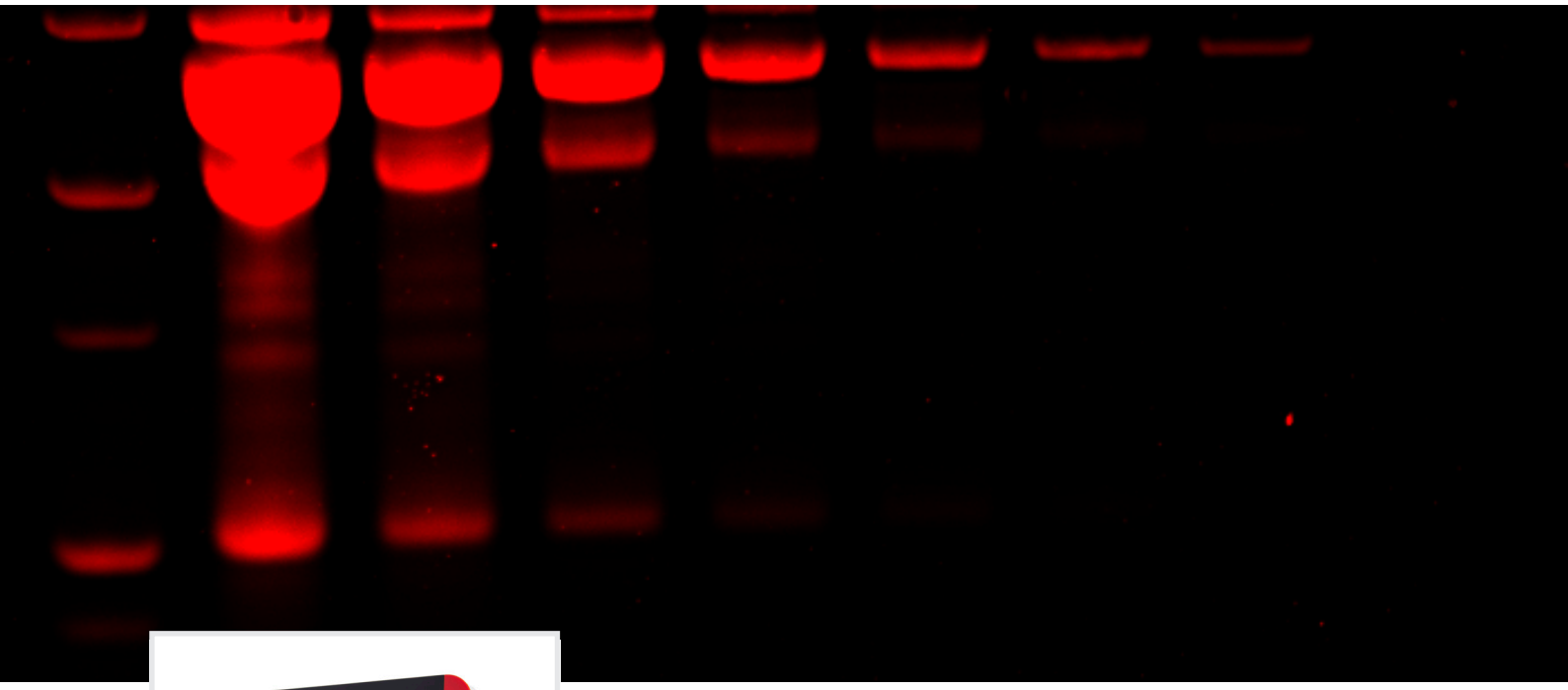

Task

Fluorscence-based gel imaging.

Solution

Multiple wavelength excitation sources combined with easily accessible filter wheel enables users to perform any fluorescence-based gel application.

