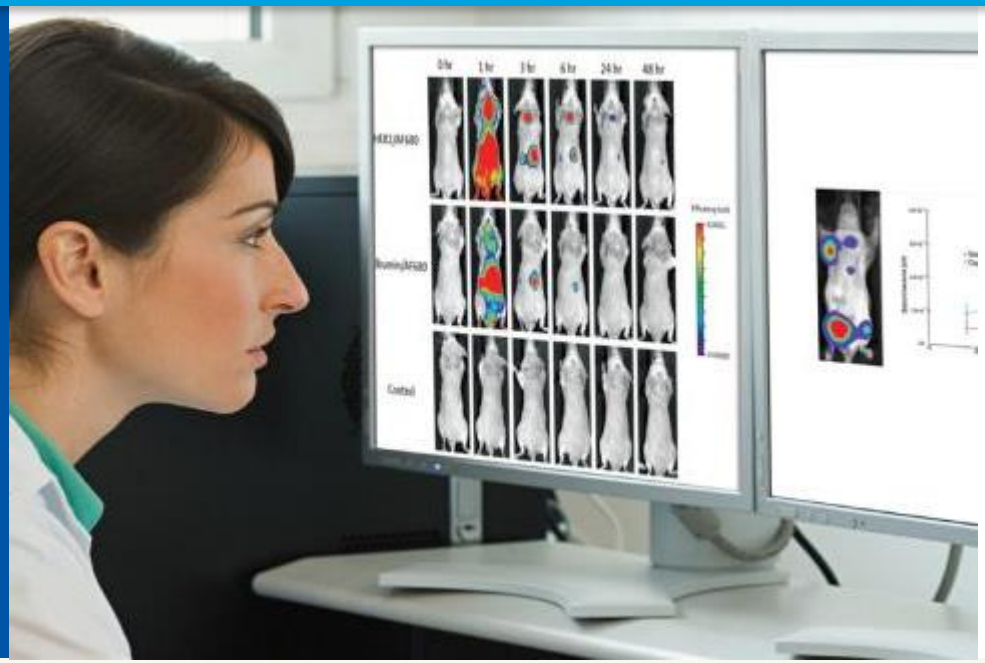
PerkinElmer
For the Better

HUMAN HEALTH | ENVIRONMENTAL HEALTH



In Vivo Optical Imaging Technology and Application

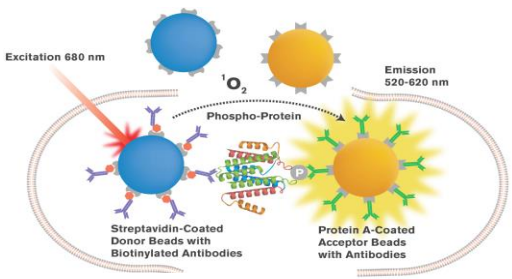
IVIS Lumina XR

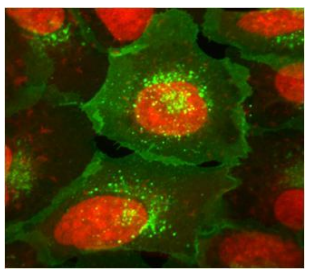
產品應用專員 曾筱筑
博克科技有限公司

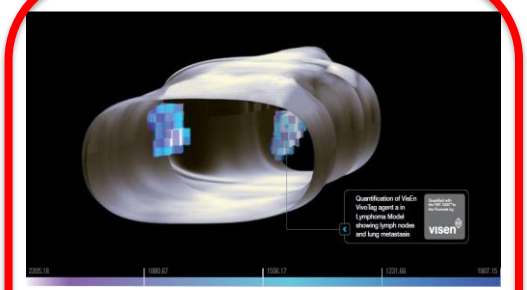
PerkinElmer Biomarker Imaging

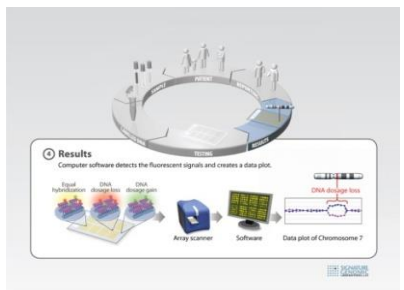
Rational

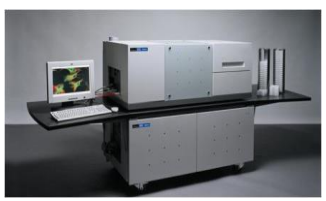
WELL → CELL → ANIMAL → HUMAN







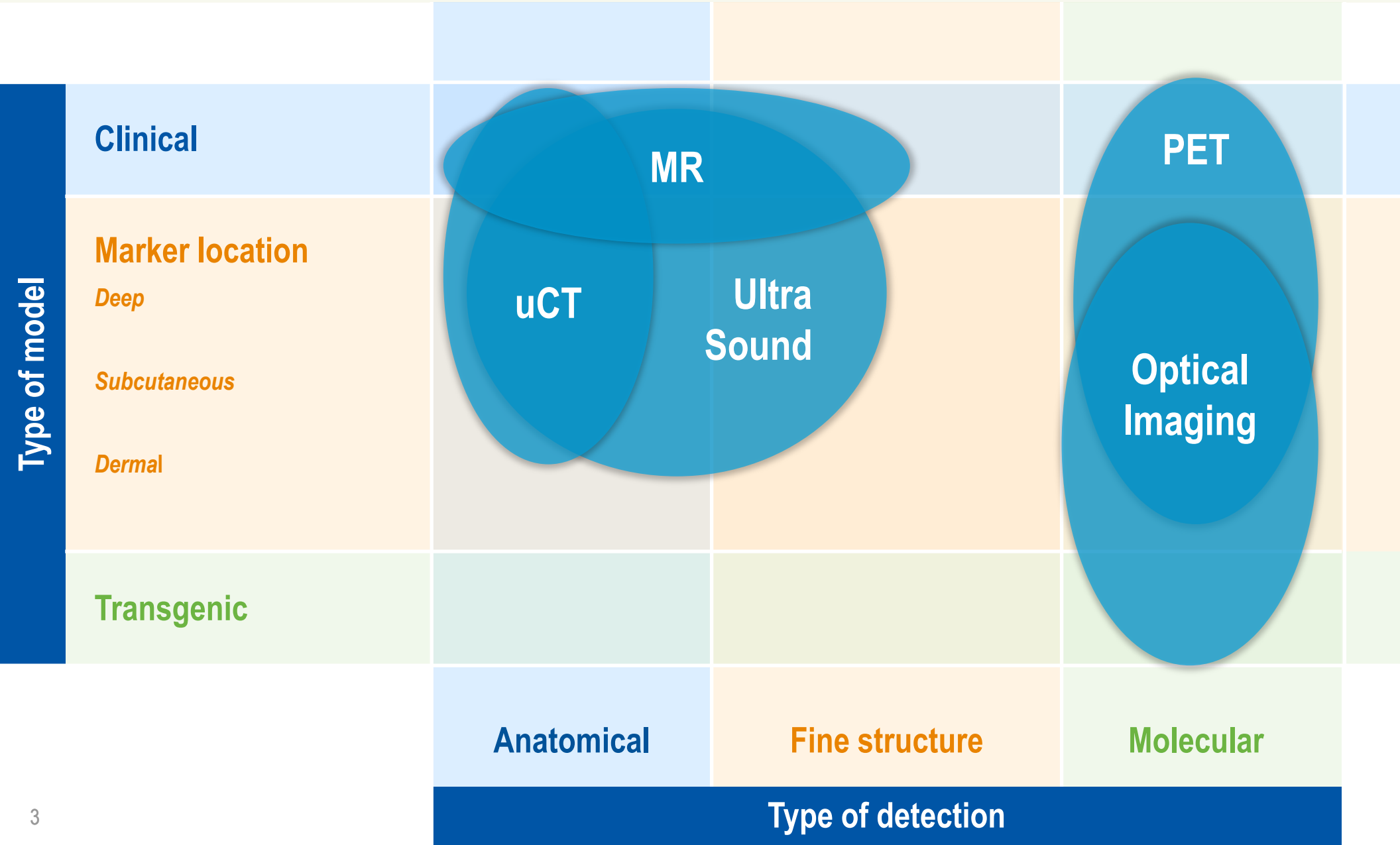




Now , Bio-discovery with the in vivo imaging platforms we provide the world leading portfolio for translational disease research, drug discovery and clinical development

.. .integrating all stages of research workflow

In Vivo Imaging Landscape



Full Range of Optical Imaging Platform (1700+ Installations worldwide)

Lumina III

Entry level bioluminescent/ fluorescent imaging

Lumina XR

Lumina with X-ray overlay

Kinetic

Fast, Real-time molecular imaging

Spectrum

Quantitative 2D & 3D bioluminescence and fluorescence imaging

Spectrum CT

Seamlessly integrates optical and micro CT imaging (multi-modal)

FMT Series

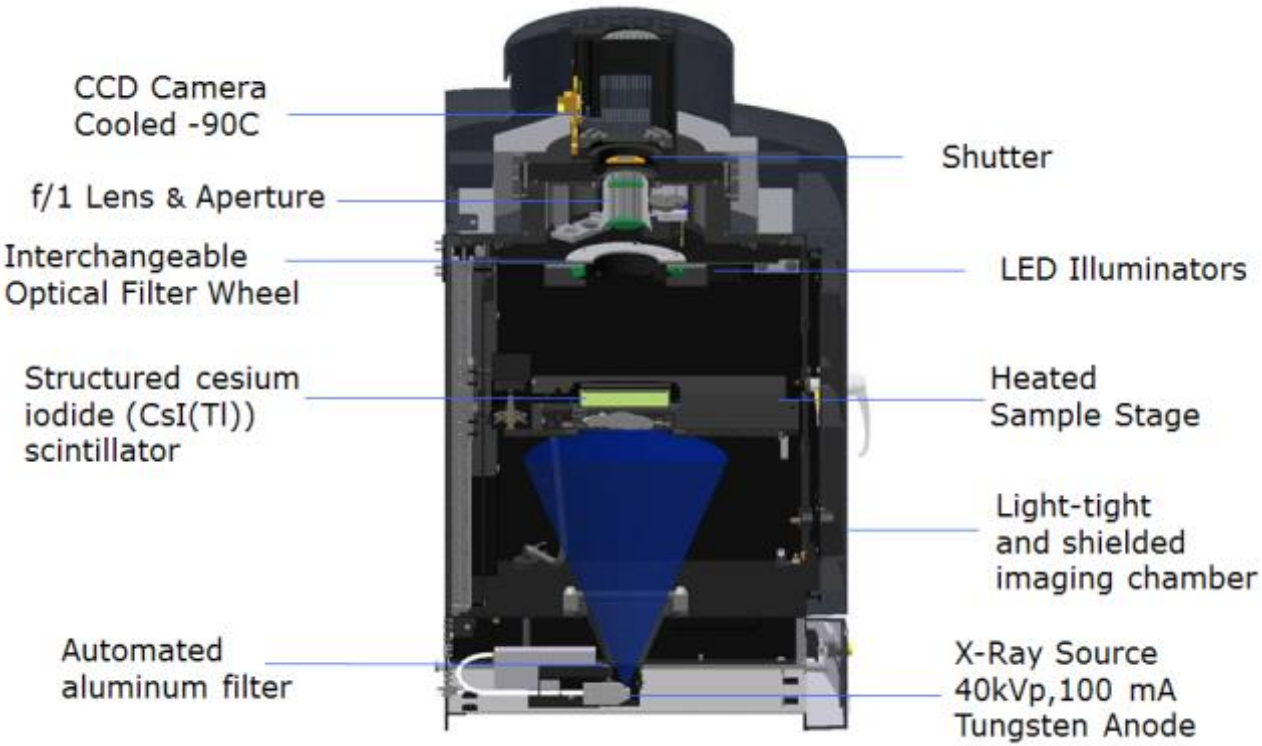
Quantitative Fluorescence 3D Tomography System

Quantum FX/GX

Fast, low dose uCT

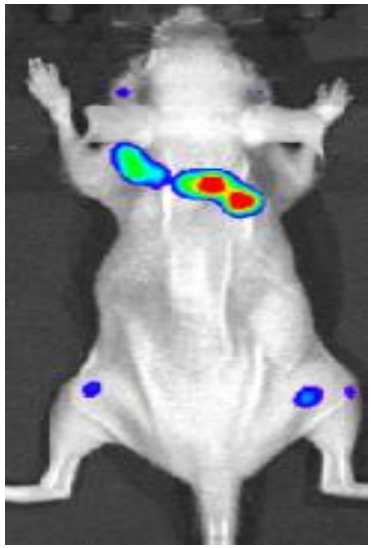
IVIS Lumina XR

Fluorescence, Bioluminescence with integrated X-Ray Imaging

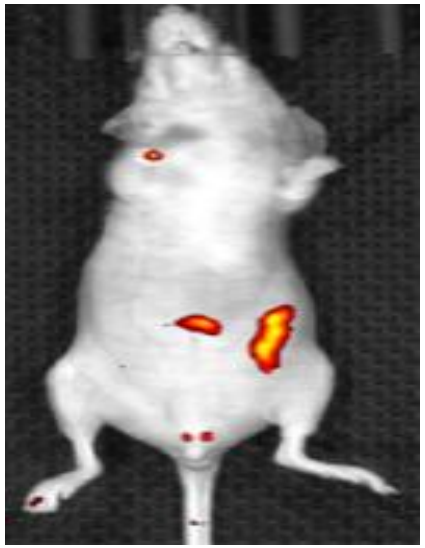


Comprehensive Imaging Function

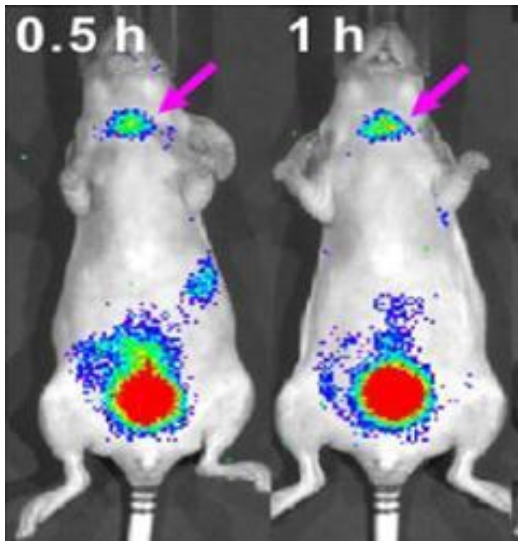
2D BLI



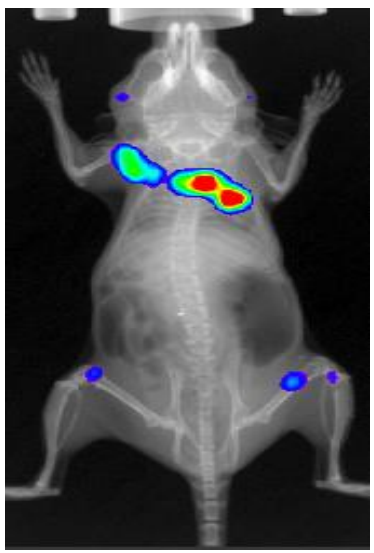
2D FLI



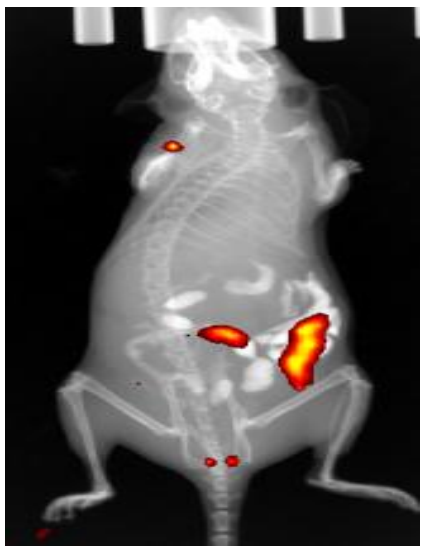
Cerenkov



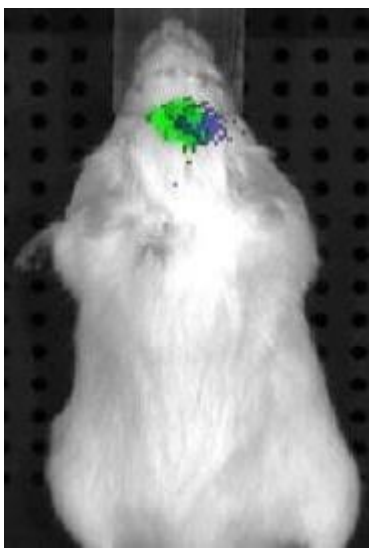
BLI-X Ray Overlay



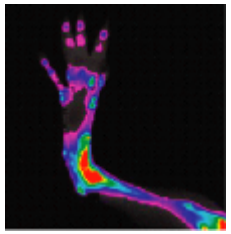
FLI-X Ray Overlay



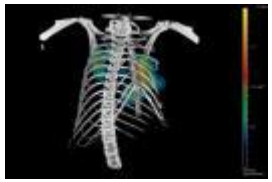
BLI-FLI Overlay



Tailored To Therapeutic Applications

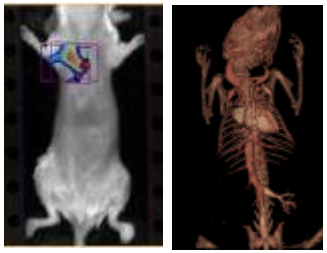


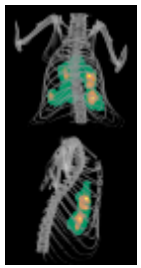
Inflammation



Oncology

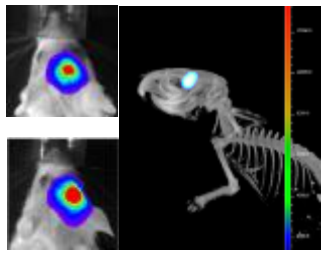
Cardiovascular Disease



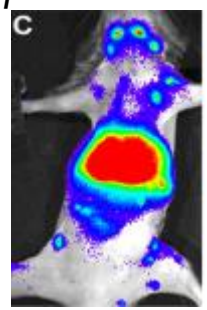


Infectious Diseases

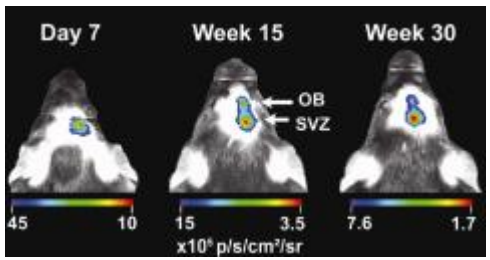
Neuroscience



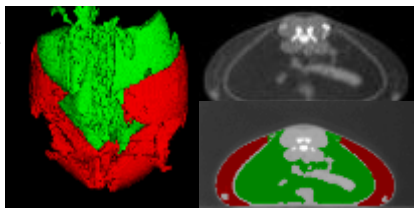
Immunology & Transplantation Biology



Stem Cells

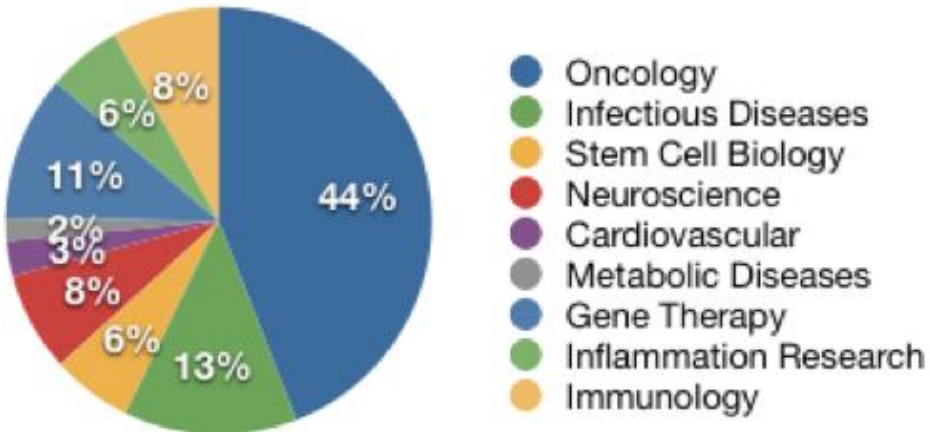


Metabolic Diseases

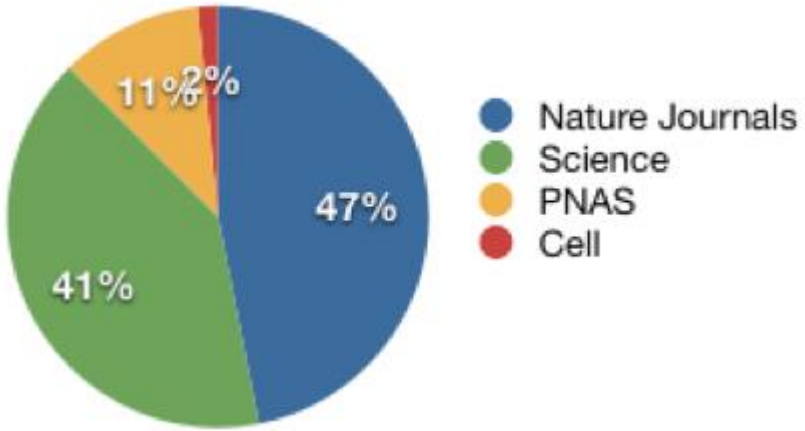


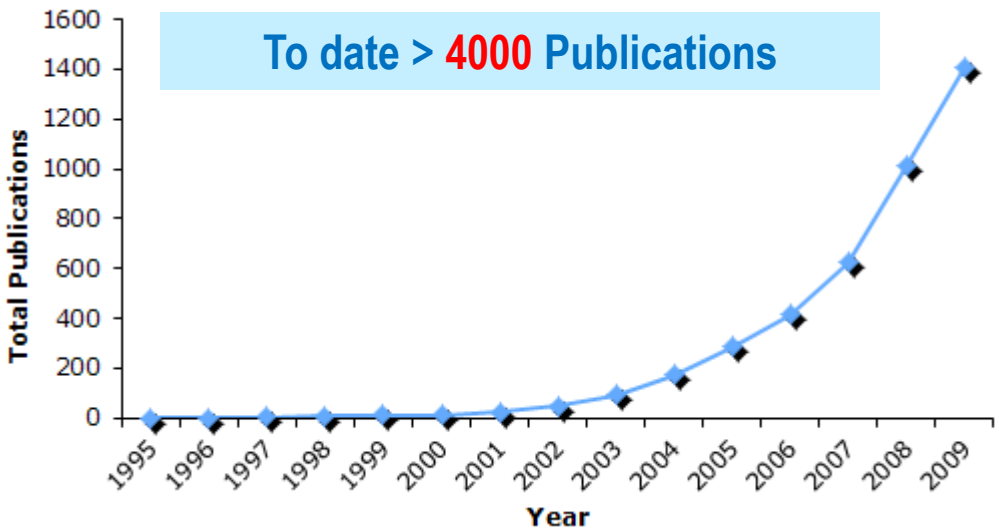
Largest Library of *In Vivo* Imaging Publications (4000+ peer-reviewed publications)

Disease Areas



Total High Impact Journal Publications > 100





Largest Superior Users In the World

IVIS Bringing Compounds to the Clinic

PKI's IVIS imaging technologies used in the preclinical evaluation of the 6 FDA approved drugs and 8 drugs currently in clinical trials.

