

# revvity

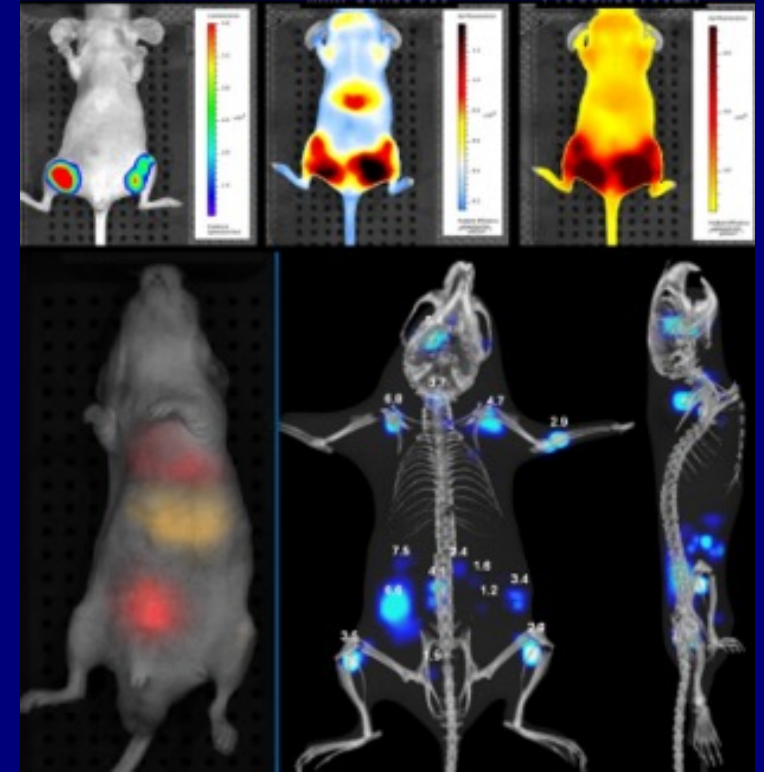
## IVIS<sup>®</sup> Spectrum 2

Gold standard for In Vivo Preclinical Imaging Solutions

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**J&H**

博克科技股份有限公司  
J&H TECHNOLOGY CO., LTD.

- **Right light** in the animal
- IVIS function and Image capture
  - software
  - Unmixing
  - 3D
  - Application

# Revvity IVIS® - In Vivo Imaging System



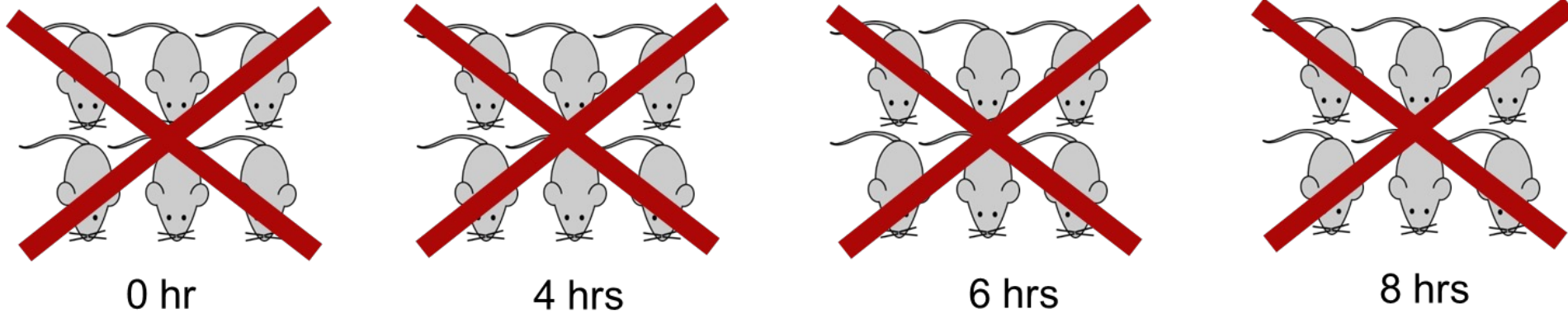
IVIS Spectrum 2 Platform



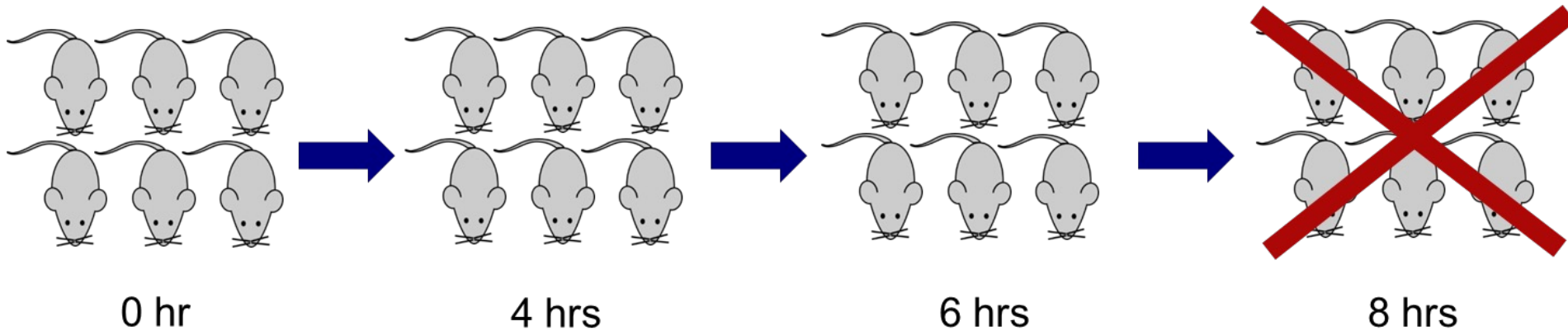
- 8500+ installation worldwide
- Sensitivities to image down to a single cell
- Offering spectrum unmix for “High-Plex” capability
- 3D multimodality – More information - High impact paper
- **Over 20 years experience in preclinical *in vivo* imaging**
- **Over 15,000 peer-reviewed publications**

# Transitional methodology vs. BPI methodology

Current Methodology = **24 animals** over four treatment points



Biophotonic imaging (BPI) Methodology = the same **6 animals** over four treatment points



**Does not require subject to be euthanized**



# What Advantage Optical In Vivo Imaging?

Gene expression results in production of luciferase or fluorescence protein

- Amount of light is proportional to number of live active cells

Powerful labeling technique

- Reveals enzyme activity, particle / drug location etc.

Non-invasive

- Does not require subject to be euthanized

Comply with 3R principle

Reduce individual differences

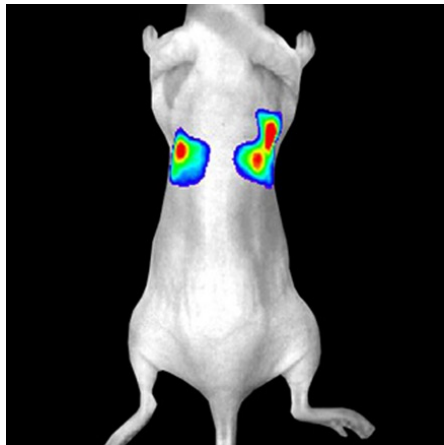
Easy Operation

Multi-function and Multiplex Bioluminescence, Fluorescence

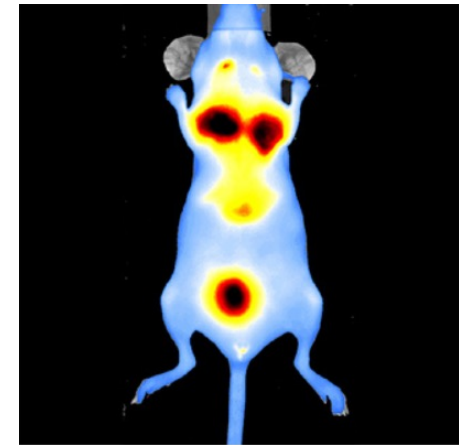
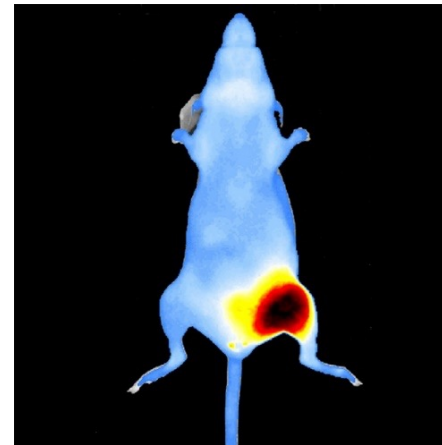
High Throughput

## Optical Imaging Approaches

- **Bioluminescence & Fluorescent Proteins**
  - Powerful approach using animals/cells with modified genetics
  - Uses promoter systems for deep understanding of underlying mechanisms

