Pearl® Impulse

SMALL ANIMAL IMAGING SYSTEM

2013 WINNER OF FROST & SULLIVAN AWARD

NEAR-INFRARED FLUORESCENT OPTICAL IMAGING

EXCEPTIONAL SENSITIVITY

ULTIMATE EASE OF USE

MULTI-TARGET IMAGING

LI-COR®

Pearl® Impulse
SMALL ANIMAL IMAGING SYSTEM

Fluorescent Optical Imaging
Visualize biological processes in living animals with IRDye® optical agents

Exceptional Sensitivity
Innovative FieldBrite™ optical design detects smaller and deeper targets

Ultimate Ease of Use
One-button image capture, simple analysis software, and ready-to-use BrightSite™ optical agents

Multi-Target Imaging
Monitor multiple biological targets simultaneously

Small Animal *In Vivo* Imaging:
The Next Step in Discovery

The same LI-COR® near-infrared (NIR) technology that brought quantitative Western blots to thousands of labs now puts small animal imaging at your fingertips. The Pearl Impulse Imaging System delivers *in vivo* molecular imaging with speed, sensitivity, and simplicity – at a sensible price.

In vivo imaging extends your *in vitro* work into animals to unravel the complex biology of your system in a relevant context. Ready-to-use BrightSite™ imaging agents and helpful educational resources make it easy to get started immediately.

Take the next step, and discover why LI-COR near-infrared fluorescent technology is not just for Westerns anymore. Near-infrared fluorescence imaging is a versatile, affordable way to pursue your molecular questions – and has the potential for translation to the clinic.

LI-COR is honored to receive the 2013 Frost & Sullivan North American *In Vivo* Molecular Imaging Technology Leadership Award. Learn more about this prestigious award at *www.licor.com/frostsullivan.*

Optical Imaging Workflow
with Pearl Impulse

Figure 1. Choose mouse model and NIR fluorescent probe

Benefits of Pearl Impulse fluorescence imaging include:

- Cost-effectiveness and affordability
- Small, easy-to-use platform
- Ready-to-use imaging agents
- No cell transfections required
- No radiation exposure
- NIR contrast agents have potential for clinical translation

In vivo Imaging and Your Research

Optical imaging with NIR fluorescent agents is surprisingly accessible. The ability to track biological events and disease progression in living animals is valuable for many areas of research, including apoptosis, angiogenesis, inflammation, bone growth, cell signaling, and many others.

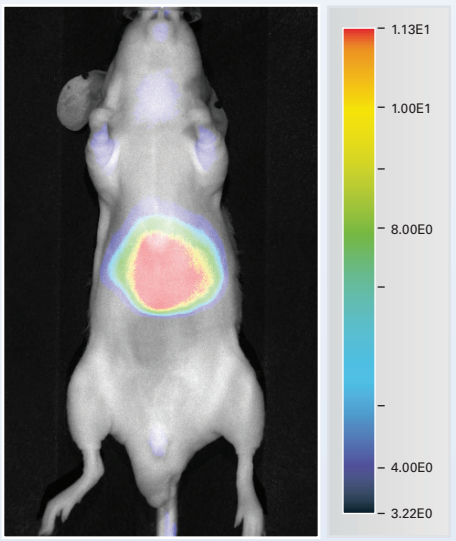
Relevant answers

In vivo analysis delivers relevant and compelling answers to your experimental questions by assessing biological processes and pathways in context. *In vivo* imaging should do more than just measure tumors – it should enhance your understanding of disease mechanisms, progression, and therapeutic response.

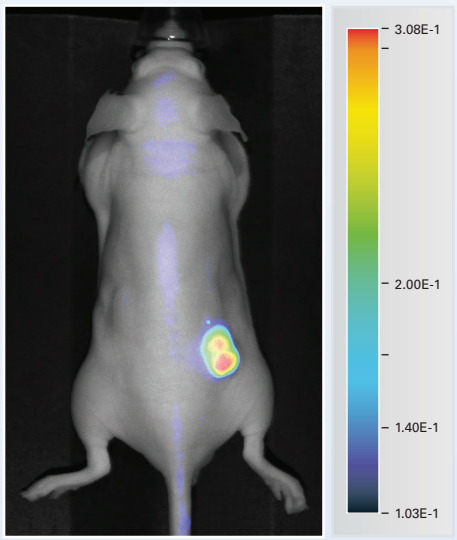
Get started right away with ready-to-use agents

Ready-to-use BrightSite™ optical agents from LI-COR Biosciences target a variety of disease characteristics, so you can get to work immediately without developing new imaging agents or transgenic animals. NIR optical agents are also available from several other sources.

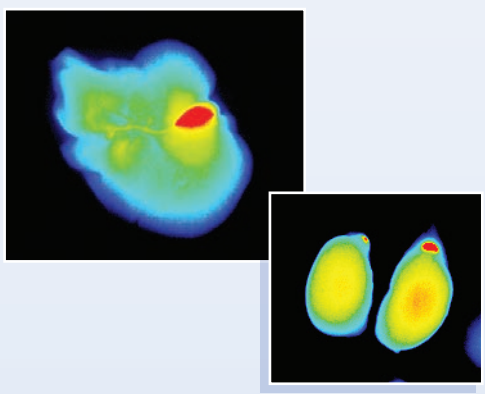
With BrightSite agents, months of development work have been done for you – so you can spend your time gathering critical data. Just confirm specificity for your model and target, and you're ready to get started (Fig. 1). After imaging, you can examine *ex vivo* tissue samples for retention of the optical agent.



Evaluate clearance and dosing



Perform *in vivo* imaging study



Ex vivo tissue analysis

Why Choose NIR Fluorescence Optical Imaging?

In disease research, preclinical imaging of animal models is used to study disease biology and evaluate therapeutic options. Modalities such as CT, ultrasound, and MRI excel at anatomical imaging but are rarely used for *in vitro* cellular assays or histology of excised tissues. Nuclear modalities such as PET and SPECT require radiolabeled probes and costly equipment. Optical imaging with fluorescent probes enables a wide range of affordable experimental formats (Fig. 2). And unlike many other methods, optical imaging targets the molecular changes that underlie disease.

Near-infrared fluorescence imaging is a versatile, affordable modality that gives relevant answers to molecular questions – *and has the potential to be translated to the clinic.*

Track disease progression

With optical fluorescence imaging, you can visualize and track disease progression in the living animal, follow the spread of a tumor, or look for drug effects. Fluorescence imaging meets a broad range of research needs – *in vitro* analysis, anatomical imaging, disease targeting, microscopy, and tissue section analysis (Fig. 2) – to investigate a variety of biological processes.

Longitudinal imaging

Longitudinal imaging studies follow the same group of animals over an extended period of time to observe changes in individual animals.

