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Protein quantification in SDS-PAGE gels by densitometry using ImageJ/Fiji (with BSA normalization)

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Chemistry Method Devel...



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Protocol status: Working

We use this protocol and it's working

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Keywords: Protein quantification, SDS-PAGE, Densitometry, ImageJ, BSA, Coomassie, Gel Analyzer, ROI Calibration, curve, Protein normalization, gel analyzer method, bsa protein standards for normalization, protein band, gel image, bsa protein standard, gel, peak areas under each band, protein, bsa normalization, calibration standard, mean gray value, calibration, consistent image processing

Abstract

This protocol provides a detailed, step-by-step guide to quantify protein bands in stained SDS-PAGE gels using ImageJ/Fiji. It explains how to prepare and scan the gel image, optimize it for analysis, and then quantify protein bands by two complementary approaches:

- **Gel Analyzer method:** generates lane profiles and integrates the peak areas under each band.
- **Static ROI (Region of Interest) method:** fixed rectangular or oval ROIs are drawn over each band and measured for mean gray value and integrated density. This approach provides a simpler, reproducible measurement when bands are regular and well separated.

Both methods allow correction of background signal and can be combined with **BSA protein standards** for normalization or calibration (curve of Area vs ng). Results can then be exported to spreadsheets for statistical analysis and graphical representation.

This protocol emphasizes the importance of consistent image processing, careful baseline setting, and use of proper controls (e.g., internal BSA reference or calibration standards). It also discusses troubleshooting (e.g., saturated bands, background correction, poor separation) and good practices for reporting.

Estimated time: 45–75 min per gel

Level: Beginner–Intermediate

Materials

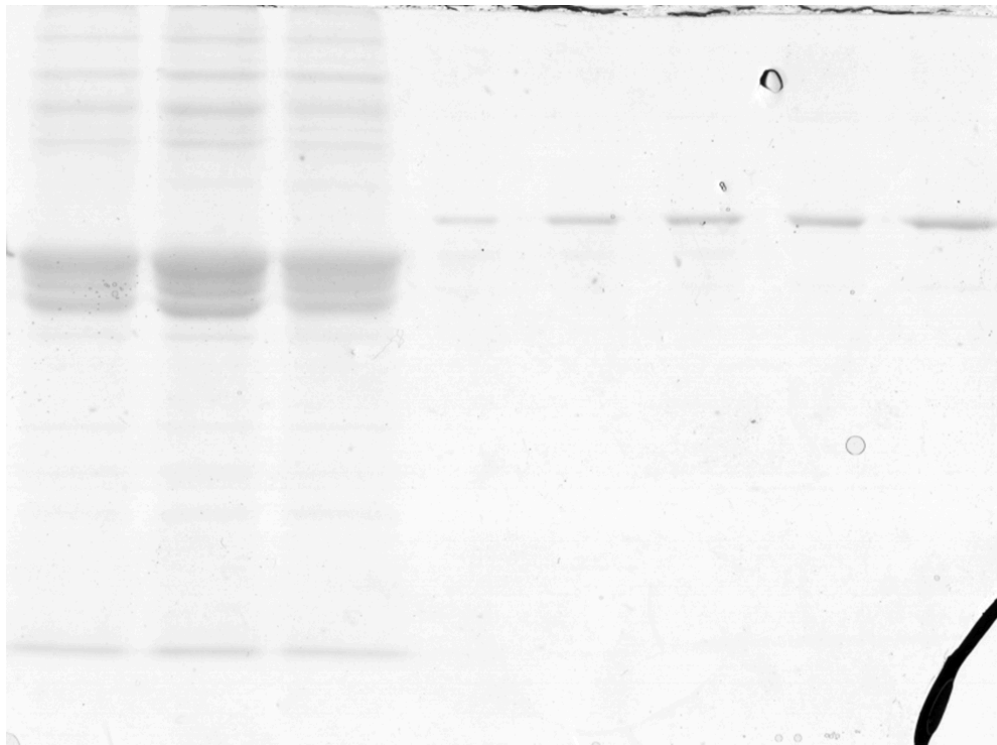
- SDS-PAGE gel with protein samples stained (e.g., Coomassie Brilliant Blue)
- **Protein samples** to be quantified
- **BSA protein standard** for calibration/normalization
- Loading buffer and molecular weight marker (protein ladder)
- Milli-Q water or equivalent

Equipment and Software

- Flatbed scanner or digital camera
- Computer with **ImageJ/Fiji** installed
- Spreadsheet software (Excel, Google Sheets)

Before you begin

- 1 Load BSA standard in at least three known amounts (e.g., 100, 250, 500 ng).
- 2 Scan the gel as grayscale, 300–600 dpi, preferably 16-bit, saved as **TIFF** (avoid JPEG).
- 3 Crop the image to include only the gel region.
- 4



A. Prepare the image in ImageJ

**5 Open the image:**

File → Open...

