Focal Points

Application Note FP-124

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Applications of a Highly Uniform UV Illumination Imaging System for Quantitative DNA/Protein Analysis: Uniformity Generates Improved Data

Overview

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. We report the development and characterization of a highly uniform UV transillumination system, the **FirstLight™ UV Illuminator**. Through use of a high density lighting system with a tuned phosphor coating, uniformity of <5% coefficient of variance (CV) across the full imaging surface has been achieved and applied to proteomic analysis.

FirstLight UV Illuminator

Introduction

Presently, the use of digital fluorescent imaging for both documentation and analysis of electrophoretic separations is commonplace in biological research laboratories [1]. Applications range from In Vivo imaging to protein and DNA gel documentation and analysis [1-5,7,8]. With the introduction of cooled low light and high-resolution CCD cameras [1], CCD 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. 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 most fluorophores [2].
- 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.

However, quantitative CCD imaging with UV has been difficult due to the lack of uniformity found in typical UV transilluminators. Accurate and repeatable UV imaging requires a uniform light source.

Uniform Illumination is critical for quantitative analysis and 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

Through the unique design of the FirstLight UV Illuminator, reproducible quantitative UV imaging is now possible.

Methods

Electrophoresis

Proteins and DNA were separated by electrophoresis and stained according to standard protocols [9,8,2].

- Proteins were stained with SYPRO Ruby (Molecular Probes) according to manufacturer's instructions
- DNA was stained with Ethidium Bromide according to standard procedures [9].
- Stained gels was imaged with an AC[1] darkroom and analyzed using LabWorks™LS software

Results

- In contrast to the typical UV transilluminator with a Coefficient of Variation (CV) of 80%, the FirstLight UV Illuminator has a Uniformity of <5% CV across the imaging surface (fig. 1,2,5,7).
- The improved uniformity of the FirstLight UV Illuminator is evident from visual inspection and band quantitation of SYPRO Ruby stained protein gels and Ethidium Bromide stained DNA gels (fig. 8, 9, 10, 11)
- The lack of illumination uniformity (CV >80%) across the filter surface of typical UV light boxes leads to inaccurate quantitation through position dependent changes in fluorescence of stained sample (figure 9,10).

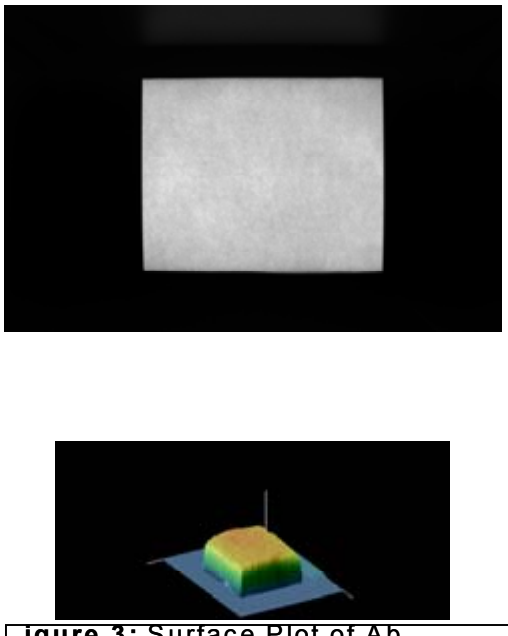


Figure 2: Surface Plot of Above Image

Figure 3: Surface Plot of Ab

Image Capture (CCD) Uniformity Profiles (Figures 1-4)

