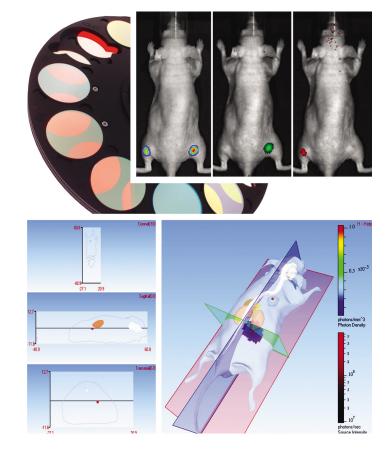
SOFTWARE MANUAL FOR USE WITH IVIS® SPECTRUM

Living Image[®] 4.5.4 Software March 2017







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1 Welcome

About This Manual What's New in Living Image 4.5.4 Software on page 2 Living Image Help on page 2 Contact Information on page 4

1.1 About This Manual

NOTE: This *Living Image 4.5.4 Software Manual* is only for use with the IVIS Spectrum Imaging System. If analyzing data acquired on a different type of IVIS Imaging System, say for example the IVIS SpectrumCT, please see the Living Image Software Manual specific for the IVIS SpectrumCT (see Table 1.1).

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

Please see the *IVIS Spectrum Hardware Manual* (PN 121450) for information on the IVIS Spectrum instrument.

Table 1.1 Living Image Software Manuals

Living Image Software Manual for:
IVIS Lumina Series III (can also be used with the IVIS Lumina LT)
IVIS Lumina XRMS Series III (can also be used with the IVIS Lumina XR)
IVIS Lumina K Series III (can also be used with the IVIS Lumina Kinetic)
IVIS Spectrum
IVIS SpectrumBL (can also be used with the IVIS 200)
IVIS SpectrumCT

1.2 What's New in Living Image 4.5.4 Software

Software improvements specific to the IVIS Spectrum Imaging System are listed below.

Item	Description	See Page
Additional operating system support	 Living Image now supports: Windows 10, 64-bit analysis. Note: Acquisition is only supported on Window 7 	
	 macOS 10.12 (Sierra). Note: OS X 10.11 (El Capitan) and 10.10 (Yosemite) are also supported. Older versions of OS X are no longer supported. 	
New image export options in sequence view	 In sequence view: Export all of the images in a sequence to one graphic file. 	89
	 Export each image of a user-selected subject to a separate graphic file. 	89

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 Living Image Software User's Manual	Press F1 or select Help \rightarrow User Guide on the menu bar and select the manual specific for your imaging system.
Living Image technical notes (see	Select Help \rightarrow Tech Notes on the menu bar.
Table 1.2 on page 2)	Note: Please see the In Vivo University download page for the most recent collection of technical notes.

Table 1.2 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 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 1.2 Technical Notes

Technical Notes
Adaptive Fluorescence Background Subtraction
Auto-Exposure
Subtracting Background ROI from a Sequence
Determine Saturation

 Table 1.2
 Technical Notes (continued)

Table 1.2 Technical Notes (continued)
Bioluminescence Tomography (DLIT) Setup and Sequence Acquisition
■ Topography
 Source Reconstruction and Analysis
Drawing ROIs
 Fluorescence Tomography (FLIT) Setup and Sequence Acquisition Topography Source Reconstruction and Analysis
High Resolution Images
Working With Image Math
Working With Image Overlay – 2D
Working With Image Overlay – 3D
Working With Imaging Wizard
Loading Groups of Images
Sending Large Files for Analysis
Spectral Unmixing
Subject ROIs
Transillumination Transillumination Fluorescence Transillumination – Raster Scan Transillumination – Normalized
Well Plate Quantification
Concept Technical Notes
Luminescent Background Sources and Corrections
Image Display and Measurement
Detection Sensitivity
Fluorescent Imaging
DLIT and FLIT Reconstruction of Sources
Planar Spectral Imaging
IVIS® Syringe Injection System

1.4 Contact Information

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Sales: CustomerCareUS@perkinelmer.com

2 Imaging Overview

Example Imaging Workflow

Overview of Image Acquisition on page 8

Overview of Living Image Tools and Functions on page 10

2.1 Example Imaging Workflow

Table 2.1 Example Imaging Workflow

 Plan the experiment. Best results are obtained using nude mice. Subjects with black or dark-colored fur and skin are not optimal and may give poor results. Determine the number of animals required: Always include control animals and replicates (for example, No disease + Probe, Disease + No Probe). Experimental animals (Disease + Probe and replicates). It may be necessary to change to low fluorescence mouse chow two weeks before the imaging study. Regular mouse chow contains chlorophyll which autofluoresces around 700 nm and can interfere with fluorophore signal. Select the type of imaging and probe: Luminescent signal is usually lower than fluorescent signal, but luminescent imaging has higher sensitivity due to low noise (instrument and animal autoluminescence). Optimal luminescence imaging is from 600 - 800 nm. Interference of the state of

Table 2.1 Example Imaging Workflow (continued)

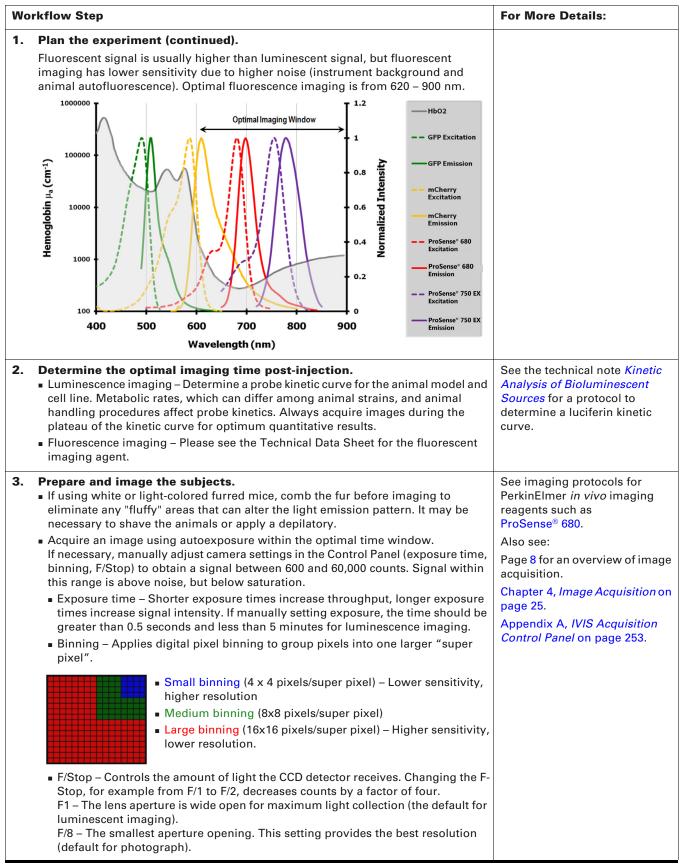


Table 2.1 Example Imaging Workflow (continued)

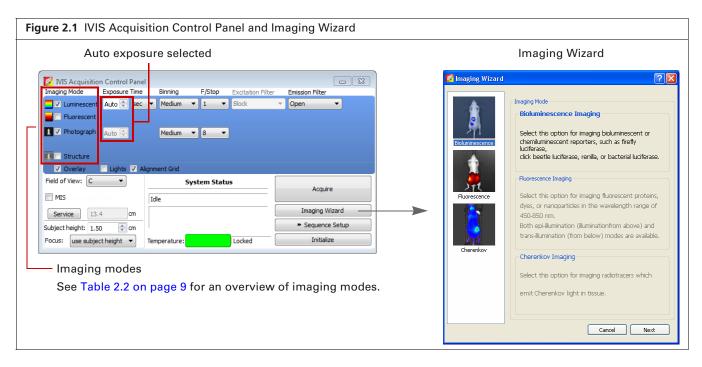
Wo	rkflow Step	For More Details:
4.	 Select images for viewing. Load the images as a group (select the images in the Living Image Browser and click Load as Group). 	See page 57 for more about the Living Image Browser.
	 Photo Adjustment Brightness: IS If necessary, adjust the photo brightness, contrast, or opacity using the Image Adjust tools. Adjust tools. Apply the same color scale to all images: Uncheck the "Individual" option. Adjust the color scale Min and Max. The changes are simultaneously applied to all images. 	See page 66 for information on the Image Adjust tools.
5.	Measure signals and analyze the data.	
	 Choose the appropriate units: "Radiance (Photons)" for luminescence "Radiant Efficiency" or "NTF Efficiency" for fluorescence. Radiance, Radiance Efficiency, and NTF Efficiency are calibrated measurements (not dependent on camera settings) that enable quantitative comparison of signals across images. "Counts" is an uncalibrated measurement (dependent 	See Concept Tech Note 2 – Image Display and Measurement for more information on measurement units (select Help → Tech Notes) .
	Place ROIs on the images in sequence view.	
	 To make changes to related ROIs in all images while in sequence view, press and hold the Ctrl key while adjusting ROI size or position in an image. This ensures that the size and position of a particular ROI are the same in all of the images. If ROIs in an individual image need adjustment, for example to account for different animal positions in the images, adjust the ROI without using the Ctrl key. 	See page 102 for instructions on measuring signal in optical images.
	Tool Palette III IIII IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	See Table 6.8 on page 118 for information on ROI measurements.
	NO1 Netwarements Image Number Foil Image Lypr Tu720050624445507.002A R011 Overlay 100 Verlay 3090+01 100 Verlay 3080+01 100 Verlay 000 Verlay 100 Verlay	

2.2 Overview of Image Acquisition

Control Panel

The control panel provides the image acquisition functions (Figure 2.1). See Appendix A on page 253 for details on the imaging parameters in the control panel.

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



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 2.1), 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 276 for more details.

Imaging Wizard

The Imaging Wizard provides a convenient option for image or sequence setup (see Figure 4.27 on page 44). The wizard guides you through a series of steps, prompting you for the information that the software needs to set up acquisition in the control panel. Table 4.4 on page 45 shows the types of images or sequences that the Imaging Wizard can set up.

Imaging Modes on IVIS Spectrum

Optical imaging detects photons in the visible range of the electromagnetic spectrum. Table 2.2 briefly explains the types of optical images that can be acquired on the IVIS Spectrum.

Table 2.2	Imaging	Modes – IVIS	SpectrumBL
	maying	100063 - 1010	opectiumbe

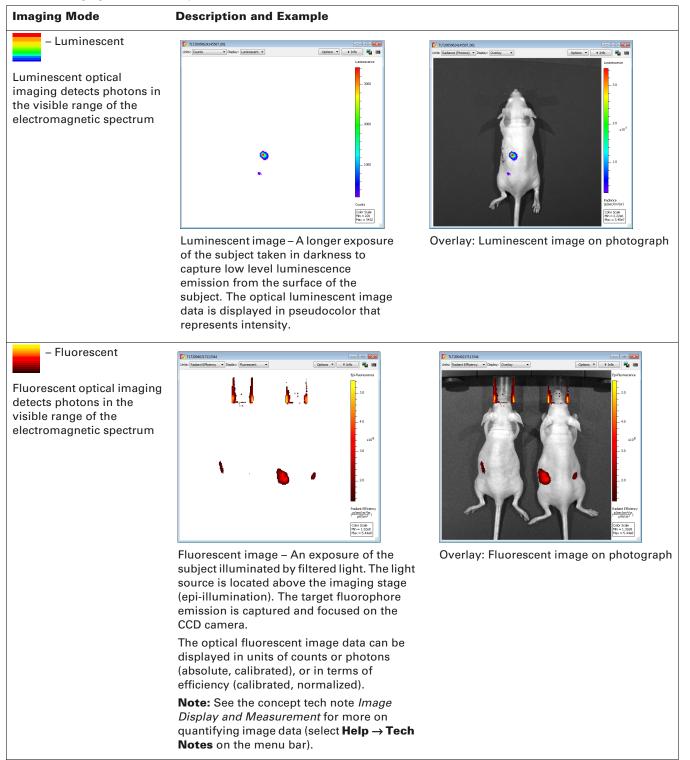
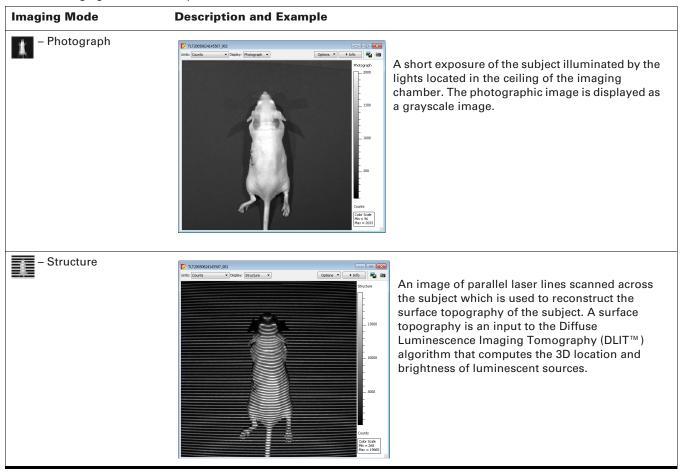


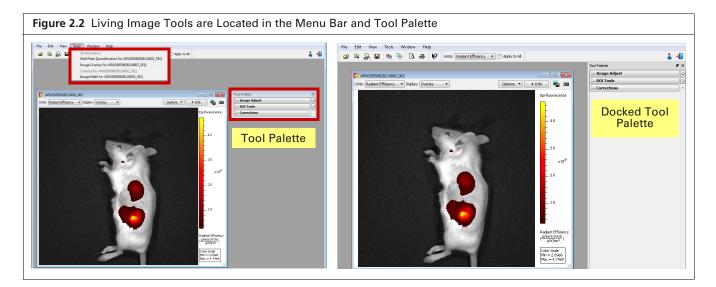
Table 2.2 Imaging Modes – IVIS SpectrumBL (continued)



2.3 Overview of Living Image Tools and Functions

Living Image tools are organized in the Tool Palette or under "Tools" in the menu bar (Figure 2.2). Some tools are for use with a single image, others require an image sequence.

The Tool Palette can be docked in the main window (click the Tool Palette title bar, then drag and drop it at either side of the main window (Figure 2.2). Docking can also be set in the general preferences (see Table E.1 on page 273).



W

Table 2.3 provides an overview of the tools available for data acquired on the IVIS Spectrum. If analyzing data acquired on a different type of IVIS instrument, say for example the IVIS Spectrum CT, please see the Living Image Software Manual specific for that imaging system.

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 Spectrum

Living Image Tools and Functi	ons	See Page
✓ Image Adjust ♥ ● ●	 Image Adjust Tools for image display (zoom, crop, or pan). Adjust photo display – Tune the photograph brightness, contrast, or opacity. Manage the color table for image display. Apply smoothing or binning to an image. View optical image data (counts or radiance) at an X,Y location. Measure distance in an image. View a line plot of pixel intensities. 	66
✓ ROI Tools ↓ □, □, □, □, ↓ ▲ Apply to Sequence Type: [Measurement ROI Save ROIs Name: ROI_1_KSA Delete Load Auto ROI Parameters Threshold %: 24	ROI Tools for 2D Image Data Specify a region of interest (ROI) in an optical image and measure the signal intensity within the ROI.	100
✓ Corrections ✓ Read Bias Subtraction ✓ Flat Field Correction ✓ Cosmic Correction	Corrections (2D Image Data) Corrections for raw data.	258
ROI Tools Image: Constraint of the second	 3D ROI Tools (3D Reconstructions or 3D Volumetric Data) Specify a 3-dimensional region of interest (3D ROI). Measure within the 3D ROI: Signal intensity of source voxels. Tissue density in a CT scan or 3D DICOM data. 	174

Living Image Tools and Functions See Page 206 Spectral Unmixing ▽ Spectral Unmixing and DyCE Analyze Results Use spectral unmixing to: Select Images Type: Emission Extract the signal of one or more fluorophores from the 605 tissue autofluorescence. 660 🔽 Analyze luminescent or fluorescent images when more 680 🔽 than one reporter is used in the same animal model. 700 🔽 720 🔽 740 🔽 760 🔽 3 **DyCE (Dynamic Contrast Enhancement)** 233 ▽ Spectral Unmixing and DyCE Analyze Results Use DyCE to: Type: Time(hh:mm:ss) 🔻 Select Images: Determine real-time pharmacokinetic (spatio-temporal biodistribution) of a probe or dye signal. Int1 00:01:34 Extract "temporal spectra" (signal intensity as a 00:01:56 🗸 function of time) from particular anatomical regions. 00:02:19 🔽 Note: DyCE acquisition and analysis tools require a 00:02:41 🔽 00:03:43 🔽 separate license. 00:04:45 🔽 00:05:47 🗸 00:06:49 🔽 00:11:51 🔽 00:16:54 🔽 Methods: Automatic Start Unmixing **3D Multi-Modality Tools** 181 ▽ 3D Multi-Modality Tools Volume Process Slice Results Note: Spectrum Imaging System does not acquire volumetric data. However Living Image 3D Multi-Modality 🏛 回 🎼 🖪 ち 🍤 🖄 tools can analyze volumetric data (CT, PET, MRI) acquired V Display Volume on a different imaging system. The 3D Multi-Modality tools Level Of Detail require a separate license. • Performance **Volume Tools** Color - Opacity Map Set color and opacity values for different intensity ranges Grays of a CT volume so that the color-opacity map shows the 1.0 Opacity 📝 🗙 🚘 🖬 👞 📃 Reverse volume regions of interest (opaque in the map) and hides unimportant regions. Co-register 3D reconstructions of luminescent or fluorescent sources (biological information) with a CT volume to provide anatomical context for interpreting biological (functional) information. **Process Tools** – Apply smoothing to a CT volume. **Slice Tools** Options for rendering slices. 10 M View slice. Slice viewing options. 0.00 Intensity 6.55e4 Logarithmic Histogram Maximum Intensity Projection (MIP) Gradient Illumination

Table 2.3 Living Image Tools Available for Data Acquired on the IVIS Spectrum (continued)

 Table 2.3
 Living Image Tools Available for Data Acquired on the IVIS Spectrum (continued)

	tions	See Page
7 3D Optical Tools	3D Optical Tools	
Surface Source Registration	Surface tools – Adjust the appearance of the reconstructed animal surface and the photon density maps.	144
Opacity: 100 😨	Source tools – Adjust the appearance of reconstructed sources, make source measurements, export voxel measurements.	147
Wavelengths * Wavelengths * intensity:* 1.00e2 Color Table Rainbow * V Reverse * Log Scale	Registration tools – Display organs on the reconstructed surface, adjust the location or scale of organs on the surface, import an organ atlas.	157
7 DLIT 3D Reconstruction	DLIT 3D Reconstruction	124
Analyze Properties Results quence: AA20090623124007_SEQ sue: Mouse Tissue Source: Firefly lect Filters: iiter Threshold % ♥ \$560 0.5 ♥ \$580 0.5 ♥ \$600 0.5 ♥ \$600 0.5	Diffuse light tomography (DLIT) analysis provides a complete 3D reconstruction of the luminescent source distribution within the subject. The 3D reconstruction is presented as volume elements called <i>voxels</i> . If a luminescent calibration database is available, the number of cells per source can be determined in addition to	
♥ 640 0.5 ♥ ♥ 660 0.5 NLS ▼ Reconstruct ▼	source intensity (photons/ sec).	
♥ ● 660 0.5	FLIT 3D Reconstruction	130
 ♥ ● 660 0.5 NLS ▼ Reconstruct ▼ 		130

Table 2.3 Living Image Tools Available	e for Data Acquired on the IVIS Spectrum (continued)	
Living Image Tools and Function	ons	See Page
Living Image Tools and Function	3D Animation Tools Select Tools → 3D Animation on the menu bar. Creates an animation from a sequence of 3D views (keyframes). For example, an animation can depict a rotating 3D scene. The animation (series of key frames) can be recorded to a movie file.	See Page 162
Play Record Frames Per Second: 10 Total Duration (secs): 5 Load Save Well Plate Quantification Window	Well Plate Quantification	260
For Sequence: EL20090414101005_SEQ Click EL20090414101005_DO ## Wel Plate Type Plucrophore Type Measurement © Dye molecules Sample Wells : 3D : 3A Ø Background Wells : Sci 5A Ø Apply to Sequence Vell Plate Well Plate Quantification Plots Results	 Select Tools → Well Plate Quantification for <sequence name=""> on the menu bar.</sequence> Generate a database of luminescence or fluorescence signal intensities by analyzing images of known serial dilutions of luminescent or fluorescent cells or dye molecules. Use the quantification database to extrapolate the 	
✓ Surface Topography Optical Surface Reconstruction Orientation: Dorsal Subject: Nude Mouse Surface Smoothing Level: Level: Medium 0 Save Results Name: SURFACE_1 Verter Load Overwrite	 Surface Topography Generate 3D reconstruction of the animal surface (topography) derived from a structured light image. A surface is a required input for: DLIT (diffuse light tomography) analysis which generates a 3D reconstruction of luminescent sources. FLIT (fluorescence imaging tomography) analysis which generate a 3D reconstruction of fluorescent sources. 	267

 Table 2.3
 Living Image Tools Available for Data Acquired on the IVIS Spectrum (continued)

 Table 2.3
 Living Image Tools Available for Data Acquired on the IVIS Spectrum (continued)

iving Image Tools and Functions		See Page
2 Image Overlay Window	Image Overlay Window	80
expanner. APV2005883:512400_5EQ Units: Badant Efficancy	Select Tools → Image Overlay for <sequence< b=""> name> on the menu bar.</sequence<>	
	View multiple fluorescent or luminescent signals in one 2-dimensional image in the Image Overlay window.	
ampr - Quint - 2.6/e8 © Color Table - 2.6/e8 © Color Table - 2.6/e8 © Color Table - 2.6/e8 Ppc - Palette Label:		
Opadity: 70 🐨 🕅 Reverse 🖉 Logarithmic		

Colorize View

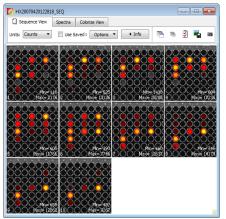
83

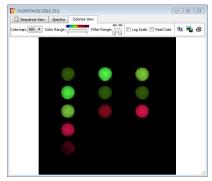
40

(Table 4.3)

Select Tools \rightarrow Colorize for <sequence name> on the menu bar.

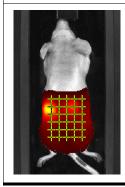
The colorize tool renders each luminescence or fluorescence image of a sequence in color, and combines them into a single image. This enables you to see both intensity and spectral information in a single view. The tool provides a useful way to visualize multiple probes or scale probe signals that are not in the visible range.





Colorize view of the combined images

Images of Quantum dot nanocrystals (700 or 800 nm) were acquired using different combinations of excitation and emission filters.



Transillumination Overview

Select Tools \rightarrow Transillumination Overview for <sequence name> on the menu bar.

The transillumination overview tool combines the images of a FLIT sequence (a fluorescence sequence acquired in transillumination mode) into a single image. All of the individual fluorescent signals are stacked over one photograph and the intensity is summed. One overview is created per filter pair. If two filter pairs were used during acquisition, then two overview images will be created.

iving Image Too	ols and Funct	ions	See Page
2 Image Math Window		Image Math Window	75
Sequence: K5 A TI/20060510114512_008A TI/20060510114512_010A	A20110305104918_5EQ	Select Tools \rightarrow Image Math for <sequence name=""> on the menu bar.</sequence>	
B 120060510114512_008A	•	Mathematically combine (add, multiply, subtract, or divide) two user-selected images.	
11720060510114512_010A	Counts	For example, subtract a blue-shifted background filter image from the primary excitation filter image to remove	
Color Scale Limits for A and B Full O Auto Result	•	tissue autofluorescence signal.	
Color Scale Limits Full Auto Min = 0 Result : A - B * k	Counts		
k : 0.787938 Compute V from ROI V with Photo from:			
Display Result For Measuring			

Table 2.3 Living Image Tools Available for Data Acquired on the IVIS Spectrum (continued)

3 Getting Started

Starting Living Image Software Initializing the Imaging System and Checking Temperature on page 19 Managing User Accounts on page 21 Tracking System and User Activity on page 24

3.1 Starting Living Image Software

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

See the Installation Guide on the Living Image CD ROM for software installation instructions. Table 3.1 shows the default software installation locations.



NOTE: If analyzing large datasets such as high resolution FLIT datasets or longitudinal studies with many subjects, a 64-bit analysis workstation with 16 GB memory capacity is recommended.-

Table 3.1	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 Spectrum 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:

PC Users: Double-click the Living Image software icon 2 on the desktop. Alternatively, click the Windows Start button 3 and select All Programs → Caliper Life Sciences → Living Image.

Macintosh Users: Double-click the Living Image icon \mathbb{Z} on the desktop or run the software from the application folder.

The main window appears (Figure 3.1).

Figure 3.1 Living Image Main Window at Startup	
File Edit View Tools Window Help	2 4
Select a user	
Select/Add User ID Image: Select Add User ID Add New User User ID: User ID: Add a new user	
Password: Confirm Password:	

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 Spectrum (Figure 3.2). For more details on the control panel, see Appendix A on page 253.



NOTE: Living Image software has optional password protection for user accounts. See page 22 for more details.

 $\overline{\mathbb{W}}$

Figure 3.2 Living Image Main Wir	ndow and IVIS Acquisition Control Panel	
Menu bar (for more details, see Appendix F on page 281). Toolbar	File Edit View Tools Acquisition Window Help Imaging Mode Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter Imaging Mode Exposure Time Medium B<	
Activity window —	Focus: use aubject height Temperature: Unlocked Initialize Information Using Image@ 4.3.0.15291 (Jan 9 2012, 10:16:03) Using Image@ 4.3.0.15291 (Jan 9 2012, 10:16:03) Information Using Image@ 4.3.0.15291 (Jan 9 2012, 10:16:03)	
		User: KSA

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

3.2 Initializing the Imaging System and Checking Temperature

The IVIS Spectrum 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 3.3).

NOTE: The control panel is only available on the PC workstation that controls the IVIS Imaging System.

Initialization moves every motor-driven component in the system (for example, imaging 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.

See the IVIS® Spectrum Hardware Manual (PN 133577) for further details on instrument operation.

Initializing the IVIS Spectrum

- 1. Start the Living Image software (double-click the 🌠 icon on the desktop).
- **2.** Click **Initialize** in the control panel that appears (Figure 3.3). After several seconds you will hear the instrument motors move.

Figure 3.3 IVIS® A	cquisition Control Pa	anel During Initializati	on
🜠 IVIS Acquisition Control I	Panel		
Imaging Mode Exposure Time	Binning F/Stop	Excitation Filter Emission Filter	
Luminescent 1.00 🗢 sec	8 🗸 1	Block 💟 Open 💟	
🚺 🗹 Photograph 🛛 Auto 🤤	4 💙 8 💙		– Red color appears in the control panel during
Structure			initialization. The color
🗸 Overlay 📃 Lights 🗸	Alignment Grid		turns blue when initialization is finished.
Field of View: C	System Status	Acquire	mitialization is mistied.
MIS	Initializing		
Service 12.8 cm	Stage temperature controller	Imaging Wizard	
Subject height: 1.50 🗘 cm		✤ Sequence Setup	
Focus: use subject height 🗸	Temperature: Unlocks	ed Initialize	
X	· · · · · · · · · · · · · · · · · · ·		

CCD Temperature

The IVIS Acquisition Control Panel indicates the temperature status of the charge coupled device (CCD) camera (see Figure 3.4 for a description of the temperature status colors). Immediately after initialization is completed, the temperature box is usually red and will turn green after several minutes. If this is not the case, contact PerkinElmer Technical Support (see page 4).

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).

Figure 3.4 Control Panel – Instrument Temperature	e Status		
VIS Acquisition Control Panel Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter V Luminescent Auto Sec Medium I Block Open Fluorescent Structure V Overlay Lights V Alignment Grid	Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter Imaging Mode Exposure Time Medium 1 Block Open Imaging Imaging Mode Auto Imaging Medium 8 Imaging Imaging Imaging Imaging Mode Exposure Time Imaging Medium 8 Imaging Imaging Imaging Mode Exposure Time Imaging Medium 8 Imaging Imaging Imaging Mode Exposure Time Imaging Medium 8 Imaging Imaging Imaging Mode Exposure Time Imaging Medium 8 Imaging Imaging Imaging Mode Exposure Time Imaging Medium 8 Imaging Imaging Imaging		
System Status Acquire Field of View: M15 Idle Service 13.4 Subject height: 1.50 Focus: use subject height Temperature: Locked Initialize	e Field of View: C V System Status Acquire MIS Demand Measured Camera Temp: 90 C V GUD Imaging Wizard Subject height: 1.50 C m		
Temperature box color: Click the temperature box to display the box to display the demand and measured temperatures White - System is not initialized. Bead - System is initialized, but CCD camera temperature is out of range and not ready for imaging. Green - System is initialized and CCD camera is at or within acceptable range of the demand temperature and locked. The system is ready for imaging.			

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 $^{\circ}$ C and is self-monitoring after the system is initialized. The imaging stage may be set to a temperature from 20 - 40 $^{\circ}$ C.

igure 3.5 Set Imaging Stage Temperature in the Control Panel			
📝 IVIS Acquisition Control Pan	el		
Imaging Mode Exposure Time	Binning F/Stop	Excitation Filter	Emission Filter
📃 🗸 Luminescent 🛛 1.00 🚔 🔤	Medium 🔻 1	 Block 	✓ Open ▼
👔 🗹 Photograph 🛛 Auto 🚔	Medium 🔻 8	-	
		_	
Structure			
🗸 Overlay 📃 Lights 🗸 /	lignment Grid		
Field of View: C 🔹	System Sta	atus	
MIS	Demand	Measured	Acquire
MIS Service 13.4 cm	Demand Camera Temp: -90 😂	-900	Acquire Imaging Wizard
		-900	

3.3 Managing User Accounts

Adding Users

New users can be created in the:

- Main window at startup (see page 18).
- User Settings dialog box (Figure 3.6).
- **1.** Select **Edit** \rightarrow **User settings** on the menu bar.
- 2. Click the Add User tab in the dialog box that appears.

Figure 3.6 User Settings – Add User	
Viser Settings Add User Add New User User ID ["]: Password: Confirm Password: Cear	Deletes all entries
	Close

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

Changing or Adding Passwords

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

Figure 3.7 User Settings – Change Password
User Settings
User ID: HLA New Password: Confirm Password:
Clear Submit
Deletes all entries

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 23 for more details on locking user accounts.
- **1.** Select **Edit** \rightarrow **User settings** on the menu bar.
- 2. Click the Delete User tab in the dialog box that appears.

Figure 3.8 User Settings – Delete User	
In this example, user accounts are locked.	
User Settings	8 23
🐍 Add User 🔮 Change Password 🐍 Delete User 🔒 Security	
Delete User	
User ID: HLA	
Enter master password	
Enter Master Password Clear	Delete
	Close
Deletes all entries	;

- **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** \rightarrow **User settings** on the menu bar.
- 2. Click the Security tab in the dialog box that appears.
- **3.** Click Lock User Accounts.

Figure 3.9 User Settings – Security	
User Settings	User Settings P Security Add User Change Password Delete User Security Currently User Accounts are Unlocked Control Lock User Accounts Delete User Delete User Enter Master Password Enter a Master Password to Lock the User Account settings. Master Password: Delete User Delete User Confirm Password: Confirm Password: Delete User Delete User Delete User Clear Lock Close Delete User Delete User

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.

Figure 3.10 User Settings – Security	
User Settings Image: Security Image: Add User Image: Change Password Image: Security Currently User Accounts are Locked Image: Unlock User Accounts Change Master Password Image: Unlock User Accounts Old Password: Image: Unlock User Accounts Old Password: Image: Unlock User Accounts Confirm Password: Image: Unlock User Accounts Clear Submit Close Image: Unlock User Accounts	User Settings 2 Add User Change Password Currently User Accounts are Locked Image: Unlock User Accounts Enter Master Password Enter the Master Password to Unlock the User Account settings. Master Password: Clear Unlock Close

3.4 Tracking System and User Activity

Activity Window

The Activity window shows the imaging system activities (Figure 3.11). The software creates and saves a log of the system activities related to data acquisition. This information may be useful for PerkinElmer 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>_2015.csv) located at C:\Program Files\Caliper Life Sciences\Living Image\Usage).

