	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ** **PRODUCTON**
Gel Analysis with ImageJ - #1 **Module Hours: 3.0**	**Effective Date: 08/15/2024** **PRQs:**	**Revision 1.0** **Author:** **D. Roberts** **Checked by Editor: M. Stowell**

Background:

ImageJ stands as a testament to the power of open-source software in the realm of image analysis. Developed in the early 1990s by Wayne Rasband at the National Institutes of Health (NIH), this Java-based program has evolved into a cornerstone tool for researchers across various disciplines.

Its versatility is showcased through its applications in analyzing a wide array of images, from Western blots and SDS-PAGE gels to fluorescent cell imaging. For Western blots and SDS-PAGE, ImageJ simplifies the quantification of protein bands, offering precise measurements of band intensity and molecular weight determination. Researchers can quantify protein expression levels and compare samples with ease, enhancing the reproducibility and accuracy of their experiments.

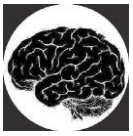
In fluorescent cell imaging, ImageJ's capabilities truly shine. It enables researchers to perform a myriad of analyses, including cell counting, colocalization studies, and tracking cellular dynamics over time. Its extensive plugin library further extends its functionality, allowing for specialized analyses tailored to specific research needs.

The beauty of ImageJ lies not only in its robust functionality but also in its accessibility. Being open-source, it is freely available to researchers worldwide, fostering collaboration and innovation within the scientific community. Furthermore, its user-friendly interface and extensive documentation make it accessible to users of all levels, from novice to expert.

As technology continues to advance, ImageJ remains at the forefront of image analysis, adapting to meet the evolving needs of researchers. Its continued development and widespread adoption ensure that it will remain an indispensable tool in the scientific toolkit for years to come.

This module will describe how to utilize ImageJ to analyze and improve the visual aesthetics of gels, both Western Blots and SDS PAGE.

1. Purpose

	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ**  **PRODUCTON**
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The purpose of this procedure is to recognize the multitude of applicable uses of ImageJ and to familiarize oneself with its most common applications.

2. Scope

- This procedure applies to qualified skills center users.

3. Responsibility

- It is the responsibility of the user to understand and perform the procedure described in this document.
- It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure (in a computer lab, document your findings using the database.)
- It is the responsibility of the user to become trained in the use of this application.

4. Definitions

- **RCSB Protein Data Bank – A database that houses information about 3D macromolecule structures, such as proteins and nucleic acids.**
- **Residue – an amino acid within a polypeptide sequence (protein)**
- **Sequence – chain of amino acids**
- **JalView – an application that allows for detailed protein, DNA, and RNA sequencing, structure visualization, and tree creation**

5. Materials/Equipment

- ImageJ application
- Google (for further research)
- Method to record results (computer file/notebook)

6. Procedures

- **6.1 Downloading the ImageJ Application:**
  - Go to the following link and click “download:” https://imagej.net/ij/

Figure 6.0

- Click the “download” tab which corresponds to your computer (windows, mac, etc.).
- Open the application from your files. You may have to select “Extract All Files” before you are able to open the application. When you have successfully downloaded ImageJ the home screen should appear:

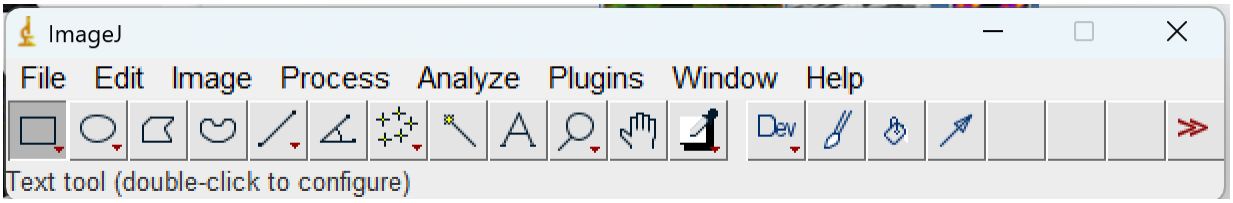


Figure 6.1

- **6.2 Improving the visual quality of your gel:**
  - Save the following image as a PNG file (HINT: “Print Screen” and then use a file converter on google).