Fluorescence Gel Imaging: Take a Walk on the Bright Side

Introduction: Something Old, Something New

Several DNA and protein detection reagents are available to researchers that vary in sensitivity, safety, and suitability for downstream applications. Chief among DNA binding dyes is ethidium bromide with detection limits as low as 0.5 ng[1]. Ethidium bromide, or homidium as referred to in the veterinary sciences, was used to effectively treat trypanosomiasis in cattle as early as the 1950s, for which the mechanism of action was unknown until recently[2]. As any molecular biologist would have expected, the dye interferes with trypanosome DNA replication[2], underscoring the mutagenic safety concerns with this and other DNA-binding dye technologies (See our Safety is a Part of Science application note). While ethidium bromide has fallen out of favor in the treatment of cattle for the aforementioned risks, ethidium bromide-agarose gel electrophoresis still remains the most common fluorescence-based DNA detection method since its conception[3]. However, several alternative fluorescence-based DNA dyes are available to investigators (See Figure 1 and Table 1).

For protein, colorimetric dyes remain the most common. Coomassie Brilliant Blue (CBB) is arguably the most popular post-electrophoresis protein dye. Several iterations of the original protocol exist with reported detection limits as low as 3 ng[4]. Alternatively, silver salt staining can increase the detection limits of protein as low as 0.1 ng. Typically, silver staining achieves 100x-1000x more sensitivity than CBB in routine protocols[5]. Still, both methods either require large volumes

of toxic reagents or laborious and time-consuming staining protocols. Fortunately, several bright fluorescence-based protein dyes are available to investigators that have vastly streamlined in-gel protein detection (Table 1).

