Performance characterization of the FluorChem® Xplor in imaging 2D protein electrophoresis dyes and chemiluminescent Western blots

Abstract

Proteomics, the large-scale study of proteins, is often used to identify proteins that change due to disease, or in response to drug treatment. Proteomic studies involve multiple experimental electrophoretic techniques, including 2D and 1D protein electrophoresis, and Western blotting. The FluorChem Xplor is an imaging system developed specifically for the workflow of proteomic studies, with the flexibility to image multiple proteomic sample types. This application note characterizes the performance of the FluorChem Xplor in imaging dyes commonly used in protein electrophoresis, as well as imaging chemiluminescent Western blots. The FluorChem Xplor is shown to match the resolution of a laser scanning imaging system, while providing much faster imaging times, for protein gels. With the additional ability to quickly and accurately image chemiluminescent Westerns, the FluorChem Xplor is a total proteomics imaging solution.

Introduction

Proteomics is the large-scale study of proteins, aimed at the identification of all the proteins in a sample (the proteome), or the identification of those proteins that change in abundance between two samples. Proteomic researchers must be able to identify and characterize hundreds or even thousands of proteins simultaneously. Multiple electrophoretic techniques are important to proteomics laboratories, including 1D and 2D gels and Western blots, and the ability to quickly and accurately image these different sample types is essential.

1D gels are frequently used to check the purity of a protein during a preparation from tissue or after recombinant expression. 1D gels are also used to generate Western blots for the characterization of changes in protein levels, or detection of post-translational modifications. 2D electrophoresis, in which proteins are separated first by charge and then by size, allows hundreds of proteins to be resolved in a single experiment. 2D gels from different samples can be compared to identify proteins of interest, which are then cut from the gel for identification, often via mass spectrometry.

In imaging both protein gels and chemiluminescent Western blots, high sensitivity and a large linear dynamic range are required to obtain accurate and quantitative data. The FluorChem Xplor is an imaging system designed specifically for proteomics workflow, able to image multiple sample types, and with acquisition and analysis software for both 1D and 2D electrophoresis. The FluorChem Xplor is equipped with a scientific-grade, 4.2 megapixel CCD camera, able to capture high-resolution images in seconds. Flexibility in choice of light source and exposure conditions allows the FluorChem Xplor to image commonly used dyes and stains for protein gels, as well as chemiluminescent Western blots, making it a single solution for multiple imaging applications.

Many stains are used to detect proteins in electrophoresis, with two of the most common being Coomassie Brilliant Blue and SYPRO® Ruby. Coomassie is a quick, inexpensive stain that provides a result visible to the naked eye. SYPRO Ruby is more sensitive than Coomassie Brilliant blue, and is more compatible with downstream applications such as immunodetection and mass spectrometry. In this application note, we compare the imaging of Coomassie-stained 1D gels, and SYPRO Ruby-stained 1D and 2D gels by the FluorChem Xplor and a laser scanning system. The FluorChem Xplor is shown to match the resolution and sensitivity of the laser scanning system, while providing much faster imaging times. We also demonstrate that the FluorChem Xplor provides fast, sensitive imaging of chemiluminescent Western blots, with a large linear dynamic range.

Materials and Methods

Electrophoresis:

1D gels: Samples containing decreasing amounts of pure BSA were mixed with 200 mM DTT and 2X Laemmli sample buffer (Bio-Rad) and the samples were boiled at 95 °C for 5 min before loading. The proteins were separated by electrophoresis on 12% SDS-PAGE gels.

2D gels: Mouse liver protein extract was prepared using 2D lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 30 mM Tris-HCl, pH 8.5). Protein concentration was determined using the Bio-Rad Protein Assay (Bio-Rad). 100 µg of mouse liver extract was used for 2D electrophoresis, using a pH 4-7 linear IPG strip.

After loading the samples into the strip holder, a 13 cm strip was placed face down, and 1 ml mineral oil placed on top of the strip. IEF was conducted according to the manufacturer's protocol (GE Healthcare) at 20 °C. Upon completion of IEF, the IPG strips were incubated in freshly made equilibration buffer 1 (50 mM Tris-HCl, pH 8.8, containing 6 M urea, 30% glycerol, 2% SDS, trace amount of bromophenol blue and 10 mg/ml DTT) for 15 minutes with slow shaking. Then the strips were rinsed in freshly made equilibration buffer 2 (50 mM Tris-HCl, pH 8.8, containing 6 M urea, 30% glycerol, 2% SDS, trace amount of bromophenol blue and 45 mg/ml DTT) for 10 minutes with slow shaking. The IPG strips were then rinsed once in the SDS-gel running buffer before being transferred onto an SDS-PAGE gel and sealed with a 0.5% (w/v) agarose solution (in SDS-gel running buffer). The SDS-gels were run at 15 °C and stopped when the dye front reached the bottom of the gel.