.

6 Convert to 8-bit (optional):

Image → Type → 8-bit

.

7 Adjust brightness/contrast:

Image → Adjust → Brightness/Contrast...

.

- Use *Auto* only if bands are not saturated.
- Apply the same adjustment for all gels in the experiment.

8 (Optional) Subtract background:

Process → Subtract Background...

(Rolling ball 50–200 px).

B. Quantification — Method 1 (Gel Analyzer)

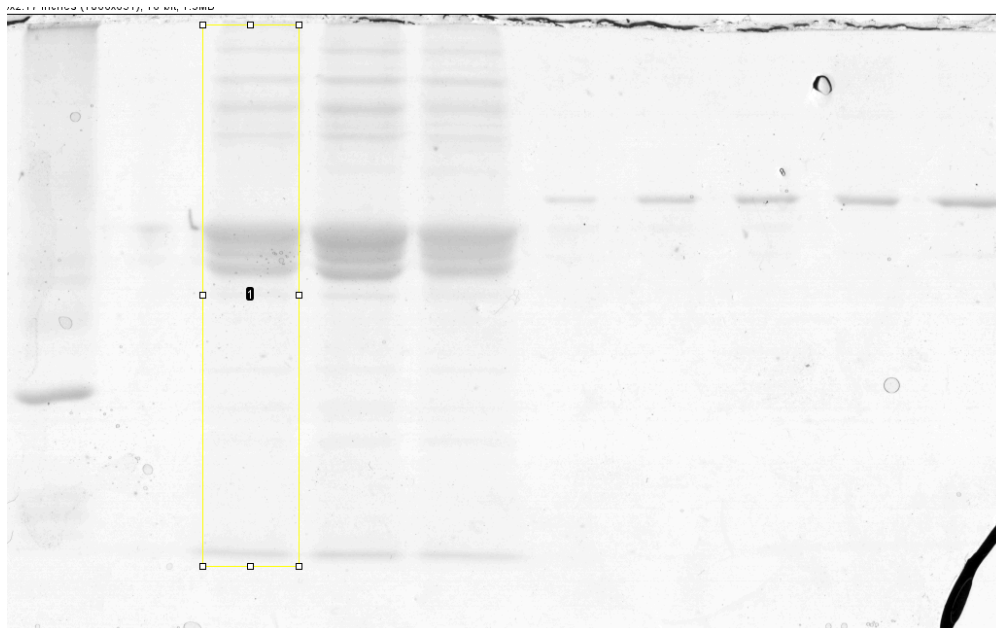
9 Select first lane: Use the rectangular tool to draw a box around the entire lane (from top to bottom, including all bands).

10 Go to

Analyze → Gels → Select First Lane

.

11

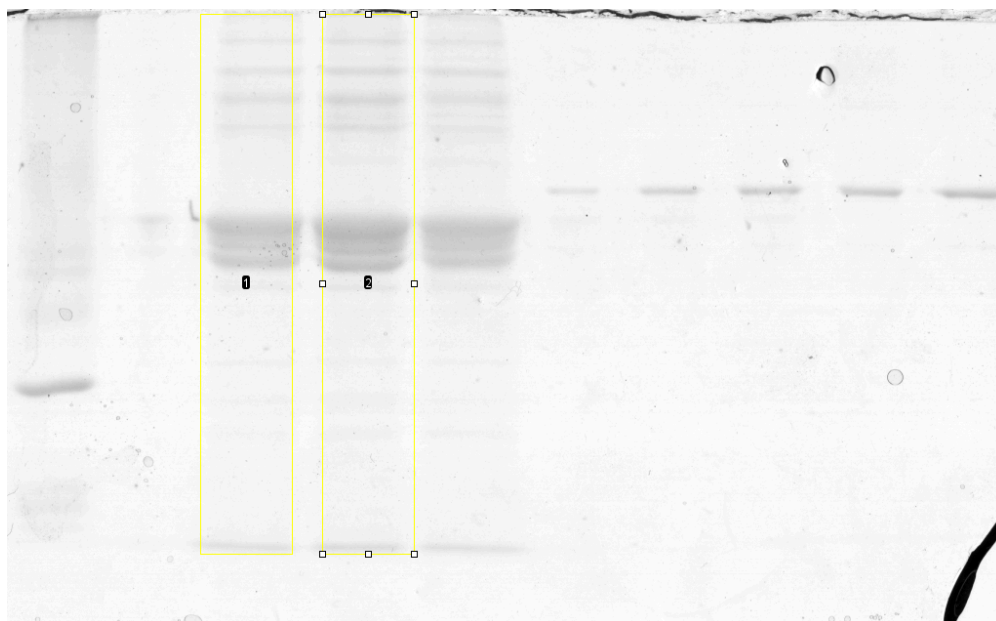


12 Move the ROI to the next lane and choose

Analyze → Gels → Select Next Lane.

