ThermoFisher SCIENTIFIC

WESTERN DETECTION

Stunningly easy western blot and gel imaging

iBright Imaging Systems

invitrogen

State-of-the-art western blot and gel imaging systems

Invitrogen™ iBright™ Imaging Systems have powerful features that help make imaging and analyzing western blots and gels easy. The high-resolution, 9.1-megapixel (MP) camera and suite of automated features can help you produce publication-quality data fast. The touchscreen interface is crafted to provide a smooth image capture experience. Our onboard software and standalone Invitrogen™ iBright™ Analysis Software are designed to streamline image analysis. Read on to see how you can empower your lab with an iBright Imaging System.

Watch now:
iBright Imaging Systems overview »

iBright Imaging Systems

Invitrogen™ iBright™ CL750 Imaging System

Essential western blot and gel imaging functions to efficiently transition from the darkroom and film

Invitrogen™ iBright™ CL1500 Imaging System

Expanded application support with many of the same high-performance specifications as the premier iBright FL1500 model

Invitrogen™ iBright™ FL1500 Imaging System

Maximum application support, including fluorescent western blot imaging with up to four fluorescence channels at a time

Smart Exposure™ automated exposure technology

High-resolution 9.1 MP camera with Smart Range™ HDR technology

Cloud connectivity via the Thermo Fisher™ Connect Platform

12.1-inch LCD touchscreen interface

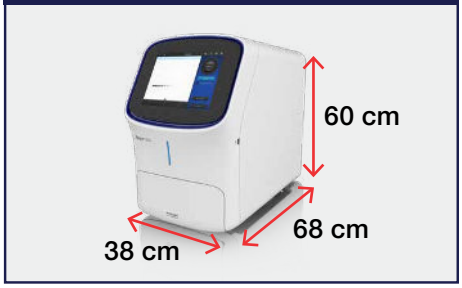
USB port

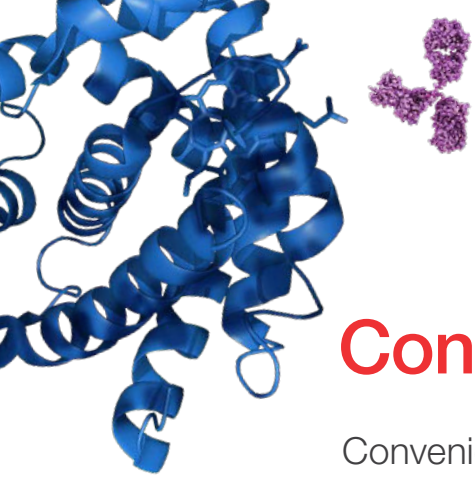
Ethernet (Wi-Fi dongle available)

Green LED-powered transilluminator

Large 18 cm x 22.5 cm imaging area

Benchtop footprint





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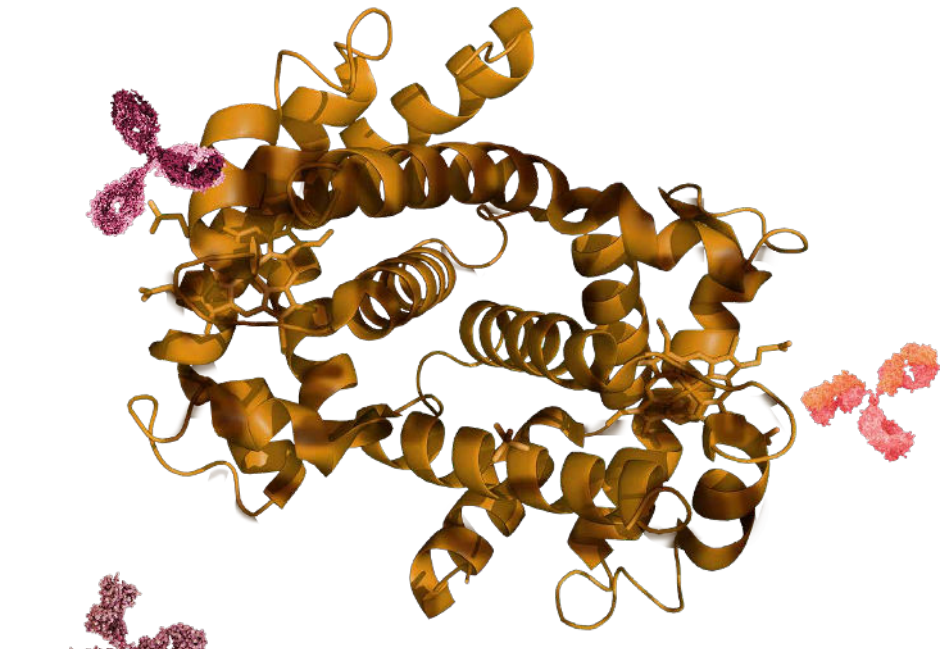


Convenient, intuitive operation and workflows

Feel at home with our touchscreen interface

The 12.1-inch capacitive LCD screens on iBright Imaging Systems respond like the high-quality touchscreens on your other devices. The interface layout is simple and easy to learn (Figure 1). Workflows for different imaging modes are similar, for a smooth imaging experience regardless of sample type.

Zoom in

Zoom out

Pan

Swipe

Mode selection: use dropdown menu to select Chemi Blots, Fluorescent Blots, Nucleic Acid Gels, Protein Gels, or Universal mode.

Camera lock: lock exposure time, zoom, and disable mechanical autorotation across multiple samples.

Help: onboard help and information.

Gallery: access previously captured images.

Settings: access general system settings, configurations, and service tools.

Drawer: open or close the sample drawer.

Sign in: create a new user account or sign in to an existing account.

Exposure dial: enter desired exposure time or turn the dial to manually change exposure time.

Color option: select color or grayscale image view.

Capture: acquire images using exposure time set by the user or the imaging system.

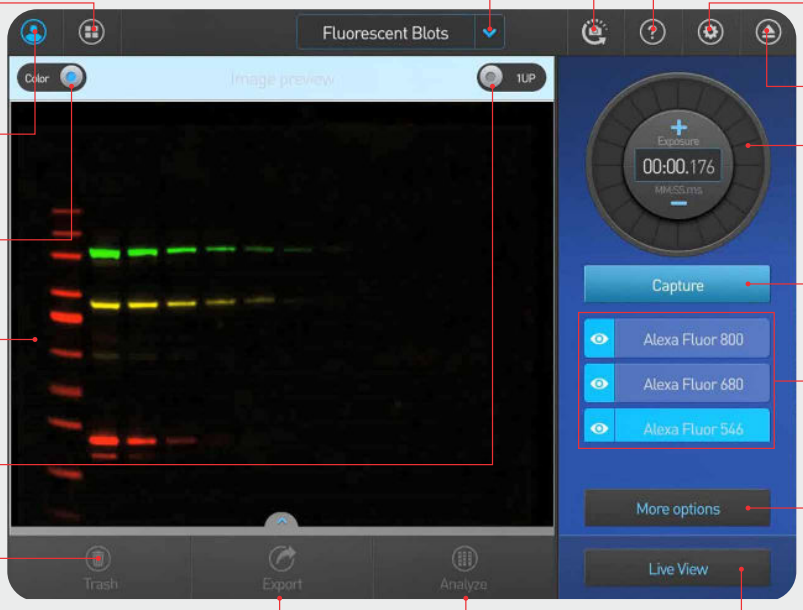
Image view window: display selected image in the window for viewing and interaction.

Edit channels: review and edit each channel for multichannel captures.

Split screen: select one-window or two-window image view.

More options: open image adjustment options.

Trash: delete current images.



Export: edit image information and export current images to destination of choice.

Analyze: initiate analysis workflow using current images.

Live view: go back to live sample view.

Figure 1. View in Fluorescent Blots mode on the iBright FL1500 Imaging System.

Powerful camera and automated technologies

Get publication-quality data fast

Capture crisp, clear, publication-quality images with the 9.1 MP cooled CCD camera. Take full advantage of the high-resolution camera or bin (combine) pixels to increase sensitivity if desired. Each image capture mode of iBright Imaging Systems has a default binning setting that balances resolution, sensitivity, and image capture speed. Binning can also be adjusted, which provides flexibility. Note that there is a trade-off between image resolution, sensitivity, and image capture speed when binning is adjusted. Take the guesswork out of capturing the optimal image with our suite of automated features and algorithms.

Watch now: Capture images fast with Smart Exposure technology »

Smart Exposure autoexposure technology

Smart Exposure technology rapidly determines the optimal exposure time, and helps minimize the risk of overexposure or underexposure, by maximizing pixel intensity while avoiding saturation (Figure 2).

- Available with all models in all five detection modes.
- The optimal exposure time for each channel is determined separately in Fluorescent Blots and Universal modes.
- Smart Exposure technology can also be applied to a specific region of a gel or blot. This can be useful when samples contain undesired artifacts that generate nonspecific signals or when certain lanes are not of interest.

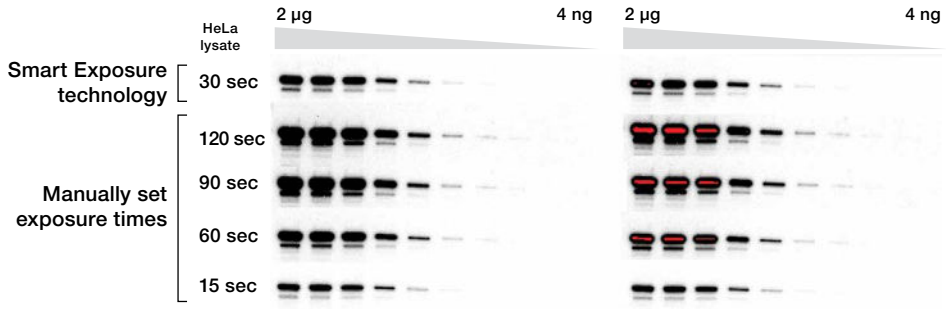


Figure 2. Minimal pixel saturation is observed when an image is captured with an exposure time determined by the algorithm used by Smart Exposure technology, and the range of data capture is maximized. The same blot was imaged with an exposure time determined by the algorithm used by Smart Exposure technology and four manually set exposure times. The images on the right were captured with the saturated pixels feature turned on. The saturated pixels are shown in red.

