WESTERN DETECTION

Stunningly easy western blot and gel imaging

Introducing Smart Range HDR technology

Introduction

Scientists performing western blots often need to capture multiple exposures of a given sample in order to maximize the signal of interest while minimizing background. The Smart Exposure™ auto-exposure feature of Invitrogen™ iBright™ Imaging Systems determines an optimal exposure time by maximizing the highest pixel intensities while avoiding saturation. Typically, medium- to high-abundance proteins will have higher signals on a western blot than low-abundance proteins, which complicates the determination of a single exposure time. Too short of an exposure time will typically result in underexposed low-abundance proteins, while too high of an exposure time will cause signal saturation from the medium- to high-abundance proteins.

Leveraging the Smart Exposure feature is excellent for capturing most western blot target proteins, as the Smart Exposure feature determines the shortest exposure recommended to balance the signal-to-noise ratio across the blot.

However, for samples containing targets with widely varying expression levels, utilizing the Smart Range™ HDR (high dynamic range) feature* is recommended. This feature leverages two different exposures of the same sample, a short exposure for capturing medium- to high-abundance proteins and a long exposure for capturing low-abundance proteins. After capture, the Smart Range algorithm normalizes and then combines different regions of the two images into a single 16-bit HDR image that provides high-quality unsaturated data for both the high-abundance and low-abundance proteins' signal intensities without compromising data quality. The long-exposure image helps separate the noise from the low-intensity signal, while the short-exposure image provides unsaturated data for the high-intensity signal. This combining of images can potentially extend the observable linear dynamic range beyond what is otherwise achievable with a single short or single long exposure time (Figures 1 and 2).The Smart Range HDR feature is enabled for chemiluminescent western blot detection.

* The Smart Range HDR feature is only available in iBright Imaging Systems running firmware 1.8 (released Nov. 15, 2022) or higher.

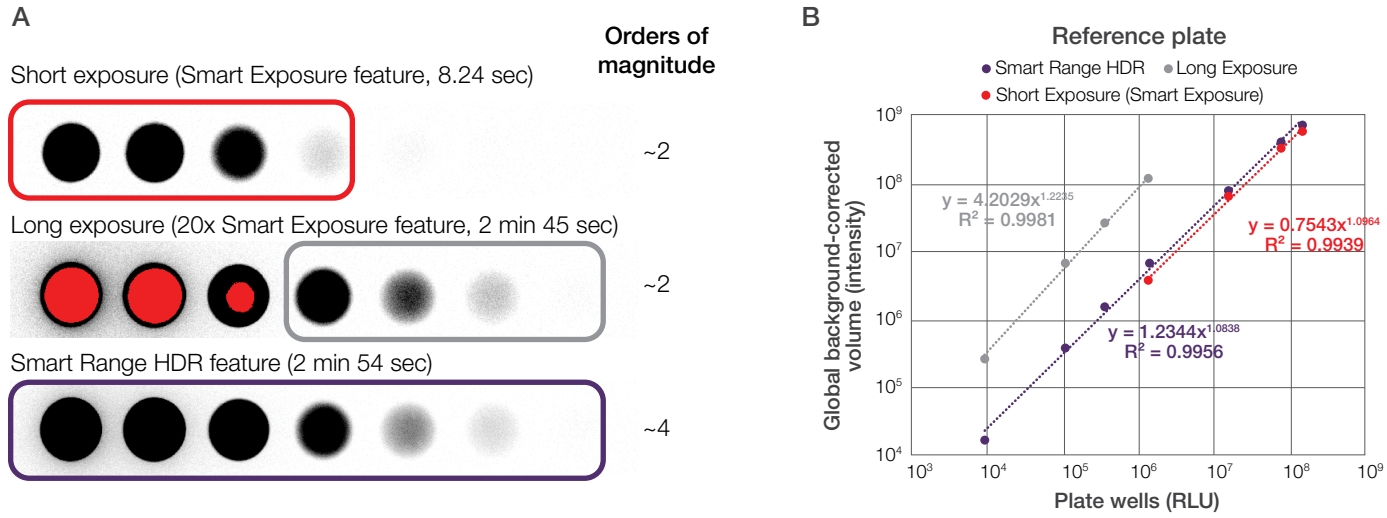

Figure 1. The Smart Range HDR feature combines short and long exposures into a single HDR image with increased linear dynamic range compared to the other imaging techniques. A luminometer reference plate was imaged on an iBright Imaging System using 3 different acquisition techniques: a short exposure (utilizing the Smart Exposure feature), a long manual exposure, and the Smart Range HDR feature. The resulting images are compared by **(A)** visual assessment and **(B)** how many data points fall within the linear dynamic range.

