



ODYSSEY[®] CLx

IMAGING SYSTEM

Tutorial Guide

Featuring Image Studio
Analysis Software
Version 3.1



LI-COR

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The logo for LI-COR, featuring the company name in a bold, italicized, sans-serif font. The letters 'LI-COR' are in a dark color, and the hyphen is also in the same color. The logo is positioned above a horizontal line.

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Chapter 1: Getting Started

Install the Software

IMPORTANT: You must be logged in using an administrator account. If necessary, log out and log back in using an Administrator account.

Windows 7, XP, or Vista

Installing via CD:

- 1) Place installation CD into drive.
- 2) If installation does not start immediately, browse to the CD and double-click on Win_Image_Studio_Installer_3.x.x.exe.
- 3) Click **Next** and follow the instructions in the Setup Wizard.

Installing via download:

- 1) Download and double-click on the Win_Image_Studio_Installer_3.x.x.exe file on your computer.
- 2) Click **Next** and follow the instructions in the Setup Wizard.

Macintosh®

Installing via CD:

- 1) Place installation CD into drive.
- 2) If installation does not start immediately, browse to the CD and double-click on MacImageStudio-3.x.dmg.
- 3) Click **Agree** to accept the license agreement.
- 4) To install, drag the Image Studio icon into the Applications folder (below).



- 5) (Optional) If you are updating Image Studio software and have purchased additional application keys, you will need to import those keys again to utilize their functionality.

Installing via download:

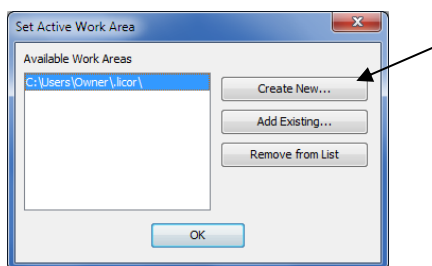
- 1) Go to your Mac System Preferences and click Security & Privacy to open the Security & Privacy window.

- 2) Click the lock in the bottom left corner of the window to allow editing, if necessary.
- 3) Under the General Tab, click **Mac App Store and identified developers** under the **Allow applications downloaded from** option.
- 4) Download, and double-click on the MacImage Studio-3.x.dmg file on your computer.
- 5) Click **Agree** to accept the license agreement.
- 6) To install, drag the Image Studio icon into the Applications folder
- 7) Open the Image Studio Application by double-clicking on the Image Studio icon.
- 8) If a window appears that says “ImageStudio.app is an application downloaded from the internet. Are you sure you want to open it?” click **Open**.
- 9) (Optional) If you are updating Image Studio software and have purchased additional application keys, you will need to import those keys again to utilize their functionality.

Set up a Work Area

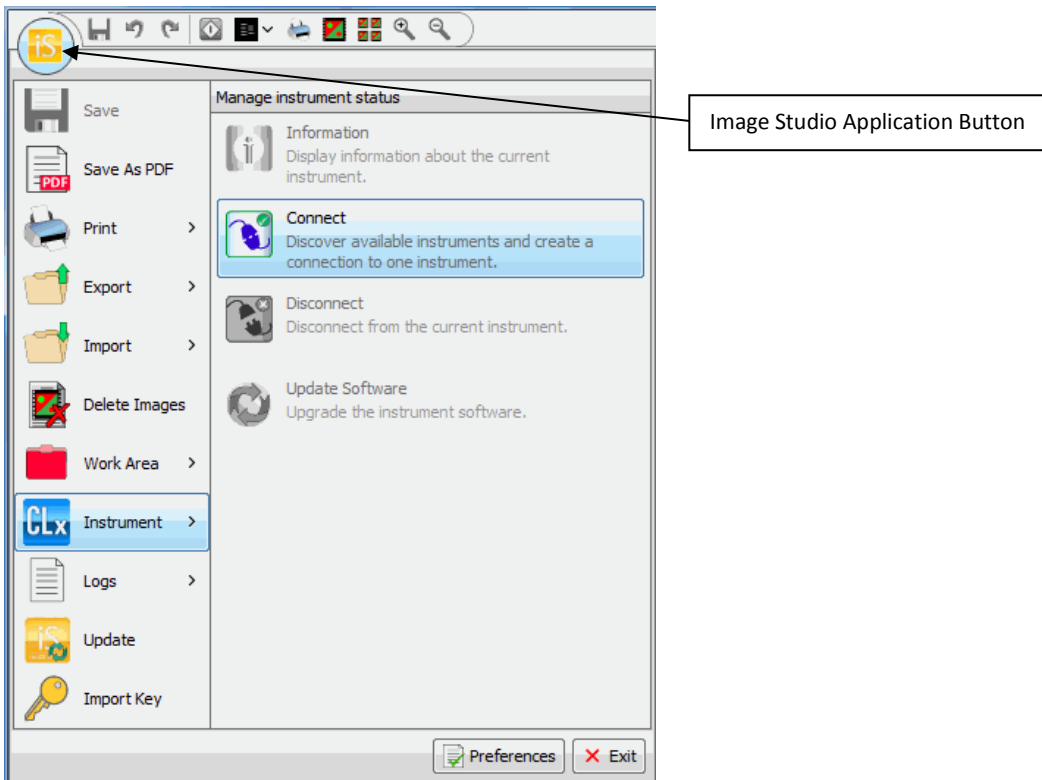
The first time you start the software you will need to create the first Work Area. The Work Area is a folder on the hard drive or network where all of the images, analyses and settings are stored. Click **Create New...** in the *Set Active Work Area* dialog box to browse to a folder on the hard drive or network to use as the Work Area. Click the New Folder icon to create a new folder for the Work Area. This folder will now appear in the *Available Work Areas* window each time the software is opened. Select the folder and click **OK** to set it as the Work Area. (To remove a Work Area from the window, select it and click **Remove from List**. Removing the folder from the window does not delete the folder or its contents.)

Note: Each user should create their own Work Area, as the previous settings for the instrument, analysis, image display, etc. from the last session will be applied to the next session in the same Work Area.



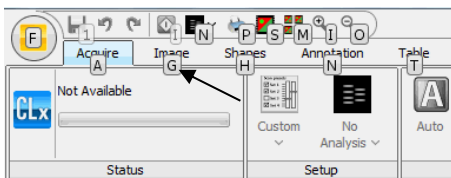
Connect to the Odyssey CLx Imager

The first time you connect, power the Odyssey® CLx Imager on and wait several minutes to allow the instrument to establish a network address. Click the Image Studio Application button, hover over **Instrument**, and click **Connect**. The software searches for and discovers the Odyssey CLx Imager on the same LAN automatically. If you experience connection problems, wait several minutes and then click **Connect** again. Check that IPv6 is enabled on the computer. The next time you connect, the software will automatically detect the Odyssey CLx Imager.



Optional Key Tips

Activate Key Tips to use the keyboard to access the ribbon tabs and other features of the Image Studio software. Press *ALT* (alt/option key on Mac computers) to display the Key Tips for all ribbon tabs and application quick launch buttons. Press the letter key next to the application quick launch buttons to select that application (e.g. press 'G' to open the Image tab, as shown below).



Press the letter key next to the ribbon tabs to view the ribbon for the selected tab and the key tips for the commands on that ribbon. Press the letter key next to the commands to activate the commands or open another menu. For Key Tips with two letters, press the keys sequentially on the keyboard.

In this manual, instructions refer to selecting tabs and commands with the mouse. Press *ALT* at any time to use the Key Tips to select the tabs and commands with the keyboard.

Chapter 2: Image Acquisition

Before starting, check that the Odyssey® CLx Imager is turned on and that the Image Studio software is running and connected to the instrument.

Place a sample on the Odyssey CLx scan bed. The Odyssey CLx Operator's Manual contains guidelines and techniques for placing membranes, gels, and microplates on the scan bed.

Acquire the Image

Click the **Acquire** tab to display the Acquire ribbon. In the *Status* group, **Ready** indicates the instrument is connected and not currently acquiring an image.



Setup

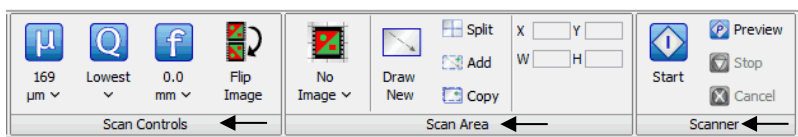
Click the first button in the Setup group to select the **Membrane** Scan Preset from the drop-down menu to quickly start a scan with default settings that are suitable for Western blots. Any change to the scan settings toggles the button to **Custom**. Save the new settings by clicking **Save Current Scan Preset...** at the bottom of the drop-down menu.

Click the second button in the Setup group to select an Analysis option from the drop-down menu to apply to the image after it is acquired. Select **No Analysis**.

Channels

Click the **Auto** button to select AutoScan for an image with wide dynamic range. Enable the check boxes next to the desired channel (700, 800, or both), indicating the channel(s) selected for image acquisition. If AutoScan is not selected, adjust the intensity of each channel manually.

Important: Do not use AutoScan when imaging live animals with the MousePOD® Accessory.



Scan Controls

Set the resolution, quality, and focus using the drop-down menus. Setting the resolution to **169 μm** and the quality to **Lowest** increases the speed of the scan while providing a quality image. Set the focus offset to **0.0 mm** for a Western blot. Select **Flip Image** when using a microplate to view the data in the same orientation as it appears on the plate.

Scan Area

Use the tools in the *Scan Area* group to define the area of the scan bed for the imager to scan. First, select **No Image** from the drop-down menu of the first button to view the scan grid without an image. Select **Draw New** to drag a new rectangle on the scan grid and erase any other scan areas. Click the scan grid at the upper left point of the new rectangle and drag to the lower right. Click **Split** to separate a selected rectangle into equal parts. Select **Add** and drag an additional rectangle on the scan grid from the upper left point to the lower right. Click **Copy** to place a replica of the selected rectangle on the scan grid.

Scanner

After the parameters have been set, click **Start** to start the scan. The imager scans the defined area and stops when finished. If necessary, click **Stop** to stop the scan before it finishes, or **Cancel** to stop the scan and delete the image.

Alternatively, you can click **Preview** to quickly obtain a low resolution scan image. You can then view this low resolution image on the scan grid to adjust the scan area. Click the first button in the *Scan Area* group and choose **Last Acquired**.

View the Image and Data

When the acquisition is complete, the image appears on the screen. The image data appear in the Images Table below. Change the Image Name to easily find the image later.

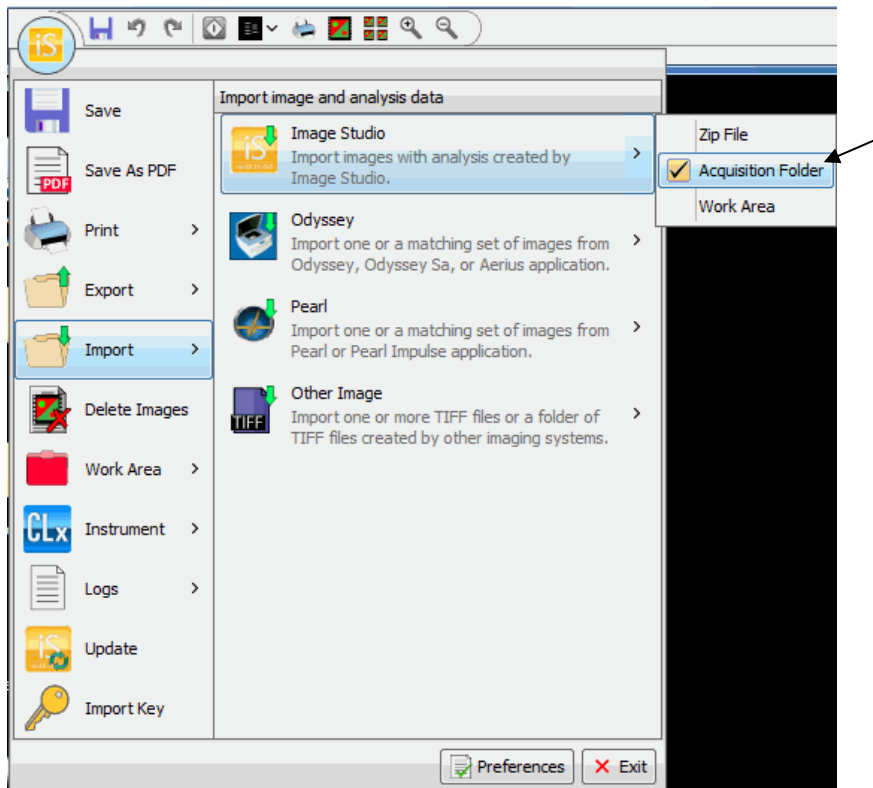
- 1) Double-click in the field under **Image Name**.
- 2) Enter a new name. Press *Enter* when finished.
- 3) Refer to [Chapter 4: Image Display](#) for complete information on adjusting the image appearance.

Chapter 3: Image Files

Import an Image

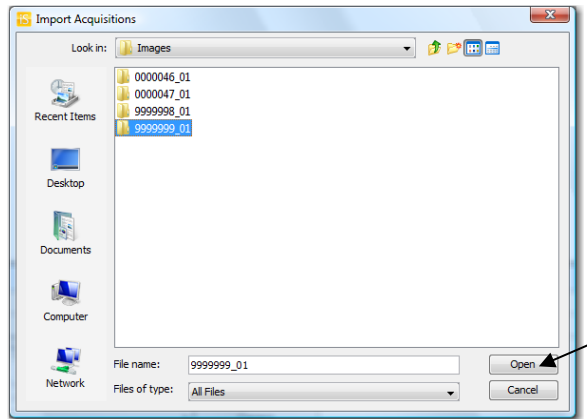
Tutorial images are provided on the Image Studio CD in the **Image Examples** folder. The folder name for the Western Image is '9999999_01'.

- 1) Click the Application Menu Button and hover over **Import**. Hover over Image Studio and click **Acquisition Folder**.



Note: Images from the Odyssey Application Software (versions 1.x, 2.x, or 3.x) can be imported into Image Studio software for analysis by clicking **Import > Odyssey**.

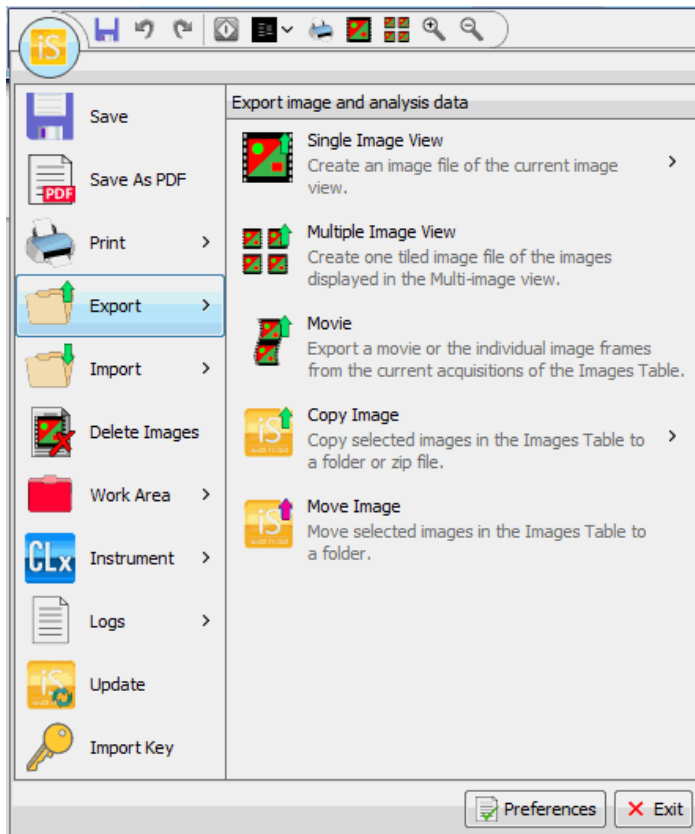
- 2) Browse to the Image Studio CD, open the **Image Examples** folder, and select the '9999999_01' folder. Click **Open** to display the image.



3) The image opens and the file appears in the Table.

Export Acquisition Data and Images

In the Images Table, click on a row to select an image acquisition. To select multiple image acquisitions, click and drag over the rows or press the *Ctrl* key on the keyboard and click on each row to select it. Click the **Image Studio Application Button** and hover over **Export** to view the *Export image and analysis data* menu.



- 1) To make a copy of the image acquisition, click **Copy Image** and choose **Folder** or **Zip File**. Click **Zip File** to save the image acquisition(s) as a compressed (zip) file. Either choice opens a window where you can select the folder to which to copy the image acquisition.

Note: Use **Copy Image** to move image acquisitions to another Work Area or to make a zip file to share with another Image Studio user.

- 2) To make a copy in another folder and delete the image acquisition from the Work Area, click on **Move Image** and click **Yes** in the *Move Selected Acquisitions* menu. Select the folder to copy to in the browse window.
- 3) To save the image to a graphics file, click **Single Image View** to view a choice of **Current Image**, **Selected Images in Images Table**, or **Color Bar Only**. Each choice opens a window where you can select the folder to copy to, as well as the file type (TIFF, PNG, or High Quality JPEG) and resolution.

Note: Images saved as these file types (TIFF, PNG, or High Quality JPEG) are for presentation purposes and should not be used for further analysis.