Smart Range HDR technology

Smart Range™ HDR (high dynamic range) technology can help maximize the linear dynamic range of chemiluminescent western blot images when sample proteins have widely varying expression levels. This feature leverages two different exposures for the same sample—a short exposure for imaging medium- to high-abundance proteins and a long exposure for imaging low-abundance proteins. The two images are then combined into a single 16-bit HDR image that captures high-, mid-, and low-intensity signals. Smart Range HDR technology effectively extends the linear dynamic range beyond what is possible with a single short or long exposure (Figure 3).

White paper: Introducing Smart Range HDR technology

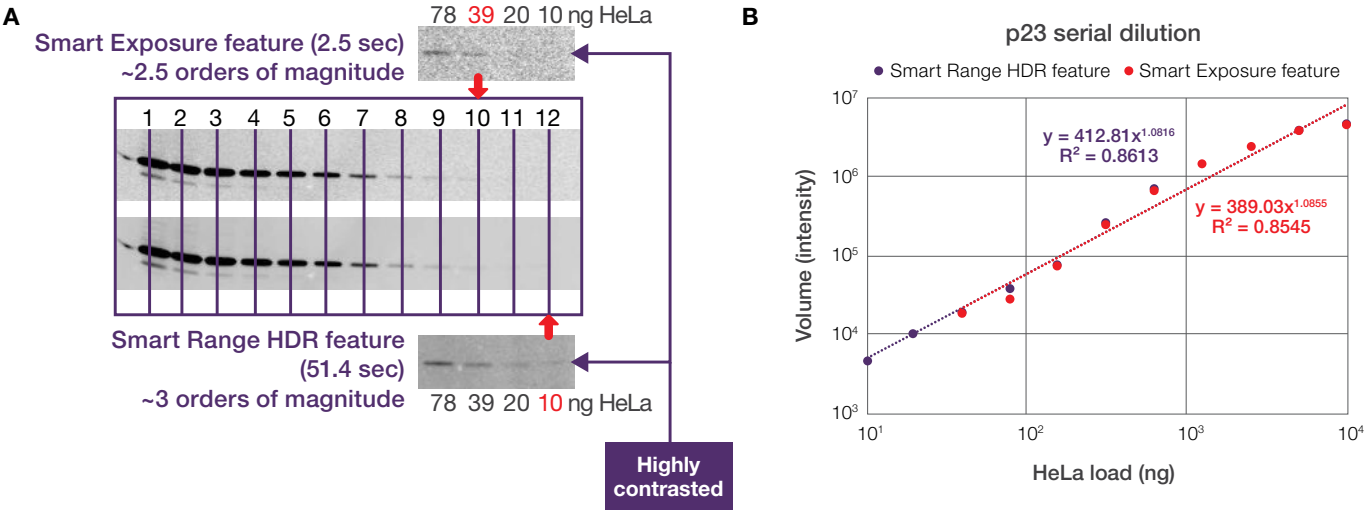


Figure 3. The Smart Range HDR feature improves the detection limit of p23 by 4-fold when compared to the Smart Exposure feature. HeLa cell lysate was serially diluted 1:1 in sample buffer from 20 µg to 10 ng, prepared for SDS-PAGE, and electrophoresed on an Invitrogen™ Novex™ WedgeWell™ 4–20% Tris-glycine gel. The protein was transferred to a nitrocellulose membrane and probed for p23. The resulting western blot was imaged on an iBright Imaging System using the Smart Exposure and Smart Range HDR features. Images were compared by **(A)** visual assessment and **(B)** how many data points fell within the linear dynamic range. The 20 µg data point was omitted from the graph because it fell outside the linear range in both the Smart Range HDR and Smart Exposure images.

Automatic sample rotation

Rather than having to open the sample drawer and repeatedly reposition your sample to achieve proper alignment, iBright CL1500 and FL1500 Imaging Systems automatically determine the sample position and can mechanically rotate the sample stage left or right by up to 10° (Figure 4). Mechanical rotation eliminates the need to digitally rotate the sample. This preserves the integrity of the data, as digital rotation can lead to data alteration.

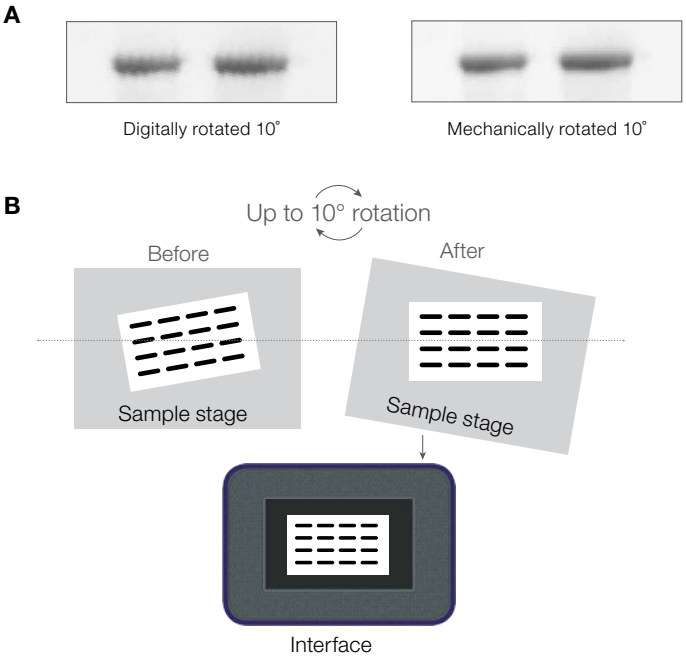


Figure 4. Digital rotation vs. mechanical rotation. (A) Pixels rotate with digital rotation, so bands appear jagged. With mechanical rotation, the sample itself rotates. Bands appear smooth because the pixels are aligned. **(B)** Depiction of the sample stage of an iBright Imaging System before and after rotation.

Autozoom and autofocus

iBright Imaging Systems automatically determine whether a sample requires zooming to fully utilize the 22.5 cm x 18.0 cm field of view. If imaging a single blot or gel, the camera will automatically zoom toward the sample up to 2x. Zooming 1–2x is done mechanically on the iBright CL1500 and FL1500 Imaging Systems and digitally on the iBright CL750 Imaging System (Figure 5). Mechanical zooming maximizes sensitivity by moving the camera closer to the sample stage, which reduces the focal length.

- The iBright CL1500 and FL1500 systems allow zooming up to 8x (1–2x mechanical zooming and 1–4x digital zooming).
- The iBright CL750 system offers 1–2x digital zooming.
- The focus is automatically adjusted at each zoom level.

1x (no zoom): The area of the field of view is 22.5 cm x 18.0 cm. This is ideal with four mini-gels or mini-blots for high-throughput imaging.

2x zoom: The field of view accommodates one mini- or midi-gel or blot.

4–8x zoom: Bands can be seen with fine detail. This may be useful for visualizing bands with molecular weights that are close together.

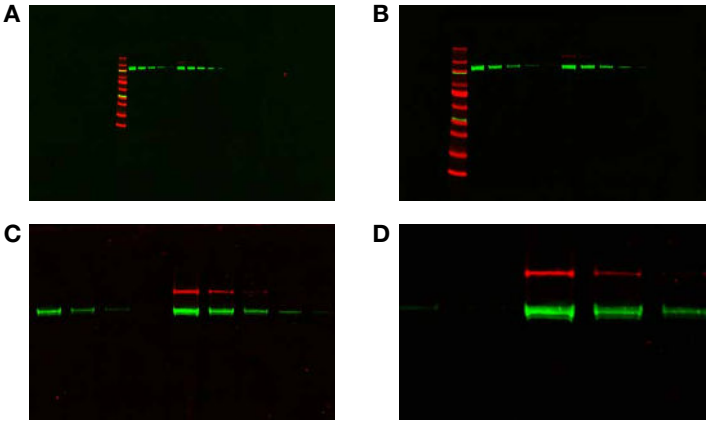


Figure 5. Zoom function. (A) Image of a fluorescent western blot without zooming. Blot zoomed **(B)** 2x, **(C)** 4x, and **(D)** 8x. The blot was not repositioned between successive zooms.

Essential imaging modes and applications

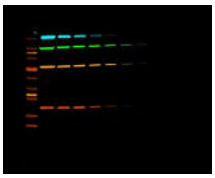
The core applications you need and the specialty applications you want

iBright Imaging Systems have up to five imaging modes to support multiple applications. Efficiently and easily capture data from protein gels, nucleic acid gels, chemiluminescent western blots, fluorescent western blots, and more. A visible image of the gel or membrane is automatically captured in each imaging mode, which can simplify image analysis workflows like molecular weight analysis.

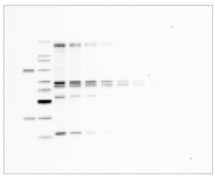
Table 1. Image capture modes on iBright Imaging Systems.

Imaging mode	What types of signals can be captured?
Protein Gels	Colorimetric signals from stains like Thermo Scientific™ Pierce™ Silver Stain or Invitrogen™ SimplyBlue™ SafeStain (gels) and Thermo Scientific™ Pierce™ Reversible Protein Stain or Ponceau S Staining Solution (membranes); fluorescence signals from stains like Invitrogen™ SYPRO™ Ruby Protein Gel Stain (gels).
Nucleic Acid Gels	Signals from ethidium bromide and a variety of fluorescent nucleic acid stains like Invitrogen™ SYBR™ stains.
Chemi Blots	Chemiluminescence from horseradish peroxidase (HRP) and alkaline phosphatase (AP) substrates like Thermo Scientific™ SuperSignal™ and Invitrogen™ WesternBreeze™ substrates.
Fluorescent Blots	Fluorescence from visible and near-infrared (NIR) fluorophores like Invitrogen™ Alexa Fluor™ and Alexa Fluor™ Plus conjugates.
Universal	Custom mode to image with multiple signals (chemiluminescence, fluorescence, colorimetric signals, and/or visible signals). Image display is similar to display in fluorescent blot mode and allows false colors to be assigned to any sample.

