PROTOCOL

Densitometric analysis of protein gels

Image acquisition and verification of scanner calibration for band quantitation and spot identification using Azure DensitoMetrics

Azure Sapphire Biomolecular Imager

RGB & Near-Infrared Scanner | Chemiluminescence | Phosphor Imaging | Densitometry

Contents

I. Materials and reagents....2
II. Technique....2
III. Context and relevance....2
IV. Objective....3
V. Theoretical basis....3
VI. Procedure....6
VII. Quantitative analysis of 1D gels....12
VIII. Results....15
IX. Conclusions....16

Image acquisition and verification of scanner OD calibration for band quantitation and spot identification using Azure DensitoMetrics™

I. Materials and reagents

- Gels stained with colorimetric total protein stain (e.g., Silver Stain, Coomassie Blue)
- Azure Sapphire Biomolecular Imager with green channel (520nm laser; 565/24nm emission filter)
- Azure DensitoMetrics™ (Sapphire Densitometry Package)
- AzureSpot Analysis Software for band quantitation

II. Technique

This note describes measurement of optical density (OD) using the Azure Sapphire Biomolecular Imager for quantification and identification of proteins separated by gel electrophoresis and stained with colorimetric dyes.

The note also explains how to verify linearity of the scanner signal using a density steps tablet.

III. Context and relevance

The identification and quantification of target proteins in complex mixtures is broadly performed through the analysis of images of gels stained with reagents for total protein detection. Among the most-used colorimetric reagents are several formulations for silver staining and many commercial preparations of Coomassie blue dyes.

Different silver stain formulations stain protein spots and bands in a variety of colors including black, blue, brown, yellow, or red, depending on their charge and experimental conditions, which is useful for differentiating overlapping spots in 2D gels.

Some of the advantages of densitometry conducted on the Azure Sapphire Biomolecular imager are:

- High-resolution scanning for small spot identification
- Large imaging area
- Variable sample thicknesses
- Direct proportionality of the signal over a range of 3.6 OD
- Intensity of illumination auto-adjustable to the optical density of the sample
- Verification of the scanner calibration

Flatbed laser scanners offer the advantage of high resolution that allows the identification of extremely small spots. The Azure Sapphire Biomolecular Imager can image samples of up to 25cm x 25cm at resolutions of 10, 25, 50, 75, 100, 150, and 200 microns.

The Azure Sapphire Biomolecular Imager accommodates gels of different thicknesses. With the software, fine adjustment of the focal plane is possible to any value up to 4 mm above the glass surface. In addition, the automatic Z-scan feature makes it easy to find the optimal imaging setting by generating multiple independent images over multiple selected focal planes on the Z-axis. The SmartScan feature determines the optimal combination of laser intensity and detector sensitivity for each sample, avoiding saturated signals.

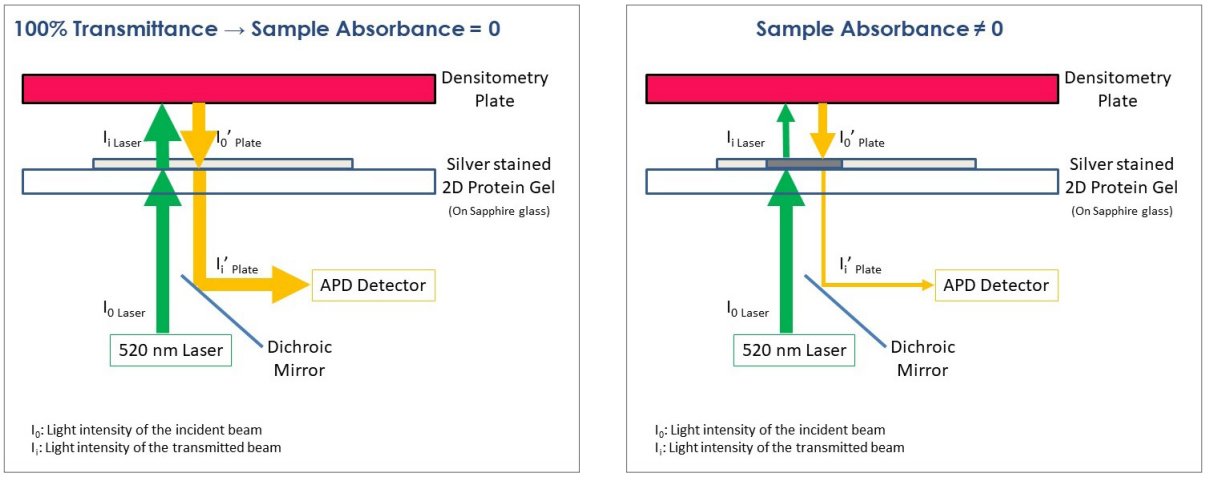
The laser scans the entire region of interest (ROI) using a fixed intensity value for even illumination across the imaging bed. Simultaneously, a high-sensitivity and linear-response detector measures the light transmitted through the sample in every step of the scan, which ensures even sensitivity across the scan surface.