File Edit View Tools Acquisition	Window Help		-
😅 😫 🔒 🖩 🐘 🛸 🖾 é			2
	📝 IVIS Acquisition Control Pane	el	
	Imaging Mode Exposure Time	Binning F/Stop	Emission Filter
	🔤 🔽 Luminescent 🛛 Auto 🚔 🔤	▼ Medium ▼ 1 ▼	Open 👻
	🚺 🗹 Photograph 🛛 Auto 🍚	Medium 🔻 8 🔻	
	Structure		
	✓ Overlay Lights ✓ A Field of View: C ✓	System Status	
		-	Acquire
	MIS	Idle	
	Service 13.4 cm		Imaging Wizard
	Subject height: 1.50 🚔 cm		* Sequence Setup
	Focus: use subject height 🔻	Temperature: Locked	d Initialize
. Two			
Type Description	0.15210 (64-bit) RC (Dec 13 2011, 13	-12-10)	
A Information Living Image@ 4.3.1 Information >>KS Logged IN << A Information MS configuration f Information Reading user prefer			ity window
Information MIS configuration f	ile found and loaded.	ACTIV	
	ile found and loaded.		

4 Image Acquisition

Luminescent Imaging

Fluorescent Imaging With Epi-Illumination on page 31 Fluorescent Imaging With Transillumination on page 38 Cherenkov Imaging on page 43 Acquire a Sequence Using the Imaging Wizard on page 44 Acquire Multiple Sequences in Batch Mode on page 50 Manually Set Up a Sequence on page 52 Manually Save Image Data on page 56 Exporting Images on page 56

4.1 Luminescent Imaging

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

- Quick guide See below.
- Detailed instructions See page 26.

See page 44 for information on acquiring a luminescent sequence using the Imaging Wizard.

Quick Guide

1. Start Living Image software and initialize the IVIS Spectrum (see page 19 for details).



NOTE: See the *IVIS Spectrum Hardware Manual* (PN 121450) for more information on the imaging system.

- 2. Place the anesthetized subjects in the imaging chamber and close the door.
- **3.** In the control panel (Figure 4.1):
 - a. Put a check mark next to Luminescent and select Auto exposure.
 - **b.** Choose **Photograph** (optional, selecting **Photograph** automatically selects **Overlay**).
 - c. Choose Structure (subject height will be automatically determined).
 - d. Select a field of view (FOV) (see Table 4.1 on page 27).
 - e. Click Acquire.

💈 IVIS Acquisition Control	Panel	Ū.	
Imaging Mode Exposure Time	Binning F/Stop	Excitation Filter Emission Filter	
🔤 🗹 Luminescent 🛛 Auto 🤤 se	c 💟 Medium 💟 1 🔍	Block 🗸 Open 🖌	
Fluorescent			
🗴 🗹 Photograph 🛛 0.20 💲	Medium 💙 8 💙		
Structure			
🗹 Overlay 📃 Lights 🚺	Alianment Grid		
Field of View: C			
Field of View: C	System Status	Acquire	
Field of View: C	System Status	Acquire Imaging Wizard	
Field of View: C	System Status		

- **4.** Select a location for the image data when prompted (optional, but strongly recommended). Image data acquired during the session will be automatically saved to this location.
- **5.** Enter experiment and subject information in the dialog box that appears (optional, but strongly recommended).

An image window and Tool Palette appear when acquisition is finished (Figure 4.2).

Figure 4.2 Image Window and Tool Palette	
Image Window	Tool Palette
Introductive Units ContaDeploy: OverlayDeploy: OverlayIntroductive Units ContaIntroductive Units Cont	Tool Palette

Acquire a Luminescent Image

This section provides detailed instructions for luminescent imaging.

- **NOTE:** The IVIS Spectrum should be initialized and the temperature locked before setting the imaging parameters in the control panel. See page 19 for more details.
- 1. Put a check mark next to Luminescent and select Auto exposure (click the next) 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** \rightarrow **Tech Notes** on the menu bar).

Alternatively, manually set the exposure, binning, and F/Stop. See Appendix A on page 253 for details on these parameters.

igure 4.3 Control	Panel			
🜠 IVIS Acquisition Contr	ol Panel			
Imaging Mode Exposure T	me Binning	F/Stop	Excitation Filt	er Emission Filter
🔁 🗹 Luminescent 🛛 Auto 🤤	sec 🔽 Medium	✓ 1 ✓	Block	🖂 Open 🛛 👻
Eluorescent				
🚺 🗹 Photograph 🛛 0.20 💲	Medium	¥8 ¥		
Structure				
Voverlay Light:	; 🗹 Alignment Gri	d		
Field of View: C		System Status		Acquire
MIS	Idle			nequiro
Service 12.8	m			Imaging Wizard
Subject height: 1,50 🗘 c	m		•	• Sequence Setup
Focus: use subject height 💊	Temperature:	Loc	ked 📃	Initialize

2. Put a check mark next to Photograph (optional).

Selecting **Photograph** automatically selects Overlay, so that an overlay image (registered photograph and luminescent image) is displayed after acquisition.

3. Select a field of view (FOV, size of the stage area to be imaged, see Table 4.1).



TIP: See the technical note *Detection Sensitivity* for more information about the field of view (select **Help** \rightarrow **Tech Notes** on the menu bar).

 Table 4.1
 Field of View (FOV) Settings – IVIS Spectrum

FOV Setting	FOV (cm)
A ¹	4
В	6.5
C ²	13
D	22.5 (19.5) ³
E	22.5 (26) ²

¹Position A is not recommended for epi-fluorescent imaging because corrections for non-uniform excitation light pattern are not available. ²Position C is the default setting.

³Some IVIS Spectrum Imaging Systems may have the FOV in parentheses. FOV 19.5 and 26 were replaced with FOV 22.5. 4. Select a focus option in the control panel (Figure 4.4).

The focal distance to the camera is set at 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. For example, if the subject height is 1.5 cm, the stage will move down 1.5 cm to set the plane of focus at the top of the subject.

Do one of the following to set the focus for the area to be imaged:

• If Structure is selected, choose the use subject height focus option.

The subject height will be automatically determined from a structured light image.

- If **Structure** is not selected, enter the subject height (cm) and choose the **use subject height** focus option.
- Choose the manual focus option and follow the instructions in Appendix A on page 257.

igure 4.4 Choose a l	Focus Opt	ion in the Co	ontrol F	anel	
🜠 IVIS Acquisition Control I	Panel				
Imaging Mode Exposure Time	Binning	F/Stop	Excitat	ion Filter Emissio	n Filter
📃 🗹 Luminescent 🛛 Auto 🤤 sec	🖌 Medium	✓ 1 ✓	Block	💟 Open	~
Eluorescent					
🚺 🗹 Photograph 🛛 0.20 🤤	Medium	v 8 v			
Overlay Lights Field of View: C	Alignment Grid	ystem Status	[
MIS	Idle			Acquire	
Service 12.8 cm				Imaging Wizan	d
Subject height: 1.50 💲 cm				 Sequence Set 	tup
Focus: use subject height 🔽	Temperature:	Locke	ed 📃	Initialize	

5. If you are using the Mouse Imaging Shuttle (MIS), select the "MIS" option (Figure 4.4). If not using the MIS, confirm that this option is cleared, otherwise the computed subject height will be inaccurate.

When the MIS option is selected, Living Image software applies a correction factor to the subject height measurement to offset the MIS platform and sets binning at 4, the optimum binning level for computing height from structured light measurements when using the MIS.

NOTE: If image data was acquired without using the MIS, but the MIS option was selected, the subject height will be 2.9 mm less than actual due to the MIS correction factor. To correct this, manually edit the subject height in the ClickInfo.txt file by adding 2.9 mm to the Height Reference measurement (Figure 4.5).

Figure 4.5 ClickInfo Text File									
		📃 ClickInfo - Notepad	- • •						
		File Edit Format View Help							
		Binning Factor: 4 Image Units: counts f Number: 8 Field of view: 13 Filter Position: 1 Emission filter: Open Exposure Time Sec: 0.2 Object radius: 0.652664 Subject size: 1.30533							
		Height Reference: 13.1, Height Reference Pixel X: Height Reference Pixel Y: Structure frequency: Medium Error Wave: No Error	240, 252,						
l		Mouse Tmaging Shuttle Enabled:	Ves -						

- 6. If you want to check the subject inside the chamber before acquisition, take a photograph (uncheck Luminescent, choose Photograph, and click Acquire). Be sure to select Luminescent 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 44 for more details on sequence setup using the Imaging Wizard).
- **8.** Enter information about the image in the dialog box that appears (optional, but strongly recommended) and click **OK**.

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 93 for details on adding information to an image after acquisition.

Figure 4.6 Enter Information About t	he Image)	
All information entered here will be saved with the image. However, only the check marked values will appear in the image label (see Figure 4.8 on page 30).	Comment2 Comment1 Comment2 Comment2 Comment2 Comment Animal Strain Animal Strain Reporter Reporter Cel Line Reporter LACLO Number LACUO Number LACUO Number CAUCION	Living Image Universal
		OK Cancel

If this is the first image of the session, you are prompted to enable the autosave function (Figure 4.7). 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 \rightarrow Auto-Save on the menu bar).

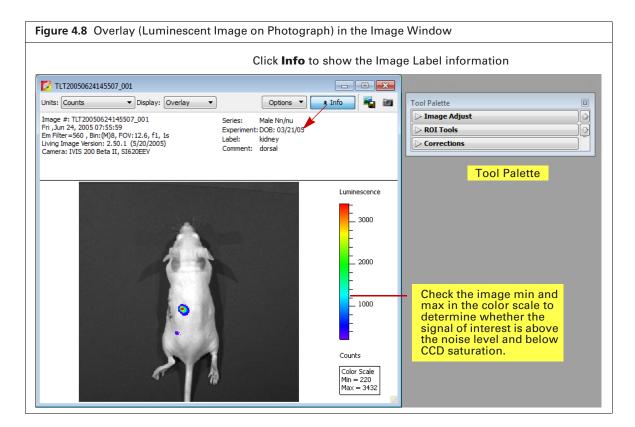
Figure	4.7 Autosave Prompt
🚺 Livin	g Image® (64-bit) - Auto Save?
?	Do you want to enable 'auto saving' of acquired data for this session? This can be changed anytime from the 'Acquisition' menu.
	Yes No

9. 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 56 for details. Image acquisition begins and the upper area of the control panel changes to red color.

Ŵ

NOTE: During acquisition, the **Acquire** button in the control panel changes to **Stop**. Click Stop to cancel acquisition and reinitialize the imaging system.

The control panel returns to blue color when acquisition is finished and the image window appears (Figure 4.8).



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

Table 4.2 Image Window

ltem	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</i> and <i>Measurement</i> for more details (select Help \rightarrow 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 9.
Info	Click to display or hide the image label. The image label includes information you enter in the Edit Image Labels dialog box (Figure 4.8) 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 4.2 Image Window

ltem	Description
0	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 57.
	🚺 Living Image® Browser
	TU20050624445507_SEQ Ckick Number EX Filter M Filter Illumination Mode User ID User Group Experiment User Group Experiment CKX CKX CKX CF750 dyc in pillows User Group Experiment CKX CKX CF750 dyc in pillows User Group Experiment CKX CKX CF750 dyc in pillows User Group Experiment CKX CKX CF750 dyc in pillows User Group Experiment CKX CKX CKX CF750 dyc in pillows User Group Experiment CKX CKX
Color Scale	Provides a reference for the pixel intensities in a luminescent or fluorescent image. Pixe 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.

4.2 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 below.
- Detailed instructions See page 33.

See page 38 for information on fluorescent imaging with transillumination (excitation light source located below the stage). See page 44 for information on acquiring a fluorescent sequence using the Imaging Wizard.



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

Quick Guide

- 1. Start Living Image software 🜠 and initialize the IVIS Spectrum (page 19).
 - **NOTE:** See the *IVIS Spectrum Hardware Manual* (PN 121450) for more information on the instrument
- 2. Place the anesthetized subjects in the imaging chamber and close the door.
- **3.** In the control panel (Figure 4.9):
 - a. Put a check mark next to Fluorescent and select Auto exposure in the control panel.
 - **b.** Select an excitation and emission filter.
 - c. Choose Photograph (optional, selecting Photograph automatically selects Overlay).
 - d. Choose Structure (subject height will be automatically determined).
 - e. Select a field of view (FOV) (see Table 4.1 on page 27).
 - f. Click Acquire.

iguic 4.5 00	ontrol Pa	nel				
💋 IVIS Acquisiti	on Control P	anel				
Imaging Mode E	xposure Time	Binning	F/Stop	Excitatio	on Filter Emission F	ilter
🔤 📃 Luminescent	1.00 🤤 sec	🔽 Medium	✓ 2 ✓	640	740	*
📕 🗹 Fluorescent	📃 Transillumin	ation			Lamp Level: High	*
🚺 🗹 Photograph	Auto 🤤	Medium	✓ 8 ✓			
Voverlay	Lights 🗸	-	l System Status			
Structure Overlay Field of View: C MIS		-		[Acquire	
✓ Overlay Field of View: C	×			[Acquire Imaging Wizard	
Overlay Field of View: C MIS	~					

- **4.** When prompted, select a location for the image data (optional, but strongly recommended). Image data acquired during the session will be automatically saved to this location.
- **5.** Enter experiment and subject information in the dialog box that appears (optional, but strongly recommended).

The image window and Tool Palette appear when acquisition is finished (Figure 4.10).

Figure 4.10 Image Window	v and Tool Palette	
Image	Window	Tool Palette
Image: Transaction Image:<	Converted Red Appoorted Red Appoorted Red Appoorted Red Appoorted Red Appoorted Red Appoorted Red Appoirted Red Ap	Tool Palette Image Adjust ROI Tools Corrections Corrections Image Adjust tools (page 66) ROI Tools (page 100) Corrections tools (page 258)
5		

Acquire a Fluorescent Image With Epi-Illumination

This section provides detailed instructions for image acquisition.

NOTE: The IVIS Spectrum should be initialized and the temperature locked before setting the imaging parameters in the control panel See page 19 for more details.

1. 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** \rightarrow **Tech Notes** on the menu bar).

Alternatively, manually set the exposure, binning, and F/Stop. See Appendix A on page 253 for details on these parameters.

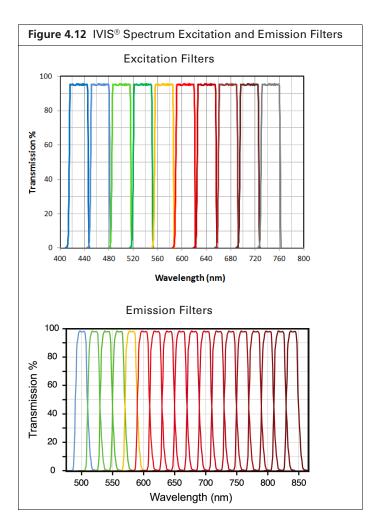
2. Put a check mark next to Photograph.

Selecting **Photograph** automatically selects Overlay, so that an overlay image (registered photograph and luminescent image) is displayed after acquisition.

Figure 4.11 Control F	anel	
🚺 IVIS Acquisition Control I	Panel	
Imaging Mode Exposure Time	Binning F/Stop	Excitation Filter Emission Filter
🗧 🗌 Luminescent 🛛 1.00 🤤 sec	: 🗸 Medium 💙 2 💙	640 🗸 740 🗸
📕 🗹 Fluorescent 📃 Transillumi	nation	Lamp Level: High 🗸 🗸
🚺 🗹 Photograph 🛛 Auto 🗘	Medium 💙 8 💙	
Structure		
🗹 Overlay 📃 Lights 🔽	Alignment Grid	
Field of View: C	System Status	
	-,	
		Acquire
	Idle	
MIS Service 12.8 cm	Idle	Acquire Imaging Wizard
	Idle	

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

The instrument has 18 narrow band emission filters (490-850nm, 20nm bandwidth) and 10 narrow band excitation filters (415-760nm, 30nm bandwidth), enabling spectral scanning over the blue to NIR wavelength region (Figure 4.12).



4. Select a field of view (FOV, size of the stage area to be imaged). See Table 4.1 on page 27 for a list of FOV settings.



TIP: See the concept technical note *Detection Sensitivity* for more information about the field of view (select **Help** \rightarrow **Tech Notes** on the menu bar).

5. Select a focus option in the control panel (Figure 4.13).

The focal distance to the camera is set at 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. For example, if the subject height is 1.5 cm, the stage will move down 1.5 cm to set the plane of focus at the top of the subject.

Do one of the following to set the focus for the area to be imaged:

• If **Structure** is selected, choose the **use subject height** focus option.

The subject height will be automatically determined from a structured light image.

- If **Structure** is not selected, enter the subject height (cm), and choose the **use subject height** focus option.
- Choose the manual focus option and follow the instructions in Appendix A on page 257.

. <u></u>	ose a Focus C	Option in the C	ontrol Par	nel
💋 IVIS Acquisition Co	ntrol Panel			
Imaging Mode Exposu	re Time Binning	F/Stop	Excitation Filte	r Emission Filter
Luminescent 1.00	🗢 sec 🔽 Medium	✓ 2 ✓	640	✓ 740 ✓
📕 🗹 Fluorescent 📃 Tra	ansillumination		Lam	p Level: High 🛛 🗸
T Photograph Auto	Medium	▼ 8 ▼		
Thursday we				
	ghts 🔽 Alignment Grid	d System Status	_	Acquire
Verlay 🗌 Li			_	Acquire
Overlay Li Field of View:	· · · · ·			Acquire maging Wizard
Overlay I Field of View: C MIS Service 12.8	Idle			

6. If you are using the Mouse Imaging Shuttle (MIS), select the "MIS" option (Figure 4.14). If not using the MIS, confirm that this option is cleared, otherwise the computed subject height will be inaccurate.

When the MIS option is selected, Living Image software applies a correction factor to the subject height measurement to offset the MIS platform and sets binning at 4, the optimum binning level for computing height from structured light measurements when using the MIS.

NOTE: If image data was acquired without using the MIS, but the MIS option was selected, the subject height will be 2.9 mm less than actual due to the MIS correction factor. To correct this, manually edit the subject height in the ClickInfo.txt file by adding 2.9 mm to the Height Reference measurement (Figure 4.14).

Figure 4.14 ClickInfo Text File	
ClickInfo - Notepad	
File Edit Format View Help	
Binning Factor: 4 Image Units: counts f Number: 8 Field of View: 13 Filter Position: 1 Emission filter: Open Exposure Time Sec: 0.2 Object radius: 0.652664 Subject size: 1.30533 Height Reference: 13.1,	
Height Reference Pixel X: Height Reference Pixel Y: Structure frequency: Medium Error Wave: No Error	240, 252,
Mouse Imaging Shuttle Enabled:	Yes -

- **7.** If you want to check the subject inside the chamber before acquisition, take a photograph: uncheck **Fluorescent**, choose **Photograph**, and click **Acquire**. Be sure to select **Fluorescent** after taking the photograph.
- 8. Click Acquire when you are ready to capture the image.
 - **NOTE:** If necessary click I mage 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 44 for more details on sequence setup).
- **9.** Enter information about the image in the Edit Image Labels box that appears (optional, but strongly recommended) and click **OK**.

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 93 for details on adding information to an image after acquisition.

Figure 4.15 Enter Information About the	e Image	
	🜠 Edit Image Labels	
	UserID: BI 👻	Living Image Universal
	Saved Labels: LABELS	1 • Ø 🖬 ×
	User	
	Group	•
	Experiment	87-MG-luc2 Intracranial Implantation 🔻
All information entered here will be saved with the image.	Comment1	Male nu/nu, day 8, Mouse #4
However, only the check marked values will appear in the image label (see Figure 4.17 on page 37).	Comment2	•
	Time Point	
	Animal Number	4
	Animal Strain	
	Animal Model	
	Sex 📃	•
	View	•
	Cell Line	•
	Reporter	
	Treatment	
	Luc Injection Time	
	IACUC Number	•
		Apply To Sequence OK Cancel

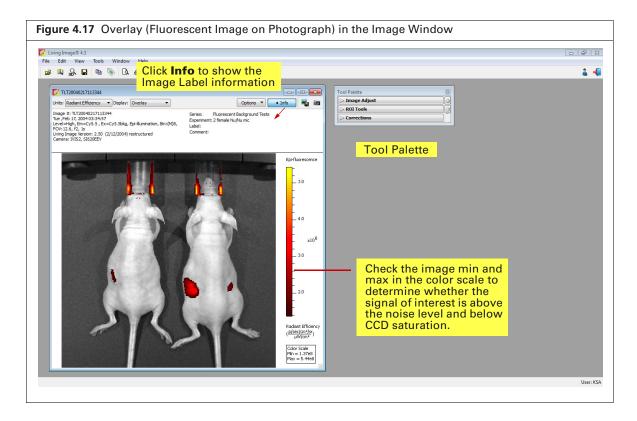
If this is the first image of the session, you are prompted to enable the autosave function (Figure 4.16). When Autosave is enabled, all images acquired during the session are automatically saved to a user-selected location. A different location can be chosen at any time (select Acquisition \rightarrow Auto-Save on the menu bar).

Figure	4.16 Autosave Prompt
🚺 Livin	g Image® (64-bit) - Auto Save?
?	Do you want to enable 'auto saving' of acquired data for this session? This can be changed anytime from the 'Acquisition' menu.
	Yes No

10. 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 56 for details. Image acquisition begins and the upper area of the control panel changes to red color.

NOTE: During acquisition, the **Acquire** button in the control panel changes to **Stop**. Click **Stop** to cancel acquisition and reinitialize the imaging system.

The control panel returns to blue color when acquisition is finished and the image window appears (Figure 4.17). See Table 4.2 on page 30 for details on the image window.



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

4.3 Fluorescent Imaging With Transillumination

Fluorescent imaging captures signals from fluorescent molecular reporters. Transillumination (excitation light source located below the stage) is recommended if the fluorescent source is deep relative to the imaged side of the animal.

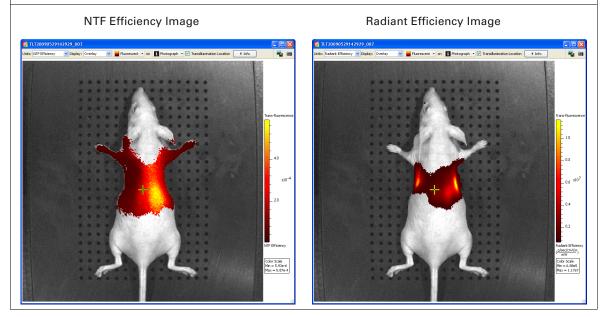
Acquisition with transillumination includes a Normalized Transmission Fluorescence (NTF) Efficiency image in which the fluorescent emission image is normalized by the transmission image measured with the same emission filter and open excitation filter (Figure 4.18).

TIP: See these tech notes for helpful information and quick guides (select **Help** \rightarrow **Tech Notes** on the menu bar):

- Transmission Fluorescence
- Transmission Fluorescence Raster Scan
- Transmission Fluorescence Normalized Transmission Fluorescence
- Transmission Fluorescence Well Plates

Figure 4.18 Fluorescent Images Acquired with Transillumination

The NTF Efficiency image in this example highlights the presence of fluorescence in the animal, while the Radiant Efficiency image shows signal ambiguous with autofluorescence.



This section explains how to acquire a single fluorescent optical image with transillumination. See page 44 for information on acquiring a fluorescent sequence.

To acquire a fluorescent image with transillumination:

NOTE: Only one mouse can be imaged at a time using transillumination.

1. Put a check next to Fluorescent and Transillumination in the control panel.

NOTE: The Normalized option is selected by default so that NTF Efficiency images can be produced. See page 38 for more information.

💋 IVIS Acquisition Control	Panel	
Imaging Mode Exposure Time	e Binning F/Stop	Excitation Filter Emission Filter
🔁 📃 Luminescent 🛛 1.00 🤤 se	ec 💌 Medium 💌 2 💌	640 🔽 740 🔽
📕 🗹 Fluorescent 🛛 🗹 Transillur	nination 🔽 Normalized 💦 Setup	Lamp Level: High 🗸 🗸
🚹 🗹 Photograph 🛛 Auto 🤤	Medium 💙 8 💙	
Structure	Alignment Grid	
✓ Overlay Lights	Alignment Grid System Status	Acquire
V Overlay Lights		Acquire
Overlay Lights	System Status	Acquire Imaging Wizard
Overlay Lights	System Status	

- 2. Choose the Auto exposure option.
- **3.** Put a check mark next to **Photograph**.



NOTE: Selecting Photograph automatically selects Overlay, so that an overlay image (registered photograph and fluorescent image) is displayed after acquisition.

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

The instrument has 18 narrow band emission filters (490-850nm, 20nm bandwidth) and 10 narrow band excitation filters (415-760nm, 30nm bandwidth), enabling spectral scanning over the blue to NIR wavelength region (Figure 4.12 on page 34).

- 5. Click Setup. Click Yes if prompted to acquire a subject photograph.
- 6. Choose the locations (select squares) for transillumination and image acquisition in the Transillumination Setup box that appears (Figure 4.20).

Figure 4.20 Open t	the Transillumination	n Setup Dialog Box
VIS Acquisition Control P Imaging Mode Exposure Time	Binning F/Stop	Excitation Filter Emission Filter
↓ Ummescent 1.00 ↓ sec ↓ ✓ Fluorescent ✓ Transillumin ↓ ✓ Photograph Auto ↓		Lamp Level: High 🔍
Structure	Alignment Grid	Transillumination Setup - Single Location Mode Move Motors To Selected Spot Mask Grid Points To Subject Grid Type: 15x23 Rasker Scan Clear Selections Cancel
Field of View: C	System Status	
	Single location mode acquires one image at the location marked by a green square <mark>to</mark> .	

Item	Description
Move Motors To Selected Spot	Transillumination motors will move the excitation light source to the grid location selected in the Transillumination Setup dialog box.
Mask Grid Points To Subject	When setting up a transillumination sequence, choose this option to automatically select only the grid locations within the subject boundaries. Grid locations outside the subject are masked out. The mask prevents the transillumination excitation source from selecting an uncovered hole. Projecting light through an open hole would saturate the camera.
Raster Scan	If the raster scan option is selected, the software takes all of the images from the transillumination locations and adds them together into one image. All of the individual fluorescent signals are stacked over one photograph and the intensity is summed.
	One overview is created per filter pair. If two filter pairs were used during acquisition, then two overview images will be created. The raster scan option may be helpful when trying to determine the optimal excitation and emission filters for a particular fluorescent probe.
	All transillumination locations are displayed simultaneously; a tool tip displays the transillumination position when you mouse over a transillumination point. An overview image is displayed by default in radiant efficiency, and if transmission images are available, in normalized transmission fluorescence efficiency. Transillumination overview images can be analyzed using the tools in the Tool Palette.
	If this option is not selected, the software generates one image per transillumination location per filter pair. For example, a sequence setup that includes 20 locations using two filters will generate 20 images. In this case, a transillumination overview can be created manually by clicking the Overview button in the Sequence window. Alternatively, select Tools \rightarrow Transillumination Overview for <name>_SEQ</name> on the menu bar.
Grid Type	9x19 grid
Update Photograph	Click to acquire a new photographic image. If the chamber door is opened during transillumination setup, you are prompted to acquire a new photograph.
Clear Selections	Clears selected/ highlighted transillumination locations on the grid.

Table 4.3 Transillumin	ation Setup Box
------------------------	-----------------

7. Confirm that the Lamp Level is set to High in the control panel.

NOTE: The lamp may be set to Low for certain applications, such as long wavelength data through thin tissue.

- **8.** Select a field of view (FOV, size of the area to be imaged). See Table 4.1 on page 27 for a list of FOV settings.
- **9.** Select a focus option in the control panel (Figure 4.21).

The focal distance to the camera is set at 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. For example, if the subject height is 1.5 cm, the stage will move down 1.5 cm to set the plane of focus at the top of the subject.

Do one of the following to set the focus for the area to be imaged:

• If Structure is selected, choose the use subject height focus option.

The subject height will be automatically determined from a structured light image.

- If **Structure** is not selected, enter the subject height (cm), and choose the "use subject height" focus option.
- Choose the **manual** focus option and follow the instructions in Appendix A on page 257.

			Control Pane	
💋 IVIS Acquisition Con	trol Panel			
Imaging Mode Exposure	Time Binning	F/Stop	Excitation Filter	Emission Filter
🔁 📃 Luminescent 🛛 1.00 🤤	sec 💙 Medium	✓ 2 ✓	640	🖌 740 🗸
📕 🗹 Fluorescent 🛛 🗹 Tran	sillumination 🛛 🗹 Norm	nalized Setup	Lamp L	.evel: High 🔽
👔 🗹 Photograph 🛛 Auto 🗘	Medium	✓ 8 ✓		
Structure				
	its 🗹 Alignment Grid	l System Status		Acquire
✓ Overlay 🗌 Ligh				Acquire
Overlay Ligh Field of View: C				Acquire
Voverlay Ligt Field of View: MIS Service 12.8	Idle			

10. If you are using the Mouse Imaging Shuttle (MIS), select the "MIS" option (Figure 4.22). If not using the MIS, confirm that this option is cleared, otherwise the computed subject height will be inaccurate.

When the MIS option is selected, Living Image software applies a correction factor to the subject height measurement to offset the MIS platform and sets binning at 4, the optimum binning level for computing height from structured light measurements when using the MIS.

5			
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1	I	I	L
1	I	I	L
	۸	•	,

W

NOTE: If image data was acquired without using the MIS, but the MIS option was selected, the subject height will be 2.9 mm less than actual due to the MIS correction factor. To correct this, manually edit the subject height in the ClickInfo.txt file by adding 2.9 mm to the Height Reference measurement (Figure 4.22).

Figure 4.22 ClickInfo Text File	
ClickInfo - Notepad	
File Edit Format View Help Binning Factor: 4 Image Units: counts f Number: 8 Field of View: 13	
Filter Position: 1 Emission filter: Open Exposure Time Sec: 0.2 Object radius: 0.652664 Subject size: 1.30533	
Height Reference: 13.1, Height Reference Pixel X: Height Reference Pixel Y: Structure frequency: Medium Error Wave: No Error	240, 252,
Mouse Imaging Shuttle Enabled:	Yes -

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

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

NOTE: If necessary click * Image Setup single image mode, the * Sequence Setup up sequence acquisition (see page 44 for more details on sequence setup). **13.** Enter information about the image in the dialog box that appears (optional, but strongly recommended) and click OK.

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 93 for details on adding information to an image after acquisition.

Figure 4.23 Enter Information About the	Image	
	Edit Image Labels UserID: BI	Living Image Universal
	Saved Labels: LABELS	
	V User	
All information entered here will be	Group	•
saved with the image.	Experiment	87-MG-luc2 Intracranial Implantation ▼
However, only the check marked values will appear in the image label (see Figure 4.25 on page 43).	Comment1	Male nu/nu, day 8, Mouse #4
	Comment2	•
	Time Point	· · · · · · · · · · · · · · · · · · ·
	Animal Number	4 🔹
	Animal Strain	•
	Animal Model	▼
	Sex	
	View	
	Cell Line	
	Reporter Treatment	• • •
	Luc Injection Time	
	IACUC Number	_
		Apply To Sequence
		OK Cancel

If this is the first image of the session, you are prompted to enable the autosave function (Figure 4.24). 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 \rightarrow Auto-Save on the menu bar).

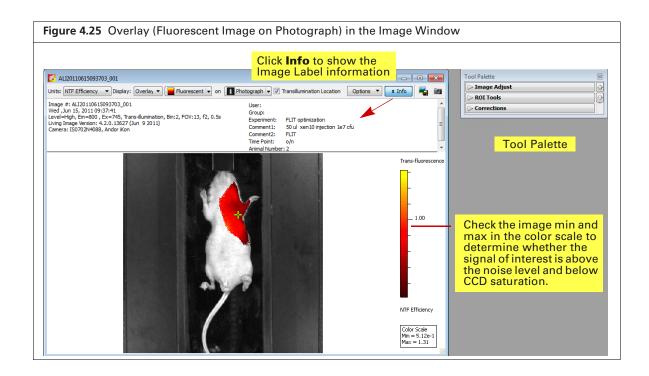
Fig	gure	4.24 Autosave Prompt
	Living	Image® (64-bit) - Auto Save?
	?	Do you want to enable 'auto saving' of acquired data for this session? This can be changed anytime from the 'Acquisition' menu.
		Yes No

14. 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 56 for details. Image acquisition begins and the upper area of the control panel changes to red color.



NOTE: During acquisition, the Acquire button in the control panel changes to Stop. Click Stop to cancel acquisition and reinitialize the imaging system.

The control panel returns to blue color when acquisition is finished and the image window appears (Figure 4.25). See Table 4.2 on page 30 for details on the image window.



TIP: See the tech note *Identify Saturated Pixels in an Image* for information on pixel measurements (select **Help** \rightarrow **Tech Notes** on the menu bar.

4.4 Cherenkov Imaging

Cherenkov luminescent imaging captures optical photons produced by Cherenkov radiation from radiotracer probes. Minutes of exposure time may be required because the Cherenkov signal can be very dim.

See page 44 for information on acquiring a Cherenkov sequence using the Imaging Wizard.

