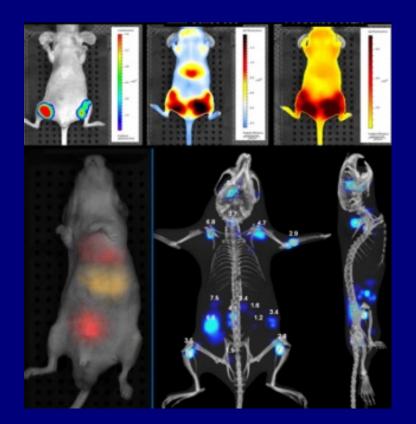
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## **IVIS® Spectrum 2**

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

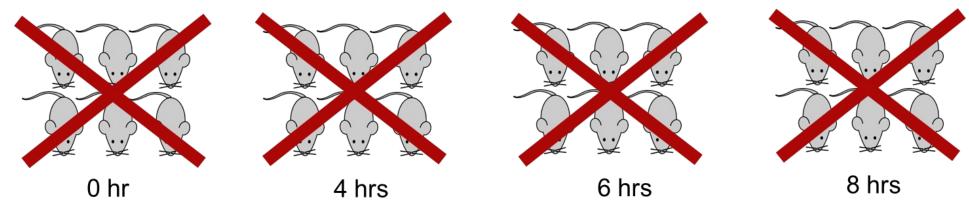
### **IVIS Spectrum 2 Platform**



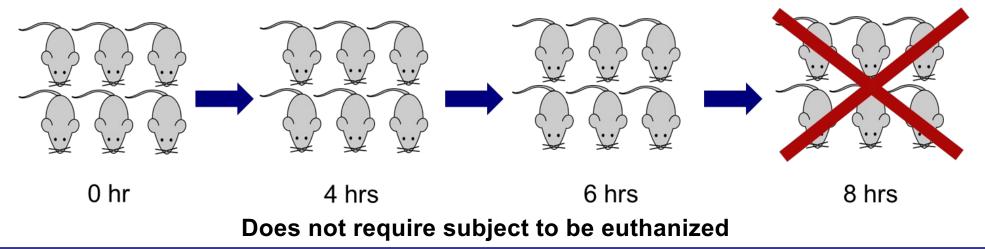
### Transitional methodology vs. BPI methodology



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

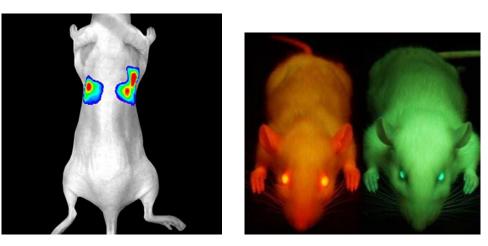
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## **Optical Imaging Approaches**

### • Bioluminescence & Fluorescent Proteins

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



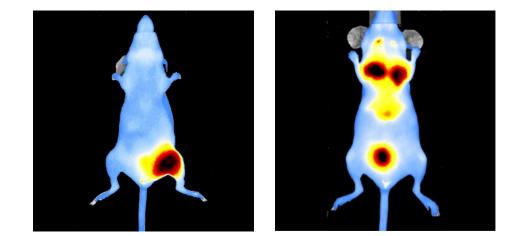
### • Fluorescent Agents (Red/NIR)

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

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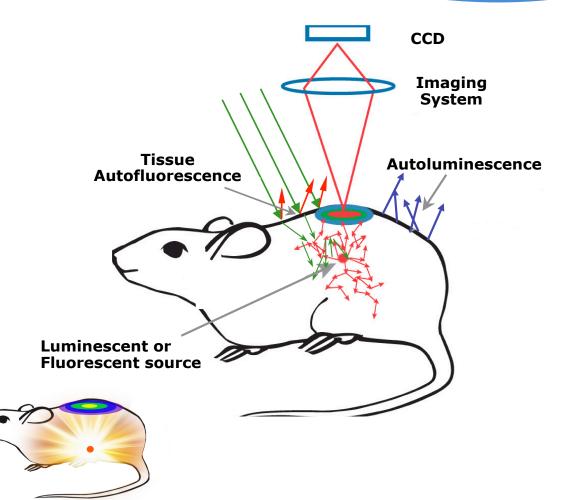
## The physics of light in tissue