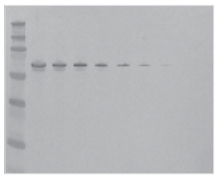
Core imaging applications



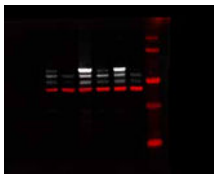
Fluorescent western blots



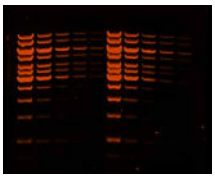
Chemiluminescent western blots



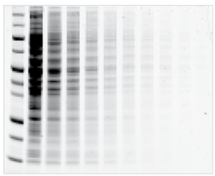
Colorimetrically stained western blots



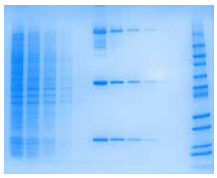
Combined fluorescent and chemiluminescent western blots



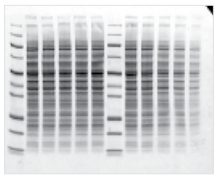
Fluorescently stained nucleic acid gels



Fluorescently stained protein gels

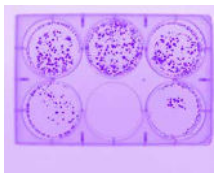


Colorimetrically stained protein gels

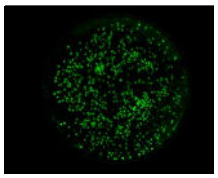


Colorimetrically stained membranes

Specialty plate–based imaging applications



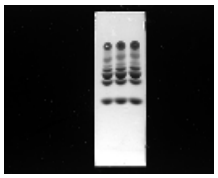
Visible colonies (e.g., stained with crystal violet dye)



Fluorescent colonies (e.g., GFP expression)

Qualitative visible imaging* applications

Leaf sections



Thin layer chromatography (TLC) plates

Figure 6. Examples of imaging applications. The data were captured in grayscale. Pseudocolor (false color) can be applied for visualization purposes.

* Enables qualitative visualization of samples and signal confirmation. Not recommended for quantitation.

Colony counting

To maximize your investment, capabilities to image multiple cell culture plates have been added to iBright Imaging Systems running firmware version 1.8* or greater. One can choose to image blue, white, chemiluminescent, fluorescent, and crystal violet–stained colonies on single- or multi-well cell culture plates (Figure 7).

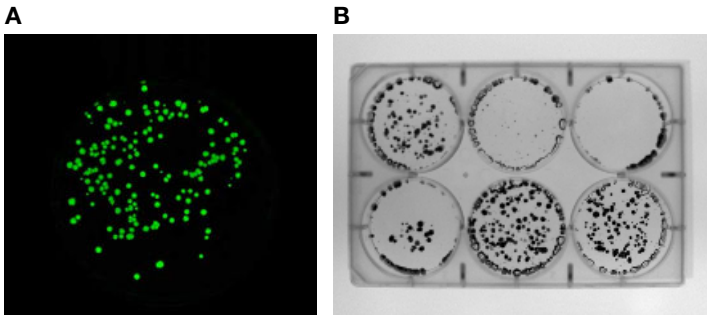


Figure 7. Single- and multi-well plates containing colonies imaged on an iBright Imaging System. (A) Colonies expressing GFP. **(B)** Colonies stained with crystal violet dye.

In addition to colony plate imaging capability, colony count analysis is available for added convenience. Colony counting can be performed on instrument, or it can be performed off instrument leveraging iBright Analysis Software.

Wells and colonies can be detected automatically with our detection algorithm, which greatly streamlines the otherwise tedious manual process (Figure 8). You can fine-tune gating parameters for colony size, average pixel intensity, and circularity. For documentation purposes, the analytical output can be exported in a complete report.

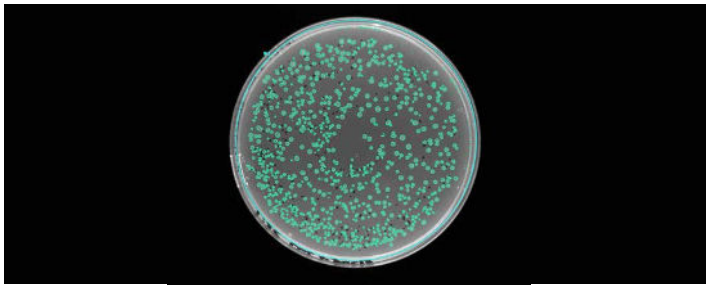
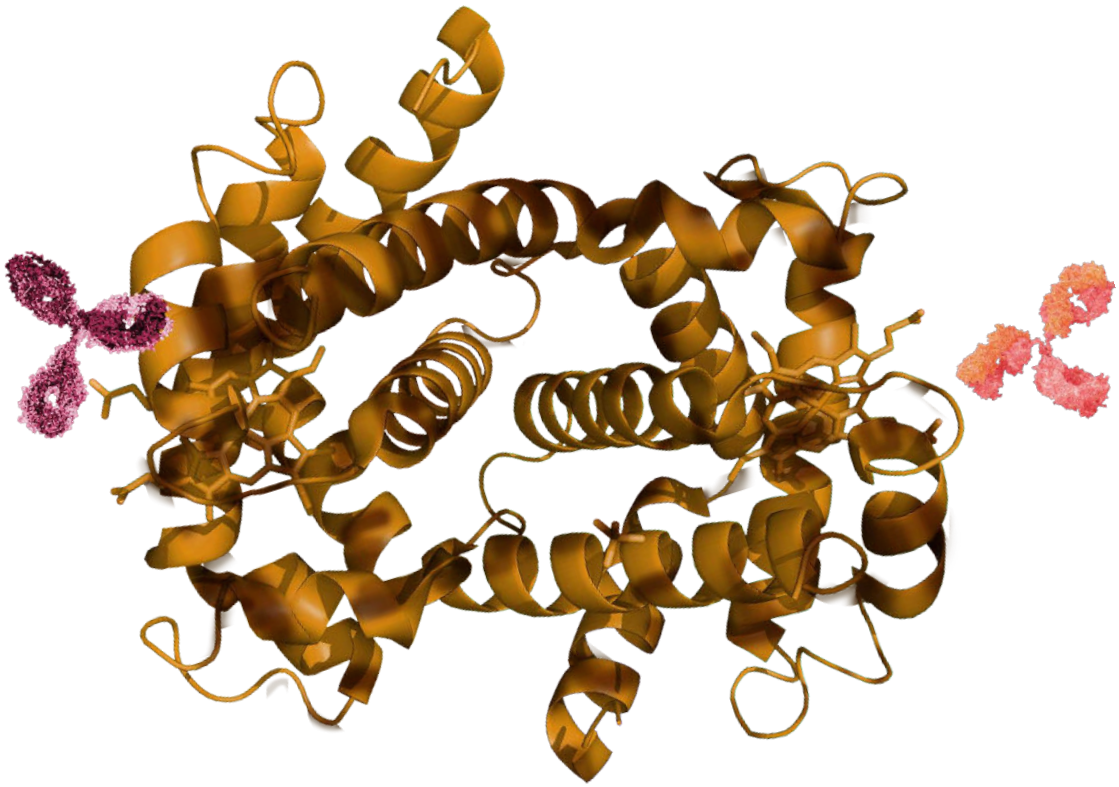


Figure 8. Colony identification using the colony counting workflow. Absence of β-galactosidase in transformed competent cells is indicated by the white colonies circled in green.

* iBright Imaging Systems Firmware 1.8 was released on November 15, 2022.

[White paper: Colony counting with iBright Imaging Systems]



E-Gel imaging and deconvolution analysis

DNA electrophoresis can be performed with Invitrogen™ E-Gel™ precast agarose gels in less than 30 minutes, and no gel preparation or liquid buffers are required. Invitrogen™ iBright™ Tray Adapters For E-Gel™ Agarose Gels have been designed for imaging and analyzing standard Invitrogen™ E-Gel™ 11-well agarose gels, E-Gel™ EX Double Comb 22-well agarose gels, E-Gel™ 48-well agarose gels, and E-Gel™ 96-well agarose gels on iBright Imaging Systems.

iBright Tray Adapters are two-component accessories (Figure 9). The bottom component centers the E-Gel cassette over the iBright transilluminator. The window in the bottom component allows light from the transilluminator to pass through the gel and enables precise and consistent zooming. The top component of the tray adapter covers the barcode and label on the E-Gel cassette. If they are not covered, the barcode and label can emit fluorescence that will interfere with the algorithm of the Smart Exposure technology used by iBright Imaging Systems (Figure 10).

A specialized deconvolution workflow has been developed to support the analysis of high-throughput E-Gel 96-well agarose gels. Deconvolution separates the lane-to-lane data for easier interpretation of results (Figure 11).

Figure 9. iBright Tray Adapters. (A) An adapter for E-Gel 11/22-well agarose gels and **(B)** E-Gel 48/96-well agarose gels. **(C)** Placement of a 48/96-well tray adapter on the iBright transilluminator.

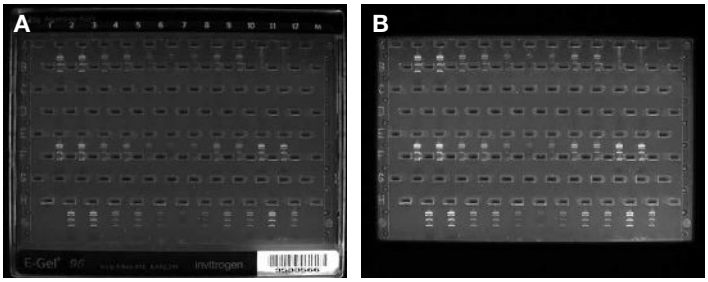


Figure 10. Imaging with iBright Tray Adapters helps prevent fluorescence emitted by the E-Gel cassette barcode and label from interfering with the algorithm used by Smart Exposure technology. Images of an E-Gel 96-well agarose gel captured **(A)** without and **(B)** with an iBright Tray Adapter.

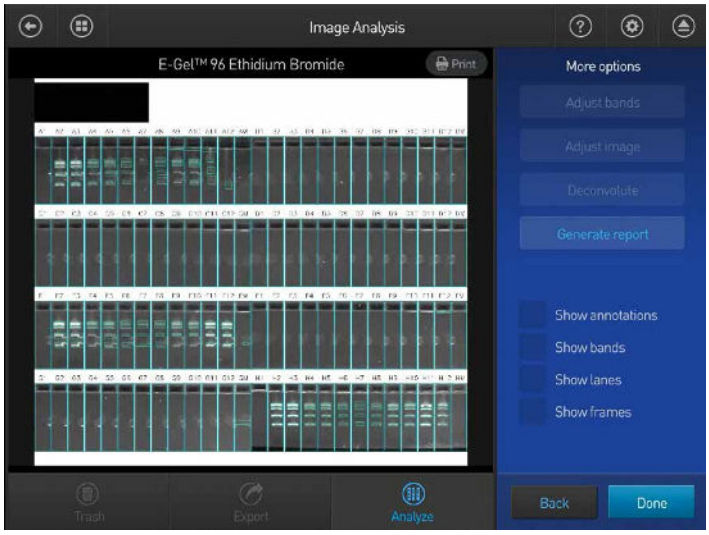


Figure 11. Image of an E-Gel 96-well agarose gel after data deconvolution.

