



In Vivo Imaging with the



Xenogen IVIS™ 200 and Living Image® Software



What will be covered?

Introduction

- Science of *in vivo* Imaging
- Xenogen IVIS™ Hardware Overview
- Living Image® Software Overview
- Fluorescence System
- Basic Imaging Examples

Training

- Hands on Training



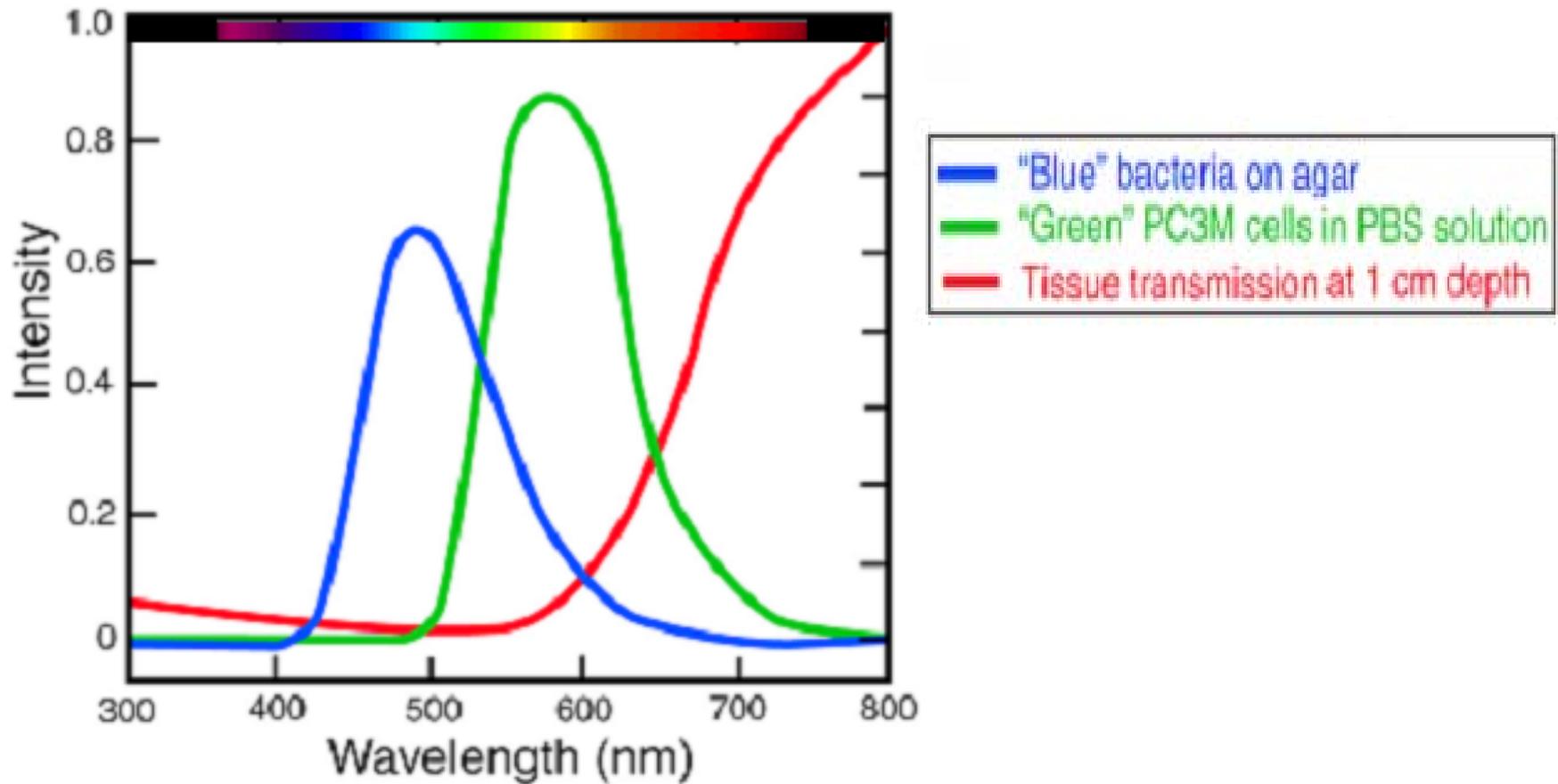
Why optical *in vivo* imaging?

- Powerful labeling technique - gene expression results in production of luciferase
 - Tracer Applications: Amount of light is proportional to number of cells
 - Functional Applications: Light is produced in response to a stimulus
- Extremely low backgrounds compared to other *in vivo* technologies
- Non-invasive – does not require subject to be euthanized
- Relatively simple instrumentation. Users can run themselves – lab instrument, not imaging center



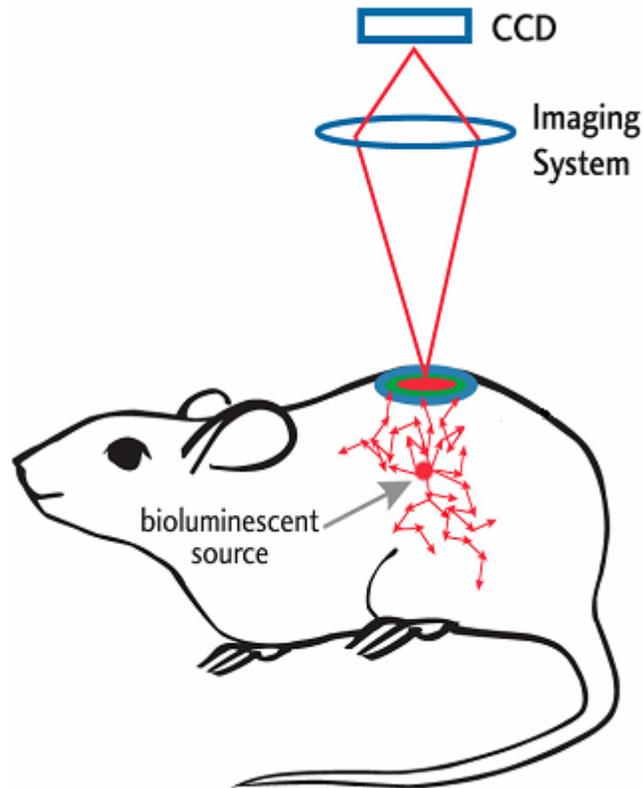
Tissue is not transparent - Light absorbance depends on wavelength

Luciferase Emission Spectra





Photons “diffuse” through tissue and the IVIS views this signal on the surface of the subject.



- Light traveling through tissue scatters many times creating a "fuzzy" image at the surface of the animal
- The IVIS™ Imaging System views the diffuse image on the surface of the subject



Calibrated Physical Units

- The amount of light reaching the detector is the Raw Signal Level. Raw signal is a function of Exposure time, Resolution (Binning), f/stop, and Field of View.
- Living Image[®] automatically accounts for differences in imaging settings and gives you the amount of light leaving the source.
- Calibrated units are expressed in Photons per unit time, per unit surface area, per unit collection angle.



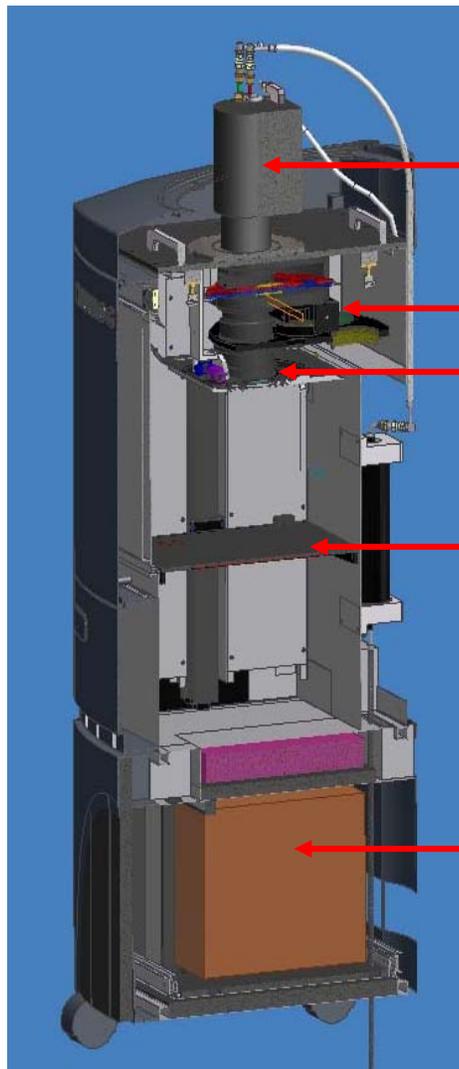
Xenogen IVIS™ Hardware



- Customized for *in vivo* imaging
- High sensitivity from 300-900 nm
- Large dynamic range
- Living Image® software



