Why You Should Leave the Darkroom

Introduction

Change is hard. It takes work to adapt to a new technology and to get used to something different. It's especially hard if you have something that already seems to work. This is a big reason people still use film and darkrooms to process chemiluminescent western blots. It's familiar, sensitive, and simple. But now, so is digital imaging. The time has come to get out of the darkroom.

Are these familiar excuses?

"I really like film. It's what I grew up with!"

We all cling to methods we're most comfortable with in the lab. New things can be expensive, they take time to learn, and they can take time to optimize for your lab's specific purposes. If it's not broken don't fix it, right? The use of film for chemiluminescent western blots might not seem like an outdated method yet, but it's quickly getting there.

Digital imaging hardware and software are becoming more popular and surpassing film-based methods for gathering data. Compared to film, digital imaging offers wider dynamic range and more accurate quantitation. With the increasing pace of discovery and tougher funding situations, everyone needs to be doing the best data analysis possible – and this means accurate quantitation.

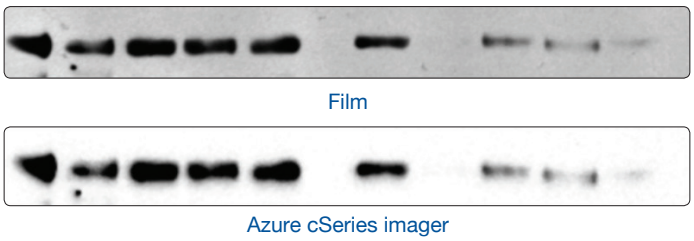

Figure 1. The bands on the top are from a chemiluminescent western blot film, scanned. The bottom bands are from the same western blot, taken on an Azure cSeries western imager about one hour later.

"I tried an imaging system years ago and I wasn't impressed by it."

Technology has advanced a lot these past few years, both in hardware and software – meaning, digital imagers now have the same or better **performance quality** as film (Figure 1).

What has caused this leap in performance? Firstly, **higher resolution** CCD cameras enable you to see fine detail in your image when you zoom in. The larger the number of pixels, the greater the resolution.

Pixel binning is also possible with digital imagers (Figure 2), which provides a **better signal to noise** ratio. A CCD camera can combine multiple pixels into a single larger pixel, or "super pixel." Binning of 1X1 means the full resolution of the camera is used to capture an image, while a binning of 2X2 means that the areas of 4 adjacent pixels are combined into one larger pixel, and the sensitivity to light is increased 4 times due to the 4 pixel contributions.

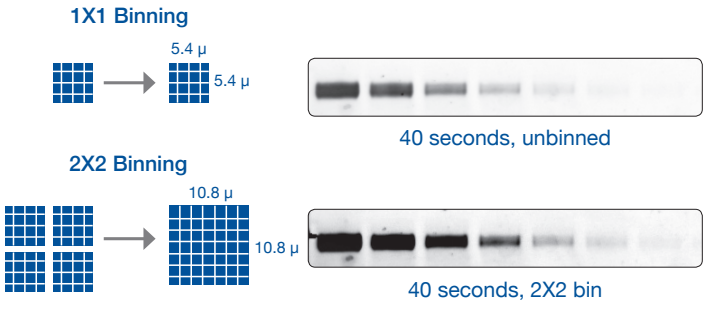

Figure 2. Binning is a powerful technique for digital imaging of chemiluminescent western blots. Binning increases sensitivity by combining pixels to make a larger, extra sensitive pixel with a better signal to noise ratio.

Secondly, improved camera properties include aspects of the lens, such as the f-stop or aperture. The f-stop is an important value to consider, especially for chemiluminescence; and the new digital imagers have a **small f-stop and wide aperture**. The smaller the f-stop value, the wider the aperture, and the more light that can be let in. This drastically reduces exposure times. Lenses are now available on CCD imaging systems at f 0.95.

Importantly, **wider dynamic range** is possible with today's systems, offering the ability to see over three orders of magnitude in one western blot (Figure 3). A wide dynamic range means lower quantities of protein can be accurately detected at the same time as proteins of much higher concentration. Digital imaging has the added benefit of detecting signal saturation, which occurs when the signal intensity is so bright that its measurement is not linear to the concentration. Without a wide dynamic range, strong signals will saturate before you can detect your weak bands, making quantitation impossible. Digital imaging systems for chemiluminescent are typically 16 bit, meaning the camera can see over 65,000 shades of grey.

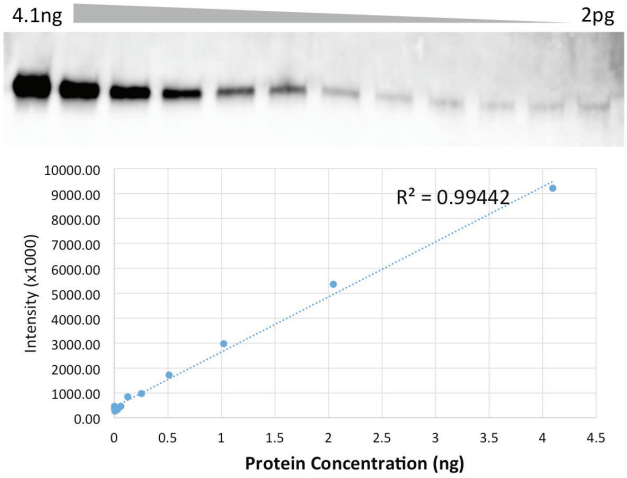

Figure 3. Digital images offer signal linearity, increased dynamic range, and a low limit of detection. The chemiluminescent signal on a western blot of a serial dilution of transferrin was imaged on an Azure c600. Quantitation of the bands demonstrates a linear signal with a dynamic range over three orders of magnitude and a limit of detection of 2pg.