Staining:

Staining with Coomassie blue R-250 (Bio-Rad) was done according to the manufacturer's protocol. The gel was immersed in about 500 ml of fix solution at room temperature for one hour, and stained for 1 hour with gentle shaking. The gel was destained in 500 ml destain solution with gentle agitation for 1-3 hours until the background was clear. Then the gel was washed twice with ultrapure water before imaging.

Staining with SYPRO® Ruby (Sigma) was done according to the manufacturer's instructions.

Imaging protein gels:

The 1D Coomassie-stained gel was imaged on three different systems: the FluorChem Xplor (Alpha Innotech), the Typhoon Trio™ (GE Healthcare), and a flatbed scanner. The SYPRO Ruby stained 1D and

2D gels were imaged on the FluorChem Xplor and the Typhoon Trio (a flatbed scanner cannot detect fluorescence). For the detailed imaging settings of each instrument, please see Table 1.

Imaging System	Imaging Settings		
	Coomassie	SYPRO Ruby 1D	SYPRO Ruby 2D
FluorChem®Xplor	XplorBright, Orange filter	XplorUV, Orange filter	XplorUV, Orange filter
Typhoon Trio™	Laser intensity 545 V (normal sensitivity), Detection in Green (532 nm), Scan resolution 100 dots/cm	Laser intensity 545 V (normal sensitivity), Excitation channel 610 BP 30 Detection in Green (532 nm), Scan resolution 100 dots/cm	Laser intensity 545 V (normal sensitivity), Excitation channel 610 BP 30 Detection in Green (532 nm), Scan resolution 100 dots/cm
Flatbed scanner	200 dpi scan	N/A	N/A

Table 1. Imaging conditions for each imaging system and each type of gel stain.

Data analysis:

The 2D SYPRO Ruby images were analyzed using Xpedition™ software (Alpha Innotech). Spots were identified using the default settings, with no spot criteria set. Two spots were chosen that were visible on both the FluorChem Xplor and the Typhoon image, and the peak height and volume of these spots was calculated by the software.

Chemiluminescent detection:

A slot blot containing a 2-fold serial dilution of HRP-IgG conjugate was imaged using ChemiGlow chemiluminescent substrate (Alpha Innotech) and the FluorChem Xplor. Binning was 1x1 (highest resolution) and exposure time was 1 minute. Analysis of the chemiluminescent signal with respect to protein concentration was conducted using the band analysis module with local background correction in AlphaView Q software (Alpha Innotech).

Results

A 1D gel was generated containing dilutions of BSA over a concentration range of 0.1 ng to 10 µg of protein per lane. The gel was stained with Coomassie blue, and imaged with three imaging systems. The resulting images are shown in Figure 1. The brightness and contrast for each image was adjusted to maximize the number of bands that could be seen by eye. The lowest protein amount band that could be visualized by eye is outlined with a red box in each panel. For each of the images, the lowest visible band corresponds to 50 ng.

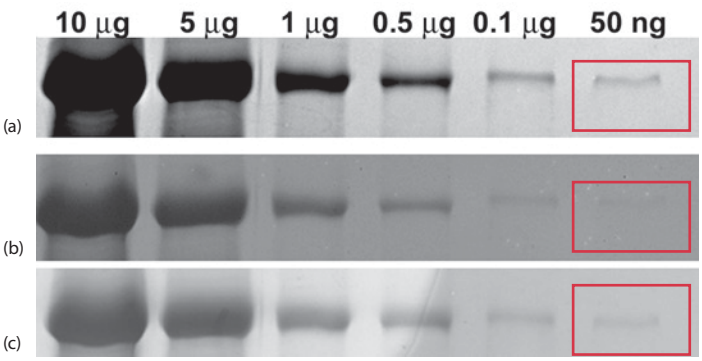


Figure 1. Detection of BSA using Coomassie stain, imaged on three different systems. The same gel was stained with Coomassie blue, and imaged on the FluorChem Xplor (a), the Typhoon (b), and a flat bed scanner (c). The band containing the least protein visible by eye is boxed for each image.

A duplicate gel was stained with SYPRO Ruby, and imaged on the FluorChem Xplor and the Typhoon Trio. As in Figure 1, the contrast and brightness of the images were adjusted to maximize the bands that could be seen by eye (Figure 2), and the band with the lowest protein amount that could be seen is boxed for each image. For SYPRO Ruby, the limit of detection was 10 ng for both imaging systems.

