Focal Points

Application Note FP-127

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Quantitative DNA and Protein Analysis with a Highly Uniform UV Illumination Imaging System and Fluorescent Calibration Step Tablet

Overview

Ultraviolet (UV) transillumination is a ubiquitous tool in Life Science research. With few exceptions, fluorescent stains used in post electrophoresis analysis of proteins and nucleic acids have significant excitation peaks with ultraviolet (300-365 nm) light, making midrange UV the excitation source of choice for high sensitivity analysis for many fluorophores. However, quantitative analysis is limited by the extreme lack of illumination uniformity across the surface of typical UV light boxes. To enable quantitative UV imaging, UVP has developed and characterized the FirstLight™ UV Illuminator, a highly uniform UV transilluminator. Through use of this high density lighting system with its tuned phosphor coating, uniformity of <5% coefficient of variance (CV) across the full imaging surface has been achieved and applied to proteomic analysis. Combined with the Fluorescent Calibration Step Tablet, the FirstLight Uniform UV Illuminator provides unparalleled accuracy in day-to-day quantitative imaging of protein and DNA gels.

Introduction

Presently, the use of digital fluorescent imaging for both documentation and analysis of electrophoretic separations is commonplace in biological research laboratories [1-5]. Applications range from In Vivo imaging to protein and DNA gel documentation and analysis -. With the introduction of cooled low light and high-resolution CCD cameras, CCD image capture has become an attractive alternative to laser scanning based approaches.

Digital Fluorescent CCD imaging has a number of advantages, including:

- Low capital cost compared to laser based scanning
- High detection sensitivity
- Wide dynamic range
- Rapid signal acquisition by low noise CCD cameras (typically msec to seconds)
- Availability of a wide range of highly sensitive stains for protein and nucleic acid analysis
- Rapid multiplex analysis of proteins (multiple fluorescent signatures from a single gel), greatly simplifying the analysis of protein expression, turnover, and posttranslational modifications after one and two-dimensional SDS PAGE separations

Although CCD cameras greatly simplify image capture, uniform UV Illumination is critical for quantitative analysis. The FirstLight UV Illuminator (Figure 1) is unique in providing highly uniform (<5% CV) excitation light across the gel. This ensures:

- Sensitivity and dynamic range are consistent across the illumination surface
- Little or no reliance on uniformity correction by software that can lead to low signal data loss
- Straight forward gel to gel comparison

Figure 1. FirstLight UV Illuminator

In comparing the FirstLight UV Illuminator to a standard UV transilluminator, Figure 2 shows the high uniformity of across the imaging surface of a FirstLight Illuminator. The improved uniformity of the FirstLight UV Illuminator is also evident from visual inspection and band quantitation of fluorescently stained gels such as SYPRO Ruby, Ethidium bromide and SYBR green. Figures 4 and 5 show data from a SYPRO Ruby stained protein gel.

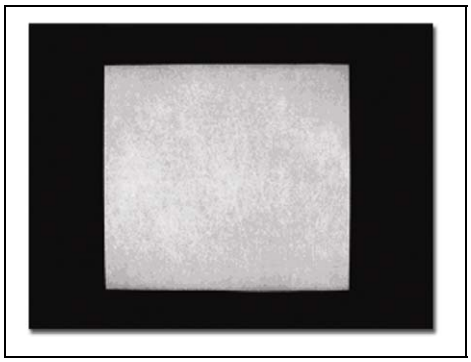

Figure 2. FirstLight UV Illuminator shows <5% CV

In contrast, the lack of illumination uniformity (CV >80%) across the filter surface of typical UV light boxes is illustrated in Figure 3. The peak intensity is associated with the individual UV bulbs typically used for UV light boxes, which leads to position dependent variability in fluorescence of stained sample (figure 5).

