

A Guide to Analyzing Western Blot Images from Jess Using AlphaView Software

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Introduction

Jess™, the newest Simple Western™ technology from ProteinSimple, allows you to not only run fully automated capillary-based Westerns, but she takes digital images of traditional chemiluminescent Western blot membranes. ProteinSimple's AlphaView® software allows you to analyze Western blot images taken on Jess so that you can prepare publication-ready figures. In this tech note, we'll show you how to export your images from Jess into AlphaView, and we'll cover the tools that you'll need to get the most out of your Westerns.



Exporting Image Files from Compass for Simple Western

To export raw images for further analysis in Compass for Simple Western, from the **File** menu, select **Export Images** (Figure 1). Then select a folder to export image files to.

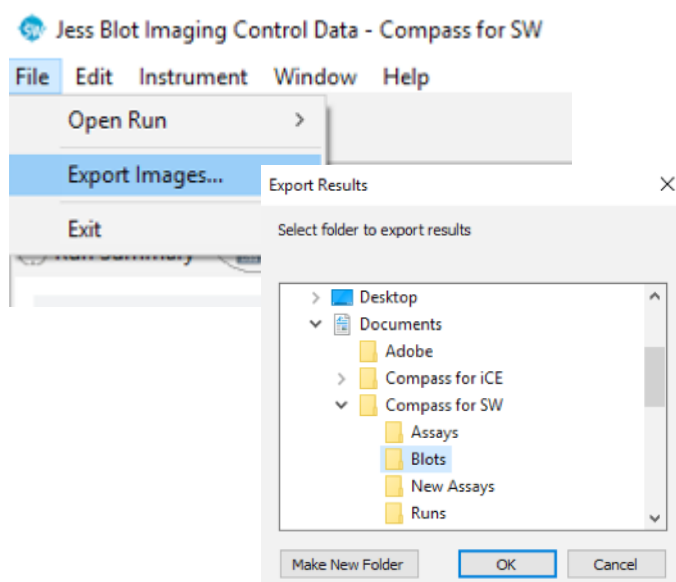


FIGURE 1. Export of image files from Compass for Simple Western.

Additionally, Jess will automatically launch a Windows Explorer window for you to access saved files. Images are saved in .png format. All raw and processed images will be saved to the selected folder. Raw chemiluminescent images will be saved as *<Results File Name>_Chemi_<Exposure Number>_<Exposure Time>*. Processed images, as shown in the Review pane, will be saved as *<Results File Name>_<Exposure Time>_view*. Raw and processed marker images will also be saved as *<Results File Name>_Markers* and *<Results File Name>_Markers_view*, if selected during the blot imaging protocol.

Importing Images into AlphaView

AlphaView is able to open most common image file formats. By default, when selecting **Open** from the **File** menu, AlphaView will only display available .tiff files. This default setting can be changed by clicking on the **Setup** menu and selecting **Preferences** (Figure 2A). From the **General** options tab, use the **Default Open** dropdown menu to select **All Image Files** (Figure 2B).

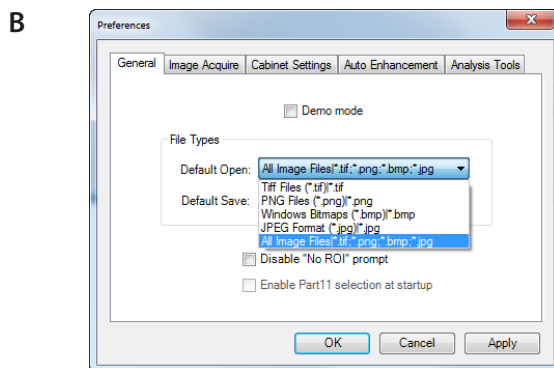
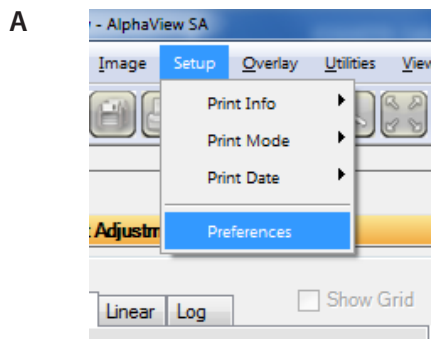


FIGURE 2. Importing images into AlphaView.

