

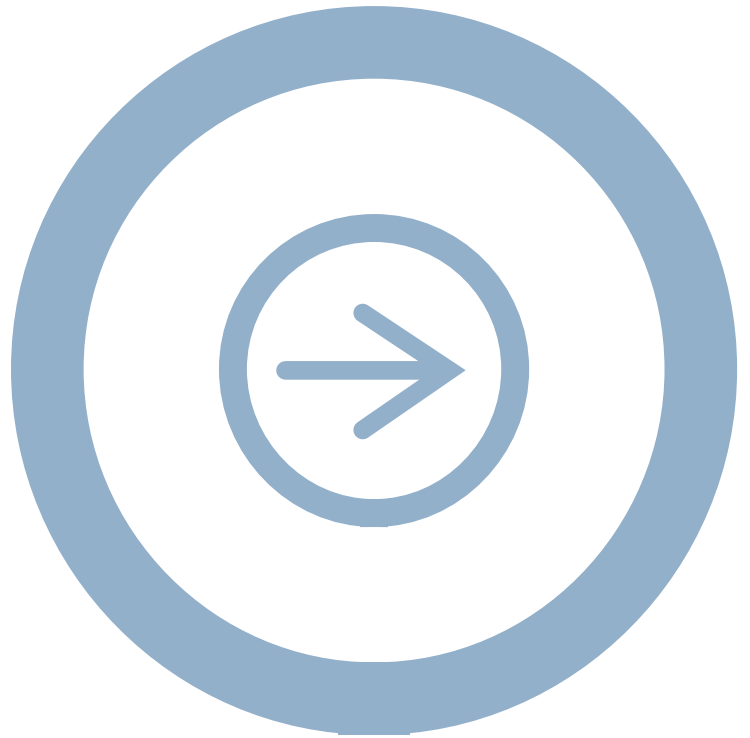
Technical Note

How to Use the Image Studio™ Software Western and MPX™ Western Analyses

Developed for:

Image Studio Software

Please refer to your manual to confirm that this protocol is appropriate for the applications compatible with your instrument model.



LI-COR®

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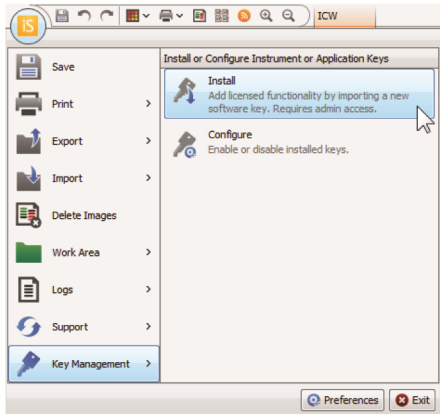
I. Western and MPX Western Analysis Quick Procedure Overview

This guide provides a quick overview of the **Western** and **MPX Western** analyses.

A graphical outline of the **Western** and **MPX Western** procedure is shown on the next page. The process is described further throughout the rest of this guide, and more detail is provided in the Image Studio Software Help.

Step 0

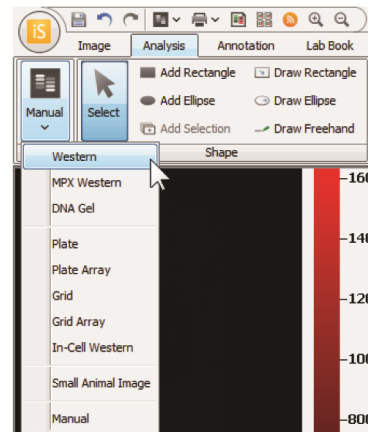
Import Analysis Key



- ◆ Keys purchased separately must be imported to access additional functionality.
- ◆ Click the Application Button > Key Management > Install

Step 1

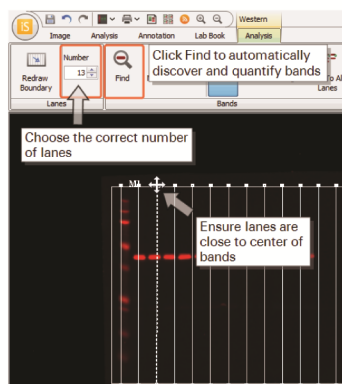
Apply the Analysis



- ◆ Click the **Analysis** tab and set the analysis type in the **Type** group to **Western** or **MPX Western**.