To acquire a Cherenkov image:

1. Put a check mark next to Luminescent and select Auto exposure in the control panel (Figure 4.26).

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

Alternatively, manually set the exposure time, binning, and F/Stop. See Appendix A on page 253 for details on these parameters. It may be helpful to increase the maximum time for Auto exposure in the user preferences to 5 minutes (see *Acquisition* on page 276).



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

Figure 4.26 Control P	anel			
🚺 IVIS Acquisition Control I	Panel			
Imaging Mode Exposure Time	Binning	F/Stop	Excitation Filter	Emission Filter
🔄 🗹 Luminescent 🛛 Auto 🤤 sec	💌 Medium	✓ 1 ✓	Block	🖌 Open 🛛 🔽
Eluorescent				
🚺 🗹 Photograph 🛛 0.20 💲	Medium	▼ 8 ▼		
Structure	Alignment Gric	1		
Field of View: C		System Status		Acquire
MIS	Idle			nequiro
Service 12.8 cm			Im	aging Wizard
Subject height: 1.50 🗘 cm				equence Setup
Focus: use subject height 💙	Temperature:	Locked		Initialize

2. Perform step 2 on page 27 to step 9 on page 29.

4.5 Acquire a Sequence Using the Imaging Wizard

The acquisition parameters for each image in a sequence must be specified. The Imaging Wizard (Figure 4.27) provides a convenient way to set up a sequence for some imaging applications (see Table 4.4 on page 45). 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 start the Imaging Wizard and acquire a sequence of luminescent, fluorescent, or Cherenkov images. A sequence can also be set up manually (see page 52 for details).



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

Start the Imaging Wizard and Setup a Sequence

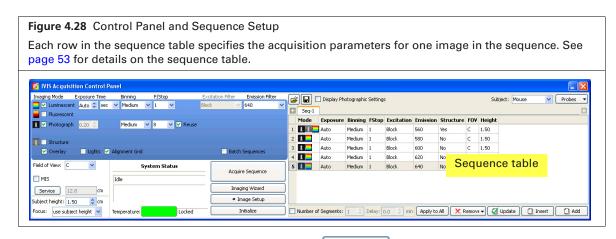
NOTE: The IVIS Spectrum should be initialized and the temperature locked before setting imaging parameters. See page 19 for more details.

1. Click **Imaging Wizard** in the control panel (Figure 4.27). If necessary, click **Restart** in the Imaging Wizard to show the first screen of the wizard.

Figure 4.27 Open the Imaging Wizard		
IVIS Acquisition Control Panel Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter Utuminescent Block Photograph 0.20 Medium 8 Structure Vovrlay Lights Alignment Grid	Imaging Wized Imaging Wized Imaging Wized Imaging the matching with the wavelength the matching with the matching with the wavelength the matching with the wavelength	
Field of View: C System Status Acquire MIS Ide Imaging Wizard Subject height: 1.50 © cm * Sequence Setup Focus: use subject height Y Temperature: Locked	Cherenkov Tinging radiotacers which ent Cherenkov light in tissue. Carvet Vect	

- 2. Double-click an imaging mode: Bioluminescence 🚺 , Fluorescence 🚺 , or Cherenkov
- **3.** Double-click an imaging option in the next screen (see Table 4.4 on page 45).
- 4. 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 4.28).



- 5. To clear the sequence, click the Remove button Remove and select All. See additional information about:
 - Editing image parameters on page 54.
 - Inserting images in a sequence on page 55.
 - Removing images from a sequence on page 55.

Table 4.4 Imaging Wizard – Imaging Mode Options for IVIS Spectrum

Imaging Mode	Options	See Page
Bioluminescence	Open Filter – Acquires a luminescent image at maximum sensitivity.	
	Spectral Unmixing – Acquires an image sequence for analysis using the Spectral Unmixing tools which use a mathematical operation to separate the signals from multiple luminescent probes.	206
	DyCE ¹ – Acquires a time series of optical images following a bolus injection of probe (radiotracer, bioluminescent, or fluorescent) to track probe biodistribution.	233
	DLIT (Diffuse Light Tomography) – Acquires an image sequence for analysis with the DLIT algorithm that reconstructs the position, geometry, and strength of 3D luminescent sources.	124
	Note: Hemoglobin (Hb and HbO ₂) has an absorption peak near 540 nm. The Imaging Wizard automatically excludes the 540 nm emission filter from DLIT sequence setup to avoid long imaging times. This helps ensure that image acquisition occurs during the optimal time post-injection (determined by the probe kinetic curve for the animal model). If you want to include acquisition at 540 nm, manually add the image to the sequence table. See <i>Inserting Images in a Sequence</i> on page 55 for instructions.	

 Table 4.4 Imaging Wizard – Imaging Mode Options for IVIS Spectrum

Imaging Mode	Options	See Page
Fluorescence	Filter Pair – Selects the best excitation and emission filters for a specific fluorescent probe. Acquires a single image and detects the fluorescent signal on the surface of the subject. Epi-illumination or transillumination may be selected.	
	 Spectral Unmixing/Filter Scan – Acquires an image sequence for analysis with the Spectral Unmixing tools to: Extract the signal of one or more fluorophores from the tissue autofluorescence. 	206
	 Determine the optimum excitation and emission filter for a probe. DyCE – Acquires a time series of optical images following a bolus injection of probe (radiotracer, bioluminescent, or fluorescent) to track probe biodistribution. 	233
	FLIT (Fluorescence Imaging Tomography) – Acquires an image sequence for analysis with the FLIT algorithm that reconstructs the position, geometry, and strength of 3D fluorescent sources. This technique is only available in transillumination mode (light source below the stage).	130
Cherenkov	Open Filter – Acquires a Cherenkov image at maximum sensitivity.	
	Spectral Unmixing – Acquires an image sequence for analysis using the Spectral Unmixing tools which use a mathematical operation to separate the signals from multiple luminescent probes.	
	DyCE – Acquires a time series of optical images following a bolus injection of probe (radiotracer, bioluminescent, or fluorescent) to track probe biodistribution.	239

¹DyCE analysis tools require a separate license.

Acquire the Sequence

- **1.** Confirm that the IVIS Spectrum is initialized and the CCD temperature is locked. (See page 19 for details.)
- **2.** If you are using the Mouse Imaging Shuttle (MIS), select the "MIS" option (Figure 4.22). If not using the MIS, confirm that this option is cleared, otherwise the computed subject height will be inaccurate.

When the MIS option is selected, Living Image software applies a correction factor to the subject height measurement to offset the MIS platform and sets binning at 4, the optimum binning level for computing height from structured light measurements when using the MIS.

NOTE: If image data was acquired without using the MIS, but the MIS option was selected, the subject height will be 2.9 mm less than actual due to the MIS correction factor. To correct this, manually edit the subject height in the ClickInfo.txt file by adding 2.9 mm to the Height Reference measurement (Figure 4.22).

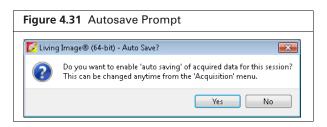
Figure 4.29 ClickInfo Text File	
(
ClickInfo - Notepad	
File Edit Format View Help	
Binning Factor: 4 Image Units: counts f Number: 8 Field of View: 13 Filter Position: 1 Emission filter: Open Exposure Time Sec: 0.2 Object radius: 0.652664	
Subject size: 1.30533 Height Reference: 13.1, Height Reference Pixel X: Height Reference Pixel Y: Structure frequency: Medium Error Wave: No Error	240, 252,
Mouse Tmaging Shuttle Enabled:	Yes -

- 3. Click Acquire Sequence in the control panel when ready to begin acquisition.
- **4.** Enter information about the image in the dialog box that appears (optional, but strongly recommended) (Figure 4.30). Click **OK**.

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 93 for details on adding information to an image after acquisition.

Figure 4.30 Enter Information About	the Image	
	[Edit Image Labels	
	UserID: BI 🔻	Living Image Universal
	Saved Labels: LABELS	_1 • 🖾 🖬 🗙
	V User	•
	Group	•
All information entered here will be	Experiment	87-MG-luc2 Intracranial Implantation 🔻
saved with the image. However, only the check marked values will appear in the image	V Comment1	Male nu/nu, day 8, Mouse #4
label (see Figure 4.32 on page 48).	Comment2	•
	Time Point	•
	Animal Number	4 🔻
	Animal Strain	•
	Animal Model	•
	Sex 📃	
	View	
	Cell Line	•
	Reporter	•
	Treatment	•
	Luc Injection Time	
	IACUC Number	•
		Apply To Sequence
		OK Cancel

If this is the first image of the session, you are prompted to enable the autosave function (Figure 4.31). 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 \rightarrow Auto-Save on the menu bar).



5. 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 56 for details. Image acquisition begins and the upper area of the control panel changes to red color.



NOTE: During acquisition, the **Acquire** button in the control panel changes to **Stop**. Click **Stop** to cancel acquisition and reinitialize the imaging system.

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 4.32).

The Image window may include multiple tabs, depending on the type of acquisition:

- Sequence View Displays the image sequence.
- 3D View Displays the 3D volume if the acquisition included DLIT or FLIT data.



TIP: See the tech note *Saturated Pixels In an Image* for information on pixel measurements.

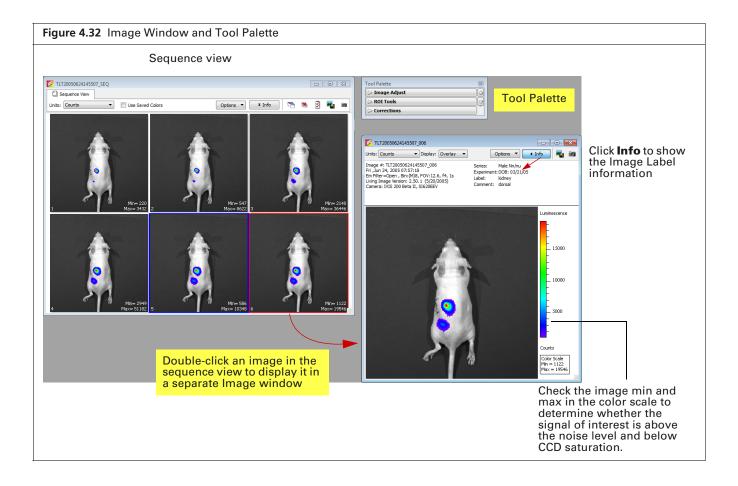


Table 4.5 Image Window - Sequence View

ltem	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 \rightarrow 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	Layout - Choose a display option for the images in a sequence (Default, Dynamic, or Film Strip). For example, here is Film Strip mode: Image: Choose a display option for the images in a sequence (Default, Dynamic, or Film Strip). For example, here is Film Strip mode: Image: Choose a display option for the 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.
Info	Click to show or hide the image label information (Figure 4.32).
r-	Opens all of the images in the sequence.
*	Closes all open images.

Table 4.5 Image Window - Sequence View (continued)

ltem	Descriptio	n								
	Enables you	Enables you to export the active image as a graphic file (for example, .png, .dcm).								
0	Browser dis	eview picture (plays when the				-			ore details	on
	🜠 Living Image® Browser									×
	TLT20050624145507_SEQ	Click Number	EX Filter	EM Filter	Illumination Mode	User ID	User	Group	Experiment	~
		▷ 459 CK20100628141050_SEQ				СК			CF750 dye in pillows	
		▶ 500 CK20091209140056_SEQ				СК	Irina	L14.0.0.9711	DLIT, subject=phantom	
		▷ 100 TLT20060510114512_SEQ				TLT				
		► 14EP TLT20050624145507_SEQ							•	+
	Hide Browse View	Close Preview Label Set: All	• 🗸	Add to List B	rowse View: Default	- Con	figure Loa	ad as Group	Load Remove Close	e
		Location: C:/Share/Caliper LS/Caliper D	ata/Sample Da	ta/IVIS200 data,	TraserBeadsPC/TLT200506	24145507_SEQ/	SequenceInfo	o.txt		А
	Preview pict	ure of the select	ed da	ta						

4.6 Acquire Multiple Sequences in Batch Mode

Use 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 4.33).

Vumnescent Auto © sec Medum ¥ 1 Block ¥ Open Florescent Wedum ¥ 8 Medum ¥ 8 Photograph 200 * Medum ¥ 8 Wedur System Status Acquire ide Imaging Weard within 15 ide Service 12.8 or me ises subject height ¥ Temperatures INS instatce (VIS Acquire Sequence Setup Total Total Control Panel **********************************	Figure 4.33 Cont	rol Panel						
Imaging Made Exposes Time Brings F1200 Exclusion Filer Ensisten Filer Unement Ludo S or Medum I V BODY Open V Procession Overlay 200° Medum I V BODY Adgement Gid Teld Ven: V System Status Adjob S of Ventor Status Adjob S of Ventor Status Adjob S of Ventor Status VIS Acquire Sta	🜠 IVIS Acquisition Control F	Panel						
Structure Overlay Upto: Aggine Acquire Ide Imaging Wicard Stopperse Time Binsging Mode Exposure Time <td>Luminescent Auto 🗢 sec</td> <td>Medium V 1 V</td> <td>Excitation Filter Emission Filter</td> <td></td> <td></td> <td></td> <td></td> <td></td>	Luminescent Auto 🗢 sec	Medium V 1 V	Excitation Filter Emission Filter					
v Overlay Lights Field of Wew: Ide Insaging Mozard * Sequence Setup * USA cquiristion Control Panel Imaging Mozard * Mosser * Mosser * None * Field of Wew: * Usabes: * None * Field of Wew: * Usabes: * None * Field of Wew: * Usabes: * None * Field of Wew: * Souture * Overlay * Stuture * Overlay * Stuture * Stuture * Souture * Souture * Stuture <	🚺 🗹 Photograph 🛛 0.20 🤤	Medium 🔽 8 🔽						
Field of Wew: System Status Acquire Ide Imaging Wizard Acquire Service: 20 of m Temperature: Italiance	Structure							
MIS Gervice 12.8 Gervice 12.8 Integration * Sequence Setup Intialize	🗹 Overlay 📃 Lights 🗹	Alignment Grid						
Ide Imaging Word Subject height: 1.50 INTS Imaging Mode Exposure Time Binning F/Stop Exclusion Filer Imaging Mode Exposure Time Binning Filed of View: System Status Acquire Sequence Ide Imaging Woard Mis Ide Imaging Woard Mis Ide Imaging Woard Initialize Number of Segments: I plessy: Initialize	Field of View: C 🛛 👻	System Status	Acquire					
ubieter height i: 1:50 ubieter height: 1:50 ubieter h	MIS	Idle						
dugez height i 130 von couse subject height Temperature: NUIS Acquisition Control Panel Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter Vinnescent Auto Sec Medum V 1 V Block V Open Flourescent Vertication Plane Course Sec Medum V 2 V Reuse Structure Vertication Filter Emission Filter Vertication Filter Emission Filter Subject height V Reuse Batch Sequences Field of View: C V System Status Acquire Sequence Field of View: C V System Status Field of View: C V Sys								
VI/S Acquisition Control Panel Imaging Mode Exclation Filter Imaging Mode Exclation Filter Exclation Filter Emission Filter Emissi								
Imaging Mode Exposure Time Bining F/Stop Exclation Filter Emission Filter Uninescent Auto © sec Medum V 1 Block Open Fluorescent Imaging Mode Seq.1 Structure Medum V 8 Reuse Structure Vorelay Lights Ide Inaging Wizard Visite Height: 1.50 © min Fourier Lister Instalize Number of Segments: 1 © Delay: 0.0 © min Apply to All © Remove ♥ Wildete ① Insert ① Add	Focus: use subject height 💌	Temperature:	Initialize	7				
Imaging Mode Exposure Time Bining F/Stop Exclation Filter Emission Filter Uninescent Auto © sec Medum V 1 Block Open Fluorescent Imaging Mode Seq.1 Structure Medum V 8 Reuse Structure Vorelay Lights Ide Inaging Wizard Visite Height: 1.50 © min Fourier Lister Instalize Number of Segments: 1 © Delay: 0.0 © min Apply to All © Remove ♥ Wildete ① Insert ① Add								
Utuninescent Auto © sec Medum 1 V Block Open Image: Comparison of the second of th	🜠 IVIS Acquisition Control I	Panel						
Imaging Wizer Structure Ide Imaging Wizerd tubject height: 1.50 @ cm requere subject height: Temperature: Initialize Number of Segments: 1 @ Delay: 0.0 @ min Apply to All X Remove @ Quick @ Linket				🕞 🔲 🗖 Display Photo	graphic Settings		Subject: None	Probes
Wedum 3 Structure Overlay Lights Alignment. Grid Batch Sequences Ide Intaging Wizard Verlay: Ide Inaging Wizard Intaging Wizard Temperature: Intalize Number of Segments: 1 Delay: 0.0 Insert Imaging Wizard Imaging Wizard <td></td> <td>: 🗸 Medium 💙 1 💙</td> <td>Block 🗹 Open 🔽</td> <td></td> <td></td> <td></td> <td></td> <td></td>		: 🗸 Medium 💙 1 💙	Block 🗹 Open 🔽					
V Overlay Lights Alignment Grid Field of View: C System Status Ide Ide Integing Wizerd vibipect height: Temperature: Locked Initialize Number of Segments: 1 Delay: 0.0 0 min Apply to All X Remove (Insert)	Photograph 0.20 0	Medium 🗸 8 🗸 🖌 Reuse						
V Overlay Lights Alignment Grid Field of View: C System Status Ide Ide Integing Wizerd vibipect height: Temperature: Locked Initialize Number of Segments: 1 Delay: 0.0 0 min Apply to All X Remove (Insert)								
Field of View: System Status MIS Ide Service 12.8 om Imaging Wizard ubject height: Installize Number of Segments: 1 © Delay: 0.0 © min Apply to All X Remove (Update (Insert (Add		Alizament Crid	Patel Comunes					
MIS Idle Imaging Wizad Service 12.8 Imaging Wizad ubject height: 1.50 cm Imaging Wizad ocuss: use subject height Imaging Wizad			Datur bequerces					
Service 12.8 cm ubject height: 1.50 cm Focus: use subject height V Cocces: use subject height V Service 12.8 cm ubject height V Focus: use subject height V Service 12.8 cm Umaging Wizard Number of Segments: 1 Delay: 0.0 min Apply to All X Remove V Update I Insert Add			Acquire Sequence					
ubject height v isse subject height v Temperature: Locked Initialize Number of Segments: 1 ↓ Delay: 0.0 ↓ min Apply to All × Remove v 《 Update ① Insert ① Add		Idle						
Encuse use subject height V Temperature: Locked Initialize Number of Segments: 1 Delay: 0.0 min Apply to All X Remove V 🖉 Update 🗋 Insert 🗋 Add		-						
		Temperatures Lacked		Number of Segments: 1	A Delay: 0.0 A min	Apply to All	Lindate [7]	Incert D Add
Batch Sequences option	use subject height	Locked	1110/01/20	I number or beginents: 1	Velay: U.U V min		M obnare	MOD L MOD
			Batch Sequences o	ntion				

- **3.** To set up the first sequence, do either of the following:
 - Click **Imaging Wizard** and step through the wizard (see page 44 for details). OR
 - Set up the sequence manually (see page 52 for details).
- **4.** To set up the next sequence:
 - If using the Imaging Wizard, repeat step 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, rightclick the selected name to view a shortcut menu of edit commands (for example, Cut, Copy, Paste).

Figure 4.34 Multiple Segu	onoo To	ha							
rigule 4.34 Multiple Sequ	ence la	05							
Three sequences are spec	fied in t	his exar	nple.						
				5	Sequenc	e tabs			Removes the active tab and its sequenc
					1				
Click to open or save a									
batch sequence setup — (.xsq)	F	🗌 Display Pl	hotographi	ic Setting	IS				Subject: Mouse V Probes V
	-🛃 Seq-1	Seq-2	Seq-3						
	Mode	Exposure	Binning	FStop	Excitation	Emission	FO¥	Height	
Adds a new tab (use with manual sequence setup)	1	Auto	Medium	1	Block	520	D	1.50	
manual sequence setup/	2	Auto	Medium	1	Block	560	D	1.50	
	3	Auto	Medium	1	Block	620	D	1.50	
			S	Seque	nce tab	le			
)		
	Number	of Segments:	1	Delay:	0.0 🤤 mi	n Apply t	D All	X Re	emove 🗸 🕼 Update 📋 Insert 📋 Add

- 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. During acquisition, the **Acquire** button in the control panel changes to **Stop**. Clicking **Stop** cancels acquisition.

The upper area of the control panel changes to red color during acquisition. The control panel returns to blue color when acquisition is finished.



NOTE: If the **Batch Sequences** option in the control panel is not selected (Figure 4.33), only the sequence in the active tab will be acquired.

- **7.** To save the batch sequence setup:
 - **a.** Click the **Save** button
 - **b.** Enter a file name (.xsq) and choose a location for the file in the dialog box that appears.

4.7 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).

See Acquire the Sequence on page 46 for details on image acquisition.

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 4.35).

The sequence table appears.

2. If necessary, click the **Remove** button **Remove** and select **All** to clear the sequence table.

Figure 4.35 Open the Sequence Table		
🜠 IVIS Acquisition Control Panel	Lion Filter Emission Filter	
Field of View: C System Status M15 Idle Service 12.8 cm Subject height: 1.50 cm Focus: use subject height v Temperature: Locked	Acquire Imaging Wizard ** Sequence Setup Initialize	
✓ IVIS Acquisition Control Panel Imaging Mode Exposure Time ✓ Luminescent Auto ♀ ✓ Luminescent Sec ♥ Medium ♥ 1 ✓ Photograph 0.20 ♀ Medium ♥ 8 ♥	tation Filter Emission Filter	
Structure Overlay Lights Field of View: C MIS Idle	Batch Sequences Acquire Sequence	
Service 12.8 Subject height: 1.50 Focus: use subject height	Imaging Wizard	Number of Segments: 1 Delay: 0.0 0 min Apply to All X Remove V Value Insert Add

3. Choose a subject and probe from the drop-down lists (Figure 4.36)

Ĩ		Display Ph	otographic	Setting	5				Subject	: Mouse 🔽	F	Probes 🔻		
1	Seq-1	1											ent Probes 🔸	Unselect Probes
ī	Mode	Exposure	Binning	FStop	Excitation	Emission	Structure	FOV	Height		_	Fluorescen	Probes	Bacteria
1	t II	Auto	Medium	1	Block	560	Yes	с	1.50			Unselect Pr	obes	CBGreen
2	1	Auto	Medium	1	Block	580	No	С	1.50					CBRed
3	X	Auto	Medium	1	Block	600	No	С	1.50					✓ Firefly hRenilla
4	<u>x</u>	Auto	Medium	1	Block	620	No	С	1.50					Tritium Bead 5
5	1	Auto	Medium	1	Block	640	No	С	1.50					XPM-2-LED
														ZnS

- **4.** Specify the imaging settings for the first image in the sequence. (See Appendix A on page 253 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 4.37), the IVIS Spectrum acquires only one photograph for the entire sequence. If **Reuse** is not chosen, the imaging system acquires a photograph for each image in the sequence.
- **5.** Click the **Add** button

The acquisition parameters appear in the sequence table (Figure 4.37).

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

Figure 4.37 Control Panel and Sequence Table with Image Settings 💋 IVIS Acquisition Control Panel Imaging Mode Exposure Time Binning Emission Filter F/Stop 🗃 📘 🗌 Display Photographic Settings Subject: Mouse V Probes Luminescent Auto 🗘 sec 🗸 Medium 🗸 1 ~ 640 Seq-1 Mode Exposure Binning FStop Excitation Emission Structure FOV Height 🚺 🗹 Photograph 🛛 0.20 🔅 Medium 🔽 8 🔽 🗹 Reuse 🗶 📃 🔤 Auto Medium 1 560 Block 1.50 Yes С Auto с Medium 1 Block 580 No 1.50 Structure 3 🚺 🔤 1.50 Auto Medium 1 Block 600 No С 🗌 Liahts 🔽 Alianment Grid Batch Sequences V Overlay с 1 Auto Medium 1 Block 620 No 1.50 Field of View: C ~ System Status 5 Auto 640 C 1.50 Medium 1 Block No Acquire Sequence 🔲 MIS Idle Imaging Wizard Service 12.8 cm * Image Setup Subject height: 1.50 🗢 cm Number of Segments: 1 🗘 Delay: 0.0 🗘 min Apply to All 🗙 Remove 🗸 🌠 Update 🗍 Insert 📋 Add use subject height 🔽 Locked Initialize cus: Temperature:

Table 4.6 Sequence Table

Item	Description
Imaging Wizard	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.
8	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 V Probes V	If a subject and probe are specified (optional), the software uses the information to automatically set parameters in the Surface Topography, DLIT, FLIT, 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 4.6 Sequence Table (continued)

Item	Description
Apply to All	Applies the selected cell value to all cells in the same column.
X Remove -	Remove Selected - Deletes the selected row from the sequence table.
Keniove	Remove All - Removes all rows from the sequence table.
🌠 Update	Updates the selected row in the sequence table with the acquisition parameters in the control panel.
🗍 Insert	Inserts a row above the currently selected row using the information from the control panel.
Add	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 4.38).

Imaging Mode Exposure Time													
	Binning F/Stop	Excitation Filter Emission Filter		🔲 Display Ph	otographic	Settings					Subject:	Nouse	Probes
Luminescent Auto 🗘 sec	V Medium V 1 V	Block 🗹 640 🗸	Seq-										
Fluorescent			Mode	Exposure	Binning	FStop	Excitation	Emission	Structure	FOV	Height		
1 Photograph 0.20 🤤	Medium 🔽 8 🔽 🗹 Reus	9	1	🔤 Auto	Medium	1	Block	560	Yes	С	1.50		
Structure			2	Auto	Medium	1	Block	580	No	С	1.50		
	Alignment Grid	Batch Sequences	3	Auto	Medium	1	Block	600	No	С	1.50		
	ring interior and	- Data Sequences	4	Auto	Medium	\frown	Block	620	No	С	1.50		
Field of View: C 🛛 👻	System Status	Acquire Sequence	5 🚺 🗖	Auto	Mediur	1 🔽	Bock	640	No	С	1.50		
MIS	Idle	Acquire Sequence				1							
Service 12.8 cm		Imaging Wizard	5		<u>ا</u>	4 8	1						
Subject height: 1.50 🗘 cm		* Image Setup	Ĩ.										
	Temperature:	Initialize		r of Segments:				Apply to			: 🗸 🚺 Update	e 🗍 Inse	ert 🗍 Add

- 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 Apply to All.
- **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 **G** Update 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 Insert 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).

ž		🗌 Display Pł	notographi	c Setting	s				Subject: Mouse Probes
1	Seq-1	Seq-2	Seq-3	Seq-4	Seq-5	Seq-6	Seq	7 St	5eq-8 Seq-9
1	Mode	Exposure	Binning	FStop	Excitation	Emission	FOV	Height	nt
	<u>x</u>	Auto	8	1	Block	560	С	1.50	
2	1	Auto	8	1	Block	580	С	1.50	
I	¥ 🔤	Auto	8	1	Block	600	С	1.50	Copy row(s)
	X	Auto	8	1	Block	620	С	1.50	Select All
	X	Auto	8	1	Block	640	С	1.50	Delete row(s)
									Replace Row(s) Paste Row(s)

Table 4.7 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

Do either of the following:

■ Select the row(s) that you want to delete. click **×** Remove, and choose **Selected** from the drop-down list.

or

• Select the row(s) of interest and right-click the sequence table to view a shortcut menu of edit commands (Figure 4.39).

4.8 Manually Save Image Data

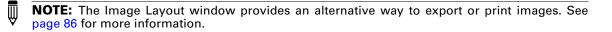
Living Image software prompts you to enable the autosave feature during the first acquisition setup of an imaging session. If autosave is enabled, all images acquired during the session are automatically saved to a user-selected location. You can choose a different location at any time (select Acquisition \rightarrow 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 \square . Alternatively, select File \rightarrow Save on the menu bar.
- **3.** Select a directory in the dialog box that appears, and click **OK**. The data includes the user ID and a date/time stamp.

4.9 Exporting Images

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



- **1.** Open an image or sequence.
- 2. Click the Export Graphics button **Figure 4.40**).
- 3. Select a directory in the dialog box that appears, enter a file name, and click Save.

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

Figure 4.40 Export Image to a Graph	nic File		
Z TLT20050624145507_006		Export Active View As	
Units: Counts Display: Overlay Options Options	🔹 Info 🤇 📸 🔤	🚱 🔍 🗢 🔀 Favorites	- 4 Search Favorites
	Luminescence	Organize 🔻	
		★ Favorites Desktop Shortcut 449 byte	t
	- 15000	Cibraries Downlos	
	-	Nonceal Strategy Stra	
2		1 Computer	
	10000	🗣 Network	
	5000		
	-	File name:	
		Save as type: PNG Image (*.png) PNG Image (*.png)	r}.
	Counts Color Scale	Hide Folders Bitmap Image (*.bmp) JPEG Image (*.jpg)	
· · · · ·	Min = 1122 Max = 19546	Postscript Enhanced Metafile (*.eps) Tagged Image File Format (*.tif)	-
		Windows Metafile Format (*.wmf) Enhanced Metafile Format (*.emf) DICOM (*.dcm)	

5 Working With Images

Loading Image Data

Adjusting Image Appearance on page 66

Viewing Intensity Data on page 70

Measuring Distance on page 73

Combining Images Using Image Math on page 75

Overlaying Multiple Images on page 80

Rendering Intensity Data in Color on page 83

Annotating or Tagging Images on page 84

Exporting an Image on page 86

Exporting an Image Sequence on page 88

Managing Image Sequences on page 94

5.1 Loading Image Data

Images can be loaded (opened):

- Using the Living Image Browser (see below).
- From the toolbar or menu bar (page 61).
- By dragging an image file or sequence folder to the Living Image main window.

Multiple datasets can be open at the same time.



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

Preview and Load Data Using 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.

- 1. Start the Living Image Browser:
 - **a.** Click the **Browse** button a. Alternatively, select **File** \rightarrow **Browse** on the menu bar.
 - **b.** Select a folder in the dialog box that appears.

The Living Image Browser appears (Figure 5.1). 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 Living Image software and click the **a** toolbar button, the software automatically returns to the last folder visited.

Figure 5.1 Opening the Living Image Bro	owser
File Edit View Tools Window Help	
Living Image Dataset Folder:	
Comparing Dataset Folder:	
Organize New folder Documents	Name Date modified
↓ Music	Name Date modified
Pictures	LTT20050624145507_SEQ 2/2/2011 8:17 AM
SW Download Installers	
Videos	
🜏 Homegroup	
Connets	E
rte Computer	
DVD RW Drive (D:) Audio CD	
🖉 KINGSTON urDrive (F:)	
Seagate Expansion Drive (G:)	4 III > 7
Folder: TraserBeadsPC	
	Select Folder Cancel
Living Image® Browser RKG20110210131022_SEQ Click Number EX Eilber EM Eil	
RKG20110210131022_SEQ Click Number EX Filter EM Filt Image:	ter Illumination Mode User ID User Group Experiment Comn * RKG Ruby et al Cherry Lab 4T1-luc2-tdTomato FDG 5:
EL20120411170231 675 720	Reflective EL PSMAdiabody VT 680XL Mal 2nmol
· · · · · · · · · · · · · · · · · · ·	*
Hide Browse View / Close Preview Label Set: All	t Browse View: Default Configure Load as Group Load Remove Gose
Location: //KATHERINE-PC/Share/Caliper LS/Caliper Data/Cerenk	tovDyCE/RKG20110210131022_SEQ/SequenceInfo.txt
	nage Browser
MC – Image	
Image with saturated pixels	
• Image sequence	
- Image exported as DICOM file	
DyCE or Cherenkov image sequer	ice

expand a sec arrow next to	quence, click the o 4슰.	b al co	row pha plun	a colum ser conte numerio nn heade ending al	ents i c orde er aga	n asce er. Clic ain to s	ending ck the sort in	rig Pr sh	ht-click	ata prop a row an es on the nenu.	nd sele
Living Image® Browser											
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preview data	a, click a row.										

- **2.** Load data by doing 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 sequence thumbnail or, if available, image thumbnail.

The image(s) and Tool Palette are displayed. Green rows in the browser indicate loaded data (Figure 5.3).



NOTE: Multiple datasets can be loaded.

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Table 5.1 Living Image Browser

ltem	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 the image information (column headers) displayed in the Living Image Browser.
Add to List	If this option is chosen, the data selected in the "Living Image Dataset Folder" dialog box (Figure 5.1 on page 58) is added to the Living Image Browser. If this option is not chosen, the data selected in the dialog box replaces the contents of the Living Image Browser, except for loaded data.
Browse	Opens the "Living Image Dataset Folder" dialog box that enables you to choose data to add to the browser (Figure 5.1 on page 58).
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 to 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.

