

# Technical Note

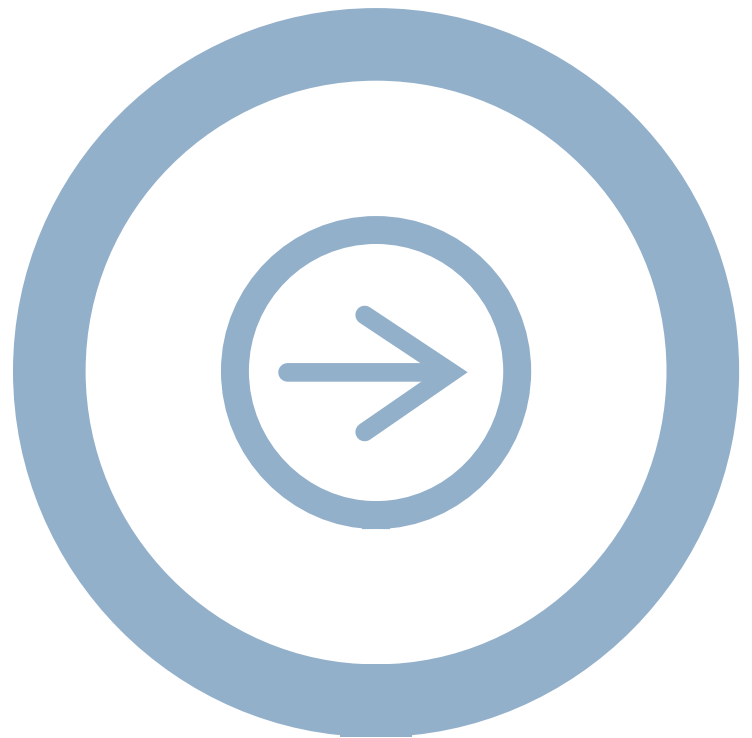
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## How to Use the Image Studio™ Software Small Animal Image Analysis

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.*



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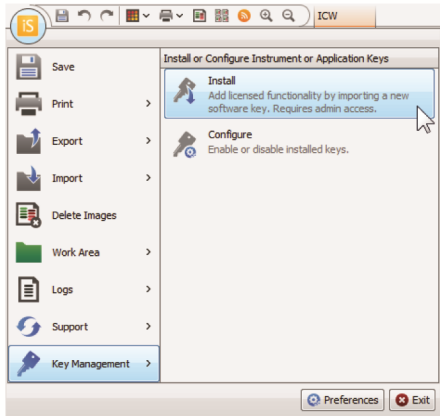
### I. Small Animal Image Analysis Quick Procedure Overview

This guide provides a quick overview of the **Small Animal Image** analysis.

A graphical outline of the **Small Animal Image** 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

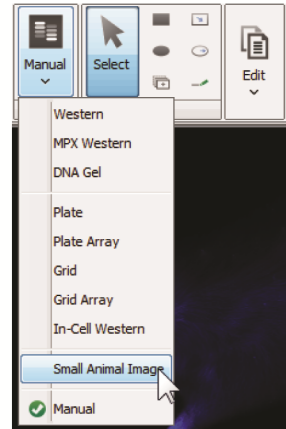
### Import Analysis Key



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

## Step 1

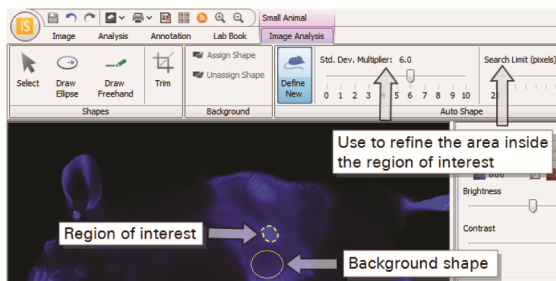
### Apply the Analysis



- ◆ Click the **Analysis** tab and set the analysis type in the **Type** group to **Small Animal Image**.