Step 2

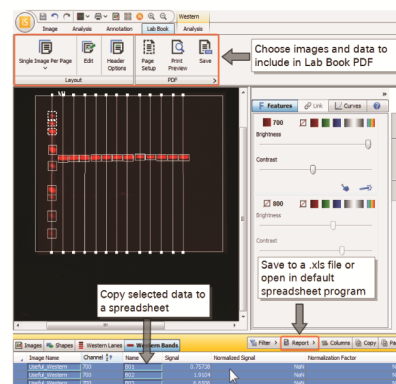
Complete the Analysis



- ◆ Choose the correct number of lanes in the **Lanes** group.
- ◆ Ensure lanes are positioned approximately in the center of the bands (lanes are positioned optimally during band finding).
- ◆ Click **Find** (bands will be located and quantified automatically).

Step 3

Export Data



- ◆ Copy data from the **Western** or **MPX Western Bands Table** to a spreadsheet program.
- ◆ To export data to a spreadsheet, click **Report**.
- ◆ To export a Lab Book PDF, open the **Lab Book** tab and choose the data and images to export.


II. Import the Western Analysis Key

If you have purchased the Western Analysis key separately, the license key file must be imported to access the additional functionality.

NOTE: The Western Analysis Key unlocks the **Western** and **MPX Western** analysis types.

NOTE: You must have administrative privileges to install an analysis key.

To import the Western Analysis Key:

1. Click the **Image Studio Application** button , point to **Key Management**, and then click **Install**.

The **Import LI-COR Key** dialog will open.



2. Navigate to the folder containing the license key, and click once to highlight the LICOR-WesternAnalysis.lke license file.
3. Click **Open**.
4. Restart Image Studio Software.

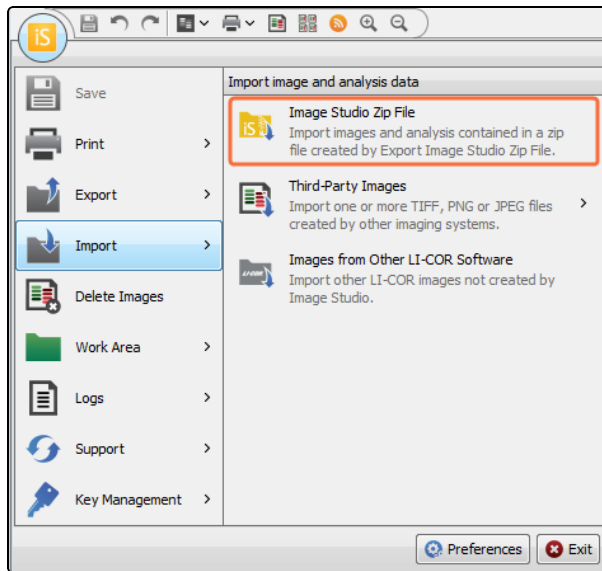
The additional analysis functionality can now be accessed in Image Studio Software.

III. Import Example Images

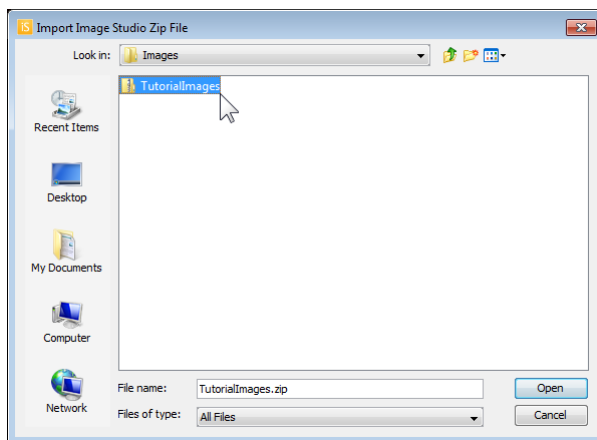
Tutorial images are provided for practice on the Image Studio CD.

To import tutorial images:

1. Click the **Image Studio Application** button , point to **Import**, and then click **Image Studio Zip File** .



2. In the **Import Image Studio Zip File** dialog, navigate to the Image Studio CD, and select the TutorialImages.zip file.