[White paper: Optimized gel imaging and analysis with iBright Tray Adapters for E-Gel Agarose Gels]

Accelerate your work with multiplex fluorescence

Expand the possibilities to get more data from each experiment

Multiplexing helps make research more efficient and productive. You can visualize the signal from a protein of interest and the signal from a loading control protein simultaneously (Figure 12), evaluate complex biological pathways (Figure 13), and differentiate proteins with similar molecular weights (Figure 14). With five fluorescence channels on the iBright FL1500 Imaging System (Table 2), researchers can multiplex with up to four fluorophores that emit in the visible and NIR ranges (Figure 15). Smart Exposure technology can further enhance acquisition of western blot multiplex fluorescence data, because the exposure time is optimized separately for each fluorescence channel.

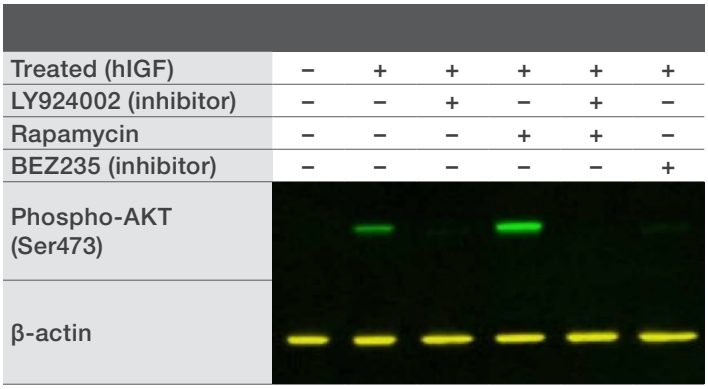
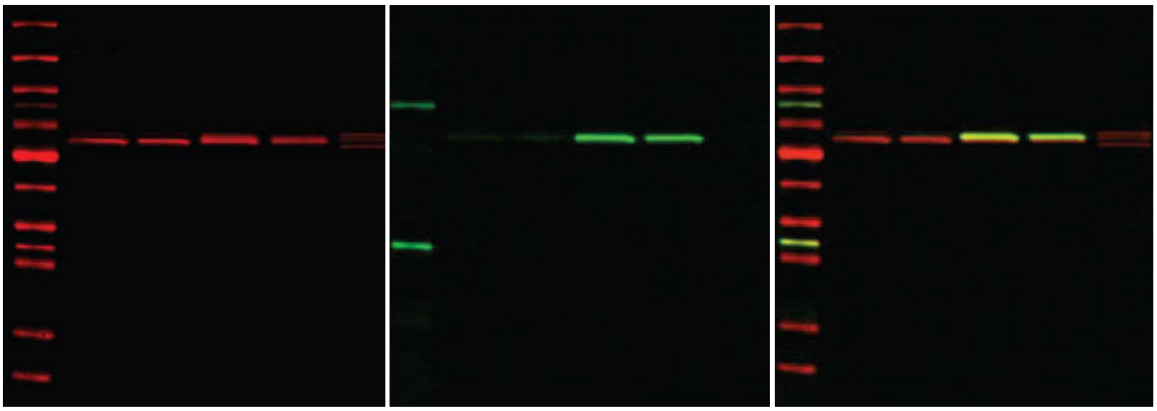


Figure 12. With fluorescence multiplexing, the signal of each protein is captured in different fluorescence channels, which enables detection of two or more proteins on the same blot without stripping and reprobing. Human colon cancer cells (HCT116) were serum-starved for 24 hours and pretreated with LY294002 (50 µM, 1 hr), rapamycin (10 nM, 1 hr) and/or BEZ235 (500 nM, 1 hr). Following pretreatment, insulin-like growth factor-1 (hIGF-1) was added to each sample (12.8 nM, 15 min). Cells were lysed and prepared for reducing SDS-PAGE and 20 µg of each sample was electrophoresed on a Novex 4-20% Tris-Glycine Gel, WedgeWell format. The protein was transferred to a PVDF membrane and the resulting blot was blocked with Thermo Scientific™ Blocker™ FL buffer and then probed with the following primary antibodies overnight: anti-pAKT rabbit mAb and anti-β-actin mouse mAb. The blot was washed and probed with the following secondary antibodies for one hour: Invitrogen™ Goat Anti–Rabbit IgG–Alexa Fluor™ Plus 546 and Goat Anti–Mouse IgG–Alexa Fluor™ Plus 800 conjugates. The blot was then washed and imaged on the iBright FL1500 imager using appropriate settings. The composite image shows the overlayed channels from each protein (p-AKT: Alexa Fluor Plus 800 conjugate and β-actin: Alexa Fluor Plus 546 conjugate).

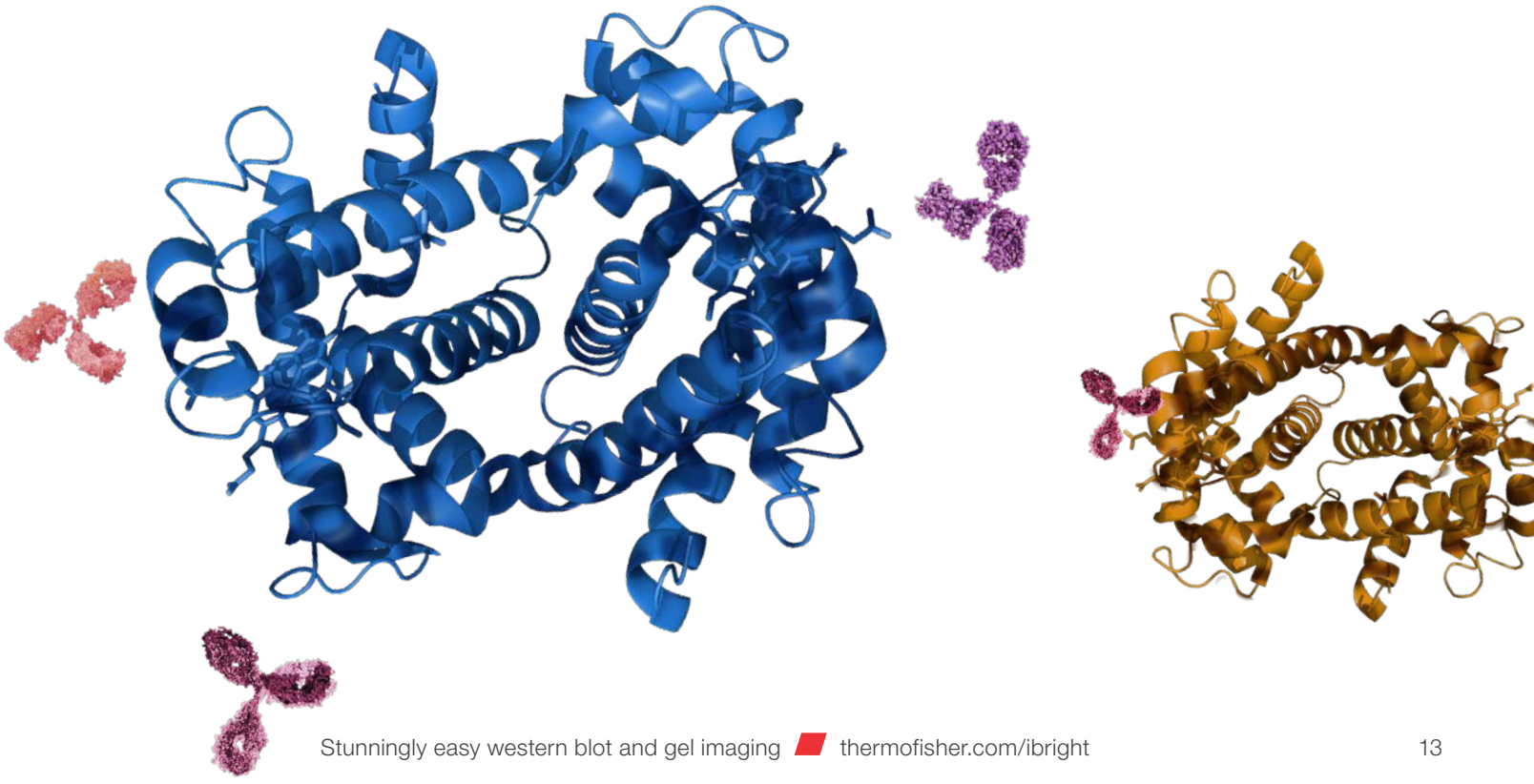
Figure 13. Leverage multiplexing to use western blotting as a tool to study complex biological pathways. Western blot analysis demonstrates the phosphorylation of AKT and GSK-3αβ after IGF-1 treatment of human colon cancer cells (HCT116). HCT116 cells were serum-starved for 24 hours and pretreated with the PI3K pathway inhibitor LY294002 (50 µM, 1 hr). Following pretreatment, insulin-like growth factor-1 (hIGF-1) was added to each sample (12.8 nM, 15 min) to activate the PI3K pathway. Cells were lysed and prepared for reducing SDS-PAGE and 30 µg of each sample was electrophoresed on a Novex 4-20% Tris-Glycine Gel, WedgeWell format. The protein was transferred to a PVDF membrane and the resulting blot was blocked with Blocker FL buffer. Blot 1 was probed overnight with primary antibodies anti-pAKT rabbit mAb and anti-AKT mouse mAb. Blot 1 was washed and probed with Invitrogen™ Goat Anti-Rabbit Alexa Fluor™ Plus 800 and Goat Anti-Mouse Alexa Fluor™ Plus 647 conjugates. Blot 1 was washed again and probed with Invitrogen™ anti-β-actin mAb DyLight™ 488 conjugate for one hour. Blot 1 was washed and imaged on the iBright FL1500 imager using appropriate settings. Blot 2 was probed overnight with primary antibodies anti-p-GSK-3αβ rabbit mAb and anti-GSK-3αβ mouse mAb. Blot 2 was washed and probed with Invitrogen™ Goat Anti–Rabbit IgG–Alexa Fluor™ Plus 647 and Goat Anti–Mouse IgG–Alexa Fluor Plus 800 conjugates. Blot 2 was washed and imaged on the iBright FL1500 imager using appropriate settings.

