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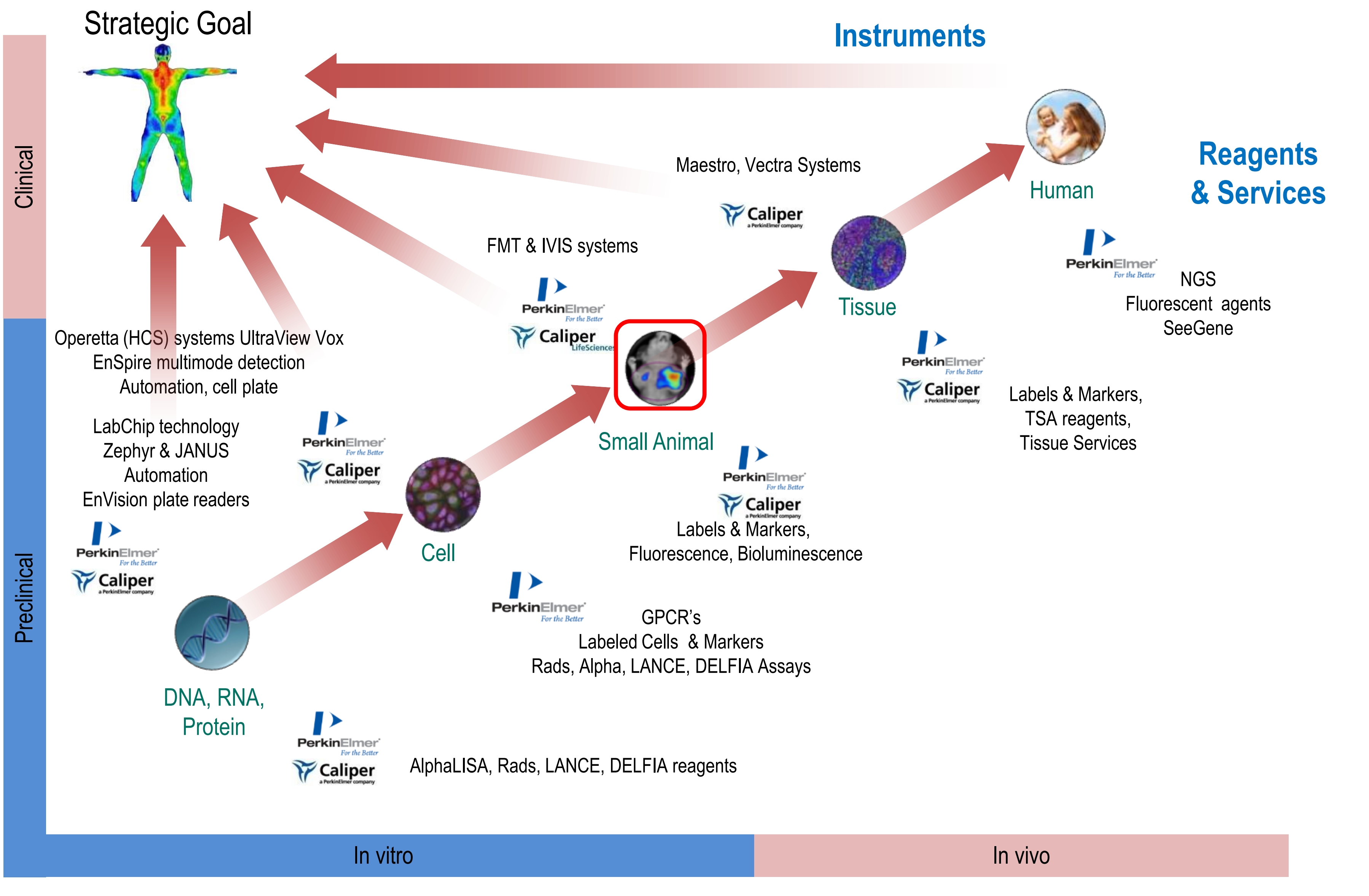
HUMAN HEALTH | ENVIRONMENTAL HEALTH

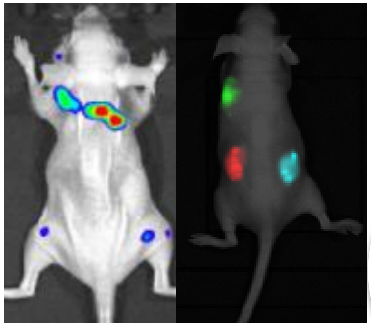
分子影像技术在转化医学研究中的应用

张　勇
15021163010

Our Platforms for Translational Research



分子影像技术概览



分子影像

功能

结构

活体显微镜

光学

CT

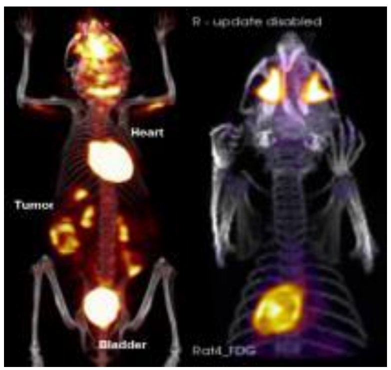
PET

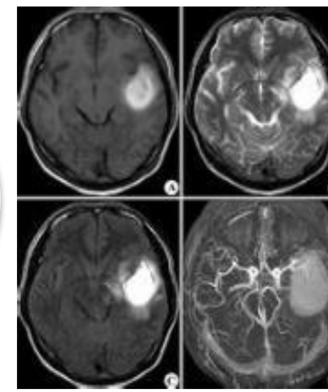
MRI

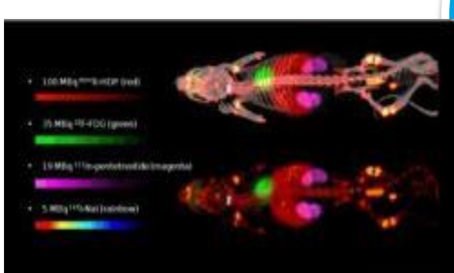
SPECT

超声

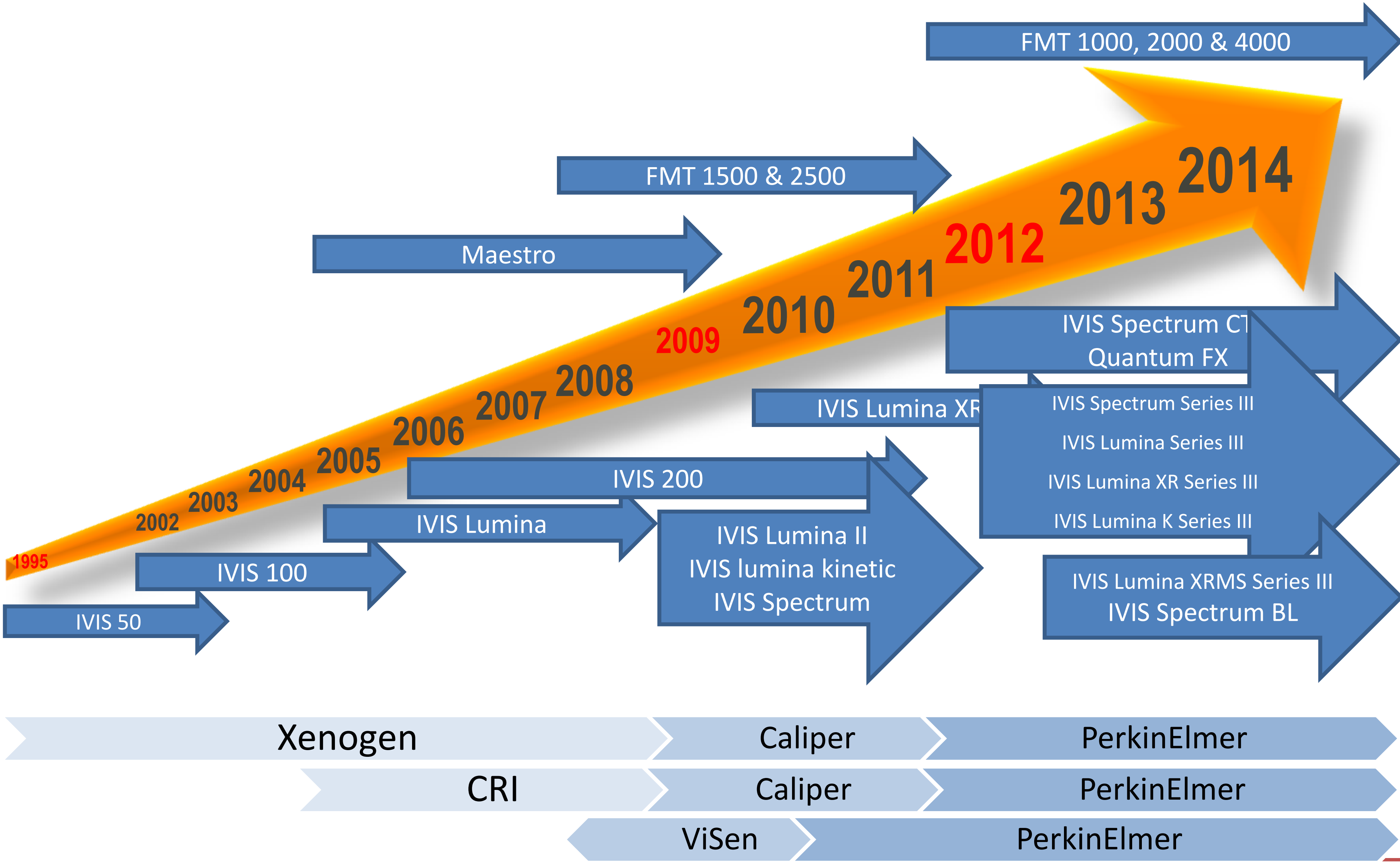
光声







History of PerkinElmer In Vivo Imaging System



成像系统——型号概览

Lumina LT

Entry level bioluminescent/ fluorescent imaging

Lumina III

Lumina with X-ray overlay

Lumina XRMS Series III

Fast, Real-time molecular imaging

Lumina S5

Fast, Real-time molecular imaging

Lumina X5

Fast, Real-time molecular imaging

Spectrum BL

Quantitative 2D & 3D bioluminescence imaging

Spectrum

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

Spectrum CT

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

FMT 4000

Quantitative Fluorescence 3D Tomography System with 4 excitation laser channels (635, 680, 750, and 790 nm)

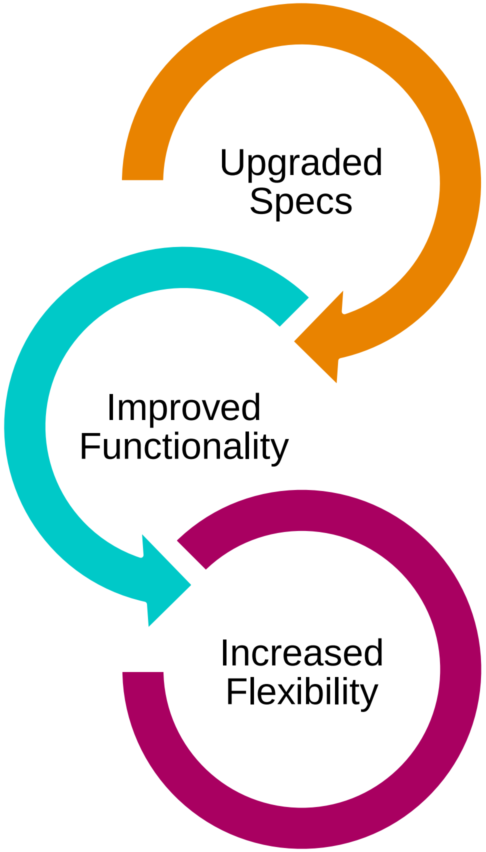
FMT 2000

Quantitative Fluorescence 3D Tomography System with 2 excitation laser channels (680 and 750 nm)

FMT 1000

Quantitative Fluorescence 3D Tomography System with 1 excitation laser channels (680 or 750 nm)

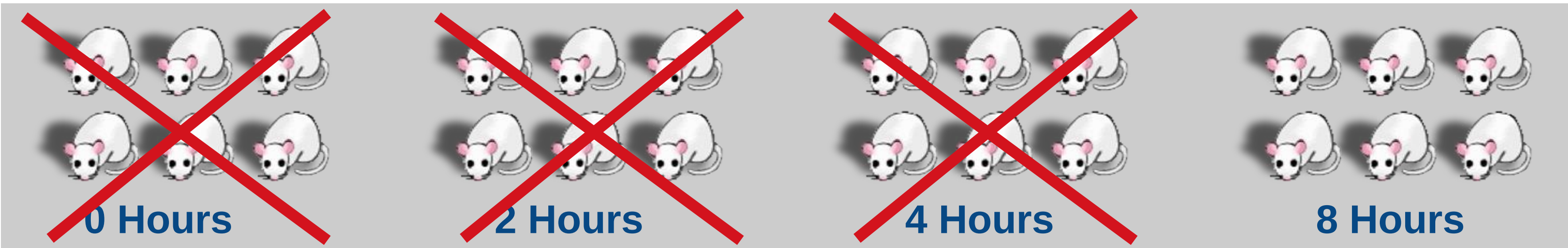
Introducing the Quantum GX2



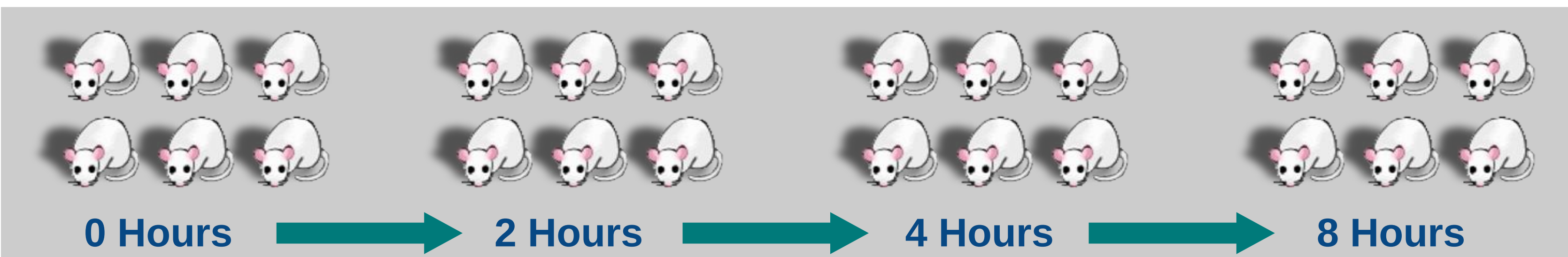
- 2.3 micron voxel resolution
- 2 additional imaging FOVs
  - 18mm for high resolution sample scanning
  - 86mm for wide field of view imaging
- X-ray filter changer with 6 x-ray filters
- Fast 3.9 second scan
- Improved cardiac and respiratory gating algorithms
- X-ray dose display (prior to initiating a scan)
- Disk storage level indicator