3. Click **Open**.

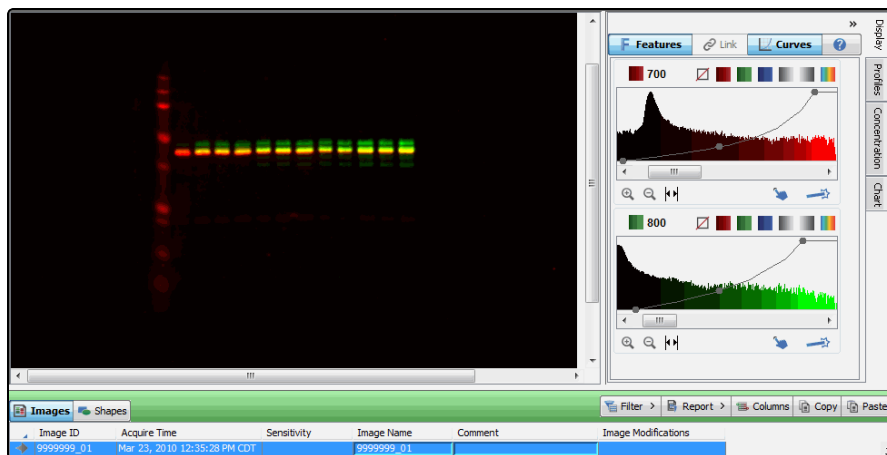
The **Zip File**, containing the following images, will be imported:

- '9999999_01' - a **Western** image
- '9999998_01' - an **MPX Western** image
- '9999996_01' - a **Small Animal** image
- '9999997_01' - an **In-Cell Western** image

IV. Apply a Western or MPX Western Analysis

Begin the **Western** or **MPX Western** analysis by opening the image and applying the desired analysis.

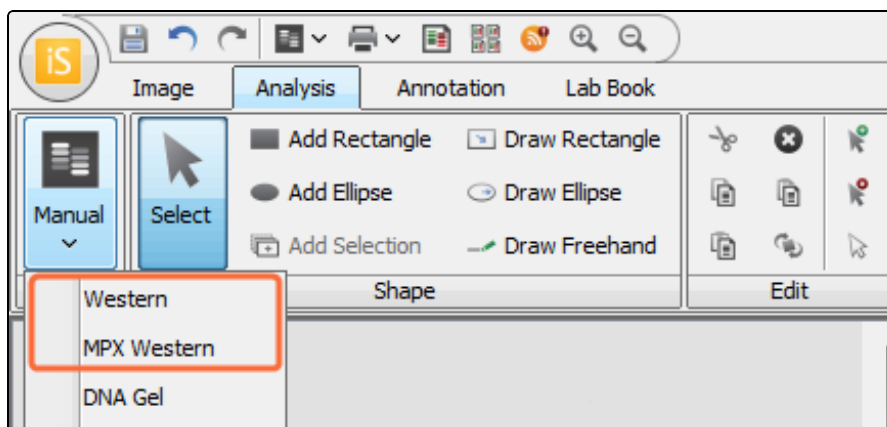
1. Display the **Western** or **MPX Western** tutorial image by clicking the '9999999_01' or '9999998_01' entry in the **Images Table**. Point to an entry in the **Images Table** to see a thumbnail of the image.



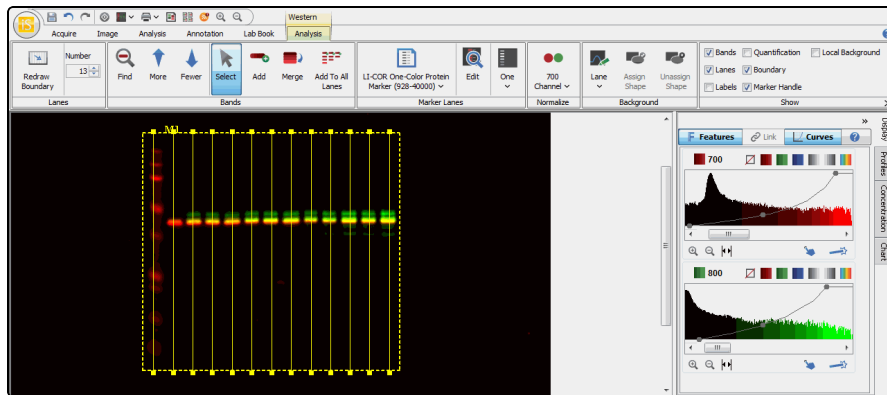
2. Click the **Analysis** tab.