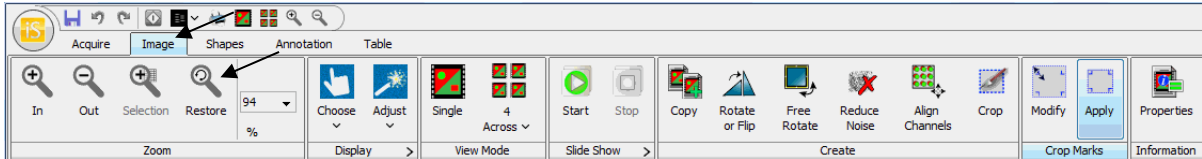
- 4) To save a tiled image file from images in the Table, click **Multiple Image View** to open a browse window where you can select the folder to copy to as well as the file type (TIFF, PNG, or High Quality JPEG) and resolution.

Note: Data can be quickly and easily exported from the Tables to a spreadsheet. Refer to Exporting Data in [Chapter 10: Tables](#).

Chapter 4: Image Display

View the Image

Click the **Image** ribbon tab to display the *Image* ribbon.

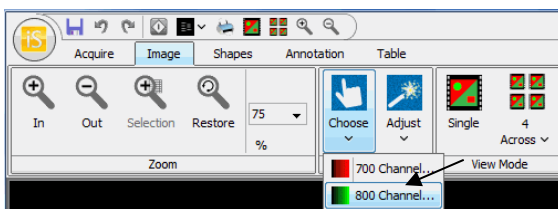


In the *Zoom* group click the magnifier icons to enlarge or reduce the displayed image. You can also scroll the mouse wheel or use the scroll function of a keypad to zoom in or out. Click **Restore** to automatically fit the image to the window.

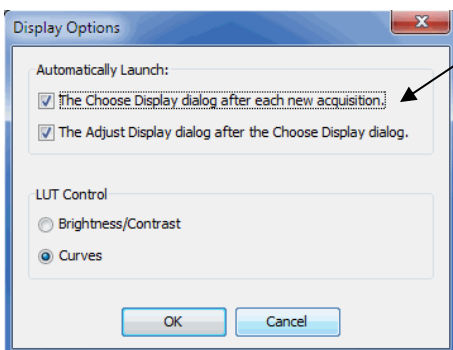
Display

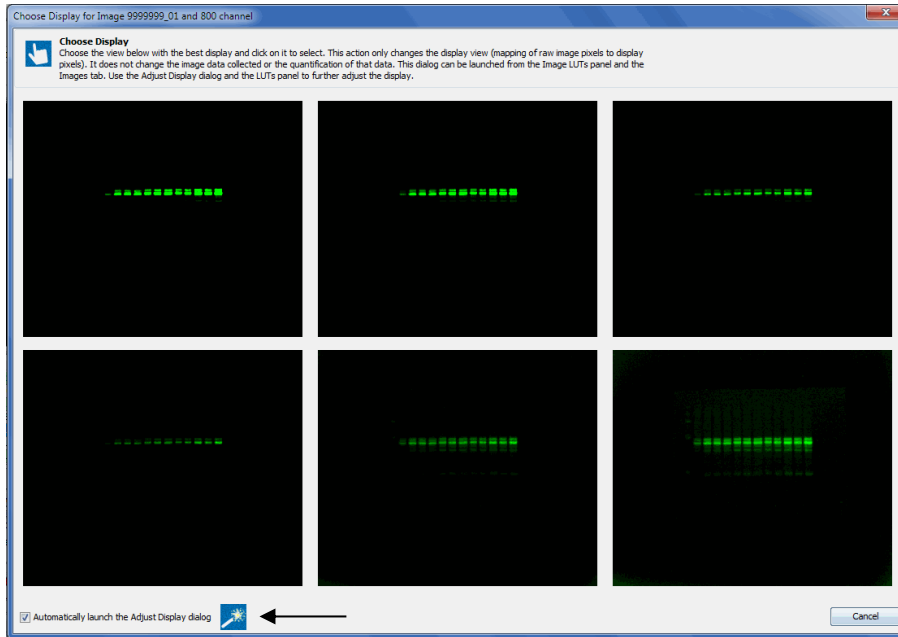
The *Choose Display* and *Adjust Display Assistants* are alternate ways to adjust the *Look Up Tables (LUTs)*.

Click **Choose** in the *Display* group and select the **800 channel** to open the Choose Display Assistant.

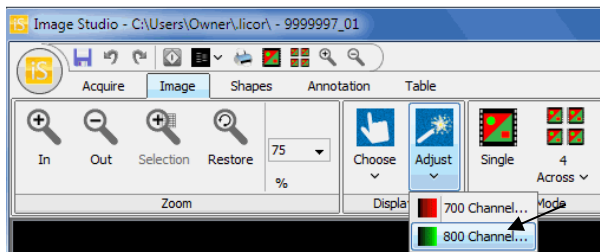


- 1) Click the view with the best display. The Adjust Display dialog will open for further adjustment. You can choose to not open the Adjust Display dialog automatically by disabling **Automatically launch the Adjust Display dialog** at the bottom of the window. You can also click the arrow (>) on the *Display* group on the *Image* ribbon to open the *Display Options* dialog and disable the automatic launch of either the Choose Display or Adjust Display dialogs.

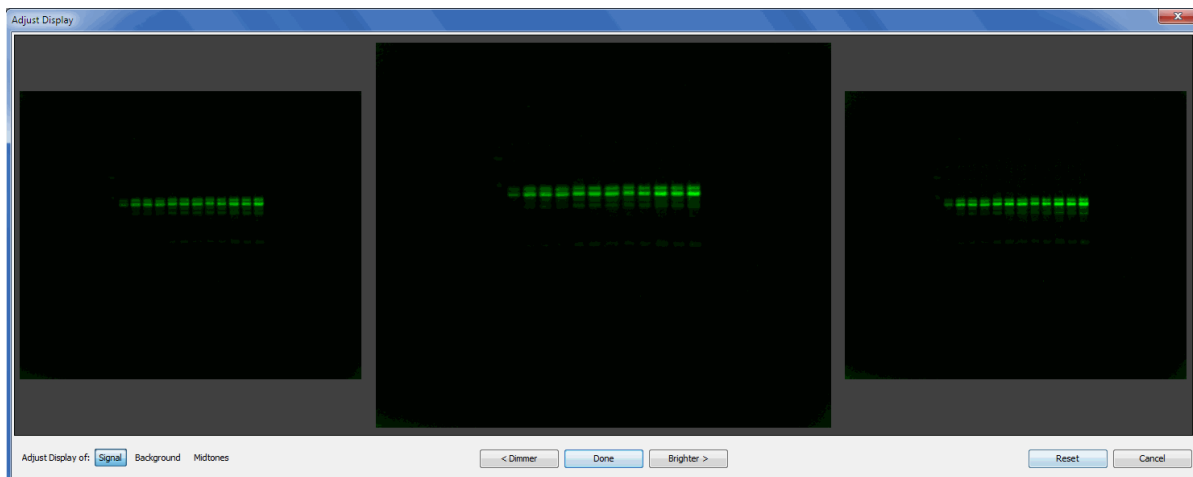




Click **Adjust** in the *Display* group and select the **800 channel** to open the Adjust Display Assistant.



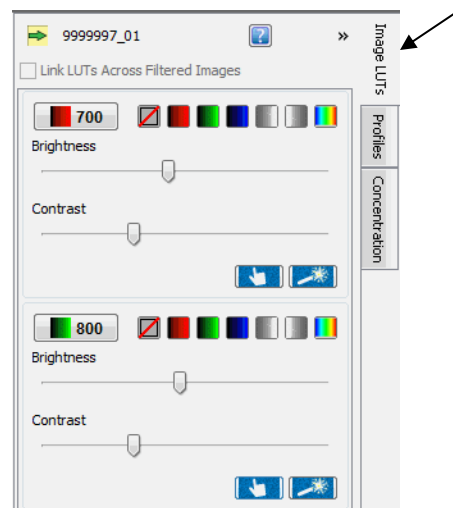
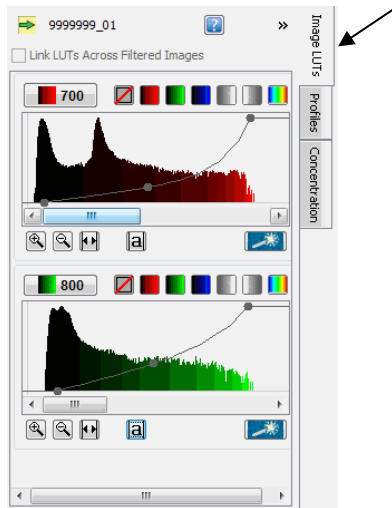
Select **Signal** at the bottom left to adjust the maximum point on the curve in the Look Up Tables, **Background** to adjust the minimum point, or **Midtones** to adjust the K value. Click **Dimmer** or **Brighter** to change the visual appearance of the image. When finished, click **Done**.



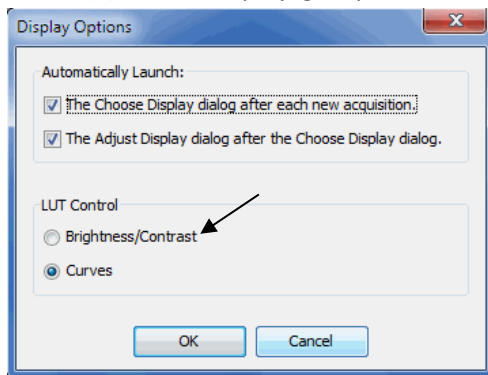
Adjust the Image Appearance

The Look Up Tables (LUTs) display histograms of the pixel intensities for each channel acquired for the image. The LUTs are on the right side of the screen.

- 1) Click the **Image LUTs** tab to view the Image Look Up Tables as either histograms overlaid with a curve, or as slider bars. To hide this view, click the double arrows in the top right corner.



- 2) To change the Image LUTs from histograms (Curves) to Brightness/Contrast sliders, click the arrow (>) on the *Display* group on the *Image* ribbon to open the *Display Options* dialog.



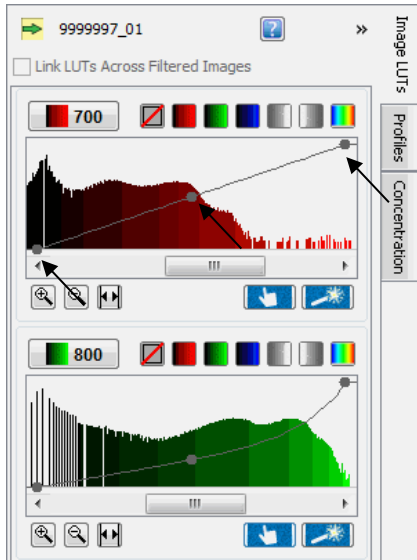
- 3) Click the appropriate colored button above the graph to select a different color scheme for each channel, or to disable a specific channel.

Note: Multiple channels can be viewed, printed, and exported in gray scale.

To adjust the intensity of the image in the histogram view,

- a. Drag the left (min) dot on each graph to adjust the lower threshold of pixel values that will shade to the same color on the viewed image. Shading the lower intensity pixels to the same color will create a visually cleaner background on the image.

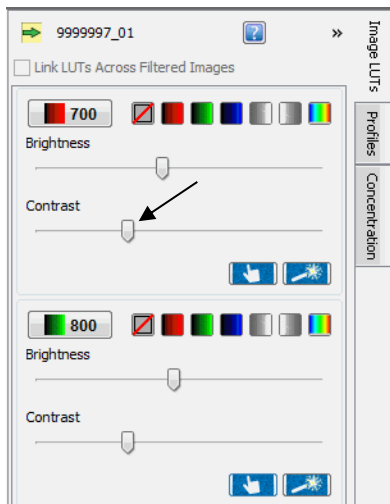
Note: Changing the visual image will not change the data collected for that image or the quantification of that data. It only changes the mapping of raw image pixels to display pixels.



- b. The rightmost (max) dot on each graph adjusts the upper threshold of pixel values that will shade to the same color on the viewed image.
- c. Click and drag the middle dot on each graph vertically to change the K value.

Note: Further explanation of the K value can be found in the online Help system by clicking the question mark in the upper right-hand corner of the screen.

To adjust the intensity of the image in the Brightness/Contrast view, click and drag each slider.



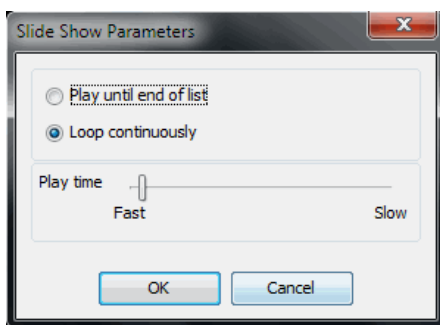
View Mode

Select **Single** in the *View Mode* group to view and analyze a single image. Click the Multi-Image View button and select the number of images to view from the drop-down menu.

Note: Analyses cannot be performed in Multi-Image View.

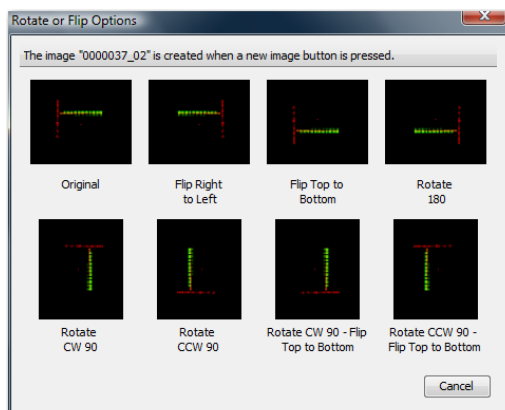
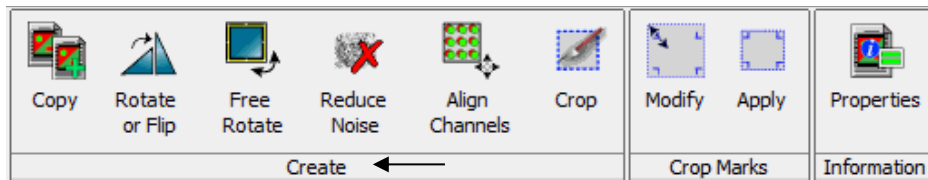
Slide Show

Click **Start** and the images in the Table will appear in the window sequentially. Click **Stop** to return to normal viewing. Click the arrow (➤) on the *Slide Show* group to open the *Slide Show Parameters* menu. Choose to display the images in the Table and stop at the end, or return to the beginning of the table and continue displaying the images (**Loop continuously**). Adjust the slider to modify the amount of time each image is displayed.

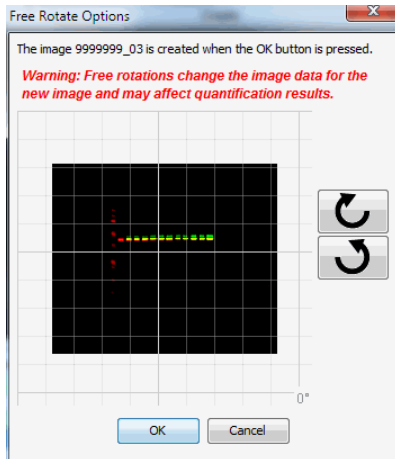


Create

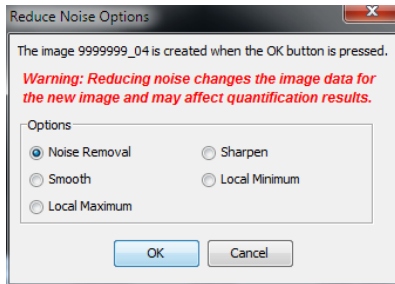
Click a button in the *Create* group to create a new image acquisition file with the designated action applied. The original image acquisition remains unchanged. Click **Copy** to create a copy of the image. Click **Rotate or Flip** to open the *Rotate or Flip Options* menu and select an orientation.



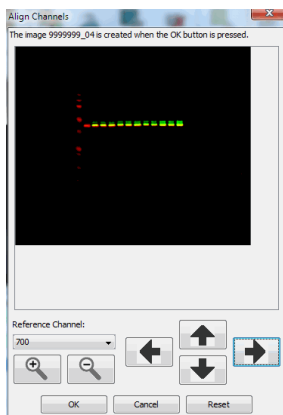
Click **Free Rotate** to open the *Free Rotate Options* dialog box and click the arrow buttons to rotate the image clockwise or counterclockwise. **Warning:** *Free rotations change the image data for the new image and may affect quantification results.*



Click **Reduce Noise** to open the *Reduce Noise Options* dialog box and select an option. Click **OK**. **Warning:** *Reducing noise changes the image data for the new image and may affect quantification results.*

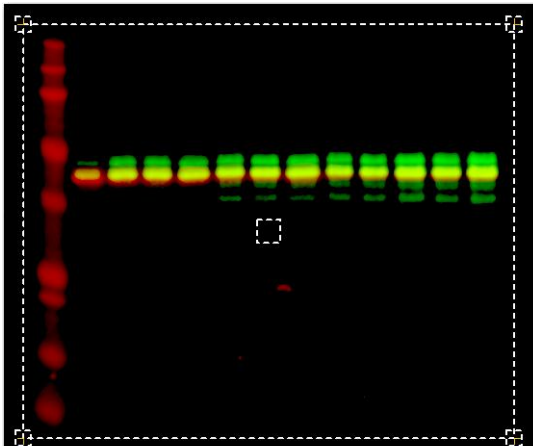


Click **Align Channels** to open the *Align Channels* menu. Select one channel as the static reference and adjust the other channel using the arrow buttons. Click **OK**.



