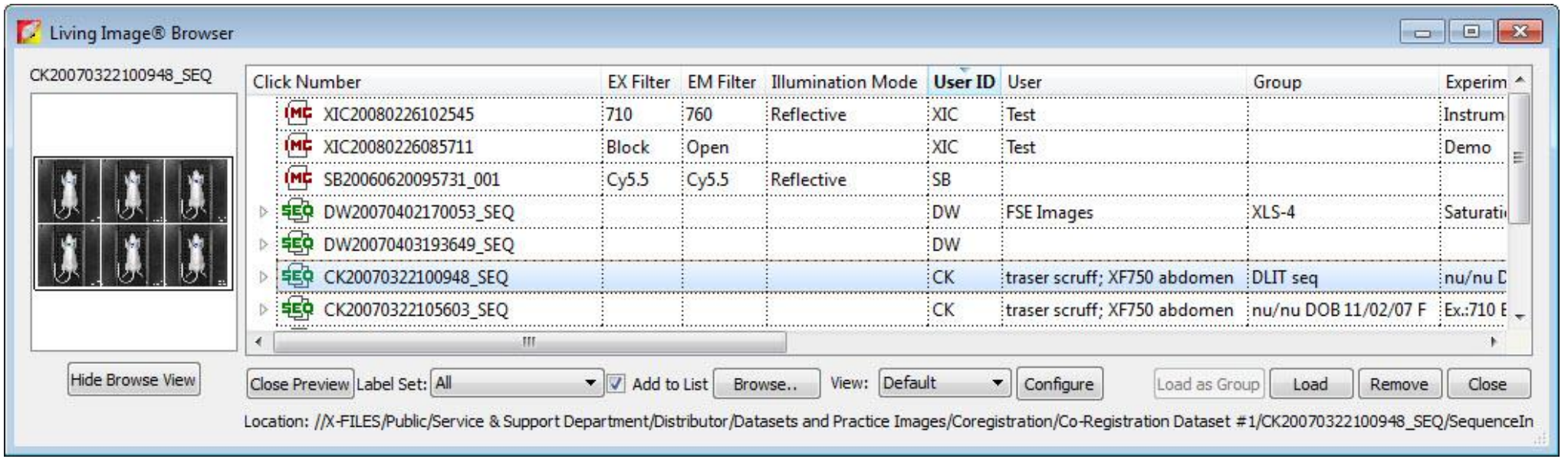


# Image Labeling

- Good labeling practices are necessary for effective data browsing
- Easily label your image while acquisition is taking place

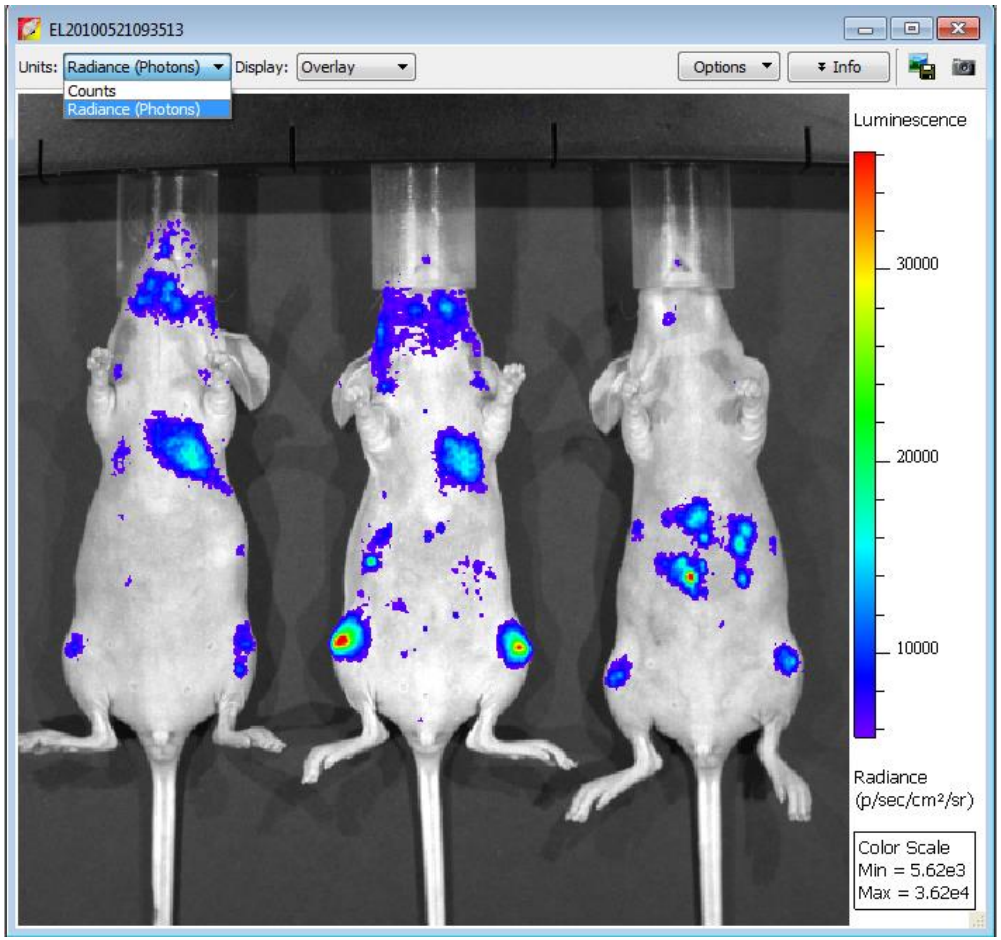
# Image Cataloging and Browsing Tools

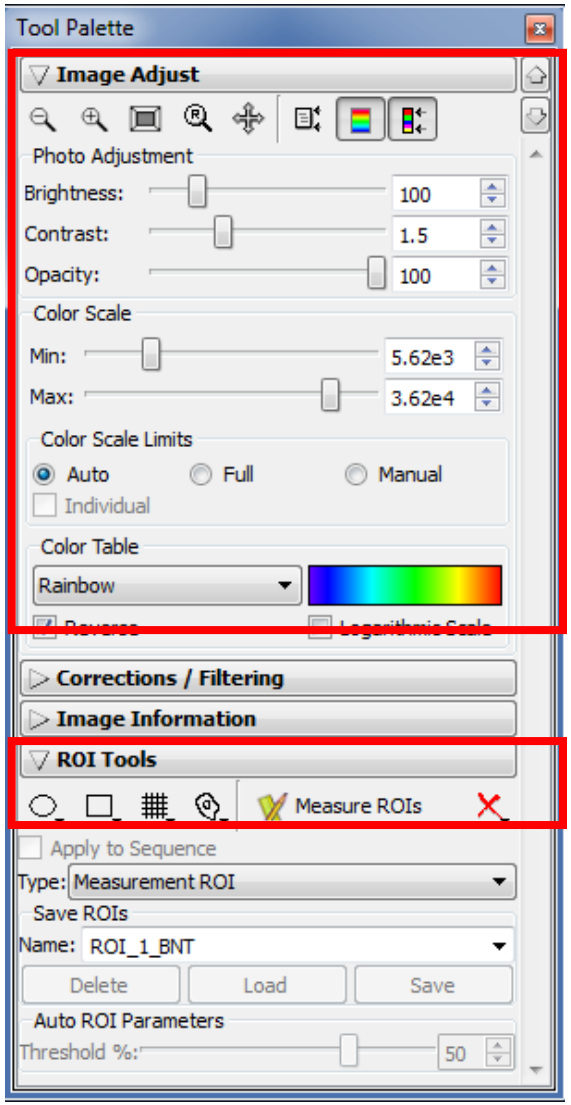



- Convenient preview window
- User defined labels listed with corresponding click number
- Sort by one or multiple columns
- Open multiple images in a single window for easier analysis with Load as Group

# Quantification

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



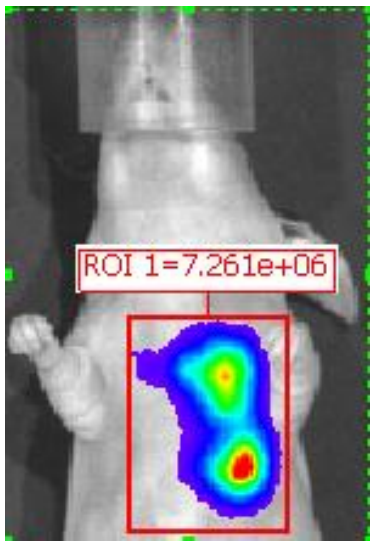



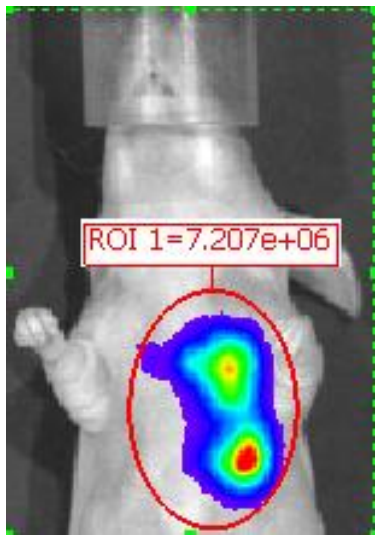
# Region of Interest (ROI) Tools

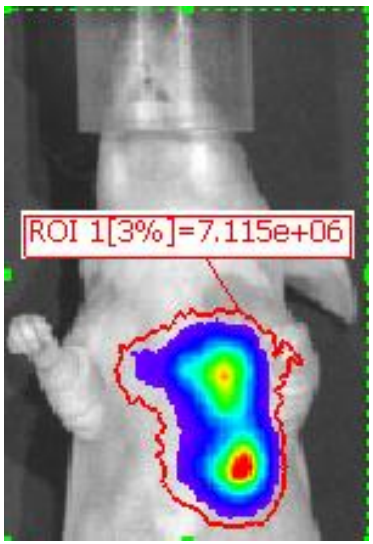
- ROI shapes available:
  - Square
  - Circle
  - Contour
  - Grid