The Biggest Advantage of In Vivo Optical Imaging Technology

Current Methodology = 24 animals over four treatment points



In Vivo Imaging= the same 6 animals over four treatment points



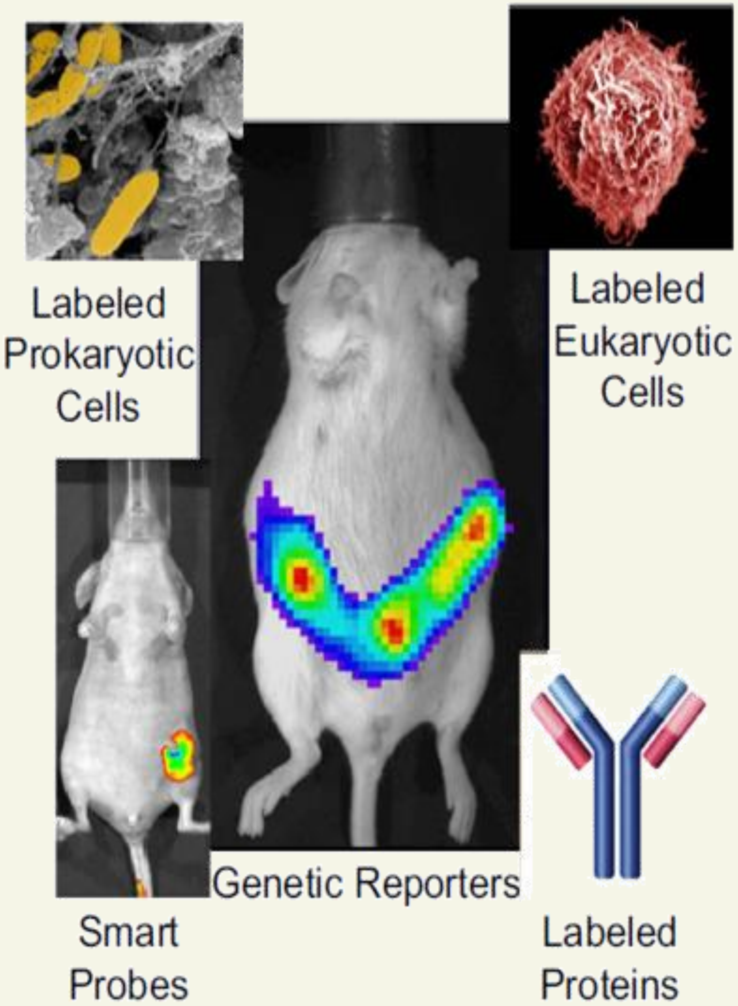
Example: 4 groups, 5 mice each group, 8 time points

Traditional methodology: **160 mice**

In vivo imaging: **20 mice**

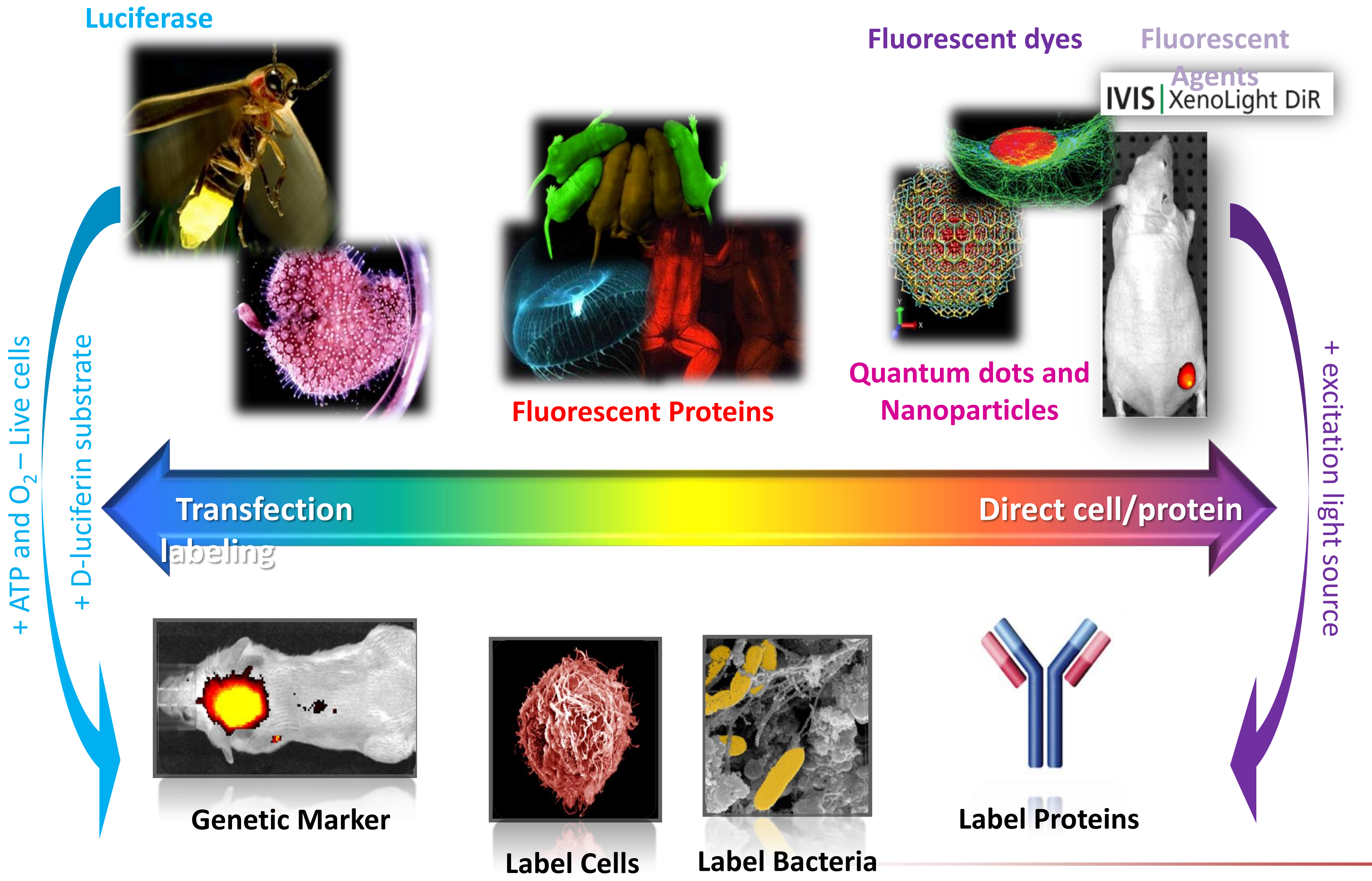
In Vivo Optical Imaging Technology

BIOLOGY



INSTRUMENTATION

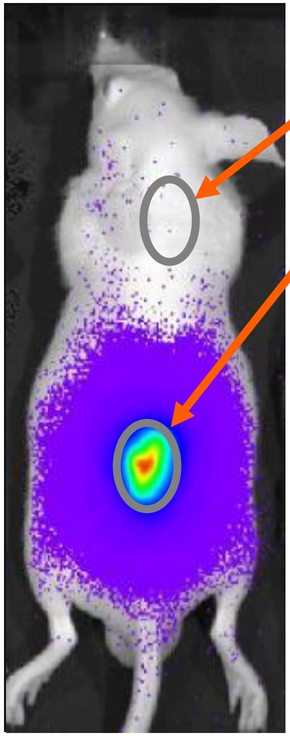
Key Technology– Reporter Molecules Labelling



Bioluminescence Imaging VS Fluorescence Imaging

- Fluorescent signal is limited by tissue autofluorescence
- The BLI signal level is ~100x lower, yet the signal to background is ~1000x higher

Bioluminescence



Autoluminescent flux ~ 9.5×10^3 p/s

Signal flux ~ 7.1×10^7 p/s

Signal/background ~ 7500

Min. detectable cells ~ 400

Autofluorescent flux ~ 1.0×10^9 p/s

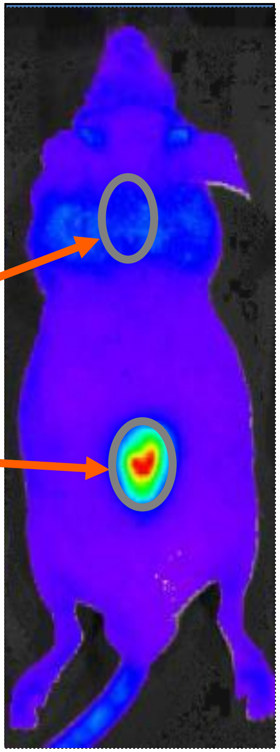
Signal flux ~ 7.8×10^9 p/s

Signal/background ~ 7.8

Min. detectable cells ~ 380,000

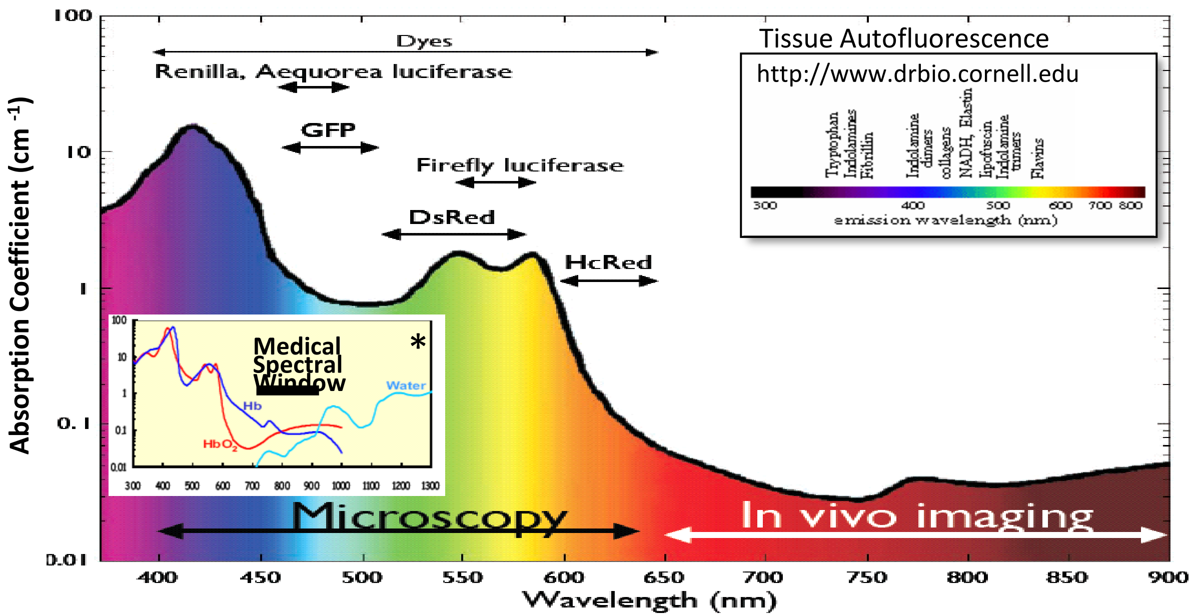
3×10^6 PC3M-luc/DsRed cells injected s.c.

Fluorescence



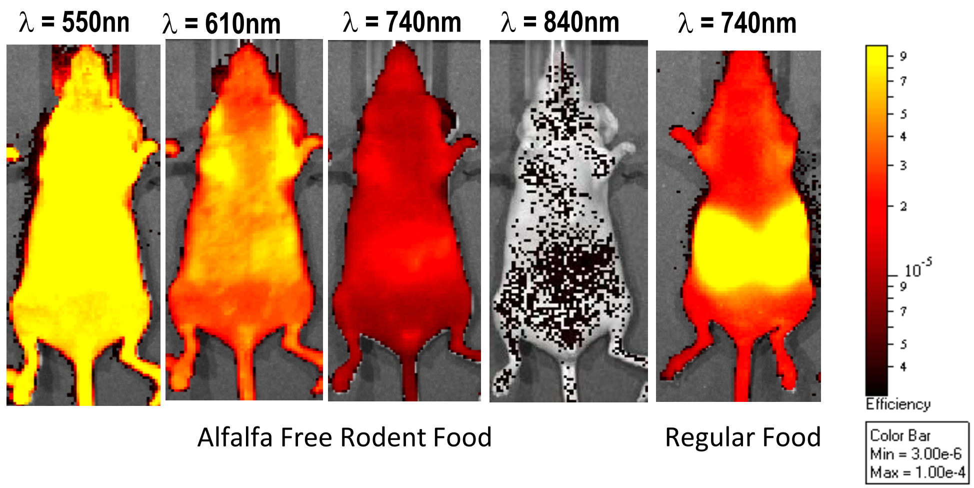
T.L. Troy, et al. (2004). Molecular Imaging 3:9-23.

Wavelength- the Key Factor of Autofluorescence and Transmission



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

SNR=Signal/Autofluorescence

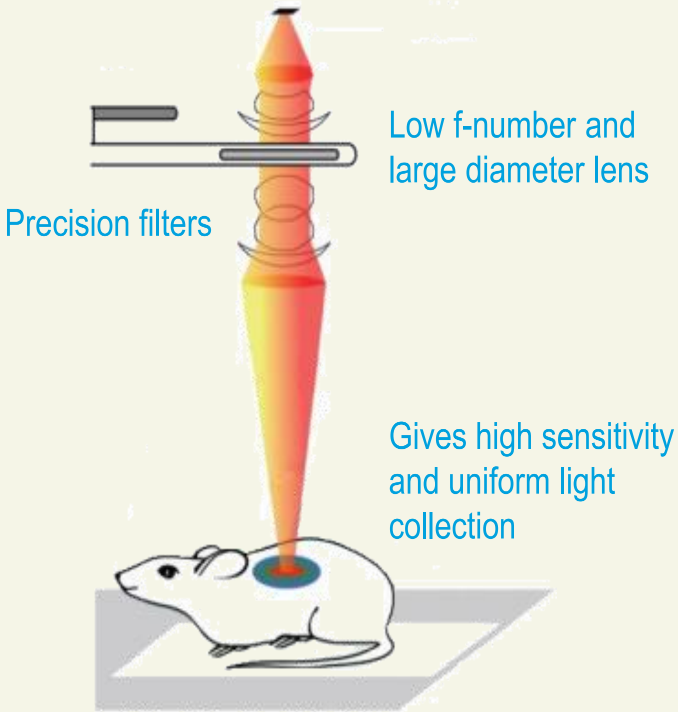


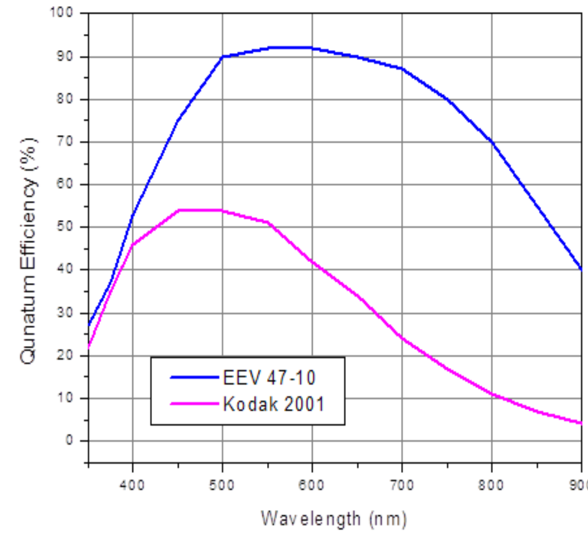
Alfalfa Free Rodent Food

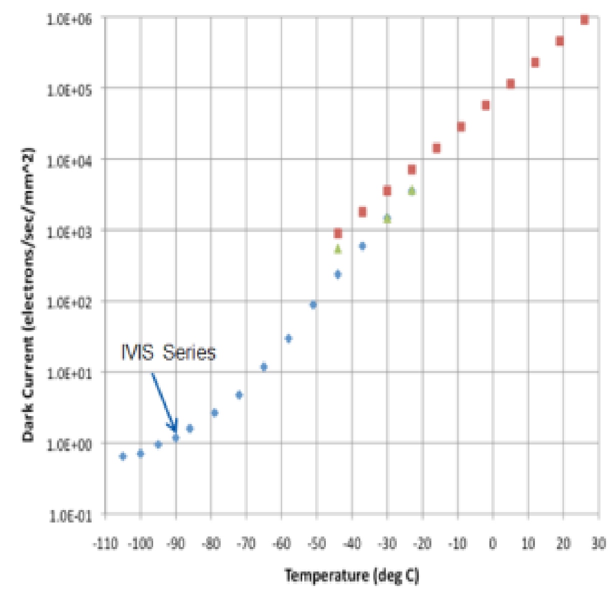
Regular Food

Bioluminescence Imaging Sensitivity

Back-thinned, back-illuminated, Grade 1 Cooled (-90C) camera with large CCD chip area for high sensitivity light detection



