

IVIS[®] Lumina Imaging System



IVIS[®] Lumina Imaging System R&D Applications

- Tracking and monitoring
- High-quality data on treatment efficacy
- Drug metabolism and toxicological screening for pharmaceutical and biotechnology companies

IVIS[®] Lumina Components: CCD Camera and Imaging Chamber, Acquisition Computer, High-Resolution Monitor, Integrated Fluorescence Capability, and Living Image[®] Software, shown with workstation option

The IVIS Lumina Imaging System allows researchers to perform in vivo optical imaging — a novel technology from Xenogen that uses real-time imaging to monitor and record cellular and genetic activity within a living organism — using fluorescent and/or bioluminescent reporters.

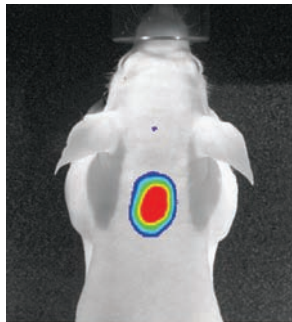
Contact Information

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E-mail: imaging@xenogen.com
Xenogen Corporation
860 Atlantic Avenue,
Alameda, California 94501
www.xenogen.com

The IVIS[®] Lumina from Xenogen provides high sensitivity, ease of use, and the flexibility to image fluorescent and/or bioluminescent reporters both *in vivo* and *in vitro*. The system includes a highly sensitive CCD camera, a light-tight imaging chamber with complete computer automation, and the Living Image[®] software package for image acquisition and analysis.

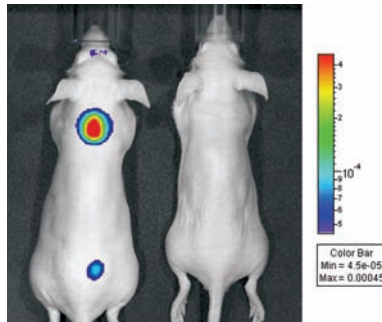
An adjustable field of view from 5–12.5 cm allows imaging of up to 3 mice or a medium size rat and also accommodates standard microtiter plates for *in vitro* imaging. The system includes premium animal handling features such as a heated sample shelf, gas anesthesia connections and manifold, and an optional full gas anesthesia option.

Imaging Results – Living Image® Software with IVIS® Lumina System



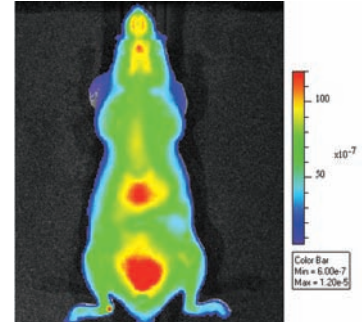
Quantum Dots 800 nm*

- SQ injection 3×10^{10} dots
- Filter set #4
- 10 sec exposure time



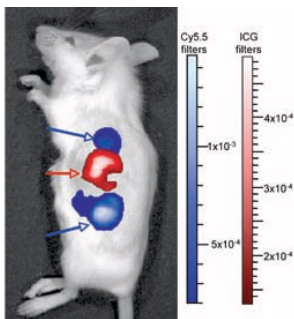
Alexa Fluor® 680 nm Dye*

- Left mouse: SQ injection 10^{13} (top) and 10^{12} (bottom) molecules
- Right mouse: control
- Filter set #3
- 1 sec exposure time



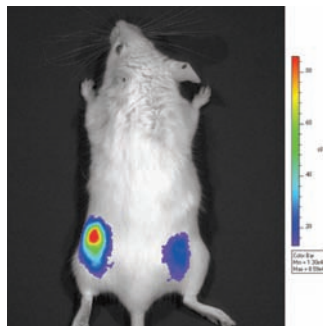
Quantum Dots 800 nm*

- IV injection 2×10^{13} dots
- 27 minutes post-injection
- Filter set #4
- 10 sec exposure time



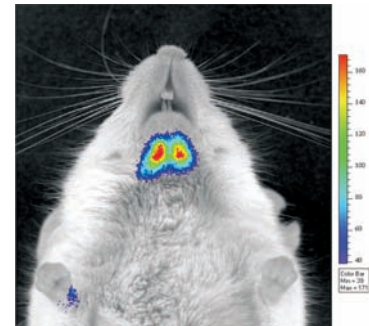
Dual Probe Imaging

- Image of two spectrally distinct fluorescent dyes using filter set #3 and filter set #4