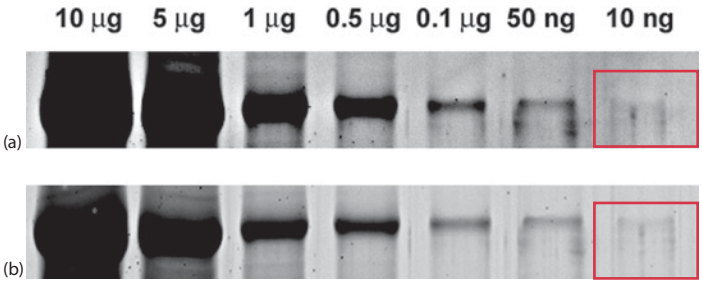


Figure 2. Detection of BSA using SYPRO Ruby stain, imaged on two different systems. The same gel was stained with SYPRO Ruby, and imaged on the FluorChem Xplor (a), and the Typhoon (b). The band containing the least protein visible by eye is boxed for each image.

Imaging System	BSA smallest band visible		Resolution	Imaging time	
	Coomassie	SYPRO Ruby		Coomassie	SYPRO Ruby
FluorChem Xplor	50 ng	10 ng	130 µm	15 sec	0.6 sec
Typhoon	50 ng	10 ng	100 µm	8 min	8 min
Flatbed scanner	50 ng	N/A	120 µm (200 dpi)	Approx 1 min	N/A

Table 2. Comparison of detection limits, resolution, and imaging times for three different imaging systems imaging 1D gels.

The detection limits, imaging times, and image resolutions for each dye and with each system are shown in Table 2.

A serial dilution of HRP-conjugated antibody was spotted onto a membrane, and the chemiluminescent signal was read using the FluorChem Xplor. The 0.3 pg band was detected with a 1-minute

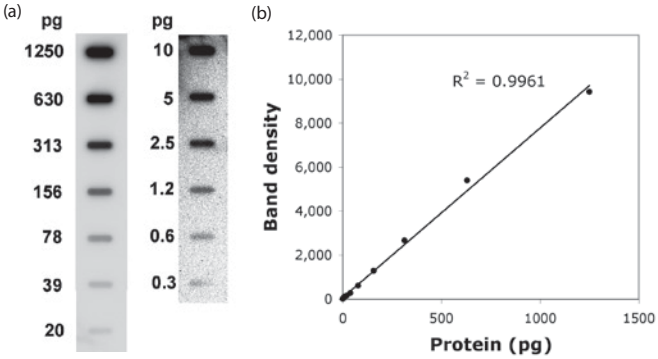
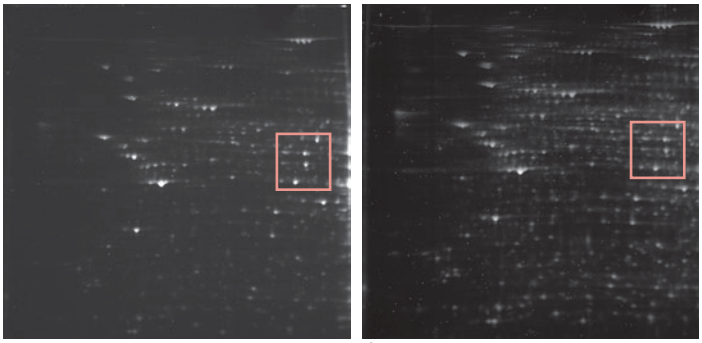
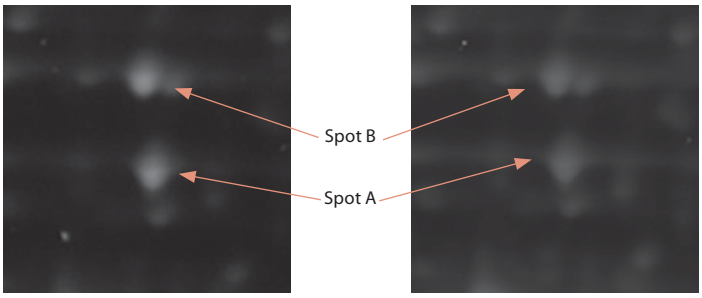


Figure 3. The FluorChem Xplor quickly acquires sensitive images of chemiluminescent signals, with a large linear dynamic range. (a) A slot blot containing a serial dilution of HRP-conjugated antibody was imaged on the FluorChem Xplor with a 1-minute exposure. Two regions of the slot blot are shown with different contrast adjustments, to allow visualization of the entire concentration range. (b) The relationship of the chemiluminescent signal to the protein concentration was linear over the range of 0.3 pg to 1.25 ng.



(a) (b)

Figure 4. Images of a SYPRO Ruby-stained 2D gel imaged with the FluorChem Xplor (a) or the Typhoon Trio (b). The FluorChem Xplor image was captured in 1.4 seconds, while the Typhoon scan took 8 minutes. The boxed areas are expanded in Figure 5, showing the location of two spots that were selected for analysis using Xpedition software.



