SPECTRAL INSTRUMENTS imaging

SPECTRAL Ami　SPECTRAL Lago

SPECTRAL AmiX　SPECTRAL LagoX

SI Imaging

小動物光學成像系統介紹

產品專員
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冷泉港生物科技股份有限公司
Cold Spring Biotech Corp.

主要內容

SI Imaging 公司簡介

SI Imaging 小動物活體成像系統特性

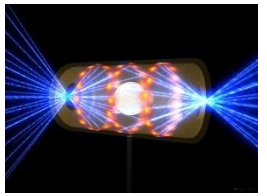
SI Imaging 應用

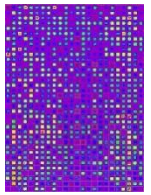
SI Imaging 公司背景

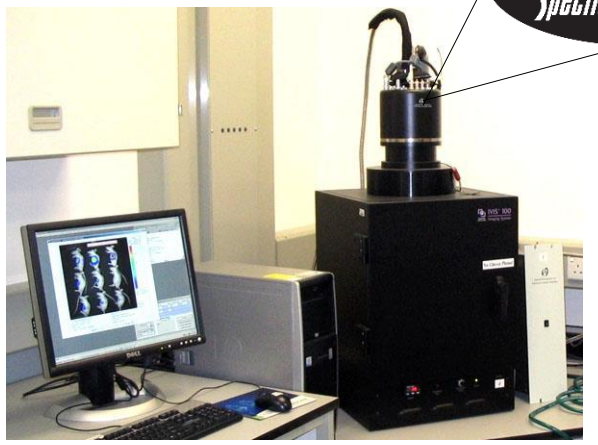
Bo Nelson and Mike Cable

SI Imaging 公司是活體成像系統研發生產領域的新貴。10多年前全球第一台小動物活體成像系統Xenogen IVIS100誕生在香港，發明人為SI Imaging公司現任的CTO-Bo Nelson，自此開啟了小動物活體成像研究之路。

Spectral Instruments (SI) 公司是世界頂級CCD製造商，是SI Imaging公司的盟友，該公司生產的CCD全球故障率最低，廣泛應用於宇宙、天文及等離子研究領域。世界上第一台高靈敏度成像系統IVIS 100即採用SI公司的CCD。





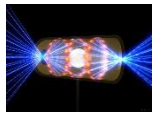


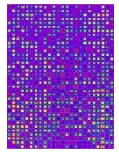
IVIS 100

SI Imaging 公司背景

Who We Are?

Spectral Instruments Imaging (SI Imaging) came about by teaming the camera expertise of Spectral Instruments with the system expertise of the developers of the first high sensitivity laboratory imaging systems designed primarily for the demanding application of bioluminescent animal imaging





Bo and Mike

Bo Nelson, Founder and Chief Technical Officer

- was in charge of the instrumentation department of Xenogen

Mike Cable, Founder and Advisor

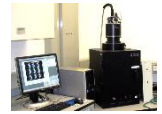
- CTO of Xenogen

Keith Copeland, Founder and CEO

- has been CEO of Spectral Instruments for 20 years

Gary Sims Ph.D., Founder and President

- has 30 years experience developing and testing solid state imaging sensors and systems



SI Imaging 小動物活體成像系統特性

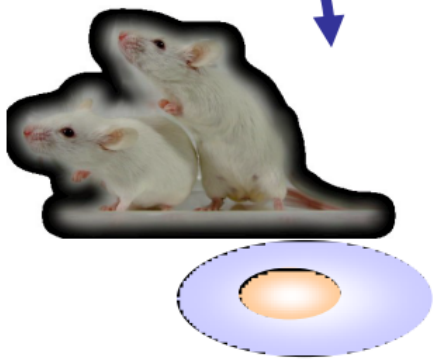
可見光成像原理介紹

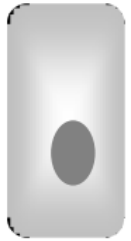
is a natural biological process by which certain organisms can generate light

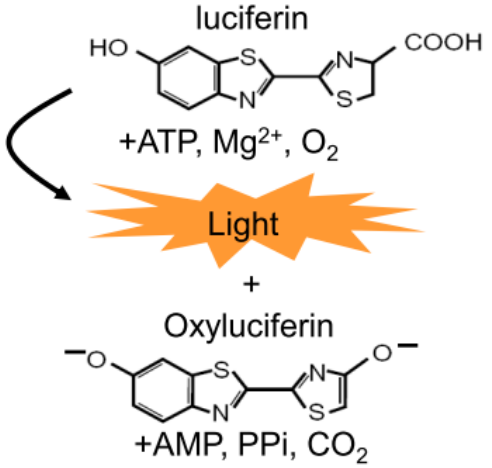
Bioluminescence

Luciferase

Renilla



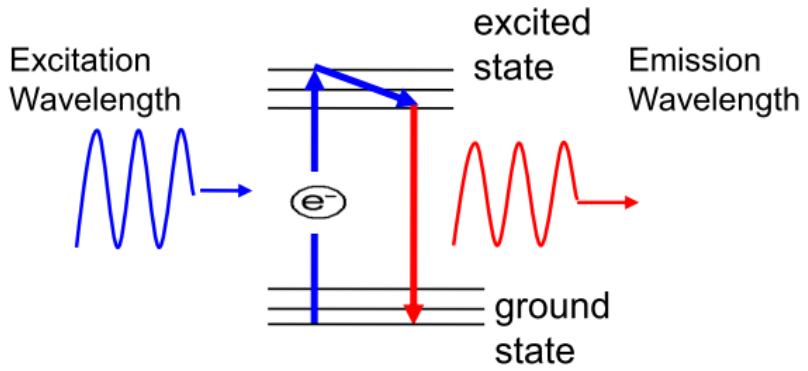




GFP

Quantum dot

NIR dye

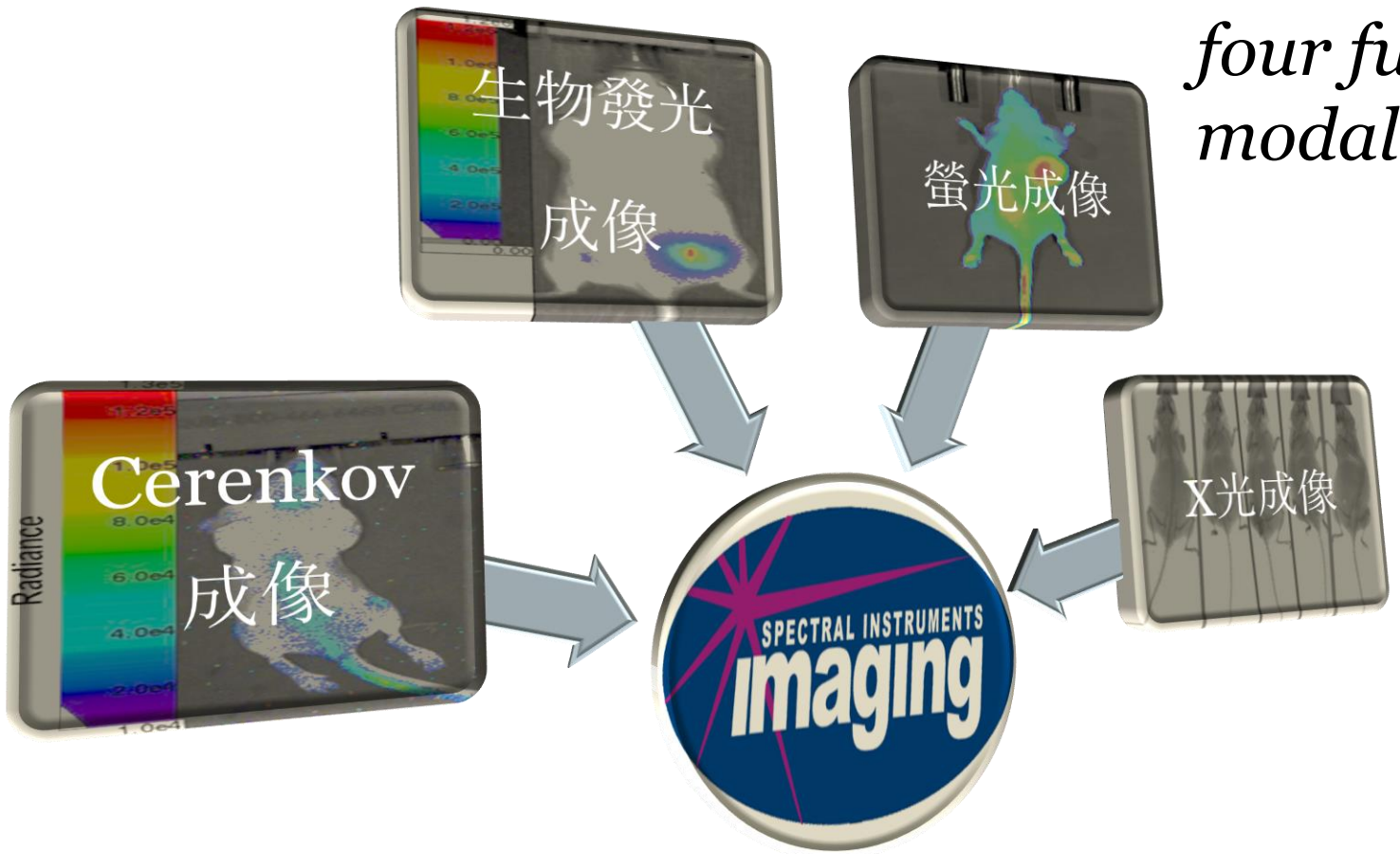


Fluorescence

protein (flurophore) generate light when “excited” by an external light source of a specific wavelength

具有多模組成像功能的活體光學系統

SI Imaging sensitive to all four functional imaging modalities.



SI Imaging 活體光學成像產品

SPECTRAL Ami / SPECTRAL AmiX

完全氣冷式CCD鏡頭

快速及高解析度的活體螢光系統

以LED為基礎的螢光活體系統

- 10個發射波長 (430, 465, 500, 535, 570, 605, 640, 675, 710, 745nm)
- 10個激發波長濾片插槽
- 20個激發濾光片可供選擇 (490, 510, 530, 550, 570, 590, 610, 630, 650, 670, 690, 710, 730, 750, 770, 790, 810, 830, 850, 870nm)

大的動態範圍

大的照野範圍

操作簡單的軟體控制系統及分析軟體

SPECTRAL Lago / SPECTRAL LagoX

完全氣冷式CCD鏡頭

快速及高解析度的活體螢光系統

以LED為基礎的螢光活體系統

- 14個發射波長 (360, 405, 430, 465, 500, 535, 570, 605, 640, 675, 710, 745, 770 and 805nm)
- 20個激發光濾片插槽
- 20個激發濾光片可供選擇 (490, 510, 530, 550, 570, 590, 610, 630, 650, 670, 690, 710, 730, 750, 770, 790, 810, 830, 850, 870nm)
- *可進行近紅外光*

大的動態範圍

市面上最大的照野範圍

簡單操作的軟體控制系統及分析軟體

產品規格

產品比較規格項目		Ami/Ami X	Lago/Lago X
CCD特性	CCD芯片類型	背照射、背部薄化CCD	
	製冷方式	完全氣冷卻方式	
	CCD溫度	-90	
	CCD尺寸	25.9 x 17.3 mm	27.6 x 27.6 mm
	鏡頭	f/1.2-f/16, 50 mm	
	像素	88 萬 （1152 x 770）	400 萬 (2048 x 2048)
	像素尺寸	22.5 um	13.5 um
	暗電流	<0.0003 e-/pixel/s	
	量子效率	>85%: 500-650 nm	
		>30%: 400-850 nm	
	最小檢測光子數	100 photons/sec/cm² /sr	45 photons/sec/cm² /sr
	FOV大小	10 x 7 cm to 25 x 17 cm	6 x 6 cm to 25 x 25 cm
螢光產品規格	螢光激發光源	LED燈	
	激發光濾光片位置數量	N/A	
	LED燈數量/激發光濾光片數量	10個特定波長的LED燈 (430, 465, 500, 535, 570, 605, 640, 675, 710, and 745 nm)	14個特定波長的LED燈 (360, 405, 430, 465, 500, 535, 570, 605, 640, 675, 710, 745, 770 and 805 nm)
	發射光濾光片位置數量	10位	20位
	發射光濾光片數量	10塊，20塊可選擇 (490, 510, 530, 550, 570, 590, 610, 630, 650, 670, 690,710, 730, 750, 770, 790, 810, 830, 850, and 870 nm)	20塊，20塊可選擇 (490, 510, 530, 550, 570, 590, 610, 630, 650, 670, 690, 710, 730, 750, 770, 790, 810, 830, 850, 870 nm)
	植物成像專用濾光片	1塊	
X光產品規格	X-ray能量範圍	10-40 kev	10-50 kev
	X-ray成像技術	Line scanning imager, TDI (Time Delay Integration)技術	
	X-ray成像視野	25 x 15 cm	25 x 23 cm
	一次X-ray成像輻射劑量	<3 mGy	
Cerenkov成像		可以執行Cerenkov成像，由CCD接受光子訊號	
操作軟體	NIST校準	有	
	光譜分離	有	
硬體規格	儀器大小	56 x 66 x 122 cm	56 x 66 x 211 cm
	影像模組	螢光、生物發光、放射性同位素成像及X光成像	
	氣體麻醉機及管線	有	
	載物台溫控範圍	20-40, 標準配備	

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SI Imaging 活體光學成像系統特點

- 具有高品質的生物發光成像CCD
- 使用新型LED燈的低背景螢光成像系統
- 獨有的線性X光成像技術
- 擁有市面上最大的成像視野，高通量成像
- 軟體操作簡單易懂，能不限次數的在不同的電腦上安裝操作

SI Imaging CCD與各家之比較

	SPECTRAL Lago	IVIS® Spectrum	Bruker Xtreme
			
Array Format	2048*2048	2048*2048	2048*2048
Pixel Size	13.5 × 13.5	13.5 × 13.5	13.5 × 13.5
Cooling Air/Water (20°C ambient)	-90°C air Absolute, Guaranteed	-80°C air -90°C liquid Absolute, Guaranteed	-65°C air (-55°C 'guaranteed')
Dark Current: Maximum (e-/p/s)	0.0003@-90°C	0.0008@-90°C	0.02@-60°C
Read Noise Maximum (e- rms)	3.5@100kHz 7.0@800kHz	4.0@50kHz 10.0@1MHz 14.0@3MHz	5.0@100kHz 16@2MHz

SI Imaging 生物發光成像特點

一級+高品質CCD，具備最佳檢測靈敏度

- 背照射、背部薄化、絕對-90度、一級+高品質CCD
- 量子轉化效率大於85%
- 極低暗電流，低系統背景雜訊，高訊噪比
- CCD超快速冷卻降溫，只需**3min**即可到達-90度
- 一次校準即可，系統無需凌晨自我校準背景

獨有CCD芯片全氣制冷技術

- 無需任何液態製冷劑，無洩漏、細菌滋生等可能性
- 溫度及濕度等環境因素對CCD影響不大
- 故障率低

一體成形，無須任何的組裝

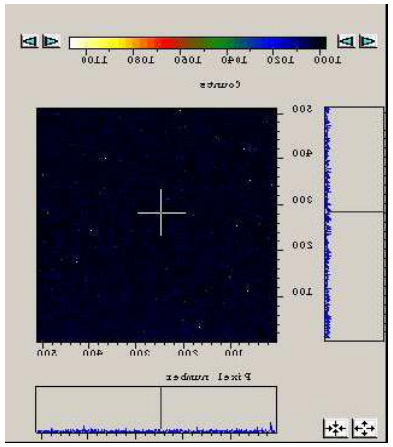
- 減少運輸、組裝過程中對精密零件的損傷

高靈敏度CCD-弱暗電流

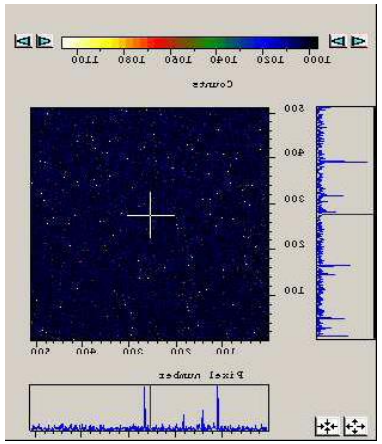
暗電流（Dark Current）

- CCD的固有特性: CCD在一定溫度下每個像素在一定時間內產生的電荷數

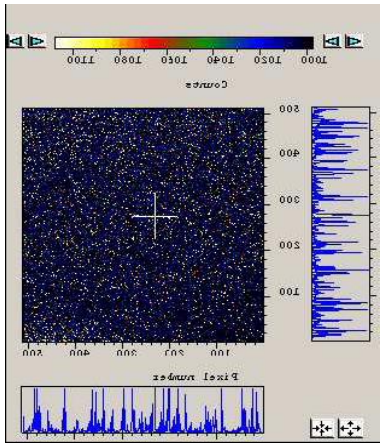
- 溫度越低暗電流越少→雜訊少



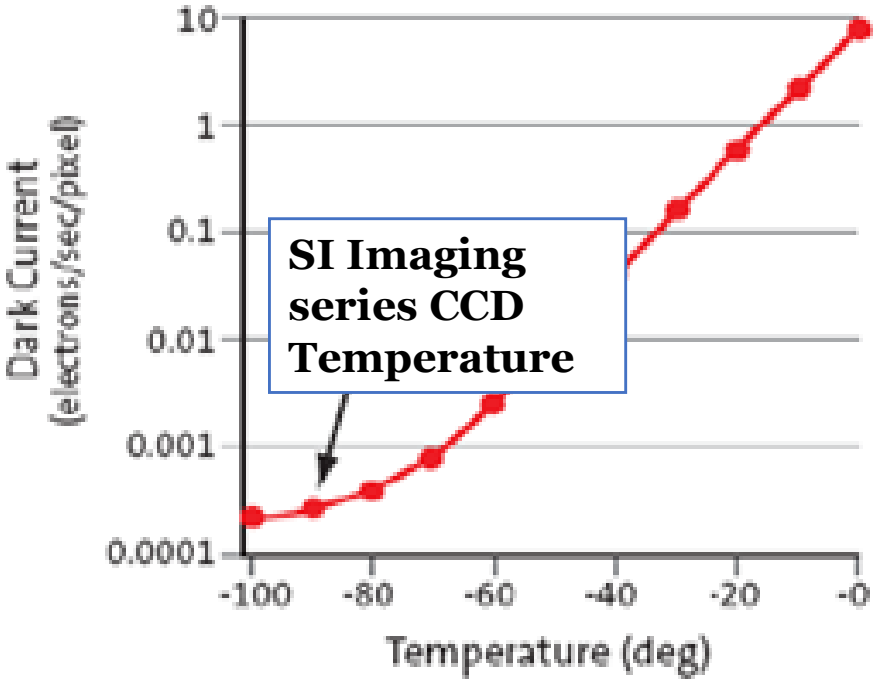
-90℃



-60℃



-30℃



EEV 47-10: Andor and our own measurements Kodak 4021 Product Sheet and http://ccd.com/pdf/AscentLifeSci.pdf and using 7°C for doubling the dark current

