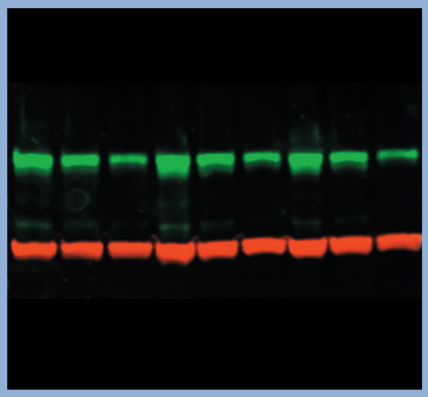
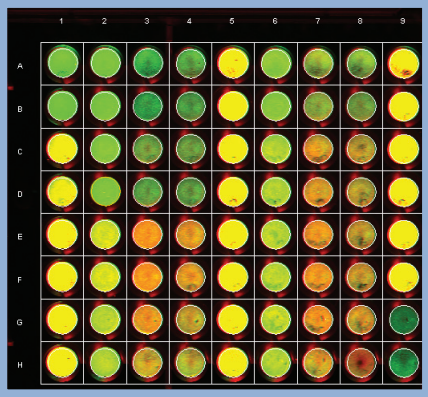
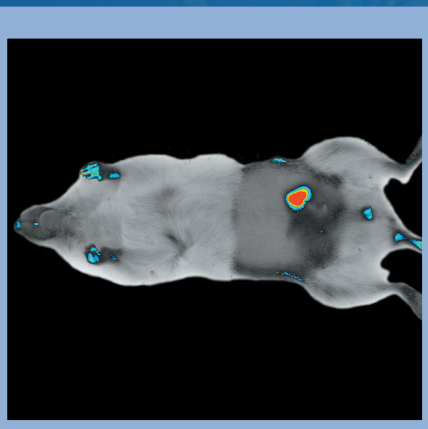
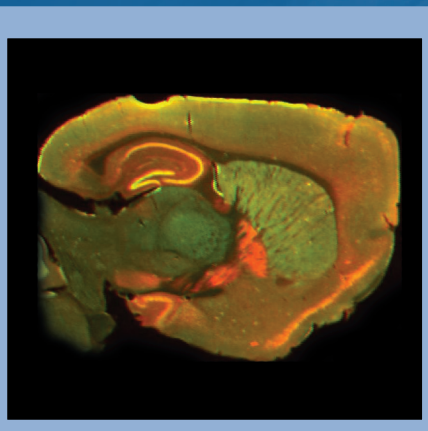








ODYSSEY®

INFRARED IMAGING SYSTEM

LI-COR®
Biosciences

2006
FROST & SULLIVAN
North American Drug Discovery Technologies Product of the Year Award

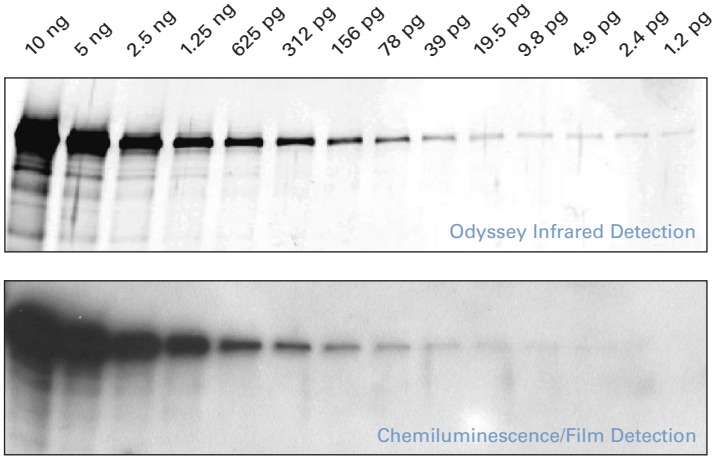
Infrared Fluorescence

A Fundamental Change in Western Blot Analysis

The **Odyssey® Infrared Imaging System** sweeps away the old paradigms of chemiluminescence and visible fluorescence, and introduces a new standard for Western blot analysis – direct infrared fluorescence detection. 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 become clear when imaged with Odyssey.