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ltem	Description
Load as Group	Enables you to select particular images that you want to view as a sequence. The images may be acquired during different sessions.
	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 in the browser Macintosh users: Press and hold the Cmd key (apple key) while you click the images in the browser.
	Note: The Load as Group option is only available when two or more images (non-kinetic) are selected in the browser.
	Tip: See the tech note Loading Groups of Images for a quick guide (select Help \rightarrow Tech Notes on the menu bar).
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.

Table 5.1	Living	Image	Browser	(continued)
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Load Data From the Menu Bar or Toolbar

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

- **1.** Click the **Open** button \overrightarrow{P} on the toolbar. Alternatively, select **File** \rightarrow **Open** on the menu bar.
- **2.** Choose a file type filter from the drop-down list in the box that appears (Figure 5.4). The default file type selection is "Click*.txt, Sequence*.txt, or *.dcm", which are the file types generally used to open a sequence or single image (see Table 5.2 on page 62).

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Organize 🔻 New folder		·		:≡ - □	
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\rm Downloads		RKG20110210131022_002	5/2/2012 3:42 PM	File folder	
🖳 Recent Places		RKG20110210131022_003	5/2/2012 3:42 PM	File folder	
		RKG20110210131022_004	5/2/2012 3:42 PM	File folder	
🗃 Libraries		RKG20110210131022_005	5/2/2012 3:42 PM	File folder	
Documents	=	RKG20110210131022_006	5/2/2012 3:42 PM	File folder	
J Music	_	RKG20110210131022_007	5/2/2012 3:42 PM	File folder	
Pictures		RKG20110210131022_008	5/2/2012 3:42 PM	File folder	
📑 Videos		RKG20110210131022_009	5/2/2012 3:43 PM	File folder	
		퉬 RKG20110210131022_010	5/2/2012 3:43 PM	File folder	
🖏 Homegroup		🕌 XML	5/1/2012 8:56 AM	File folder	
		SequenceInfo	4/16/2012 10:03 AM	Text Document	
💺 Computer					
🚢 Local Disk (C:)					
🖥 Network	-	•			
File name:			Living	mage Files(Click*.txt	Sequence*.txt *.de
				Image Files(Click*.txt age Files(*.tif;*.tiff)	Sequence*.txt *.de

Table J.2 The Thees	
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.

Table 5.2 File Filters

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

About the Image Window and Tool Palette

An image or image sequence is displayed in an image window (Figure 5.5). Multiple image windows can be open at the same time.

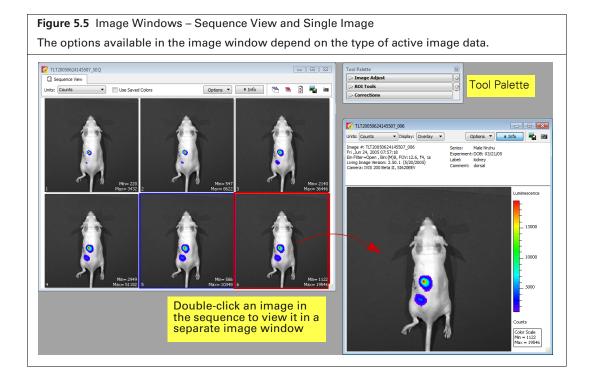


Table 5.3 Image Window

ltem	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 \rightarrow Tech Notes on the menu bar).					
	Counts – An uncalibrated measurement of the photons incident on the CCD camera. Recommended for image acquisition to ensure that the camera settings are properly adjusted. Proper image parameter adjustment should avoid image saturation and ensure sufficient signal (greater than a few hundred counts at maximum).					
	Radiance (photons) – A calibrated measurement of the photon emission from the subject. Radiance is in units of "photons/second/cm ² /steradian". Recommended for luminescence measurements.					
	 Radiant Efficiency (fluorescence) – Recommended for fluorescence measurements. Epi-fluorescence - A fluorescence emission radiance per incident excitation power. Transillumination fluorescence - Fluorescence emission radiance per incident excitation power. 					
	Efficiency (epi-fluorescence) – Fluorescent emission normalized to the incident excitation intensity (radiance of the subject/illumination intensity). Recommended for epi-fluorescence measurements.					
	NTF Efficiency – Fluorescent emission image normalized to the transmission image which is measured with the same emission filter and open excitation filter. Recommended for transillumination fluorescent measurements.					
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.					
Options (image sequence)	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: 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. 					

Item Description Display - Choose the types of information to display with each image. Z TLT20050624145507_SEQ . . . Sequence View Spectra Units: Counts • Use Saved Cold 🕾 🐘 🗿 📲 🖮 tions 🔻 * Info Displa Exposure Time Labels **Binning Factor** tion filter **Emission filter** f Number Field of Viev Select All Clear All sure Time to Eactor : In this example, exposure time (and binning factor are displayed on each image Labels - Enables you to select the information to include in the image label. Z TLT20050624145507_005 - - -Units: Counts 🔹 Display: Overlay 🔻 Options 🔻 主 Info 🛛 🖏 🗃 Image #: TLT20050624145507_005 Fri ,Jun 24, 2005 07:56:46 Em Filter=640 , Bin:(M)8, FOV:12.6, f2, 1s Camera: IVIS 200 Beta II, SI620EEV : Male Nn/nu iment: DOB: 03/21/05 kidney ent: dorsal Series: Experin Label: -Image label 10000 8000 6000 4000 2000 lounts Color Scale Min = 586 Max = 10348 Info Click to show or hide the image label. The image label includes information you enter in the Edit Image Labels dialog box (see page 29) and other information automatically recorded by the software. P 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 4 sequence display. Opens a dialog box that enables you to export the active view as a graphic file. E

Table 5.3 Image Window (continued)

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Table 5.3 Image Window (continued)

ltem	Description								
Τ.	Takes a "snapshot" that is displayed with the data in the Living Image Browser. See page 57 for more details on the browser.								;
	🚺 Living Image® Browser								×
	TLT20050624145507_SEQ	Click Number	EX Filter	EM Filter	Illumination Mode	User ID	Experiment	Comment1	~
		▶ 50 CK20100628141050_SEQ				СК	CF750 dye in pillows	0.25 pmol/uL, 2 uL bead+1	12
		▶ € CK20050420175030_SEQ				СК			
		▶ 50 TLT20060510114512_SEQ				TLT			
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	<u></u>	<						,	+
	Hide Browse View	Close Preview Label Set: All	-	Add to List B	rowse View: Default	▼ Cor	figure Load as Group	Load Remove Clos	se
		Location: C:/Share/Caliper LS/Caliper D	ata/Sample Da	ta/IVIS200 data	TraserBeadsPC/TLT2005062	4145507_SEQ/	SequenceInfo.txt		
	Snapshots of	an image seque	nce						

The Tool Palette appears when an image or sequence is loaded (Figure 5.6). The options available in the Tool Palette depend on the type of active image data. A tool is only available if the dataset includes the components that the tool requires to perform the analysis.

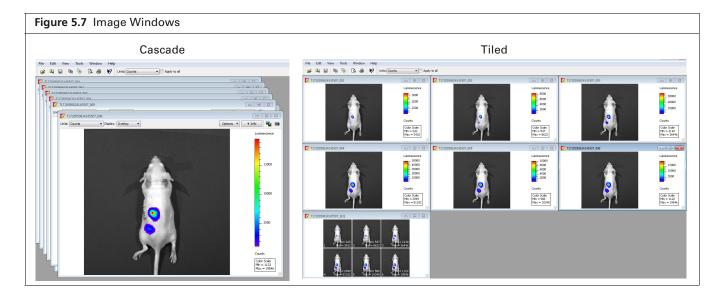
NOTE: The 3D Multi-Modality tools and DyCE tools require a separate license.

Figure 5.6 Tool Palette	
See page 11 for an overview of Living Image tools.	
Tool Palette Image Adjust ROI Tools Corrections	a tool Tool Palette Image Adjust Image Adjust Image Adjust Image Data: 6 counts Image Data: 6 counts

Organizing Images

If multiple image windows are open, they can be organized in a cascade or tile arrangement.

Choose **Window** \rightarrow **Cascade** or **Window** \rightarrow **Tile** on the menu bar.



5.2 Adjusting Image Appearance

Use the Image Adjust tools to adjust image display (Figure 5.8). Most of the Image Adjust tools do not change the image data (for example, adjusting the color scale or color table). However, binning and smoothing may slightly change image data, and therefore should only be applied after image data have been analyzed.



NOTE: Not all tools are available for all image display modes. Some tools are available for single images, but not an image sequence and vice versa.

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its: Radiant Efficiency Display: Overlay	Doptions ▼ ▼ Info 🎽 📷 🛛 🔍 🕀 🖾 🕃	3
	Epi-fluorescence Photo Adjustment	~
	Brightness: 100 🜩	
	Contrast: 1.5	
	4.0 Opacity:	
	Color Scale	
	Min:	
	- 3.0 Max:	
	x109 Color Scale Limits	
	Auto 🔿 Full 🔿 Manual	
	_ 2.0	
	Color Table	
	YellowHot 🔻	
	_ 1.0 Reverse Dogarithmic Scale	
	Smoothing: None 🔻 😝 Binning: 2 (*) 💌 👆	
	Image X, Y: 9.265, 8.474 cm Units: Cm 🔻	
	(p/sec/cm ² /sr)	
	µW/cm² / Distance: 0.00	
P	Color Scale	
	Min = 2.5988 Max = 4.74e9	Ŧ
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Table 5.4 Image Adjust Tools

ltem	Description
Q	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).
Q	Click this button to incrementally zoom in on the image (incrementally magnifies the image in the image window).
	Click this button, then draw a box on the image to magnify the area inside the box. (Sets the dimensions of the magnified area equal to image window dimensions.) See <i>Zooming or Panning</i> on page 68 for more information.
<u> 151</u>	Click this button to draw a box on an image that can be used to: Make measurements (see page 74)
	 Select an area of the image to copy to the system clipboard.
R	Click this button to return the image to the default display magnification.
t.	Click this button to move a magnified image (<i>pan</i>) in the image window. For more details, see page 68.
	Click this button to hide or display the image min/max information in the image window
	Click this button to hide or show the color scale in the image window.
Photo Adjustment	 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. 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.) 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.
Color Scale	Min – The minimum pixel intensity associated with the color scale for an image. Pixels less than the minimum value are not displayed. 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.
Color Scale Limits	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.
	Full – Choose this option to set the Max and Min values to the maximum and minimum data values in the image.
	Manual – Choose this option to enter Max and Min values for the image display.
	Individual – Applies a separate color scale limit to each image in a sequence. Note: This option is only available when an image sequence is active.
Color Table	Rainbow \bullet 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 the color table (select Help \rightarrow Tech Notes on the menu bar).
	Reverse – Choose this option to reverse the selected color table.
	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.

Table 5.4	Image	Adjust	Tools	(continued)
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ltem	Description
Smoothing	Computes the average signal of a specified number of pixels (for example, 5 x 5) and replaces the original signal with the average signal (see Figure 5.9). Smoothing removes signal noise without changing pixel size. Smoothing can be applied to an image or a sequence.
	Click this button to return smoothing to the previous setting and update the image.
Binning	Specifies the number of pixels in the image data that are grouped together to form a larger pixel (called <i>soft</i> binning). Binning changes the pixel size in the image (see Figure 5.9). Binning can be applied to an image or a sequence. See the tech note <i>Detection Sensitivity</i> for more details on binning (select Help \rightarrow Tech Notes on the menu bar).
	Click this button to return binning to the previous setting and update the image.
lmage X,Y	The x,y pixel coordinates of the mouse pointer location in an image and the intensity (counts or photons) at that location. Note: This tool is only available when an image is active.
0	Click this button to display a line profile (see page 71.) Note: This tool is only available when an image is active.
/	Click this button to display the distance measurement tool in the image window (see page 73). Note: This tool is only available when an image is active.

Zooming or Panning

To incrementally zoom in or out on an image:

Click the Q or Q 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 R 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. Panning is only available if the image has been magnified.

1. Click the 🕀 button.

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2. When the pointer becomes a \bigoplus , click and hold the pointer while you move the mouse.

Smoothing and Binning

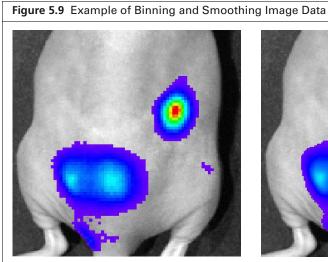


TIP: See the technical note *Detection Sensitivity* for helpful information about binning and smoothing (select **Help** \rightarrow **Tech Notes** on the menu bar).

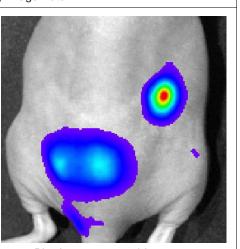
Smoothing computes the average signal of a specified number of pixels (for example, $5 \ge 5$) and replaces the original signal with the average signal (Figure 5.9). Smoothing removes signal noise without changing pixel size

Binning specifies the number of pixels in the image data that are grouped together to form a larger pixel (called soft binning) (Figure 5.9). Binning changes the pixel size in the image.

Smoothing and binning can be applied to a single image or all of the images in a sequence.



Binning at acquisition = 8, no smoothing



Binning = 2, smoothing = 5x5

To set smoothing and/or binning:

- **1.** Load an image or a sequence.
- Make a selection from the Smoothing and/or Binning drop-down lists in the Image Adjust tools (Figure 5.10).

The image or all images in the sequence will be updated.

3. Click the 🔄 button to return the smoothing or binning to the previous setting and update the image or sequence images.

Figure 5.10 Select Smoothing ar	nd/or Binning	Levels
Tool Palette	×	
✓ Image Adjust		
Q ⊕ □ □ Q ⊕ □; □;		
Brightness:		
Contrast:		
Opacity: 100		
Min: 0		
Max: 0	A V	
Color Scale Limits Auto C Full Manual		
☑ Individual		
Color Table		
Rainbow		
Reverse Logarithmic Sci	ale	
Smoothing: None 🔻 🦛 Binning: 1 💌	•	
Image X, Y 3x3 Units: Cm		
5x5 N		
Image Data 7x7		
++ / 11x11 0.00		
► ROI Too 13x13 15x15		
> Spectral Unmixing and DyCE		
Surface Topography		
▷ 3D Multi-Modality Tools		
DLIT 3D Reconstruction	-	

5.3 Viewing Intensity Data

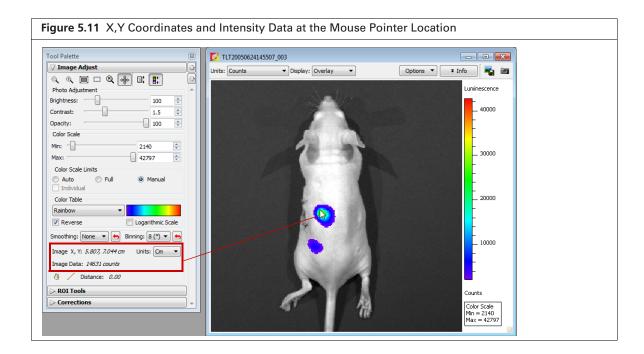
You can view intensity data:

- At a particular x,y location.
- Along a line drawn on the image.
- Within a user-selected *region of interest* (ROI). See Chapter 6 on page 98 for information on measuring signal in 2D images using an ROI.

X,Y Coordinates and Intensity Data

- 1. Open an image and choose Cm or Pixels from the Units drop-down list in the Image Adjust tools.
- 2. Put the mouse pointer over the image to view the:
 - x,y pixel coordinates of the mouse pointer location in the image (Figure 5.11).
 - Intensity at the pixel location of the mouse pointer. The intensity is represented in the units currently selected for the image.

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Line Profile

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The line profile plots intensity (y-axis) at each pixel (x-axis) along a user-specified line on an image. It is particularly useful for inspecting the detailed character of the image data.

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 a line profile:

Open an image and click the Line Profile button A in the Image Adjust tools (Figure 5.12).
 A line appears on the image and the Line Profile window opens. See Table 5.5 on page 72 for details on the Line Profile window.

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igure 5.12 View a Line Profile of F	Pixel Intensities
Tool Palette B V Image Adjust Image Adjust Image Data: 14 counts Image Adjust Image Data: 14 counts Image Adjust Image Data: 14 counts Image Adjust Image Data: 100 Image Adjust	ItIZ20050624145507_003 Units: Display: Options Info Image: Counts Image: Counts Image: Counts Image: Counts
	✓ Line Profile Window Line Orientation: Horizontal ♥ Width: 1 ♥ Position: (137) ↓ ♥ ● ● X Min: 0 ● X Max; 239 ♥ Y Min: -21 ♥ Max; 58 ♥ ♥ Full Scale Logarithmic Scale x10 ⁴ Counts 4.00 − 3.00 − 2.00 − 1.00 − 0 − 50 − 100 − 100 − 100 − 100 − 100 − 100 − 150 − 200 −

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 line segment colored blue 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.

ltem	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).
Ľ.,	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.
Ē	Copies the line profile graph to the system clipboard.

ltem	Description
4	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.
(-)	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.

Table 5.5	Line Profile	Window	(continued)
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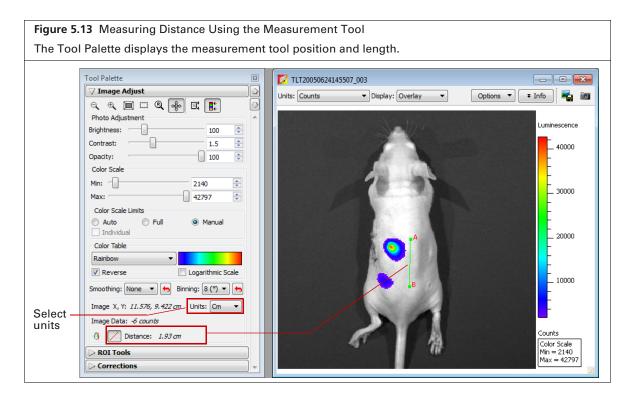
5.4 Measuring Distance

Measure distance in an image using the distance measurement tool or image crop box.

Distance Measurement Tool

Open an image, select Cm or Pixels from the Units drop-down list in the Image Adjust tools. and click the Distance Measurement button

A measurement tool (**_A**____**B**) appears on the image (Figure 5.13). The Tool Palette displays the length of the cursor.



2. Change the cursor position or size by dragging 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. Click the *intermediate button to hide the cursor.*

Image Crop Box

W

- 1. Open an image and select Cm or Pixels from the Units drop-down list in the Image Adjust tools.
- **2.** Click the **Image Crop** button in the Image Adjust tools (Figure 5.14). The mouse pointer changes a "+".
- **3.** Draw a rectangle on the image using the mouse pointer. The length of the crop box diagonal is displayed.
- **4.** Change the size or position of the crop box by dragging a handle on the crop box.

NOTE: Only the area within the crop box will be exported when you click the **E** button.

5. Click the **button** to hide the crop box.

Figure 5.14 Measuring Distance Using	a Crop Box	
Tool Palette	TLT20050624145507_003	
✓ Image Adjust ☑	Units: Counts Display: Overlay	Options 🔻 🖛 🕅
୍ କ୍ 🔲 🛄 🔍 🚸 🗉 📑 😥		
Photo Adjustment		Luminescence
Brightness: 100 荣		Lanimescence
Contrast: 1.5		40000
Opacity: 100		
Color Scale		
Min: 2140	7	L 30000
Max: 42797	and the second	
Color Scale Limits		
O Auto O Full O Manual		
		_ 20000
Color Table		
Reverse Cogarithmic Scale		
Smoothing: None 🔻 😝 Binning: 🛚 (*) 💌 😝		
Image X, Y: <i>3.227, 11.818 cm</i> Units: Cm 💌		
Image Data: 16 counts		
Distance: 2.69 cm		Counts
ROI Tools	5 71	Color Scale Min = 2140
		Max = 42797
Corrections		

5.5 Combining Images Using Image Math

The Image Math tool mathematically combines two images to create a new image. Image math is primary used to subtract tissue autofluorescence background from signal. It provides an alternative to spectral unmixing for autofluorescence background subtraction.

To perform image math, open an image sequence or a group of images. See page 96 for more details on creating a sequence from individual images.



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

Subtracting Tissue Autofluorescence

To remove tissue autofluorescence from image data, you can use a subtraction method which uses a second excitation filter that 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:

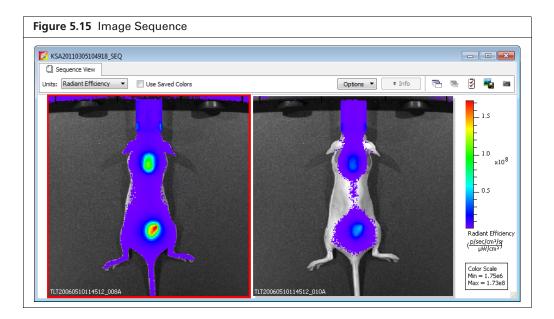
- A = primary image (acquired using the excitation filter)
- B = background image (acquired using the background filter)
- k = (primary signal/background signal)

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.

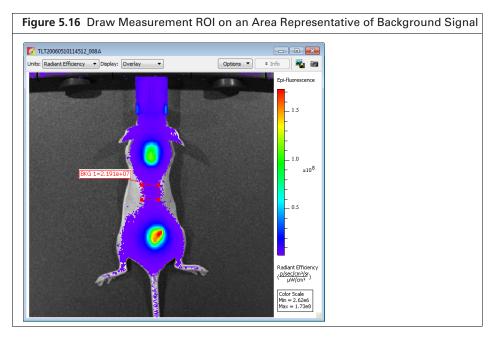
After you acquire an image sequence that includes a primary and background image, use the image math tool to subtract tissue autofluorescence. (See page 44 for more details on acquiring an image sequence.)

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:
 - **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, Figure 5.16).
- **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** \rightarrow **Image Math for <name>_SEQ** on the menu bar.
- In the Image Math window that appears, select the primary image in box A. Select the background image in box B (Figure 5.17).
 See Table 5.6 on page 70 more details on the Image Math window.

See Table 5.6 on page 79 more details on the Image Math window.

5. Select the math function 'A-B*k' in the Result drop-down list.

Image Math Window		TLT_M20060510114512_008	
	20110305104918_SEQ	Units: Counts V Display: Overlay V Option:	s 🔻 🖛 Info 🛛 📲 🛍
Α			
T20060510114512_008A T20060510114512_010A	181	TLT20060510114512_008A - TLT20060510114512_010A * 0.7	37938
120080310114312_010A			
	•		
	•		
В			Epi-fluorescence
T20060510114512_008A			
T20060510114512_010A	Counts		
			E 5000
	101		
			L 4000
olor Scale Limits for A and B			
Full O Auto			
esult			L 3000
Color Scale Limits	Counts		
Full 🔿 Auto 📄 Min = 0	Counts		
Result : A - B * k			L 2000
Result. A-D K			Counts
k: 0.787938			Counts
Compute 'k' from ROI 🔻		57	
	9		Color Scale
with Photo from:			Min = 1773 Max = 6087
Display Result For Measuring			[Indx = 0007]

- 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 \square . Alternatively, select File \rightarrow 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.

Creating a New Image

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

	Window Help JD Animation Longitudinal Study Well Plate Quantification for TI Image Overlay for TLT20060510 Colorize Image Math for TLT2006051011 Memory Tracker	114512_SEQ				
💈 Image Math Window		60510114512_SEQ	TLT20060510114512_SEQ			
Result Color Scale Limits Full Auto Result : A k : 1.4	Min = 0 = B * k • 00 ompute ½ from ROI • A •	Radiart Efficiency Radiart Efficiency	Units: Redent Efficiency	Use Saved Colors	Options 1 Info Mme 8.0007 Mme 1.7160 Mixe 1.7160 Mixe 1.7160 Mixe 2.6468	Mm= 9.0667 Mix= 1.7989

3. In the Image Math window that appears, select an image from box A and from box B (Figure 5.19).

The Image Math window shows a thumbnail of image A, image B, and the new image.

Figure 5.19 Image Math Window and New Imag	ge
	Click to export the image to a graphic file
🚺 Image Math Window	TLT_M20060510114512_005
Sequence: TLT20060510114512_SEQ	Units: Radiant Efficiency 👻 Display: Overlay 🔹 Options 👻 🖛 Info
A 11/2006/01/014512_001 11/2006/01/014512_002 11/2006/01/014512_003 11/2006/01/014512_004 11/2006/051014512_004 11/2006/051014512_006 11/2006/051046 11/2006/05104 11/2006/05104 11/2006/05104 11/2006/05104 11/2006/0510	TIT20060510114512_005 - TIT20060510114512_001 * 1.00 Epifluorescence 4.0 ×10 ⁹
Color Scale Limits Radiant Efficiency	2.0
○ Full ● Auto Min = 0 Nodek Enterty Result: A - B * k ▼ k: 1.00 ● ● ✓ with Photo from: ▲ ▼ ● Dipley Result For Measuring ● ● ●	Radark Efficiency (<u>p)sec(cm)ar</u>) (Color Scale Max = 7.7768

T T

NOTE: For more details on items in the Image Math window, see Table 5.6, page 79

- **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. Click Display Result for Measuring to view the new image.
- **7.** To save the new image:
 - **a.** Click the Save button \blacksquare . Alternatively, select File \rightarrow 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).

- 8. To export the image to a graphic file:
 - a. Click the Export button **•** (Figure 5.19).
 - **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 5.6 Image Math Window

ltem	Description
Color Ranges for A and B	Full - Choose this option to set the Max and Min values to the maximum and minimum data values in the image.
	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.
	Note: The color scale does not affect the image math result.
Color Ranges for Result	Full - See above.
Image	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.

5.6 Overlaying Multiple Images

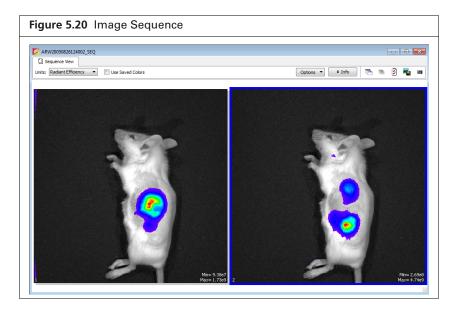
The image overlay tool provides a convenient way to view multiple reporters in one image. You can use the image overlay tool to display multiple luminescence or fluorescence images on one photographic image.



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

To overlay 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 96.)
- **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 \rightarrow 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.

🜠 Image Overlay Window		- • ×
Sequence: ARW20050826124002_SEQ Photograph: ARW20050826124002_001 ARW20050826124002_001 ARW20050826124002_002 K	Units: Radant Efficiency	
Min: 9.3867 CC	Table JorScale Type: Palette Label: ed Reverse Logarithmic Scales per Column: 3	

5. 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.

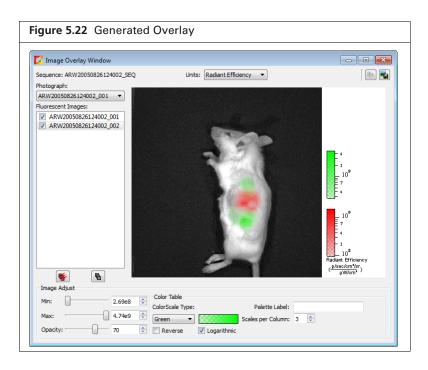


Table 5.7 Image Overlay Window

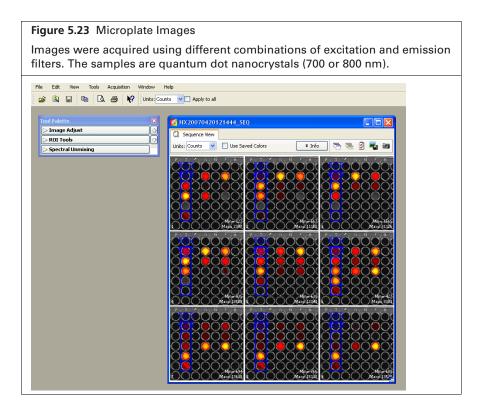
Item	Description
Units	Choose the type of units for displaying the fluorescent or luminescent image. See the concept tech note <i>Image Display and Measurement</i> for more details on measurement units.
Photograph	A drop-down list of the photographs in the image sequence.
Fluorescent or Luminescent Images	The sequence images.
RA .	Copies the overlay to the system clipboard.
-	Click to export the overlay to a graphic file.
5	Click to include all fluorescent or luminescent images in the overlay.
6	Click to remove all fluorescent or luminescent images from the photograph.
Image Adjust	Tools for adjusting the appearance of the highlighted fluorescent or luminescent image. Adjustments can only be made on one image at a time.
	Min – The minimum pixel intensity associated with the color scale for an image. Pixels less than the minimum value are not displayed.
	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.
	Opacity – Controls the opacity of the fluorescent or luminescent image.
Color Table	Tools for selecting and modifying the color scale associated with an image.
	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.
	Red \checkmark 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 \rightarrow Tech Notes on the menu bar).
	Reverse – Choose this option to reverse the selected color table.
	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.
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.

5.7 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.

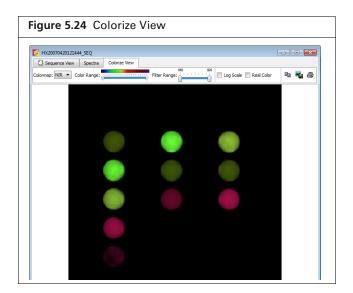
To view colorized intensity data:

1. Load an image sequence.



2. Select **Tools** \rightarrow **Colorize** on the menu bar.

The software renders each luminescent or fluorescent image in color and combines them into a single image (Figure 5.24).



	Т	able	5.8	Colorize	Tools
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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.
Ê	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).
4	Click this button to print the colorize view.

5.8 Annotating or Tagging Images

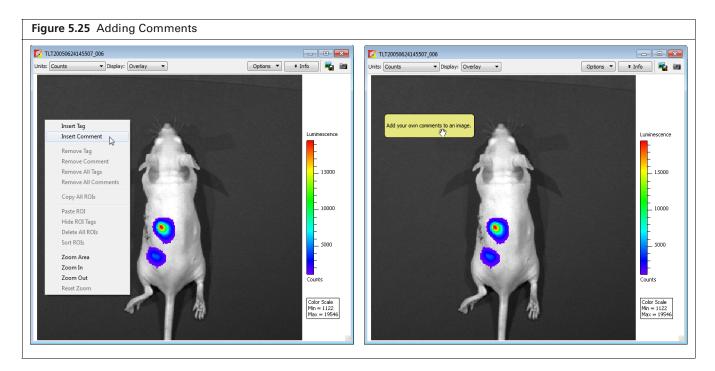
Adding Comments

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

To add comments:

- 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 5.25).
- **3.** To move a comment in an image:
 - **a.** Position the mouse pointer over the comment.
 - **b.** When the hand tool appears $\langle h \rangle$, drag the comment box, then click the mouse to set the location.
- **4.** Remove comments by doing either of the following:
 - Right-click a 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.

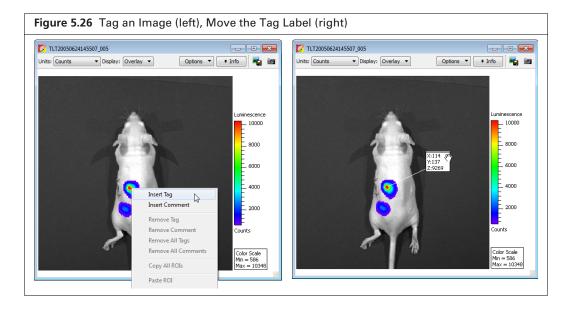


Applying Tags

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

To apply a tag:

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

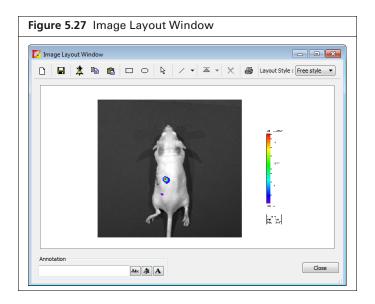


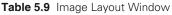
- **3.** To move a tag:
 - **a.** Position the mouse pointer over the tag.
 - **b.** When the hand tool appears $\langle \uparrow \rangle$, drag the tag, then click the mouse to set the tag location. A line between the pixel and the tag identifies the location associated with the tag.

5.9 Exporting an Image

The Image Layout window (Figure 5.27) provides an alternative way to:

- Annotate and export an image (for example, .bmp)
- Print an image
- Copy an image to the system clipboard
- Load an image or image sequence and 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 \blacksquare at a corner of the image to resize the image.
- **4.** Drag the image to reposition it in the window.





ltem	Description
E h	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.