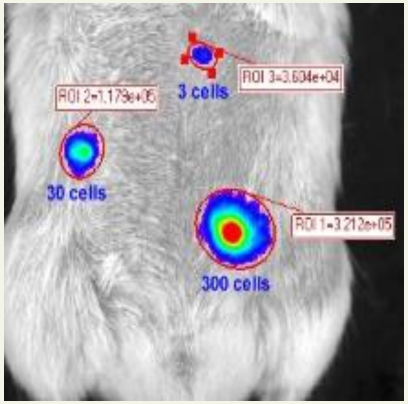




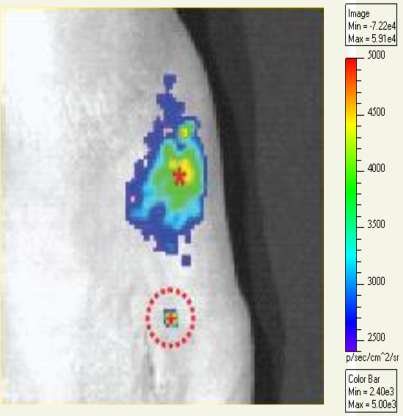
Super Sensitivity of BLI

"This approach has several advantages over conventional tumor models: the sensitive of cell detection in vivo is surprisingly high and exceeds even the sensitivity of detection by flow cytometry ex vivo. As few as 7×10^3 cells are detectable in the lungs early after injection, 2-2.5 $\times 10^4$ cells within liver or spleen… In other experiments using cells with even higher luciferase expression, as few as 100 cells can be reliably detected in the peritoneal cavity of living animals." Since cells are detectable even from deep within tissues, tumor cell trafficking, engraftment in different organs, and metastasis could be visualized without perturbing intact organ systems. ------ *Blood 2003, 15(101):640-648*

Most sensitive system available
Resolves multiple bioluminescent reporters
Detects down to even a single cell *in vivo*



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)

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IMAGING

Single-cell bioluminescence imaging of deep tissue in freely moving animals

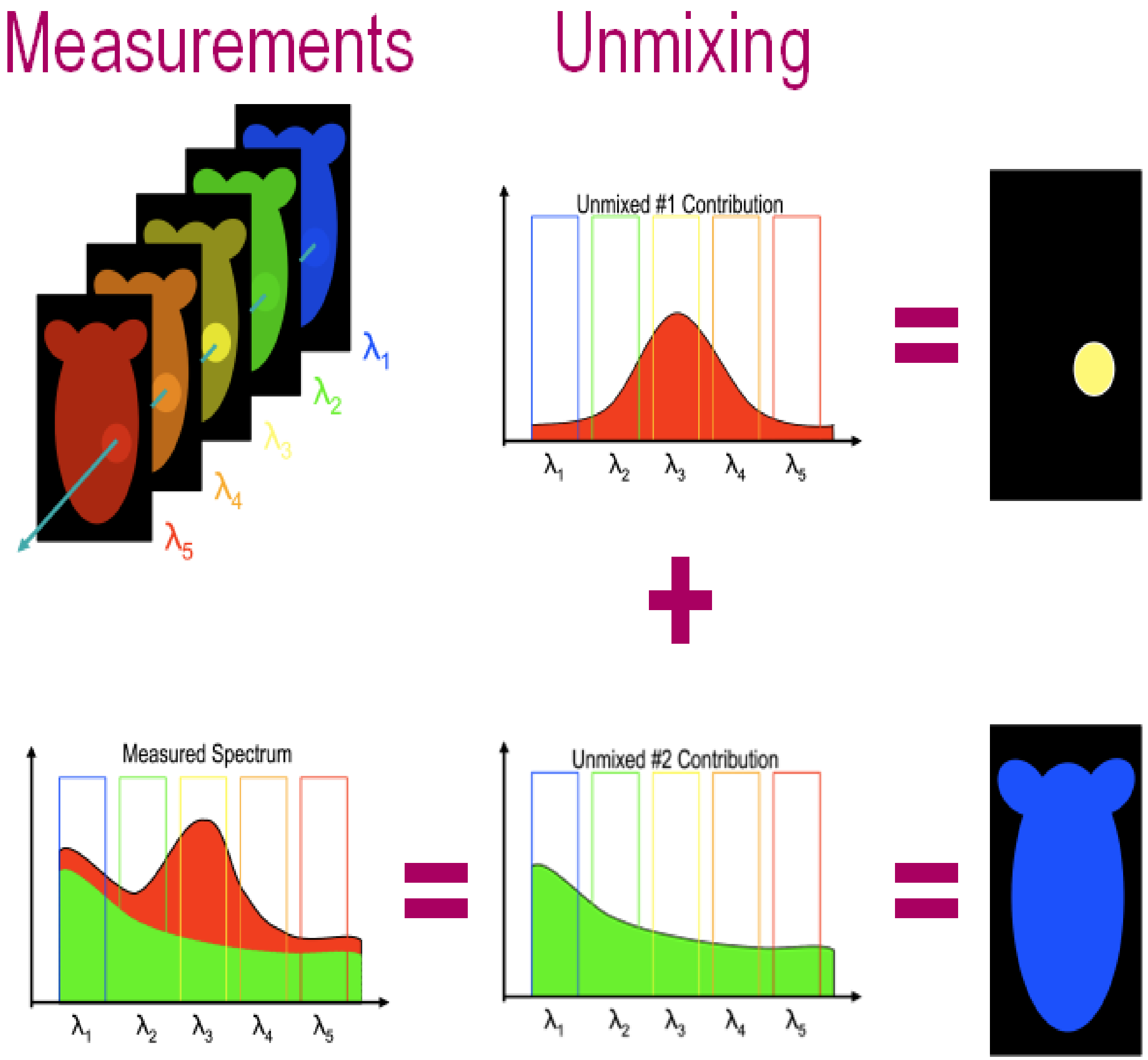
Satoshi Iwano,[1] Mayu Sugiyama,[1] Hiroshi Hama,[1] Akiya Watakabe,[2] Naomi Hasegawa,[2] Takahiro Kuchimaru,[3] Kazumasa Z. Tanaka,[4] Megumu Takahashi,[5] Yoko Ishida,[5] Junichi Hata,[6] Satoshi Shimozono,[1] Kana Namiki,[1] Takashi Fukano,[1] Masahiro Kiyama,[7] Hideyuki Okano,[6] Shinae Kizaka-Kondoh,[3] Thomas J. McHugh,[4] Tetsuo Yamamori,[2] Hiroyuki Hioki,[5] Shojiro Maki,[7] Atsushi Miyawaki[1,8]*

Bioluminescence is a natural light source based on luciferase catalysis of its substrate luciferin. We performed directed evolution on firefly luciferase using a red-shifted and highly deliverable luciferin analog to establish AkaBLI, an all-engineered bioluminescence in vivo imaging system. AkaBLI produced emissions in vivo that were brighter by a factor of 100 to 1000 than conventional systems, allowing noninvasive visualization of single cells deep inside freely moving animals. Single tumorigenic cells trapped in the mouse lung vasculature could be visualized. In the mouse brain, genetic labeling with neural activity sensors allowed tracking of small clusters of hippocampal neurons activated by novel environments. In a marmoset, we recorded video-rate bioluminescence from neurons in the striatum, a deep brain area, for more than 1 year. AkaBLI is therefore a bioengineered light source to spur unprecedented scientific, medical, and industrial applications.

Iwano et al., Science 359, 935–939 (2018) 23 February 2018

The Optimized Solution for Fluorescent Imaging

Spectral Unmixing Concept



唯一成熟的专利荧光光谱分离技术

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超过500篇的光谱分离文献，技术非常成熟！

Journal of Biomedical Optics 14(6), 064011 (November/December 2009)

Nat Methods. 2013 August ; 10(8): 751–754. doi:10.1038/nmeth.2521.

Stafford *et al. Nutrition & Metabolism* 2010, 7:74
http://www.nutritionandmetabolism.com/content/7/1/74

NIH Public Access

NIH-PA Author Manuscript

US006930773B2

(12) **United States Patent**
Cronin et al.

(10) **Patent No.:** **US 6,930,773 B2**
(45) **Date of Patent:** **Aug. 16, 2005**

(54) **SPECTRAL IMAGING**

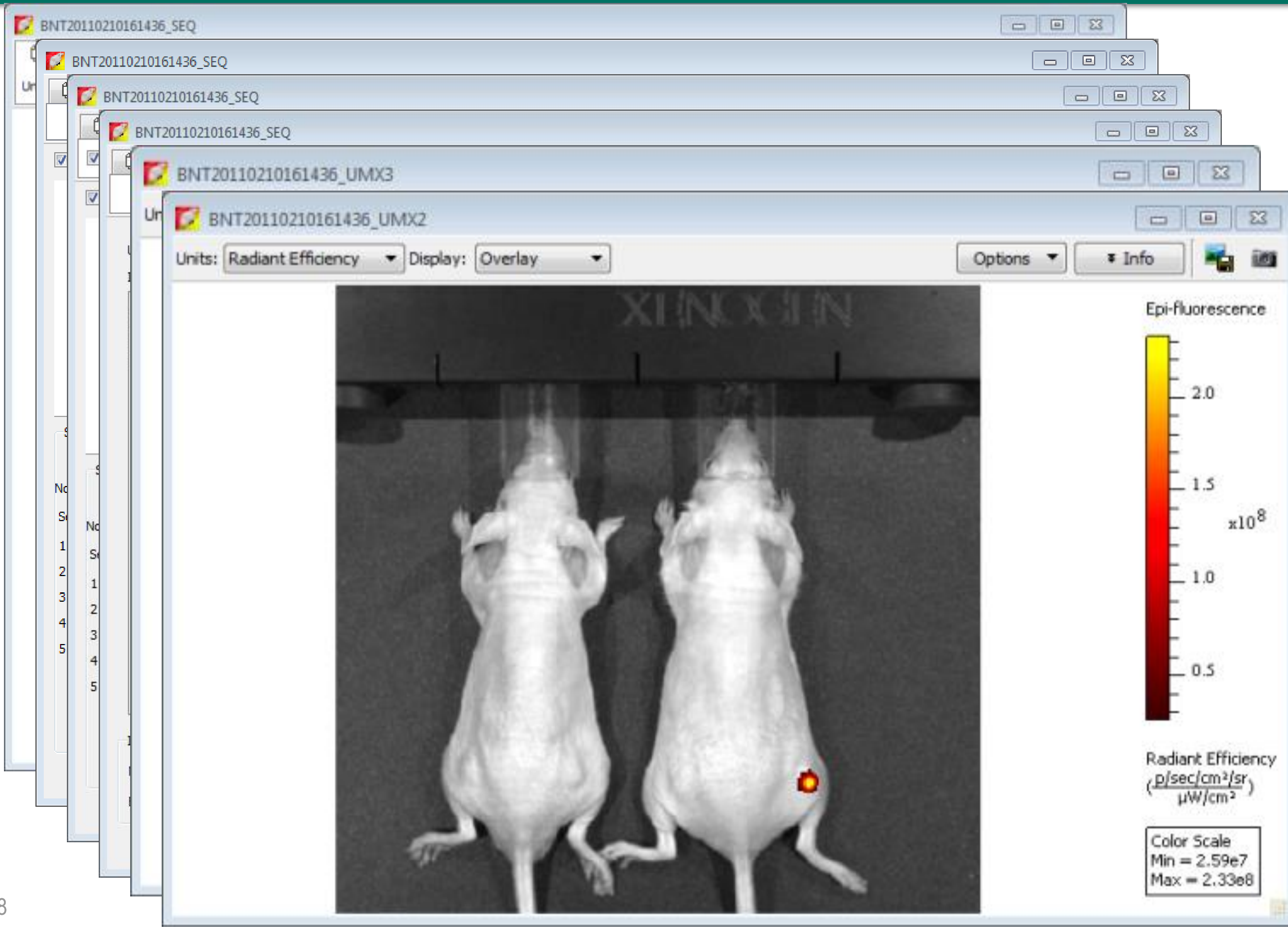
(75) Inventors: **Paul J. Cronin**, Charlestown, MA (US); **Peter J. Miller**, Newburyport, MA (US)

(73) Assignee: **Cambridge Research and Instrumentation, Inc.**, Woburn, MA (US)

5,424,545	A		6/1995	Block et al.	
5,433,197	A		7/1995	Stark	
5,435,309	A	*	7/1995	Thomas et al.	600/310
5,539,517	A		7/1996	Cabib et al.	
5,567,937	A		10/1996	Pinkus	
5,608,213	A		3/1997	Pinkus et al.	
5,719,024	A		2/1998	Cabib et al.	
5,731,581	A	*	3/1998	Fischer et al.	250/339.13
5,732,150	A		3/1998	Zhou et al.	