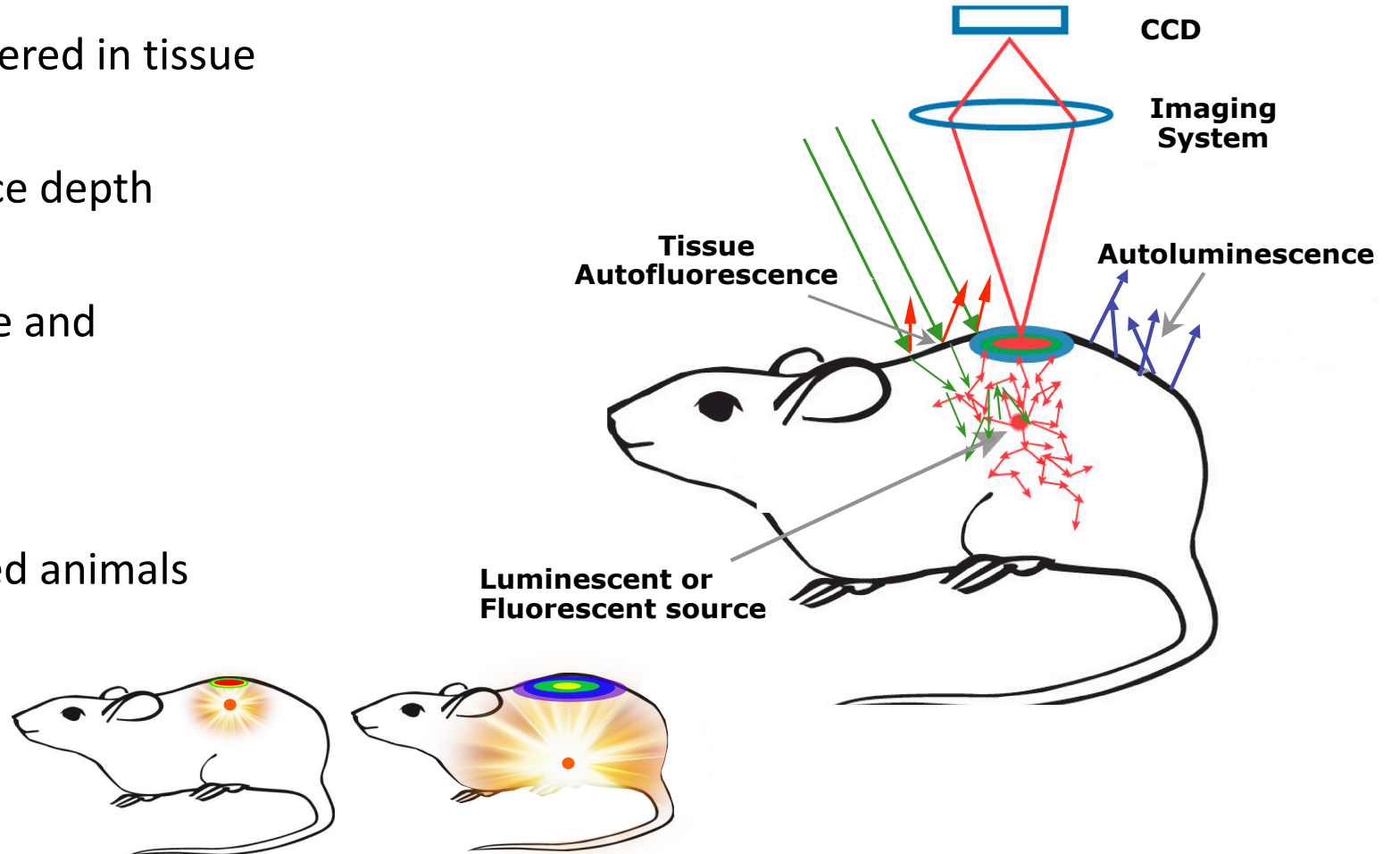


- **Fluorescent Agents (Red/NIR)**
  - Standard disease biology/models
  - Injectable drug-like imaging agents to view biology

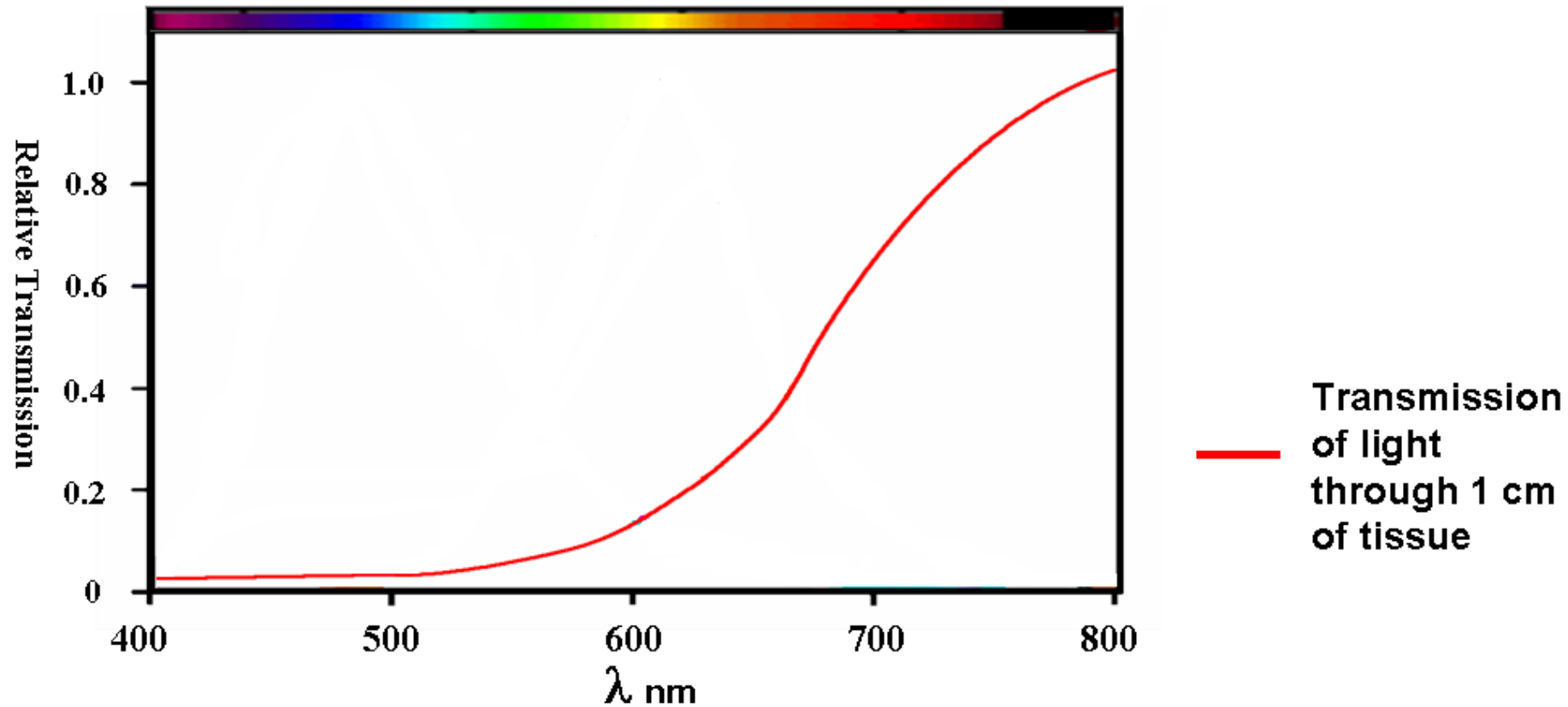


# The physics of light in tissue

- Photons are absorbed and scattered in tissue
- Surface signal depends on source depth
- Tissue is both autoluminescence and autofluorescence
- Use hairless mice or white-furred animals and depilate or shave

