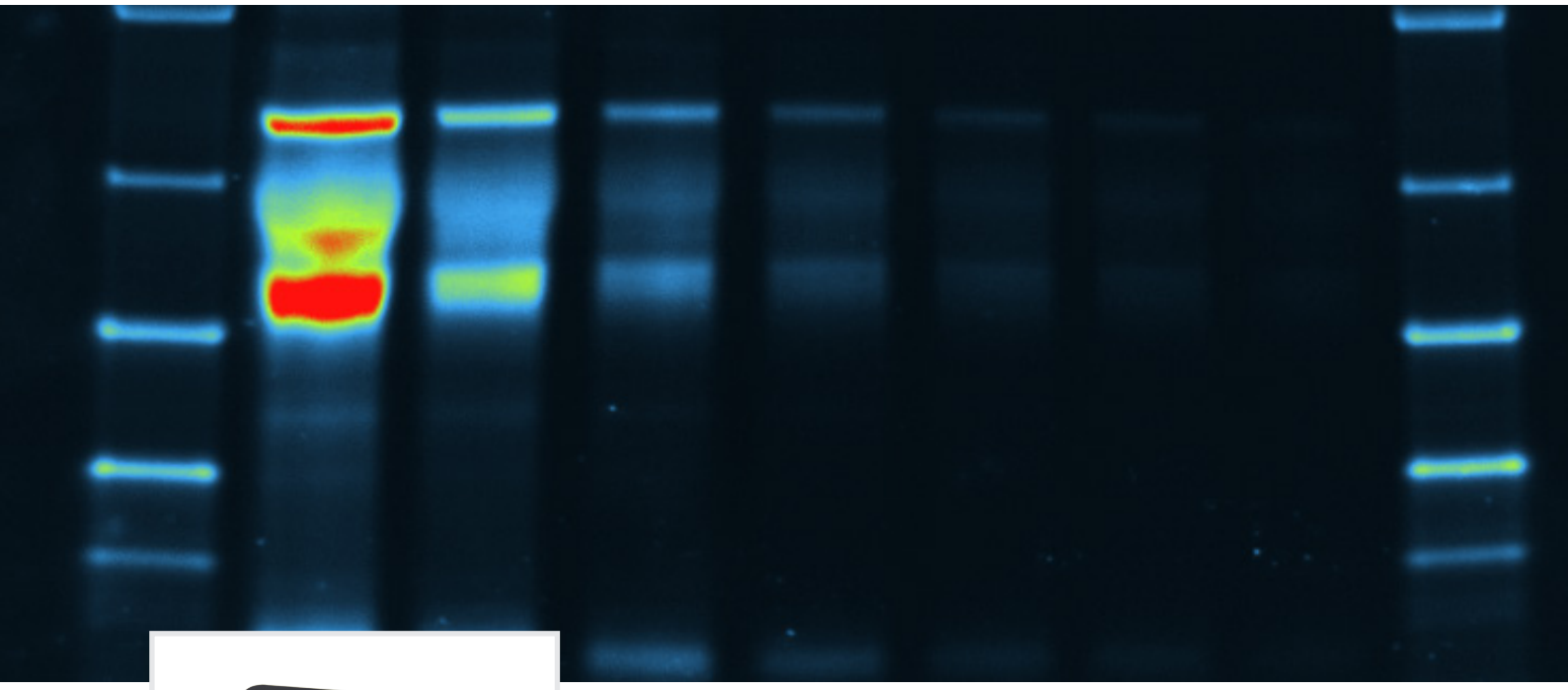

Challenge

Time-consuming protein staining protocols

Solution

Stain-Free™ Imaging on the UVP ChemStudio

Protein Fluorescence: A New Twist on a Classic Phenomenon

Introduction

In the early 1900s, protein analysis in electrophoresis systems was not possible by eye and instead relied on chemical analysis—positions along a gradient could be sampled and subsequently assayed for protein[1]. Visualization of proteins, under most circumstances, was impossible. For example, samples that contained proteins may appear slightly more turbid than solutions without protein, but below certain concentrations, the turbidity was indiscernible by eye. A breakthrough occurred when researchers realized that proteins fluoresce when exposed to ultraviolet (UV) light and that fluorescence was enhanced by using high energy UV[1]. However, this presented a problem. High energy UV could damage the researcher's eyes. Therefore, in order to observe protein fluorescence under these conditions, protective eyewear was required. This inherently made fluorescence from the protein less obvious. The challenge was overcome by the development of indirect visualization methods using photographic imaging (Figure 1). After electrophoresis in a U-tube, researchers could image where the protein had migrated using a camera with a high-sensitivity photographic plate made of glass. Ultraviolet light was directed at an electrophoresed protein sample in a U-tube. Where the solution was devoid of protein, UV light would pass through the tube and into the camera. Positions in the U-tube where protein was present would absorb the UV light, fluoresce, and thus, transmit less light to the camera, effectively creating a negative image of the protein sample in the U-tube. The first demonstration of imaging UV-mediated protein fluorescence was published in 1923 by Theodore Svedburg[1], eponym of the Svedburg unit and awardee of the 1926 Nobel Prize in Chemistry for his invention of the ultracentrifuge[2].

Svedburg's early work on electrophoresis would intrigue his pupil Arne Tiselius, who would go on to develop this technology further and attain the 1948 Nobel Prize in Chemistry for his contributions to protein electrophoresis.

Protein separation and visualization would experience several significant improvements through the 1960s, 1970s, and again in the 1990s that included detergent-based denaturation[3], polyacrylamide-based slab separation[4], and colorimetric and fluorescent dye detection[5,6] (discussed in SDS-PAGE App Note and Fluorescence App Note).

