Densitometry and Gel Imaging with the chemiSOLO

Introduction

Polyacrylamide gel electrophoresis is an essential analytical technique for labs studying proteins. Proteins are driven by an electric current to migrate through a gel matrix, separating them by size. The separated proteins are then detected using total protein stains. Many of the most commonly used stains are colorimetric stains, such as Coomassie Brilliant Blue or silver stains. Using stains visible to the eye allows for quick visual inspection of gels to gauge sample composition and easy documentation using a gel documentation system. Protein gels stained with visible stains are often used to follow expression and purification of recombinant proteins used in physical, enzymatic, or structural studies.

Imaging and documenting stained gels allows for quick, qualitative analysis of protein samples. The same gels can then be analyzed quantitatively using densitometry, the process of quantitating sample amounts based on their absorbance of light. The optical density of a sample is related to the percent of incident light that is transmitted through the sample. Using analysis software such as AzureSpot Pro, the concentration of a sample band can be determined by comparing its optical density to the optical densities of known standards.

Figure 1. chemiSOLO, a cutting-edge imager capable of highly sensitive chemiluminescent and visible imaging, all within a compact design. Details at azurebiosystems.com/chemiSOLO.

Azure Biosystems has developed the chemiSOLO, a digital chemiluminescence imager that also conducts high-resolution visible protein gel imaging and quantitative densitometric analysis, all within a space-efficient design.

Densitometric Gel Imaging

The chemiSOLO is designed to provide sensitive imaging of visibly stained gels via a six megapixel, back-illuminated, Peltier-cooled CMOS camera. The gel is evenly illuminated over the entire imaging area by transillumination LEDs imbedded within the lid of the chemiSOLO. The even illumination maximizes both visual image quality and quantitative accuracy.

The chemiSOLO is compact and space-efficient. With physical dimensions of 11.5” x 8.75” x 17.0” (29.2 x 22.2 x 43.2 cm), the chemiSOLO fits neatly into busy lab spaces.

Simple, Intuitive Controls

The chemiSOLO is controlled remotely through a unique web browser interface. The instrument is seamlessly controlled through the user’s own external computer, tablet, or mobile device (Figure 2). This feature maximizes the chemiSOLO’s accessibility and useability.

In this application note, we will demonstrate high-quality gel imaging and densitometric analysis using the chemiSOLO.

Figure 2. chemiSOLO Connection. Through its novel browser-based software, the chemiSOLO can be controlled by computers, tablets, or mobile devices with no downloads required.

Materials and methods

Densitometric Analysis with the chemiSOLO

Densitometry was conducted using a chemiSOLO outfitted with the chemiSOLO Densitometry Package [CS1004]. Analysis of images was carried out using AzureSpot Pro Analysis Software for 1D gel analysis and the chemiSOLO Densitometry Calculator in Microsoft® Excel®.

First, the calibration of the chemiSOLO was verified using the 21-Step Tablet included in the chemiSOLO Densitometry Package [CS1004]. The 21-Step Tablet is manufactured with regions of precise optical densities (OD) between 0 and 2.1 in increments of 0.1 OD. The 21-Step Tablet was imaged on the chemiSOLO and captured as a 16-bit greyscale image using the Greyscale Imaging function in the Visible Imaging section of the Home Screen. Lanes were defined and calibration plotted using AzureSpot Pro Analysis Software for 1D gel analysis and the chemiSOLO Densitometry Calculator in Microsoft® Excel® as described in the Azure Biosystems protocol Densitometric Analysis of Protein Gels.

To demonstrate densitometric analysis with the chemiSOLO, two SDS-PAGE gels were run. The first contained a serial dilution of bovine serum albumin (BSA) from 5 µg to 0.136 µg. The second contained a serial dilution of HeLa cell extract. The gels were stained using SimplyBlue SafeStain (Invitrogen, #465034) according to the manufacturer's instructions. Gels were imaged on the chemiSOLO and captured as a 16-bit greyscale images by selecting Greyscale Imaging in the Visible Imaging section of the Home Screen. Bands were drawn manually, minimizing the capture of unstained area surounding each band. Using the auto-select function in AzureSpot Pro, which results in bands of identical area across all samples, is not recommended for densitometric analysis based on transmittance.