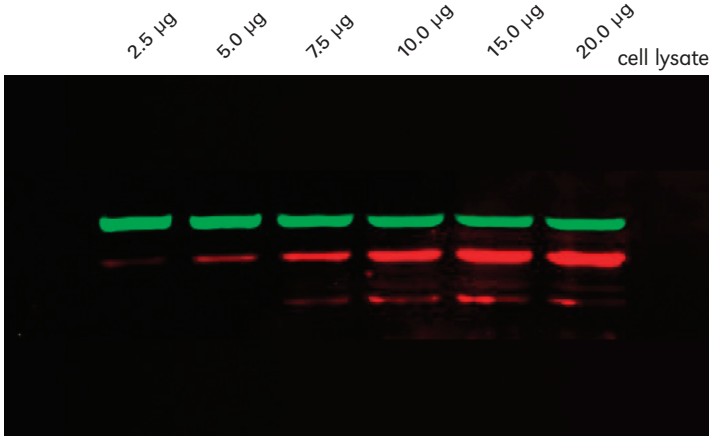
Odyssey is uniquely equipped with two infrared channels for direct fluorescence detection on membranes. With two detection channels, you can probe two separate targets in the same experiment. Quantification accuracy is increased when the second color is used for normalization.

Odyssey 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.



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).

Two Color Simultaneous Detection of Tubulin & IkappaB

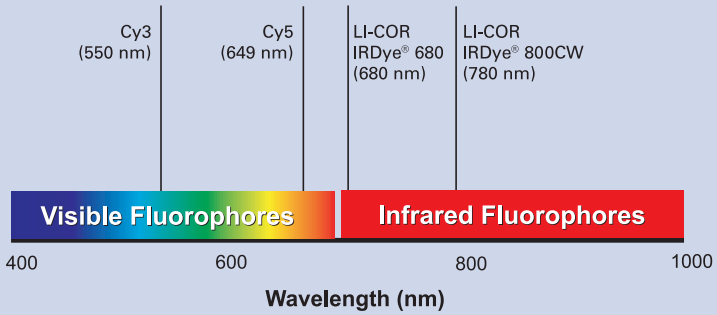


In this pseudocolored image, 700 nm fluorescence (IkappaB) is shown in red and 800 nm fluorescence (Tubulin) is shown in green. The two colors were imaged simultaneously in a single scan.

Data courtesy of Dr. Catrin Albrecht, IUF, Germany

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.

Infrared dyes are the key to this performance advantage, and LI-COR Biosciences is a pioneer in the use of infrared fluorophores for imaging applications. We offer a unique family of IRDye Infrared Dyes synthesized with reactive functional groups that enable easy covalent coupling to antibodies and other biomolecules.

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

Clear Data

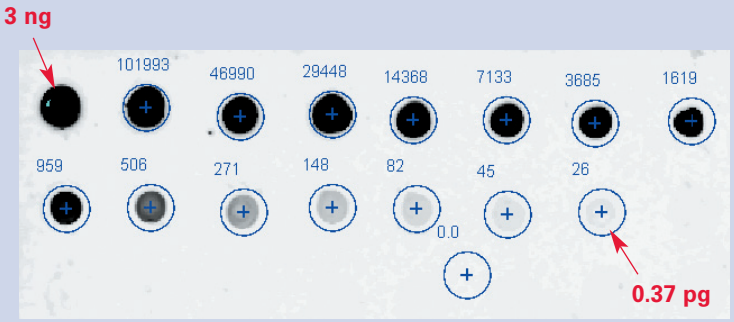
No loss of weak bands due to overexposed bands