FDA Approved Drugs

- Sutent (Pfizer): subcutaneous tumer xenograft
- Dasatinib (Bristol-Myers Squibb): chronic myelogenous leukemia
- Tasigna (Novartis): leukemia/metastasis model
- Cubicin (Cubist Pharmaceuticals): bacterial peritonitis model
- Aflibercept (Sanofi-Aventis): orthotopic renal cancer
- Velcade (Millennium Pharmaceuticals): multiple myeloma

Drugs currently in clinical trials

- ABT-888 (Abbott): multiple diverse tumor models
- RANKL Inhibitor (Amgen): denosumab and bone metastasis models
- Panzem (EntreMed Pharmaceuticals): orthotopic gliosarcoma
- AEE788 (Novartis): intraperitoneal tumor model
- IT-101/CRLX 101 (Insert Therapeutics, Cerulean Pharma Inc.): Ewings sarcoma
- CHIR-258 (Novartis): orthotopic multiple myeloma model
- NPI-0052 (Nereus Pharmaceuticals): subcutaneous tumor model
- CG0070 (Cell Genesys): orthotopic bladder cancer (*status of trial unknown)

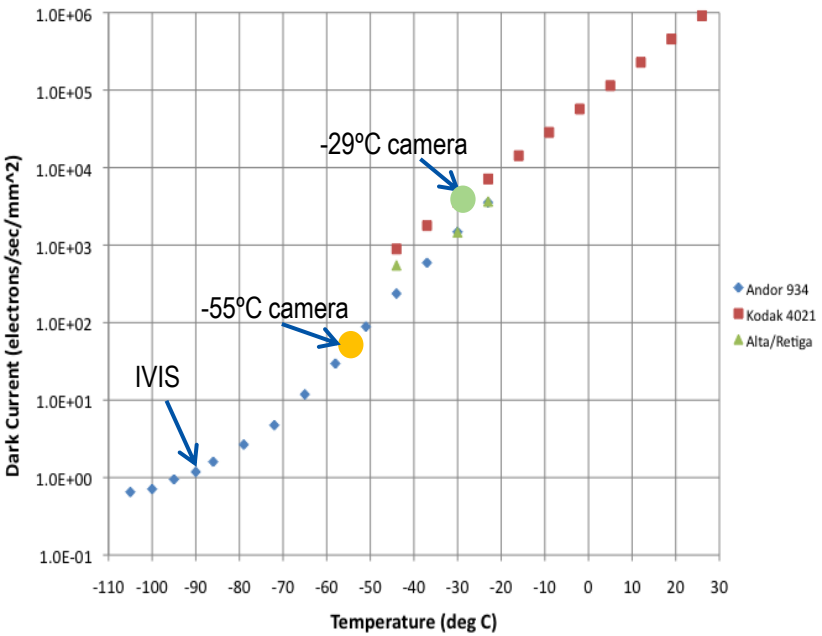
Characteristics of Lumina XR

The Most Sensitive Bioluminescent Imaging

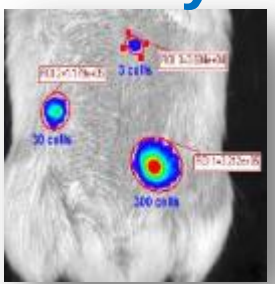
BIOLUMINESCENCE

Proven Leading Sensitivity *In-Vivo*

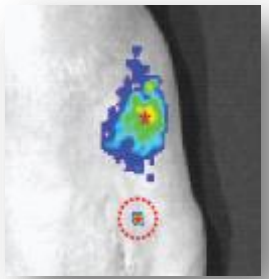
Cooled (-90ºC) camera with large CCD chip area and low F-stop for high sensitivity bioluminescent light detection



Firefly Luciferase



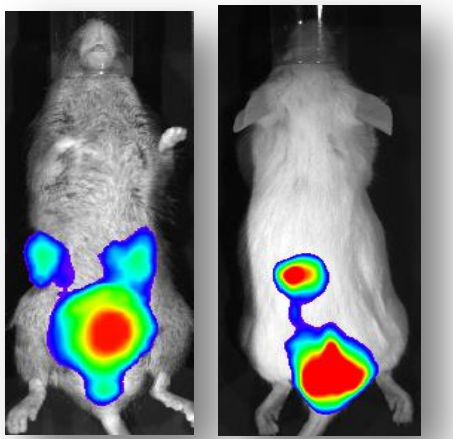
Rabinovich et al, PNAS,2008



Kim et al, pLOS One et al, 2010

In vivo imaging of s.c. implanted T cells transduced with optimized firefly luciferase (left) and a 'single' 4T1 breast cancer cell (right)

Bacterial Lux

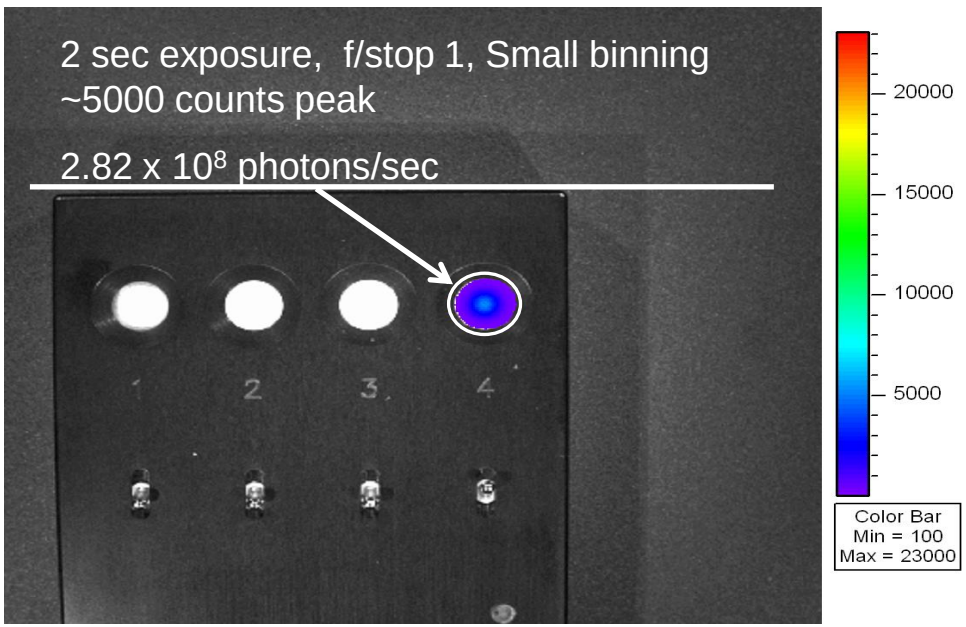


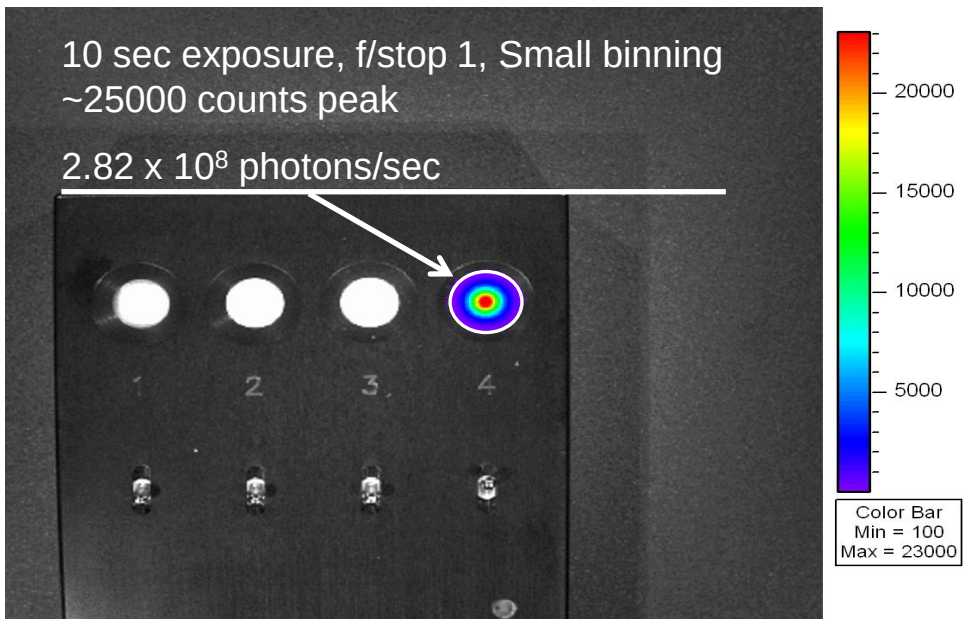
Imaging kidneys from bacterial lux expressing Proteus strain (even in black mice), 5 minute exposure

- Most sensitive system available
- Resolves multiple bioluminescent reporters
- In vivo systems cooled to -55ºC have 25 times higher dark current than IVIS
- IVIS systems are 5x more sensitive than cameras cooled to -55ºC
- Detects down to even a single cell in vivo

Patented Absolute Intensity Determination

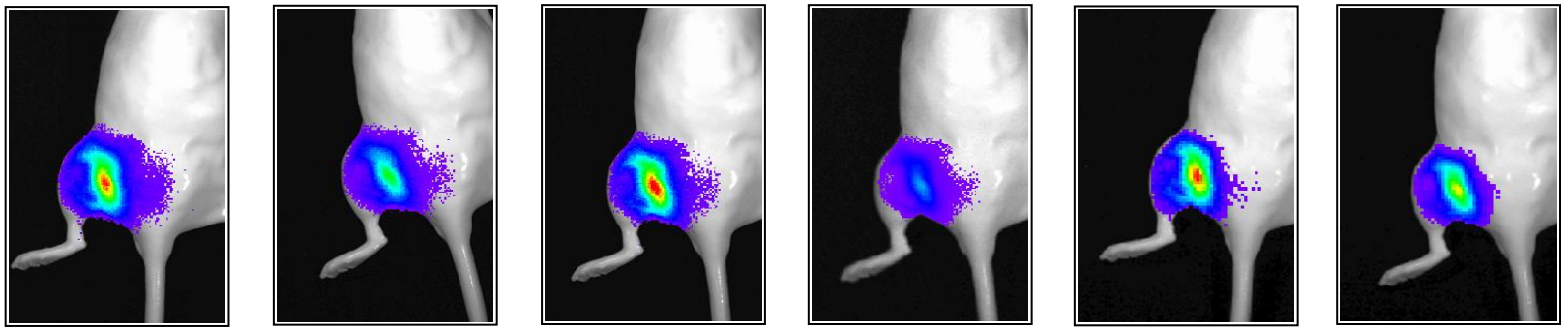
- Living Image® automatically compensates for device settings: Exposure time, *f*/stop, binning and field of View.
- Calibrated units are Photons per Second, representing the flux radiating omni-directionally from a user-defined region
- **NIST standard**





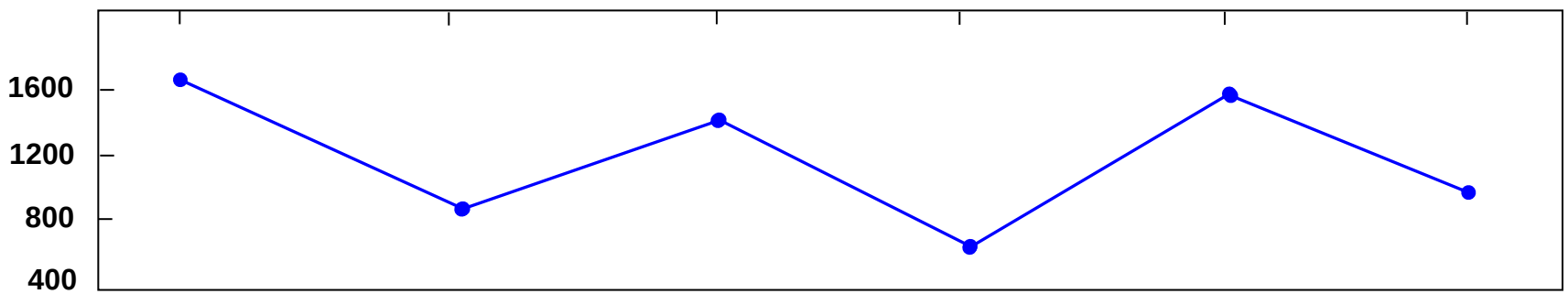
Calibrated Physical Units vs. Raw Signal

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

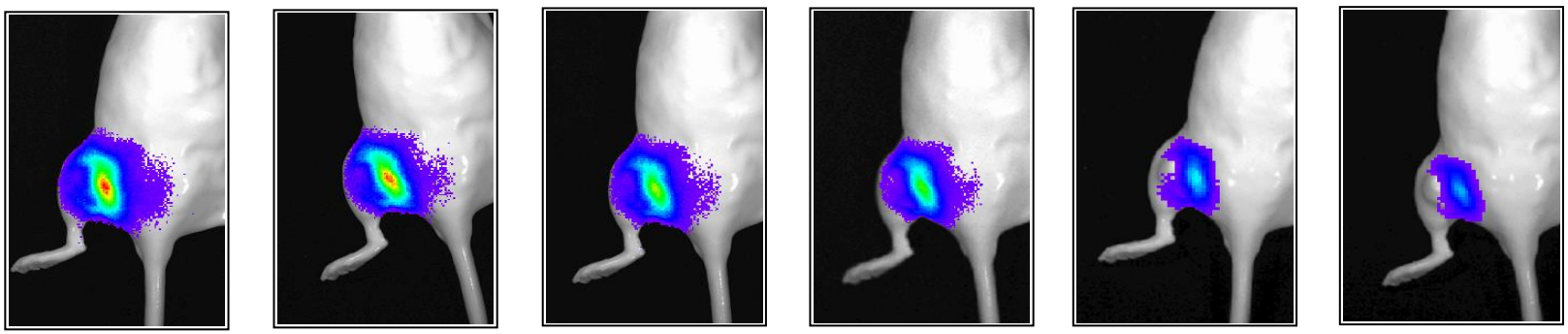


Calibrated Physical Units vs. Raw Signal

- *Mechanics*

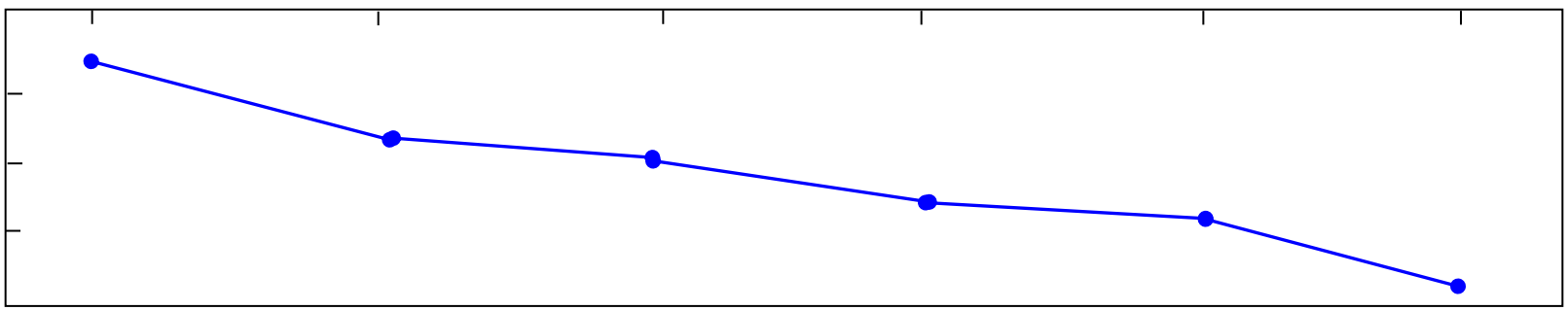
Calibrated Signal

(Photons per second)

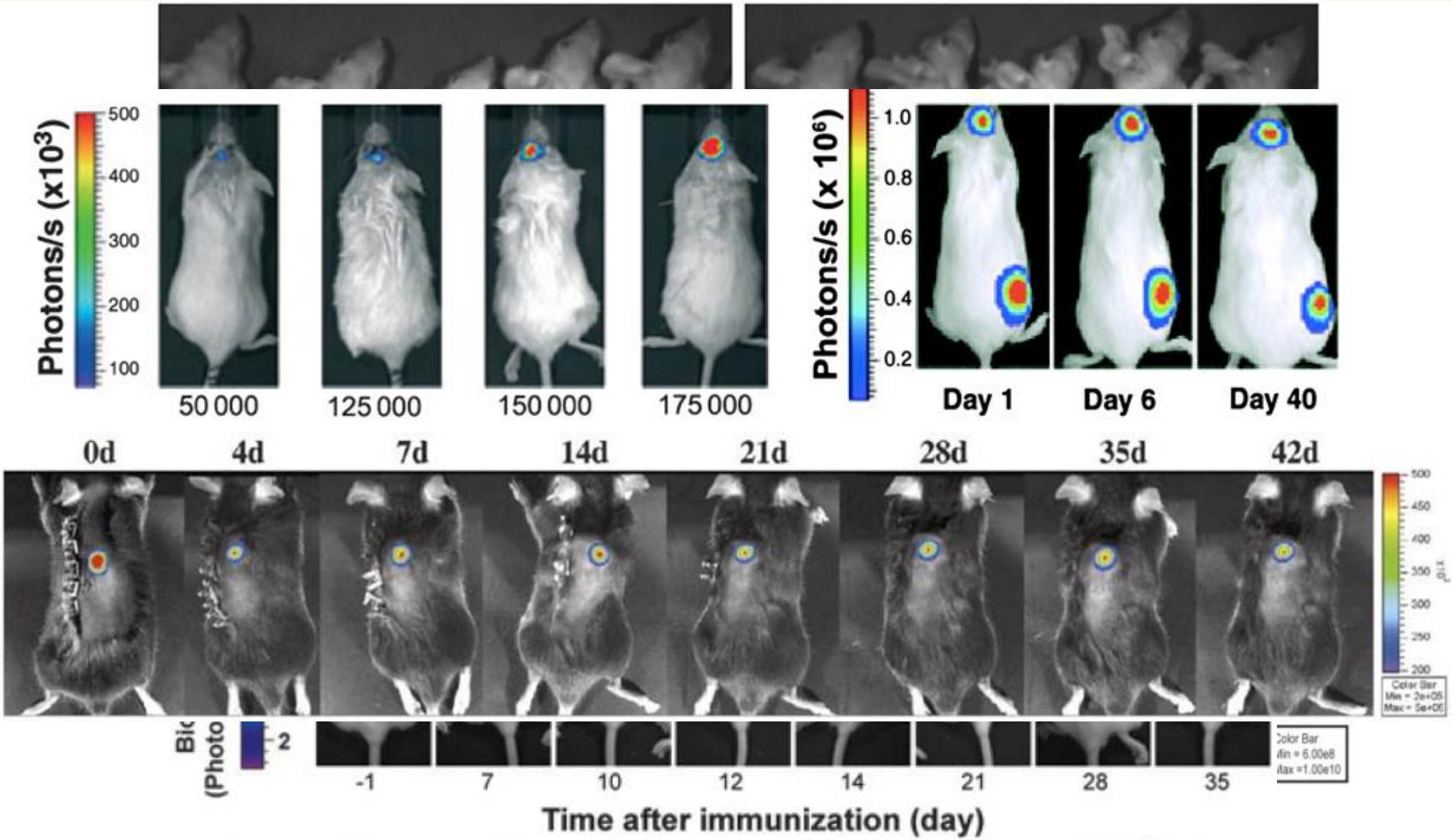


Exp time:	30 sec	30 sec	60 sec	60 sec	60 sec	60 sec
Binning:	small	small	small	small	medium	medium
Day:	1	2	3	4	5	6

