

Living Image® Software User's Manual

Version 4.3.1

For the IVIS® Lumina II

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PN CLS135289

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1 Welcome

About This Manual

What's New in the Living Image® 4.3.1 Software on page 2

Living Image® Help on page 3

Caliper Technical Support on page 4

1.1 About This Manual



NOTE: This *Living Image® Software 4.3.1 User's Manual* (part no. CLS135289) is only for use with the IVIS® Lumina II instrument.

This user manual explains how to acquire optical image data on the IVIS® Lumina II and analyze the data using the Living Image® software. The manual provides detailed instructions and screenshots for the Living Image software tools that are available for data acquired on the IVIS Lumina II. Sometimes the screenshots in the manual may not exactly match those displayed on your screen.

When analyzing data acquired on a different type of IVIS instrument, say for example the IVIS Spectrum CT, please see the Living Image Software User's Manual specific for the IVIS Spectrum CT.

Please see the *IVIS Lumina II Hardware Manual* (part no. 117110_Rev01) for information on the instrument.

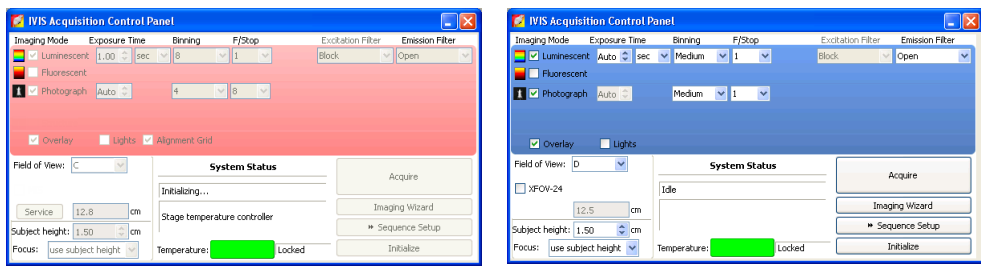
Table 1.1 Living Image 4.3.1 software manuals

Living Image Software Manual for the:	Part No.
IVIS Lumina II	CLS135289
IVIS Lumina XR	CLS135290
IVIS Kinetic	CLS135288
IVIS 200	CLS135287
IVIS Spectrum	CLS135291
IVIS Spectrum CT	CLS135292

1.2 What's New in the Living Image® 4.3.1 Software


The Living Image 4.3.1 software controls image acquisition on the IVIS® instrument and provides tools for optimizing image display and analyzing images. [Table 1.2](#) shows the major new or improved software features that are applicable to acquisition and analysis of IVIS Lumina II image data.

Table 1.2 New or improved features of Living Image 4.3.1 software

New or Improved Features	See Page
<p>New DyCE (Dynamic Contrast Enhancement) Acquisition and Analysis Tools</p> <p>Note: DyCE acquisition and analysis features require a separate license.</p> <p>DyCE acquisition supports Cerenkov (radioactive), luminescent or fluorescent imaging.</p> <p>DyCE acquisition and analysis tools enable real-time pharmacokinetic (spatio-temporal biodistribution) studies of probe or dye signal.</p>	<p>140</p>
<p>Improved Spectral Unmixing Tools</p> <p>Choose from four methods of spectral unmixing, depending on your knowledge of the probe spectral response and the probe location.</p> <p>If a user-created spectrum library (reference spectral data from known probes at known locations) is available, it can be selected in the Imaging Wizard during sequence setup. This provides a convenient way to select filters.</p> <p>Ability to subtract compute "pure" spectra by subtracting unmixed spectra which overlap</p> <p>Export unmixing results as an image or sequence which can be analyzed using image analysis tools.</p>	<p>119</p> <p>120</p> <p>133</p> <p>138</p>
<p>ROIs</p> <p>Mirror ROIs for optical data support measurements on the left and right views of images acquired using the Side Imager accessory.</p>	<p>92</p>
<p>View a histogram of measurement ROI pixel intensities.</p>	<p>98</p>
<p>Improved Acquisition Features</p> <p>The color of the upper control panel is an indicator of instrument activity. Red color means the instrument is initializing or acquiring images; blue color means the instrument is idle.</p> 	<p>40</p>
<p>Save a batch sequence setup (.xsq).</p>	<p>40</p>

1.3 Living Image® Help

There are several ways to obtain help on the software features and related information.

To view:	Do this:
A tooltip about a button function	Put the mouse cursor over the button.
A brief description about an item in the Living Image user interface	Click the  toolbar button, then click the item.
The <i>Living Image Software User's Manual</i>	Press F1 or select Help → User Guide on the menu bar and select the manual specific for your imaging system.
Living Image technical notes (see Table 1.3 on page 3)	Select Help → Tech Notes on the menu bar. Note: Please see the IVIS University download page for the most recent collection of technical notes.

[Table 1.3](#) lists the tech notes that are available under the Help menu. There are three types of tech notes:

- Tech Notes – Quick guides for tasks using the Living Image software tools.
- Biology Tech Notes – Protocols and procedures related to animal subjects.
- Concept Tech Notes – Background information on *in vivo* imaging topics.

Table 1.3 Tech Notes

Tech Notes	Title
1	Adaptive Fluorescence Background Subtraction
2	Auto-Exposure
3	Determine Saturation
4	Bioluminescence Tomography (DLIT) <ul style="list-style-type: none"> ■ 4a – Setup and Sequence Acquisition ■ 4b – Topography ■ 4c – Source Reconstruction and Analysis Note: DLIT analysis is not available for data acquired on the IVIS® Lumina II.
5	ROIs (optical data) <ul style="list-style-type: none"> ■ 5a – Drawing ROIs ■ 5b – Subtracting Background ROI from Sequence ■ 5c – Subject ROIs
6	Fluorescence Tomography (FLIT) <ul style="list-style-type: none"> ■ 6a – Setup and Sequence Acquisition ■ 6b – Topography ■ 6c – Source Reconstruction and Analysis Note: FLIT analysis is not available for data acquired on the IVIS® Lumina II.
7	High Resolution Images
8	Image Math
9	Image Overlay – 2D
10	Image Overlay – 3D Note: This type of analysis is not available for data acquired on the IVIS® Lumina II.
11	Imaging Wizard

Table 1.3 Tech Notes (continued)

12	Load Groups of Images
13	Spectral Unmixing
14	Transillumination <ul style="list-style-type: none"> ■ 14a – Transillumination Fluorescence ■ 14b – Raster Scan ■ 14c – Normalized Transmission Fluorescence ■ 14d – Well Plates Note: This type of analysis is not available for data acquired on the IVIS® Lumina II.
15	Well Plate Quantification
Biology Tech Notes	
1	d-luciferin Prep Sheet
2	Kinetic Analysis of Bioluminescent Sources Note: This type of analysis is not available for data acquired on the IVIS® Lumina II.
3	Imaging Protocol Guide
4	Imaging Procedure
5	Intraperitoneal Injections
Concept Tech Notes	
1	Luminescent Background Sources and Corrections
2	Image Display and Measurement
3	Detection Sensitivity
4	Fluorescent Imaging
5	DLIT and FLIT Reconstruction of Sources Note: DLIT or FLIT analysis is not available for data acquired on the IVIS® Lumina III.

1.4 Caliper Technical Support

For technical support, please contact Caliper at:

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2 Getting Started

Starting the Living Image® Software

Initializing the System and Checking Temperature on page 7

Overview of Image Acquisition on page 9

Overview of Living Image® Tools and Functions on page 11

Managing User Accounts on page 16

Tracking System and User Activity on page 19

2.1 Starting the Living Image® Software

The Living Image® software on the PC workstation that controls the IVIS® Lumina II includes both the acquisition and analysis features. The Living Image software on other workstations includes only the analysis features.

For information on installing the software, see the Installation Guide included on the Living Image CD ROM. [Table 2.1](#) shows the default software installation locations.

Table 2.1 Living Image software installation locations

Living Image Software	Operating System	Installation Location
32-bit version	32-bit Windows	C:\Program Files\Caliper Life Sciences\Living Image
	64-bit Windows	C:\Program Files(x86)\Caliper Life Sciences \Living Image
64-bit version	64-bit Windows	C:\Program Files\Caliper Life Sciences\Living Image



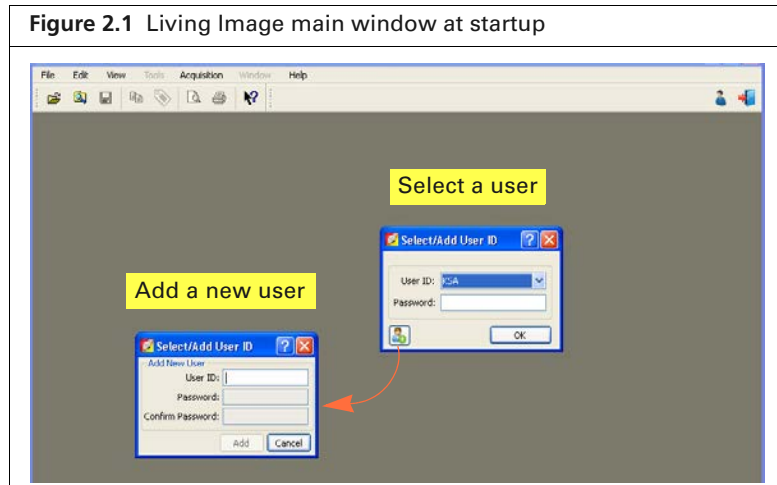
NOTE: All components of the IVIS Lumina imaging system should be left on at all times due to the long cooling time required to reach operating (demand) temperature. It is also important to leave the system on to enable automatic overnight electronic background measurements. Periodically rebooting the computer is permissible and does not affect the camera operation.


To start the software:

- 1. PC Users:** Double-click the Living Image software icon  on the desktop. Alternatively, click the Windows Start button  and select **All Programs → Caliper Life Sciences → Living Image**.

Macintosh Users: Double-click the Living Image icon  on the desktop or run the software from the application folder.

The main window appears ([Figure 2.1](#)).

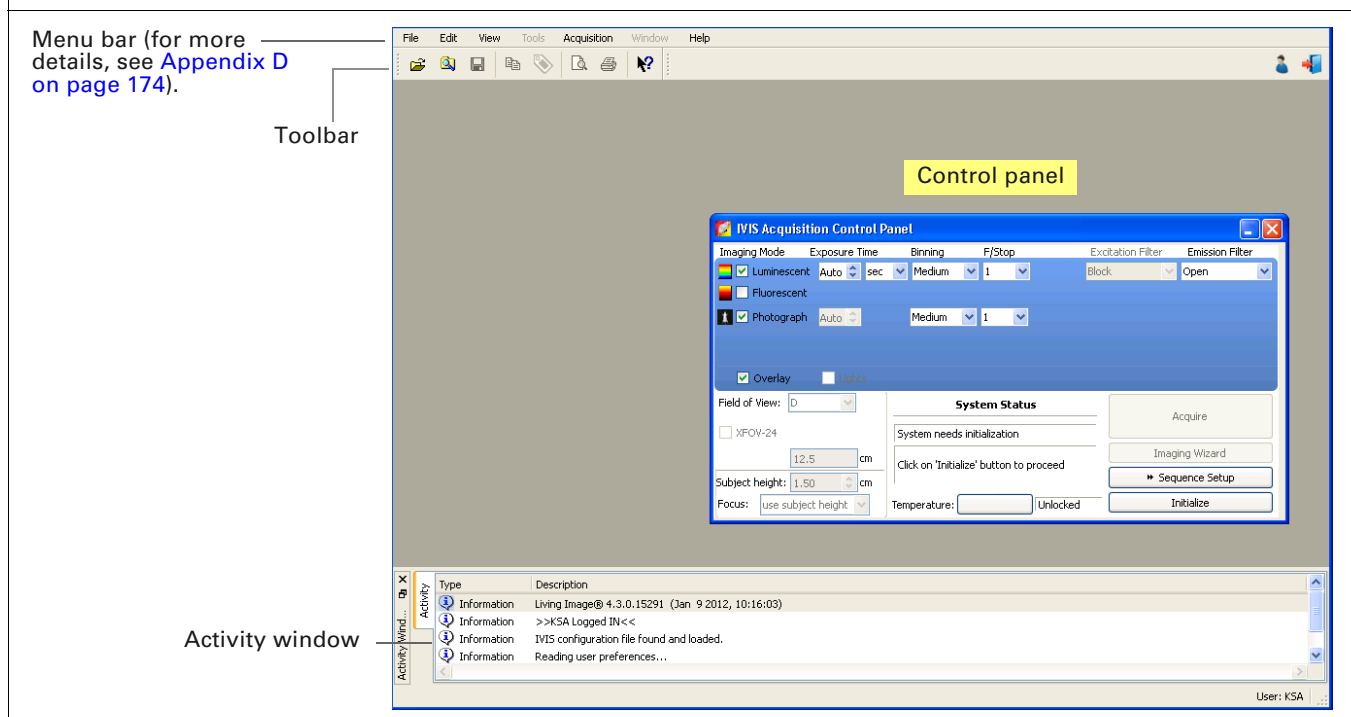


- 2.** In the dialog box that appears, select a user ID from the drop-down list. If the user ID is password protected, enter the password and click **OK**.
Alternatively, create a new user ID:
 - a.** In the Select/Add User ID box, click the  button.
 - b.** Enter a user ID.
 - c.** Enter and confirm a password. This is optional.
 - d.** Click **Add** and **OK**.

The control panel appears if the workstation controls the IVIS® Lumina II ([Figure 2.2](#)). For more details on the control panel, see [Appendix B on page 164](#).



NOTE: The Living Image® software has optional password protection for user accounts. See [page 16](#) for more details.

Figure 2.2 Living Image® main window and IVIS Acquisition Control Panel

NOTE: The Living Image software on the PC workstation that controls the instrument includes both the acquisition and analysis features. The Living Image software on other workstations includes only the analysis features. Macintosh users have access to the analysis features only.

2.2 Initializing the System and Checking Temperature

The IVIS® Lumina II must be initialized each time Living Image® software is started, or if the power has been cycled to the imaging chamber.

The initialization procedure is started from the control panel ([Figure 2.3](#)).




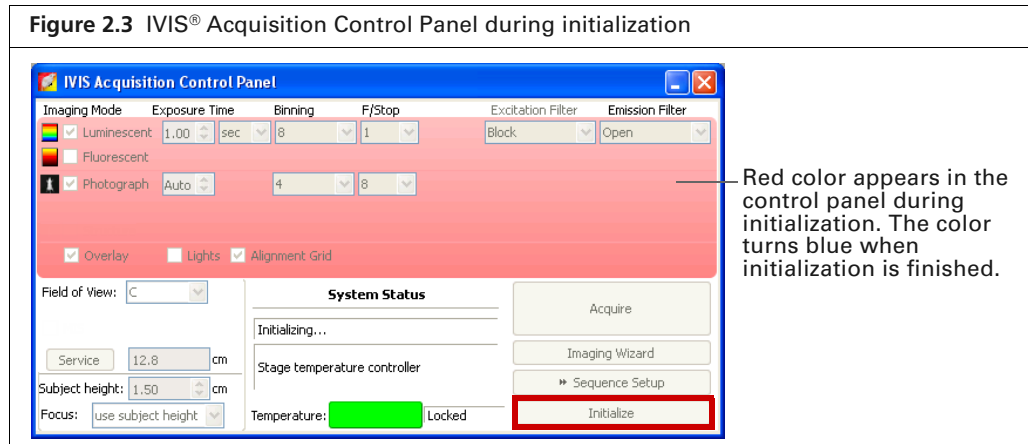
NOTE: The control panel is only available on the PC workstation that controls the IVIS Lumina II. The items available in the control panel depend on the imaging mode selected and the type of acquisition (Image Setup or Sequence Setup).

Initialization moves every motor-driven component in the system (for example, stage and lens) to a home position, resets all electronics and controllers, and restores all software variables to the default settings. Initialization may be useful in error situations.

For further details on instrument operation, see the *IVIS Lumina II Hardware Manual* (part no. 117110_Rev01).

Initializing the IVIS® Lumina II

1. Start the Living Image software (double-click the  icon on the desktop).
2. Click **Initialize** in the control panel that appears (Figure 2.3).
After several seconds you will hear the instrument motors move.

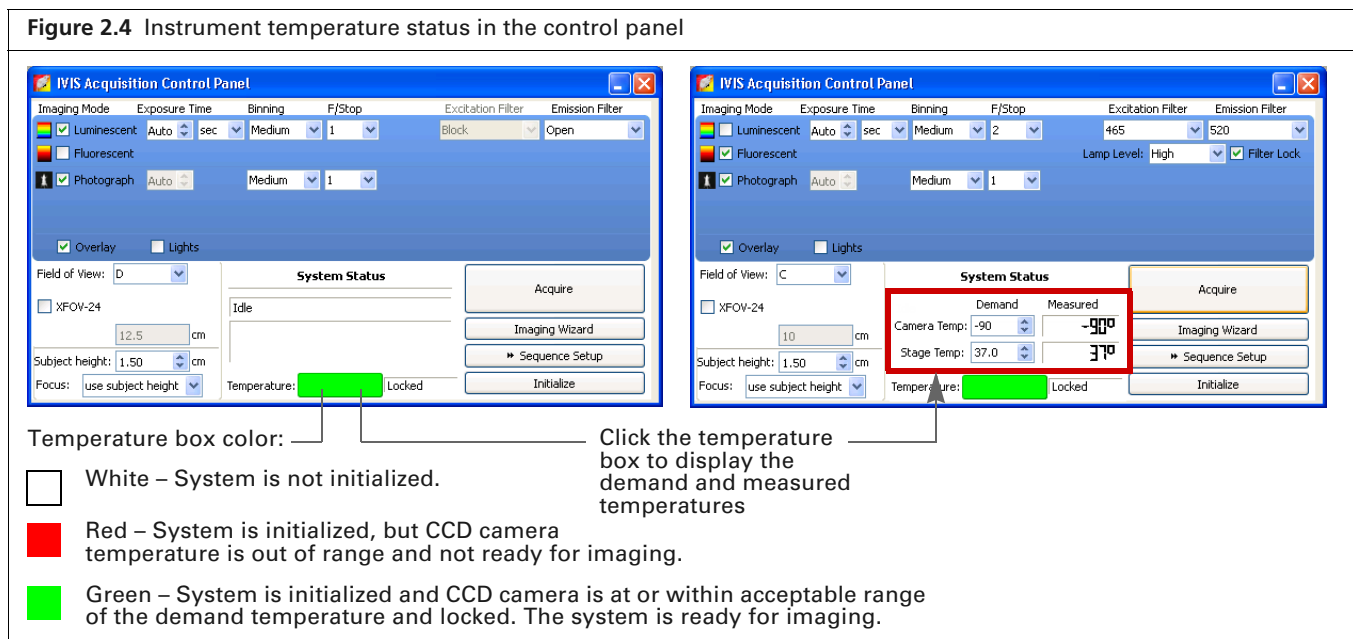


CCD Temperature

The IVIS Acquisition Control Panel indicates the temperature status of the charge coupled device (CCD) camera (Figure 2.4). The green temperature box indicates that the instrument is ready for operation and image acquisition.

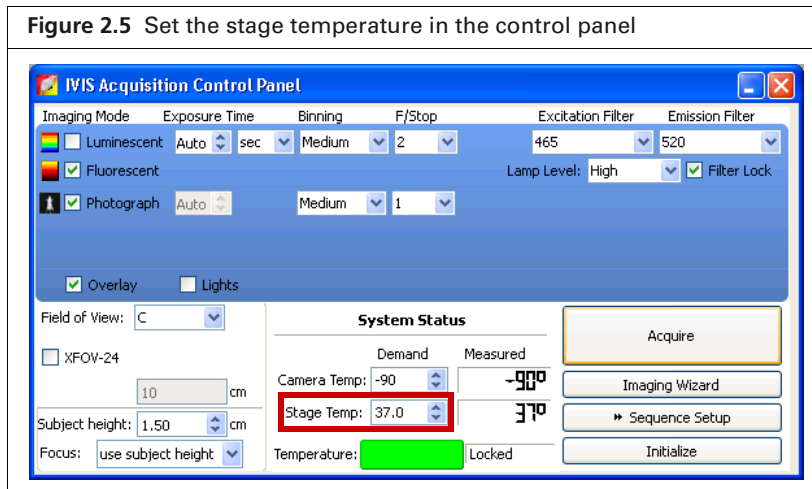
The demand temperature for the CCD camera is preset and generally should not be changed. Electronic feedback control maintains the CCD camera temperature to within a few degrees of the demand temperature.

The instrument is ready for imaging after the system is initialized and the operating (demand) temperature of the CCD camera is reached (locked).



Stage Temperature

The stage is temperature-controlled to keep subjects warm during imaging. The temperature control is enabled after the instrument is powered on and initialized from the Living Image® software. The default temperature is 37 °C and is self-monitoring after the system is initialized. The stage may be set to a temperature from 20 - 40 °C.

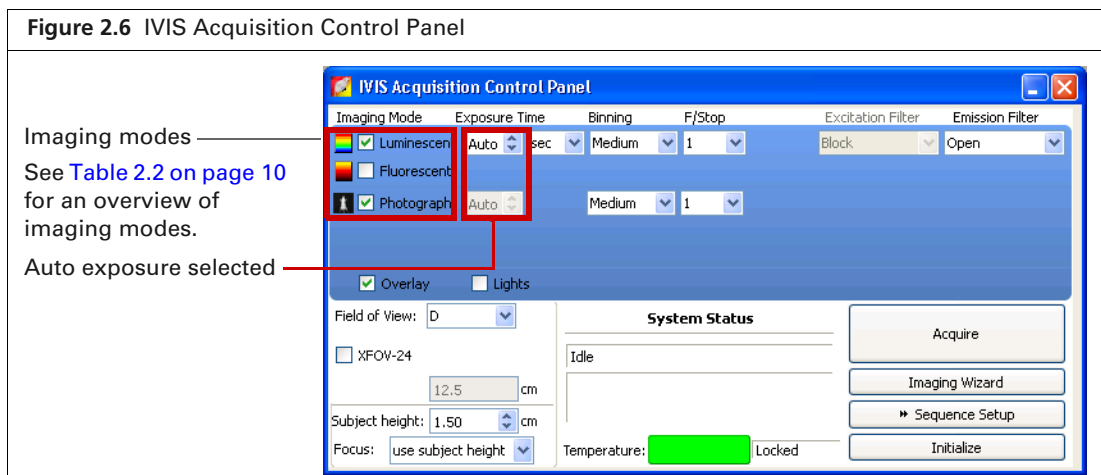


2.3 Overview of Image Acquisition

Control Panel

The control panel provides the image acquisition functions (Figure 2.6). See Appendix B on page 164 for details on the imaging parameters in the control panel.

NOTE: The control panel is only available on the PC workstation that controls the instrument. The items available in the control panel depend on the selected imaging mode (luminescent or fluorescent) and acquisition mode (Image Setup or Sequence Setup).



Auto Exposure Feature

The Auto exposure setting is useful in situations where the signal strength is unknown or varies widely, for example during a time course study. If Auto exposure is chosen (Figure 2.6), the system acquires an image at maximum sensitivity, then calculates the required settings to achieve, as closely as possible, an image with a user-specified target max count. If the resulting image has too little signal or saturated pixels, the software adjusts the parameters and takes another image.

In most cases, the default auto exposure settings provide a good luminescent or fluorescent image. However, you can modify the auto exposure preferences to meet your needs. See page 170 for more details.

Imaging Modes on the IVIS® Lumina II

Table 2.2 briefly explains the types of images that can be acquired on the IVIS® Lumina II.

Table 2.2 Imaging modes on the IVIS Lumina II


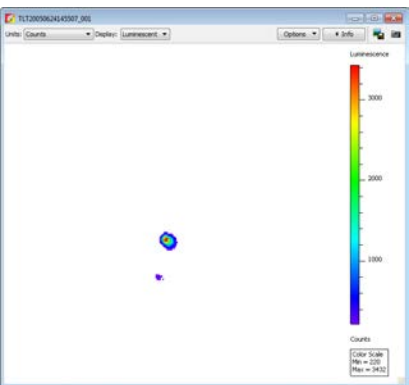
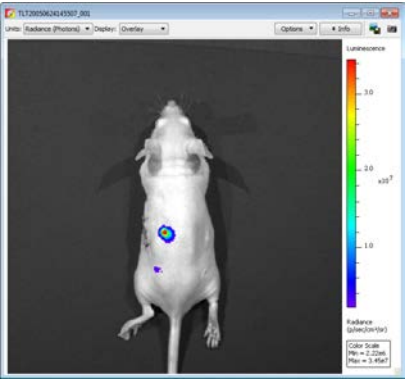

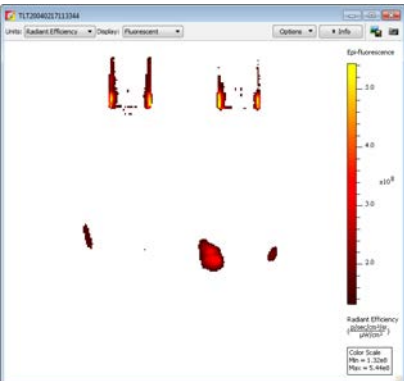



Imaging Mode	Description	Example
Luminescent optical imaging 	A longer exposure of the subject taken in darkness to capture low level luminescence emission from the surface of the subject. The optical luminescent image data is displayed in pseudocolor that represents intensity.	
		Luminescent image
		
		Overlay: Luminescent image on photograph

Table 2.2 Imaging modes on the IVIS Lumina II (continued)

Imaging Mode	Description	Example
Fluorescent optical imaging 	<p>An exposure of the subject illuminated by filtered light. The light source is located above the stage (epi-illumination). The target fluorophore emission is captured and focused on the CCD camera.</p> <p>The optical fluorescent image data can be displayed in units of counts or photons (absolute, calibrated), or in terms of efficiency (calibrated, normalized).</p> <p>Note: See the concept tech note Image Display and Measurement for more on quantifying image data (select Help → Tech Notes on the menu bar).</p>	
		<p>Fluorescent image</p>  <p>Overlay: Fluorescent image on photograph</p>
Photograph 	<p>A short exposure of the subject illuminated by the lights located in the ceiling of the imaging chamber. The photographic image is displayed as a grayscale image.</p>	

2.4 Overview of Living Image® Tools and Functions

The Living Image tools are organized in the Tool Palette and the Tools menu (Figure 2.7). Some tools are for use with a single image, others require an image sequence.

Table 2.3 provides an overview of the tools available for data acquired on the IVIS® Lumina II. If analyzing data acquired on a different type of IVIS instrument, say for example the IVIS Spectrum CT, please see the Living Image Software User's Manual specific for the IVIS Spectrum CT.



NOTE: The tools available in the Tool Palette or menu bar depend on the active image data.

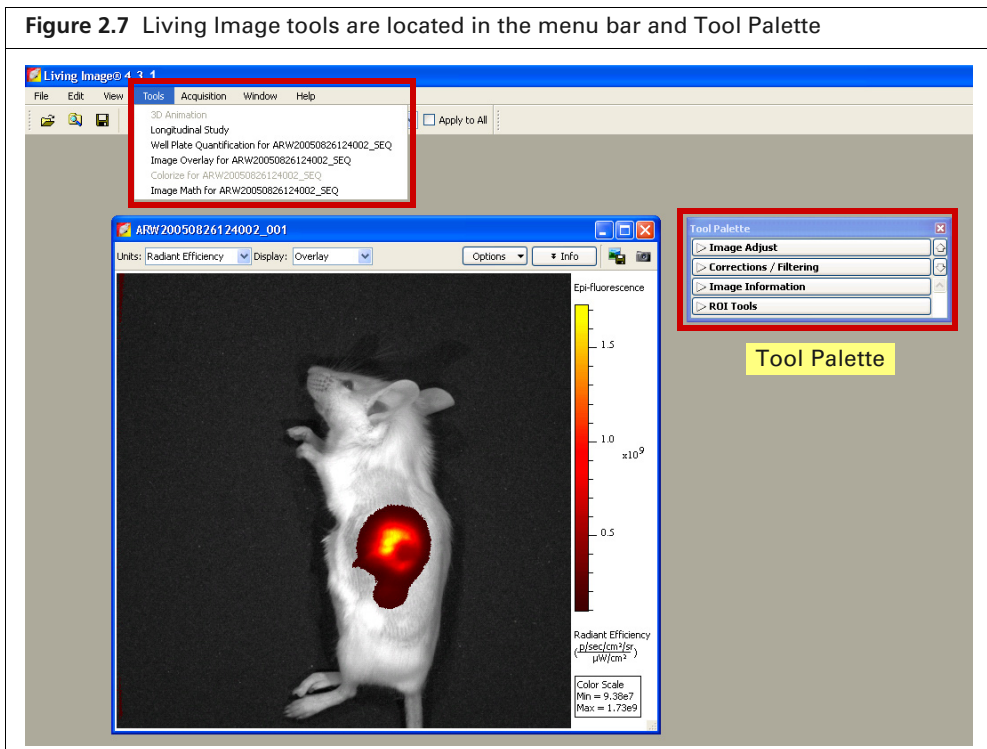


Table 2.3 Living Image tools available for data acquired on the IVIS Lumina II

Living Image Tools and Functions	See Page
<p>The screenshot shows the 'Image Adjust' tool palette. It includes a 'Photo Adjustment' section with sliders for 'Brightness' (set to 100), 'Contrast' (set to 1.5), and 'Opacity' (set to 100). Below this is a 'Color Scale' section with 'Min' (2140) and 'Max' (36446) sliders, and radio buttons for 'Auto', 'Full', and 'Manual'. The 'Auto' option is selected. There is also a 'Color Table' dropdown set to 'Rainbow' and a 'Reverse' checkbox which is checked. A 'Logarithmic Scale' checkbox is present and unchecked.</p>	<p>Image Adjust 61</p> <p>Tune the photograph brightness, contrast, or opacity.</p> <p>Change the minimum or maximum of the color scale applied to the optical image data.</p> <p>Select a color table for image display.</p>
<p>The screenshot shows the 'Corrections / Filtering' tool palette. It lists several correction options: 'Read Bias Subtraction' (checked), 'Flat Field Correction' (checked), 'Cosmic Correction' (checked), and 'Adaptive FL Background Subtraction' (unchecked). Below these are 'Binning' (set to 8) and 'Smoothing' (set to None) controls. Other sections visible include 'Image Information', 'ROI Tools', and 'Spectral Unmixing and DyCE'.</p>	<p>Corrections/Filtering (Optical Data) 63</p> <p>Apply corrections or filters to the raw data.</p>

Table 2.3 Living Image tools available for data acquired on the IVIS Lumina II (continued)

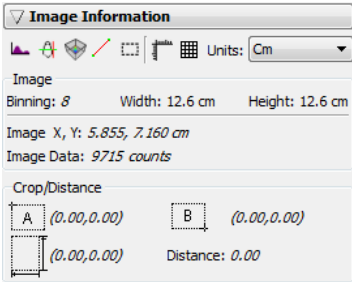
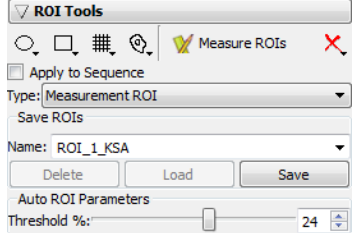
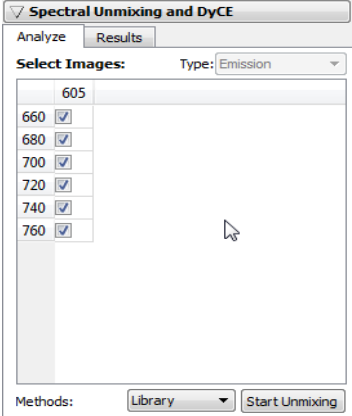
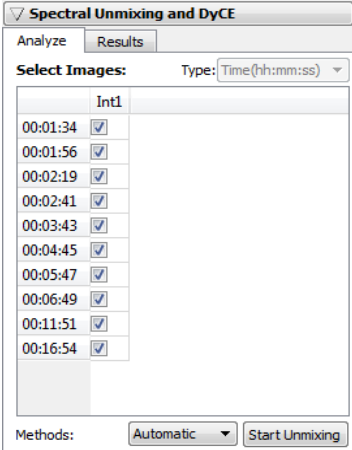
Living Image Tools and Functions	See Page
	<p>Image Information 87</p> <p>View information about an optical image (for example, a pixel histogram or a 3D plot of pixel intensities).</p> <p>Make measurements in the image.</p>
 	<p>ROI Tools for Optical Data 82</p> <p>Specify a region of interest (ROI) in an optical image and measure the signal intensity within the ROI.</p> <p>Spectral Unmixing 119</p> <p>Use spectral unmixing to:</p> <ul style="list-style-type: none"> ■ Extract the signal of one or more fluorophores from the tissue autofluorescence ■ Analyze luminescent or fluorescent images when more than one reporter is used in the same animal model
	<p>DyCE (Dynamic Contrast Enhancement) 140</p> <p>Use DyCE to:</p> <ul style="list-style-type: none"> ■ Determine real-time pharmacokinetic (spatio-temporal biodistribution) of a probe or dye signal ■ Extract “temporal spectra” (signal intensity as a function of time) from particular anatomical regions. <p>Note: DyCE acquisition and analysis tools require a separate license.</p>

Table 2.3 Living Image tools available for data acquired on the IVIS Lumina II (continued)

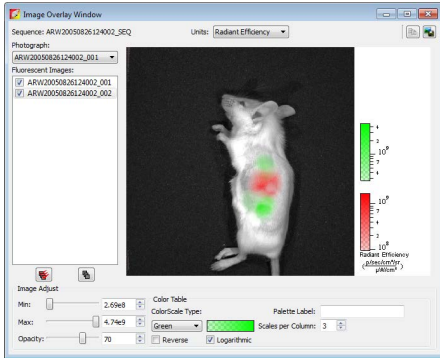
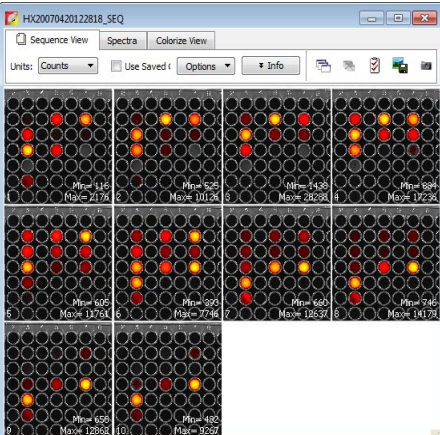
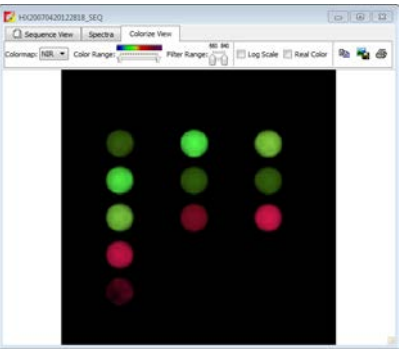
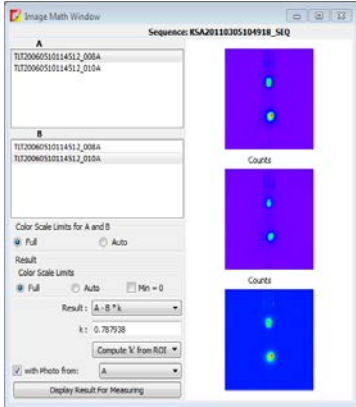
Living Image Tools and Functions	See Page
	<p>Image Overlay 73</p> <p>Select Tools → Image Overlay for <sequence name> on the menu bar.</p> <p>View multiple fluorescent or luminescent signals in one 2-dimensional image in the Image Overlay window.</p>
	<p>Colorize View 76</p> <p>Select Tools → Colorize for <sequence name> on the menu bar.</p> <p>The colorize tool renders each luminescence or fluorescence image of a sequence in color, and combines them into a single image. This enables you to see both intensity and spectral information in a single view.</p> <p>The tool provides a useful way to visualize multiple probes or scale probe signals that are not in the visible range.</p>
<p>Images of Quantum dot nanocrystals (700 or 800 nm) were acquired using different combinations of excitation and emission filters.</p>  <p>Colorize view of the combined images</p>	

Table 2.3 Living Image tools available for data acquired on the IVIS Lumina II (continued)

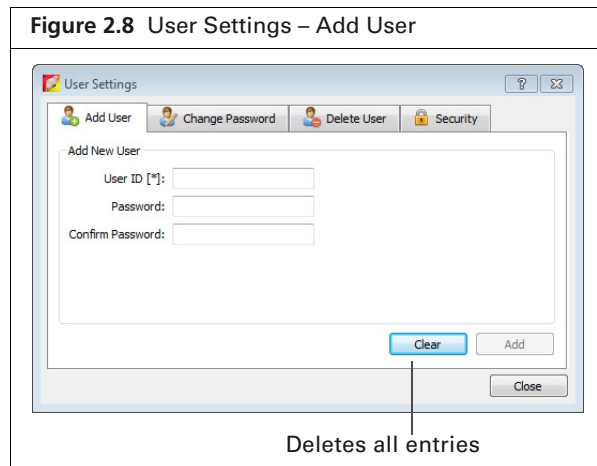
Living Image Tools and Functions	See Page
	<p>Image Math Window 127</p> <p>Select Tools → Image Math for <sequence name> on the menu bar.</p> <p>Mathematically combine (add, multiply, subtract, or divide) two user-selected images.</p> <p>For example, subtract a blue-shifted background filter image from the primary excitation filter image to remove tissue autofluorescence signal.</p>

2.5 Managing User Accounts

Adding Users

New users can be created in the:

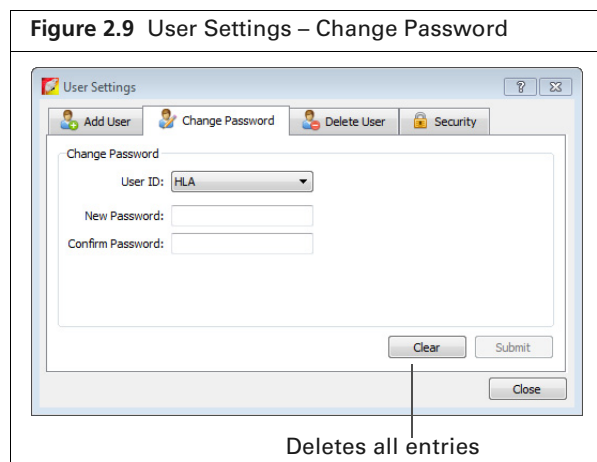
- Main window at startup (see [page 6](#)).
 - User Settings dialog box ([Figure 2.8](#)).
1. Select **Edit** → **User settings** on the menu bar.
 2. Click the Add User tab in the dialog box that appears.



3. Enter a user ID.
4. Optional: enter and confirm a password.
5. Click **Add**.

Changing or Adding Passwords

1. Select **Edit** → **User settings** on the menu bar.
2. Click the Change Password tab in the dialog box that appears.



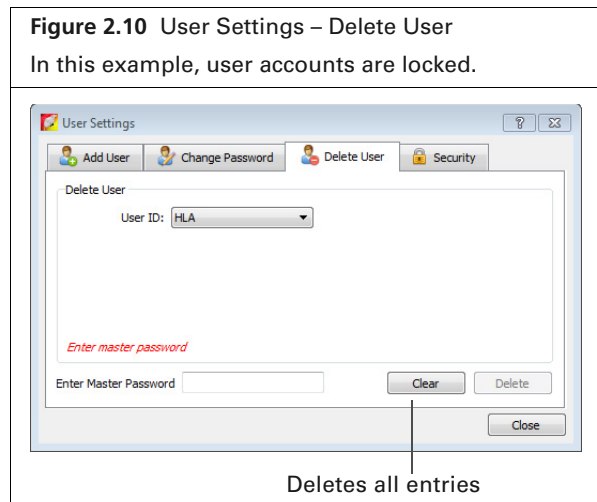
3. Select a User ID, enter and confirm a new password, and click **Submit**.

Deleting Users



NOTE: User accounts can be locked. If this security is applied, a master password is required to delete users from the system. See [page 17](#) for more details on locking user accounts.

1. Select **Edit → User settings** on the menu bar.
2. Click the Delete User tab in the dialog box that appears.



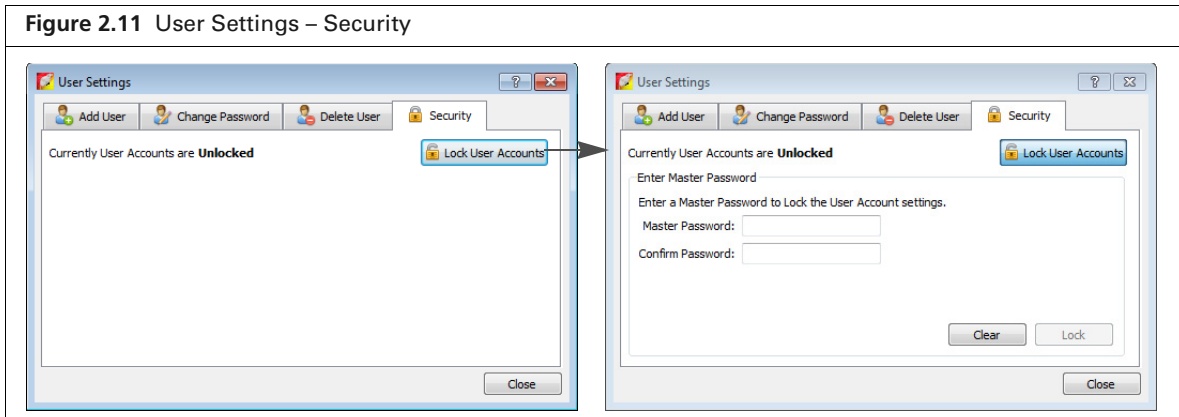
3. Select a User ID.
4. If the accounts are locked, enter the master password.
5. Click **Delete** and **Close**.

Locking User Accounts

If user accounts are locked, a master password is required to change user passwords, delete users, or unlock user accounts.

To lock user accounts:

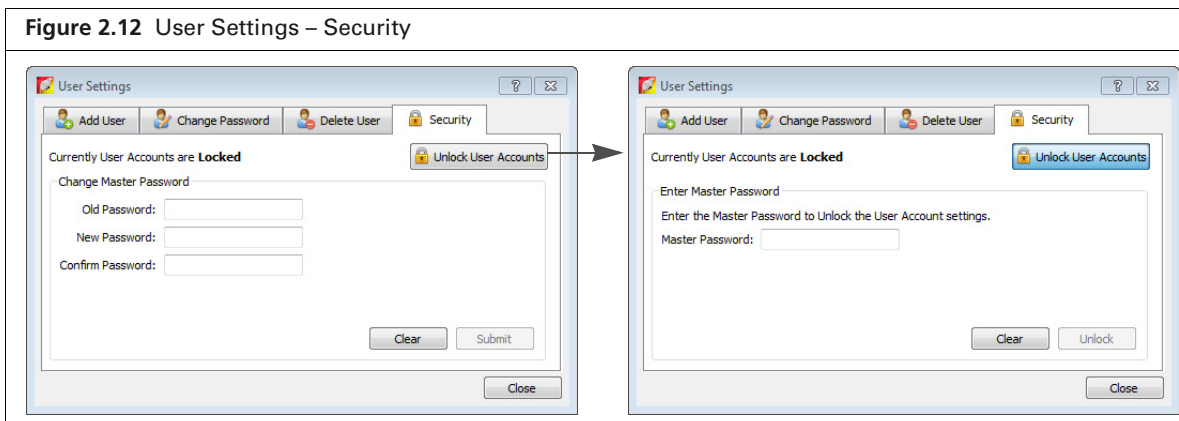
1. Select **Edit → User settings** on the menu bar.
2. Click the Security tab in the dialog box that appears.
3. Click **Lock User Accounts**.



4. Enter and confirm a master password. Click **Close**.
The master password will be required to delete users.

To unlock user accounts:

1. In the Security tab, click **Unlock User Accounts**.
2. Enter the master password and click **Unlock**. Click **Close**.

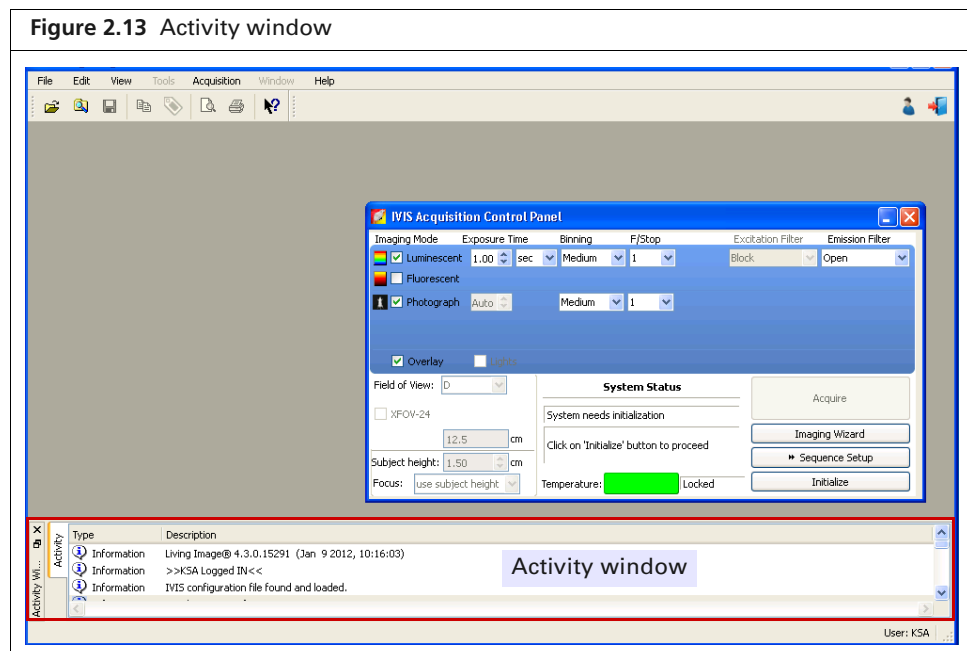


2.6 Tracking System and User Activity

Activity Window

The Activity window shows the imaging system activities (Figure 2.13). The software creates and saves a log of the system activities related to data acquisition. This information may be useful for Caliper field service engineers to understand the imaging system behavior over time or for troubleshooting. The activity log is located at C:\Program Files\Caliper Life Sciences\Living Image.

The software tracks user time on the system (hr/min/sec per user ID) from logon until switching users or system shut down. The software creates a separate record for each month (for example, LI_USAGE_<MONTH>_2011.csv) located at C:\Program Files\Caliper Life Sciences\Living Image\Usage).



3 Image Acquisition

Luminescent Imaging

Fluorescent Imaging With Epi-Illumination on page 26

Acquire a Sequence Using the Imaging Wizard on page 33

Acquire Multiple Sequences in Batch Mode on page 39

Manually Set Up a Sequence on page 41

High Resolution Imaging on page 45

Manually Saving Image Data on page 46

Exporting Images on page 46

3.1 Luminescent Imaging


Luminescent imaging captures signals from luminescent molecular reporters. This section explains how to acquire a single luminescent optical image:

- Quick guide – See [Figure 3.1](#) on page 21.
- Detailed instructions – See [page 22](#).

See [page 33](#) for information on acquiring a luminescent sequence.

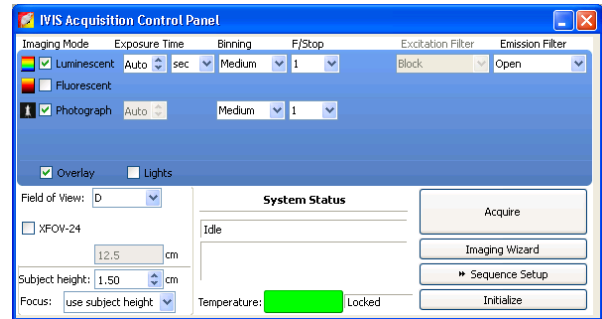
Quick Guide: Acquire a Luminescent Image

Figure 3.1 Quick Guide: Acquire a luminescent image

1. Start the Living Image® software  and initialize the IVIS® Lumina II (page 5).
Note: See the *IVIS Lumina II Hardware Manual* (part no. 117110_Rev01) for more information on the instrument.
2. Place the anesthetized subjects in the imaging chamber and close the door.



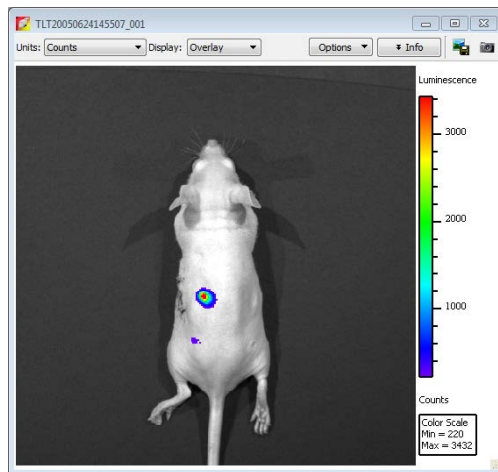
3. Put a check mark next to “Luminescent” and select “Auto” exposure.
4. Choose “Photograph” (optional). Selecting “Photograph” automatically selects “Overlay”.
5. Select a field of view (see Table 3.4 on page 29).
6. Select “Use subject height” and enter the height in centimeters.
7. Click **Acquire**.



8. When prompted, select a location for the image data (optional). Image data acquired during the session will be automatically saved to this location.
9. Enter experiment and subject information in the "Edit Image Labels" dialog box that appears (optional).

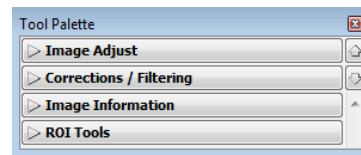
The image window and tool palette appear when acquisition is finished.

Image Window



See Table 3.2 on page 25 for more details on the image window.

Tool Palette



The Tool Palette includes the:


- Image Adjust tools (page 61)
- Corrections/Filter tools (page 63)
- Image Information tools (page 57)
- ROI Tools (page 82)

Acquire a Luminescent Image

This section provides detailed instructions for luminescent image acquisition.



NOTE: The IVIS® Lumina II should be initialized and the temperature locked before setting the imaging parameters in the control panel. See [page 7](#) for more details.

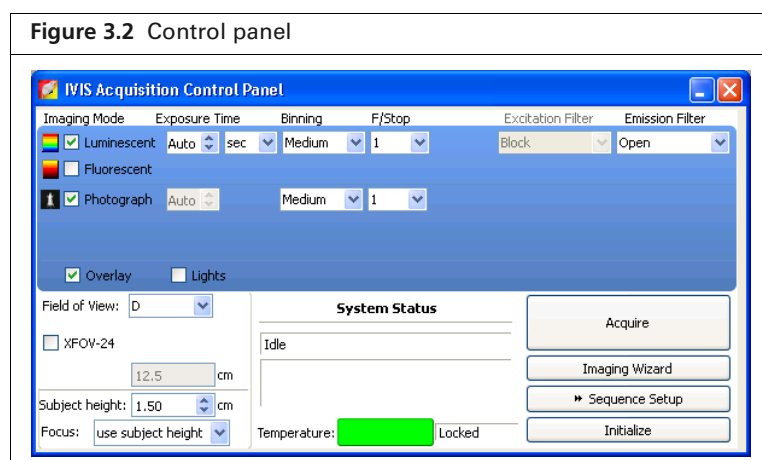
1. Put a check mark next to **Luminescent** and select **Auto** exposure (click the  arrows) in the control panel.

The software automatically determines the binning and F/Stop settings.



TIP: See the tech note *Auto-Exposure* for helpful information (select **Help** → **Tech Notes** on the menu bar).

Alternatively, manually set the exposure, binning, and F/Stop. See [Table B.1 on page 164](#) for details on these parameters.



2. Put a check mark next to Photograph.
3. Select a Field of View (size of the stage area to be imaged).



TIP: See the technical note *Detection Sensitivity* for more information about the Field of View (select **Help** → **Tech Notes** on the menu bar).

Table 3.1 Field of View (FOV) settings

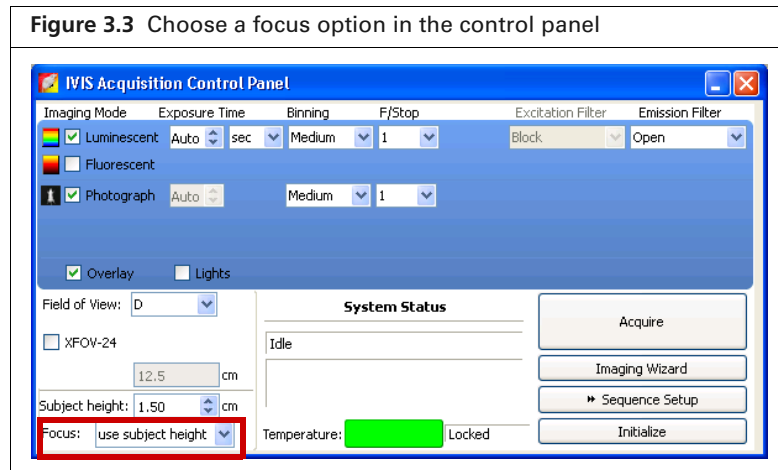
FOV Setting	FOV (cm)
A	5
B	7.5
C	10
D	12.5
E ¹	24
Z ²	2.6

¹Position E is available when the XFOV-24 lens (part no. 123177) is installed.

²Position Z is available when the ZFOV-2.6 lens (part no. 127285) is installed.

4. Select a focus option in the control panel (Figure 3.3).

The focal distance to the camera is set a stage $z = 0$ for each field of view. To focus at the top of the animal, the stage moves down so that the top of the animal is at $z = 0$. You can enter the height of the animal and select the “use subject height” option or use the manual focus option to determine the proper subject height for the area to be imaged. See Appendix B on page 167 for manual focus instructions.



5. Select Overlay to view an overlay image (registered photograph and luminescent image) after acquisition.



NOTE: If you want to check the subject inside the chamber before acquisition, take a photograph—uncheck the Luminescent option, choose the Photograph option, and click **Acquire**. Be sure to select the Luminescent option after taking the photograph.

6. Click **Acquire** when you are ready to capture the image.

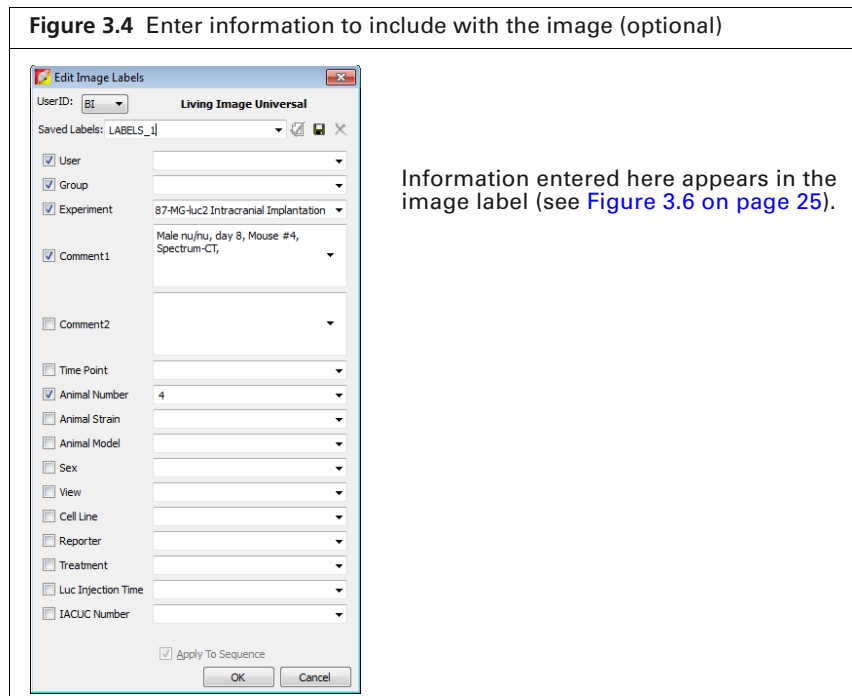


NOTE: If necessary click **Image Setup** in the control panel to operate in single image mode. In single image mode, the **Sequence Setup** button appears in the control panel. Use this button to set up sequence acquisition (see page 33 for more details on sequence setup).

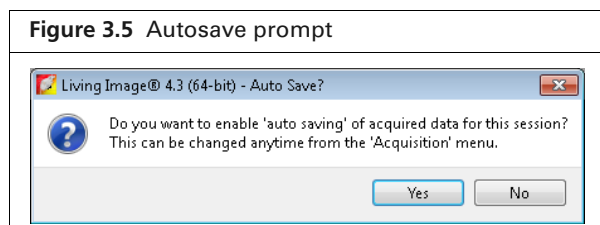
7. Enter information about the image in the Edit Image Labels box that appears (optional). Click **OK**.



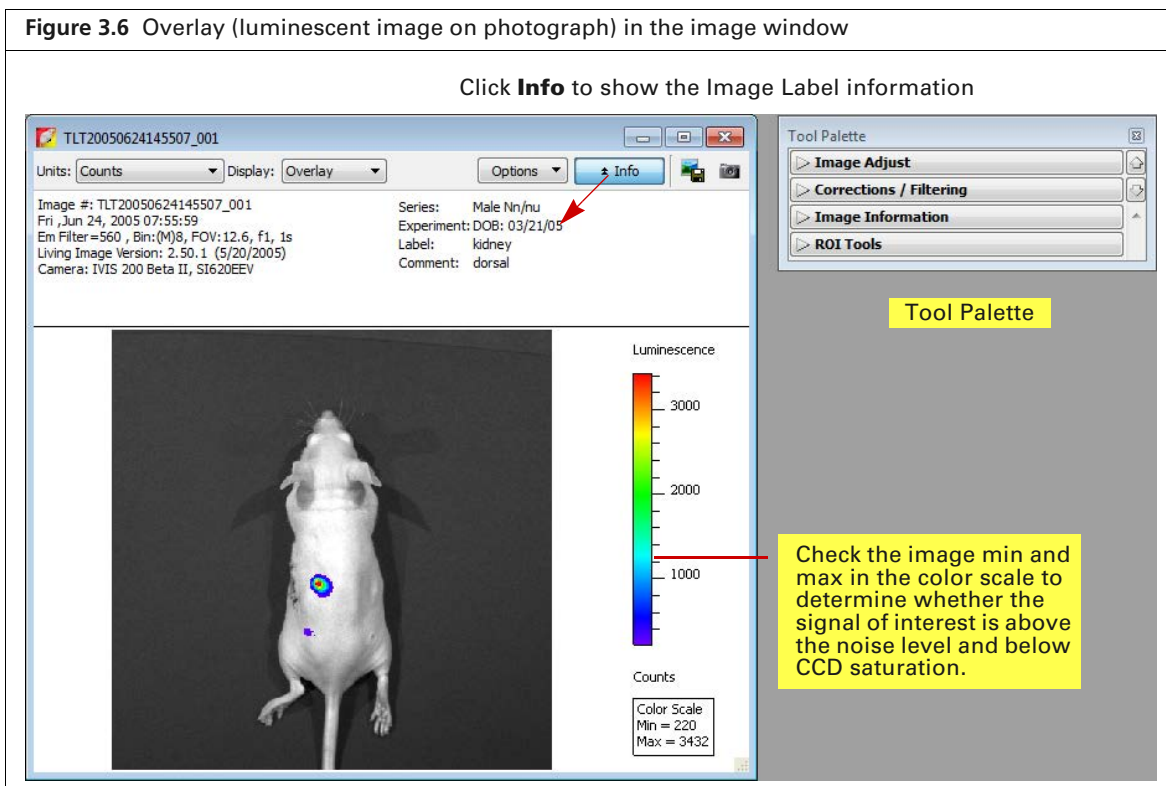
NOTE: You can enter image label information at any time during or after acquisition. If you do not want to enter image information, click **Cancel**. See page 58 for details on adding information to an image after acquisition.



If this is the first image of the session, you are prompted to enable the autosave function ([Figure 3.5](#)). When Autosave is enabled, all images acquired during the session are automatically saved to a user-selected location. A different location can be chosen at any time (select **Acquisition** → **Auto-Save** on the menu bar).



8. Click **Yes** in the prompt to enable autosave, then choose a location in the dialog box that appears. Alternatively, click **No** in the prompt and manually save the image data. See [page 46](#) for details. Image acquisition begins and the upper area of the control panel changes to red color. During acquisition, the **Acquire** button in the control panel becomes a **Stop** button. Click **Stop** to cancel acquisition. The control panel returns to blue color when acquisition is finished and the image window appears ([Figure 3.6](#)).



TIP: See the tech note *Determine Saturation* for information on pixel measurements (select **Help** → **Tech Notes** on the menu bar).

Table 3.2 Image window



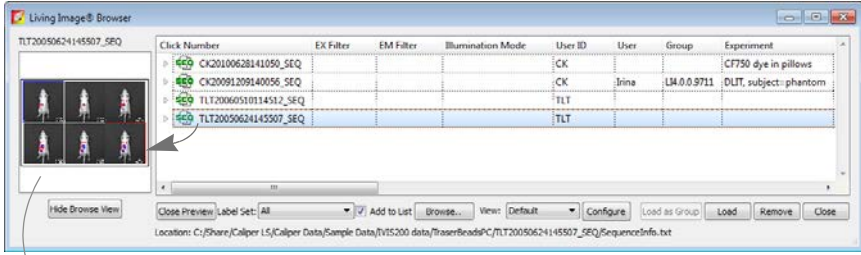
Item	Description
Units	Select the measurement units for the image display from this drop-down list. The available units depend on the type of image data. See the concept tech note <i>Image Display and Measurement</i> for more details (select Help → Tech Notes on the menu bar).
Display	A list of image types available for display, for example, overlay. For more details on the different types of image displays, see Table 2.2 on page 10 .
Info	Click to display or hide the image label. The image label includes information you enter in the Edit Image Labels dialog box (Figure 3.6) and other image information automatically recorded by the software.
	Opens a dialog box that enables you to export the active view as a graphic file.

Table 3.2 Image window (continued)

Item	Description
	Creates a preview picture (<i>snapshot</i>) of the image or thumbnails that the Living Image Browser displays when the data are selected in the browser. For more details on the browser, see page 48 .
	Preview picture of the data selected in the browser (blue row)
Color Scale	Provides a reference for the pixel intensities in a luminescent or fluorescent image. Pixels less than the color scale minimum do not appear in the image. Pixels greater than the color scale maximum are displayed in the maximum color.

