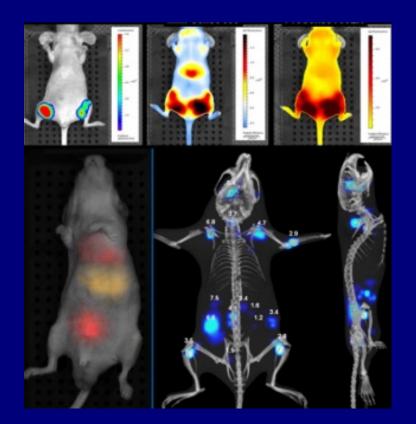
revvity

## **IVIS® Spectrum 2**

Gold standard for In Vivo Preclinical Imaging Solutions

徐明嘉 Buzz Hsu 連絡電話:0800-898-178 E-mail:support@jnhtech.com.tw









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

## Revvity IVIS® - In Vivo Imaging System





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

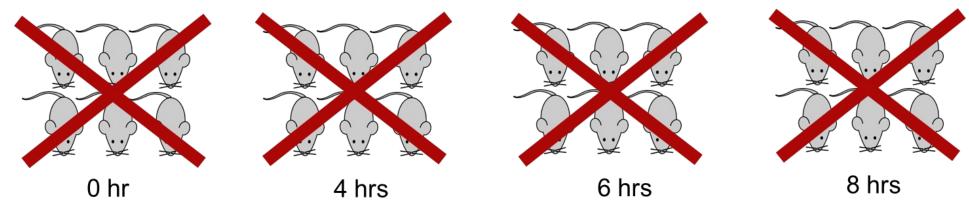
### **IVIS Spectrum 2 Platform**



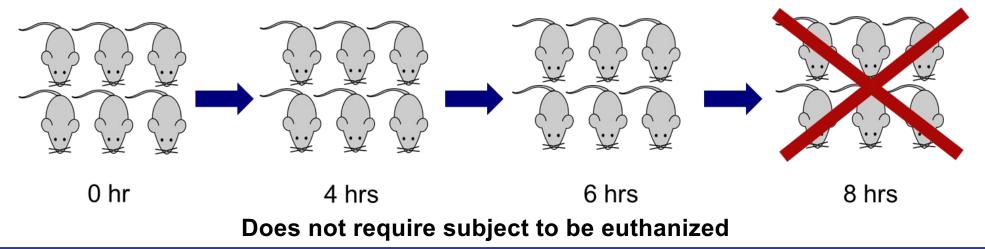
### Transitional methodology vs. BPI methodology



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



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





## What Advantage Optical In Vivo Imaging?

Gene expression results in production of luciferase or fluorescence protein • Amount of light is proportional to number of live active cells	Powerful labeling technique • Reveals enzyme activity, particle / drug location etc.	Non-invasive • Does not require subject to be euthanized	Comply with 3R principle
Reduce individual differences	Easy Operation	Multi-function and Multiplex Bioluminescence, Fluorescence	High Throughput

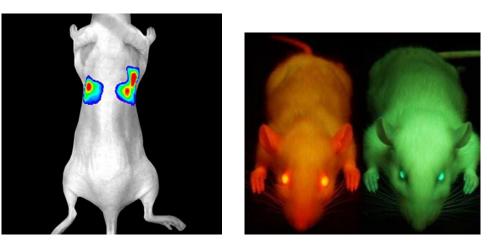
### 專業服務,誠心以赴 Always for you

### ntroduction

## **Optical Imaging Approaches**

### • Bioluminescence & Fluorescent Proteins

- Powerful approach using animals/cells with modified genetics
- Uses promoter systems for deep understanding of underlying mechanisms



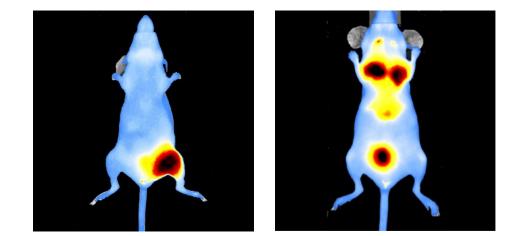
### • Fluorescent Agents (Red/NIR)