Sample		NIH-3T3				HeLa		NIH-3T3				HeLa		NIH-3T3				HeLa
Load (µg)		20	10	20	10	10		20	10	20	10	10		20	10	20	10	10
PDGF		–	–	+	+	–		–	–	+	+	–		–	–	+	+	–

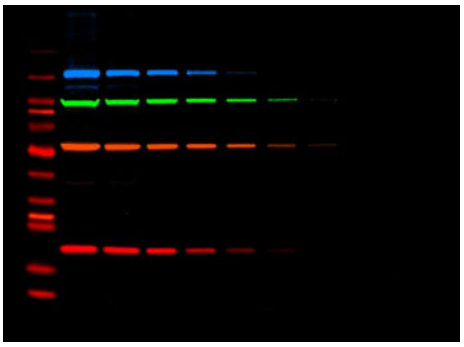


AKT Phospho-AKT Composite

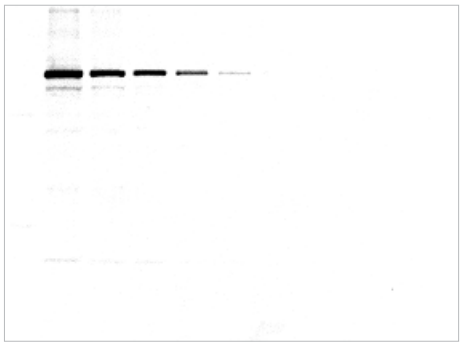
Figure 14. Fluorescent multiplexing allows for clear distinction of multiple targets on the same blot, even when they have similar molecular weights. A composite image is shown along with images showing the single-color signals of individual proteins. Visualizing the individual signals can enable assessment of details that may be harder to see in a composite. PDGF was used to induce phosphorylation of AKT (Ser 473) in mouse embryo fibroblast cells (NIH-3T3). The cells were lysed and prepared for reducing SDS-PAGE and electrophoresed on a Novex 4-20% Tris-Glycine Gel, WedgeWell format. The proteins were transferred to a PVDF membrane and the resulting blot was blocked with Blocker FL buffer and probed with the following primary antibodies overnight: anti-pAKT rabbit mAb and anti-AKT mouse mAb. The blot was washed and probed with the following secondary antibodies for one hour: Goat Anti–Rabbit IgG–Alexa Fluor Plus 647 and Goat Anti–Mouse IgG–Alexa Fluor Plus 800 conjugates. The blot was washed and imaged on the iBright FL1500 imager using appropriate settings.



Multiplex with up to four fluorophores and simultaneously capture a membrane image



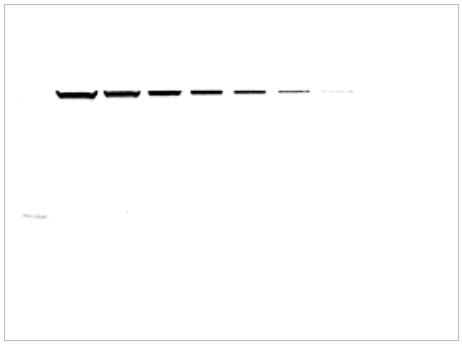
Four-color fluorescent blot



Alexa Fluor Plus 488 dye—RB1 protein with GST and HA tag (134 kDa)

Alexa Fluor 546 dye—calreticulin protein (55 kDa)

Alexa Fluor Plus 680 dye—p23 protein (23 kDa)



Alexa Fluor Plus 800 dye—HSP90 protein (90 kDa)

Membrane—iBright prestained marker

Figure 15. Four-color multiplex fluorescent blot. A false-color composite is shown at upper left. Individual channels are shown in grayscale.

Table 2. Filter sets for the iBright FL1500 Imaging System.

Excitation channel	Filter range (nm)	Emission channel	Filter range (nm)	Compatible fluorophores
EX1	455–485	EM1	508–557	Alexa Fluor 488, Alexa Fluor Plus 488 dyes
EX2	515–545	EM2	568–617	Alexa Fluor 546, Alexa Fluor Plus 555 dyes
EX3	608–632	EM3	675–720	Alexa Fluor 647, Alexa Fluor Plus 647 dyes
EX4	610–660	EM4	710–730	Alexa Fluor 680, Alexa Fluor Plus 680 dyes
EX5	745–765	EM5	800–850	Alexa Fluor 790, Alexa Fluor Plus 800 dyes
EX (Green Trans)	490–520	EM2	568–617	Ethidium bromide, SYBR Safe, SYBR Gold, SYPRO Ruby, SYPRO Orange dyes

Note: Do not use the EX3/EM3 and EX4/EM4 channels in the same multiplex experiment. There will be significant spectral overlap of the dyes that would be captured in these channels.

Streamlined image analysis

Complete the image acquisition experience with easy on-instrument data analysis and iBright Analysis Software

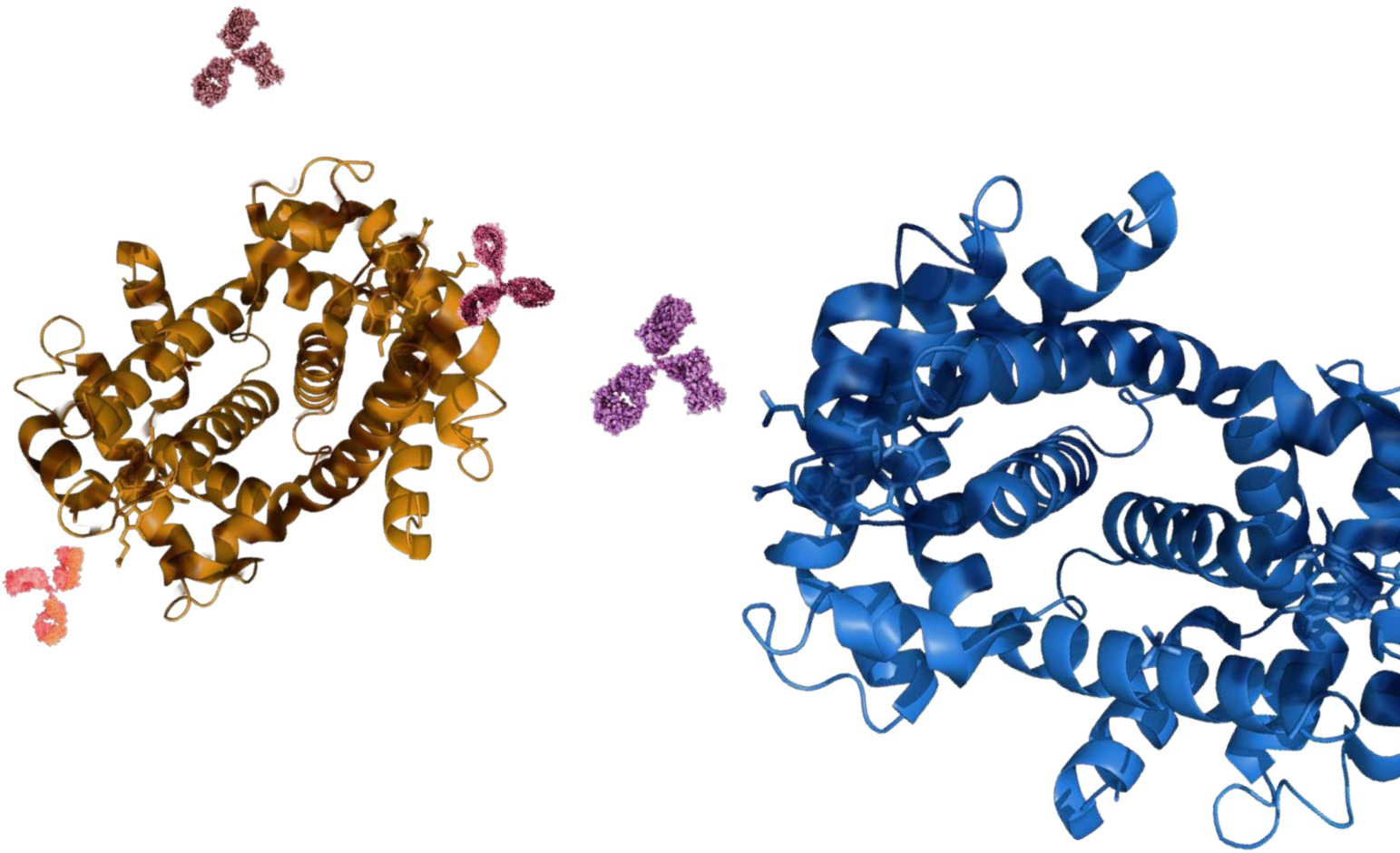
iBright Analysis Software is free software for organizing and analyzing images captured on iBright Imaging Systems. iBright Analysis Software is designed to build on the analysis functions that can be performed with the instrument's onboard software with the convenience of multiple software formats for different preferences and needs.

Standard iBright Analysis Software is available in a desktop version that is compatible with Windows™ and macOS™ operating systems. A cloud-based version can also be accessed on the Thermo Fisher Connect Platform. We also offer a third version of iBright Analysis Software called iBright Analysis Software—Secure. It is adapted from the desktop version and supports 21 CFR Part 11 compliance as part of our 21 CFR Part 11 compliance support package.

Whether making a simple adjustment for a presentation or comparing subtle differences between important samples, our software has you covered. We regularly update our software to add new features and continuously improve the user experience.

Watch now: Analyzing images on iBright Imaging Systems »

For details about the Thermo Fisher Connect Platform and data security, go to **thermofisher.com/cloudsecurity**



iBright Analysis Software workspaces

Manage your images with ease

Sort images in the gallery by date, month, name, mode, size, or recent use to make your data readily retrievable and to stay organized. The image preview can be adjusted, so you can scan through closely related data without opening each individual image (Figure 16).