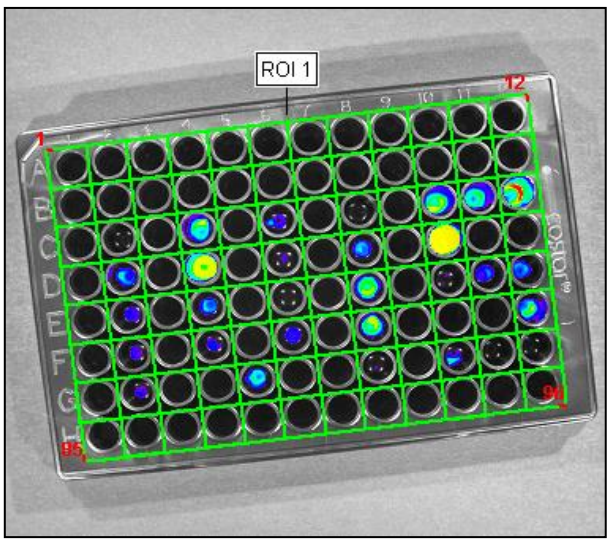
ROI's can be created:

- Manually
- Automatically
- Free Draw

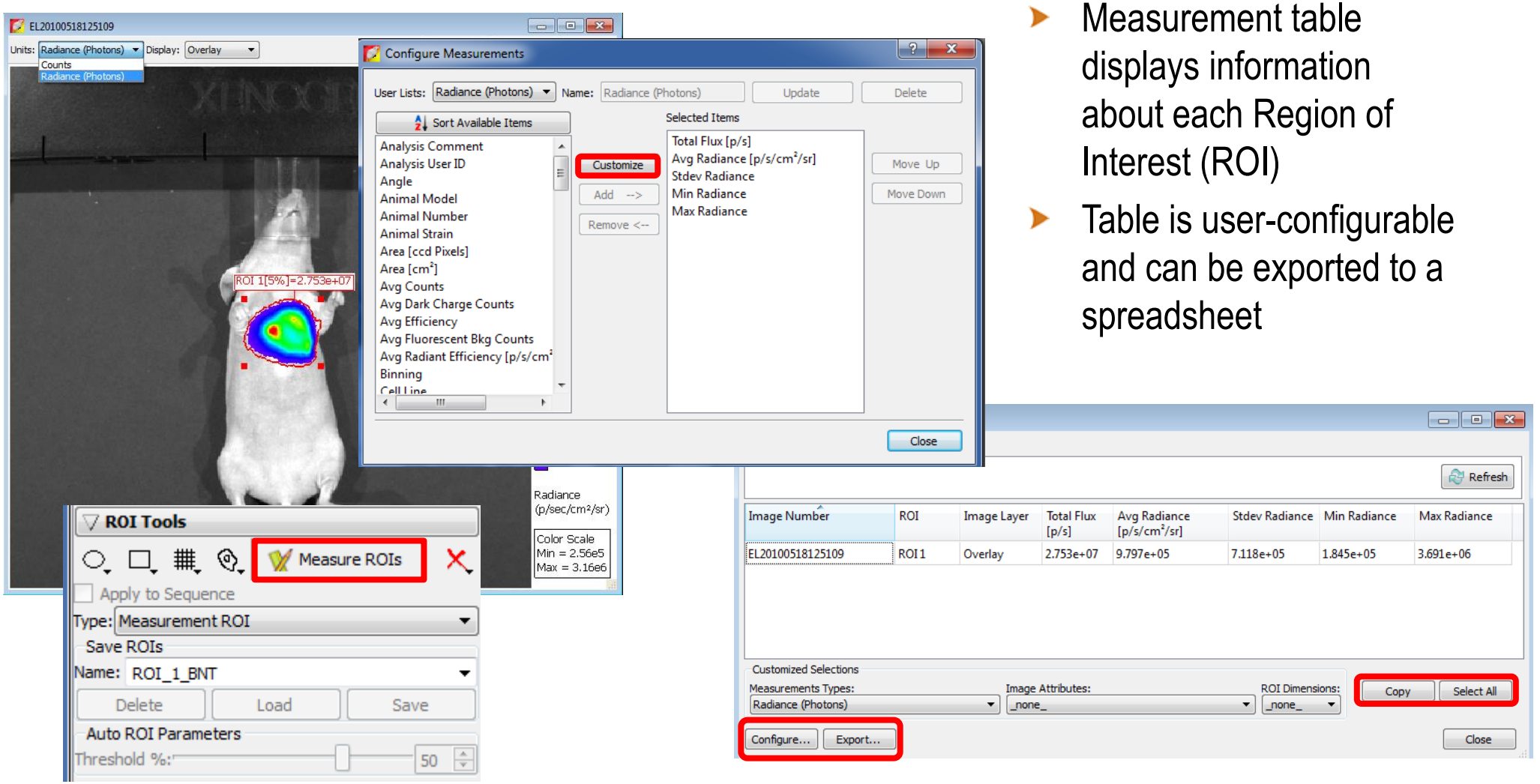


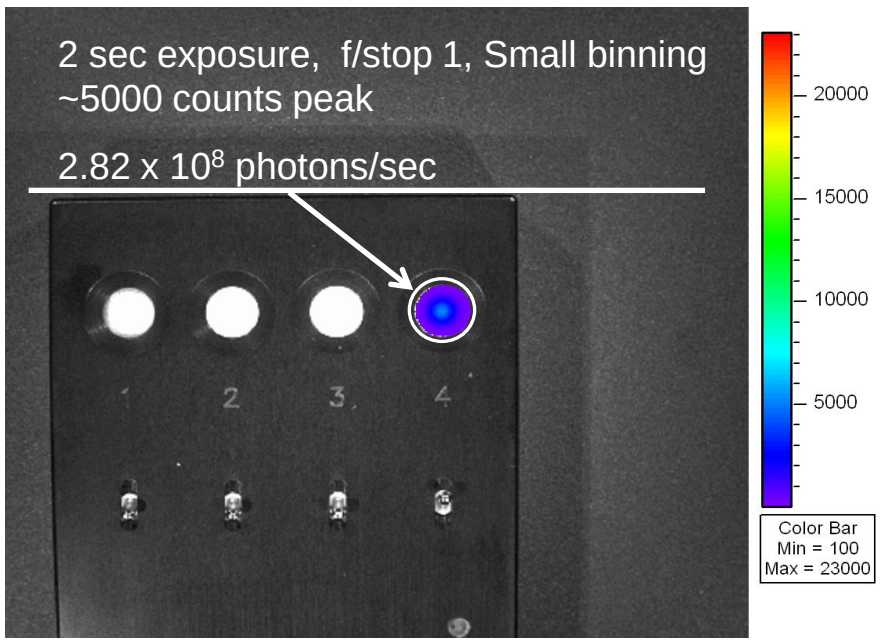
# Measurement Table

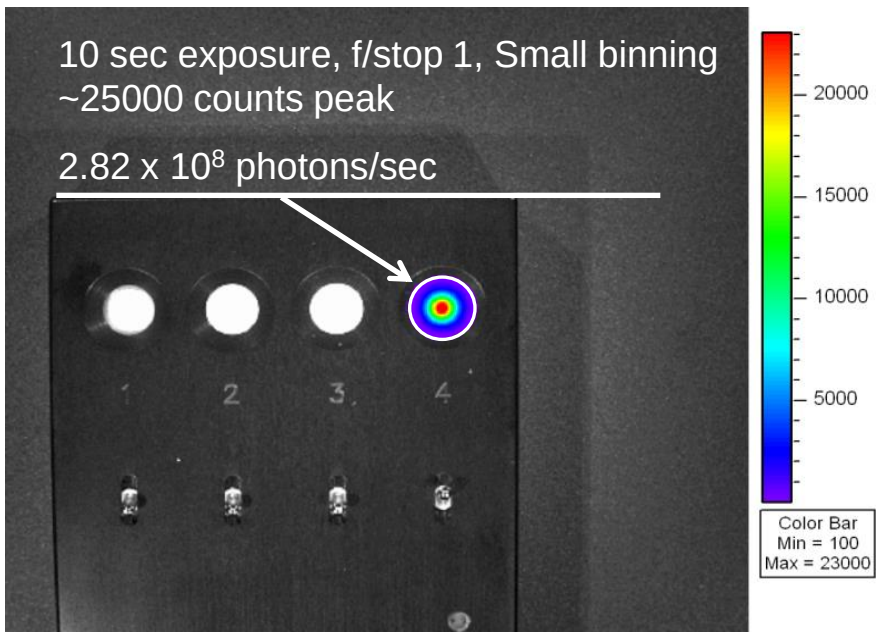



- Measurement table displays information about each Region of Interest (ROI)
- Table is user-configurable and can be exported to a spreadsheet

