



Focal Points

Application Note FP-112

UVP

Biolmaging Systems

ENHANCEMENT OF CHEMILUMINESCENT IMAGES

When capturing western blots with any CCD camera-based system, the detection of the signal that is produced is not typically visible without software enhancement. This is not a problem with the system or the camera, but simply a function of the very low light emission from the chemical reaction. Many times the image will look completely black (Fig. 1) without modification to the image's display range.

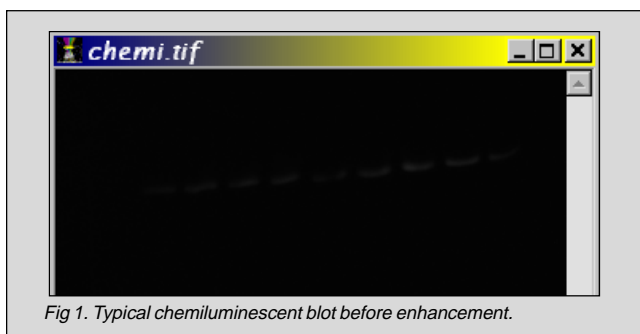


Fig 1. Typical chemiluminescent blot before enhancement.

Therefore, LabWorks software includes a specific feature to enhance low light chemiluminescent images. This feature is called **Display Range** and it can be found under the **Edit** menu (Fig. 2).

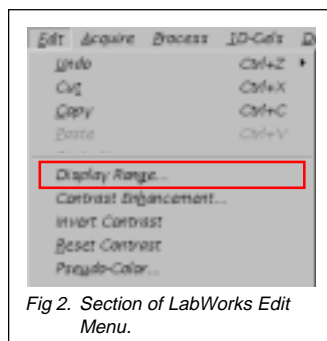


Fig 2. Section of LabWorks Edit Menu.

After capturing the image in LabWorks, click on **Display Range** from the **Edit** menu and the display range profile window of the image will appear (Fig. 3). This is a graphical representation of the total pixels in the image and their gray scale values.

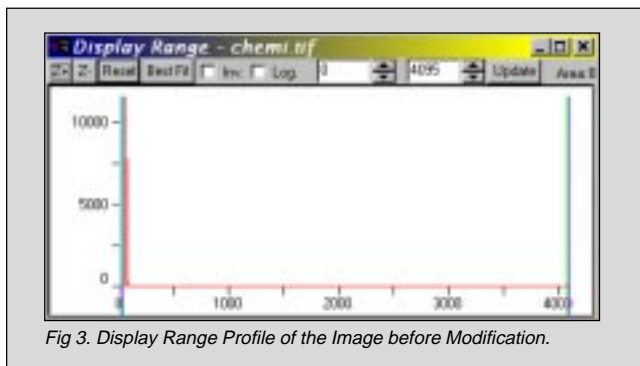


Fig 3. Display Range Profile of the Image before Modification.

Using the right mouse button, move the green and blue bars on the right side of the graph to the left until you can see the blot on the image (Fig. 4).

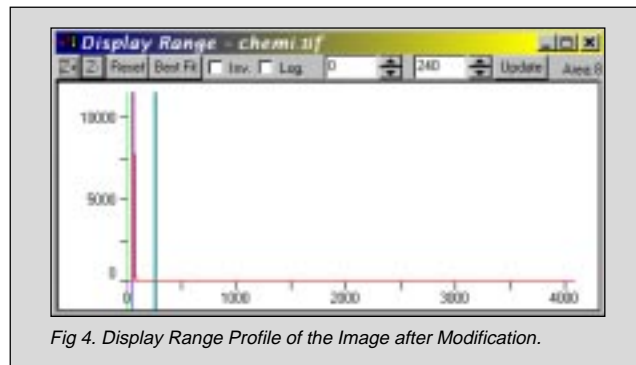


Fig 4. Display Range Profile of the Image after Modification.

The blot is now visible in the image (Fig. 5).

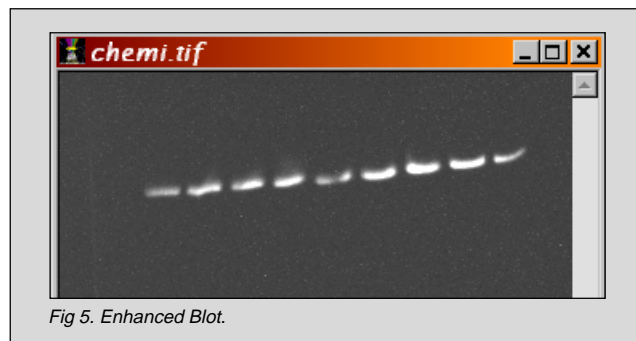


Fig 5. Enhanced Blot.

Now click on the **Z+** button on the **Display Range** window. This rescales the graph to show more detail in the specific area of the image where pixels of the blot are concentrated (Fig. 6).