- 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



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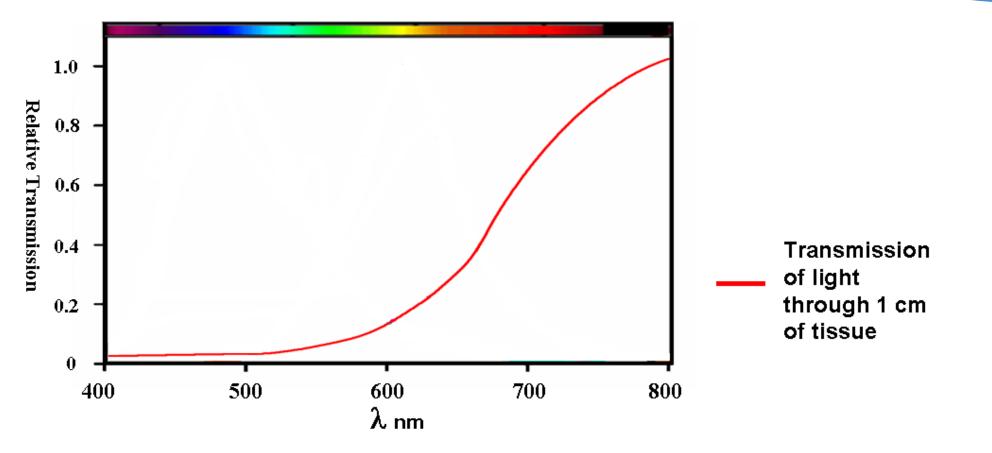
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## **Light in Tissue**



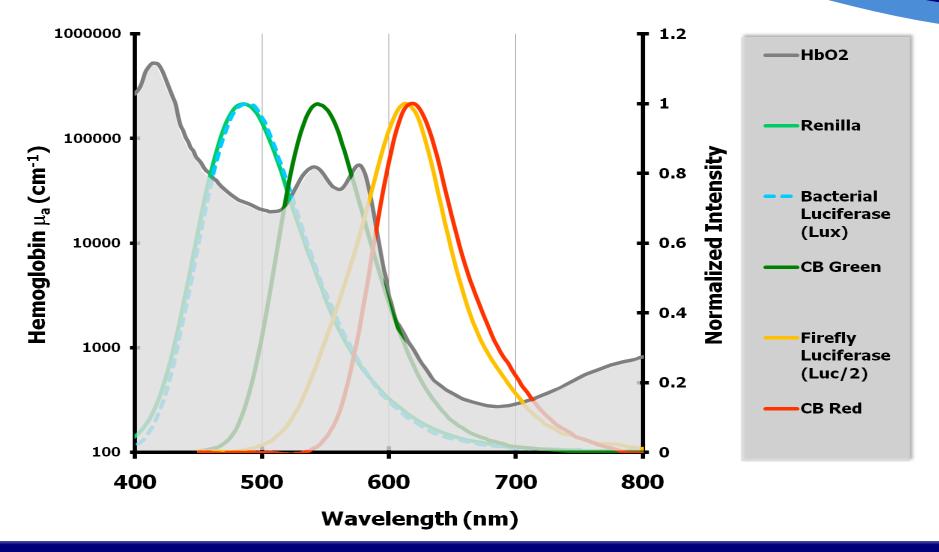
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Tissue Is Not Transparent – Light Absorbance Depends on Wavelength