# Calibrated Physical Units

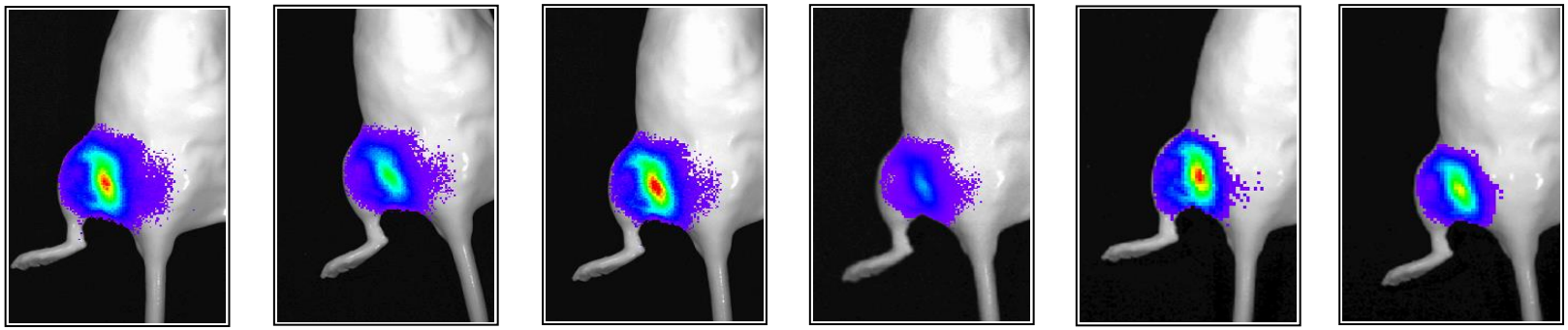
- Living Image® automatically compensates for device settings: Exposure time, *f*/stop, binning and field of View.
- Calibrated units are Photons per Second, representing the flux radiating omni-directionally from a user-defined region



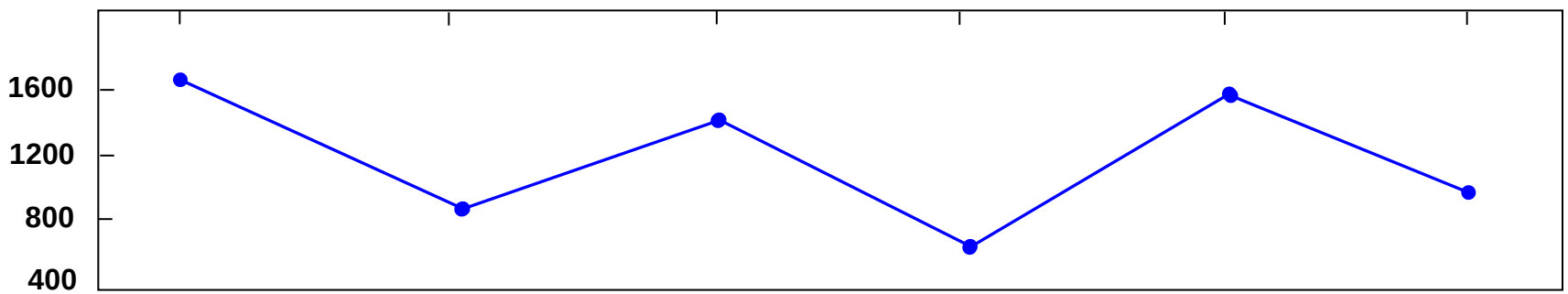
# Calibrated Physical Units vs. Raw Signal