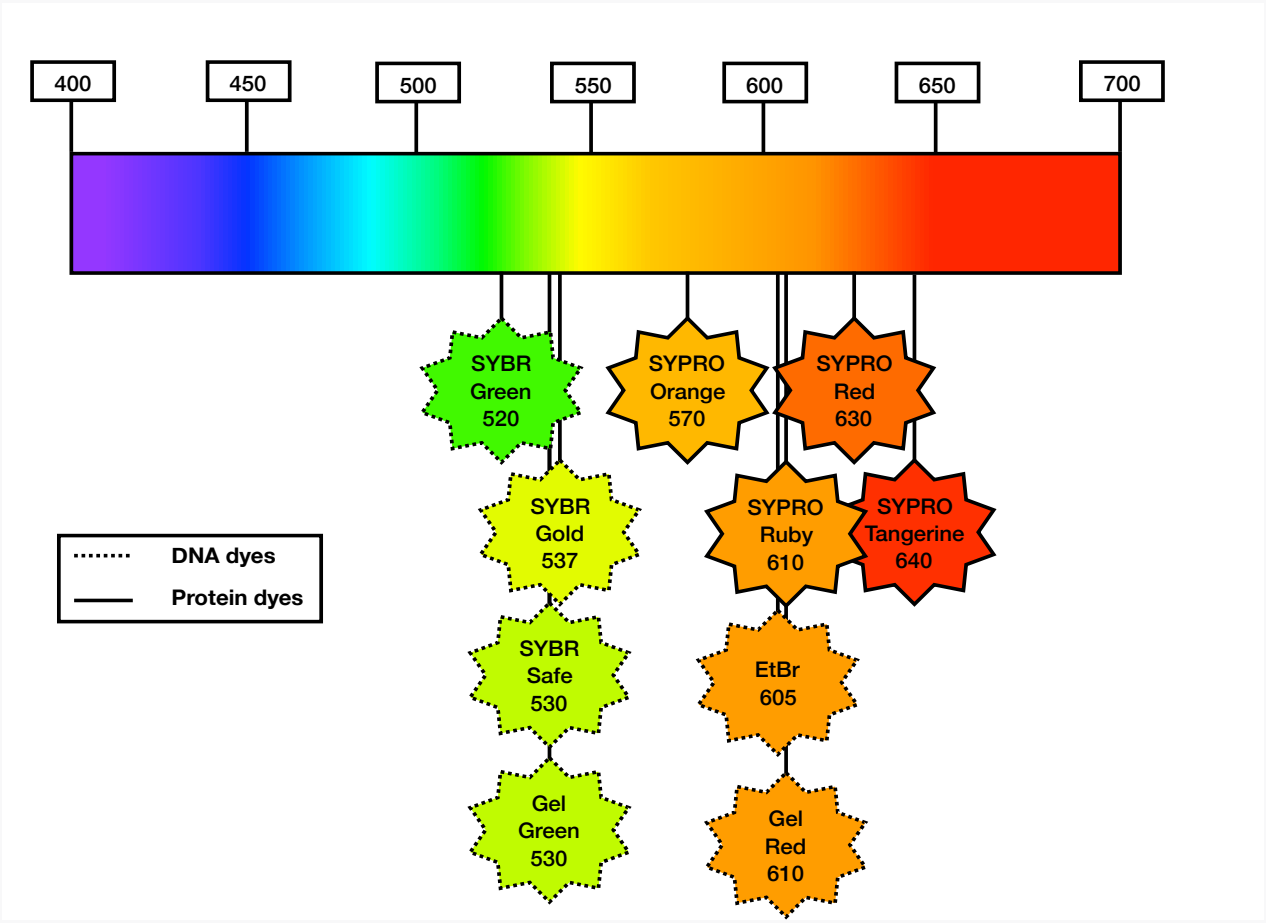

Figure 1: Popular fluorescent dyes for DNA and protein detection within the visible spectrum. SYBR Green, SYBR Gold, SYBR Safe, GelGreen, GelRed, and ethidium bromide (EtBr) are considered the most popular DNA binding dyes for in-gel fluorescence detection. SYPRO Orange, SYPRO Ruby, SYPRO Red, and SYPRO Ruby are the most popular protein binding dyes for in-gel fluorescence detection. SYBR Safe, GelRed, and GelGreen are considered safer alternatives to EtBr. SYBR Gold and SYPRO Ruby are considered the most sensitive dyes for DNA and protein detection, respectively.

At Analytik Jena, we have designed our instruments to accommodate technologies both old and new. Importantly, all our instruments are capable of imaging gels stained with the most popular fluorescent dyes on the market (Table 1). These include safer DNA dyes like GelRed, GelGreen, and SYBR safe as well as high sensitivity SYBR Gold and SYPRO Ruby for DNA and protein detection, respectively. Below we demonstrate the diversity of dyes that can be captured with our imagers.

Fluorescence Imaging with Analytik Jena's UVP GelStudio

The UVP GelStudio Series imagers are equipped with a high-performance 5 megapixel camera to capture the finest details in your samples. With a UV transilluminator, RGB LED epi-illumination, an extensive library of emission filters, an easily accessible filter wheel, the UVP GelStudio can image any fluorescent dye technology to fit your research needs. Below we have demonstrated the flexibility of the UVP GelStudio by imaging with several DNA and protein fluorescent dye technologies.

Table 1: DNA and Protein Binding Dye Specifications and Filter Requirements.

DNA Dye	Peak Excitation (nm)	Peak Emission (nm)	AJ Filter Part Number
Ethidium Bromide	300/360	590	38-0220-01
GelGreen®	254/488	530	38-0352-01
GelRed®	300	590	38-0220-01
SYBR™ Gold	300/495	537	38-0352-01
SYBR™ Green	254/497	520	38-0352-01
SYBR™ Safe	280/502	530	38-0352-01

Protein Dye	Peak Excitation (nm)	Peak Emission (nm)	AJ Filter Part Number
SYPRO® Orange	300/470	570	38-0344-01
SYPRO® Red	300/550	630	38-0341-01
SYPRO® Ruby	280/450	610	38-0344-01 or 38-0220-01
SYPRO® Tangerine	300/490	640	38-0341-01

Methods

DNA Gels and Protein Gels

DNA samples were separated on 2% agarose at 120 volts for at least 45 minutes in 1X TAE buffer. Biotium 1KB ladder was diluted 1:2 and 20 ul was loaded per well. All dyes were homogenized with agarose prior to loading DNA. No post-staining or destaining was performed. Dye concentrations used were per the manufacturer's recommendations.

Protein samples were boiled at 85°C for 5 minutes in Laemlli Buffer and separated on pre-cast NuPage 4-12% Bis-Tris protein gels. Samples were diluted 1:10 (from 40 ug to 40 ng) for SYPRO Ruby detection and 1:2 (from 20 ug to 156 ng) for SYPRO Red. SYPRO dyes were used per the manufacturer's recommendations. For SYPRO Ruby, the rapid protocol was used for this note.