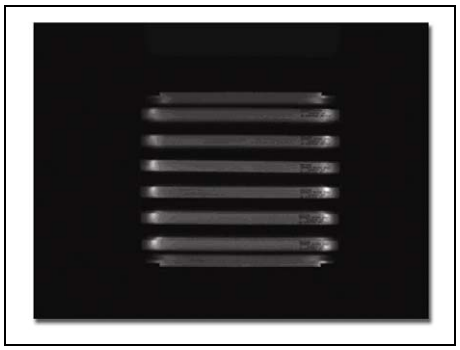

Figure 3. M-26X Transilluminator is very uneven and shows 81.8% CV Full Area, 84% CV Inner Area

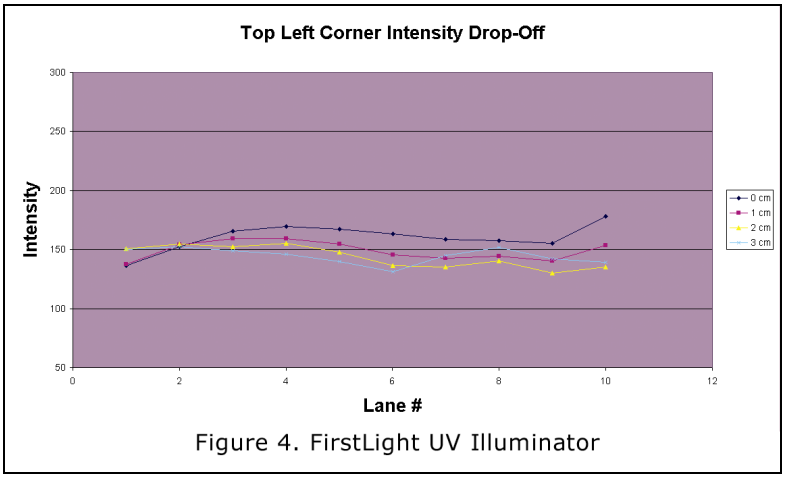

Figure 4. FirstLight UV Illuminator

Figure 4 and 5. Intensity Profile of electrophoretically separated Protein Standards: Comparison of FirstLight Illuminator and 8-Watt Transilluminator. Equal amounts of protein were loaded per lane, stained with SYPRO Ruby, and the individual protein fluorescence intensity was quantitated. Note the severe loss of signal toward the edge of the typical nonuniform UV light table (Figure 5) compared to the uniform FirstLight illuminator (Figure 4).

Figure 5. Eight Watt Transilluminator

With the availability of a highly uniform UV illuminator, calibration standards can be used accurately to monitor the day-to-day reproducibility of the imaging system. UVP's Fluorescent Calibration Step Tablet (Figure 6) is ideal for confirmation of capture settings and day-to-day normalization of the images. The step tablet provides a calibrated reference with a 21-step gradation with density from total clear (step 1) to totally opaque (step 21).

The step tablet offers consistent imaging results allowing performance verification of:

- Lens settings
- Exposure settings
- Illumination intensity
- Sample integrity
- Sample amount
- Staining chemistry

The method below describes the use of the Fluorescent Calibration Step Tablet.

Equipment

- Fluorescent Calibration Step Tablet (Figure 6)
- FirstLight™ Uniform UV Illuminator
- Biospectrum®AC Imaging System
- VisionWorks®LS Analysis Software

Figure 6

Electrophoresis. DNA standards were separated by agarose gel electrophoresis and stained with ethidium bromide according to standard protocols (5). The BioSpectrumAC was used with VisionWorksLS analysis software to automatically set and control the imaging darkroom, camera, filters and software acquisition and analysis parameters.

Procedure

Using the Step Tablet for DNA gel analysis using the Firstlight UV Illuminator. The fluorescent calibration step tablet is placed on the UV Transilluminator next to a gel with electrophoretically separated and fluorescently stained nucleic acids or proteins. The UV light causes the step tablet to luminesce, giving a series of intensity calibration steps as a reference in each image.

Ideally, for a standard separation, the conditions should be the same from image to image. However, changes in lens settings (e.g., aperture, focal length, focus), emission light filtration, UV excitation light intensity, can all affect the intensity of the DNA bands on the gel being analyzed. Fortunately the step tablet

responds in a linear fashion to the same factors as the fluorescent stained bands on the gel. Thus, any changes in the calibration step intensities would indicate a variance from the standard.