Repeat for all lanes.

13

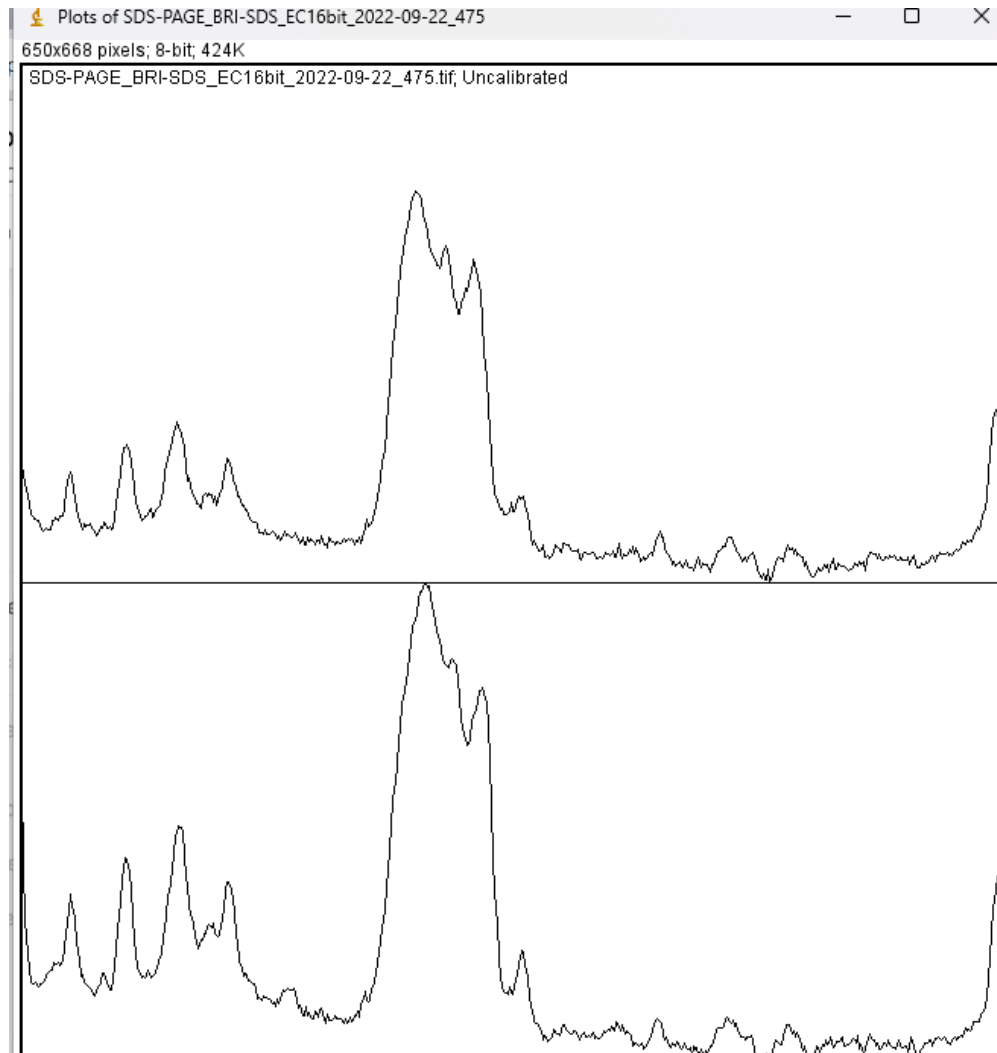


14 Plot lane profiles:

Analyze → Gels → Plot Lanes.