## Step 2

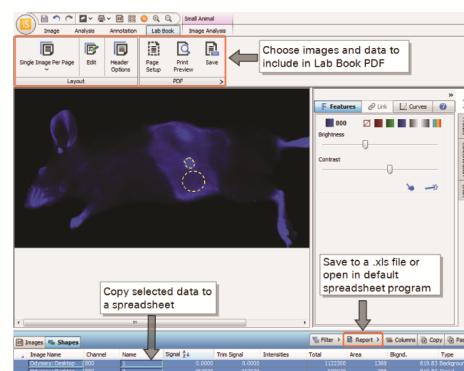
### Complete the Analysis



1. Draw a background shape using **Draw Ellipse** and then assign it by clicking **Assign Shape**.
2. Click **Define New** and click on the region of interest in the image. The region will be located and quantified automatically.
3. Adjust the **Std. Dev. Multiplier** and **Search Limit** to refine the region of interest.

## Step 3

### Export Data




- ◆ Copy data from the **Shapes Table** and paste in 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 Small Animal Analysis Key

If you have purchased the Small Animal Image 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 Small Animal Image 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-SmallAnimalAnalysis.lke license file.
3. Click **Open**.
4. Restart Image Studio Software.

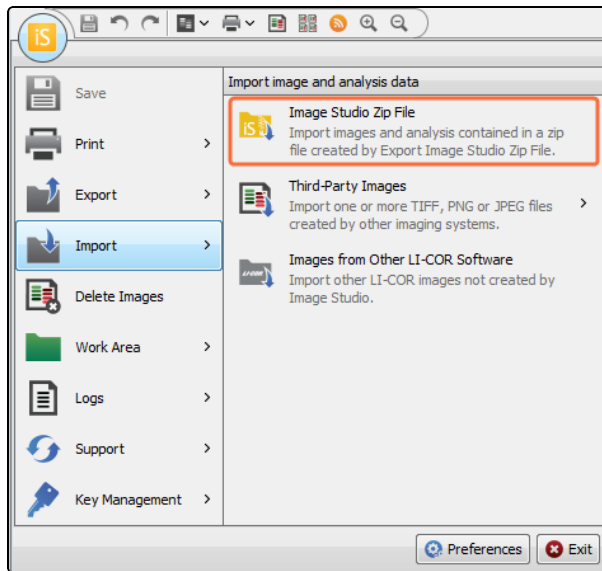
The additional analysis functionality can now be accessed in Image Studio Software.

## III. Import Example Images

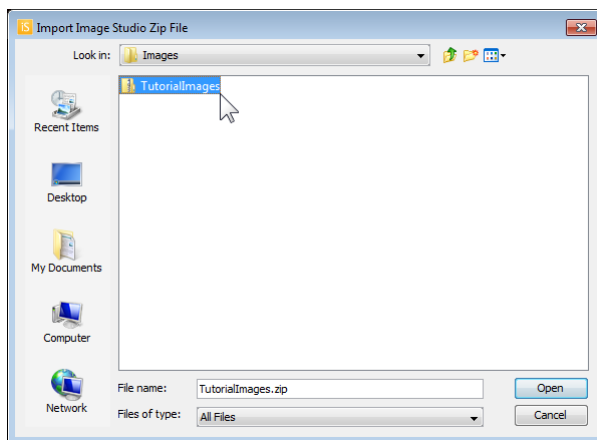
Tutorial images are provided for practice on the Image Studio CD.

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**.

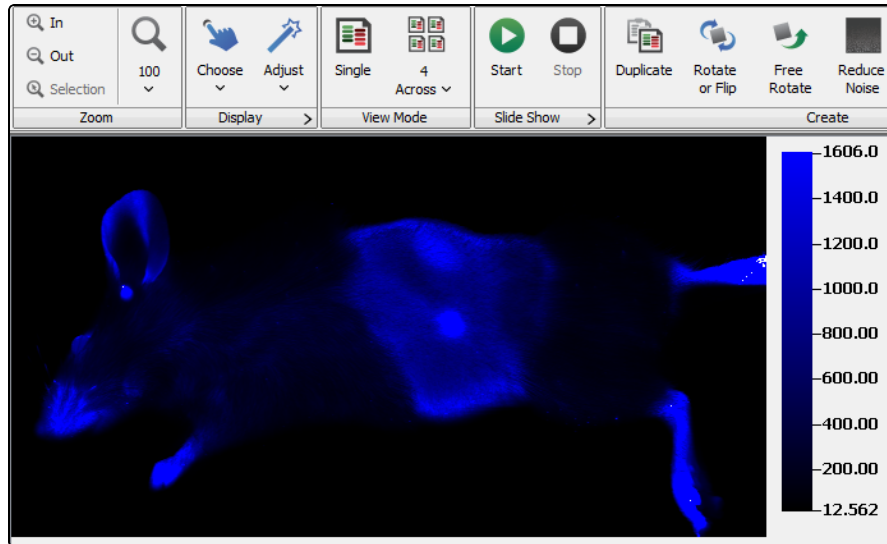
The **Zip File**, containing the following images, will be imported:

