ODYSSEY®

INFRARED IMAGING SYSTEM

ACCURATE QUANTIFICATION

MULTIPLEX DETECTION

HIGH SENSITIVITY

DIRECT DETECTION

CLEAR DATA

WIDE RANGE OF APPLICATIONS

LI-COR® Biosciences

ODYSSEY®
INFRARED IMAGING SYSTEM

Accurate Quantification
Wide linear dynamic range

Multiplex Detection
Normalization increases quantification accuracy

High Sensitivity
Equal to or better than chemiluminescence

Direct Detection
No film, darkroom, or messy substrates
No multiple exposures, re-scan membrane at any time

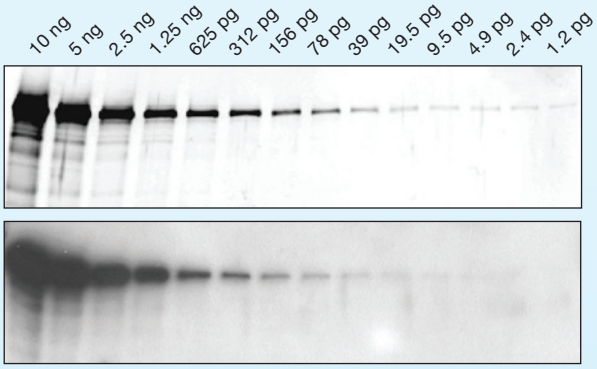
Clear Data
No loss of weak bands due to overexposed bands

Wide Range of Applications
Western blots, In-Cell Westerns™, Coomassie® stained gels, DNA Gels,fluorescent gel-shift assays, tissue imaging, in vivo imaging, whole organ imaging, protein arrays, and more

Infrared Fluorescence
Upgrade to Quantitative Westerns

Direct infrared fluorescence detection on the Odyssey Infrared Imaging System provides the established standard for Western blot analysis that can't be equaled with chemiluminescence and visible fluorescence. Infrared detection gives you the quantitative analysis and wide linear dynamic range that chemiluminescence cannot.

Strong and weak bands on the same blot are accurately detected without the uncertainty and inconvenience of multiple exposures, and without spending time in the darkroom. The Odyssey System gives you clear, sharp, reproducible bands without fuzziness or "blowout". Bands hidden by overexposure with chemiluminescence are clear in Odyssey images.



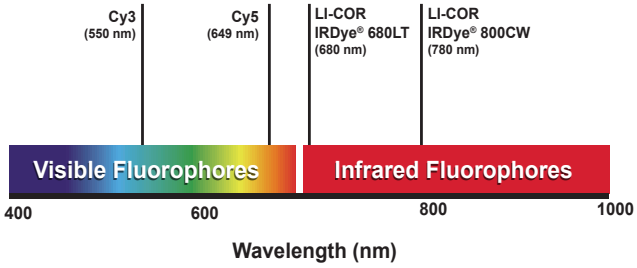
Serial dilutions (10 ng to < 1 pg) of purified human transferrin (Tf) were used to assess Western sensitivity. The Odyssey System, using infrared fluorescence detection, reproducibly detected 1.2 pg of Tf, while only 4.9 - 9.8 pg was detected with chemiluminescence. Infrared detection sensitivity was approximately 200-fold greater than previous studies with visible fluorophores (Cy®3, Cy®5, or FITC).

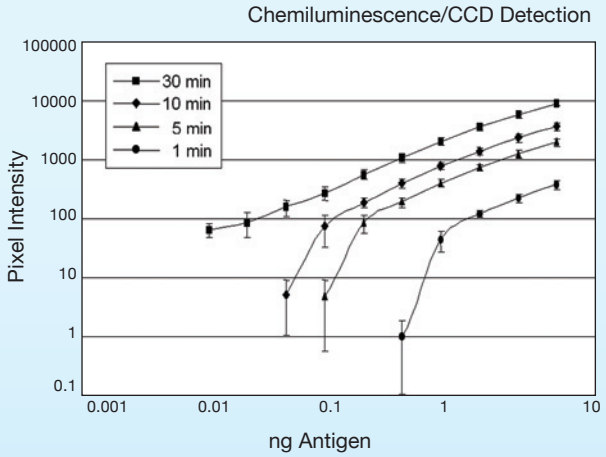
The Odyssey System provides a flexible, multifunctional platform to accommodate a variety of applications, so you get results faster. One example is the In-Cell Western™ assay, an immunocytochemical technique performed with cultured cells in microtiter plates. This assay uses target-specific antibodies to quantify protein levels in fixed cells. Accuracy, reproducibility, and throughput are all increased by eliminating time-consuming, error prone steps such as lysate preparation and gel electrophoresis.

