PerkinElmer
For the Better

HUMAN HEALTH | ENVIRONMENTAL HEALTH

In Vivo Imaging
IVIS Lumina XRMS

曾筱筑
博克科技有限公司

What Will Be Covered?

Introduction

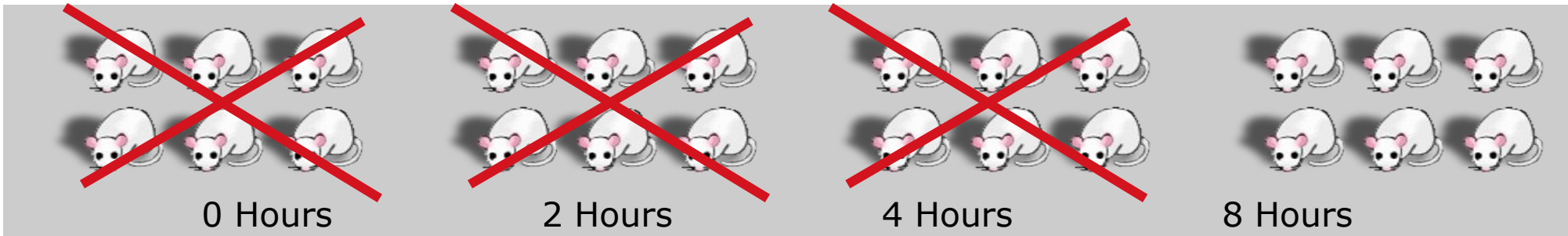
- Principles of Optical In Vivo Imaging
- Key IVIS® Hardware Components
- Overview of Living Image® Software
- Fluorescence and X-Ray Options

Training

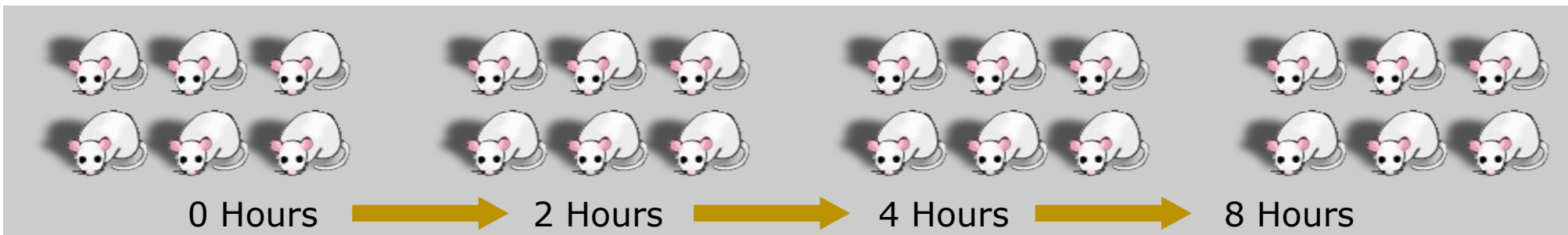
- Hands-on Training

Current vs. BPI methodology

Current Methodology = 24 animals over four treatment points



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

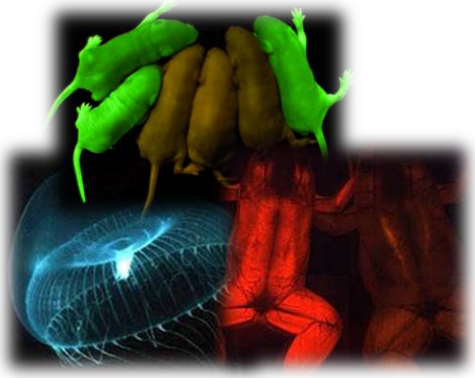


Same group of anesthetized test animals at each time point of an experiment uses far fewer animals than current methodology.

Optical Imaging Approaches

Luciferase

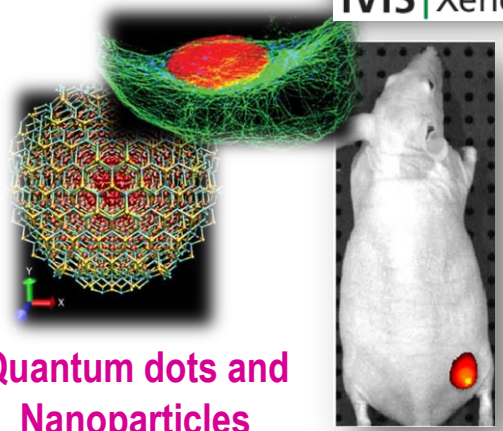
Fluorescent Proteins



Fluorescent dyes

Fluorescent Agents

IVIS | XenoLight DiR



Quantum dots and Nanoparticles

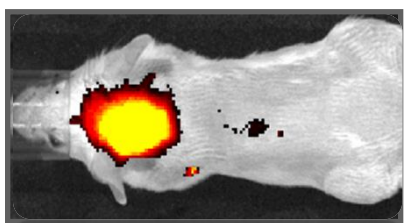
+ ATP and O_2 – Live cells

+ D-luciferin substrate

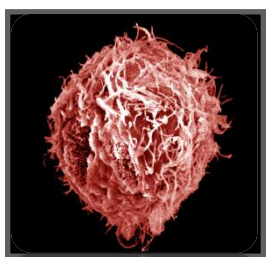
+ excitation light source

Transfection

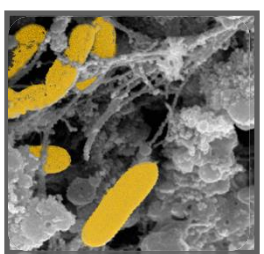
Direct cell/protein labeling



Genetic Marker



Label Cells



Label Bacteria

Label Proteins

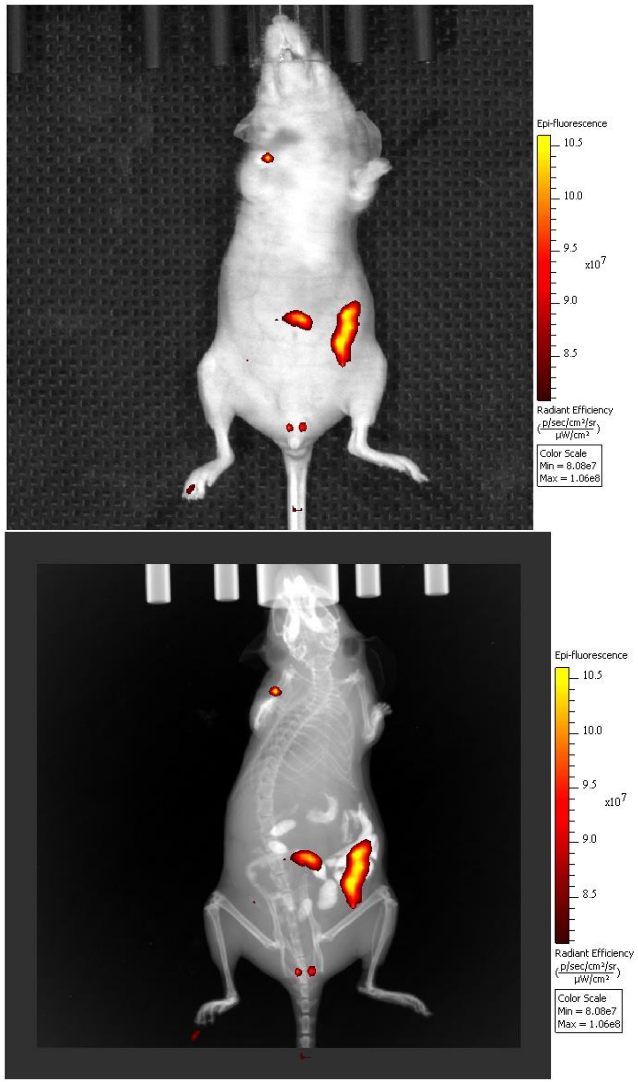
Why Optical In Vivo Imaging?

- 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

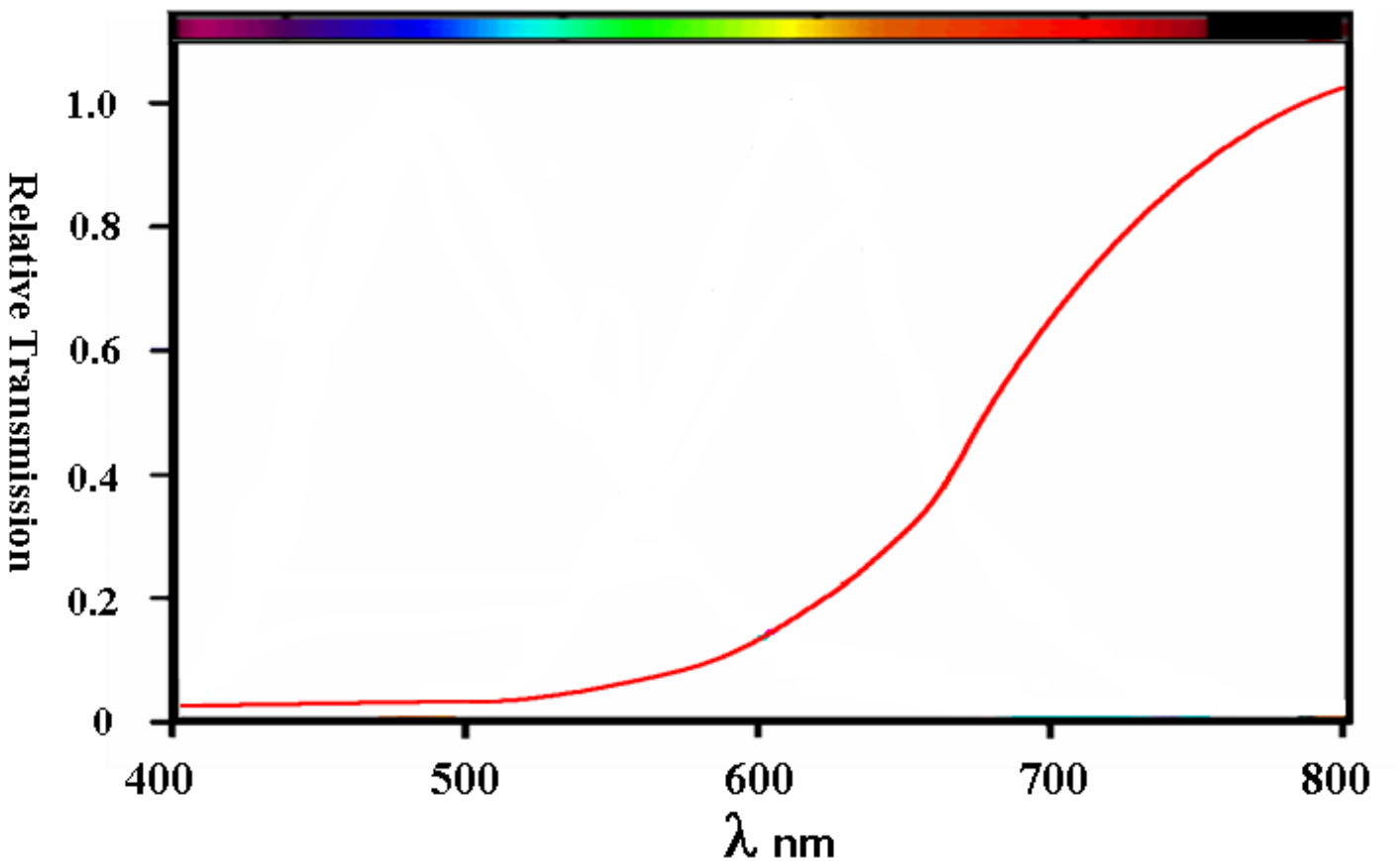
Value of X-Ray with Optical

X-Ray Provides an Anatomical Reference
to the Functional Optical Reporter

- The Question: Where is the source origin relative to the surface signal?
- The Problem: Tissue attenuation/ scattering makes 2D optical signals difficult to locate at a defined location.
- The Solution: A co-registered X-ray image provides a fixed anatomical reference, defining skeletal structure and soft tissue organs and enabling better localization of the optical signal.

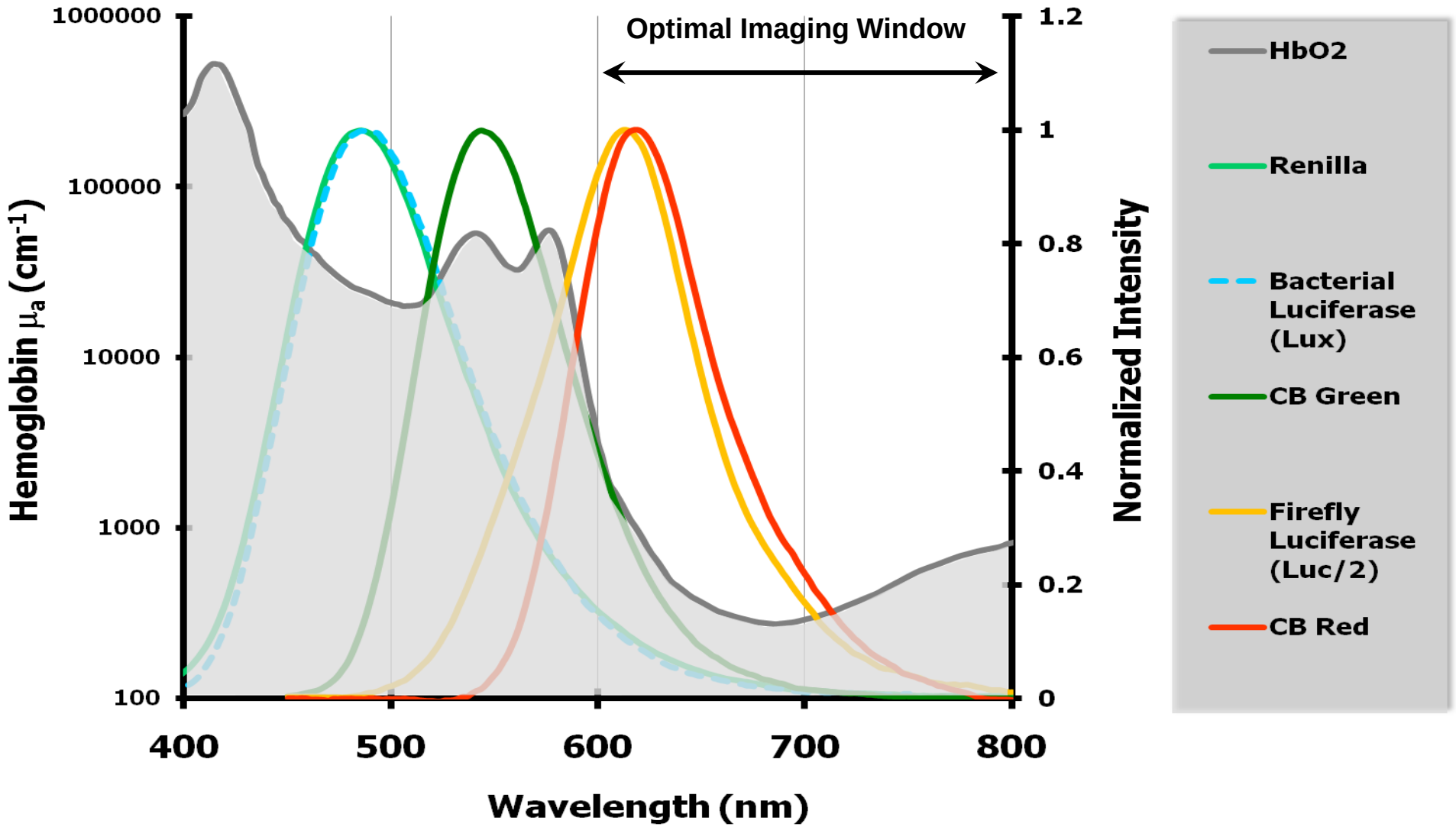


Tissue Is Not Transparent – Light Absorbance Depends on Wavelength

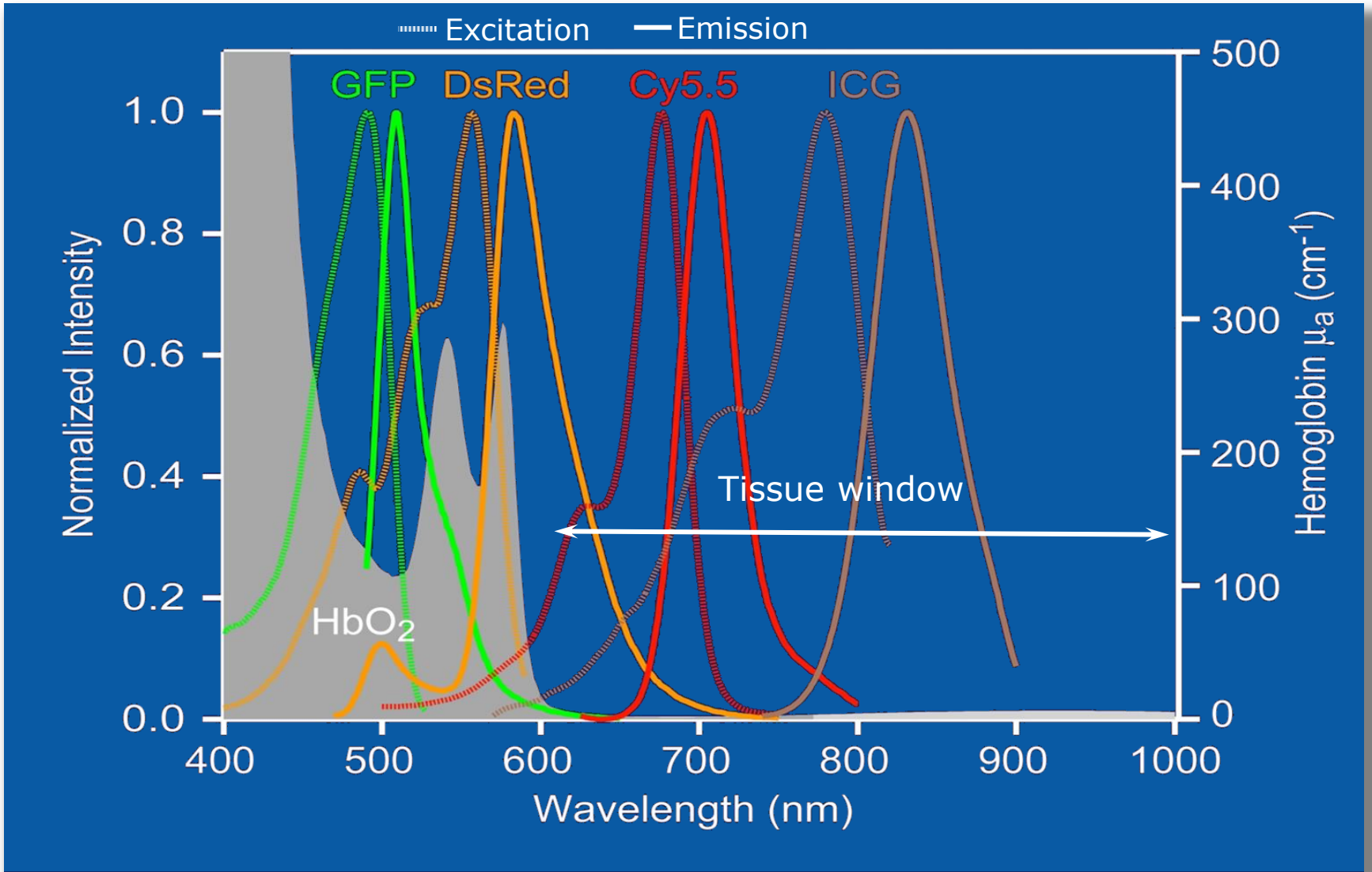


Transmission of light through 1 cm of tissue

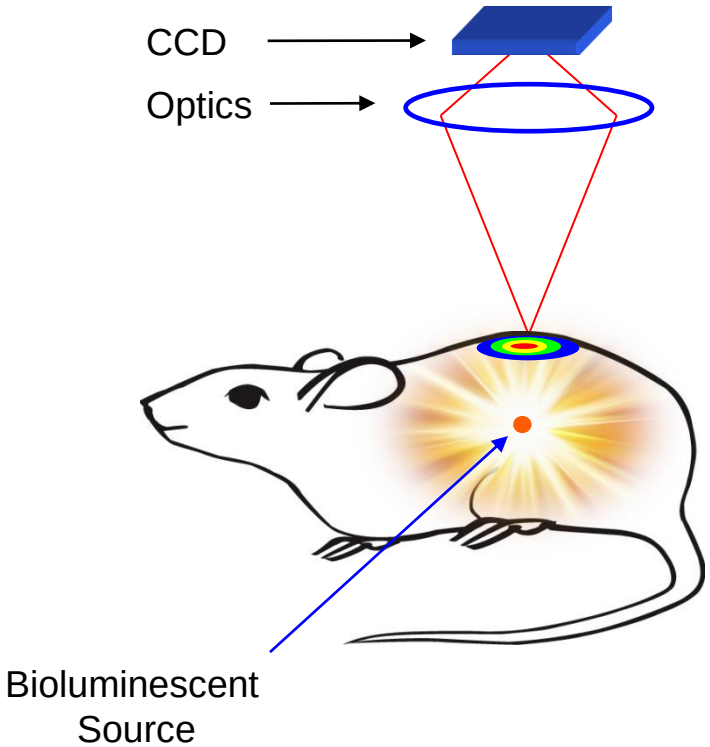
Bioluminescent Spectra and Tissue Penetration



Emission Spectra of Common Fluorophores

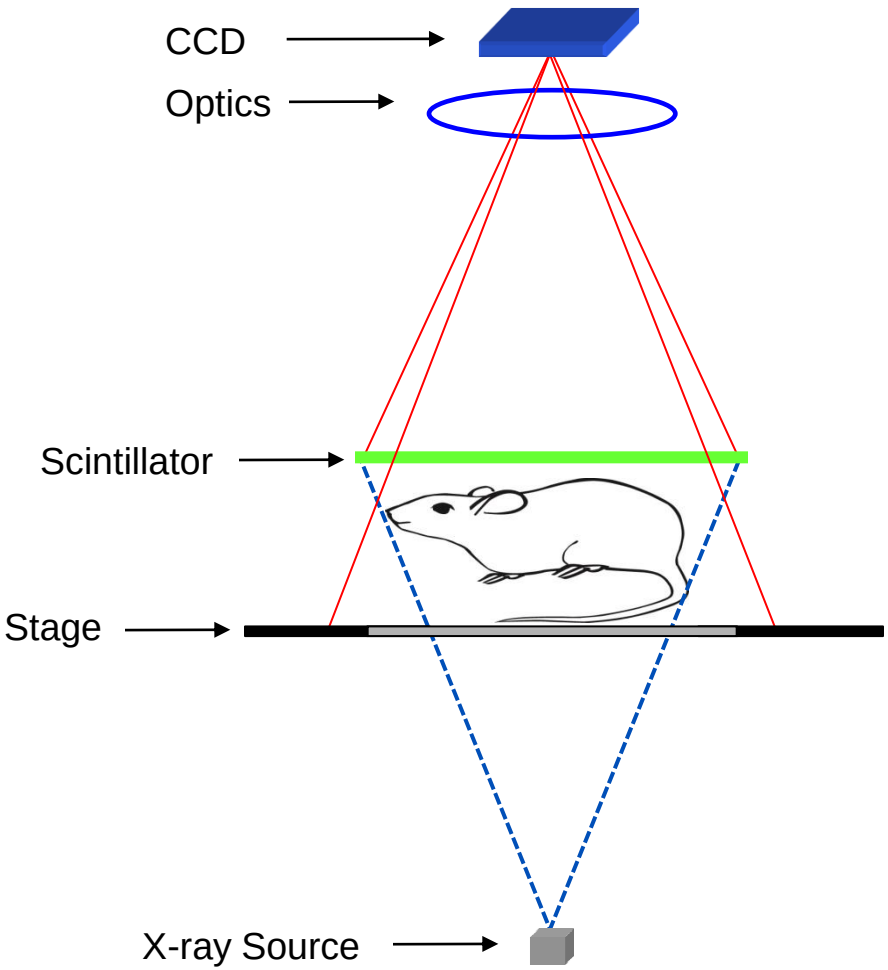


Photons Diffuse Through Tissue – Surface Light Pattern is Recorded



- 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

How an X-Ray Image is Acquired



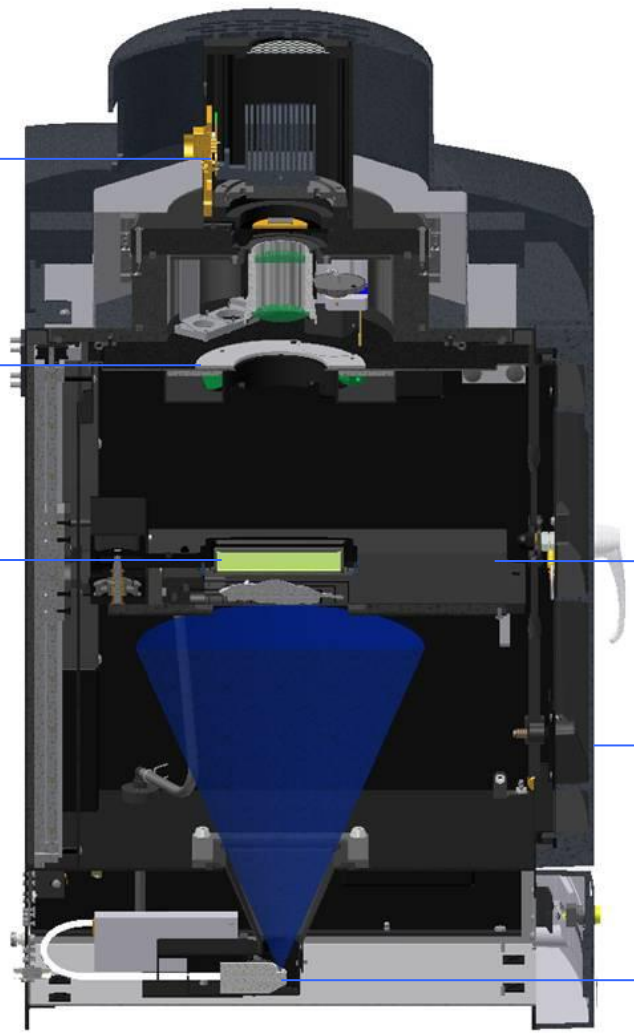
- X-rays will be attenuated in tissue differently resulting in an image on the scintillator
- The CCD views the scintillator resulting in a planar X-ray image
- X-ray and Optical images have different path lengths. To correct this geometrical difference, the X-ray image is registered to the optical image

Close Up of Scintillator in Position

IVIS Lumina XRMS Imaging System - Hardware

- Customized for *in-vivo* imaging
- High sensitivity from 300-900 nm
- Large dynamic range