IVIS 200 Hardware



CCD camera

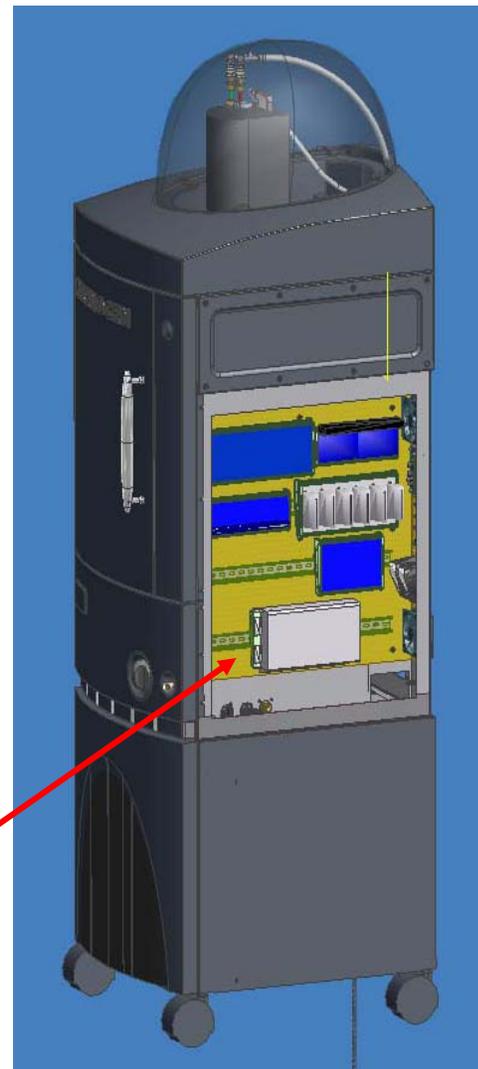
Filter Wheels

Lens

Heated Sample Stage

Cryotiger and Camera controller

Electronics





IVIS™ Fluorescence System

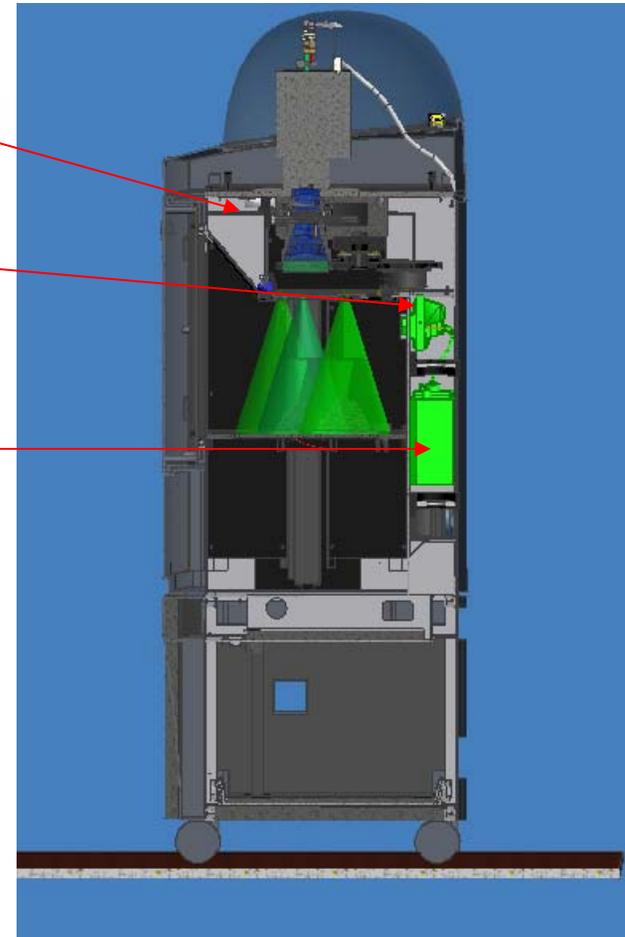
Twenty Two position computer controlled
Emission filter wheels

Six position computer controlled Excitation
filter wheel

150 Watt Tungsten/Halogen lamp with
computer controlled intensity

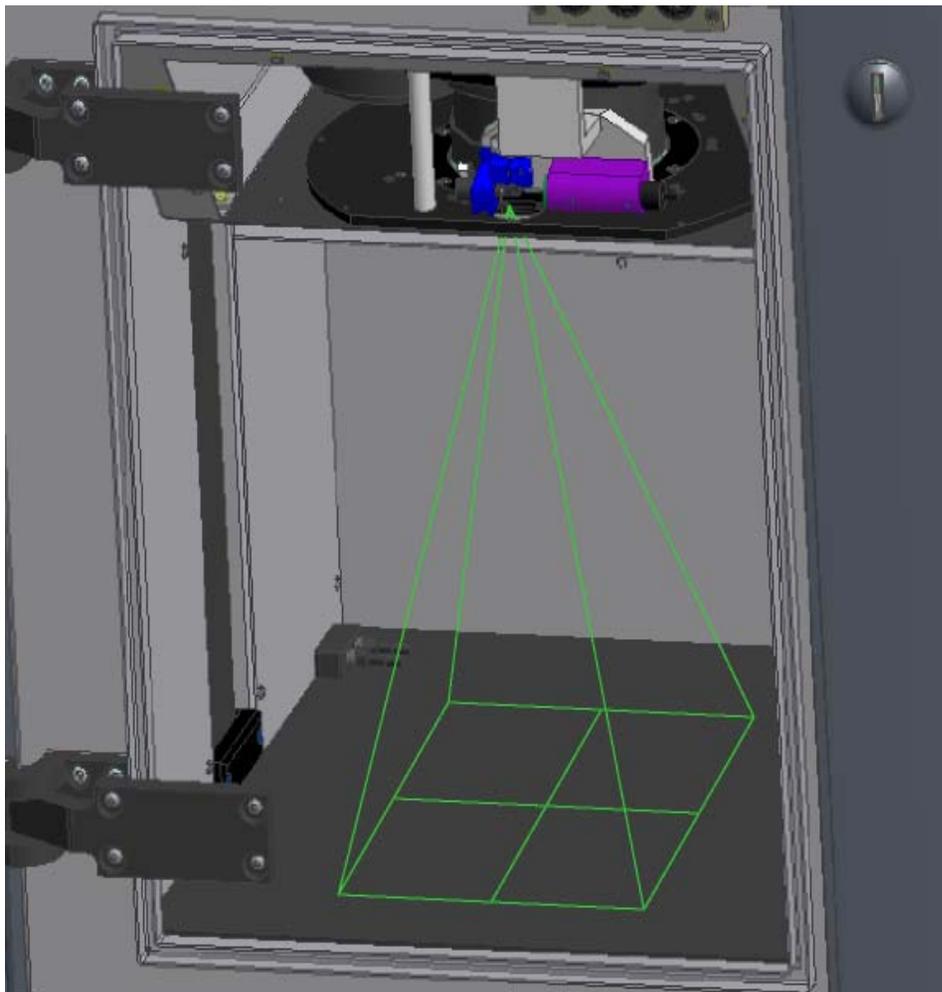
Low Auto Fluorescence optics
and fiber optics

Ultra-high out-of-band blocking
filters with steep edges on
bandpass



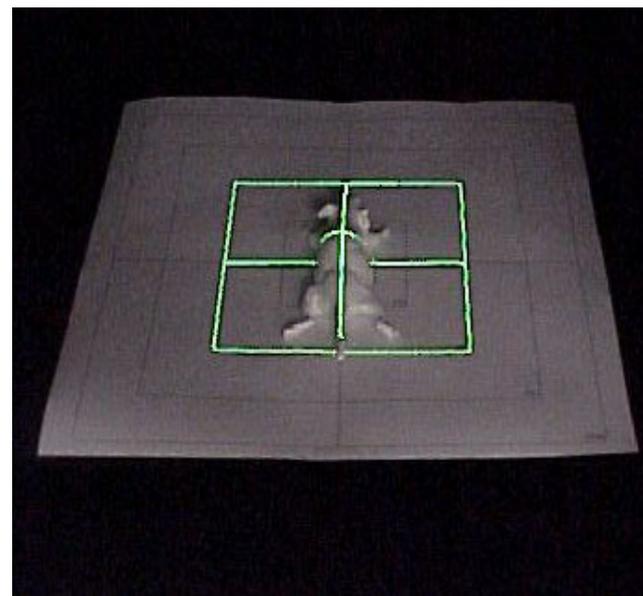


Alignment Light Projector



Size changes with FOV setting

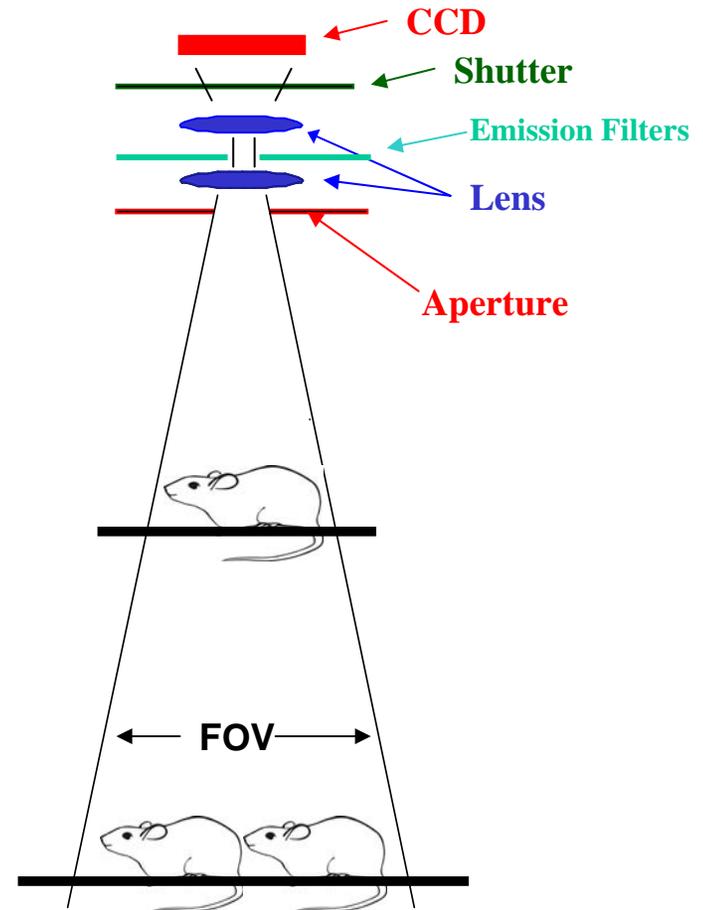
Allows rapid, accurate, and reproducible positioning of subjects