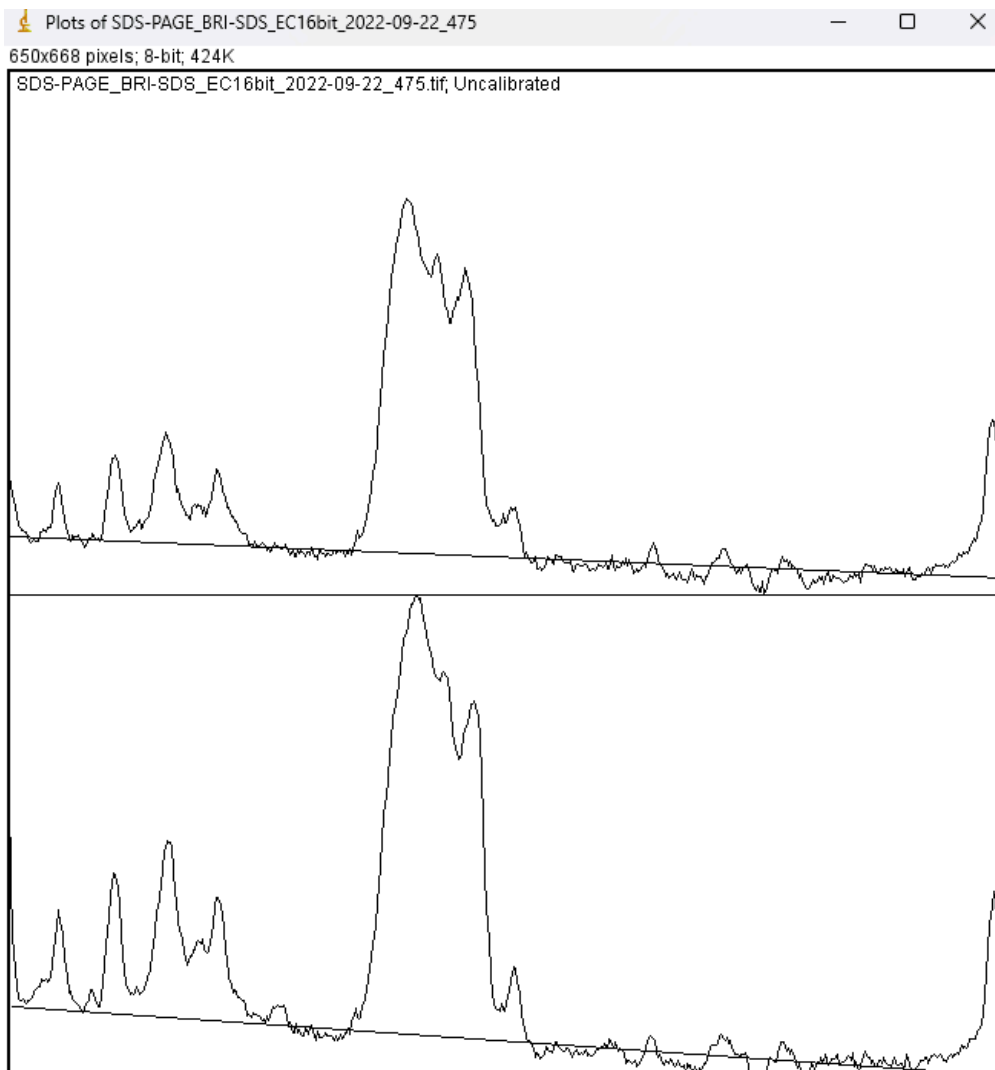
This generates a density profile with peaks representing bands.

