Focal Points

Application Note FP-124

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Applications of a Highly Uniform UV Ilumination Imaging System for Quantitative DNA and Protein Analysis

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

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

Intensity Profile of the FirstLight UV Illuminator Image Capture Method

Equipment (shown in figure 1):

- AC[1] AutoChemi darkroom 95-0351-01
- FirstLight UV Illuminator
- CCD BioChemi camera
- 8.5mm, motorized zoom 1:1.5 lens
- LabWorks software

Procedure:

- Exposure was set at 150 msec and binning was set at 4.
- Aperture was adjusted to give full range of signal
- The CV [6] was calculated for the entire filter surface (full area) and for an area one centimeter in from each edge (inner area).

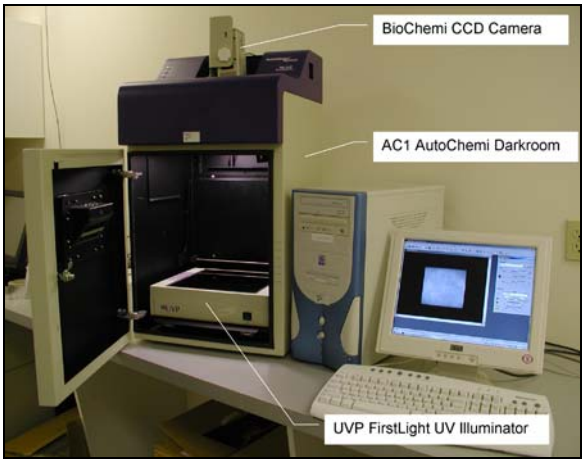


Figure 1. Image Capture Equipment

UV Intensity Method

Equipment Used (shown in figure 2):

- X-Y stage and controller (Velmex)
- 1700 UV meter (ILC)
- SED005 300nm sensor (ILC)
- Aperture Cones (see inset, figure 2)

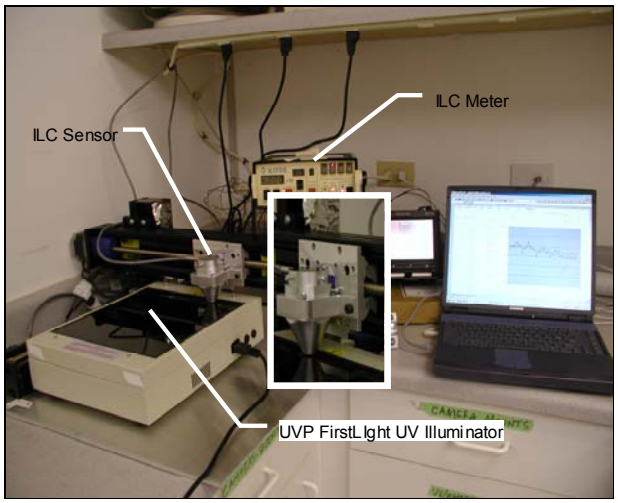


Figure 2. UV Intensity Equipment

Procedure:

- Aperture cones were installed over the sensor and this assembly was mounted on the X-Y stage with the aperture 1mm from the filter surface.
- The X-Y stage was programmed to travel across the surface of the Illuminator filter and stop for 3 seconds at each of 100 equally spaced points.
- At each point the UV intensity was measured by the ILC meter, and exported to Excel to calculate the CV.

Results

- In contrast, the FirstLight UV Illuminator has a Uniformity of <5% CV across the imaging surface (figure 3-4, 7, 9).
- The improved uniformity of the FirstLight UV Illuminator is evident from visual inspection and band quantitation of SYPRO Ruby stained protein gels (figure 10 and 11).
- The lack of illumination uniformity (CV >80%) across the filter surface of typical UV light boxes has been confirmed (figure 9). The peak intensity is associated with the individual UV bulbs typically used for UV light boxes (figures 5-6, 8) and leads to position dependent changes in fluorescence of stained sample (figure 10).

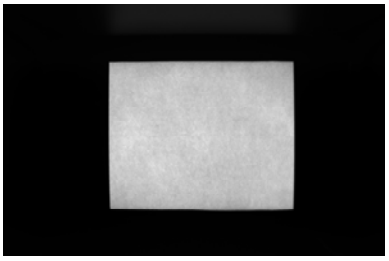


Figure 4
FirstLight™ UV Illuminator at 10 minutes
6.0%CV Full Area, 4.6% CV Inner Area