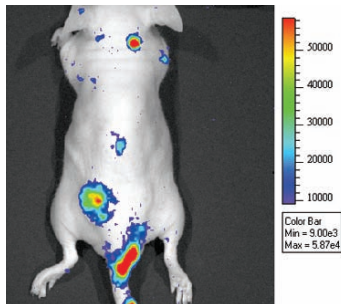
B16-F10-luc-GF

- Melanoma model xenograft
- Firefly luciferase
- SQ injection, Left 1×10^6 , Right 2×10^5 cells
- 2 min exposure time



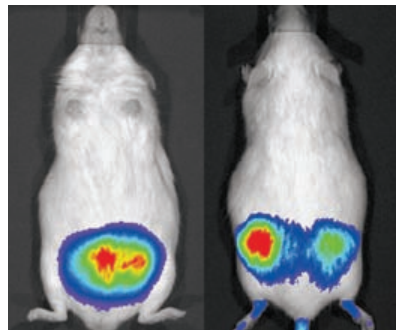
B16-F10-luc-GF

- Melanoma metastasis model, day 16
- Firefly luciferase
- IV injection 1×10^6 cells
- 1 min exposure time



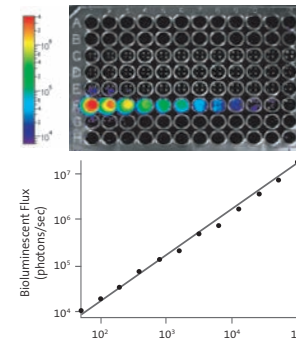
PC3M-Vegf-luc/SV40

- Prostate metastasis model
- Renilla luciferase
- IV injection 4×10^6 cells
- 1 min exposure time



PSA-luc Transgenic Mouse

- Endothelial prostate cells expressing firefly luciferase
- Left: ventral view; Right: dorsal view
- 5 min exposure time



PC3M-luc

- *In vitro* well plate study
- Prostate cancer cells transfected with firefly luciferase
- Serial dilution from 1.0×10^5 to 50 cells
- 1 minute exposure time

* 800 nm quantum dots and Alexa Fluor® 680 dye are provided by Invitrogen

CCD Camera

- The IVIS Lumina CCD is 13 x 13 mm square, with 1024 x 1024 pixels 13 micron in width, yields higher imaging resolution
- Back-thinned, back-illuminated grade 1 CCD provides high quantum efficiency over the entire visible to near-infrared spectrum
- 16 bit digitizer delivers broad dynamic range
- The CCD is thermoelectrically (Peltier) cooled to -90°C ensuring low dark current and low noise

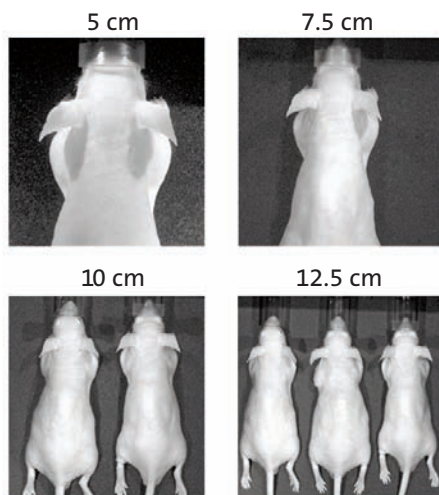
Imaging Chamber

- Light-tight imaging chamber
- High light collection lens, f/0.95 – f/16
- 8 position emission filter wheel
- LED lamps for photographic images
- Heated stage to maintain optimum body temperature
- Motor controlled stage, filter wheel, lens position, and f-stop

