Enhanced Chemiluminescent Western Imaging, at an Economical Price

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With the cSeries digital imaging family Azure Biosystems has been delivering industry-leading performance, coupled with a user-friendly experience across a wide range of applications. In this note we are delighted to introduce the newest member of the cSeries family, the Azure Biosystems c280 imaging system; and demonstrate how it provides the ideal entry point to digital chemiluminescent imaging.

Introduction

Many scientists still use a darkroom to expose their chemiluminescent Western blots to film in a process not far removed from that used when Western blotting was first described in the 1970's. However, rapid progress in the field of digital chemiluminescent imaging means that now really is the time to consider switching, with the Azure Biosystems c280 imager being an ideal entry point. We discuss the benefits that digital chemiluminescent imaging can bring to your Western blot workflow in detail in our Why You Should Leave the Darkroom app note.

Briefly, film and the associated chemicals and developing equipment are expensive, and with fewer and fewer suppliers these prices are only likely to increase; additionally, the user-friendly nature of digital imaging systems allows for rapid image acquisition and analysis. Film has a small dynamic range when compared to modern high resolution, sensitive digital imagers. Furthermore, the increased dynamic range achievable with digital chemiluminescent imaging allows for more accurate quantification of Western blots featuring a wide range of signal intensity.

In this app note we demonstrate the above improvements using an Azure Biosystems c280 imaging system.

Materials and Methods

Sample preparation and Western blotting

Purified transferrin was serially diluted from a starting concentration of 500pg through to 61fg, and separated by gel electrophoresis. After electrophoresis and separation, proteins were transferred to a low fluorescence PVDF membrane using Azure Transfer Buffer. Blots were blocked, probed with a chicken-anti-transferrin primary antibody, washed and then incubated with a goat-anti-chicken HRP conjugated secondary antibody; all diluted in Azure Chemi Blot Blocking Buffer. Signal was detected using Azure Radiance chemiluminescent substrate.

Imaging

Blots were imaged using the Azure Biosystems c280 digital imager, along with traditional exposure to Lucent Blue X-ray film for a variety of time points.

Results

Figure 1 shows representative images and quantification of signal intensity and background, for the same blot imaged for 30 seconds using either the c280 set at its lowest sensitivity (1x1 binning) or film. A far greater dynamic range was observed when using the c280 compared with film, making accurate quantification between bands possible, even at the higher intensity regions. Similarly, detected background was reduced across the blot allowing for a further improvement in quantification.

Whilst the above example demonstrates the innate improvements that digital chemiluminescent imaging can bring to your Western blotting, its true power lies in the ability to alter exposure options to maximize dynamic range and prevent signal saturation.

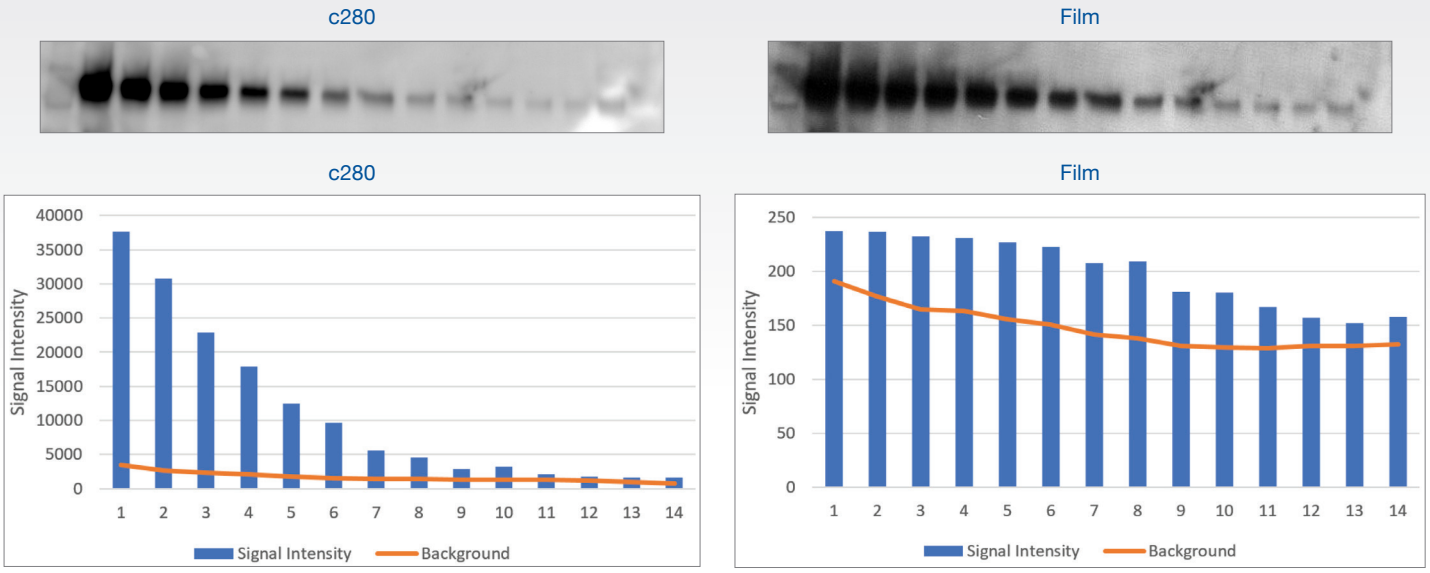

Figure 1. Improved signal detection and reduced background when using the c280. Plotted signal intensity values include background signal.