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Table 5.9 Image Layout Window (continued)

Item	Description
Ê	Pastes the contents of the system clipboard to the Image Layout window.
	Rectangle drawing tool
0	Ellipse drawing tool
6	Pointer tool
~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	Arrow and line drawing tool
Fing to front Bring forward Send backward Send to back	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 a drop-down list.
×	Deletes the selected image.
Layout Style : Layout 2x2 👽	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.
Annotation (As) (A)	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.
Abc	Opens a dialog box that enables you to select a font or edit the font style and size.
A	Opens a color palette that enables you to select a font color or specify a custom font color.
Α	Opens a text editor that enables you to edit the selected text.

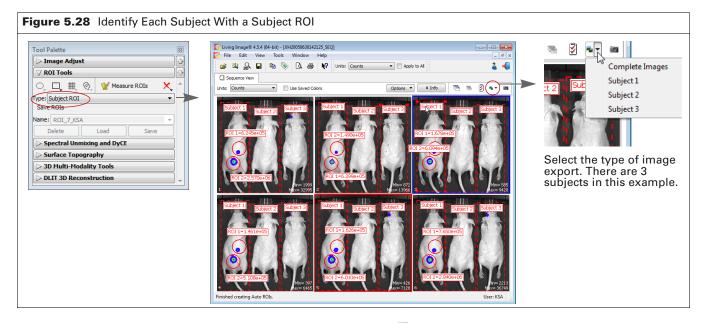
5.10 Exporting an Image Sequence

You can export sequence images or images of a user-selected subject from a sequence to a graphic file.

Preparing for Export

A subject ROI must be applied to each subject before exporting sequence images.

- **1.** Load an image sequence.
- 2. Identify each subject with a subject ROI (Figure 5.28).
 - a. Choose "Subject ROI" from the Type drop-down menu in the ROI tools
 - **b.** Click the button and select **Auto All**.

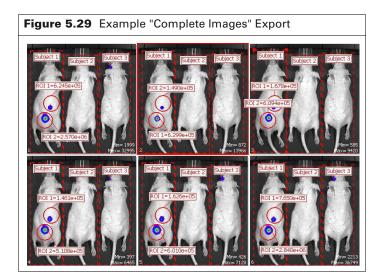


- 3. Click the Export Graphics button down arrow 📲 ▼ and choose an option from the drop-down list.
 - Export All Images All images will be exported to one file (Figure 5.29 on page 89). See below for instructions.
 - Subject "N" Images of the selected subject can be exported to either:
 - One file (Figure 5.32 on page 90). See page 89 for instructions. or
 - Separate files (Figure 5.35 on page 92). See page 91 for instructions.

Export All Images

All images of the sequence will be exported to one file (Figure 5.29).

- **NOTE:** Be sure to apply subject ROIs to the images before proceeding with the export. See *Preparing for Export* on page 88 for instructions.
- 1. Click the Export Graphics button down arrow **■ ▼** and choose "Complete Images" from the drop-down list (Figure 5.28).
- 2. Select the file location, file type, and enter a file name in the dialog box that appears. Click Save. Figure 5.28 shows an example of the exported image.



Export Images of a Subject

NOTE: Be sure to apply subject ROIs to the images before proceeding with the export. See *Preparing for Export* on page 88 for instructions.

Each image of a subject can be exported to either:

- A single file Images of the selected subject will be cropped to the subject ROI and placed sideby-side in a single row in the order in which they were acquired (Figure 5.32 on page 90).
- One image per file Each image of the selected subject will be cropped to the subject ROI and exported to separate graphic file (Figure 5.35 on page 92).

Exporting the Images to a Single File

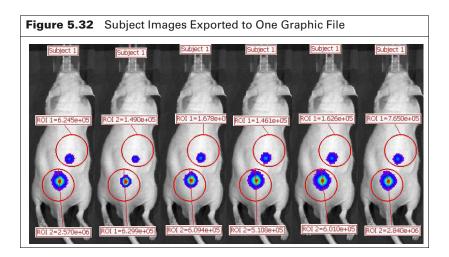
 Click the Export Graphics button down arrow s and select a subject from the drop-down list. The Export Graphics Options dialog box appears (Figure 5.30).

Figure 5.30 Export Graphics Option	
Export Graphics Options	? 💌
Entire sequence in one file	
One file per image	
Save as type JPEG Image (*.jpg)	
ОК	Cancel

- **2.** Choose the "Entire sequence in one file" option, select a graphic file type, and click **OK** (Figure 5.30).
- **3.** Select a location and enter a name for the file in the next dialog box that appears (Figure 5.31). Click **Select Folder**.

The software will export the image to a subfolder (named with the sequence identifier "xxx_SEQ") in the selected folder.

igure 5.31 Sele	ct a Folder for	the Export		
Export ROI Subject 1 where ?	▶ Temp docs ▶	- 47 Search Temp	docs P	
Organize 👻 New folder)== • 🔞	
 ☆ Favorites Desktop Desktop Recent Places Domboads trophox Cloud Drive Cloud Photos Cloud Photos 	Name	*		
Documents			×	
Folder: Exp	orted Images	Select Folder	Cancel	



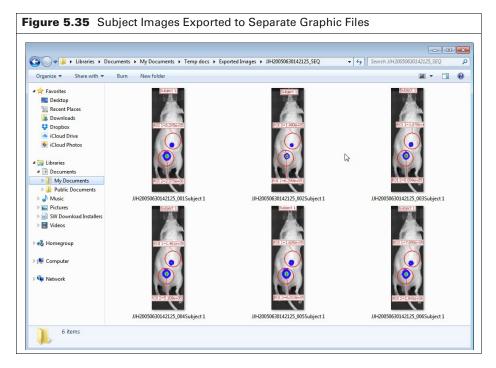
Exporting Each Image to a Separate File

 Click the Export Graphics button down arrow s and select a subject from the drop-down list. The Export Graphics Options dialog box appears (Figure 5.30).

Figure 5.33 Exp	ort Graphics Option
Export Graphics Optic	ons ? 💌
Entire sequence in or	ne file
One file per image	
Save as type	JPEG Image (*.jpg)
	OK Cancel

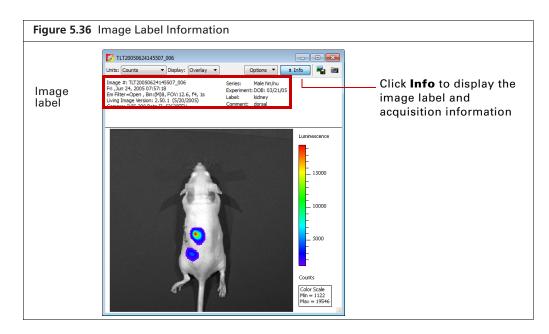
- 2. Choose the "One file per image" option, select a graphic file type, and click OK (Figure 5.33).
- **3.** Select a folder location in the next dialog box that appears. Click **Select Folder** (Figure 5.34). The software will export each image to a subfolder (named with the sequence identifier "xxx_SEQ") in the selected folder (Figure 5.35).

port ROI Subject 1 where ?	N	
🔾 🗸 🕌 « My Documents 🕨 Temp docs 🕨	👻 🍫 Search Temp	docs 🔎
ganize 🔻 New folder		8== • 🕢
Favorites Name Sector Places S	^ Images	
Documents Music Pictures		



5.11 Managing Image Information

At acquisition, the software captures image information such as camera parameters and any image label information you entered at acquisition time (Figure 5.36).

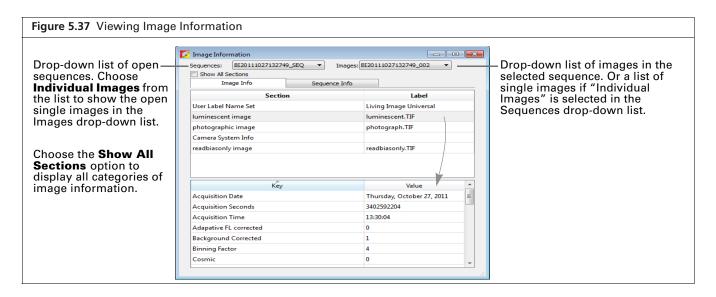


Viewing Image Information

Detailed information about images is available in the View menu.

- **1.** Open an image or sequence.
- 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 5.37).



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 (Figure 5.38).

Figure 5.38 Image Information	
✓ TLT20050624145507_005 Units: Outplay: Options Info Image Image #: TLT20050624145507_005 Series: Male Nn/nu Image #: TLT20050624145507_005 Series: Male Nn/nu Fri , Jan 24, 2005 0756-66 Series: Male Nn/nu Em Filter =640, Bint(M)8, FOV:12.6, f2, is Series: Male Nn/nu Camera: IVIS 200 Beta II, SI620EEV Series: Male Nn/nu Comment: Ide North Comment: dorsal	—Edit an entry. For example, revise the comment.
Luminescence 10000 6000 4000 2000 Counts Counts Color Scale Max = 10348	

3. Edit the label information.

To add information to the image label:

- **1.** Click the \bigotimes toolbar button. Alternatively, select **Edit** \rightarrow **Image Labels** on the menu bar.
- **2.** Select information and/or enter a comment in the Edit Image Labels box that appears (Figure 5.39).

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.

Figure 5.39	Edit Image Labels
🜠 Edit Image Labels	
UserID: BI 💌	Living Image Universal
Saved Labels: LABELS_	1 - 🐼 🖬 🗙
User	
Group	•
Z Experiment	87-MG-luc2 Intracranial Implantation 💌
Comment1	Male nu/nu, day 8, Mouse ≢4 ▼
Comment2	•
Time Point	•
Animal Number	4 🔹
Animal Strain	_
Animal Model	_
Sex Sex	· · · · · ·
View	· · · · ·
Cell Line	•
Reporter	
Treatment	_
Luc Injection Time	· · · · · · · · · · · · · · · · · · ·
IACUC Number	-
	Apply To Sequence OK Cancel

3. Click **OK** when finished.

The image information is updated.

Save the image to save the updated image label (select File → Save or File → Save As on the menu bar).

5.12 Managing Image Sequences

Editing a 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 images in the Living Image browser. (See page 57 for more details on browsing.)
- **3.** Click the **Edit** button in the image window(Figure 5.40).

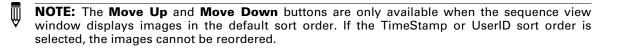
95

Figure 5.40 Editing a Sequence		
	Single image that can be a	s in the Living Image Browser dded to the sequence
🔽 TLT20050624145507_SEQ	🔀 Edit Sequence	
Sequence View Spectra	Sequence Clicks	Browser Images
Units: Counts 🔹 🔲 Use Saved Colors 🛛 Options 💌 🖛 Info	TLT20050624145507_001 TLT20050624145507_002	JJH20050630142125_001 JJH20050630142125_002
	TLT20050624145507_003 TLT20050624145507_004	JJH20050630142125_003
	TLT20050624145507_005	JJH20050630142125_004
	TLT20050624145507_006	
	Images in the	
	active sequence	Retired Images
		Retire >> Images that have
1 Min= 220 Min= 547 Min= 2140 Max= 3432 2 Max= 8622 3 Max= 36446		<< Reactivate been removed from the active
		Move Up rom the active sequence
		Move Down
		Close
4 Min= 2949 4 Min= 5182 5 Max= 10348 6 Max= 1954		

4. Choose the image(s) to add or remove (*retire*) from the sequence in the Edit Sequence box that appears (Figure 5.40).

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.



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

Creating a Sequence From Individual Images

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

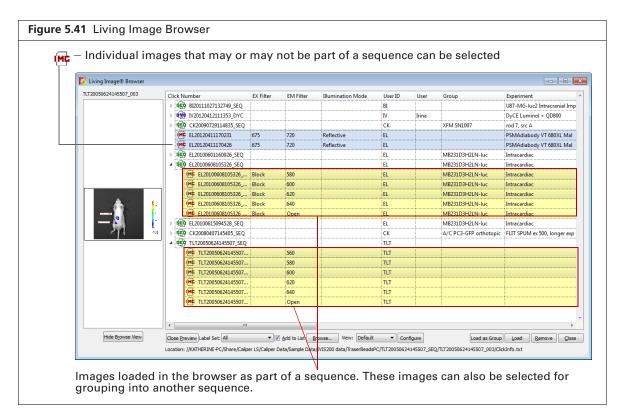


TIP: Also see the tech note *Loading Groups of Images* for helpful information (select Help \rightarrow Tech Notes on the menu bar).

1. Browse for the images of interest using the Living Image Browser. (See page 57 for more details on browsing.)



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



2. In the browser, select the images that you want to group together (Figure 5.41).

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.

- **NOTE:** Images loaded as a group are displayed by default in Counts units with the Luminescent color table (set in the Preferences). If working with fluorescent images, be sure to change the units to Radiant Efficiency and, if desired, select a different color table.
- **4.** Save the images as a sequence:
 - **a.** Click the Save button \blacksquare . Alternatively, select File \rightarrow Save on the menu bar.
 - **b.** Select a folder in the dialog box that appears and click **OK**.

6 Measuring Signal in 2D Image Data

About ROIs

Overview of ROI Tools on page 100 Measuring Signal on page 102 Measuring Background-Corrected Signal on page 106 Measuring Signals Obtained Using the Side Imager on page 109 Managing ROIs on page 112 ROI Measurements on page 118

6.1 About ROIs

This chapter explains how to measure the signal (surface intensity) within a *region of interest* (ROI) in 2D image data. Four types of ROIs are available for images (Table 6.1).

ROI Name	Description	ROI Line and Available Shapes	See Page
Measurement ROI	Measures the signal (surface intensity) in an area of an image. ROI 1[25%]=1.073e+06 ROI 2[25%]=5.324e+05	ROI 1=1.385e+02 Circle, square, grid, or contour	102
Mirror ROI	Images acquired using the Side Imager have three views: left, right, and center. Left or right images – Measure signal using a mirror ROI. Center image (non-reflected view) – Measure signal using a measurement ROI.	Mirror 1=8.755e+01	109

 Table 6.1
 Types of ROIs

Table 6.1	Types of ROIs	(continued)
-----------	---------------	-------------

ROI Name	Description	ROI Line and Available Shapes	See Page
Average Background ROI	Measures the average signal (surface intensity) in a user-specified area of an image that is considered background. Only available in image view.	BKG 1=7.033e+02	106
	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.	Circle or square	
	BKG 1=7.7819+02		
Subject ROI	Identifies a subject in an image.	Subject 1	108
	 Note: Using this type of ROI is optional. A subject ROI enables you to: Automatically associate (link) a measurement and average background ROI for background-corrected ROI measurements when there is significant autoluminescence or autofluorescence. Show the subject in which an ROI appears in the ROI table. This is helpful when one image includes multiple subjects and signals. 		

6.2 Overview of ROI Tools

The ROI tools that appear in the Tool Palette depend on the type of ROI selected from the Type dropdown list, and whether an image or sequence is active. Table 6.2 provides a description of the ROI tools. Some ROI parameters are only available if "Show Advanced Options" is selected in the General Preferences.

ROI measurements and measurement statistics are available in the ROI Measurements table which provides a convenient way to review or export ROI information. See *ROI Measurements* on page 118 for more information.

Figure 6.1 ROI Tools	
Tool Palette	
▷ Image Adjust ♥ ROI Tools	
् 🖵 🗮 🎯 🏏 Measure ROIs 🛛 🗙 📩	
Apply to Sequence Type: Measurement ROI	– Type of ROI
Save ROIs	
Name: ROI_1_WLA Delete Load Save	Adjustment for "Auto" ROIs
Auto ROI Parameters Threshold %: 23 🜩	
Lower Limit: 1.0 荣	These Auto DOL server store and service its his if #Channel
Minimum Size: 20 - 20 - 20 - 20 - 20 - 20 - 20 - 20	These Auto ROI parameters are available if "Show Advanced Options" is selected in the General
Restore Defaults Save / Load	Preferences. See Appendix E on page 273 for more details on setting Preferences.
Corrections	

Table 6.2 ROI Tools

Item	Description
Q.	Click to apply circle ROIs to an image or sequence.
D,	Click to apply square ROIs to an image or sequence.
₩.	Click to specify the grid ROI to an image or sequence. This tool is useful for an image of a multi-well culture plate or microplate.
ବ୍	Click to apply contour ROIs to an image or sequence. Select Auto All to automatically draw ROIs on an image or sequence 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. See Table 6.3 on page 104 for more details on using the auto ROI features.
W Measure ROIs	Click to display the ROI Measurements table or compute intensity signal in an ROI. See <i>ROI Measurements</i> on page 118 for more information.
Selected All	Click X to display a drop-down list of ROI delete options for the active image data. These commands delete ROIs from image data. If an image is active and the "Apply to Sequence" option is selected, the delete operation is applied to all images in the sequence.
All Measurements All Autos All BKGs All Subjects All Mirrors	Note: These commands do not delete named ROIs that are saved to the system (ROIs in the Name drop-down list).

Table 6.2 ROI Tools (continued)

ltem	Description			
Apply to Sequence	 This option is available when an image of a sequence is open. If this option is selected: ROIs created on the active image will also be created on the other images of the sequence. Adjustments to an ROI in the image will be applied to related ROIs in the other images of the sequence. See Table 6.3 on page 104 for more information about related ROIs. Deleting an ROI in the image will delete related ROIs from the other images of the sequence. 			
Туре	Choose the ROI type from the Type 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 the left or right views of an image acquired using the Side Imager, taking mirror reflection effects into account.			
Save ROIs to the	Name of an ROI or a set of ROIs			
system	Save ROIs			
	Name: ROI_5_KSA These ROI tools are only available			
	Delete Load Save when an image is active.			
	ROIs (parameters only such as coordinates, type, shape, location) can be saved to the system (per user) and used to apply the ROIs to other images. These ROIs appear in the Name drop-down list. See page 112 for instructions. Note: ROIs can also be saved with the image data. The software prompts you to save ROIs before closing image data. ROIs saved with the image do not appear in the Name drop-down list.			
Auto ROI	Parameters that specify how the auto ROI tool draws an ROI.			
Parameters	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.			
	Auto ROI Parameters Threshold %: Lower Limit: Minimum Size: Preview Use Bkg Offset Replace ROIs Restore Defaults Save / Load			
	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.			
	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.			
	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.			
	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. See page 106 for more details.			
	Replace ROIs – If this option is chosen, all auto ROIs are replaced when new ROI(s) are created.			
Auto ROI	Restore Defaults – Restores the factory-set defaults for the auto ROI parameters.			
Parameters	Save/Load – Click to save auto ROI parameter settings to the system (per user) or load parameter settings selected from the Name drop-down list.			

6.3 Measuring Signal

This section explains how to measure signal (surface intensity) within an ROI.

In sequence view, ROIs will be applied to all images of the sequence. If you want to apply ROIs to only one image of a sequence, open the image.

If an image of a sequence is active, selecting the "Apply to Sequence" option in the ROI tools will create related ROIs in the other images of the sequence as well.

ROIs that are applied to an image using the Auto All command are numbered from 1 to n (ROI 1 = brightest signal). If an image has multiple subjects and signals, it is helpful to first apply a subject ROI to each subject, then apply measurement ROIs. The ROI table will list the subject which contains each ROI.



 $\overline{\mathbb{W}}$

NOTE: Ensure that the ROI table configuration includes "Subject" and/or "Subject Label" (if the subject ROI label was renamed). See *Creating a Custom ROI Table Configuration* on page 120 for instructions.

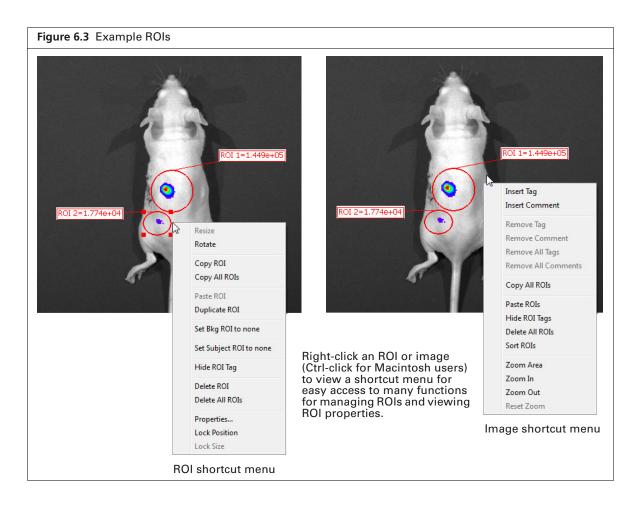
- 1. If there are multiple subjects in an image, apply subject ROIs. If not, skip to step 2.
 - a. Select Subject ROI from the Type drop-down list (Figure 6.2).
 - **b.** Click the square ROI shape , and select **Auto All** from the drop-down list.

Figure 6.2 ROI Tools – Apply Subject ROIs	
> Image Adjust > ROI Tools > □ ## ? ROI Tools > □ ## ? Measure ROIs ? Apply to Sequence ## ? ? ## ? Type: Measurement ROI ~ ? ? ## ? Sav Measure Bkg ROI Name 3 Name 3 Name Auto ROI Parameters Save 5 Save Save 5	

- 2. Select Measurement ROI from the Type drop-down list
- 3. Click an ROI shape (Circle ○, Square □, Grid ∰, or Contour ④) and make a selection from the drop-down list. If applying a grid ROI, choose the grid dimensions.

The ROIs appear on the image(s) (Figure 6.3).

NOTE: If using subject ROIs, ensure that all of the measurement ROIs for a subject are
completely within the subject ROI by resizing the subject ROI if necessary (see page 105 for
instructions on resizing ROIs). Measurement ROIs not completely within a subject ROI will not
be counted as part of the subject.



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

Table 6.3 ROI Drawing Tools

Item	Description
	Select the number of ROIs to apply. The software places up to five ROIs on an image or each image of a sequence, whichever is the active view. ROI position and size will need manual adjustment. See Table 6.4 on page 105 for instructions on adjusting ROIs.
3 4 5 Auto All Auto 1 Free Draw	Using this method in sequence view creates identical ROIs in each image of the sequence. The example below shows two ROIs that were added in sequence view. Each image of the sequence has ROI 1 and ROI 2. All ROIs named "ROI 1" in the sequence are "related" and can be moved or resized as a group in sequence view. Similarly, all of the ROIs named "ROI 2" are related. Moving or resizing related ROIs as a group is optional, an ROI can also be individually adjusted.
	ROI 1=1.446e+05 ROI 1=1.446e+05 ROI 1=4.188e+05 ROI 2=1.873e+04 Min= 2420 Min= 547 Min= 547 Min= 547
Auto All	The software automatically applies ROIs by locating the peak pixel intensities in the image and searching 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.
	ROI 2[7%]=5.134e+0 ROI 1[7%]=1.935e+06 1 ROI 4[7%]=1.331e+05 ROI 3[7%]=5.121e+05 ROI 3[7%]=5.121e+05 ROI 3[7%]=5.121e+05 ROI 3[7%]=4.816e+05 ROI 5[7%]=4.816e+05 ROI 5[7
	ROIs created in sequence view using Auto All are numbered in ascending order starting in image 1. The numbering continues from left to right across the sequence images.
	ROIs created in image view using Auto All are numbered in ascending order where ROI 1 contains the highest maximum signal and the last ROI contains the lowest maximum signal.
	Note: Manually adding ROIs afterward may affect ROI numbering. If necessary, ROIs can be renumbered by editing the ROI labels. See <i>Managing ROIs</i> on page 112 for more information.
Auto 1	Only available in image view. Automatically identifies signal and applies an ROI using the auto ROI parameter thresholds at a user-selected location.
	Drag the ring to the location for the ROI and click Create. Click Done when you finish drawing all ROIs.
Free Draw	 Only available in image view. To draw a: Circle O. or square D. ROI – Drag the pointer (+) to draw and size the ROI around the signal.
	 Contour ROI – Draw line segments around the signal by clicking the mouse pointer (+) at points that define the ROI perimeter. Right-click when the last point is near the first point of the ROI.

- 4. Adjust ROI size or position if necessary (see Table 6.4).
 - **NOTE:** The position and size of auto ROIs (ROIs created using Auto All or Auto 1) are locked by default. To unlock auto ROIs:
 - Sequence view Right-click the ROI and select **Properties** on the shortcut menu. Clear the lock options in the ROI Properties dialog box that appears (see Figure 6.12 on page 114).
 - Image view Right-click the ROI and select unlock options from the shortcut menu.

Table 6.4	Select and	Adjust ROIs
-----------	------------	-------------

Operation	Single ROI	Multiple ROIs	Related ROIs Applied in Sequence View
Select	Click the ROI border. This will clear a previous ROI selection in the image.	Shift-click the border of each ROI. This does not clear a previous ROI selection in the image.	Press and hold the Control key while you click an ROI border.
Move	Put the mouse pointer over the ROI border. Drag the ROI when the pointer changes to \bigoplus .	Select multiple ROIs and drag them when the pointer changes to $.$	Press and hold the Control key while you move a selected ROI.
	Note: If an image of a sequence is active, choose the "Apply to Sequence" option in the ROI tools to move related ROIs in the other images of the sequence as well.	Note: If an image of a sequence is active, choose the "Apply to Sequence" option in the ROI tools to move related ROIs in the other images of the sequence as well.	
Resize	 Circle, square, or contour ROI – Select the ROI. Put the mouse pointer over an ROI handle and drag the handle when the pointer changes to . Grid ROI – Select the ROI. To move the entire grid, put the mouse pointer over the grid perimeter. Drag the grid when the pointer changes to . To adjust width or height, put the mouse pointer over a grid handle and drag the handle when the pointer changes to . 		Put the mouse pointer over the ROI border and click the ROI when the pointer changes to . Put the mouse pointer over an ROI handle . When the pointer changes to . , press and hold the Control key while you drag the handle.

Click the Measure button ^{[™] Measure ROIs} in the ROI tools to show the ROI Measurements table (Figure 6.4).

The ROI Measurements table shows data for all ROIs created in images or sequences during a session (one ROI per row). The table display is automatically updated when new ROIs are created.

The table provides a convenient way to review and export ROI data. See *ROI Measurements* on page 118 for more details.

ROI Measurements							C	
ROI Measurements								
								Refresh
Image Number	ROI	Image Laye	Total Coun	Avg Count	Stdev Cour	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	Overlav	7.273e+05	8.456e+03	1.784e+03	6.007e+03	1.199e+04	-
Customized Selections Measurements Types:		Image Attribu	itee:		ROI Dimer	ncione:		Select All
Counts	•	_none_	10231		 _none_ 	▼	Сору	Select All

6. Click Yes in the prompt when closing the data to save the ROIs with the image data. Alternatively, select File \rightarrow Save on the menu bar.

The ROIs will be displayed the next time the image data is loaded.

NOTE: ROIs can also be saved to the system (per user) and applied to other images. See page 117 for instructions.

6.4 Measuring Background-Corrected Signal

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

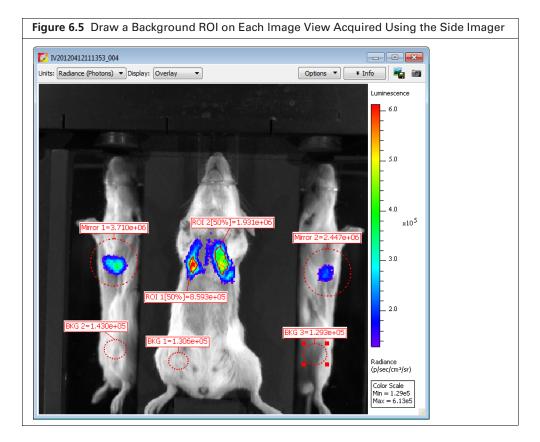
Background-corrected intensity signal = Signal in the measurement ROI - Average signal in the average background ROI

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 *Viewing Intensity Data* on page 70 and *Zooming or Panning* on page 68.

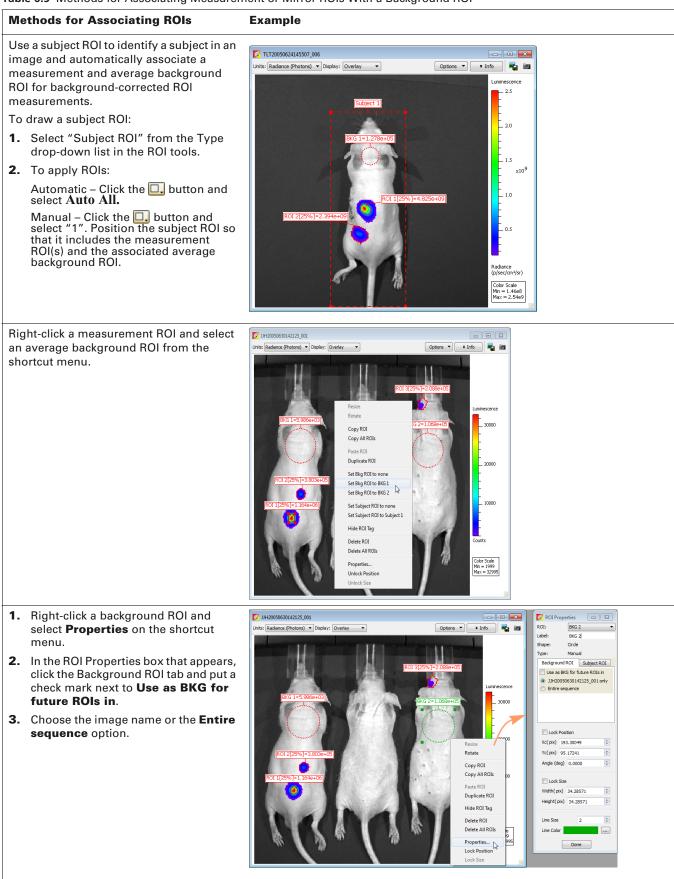
To measure background-corrected signal:

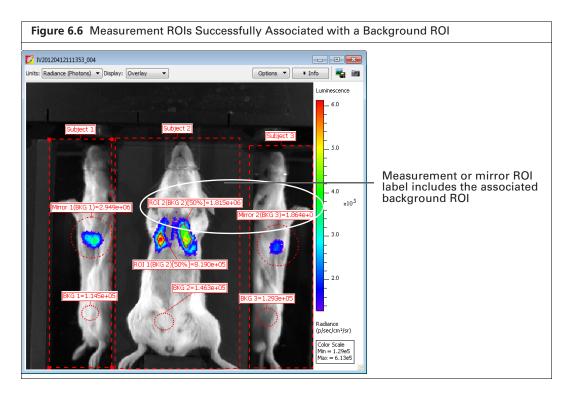
- 1. Open an image and draw one or more measurement ROIs on the subject (see page 102 for instructions).
- 2. Draw an average background ROI on the subject:
 - **a.** Select Average Bkg ROI from the Type drop-down list.
 - b. Click the Square , or Circle , button and select 1.
 The ROI is added to the image.
 - c. Adjust the ROI position or dimensions (see Table 6.4 on page 105 for instructions).
 - **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. If the image was acquired using the Side Imager, draw a background ROI on each view (Figure 6.5).



3. Associate each background ROI with a measurement ROI(s) or mirror ROI(s) using one of the methods in Table 6.5.

Table 6.5 Methods for Associating Measurement or Mirror ROIs With a Background ROI





6.5 Measuring Signals Obtained Using the Side Imager

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 102 for instructions on drawing a measurement ROI.

- **NOTE:** Fluorescent image data acquired in reflectance/epi-illumination mode must include a photograph. Do not apply mirror ROIs on the center view or measurement ROIs on the left or right mirror-reflected views. This will result in incorrect ROI measurements.
- 1. Open an image or image sequence acquired with the Side Imager.
- **2.** Select "Mirror ROI" from the Type drop-down list in the ROI tools (Figure 6.7). If analyzing a fluorescent image, choose the Photo Mask option.

Tool	Palette
	(mage Adjust
∇	ROI Tools
0	🛛 🖵 🏢 🍕 🦞 Measure ROIs 🛛 🗙 🖌
A	pply to Sequence
Туре	Mirror ROI 🔹
Sav	/e ROIs
Name	ROI_1_WLA
	Delete Load Save

- **3.** Select the ROI shape:
 - **a.** Click the **Circle O**, or **Square D**, 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 6.8).

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 6.6).

b. Click **OK**.

The mirror ROIs and intensity measurements appear on the image (Figure 6.9 on page 111). 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.

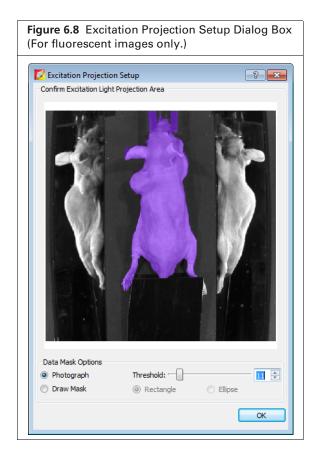


Table 6.6 Data Mask Options

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.