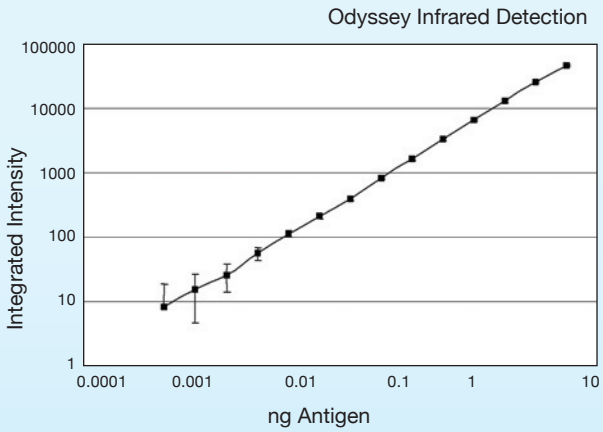
The Infrared Advantage

In the visible wavelength range used by most fluorescent imaging systems, membranes and plastics produce high background due to light scattering and autofluorescence.

This high background limits the sensitivity of visible fluorescent systems and makes it nearly impossible to detect low-abundance proteins at endogenous levels. At the infrared wavelengths detected by Odyssey, both autofluorescence and light scatter are dramatically reduced. The result is the cleanest background, highest signal-to-noise ratios, and best detection sensitivity available with a fluorescent system.