15



16 Mark baseline: With the **Straight Line** tool, draw a baseline between adjacent peaks.

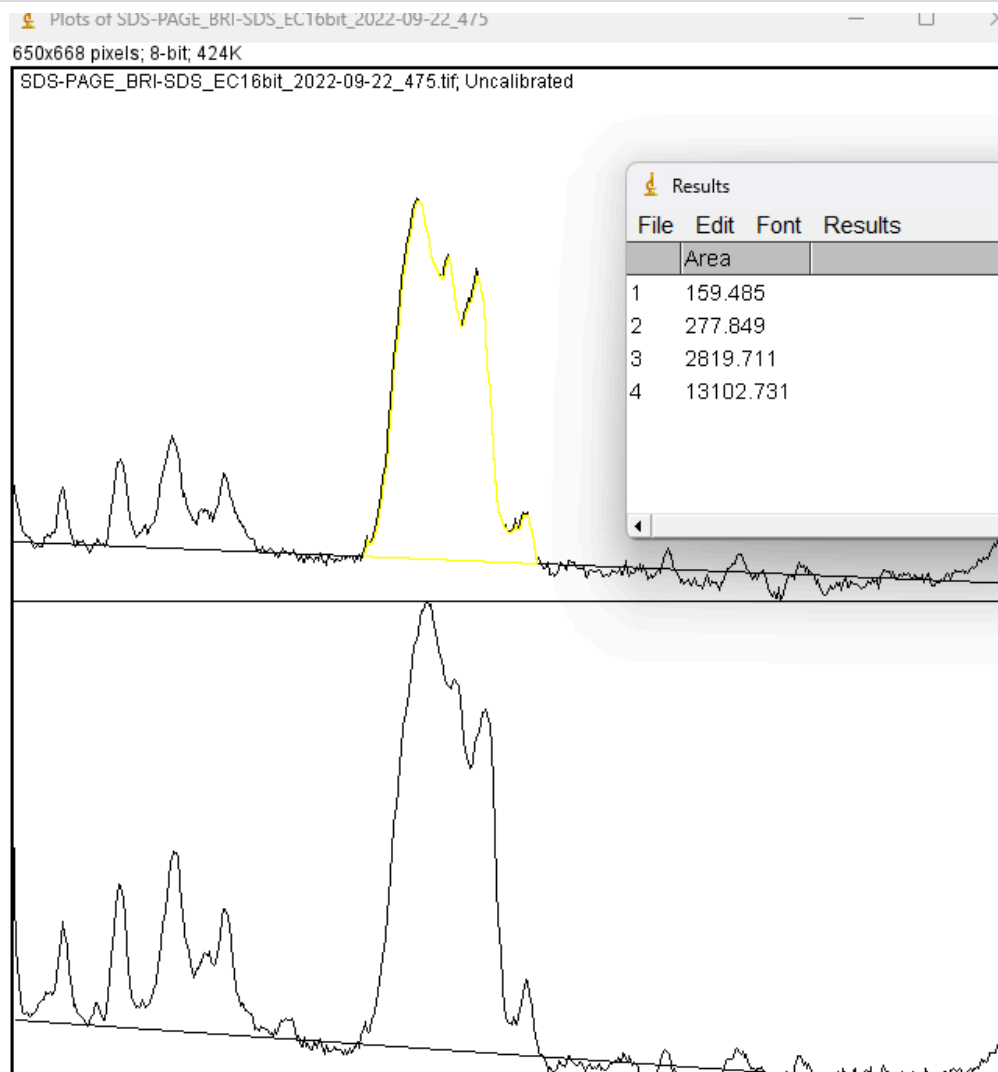
17



18 **Select peaks:** Use the **Wand Tool (tracing)** to click inside each peak. The area under the curve is measured.

19 **List results:** Click **List** in the profile window to send integrated values to the Results table.

20



C. Quantification — Method 2 (Static ROI)

21 Draw a **ROI** (rectangle/oval) around each band.

22 Measure:

Analyze → Measure

(shortcut **M**). This records **Mean Gray Value**, **Area**, and **Integrated Density**.

23



Results							
File Edit Font Results							
Label		Area	Mean	Min	Max	IntDen	RawIntDen
1 Plots of SDS-PAGE_BRI-SDS_EC16bit_2022-09-22_475		12730	255	255	255	3246150	3246150

- 24 Copy/paste the same ROI to equivalent bands to maintain identical size.
- 25 Correct background: measure a nearby background ROI and subtract it from band values (*Corrected Integrated Density = Integrated Density – Mean_bg × Area*).

D. Normalization and quantification

- 26 **Internal control:** Normalize each sample band against a constant reference (e.g., constant BSA load).
- 27 **Calibration curve:** Use multiple BSA standards to build a curve *Area vs ng*.
- 28 Export results from ImageJ (CSV) and calculate:
- Regression equation ($y = a \cdot x + b$) from BSA standards.
 - Convert unknown band areas into ng protein.

Step-by-step: Marking bands in the gel

- 29 **First lane selection:** Use the rectangular tool →

Analyze → Gels → Select First Lane

- 30 **Subsequent lanes:** Move the ROI horizontally to the next lane →

Select Next Lane

Repeat until all lanes are selected.

- 31 **Plotting:** After all lanes are defined →

Analyze → Gels → Plot Lanes

- 32 **Baseline drawing:** Use the straight line tool to mark the valley between peaks.
- 33 **Peak selection:** Click with the Wand Tool inside each peak to record integrated density.

Expected output

- 34
- Table of integrated area values for each band.
 - Normalized values (internal control) or converted to ng protein (calibration curve).

Troubleshooting

- 35
- **Saturated bands:** rescan gel with lower exposure.
 - **High background:** increase rolling ball radius or improve destaining.
 - **Poor band separation:** run gel longer or load less protein; adjust baseline manually.

Notes

- 36
- Always report dpi, bit depth, and method used.
 - Save and archive the raw image file (TIFF).

Protocol references

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