NOTE: Delete any shapes on the image before changing the analysis type to **Western** or **MPX Western**.

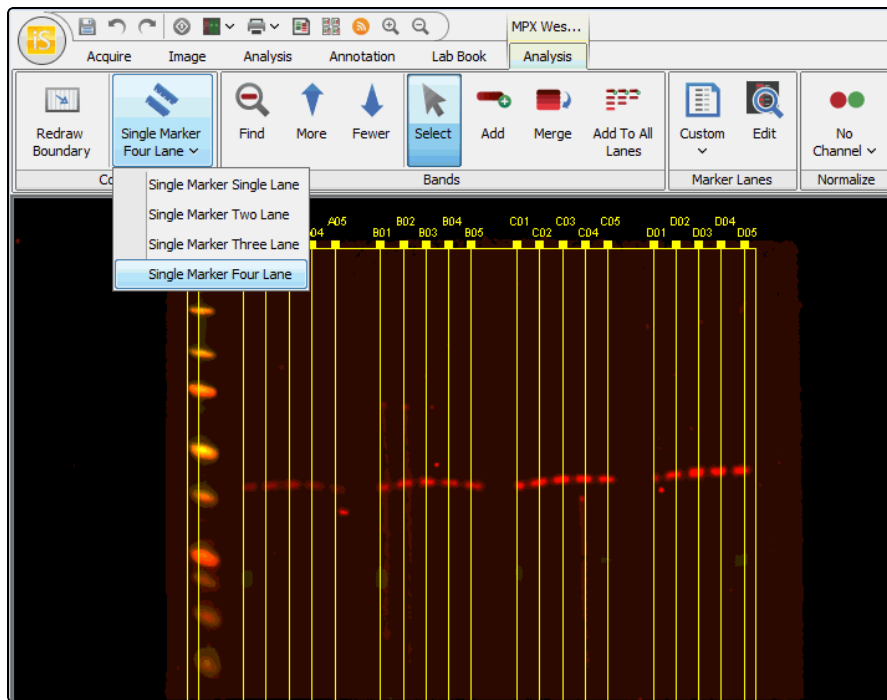
3. In the **Type** group, click the **Analysis Type** list and then click **Western** or **MPX Western**.



The analysis will be applied to the image using settings from the most recent **Western** or **MPX Western** analysis in your **Work Area**.



4. **For an MPX Western Analysis:** Ensure the correct template is selected in the comb group. For the '9999998_01' example image, select **Single Marker Four Lane**.




NOTE: To automatically apply a **Western** or **MPX Western** analysis after image acquisition, choose **Western** or **MPX Western** analysis in the **Setup** group on the **Acquire** tab when starting the acquisition.

V. Adjust the Boundary

After a **Western** or **MPX Western** analysis is applied, the boundary box surrounding the lanes and shapes on an image can be adjusted or redrawn.

NOTE: **Boundary** must be selected in the **Show** group of the **Western** or **MPX Western Analysis** tab for the boundary box to be visible.


Redraw Boundary

1. Click **Redraw Boundary**  (in the **Lanes** group for a **Western** analysis or the **Comb** group for an **MPX Western** analysis).

The cursor will become a cross-hair .

2. Click and drag a rectangular shape around all the shapes to be analyzed.
3. The boundary will be re-drawn and lanes will be placed in an approximate location within the boundary (lanes are set during band finding, see [Find Bands Automatically](#) on page 12).

Adjust the Boundary


1. Click the boundary to select it (the sides of the box will become dashed lines).
2. Point to the midpoint of a side or to a corner of the boundary box to display a double-headed arrow .
3. Click and drag the arrow to adjust the boundary box.
4. Set the boundary box by clicking anywhere outside the box.

Repeat this procedure until you are satisfied that all the shapes to be quantified are inside the box.

NOTE: Adjusting the boundary will also adjust the lanes inside the boundary.