Wide Range of Applications

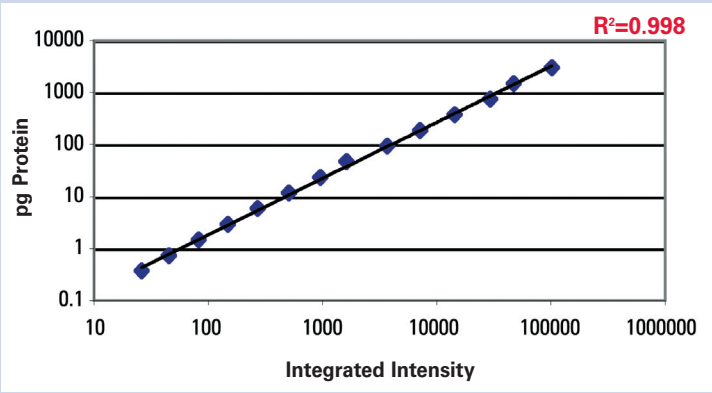
Western blots, Coomassie® stained gels, cell-based assays, fluorescent gel-shift assays, tissue imaging, *in vivo* imaging, protein arrays, and more.

Accurate Quantification

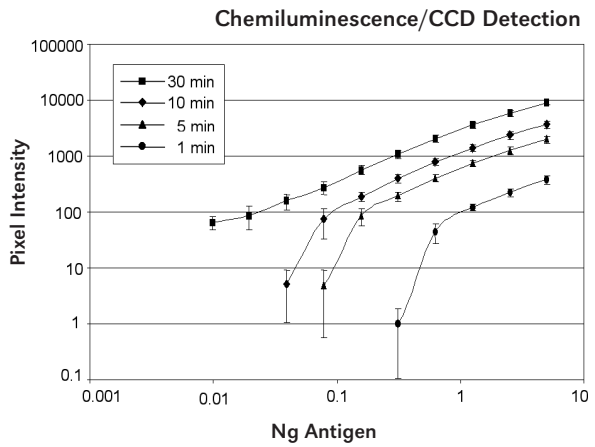
Through its innovative use of fluorescently labeled antibodies rather than enzyme labels, Odyssey provides a broad, linear dynamic range to accurately detect strong and weak bands on the same Western blot. In contrast, the dynamic enzymatic nature of chemiluminescence only allows you to capture a "snapshot" 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 Odyssey detection allow you to be confident about differences you see in protein levels, and your blots can be archived and imaged again months later, if needed.

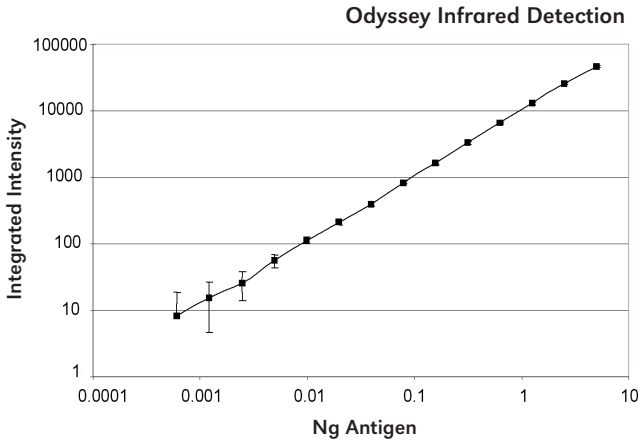


Two-fold serial dilutions of labeled antibody (6 ng to 0.19 pg) were spotted on nitrocellulose and imaged in the 700 nm channel.



Linear Range Comparison

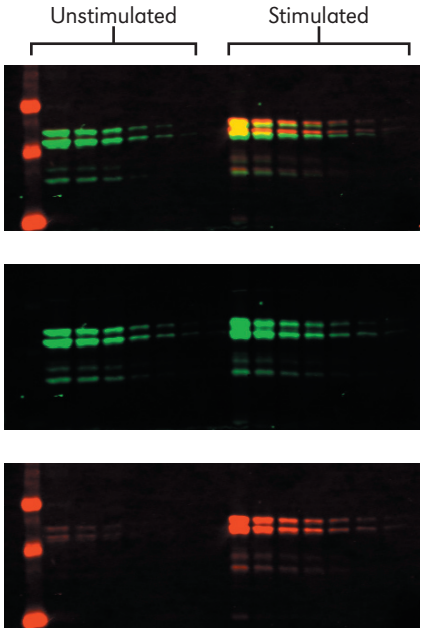




A dot blot assay was used to compare the linear ranges of chemiluminescent and infrared fluorescent detection. Dilutions of mouse antibody were spotted as antigen, and detected with HRP- or infrared-labeled goat anti-mouse antibodies. Chemiluminescent data were collected using ECL substrate and a CCD 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.

