Sensitive Chemiluminescent Imaging with the chemiSOLO

Introduction

Chemiluminescence is a well-established, light-based detection method used in numerous molecular biology applications. Detection of a target molecule on a membrane typically is achieved through a labeled antibody specific to the target. For chemiluminescent detection, the antibody is labeled with an enzyme such as horseradish peroxidase (HRP) or alkaline phosphatase (AP) that produces light as a byproduct of acting on a substrate. Highly sensitive detection is possible because light is produced by an enzymatic process, which amplifies the presence of a small number of target molecules.

Chemiluminescence is used to detect proteins and nucleotides for a variety of applications. For example, digoxigenin (DIG)-labeled nucleic acids can be detected using anti-DIG antibodies for chemiluminescent detection of Southern or Northern blots, gel shift assays, electrophoretic mobility shift assays, and more. However, chemiluminescence most commonly is used to detect proteins on Western blots. Western blotting is used to detect the presence of a protein in a sample and to compare the relative amounts of a protein between samples.

In the past, chemiluminescence has been detected using X-ray film, but the emergence of digital imaging has provided a modern solution for chemiluminescent detection. Developing film can be time consuming, requires access to a dedicated darkroom with appropriate equipment, and necessitates repeated purchase of reagents and single-use film. Digital imaging circumvents the development process altogether and allows labs to leave the darkroom behind. In addition to reducing the waste associated with developing film, digital imaging is more sensitive and provides a larger linear dynamic range than X-ray film. These attributes allow quantitative information to be obtained from Western blots.

Azure Biosystems developed the chemiSOLO Personal Western Blot Imager to make digital chemiluminescent imaging accessible to every lab. The chemiSOLO also captures visible light images, and makes it straightforward to overlay visible images of multicolor protein markers with chemiluminescent images of blots to facilitate gel annotation and band identification. The chemiSOLO is a digital imager that provides superior imaging quality and ease of use in a space-efficient design.

Sensitive Chemiluminescent Imaging with Space-Efficient Design

The chemiSOLO is designed to provide sensitive detection of chemiluminescent signals via a six megapixel, back-illuminated, Peltier-cooled CMOS camera. It includes an Extended Dynamic Range (EDR) function that is able to capture images at a higher bit depth, avoiding pixel saturation and maximizing the linear dynamic range.

Figure 1. The chemiSOLO, a cutting-edge imager capable of highly sensitive chemiluminescent and visible-light imaging with a compact design.

Compared to the space needed for X-ray film development, or even with competing digital imagers, the chemiSOLO is compact and space-efficient. Its dimensions of 11.5" x 8.75" x 17.0" (29.2 x 22.2 x 43.2cm) allow the chemiSOLO to fit neatly into busy lab spaces.

Simple, Intuitive Controls

The chemiSOLO is controlled remotely through a unique web browser system. The instrument is seamlessly controlled through the user's own external computer, tablet, or mobile device. This feature maximizes the chemiSOLO's accessibility and useability.

In this application note, we will demonstrate the femtogram sensitivity of the chemiSOLO when imaging chemiluminescent Western blots and demonstrate maximization of the linear dynamic range using the EDR function.

Materials and methods

chemiSOLO Limit of Detection

A dot blot was prepared containing a serial dilution of bovine serum albumin (BSA) spanning the concentration range of 150 pg to 342 fg. The BSA samples were spotted onto nitrocellulose membrane using a dot blotter with vacuum manifold (Bio-Dot®, Bio-Rad).

The dot blot was probed with an anti-BSA primary antibody followed by an HRP-conjugated secondary antibody. Blots were detected using WesternBright Sirius ECL substrate (Advansta). The blot was imaged on the chemiSOLO using the chemiluminescent imaging setting, 3x3 binning, and a manual exposure time of 5 minutes.

Figure 2. Through its novel browser-based system, the chemiSOLO can be controlled by computers, tablets, or mobile devices with no downloads required.