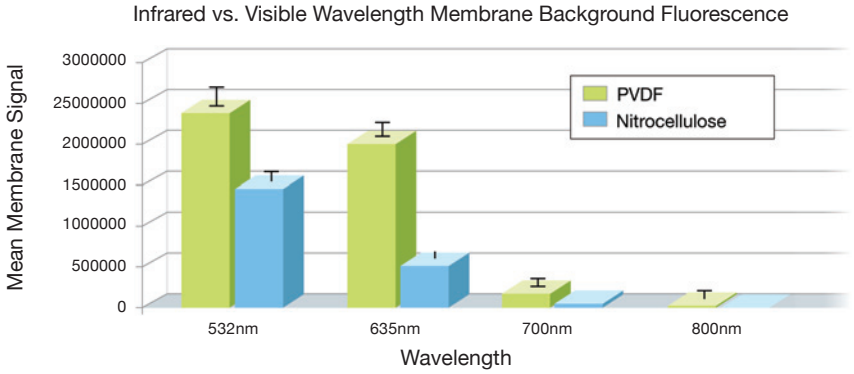




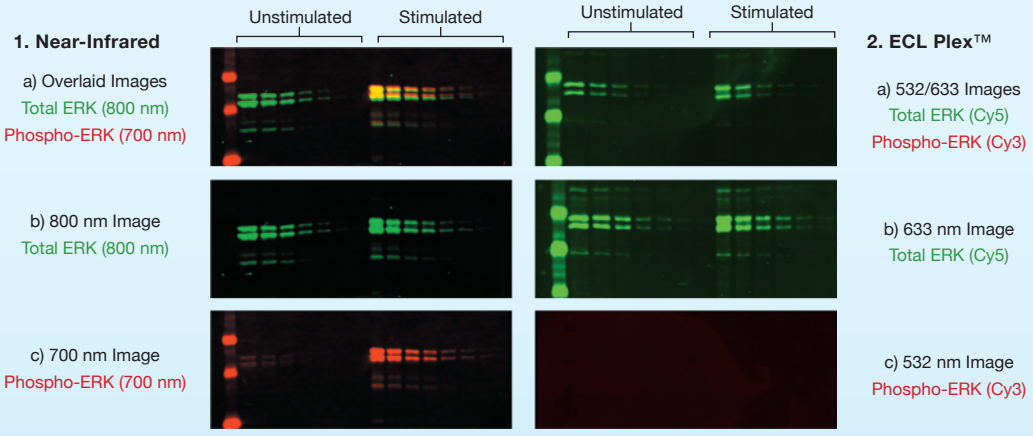
A dot blot assay was used to compare the linear ranges of chemiluminescent and infrared fluorescent detection. Dilutions of mouse antibody were spotted as antigen, and detected with HRP- or IRDye infrared dye-labeled goat anti-mouse antibodies. Chemiluminescent data were collected using ECL substrate and a CCD camera with varying exposures; the infrared image was obtained in a single scan with the Odyssey System. For a 30 minute chemiluminescent exposure, the data set was linear over a 250-fold range. In contrast, infrared detection displayed a quantitative linear range greater than 4000-fold (3.6 orders of magnitude). A paper detailing this study can be downloaded at www.licor.com/chemiccd.

Multiplex Western Detection

The two infrared fluorescent detection channels of the Odyssey System enable simultaneous two-color target analysis – an advantage that's not available with chemiluminescent or radioactive methods. Two-color Western analysis makes normalization easy and eliminates error introduced by stripping and reprobing or by comparison of separate blots. Superior image clarity and detail make it easier to detect subtle mobility shifts caused by protein modifications such as phosphorylation.



Nitrocellulose and PVDF membranes were scanned on the Odyssey Infrared Imaging System at an Intensity = 5 for both 700 and 800 nm wavelengths. The same membranes were scanned at a 532 nm and 635 nm wavelength with a PMT=500 on a GenePix® 4100A (Molecular Devices).



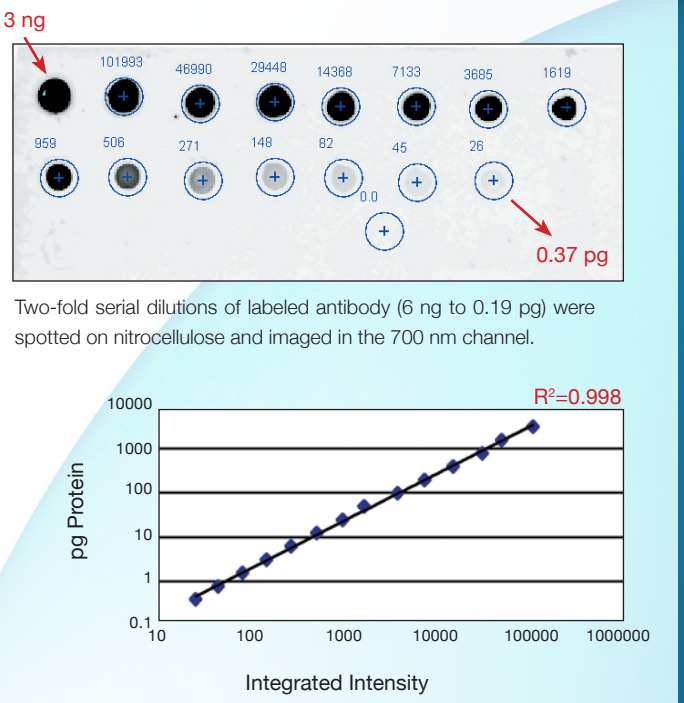
Western Blot Analysis of ERK Activation

Comparison of Odyssey Detection to Visible Fluorescence

ERK1/2 and phospho-ERK were detected in lysates of unstimulated and EGF-stimulated A431 cells. Two fold serial dilutions of lysate are shown.

A. Odyssey Near-Infrared Images: The single-color images (1b & 1c) were collected simultaneously. Images were overlaid (1a) to show both total ERK and phospho-ERK (yellow color indicates overlap of red and green signals). The mobility shift caused by phosphorylation can be seen in the EGF-stimulated lysate.