Camera and lens settings are analogous to those used in standard photography

- Light collected is proportional to the time the shutter is open (exposure time)
- Field of View (FOV) is dependent on the distance from the lens to the sample
- Zooming
- Auto Focus
- Adjustable Resolution



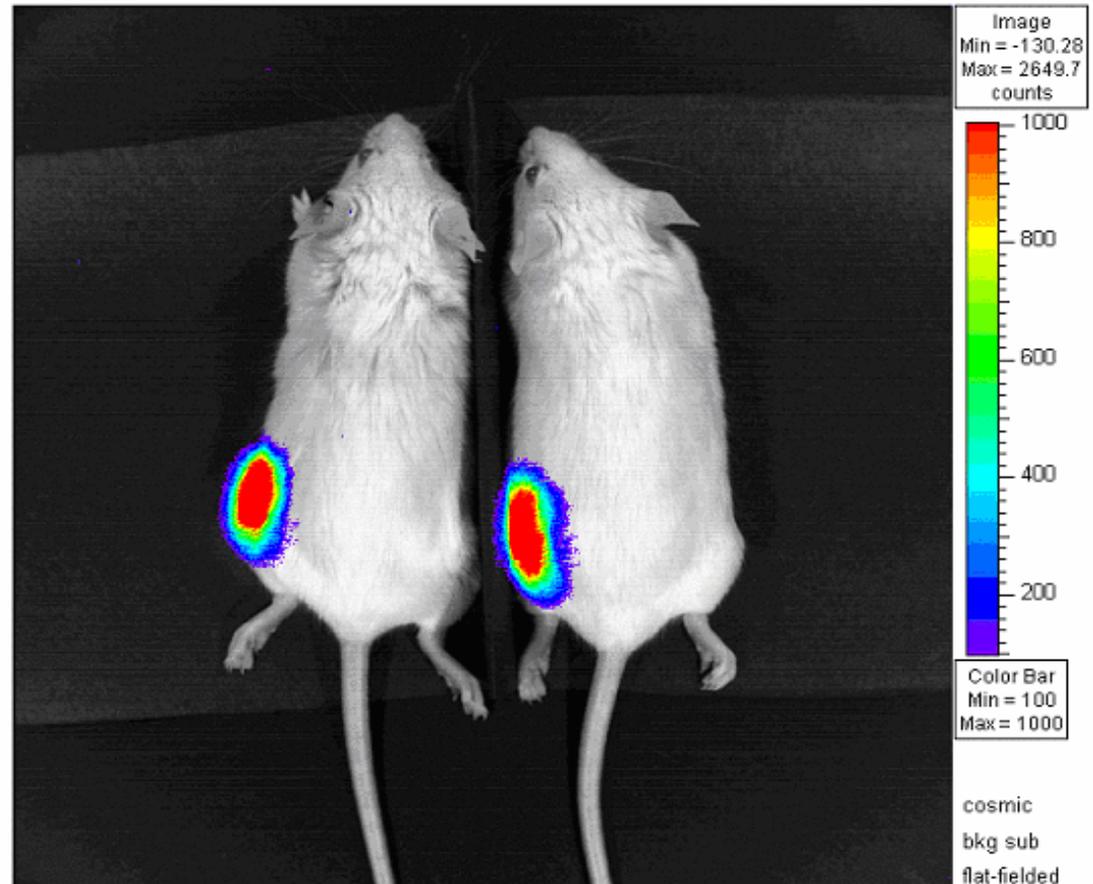
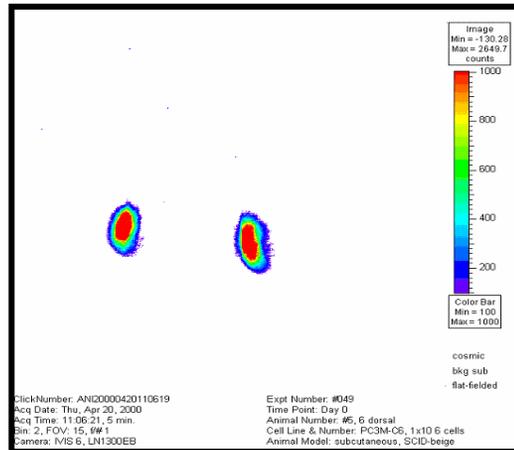


Xenogen's Living Image[®] software provides the interface for imaging and analysis

- Living Image[®]
 - Controls all system components and acquires images
 - Allows definition and quantitation of regions of interest (ROIs) in the image
 - Provides simplified image cataloging and browsing tools – good labeling practices improve data browsing
- Images can be analyzed at the IVIS[™] computer or at a user's computer running Living Image[®] analysis software



Standard Images are composed of two images Photographic + Luminescent/Fluorescent → Overlay





Living Image Control Panel

IVIS System Control

Imaging Mode	Exposure Time	Binning	f/stop	Excitation Filter	Emission Filter
<input type="checkbox"/> Luminescent <input checked="" type="checkbox"/> Fluorescent	1 <input type="text"/> sec	Medium	2	Block	Open
<input checked="" type="checkbox"/> Photographic	0.2 <input type="text"/>	Medium	8		
<input type="checkbox"/> Structure		<input type="checkbox"/> Enable alignment grid		Fluorescence level	High
<input checked="" type="checkbox"/> Overlay	<input type="checkbox"/> Lights On			<input checked="" type="checkbox"/> Auto Filter Lock	

Field of View: E 25 cm

Subject size (cm): 1.5

Focus: use subject size

System Status
Temperature ■
Idle

X E N O G E N

Acquire

Acquire continuous photos

Select sequential mode

Initialize IVIS system



Regions of Interest and Electronic Lab Book

Igor Pro 4.02A

File Edit Data Analysis Macros Windows Notebook Misc Help Living Image LI Tools

ANI20000501120643

Max Bar: 3.3917e+07
Min Bar: 1.0175e+06

Create Measure Remove photons Overlay

1 All All Print

Bright 100 Gamma 1.5

Circle Histogram

Bkg Sub
 Flat Field
 Cosmic

Total Area Flux = 1.43831e+08

Image
Min = -2.9155e+05
Max = 4.8898e+07
p/sec/cm²/sr

Color Bar
Min = 1.0175e+06
Max = 3.3917e+07

cosmic
bkg sub
flat-fielded

ClickNumber: ANI20000501120643 Series: Training
Acq Date: Mon, May 01, 2000 Experiment: Demo Expt 1
Acq Time: 12:06:46, 30 sec. Label: Mouse 4, 6
Bin: 2, FOV: 15, #/1 Comment: Dorsal view
Camera: MS 5, LNI300EB Analysis Comment: subcu left thighs

Lab Book

from: J:Training Presentation:ANI20000501120643A:

Measurement on calibrated Luminescent image (flat-field, background-subtracted and cosmic-corrected).

Click Number	Exp(s)	Binning	FOV	f/stop	Filter	Units
ANI20000501120643	30	2	15	1	(unknown)	photons

ROI	Total Flux (photons/sec)	ROI Pixels	Avg Radiance (p/s/cm ² /sr)	Stdev (p/s/cm ² /sr)	Min (p/s/cm ² /sr)	Max (p/s/cm ² /sr)
ROI 1	2.744e+07	2.734e+03	1.500e+06	1.968e+06	-4.264e+04	9.689e+06

Shape	Area (cm ²)	Xc (cm)	Yc (cm)	Width (cm)	Height (cm)	Angle
Square	1.536e+00	3.073e+00	1.006e+01	9.330e-01	1.646e+00	11.2

ROI	Total Flux (photons/sec)	ROI Pixels	Avg Radiance (p/s/cm ² /sr)	Stdev (p/s/cm ² /sr)	Min (p/s/cm ² /sr)	Max (p/s/cm ² /sr)
ROI 2	1.164e+08	3.522e+03	4.938e+06	8.656e+06	-3.039e+04	4.870e+07

Shape	Area (cm ²)	Xc (cm)	Yc (cm)	Width (cm)	Height (cm)	Angle
Circle	1.936e+00	8.072e+00	9.979e+00	1.152e+00	2.140e+00	8.31

	Total Counts	Pixels	Avg	Min	Max
Total	1.438e+08	6.256e+03	2.299e+04	-4.264e+04	4.870e+07

Ready



Image Cataloging & Browsing Tools

Browser Sort

Sort by:

Then by:

Then by:

Reverse Sort

Change Info for AH20010607154943

Known User ID: Other User ID:

Display any 5:

Series:

Experiment:

Label:

Comment:

Analysis Comment:

LivingImage Browser

Click Number	User ID	Series	Experiment	Label	Comment	Analysis Comment	Date and Time	Binning	Exposure	Field of View
AOC20031010104524	AOC	IkB Line 26	Prescreen	Males	1,2,3,4,5	Dorsal - 6min post luc	10/10/2003 10:45:52	5	30	20
AOC20031010104708	AOC	IkB Line 26	Prescreen	Males	1,2,3,4,5	Dorsal - 7min post luc	10/10/2003 10:47:40	5	10	20
AOC20031010104857	AOC	IkB Line 26	Prescreen	Males	1,2,3,4,5	Dorsal - 7min post luc	10/10/2003 10:49:29	5	10	20
AOC20031010105125	AOC	IkB Line 26	Prescreen	Males	1,2,3,4,5	Dorsal - 10min post luc feet covered	10/10/2003 10:51:57	5	10	20
AOC20031010105340	AOC	IkB Line 26	Prescreen	Males	1,2,3,4,5	Ventral - 10min post luc feet covered	10/10/2003 10:54:10	5	10	20
AOC20031010105743	AOC	IkB Line 26	Prescreen	Males*, Females	6*,7*,8,9,10	Ventral - 10min post luc feet covered	10/10/2003 10:58:13	5	10	20
AOC20031010110149	AOC	IkB Line 26	Prescreen	Females	9	Ventral - 10min post luc feet covered	10/10/2003 11:02:19	5	10	20
AOC20031010110603	AOC	IkB Line 26	Prescreen	Females	11,12,13,14,15	Ventral-3 min post luc	10/10/2003 11:06:34	5	10	20
AOC20031010111257	AOC	IkB Line 26	Prescreen	Females	11,12,13,14,15	Ventral-10min post luc	10/10/2003 11:13:28	5	10	20
AOC20031010112602	AOC	IkB Line 26	Prescreen	Females	16,17,18,19,20	Ventral-8min post luc	10/10/2003 11:26:32	5	10	20
AOC20031010113526	AOC	IkB Line 26	Prescreen	Females	21,22	Ventral-8min post luc	10/10/2003 11:35:56	5	10	20
AOC20031013102738	AOC	IkB Line 26 LPS	2mg/kg - 0hr	1,2,3,4,5	Male	Ventral	10/13/2003 10:28:05	5	10	20
AOC20031013102738	AOC	IkB Line 26 LPS	2mg/kg - 0hr	1,2,3,4,5	Male	Ventral	10/13/2003 10:27:40	2	10	20
AOC20031013104529	AOC	IkB Line 26 LPS	0mg/kg - 0hr	6*,7*,14,15,22	Male*, Female	Ventral	10/13/2003 10:45:33	2	10	20
AOC20031013104529	AOC	IkB Line 26 LPS	0mg/kg - 0hr	6*,7*,14,15,22	Male*, Female	Ventral	10/13/2003 10:45:59	5	10	20
AOC20031013105457	AOC	IkB Line 26 LPS	2mg/kg - 0hr	16,17,18,20,21	Female	Ventral	10/13/2003 10:55:28	5	10	20
AOC20031013105457	AOC	IkB Line 26 LPS	2mg/kg - 0hr	16,17,18,20,21	Female	Ventral	10/13/2003 10:55:01	2	10	20
AOC20031013110448	AOC	IkB Line 26 LPS	0mg/kg - 0hr	8,10,11,12,13	Female	Ventral	10/13/2003 11:05:19	5	10	20
AOC20031013110448	AOC	IkB Line 26 LPS	0mg/kg - 0hr	8,10,11,12,13	Female	Ventral	10/13/2003 11:04:52	2	10	20

Columns:



Standard Label Sets

Change Info for DW20020107092023

Known User ID: Other User ID:

Display any 5 Label Name Set:

- Group ID:
- Expt ID:
- Animal Model:
- Animal Strain:
- Pathogen:
- Route of Infection:
- Dose:
- Treatment:
- Animal Number:
- Time Point:
- Comment1:
- Comment2:
- IACUC Number:

Change Info for DW20020107092023

Known User ID: Other User ID:

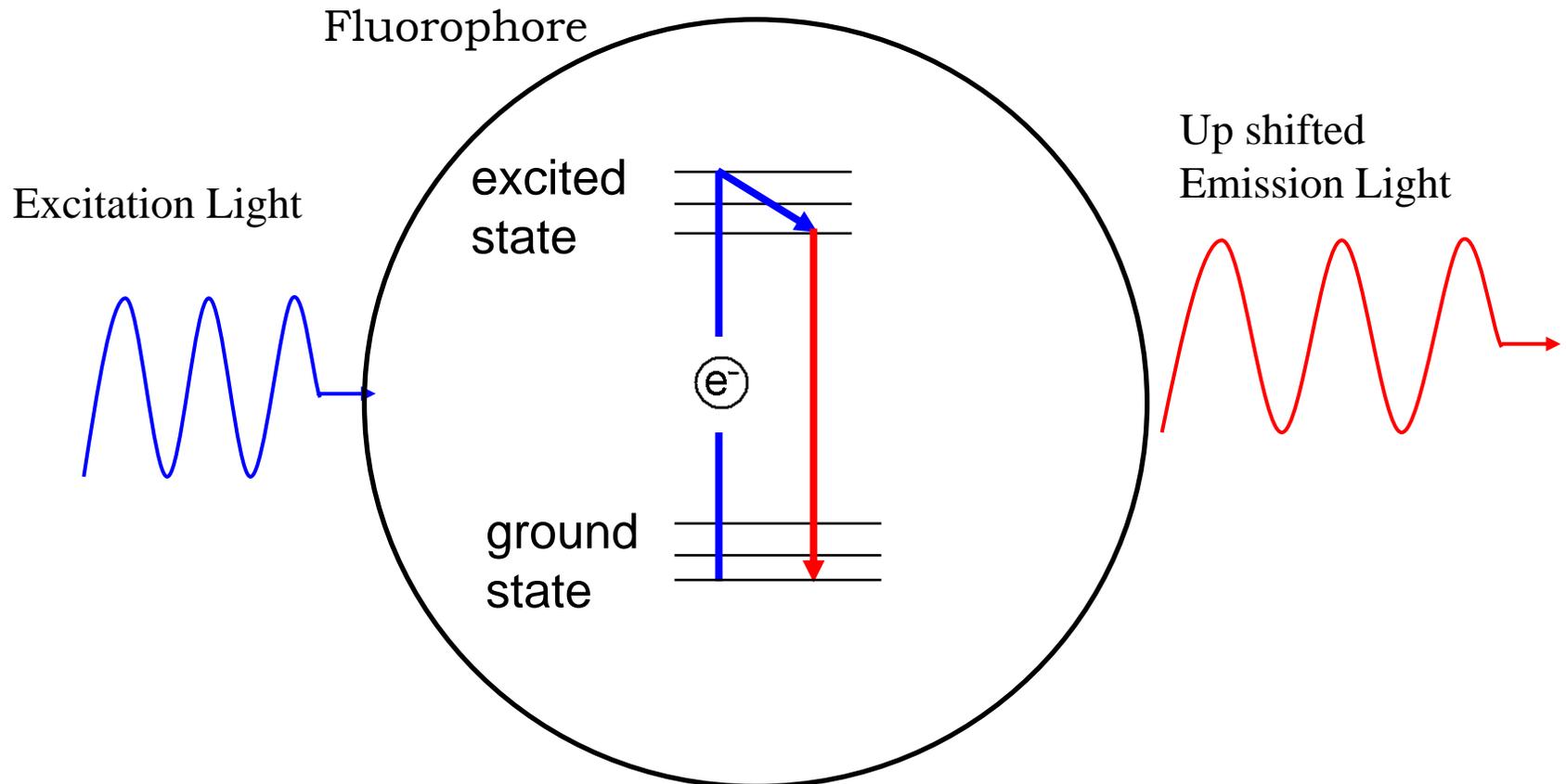
Display any 5 Label Name Set:

- Group ID:
- Expt Number:
- Time Point:
- Animal Number:
- Cell Line & Number:
- Animal Model:
- Comment1:
- Comment2:
- IACUC Number:
- Animal Strain:
- User:



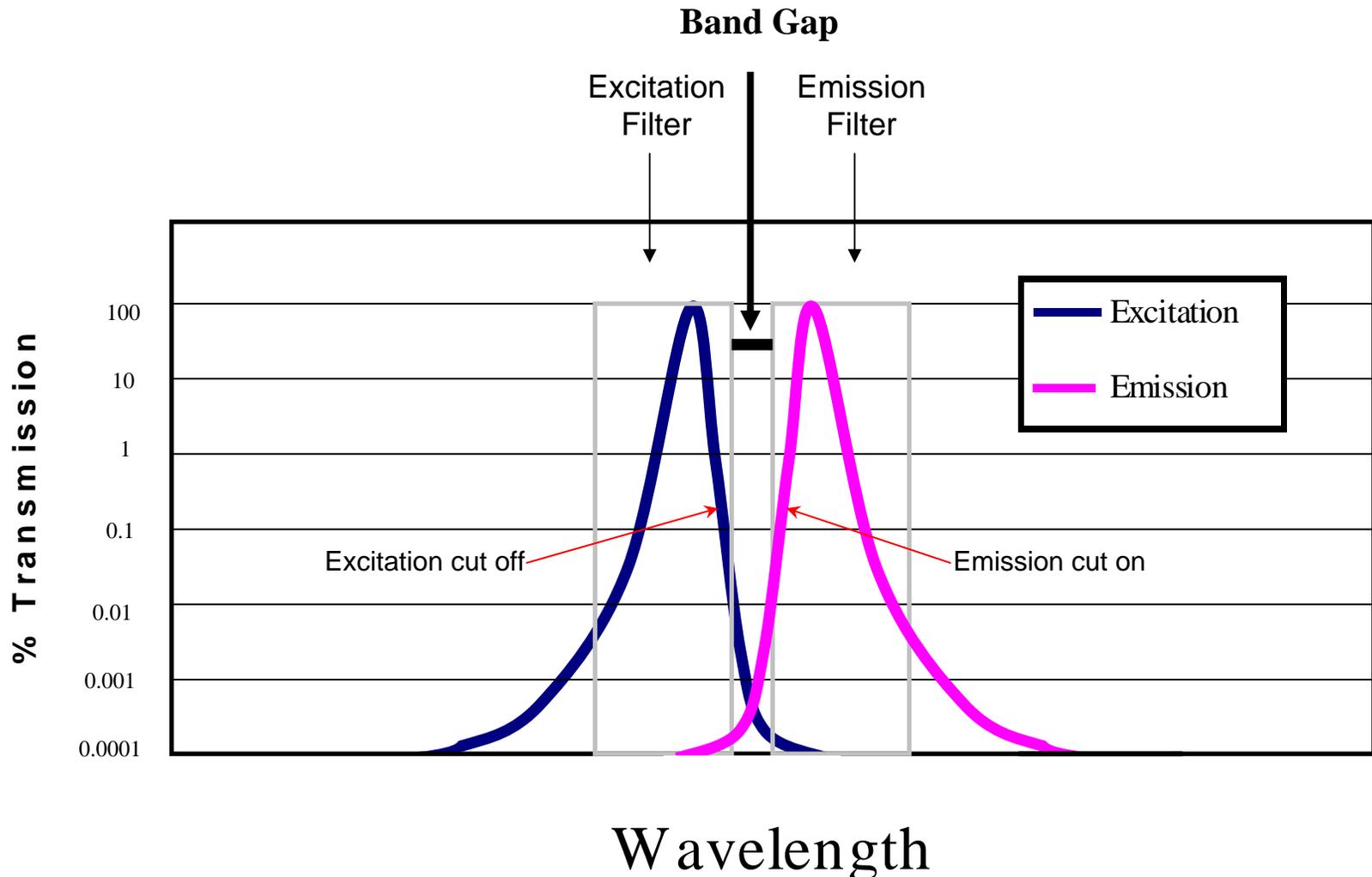
Fluorescence Process

$$\lambda_{em} - \lambda_{ex} = \text{Stokes shift}$$





Idealized Filter Transmission





IVIS 200 Fluorescence Filters

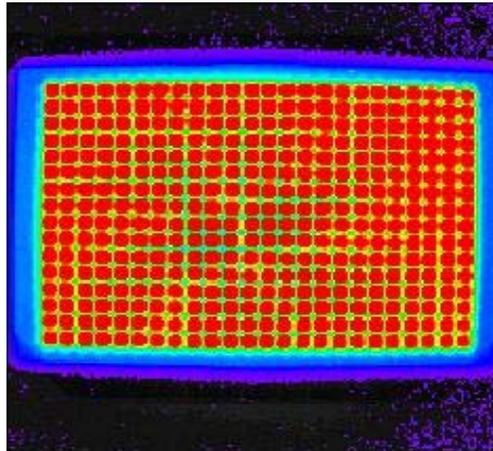
Fluorophore Excitation cut off (nm) Full Width at Half Max (nm) Emission cut on (nm) Full Width at Half Max (nm)

GFP	490	45	515	60
DsRed	550	50	575	75
Cy5.5	665	50	695	75
ICG	760	50	810	65

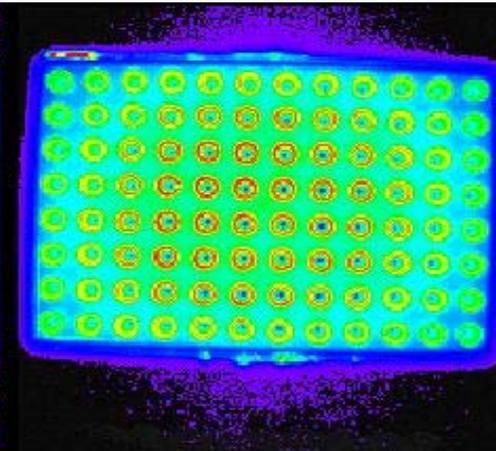


In Vitro Auto Fluorescence

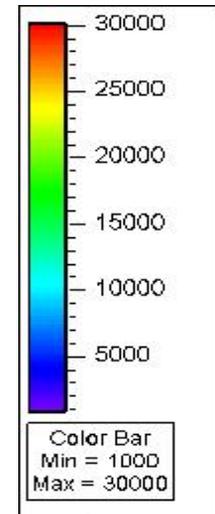
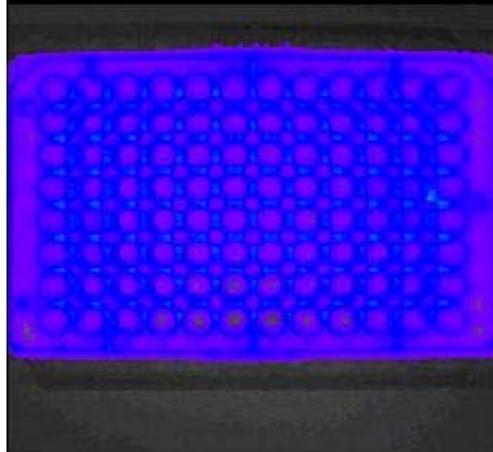
white
polystyrene



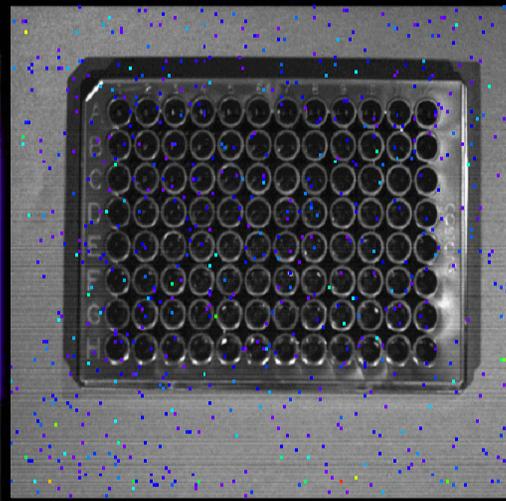
clear
polypropylene



clear
polystyrene



black
polystyrene





Autofluorescence of Control Mice

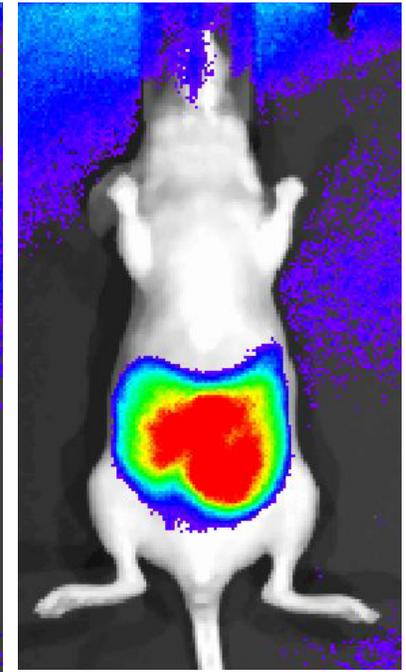
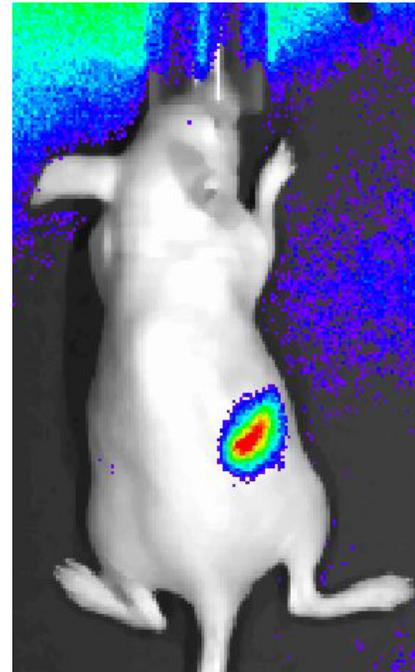
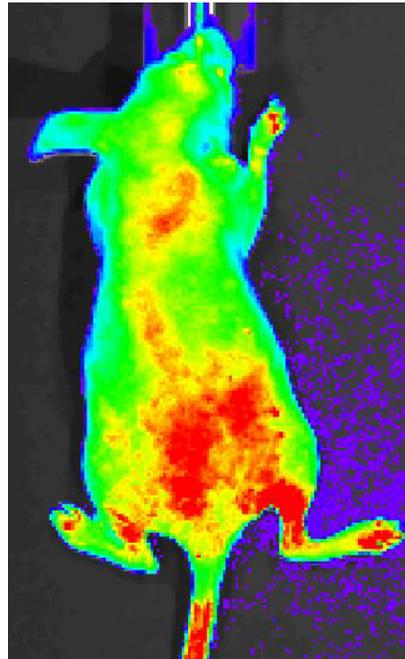
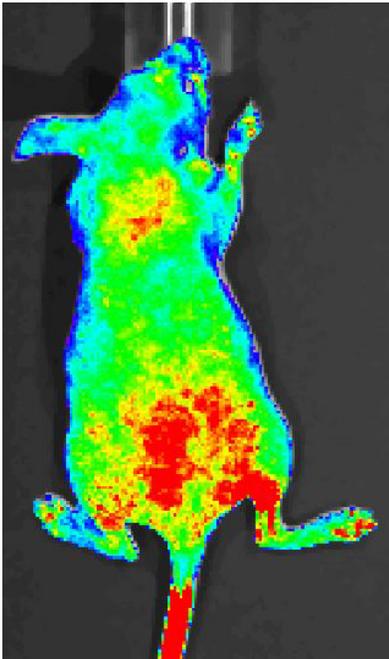
Infrared Filters