"I like having that physical image on the film."

It's a great feeling, seeing crisp bold bands stand out on a warm film fresh out of the developer. On the way back to the lab you can proudly wave your film around to show labmates new data and then slide it into your thick binder of previous films.

But that's not a good excuse when a thermal printer can be hooked up to your digital imager to provide the immediate satisfaction of physical data. Film is bulky and hard to file in a standard notebook. And what about the growing trend of digital lab notebooks? Moreover, with a digital image you have **publication quality** data ready for immediate analysis, without the extra step of scanning your film.

What are the real advantages of digital imaging?

"What can a digital imager do that film can't?"

Quick and easy **quantitation** is one of the main reasons to leave film behind. With today's technology and the increasingly detailed questions being asked in biology, people want quantitative answers. That western blot film might hint at a difference in protein expression levels, but in order to determine the significance of those bands, you would need to scan the film, open an image analysis program, and measure density of the bands. With digital imaging there are no additional steps between obtaining the data and beginning quantitative comparisons.

Besides the convenience of forgoing the film development and scanning steps, how do you know whether or not the **bands are saturated**? On film, chemiluminescent signals are easily saturated because of the small dynamic range of film, and this prevents accurate quantitative analysis. Digital imaging software can allow you to visualize saturation and adjust your exposure time accordingly (Figure 4). With film, however, there is no way to know that a signal is saturated.

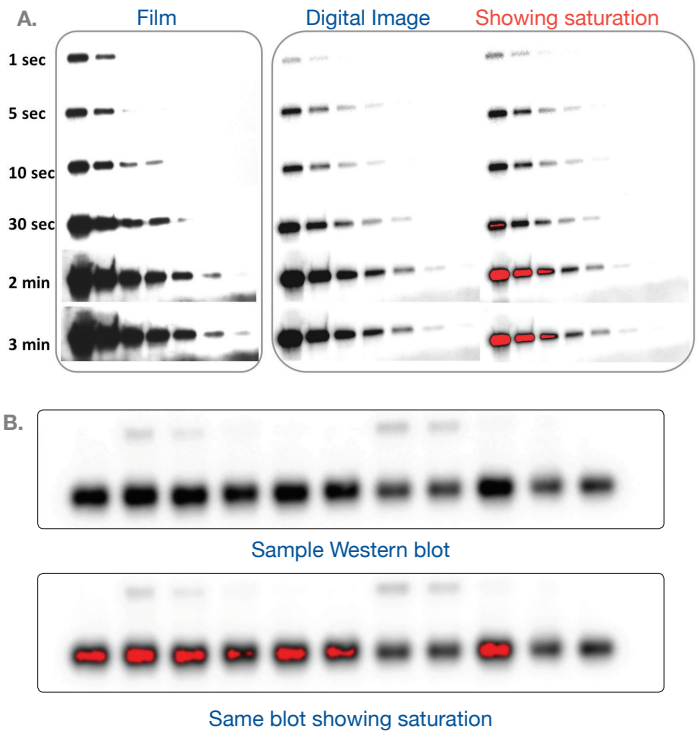

Figure 4. Illustrated here is an examlpe of band saturation after prolonged exposure of a chemiluminescent signal, which is easily revealed with a digital imager. A. The same blot was imaged on both x-ray film and the Azure c600. The Azure c600 detects when saturation occurs and calculates an auto-exposure time to avoid saturated bands. B. On the top is a sample western blot showing a variety of band intensities. You may want to compare intensities, but by viewing saturation on an imager, we can see that this blot needs a shorter exposure before accurate comparison can be made.

This brings us to the issue of detecting signals with widely different intensities. What if a small population of your protein of interest has post-translational modifications or small cleavage products? It can be a guessing game finding the right exposure time with film to prevent saturation and avoid interference with nearby bands. Digital imaging provides a **wider dynamic range than film**, so low- and high-intensity bands can be imaged simultaneously (Figure 3).

The ability to obtain signals within a wide dynamic range without saturation is what makes digital imagers superior for quantitative analysis of western blot data. Figure 5 demonstrates the difference between film and digital imagers for quantitation. The brightest bands on the film are saturated because the measured signal is no longer linear with its concentration when quantified. Data from the same conditions obtained with the digital imager, however, is still in linear range and can be accurately quantified.