Move the Boundary

1. Click the boundary to select it (the sides of the box will become dashed lines)
2. Move the pointer so it is within the boundary box.

The pointer will become a four-pointed cursor .

3. Click and drag using the four-pointed cursor to move the boundary box.
4. Set the boundary box by clicking anywhere outside the box.

Repeat the procedure until you are satisfied that all the shapes to be quantified are inside the box.

Repeat the procedure until you are satisfied that all the shapes to be quantified are inside the box.

VI. Set the Lanes

Lanes are represented by vertical lines that transect bands in a lane.

NOTE: Lanes must be selected in the **Show** group of the **Western** or **MPX Western Analysis** tab for the lanes to be visible.

The correct number of lanes must be designated in the **Lanes** group. Lanes only need to be positioned close to the center of a band, because the lanes will be optimally positioned by the software during band finding.

To adjust a lane's position:

1. Click the lane to select it (selected lanes are dashed lines).
2. Click and drag the anchor point (dashed square) at the top or bottom of the lane to move only the top or bottom end point of the lane.

Hold SHIFT while moving an anchor to shift the entire lane.

3. Click anywhere away from the lane to set it.


Repeat this procedure until you are satisfied that the lanes are positioned close to the center of the bands. Lanes will be positioned optimally during band finding.

VII. Size Bands Using Markers

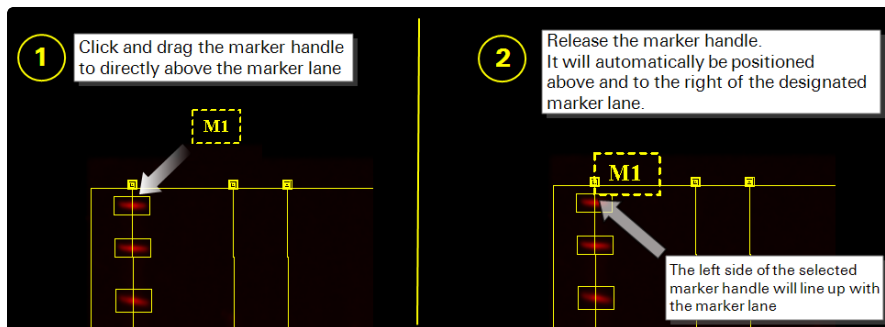
Follow this procedure to estimate unknown band sizes based on protein markers.

1. Click the **Select Marker Lanes Set** list  on the far left of the **Marker Lanes** group and select the appropriate marker.

A marker handle will appear above the blot **M1**.

NOTE: To designate more than one marker lane, click the **Number of Marker Lanes** list  and select the number of marker lanes being used.

2. Click and drag the marker handle directly above the marker lane. After releasing the marker handle, it will automatically be positioned above and to the right of the designated marker lane.



3. The estimated band sizes will appear in the **Size** column in the **Western Bands Table**.


Image Name	Channel	Name	Size	Signal
			160	6.56E-1
			90.0	1.69E0
			50.0	6.45E0
			30.0	7.66E0
Useful_Western	700	B05	15.0	2.15E0
Useful_Western	700	B06	8.00	5.61E0

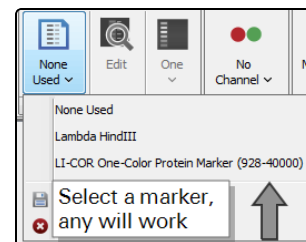
The **Size** column. Columns can be moved and may not appear in this order.

VIII. Add, Save, or Delete Markers

Molecular weights for a protein marker can be added and saved to a **Work Area**. The marker can then be used to size unknown bands. Use the following procedures to add and save a marker to a **Work Area** or delete an existing marker.


Add a New Marker

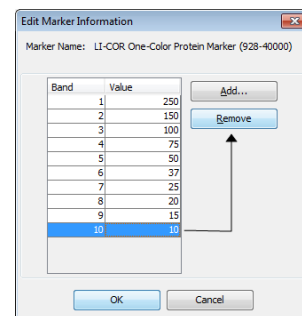
1. In the **Marker Lanes** group, select any Molecular Weight Marker from the **Select Marker Lanes Set** list .




2. Click **Edit** . The **Edit Marker Information** dialog will open.

NOTE: Changes made in this procedure will not overwrite the current marker, unless you save the changes with the current marker's name.