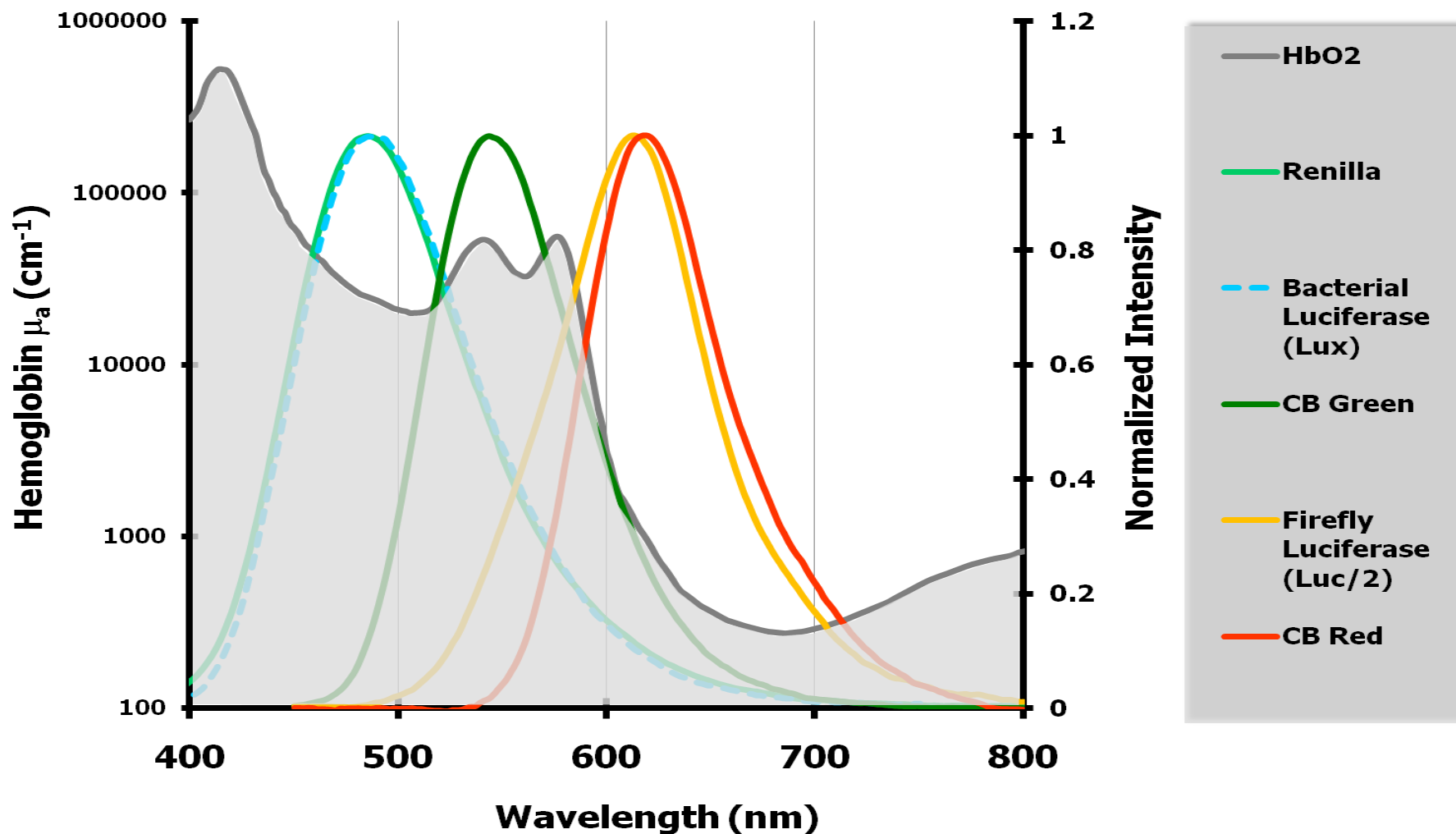


# Light in Tissue

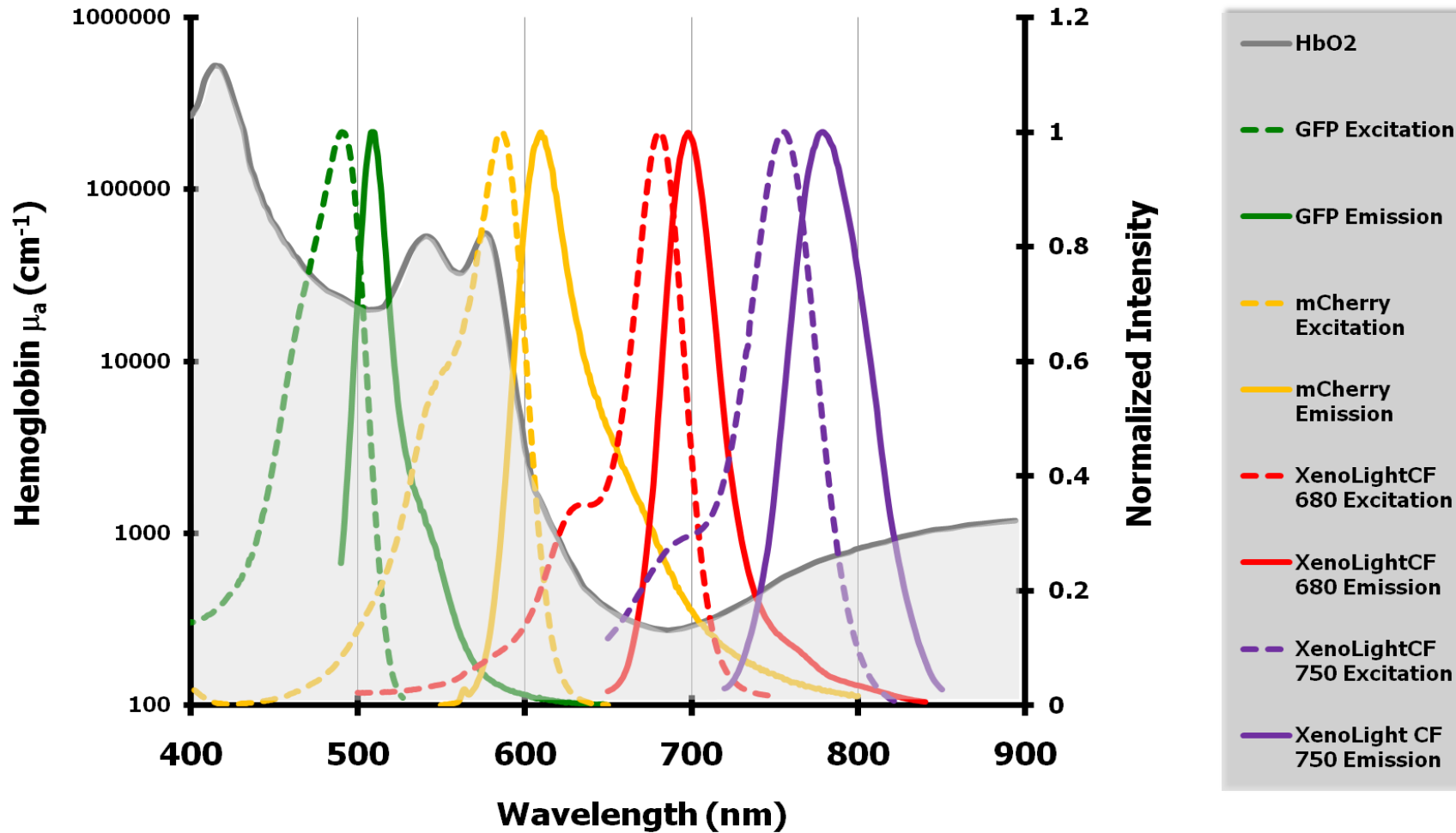


Tissue Is Not Transparent – Light Absorbance Depends on Wavelength

# Bioluminescent Spectra and Tissue Penetration

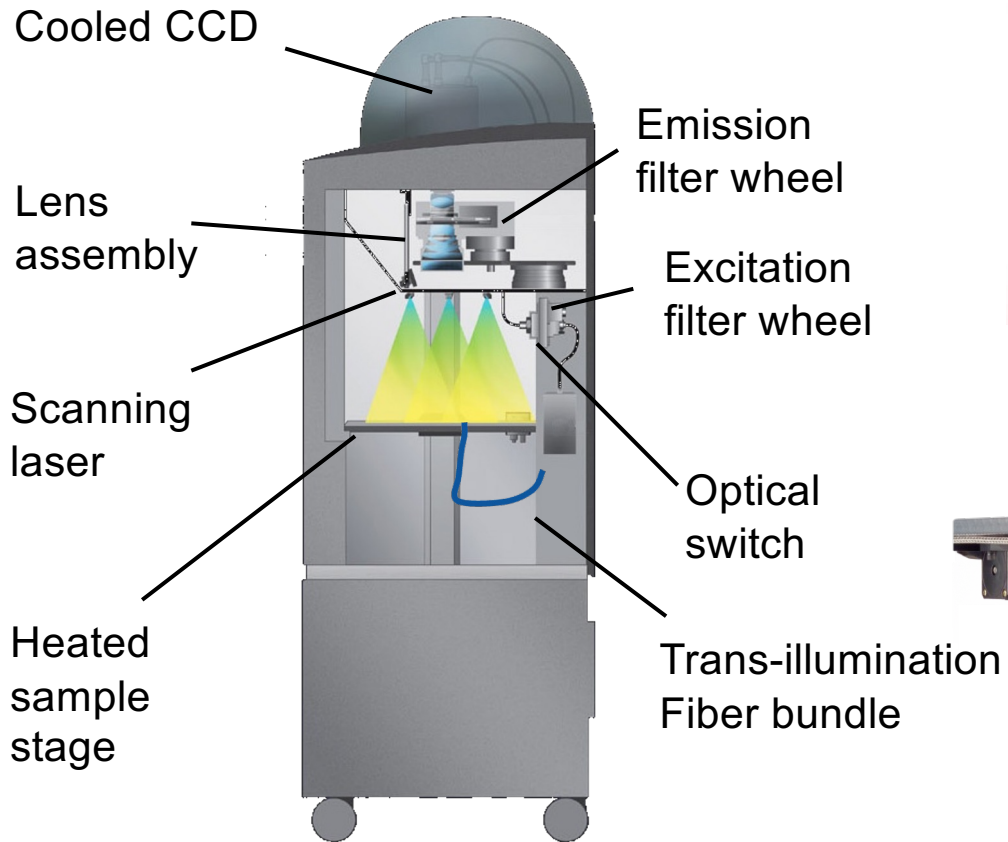


# Fluorophore Spectra and Light Diffusion Through Tissue

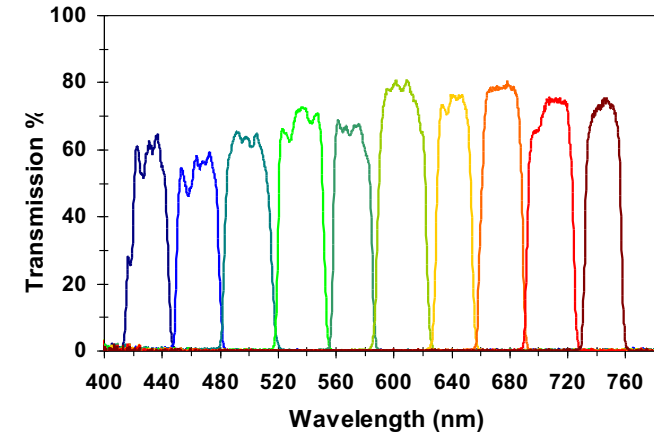




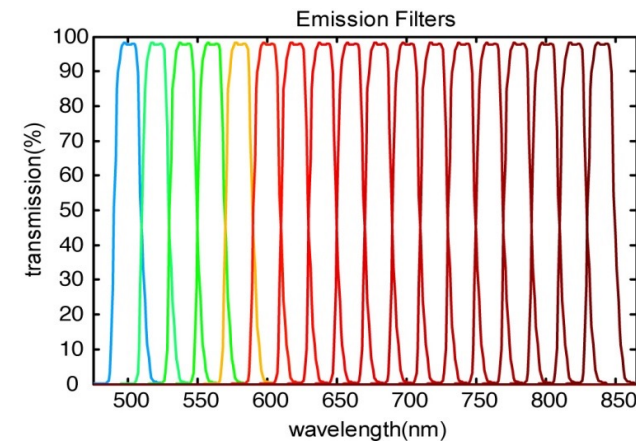
# IVIS<sup>®</sup> Spectrum 2



10 excitation filters

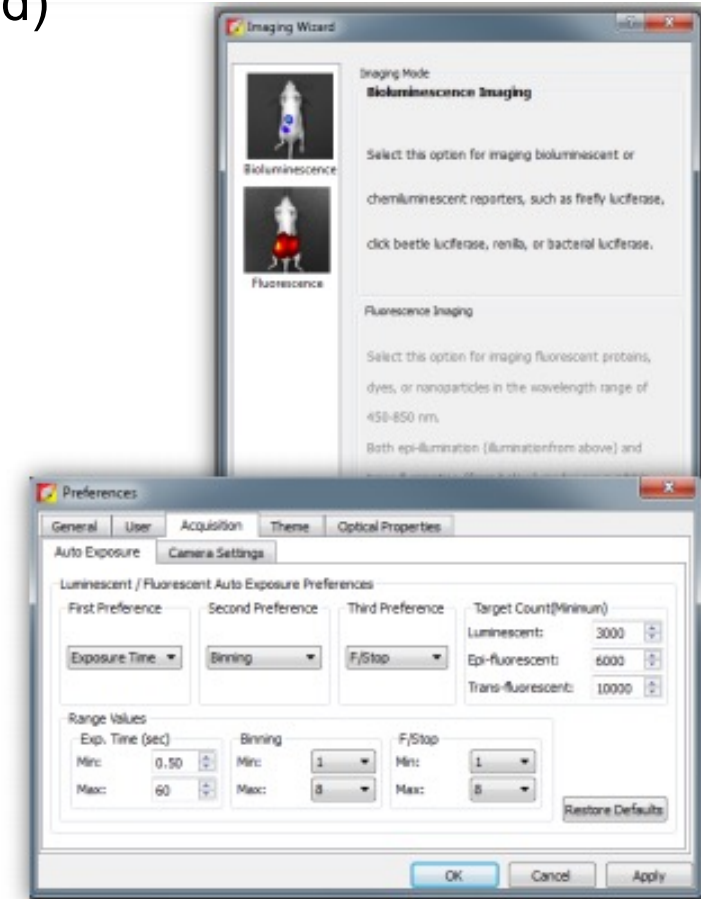


18 emission filters



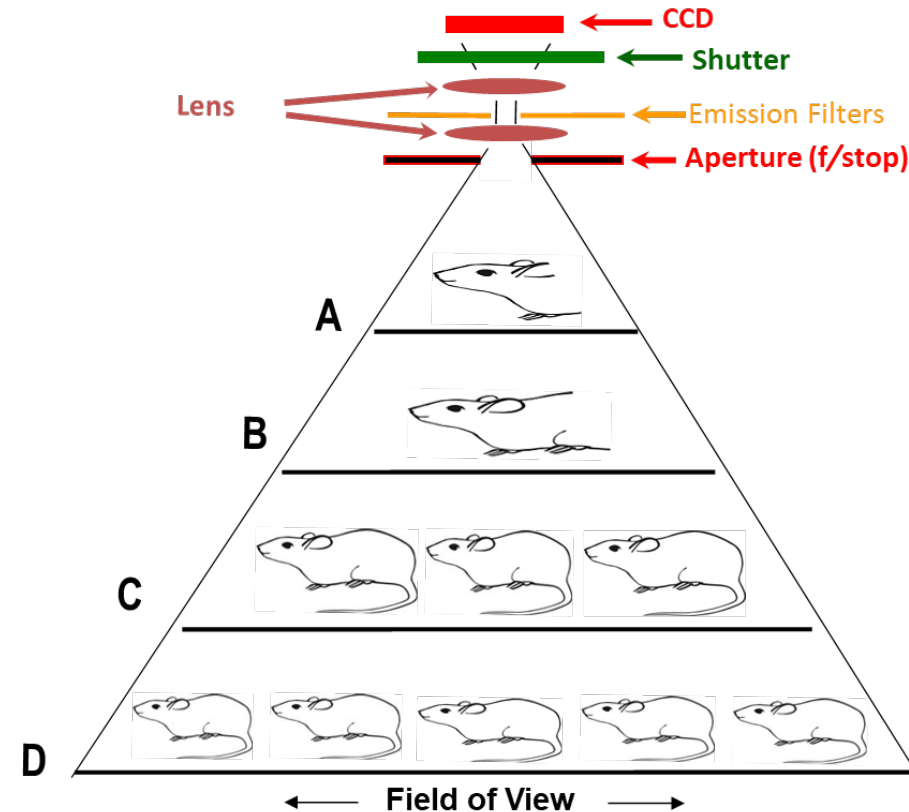
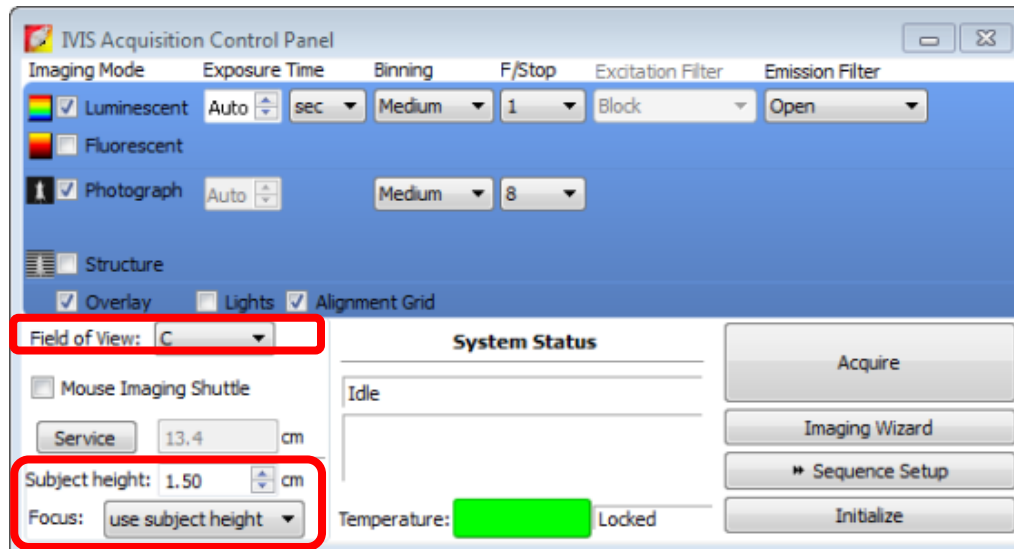
## Living Image<sup>®</sup> Software

- Controls all settings in the IVIS<sup>®</sup> system (fully computer controlled)
- Provides advanced **cataloging and browsing** tools
- Provides analysis tools for **quantification**