GFP filters

DsRed filters

Dorsal

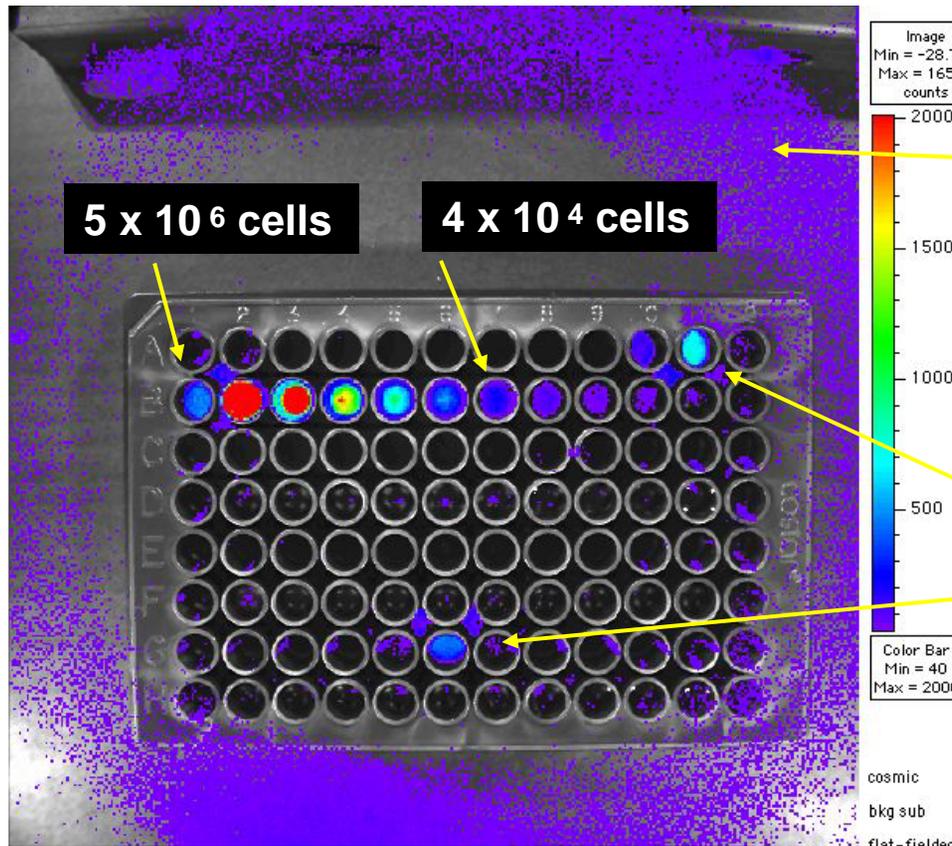
Ventral





Fluorescence *In Vitro* Example

Fluorescence - PKH26



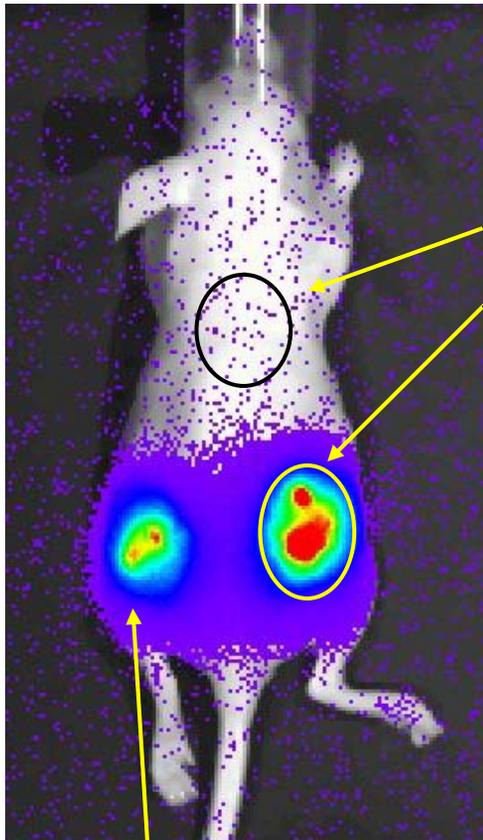
Background
Autofluorescence –
Software will subtract
this

Specular
Reflections



Comparison of Fluorescence and Bioluminescence *In Vivo*

Bioluminescent Image

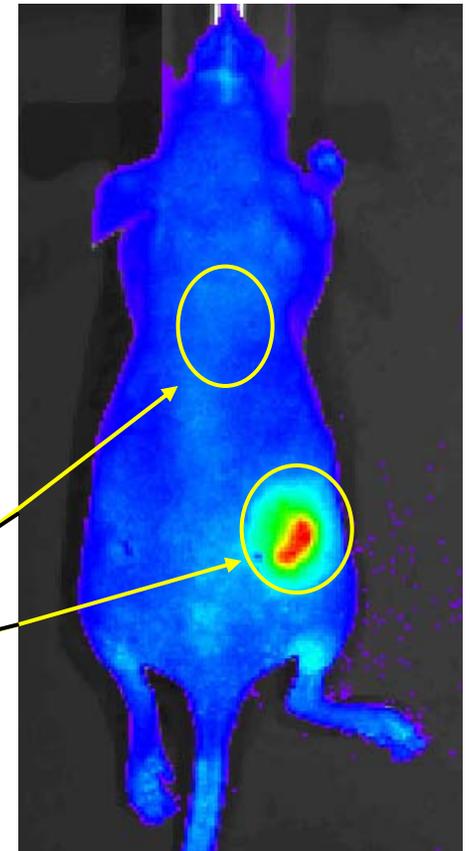


PC3M-luc (no DsRed)

Bioluminescence (10^7 cells):
Background flux $\sim 4.3 \times 10^3$ p/s
Signal flux $\sim 1.2 \times 10^7$ p/s
Signal/background ~ 2700

DsRed transient transfection (10%):
Background flux $\sim 5.1 \times 10^9$ p/s
Signal flux $\sim 1.1 \times 10^{10}$ p/s
Signal/background ~ 2.2

Fluorescent Image





In Vivo Fluorescence/Bioluminescence Comparison

Fluorescence Pros

Shorter Exposure Times

Multi-wavelength tags available

Proteins and dyes available

No luciferin

Cons

Autofluorescence limits sensitivity

Harder to get light in and out at short wavelengths (GFP and DsRed)



What we've covered . . .

- **Science**

- Light is scattered and absorbed by tissue dependant on λ
 - Calibrated physical units compensate for device settings

- **Hardware**

- Custom designed for *in vivo* bioluminescent imaging
 - Settings are analogous to a camera

- **Software**

- Images are acquired in a two step process
 - Living Image controls IVIS and provides image analysis tools

- **Fluorescence**

- Attention must be paid to Auto Fluorescence issues
 - Filter selection and lamp are controlled by Living Image



Basic Imaging Examples

- Bioluminescent *In Vivo* Acquisition
- Bioluminescent *In Vivo* Analysis



In Vivo Acquisition Example: Start Up Screen

The screenshot displays the Igor Pro 4.06A software interface. The main window is titled "Igor Pro 4.06A" and contains a menu bar with options: File, Edit, Data, Analysis, Macros, Windows, Notebook, Misc, Help, Living Image, and LI Tools. A "Lab Book" window is open in the upper right, showing a ruler from 0 to 4, a font style of "Normal", and the text "User ID: XQA". The "IVIS System Control" window is open in the lower right, featuring a control panel with the following settings:

Imaging Mode	Exposure Time	Binning	f/stop	Excitation Filter	Emission Filter
<input checked="" type="checkbox"/> Luminescent <input type="checkbox"/> Fluorescent	2 sec	Small (Hi Res)	1	Block	Open
<input checked="" type="checkbox"/> Photographic	0.2	Medium	8		
<input type="checkbox"/> Structure		<input type="checkbox"/> Enable alignment grid			
<input checked="" type="checkbox"/> Overlay	<input type="checkbox"/> Lights On				

Additional controls in the IVIS System Control window include:

- Field of View: E, 25 cm
- Subject size (cm): 1.5
- Focus: use subject size
- System Status: Temperature (green indicator), Idle
- Buttons: Acquire, Acquire continuous photos, Select sequential mode, Initialize IVIS system
- Text: X E N O G E N

The status bar at the bottom left of the main window shows "Ready".