IVIS IVIS XR Imaging System - Chamber



CCD Camera
Cooled -90C

Interchangeable
Emission Filter Wheel

Cesium Iodide
(CsI(Tl)) scintillator

Heated
Sample Stage

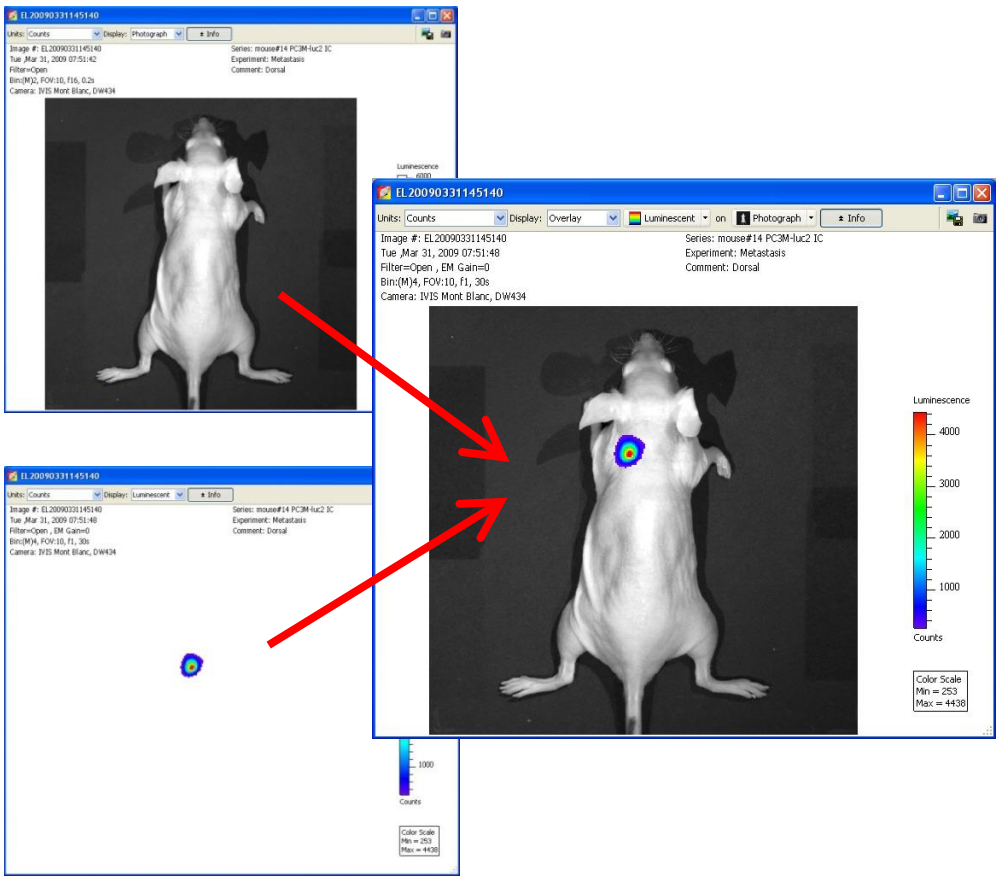
Light-tight
and shielded
imaging chamber

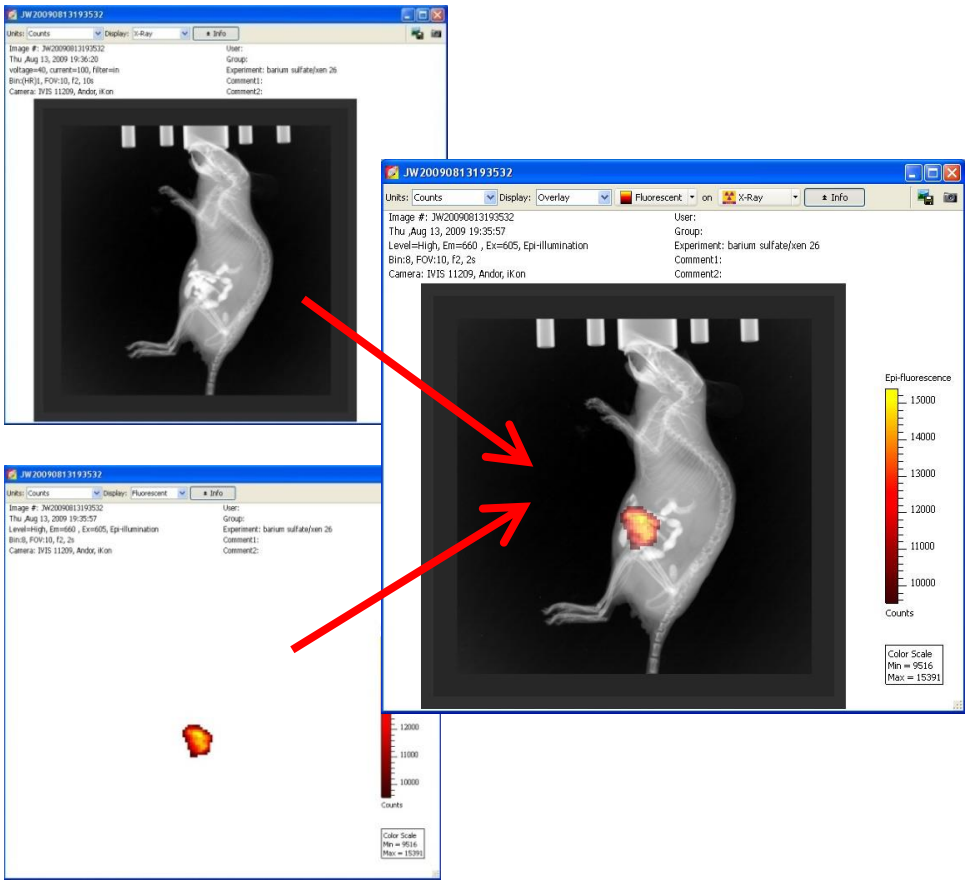
X-Ray Source

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

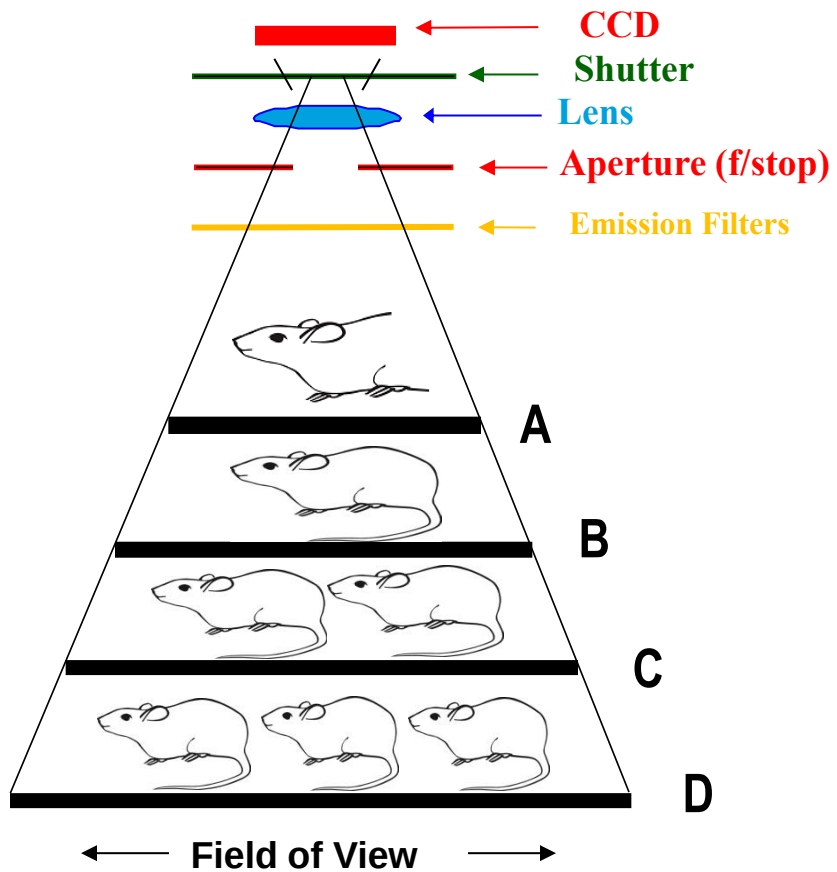
Photographic /X-Ray + Optical Image = Overlay





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

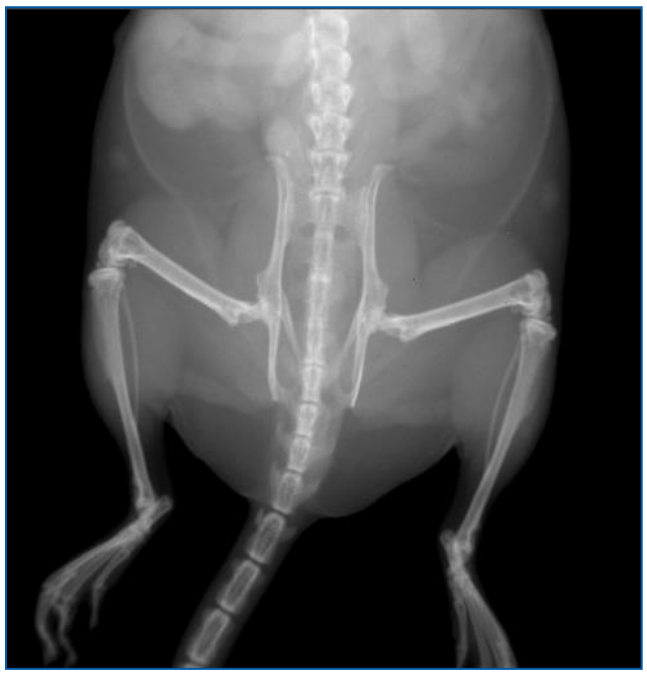
- Field of View (FOV) is dependent on the distance from the lens to the sample
- Light collected is proportional to how long the shutter is open (exposure time)
- Aperture (f/stop) controls the amount of light collected
- Digital pixel binning possible with CCD - for further increase in sensitivity



Field of View (X-Ray)

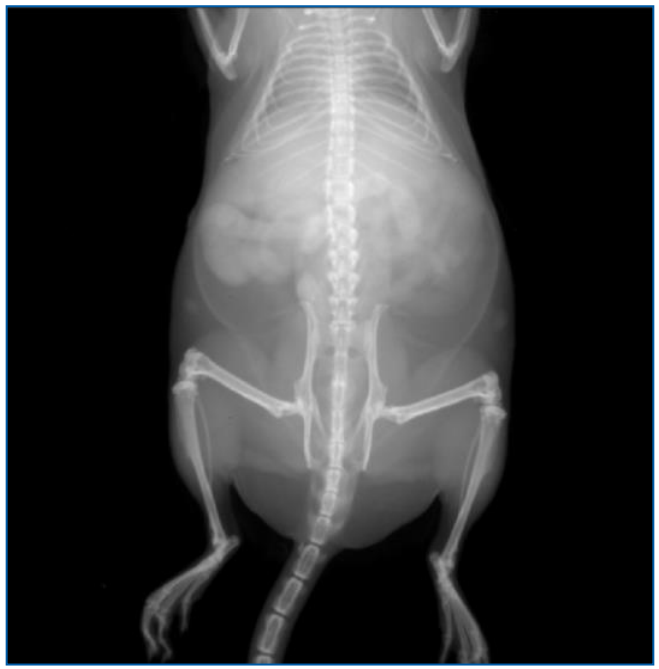
FOV A

5 x 5 cm



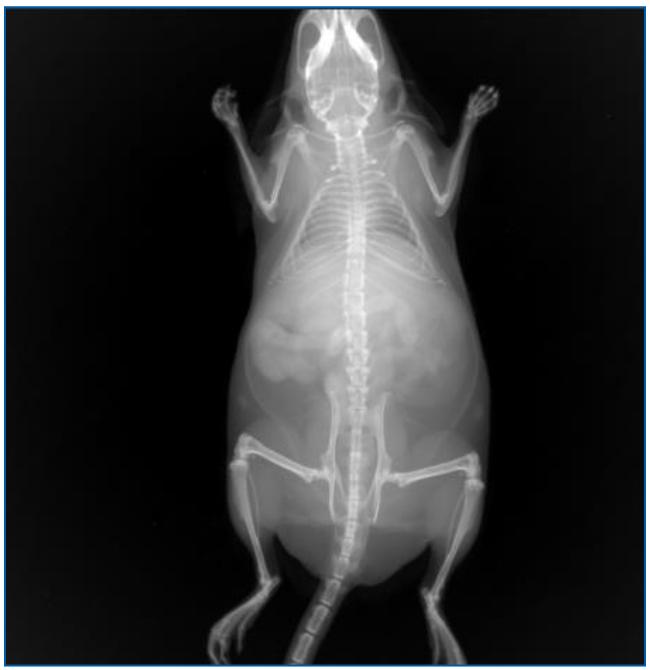
FOV B

7.5 x 7.5 cm



FOV C

10 x 10 cm

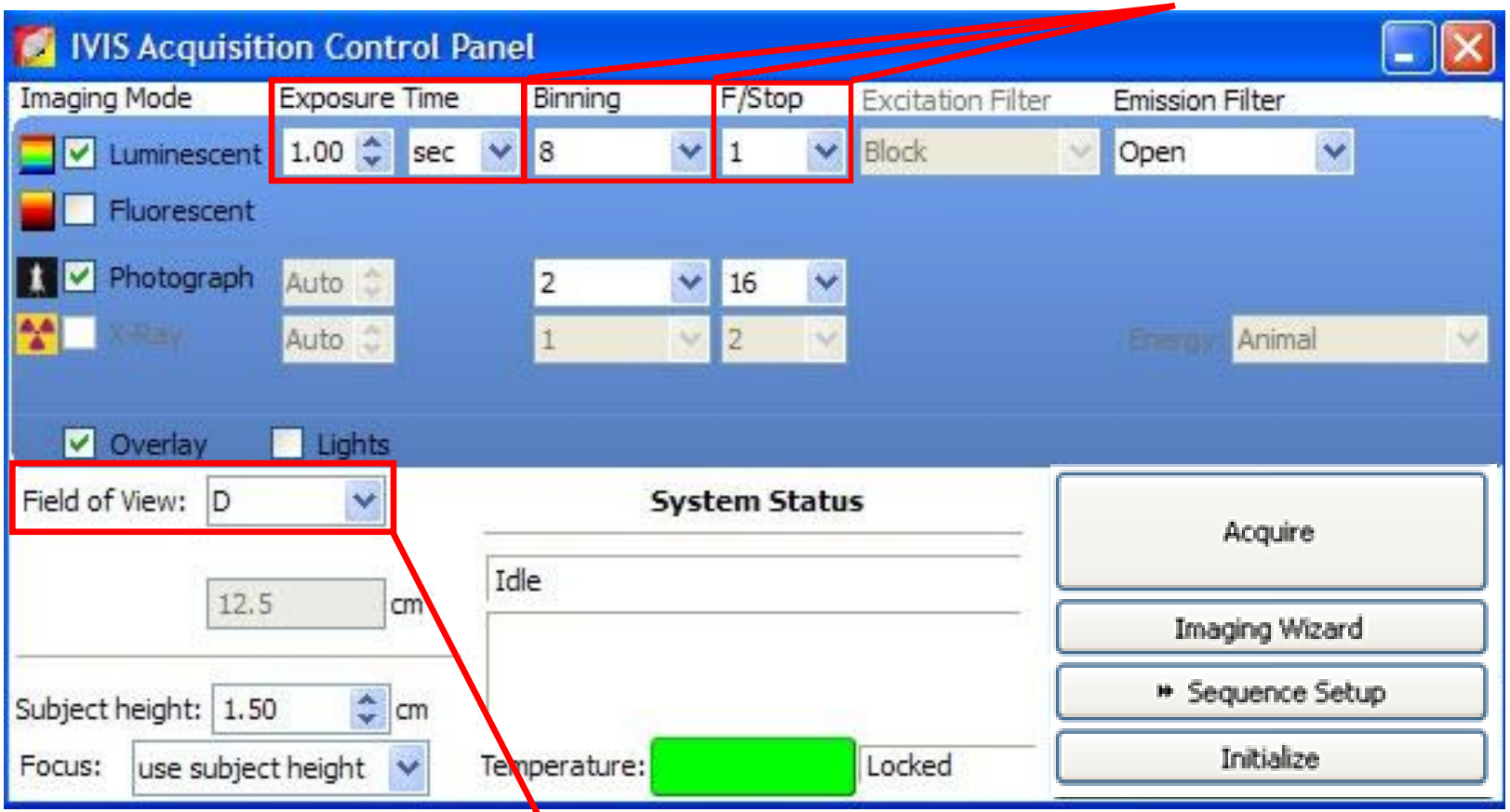


Setting Sensitivity – Signal Level

- 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

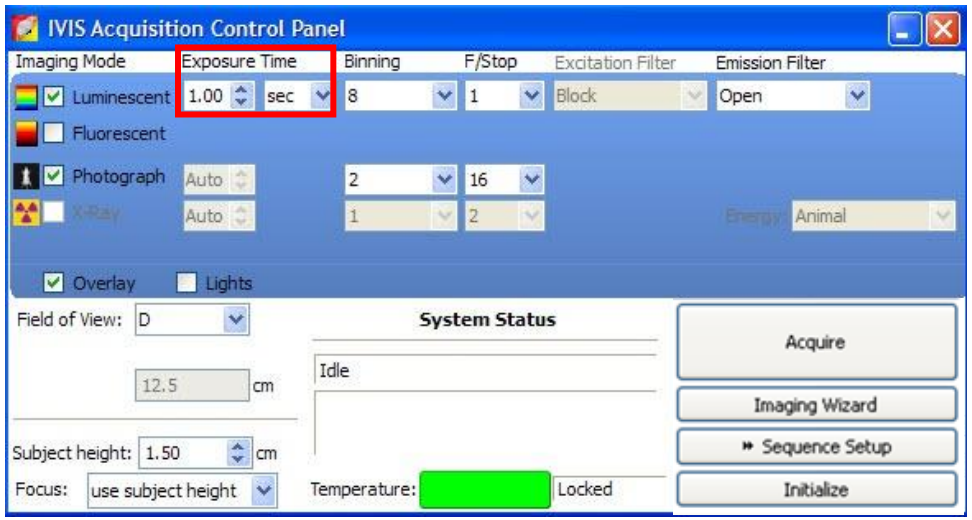
Controls Sensitivity

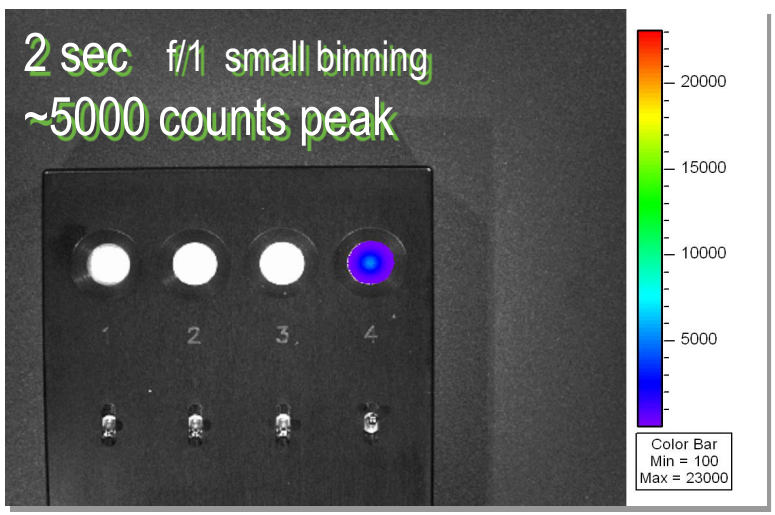


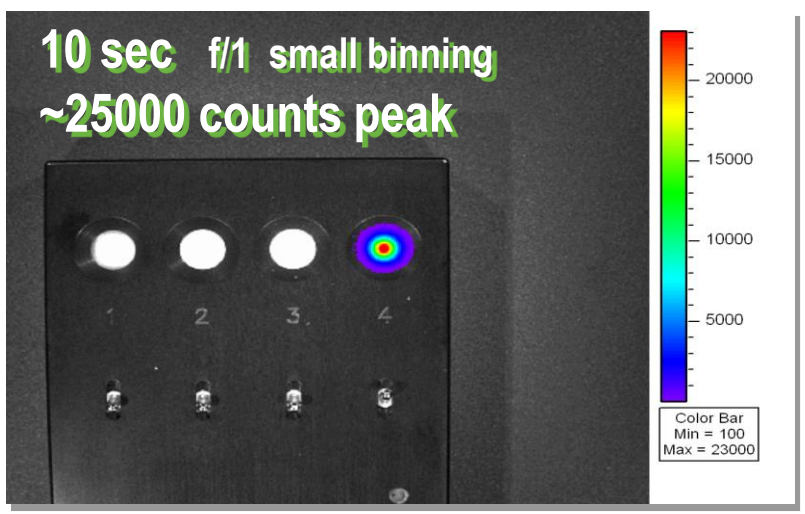
Affects Sensitivity

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

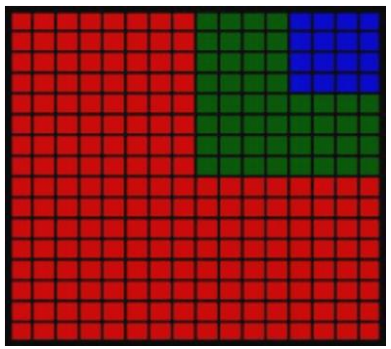




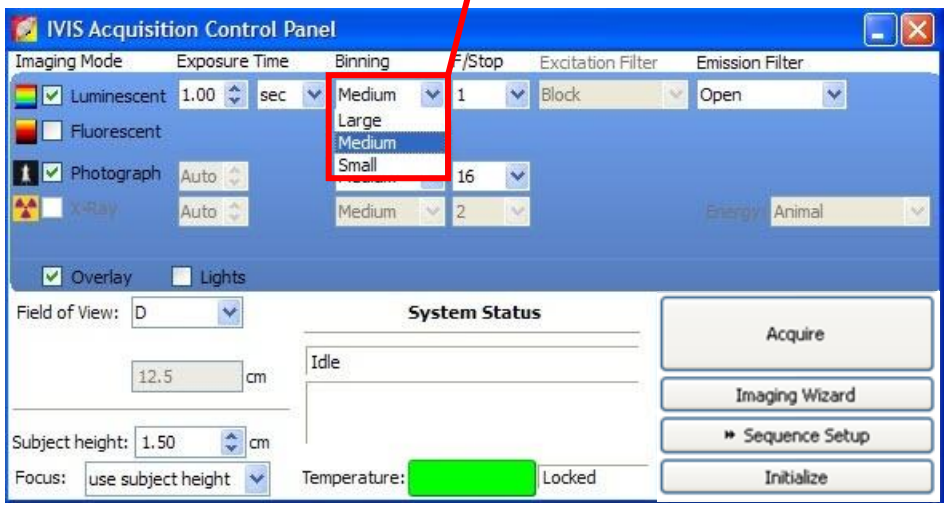


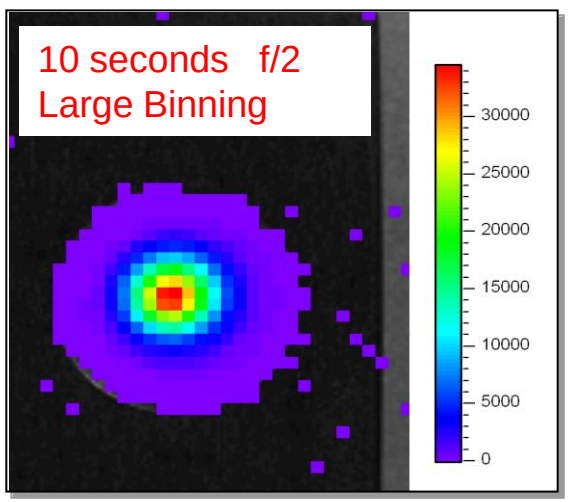
Pixel Binning (CCD Resolution)

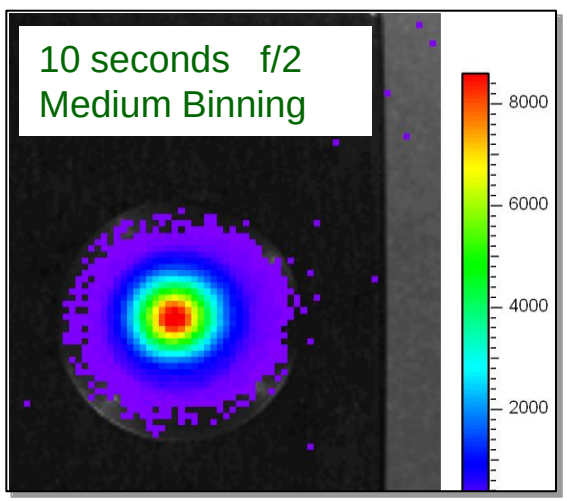
- Binning is the grouping of pixels into a larger super-pixel
- Changing binning settings changes counts by a factor of 4
- Large Binning (16)
  Higher Sensitivity/Lower Resolution
- Medium Binning (8)
- Small Binning (4)
  Lower Sensitivity/Higher Resolution

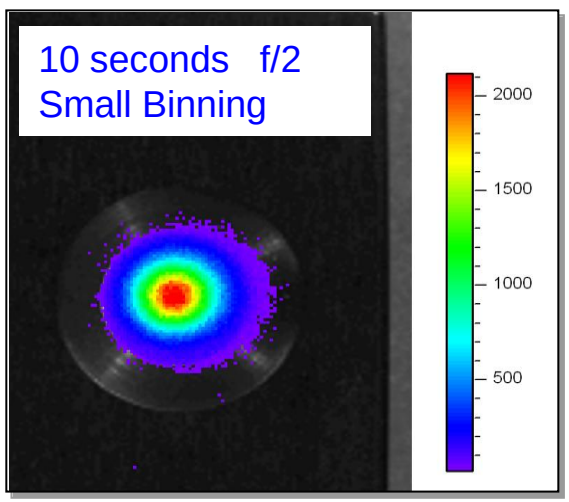


Pixel binning setting





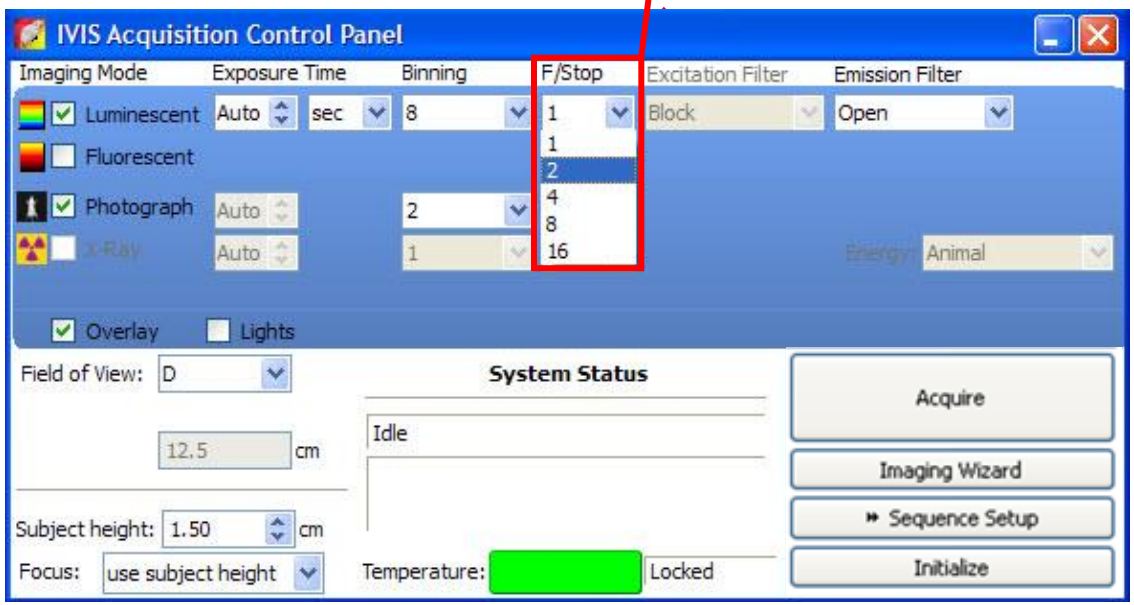




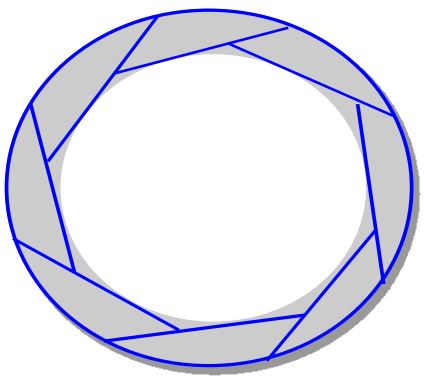
f/stop (Lens Aperture)