活體生物發光成像比較

4T1-luc2 cells implanted subcutaneously on dorsal side of mice

2 hours later, luciferin (150 mg/kg) was injected and mice were imaged

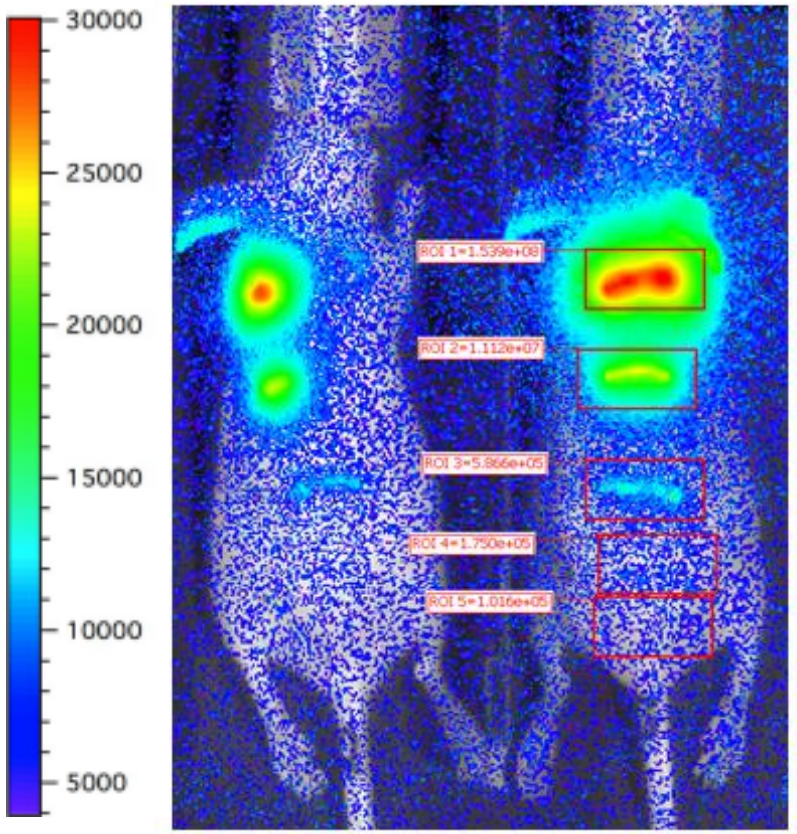
ROI 1=1.539e+08

ROI 2=1.112e+07

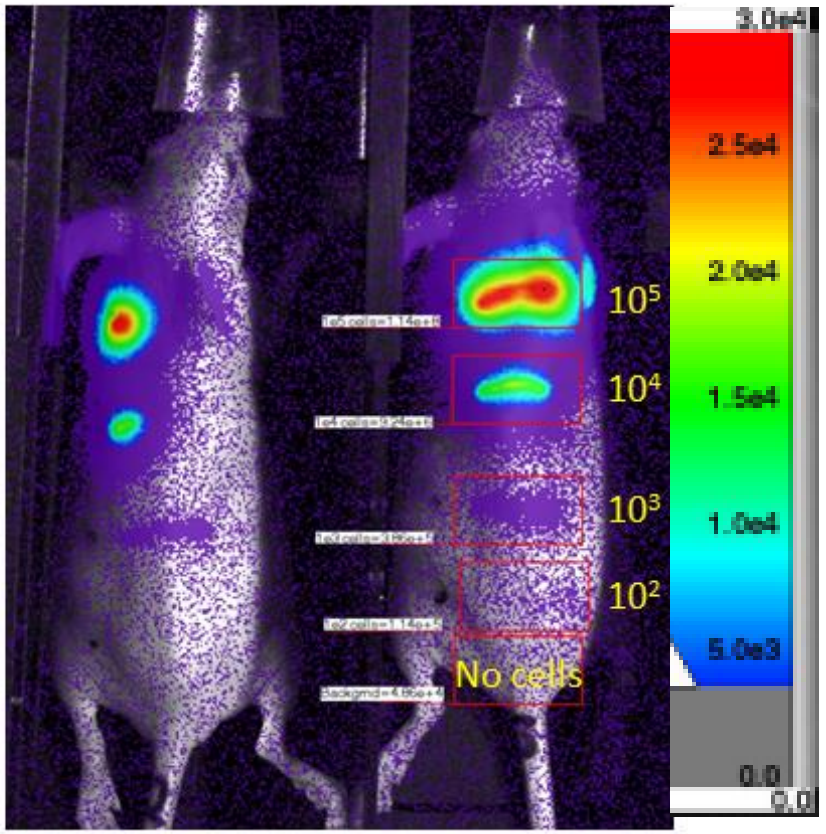
ROI 3=5.866e+05

ROI 4=1.750e+05

ROI 5=1.016e+05



IVIS Spectrum image



SI Imaging Ami X image

ROI 1=1.14e+08

ROI 2=9.24e+06

ROI 3=3.66e+05

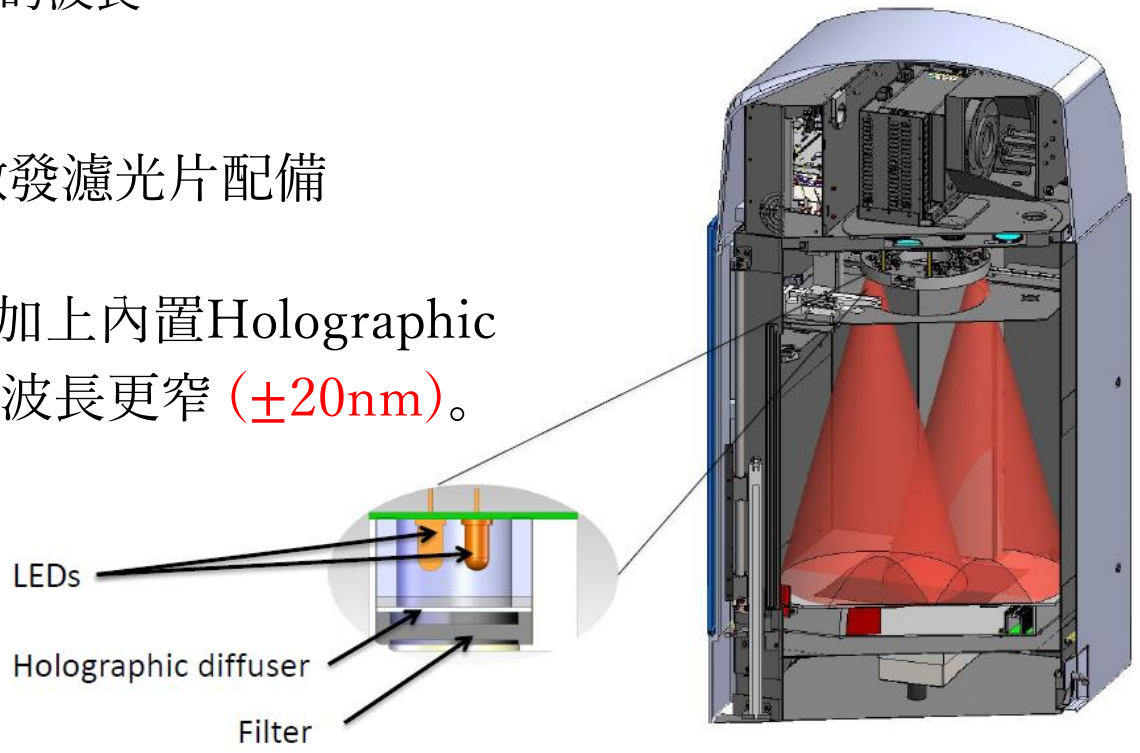
ROI 4=1.14e+05

ROI 5=4.66e+04

SI Imaging 螢光成像特點

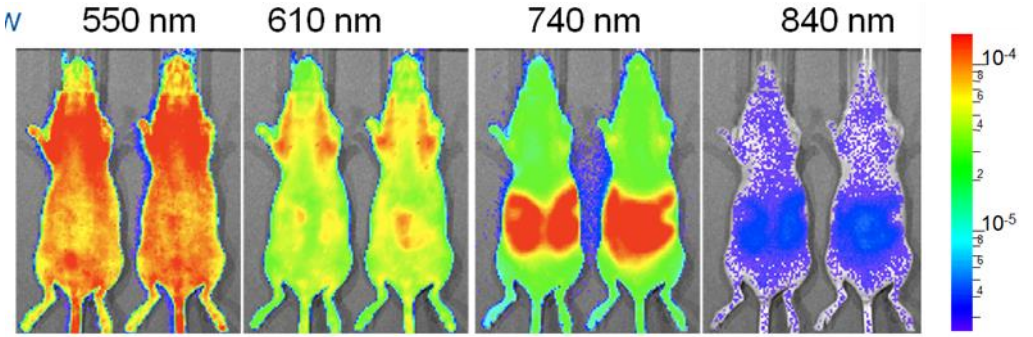
新型LED燈的螢光成像系統

- Light Emitting Diodes (LED)
  - → 使用壽命長，不易耗損
  - → 波長單一，在不加濾光器下可提供多種單純的波長
  - → 穿透力強
- 一個LED燈對應一個特異波段，無須額外激發濾光片配備
- LED發出的光波長範圍集中 (±50nm)，再加上內置Holographic diffusers及高品質notch filter，使激發光的波長更窄 (±20nm)。
- LED燈發射波長選擇多:
  - → Ami/AmiX: 10個LED wavelength
  - → Lago/LagoX: 14個LED wavelength



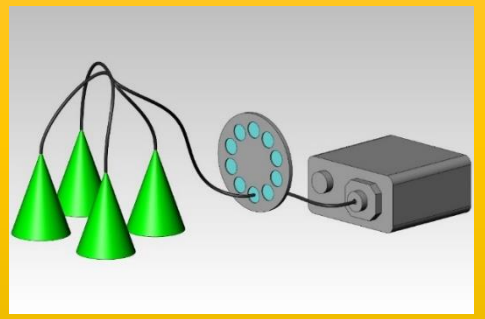
SI Imaging 螢光成像特點

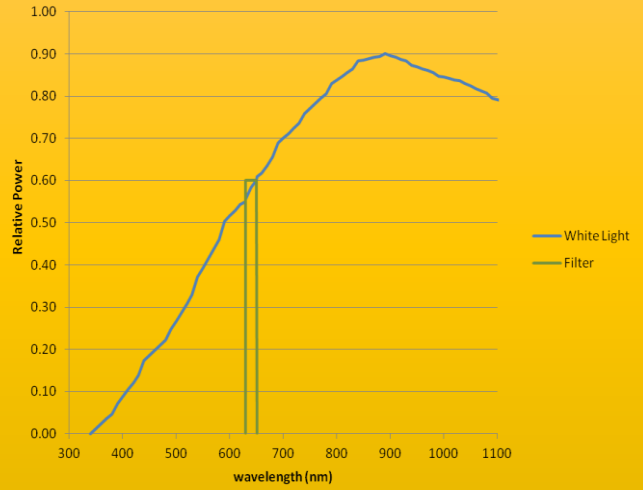
SI Imaging的LED燈發出不同的特定波長，這些波長的單一性要遠遠高於**鹵素燈**發出的**全光譜光源加上普通濾光片**的效果。後者總是無法避免特定波長之外的光透過濾光片而產生非訊號波段的背景螢光。



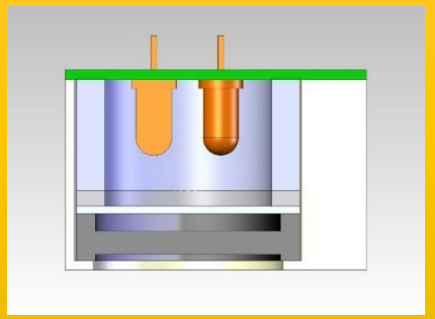
不同波段的光激發裸鼠

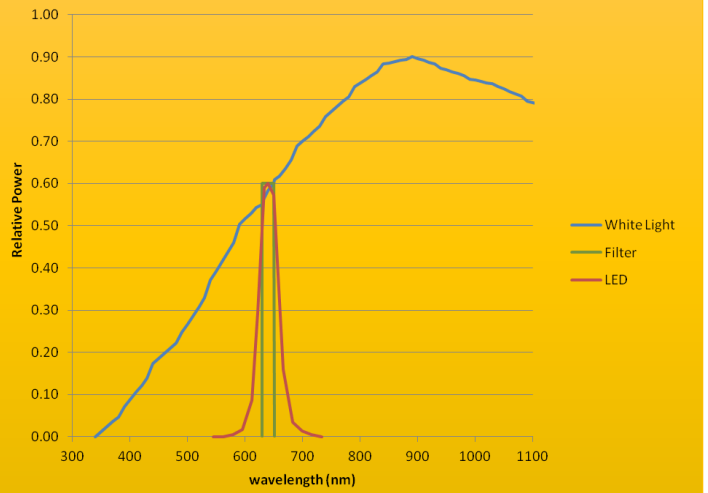
White Light Source Design





LED design

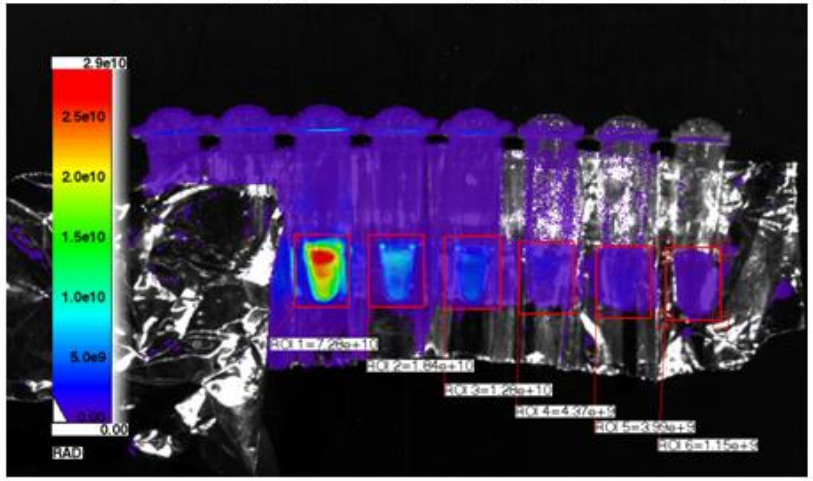


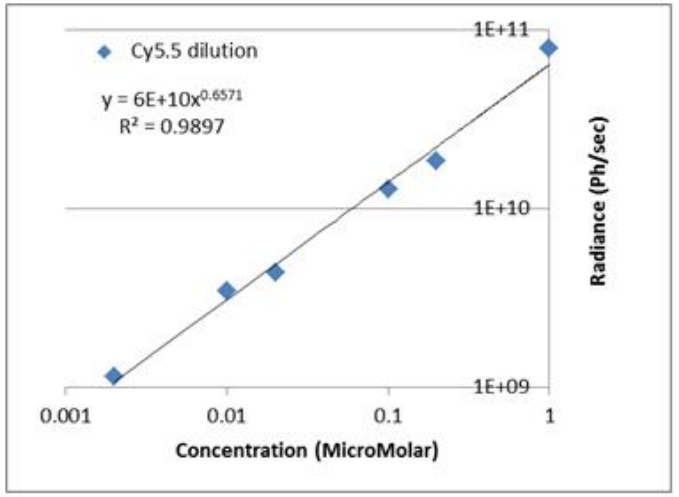


體外螢光成像結果比較

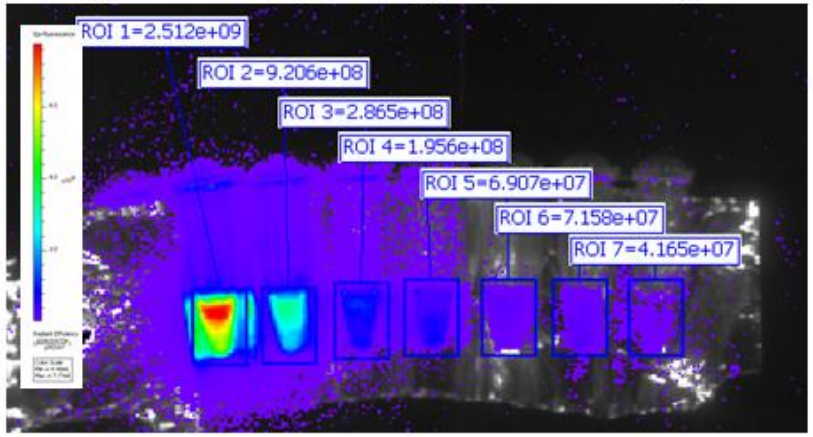
- Alexafluor 700 and Cy5.5 dyes serially diluted in PCR tubes for sensitivity measurements (1x, 5x, 10x, 50x, *etc.* dilutions)
- Tubes imaged on foil background with foil "walls" between tubes to prevent cross-illumination