IV. Objective

To describe a standard procedure for an easy and reliable densitometric quantification of 1D and 2D protein gels stained with colorimetric dyes.

V. Theoretical basis

The laser light of the point scanner excites the densitometry plate placed on top of the sample. The plate's fluorescent emission provides the light that passes through the sample towards the detector. The absorbance of light by the colorimetric stain in the sample is proportional to the concentration of the stain bound to the target.



Verification of the scanner's OD calibration (analytical instrument qualification, AIQ)

For Operational Qualification (OQ), the verification of the scanner linearity for densitometry measurements is performed using a neutral density filter array (21-Step Tablet, delta OD 0.15 per step). The user should decide the frequency of this procedure. Some laboratories perform the verifications every 6 months, while others repeat it every day a densitometry experiment is performed.

a. Light transmittance (T):

$T_i = I_i / I_0$

$T_i\% = T_i \times 100$

Where:

T_i: Transmittance of the sample for an individual pixel

I_0: Light intensity of the incident beam (T%= 100%)

I_i: Light intensity of the transmitted beam (after passing through the sample)

b. Relation between light absorption and optical density (OD):

$$OD_i = -\text{Log}(T_i) = -\text{Log}(I_i/I_0) = \text{Log}(I_0) - \text{Log}(I_i)$$

Where:

ODi: Optical density of the sample for an individual pixel

Thus, for:

T% = 100%	→ T= 1	→ OD= 0
T% = 10%	→ T= 0.1	→ OD= 1
T% = 1%	→ T= 0.01	→ OD= 2
T% = 0.1%	→ T= 0.001	→ OD= 3

c. Before hitting the densitometry plate, the laser light is absorbed by the sample (first pass, laser attenuation). The light intensity of the laser is reduced by the sample OD:

$$\text{Log}(I_{i\ LASER})_{ODi} = \text{Log}(I_{0\ LASER}) - OD_i$$

Where:

$\text{Log}(I_{0\ LASER})$: Logarithm of the incident intensity of the laser

$\text{Log}(I_{i\ LASER})_{ODi}$: Logarithm of the transmitted intensity of the laser after passing through the sample

d. That attenuation in the laser intensity affects the fluorescence of the densitometry plate directly. The intensity of the **plate emission** is reduced by the same OD_i magnitude as the laser beam.

$$\text{Log}(I_i'_{\ PLATE})_{Emi} = \text{Log}(I_0'_{\ PLATE})_{OD=0} - OD_i$$

Where:

$\text{Log}(I_i'_{\ PLATE})_{Emi}$: Logarithm of the intensity of the plate emission linked to the laser attenuation

$\text{Log}(I_0'_{\ PLATE})_{OD=0}$: Logarithm of intensity of the plate emission when there's no laser attenuation

e. The **light emitted by the densitometry plate** is absorbed as it passes through the sample (second pass, plate light attenuation).

The intensity of the **transmitted plate-light** after passing through the sample decreases by ($-OD_i$). The double path of light through the sample increases the sensitivity of detection specially for faint spots and bands.

The neutral density filters are designed to absorb light of all visible wavelengths equally. Then, the OD of the sample in a certain position is the same for both the laser beam and the densitometry plate emission.

Replacing the equation from (d):

$$\text{Log}(I_i'_{\ PLATE})_{ODi} = \text{Log}(I_i'_{\ PLATE})_{Emi} - OD_i = \text{Log}(I_0'_{\ PLATE})_{OD=0} - OD_i - OD_i = \text{Log}(I0'_{\ PLATE}) - 2OD_i$$

Where:

$\text{Log}(I_i'_{\ PLATE})_{ODi}$: Logarithm of the intensity of the plate emission after attenuation by the sample.

f. Using the OD values of the calibrated densitometry 21-Step Tablet supplied in the Azure DensitoMetrics™ package, the theoretical OD of the steps in densitometry can be calculated by multiplying each value of the step tablet by 2.

These theoretical OD values correlate with the experimental optical density over a range of 0.00 – 3.60 OD. The linearity is verified in a scatter plot of the theoretical and experimental ODs with $R^2 > 0.97$.