- *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

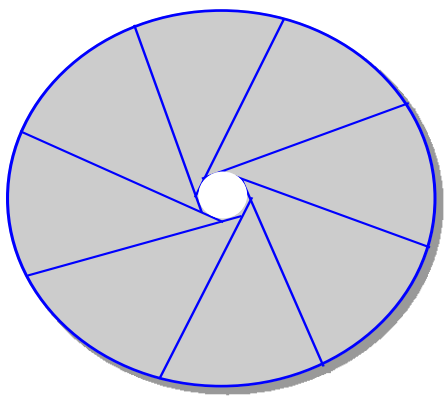
***f*/stop (aperture) setting**



***f*/1**

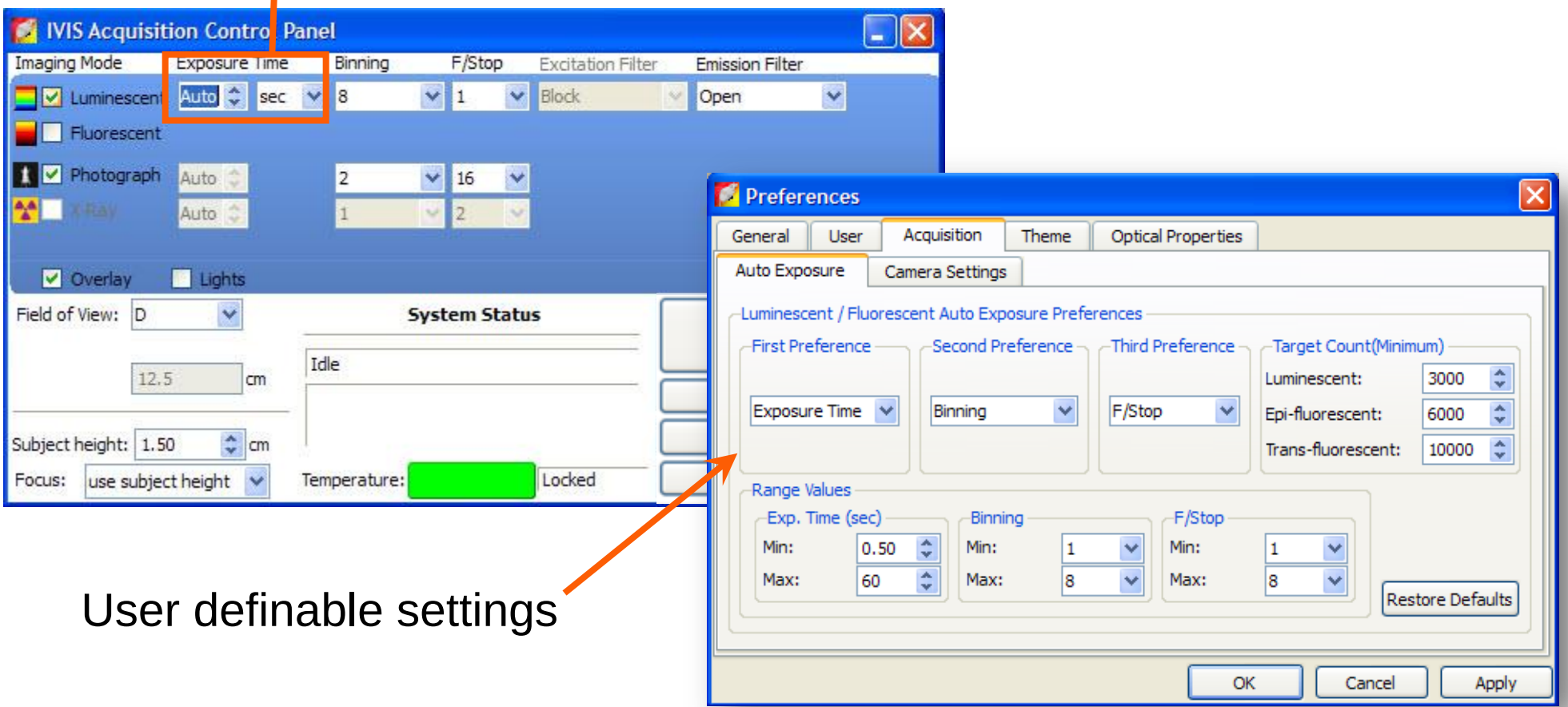


***f*/8**



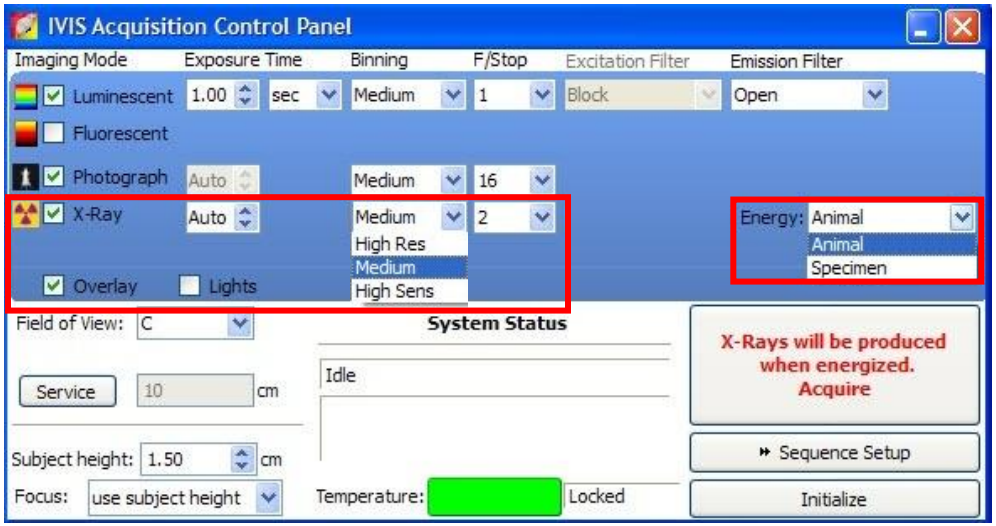
Auto-Exposure

Auto-exposure feature available for bioluminescence and fluorescence



User definable settings

X-Ray Parameters



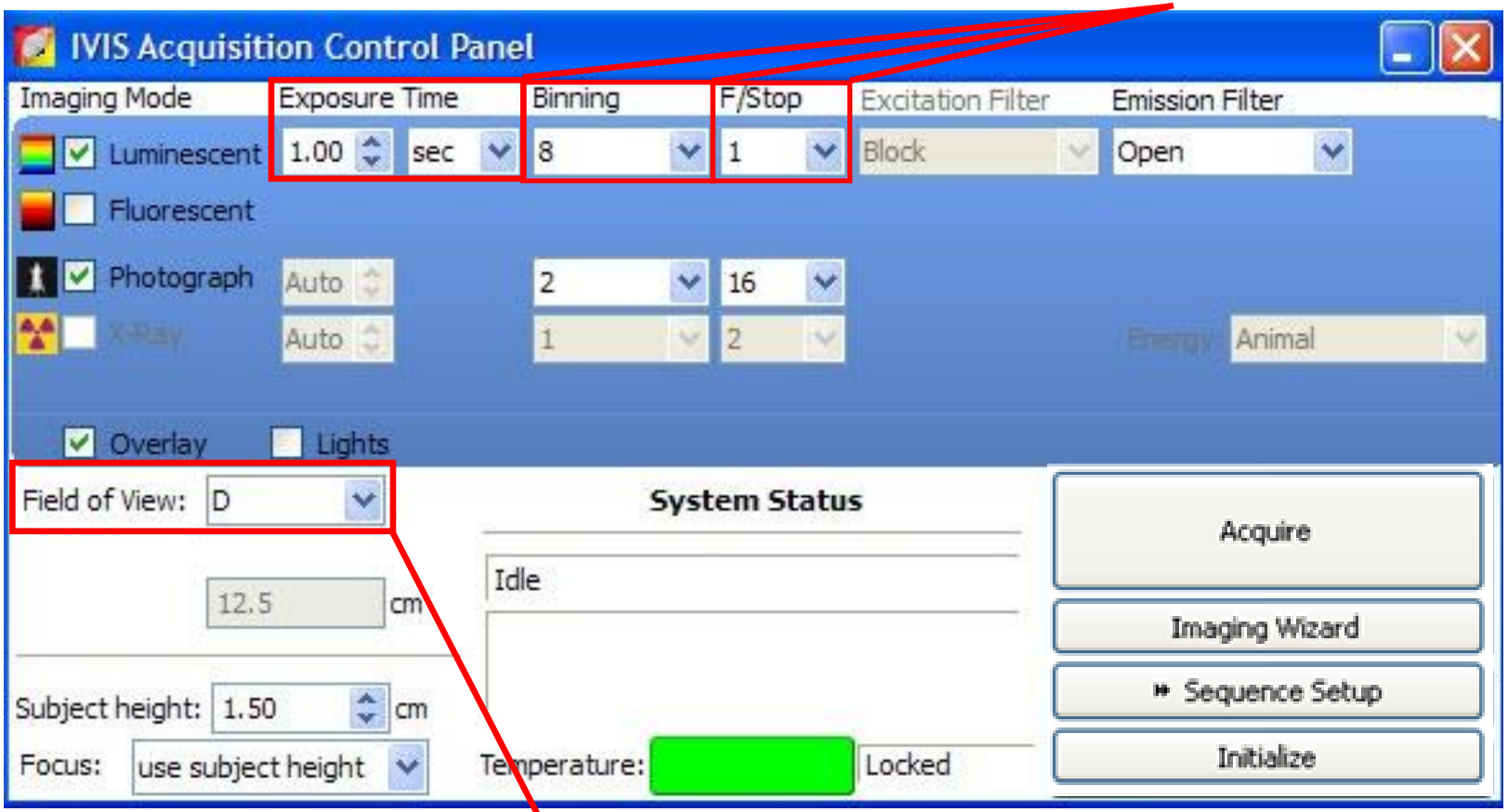
Binning controls resolution

Two Energy Settings:

- Animal: Tuned for live animal imaging, filter in place to reduce dose
- Specimen: Tuned for thin tissue samples, filter out to increase contrast

Summary – Basic Camera Settings

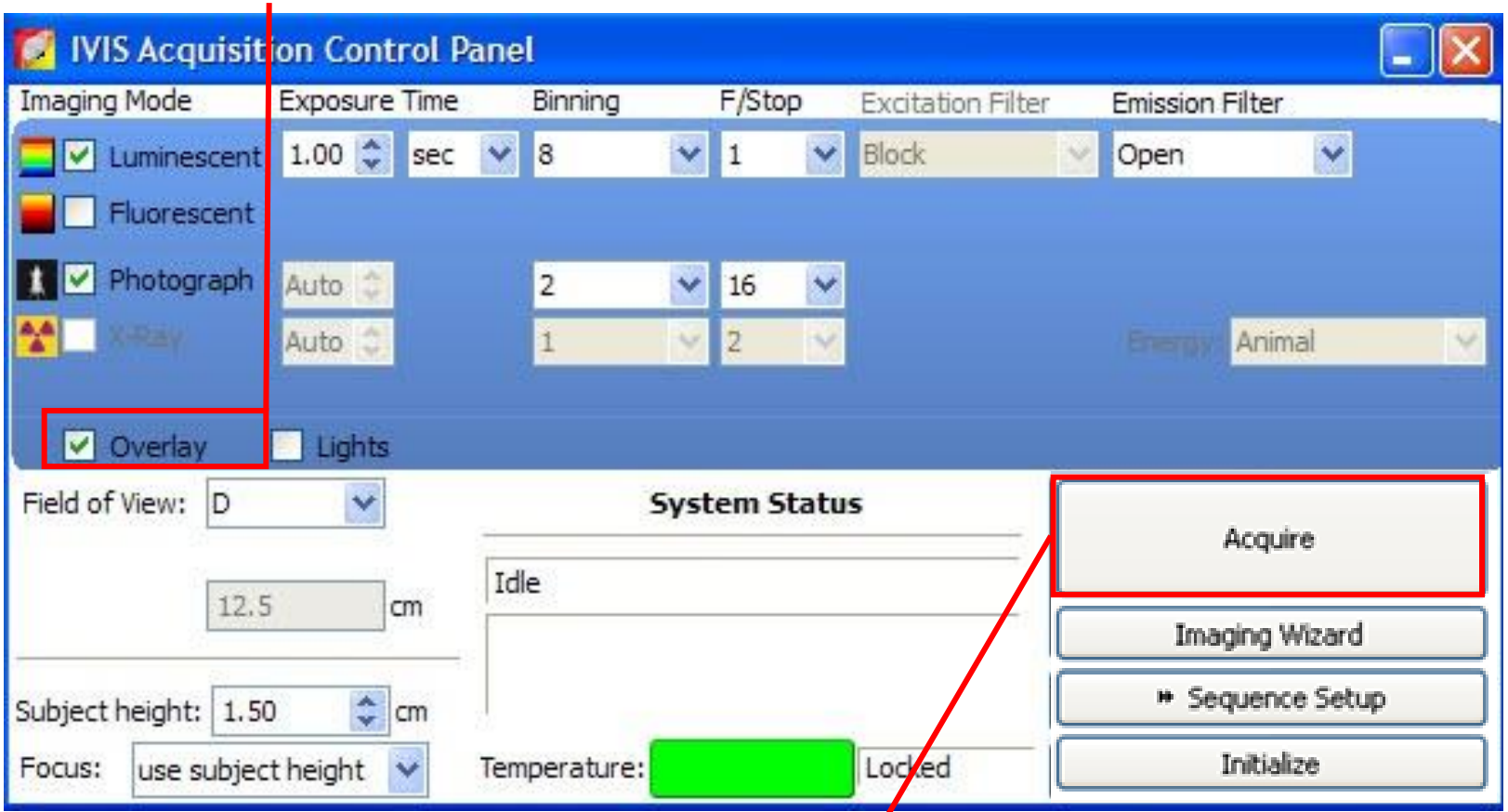
Controls Sensitivity



Affects Sensitivity

Single Image Acquisition

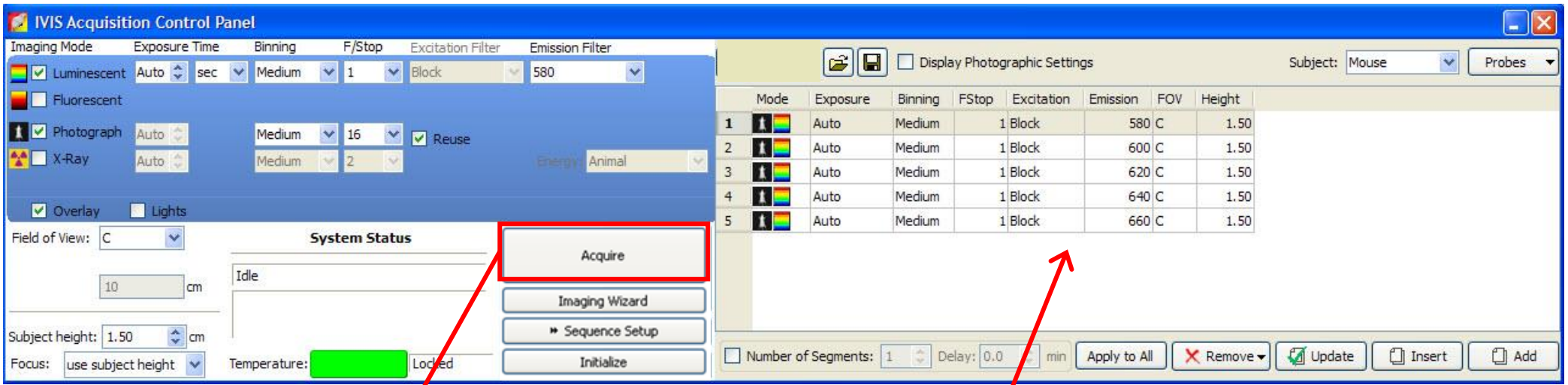
Overlay will automatically take Photo + Luminescent



Single Image Acquisition

Sequence (or Imaging Wizard) Acquisition

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



Starts Sequential
Image Acquisition

User Friendly
Sequence Editor

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

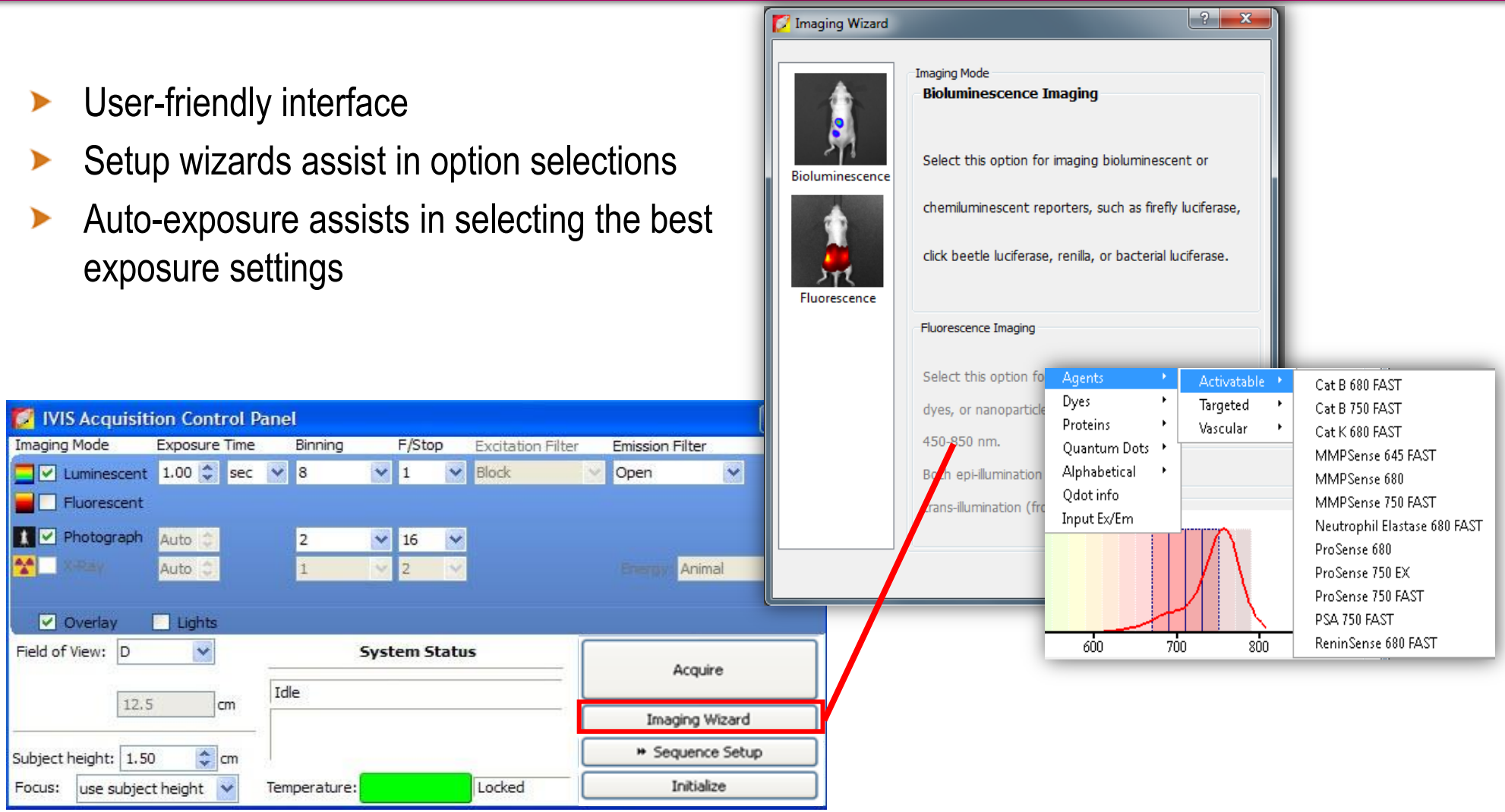
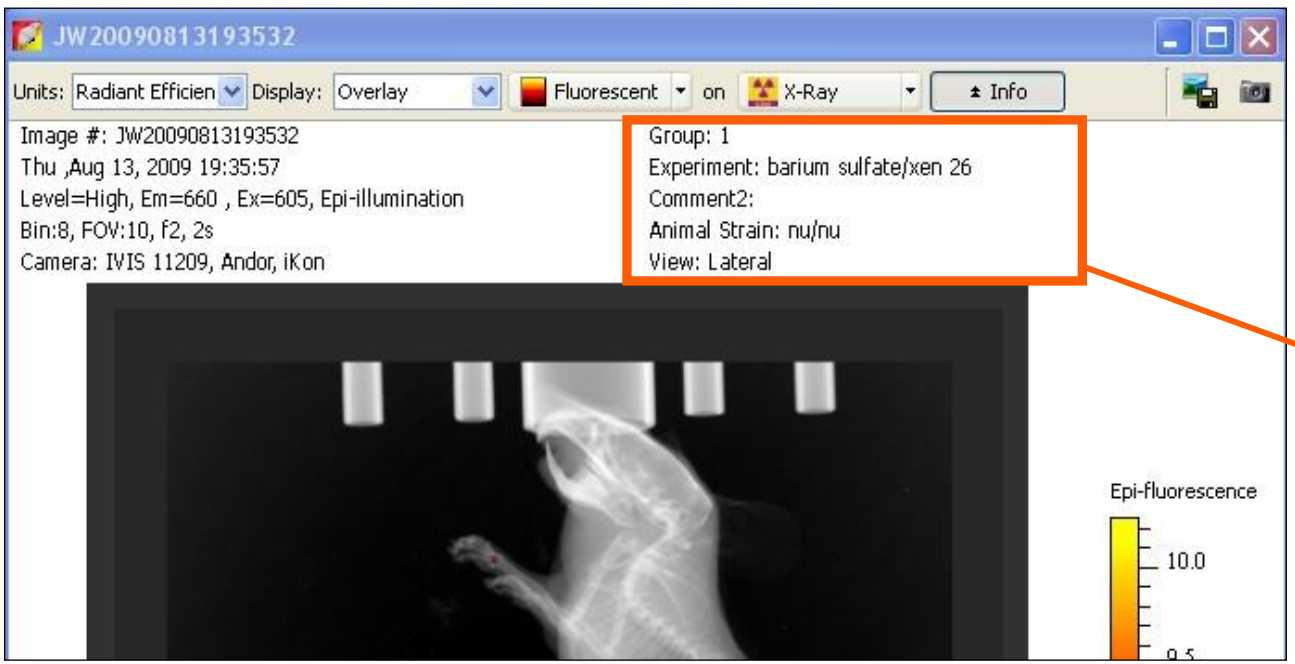


Image Labeling

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



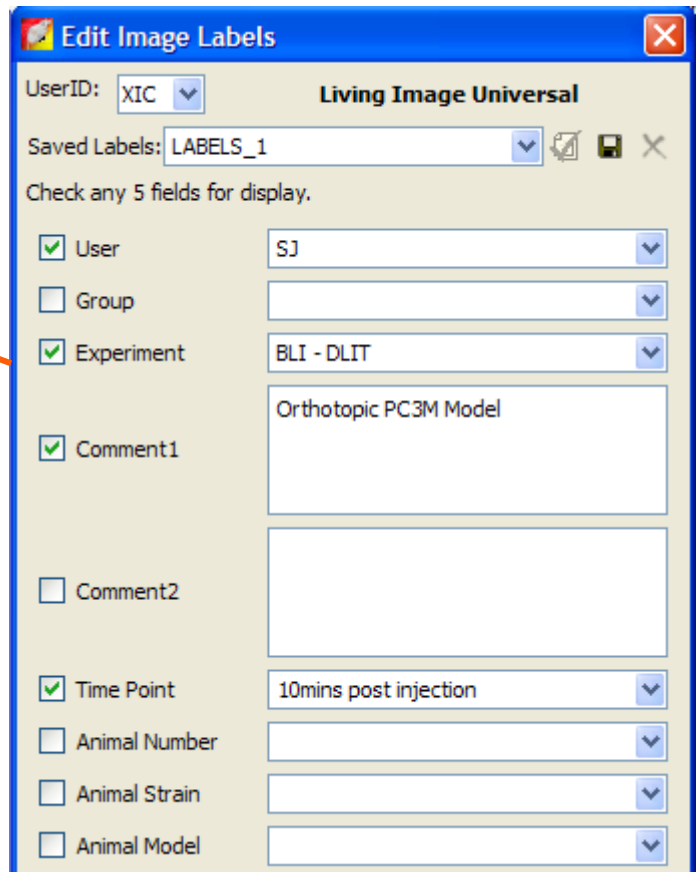
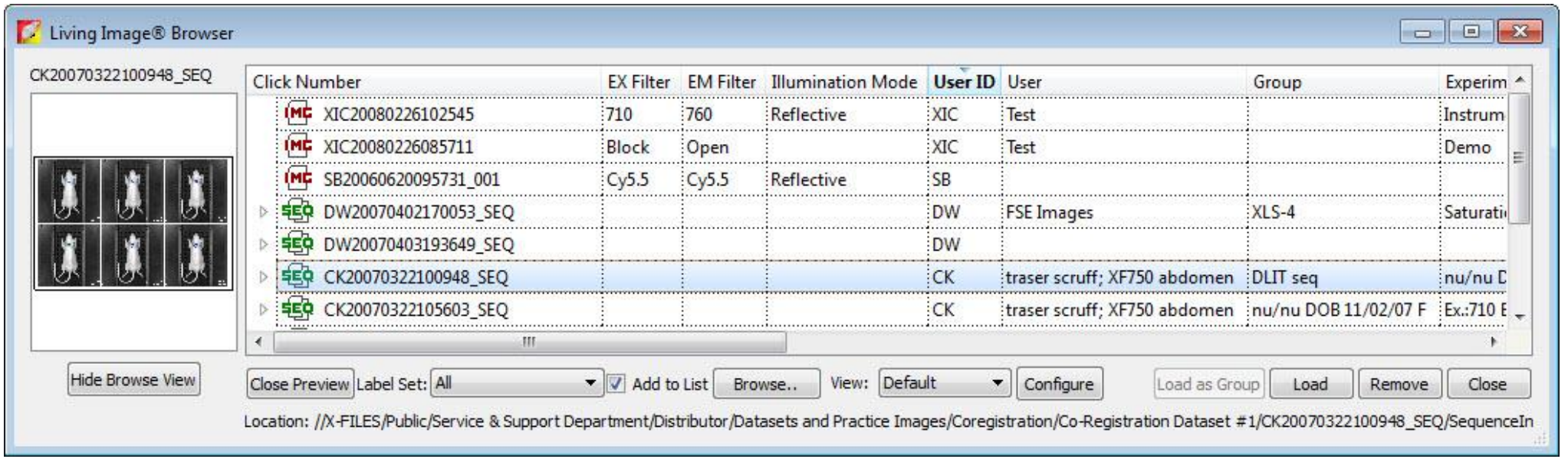