Radiance: ***Photons per second***



Benefits of High BLI Sensitivity

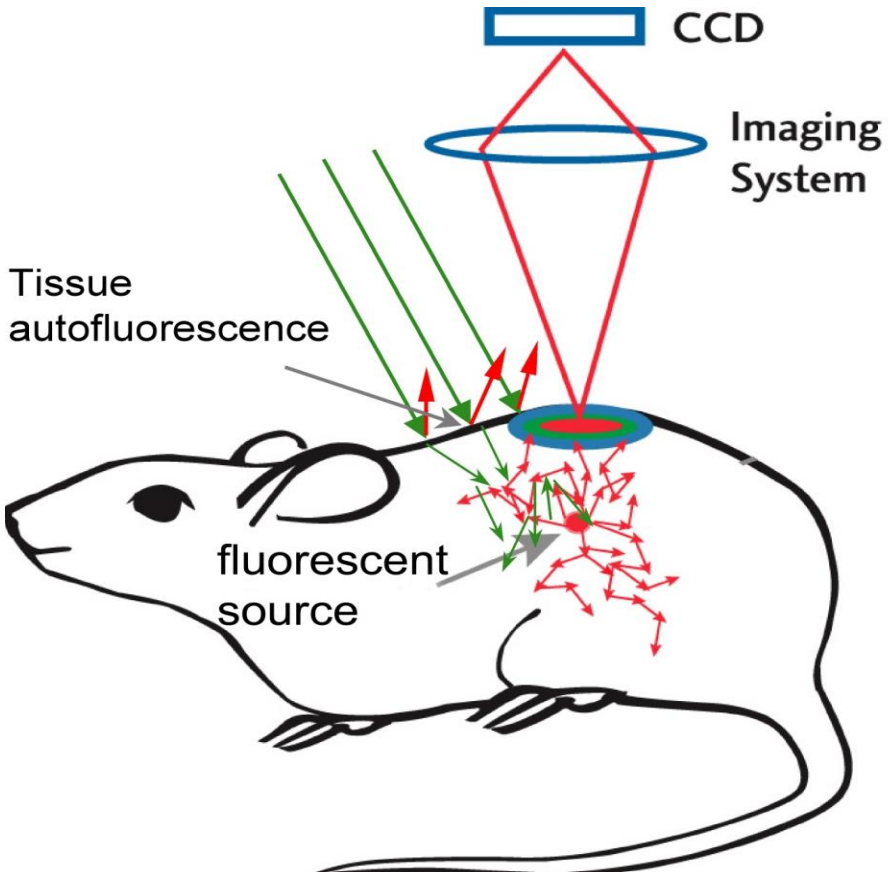


Early activation of TGF-β signaling in brains of SBE-luc

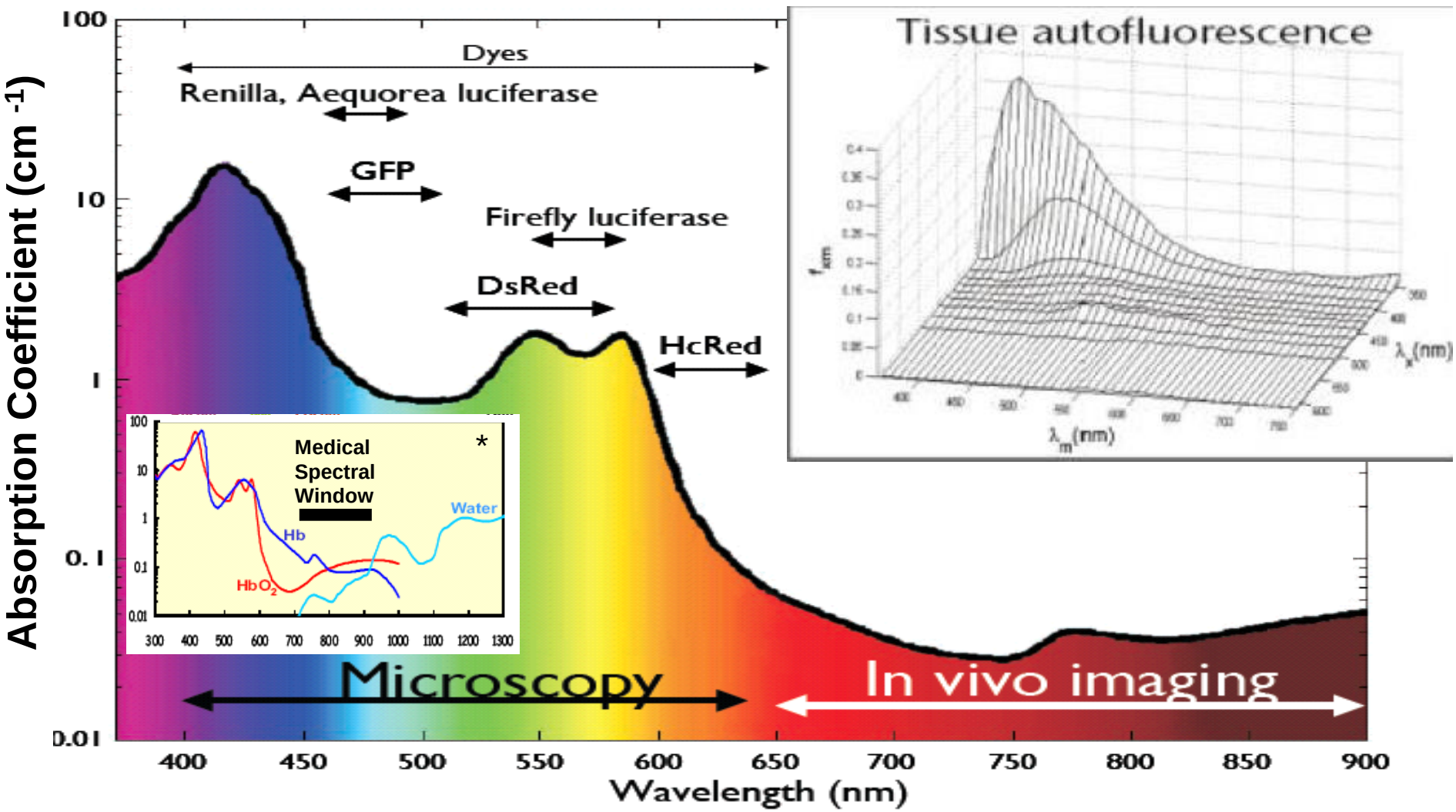
The Optimized Solution for Fluorescent Imaging

Challenges of Fluorescent Imaging

- Absorption
- Tissue Autofluorescence



Imaging Window of Opportunity

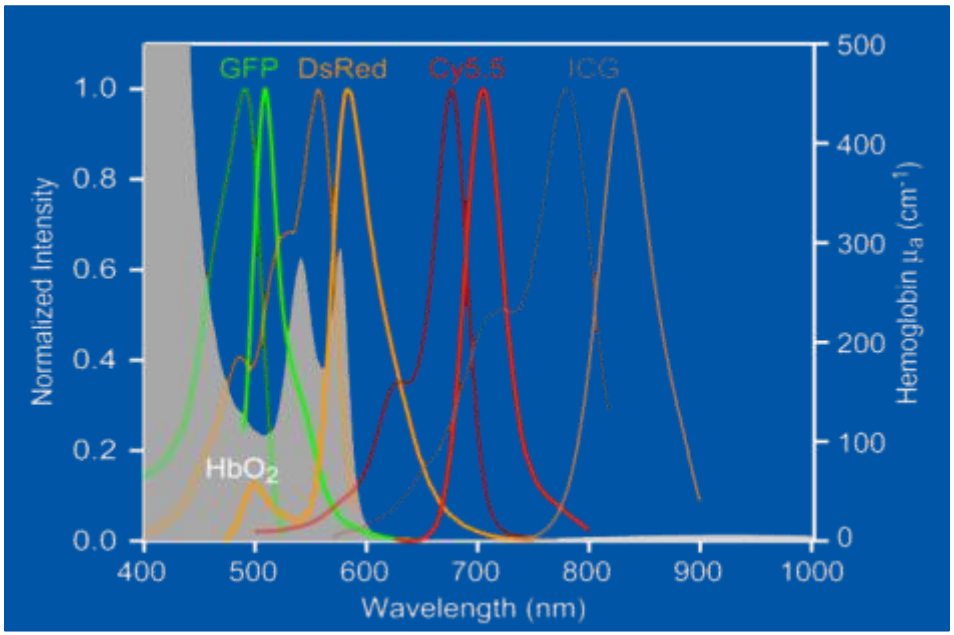


*http://ase.tufts.edu/biomedical/research/Fantini

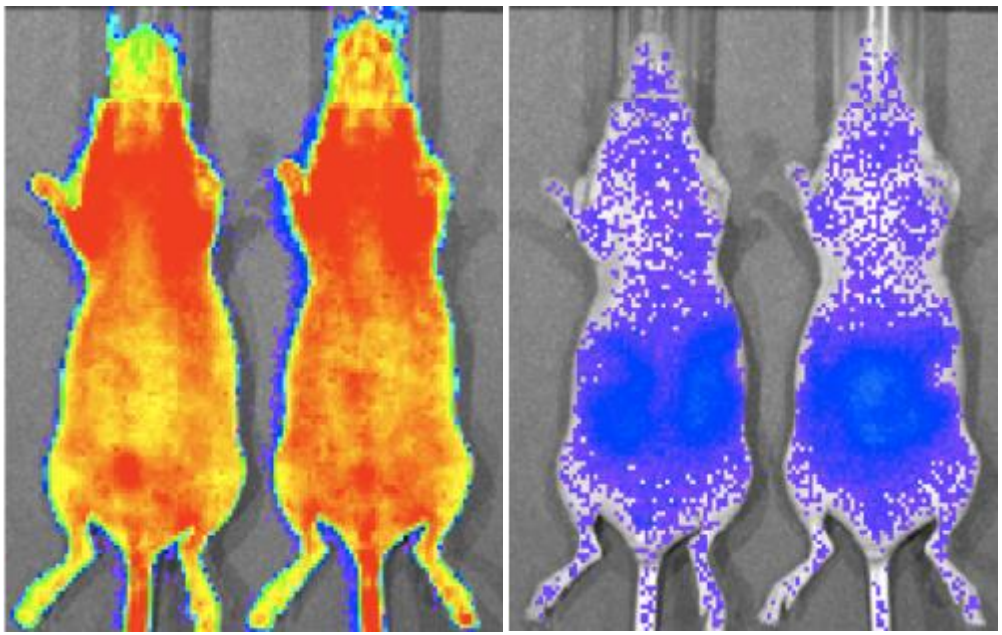
Imaging Window of Opportunity

Why red-shifted/near-IR fluorescent reporters? Why not GFP?

Hemoglobin absorption



Autofluorescence



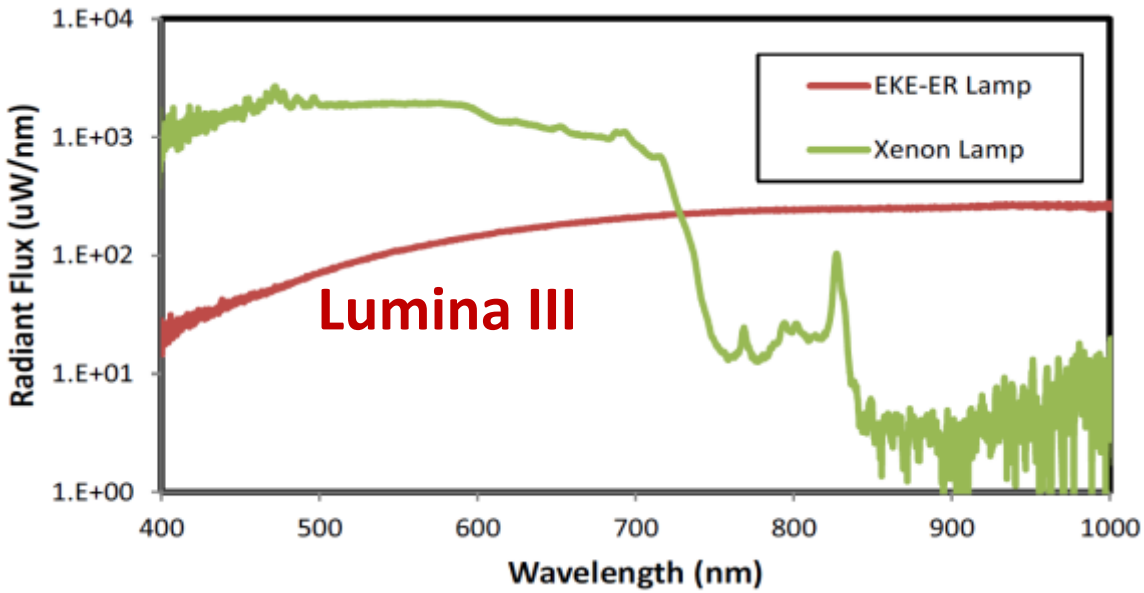
550nm

840nm

New Illumination System- NIR application

- 150 W EKE bulb with Extended Range (ER) coating

Xenon Lamp does not have an extended NIR range



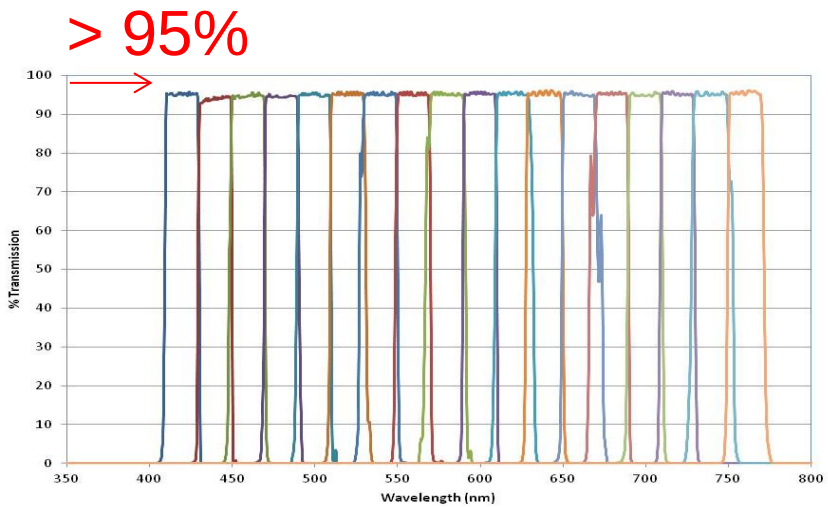
- **Optimized illumination for NIR imaging**
- Increased power at NIR wavelengths
- New high temperature fiber bundle (high temperature epoxy)

FLUORESCENCE

Specially designed filters- last longer

- **Hard Coated filters- last longer**

- Transmission values that commonly exceed 95%.

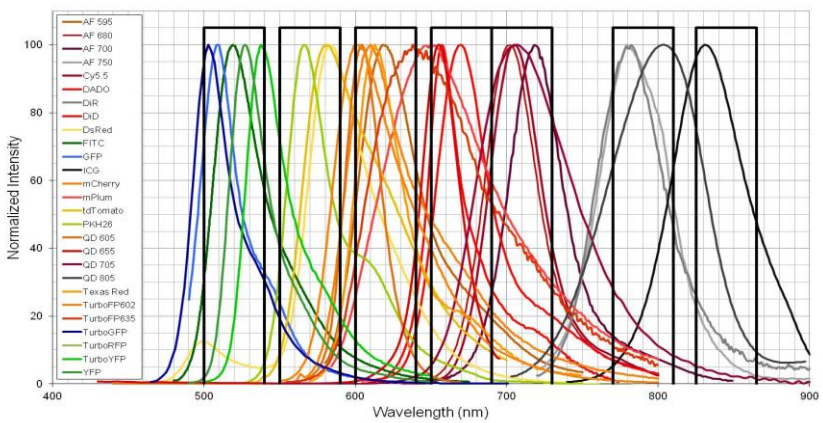


- **Excitation and Emission filters**
  - 19 Excitation filters go deep in the NIR range
  - IVIS offers 7 emission filters in Lumina Series III

- **Patented Absolute Calibration**

- IVIS is calibrated to NIST® Standards.

- Absolute units allows for the comparison of data from other labs as well as data taken on different types of Instruments.

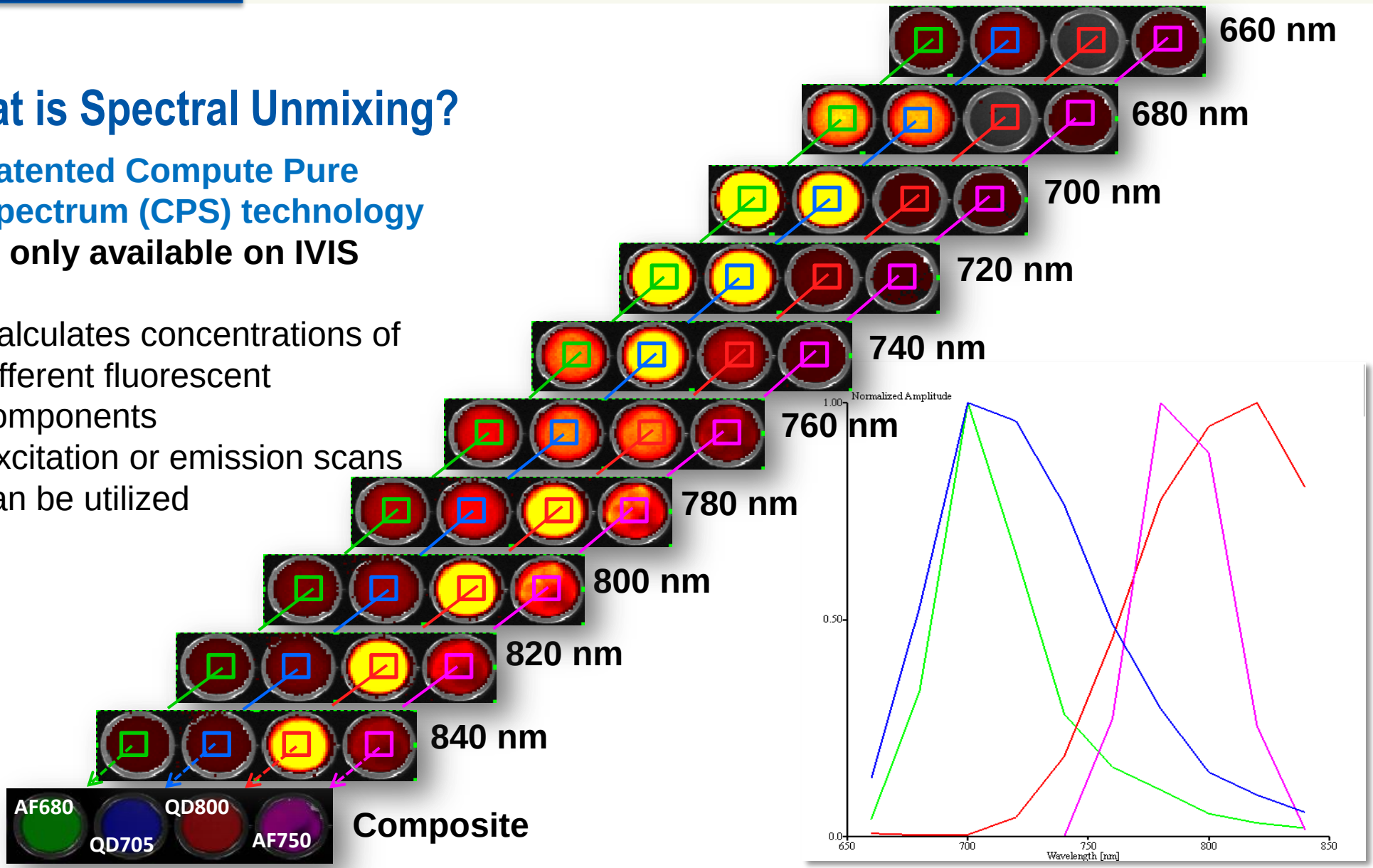


Multispectral Unmixing Imaging in the IVIS

What is Spectral Unmixing?