Results

The calibration of the chemiSOLO was verified by analyzing the visible detection of a 21-Step Tablet. As shown in Figure 3, the optical densities measured by the chemiSOLO were highly correlated with the expected values, with a linear relationship and R^2 value of 0.9959.

The densitometry capabilities of the chemiSOLO were demonstrated by analyzing a gel containing a highly purified sample of commercially available BSA, and a gel containing a complex sample of total cell lysate. First analyzed was a gel containing a serial dilution of BSA and stained with Coomassie. The gel was imaged on the chemiSOLO in greyscale (Figure 4).

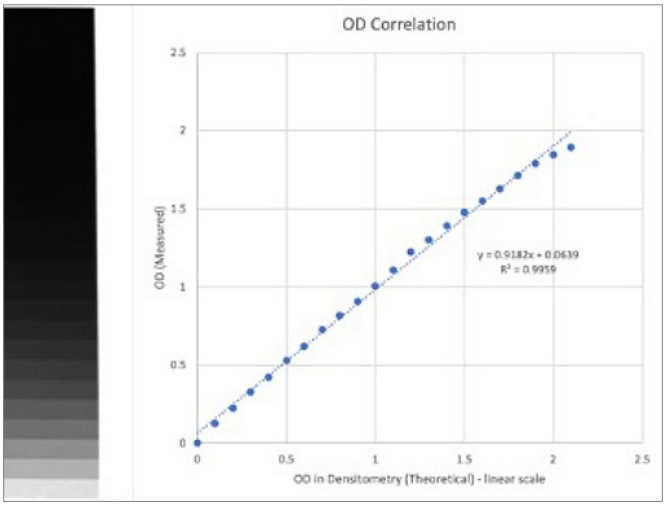

Figure 3. Image capture and quantification of the 21-Step Tablet on the chemiSOLO confirming calibration over a wide range in optical density.

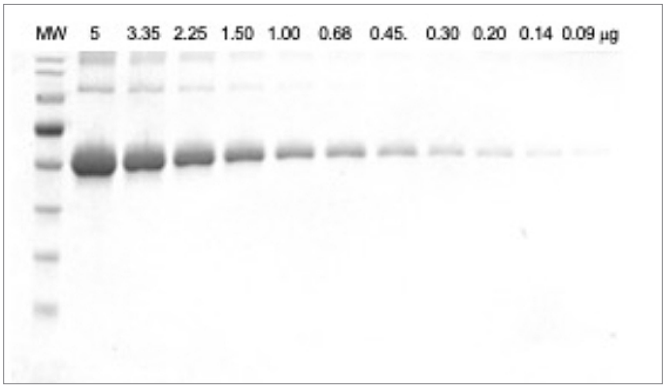

Figure 4. Coomassie-stained gel with serial dilution of BSA imaged on the chemiSOLO.

The lid of the chemiSOLO contains LEDs that uniformly illuminate the gel area during imaging. Light is absorbed as it passes from the lid through stained regions of the gel on its way to the detector. The amount of light absorbed is proportional to the concentration of stain bound to the sample. Therefore, the amount of sample in a band can be quantified by measuring the light absorbed by the band and comparing it to absorbance of bands containing known amounts of protein (Figure 5).

Densitometric analysis was performed using AzureSpot Pro Analysis Software. The 1D module was selected in AzureSpot Pro and the image opened. A box was manually drawn to represent the background (Ti) signal, and lanes and bands were identified within the software (Figure 6).

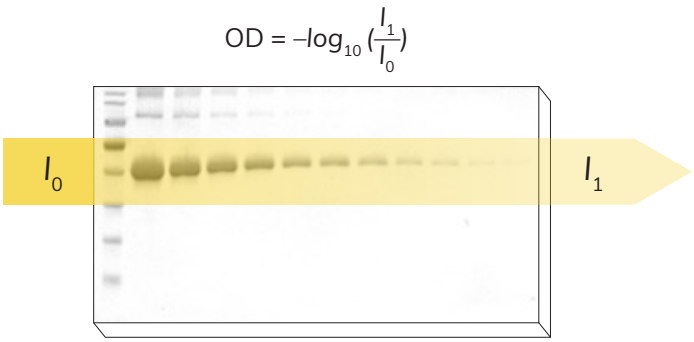

Figure 5. The principal of densitometry based on measurement of light transmittance.