- Standard disease biology/models
- Injectable drug-like imaging agents to view biology

revvit

**J&H** 

<u>博</u>克科技



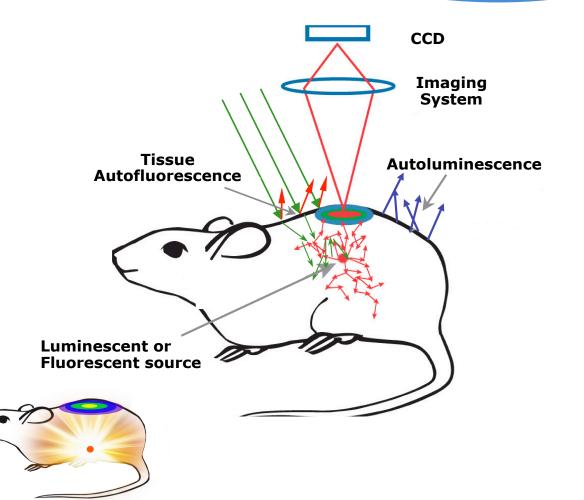
## The physics of light in tissue

- Photons are absorbed and scattered in tissue
- Surface signal depends on source depth
- Tissue is both autoluminescence and

autofluorescence

• Use hairless mice or white-furred animals

and depilate or shave



revvity

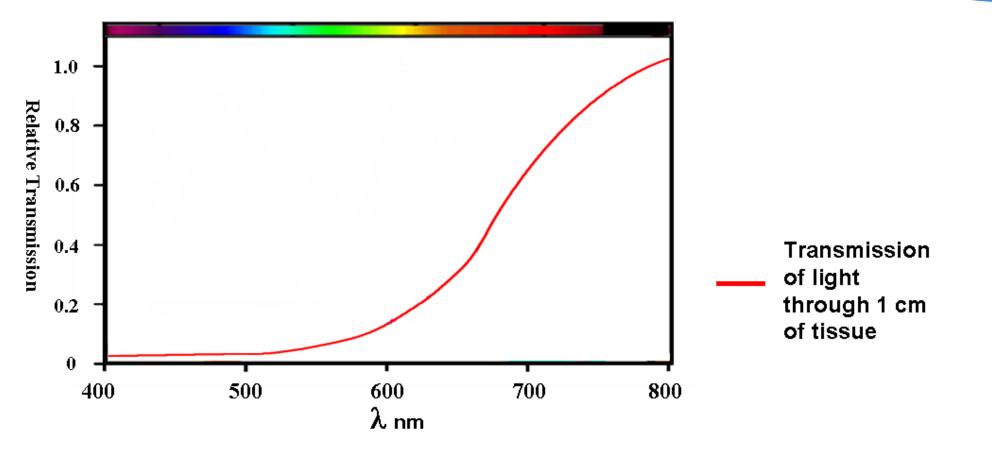
J&H

博克科技

### 專業服務,誠心以赴 Always for you

### ntroduction

## **Light in Tissue**



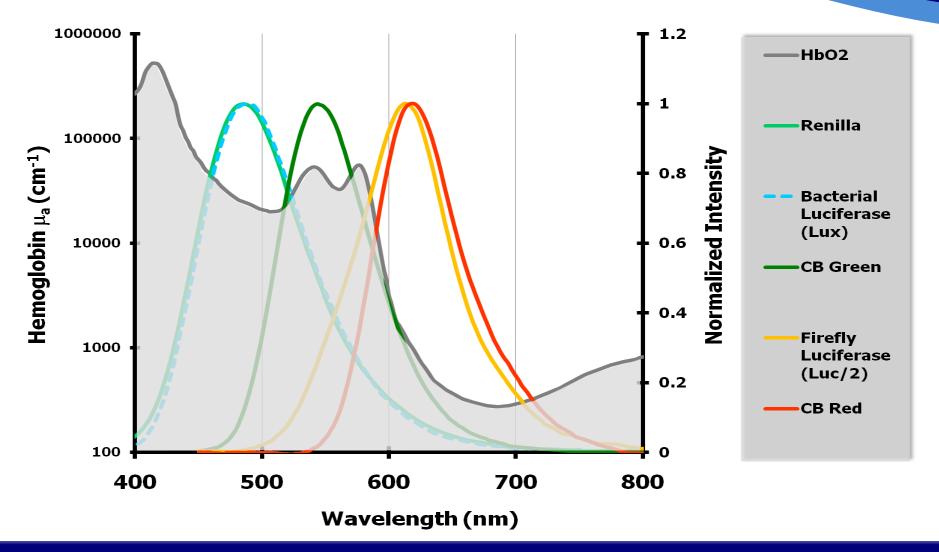
revvity

J&H

博克科技

Tissue Is Not Transparent – Light Absorbance Depends on Wavelength