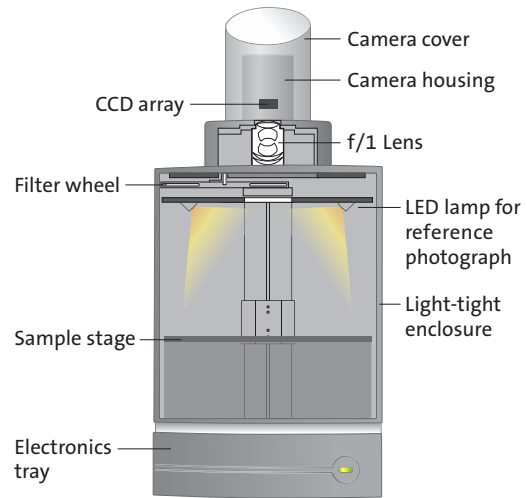
Integrated Gas Anesthesia

- Gas anesthesia ports and 5 position manifold within imaging chamber allow anesthesia to be maintained during imaging sessions

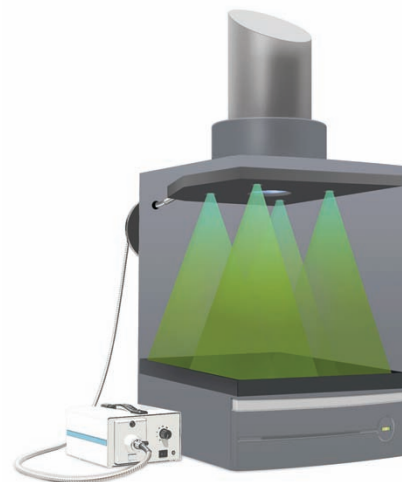
Field of View



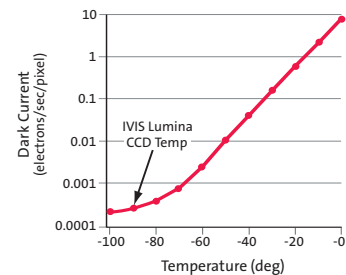
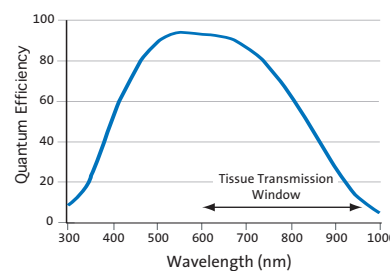
The IVIS Lumina Imaging system provides 4 fields of view



Cross sectional view of IVIS[®] Lumina Imaging System



Fluorescence features include a tungsten halogen lamp, 12-position excitation filter wheel, and 8-position emission filter wheel. Specimen illumination is from above in reflectance mode.

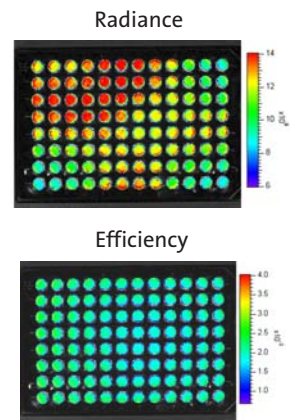


High quantum efficiency (left) and low dark current (right) provide excellent sensitivity for imaging at low light levels

Units for Fluorescent Imaging

All IVIS Imaging Systems are calibrated relative to a NIST traceable radiance standard. Images measured in relative light units (CCD camera counts) can be converted to physical units of surface radiance (photons/sec/cm²/sr). In fluorescent imaging the surface radiance depends on the illumination intensity which can vary depending on field-of-view and wavelength.

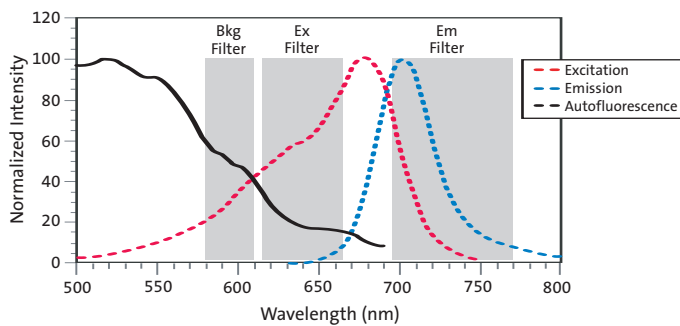
To eliminate the effect of illumination intensity, fluorescent images are normalized by dividing the fluorescent image by a reference illumination image. The resulting “normalized” fluorescent image is unitless and is called a fluorescent efficiency image. The value of each pixel in an efficiency image represents the fractional ratio of fluorescent emitted photons per incident excitation photons.