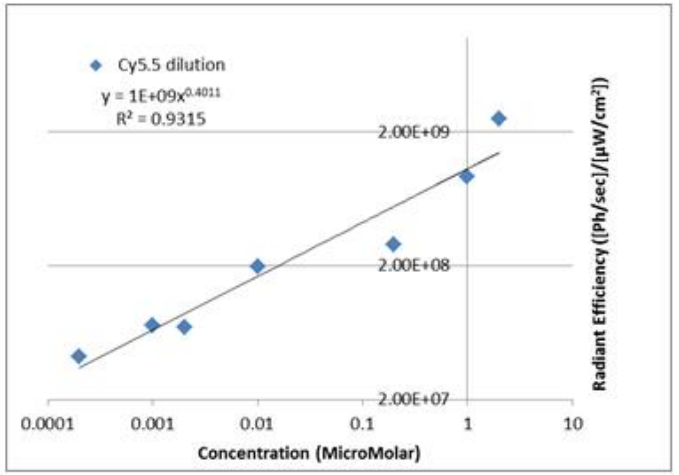
AMI-X (Cy5.5, λ_{ex} = 640nm, λ_{em} = 710nm)





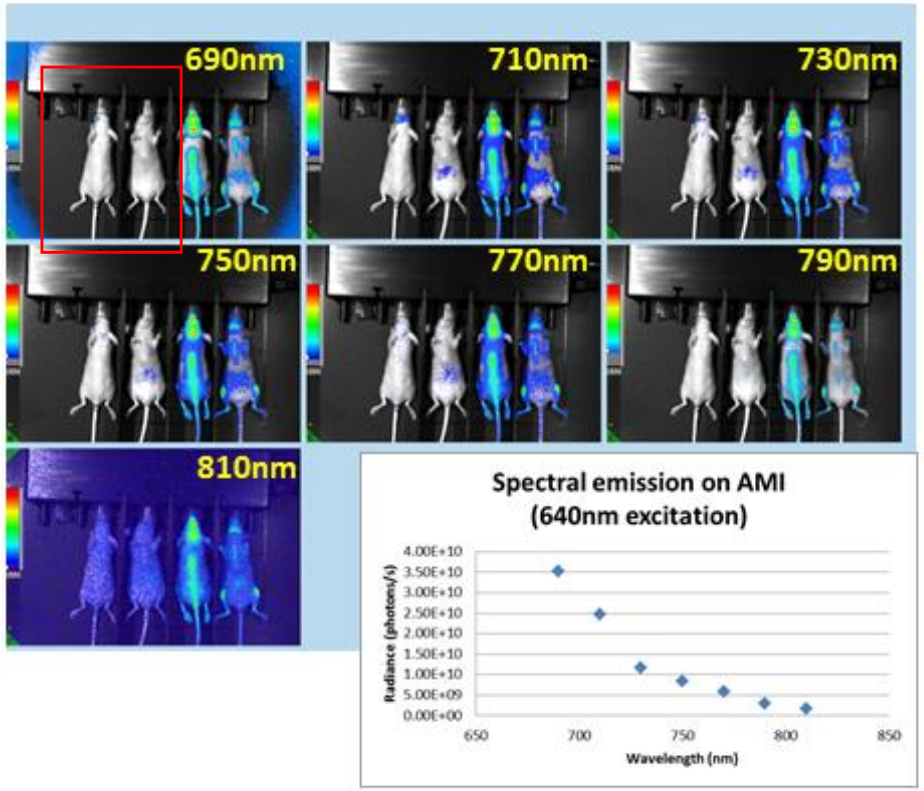
IVIS Spectrum (Cy5.5, λ_{ex} = 640nm, λ_{em} = 720nm)



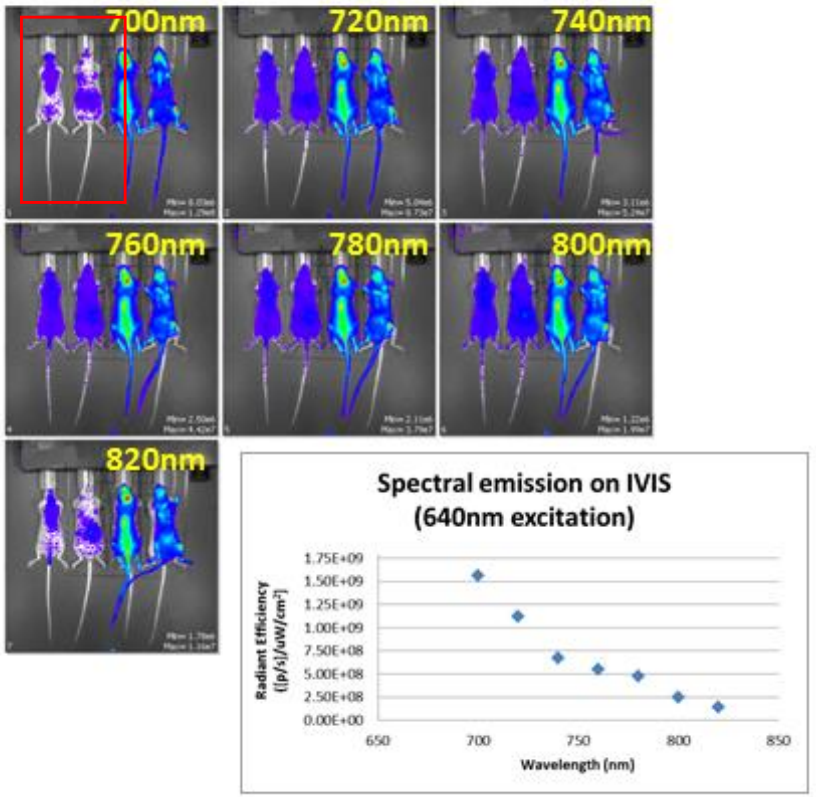


螢光成像結果比較

AMI-X emission spectral images



IVIS Spectrum emission spectral images



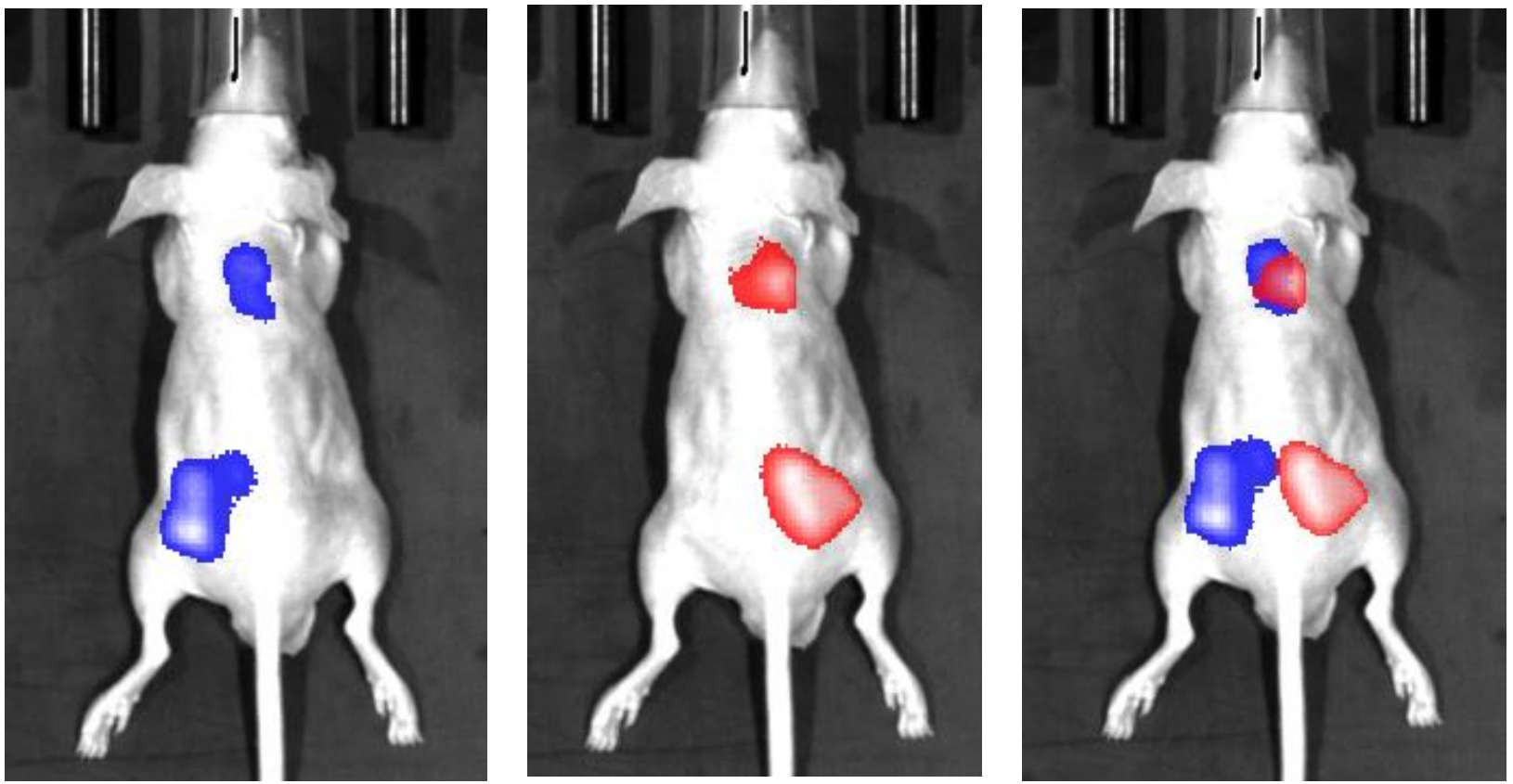
These are the same mice imaged in both an Ami X and a Spectrum. Mice 1 and 2 are controls, the have no dye. Note the background in the control mice on the Spectrum. **CALP/PE has been telling users this is autofluorescence but it's really system background**.

SI Imaging 螢光成像特點

軟體中具備降低背景雜訊的演算法

- ✓背景扣除
- ✓光譜分離

兩種螢光染料活體成像結果



Rhodamine B　　Cy5　　Rhodamine B and Cy5

SI Imaging 系統視野大小

SPECTRAL Ami

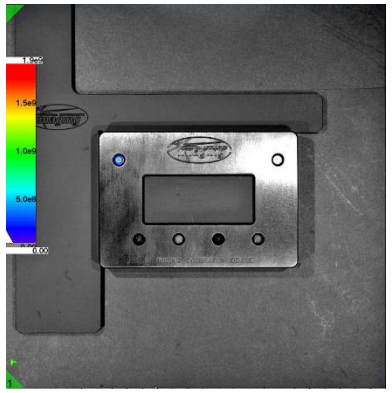
25cm FOV

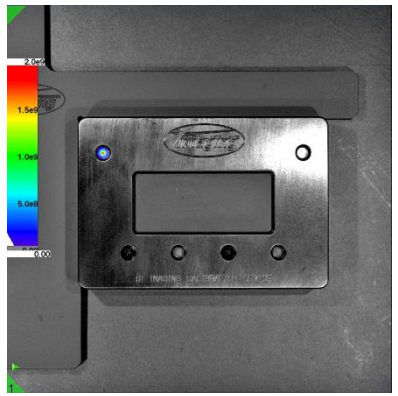
20cm FOV

SPECTRAL AmiX

15cm FOV

10cm FOV

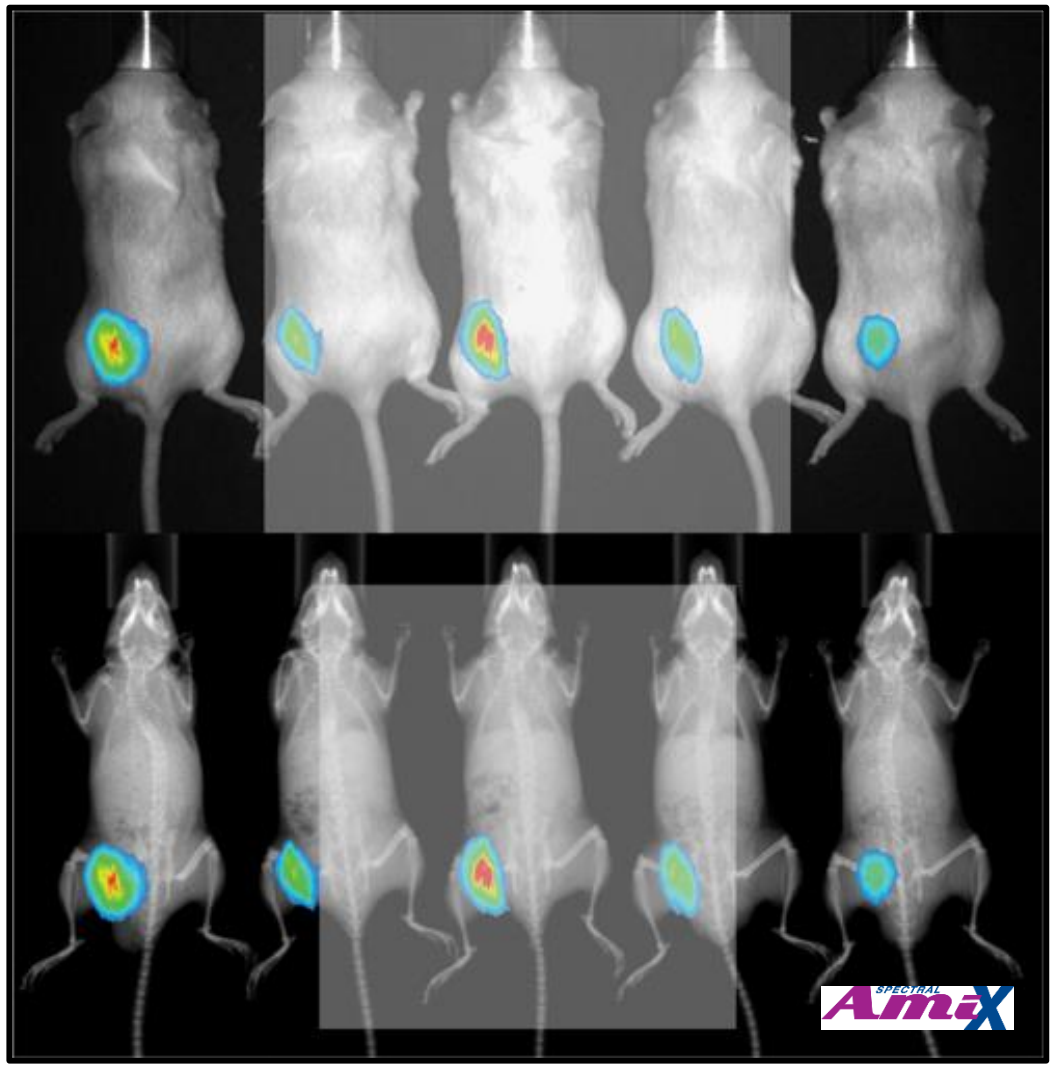




SPECTRAL Lago

SPECTRAL LagoX

SI Imaging AmiX視野大小



具備大的視野範圍

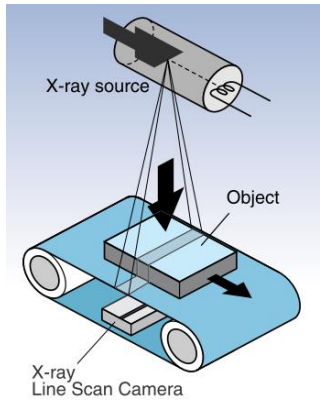
黑色底: Aim/AimX的視野範圍大小

灰色底: Lumina視野範圍大小

	SPECTRAL Ami	SPECTRAL Lago	IVIS Spectrum Series	IVIS Lumina Series III
Imaging field of view	25x17	25x25	23x23	12.5 x 12.5