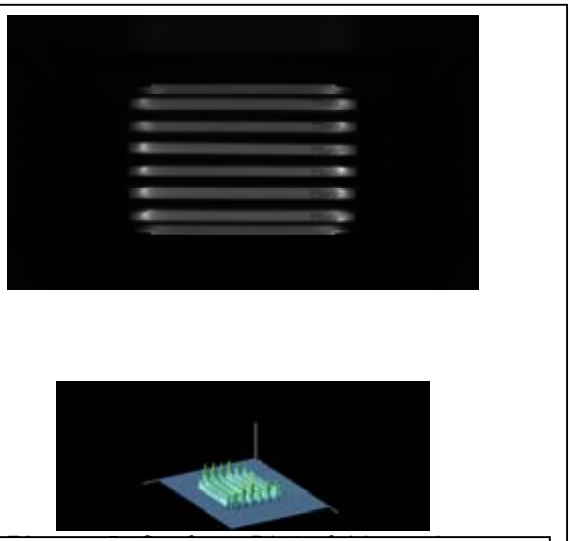
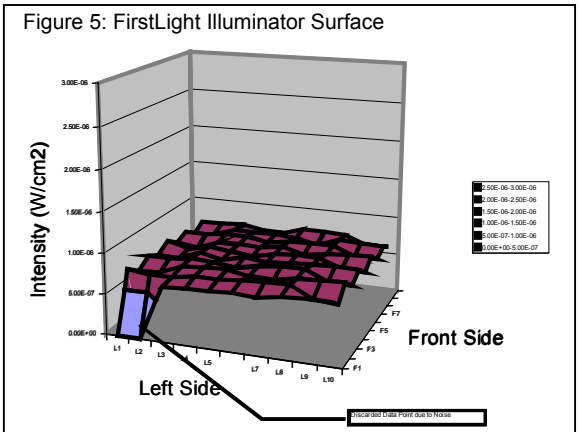
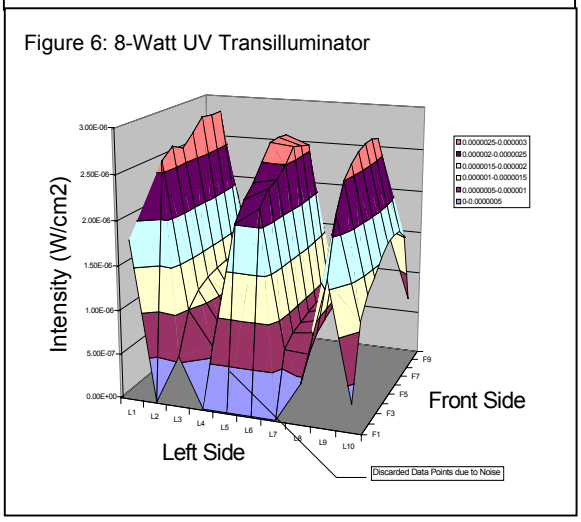


Figure 4: Surface Plot of Above Image





Figures 5 and 6. Intensity Profile Comparison of FirstLight Illuminator and 8-Watt Transilluminator

Image Intensity (Light Meter) Uniformity Profiles (Figures 5-7)

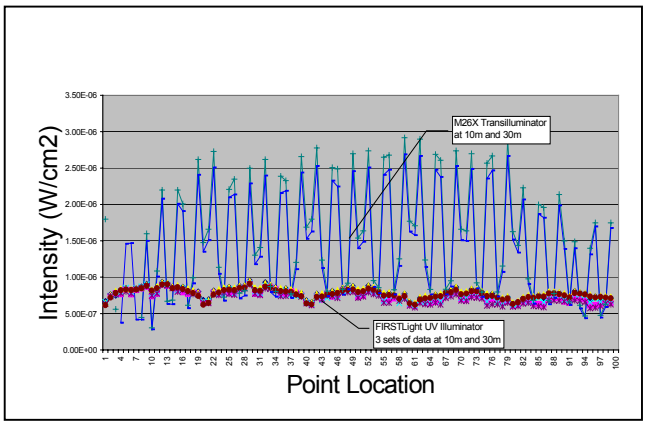
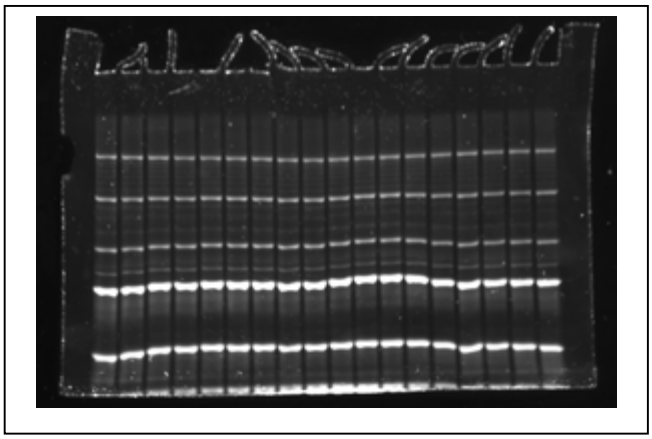