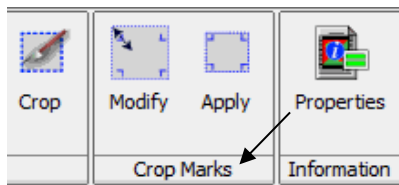
Crop the Image

Click **Crop** to create an image that contains only the area within the bounding box. Adjust the crop area by dragging the boxes in the corners or at the center. Click **OK** to crop the image.



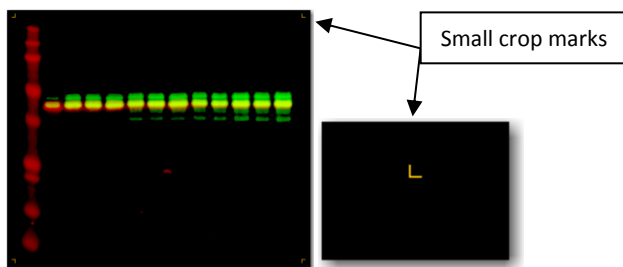
Crop Marks

Place crop marks on the image to indicate the area of the image to print or export. These crop marks can apply to the selected image, or all images in the Images table.



- 1) Click **Apply** and marks indicating the corners of the cropped area appear on the image.
- 2) Click **Modify** to open the *Edit Image Crop Marks* dialog. Two boxes appear as dotted lines on the image.
- 3) Drag the small box in the middle of the image to center the crop marks. Drag the corners of the large box to surround the marker and bands.
- 4) Click **Current Image** to mark this image with these crop marks and click **OK**. Small crop marks indicating the corners of the cropped area appear on the image at the new location.

Note: If **Images in images table** is selected, this crop definition applies to all images in the *Images Table*.

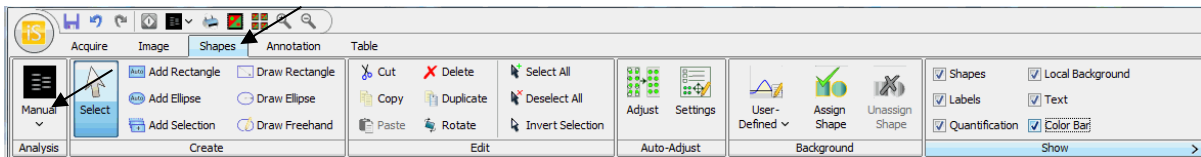


Note: *These small crop marks will appear on the image as long as **Apply** is selected. The crop area will apply to any print or export action. To remove the small crop marks and print or export the full image, click **Apply** again to toggle it off.*

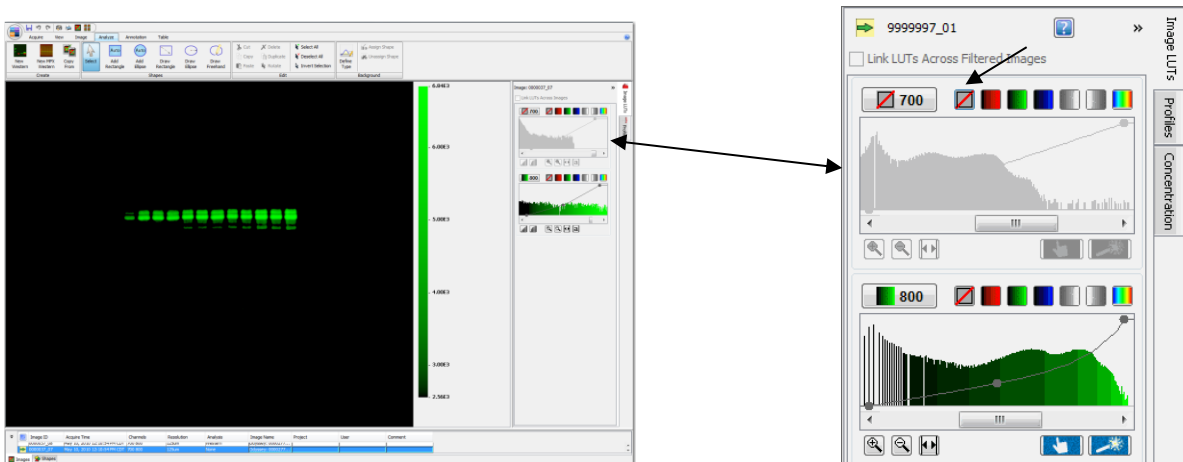
Chapter 5: Data Analysis

Add and Manipulate Shapes Manually

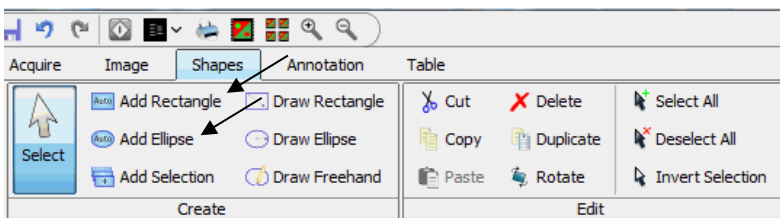
- 1) Click the *Shapes* tab to open the *Shapes* ribbon. Set the Analysis Type to **Manual**.



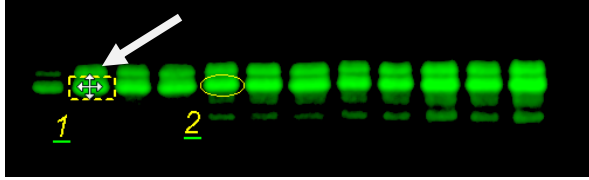
- 2) To use the **Auto Add Rectangle** or **Auto Add Ellipse** tools, only one channel can be selected. Click the *Don't show this channel* thumbnail in the Image LUTs for the 700 channel, leaving only the 800 channel displayed on the image.



- 3) Click **Auto Add Rectangle** to add a rectangle to the image or **Auto Add Ellipse** to add an ellipse. Click in the center of a feature to place the rectangle or ellipse around it. After adding the desired shapes, click **Select** or press the *Esc* key on your keyboard to return the cursor to the selection tool.

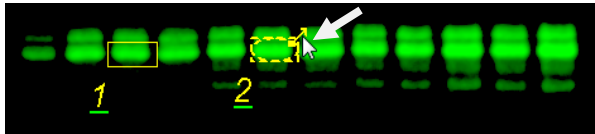


- 4) To move or resize a shape, first select the shape by clicking on it once. Dotted lines indicate the shape is selected.
 - a. To move the shape, drag the four-pointed cursor within the selection shape.



- b. To resize the shape, hover over any corner or side of the shape. Drag the double-sided yellow arrow to resize.

Note: To find the double-sided yellow arrow, the cursor must be on the selection tool (click **Select** in the Shapes group or push the Esc key on the keyboard).



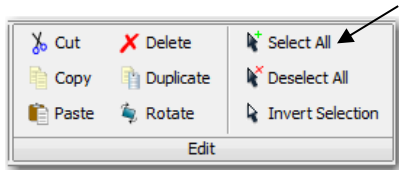
- c. To view the shapes and labels on the image, enable the **Shapes** and **Labels** check boxes in the **Show** group. Disable the check marks to remove the shapes and labels from the image.
- d. To edit the label, click the **Shapes** tab at the bottom of the screen to view the Shapes Table. Under the column **Name**, double-click on the cell for this shape and edit the text. Press **Enter** and the new text will appear on the image. To change the location of the label, click the small arrow on the **Show** group to open the **Image View Labels** menu.

Note: You must press **Enter** or click on another cell in the Table to save the change to the Table cell before saving the acquisition.

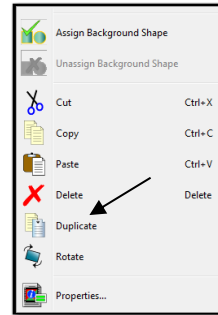
Image ID	Image Name	Channel	Name	Signal	To
000033_01	0000277_01 0000277_01	800	14	1.09E6	
000033_01	0000277_01 0000277_01	800	15	1.02E6	
000033_01	0000277_01 0000277_01	800	17	1.02E6	
000033_01	0000277_01 0000277_01	800	30	1.18E6	
000033_04	0000277_01 0000277_01	800	2	1.50E6	
000033_09	0000277_01 0000277_01	800	2	1.05E6	
000033_05	0000277_01 0000277_01	800	11	6.92E5	
000033_09	0000277_01 0000277_01	800	1	1.38E6	
000033_03	0000277_01 0000277_01	700	13	2.00E7	

Note: Select **Draw Rectangle**, **Draw Ellipse**, or **Draw Freehand** to manually draw Shapes. Drag the mouse to create the shape. The resulting shape can be moved or resized in the same way that an auto shape is moved or resized.

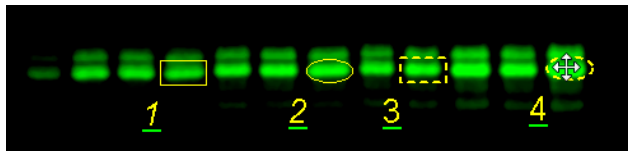
- 5) To copy the same shapes to the 700 channel:
 - a. Click **Select All** in the *Edit* group to select the shapes. Dotted lines indicate the shapes are selected.
 - b. Click **Duplicate** in the *Edit* group or right-click on the image and select **Duplicate** from the pop-up menu.
 - c. Click the *Show this Channel in Red* thumbnail for the 700 channel to see the bands.



Right-click menu



- 6) To quickly add shapes in one or both channels, use the **Add Selection** tool.
 - a. Drag a rectangle around the shapes to select them. Dotted lines indicate the shapes are selected.
 - b. Click **Add Selection** in the *Create* group.
 - c. Click the image to add the shapes to the image.
 - d. To move the shapes, drag the four-pointed cursor that appears within either shape. The selected shapes move together.

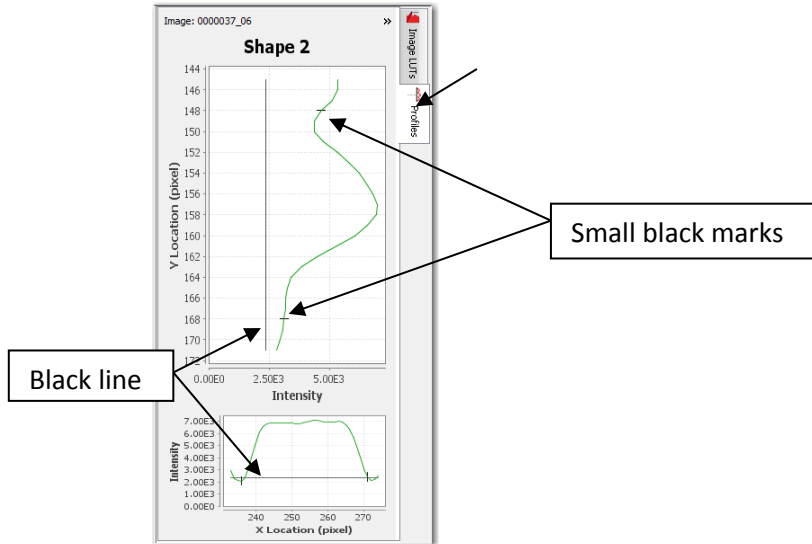


- 7) To rotate the shapes in the 800 channel...
 - a. Click the *Don't show this channel* thumbnail for the 700 channel, leaving only the 800 channel displayed on the image.
 - b. Drag a rectangle around the shapes to select them. Dotted lines indicate the shapes are selected.
 - c. Click **Rotate** in the *Edit* group or right-click on the image and select **Rotate** from the pop-up menu.
 - d. Drag inside the circle to rotate the selected shapes.

Note: You can also rotate shapes by selecting them, pressing the *Ctrl* key, and scrolling with the mouse.

Profiles Tab

- 1) Select 'Shape 2' by clicking on the shape with the selection arrow until it changes to a dashed line.
- 2) On the right side of the screen, click the **Profiles** tab to view two graphs of the pixels within a selected shape. The top graph is a cross section of the pixel intensities from top to bottom. The bottom graph is a cross section of the pixel intensities from left to right.



- 3) Adjust the size of 'Shape 2' by dragging the arrows on the corners of the shape. Notice how the graph changes.

Note: The small black marks indicate the boundaries of the shape. Move the shape around the band so that the small black marks are close to the edges of the peak to capture the entire signal for that band.

Auto Adjust

The software moves selected shapes to fully enclose areas of fluorescence near the shapes.

- 1) Drag a rectangle around the shapes to select them. Dotted lines indicate the shapes are selected.
- 2) Click **Auto Adjust** and the shapes will move slightly to better enclose nearby areas of fluorescence.

Subtract the Background

Subtract the background of the blot or gel from the shapes to obtain consistent data. The software will not calculate **Signal** for the shapes if a background method is not selected.

- 1) Click the first button in the *Background* group and select **Median** from the drop-down menu to subtract the median value of the pixels in the background segment. This method is useful if the pixel intensities in the background segment are not uniform.

Note: Select **Average** for the Background Method to subtract the average value of the pixels in the background segment.



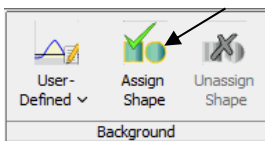
- 2) In the pop-up window set the *Border Width* to **3** and select **Right/Left** to select the segments on the left and right sides of the shape.
- 3) Click **Save**.

Note: The black line in the **Profiles** indicates the background that will be subtracted.

Subtract a User-Defined Background

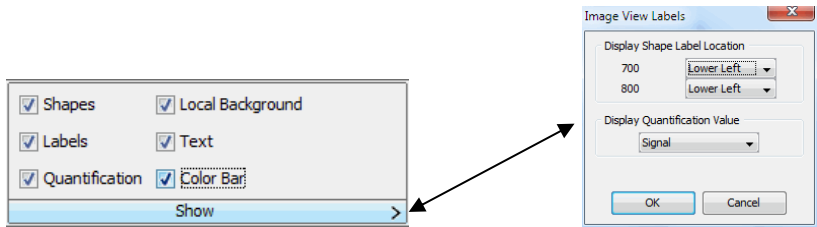
A region of the image can be chosen for the background.

- 1) Click the first button in the *Background* group and select **User-Defined** from the drop-down menu.
- 2) Using what you've learned from *Add and Manipulate Shapes Manually* in this chapter, place a shape on the image in the region that you have chosen as the background.
- 3) Select the shape by clicking on it with the selection arrow until the shape outline becomes a dashed line.
- 4) Click **Assign Shape** in the *Background* group.



Show

In the Show group, enable all check boxes, indicating all items will appear on the image. To specify the location of the labels, click on the arrow at bottom right to open the Image View Labels menu.



Concentration

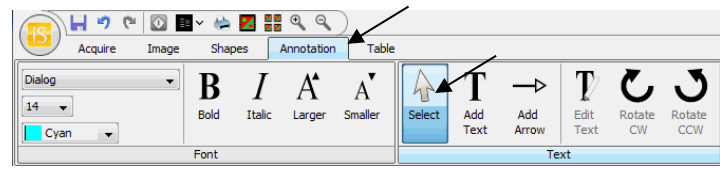
You can assign concentration values to two or more shapes or Bands and the Image Studio software will assign a value to the other shapes or Bands based on their relative Signal.

- 1) Click the Shapes tab at the bottom of the table to open the Shapes Table, if necessary.
- 2) Double-click the *Conc. Std.* cell for shape **1** and enter **1**.
- 3) Double-click the *Conc. Std.* cell for shape **4** and enter **9**.
- 4) Click the **Concentration** tab on the right side of the screen to view the assigned concentration standards.
- 5) Click the down arrow under *Interpolation Method* and click **Linear** from the drop-down menu to assign a linear interpolation.
- 6) You can see the concentration values assigned to Shapes 2 and 3 in the *Concentration* column in the Shapes Table.

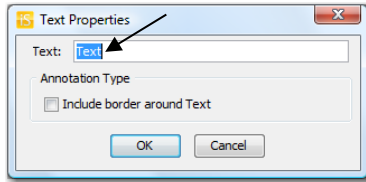
Image Name	Channel	Name	Signal	Total	Area	Bkgnd.	Type	Conc. Std.	Concentration
9999999_01	800	1	4.88E-1	5.52E-1	560	1.14E-4	Signal	1.00E0	1.00E0
9999999_01	800	2	7.45E-1	8.10E-1	530	1.23E-4	Signal		3.66E0
9999999_01	800	3	6.21E-1	6.91E-1	560	1.24E-4	Signal		2.38E0
9999999_01	800	4	1.26E0	1.31E0	530	9.57E-5	Signal	9.00E0	9.00E0

Annotate the Image

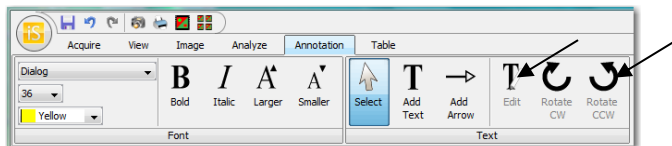
Click the **Annotation** tab to open the *Annotation* ribbon.



Click **Add Text** in the *Text* group and click on the image. Type the desired text in the *Text:* box. Click **OK** to add the text to the image.