NIH-PA Auth

The most powerful spectral unmixing method

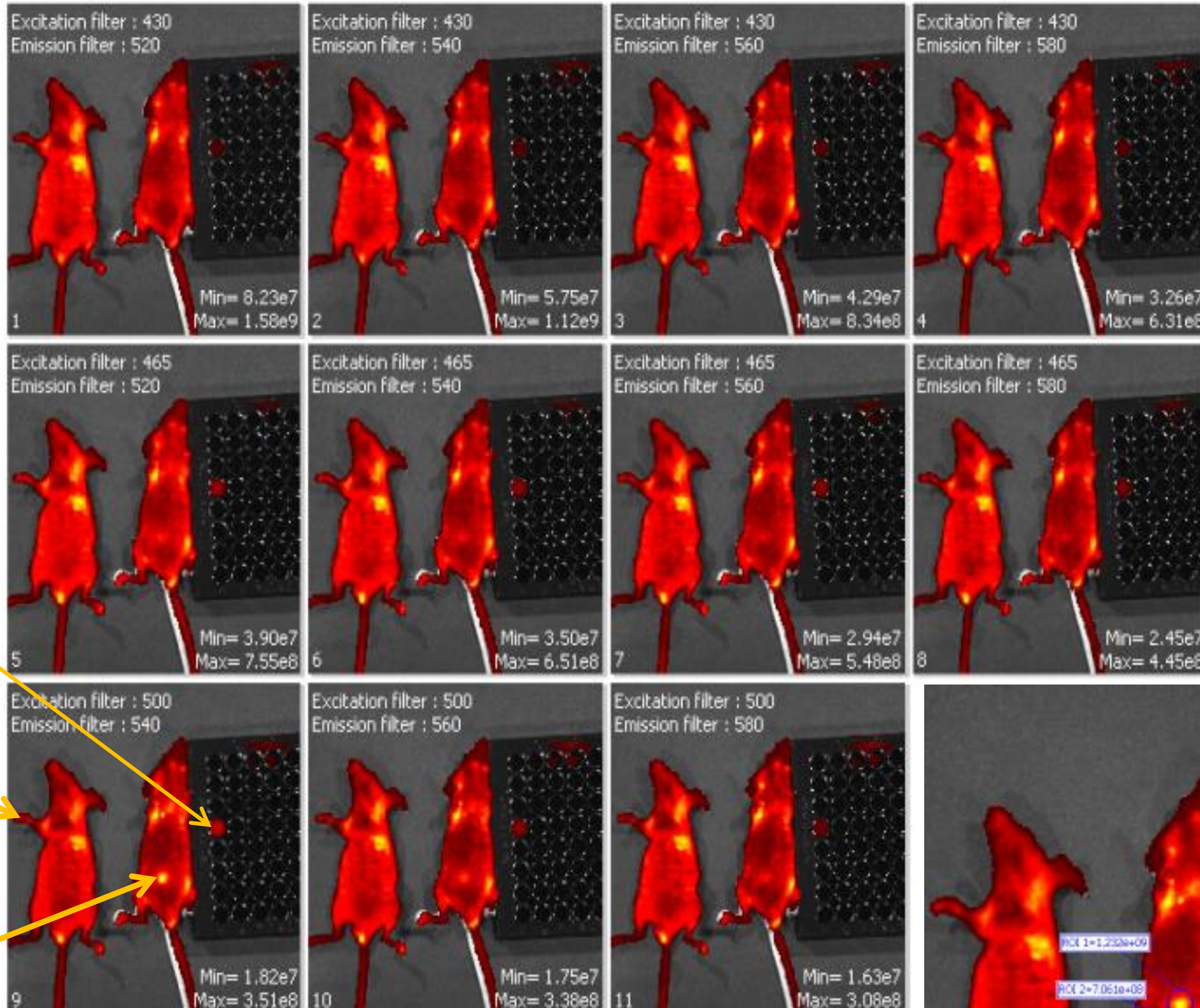


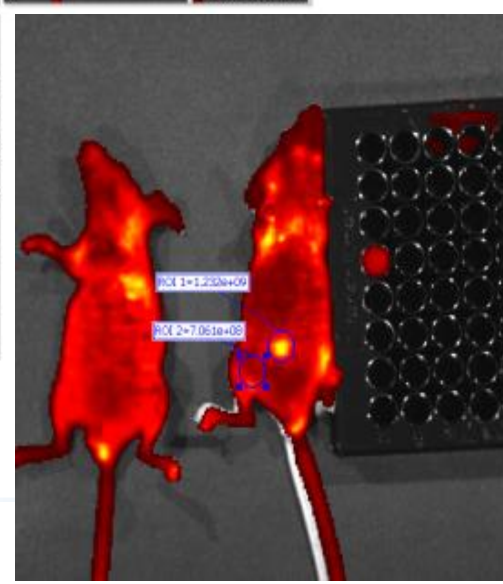
Spectral Unmixing Using FITC

0.05 µg/ml FITC

阴性对照鼠

5 ng FITC





Raw data（光谱分离前），5 ng FITC的信号隐约可见，但背景荧光高，信噪比低（小于2:1），3 ng FITC的信号难以辨别

Golden Standard Bioluminescence Quantification Method

Journal of Biomedical Optics 6(4), 432–440 (October 2001)

In vivo imaging of light-emitting probes

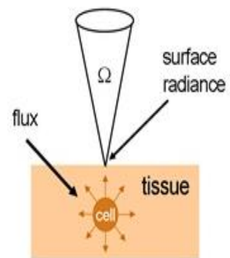
B. W. Rice
M. D. Cable
M. B. Nelson
Xenogen Corporation
860 Atlantic Avenue
Alameda, California 94501

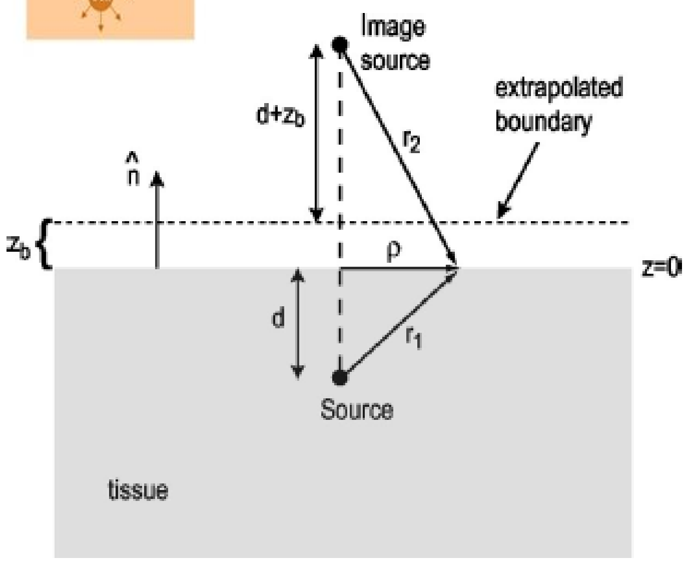
Abstract. *In vivo* imaging of cells tagged with light-emitting probes, such as firefly luciferase or fluorescent proteins, is a powerful technology that enables a wide range of biological studies in small research animals. Reporters with emission in the red to infrared (>600 nm) are preferred due to the low absorption in tissue at these wavelengths. Modeling of photon diffusion through tissue indicates that bioluminescent cell counts as low as a few hundred can be detected subcutaneously, while ~10^6 cells are required to detect signals at ~2 cm depth in tissue. Signal-to-noise estimates show that cooled back-thinned integrating charge coupled devices (CCDs) are preferred to image-intensified CCDs for this application, mainly due to their high quantum efficiency (~85%) at wavelengths >600 nm where tissue absorption is low. Instrumentation for *in vivo* imaging developed at Xenogen is described and several examples of images of mice with bioluminescent cells are presented. [DOI: 10.1117/1.1413210]

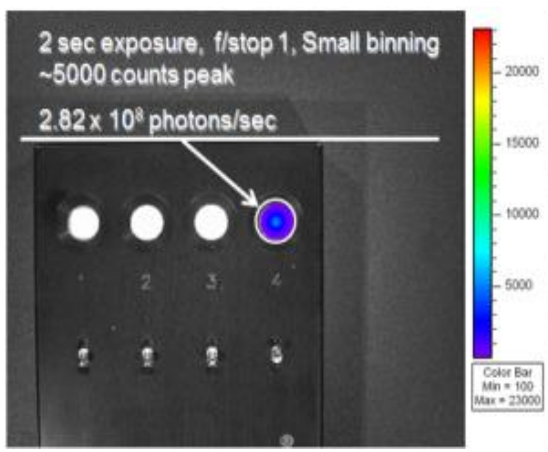
Keywords: imaging; bioluminescence; luminescence; fluorescence; luciferase; *in vivo*.

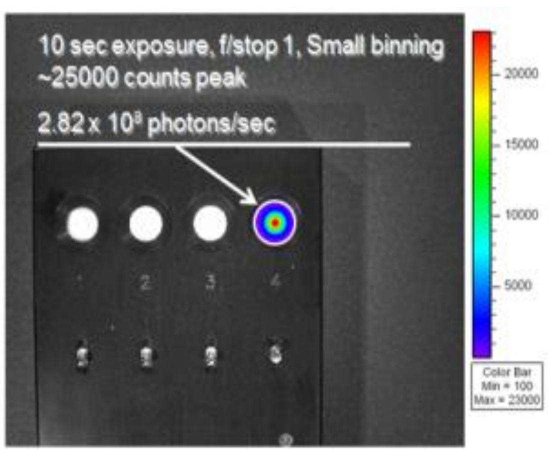
Paper 080179 received Jan. 26, 2001; revised manuscript received July 16, 2001; accepted for publication July 17, 2001.

Total Flux: *Photons/second/cm2/sr*









Calibrated Fluorescent Units: Radiant Efficiency

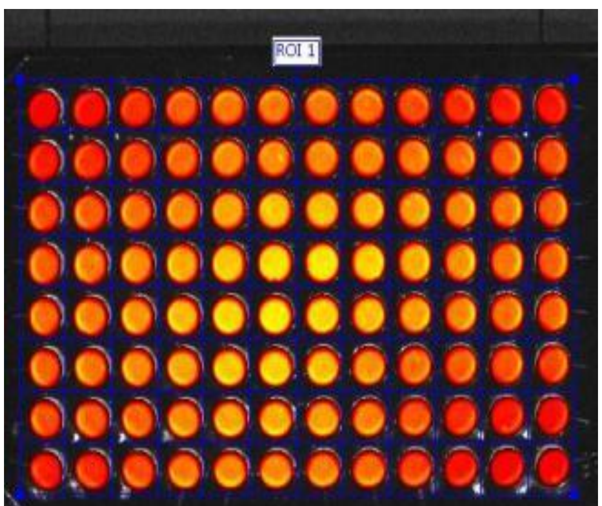
Excitation Light Pattern

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

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

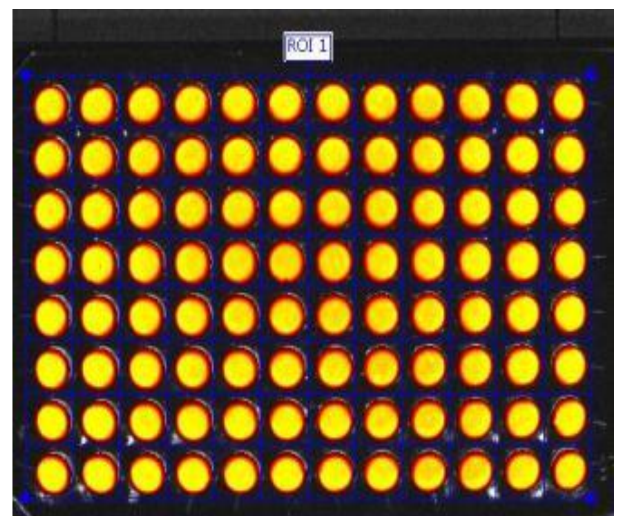
GFP Well Plate Uncorrected

Counts



vs.

GFP Well Plate Corrected

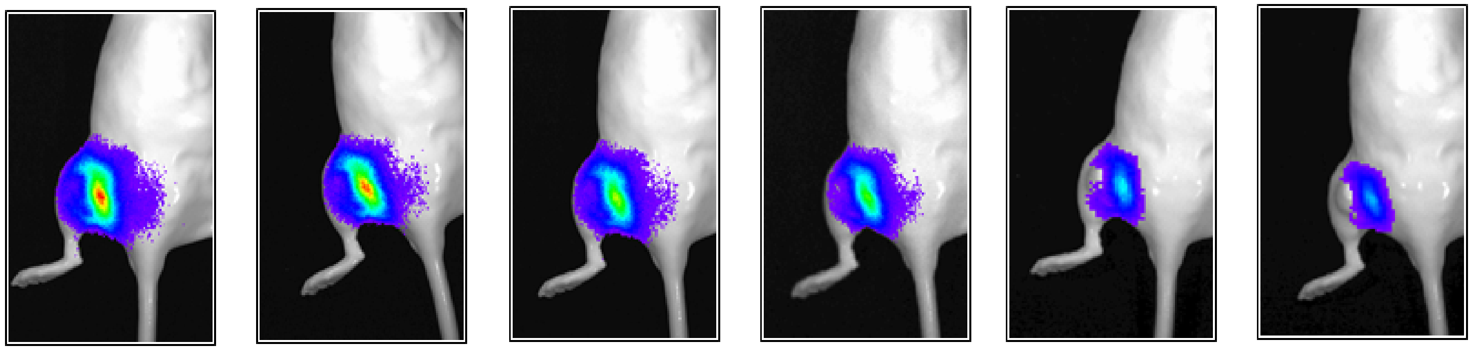


Radiant Efficiency

Calibrated Physical Units vs. Raw Signal

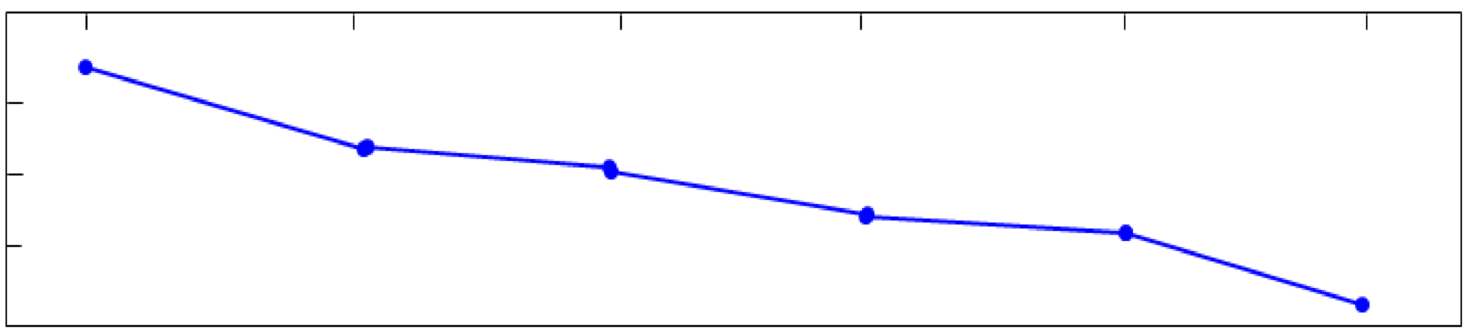
Calibrated Signal

(Photons per second)

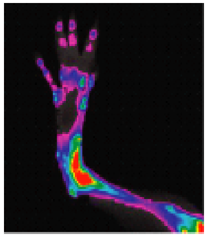


Exp time:	30 sec	30 sec	60 sec	60 sec	60 sec	60 sec
Binning:	small	small	small	small	medium	medium
Day:	1	2	3	4	5	6