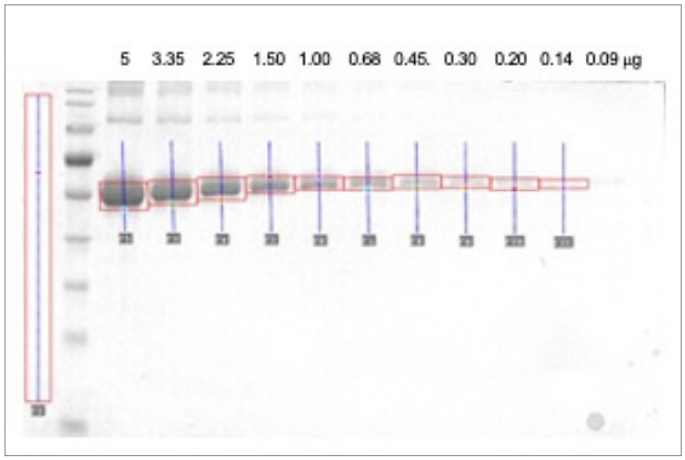

Figure 6. The image from Figure 4 with bands and region used to define Ti highlighted in AzureSpot Pro software in preparation for densitometric analysis.

The measurements for band area and volume were copied from AzureSpot Pro and pasted into the "chemiSOLO Densitometry Calculator" Excel® sheet and the loaded standard values were entered in the appropriate column. Within the spreadsheet, a standard curve was automatically created, plotting the optical density vs the loaded standard amount and a line of best fit.

As shown in Figure 7, the optical density measured for each band varied linearly with protein loaded over the entire range, with an R^2 value of 0.984. If unknown samples had been included on the gel, their concentration would be automatically calculated within the Excel sheet.

To demonstrate the potential to carry out densitometric analysis on a more complex sample, a band within a more complex sample was analyzed (Figure 8).

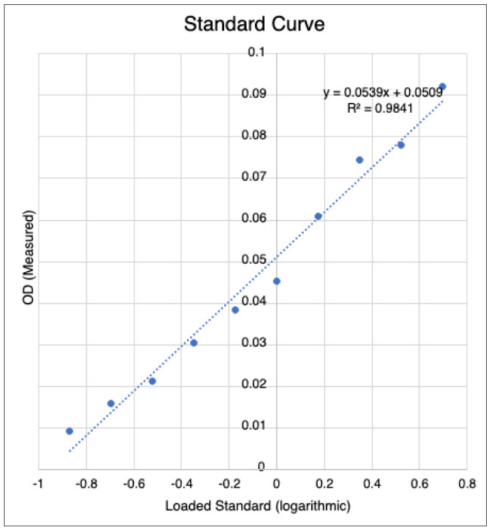

Figure 7. Plot of optical density (OD) vs protein loaded for the gel in Figure 6.

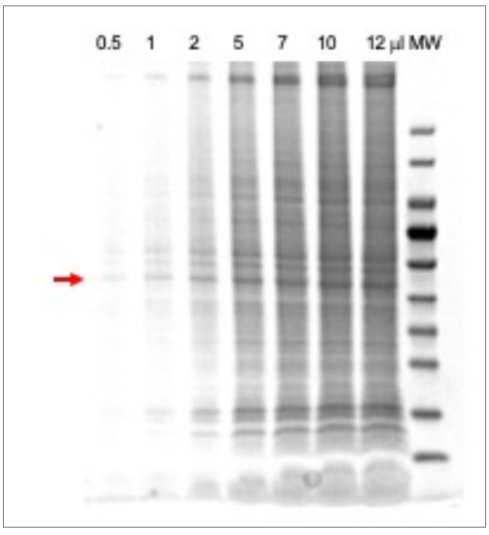

Figure 8. Coomassie-stained gel containing a serial dilution of HeLa cell lysate. The band indicated by the red arrow was analyzed.