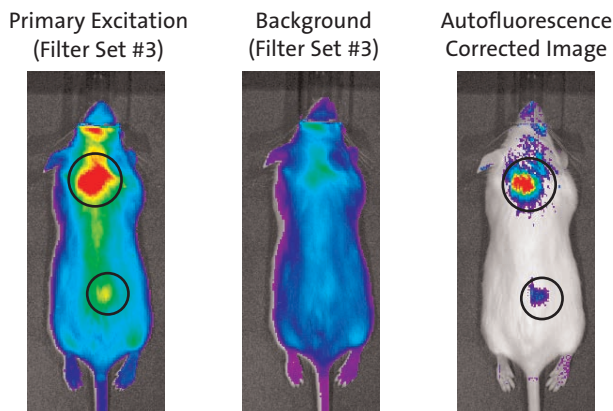
Standard Fluorescent Filter Sets

Filter Set	Label	Background Passband (nm)	Excitation Passband (nm)	Emission Passband (nm)	Dyes, Fluorescent Proteins, and Quantum Dots
1	Green	410 – 440	445 – 490	515 – 575	GFP, EGFP, FITC
2	Red	460 – 490	500 – 550	575 – 650	DsRed, PKH26, Qdot® 605
3	Far-red	580 – 610	615 – 665	695 – 770	Cy5.5, Alexa Fluor®, Qdot® 705
4	NIR	665 – 695	705 – 780	810 – 885	ICG, Qdot® 800

Autofluorescent Subtraction Using Background Filters

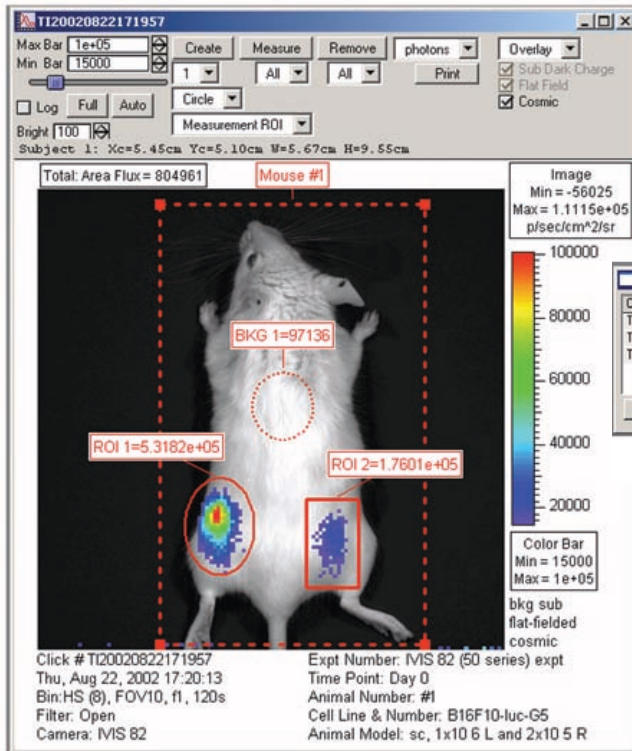


Graph of the excitation and emission spectrum of Alexa Fluor® 680 and the autofluorescent excitation spectrum of mouse tissue. Also included are the spectral passbands for filter set #3.



The autofluorescence corrected image is the result of subtracting the scaled background filter image (multiply by 1.85) from the primary filter image. The FVB mouse was injected in the scruff and lower back with 20 µL of Alexa Fluor® 680 solution of a concentration of 1x10¹³ and 1x10¹² molecules of dye, respectively.

Living Image® Imaging Software Control and Analysis



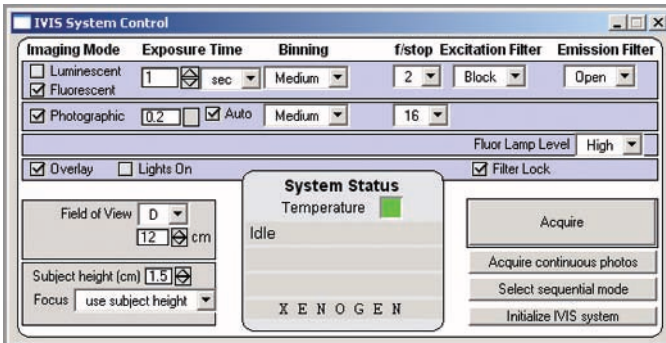
Living Image® Software

Living Image software from Xenogen controls image acquisition, analysis, and archiving. Data analysis versions of Living Image software run on both Windows® and Macintosh® platforms.