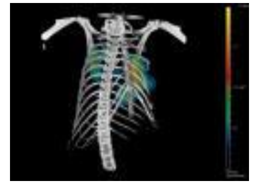
Radiance: *Photons per second*



Tailored To Therapeutic Applications

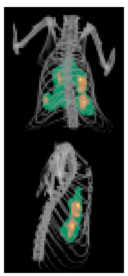


Inflammation



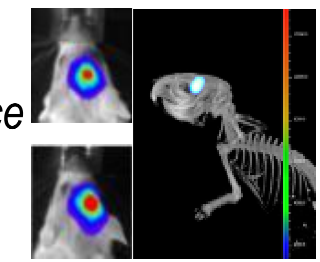
Oncology

Cardiovascular Diseas

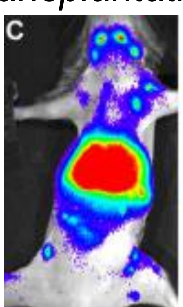


Infectious Diseases

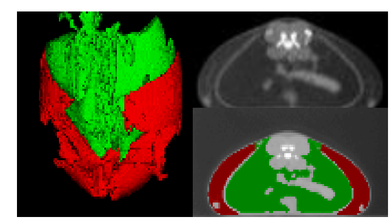
Neuroscience



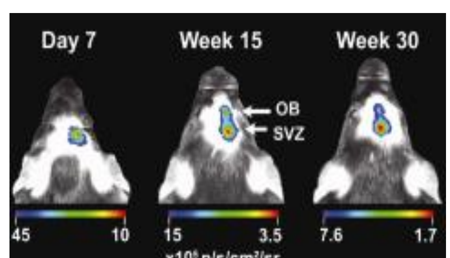
Immunology & Transplantation



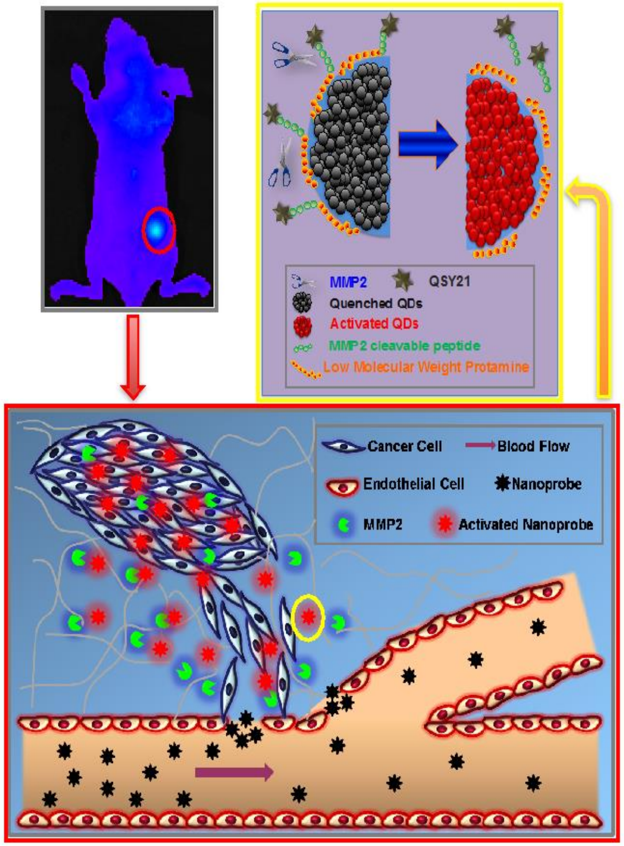
Metabolic Diseases

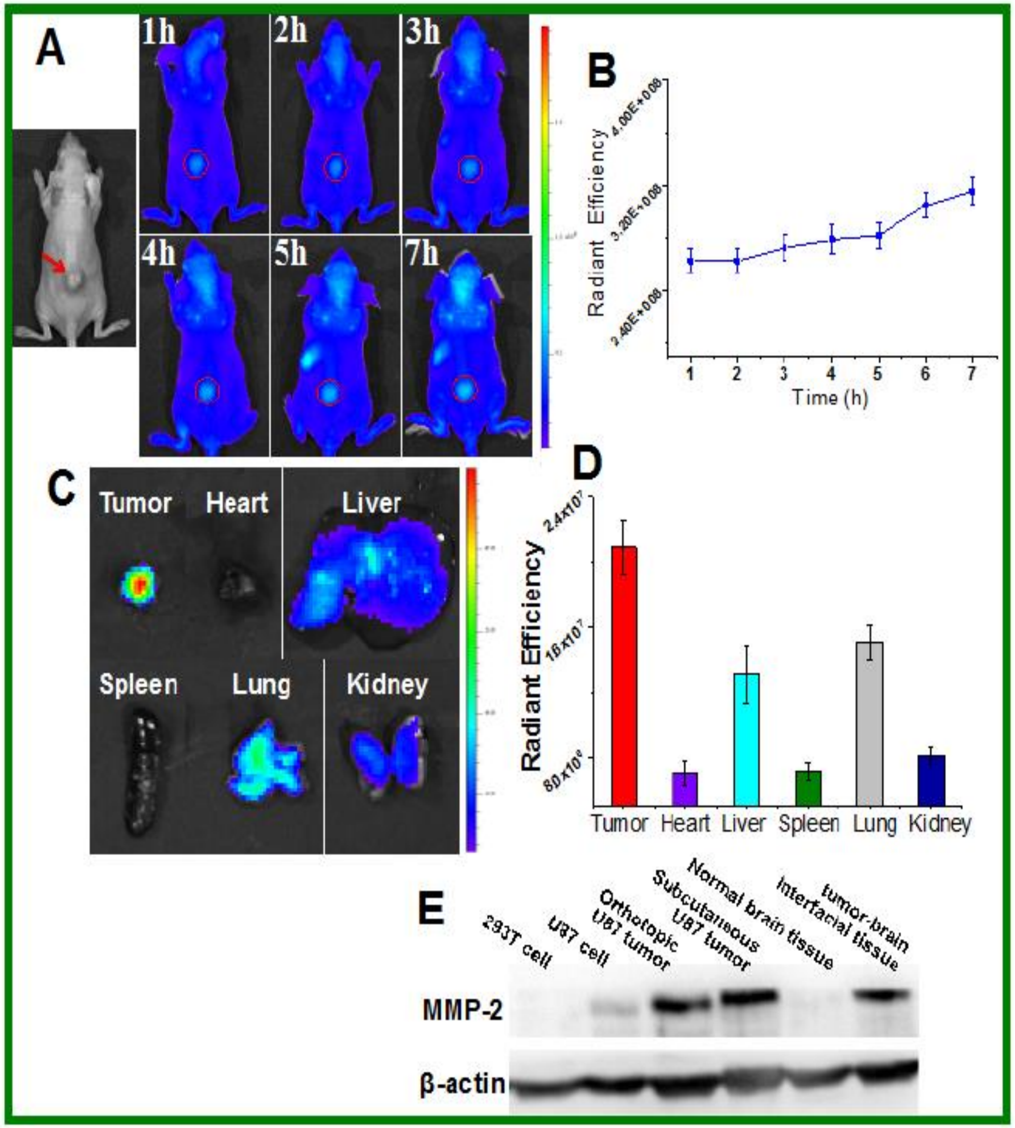


Stem Cells



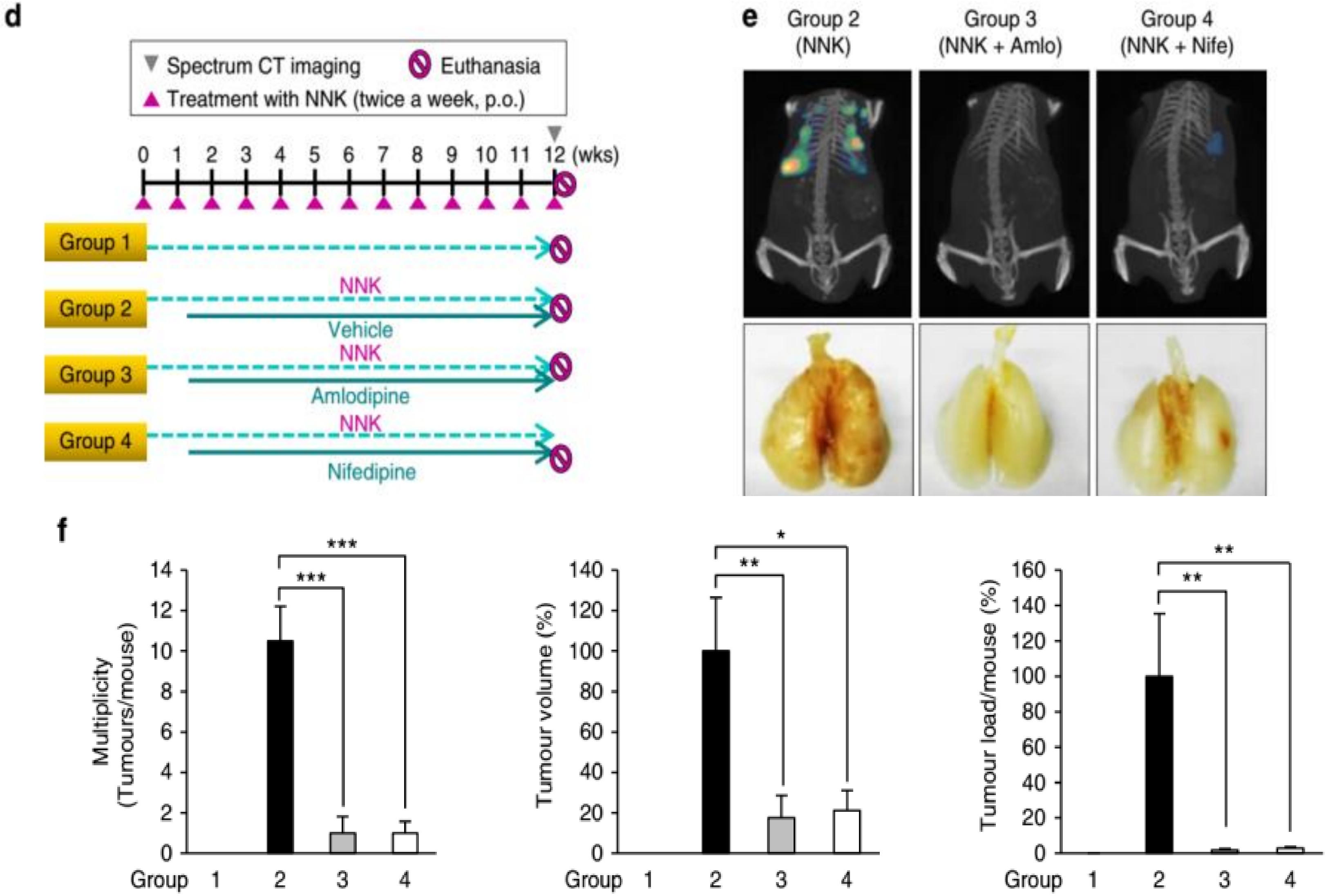
基于量子点设计的荧光探针，特异性检测MMP-2，用于肿瘤检测。





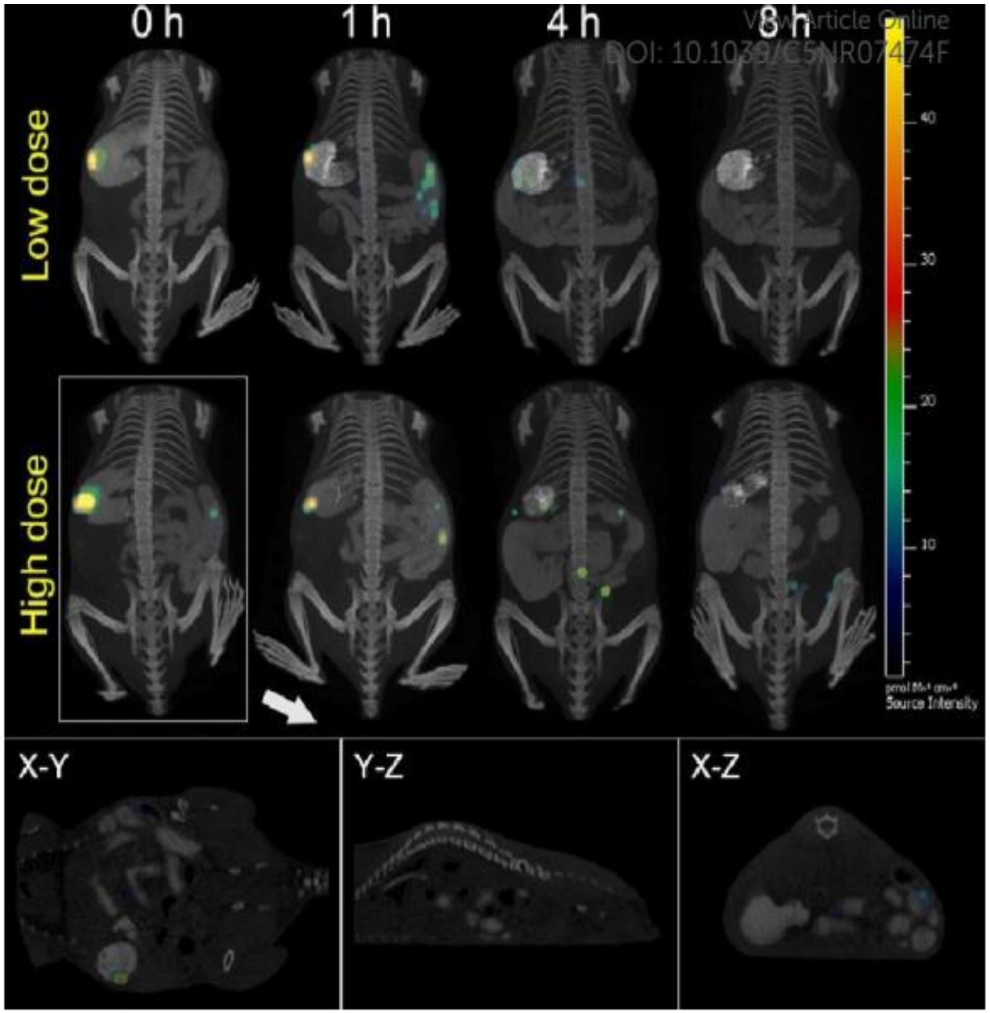
Yaping Wang et al, Theranostics,2015

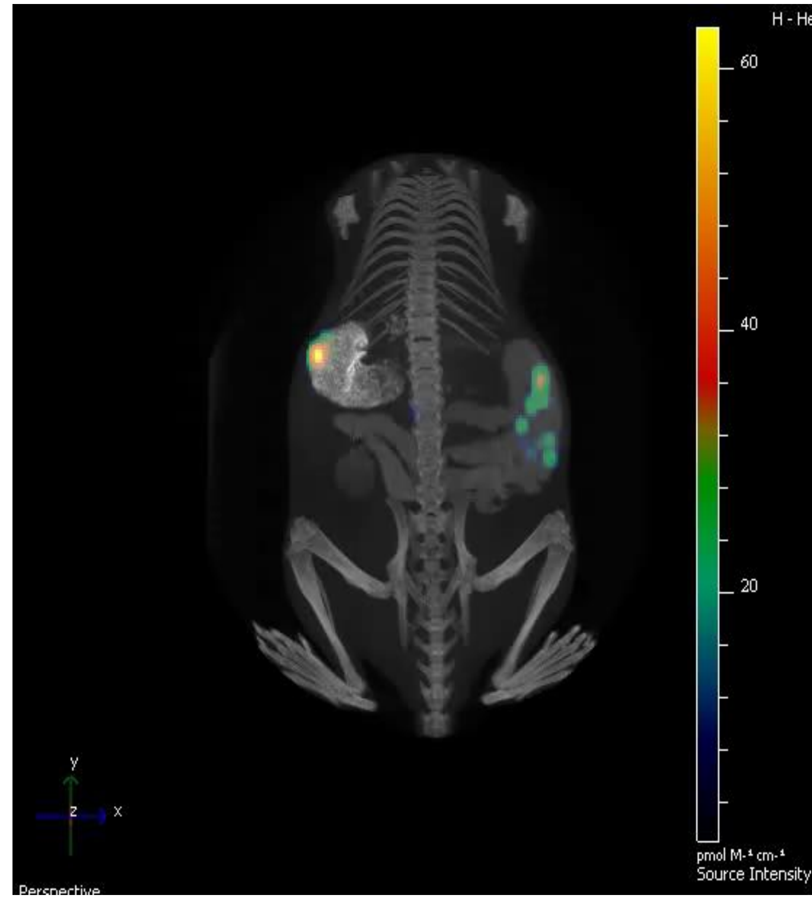
应用举例——荧光探针开发（酶敏感型探针）



Hye-Jin Boo. et al., **NATURE COMMUNICATIONS.2016**

应用举例——纳米药物载体研究（吸收代谢）





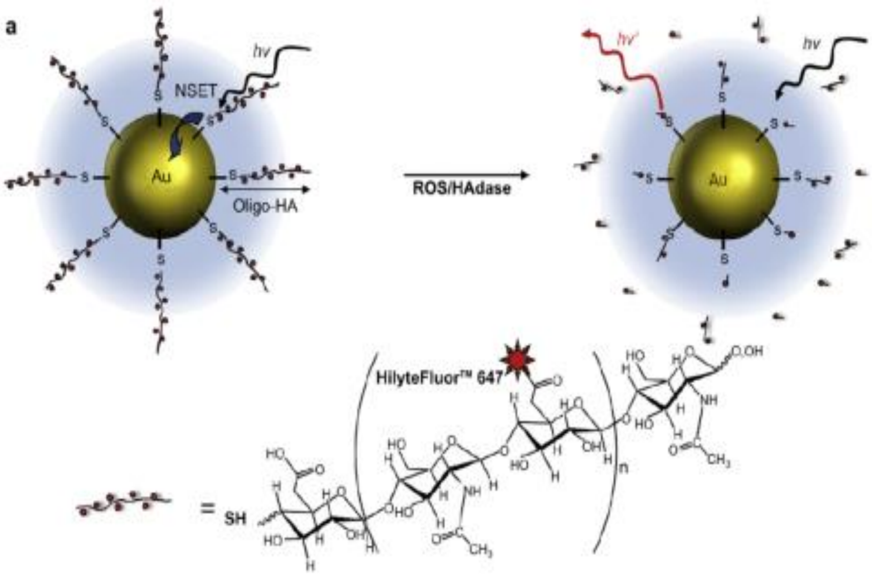
Xiongwei Hu *et al*,，Nanoscale, 2015, 00, 1-13 ,1