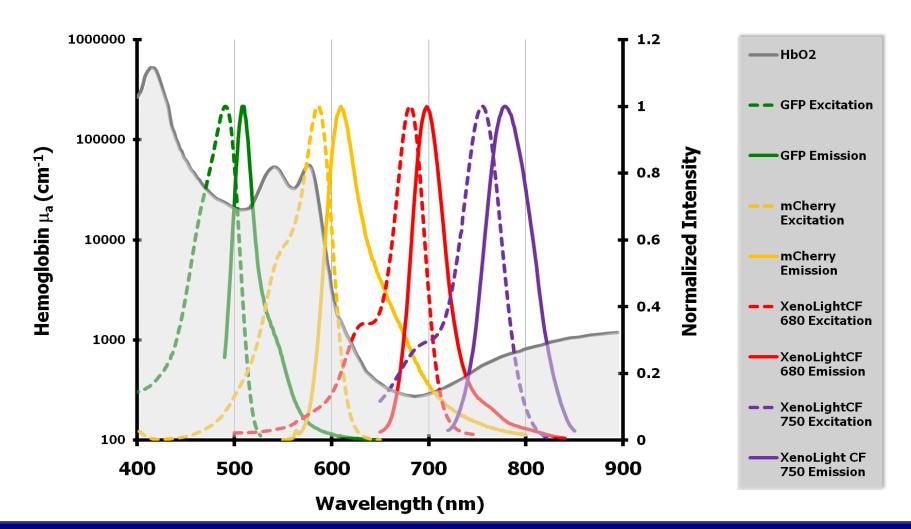
### **Bioluminescent Spectra and Tissue Penetration**



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### Fluorophore Spectra and Light Diffusion Through Tissue



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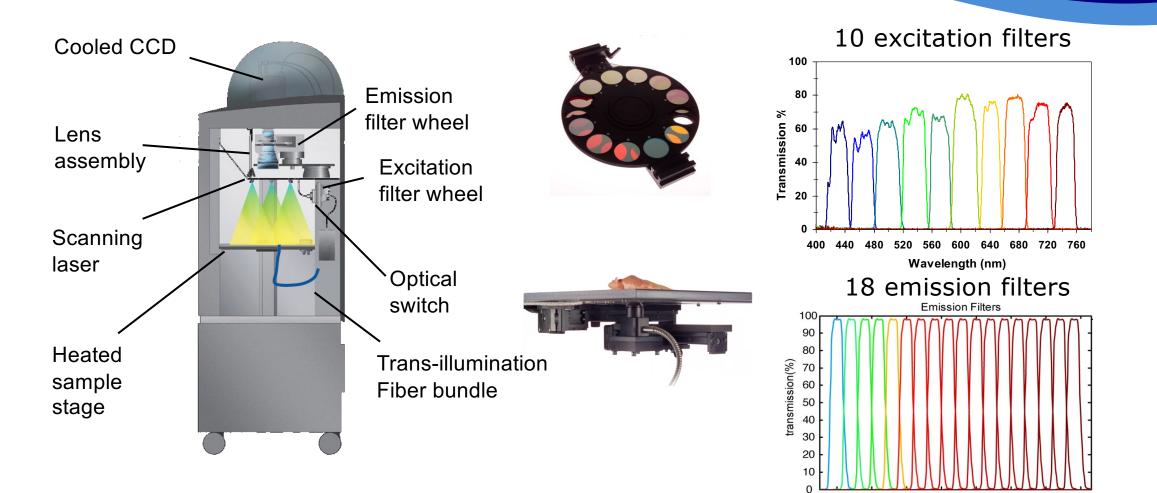
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## **IVIS<sup>®</sup> Spectrum 2**

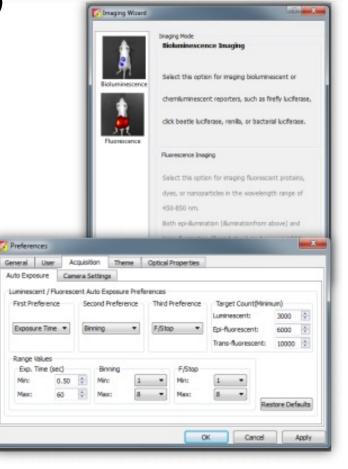


wavelength(nm)