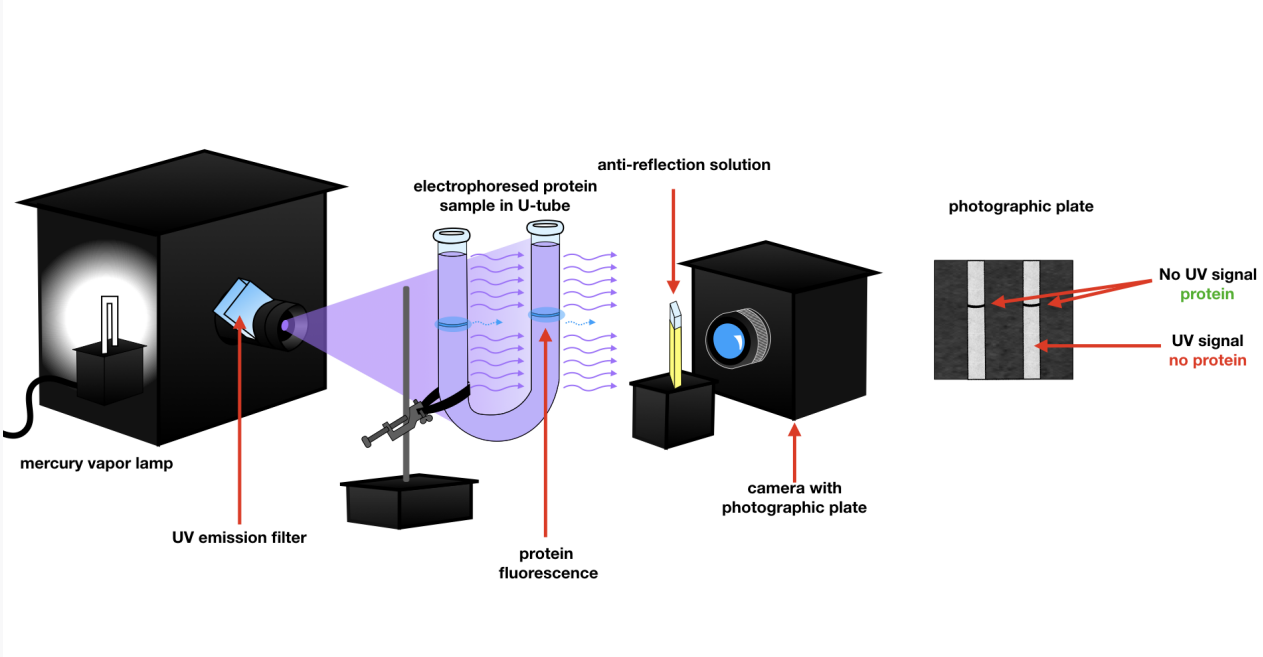

Figure 1: U-tube Electrophoresis and Imaging Apparatus. (From left to right) A mercury vapor lamp enclosed in a box is focused on an electrophoresed sample containing protein. The light emitted from the box first passes through an excitation filter that only transmits ultraviolet (UV) wavelengths. When the UV light strikes the U-tube, regions devoid of protein will transmit the UV light. The electrophoresed protein creates a tight band in the U-tube and absorbs the UV light and emits fluorescence as a result. Transmitted light from the U-tube passes through a cuvette containing an organic acid solution that blocks reflections from the glassware and subsequently passes into the camera. A photographic glass plate is used to detect emissions from the sample. The image produced on the photographic plate is effectively a negative of the sample since the UV light was absorbed by the protein and very little light is transmitted beyond the U-tube.

More recently, researchers have revisited UV-mediated protein fluorescence from the early 1900s, with a twist. In 1993, Pinchuk and Vorobei discovered that in the presence of haloalkanes, tryptophan decomposed and emitted fluorescence as a byproduct[7]. The researchers reasoned that the degradation of the indole moiety was the cause of the fluorescence emission. In the early 2000s, researchers exploited this phenomenon for a post-electrophoresis stain[8] using chloroform or trichloroacetic acid, and later as an in-gel stain with tricholorethanol[9]. Briefly, proteins