B. Visible Fluorescence Images: 532 nm and 633 nm images were collected separately. High background obscures visual observation of phospho-ERK and the mobility shift in the EGF-stimulated lysate.



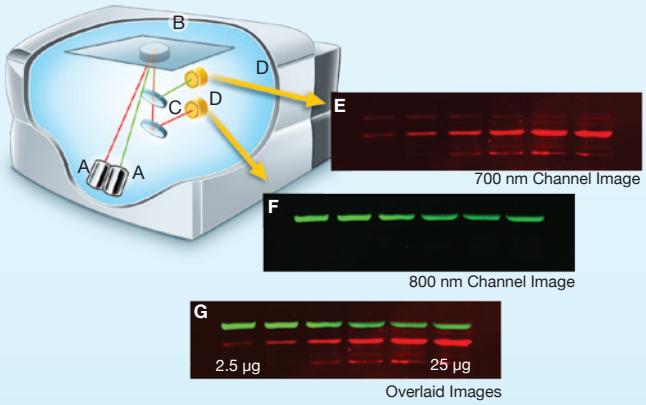
Two-fold serial dilutions of labeled antibody (6 ng to 0.19 pg) were spotted on nitrocellulose and imaged in the 700 nm channel.

Accurate Quantification

Through the innovative use of infrared fluorescent labeled antibodies rather than enzyme labels, the Odyssey System provides a broad, linear dynamic range to accurately detect strong and weak bands on the same Western blot. In contrast, the dynamic enzymatic nature of chemiluminescence allows you to capture only a "snapshot" of the enzymatic reaction and is highly dependent on timing and exposure, limiting linear range and offering only qualitative or semi-quantitative results. The accuracy and linearity of the Odyssey System detection allows you to be confident about differences you see in protein levels, and your blots can be archived and imaged again months later, if needed.

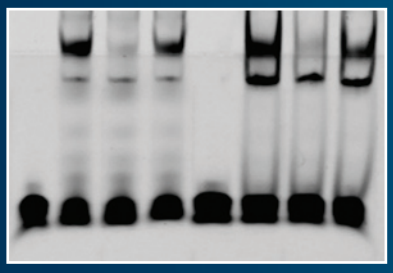
Two Independent Infrared Detection Channels

Two separate lasers and detectors simultaneously detect both fluorescent signals. The optical system employs diode lasers and solid-state detectors due to their long lifetimes and very low maintenance requirements. Infrared laser excitation outperforms systems that use white light and filter wheels by delivering higher intensity excitation light to the fluorophore. A variety of fluorescent dyes and stains are compatible with the 685 and 785 nm excitation wavelengths of the Odyssey System's two diode lasers. Spectral overlap is minimized by the 100 nm separation of the two detection channels, and optical filtering assures that each detector measures fluorescence from only one of the infrared dyes.

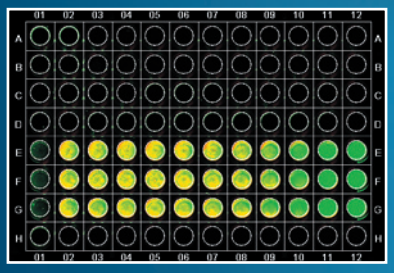


Beams from solid-state 685 and 785 nm lasers (A) are focused to form an excitation spot on the scanning surface. A microscope objective (B), focused on the excitation spot, collects light from both fluorescing infrared dyes. Light from the microscope objective is passed through a dichroic mirror (C) that splits the light into two fluorescent signals. The fluorescent signals travel through two independent optical paths and are focused on separate silicon avalanche photodiodes (D) and detected. In this example, 700 nm fluorescence (IkappaB) is shown in red (E) and 800 nm fluorescence (Tubulin) is shown in green (F). The two colors were imaged simultaneously in a single scan and can be displayed separately or together in a single image (G).