The experimental OD values of the 21-Step Tablet can be calculated as follows:

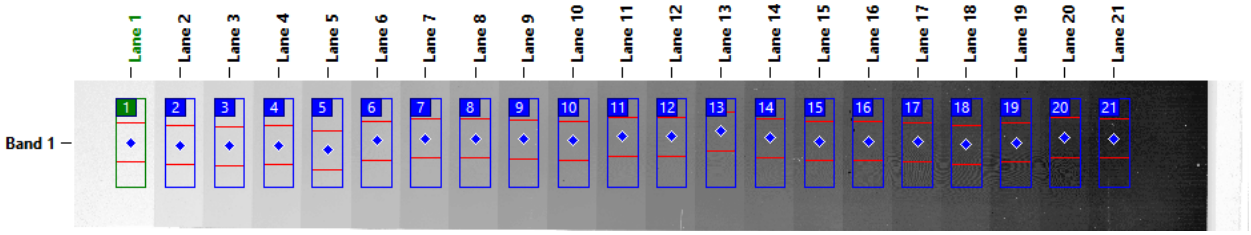
i. If the measurements of the intensities are determined using boxes of the same area in the analysis software:

$$OD_i \text{ (Measured)} = -\text{Log } (T_i) = -\text{Log } (I_i/I_0) = \text{Log (Signal }_{\text{STEP OD=0}}) - \text{Log (Signal }_{\text{STEPi}})$$

ii. If the measurements of the intensities are determined using boxes of the different size in the analysis software:

$$OD_i \text{ (Measured)} = -\text{Log } (T_i) = -\text{Log } (I_i/I_0) = \text{Log (Signal }_{\text{STEP OD=0}}) / \text{Area }_{\text{STEP OD=0}} \text{ x Area }_{\text{STEPi}} - \text{Log (Signal }_{\text{STEPi}})$$

Example 1. 21-Step Tablet analyzed in AzureSpot Analysis Software.



For Step OD = 0.30 → Theoretical OD_{Densit} = 0.60

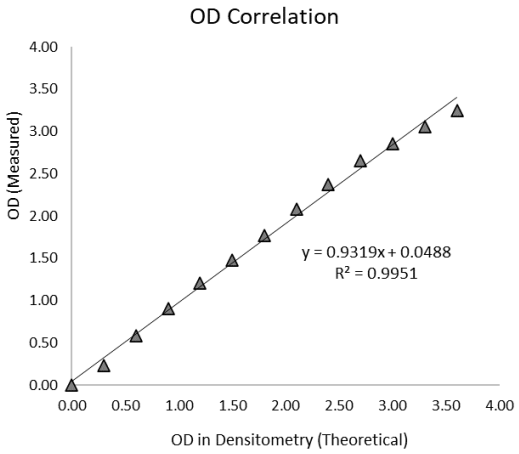
Measurements:

$T_{0.60}$ = 8,763,452 / 33,632,900 = 0.26

$OD_{0.60}$ = – Log (8,763,452 / 33,362,900) = 0.58

OD_i of Steps Tablet	OD_i in Densitometry (Theoretical)	Measured Signal (Counts)	T_i (Measured)	OD_i (Measured)
0.00	0.00	33,632,900	1.00	0.00
0.15	0.30	19,803,258	0.59	0.23
→ 0.30	0.60	8,763,452	0.26	0.58
0.45	0.90	4,209,248	0.13	0.90
0.60	1.20	2,101,386	0.06	1.20
0.75	1.50	1,114,388	0.03	1.48
0.90	1.80	569,647	0.02	1.77
1.05	2.10	278,103	0.01	2.08
1.20	2.40	141,150	0.00	2.38
1.35	2.70	74,137	0.00	2.66
1.50	3.00	47,380	0.00	2.85
1.65	3.30	29,390	0.00	3.06
1.80	3.60	19,210	0.00	3.24

Table 1. Theoretical and experimental OD.



Graphic 1. Scatter plot for the ODs.

VI. Procedure

Procedure for gel analysis

The following procedure describes how to use the Sapphire Capture software to acquire the images of 1D or 2D gels for densitometric analysis. Instructions below explain how to:

1. Acquire an image of your sample to be analyzed
2. Acquire and (3) analyze an image of the 21-Step Tablet to verify instrument calibration

You will then analyze the image of your sample using one of the following:

- AzureSpot Analysis Software: 1D gel analysis
- SameSpots 2D Analysis Software: 2D single stain and DIGE analysis
- SpotMap Analysis Software: Host Cell Protein (HCP) Coverage

Section VII will describe analysis of the image using AzureSpot Analysis Software for 1D gel analysis. For 2D gel analysis, follow the instructions in the software manual.