- Image animals at multiple time points throughout your study
- Track changes – even small or early ones – in the same animal to avoid variability
- Dramatically reduce the number of animals needed for each study, with considerable time and cost savings

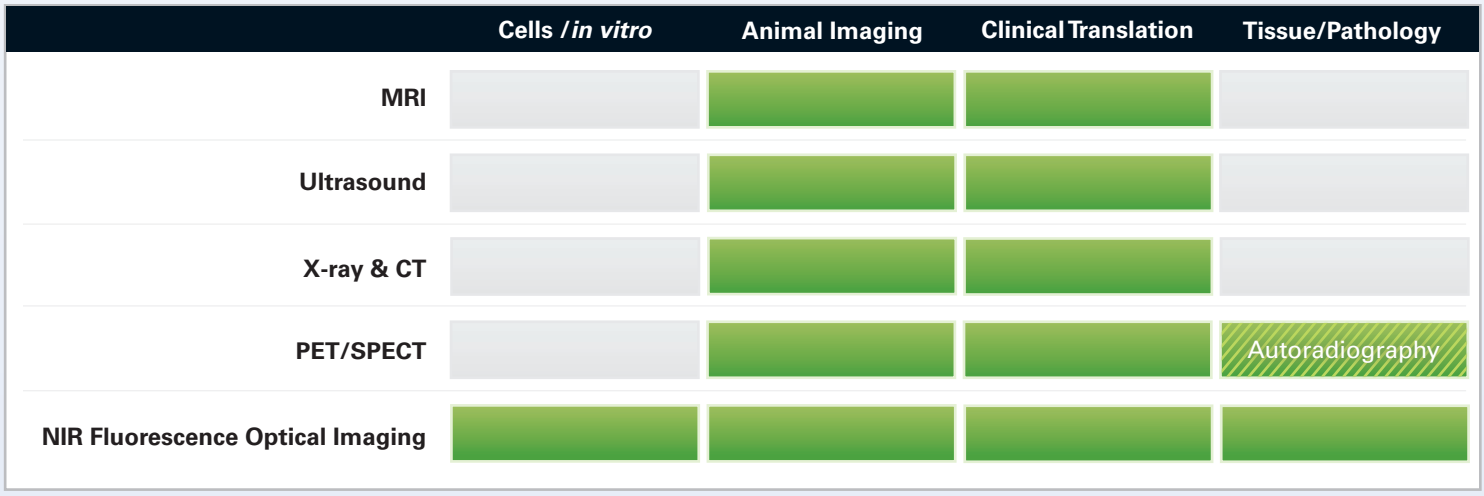


Figure 2. Optical imaging supports a wide range of research applications.

Beyond Bioluminescence and Fluorescent Proteins
with the Pearl Impulse Imaging System

What is the best optical imaging modality for your research? Important factors include sensitivity, reproducibility, simplicity – and the versatility to go wherever the results take you.

Bioluminescent (luciferase) reporters and fluorescent proteins are commonly used but present several challenges (Table 1).

- Genetic modification is required to introduce the reporter gene
- Unmodified endogenous proteins cannot be detected
- Not suitable for translation to clinic

Fluorescent proteins in the visible light spectrum also present challenges. Detection efficiency of shorter wavelengths is affected by absorbance and scatter of light, which limit depth of penetration. Tissue autofluorescence is also a concern and can obscure the desired signal.

High performance with NIR fluorescence

With NIR fluorescence detection, background is very low, and tissue autofluorescence does not limit performance. Light absorbance and scatter are reduced, increasing depth of penetration. Histology and microscopy studies can be performed with the same probes, and clinical translation is a possibility. Laser illumination can provide sensitivity comparable to bioluminescent imaging without the lengthy exposure times. There's no need for laborious transfections or transgenic animals, so you can get started immediately.

Table 1. Advantages of NIR fluorescent dyes for optical imaging.

	Fluorescent Proteins	Bioluminescence (luciferase)	Fluorescent Dyes (visible)	**Fluorescent Dyes** (NIR)
In vitro				
No cell transfection required	☐	☐	☑	☑
Can probe endogenous proteins	☐	☐	☑	☑
Multi-target imaging	☑	☐	☑	☑
In vivo				
No genetic modification of cells or animals	☐	☐	☑	☑
Low autofluorescence of animal tissue	☐	☐	☐	☑
Imaging time	msec - sec	min	msec - sec	msec - sec
Clinic				
Likelihood of clinical translation	–	–	+	+++
Pathology				
Histology and *ex vivo* analysis	–/+	–/+	+++	+++

Keys to Successful Fluorescence Imaging

Wavelength: *Light Scatter and Autofluorescence*

Animal tissue strongly absorbs and scatters visible light. This limits penetration of excitation light and escape of emitted fluorescence for detection. At near-infrared wavelengths, tissue absorption is dramatically reduced (Fig. 3) – improving light penetration and detection sensitivity.

Near-infrared light also boosts sensitivity for early detection of small tumors. Tissue autofluorescence is significantly reduced in this range (Fig. 4), so the desired signals are not obscured. Smaller doses of optical agents can typically be used, and detection of the early stages of disease – and even metastasis – becomes possible.

Optical System: *Excitation and Detection*

The success of *in vivo* imaging hinges on the optical system. An excellent optical system minimizes background, maximizes excitation of the fluorophore, and efficiently detects the desired fluorescent emission. Pearl Impulse's FieldBrite™ Xi optical system offers all these advantages.

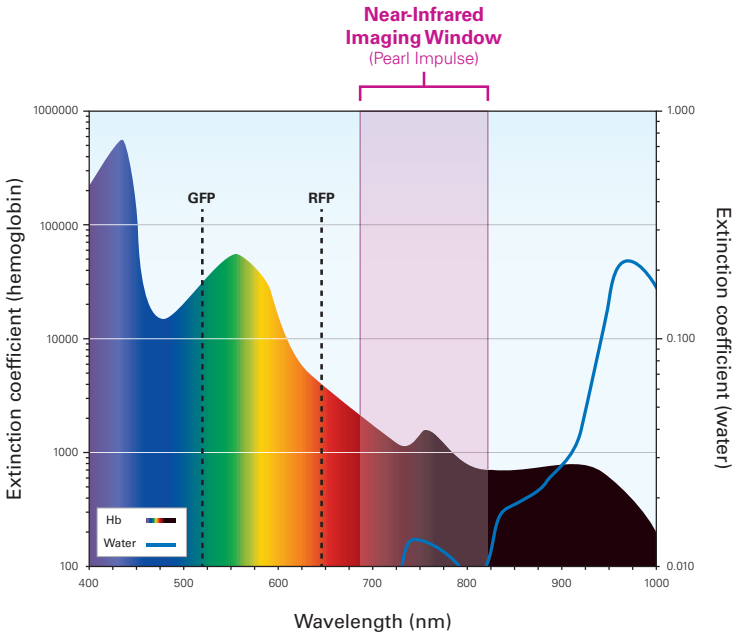


Figure 3. Hemoglobin (Hb) and other tissue components strongly absorb visible light. In the near-infrared region, tissue absorbance is dramatically reduced. Above 820 nm, light absorbance by water begins to increase.

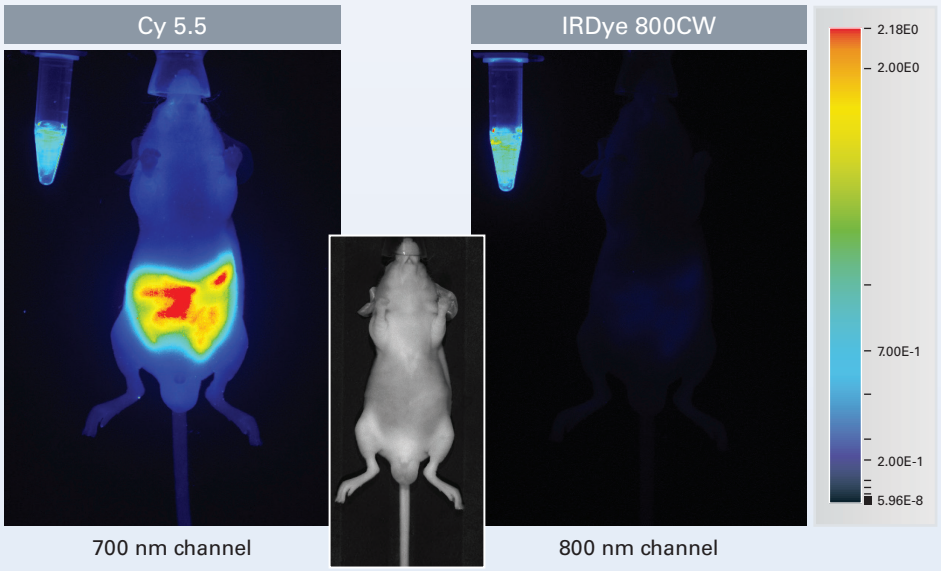
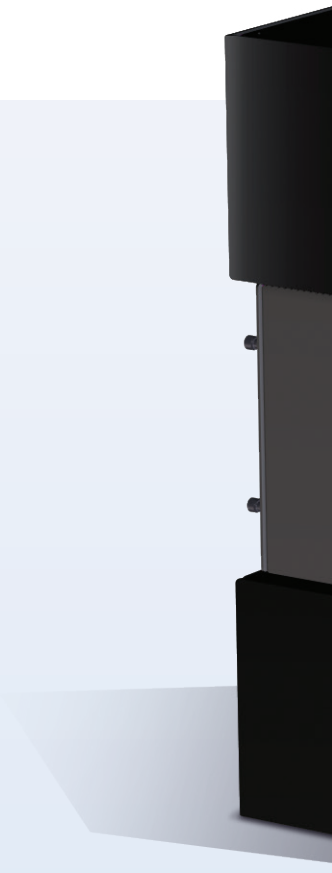