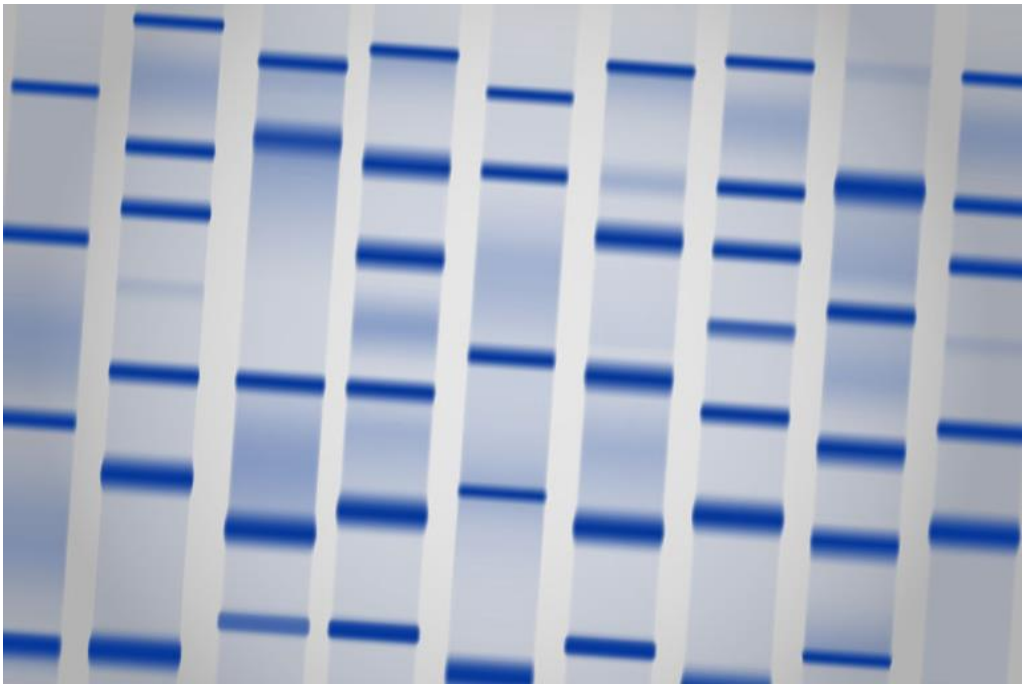


Figure 6.2

- Rotating the Gel: Select Image -> Transform -> Rotate: select the angle you wish to rotate it (you may have to do this multiple times to get the correct angle, and you have to use a negative value to rotate the gel counterclockwise).
- Increasing the contrast of the gel: Image -> Adjust -> Brightness/Contrast. Adjust the contrast and brightness bars so that only the darker bands are visible.
- Convert the image to 32-bit: Select Image -> Type -> 32-bit: this will aid in future steps of the module.

