Analytical Biochemistry xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Notes & Tips

# Acquisition of chemiluminescent signals from immunoblots with a digital single-lens reflex camera

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ARTICLE INFO

*Article history:*
Received 31 July 2009
Available online xxxx

ABSTRACT

We found that certain mid-range consumer-level digital single-lens reflex (SLR) cameras using full-frame complementary metal oxide semiconductor (CMOS) sensors outperform X-ray film in acquiring signals from immunoblots that use enhanced chemiluminescence for detection. These cameras exhibit a sensitivity comparable to X-ray film, yet they provide a 3-fold increase in linear dynamic range and substantial cost savings over time, are more convenient to use, and eliminate the chemical waste associated with processing film.

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Due to its high sensitivity, enhanced chemiluminescence (ECL)[1] is currently the dominant signal-generating method for immunodetection of proteins via Western blots and dot blots [1]. Signal acquisition is typically accomplished with either X-ray film or specialized cameras that employ cooled charge-coupled device (CCD) sensors. X-ray film is more popular because of its greater sensitivity and/or resolution for a given exposure time and because it requires only a modest expenditure for equipment. The main drawbacks of film are its limited and nonlinear dynamic range and the fact that automated film processors are expensive to maintain and generate chemical waste. Dedicated CCD-based imagers exhibit a significantly better dynamic range and linearity than film and do not generate chemical waste, but their combination of sensitivity and pixel resolution is generally inferior to film. Moreover, the cost of these imagers ($30,000–60,000 US) is out of the range of many academic research labs.

Several new single-lens reflex (SLR) digital cameras in the mid-range consumer market have been shown to achieve remarkable low-light sensitivity using high-resolution, full-frame 35-mm sensors based on complementary metal oxide semiconductor (CMOS) technology in combination with advanced noise reduction firmware. Here we report a comparison of one such camera versus X-ray film for acquiring ECL signals from dot blots and Western blots.

In our first test, we spotted serial dilutions (in triplicate) of horseradish peroxidase-coupled antibody (HRP–Ab) directly onto two separate, but identically treated, nitrocellulose membranes. The membranes were developed with a commercial ECL reagent kit. One was imaged by a 4.0-min exposure to a Nikon D700 SLR camera fitted with a 50-mm f/1.4 lens and a 12-mm extension tube, and the other one was imaged by a 4.0-min exposure to X-ray film. To process the SLR image, the resulting 14-bit RAW file (produced in parallel with an 8-bit JPEG file) was opened in the Camera RAW application that is included with Adobe Photoshop CS3. Exposure and black levels were optimized, and the image was then ported into the main Photoshop application where the blue light channel only was used to create a grayscale white-on-black TIFF. The TIFF was opened in the ImageJ (National Institutes of Health) application for quantitative analysis of dot densities. A reversed (black-on-white) version of the TIFF (Fig. 1A) was also generated in Photoshop for visual comparison with the X-ray film. To digitize the film image, it was photographed with transillumination using the same D700 SLR camera. The resulting 14-bit RAW image was opened in Camera RAW, and the default exposure and black level settings were accepted prior to importing the image into the main Photoshop program. The film image was then converted to a grayscale TIFF (with no color filtering) (Fig. 1B) and also to a reversed (white-on-black) version of the same TIFF for quantitative analysis in ImageJ. The image processing procedures outlined above were designed to achieve maximum dynamic range and linearity of the integrated density data and are described in more detail in the Supplementary material.

The greater dynamic range of the SLR camera compared with X-ray film is apparent by comparing dots containing 0.156 and 1.25 nl of HRP–Ab in Figs. 1A and B. At 1.25 nl, the film image appears to be darker than the SLR image. In contrast, the 0.156-nl dot from the SLR appears to be darker than the corresponding dot on the X-ray film. These subjective impressions were confirmed by ImageJ

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[1] *Abbreviations used:* ECL, enhanced chemiluminescence; CCD, charge-coupled device; SLR, single-lens reflex; CMOS, complementary metal oxide semiconductor; HRP–Ab, horseradish peroxidase-coupled antibody; CRMP2, collapsin response mediator protein 2.