- Instrument settings are analogous to photography
- Imaging Wizard assists in choosing optimal setup and analysis parameters
- Auto-exposure for optimal image capture settings



## Field of View (FOV)

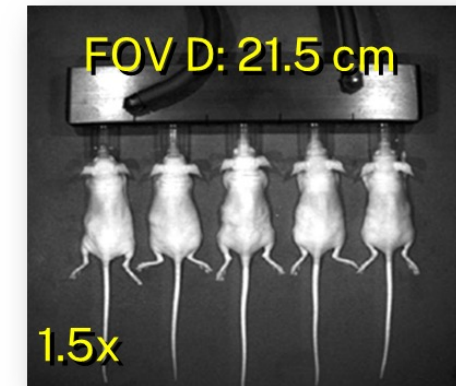
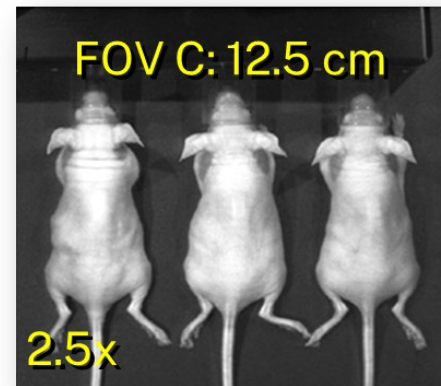
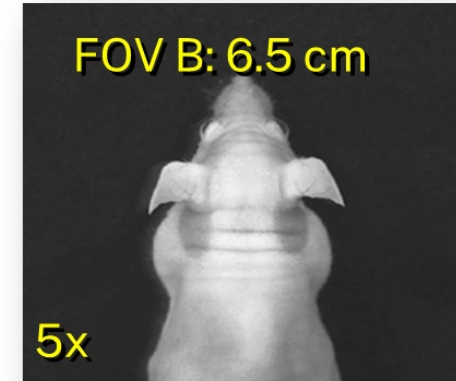
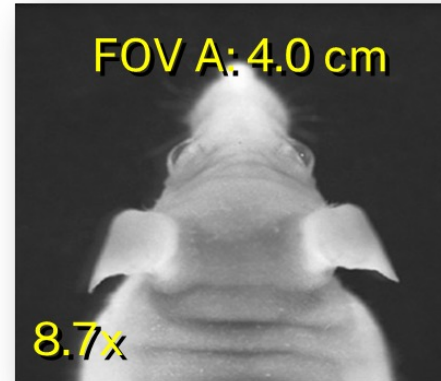
- Sets the size of the stage area to be imaged by adjusting the position of the stage and lens
- A smaller FOV gives a higher sensitivity measurement



## Field of View (FOV)

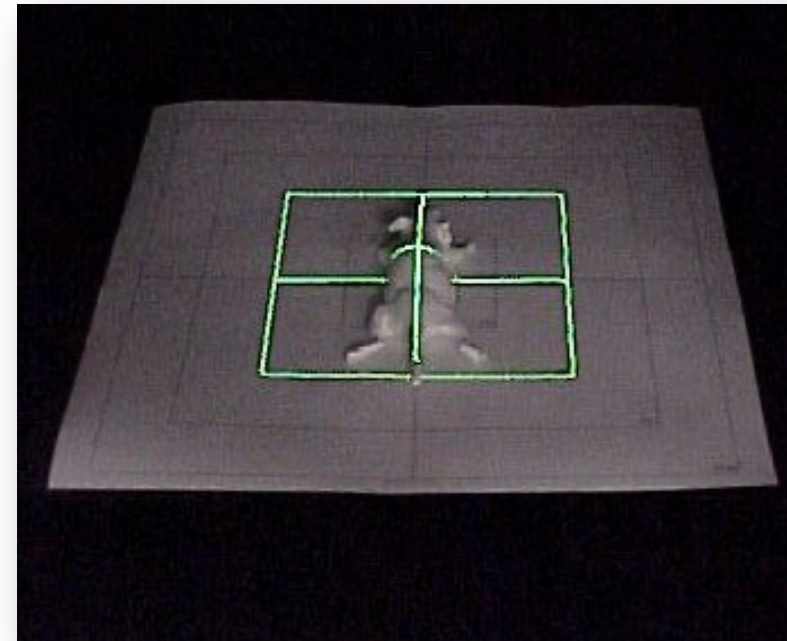
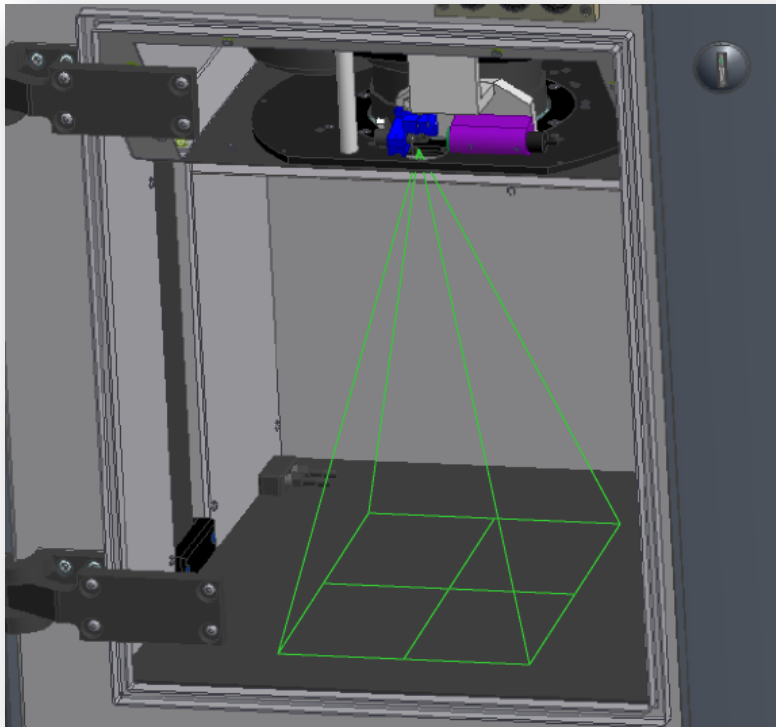
- 1 Position A is **not** recommended for **epi-fluorescent** imaging because corrections for non-uniform excitation light pattern are not available.
- 2 Position C is the default setting.