## 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



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## Field of View (FOV)

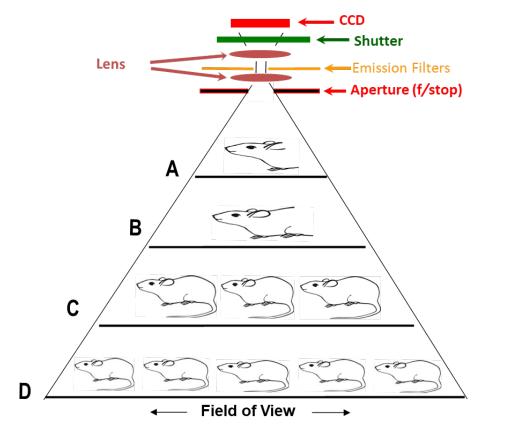
• Sets the size of the stage area to be imaged by

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



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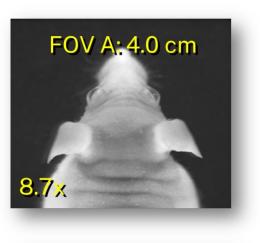
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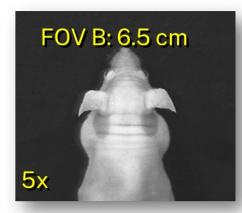
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## **Field of View (FOV)**

- 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

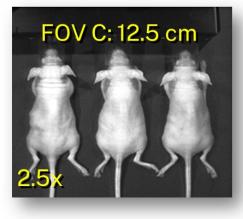


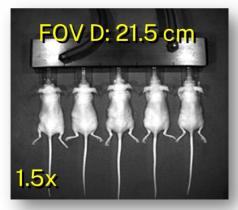


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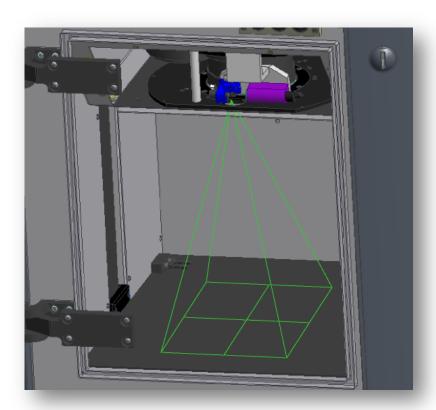


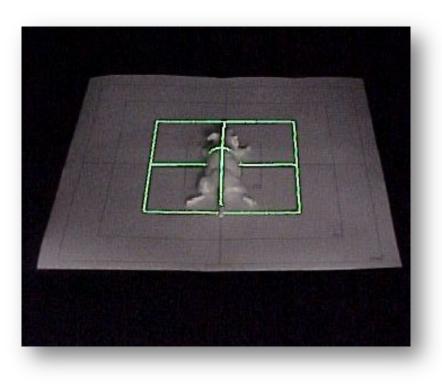


## **Alignment Light Projector**



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

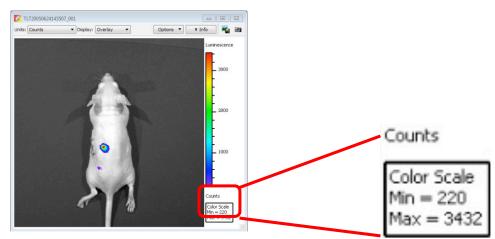






## **Setting Sensitivity – Signal Level**

- 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)



