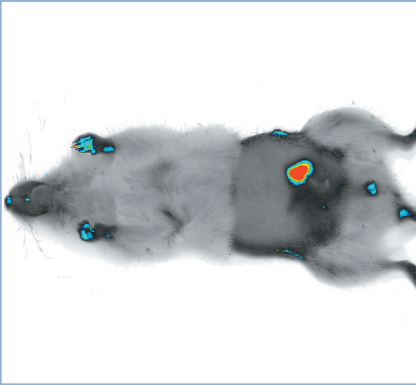


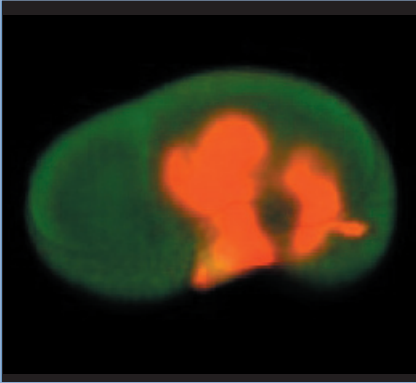
PROBE CHARACTERIZATION

APPLICATION OVERVIEW

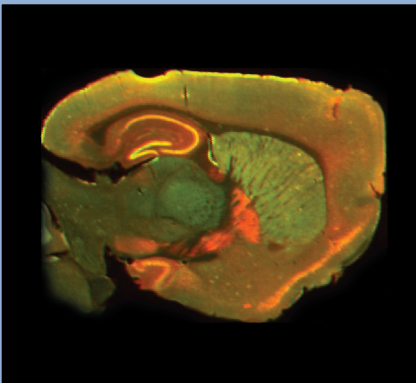


SMALL ANIMAL IMAGING

Molecular Imaging on the Odyssey[®]



WHOLE ORGAN IMAGING



TISSUE SECTION IMAGING

LI-COR[®]
Biosciences

One Probe Simplifies Discovery

Small animal *in vivo* imaging has important significance in biological studies, including human cancer research. The ability to track and visualize a biological probe tagged with an infrared dye *in vivo* yields excellent data for these studies. LI-COR's Odyssey® Infrared Imaging System and IRDye® infrared dyes provide the unique ability to select and use the same probe for every step of the molecular imaging process.

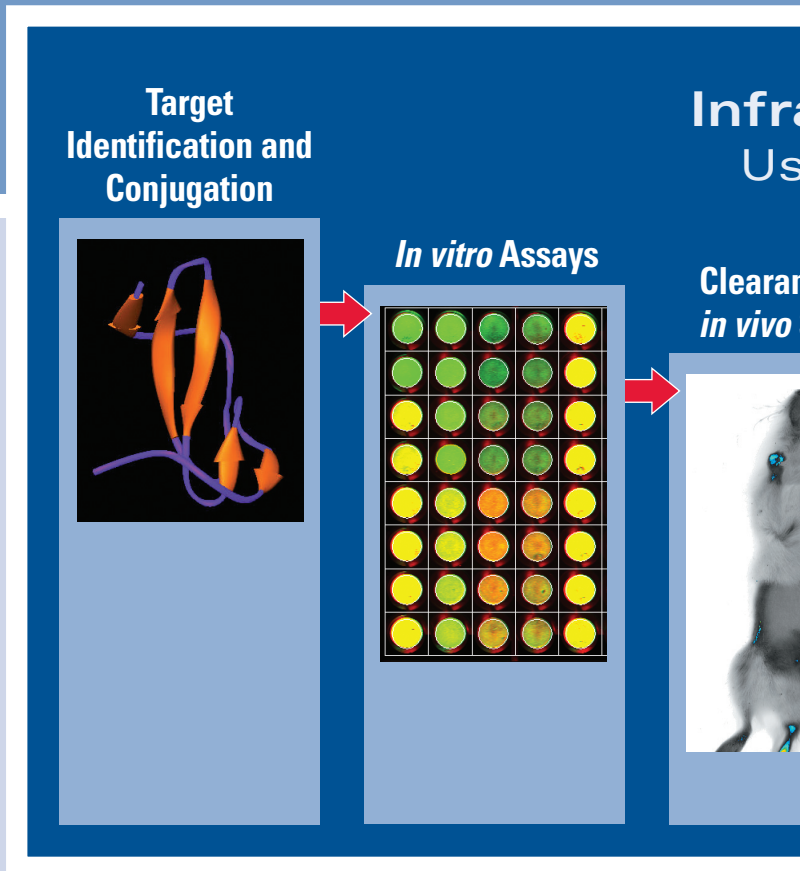
Additional advantages of molecular imaging with IRDye-labeled probes include:

- Low autofluorescence from tissues, plates, cells, biological materials and drug compounds in the near-infrared (NIR).
- IRDye® 800CW absorption/emission near 800 nm matches NIR absorption minima for bodily fluids and tissues.
- Low tissue autofluorescence in the 800 nm region results in excellent depth of tissue penetration (>2 cm).
- High signal-to-noise ratio.

The Odyssey System is a complete solution for *in vitro*, *in vivo*, and tissue section analysis – all on a single platform. Features such as simultaneous two-color detection make it possible for ratiometric analysis *in vitro*, enabling easier and more cost effective tests for probe specificity before *in vivo* imaging. Additional solutions such as the Odyssey MousePOD™ streamline your molecular imaging workflow and make it easier to achieve your research goals.