Figure 4. Autofluorescence is nearly eliminated at 800 nm. Untreated nude mouse was imaged at 700 nm and 800 nm for tissue autofluorescence. Tubes of fluorescent dye (Cy® 5.5 or IRDye® 800CW) are shown in the upper left; white light image is shown (inset). At 700 nm, strong autofluorescence is contributed by plant material in the digestive tract. In contrast, the 800 nm image shows extremely low autofluorescence.



FieldBrite™ Xi Optics:
Exceptional Sensitivity and Performance

In contrast to traditional optical systems, the Pearl Impulse system and FieldBrite Xi technology are optimized for uniform illumination, high sensitivity, low background noise, and wide dynamic range.

Excitation light source

NIR lasers provide targeted, high-quality excitation light for high-performance imaging of small or deep targets (Fig. 5). Two laser wavelengths (685 and 785 nm) allow you to detect and discriminate multiple targets simultaneously. Many imagers being developed for clinical use also employ NIR laser excitation.

Detection of fluorescence emission

Exceptional sensitivity is achieved without lengthy exposure times. The proprietary FieldBrite Xi filtering scheme prevents leakage of excitation light, reducing noise and delivering superior imaging sensitivity (Fig. 5). There is no need to change filters or adjust image capture settings, so you can concentrate on your science rather than the instrumentation.

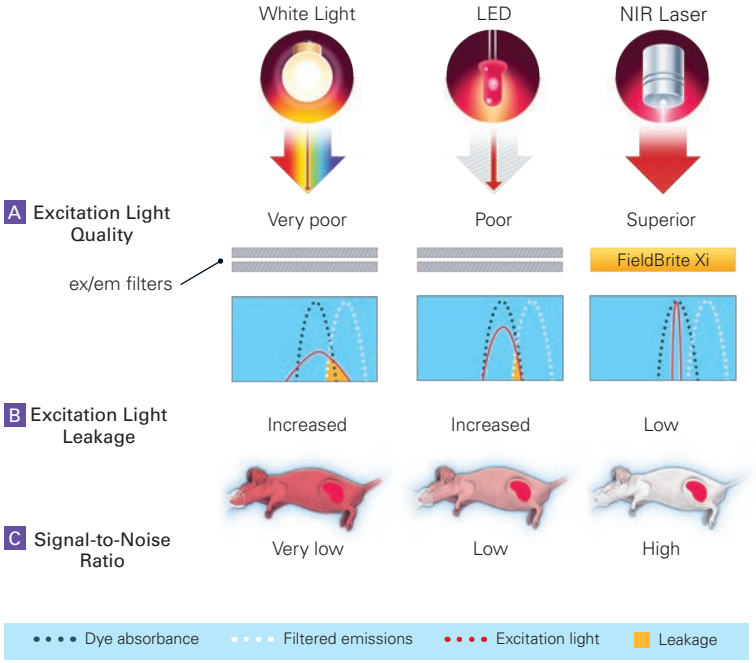
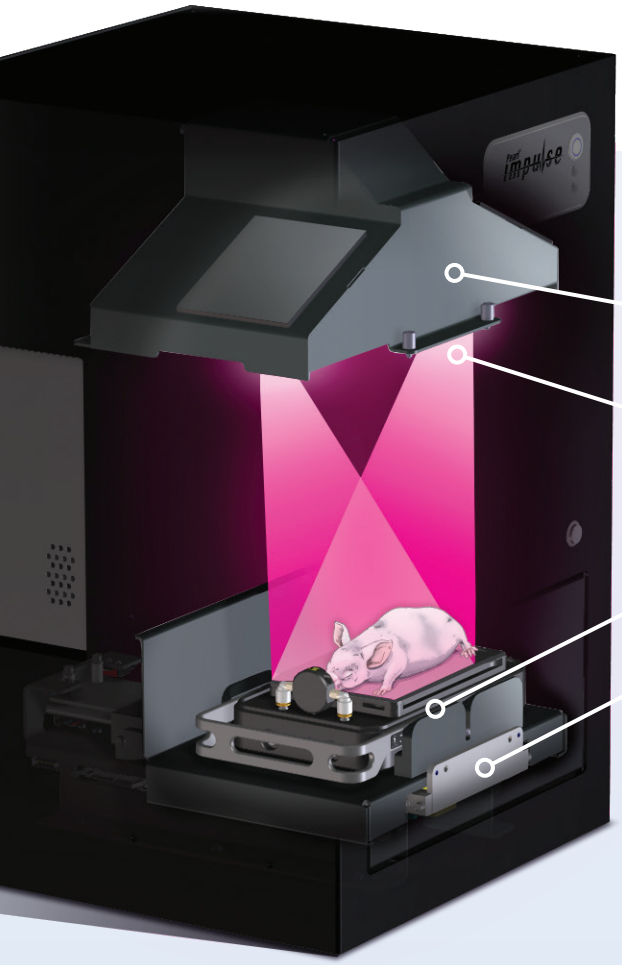


Figure 5. Improved performance with FieldBrite Xi optics. A) NIR laser delivers excitation light in the narrow wavelength band desired, unlike LED or white light sources. B) Leakage of excitation light (yellow shading) increases image background. C) Proprietary FieldBrite Xi filtering technology dramatically reduces excitation light leakage, for decreased image background and earlier detection of small or deep targets.



Pearl Impulse Design Features

FieldBrite Xi Optics
for low image background

Dual Laser Modules
for uniform illumination

Removable Imaging Bed
with temperature control and anesthesia nose cone

Unique Imaging Drawer
brings the mouse out to you

Ultimate Ease of Use

Research is complicated, but animal imaging doesn't have to be. Pearl Impulse delivers ultimate ease-of-use and powerful performance, whether you are new to optical imaging or highly experienced.

- One-button image acquisition without optimization of camera settings
- High sensitivity to detect small and deep targets
- Wide dynamic range to prevent image saturation
- Acquire multi-channel images in <30 sec (as little as 500 msec for single-channel images)
- Uniform illumination for enhanced reproducibility
- Easy analysis of raw data without spectral unmixing
- Quick normalization of image groups with linked lookup table options

Easy image capture

One-button image acquisition saves you time by automatically optimizing the imaging parameters. Repeated exposures are unnecessary. The acquisition settings you use today will be suitable for your entire study.

Sensitivity that's more than skin deep

Pearl Impulse is sensitive enough to detect small or deep tumors that are not yet palpable, both subcutaneous and orthotopic (Fig. 6, 10, 11). Less probe is needed, making your experiments more cost effective.