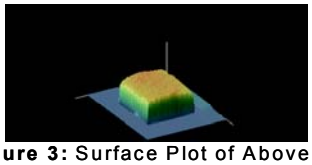
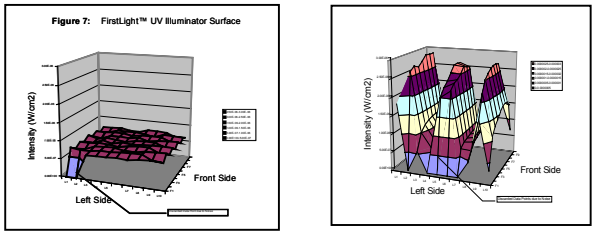


Figure 3: Surface Plot of Above Image

Image Capture (CCD) Uniformity Profiles (Figures 3-6)

Figure 5: Surface Plot of Above Image



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

Image Intensity (Light Meter) Uniformity Profiles (Figures 7-9)

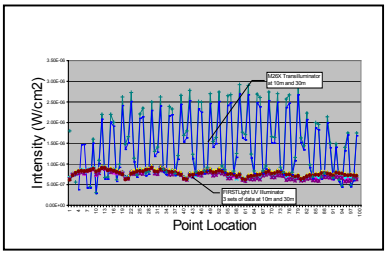


Figure 9. Intensity Profile Comparison of FirstLight Illuminator and 8-Watt Transilluminator

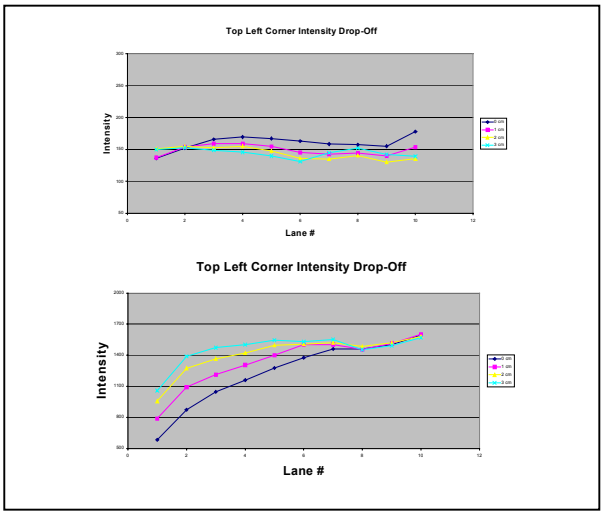


Figure 10. Intensity Profile of Protein Standards: Comparison of FirstLight Illuminator and 8-Watt Transilluminator. Equal amounts of protein were loaded per lane, and the individual protein fluorescence intensity was quantitated. Note the severe loss of signal toward the edge of the typical UV light table (bottom graph) compared to the FirstLight illuminator (top graph).

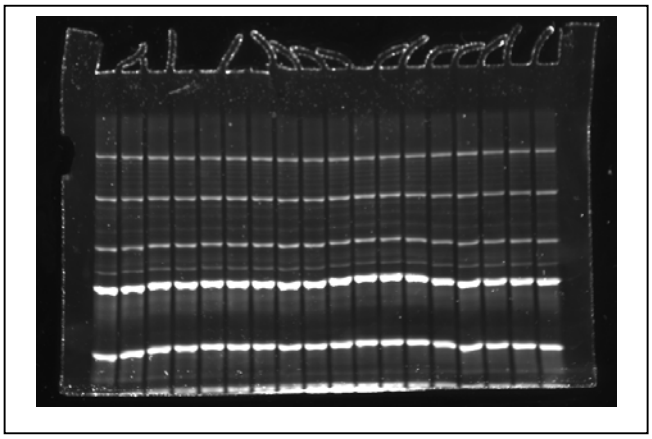


Figure 11. Protein Separation Imaged with the FirstLight UV Illuminator. Protein standards stained with SYPRO Ruby.

Conclusion

The FirstLight UV Illuminator represents a unique highly uniform excitation source for quantitative fluorescent imaging:

Uniformity was confirmed with 3 methods:

- CCD image capture
- Intensity profiling with automated X-Y stage
- Electrophoretic separation and quantitation

Quantitative UV fluorescent imaging has 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

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. Patton, W.F. and J.M. Beechem, *Rainbow's end: the quest for multiplexed fluorescence quantitative analysis in proteomics.* Curr Opin Chem Biol, 2002. 6(1): p. 63-9.
4. Yan, J.X., et al., *Fluorescence two-dimensional difference gel electrophoresis and mass spectrometry based proteomic analysis of Escherichia coli.* Proteomics, 2002. 2(12): p. 1682-98.
5. Patton, W.F., *Detection technologies in proteome analysis.* J Chromatogr B Analyt Technol Biomed Life Sci, 2002. 771(1-2): p. 3-31.
6. Steele, R.G.D. and J.H. Torrie, *Principles and procedures of statistics*. Second ed. 1980: McGraw-Hill Book Company.
7. 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.
8. 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.

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