(a) (b)

Figure 5. Figure 5. Enlarged images of region of a SYPRO Ruby-stained 2D gel imaged with the FluorChem Xplor (a) or the Typhoon Trio (b) containing two spots that were selected for analysis using Xpedition software.

exposure (Figure 3a), and the signal was linear with respect to protein concentration over the range of 0.3 pg to 1.25 ng, a range of 4000:1 (Figure 3b).

A SYPRO Ruby stained 2D gel was imaged with the FluorChem Xplor (Figure 4a) and the Typhoon Trio (Figure 4b). The imaging times were 8 minutes for the Typhoon, and 1.4 seconds for the FluorChem Xplor.

The SYPRO Ruby images were analyzed using Xpedition software. Spot identification was run using the default settings, and two spots were selected for further analysis (Figures 5a and 5b, Table 3). The spot volumes and peak heights were very similar for the two images (Table 3).

Instrument	Spot A (Volume)	Spot B (Volume)	Ratio spots A/B
FluorChem Xplor	2.46E+06	2.33E+06	1.06
Typhoon Trio	2.93E+06	2.79E+06	1.05

Table 3. Volume measurements for two spots from the images of a 2D gel stained with SYPRO Ruby.

Discussion

Proteomics projects involve multiple sample types, including 1D and 2D gels stained with a variety of colorimetric and fluorescent dyes, as well as chemiluminescent Western blots. This note demonstrates the flexibility of the FluorChem Xplor in providing rapid, accurate imaging of each of these samples. Many projects involve imaging multiple samples and comparing multiple images to identify proteins of interest for further study. The FluorChem Xplor captures high-resolution images of 1D and 2D gels in seconds, making it hundreds of times faster than laser scanning systems, improving workflow and throughput.

Coomassie-stained gels can be imaged with many imaging systems, including flat bed scanners, since Coomassie dye is visible to the naked eye. Of the three systems tested in this note, the FluorChem Xplor was fastest in imaging a Coomassie-stained gel (Figure 1) while providing resolution suitable for publication. Speed is important in proteomic research, where multiple samples must be imaged and high throughput is necessary. Additionally, an 8-minute scan time, which was required by the laser scanner, is a very long time to have a large polyacrylamide gel exposed to air. There is no risk of gel drying, shrinking, or cracking with the 1-second imaging time provided by the FluorChem Xplor.

The FluorChem Xplor also matched the Typhoon Trio laser scanner in imaging a 1D gel stained with the fluorescent dye SYPRO Ruby (Figure 2). The FluorChem Xplor image was captured 800 times faster, with an exposure time of 0.6 seconds compared to the 8 minutes required for the Typhoon scan. In both images, the 10 ng band of BSA can be visualized demonstrating high sensitivity as well as resolution.

The FluorChem Xplor provided sensitive imaging of chemiluminescent signals, with a large linear dynamic range (Figure 3). A 0.3 pg band was visible with only a 1-minute exposure (Figure 3a), providing results in less time than would be required if using film, which must be exposed and then developed. Digital images have a larger linear dynamic range than film, providing more quantitative data. Chemiluminescent Western blotting is a common technique used in proteomic studies, and the ability to image these experiments on the FluorChem Xplor means labs can do away with darkrooms, expensive film, and the toxic chemicals used to develop film.

Figures 4 and 5 show images of a 2D gel captured with the FluorChem Xplor (Figures 4a and 5a) and the Typhoon Trio (Figures 4b and 5b). The images are very similar resolution, but the FluorChem Xplor images were captured in seconds, while the Typhoon images required 8 minute scans. This difference in time is significant, especially to laboratories conducting projects that require imaging multiple gels.

The FluorChem Xplor is packaged with Xpedition 2D analysis software, which automatically identifies spots based on user-defined criteria, and guides the user through the alignment of images for comparison, and the identification of spots that differ between images.

Two spots were chosen and the spot volumes measured using Xpedition software for the images captured by the FluorChem Xplor and the Typhoon (Figure 5, Table 3). The measured values are very similar for the two images, indicating the similarity of the quantitative nature of the two images.

In conclusion, the FluorChem Xplor is demonstrated to be a sensitive, fast imaging system with the flexibility to image multiple proteomic applications including Coomassie-stained gels, SYPRO-Ruby stained gels, and chemiluminescent Western blots. Rapidly providing high-resolution images of multiple sample types, the FluorChem Xplor is a complete imaging solution for the proteomics laboratory.

Acknowledgements

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The FluorChem Xplor imaging system is designed to be a total solution for the proteomics laboratory, offering rapid, high-resolution, and quantitative imaging of 1D and 2D gels, as well as chemiluminescent Western blots. Application-driven image acquisition software allows switching between different sample types with a click of a button. Xpedition™ 2D analysis software guides the user through a streamlined workflow to process, compare and analyze 2D gel data.

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