Figure 7. Intensity Profile Comparison of FirstLight Illuminator and 8-Watt Transilluminator



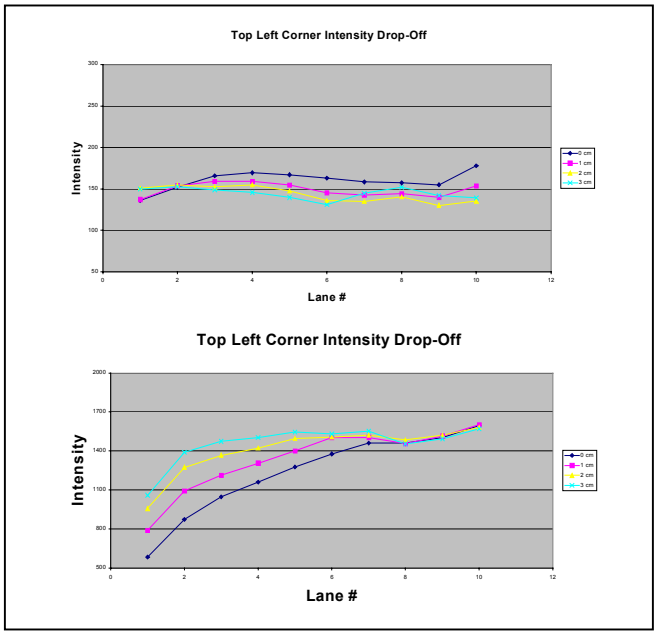
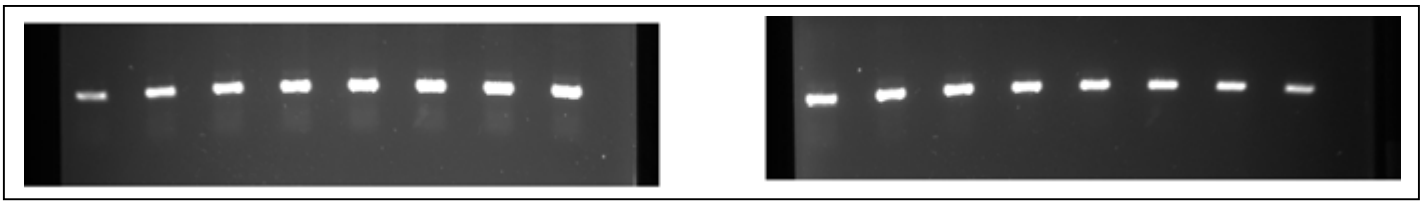
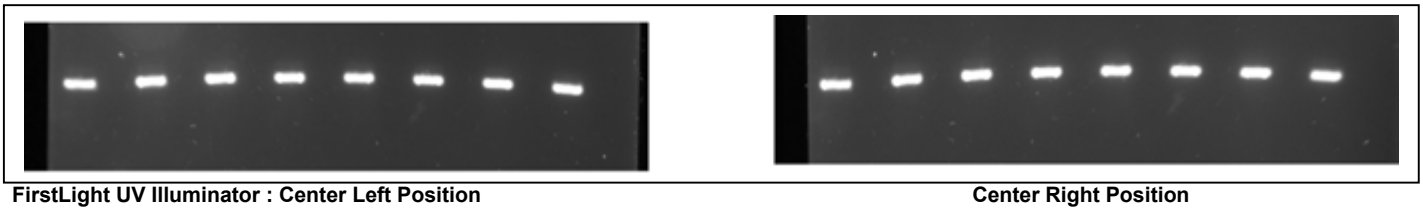


Figure 8 (above). Intensity Profile of Protein Standards: Comparison of FirstLight Illuminator and 8-Watt Transilluminator. Equal amounts of protein were loaded per lane, and the individual protein fluorescence intensity was quantitated. Note the severe loss of signal toward the edge of the typical UV light table (bottom graph) compared to the FirstLight illuminator (top graph).

Figure 9 (left). Protein Separation Imaged with the FirstLight UV Illuminator. Protein standards stained with SYPRO Ruby.