3. In the **Edit Marker Information** dialog, click to select a weight and click **Remove**. Repeat this step until all weights have been removed.
4. After all current weights have been removed, click **Add** to enter all the appropriate weight markers.
5. After all appropriate weights have been added, click **OK**. The **Select Marker Lanes Set** list  will display **Custom**.




6. To save the newly added marker:
 - a. In the **Marker Lanes** group, click the **Select Marker Lanes Set** list .
 - b. Click **Save Current Marker Lane Set**.
The **Marker Set Name** dialog will open.
 - c. Enter the name of the marker and specify the channel (e.g. "Chameleon 700" or "Chameleon 800").
 - d. Click **OK**.

The new marker has been added to the **Work Area** and saved.

Repeat this process for each channel if using the Chameleon™ Duo Protein Marker or Chameleon Kit Protein Marker.

Delete a Marker

1. Select **Delete Marker Lane Set...** from the **Select Marker Lanes Set** list .

The **Delete Marker Lane Set** dialog will open.

2. Select the marker lane to delete and click **Delete**.

The marker will be deleted from the **Work Area**.

IX. Find Bands Automatically

To find bands automatically in all channels:




1. In the **Bands** group, click **Find** .

Image Studio Software will automatically discover bands in a lane and designate bands by placing a box around them.

NOTE: Turning off labels may make bands easier to see. To turn off labels, clear the **Labels** check box in the **Show** group.

2. Click **Fewer**  to increase sensitivity and decrease the number of bands, or click **More**  to decrease sensitivity and increase the number of bands.

X. Edit Bands Manually

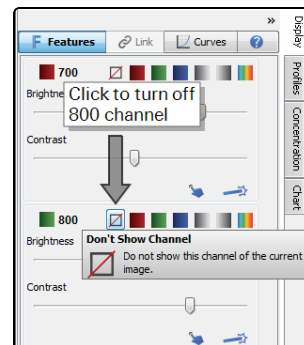
Bands can be manually added, edited, or deleted.

Bands Can Be Edited Manually From Only One Channel at a Time

To add, edit, or delete bands, ensure only one channel is visible. For this section of the tutorial, ensure only the 700 channel is visible:



Click the **Don't Show Channel** button  in the 800 channel section of the **Display** tab to turn off 800 channel visibility.

Only the 700 channel will be visible.



Add a Band


Bands can only be added to one channel at a time.

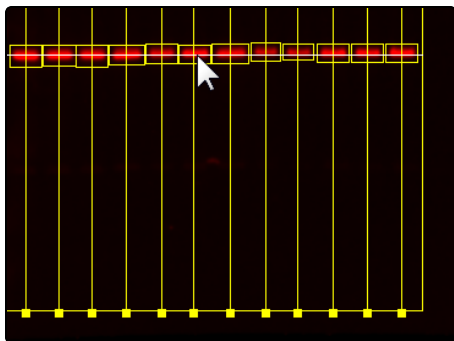
1. In the **Bands** group, click **Add** .
2. Click the band that needs to be added. A box will appear around the band.
3. Once you are done adding bands, press ESC or click **Select**  in the **Bands** group.

The cursor can now be used to move or re-size bands.


Add Band in All Lanes

To add the equivalent band to every lane (when only one channel is visible):

1. In the **Bands** group, click **Add to All Lanes** .
2. Point the cursor to the row of bands that need to be added. A horizontal line will appear.




3. Once the horizontal line is set correctly, click to add bands. Bands will be located in every lane where the horizontal line crosses the lane.

Press ESC or click **Select**  to exit the **Add to All Lanes** mode.

Re-size or Move a Band

Bands can be re-sized or moved (if only one channel is visible).


1. Click a band to select it (selected bands will be designated by dashed lines).
 - Move a band by placing the cursor inside the shape. The cursor will become a four-pointed cursor  that can be used to move the band.

NOTE: The band cannot be moved away from the lane it is in.

- Re-size a band by pointing the cursor to the midpoint of a side of the box or to the corner of the box. A double-headed arrow will appear that can be used to adjust the size of the box.
2. Set the changes by clicking outside the shape.

Merge Bands

Two or more bands can be merged into one band.