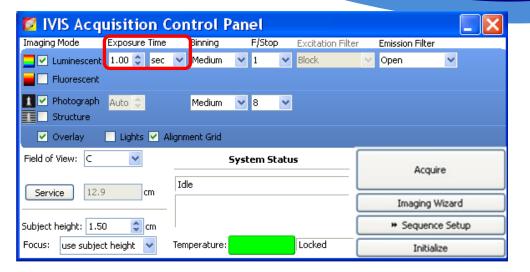
Instrument is calibrated to automatically compensate for changes in sensitivity settings when count levels are within the linear range

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### 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

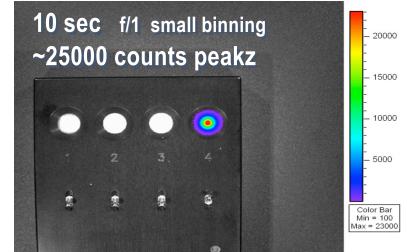


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## **Auto-Exposure**

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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

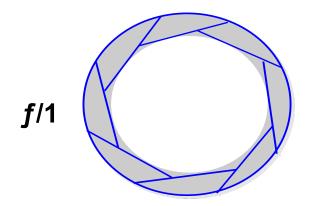
## revvity



## *f*/stop (Lens Aperture)

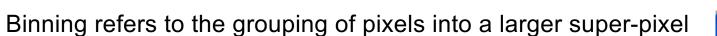
- *f*/stop controls the amount of light received by the ۲ CCD detector
- f/1 is wide open, maximum light collection ۲ - default for luminescent
- f/8 is smallest aperture, best resolution ۲ - default for photo
- Changing f/stop changes counts by a factor of 4

🚺 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

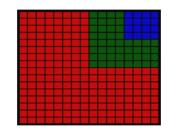


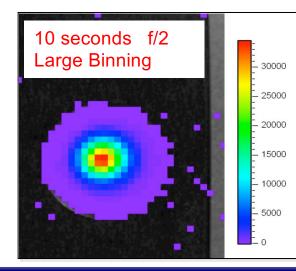


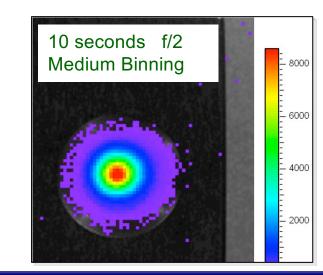
## **Pixel Binning (CCD Resolution)**

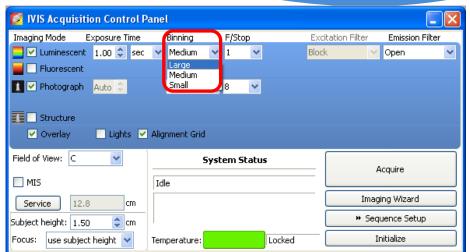


- Changing binning settings changes counts by a factor of 4
  - Large Binning (16)
     Higher Sensitivity/Lower Resolution
  - Medium Binning (8)
  - Small Binning (4)
     Lower Sensitivity/Higher Resolution





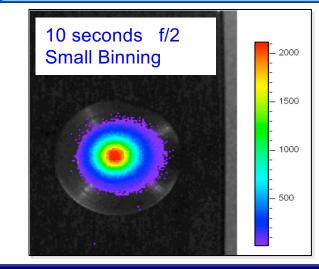




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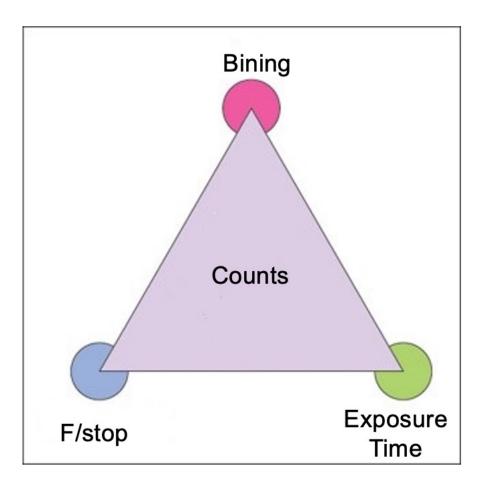