SI Imaging X-Ray成像特點

HAMAMATSU
PHOTON IS OUR BUSINESS



improve both speed and signal-to-noise ratio

採用線性掃描X光成像技術

使用日本知名廠家: **HAMAMATSU**

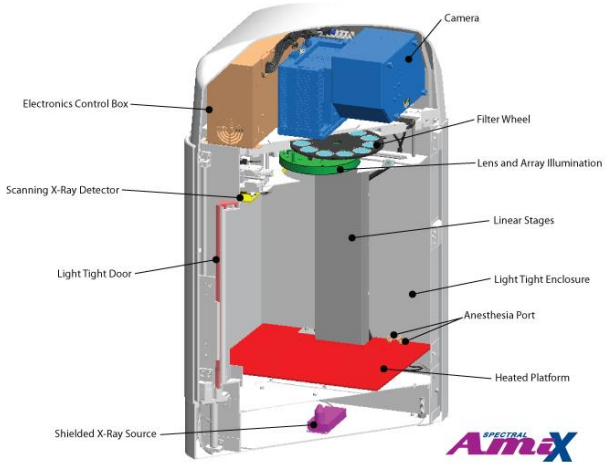
使用Time Delay Integration掃描技術→提升掃描速度及增加訊雜比

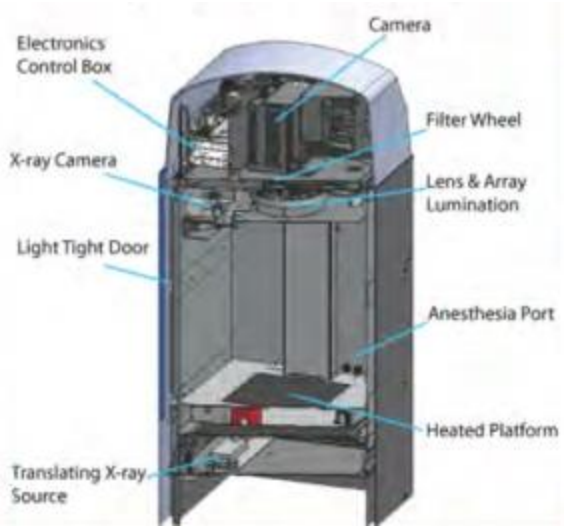
靈敏度高

快速影像擷取→20s成像一次

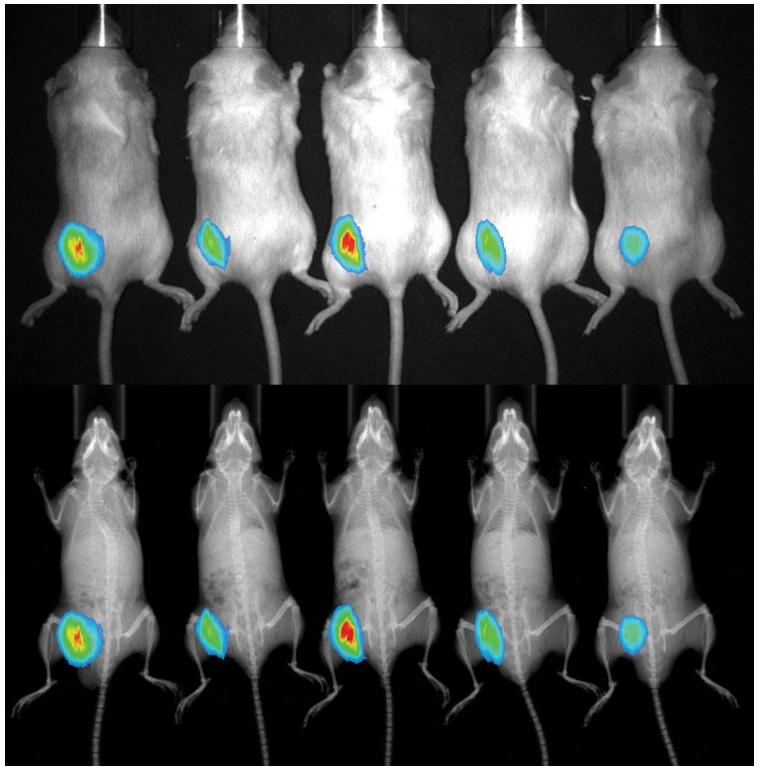
低輻射劑量，安全性高→一次X光成像的輻射劑量小於3mGy.

高通量的X光成像→同時5只小鼠掃描





X-Ray 活體成像實驗結果



	Ami	Lago	IVIS Lumina XRMS Series III
X-ray source	10-40 keV	10-50 keV	10-40 keV
X-ray field of view	25 x 15 cm	25 x 23 cm	10 x 10 cm

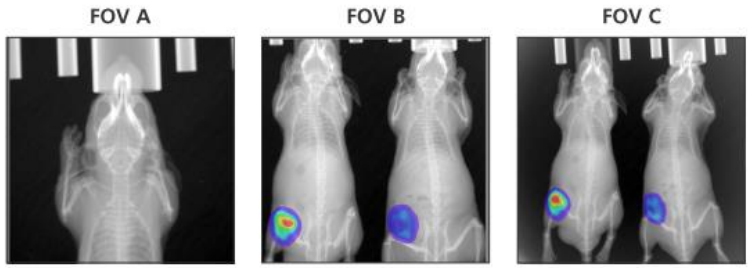
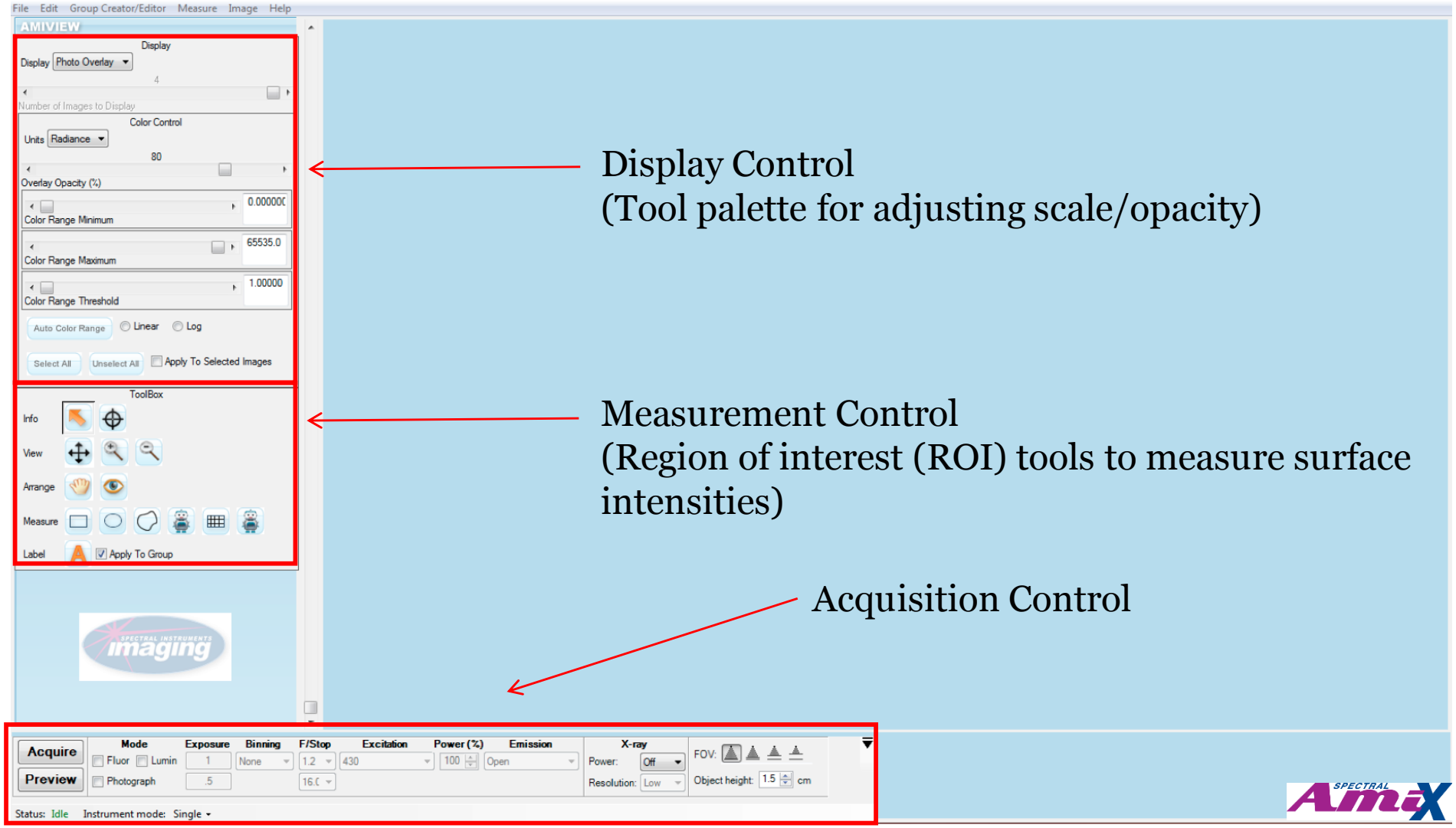
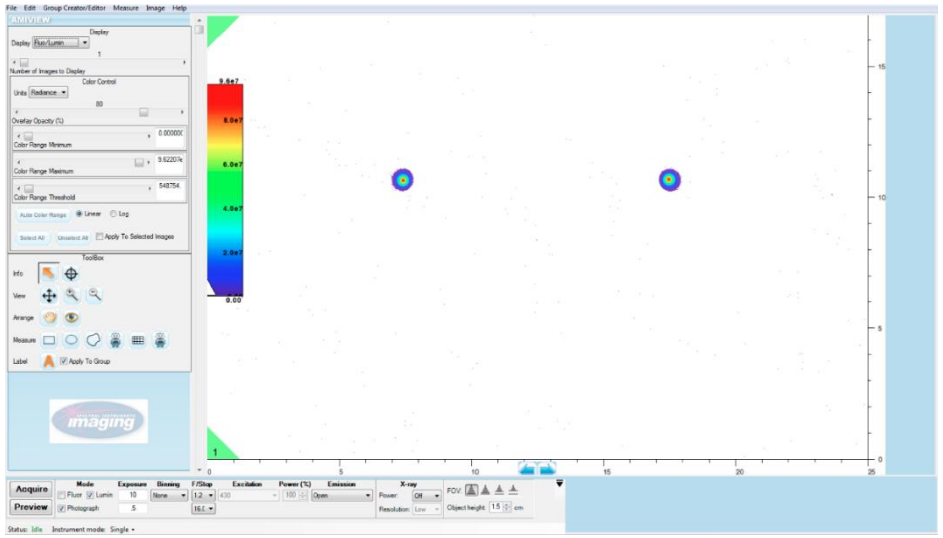


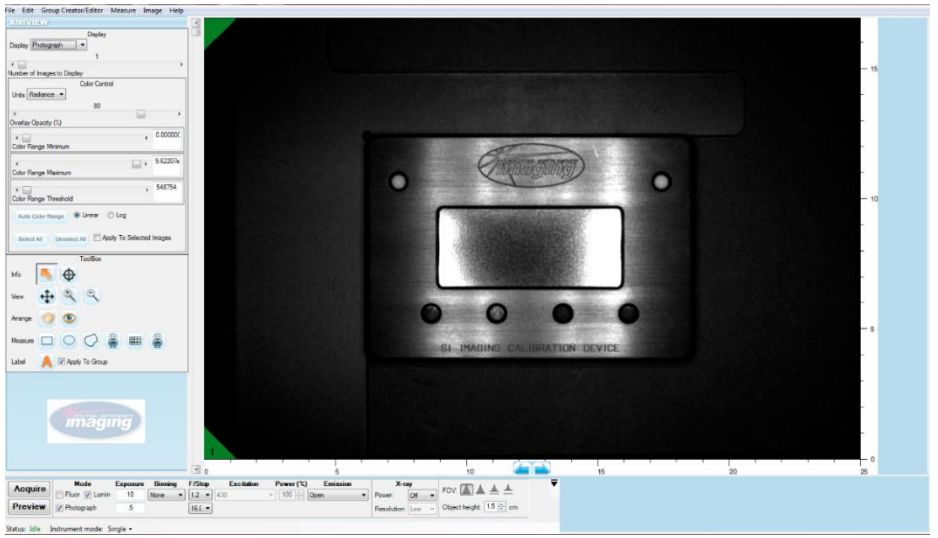
Figure 2. Optical overlay of bioluminescent signal with X-ray image at multiple FOV's in mice.