If you again select **Open** from the **File** menu, AlphaView will now display all image file types by default.

To open a Jess image for analysis, click on the **File** menu and select **Open**. Click on the desired image file name.

Adjusting Images in AlphaView

AlphaView offers multiple image adjustment tools (Figure 3). Contrast adjustments can be made using the sliders to adjust black, white and gamma levels (Figure 4). There is also an Auto Contrast option, as well as nine different pre-set Auto Enhance Levels that can be applied.

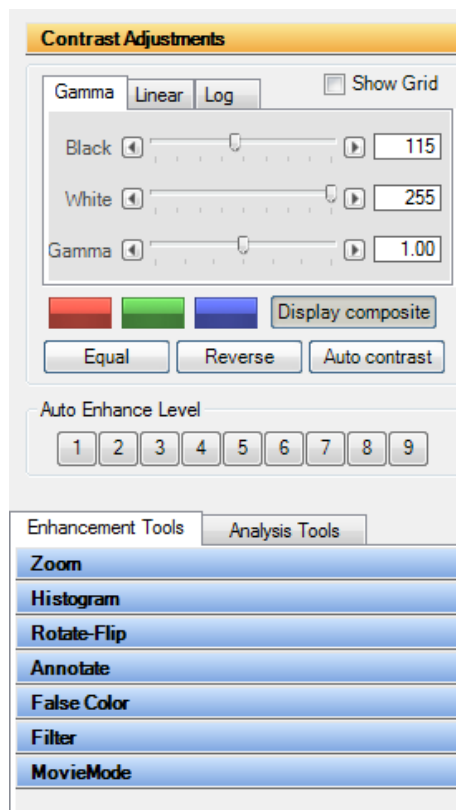


FIGURE 3. Adjustments tool panel.

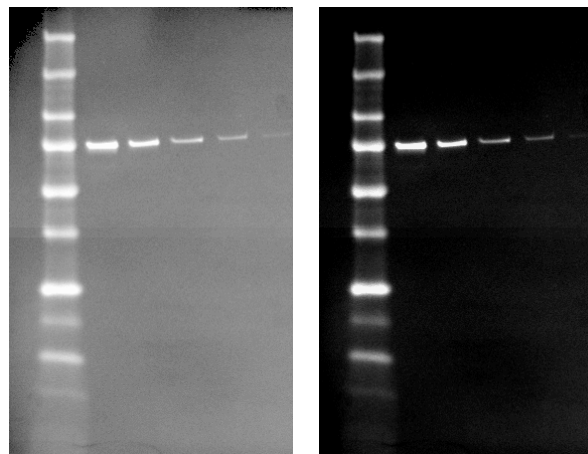


FIGURE 4. Blot image with contrast adjustments. Left, black level set at 0; right, black level set at 50.

Additional adjustment tools include the ability to rotate and flip images, apply sharpening and noise filters and annotate with text and shapes.

In AlphaView, image files can be saved as either Original or Modified file types (**Figure 5**). Original files refer to those that contain data saved in an unaltered form. This file type is best suited for images to be analyzed later. If black, white, or gamma levels have been adjusted, the new values are saved, but the pixel values are not altered. When re-opening the image file in AlphaView, the software will display the image with the values that were set when the image was saved. Selecting **Reset** in the tool bar will revert the image back to its original raw file format.

Selecting **Save Modified** from the **File** menu will save the image file as an 8-bit image that will permanently retain the changes made to the contrast settings. Any annotations and filters that were applied will also be permanently saved.

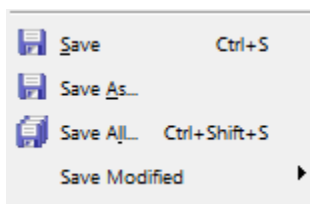


FIGURE 5. Available Save options in AlphaView.