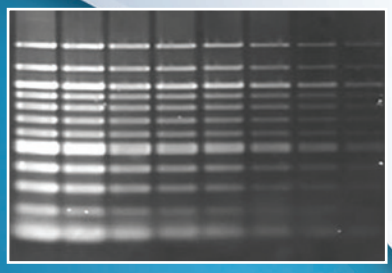
** Data courtesy of Dr. Catrin Albrecht, IUF, Germany*



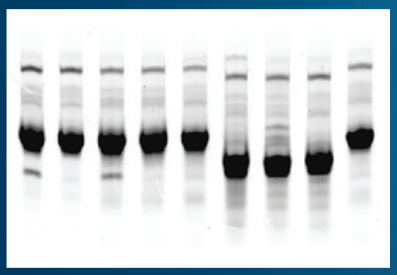
EMSA/GEL SHIFT ASSAYS



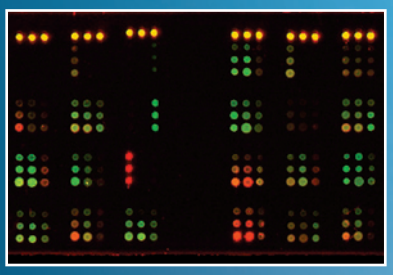
IN-CELL WESTERN ASSAYS



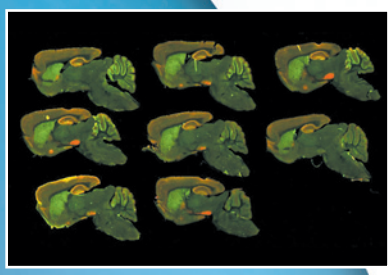
DNA GEL STAINING



COOMASSIE-STAINED GELS



PROTEIN ARRAYS



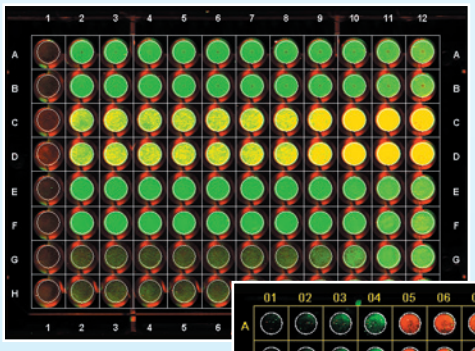
TISSUE SECTION IMAGING

Data courtesy C. Kearns, University of Washington

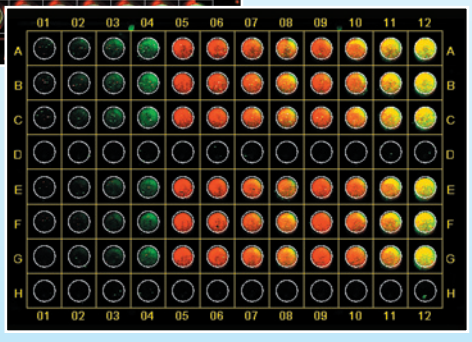
In-Cell Western™ Assay

For Ratiometric Protein Analysis

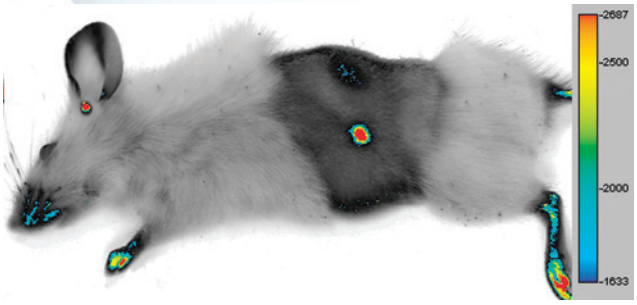
The In-Cell Western is an immunocytochemical assay performed in microplate format. Target-specific primary antibodies and infrared-labeled secondary antibodies are used to detect target proteins in fixed cells, and fluorescent signal from each well is quantified. Accuracy is enhanced and data are more meaningful because proteins are detected in their cellular context.



Adherent



Suspension



Odyssey® Applications

Featuring IRDye® Infrared Dyes

Infrared dyes are a key performance advantage of the Odyssey System. LI-COR Biosciences pioneering family of IRDye infrared dyes are synthesized with reactive functional groups that enable easy covalent coupling to antibodies and other biomolecules.