Densitometric analysis was carried out within AzureSpot Pro (Figure 9) and the data exported to the "chemiSOLO Densitometry Calculator" Excel® sheet. As shown in Figure 10, the optical density measured for the band varied linearly with protein loaded over the entire range, with an R2 value of 0.983 (Figure 10).

In addition to greyscale imaging, the chemiSOLO can capture full-color images, such as that shown in Figure 11. Color images are particularly useful when interpreting protein molecular weight markers that contain differently colored bands. Full-color images are also helpful when interpreting gels stained with a stain that reacts differently with different proteins, as can occur with some silver stain protocols. In these situations, a color image can help identify bands or spots of interest.

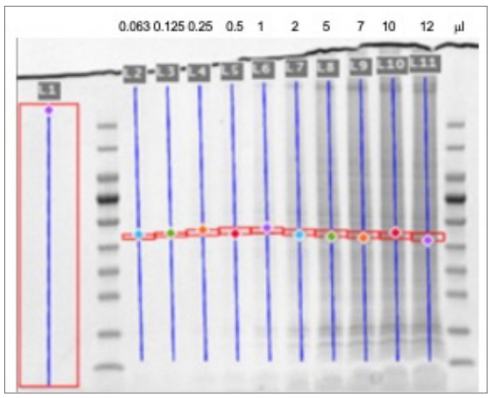

Figure 9. The image from Figure 8 with bands and region used to define Ti highlighted in AzureSpot Pro software in preparation for densitometric analysis.

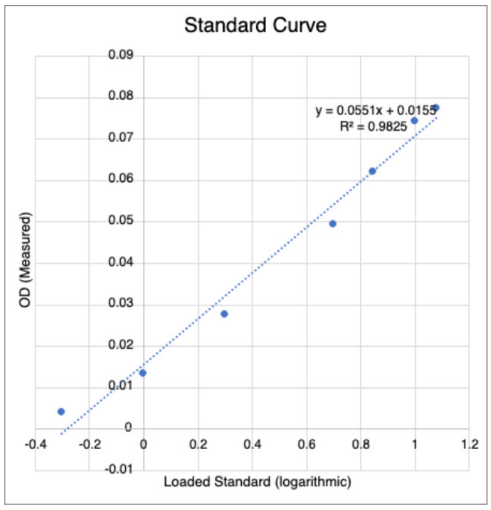

Figure 10. Plot of optical density (OD) vs protein loaded for the gel in Figure 9.

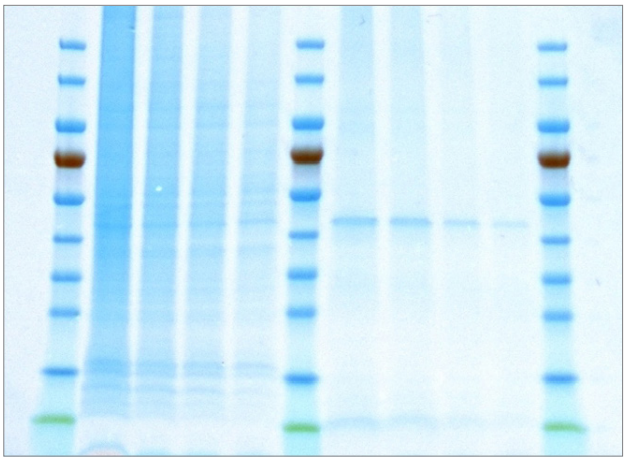

Figure 11. Coomassie-stained gel showing a color molecular weight marker.

Conclusion

The chemiSOLO provides fast, intuitive white-light imaging and densitometric analysis of colorimetrically stained gels. Images may be stored as full-color images or as greyscale images. Characteristics of the chemiSOLO that facilitate densitometry include:

- High-resolution imaging for identification of fine bands on 1D gels or small spots on 2D gels
- A linear range over 2.1 OD
- Even transillumination over the entire 15 cm x 10 cm imaging area to accommodate most common gel sizes
- Color and greyscale imaging for the most informative qualitative and quantitative analysis

For more information, visit azurebiosystems.com/chemisolo.

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