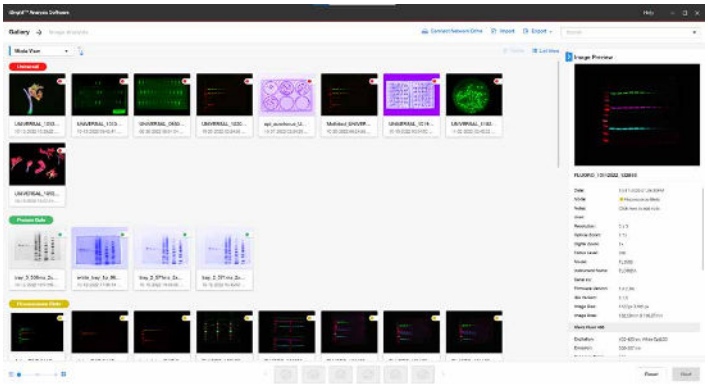


Figure 16. Gallery workspace in iBright Analysis Software.

Annotate for a more complete picture

Annotate images to add necessary experimental details for visualization purposes (Figure 18). Annotations will be shown in magenta in the viewport. All annotations added using the annotation tools and lane labels added using the annotation table will be included in exported images. All annotations are also included in image reports.

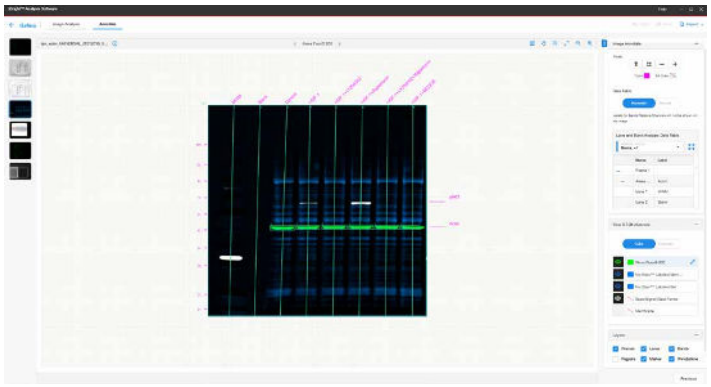


Figure 18. Annotation workspace in iBright Analysis Software. Lane labels were added in a data table. The remaining labels were added using annotation tools.

Analyze your data quickly

Image analysis can be complicated and cumbersome, but it can be simplified to just a few clicks. Automatically analyze up to four mini-blots or mini-gels at a time from a single image to increase throughput (Figure 17). The powerful algorithms are designed to consistently and accurately select and identify lanes and band boundaries for molecular weight estimation, densitometry, or normalization. Images can also be analyzed manually if desired.

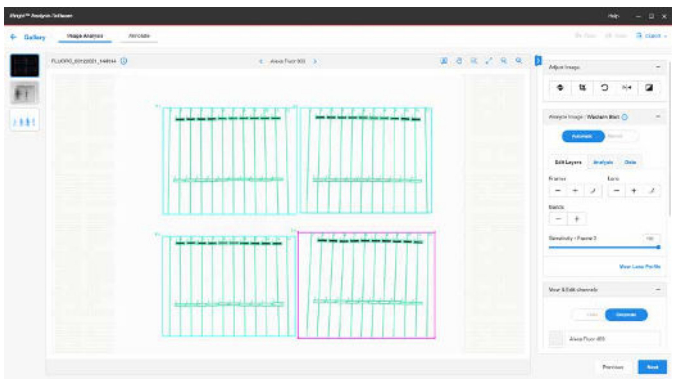


Figure 17. Analysis workspace in iBright Analysis Software. Four separate blots were analyzed using the automatic analysis feature. The blots were identified in four independent analysis frames that could be edited and analyzed separately.

Bring it all together with a complete report

After analysis, view your images alongside your data report (Figure 19). Data can be filtered to view only the desired parameters. Reports can be exported in PDF format, and the raw data can be exported to Excel™ for further analysis.

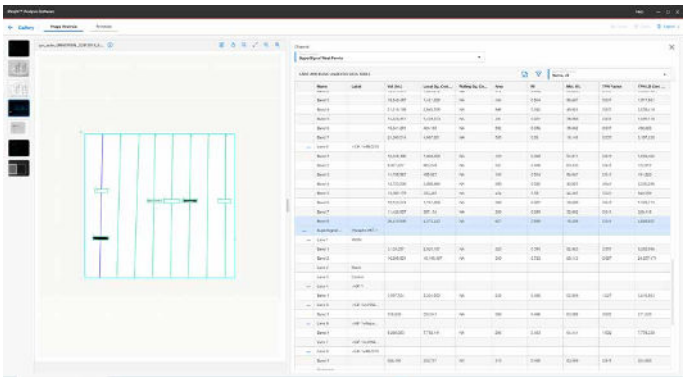


Figure 19. Image report generated with iBright Analysis Software.

Integrated 21 CFR Part 11 compliance support

Flexible software with security, auditing, and electronic signature (SAE) features

The FDA issued the Electronic Records and Signatures Rule, known as 21 CFR Part 11, in August 1997. 21 CFR Part 11 defines the requirements for using electronic documents instead of paper documents. The law specifies the system elements, controls, and procedures needed to ensure the reliability of electronically stored records.

21 CFR Part 11 encompasses both procedural and technical requirements. Procedural requirements pertain to the standard operating procedures instituted by the end user. Technical requirements pertain to the functional characteristics of the compliance management software used. Satisfying technical requirements alone does not guarantee 21 CFR Part 11 compliance. Compliance encompasses the work process of the end user and the systems used.

* An iBright SAE Software license is required to activate SAE mode and iBright Analysis Software–Secure, allowing communication with the SAE Module. **Note:** We recommended that specific personnel who are responsible for 21 CFR Part 11 compliance procedures at their institutions manage the SAE Module.

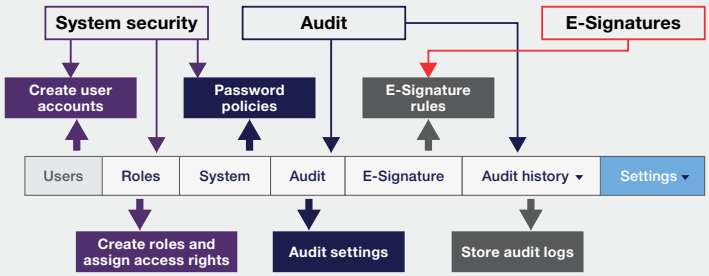
Invitrogen™ iBright™ SAE Software—Secure for 21 CFR Part 11 support is a flexible solution that enables users to satisfy SAE requirements. Our package includes the following:

1. SAE Module software that includes the SAE Administrator Console installed on a networked computer with the iBright Imaging System–specific application profile

   Note: Any instrument or software configured through the SAE Administrator Console is considered an application and requires installation of the appropriate application profile.

2. iBright SAE Software license
3. iBright Imaging System with SAE mode activated (firmware v1.5.0 or above)*
4. iBright Analysis Software–Secure* (version of iBright Analysis Software designed to support 21 CFR Part 11 compliance)

SAE settings can be configured to meet user-specific requirements through the SAE Administrator Console. The SAE Module utilizes an SAE server that runs in the background and stores SAE settings, accounts, and records (Figure 20). The SAE server is installed on the same computer as the SAE Administrator Console by default. The computer should be a networked computer with a static IP address.



Function	Description
System security	Controls user access to an application by allowing creation of user account and defining user privileges through roles and managing password policies.
Auditing	Tracks actions performed by users, changes to the SAE module settings, and creation of audit reports.
Electronic signature	Determines whether users are required to fulfill signature requirements before performing specific functions.

Figure 20. High-level overview of SAE Administrator Console software functions.

Flexible protein normalization

Get a better understanding of your data with our normalization workflow

Data validation and normalization are key in any experiment. Scientific experiments are typically designed with built-in controls or checkpoints to monitor or correct for the inherent variability of samples and experiments. Variability in western blotting is usually due to unequal protein concentrations, inconsistent sample loading onto the gel, and/or irregularities during transfer.

These sources of inconsistency can be monitored with visible or fluorescent gel- and membrane-based labeling methods followed by quantitation of total protein in each lane, or by using exogenous loading controls. Sample consistency and health can also be evaluated using a housekeeping protein as an internal control, such as GAPDH, β-tubulin, β-actin, or cyclophilin B.

iBright Imaging Systems and iBright Analysis Software enable automated and customizable quantitation and normalization to monitor or mathematically compensate for experimental or sample variability. This includes normalization based on housekeeping proteins (HKPs) and total lane protein.

It used to be assumed that HKPs were expressed constitutively at the same levels across experiments. However, recent studies have shown that expression of housekeeping proteins can differ across different cell types and biological conditions. For this reason, some scientific publishers and funding agencies require other forms of normalization or validation of normalization controls to ensure that quantitative western blotting results are accurate and reproducible. With total protein normalization (TPN), the quantity of the target protein is normalized to the total amount of protein in each lane rather than a single loading control protein.

Invitrogen™ No-Stain™ Protein Labeling Reagent is designed to support total lane protein labeling of post-transfer membranes for TPN-based normalization and can be imaged on iBright Imaging Systems, as shown in Figures 21 and 22.

See how easy it is to perform total protein normalization on an iBright Imaging System »

No-Stain Protein Labeling Reagent for total lane protein–based normalization

- **Simple protocol**—Just mix and add reagents to label proteins in 10 minutes.
- **Flexible visualization**—No-Stain Protein Labeling Reagent can be used with a wide range of imagers with UV or 488 nm excitation sources, green LED transilluminators, or blue LED transilluminators.
- **Accurate total protein normalization**—Broad linear range enables detection of 1–80 µg total protein.
- **Sensitive and stable signal**—Detect as little as 20 ng per band with a stable signal. No-Stain Protein Labeling Reagent is also compatible with downstream chemiluminescent and fluorescent antibody detection.