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

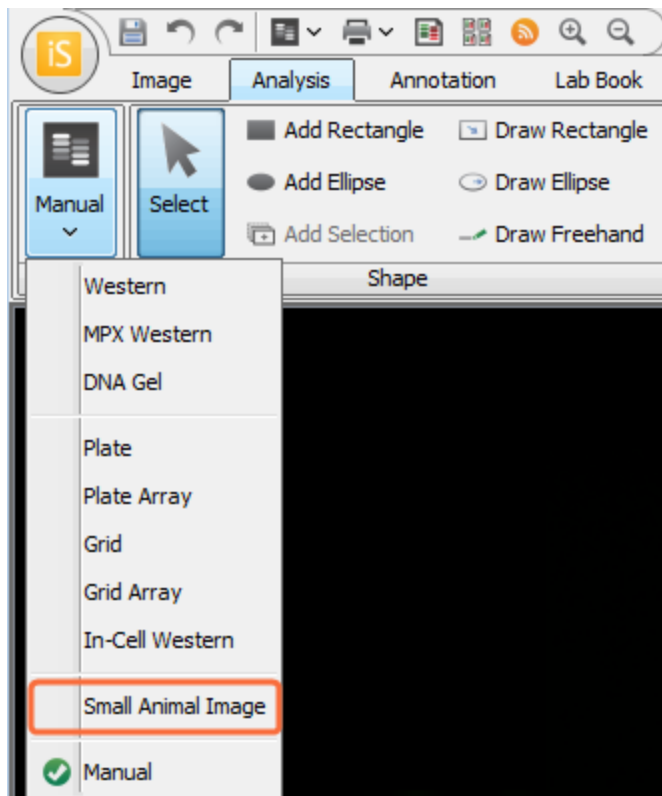
## IV. Apply a Small Animal Image Analysis

Begin the **Small Animal Image** analysis by opening the image and applying the desired analysis.

1. Display the **Small Animal Image** tutorial image by clicking the '9999996\_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.
3. In the **Type** group, click the **Analysis Type** list and then click **Small Animal Image**.




The analysis will be applied to the image using settings from the most recent **Small Animal Image** analysis in your **Work Area**.

**NOTE:** To automatically apply a **Small Animal Image** analysis after image acquisition, choose **Small Animal Image** analysis in the **Setup** group on the **Acquire** tab when starting the acquisition.

## V. Set the Background Shape

The **Small Animal Image** application requires a user-defined background shape to be placed and assigned prior to defining the region to be quantified.

To draw and assign a background shape:


1. In the **Shapes** group, click **Draw Ellipse** .

The cursor will become a cross-hair .

2. Use the cross-hair cursor to draw a background shape on the image by clicking

and dragging to form the shape.

**MORE INFO:** For best practices on where to place the background shape, see [Best Practice for Where to Place the Background Shape](#) below.

3. Once the shape has been drawn and while it is still selected (shown as a dashed line), click **Assign Shape**  in the **Background** group.

## Best Practice for Where to Place the Background Shape

- The background shape should be placed on a shaved part of the animal, ideally in the same location but on the contralateral side of the feature to be quantified.
- If the background shape cannot be placed on the animal's contralateral side, place it close to the feature to be quantified.
- Some organs may retain probe after 24 hours, be careful not to place the background shape on these bright areas.
- If the region drawn by the **Define New** feature encompasses too much area, the background shape can be moved so it incorporates a small region of bright space surrounding a feature to be quantified. This will raise the average background value and decrease the area of the region after the region is redrawn using the **Define New** feature. However, only try moving the background shape this close to the feature of interest if adjusting the **Std. Dev. Multiplier** and **Search Limit** does not produce the desired boundary.

## VI. Define the Region of Interest Using the Auto Shape Tool

After the background shape has been set, the region of interest needs to be defined.

### Define a Region of Interest

1. In the **Auto Shape** group, click **Define New** .


The cursor will become a cross-hair .

2. Click the center of the region of interest with the cross-hair cursor.

A dashed line will appear around the region of interest.