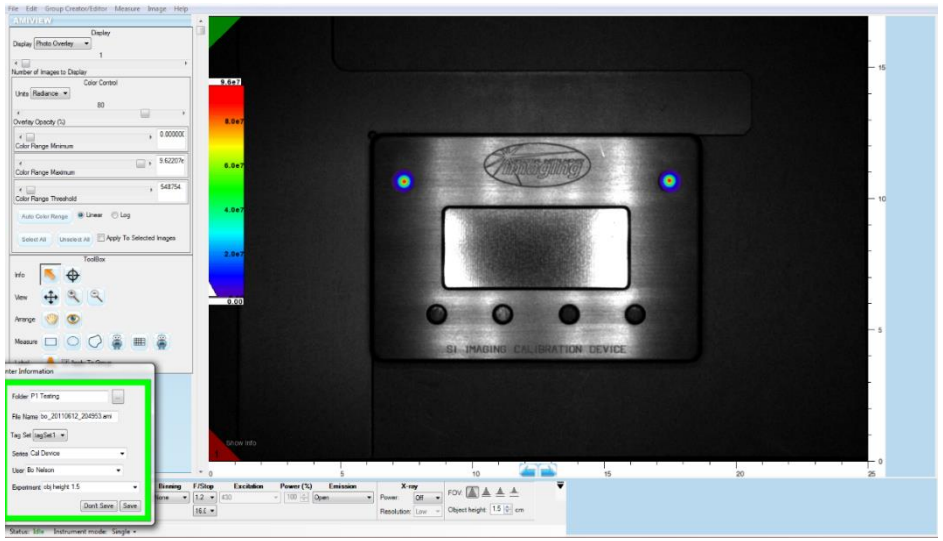
SI Imaging AMIView 軟體介紹



SI Imaging AMIView 軟體介紹







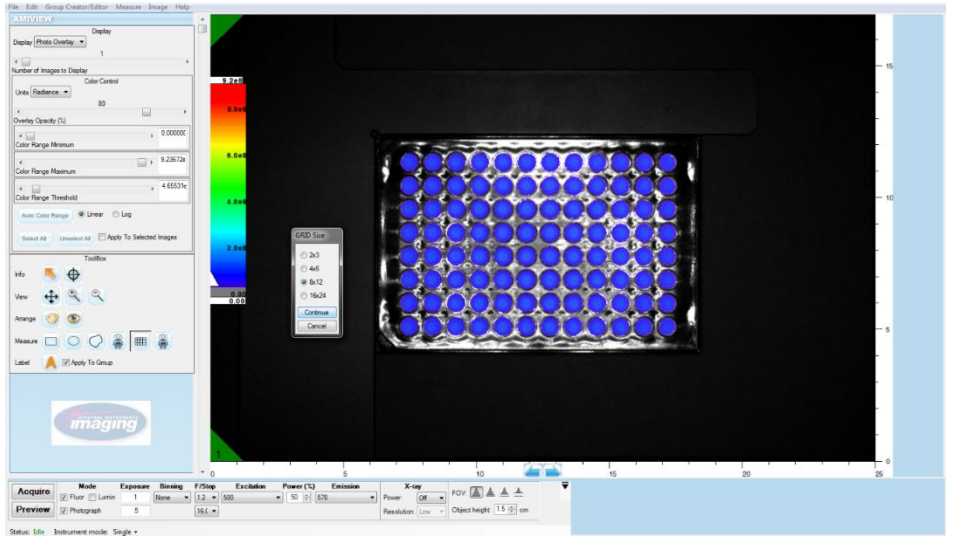
量測及感興趣區域圈選

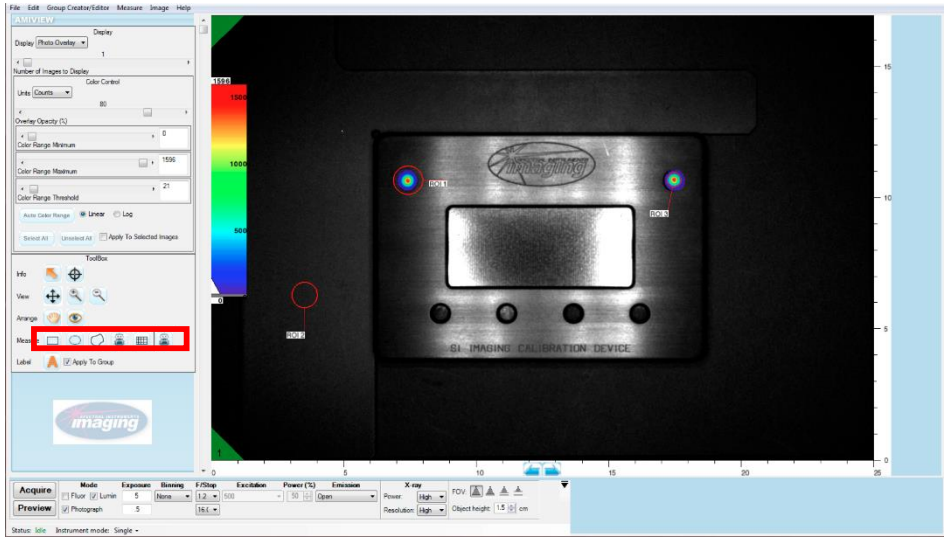
可選擇圈選ROI之形狀

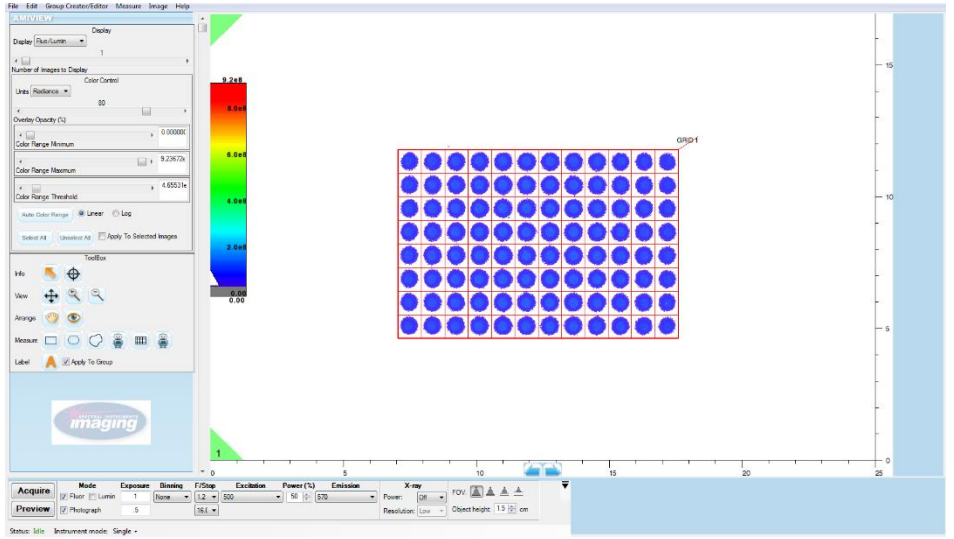
- 方形
- 圓形
- 輪廓狀
- 網格狀

ROIs產生的方式

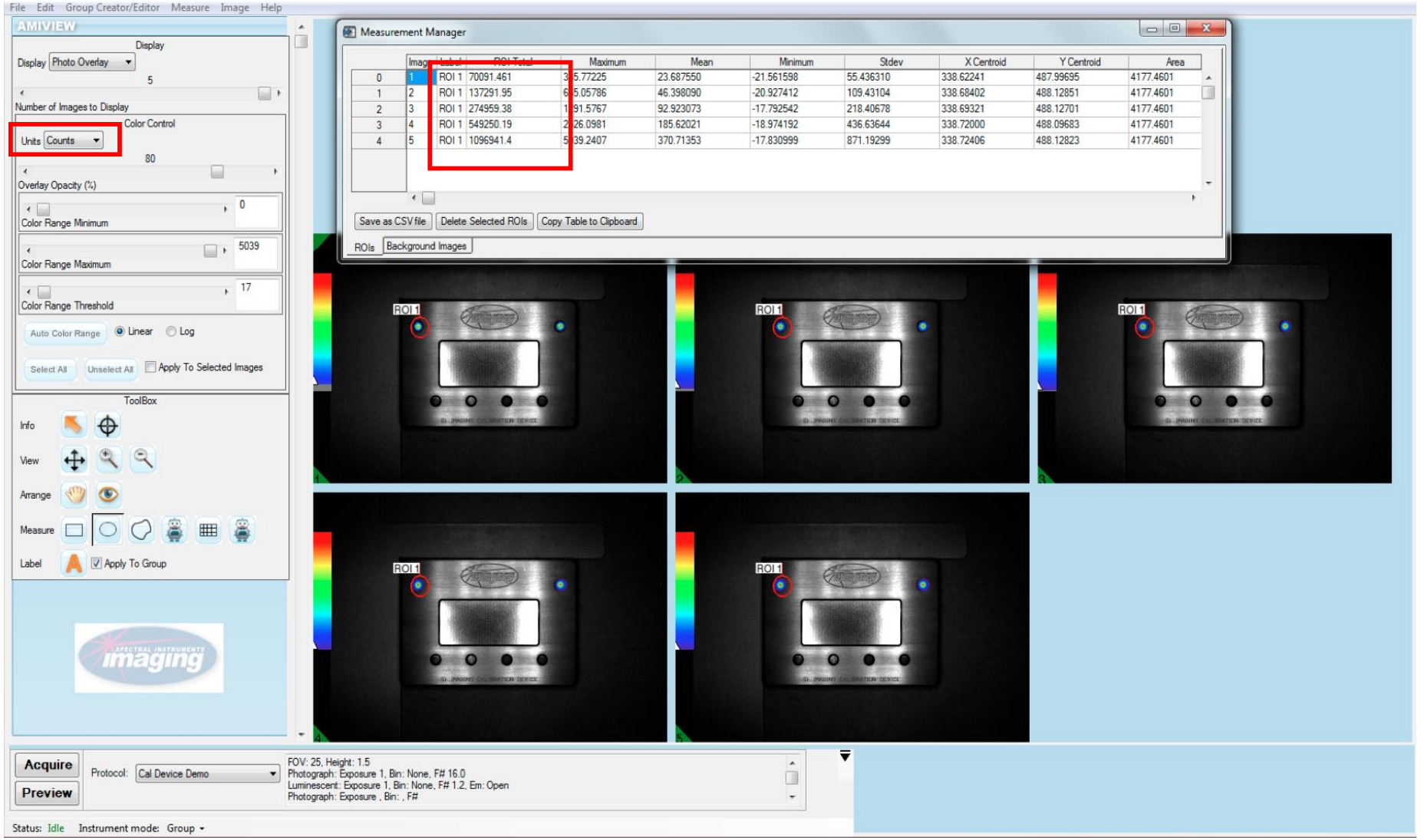
- 手動圈選
- 自動圈選
- 自由圈選







群組感興趣區域圈選及呈現



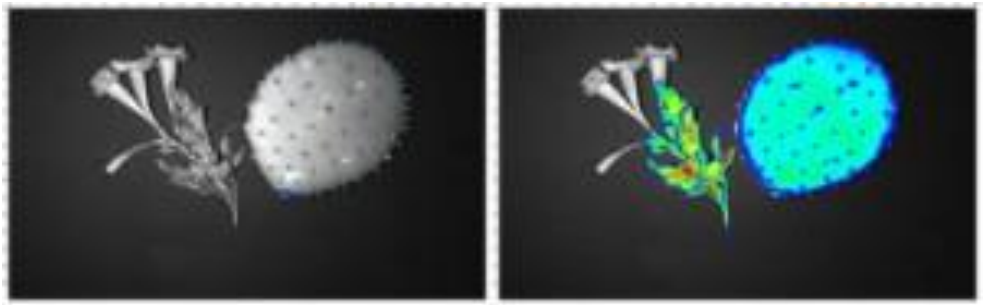
SI Imaging 公司簡介

SI Imaging 小動物活體成像系統特性

SI Imaging 應用

Plants Imaging

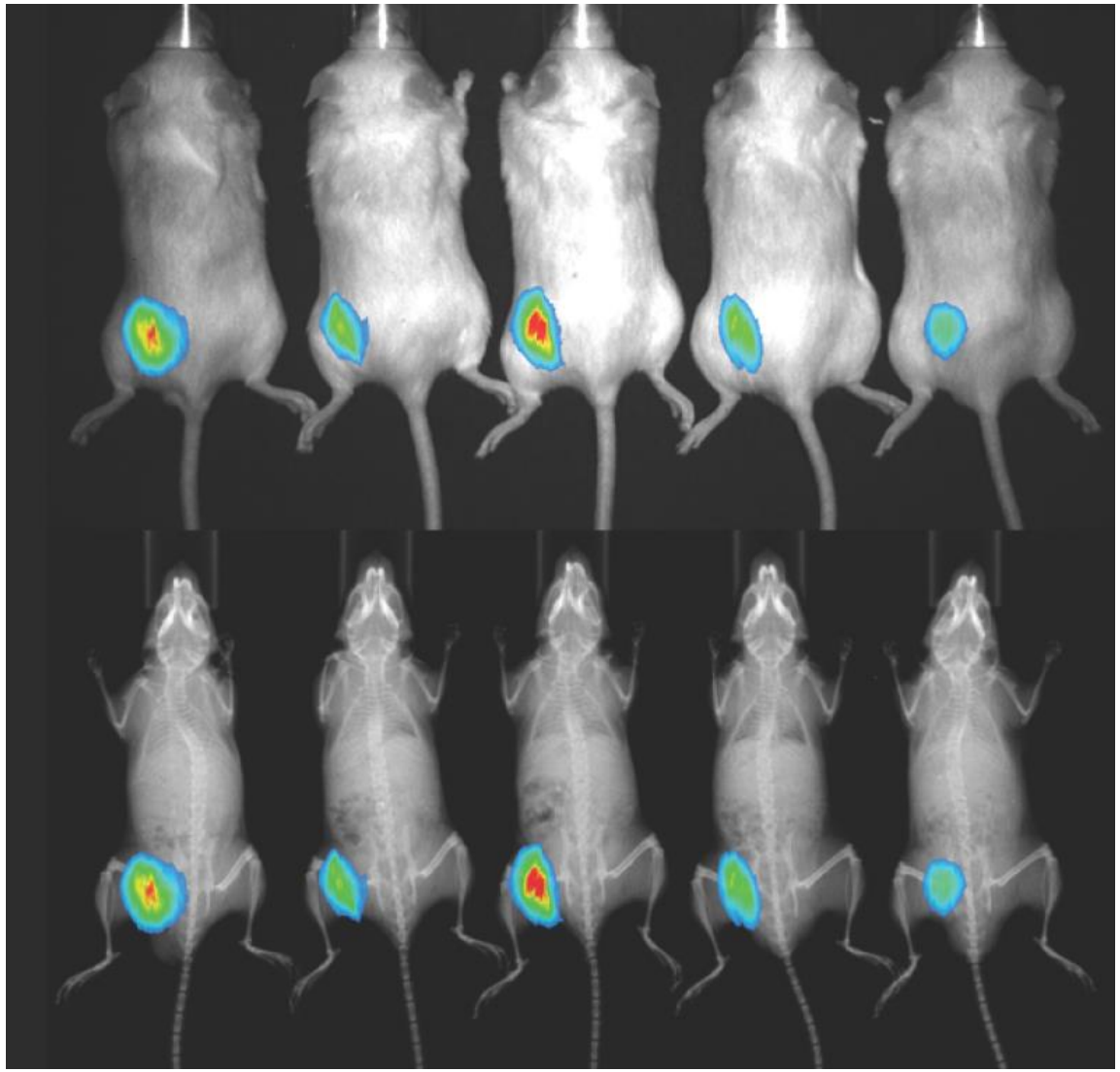
Fluorescent imaging of plants and seedlings has become a valuable tool to investigate multiple aspects of plant biology and genetics. The localization of fluorescent indicators to help in the understanding of plant gene expression, growth and development, circadian rhythm, chemical resistance, and stress response.



Fluorescent emissions from desert flora. A flowering plant and cactus were imaged for 20 sec with 465 nm excitation. Pseudocolor fluorescent signals were overlaid onto a photographic image. Panel A was imaged at 550 nm emission; panel B was imaged at 735 nm emission

10s images of tomato plant in group mode. Psuedocolor luminescence imaging overlaid onto photographic image. In support of food safety program with the University of Arizona Dept. of Veterinary Science and Microbiology.

In-vivo imaging

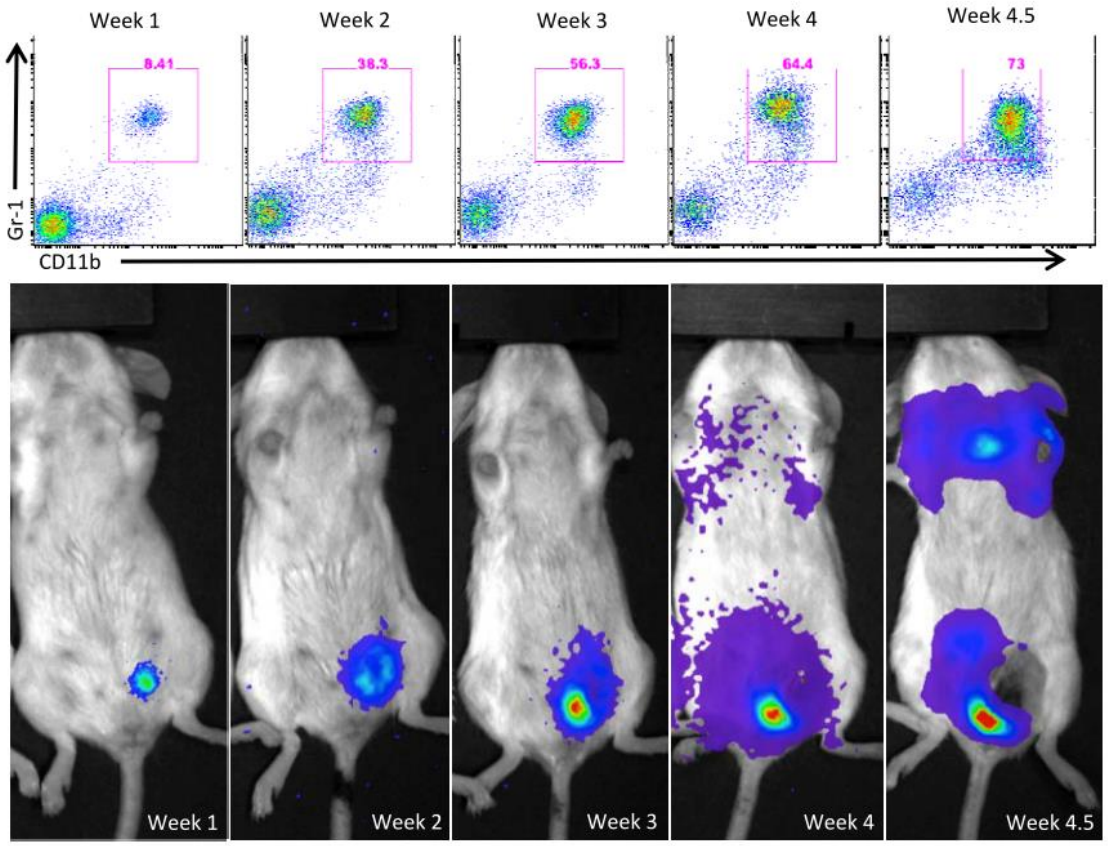


The top image displays the detection of fluorescent signals within mice. Five mice were subcutaneously injected with LiCor IRDye 800 CW Carboxylate and imaged for 20 seconds.

Fluorescent signals are shown overlaid onto a photographic image. [745 nm excitation, 845 nm emission, f/2].