Labeling with No-Stain Protein Labeling Reagent can help you achieve better accuracy than normalizing to an HKP

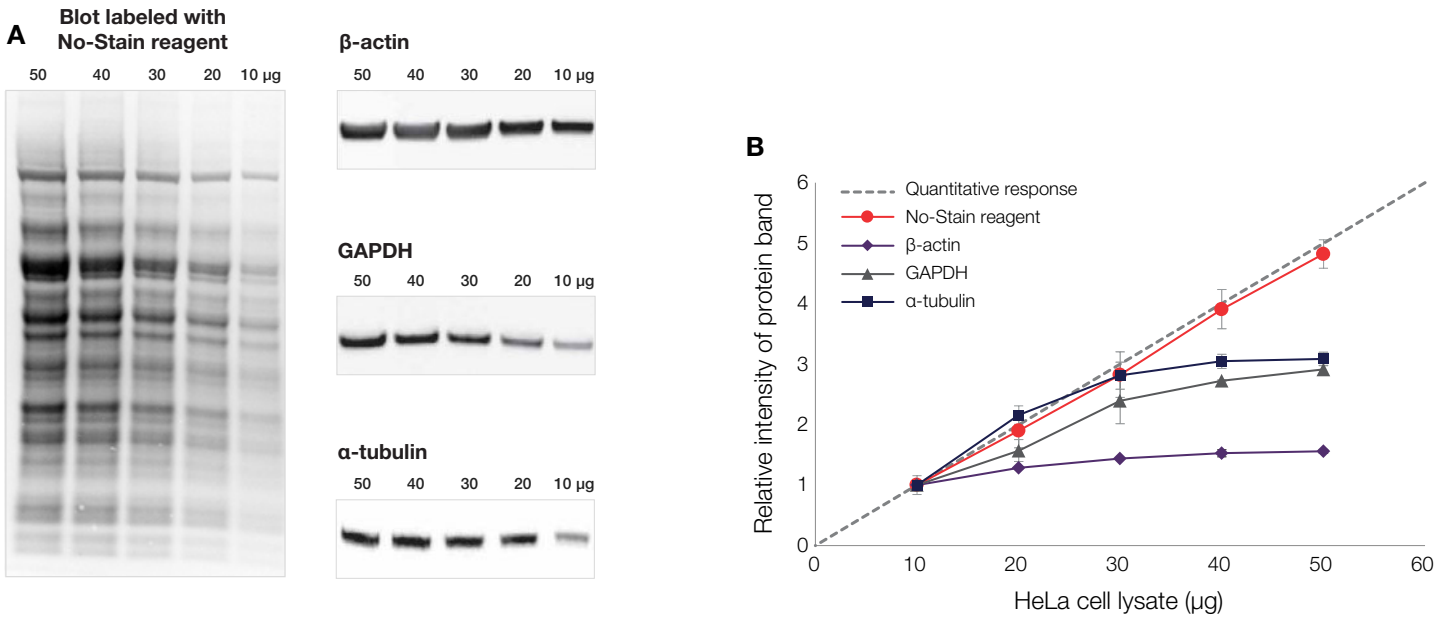


Figure 21. Signals from housekeeping proteins become saturated as protein load increases. (A) Western blots of HeLa cell lysates labeled with No-Stain reagent or β-actin, GAPDH, or α-tubulin probes. **(B)** Densitometric analysis of the blots in panel A. The signal from total protein in the HeLa cell lysate labeled with No-Stain Protein Labeling Reagent stays linear with increasing protein load.

No-Stain Protein Labeling Reagent–based TPN provides excellent concordance with protein load

Normalization method	Percent error relative to the predicted response for HeLa lysate protein loaded in a gel					
	10 µg	20 µg	30 µg	40 µg	50 µg	Average (20–50 µg)
No-Stain reagent	0.0%	5.1%	5.8%	2.3%	3.6%	4.2%
β-actin	0.0%	35.9%	51.9%	61.9%	68.8%	54.6%
GAPDH	0.0%	21.4%	20.1%	31.8%	41.7%	28.8%
α-tubulin	0.0%	7.9%	6.3%	23.9%	38.3%	19.1%

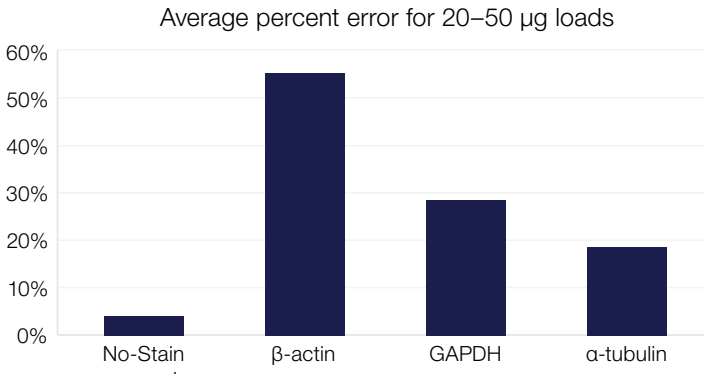


Figure 22. Total protein normalization with No-Stain Protein Labeling Reagent enables higher accuracy than HKP-based normalization. Variance from the predicted response was calculated and compared. The relative intensities were normalized by setting the relative densitometric signals equal to the predicted densitometric signals for 10 µg loads. The larger the percent error, the further the densitometric signal was from the predicted response.

Go green

Green LEDs—our alternative to UV transilluminators

iBright Imaging Systems have green LED–powered transilluminators, which effectively excite common DNA stains such as ethidium bromide and Invitrogen™ SYBR™ Green dyes.

No harmful UV rays

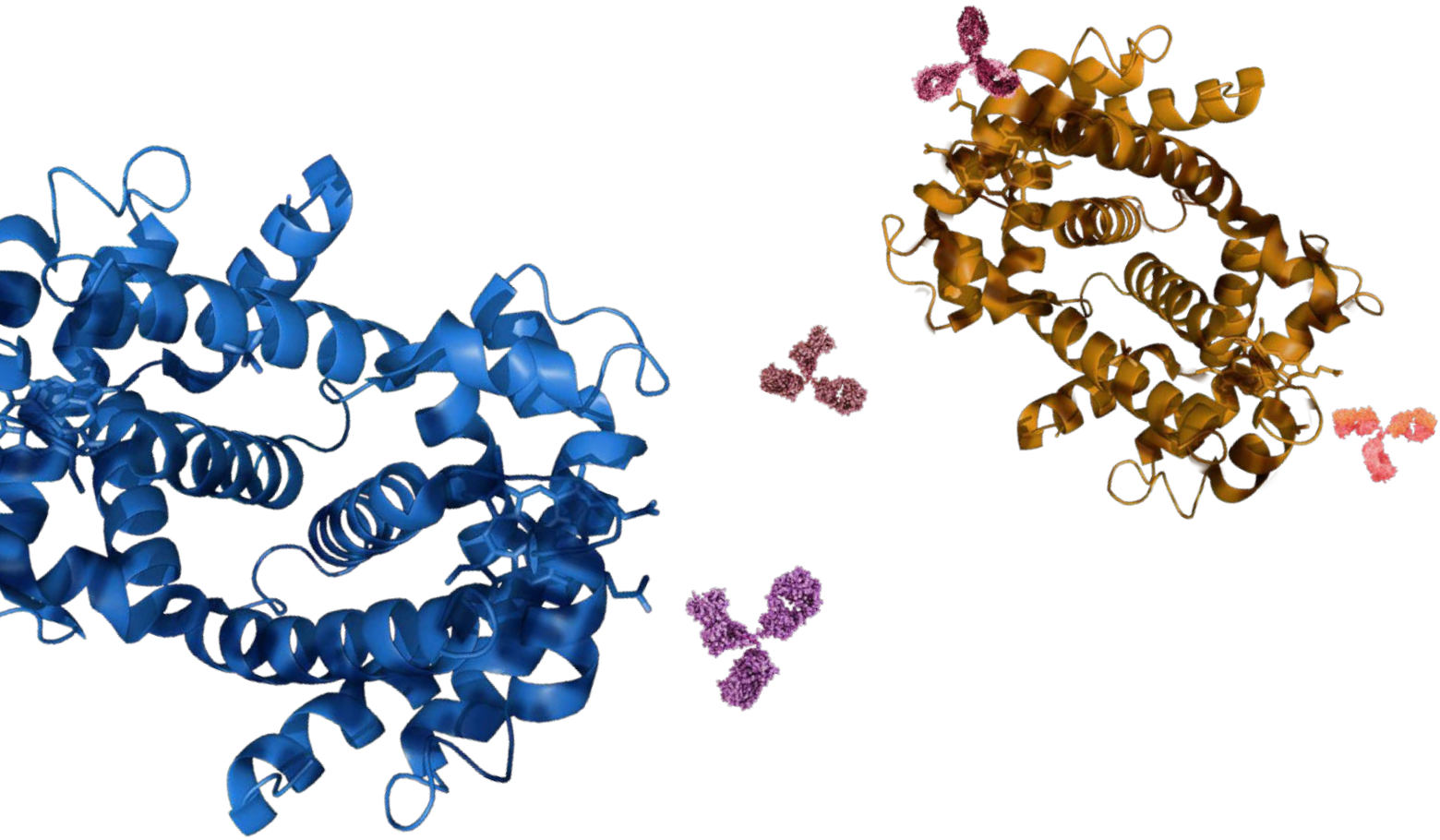
While UV light effectively excites many fluorescent dyes and stains, it is a health hazard. Prolonged exposure to UV light can damage DNA and may compromise the integrity of samples intended for use in downstream applications such as subcloning.

No mercury waste

Fluorescent bulbs may contain hazardous mercury, so they require special care for handling and disposal.

Longer lifetime

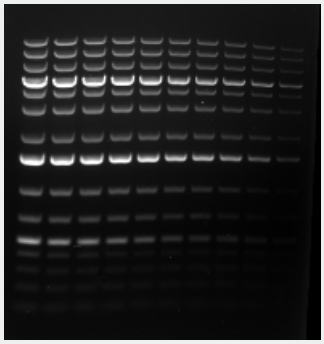
LED bulbs have significantly longer lifetimes than UV bulbs, which can reduce costs considerably over the lifetime of the instrument.



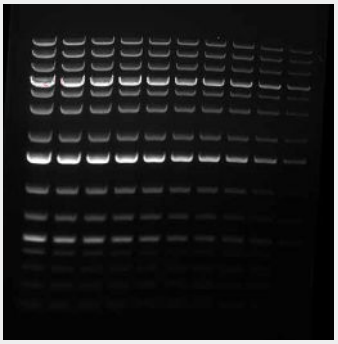
Emission range of the green LED iBright transilluminator

The emission range of the green LED transilluminator (490–520 nm) covers the excitation peaks of SYBR Green and Invitrogen™ SYBR™ Gold dyes as well as the secondary excitation range of ethidium bromide (Figure 23). While the transilluminator covers the secondary excitation range of ethidium bromide, the high-intensity energy of the LED source compensates and enables visualization that is comparable to visualization with a UV transilluminator.