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doi:10.1016/j.ab.2009.09.041

Please cite this article in press as: M.K. Khoury et al., Acquisition of chemiluminescent signals from immunoblots with a digital single-lens reflex camera, Anal. Biochem. (2009), doi:10.1016/j.ab.2009.09.041

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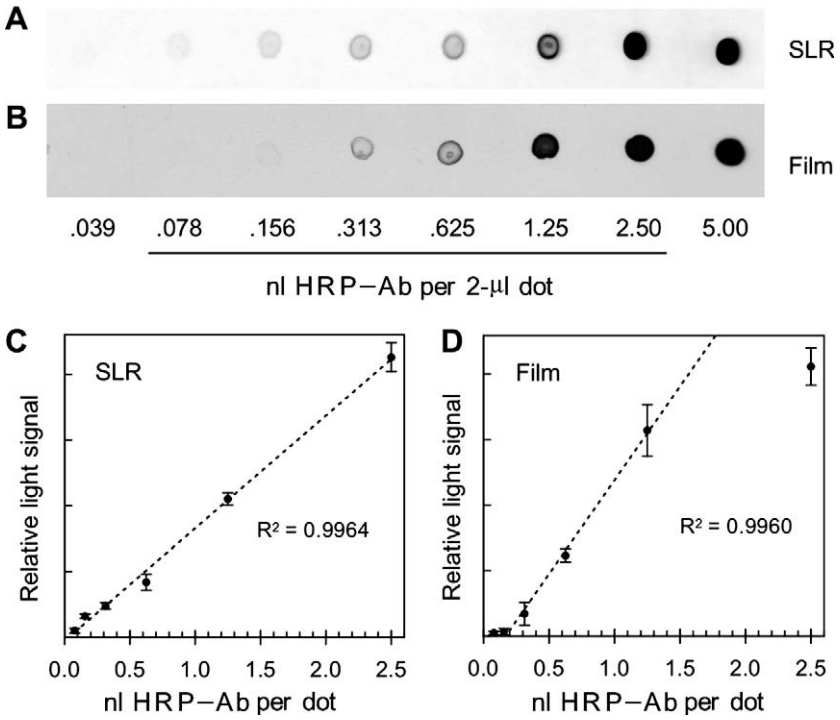


**Fig. 1.** Comparison of dot blot data obtained with the D700 SLR camera versus X-ray film. Serial dilutions of HRP–Ab were spotted onto nitrocellulose and processed for ECL as described in the text. The ECL signals were recorded in 4.0-min exposures using either the D700 SLR camera (A and C) or X-ray film (B and D). (A and B) Representative dot blot images obtained with the camera (A) and X-ray film (B). These are black-on-white versions of the same TIFFs used for the ImageJ analyses that generated the plots in panels (C and D). The underlined dilution numbers indicate the range of data that were plotted. The highest dilution (0.039 nl HRP–Ab) was not plotted because it was below the limit of detection in both sets of dot blots. The lowest dilution (5.0 nl HRP–Ab) was not plotted because the images were oversaturated in both sets of dot blots. (C) Plot of ECL signal versus amount of HRP–Ab recorded with the camera. Each point is the average signal of three blank-corrected triplicate dots, with standard deviations indicated by error bars. The digital RAW image from the camera was processed for maximum dynamic range (as described in the text), and the resulting TIFF was analyzed by ImageJ. (D) Plot of ECL signal versus amount of HRP–Ab recorded on X-ray film. Each point is the average signal of three blank-corrected triplicate dots, with standard deviations indicated by error bars. The film was digitized using the SLR camera with transillumination, and the resulting RAW image was converted to a white-on-black TIFF for analysis by ImageJ. The regression coefficient ($R^2$) shown in panel (C) is based on a fit to all six data points relative to the dashed line; this was generated by the linear trend line function in Microsoft Excel. The same applies to panel (D) except that the lowest and highest points in that plot were excluded from the calculation.

density analysis. The SLR camera produced a plot of integrated density signal versus amount of HRP–Ab spotted that was linear over a 32-fold range (0.078–2.50 nl) of HRP–Ab (Fig. 1C). In contrast, the X-ray film produced a plot that was markedly sigmoidal, with a linear range of only 10-fold (0.156–1.50 nl) (Fig. 1D).

