

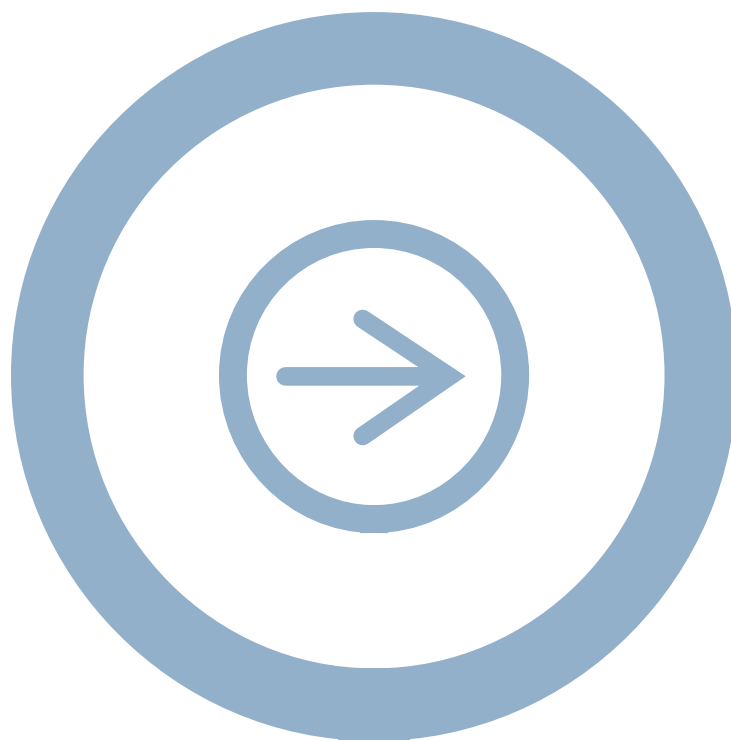
Software Guide

How to Use the Image Studio™ Software In-Cell Western Analysis

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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Table of Contents

	Page
I. In-Cell Western Analysis Quick Procedure Overview	2
II. Import the In-Cell Western Analysis Key	2
III. Import Example Images	3
IV. Apply an In-Cell Western Analysis	4
V. Adjust the Grid Border	6
VI. Assign Well Types	7
VII. Assign Linked Wells	8
VIII. Review Data	10
IX. Export Data	12
Export Data	12

I. In-Cell Western Analysis Quick Procedure Overview

This guide provides a quick overview of the **In-Cell Western** analysis.

To practice performing an **In-Cell Western** analysis, follow the procedure outline on the next page and the tutorial images throughout the guide. Additional detail is provided in the Image Studio Software Help.

II. Import the In-Cell Western Analysis Key

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

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

To import the In-Cell 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-IcwAnalysis.lke license file.
3. Click **Open**.
4. Restart Image Studio™ Software.

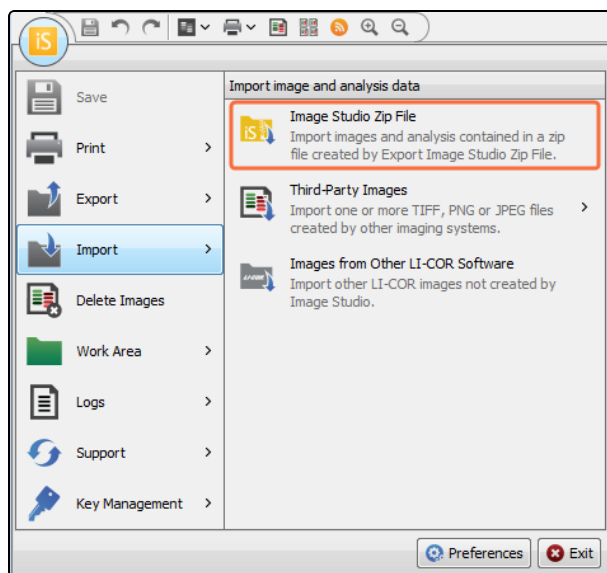
The additional analysis functionality can now be accessed in Image Studio™ Software.