1. Sample Image Acquisition

1.	Turn on the Azure Sapphire Biomolecular Imager with green laser channel (520nm laser).
	Launch the Sapphire Capture Software. In the Imaging menu, go to FLUORESCENCE.
	If the 21 CFR Part 11 version of the Sapphire Capture software is installed, a user, password and specific privileges will be required to operate the instrument.
2.	Place the sample (e.g., silver-stained protein gel, 21-Step Tablet) on the clean glass surface of the scanner.
	A broad variety of colorimetric stains and samples can be analyzed. Samples can be fresh gels or dried gels wrapped in cellophane.
3.	Place the densitometry plate on top of the sample. Make sure there are no air bubbles in between the gel and the plate. The wash buffer of the stain technique could be used on top of the gel to minimize the presence of air bubbles. Note that the scan surface is water sealed. The densitometry plate must cover the entire sample.
	Use a clean densitometry plate. Protect the plate form scratches. Wash immediately with distilled/deionized water.

4. On the right-hand side of the FLUORESCENCE tab, select the protocol for densitometry plates.

   Set the desired parameters for scanning (pixel size, scan speed, and focal plane).

   Assign a pseudocolor to the channel(s).

   The L1 intensity value displayed for the available channel is the optimal preset value for the protocol.

   The preset L1 intensity value produces enough illumination to detect faint spots and bands, while allowing enough contrast of darker ones.

   The intensity preset produces no saturation of the detectors in sample regions that are totally translucent (OD = 0; T% = 100%).

   The focal plane is approximately in the middle of the gel thickness. The Z-Scan functionality of the scanner can be used to determine the focal plane for optimal imaging.

5. Select the region of interest (ROI) to be scanned by clicking and dragging the green corners of the ROI.

   The ROI can be adjusted to the sample size after acquiring a PREVIEW image, or by using the rulers adjacent to the scanner glass. Allow some extra frame around the sample to verify the intensity when there's no absorbance from the sample (100% of transmittance).

6. Assign a name and location to save the image.

   Acquire the image using the preselected intensity level of the channel (recommended) by clicking SCAN.

   For gels with intense color, it is possible to increase the channel's intensity value in order to visualize darker regions.

   The use of SmartScan is recommended in these cases (algorithm that determines the highest intensity value while avoids saturation of pixels).

   Manual selection of an intensity value followed by SCAN is also an available option. For gels with faint bands or spots, the use of lower intensity values will allow the detection of such bands. For gels with intense color, higher intensity values will allow resolution of spots and bands—make sure that the signal is not saturated.

7. Once the scan is finished, the image will be displayed in the Gallery menu of the Sapphire Capture Software.

   Adjust the contrast of the image and do a visual analysis.

   Visually compare the gel to the densitometry image.

   Look for missing bands or spots in the image. If that were the case, rescan using lower intensity values.

   If the bands or spot were not resolved, rescan using higher intensity values.

   Verify there is no saturation of pixels by activating the SATURATION button in the Gallery.

8. **Optional:**

Remove the densitometry plate that was placed on top of the sample and rescan the gel using the same settings used for the densitometry.

Look for the presence of fluorescent bands or spots. Those shouldn't be present.

The presence of fluorescence signals from the gel affects the OD values. If fluorescent bands or spots were present, consider the use of lower intensity values.

The densitometry plates have been selected to be efficient at low laser intensity levels (low laser power, Level L1), which also keeps the autofluorescence of the samples at low levels.

9. Remove the gel from the scanning bed and clean the surface with a soft, lint-free paper being careful to avoid leaving water marks.

Distilled water or ethanol can be used to clean the glass, if necessary.

DO NOT leave a gel sitting on the glass surface of the scanner. Doing so can cause permanent staining of the glass.

DO NOT use solvents to clean the glass surface of the scanner. Doing so can permanently damage the plastic surfaces and the water-tight seal of the glass.

10. Proceed with the desired image analysis using:

- AzureSpot Analysis Software, Azure Biosystems' standard analysis software for 1D gel analysis. For quantitative analysis see Section VII.
- Premium Azure Analysis Software for 2D gel analysis (SameSpots 2D Analysis Software, and SpotMap Analysis Software).

Single channel images, the original .tif files generated in the Sapphire Capture Software, can be opened directly in the AzureSpot Analysis Software using the **Open Image** button.

2. Verification of the scanner's OD calibration. Image acquisition:

11. Place the 21-Step Tablet on the clean glass surface of the scanner:

- Make sure the glossy side is facing down (in contact with the glass).
- Align the longer side of the step tablet with the X-axis of the scanner's imaging area.
- Orient the darker steps towards the right-hand side of the imaging area.

Use gloves. Do not touch the surface of the neutral filter steps tablet.

12. Place the densitometry plate on top of the 21-Step Tablet.

The densitometry plate must cover the Steps Tablet entirely.

Use the same densitometry plate that was used for the sample(s).

13. On the right-hand side of the FLUORESCENCE tab of the Sapphire Capture Software, select the same settings previously used with the sample, **except for the focal plane that must be 0 cm**—under the FOCUS dropdown menu select “MEMBRANE (0)”.