Click **Add Arrow** and click on the image to add an arrow to the image. Click **Select** or press the *Esc* key on the keyboard to toggle the cursor back to the selection tool. Click the text or arrow that was added to the image with the selection tool. A dashed box appears around the text or arrow. Use the tools in the *Font* group to change the appearance of the text or arrow.



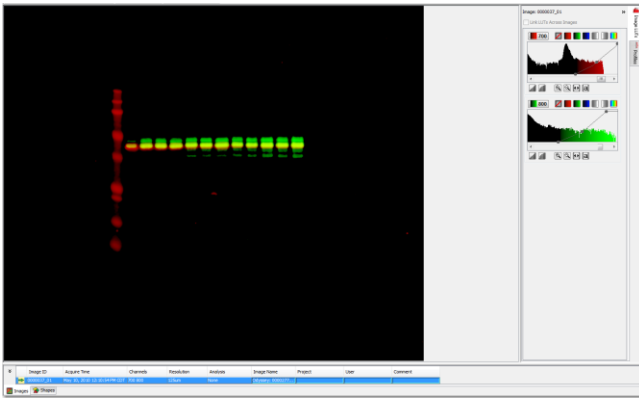
Click **Rotate CW** or **Rotate CCW** in the *Text* group to rotate the text or arrow. Click **Edit** in the *Text* group to view the *Text Properties* menu for the selected item. Insert different text or add a border to the text with this menu. If the selected item is an arrow, click and drag the sides of the dashed box to change the size of the arrow.

Chapter 6: Western and MPX™ Western Analyses

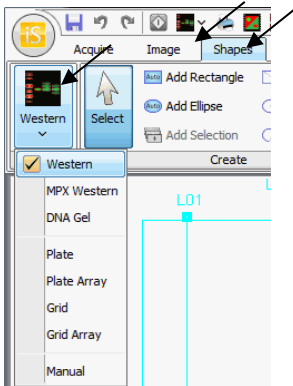
The Western and MPX Western Analyses quickly place lines and boxes over the image to designate Lanes and Bands for analysis.

Apply a Western Analysis

- 1) Open the Western image imported in [Chapter 3: Image Files](#) by clicking the appropriate row in the *Images Table*. To quickly find this image, hover over the *Images Table* to view a thumbnail of each image.

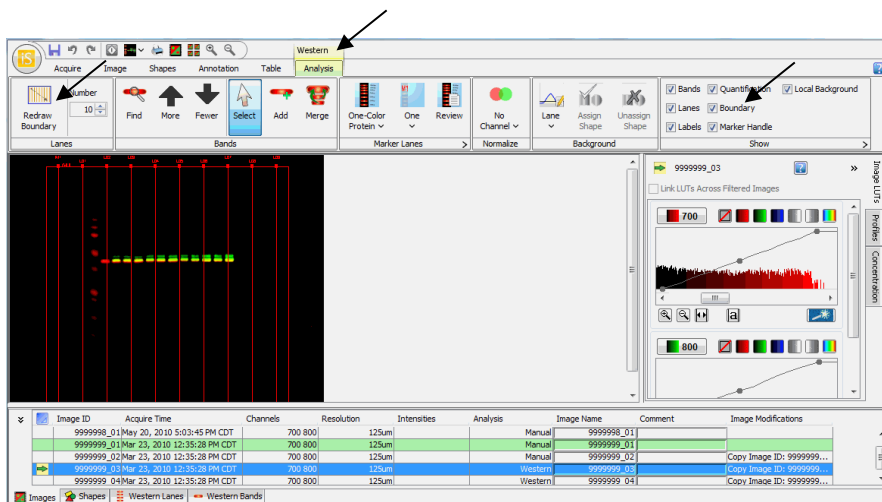


- 2) Click the **Shapes** tab to view the *Shapes* ribbon. **Note:** Delete any Shapes on the image.



- 3) Click **Western** to open the *Western Analysis* ribbon and apply the Western analysis to the image. Or, click the quick launch button at the top of the window and select Western from the drop-down menu.

Note: To automatically apply the Western Analysis after image acquisition, click **Western** in the Setup group on the Acquire ribbon when starting the acquisition.



Settings from the most recent Western analysis in your Work Area are applied to the image.

Set the Boundary

The Boundary is the box that surrounds the lanes and bands on the image.

- 1) To view the Boundary on the image, click **Boundary** in the *Show* group so that the check box is enabled. To hide the Boundary, click to disable the check box.
- 2) Click on the Boundary to select it. The dashed line indicates it can be adjusted.
- 3) To adjust the applied boundary, hover over the corners or the midpoints of the sides to enable a double-sided arrow. Drag the arrow to move the corner or side.
- 4) To move the entire Boundary, drag the four-pointed cursor that appears inside the Boundary. Set the vertical edges of the Boundary to just outside the outermost lanes.
- 5) To draw a new Boundary, click on **Redraw Boundary** in the *Lanes* group. Click and drag a rectangular shape on the image. Repeat the above steps to adjust the Boundary.
- 6) To set the Boundary, click on a different spot on the screen. The Boundary toggles back to a solid line.

Set the Lanes

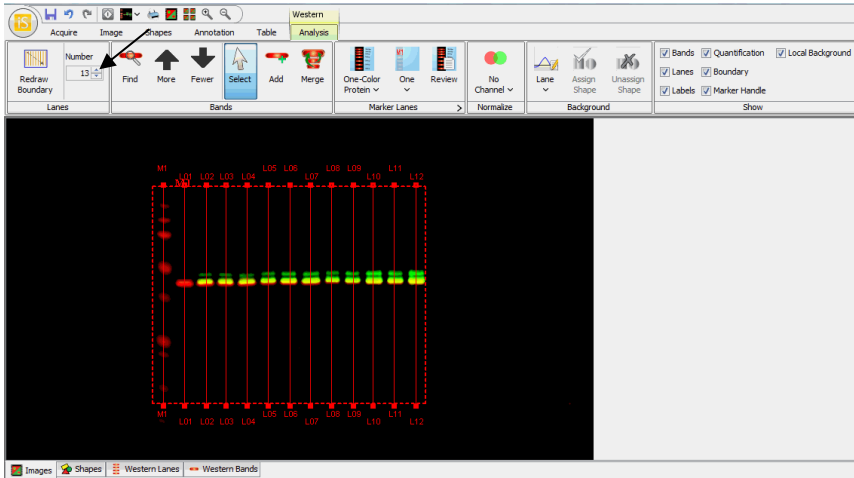
The Lanes are vertical markers that overlay and transect the bands (below).

- 1) To view the Lanes on the image, click **Lanes** in the *Show* group to enable the check box next to it. To hide the Lanes, disable the check box.
- 2) Count the total number of lanes in the image (including empty lanes). Adjust the **Number** in the *Lanes* group by clicking the up or down arrows next to the box to match the total number of lanes in the image.

Note: The Lanes are labeled with 'L' plus a number, starting with '01' on the left. To view the labels, click **Labels** in the *Show* group to enable the check box next to it. To hide the labels, click to disable the check box.

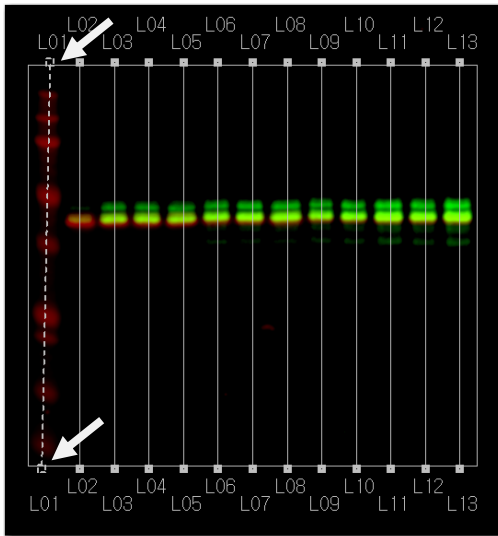
- 3) To easily adjust the Lanes to transect the image lanes, follow the steps at *Setting the Boundary* to adjust the sides of the Boundary.

Note: The Lanes are anchored to the Boundary at the top and bottom of the Lane. Adjusting the Boundary also adjusts the Lanes.



- 4) To further adjust a single Lane, click on it so that a dashed line appears. Drag the anchor points at the top and bottom of the Lane to move only the end points of the lane. Hold the *Shift* key on the keyboard while dragging the anchor points to move the entire lane.

Note: The Lanes only need to be close to the centers of the bands, as they will be optimally adjusted by the software during band-finding.



- 5) To set the Lane marker, click on a different spot on the screen. The Lane marker toggles back to a solid line.

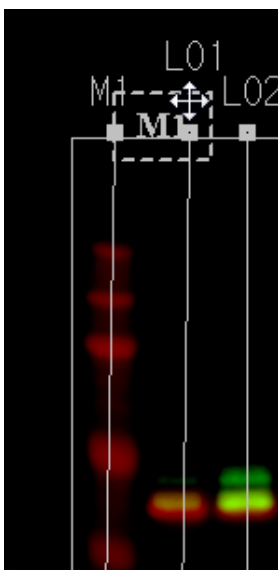
Set the Marker

Each Marker Lane is designated by 'M' followed by 1, 2, or 3.

- 1) Click the first button in the *Marker Lanes* group and choose **One-Color Protein** from the drop-down menu.
- 2) Click the second button in the *Marker Lanes* group and select **One** from the drop-down menu.
- 3) To change the location of the Marker Lane, click the Marker Handle to toggle the rectangle to a dashed line.

Note: The Marker Handle is a rectangle with 'M1', 'M2', or 'M3' inside. It designates the leftmost lane adjacent to it as a marker lane. To view the Marker Handle, click **Marker Handle** in the Show group to enable the check box. To hide the Marker Handle, click to disable the check box.

- 4) Drag the four-pointed cursor on the Marker Handle over any lane to designate that lane as the Marker. The name of selected lane changes to 'M1' and the other lanes are renumbered to exclude that lane.



- 5) Click **Review** in the *Marker Lanes* group to see the assigned values for the bands in the One-Color Protein Marker. Click **OK** to close the box.

Create a New Marker

- 1) Click the arrow on the *Marker Lanes* group to open the *Create New or Delete Marker* menu.
- 2) Click **New...** to open the *Name Marker* menu. Enter a name for the Marker in the box and click **OK**. The *New Marker* menu will open.
- 3) Click **Add...** to open the *Add Band Value* menu. Enter a numerical value for a Marker band in the box and click **OK**.
- 4) Repeat step 3 to add all of the values for the bands in the Marker. The numerical values will be sorted from high to low. To delete a value, select the value and click **Remove**.
- 5) Click **OK** to close the *New Marker* menu.

Note: The name of the new Marker will appear in the *Select Marker Lanes Set drop-down menu* in the *Marker Lanes* group. Select the Marker from the menu and click **Review** to view the details. Click the arrow on the *Marker Lanes* group to open the *Create New or Delete Marker* menu and click **Delete** to remove the Marker.

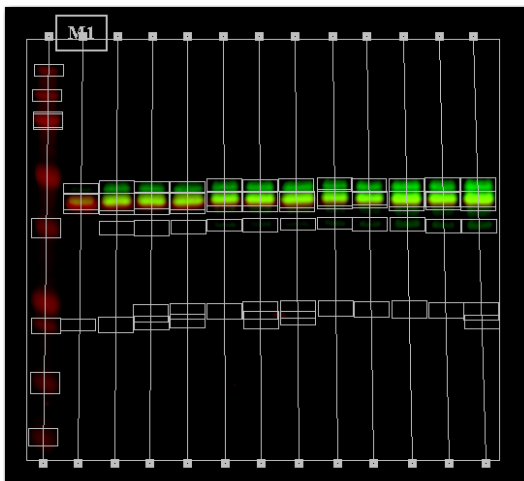
Find the Bands Automatically

Bands will be found in all channels.

- 1) In the *Bands* group, click **Find** to automatically place Bands on areas of fluorescence on the Lanes.

Note: Removing the Labels makes the Bands easier to see. Click **Labels** in the *Show* group to disable the check box next to it.

- 2) Click **Fewer** to decrease the sensitivity, resulting in fewer Bands. Click **More** to increase the sensitivity and find more Bands.

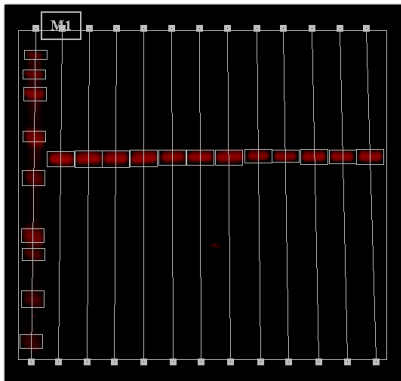


Edit the Bands Manually

- 1) To manually edit the Bands, only one channel can be selected. In the Image LUTs click the **Don't show this channel** thumbnail for the 800 channel, leaving only the 700 channel displayed on the image.

- 2) To manually add a Band,
 - a. Click **Add** in the *Bands* group and then click on a Lane to place the Band.
 - b. Click **Select** or press the *Esc* key on the keyboard to toggle the cursor back to the selection tool.
- 3) To manually delete a Band,
 - a. Select the Band by clicking it once with the selection tool. Dotted lines indicate the Band is selected.
 - b. Press *Ctrl+X* or the *Delete* key on the keyboard (or right-click and select **Cut** or **Delete**) to delete the selected Band.

Note: Multiple Bands can be deleted or moved at one time. Select multiple Bands by pressing the *Ctrl* key on the keyboard and clicking once on each Band, or use the selection tool to drag a bounding box over the Bands.



- 4) To move a Band,
 - a. Select the Band by clicking on it once with the selection tool. Dotted lines indicate the Band is selected.
 - b. Drag the four-pointed cursor that appears on the selected Band.

Note: The Band is anchored to the Lane it appears on and cannot be moved to another Lane.

- 5) To resize a Band,
 - a. Select the Band by clicking on it once with the selection tool. Dotted lines indicate the Band is selected.
 - b. Hover the mouse over any side of the Band. Drag the double-sided arrow to make the selected Band larger or smaller.
- 6) To merge Bands,
 - a. Use the selection tool to drag a rectangle over two or more Bands that appear on the same Lane, or press *Ctrl* on the keyboard and click once on each Band. Dotted lines indicate the Bands are selected.

- b. Click **Merge** in the *Band* group. One Band now surrounds the area in and between the previous Bands.

Subtract the Background

Subtract the background of the blot from the Bands to obtain consistent data. The software will not calculate **Signal** for the shapes if a background method is not selected.

Select **Lane** from the drop-down menu in the *Background* group to subtract the mean background in the Lane from the Bands that appear on that Lane. **Lane** is the recommended setting for background subtraction in the Western Analysis application. Refer to *Subtract the Background* in [Chapter 5: Data Analysis](#) for other background subtraction options.

Normalize the Signal in One Channel

The Band with the largest Signal in the normalization channel is assigned a value of 1, and the Signals from each of the other Bands in the normalization channel are divided by the largest Signal to obtain each Band's Normalization Factor. The Signal for each Band in the other channel is divided by the Normalization Factor of the Band in the same Lane.

- 1) Apply a Western Analysis to the image. Assign **only one** Band per Lane for the sample Lanes when normalizing the Signal for these Lanes.
- 2) Select **Lane** in the *Background* group.
- 3) Click the **Normalize** button and select **700 channel** from the drop-down menu.
- 4) Look at the *Normalized Signal* column in the *Western Bands* Table. The Bands in the Marker will show the text 'NaN'. The Bands in the Normalization Channel will show the value of the Signal of the Band with the largest Signal. The Bands in the other channel will show the value of the Signal of each Band divided by the Normalization Factor of the Band in the Normalization Channel in the same Lane.

Image Name	Channel	Lane Name	Name	Signal	Size	Area	Conc. Std.	Concentration	Normalized Signal
9999999_01	700	M1	B07	8.67E-1	25.0	560		NaN	NaN
9999999_01	700	M1	B08	1.36E0	20.0	616		NaN	NaN
9999999_01	700	L01	B01	1.95E1	59.6	748		NaN	1.95E1
9999999_01	700	L02	B01	1.78E1	60.2	748		NaN	1.95E1
9999999_01	700	L03	B01	1.65E1	60.2	816		NaN	1.95E1
9999999_01	700	L04	B01	1.82E1	60.7	748		NaN	1.95E1
9999999_01	700	L05	B01	1.07E1	61.2	748		NaN	1.95E1
9999999_01	700	L06	B01	1.50E1	61.2	680		NaN	1.95E1
9999999_01	700	L07	B01	1.46E1	61.8	680		NaN	1.95E1
9999999_01	700	L08	B01	7.32E0	62.4	680		NaN	1.95E1
9999999_01	800	L02	B01	4.32E-1	61.8	612		NaN	4.72E-1
9999999_01	800	L03	B01	4.79E-1	61.8	680		NaN	5.65E-1
9999999_01	800	L04	B01	5.06E-1	61.8	680		NaN	5.39E-1
9999999_01	800	L05	B01	5.17E-1	62.9	612		NaN	9.37E-1
9999999_01	800	L06	B01	6.82E-1	62.9	578		NaN	8.84E-1
9999999_01	800	L07	B01	7.40E-1	62.9	578		NaN	9.84E-1

Review the Western Lanes Table

Click on the **Western Lanes** tab at the bottom of the screen to view the Western Lanes Table. The Western Lanes Table shows the Lanes from the current single image.