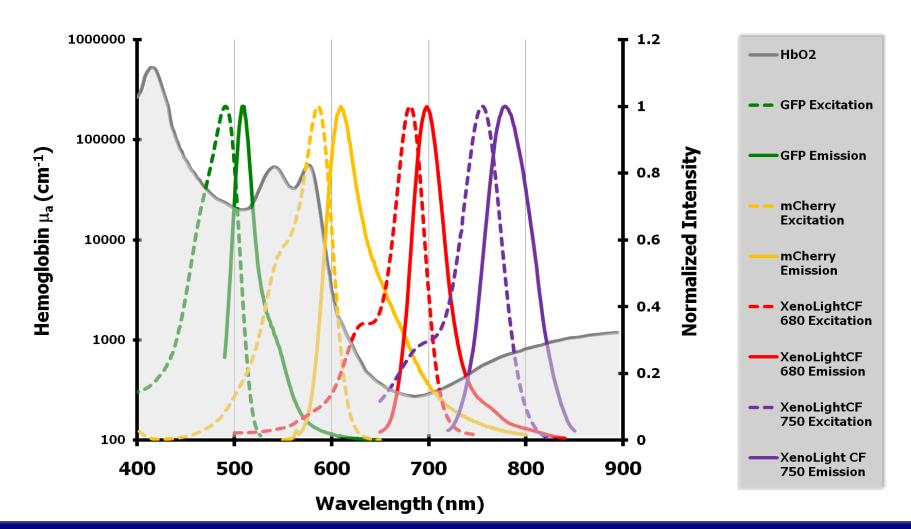
### **Bioluminescent Spectra and Tissue Penetration**



revvty J&H 博克科技

專業服務, 誠心以赴 Always for you

### Fluorophore Spectra and Light Diffusion Through Tissue



revvity

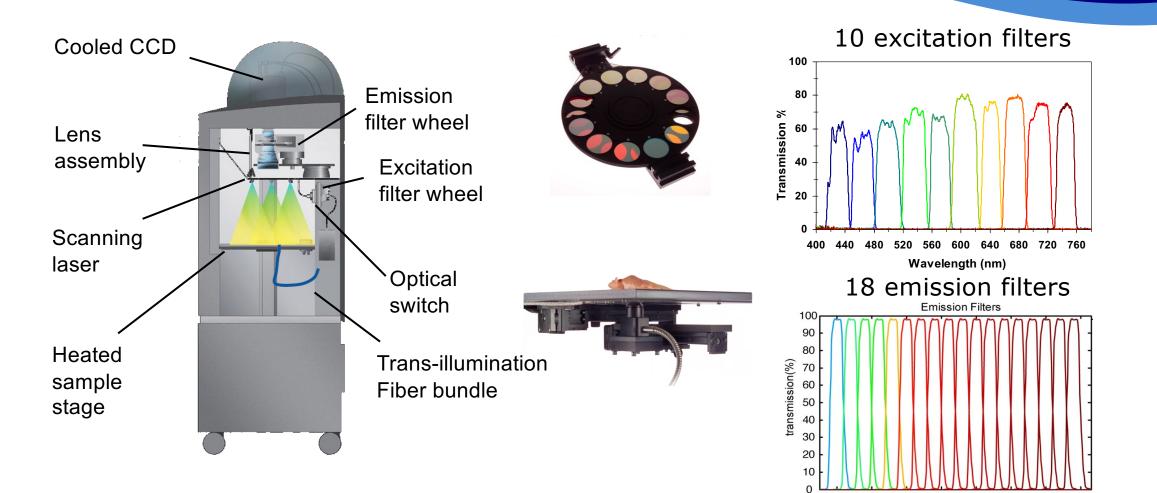
J&H

博克科技

## **IVIS<sup>®</sup> Spectrum 2**

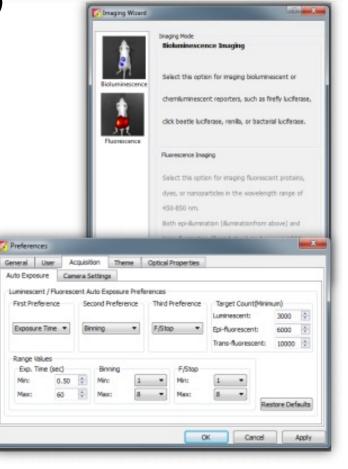


wavelength(nm)



## Living Image<sup>®</sup> Software

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



**re**√

J&H

### 專業服務,誠心以赴 Always for you

## Field of View (FOV)

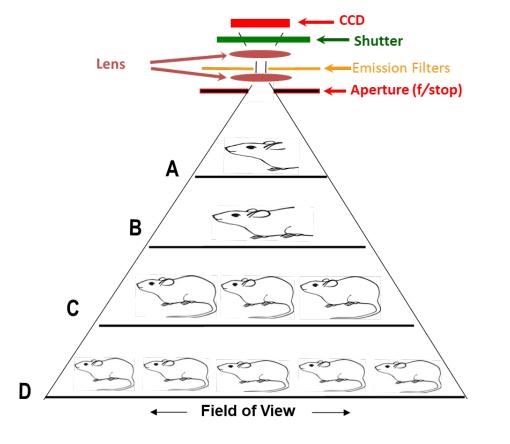
• Sets the size of the stage area to be imaged by