Effect of EDR on Dynamic Range

To demonstrate the effect of the EDR function on the dynamic range of the chemiSOLO when imaging chemiluminescence, a dot blot was prepared containing a serial dilution of BSA spanning the concentration range of 228 pg to 66 ng. The blot was imaged on the chemiSOLO using the chemi setting, 1x1 binning, and a manual exposure time of 1 minute, and also using the chemiluminescent imaging setting, 1x1 binning, and EDR;24-bit.

Overlay of Visible Image of Multicolor Marker and Chemiluminescent Blot Image

To demonstrate the ability to capture and overlay visible and chemiluminescent images of a blot, a Western blot was created from a gel containing Fisher BioReagents™ EZ-Run prestained protein ladder (Fisher Scientific) and Precision Plus Protein™ Dual Color Prestained Standards (Bio-Rad) and a serial dilution of cell lysate. The blot was probed with an anti-GAPDH antibody and HRP-conjugated secondary antibody. The gel was imaged on the chemiSOLO with the chemiluminescent imaging setting, 1x1 binning, RapidCapture (auto-exposure), with the "Color Marker" option selected.

Results

As shown in Figure 3, the chemiSOLO can detect proteins at the femtogram level using chemiluminescent detection. Chemiluminescence detection limits will depend in part on the chemiluminescent substrate used and imaging variables such as exposure time. The Peltier-cooled camera in the chemiSOLO provides excellent signal-to-noise ratio allowing sensitive detection of low-abundance samples with low background. Additionally, the chemiSOLO's camera is a back-illuminated CMOS, which enables higher sensitivity through optimization of the internal components' layout.

Figure 4 demonstrates the effect of the EDR function on the linear dynamic range of the chemiSOLO when detecting chemiluminescence. When a broad range of protein amounts is included on a single blot, it can be difficult to detect the lowest abundance bands without saturating the high abundance bands. Using EDR, a 24-bit image is captured instead of the default 16-bit image, avoiding pixel saturation and allowing for a wider range of linear quantitation (Figure 4).

The “Saturation” function in the chemiSOLO control software will colorize an image to reveal any saturated pixels. Using the “Saturation” icon shown in Figure 5, the difference in saturated pixels (indicated in red) between the 16-bit image and the 24-bit EDR image in Figure 4 is readily apparent.

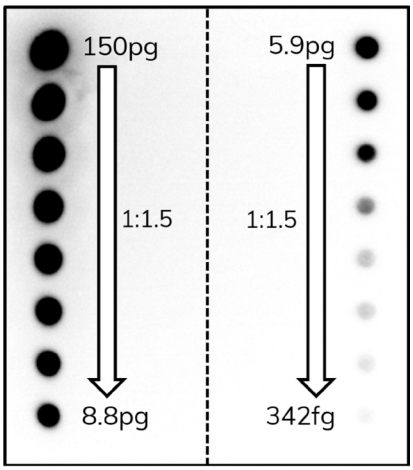

Figure 3. When imaging chemiluminescence, the chemiSOLO is capable of detecting proteins at the femtogram level.

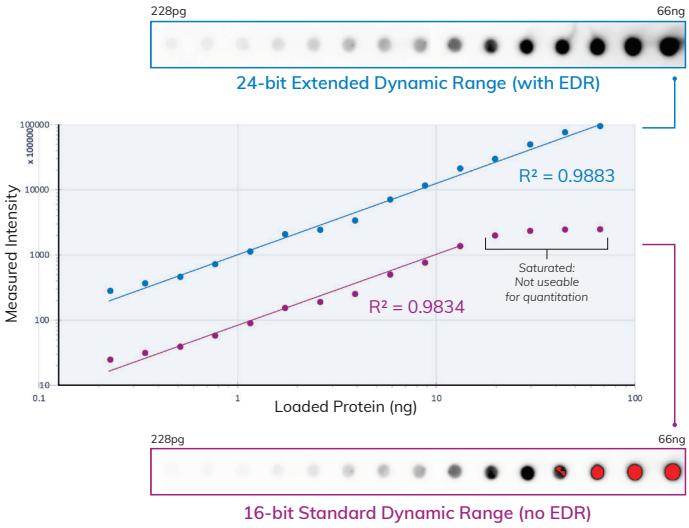

Figure 4. Using the extended dynamic range (EDR) function, the chemiSOLO takes images with >16-bit depth, allowing for a wider linear dynamic range without saturation for a wider range of quantitation.