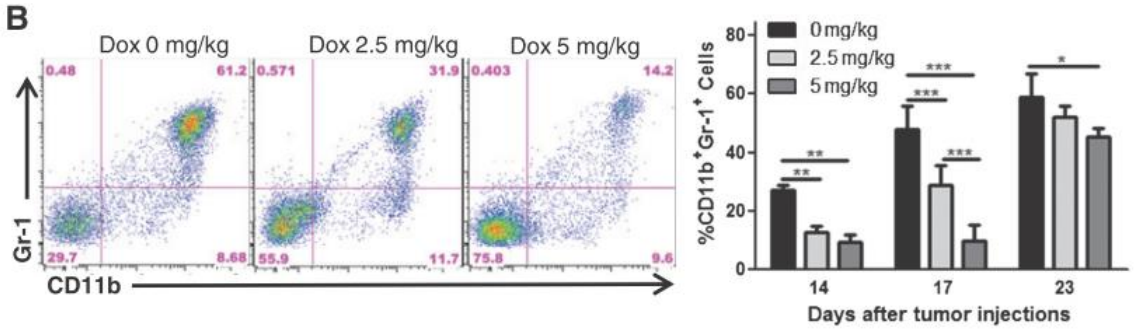
The bottom portion of the image shows the X-ray image of mice with fluorescent signal overlay. [40 kV, 20 sec x-ray exposure]

Doxorubicin Eliminates Myeloid-Derived Suppressor Cells and Enhances the Efficacy of Adoptive T-Cell Transfer in Breast Cancer



Cancer Research IF: 9.284

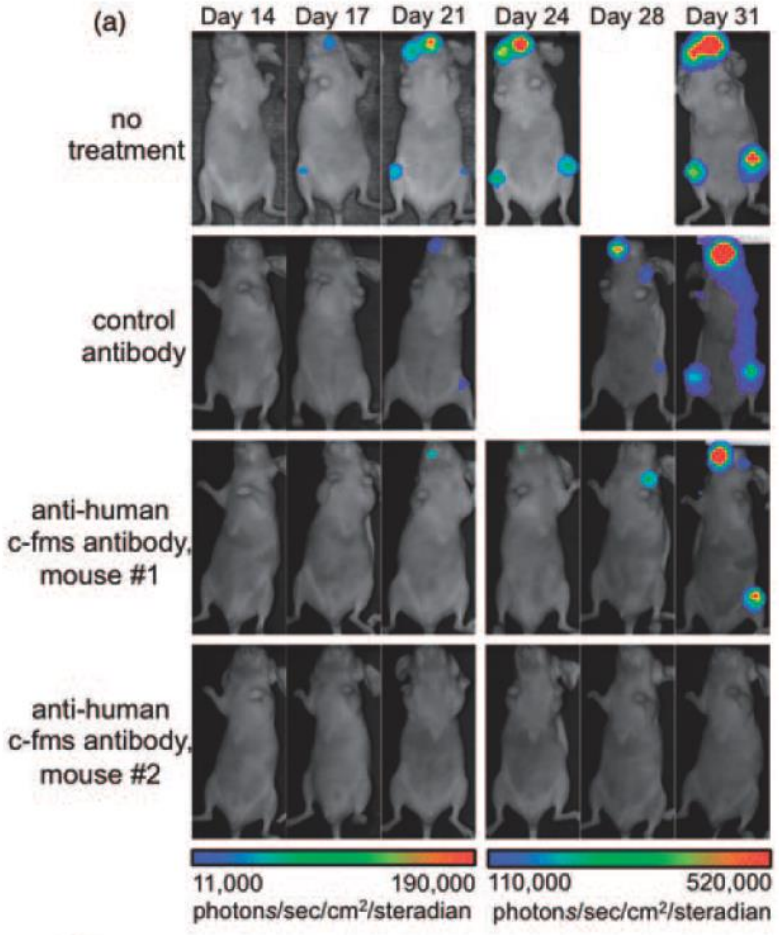
Darya Alizadeh, Malika Trad, Neale T. Hanke, et al.



Six to eight week-old Balb/c mice were injected with 1×10^6 4T1 cells (mammary fat pad). MDSC ($CD11b^+Gr\text{-}1^+$) frequency was analyzed 1, 2, 3, 4 and 4.5 weeks post-tumor injection. Percentage of $CD11b^+Gr\text{-}1^+$ cells in the spleen of mice bearing 4T1 tumors (upper panels). Bioluminescent imaging of 4T1 tumor progression and lung metastases at the same time points (lower panels). n=4 mice per time point. One representative mouse out of 4 is shown. Two independent experiments were performed.

Mice were anesthetized using isoflurane (1.5 L/min oxygen, 4% iso fl urane) and kept in an induction chamber. Images were captured with an AMI1000 imager (Spectral Instruments Imaging). Light emission was measured over an integration time of 1 minute, 10 minutes after injection of luciferin.

Autocrine inhibition of the c-fms proto-oncogene reduces breast cancer bone metastasis assessed with in vivo dual-modality imaging



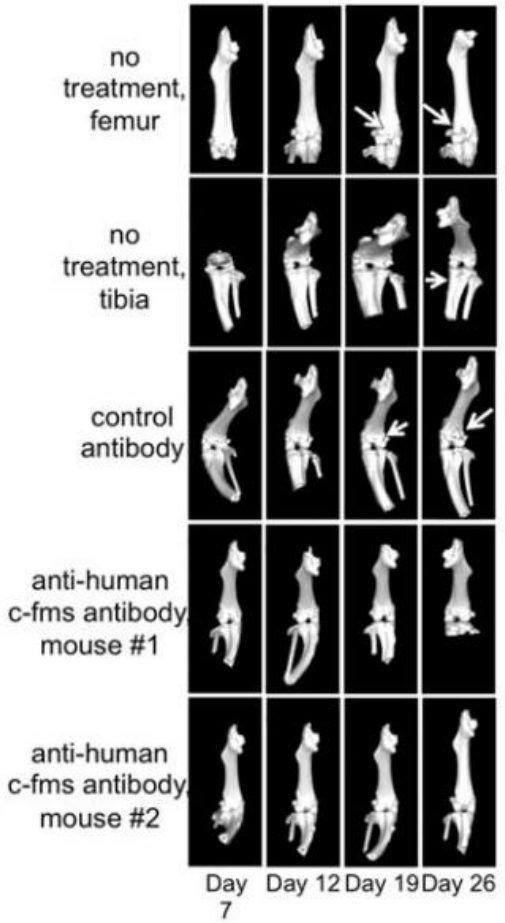
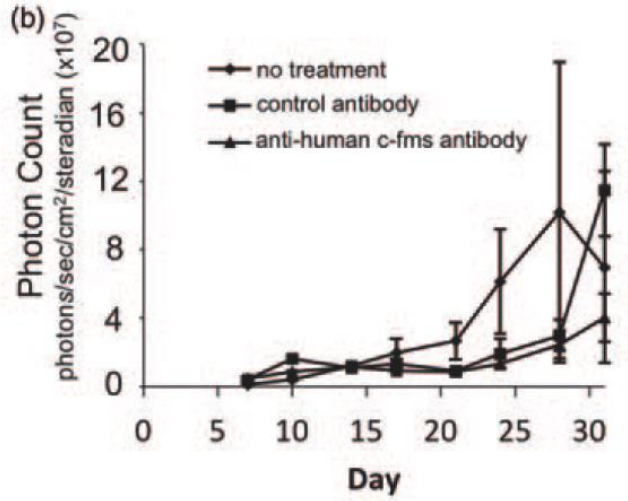


Figure3 (a) Untreated mice showed measureable light at day 17, with substantial growth of metastases at day 31. Mice treated with control antibody also showed metastasis at day 31, although the level of metastasis was less than metastases detected in untreated mice. Mice treated with anti-human c-fms antibody emitted the least amount of light over time, and five of the nine mice in this treatment group had negligible light emission. (b) The measurement of the mean photon count (±SEM) taken from bilateral femurs over time for each of the three groups showed a significant difference between the control antibody and anti-human c-fms antibody group on day 31 ($P < 0.05$).



Scans were acquired with 30s exposure time, high eight-fold binning, 1.2 f/stop aperture, and 25 cm FOV

X-ray skeleton imaging in conjunction with bioluminescence imaging does not alter pancreatic tumor

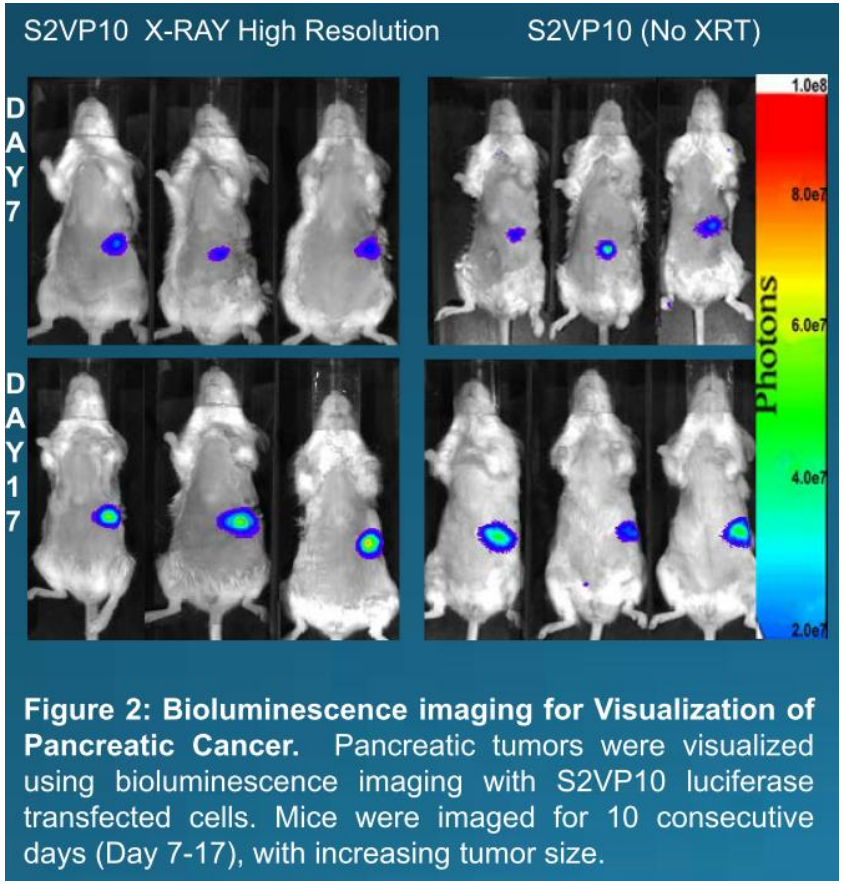


Figure 2: Bioluminescence imaging for Visualization of Pancreatic Cancer. Pancreatic tumors were visualized using bioluminescence imaging with S2VP10 luciferase transfected cells. Mice were imaged for 10 consecutive days (Day 7-17), with increasing tumor size.

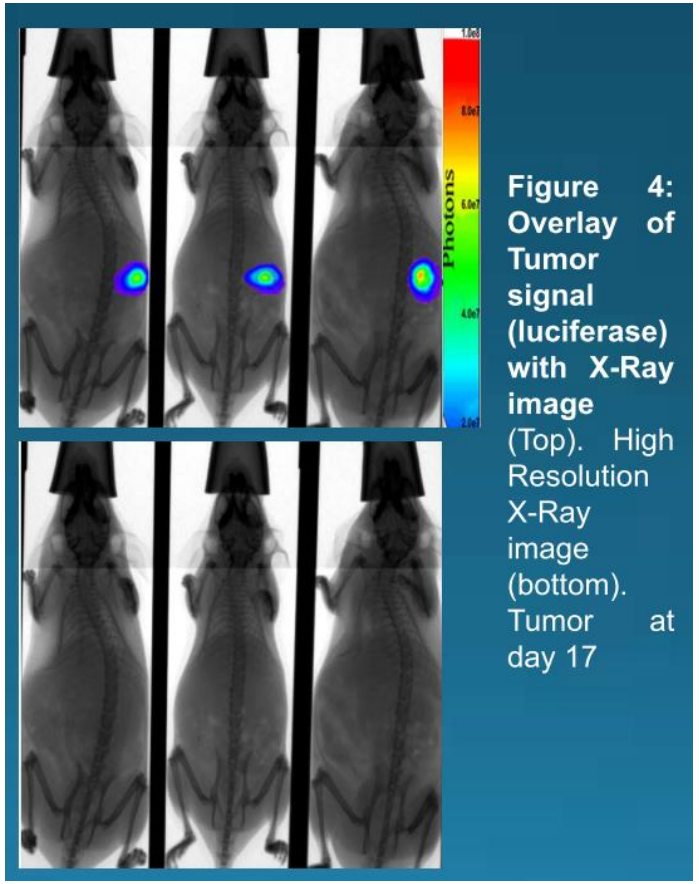
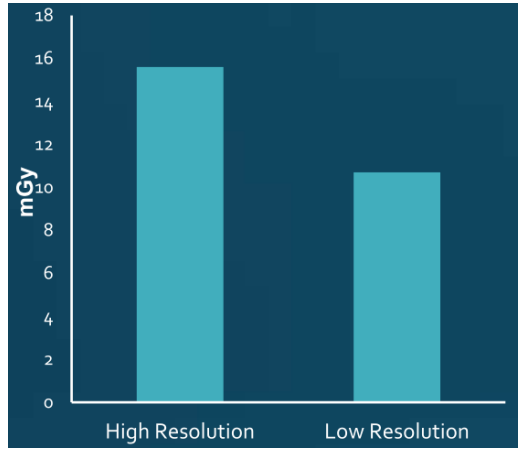


Figure 4: Overlay of Tumor signal (luciferase) with X-Ray image (Top). High Resolution X-Ray image (bottom). Tumor at day 17



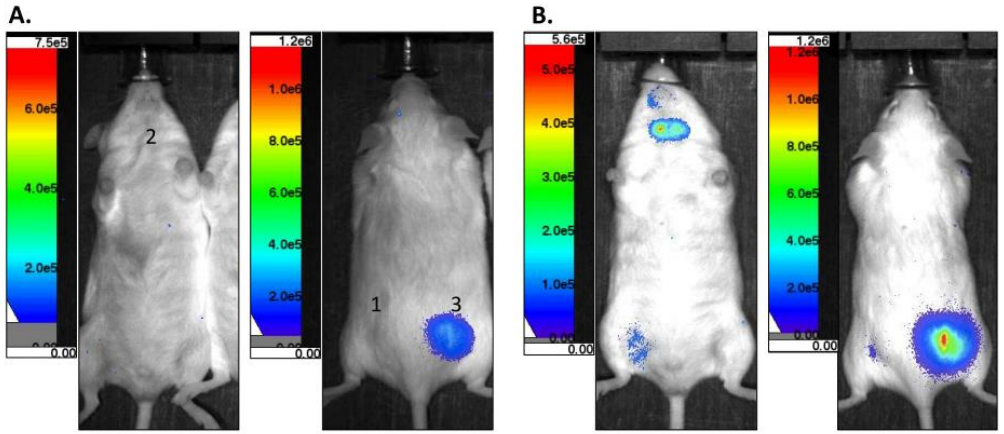
Exposure to radiation during the imaging of cancer was studied to determine secondary effects. This study found:

- There were no signs of increased DNA damage.
- Tumor size of x-ray exposed mice were statistically similar to control group.
- Immunohistochemistry showed minimal upregulation of radiation-sensitive markers.

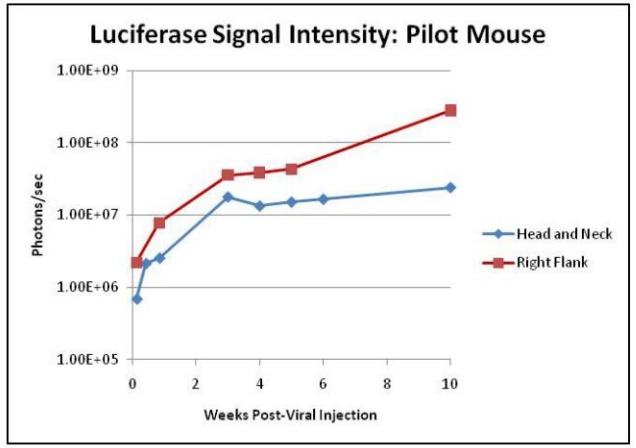
Conclusion:
Radiation exposure obtained during animal imaging does not increase DNA damage, up-regulate radiation sensitive markers, or induce cell stress.

Development of a Tongue Carcinoma Model Using Real-Time in Vivo Molecular Monitoring

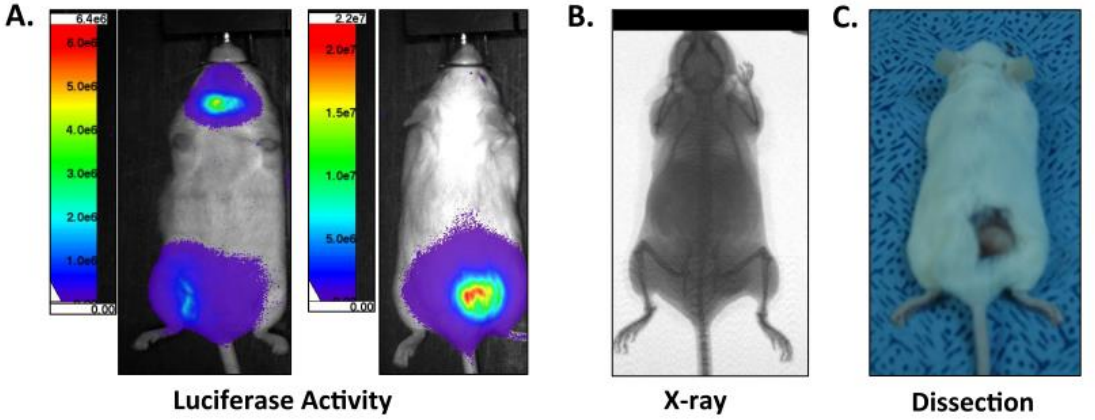
Increase in Luciferase Signal Intensity Post-Injection: Pilot Mouse



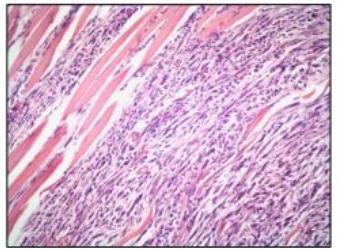
C.