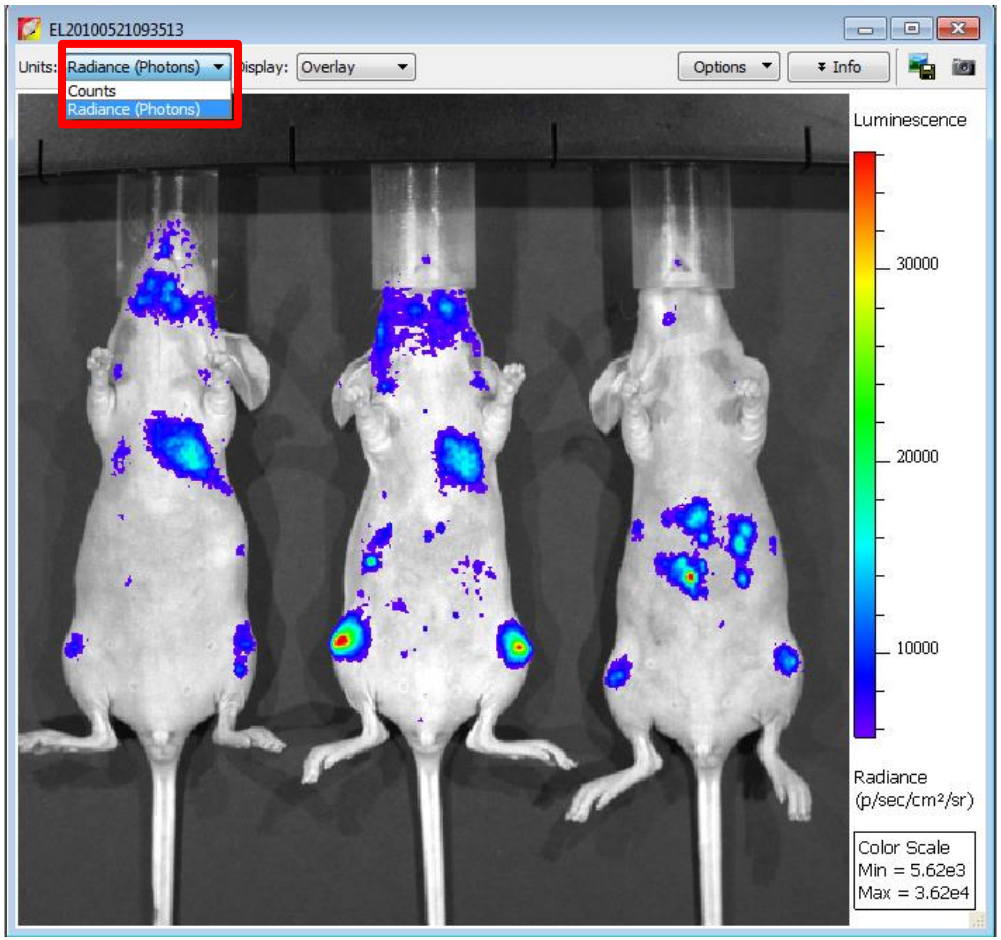
Image Cataloging and Browsing Tools

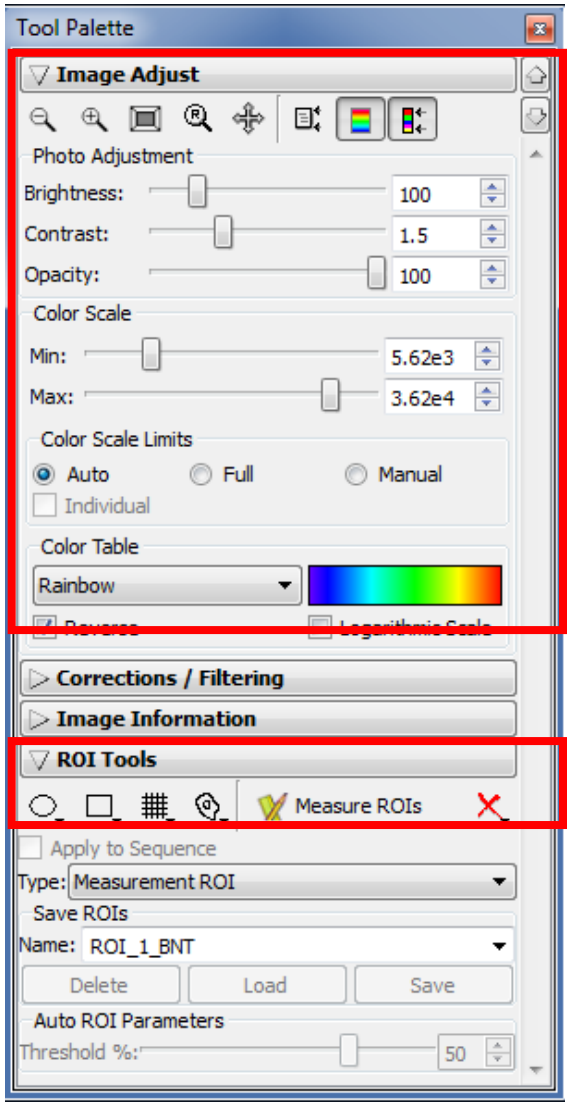


- Convenient preview window
- User defined labels listed with corresponding click number
- Sort by one or multiple columns
- Open multiple images in a single window for easier analysis with Load as Group

Quantification

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



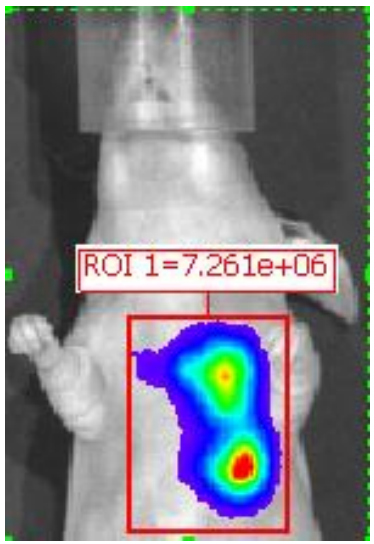


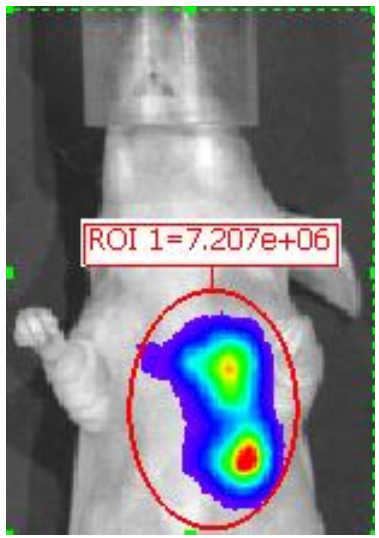
Region of Interest (ROI) Tools

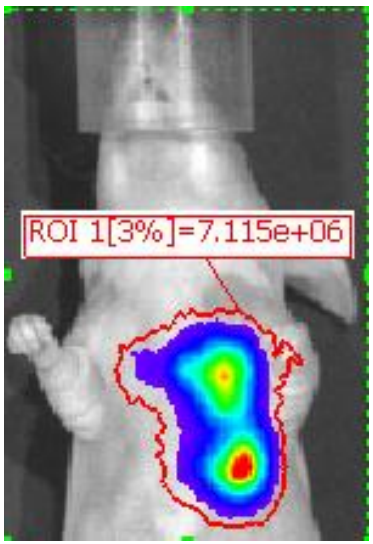
- ROI shapes available:
  - Square
  - Circle
  - Contour
  - Grid

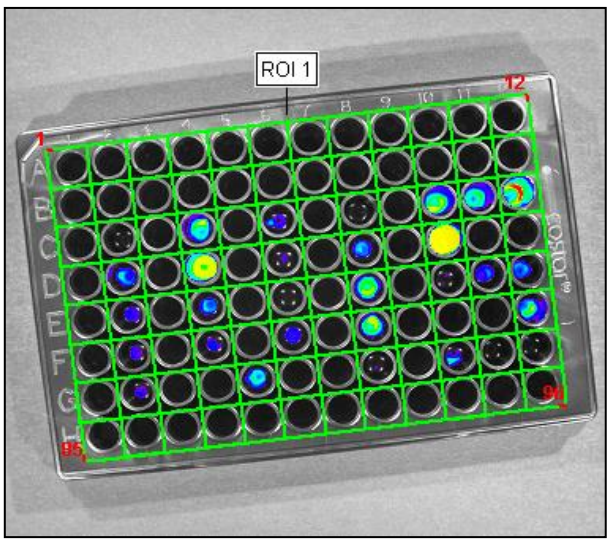
ROI's can be created:

- Manually
- Automatically
- Free Draw

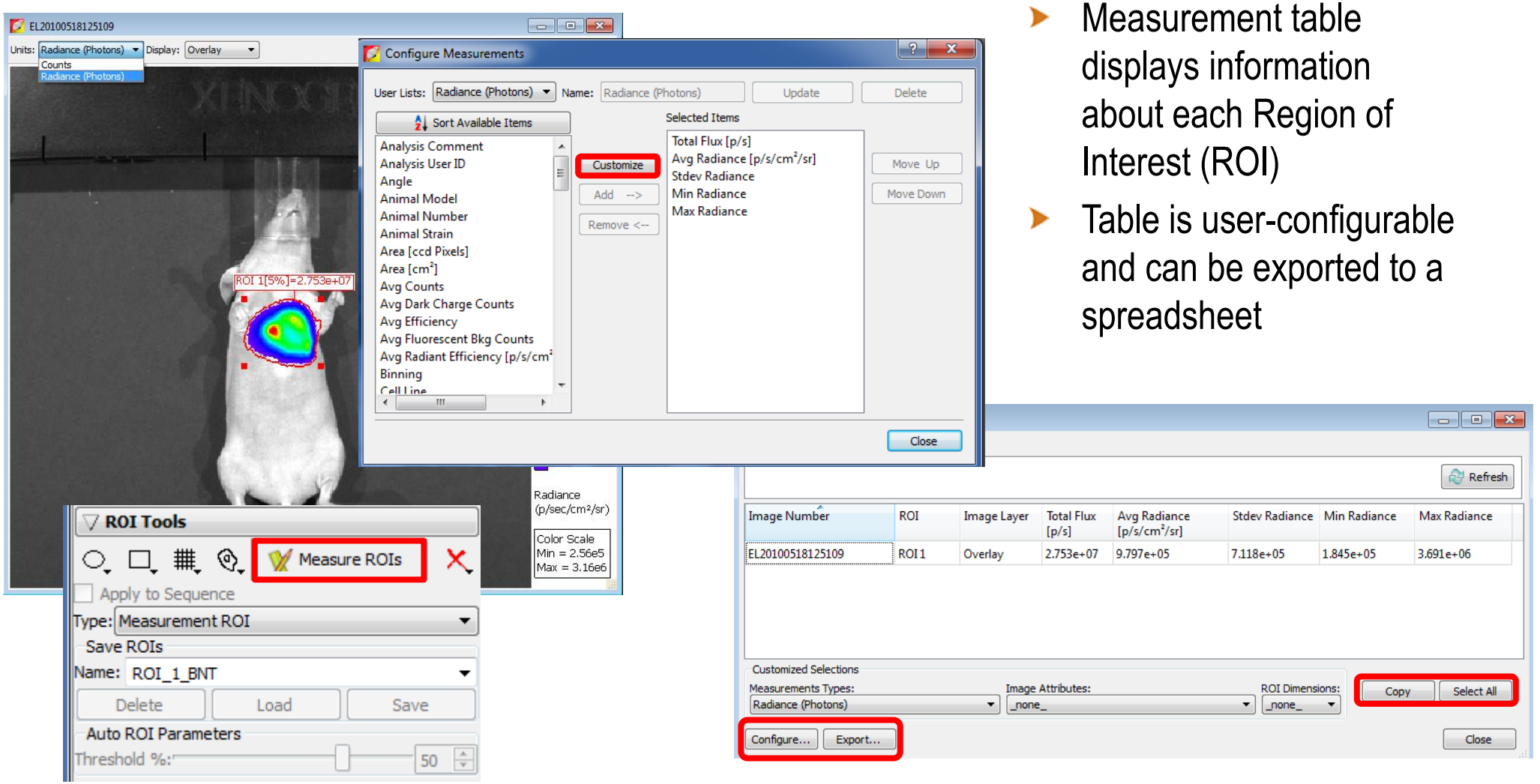








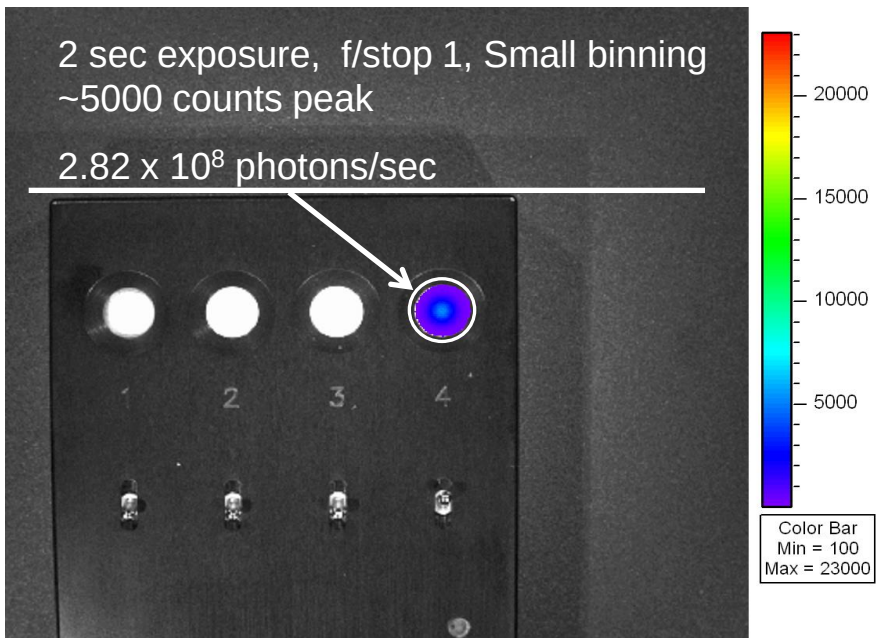
Measurement Table

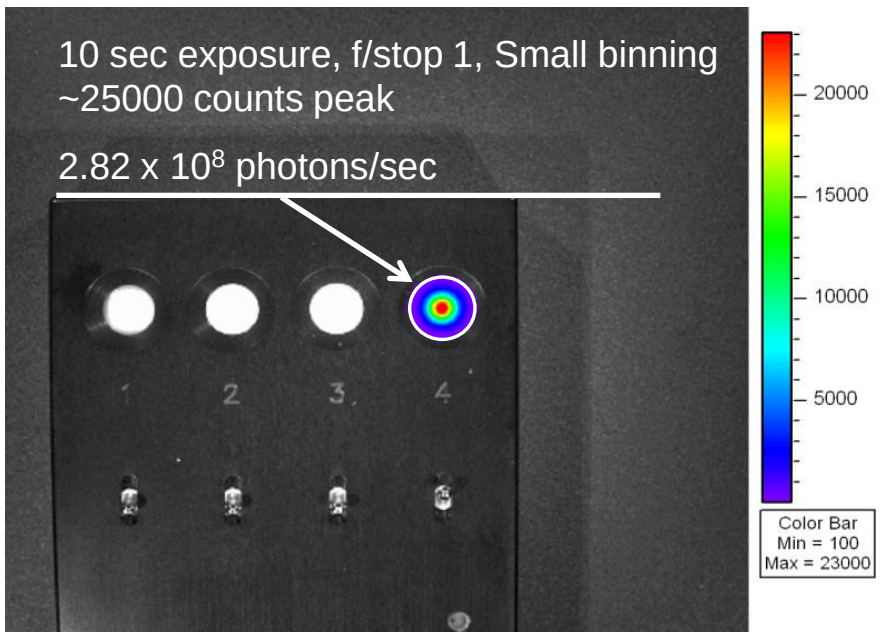


- Measurement table displays information about each Region of Interest (ROI)
- Table is user-configurable and can be exported to a spreadsheet

Calibrated Physical Units

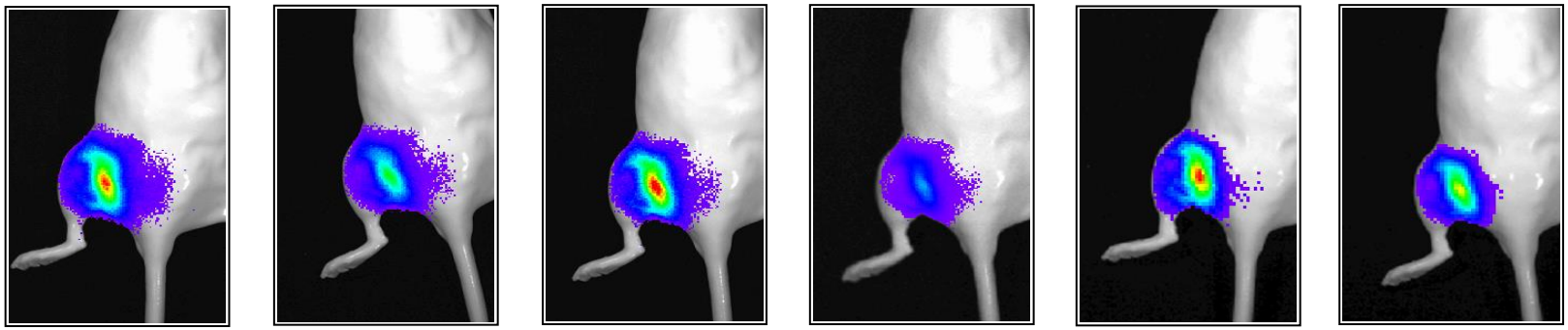
- Living Image® automatically compensates for device settings: Exposure time, *f*/stop, binning and field of View.
- Calibrated units are Photons per Second, representing the flux radiating omni-directionally from a user-defined region





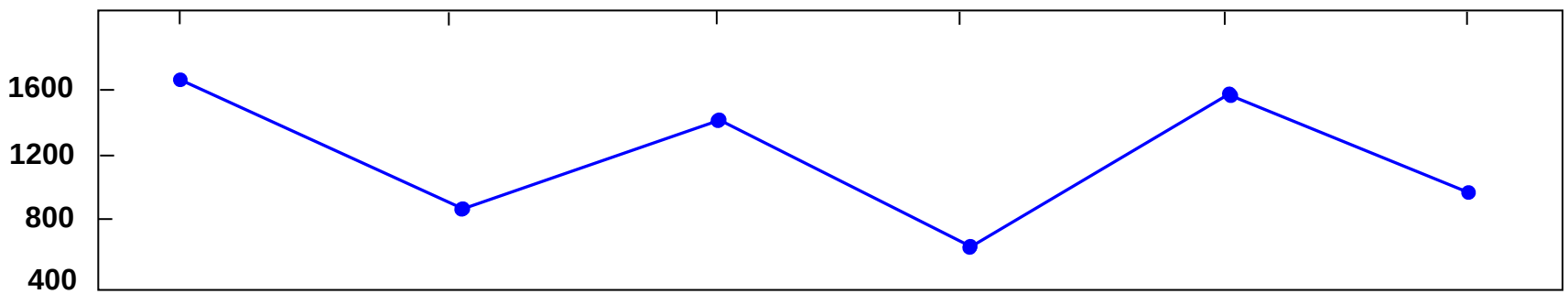
Calibrated Physical Units vs. Raw Signal

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

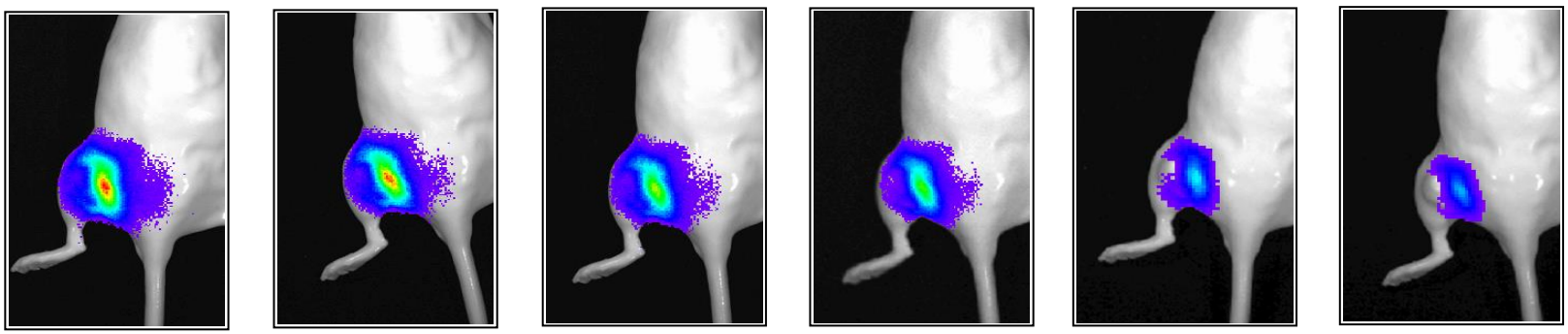
Peak Counts



Calibrated Physical Units vs. Raw Signal

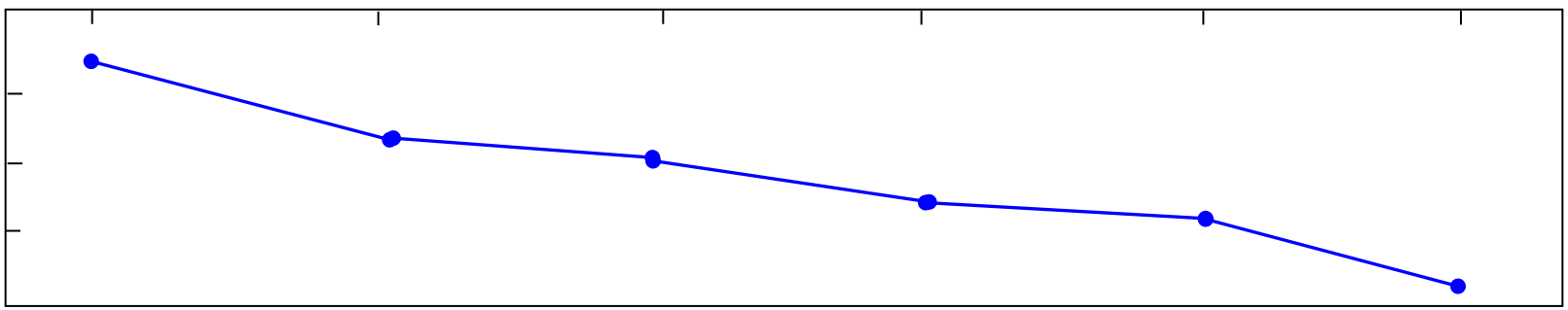
Calibrated Signal

(Photons per second)

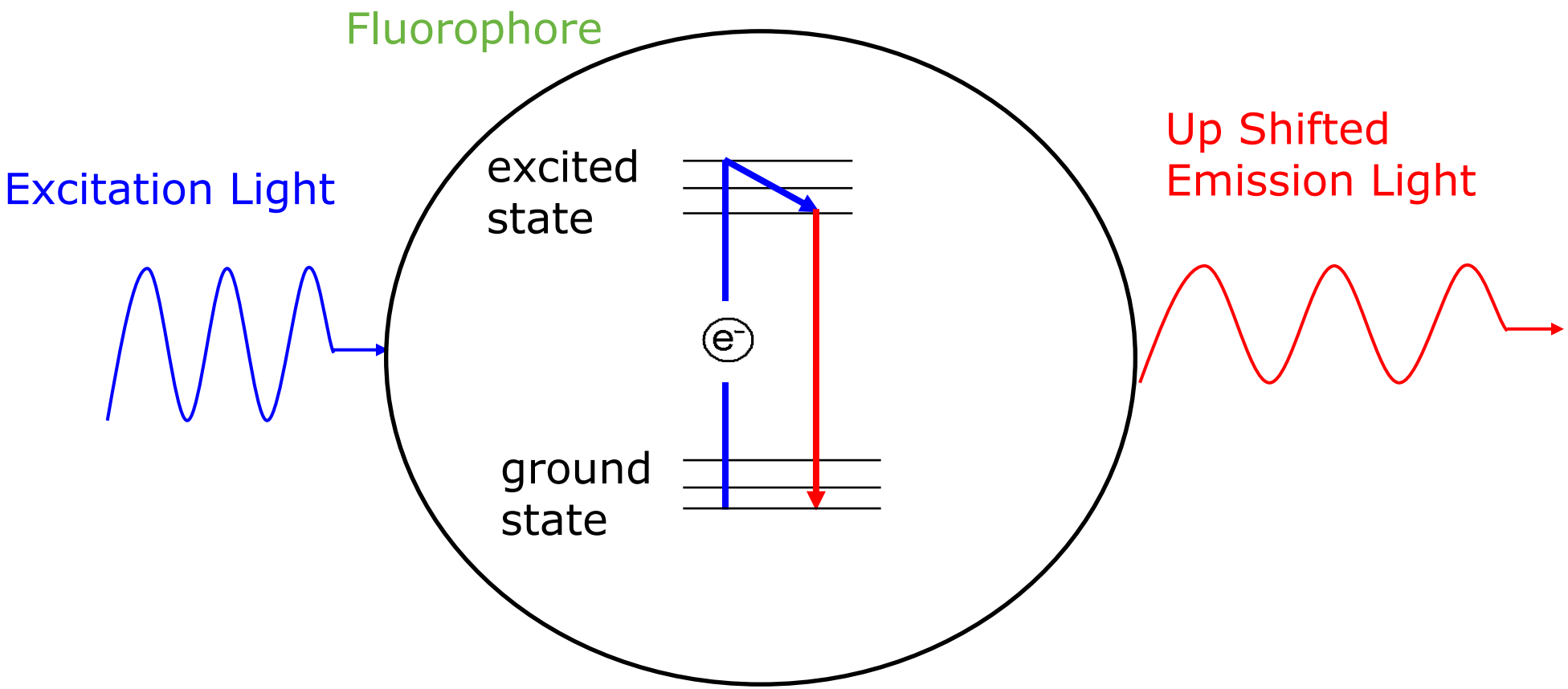


Exp time:	30 sec	30 sec	60 sec	60 sec	60 sec	60 sec
Binning:	small	small	small	small	medium	medium
Day:	1	2	3	4	5	6