1. Select both bands by clicking and dragging a rectangle around the bands or CTRL+clicking each band.
2. In the **Bands** group, click **Merge** .

The space inside and between each band will be merged into one band.

Delete a Band

Bands can only be deleted if only one channel is visible. To delete a band:

1. Click a band to select it (selected bands will be designated by dashed lines).
2. Press **Delete** or **CTRL + X**.


The box around the band will be deleted and the band will no longer be used in analysis.

XI. Normalize One Channel at a Time

The **Signal** for each band in the channel designated for normalization is divided by the band with the largest **Signal** value to obtain a **Normalization Factor** (the

Normalization Factor for the largest band will, of course, be 1). The **Signal** for each band in the opposite channel will be divided by the **Normalization Factor** of the band in the same lane from the normalization channel.

Follow this procedure to normalize signal values (this example will normalize the 800 channel to the 700 channel):

1. Ensure only one band is present in each sample lane for the normalization channel.
2. In the **Normalize** group, select **700 channel** from the **Normalize Channel** list.
3. Choose a background method in the **Define Type** list . See the Image Studio Software Help for more information on which background method to choose.

NOTE: Changing the background method will change the quantification values. When analyzing repetitions of the same assay, choose one background method to use each time repetitions of the same assay are analyzed.

4. You should see the following in the **Normalized Signal** column of the **Western Bands Table**:
 - a. Rows corresponding to bands in the marker lane will display "NaN".
 - b. Rows corresponding to bands in the normalization channel will display the value of the band with the greatest **Signal** value.
 - c. Rows corresponding to bands in the opposite channel will show each band's **Signal** divided by the **Normalization Factor** from the band in the normalization channel in the same lane.

Image Name	Channel	Lane Name	Name	Signal	Size	Area	Normalized Signal	Normalization Factor
9999999_01	700	M1	B09	1.06	15.0	840	NaN	NaN
9999999_01	700	M1	B08	1.56	20.0	840	NaN	NaN
9999999_01	700	M1	B07	1.41	25.0	532	NaN	NaN
9999999_01	700	M1	B06	6.43	37.0	784	NaN	NaN
9999999_01	700	M1	B05	2.70	50.0	784	NaN	NaN
9999999_01	700	M1	B04	8.09	75.0	728	NaN	NaN
9999999_01	700	M1	B03	7.19	100	560	NaN	NaN
9999999_01	700	M1	B02	2.56	150	504	NaN	NaN
9999999_01	700	M1	B01	1.43	250	672	NaN	NaN
9999999_01	800	L12	B03	0.0773	50.9	612	0.107	0.725
9999999_01	700	L12	B01	14.0	62.1	680	19.3	0.725
9999999_01	800	L12	B02	1.30	63.2	714	1.79	0.725
9999999_01	800	L12	B01	0.403	69.9	408	0.555	0.725
9999999_01	800	L11	B03	0.0466	50.9	544	0.0792	0.588
9999999_01	700	L11	B01	11.4	62.1	680	19.3	0.588
9999999_01	800	L11	B02	0.841	63.2	714	1.43	0.588
9999999_01	800	L11	B01	0.255	69.9	408	0.433	0.588
9999999_01	800	L10	B03	0.0635	51.2	544	0.0970	0.655
9999999_01	700	L10	B01	12.6	62.1	680	19.3	0.655
9999999_01	800	L10	B02	1.12	63.2	714	1.71	0.655

XII. Review Data


Results for the **Western** or **MPX Western** analysis will be displayed in the **MPX Western Bands/Western Bands Table** or the **MPX Western Lanes/Western Lanes Table**.


- Click **Western Bands** or **MPX Western Bands** at the lower left side of the Image Studio Software window to view a table with analysis data for each band (the **Western Bands Table** is shown).