III. Import Example Images

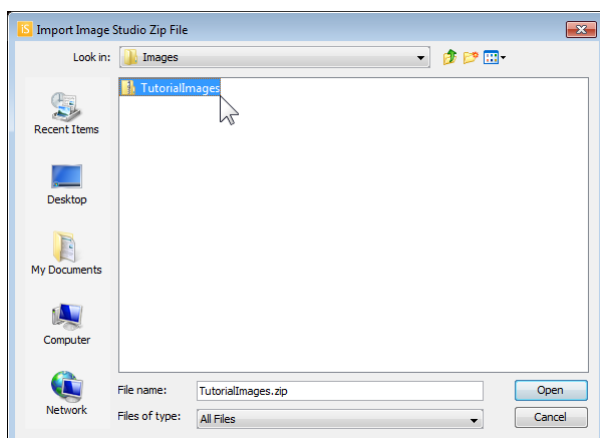
Tutorial images are provided for practice on the Image Studio CD or can be downloaded with the following link: licor.com/ImageStudioImages.

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:

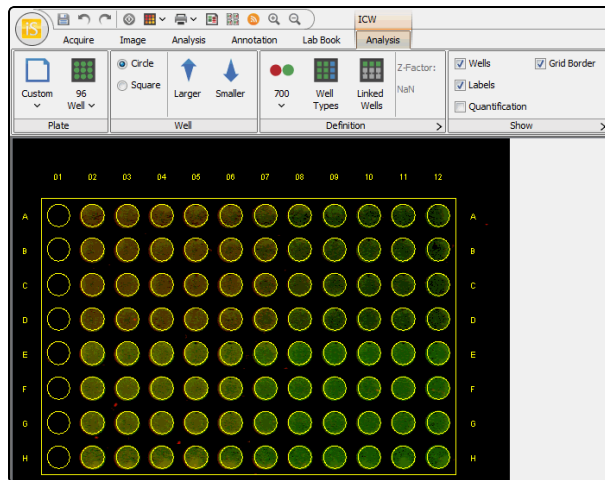
- '9999999_01' - a **Western** image
- '9999998_01' - an **MPXWestern** image
- '9999997_01' - an **In-Cell Western** image
- '9999996_01' - a **Small Animal** image
- '9999995_01' - an **Odyssey® Loading Indicator** image
- '9999994_01' - a **target for Revert™ 700 Total Protein Stain normalization** image
- '9999993_01' - a **Revert™ 700 Total Protein Stain** image
- '0010000_01' - a **chemiluminescence blot** image

IV. Apply an In-Cell Western Analysis

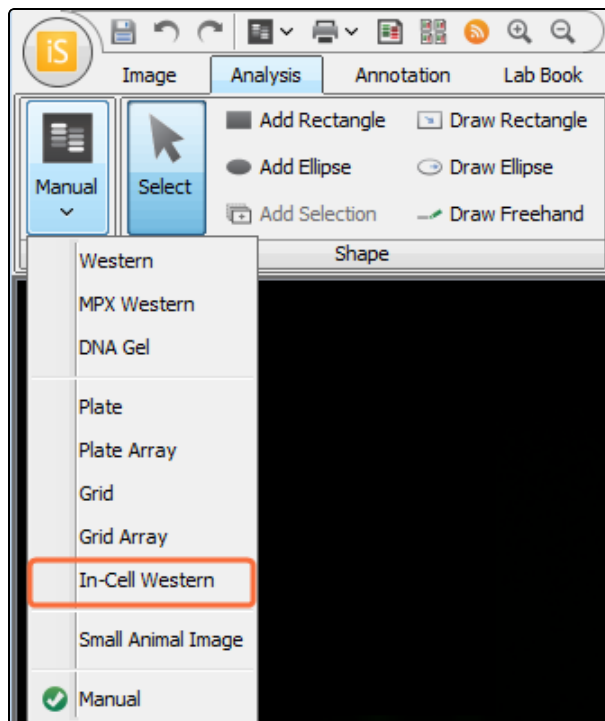
Begin the **In-Cell Western** analysis by opening the image and applying the desired analysis.