Image Acquisition & Labeling

The screenshot displays the Igor Pro 4.06A software interface. The main window shows a grayscale image of a white mouse with a small, multi-colored spot on its hip. A 'Change Info for XQA20030217140733' dialog box is open, allowing for metadata entry. The 'Lab Book' window shows acquisition parameters, and the 'IVIS System Control' window shows real-time system status and control options.

Igor Pro 4.06A
File Edit Data Analysis Macros Windows Panel Misc Help Living Image LI Tools

XQA20030217140733
Max Bar: 16343, Min Bar: 1634, Bright: 100, Gamma: 1.5
Buttons: Create, Measure, Remove, counts, Overlay, Print, Bkg Sub, Flat Field, Cosmic, Histogram

Change Info for XQA20030217140733
Known User ID: XQA, Other User ID:
Label Name Set: Xenogen Default
Series: Presentation
Experiment: Plastic Mouse
Label: LED in Hip
Comment:
Analysis Comment:
Done

Lab Book
fNumber: 8, Exposure Time Sec: 0.2
luminescent image: LuminWave
Acquisition Date: Monday, February 17, 2003
Acquisition Time: 14:08:28
Acquisition Seconds: 3128335708
Width: 4, Height: 4
Units: counts
luminescent Exposure (Seconds): 2
Ambient Temperature: -105
Sample Temperature: -105.25
Wave: No Error
Field of View: 25
Channel: 1
luminescent Exposure Units: sec
Position: 1
Open
Number: 1

IVIS System Control
Imaging Mode: Luminescent, Fluorescent
Exposure Time: 2 sec
Binning: Small (Hi Res)
f/stop: 1, Excitation Filter: Block, Emission Filter: Open
 Photographic, 0.2, Medium, 8
 Structure, Enable alignment grid
 Overlay, Lights On
System Status: Temperature (green), Idle
Acquire, Acquire continuous photos, Select sequential mode, Initialize IVIS system
Field of View: E, 25 cm
Subject size (cm): 1.5, Focus: use subject size

Color Bar: Min = 1634, Max = 16343
cosmic, bkg sub, flat-fielded
ClickNumber: XQA20030217140733
Acq Date: Mon, Feb 17, 2003
Acq Time: 14:08:28, 2 sec.
Bin: HR (4), FOV: 25, f/# 1
Camera: IVIS 103, S1620EEV



Field of View (FOV) Change, Lab Book Editing

The screenshot displays the Igor Pro 4.06A software interface. The main window shows a grayscale image of a mouse with a color overlay on its hip. The image is titled 'XQA20030217140733'. The 'Image' statistics show a minimum of -196.65 and a maximum of 16343 counts. A color bar on the right indicates a scale from 2000 to 16000 counts. The 'Lab Book' window displays acquisition parameters: Acquisition Time: 14:08:28, Acquisition Seconds: 3128335708, Pixel Width: 4, Pixel Height: 4, Image Units: counts, Luminescent Exposure (Seconds): 2, Demand Temperature: -105, Measured Temperature: -105.25, Error Wave: No Error, Field of View: 25, Cosmic: 1, Luminescent Exposure Units: sec, Filter Position: 1, Filter: Open, fNumber: 1. The 'IVIS System Control' window shows imaging mode settings: Luminescent checked, Exposure Time: 2 sec, Binning: Small (Hi Res), f/stop: 1, Emission Filter: Open. The 'System Status' section shows Temperature: Idle. A dropdown menu for 'Field of View' is open, showing options A, B, C (selected), D, E, Service, and Load.

Max Bar: 16343
Min Bar: 1634
Bright: 100
Gamma: 1.5

Image
Min = -196.65
Max = 16343
counts

Color Bar
Min = 1634
Max = 16343

cosmic
bkg sub
flat-fielded

ClickNumber: XQA20030217140733
Acq Date: Mon, Feb 17, 2003
Acq Time: 14:08:00, 2 sec.
Bin: HR (4), FOV: 25, f/# 1
Camera: IVIS 103, S1620EEV

Series: Presentation
Experiment: Plastic Mouse
Label: LED in Hip
Comment:
Analysis Comment:

Lab Book

Acquisition Time: 14:08:28
Acquisition Seconds: 3128335708
Pixel Width: 4
Pixel Height: 4
Image Units: counts
Luminescent Exposure (Seconds): 2
Demand Temperature: -105
Measured Temperature: -105.25
Error Wave: No Error
Field of View: 25
Cosmic: 1
Luminescent Exposure Units: sec
Filter Position: 1
Filter: Open
fNumber: 1

*** User Label Name Set: Xenogen Default
Series: Presentation
Experiment: Plastic Mouse
Label: LED in Hip

IVIS System Control

Imaging Mode	Exposure Time	Binning	f/stop	Excitation Filter	Emission Filter
<input checked="" type="checkbox"/> Luminescent <input type="checkbox"/> Fluorescent	2 sec	Small (Hi Res)	1	Block	Open
<input checked="" type="checkbox"/> Photographic	0.2	Medium	8		
<input type="checkbox"/> Structure					
<input checked="" type="checkbox"/> Overlay					
<input type="checkbox"/> Lights On					

System Status
Temperature: Idle

Field of View: A, B, C, D, E, Service, Load

Subject size (cm):
Focus: use:

Acquire
Acquire continuous photos
Select sequential mode
Initialize IVIS system



Field of View Comparison, Image Saving

The screenshot displays two windows from the Igor Pro 4.05A software, comparing different field of view (FOV) settings for a mouse image. The left window, titled 'XQA20030217141129', shows a zoomed-in view of the mouse's rear. The right window, titled 'XQA20030217140730', shows a wider field of view of the same mouse. Both windows include a color scale on the right side of the image, a 'Color Bar' with 'Min' and 'Max' values, and a metadata section at the bottom.

Left Window (Zoomed Image):

- ClickNumber: XQA20030217141129
- Acq Date: Mon, Feb 17, 2003
- Acq Time: 14:11:51, 2 sec
- Bin: HR (4), FOV: 15, #1
- Camera: IVIS 103, S620EEV
- Series: Presentation
- Experiment: Plastic Mouse
- Label: LED in Hip
- Comment: Zoomed Image
- Analysis Comment:

Right Window (Wider Field of View):

- ClickNumber: XQA20030217140730
- Acq Date: Mon, Feb 17, 2003
- Acq Time: 14:09:00, 2 sec
- Bin: HR (4), FOV: 25, #1
- Camera: IVIS 103, S620EEV
- Series: Presentation
- Experiment: Plastic Mouse
- Label: LED in Hip
- Comment:
- Analysis Comment:



In Vivo Analysis Example: Image Retrieval

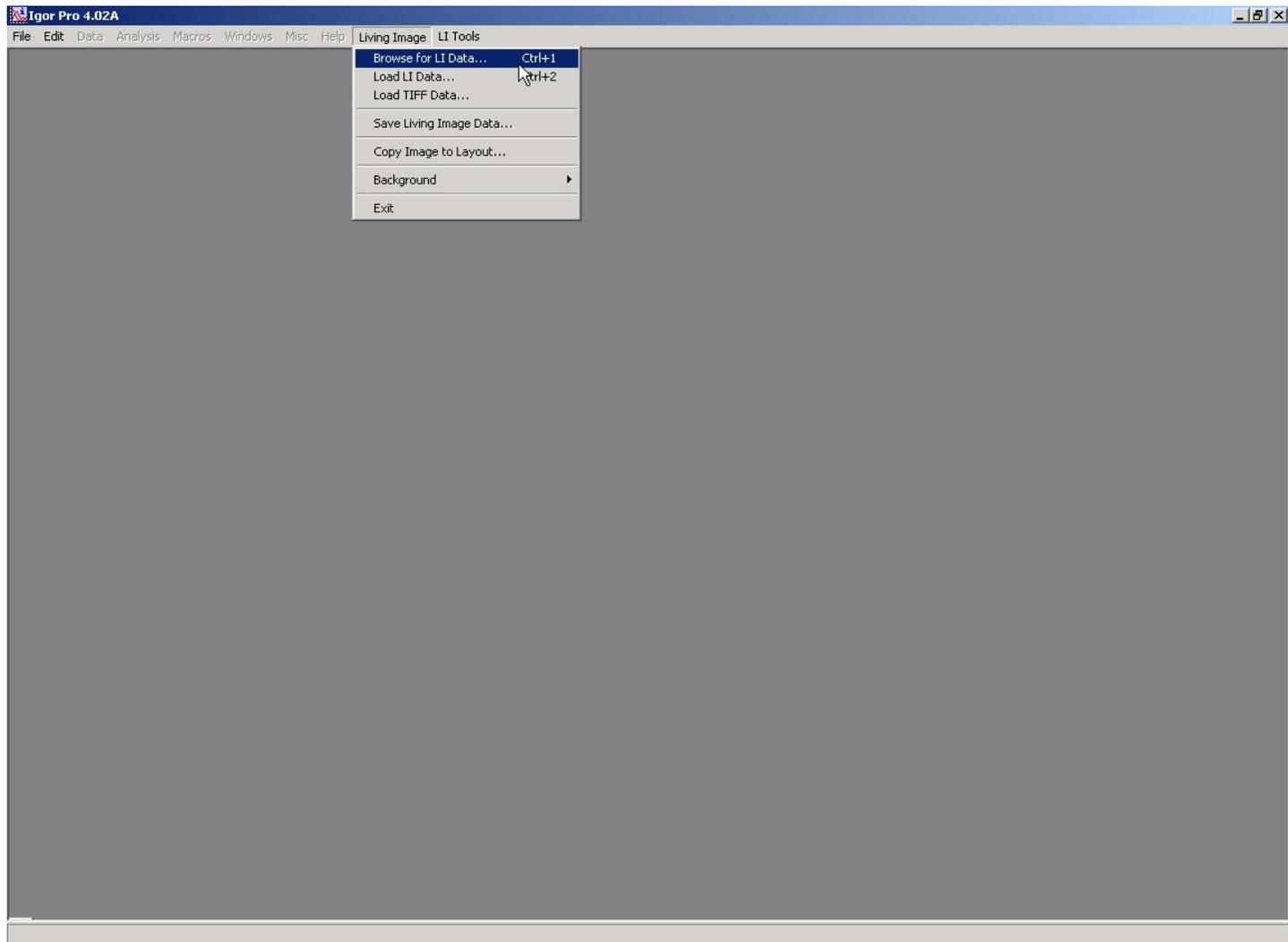




Image Browser

Igor Pro 4.08

File Edit Data Analysis Macros Windows Panel Misc Help Living Image LI Tools

LivingImage Browser

Click Number	User ID	Series	Experiment	Label	Comment	Analysis Comment	Date and Time	Binning	Exposure	Field of View	f-Stop	Fit
INV20020814102030	INV	A549 Drug Study	A549-C8,HT-29-D6 + Dox/Taxol	T=24hr 4,000 cells	60s, 2bin		8/14/2002 10:21:22	2	60	15	1	0
INV20020815100938	INV	A549 Drug Study	A549-C8,HT-29-D6 + Dox/Taxol	T=48hr 4,000 cells	60s, 10bin		8/15/2002 10:10:18	10	60	15	1	0
INV20020815101152	INV	A549 Drug Study	A549-C8,HT-29-D6 + Dox/Taxol	T=48hr 4,000 cells	60s, 2bin		8/15/2002 10:12:45	2	60	15	1	0
INV20020815101449	INV	A549 Drug Study	MCF-7-F5,PC3M-C6 + Dox/Taxol	T=48hr 4,000 cells	60s, 2bin		8/15/2002 10:15:41	2	60	15	1	0
INV20020815101758	INV	A549 Drug Study	MCF-7-F5,PC3M-C6 + Dox/Taxol	T=48hr 4,000 cells	60s, 10bin		8/15/2002 10:18:41	10	60	15	1	0
INV20020815102525	INV	A549 Drug Study	A549-C8,HT-29-D6 + Dox/Taxol	T=48hr 4,000 cells	60s, 2bin	4,000 plate	8/15/2002 10:26:18	2	60	15	1	0
INV20020815102849	INV	A549 Drug Study	A549-C8,HT-29-D6 + Dox/Taxol	T=48hr 4,000 cells	60s, 10bin	4,000 plate	8/15/2002 10:29:32	10	60	15	1	0
INV20020815103121	INV	A549 Drug Study	MCF-7-F5,PC3M-C6 + Dox/Taxol	T=48hr 4,000 cells	60s, 10bin	4,000 plate	8/15/2002 10:32:04	10	60	15	1	0
INV20020815103346	INV	A549 Drug Study	MCF-7-F5,PC3M-C6 + Dox/Taxol	T=48hr 4,000 cells	60s, 2bin	4,000 plate	8/15/2002 10:34:38	2	60	15	1	0
INV20020816102252	INV	A549 Drug Study	A549-C8, HT-29-D6; +Dox, Taxol	T=72hr plate 1	60s, 2bin		8/16/2002 10:23:41	2	60	15	1	0
INV20020816102700	INV	A549 Drug Study	MCF-7-F5,PC3M-C6; +Dox, Taxol	T=72 hr plate 1	60s, 2bin		8/16/2002 10:27:52	2	60	15	1	0
INV20020816103330	INV	A549 Drug Study	A549-C8, HT-29-D6; +Dox, Taxol	T=72 hr plate 2	60s, 2bin		8/16/2002 10:34:23	2	60	15	1	0
INV20020816103714	INV	A549 Drug Study	MCF-7-F5, PC3M-C6; +Dox, Taxol	T=72 hr plate 2	60s, 2bin		8/16/2002 10:38:06	2	60	15	1	0
XQA20021125202819	DW						11/25/2002 20:28:44	4	300	15	1	0
XQA20021125205615	DW						11/25/2002 20:56:44	4	300	10	2	0
XQA20021125212410	DW						11/25/2002 21:24:39	4	300	25	2	0
XQA20021125215205	DW						11/25/2002 21:52:35	4	300	20	1	0
XQA20030217140733	XQA	Presentation	Plastic Mouse	LED in Hip			2/17/2003 14:08:28	4	2	25	1	0
XQA20030217141129	XQA	Presentation	Plastic Mouse	LED in Hip	Zoomed Image		2/17/2003 14:12:11	4	2	15	1	0

Add Remove Remove All Sort Columns: All Populated Values Export Measurements Load As Group Load

Ready



Region of Interest (ROI)

Igor Pro 4.06A

File Edit Data Analysis Macros Windows Graph Misc Help Living Image LI Tools

XQA20030217141129

Max Bar 31571 Create Measure Remove counts Overlay
Min Bar 3157 1 All All Print Bkg Sub Flat Field Cosmic

Full Auto Circle Histogram

Bright 100 Gamma 1.5 ROI 1: Xc=2.14cm Yc=7.50cm W=2.14cm H=2.14cm

Image
Min = -124.76
Max = 31571
counts

30000
25000
20000
15000
10000
5000

Color Bar
Min = 3157
Max = 31571
cosmic
bkg sub
flat-fielded

ClickNumber: XQA20030217141129 Series: Presentation
Acq Date: Mon, Feb 17, 2003 Experiment: Plastic Mouse
Acq Time: 14:11:51, 2 sec. Label: LED in Hip
Bin: HR (4), FOV: 15, f/# 1 Comment: Zoomed Image
Camera: IMS 103, S1620EEV Analysis Comment:

ROI Dimensions

ROI ROI 1

ROI Label ROI 1

Xc 2.13895
Yc 7.49609

Locked Size

Width 2.14286
Height 2.14286

Angle (deg) 0

Unselected Trace Color
BlueRedGreen

Line Size 2

Done

Ready



Analysis in Counts

Igor Pro 4.06A

File Edit Data Analysis Macros Windows Notebook Misc Help Living Image LI Tools

XQA20030217141129

Max Bar 31571 Min Bar 3157

ROI 1: Xc=5.83cm Yc=9.48cm W=1.55cm H=1.90cm Angle=-17.00

Image Min = -124.76 Max = 31571 counts

Color Bar 30000 25000

XQA20030217140733

Max Bar 16343 Min Bar 1634

ROI 1: Xc=10.70cm Yc=14.47cm W=1.55cm H=1.90cm Angle=-17.0

Image Min = -196.65 Max = 16343 counts

Color Bar 16000 14000 12000 10000 8000 6000 4000 2000

Color Bar Min = 1634 Max = 16343

cosmic bkg sub flat-fielded

Lab Book

Measurement on uncalibrated Luminescent image (flat-field, background-subtracted)

Click Number	Exp(s)	Binning	FOV	f/stop	Filter	Unit
XQA20030217141129	2	4	15	1	Open	counts

ROI	User Label	Total Counts	ROI Pixels	Avg
ROI 1	ROI 1	1.407e+07	2.288e+03	6.150e+03

Shape	Area (ecd pixels)	Xc	Yc	Width
Circle	3.791e+04	7.465e+02	1.213e+03	1.980e+02

ClickNumber: XQA20030217141129
Acq Date: Mon, Feb 17, 2003
Acq Time: 14:11:51, 2 sec.
Bin:HR (4), FOV:15, f/# 1
Camera: IMS 103, SI620EEV

Measurement on uncalibrated Luminescent image (flat-field, background-subtracted)

Click Number	Exp(s)	Binning	FOV	f/stop	Filter	Unit
XQA20030217140733	2	4	25	1	Open	counts

ROI	User Label	Total Counts	ROI Pixels	Avg
ROI 1	ROI 1	2.800e+06	8.040e+02	3.483e+03

Shape	Area (ecd pixels)	Xc	Yc	Width
Circle	1.365e+04	8.217e+02	1.111e+03	1.188e+02

A20030217140733 Series: Presentation
Feb 17, 2003 Experiment: Plastic Mouse
28, 2 sec. Label: LED in Hip
25, f/# 1 Comment:
3, SI620EEV Analysis Comment:

Ready



Imaging Examples - Summary

- Exploit the Field of View

- Living Image® Menus
 - Saving Imaging
 - Browsing

- Region of Interest
 - Create
 - Analysis



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