Image Analysis

AlphaView has many analysis tools available for doing blot image analysis. These tools can be found in the **Analysis Tools** tab (**Figure 6**), where common features such as molecular weight determination and densitometry analysis are also found. More analysis tools can also be enabled. See AlphaView Software User Guide for more information.

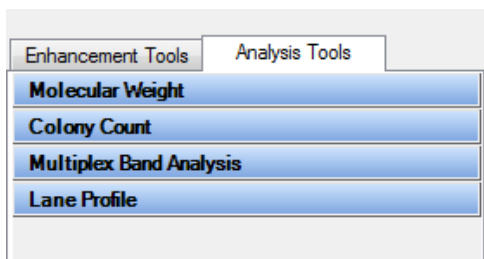


FIGURE 6. Analysis Tools tab options in AlphaView software.

Molecular Weight Analysis

Molecular Weight analysis allows you to assign molecular weight values to the ladder(s) present, save the assigned markers for later use and load previously saved ladders (**Figure 7**). Bands of interest can then be manually or automatically detected, from which the software will automatically calculate their molecular weights (**Figure 8**).

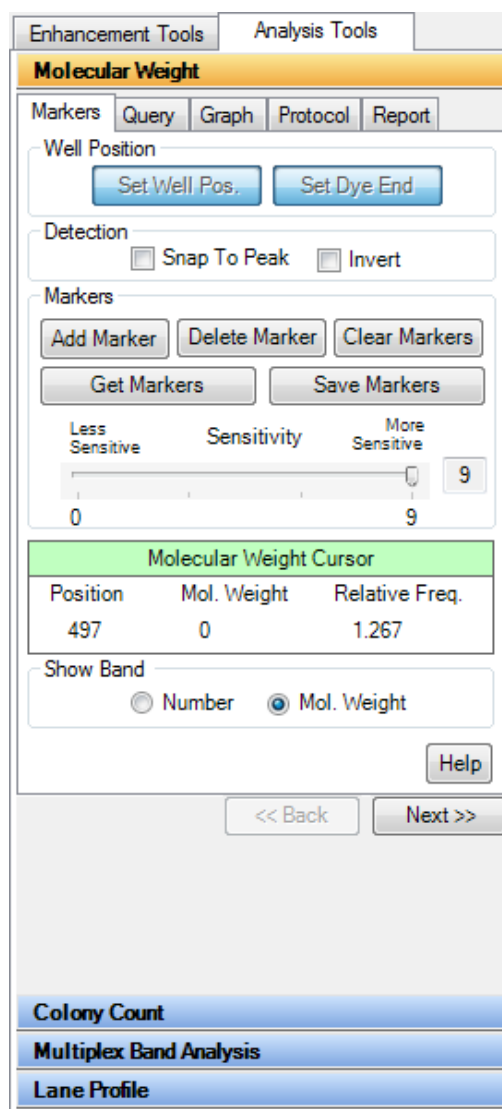


FIGURE 7. Analysis in AlphaView uses a step-by-step wizard to guide you through the process.