exposed to UV in the presence of trihaloalkanes emit a blueish glow (Figure 2). Today, Bio-Rad manufactures and sells these trihaloalkane-based precast gels under the TGX Stain-Free™ product name. Below, we have highlighted the flexibility of our UVP ChemStudio imager to capture this new twist on a classic phenomenon.

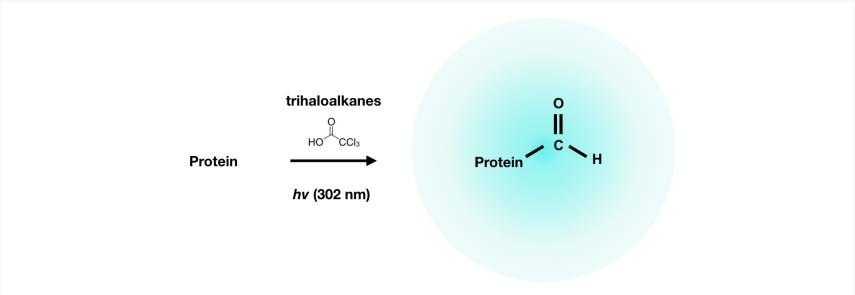

Figure 2: UV and trihaloalkane mediated protein fluorescence. When exposed to UV, in the presence of trihaloalkanes, tryptophan moieties in protein are formylated, which produce fluorescence when excited with UV light.

Imaging with the UVP ChemStudio

In principle, there are no differences when imaging Stain-Free™ gels on our instrument in comparison to other gel imaging strategies. The fundamental recipe is the same. You need an excitation light source, emission filter, high-sensitivity camera, and software to analyze the data. Our UVP ChemStudio is Stain-Free™ ready (Figure 3).

Every UVP ChemStudio comes standard with a high-power UV transilluminator that can produce variable intensity 302nm UV light. Every UVP ChemStudio comes standard with an emission filter that blocks UV light and captures the intense protein fluorescence produced by Stain-Free™ gels. Every UVP ChemStudio comes with a cooled, high-sensitivity camera to dramatically increase the signal-to-noise ratio, enabling researchers to capture the finest details in their protein gel samples. Lastly, every UVP ChemStudio comes with our VisionWorks software for image capture, processing, and analysis.