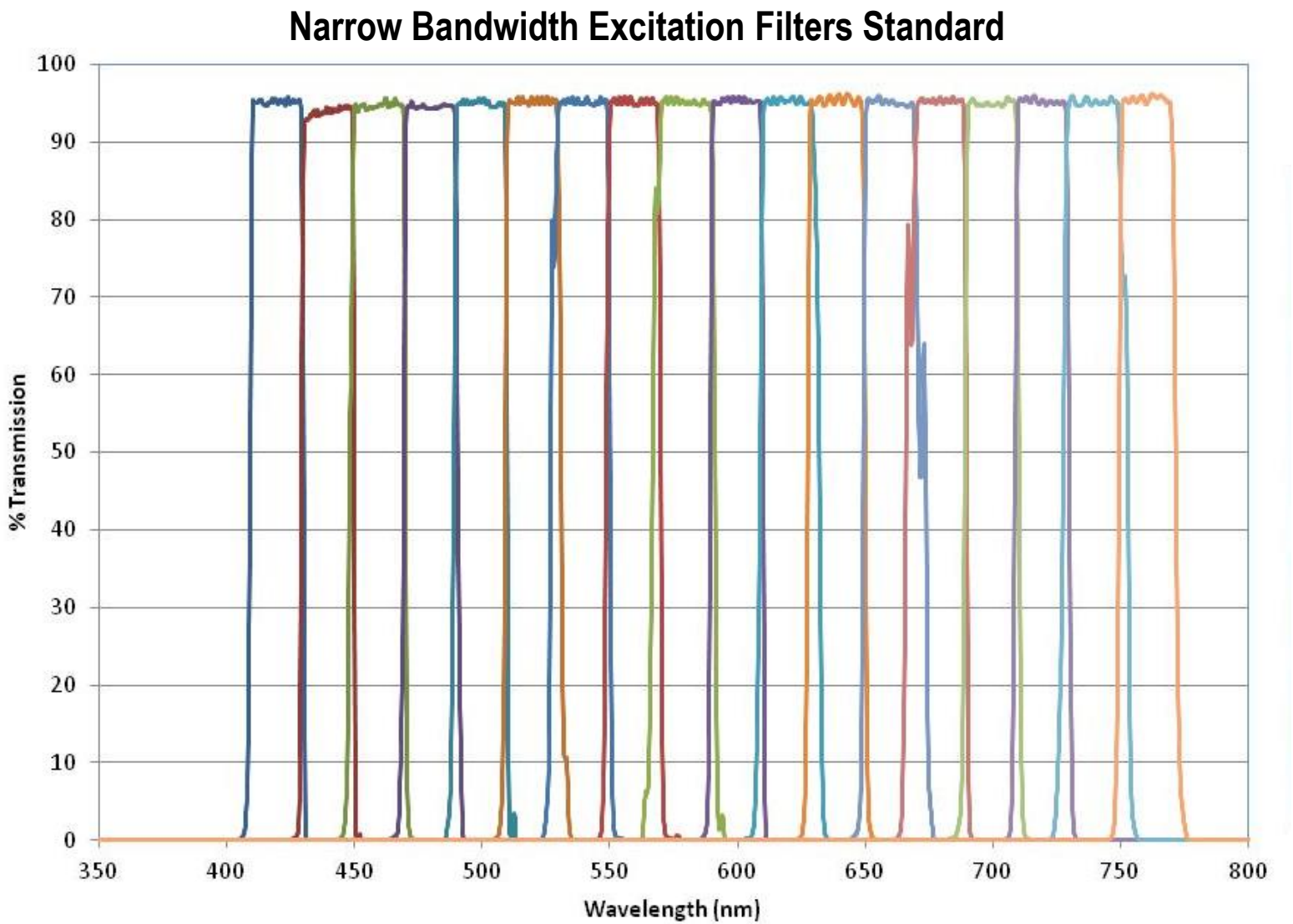
Radiance:
Photons per second



Fluorescence Process



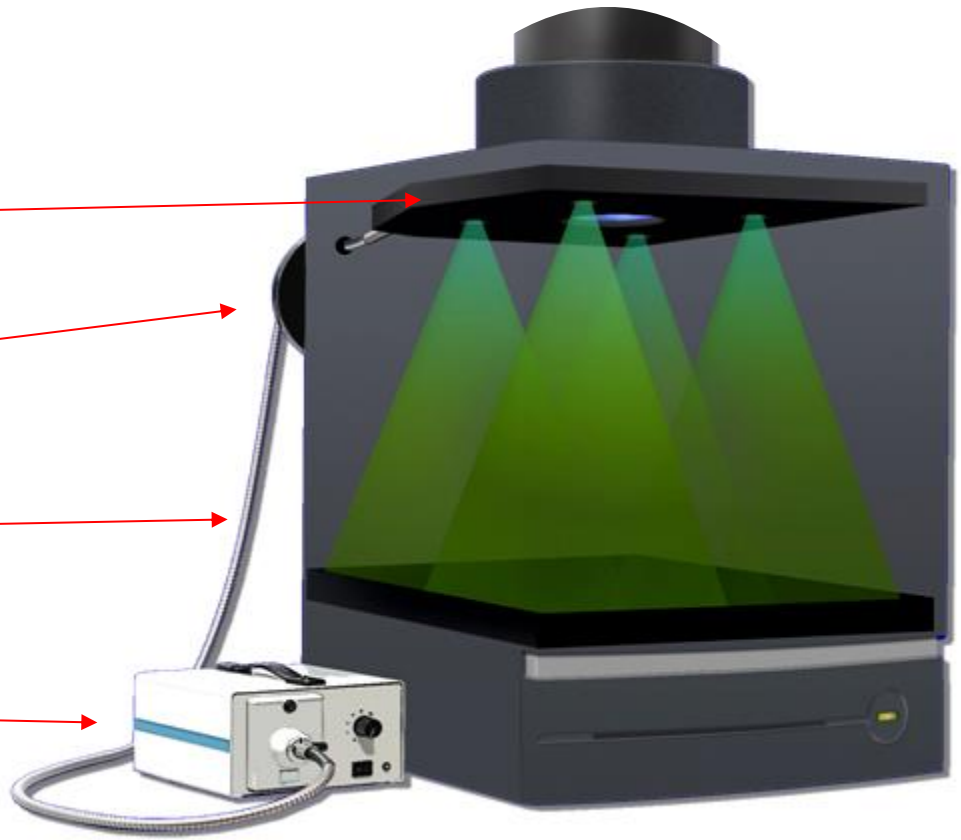
Fluorescence Filters



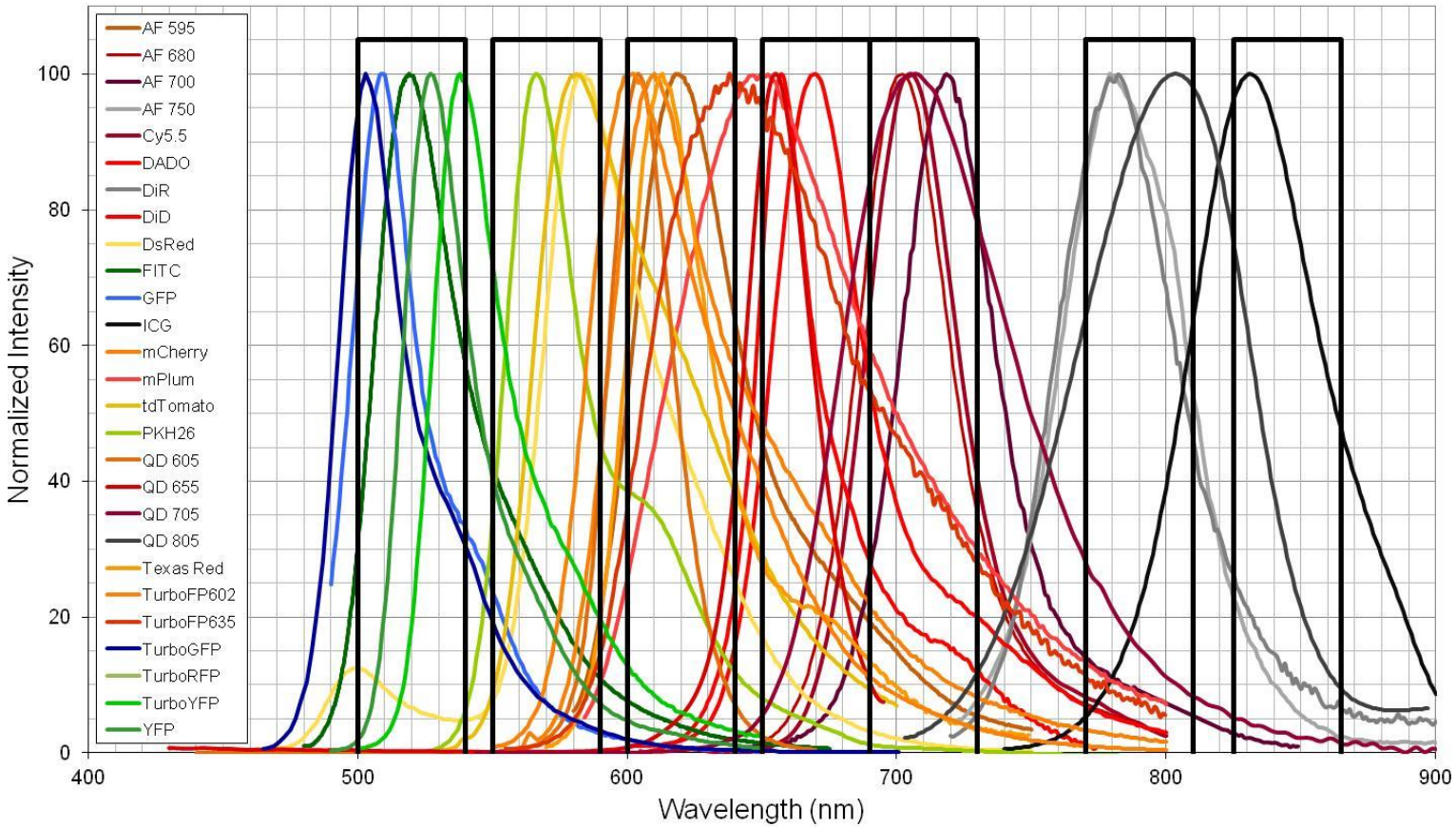
Center Λ	Band pass
420	20
440	20
460	20
480	20
500	20
520	20
540	20
560	20
580	20
600	20
620	20
640	20
660	20
680	20
700	20
720	20
740	20
760	20
780	20

IVIS® Lumina Series Fluorescence Components

- Fully computer controlled
- Seven position emission filter wheel
- Nineteen position Excitation filter wheel
- Low Auto-Fluorescence optics and fibers
- 150 Watt Tungsten/Halogen lamp with computer controlled intensity



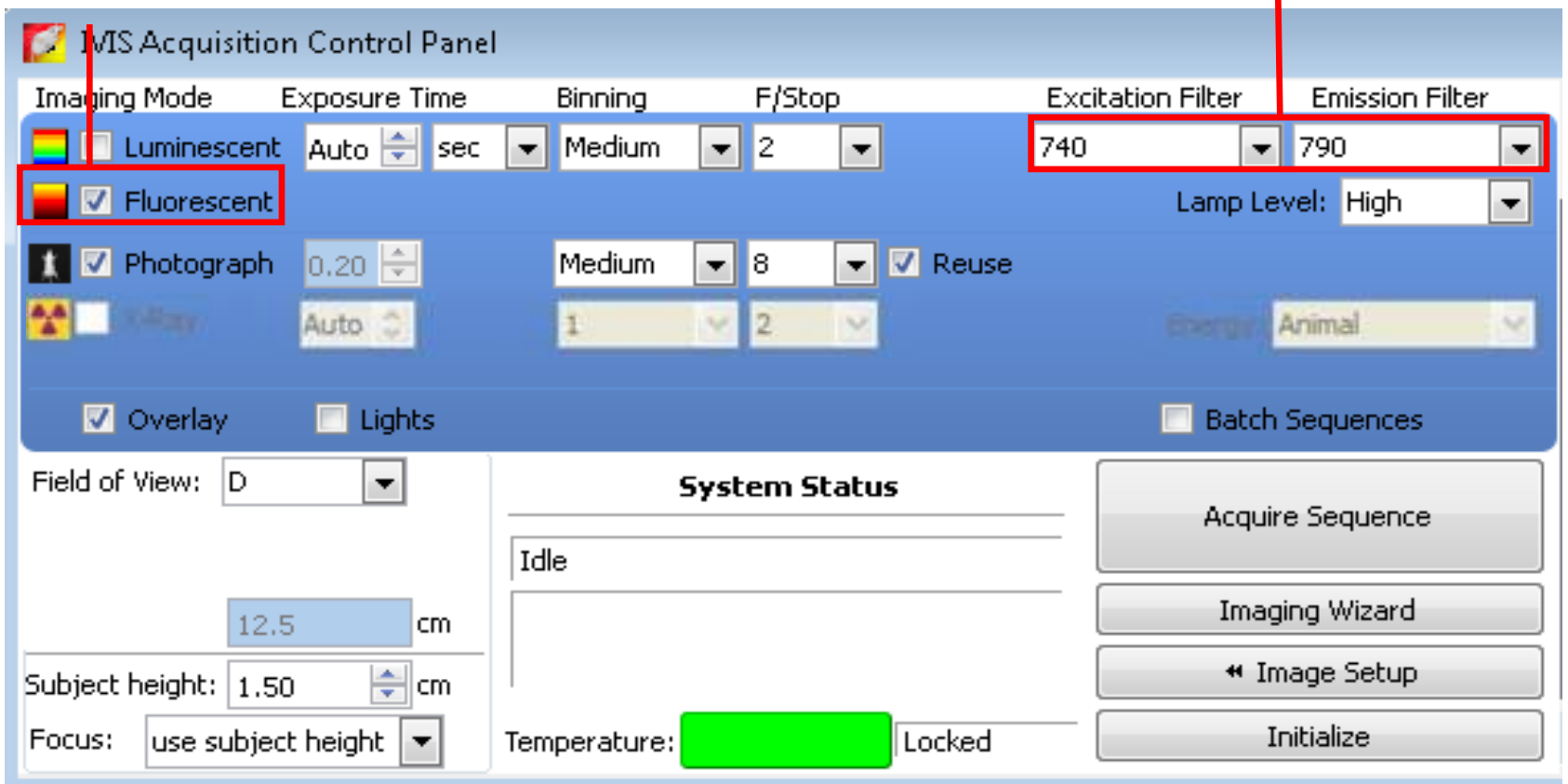
High Efficiency Emission Filters Centered Around Common Fluorophores



Center Λ	Band pass
520	40
570	40
620	40
670	40
710	40
790	40
845	40

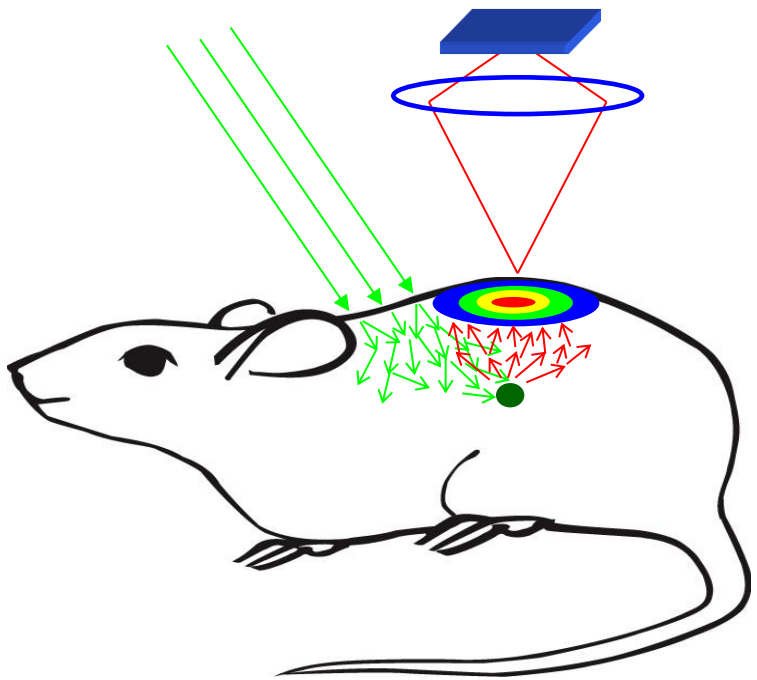
Select Fluorescent Imaging Mode

Select filters



Epi-illumination Unit

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

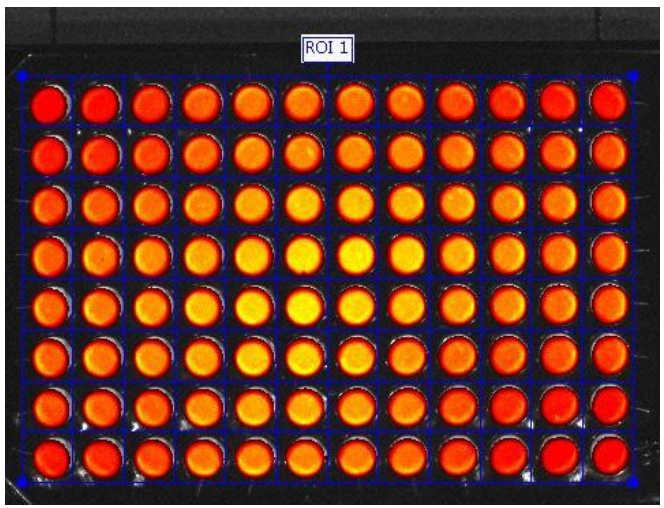


Fluorescent Calibrated Units: Radiant Efficiency

Excitation Light Pattern

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

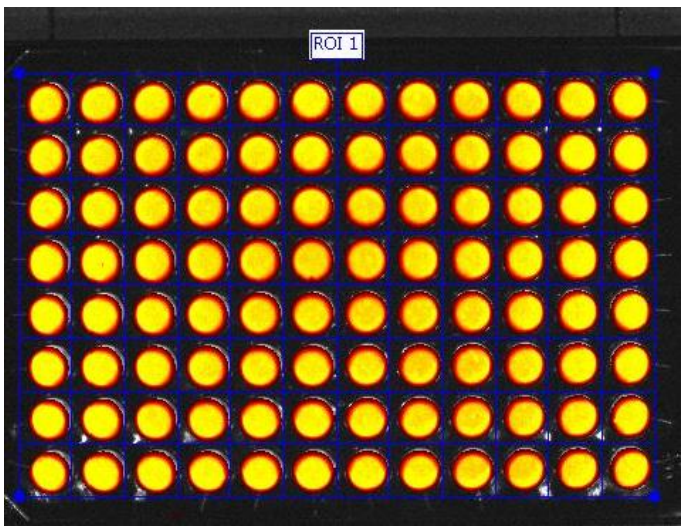
GFP Well Plate Uncorrected



Counts

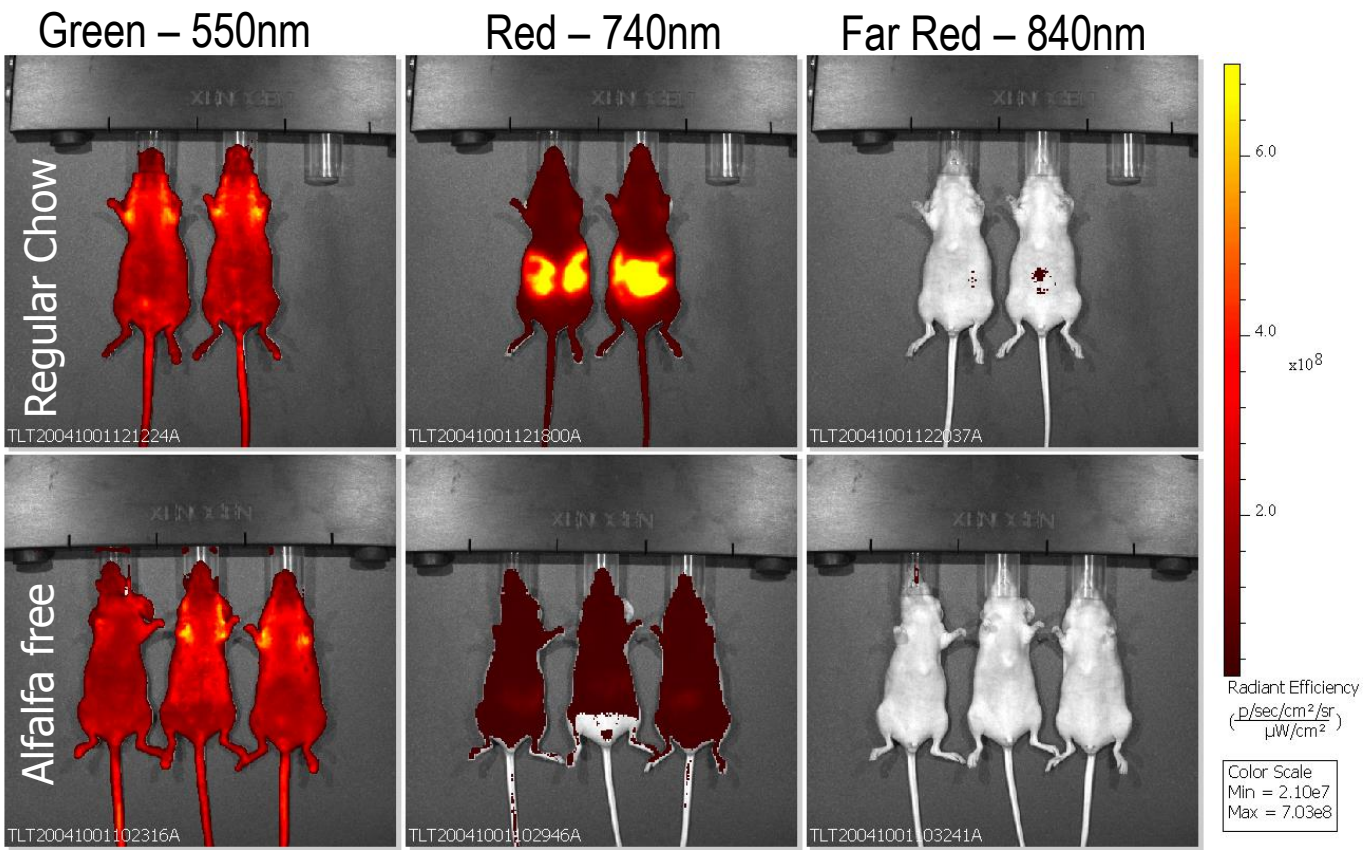
vs.

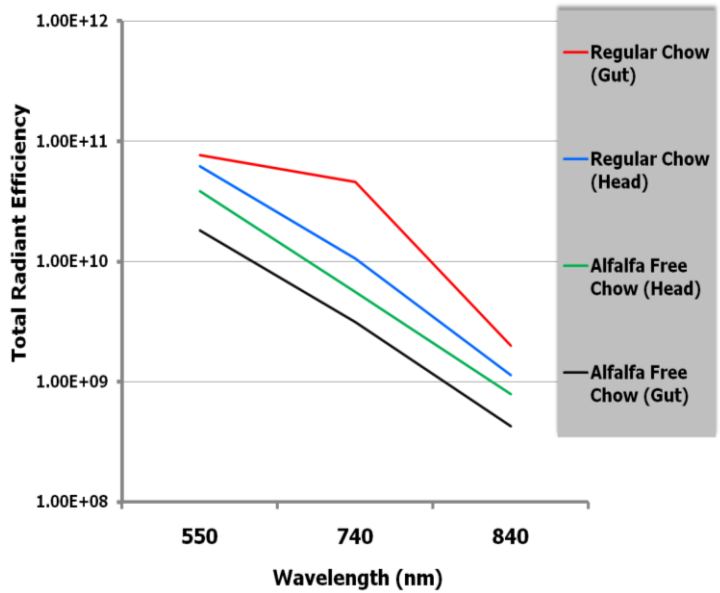
GFP Well Plate Corrected



Radiant Efficiency

Autofluorescence in Negative Control Mice





Research Diets
http://www.researchdiets.com

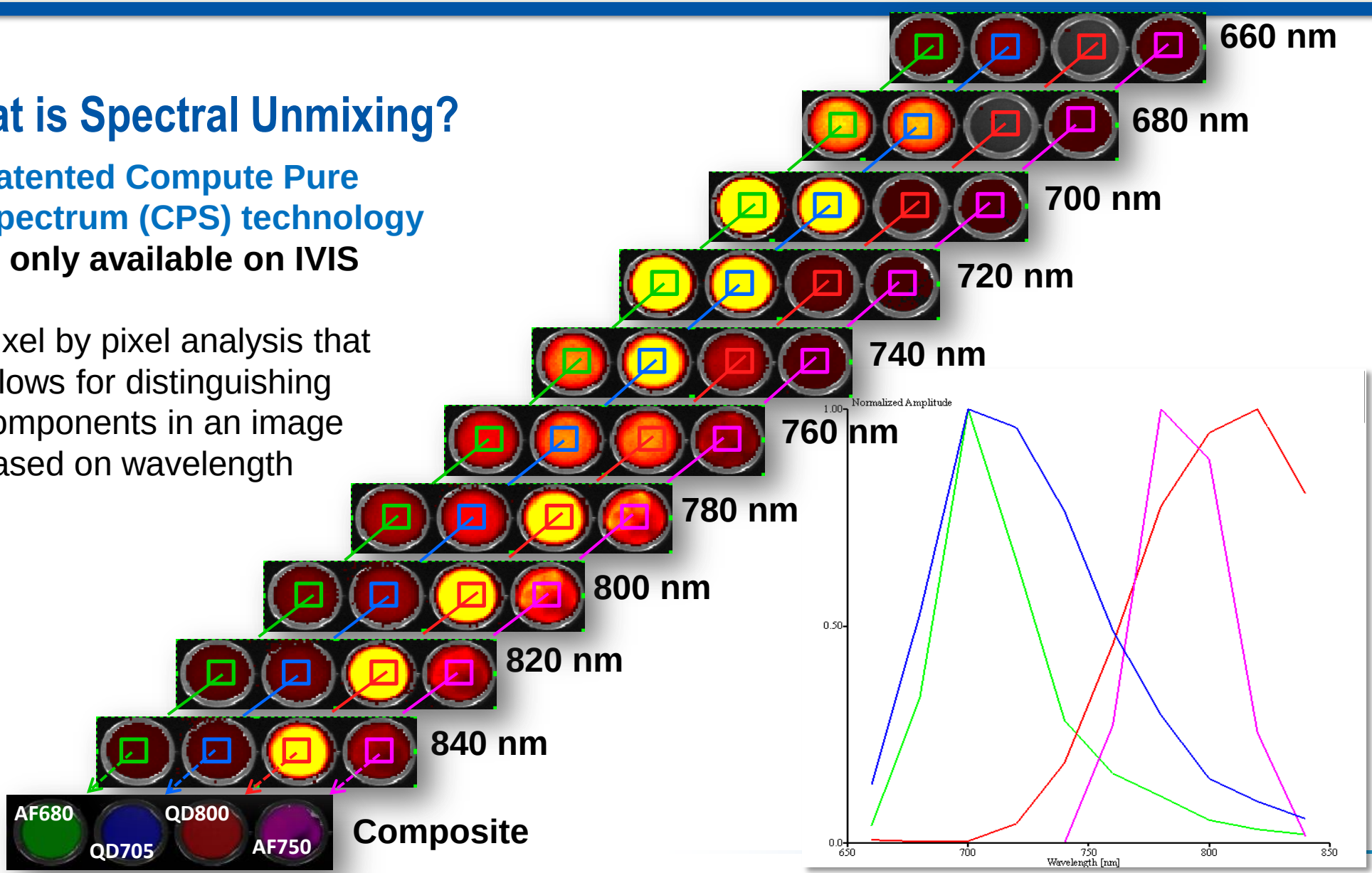
AIN-76A (D10001i) – alfalfa free

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

Multispectral Unmixing Imaging in the IVIS

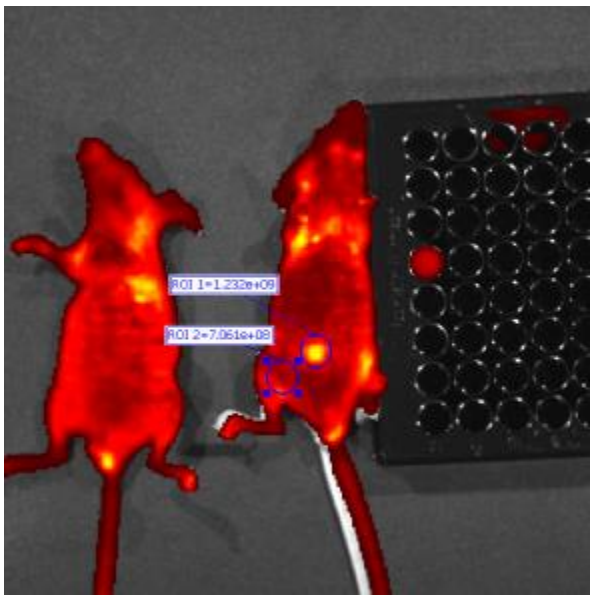
What is Spectral Unmixing?