1. Display the **In-Cell Western** tutorial image by clicking the '9999997_01' entry in the **Images** table.

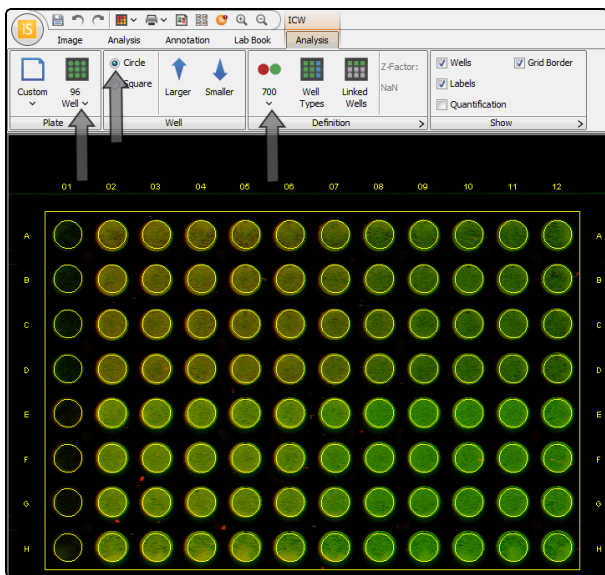
Note: Hover over an entry in the **Images** table to view a thumbnail of the image.



2. Click the **Analysis** tab.
3. In the **Type** group, click the **Analysis Type** list and then click **In-Cell Western**.



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




4. In the **Plate** group, select 96 from the **Define Plate Type** list.
5. In the **Well** group, select **Circle** for the shape.
6. In the **Definition** group, select 700 in the **Select ICW Normalize Channel** list.

Note: To automatically apply an **In-Cell Western** analysis after image acquisition, choose **In-Cell Western** analysis in the **Setup** group on the **Acquire** tab when starting the acquisition.

V. Adjust the Grid Border


The Grid Border is the box that surrounds the wells. All wells should be inside the Grid Border.

To move the Grid Border:

1. Click the Grid Border 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 wells are inside the box.

To adjust the Grid Border:

1. Click the Grid Border 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 Grid Border to display a double-headed arrow .
3. Click and drag the arrow to adjust the Grid Border.
4. Set the Grid Border by clicking anywhere outside the box.

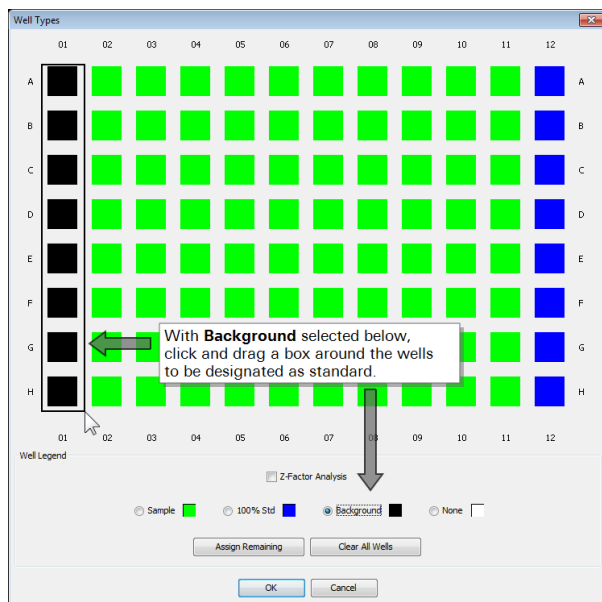
Repeat this procedure until you are confident the Grid Border has been positioned correctly.

VI. Assign Well Types

Use the following procedure to designate Well Types (**Background**, **100% Standard**, and **Sample**) for an **In-Cell Western** analysis:

1. In the **Definition** group, click **Well Types** .

The **Well Types** dialog will open. In the **Well Types** dialog, a grid of squares is used to represent the wells in the image.



2. At the bottom of the **Well Types** dialog, select **Sample**, **100% Standard**, or **Background** to designate the Well Type to be assigned.
 - **100% Standard**: wells that are incubated with primary and secondary antibodies. They receive appropriate treatment to elicit maximum response.

- **Background**: wells that are incubated with secondary antibody and without primary antibody. They are used to identify non-specific secondary antibody binding.
3. Assign wells as the selected type by clicking and dragging a box around the squares that represent the appropriate wells.
 - Squares designated as **Background** will be highlighted black.
 - Squares designated as **Sample** will be highlighted green.
 - Squares designated as **100% Standard** will be blue.


Repeat this procedure for the other Well Types.