3.2 Fluorescent Imaging With Epi-Illumination

Fluorescent imaging captures signals from fluorescent molecular reporters. This section explains how to acquire a single fluorescent optical image with epi-illumination (excitation light source located above the stage):

- Quick guide – See [Figure 3.7 on page 27](#).
- Detailed instructions – See [page 22](#).


See [page 33](#) for information on acquiring a fluorescent sequence.



TIP: See the concept tech note *Fluorescent Imaging* for more about fluorescence imaging theory (select **Help** → **Tech Notes** on the menu bar).

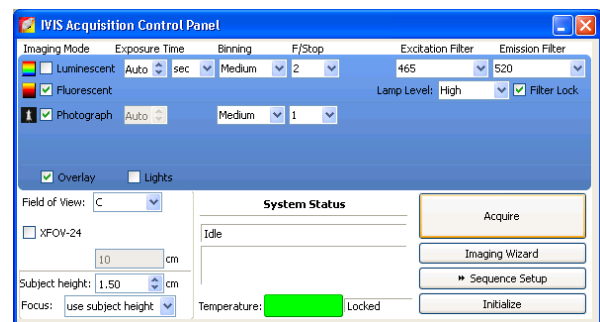
Quick Guide: Acquire a Fluorescent Image With Epi-Illumination

Figure 3.7 Quick Guide – Acquire a fluorescent image with epi-illumination

1. Start the Living Image® software  and initialize the IVIS® Lumina II (page 5).
Note: See the *IVIS Lumina II Hardware Manual* (part no. 117110_Rev01) for more information on the instrument.
2. Place the anesthetized subjects in the imaging chamber and close the door.

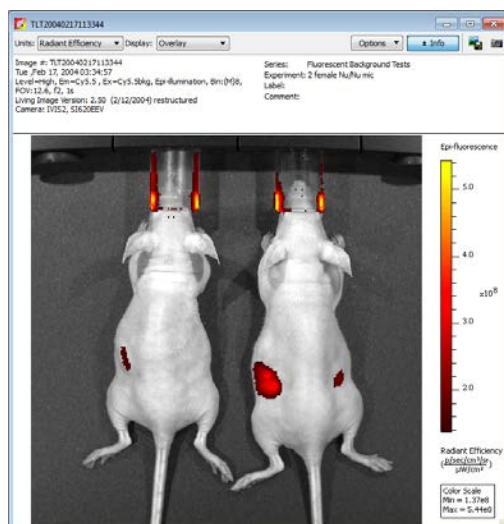


3. Put a check mark next to “Fluorescent” and select “Auto” exposure.
4. Select an excitation and emission filter.
5. Choose “Photograph” and “Overlay”.
6. Select “Use subject height” and enter the height in centimeters.
7. Click **Acquire**.



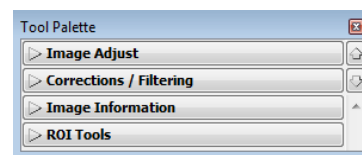
8. When prompted, select a location for the image data (optional). Image data acquired during the session will be automatically saved to this location.
9. Enter experiment and subject information in the dialog box that appears (optional).
The image window and tool palette appear when acquisition is finished.

Image Window



See [Table 3.2 on page 25](#) for more details on the image window.

Tool Palette



The Tool Palette includes the:

- Image Adjust tools ([page 61](#))
- Corrections/Filter tools ([page 63](#))
- Image Information tools ([page 57](#))
- ROI Tools ([page 82](#))

Acquire a Fluorescent Image

This section provides detailed instructions for fluorescent image acquisition with epi-illumination (excitation light source located above the stage).



NOTE: The IVIS® Lumina II should be initialized and the temperature locked before setting the imaging parameters in the control panel. See [page 7](#) for more details.

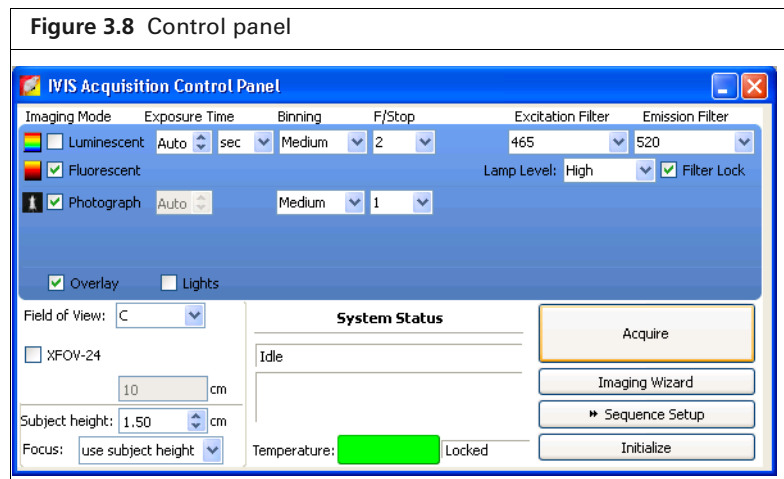
- Put a check mark next to **Fluorescent** and select **Auto** exposure (click the arrows) in the control panel.

The software automatically determines the binning and F/Stop settings.



TIP: See the tech note *Auto-Exposure* for helpful information (select **Help** → **Tech Notes** on the menu bar).

Alternatively, manually set the exposure, binning, and F/Stop. See [Table B.1 on page 164](#) for details on these parameters.



- Select an excitation and emission filter from the drop-down lists.

The instrument has 10 narrow band excitation filters that span 415-760nm with a 30nm bandwidth. There are five emission filter wheel options.

[Table 3.3](#) shows the wavelength range for the standard filters set and the center wavelength for the low range, mid range, mid-high range and high range emission filters which have a 20nm bandwidth.

Table 3.3 Standard filter sets for use with the IVIS® Lumina II

Standard High Resolution Excitation Filter Set (nm)	Emission Filter Option (nm)
430	Standard: 515 – 575, 575 – 650, 695 – 770, 810– 875
465	Low Range: 500, 520, 540, 560, 580, 600, 620
500	Mid Range: 580, 600, 620, 640, 660, 680, 700
535	Mid High Range: 640, 660, 680, 700, 720, 740, 760
570	High Range: 720, 740, 760, 780, 800, 820, 840
605	
640	
675	
710	
745	

- Put a check mark next to Photograph.
- Select a Field of View (size of the stage area to be imaged).



TIP: See the concept tech note *Detection Sensitivity* for more information about the Field of View (select **Help** → **Tech Notes** on the menu bar).

Table 3.4 Field of View (FOV) settings

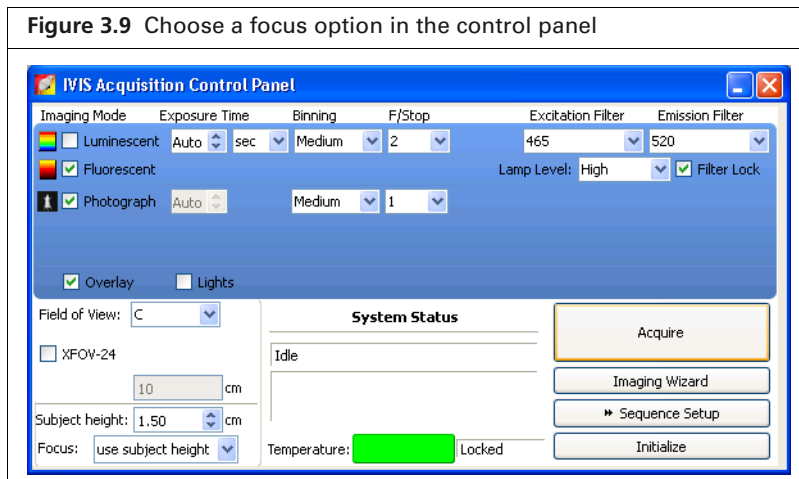
FOV Setting	FOV (cm)
A	4
B	6.5
C	13
D	19.5
E ¹	24
Z ²	2.6

¹Position E is available when the XFOV-24 lens (part no. 123177) is installed.

²Position Z is available when the ZFOV-2.6 lens (part no. 127285) is installed.

- Select a focus option ([Figure 3.9](#)).

The focal distance to the camera is set a stage $z = 0$ for each field of view. To focus at the top of the animal, the stage moves down so that the top of the animal is at $z = 0$. You can enter the height of the animal and select the “use subject height” option or use the manual focus option to determine the proper subject height for the area to be imaged. See [Appendix B on page 167](#) for manual focus instructions.



6. Select **Overlay** to view an overlay image (registered photograph and fluorescent image) after acquisition.



NOTE: If you want to check the subject inside the chamber before acquisition, take a photograph—uncheck the Fluorescent option, choose the Photograph option, and click **Acquire**. Be sure to check the Fluorescent option after taking the photograph.

7. Click **Acquire** when you are ready to capture the image.

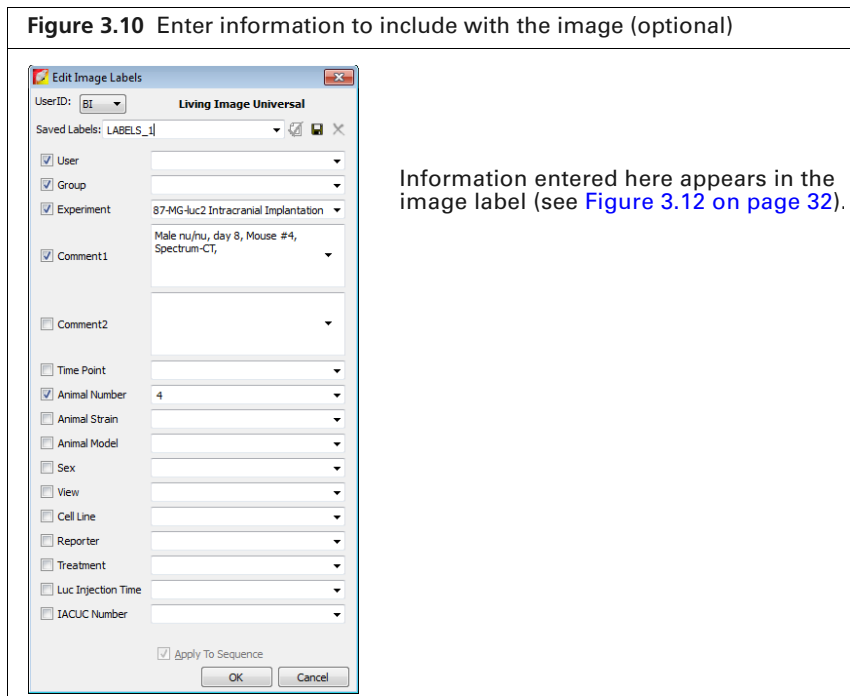


NOTE: If necessary click **Image Setup** in the control panel to operate in single image mode. In single image mode, the **Sequence Setup** button appears in the control panel. Use this button to set up sequence acquisition (see page [page 33](#) for more details on sequence setup).

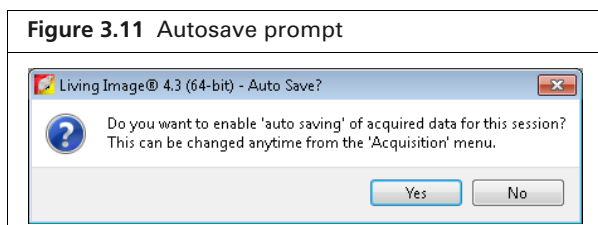
8. Enter information about the image in the Edit Image Labels box that appears (optional). Click **OK**.



NOTE: You can enter image label information at any time during or after acquisition. If you do not want to enter image information, click **Cancel**. See [page 58](#) for details on adding information to an image after acquisition.

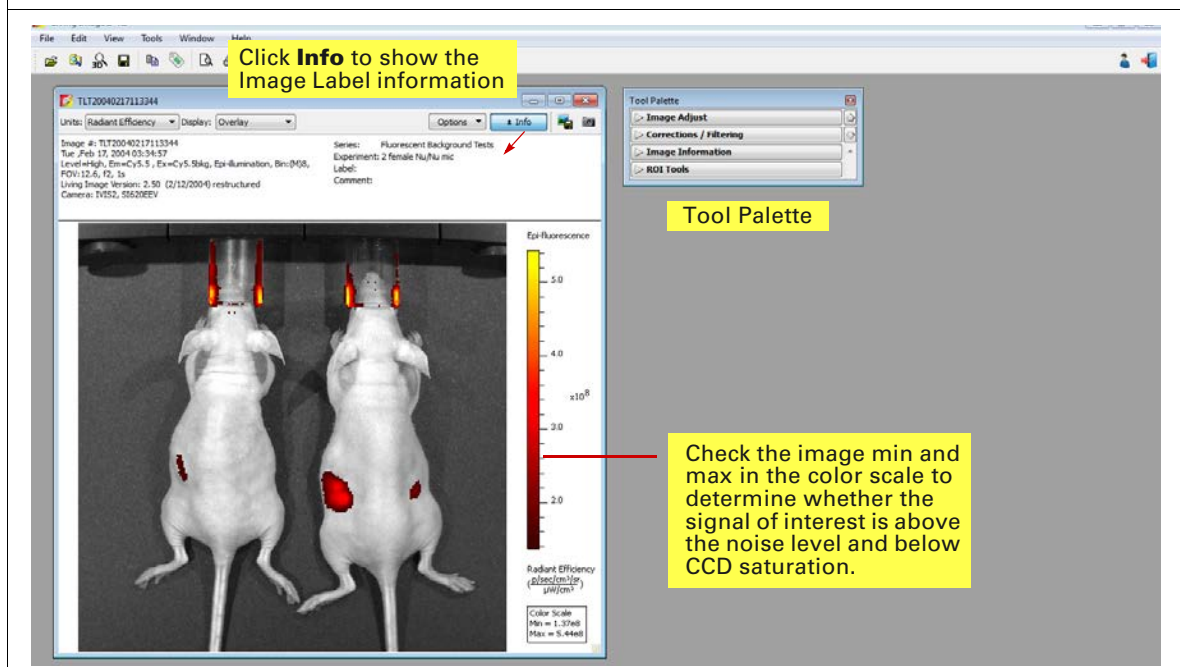


If this is the first image of the session, you are prompted to enable the autosave function ([Figure 3.11](#)). When Autosave is enabled, all images acquired during the session are automatically saved to a user-selected location. A different location can be chosen at any time (select **Acquisition** → **Auto-Save** on the menu bar).



- Click **Yes** in the prompt to enable autosave, then choose a location in the dialog box that appears. Alternatively, click **No** in the prompt and manually save the image data. See [page 46](#) for details. Image acquisition begins and the upper area of the control panel changes to red color. During acquisition, the **Acquire** button in the control panel becomes a **Stop** button. Click **Stop** to cancel acquisition. The control panel returns to blue color when acquisition is finished and the image window appears ([Figure 3.12](#)).

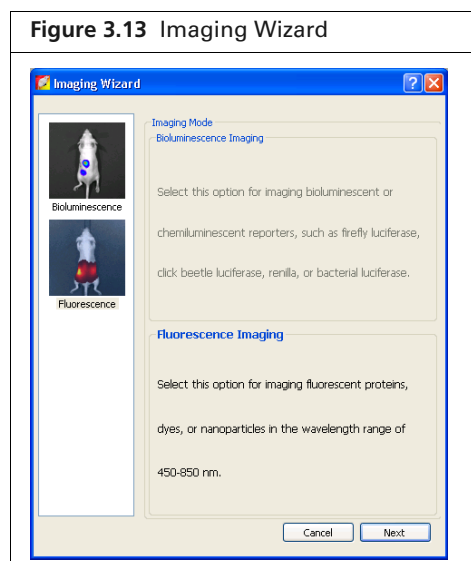
Figure 3.12 Overlay (fluorescent image on photograph) in the image window
See [Table 3.2 on page 25](#) for details on the image window.



TIP: See the tech note *Determine Saturation* for information on pixel measurements (select **Help** → **Tech Notes** on the menu bar).

3.3 Acquire a Sequence Using the Imaging Wizard

The Imaging Wizard (Figure 3.13) provides a convenient way to set up a sequence for some imaging applications (see Table 3.5 and Table 3.6 on page 35). The acquisition parameters for each image in a sequence must be specified. The wizard guides you through a series of steps, prompting you for the information that the software needs to set up the sequence.



This section explains how to use the Imaging Wizard and acquire a sequence of luminescent or fluorescent images. A sequence can also be set up manually (see page 41 for details).



TIP: See the *Imaging Wizard* tech note for a quick guide (select **Help** → **Tech Notes** on the menu bar).

Set Up a Sequence



NOTE: The IVIS® Lumina II should be initialized and the temperature locked before setting up the imaging parameters. See page 7 for more details.



1. Click **Imaging Wizard** in the control Panel (Figure 3.14).
2. If necessary, click **Restart** in the Imaging Wizard to show the first page of the wizard.
3. Double-click Bioluminescence  or Fluorescence  imaging.

Figure 3.14 Opening the Imaging Wizard

If this screen does not appear when the wizard starts, click **Restart Wizard** (on the wizard screen) to restart the wizard.

- Click **Next** in the wizard and choose the type of image sequence to acquire. See [Table 3.5](#) and [Table 3.6](#) on page 35 for more information on the imaging options.



NOTE: DyCE acquisition requires a separate license. The DyCE option appears in the Imaging Wizard if the software is appropriately licensed. Select **Help** → **License Information** to view the Living Image® license details.

Figure 3.15 Choose the type of image sequence

Imaging Wizard – Bioluminescence options

Imaging Wizard – Fluorescence options

Table 3.5 Imaging Wizard – bioluminescence imaging options

Option	Description	See Page
Open Filter	Acquires a luminescent image at maximum sensitivity.	
DyCE	Acquires a time series of optical images following a bolus injection of probe (radiotracer, bioluminescent, or fluorescent) to track probe biodistribution. Note: DyCE imaging and analysis requires a separate license.	140

Table 3.6 Imaging Wizard – fluorescence imaging options

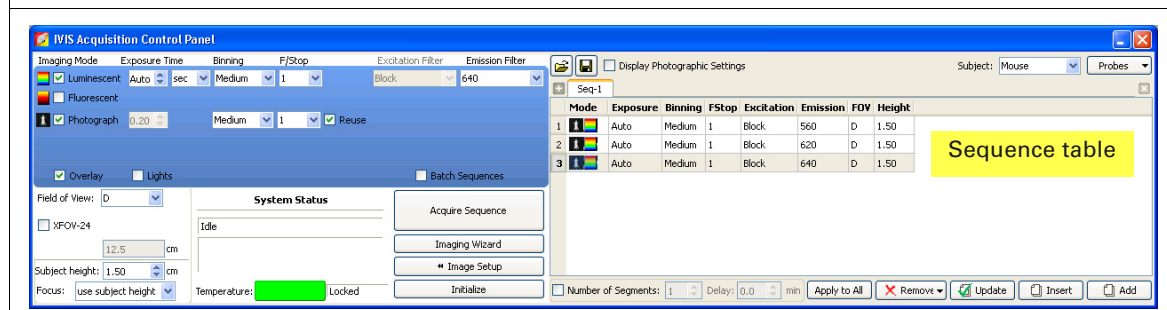
Option	Description	See Page
Filter Pair	Choose this option to acquire measurements of one or more fluorescent probes.	
Spectral Unmixing/ Filter Scan	Acquires an image sequence for analysis when more than one reporter is used in the same animal model. Acquires an image sequence for analysis with the Spectral Unmixing tools to: <ul style="list-style-type: none"> ■ Extract the signal of one or more fluorophores from the tissue autofluorescence. ■ Determine the optimum excitation and emission filter for a probe. 	119
DyCE	Acquires a time-series of optical images following a bolus injection of radiotracer to enable detection of radiotracer distribution by tracking Cerenkov emission from charged decay products. Note: DyCE imaging and analysis requires a separate license.	140

5. Step through the rest of the wizard.

Each page of the wizard guides you with step-by-step instructions and descriptions. When you finish the wizard, it sets up the sequence to acquire (Figure 3.16).

Figure 3.16 Control panel and sequence setup

Each row in the sequence table specifies the acquisition parameters for one image in the sequence. See page 42 for details on the sequence table.



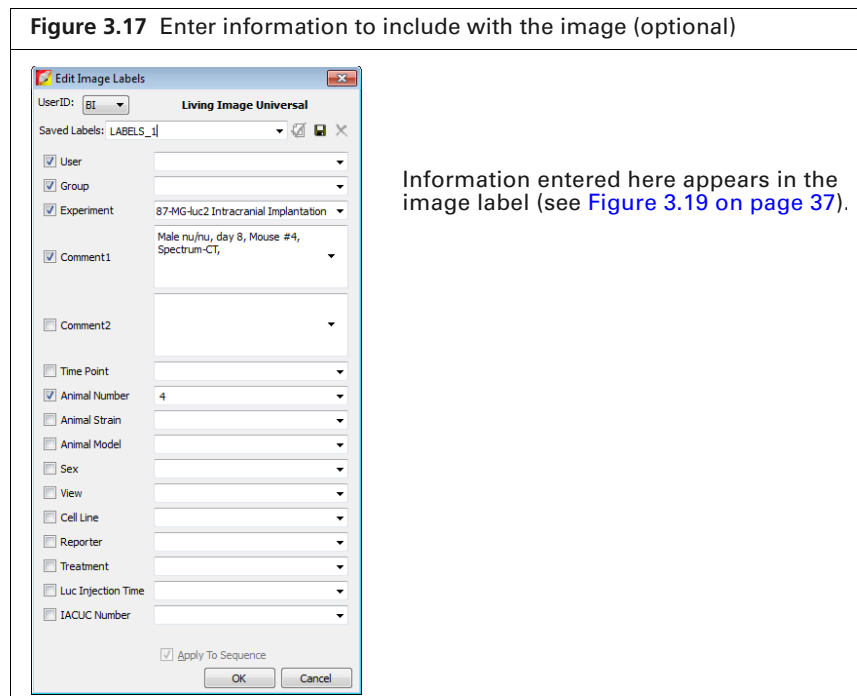
6. To clear the sequence, click the **Remove** button  and select **All**.

Acquire the Sequence

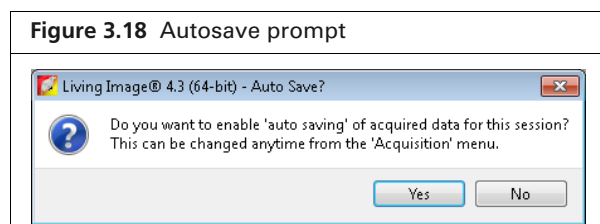
1. Confirm that the IVIS® Lumina II is initialized and the CCD temperature is locked. (See [page 7](#) for details.)
2. Click **Acquire Sequence** in the control panel when ready to begin acquisition.
3. Enter information about the image in the Edit Image Labels box that appears (optional). Click OK ([Figure 3.17](#)).



NOTE: You can enter image label information at any time during or after acquisition. Click **Cancel** if you do not want to enter image information.



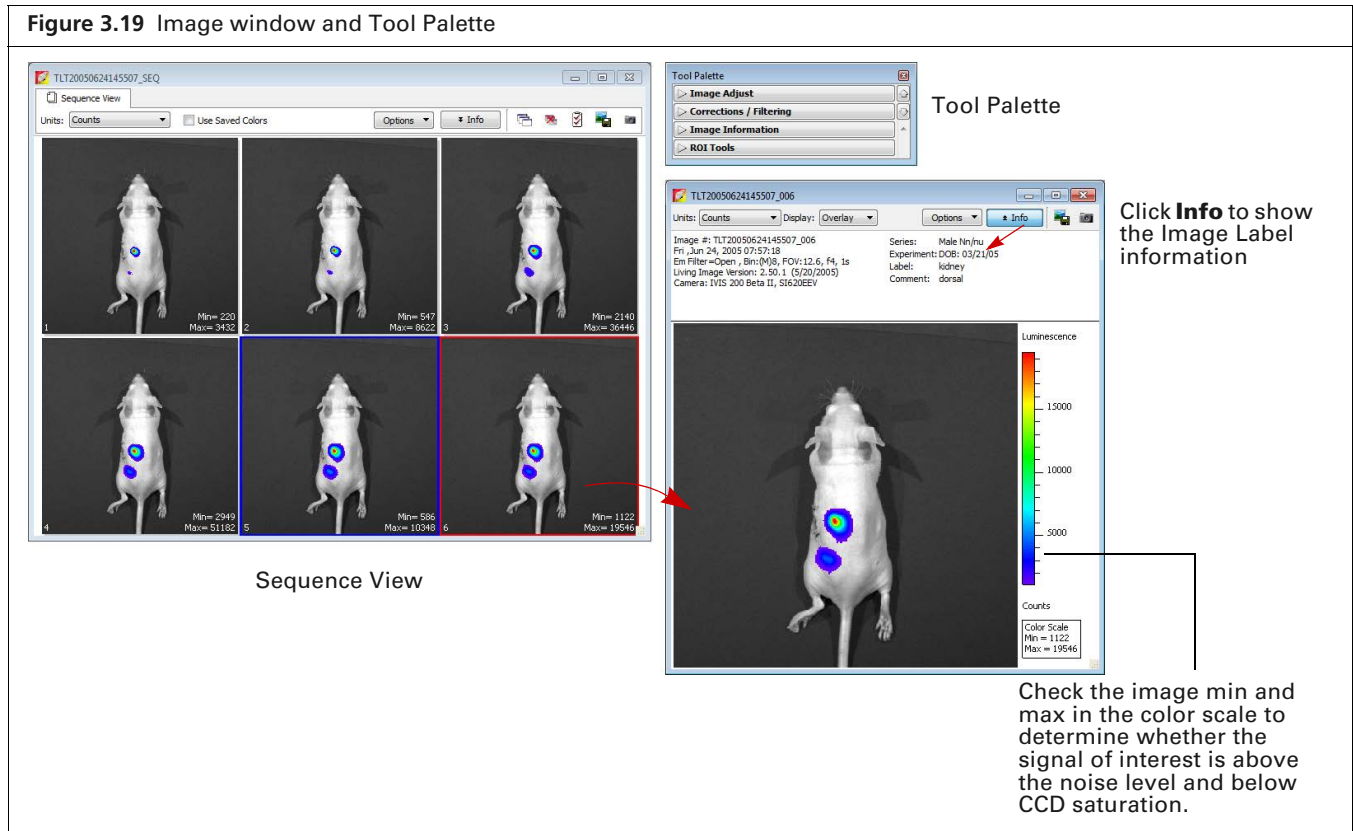
If this is the first image of the session, you are prompted to enable the autosave function ([Figure 3.18](#)). When Autosave is enabled, all images acquired during the session are automatically saved to a user-selected location. A different location can be chosen at any time (select **Acquisition** → **Auto-Save** on the menu bar).



4. Click **Yes** in the prompt to enable autosave, then choose a location in the dialog box that appears. Alternatively, click **No** in the prompt and manually save the image data. See [page 46](#) for details. Image acquisition begins and the upper area of the control panel changes to red color. During acquisition, the **Acquire** button in the control panel becomes a **Stop** button. Click **Stop** to cancel acquisition.

The image window displays the images as they are acquired. The control panel returns to blue color when acquisition is finished and the Tool Palette appears (Figure 3.19).

Figure 3.19 Image window and Tool Palette



TIP: See the tech note *Saturated Pixels In an Image* for information on pixel measurements (select **Help** → **Tech Notes** on the menu bar).

Table 3.7 Sequence View window

Item	Description
Units	Select the measurement units for the image display from this drop-down list. The available units depend on the type of image. See the concept tech note <i>Image Display and Measurement</i> for more details (select Help → Tech Notes on the menu bar).
Use Saved Colors	Choose this option to display the image data using the color table that was specified in the Preferences at the time of acquisition. If this option is not selected, image data are displayed using the color table currently specified in the Preferences.

Table 3.7 Sequence View window (continued)

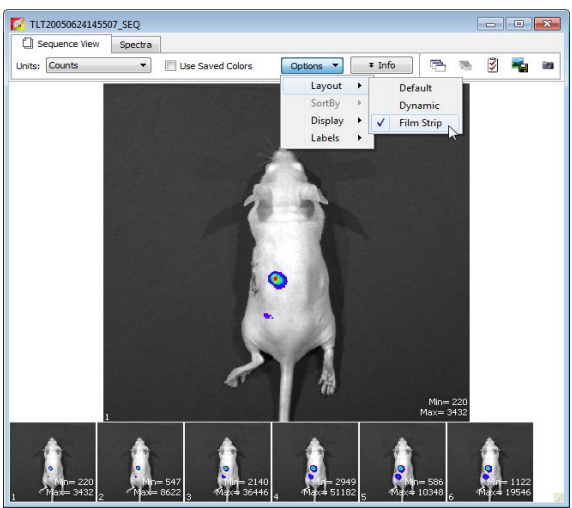
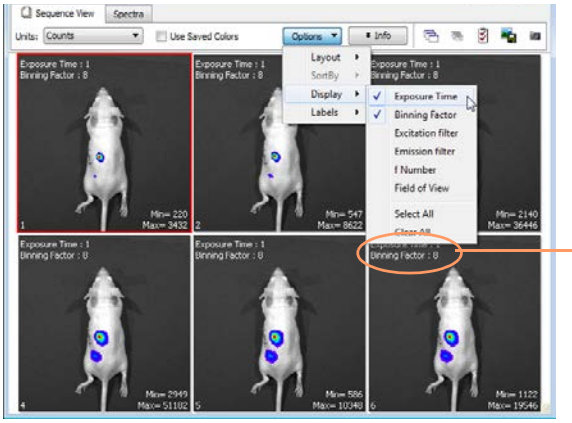





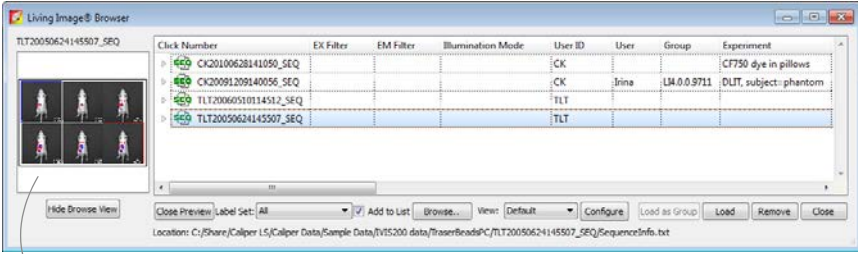
Item	Description
Options	Layout - Choose a display option for the images in a sequence (Default, Dynamic, or Film Strip). For example, here is Film Strip mode:
	
Sort by - Options for ordering images in the sequence window. This option only applies to images that were opened using the “Load as Group” function in the Living Image browser. Default - Order in which the images are stored in the folder. TimeStamp - Ascending order of the image acquisition time. UserID - Ascending alphanumeric order of the user ID	
Display - Choose the types of information to display with each image.	
	
In this example, exposure time and binning factor are displayed on each image	
Info	Click to show or hide the image label information (Figure 3.19).
	Opens all of the images in the sequence.
	Closes all open images.
	Opens the Edit Sequence dialog box that enables you to add or remove images from the sequence.
	Enables you to export the active image as a graphic file (for example, .png, .dcm).

Table 3.7 Sequence View window (continued)

Item	Description
	Creates a preview picture (<i>snapshot</i>) of the image or thumbnails that the Living Image Browser displays when the data are selected. See page 48 for more details on the browser.
	Preview picture of the selected data

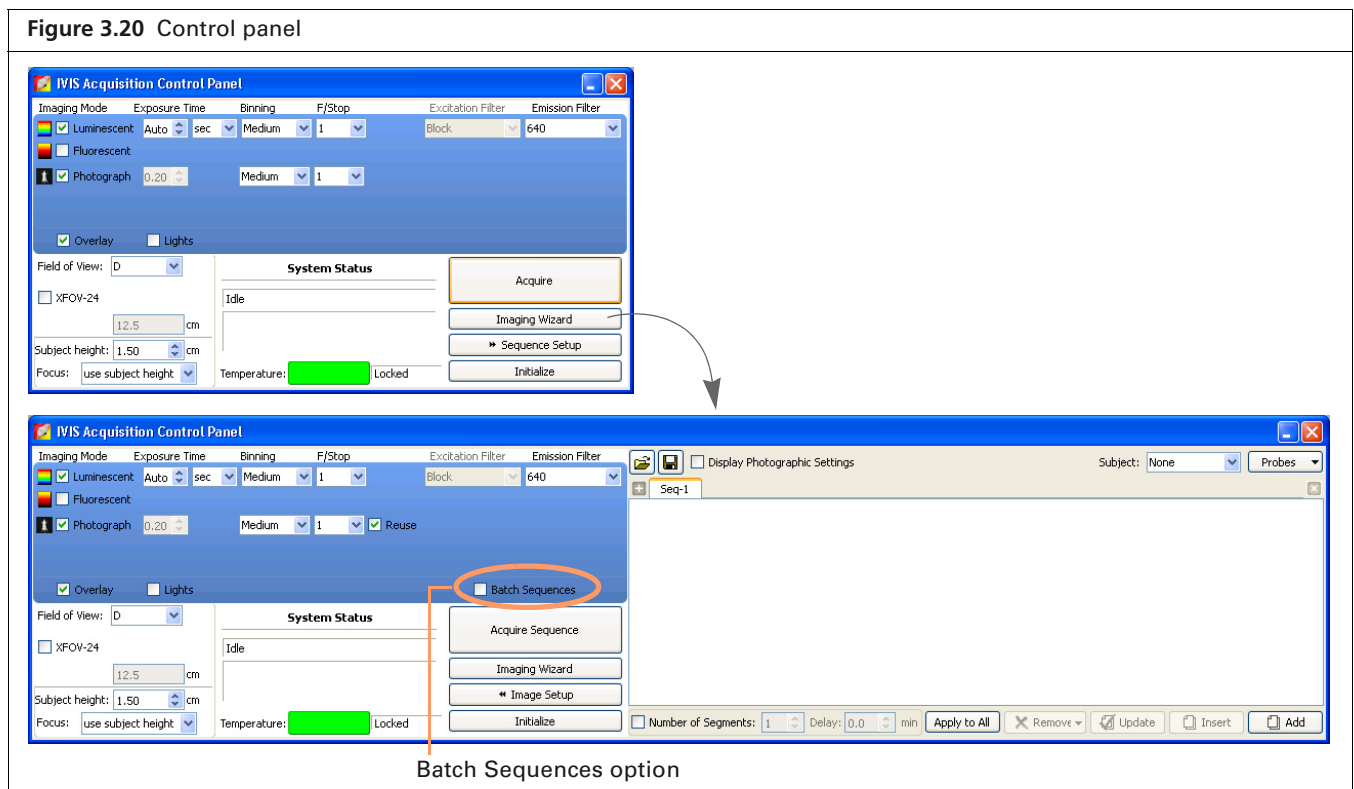
3.4 Acquire Multiple Sequences in Batch Mode

Use the batch mode to set up multiple, separate sequences which will be automatically acquired, one after another, without manual intervention.

To setup and acquire sequences in batch mode:


1. Click **Sequence Setup** in the control panel.
2. Choose the Batch Sequences option ([Figure 3.20](#)).

Figure 3.20 Control panel



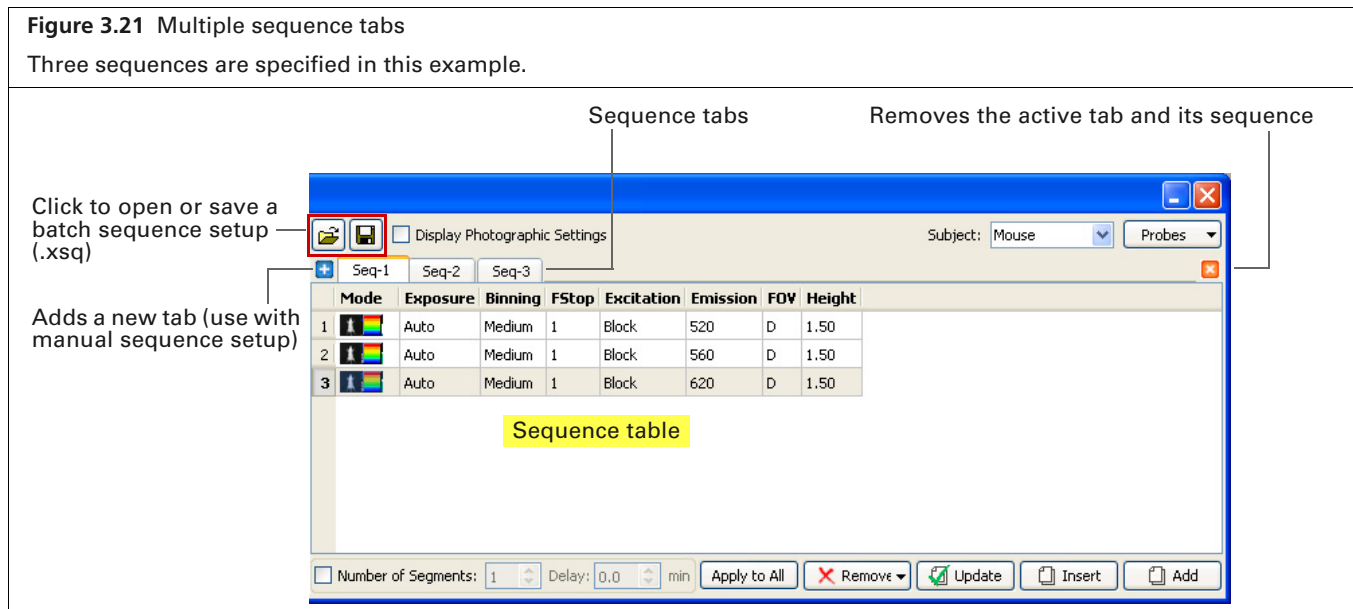
3. To set up the first sequence, do either of the following:
 - Click Imaging Wizard and step through the wizard (see [page 33](#) for details).


OR

- Set up the sequence manually (see [page 41](#) for details).
4. To set up the next sequence:
 - If using the Imaging Wizard, repeat [step 3](#).
Each sequence is displayed in a separate tab.
 - If setting up the sequence manually, click the button  in the sequence table to add a new tab, then proceed with manual setup in the new tab.



NOTE: Sequence tabs can be renamed. Double-click a tab name to edit it. Alternatively, right-click the selected name to view a shortcut menu of edit commands (for example, Cut, Copy, Paste).




5. To remove a sequence, click the sequence tab and then click the  button.
6. Click **Acquire Sequence** when you are ready to capture the sequences.
Image acquisition proceeds with no intervening time delay between sequences.



NOTE: If the check mark is removed next to the Batch Sequences option in the control panel ([Figure 3.20](#)), only the sequence in the active tab will be acquired.

To save the batch sequence setup:

1. Click the Save button .
2. Enter a file name (.xsq) and choose a location for the file in the dialog box that appears.

3.5 Manually Set Up a Sequence

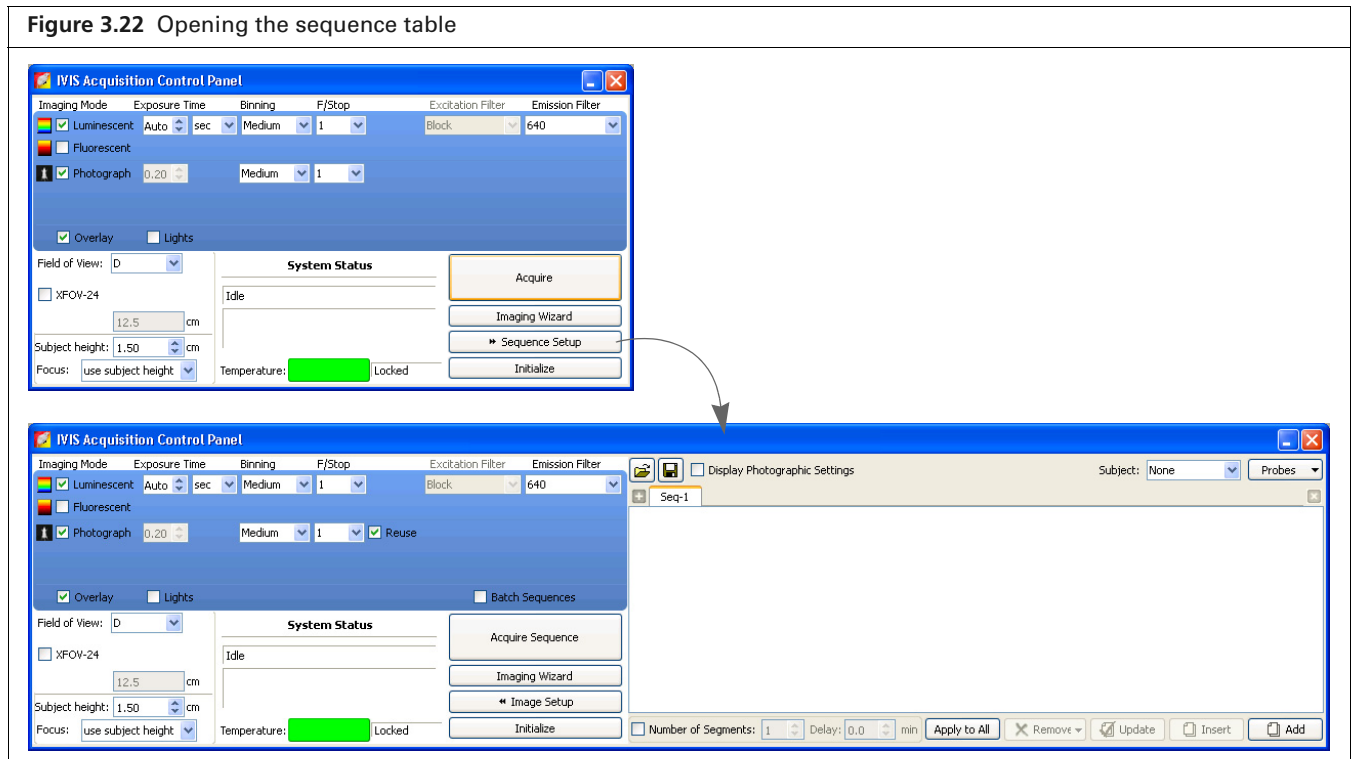
This section explains how to set up an image sequence if you do not use the Imaging Wizard. The sequence parameters in the sequence table can be saved as a Living Image Sequence Setup file (.xsq).

For details on image acquisition, see *Acquire the Sequence* on page 36.

TIP: It may be convenient to create an image sequence by editing a sequence setup generated with the Imaging Wizard or an existing sequence setup (.xsq). Save the modified sequence setup to a new name.

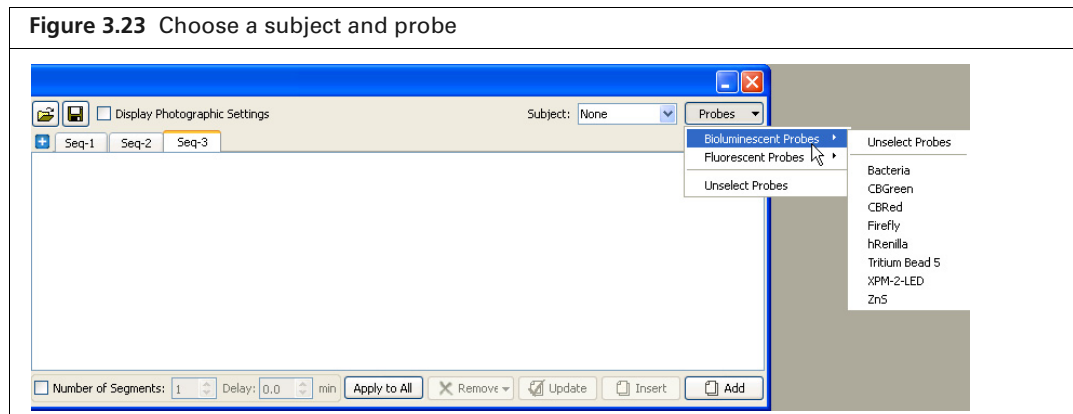
1. Click Sequence Setup in the control panel (Figure 4.30).
The sequence table appears.
2. If necessary, click the **Remove** button  and select **All** to clear the sequence table.

Figure 3.22 Opening the sequence table



3. Choose a subject and probe from the drop-down lists (Figure 3.23)

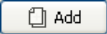
Figure 3.23 Choose a subject and probe



- Specify the imaging settings for the first image in the sequence. (See [Appendix B](#) on page 164 for details on the imaging parameters in the control panel.)



NOTE: If you selected Photograph and the photograph Reuse option in the control panel ([Figure 3.24](#)), the IVIS® Lumina II acquires only one photograph for the entire sequence. If this option is not chosen, the system acquires a photograph for each image in the sequence.

- Click the **Add** button .


The acquisition parameters appear in the sequence table ([Figure 3.24](#)).
- Repeat [step 4](#) to [step 5](#) for each image in the sequence.
- To set a time delay between each acquisition, enter a time (minutes) in the Delay box in the sequence table.
- To save the sequence setup information (.xsq):
 - Click the **Save** button  in the sequence table.
 - Select a directory, enter a file name, and click **Save** in the dialog box that appears.

Figure 3.24 Control panel and sequence table with image settings

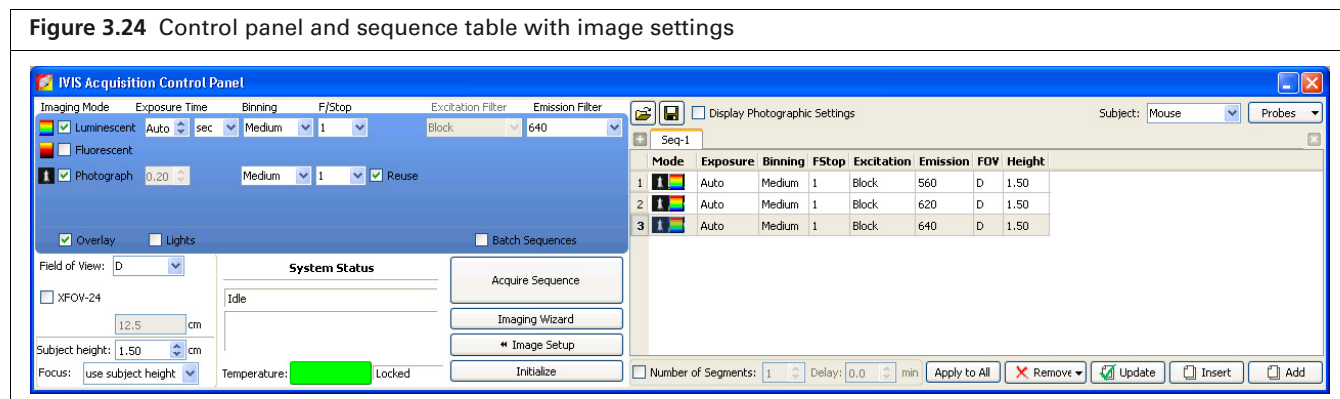


Table 3.8 Sequence table

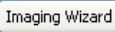




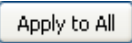


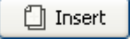
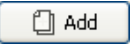
Item	Description
	Starts the Imaging Wizard.
	Displays a dialog box that enables you to select and open a sequence setup (.xsq), sequenceinfo.txt, or clickinfo.txt file.
	Displays a dialog box that enables you to save the information in the sequence table to a sequence setup file (.xsq).
Display Photographic Settings	Choose this option to include the photograph exposure time, binning, and F/Stop in the sequence table.
Subject:  Mouse  Probes	If a subject and probe are specified (optional), the software uses the information to automatically set parameters in the Spectral Unmixing and Planar Spectral Imaging tools. If a subject or probe is not selected here, the default parameters appear in the Tool Palette.
Number of Segments	The sequence specified in the sequence table is called a <i>segment</i> . Choose this option to set the number of segments to acquire and the time delay between segments. This is useful for acquiring data for kinetic analysis.
Delay	Specifies a time delay between each segment acquisition.

Table 3.8 Sequence table (continued)

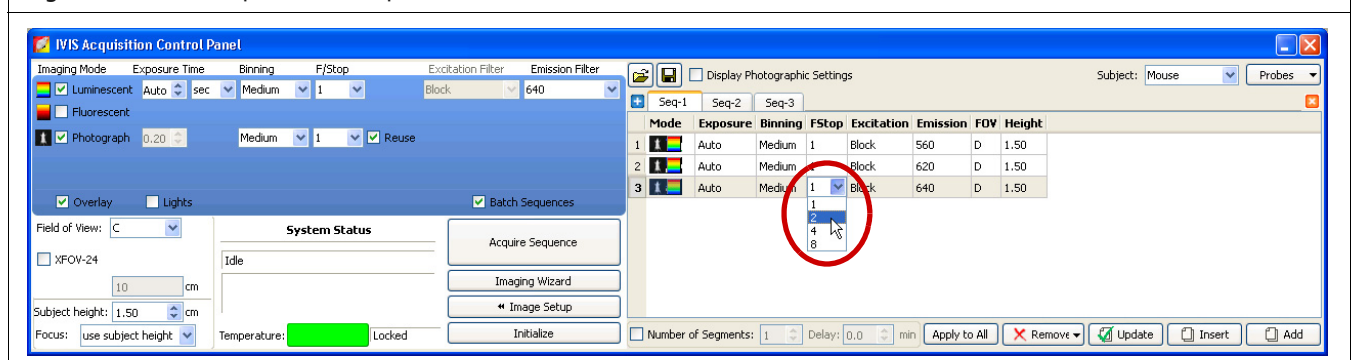
Item	Description
	Applies the selected cell value to all cells in the same column.
	Remove Selected - Deletes the selected row from the sequence table. Remove All - Removes all rows from the sequence table.
	Updates the selected row in the sequence table with the acquisition parameters in the control panel.
	Inserts a row above the currently selected row using the information from the control panel.
	Adds a new row at the end of the sequence setup list.

Editing Image Parameters

You can edit imaging parameters in the sequence table or in the control panel.


To edit a parameter in the sequence table:

1. Double-click the cell that you want to edit (Figure 3.25).

Figure 3.25 Control panel and sequence table


2. Enter a new value in the cell or make a selection from the drop-down list. To apply the new value to all of the cells in the same column, click .
3. Click outside the cell to lose focus.

To edit a parameter in the control panel:

1. Select the row that you want to modify in the sequence table.
2. Set new parameter values and/or imaging mode in the control panel.
3. Click  in the sequence table.

Inserting Images in a Sequence

Method 1:

1. Select the sequence table row that is below where you want to insert a new image (row).
2. Set the imaging mode and parameters in the control panel.
3. Click  to insert the new image above the selected row,

Method 2:

1. Select the row(s) of interest and right-click the sequence table to view a shortcut menu of edit commands (Figure 4.34 on page 57).

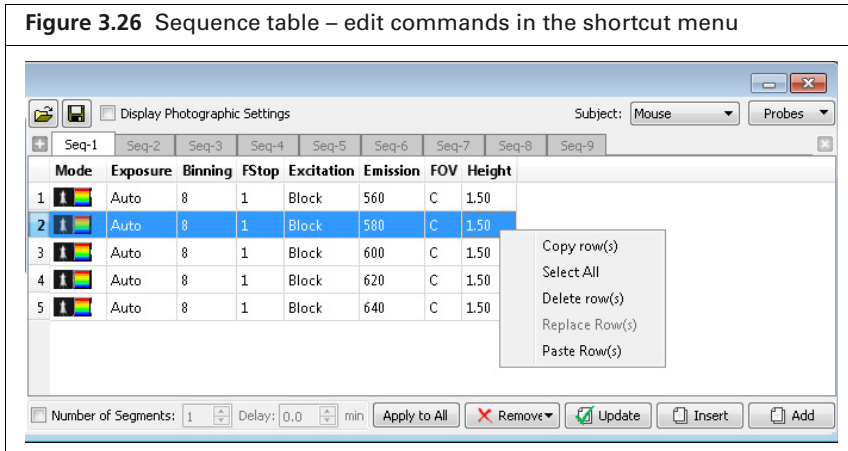


Table 3.9 Sequence table – shortcut menu edit commands

Command	Description
Copy row(s)	Copies the selected row(s) to the system clipboard.
Select All	Selects all rows in the sequence table.
Delete row(s)	Deletes the selected row(s) from the sequence table.
Replace Row(s)	Replaces the row(s) selected in the sequence table with the rows in the system clipboard. Note: The Replace function is only available when the number of rows in the system clipboard is the same as the number of rows selected in the sequence table.
Paste Row(s)	Adds copied rows to end of the sequence.

Removing Images From a Sequence

Method 1:

1. Select the row(s) that you want to delete.
2. Click and choose **Selected** from the drop-down list.

Method 2:

Select the row(s) of interest and right-click the sequence table to view a shortcut menu of edit commands (Figure 3.26).

3.6 High Resolution Imaging

The optional Optical Zoom Lens attachment enables close up and high resolution imaging on the IVIS Lumina, IVIS Lumina XR, and IVIS Kinetic Imaging Systems. When the Zoom lens attachment is installed, only the “Z” field of view setting (2.6 cm) is available for single-image or sequence acquisition.

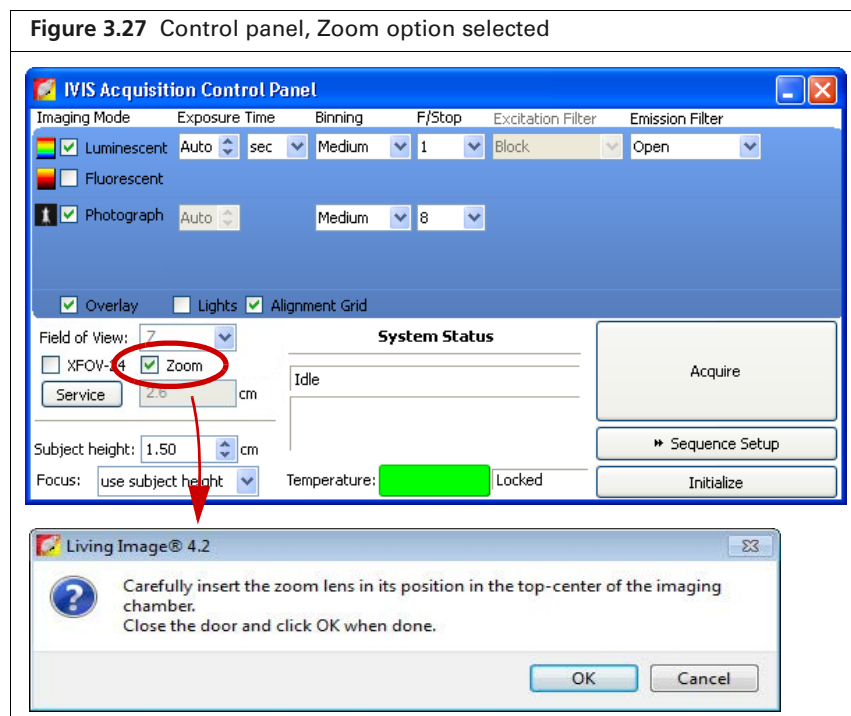
The imaging system is set to the Z field of view position until the Zoom lens attachment is removed. If the Zoom lens attachment is installed when the Living Image software is closed, the stage will move to the Z position when the system is initialized.



NOTE: Avoid touching the optical glass when installing or removing the Optical Zoom Lens attachment.

Installing the Zoom Lens Attachment

1. Choose the Zoom option in the control panel (Figure 3.27).
You are prompted to insert the Zoom Lens attachment.



2. Click **OK** in the prompt after you install the Zoom Lens attachment in the imaging chamber.
The stage moves to the “Z” field of view position.



NOTE: Single images or sequential images can only be acquired at the Z field of view setting when the Zoom Lens attachment is installed. During sequence setup, if you attempt to change away from the Z setting, the sequence table will be cleared. If you attempt to change from another field of view setting to the Z setting, the sequence table is cleared of all previous settings before the camera settings for the Z position are added.

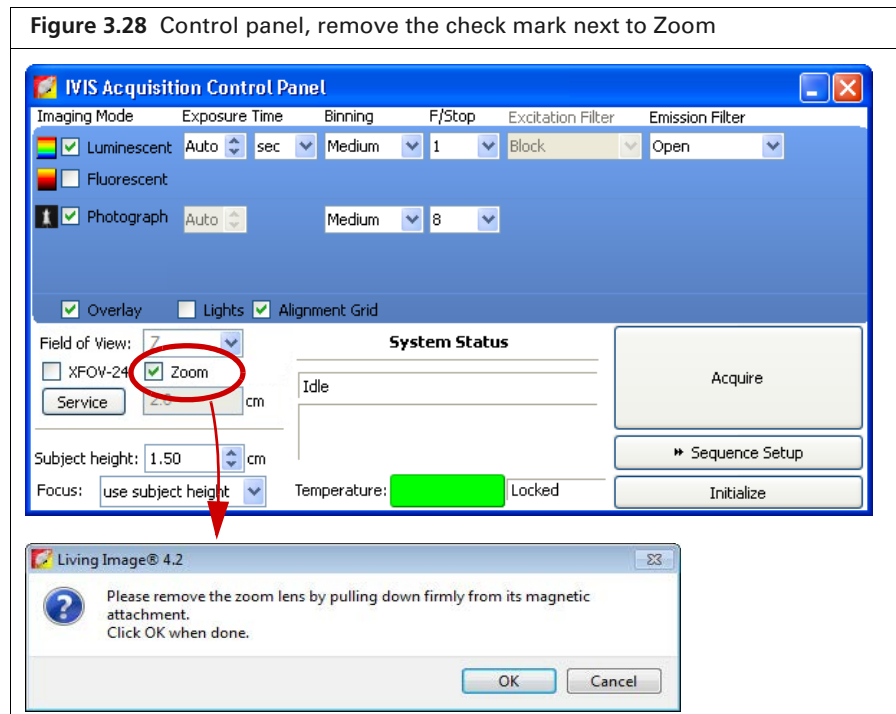
Removing the Zoom Len Attachment

The imaging system is set to the "Z" field of view until the Zoom Lens attachment is removed.

1. Remove the check mark next to Zoom in the control panel (Figure 3.28).
The stage moves to position C, then you are prompted to remove the lens attachment.
2. Click **OK** in the prompt after you remove the Zoom Lens attachment.




NOTE: Always store the lens wrapped in its protective container.



3.7 Manually Saving Image Data

When you acquire the first image(s) of a session, you are prompted to enable the autosave feature. If autosave is enabled, all images acquired during the session are automatically saved to a user-selected location. You can choose a different location at any time (select **Acquisition** → **Auto-Save** on the menu bar).

This section explains how to manually save data if you do not want to use the autosave feature.

1. Turn off the autosave feature (select **Acquisition** on the menu bar and remove the check mark next to **Auto Save**).
2. After image or sequence acquisition, click the **Save** button . Alternatively, select **File** → **Save** on the menu bar.
3. Select a directory in the dialog box that appears, and click **OK**.

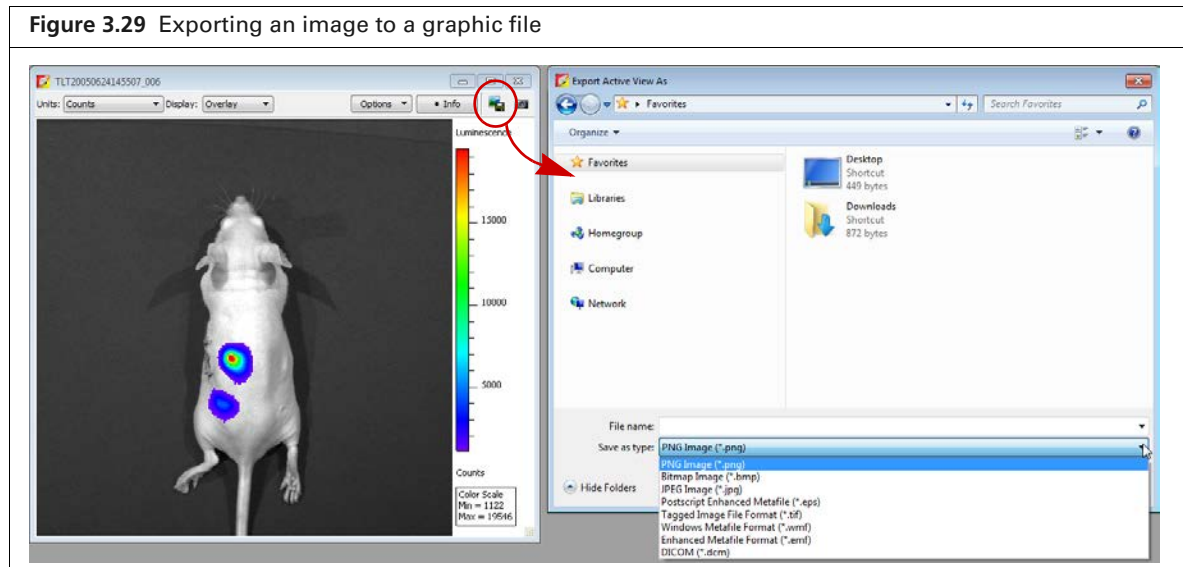


NOTE: The software automatically includes the user ID, and a data and time stamp with the data.

3.8 Exporting Images

The active image view can be saved in different file formats (for example, .bmp, .dcm).

1. Open an image or sequence.
2. Click the **Export Graphics** button  (Figure 3.29).



3. Select a directory in the dialog box that appears and enter a file name.
4. Click **Save**.



NOTE: To export a sequence to DICOM (.dcm) format, select **Export → Image/Sequence** as DICOM on the menu bar. This creates a directory that contains the .dcm files and a SequenceInfo.txt.

4 Working With Optical Image Data

Loading Optical Image Data

About the Image Window and Tool Palette on page 54

Viewing Image Information on page 57

Adding Comments or Tags to an Image on page 59

Adjusting Image Appearance on page 61

Correcting Optical Image Data on page 63

Viewing Intensity Data and Making Measurements on page 65

Overlaying Multiple Images on page 73

Rendering Intensity Data in Color on page 76

Exporting or Printing Images on page 77

Editing an Image Sequence on page 79

Creating an Image Sequence from Individual Images on page 80

4.1 Loading Optical Image Data

You can load (open) optical images from the:

- Living Image Browser (see below)
- Toolbar or menu bar ([page 52](#))

Multiple data sets can be open at the same time.




NOTE: Select **File** → **Recent Files** on the menu bar to view recently opened files.

Loading Optical Images From the Living Image Browser

The Living Image Browser provides a convenient way to browse and preview optical data, view information about the data, and load the data.