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### Sensitivity Controls – Relationship of F/Stop, Exposure Time and Bining



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

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 Excitation light intensity

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

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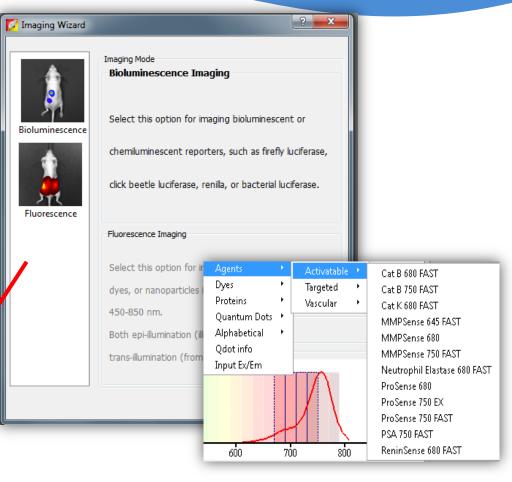
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## **Acquisition - Sequential mode**

- 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		



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## **Quantification**

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

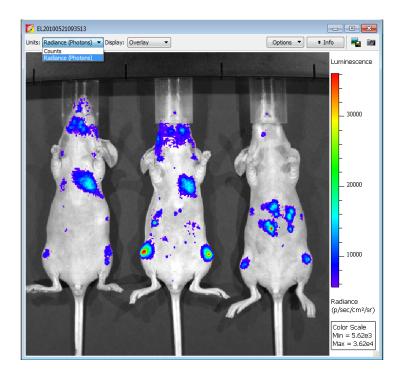


Image Adjust   Image Adjust   Photo Adjustment   Brightness:   100   Contrast:   1.5   Opacity:   100   Color Scale   Min:   5.62e3   Max:   3.62e4   Color Scale Limits   Auto   Full   Manual   Individual   Color Table   Rainbow   Deveree   Logarithmic Scale   Namual   Individual   Corrections / Filtering   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 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	
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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		
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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 👻 🔻

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### Analysis



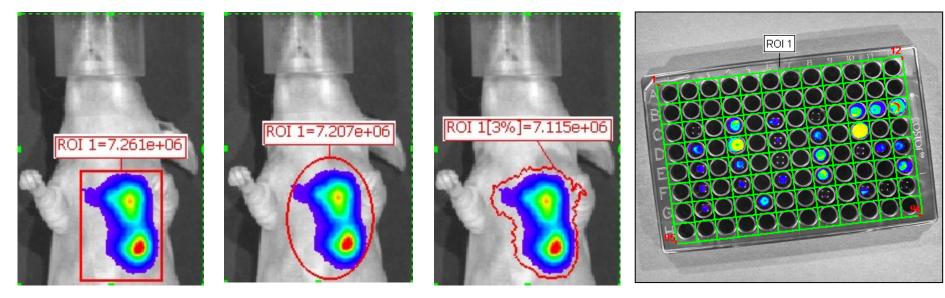


## **Region of Interest (ROI) Tools**

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

►ROI shapes available:

- Square
- Circle
- Contour
- Grid

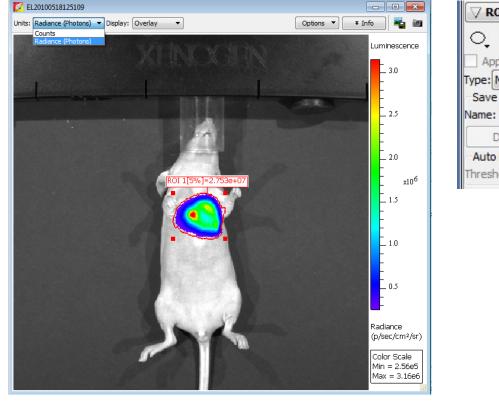


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### Analysis

### **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

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