Image Name	Channel	Lane Name	Name	Signal	Size	Area	Normalized Si...	Normalization ...
UseM_Western	800	M1	B02	0.0220	250	646	NaN	NaN
UseM_Western	800	L01	B01	0.116	NaN	476	NaN	NaN
UseM_Western	800	L01	B02	0.381	0.00	578	NaN	NaN
UseM_Western	800	L02	B01	0.147	NaN	476	NaN	NaN
UseM_Western	800	L03	B02	0.486	0.00	714	NaN	NaN
UseM_Western	800	L03	B03	0.0363	NaN	544	NaN	NaN
UseM_Western	800	L04	B01			476	NaN	NaN
UseM_Western	800	L04	B02			686	NaN	NaN
UseM_Western	800	L04	B03	0.0281	NaN	612	NaN	NaN
UseM_Western	800	L05	B01	0.210	NaN	476	NaN	NaN
UseM_Western	800	L05	B02	0.575	0.00	714	NaN	NaN
UseM_Western	800	L05	B03	0.0244	NaN	612	NaN	NaN
UseM_Western	800	L06	B01	0.144	NaN	442	NaN	NaN
UseM_Western	800	L06	B02	0.480	0.00	782	NaN	NaN
UseM_Western	800	L06	B03	0.0246	NaN	544	NaN	NaN
UseM_Western	800	L07	B01	0.332	NaN	476	NaN	NaN
UseM_Western	800	L07	B02	0.974	0.00	714	NaN	NaN
UseM_Western	800	L07	B03	0.0526	NaN	612	NaN	NaN
UseM_Western	800	L08	B01	0.253	NaN	510	NaN	NaN
UseM_Western	800	L08	B02	0.746	0.00	714	NaN	NaN
UseM_Western	800	L08	B03	0.0343	NaN	544	NaN	NaN
UseM_Western	800	L09	B01	0.388	NaN	510	NaN	NaN
UseM_Western	800	L09	B02	1.14	0.00	714	NaN	NaN
UseM_Western	800	L09	B03	0.0557	NaN	612	NaN	NaN

- Click **Western Lanes** or **MPX Western Lanes** below and to the left of the image to view a table with data related to lanes.


Columns can be re-ordered, sorted, filtered, and new columns can be added:

- Click and drag a column header to move the column's position within the table.
- Click a column header to quickly sort the column alphabetically or numerically (clicking repeatedly toggles the sort from ascending to descending).
- Right click a column header to view more options.
- Click **Columns**  above and to the right of the table to choose additional columns that can be added to the table.







MORE INFO: For more details on how to use these tables, visit the Image Studio Software Help System by clicking the blue question mark  in the upper right corner of the window.

XIII. Export Data

Data can be exported in three ways.

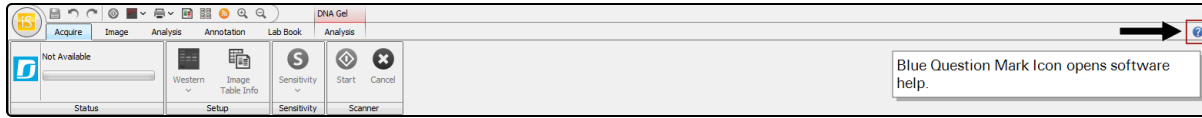
- **Copy and Paste:** Right click selected data in the **Western Bands Table/MPX Western Bands Table** or **Western Lanes Table/MPX Western Lanes Table** and then click copy (or press CTRL+C). Paste the data into a spreadsheet.
- **Export table data to a spreadsheet:** Click **Report**  above and to the right of the table view and then choose whether to launch the data in an external spreadsheet program or save the data.

Use **Options**  at the bottom of the **Report** menu to:

- Change whether data is saved as a .xls file or a tab-separated text document.
- Change whether the entire table is exported or just selected rows.
- **Export data and images to a Lab Book PDF:** Open the **Lab Book** tab.
 - On the far left of the **Lab Book** tab, click the **Layout Template** list  and choose a layout close to what is needed (the **Western Lab Book** or **MPX Western Lab Book** is probably a good starting point).
 - Click **Edit**  to choose which images and data will be included in the PDF. The layout and any changes will appear in a layout preview.
 - Click **Header Options**  to choose a logo and text to appear at the top of the exported **Lab Book** page.
 - Click **Page Setup**  to change the paper size, page setup, and margins. Use **Print Preview**  to preview how all changes will appear.
 - Click **Save**  to save the **Lab Book** page.

XIV. Further Questions

1. See the software help. Click the blue question mark in the upper right corner of the window to open the help. The upper right corner of the software help has a search box for quickly finding answers.



2. Contact LI-COR Technical Support (details in the footer of this document).

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