FOV Setting	FOV (cm)
A <sup>1</sup>	4
B	6.5
C <sup>2</sup>	13
D	22.5



## Alignment Light Projector

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



## Setting Sensitivity – Signal Level

- The IVIS<sup>®</sup> 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



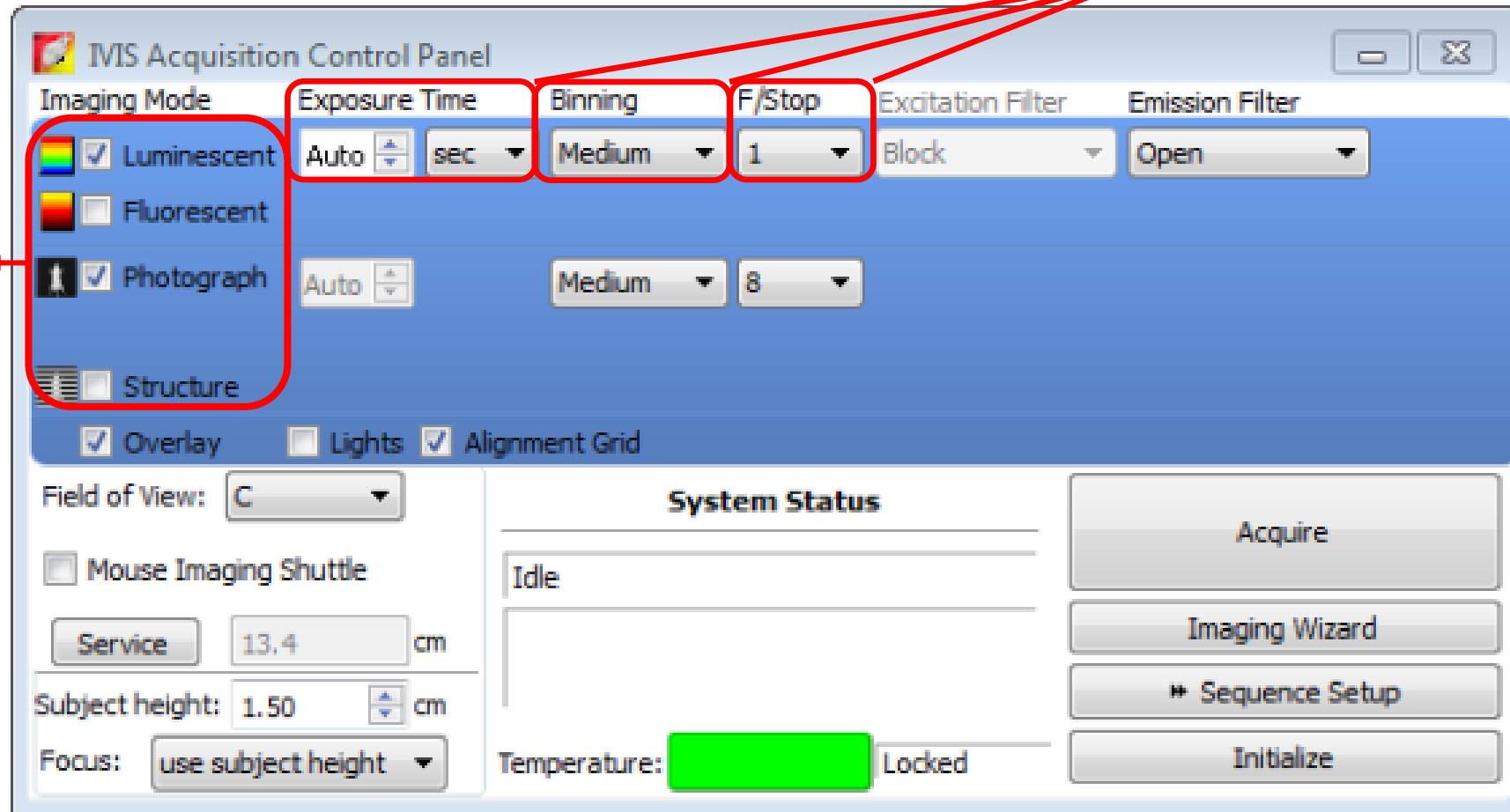


# Living Image® Control Panel

- Software

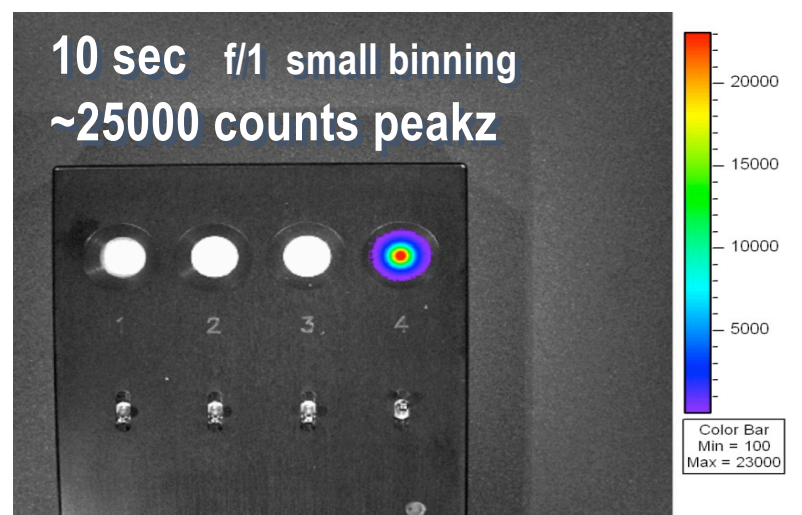
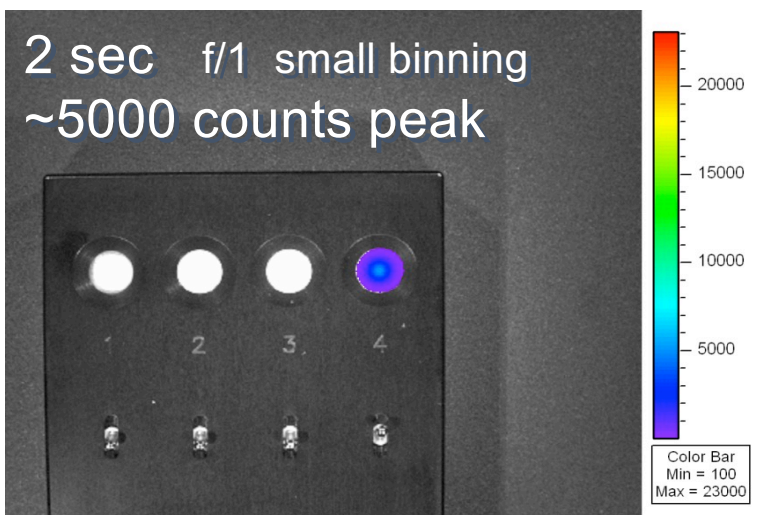
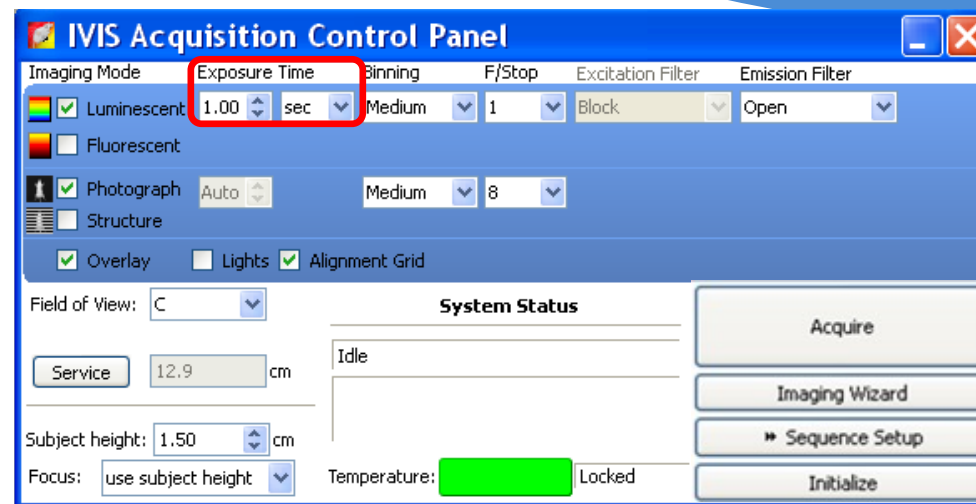
Controls Sensitivity

Imaging Modes



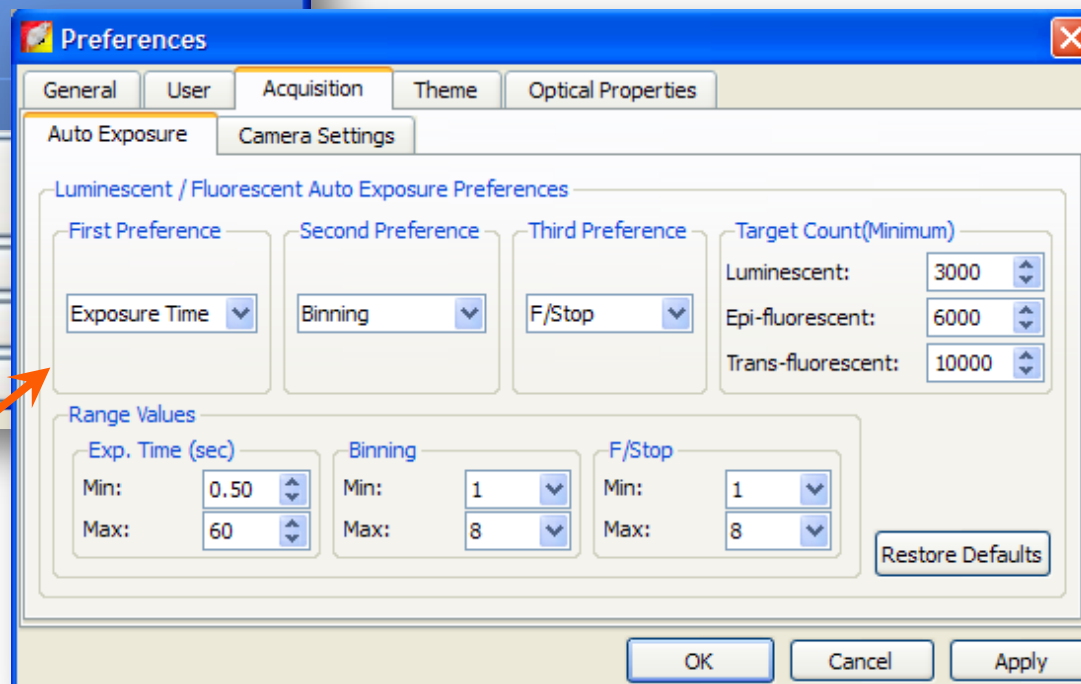
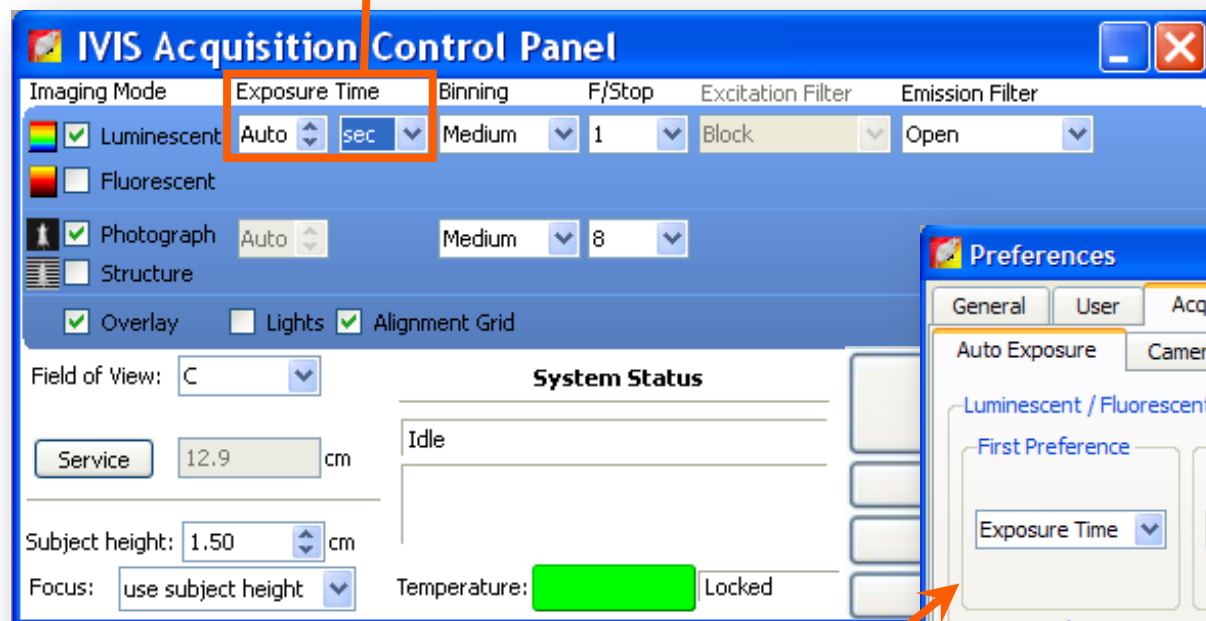
# Exposure Time