- **Patented Compute Pure Spectrum (CPS) technology is only available on IVIS**

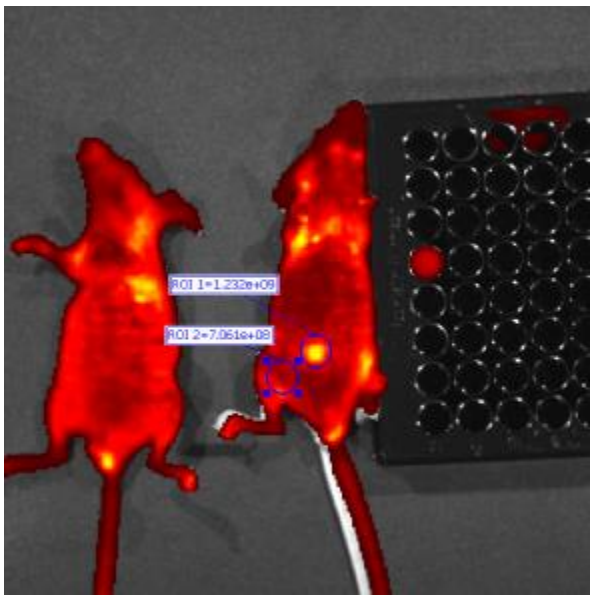
- Calculates concentrations of different fluorescent components
- Excitation or emission scans can be utilized



Powerful Fluorescence--Remove Autofluorescence with Spectral Unmixing

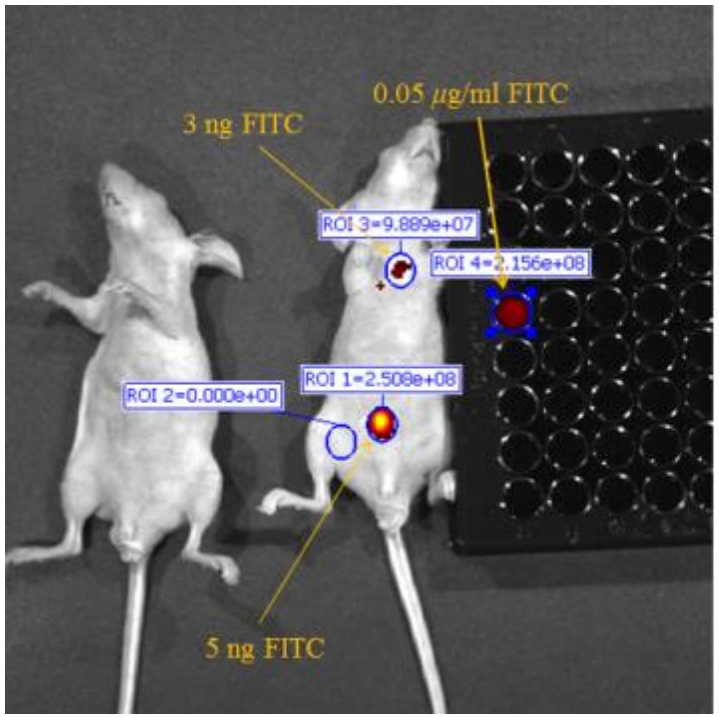
PerkinElmer
For the Better

Autofluorescence



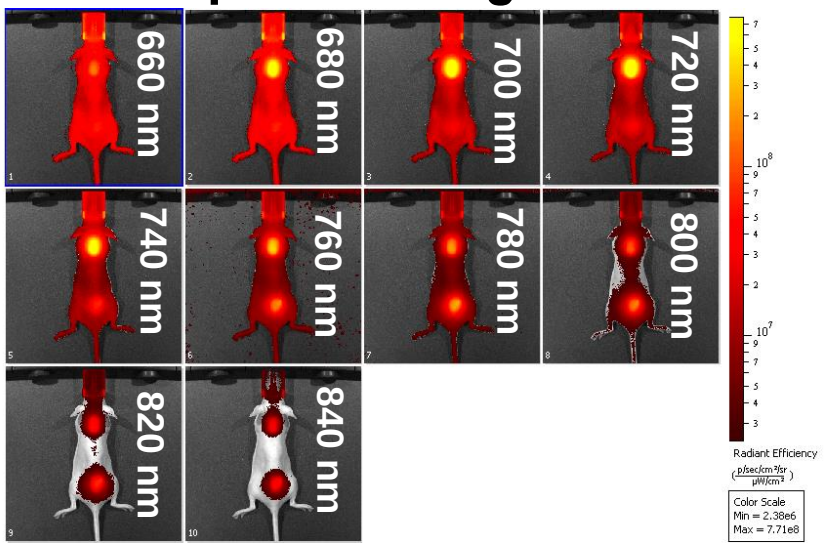
Where is the Signal?

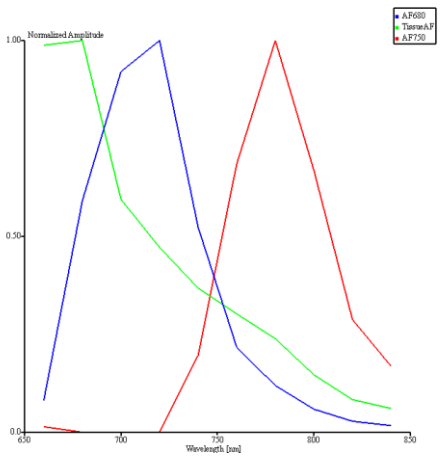
Spectral Unmixing



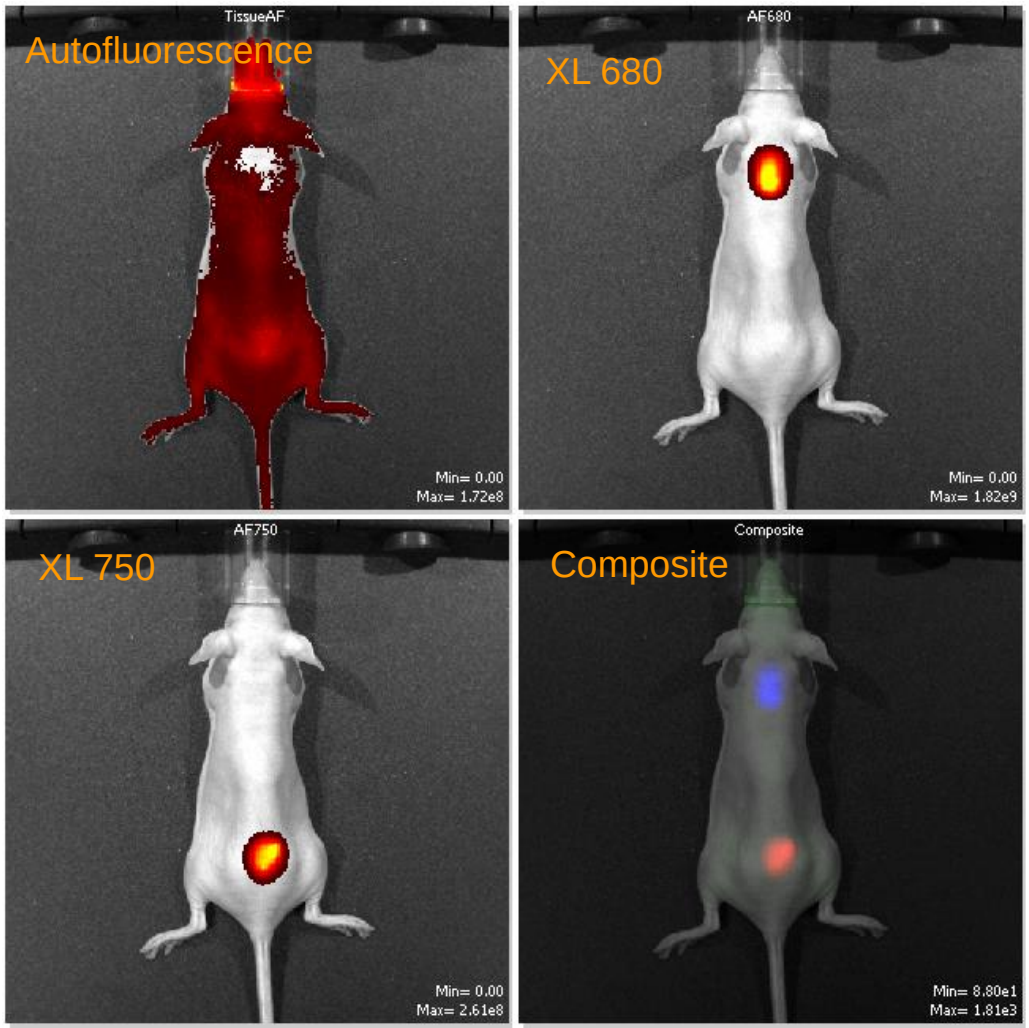
XenoLight 680/750 in a Mouse Using spectral unmixing

Raw Spectral Images



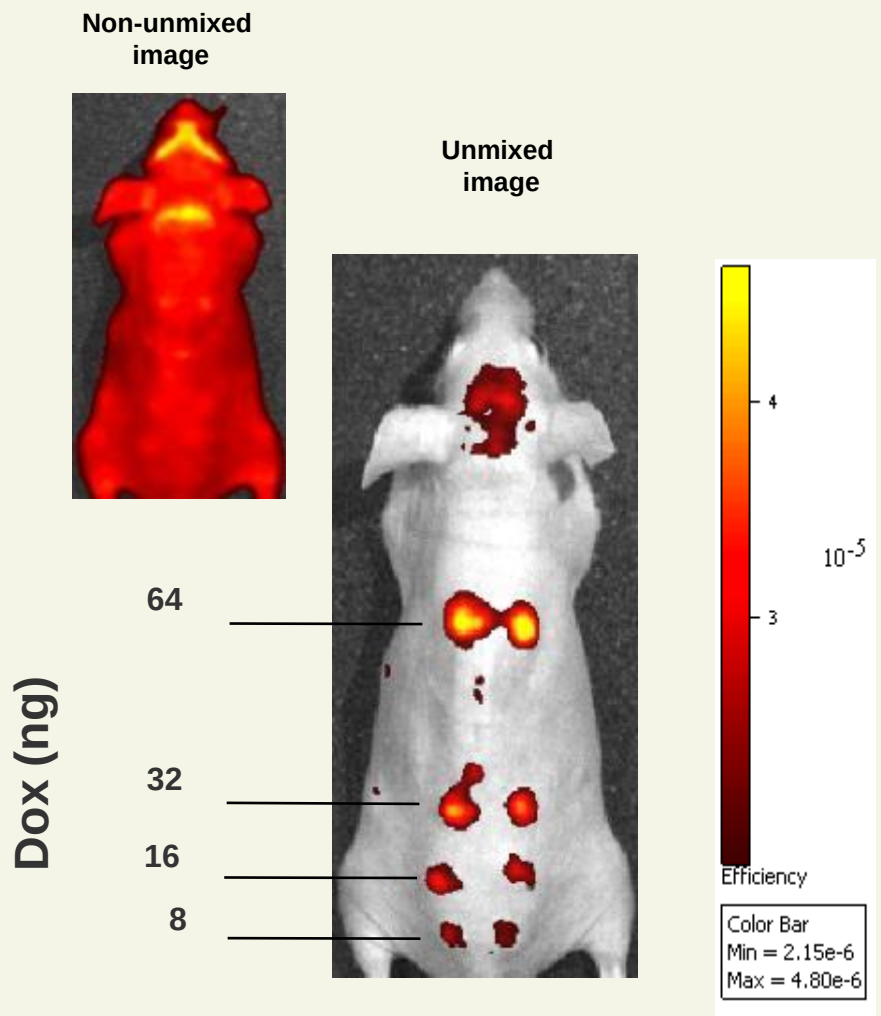


- Subcutaneous injections of 10^{14} molecules of XenoLight 680 (scruff)
- Subcutaneous injection of 10^{14} molecules of XenoLight 750 (lower dorsal region)
- 605nm excitation filter



When is Spectral Unmixing Most Useful?

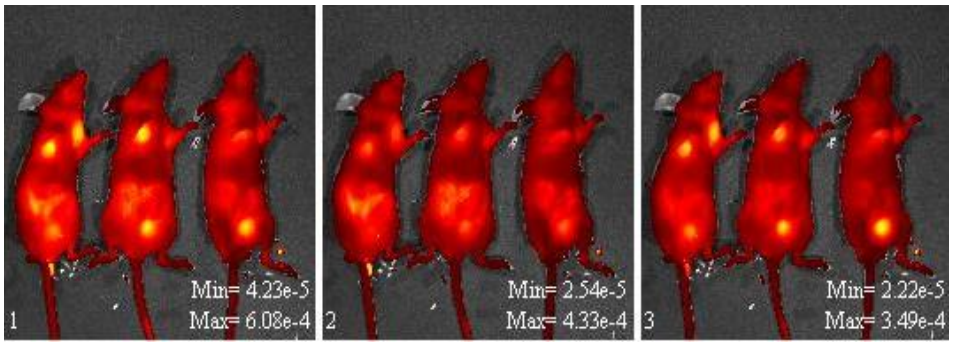
Improve Quantification Sensitivity and Accuracy



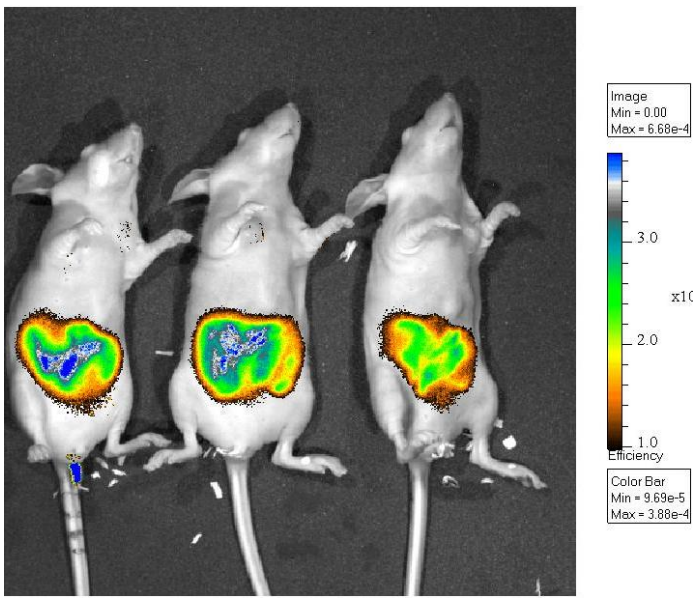
Spectral unmixing of activatable probes

4T1 cells

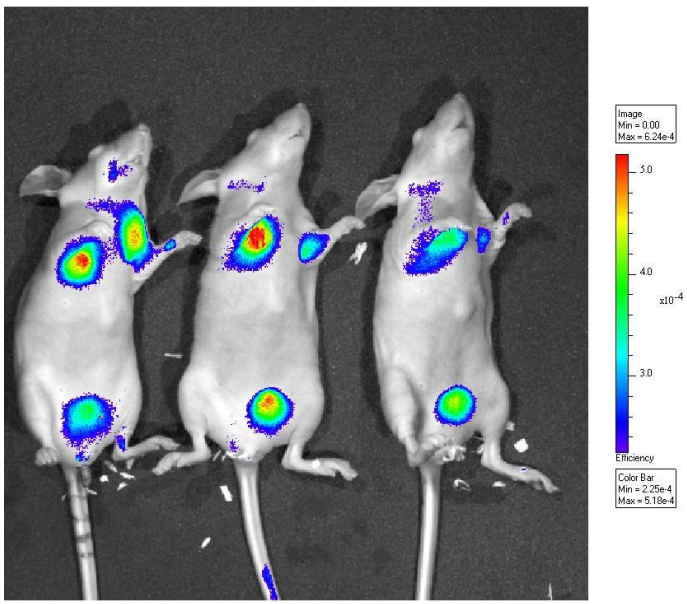
Raw Data



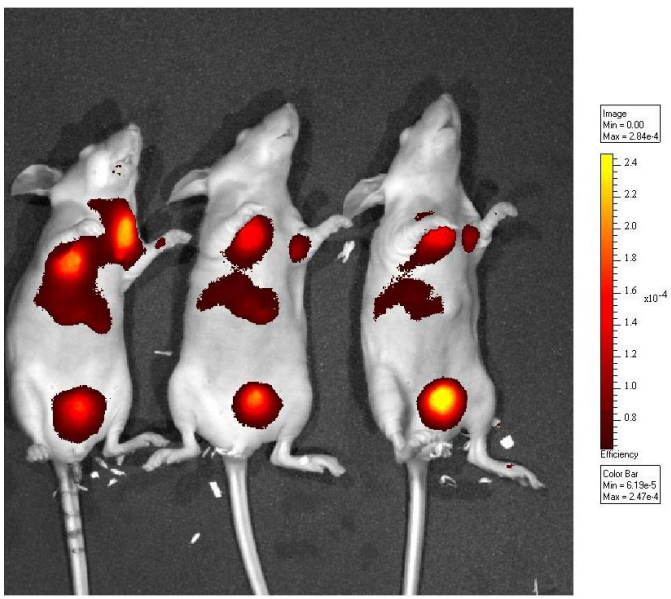
Food Background



Unmixed ProSense 680

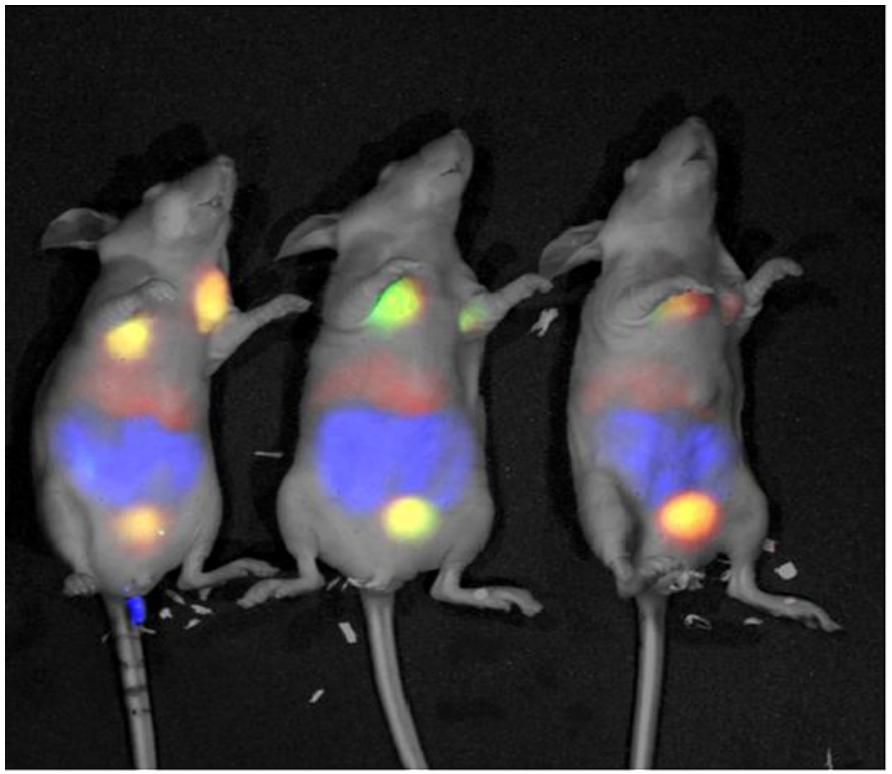


Unmixed MMPSense 750



Overlaid images

Spectral Unmixing



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

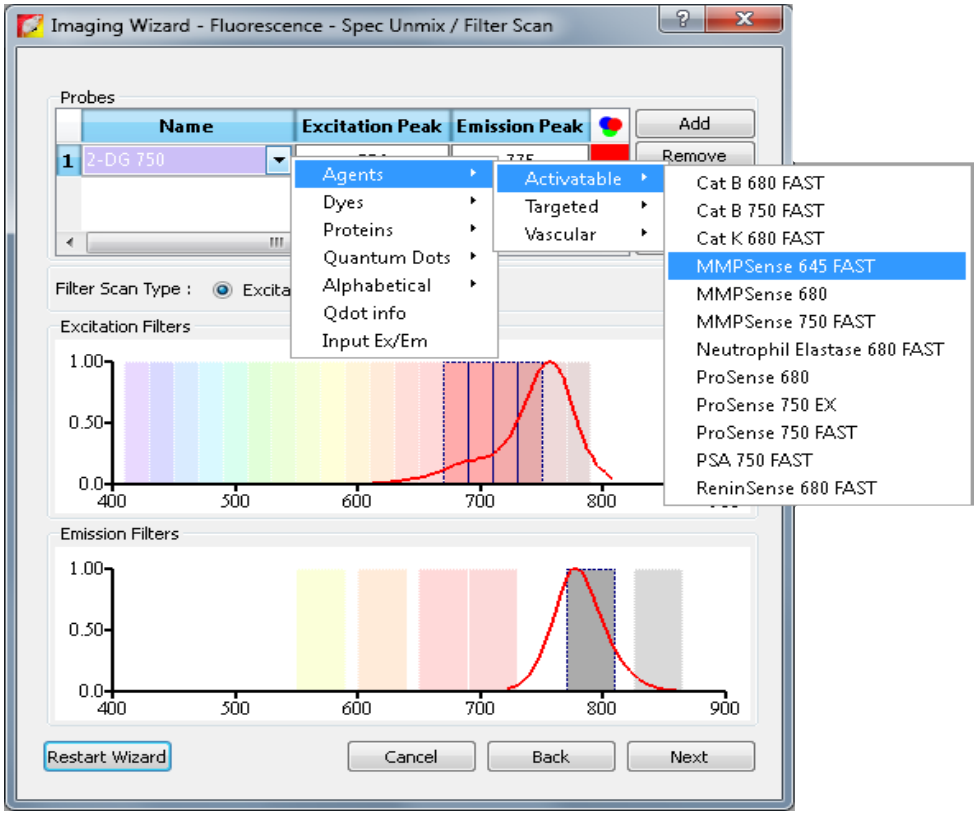
Green: ProSense680

Red: MMPSense750

Blue: Chlorophyll signal in Food

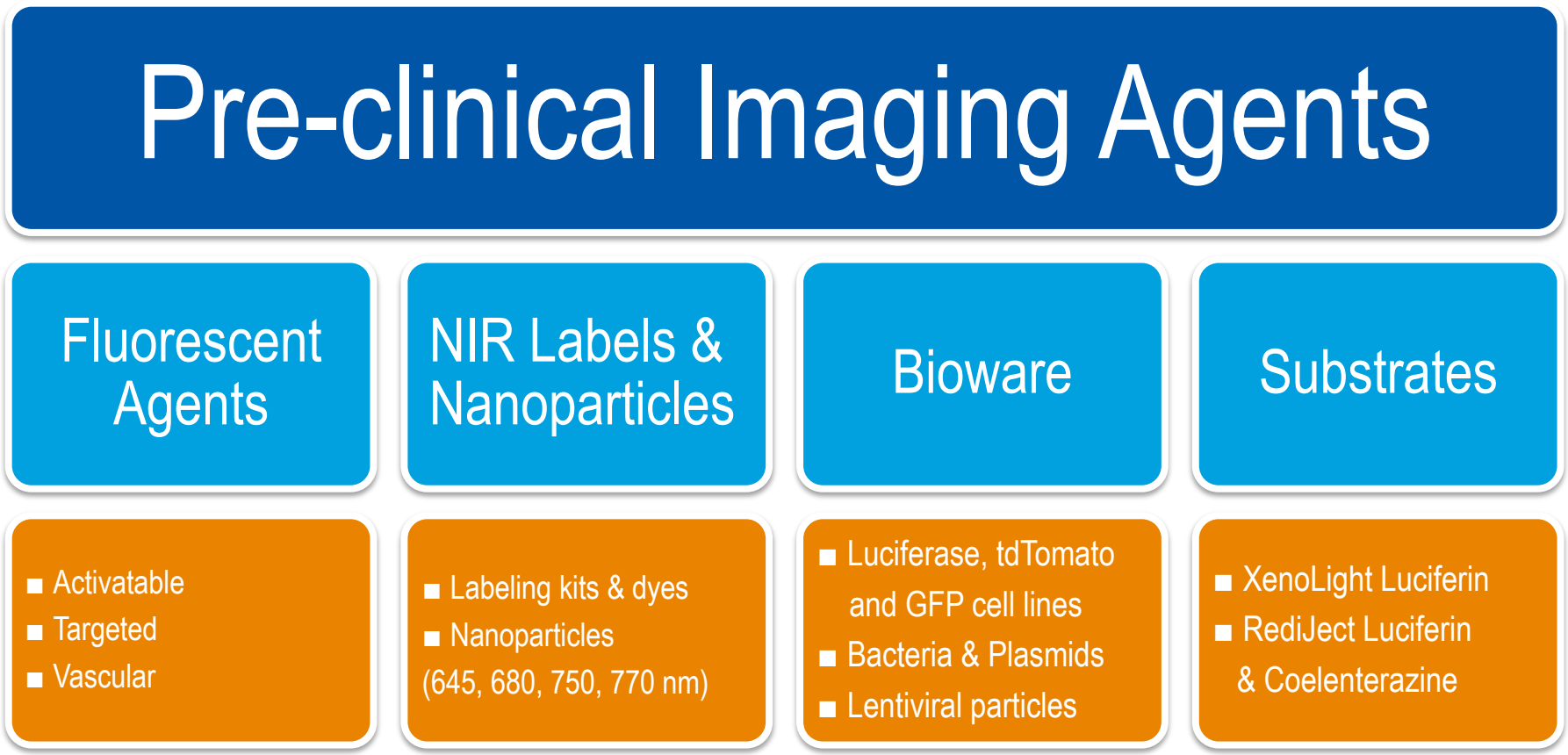
A complete probe library for easier use

- Incorporated 99 probes into Living Image software (only in LI4.3.1 SP2)
  - 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