Image Name	Channel	Lane Name	Sample
9999999_01	700	M1	
9999999_01	700	L01	
9999999_01	700	L02	
9999999_01	700	L03	
9999999_01	700	L04	
9999999_01	700	L05	
9999999_01	700	L06	
9999999_01	700	L07	
9999999_01	700	L08	
9999999_01	700	L09	
9999999_01	700	L10	
9999999_01	700	L11	
9999999_01	700	L12	
9999999_01	800	M1	
9999999_01	800	L01	
9999999_01	800	L02	

Note: The columns in the Western Lanes Table can be moved, added, deleted, sorted, and filtered in the same ways as the columns in the Images Table. Refer to [Chapter 10: Tables](#) for more information.

Review the Western Bands Table

- 1) Click the **Western Bands** tab at the bottom of the screen to view the Western Bands Table. The Western Bands Table shows the Bands from the current single image.

Image Name	Channel	Lane Name	Name	Signal	Size	Area	Conc. Std.	Concentration	Normalized Signal
9999999_01	700	M1	B01	5.90E-1	250	672		NaN	NaN
9999999_01	700	M1	B02	1.69E0	150	504		NaN	NaN
9999999_01	700	M1	B03	6.49E0	100	560		NaN	NaN
9999999_01	700	M1	B04	7.62E0	75.0	784		NaN	NaN
9999999_01	700	M1	B05	2.13E0	50.0	672		NaN	NaN
9999999_01	700	M1	B06	5.59E0	37.0	728		NaN	NaN
9999999_01	700	M1	B07	8.67E-1	25.0	560		NaN	NaN
9999999_01	700	M1	B08	1.36E0	20.0	616		NaN	NaN
9999999_01	700	L01	B01	1.95E1	59.6	748		NaN	1.95E1
9999999_01	700	L02	B01	1.78E1	60.2	748		NaN	1.95E1
9999999_01	800	L02	B01	4.32E-1	61.8	612		NaN	4.72E-1
9999999_01	700	L03	B01	1.65E1	60.2	816		NaN	1.95E1
9999999_01	800	L03	B01	4.79E-1	61.8	680		NaN	5.65E-1
9999999_01	700	L04	B01	1.82E1	60.7	748		NaN	1.95E1
9999999_01	800	L04	B01	5.06E-1	61.8	680		NaN	5.39E-1
9999999_01	700	L05	B01	1.07E1	61.2	748		NaN	1.95E1

Note: The columns in the Western Bands Table can be moved, added, deleted, sorted, or filtered in the same ways as the columns in the Images Table. Refer to [Chapter 10: Tables](#) for more information.

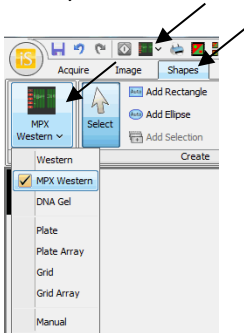
- 2) Notice the first eight Bands are in the Marker Lane (M1) and the values from the selected Marker are in the column **Size**. The rest of the Bands are assigned a Size in reference to the values of the Marker Bands.

Apply the MPX™ Western Analysis

Tutorial images are provided on the Image Studio CD in the **Image Examples** folder. The folder name for the MPX Western Image is '9999998_01'.

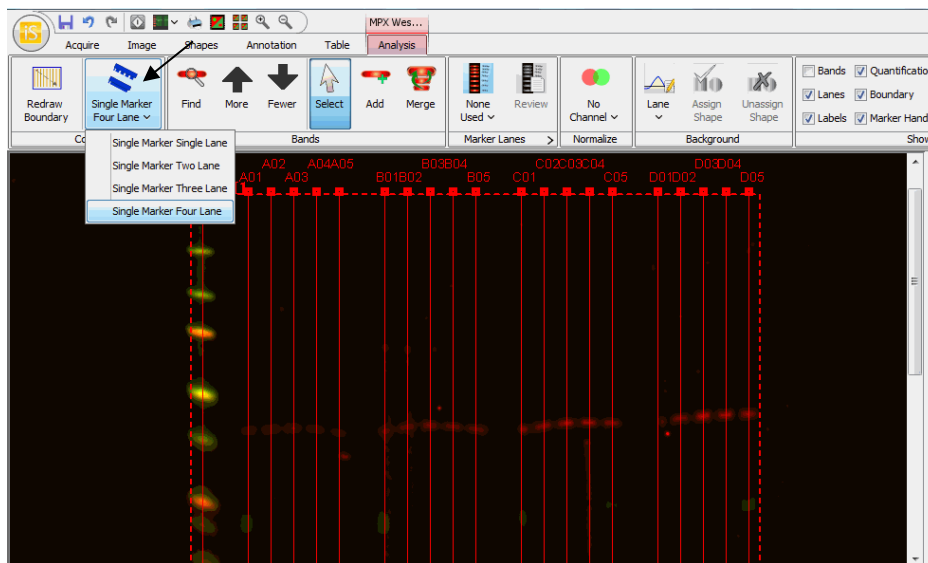
- 1) Import '9999998_01' from the **Image Examples** folder on the Image Studio software CD. Refer to [Chapter 3: Image Files](#) for more information.

- a. Click the *Application Menu* button and hover over **Import**. Click **Copy From (Import Acquisition)** in the *Import* menu.
 - b. Select the file '9999998_01' in the **Image Examples** folder on the Image Studio software CD. Click **Open** to display the image. The file will appear in the Images Table.
- 2) Adjust the look of the table in the **Image LUTs** on the right side of the screen. Refer to [Chapter 4: Image Display](#) for more information.
 - 3) Click the **Shapes** tab to view the *Shapes* ribbon.



- 4) Click **MPX Western** to open the *MPX Western Analysis* ribbon and apply the MPX™ Western analysis to the image, or click the quick launch button at the top of the window and select **MPX Western** from the drop-down menu.

Note: To apply the *MPX Western Analysis* automatically after image acquisition, click **MPX Western** in the *Setup* group on the *Acquire* ribbon when starting the acquisition.



Parameters from the most recent *MPX Western* analysis will be applied to the image.

- 5) Click **Select Comb for MPX Western** to open the drop-down menu. Click **Single Marker Four Lane** to apply a template with a single marker followed by four sets of five Lanes each.

- 6) Adjust the Boundary so the Lanes transect the image lanes. Refer to *Set the Boundary* in this chapter for more information.
- 7) Click *Select Marker Lanes Set* in the *Marker Lanes* group to view the drop-down menu. Click ***Two-Color Protein***. Refer to *Set the Marker* in this chapter for more information.
- 8) Click ***Find*** in the *Bands* group to find the Bands automatically. Refer to *Find the Bands Automatically* and *Edit the Bands Manually* in this chapter for more information.
- 9) Click ***Lane*** in the *Background* group to set the background subtraction. Refer to *Subtract the Background* in this chapter for more information.

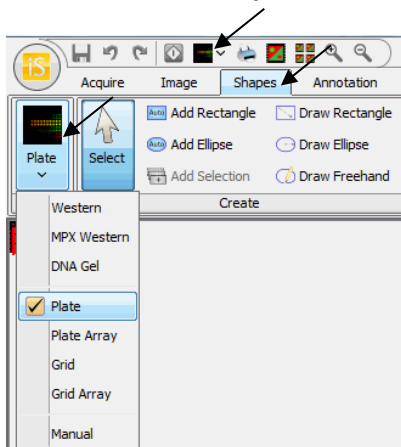
Chapter 7: Plate & Grid Analyses

The Plate & Grid Analyses place a pattern of shapes on the image to make data analysis faster and easier. Analyze microplates with the Plate analysis, or use the Plate Array analysis for analyzing patterns of shapes inside Plate Wells. Use the Grid Analysis for any pattern of shapes, or analyze a pattern of shapes inside another pattern with the Grid Array analysis. Following are detailed instructions for applying a Plate Analysis.

Apply a Plate Analysis

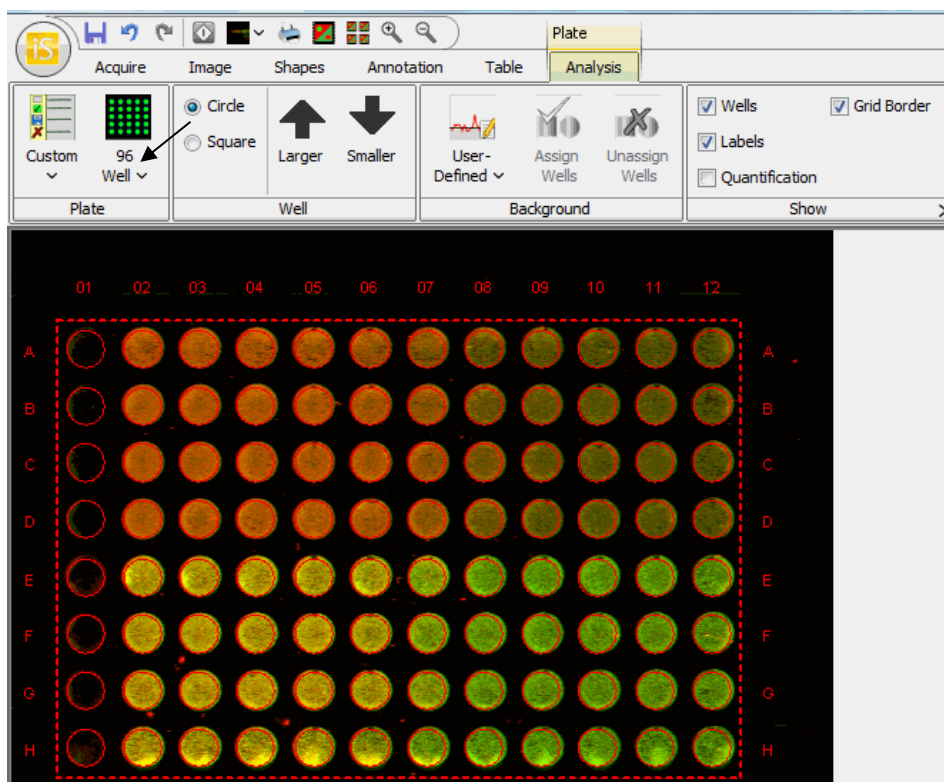
Tutorial images are provided on the Image Studio CD in the **Image Examples** folder. The folder name for the Plate Image is '9999997_01'.

- 1) Import '9999997_01' from the **Image Examples** folder on the Image Studio software CD. Refer to [Chapter 3: Image Files](#) for more information.
 - a. Click the **Application Menu** button and hover over **Import**. Click **Copy From (Import Acquisition)** in the **Import** menu.
 - b. Select the file '9999997_01' in the **Image Examples** folder on the Image Studio software CD. Click **Open** to display the image. The file will appear in the Images Table.
- 2) Adjust the look of the table in the **Image LUTs** on the right side of the screen. Refer to [Chapter 4: Image Display](#) for more information.
- 3) Click the **Shapes** tab to view the **Shapes** ribbon.



- 4) Click **Plate** to open the **Plate Analysis** ribbon and apply the Plate analysis to the image. Or, click the quick launch button at the top of the window and select **Plate** from the drop-down menu.

Note: To apply the Plate Analysis automatically after image acquisition, click **Plate** in the Setup group on the Acquire ribbon when starting the acquisition.



- 5) Select **96 Well** from the drop-down menu and **Circle** for the shape type.

Set the Grid Border

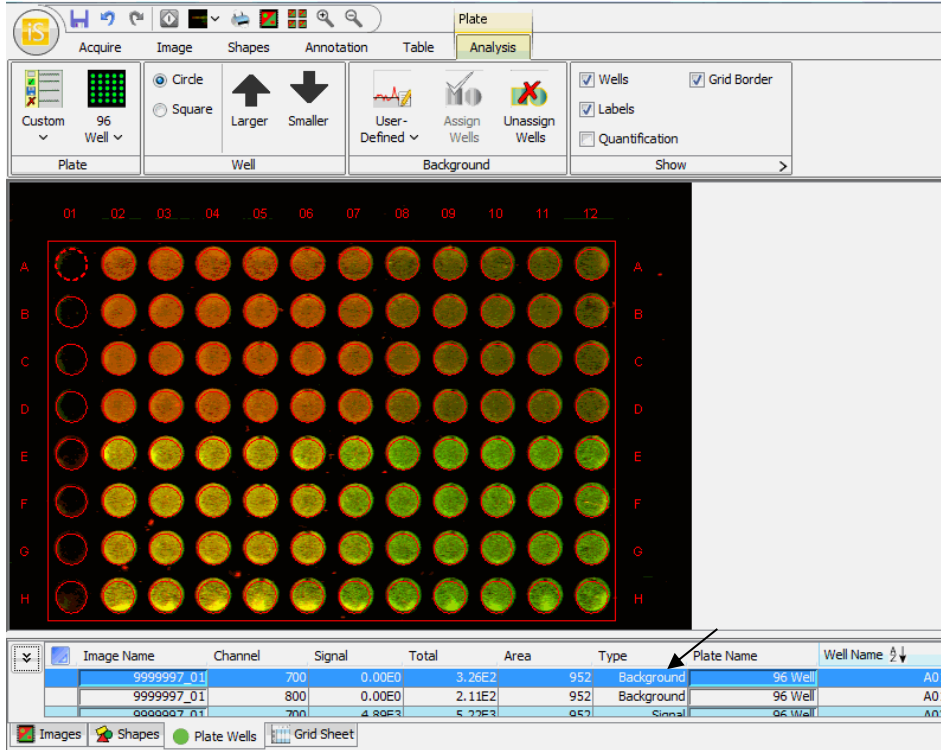
The Grid Border is the bounding box that surrounds the Wells on the image.

- 1) To view the Grid Border on the image, click **Grid Border** in the *Show* group to enable the check box next to it. To hide the Grid Border, click to disable the check box.
- 2) To select the Grid Border, click on it once. The line toggles to a dashed line, indicating it can be adjusted.
- 3) To adjust the applied Grid Border, hover over the corners or the midpoints of the sides to display a double-sided arrow. Drag the arrow to move a corner or side.
- 4) To move the entire Grid Border, drag the four-pointed cursor that appears inside the Grid Border.
- 5) To set the Grid Border, click on a different spot on the screen. The Grid Border line toggles back to solid.

Set the Background Shape

- 1) Select **User-Defined** from the drop-down menu on the first button in the *Background* group.
- 2) Define a Well in the Plate as a Background Shape.

- a. Click on the Well once and the circle becomes dashed, indicating the Well is selected.
 - b. Click **Assign Well** in the *Background* group.
- 3) The Well is assigned as a Background Shape in the Plate Wells Table.

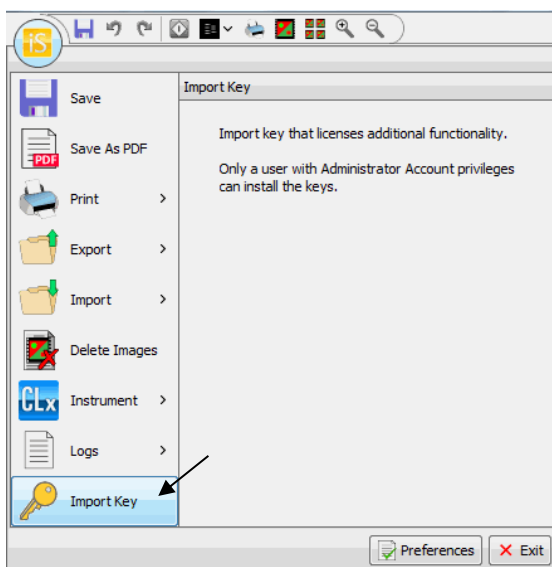


Chapter 8: In-Cell Western™ Analysis

Import the In-Cell Western key to add this analysis to Image Studio.

Import the In-Cell Western Key

- 1) If your computer operating system is Windows® Vista or Windows 7, start the application by right clicking on the Image Studio icon and select “Run as Administrator.”
- 2) Click the Application Menu Button and click **Import Key**.

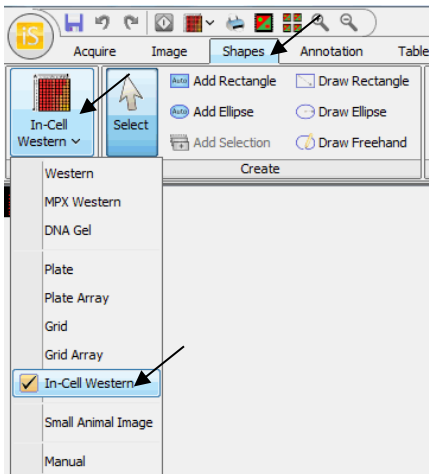


- 3) Browse to the In-Cell Western key file and click **Open**.
- 4) Click **OK** in the pop-up window and restart Image Studio software.