Tumor Growth: Pilot Mouse

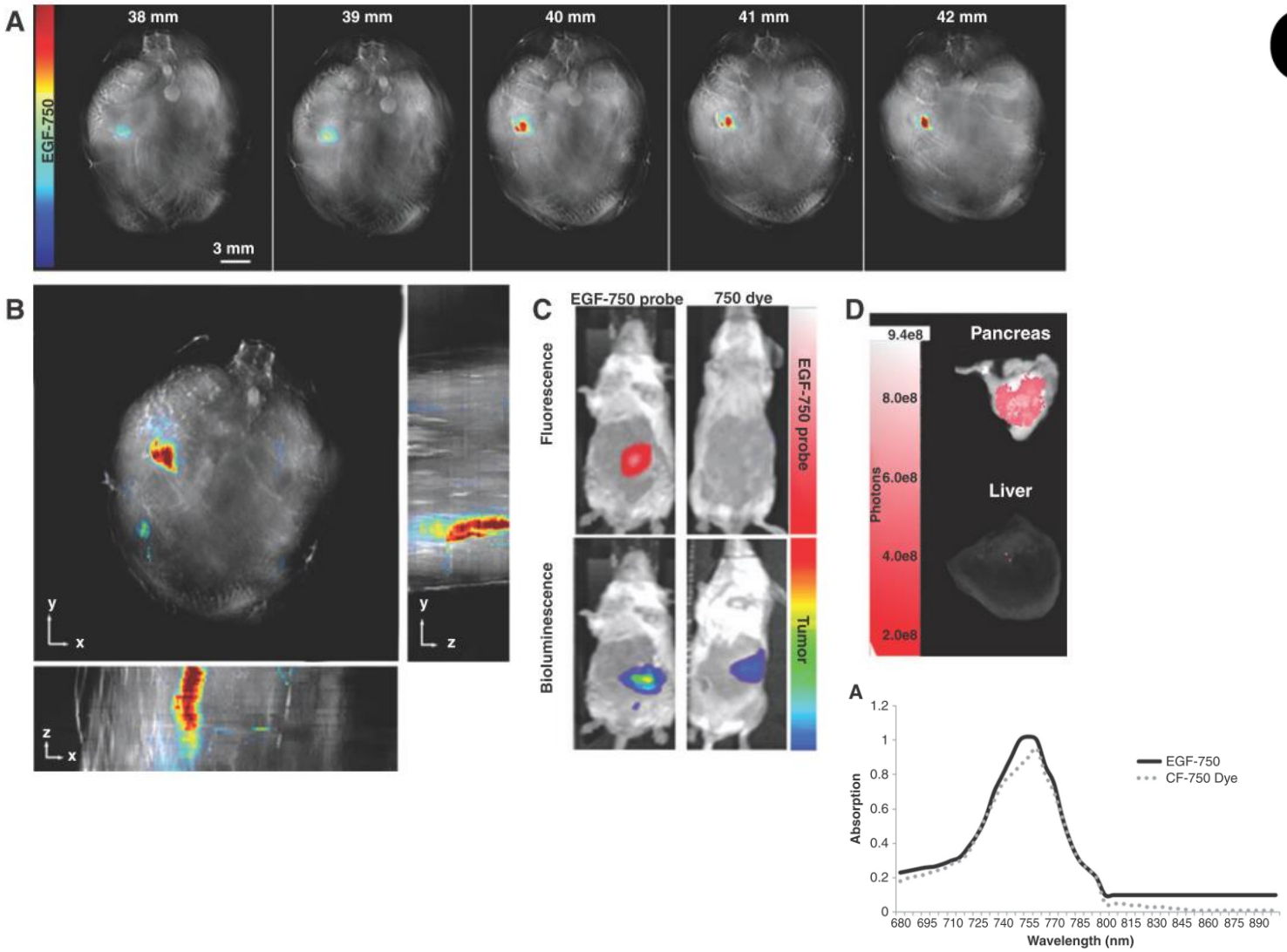


D.



Successful development of this model will help us gain a better understanding of HNSCC initiation and progression, and real-time molecular imaging of cancer cells in vivo will allow us to monitor tumor dynamics and metastasis

Targeted Noninvasive Imaging of EGFR-Expressing Orthotopic Pancreatic Cancer Using Multispectral Optoacoustic Tomography

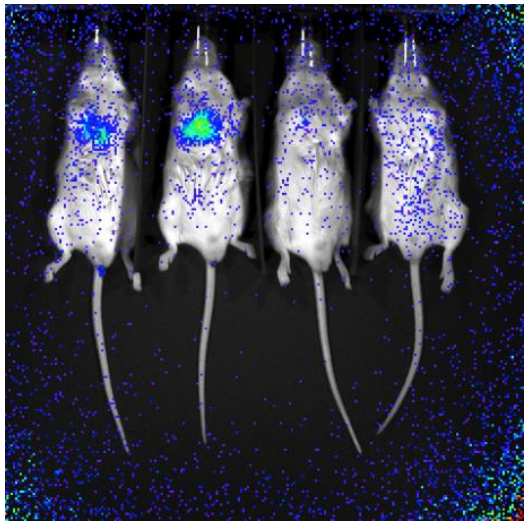


Cancer Research

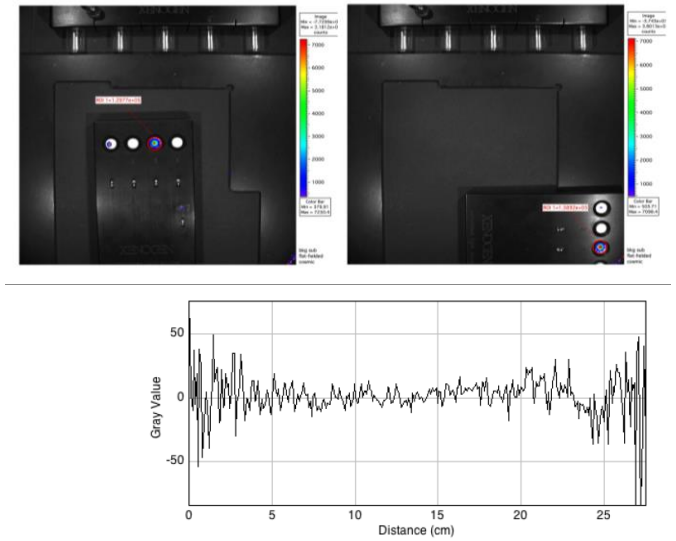
C, top, fluorescence imaging of EGF-750 probe-labeled pancreatic tumor;as a control, unconjugated inert CF-750 dye was shown to not accumulate preferentially in the tumor. Bottom, detection of pancreatic tumor was confirmed using S2VP10L cells (Luc positive) through bioluminescence imaging on the AMI.

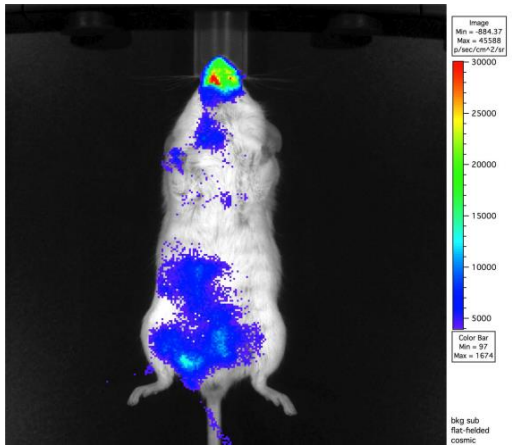
D, in vivo MSOT images were further validated through fluorescence imaging detection of EGF-750 probe in pancreatic tumor and liver tissues analyzed ex vivo on the AMI; ex vivo scan of organs con fi rmed the accumulation in the pancreas and lack of fluorescent EGF-750 probe distribution in the liver, corresponding to MSOT signal quantified for the liver and kidney at < 10 MSOT signal units (a.u.).

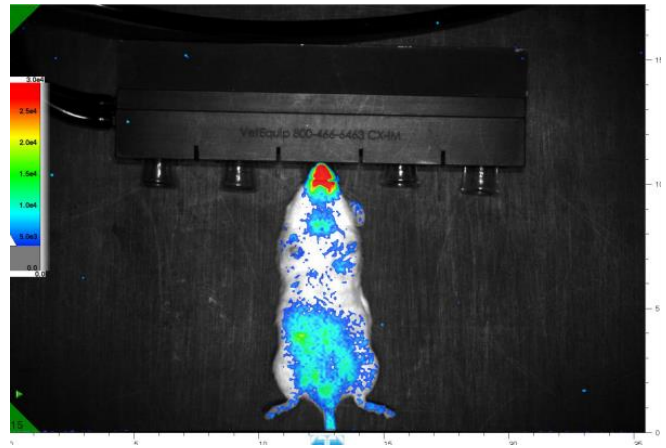
Comparison of High Sensitivity BLI Imaging Systems for Ultra-weak Signal Applications



Corner vs. Center Measurements







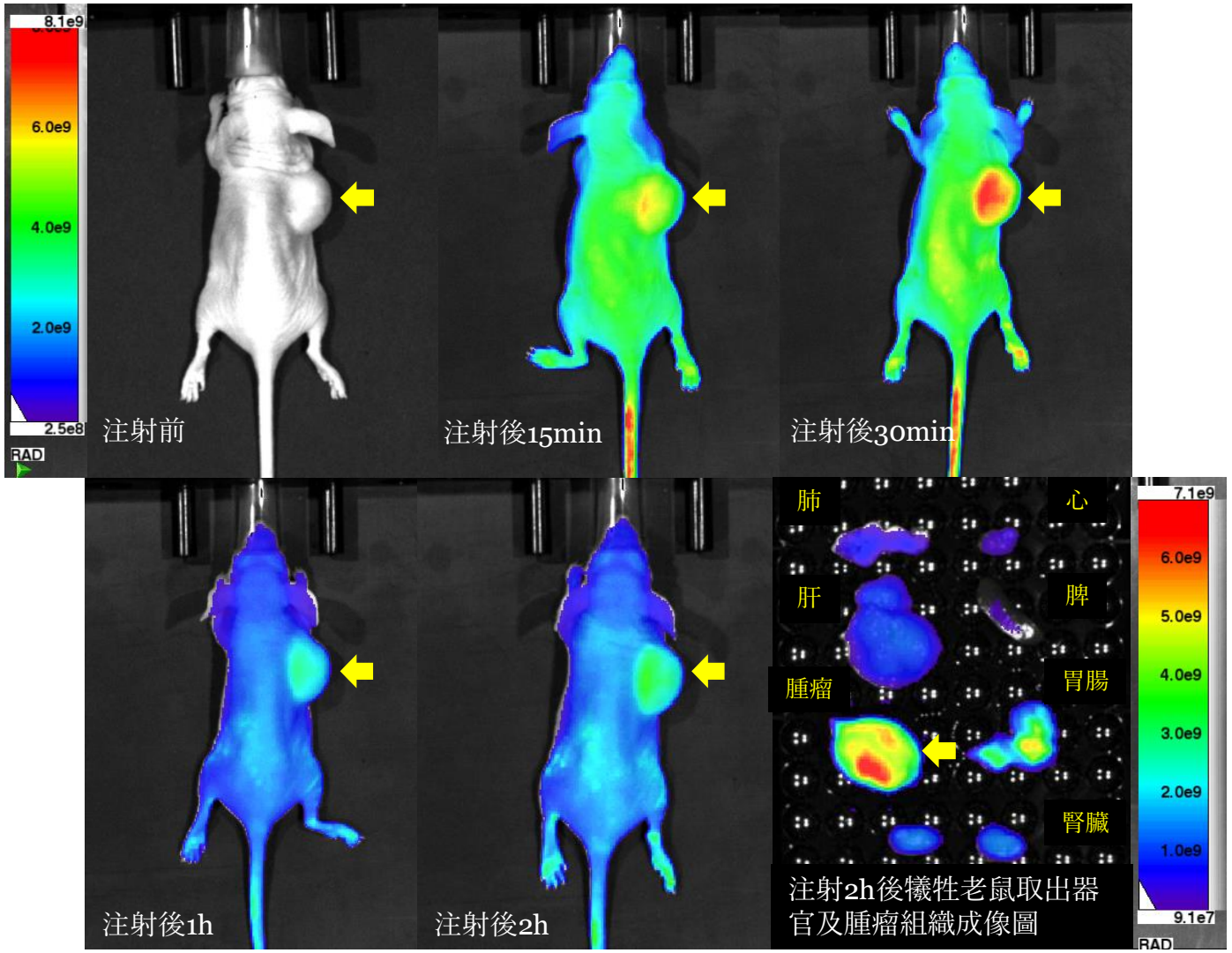
High sensitivity BLI image taken with an IVIS 200 and Ami X system. The same C57BL/6 LSL-luciferase $TP53^{fl/fl}$ $PTEN^{fl/fl}$ mouse 16 weeks post-viral exposure was imaged following D-luciferin intraperitoneal injection

Center vs. Corner Measurements for Three High Sensitivity BLI Systems

	corner	center	ratio	FF
IVIS 100	1.59E+05	1.30E+05	0.82	on
	2.63E+04	1.29E+05	4.90	off
IVIS 200	4.00E+05	3.83E+05	0.96	on
	2.33E+05	3.80E+05	1.63	off
Ami X	2.89E+05	2.78E+05	0.96	on
	1.83E+05	2.78E+05	1.52	off

All three instruments achieve color range required for high sensitivity BLI imaging, but IVIS 200 and Ami X lenses require less correction resulting in no halo

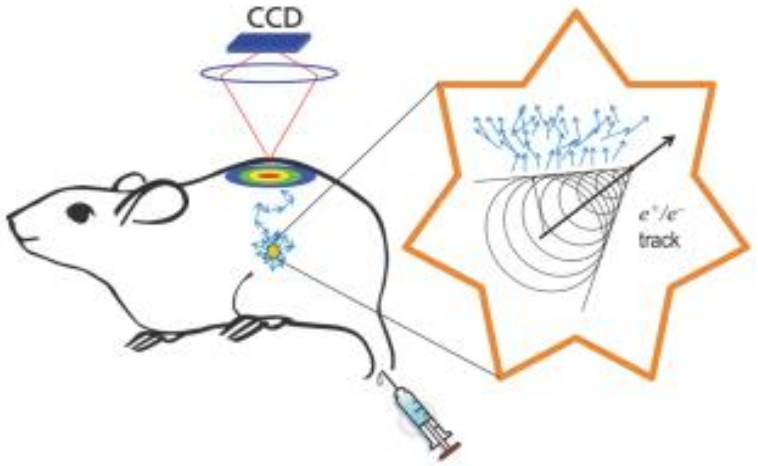
AmiX 觀察螢光探針Cy7在腫瘤裸鼠體內的螢光成像



※ 螢光物質在注射後的30min時間，於腫瘤處的攝取值達到最高峰，而身體其他地方的攝取則則相對低許多，顯示此螢光物質具有高度的靶向性。

※ 注射後2h犧牲老鼠後，取出腫瘤及身體其他組織器官比較的結果，顯示腫瘤處的確擁有非常高的螢光信號，與螢光活體影像的結果一致性的吻合。

Cerenkov Luminescence Imaging



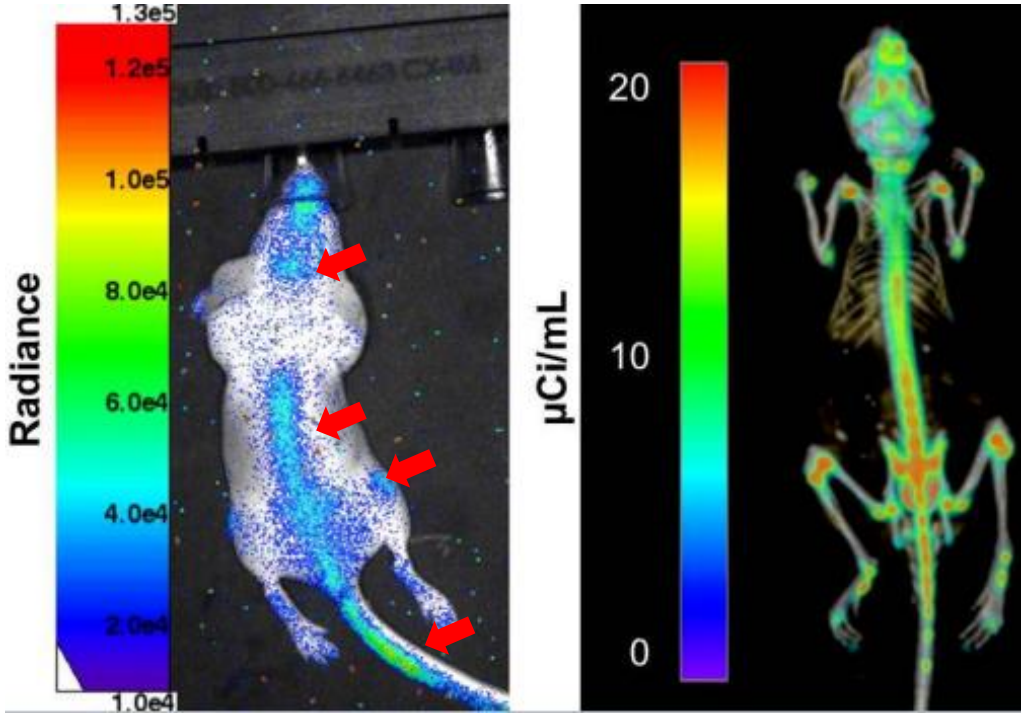
Cerenkov radiation: It is possible that the beta particle (electron or positron) is relativistic, traveling faster than the speed of light ($v>c/n$) in the tissue.