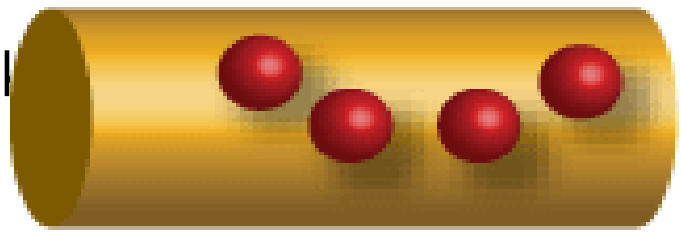
In Vivo Imaging Agent Platforms:

Agent Categories

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

Vascular Agents

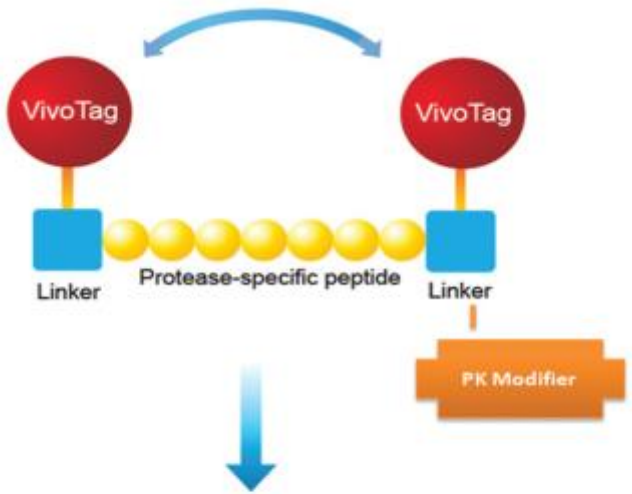
- Vascular agents circulate with the blood, but have no target selectivity
- Vascular agents will accumulate in areas of vascular leakage associated with tumorigenesis and inflammation
- Used to image vascular disease processes in oncology, inflammation, pulmonary disease and arthritis
- Superhance is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

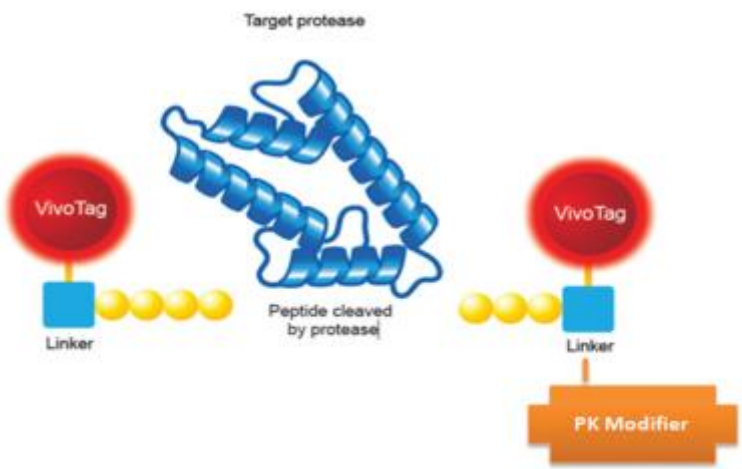


Monitor the integrity of the vascular system

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

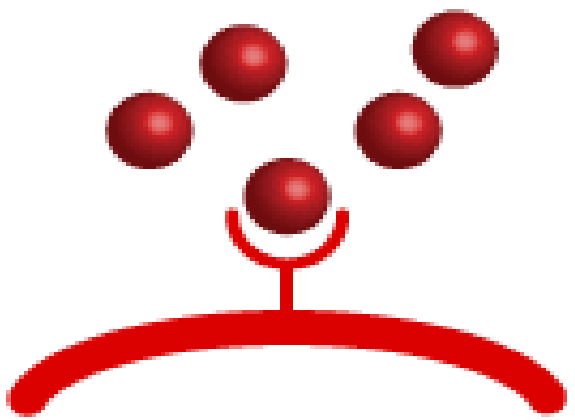




Monitor protease activity associated with disease state

Targeted Agents

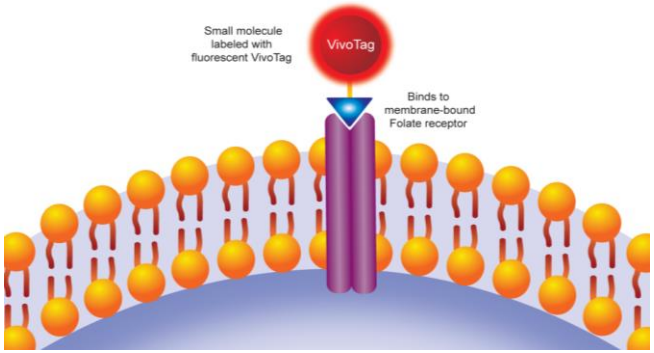
- Optimized agents that actively target and bind to specific biomarkers
  - Designed for *in vivo* use
  - *Emerging In vitro applications*

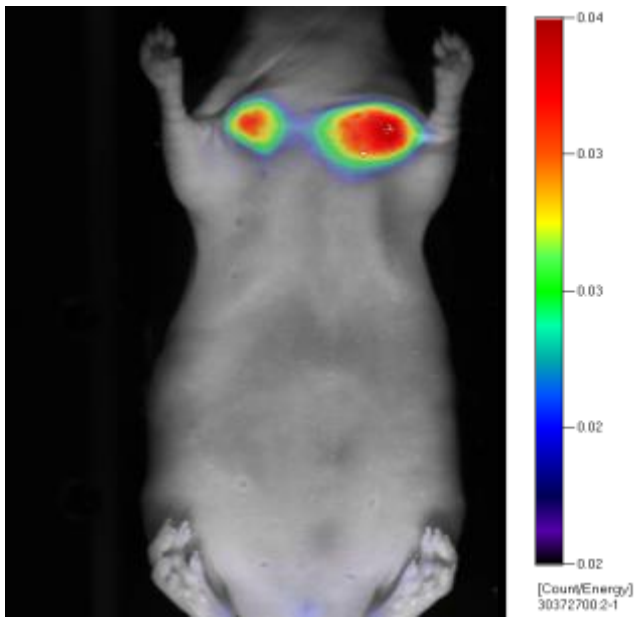


Target specific biomarkers

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:

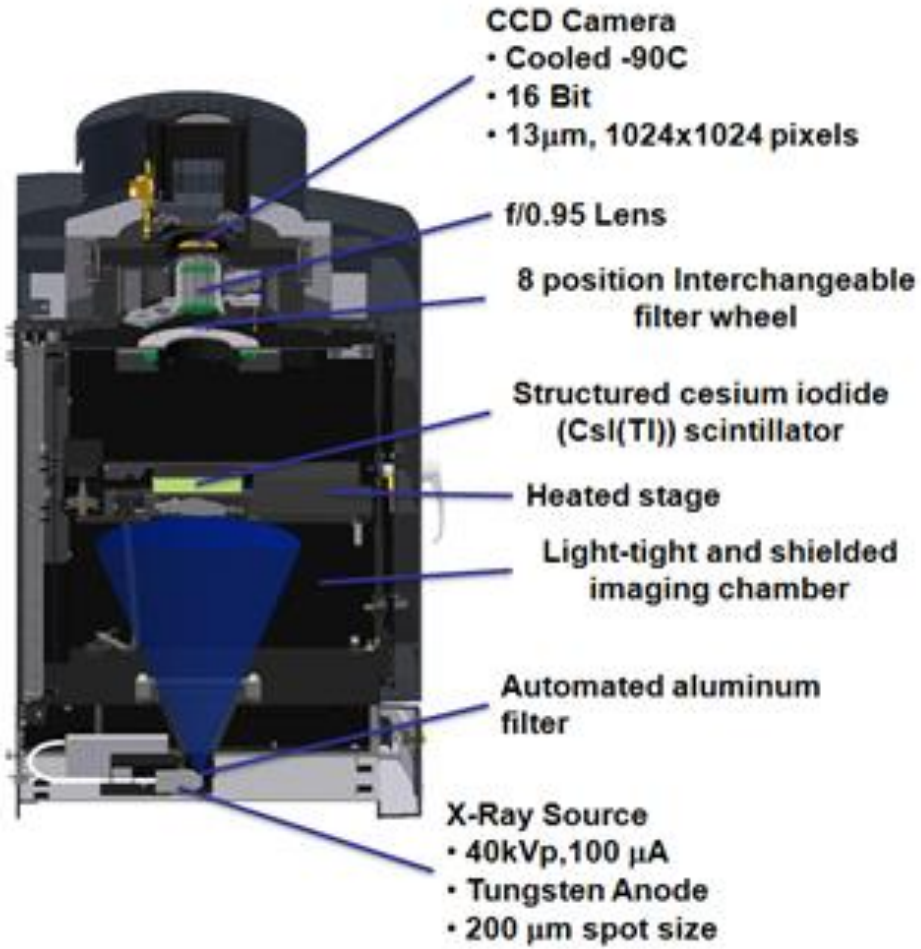
LABELS and NANOPARTICLES

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.

The Integrated X-Ray Imaging Capability

X-Ray Imaging Configuration



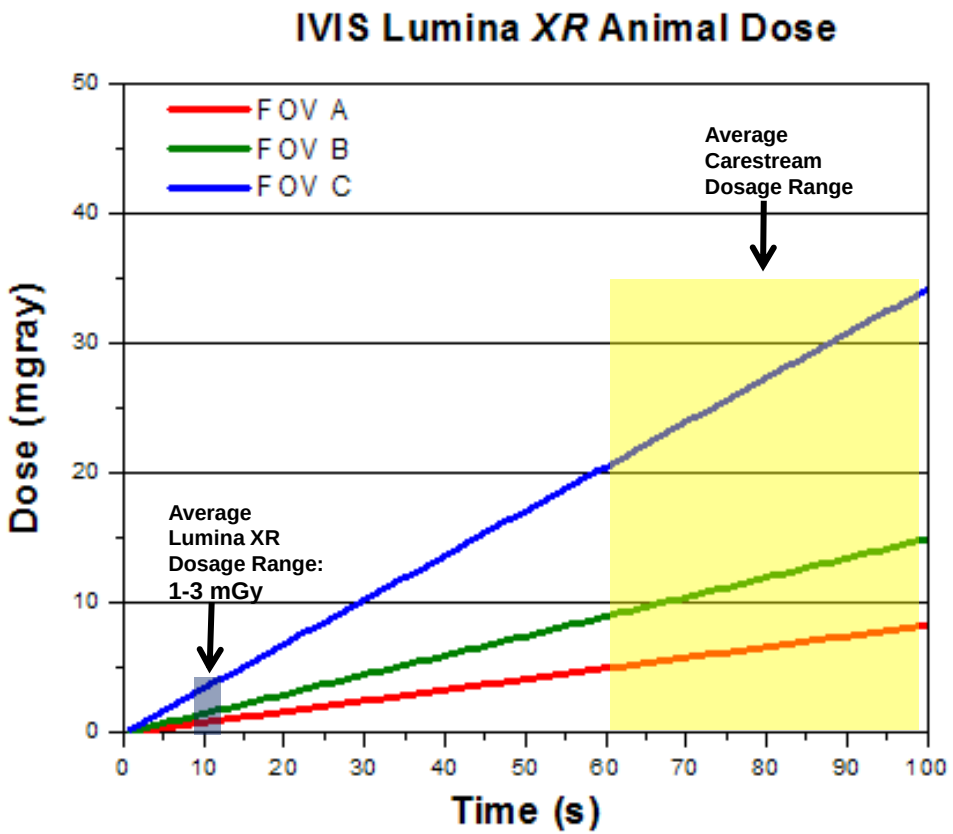
Typical X-Ray Imaging Settings:
Acquisition Time: 10 sec
Voltage: 35kV
Current: 100µA

Additional Information

- X-ray field of view (FOV) range:10cm, 7.5cm, 5cm
- Aluminum filter cutoff 13 KeV
- Low dose, typical X-ray imaging time 10 seconds

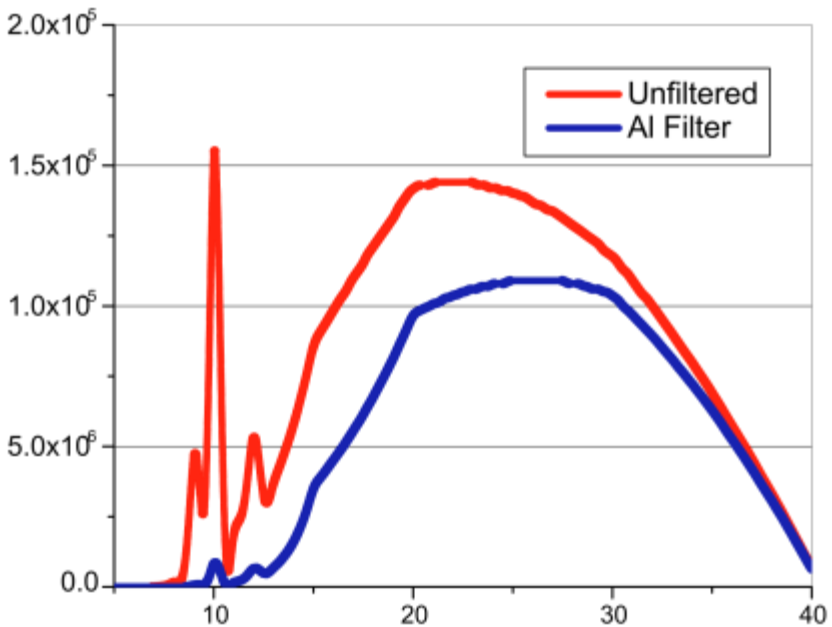
Low Radiation Dosage

- IVIS Lumina XR's high sensitivity camera enables fast X-ray acquisition times, minimizing radiation dosage to the animal.



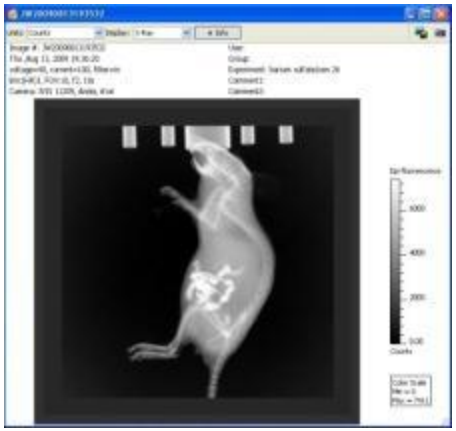
Filters Reduce Exposure and Noise

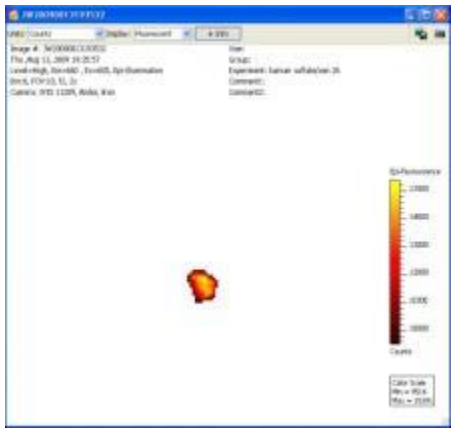
0.4 mm Aluminum filter cuts out low energy x-rays and reduces exposure in animals