Wide Range of Applications

IRDye® Infrared Dye secondary antibodies and conjugates are optimized for a wide variety of applications, including:

- Western Blots: Two-color and In-Gel
- Cell-based Assays: In-Cell Westerns™ and On-Cell Westerns
- Protein Detection: Coomassie Stained Gels, Membrane and Slide Arrays
- Imaging: *In Vivo*, Whole Organ and Tissue Section
- Nucleic Acids: Mobility Shift Assays, DNA Gel Staining, Arrays
- Microwell Assays: ELISA/FLISA, Transcription Factors, Protein Arrays

In-Cell Western Assay Workflow

Grow Adherent/Suspension Cells in Microplate

↓

Fix and Permeabilize Cells, Block

↓

Primary Antibody Incubation

↓

Secondary Antibody Incubation (DNA Staining, if desired)

↓

Image and Quantify

Advantages of the In-Cell Western Assay

- Simultaneous, two-target detection enables precise quantification and accurate measurement of abundance or phosphorylation of one target by normalization against another target or DNA stain.
- Direct detection of proteins in their cellular context eliminates variabilities and artifacts caused by cell lysis. In-cell detection can provide more relevant results than enzyme assays with purified proteins.
- Near-infrared probes yield high sensitivity for measuring small changes in protein amount or modification.
- Analyze multiple targets in 96- or 384-well plates.
- Fast, microplate-based assay – lysate preparation, gel loading, electrophoresis, and membrane transfer are eliminated.
- Ideal for screening cell treatments or drug candidates for effects on target proteins.

Western Blotting Accessories

LI-COR Blot Washer configured for washing and secondary antibody incubation of four blots in incubation boxes. Shown with optional shaker (The Belly Dancer®, Stovall Life Science Inc.).

Blot Washer

Operating the LI-COR® Blot Washer is simple. Select a stored wash sequence, press the Start button, and your Western blots are processed without the constant interruptions necessary to wash blots manually. For further automation, stored sequences can also include secondary antibody incubation.

Blot Washer is compatible with most fluorescent and chemiluminescent Western blotting systems, as well as LI-COR Odyssey® and Aerius® Infrared Imaging Systems.

FEATURES:

- Automates washes and secondary antibody incubation
- Eliminates workday interruptions to process blots
- Increases throughput
- Improves reproducibility
- Processes up to four blots simultaneously

Western Cost Comparison

Infrared Detection vs. Chemiluminescence

Cost Savings: Reagents	IR Detection (2 Targets)	Chemiluminescence (1 Target)	Chemiluminescence (strip and reprobe for second target)
Secondary Antibody (15 mL) (Recommended Dilutions: 1:15,000 for IR; 1:2,500 for Chemi)	$0.68	$0.32	$0.64
Chemiluminescent Substrate (12 mL)	---	$4.97 (2 mL)	$9.88 (2 mL)
Film (2/4 films/blot)	---	$7.48	$14.96
Protein Markers Odyssey Two-Color Protein Marker for IR (2 µL) Standard Protein Marker for Chemi (10 µL)	$1.16	$4.68	No charge to reuse marker
Cost	**$1.84**	**$17.42 (2 mL)**	**$25.48 (2 mL)**
Extra Cost Per Blot Compared to IR*		**$15.58**	**$23.64**

**Based on GE Healthcare pricing, April 26, 2010. Assumes 10 x 10 cm blot.*

The increased sensitivity of IRDye Infrared Dye labeled reagents reduces consumption of costly secondary antibodies and protein markers per blot. Chemiluminescent substrates, film and related labor are completely eliminated.

Calculate your savings at:
www.licor.com/compare

MPX Blotting System and representative Western Blot image with multiple Primary and Secondary antibody concentrations. (Pipette not included.)

MPX™ Blotting System

The MPX Blotting System is ideal for nearly all multiple-target Western blot procedures that utilize PVDF or Nitrocellulose membranes (7 x 8.5 cm). Low-volume channel ports (up to 160 µl) conserve antibody and reduce costs. Twenty-four channel ports are conveniently spaced, staggered, and beveled creating an efficient workflow using standard or multi-channel pipettes. Forty-eight targets on a single membrane are possible with Odyssey 2-color detection. The MPX Blotting System is compatible with many single well, precast gels.