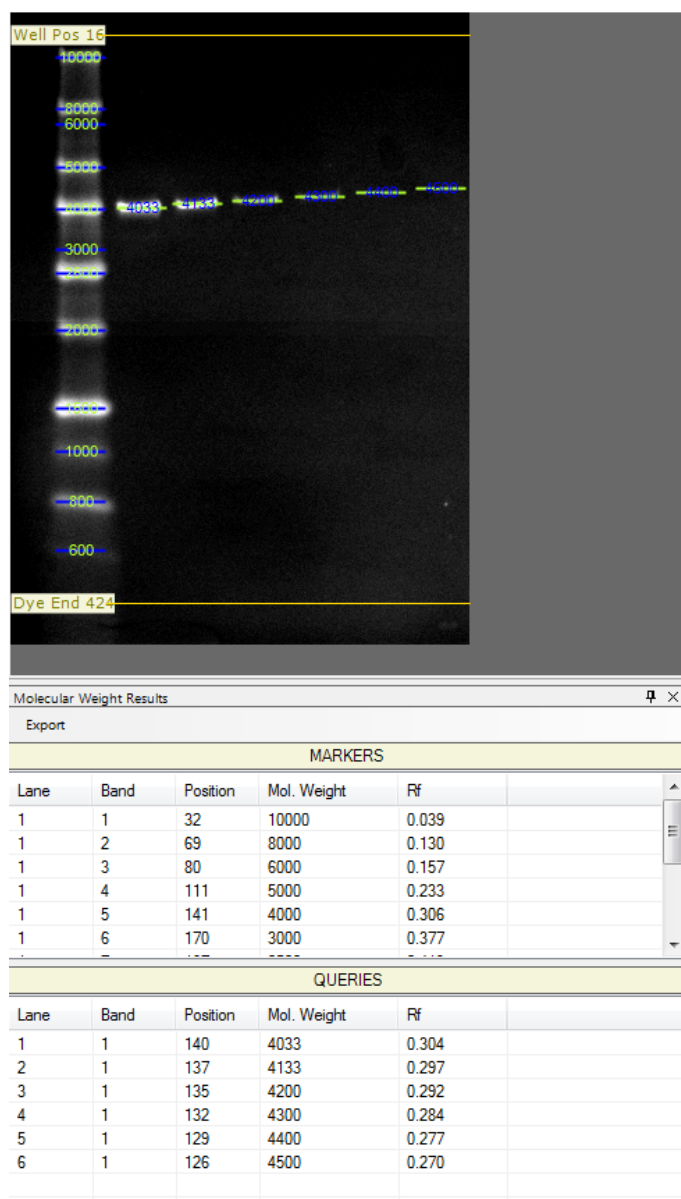


FIGURE 8. Molecular weight analysis of a blot imaged on Jess.

When images are exported from Jess, multiple image files will appear in the export folder. Select the desired image file containing the overlay of ladder(s) and sample bands to continue with Molecular Weight analysis.

Lane Profiling

The Lane Profile tool (Figure 9) makes it easy to do automatic molecular weight, mass and densitometry analyses of bands in the lane view format. Note: for quantitative analyses, image overlays should NOT be used. For instance, if the blot uses colorimetric or fluorescent ladder markers, and chemiluminescent detection for samples, only the chemiluminescent image should be used to do sample analysis, and not the combined ladder/sample image, due to differences in exposure settings and background. The Lane Profile tool can automatically detect lanes containing loaded samples, as well as bands within the lanes.

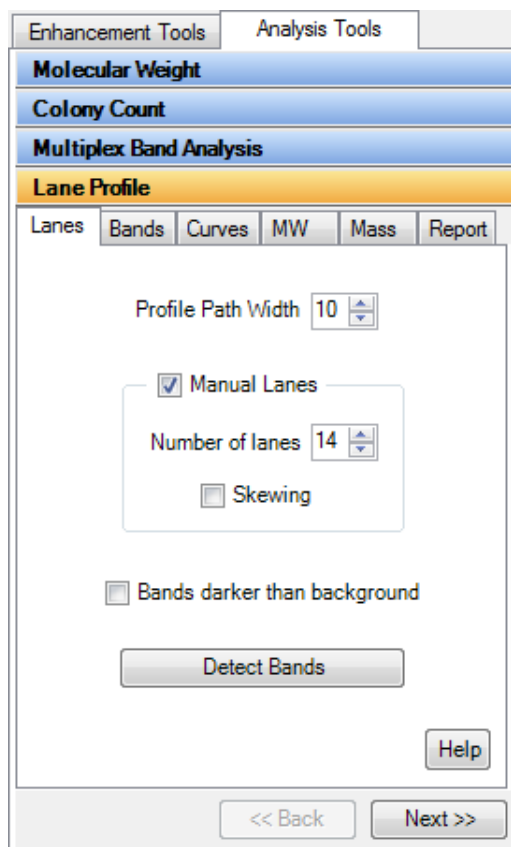


FIGURE 9. Lane Profile analysis tool.