To start the browser:

1. Click the **Browse** button . Alternatively, select **File** → **Browse** on the menu bar.
2. In the dialog box that appears, select the folder of interest and click **OK**.

The Living Image Browser appears ([Figure 4.1](#)). It displays all Living Image data located in the folder and its subfolders, along with the user ID, label information, and camera configuration information.



NOTE: The next time you start the Living Image software and open the Browse For Folder box, the software automatically returns to the last folder visited.

Figure 4.1 Opening the Living Image Browser

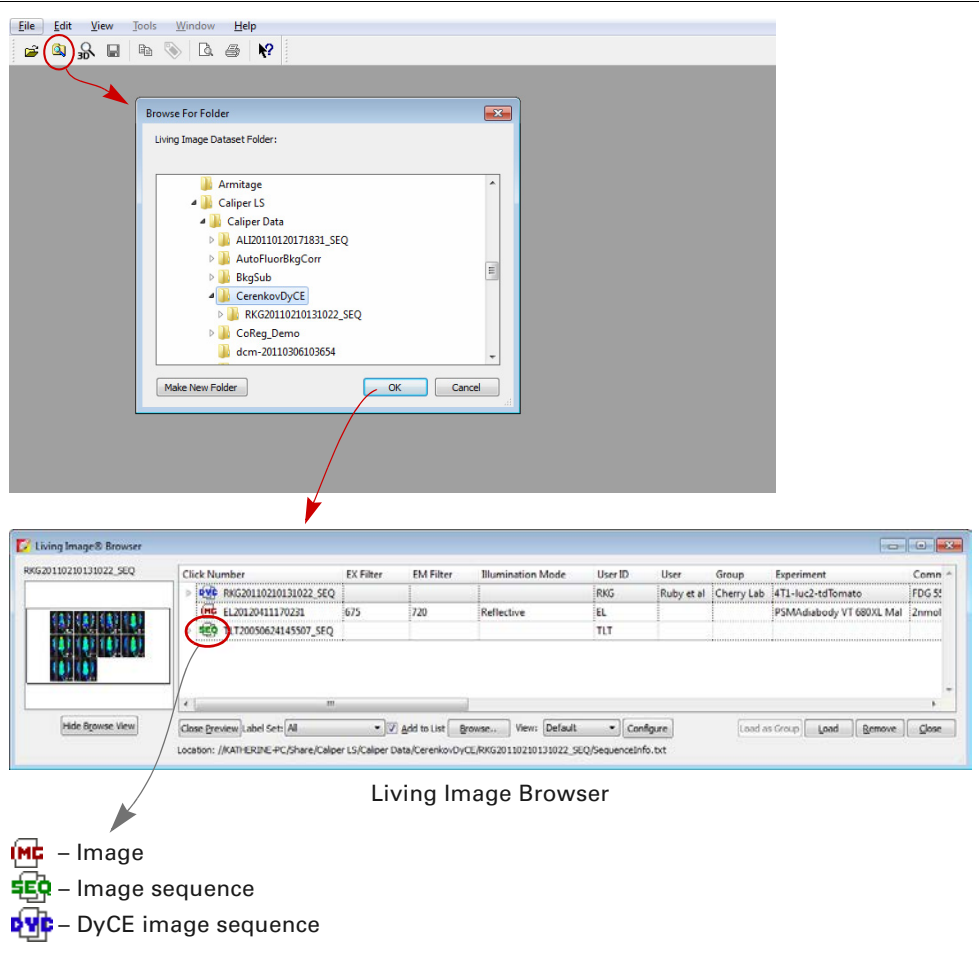


Figure 4.2 Living Image Browser

To expand a sequence, click the arrow next to SEQ.

Click a column header to sort the browser contents in ascending alpha numeric order. Click the column header again to sort in descending alpha numeric order.

To view data properties, right-click a row and select **Properties** on the shortcut menu.

The screenshot shows the Living Image Browser window with a table of sequences. The table has columns: Click Number, EX Filter, EM Filter, Illumination Mode, User ID, User, Group, Experiment, and Comment. A Properties dialog box is open over the table, showing details for a selected sequence: Click No.: CK20090729114835_SEQ, Dataset Type: Sequence Dataset, No. of clicks: 16, Location: C:/Share/Caliper LS/Caliper Data/Sample Data/FLIT/CK20090729114835_SEQ/SequenceInfo.txt.

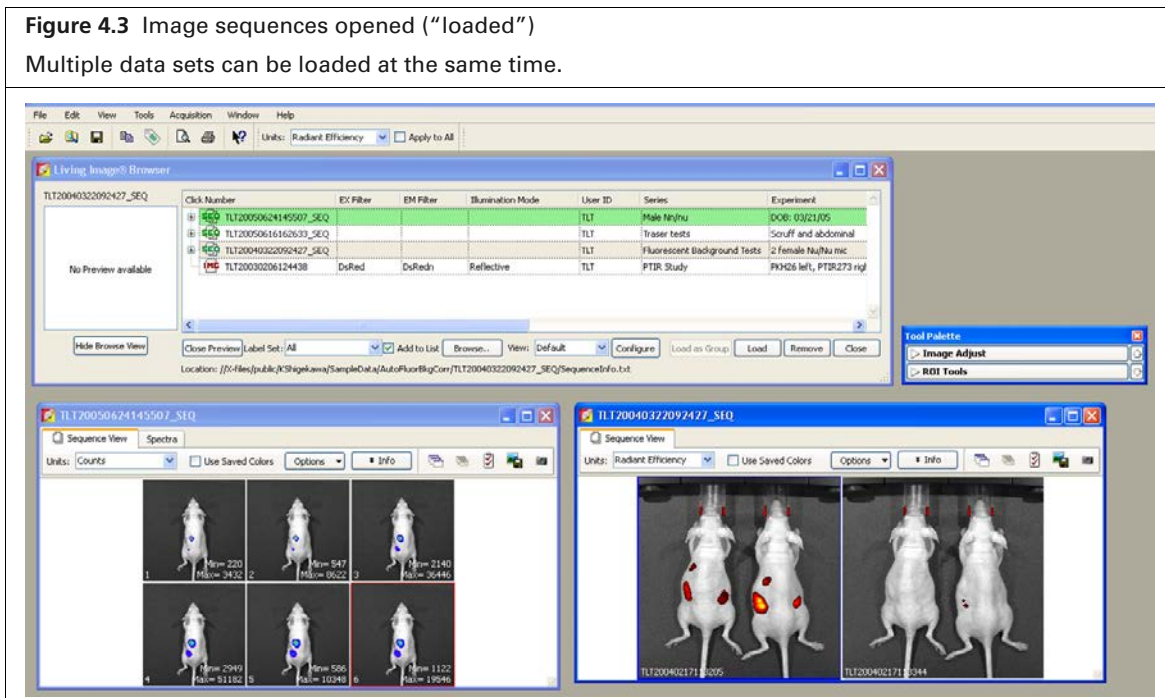
To preview data, click a row.

Note: A preview snapshot is automatically taken at the time of image or sequence acquisition. A snapshot can also be captured manually (see page 26 for more details).

3. To load data, do one of the following:

- Double-click the data row.
- Right-click the data name and select **Load** on the shortcut menu.
- Select the data row and click **Load**.
- Double-click the thumbnail.

The image(s) and Tool Palette are displayed. Green rows in the browser indicate loaded data (Figure 4.3).

**Table 4.1** Living Image Browser

Item	Description
Hide Browse View	Closes the browser table.
Close Preview	Closes the image preview box.
Label Set	A drop-down list of the available label sets which specify image information (column headers) that is displayed in the Living Image Browser.
Add to List	If this option is chosen, the data selected in the Browse for Folder box is added to the Living Image Browser. If this option is not chosen, the data selected in the Browse for Folder box replaces the contents of the Living Image Browser, except for the loaded data.
Browse	Opens the Browse For Folder box.
View	The name of the Living Image Browser configuration (the column headers and their order in the browser).
Configure	Opens a dialog box that enables you create and save custom Living Image Browser configurations. Note: To reorder a column in the browser, click the column header, then press the mouse key while you drag the header left or right. Release the mouse key to set the new position.

Table 4.1 Living Image Browser (continued)

Item	Description
Load as Group	<p>Enables you to select particular images that you want to view as a sequence. The images may be acquired during different sessions.</p> <p>To select adjacent images in the browser, press and hold the Shift key while you click the first and last file in the selection.</p> <p>To select non-adjacent images in the browser:</p> <ul style="list-style-type: none"> PC users: Press and hold the Ctrl key while you click the images in the browser Macintosh users: Press and hold the Cmd key (apple key) while you click the images in the browser. <p>Note: The Load as Group option is only available when two or more images (non-kinetic) are selected in the browser.</p> <p>Tip: See the tech note Loading Groups of Images for a quick guide (select Help → Tech Notes on the menu bar).</p>
Load	Opens the selected image or image sequence.
Remove	Removes a user-selected image sequence(s) from the browser.
Close	Closes the Living Image Browser.

Opening Data from the Menu or Toolbar



NOTE: To open a recently viewed file, select **File** → **Recent Files** on the menu bar.

1. Click the **Open** button  on the toolbar. Alternatively, select **File** → **Open** on the menu bar.
2. In the box that appears, choose a file type filter from the drop-down list (Figure 4.4).

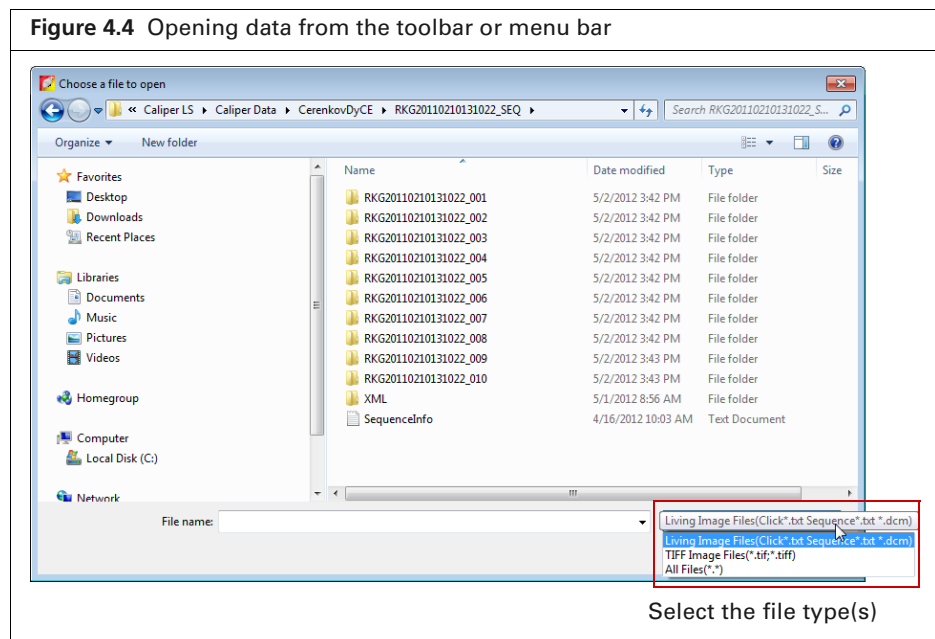


Table 4.2 File filters

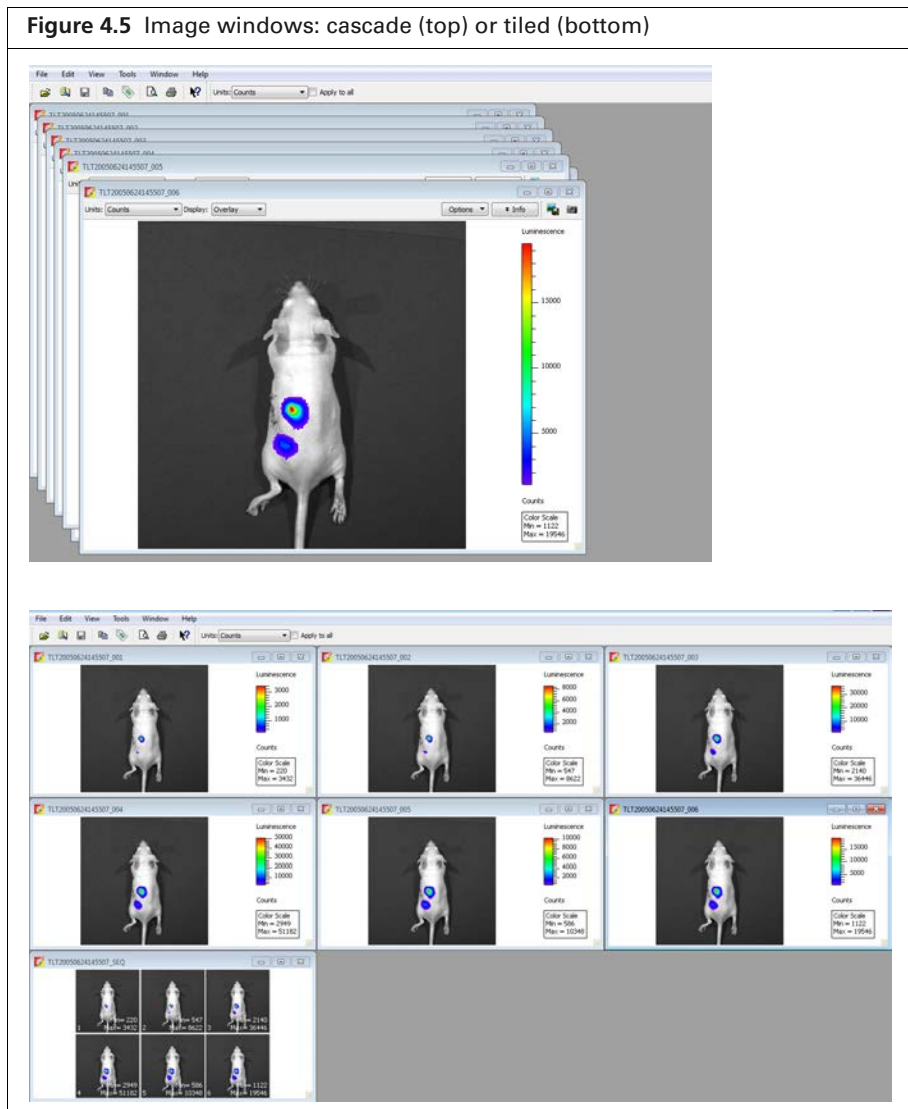
File Type Filter	Shows:
Living Image files	Click*.txt – an image (Living Image file format). Sequence*.txt – an image sequence (Living Image file format). *.dcm – kinetic data or an image that was exported to a DICOM file.
TIFF Image Files	Graphic files (*.tif, *.tiff).
All Files (*.*)	All file types.

3. Navigate to the file and click double-click it. Alternatively, select the data and click **Open**.

Organizing Images

When multiple image windows are open, you can organize them in a cascade or tile arrangement.

Choose **Window** → **Cascade** or **Window**→ **Tile** on the menu bar.



4.2 About the Image Window and Tool Palette

Image Window

An image or image sequence is displayed in an image window. Multiple image windows can be open at the same time.

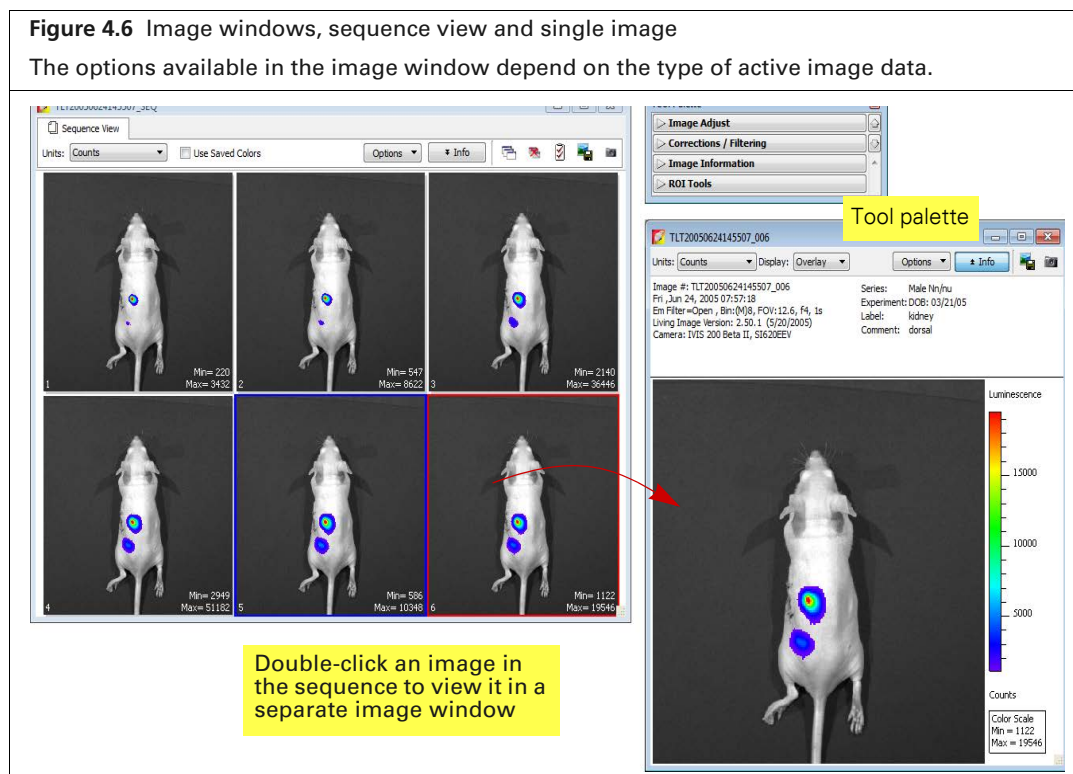


Table 4.3 Image window

Item	Description
Units	Select the measurement units for the image display from this drop-down list. The available units depend on the type of image data. See the concept tech note <i>Image Display and Measurement</i> for more details on measurement units (select Help → Tech Notes on the menu bar).
Use Saved Colors (image sequence)	Choose this option to display the image data using the color table that was specified in the Preferences at the time of acquisition. If this option is not selected, image data are displayed using the color table currently specified in the Preferences.

Table 4.3 Image window (continued)

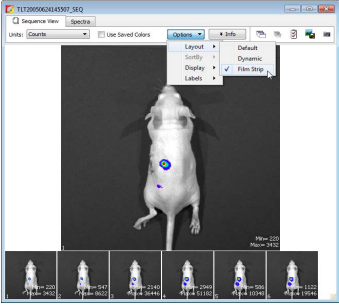
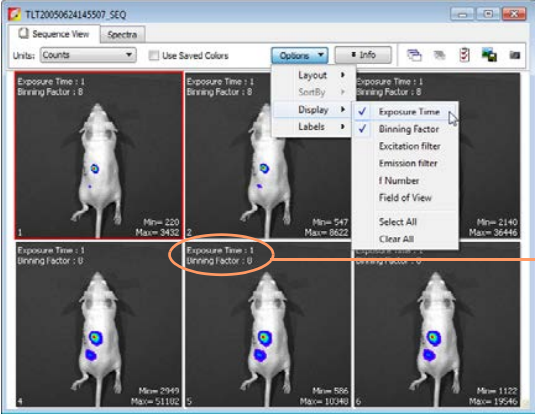
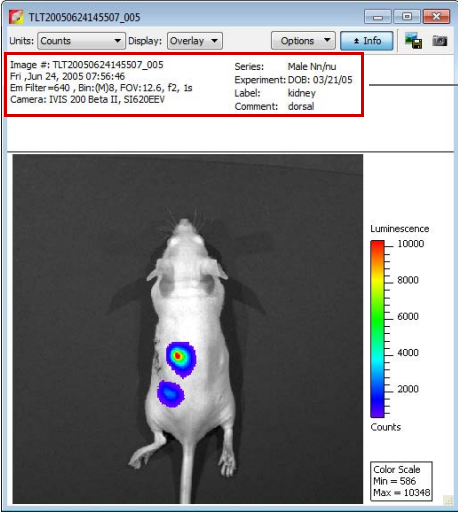





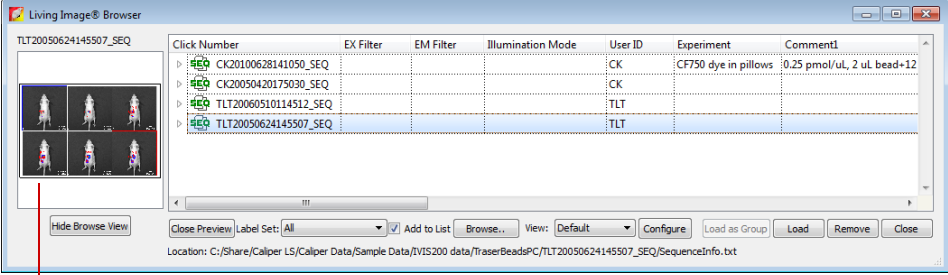
Item	Description
Options (image sequence)	<p>Layout – Choose a display option for the images in a sequence (Default, Dynamic, or Film Strip). For example, here is Film Strip mode:</p> 
	<p>Sort by – Options for ordering images in the sequence window:</p> <ul style="list-style-type: none"> ■ Default – Order in which the images are stored in the folder. ■ TimeStamp – Ascending order of the image acquisition time. ■ UserID – Ascending alphanumeric order of the user ID.
	<p>Display - Choose the types of information to display with each image.</p>  <p>In this example, exposure time and binning factor are displayed on each image</p>
	<p>Labels – Enables you to select the information to include in the image label.</p>  <p>Image label</p>
Info	<p>Click to show or hide the image label. The image label includes information you enter in the Edit Image Labels dialog box (see page 25) and other information automatically recorded by the software.</p>

Table 4.3 Image window (continued)

Item	Description
	Opens all of the images in a sequence.
	Closes all open images of a sequence.
	Opens the Edit Sequence dialog box that enables you to add or remove images from the sequence display.
	Opens a dialog box that enables you to export the active view as a graphic file.
	Takes a “snapshot” that is displayed with the data in the Living Image Browser. See page 48 for more details on the browser.



Snapshots of an image sequence

Tool Palette

The Tool Palette appears when you open an image or sequence. The options available in the Tool Palette depend on the type of active image data. A tool is only available if the data set includes the components that the tool requires to perform the analysis.



NOTE: The DyCE tools require a separate license.


Figure 4.7 Tool palette

See [page 11](#) for an overview of the Living Image tools.

Tool Palette

- ▶ Image Adjust
- ▶ Corrections / Filtering
- ▶ Image Information
- ▶ ROI Tools
- ▶ Spectral Unmixing and DyCE

Click to expand a tool



Tool Palette

Image Adjust

Photo Adjustment

Brightness:

Contrast:

Opacity:

Color Scale

Min:


Max:

Color Scale Limits

Auto Full Manual

Individual

Color Table

Yellow-Hot 

Reverse Logarithmic Scale

▶ Corrections / Filtering

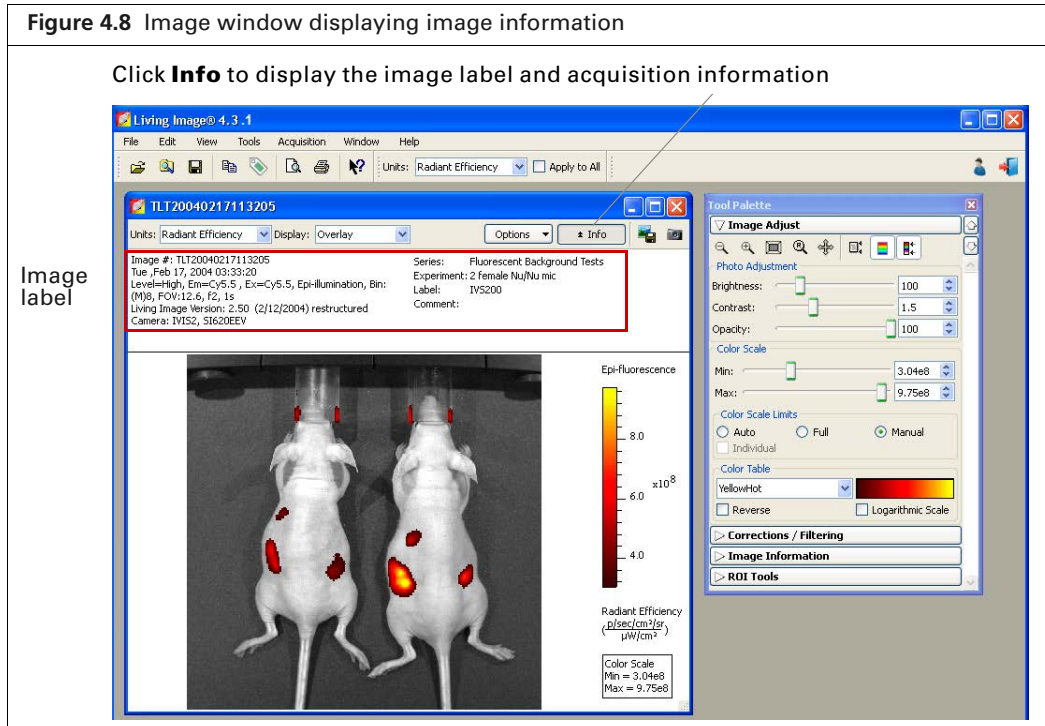
▶ Image Information

▶ ROI Tools

▶ Spectral Unmixing and DyCE

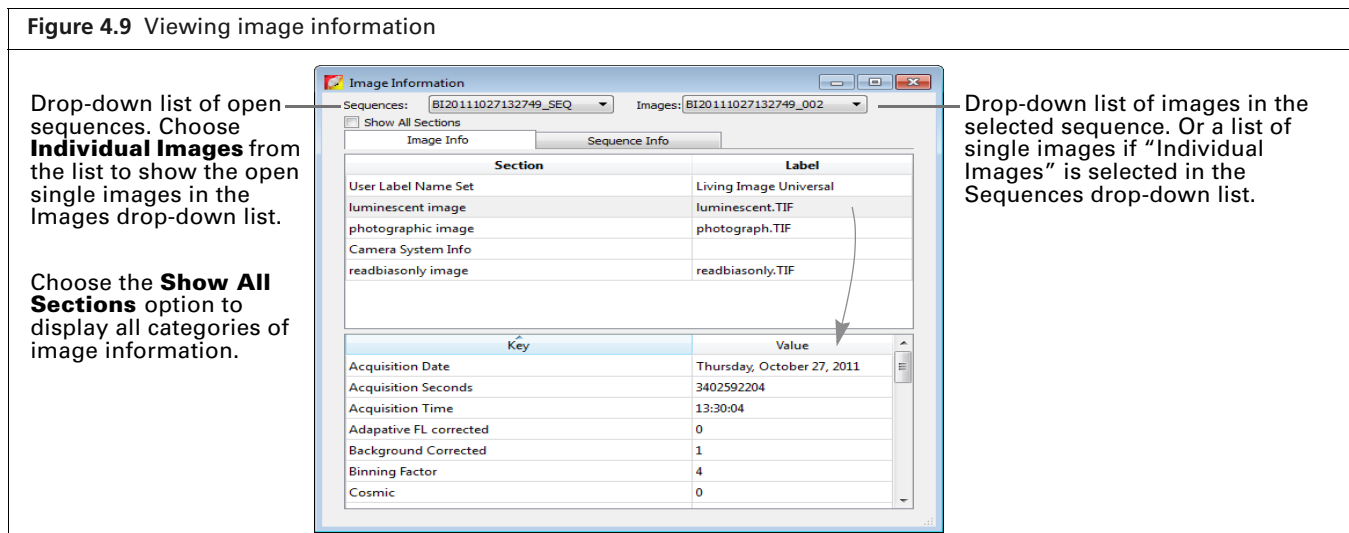
4.3 Viewing Image Information

At acquisition, the software captures image information such as camera parameters and any image label information you entered at acquisition time (Figure 4.8).



Detailed information about images is available in the View menu.

1. Open an image or sequence.
2. Select **View** → **Image Information** on the menu bar.
The Image Information window appears.
3. Choose an image by making a selection from the Sequences drop-down list and the Images drop-down list (Figure 4.9).



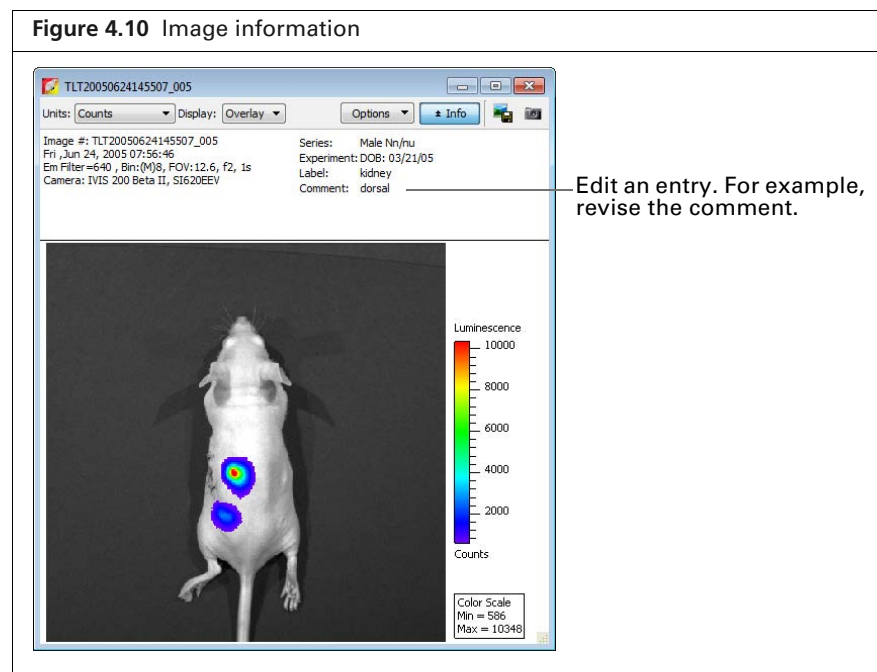
4. To view particular information, select a category in the upper box to show the associated information in the lower box. For example, select luminescent image in the upper box to show the luminescent image acquisition parameters.

Editing the Image Label

You can edit image label information or add information to the label after acquisition.


To edit the image information:

1. Open an image or sequence.
2. Click **Info** to display the image label.



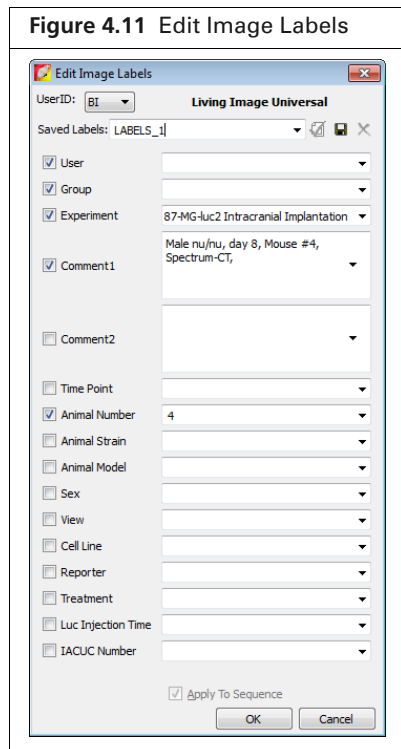
3. Edit the label information.

To add information to the image label:

1. Click the  toolbar button. Alternatively, select **Edit** → **Image Labels** on the menu bar.
2. In the Edit Image Labels box that appears, select information and/or enter a comment (Figure 4.11).



NOTE: If a single image is active, changes are applied to that image only. If a sequence is active, changes are applied to each image of the sequence.



3. Click **OK** when finished.
The image information is updated.
4. Save the image to save the updated image label (select **File** → **Save** or **File** → **Save As** on the menu bar).

4.4 Adding Comments or Tags to an Image

Adding Comments

Comments can be added to an image and saved with the image.

1. Open an image.
2. Right-click the image and select **Insert Comment** on the shortcut menu. Enter comments in the yellow box that appears (Figure 4.12).

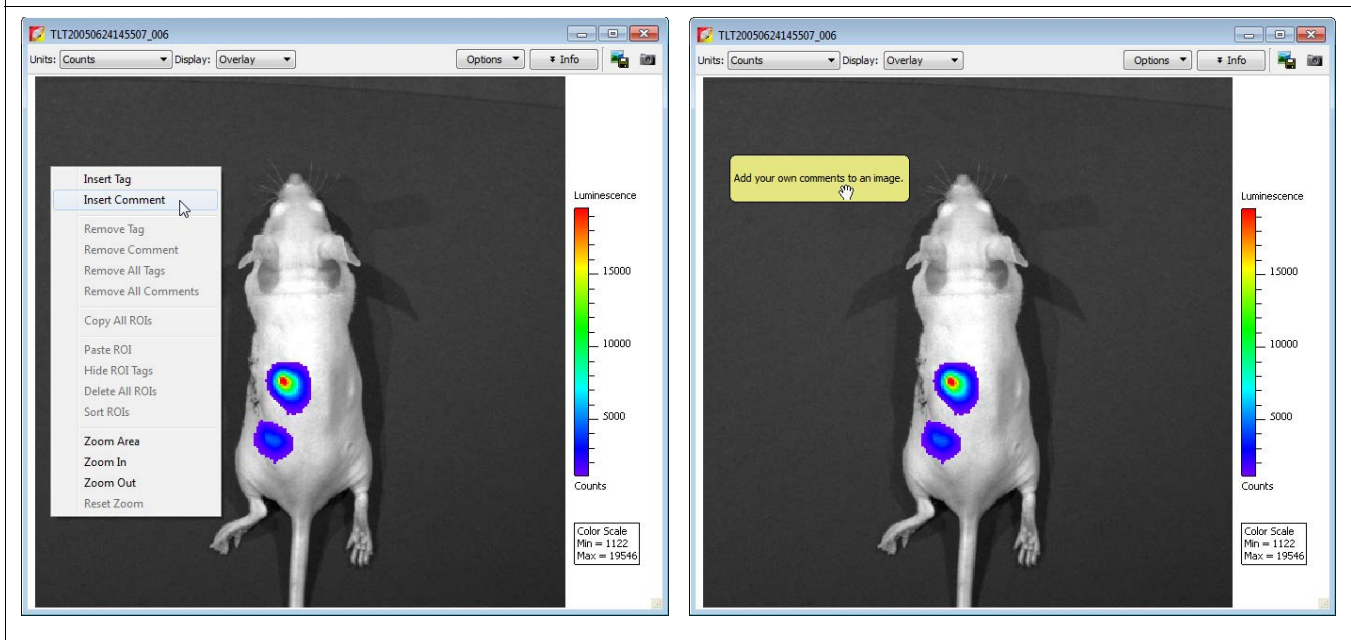
To reposition a comment:

1. Position the mouse pointer over the comment.
2. When the hand tool appears , use a click-and-drag operation to move the comment box, then click the mouse to set the location.

To remove a comment(s):

- To remove a comment, right-click the comment and select **Remove Comment** on the shortcut menu.
- To remove all comments, right-click the image and select **Remove All Comments** on the shortcut menu.

Figure 4.12 Add a comment to an image



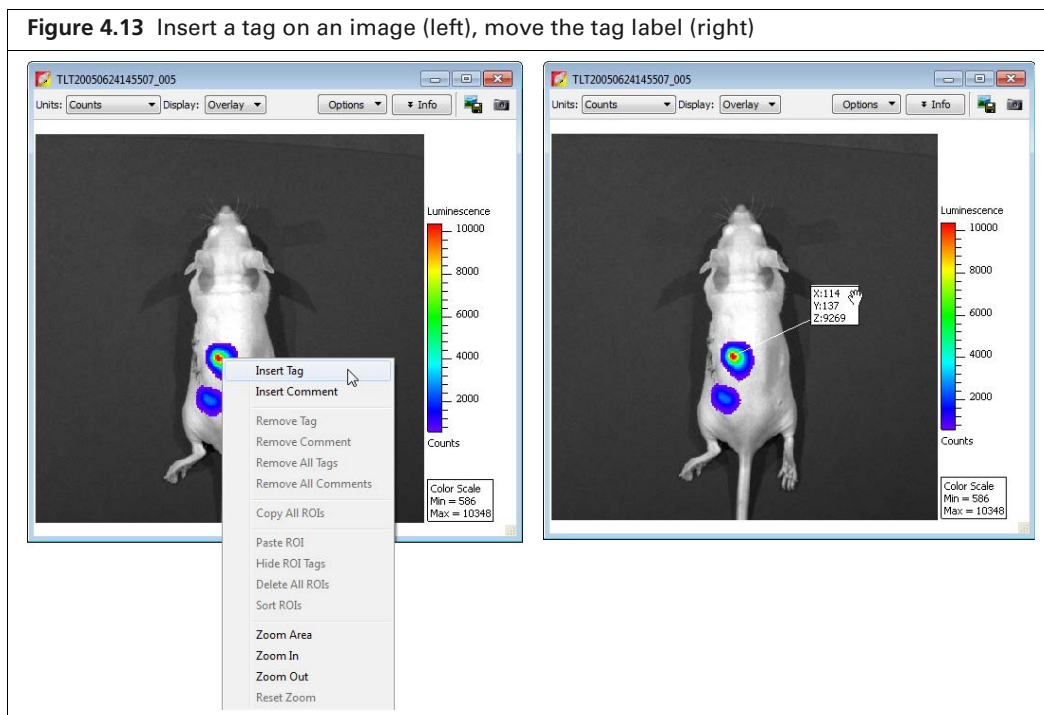
Tagging an Image

An image tag displays the x,y pixel coordinates of the location, and the pixel intensity (z, counts or photons). You can apply a tag at a user-selected location in an image.


To apply a tag:

1. Right-click a location in the image.
2. Select **Insert Tag** on the short cut menu.

Figure 4.13 Insert a tag on an image (left), move the tag label (right)



To move a tag:

1. Position the mouse pointer over the tag.
2. When the hand tool appears , use a click-and-drag operation to move the tag, then click the mouse to set the tag location.
3. A line between the pixel and the tag identifies the location associated with the tag.

4.5 Adjusting Image Appearance

Use the Image Adjust tools to adjust the appearance of an image (Figure 5.14).



NOTE: Not all tools are available for all image display modes. Some tools are available for single images, but not image sequence and vice versa. For example, the Correction/Filtering and Image Information tools are available for an image, but not for an image sequence.

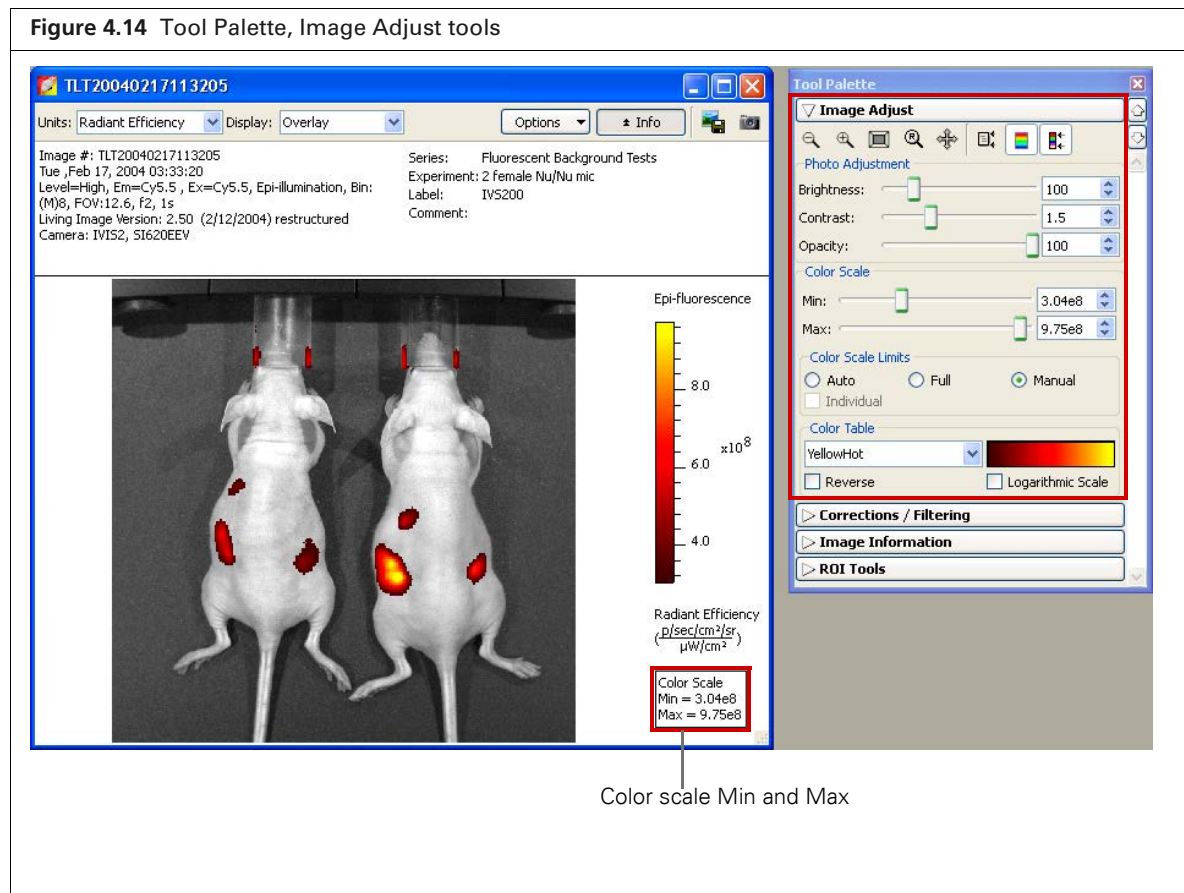


Table 4.4 Image Adjust tools










Item	Description
	Click this button to incrementally zoom out on the image (reduces the image dimensions in the image window). Note: The zoom tools are also available in the shortcut menu when you right-click the image (Cmd-click for Macintosh users).
	Click this button to incrementally zoom in on the image (incrementally magnifies the image in the image window).

Table 4.4 Image Adjust tools (continued)


Item	Description
	Click this button to magnify the area inside a rectangle that you draw using a click-and-drag operation. (Sets the dimensions of the magnified area equal to image window dimensions.)
	Click this button to return the image to the default display magnification.
	Click this button to move a magnified image (<i>pan</i>) in the image window. For more details, see page 63 .
	Click this button to hide or display the image min/max information in the image window
	Click this button to hide or display the color scale in the image window
	Click this button to hide or display the color scale min/max information in the image window
Photo Adjustment	<p>Brightness – Click and move the slider left or right to adjust the brightness of an image displayed in overlay or photograph mode. Alternatively, enter a brightness value.</p> <p>Contrast – Click and move the slider left or right to adjust the <i>gamma</i> of an image displayed in overlay mode. Alternatively, enter a gamma value. (Gamma is related to image contrast.)</p> <p>Opacity – Click and move the slider left or right to adjust the opacity of the pseudocolor luminescent data of an image displayed in overlay mode. Alternatively, enter an opacity value.</p>
Color Scale	<p>Min – The minimum pixel intensity associated with the color scale for an image. Pixels less than the minimum value are not displayed.</p> <p>Max – The maximum pixel intensity associated with the color scale for an image. Pixels greater than the maximum value are displayed in the maximum color.</p>
Color Scale Limits	<p>Auto – If this option is chosen, the software sets the Min and Max values to optimize image display and suppress background noise. The Min and Max settings can be manually adjusted to further optimize the image display for your needs.</p> <p>Full – Choose this option to set the Max and Min values to the maximum and minimum data values in the image.</p> <p>Manual – Choose this option to enter Max and Min values for the image display.</p> <p>Individual – Applies separate scale limits to each image in a sequence. Note: This option is only available when an image sequence is active.</p>
Color Table	<p></p> <p>Click the drop-down arrow to select a color table for the image data. See the concept tech note <i>Image Display and Measurement</i> for more details on color tables (select Help → Tech Notes on the menu bar).</p> <p>Reverse – Choose this option to reverse the selected color table.</p> <p>Logarithmic Scale – Choose this option to apply a log scale to the relationship between numerical data and the color range in the color table. A log scale improves the visibility of dark areas in an image.</p>

Magnifying or Panning in the Image Window


To incrementally zoom in or out on an image:

Click the  or  button. Alternatively, right-click the image and select **Zoom In** or **Zoom Out** on the shortcut menu.

To magnify a selected area in an image:

1. Click the  button. Alternatively, right-click the image and select **Area Zoom** on the shortcut menu.
2. When the pointer becomes a +, draw a rectangle around the area that you want to magnify. The selected area is magnified when you release the mouse button.



To reset the magnification (remove magnification):

Click the  button. Alternatively, right-click the image and select **Reset Zoom** on the shortcut menu.

To pan the image window:



NOTE: Panning helps you view different areas of a magnified image. If the image has not been magnified, you cannot pan the image.

1. Click the  button.
2. When the pointer becomes a , click and hold the pointer while you move the mouse.

4.6 Correcting Optical Image Data

Use the Corrections/Filtering tools to subtract background or apply corrections to the optical image data. You can also apply smoothing and soft binning to the image data.



TIP: See these technical notes for helpful information (select **Help** → **Tech Notes** on the menu bar).

- *Detection Sensitivity* (includes information about binning and smoothing).
- *Luminescent Background Sources and Corrections*.
- *Fluorescent Imaging* for more about fluorescent background.

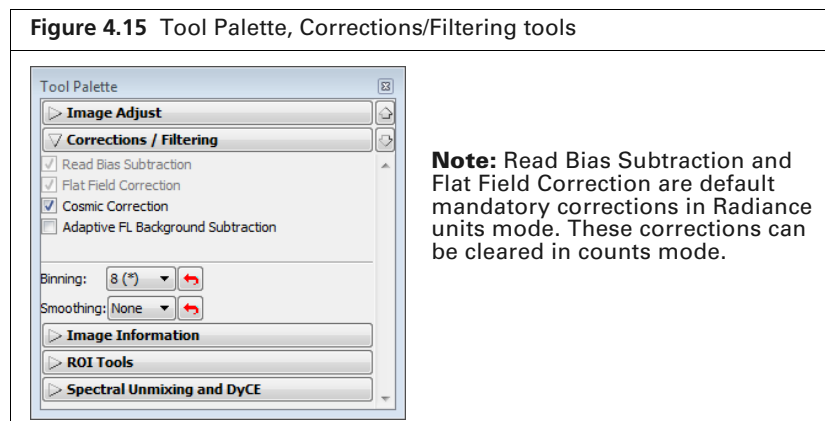


Table 4.5 Corrections/Filtering tools


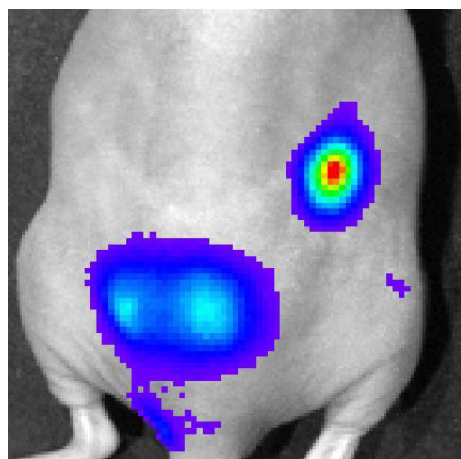
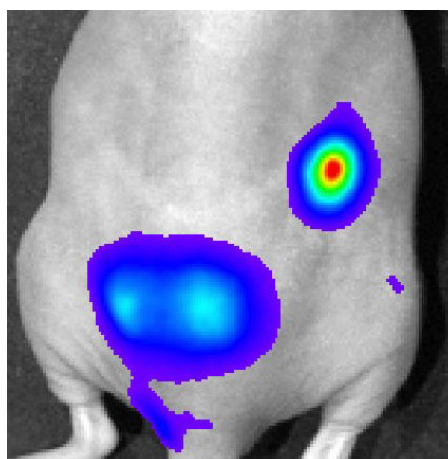
Item	Description
Adaptive FL Background Subtraction	Opens the Photo Mask Setup box that enables you to set the photo mask for adaptive fluorescent background subtraction. Tip: See the tech note <i>Adaptive Fluorescence Background Subtraction</i> (select Help → Tech Notes on the menu bar).
Read Bias Subtraction/Dark Charge Subtraction	Select this check box to subtract dark background from the image data. If a dark charge image is available for the imaging conditions, the dark background image, including read bias noise, will be subtracted. Otherwise, only read bias noise will be subtracted. Note: In Radiance (Photons) mode, dark background or read bias subtraction is a mandatory default. In counts mode, the check box can be cleared. Tip: See the tech note <i>Luminescent Background Sources and Corrections</i> (select Help → Tech Notes on the menu bar).
Flat Field Correction	Select this check box to apply flat field correction to the image data. Note: In photons mode, flat field correction is a mandatory default. In counts mode, the check box can be cleared.
Cosmic Correction	Select this check box to correct image data for cosmic rays or other ionizing radiation that interact with the CCD. See the tech note <i>Image Data Display and Measurement</i> for more about cosmic correction (select Help → Tech Notes on the menu bar).
Binning	Specifies the number of pixels in the image data that are grouped together to form a larger pixel (called <i>soft binning</i>). Binning changes the pixel size in the image (Figure 4.16). See the tech note <i>Detection Sensitivity</i> for more details on binning (select Help → Tech Notes on the menu bar).
Smoothing	Computes the average signal of the specified number of pixels and replaces the original signal with the average signal (Figure 4.16). Smoothing removes signal noise without changing pixel size.
	Click this button to return the binning or smoothing to the previous setting and update the image.

Figure 4.16 Example of binning and smoothing image data

Binning at acquisition = 8, no smoothing



Binning = 2, smoothing = 5x5

4.7 Viewing Intensity Data and Making Measurements

The Image Information tools enable you to view intensity data and measure distance on an image. Pixel data can be viewed in different formats:

Image Information	Description	See Page
x,y coordinates and associated intensity	The x,y pixel coordinates of the mouse pointer location in the image and the intensity (counts or photons) at that location.	67
Histogram	Histogram of pixel intensities in an image.	67
Line profile	Plots a line graph of intensity data at each pixel along a user-specified horizontal or vertical line in the image.	68

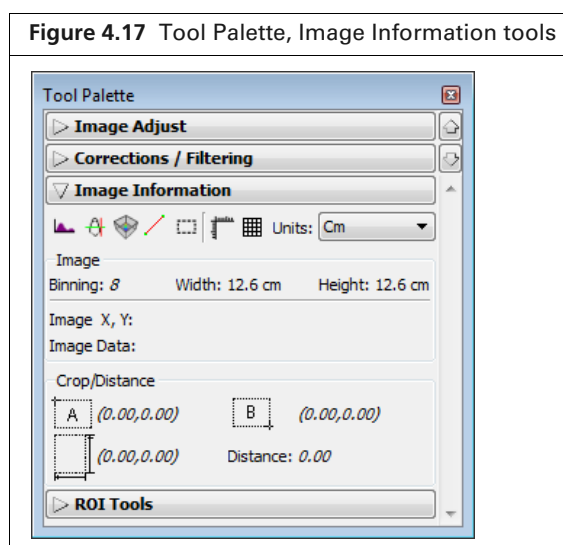


Table 4.6 Image Information tools











Item	Description
	Click this button to display a histogram of pixel intensity (see page 67).
	Click this button to display a line profile (see page 68 .)
	Click this button to display a 3D representation of signal intensity (see page 70).
	Click this button to display the distance measurement cursor in the image window (see page 71).
	Click this button to draw and measure a rectangle on an image (see page 72).
	Click this button to display/hide a scale on the x and y-axis of the image window.
	Click this button to display/hide a grid the image window.

Table 4.6 Image Information tools

Item	Description
Units	Choose the units (cm or pixels) for distance measurements in the image window.
Image	<p>Binning – The binning applied to the image. Note: If soft binning is applied to the image data, and the binning level is changed from 8 to 16, the new binning is indicated as 8x2.</p> <p>Width/Height – The FOV dimensions. Note: If “Pixels” is selected from the Units drop-down list, the dimensions are provided in terms of binned pixels.</p> <p>Image X,Y – The x,y pixel coordinates of the mouse pointer location in the image.</p> <p>Image Data – The intensity at the pixel location of the mouse pointer. The intensity is represented in the units currently selected for the image.</p>
Crop/Distance	<p>The x,y pixel coordinates at the upper left corner of the crop tool.  A</p> <p>OR</p> <p>The x,y pixel coordinates at the “A” end of the distance.  B</p> <p>The x,y pixel coordinates at the lower right corner of the crop tool.</p> <p>OR</p> <p>The x,y pixel coordinates at the “B” end of the distance.</p> <p>The width and height of the image crop tool. </p> <p>OR</p> <p>Δx, Δy from the A to B end of the distance measurement cursor.</p> <p>For more details, see page 71 and 72.</p>

Viewing X,Y Coordinates and Intensity Data

1. Open an image, and the Image Information tools, choose Cm or Pixels from the Units drop-down list.
2. Put the mouse pointer over a location in the image.
The x,y coordinates and intensity data are displayed in the Tool Palette.

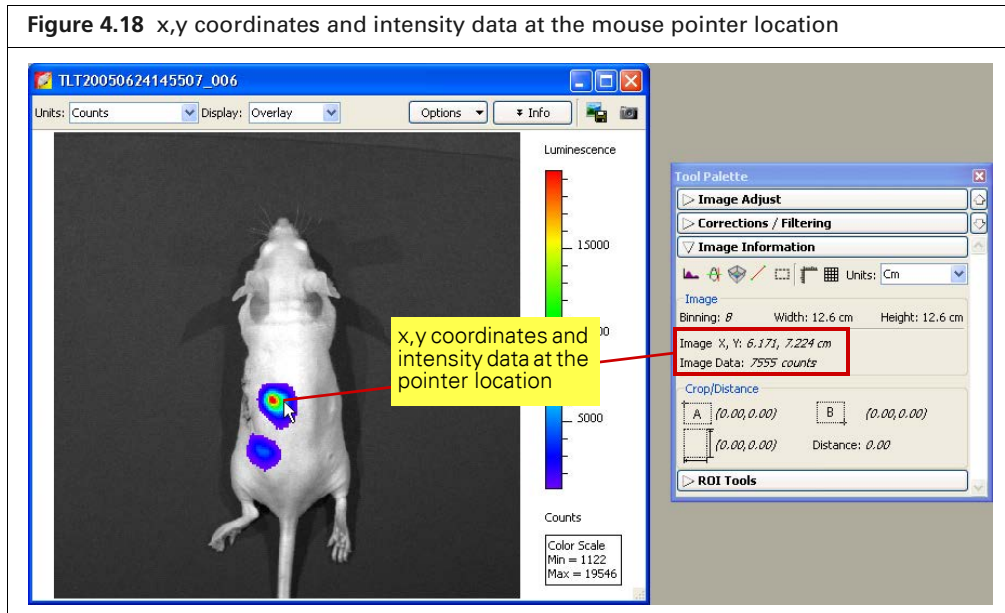

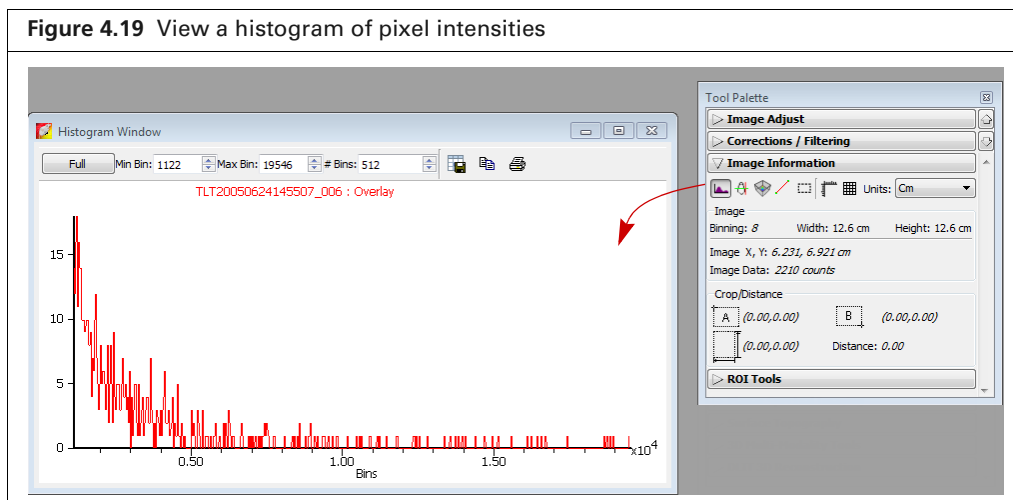


Image Histogram

The image histogram plots a frequency distribution of the pixel intensities in an image. The software sorts the intensities into groups or *bins* (x-axis) and plots the number of pixels per bin (y-axis).



To view the image histogram:

1. Open an image, and in the Image Information tools, click the **Image Histogram** button .








NOTE: By default the Auto min/max range of the image data determines the histogram range and bins (the software sets the min and max values to optimize image display and suppress background noise). To display the histogram using the full intensity range of the image, click **Full** in the Histogram window.

2. To edit the minimum or maximum bin intensity, enter a new value in the Min Bin or Max Bin box, or click the  arrows.
3. To edit the number of bins, enter a new value in the # Bins box or click the  arrows.



NOTE: In the Overlay display mode, the histogram plots the luminescent data. To obtain a histogram of the photograph, select Photograph from the Display drop-down list.

Table 4.7 Histogram window

Item	Description
Full	Displays the histogram using the full intensity range of the image.
Min Bin	The lowest intensity bin.
Max Bin	The highest intensity bin.
# Bins	The total number of bins.
	Opens a dialog box that enables you to export the histogram (.csv).
	Copies the histogram to the system clipboard.
	Opens the print dialog box.


Line Profile

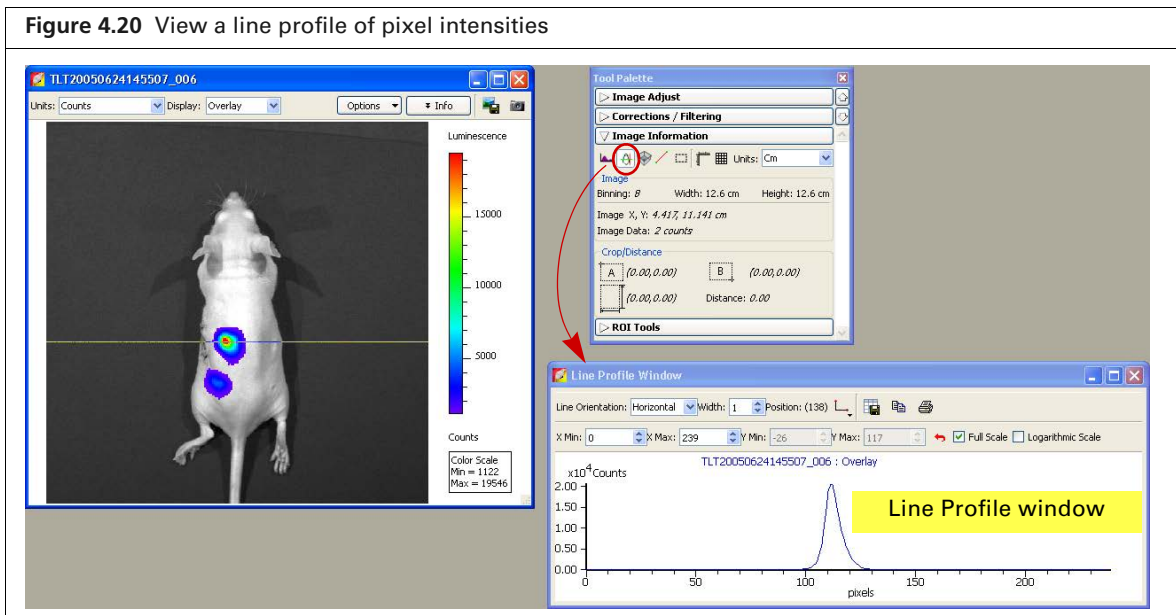
The line profile plots intensity (y-axis) at each pixel (x-axis) along a user-specified line in the image. It is particularly useful for inspecting the detailed character of the image data. The line profile is automatically updated when you change the line position.



NOTE: In the Overlay display mode, the line profile plots the luminescent data. To obtain a histogram of the photograph, select Photograph from the Display drop-down list.

To display the line profile:

1. Open an image, and in the Image Information tools, click the **Line Profile** button . A line appears on the image and the Line Profile window appears. The Line Profile window displays the average of the pixel values included in the line width.




- To view the line profile at another location in the image, put the mouse pointer over the line. When the pointer becomes a , drag the line over the image. The blue part of the line indicates the pixel intensities that are plotted in the line profile graph. The line profile is updated as you move the line move over the image.

Table 4.8 Line Profile window









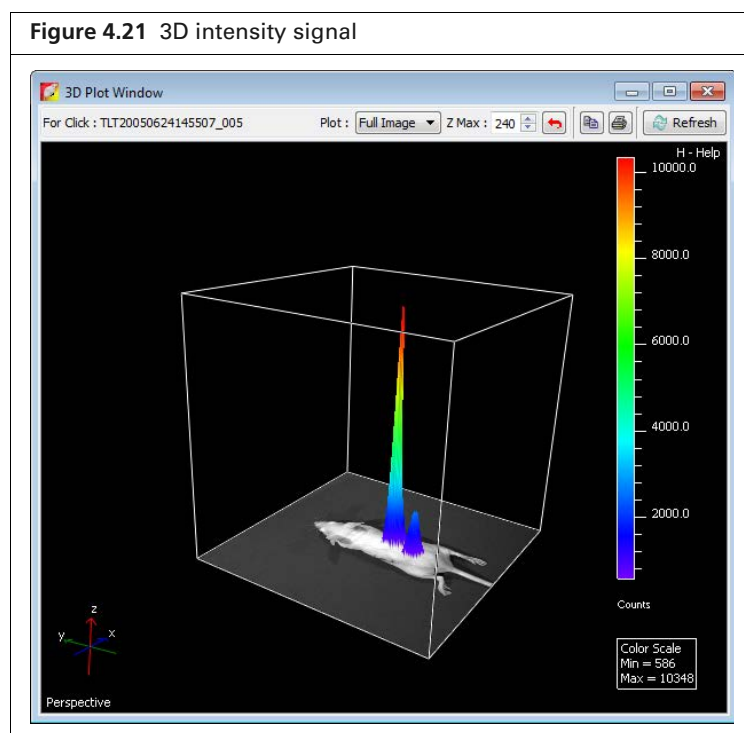
Item	Description
Line Orientation	Choose Vertical, Horizontal, or Free Hand from the drop-down list to set the orientation of the line in the image window. The Free Hand orientation enables you to drag each line segment endpoint to a user- selected position.
Width	Sets the line width. The Line Profile window displays the average of the pixel values included in the line width.
Position	Line position (pixels).
	Enables you to choose the grid line pattern to display in the line profile window.
	Exports the line profile data to a .csv or .txt file.
	Copies the line profile graph to the system clipboard.
	Opens the Print dialog box.
X Min X Max	Displays the minimum and maximum value of the x-axis. Use the  arrows to change the x-axis min or max. If a calibrated unit such as “radiance” is selected in the image window, the x-axis units = cm. If “counts” is selected in the image window, the x-axis units = pixels. To display the range available for the Min or Max, place the mouse pointer over the Min or Max edit box.
Y Min Y Max	Displays the minimum and maximum value of the y-axis. Use the  arrows to change the y-axis min or max. To display the range available for the Y Min or Y Max, place the mouse pointer over the Min or Max edit box.