3. If the dashed shape needs to be adjusted so it more accurately represents the region of interest, adjustments can be made while the shape is still selected. See [Adjust the Region of Interest](#) on the facing page.



4. If the dashed shape represents the correct region to be quantified, set the shape by clicking **Select**  and then clicking anywhere on the image outside the dashed shape.

## Adjust the Region of Interest

The **Standard Deviation Multiplier** or **Search Limit** in the **Auto Shape** group can be used to refine how the dashed shape fits the region of interest.

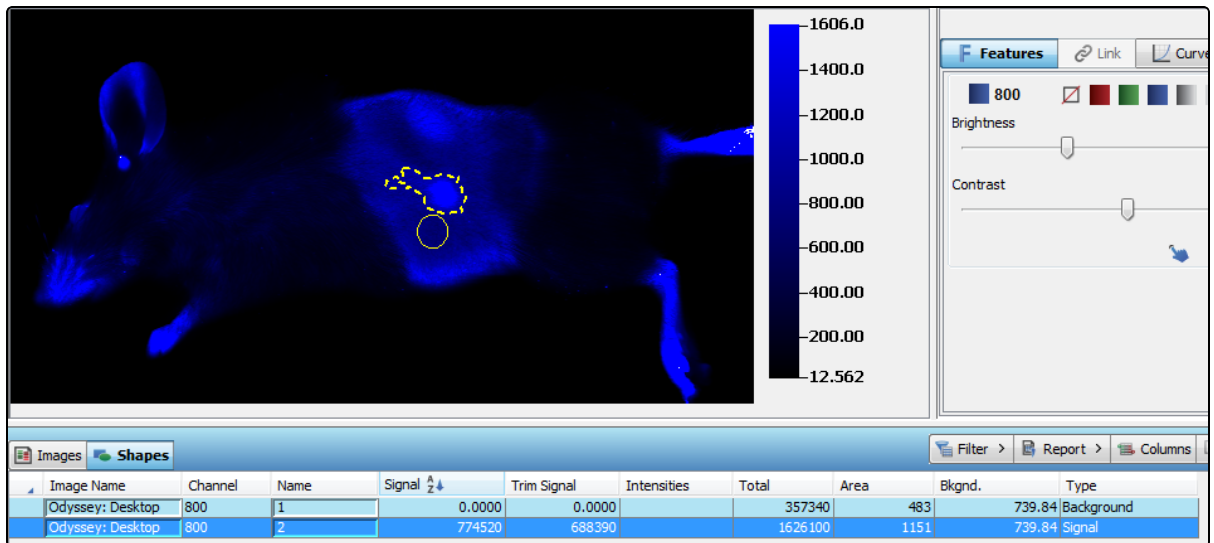
**NOTE:** Use the same value for the **Standard Deviation Multiplier** and **Search Limit** when comparing multiple images.

1. **Standard Deviation Multiplier** - Adjust the **Standard Deviation Multiplier** slider to change the sensitivity threshold used to define the region of interest.
  - Increasing the **Standard Deviation Multiplier** will increase the sensitivity and reduce the size of the region of interest.
  - Decreasing the **Standard Deviation Multiplier** will decrease the sensitivity and increase the size of the region of interest.
2. **Search Limit** - The Search Limit adjusts the size of the image area searched to find a region of interest.


## VII. Review Data


Results for the **Small Animal Image** analysis will be displayed in the **Shapes Table**.

- Click **Shapes** below and to the left of the image to view a table with data for the background shape and regions of interest.




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.

## VIII. Export Data

Data can be exported in three ways.

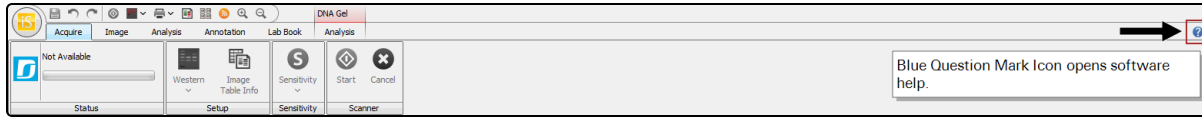
- **Copy and Paste:** Right click selected data in the **Shapes Table** and then click copy (or press CTRL+C). Paste the data into a spreadsheet.
- **Export table data to a spreadsheet:** Click **Report**  above and to the right of the table view and then choose whether to launch the data in an external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

## IX. 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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