The response of the tablet to changes in both exposure and UV intensity is linear (Fig. 7 and 8), so the tablet can also be used to normalize day-to-day variation. The protocol below illustrates how normalization is accomplished.

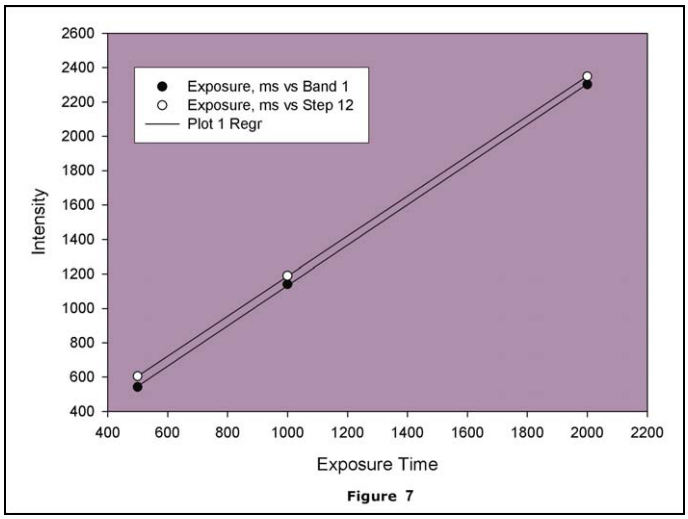

Figure 7. The step tablet responds linearly to increases in UV exposure. Exposure time and intensity comparison of DNA Band 1 (lane 2) from the gel and Step 12 from the Fluorescent Calibration Step Tablet in figures 9 and 10 above. Note the increased exposure leads to simultaneously a brighter image of the DNA fragment and Step 12 of the calibration tablet. Step 12 is also good substitute for a typically stained fragment of DNA.

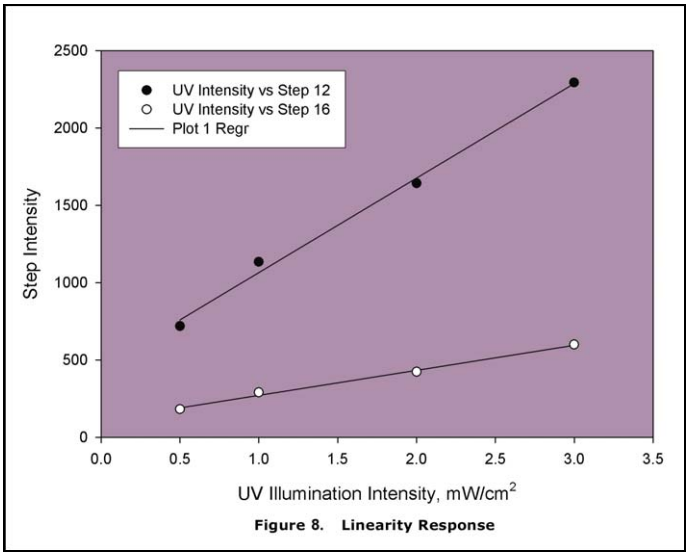

Figure 8. The step tablet responds linearly to increases in UV intensity. The response of steps 12 and 18 to increased UV intensity using a variable intensity FirstLight Illuminator are shown. UV Intensity at 302 nm was measured using a UVP NIST traceable UV calibration meter (**UVX-31).**

To use the step tablet:

1. Place step tablet on the FirstLight Illuminator next to the gel being analyzed.
2. Select the appropriate emission filter for the fluorescent stain in use (e.g., ethidium bromide would use the 605 nm filter). When performing day-to-day calibrations, always use the same emission filter.
3. Capture the fluorescent image of the gel and step tablet using the appropriate filter, exposure time and aperture. Note the settings and presets so the same conditions can be used in subsequent image capture.
4. Using VisionWorks Area Density Tool, determine the average pixel intensity of step 12. Depending on the brightness of the fluorescent DNA or protein image, other steps (typically from 9-15) can be used as the day-to-day reference.
5. Using VisionWorksLS Area Density Tool, determine the average pixel intensity of DNA band of interest in the same image as the calibration step tablet.

