

HUMAN HEALTH | ENVIRONMENTAL HEALTH



In Vivo Imaging IVIS Spectrum

Tarik Harb

Field Service Engineer Mid-Atlantic States Washington D.C.

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Introduction

- Principles of Optical In Vivo Imaging
- ► Key IVIS[®] Hardware Components
- Overview of Living Image[®] Software
- Fluorescence Options

Training

Hands-on Training





Why Optical In Vivo Imaging?

- Powerful labeling technique gene expression results in production of luciferase
 - Amount of light is proportional to number of live active cells
 - Typical applications range from oncology studies, infectious diseases, imaging transgenic animals, stem cell development

- Non-invasive
 - Does not require subject to be euthanized

Relatively simple instrumentation





Overview





Luciferase Emission Spectra and Tissue Transmission

Overview







Light Transmission Through Clear Media





Light will not scatter or diffuse traveling through pure water.

7





















Bioluminescent Source

- Light traveling through tissue scatters many times creating a "fuzzy" light diffusion pattern on the surface of the animal
- The IVIS[®] views the diffuse light on the camera-facing (top) surface of the subject
- Not all light from the source will make it to the camera – light absorption will occur as signal exits the animal







- Customized for *in vivo* imaging
- Highly sensitive camera with a large dynamic range

IVIS Spectrum Imaging System - Hardware











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







- Controls all settings in the IVIS[®] system (fully computer controlled)
- Provides advanced cataloging and browsing tools
- Provides analysis tools for quantification
- Instrument settings are analogous to photography
- Images are acquired in a two-step process



Photographic + Luminescent = Overlay











Camera and Lens Settings are Analogous to Photography

- Field of View (FOV) is dependent on the distance from the lens to the sample
- Light collected is proportional to how long the shutter is open (exposure time)
- Aperture (*f*/stop) controls the amount of light collected
- Digital pixel binning is possible on the CCD – alters sensitivity/resolution







- The IVIS[®] CCD camera has a raw signal range of 0 to 65,535 Analog to Digital counts (2¹⁶ 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



Controls Sensitivity

🜠 IVIS Acqu	isition (Control P	ap	e	-				
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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



🚺 IVIS Acquisition Control Panel . Imaging Mode Exposure Time Emission Filter Binning F/Stop Excitation Filter 🔤 🔽 Luminescent 1.00 🗘 sec 🗸 ~ Medium Block Open ¥ Eluorescent 🚹 🗹 Photograph 🛛 Auto 😂 Medium 🔽 8 ¥ Structure V Overlay 📃 Lights 🔽 Alignment Grid Field of View: C System Status Acquire Idle 12.9 Service cm. Imaging Wizard 😂 cm Subject height: 1.50 Sequence Setup Focus: use subject height 🗸 Temperature: Locked Initialize



Exposure time setting

Pixel Binning (CCD Resolution)



- Binning refers to the grouping of pixels into a larger super-pixel
- 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



Pixel binning setting









Software



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

🜠 IVIS Acquisition Control P	anel							
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f/8









Auto-exposure feature available for bioluminescence and fluorescence

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Controls Sensitivity

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Overlay will automatically take Photo + Luminescent

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Single Image Acquisition





Allows automatic acquisition of a series of images separated by fixed time points.

(useful option for kinetic studies and DLIT 3D reconstruction)



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Living Image

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

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Image Labeling



- Good labeling practices are necessary for effective data browsing
- Easily label your image while acquisition is taking place

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- Convenient preview window
- User defined labels listed with corresponding click number
 - Sort by one or multiple columns
- Open multiple images in a single window for easier analysis with Load as Group

Quantification



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



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Software



►ROI shapes available:

- Square
- Circle
- Contour
- Grid

ROI's can be created:

- Manually
- Automatically
- Free Draw









- Measurement table displays information about each Region of Interest (ROI)
- Table is user-configurable and can be exported to a spreadsheet

	Close						Refresh
	ROI	Image Layer	Total Flux [p/s]	Avg Radiance [p/s/cm²/sr]	Stdev Radiance	Min Radiance	Max Radiance
109	ROI 1	Overlay	2.753e+07	9.797e+05	7.118e+05	1.845e+05	3.691e+06
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ypes: ons)		Image	Attributes: e_		ROI Dimens	ions: Copy	y Select All

- PerkinElmer For the Better
- Living Image[®] automatically compensates for device settings: Exposure time, *f*/stop, binning and field of View.
- Calibrated units are Photons per Second, representing the flux radiating omni-directionally from a user-defined region







Raw Signal (Counts)











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

Peak Counts





Calibrated **Signal** (Photons per second) Exp time: 30 sec 30 sec 60 sec 60 sec 60 sec 60 sec **Binning**: small small small medium medium small 2 3 4 5 6 Day: 1 **Radiance: Photons per** second





IVIS® Spectrum



















GFP Well Plate Uncorrected



VS.