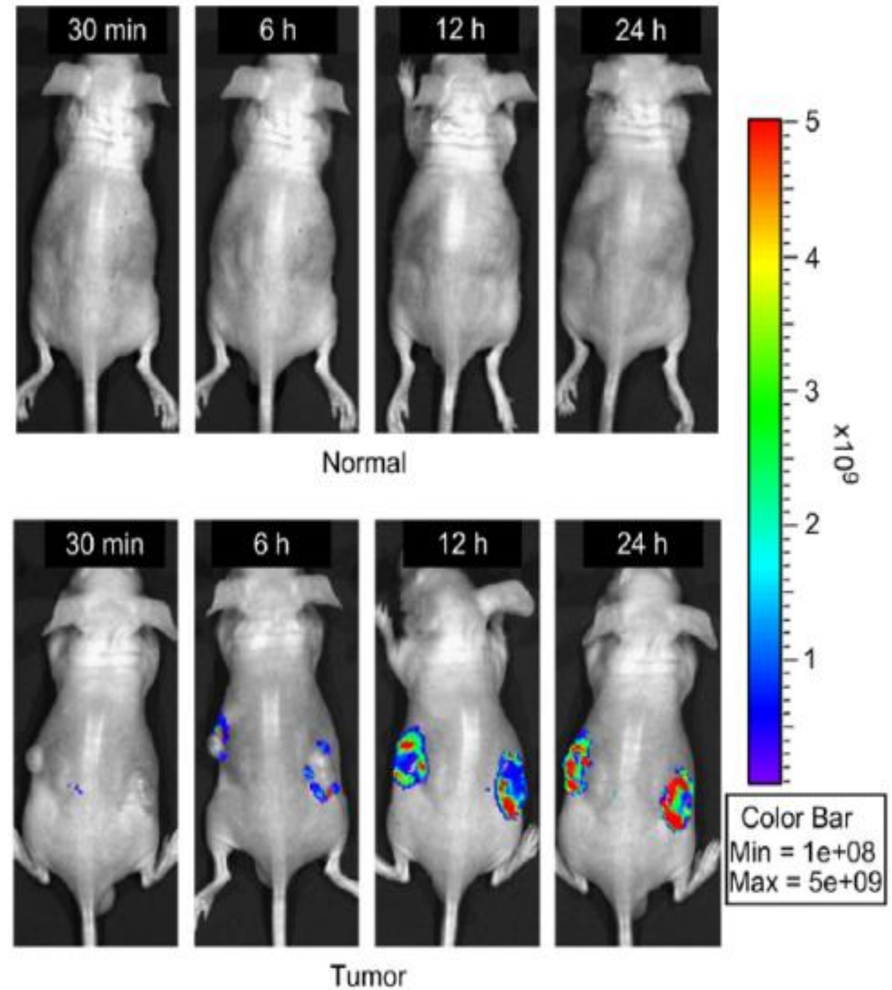
应用举例——监测疾病发展（癌症）

3D bioluminescent were co-registered with the the subject's skeletal anatomy

NIRF-HA based gold nanoprobes detect reactive oxygen species (ROS) and hyaluronidase

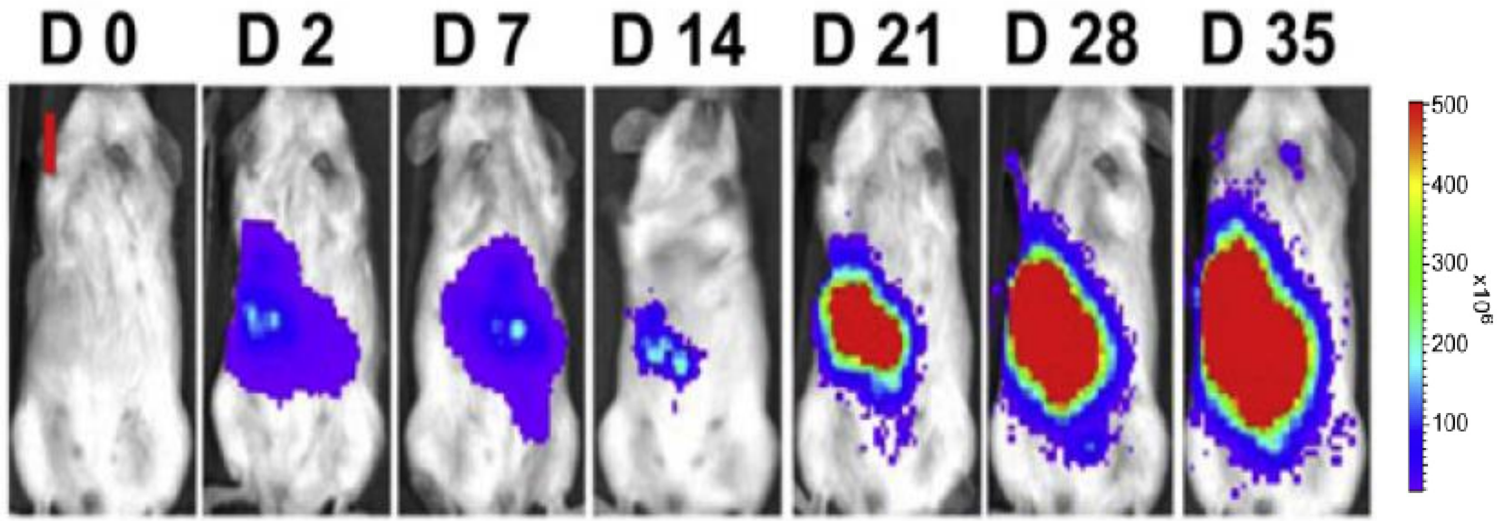
RA and highly metastatic tumor are known to express high levels of ROS and HAdase, respectively, inducing the progressive degradation of local HA in the extracellular matrix

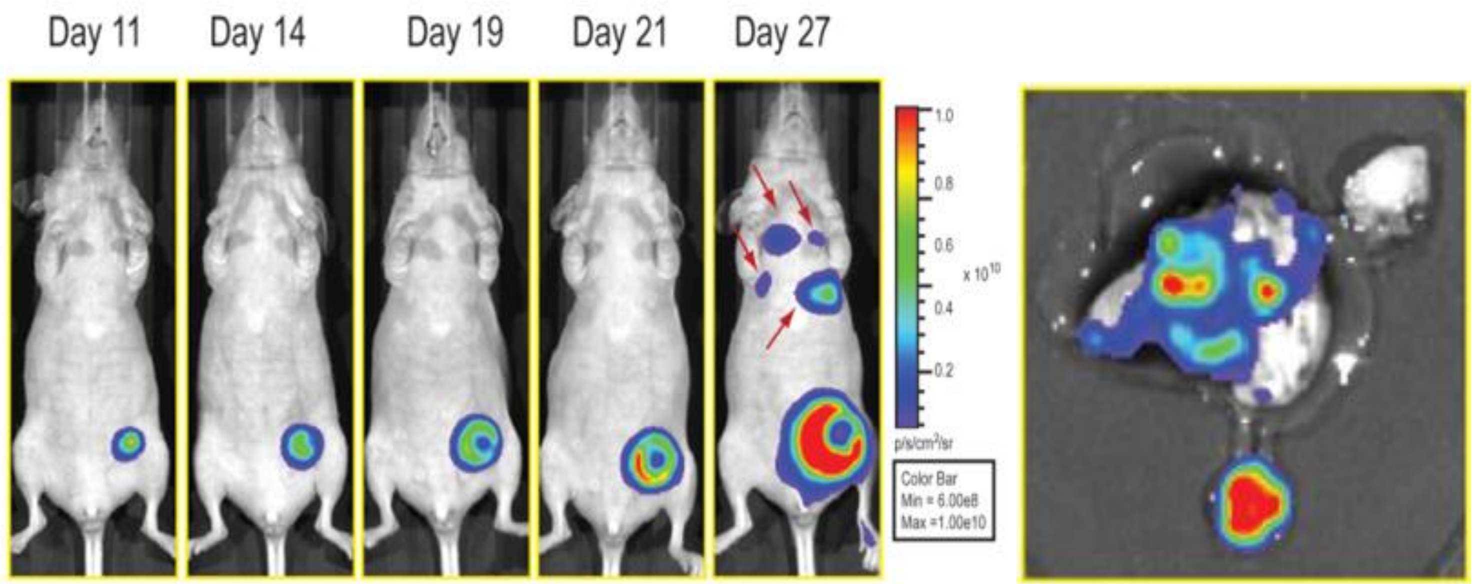




Lee *et al*,Biomaterials,2008

应用举例——监测疾病发展（癌症）



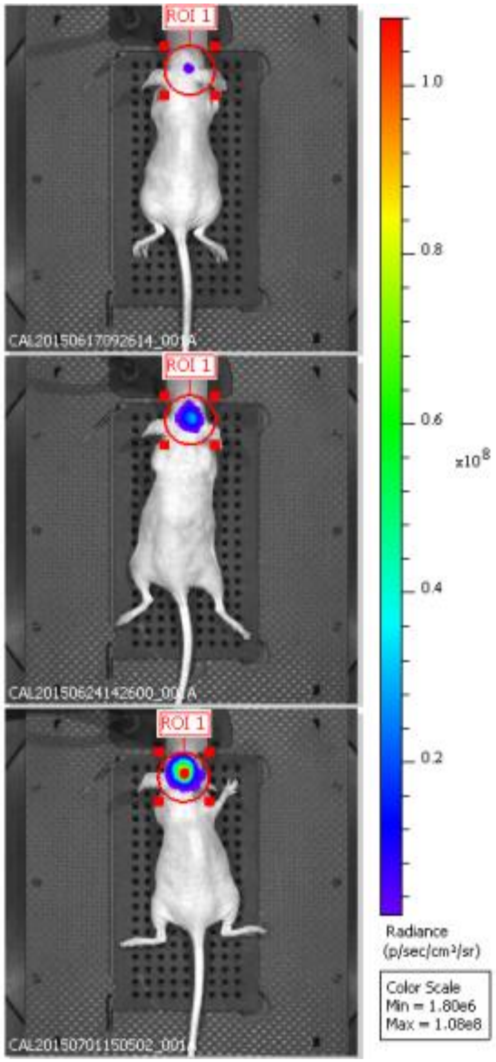


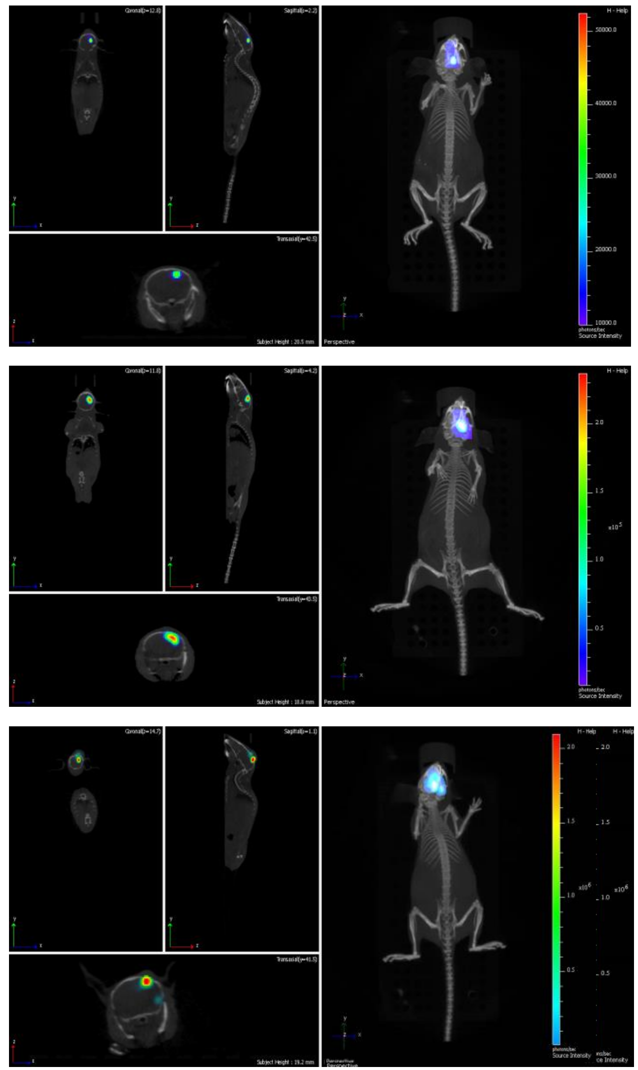
Orthotopic xenograft model of brain tumor