Multiplex Detection

Western blot analysis of ERK activation

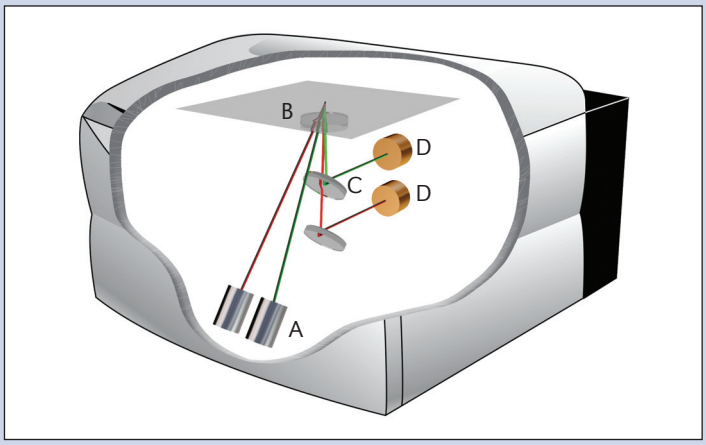


The Odyssey System uses two infrared fluorescent channels for detection, enabling simultaneous two-color target analysis – an advantage that's not available with chemiluminescent or radioactive methods. Two-color Western analysis makes normalization easy and eliminates error introduced by stripping and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

ERK1/2 and phospho-ERK were detected simultaneously in lysates of unstimulated and EGF-stimulated A431 cells. Two fold serial dilutions of lysate are shown. The single-color images can be overlaid 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.

Two Independent 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 Odyssey'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 on focused on separate silicon avalanche photodiodes (D) and detected.

Application Flexibility

Western Detection
Two Color Blots
In-Gel Westerns

Tissue and Tissue Sections

Microwell Assays
ELISA/FLISA
Transcription Factors

Nucleic Acids
EMSA/Gel Shift
DNA Staining

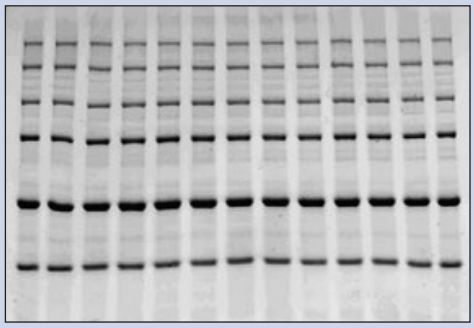
Odyssey
Infrared Imaging System

Cell-Based Assays
In-Cell Westerns™
On-Cell Westerns

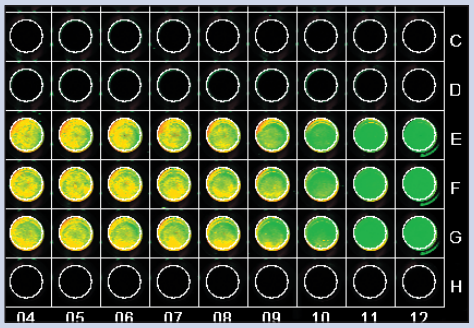
Small Animal Imaging

Protein Detection
1-D/2-D Coomassie
Membrane, Slide Arrays

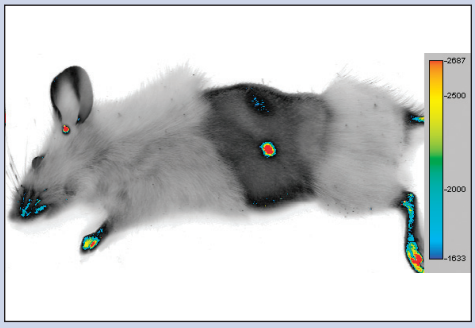
Odyssey provides a flexible, multifunctional platform to accommodate a variety of applications, so you get results faster.