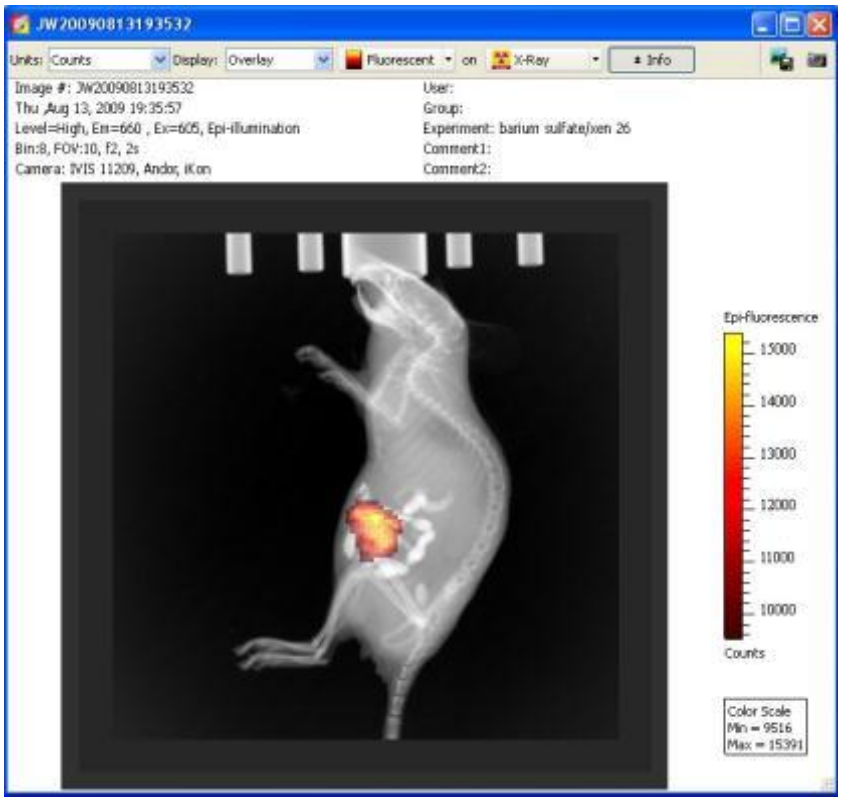


Optimized for mice
- 40 kV, 100 uA
- Dose < 5 mGy

Automatic Overlay of Optical and X-Ray Image

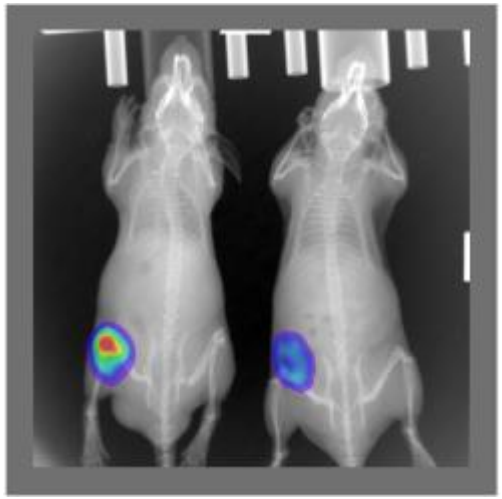




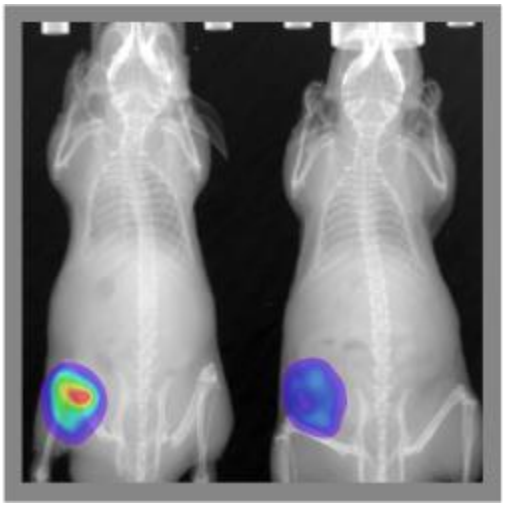


Imaging Bones and Skeletal Structure

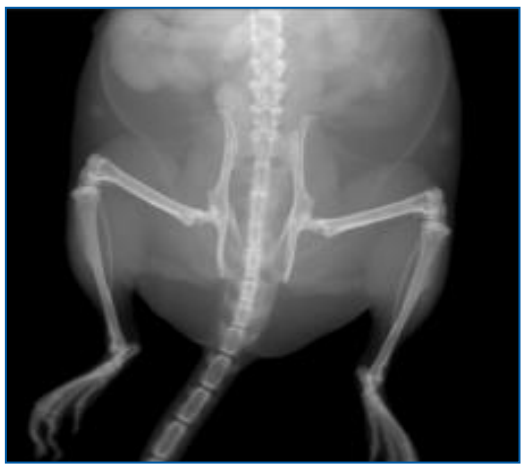
FOV C = 10 x 10 cm



FOV B = 7.5 x 7.5 cm

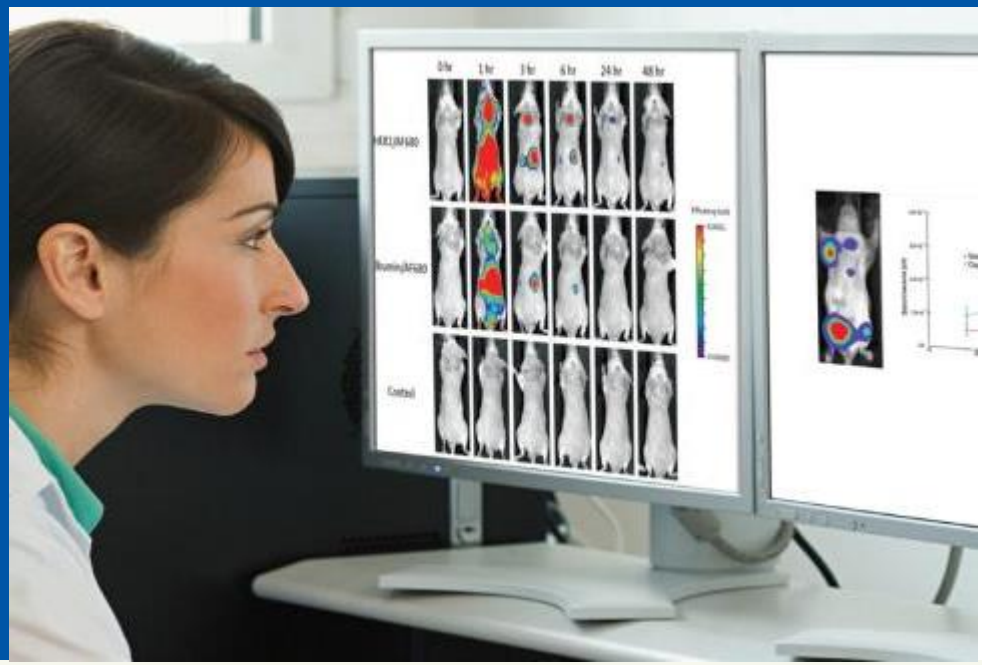


FOV A = 5 x 5 cm



ZOOM = 2.4 x 2.4 cm

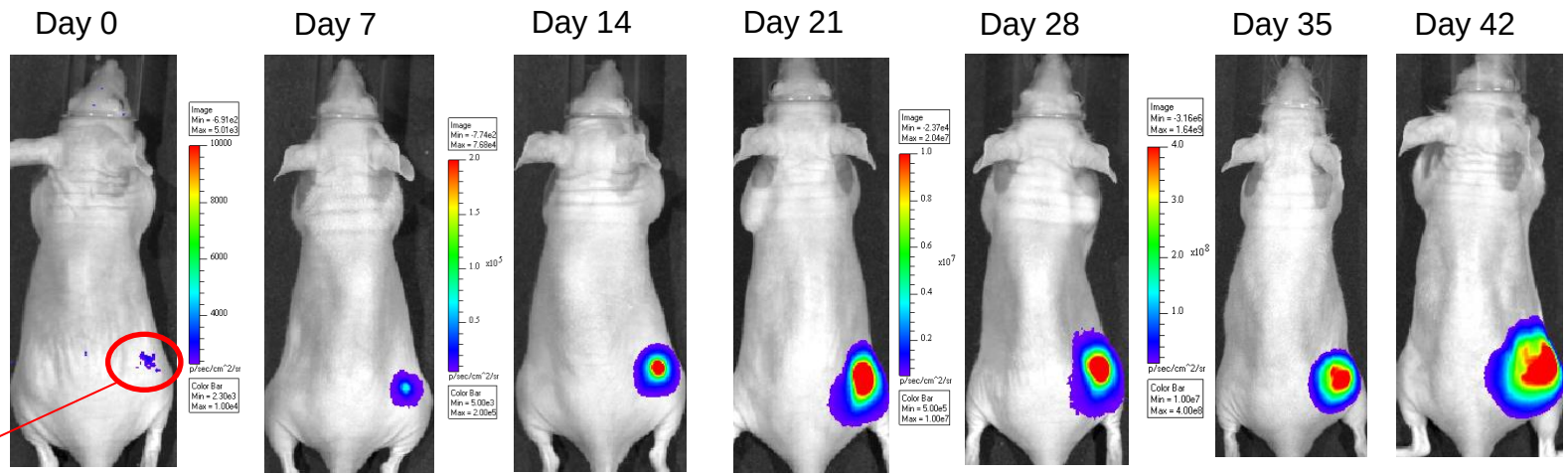
PerkinElmer
For the Better

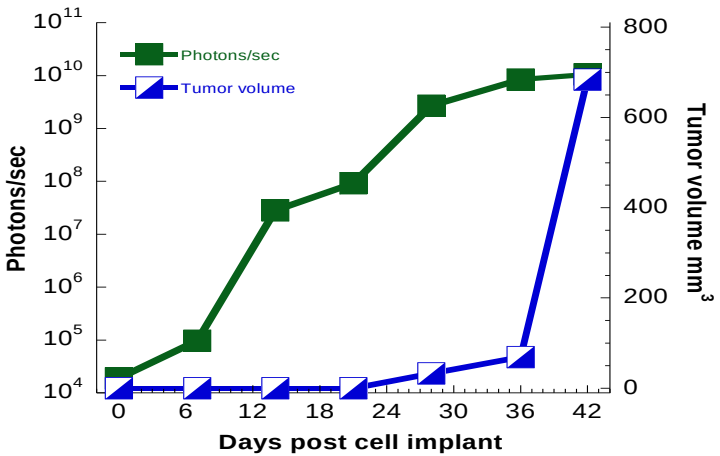


PerkinElmer's In Vivo Imaging Applications

Longitudinal monitoring of tumor development

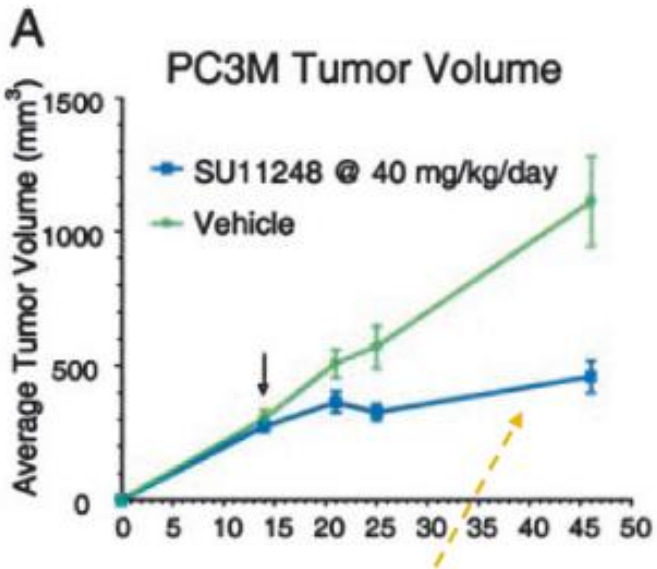
Bioware Ultra: 4T1-luc2



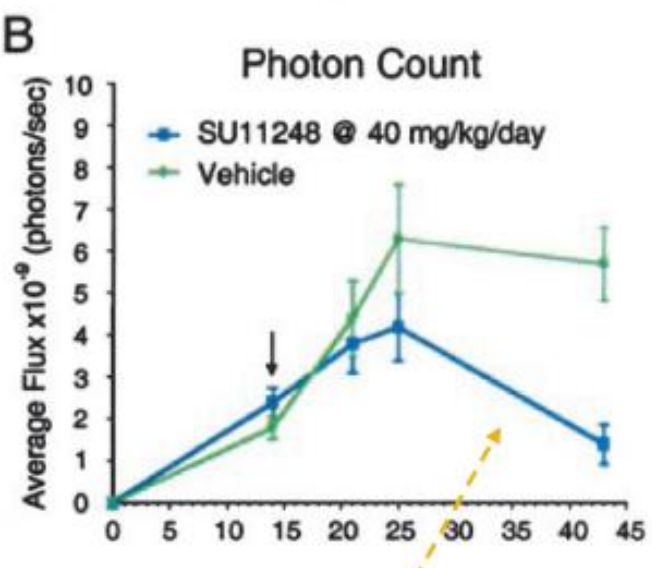


With Bioware Ultra one can start collecting data from Day 0, while with caliper measurements one has to wait at least 28 days to see any tumor growth

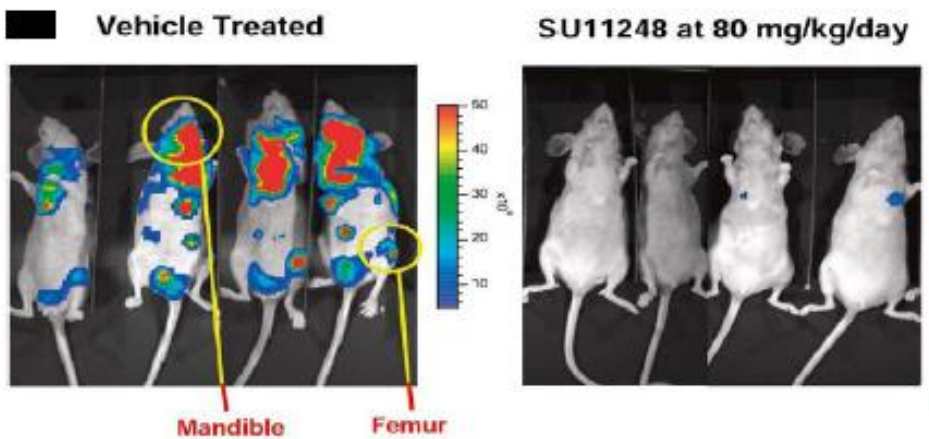
Sutent – Fast Tracked FDA Approval



Physical measurement
(tumor still getting bigger)

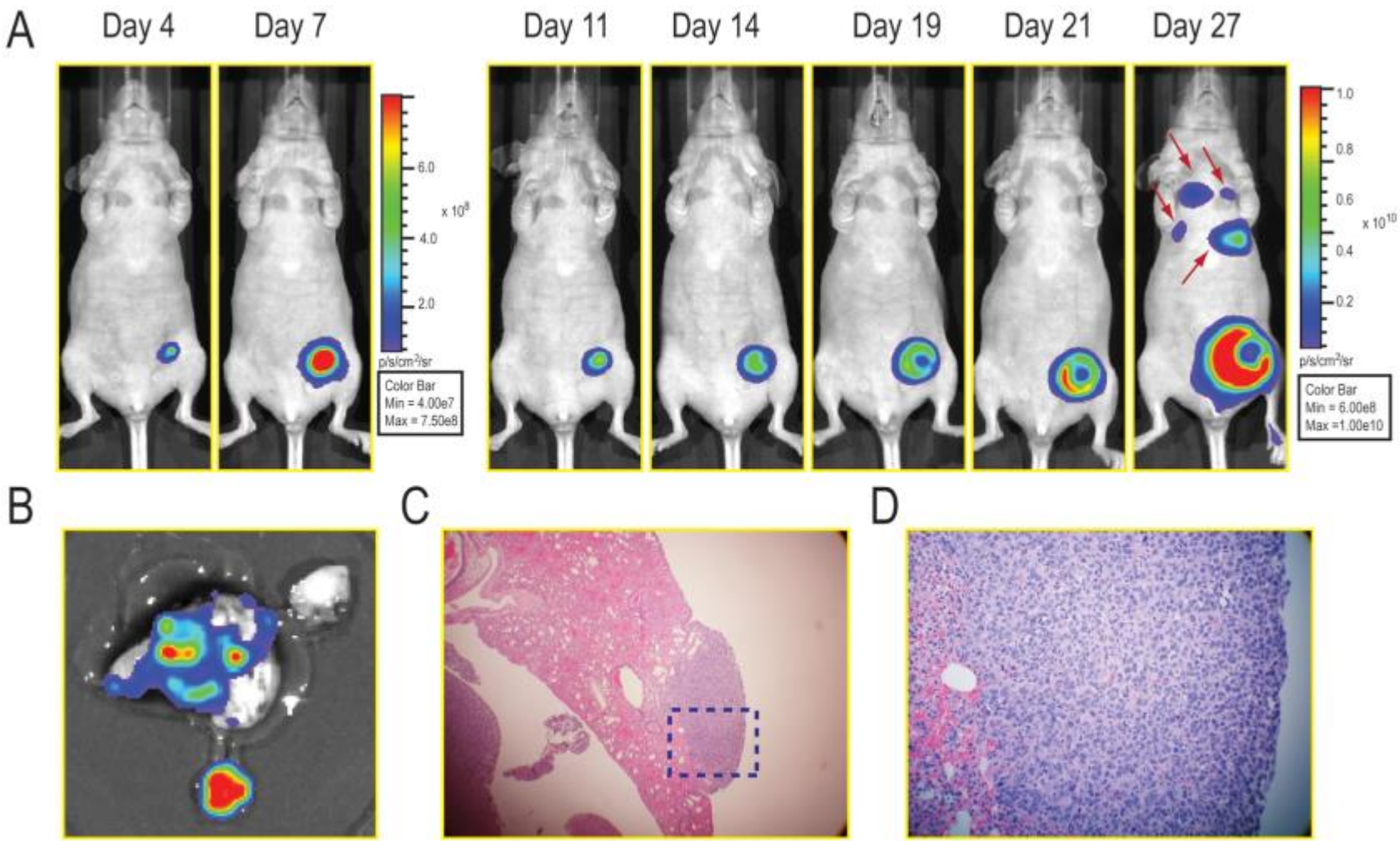


Biophotonic imaging
(tumor cells being killed)



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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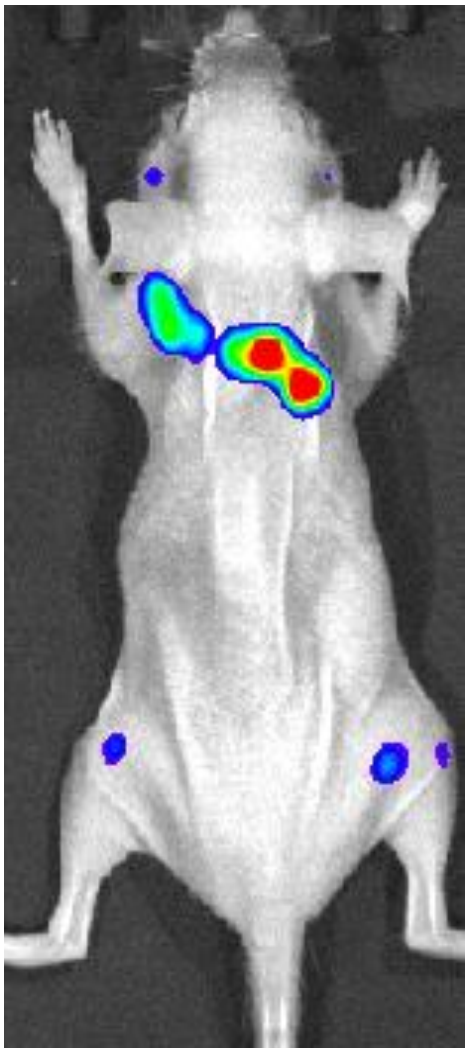
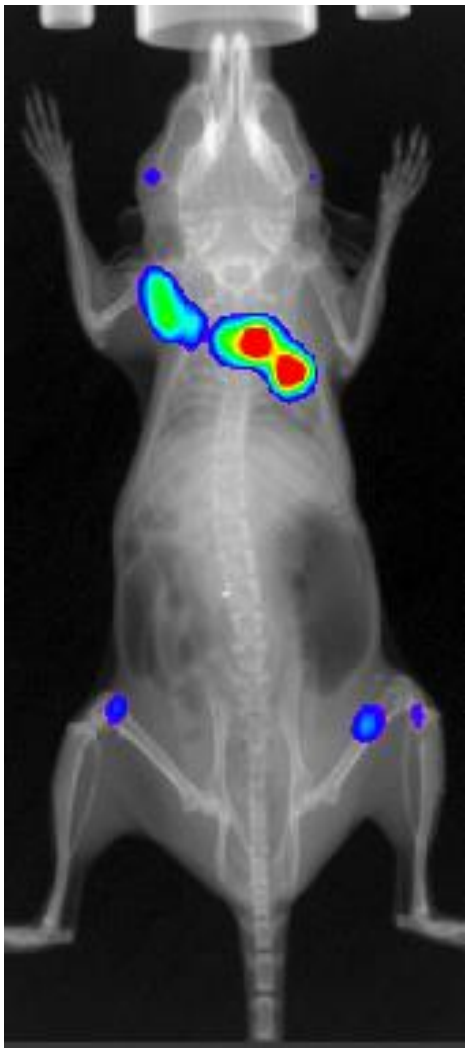
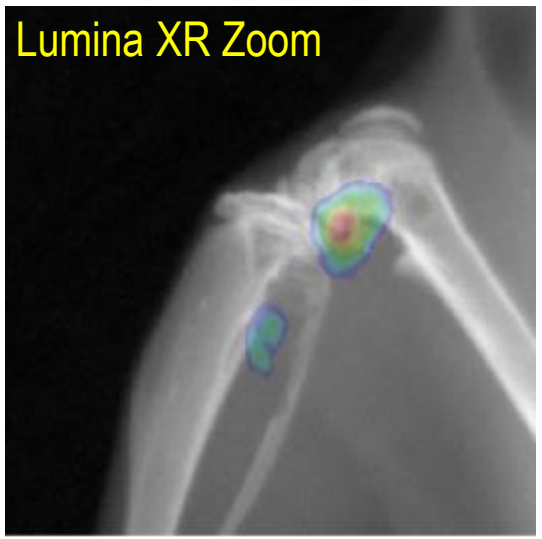
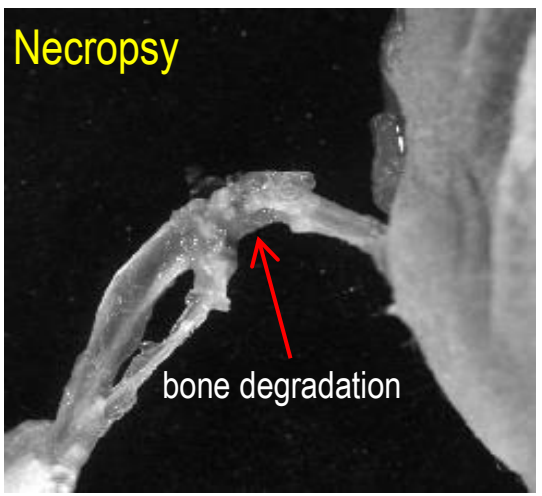
Imaging Cancer Metastases (validation with X-Ray)