**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 |

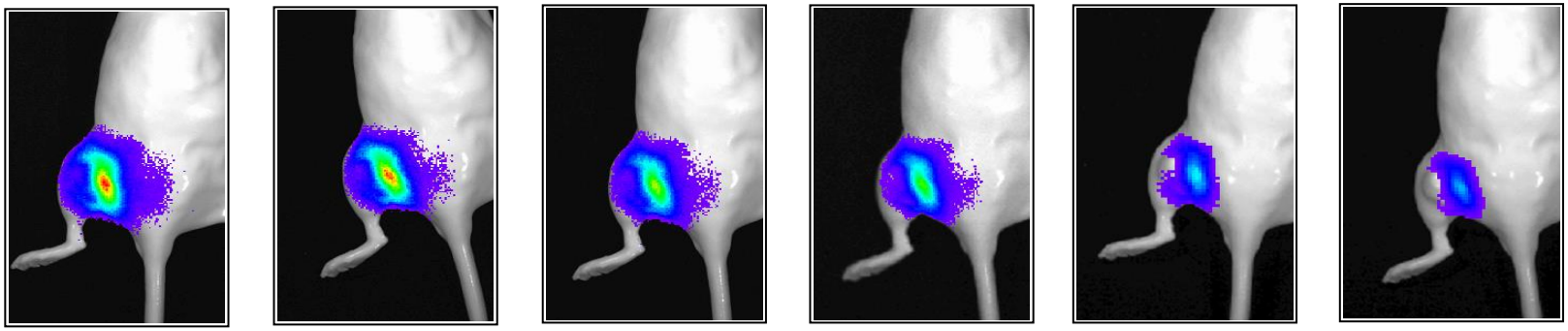
***Peak Counts***



# Calibrated Physical Units vs. Raw Signal

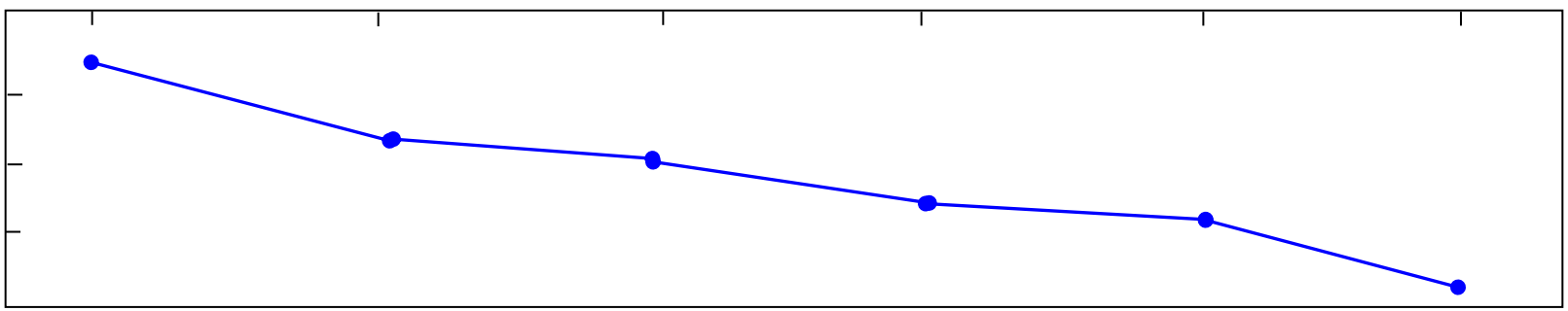
**Calibrated Signal**

***(Photons per second)***

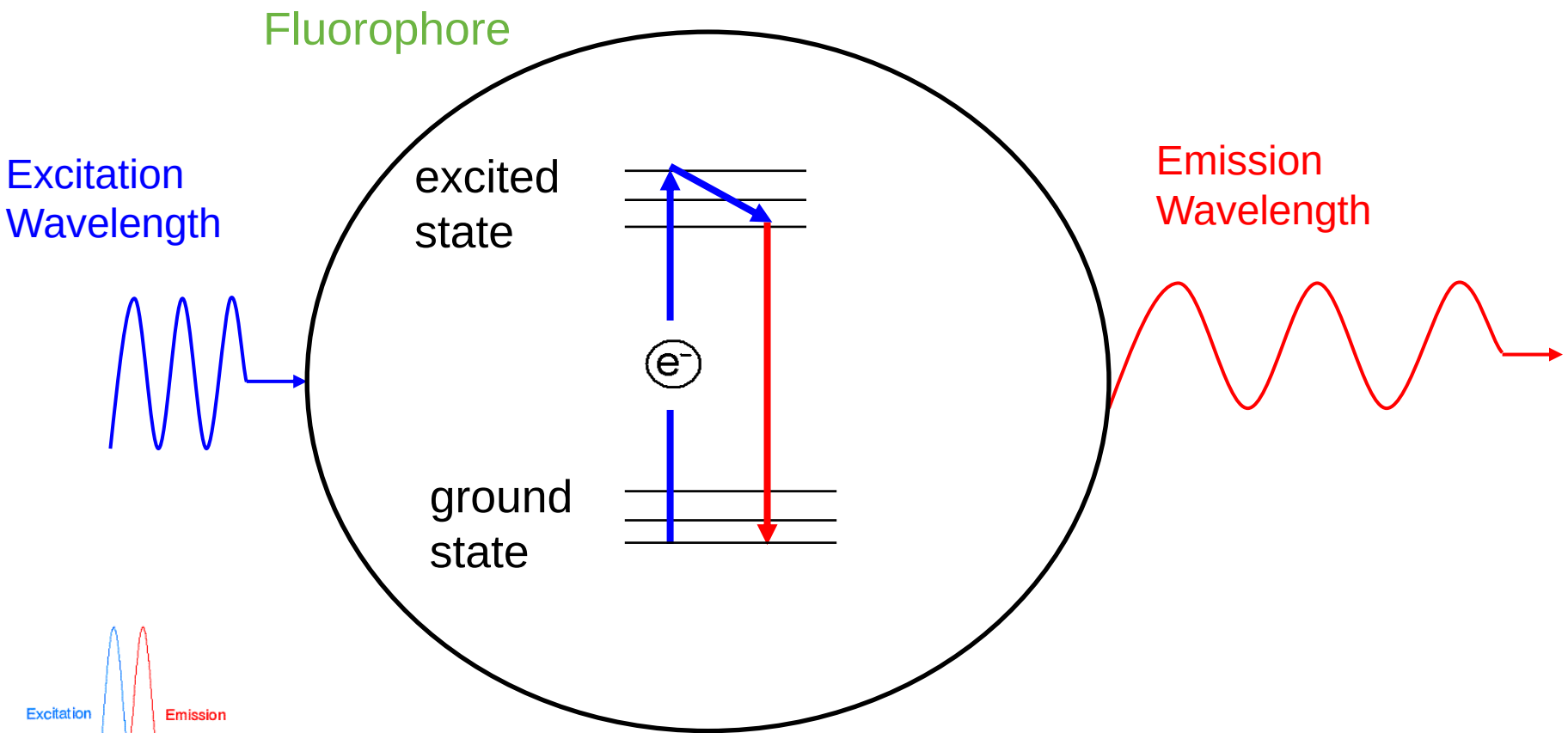


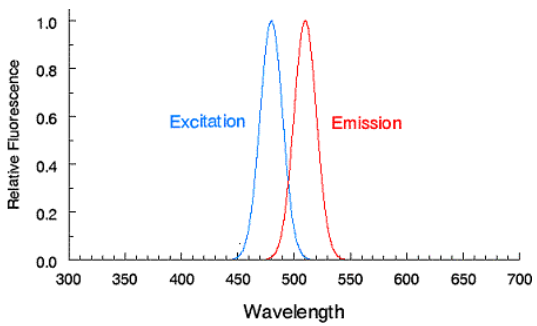
| | | | | | | |
|---|---|---|---|---|---|---|
| **Exp time:** | 30 sec | 30 sec | 60 sec | 60 sec | 60 sec | 60 sec |
| **Binning:** | small | small | small | small | medium | medium |
| **Day:** | 1 | 2 | 3 | 4 | 5 | 6 |