Units of 'Radiant Efficiency' compensates for nonuniform excitation light pattern

GFP Well Plate Corrected







Unrefined chlorophyll-containing ingredients, particularly alfalfa, responsible for gut signal



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Trans-illumination Fluorescence









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32

Batch Sequences

Acquire Sequence

Imaging Wizard

Image Setup

Initialize

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Temperature Status

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360

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Camera Temp: -90

Temperature

Stage Temp: 37.0

Single image taken for each point





Photograph 0.20

13.5

Focus: use subject height 🔻

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Overlay

Field of View: C

Subject height: 1.50

Service





Imaging units are defined as **Radiant Efficiency**;

Emission Radiance \div Excitation light power



Quantification not comparable to Epi-Fluorescence images;

Emission Radiance ÷ Excitation power density (per area)





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Preview of Advanced Topics

3D Tomography (Luminescence)





29 days after i.c. injection of 2x10⁶ cells

l = 580, 600, 620 nm

С	hest Cavity	Peritoneal Cavity			
Depth [mm]	Flux [photons/sec]	Depth [mm]	Flux [photons/sec]		
2.1	2.43×10 ⁸	3.2	1.44×10 ⁸		

3D Tomography (Fluorescence)



Ex: 745nm Em: 800 nm



50 μg XLCF750 dye Herceptin conjugate Injected IV on Day 20 Imaged on Day 22, T=48 hour







Software

Spectral Unmixing





- Calculates concentrations of different fluorescent components
- Requires images acquired at multiple wavelengths to perform the spectral analysis

Composite image of 4T1 murine mammary tumor cells implanted in mammary fat pads:

- ProSense 680 (Yellow) activated by cathepsins in tumor cells and accumulates in bladder
- MMPSense750 (Red) activated by metalloproteinases in tumor cells and liver accumulates in bladder
- Auto-fluorescence from chlorophyll in food (blue) and animal tissue background (green)

Summary



>Imaging principles

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

►Hardware

- Custom designed for *in-vivo* bioluminescent & fluorescent imaging
- 28 filters make IVIS Spectrum ideal for imaging multiple probes
- Settings are analogous to photography

►Software

- Living Image[®] used for acquisition and analysis
- Images are acquired in a two step process
- Sensitivity is controlled by Exposure time, f/stop and binning

► Fluorescence

- Two modes of illumination: Reflection (epi) or Transillumination
- Tissue and Instrument Auto-fluorescence can be subtracted



- 1. Choose reporters that maximize signal-to-noise (S:N) ratio
- 2. Consider the appropriate control groups and imaging time points necessary
- 3. Use hairless mice or white-furred animals and depilate or shave
- 4. Switch to autofluorescence-free mouse diet
- 5. Closely map the kinetics of your biological bioluminescent model
- 6. Animal handling can significantly affect kinetics
- 7. Image in the animal orientation that yields the highest signal intensity
- 8. Cover intense signal to allow dimmer signals to dictate auto-exposure
- 9. Utilize guards to prevent reflection off neighboring animals
- 10. Use black well plates when doing in vitro experimentation



Through thorough engineering, it may be possible to resolve as few as 3 bioluminescent cells





Optical Imaging Bioware and Reagents



Activateable

- ✓ CatB 680 and 750
- ✓ CatK 680
- ✓ MMPSense 680, 750
- ✓ Neutrophil Elastase 680
- ✓ ProSense 680, 750
- ✓ ReninSense 680

Targeted

- ✓ 2-DG Probe
- ✓Annexin-Vivo 750
- ✓ BacteriSense 645
- ✓ Bacterial Detection Probe 750
- ✓ COX-2 Probe
- ✓ FolateSense 680
- ✓IntegriSense 680, 750, 645
- ✓HypoxiSense 680
- ✓Inflammation Probe
- ✓ OsteoSense 680, 750, 800
- ✓TLectinSense 680

Vascular

- AngioSense 680 and 750
 AngioSPARK 680 and 750
- ✓ Superhance 680

Bioware

- ✓ Cell lines
- ✓ Microorganisms
- ✓ Bioware Ultra
- ✓ Bioware Ultra Red

Substrates

D-Luci

- ✓ D-Luciferin Substrate
- ✓ Coelenterazine
- ✓ RediJect D-Luciferin
- ✓ RediJect D-Luciferin Ultra

Overview

For an In-Depth Study



IVIS Software Manual





Living Image[®] Software User's Manual Version 4.3.1

For the IVIS® Spectrum

IVIS University Web page http://www.perkinelmer.com/pages/020/imaging /invivouniversity.xhtml



Overview



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Thank you for your attention!

- On Call Services Urgent Hardware Issues
- Technical Support (508) 435-9761
 <u>Global.techsupport@perkinelmer.com</u>
- Tarik Harb
 (510)-229-7272
 tarik.harb@perkinelmer.com

- Brad Taylor, Ph.D. (630) 435-9761
 <u>brad.taylor@perkinelmer.com</u>
- Alexandra De Lille, Ph.D. (970) 214-8758 alexandra.delille@perkinelmer.com