Apply an In-Cell Western Analysis

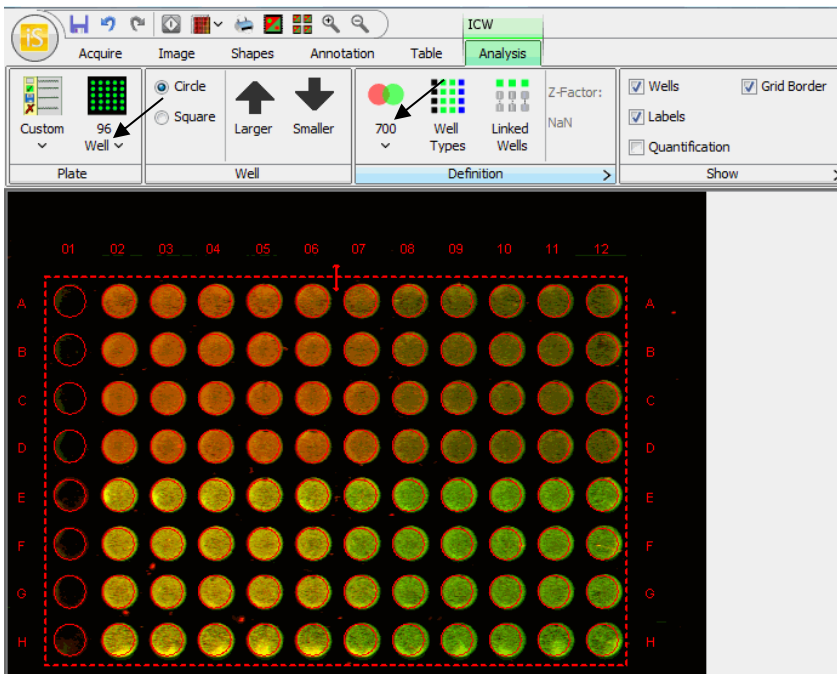
Tutorial images are provided on the Image Studio CD in the **Image Examples** folder. The folder name for the Plate Image is '9999997_01'.

- 1) Import '9999997_01' from the **Image Examples** folder on the Image Studio software CD. Refer to [Chapter 3: Image Files](#) for more information.
 - a. Click the **Application Menu** button and hover over **Import**. Click **Copy From (Import Acquisition)** in the **Import** menu.
 - b. Select the file '9999997_01' in the **Image Examples** folder on the Image Studio software CD. Click **Open** to display the image. The file will appear in the Images Table.
- 2) Adjust the look of the table in the **Image LUTs** on the right side of the screen. Refer to [Chapter 4: Image Display](#) for more information.
- 3) Click the **Shapes** tab to view the **Shapes** ribbon.



- 4) Click **In-Cell Western** to open the *In-Cell Western Analysis* ribbon and apply the In-Cell Western™ analysis to the image, or click the quick launch button at the top of the window and select **In-Cell Western** from the drop-down menu.

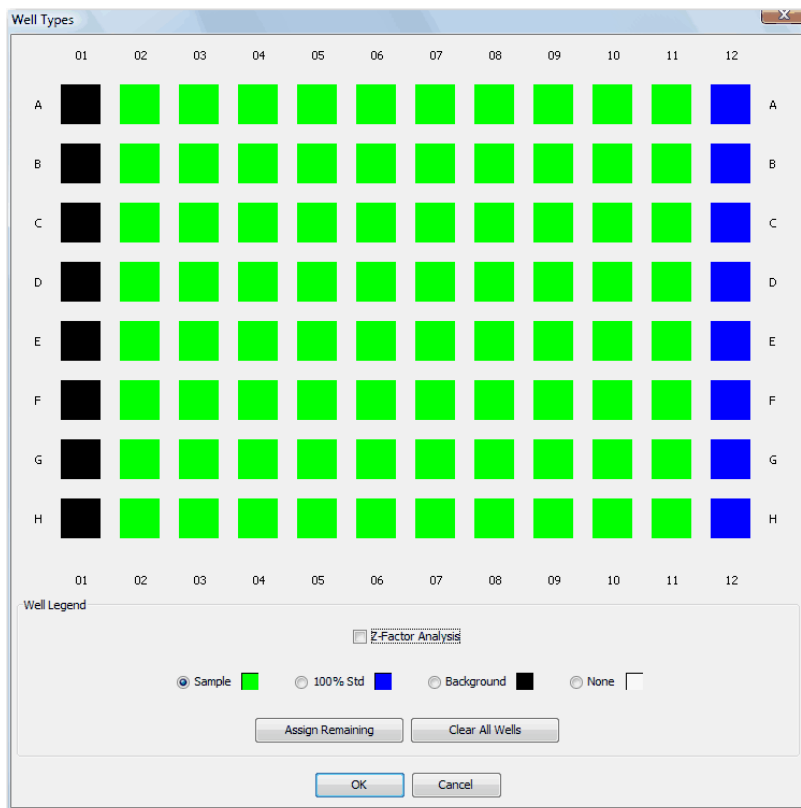
Note: To apply the In-Cell Western Analysis automatically after image acquisition, click **In-Cell Western** in the Setup group on the Acquire ribbon when starting the acquisition.



- 5) Select **96 Well** from the drop-down menu and **Circle** for the shape type.
- 6) Select **700** as the Normalization Channel from the drop-down menu.

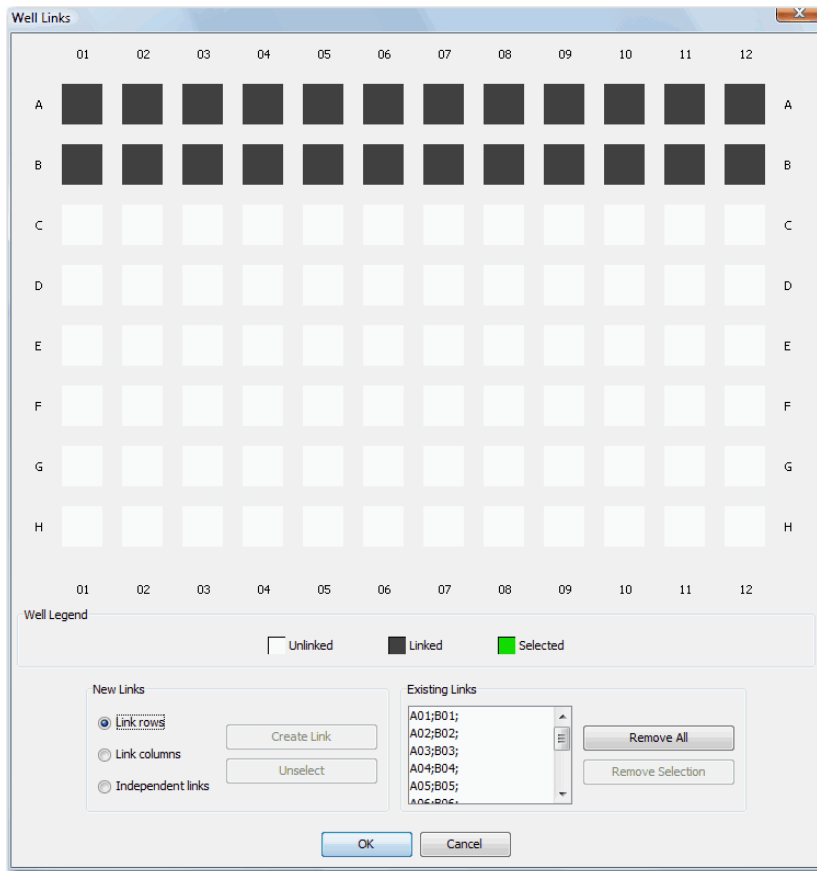
Assign Well Types

- 1) Click **Well Types** in the *Definition* group to open the *Well Types* menu.
- 2) Assign the Background Wells.
 - a. Click **Background** in the Well Legend at the bottom of the *Well Types* menu.
 - b. Drag a bounding box through the first column of Wells.
- 3) Assign the 100% Standard Wells.
 - a. Click **100% Std** in the Well Legend at the bottom of the *Well Types* menu.
 - b. Drag a bounding box through the last column of Wells.
- 4) Assign the Sample Wells.
 - a. Click **Sample** in the Well Legend at the bottom of the *Well Types* menu.
 - b. Drag a bounding box through the Wells between the first and last column.
- 5) Click **OK**.



Assign Linked Wells

- 1) Click **Linked Wells** in the *Definition* group to open the *Well Links* menu.
- 2) Drag a bounding box through the top two rows of Wells.
- 3) Select **Link Rows** and click **Create Link** in the New Links at the bottom of the *Well Links* menu.
- 4) Click **OK**.



ICW Wells Table

Click the *ICW Wells* tab at the bottom of the window to open the ICW Wells table.

Image Name	Channel	Spot Name	Total	ICW Type	ICW Relative	ICW % Response
9999997_01	800	A01	2.20E2	Background	0.00E0	0.00
9999997_01	800	A02	4.26E3	Sample	1.26E1	16.9
9999997_01	800	A03	4.41E3	Sample	1.27E1	17.0
9999997_01	800	A04	4.25E3	Sample	1.30E1	17.3
9999997_01	800	A05	4.22E3	Sample	1.37E1	18.4
9999997_01	800	A06	4.45E3	Sample	1.62E1	21.7
9999997_01	800	A07	4.22E3	Sample	2.65E1	35.5
9999997_01	800	A08	4.29E3	Sample	5.29E1	70.8
9999997_01	800	A09	4.96E3	Sample	7.13E1	95.4
9999997_01	800	A10	4.12E3	Sample	7.35E1	98.4
9999997_01	800	A11	4.15E3	Sample	7.89E1	106
9999997_01	800	A12	3.74E3	100% Std	7.47E1	100
9999997_01	800	B01	2.21E2	Background	0.00E0	0.00
9999997_01	800	B02	5.05E3	Sample	1.26E1	16.9

Grid Sheet

Click *Grid Sheet* at the bottom of the screen to view the values for each of the Wells in a format similar to a plate layout. Select the field to view from the drop-down menu.

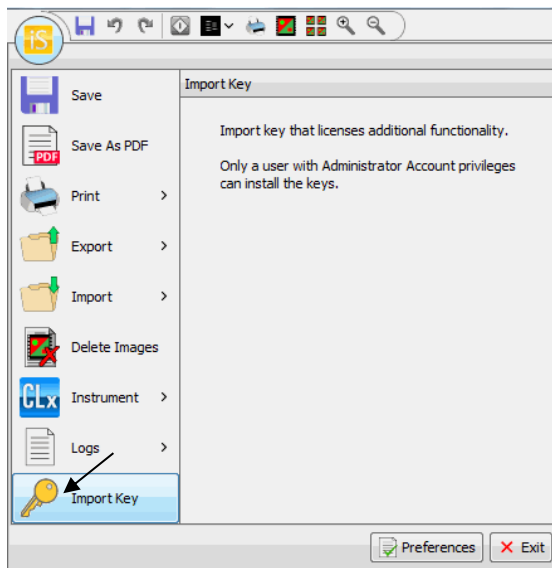
	01	02	03	04	05	06	07	08	09	10	11	12
A	0.00E0	1.26E1	1.27E1	1.30E1	1.37E1	1.62E1	2.65E1	5.29E1	7.13E1	7.35E1	7.89E1	7.47E1
B	0.00E0	1.26E1	1.27E1	1.30E1	1.37E1	1.62E1	2.65E1	5.29E1	7.13E1	7.35E1	7.89E1	7.47E1
C	0.00E0	1.26E1	1.27E1	1.30E1	1.37E1	1.62E1	2.65E1	5.29E1	7.13E1	7.35E1	7.89E1	7.47E1
D	0.00E0	1.26E1	1.27E1	1.30E1	1.37E1	1.62E1	2.65E1	5.29E1	7.13E1	7.35E1	7.89E1	7.47E1
E	0.00E0	1.26E1	1.27E1	1.30E1	1.37E1	1.62E1	2.65E1	5.29E1	7.13E1	7.35E1	7.89E1	7.47E1
F	0.00E0	1.26E1	1.27E1	1.30E1	1.37E1	1.62E1	2.65E1	5.29E1	7.13E1	7.35E1	7.89E1	7.47E1
G	0.00E0	1.26E1	1.27E1	1.30E1	1.37E1	1.62E1	2.65E1	5.29E1	7.13E1	7.35E1	7.89E1	7.47E1
H	0.00E0	1.26E1	1.27E1	1.30E1	1.37E1	1.62E1	2.65E1	5.29E1	7.13E1	7.35E1	7.89E1	7.47E1

Chapter 9: Small Animal Image Analysis

Import the Small Animal Image Analysis key to add this analysis to Image Studio.

Import the Small Animal Image Analysis Key

- 1) If your operating system is Windows® Vista or Windows 7, start the application by right clicking on the Image Studio icon and select “Run as Administrator.”
- 2) Click the Application Menu Button and click **Import Key**.



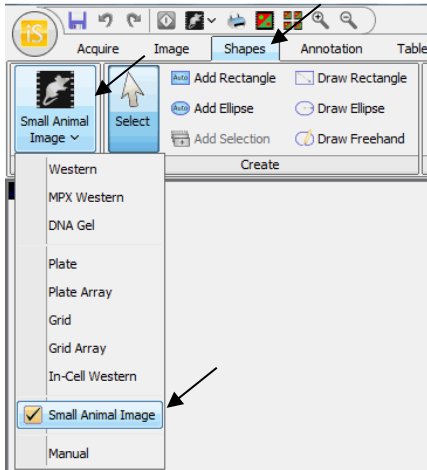
- 3) Browse to the Small Animal Image Analysis key file and click **Open**.
- 4) Click **OK** in the pop-up window and restart Image Studio software.

Apply a Small Animal Image Analysis

*Tutorial images are provided on the Image Studio CD in the **Image Examples** folder. The folder name for the Small Animal Image is '0000192_01'.*

- 1) Import '0000192_01' from the **Image Examples** folder on the Image Studio software CD. Refer to [Chapter 3: Image Files](#) for more information.
 - a. Click the *Application Menu* button and hover over **Import**. Click **Copy From (Import Acquisition)** in the *Import* menu.
 - b. Select the file '0000192_01' in the **Image Examples** folder on the Image Studio software CD. Click **Open** to display the image. The file will appear in the Images Table.

- 2) Adjust the look of the table in the **Image LUTs** on the right side of the screen. Refer to [Chapter 4: Image Display](#) for more information.
- 3) Click the **Shapes** tab to view the **Shapes** ribbon.



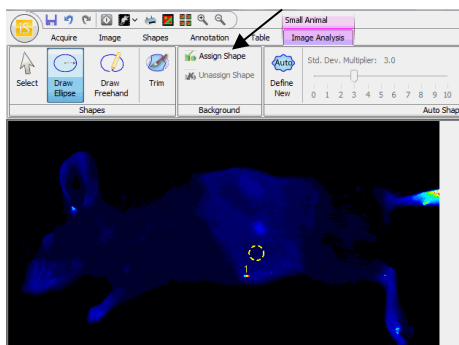
- 4) Click **Small Animal Image** to open the **Small Animal Image Analysis** ribbon and apply the Small Animal Image analysis to the image. Or, click the quick launch button at the top of the window and select **Small Animal Image** from the drop-down menu.

Note: To apply the Small Animal Image Analysis automatically after image acquisition, click **Small Animal Image** in the Setup group on the Acquire ribbon when starting the acquisition.

Set the Background Shape

Draw a shape on the animal and designate it as the background shape. Draw the shape with an area similar to the area of fluorescence. For example, if the area of fluorescence is on a shaved part of the mouse, draw the background shape on a shaved part of the mouse.

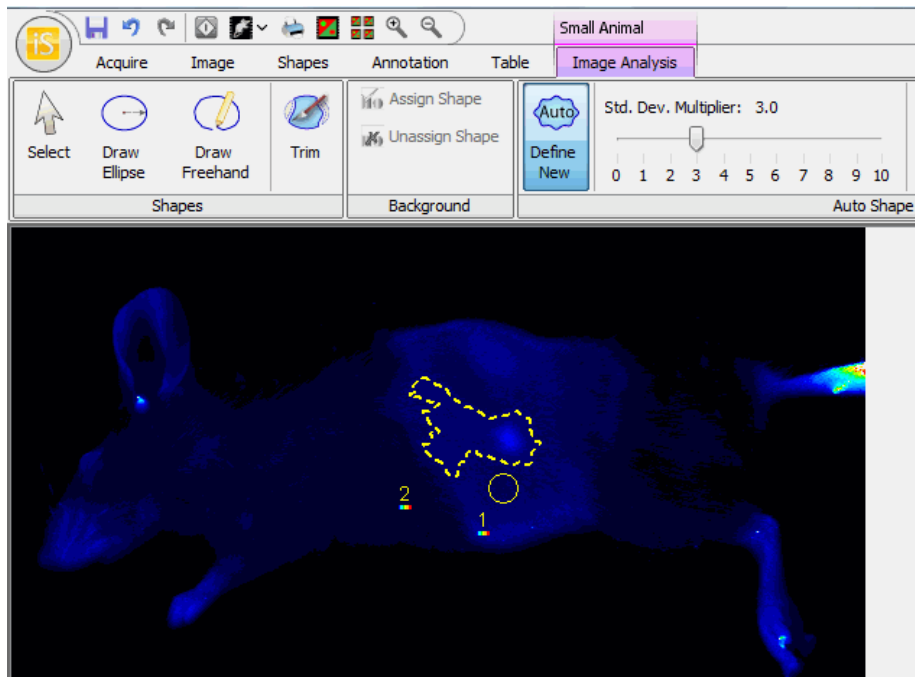
- 1) Click **Draw Ellipse** in the **Shapes** group.
- 2) Drag a shape onto the image as shown below.



- 3) Click **Assign Shape** in the **Background** group to assign the shape as the background.

Use the Auto Shape Tool

- 1) Click **Define New** in the *Auto Shape* group.
- 2) Click the center of the area of fluorescence.