VisionWorks Software Image Capture

Images were captured using the VisionWorks ver. 9.0 software package following the steps below (See Figure 2).

1. Select the appropriate emission filter for the application by navigating to the Devices menu and then the Filters sub-menu. Up to five preset filter options can be programmed.
2. Select excitation light in the Lights pane.
3. Adjust the exposure time in the Camera sub-menu by selecting Manual (M) and toggling the arrows. For most gel applications, we recommend starting with a 200 ms exposure.
4. Select Live View to begin preview. During Live View, navigate to the Lens tab and adjust the Focus and Brightness to your desired level. Once you are satisfied, select Capture in the Camera sub-menu.
5. After capturing an image, navigate to the Image tab and select Histogram (Hist). We recommend selecting Auto. Alternatively, you can select Manual (M) and adjust the black, white, and gray levels to your liking. Briefly, the histogram indicates the pixel counts in regions of the black part of the spectrum (left), in the white part of the spectrum (right) and in the gray part of the spectrum (middle or everything in between). As with most gel

applications, the majority of the signal will be in the black regions, since the gel background is black with fluorescent signal. This will be indicated by a right skewed histogram (Figure 2, Histogram Adjustment). Adjusting the black slider to the right will drop off background in the image as will sliding the gray slider to the left. Moving the white slider to the left will bring up the white levels (e.g. DNA bands) as well as any background signal. Importantly, none of these modifications change the metadata of the image, so consider this a tool to help you locate and demarcate bands for subsequent analysis or for simple image beautification.

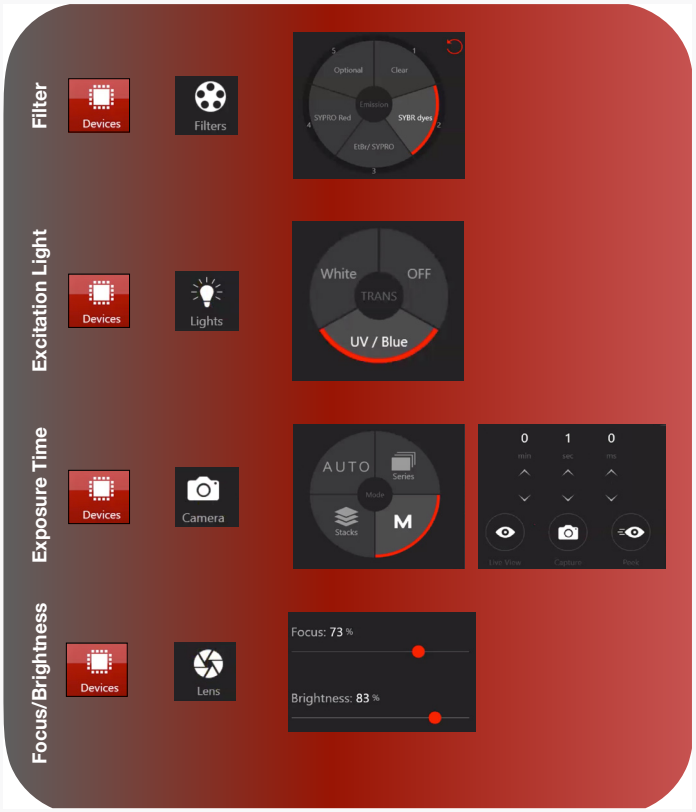

Figure 2a: Capturing a fluorescent gel image with VisionWorks software ver. 9.0. In the DNA or Protein application, navigate to Devices menu and the Filters sub-menu to select the emission filter for your application. Navigate to the Lights sub-menu and select the excitation light source. Both epi and trans illumination are available (trans lighting option shown above). Next, navigate to the Camera sub-menu, select manual capture (M), and adjust the exposure time as determined empirically (continued in Figure 2b).

Figure 2b: Starting with 200 ms is recommend. Next, select Live View. During Live View, adjust the Focus and Brightness settings until you can clearly see bands. You may need to increase your exposure time to improve visualization. Once the preview image is optimized, select Capture. You can begin post-processing. You want to adjust the histogram to improve the aesthetics of the image. Moving the black slider increases the black levels. Moving the white slider left, increases the white levels. Adjusting the gray slider will change the midpoint of the scale. The gray slider is especially useful if you use a dye with high background fluorescence. Lastly, navigate to the Devices sub-menu Pcolor to apply a pseudocolor to the image.