**Radiance:** ***Photons per second***

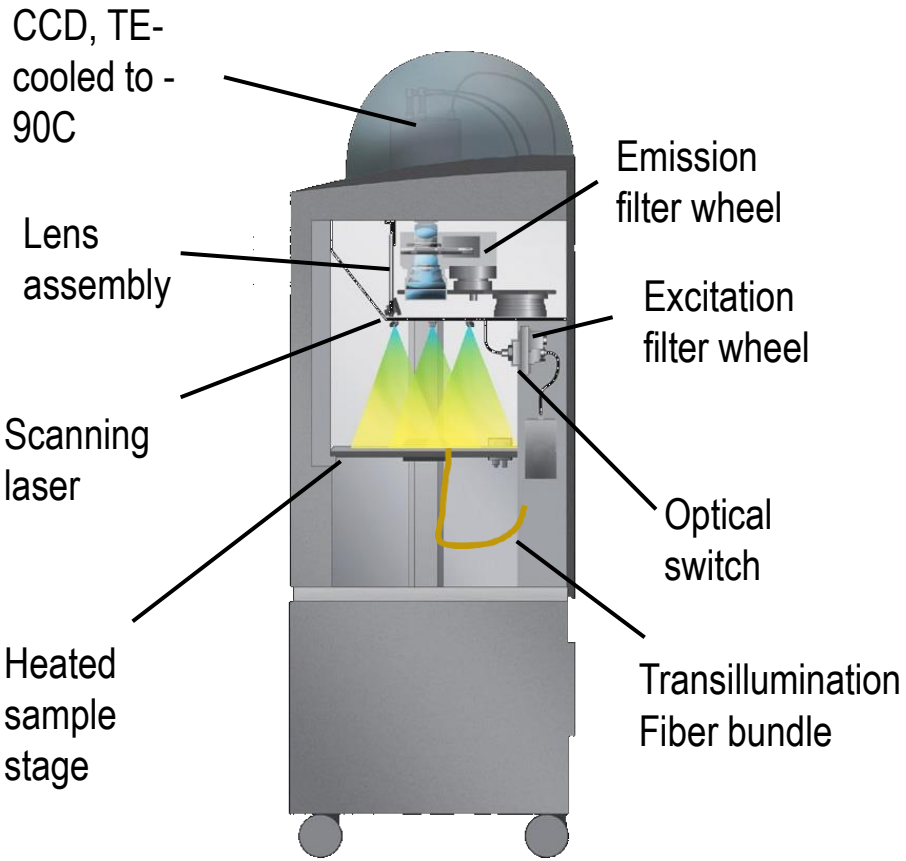


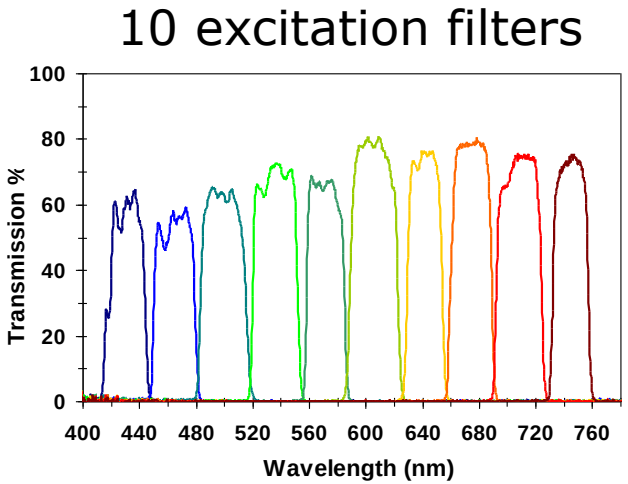
# Fluorescence

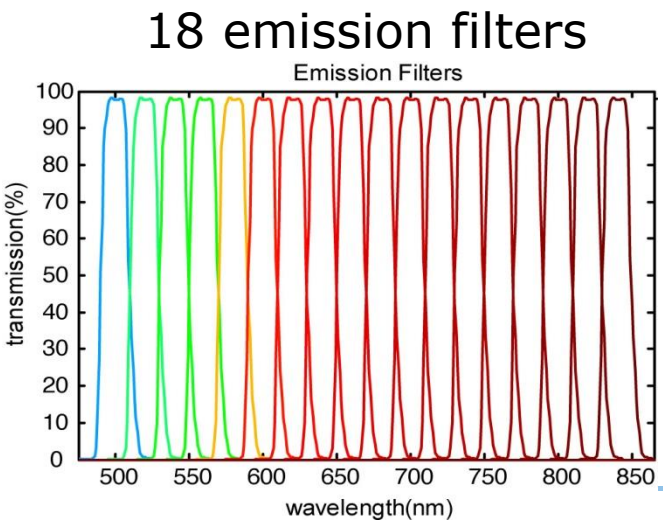


# IVIS® Spectrum



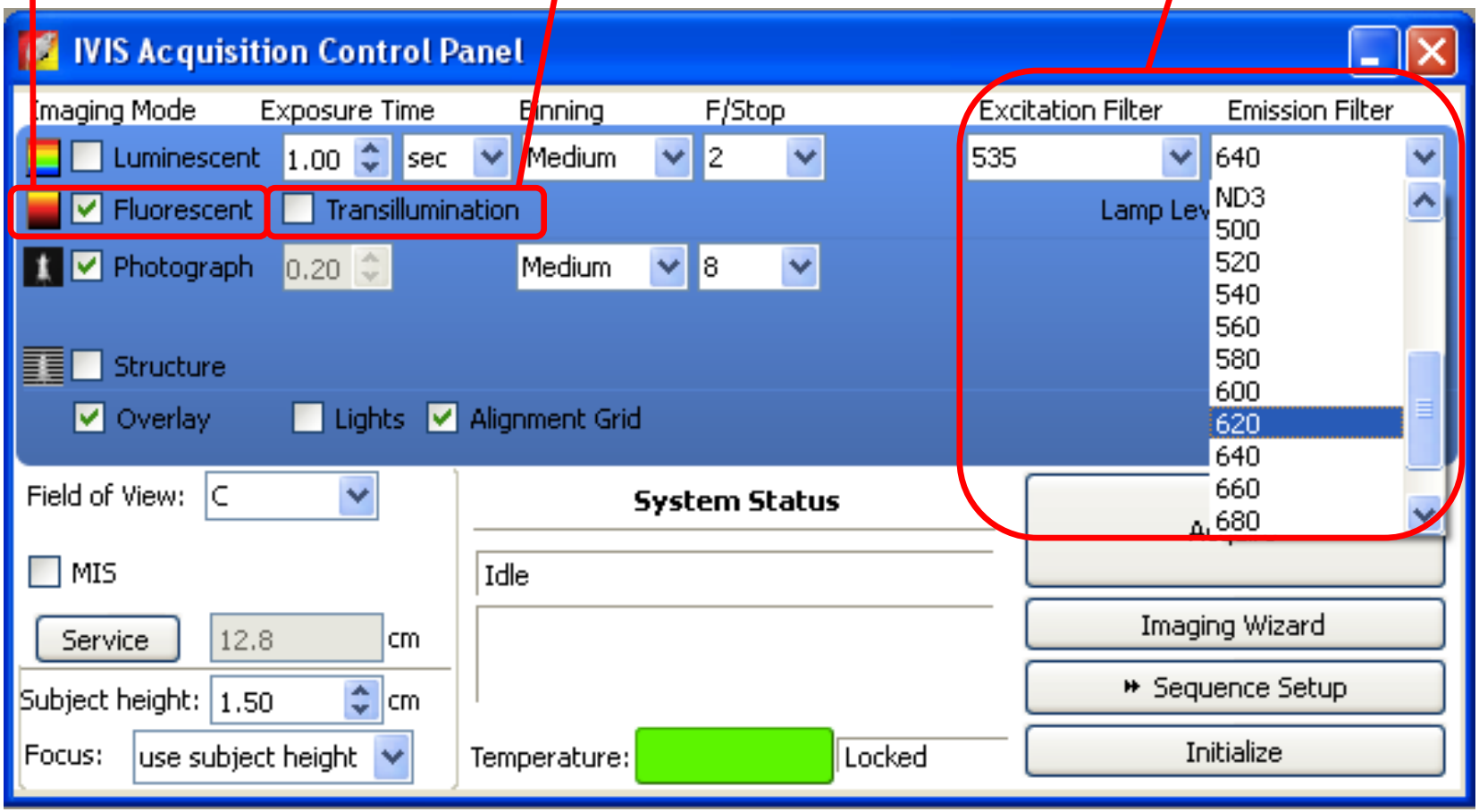



# Fluorescence Acquisition

Select Fluorescent Imaging Mode

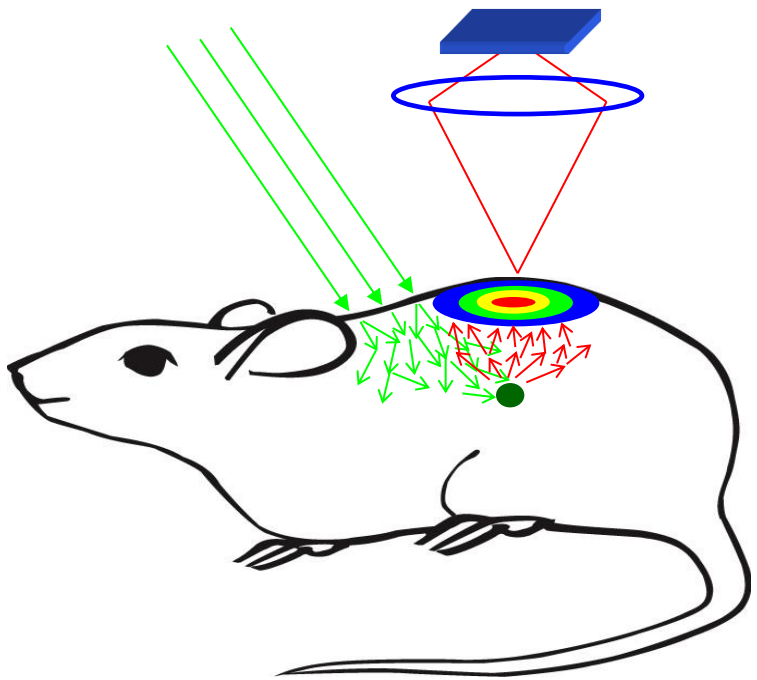
Trans-illumination

Select filters



# Epi-illumination Unit

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

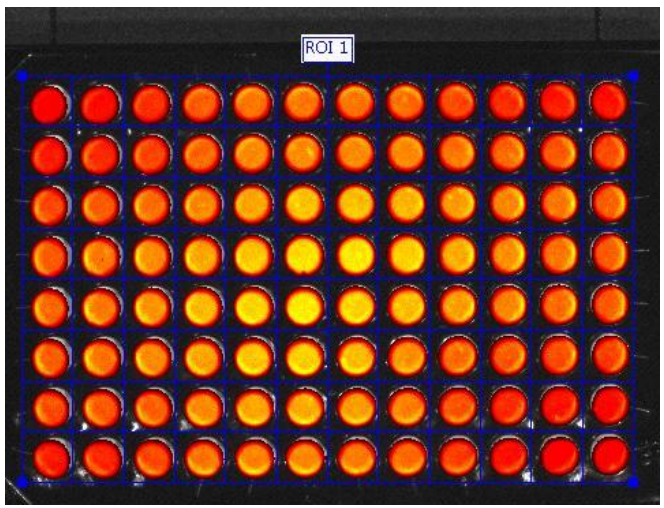


# Fluorescent Calibrated Units: Radiant Efficiency

Excitation Light Pattern

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

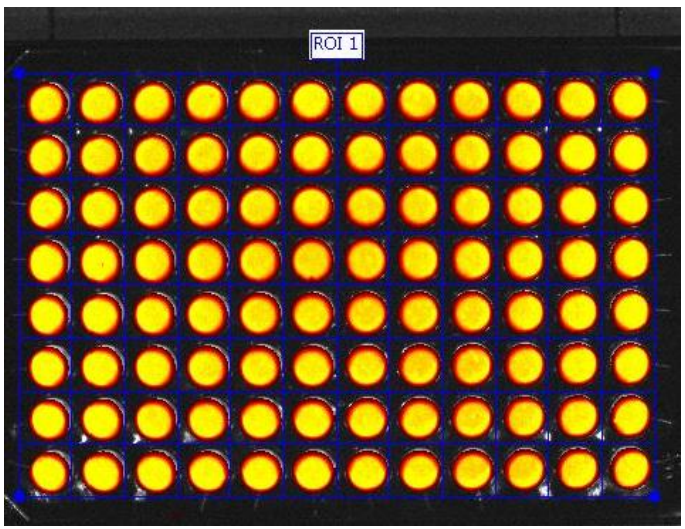
GFP Well Plate Uncorrected




Counts

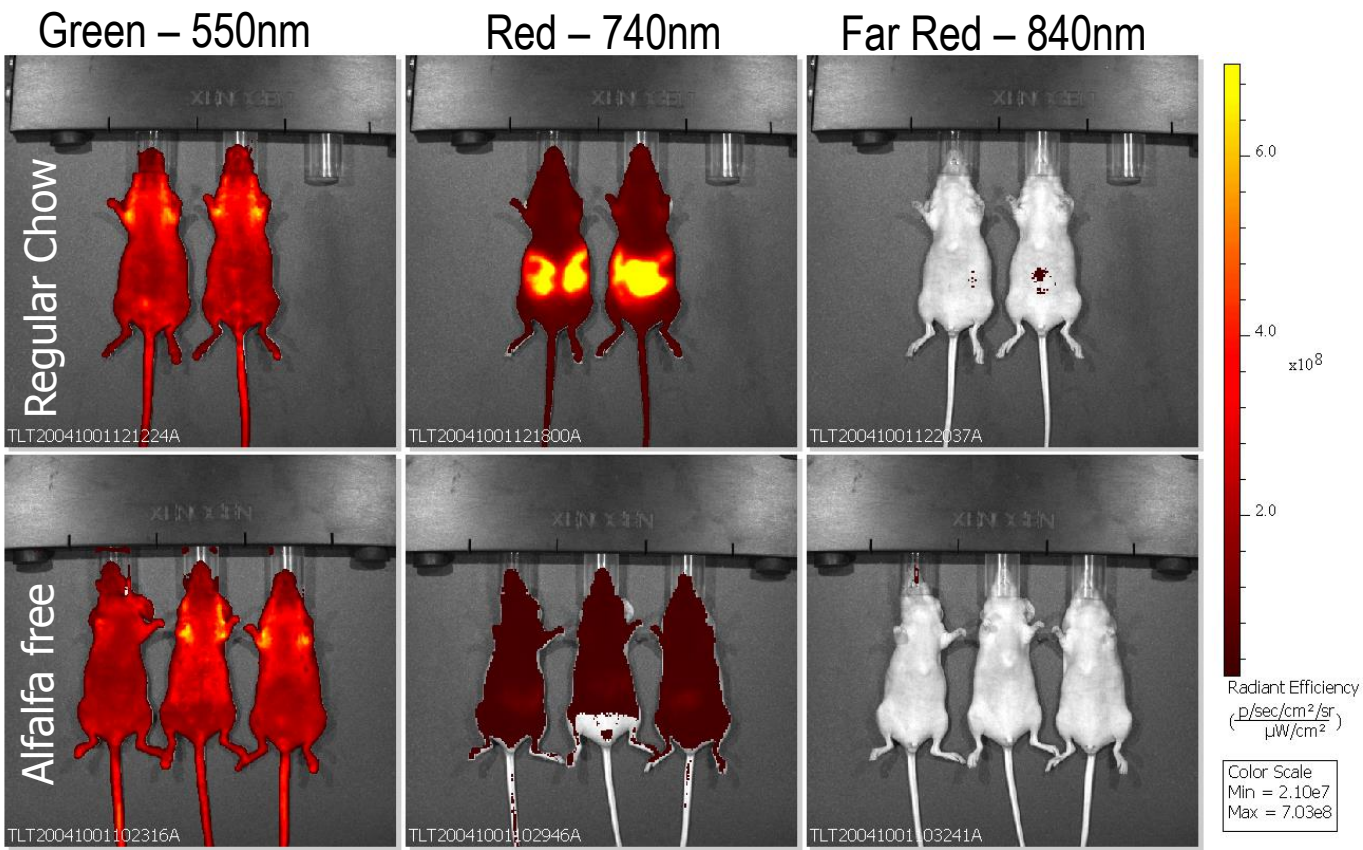
**vs.**

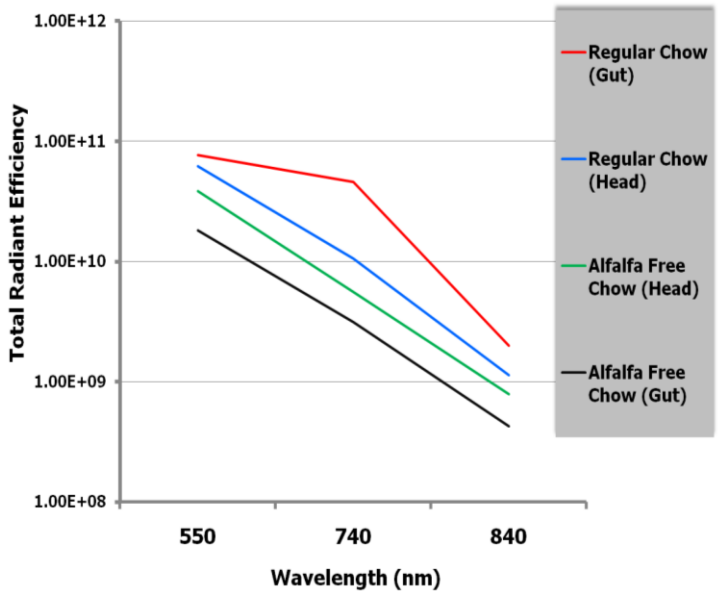
GFP Well Plate Corrected




Radiant Efficiency

# Autofluorescence in Negative Control Mice




**Research Diets**
**http://www.researchdiets.com**

**AIN-76A (D10001i) – alfalfa free**

- **Unrefined chlorophyll-containing ingredients, particularly alfalfa, responsible for gut signal**