adjusting the position of the stage and lens

• A smaller FOV gives a higher sensitivity

### measurement

🚺 IVIS Acquisition	n Control Panel					- 8
Imaging Mode	Exposure Time	Binning	F/Stop	Excitation Filter	Emission Filter	
🔤 🗹 Luminescent	Auto 🚔 🛛 sec 📑	Medium 🕚	• 1 •	Block	<ul> <li>Open</li> </ul>	•
Fluorescent						
🚺 🗸 Photograph	Auto ≑	Medium	- 8 -	]		
Structure	📃 Lights 🗸 Aligr	nment Grid				
Field of View: C	-		stem Stati	us	Acquir	•
Mouse Imaging S	Shuttle	Idle			Acqui	-
Service 13.4	4 cm				Imaging W	lizard
Subject height: 1.50	) 🌩 cm			[	Sequence	Setup
Focus: use subject	theight 🔻	emperature:		Locked	Initializ	e



revvity

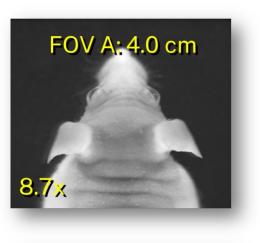
J&H

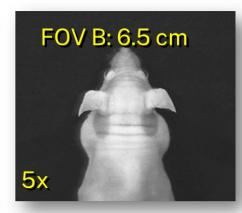
<u>博</u>克科技

## **Field of View (FOV)**

- 1 Position A is not recommended for epifluorescent imaging because corrections for nonuniform excitation light pattern are not available.
- 2 Position C is the default setting.

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

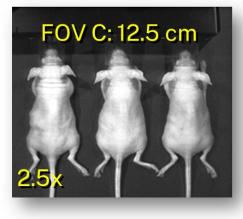


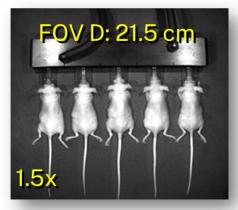


revvity

J&H

博克科技

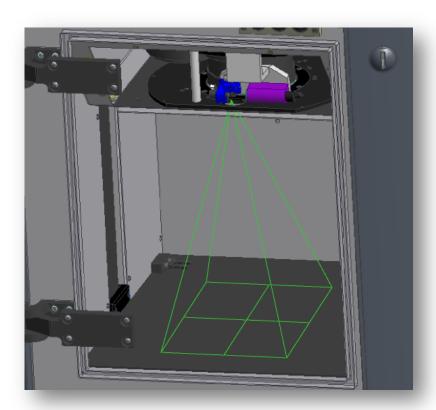


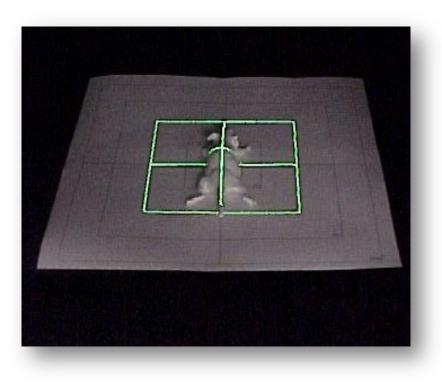


## **Alignment Light Projector**



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

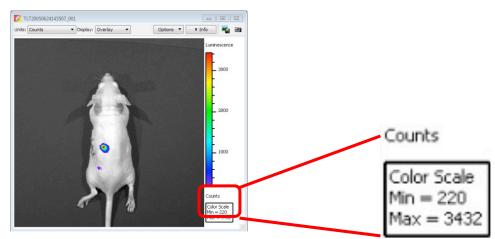






## **Setting Sensitivity – Signal Level**

- The IVIS<sup>®</sup> CCD camera has a raw signal range of 0 to 65,535 Analog to Digital counts (2<sup>16</sup> or 16-bit)
- Adjust camera settings to obtain a signal level of <u>600 to 60,000 counts</u> to be within the linear range of the detector
- Settings that control signal level are:
  - Exposure time
  - Pixel binning (CCD resolution)
  - *f*/stop (aperture)



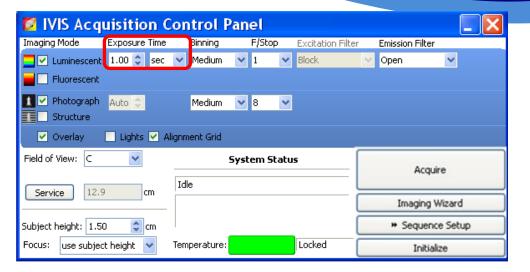
Instrument is calibrated to automatically compensate for changes in sensitivity settings when count levels are within the linear range