Results and Conclusion

Some of the most popular dyes used for fluorescence-based DNA and protein detection are readily captured by the UVP GelStudio imager. In Figure 3, we highlight the flexibility of the GelStudio imager by using several fluorescent dyes covering the visible spectrum from green to red.

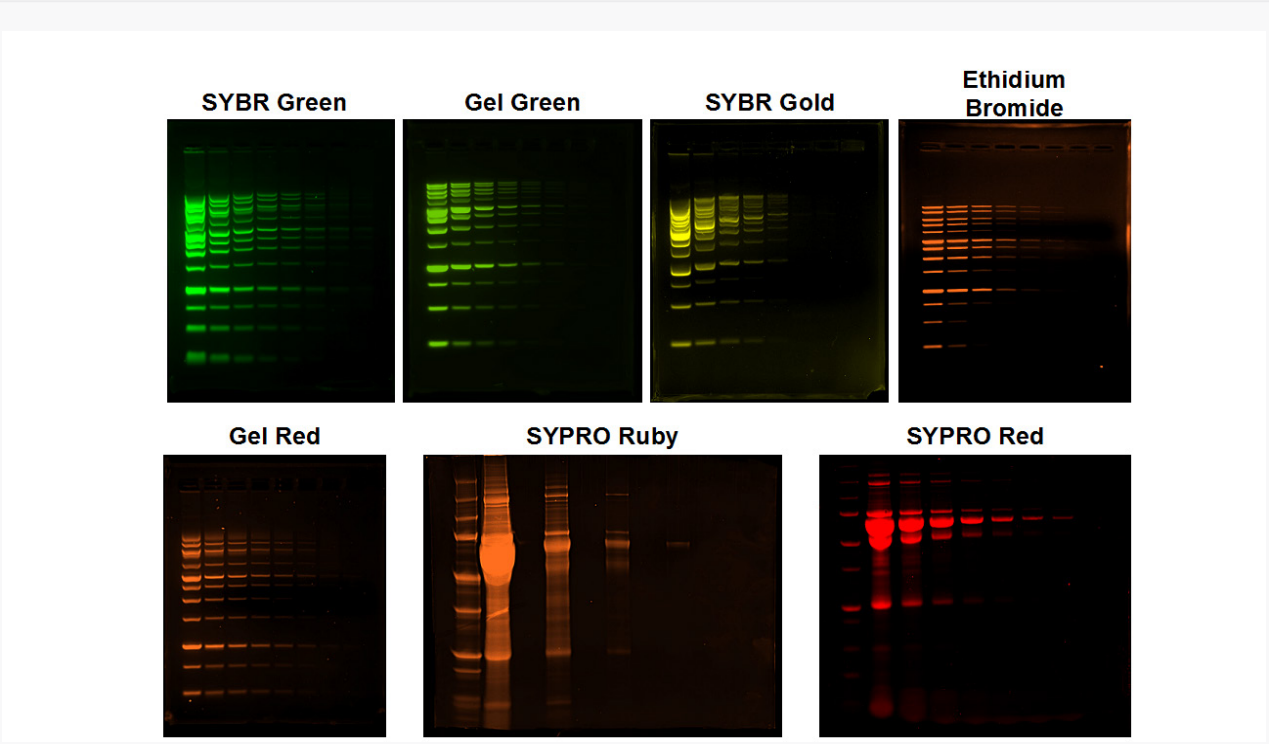

Figure 3: Fluorescent DNA and Protein Gel imaging with the UVP GelStudio. All gels were imaged using a 302 nm UV transilluminator. SYBR Green, Gel Green, and SYBR Gold were imaged using AJ emission filter 38-0352-01. Ethidium bromide and SYPRO Ruby were imaged using AJ Filter 38-0220-01 and SYPRO Ruby were imaged using AJ Filter 38-0341-01. All images were pseudocolored to reflect the emission wavelength. All image processing was completed on Analytik Jena's VisionWorks Data Acquisition and Analysis software version 9.0.

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