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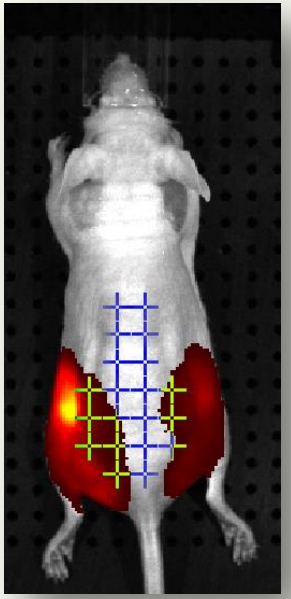
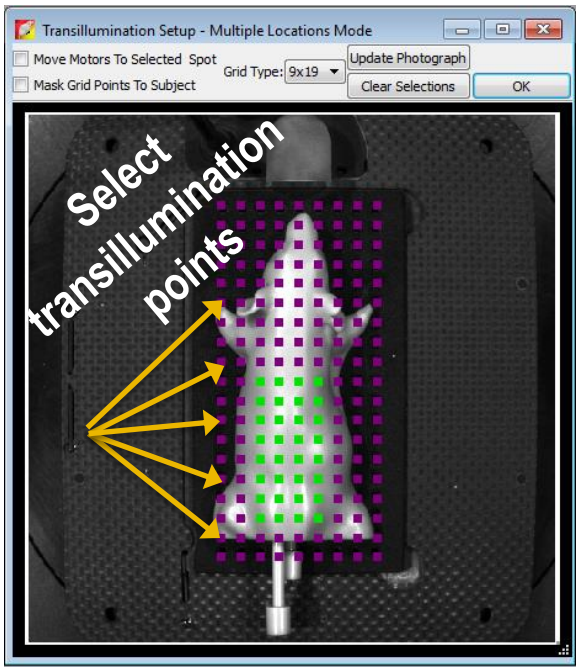
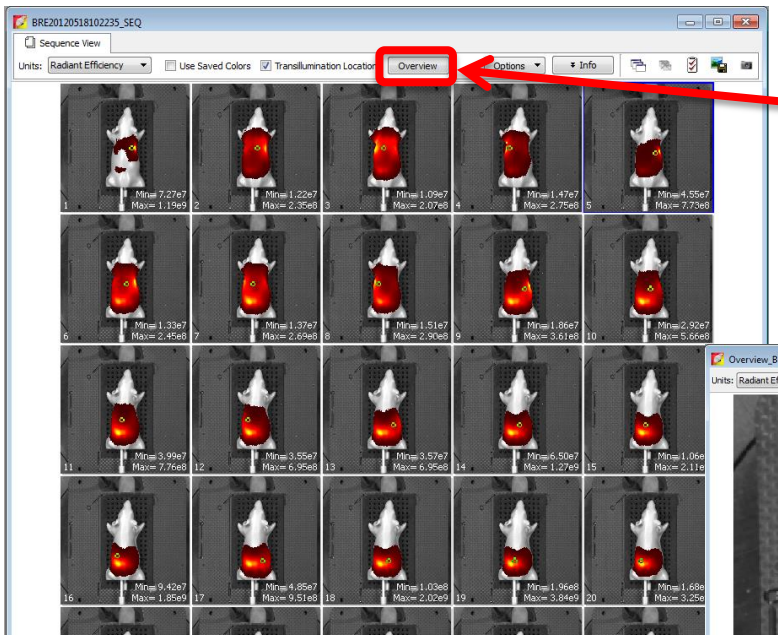
# Trans-illumination Fluorescence