A second test of ECL image acquisition with the D700 SLR versus X-ray film was carried out on a Western blot that employed a series of dilutions of mouse brain extract immunostained for collapsin response mediator protein 2 (CRMP2). In this experiment, the same blot was imaged on film for 20 s (Fig. 2B) and, a few minutes later, was imaged with the D700 SLR for 60 s (Fig. 2A). A RAW-to-TIFF conversion of the SLR image file (Fig. 2A, upper panel) was carried out as described above and then analyzed with ImageJ to generate a plot of signal intensity versus protein load (Fig. 2C). Similarly, a TIFF file of the corresponding X-ray film image was generated as above and used to create the plot shown in Fig. 2D. The SLR data were linear over a 32-fold range of protein load (0.125–4.0 µg), whereas the film data were linear over a 10-fold range (0.25–2.5 µg).

For higher protein loads (0.50–8.0 µg), the SLR TIFF image showed less intense bands than the corresponding film image. This is due to desaturation applied to the SLR RAW file using the exposure control available in the Camera RAW application. A contrast-enhanced JPEG image of the same SLR exposure is shown in the bottom panel of Fig. 2A. This image closely resembles that of the X-ray film (Fig. 2B) but, like the film, has a reduced range of linearity. For qualitative comparison or detection of antigen bands, enhanced JPEGs from the SLR are advantageous.

The camera used in our study has adjustable ISO (light sensitivity) settings that range from 100 to 25,600. To see how light sensitivity and overall image quality are interrelated, we took a series of 1.0-min exposures of a single Western blot (similar to that shown in Fig. 2) at four different ISO settings (800, 1600, 6400, and 25,600) from the high end of the available range. The results are shown in the Supplementary material (Fig. S2-1). ISO 25,600 was most sensitive by a small margin, but ISO 6400 gave the best overall ratio of signal to background. We were surprised to find that a qualitatively usable image could be obtained even at an ISO setting as low as 800.

We have demonstrated that a moderately priced digital SLR camera designed for the mid-range consumer/professional photography market is comparable to X-ray film in terms of sensitivity and is clearly superior in dynamic range. Dedicated CCD-based imagers with similar performance have been available for several years, but their cost is typically 10–15 times that of the type of camera used here. The key factors in successful application of this technology are (i) a camera with minimal sensor noise at high ISO settings, (ii) a wide aperture lens and a short subject-to-camera distance so as to maximize light capture, (iii) effective noise reduction firmware in the camera, and (iv) the ability to capture and process RAW images. We presume that there are several digital SLRs on the market that can be used to record ECL signals with good sensitivity and integrity, but not all cameras with equivalent high ISO settings alone will produce equally good images. Sensor design and noise reduction firmware vary between brands and models,