6. Repeat steps 1-3 for each new fluorescent DNA or protein gel. The reference step in this example, Step 12, can then be used to normalize (adjust) the intensity of the band of interest up or down. For example, if the reference step is 10% higher, then the intensity of the band of interest should be adjusted up 10% in order to get an accurate comparison to the gels from previous images. This particularly useful for day-to-day and month-to-month calibration checks. Note that the step tablet compensates for both changes in exposure and excitation light intensity. The identical zoom, aperture, and exposure time settings should give the same intensity on a specific step of the step tablet, assuming the UV light is the same intensity. If the step intensity is the same, any differences in the nucleic acid intensity will indicate variation in the staining process, more or less nucleic acid or protein, or degraded reagents.

Figures 9 and 10 show the image of the step tablet along side the same gel captured at two different exposure times.

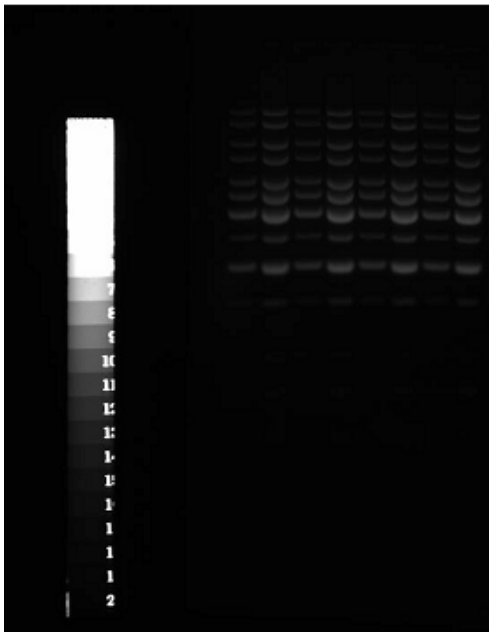

Figure. 9. Captured image showing sample ethidium bromide stained DNA gel and calibration step tablet at 0.5 second exposure.

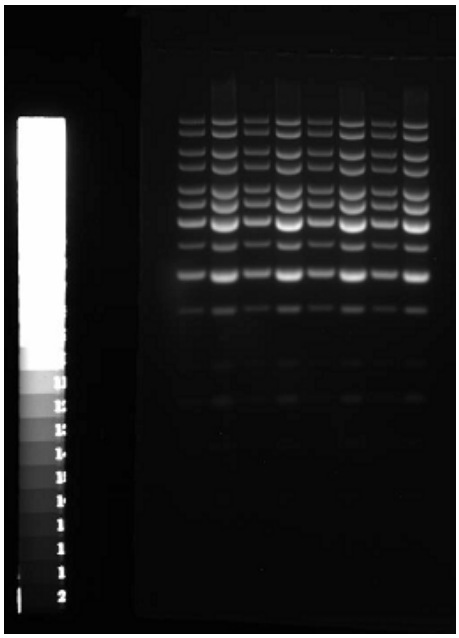

Figure 10. Captured image of the same sample DNA gel and calibration step tablet from figure 9 above except with a longer 2-second exposure. Note both the DNA bands and the step tablet increased by the same percentage (figure 7).

References

1. Gallagher, S.R., B. Moomaw, and S. Medberry, *Digital electrophoresis analysis*, in *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., Editors. 2004, John Wiley & Sons: New York.
2. Haugland, R.P., *Handbook of Fluorescent Probes and Research Products.* 2003.
3. Gallagher, S.R., et al., *Immunoblotting and immunodetection*, in *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., Editors. 2004, John Wiley & Sons: New York.
4. Sasse, J. and S.R. Gallagher, *Staining proteins in gels*, in *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., Editors. 2003, John Wiley & Sons: New York. p. 10.6.
5. Voytas, D. *Agarose gel electrophoresis*, in *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., Editors. 2003, John Wiley & Sons: New York. p. 10.6.