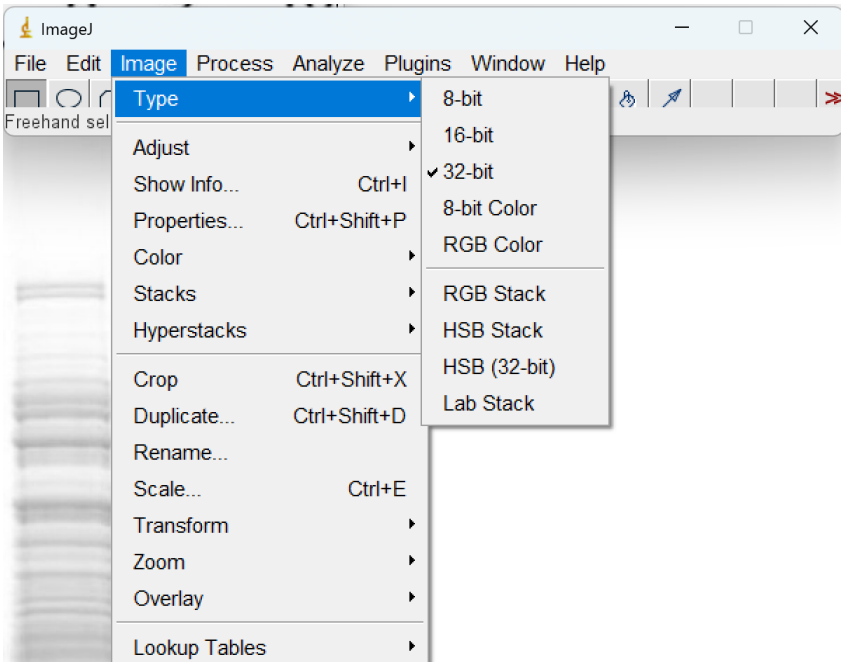


Figure 6.3

- **6.3 Gel Quantification**
  - Using the rectangle tool found at the top left of the program (just under “file”), click and drag so that the entirety of a single band is selected: select analyze -> gels -> select first lane
  - Now to duplicate select analyze -> gels -> select next lane. A second box should appear with a new box number. Drag it over a second band.

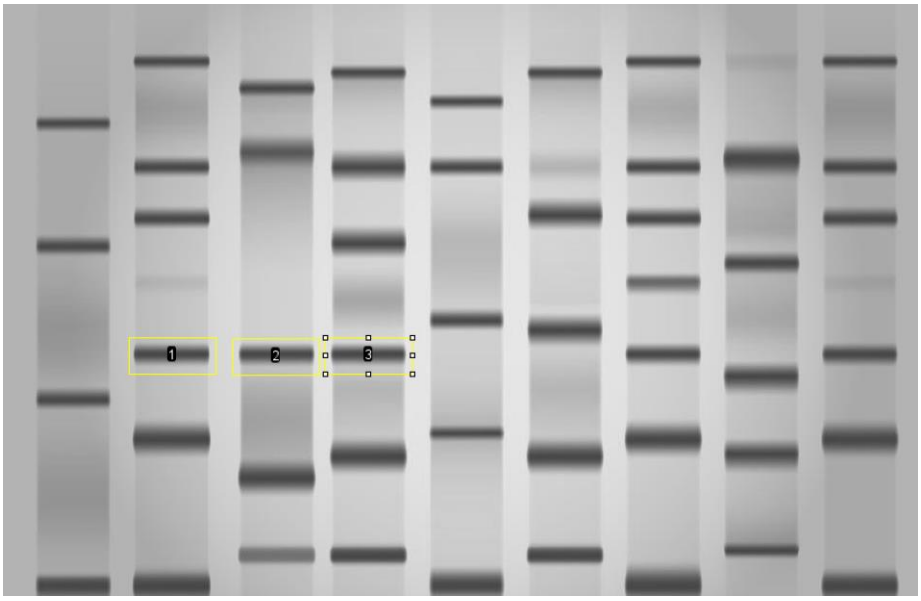


Figure 6.4

- Once all the bands have been selected, click Analyze -> gels -> plot lanes. You should see a third window appear with graphs of each bands' area.
- Select the line tool (located under process and close off your chart's curve.

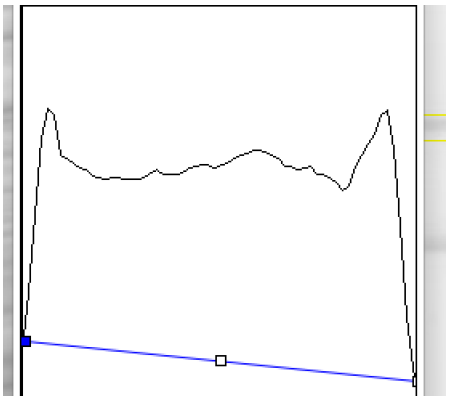
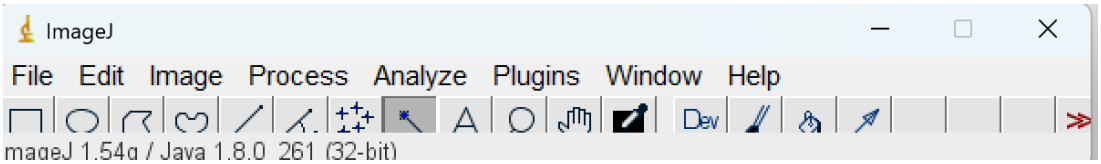


Figure 6.5

- Select the wand tool (located under analyze). Select the curve. Your charts should be highlighted in yellow.