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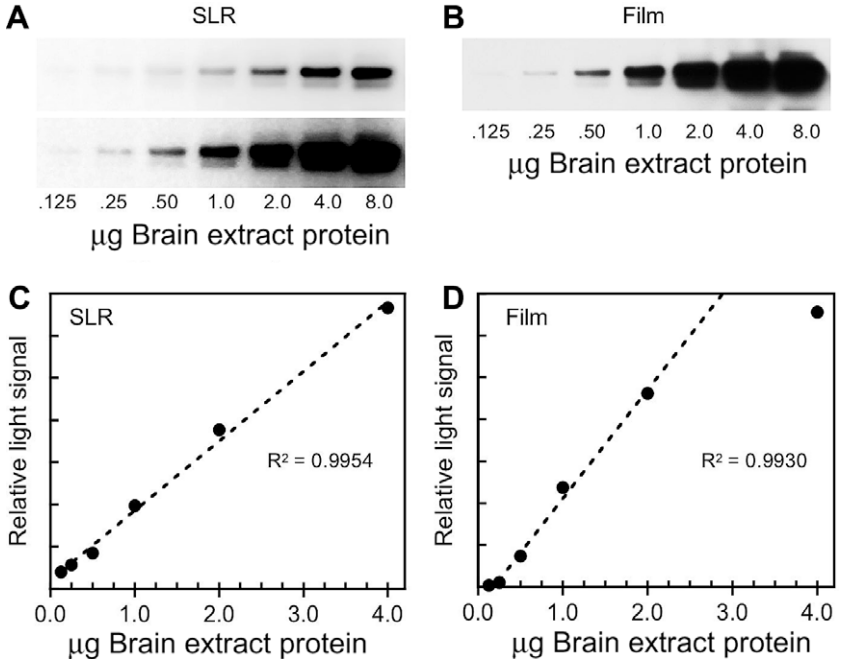


**Fig. 2.** Comparison of Western blot data obtained with the SLR camera versus X-ray film. Serial dilutions of mouse brain extract were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blotting for CRMP2 by ECL as described in the text. The same blot was used in all four panels. It was first exposed to X-ray film for 20 s and, approximately 5 min later, was imaged with the camera in a 60-s exposure. (A) Upper panel: Image file recorded by the camera and processed for maximum dynamic range for the plot shown in panel (C). Lower panel: contrast-enhanced JPEG version of the same exposure. (B) Digitized X-ray film image used to generate the plot in panel (D). (C) Plot of ECL signal versus amount of brain extract recorded with the camera. (D) Plot of ECL signal versus amount of brain extract recorded on X-ray film. The regression coefficient ($R^2$) shown in panel (C) is based on a fit to all six data points relative to the dashed line; this was generated by the linear trend line function in Microsoft Excel. The same applies to panel (D) except that the lowest and highest points in that plot were excluded from the calculation.

and these factors play an important role in image quality, especially at the higher ISO settings.

A sometimes forgotten disadvantage of imaging blots on X-ray film is the cost of maintaining and servicing processors that are often used to develop the film. In our recent experience, this has averaged $4700 per year per machine. Over a 5-year period, replacing one film processor with a $3500 digital camera/lens setup is expected to generate a net savings of approximately $20,000 (not including the extra savings in reduced X-ray film purchase). As our university campus shifts from film to SLR cameras for ECL, we also expect to eliminate 70% to 80% of the 1000 gallons per year of silver-laden hazardous waste generated in the form of spent fixer and to save significant amounts of water that continuously pass though these machines when they are on. The elimination of X-ray film will also decrease the need for darkrooms. Although we currently use a darkroom to capture ECL signals with the SLR camera, it would be easy to make a portable light-tight enclosure (similar to those that house dedicated CCD imagers) so that image acquisition can be carried out on the bench of a normally lit laboratory. Moreover, some digital SLR cameras in this performance class can be connected directly to a computer, by either USB cable or wireless adapter, allowing remote control of the camera settings as well as rapid image download.

## Acknowledgments

This work was supported by National Institutes of Health (NIH) Grant NS17269 and funds from the Faculty Research and Travel Committee, School of Biological Sciences, University of California, Irvine. D.W.A. thanks Chris Benes for initially suggesting that recent advances in digital camera technology might provide the sensitivity needed to image the ECL reaction, and all of the authors thank Kelsey Goodman and Kyle Horvath for preparing Western blots used in an initial proof-of-concept experiment. None of the authors has any financial interest in or business relationship with any of the companies whose products are mentioned in this article.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ab.2009.09.041. Q1

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