- 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



# Auto-Exposure

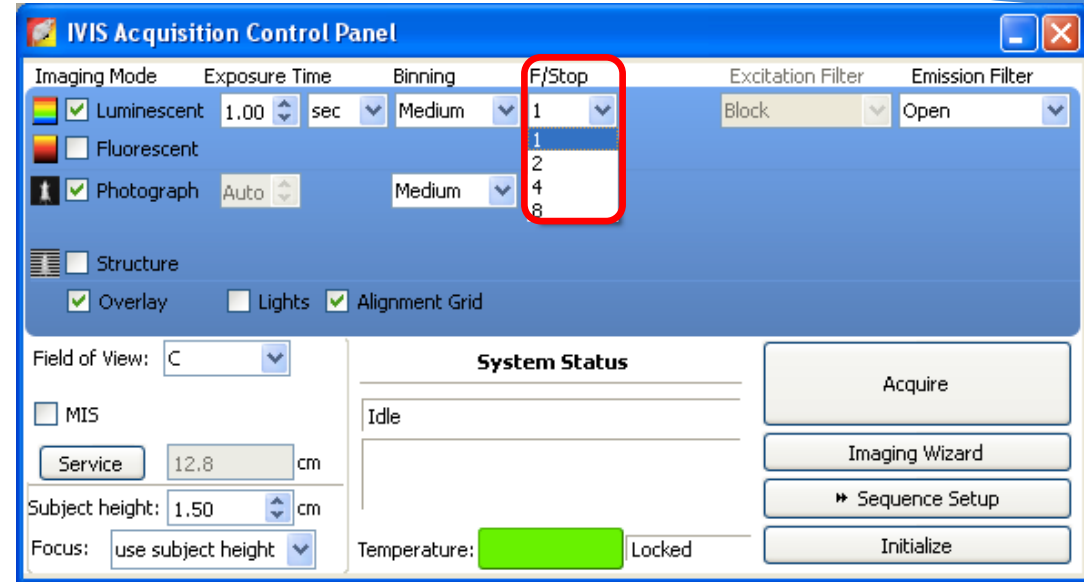
Auto-exposure feature available for bioluminescence and fluorescence



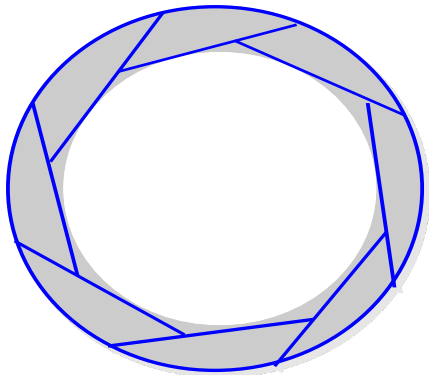
User definable settings

## $f$ /stop (Lens Aperture)

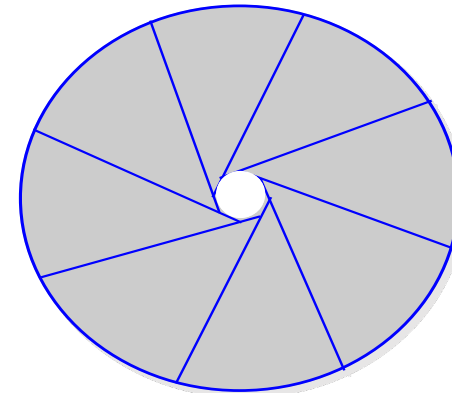
- $f$ /stop controls the amount of light received by the CCD detector
- $f/1$  is wide open, maximum light collection  
– default for luminescent
- $f/8$  is smallest aperture, best resolution  
– default for photo
- Changing  $f$ /stop changes counts by a factor of 4



$f/1$



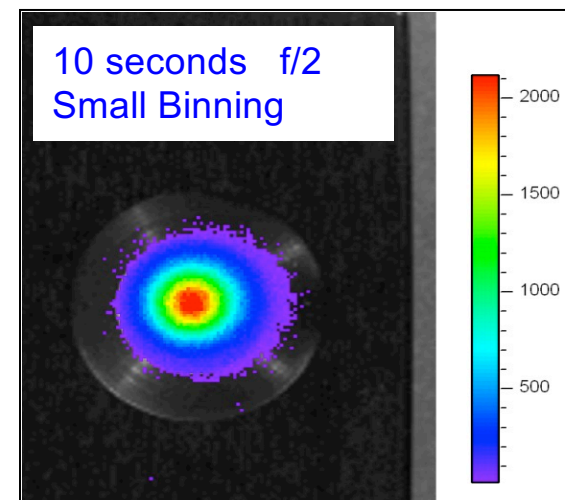
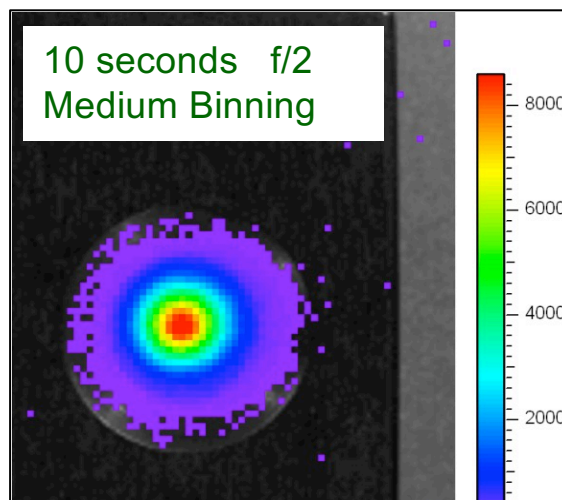
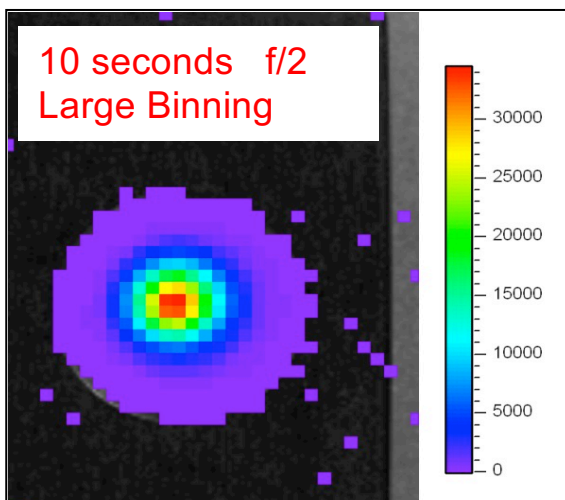
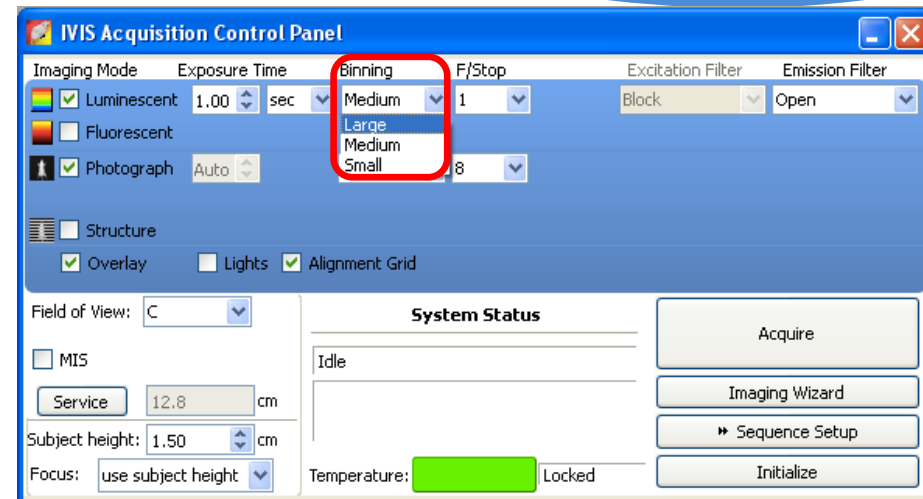
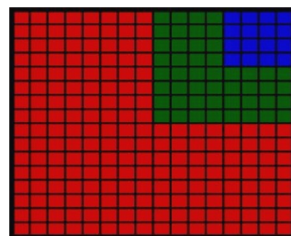
$f/8$