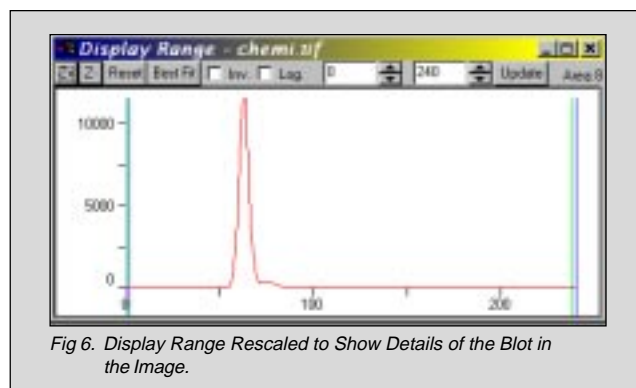
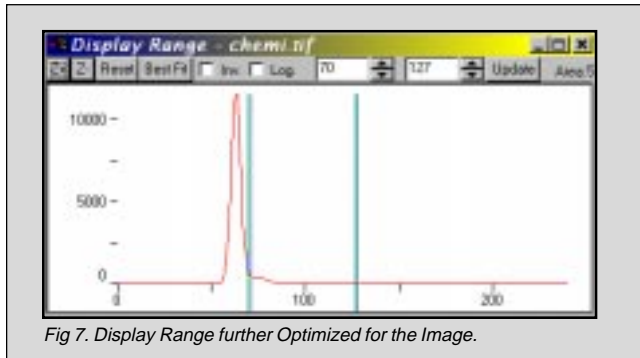


Fig 6. Display Range Rescaled to Show Details of the Blot in the Image.

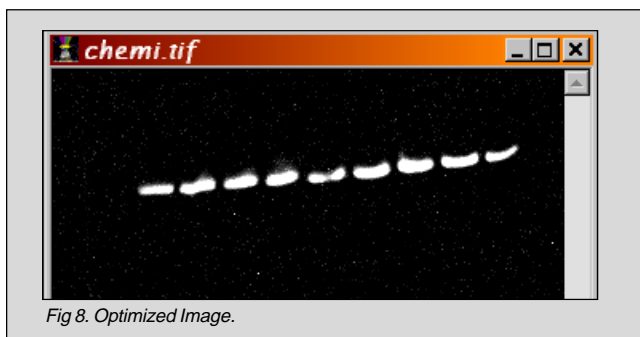


Move both the left hand and right bars to further enhance the blot to your visible preference (Fig. 7).



The large red spike of pixels is the background of the image. You can always check the value of any pixel in any image by placing the mouse pointer on the pixel and looking at the bottom toolbar of LabWorks. Depending on whether or not the image is 8-bit, 12-bit or 16-bit, the values of these pixels and the display range will be 0 - 255, 0 - 4,095, or 0 - 65,535, respectively. Values of 0 are completely black and maximum values are completely white.

After optimization of the display range, the image should look as shown in Fig. 8.



Process filters under the **Process** menu (Fig. 9) also allow sharpening of the image and subtraction of hot pixels from the image. These filters should only be applied for image enhancement and not for quantitative analysis. Filters can be removed under the **Edit Undo** menu.

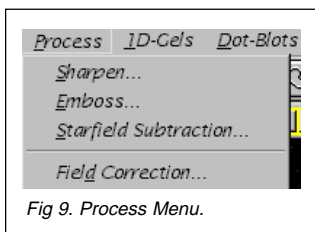
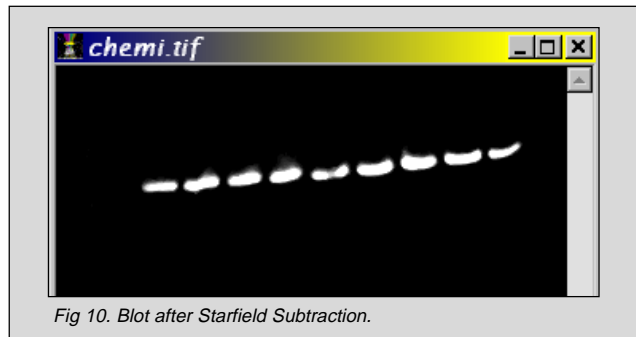
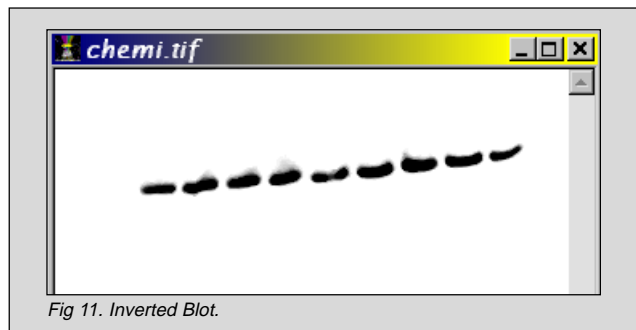


Figure 10 shows the image after the **Starfield Subtraction Filter** has been applied.



It is also possible to view the image as if it were captured on film. Click on the **Invert** button on the display range window. The resulting image will appear like Fig. 11.



Optimization of the display range of western blot images captured with the **Chemi, BioChemi or OptiChemi Systems** may always be required, even though these systems use highly sensitive cameras. This is primarily because so little signal is produced from the chemical reaction of most chemiluminescent substrates. This directly affects the dynamic range of the image and what is displayed on the image profile. Modifying the boundaries of the display range of the image simply allows the human eye the ability to focus in the range of pixels that are visible.

We recommend UVP's **BioWest Extended Duration Chemiluminescent Substrate Kit** to produce more signal from Western Blots. BioWest is specifically formulated for use with CCD camera-based systems and film. Please call UVP or go to <http://www.uvp.com/biowest.html> for details.

Contact UVP's technical support if you need any further assistance in visualizing your chemiluminescent images.



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