To facilitate blot annotation and band identification, the chemiSOLO has a built-in function to image a chemiluminescent blot twice, first as a chemiluminescence image, then with visible light to document a stained maker, as shown in Figure 6. The images are easily overlayed as shown in Figure 7, which depicts a chemiluminescent blot with a color image of pre-stained molecular weight markers. Other imaging options include capturing a grayscale image of a marker or imaging a blot with no marker image.

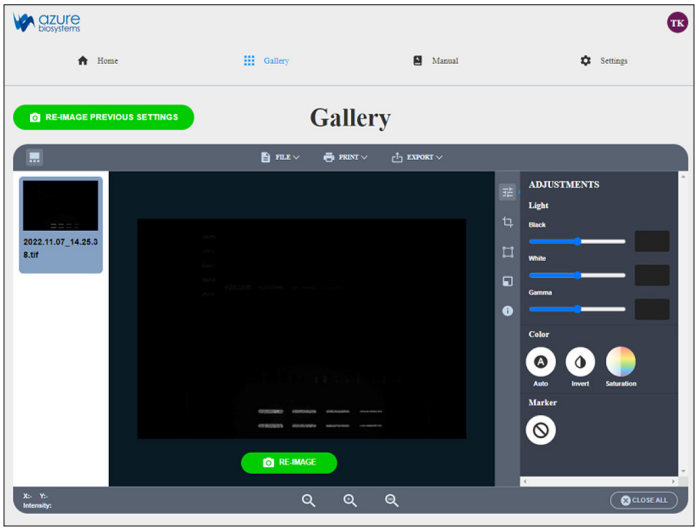

Figure 5. The unique browser-based controls for the chemiSOLO simplify detection of pixel saturation.

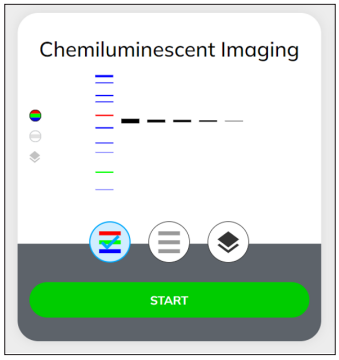

Figure 6. Color and black-and-white marker options in the chemiSOLO’s chemiluminescent imaging control screen.

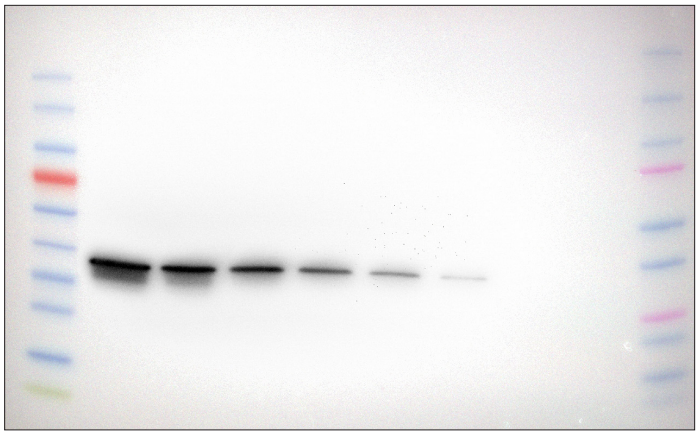

Figure 7. Chemiluminescent blot imaged using the visible color marker option under the chemiluminescent imaging control screen on the chemiSOLO.

Conclusions

The chemiSOLO's advanced imaging technology, intuitive controls, and compact design provide users with a personal, high-quality chemiluminescent imager that will fit neatly into their busy lab spaces. It provides sensitive chemiluminescent imaging with femtogram protein detection capability and up to 24-bit imaging to capture images with increased dynamic range for more quantitative Western blots. The flexibility of controling the imager using a wide range of devices, including tablets and mobile devices, through a unique browser-based system allows the chemiSOLO to effortlessly bring digital chemiluminescent imaging to every lab. For more information, visit azurebiosystems.com/chemisolo.