Option	Description
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:** Manual ROIs are numbered in the order they were created. You may want to arrange ROIs in a known order for easier comparison between images. To renumber ROIs, right-click the image and select **Sort ROIs** from 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 each image of the sequence. Sort options are only available if the ROIs have not been previously sorted.
- 5. Adjust ROI position or size following the instructions for a single ROI in Table 6.4 on page 105,
- 6. Click the Measure button V Measure ROIS.
 - The ROI measurements and table appear. See ROI Measurements on page 118 for more details.
- Click Yes in the prompt when closing the dataset to save the ROIs with the data. Alternatively, select File → Save on the menu bar.

The ROIs will be displayed the next time the image data is loaded.

NOTE: ROIs can also be saved to the system (per user) and applied to other images. See *Save ROIs to the system* on page 101 for instructions.

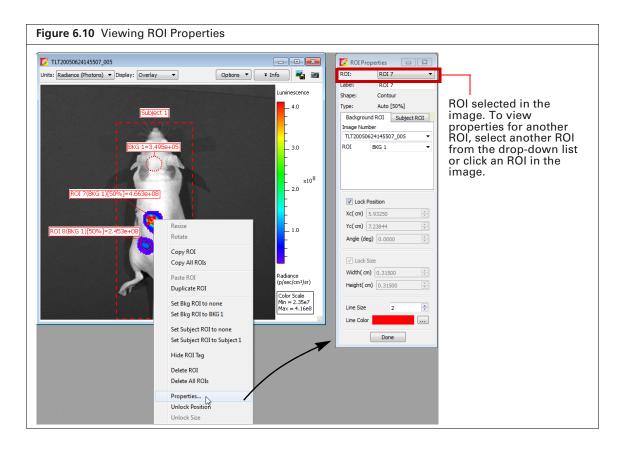
6.6 Managing ROIs

This section explains how to:

- View information about an ROI.
- Change the position of the ROI on the image.
- Edit the ROI label or line characteristics.

ROI Properties

- **1.** Do one of the following view ROI properties:
 - 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 6.12).
- **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 6.10).



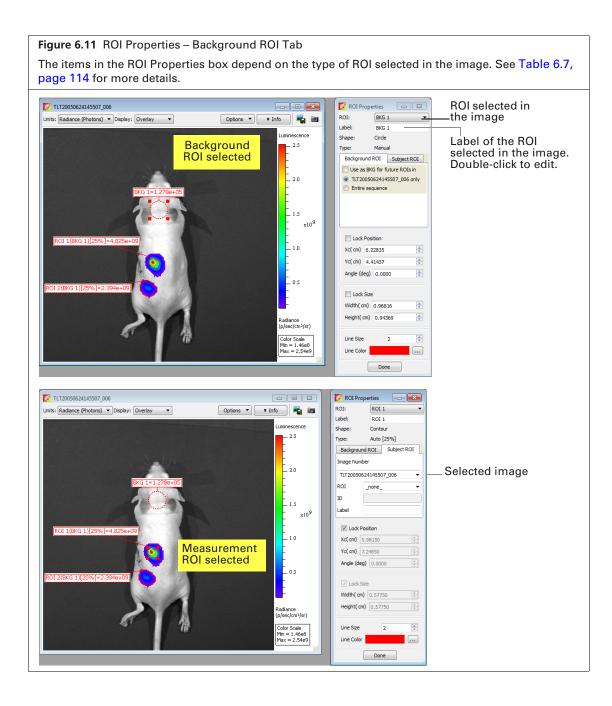


Figure 6.12 ROI Properties -	Figure 6.12 ROI Properties – Subject Tab		
The items in the ROI Propert image. See Table 6.7 for mo	ies box depend on the type of ROI selected in the		
intage. See Table 6.7 for the			
ROI Properties 📃 💌			
ROI 1	— Drop-down list of subject ROIs in the image		
Label: ROI 1			
Shape: Circle	ROI label name. Edit the label here.		
Type: Auto [24%]			
Background ROI Subject ROI			
Image Number			
TLT20050624145507_002 -			
ROI			
ID	— Enter information about the		
Label	selected ROI (optional)		
Lock Position			
Xc(pix) 113.76990			
Yc(pix) 138.05561	— Position of the selected ROI which can be adjusted using these controls		
Angle (deg) 38.6197 🚔	adjusted using these controls.		
Lock Size			
Width(pix) 8.82149	— Dimensions of the selected ROI which can also be adjusted using these controls.		
Height(pix) 7.06693			
Line Size 2			
Line Color			
Done			

Table 6.7 ROI Properties

ltem	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).
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.

Table 6.7 ROI Properties (continued)

ltem	Description
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 (see <i>ROI Dimensions</i> on page 119 for more details on setting the units). Edit this value to resize an ROI, except for ROIs applied using the "Auto AII" or "Auto 1" commands. Use the Threshold% slider to resize auto ROIs.
Height	Height (pixels or cm) of the ROI selected in the image. Edit this value to resize an ROI, except for ROIs applied using the "Auto AII" or "Auto 1" commands. Use the Threshold% slider to resize auto ROIs.
Line Size	Specifies the ROI line thickness. To change the line thickness, enter a new value or click the up/down arrows 4 .
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: Linkage between a measurement ROI and subject ROI (See Table 6.5 on page 108 for more details).
	 ROI size dimensions or position. Subject ROI ID information.

ROI Line

1. Double-click the ROI that you want to edit. The ROI Properties box appears (Figure 6.13).

Figure 6.13 Editi	ng ROI Properties		
KOI Properties Image: Colored State ROI: Image: Colored State Label: BKG 1 Share: Cride Type: Manual Badground ROI Subject ROI: Use as BKG for future ROIs in TUT20050621145007_005 only Entire sequence Entire sequence Lock Position Xc(pix) Xc(pix) 97.93335 Angle (deg) 0.0000 Lock Size Width(pix) Unc Size 2 Line Size 2 Line Color main Done Done	Select Color Ber color Cuton colors	Hut: 104 5 Red: 109 5 5ab 199 5 Green 253 5 100 225 5 Back 16 5	Brightness slider Cross hairs in the custom color field

- 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:

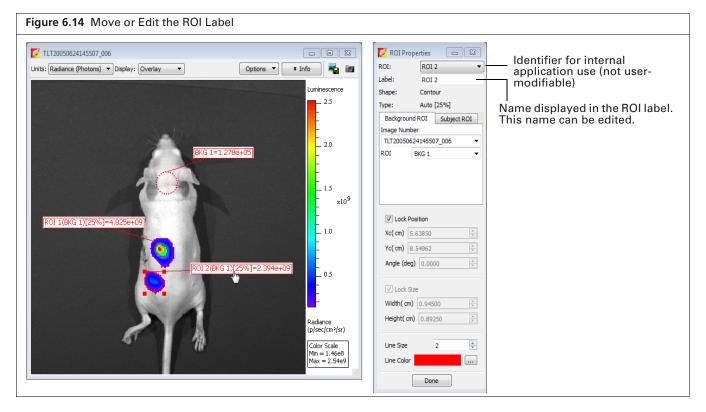
The Select Color box appears.

- **b.** Select a basic color or create a custom color for the ROI line:
 - Select a basic color Click a basic color swatch and click **OK**.
 - Define a custom color Drag the crosshairs in the custom color field, adjust the brightness slider, and click Add to Custom Colors. Click a custom color swatch and click OK.

ROI Label

To move the ROI label:

- 1. Put the mouse pointer over the ROI label.
- When the pointer becomes a th, drag the label, then click to release the label at the new location (Figure 6.14).



To edit the ROI label:

- **1.** Double-click the ROI. Alternatively, right-click the ROI (**Ctrl**-click for Macintosh users) and select Properties on the shortcut menu.
- 2. Edit the name in the Label field in the ROI Properties box that appears (Figure 6.14).

Saving ROIs to the System

Living Image software saves ROIs with an image (the software prompts you to save before closing the image). ROI measurements are saved in the AnalyzedClickInfo.txt file associated with the image.

Additionally, ROI parameter values (for example, Threshold%, Lower Limit, Minimum Size) can be saved to the system (per user) as a "named" ROI and used to apply ROIs to other images (Figure 6.15). This section explains how to save ROIs to the system.

NOTE: Before closing a dataset, the software prompts you to save ROIs with the dataset. ROIs saved with an image do not appear in the "Name" drop-down list.

- **1.** After one or more ROIs are applied to an image:
 - a. Confirm the default name or enter a new name for the ROI in the Name drop-down list.
 - **b.** Click Save (Figure 6.15).

Figure 6.15 Name and Save ROIs to the S	ystem
Tool Palette	0
Dimage Adjust	
ROI Tools	
् 🖵 🏥 🚱 💥 Measure ROIs 🛛 🗙 🖌	
Apply to Sequence	
Type: Measurement ROI 🔹	
Save ROIs	
Name: ROI_KSA	"Named" ROIs specify ROI parameter values that are used to apply ROIs to an image.
Delete Load Save	that are used to apply ROIs to an image.
Auto II/AL Docomotore	

2. To apply a "named" ROI to an image, make a selection from the Name drop-down list and click **Load** (Figure 6.15).

NOTE: If you load an ROI, then apply or delete ROIs, the **Save** button changes to **Overwrite**. Click **Overwrite** to save the ROIs using the existing name. Alternatively, enter a new name and click **Save**.

3. To delete a "named" ROI from the system (per user), select the ROI from the Name drop-down list and click **Delete** (Figure 6.16).

Figure 6.16 Delete ROIs From the System
Tool Palette ☑ ▶ Image Adjust ↓ ♥ ROI Tools ↓ ♥ □ ## • ↓ ↓ ★ ↓ Apply to Sequence ▼ Type: Measurement ROI ▼ Save ROIs ▼
Name: ROI_1

6.7 ROI Measurements

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.



TIP: See the technical note *Quantifying Image Data* for more details (select **Help** \rightarrow **Tech Notes** on the menu bar).

Viewing ROI Measurements

- 1. Load an image or sequence that includes ROIs.
- **2.** Click the <u>W Measure ROIs</u> button in the ROI tools to display the ROI measurement table (Figure 6.17). Alternatively, select View \rightarrow ROI Measurements on the menu bar.

ROI Measurements												
ROI Measurements Gr	id ROI Measu	rements									R R	efresh
Image Number	ROI	Image Laye	Total Coun	Avg Count	Stdev Cour	Min Count	Max Count	ROI Pixels	Area [ccd Pixels]	Xc [pixels]	Yc [pixels]	Widt ^ [pixe
HX20070420121444_001	ROI 1	Overlay	1.425e+06	4.167e+02	6.726e+02	-8.462e+00	2.545e+03	3420	2.189e+05	4.141e+02	1.020e+03	2.876
HX20070420121444_001	ROI 2	Overlay	1.489e+06	3.309e+02	6.061e+02	-8.642e+00	2.545e+03	4500	2.880e+05	4.168e+02	9.001e+02	2.823
HX20070420121444_001	ROI 3	Overlay	1.676e+06	3.637e+02	5.988e+02	-1.056e+01	2.545e+03	4608	2.949e+05	4.168e+02	1.152e+03	2.849
HX20070420121444_002	ROI 1	Overlay	7.261e+06	2.123e+03	3.445e+03	-2.181e+01	1.031e+04	3420	2.189e+05	4.141e+02	1.020e+03	2.876
HX20070420121444_002	ROI 2	Overlay	7.572e+06	1.683e+03	3.107e+03	-2.181e+01	1.031e+04	4500	2.880e+05	4.168e+02	9.001e+02	2.823
•												•
Customized Selections Measurements Types: Counts				age Attributes Possible Value				ROI • Pix	Dimensions: els 🔹	Сору	Selec	ct All

Table 6.8	ROI	Measurements	Table

ltem	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. Custom ROI table configurations also appear in this drop-down list. See <i>Configuring the ROI Measurements Table</i> on page 120 for instructions on creating a custom table.
None	Excludes ROI measurements from the table.
Counts (luminescence and	Includes Total Counts, Avg Counts, Stdev Counts, Min Counts, and Max Counts in the table.
fluorescence)	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 Image <i>Display and Measurement</i> for more details on count units (select Help \rightarrow Tech Notes on the menu bar).

Table 6.8	ROI Measurements	Table	(continued)
-----------	-------------------------	-------	-------------

Item	Description
Radiance (Photons) (fluorescence and	Total Flux (photons/sec) = the radiance (photons/sec/cm ² /steradian) in each pixel summed or integrated over the ROI area (cm ²) x 4π .
luminescence)	Average Radiance = the sum of the radiance from each pixel inside the ROI/ number of pixels or super pixels (photons/sec/cm²/sr).
	Stdev Radiance = standard deviation of the pixel radiance inside the ROI
	Min Radiance = lowest radiance for a pixel inside the ROI.
	Max Radiance = highest radiance for a pixel inside the ROI.
	Tip: See the tech note <i>Image Display and Measurement</i> for more details on photon units (select Help \rightarrow Tech Notes on the menu bar).
Radiant Efficiency (fluorescence)	Epi-fluorescence - Fluorescence emission radiance per incident excitation intensity: p/sec/cm²/sr/ μ W/cm²
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.
Сору	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	Displays the Save Measurements box so that the data can be saved to a .txt or .csv file.
	Note: Grid ROI measurements exported to a .csv file can be opened in a spreadsheet application like Microsoft [®] Excel [®] .
Close	Closes the ROI Measurements table.

W

Configuring the ROI Measurements Table

You can customize the data and information (column headers) in the ROI Measurements table (Figure 6.18). 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.

ROI Measurements												2 23
ROI Measurements Grid	d ROI Measu	urements										
											R	efresh
mage Number	ROI	Image Laye	Total Coun	Avg Count	Stdev Cour	Min Count	Max Count	ROI Pixels	Area [ccd Pixels]	Xc [pixels]	Yc [pixels]	Widt 1
HX20070420121444_001	ROI 1	Overlay	1.425e+06	4.167e+02	6.726e+02	-8.462e+00	2.545e+03	3420			1.020e+03	2.876
HX20070420121444_001	ROI 2	Overlay	1.489e+06	3.309e+02	6.061e+02	-8.642e+00	2.545e+03	4500	2.880e+05	4.168e+02	9.001e+02	2.823
HX20070420121444_001	ROI 3	Overlay	1.676e+06	3.637e+02	5.988e+02	-1.056e+01	2.545e+03	4608	2.949e+05	4.168e+02	1.152e+03	2.849
HX20070420121444_002	ROI 1	Overlay	7.261e+06	2.123e+03	3.445e+03	-2.181e+01	1.031e+04	3420	2.189e+05	4.141e+02	1.020e+03	2.876
HX20070420121444_002	ROI 2	Overlay	7.572e+06	1.683e+03	3.107e+03	-2.181e+01	1.031e+04	4500	2.880e+05	4.168e+02	9.001e+02	2.823
•												Þ
Customized Selections												
Measurements Types: Counts				ge Attributes Possible Value				▼ RO	Dimensions:	Сору	Sele	ct All

- **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 6.18). 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. Click **Configure** in the ROI Measurements table.

The Configure Measurements box appears (Figure 6.19).

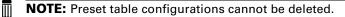
Figure 6.19 Configure Meas	surements Dialog Bo	X		
Configure Measurements User Lists: Counts National Strain Analysis Comment Analysis Comment Analysis User ID Angle Animal Model Animal Model Animal Strain Area [ccd Pixels] Area [cm ²] Avg Dark Charge Counts Avg Efluorescent Bkg Counts Avg Radiante [p/s/cm ² , sr] Avg Radiant Efficiency [p/s/cm ² , Binning Cell Line	me: Counts Customize Add> Remove <	Update (Pelete Move Up Move Down Close	—— Column headers in the active ROI table

- 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.

The custom configuration is the added to the Measurements Types drop-down list in the ROI Measurements table (Figure 6.18). If a custom configuration is saved with the data, it becomes the default configuration.

To delete a custom table configuration:

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



Copying or Exporting ROI Measurements

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 (Figure 6.20).
- 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.

ROI Measurements	etic ROI Mea	surements Plo	t Kinetic ROI Me	asurements					~
								Refres	h
mage Number	ROI	Image Layer	Total Counts	Avg Counts	Stdev Counts	Min Counts	Max Counts	Threshold	Ţ
TLT20050624145507_001	ROI 1	Overlay	6.536e+03	2.334e+02	4.395e+01	1.723e+02	3.301e+02	50	1
TLT20050624145507_002	ROI 2	Overlay	2.653e+04	6. <u>982e+02</u>	1.402e+02	4.730e+02	9.393e+02	50	
LT20050624145507_003	ROI 3	Overlay	8.290e+05	2. Copy	Ctrl+C	209e+04	4.280e+04	50	ļ
LT20050624145507_003	ROI 4	Overlay	2.687e+05	 Select 	t All Ctrl+A	042e+03	6.048e+03	50	
LT20050624145507_004	ROI 5	Overlay	1.621e+06	4.053e+04	8.599e+03	2.976e+04	5.899e+04	50	
LT20050624145507_004	ROI 6	Overlay	7.273e+05	8.456e+03	1.784e+03	6.007e+03	1.199e+04	50	
•								4	
Customized Selections Measurements Types: Counts			ttributes: ble Values		ROI Dir	nensions:	Сору	Select All	_

7 Reconstructing 3D Sources

About 3D Reconstruction Reconstructing Luminescent Sources on page 124 Reconstructing Fluorescent Sources on page 130 Manual 3D Reconstruction on page 134 3D Reconstruction Results on page 139 Checking 3D Reconstruction Quality on page 141 Troubleshooting on page 145

7.1 About 3D Reconstruction

Living Image DLIT and FLIT algorithms analyze 2-dimensional optical image data to render 3dimensional (3D) reconstructions of luminescent or fluorescent sources located inside an animal (*tomographic analysis*).

3D Reconstruction Algorithm	Description	See Page
Diffuse Tomography (DLIT)	DLIT provides a complete 3D reconstruction of the luminescent source distribution within a subject. DLIT places no constraints on the geometry or spatial variation of the source strength throughout the volume. DLIT is well-suited for analyzing complex and spatially extended luminescent sources. The 3D reconstruction is presented as voxels. If a luminescent quantification database is available, the number of cells per source can be determined in addition to source intensity (photons/sec).	127
Fluorescent Tomography (FLIT)	FLIT provides a complete 3D reconstruction of the fluorescent source distribution within a subject. The 3D reconstruction is presented as voxels. If a fluorescent quantification database is available, the number of fluorophore molecules or cells per source can be determined in addition to the total fluorescence yield.	132

Input data for 3D reconstruction of luminescent or fluorescent light sources include:

- An appropriate image sequence. Use the Imaging Wizard to set up and acquire a DLIT or FLIT sequence (acquisition instructions for DLIT on page 124, FLIT on page 130). If not using the Imaging Wizard, see *Manual Sequence Setup* on page 134 for instructions.
- A *surface* (a 3D reconstruction of the animal surface, also called a *topography*). Living Image software will automatically generate a surface during reconstruction or you can manually create and/or select a surface using the Surface Topography tools.
- A quantification database (optional). If a quantification database is available, it is possible to determine the number of cells in a luminescent source or the number of cells or dye molecules in a fluorescent source. A quantification database is derived from an analysis of images of known serial dilutions of luminescent cells, fluorescent cells, or dye molecules in a well plate. See Appendix C on page 260 for more details on generating a database.



TIP: See the technical note *DLIT and FLIT Reconstruction of Sources* for more details on the DLIT or FLIT algorithm (select **Help** \rightarrow **Tech Notes** on the menu bar).

There are two reconstruction options:

• "Single-Click" – Uses the current settings in the Properties tab of the 3D Reconstruction tools (Figure 7.6). You do not need to manually create or select a surface (as in Living Image 4.4 and earlier versions).

for "

- **NOTE:** Living Image software uses the following rules to automatically generate or select a surface for "single-click" reconstruction:
- If the dataset has not yet been analyzed (no 3D reconstruction results or surface saved with the data), the software will automatically create a surface. When the analysis results are saved, the surface will be saved (available as "SURFACE_1" in the Surface Topography tools).
- If the dataset was previously analyzed, but no surface is loaded, the software loads and uses the surface most recently saved with the dataset.
- If the dataset was previously analyzed and a surface is loaded and displayed in the 3D View, the software uses that surface for the reconstruction.
- Manual Reconstruction This is the method used in Living Image 4.4 software and earlier versions. Use manual reconstruction if you want to select particular pixel data to reconstruct or choose a surface. See *Manual 3D Reconstruction* on page 134 for instructions.

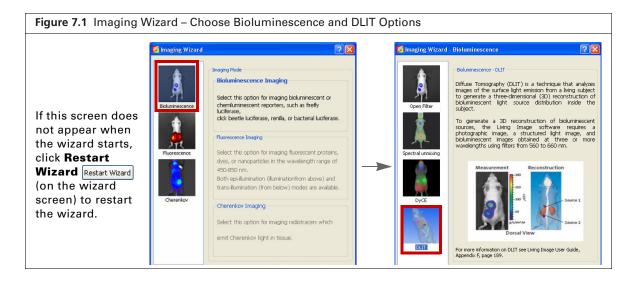
7.2 Reconstructing Luminescent Sources

NOTE: It is important to consider the luciferin kinetic profile when planning the image sequence acquisition. The DLIT algorithm assumes a stable luciferin kinetic profile. Therefore, to optimize the signal for DLIT 3D reconstruction, carefully plan the start and finish of image acquisition and ration the exposure time at each emission filter so that the sequence is acquired during the flattest region of the luciferin kinetic profile. See the technical note *Kinetic Analysis of Bioluminescent Sources* for a protocol to determine a kinetic curve for luciferin.

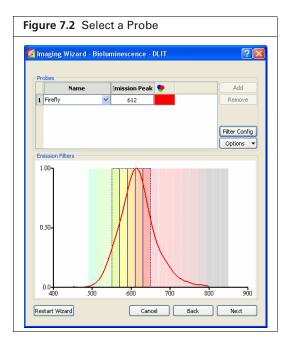
Acquire a Luminescent Sequence

NOTE: The IVIS Spectrum should be initialized and the temperature locked before setting the imaging parameters. See page 19 for more details.

- 1. Start the Imaging Wizard. See page 44 for instructions.
- **2.** Double-click the Bioluminescence option. Double-click the DLIT option in the next screen (Figure 7.1).



3. Select a probe from the Name drop-down list and click Next (Figure 7.2).



- **4.** In the next screen (Figure 7.3):
 - a. Select the type of imaging subject.
 - **b.** Choose the Auto Settings option for the exposure parameters.
 - c. Select a field of view from the drop-down list.

Figure 7.3 Imaging Wizard – Bioluminescence	DLIT
🚺 Imaging Wizard - Bioluminescence - DLIT	
Imaging Subject: Mouse Exposure Parameters Auto Settings Manual Settings	
Uminescent	
Field of View C - 12.8 cm Focus	
Subject Height: 1.50 Cm Focus: use subject height Options Time Series Study Total number of segments: 1 Delay between segments: 0,0 Mins	
Restart Wizard Cancel Back Next	

- **5.** 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.

6. If performing a time series study, choose the Time Series Study option. Enter the number of segments to acquire and the delay between segment acquisition. A segment is an image sequence.

7. Click Next.

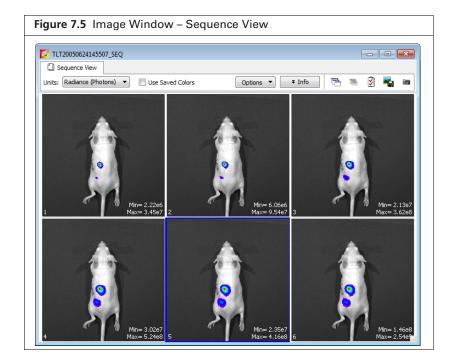
The specified sequence appears in the sequence table (Figure 7.4).

Figure 7.4 Sequence Setup	Complete
Imaging Wizard - Biolaminescence - Di II Topore Remains O Ando Settings Linescore Unescore	
Field of Yeav C - 12.8 cm V Focus Subject Height 1.50 C cm Focus: use subject height V	Imaging Node Exposure Time Brining F/Stop Exclusion Filter Emission Filter Imaging Node Exposure Time Subject: Mouse Problems v Imaging Node Exposure Time Brining F/Stop Exclusion Filter Imaging Node Exposure Time Subject: Mouse v Problems v Imaging Node Exposure Time Brining F/Stop Exclusion Filter Imaging Node Exposure Time Problems v Problems v Imaging Node Exposure Time Brining F/Stop Exclusion Filter Imaging Node Exposure Time Problems v Problems v Imaging Node Exposure Time Brook 640 Imaging Node Exposure Time E
Options Total number of segments: Delay between segmen	Image: Constraint of the state of
	Ughts Z Algoment Grid Batch Sequences 3 1 2 Advo Medium 1 Block 600 No C 1.50
	Field of Verv: C V System Status Acquire Sequence Acquire Sequence
	Service 12.8 cm Imaging Woard Subject health: [1.50 C cm 4 Image Solution
Restart Wizard Cancel Back Next	Focus: Les subject height 👻 Temperature: Locked Locked Initialize Number of Segments: 1 C Delay: 0.0 C min Apply to All X Remove V 🖉 Update C Insert C Add

8. Acquire the sequence following the instructions on page 46.

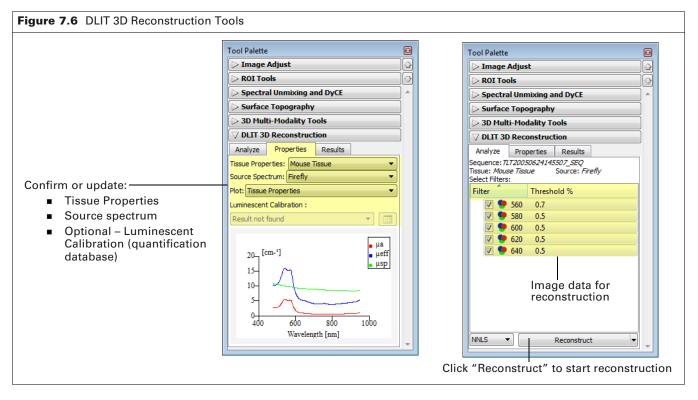
The image window appears when acquisition is completed (Figure 7.5). See Table 4.2 on page 30 for more details on the Image window.

Next, proceed to "Single-Click" DLIT 3D Reconstruction on page 127.



"Single-Click" DLIT 3D Reconstruction

- **NOTE:** If the image sequence includes multiple subjects, for example two mice, perform manual 3D reconstruction for each subject separately (see page 135 for instructions). "Single-click" reconstruction is not available for this type of image data.
- **1.** Load a luminescent image sequence.
- **2.** Confirm that the correct tissue properties, source spectrum, and if using, quantification database are selected in the Properties tab (Figure 7.6). If necessary, change a property by making a selection from the drop-down lists.
- **3.** Click **Reconstruct** (Figure 7.6).
- 4. Save reconstruction results (see *Managing 3D Reconstruction Results* on page 140).



Reconstruction usually requires less than one minute, depending on the reconstruction volume, parameter settings, and computer performance. After the analysis is complete, the:

- 3D View window displays the animal surface and the reconstructed sources (Figure 7.7).
- 3D Optical Tools appear in the Tool Palette (see Table 7.1 for an overview of the tools).
- Results tab of the DLIT 3D Reconstruction tools displays the results data and the algorithm parameter values (see Table 7.6 on page 139 for more details).

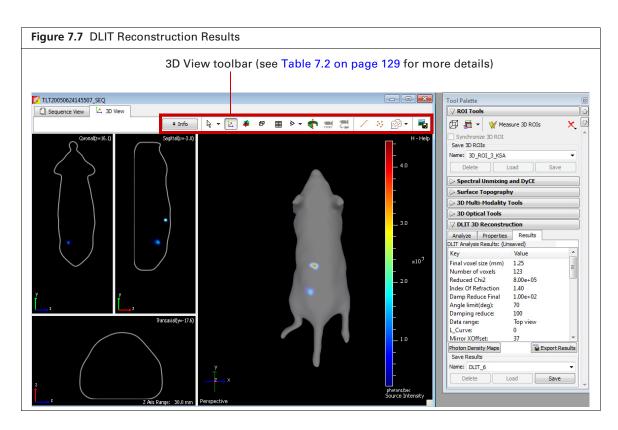


Table 7.1 3D Optical Tools

Tool Palette	3D Optical Tools	Functions	See Page
Tool Palette	Surface Tools	Adjust the appearance of the reconstructed animal surface and photon density maps.	143
Spectral Unmixing and DyCE Surface Topography 30 Multi-Modality Tools Joptical Tools	Source Tools	Make source measurements, adjust the appearance of reconstructed sources, export voxel measurements.	146
Surface Source Registration	Registration Tools	Display organs on the reconstructed surface, adjust the location or scale of organs on the surface, import an organ atlas.	156
Clisplay Photon Density Map Apply: Simulated Wavelengths 560 Intensity: Color Table Rainbow Reverse Log Scale	Animate Tools Select Tools \rightarrow 3D Animation on the menu bar to view these tools.	Display preset animations of the 3D View scene. Enables you to create custom animations and record an animation to a movie file.	162

Figure 7.8 3D View Toolbar							
म Info 🛛 🔓 👻 💆	ø	Ⅲ	» - 💠	= [/	22	🔊 •

Table 7.2 3D View Toolbar

Tool	Description
Info	Click to show or hide the image label. The image label includes information you enter in the Edit Image Labels dialog box (see Figure 4.6 on page 29) and other information automatically recorded by the software.
Image Tools	A drop-down list of tools for viewing and working with the surface or 3D reconstruction.
L ₂	$\langle \!\!\!\! \P \rangle$ or $\left. \!$
Q	\bigoplus - Moves the surface in the x or y-axis direction.
+ (1)	\bigcirc - Zooms in or out on the image. To zoom in, right-click (Cmd key (apple key) +click for Macintosh users) and drag the \oplus toward the bottom of the window. To zoom out, right-click and drag the \ominus toward the top of the window.
乜	Displays the x,y,z-axis display in the 3D view window.
4	Displays coronal, sagittal, and transaxial cross-sections through the subject in the 3D view window.
Ø	Displays a bounding box around the subject.
	Displays a grid under the subject.
¢ -	Select this tool from the drop-down list to change the view perspective (top, bottom, left, right, front, back, or perspective view). See Figure 8.14 on page 160 for examples.
	Select this tool from the drop-down list to display the perspective view.
†	Rotates the 3D reconstruction in the 3D view window (<i>3D scene</i>). Click the + or - key to increase or decrease the rotation speed. To stop the rotation, click the 3D scene or the the state of the stat
5	If multiple 3D reconstructions are loaded, click this button in one of the 3D views to turn on synchronization. All other 3D views will reset to display the same perspective as the active data. Click the button again to turn off synchronization. See page 154 for more details on synchronizing 3D views.
	Note: This button is only available if multiple 3D reconstructions are loaded.
-	Applies the 3D view perspective of the active dataset to all loaded 3D reconstructions.
G	Note: This button is only available if multiple 3D reconstructions are loaded and synchronization is turned off.
/	Click to show or hide measurement tools in the coronal, sagittal, or transaxial views. Click and drag the green handle () at either end of a measurement tool to resize and reposition it.
***	Use this tool to obtain source measurements (for example, total flux, volume, center of mass, host organ). See <i>Source Quantitation</i> on page 149 for instructions.
- G	Copies or pastes voxels or a source surface so that DLIT and FLIT reconstructions can be displayed on one surface. See page 155 for more details.
-	Enables you to save the 3D view to a graphic file (for example, .jpg).

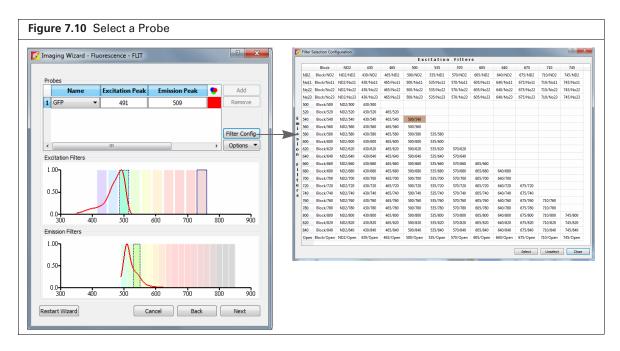
7.3 Reconstructing Fluorescent Sources

Acquire a Fluorescent Sequence

- **NOTE:** The IVIS Spectrum should be initialized and the temperature locked before setting the imaging parameters. See page 19 for more details.
- 1. Start the Imaging Wizard. See page 44 for instructions.
- **2.** Double-click the Fluorescence option. Double-click the FLIT option in the next screen (Figure 7.9).