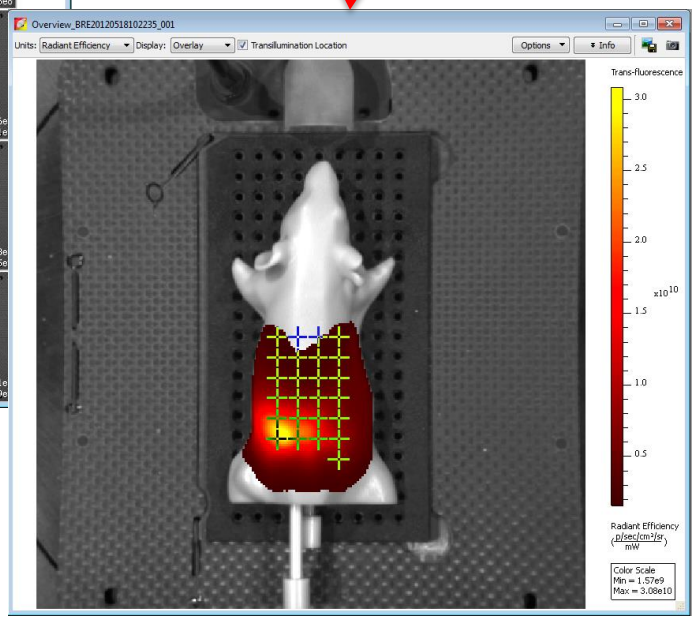
# Point-by-Point Transillumination

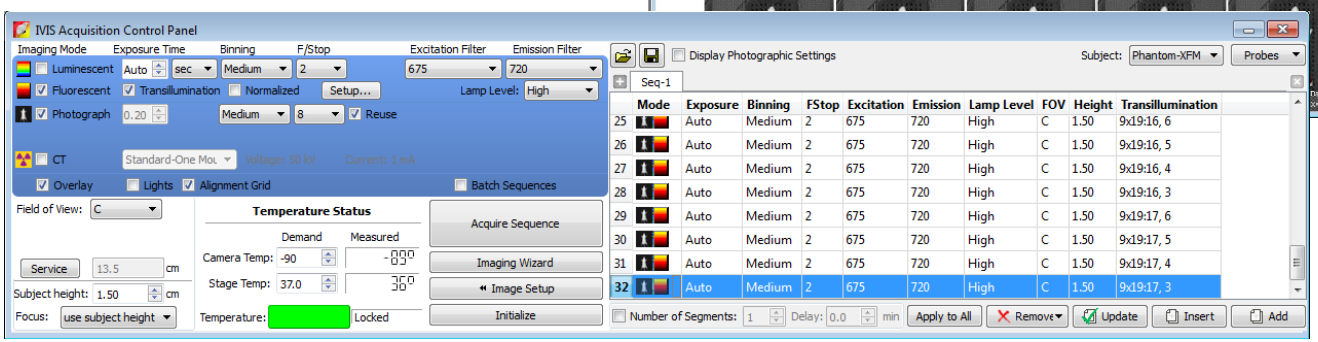



**Single image taken for each point**

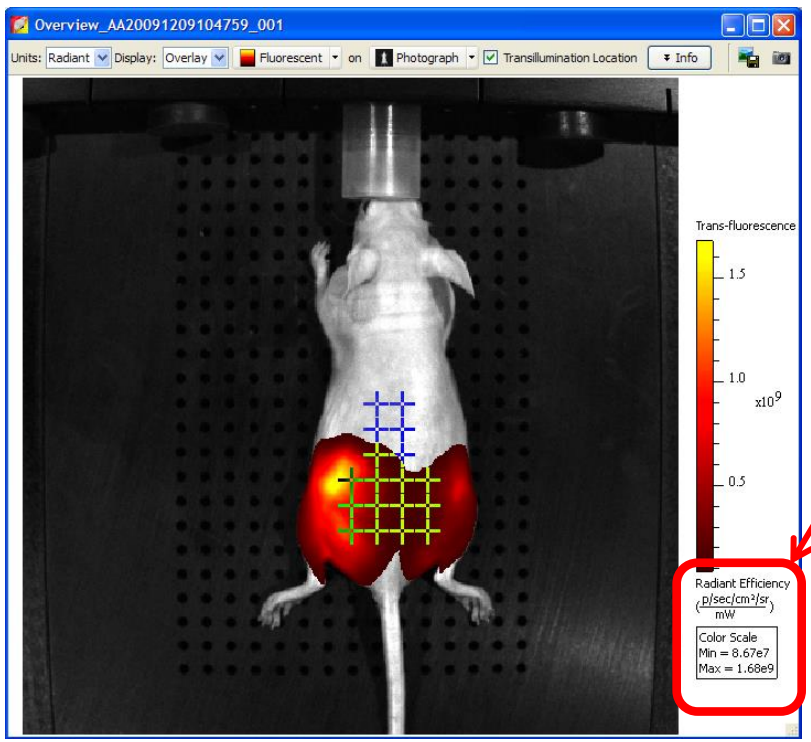



**Overview combines points into one image**



# Transillumination 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)

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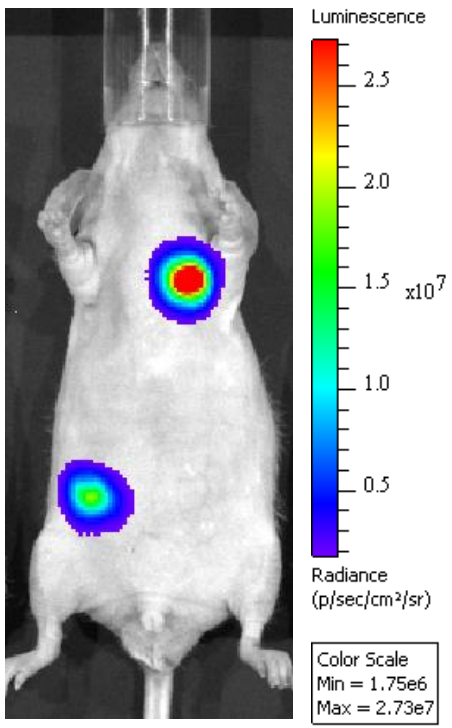
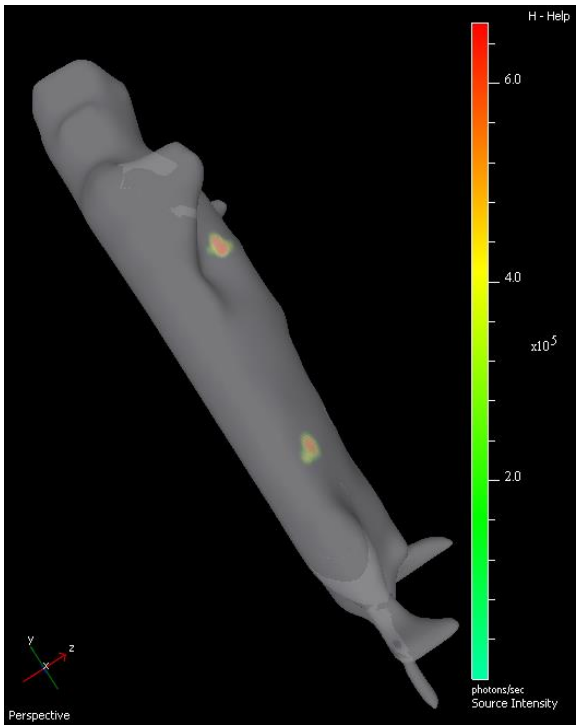
# Preview of Advanced Topics

# 3D Tomography (Luminescence)



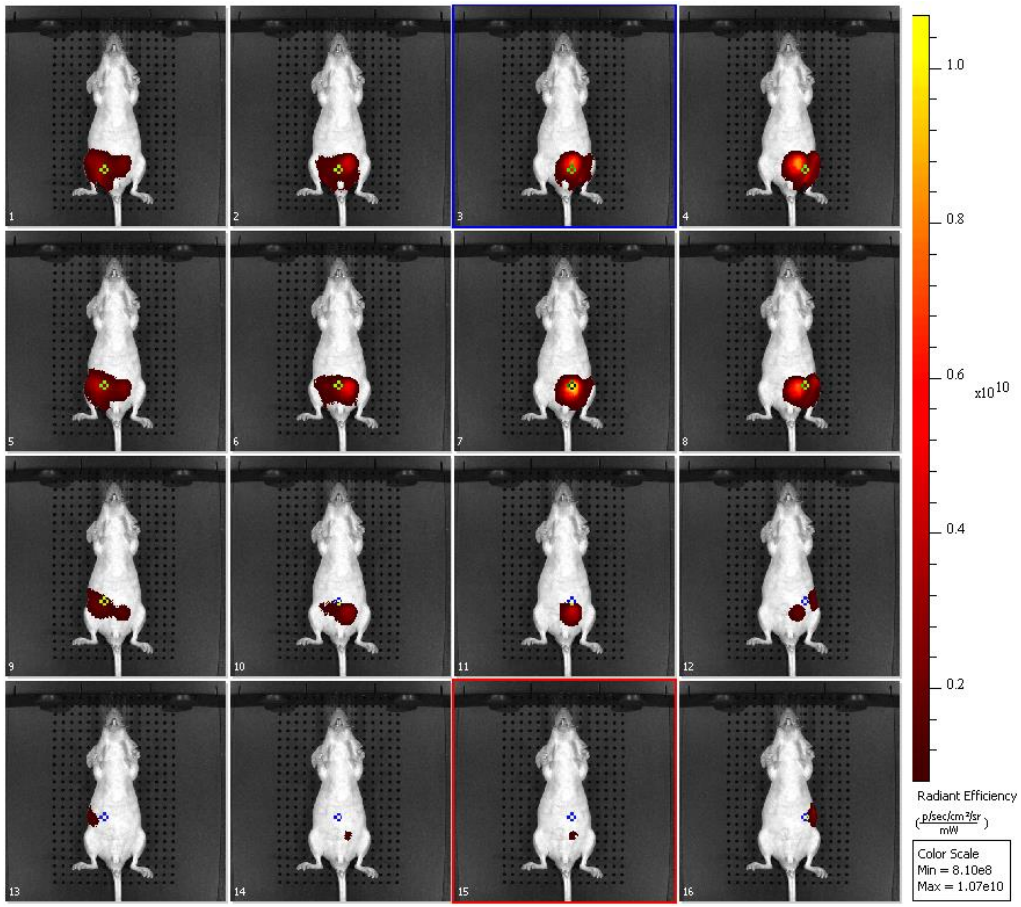

29 days after i.c. injection of $2x10^6$ cells

I = 580, 600, 620 nm

| Chest Cavity | | Peritoneal Cavity | |
|---|---|---|---|
| Depth [mm] | Flux [photons/sec] | Depth [mm] | Flux [photons/sec] |
| 2.1 | $2.43\times10^8$ | 3.2 | $1.44\times10^8$ |

# 3D Tomography (Fluorescence)

Ex: 745nm  Em: 800 nm

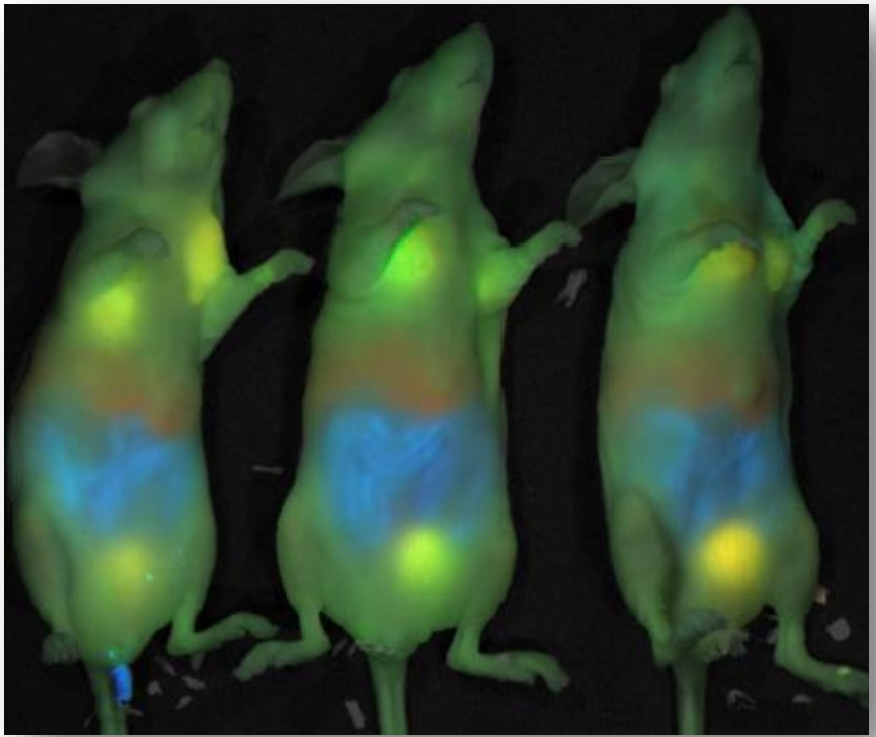



50 μg XLCF750 dye Herceptin conjugate
Injected IV on Day 20
Imaged on Day 22, T=48 hour

.