Odyssey MousePOD™ Accessory (open), showing anesthesia nose cones.



Molecular Imaging Workflow

Target Identification: *In vivo* imaging projects typically begin with identification of a possible tracking agent or probe, such as a ligand to a receptor or specific antibody.

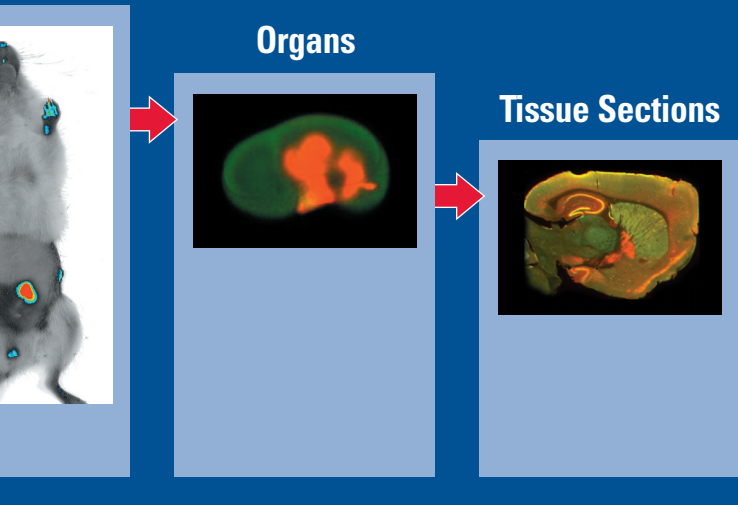
Label/Conjugation: The probe of choice can be labeled via NHS ester conjugation with IRDye® 800CW infrared dye for imaging on the Odyssey System. Protein labeling kits are available that use IRDye® 800CW NHS ester to label antibodies and proteins. Alternatively, IRDye® 800CW EGF is a labeled targeting agent for use in common human cancers that over-express EGFR.

In vitro Assays: Before starting a costly and time consuming *in vivo* experiment, probe specificity can be determined *in vitro* on the Odyssey System using an immunocytochemical assay performed in microplate format such as the cost effective In-Cell Western (ICW) assay.

Clearance and Characterization Studies in vivo: The labeled targeting agent should be evaluated in “tumor-negative” and “tumor positive” animals. Clearance studies address important questions such as:

Advanced Molecular Imaging Using the Odyssey® System

Preclinical and Bench Studies



- 1) Is the probe retained beyond the intended targets (e.g. liver, kidneys, or bladder)?
- 2) How long does it take for the targeting agent to clear the animal?
- 3) What is the ideal probe concentration?
- 4) When is the best time post-injection to achieve the best signal-to-noise ratio?

Once probe clearance is determined, a full *in vivo* experiment can be performed with greater confidence. Up to three mice can be scanned and the IRDye-labeled probes visualized using the Odyssey System and MousePOD accessory.

Organs: More focused analysis of the probe's localization can be done on the Odyssey by excising and scanning the target organ(s). Scans of organs provide a semi-quantitative estimate of signal and quickly show whether the targeting agent is present.

Tissue Sections: To determine exact tissue localization, frozen or paraffin embedded tissue sections can be scanned on the Odyssey at high resolution. Tissue sections can also be examined using a microscope equipped with an infrared filter.

Products for Molecular Imaging

MousePOD™ Imaging Accessory

- Fits on the Odyssey scanning surface and accommodates up to three mice.
- Delivers gas anesthesia to animals via nose cones (external anesthesia system not included).
- Regulates air temperature to maintain animal's temperature during scanning.
- Includes new Auto Shape tool for Odyssey software to quickly mark tumors, organs, and other regions of interest. Pseudo color display style helps to quickly isolate regions of interest.



MousePOD™ (closed)

IRDye® 800CW Labeling Reagents and Probes

- IRDye® 800CW NHS ester in 0.5 and 5.0 mg pack sizes.
- IRDye® 800CW Labeling Kit for proteins and antibodies. Optimized for 3 conjugations of 1 mg IgG (MW~150,000) or other proteins.
- IRDye® 800CW EGF Optical probe for general, solid-tumor characterization.



IRDye 800CW EGF Optical Probe

Products for Molecular Imaging (continued)

Odyssey® Infrared Imaging System

- Very accurate quantification for Western Blots due to a wide, linear detection range.
- High Sensitivity – equal to or better than chemiluminescence.
- Simultaneous detection in two channels (700 and 800 nm) enables ratiometric analysis in which the one channel is used for normalization.
- Direct infrared detection on membranes eliminates film, darkrooms and messy substrates.
- Wide range of compatible dyes and stains, including LI-COR's IRDyes.
- Familiar software tools for band sizing and quantification. Optional software accessory packages for *in vivo* imaging (included with MousePOD) and In-Cell Western assays (*in vitro* analysis).

Odyssey® Software Accessory Packages

- In-Cell Western Analysis Software for *in vitro* assays in microplate format. Automatically finds wells for 96- and 384-well microplates, and performs ratiometric calculations using the two imaging channels of the Odyssey System.



Odyssey® Infrared
Imaging System

Odyssey® Applications

- Quantitative Western blots
- Coomassie® stained gels
- Cell-based assays
- Fluorescent gel-shift assays
- Tissue imaging
- *In vivo* Imaging
- Protein Arrays

LI-COR®

Biosciences

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Tissue section images courtesy of C. Kearn, University of Washington.

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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