Figure 7.9 Imaging Wizard – C	noose Fluorescence and FLIT (Options	
If this screen does not appear when the wizard starts, click Restart Wizard (on the wizard screen) to restart the wizard.	Timaging Mode Bioluminescent reporters, such as freihy Locificase, Locificas	Processor File Far Spectral Universe Option Option	ructing This oode.

3. Select a probe from the Name drop-down list and click Next (Figure 7.10).



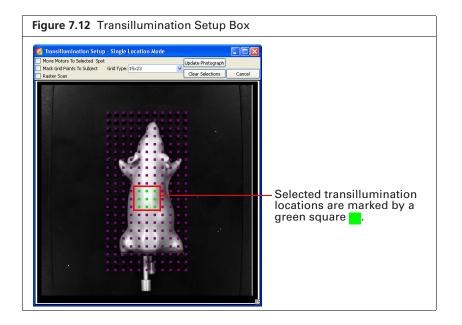
- **4.** In the next screen (Figure 7.11):
 - a. Select the type of imaging subject.

- **b.** Choose the Auto Settings option for the exposure parameters.
- c. Select a field of view from the drop-down list.

Figure 7.11 Imaging W	2.1.1
Imaging Subject: Mouse	
 Auto Settings 	O Manual Settings
Fluorescent	
🚺 🗹 Photograph	
Field of View	
C - 12.8 cm	
Subject Height: 1.50 🗘 cm	Focus: use subject height 💙
Options Time Series Study Total number of segments:	
Fluorescence	
Transilumination Setup Trans-Illumination locati	ons selected - 35
Restart Wizard Cancel	Back Next

- 5. 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.
- **6.** If performing a time series study, choose the Time Series Study option. Enter the number of segments to acquire and the delay between segment acquisition. A segment is an image sequence.
- 7. Select the transillumination locations.
 - a. Click Transillumination Setup.
 - **b.** Choose the transillumination locations in the Transillumination Setup box that appears (Figure 7.12).

See Table 4.3 on page 40 for more details on Transillumination Setup.



8. Click Next.

The specified sequence appears in the sequence table (Figure 7.13).

Imaging Wizard - Fluorescence - FLIT									
maging Subject: Mouse									
Exposure Parameters Auto Settings Manual Settings									
European Fluorescent									
1 🗹 Photograph									
Field of Wew	🜠 IVIS Acquisition Control Panel								
C - 12.8 cm V ious subject Height: 1.50 C cm Focus: use subject height V	Imaging Mode Exposure Time Binning F/Stop	5/0 • 020 •	Display F	hotographic Settin	gs		Sub	ect: Mouse	Probes
otions	Fluorescent V Transilumination V Normalized Setup		Mode Exposu	re Binning FSto	o Excitation En	hission Lamp L	evel Structure	FOV Heia	nt Transillumination
Time Series Study Total number of segments:	🚺 🗹 Photograph 0.20 🗘 🛛 Medium 👻 8 💌 🗹	Reuse	1 Auto	Medium 2	570 62		Yes	C 1.50	15×23:8, 10
Delay between segments: 0.0 C Mins			2 Auto	Medium 2	570 62		No	C 1.50	15x23:8, 9
	Structure		3 Auto	Medium 2	570 62		No	C 1.50	15×23:8, 8
	🗹 Overlay 📄 Lights 🗹 Alignment Grid	Batch Sequences	4 Auto	Medium 2	570 62	-	No	C 1.50	15×23:8, 7
Flucrescence			1 1000		570 62		No	C 1.50	15x23:8, 6
Fluorescence	Field of View: C 💌 System Status		E E		570 02		No		15x23:8, 5
Flucrescence	Field of Yiew: C System Status	Acquire Sequence	S Auto	Medium 2	F70 (2				
Fluxescence	Field of Yiew: C System Status MIS Idle	Acquire Sequence	6 🚺 Auto	Medium 2	570 62	-		C 1.50	
Flucrescence		Acquire Sequence Imaging Wizard	6 1 Auto 7 1 Auto	Medium 2 Medium 2	570 62) High	No	C 1.50	15×23:8, 4
Fluxescence	MIS Idle		6 🚺 Auto	Medium 2		D High D High			

9. Acquire the sequence following the instructions on page 46. The image window appears when acquisition is completed (Figure 7.5). See Table 4.2 on page 30 for more details on the Image window.

Next proceed to "Single-Click" FLIT 3D Reconstruction on page 132.

"Single-Click" FLIT 3D Reconstruction

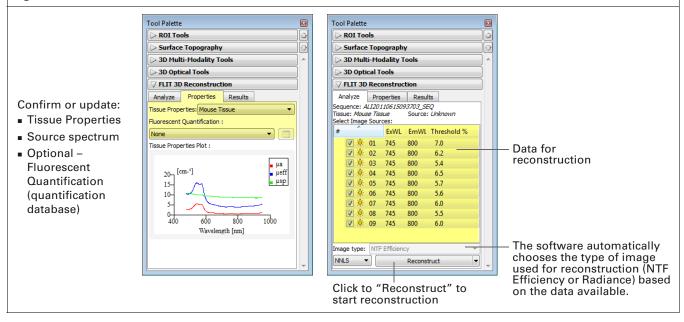
NOTE: If working with large datasets (e.g. high resolution FLIT datasets, longitudinal studies with many subjects), a 64-bit analysis workstation with 16 GB memory capacity is recommended.

- **NOTE:** If the image sequence includes multiple subjects, for example two mice, perform manual 3D reconstruction for each subject separately (see page 135 for instructions). "Single-click" reconstruction is not available for this type of image data.
- **1.** Load a fluorescent image sequence.
- **2.** Confirm that the default properties for reconstruction are correct (tissue properties and, if using, fluorescent quantification database) (Figure 7.6). If necessary, change a property by making a selection from the drop-down lists.



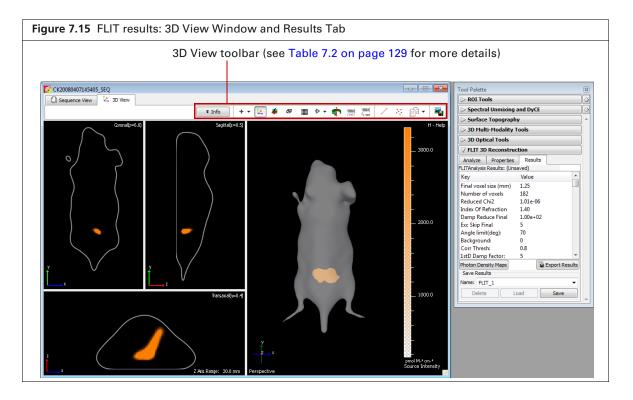
- **NOTE:** The software automatically chooses the type of image used for reconstruction (NTF Efficiency or Radiance) based on the available image data (Figure 7.14).
- NTF Efficiency image is the default, if both types of image data are available.
- Radiance image is selected if an NTF Efficiency image (transmission.tif) is not available in the click image folder.
- If both types of image data are available and you want to use Radiance images for reconstruction, perform a manual reconstruction (see page 135 for instructions).
- **3.** Click **Reconstruct** (Figure 7.6).
- 4. Save reconstruction results (see *Managing 3D Reconstruction Results* on page 140).





Reconstruction time depends on the reconstruction volume, parameter settings, and computer performance. After the analysis is finished, the:

- 3D View window displays the animal surface and the reconstructed sources (Figure 7.15).
- 3D Optical Tools appear in the Tool Palette (see Table 7.1 on page 128 for an overview of the tools).
- Results tab displays the results data and the algorithm parameter values (see Table 7.6 on page 139 for more details).



7.4 Manual 3D Reconstruction

This section explains how to:

- Manually set up a luminescence or fluorescence image sequence if you will not be using the Imaging Wizard.
- Perform DLIT or FLIT "manual reconstruction" which enables you to create or select a particular surface and include or exclude user-selected image data from the reconstruction.

Manual Sequence Setup

Table 7.3 lists the IVIS Spectrum filters available for DLIT or FLIT tomography.

Table 7.3 IVIS Spectrum Filters Available for Luminescence Tomography

Filters	Range	Bandwidth
10 excitation filters	415-760 nm	30 nm
18 emission filters	490-850 nm	20 nm

Luminescence Sequence for DLIT

Set up a sequence that includes:

- A structured light image.
- Optical data from at least two different emission filters (560 660 nm), at a minimum:
 - Emission filter #1: Photographic, luminescent.
 - Emission filter #2: Luminescent image.

Analyzing more optical images usually produces more accurate results. Table 7.4 shows the recommended optical image sequence.

Table 7.4 Manual Sequence Setup – Recommended DLIT Optical Image Sequence

Image Type	Emission Filter Options									
	560	580	600	620	640	660				
Photograph	1	Select the Reuse option in the control panel.								
Luminescent	1	1	1	1	1	~				

NOTE: It is recommended to set the same binning level for all of the luminescent images.

Fluorescence Image Sequence for FLIT

Use transillumination on the IVIS Spectrum and the same excitation and emission filters at a minimum of four source locations which form a rectangle. Acquire the following images:

- Fluorescent image and photograph at the first transillumination location.
- Fluorescent image at the remaining transillumination locations.
- A structured light image.

Figure 7.16 shows an example image sequence.

2	Seq-1	Display Ph	otographic :	Settings					Subjec	t: Mouse 🔻	Probes
	Mode	Exposure	Binning	FStop	Excitation	Emission	Lamp Level	FO¥	Height	Transillumination	
1	* -	Auto	Medium	2	675	720	High	С	1.50	9×19:4, 8	
2	*	Auto	Medium	2	675	720	High	С	1.50	9×19:4, 7	
3	*	Auto	Medium	2	675	720	High	С	1.50	9×19:4, 6	
4	*	Auto	Medium	2	675	720	High	С	1.50	9×19:4, 5	
5	X	Auto	Medium	2	675	720	High	С	1.50	9×19:4, 4	
6	x	Auto	Medium	2	675	720	High	С	1.50	9×19:4, 3	
7	*	Auto	Medium	2	675	720	High	С	1.50	9×19:4, 2	
8	1	Auto	Medium	2	675	720	Hiah	С	1.50	9×19:5.8	

Manual 3D Reconstruction

- 1. Load a DLIT or FLIT image sequence.
- **2.** Generate or load a surface using the Surface Topography tools. See Appendix D on page 267 for instructions.

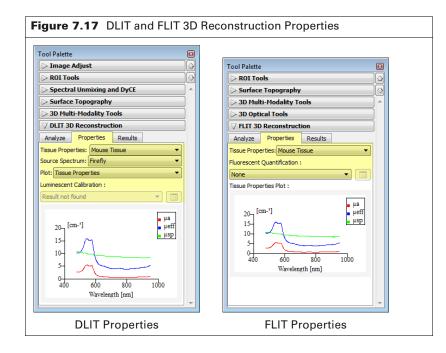


NOTE: If the image sequence includes multiple subjects, you will need to reconstruct each subject separately. This requires a manually generated surface for each subject.

- **3.** Select 3D reconstruction properties from the Properties drop-down lists (Figure 7.17):
 - DLIT Select Tissue Properties, Source Spectrum, and if using, a quantification database ("Luminescent Calibration").
 - FLIT Select Tissue Properties and, if using, a quantification database ("Fluorescent Quantification").



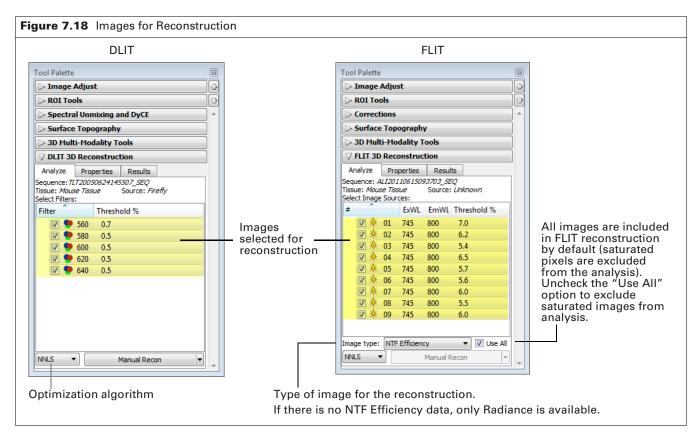
NOTE: See Appendix C on page 260 for more details on quantification databases.



4. Confirm the images selected for reconstruction (Figure 7.18).

For FLIT, images with saturated pixels are included by default in the analysis. However, saturated pixels will be excluded from the analysis. Uncheck the "Use All" option to exclude saturated images from the analysis (Figure 7.18).

5. Choose the type of image to use in the 3D reconstruction from the "Image type" drop-down list (NTF Efficiency or Radiance) (Figure 7.18).



- 6. Click Manual Recon in the Analyze tab (Figure 7.18).
- 7. Select an option in the prompt that appears (Figure 7.19).
 - **OK** Reconstruction will use the surface that was last generated by the software. If this surface is not appropriate for the active data, the software automatically generates a new surface.
 - **Cancel** You will create (or select) and load a surface using the Surface Topography tools. See Appendix D on page 267 for instructions.

NOTE: If the image sequence includes multiple subjects, choose "Cancel" and select the surface (created in step 2) for the subject that you want to reconstruct.

Figure 7.19 Prompt to Select a Surface	
🚺 Living	g Image® 4.5 (64-bit)
?	No valid surface is available to perform this operation. Living Image will automatically generate a 3D surface for use with reconstruction, or you can cancel the reconstruction and manually load or generate a surface using the Surface Topography tools.
	OK Cancel

- **8.** Select pixels to include or exclude from the reconstruction in the Data Preview window that appears (Figure 7.20). There are two ways to do this:
 - Change the Threshold % value (see page 137) Applying a Threshold % value excludes or includes some pixels from the reconstruction. The software computes the minimum and maximum pixel values of an image based on an histogram of pixel intensities. For example, if Threshold % = 0.5%, then pixels with intensity less than 0.5% of the maximum intensity value are excluded from the reconstruction. The Threshold % can be edited for individual images. The Data Preview window is updated when you change the Threshold % value. Min Counts translates the Threshold % to the minimum counts required for reconstruction. Keep the minimum counts > 200.
 - Select regions on an image (see page 138) Use the pencil tool to mark particular regions to
 include in the reconstruction. This may be useful for noisy images with high intensity pixels
 where changing the Threshold % value is not helpful. This method also provides a useful way
 to focus on particular sources to reconstruct and ignore others.

To change Threshold %:

1. Click an image in the Data Preview window (Figure 7.20).

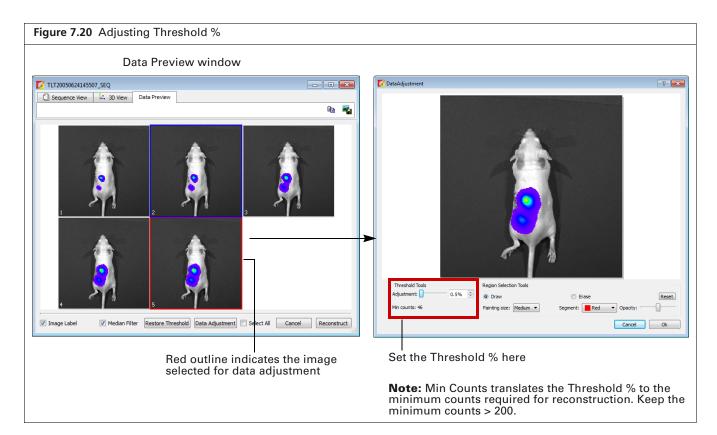


NOTE: Changes to Threshold % are applied to the selected image only. To apply the change to all images, choose the **Select All** option.

- 2. Click Data Adjustment.
- **3.** Enter a new Threshold % value in the window that appears.

The new Threshold % appears in the Analyze tab. If necessary, click **Restore Threshold** to reset the Threshold % to the default value (for the selected images).

4. Click Reconstruct after you set the Threshold%.



To select particular regions for reconstruction:

- 1. Open the Data Preview window as shown in Figure 7.20.
- 2. Click Data Adjustment.
- 3. Choose the Draw option In the window that appears, and put the mouse pointer over the image so that the pencil tool
- **4.** To automatically select all pixels in a source, right-click within the region with the pencil tool. Alternatively, put the pencil over the image and click the mouse key or press and hold the mouse button while moving the pencil over an area of the image.



NOTE: Pencil tool markings will be applied to all images of a sequence. If pencil tool markings are applied, only the marked pixels are included in the analysis.

5. Click Reconstruct after you are done marking the regions.

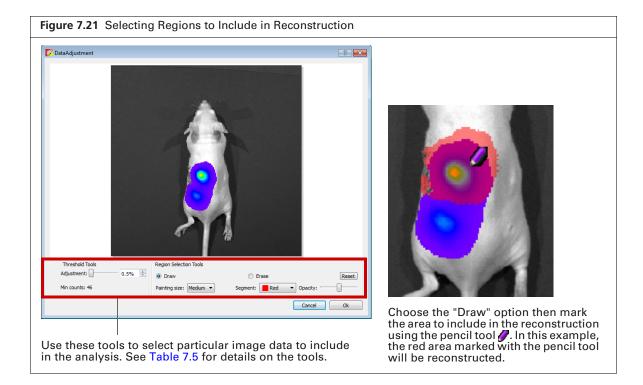


Table 7.5	Region	Selection	Tools
	negion	Selection	10015

ltem	Description
Draw	Choose this option to display the pencil tool 🖉 when the mouse pointer is over the data adjustment image. Use this tool to apply markings that select regions to include in the reconstruction.
Erase	Choose this option to display the eraser tool. Use the eraser to remove pencil tool markings (exclude pixels from the image).
Painting size	Adjusts the width of the pencil tool mark or the eraser tool.
Segment	Colors available for the pencil tool.
Opacity	Adjusts the opacity of the pencil tool markings.
Reset	Removes all pencil tool markings.

7.5 3D Reconstruction Results

The Results tab displays information about the photon density, voxels, and algorithm parameters.

DLIT or FLIT Results

NOTE: For more details on DLIT, see the see the reference article *DLIT and FLIT Reconstruction of* Sources (select **Help** \rightarrow **References** on the menu bar). Sometimes adjusting the DLIT algorithm parameters improves the fit of the simulated photon density to the measured photon density data.

Figure 7.22 Examp	ole DLIT 3D Reco	onstruction Resul	ts
	Tool Palette		X
	> ROI Tools		
	> Spectral Unmixing	and DvCF	i.
	Surface Topograph	-	
	> 3D Multi-Modality	-	
		TOOIS	
	> 3D Optical Tools		
	☑ ✓ DLIT 3D Reconstru	ction	
	Analyze Properties	Results	
	DLIT Results: DLIT_1 (Loa	ded)	1
	Key	Value ^	
	Final voxel size (mm)	1.75	
	Number of voxels	34 ⋿	
	Reduced Chi2	1.71e+02	
	Starting vsize (best)	7.00	
	Kappa (best)	4.00	
	Nsurf (best)	105	
	Total surf samples	525	
	Threshold angle	70.00	
	Kappa limits	0.50 - 4.00	
	Nsurface limits	200.00 - 200.00 -	
	Photon Density Maps	Export Results	
	Save Results		
Results name —	Name: DLIT_1	-	
	Delete L	oad Overwrite	
			*

Table 7.6 DLIT or FLIT 3D Reconstruction Results

Item	Description
Final voxel size (mm)	The voxel size (length of a side, mm) that produces the optimum solution to the DLIT or FLIT analysis.
Number of voxels	The number of voxels that describe the light source(s).
Reduced Chi2	A measure of the difference between the computed and measured photon density maps at the optimum solution. A smaller χ^2 value indicates a better quality of fit.
Index of Refraction	Refractive index of light for the imaged subject.
Angle Limit(deg)	Angle limit of surface normal to optical axis, above which data will not be used in the reconstruction.
Damping reduce	The damping parameter is calculated from this reduction factor, relative to the maximum singular value of the system matrix.

ltem	Description
Data range	For multi-view data, the image views used in the reconstruction.
Mirror XOffset	For multi-view data, the mirror location from the x center line.
Starting voxel size	The voxel size at the start of the analysis. The length of the side of the voxel cube in mm units for the coarsest initial grid size in the adaptive gridding scheme.
Total # of data pts	The total number of data points used in the reconstruction.
Median Filter	Indicates whether or not a median filter was applied to the data.
Image Threshold	The percentage of the minimum radiance at each wavelength (DLIT) or source location (FLIT) is of the maximum radiance. This defines the minimum intensity included in the data.
Samples of Image	The data in each image is sampled. This parameter shows the number of pixels sampled from each image.
Tissue Properties	The tissue properties for modeling the photon propagation.
Source Spectrum	The emission spectrum of the type of luminescent source.
Quantification Selection	A user-selected quantification database used in the reconstruction to convert reconstruction voxel units to 'cells' or 'picomoles' units.
Sequence name	Image data sequence name.
Version	Living Image [®] software version

Table 7.6 DLIT or FLIT 3D Reconstruction Results (continued)

Managing 3D Reconstruction Results

Item in the DLIT/FLIT 3D Reconstruction Results Tab	Description
Name	The name for the active DLIT or FLIT results. Select results from this drop-down list.
Delete	Deletes the selected DLIT or FLIT results.
Load	Opens the selected reconstruction results in the 3D View.
Save	Saves the active DLIT or FLIT results to 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 saved results, saves the new results and overwrites the previous results.
Export Results	Saves the results to a .csv file.

Copying Results to the System Clipboard

- **1.** To copy all results:
 - a. Right-click the results and chose Select All from the shortcut menu.
 - **b.** Right-click the results again and select **Copy** from the shortcut menu.

Fool Palette		
> ROI Tools		
Spectral Un	mixing and DyCE	
> Surface Top	ography	^
⊳ 3D Multi-Me	dality Tools	
> 3D Optical 1	ools	
√ DLIT 3D Re	construction	
Analyze Pr	perties Results	
DLIT Results: DLI	perces	
Key	Value	*
Final voxel size	(mm) 1.75	
Number of vox		E
Reduced Chi2	1.71e+02	
Starting vsiz	Сору	
Kappa (best	C 1 1 AU	
Nsurf (best)	Select All	
Total surf sa Threshold a	Export Results	
Kappa limits	0.50 - 4.00	
Nsurface limits	200.00 - 200.00	-
Photon Density N	laps 🛛 🙀 Export Resul	lts
Save Results		
Name: DLIT_1		-
Delete	Load Overwrite	

- **2.** To copy user-selected results:
 - **a.** Select the results.
 - **b.** Right-click the selection and choose **Copy** from the shortcut menu.

7.6 Checking 3D Reconstruction Quality

Comparing the measured and simulated photon density plots is a useful way to check the reconstruction .

Photon density is closely related to the measured radiance. Photon density is the steady state measure of the number of photons in a cubic millimeter. Light sources inside the tissue contribute to photon density in other portions of the tissue.

The reconstruction algorithm first converts the luminescent or fluorescent image of surface radiance to photon density just inside the animal surface because this is what can be observed. The algorithm then solves for intensity values at locations inside the tissue which would produce the observed photon density near the surface.

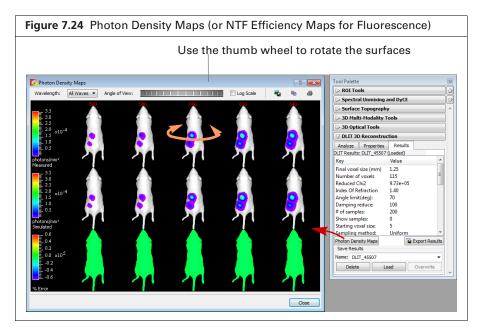
For fluorescence reconstructions using NTF Efficiency data, the photon density of the fluorescence image is divided by the photon density of the transmission image, giving the NTF Efficiency. The NTF Efficiency values are the data just inside the animal surface for this type of dataset.

Viewing Photon Density or NTF Efficiency Maps

1. After the reconstruction is finished or results are loaded, click **Photon Density or NTF Efficiency Maps** in the Results tab.

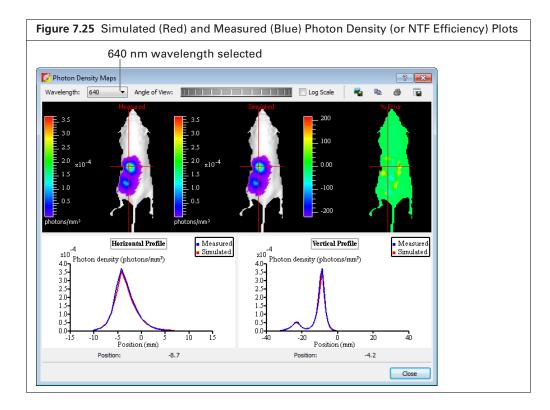
The photon density maps or NTF Efficiency maps for all image data are displayed (Figure 7.24).

2. To rotate the surface and view it from a different angle, move the thumb wheel to the left or right.



3. Select a wavelength from the drop-down list

The photon density or NTF Efficiency profiles at the crosshairs location are displayed. In a good reconstruction, the simulated photon density or NTF Efficiency curves (red) closely resemble the measured photon density or NTF Efficiency curves (blue).



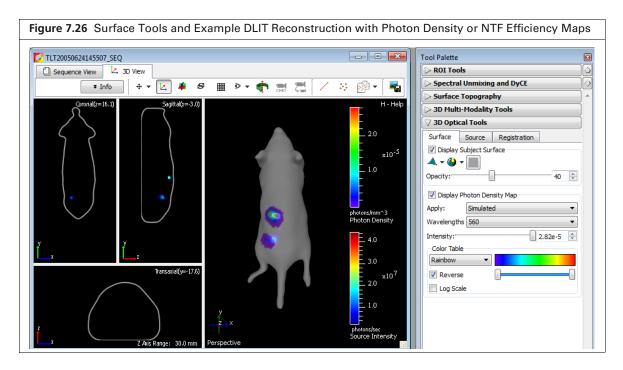
4. To view the photon density or NTF Efficiency profile at another location on the animal surface, drag the cross hairs or click a point on the photon density or NTF Efficiency map.

Table 7.7	Photon	Density	Maps	Window
	1 1101011	Dononcy	iviupo	v v ii i GO v v

ltem	Description
Image sources	A list of images used in the reconstruction. Select all images or a particular image number to display.
Angle of View	The thumb wheel position. Turn the thumb wheel to rotate the surface on the vertical axis.
Log Scale	Choose this option to display the photon density or NTF Efficiency using a log scale.
Simulated	The photon density or NTF Efficiency computed from DLIT or FLIT source solutions which best fit the measured photon density or NTF Efficiency.
Measured	The photon density or NTF Efficiency determined from the image measurements of surface radiance.
Horizontal Profile	The photon density or NTF Efficiency line profile at the horizontal plane through the subject at the crosshairs location.
Vertical Profile	The photon density or NTF Efficiency line profile at the vertical plane through the subject at the crosshairs location.
Position (mm)	Horizontal Profile: The y-axis position of the crosshairs horizontal line. Vertical Profile: The x-axis position of the crosshairs vertical line. The x-y positions are relative to the center of the FOV (where $x = 0$ and $y = 0$).

Adjusting Surface and Photon Density Appearance

Use the 3D Optical Surface tools to adjust the appearance of the reconstructed animal surface and photon density maps.



ltem	Description			
Display Subject Surface	Choose this option	to display the surface	in the 3D View windov	V.
	Drawing styles for			00
	• Point cloud	A Wire frame	Surface face	🙏 Wire frame & surface
	Shading styles for	Smooth surface	Image: Second system Image: Second system	Reflect smooth surface face
	Click to open the c and the cross secti		you can select a displ	ay color for the surface
Opacity	Adjusts the surface	e opacity.		
Display Photon Density or NTF Efficiency Map	Choose this option	to display the photon	density or NTF Efficien	icy on the surface.
Apply	Choose measured	or simulated photon de	ensity or NTF Efficiency	y maps for display.
Wavelengths (DLIT) Images (FLIT)	Choose the data to	o display in the photon o	density or NTF Efficien	cy map.
Intensity	Set the maximum or by entering a va		density or NTF Efficien	cy map using the slider
Color Table	Color scheme for t	he photon density or N	TF Efficiency map.	
Reverse	example, the Red of color scale from tra	to apply the colors of t color table represents th ansparent to red. If Rev e ted using the color sca	he mapped intensity fr e rse is chosen, the ma	om low to high using a oped intensity from low
Log Scale	Choose this option scale.	to apply a logarithmic	scale to the photon de	ensity or NTF Efficiency

7.7 Troubleshooting

Issue	Solution
No sources in solution	This can occur in DLIT or FLIT if the surface is not correct. For example, if a surface is imported into the 3D View from another source other than a Surface Topography analysis.
Surface has spikes	The most common source of spiky surfaces are folds in the animal skin or fur which corrupt the desired smooth lines projected on the animal from the laser galvanometer. Choose the 'Fur Mouse' option for 'Subject'. Smoothing the surface by using the 'Smooth' feature in the Surface Topography tools can help improve the surface. Tool Palette ROI Tools Suface Topography Generate Surface Surface Resonstruction Generate Surface Surface Smoothing Level: Low C Restore Surface Topography Centre Load Correntation
Bad Photon Density or NTF Efficiency fit	The optical properties or source spectrum may have been incorrectly chosen. For example, 'Mouse Tissue' optical property is appropriate or mice, but 'XPM-2/XFM-2' is only appropriate for the mouse phantom.

8 Working With 3D Reconstructions

Adjusting Source Appearance and Making Measurements Synchronizing 3D Views on page 154 Viewing Luminescent and Fluorescent Sources on One Surface on page 155 Displaying Organs With a Reconstruction on page 156 3D Animation on page 162 Exporting a 3D Scene as DICOM on page 167

8.1 Adjusting Source Appearance and Making Measurements

Use the Source tools to:

- Adjust the appearance of sources in 3D reconstructions.
- Make source measurements.
- Export voxel measurements (.csv)

ool Palette		83
> ROI Tools		
> Surface Topog	jraphy	- C
> 3D Multi-Moda	ality Tools	
7 3D Optical Too	ols	
Surface Source	e Registration	_
elect Source: Orig	inal 🗸	X
Display Source		
A - Q - I		
	100	
Opacity:	100	
Display Voxels	the Device Prov	
Maximum Inten	9.94e-7	
Gradation:	50	
Voxel Size: 0.31	Display Voxels	
Smoothing: 5x5 Color Scale	Texture	-
Voxel Min:	3.67e1 🖨 🚺	
Color Table	3.6/e1 💌	2
Jet 👻		
Reverse		
Log Scale	Min 3.67e1 🖨 Max 7.34e2 🖨	
Reset Each	Reset Global Apply to All	1
Measured Sources		
Measured Sources	Value	
Quantification	0.00 pmol M-1 cm-1	
Volume	0.00 mm^3	
Depth	0.00 mm	
Center of Mass	0.00, 0.00, 0.00	
Host Organ	Unknown	
	Center of M	
Export Voxels		ass

Table 8.1 3D Optical Tools, Source Tab

ltem	Description
Select Source	A drop-down list of available sources.
	Original – Results saved with the data.
	<sequence namesourcevoxels=""> – Pasted voxels. (Click the X button to remove pasted voxels from the surface.) See Viewing Luminescent and Fluorescent Sources on One Surface on page 155 for more details on copying and pasting sources from one sequence to another.</sequence>
Display Source Surface	Choose this option to display the source surfaces reconstructed using DLIT or FLIT. A surface will be wrapped around the currently displayed voxels. Adjust the voxel display by moving the Threshold slider.
	Note: Choosing "Display Source Surface" automatically turns off the "Display Voxels" option, and vice versa. If you uncheck "Display Source Surface", neither the surface nor voxels will be visible. Put a check mark next to "Display Voxels" to see voxels.
	Drawing styles for the source surface (see " <i>Display Source Surface</i> " above).
 ✓ ✓	Shading styles for the source surface (see "Display Source Surface" above).
	Click to open the color palette from which you can select a display color for the source surface.
Opacity	Adjusts the source surface opacity.
Display Voxels	Choose this option to display the sources reconstructed using DLIT or FLIT.
	Note: Choosing "Display Voxels" automatically turns off the "Display Source Surface" option, and vice versa. If you uncheck "Display Voxels", neither the voxels nor source surfaces will be visible. Put a check mark next to one of these display options to see voxels or source surfaces.
Maximum Intensity Projection	Choose this option to project all maximum intensity voxels in the view along the viewing direction into the viewing plane.
Threshold (DLIT/FLIT)	Choose this option to apply a minimum threshold intensity to the voxel display.
	Loo this plider to get a threshold for the negative request interview shows which we shall
Gradation (DLIT/FLIT)	Use this slider to set a threshold for the percentage voxel intensity above which voxels are opaque and below which voxels will gradually face to transparent. The percentage voxel intensity is the percentage relative to the maximum intensity.
Voxel size	The 3D grid-spacing size for interpolation of the reconstructed source.
Smoothing	The smoothing box filter size.
Display voxels as	The voxel display mode (cubes, spheres, points, or texture).