Image Analysis Panel

- Photo brightness and color bar adjustment
- Image annotation tools
- Region of interest (ROI) measurements
 - Subject, background, measurement ROIs
 - ROI measurements are organized in a measurements table
- Absolute calibration of image intensity against NIST radiance standards
- Corrections for cosmic rays, dark charge, and flat field
- Printing, layout, and image export functions

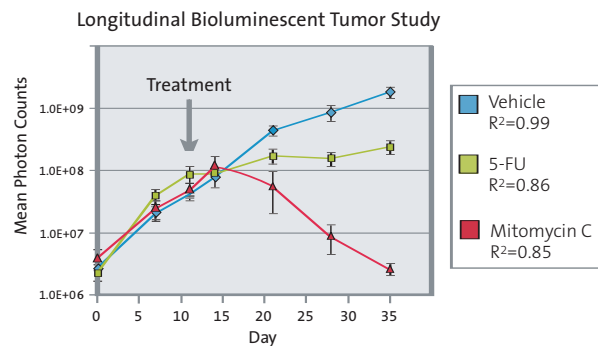
Between one and three subjects can be imaged per session



IVIS System Control Panel

- Controls for both fluorescence and bioluminescence imaging
- Manual and auto focus
- Setting of exposure time, binning, f/stop, and field of view
- Fluorescence lamp and filter controls
- Acquisition setup of a single image or an image sequence

Imaging Controls – Living Image® software controls both fluorescent and bioluminescent options



The wide range of IVIS system instrument settings, combined with absolute calibration of each setting, allows users to track signals during longitudinal studies that vary by many orders of magnitude. In this drug study, tumor signals vary by three orders of magnitude during the course of a 35 day experiment. The capability of Living Image software makes this type of analysis simple for the user in both fluorescent and bioluminescent modes.

IVIS® Lumina Imaging System Accessories



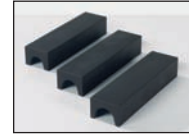
XGI-8 Gas Anesthesia System



XWS-260 Workstation Option



XLS-4 Calibrated Light Source



XAS-3 Animal Shield Kit



XPP-1 Plate Positioner

Imaging System Components	Specifications
Camera Sensor	Back-thinned, back-illuminated, cooled Grade 1 CCD
CCD Size	1.3 x 1.3 cm
Imaging Pixels	1024 x 1024
Quantum Efficiency	> 85% at 500–700 nm, > 30% at 400–900 nm
Pixel Size	13 microns
Min. Detectable Radiance	100 photons/s/sr/cm ²
Min. Field of View (FOV)	5 x 5 cm
Max. Field of View (FOV)	12.5 x 12.5 cm
Min. Image Pixel Resolution	50 microns
Read Noise	< 3 electrons for bin=1, 2, 4; < 5 electrons for bin=8, 16
Dark Current (Typical)	<120 electrons/s/cm ² ; or 2 x 10 ⁻⁴ electrons/s/pixel
Min. Detectable Radiance	100 photons/s/sr/cm ²
Lens	f/.95 – f/16, 50 mm
Fluorescence Capability	Standard
Fluor. Excitation Filter Slots	12
Fluor. Emission Filter Slots	8
Excitation Fluorescence Filters	8
Emission Fluorescence Filters	4
Fluor. Bkg. Subtraction Filters	Yes
CCD Operating Temp.	-90°C
Imaging System Space Requirement	48 x 71 x 104 cm (W x D x H)
Imaging Chamber Interior Dimension	43 x 38 x 43 cm (W x D x H)
Power Requirements	6A at 120V
Stage Temperature	20 – 40°C
Computer (Minimum specifications)	2.8 GHz, 1 GB RAM, RW CD/DVD, 80 GB HD, 20" flat screen monitor