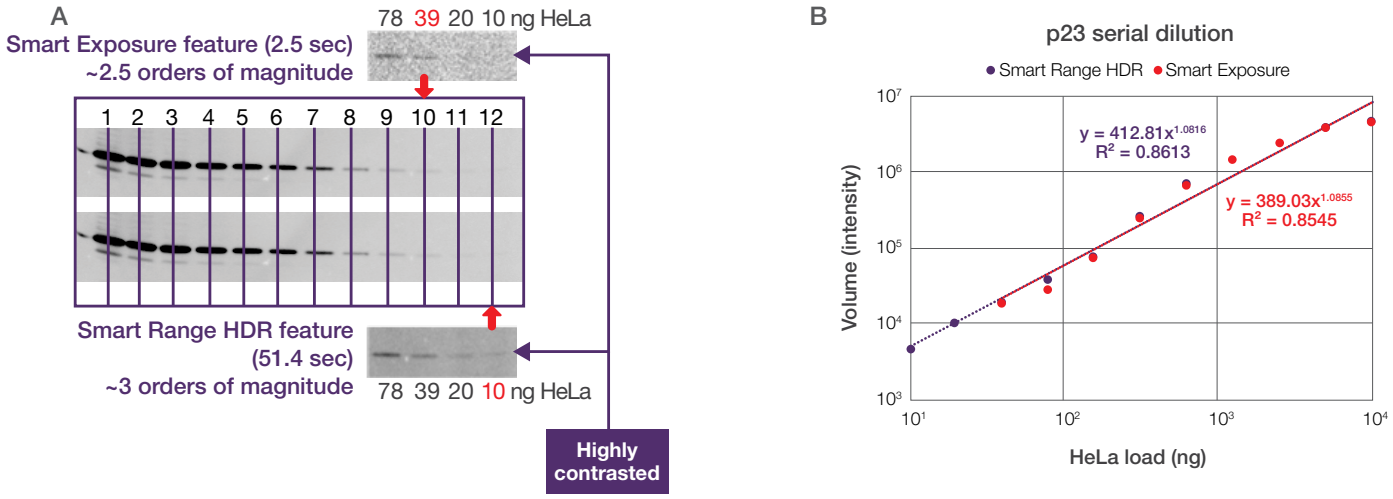

Figure 2. The Smart Range HDR feature improves the detection limit of p23 by 4-fold compared to the Smart Exposure feature. HeLa lysate was serially diluted 1:1 in sample buffer (from 20 µg to 10 ng), prepared for SDS-PAGE, and electrophoresed on an Invitrogen™ Novex™ WedgeWell™ 4–20% Tris-glycine gel. 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 are compared by **(A)** visual assessment and **(B)** how many data points fall within the linear dynamic range. The 20 µg data point is omitted from the graph because it falls outside the linear range in both the Smart Range HDR and Smart Exposure images.

How to capture an HDR image on an iBright Imaging System

Capturing an HDR image on an iBright Imaging System is easy and can be completed in just a few simple steps:

1. Open the system drawer and insert a chemiluminescent western blot. Touch the **Close** button to close the system drawer and enter **Chemi Blots Mode**.
2. Touch the **Smart Exposure** button. (This will generate a preview of how the resulting blot image will appear after capture.)
3. Touch the **Smart Range HDR** checkbox located on the Smart Exposure preview screen.

   When the box is checked, the total HDR exposure time (short exposure plus long exposure) will be displayed on the exposure time dial, but the image preview will not be updated to reflect how the final HDR image will appear. The final HDR image will only become visible after capture, in step 4.
4. Touch **Capture** to acquire the HDR image.

Notes

A Smart Range HDR image can be captured after capturing the Smart Exposure image; the exposure time can be adjusted within the Smart Exposure feature, or by manually entering an exposure time.

Expanded linear dynamic range improves the detection of low-abundance proteins

In the following example (Figure 3), the Smart Range HDR feature noticeably improves detection of low-expressing phosphorylated proteins compared to capture of the blot signals using the Smart Exposure feature alone. The extended range of the results can enable more robust overall interpretation.