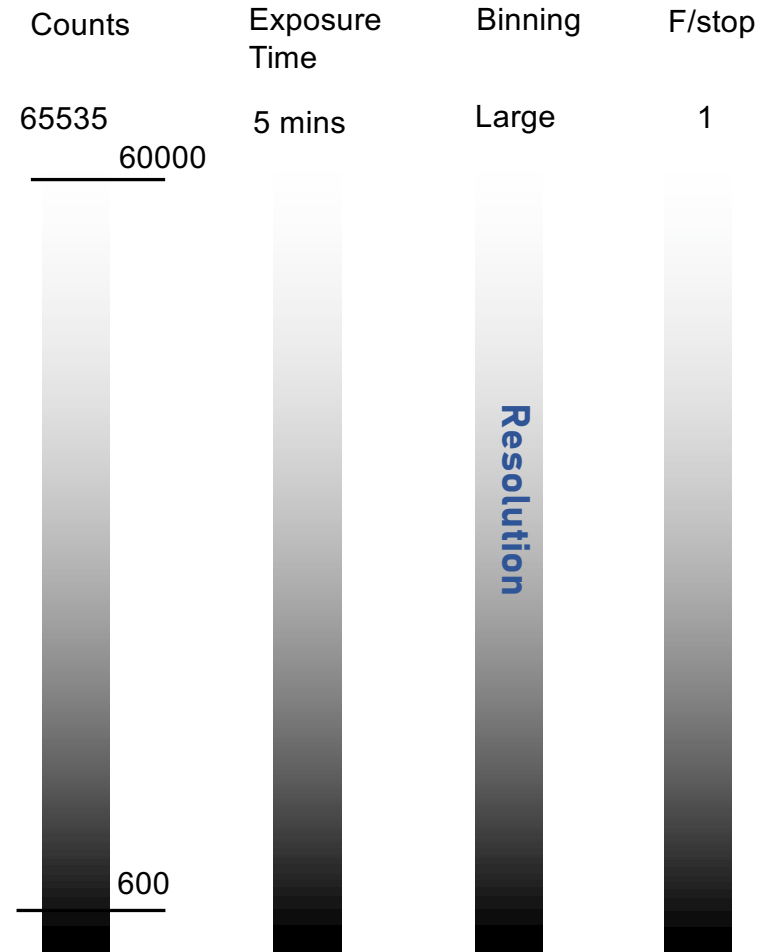
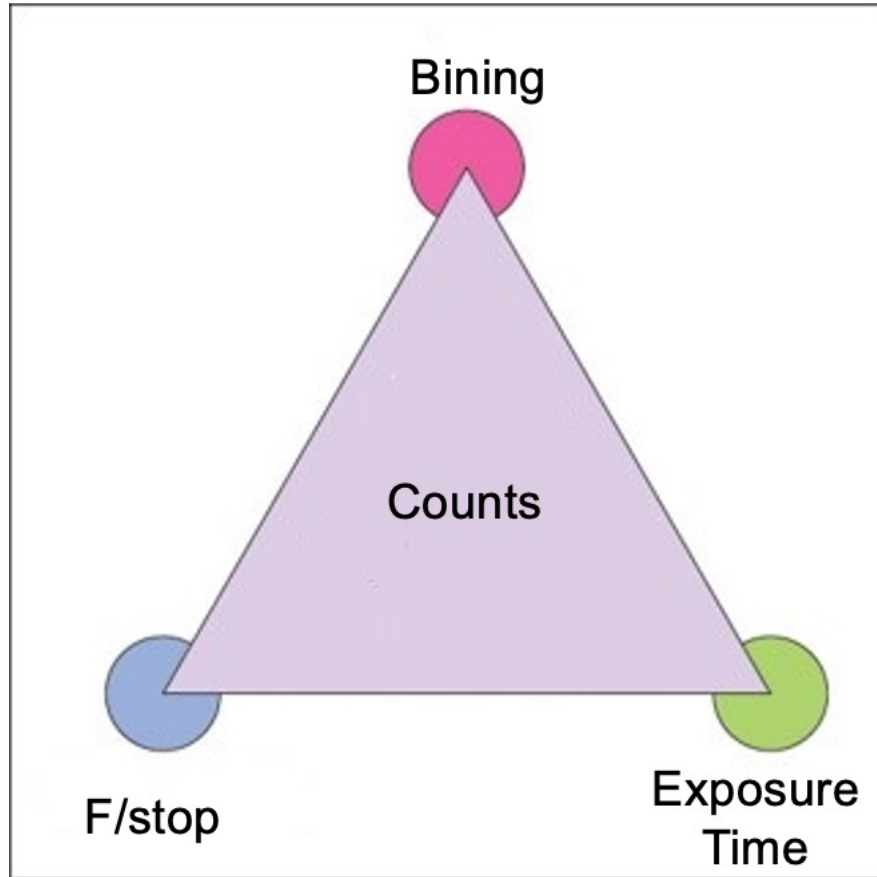
# Pixel Binning (CCD Resolution)

- 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

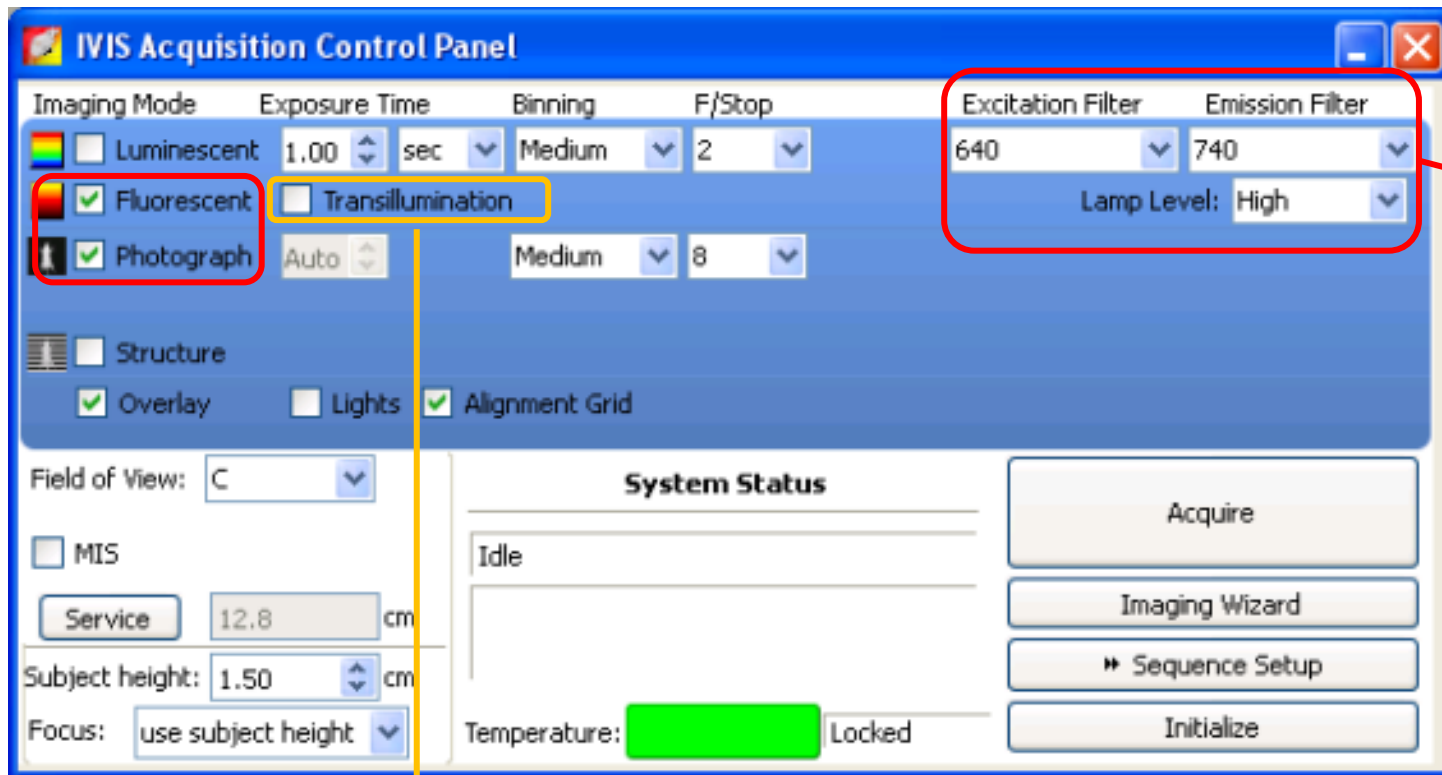


# Sensitivity Controls – Relationship of F/Stop, Exposure Time and Bining





## Acquisition - Single Image (Fluorescent)

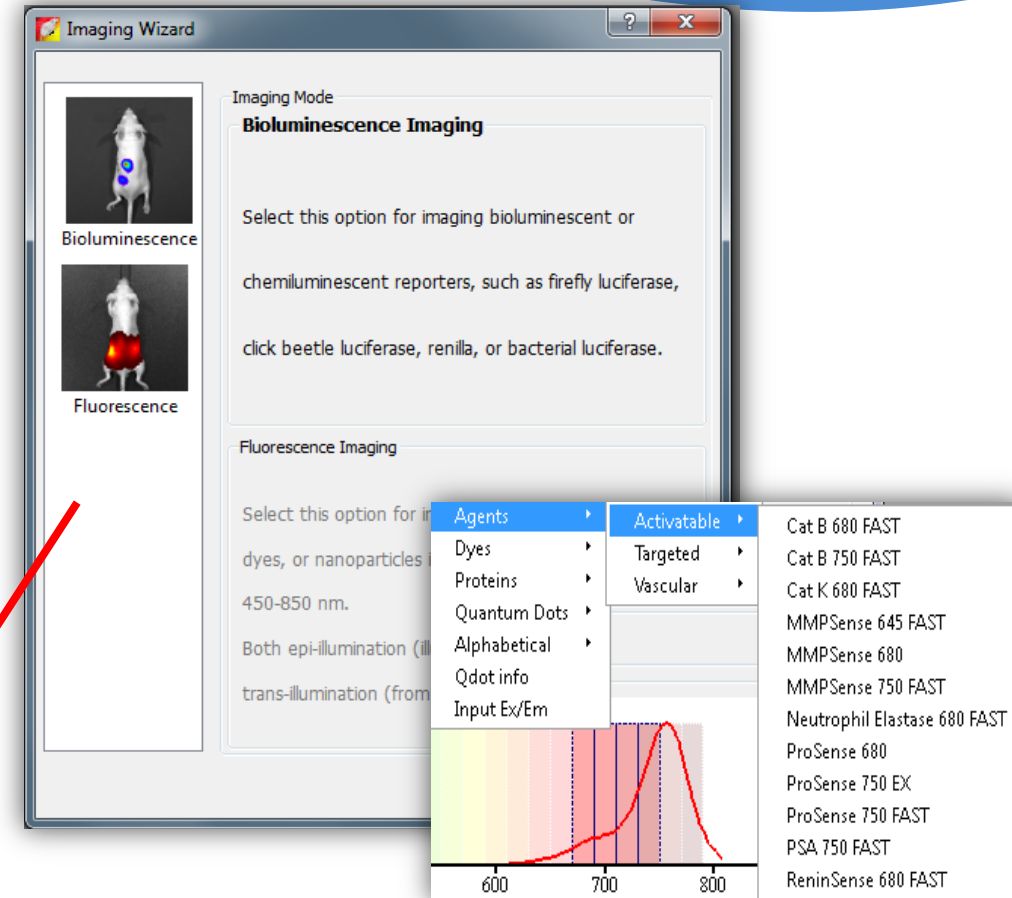
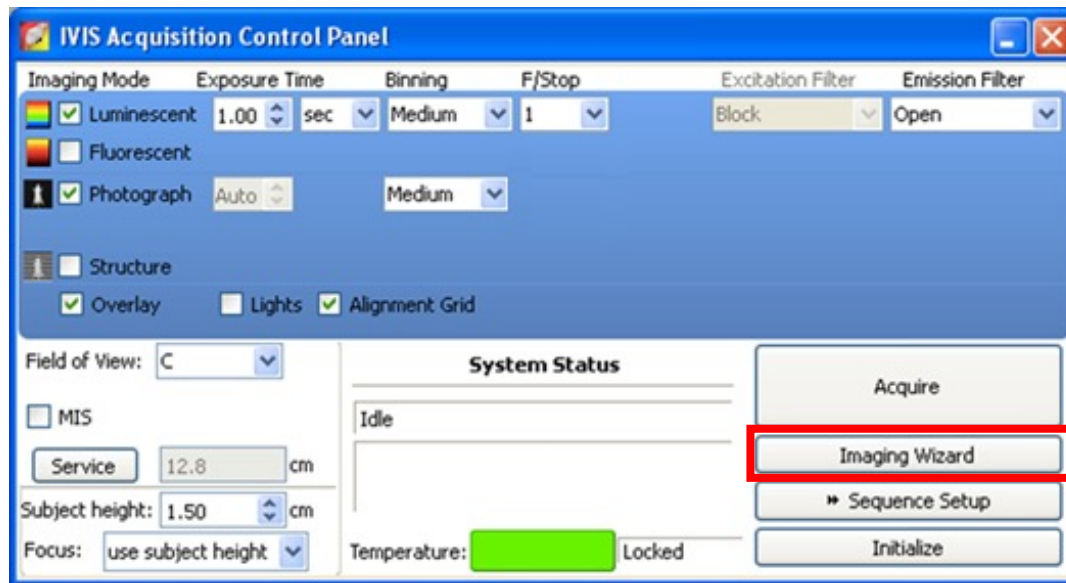


