

# Model 4300

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DNA Analysis System

## Technical Note

### Combining LI-COR<sup>®</sup> TIFF Images For TILLING<sup>®</sup> Applications

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**LI-COR<sup>®</sup>**

Biosciences

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# I. Introduction

During a run, LI-COR DNA analyzers create one 16-bit grayscale TIFF image file for each detection channel. Adobe® Photoshop® version 6 or 7 is capable of opening LI-COR image files, as are some older versions. Adobe Photoshop Elements 2.0 (a less expensive program) does not recognize 16-bit TIFF images, but it does offer to convert them to 8-bit images. This actually saves time because the images need to be converted to 8 bits per pixel anyway.

When LI-COR images are opened in Photoshop they initially appear very dark. The procedure below describes how to open the images in Photoshop, adjust them so they have a normal appearance, and then combine images using layers. The instructions apply to both Photoshop and Photoshop Elements, except where noted. Keyboard shortcuts are listed Adobe Photoshop for Windows® only.

# II. Combining Images

1.	Open the 700 channel image by selecting <b>Open</b> from the Photoshop <b>File</b> menu (Ctrl+O). If you are using Photoshop Elements, click <b>Yes</b> when asked if you want to convert the image to the default color depth.
2.	If you are using Photoshop, select <b>Adjustments</b> ⇒ <b>Auto Levels</b> on the <b>Image</b> menu (Shift+Ctrl+L). If you are using Photoshop Elements, select <b>Auto Levels</b> on the <b>Enhance</b> menu. This changes how the grayscales in the image are mapped to the monitor and makes the image appear brighter.
3.	Select <b>Adjustments</b> ⇒ <b>Invert</b> on the <b>Image</b> menu to invert the image (Ctrl+I).
4.	If the image needs further adjustment, select <b>Adjustments</b> ⇒ <b>Brightness/Contrast</b> on the <b>Image</b> menu.
5.	If you are using Photoshop, select <b>Mode</b> ⇒ <b>8 Bits/Channel</b> on the <b>Image</b> menu. If you are using Photoshop Elements skip to step 6.
6.	Repeat steps 1 - 5 for the 800 channel image.
7.	With the 800 channel image window active, select <b>New</b> ⇒ <b>Layer</b> from the Photoshop <b>Layer</b> menu (Shift+Ctrl+N). (The 700 channel image can be used rather than the 800 channel image if you prefer.)
8.	In the New Layer window, name the layer "700 Channel", set the <b>Mode</b> to <b>Multiply</b> , and click <b>OK</b> .
9.	Switch to the 700 channel image window and select <b>All</b> on the <b>Select</b> menu to select the entire image (Ctrl+A).
10.	Select <b>Copy</b> on the <b>Edit</b> menu to copy the image (Ctrl+C).
11.	Switch to the 800 channel image window and select <b>Paste</b> on the <b>Edit</b> menu (Ctrl+V) to paste the image into the layer created in steps 7 and 8.
12.	Next, flip the image. In Photoshop, select <b>Rotate Canvas</b> ⇒ <b>Flip Canvas Vertical</b> from the <b>Image</b> menu. In Photoshop Elements, select <b>Rotate</b> ⇒ <b>Flip Vertical</b> from the <b>Image</b> menu.

13.	Bands from both images are now visible. Select <b>Show Layers</b> (Photoshop) or <b>Layers</b> (Photoshop Elements) from the <b>Window</b> menu to open the Layers palette. In the Layers palette, click the eye icon on one of the image layers to make it invisible, then click again to make it visible and show the combined image. Compare the single and combined images to make sure all the bands on the single image are visible on the combined image. On the combined image, there should be two bands visible in any lane containing a mutant. Repeat the test for the other image. If some weak bands are hard to see on the combined image, try changing the opacity of the upper layer using the Layers palette.
14.	<p>Select <b>Save As</b> from the <b>File</b> menu to save the combined image to a new file in Photoshop (*.psd) format.</p> <p><b>Notes:</b></p> <ul style="list-style-type: none"> <li>• This new TIFF file can no longer be opened in LI-COR software.</li> </ul>

### III. Identifying Lanes

One of the difficulties with 96-well images is determining the lane number of lane(s) of interest. Unfortunately, there is not an easy way to do this in Photoshop, but there are two commonly used methods that may help. First, with the full version of Photoshop, you can place guide lines in every fifth or tenth lane to help you quickly identify lane numbers. To place a guide line, turn on the rulers by selecting **Rulers** from the **View** menu (Ctrl+R). Next, click and hold down the mouse button with the cursor inside the vertical ruler on the left side of the window. Drag to the right and a guide line will appear under the cursor as you drag onto the image. Drag the line out to the middle of the fifth (or tenth) lane and release the mouse button to position the line. Repeat this for every fifth or tenth lane. If you need to move a guide line, hold down the **Control** key (Windows) or **Command** key (Macintosh), then click and drag the guide line. The guide line feature is not available in Photoshop Elements, so the second approach must be used.

The second approach is to use the text tool to place lane numbers in the lanes on the image. This can be done with either Photoshop or Photoshop Elements. To number a lane, select the text tool from the tool palette, find the fifth (or tenth) lane, click and type the lane number. If the number is not positioned in the middle of the lane, use the move tool in the tool palette to reposition the type. Repeat every fifth or tenth lane.

If the lane numbers are not easy to see, make sure all type layers are above the image layers in the Layers palette. If the numbers are still hard to see, you may want to change the image to RGB so that you can use colored numbers (red usually is easy to distinguish). To change the image from grayscale to RGB, select **Mode** ⇒ **RGB Color** on the **Image** menu. Click **Don't Flatten** or **No** when asked if you want to flatten the image. Change the text color on the tools palette and then use the text tool to apply colored numbers.

### IV. Determining Approximate Fragment Size

If molecular weight markers were run in at least one lane, the horizontal guide lines in the full version of Photoshop can be used to visually determine the approximate molecular weight of DNA fragments. Assuming the rulers are turned on (see above), click and hold down the mouse button with the cursor inside the horizontal ruler at the top of the window. Drag downward and a guide line will appear under the cursor as you drag onto the image. Drag the line downward until it is positioned over one of the molecular weight

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marker bands and release the mouse button. Repeat for all the other molecular weight marker bands if desired. If you need to move a guide line, hold down the **Control** key (Windows®) or **Command** key (Macintosh®), then click and drag the guide line. The text tool can be used to annotate each line with the appropriate molecular weight, if desired. After the lines are drawn, you can visually determine the approximate size of bands by identifying the two nearest molecular weight marker bands.

Photoshop Elements does not have guide lines, but the pencil tool can be used to draw horizontal lines on the image that pass through the middle of each molecular weight marker band. First, create a new layer (Shift+Ctrl+N). Next, use the pencil tool to draw a horizontal line through each molecular weight marker band by holding down the **Shift** key before clicking the mouse button and moving the mouse horizontally.

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