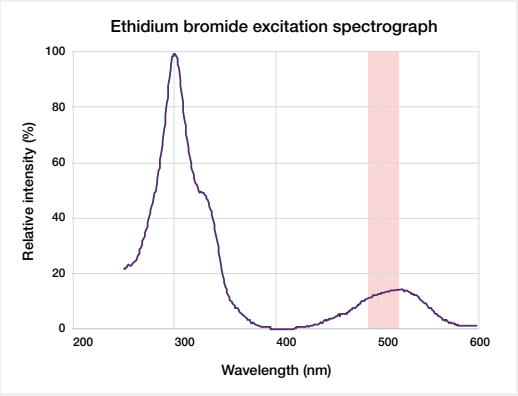
A



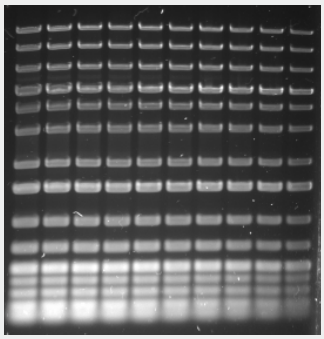
Gel stained with ethidium bromide and imaged on an iBright Imaging System



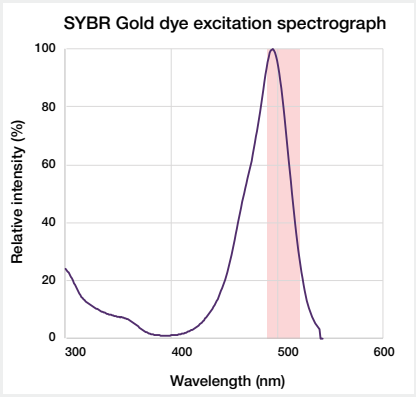
Gel stained with ethidium bromide and imaged using a UV transilluminator–based imaging system



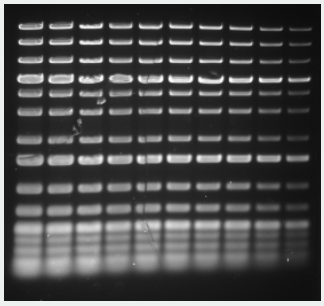
B



Gel stained with SYBR Gold dye and imaged on an iBright Imaging System



C



Gel stained with SYBR Green dye and imaged on an iBright Imaging System

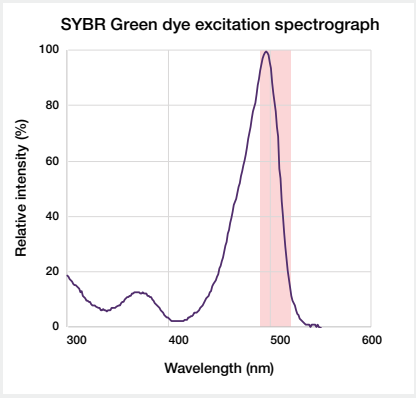


Figure 23. The iBright green LED transilluminator effectively excites common DNA stains. Excitation spectrographs of **(A)** ethidium bromide, **(B)** SYBR Gold dye, and **(C)** SYBR Green dye (purple lines). The 490–520 nm emission range of the iBright transilluminator is indicated by the light red boxes.

Get exceptional service and support to maximize your investment

Keep discovering. We've got your back.

A SmartStart Orientation can help enable your success

Every iBright Imaging System includes a SmartStart™ Orientation. SmartStart Orientations for the iBright FL1500 and CL1500 Imaging Systems are given on-site, and a digital orientation is available for the iBright CL750 Imaging System.* Led by professional trainers, the interactive on-site orientation includes application-specific lectures, hands-on experiment preparation, instrument and software setup, and basic data analysis. The digital SmartStart Orientation for the iBright CL750 Imaging System is a convenient option that can help you quickly and efficiently install, operate, and maintain your instrument.

To access the self-paced digital SmartStart course, please visit **thermofisher.com/digitalsmartstartCL750**

Comprehensive warranty and service plans help maximize system uptime

Every new iBright Imaging System comes with a standard factory warranty. The iBright CL750 Imaging System comes with a one-year warranty that covers all costs for travel, labor, and parts for repairs. The iBright FL1500 and CL1500 Imaging Systems come with a two-year warranty that includes a planned maintenance (PM) visit in the second year. Extended coverage service plans are also available at the time of purchase. Our service plans can help you maximize system uptime, reduce overall repair costs, get fast repair by a manufacturer-trained and certified field service engineer (FSE), and extend the life of your instrument.

To build your personalized service quote, go to **thermofisher.com/ibrightserviceselector**

AB Assurance Plan

The AB Assurance Plan is designed for customers like research laboratories, core service facilities, and hospitals who require predictability and productive uptime. This service plan will help keep your laboratory running smoothly with preventative maintenance, proactive instrument monitoring, and a rapid response should one of your instruments require repair.

Find out more about the AB Assurance Plan at **thermofisher.com/abassurance**

Qualification services

With our instrument installation qualification (IQ) and operational qualification (OQ) services, you can be confident that your iBright Imaging System is installed and operating according to manufacturer specifications.

To learn more, contact an instrument qualification specialist at **thermofisher.com/iqoqpq**

* On-site SmartStart Orientation is not available in all regions. Connect with your sales representation for more details.

Explore our services and support solutions at **thermofisher.com/instrumentservices**

Find the right model for your research

Compare models

Table 3. Specifications for iBright Imaging Systems.

	iBright CL750 Imaging System	iBright CL1500 Imaging System	iBright FL1500 Imaging System
	Essential western blot and gel imaging—transition from darkroom and film-based detection with ease	Expanded application support with many of the same high-performance specifications as the iBright FL1500 Imaging System	Maximum application support, including fluorescent western blot imaging with up to 4 fluorophores at a time
Camera			
Detector	Cooled 16-bit CCD; 65,535 shades of gray		
Resolution	9.1 megapixels		
Lens	Fixed, 25 mm, f/0.95		
Field of view	22.5 x 18.0 cm (W x D); image up to 4 mini blots or gels		
Binning modes	1 x 1, 2 x 2, 4 x 4 (high resolution and sensitivity settings)	1 x 1, 2 x 2, 3 x 3, 4 x 4, 5 x 5, 6 x 6, 8 x 8 (maximum flexibility for adjusting resolution and sensitivity)	
Zoom	1–2x, digital (digital zoom reduces the effective resolution of the zoomed image)	1–8x (1–2x mechanical, 1–4x digital); mechanical zooming improves sensitivity by moving the camera closer to the sample stage and reducing the focal length	
System interface			
Touchscreen	12.1-inch capacitive LCD display; 1,024 x 768 pixels		
Storage and connectivity			
USB	2 x USB 2.0		
Networking	Ethernet port, cloud-based connectivity; optional Wi-Fi adapter sold separately		
Image file formats	G2i (proprietary), TIFF, JPG, PNG		
Hard drive	64 GB SSD	256 GB SSD	
System hardware			
Sample drawer and stage	Manually operated drawer with fixed stage	Automatic drawer with automatic rotating sample stage	
Filter sets	2 filters (0 excitation, 2 emission)	4 filters (2 excitation, 2 emission)	12 filters (6 excitation, 6 emission)
Illumination sources	• Green LED transilluminator	• Green LED transilluminator • Epi white LED	• Green LED transilluminator • Epi white LED • Epi near-IR LED
System software			
Automated features and algorithms	• Automatic zoom • Automatic focus • Automatic exposure (Smart Exposure) • Automatic onboard image analysis • High dynamic range (HDR) image capture capability (Smart Range HDR*)		
Standalone analysis applications	iBright Analysis Software (desktop version for macOS or Windows operating system), Thermo Fisher Connect Platform (cloud-based), and iBright Analysis Software—Secure (desktop version supporting 21 CFR Part 11 compliance)		
Core imaging applications			
Colorimetric protein gel imaging	•	•	•
Fluorescent protein gel imaging	•	•	•
Fluorescent nucleic acid gel imaging	•	•	•
Colorimetric membrane imaging	Limited**	•	•
Chemiluminescent western blot imaging	•	•	•
Colorimetric western blot imaging	–	•	•
Fluorescent western blot imaging	–	–	•
Specialty plate–based imaging applications			
Fluorescent colony counting	•	•	•
Visible colony counting	•	•	•
Qualitative visible imaging applications†			
Opaque objects	–	•	•
Shipping, warranty, and upgrade			
Upgrade option	No	Upgrade to the iBright FL1500 system‡	Not applicable
Weight	Approximately 47 kg (105 lb)	Approximately 50 kg (110 lb)	
Warranty	1 year from date of purchase	2 years from date of purchase	
Dimensions (L x W x H)	68 x 38 x 60 cm		

*Smart Range HDR available in chemiluminescent blot mode only.

** Membranes must be imaged when wet using Thermo Scientific™ Pierce™ Reversible Protein Stain Kit for Nitrocellulose Membranes or Thermo Scientific™ Pierce™ Reversible Protein Stain Kit for PVDF Membranes.

† Applications enable qualitative visualization of objects or confirmation of signal. Not recommended for quantitation.

‡ Upgrades not available in all regions. Please check with your sales representative for upgrade details.

Whether you have a classical approach to gel electrophoresis and western blotting or a more efficient modern approach, we offer solutions across the workflow.

Ordering information

Product	Cat. No.
iBright FL1500 Imaging System	
1 instrument, including SmartStart Orientation and 2-year warranty	A44241
1 instrument, including SmartStart Orientation, 2-year warranty, and license for iBright SAE Software for 21 CFR Part 11 support	A44241CFR
1 instrument, including 1-year warranty	A44115
iBright CL1500 Imaging System	
1 instrument, including SmartStart Orientation and 2-year warranty	A44240
1 instrument, including SmartStart Orientation, 2-year warranty, and license for iBright SAE Software for 21 CFR Part 11	A44240CFR
1 instrument, including 1-year warranty	A44114
iBright CL750 Imaging System	
1 instrument, including 1-year warranty and digital SmartStart Orientation	A44116
iBright SAE Software for 21 CFR Part 11	
1 license (one license required per instrument)	A49208
iBright Tray Adapters for E-Gel Precast Agarose Gels	
iBright Tray Adapter for E-Gel Agarose Gels, 11/22 well	A56600
iBright Tray Adapter for E-Gel Agarose Gels, 48/96 well	A56599

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