- **Patented Compute Pure Spectrum (CPS) technology is only available on IVIS**
- Pixel by pixel analysis that allows for distinguishing components in an image based on wavelength



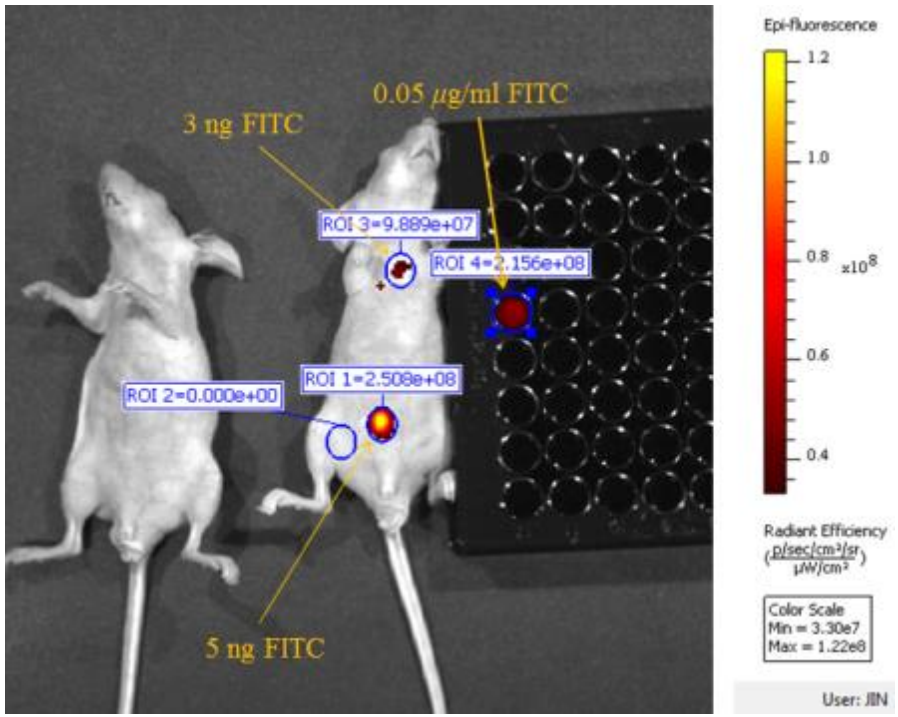
Remove Autofluorescence with Spectral Unmixing

Autofluorescence



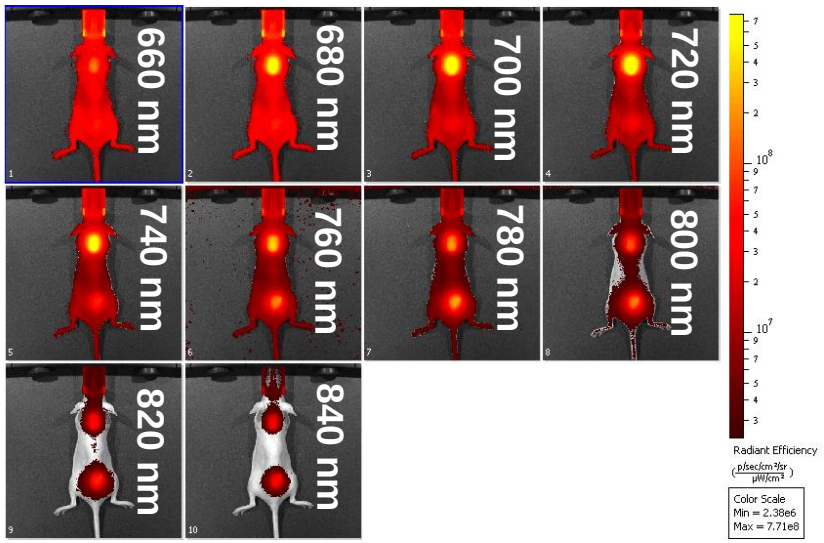
Where is the Signal?

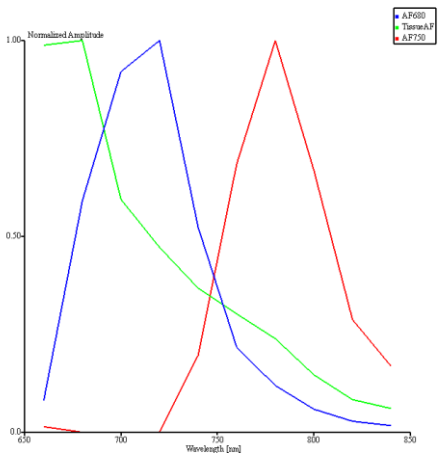
Spectral Unmixing



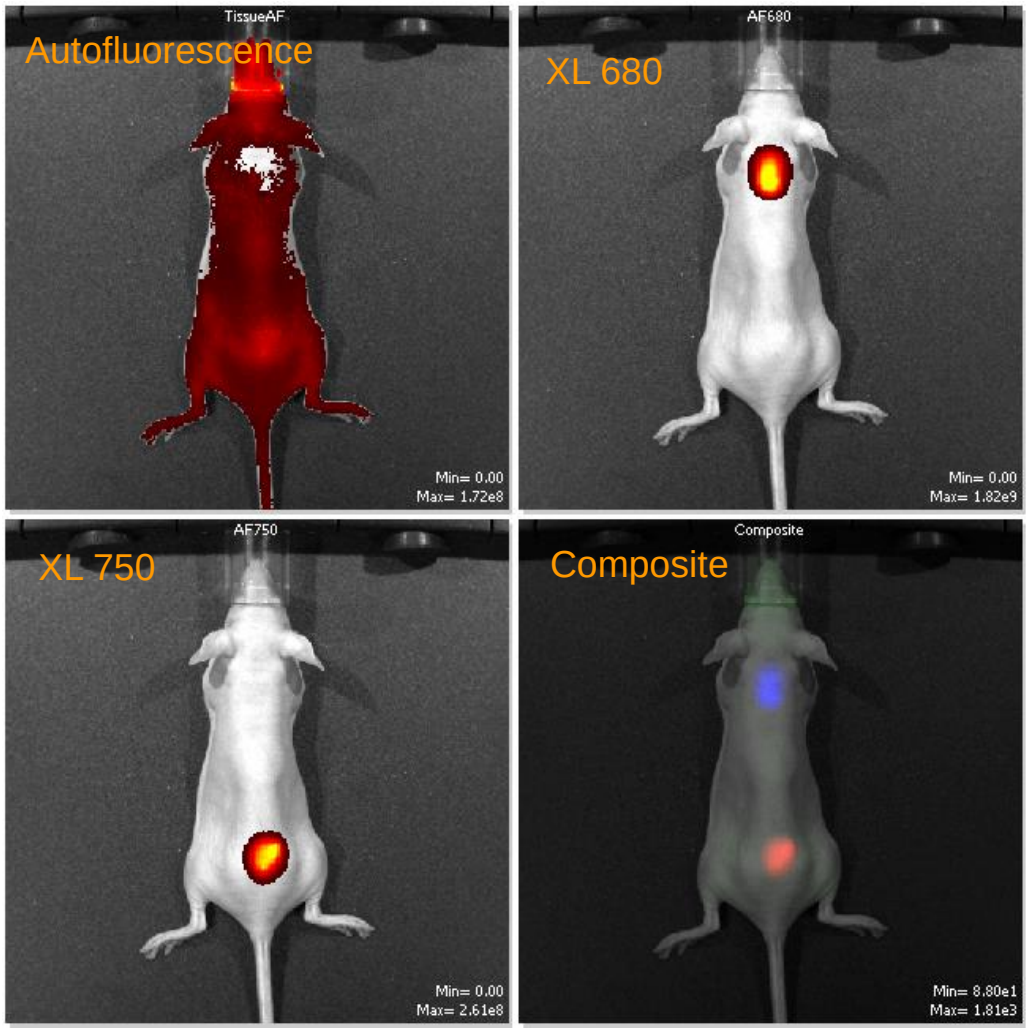
Powerful Fluorescence- Separate multiple reporters in the same subject.

Raw Spectral Images



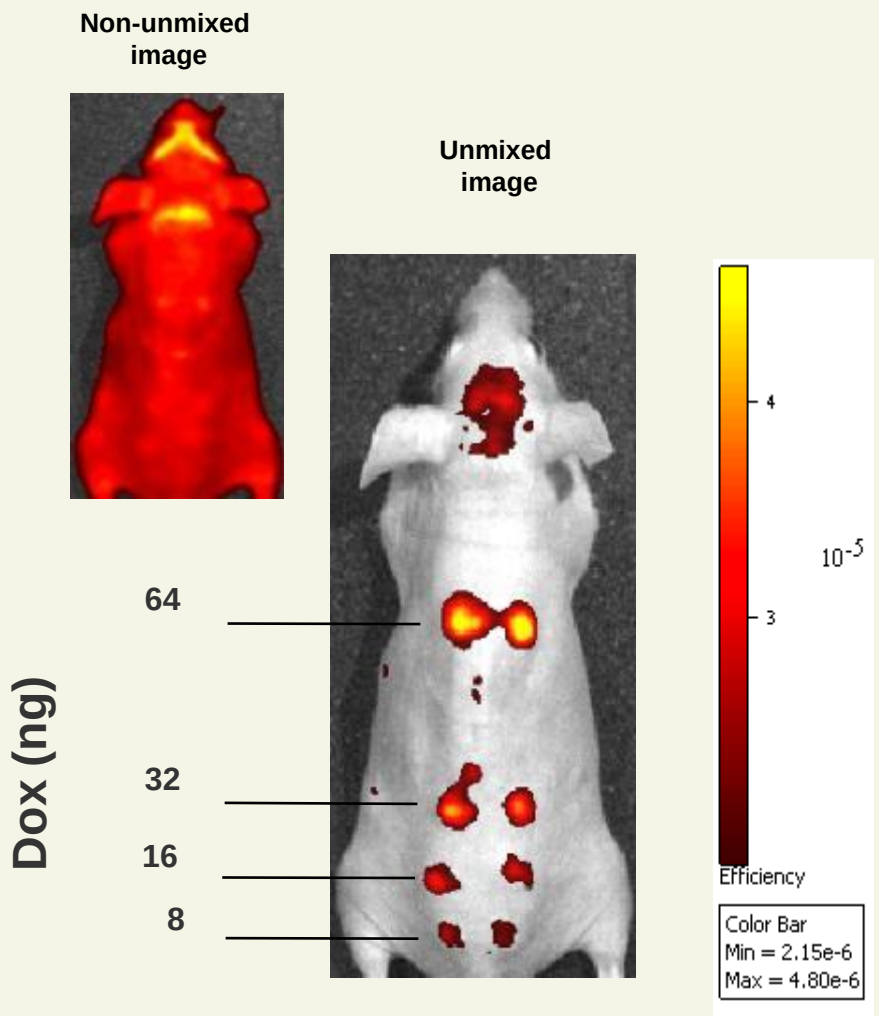


- Subcutaneous injections of 10^{14} molecules of XenoLight 680 (scruff)
- Subcutaneous injection of 10^{14} molecules of XenoLight 750 (lower dorsal region)
- 605nm excitation filter



When is Spectral Unmixing Most Useful?

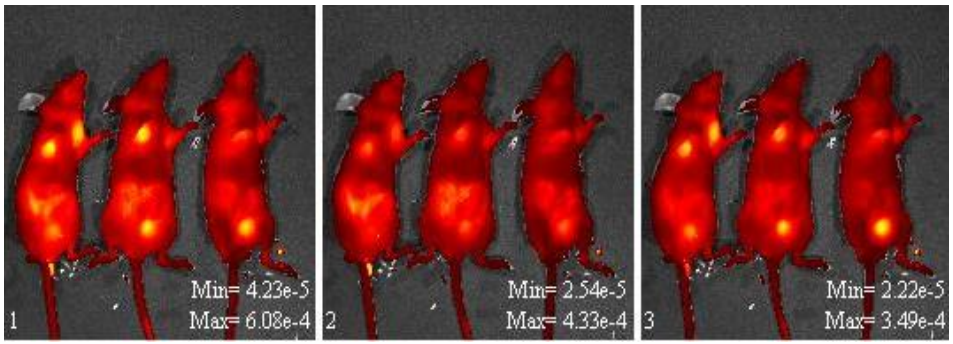
Improve Quantification Sensitivity and Accuracy



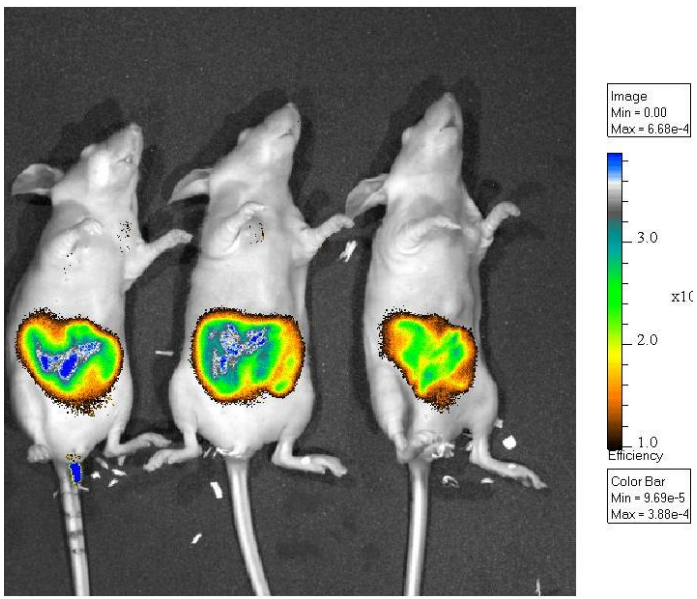
Spectral unmixing of activatable probes

4T1 cells

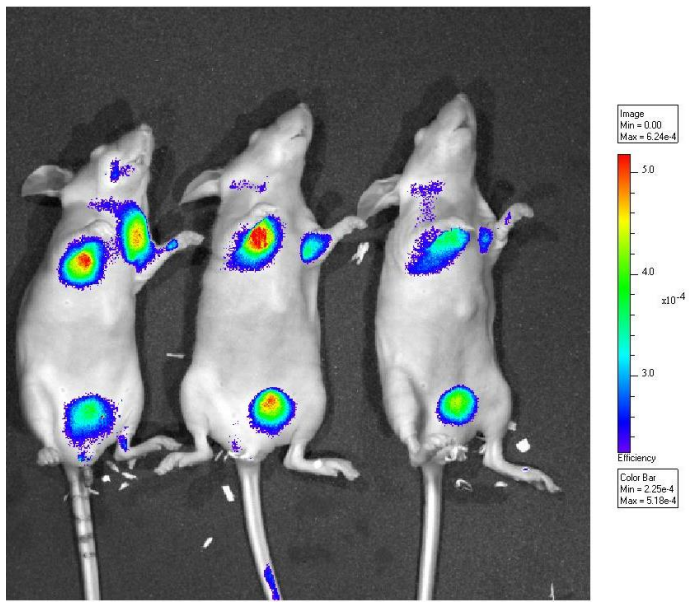
Raw Data



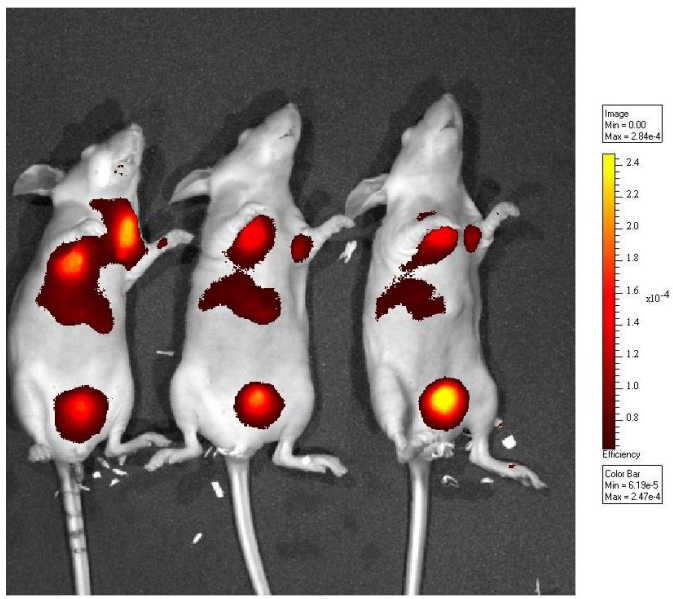
Food Background



Unmixed ProSense 680

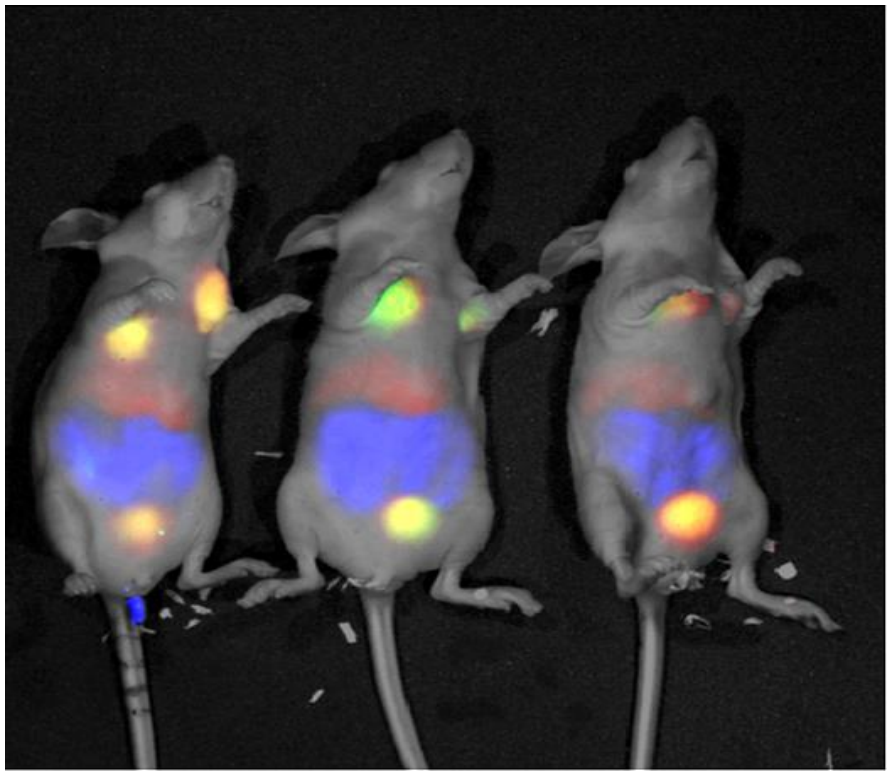


Unmixed MMPSense 750



Overlaid images

Spectral Unmixing



4T1 murine mammary tumor cells implanted in mammary fat pads labeled with:

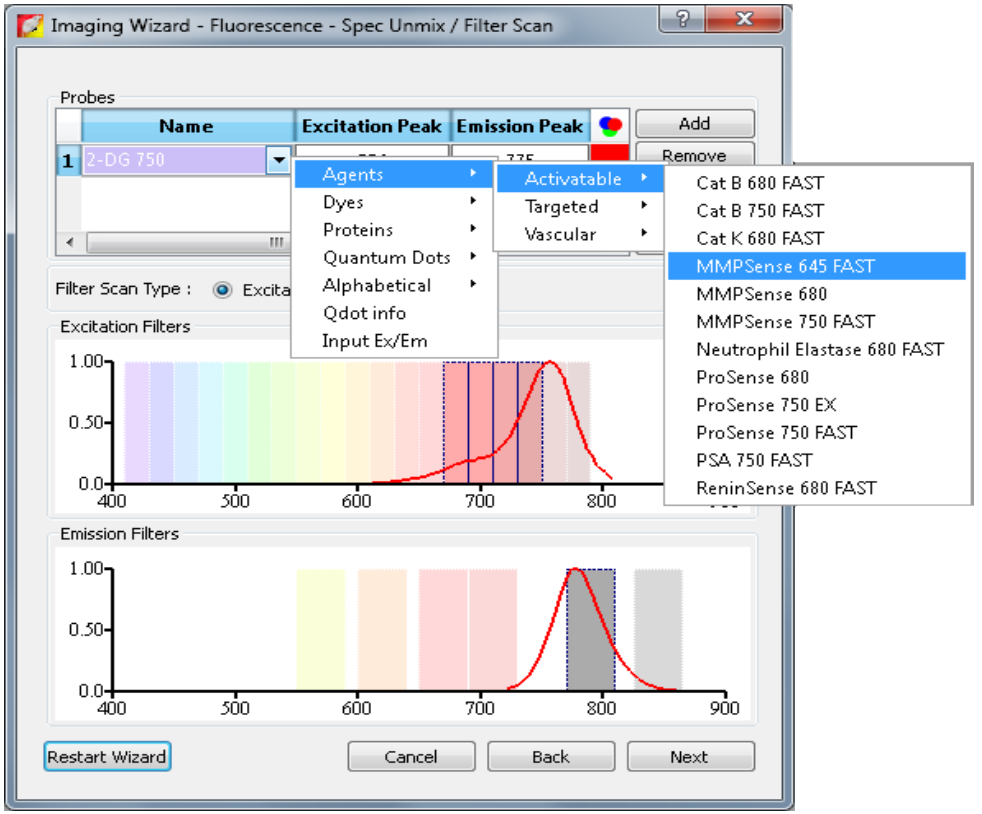
Green: ProSense680

Red: MMPSense750

Blue: Chlorophyll signal in Food

A complete probe library for easier use

- Incorporated 99 probes into Living Image software
- Contains all the Perkin Elmer fluorescent Agent and Dyes



- Contains commonly used Probes
  - Dyes
    - Alexafluor dyes
    - Cyanine dyes
    - VivoTag
    - Miscellaneous
  - Proteins
  - Quantum dots
- Data base can be expanded as needed
  - Input Ex/Em and Qdot info will allow user to input peaks if their probe isn't in database

Total solution- Pre-clinical *in vivo* Imaging Agents

PerkinElmer
For the Better

Pre-clinical Imaging Agents

Fluorescent Agents	NIR Labels & Nanoparticles	Bioware	Substrates
■ Activatable ■ Targeted ■ Vascular	■ Labeling kits & dyes ■ Nanoparticles (645, 680, 750, 770 nm)	■ Luciferase, tdTomato and GFP cell lines ■ Bacteria & Plasmids ■ Lentiviral particles	■ XenoLight Luciferin ■ RediJect Luciferin & Coelenterazine

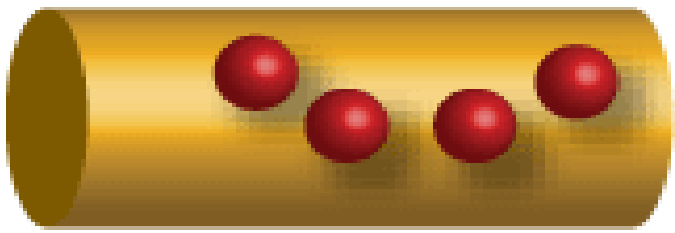
In Vivo Imaging Agent Platforms:

Agent Categories

	Vascular	Targeted	Activatable
Mechanism			
Detection	*Physiology*	*Biological Structure/Expression*	*Biological Function*
Value	Increasing value of molecular information		

Vascular Agents

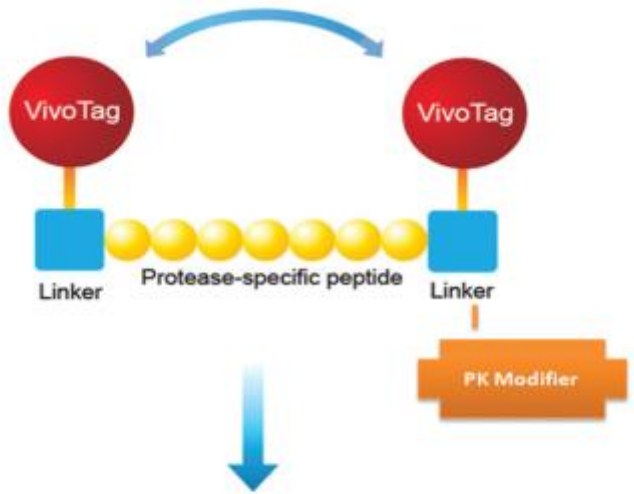
- Vascular agents circulate with the blood, but have no target selectivity
- Vascular agents will accumulate in areas of vascular leakage associated with tumorigenesis and inflammation
- Used to image vascular disease processes in oncology, inflammation, pulmonary disease and arthritis
- Superhance is a low molecular weight agent, AngioSense® is a high molecular weight agent, and AngioSPARK is 30-50 nm nanoparticles
- Each agent differs significantly in pharmacokinetics, biodistribution and tissue clearance rates

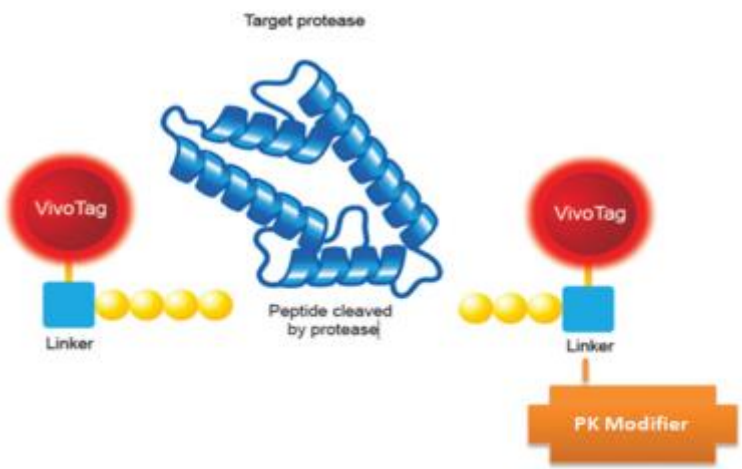


Monitor the integrity of the vascular system

Activatable fluorescent Agents

Agent	Agent Description
MMPSense™ 680	MMPSense™ 680: Activated by matrix metalloproteinases including MMP's MMP-2, -3, -9 and -13
MMPSense™ 645, 750 FAST	MMPSense™645m 750 FAST (Fluorescent Activatable Sensor Technology) is an MMP activatable agent
ProSense® 680/750	ProSense® 680/750: Activated by proteases: cathespins B, L, S, and plasmin
Neutrophil Elastase 680 FAST™	FAST agent activated by elastase produced by neutrophil cells
Cat B 680/750 FAST™	Cathepsin B selective FAST activatable agent
Cat K 680 FAST™	Cat K 680 FAST (Fluorescent Activatable Sensor Technology) is a Cathepsin K activatable agent
ReninSense680 FAST™	ReninSense680 FAST™ (Fluorescent Activatable Sensor Technology) is a renin activatable agent
PSA 750 Fast NEW!	Activatable agent that detects active PSA in vivo