# 3D Tomography Co-registration Options

# Spectral Unmixing



- Calculates concentrations of different fluorescent components
- Requires images acquired at multiple wavelengths to perform the spectral analysis

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)

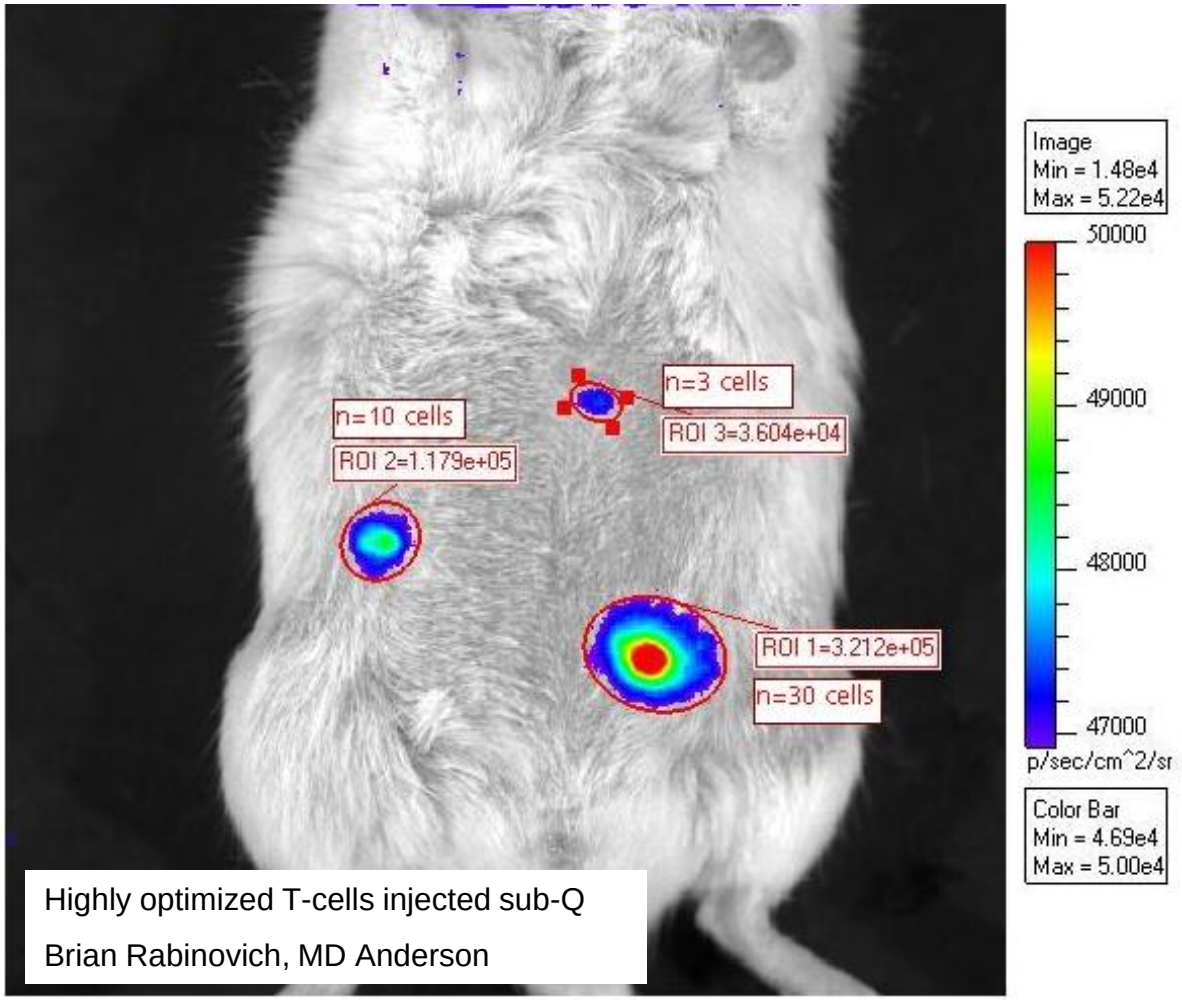
# Summary

- 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 Transillumination
  - Tissue and Instrument Auto-fluorescence can be subtracted

# Top Ten Tips for Optical Imaging

1. Choose reporters that maximize signal-to-noise (S:N) ratio
2. Consider the appropriate control groups and imaging time points necessary
3. Use hairless mice or white-furred animals and depilate or shave
4. Switch to autofluorescence-free mouse diet
5. Closely map the kinetics of your biological bioluminescent model
6. Animal handling can significantly affect kinetics
7. Image in the animal orientation that yields the highest signal intensity
8. Cover intense signal to allow dimmer signals to dictate auto-exposure
9. Utilize guards to prevent reflection off neighboring animals
10. Use black well plates when doing in vitro experimentation

# Through thorough engineering, it may be possible to resolve as few as 3 bioluminescent cells




Highly optimized T-cells injected sub-Q

Brian Rabinovich, MD Anderson

# Optical Imaging Bioware and Reagents

## Activateable

- ✓CatB 680 and 750
- ✓CatK 680
- ✓ MMPSense 680, 750
- ✓Neutrophil Elastase 680
- ✓ProSense 680, 750
- ✓ReninSense 680

## Targeted

- ✓2-DG Probe
- ✓Annexin-Vivo 750
- ✓BacteriSense 645
- ✓Bacterial Detection Probe 750
- ✓COX-2 Probe
- ✓FolateSense 680
- ✓IntegriSense 680, 750, 645
- ✓HypoxiSense 680
- ✓Inflammation Probe
- ✓OsteoSense 680, 750, 800
- ✓TLectinSense 680

## Vascular

- ✓AngioSense 680 and 750
- ✓AngioSPARK 680 and 750
- ✓Superhance 680

## Bioware

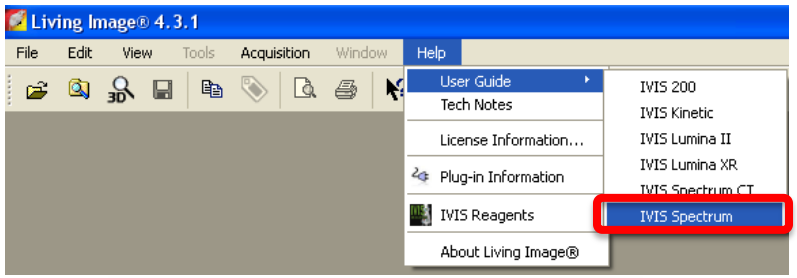
- ✓ Cell lines
- ✓ Microorganisms
- ✓ Bioware Ultra
- ✓ Bioware Ultra Red

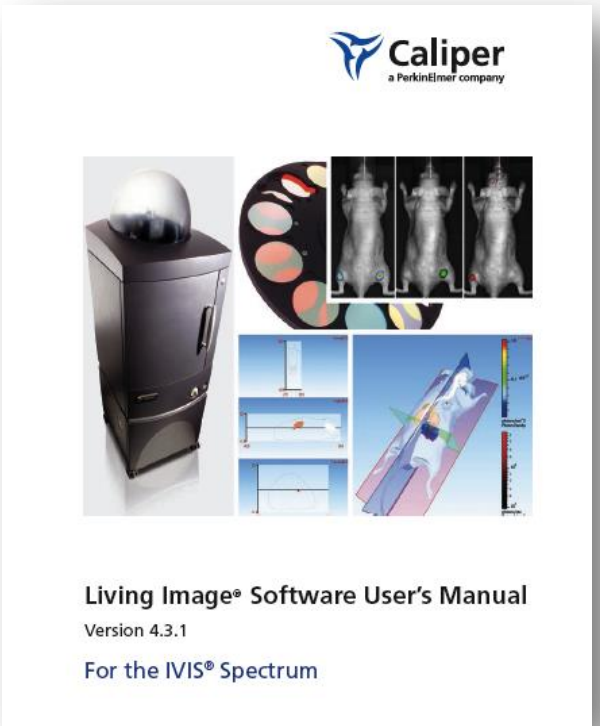
## Substrates


- ✓ D-Luciferin Substrate
- ✓ Coelenterazine
- ✓ RediJect D-Luciferin
- ✓ RediJect D-Luciferin Ultra

# For an In-Depth Study

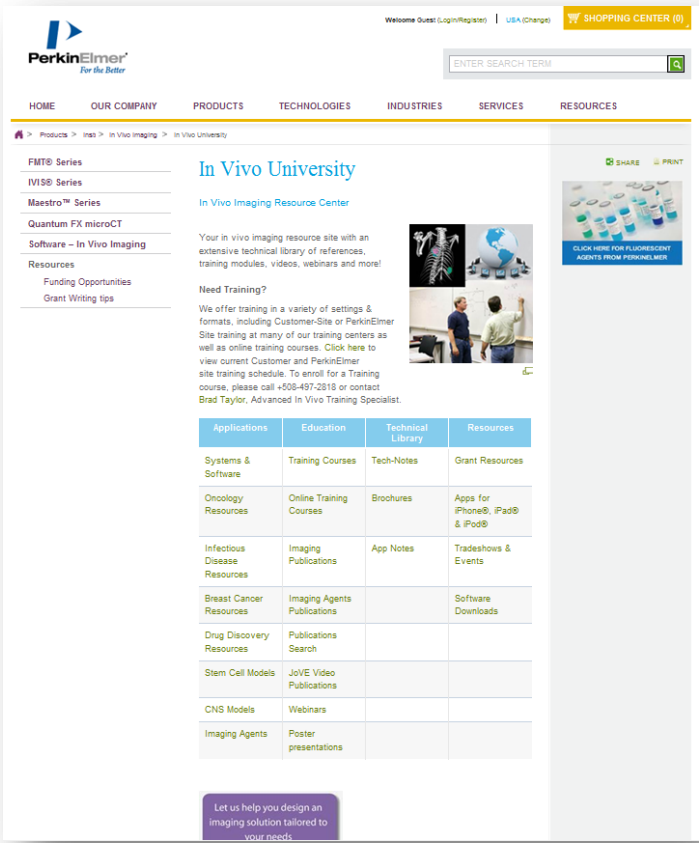
## IVIS Software Manual




## IVIS University Web page

http://www.perkinelmer.com/pages/020/imaging/invivouniversity.xhtml



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# Thank you for your attention!

## On Call Services – Urgent Hardware Issues

- Technical Support
  (508) 435-9761
  Global.techsupport@perkinelmer.com

- Tarik Harb
  (510)-229-7272
  tarik.harb@perkinelmer.com

- Brad Taylor, Ph.D.
  (630) 435-9761
  brad.taylor@perkinelmer.com

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  (970) 214-8758
  alexandra.delille@perkinelmer.com