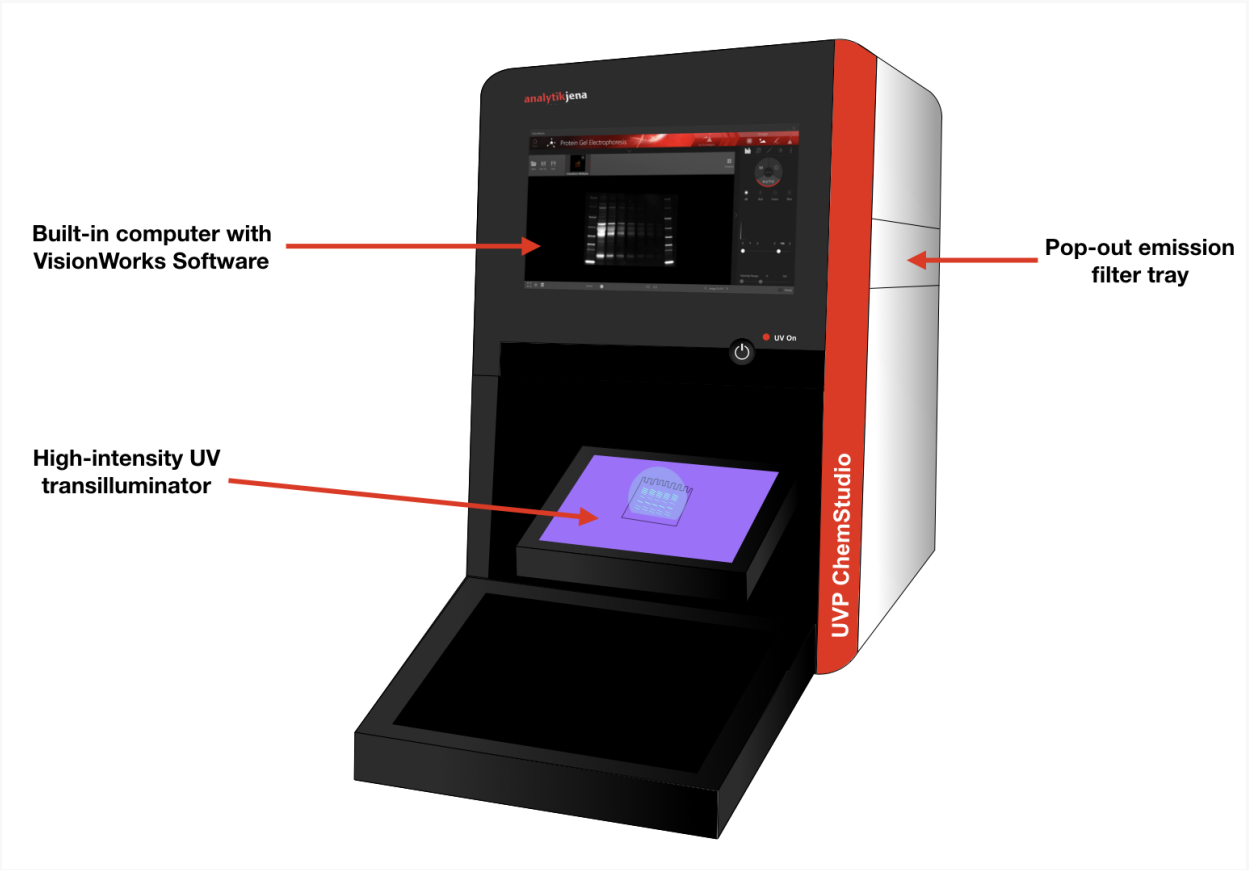

Figure 3: UVP ChemStudio. The UVP ChemStudio has a pop-out emission filter tray to enable researchers to add filters at will, for custom applications. This instrument comes standard with a high-intensity UV transilluminator, which is critical for many applications including Stain-Free™ gels. Each instrument comes with VisionWorks software for image capture, processing, and analysis. Researchers have the option to purchase an instrument with or without an on-board computer as depicted above.