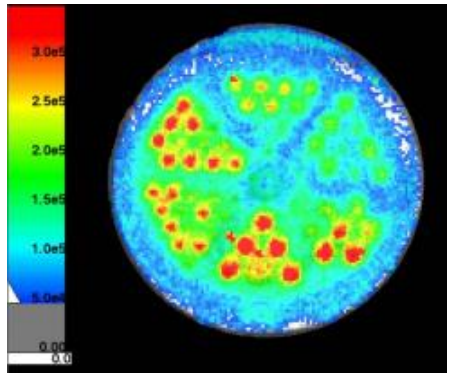
- *c= speed of light in a vacuum*
- *n= index of refraction of the medium*

In vitro and *in vivo* studies of Cerenkov Luminescence Imaging

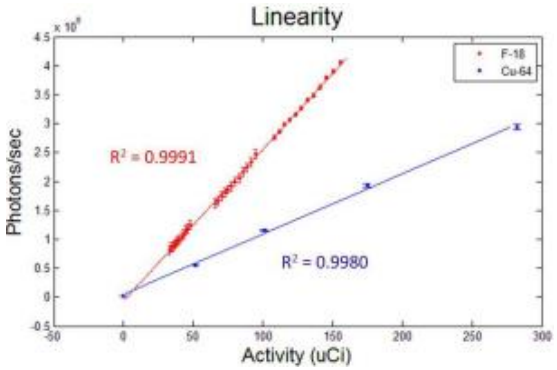
Michael Dykstra[1,2], Anderson Peck[2], Anthony Chang[2]
Grand Valley State University[1], Van Andel Research Institute[2]

The CLI image and the PET image both show uptake in the spine and growth plates in the knees.





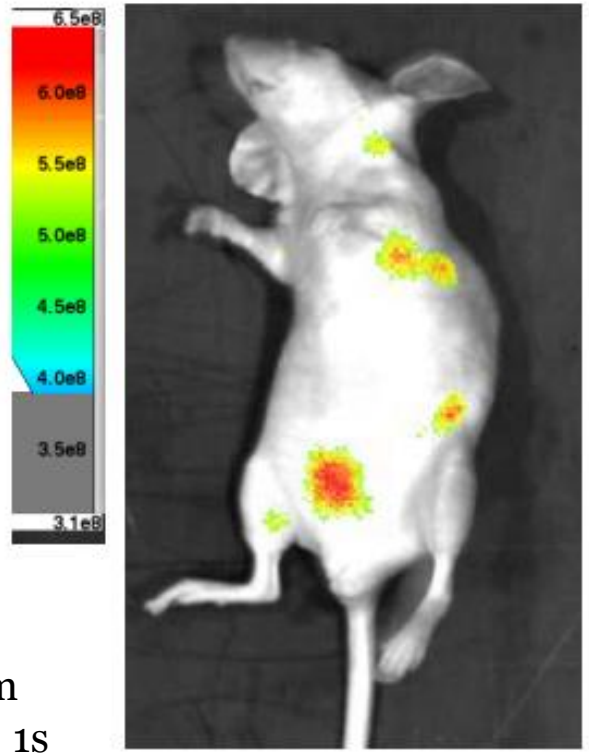
Visual analysis yielded a spatial resolution of 1.2 mm.



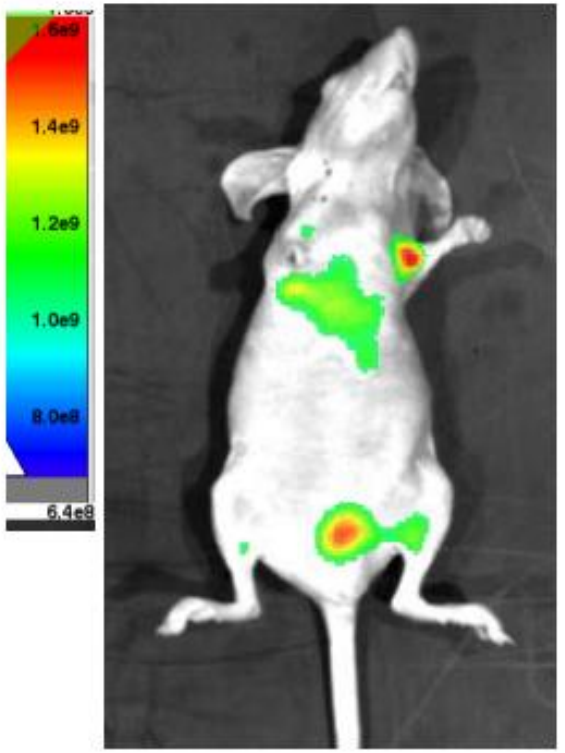
Linearity was assessed by placing samples of varying activities in the AMI-1000 and performing a longitudinal scan

AmiX 阿黴素體內代謝的螢光成像

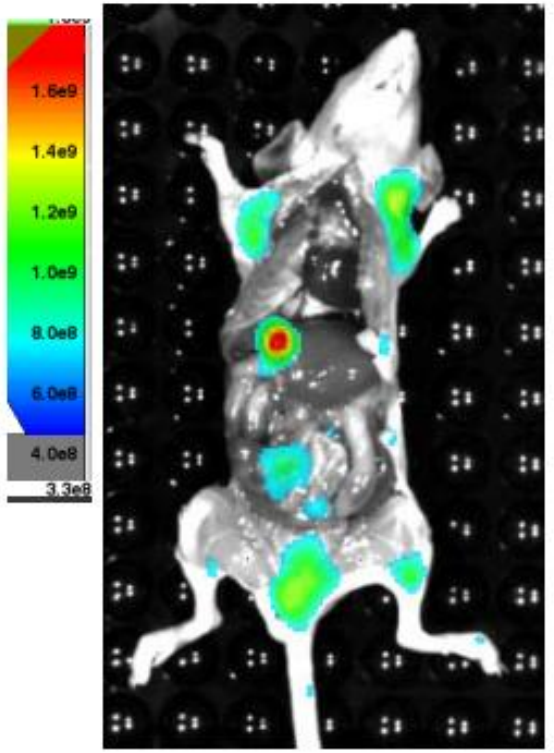
對照組小鼠



注射阿霉素後24h影像



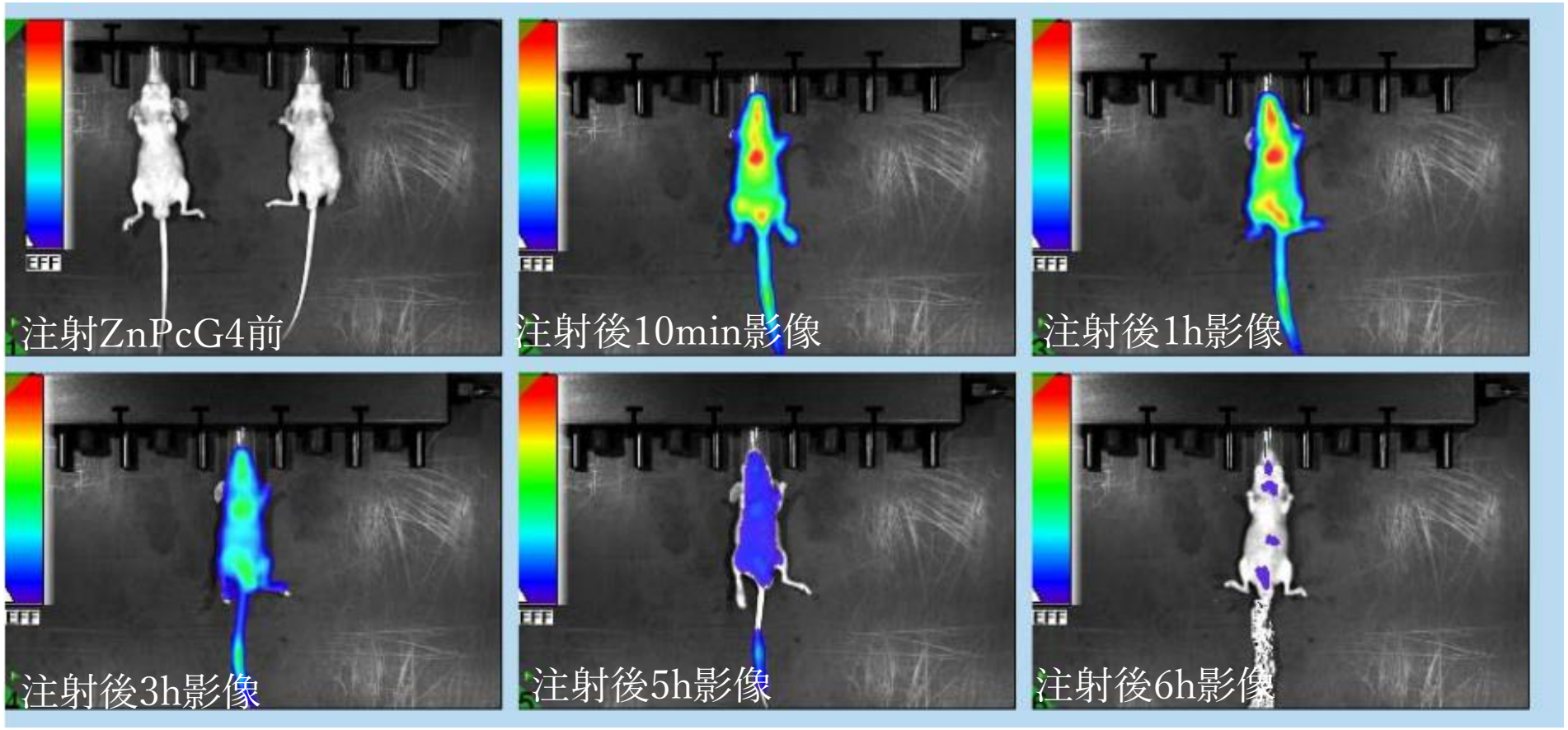
小鼠解剖影像



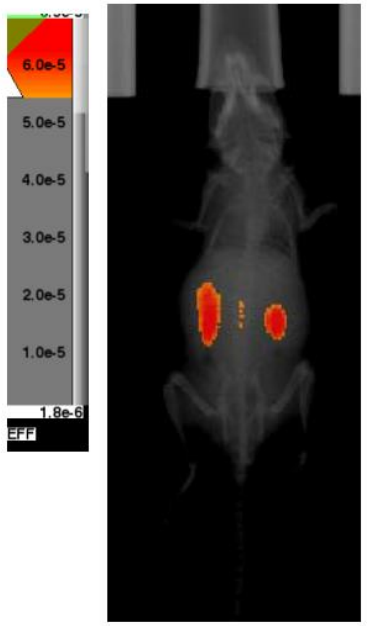
Ex: 465 nm
Em: 630 nm
Scan time: 1s

對照小鼠在阿黴素注射前先進行影像掃描擷取，會發現其腋下和下腹部出現訊號，判定為小鼠的自發螢光現象。在通過尾靜脈注射阿黴素後約**24小時成像**，發現了上腹部出現了明顯的螢光訊號。藉由動物解剖結果得知，上腹部的螢光訊號主要來自於**膽囊**，與阿黴素主要通過膽汁排泄的代謝路徑相符合。**由上述影像結果可以清楚看出，使用新型LED燈做為激發光，老鼠自我產生的自發螢光是極低的，因此不會影響實驗結果及分析數據**。

AmiX 觀察ZnPcG4在裸鼠體內的螢光成像



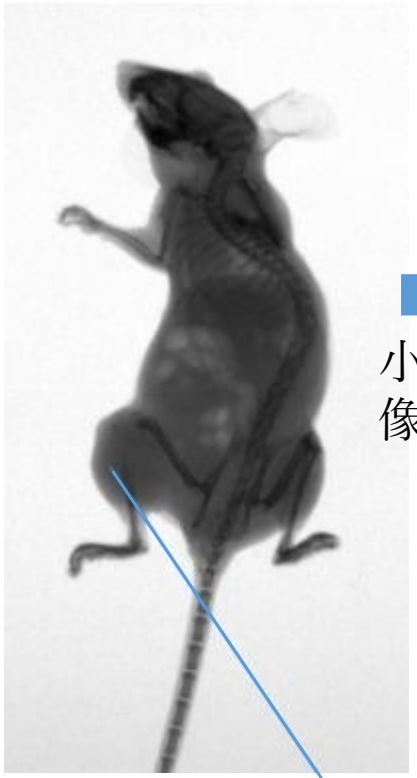
注射後6h影像（俯臥位）

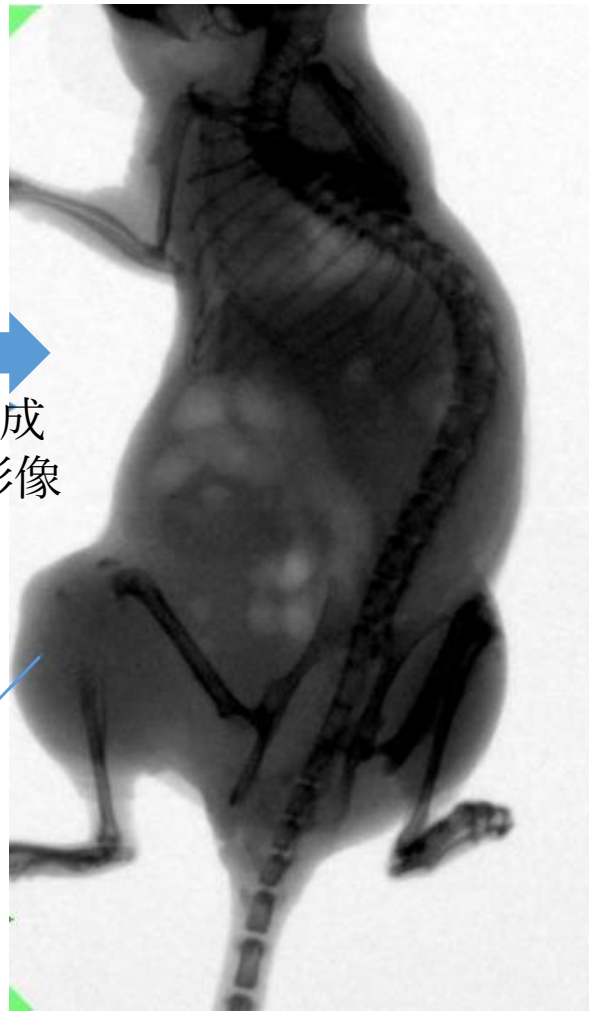


注射ZnPcG4後於老鼠體內代謝分布的結果。將各個時間點的color map以統一的方式呈現，從影像中可以清楚看到注射了ZnPcG4後，10min-1h的胸部訊號最強；注射後3h胸部訊號逐漸減弱直到6h後胸部訊號完全消失。在6h的主要訊號經由X光結果證實來自於腎臟。

AmiX系統X光成像小鼠結果

大視野X光成像小鼠影像

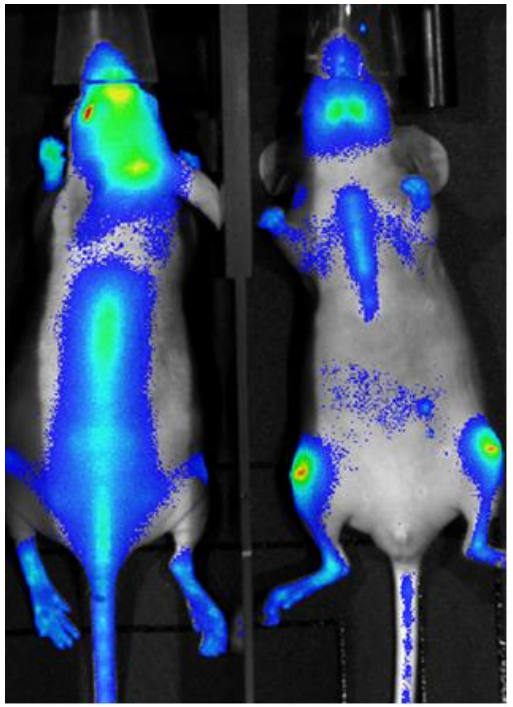




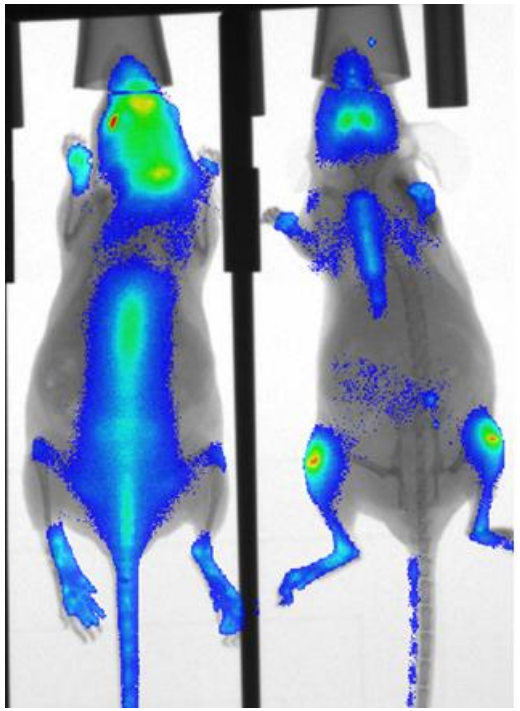
小視野X光成像後小鼠影像

腿部骨腫瘤

Photograph overlay



X-ray overlay



Images of mice 48 hours post injection of Osteosense™ 680

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Thanks a lot for your attention!