Table 4.8 Line Profile window

Item	Description
	Click to reset the X and Y Min and Max values to the defaults.
Full Scale	Select this option to display the full X and Y-axis scales.
Logarithmic Scale	Select this option to apply a log scale to the y-axis.

Viewing 3D Signal Intensity

1. Open an image and then click the Plot 3D button  in the Image Information tools.
A 3D representation of all signals in the image is displayed in the 3D Plot window (Figure 4.21).



2. To change the display, make a selection from the Plot drop-down list and click the **Refresh** button

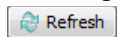


Table 4.9 3D Plot window




Item	Description
Plot	Full Image – Displays all signals in the image. ROI <ROI number or name> – Displays the signal within the selected ROI. All ROIs – Displays the signal within all ROIs in the image.
Z Max	Height of the z-axis. Use the up/down arrows to change the height of the z-axis.
	Click to reset the z-axis to the default setting.
	Copies the 3D window to the system clipboard.


Table 4.9 3D Plot window (continued)

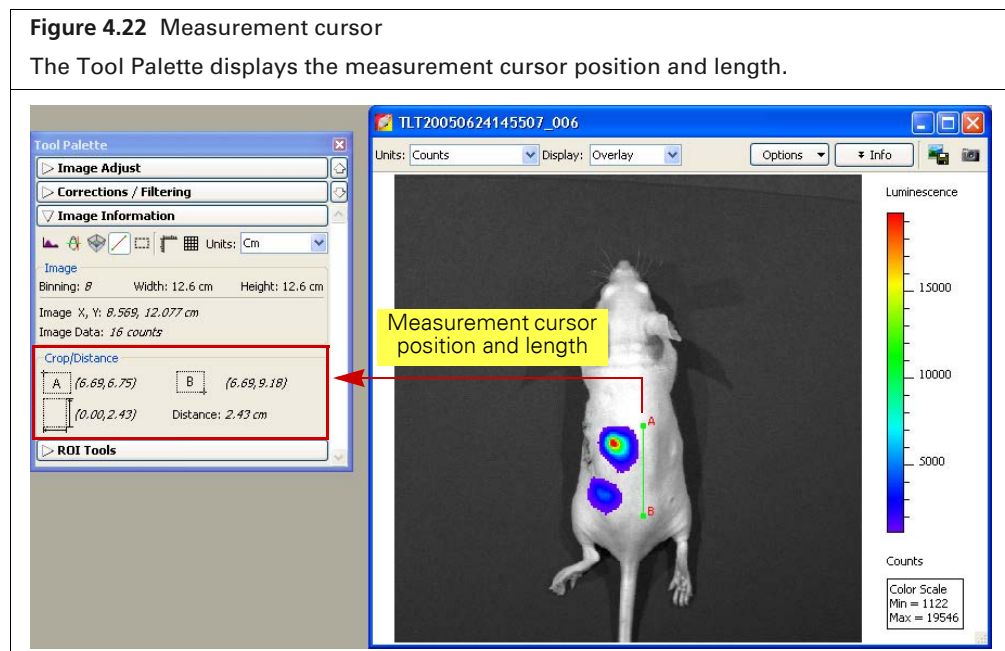
Item	Description
	Opens a Print dialog box that enables you to print the 3D window.

Making Measurements

To measure distance with the measurement cursor:

1. Open an image and click the **Distance Measurement Cursor** button  in the Image Information tools.

A measurement cursor () appears on the image ([Figure 4.22](#)). The Tool Palette shows the position and length of the cursor.








2. To change the cursor position or size, drag the A or B end of the cursor to a new location on the image.
The measurement information in the Tool Palette is updated.
3. To hide the cursor, click the  button.

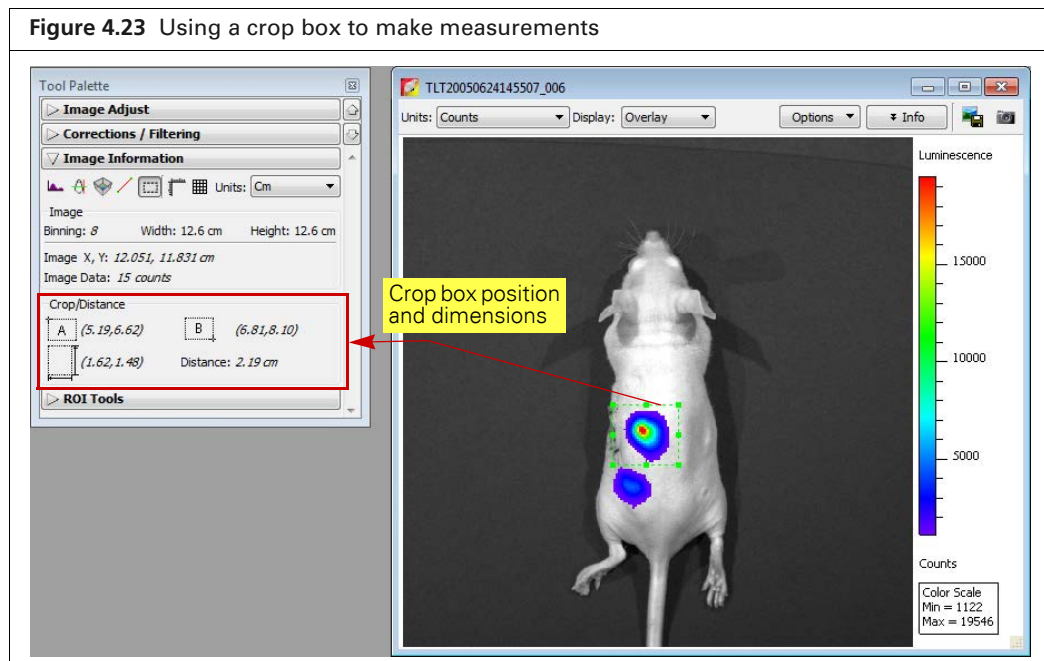
Table 4.10 Measurement cursor position and length

Item	Description*
	Pixel x,y coordinates of position A on the cursor. Note: Measurements are report in pixels or cm, whichever is selected from the Units drop-down list in the Image Information tools (Figure 4.23).
	Pixel x,y coordinates of position B on the cursor.
	Length of the cursor from A to B (number of pixels*), vertical distance from A to B (number of pixels*).
Distance	Length of the cursor from A to B (number of pixels*).

*Measurements are reported in pixels or cm, whichever is selected from the Units drop-down list in the Image Information tools (Figure 4.23).

To measure distance using the crop box:

1. Open an image, and in the Image Information tools, click the **Image Crop** button .








2. When the mouse pointer changes to a +, draw a rectangle on the area of interest.
3. To change the size or position of the crop box, drag a handle  at a corner or side of the box.
4. To delete the crop box from the image, click the  button.

Table 4.11 Crop box position and dimensions

Item	Description
	x,y coordinates at the upper left corner of the box.
	x,y coordinates of lower right corner of the box.
	Box width and height.
Distance	Length of the diagonal from the upper left to lower right corner of the box.

4.8 Overlaying Multiple Images

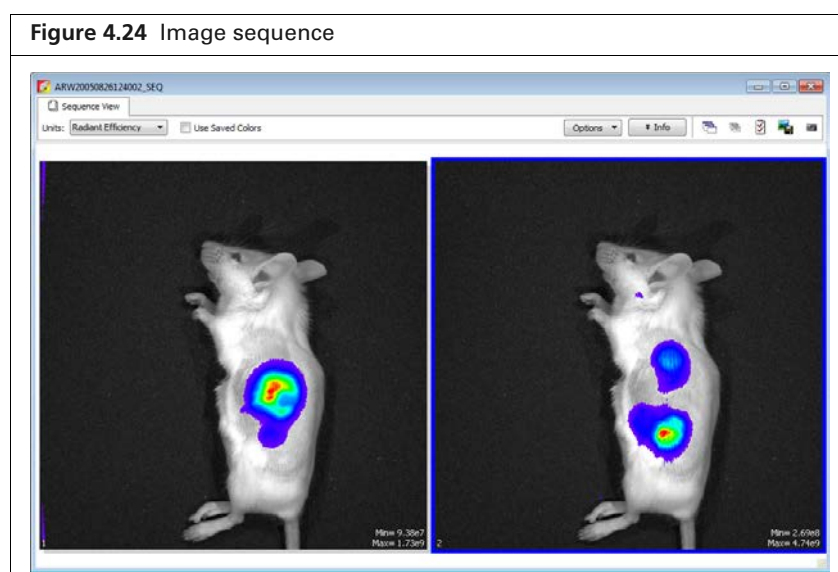
The image overlay tool provides a convenient way to view multiple reporters in one image. You can use the image overlay tool to display multiple luminescence or fluorescence images on one photographic image.



TIP: See the technical note *Image Overlay – 2D* for a quick guide (select **Help** → **Tech Notes** on the menu bar).

To coregister multiple images:

1. Acquire an image sequence using the appropriate filters for each reporter. Alternatively, create a sequence from images acquired during different sessions. (For more details, see [page 80](#).)
2. Load the image sequence.

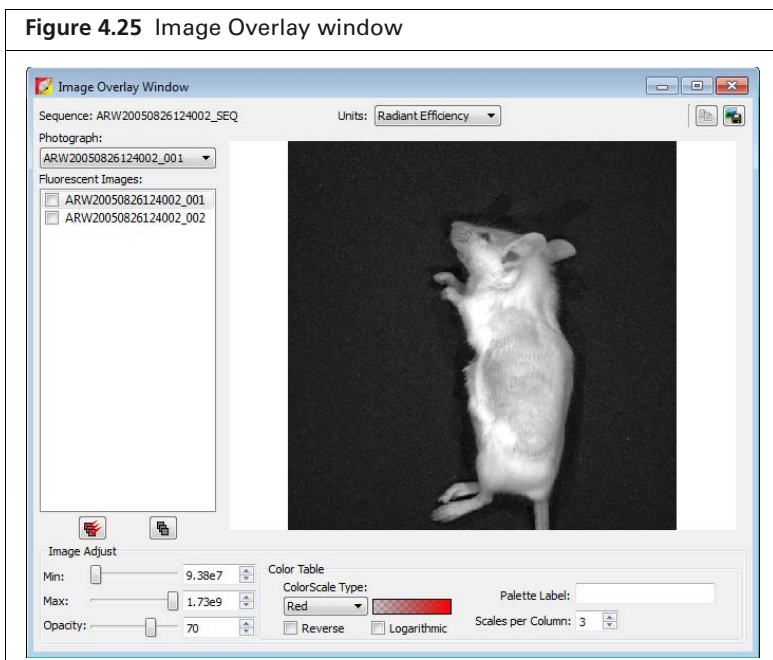



3. Open one of the images and optimize the image display using the color scale Min and Max sliders in the Image Adjust tools.

To view all images in the sequence, click the **Display All** button  to open each image (overlay mode) in a separate image window.

4. Select **Tools** → **Image Overlay for <sequence name>_SEQ** on the menu bar.

The image overlay window appears and shows the first photograph in the sequence. To view a different photograph, make a selection from the photograph drop-down list.



- To overlay all images, click the  button.
The overlay appears. The photograph is at the bottom of the stack and the last fluorescent or luminescent image in the list is at the top of the stack.

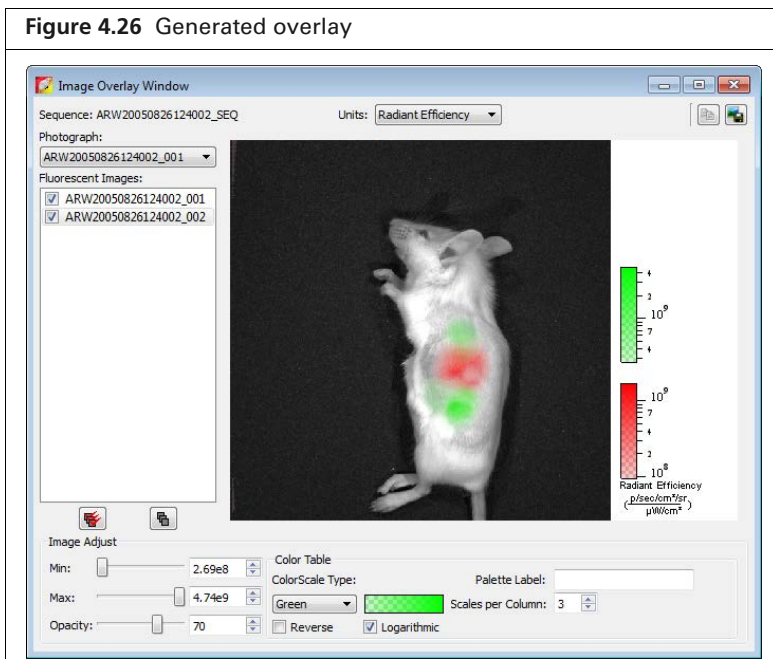







Table 4.12 Image Overlay window

Item	Description
Units	Choose the type of units for displaying the fluorescent or luminescent image. See the concept tech note <i>Image Display and Measurement</i> for more details on measurement units (select Help → Tech Notes on the menu bar).

Table 4.12 Image Overlay window (continued)

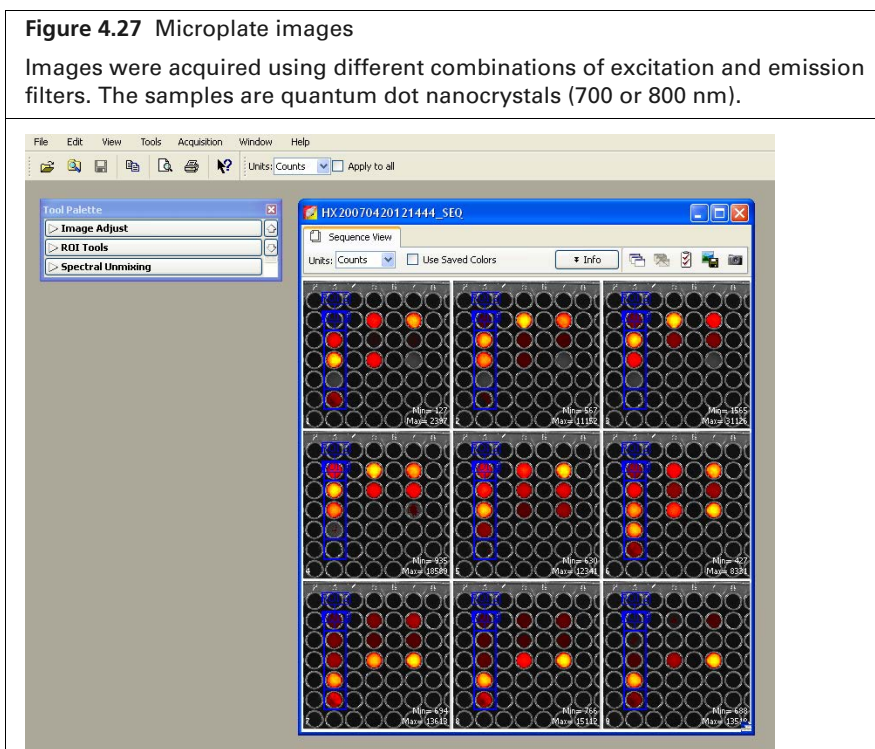
Item	Description
Photograph	A drop-down list of the photographs in the image sequence.
Fluorescent or Luminescent Images	The sequence images.
	Copies the overlay to the system clipboard.
	Click to export the overlay to a graphic file.
	Click to include all fluorescent or luminescent images in the overlay.
	Click to remove all fluorescent or luminescent images from the photograph.
Image Adjust	<p>Tools for adjusting the appearance of the highlighted fluorescent or luminescent image. Adjustments can only be made on one image at a time.</p> <p>Min – The minimum pixel intensity associated with the color scale for an image. Pixels less than the minimum value are not displayed.</p> <p>Max – The maximum pixel intensity associated with the color scale for an image. Pixels greater than the maximum value are displayed in the maximum color.</p> <p>Opacity – Controls the opacity of the fluorescent or luminescent image.</p>
Color Table	<p>Tools for selecting and modifying the color scale associated with an image.</p> <p>Color Scale Type – Choose BlackLevel to show black at the low end of the color scale. Choose WhiteLevel to show white at the low end of the color scale.</p> <p> Click the drop-down arrow to select a color table for the image data. See the concept tech note <i>Image Display and Measurement</i> for more details on measurement units (select Help → Tech Notes on the menu bar).</p> <p>Reverse – Choose this option to reverse the selected color table.</p> <p>Logarithmic – Choose this option to apply a log scale to the relationship between numerical data and the color range in the color table. A log scale improves the visibility of dark areas in an image.</p>
Palette label	To include a brief line of text next to the color scale, enter text in the palette label box, then press the Enter key. To remove the text from the image window, delete the text in the palette label box and press Enter .
Scales per Column	Sets the number of color scales to display in a column.

4.9 Rendering Intensity Data in Color

The colorize tool renders luminescence or fluorescence data in color, enabling you to see both intensity and spectral information in a single view. The tool provides a useful way to visualize multiple probes or scale probe signals that are not in the visible range.

To view colorized intensity data:

1. Load an image sequence.



2. Select **Tools** → **Colorize** on the menu bar.

The software renders each luminescent or fluorescent image in color and combines them into a single image (Figure 4.28).

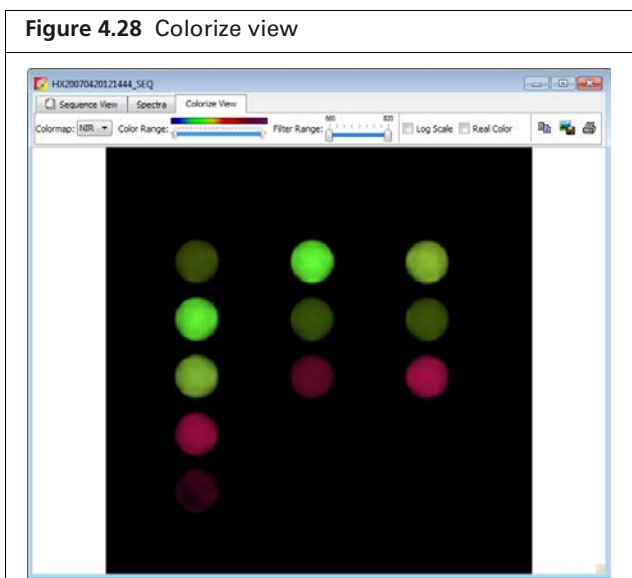







Table 4.13 Colorize tools

Item	Description
Colorize View	
Color Map	NIR – A special camera setup that extends the color response into the near infrared range. Near infrared fluorophores appear red to purple using the NIR camera setup. VIS – Regular camera setup that mainly renders color in the visible range. It is similar to the color response of a commercial digital camera. NIR fluorophores appear dark red to invisible using the VIS camera setup.
Color Range	The color map indicates the color range of the selected camera setup from short to long wavelength. The two sliders determine the lower and upper limits of the color range that is used to render color. The parts of the color map outside the selected range are not used in the color rendering process. By default, the entire color range is selected.
Filter Range	The wavelength range of the luminescent images in the sequence. The two sliders determine the lower and upper end of the filter range. Only the parts of the image that are within the selected wavelength range are colorized. By default, the entire filter range is selected.
Log Scale	If this option is chosen, the dynamic range of the brightness in the image is compressed using a log scale. This improves the visibility of dark areas in the image.
Real Color	If this option is chosen, the colors are rendered using the wavelengths that directly correspond to the camera setup. For example, GFP appears green using real color rendering. If this option is not chosen, the original wavelength range of the image is modified to include the entire visible wavelength range of the camera setup. This helps improve the color contrast.
	Click this button to copy the colorize view to the system clipboard.
	Click this button to export the colorize view as a graphic file (for example, .jpg).
	Click this button to print the colorize view.

4.10 Exporting or Printing Images

The Image Layout window (Figure 4.29) provides an alternative way to:

- Annotate and export an image (for example, .bmp)
- Print an image
- Copy an image to the system clipboard

1. Select **View** → **Image Layout Window** on the menu bar to open the Image Layout window.
2. Click the  button to paste the active image into the Image Layout window.
3. Drag a handle  at a corner of the image to resize the image.
4. Drag the image to reposition it in the window.

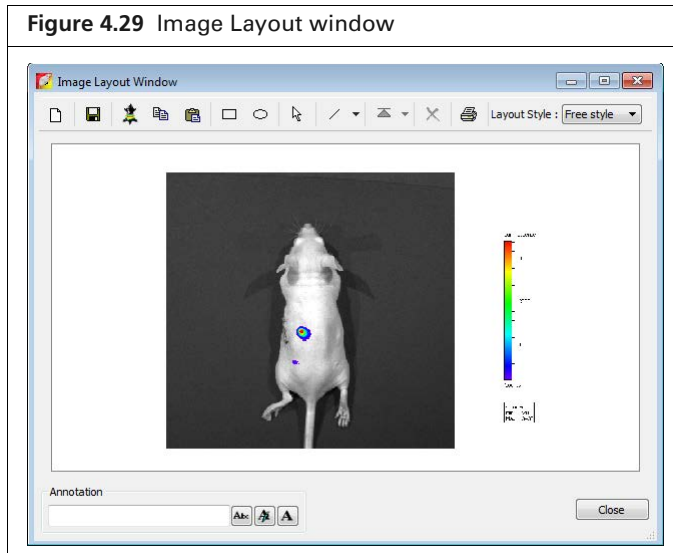


Table 4.14 Image Layout window









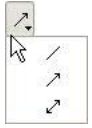
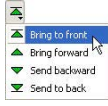







Item	Description
	Clears the Image Layout window. Note: If you do not clear the layout (click the button) before you close the Image Layout window, the same window contents are displayed the next time the window is opened
	Opens a dialog box that enables you to save the Image Layout window contents to a graphic file.
	Pastes an image of the active data in the Image Layout window.
	Copies the contents of the Image Layout window to the system clipboard.
	Pastes the contents of the system clipboard to the Image Layout window.
	Rectangle drawing tool
	Ellipse drawing tool
	Pointer tool
	Arrow and line drawing tool
	Select an the item in the Image Layout window. To move the item to the front or back in the window, choose an option from the  drop-down list.

Table 4.14 Image Layout window (continued)

Item	Description
	Deletes the selected image.
	A drop-down list of formatting options for the Image Layout window. For example, the 2x2 layout style provides 4 separate layout areas in the window. A different image can be pasted into each layout area.
	To apply notes to an image, enter text in the annotation box and press Enter . Drag the text to the location of interest in the image.
	Opens a dialog box that enables you to select a font or edit the font style and size.
	Opens a color palette that enables you to select a font color or specify a custom font color.
	Opens a text editor that enables you to edit the selected text.

4.11 Editing an Image Sequence

You can add or remove individual images from a sequence. Only individual images, not an image sequence, can be added to a sequence.


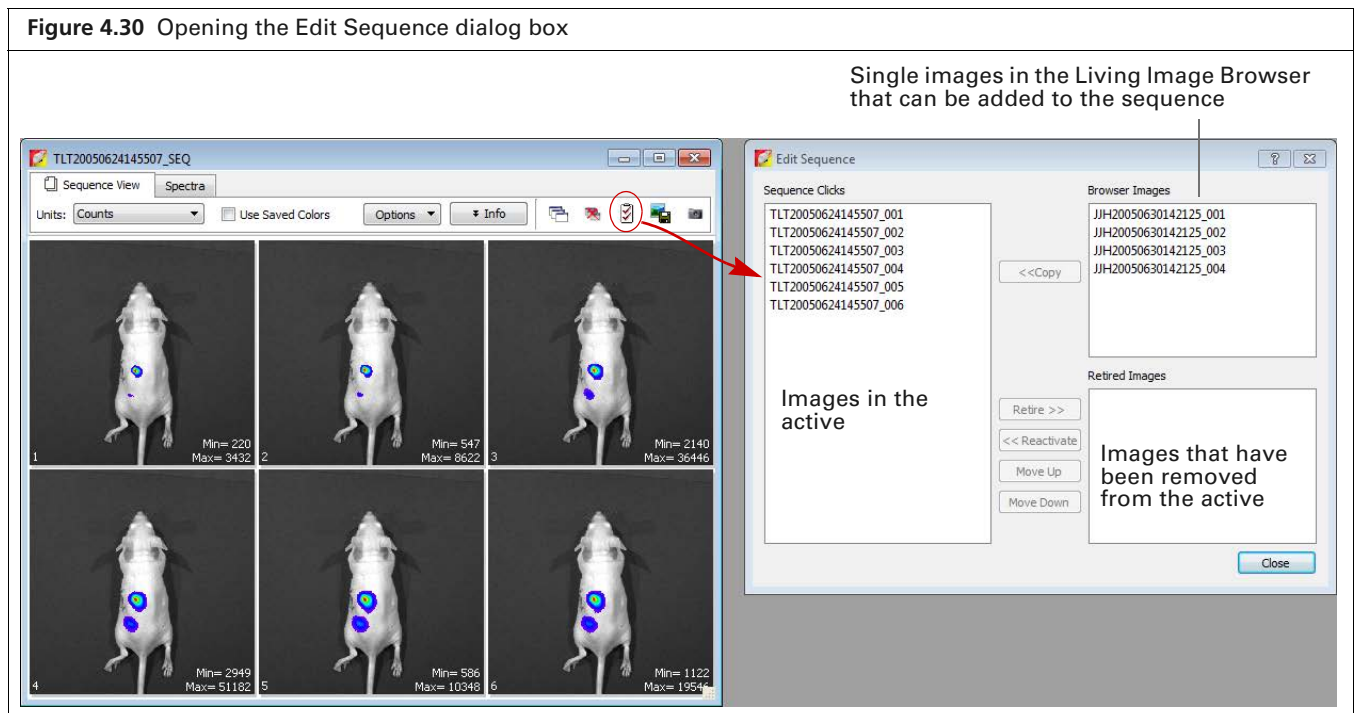
1. Open the image sequence that you want to edit.
2. If you plan to add images to the sequence, browse for the images that you want to add in the Living Image® browser. (See page 48 for more details on browsing.)
3. Click the **Edit** button  in the image window (Figure 4.30).

Figure 4.30 Opening the Edit Sequence dialog box



4. Choose the image(s) to add or remove (*retire*) from the sequence in the Edit Sequence box that appears (Figure 4.30).

To add an image to the sequence, select an image from the “Browser Images” and click **Copy**. To remove an image from the sequence, choose an image from “Sequence Clicks” and click **Retire**.

5. To restore a retired image to the sequence, select the retired image and click **Reactivate**.
6. To reorder the sequence, select an image and click **Move Up** or **Move Down**.

NOTE: The **Move Up** and **Move Down** buttons are only available when the sequence view window displays images in the default sort order. If the TimeStamp or UserID sort order is selected, the images cannot be reordered.

7. Click **Close** when you are finished editing the sequence.
The updated image sequence is displayed.

4.12 Creating an Image Sequence from Individual Images

This section explains how to create a sequence from images acquired during different sessions.

TIP: Also see the tech note *Loading Groups of Images* for helpful information (select Help → Tech Notes on the menu bar).

1. In the Living Image Browser, browse for the images of interest. (See page 48 for more details on browsing.)


NOTE: Browse for individual images (which may or may not be part of a sequence), not image sequences.

Figure 4.31 Living Image Browser

Individual images that may or may not be part of a sequence can be selected

Click Number	EX Filter	EM Filter	Illumination Mode	User ID	User	Group	Experiment
BI20111027132749_SEQ				BI			U87-MG-luc2 Intracranial Imp
IV20120412111353_DVC				IV	Irina		DyCE Luminol + QD800
CK20090729114835_SEQ				CK		IFM SNI007	rod 7, src A
EL20120411170231	675	720	Reflective	EL			PSMAAdiabody VT 680XL Mal
EL20120411170426	675	720	Reflective	EL			PSMAAdiabody VT 680XL Mal
EL20100601160926_SEQ				EL		MB231D3H2LN-luc	Intracardiac
EL20100608105326_SEQ				EL		MB231D3H2LN-luc	Intracardiac
IMC EL20100608105326 ...	Block	580		EL		MB231D3H2LN-luc	Intracardiac
IMC EL20100608105326 ...	Block	600		EL		MB231D3H2LN-luc	Intracardiac
IMC EL20100608105326 ...	Block	620		EL		MB231D3H2LN-luc	Intracardiac
IMC EL20100608105326 ...	Block	640		EL		MB231D3H2LN-luc	Intracardiac
IMC EL20100608105326 ...	Block	Open		EL		MB231D3H2LN-luc	Intracardiac
EL20100615094528_SEQ				EL		MB231D3H2LN-luc	Intracardiac
CK20080407145405_SEQ				CK		A/C PC3-GFP orthotopic	FLIT SPUM ex 500, longer exp
TLT20050624145507_SEQ				TLT			
IMC TLT20050624145507 ...		560		TLT			
IMC TLT20050624145507 ...		580		TLT			
IMC TLT20050624145507 ...		600		TLT			
IMC TLT20050624145507 ...		620		TLT			
IMC TLT20050624145507 ...		640		TLT			
IMC TLT20050624145507 ...		Open		TLT			

Images loaded in the browser as part of a sequence. These images can also be selected for grouping into another sequence.

2. In the browser, select the images that you want to group together.
To select adjacent images in the browser, press and hold the **Shift** key while you click the first and last file in the selection.
To select non-adjacent images in the browser:
 - PC users - Press and hold the **Ctrl** key while you click the images of interest in the browser.
 - Macintosh users - Press and hold the **Cmd** key (apple key) while you click the images of interest in the browser.
3. Click **Load as Group**.
The image thumbnails are displayed together in an image window.
4. Save the images as a sequence:
 - a. Click the Save button . Alternatively, select **File** → **Save** on the menu bar.
 - b. In the dialog box that appears, select a folder and click **OK**.

5 ROI Tools for Optical Data

About ROIs

Quick Guide: Drawing Measurement ROIs on an Optical Image or Sequence on page 85

ROI Tools for Optical Images on page 86

Measurement ROIs on page 88

Mirror ROIs on page 92

Measuring Background-Corrected Signal on page 95

ROI Histogram on page 98

Managing ROI Properties on page 100

Managing the ROI Measurements Table on page 109

5.1 About ROIs

This chapter explains how to draw and measure signal within a *region of interest* (ROI) on an optical image. Four types of ROIs are available for optical data (Table 5.2).

Table 5.1 Types of ROIs for optical images

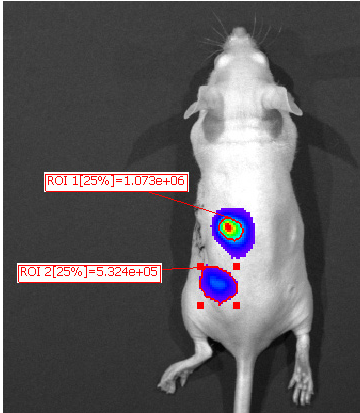
ROI Name	Description	Shape	See Page
Measurement ROI for optical data	Measures the signal intensity in an area of an optical image.	Circle, square, grid, or contour	85 (Quick Guide) 88 (detailed steps)
			

Table 5.1 Types of ROIs for optical images (continued)

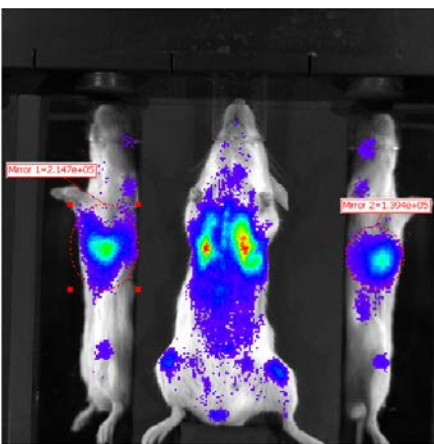
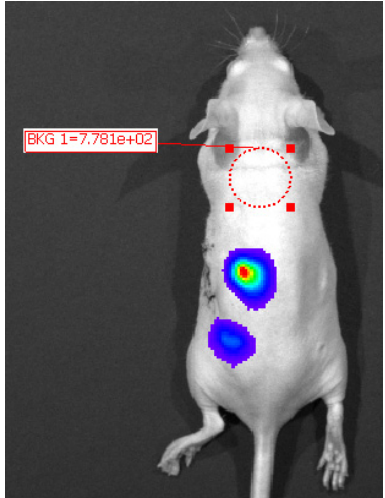
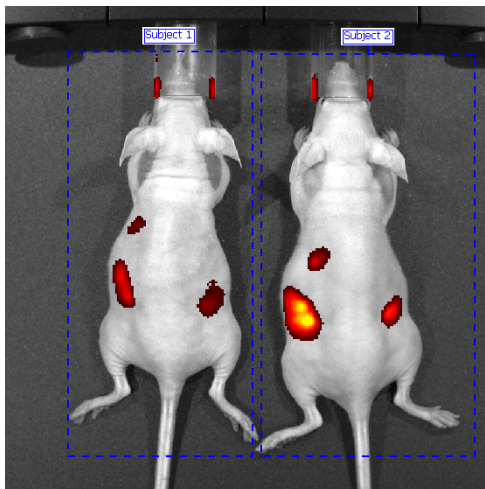
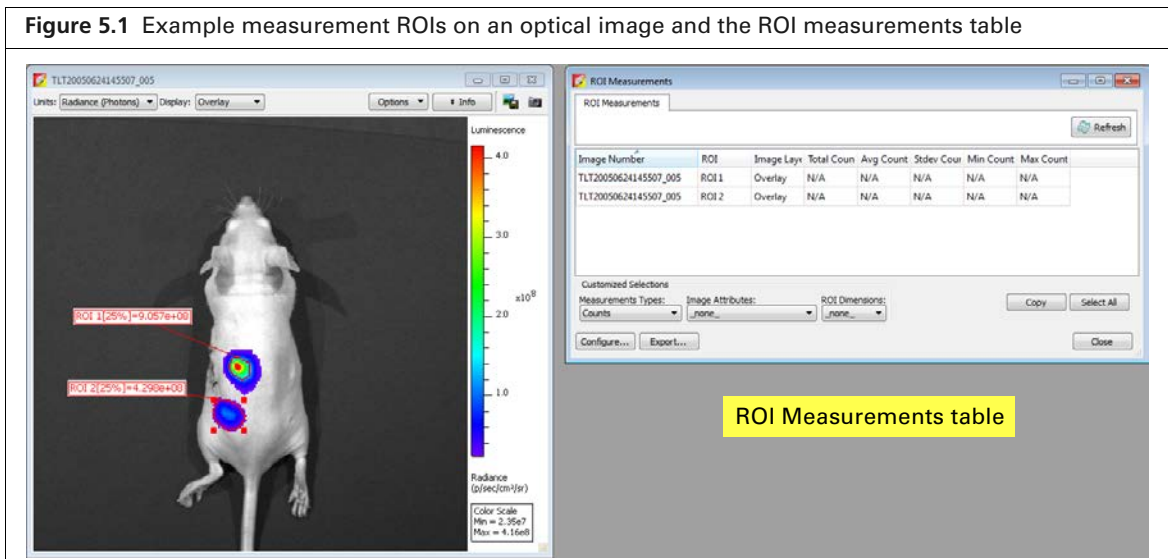
ROI Name	Description	Shape	See Page
<p>Mirror ROI for left or right views of optical data obtained using the Side Imager</p>	 <p>Images acquired using the Side Imager have three views: left, right, and center.</p> <p>Note: Use mirror ROIs to measure signal in the left or right views which are reflected from the mirrors. Use measurement ROIs to measure signal in the direct, non-reflected center view only.</p>	<p>Circle or square</p>	<p>92</p>
<p>Average Background ROI for optical data</p>	<p>Measures the average signal intensity in a user-specified area of an optical image that is considered background.</p> <p>Note: Using this type of ROI is optional. If the animal has significant autoluminescence or autofluorescence, you can determine a background-corrected signal in a measurement ROI by subtracting an average background ROI from a measurement ROI.</p> 	<p>Circle or square</p>	<p>95</p>

Table 5.1 Types of ROIs for optical images (continued)

ROI Name	Description	Shape	See Page
Subject ROI for optical data	Identifies a subject animal in an optical image. Note: Using this type of ROI is optional. It provides a convenient way to automatically associate (link) a measurement and average background ROI for background-corrected ROI measurements when there is significant autoluminescence or autofluorescence.	Square	95




The Living Image software records information about the ROIs you create during a session and computes statistical data for the ROI measurements. The ROI Measurements table displays the data and provides a convenient way to review or export ROI information (Figure 5.1).



5.2 Quick Guide: Drawing Measurement ROIs on an Optical Image or Sequence

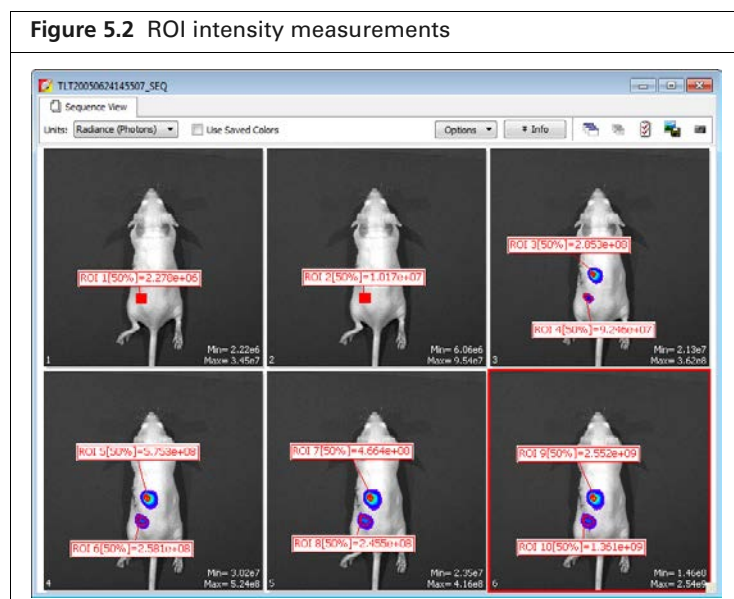
These steps provide a quick guide on how to apply a measurement ROI on an optical image or image sequence. See [page 88](#) for details on measurement ROIs.


1. Open an image or sequence and click ROI Tools in the Tool Palette.
2. Select Measurement ROI from the Type drop-down list.
3. Click the  button and select Auto All on the drop-down list.

The software automatically draws measurement ROIs on all images. The ROI label shows the total intensity in the ROI and the Threshold % ([Figure 5.2](#)).



NOTE: Auto ROIs are created and numbered in order from highest to lowest maximum signal within the ROI (ROI 1 contains the highest maximum signal). You may want to arrange the ROIs in a known order for easier comparison between images. To renumber the ROIs (ascending order from right to left), right-click the image and select Sort ROIs on the shortcut menu. If the "Apply to Sequence" option is selected in the ROI tools, choose "Sort ROIs in Sequence" to sort all of the ROIs in the sequence. The sort options are only available if the ROIs have not been sorted.

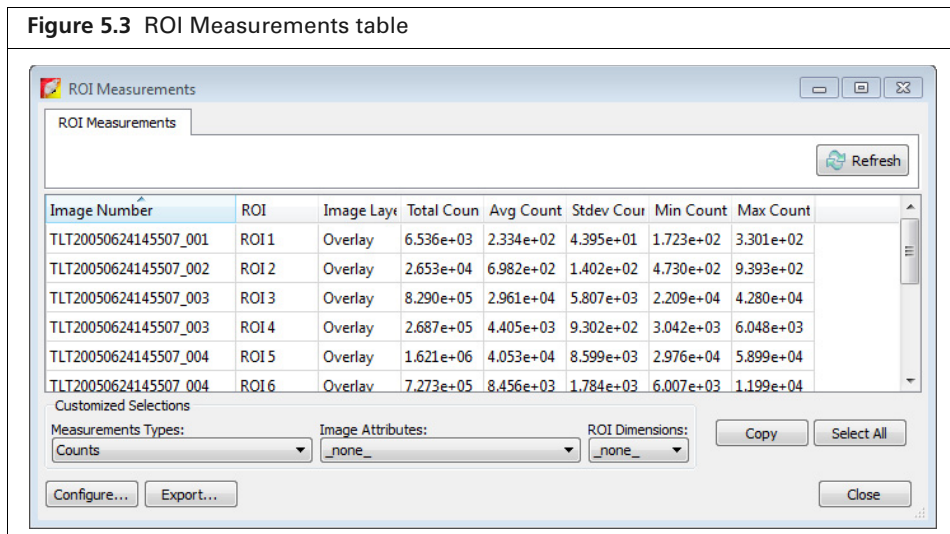


4. Use the Threshold % slider or  arrows to adjust the ROI boundaries.



NOTE: After the ROIs have been created, right-click an ROI to view a shortcut menu of ROI commands (Ctrl-click for Macintosh users). The shortcut menu provides easy access to many functions for managing ROIs and viewing ROI properties.

5. Click the **Measure** button  in the ROI tools to show the ROI Measurements table.



The ROI Measurements table displays data for all ROIs created in images or sequences during a session (one ROI per row). The table provides a convenient way to review and export ROI data. For more details on the table, see “ROI Measurements table,” page 109.

6. Click **Yes** in the save prompt when closing a data set, to save the ROIs with the data.

5.3 ROI Tools for Optical Images

This section provides an overview of the ROI tools for optical images (Table 5.2). The ROI tools that appear in the Tool Palette depend on the type of ROI selected from the ROI Type drop-down list, and whether an image or sequence is active. Some ROI parameters are only available if “Show Advanced Options” is selected in the General Preferences (Figure 5.4).

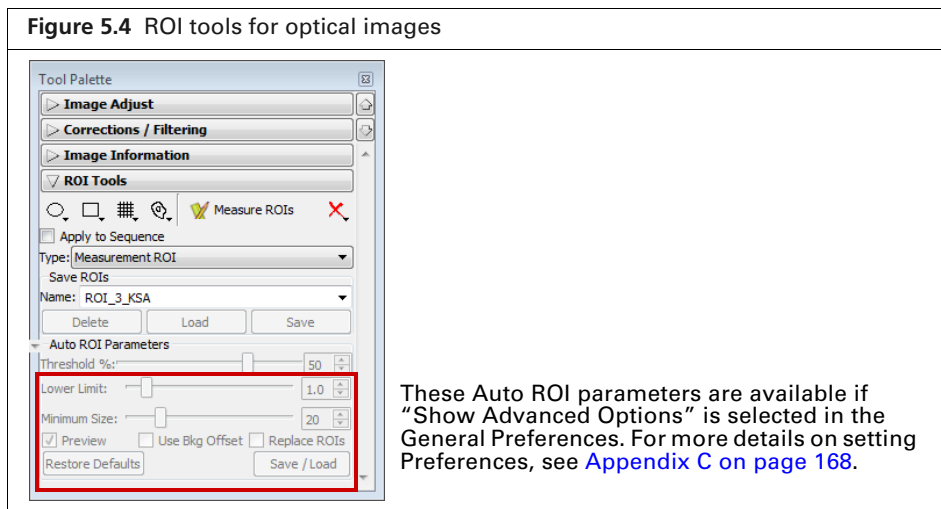


Table 5.2 ROI tools for optical images

Item	Description
	Click to select the number of circle ROIs to add to the active image.

Table 5.2 ROI tools for optical images (continued)




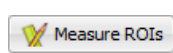

Item	Description
	Click to select the number of square ROIs to add to the active image.
	Click to specify the grid pattern for a measurement ROI that you want to add to the active image. This tool is useful for an image of a multi-well culture plate or microplate.
	Click and select Auto All to automatically draw ROIs in the image using the auto ROI parameters. Click and select Auto 1 to automatically draw one ROI at a user-selected location using the auto ROI parameters. For more details on using the auto ROI features, see page 89 .
	Click to display the ROI Measurements table or compute intensity signal in an ROI.
	Click to display a drop-down list of options to delete an ROI(s) in the active image. For more details, see page 108 . Note: These commands do not delete the ROIs that are saved to the system (listed in the Menu Name drop-down list).
Apply to Sequence	Choose this option to apply the selected ROI to all images in a sequence.
Type	Choose the ROI type from the drop-down list: Measurement – Measures the signal intensity in an area of an image. Average Bkg – Measures the average signal intensity in a user-specified area of the image that is considered background. Subject ROI – Identifies a subject animal in an image. The software automatically associates a measurement and an average bkg ROI that are included in the same subject ROI. Using this type of ROI is optional. Mirror ROI – Measures the signal intensity in an area of an image acquired using the Side Imager, taking mirror reflection effects into account
Save ROIs	Creates a file that includes the ROI parameters (for example, the X,Y coordinates, type of ROI, color, shape, width/height). ROIs that have been saved to file can be recalled and applied for another image at any time. Name – The name of the selected ROI set or the default name for a new ROI set. Delete – Deletes the selected ROI set from the system. Note: This permanently removes the ROI from the system. Load – Applies the ROI set selected from the Name drop-down list to the active image. Save – Saves the ROI set in the active image. Note: This is a global save (the ROI is saved to the system) and the ROI set can be loaded onto any image. If you use the File → Save commands to save an image that includes an ROI, the ROI is saved with the image only (not a global save) and is not available for loading onto other images. For more details, see Save, Load, or Delete ROIs, page 107 .
Auto ROI Parameters	Parameters that specify how the auto ROI tool draws an ROI. Threshold % – If the Auto All or Auto 1 method is selected, the Threshold % specifies the minimum percent of peak pixel intensity that a pixel must have to be included in an ROI identified by the software. After ROIs are drawn on an image, if you modify the Threshold% (move the slider or enter a new value), the software automatically updates the ROIs.

Table 5.2 ROI tools for optical images (continued)

Item	Description
	<p>Note: The following Auto ROI parameters are only available if “Show Advanced Options” is selected in the general preferences. For more details on setting Preferences, see Appendix C, page 168.</p> <p>Lower Limit – Specifies a multiple (1 to 10) of the color scale minimum that sets the lower threshold for identifying an ROI. For example, if the lower limit = 2 and the color scale minimum = 1000 counts, then the auto ROI tool will only draw an ROI on areas of 2000 counts or greater. This helps create ROIs only within pixels visible on the image.</p> <p>Minimum Size – Sets the minimum size of an ROI (measured in pixels). For example if the minimum size is set at 50, then ROIs created on the image must be greater than 50 pixels in size.</p> <p>Preview – If this option is chosen, the software draws the ROI each time a parameter is changed. ROI parameters can be saved without drawing the ROI.</p> <p>Use Bkg Offset – Choose this option to measure background-corrected signal. This is typically used to remove natural animal background luminescence, and should not be confused with the dark-charge and read-bias 'background' corrections that are applied (by default) to the raw CCD data to remove electronic noise before any measurements. For more details, see page 95.</p> <p>Replace ROIs – If this option is chosen, all auto ROIs are replaced when new ROI(s) are created.</p> <p>Restore Defaults – Restores the factory-set defaults for the auto ROI parameters.</p>
Save/Load	Click to display or hide the tools that enable you to save, load, or delete auto ROIs in the active data. Note: The save function saves parameters, the not actual ROIs. This means that when you load saved auto ROI parameters, the software draws a new ROI using the saved values (Threshold%, Lower Limit, Minimum Size).

5.4 Measurement ROIs

This section explains in detail how to draw a measurement ROI on an optical image to obtain the intensity signal in a user-specified area. [Table 5.3](#) lists the three methods for drawing measurement ROIs on an image.



NOTE: See [page 85](#) for a quick guide to drawing measurement ROIs on an optical image or sequence.




Table 5.3 Methods for drawing a measurement ROI

Drawing Method	Description	See Page
Automatic	The software automatically locates and draws an ROI(s) on the image. To do this, the software locates the peak pixel intensities in the image and searches the neighborhood around a peak pixel. A pixel is included in the ROI if the pixel intensity is greater than the threshold%, a user-specified percentage of the peak pixel intensity.	89
Manual	Places one or more ROIs (circular, square, or grid shape) on the image.	85
Free draw	Draw line segments that define the ROI.	91

Drawing Measurement ROIs Automatically

The Living Image® software can automatically identify all of the ROIs in an image or image sequence that meet the auto ROI parameter thresholds or draw one ROI at a user-specified location.

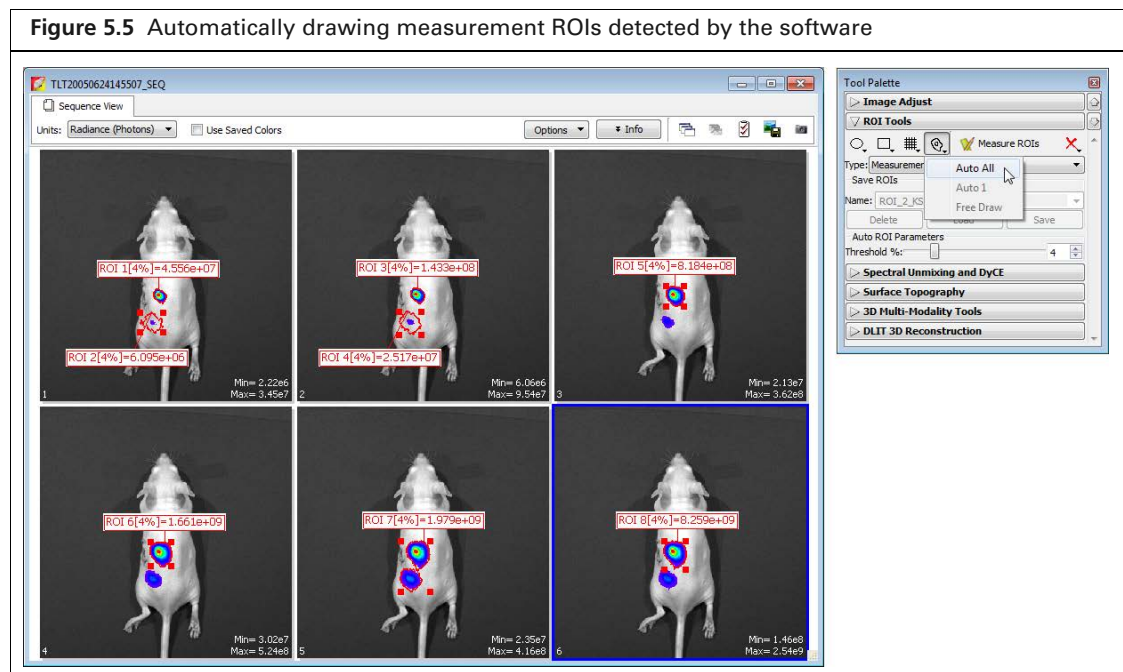
To automatically identify and draw all ROIs:

1. Open an image or image sequence, and in the ROI tools, select Measurement ROI from the Type drop-down list.
2. Click an ROI shape button (Circle , Square , or Contour ) and select **Auto All** from the drop-down list.

The ROIs appear on the image or sequence thumbnails. The ROI label includes the ROI intensity threshold (Threshold%) and intensity measurement.



NOTE: Auto ROIs are created and numbered in order from highest to lowest maximum signal within the ROI (ROI 1 contains the highest maximum signal). You may want to arrange the ROIs in a known order for easier comparison between images. To renumber the ROIs (ascending order from right to left), right-click the image and select Sort ROIs on the shortcut menu. If the "Apply to Sequence" option is selected in the ROI tools, choose "Sort ROIs in Sequence" to sort all of the ROIs in the sequence. The sort options are only available if the ROIs have not been sorted.



3. Click the **Measure** button  in the ROI tools to show the ROI Measurements table.

Figure 5.6 ROI Measurements table

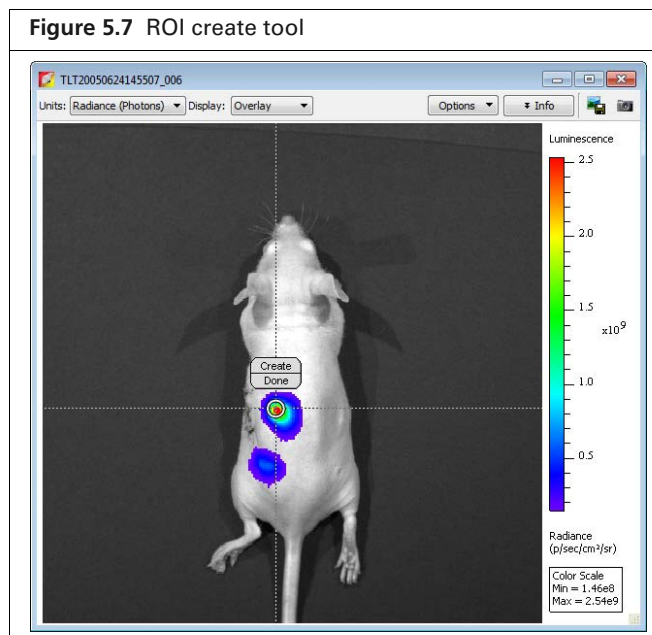
Image Number	ROI	Image Layer	Total Count	Avg Count	Stdev Count	Min Count	Max Count
TLT20050624145507_001	ROI 1	Overlay	6.536e+03	2.334e+02	4.395e+01	1.723e+02	3.301e+02
TLT20050624145507_002	ROI 2	Overlay	2.653e+04	6.982e+02	1.402e+02	4.730e+02	9.393e+02
TLT20050624145507_003	ROI 3	Overlay	8.290e+05	2.961e+04	5.807e+03	2.209e+04	4.280e+04
TLT20050624145507_003	ROI 4	Overlay	2.687e+05	4.405e+03	9.302e+02	3.042e+03	6.048e+03
TLT20050624145507_004	ROI 5	Overlay	1.621e+06	4.053e+04	8.599e+03	2.976e+04	5.899e+04
TLT20050624145507_004	ROI 6	Overlay	7.273e+05	8.456e+03	1.784e+03	6.007e+03	1.199e+04


The ROI Measurements table displays data for all ROIs created in images or sequences during a session (one ROI per row). The table provides a convenient way to review and export ROI data. For more details on the table, see [“Managing the ROI Measurements Table,” page 109](#)

To automatically draw an ROI at a user-specified location:




1. Open an image.
2. Click an ROI shape button (Circle , Square , or Contour ) and select **Auto 1** from the drop-down list.

The create tool appears on the image.




3. Use the ring  to move the create tool to the area where you want to draw the ROI, then click **Create**.
The ROI appears on the image and the ROI label displays the intensity signal.
4. To draw another ROI on the image, repeat step 2. to step 3.
For information on how to save ROIs, see [page 107](#).

Drawing Measurement ROIs Manually

1. Open an image or image sequence, and in the ROI tools, select Measurement ROI from the Type drop-down list.
2. Select the ROI shape:
 - a. Click the **Circle** , **Square** , or **Grid**  button.
The grid shape is useful for drawing a grid of ROIs on an image of a well plate.
 - b. On the drop-down list that appears, select the number of ROIs that you want to add to the image or the grid ROI dimensions.
The ROIs and intensity measurements appear on the image.






NOTE: Manual ROIs are numbered in the order they are created. You may want to arrange the ROIs in a known order for easier comparison between images. To renumber the ROIs (ascending order from right to left), right-click the image and select Sort ROIs on the shortcut menu. If the "Apply to Sequence" option is selected in the ROI tools, choose "Sort ROIs in Sequence" to sort all of the ROIs in the sequence. The sort options are only available if the ROIs have not been sorted.

3. Adjust the ROI position:
 - a. Place the mouse pointer over the ROI. When the pointer becomes a , click the ROI.
 - b. Drag ROI(s).



NOTE: To move multiple ROIs at the same time, press and hold the Shift key while you click the ROIs, and then drag them to a new location. Contour ROIs () cannot be moved using this method.

4. Adjust the ROI dimensions:
 - a. Place the mouse pointer over the ROI. When the pointer becomes a , click the ROI.
 - b. Place the mouse pointer over an ROI handle  so that it becomes a . Drag the handle to resize the ROI.










NOTE: You can also change the ROI position or size using the adjustment controls in the ROI Properties box (see [Moving an ROI, page 103](#) and [Editing ROI Dimensions, page 104](#)).

5. Click the **Measure** button .

The ROI measurements and table appear. For more details on the table, see “[Managing the ROI Measurements Table,](#)” page 109.

For information on how to save ROIs, see [page 107](#).

Drawing ROIs Using the Free Draw Method

1. Open an image, and in the ROI tools, select the type of ROI that you want to draw from the Type drop-down list.
2. Click an ROI shape button (**Circle** , **Square** , or **Contour** ) and select **Free Draw** from the drop-down list. In this example, the Contour shape  was selected for the free draw method. The ROI shapes that are available depend on the type of ROI selected.
3. If you selected:
 -  or  - Use the pointer (+) to draw the ROI.
 -  - Use the pointer (+) to click around the area of interest and draw line segments that define the ROI. Right-click when the last point is near the first point in the ROI.

4. Click the **Measure** button .

The ROI measurements and table appear. For more details on the table, see “[Managing the ROI Measurements Table](#),” page 109.

For information on how to save ROIs, see page [page 107](#).

5.5 Mirror ROIs

Use a mirror ROI to measure bioluminescence or fluorescence in the right or left mirror-reflected view of images acquired using the Side Imager. Measure signals in the center view using a measurement ROI. See [page 88](#) for more details on drawing a measurement ROI.



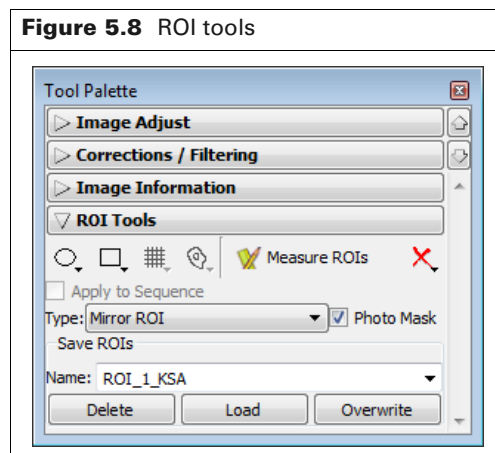
NOTE: Do not apply mirror ROIs on the center view or measurement ROIs on the left or right mirror-reflected views. Placing an ROI on the wrong view will result in incorrect ROI measurements.



1. Open an image or image sequence acquired with the Side Imager.

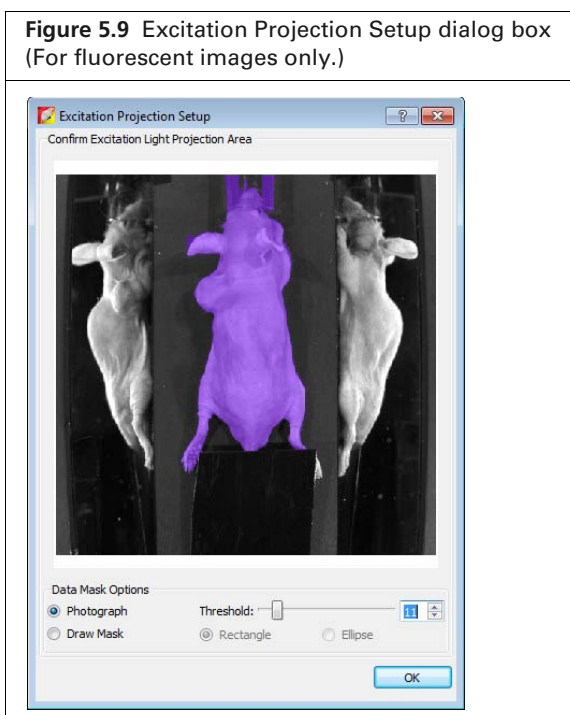



NOTE: Fluorescent image data acquired in reflectance/epi-illumination mode must include a photograph.

2. Select "Mirror ROI" from the Type drop-down list in the ROI tools. If analyzing a fluorescent image, choose the Photo Mask option.



3. Select the ROI shape:
 - a. Click the **Circle** , or **Square** , button.
 - b. Select the number of ROIs to add to the image on the drop-down list that appears.
 - If analyzing a reflectance/epi-illumination fluorescent image, go to [step 4](#); otherwise, go to [step 5](#).
4. For reflectance/epi-illumination fluorescent images only:
 - a. Confirm the purple data mask in the dialog box that appears ([Figure 5.9](#)).
The data mask includes the entire subject by default and defines the area of excitation light projection onto the animal. If you do not want to analyze the entire subject, select the Data Mask option and mask a particular area using the data mask options ([Table 5.4](#)).
 - b. Click **OK**.
The mirror ROIs and intensity measurements appear on the image ([Figure 5.10](#)).

**Table 5.4** Data mask options




Option	Description
Photograph	If this option is chosen, the software automatically draws the data mask by using higher intensities in the photograph. The mask selects high-valued photograph image pixels which are located continuously and centrally in the photograph image. The photograph mask works best with light-colored subjects.
Threshold	If necessary use the threshold slider or  arrows to adjust the mask so that it matches the underlying subject photograph as closely as possible without including any area outside the subject image.
Draw Mask	Choose this option to manually draw a data mask on an area of the photograph.
Rectangle	Specifies a rectangular shape for the manual data mask.
Ellipse	Specifies an elliptical shape for the manual data mask.



NOTE: The ROIs are numbered in the order they are created. You may want to arrange the ROIs in a known order for easier comparison between images. To renumber the ROIs (ascending order from right to left), right-click the image and select Sort ROIs on the shortcut menu. If the "Apply to Sequence" option is selected in the ROI tools, choose "Sort ROIs in Sequence" to sort all of the ROIs in the sequence. The sort options are only available if the ROIs have not been sorted.

5. Adjust the ROI position:
 - a. Place the mouse pointer over the ROI. Click the ROI when the pointer becomes a .
 - b. Drag ROI(s).