Very broad dynamic range

Sensitivity is most powerful when combined with wide dynamic range. Pearl Impulse's unprecedented 22-bit dynamic range captures both weak and strong signals optimally in a single exposure, without signal saturation (Fig. 6 and 7). Dynamic range is critical, because tumor signals can vary by several orders of magnitude across a longitudinal study.

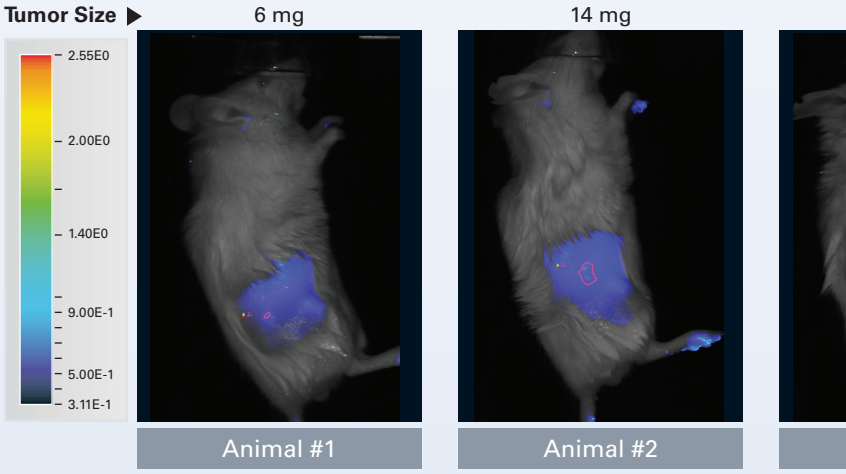


Figure 6. Imaging of small and large tumors without signal saturation. Subcutaneous prostate tumors were established in 7 different NOD/SCID mice. Tumor size ranged from 6 - 424 mg. Tumors were imaged after injection of IRDye® 800CW 2-DG probe (15 nmol), to detect increased glucose metabolism. Large and small tumors were imaged with the same acquisition settings, without signal saturation. After imaging, the tumors were excised and weighed.

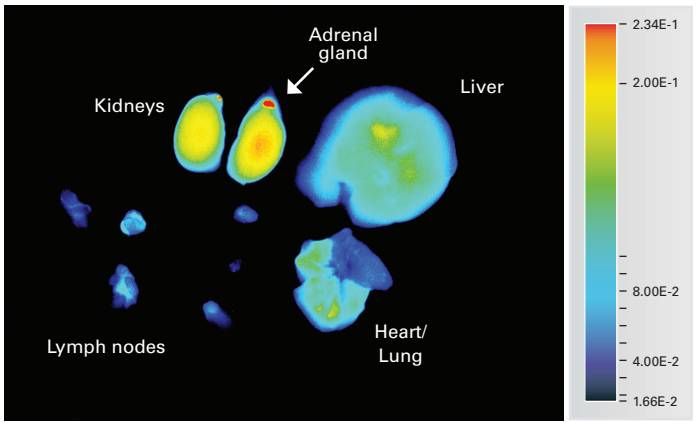


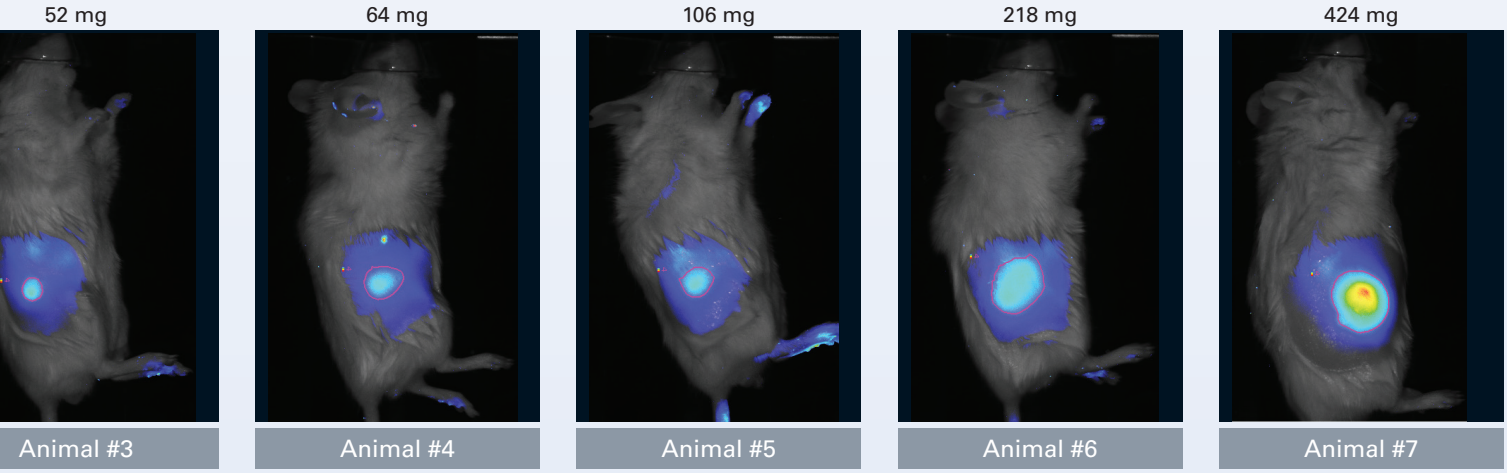
Figure 7. Imaging of strong and weak signals in excised tissues. Excised organs may be imaged to examine the distribution and clearance of an optical agent. Imaging of low-signal targets such as excised lymph nodes alongside high-signal organs such as kidneys requires a very wide dynamic range.

You can normalize and compare many animals with different tumor sizes (Fig. 6), without saturation of larger targets or inability to detect smaller targets. And you'll never have to change image capture settings to accommodate signal increases. This simplifies longitudinal imaging studies and eliminates the need for multiple exposures.

Other optical imaging systems are limited to 12- or 16-bit dynamic ranges. These ranges are insufficient for the diversity of signal strength observed in small animal imaging, so signals are easily saturated and image capture requires trial and error – and you waste time taking multiple exposures of each animal.

But Pearl Impulse, with FieldBrite™ Xi, removes the guesswork. Its 22-bit dynamic range – more than 6 orders of magnitude – lets you capture a high-quality image every time. The entire range is available in every image, without adjustment of image capture settings. Both strong and weak signals are accurately recorded without saturation.

Brighter signals than you expected? Extended longitudinal study? Excised organs? Large target and small target? No problem. Publication-quality images are fast and easy to obtain, regardless of signal intensity – even for longitudinal studies.



(*Ultimate Ease of Use - Continued*)

Uniform illumination for better imaging reproducibility

Reproducibility is critical. Because *in vivo* imaging can be performed longitudinally, changes in signal must reflect biological change rather than limitations of the optical system. Pearl Impulse delivers uniform illumination across the entire imaging area for exceptional reproducibility (coefficient of variation < 3%; Table 2). Laser illumination is stable throughout the course of your study, so you can feel confident of the biological phenomena you observe.

Reproducibility of Imaging		
Same position	n = 3	CV = 0.04%
Different positions	n = 8	CV = 2.24%

Table 2. Illumination and imaging are uniform across the entire field of view. A brain tissue sample containing IRDye® 800CW probe was imaged 3 times in the same position, then imaged 8 times in 8 different positions within the 8 x 10 cm field of view. Signal intensity was very consistent and did not show positional variation.

Quick normalization of image display with linked lookup tables

Lookup tables (LUTs) are used to adjust how an image is displayed without changing the raw data or quantification results. To visually compare related animal images across a study, all images must be viewed with the same LUT. It's easy with linked LUTs – just click to normalize a group of images, and you can compare signals over the entire course of your project (Fig. 8).