The information on the intensity level used to scan the sample is available in its image in the Gallery menu, under the INFO tab.

14. Select the region of interest (ROI) to be scanned by clicking and dragging the green corners of the ROI.

The ROI can be adjusted to the sample size after acquiring a PREVIEW image, or by using the rulers adjacent to the scanner glass.

15. Assign a name and location to save the image.

Acquire the image using SCAN, verifying that the **parameter settings of the scanner are the same** as the ones used for scanning the sample.

High intensity levels use high laser power that might damage the Steps Tablet.

The densitometry plates have been selected to be efficient at low laser intensity levels (low laser power, Level L1), which also keeps the autofluorescence of the samples at low levels.

16. Once the scan is finished, the image will be displayed in the Gallery menu of the Sapphire Capture Software.

Verify there is no saturation of pixels by activating the SATURATION button. If saturated pixels are present, register which steps are saturated to exclude them from the calculations.

17. **Optional:**

Remove the densitometry plate that was placed on top of the Steps Tablet and rescan the Steps Tablet using the same settings used with the densitometry plate.

The presence of any fluorescence signals from the Steps Tablet affects the OD values. If fluorescent signals are present, consider the use of lower intensity values.

The densitometry plates have been selected to be efficient at low laser intensity levels (low laser power, Level L1), which also keeps the autofluorescence of the samples at low levels.

18. Proceed with the 21-Step Tablet image analysis using AzureSpot Analysis Software (the Azure Biosystems’ standard analysis software for 1D gel analysis).

Single channel images, the original .tif files generated in the Sapphire Capture Software, can be opened directly in the AzureSpot Analysis Software using the **Open Image** button.

3. Verification of the scanner's OD calibration. Image analysis:

19. Launch the AzureSpot Analysis Software: 1D Gel / Western Blot Analysis module.

If the 21 CFR Part 11 version of the AzureSpot Analysis Software is installed (AzureSpot Version Control), a user, password and specific privileges will be required to operate the software.

20. Open the densitometry image of the 21-Step Tablet. The image should not have saturated pixels (See item #7).

Adjust the contrast to visualize the differences of signal intensities of the steps.

To visualize all the steps of the tablet:

- Open the Contrast menu.
- Select the checkbox for 'Invert Display'.
- Change the default contrast from Manual to Hi-Contrast.
- Move the contrast slider from Mid to High. Adjust the visualization by typing 30,000 in the field named "High: 65,535".

The image should display all the shades of gray in the step tablet.

21. Create a lane for each step of the 21-Step Tablet as indicated in the Example 1 (See above: item V. Theoretical basis, sub-item f).

- Go to the 'Create Lane' tab in AzureSpot.
- Make sure that the checkbox for 'Invert Intensity (white bands)' **is selected accordingly** on the left-hand side menu. The signal around the sample (see item #5) should be higher than the signal from the steps on the right-hand side of the image (steps seen as valleys, not peaks).
- Select the 'Draw New Lanes' icon from the left-hand side menu.
- Change the value in the field 'Lanes' to 21.
- Change the value in the field 'Lane % Width' to 80%.
- Add the 21 lanes by clicking and dragging from the upper left corner of the first step on the left side of the image to the lower right corner of the last step on the right side of the image.
- Use 'Move Lane Box Edges' button to adjust and fit the lanes to the steps by clicking on the edges and dragging.

The template of lanes can be saved in a file as a 'Lane Object', which could be imported and reused in another analysis:

- Once the lanes are created and adjusted to the steps, go to the 'Export' tab on the top of the software plate and select 'Export Lane Object'.
- Save the .lan file for later use.
- To import a previously created Lane Object, go to 'Export' and select 'Import Lane Object'. When repeating this analysis for other experiments, place the Steps Tablet in the exact same position and select the same ROI to scan the Steps Tablet. This would help you avoid adjusting the lane box edges to fit the lanes on the steps.

22. Create a box manually for each lane of the template:

- Go to the 'Detect bands' tab in AzureSpot.
- Select 'Add/Delete Bands' from the left-hand side menu.
- Click on 'Advanced Options' button.
- Under 'Edge detection' select 'Fixed Width' and set a value for the width in pixels, e.g., 10 pixels for an image of 200-micron pixel size (resolution); 20 pixels for a 100-micron resolution image; etc.
- Add one box per lane manually. Left-click to add a box. Right-click to delete a box.

The number of pixels set for the fixed width of the boxes could be different from the suggested values.

To simplify the calculations, make sure that all the boxes are the same size, which can be verified in the 'Area" column of the measurements table below the image. The steps described above will produce areas of equal size.

23. Correct the measured signal applying a background subtraction:

- Go to the 'Background' tab in AzureSpot.
- Select 'Image Rectangle' from the left-hand side menu.
- Click and drag to draw a box inside the darkest lane of the image.