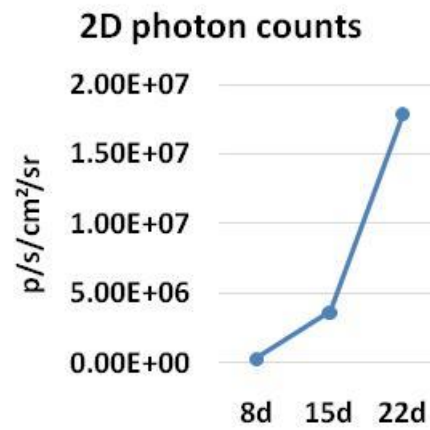
Day 8

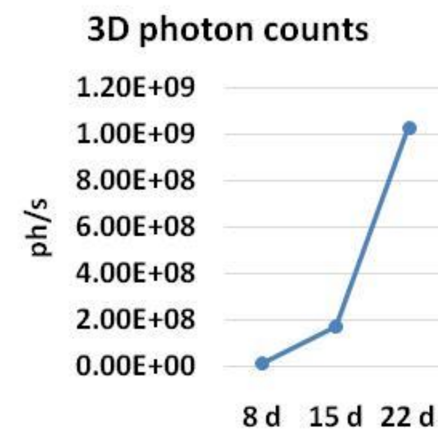
Day 15

Day 22

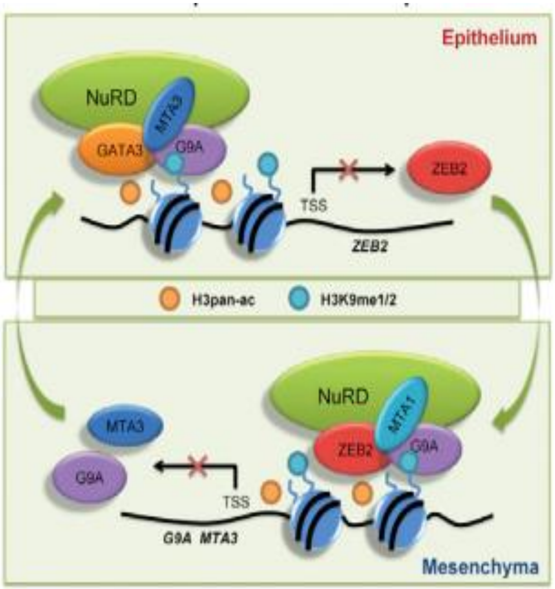


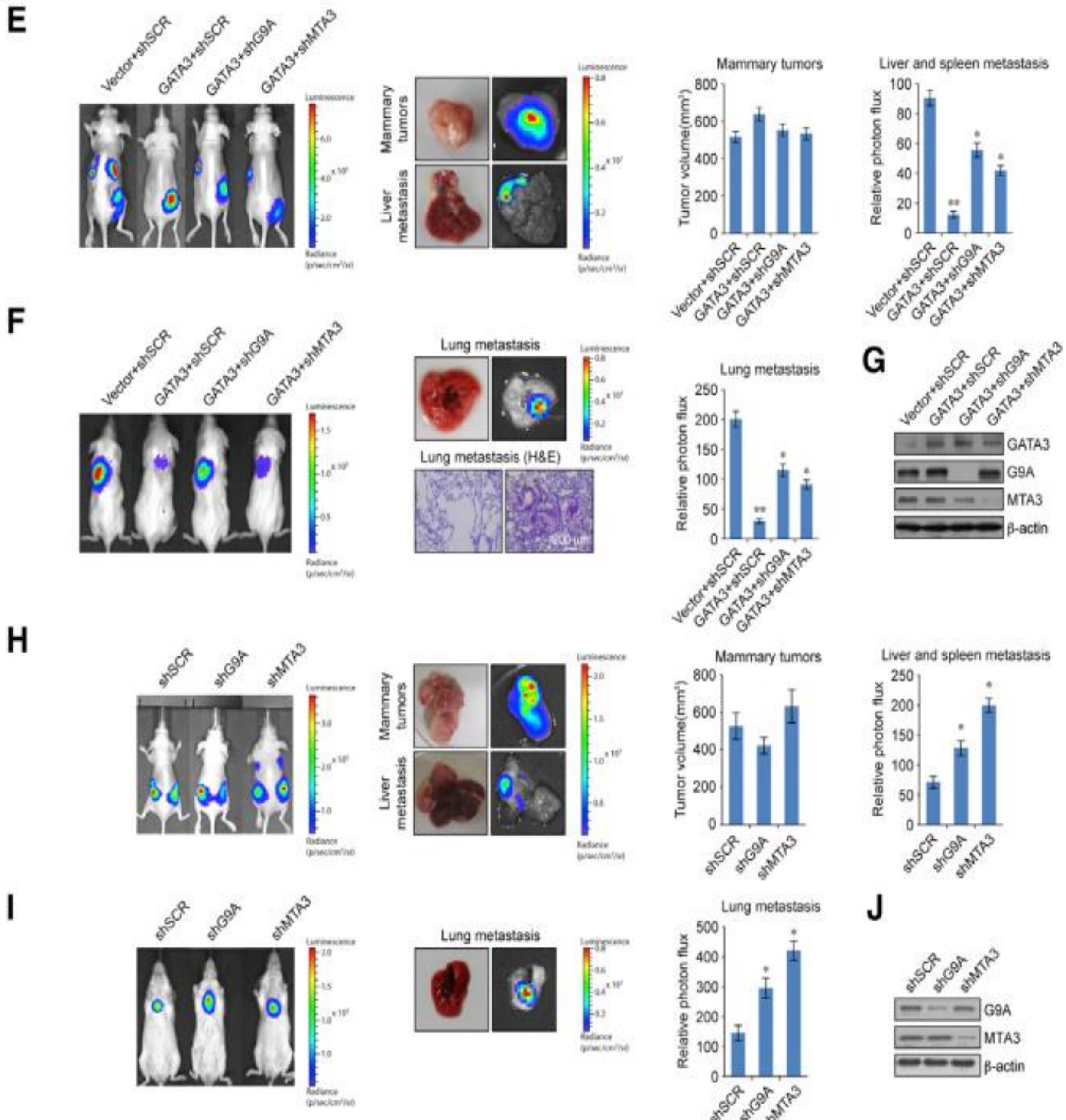






应用举例——肿瘤机制相关研究





Si et al. , Cancer Cell, 2015

应用举例——肿瘤疗效评价

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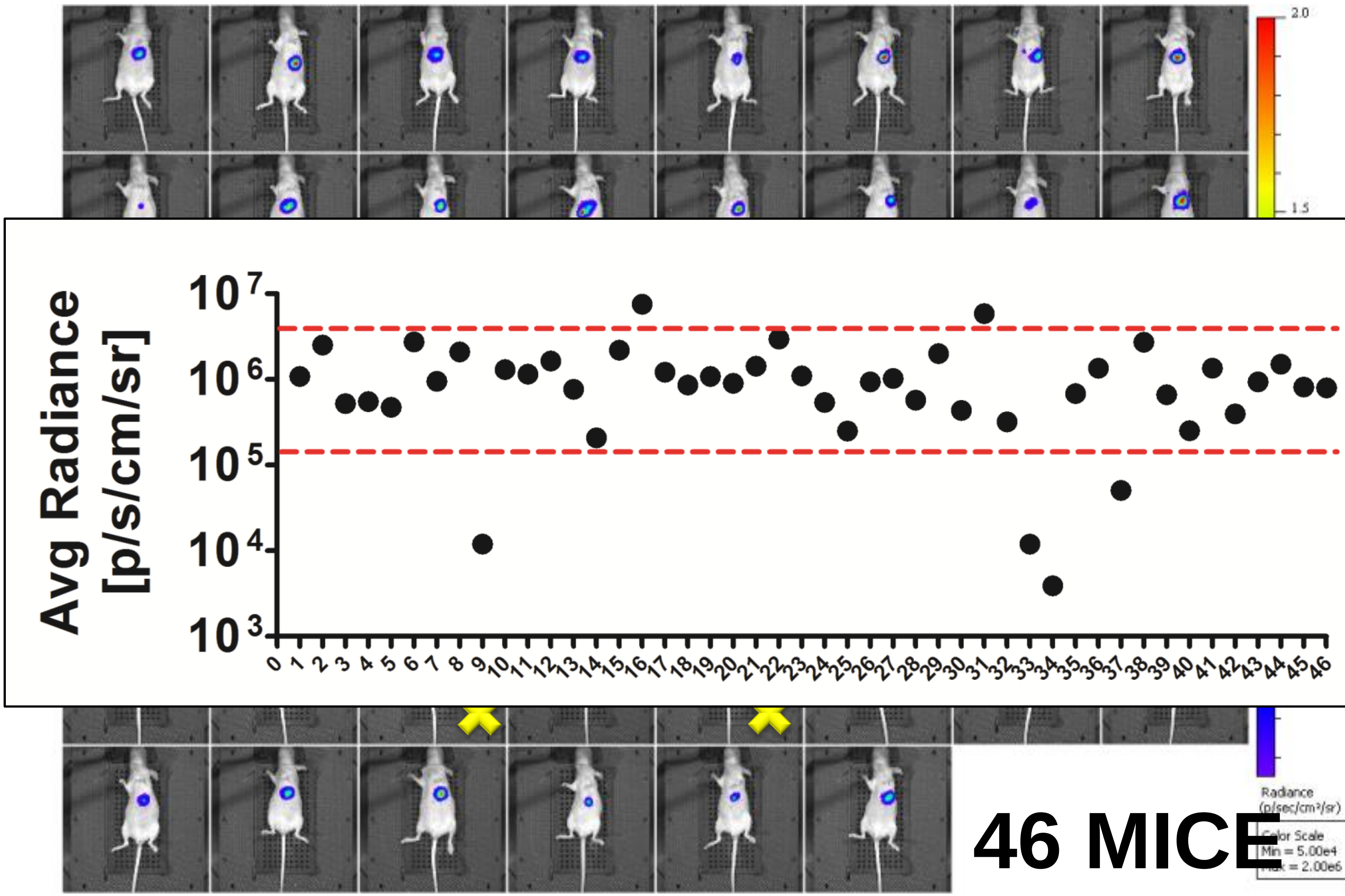
Orthotopic xenograft models of liver tumor

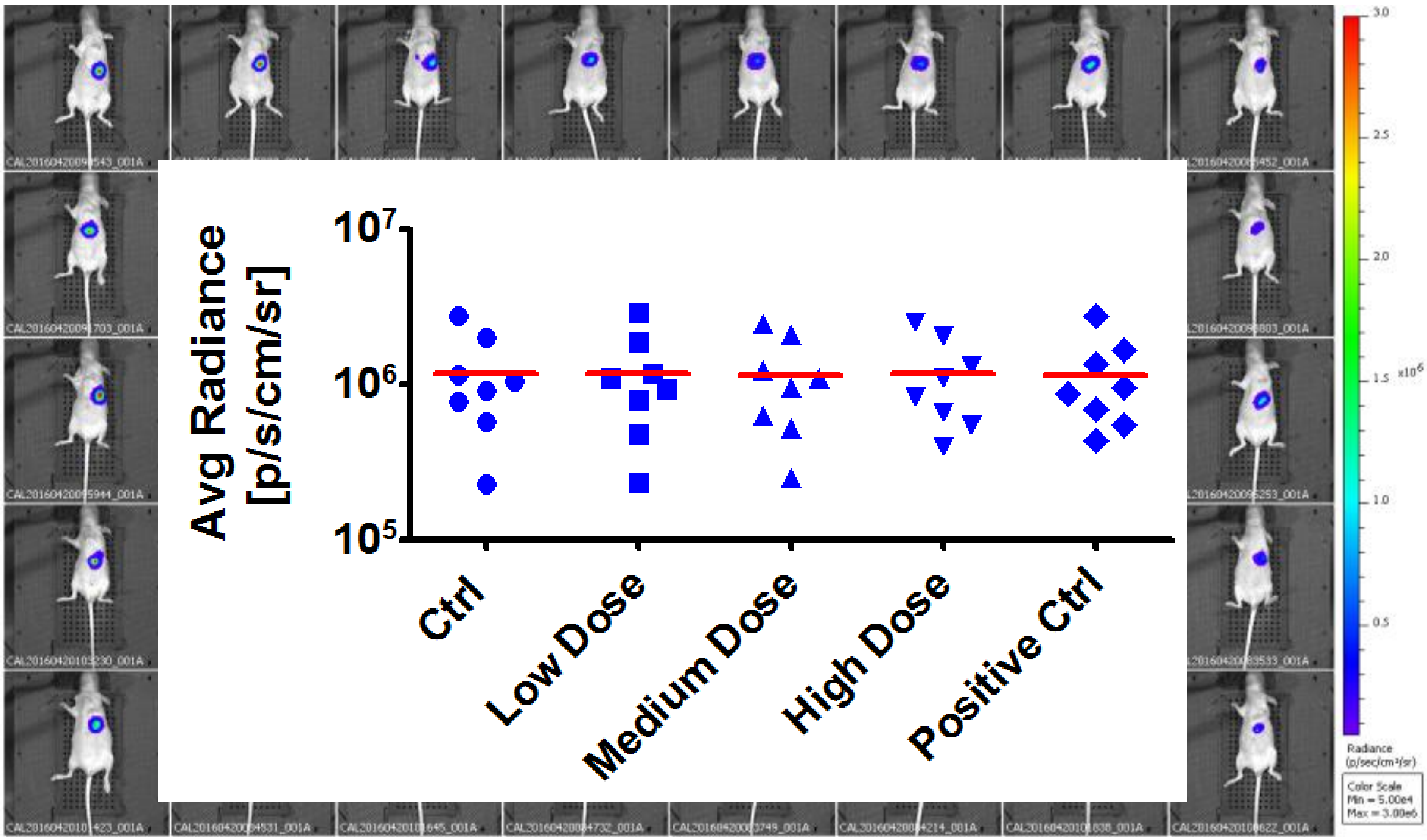
(BLI data before randomized grouping)



46 MICE

Orthotopic xenograft models of liver tumor

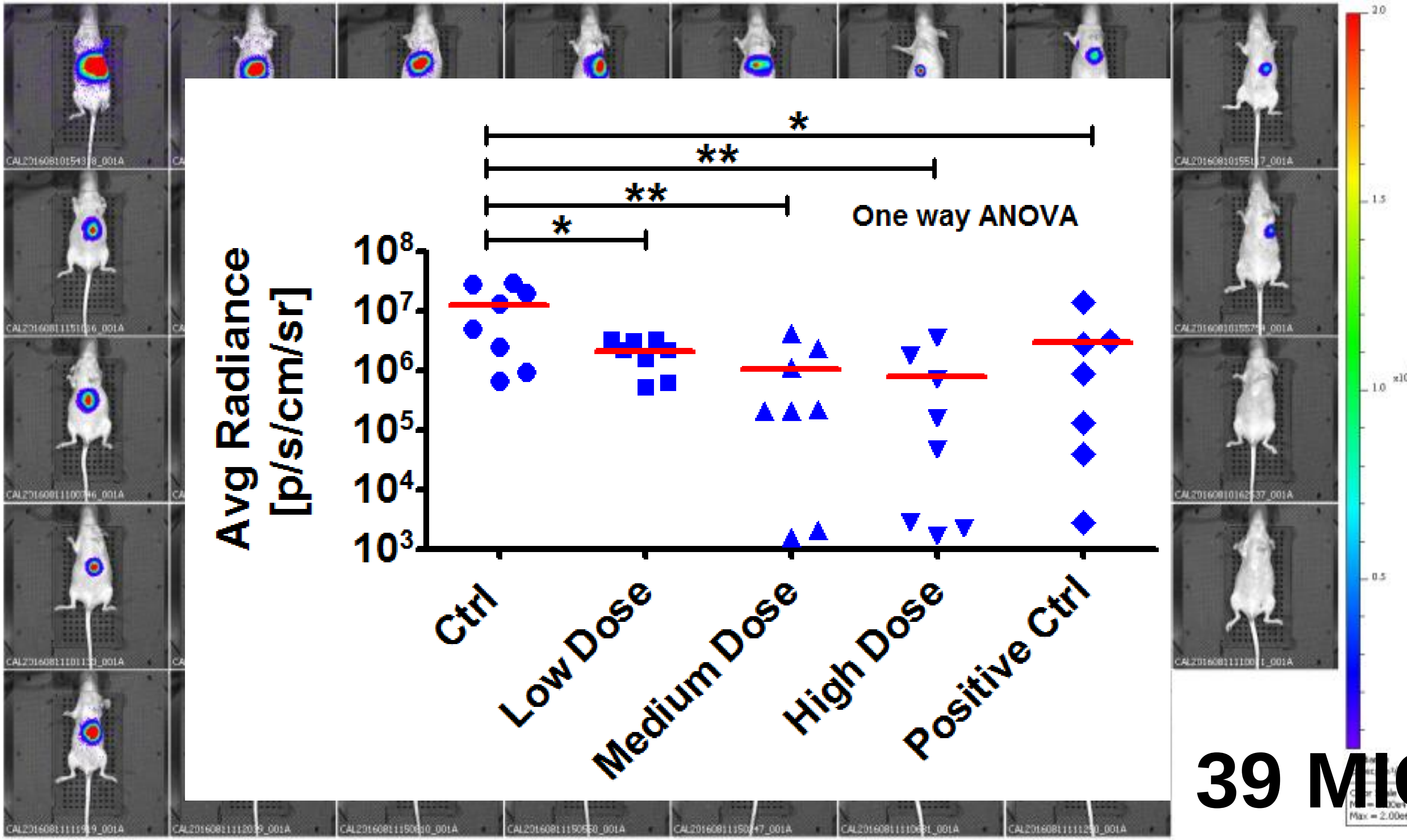
(Randomized grouping before treatment)