Typical Transilluminator: Top Left Position **Top Right Position**



FirstLight UV Illuminator : Center Left Position **Center Right Position**

Figure 10. Intensity profile of DNA standards. Equal amounts of DNA were loaded per lane and the gel stained with Ethidium Bromide. The gel was imaged at two positions on the illuminators filter top: top left and right positions and the left and right center edges. Each band in each lane was then quantitated with LabWorks imaging software. A typical transilluminator shows edge fall off of band intensity due to uneven illumination compared to the FirstLight. Note even intensity when using the highly uniform FirstLight illuminator.

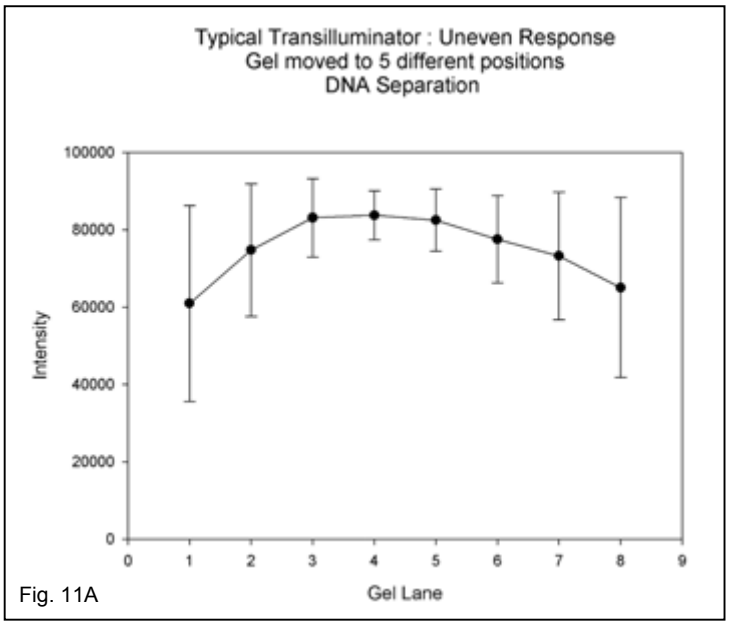


Fig. 11A

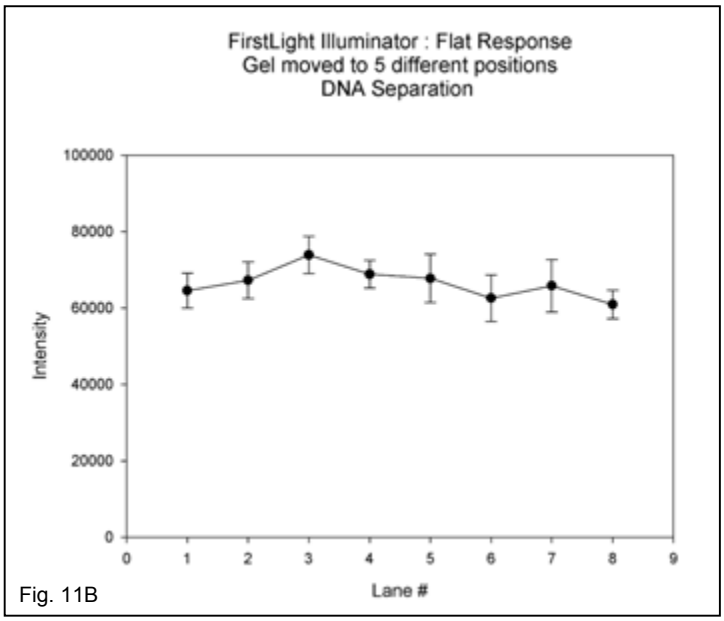


Fig. 11B

Figures 11A, 11B. DNA standards separated by electrophoresis and imaged with the FirstLight shows excellent uniformity (Fig. 11B). The mini gel was moved to five separate positions on the transilluminator surface and the separate band intensities were then quantitated with LabWorksLS Analysis Software.

Conclusion

The FirstLight UV Illuminator represents a unique highly uniform excitation source for quantitative fluorescent imaging with a wide range of genomic and proteomic applications including:

- Electrophoretic separation and quantitation of 1D and 2D protein separations
- Solid phase immunoassay
- DNA quantitation
- RNA quantitation

Through the patented design of the FirstLight UV Illuminator, quantitative ultraviolet multispectral fluorescent CCD imaging is now possible.

References

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