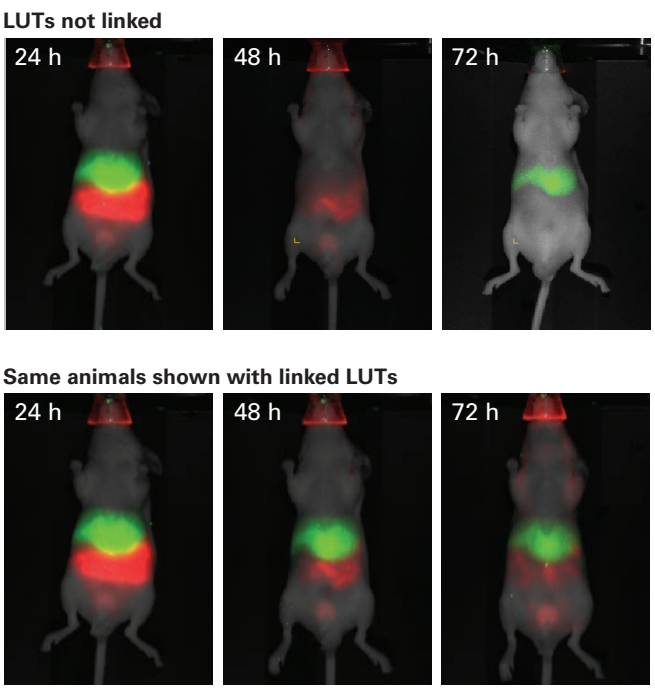


Figure 8. Linked lookup table option for image analysis. Animal was injected with IRDye 800CW imaging agent (green; 800 nm). Red indicates autofluorescence of food material (700 nm) in the animal's gut. A) BEFORE: Images displayed with different LUTs. B) AFTER: When images were normalized to a common LUT, clearance of the optical agent (green) through the liver was seen over time. Signal in gut (red) decreased when animal was fed a purified diet.

Correlation Between Fluorescent Signal and Tumor Size

Although optical imaging does not offer the quantitative power of PET, it can be extremely useful for noninvasive estimation of tumor burden. For example, in a subcutaneous tumor model, IRDye 800CW 2-DG probe was used to detect increased glucose metabolism. After *in vivo* imaging of fluorescent signal, tumors were excised and wet weight measured.

For this subcutaneous tumor model and probe, 97% of the variance in fluorescence intensity could be explained by tumor wet weight (Fig. 10) – indicating that NIR optical imaging can reliably estimate tumor burden without sacrificing the animal. In a separate study with an orthotopic prostate tumor model (Fig. 11), the correlation between signal and wet weight was 91% (*Kovar et al (2006) Am. J. Pathol 169:1415*).

Multi-Target Imaging

In the living animal, many biological processes occur simultaneously. Imaging one probe at a time may not tell the whole story. With multi-target imaging, you can monitor multiple biological processes simultaneously in the same animal (Fig. 9). You can obtain both structural and molecular information, or target two different molecular mechanisms.

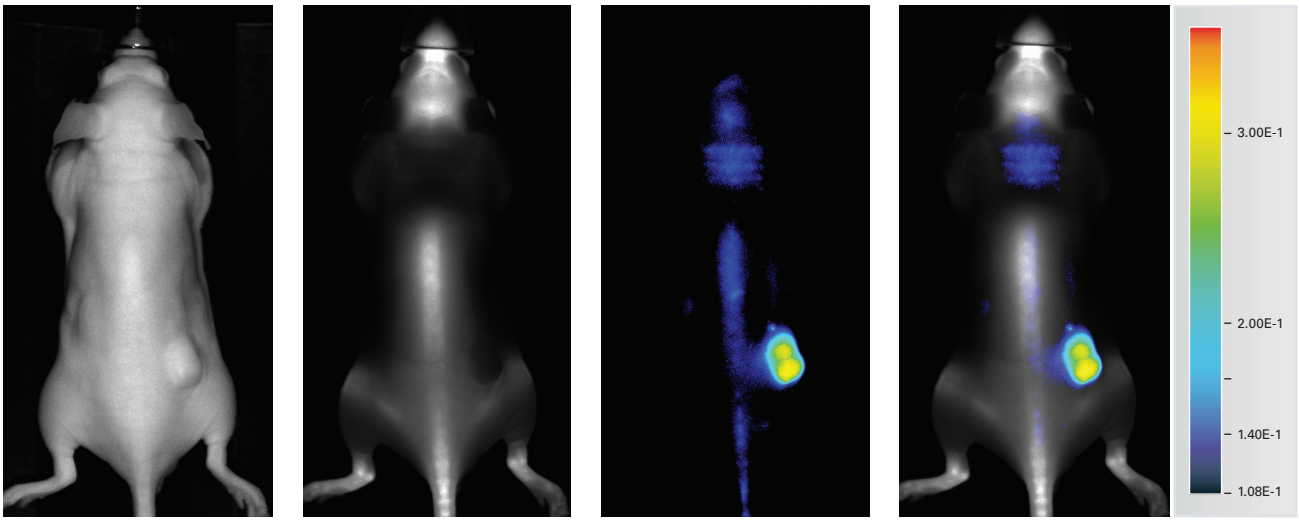


Figure 9. Imaging of multiple targets in the same animal. IRDye® 680 BoneTag™ agent reveals skeletal features (700 nm; greyscale). IRDye 800CW EGF (800 nm; pseudocolor) was used to image A431 tumor on the right hip. Overlay shows combined 700 nm and 800 nm images.

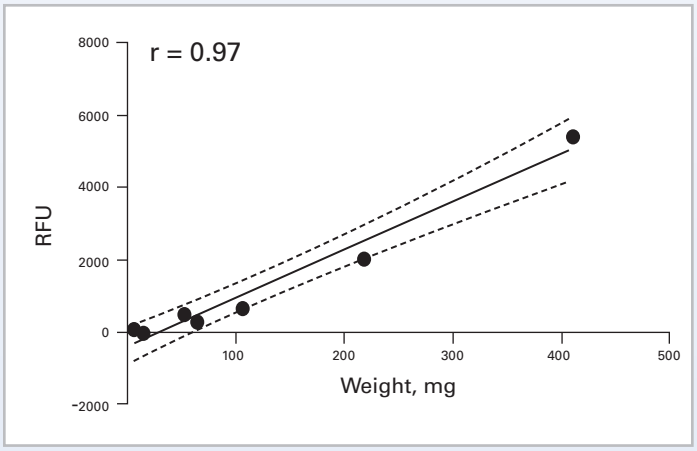


Figure 10. Fluorescent signal of subcutaneous tumors is correlated with tumor weight. NOD/SCID mice (n=7) were implanted with subcutaneous prostate tumors. Animals were injected with IRDye 800CW 2-DG probe (15 nmol) for tumor imaging (shown in Fig. 6). Tumors were then excised and weighed. Linear regression analysis was performed. Correlation (Pearson r) for intensity to weight was 0.97; P-value (two-tailed) = P< 0.0001.

