



# IVIS | Spectrum

In Vivo and In Vitro 3D Molecular Imaging Bioluminescence & Fluorescence Spectral Unmixing Transmission & Reflectance Imaging

# World-Leading Optical Imaging System: Uncompromising Sensitivity and Flexibility

### **Transmission and Epi-illumination Imaging**

Emitted light from the excitation filter wheel feeds through a fiber optic bundle to illuminate the specimen from either the top, in epi-illumination (reflectance) mode, or from underneath the stage, by means of an automated bundle switch. Transilluminating the subject from below at precise x,y-locations allows for transmission imaging, enabling more sensitive detection and accurate quantification of deep sources. Transmission fluorescence imaging also reduces the effects of autofluorescence.



## Inside the IVIS Spectrum



### Narrow Band Excitation and Emission Filters

The IVIS Spectrum excitation and emission filters enable spectral scanning over the blue to NIR wavelength region.

- 10 narrow band excitation filters: 415 nm -760 nm (30 nm bandwidth)
- 18 narrow band emission filters: 490 nm -850 nm (20 nm bandwidth)



### **Imaging Chamber**

- Light-tight imaging chamber
- Heavy-duty castors
- Integrated gas anesthesia
- Integrated fluorescence
- LED lamps for photographic images
- Heated stage to maintain optimum body temperature
- Electromagnetic door latch
- Motor-controlled stage, filter wheel, lens position, and f-stop
- Scanning laser for mouse alignment and surface topography

#### CCD Camera

- Back-thinned, back-illuminated grade 1 CCD provides high quantum efficiency over the entire visible to near-infrared spectrum
- 13.5 micron pixels, 2048 x 2048
- 16-bit digitizer delivers broad dynamic range
- CCD is thermoelectrically (Peltier) cooled to -90 °C , ensuring low dark current and low noise

#### **Custom-Designed Lens**

- 6-inch diameter optics, f/1- f/8
- High-resolution down to 20 microns
- Emission filter wheel with 24 slots

### Versatile Field-of-View - Single-Cell to Five Mice

From 20 microns to localize single cells to 5 whole mice, the IVIS Spectrum gives you the automated flexibility, throughput and resolution required the quantitate functional developments in whole animals down to single cells.





FOV 3.9 cm

20 microns







Single Cells In Vitro

## **Multimodal Imaging**

The IVIS Spectrum is capable of imaging both fluorescent and bioluminescent reporters, as well as with most fluorescent probes. Absolute calibration affords you consistent and reproducible results, independent of magnification and filter selection. Data can be shared between IVIS instruments within an organization, or around the world. The Living Image software yields reproducible, quantitative results by incorporating instrument calibration, background subtraction and image processing algorithms.

Inoculation with Klebsiella pneumoniae Expressing luxCDABE and GFP or RFP



**IUXCDABE** Bioluminescence RFP

### Fluorescence Imaging - Versatility in Fluorescence

The IVIS Spectrum can image and quantify all commonly used fluorophores, including fluorescent proteins, dyes and conjugates. The Spectrum achieves superior spectral unmixing through a wide range of high resolution, short cut-off filters and advanced spectral unmixing algorithms. Spectral unmixing not only allows detection and separation of multiple reporters, but greatly reduces the effects of tissue auto-fluorescence.



# Living Image 3.1 Software

# Acquire, Analyze & Discover

# **Multimodality Co-Registration**

### Fluorescence - CT Co-Registration Analysis

The IVIS Spectrum is the most advanced *in vivo* imaging system available on the market today - not only can it quantitate and localize 3D fluorescent and bioluminescent sources *in vivo*, but it can import and automatically co-register CT or MRI images yielding anatomical context. No need to confine or morph your subject, structured light measurements provides clear anatomical reference for co-registration.





2D Fluorescent Image and Photograph Overlay

2D Fluorescent Image and Co-r X-Ray Overlay of F (Single CT Image) Sou

t 3D Co-registration of Fluorescent Source and CT





The example above shows co-registration of a CT scan from the SkyScan 1178 imaging system

# Spectral Unmixing Algorithms

The Living Image 3.1 software provides advanced spectral unmixing algorithms that:

- Allow detection and separation of multiple reporters
- Greatly reduce the effects of tissue autofluorescence
- Effectively reduce cross talk between reporters



**Filtered Images** 



Unmixed Autofluorescence



Unmixed VivoFluor 680



Unmixed VivoFluor 750



Composite Image

# **Imaging Wizard - Making it Simple**

### Advanced Imaging with Confidence

Living Image 3.1 now offers a step by step wizard to guide you through the imaging process. The imaging wizard will help you design imaging settings for fluorescence and bioluminescence protocols. Whether you require spectral unmixing of multiple fluorophores or you're performing 3D imaging techniques, Living Image will recommend the optimal settings and simplify the process from start to finish.