Figure 6.6



- A fourth window should also appear with a table including the area of each chart. You can transfer this data to programs like excel for further graphical analysis.

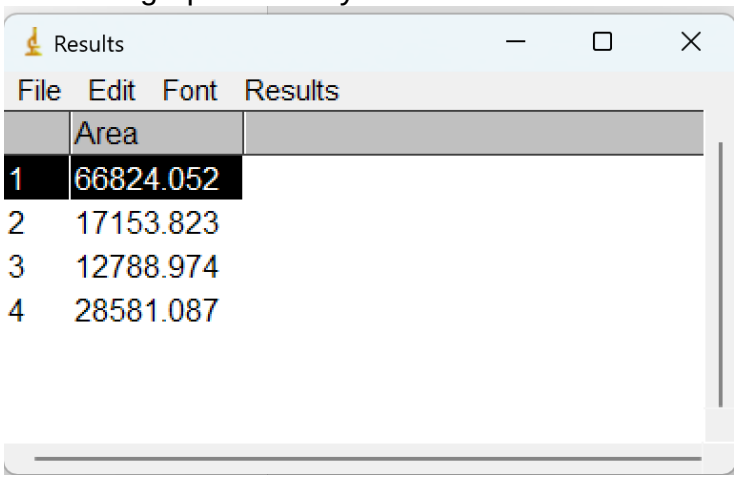


Figure 6.7

- **6.4 Calculating Gel**

Intensity Density:

- Invert your gel by selecting Edit -> invert: your bands should be white, and the background is black
- Use the rectangle tool to draw a box around the entire lane.
- Select Analyze -> Set measurements -> only check the box that says, “integrated density.”
- Select Analyze -> Measure. A third window should pop up giving numerical value for the lane’s density.
- Click and drag the box to the next lane and select Analyze -> Measure.

Figure 6.8

- After selecting and measuring 3 lanes, select three individual bands (one in each lane) at the same height. From the 6 intensities collected, you can use programs like excel to further analyze the data.

7. Troubleshooting:

- Always make sure the file is a PNG, 32-bit. The procedure might not work otherwise.
- Ensure your gels are horizontal when analyzing.
- Make sure you have the correct tool selected.
- In Procedure 6.4, if you get negative values, just take the absolute value of them for your data.

8. References:

"Introduction to ImageJ." *Image Analysis Modules*, 29 Apr. 2024, serc.carleton.edu/earth_analysis/image_analysis/intro_imageJ.html.

9. Module Methods Task

9.1 Use the image from 6.2 for the following tasks: be sure to convert the file to PNG!
9.2 Rotate the gel image so that it is no longer diagonal. Submit a resulting image.
9.3 Increase the contrast of your gel. Submit a resulting image. How does this tool help with visual analysis?
9.4 Analyze the area of the three bands shown in Figure 6.4. Submit one of the resulting charts (don't forget to close them off).
9.5 Submit an excel table with the band number, area values, and the fractional value of the area divided by Band 1's area. Your table should look something like this:

Band	Area	Band Area/Band 1 Area (Fractional Area)
1		
2		
3		

9.6 You know the concentration of the band in the first lane to be 2.0 mM. Using ImageJ and excel, predict the concentration of the adjacent bands. How did you use this technique to make your prediction?
9.7 Using the procedure from 6.4, calculate the intensity of lanes 2, 3 and 4 of the example gel. For your band, use the same 3 bands in figure 6.4. Create an excel file from the data collected. Compare the intensity of the band to the intensity of the lane. Your table should look something like this:

Lane/Band	Intensity	Comparison (B1/L1)
L1		
L2		
L3		
B1		
B2		
B3		

9.8 Which lane's protein is the purest? Why?

9.9 What is an application for intensity calculations? When might understanding the relative intensity/purity of a band to a lane be helpful?