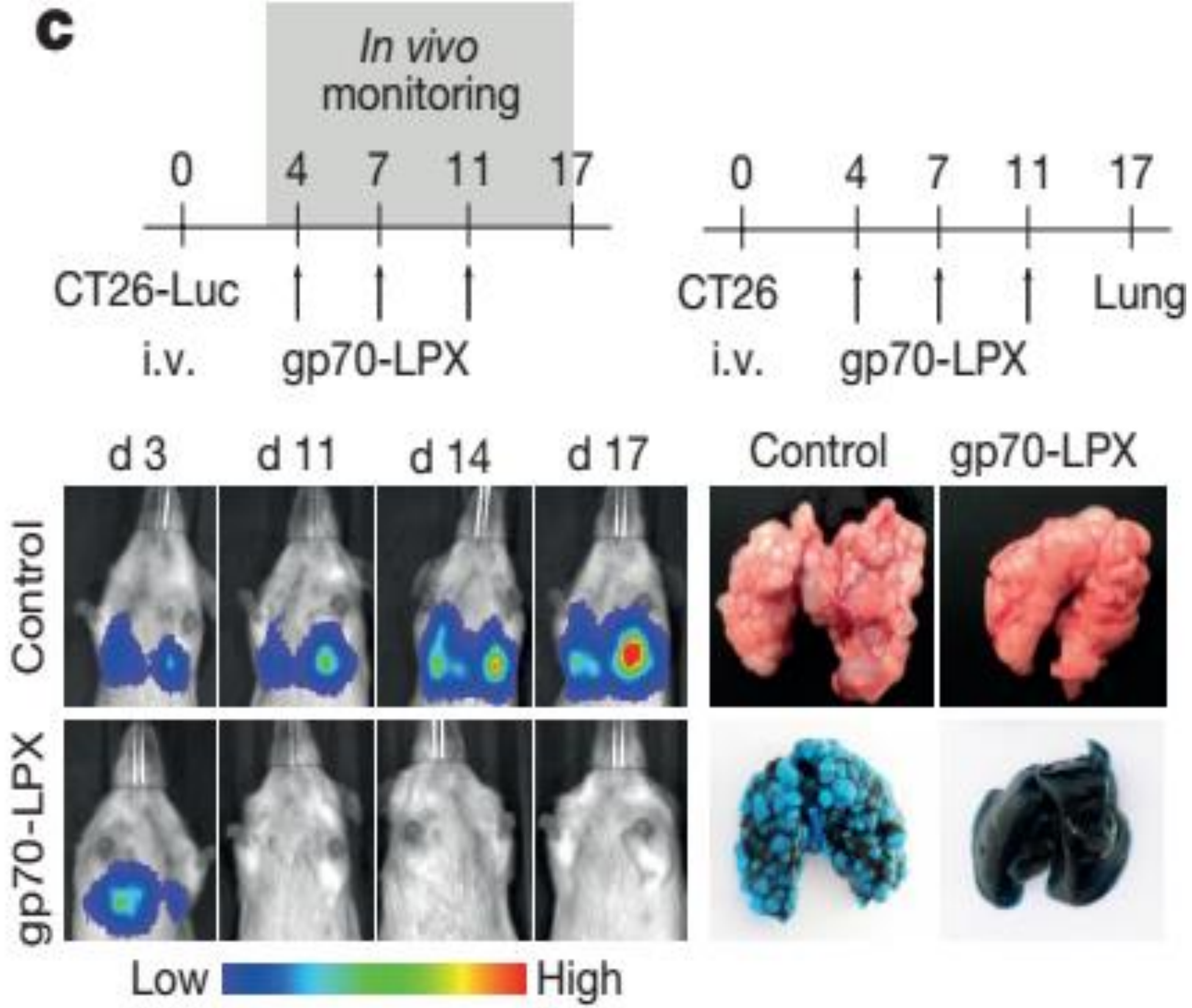
40 MICE

应用举例——肿瘤疗效评价

Orthotopic xenograft models of liver tumor
(BLI data after treatment)

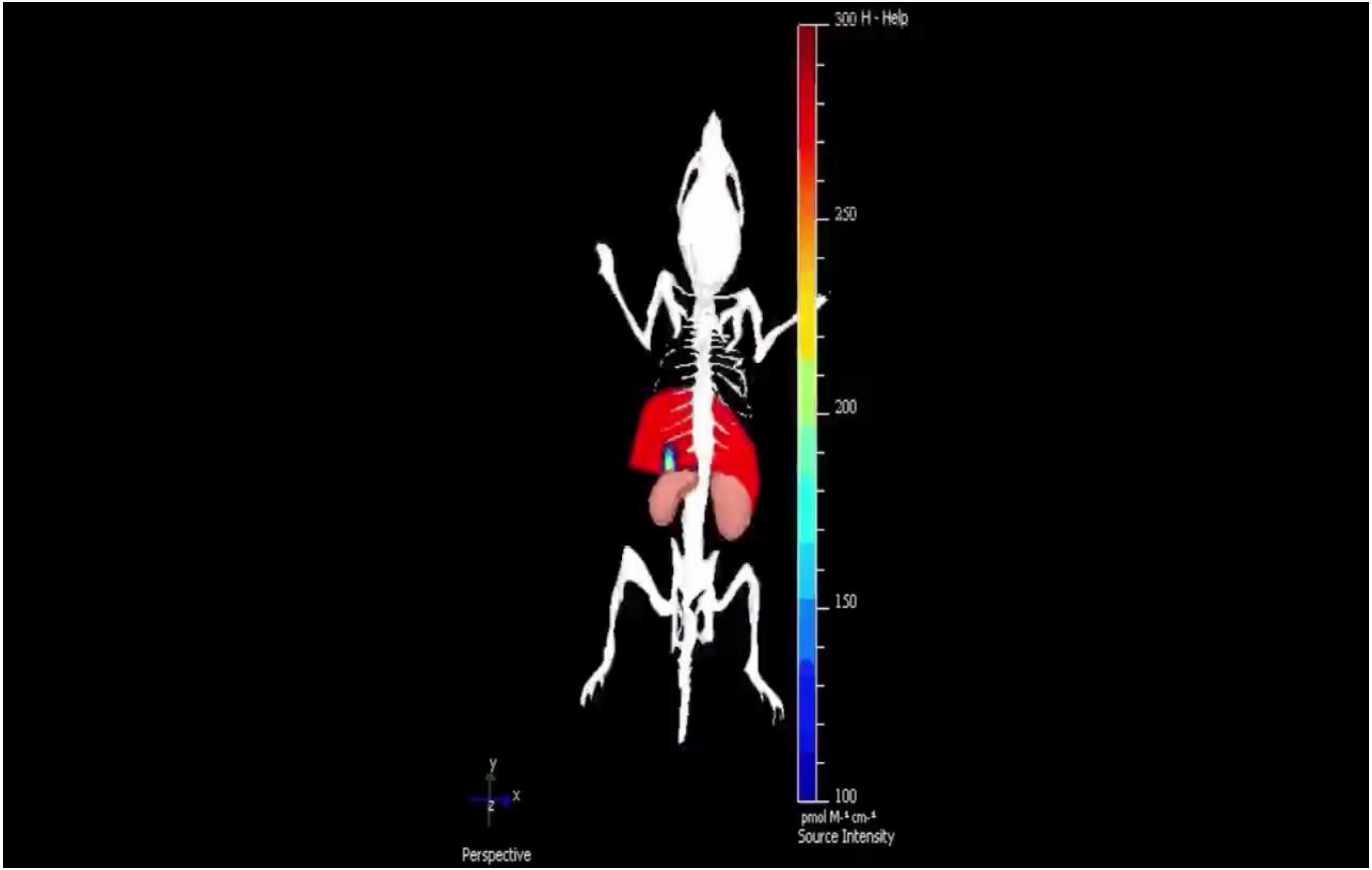


39 MICE


c
In vivo monitoring
0 4 7 11 17
CT26-Luc
i.v.
gp70-LPX
0 4 7 11 17
CT26
i.v.
gp70-LPX
Lung
d 3
d 11
d 14
d 17
Control
gp70-LPX
Control
gp70-LPX
Low
High

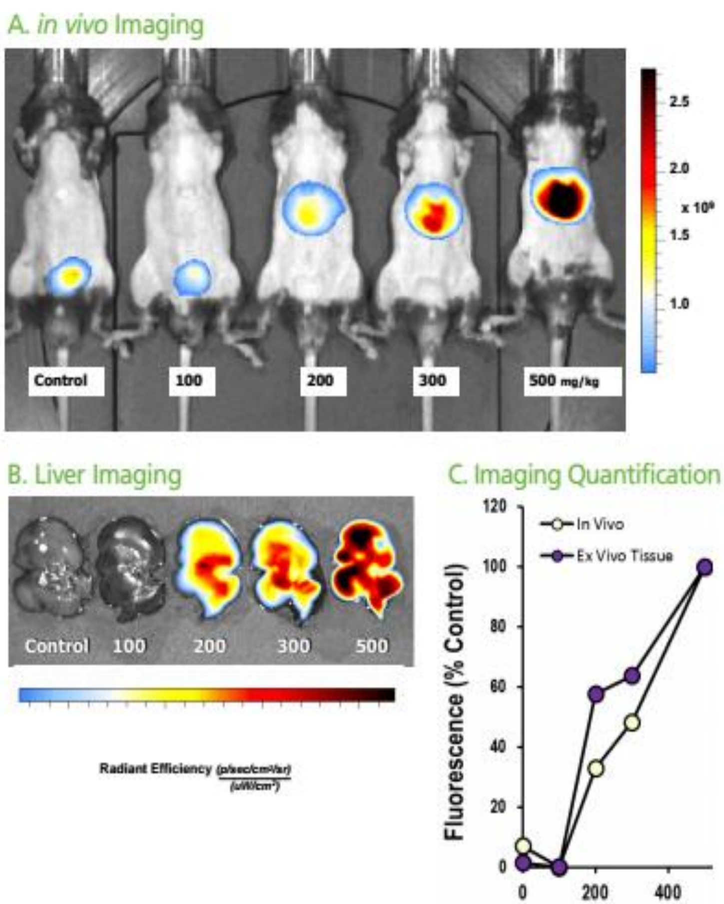
疫苗治疗效果分析

毒性评价——肝脏毒性（肝脏纤维化）



Saimir Luli， et al, Journal of Hepatology,2016

毒性评价——肝脏毒性（肝脏细胞凋亡）

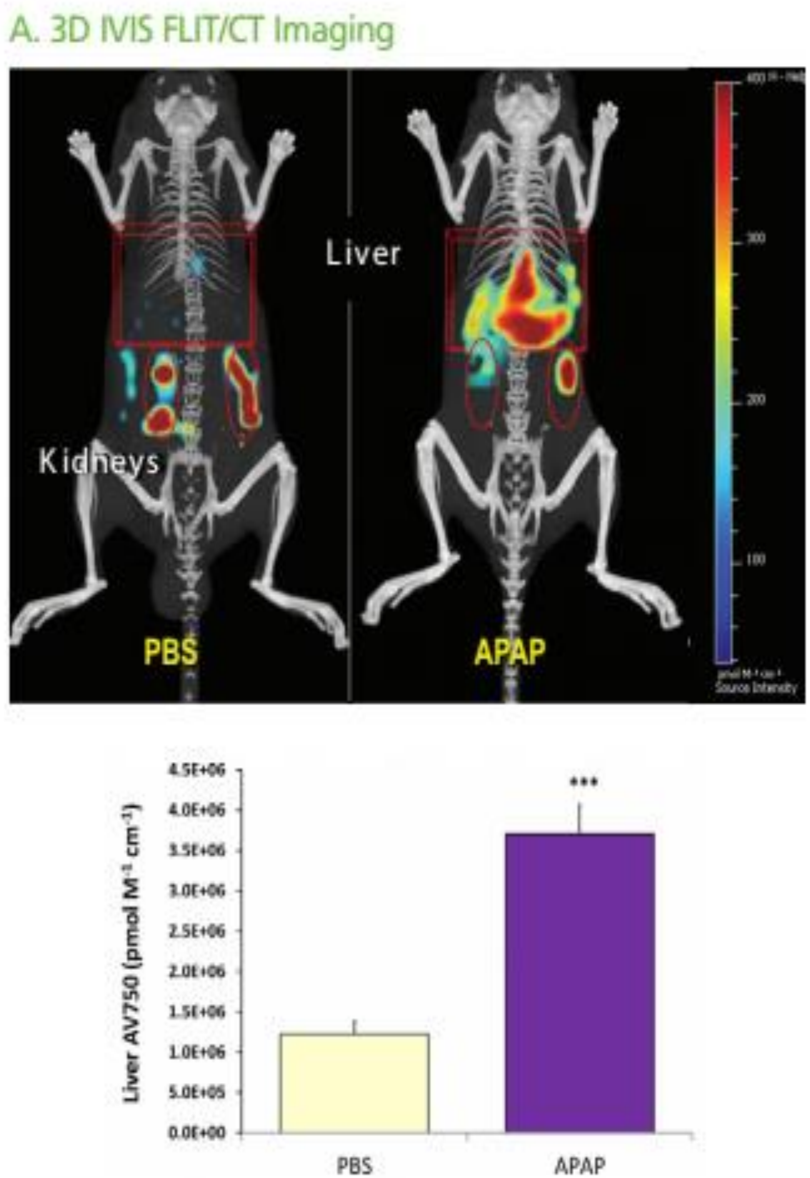


Whole mouse epifluorescence imaging was used to detect accumulation of AV750 following APAP treatment.

A. Epifluorescence images of mice receiving different doses of APAP 24 h prior to imaging shows an increase in signal with APAP dose.

B. Epifluorescence imaging of excised livers from APAP treated mice.

C. Quantification of liver signal from non-invasive imaging and ex vivo imaging was determined by ROI placement to capture the entire liver, and results were represented as the percent of the 500 mg/kg group.



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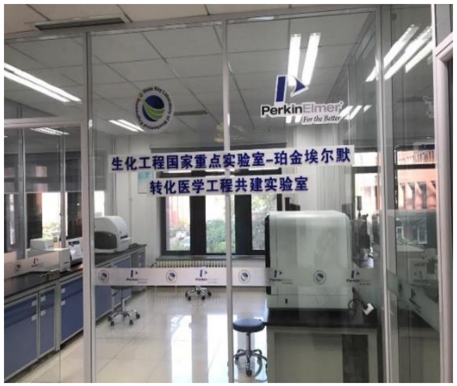





整体解决方案--完备的技术服务平台及应用支持团队

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北京大学 PEKING UNIVERSITY 1898

复旦大学 Fudan University

中国科学技术大学 1958 University of Science and Technology of China

南开大学

上海交通大学 Shanghai Jiao Tong University

南京大学 NANJING UNIVERSITY

吉林大学 JILIN UNIVERSITY CHINA 1946

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中山大学 SUN YAT-SEN UNIVERSITY

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Cell

LSD1 Is a Subunit of the NuRD Complex and Targets the Metastasis Programs in Breast Cancer

Yan Wang,[1] Hua Zhang,[1] Yupeng Chen,[1] Yimin Sun,[1] Fen Yang,[1] Wenhua Yu,[1] Jing Liang,[1] Luyang Sun,[1] Xiaohan Yang,[1] Lei Shi,[1] Ruifang Li,[1] Yanyan Li,[1] Yu Zhang,[1] Qian Li,[1] Xia Yi,[1] and Yongfeng Shang[1,*]
[1]Key Laboratory of Carcinogenesis and Translational Research, Ministry of Education, Department of Biochemistry and Molecular Biology, Peking University Health Science Center, Beijing 100191, China
*Correspondence: yshang@hsc.pku.edu.cn
DOI 10.1016/j.cell.2009.06.050

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