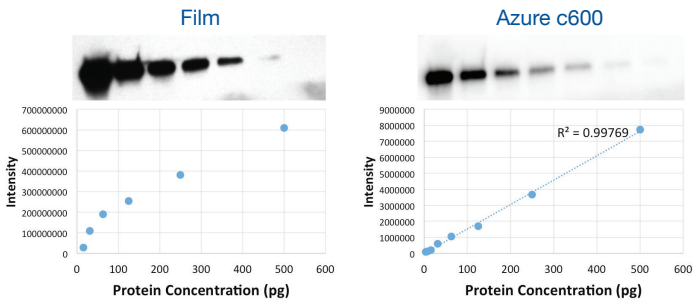

Figure 5. An illustration of the difference in linearity between a digital image and x-ray film. For accurate quantitation, signals must be within linear range of each other – the bright signals from the film are saturated and no longer linear. The same blot was imaged on both Lucent Blue X-ray Film and the Azure c600 for 1 minute, and the correlation between the signal and protein concentration is indicated in the two diagrams.

Then there are the advantages of **multiplexing**. Truly quantitative multiplexing using a single blot is something you can only perform with digital imaging systems, where the different channels allow you to use different fluorescent probes to recognize and image different proteins of interest on the same blot at the same time (Figure 6). Besides convenience and time saved by not needing to strip and reprobe the blot, multiplexing lets you distinguish between signals on a small blot like film never could.

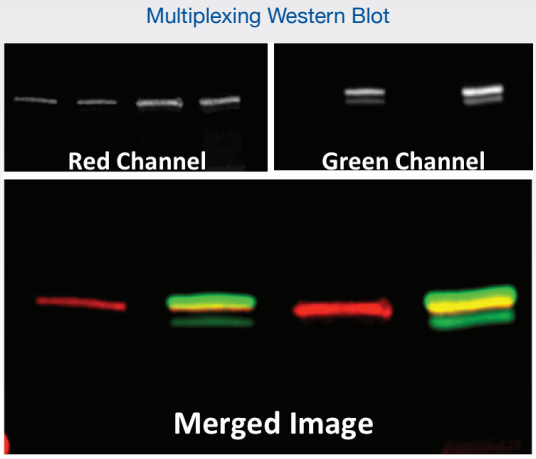

Figure 6. Multiplexing on a single blot is a unique method available with digital imagers. Here, a western blot has been probed for both STAT1 (red channel) and phosphorylated STAT1 (green channel) using fluorescently conjugated secondary antibodies and imaged using infrared detection on the Azure c600. This information would involve many more steps and much more time using film.

And what about costs?

"But a digital imager is too expensive."

The initial purchase of an imager is an investment, but there are a number of things to consider when talking costs, such as the cost of chemical waste. Film developers generate gallons of waste that either go down the drain or have to be specially disposed of as hazardous materials.

Film can get expensive when you add it up (Table 1), and its light-sensitive nature means one accidental flip of the light switch in the dark room and the whole pack could be ruined. Additionally, as institutions move away from darkrooms, film and developers are becoming scarce and increasing in price.

Lab costs for film	**(200 – 800 sheets per year)**
Film	$3,091 – $12,355
Dark room fees ($2 per blot)	$400 – $1,600
TOTAL	**~ $3,000 – $14,000**
Department costs for film processor	
Maintenance contract	
Chemicals	**>$2,500 per year**
Extra repairs	

Table 1. The costs of film-based western blots.

Furthermore, when it comes to maintaining a darkroom, it can be a headache for the person and department in charge. For Brenda Franklin and the Microbiology department at the University of Michigan, they realized they were starting to lose money maintaining their own darkroom and are in the process of removing it.

"The service contract on the processor includes monthly cleaning and inspection, and then you have to purchase chemicals. In total it's about $2500 per year. But the contract doesn't cover user error. We are constantly repairing the machine and usually have about one extra service bill per month. About half the time it is because it's old and half the time its user error, like someone jams the machine or a roller breaks. The repairs come from the high turnover of new users that just don't get properly trained. Another pitfall of the darkroom is dealing with the fixer, which adheres to the drain and builds up quickly. If we don't have it snaked out once a month, the drain malfunctions and can destroy property. The other option is to collect the waste and it has to be handled as chemical waste and go through hazmat… There just aren't enough users for our darkroom to be worth it. Everyone is going digital, so they are really becoming obsolete."

Final Word

A final point that shouldn't be overlooked is the convenience of options you have with a digital imager. Not only can you image and analyze chemiluminescent western blots, but imaging options can include fluorescently labeled probes, UV imaging for DNA gels, and visible light for other gel types. One digital imager can replace the need for a darkroom, a UV or visible light box, and a separate scanner and image analysis software.

The major advantages of a digital imager over film are the ability to detect saturation and the wider dynamic range that helps avoid saturation in the first place. Both of these features provide more accurate quantitative comparisons between bands than could be achieved from data on film. Furthermore, compared to ancestral versions, the new digital imagers have greatly improved performance that can compete with or even surpass the sensitivity of film. In terms of cost, a digital imager will pay for itself in a couple years by eliminating darkroom and film expenses, and better data is priceless.

While film has been a stalwart companion, darkrooms are becoming obsolete. It it time to step into the future – into the new age of digital imaging.

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