### 專業服務,誠心以赴 Always for you

### J&H revvity 博克科技 Living Image<sup>®</sup> Control Panel **Controls Sensitivity** • Software - 23 📝 IVIS Acquisition Control Panel Exposure Time Imaging Mode Binning F/Stop Emission Filter Excitation Filter 🔽 Luminescent 🛛 Auto 🚔 🛛 sec 🔫 Medium Open Ŧ Block $\overline{\mathcal{T}}$ Fluorescent Imaging Modes • 🚺 🔽 Photograph Auto ≑ Medium 🔻 8 Ŧ Structure 📃 Lights 🔽 Alignment Grid V Overlay Field of View: C System Status Ŧ Acquire Mouse Imaging Shuttle Idle Imaging Wizard 13.4 Service cm Sequence Setup - A. . W Subject height: 1.50 cm Initialize Focus: use subject height 💌 Temperature: Locked

## **Exposure Time**

- Signal level is directly proportional to exposure time (1:1)
- Shorter exposure time improves throughput
- Recommended minimum exposure time > 0.5 seconds
- Longer exposure times increase signal intensity
- Recommended maximum exposure time < 5 minutes

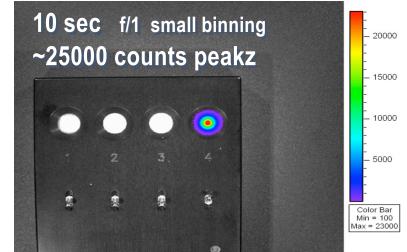


revvity

J&H

博克科技





專業服務,誠心以赴 Always for you

## **Auto-Exposure**

revvity



Auto-exposure feature available for bioluminescence and fluorescence

<b>VIS Acquisition</b> Control Panel	
Imaging Mode Exposure Time Binning F/Stop Excitation Filter En	nission Filter
📃 🗹 Luminescent Auto 🗘 sec 🗸 Medium 🔽 1 🔽 Block 💟 O	open 🗸
Photograph Auto 🗢 Medium 🔽 8 🔽	Preferences
🗸 Overlay 🔄 Lights 🗸 Alignment Grid	General User Acquisition Theme Optical Properties
Field of View: C V System Status	Auto Exposure Camera Settings
Service 12.9   Subject height: 1.50   Focus: use subject height   Temperature: Locked	Luminescent / Fluorescent Auto Exposure Preferences         First Preference       Second Preference         Exposure Time       Binning         F/Stop       Epi-fluorescent:         6000 \$         Trans-fluorescent:       10000 \$
User definable settings	Range Values         Exp. Time (sec)         Min:       1         Max:       60         Max:       8         Max:       8         OK       Cancel

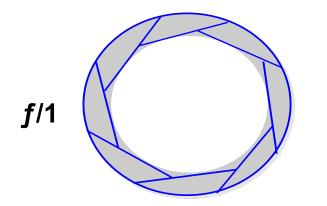
## revvity



## *f*/stop (Lens Aperture)

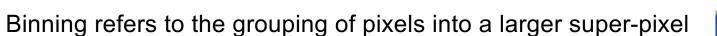
- *f*/stop controls the amount of light received by the ۲ CCD detector
- f/1 is wide open, maximum light collection ۲ - default for luminescent
- f/8 is smallest aperture, best resolution ۲ - default for photo
- Changing f/stop changes counts by a factor of 4

🚺 IVIS Acquisition Contr	ol Panel		
Imaging Mode Exposure Tir	me Binning F,	<b>'Stop</b> Exc	citation Filter Emission Filter
📃 🗹 Luminescent 🛛 1,00 🤤	sec 🔽 Medium 🔽 1	Bloc	:k 🔽 Open 💉
📕 📃 Fluorescent	1		
🚺 🗹 Photograph 🛛 Auto 😂	Medium 🔽 4		
Structure     Overlay     Lights Field of View: C	Alignment Grid	- Chalue	
MIS	Idle	n Status	Acquire
Service 12.8			Imaging Wizard
Subject height: 1.50 💲 cr			+ Sequence Setup
Focus: use subject height 🗸	Temperature:	Locked	Initialize