In Figure 2 the same Western blot was exposed to film (a) imaged using the c280 at normal sensitivity (b) or imaged using the c280 at its lowest sensitivity setting (c). Signal saturation limits the ability to accurately quantify Western blots, and with film there is no way to determine if or when a signal is saturated. When comparing the 10 and 30 second exposures of (a) and (b) the improvements in signal intensity and reduced background achievable by the c280 are apparent. However, whilst the lower concentration bands become visible at 10 and 30 seconds, oversaturation of the higher concentration bands is also apparent (shown in red). This limits the ability to accurately quantify the two extremes of signal.

If using film, this is when a complicated cycle of sample dilution and assessment would be required. However, with the c280 a simple reduction in sensor sensitivity, coupled with a longer exposure dramatically increased the dynamic range of the blot. As such the 2-minute exposure of the blot shown in (c) demonstrates clear expression of all bands, with low background and no saturation, allowing for more accurate quantification across the whole blot (500pg – 61fg).

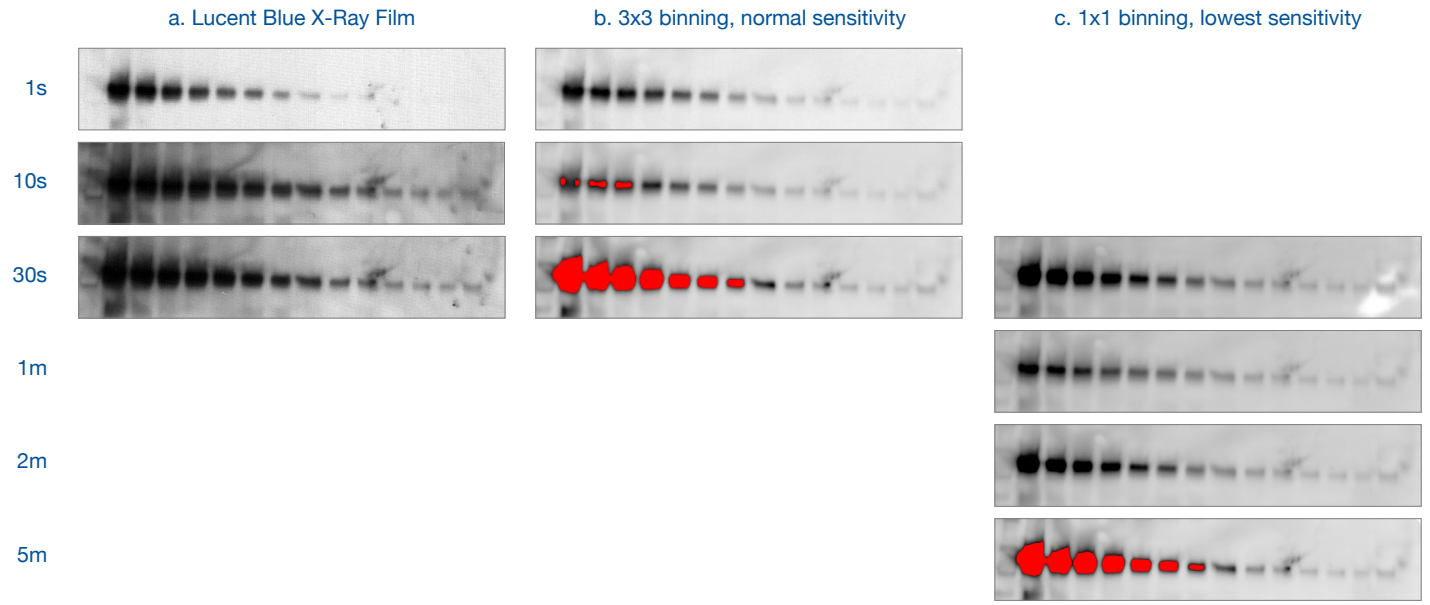

Figure 2. Imaging flexibility, and real-time saturation detection when using the c280 allows for accurate Western blot quantification across a wide dynamic-range. Red indicates signal saturation in digital images. a) Blot exposed to x-ray film. b) Images of the same blot taken using normal sensitivity on the c280 (3x3 binning). c) Images of the same blot taken using lowest sensitivity on the c280 (1x1 binning).

The c280 also allows for simultaneous acquisition of chemiluminescence and visible light color-images. This allows for the overlay of protein standards and molecular weight markers with chemiluminescent bands (Figure 3) allowing for more accurate, consistent and reproducible documentation of molecular weight estimation.

Conclusions

With a real-time display of saturation and a much greater dynamic range when compared to film, digital chemiluminescent imaging opens the door to accurate quantification of Western blots. Furthermore, the user-friendly controls of our cSeries digital imagers and increased sensitivity achievable through digital chemiluminescent imaging can introduce significant time and cost savings into your Western blot workflow.

As such the Azure Biosystems c280 represents an ideal entry point into digital chemiluminescent imaging; bettering the sensitivity of film at an economical price.

Figure 3. Full color image capture of molecular weight standards.

Step	Product	Part number
Electrophoresis & transfer	4-15% Mini-Protean TGX gel	BioRad, 4561086
	PVDF Membrane	Azure, AC2105
	Azure Transfer Buffer	Azure AC2127
Blocking & antibodies	Azure Chemi Blot Blocking Buffer	Azure, AC2148
	Primary Antibody	Millipore, AB3487
	Secondary Antibody, Goat anti-chicken HRP	Azure, AC2117
Imaging	Lucent Blue X-ray film	Advansta, L-07013-100
	Azure Biosystems c280	Azure, AC2801

Table 1. Material and product numbers.

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