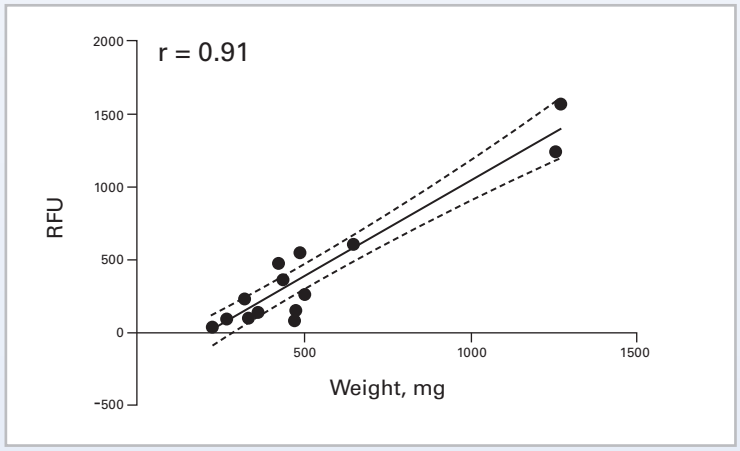


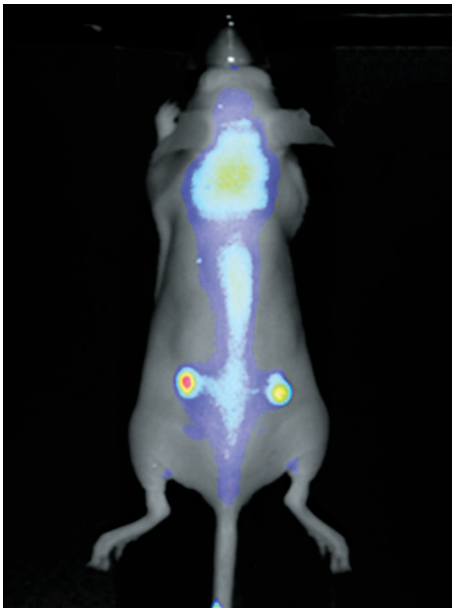
Figure 11. Fluorescent signal of orthotopic prostate tumors is correlated with tumor weight. NOD/SCID mice (n=14) were orthotopically implanted with prostate tumor cells. After 6 weeks, animals were injected with IRDye 800CW probe (1 nmol) and imaged. Tumors were then excised and tumor wet weight determined. Linear regression analysis was performed. Correlation (Pearson r) for total intensity to wet weight was 0.91; P-value (two-tailed) = P< 0.0001.

Adapted from Kovar et al (2006) Am. J. Pathol 169:1415.

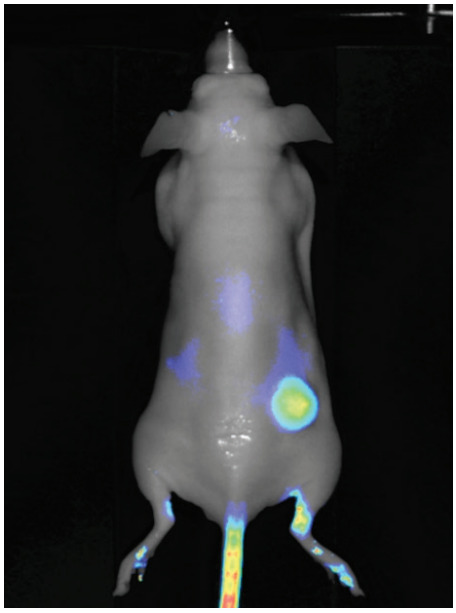
In Vivo Imaging Gallery

An updated list of LI-COR® Biosciences' growing portfolio of BrightSite™ IRDye® imaging agents and applications can be found at *www.licor.com/brightsite*.

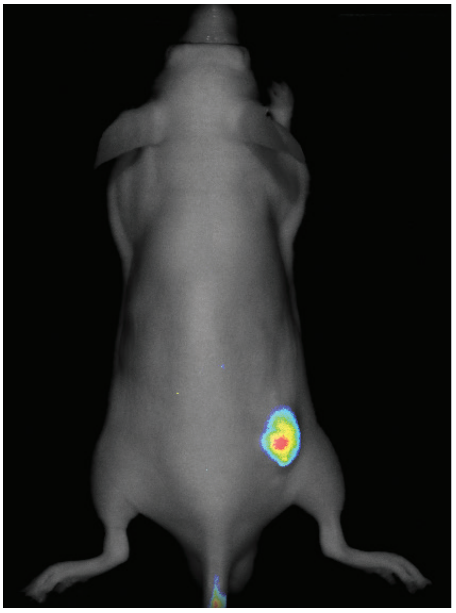
Tumor Imaging



Integrin overexpression imaged with IRDye 800CW RGD. Subcutaneous tumors were detected in a nude mouse (left hip, U87; right hip, A431).

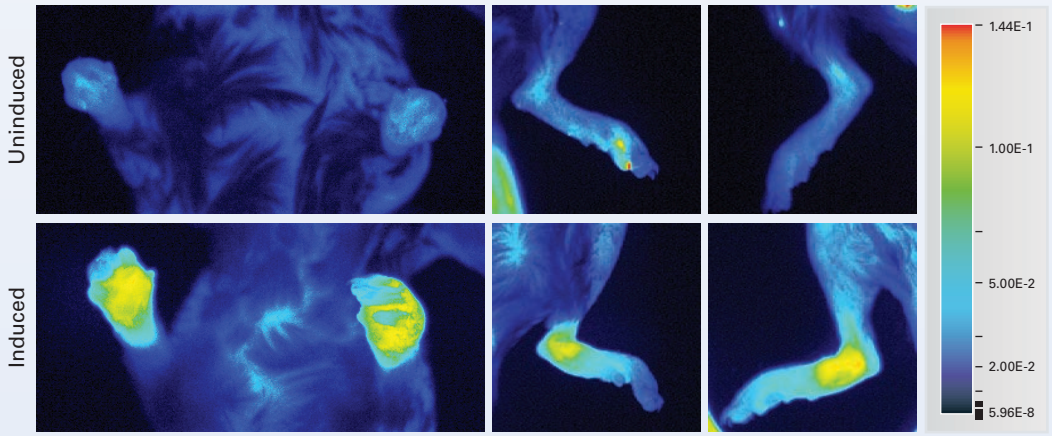


Increased glucose metabolism imaged with IRDye 800CW 2-DG. Subcutaneous A431 tumor was detected. *For detailed discussion, see: Kovar et al., Anal Biochem 384:254 (2009).*



EGF overexpression imaged with IRDye 800CW EGF. Subcutaneous prostate tumor was detected.

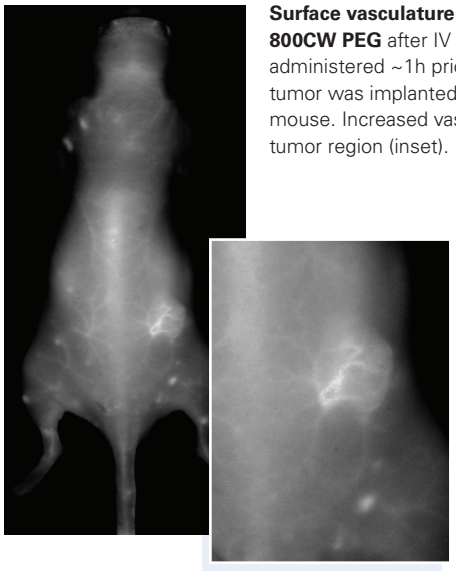
Arthritis and Inflammation



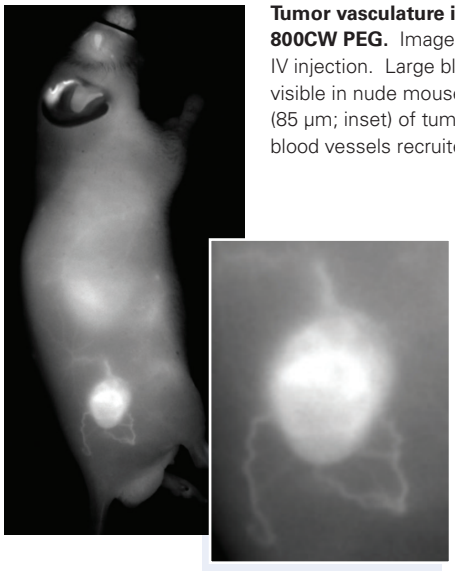
Arthritis imaging with IRDye 800CW 2-DG in DBA Collagen antibody-induced arthritis model. Uninduced and induced animals were injected with IRDye 800CW 2-DG probe. Probe was retained in affected areas (feet and ankles) of induced animal.