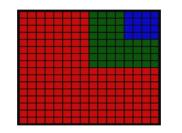


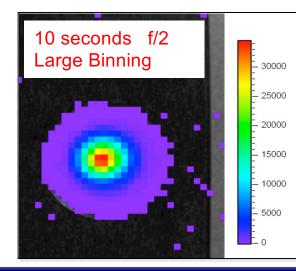


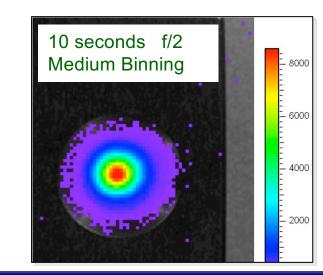
## **Pixel Binning (CCD Resolution)**

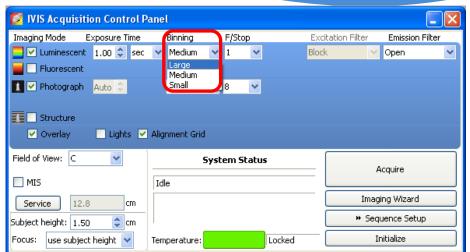


- Changing binning settings changes counts by a factor of 4
  - Large Binning (16)
     Higher Sensitivity/Lower Resolution
  - Medium Binning (8)
  - Small Binning (4)
     Lower Sensitivity/Higher Resolution





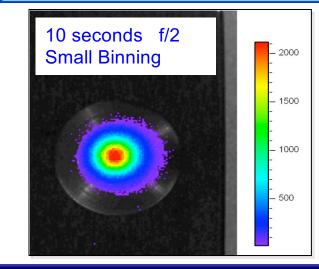




revvity

J&H

博克科技

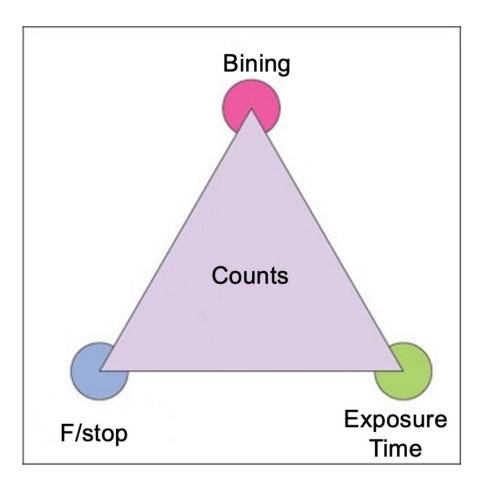


專業服務,誠心以赴 Always for you





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



Counts	Exposure Time	Binning	F/stop
65535 <u>600</u> 00	5 mins	Large	1
600		Resolution	

# Acquisition - Single Image (Fluorescent)

💋 IVIS Acquisition Contro	l Panel	
Imaging Mode Exposure Tim	e Binning F/Stop	Excitation Filter Emission Filter
🔁 🗌 Luminescent 🛛 1.00 🤤 s	ec 🕶 Medium 💌 2 💌	640 🛛 740 🖌
📕 🗹 Fluorescent 📃 Transillu	mination	Lamp Level: High 🛛 🛩
🚺 🗹 Photograph 🛛 Auto 😂	Medium 🔽 8 🛩	
Structure		
🗹 Overlay 📃 Lights	<ul> <li>Alignment Grid</li> </ul>	
Field of View: C	System Status	Acquire
MIS	Idle	
Service 12.8 cm		Imaging Wizard
Subject height: 1.50 🗘 cm		* Sequence Setup
Focus: use subject height 💙	Temperature: Locked	Initialize

 Choose a suitable filter for your fluorescent

J&H

博克科技

 Excitation light intensity

revvity

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

### 專業服務,誠心以赴 Always for you

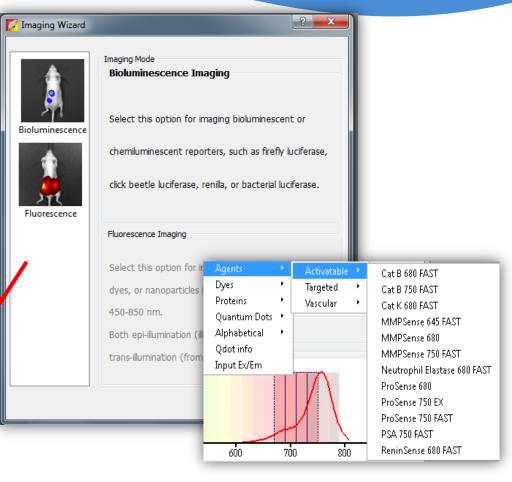
## revvity



## **Acquisition - Sequential mode**

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

🜠 IVIS Acquisition Control	Panel				[	
Imaging Mode Exposure Time	Binning	F/Stop	Excitatio	on Filter	Emission Fil	ter
🔜 🗹 Luminescent 1.00 🗘 sec	: 🖌 Medium	✓ 1	Block	×	Open	~
Eluorescent						
🚺 🗹 Photograph 🛛 Auto 💲	Medium	~				
Structure						
Overlay Lights	Alignment Grid	ystem Status	[	A	cquire	
Overlay Lights	1	ystem Status	_	A	cquire	
Overlay Lights Field of View: C	5	ystem Status			cquire ng Wizard	
Overlay Lights Field of View: MIS	5	ystem Status		Imagi		