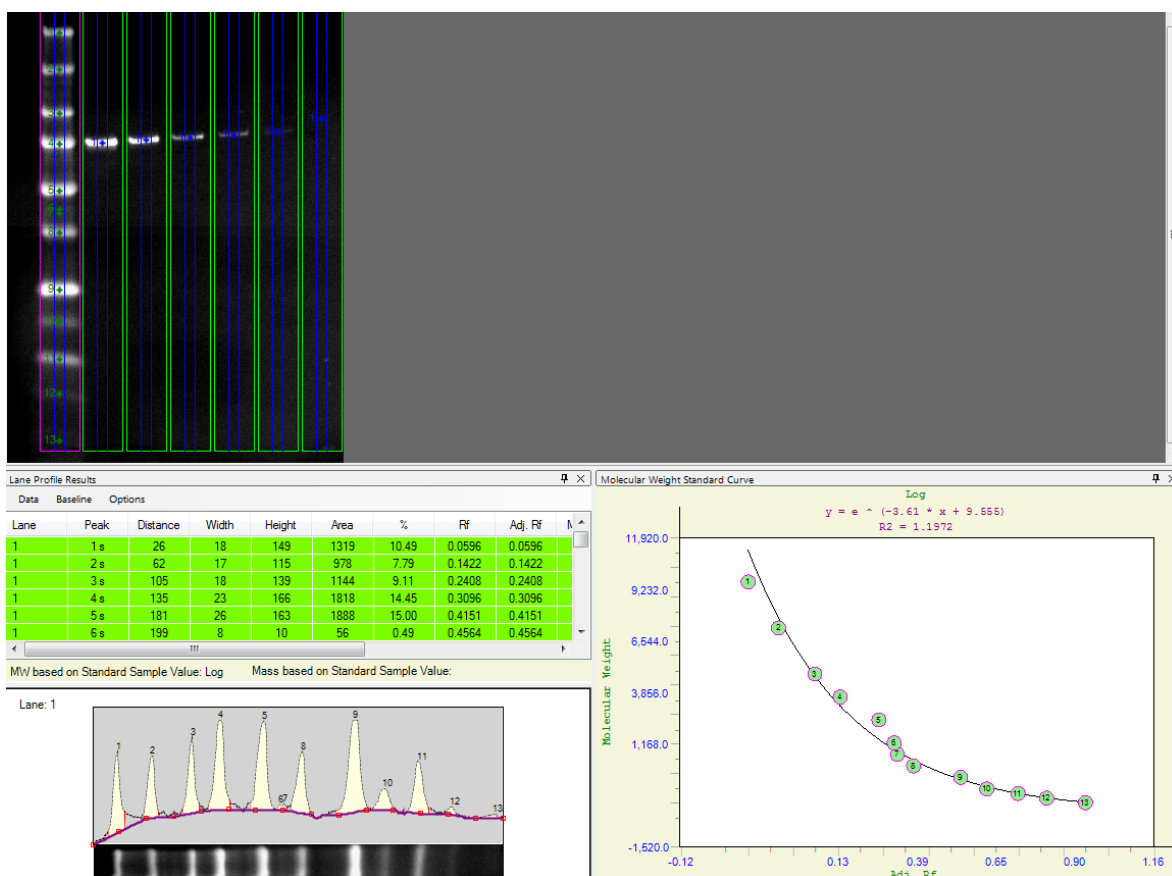


FIGURE 10. Lane Profile Analysis.

Lane Profile Analysis allows you to adjust the number of lanes, their width and straightness, if your gel runs askew. There is also a smile correction feature, which allows you to correct for lanes running at different speeds. Bands in lanes will automatically be detected, and can be manually added or deleted. Molecular weight or mass calculations for samples are shown in the data table, and a graph of the standard curve for molecular weight or mass is also displayed. Finally, a graph showing the migration distance and band intensity in each lane is also included.

Once analysis is complete, a report displaying the blot image and data analyses performed can be generated and saved (**Figure 11**). Analysis protocols can also be saved for future use.