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

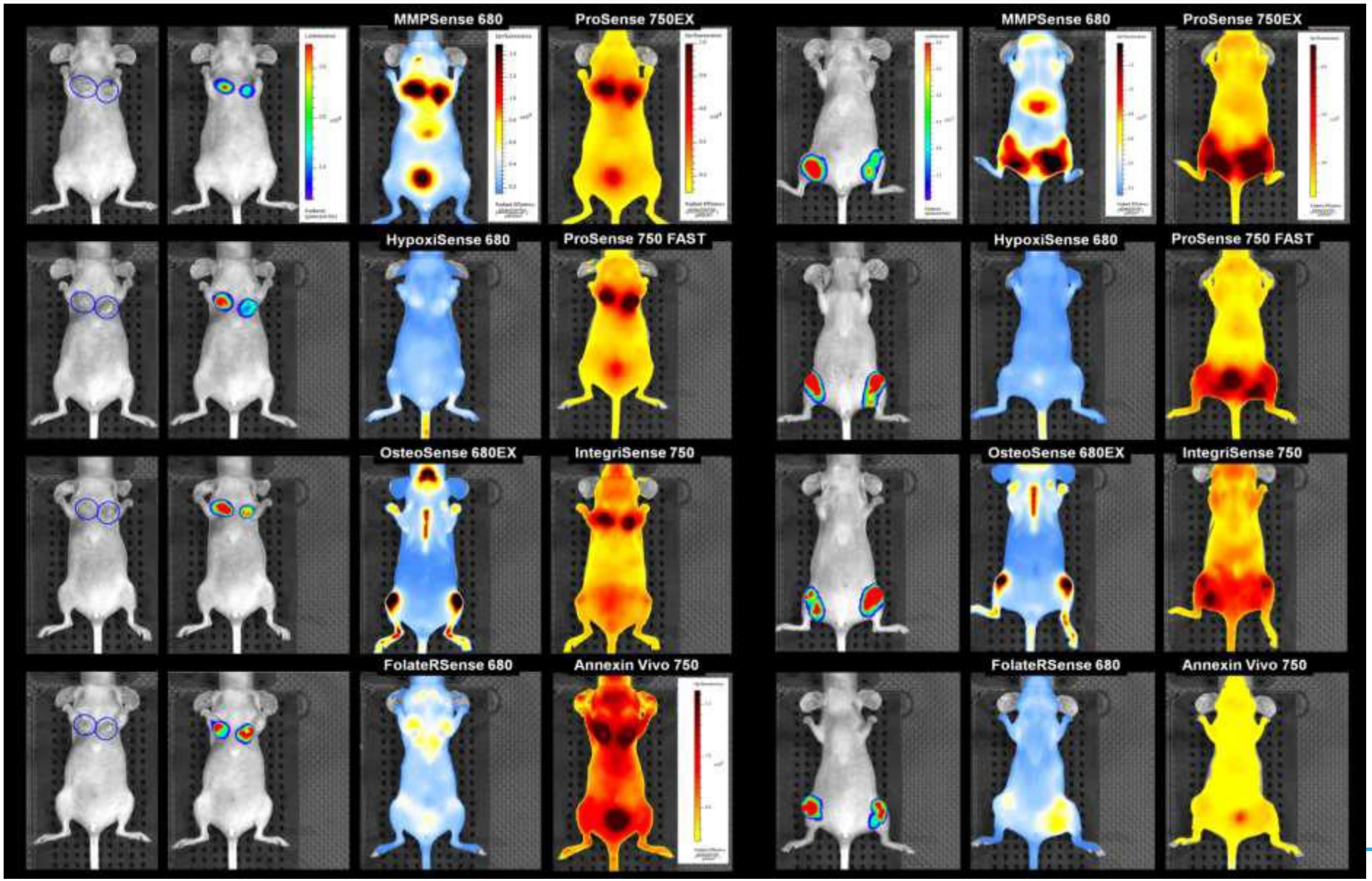








4T1-luc Tumors: Orthotopic vs Bone Metastases Profiling by IVIS



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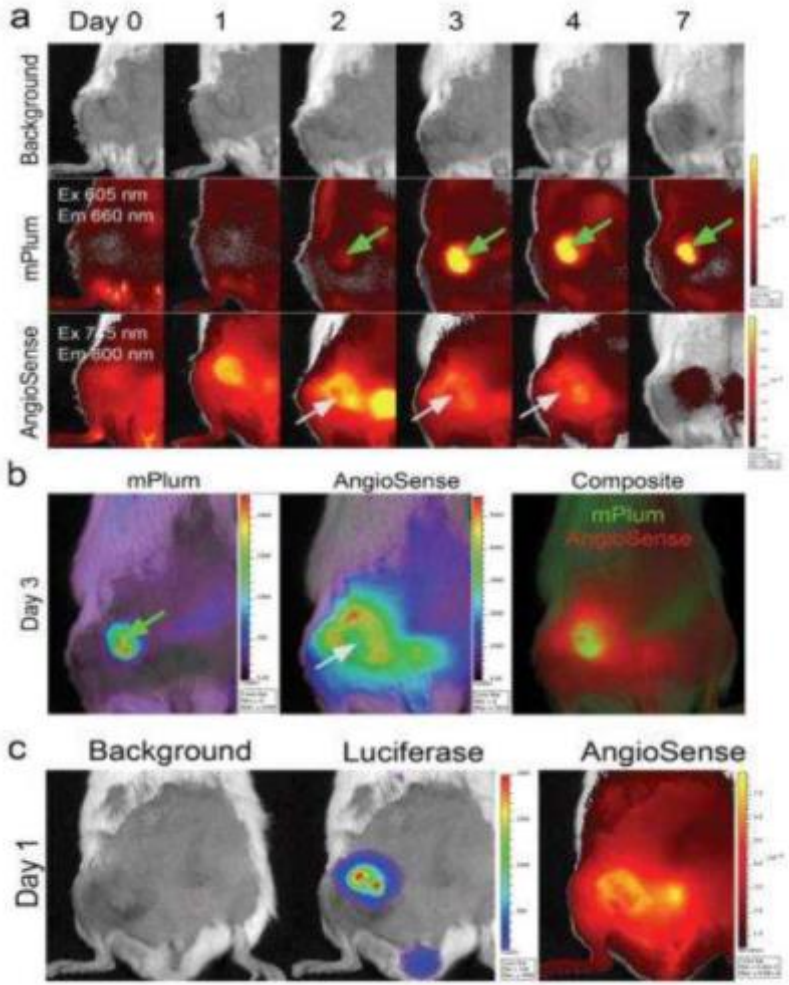
Near-infrared (NIR) fluorescent imaging of tumor vessel leakiness *in vivo*



(**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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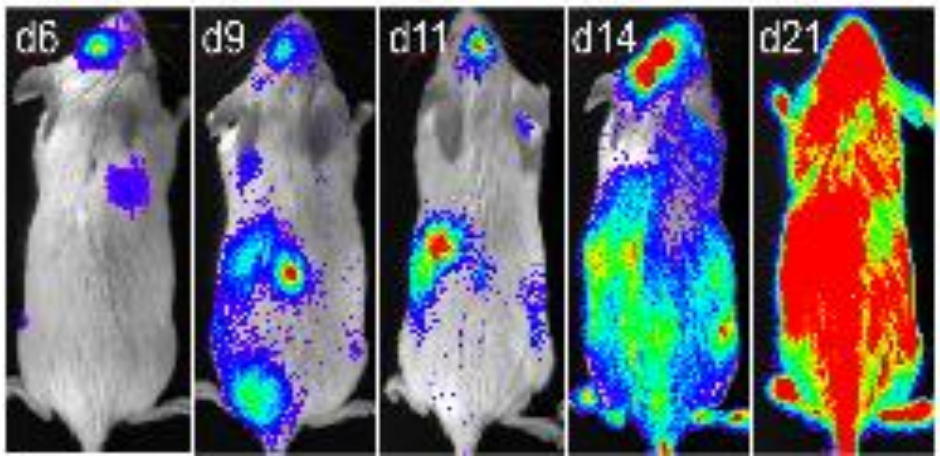
Stem cell application

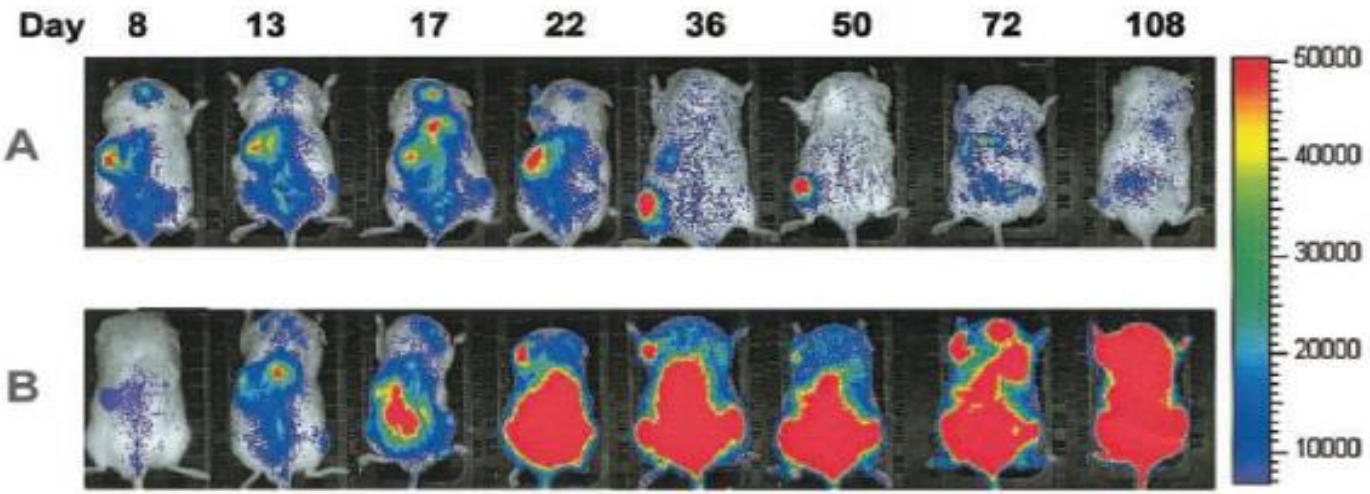
Cell Transplantation and Trafficking Patterns

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

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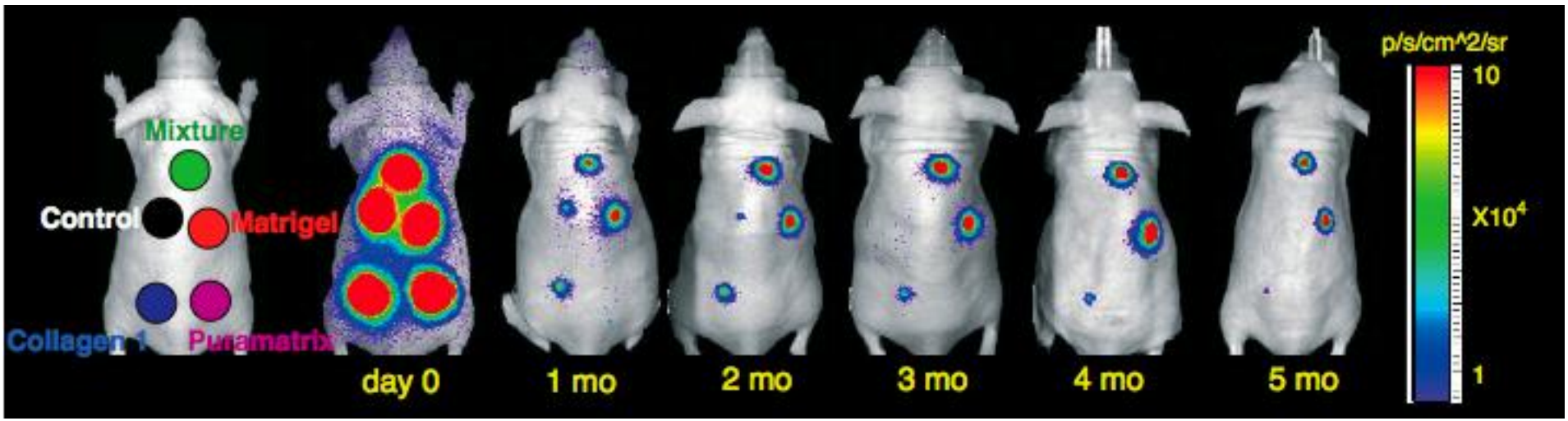
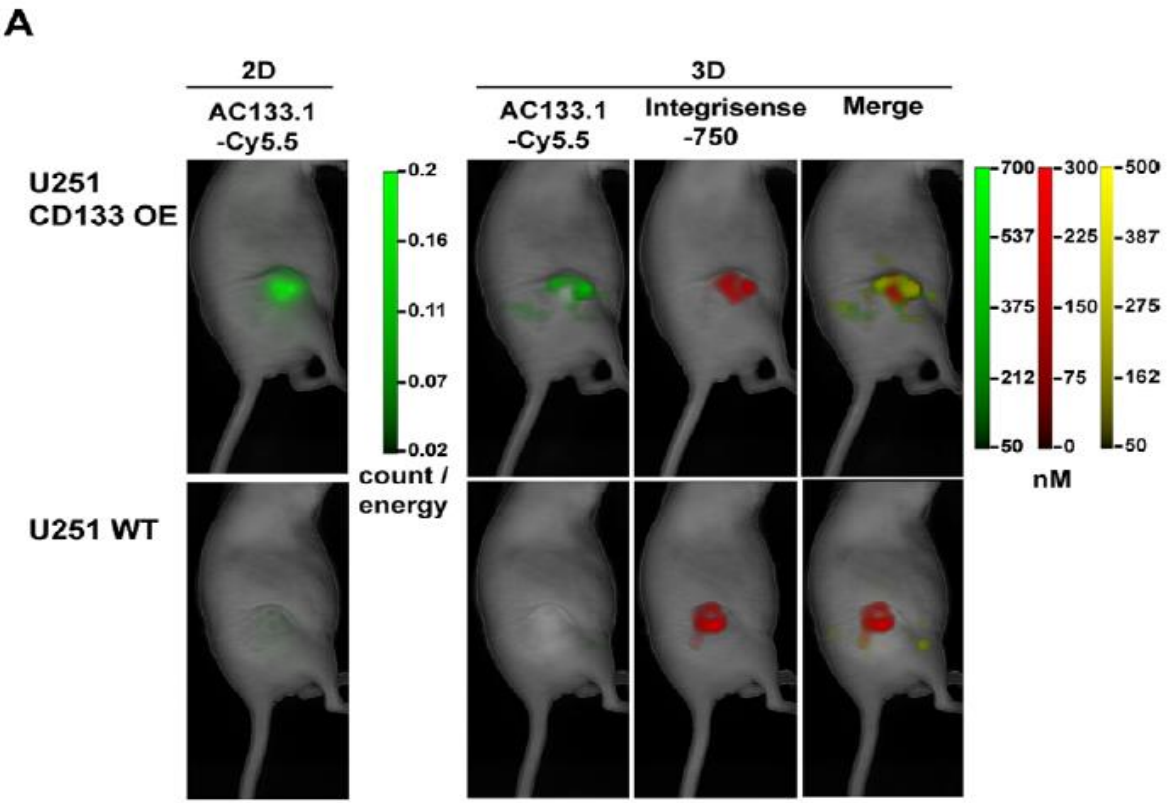
Effect of Biomatrices on Stem Cell Viability

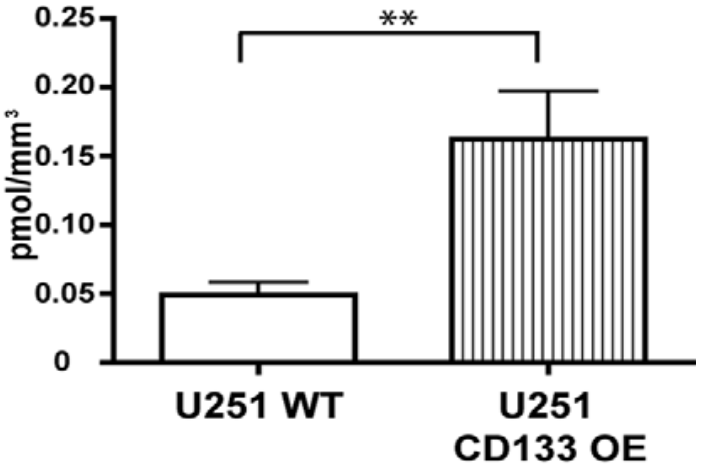
Cao *et al*, J Tissue Eng Reg Med, 2007

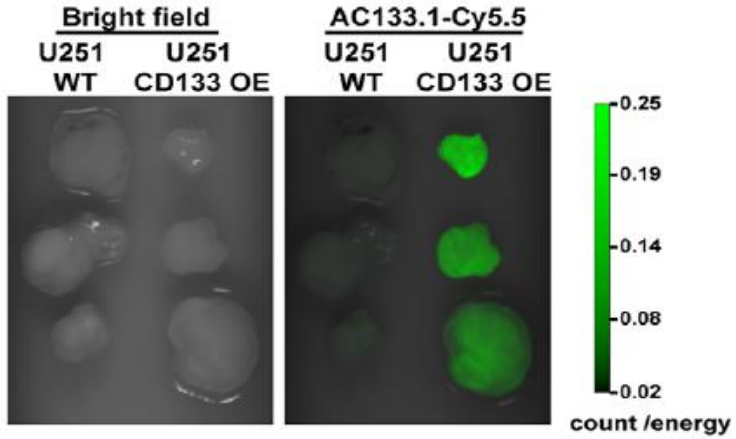


Non-Invasive In Vivo Imaging of Tumor-Associated CD133/Prominin.



In vivo imaging of U251 xenografts in mice injected with Cy5.5-labeled AC133.1 antibody.





Tsurumi C, Esser N, Firat E, Gaedicke S, Follo M, et al. (2010) PLoS ONE 5(12):e15605. doi:10.1371/journal.pone.0015605

Pulmonary Disease applications

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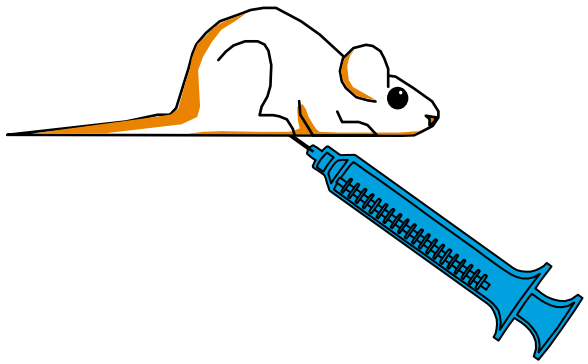
Ovalbumin (OVA)-induced Asthma

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)

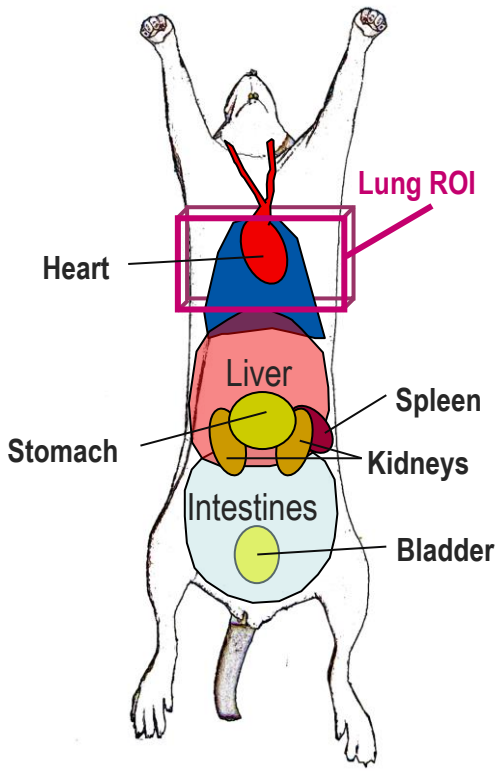
5nmol ProSense or MMPSense

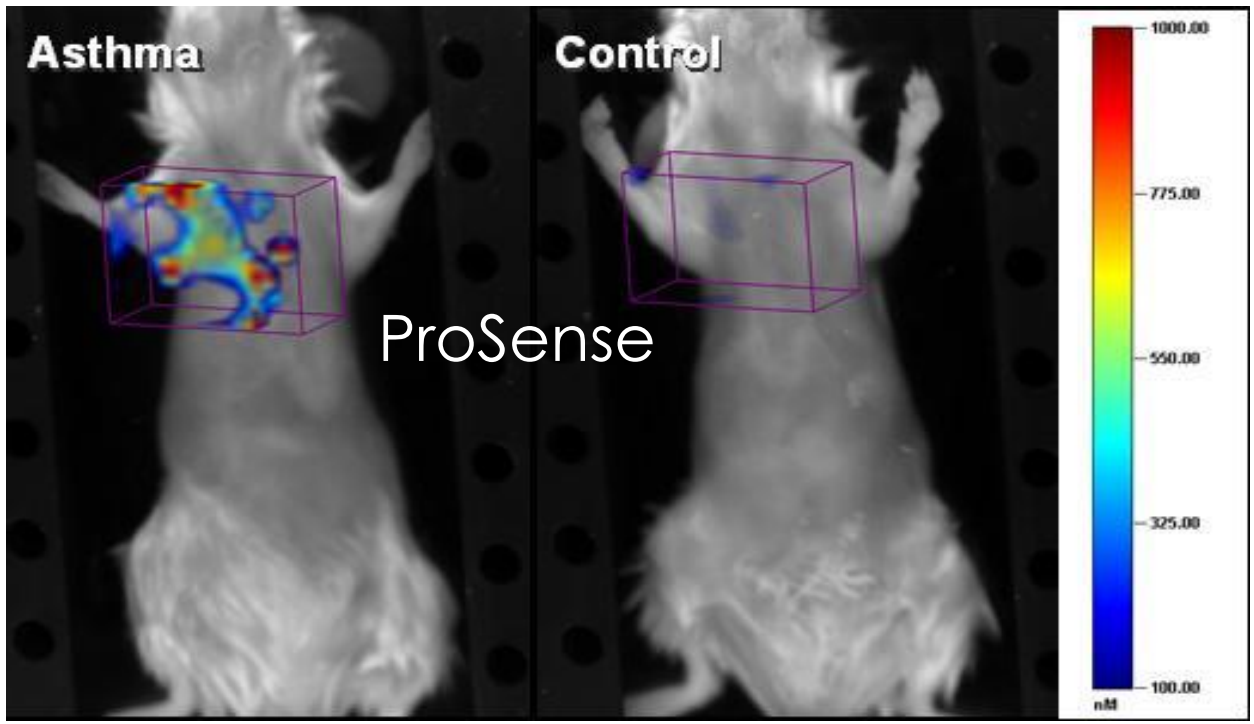
We adopted a standard model of murine asthma used in multiple research labs