4. After the necessary Well Types have been assigned, click **OK**.

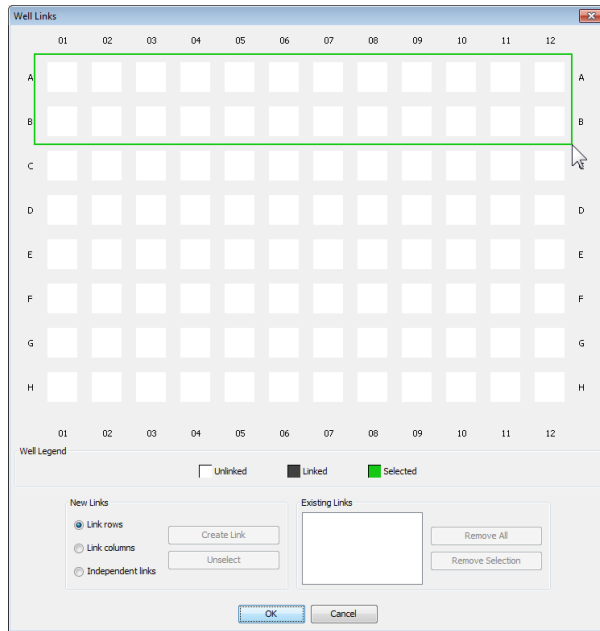
VII. Assign Linked Wells

Linked wells contain identical samples. The response values for linked wells will be averaged together.

To link wells:

1. In the **Definition** group, click **Linked Wells** .
- The **Linked Wells** dialog will open. In the **Linked Wells** dialog, a grid of squares is used to represent the wells in the image.

2. If you need to link rows or columns, ensure the **Link rows** or **Link Columns** check box is selected. To link individual wells, select **Independent links**.
3. Assign linked wells by clicking and dragging a box around the squares that represent the wells to be linked. Select individual wells by clicking a well and then holding **CTRL** while clicking additional wells.

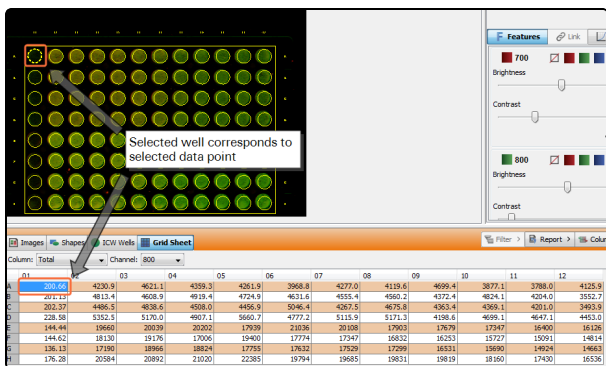


4. Link selected wells (highlighted in green) by clicking **Create Link**.
5. Once all necessary wells have been linked, click **OK**.

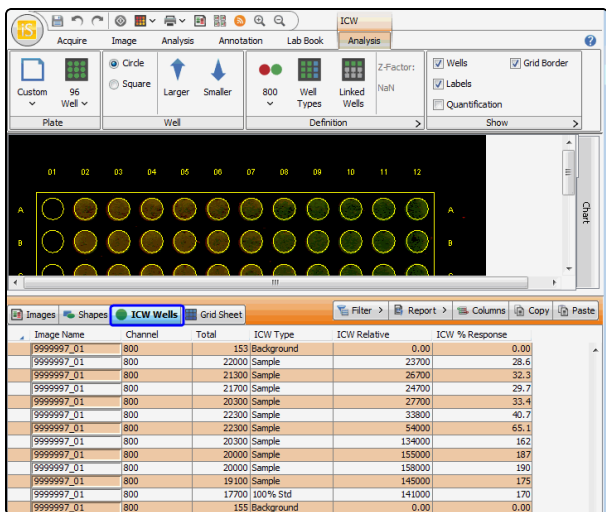
VIII. Review Data

Results for the **In-Cell Western** analysis will be displayed in the **ICW Wells Table** or the **Grid Sheet** table .

- Click **Grid Sheet** below and to the left of the image to view data in a grid format that corresponds to the layout of wells in the image.





- Click **ICW Wells** to view a table with analysis data for each well.



Use the **Column** list to select the data value to display.

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.







IX. Export Data

Data can be exported in three ways.

Export Data

- **Copy and Paste:** Right click selected data in the table you want to export data from, 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 you need.
 - 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.



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