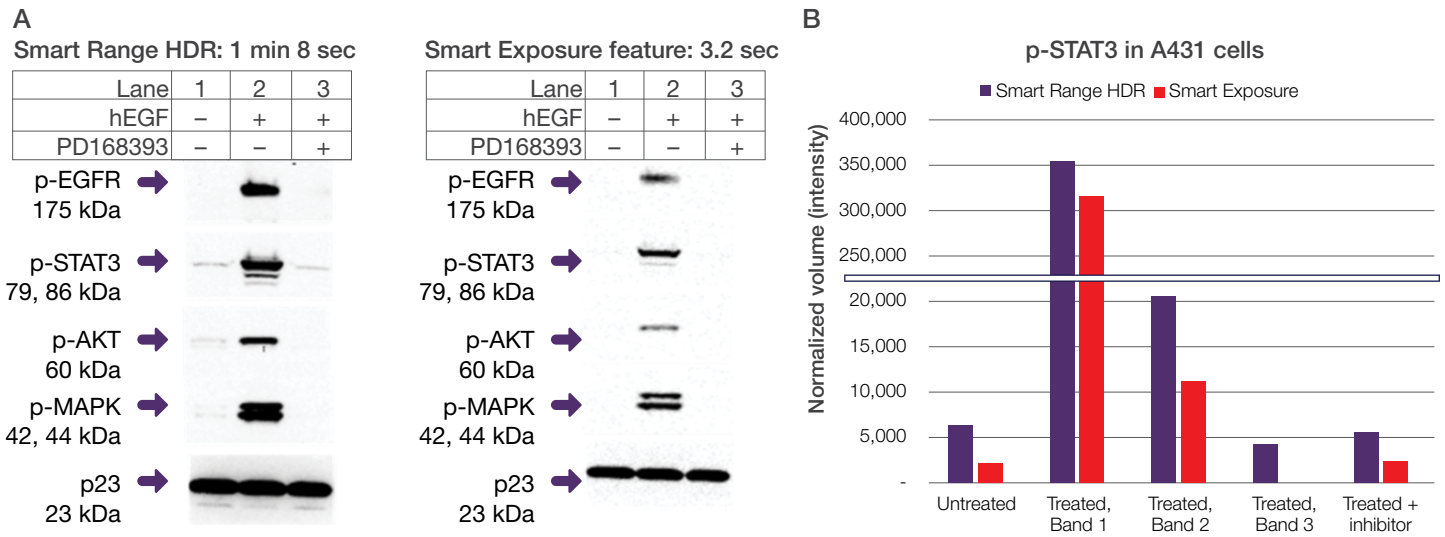

Figure 3. The Smart Range HDR images have much more detail, with the lowest-signal bands now distinguishable above background and the highest-signal bands unsaturated, when compared to the images acquired with the Smart Exposure feature and the long-exposure (overexposed) images (not shown), respectively. Human epidermoid carcinoma cells (A431) were either left untreated, or treated with human epidermal growth factor (hEGF), or hEGF plus PD168393 (acute inhibitor of EGFR). The cells were lysed and prepared for SDS-PAGE. From each sample, 10 µg was loaded in triplicate into two 12-well Novex WedgeWell 4–20% Tris-glycine gels. The protein was transferred to a nitrocellulose membrane and then cut into 3 identical strips, each having the same sample layout, and the resulting blots were blocked with Pierce™ Clear Milk Blocking Buffer and then probed for 4 different phosphoproteins in the EGF (epidermal growth factor) receptor pathway and p23 (housekeeping protein). The four different blots were incubated with secondary antibody conjugated to HRP, washed, and then placed in SuperSignal West Dura substrate. **(A)** The resulting chemiluminescent blots were imaged on the iBright FL1500 Imaging System using the Smart Exposure and Smart Range HDR features. **(B)** Background-corrected band volumes for p-STAT3 were normalized to those of the housekeeping protein p23 and plotted for comparison of the signal acquisition technologies.

How to achieve the best results when leveraging the Smart Range HDR feature

When optimizing your western blot protocol, consider the following:

- ☑ Optimize the protein load based on the expected target abundance, so that the primary antibody binds to the target protein in a linear fashion.
- ☑ Start with the supplier's recommended primary antibody concentration, and then optimize the antibody concentration to obtain a signal that is high but below saturation. If too much primary antibody is used, the higher loads of sample containing the target of interest often become saturated and nonlinear. However, if the primary antibody is too dilute, sensitivity is compromised.
- ☑ Follow the chemiluminescent substrate supplier's recommendation for the secondary antibody concentration. If the secondary antibody's concentration is too high for the substrate, signal burnout may occur, but if the secondary antibody is too dilute, sensitivity is compromised.
- ☑ Choose a long-lasting chemiluminescent substrate with a slow decay rate, such as the Thermo Scientific™ SuperSignal™ West Dura Extended Duration Substrate. Because an HDR image can sometimes take up to 50 minutes to acquire, the luminescent signal needs to be consistent for that amount of time.
- ☑ Make sure that the signal from the protein of interest does not have any saturated pixels (false-colored red) in the short exposure. Saturated pixels in the short exposure will result in saturated pixels in the HDR image. Saturated pixels fall outside of the linear dynamic range.
- ☑ (Optional) Use the region of interest (ROI) function to focus the Smart Exposure algorithm on your protein of interest. The ROI Smart Exposure feature allows you to direct the Smart Exposure algorithm to a specific region on the sample that contains your proteins of interest, instead of the whole sample. Although some off-target pixels may become saturated, the pixels comprising the bands for the protein of interest should not be saturated.

Definitions

- **Pixel saturation:** Imagine that each pixel on the camera sensor is a bucket or well. When the sensor is exposed to light, each well fills up with photons that are converted to the electronic signal. As the well nears capacity, the relationship between light and signal output becomes less linear and eventually reaches saturation (65,535 for a 16-bit camera sensor). On an iBright Imaging System, saturated pixels are displayed in a false red color.
- **Dynamic range:** A 16-bit CCD camera sensor can distinguish 65,535 shades of gray between black (0, no signal) and white (65,535, maximum signal). Dynamic range is defined as the full well capacity (65,535) divided by the background noise of the camera.
- **Linear range:** The linear range is the portion of the dynamic range where light intensity and camera signal have a linear relationship. Only data that fall within the linear range are quantifiable.

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	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 digital SmartStart Orientation and 1-year warranty	A44116
iBright SAE Software for 21 CFR Part 11	
1 license (single license required per instrument)	A49208
SuperSignal West Dura Extended Duration Substrate	
100 mL	34075
200 mL	34076
20 mL	37071

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