Sample Preparation and Running Conditions

Diluted mouse serum was quantified using the Bradford Assay and measured on an Analytik Jena Specord 250 UV/Vis spectrophotometer. The protein sample was serially diluted 1:2 from 2 µg/µl to 31 ng/µl, solubilized 1:2 in 2x Laemmli Buffer from Bio-Rad (Hercules, CA), supplemented with β-mercaptoethanol per the manufacturer's instructions, and boiled at 85°C for 5 minutes. 20 µl of boiled samples and 5 µl of ThermoFisher's (Waltham, MA) PageRuler Unstained Broad Range Protein Ladder were loaded onto a Bio-Rad Mini-PROTEAN® TGX Stain-Free™ 4-12% gradient pre-cast gel and run at 200V for 40 minutes in a Bio-Rad Mini-PROTEAN Tetra Vertical Electrophoresis cell with Bio-Rad's 10X Tris/Glycine/SDS Buffer diluted to a 1x working concentration.

Gel Activation, Image Capture and Processing

TGX Stain-Free™ gels require a brief activation under UV light, which triggers the photomodification of tryptophan residues and subsequent fluorescence[7] (Figure 2). The gel was exposed to high-intensity 302 nm UV for 30 seconds prior to imaging. After activation, the gel image was captured on a UVP ChemStudio using our VisionWorks software.

Results and Conclusion

Stain-Free™ gels are readily imaged with the UVP ChemStudio (Figure 3). For easier visualization in Figure 4, the original image was cropped (Figure 4A), inverted (Figure 4B) and pseudocolored (Figure 4C) after capture using our VisionWorks software.

Our UVP ChemStudio has been designed with flexibility in mind. To anticipate new technologies, we have designed our instrument to have the most common excitation light sources standard on the instrument including UV transillumination, white and RGB LED epi-illumination. For custom applications, the UVP ChemStudio can be configured to accommodate our high-power multispectral xenon light source and near-infrared laser modules. Our easily accessible filter tray enables researchers to select only the emission filters they need from our library, saving thousands of dollars relative to our competitors. Whether you are imaging protein gels with Coomassie Brilliant Blue or Stain-Free™ strategies as demonstrated here, our instruments can accommodate technologies old and new.

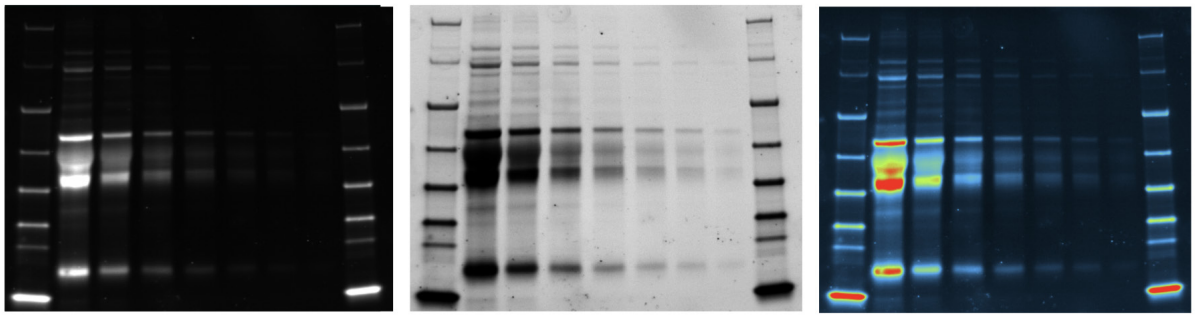

Figure 4: Imaging Stain-Free™ gel with the UVP ChemStudio. The gel was activated for 30 seconds prior to imaging. After imaging, the original image was cropped (A), inverted (B), and pseudocolored (C). We have several pseudocolor options available for customers, which can aid in visualization, especially for low abundance bands. Auto histogram was used in all the images, yet the bands were more obvious in the inverted (B) and pseudocolored (C) images. Importantly, the contrast of the low abundance bands can be augmented by adjusting the histogram in the VisionWorks software, and it does not affect quantitation (data not shown here).

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