The average intensity of the pixels in the background area will be subtracted automatically, pixel by pixel, from each band. This can be verified in the 'Background' column of the measurements table below the image.

24. Export the correct measurements table ('All lanes' tab):

Go to the 'Export' tab on the top of the software plate and select 'Export to file/Measurements Table'.

Another option is to select 'Export measurements to Excel', if Microsoft Excel is installed in the computer running AzureSpot Analysis Software.

Make sure that the table is organized in columns, and the necessary information for the calculations is displayed.

The table that AzureSpot exports is the table displayed in the software plate. Note that there are several measurements table tabs to select:

- Select 'All Lanes' tab.
- On the top left-corner of the table there are three icons. Click on the first icon and select the checkboxes for 'band index', 'band name', 'volume', 'volume + background', 'background', 'area', and 'band width'. These fields are necessary for the calculations.
- Click on the second icon to select it as active. The information will be displayed in columns for the fields selected above. The first column should display the lane numbers.

25. Calculate the experimental OD for each step using the data of the measurements table as indicated in the Example 1 (See above: item V. Theoretical basis, sub-item f).

In a scatter plot of $OD_{Measured} = f(OD_{Theoretical})$ in Densitometry):

- The trendline should be linear with low dispersion of the values ($R^2 > 97\%$)
- The slope should be approximately 1 (0.9–1.0)

Use a spreadsheet to create the graphs, e.g., Microsoft Excel.

VII. Quantitative analysis of 1D gels

4. Image analysis using AzureSpot Analysis Software

26. Launch the AzureSpot Analysis Software: 1D Gel / Western Blot Analysis module.

If the 21 CFR Part 11 version of the AzureSpot Analysis Software is installed (AzureSpot Version Control), a user, password and specific privileges will be required to operate the software.

27. Open the densitometry image of 1D gel. The image should not have saturated pixels (See item #7).

The lanes must be oriented in a vertical position.

Adjust the contrast to visualize the bands.

To visualize all the bands:

- Open the Contrast menu.
- Select the checkbox for 'Invert Display' accordingly. Dark bands should be displayed in a clear background.

28. Create a lane for each sample, control, and empty lane in the gel:

- Go to the ‘Create Lane’ tab in AzureSpot.
- Make sure that the checkbox for ‘Invert Intensity (white bands)’ **is selected accordingly** on the left-hand side menu. The background signal should be lower than the signal from the bands (bands seen as peaks).
- Select the ‘Draw New Lanes’ icon from the left-hand side menu.
- Change the value in the field ‘Lanes’ to match the number of lanes in the gel.
- In the field ‘Lane % Width’, use a value to include the full width of the bands, e.g., 100%.
- Add the lanes by clicking and dragging from the upper left corner of the first lane on the left side of the image to the lower right corner of the last lane on the right side of the image.
- Use ‘Move Lane Box Edges’, ‘Move Lane’, ‘Bend/Resize’, and the other buttons to adjust and fit the template lanes to the gel lanes and bands.

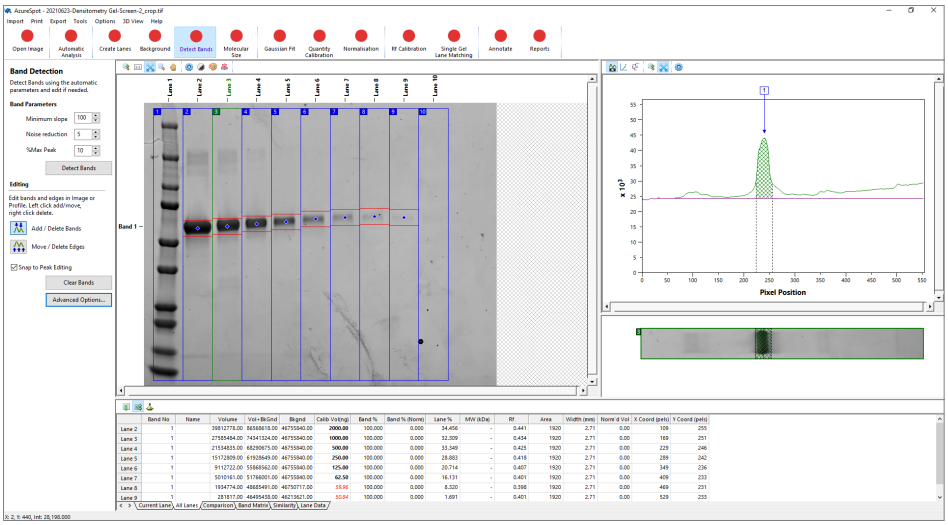
In densitometry, the absorbance of the bands is visualized as a valley instead of a peak.