NOTE: To move multiple ROIs at the same time, press and hold the Shift key while you click the ROIs, and then drag them to a new location. Contour ROIs cannot be moved using this method.

6. Adjust the ROI dimensions:
 - a. Place the mouse pointer over the ROI. Click the ROI when the pointer becomes a .
 - b. Place the mouse pointer over an ROI handle  so that it becomes a . Drag the handle to resize the ROI.

NOTE: You can also change the ROI position or size using the adjustment controls in the ROI Properties box (see [Moving an ROI, page 103](#) and [Editing ROI Dimensions, page 104](#)).

7. Click the **Measure** button .

The ROI table appears. For more details on the table, see ["Managing the ROI Measurements Table," page 109](#).

5.6 Measuring Background-Corrected Signal

If a subject has significant autoluminescence or autofluorescence, you can obtain a background-corrected ROI measurement by subtracting an average background ROI from a measurement ROI. The software computes:

Background-corrected intensity signal = Signal in the measurement ROI - Average signal in the average background ROI




NOTE: This is an optional "background" correction that is applied in addition to the electronic dark-charge and read-bias corrections that are applied to the raw CCD data.

The Image Adjust tools and zoom feature are helpful for selecting an appropriate area for an ROI. By setting the image minimum close to zero and zooming in on a background area in the image, you can determine where naturally occurring background luminescence or autofluorescence is present. For more details on the Image Adjust tools and the zoom feature, see [Adjusting Image Appearance](#), page 61 and [Magnifying or Panning in the Image Window](#), page 63.

Subject ROIs

A subject ROI identifies a subject animal in an image. It provides a convenient way to automatically associate (link) a measurement and average background ROI for background-corrected ROI measurements when there is significant autoluminescence or autofluorescence. Using a subject ROI is optional.


To draw a subject ROI using the auto ROI feature:

1. Select Subject ROI from the Type drop-down list.
2. Click the  button.
3. Select **Auto All**.



To manually draw a subject ROI:



NOTE: If the image was acquired using the Side Imager, draw three subject ROIs, one for each view.

1. Select Subject ROI from the Type drop-down list.
2. Click the  button, and select **1**.
3. Position the subject ROI so that it includes the measurement ROI(s) and the associated average background ROI.

Measuring Background-corrected Signal

1. Draw one or more measurement ROIs on the subject (see [page 88](#) for more details).
2. Draw an average background ROI on the subject.
 - a. Select Average Bkg ROI from the Type drop-down list.
 - b. Click the **Square**  or **Circle**  button and select **1**.
The ROI is added to the image. For more details on adjusting the ROI position or dimensions, see [page 103](#) and [page 104](#).

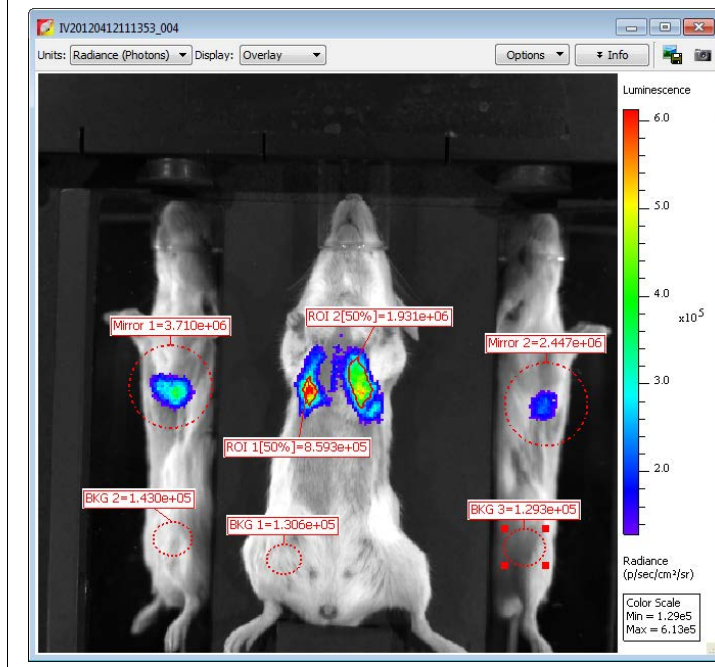


NOTE: The average background ROI and measurement ROI do not need to be the same shape or size because the software computes the average intensity signal in each ROI.



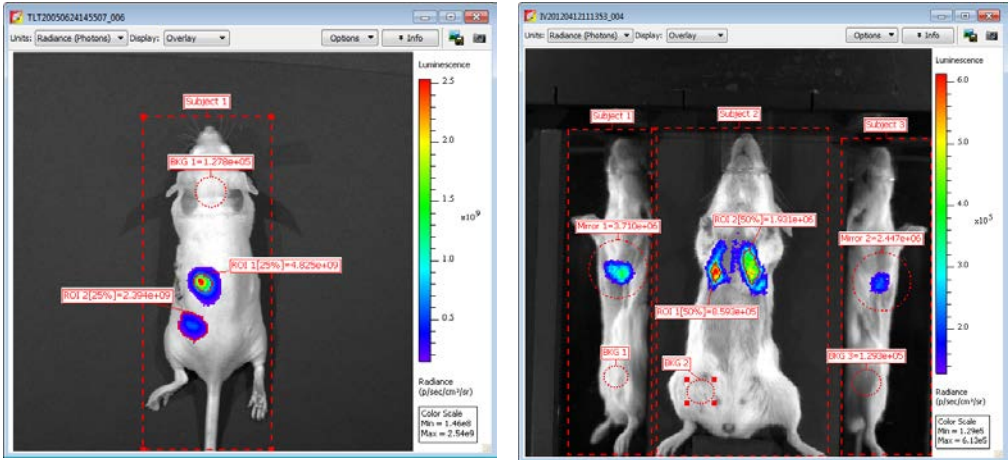
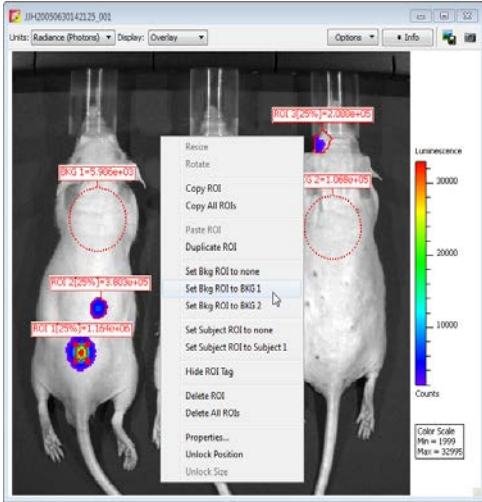

NOTE: If the image was acquired using the Side Imager, draw a background ROI on each view (Figure 5.11).

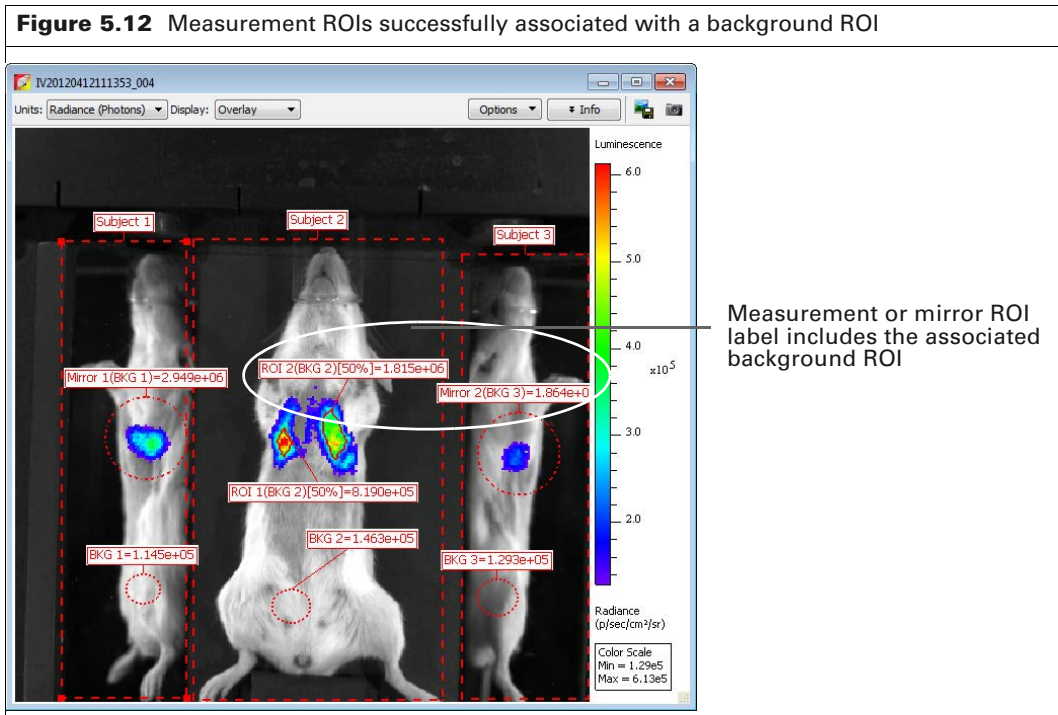
Figure 5.11 Draw a background ROI on each view in an image acquired using the Side Imager



3. Associate each background ROI with a measurement ROI(s) or mirror ROI(s) using one of the methods in [Table 5.5](#).

Table 5.5 Methods for associating measurement or mirror ROIs with a background ROI

Method	Description
<p>Draw a subject ROI See page 95 for details.</p>	
<p>Right-click a measurement ROI and select an average background ROI from the shortcut menu.</p>	
<ol style="list-style-type: none"> Right-click a background ROI and select Properties on the shortcut menu. In the ROI Properties box that appears, click the Background ROI tab and put a check mark next to Use as BKG for future ROIs in. Choose the image name or the Entire sequence option. 	



5.7 ROI Histogram

The ROI histogram plots a frequency distribution of pixel intensity. The histogram sorts the pixels into groups or *bins* (x-axis coordinate) and plots the number of pixels in each bin (y-axis coordinate).

To view the ROI histogram:


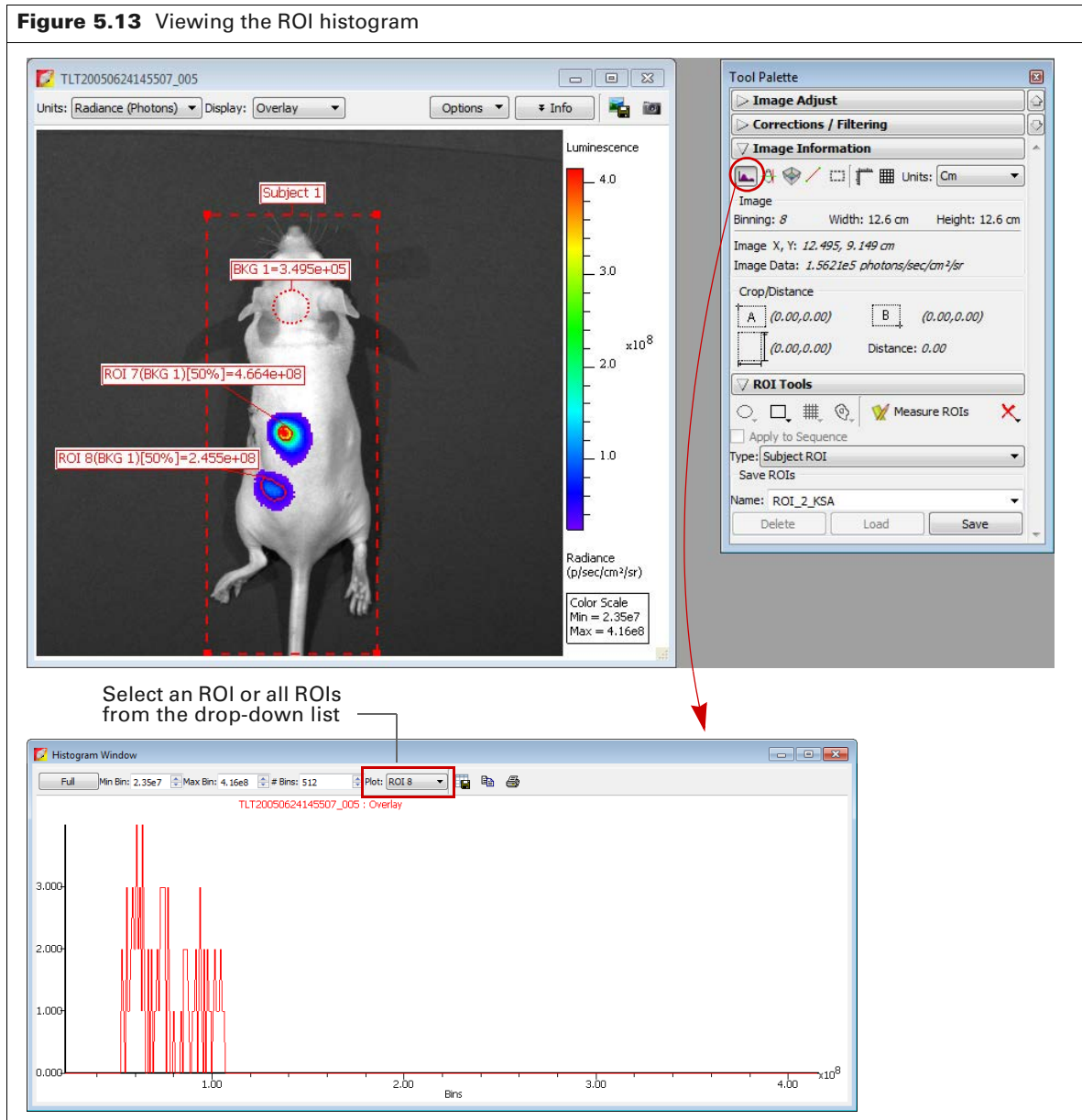
1. Open an image that includes measurement ROIs.
2. Click the histogram button  in the Image Information tools.
3. Select an ROI or "All ROIs" from the Plot drop-down list of the histogram that appears.

Figure 5.13 Viewing the ROI histogram



5.8 Managing ROI Properties

In the ROI Properties box, you can view information about an ROI, change the position of the ROI on the image, and edit the ROI label or line characteristics.

Viewing ROI Properties

1. To view ROI properties, do one of the following:
 - Double-click an ROI in the image.
 - Right-click the ROI and select **Properties** from shortcut menu that appears.
 - Select the ROI, then select **View** → **Properties** on the menu bar.The ROI Properties box appears (for more details see [Figure 5.16](#)).
2. To view properties for another ROI, click the ROI in the image. Alternatively, select an ROI from the ROI drop-down list in the ROI Properties dialog box ([Figure 5.14](#)).

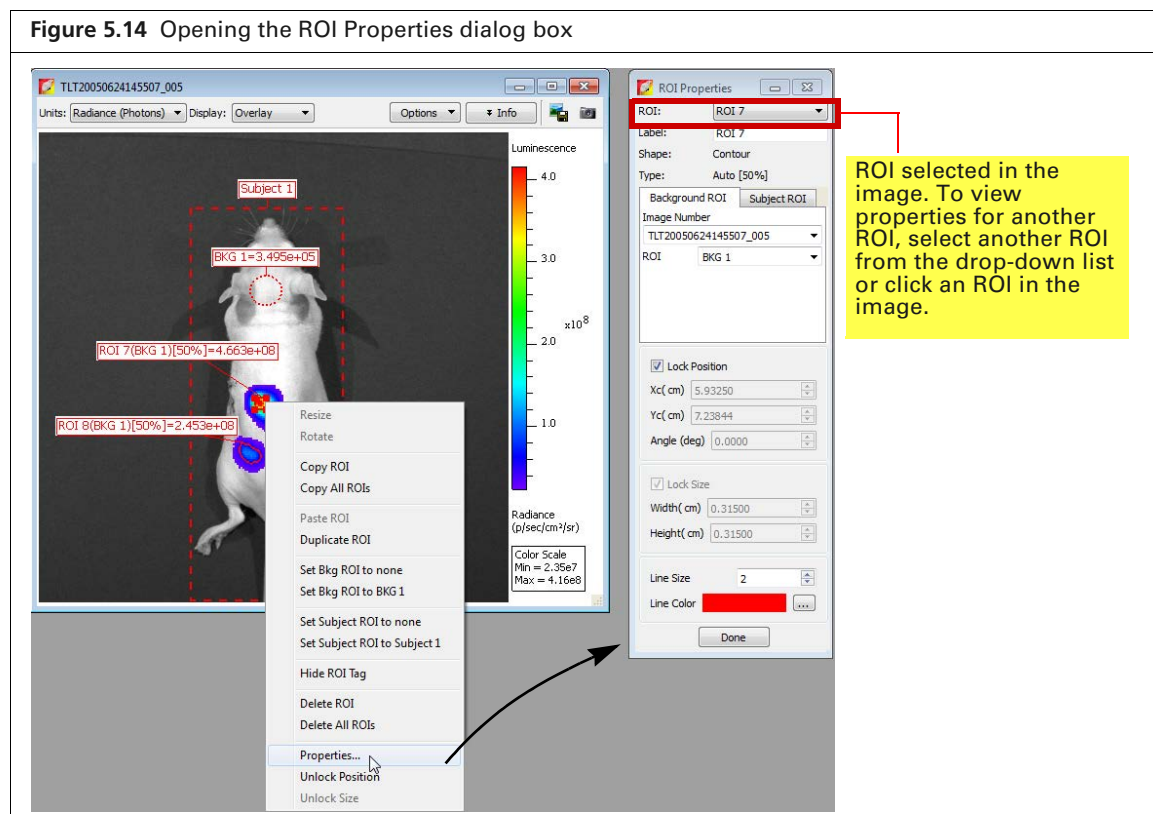
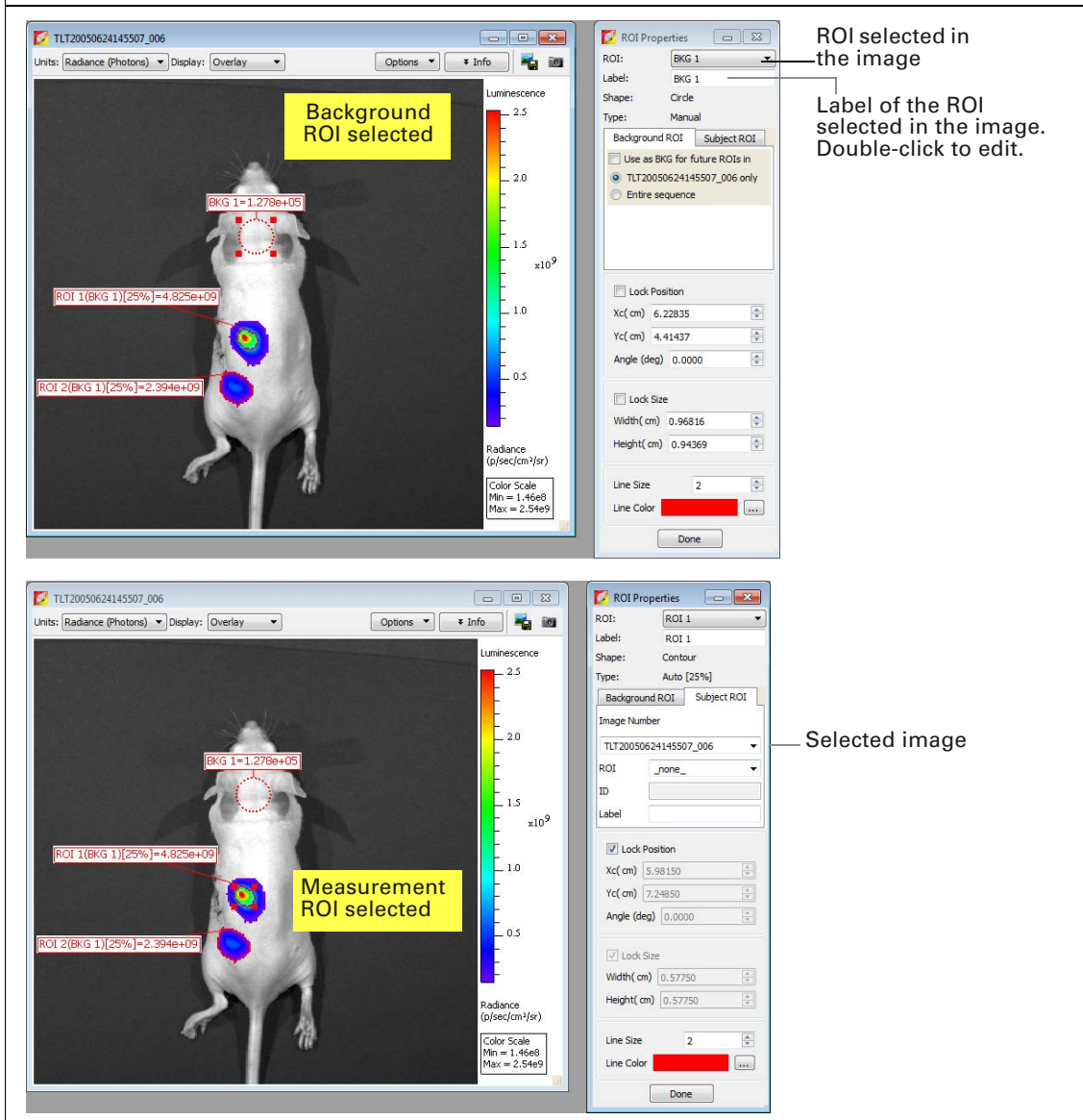
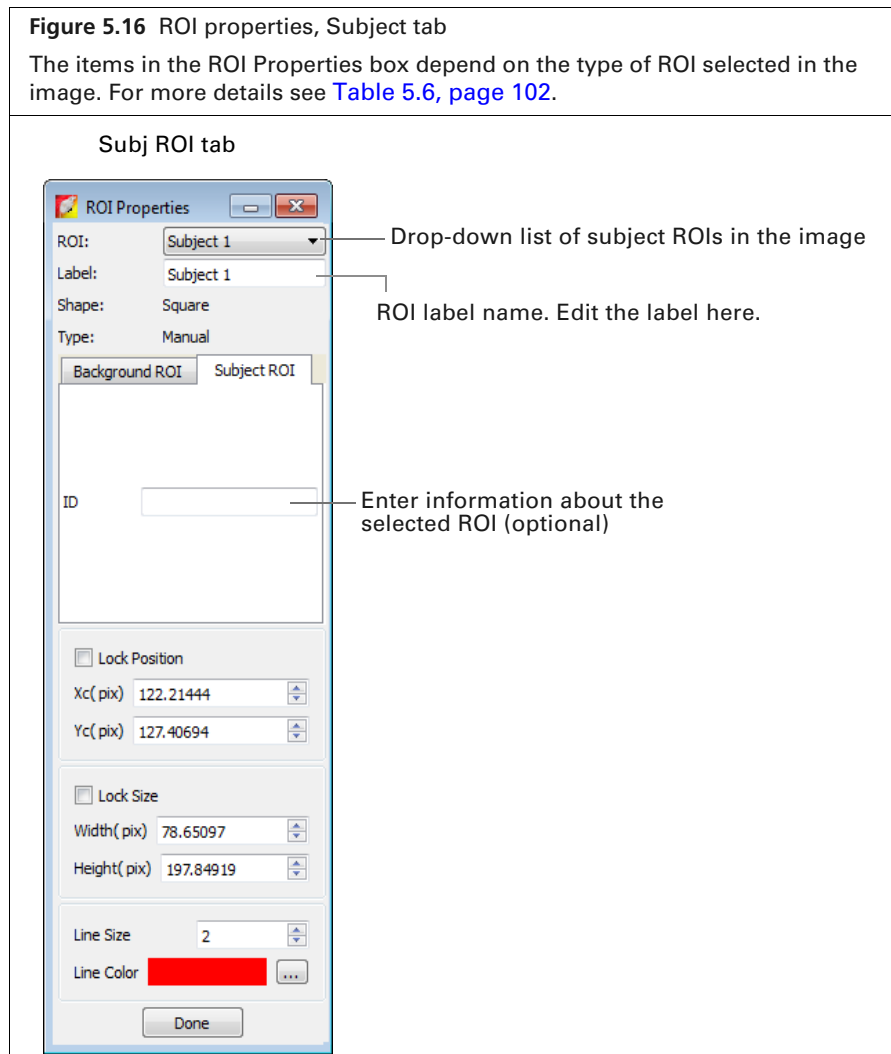


Figure 5.15 ROI Properties, Background ROI tab



The items in the ROI Properties box depend on the type of ROI selected in the image. For more details see [Table 5.6, page 102](#).



**Table 5.6** ROI Properties

Item	Description
ROI	A drop-down list of ROIs in the active image or image sequence. To select an ROI, double-click the ROI in the image or make a selection from the drop-down list. Shape – The shape of the ROI (circle, square, grid, or contour) selected in the image. Type – Indicates the method that was used to draw the selected ROI (automatic, manual, or free draw).
ROI Label	Click to edit the selected ROI label name.
Image Number	A drop-down list of open images.
Background ROI tab	The Background ROI tab shows a drop-down list shows all average background ROIs in active image that can be linked to a user-specified measurement ROI or subject ROI (selected from the drop-down list at the top of the dialog box).

Table 5.6 ROI Properties (continued)

Item	Description
Subj ROI	The Subject ROI tab shows a drop-down list of all subject ROIs in the image number selected above that can be linked to a user-specified measurement ROI or average background ROI (selected from the drop-down list at the top of the dialog box). The Background ROI tab shows a drop-down list of all average background ROIs in the click number selected above that can be linked to a user-specified measurement ROI or subject ROI (selected from the drop-down list at the top of the dialog box).
ID	User-entered information about a subject ROI.
Label	Label name of the selected subject ROI.
Lock Position	Choose this option to lock the position of the ROI selected in the image.
Xc	x-axis coordinate at the center of the ROI selected in the image.
Yc	y-axis coordinate at the center of the ROI selected in the image.
Lock Size	Choose this option to lock the dimensions of the ROI selected in the image.
Width	Width (pixels or cm) of the ROI selected in the image (for more details on setting the units, see ROI Dimensions, page 110).
Height	Height (pixels or cm) of the ROI selected in the image.
Line Size	Specifies the ROI line thickness. To change the line thickness, enter a new value or click the up/down arrows  .
Line Color	Specifies the color of the ROI line. To select a line color, click the Browse button  .
Done	Click to close the ROI Properties box and apply any new settings, including: <ul style="list-style-type: none"> ■ Linkage between a measurement ROI and subject ROI (for more details, see Drawing ROIs Using the Free Draw Method, page 91). ■ ROI size dimensions or position ■ Subject ROI ID information

Moving an ROI


To move an ROI on an image, select it and do one of the following:

- Press a keyboard arrow key
- Drag the ROI
- Edit the settings in the ROI Properties box



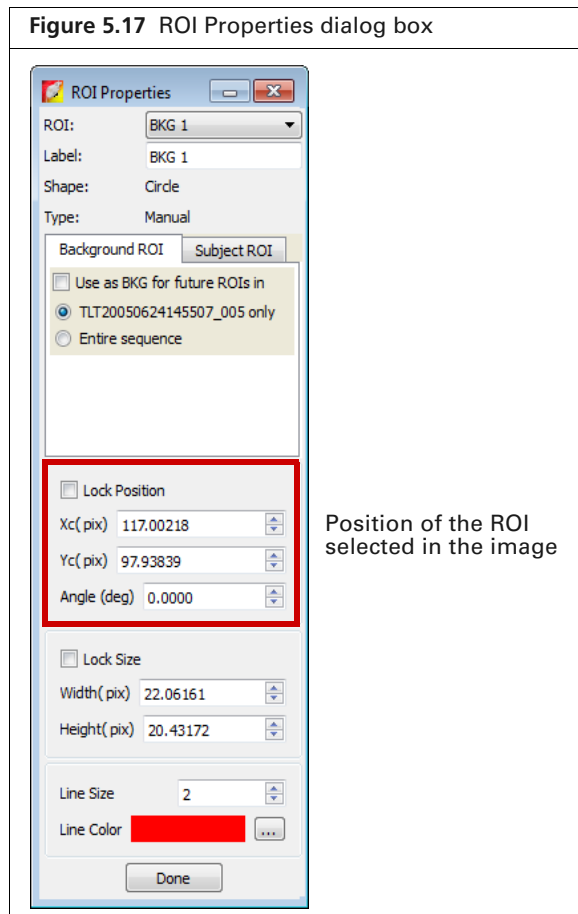
NOTE: An ROI cannot be moved if it was created using the auto ROI tool or if the ROI position is locked.

To drag an ROI:

1. Put the mouse pointer over the ROI so that it becomes a  arrow.
2. Drag the ROI.
3. Release the mouse button when the ROI is properly positioned.

To move an ROI using the ROI Properties dialog box:

1. Double-click the ROI in the image.
The ROI Properties box appears and displays the position and dimensions of the selected ROI.



2. To set ROI position, enter new coordinates for the center of the ROI (Xc (pix or cm) and Yc (pix or cm values)) in the ROI Properties box.
3. To rotate the ROI clockwise, enter the degrees in the Angle (deg) box and click outside the box.
4. To lock the current ROI position, choose the Lock Position option.



NOTE: The ROI position cannot be changed until the Lock Position option is cleared.

Editing ROI Dimensions

There are two ways to resize a circle or square ROI:

- Drag a handle on the ROI
- Edit the settings in the ROI Properties box



NOTE: You cannot change the size of an ROI that was created using the auto ROI or free draw tool.

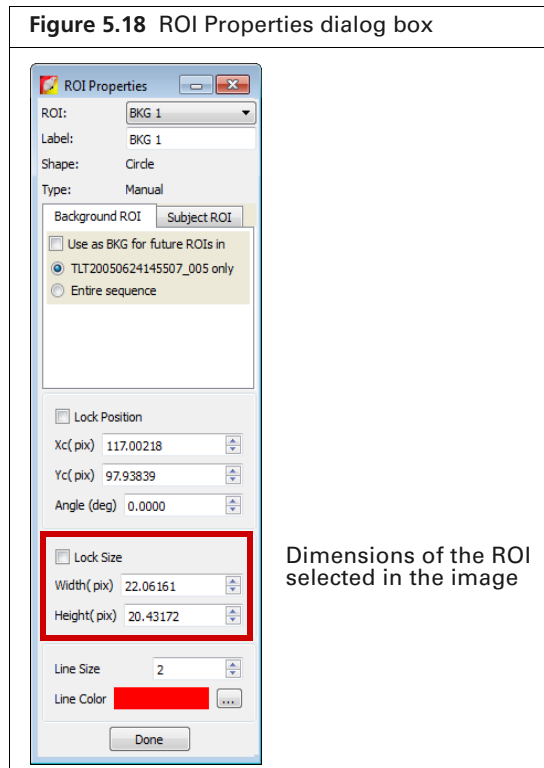
To resize an ROI using a handle:

1. Select the ROI and put the mouse pointer over a handle (■) on the ROI.
2. When the pointer becomes a ↖ arrow, drag the handle.

To resize an ROI using the ROI Properties box:

1. Double-click the ROI in the image.

The ROI Properties box appears and displays the positions and dimensions of the selected ROI.



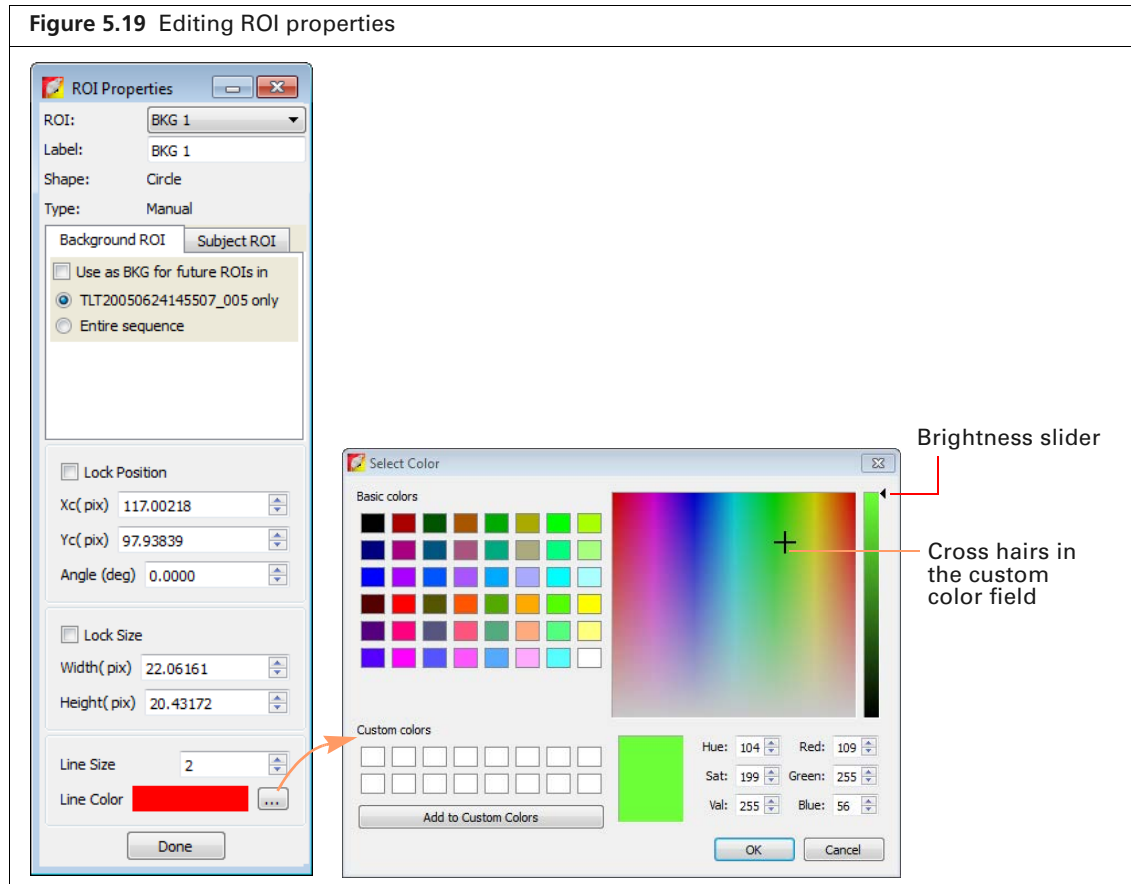
2. Enter a new width or height value in the ROI Properties box.
3. To lock the current ROI size, choose the Lock Size option.





NOTE: The ROI size cannot be changed until the Lock Size option is cleared.

Editing the ROI Line


1. Double-click the ROI that you want to edit.
The ROI Properties box appears (Figure 5.19).

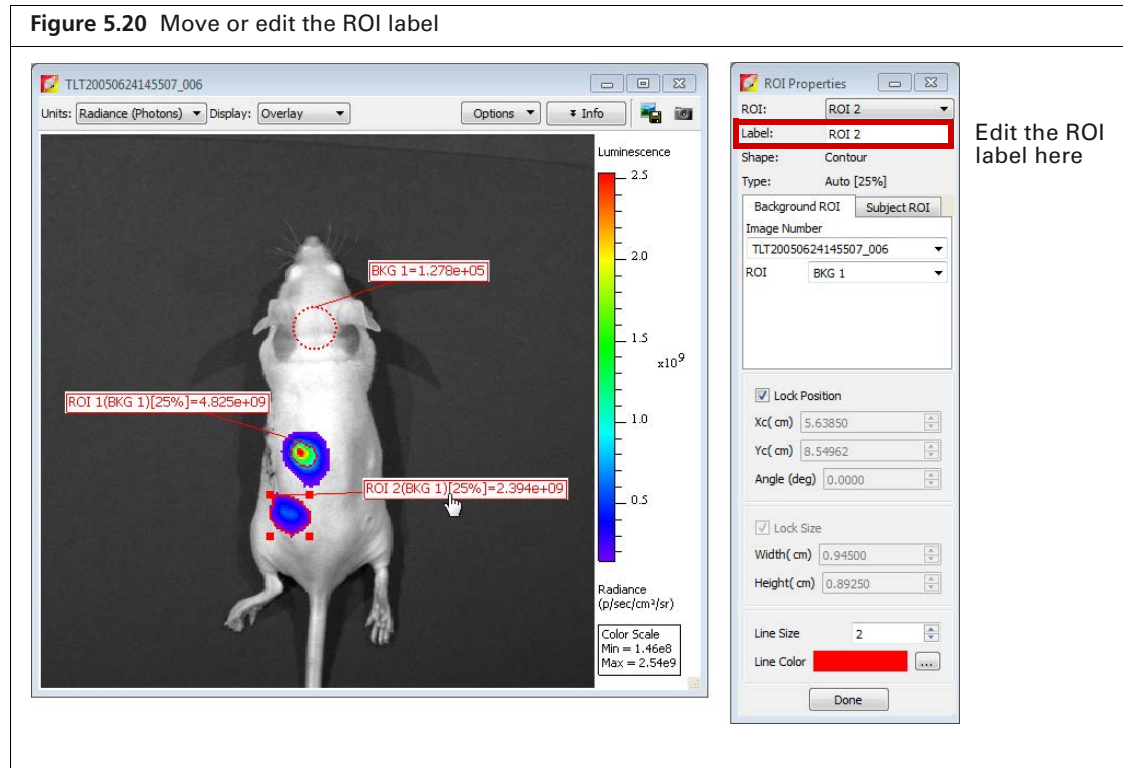


2. To edit the ROI line thickness, enter a new value in the Line Size box. Alternatively, click the  arrows.
3. To change the ROI line color:
 - a. Click the **Browse** button . The Select Color box appears.
 - b. To select a basic color for the ROI line, click a basic color swatch, and click **OK**.
 - c. To define a custom color, drag the crosshairs in the custom color field, adjust the brightness slider, and click **Add to Custom Colors**.
 - d. To select a custom color for the ROI line, click a custom color swatch, and click **OK**.

Move or Edit the ROI Label

To move the ROI label:

1. Put the mouse pointer over the ROI label.
2. When the pointer becomes a , drag the label, and then click to release the label at the new location (Figure 5.20).



To edit the ROI label:

1. Double-click the ROI of interest. Alternatively, right-click the ROI (**Ctrl-click** for Macintosh users) and select Properties on the shortcut menu.
2. In the ROI Properties box that appears, edit the name in the ROI Label box and click **Done** (Figure 5.20).

Save, Load, or Delete ROIs

The software automatically saves ROIs with an image. The ROI measurements are saved in the AnalyzedClickInfo.txt file associated with the image. ROIs are saved per user and can be applied to other sequences. Additionally, ROI parameters can be saved per user and applied to other sequences.

To save ROIs to the system:

1. In the Name drop-down list, confirm the default name or enter a new name for the ROI(s).
2. Click **Save**.

The ROI(s) from the image are saved to the system and can be selected from the Name drop-down list.

To load ROIs on an image:

1. Open an image.

- In the ROI tools, make a selection from the Name drop-down list and click **Load**.



NOTE: If you load ROI(s) onto an image, then draw additional ROIs, the Save button changes to Overwrite. If you want to save this collection of ROIs using the existing name, click Overwrite.

To delete ROIs from an image:

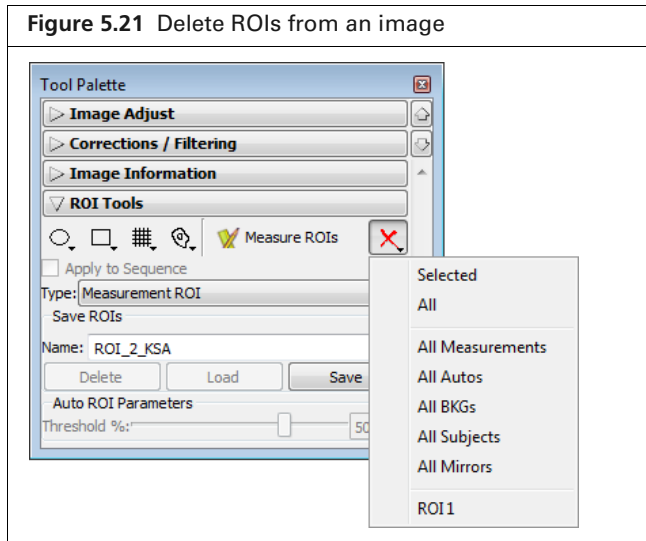


NOTE: This does not delete ROIs saved to the system (global save).

- Select the ROI and press the Delete key.

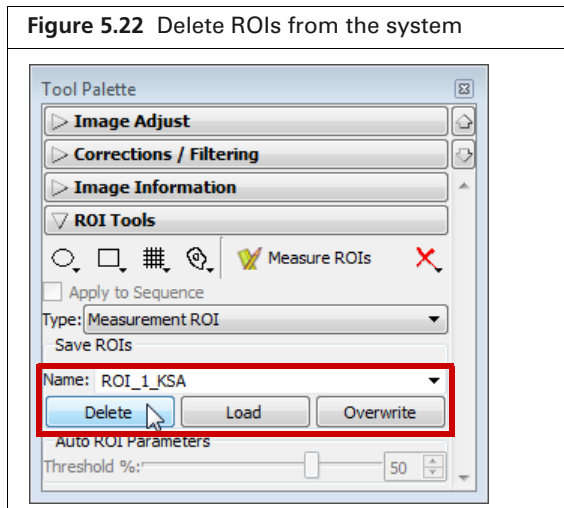
OR

- Click the  button in the ROI tools and select a delete command from the drop-down list.



To permanently delete ROIs from the system:

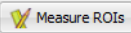
- Select the ROI(s) that you want to delete from the drop-down list of saved ROIs.
- Click **Delete**.



5.9 Managing the ROI Measurements Table

The ROI Measurements table shows information and data for the ROIs created during a session. The ROI measurements can be displayed in units of counts, radiance, Radiant Efficiency, Efficiency, or NTF Efficiency, depending on the type of image data. See the technical note *Quantifying Image Data* for more details (select **Help** → **Tech Notes** on the menu bar).

Viewing the ROI Measurements Table

Click the  button to display the ROI measurement table. Alternatively, select **View** → **ROI Measurements** on the menu bar.

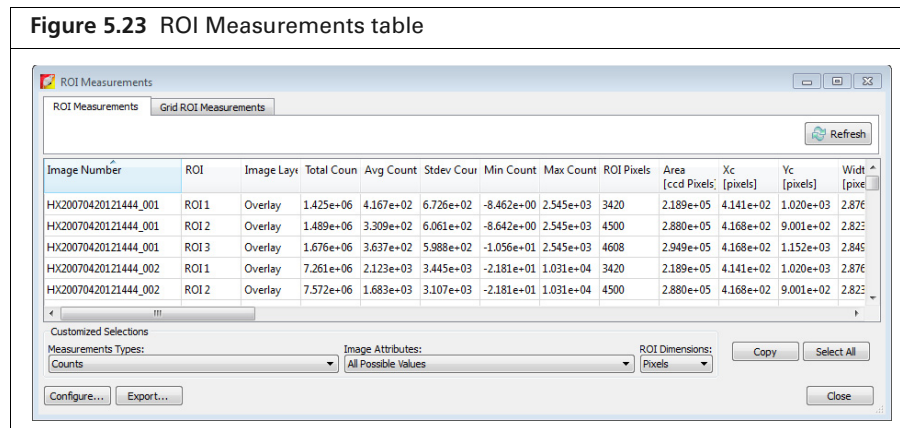


Table 5.7 ROI Measurements table

Item	Description
Measurement Types	Make a selection from the this drop-down list to select the type of image unit for the ROI measurements in the table.
None	Excludes ROI measurements from the table.
Counts (luminescence and fluorescence)	Includes Total Counts, Avg Counts, Stdev Counts, Min Counts, and Max Counts in the table. Total Counts = the sum of all counts for all pixels inside the ROI. Avg Counts = Total Counts/Number of pixels or super pixels. Stdev Counts = standard deviation of the pixel counts inside the ROI Min Counts = lowest number of counts in a pixel inside the ROI. Max counts = highest number of counts in a pixel inside the ROI. Note: These numbers are displayed if the units selected in the ROI Measurements table and the image are the same. Otherwise, N/A appears in each column. Tip: See the tech note <i>Image Display and Measurement</i> for more details on count units (select Help → Tech Notes on the menu bar).

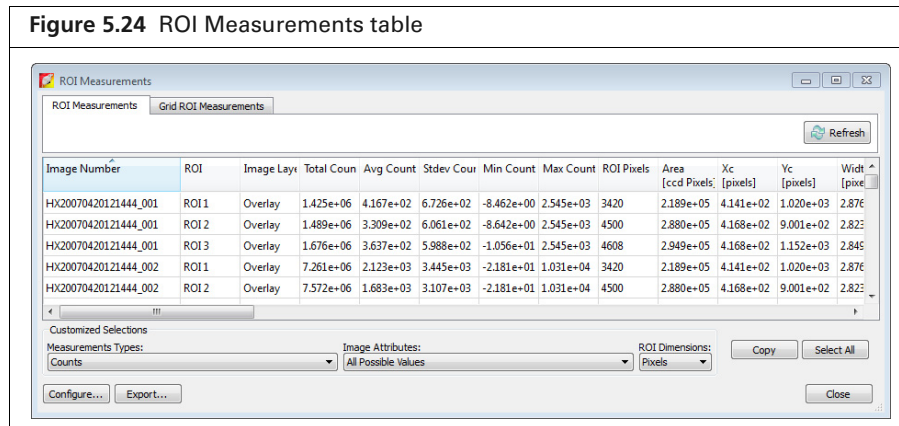
Table 5.7 ROI Measurements table (continued)

Item	Description
Radiance (Photons) (fluorescence and luminescence)	<p>Total Flux (photons/sec) = the radiance (photons/sec/cm²/steradian) in each pixel summed or integrated over the ROI area (cm²) x 4π.</p> <p>Average Radiance = the sum of the radiance from each pixel inside the ROI/ number of pixels or super pixels (photons/sec/cm²/sr).</p> <p>Stdev Radiance = standard deviation of the pixel radiance inside the ROI</p> <p>Min Radiance = lowest radiance for a pixel inside the ROI.</p> <p>Max Radiance = highest radiance for a pixel inside the ROI.</p> <p>Tip: See the tech note <i>Image Display and Measurement</i> for more details on photon units (select Help → Tech Notes on the menu bar).</p>
Radiant Efficiency (fluorescence)	Epi-fluorescence - Fluorescence emission radiance per incident excitation intensity: p/sec/cm ² /sr/μW/cm ²
Efficiency (epi-fluorescence)	Fluorescent emission yield normalized to the incident excitation intensity (radiance of the subject/illumination intensity)
Image Attributes	Make a selection from the drop-down list to specify the click number (image file) information to include in the table. Click attributes include label name settings and camera settings.
None	Excludes image attributes from the table.
All Possible Values	Includes all of the image attributes (for example, label name settings and camera settings) in the table.
All Populated Values	Includes only the image attributes with values in the table.
Living Image Universal	Includes all Living Image Universal label name settings in the table.
ROI Dimensions	Make a selection from the drop-down list to specify the ROI dimensions to include in the table.
None	Excludes the ROI area, x,y-coordinates, and dimensions from the table.
Pixels	Includes ROI area, x,y-coordinates, and dimensions (in pixels) in the table.
cm	Includes ROI area, x,y-coordinates, and dimensions (in cm) in the table.
Copy	Copies the selected row(s) in the table to the system clipboard.
Select All	Copies all rows in the table to the system clipboard.
Refresh	Updates the ROI Measurements table (for example, after you draw new ROIs, move an ROI, and close or open image data).
Configure	Displays the Configure Measurements box that enables you to specify and organize the data categories (column headers) for the table.
Export	<p>Displays the Save Measurements box so that the data can be saved to a .txt or .csv file.</p> <p>Note: Grid ROI measurements exported to a .csv file can be opened in a spreadsheet application like Microsoft® Excel®.</p>
Close	Closes the ROI Measurements table.

Configuring the ROI Measurements Table

You can customize the data and information (column headers) in the ROI Measurements table. Several preset categories are available in the Measurement Types, Click Attributes, and ROI Dimensions drop-down lists.

1. Drag a column header (left or right) in the table to reorder the columns.
2. Make a selection from the Measurement Types drop-down list to change the measurement units.



3. Make a selection from the Image Attributes drop-down list to include image information in the ROI table.
4. Select units (Pixels or cm) from the ROI Dimensions drop-down list to include ROI dimensions in the table.

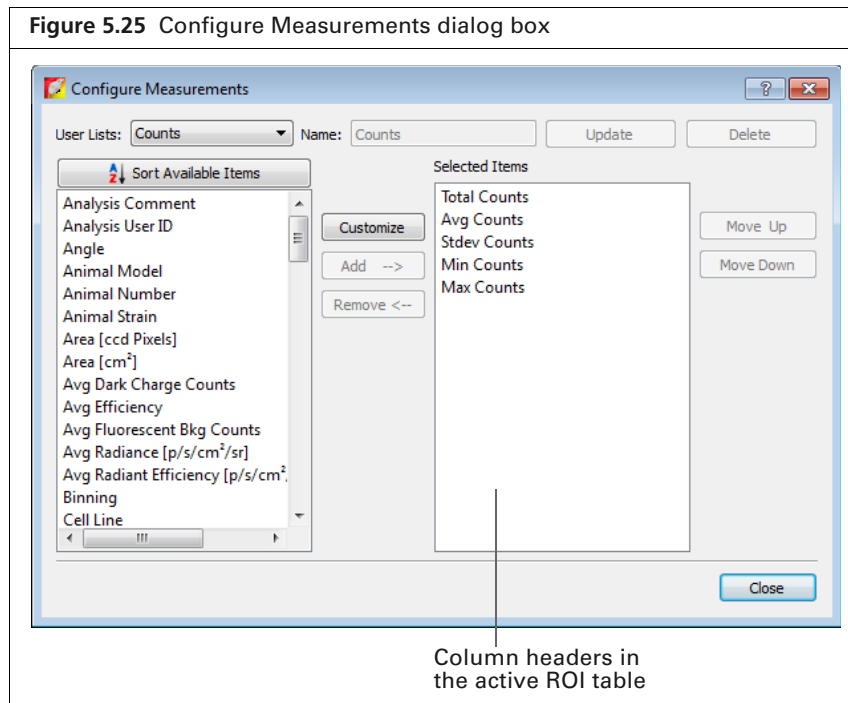
Creating a Custom ROI Table Configuration

A table configuration specifies the column headers in the ROI table. Several preset configurations are available (selected from the Measurements Types drop-down list in the ROI table, [Figure 5.24](#)). You can also create a custom table configuration.



NOTE: Preset table configurations cannot be edited. You can modify a preset configuration and save it to a new name.

1. In the ROI Measurements table, click **Configure**.
The Configure Measurements box appears.



2. Select a configuration from the User Lists drop-down list and click **Customize**.
3. To add column header to the ROI table, make a selection from the “Available Item” list and click **Add**.
4. To remove column header from the ROI table, select the item that you want to remove in the Selected Items list, and click **Remove**.
5. To reorder an item in the Selected Items list, select the item and click **Move Up** or **Move Down**. The columns in the ROI Measurements table are updated.
6. Enter a name for the custom configuration in the Name box and click **Save**.

To delete a custom table configuration:

Select the configuration from the User Lists drop-down list and click **Delete**.



NOTE: Preset table configurations cannot be deleted.

Copying or Exporting the ROI Measurements Table

To export the table:

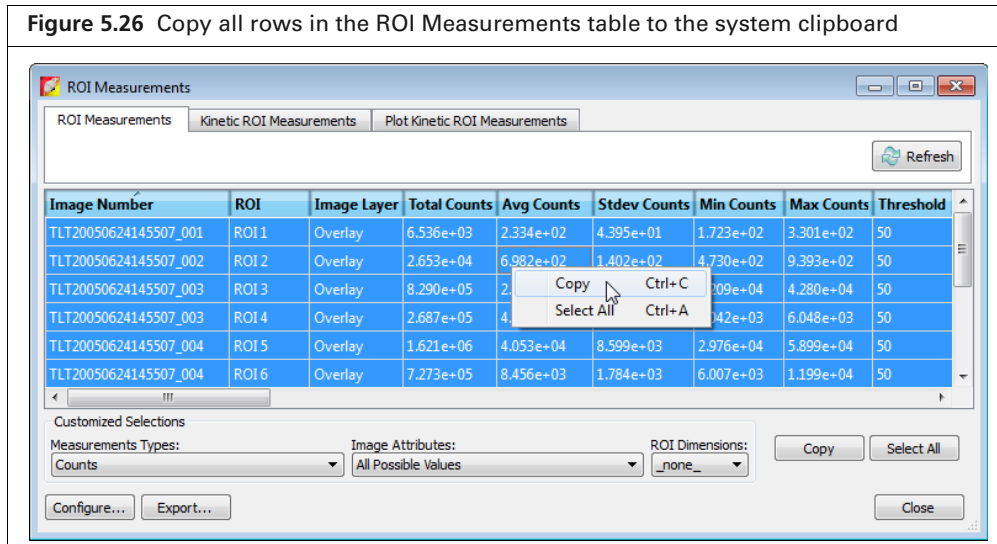
1. Click **Export** in the ROI Measurements table.
2. In the dialog box that appears:
 - a. Select a folder and enter a name for the file.
 - b. Select a file type (.txt or ,csv) and click **Save**.

To copy the table to the system clipboard:

- Copy selected rows – Select the rows of interest and click **Copy**. Alternatively, select the rows, then right-click the table and choose Copy on the shortcut menu.

- Copy all rows – Click **Select All** and click **Copy**. Alternatively, press **Ctrl+A**, then right-click the table and choose **Copy** on the shortcut menu.

Figure 5.26 Copy all rows in the ROI Measurements table to the system clipboard



6 Image Math

Creating a New Image Using Image Math

Subtracting Tissue Autofluorescence on page 116

6.1 Creating a New Image Using Image Math

The Image Math tool is used to mathematically combine two images to create a new image. Image math is primarily for subtracting tissue autofluorescence background from signal.

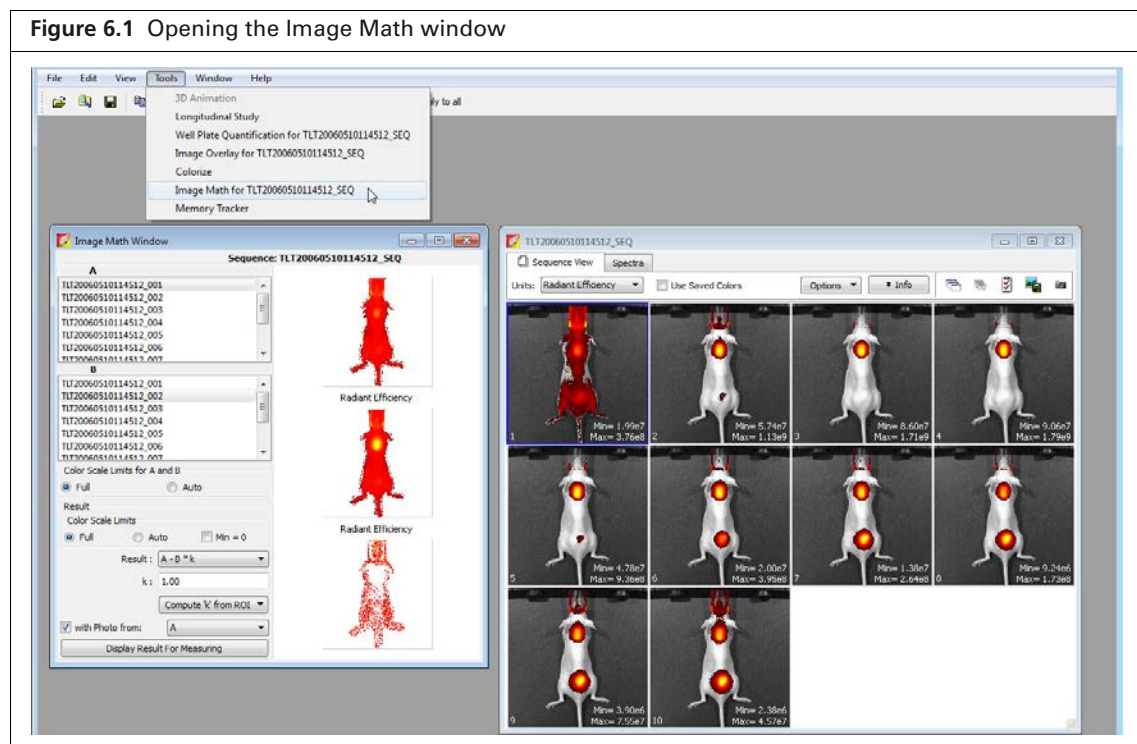
To perform image math, open an image sequence or a group of images. For more details on creating a sequence from individual images, see [page 80](#).



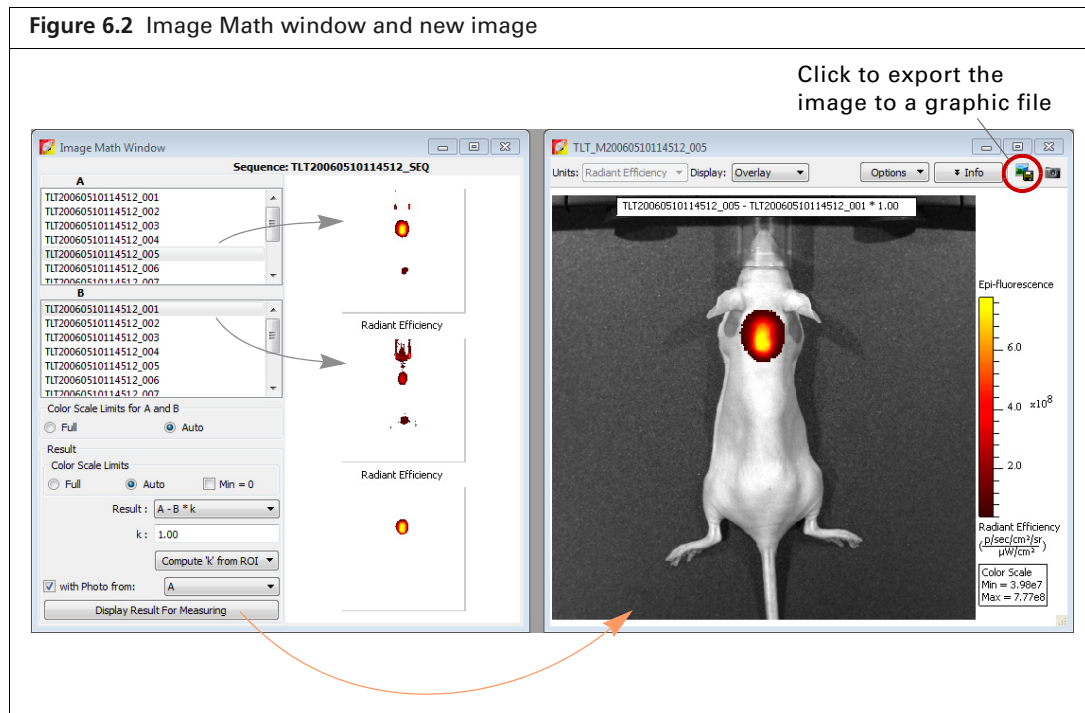
TIP: See the tech note *Image Math* for a quick guide (select **Help** → **Tech Notes** on the Help menu).

To mathematically combine images:

1. Load an image sequence.
2. Select **Tools** → **Image Math for <name>_SEQ** on the menu bar.



3. In the Image Math window that appears, select an image from box A and from box B. The Image Math window shows a thumbnail of image A, image B, and the new image.



NOTE: For more details on items in the Image Math window, see [Table 6.1, page 115](#)

4. Select a mathematical function from the Result drop-down list.
5. To include a scaling factor (k) in the function, enter a value for k.
6. To view the new image, click **Display Result for Measuring**.
7. To save the new image:
 - a. Click the **Save** button . Alternatively, select **File** → **Save** on the menu bar.
 - b. In the dialog box that appears, select a directory, and click **Save**.
A folder of data is saved to the selected location (AnalyzedClickInfo.txt, ClickInfo.txt, luminescent and photographic TIF images).
8. To export the image to a graphic file:
 - a. Click the **Export** button (Figure 6.2).
 - b. Select a directory in the dialog box that appears, enter a file name, and select the file type from the “Save as type” drop-down list.
 - c. Click **Save**.

Table 6.1 Image Math window

Item	Description
Color Ranges for A and B	<p>Full - Choose this option to set the Max and Min values to the maximum and minimum data values in the image.</p> <p>Auto - When this option is chosen, the software sets the Min and Max values to optimize image display and suppress background noise. The Min and Max settings can be manually adjusted to further optimize the image display for your needs.</p> <p>Note: The color scale does not affect the image math result.</p>

Table 6.1 Image Math window (continued)

Item	Description
Color Ranges for Result Image	Full - See above. Auto - See above. Min = 0 - Choose this option to set the minimum data value to zero.
Results	Drop-down list of mathematical functions that can be used to generate the new image, including: A - B*k A + B*k A * B*k A/B if Counts(B)>k (Useful for fluorescence tomography.) (A/B)*k
k, Image Math window	A user-specified scaling factor applied in the results function.
Compute 'k' from ROI	This option is useful for subtracting fluorescence background. Draw one ROI in an image on an area considered background. In the "Compute 'k' from ROI" drop-down list, select the this ROI.
with Photo from	Choose this option to display the new image in overlay mode using the selected photographic image. (This option is only available if one of the selected images is an overlay.)
Display Result for Measuring	Opens the image generated by image math in an image window.

6.2 Subtracting Tissue Autofluorescence

To remove tissue autofluorescence from image data, you can use a subtraction method that uses a second excitation filter which is blue-shifted (a background filter) from the primary excitation filter.

The objective of using a background filter is to excite the tissue autofluorescence without exciting the fluorophore. To reduce autofluorescence signal in the primary image data, use the image math tool to subtract the background filter image from the primary excitation filter image.