Courtesy of Dr. Andrea Augello (De Bari lab, Musculoskeletal Programme, Institute of Medical Sciences, University of Aberdeen, UK).

Vasculature

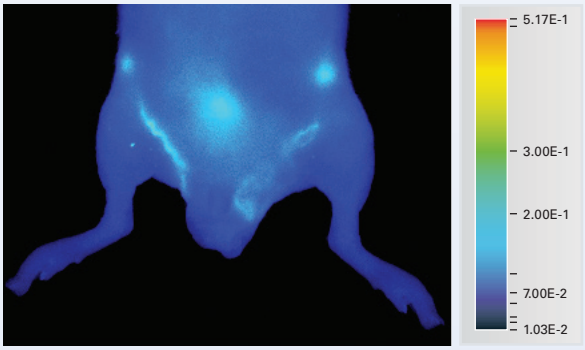


Surface vasculature imaged with IRDye® 800CW PEG after IV injection. Agent was administered ~1h prior to imaging. A431 tumor was implanted on the right flank of nude mouse. Increased vasculature is seen in the tumor region (inset).

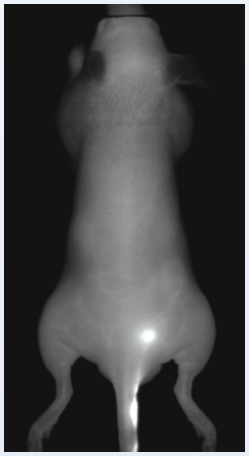


Tumor vasculature imaged with IRDye 800CW PEG. Images were captured 3.5h after IV injection. Large blood vessels and tumor are visible in nude mouse. High resolution image (85 µm; inset) of tumor region shows large blood vessels recruited to feed the tumor.

Lymphatics

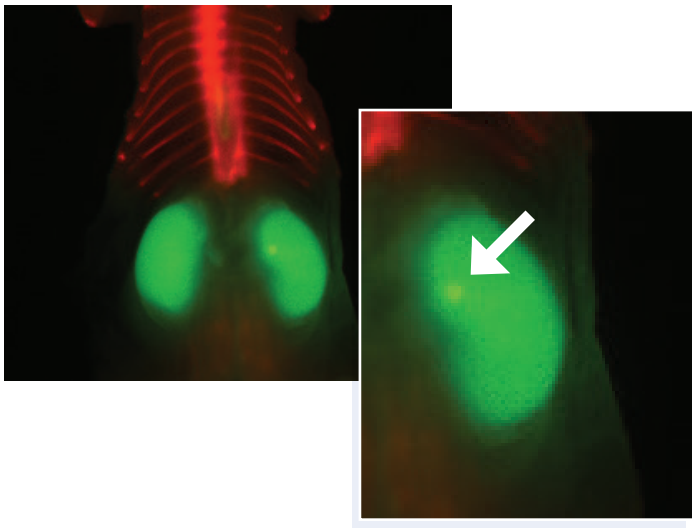


Imaging of lymph vessels leading to subiliac lymph nodes after intradermal injection of IRDye 800CW HA.



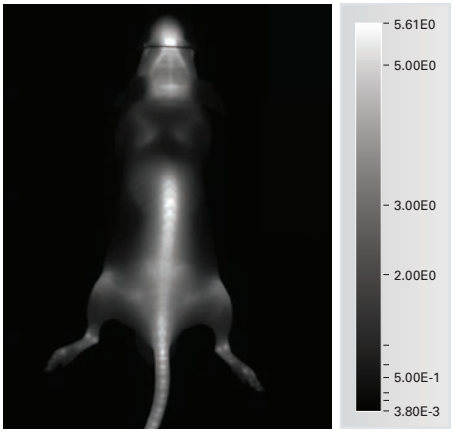
Lymph tracking with IRDye 800CW PEG after intradermal injection on right side of tail. Draining of agent to sciatic lymph node is detected.

Metastasis

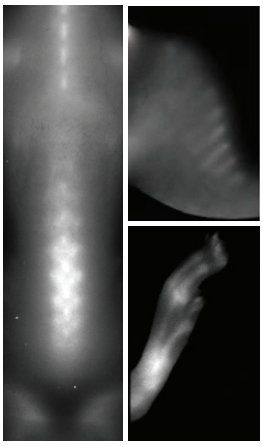


Micro-metastasis to kidney (intraoperative imaging). Animal received tumor cells intracardially. After 10 weeks, IRDye® 800CW EGF probe (green) was injected 48h prior to final imaging. Animal was surgically examined in more detail after sacrifice. Skeleton was visualized with IRDye 680 BoneTag™ agent (red). Micrometastasis is seen on one kidney (arrow).

Bone Imaging

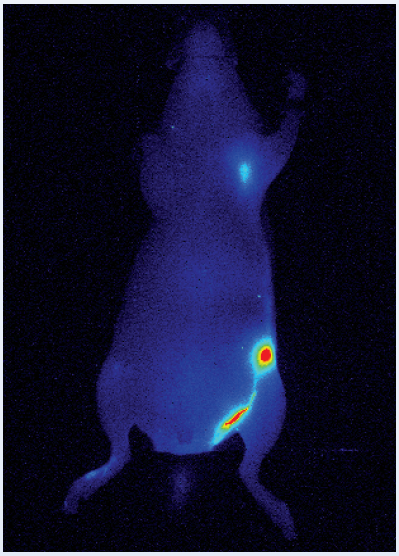


IRDye BoneTag agent for imaging of bone structure and remodeling. Tetracycline-derived probe detects skeletal structure, and signal is stable for weeks. Dorsal view of mouse imaged with IRDye 680 BoneTag.

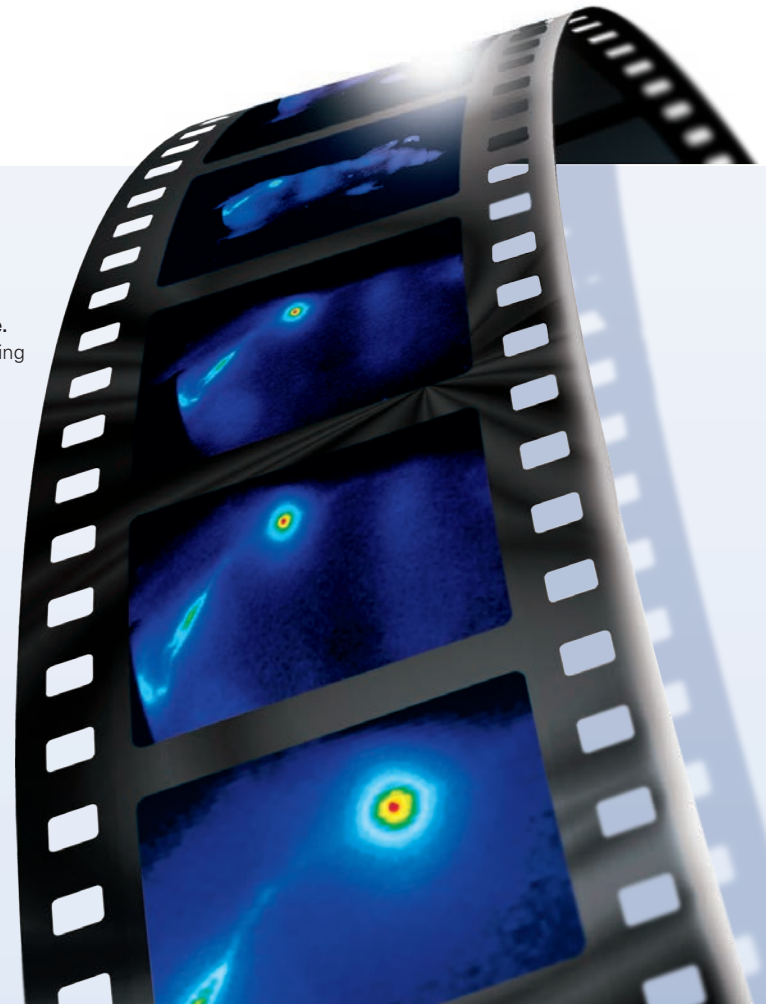


Imaging of skeletal details with IRDye 680 BoneTag agent. Spine, ribs, and foot of nude mouse were imaged at 85 µm resolution.