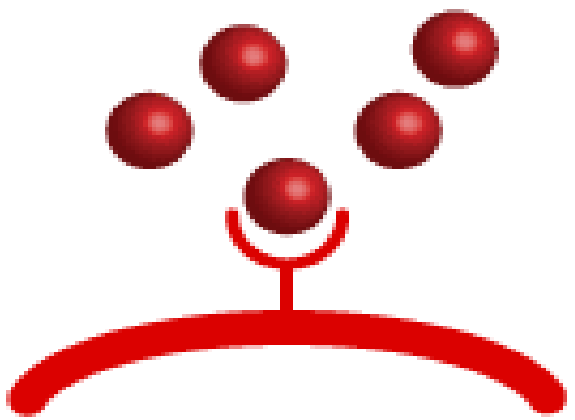




Monitor protease activity associated with disease state

Targeted Agents

- Optimized agents that actively target and bind to specific biomarkers
  - Designed for *in vivo* use
  - *Emerging In vitro applications*

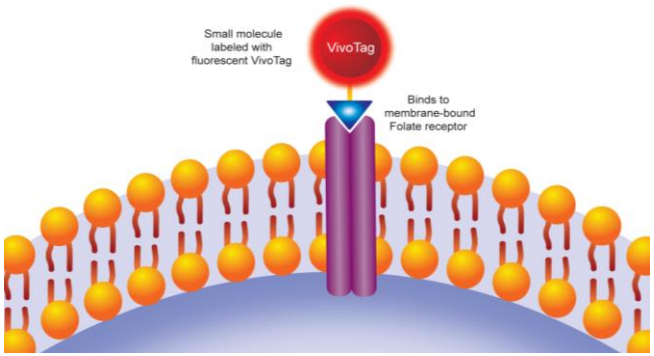


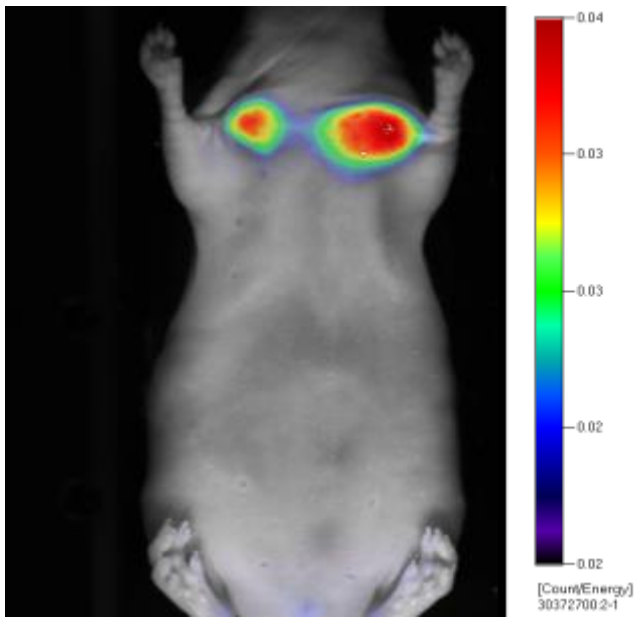
Target specific biomarkers

58

Targeted Fluorescent Agents

Agent	Binds to …
BombesinRSense 680	Bombesin receptors
HER2Sense 645	HER2/Neu receptor
FolateRSense 680	Folate Receptor Protein
TlectinSense 680	Vascular Endothelial cells (N-actelyglucosamines)
OsteoSense® 680/750/800	Hydroxyapatite
IntegriSense™ 655/680/750	Integrin αvβ3 antagonist
BacteriSense 645	Negatively charged phospholipids in Bacterial membrane
Annexin-Vivo 750	Phosphatidylserine during early apoptosis
HypoxiSense 680	Carbonic Anhydrase IX in hypoxic tissue and cells
COX-2 Probe	Cyclooxygenase-2 (COX-2)
2-DG 750	Glucose uptake Imaging
Transferrin-vivo	Transferrin receptors





HER2/Neu+ tumor targeting by HER2Sense 645

Targeted Agents – application

agent	application
IntegriSense	• Angiogenesis • Atherosclerosis • Oncology • Neurological
Annexin-Vivo	• Apoptosis • Atherosclerosis • Inflammation • Oncology • Neurological
OsteoSense	• Arthritis • Atherosclerosis • Bone Turnover • Skeletal • Oncology
HypoxiSense	• Oncology
FolateR-Sense	•cancer and inflammation
BacteriSense	•infection
Transferrin-Vivo	• Oncology • Inflammation

Target specific biomarkers

PerkinElmer offers four categories of fluorescent *in vivo* imaging agents:

LABELS and NANOPARTICLES

VivoTag™ 680XL Protein Labeling Kit : designed for preparing fluorescently labeled antibodies, proteins or peptides for small animal in vivo imaging applications.

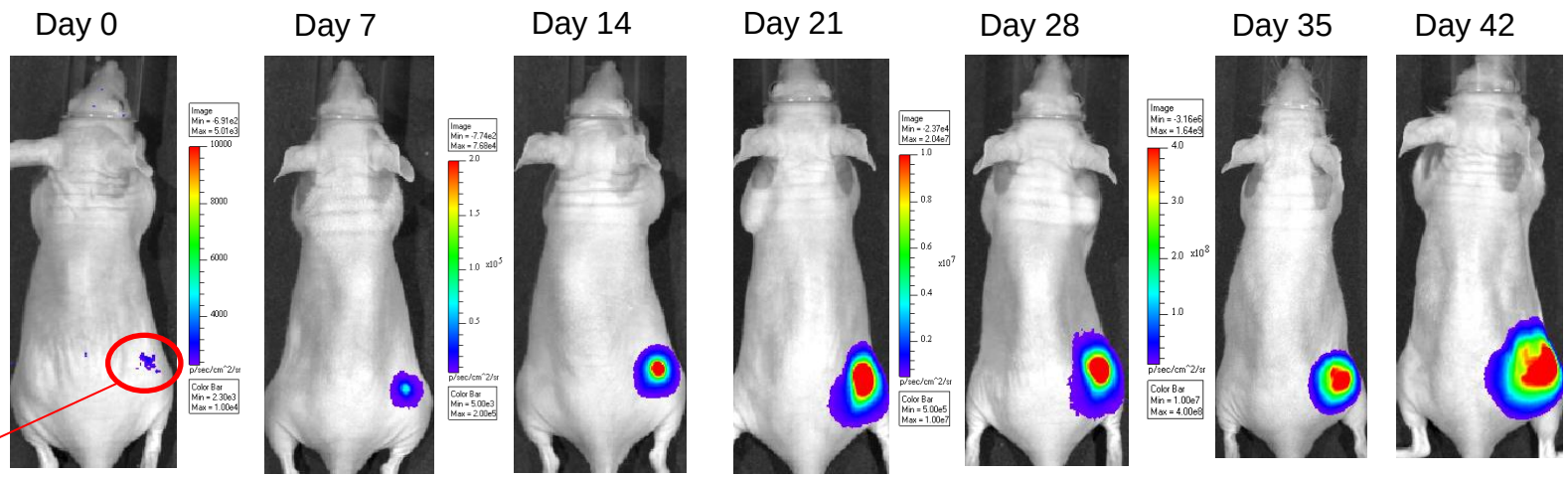
VivoTrack 680 : cell labeling agent that intercalates into the plasma membrane of primary cells and cell lines.

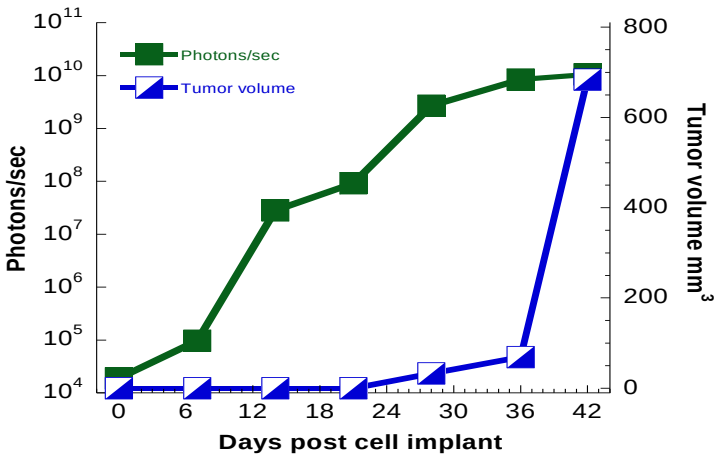
Top Ten Tips for Optical Imaging

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

Longitudinal monitoring of tumor development

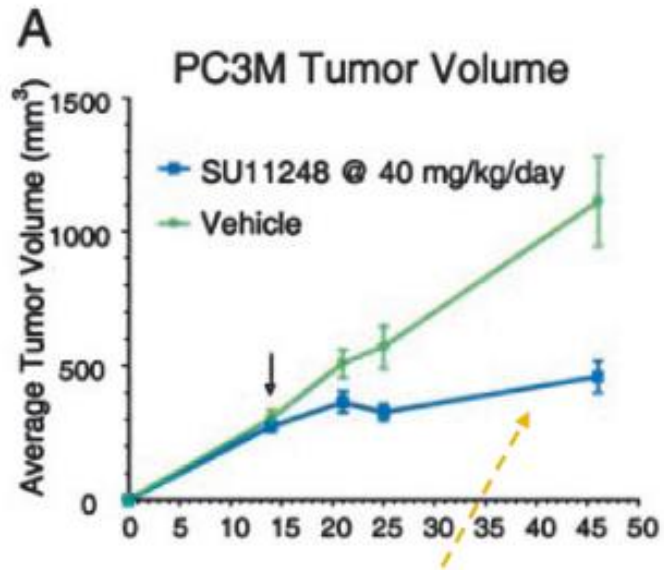
Bioware Ultra: 4T1-luc2



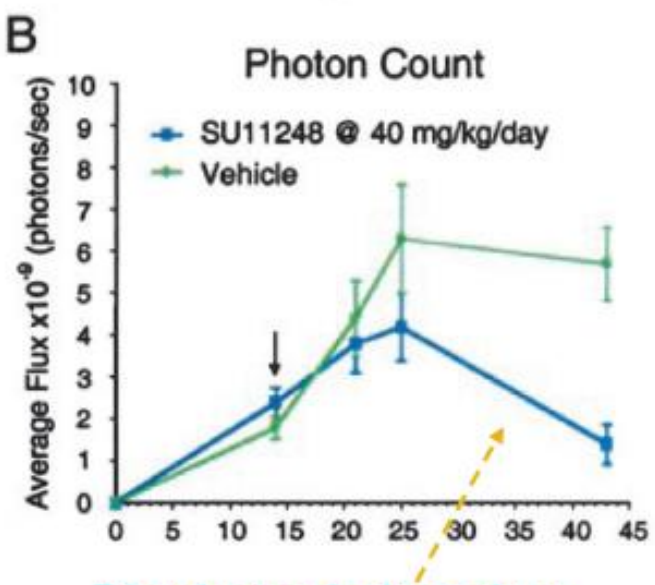


With Bioware Ultra one can start collecting data from Day 0, while with caliper measurements one has to wait at least 28 days to see any tumor growth

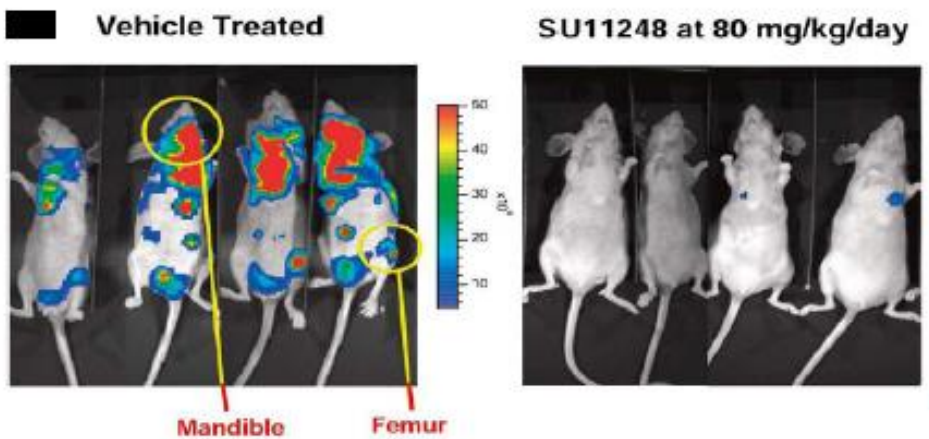
Sutent – Fast Tracked FDA Approval



Physical measurement
(tumor still getting bigger)

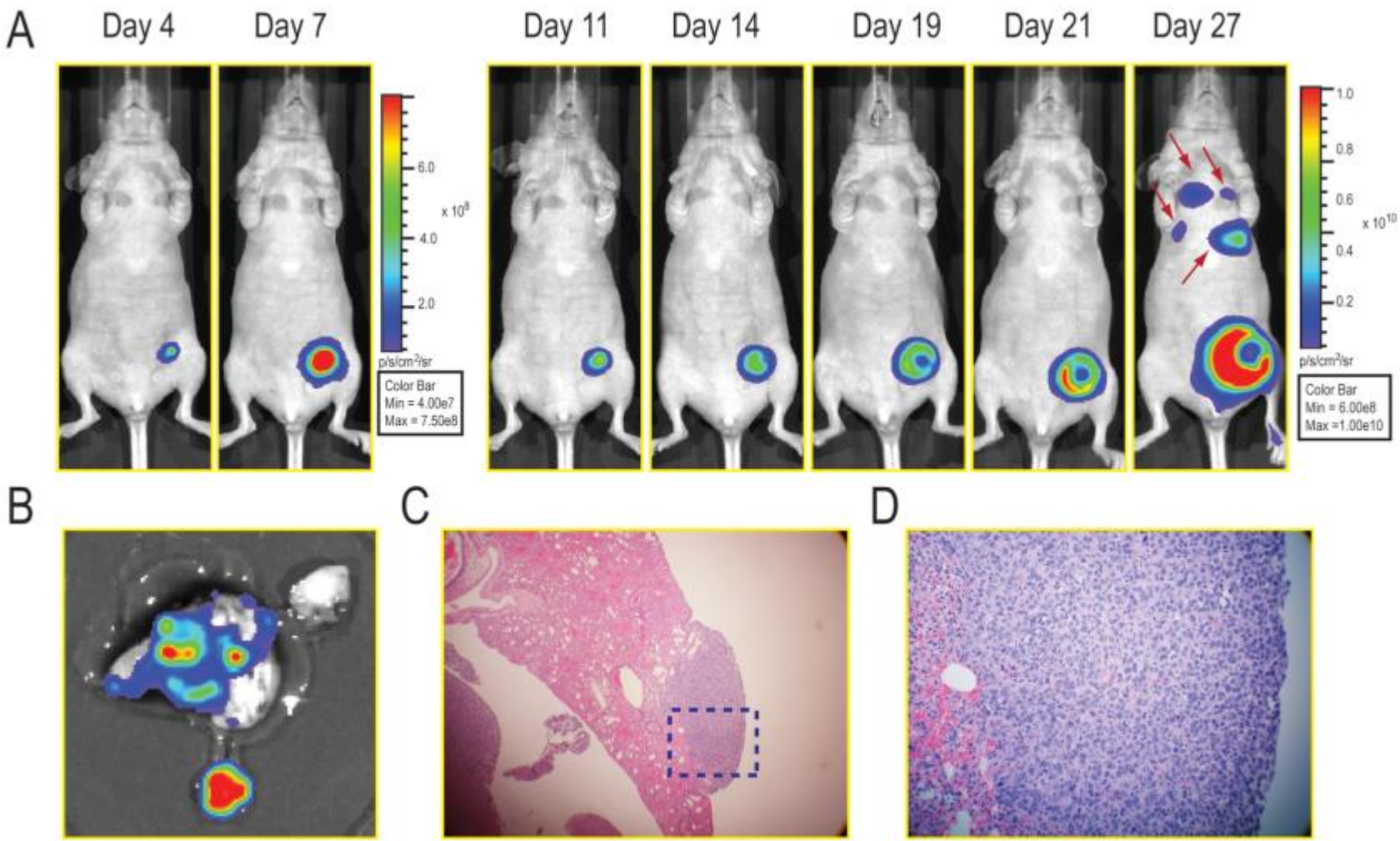


Biophotonic imaging
(tumor cells being killed)



Murray et al 2003

Non-Invasive Detection of Micrometastasis



5×10^5 4T1-luc2-1A4 cells orthotopically into the abdominal mammary fat pads

Kim et al, PLoS ONE (2010)

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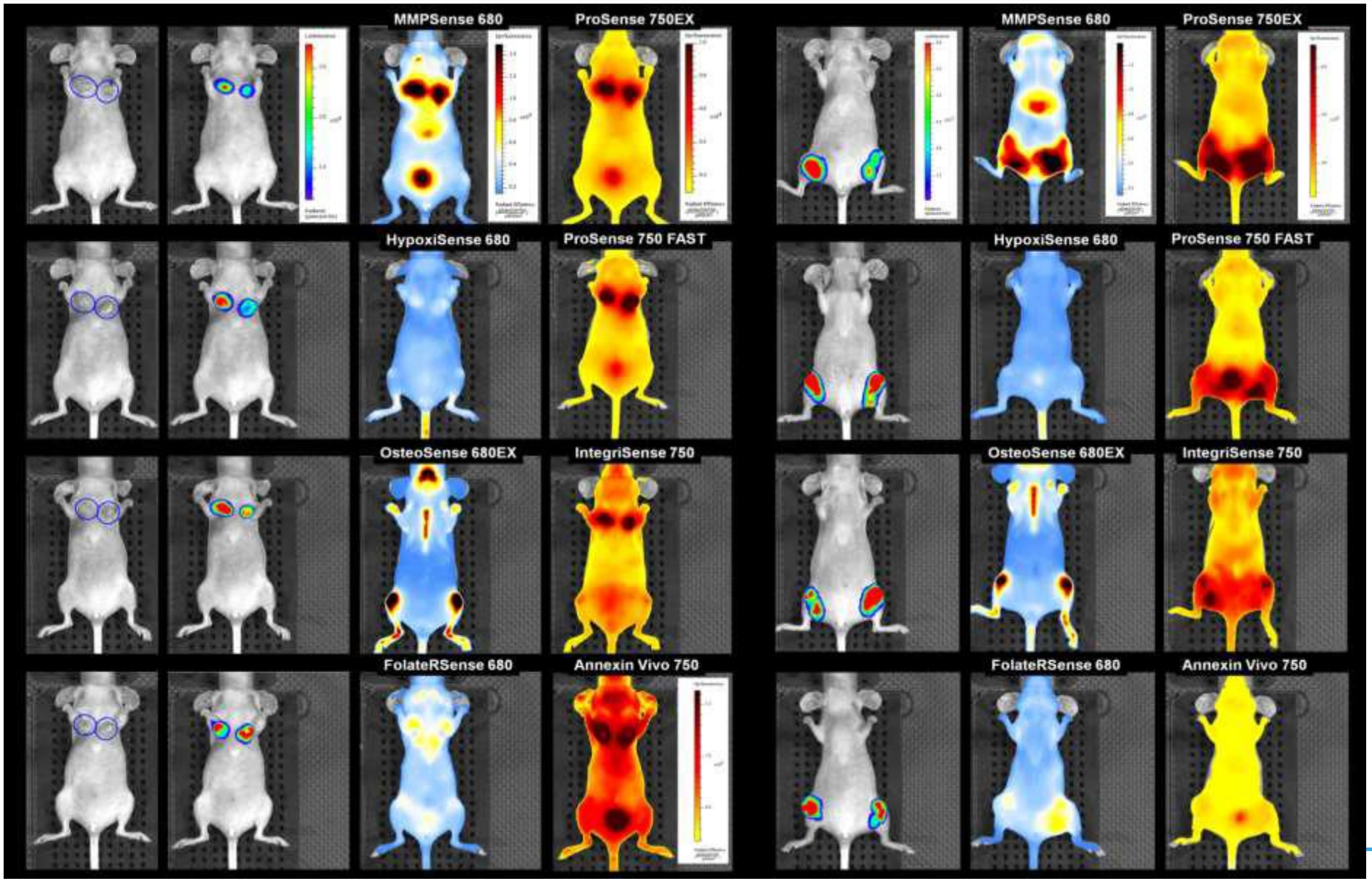
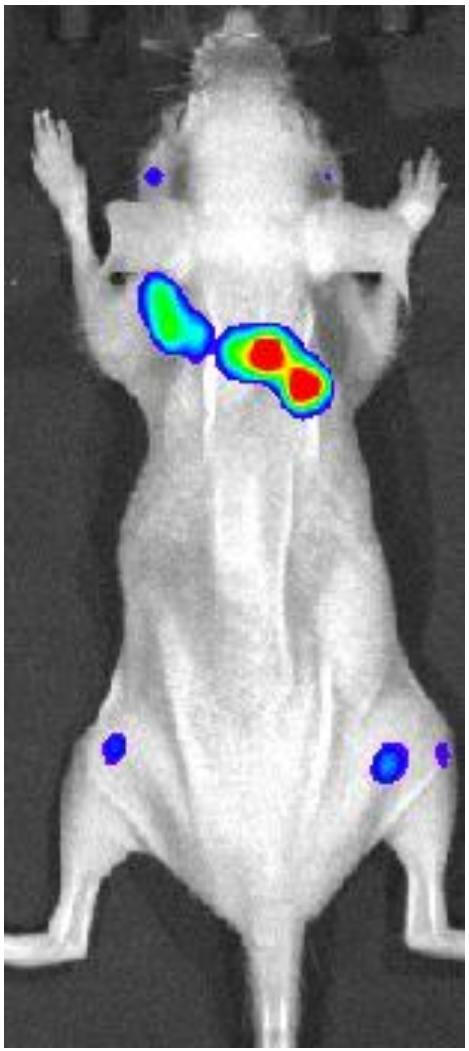
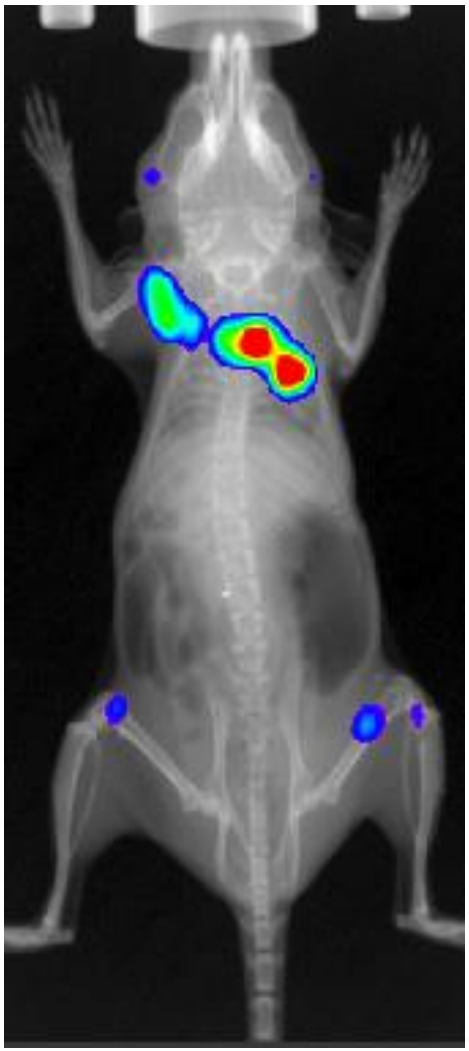
4T1-luc Tumors: Orthotopic vs Bone Metastases Profiling by IVIS

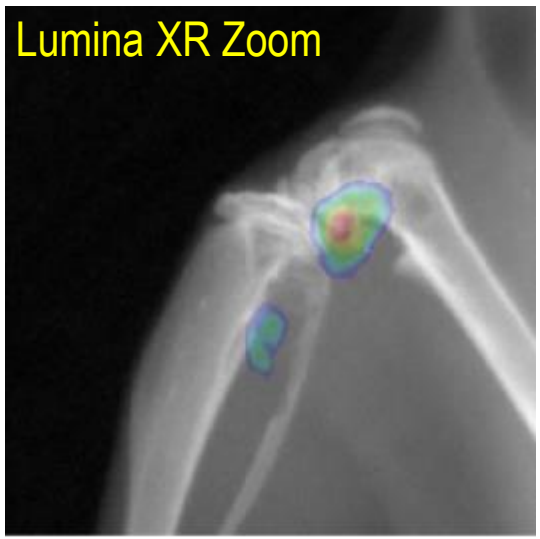


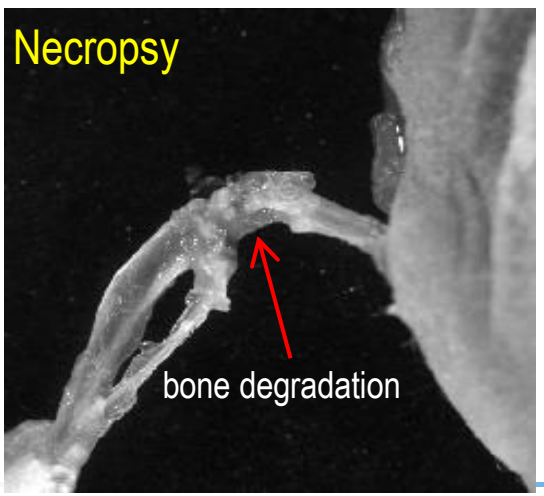
Imaging Cancer Metastases (validation with X-Ray)

IC delivery of MDA-MB-231 cells into immune-deficient mice



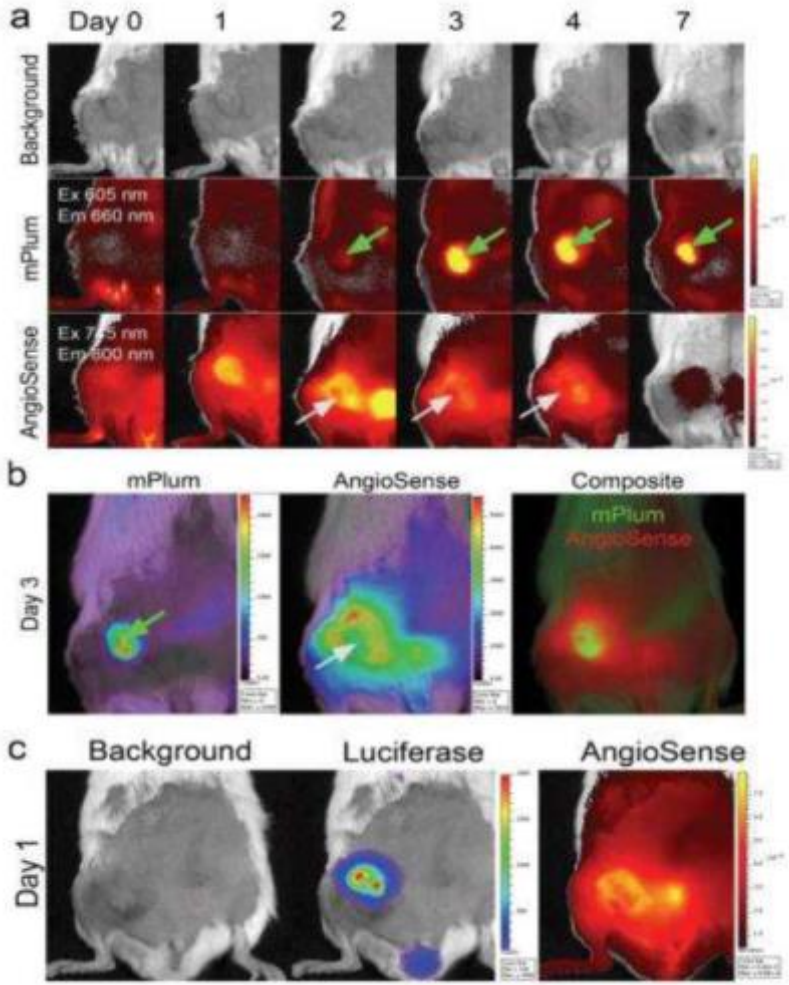






Monitoring Vascular leakiness with AngioSense

Near-infrared (NIR) fluorescent imaging of tumor vessel leakiness *in vivo*



(**a**) Kinetic images of s.c. tumors after i.v. injection of AngioSense and RD-Sindbis/mPlum (~107 particles) on day 0.
Green arrows indicate positive mPlum fluorescent signals, and gray arrows indicate tumor necrosis resulted from Sindbis-induced apoptosis.