BAL = Bronchoalveolar Lavage

Imaging Ovalbumin (OVA)-induced Asthma

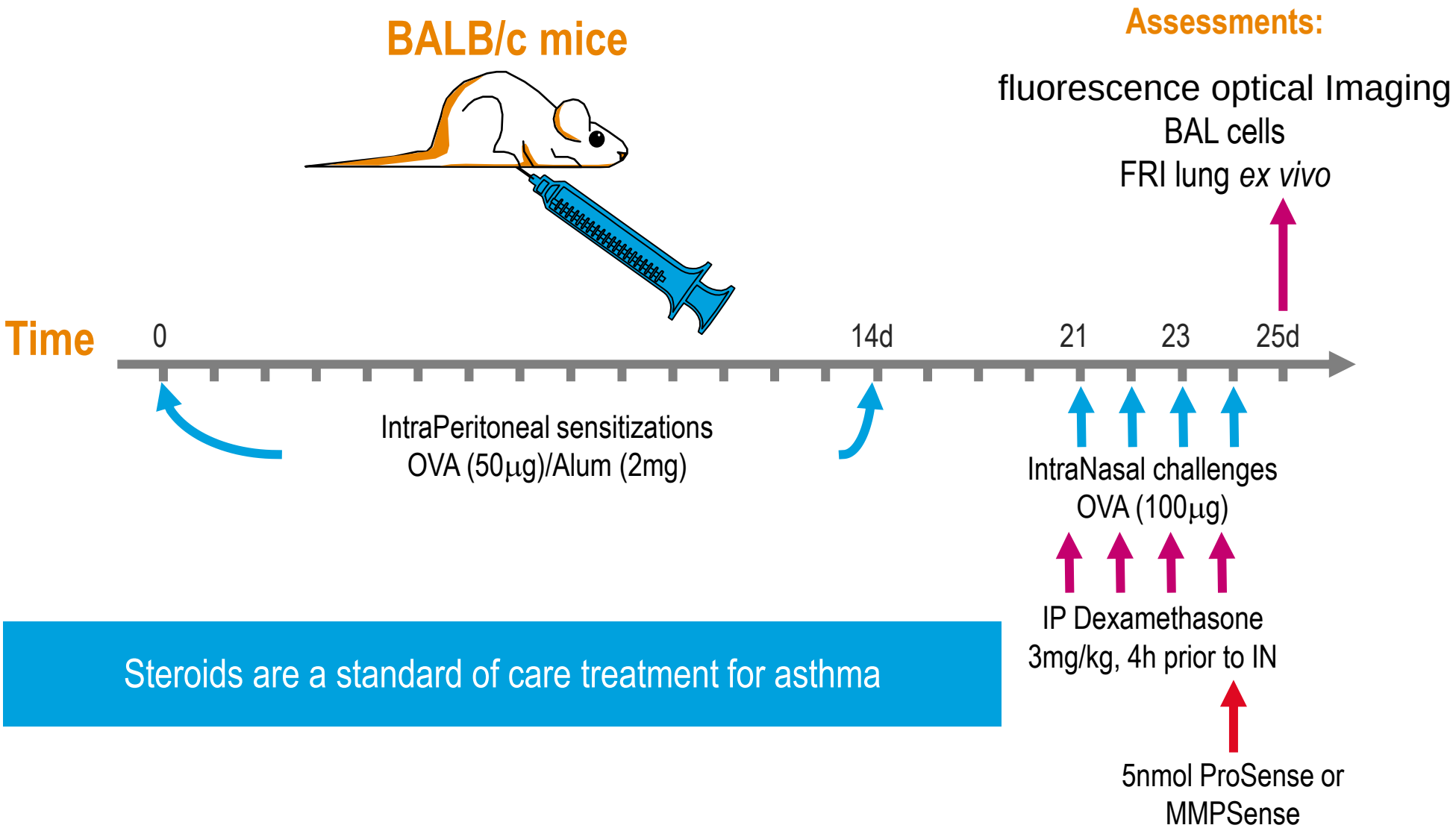
In vivo Fluorescence Imaging





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

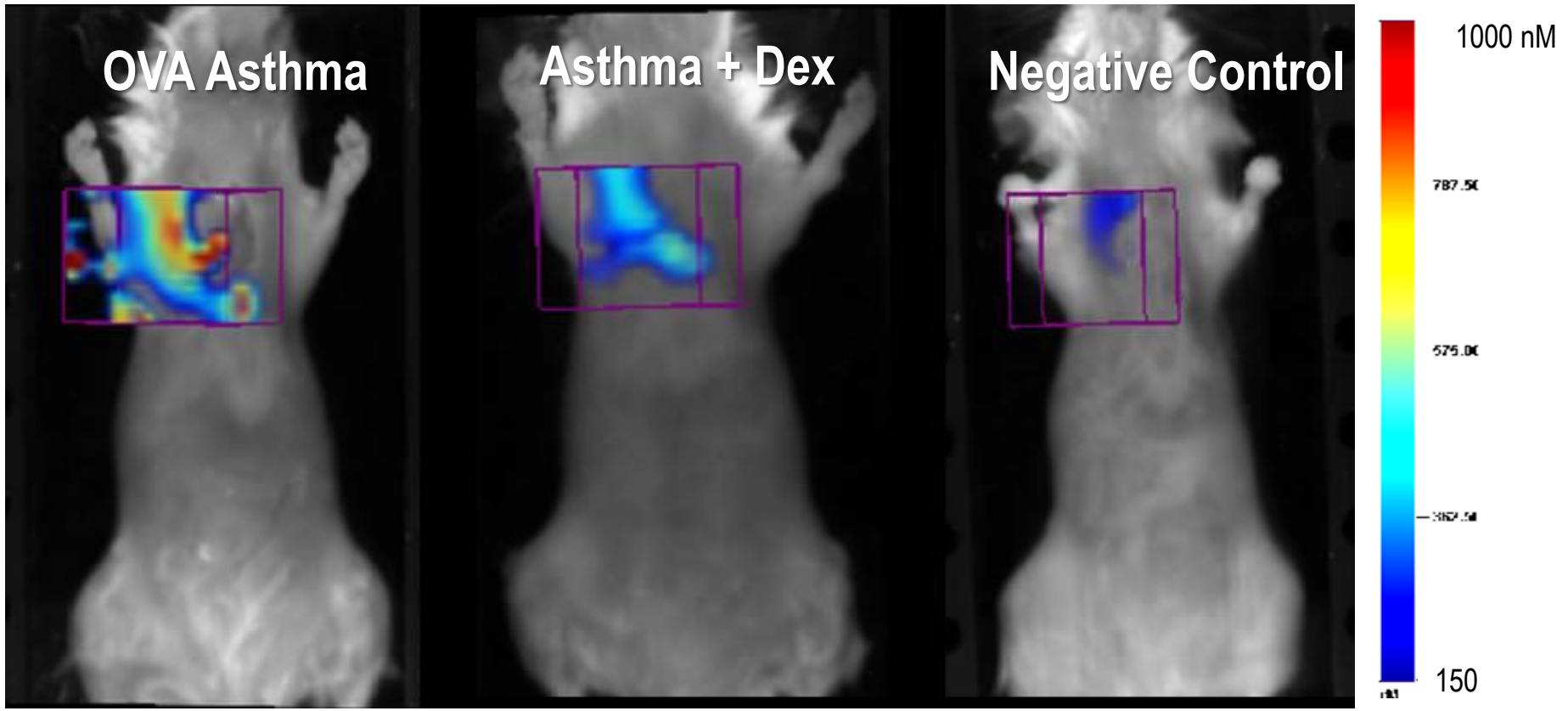
Steroid inhibition of OVA-induced Asthma



Steroids are a standard of care treatment for asthma

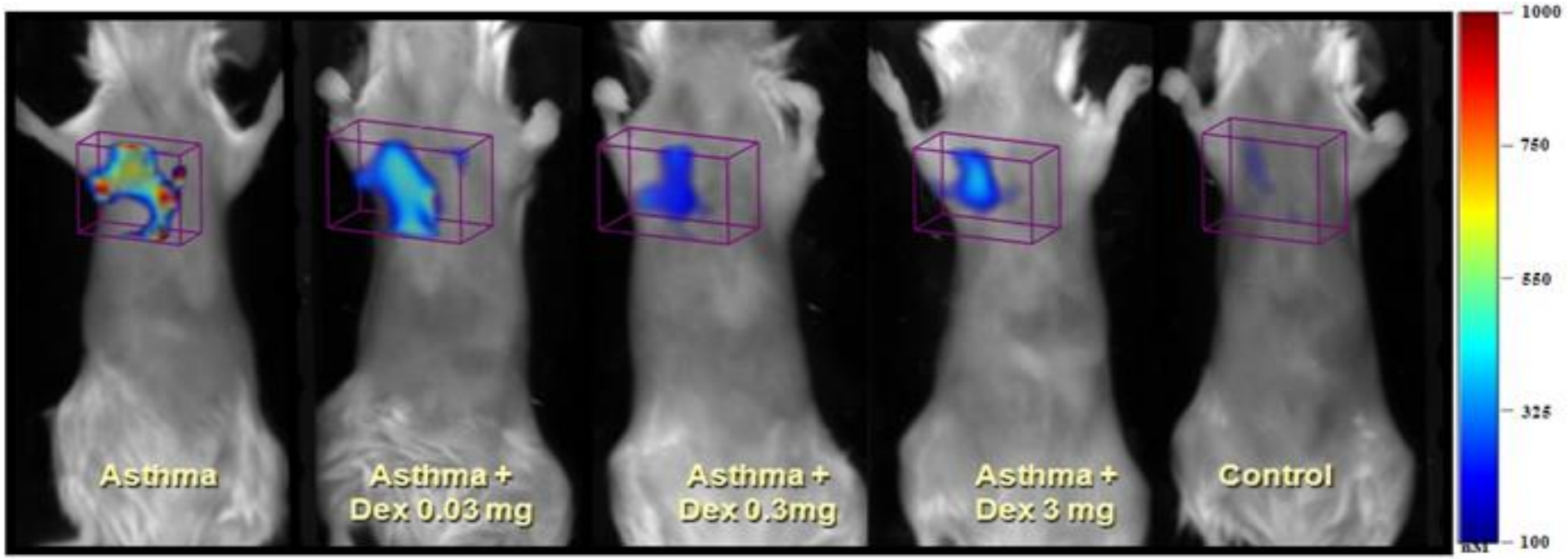
BAL = Bronchoalveolar Lavage

Dexamethasone Therapeutic Activity in Asthma



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

Dexamethasone Dose Response in Asthma



Arthritis applications

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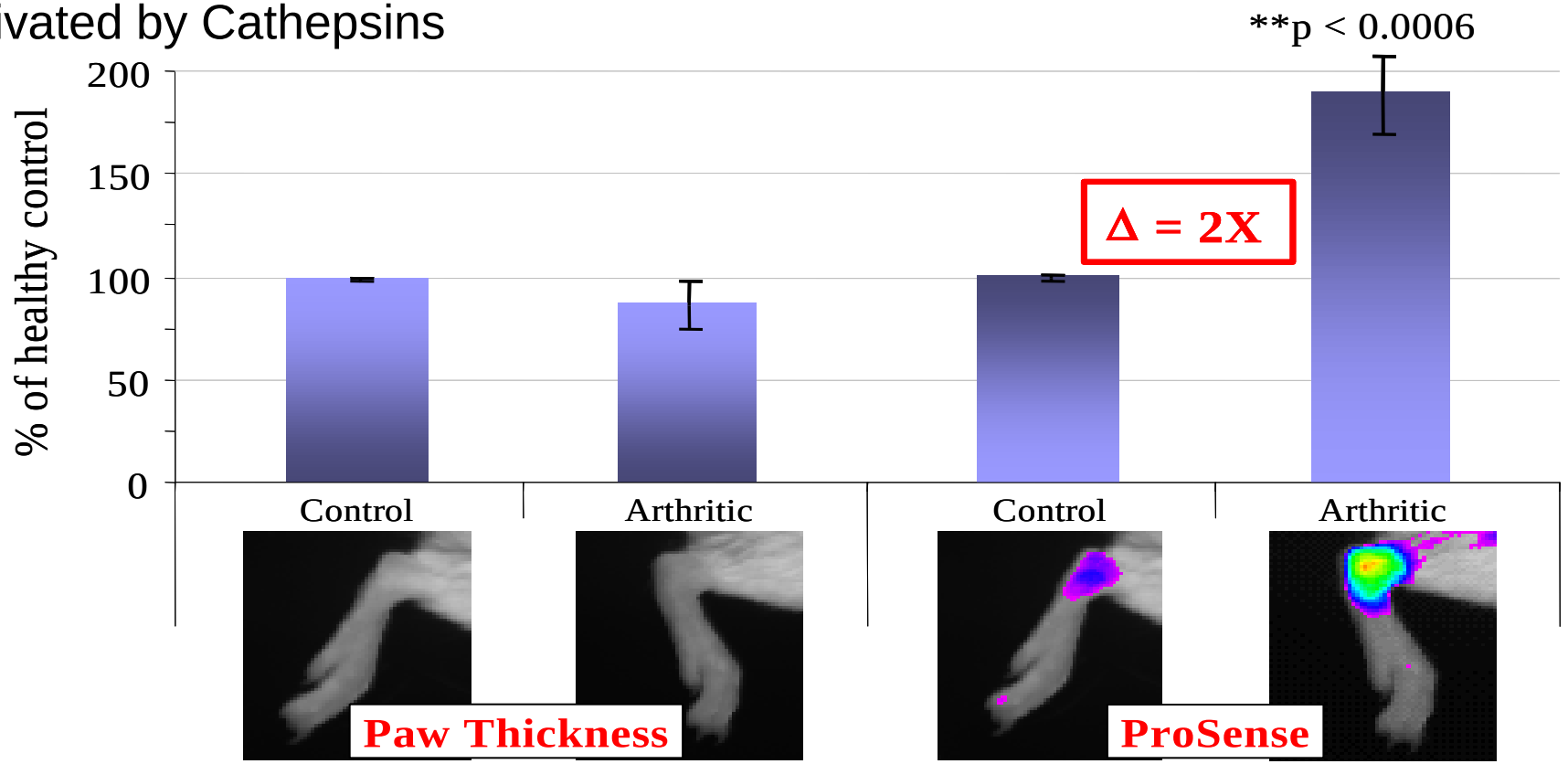
CAIA Model : ProSense & Early Disease (Day 4)

- 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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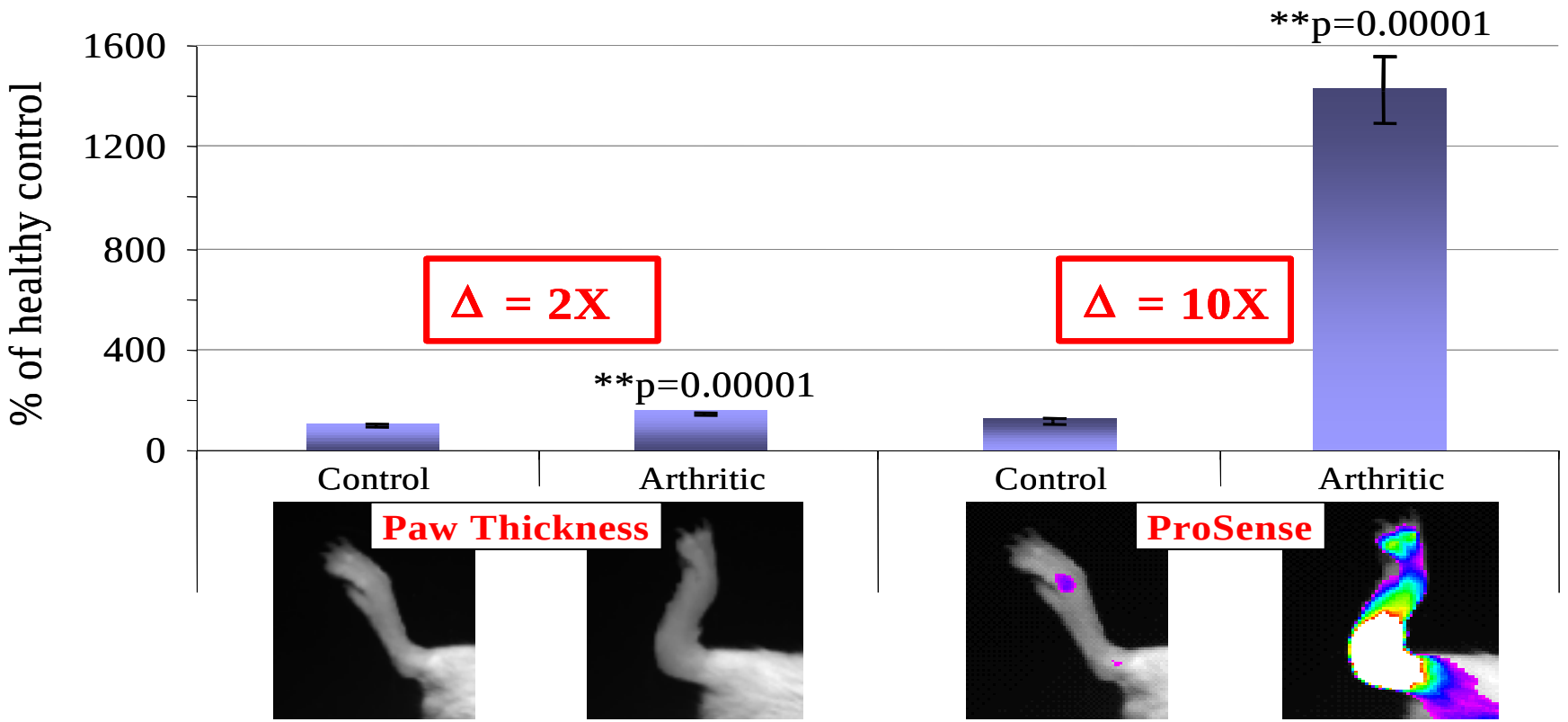
CAIA Model : ProSense & Late Disease (Day 8)

- 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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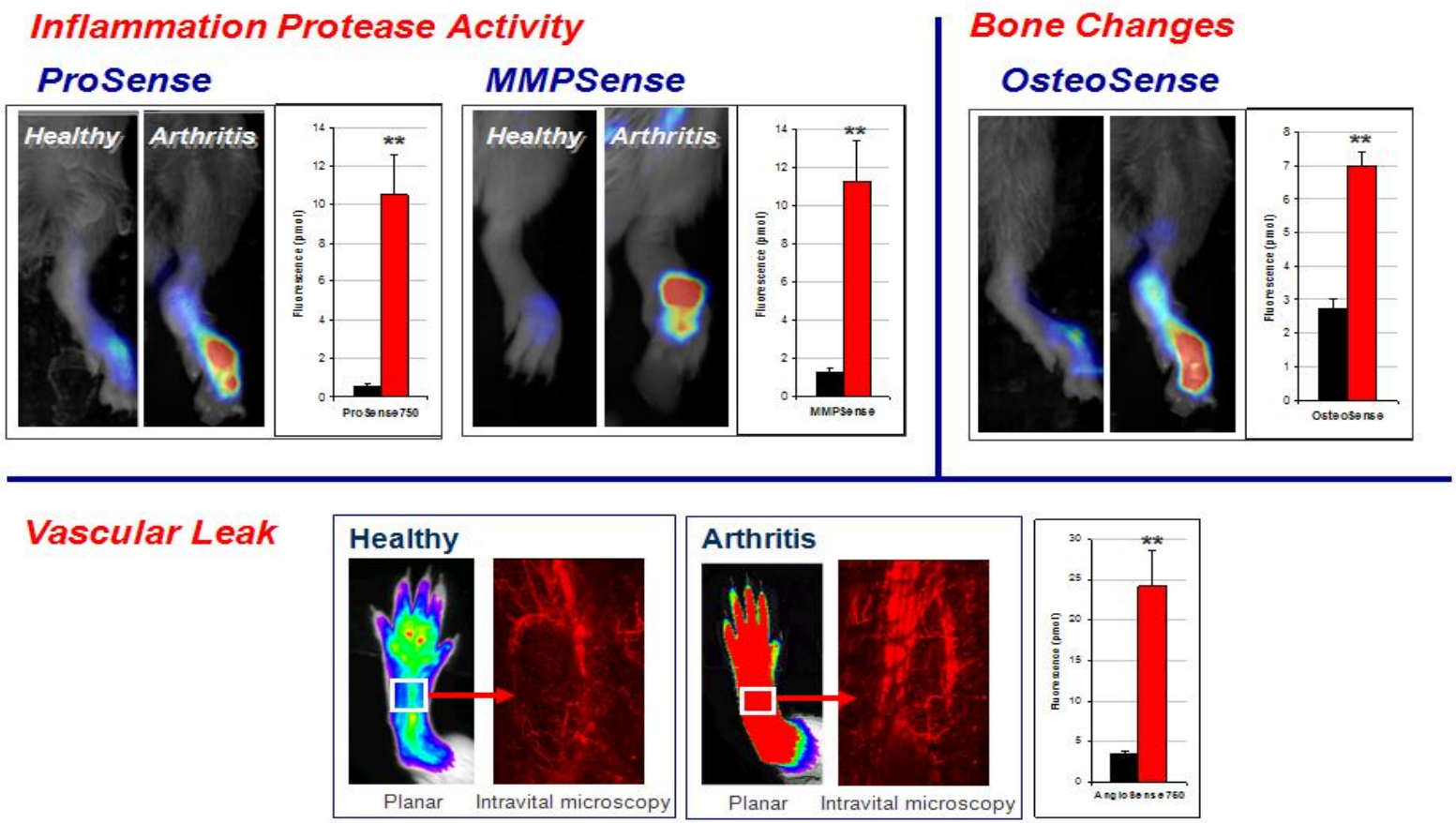
Targeted Agents – Inflammation: Multiplex Arthritis

Multiplex fluorescence Imaging



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