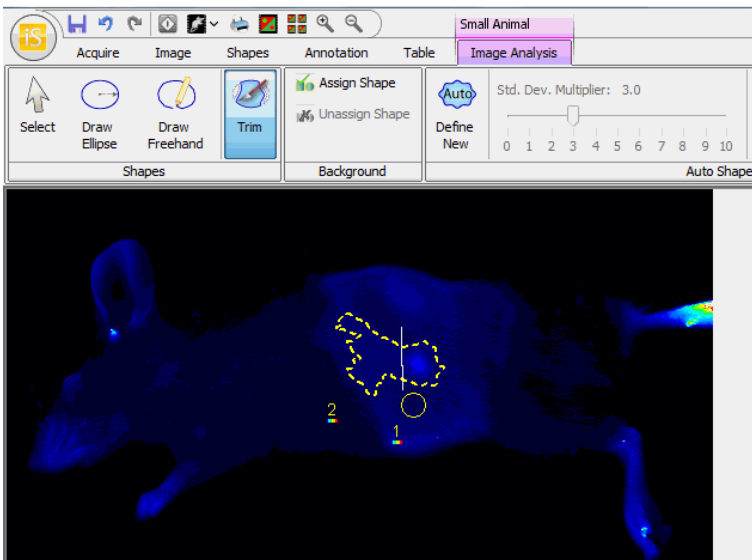


- 3) Click **Select** in the *Shapes* group to toggle back to the selection tool.
- 4) Click anywhere on the image to set the Auto Shape indicated by a solid line.

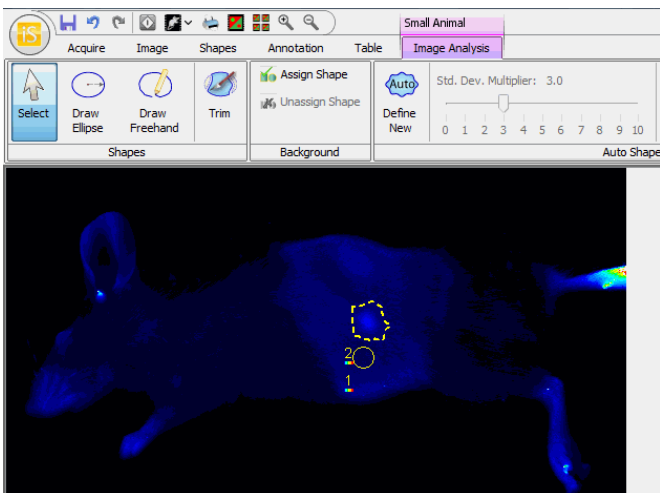
Note: The feature created by the Auto Shape tool can be changed interactively by adjusting the **Std. Dev. Multiplier** in the Auto Shape group. In general, larger **Std. Dev. Multipliers** increase the threshold, reducing the size of the feature on the image by excluding more pixels of lower intensity. Similarly, decreasing the **Std. Dev. Multiplier** increases the size of the feature on the image.

Use the Trim Tool

- 1) Click Shape 2 to select it. The dashed line indicates it is selected.
- 2) Click **Trim** in the *Shapes* group.
- 3) Drag a line that transects the shape in two places as shown below.



- 4) Click on the image to the left of the line to remove that side of the shape as shown below.



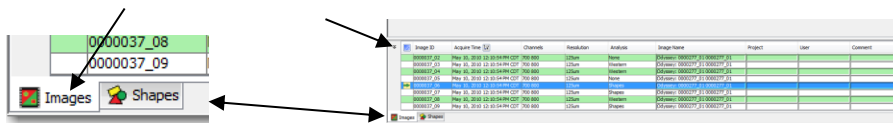
- 5) Click anywhere on the image to set the shape indicated by a solid line.

Chapter 10: Tables

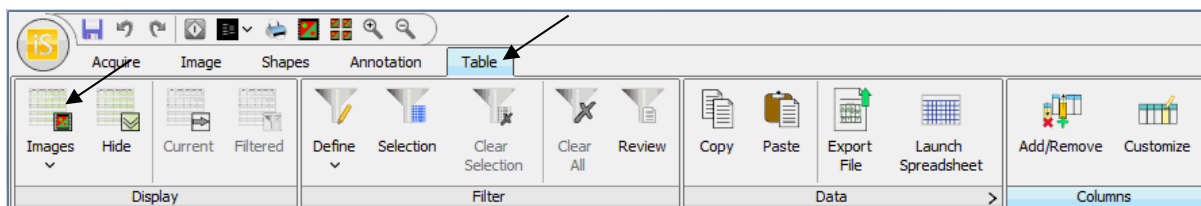
Image data are stored in the folder selected as the Work Area when Image Studio is opened. The data are easily accessed in the Tables below the image window. In the Images Table, each row represents one image. Various fields in that row contain identifying information about the image. These fields are grouped as columns that can be moved, added, deleted, sorted, or filtered.

Images Table

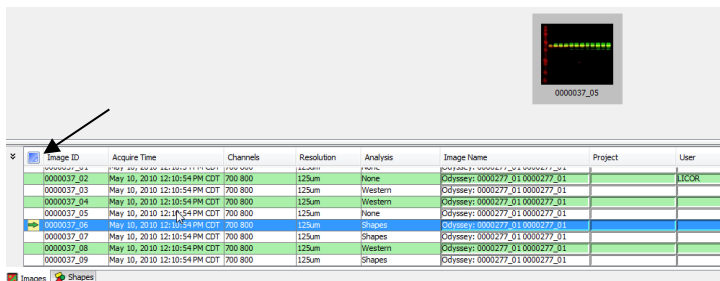
- 1) Click the **Images** tab at the bottom of the screen to show the Images Table. Click the double arrow to hide the Table from view.



Or, click **Table** to open the **Table** ribbon and select **Images** from the drop-down menu on the first button in the **Display** group. Click **Hide** to hide the Table from view.



- 2) Hover the mouse over any row to display a thumbnail picture of that image.



- 3) Click any cell to select the entire row and display that image in the window.
- 4) Click the corner button to select all of the images or press **Ctrl+A** on the keyboard.
- 5) Right-click any cell to view a menu with options that include copy an image, delete images, or view the properties of an image.
- 6) To move a column, drag the column header to the new location.

User	Project	Comment

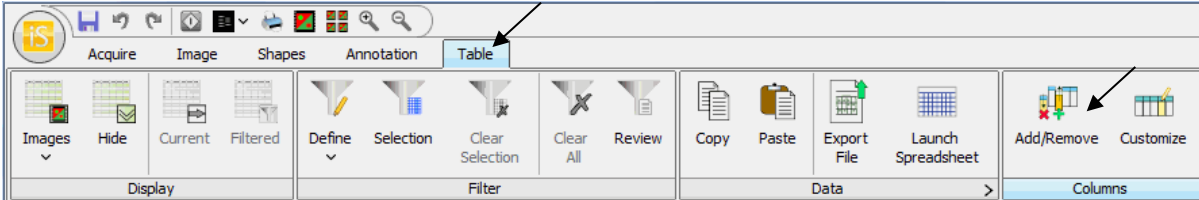
The fields in each row contain information about the image and are organized as columns across all of the images. Some of the columns are properties of the image and cannot be edited. Examples of these columns include Image ID, Acquire Time, Channels, Resolution, and Instrument Name. Other columns can be edited with information for that image.

- 7) To edit a field, double-click on the cell so that a cursor appears. If a cursor does not appear, that field cannot be edited.
- 8) Add text where the cursor appears. Press *Enter* when complete.

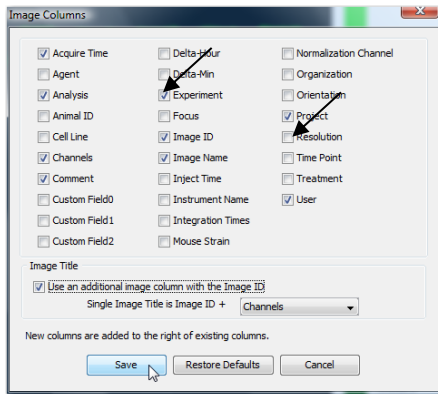
Note: To easily find images later, enter identifying information in one or more fields after acquiring an image.

Add or Remove a Column

- 1) Click the **Table** tab to view the *Table* ribbon.
- 2) Click **Add/Remove** in the *Columns* group.



- 3) To add the *Experiment* column, click the column description to enable the check box, indicating that column will appear in the table.
- 4) To remove the *Resolution* column, click the column description to disable the check box and remove that column from the table.

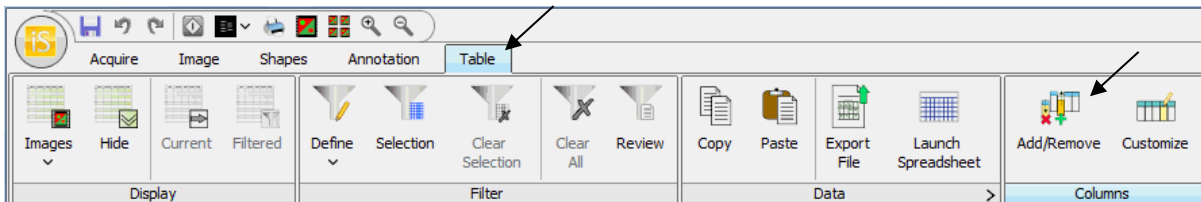


5) Click **Save**. The table now appears as shown below.

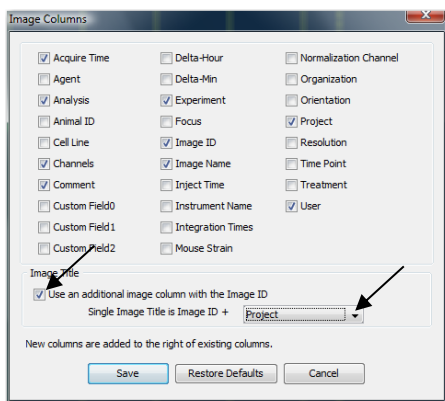
Image ID	Acquire Time	Channels	Analysis	Image Name	User	Project	Comment	Experiment
0000037_06	May 10, 2010 12:10:54 PM CDT	700 800	Shapes	Odyssey: 0000277_01 0000277_01				
0000037_07	May 10, 2010 12:10:54 PM CDT	700 800	Shapes	Odyssey: 0000277_01 0000277_01				
0000037_09	May 10, 2010 12:10:54 PM CDT	700 800	Shapes	Odyssey: 0000277_01 0000277_01				
0000037_01	May 10, 2010 12:10:54 PM CDT	700 800	None	Odyssey: 0000277_01 0000277_01				
0000037_02	May 10, 2010 12:10:54 PM CDT	700 800	None	Odyssey: 0000277_01 0000277_01				
0000037_05	May 10, 2010 12:10:54 PM CDT	700 800	None	Odyssey: 0000277_01 0000277_01				
0000038_01	May 14, 2010 9:11:07 AM CDT	700 800	None	Odyssey: 0000002_01 0000002_01				
0000039_01	May 14, 2010 3:10:33 PM CDT	700 800	None	Odyssey: 0000002_01 0000002_01				

Edit the Image Title

- 1) Click the **Table** tab to view the **Table** ribbon.
- 2) Click **Add/Remove** in the **Columns** group.



3) Click *Use an additional image column with the Image ID* to enable the check box.

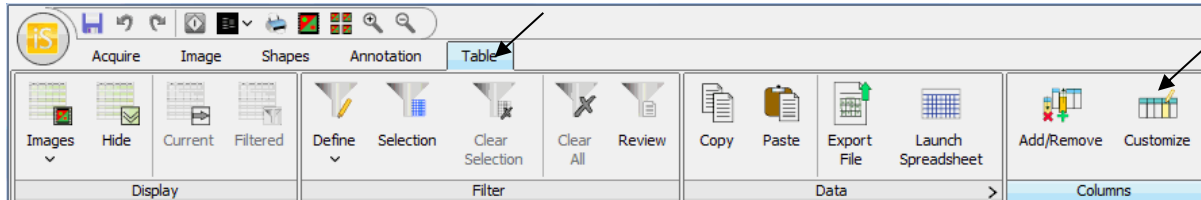


4) Click the down arrow in the selection box to choose a column to add to the Image ID to form the Image Title.

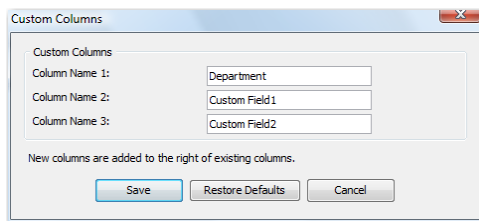
- 5) Click **Project**. Text in the *Project* column will be added to the title of every Image.
- 6) Click **Save**.

Add Custom Columns

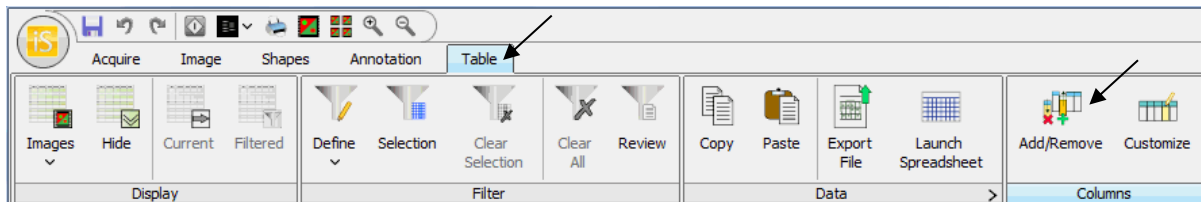
- 1) Click the **Table** tab to view the *Table* ribbon.
- 2) Click the **Customize** icon in the *Columns* group to view the *Custom Columns* menu.



- 3) In the *Custom Columns* menu, replace *Custom Field0* with *Department*.



- 4) Click **Save**.
- 5) To add this custom column to the table, click the **Table** tab to view the *Table* ribbon.
- 6) Click **Add/Remove** in the *Columns* group.



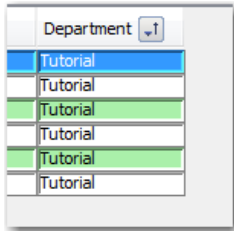
- 7) To add the *Department* column, click the column description to enable the check box, indicating that column will be in the table.
- 8) Click **Save**. The *Department* column will be added to the right of the existing columns.

Paste Data in a Column

Use **Paste** in the *Data* group to quickly fill a column with information.

- 1) Double-click the first cell in the *Department* column so that a cursor appears in the cell.
- 2) Type 'Tutorial' at the cursor position using the keyboard.
- 3) Drag the mouse over 'Tutorial' to highlight it. The cursor should still appear in the cell.
- 4) Press **Ctrl+C** on the keyboard to copy the data to the clipboard.

- 5) Drag the mouse over the cells in the *Department* column (or press the *Ctrl* key while clicking each cell once) to select which cells to fill with 'Tutorial'.
- 6) Click **Paste** in the *Data* group, press *Ctrl+V* on the keyboard, or use the right-click menu to paste the data in the selected cells.



'Tutorial' added to cells in Department column.

Paste Data from a Spreadsheet to a Column

- 1) In a spreadsheet, select data and press *Ctrl+C* on the keyboard to copy the data to the clipboard.
- 2) In the Table, drag the mouse over the cells in the *Department* column (or press the *Ctrl* key while clicking each cell once) to select which cells in a column to place the data.
- 3) Click **Paste** in the *Data* group, press *Ctrl+V* on the keyboard, or use the right-click menu to paste the data in the selected cells.

Copy Data to a Spreadsheet

Use **Copy** in the *Data* group to quickly copy rows to a spreadsheet.

- 1) Drag the mouse over the rows or press the *Ctrl* key while clicking on each row once to select which rows to copy. To copy all rows, press *Ctrl+A* on the keyboard.
- 2) Click **Copy** in the *Data* group, press *Ctrl+C* on the keyboard, or use the right-click menu to copy the data to the clipboard.
- 3) In a spreadsheet, press *Ctrl+V* on the keyboard to paste the data in the selected cells.

Note: The column headings will be pasted above the data.

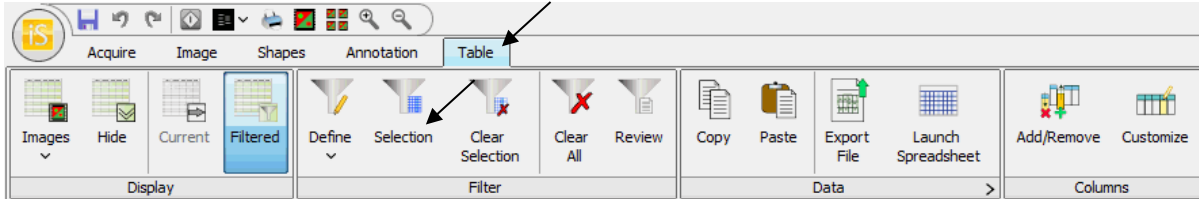
Sort by Column

- 1) To sort by any column, click once on the header. The table will be sorted by that column.
Note: No change will be made if the fields in a column are all the same.
- 2) To change the direction of sorting, click the small box in the header with the up or down arrow. Or, right-click on the header and select **Sort Ascending** or **Sort Descending**.