In densitometry, the transmittance of a sample is proportional to its concentration being this proportionality described by -Log (T_i) = A_i. The bands in a gel are not homogeneous, and have diffuse edges and irregular shapes, which makes it difficult to accurately quantify the transmittance from the band signals.

The procedure described here uses the inversion of the intensity of the bands to convert the valleys to peaks to allow the quantification using standards of concentration. The peaks are not directly proportional to the concentration, which requires either some transformation of the data in order to obtain a linear correlation (in a manual procedure not described in this application note) or the use of software to determine an algorithm that correlates signal with concentration (described in item 31).

The template of lanes can be saved in a file as a ‘Lane Object’, which could be imported and reused in another analysis:

- Once the lanes are created and adjusted to gel, go to the ‘Export’ tab on the top of the software plate and select ‘Export Lane Object’.
- Save the .lan file for later use.
- To import a previously created Lane Object, go to ‘Export’ and select ‘Import Lane Object’.



29. Detect the bands automatically or create the boxes manually for each lane of the template.

For manual creation of the boxes:

- Go to the 'Detect bands' tab in AzureSpot.
- Select 'Add/Delete Bands' on the left-hand side menu.
- Optional: Click on 'Advanced Options' button. Under 'Edge detection' select 'Fixed Width' and set a value for the width in pixels to be the same for all the boxes.
- Add the boxes for all the bands of interest manually. Left-click to add a box. Right-click to delete a box.

It is necessary to include each entire band in a box.

The size of the boxes is required to be the same for different bands. The background correction will be used to isolate the specific signal of each band ('Volume', displayed as peaks).

30. Correct the measured signal applying a background subtraction to obtain the value of each peak.

For gels with even background:

- Go to the 'Background' tab in AzureSpot.
- Select 'Image Rectangle' from the left-hand side menu.
- Click and drag to draw a box representative background area (clear color).

When using 'Image Rectangle', the average intensity of the pixels in the background area will be subtracted automatically, pixel by pixel, from each band. This can be verified in the 'Background' column of the measurements table below the image.

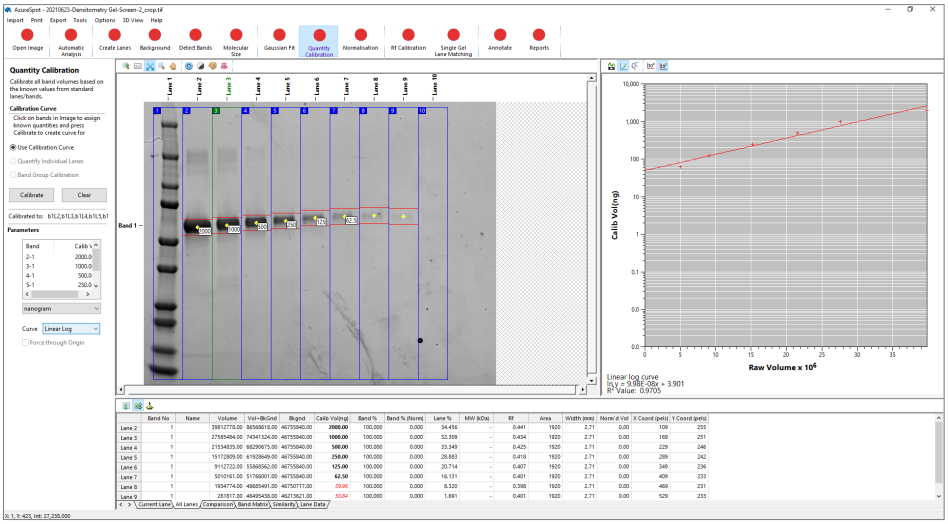
For gels with uneven background use a background subtraction method that allows the isolation of the peak.

31. Automated quantification of bands of unknown mass using 'Quantity Calibration' tab in AzureSpot:

- Go to 'Quantity calibration' tab in AzureSpot.
- Select 'Use Calibration Curve' from the left-hand side menu.
- Add the corresponding mass of each standard by left-clicking within the box of each standard band.
- Select the corresponding units from the dropdown menu on the left-hand side menu (e.g., picogram).
- Select 'Linear-Log' or 'Cubic Spline' from the dropdown menu for curve on the left-hand side of the menu.
- Click on 'Calibrate' button on the left-hand side menu.
- The mass of each unknow sample will be populated in the table in the column 'Calibrated Volume (units)'.

The table can be exported from the ‘Export’ tab on the top of the software window.

To express as concentration, calculate the ratio: Mass of each sample divided by the volume of sample loaded in the gel.