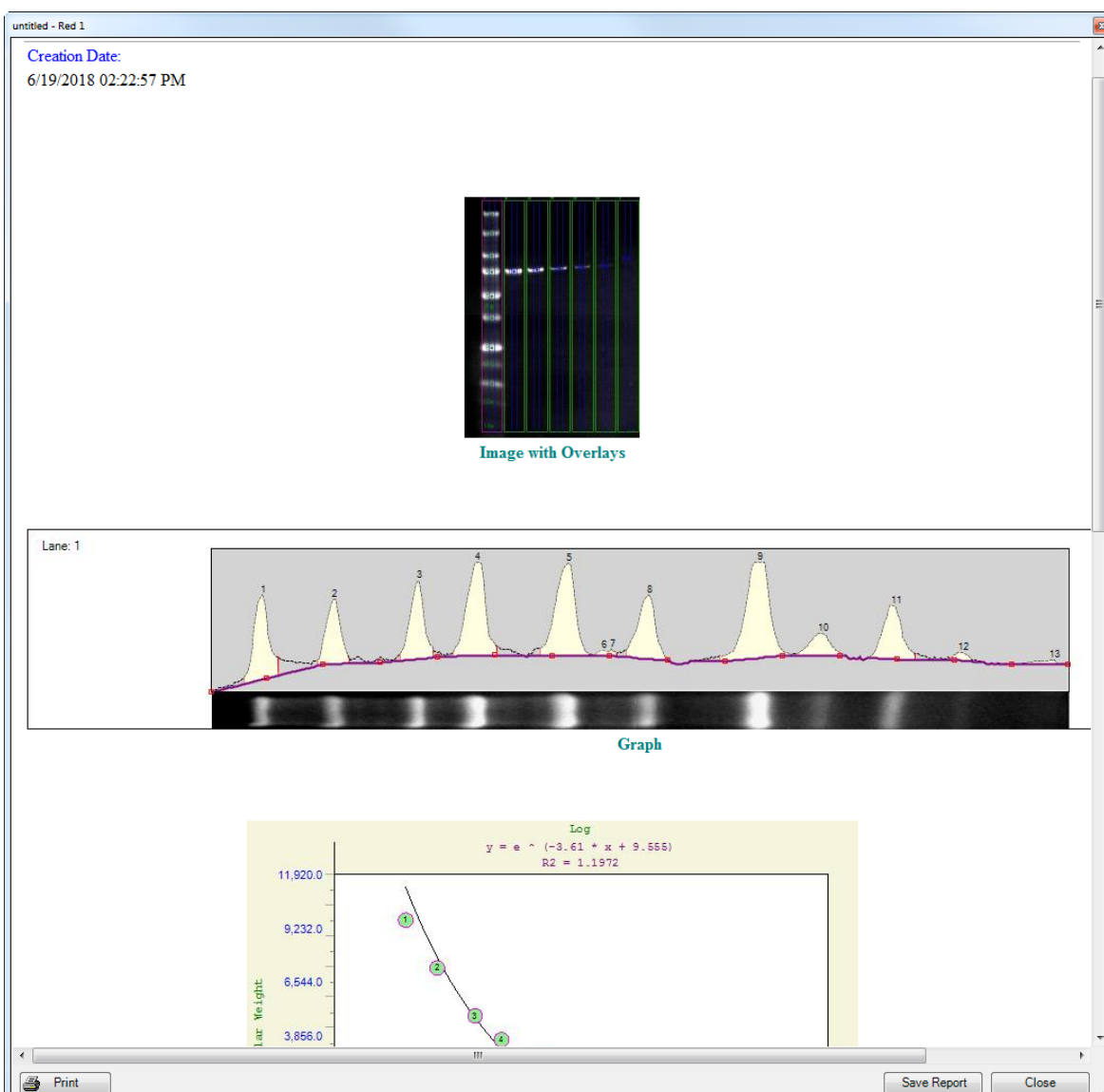


FIGURE 11. Analysis report.

Summary

AlphaView provides many tools for analyzing and enhancing blot images exported from Jess to give you the best publication-ready images and data. More information about processing Western blot images using AlphaView can be found in the AlphaView Software User Guide.