### 專業服務, 誠心以赴 Always for you

## **Quantification**

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

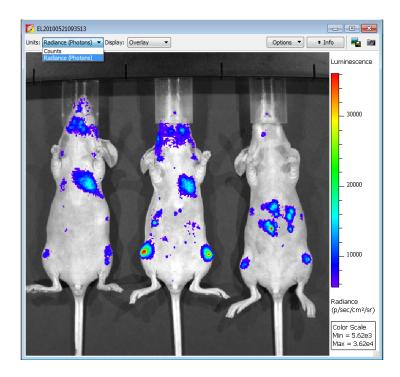


Image Adjust   Image Adjust   Photo Adjustment   Brightness:   100   Contrast:   1.5   Opacity:   100   Color Scale   Min:   5.62e3   Max:   3.62e4   Color Scale Limits   Auto   Full   Manual   Individual   Color Table   Rainbow   Deveree   Logarithmic Scale   Namual   Individual   Corrections / Filtering   Image Information   ROI Tools   Image Information   Apply to Sequence		
Image Information     Image Information <th>Tool Palette</th> <th>×</th>	Tool Palette	×
Photo Adjustment Brightness:  Description  Description  Description  Description  Description  Description  Pull  Description  Descript	🗸 Image Adjust	) [4]
Brightness: 100 Contrast: 1.5		
Contrast: 1.5 Opacity: 100 Color Scale Min: 5.62e3 Max: 3.62e4 Color Scale Limits Auto Full Manual Individual Color Table Rainbow Corrections / Filtering Deverse Corrections / Filtering Manual Color Table Rainbow Corrections / Filtering Manual Color Table Rainbow Corrections / Filtering Measure ROIs Apply to Sequence Measure ROIs Save ROIs Name: ROI_1_BNT Delete Load Save Auto ROI Parameters		
Opacity: 100 ♀   Color Scale   Max: 5.62e3 ♦   Max: 3.62e4 ♦   Color Scale Limits   Auto Full   Manual   Individual   Color Table   Rainbow   Prevent   Corrections / Filtering   Image Information   V ROI Tools   Image Information   V ROI Tools   Prevent   Name:   ROI_1_BNT   Delete   Load   Save   Auto ROI Parameters		
Color Scale  Min: 5.62e3  Max: 3.62e4  Color Scale Limits  Auto Full Manual  Individual  Color Table  Rainbow  Corrections / Filtering  Corrections / Filtering  Corrections / Filtering  Manual  Color Table  Rainbow  Corrections / Filtering  Manual  Color Table  Corrections / Filtering  Manual  Color Table  Rainbow  Corrections / Filtering  Manual  Color Table  Rainbow  Corrections / Filtering  Manual  Color Table  Rainbow  Corrections / Filtering	Contrast:	1.5 ≑
Min: 5.62e3 Max: 3.62e4 Color Scale Limits Auto Full Manual Individual Color Table Rainbow Provess Corrections / Filtering Corrections / Filtering Corrections / Filtering Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manual Manua	Opacity:	100 🚖
Max: 3.62e4 Color Scale Limits Auto Full Manual Individual Color Table Rainbow Corrections / Filtering Corrections / Filtering Corrections / Filtering Corrections / Filtering Maximum Content of the sele Maximum Content of the sele Auto ROI Parameters	Color Scale	
Color Scale Limits  Auto Full Manual Color Table Rainbow Color Table Rainbow Corrections / Filtering Corrections / Filtering Corrections / Filtering Corrections / Filtering Corrections C	Min:	5.62e3 ≑
Auto Full   Individual   Color Table   Rainbow   Rainbow   Proverse   Deverse   Corrections / Filtering   Image Information   ROI Tools   ROI Tools   Image Information   ROI Tools   Save ROIs   Name: ROI_1_BNT   Delete   Load   Save   Auto ROI Parameters	Max:	3.62e4 ≑
Individual   Color Table   Rainbow   Image Information   ✓ ROI Tools   ✓ ROI Tools   ✓ Image Information   ✓ ROI Tools   ✓ Delete   Logarithmic Scale   ✓ ROI Tools   ✓ Delete   Logarithmic Scale	Color Scale Limits	
Rainbow     Rainbow     Corrections / Filtering     Image Information     ROI Tools     ROI Tools     Image Information     ROI Tools     Image Information     Rowsee     Image Information     Roinge Information     Roinge Information     Image Information     Image Information     Roinge Information     Image Information	Auto  Full  M	1anual
Corrections / Filtering      Image Information      ROI Tools      Measure ROIs      Apply to Sequence      Ype: Measurement ROI     Save ROIs Name: ROI_1_BNT     Delete     Load     Save      Auto ROI Parameters	Color Table	
> Image Information         ✓ ROI Tools         ○ □ Ⅲ ④ ✔ Measure ROIs         Apply to Sequence         ype: Measurement ROI         Save ROIs         Name: ROI_1_BNT         Delete       Load         Auto ROI Parameters	Rainbow -	
> Image Information         ✓ ROI Tools         ○ □ Ⅲ ④ ✔ Measure ROIs         Apply to Sequence         ype: Measurement ROI         Save ROIs         Name: ROI_1_BNT         Delete       Load         Auto ROI Parameters		itheria Casla
> Image Information         ✓ ROI Tools         ○ □ Ⅲ ④ ✔ Measure ROIs         Apply to Sequence         ype: Measurement ROI         Save ROIs         Name: ROI_1_BNT         Delete       Load         Auto ROI Parameters		
ROI Tools   Image: Constraint of the second state of the		
Apply to Sequence          Apply to Sequence         ype:         Measurement ROI         Save ROIs         Name:       ROI_1_BNT         Delete       Load         Auto ROI Parameters		
Apply to Sequence          ype:       Measurement ROI         Save ROIs         Name:       ROI_1_BNT         Delete       Load       Save         Auto ROI Parameters       Image: Content of the second of the		
ype: Measurement ROI   Save ROIs Name: ROI_1_BNT   Delete Load Save Auto ROI Parameters	् 🔲 🏭 🍕 🏏 Measure R	OIs 🗶
Save ROIs Vame: ROI_1_BNT  Delete Load Save Auto ROI Parameters		
Vame: ROI_1_BNT   Delete Load Save Auto ROI Parameters	Type: Measurement ROI	-
Delete Load Save		
Auto ROI Parameters		
		Save
	Inresnoid %:"	50 👻 🔻