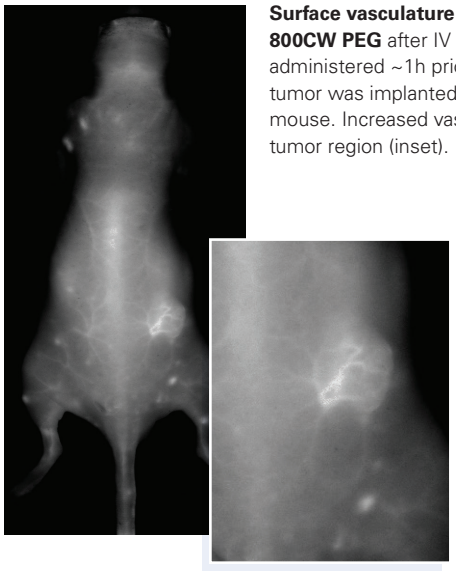
Rapid Time-Lapse Imaging



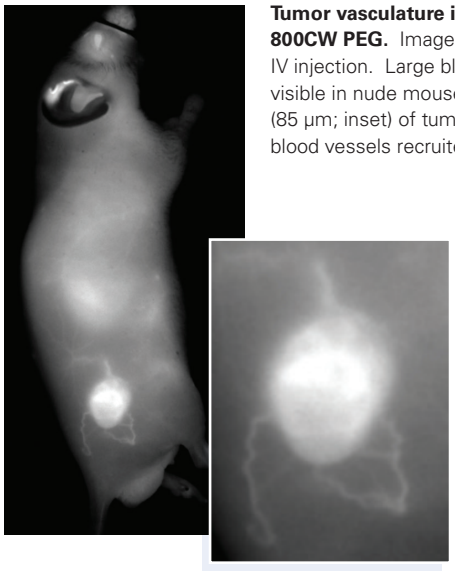
Visualize imaging agents dynamically with rapid time-lapse imaging software. IRDye 800CW PEG agent is shown pulsating to the lymph node.



Vasculature

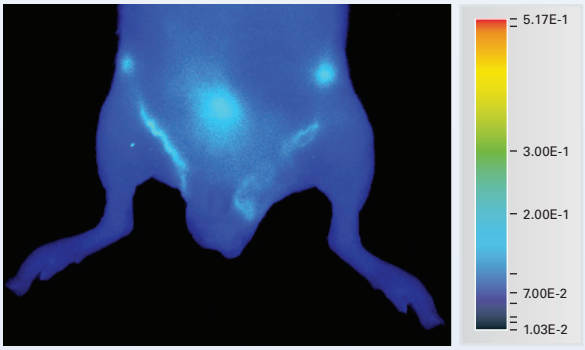


Surface vasculature imaged with IRDye® 800CW PEG after IV injection. Agent was administered ~1h prior to imaging. A431 tumor was implanted on the right flank of nude mouse. Increased vasculature is seen in the tumor region (inset).

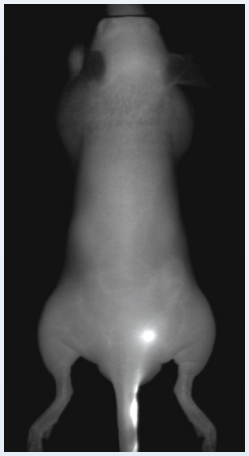


Tumor vasculature imaged with IRDye 800CW PEG. Images were captured 3.5h after IV injection. Large blood vessels and tumor are visible in nude mouse. High resolution image (85 µm; inset) of tumor region shows large blood vessels recruited to feed the tumor.

Lymphatics



Imaging of lymph vessels leading to subiliac lymph nodes after intradermal injection of IRDye 800CW HA.



Lymph tracking with IRDye 800CW PEG after intradermal injection on right side of tail. Draining of agent to sciatic lymph node is detected.

Safe Imaging Environment

Animal welfare is a key consideration. Pearl Impulse minimizes animal stress and handling:

- Detachable imaging beds with temperature-controlled surfaces and gas anesthesia ports
- Stand-alone Pearl Docking Station provides temperature and anesthesia gas control to an imaging bed at a secondary location and fits in laminar flow hood
- Clean Box for transport of immunocompromised mice from docking station to imager in a HEPA-filtered environment
- SmartFlow Anesthesia Suite and Surgical Suite accurately administers gas to all breathing devices with the flip of a switch, maintaining comfort and safety for the animal and lab personnel
- Imaging drawer brings the animal out to you, reducing your exposure to anesthesia gas

Docking Station and Clean Box

SmartFlow Anesthesia Suite

Clinical Translation

LI-COR® Clinical Translation seeks to facilitate the use of IRDye® 800CW-labeled imaging agents in clinical studies for detection of disease and its progression, intraoperative imaging, and monitoring of treatment and drug efficacy.

We have performed a study examining the toxicity of IRDye 800CW in Sprague-Dawley rats. The study was performed in a manner compatible with that needed for a Phase 0/eIND*(Marshall, M. et al. (2010) Molecular Imaging and Biology 12:583)*.

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View a complete list of our international distributors at:
www.licor.com/bio/distributors

System Specifications

Detector Type:
CCD. Thermoelectrically cooled.

Acquisition Speed:
~20-40 sec (multiple colors)
~500 msec - 10 sec (single color rapid time-lapse)

Wavelength Maxima (Ex/Em):
700 nm Channel: Ex 685 nm, Em 720 nm
800 nm Channel: Ex 785 nm, Em 820 nm
White Light Channel: Ex white, Em NA

Dye Compatibility:
IRDye® 680, IRDye 700DX, IRDye 800RS, IRDye 800CW, Alexa Fluor® 680, Alexa Fluor 750, Cy® 5.5, Cy 7, and others

Resolution:
85, 170, and 255 micron

Image Display Options:
Pseudocolor, greyscale, single color (red, green, or blue), or two colors with overlapping fluorescence displayed in a third color

Capacity:
One animal, with linked look-up tables for image normalization

Imaging Bed:
16.8 cm W x 12 cm D (6.6″ W x 4.75″ D). Vertical clearance to top of drawer is 3.8 cm (1.5″).

Field of View:
11.2 cm W x 8.4 cm D (4.4″ W x 3.3″ D) at surface of imaging bed

Imaging Bed Temperature Range:
32-42°C

Gas Anesthesia:
Inlet and outlet ports flow anesthesia gas through nose cone in imaging drawer. Rotameter controls flow rate.

Size:
41 cm W x 41 cm D x 66 cm H (16″ W x 16″ D x 26″ H). Depth with imaging drawer open is 63.5 cm (25″).

Weight:
23 kg (50 lb)

LI-COR

The LI-COR board of directors would like to take this opportunity to return thanks to God for His merciful providence in allowing LI-COR to develop and commercialize products, through the collective effort of dedicated employees, that enable the examination of the wonders of His works.

"Trust in the LORD with all your heart and do not lean on your own understanding. In all your ways acknowledge Him, and He will make your paths straight."
—Proverbs 3:5,6

LI-COR is honored to receive this prestigious award. Learn more at www.licor.com/frostsullivan.

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