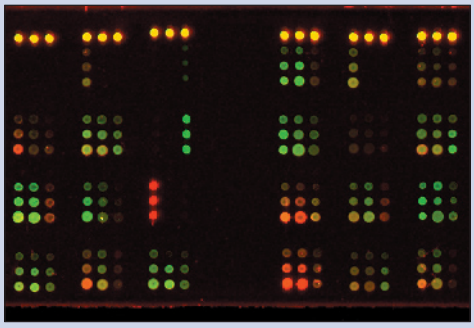
Coomassie-Stained Gels



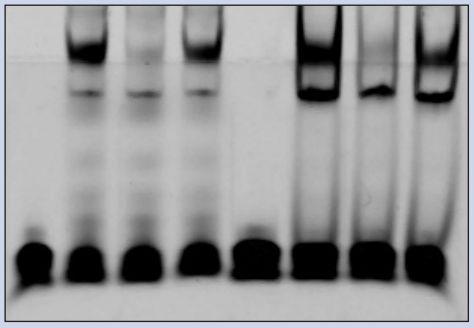
In-Cell Western Assays



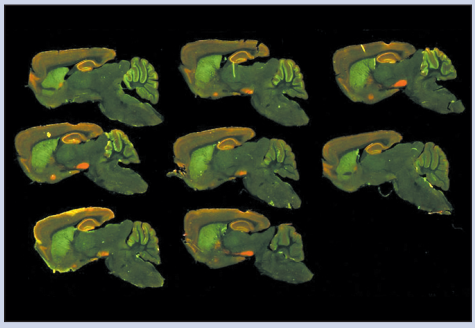
In Vivo Imaging



Protein Arrays



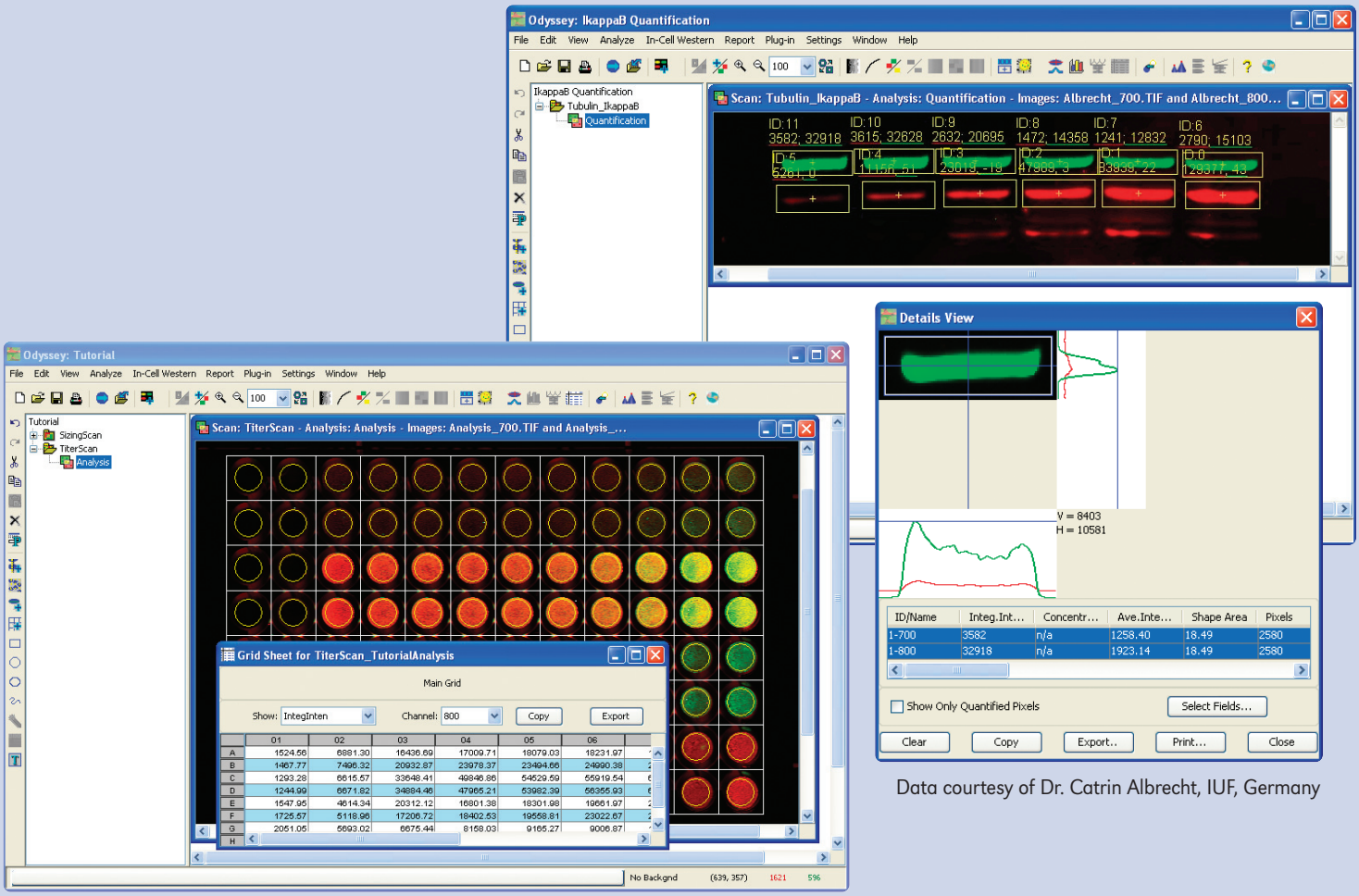
EMSA/Gel Shift Assays



Tissue Section Imaging

Data Courtesy C. Kearn, University of Washington

Scanning and Image Analysis



Data courtesy of Dr. Catrin Albrecht, IUF, Germany

Inside Odyssey, a server dedicated to scan control and data storage completely controls each scan. Important scan parameters like resolution (21 - 337 µm) can be loaded from stored files or changed manually at the beginning of a scan. At the end of a scan, image files are stored on Odyssey's internal hard disk and copied to the computer for analysis. Odyssey can connect directly to your network via TCP/IP, or it can be configured to operate independently.

Two-color scans simultaneously generate two 16-bit grayscale TIFF images. Selectable image display settings let you view the images separately in grayscale or color, or as an overlaid image with both colors displayed. When signals are in the same position on overlaid images, the two colors are combined into a third color for easy identification of overlap.

Odyssey software uses a variety of familiar tools for quick and easy band sizing and quantification. Flexible lane tools with automatic band finding make it easy to analyze gel images with bands in lanes. Smiles are quickly and accurately corrected. Array and microplate images are rapidly analyzed using grid templates that add arrays of circles or squares to define the areas to be quantified. Sizing and quantification data can be viewed in data tables, output in a formatted report, or stored in a data file for further analysis.

Odyssey Specifications

Laser Lifetime: 40,000 hours typical

700 Channel Laser Source: Solid-state diode laser at 680 nm

800 Channel Laser Source: Solid-state diode laser at 780 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

LI-COR® Biosciences

4647 Superior St. • P.O. Box 4000 • Lincoln, Nebraska 68504 USA
North America: 800-645-4267 • International: 402-467-0700 • FAX: 402-467-0819
LI-COR GmbH (Germany, Austria, Switzerland, Czech Republic, Hungary, and Slovakia):
+49 (0) 6172 17 17 771 • LI-COR UK Ltd.: +44 (0) 1223 422104 • www.licor.com

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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