(**b**) Reconstructed concentration maps for mPlum and AngioSense of the day 3 images. The mPlum signals are well associated with necrotic tumor tissue that shows little AngioSense signals.

(**c**) Using a RD-Sindbis/Fluc vector that carries a firefly luciferase, instead of a mPlum gene, enables detection of vector infection and its correlation with vascular leakiness as early as day 1.

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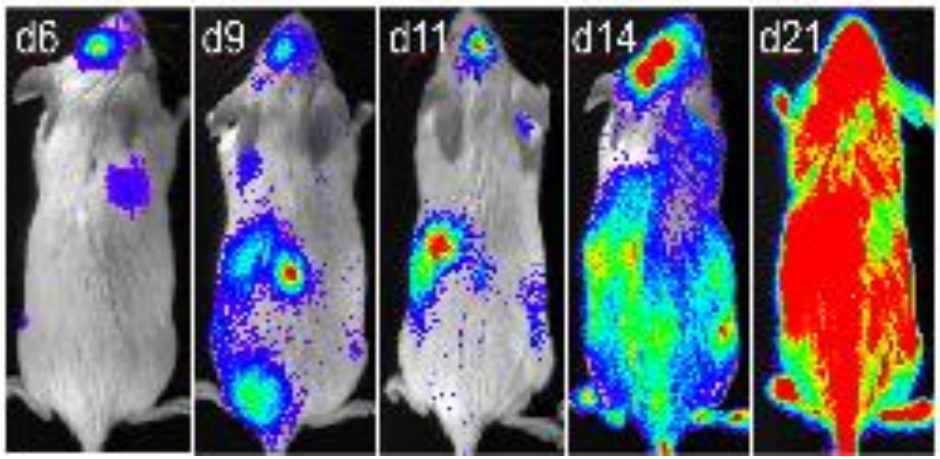
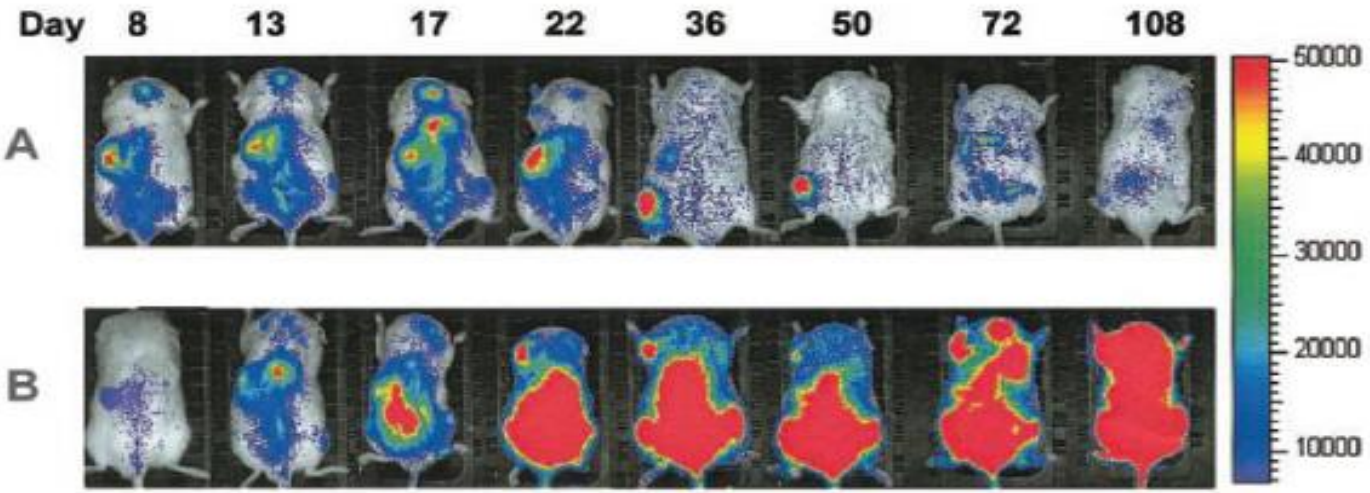
Cell Transplantation and Trafficking Patterns

HSC Hematopoiesis

Cao *et al*, Stem Cells, 2004



Transplantation of 250 Luc+ HSC into Lethally Irradiated Hosts

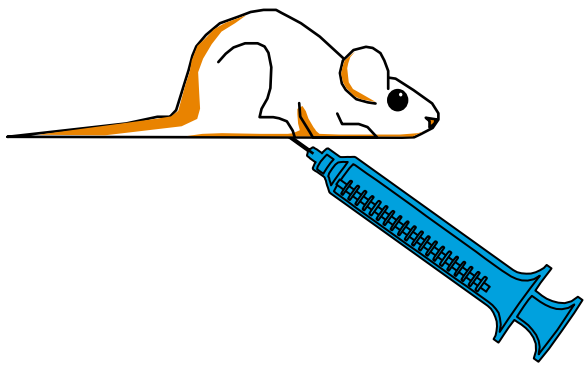


CD34+ HSC-luc(A)
or CD34+CD38- HSC-luc(B)
Tail vein inject to NOD/SCID mice
Monitor the viability and proliferation of the cells

Blood,2003

Ovalbumin (OVA)-induced Asthma

BALB/c mice



Assessments:
fluorescence optical Imaging
BAL cells
FRI lung *ex vivo*

Time 0 14d 21 23 25d

IntraPeritoneal sensitizations
OVA (50µg)/Alum (2mg)

IntraNasal challenges
OVA (100µg)

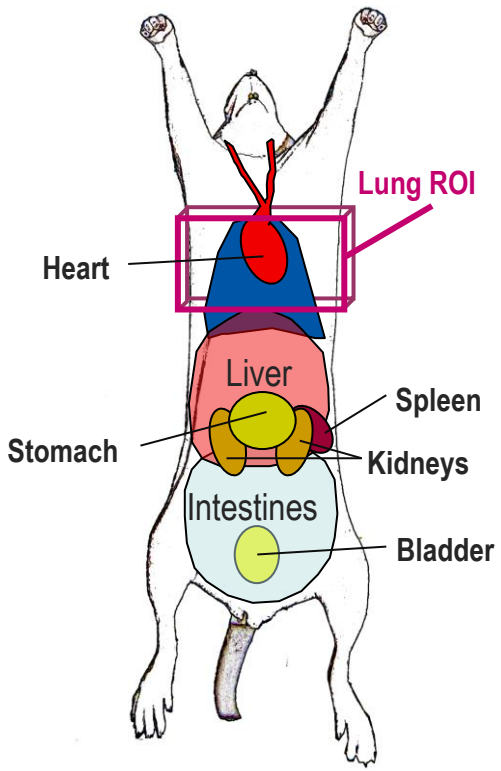
5nmol ProSense or
MMPSense

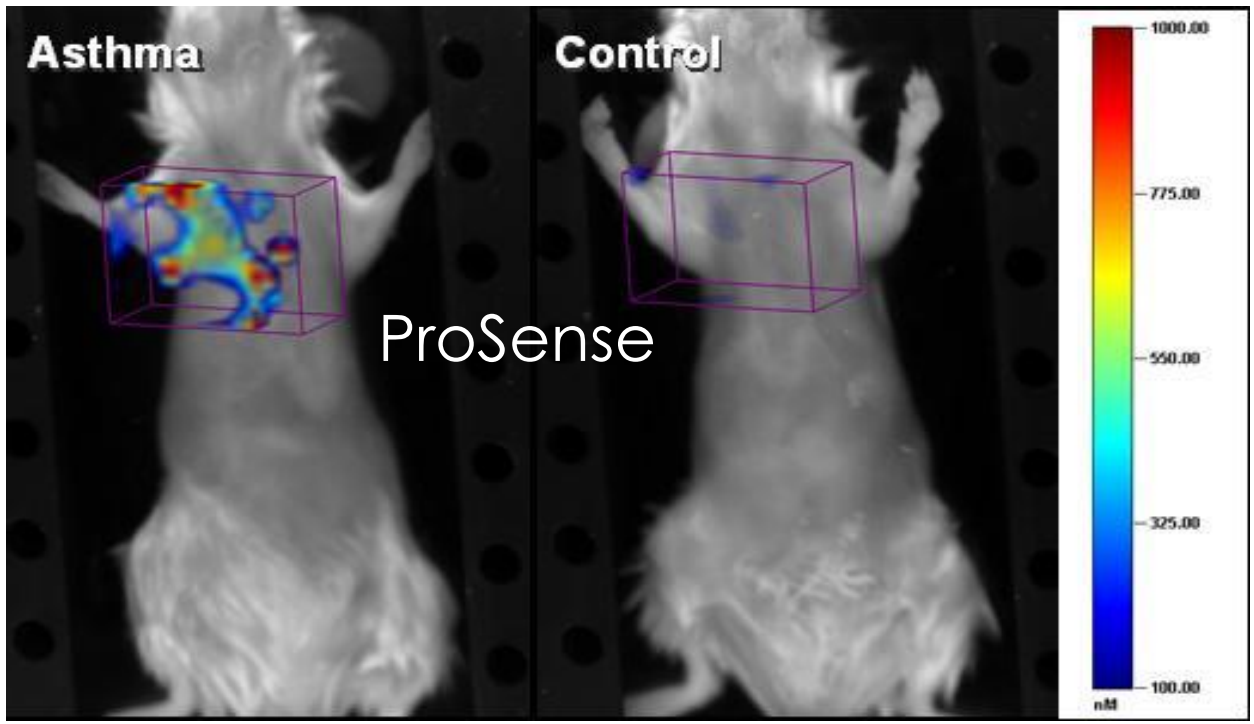
We adopted a standard model of murine asthma used in multiple research labs

BAL = Bronchoalveolar Lavage

Imaging Ovalbumin (OVA)-induced Asthma

In vivo Fluorescence Imaging

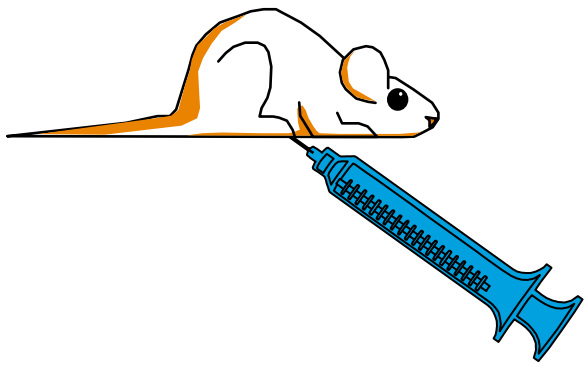




Asthma lung signal using ProSense is considerably higher and involves a larger volume than in control mice

Steroid inhibition of OVA-induced Asthma

BALB/c mice



Assessments:

fluorescence optical Imaging
BAL cells
FRI lung *ex vivo*

Time

0 — 14d — 21 — 23 — 25d

IntraPeritoneal sensitizations
OVA (50µg)/Alum (2mg)

IntraNasal challenges
OVA (100µg)

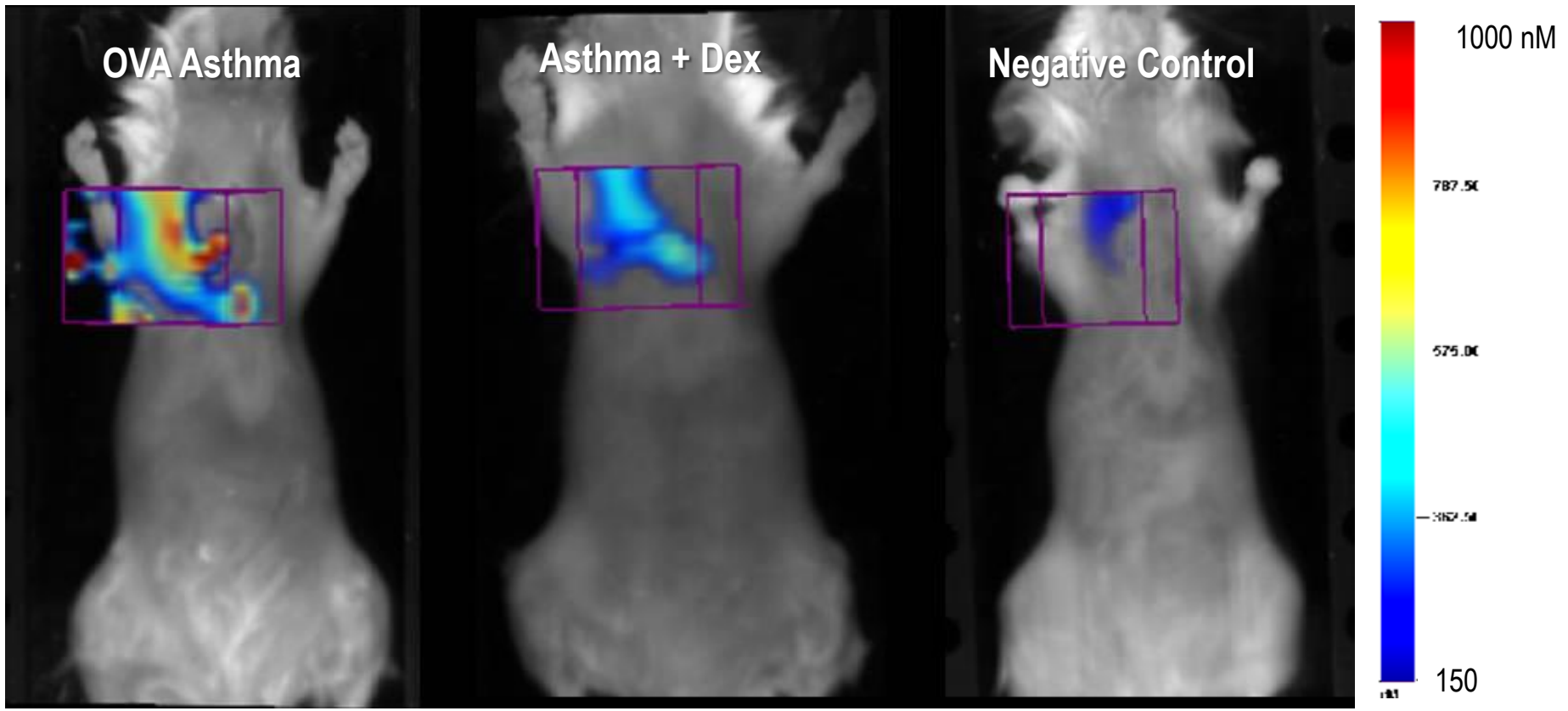
IP Dexamethasone
3mg/kg, 4h prior to IN

5nmol ProSense or
MMPSense

Steroids are a standard of care treatment for asthma

BAL = Bronchoalveolar Lavage

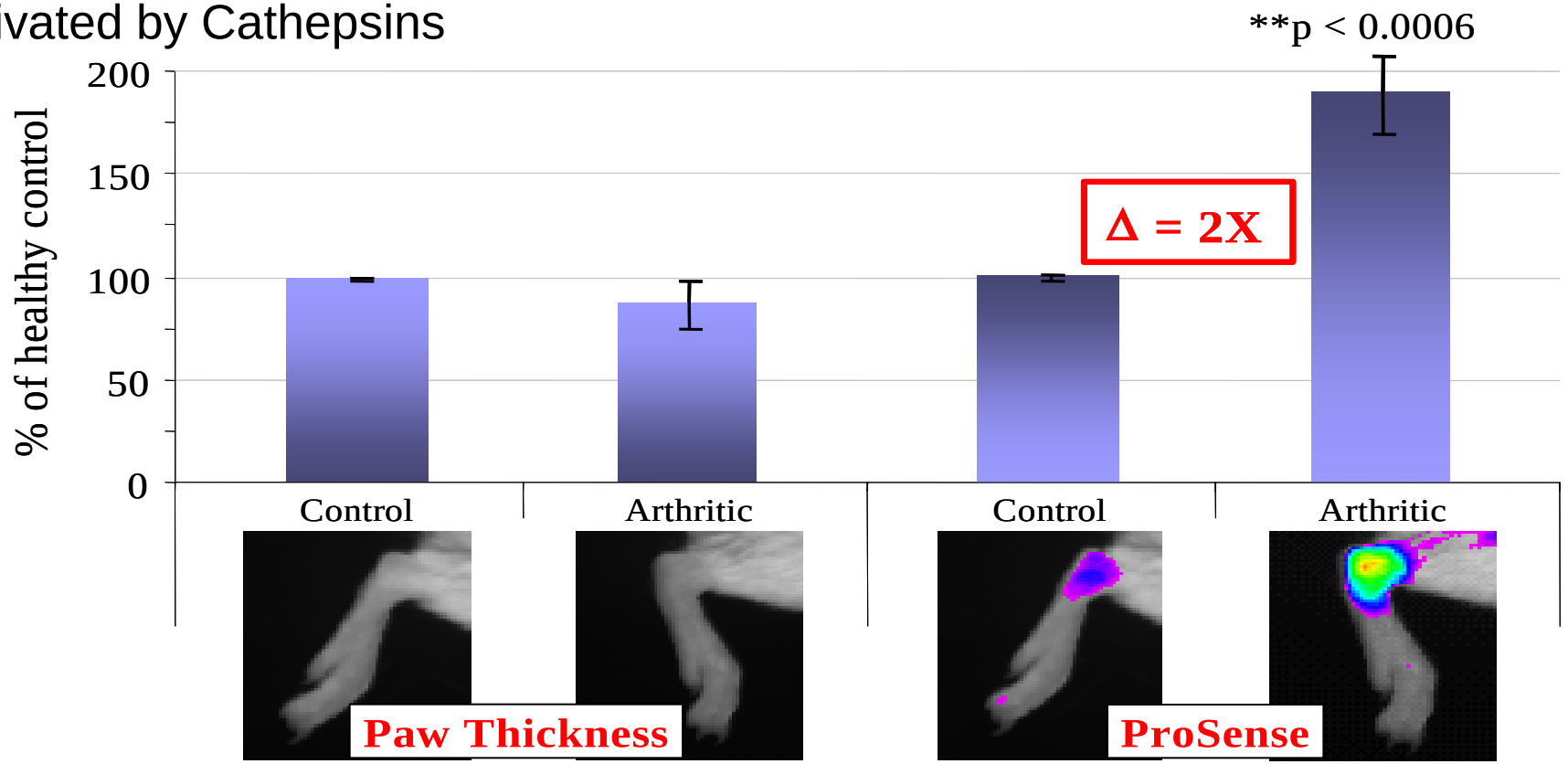
Dexamethasone Therapeutic Activity in Asthma



Fluorescence images readily show clear differences in asthmatic, treated, and control mice

CAIA Model : ProSense & Early Disease (Day 4)

- Arthritis is not clinically detectable
- 24 hrs after ProSense probe injection
- Activated by Cathepsins



imaging with ProSense can detect disease at earlier time points, prior to detection by paw thickness

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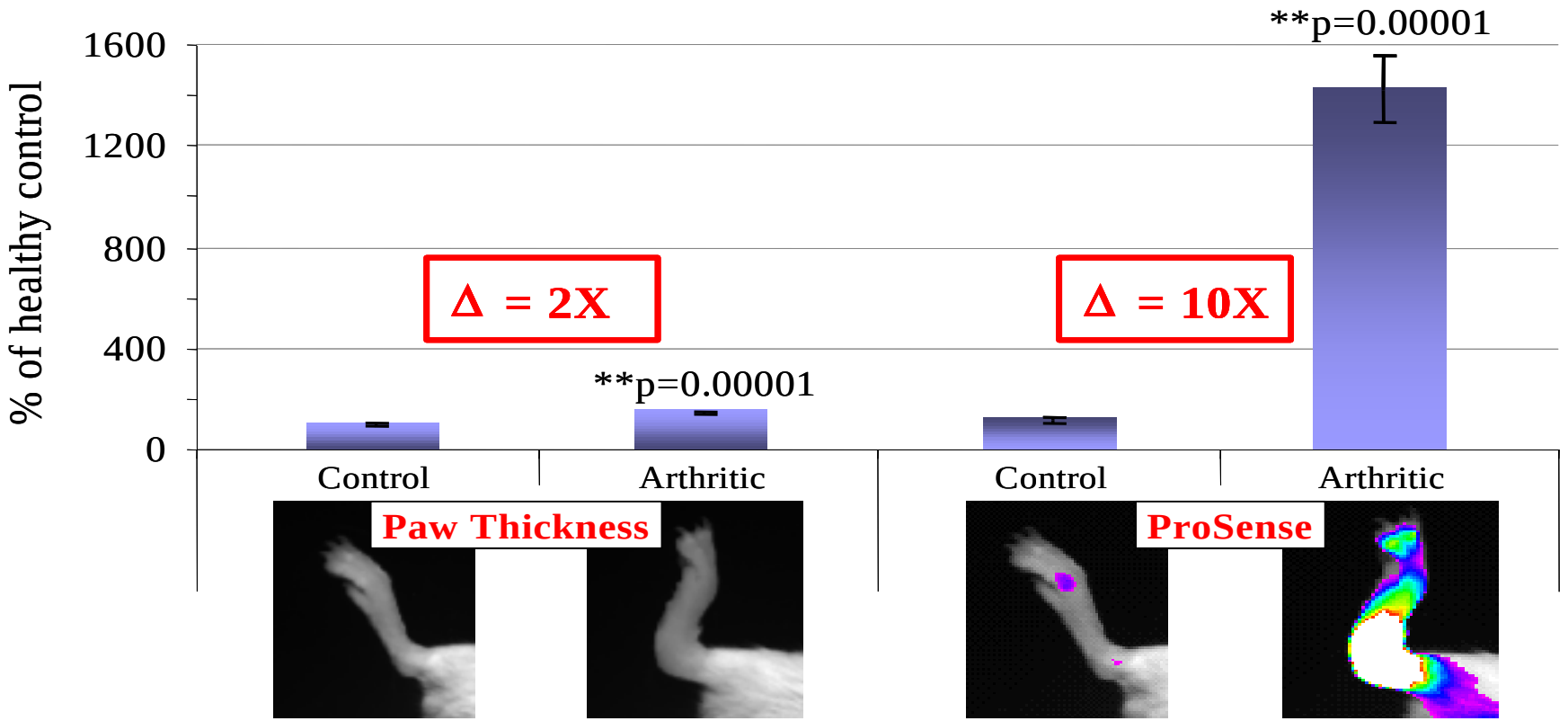
CAIA Model : ProSense & Late Disease (Day 8)

- Arthritis is clinically detectable and at its peak
- 24 hrs after ProSense probe injection



imaging with ProSense provides a 10-fold signal over control animals at the peak of the disease

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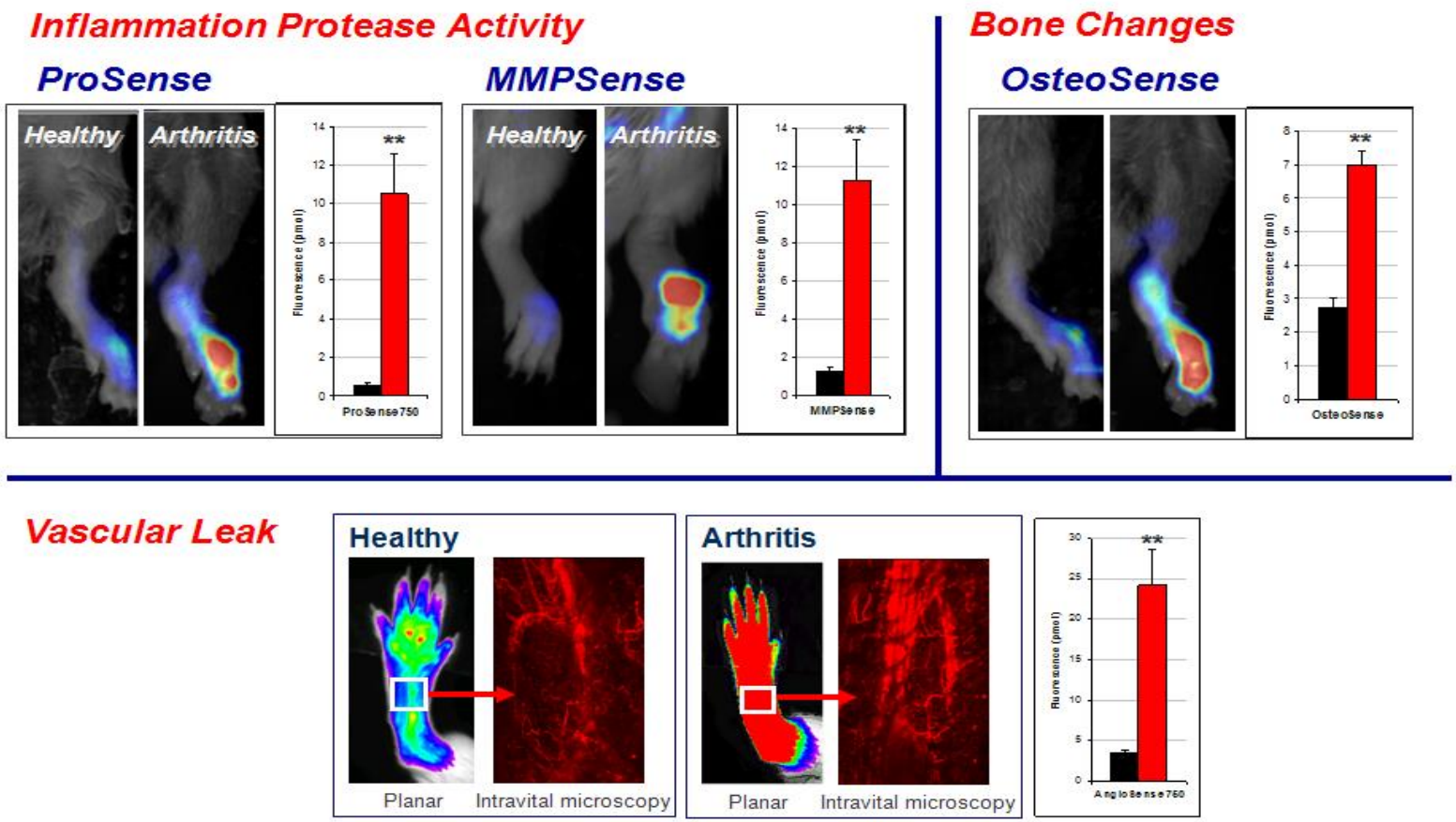
Multiplex fluorescence Imaging



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Thank you for your attention!

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