The software computes the signal corrected for background: $(A - B) \times k$, where:

A = primary image (acquired using the excitation filter)

B = background image (acquired using the background filter)

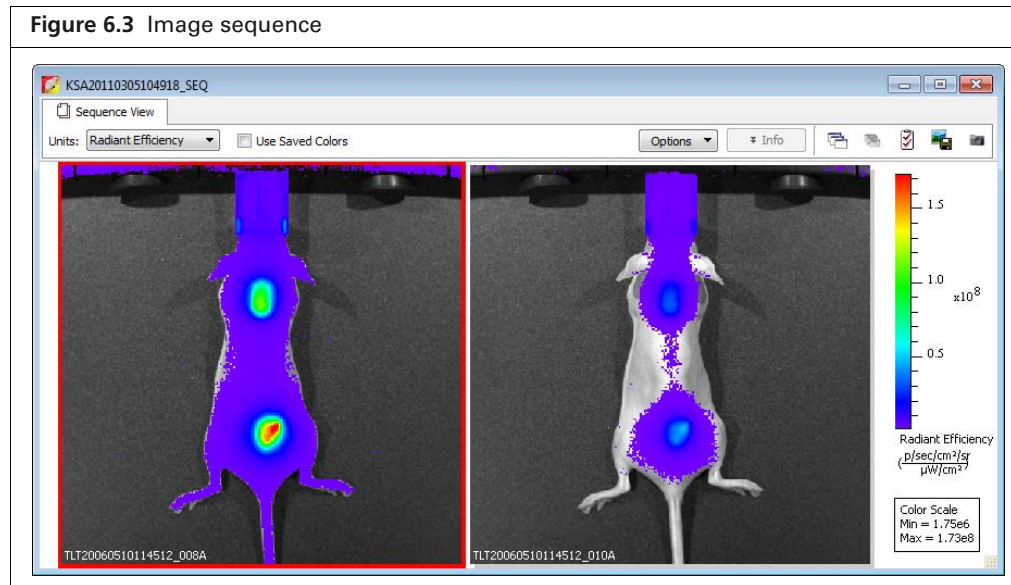
k = (primary signal/background signal)

The background signal is obtained from a measurement ROI that is located in an area where no fluorophore signal is present. The scale factor k accounts for different levels of tissue autofluorescence due to different excitation wavelengths and filter transmission characteristics.

After you acquire an image sequence that includes a primary and background image, use the image math tool to subtract tissue autofluorescence. (For more details on acquiring an image sequence, see [page 33](#).)

To subtract tissue autofluorescence:

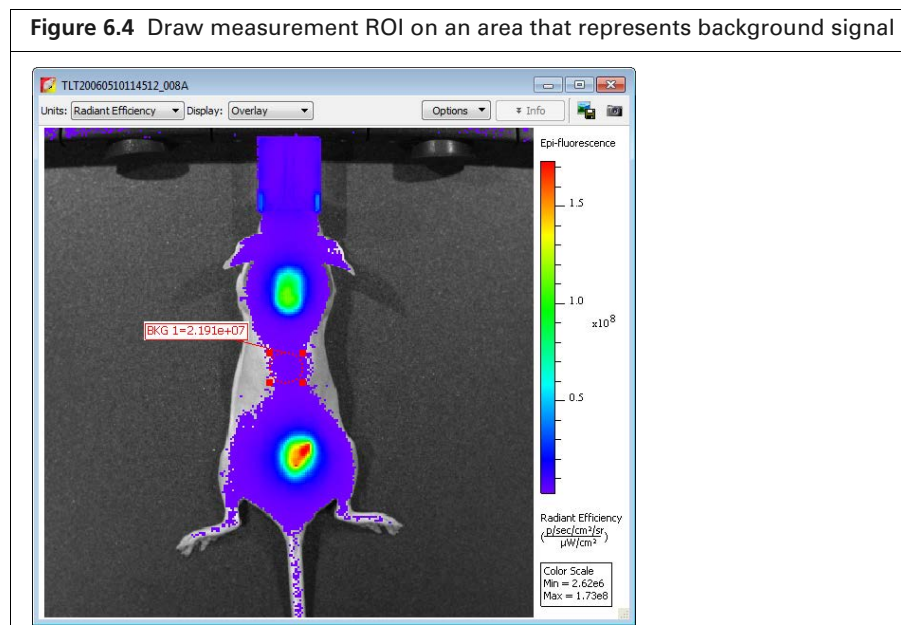
1. Load the image sequence that includes the primary and background fluorescent images.



2. Open either the primary or background image and:
 - a. Optimize the image display using the color scale Min and Max sliders in the Image Adjust tools.
 - b. Draw a measurement ROI on an area of the animal that represents background signal (area where no fluorophore signal is present).

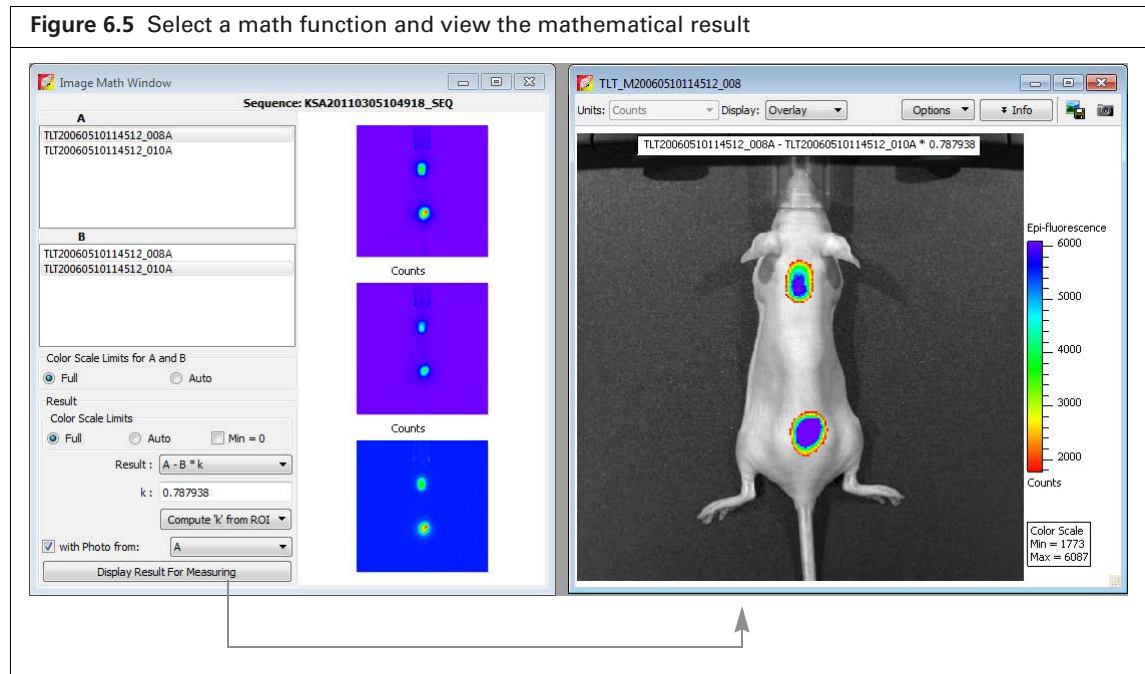


NOTE: You only need to draw the ROI on one of the images. The software copies the ROI to the other image.



3. Select **Tools** → **Image Math** for <name>_SEQ on the menu bar.

4. In the Image Math window that appears, select the primary image in box A. Select the background image in box B.
For more details on items in the Image Math window, see [Table 6.1, page 115](#).
5. Select the math function 'A-B*k' in the Result drop-down list.



6. Click **Compute 'k' from ROI** and select the ROI (created in step 2) from the drop-down list.
The background-corrected signal is displayed.
7. To view the mathematical result (overlay mode) in a separate image window, click **Display Result For Measuring**.
If necessary, use the Color Scale Min and Max sliders in the Image Adjust tools to adjust the image display.
8. To save the new image:
 - a. Click the **Save** button . Alternatively, select **File** → **Save** on the menu bar.
 - b. Select a directory in the dialog box that appears and click **Save**.
A folder of data is saved to the selected location (AnalyzedClickInfo.txt, ClickInfo.txt, luminescent and photographic TIF images).
9. To export the new image to a graphic file:
 - a. Click the **Export** button .
 - b. Select a directory in the dialog box that appears, enter a file name, and select the file type from the "Save as type" drop-down list.
 - c. Click **Save**.

7 Spectral Unmixing

About Spectral Unmixing

Spectral Unmixing Methods on page 120

Correcting Spectra on page 133

Spectral Unmixing Results on page 135

7.1 About Spectral Unmixing

The Living Image software applies spectral unmixing to distinguish the spectral signatures of different fluorescent or luminescent reporters and calculate the respective contribution of each on every pixel of an image. Use spectral unmixing to:

- Extract the signal of one or more fluorophores from the tissue autofluorescence. Images are acquired using epi-illumination (excitation light above the stage)
- Analyze luminescent or fluorescent images when more than one reporter is used in the same animal model

Image Requirements

Use the Imaging Wizard to set up an image sequence for spectral unmixing. See [page 33](#) for more details on the wizard. If you generated a spectrum library, you can select it in the Imaging Wizard ([Figure 7.1](#)).

Figure 7.1 Select a spectrum library in the Imaging Wizard

Open the Filter Section Configuration dialog box

Excitation Filters											
	Block	430	465	500	535	570	605	640	675	710	745
ND3	Block/ND3	430/ND3	465/ND3	500/ND3	535/ND3	570/ND3	605/ND3	640/ND3	675/ND3	710/ND3	745/ND3
GFP	Block/GFP	430/GFP	465/GFP								
DsRed	Block/DsRed	430/DsRed	465/DsRed	500/DsRed	535/DsRed						
Cy5.5	Block/Cy5.5	430/Cy5.5	465/Cy5.5	500/Cy5.5	535/Cy5.5	570/Cy5.5	605/Cy5.5	640/Cy5.5			
ICG	Block/ICG	430/ICG	465/ICG	500/ICG	535/ICG	570/ICG	605/ICG	640/ICG	675/ICG	710/ICG	745/ICG
Open	Block/Open	430/Open	465/Open	500/Open	535/Open	570/Open	605/Open	640/Open	675/Open	710/Open	745/Open

Click "Select by Spectrum Library" and choose a



TIP: See the *Imaging Wizard* tech note for a quick guide on sequence acquisition (select **Help** → **Tech Notes** on the menu bar).

If you do not use the Imaging Wizard to set up the image sequence, it is recommended that the sequence include images acquired using several filters that sample the emission and/or excitation spectra at multiple points across the entire range. Make sure that the band gap between the excitation and emission filters is sufficiently large (for example, >35 nm) so that the excitation light does not leak through the emission filter where it can be detected by the CCD.

If a data set includes multiple excitation and emission filter scans, the software automatically unmixes signal according to the filter type with the most entries. For example, a data set acquired using three excitation filters and four emission filters will be unmixed by emission wavelength.

7.2 Spectral Unmixing Methods

The Living Image software provides four spectral unmixing methods (Table 7.1).

Table 7.1 Spectral unmixing methods

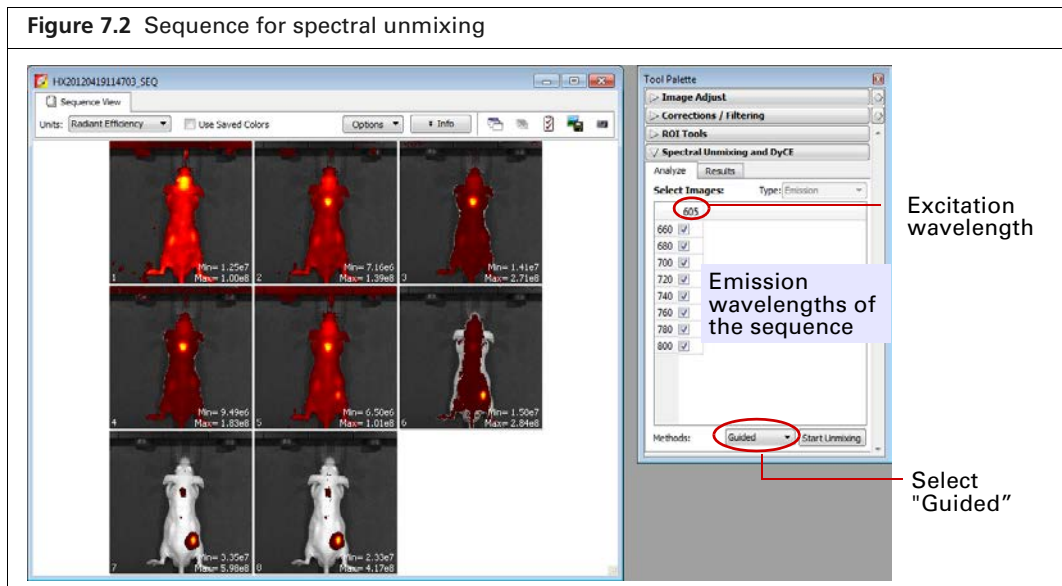
Method	Description	See Page
Guided	<p>Use this method when:</p> <ul style="list-style-type: none"> ■ Probe locations are known. ■ Probe signals are mixed with background signal, but not other probe signals. <p>Note: This method is not recommended if probe signals are overlapping.</p> <p>Use this method to generate a spectrum library (a set of reference spectra) for probes with known spectra and known locations.</p>	120
Library	<p>This method requires a user-generated spectrum library. The library method identifies pixels in the data with spectral characteristics that match the spectrum library.</p> <p>Note: The data being analyzed must be acquired using the same, or a subset of, the excitation/emission filter pairs of the spectrum library. The probe depth in the data being analyzed and the spectrum library data set should be similar for optimum analysis results. For example, do not use a spectrum library generated from <i>in vivo</i> data to analyze <i>in vitro</i> data and vice versa.</p>	120
Automatic	Use this method when the probe locations are unknown.	126
Manual	If necessary, perform a manual analysis after an automatic analysis to identify additional probe locations.	130

Guided Method

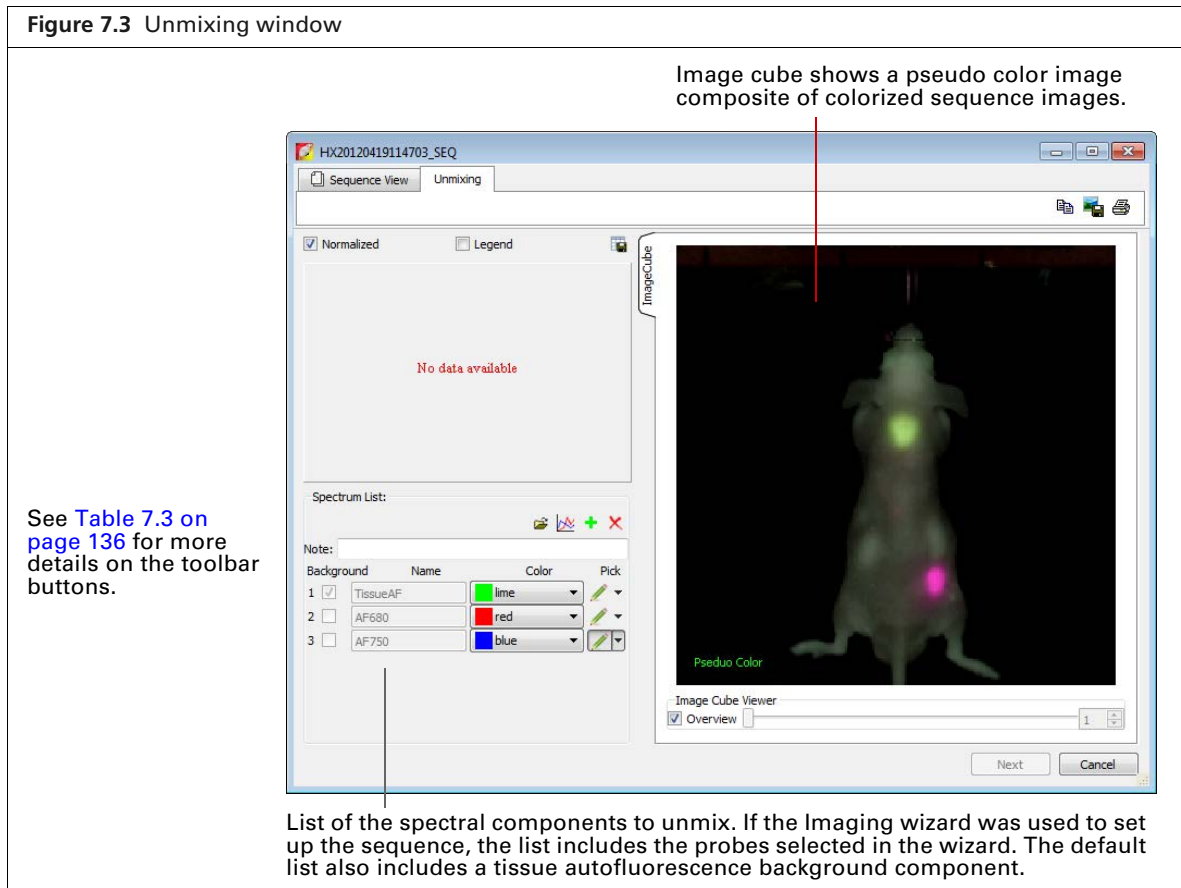
Use the guided method:

- When the probe locations are known and probe signals do not overlap.
 - To generate a spectrum library for probes with known spectra and known locations
1. Load the image sequence.

In Figure 7.2, the fluorophores are Alexa Fluor 680 and Alexa Fluor 750. Images were acquired using a 605 nm excitation filter and emission filters from 660 to 800 nm in 20 nm increments.



2. Click the Analyze tab of the Spectral Unmixing and DyCE tools.
By default, all wavelengths are included in the analysis. Remove the check mark next to wavelengths that you want to exclude from the analysis.
3. Select "Guided" from the Methods drop-down list and click **Start Unmixing**.
The Unmixing window appears (Figure 7.3).



The image cube represents a “stack” of the sequence images (sorted according to the spectral axis). When the Overview option is selected, the image cube shows a pseudo color image that is a composite of the stack images which have been colorized to encode spectral information.

The entire image cube is calibrated and visualized on the same scale. To view a particular image, remove the check mark next to the Overview option and move the slider or enter an image number.



NOTE: In the Guided method, the Tissue AF component is preset as background. After you define the Tissue AF component (mark a region of tissue autofluorescence only on the image cube), the spectra of the other components that you mark on the image cube will be background-subtracted, not raw spectra from the data.


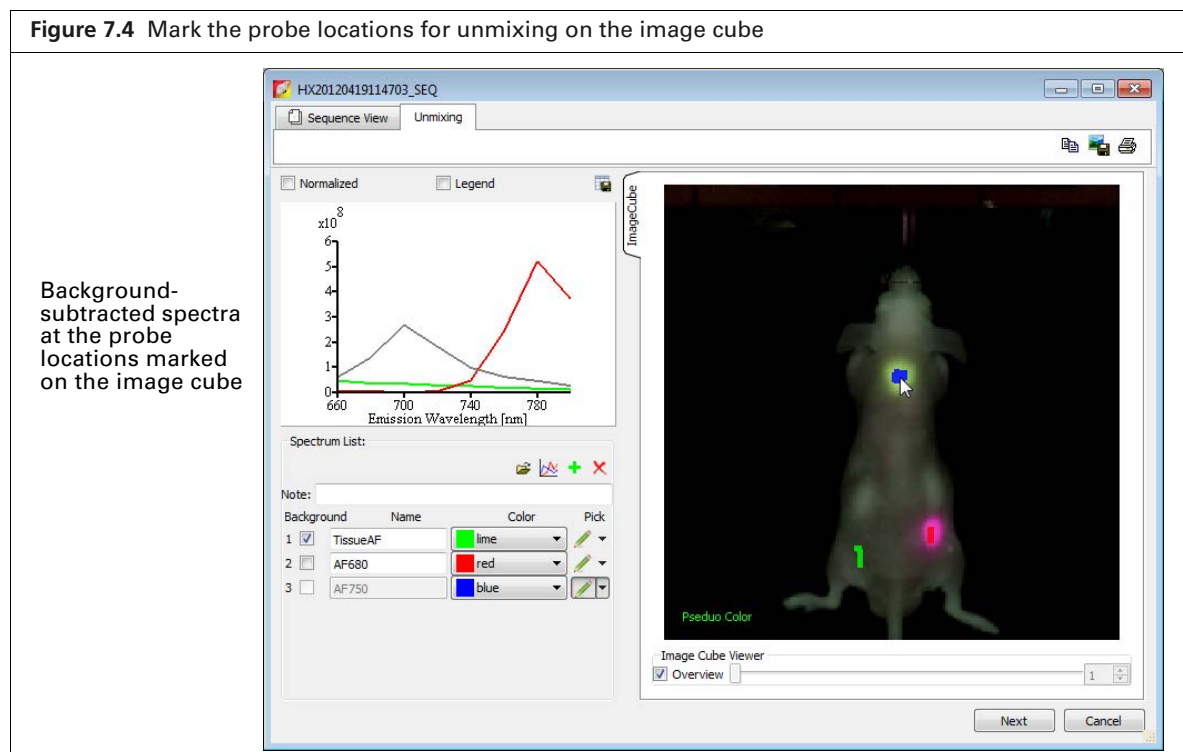
4. Move the mouse pointer over the image cube to see the spectrum at a particular location. The raw spectrum at the pointer location is updated as you move the pointer.
5. To specify a probe location for unmixing:
 - a. Click the  button for a spectrum.
 - b. Using the mouse, draw a mark on an area of the image cube which represents the probe signal. The software plots a background-subtracted spectrum of the signal ([Figure 7.4](#)).
6. If necessary, right-click the image cube to erase the mark.
7. Repeat step [step 5](#) to specify other probe locations.

Figure 7.4 Mark the probe locations for unmixing on the image cube




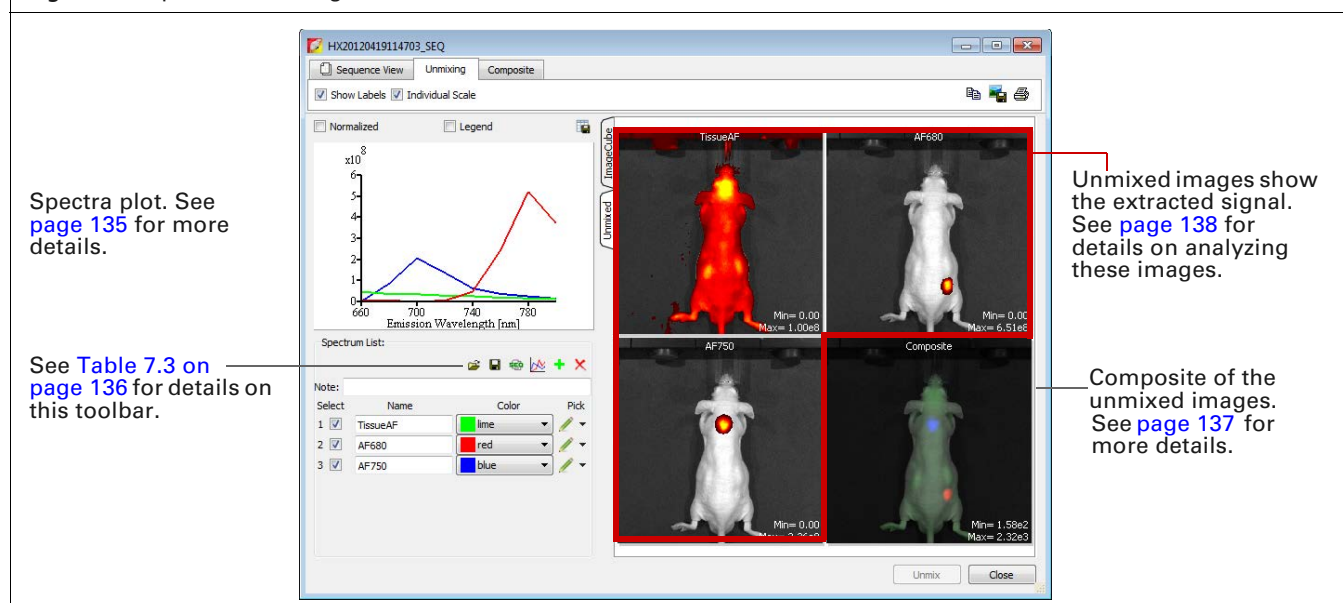
8. Click **Next** after you finish marking the probe locations. The Unmixing window shows the analysis results which include unmixed spectra corrected for tissue autofluorescence, unmixed images, and a composite of the unmixed images ([Figure 7.8](#)). See [Spectral Unmixing Results](#), page 135 for information about the results.
9. To save the results as a spectrum library:
 - a. Click the  button in the Spectrum List toolbar ([Figure 7.5](#)).
 - b. Enter a file name in the dialog box that appears and click **Save**.

Figure 7.5 Spectral unmixing results



Library Method

The library method uses a user-generated spectrum library to analyze a data set. If you plan to analyze data by this method, the data must be acquired using the same, or a subset of, the excitation/emission filter pairs of the spectrum library.

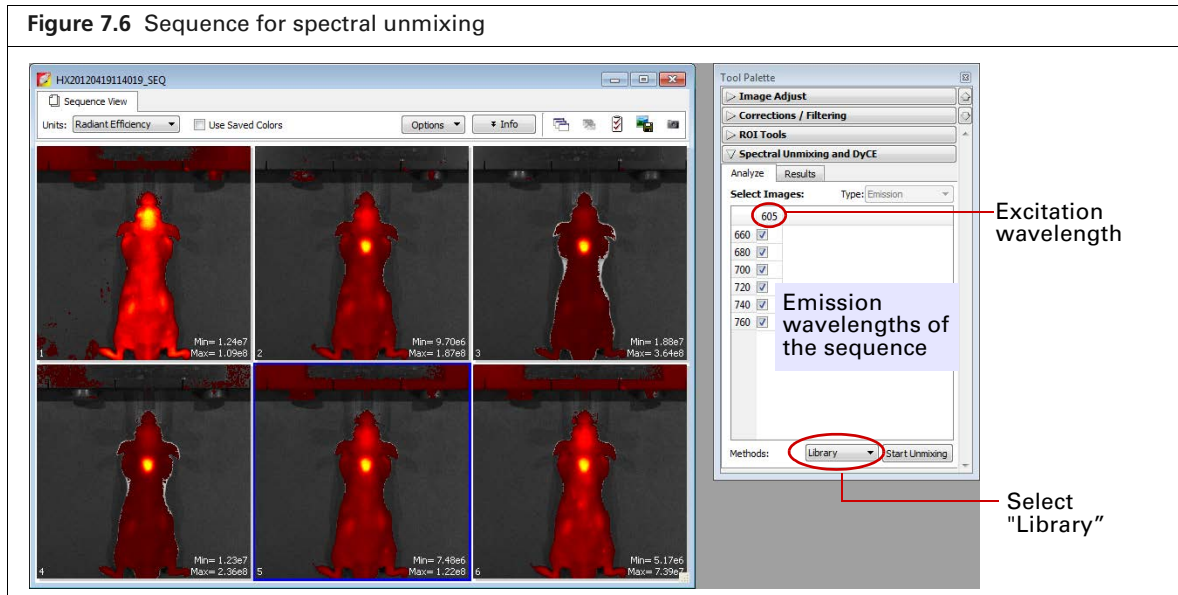
The probe depth in the data set being analyzed and the spectrum library data set should be similar for optimum analysis results. For example, do not use a spectrum library generated from in vivo data to analyze in vitro data.



NOTE: Use the "guided" method to generate a spectrum library of known probes with known locations (see [page 120](#) for more details on the guided method).

1. Load the image sequence.

In [Figure 7.6](#), the fluorophores are Alexa Fluor 680 and Alexa Fluor 750. Images were acquired using a 605 nm excitation filter and emission filters from 660 to 800 nm in 20 nm increments.



2. Click the Analyze tab of the Spectral Unmixing and DyCE tools.
By default, all wavelengths are included in the analysis. Remove the check mark next to wavelengths that you want to exclude from the analysis.
3. Select "Library" from the Methods drop-down list and click **Start Unmixing**.
4. Select a reference spectral library in the dialog box that appears and click **Apply** (Figure 7.7).
The software identifies pixels with spectral characteristics that match the spectrum library. The Unmixing window shows the analysis results which include unmixed spectra, unmixed images, and a composite of the unmixed images (Figure 7.8).
See *Spectral Unmixing Results*, page 135 for information about the results.

Figure 7.7 Select a reference spectral library

Choose this option to show only spectrum libraries with excitation/emission filters that match the data set being analyzed

Available spectrum libraries

Select	Name	Color
<input checked="" type="checkbox"/>	TissueAF	lime
<input checked="" type="checkbox"/>	AF680	red
<input checked="" type="checkbox"/>	AF750	blue

Use these controls to include or exclude spectra from the analysis, rename a spectrum, or change the spectrum plot color

Figure 7.8 Spectral unmixing results

Spectra plot. See page 135 for more details.

See Table 7.3 on page 136 for details on this toolbar.

Unmixed images show the extracted signal. See page 138 for details on analyzing these images.

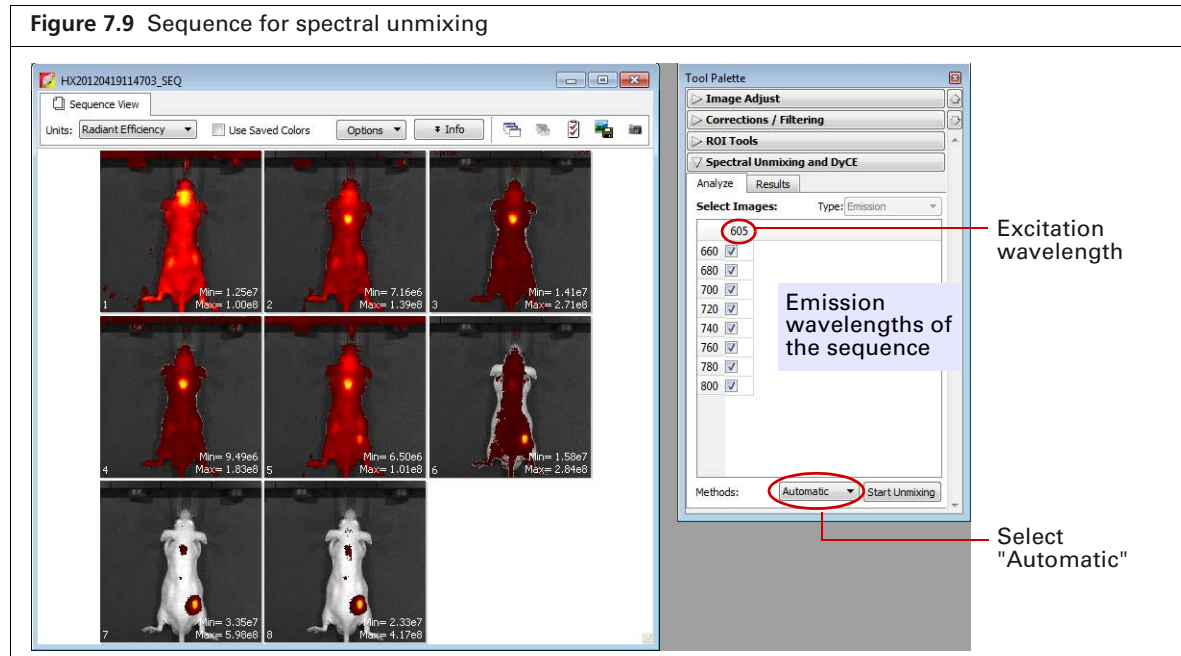
Composite of the unmixed images. See page 137 for more details.

Automatic Method

Use the automatic method to analyze data when the probe locations are unknown.

1. Load the image sequence.

In [Figure 7.9](#), the fluorophores are Alexa Fluor 680 and Alexa Fluor 750. Images were acquired using a 605 nm excitation filter and emission filters from 660 to 800 nm in 20 nm increments.

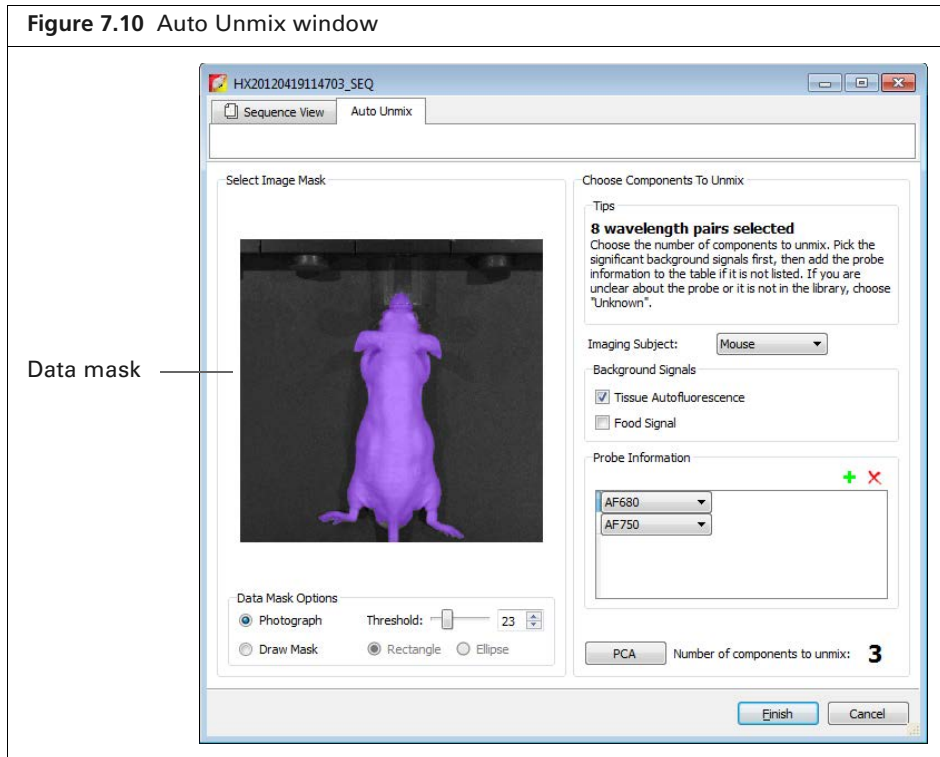


2. Click the Analyze tab of the Spectral Unmixing and DyCE tools.

By default, all wavelengths are included in the analysis. Remove the check mark next to wavelengths that you want to exclude from the analysis.

3. Select "Automatic" from the Methods drop-down list and click **Start Unmixing**.

The Auto Unmix window appears. The purple data mask shows the data that will be included in the analysis (the entire subject is included by default).



4. If you do not want to analyze the entire subject, draw a mask on a particular area (Figure 7.11).

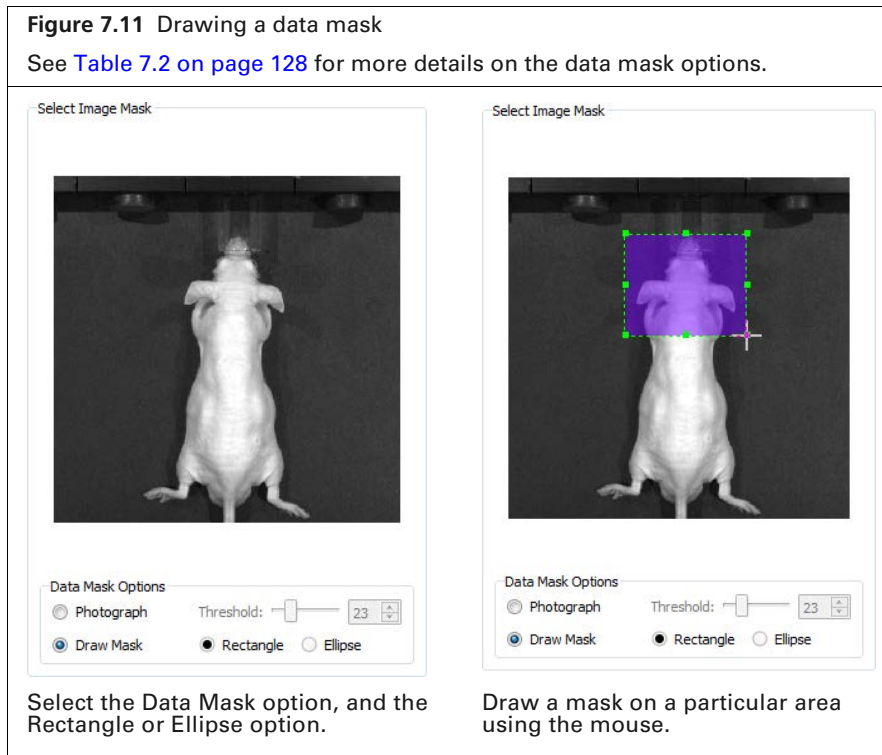

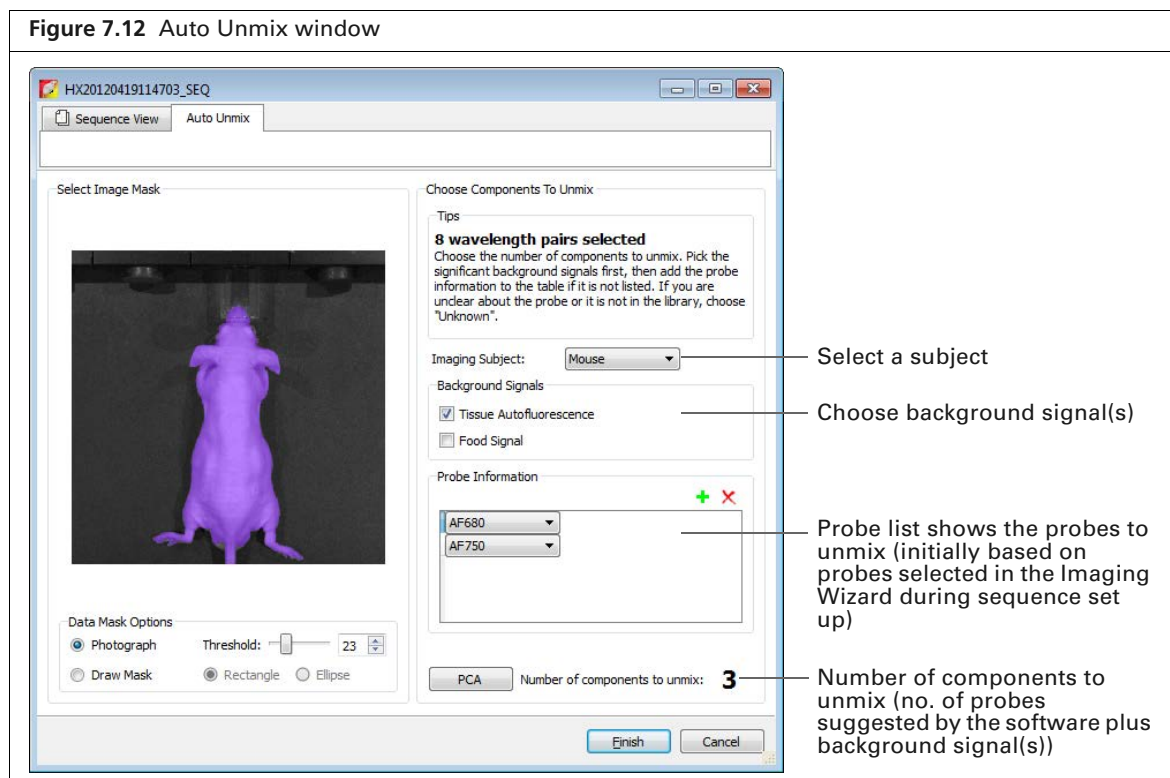


Table 7.2 Data mask options

Option	Description
Photograph	If this option is chosen, the software automatically draws the data mask so that it includes the entire photograph.
Threshold	If necessary use the threshold slider or  arrows to adjust the mask so that it matches the underlying subject photograph as closely as possible without including any area outside the subject image.
Draw Mask	Choose this option to manually draw a data mask on an area of the photograph.
Rectangle	Specifies a rectangular shape for the manual data mask.
Ellipse	Specifies an elliptical shape for the manual data mask.

5. Choose an imaging subject and background signal(s).

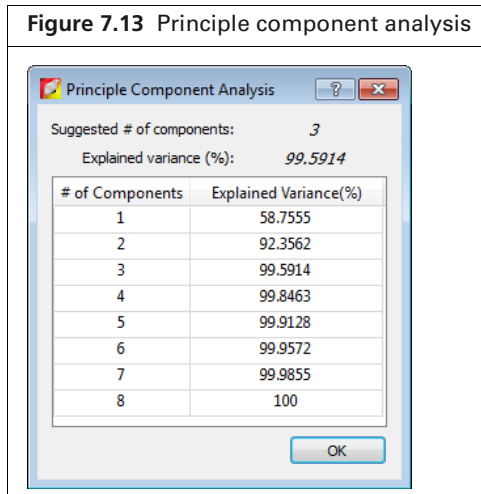


6. Click the PCA button.

The Principle Component Analysis window shows the amount of signal explained by the suggested components (Figure 7.13). The three components in this example (tissue autofluorescence, probe AF680, and probe AF750) explain more than 99.5% of the signal. The small residual is due to noise.

If the explained variance is low, add more components (probes) to unmix using the  button.

Figure 7.13 Principle component analysis



7. Click Finish.

The Unmixing window shows the analysis results which include unmixed spectra, unmixed images, and a composite of the unmixed images (Figure 7.8).

See *Spectral Unmixing Results*, page 135 for information about the results.

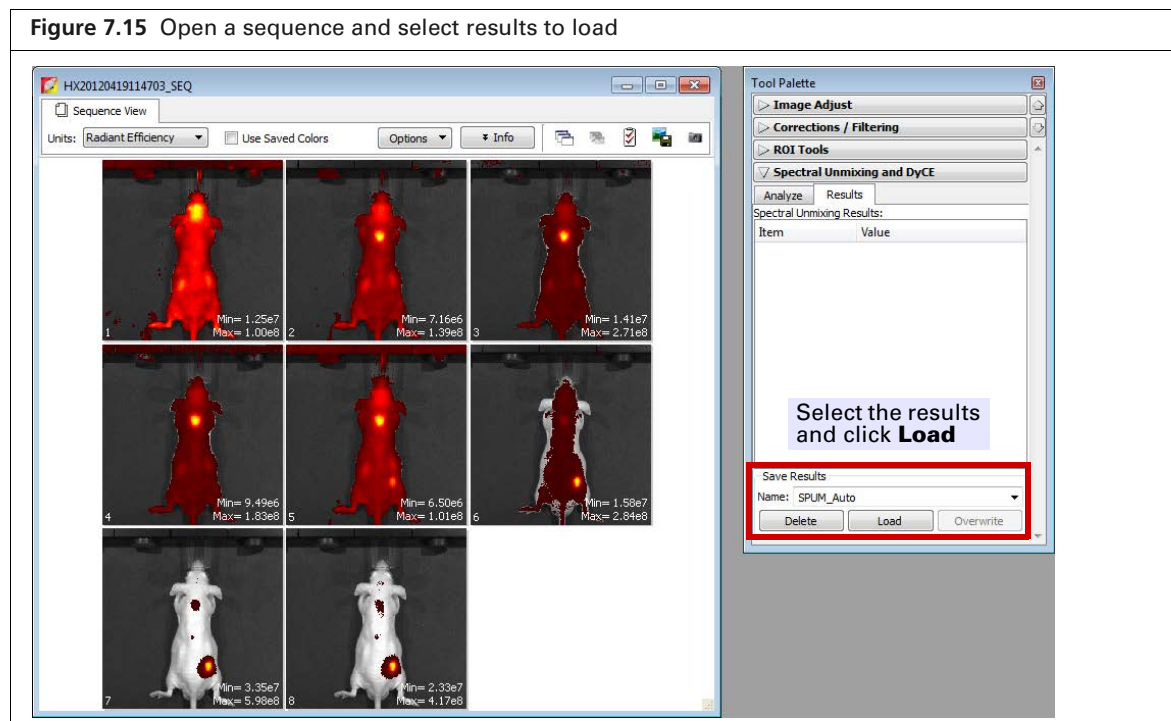
Figure 7.14 Spectral unmixing results



Manual Method

Sometimes you may want to manually analyze results, for example, if the explained variance of the principle component analysis of an automatic analysis seems low. The example in this section shows how to manually analyze results from a previous analysis.

1. Open the image sequence.
2. Select the results and click **Load**.

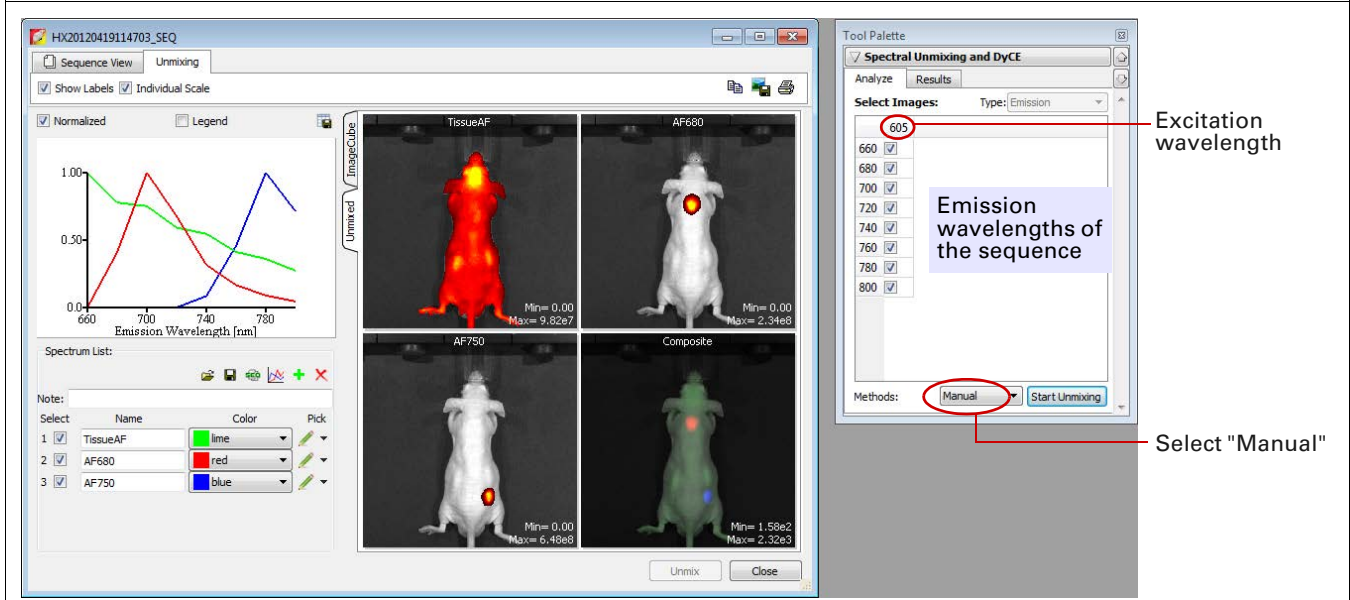


3. Click the Analyze tab of the Spectral Unmixing and DyCE tools.

All wavelengths are selected by default. Remove the check mark next to wavelengths that you want to exclude from the analysis.

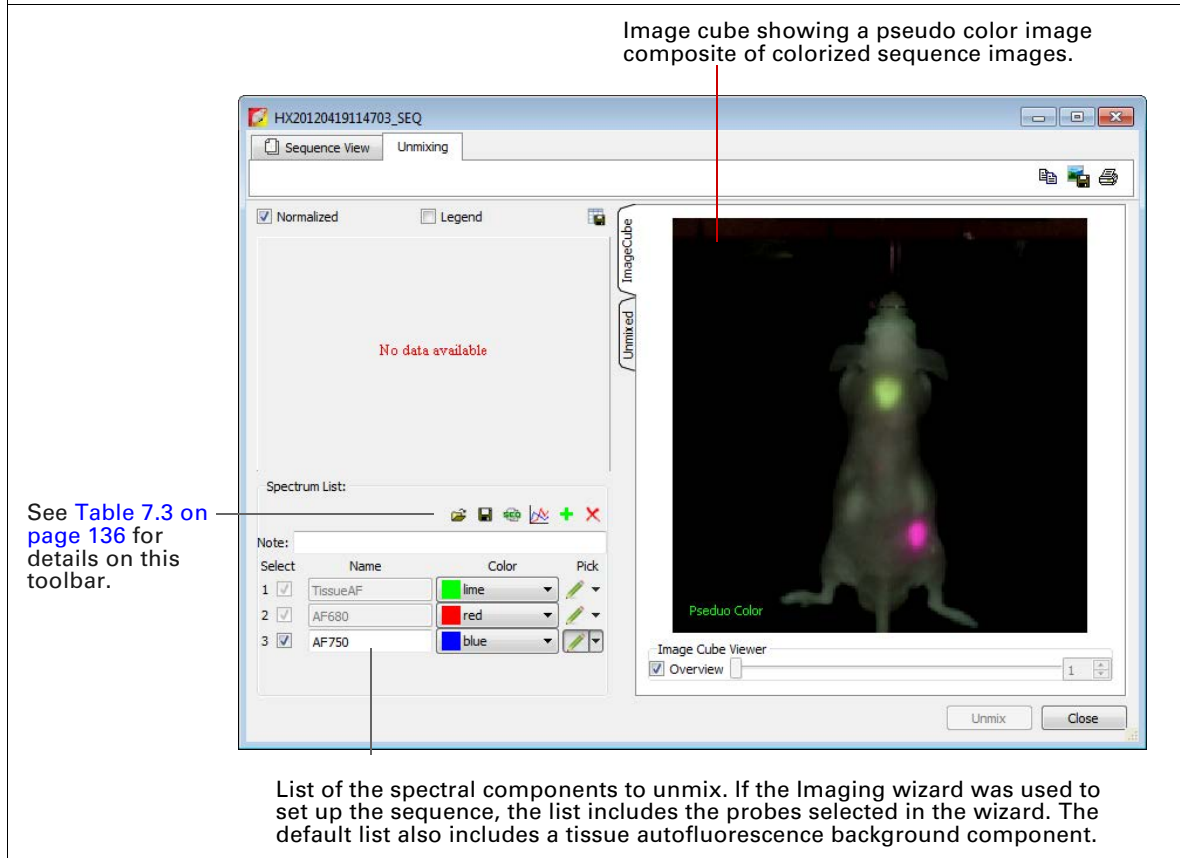
In [Figure 7.16](#), the fluorophores are Alexa Fluor 680 and Alexa Fluor 750. Images were acquired using a 605 nm excitation filter and emission filters from 660 to 800 nm in 20 nm increments.

Figure 7.16 Unmixing window showing loaded results



4. Select "Manual" from the Methods drop-down list and click **Start Unmixing**. The Unmixing window appears (Figure 7.3).

Figure 7.17 Unmixing window



The image cube represents a “stack” of the sequence images (sorted according to the spectral axis). When the Overview option is selected, the image cube shows a pseudo color image that is a composite of the stack images which have been colorized to encode spectral information.

The entire image cube is calibrated and visualized on the same scale. To view a particular image, remove the check mark next to the Overview option and move the slider or enter an image number.



NOTE: Mark a region of tissue autofluorescence only on the image cube for the Tissue AF component. The spectra of components that you mark on the image cube are raw spectra from the data when using the manual method.


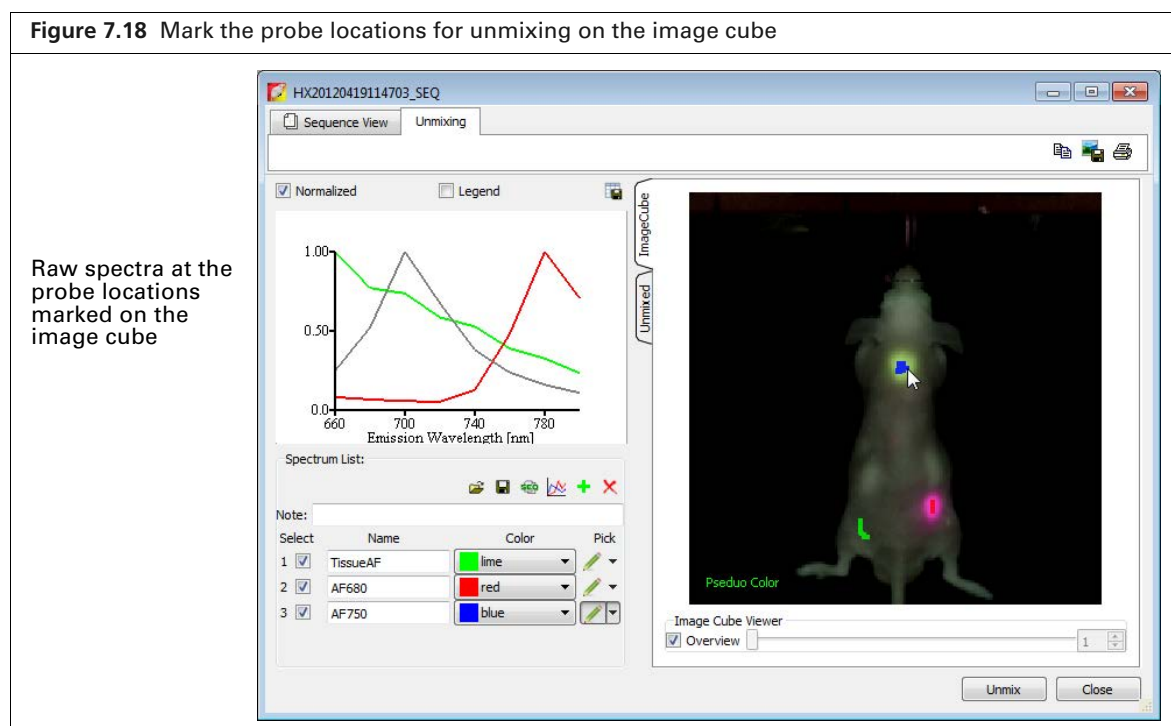
5. Move the mouse pointer over the image cube to see the spectrum at a particular location. The spectrum at the pointer location is updated as you move the pointer.
6. To specify a probe location for unmixing:
 - a. Click the  button for a spectrum.
 - b. Using the mouse, draw a mark on an area of the image cube which represents the probe location. The software plots a normalized spectrum of the signal ([Figure 7.18](#)).
 - c. If necessary, right-click the image cube to erase the mark.
7. Repeat [step 5](#) to specify other probe locations.
8. Manually subtract autofluorescence background. See [Correcting Spectra](#), page 133 for instructions.

Figure 7.18 Mark the probe locations for unmixing on the image cube

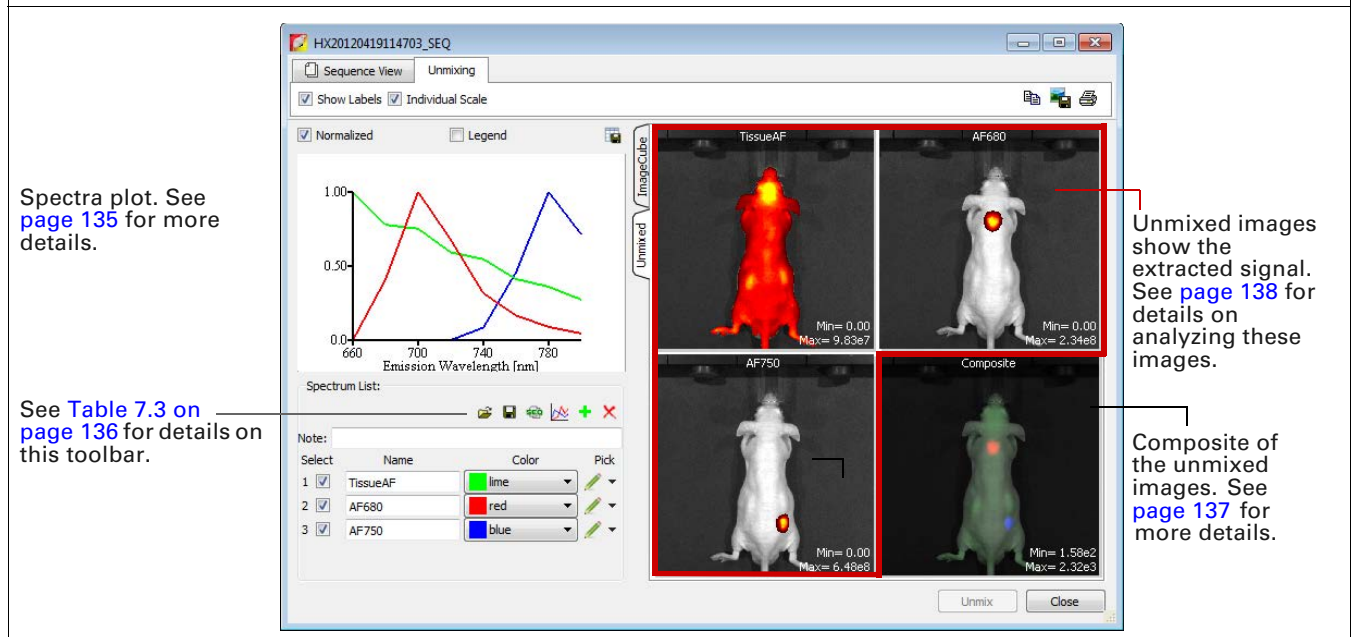


9. Click **Unmix** after you finish marking the probe locations and correct spectra for tissue autofluorescence.

The Unmixing window shows the analysis results, unmixed images, and a composite of the unmixed images ([Figure 7.19](#)).


See [Spectral Unmixing Results](#), page 135 for information about the results.

Figure 7.19 Spectral unmixing results



7.3 Correcting Spectra

Spectra can be corrected for overlapping signal by subtracting one spectrum from another.

1. Click the  button in the Unmix window.
2. Choose the spectra to subtract in the dialog box that appears. (Figure 7.20).
3. Click **Apply** to add the computed spectrum to the spectrum plot and list in the Unmixing window. Alternatively, select an existing spectrum from the Name drop-down list and click **Apply** to overwrite the results.

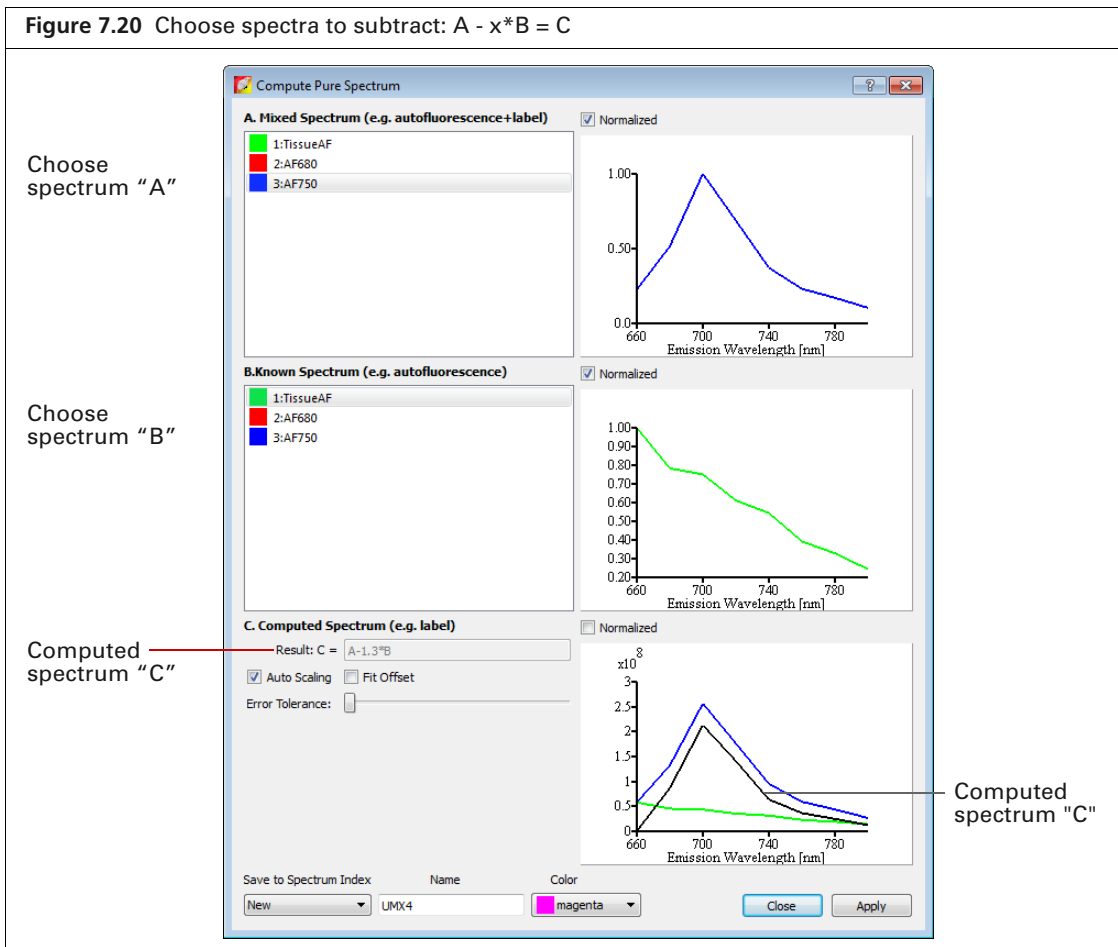
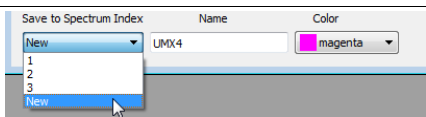
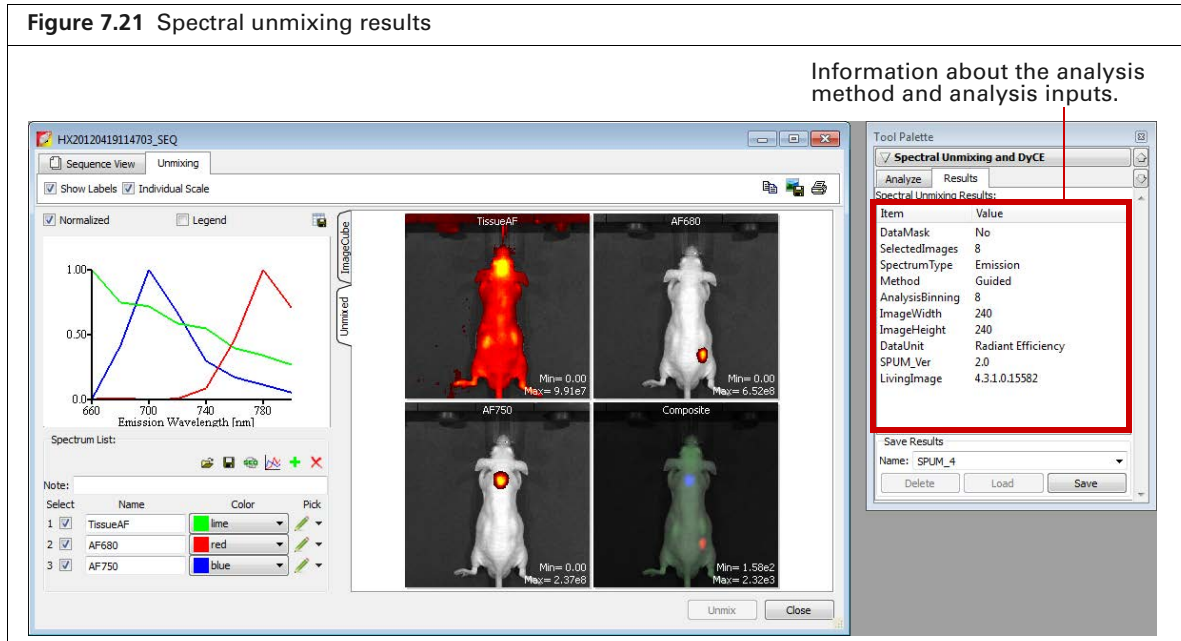


Table 7.1 Computed spectrum

Item	Description
Normalized	Choose this option to display spectra normalized on a scale from zero to one.
Result: $C = A - x*B$	The subtraction performed by the software where "x" is a factor that ensures the residual signal is positive.
Autoscaling	Choose this option to display computed results on a normalized scale starting a zero.
Fit Offset	If this option is chosen, the software computes and removes an intensity baseline from the spectra.
Error Tolerance	The software computes a default error tolerance (the factor "x" for $A - x*B$) such that signal B is maximally removed from signal A with no negative result. Moving the slider adjusts the error tolerance and automatically updates the computed spectrum.
	<p>Choose "New" to save computed spectrum with the specified name and color. Click Apply to add the computed spectrum to the spectrum plot and list in the Unmixing window.</p> <p>Choose a spectrum number from the drop-down list to overwrite that spectrum with the computed spectrum when you click Apply.</p>

7.4 Spectral Unmixing Results

The results include a signal distribution map of each unmixed result and a composite image of all signals, each displayed in a different color.