# Living Image 3.1 Features

- Integrated control panels for instrument settings, acquisition parameters and post image processing quatitation
- Instrument control and image acquisition controls the IVIS Imaging System and acquires images
- Automatically archive camera information and user-specified annotations with each image
- Automatically calibrate photon radiance to NIST standards
- Automatically draw ROIs and compute photon flux
- Create a composite image to evaluate dual reporters in a single experiment
- Measure distances (cm or pixels) and view pixel data in histogram or line graph formats
- Quantify the depth, point location, and brightness of a biophotonic source using Planar Spectral Imaging
- Quantify the depth, geometry, and brightness of a biophotonic source in 3-dimensional space using DLIT or FLIT tomography
- Co-register organs from the Digital Mouse Atlas on a 3D image
- Import and co-register CT or MRI radiographs on a 3D image
- Export 2D image to DICOM-compliant format
- Imaging Wizard User Guidance

### **Recommended Minimum Hardware & Software Requirements**

Operating System ...... Windows XP and Windows 2000, Macintosh OS 10.4 or later

RAM ..... 1 GB or larger

Available Hard Drive ..... 120 mB for Installation

Graphics Card . . . . . . . . . . Open GL 1.5 or later

### **Absolute Localization in Optical Imaging - 3D Analysis**

Now you can look deeper, see further, and take your science to a new level of sophistication with the 3D technology from Caliper Life Sciences. 3D diffuse tomography utilizes structured light data with bioluminescence or fluorescence images to reconstruct three dimensional representations of light emitting reporters and compute signal strength. Take the next step and analyze 3D sources in an anatomical context with the Digital Mouse Atlas.





Bioware Ultra 4T 1-Luc2 were implanted and determined to have an estimated 6500 ph/s/cell *in vitro*. The source flux is corrected for tissue depth to estimate that tumor #1 contains 800,000 live cells and tumor #2 contains 390,000 live cells.

### Advanced Tomographic 3D Analysis on the IVIS Spectrum

- Determine geometry and quantify the depth and intensity and of fluorescent sources in 3D space using FLIT (Fluorescent Imaging Tomopgraphy) or bioluminescent sources using DLIT (Diffused Luminescent Imaging Tomography)
- View biophotonic sources in an anatomical context. Automatically co-register organs of interest from the Digital Mouse Atlas on a 3D image.
- Import and automatically co-register a CT or MRI radiograph (Open Inventor format) on a 3D image
- Export a 2D image to DICOM compliant format
- View sagittal, coronal and transaxial sections through a 3D image
- View biophotonic sources from multiple perspectives by converting a static 3D image into a movie of a rotating subject



### **IVIS Spectrum - Complete Imaging Solutions**

Accessories



Anesthesia System Cat No. 118918 XGI-8 120V Cat No. 118919 XGI-8 230V Cat No. 118957 XGI-8 100V



Cat No. 123997 XIC-3 Animal Isolation Chamber Kit



118897 XLS-4 Calibrated Light Source



Cat No. 119207 XWS-360 Workbench



Cat No. 119002 XAS-3 Animal Shield Kit

Reagents



Cat No. 121365 XFM-2 Phantom Mouse For Fluorescent Imaging



Cat No. 118993 XPM-2 Phantom Mouse For Bioluminescent Imaging



In Vivo Grade Luciferin

Plasmids

Light Producing Animal Models

Gram Positive Bacteria

**Gram Negative Bacteria** 

**Bioware Ultra Cell Lines** 

*For more information on these accessories and other available accessories for the IVIS platform on the Caliper Store at https://store.caliperls.com* 

IMAGING SYSTEM COMPONENTS	SPECIFICATIONS
Camera Sensor	Back-thinned, back-illuminated Grade 1 CCD
CCD Size	2.7 x 2.7 cm
Imaging Pixels	2048 x 2048
Quantum Efficiency	>85% 500 – 700 nm; >30% 400 – 900nm
Pixel Size	13.5 microns
Min. Detectable Radiance	70 photons/s/sr/cm <sup>2</sup>
Min. Field of View (FOV)	3.9 x 3.9 cm
Max. Field of View (FOV)	23 x 23 cm
Min. Image Pixel Resolution	20 microns
Lens	f/1 – f/8; 1.5x, 2.5x, 5x, 8.7x magnifications
Read Noise	< 3 electrons for bin=1,2,4; < 5 electrons for bin=8,16
Dark Current (Typical)	<100 electrons/s/cm <sup>2</sup>
Fluor. Excitation Filter Slots	12
Fluor. Emission Filter Slots	24
Excitation Fluorescence Filters	10
Emission Fluorescence Filters	18
Fluor. Bkg. Subtraction Filters	Yes
Heated Stage	Yes
Diffuse Tomography Software	Yes
Gas Anesthesia	Yes
Workbench	Yes
CCD Operating Temp	-90 °C
Imaging Chamber Interior Size	43 x 50 x 60 cm (W x D x H)
Imaging System Space Requirement	203 x 163 x 214 cm (W x D x H)
Power Requirements	20 Amps for 120 VAC or 10 Amps for 230 VAC
Stage Temperature	20 – 40 °C
Computer (Min. Specifications)	2.8 GHz, 1 GB RAM, RW CD/DVD, 80 GB HD, 20" flat screen monitor



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