- ▶ Choose a suitable filter for your fluorescent
- ▶ Excitation light intensity

- ▶ Recommend the fluorescent source that is deep relative to the image side of the animal
- ▶ Excitation light source located below the stage

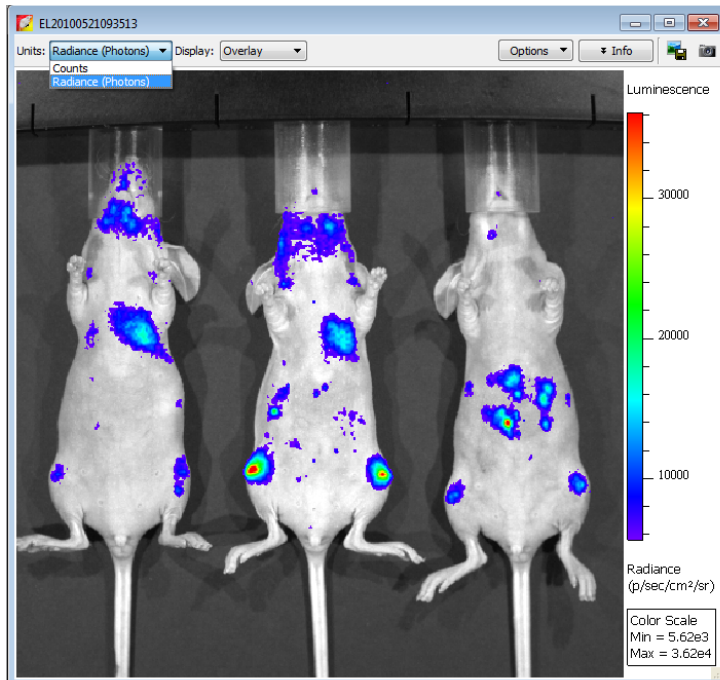
# Acquisition - Sequential mode

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



# Quantification

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



Tool Palette

Image Adjust

Photo Adjustment

Brightness: 100

Contrast: 1.5

Opacity: 100

Color Scale

Min: 5.62e3

Max: 3.62e4

Color Scale Limits

Auto Full Manual

Individual

Color Table

Rainbow

Reverse Logarithmic Scale

Corrections / Filtering

Image Information

ROI Tools

Measure ROIs

Apply to Sequence

Type: Measurement ROI

Save ROIs

Name: ROI\_1\_BNT

Delete Load Save

Auto ROI Parameters

Threshold %: 50

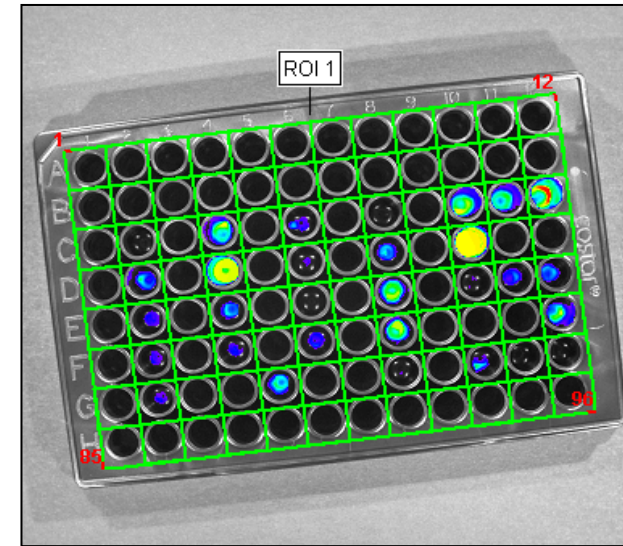
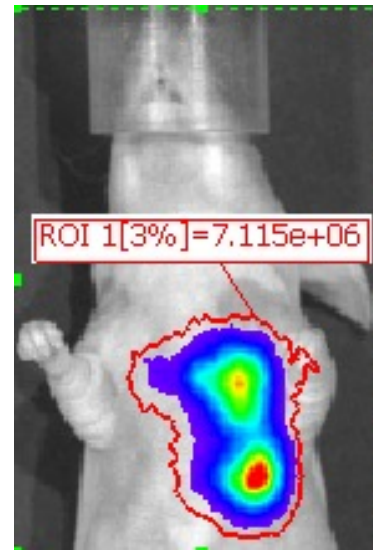
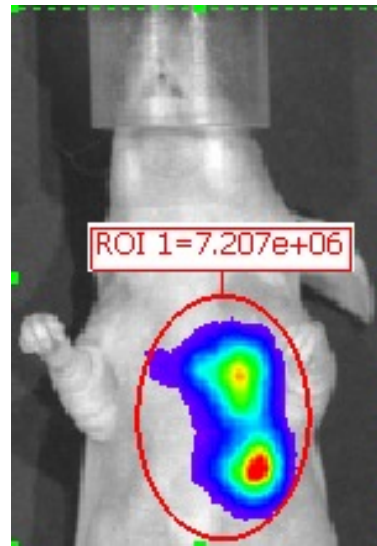
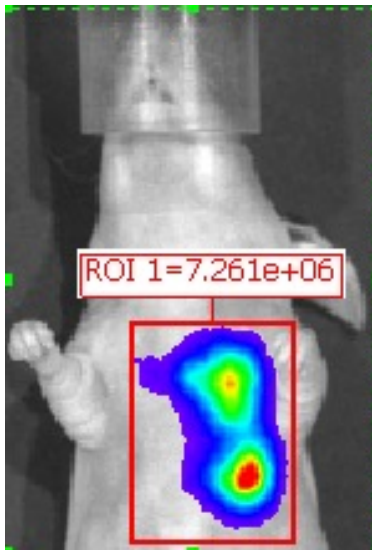
## Region of Interest (ROI) Tools

- ROI's can be created:

- Manually
- Automatically
- Free Draw

- ▶ ROI shapes available:

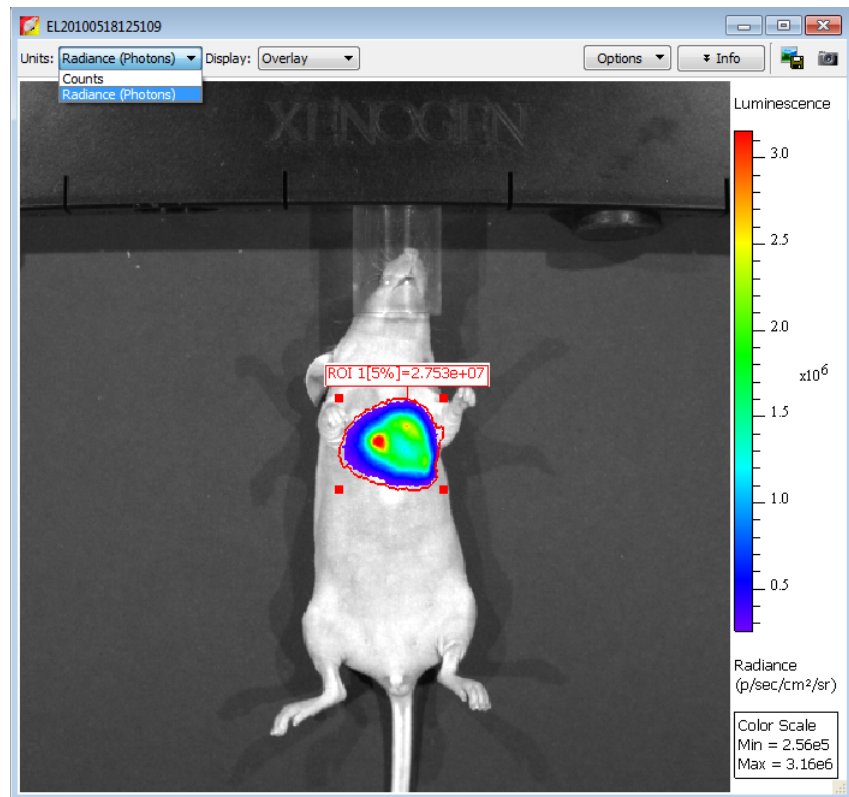
- Square
- Circle
- Contour
- Grid





# Measurement Table

- Measurement table displays information about each Region of Interest (ROI)



**ROI Tools**

Measure ROIs

Apply to Sequence

Type: Measurement ROI

Save ROIs

Name: ROI\_1\_BNT

Delete Load Save

Auto ROI Parameters

Threshold %: 50

ROI Measurements

Image Number	ROI	Image Layer	Total Flux [p/s]	Avg Radiance [p/s/cm²/sr]	Stdev Radiance	Min Radiance	Max Radiance
EL20100518125109	ROI1	Overlay	2.753e+07	9.797e+05	7.118e+05	1.845e+05	3.691e+06

Customized Selections

Measurements Types: Radiance (Photons) Image Attributes: \_none\_ ROI Dimensions: \_none\_