revvity



### 專業服務,誠心以赴 Always for you

### Analysis



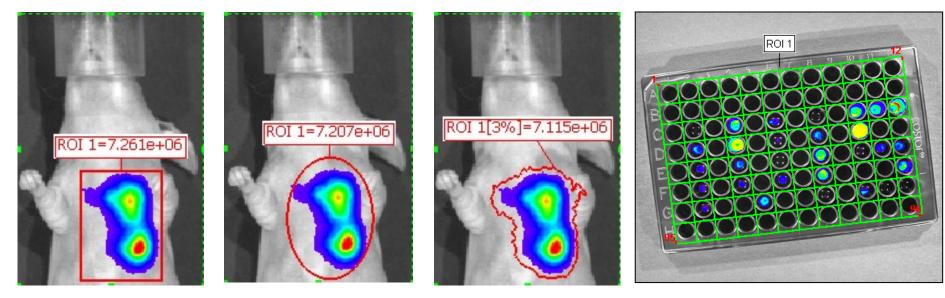


## **Region of Interest (ROI) Tools**

- ROI's can be created:
  - Manually
  - Automatically
  - Free Draw

►ROI shapes available:

- Square
- Circle
- Contour
- Grid

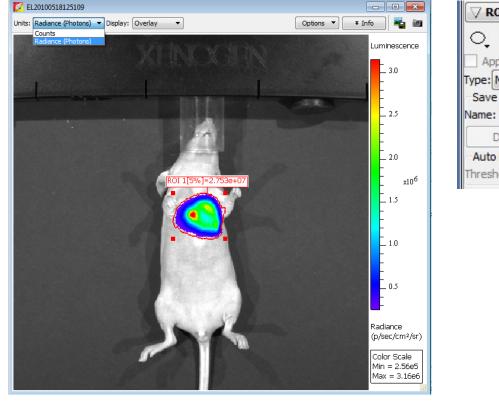


### 專業服務,誠心以赴 Always for you

### Analysis

### **Measurement Table**

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



7 ROI Tools	5							
् 🗆 🕷	🎚 💽 💘 Me	asure RO	Is 🗙					
Apply to Se	quence							
pe: Measure	ment ROI		•					
Save ROIs								
ame: ROI_1	BNT		-					
Delete	Load		Save					
Auto ROI Par nreshold %:	ameters	-0	50 🔺					
	Image Number	ROI	Image Layer	Total Flux [p/s]	Avg Radiance [p/s/cm²/sr]	Stdev Radiance	Min Radiance	Refresh Max Radiance
	EL20100518125109	ROI 1	Overlay	2.753e+07	9.797e+05	7.118e+05	1.845e+05	3.691e+06
	Customized Selections Measurements Types: Radiance (Photons)		Image	Attributes:		ROI Dimens	ions:	
	Configure Export.							Close

J&H

博克科技

revvity