Spectra Plot

The spectra plots shows the unmixed spectra.

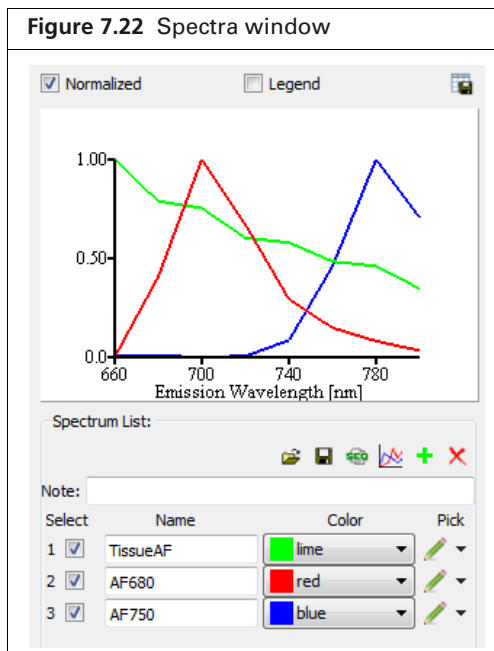










Table 7.3 Spectra window

Item	Description
Normalized	Choose this option to display signals normalized on a scale from zero to one.
Legend	Choose this option to display a key for the spectra plot.
	Opens a dialog box that enables you to export the spectra plot data to a .csv file.
	Opens a dialog box that enables you to select and load a spectrum library.
	Opens a dialog box that enables you to save spectral unmixing results as a reference spectrum library for use with the "library" method of spectral unmixing. See page 123 for more details on the library method.
	Enables you to view and save the unmixed images as a sequence data set which can be analyzed using the tool palette.
	Opens a dialog box that enables you to correct a spectrum for overlapping signal by subtracting one spectrum from another (see page 133).
	Adds a component to the spectrum list.
	Deletes the last spectrum in the spectrum list.

Adding Spectra to the Plot

To Add:	Do This:
A spectrum library	Click the  button and select a spectrum library in the dialog box that appears. Note: A spectrum library is a user-created set of reference spectra generated by analyzing probes with known spectra and known locations.
A spectrum from a user-defined region	Add a new spectrum to the list in the Unmix window and identify the region by drawing a mark on the image cube. See page 131 for more details.

Composite Image

The composite image includes all of the signals, each displayed in a different color. Double-click the composite image to view it in a separate window (Figure 7.23).

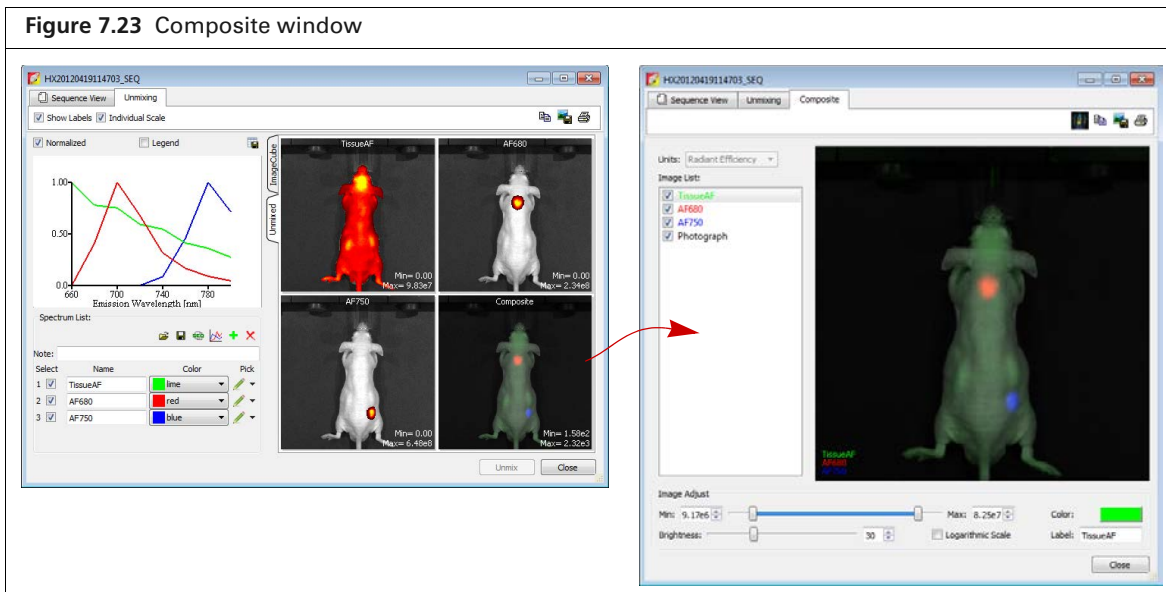







Table 7.2 Composite window

Item	Description
Units	The type of data displayed in the composite image.
Image list	A list of the images that comprise the composite (background component(s), probe(s), and a photograph).
Min/Max	Sets the minimum and maximum count to display in the image.
Brightness	Adjusts the brightness of the component signals.
Logarithmic Scale	Choose this option to display signals using a logarithmic scale. This may be useful when probe signal strengths differ significantly, for example, a bright source and a dim source.
Color	Shows the color of the figure legend for the image selected in the image list. Click the color swatch to open a color palette that enables you to select a new color for the figure legend.
Label	The name of the image selected in the image list. To edit the name, double-click the name in this box. Right-click the label name to show a short-cut menu of edit commands (for example, Cut, Copy, Paste).
	Sends the composite image to the “top” of the image cube. This helps improve the pseudo color visualization of the image cube.
	Copies the composite image to the system clipboard.
	Click to export the composite image to a graphic file (for example, .jpg).
	Opens the Print dialog box.

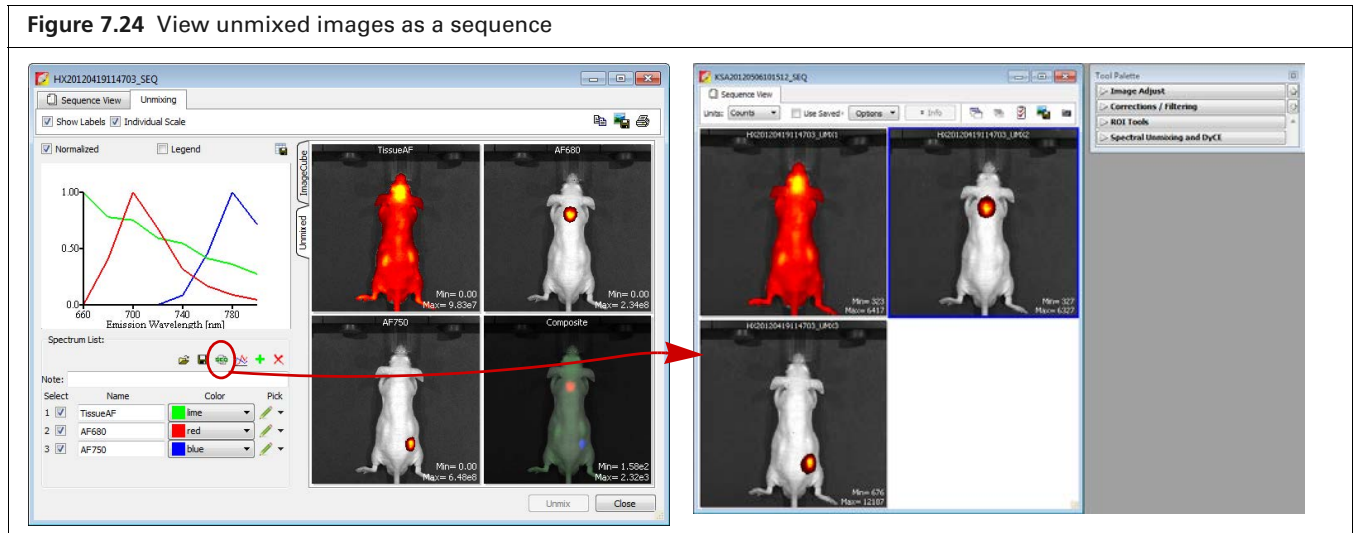
Analyzing Images

Do either of the following:

- Click the  button toolbar button to view all images as a sequence.
- Double-click a particular unmixed image.

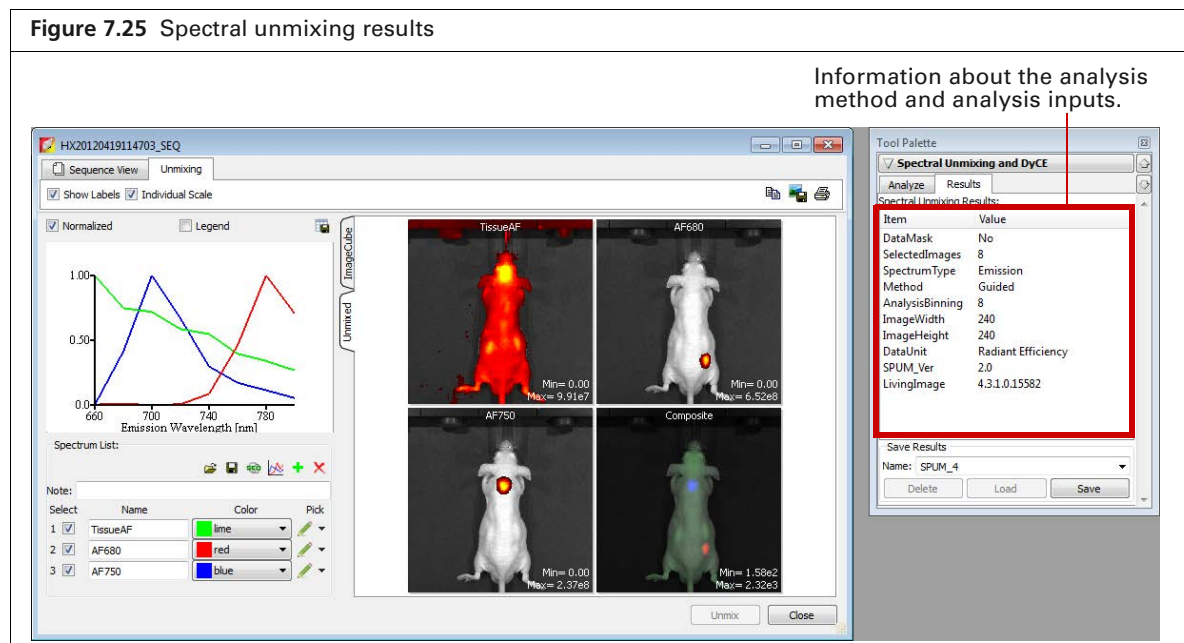
The image(s) appear in a separate window and the tool palette is available for image analysis. When closing the window, the software prompts you to save the sequence or image.

Figure 7.24 View unmixed images as a sequence



Managing Spectral Unmixing Results

Figure 7.25 Spectral unmixing results



Items in the Results Tab	Description
Name	The name for the active spectral unmixing results. Select results from this drop-down list.
Delete	Deletes the selected results.
Load	Opens the selected results in the Unmixing window.
Save	Saves the active results using the selected name. The results are saved to the sequence click number folder and are available in the Name drop-down list.
Overwrite	If you reanalyze results, saves the new results and overwrites the previous results.

8 DyCE Imaging and Analysis

About DyCE (Dynamic Contrast Enhancement)

Acquire a DyCE Sequence on page 141

DyCE Analysis on page 147

DyCE Results on page 153

8.1 About DyCE (Dynamic Contrast Enhancement)



NOTE: The DyCE acquisition and analysis features of the Living Image® software require a separate license.

DyCE imaging and analysis is intended for biodistribution studies. DyCE imaging captures a time series of optical images immediately following a bolus injection of a probe or dye. The Living Image® software “temporally unmixes” the data on a pixel-by-pixel basis for each image of the time series and determines real-time spatio-temporal distribution of the probe or dye signal.

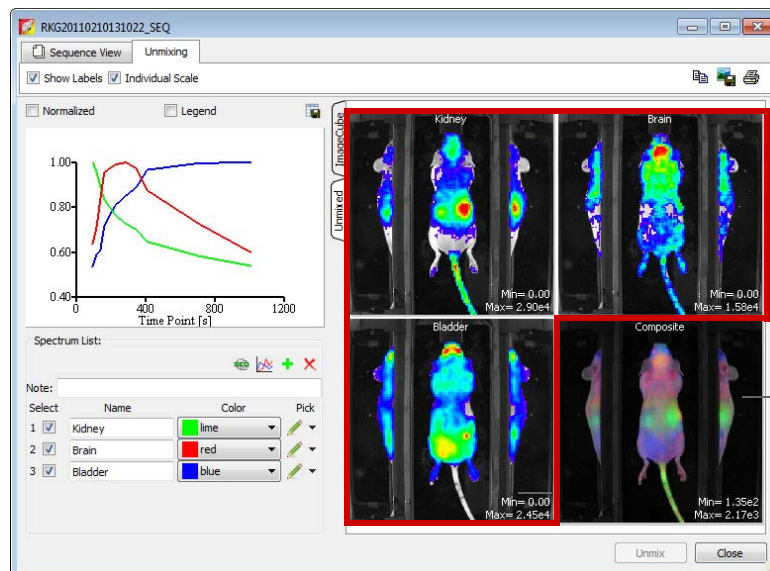
The Living Image® software presents the spatio-temporal information as:

- Temporal spectra – Line plots of signal intensity as a function of time. Each line plot represents the signal time course within a particular anatomical region.
- An unmixed image – An image that represents the peak signal time point for a particular temporal spectrum.
- A composite image – An overlay of the unmixed images.

Figure 8.1 Example DyCE results

Images were obtained using the Mouse Side Imaging Kit.

Temporal spectra show signal time course of different anatomical regions



Unmixed images. Each image is a representation of a temporal spectrum at the peak signal time point.

Composite of the unmixed images

8.2 Acquire a DyCE Sequence

A DyCE sequence includes images acquired with a user-specified time delay between exposures. An acquisition can include up to three different time intervals where each interval is defined by duration and the delay between exposures.

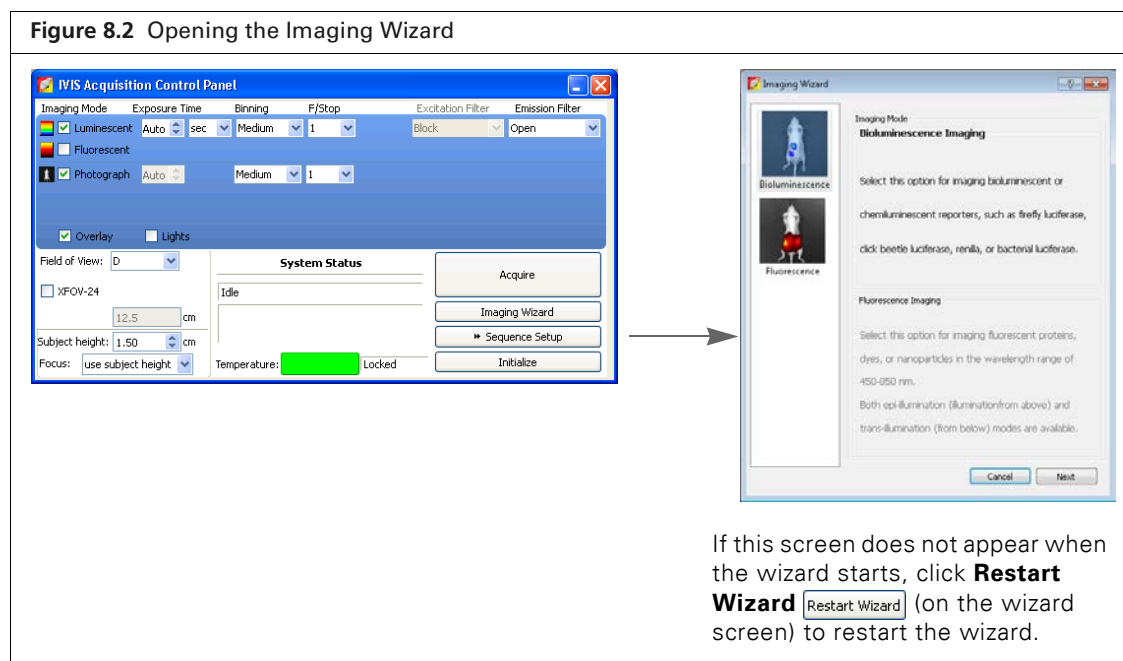


NOTE: For optimum DyCE analysis results, acquire images using the Side Imager accessory (PN CLS135111).



NOTE: The IVIS® Lumina II should be initialized and the temperature locked before setting up the imaging parameters. See [page 7](#) for more details.

1. Click **Imaging Wizard** in the control Panel ([Figure 8.2](#)).
2. If necessary, click **Restart** in the Imaging Wizard to show the first page of the wizard.

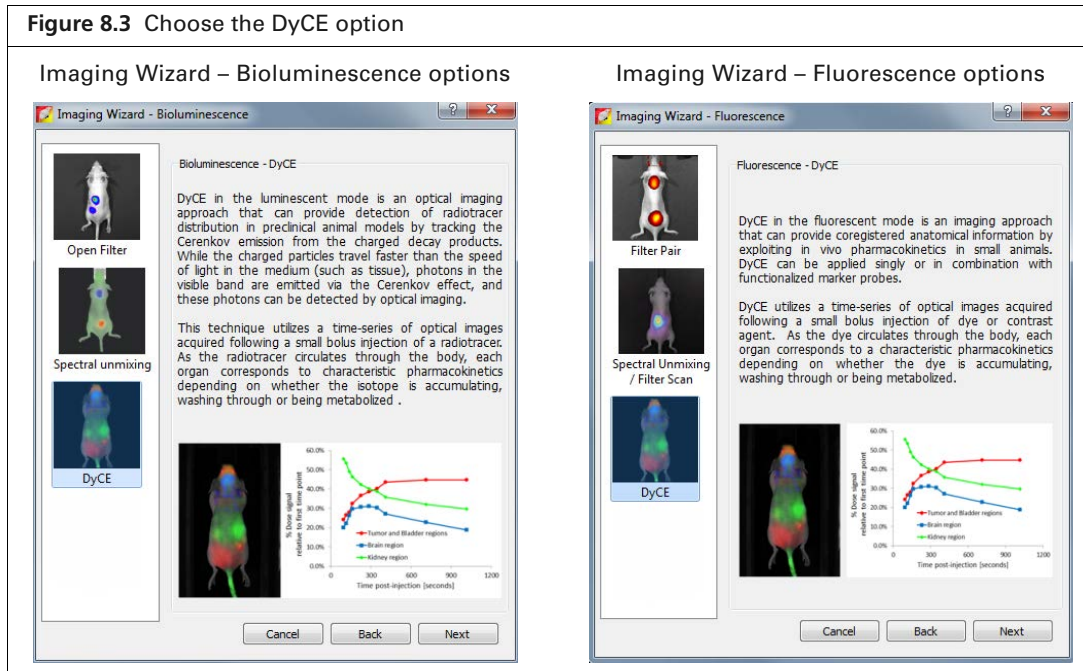


3. Double-click Bioluminescence  or Fluorescence  imaging in the wizard.

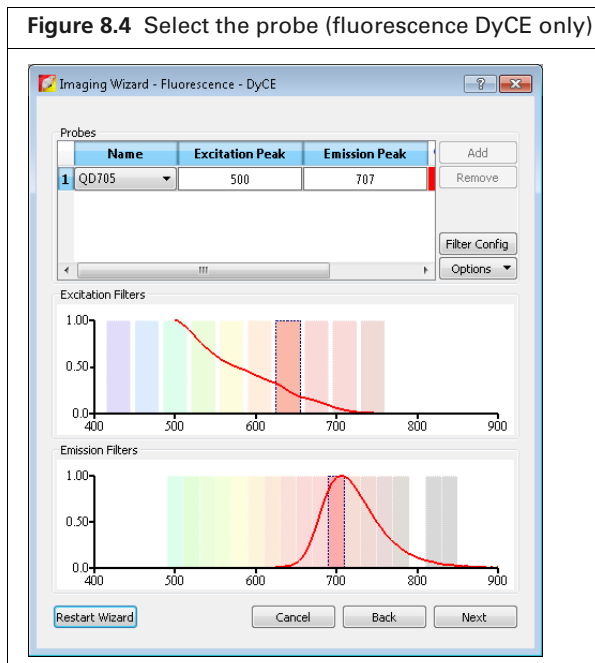


NOTE: Choose Fluorescence imaging if using near infrared probes. Choose Bioluminescence imaging if using a Cerenkov radioactive probe.

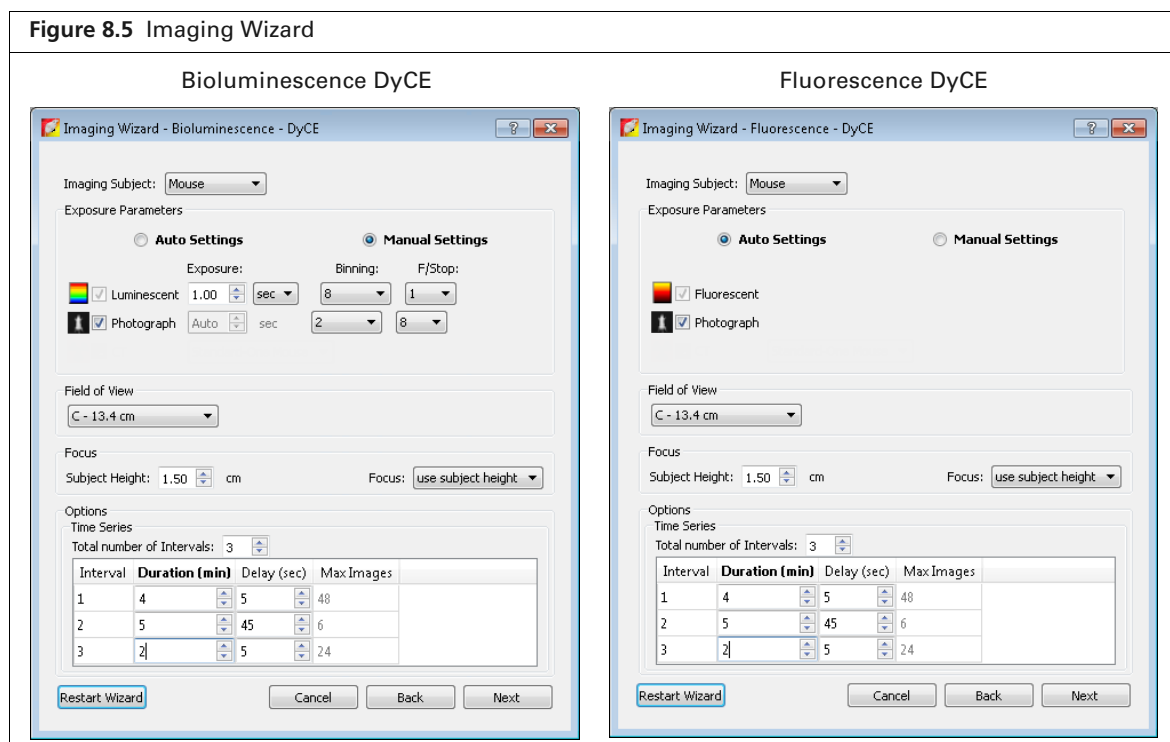
4. Choose the DyCE option ([Figure 8.3](#)) and click **Next**.



- For fluorescence DyCE only: select a probe from the Name drop-down list. For bioluminescence DyCE proceed to [step 6](#).
If your fluorescent probe is not in the list, select “Input” and enter the fluorescence excitation and emission peak wavelengths. Click **Next**.



- Select the type of imaging subject ([Figure 8.5](#)).



7. Set the exposure parameters:
 - Bioluminescence DyCE: Choose “Manual Settings” and set appropriate exposure parameter values for your probe.
 - Fluorescence DyCE: Choose the Auto Settings option.
8. Select a field of view from the drop-down list.
9. Set the focus by doing either of the following:
 - Enter a subject height and choose the “use subject height” focus option.
 OR
 - Choose the “manual focus” option from the Focus drop-down list and set the focus parameters in the Manual Focus Window that appears.



NOTE: If using the Side Imaging accessory for bioluminescence DyCE, set the subject height = 0.0 cm and FStop = 4 (or larger). If using the Side Imaging accessory for fluorescence DyCE, choose the Manual Settings options and set the subject height = 0.0 cm and FStop = 2 (or larger).

10. Specify the time series:



NOTE: A time series can include up to three intervals. Each interval is defined by duration (minutes) and delay between images (seconds). The time series can include a maximum of 100 images.

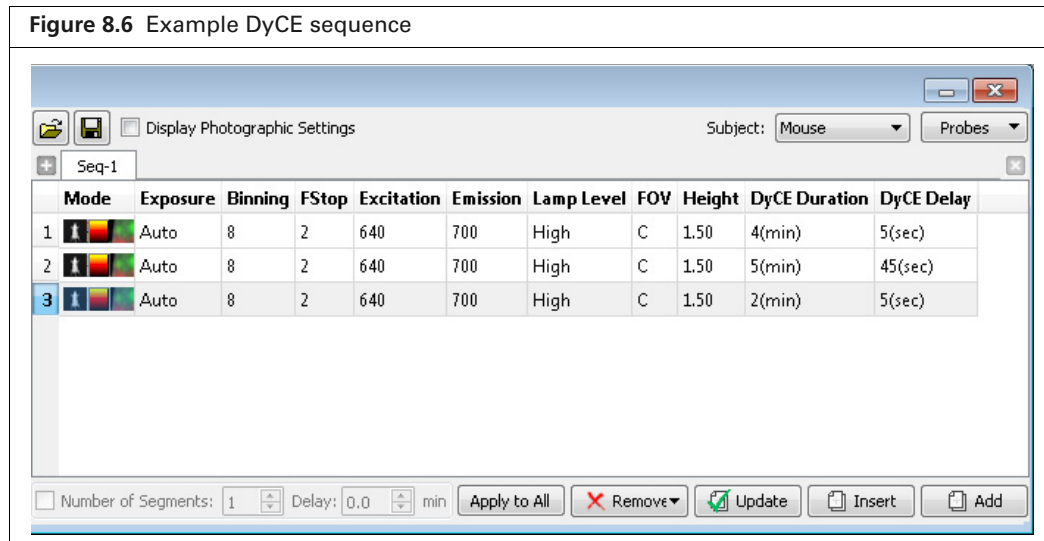
- a. Enter the number of intervals.
- b. Enter the duration of the first interval and the delay between images.
The software computes the number of images to acquire during the interval.
- c. Repeat [step b](#) for each interval.



NOTE: The software alerts you if the number of images in the time series exceeds 100. If necessary, adjust the duration or delay between images of one or more intervals to reduce the number of images.

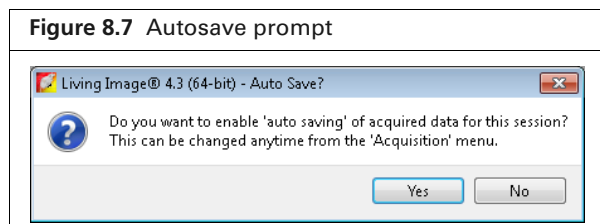
d. Click **Next**.

The specified sequence appears in the sequence table.



11. Click **Acquire** when you are ready to capture the image.

If this is the first acquisition of the session, you are prompted to enable the autosave function (Figure 8.7). When Autosave is enabled, all images acquired during the session are automatically saved to a user-selected folder. A different folder can be chosen at any time (select **Acquisition** → **Auto-Save** on the menu bar).



12. Click **Yes** in the prompt to enable autosave, then choose a folder in the dialog box that appears. Alternatively, click **No** in the prompt and manually save the image data. See page 46 for details.

13. Enter information about the image in the Edit Image Labels box that appears (optional). Click **OK**.



NOTE: You can enter image label information at any time during or after acquisition. If you do not want to enter image information, click **Cancel**. See page 58 for details on adding image information after acquisition.

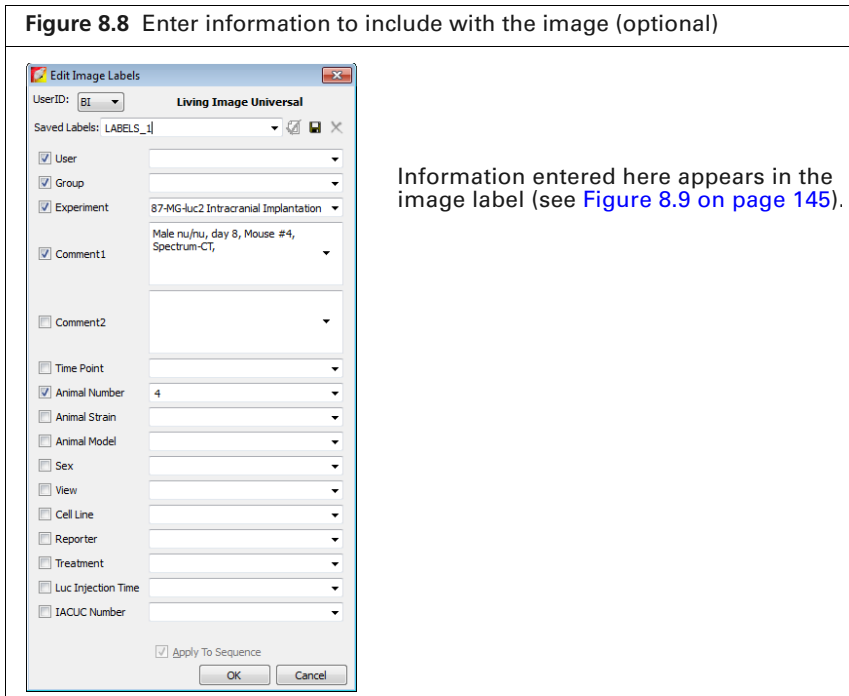


Image acquisition begins. During acquisition, the **Acquire** button in the control panel becomes a **Stop** button. Click **Stop** to cancel acquisition.

The image window appears when acquisition is completed ([Figure 8.9](#)). See [Table 3.2 on page 25](#) for more details on the image window.

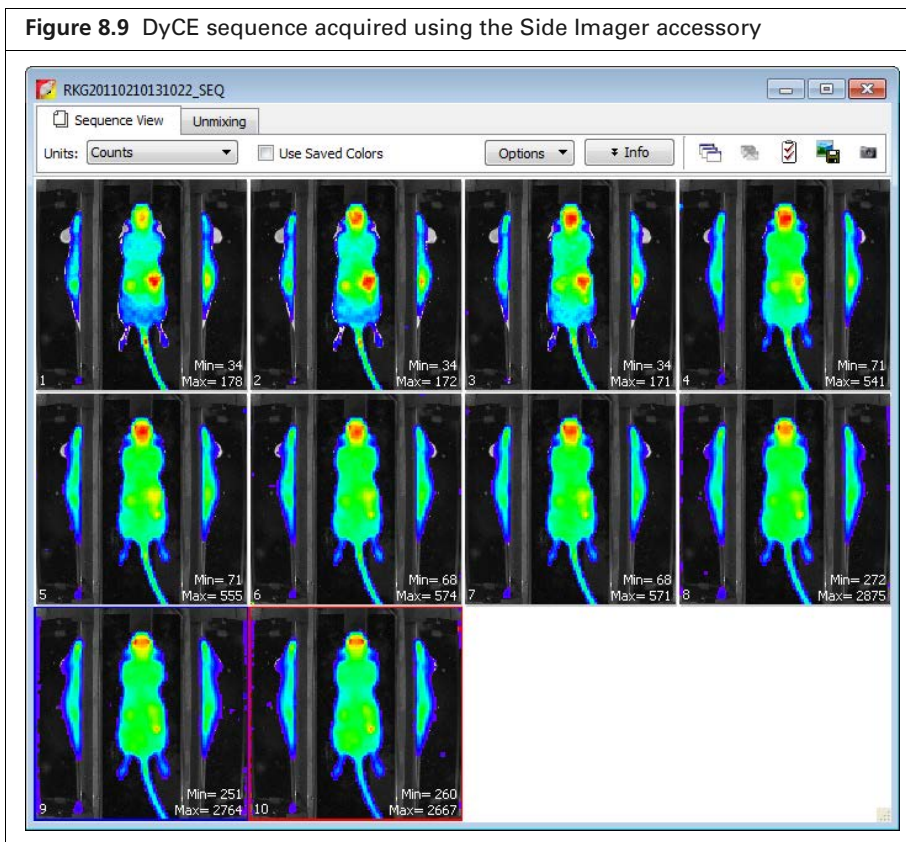


Table 8.1 Sequence View window

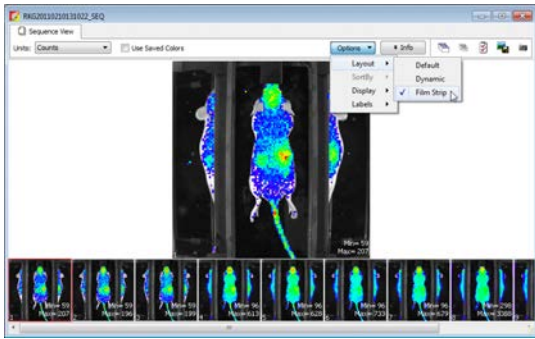
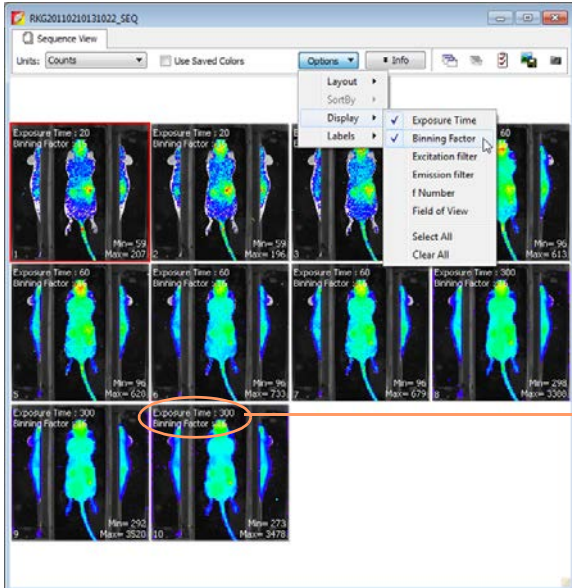





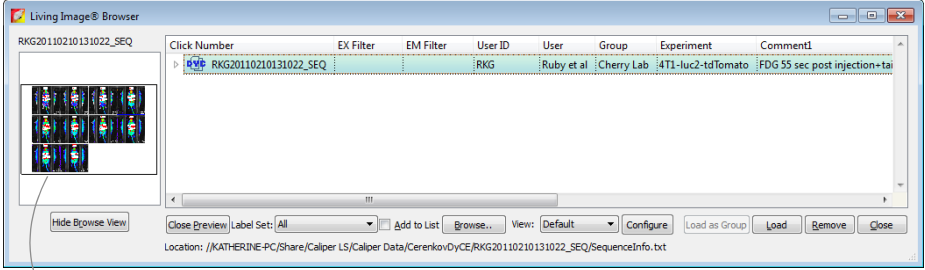
Item	Description
Units	Select the measurement units for the image display from this drop-down list. The available units depend on the type of image data. See the concept tech note <i>Image Display and Measurement</i> for more details (select Help → Tech Notes on the menu bar).
Use Saved Colors	Choose this option to display the image data using the color table that was specified in the Preferences at the time of acquisition. If this option is not selected, image data are displayed using the color table currently specified in the Preferences.
Options	<p>Layout - Choose a display option for the images in a sequence (Default, Dynamic, or Film Strip). For example, here is Film Strip mode:</p>  <p>Sort by - Options for ordering images in the sequence window. This option only applies to images that were opened using the “Load as Group” function in the Living Image browser.</p> <p>Default - Order in which the images are stored in the folder.</p> <p>TimeStamp - Ascending order of the image acquisition time.</p> <p>UserID - Ascending alphanumeric order of the user ID</p> <p>Display - Choose the types of information to display with each image.</p>  <p>In this example, exposure time and binning factor are displayed on each image</p>
Info	Click to show or hide the image label information.
	Opens all of the images in the sequence.
	Closes all open images.

Table 8.1 Sequence View window (continued)

Item	Description
	Opens the Edit Sequence dialog box that enables you to add or remove images from the sequence.
	Enables you to export the active image as a graphic file (for example, .png, .dcm).
	Creates a preview picture (<i>snapshot</i>) of the image or thumbnails that the Living Image Browser displays when the data are selected. See page 48 for more details on the browser.



Preview picture of the selected data


8.3 DyCE Analysis

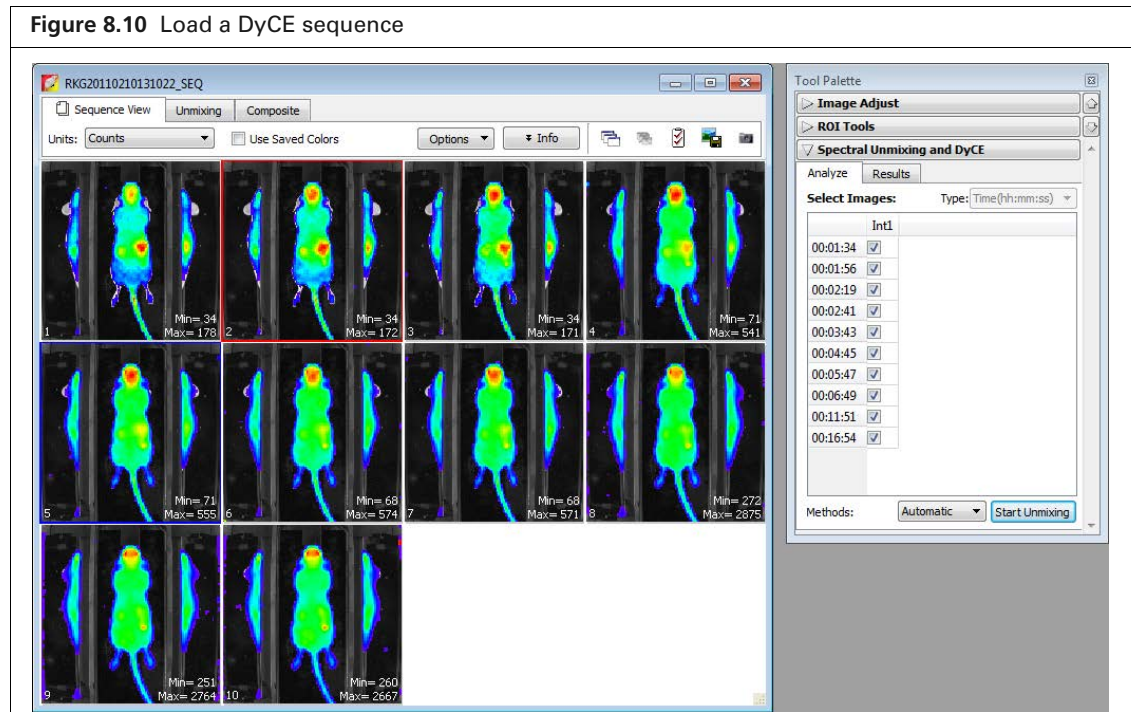
Automatic or manual DyCE analysis is available. Caliper recommends performing an automatic analysis first, followed by manual analysis to identify possible additional temporal components.

Automatic DyCE Analysis

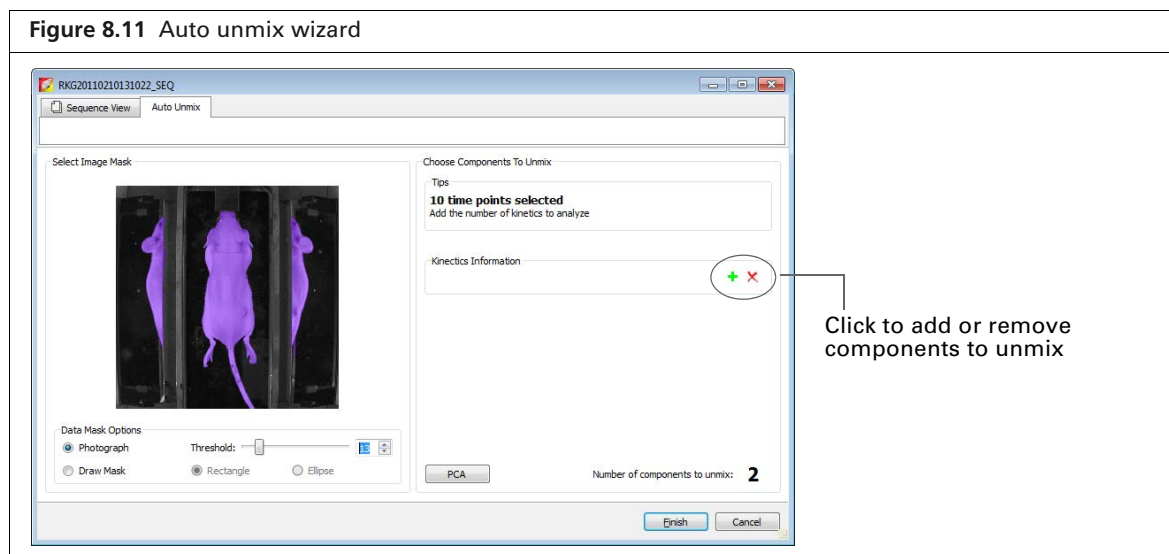
1. Load a DyCE sequence.



NOTE: The  icon in the Living Image browser indicates a DyCE sequence.



2. Click the Analyze tab in the Spectral Unmixing/DyCE tools.
3. Select **Automatic** from the Methods drop-down list and click **Start Unmixing**.
The Auto Unmix Wizard appears and shows the purple data mask that specifies the analysis area (Figure 8.11). The data mask includes the entire subject by default.
4. If necessary, change the threshold level to adjust the mask so that it matches the underlying subject photograph as closely as possible without including any area outside the subject image.



5. If you do not want to analyze the entire subject, draw a data mask on a particular area using the data mask options.
 - a. Select **Draw Mask** and choose the **Rectangle** or **Ellipse** option.
 - b. Draw a mask over an area using the mouse. If necessary, click the mask to discard it, and redraw the mask.
6. Select a subject type from the drop-down list.
7. Click the **+** button to add components to unmix.



NOTE: Two or three components are recommended for the initial automatic analysis. The DyCE results obtained from the automatic analysis can be manually analyzed to identify possible additional components (see page 150 for details on manual analysis).

8. Click **Finish**.
The Unmixing window shows a time plot of the temporal spectra, unmixed images, and a composite of the unmixed images (Figure 8.12).

Figure 8.12 DyCE results showing three temporal components

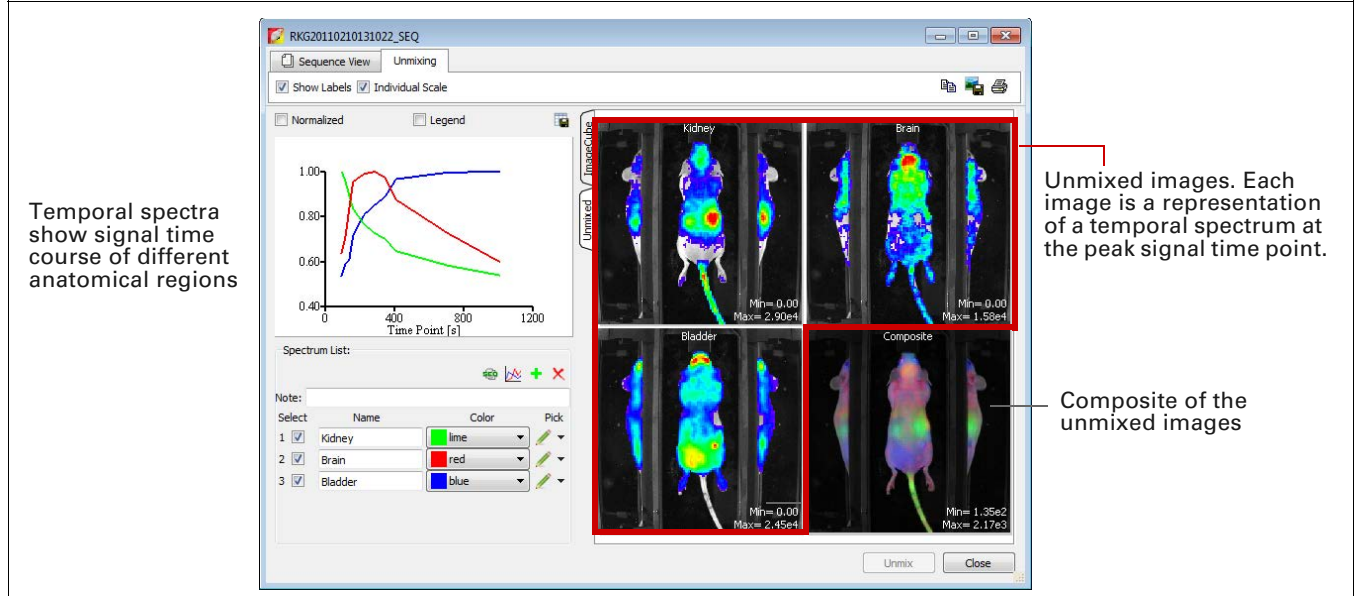
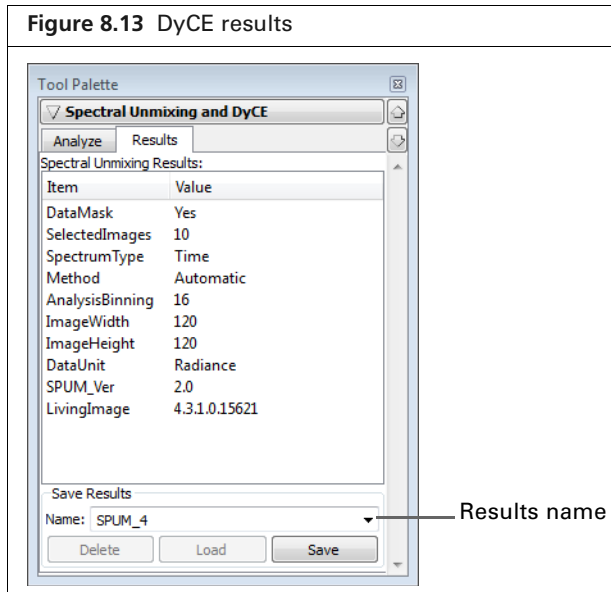


Table 8.1 Spectrum list toolbar

Item	Description
	Enables you to view and save the unmixed images as a sequence data set. The image adjust, corrections/filtering, image information, or ROI tools are available for the images.
	Enables you to subtract one spectrum from another (see page 156).
	Adds a temporal component to the spectrum list when performing a manual analysis. See page 150 for more details on manual analysis.
	Deletes the last component in the spectrum list. Click Unmix after deleting a spectrum to view updated DyCE results.

9. To save the results:

- a. Enter a name in the Results tab of the tool palette (Figure 8.13).
- b. Click **Save**.

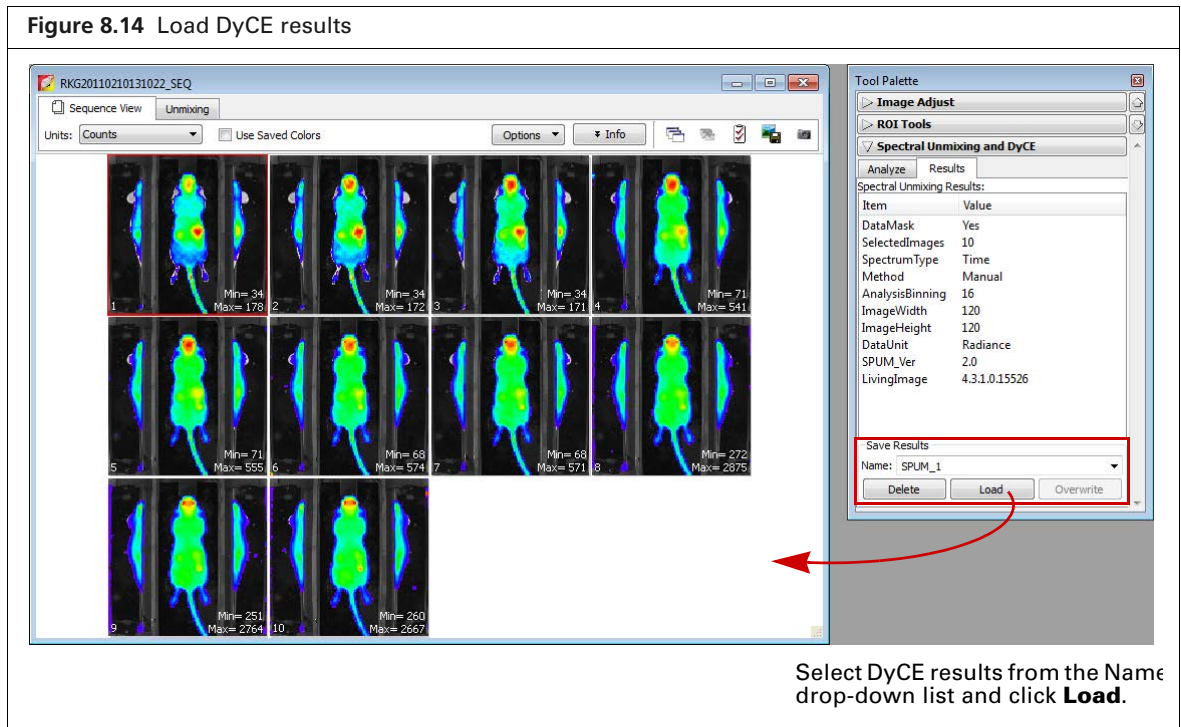


Manual DyCE Analysis

1. Load a DyCE image sequence. Alternatively, load DyCE results obtained from an automatic analysis (Figure 8.14).



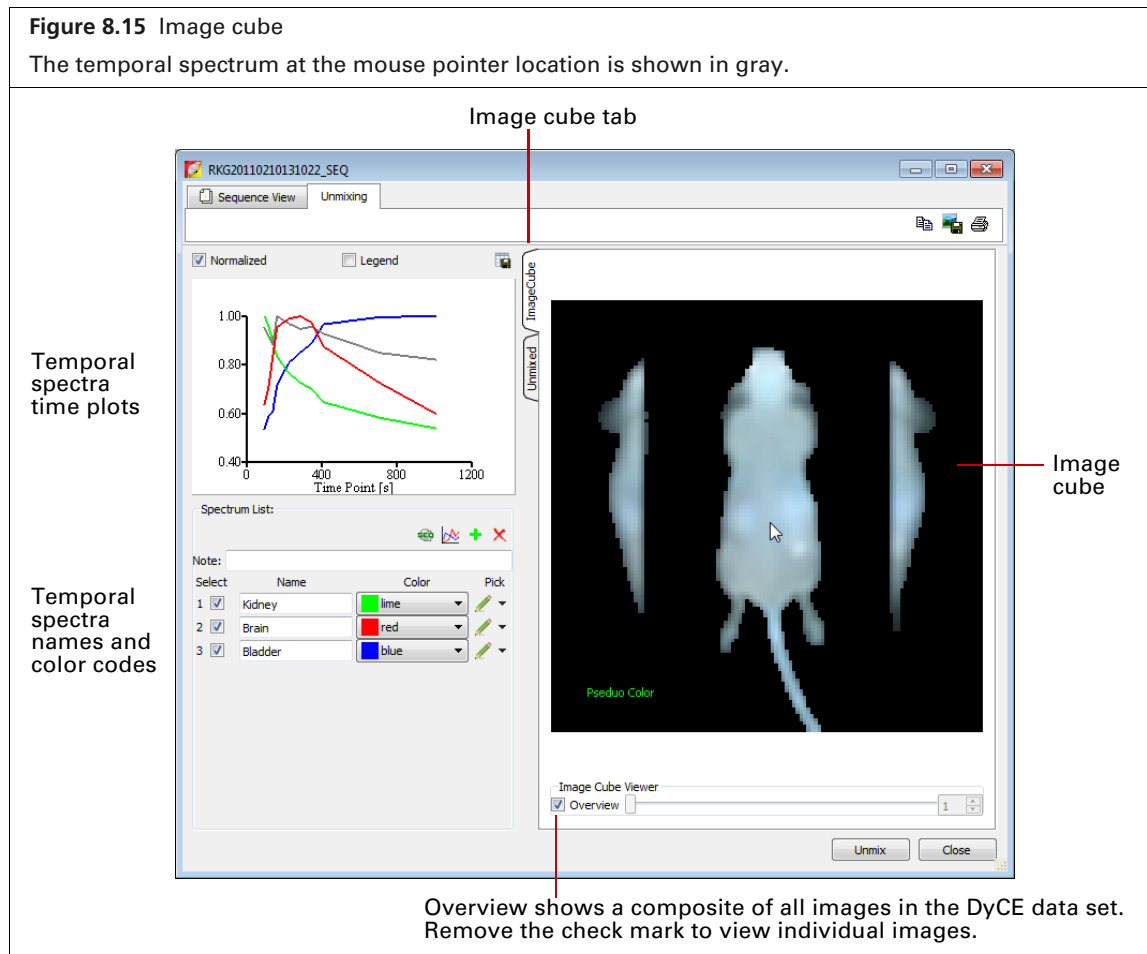
NOTE: This section illustrates manual analysis of DyCE results obtained from an automatic analysis.



Select DyCE results from the Name drop-down list and click **Load**.

2. Click the Image Cube tab (Figure 8.15).

The image cube represents a “stack” of the DyCE sequence images. If the Overview option is selected, the image cube shows a composite of all images. To view a particular image, remove the check mark next to Overview and move the slider or enter an image number.



3. Move the mouse pointer over the image cube to see the temporal spectrum at a particular location. The temporal spectrum at the pointer location is updated as you move the pointer.

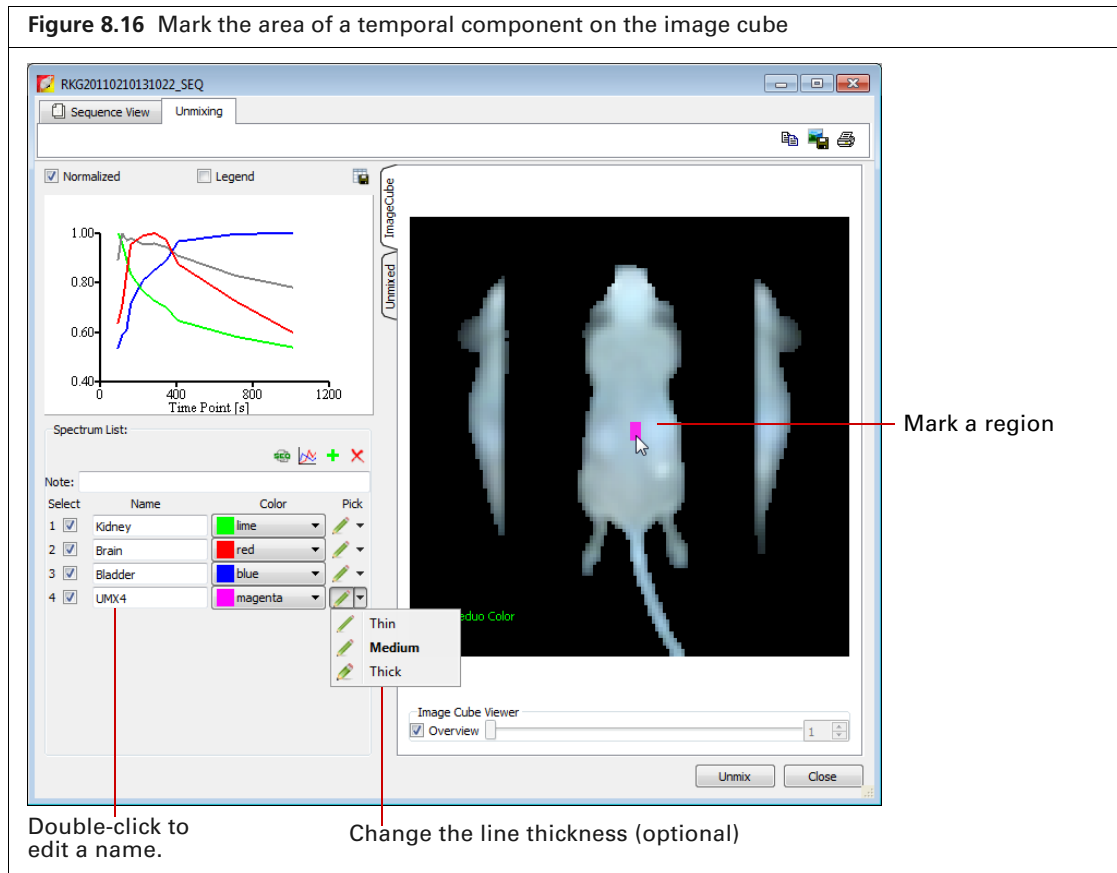


NOTE: If analyzing DyCE results, the Normalized option for the spectrum plot must be checked to see all of the temporal spectra when the mouse pointer is over the image cube.

4. To add another component to unmix:
 - a. Click the **+** button.
A new name appears in the spectrum list (Figure 8.16)
 - b. Specify the region by using the mouse to draw a mark on the image cube. If necessary, click the button next to the spectrum name to select a different line thickness from the drop-down list.
 - c. If necessary, right-click the image cube to erase the mark.
5. Repeat step 4 to specify additional temporal components.







NOTE: A maximum of 10 components can be unmixed.



6. Click **Unmix** after you finish marking the regions.

The software generates unmixed images for the new temporal spectra and updates the composite image with these components.

Table 8.2 Spectrum list toolbar

Item	Description
	Enables you to view and save the unmixed images as a sequence data set. The image adjust, corrections/filtering, image information, or ROI tools are available for the images.
	Enables you to subtract one spectrum from another (see page 156).
	Adds a component to the spectrum list.
	Deletes the last spectrum in the spectrum list.

8.4 DyCE Results

The Unmixing window shows the DyCE results. The example in [Figure 8.17](#) shows three “temporal spectra” (signal as a function of time).

Figure 8.17 DyCE results showing three temporal spectra



Viewing Unmixed Images

An unmixed image shows the maximum signal for a temporal spectrum.


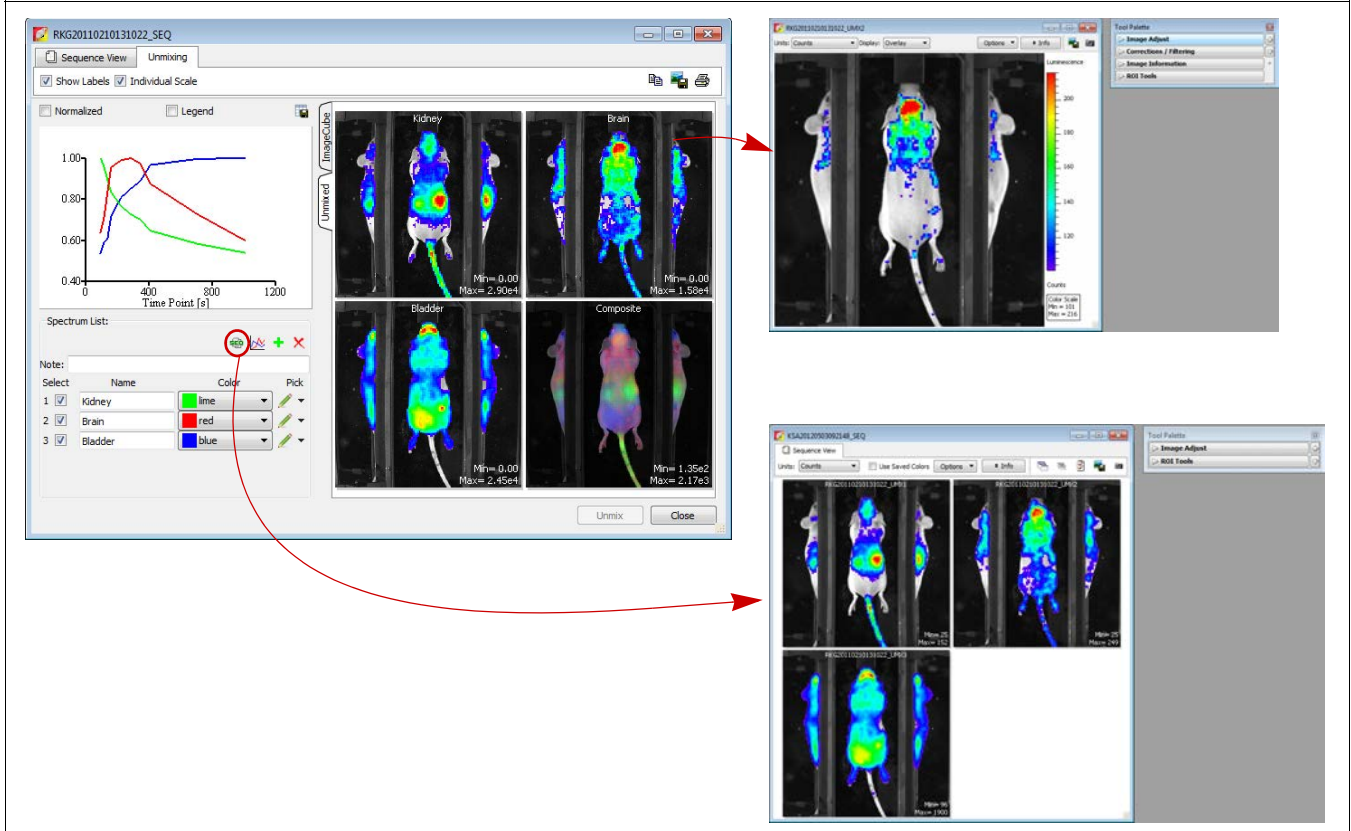
- Double-click an unmixed image to view it in an image window ([Figure 8.18](#)). The tool palette is available for viewing and analyzing the image.
- Click the  button to view the unmixed images as a sequence ([Figure 8.18](#)). The tool palette is available for viewing and analyzing the sequence. The software prompts you to save the sequence when closing the Sequence View window.