VIII. Results

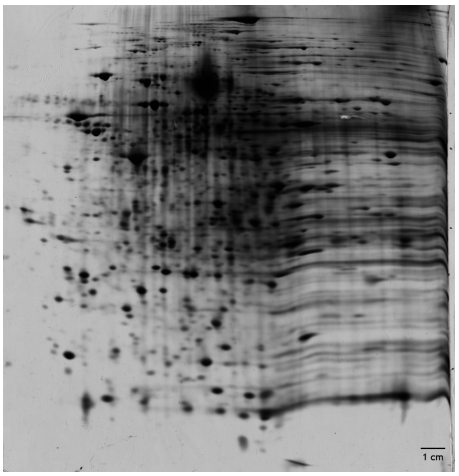


Image 1. 2D Silver Stain of a rat liver lysate.
Intensity L1 in 520nm laser channel.

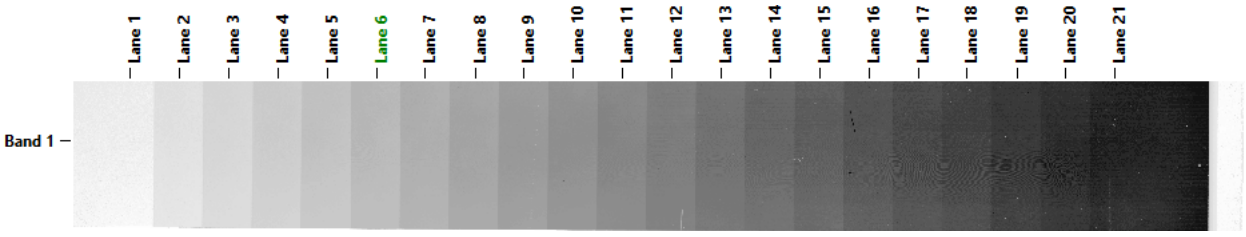


Image 2. 21-Step Tablet. Intensity L1 in 520nm laser channel.

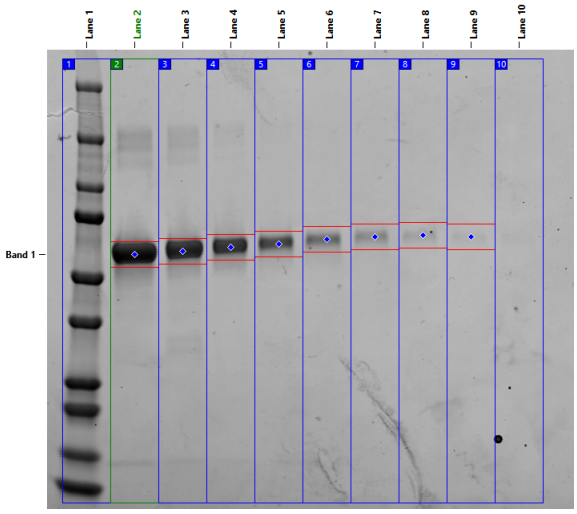


Image 3. Coomassie blue stain of a 1D SDS-PAGE. Two-fold dilutions starting at 2pg of BSA. Intensity L1 in 520nm laser channel.

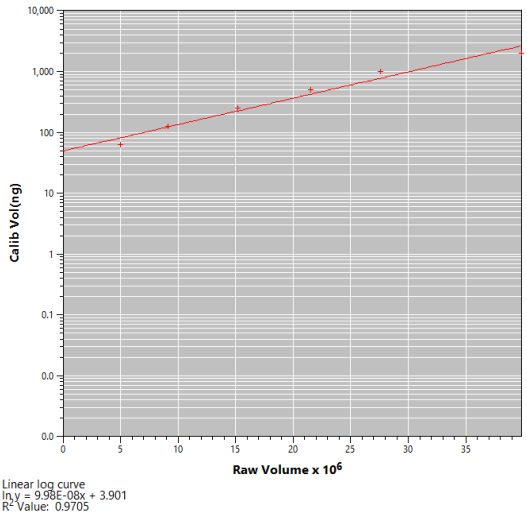


Image 4. Linear-log scatter plot of a Coomassie blue gel (Image 3).

IX. Conclusions

The Azure Sapphire Biomolecular Imager was designed for sensitive densitometric quantification of colorimetric gels, allowing assessment of a wide variety of gel stains. Its particular detection modality, in which the light passes twice through the sample, produces a high-contrast detection of faint bands and spots. The broad dynamic range of 0.00–3.90 OD (Transmittance range 100% to 0.013%) allows the detection of both strong- and weak-signal spots. The high sensitivity, linearity, and multiple stain detection capabilities make the Azure Sapphire Biomolecular Imager an ideal choice for densitometry.

Copyright © 2021 Azure Biosystems. All rights reserved. The Azure Biosystems logo, Azure Biosystems®, and Sapphire™ are trademarks of the Company. All other trademarks, service marks and trade names appearing in this brochure are the property of their respective owners.

MM-0035 R1