



Focal Points

Application Note FP-118

UVP

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Acquiring and Calibrating Chemiluminescent Blots with Visible Light Markers

Acquire an image of the blot (Figure 1) with its visible marker by using the overhead epi white lighting of the EpiChemii Darkroom.

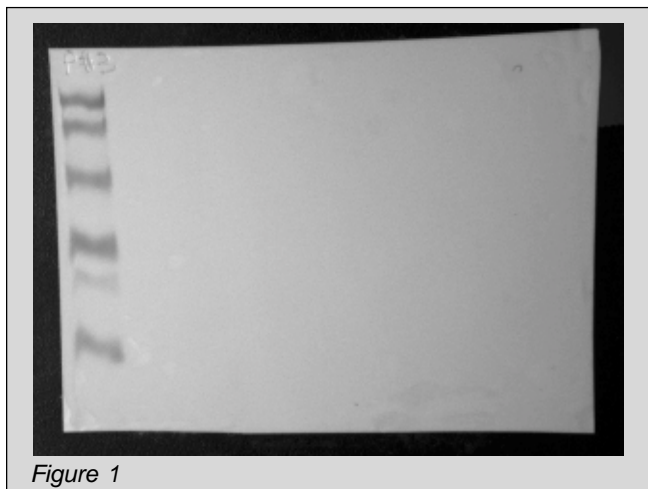


Figure 1

Without moving the membrane in the darkroom or adjusting the zoom setting on the lens, acquire an image of the chemi blot at the same resolution as the image of the visible marker (Figure 2).

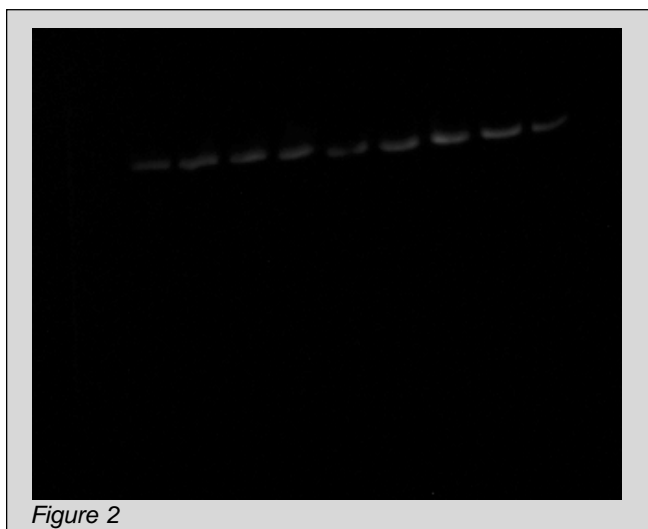


Figure 2

Save both images with original file names. With the image of the blot highlighted, open the **Display Range** feature of LabWorks under the **Edit** menu and optimize

the image (see Focal Points Application Note FP-112). Next, Invert the image of the blot so that the bands are dark and the background is light. This will make both images the same type. Access the **Image Enhancement** icon in LabWorks and **Apply** the changes to the lookup table of the image (Figure 3).

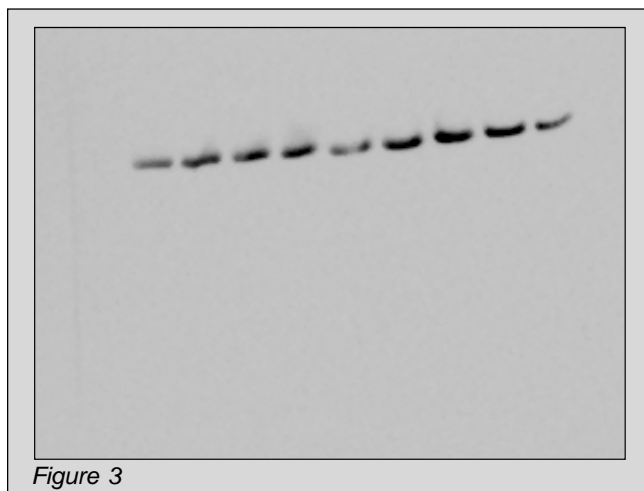


Figure 3

Highlight the image of the visible light marker and select **Edit** and **Copy** from the LabWorks menu. Then highlight the image of the blot and select **Edit** and **Paste**. This will overlay the visible light marker onto the image of the blot in exactly the same spot it appears on the membrane (Figure 4).

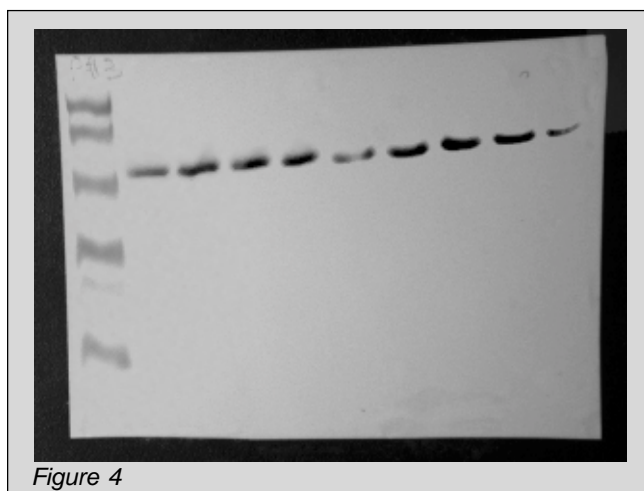


Figure 4



Without saving this image or applying any changes, select **Edit** and **Paste Options**. Check both the **Preview** and **Apply with blend** options in this dialogue box (Figure 5).

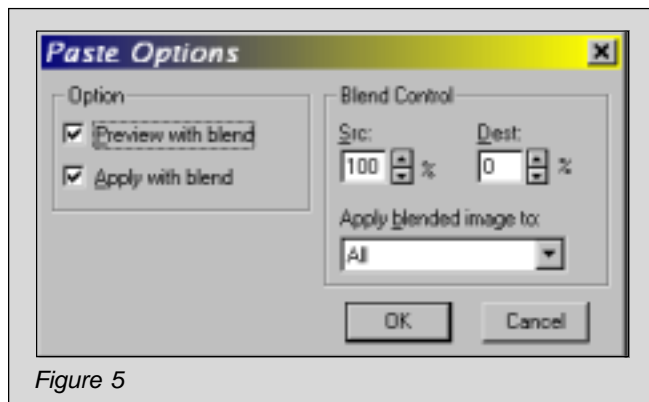


Figure 5

Change the values in the **Blend Control Box** to 50% for each and then adjust these values until the marker and blot are of similar intensity. Click **OK** to preview changes in the image.

Once you are satisfied with the combined image of the visible light marker and the chemiluminescent blot, right mouse click on the image to completely merge both images into one. Now you are ready to proceed to the **1D Tool Bar** for MW and/or mass calibration.



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