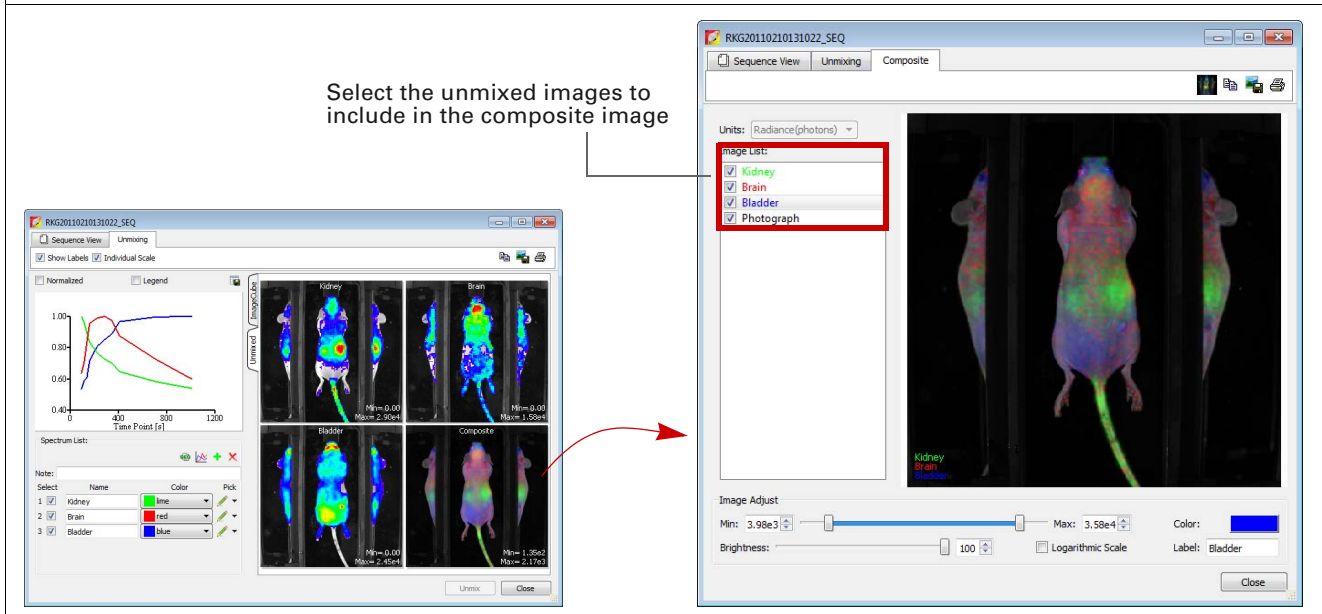
Figure 8.18 View an unmixed image in an image window or view all unmixed images as a sequence



Viewing the Composite Image



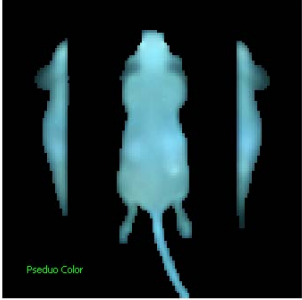

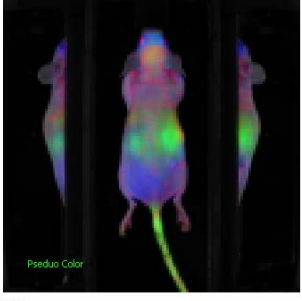




1. Double-click the composite thumbnail.
The Composite window opens.

Figure 8.19 Open the Composite window



2. Add or remove the check mark next to an image to include or exclude the data from the composite image.
3. Use the image adjust tools at the bottom of the Composite window to adjust the appearance of the composite image.


Table 8.3 Composite window

Item	Description
	<p>Sends the composite image to the “top” of the image cube.</p> <div style="display: flex; align-items: center;"> <div style="margin-right: 10px;">  </div> <div style="text-align: center;">  </div> <div style="margin-left: 10px;"> <p>Click the Image Cube tab in the Unmixing window to view the image cube. See Figure 8.15 on page 151 for more details on the image cube.</p> </div> </div> <div style="display: flex; align-items: center; margin-top: 10px;"> <div style="margin-right: 10px;">  </div> <div style="text-align: center;">  </div> <div style="margin-left: 10px;"> <p>Composite image displayed on top of the image cube.</p> </div> </div>
	<p>Copies the Composite window to the system clipboard.</p>
	<p>Opens a dialog box that enables you to export the composite image to a graphic file (for example, .png).</p>
	<p>Opens the print dialog box.</p>
<p>Color: </p>	<p>Shows the color of the data for the highlighted image.</p> <div style="border: 1px solid gray; padding: 5px; margin-bottom: 10px;"> <p>Image List:</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> Kidney <input checked="" type="checkbox"/> Brain <input checked="" type="checkbox"/> Bladder <input checked="" type="checkbox"/> Photograph </div> <p>Click the color swatch to open the color palette which can be used choose a color for the selected image data.</p>
<p>Label: <input type="text" value="Bladder"/></p>	<p>Data name for the highlighted image. Double-click the name to edit it.</p>

Correcting Temporal Spectra

Temporal spectra can be corrected for overlapping spectra, for example, correcting for tissue autofluorescence.

NOTE: If correcting for tissue autofluorescence, one of the unmixed components of the data set should be tissue autofluorescence signal only.

1. Click the  button in the Unmixing window.
2. In the dialog box that appears, choose the spectra to subtract (Figure 8.20).

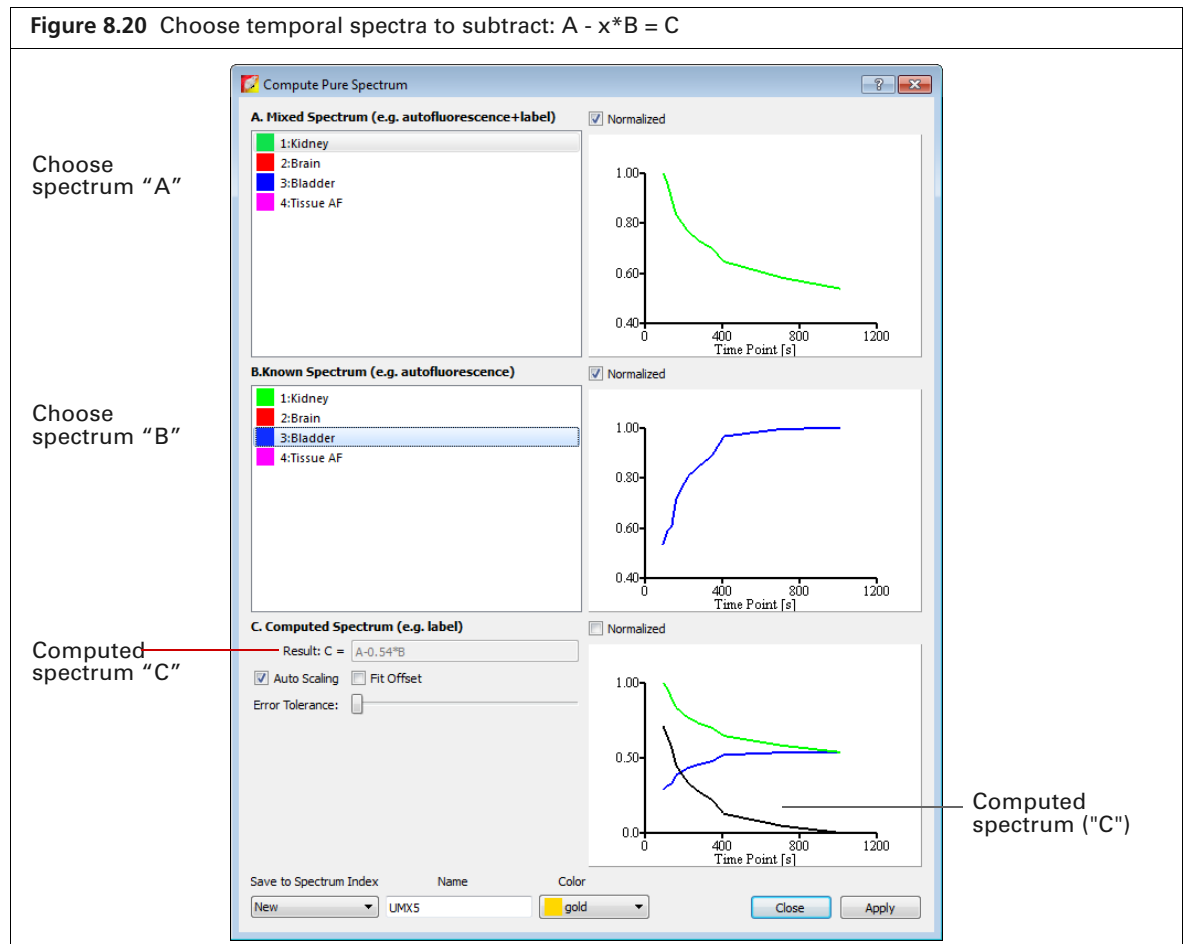
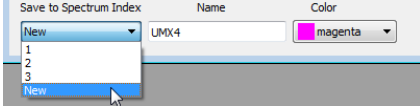


Table 8.4 Computed spectrum

Item	Description
Normalized	Choose this option to normalize the spectra with respect to time zero.
Result: $C = A - x*B$	The subtraction performed by the software where "x" is a factor that ensures the residual signal is positive.
Autoscaling	Choose this option to normalize spectra signal on a scale of zero to one.
Fit Offset	If this option is chosen, the software computes and removes an intensity baseline from the spectra.

Table 8.4 Computed spectrum (continued)

Item	Description
Error Tolerance	The software computes a default error tolerance (the factor "x" for $A - x*B$) such that signal B is maximally removed from signal A with no negative result. Moving the slider adjusts the error tolerance and automatically updates the computed spectrum.
	<p>Choose "New" to save computed spectrum with the specified name and color. Click Apply to add the computed spectrum to the line plot and spectrum list in the Unmixing window.</p> <p>Choose a spectrum number from the drop-down list to overwrite that spectrum with the computed spectrum when you click Apply.</p>

Appendix A Planar Spectral Image Analysis

About Planar Spectral Image Analysis

Image Sequence Requirements

Planar Spectral Image Analysis on page 159

Viewing Graphical Results on page 162

Managing Planar Spectral Imaging Results on page 163

A.1 About Planar Spectral Image Analysis



NOTE: The Low Range or Medium Range filter set is required for planar spectral imaging analysis. The Planar Spectral Imaging tools are only available if “Show Advanced Options” is selected in the user preferences. See [page 168](#) for details.

The Living Image software applies planar spectral image analysis to a sequence to determine the average depth and total photon flux of a luminescent point source in a user-specified region of interest.



TIP: See the tech note *Planar Spectral Imaging* for theory and examples of planar spectral imaging. Select **Help** → **Tech Notes** on the menu bar.

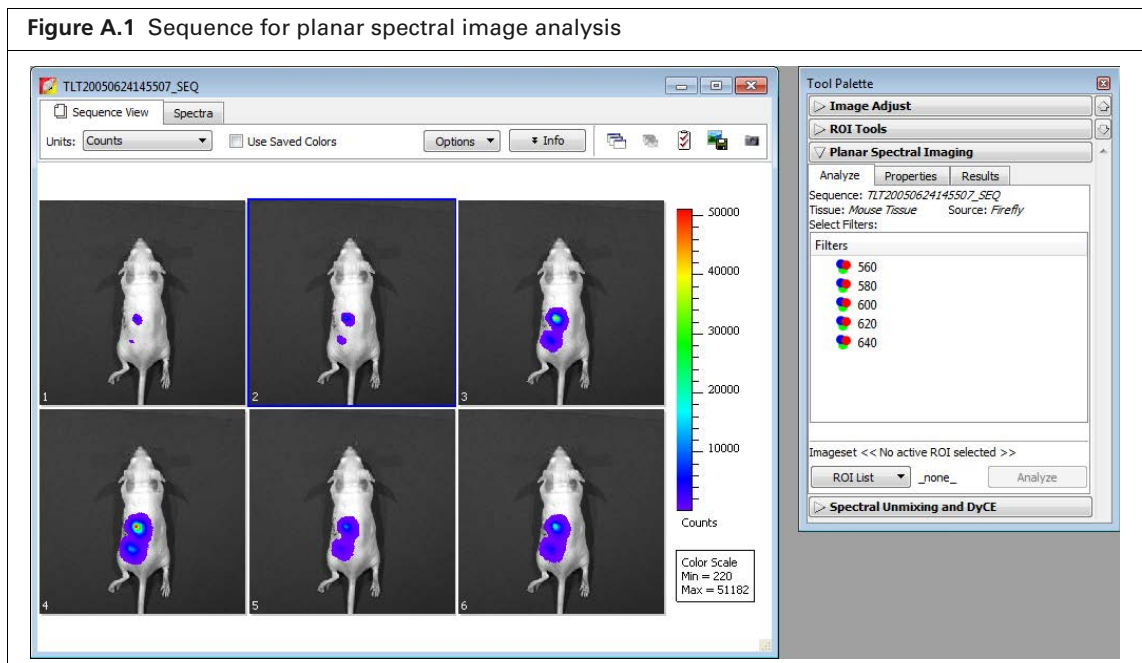
A.2 Image Sequence Requirements

Use the Imaging Wizard to set up the image sequence required for planar spectral image analysis. See [page 33](#) for more details on the Imaging Wizard.

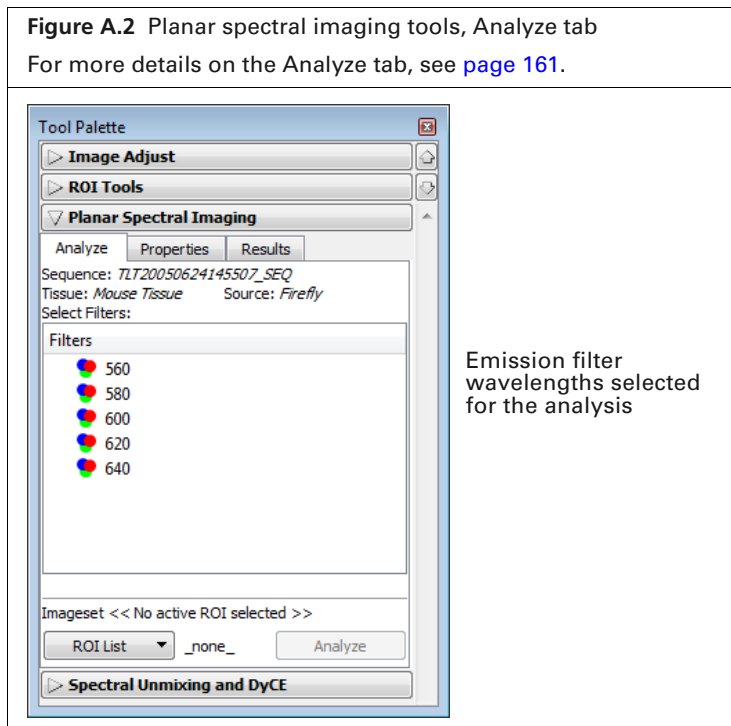
At a minimum, the sequence must include a photographic and luminescent image at the first wavelength and a luminescent image at a second wavelength. The data must be acquired in the wavelength range from 560-660 nm.

A.3 Planar Spectral Image Analysis

1. Load the image sequence that you want to analyze.

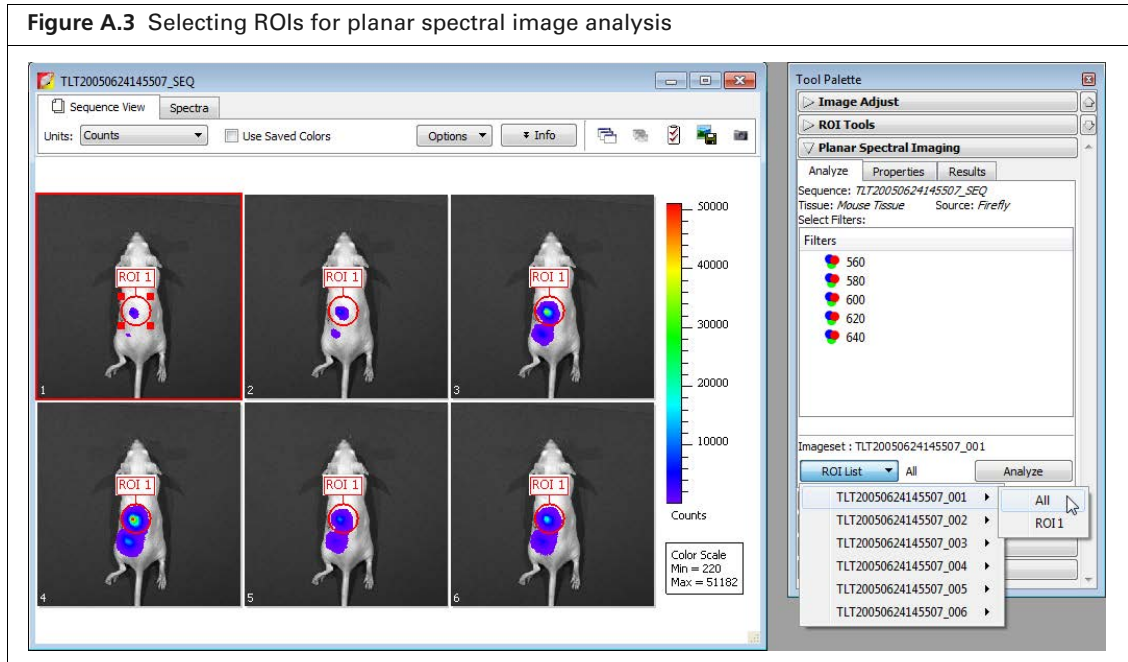


2. Click Planar Spectral Imaging in the Tool Palette.
3. Select the emission filter wavelengths for the analysis in the Analyze tab (Figure A.2).
 It is recommended that you do not include a wavelength in the analysis if the signal is less than or equal to the autoluminescent background. If autoluminescent background is a concern, you can create a background ROI and link it to the measurement ROI prior to planar spectral analysis. (See *Measuring Background-Corrected Signal*, page 95 for more details.)



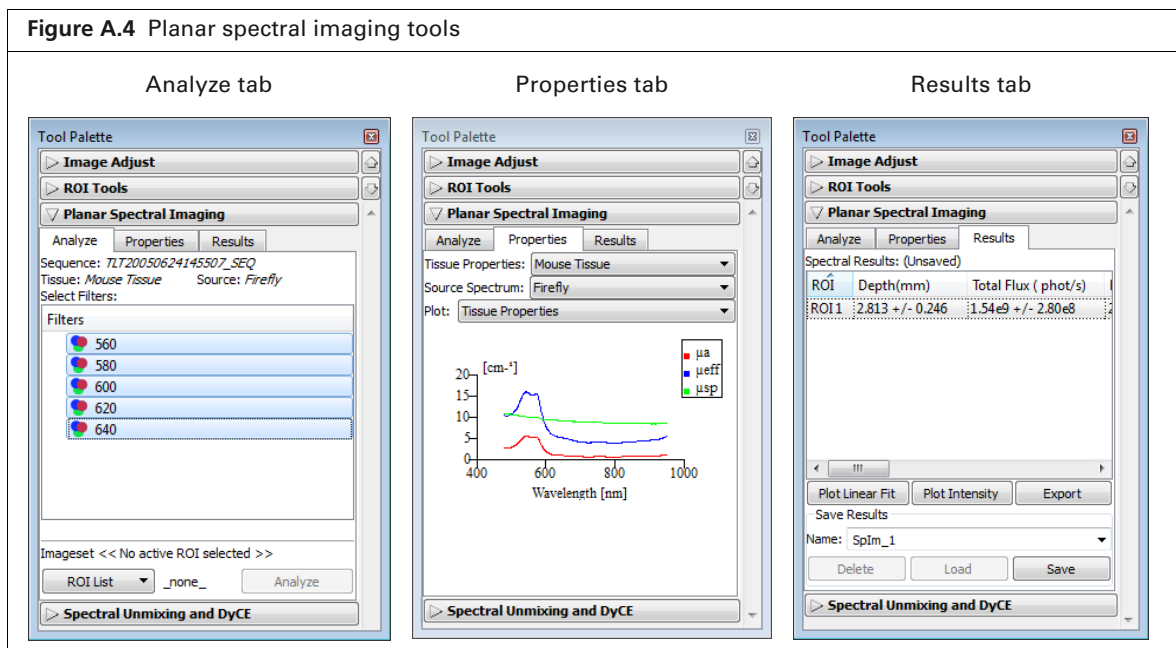
- In the ROI List drop-down, select **All** or a particular ROI in an image for the analysis. If there is no measurement ROI, draw an ROI that includes the area for analysis. (See [page 85](#) for more details on drawing ROIs.)

You only need to draw the ROI(s) on one image in the sequence. The software copies the ROI(s) to all other images of the sequence during the analysis. The ROI should include as much of the light emission from a single source as possible.



- Choose the tissue properties. In the Properties tab, choose Mouse Tissue or XPM-2/XFM-2 (mouse phantom) from the Tissue Properties drop-down list.

The software automatically sets the internal medium index of refraction based on the selection in the Tissue Properties list



6. Make a selection from the Source Spectrum drop-down list (Firefly in this example).
7. Click **Analyze** in the Analyze tab.

The Results tab displays the computed average depth (mm) and total flux (photon/sec) of the luminescent point source in the specified ROI(s). For more details on the results, see [Table A.1](#).

Table A.1 Planar spectral imaging tools

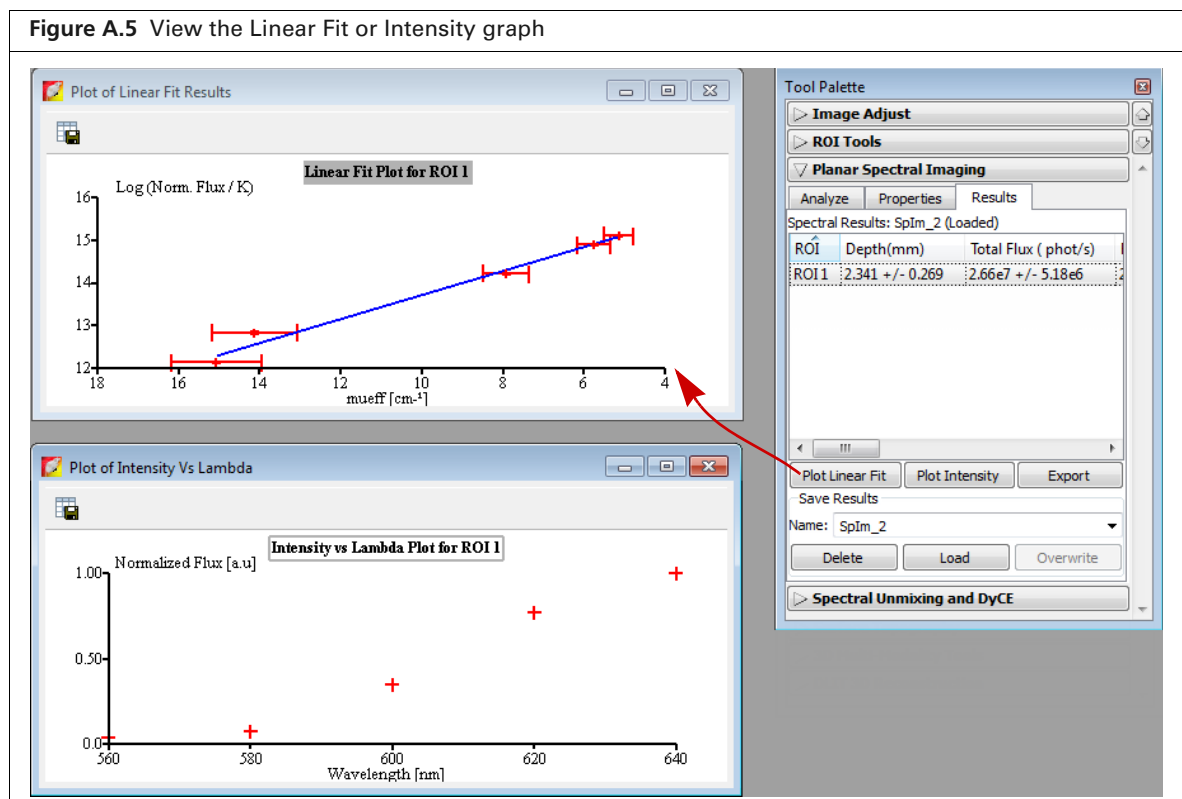
Item	Description
Analyze tab	
Sequence	Name of sequence used for the analysis.
Tissue, Source	The tissue properties and source spectrum selected in the Properties tab.
Select Filters	In the Filter box, select the acquisition wavelengths for the images in the selected sequence. To select non-adjacent wavelengths, press and hold the Ctrl key while you click the wavelengths. (Macintosh users, press and hold the Cmd key while you click the wavelengths.)
ROI List	A drop-down list of the ROIs in the active image.
Analyze	Click to perform the spectral analysis.
Properties tab	
Tissue Properties	Drop-down list of the absorption and scattering properties for Mouse Muscle or XPM-2/XFM-2 (mouse phantom). Note: If a result from an earlier version of Living Image software is loaded, the tissue type will be listed in this drop-down list.
Source Spectrum	Drop-down list of luminescent sources.
Plot	Tissue Properties - Click to display graphs (cm^{-1} vs. nm) of the absorption coefficient (μ_a), effective attenuation coefficient (μ_{eff}), and reduced scattering coefficient (μ_s). Source Spectrum - Click to display the spectrum of the selected luminescent source (intensity versus wavelength, normalized to one).
Results tab	
Spectral Results	ROI - Name of the analyzed ROI. Depth (mm) - Estimated depth of the point source. Total Flux (phot/s) - Estimated total photon flux from the point source.
Plot Linear Fit	Displays a graph of normalized intensity versus the effective attenuation coefficient (μ_{eff} , the optical property of the tissue selected in the Tissue Properties drop-down list) along with the linear fit to these data determined by the spectral analysis code.
Plot Intensity	Displays a graph of normalized intensity versus wavelength. Intensity is normalized by the selected source spectrum and filter transmission properties.
Export	Opens a dialog box that enables you to save the results to a text file (.txt).
Save Results	Name - A drop-down list of saved results. Includes the default name for new unsaved analysis results (Splm_<name>). Delete - Deletes the selected results. Load - Opens the selected results. Save - Saves the analysis results. Results name appears in the Name drop-down list).

A.4 Viewing Graphical Results


1. In the Results tab, select an ROI.
2. Click **Plot Intensity** or **Plot Linear Fit** (Figure A.5).

Linear fit graph – Plots the logarithm of the intensity, normalized to the selected source spectrum and the filter transmission properties, against the optical property of the tissue (μ_{eff}). The slope of the line is the source depth. If any of the measured points (in red) deviate significantly from the straight line fit, then the analysis results may be suspect. The horizontal error bars represent the uncertainty in the optical properties (usually estimated at $\pm 10\%$). The vertical error bars represent noise in the image.

Intensity graph – Displays a graph of the measured intensity in the selected ROI at each wavelength in the analysis. The intensity is normalized to the selected source spectrum and the filter transmission properties.

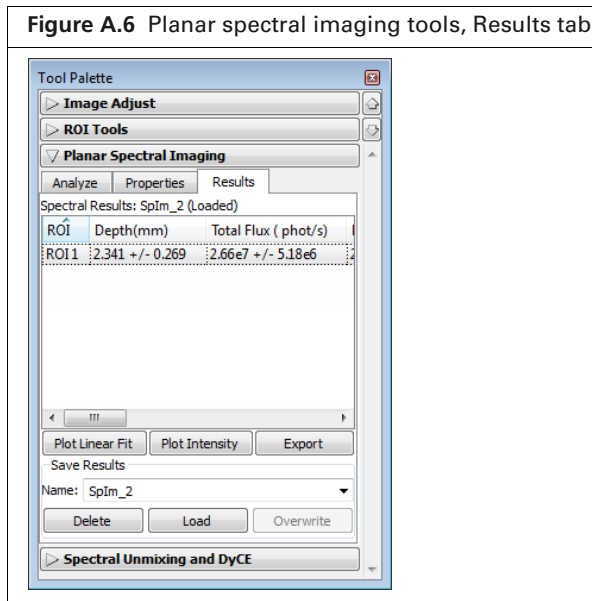


To export graph data:

1. Click the **Export Data** button .
2. In the dialog box that appears, select a directory for the data and enter a file name (.csv).
The data can be opened in a spreadsheet application such as Microsoft® Excel®.

A.5 Managing Planar Spectral Imaging Results

Go to the Results tab to select results that you want to view or manage.



Saving Results

1. Select results (SpIm_<name>) from the Name drop-down list.
2. Click **Save**.

The planar spectral imaging results are saved with the image.

Viewing Results

1. Select results from the Name drop-down list.
2. Click **Load**.

Deleting Results

1. Select the results that you want to delete from the Name drop-down list.
2. Click **Delete**.

Copying Results

1. To copy selected results, right-click the results (row in the Results tab) and select **Copy** from the shortcut menu that appears.

The selected results are copied to the system clipboard.

2. To copy all results, right-click the results table and choose **Select All** from the shortcut menu that appears.

All of the results are copied to the system clipboard.

Exporting Results

1. Right-click the results table and select **Export Results** from the shortcut menu that appears.
2. In the dialog box that appears, choose a folder for the results, enter a file name (.txt), and click **Save**.

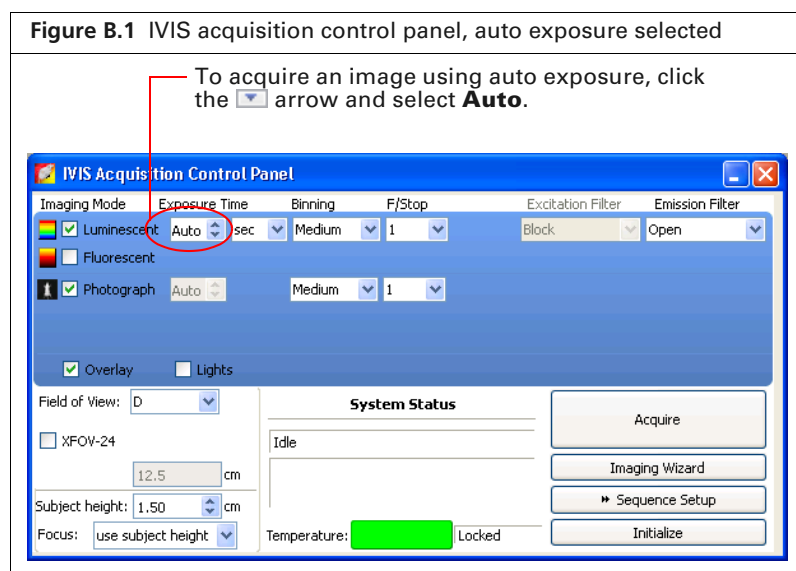
Appendix B IVIS Acquisition Control Panel

Control Panel

Manually Setting the Focus on page 167

B.1 Control Panel

The control panel provides the image acquisition functions (Figure B.1).



NOTE: The options available in the IVIS acquisition control panel depend on the selected imaging mode, the imaging system, and the filter wheel or lens option that are installed.

Table B.1 IVIS acquisition control panel




Item	Description
 Luminescent	Choose this option to acquire a luminescent image.
 Fluorescent	Choose this option to acquire a fluorescent image.
 Photograph	Choose this option to automatically acquire a photograph. The illumination lights at the top of the imaging chamber are on during a photographic image so that the system can acquire a black and white photograph of the sample(s). Note: You can adjust the appearance of the photographic image using the Bright and Contrast controls (see Adjusting Image Appearance, page 61).

Table B.1 IVIS acquisition control panel (continued)

Item	Description
Exposure time	<p>The length of time that the shutter is open during acquisition of an image. The luminescent or fluorescent signal level is directly proportional to the exposure time. The goal is to adjust the exposure time to produce a signal that is well above the noise (>600 counts recommended), but less than the CCD camera saturation of ~60,000 counts.</p> <p>Luminescent exposure time is measured in seconds or minutes. The minimum calibrated exposure time is 0.5 seconds. The exposure time for fluorescent images is limited to 60 seconds to prevent saturation of the CCD. There is no limit on the maximum exposure time for luminescent images; however, there is little benefit to exposure times greater than five minutes. The signal is linear with respect to exposure time over the range from 0.5 sec to 10 minutes. Integration times less than 0.5 seconds are not recommended due to the finite time required to open and close the lens shutter.</p>
Binning	<p>Controls the pixel size on the CCD camera. Increasing the binning increases the pixel size and the sensitivity, but reduces spatial resolution. Binning a luminescent image can significantly improve the signal-to-noise ratio. The loss of spatial resolution at high binning is often acceptable for <i>in vivo</i> images where light emission is diffuse. For more details on binning, see the reference article <i>Detection Sensitivity</i> (select Help → References on the menu bar).</p> <p>Recommended binning: 1-4 for imaging of cells or tissue sections, 4-8 for <i>in vivo</i> imaging of subjects, and 8-16 for <i>in vivo</i> imaging of subjects with very dim sources.</p>
F/stop	<p>Sets the size of the camera lens aperture. The aperture size controls the amount of light detected and the depth of field. A larger f/stop number corresponds to a smaller aperture size and results in lower sensitivity because less light is collected for the image. However, a smaller aperture usually results in better image sharpness and depth of field.</p> <p>A photographic image is taken with a small aperture (f/8 or f/16) to produce the sharpest image and a luminescent image is taken with a large aperture (f/1) to maximize sensitivity. For more details on f/stop, see the reference article <i>Detection Sensitivity</i> (select Help → References on the menu bar).</p>
Excitation Filter	<p>A drop-down list of fluorescence excitation filters. For fluorescent imaging, choose the appropriate filter for your application. For luminescent imaging, Block is selected by default. If you select Open, no filter is present. For systems equipped with spectral imaging capability, choose the appropriate emission filter for your application.</p> <p>Note: On some models with standard filter sets, the excitation filter selection automatically sets the emission filter.</p>
Emission Filter	<p>A drop-down list of fluorescence emission filters located in front of the CCD lens. The emission filter wheel is equipped with filters for fluorescence or spectral imaging applications. The number of filter positions (6 to 24) depends on the system. For luminescent imaging, the Open position (no filter) is automatically selected by default.</p>
Lamp Level	<p>Sets the illumination intensity level of the excitation lamp used in fluorescent imaging (Off, Low, High, and Inspect). The Low setting is approximately 18% of the High setting. Inspect turns on the illumination lamp so that you can manually inspect the excitation lamp.</p> <p>Note: Make sure that the filters of interest are selected in the filter drop-down lists before you select Inspect. The Inspect operation automatically positions the selected filters in the system before turning on the lamp. Subsequent changes to the filter popup menus will have no effect until another Inspect operation is performed.</p>
Overlay	<p>If this option is chosen, the system automatically displays the overlay after acquisition is completed (for example, luminescent image on photograph).</p>
Lights	<p>Turns on the lights located at the top of the imaging chamber.</p>

Table B.1 IVIS acquisition control panel (continued)




Item	Description
Field of View	Sets the size of the stage area to be imaged by adjusting the position of the stage and lens. The FOV is the width of the square area (cm) to be imaged. A smaller FOV gives a higher sensitivity measurement, so it is best to set the FOV no larger than necessary to accommodate the subject or area of interest. The FOV also affects the depth of field (range in which the subject is in focus). A smaller FOV results in a narrower depth of field. Select the FOV by choosing a setting from the drop-down list. See Table B.2 for more details on the calibrated FOV positions.
Mouse Imaging Shuttle	Choose this option if the subject will be contained in the Mouse Imaging Shuttle during image acquisition.
Load	Moves the stage from the cleaning position back to the home position.
Subject height (cm)	Sets the position of the focal plane of the lens/CCD system by adjusting the stage position. The subject height is the distance above the stage that you are interested in imaging. For example, to image a mouse leg joint, set the subject height to a few mm. To image the uppermost dorsal side of a mouse, set the subject height to the 1.5 - 2.0 cm. The default subject height is 1.5 cm.
Focus	Drop-down list of focusing methods available: Use subject height – Choose this option to set the focal plane at the specified subject height. Manual – Choose this option to open the Focus Image window so that you can manually adjust the stage position. For more details on manual focusing, see page 167 .
Batch Sequences	Choose this option if you want to specify multiple, separate image sequences for batch acquisition (multiple image sequences are automatically acquired, one after another, without user intervention). See page 39 for more details.
Temperature   	The temperature box color indicates the temperature and status of the system: <ul style="list-style-type: none"> ■ White box – System not initialized. ■ Red box – System initialized, but the CCD temperature is out of range. ■ Green box – System is initialized and the CCD temperature is at or within acceptable range of the demand temperature and locked. The system is ready for imaging. Click the temperature box to display the actual and demand temperature of the CCD and stage. See page 8 for more details.
Acquire	Click to acquire an image using the settings and options selected in the control panel or to acquire an image sequence specified in the Sequential Setup table.
Imaging Wizard	Click to start the Imaging Wizard
Sequence Setup	Click to display the sequence table so that you can specify and manage sequence acquisition parameters, or open sequence acquisition parameters (xsq). See page 33 for more details on setting up an image sequence,.
Image Setup	Click to close the sequence table.
Initialize	Click to initialize the IVIS® Lumina II. See page 7 for more details on initializing the system.

Table B.2 Field of View (FOV) settings

FOV Setting	FOV (cm)
A	5
B	7.5
C	10

Table B.2 Field of View (FOV) settings (continued)

FOV Setting	FOV (cm)
D	12.5
E ¹	24
Z ²	2.6

¹Position E is available when the XFOV-24 lens (part no. 123177) is installed.

²Position Z is available when the ZFOV-2.6 lens (part no. 127285) is installed.

B.2 Manually Setting the Focus

The IVIS Imaging System automatically focuses the image based on subject height. If you do not want to use the automatic focus feature, you can manually set the focus.

1. In the control panel, choose **Manual Focus** in the Focus drop-down list.
The Manual Focus window appears.



2. To mark the center of the camera in the window, put a check mark next to Display CCD Center.
3. Select the size of the step increment that the stage moves: **Coarse**, **Normal**, or **Fine**.
4. Click **Up** or **Down** to move the stage and change the focus.
5. If necessary, select another F/stop setting from the drop-down list and adjust the light level using the arrows.
6. Click **Update** to apply the settings.
The resulting focal plane (cm above the stage) is automatically entered in the Subject height box.
7. Click **OK** when the image is focused.

Appendix C Preferences

General Preferences

[Options on page 170](#)

[Acquisition on page 170](#)

[Theme on page 172](#)

[Optical Properties on page 173](#)

You can manage user IDs and specify defaults for some parameters that are associated with the user ID selected at the start of a new session.

After you log on, select **Edit** → **Preferences** on the menu bar to view the user-modifiable preferences.



NOTE: Any changes made to the Preferences are implemented at the start of the next session. The Acquisition tab is only available in the Living Image software that controls the IVIS Imaging System.

C.1 General Preferences

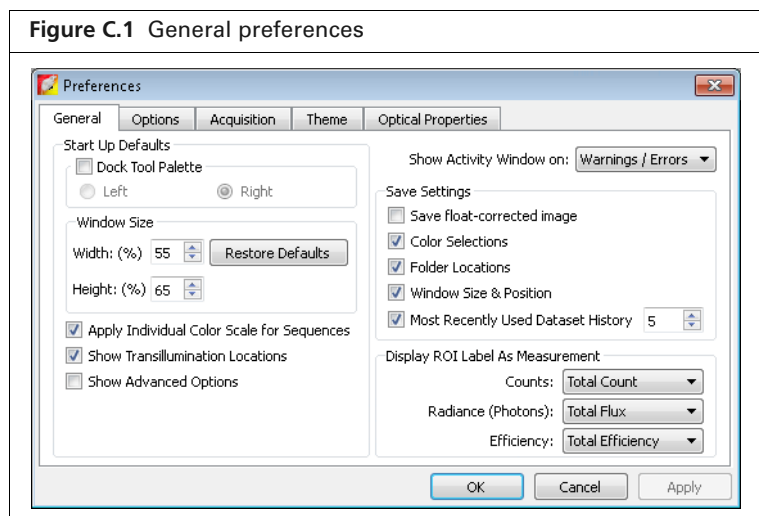



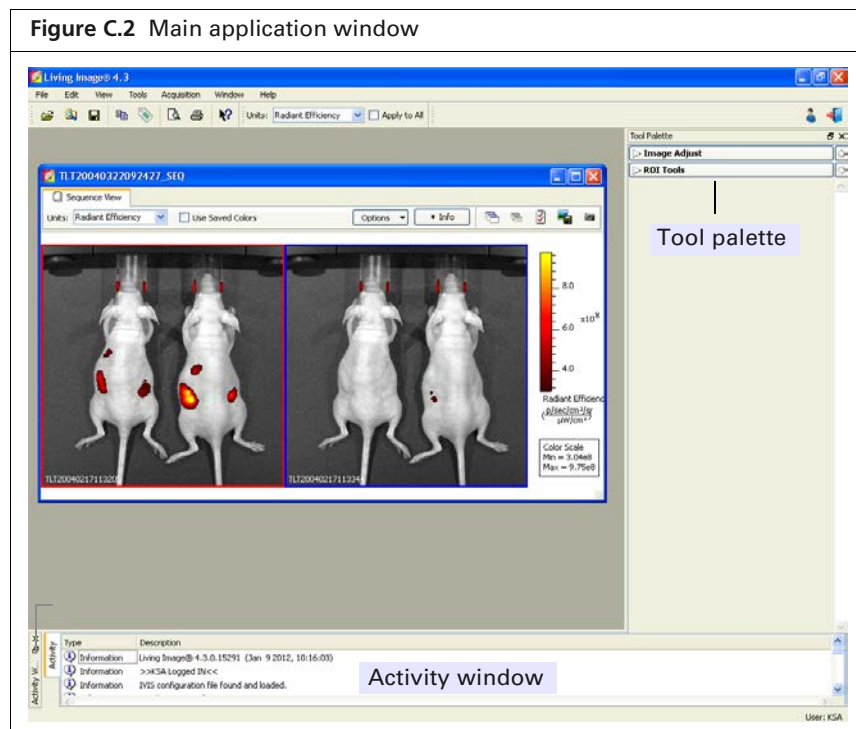
Table C.1 General preferences

Item	Description
Start Up Defaults	Dock Tool Palette - Choose this option to set the position of the Tool Palette in the application window. Choose left or right. Note: To undock the Tool Palette, click on the palette title bar and drag it a distance greater than its width.
Window Size	Specifies the dimensions of the main application window. Width, Height - Sets the dimensions of the image window. Restore Defaults - Click to apply the default settings.

Table C.1 General preferences (continued)

Item	Description
Apply Individual Color Scale for Sequences	Choose this option to apply a separate color scale to each thumbnail of a sequence. If this option is not chosen, all of the thumbnails are displayed using the same color scale.
Show Advanced Options	If this option is selected, advanced features are available in the menu bar and Tool Palette, including: <ul style="list-style-type: none"> ■ Additional ROI functionality for Auto ROI parameters. ■ Planar Spectral Imaging tools in the Tool Palette.
Show Activity Window on:	A drop-down list of options for when to display the activity log (Figure C.2).
Save Settings	Save float-corrected image - Saves an image after all corrections are applied (read bias subtraction, flat field correction, cosmic correction). Color Selections - Applies the color settings of the active image data to subsequently opened image data. Folder Locations - Sets the default folder path to the current folder path setting. Click the Export button  in the image window to view the current folder path setting (Figure C.2). Window Size & Position - Applies the active image window size and position settings to subsequently opened image data. Most Recently Used Dataset History - Defines the number of recently opened data sets to remember and display when you select File → Recent Files → Menu .
Display ROI Label As Measurement	Sets the type of measurement in counts, radiance (photons), or efficiency to show in the ROI label

Some of the general preferences specify how the main application window is organized. To undock the Tool Palette, click on the palette title bar and drag it a distance greater than its width. To dock the Tool Palette in the main window, drag the palette to the right or left side of the window and release.



C.2 Options

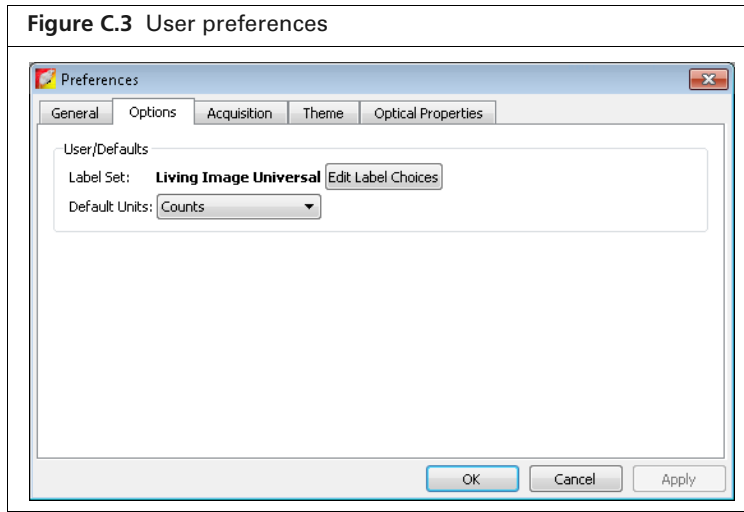


Table C.2 User preferences

Item	Description
Edit label Choices	Opens a dialog box that enables you to edit the Living Image Universal label set.
Default Units	Choose counts or radiance (photons) for image display.

C.3 Acquisition

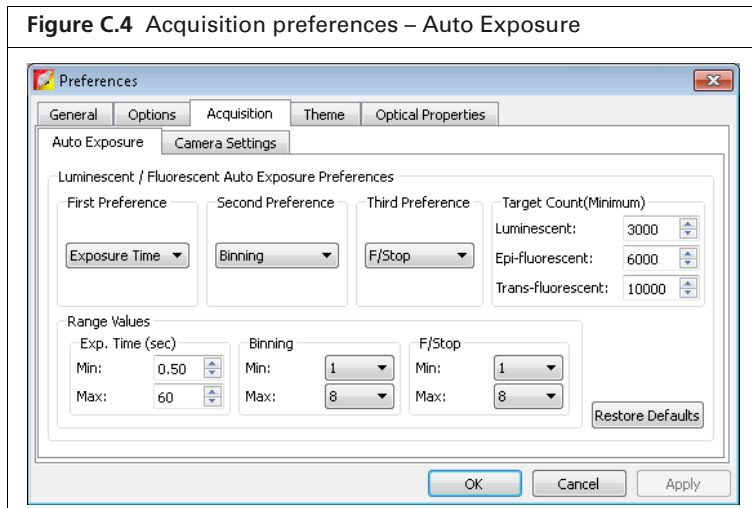


Table C.3 Auto exposure settings

Item	Description
Luminescent/Fluorescent Auto Exposure Preferences	

Table C.3 Auto exposure settings (continued)

Item	Description
First Preference Second Preference Third Preference	During auto exposure, the software acquires a luminescent or fluorescent image so that the brightest pixel is approximately equal to the user-specified Target Count (Minimum). If the target minimum count cannot be closely approximated by adjusting the first preference (for example, exposure time), the software uses the first and second or first, second and third preferences to attempt to reach the target max count during image acquisition.
Target Count (Minimum)	A user-specified intensity.
Range Values Exp Time (sec) Binning F/Stop	The minimum and maximum values define the range of values for exposure time, F/Stop, or binning that the software can use to attempt to reach the target max count during image acquisition.
Restore Defaults	Click to apply default settings.

Figure C.5 Acquisition preferences – Camera Settings

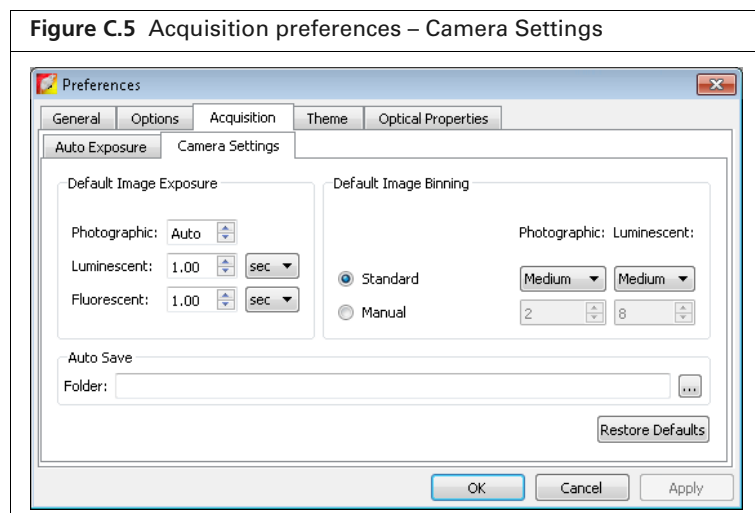



Table C.4 Camera settings

Item	Description
Default Image Exposure	Sets the default exposure settings that appear in the IVIS acquisition control panel.
Default Image Binning	Standard - Binning choices include Small, Medium and Large. These are predetermined, factory-loaded binning values that depend on the imaging system camera. Manual - Allows the user to choose a binning value (1, 2, 4, 8 or 16).
Auto Save	Specifies the folder where images are automatically saved. Click the  button to select a folder.
Restore Defaults	Click to apply the default settings.

C.4 Theme

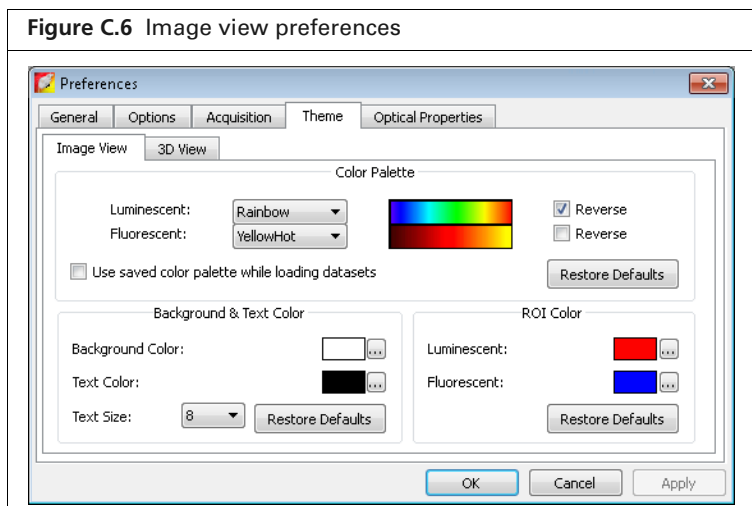

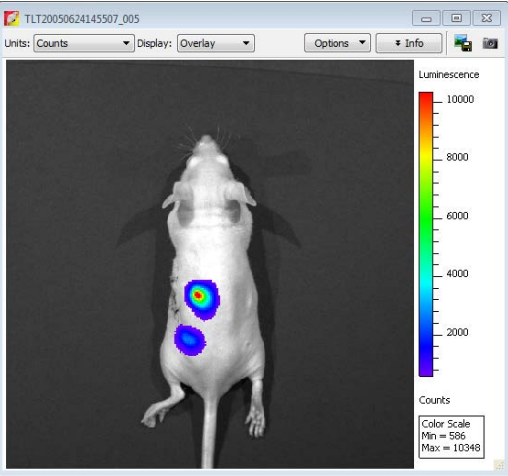



Table C.5 Image view preferences

Item	Description
Color Palette	Use these controls to select a color table for luminescent and fluorescent image data. Choose the Reverse option to reverse the min/max colors of the selected color table.
Use saved color palette while loading datasets	If this option is chosen, data are displayed using a user-specified color palette. For example, after you load data, specify a color table in the Image Adjust tools, and save the data. The user-specified color table is automatically applied whenever the data are loaded.
Background & Text Color	<p>Sets the color of the:</p> <ul style="list-style-type: none"> Background in the image window (shown below) Text for the color bar <p>To change a color, click the  button that opens the color palette.</p> 
ROI Color	<p>Sets the colors for the ROI outline. To change a color, click the  button that opens the color palette.</p> <p>Luminescent - Color of the ROI outline on a luminescent image. Fluorescent - Color of the ROI outline on a fluorescent image.</p>
Restore Defaults	Click to apply the default settings.

C.5 Optical Properties



NOTE: Some of the Optical Properties preferences (Figure C.7) are not applicable to data acquired on the IVIS® Lumina II.

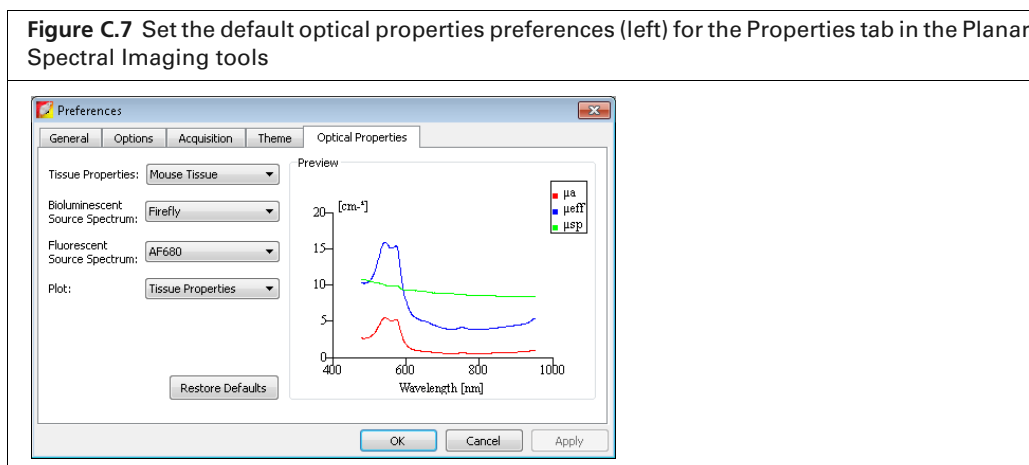


Table C.6 Tissue properties preferences

Item	Description
Tissue Properties	Choose a default tissue type that is most representative of the area of interest. This tissue type will be used if a Subject Type is not selected in the Imaging Wizard and saved during acquisition.
Restore Defaults	Click to restore the defaults in the Optical Properties tab.

Appendix D Menu Commands, Toolbars, and Shortcuts

Figure D.1 Living Image toolbar

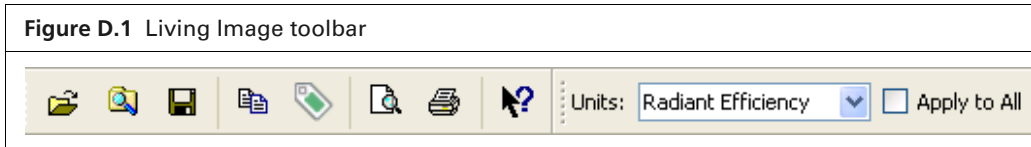


Table D.1 Menu bar commands and toolbar buttons








Menu Bar Command	Toolbar Button	Description
File → Open		Displays the Open box so that you can select and open an image data file. Double-click a SequenceInfo.txt file or ClickInfo.txt file to open the image data file (see page 52).
File → Browse		Displays the Browse For Folder box so that you can select and an image data folder. The selected folder is displayed in the Living Image Browser.
File → Save		Saves (overwrites) the AnalyzedClickInfo text file to update the analysis parameters, but the original image data files are not altered.
File → Save As		Displays the Browse For Folder box so that you can specify a folder in which to save the image data. The original data is not overwritten.
File → Import → Atlas		Opens a dialog box that enables you to import an organ atlas (.iv, .dxf, .stl).
File → Print		Displays the Print box.
File → Print Preview		Displays the Print Preview box that shows what will be printed.
File → Recent Files		Shows recently opened data sets. Note: The number of files displayed can be set in the Preferences box (select Edit → Preferences and click the General tab).
File → Logout		Opens the Select/Add User ID dialog box so that another user can logon or a new user ID can be added to the system.
File → Exit		Closes the Living Image software.
Edit → Copy		Copies the active image window to the system clipboard.
Edit → Image Labels		Opens the Edit Image Labels dialog box that enables you to edit the label set information for the active data (see Figure 4.10 on page 58).
Edit → Preferences		Opens the Preferences box (see page 168).
View → Tool Bar		Choose this option to display the toolbar.
View → Status Bar		Choose this option to display the status bar at the bottom of the main window.
View → Tool Palette		Choose this option to display the Tool Palette.
View → Activity Window		Displays the Activity window at the bottom of the main application window. The Activity window shows a log of the system activity.

Table D.1 Menu bar commands and toolbar buttons (continued)

Menu Bar Command	Toolbar Button	Description
View → Image Information		Displays the Image Information box that shows the label set and image acquisition information for the active data.
View → ROI Properties		Displays the ROI Properties dialog box (see page 100).
View → ROI Measurements		Displays the ROI Measurements table.
View → Image Layout Window		Opens the Image Layout window that enables you to paste an image of the active data in the window.
Tools → Well Plate Quantification for ...		Opens the Well Plate Quantification window.
Tools → Image Overlay for...		Opens the Image Overlay window for the active data.
Tools → Colorize		Opens the Colorized View tab for the active sequence.
Tools → Image Math for...		Opens the Image Math window for the active data.
Acquisition → Background → Measure Dark Charge		Opens a dialog box that enables you to acquire a dark charge measurement.
Acquisition → Background → Add or Replace Dark Charge		Opens a dialog box that enables you to select an instrument luminescent background. This background measurement is subtracted from luminescent images.
Acquisition → Background → Measure and Replace Dark Charge		Measures the dark charge under the same conditions as the currently selected image. When the measurement is complete, the newly acquired dark charge image will be included in the dataset of the current image, replacing any existing dark charge image that may be present in the dataset.
Acquisition → Background → View Available Dark Charge		Opens a dialog box that enables you to view the dark charge measurements for the system.
Acquisition → Background → Clear Available Dark Charge		Clears all dark charge images from the system.
Acquisition → Background → Auto Background Setup		Opens a dialog box that enables you to acquire background images, or schedule or disable automatic background acquisition.
Acquisition → Fluorescent Background → Measure Fluorescent Background		Starts a measurement of the instrument fluorescent background.
Acquisition → Fluorescent Background → Add or Replace Fluorescent Background		Opens a dialog box that enables you to select an instrument fluorescent background measurement for the active image data. If the "Fluorescent Background" Subtraction option is chosen in the Corrections/Filtering tool palette, the background measurement is subtracted from the image data.
Acquisition → Fluorescent Background → Measure and Replace Fluorescent Background		Measures fluorescent background under the same conditions as the currently selected image. When the measurement is complete, the newly acquired background image will be included in the data set of the current image, replacing any existing background image that may be present in the data set.
Acquisition → Fluorescent Background → View Available Fluorescent Background		Opens a dialog box that displays the fluorescent background measurements for the system. If a fluorescent background is selected, the "Fluorescent Background Subtraction" option appears in the Corrections/Filtering tool palette. Choose the "Fluorescent Background Subtraction" option to subtract the user-specified background measurement from the image data.
Acquisition → Fluorescent Background → Clear Available Fluorescent Background		Opens a dialog box that enables you to remove the fluorescent background measurements from the system.

Table D.1 Menu bar commands and toolbar buttons (continued)



Menu Bar Command	Toolbar Button	Description
Acquisition → Auto-Save		If Auto-Save is selected, all images are automatically saved to a user-selected folder.
Acquisition → Auto-Save To		Opens a dialog box that enables you to select a folder where images will be saved to automatically.
Window → Close		Closes the active image window.
Window → Close All		Closes all image windows.
Window → Cascade		Organizes the open image windows in a cascade arrangement (see page 53).
Window → Tile		Organizes the open image windows in a tiled arrangement (see page 53).
Window → 1. <Image or Sequence name> Window → 2. <Image or Sequence name> Window → etc.		A list of the open image windows. Click a window in the list to make it the active window (indicated by a check mark).
Window → Other Windows → <window name>		Lists other windows that are open. For example, If the Living Image Browser is open, use these commands to make the browser the active window and display it on top of all other open windows.
Help → User Guide		Displays the Living Image User Manual.
Help → Tech Notes		Displays a folder of technical notes. Note: For the most recent collection of technical notes, please see the IVIS University download page .
Help → License information		Displays the license information.
Help → Plug-in Information		Displays a list of tool plug-ins and Tool Palette plug-ins.
Help → IVIS Reagents		Opens the Caliper LS web page for In Vivo Imaging Reagents.
Help → About Living Image		Displays information about the Living Image software and Caliper technical support contact information.
		Click this button, then click an item in the user interface to display information about the item.

Table D.2 Keyboard shortcuts

Keys	Shortcut Description
Ctrl + B	Opens the Living Image Browser.
Ctrl + C	Copies the active image to the system clipboard.
Ctrl + D	Arranges open windows in a cascade.
Ctrl + O	Displays a dialog box that enables you to open data.

Table D.2 Keyboard shortcuts

Keys	Shortcut Description
Ctrl + P	Open the Print dialog box.
Ctrl + S	Saves the active file or window.
Ctrl + T	Tiles the open windows.
Ctrl + W	Closes the active window.
Shift + F1	Changes the mouse pointer to the "What's This" tool  . Click this button, then click an item in the user-interface to display information about the item.



NOTE: Macintosh users use the Cmd key (apple key) instead of the Ctrl key.

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