FEATURES:

- Screen a single sample and multiple targets on the same blot
- Screen up to 48 targets with the Odyssey System (2-color)
- Conserves antibody and reagents

MousePOD®
Imaging Accessory

- Fits on the Odyssey scanning surface and accommodates up to three mice
- Delivers gas anesthesia to animals via nosecones (external anesthesia system and rotameter not included)
- Regulates air temperature to maintain animal's temperature during scanning
- Includes 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 Module Software also sold separately)

MousePOD® (closed)

odysseyimager.com

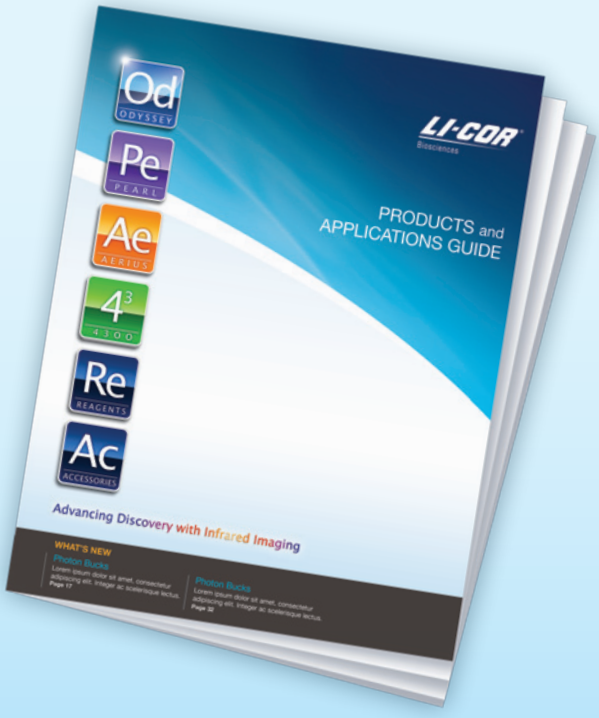
- Published papers referencing Odyssey data
- Posters related to the Odyssey System
- Upcoming and archived webinars
- LI-COR Products and Applications Guide
- Odyssey Protocols
- Odyssey Software Power Users Guide (video training)
- Related Products and Brochures

- Odyssey MousePOD® for *in-vivo* molecular imaging
- In-Cell Western™ Assay Application Overview
- Aerius® Automated Infrared Imaging System for high throughput In-Cell Western™ assays
- Pearl® Impulse Imager - Near-Infrared Animal Imaging System

Products and Applications Guide

Check out the Products and Applications Guide for a list of instruments, reagents and accessories designed for your infrared imaging needs.

Available online at:
www.licor.com/bio



Westerns on the Odyssey System

Better Data, Cleaner Planet

Infrared imaging on the Odyssey System does not require film, excessive use of fresh water, or any of the hazardous chemicals associated with film development.

For more details, visit:
www.licor.com/green

System Specifications

Laser Lifetime:
40,000 working hours typical

700 Channel Laser Source:
Solid-state diode laser at 685 nm

800 Channel Laser Source:
Solid-state diode laser at 785 nm

Detectors:
Silicon avalanche photodiodes

Scanning Speed:
5-40 cm/s

Resolution:
21-337 µm

Focusing:
Scan bed is movable in the Z-dimension, allowing the fluorescence detection microscope to be aligned to the top surface of the glass to obtain the best signal-to-noise ratio

Operating Conditions:
15-35°C and dew point no greater than 20°C

Power Requirements:
Automatic voltage selection at 90-250 VAC and 47-63 Hz; 1.1 Amps at 120 V; 200 watts maximum

Dimensions:
37 h x 53 w x 58 d cm (14.5 x 21 x 23 inches)

Weight:
33 kg (72 lbs)

Data Storage Capacity:
80 GB

Network Protocol:
TCP/IP

Network Connection:
Cat. 5 RJ-45, 10 Base-T/100 Base-TX

Security:
Password protected access

UL/CL approved

Locations WorldWide

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LI-COR®
Biosciences

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

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