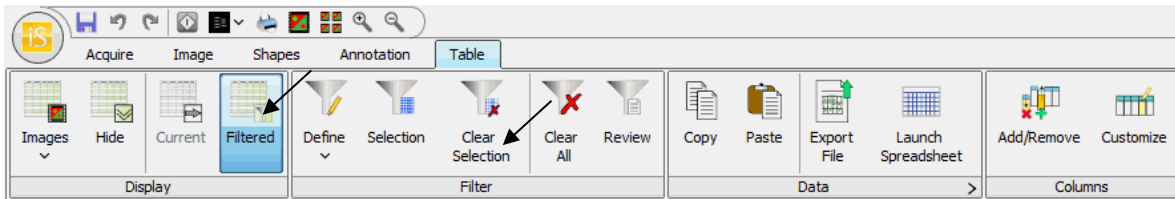
Image ID	Acquire Time	Channels	Analysis	Image Name
0000037_06	May 10, 2010 12:10:54 PM CDT	700 800	Shapes	Odyssey: 0000277_01
0000037_07	May 10, 2010 12:10:54 PM CDT	700 800	Shapes	Odyssey: 0000277_01
0000037_09	May 10, 2010 12:10:54 PM CDT	700 800	Shapes	Odyssey: 0000277_01
0000037_01	May 10, 2010 12:10:54 PM CDT	700 800	None	Odyssey: 0000277_01
0000037_02	May 10, 2010 12:10:54 PM CDT	700 800	None	Odyssey: 0000277_01
0000037_05	May 10, 2010 12:10:54 PM CDT	700 800	None	Odyssey: 0000277_01
0000038_01	May 14, 2010 9:11:07 AM CDT	700 800	None	Odyssey: 0000002_01
0000039_01	May 14, 2010 3:10:33 PM CDT	700 800	None	Odyssey: 0000002_01

Filter the Table Manually

- 1) To manually choose images, drag to highlight the rows to select the desired images. Or, select the desired images by pressing *Ctrl* on the keyboard and clicking once on each row.
- 2) Click the **Table** tab to choose the *Table* ribbon.



- 3) Click **Selection** to show only the selected images in the table. The **Filtered** button in the *Display* group will be highlighted.
- 4) Click the **Filtered** button to toggle between showing only the selected images and showing all images in the table.

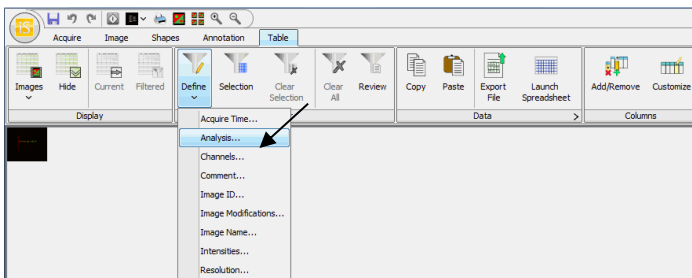


- 5) To remove the filter, click **Clear Selection** to remove only the Selection filter, or **Clear All** to remove all filters currently applied.

Define Filters

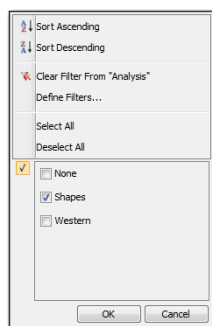
One or more defined filters can be applied to the table to show only the images of interest.

- 1) Click the **Table** tab to choose the *Table* ribbon.
- 2) Click **Define** to view the drop-down menu of all of the column headings. Select **Analysis** to filter using the *Analysis* column.



Note: *Right-click on any column header in the Table to drop down a menu for that column.*

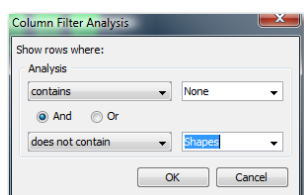
- 3) Deselect **None** and **Western** by disabling their respective check boxes.



4) Click **OK**. Only images with *Shapes* in the *Analysis* column will appear in the Table.

For more Filter Options...

- 1) Click **Define Filters...** in the *Filter* menu.
- 2) Click one of the filtering phrases to show the *Column Filter Analysis* menu.
- 3) Select filtering phrases from the drop-down menu by clicking on the down arrows next to each box, or type your own phrase.

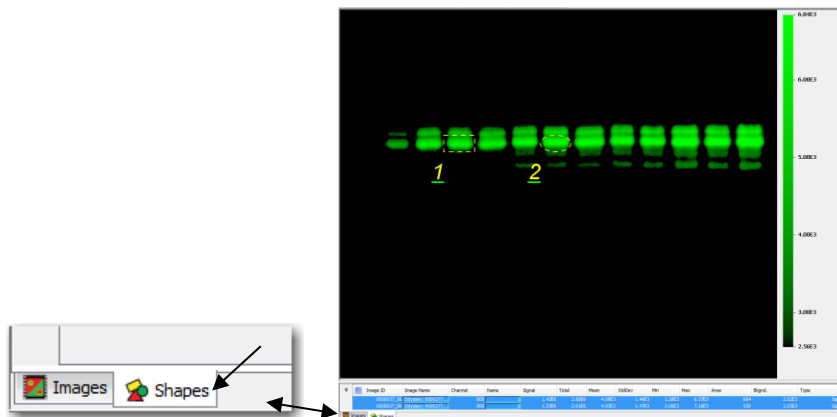


4) Click **OK**.

Note: If you close Image Studio, your filtering criteria are preserved.

View the Shapes Table

- 1) Click the **Shapes** tab in the Table to view the data for each shape.



- 2) The default columns are defined below.
 - a. *Image Name*- Original acquisition name
 - b. *Channel*- 700, 800, or Chemi

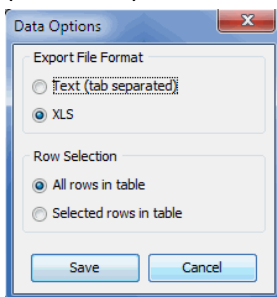
- c. *Name*- Shapes are assigned a number in sequential order. Double-click on the number in the column to edit the shape name.
 - d. *Signal*- The Signal is the sum of the individual pixel intensity values (**Total**) for a shape minus the product of the average intensity values of the pixels in the background (**Bkgnd.**) and the total number of pixels enclosed by the shape.
 - e. *Total*- Sum of the individual pixel intensities for the shape
 - f. *Area*- Total number of pixels enclosed by a shape
 - g. *Bkgnd.*- Designated background subtraction
 - h. *Type*- Signal or Background
 - i. *Conc. Std.*- Value of the concentration defined for this shape
 - j. *Concentration*- Calculated value of the concentration based on the standards for the shape
- 3) Move, add, delete, sort, and filter the columns in the same way as in the Images Table.

Exporting Data

Use **Export File** or **Launch Spreadsheet** in the Data group to prepare or open an '.xls' file with the selected data.

Export File

- 1) Click the arrow (>) on the Data group to view the *Data Options* menu. Choose the file format (text or xls) and whether to export all rows or selected rows. Click **Save**.



- 2) In the Table ribbon, click **Export File** to open the *Table Export* menu.
- 3) Select the folder destination. Click **Save**.
- 4) The file will be saved in the selected folder.

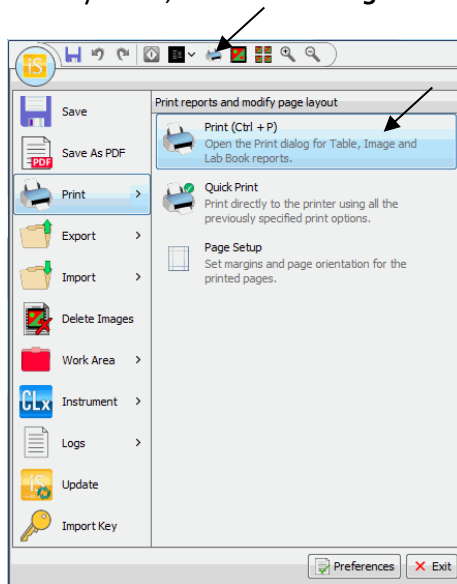
Launch Spreadsheet

- 1) Click the arrow (>) on the Data group to view the *Data Options* menu (see Step 1 above). Select to either export all rows or selected rows. Click **Save**. If necessary, select the rows in the table to export.
- 2) In the Table ribbon, click **Launch Spreadsheet** to open the *Launch Spreadsheet* menu.
- 3) Select the folder destination. Click **Save**.
- 4) An application that uses these types of files will open with the selected data and corresponding column headings.

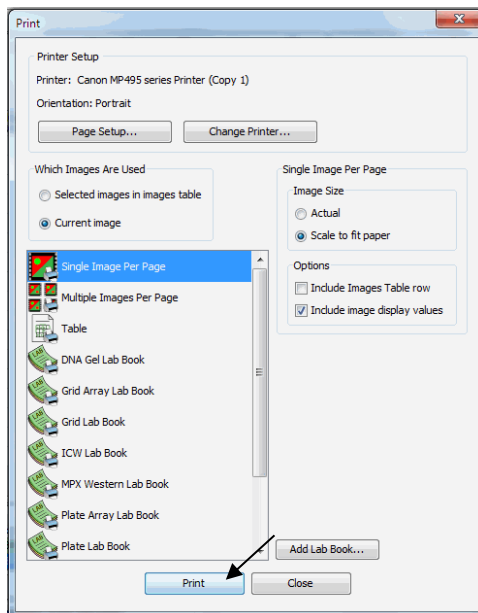
Chapter 11: Print Images and Create Reports

Print an Image

- 1) To open the *Print* menu, click the printer icon in the quick access tool bar, press *Ctrl+P* on the keyboard, or click the **Image Studio Application Button**, hover over **Print**, and click **Print**.

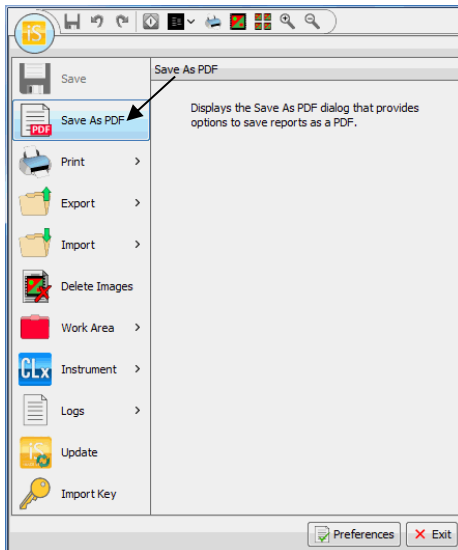


- 2) Click **Print** to print the currently displayed image.

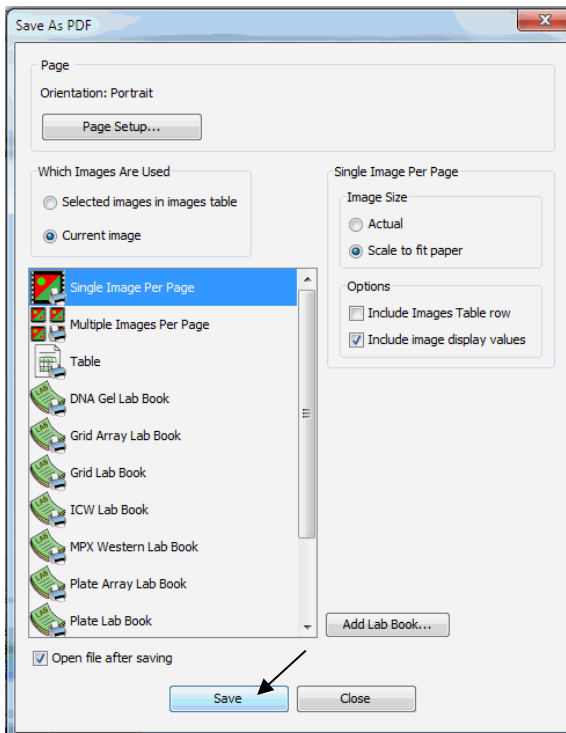


Save an Image as a PDF File

- 1) Click the **Image Studio Application Button** and select **Save As PDF**.

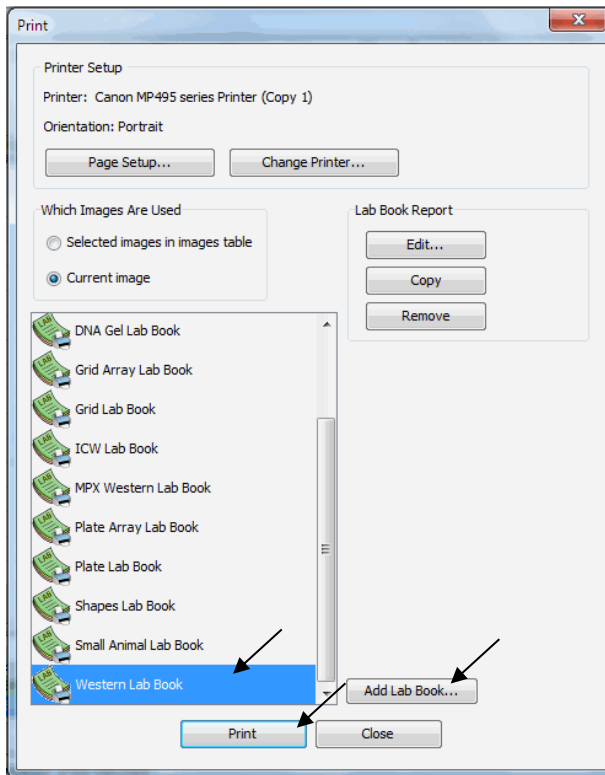


- 2) Click **Save** to save the current image in pdf format.

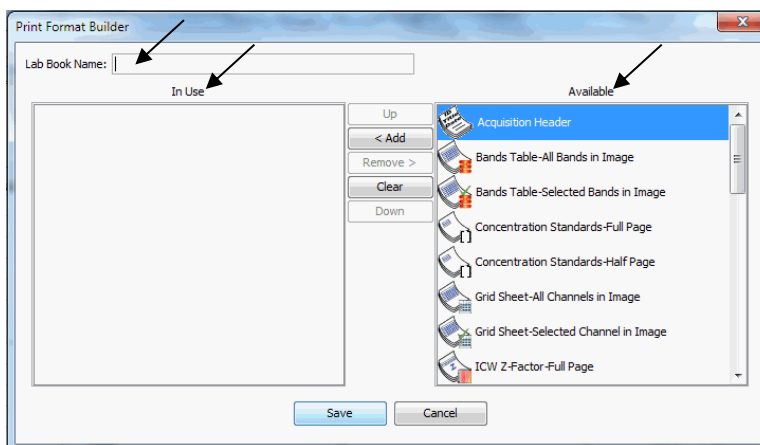


Print a Report

In the *Print* menu, select **Western Lab Book** and click **Print** to print the default Western report for the current image.



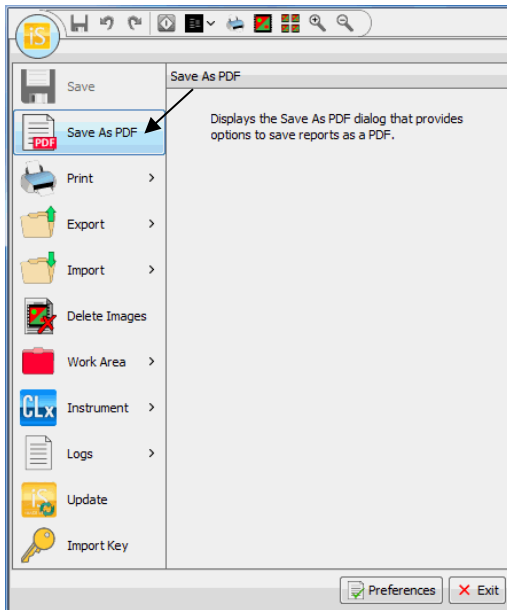
To create a custom report, click **Add Lab Book...** to open the **Print Format builder** menu.



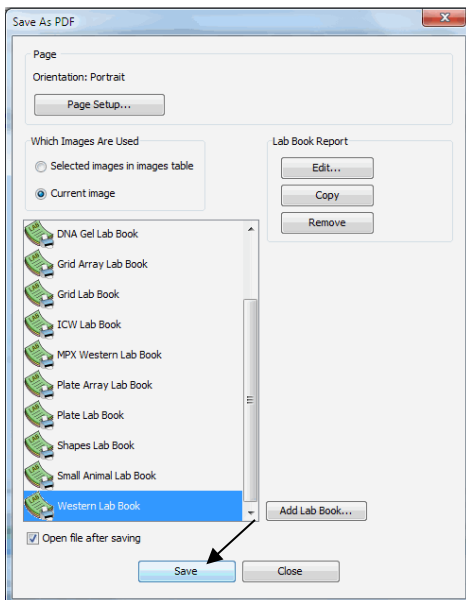
- 1) Type a report name in the *Lab Book Name:* field.
- 2) Items that are available to add to the report appear under *Available* in the right-hand column. Select an item and click **Add** to move it to the *In Use* column on the left.
- 3) Click **Save** to include the items in the *In Use* column in the report.
- 4) In the *Print* menu, click **Print** to print the custom report for the current image.

Save a Report as a PDF File

- 1) Click the **Image Studio Application Button** and select **Save As PDF**.



- 2) In the **Save as PDF** dialog box, select **Western Lab Book** and click **Save** to save the default Western report for the current image.



To create a custom report, click **Add Lab Book...** to open the **Print Format builder** menu. Follow the example above to create a custom report. Click **Save**.