Table 8.1 3D Optical Tools, Source Tab (continued)

ltem	Description
Color Scale and Color Table	Color Scale Voxel Min: 4.54e5 + 5 Color Table Jet Reverse Log Scale Min 4.54e5 Max 9.09e6 + Reset Each Reset Global Apply to All
	Voxel Min – Use the slider, up/down arrows, or enter a value to set the minimum value of the source color scale. Voxels with intensities less than the color scale minimum are not displayed in the reconstruction.
	Color Table – Color scheme for voxel display. Use the left and right sliders, up/down arrows, or enter values to set the minimum and maximum colors. The Color Table Max is allowed up to 100 times the voxel maximum.
	Reverse – Choose this option to apply the colors of the selected color table in reverse order to the source voxel scale. For example, the Red color table represents the source intensity from low to high using a color scale from transparent to red. If Reverse is chosen, the source intensity from low to high is represented using the color scale from red to transparent.
	Log scale – Choose this option to apply a logarithmic scale to the color table.
	Note: The following items are available if multiple 3D reconstructions are loaded. Reset Each – Displays each 3D view using the default color table and scale for the dataset.
	Reset Global – Applies the color table of the active data and an aggregate scale to all 3D views.
	Apply to All – Applies the color table and scale of the active data to all 3D views. The software will alert you if the loaded results have different units, for example, a DLIT and FLIT reconstruction.
Measured Sources	Quantification (DLIT) – For uncalibrated sources, the total flux measured for the sources selected using the Measure Source tool . For calibrated sources, this unit will be in [cell] units. For details on using this tool, see page 149.
	Quantification (FLIT) – For uncalibrated sources, the fluorescence yield measured for the voxels selected using the Measure Source tool . Fluorescence yield is expressed in units of [pmol M ⁻ ¹ cm ⁻¹] here for uncalibrated sources. For calibrated sources, this unit will be in either [cells] or [pmol]. For details using this tool, see page 149.
	Volume – Volume of the selected source (mm ³).
	Depth – Perpendicular distance from the source center of mass to dorsal surface.
	Center of Mass (DLIT or FLIT) – The weighted average x , y , and z -coordinates of the selected voxels, where the weights are the flux of each highlighted voxel.
	Host Organ – If reference atlas organs are displayed with the reconstruction, the organ that is closest to the source will be reported here. See page 156 for instructions on displaying organs on a reconstruction.
Export Voxels	Enables export of voxel measurements in their x-, y-, and z-coordinates and source intensities (.csv file).
Center of Mass	Click to compute the center of mass for the source selected with the Measure Source tool . For details using this tool, see page 149.

Source Quantitation

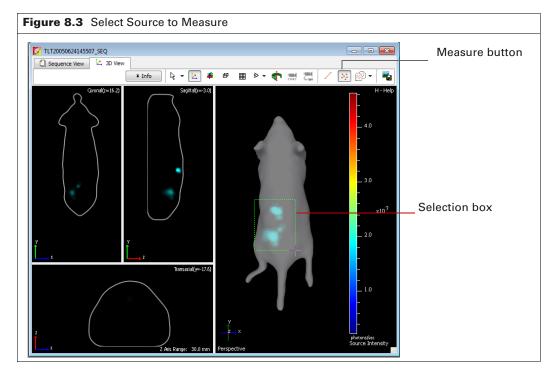
This section explains a convenient way to measure the source (voxels), total flux or total florescence yield, or if calibrated, the abundance in cells or picomoles. The volume, center of mass, and depth at the center of mass are also reported in the 3D Optical tools.



NOTE: Use 3D ROIs for more precise measurements. See page 174 for instructions.

- **1.** Click the Source tab in the 3D Optical tools.
- 2. If the surface includes voxels pasted from other results, select a source from the drop-down list (Figure 8.2).
- 3. Confirm that "Display Voxels" is selected, not "Display Source Surface".

4. Click the Measure Source button 🔅, then draw a box around the source (Figure 8.3).



5. Click **Center of Mass** to obtain the measurements (Figure 8.4). See Table 8.2 on page 151 for a description of the measurements.

The coronal, sagittal, and transaxial planes intersect at the center of mass of the selected source (see Figure 8.5 on page 151).

Surface Source Registration elect Source: Display Source Surface Display Source Surface Display Voxels Maximum Intensity Projection threshold: Display Voxels Maximum Intensity Projection threshold: So Sole Sociel Size: Color Stale Set S C C Color Scale Reverse Log Scale Min 8.65e5 C C Reverse Log Scale Min 8.65e5 C C Reverse Log Scale Min 8.65e5 C C Reverse So Sole Min 8.65e5 C C Color Table Set S C C		
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Export Voxels Center of Mass measurements (see Table 8.2 on	Host Organ Unknown	Click to display source
	Export Voxels Center of Mass	measurements (see Table 8.2 on page 151).

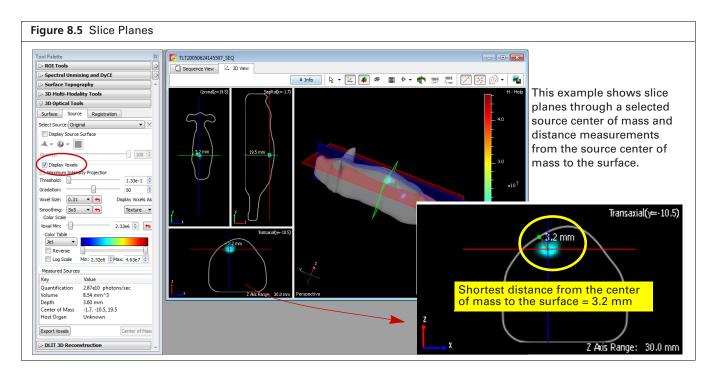
Source Measurement	Description
Quantification	The integrated intensity within the selected sources.
Volume	The total volume of the selected sources.
Depth	The perpendicular distance from the source center of mass to dorsal surface.
Center of Mass	The weighted average x, y, and z-coordinates of the selected voxels, where the weights are the flux of each highlighted voxel.
Host Organ	The reference atlas organ in which the selected sources are located. This information is available if organs are displayed with the reconstruction. See <i>Displaying Organs With a Reconstruction</i> on page 156 for more details.

Table 8.2 Source Me	asurements
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Source Depth

This section explains another way to measure source depth. Follow the steps below after reconstruction is finished or results are loaded.

- 1. Click the Source tab in the 3D Optical tools.
- 2. If the surface includes voxels pasted from other results, select a source from the drop-down list.
- 3. Confirm that "Display Voxels" is selected, not "Display Source Surface".
- **4.** Click the measurement tool button */*.
 - The distance from the center of mass to the surface is measured in the three planes.
 - Coronal and transaxial planes display the shortest distance from the center of mass to the surface.
 - The sagittal plane displays the distance from the center of mass to the bottom of the subject.
- 5. Click the **p** button to display slice planes through the center of mass. See page 152 for more information on planes.

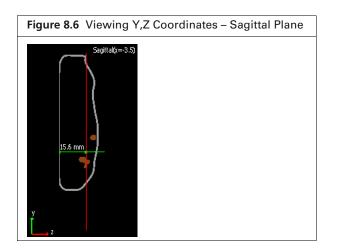


Viewing Location Coordinates

Click a location in the reconstruction slice in the Coronal, Sagittal, or Transaxial windowpane.

The coordinates (mm) of the position are displayed (Figure 8.6). The coordinates are updated when you press and hold the mouse button while you drag the cursor.

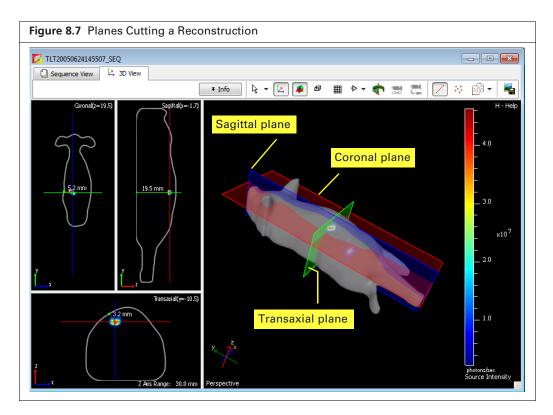
Slice Plane	Displays
Coronal	The x-y coordinates of a position.
Sagittal	The y-z coordinates of a position.
Transaxial	The x-z coordinates of a position.



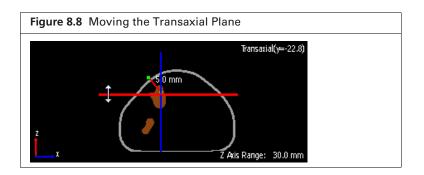
Displaying Slices Through a Reconstruction

- 1. Click a location on a source. Alternatively, click the **** toolbar button, draw a box around a source, then click **Center of mass** in the 3D Source tools.
- 2. Click the 🏄 toolbar button.

The Coronal, Sagittal, and Transaxial windowpanes show a slice through the surface taken by the associated plane (Figure 8.7).



To move a plane, put the mouse cursor over a line in the coronal, sagittal, or transaxial windowpane. When the cursor becomes a ↓ or ↔ arrow, drag the line.
 The view is updated in the windowpanes as you move the line.



8.2 Synchronizing 3D Views

Synchronizing 3D views across multiple datasets provides a convenient way to compare results, for example in a longitudinal study.

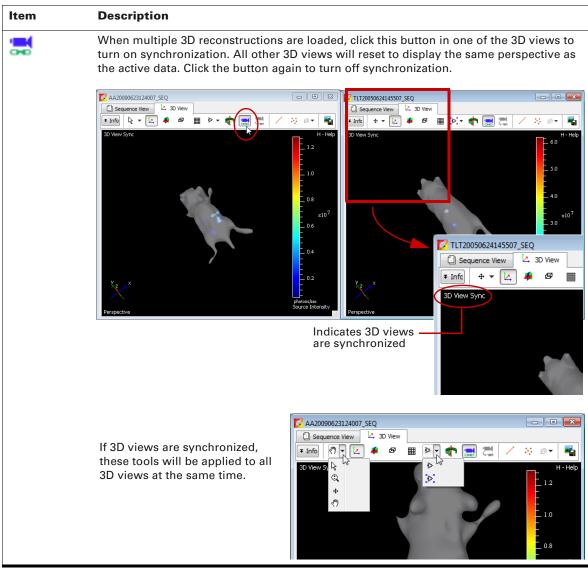
Synchronized 3D ROIs can be also be applied across the datasets (see *Measuring Sources* on page 174 for more information).

- **NOTE:** The 3D view synchronization feature replaces the Longitudinal Study window found in Living Image Software 4.4 and earlier versions.
- Load multiple 3D reconstruction results and tile the windows (select Windows → Tile on the menu bar).
- 2. Choose from the 3D view synchronization options show in Table 8.3.



NOTE: 3D view synchronization options are only available if multiple 3D reconstructions are loaded. Synchronized views are only valid during a session and cannot be saved.

Table 8.3 3D View Synchronization Options



ltem	Description
2	Applies the perspective of the active 3D view to all 3D views. Note: This button is only available if multiple DLIT or FLIT results are loaded and synchronization is turned off.
Color Table Options in 3D Optical Tools (Source tab)	30 Optical Tools Surface Source Diploy Source Surface Name Color Table Diploy Source Surface Name Diploy Source Surface Diploy Source Surface Name D
	views. See <i>Color Scale and Color Table</i> on page 148 for instructions on setting the min and max color table values using the 3D Optical Source tools. The software will alert you if the loaded results have different units, for example, a DLIT and FLIT reconstruction.
	Apply to All – Applies the color table and scale of the active data to all 3D views.
	Note: These options are only available if at least two 3D reconstruction results are loaded.

8.3 Viewing Luminescent and Fluorescent Sources on One Surface

When an experiment includes luminescent and fluorescent reporters, DLIT and FLIT reconstructions can be displayed on one surface if the luminescent and fluorescent imaging is done in the same imaging session, without moving the animal.

NOTE: If the DLIT and FLIT image sequences are acquired during the same session, the generated surfaces are nearly identical.

- 1. Load the DLIT and FLIT 3D reconstructions.
- 2. Choose one of the reconstructions, click the plan button in the 3D View toolbar, and select Copy source voxels.
- **3.** In the other reconstruction, click the provide button and choose **Paste source voxels**.



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NOTE: Pasted voxels can be measured. See page 149 for more details on measuring sources.

8.4 Displaying Organs With a Reconstruction

Mouse anatomy reference atlases are available for registration with 3D reconstructions. A mouse anatomy reference atlas is used when volumetric data from another imaging modality is not available. A reference atlas provides guidance for the luminescent or fluorescent source anatomical location.

Use the 3D Optical Registration tools to:

- Display organs in the surface (see below)
- Manually adjust the location or scale of organs in the surface ((page 158))
- Check the organ fit (page 160)
- Import an organ atlas (page 161)

To display organs:

- 1. Load reconstruction results and confirm that the surface is in the perspective view (click the 📚 toolbar button in the 3D View window or press the **R** key).
- 2. Click the Source tab in the 3D registration tools and choose the "Display Organs" option (Figure 8.9).

gure 8.9 3D Optical Registration Tool	3
Tool Palette	
> ROI Tools	
Spectral Unmixing and DyCE	
Surface Topography	
> 3D Multi-Modality Tools	
✓ 3D Optical Tools	
Surface Source Registration	
Registration Tools	
6 8 8 5	
V Display Organs	
🔺 🗸 🤹 🚱 🕶	
Opacity: 99 荣	
Organ Atlas:	
Female-Dorsal	
Organs	
adrenal	
V bladder	
✓ bone	
V brain	
Cecum	
🔽 📃 colon	
eyesExterior	

- **3.** Make a selection from the Organ Atlas drop-down list (for example, "Female–Dorsal").
- The organs in the selected atlas appear on the surface (Figure 8.10). To fit the organs to the 4. surface, click a registration tool:

- Rigid registration: Performs linear transformation, but keeps the shape of the atlas surface.



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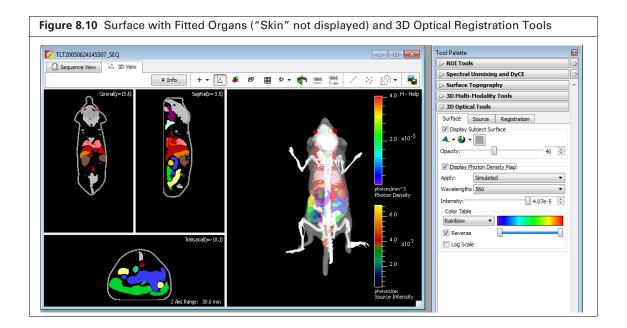
I – Full registration: Performs linear transformation and volume deformation.

NOTE: For an optimum fit when there is a large difference between the orientation or size of the atlas organs and surface, first use the transformation tool to manually register the surface and atlas organs, then click a registration tool to automatically fit the organs. (See Manually Adjusting Scale or Location of Organs on page 158 for more details.)

- **5.** If necessary, adjust the opacity of the organs using the slider or enter a number in the box. The organs are easier to view if you uncheck "Skin" in the Organs list. (Figure 8.10)
- 6. To clear all organs from the surface, click the **Deselect All** button **b**. To hide a particular organ, remove the check mark next to the organ name.



NOTE: After fitting organs to the surface using the g or tool, if necessary, you can click Reset button + to restore the default fit.



Item	Description
õ	Use this tool to manually adjust the scale of location of organs. See page 158 for more details.
8	Fits the organs to the surface using a linear transformation that keeps the shape of the atlas surface.
2	Fits the organs to the surface using linear transformation and volume deformation.
+	After fitting organs to the surface using the 🙀 or 🚺 tool, if necessary, click this button to restore the default fit.
Display Organs	Choose this option to display the organs on the surface. Organs that are check marked will be displayed. See for more details.

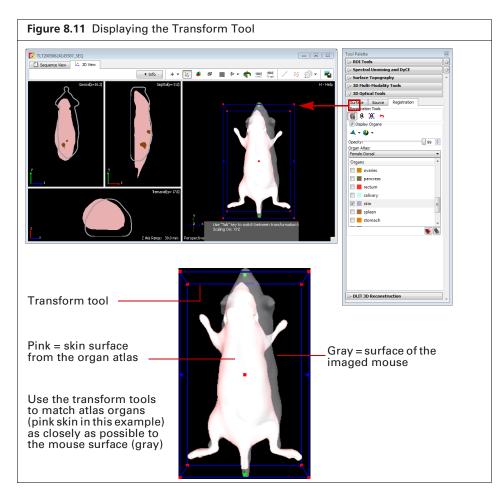
Item	Description
	Drawing styles for the organs (see " <i>Display Organs</i> " above).
	Shading styles for the organs (see " <i>Display Organs</i> " above).
Opacity	Adjusts the opacity of the organ display.
Organ Atlas	Choose a type of organ atlas.
5	Click to select all organs in the database and display them on the surface.
6	Click to clear the selected organs and remove all organ diagrams from the surface.

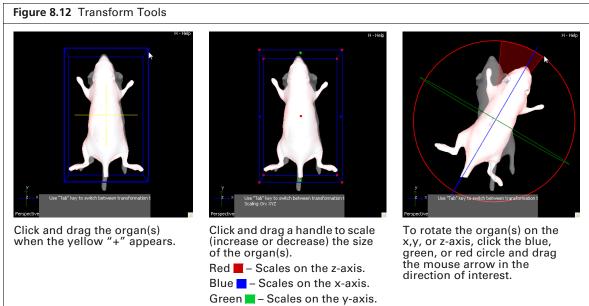
Manually Adjusting Scale or Location of Organs

1. Load reconstruction results and display organs (see *Displaying Organs With a Reconstruction* on page 156 for instructions).

The organs in the selected atlas appear on the surface. Only "Skin" is selected in Figure 8.11.

- Click the Transform tool button .
 The transform tool appears. Figure 8.12 explains the tool functions.
 - $\overline{\mathbb{W}}$
- **NOTE:** It may be helpful to view the 3D image from different perspectives to check the organ position and size. To turn and rotate the 3D image:
 - Click outside the transform tool box, then press and hold the left mouse key.
 - Drag the mouse when the hand $\langle n \rangle$ appears.





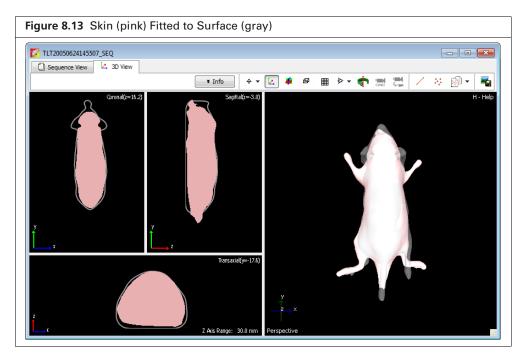
3. Press the Tab key to switch between the transform tools (Figure 8.12).

The position of the organ(s) is updated in the slice windowpanes (coronal, sagittal, and transaxial views) after each adjustment.

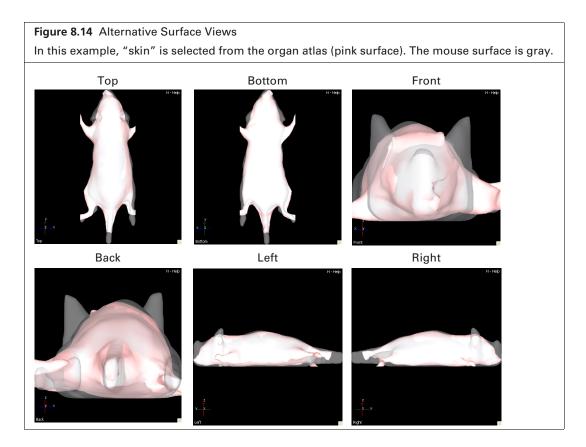
4. Turn off the transform tool when you are done adjusting the position of the organ(s) (click the button).

Checking the Organ Fit

- 1. Check the fit in the coronal, sagittal, and transaxial windowpanes.
- Click the Change view toolbar button .
 The Top view is displayed (Figure 8.13).



3. Press the V key or the **v** button to display alternative views of the surface.



Importing an Organ Atlas

An organ atlas (.iv, .dxf, or .stl, one organ per file) consisting of segmented organ surfaces derived from an MRI or CT scan can be imported into the Living Image software for registration with the animal surfaces derived from IVIS data. Organ files must be segmented from MRI or CT 3D volumetric data in third party medical imaging analysis software.

NOTE: The imported atlas must include a surface (skin) file which delineates the animal surface. The file name must include the word "skin", for example *rat skin.iv*.

- 1. Load a DLIT or FLIT image sequence that is associated with the mouse comprising the organ files in *.iv, *.dxf or *.stl format.
- **2.** Select File \rightarrow Import \rightarrow Organ Atlas on the menu bar.
- 3. In the dialog box that appears, click Add Organ Files (Figure 8.15).

Figure 8.15 Importing an Or	gan Atlas
Import Organ Atlas Organ Files: Generate Generate	E3 n Mesh: ▼ e Mesh Co-efficients
Organ Atlas	Documents Claining Documents Claining Image: Claining Image: Claining Image: Claining<
	Places Files of type: All Mesh Formats(".iv ".duf ".stl) Cancel Import Organ Atlas Import Organ Atlas Import Organ Atlas Import Organ Atlas Organ Files: Select Skin Mesh: Import Organ Atlas Import Organ Atlas Uterus.iv Import Organ Atlas Import Organ Atlas Import Organ Atlas Dones.iv Import Organ Atlas Import Organ Atlas Import Organ Atlas Index:iv Import Organ Atlas Name: Import Organ Atlas Name: Import Organ Atlas Import Organ Atlas Index:iv Import Organ Atlas Import Organ Atlas Import Organ Atlas Import Organ Atlas

4. In the next dialog box that appears, select all of the files (.iv, .dxf, .stl) that you want to include in the atlas (one file per organ) and click **Open**.

- **5.** In the Select Skin Mesh drop-down list, select the skin organ file, which must include 'skin' in the file name.
- 6. Click Generate Mesh Coefficients.
- 7. Enter a name for the atlas and click Save Organ Atlas.

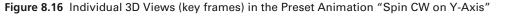
The organ atlas (.atlas) is created and is added to the Organ Atlas drop-down list (in the 3D Optical Tools, Registration tab).

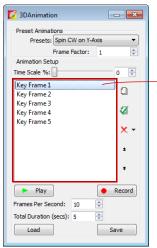
8.5 3D Animation

Living Image software can create an animation from a sequence of 3D views (*key frames*). For example, an animation can depict a rotating 3D scene (Figure 8.16). The animation (series of key frames) can be recorded to a movie file (.mov, .mp4, or .avi).

Use the animation tools to:

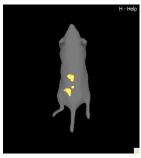
- View a preset animation (generated from a factory-loaded animation setup, page 164).
- Create a custom animation (created from your custom animation setup, page 165).
- Save an animation setup (page 166).
- Record an animation to a movie file (page 166).
- Edit an animation setup (page 166).





The box shows the key frames in the current animation setup. Click a key frame in this box to display the associated 3D view and time stamp (position in the time scale (0-100) at which the frame occurs in the animation).

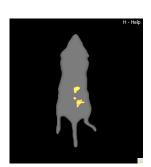




Keyframe 1



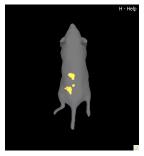
Keyframe 2



Keyframe 3



Keyframe 4



Keyframe 5

Table 8.5 3D Animation Tools

ltem	Description
Time Scale%	The time stamp of a key frame in the animation on a time scale of 0-100. For example, if the animation is 10 sec long and includes five key frames:
	Key frame 1: Time stamp= 0; first frame of the animation.
	Key frame 2: Time stamp = 25%; frame occurs 2.5 seconds after the start of animation.
	Key frame 3: Time stamp = 50%; frame occurs 5.0 seconds after the start of animation.
	Key frame 4: Time stamp = 75%; frame occurs 7.5 seconds after the start of animation.
	Key frame 5: Time stamp = 100%; last frame of the animation.
Presets	A drop-down list of predefined animation setups.
Key frame	A 3D view. The software interpolates the key frames to create intermediate frames in real time, then generates an animated sequence from all of the frames. Each successive key frame in a sequence should differ slightly from the preceding one, so that motion is smoothly depicted when the frames are shown at a proper frame rate (frames/second). The Living Image software provides preset key frames or you can specify the 3D views for the key frames.
Preset Key Frame Factor	Determines how many key frames are used to generate one revolution in a spinning animation (No. of frames = $(4 \times \text{Key Frame Factor}) + 1$). Increasing the key frame factor reduces the time period between key frames and creates the appearance of finer movement. Decreasing the key frame factor increases the time period between key frames and creates the appearance of coarser movement.
FPS	Frames displayed per second in the animation sequence.
1	Creates a new key frame from the current 3D view.
Ø	Updates the selected key frame to the current 3D view.
X	Deletes a selected or all key frames from the key frame box.
±	Moves a selected key frame up in the key frame box.
Ŧ	Moves the selected key frame down in the key frame box.
Total Duration	The total time of the animation sequence.
Play	Click to view the animation sequence defined by the current key frames and animation parameters.
Record	Displays a dialog box that enables you to save the current animation to a movie (.mov, .mp4, or .avi, .mpg).
Animation Setup	
Load	Displays a dialog box that enables you to open an animation setup (.xml).
Save	Displays a dialog box that enables you to save the current key frames and animation parameters to an animation setup (.xkf).

Viewing a Preset Animation

Preset animations are factory-loaded animation setups. They include predefined key frames which are used to generate the animation.

To view a preset animation:

- 1. Open an image sequence and load 3D reconstruction results.
- **2.** Select properties to display in the 3D View window (for example, organs, sources, surface, or photon density maps).
- **3.** Select **Tools** \rightarrow **3D Animation** on the menu bar.
- 4. In the 3D Animation tools that appear:
 - **a.** Clear the key frame box if necessary (click the X button and select **Delete All**).
 - **b.** Make a selection from the Presets drop-down list. See Table 8.5 on page 163 for a description of the preset animations.

After a preset animation is selected, a list of the key frames appears.

NOTE: You can view multiple animations sequentially. For example, if you select Spin CW on X-Axis and Spin CW on Y-axis from the Presets drop-down list, the animation shows the 3D reconstruction spinning clockwise on the x-axis, then spinning clockwise on the y-axis.

5. Click Play to view the animation.

Figure 8.17 3D Animation Tools\ See Table 8.5, page 163 for deta	
See Table 8.5, page 163 for deta	— Select a preset animation — Keyframes box

Creating a Custom Animation

To create an animation, specify a custom animation setup or edit an existing setup.

- 1. Open an image sequence and load 3D reconstruction results.
- **2.** Select properties to display in the 3D View window (for example, organs, sources, surface, or photon density maps).
- **3.** Select View \rightarrow **3D** Animation on the menu bar.

The 3D Animation tools appear (Figure 8.18).

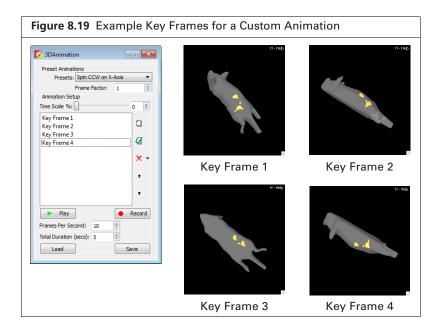
4. Clear the key frame box if necessary (click the 🔀 button and select Delete All).

7 3D Tool	5			
Surface	Source	Registra	tion	Animate
Preset Anir	mations			
	Presets: S	pin CW on >	X-Axis	1
	Fr	ame Factor	: 1	3
Animation :	Setup			
Time Scale %	/6: 🚺 🚽 🚽			- o 😂
				D
				12
				\times
				±
				Ŧ
🕨 Pla	y I		٠	Record
Frames Per	Second: 10		-	
	on (secs): 5		A	

- **5.** To capture the first key frame, click the D button. The first key frame is added to the key frame box.
- Adjust the position of the reconstruction in the 3D View using an image tool (for example,
 or <a>

 Q
 See Table 7.2 on page 129 for more details on the image tools.
- 7. Click the 📋 button.

The second key frame is added to the key frame box.



8. Repeat step 6 to step 7 until all of the key frames are captured. See *Edit an Animation Setup* below for details on how to edit the key frame sequence.

Click a key frame to display the associated 3D view and the time stamp (position in the time scale (0-100) at which the frame occurs in the animated sequence).

9. Confirm the defaults for FPS (frames per second) and Total Duration (length of animation) or enter new values.

FPS \times Total Duration = No. of frames generated to create the animation. The number of generated frames should be \geq to the number of key frames. Otherwise, the frames may not be properly animated.

10. To view the animation, click Play. To stop the animation, click Stop.

An animation setup (series of key frames) can be saved (.xkf) or recorded to a movie (.mov, mp4, .avi, mpg).

Managing Animation Setups

Save an Animation Setup

- 1. Click Save.
- 2. Select a directory and enter a file name (.xkf) in the dialog box that appears.

Record an Animation to a Movie

- 1. Click Record.
- **2.** Choose a directory, enter a file name (.mov, mp4, .avi), and click **Save** in the dialog box that appears.

Edit an Animation Setup

- **1.** Open an image sequence and load a reconstruction.
- **2.** Open an animation setup:

To select a predefined setup, make a selection from the Preset drop-down list. To select a saved user-defined setup:

- a. Click Load.
- **b.** Select an animation setup (.xkf) in the dialog box that appears.

Z 3DAnimation	×	
Preset Animations Presets: Spin CW on Y-Axis		
Frame Factor: 1 Animation Setup Time Scale %:	- 0 -	
Key Frame 1 Key Frame 2 Key Frame 3 Key Frame 4 Key Frame 5	↓ ↓ ↓ ↓	
Play Frames Per Second: 10 Total Duration (secs): 5	Record	

- **3.** To add a key frame:
 - a. Adjust the position of the reconstruction in the 3D view using an image tool (for example, $\langle \gamma \rangle$, or $\langle \cdot \rangle$). See Table 7.2 on page 129 for more details on the image tools.
 - **b.** Click the 🗍 button.
 - **c.** To reorder a key frame in the sequence, select the key frame and click the \pm or \mp arrow.
- **4.** To update a key frame:
 - a. Select the key frame and adjust the 3D view.
 - **b.** Click the 🌠 button.
- **5.** To delete a key frame:
 - **a.** Select the key frame that you want to remove.
 - **b.** Click the **X** button and select **Delete Current**.

8.6 Exporting a 3D Scene as DICOM

The items in the perspective 3D View are called a *3D scene*. For example, the 3D scene in Figure 8.21 includes a surface and voxels. The 3D scene can be exported to DICOM format and viewed in the Living Image DICOM Viewer or third party software.



To export the 3D scene:

- **1.** Load the results that you want to export.
- **2.** Select File \rightarrow Export \rightarrow 3D Scene as DICOM on the menu bar.
- **3.** In the dialog box that appears, set the export options, and click **Export** (Figure 8.22). For more details on the 3D Scene Exporter, see Table 8.6 on page 168.

Figure 8.22 3D Scene Exporter Dialog Box		
Save DICOM as: Save DICOM as: Sice Orientation : Export voxels using original resolution Parameters Sice Resolution: Total slices: 256 Sice spacing (mm): 0.3977 Approx size: 2.9 MB Pixel spacing (mm): 0.2891 Solid mesh Export	Export DICOM where ? Crganize New folder Favorites Desktop Recent Places Downloads Dopbox Hightail Documents Music Pictures SW Download Installers Folder: Select Folder	Date modified

4. In the next dialog box that appears, choose a folder for the DICOM files and click **Select Folder**. During the export operation, the 3D View window displays the each slice in the export. For example, if Transaxial Slice is selected for export, then the transaxial windowpane cycles through a display of each exported slice.

Table 8.6	3D	Scene	Exporter	Dialog Box
10010-0.0	00	000110	LAPOILOI	Dialog Dox

ltem	Description
Save DICOM as:	Single-Frame DICOMs - Exports multiple files that contain a single frame each.
	Multi-Frame DICOM - Exports a single file that contains multiple frames.
	Note: Choose the Single-Frame or Multi-Frame DICOM option, depending on the third party software you will use to import and view the 3D scene. Some applications cannot reconstruct multi-frame DICOM files.
Slice Orientation	Choose transaxial, coronal, or sagittal slices for the export.
Export voxels using original resolution	Choose this option to export source voxels without any smoothing or binning. The original resolution of the source voxels is the resolution obtained after DLIT or FLIT reconstruction (approximately 1mm resolution).
Slice Resolution	Sets the number of slices required to accommodate the slice orientation with good slice sampling/spacing.
Total Slices	Parameters that determine the number and resolution of the slices to export.
Slice spacing	
Pixel spacing	
Solid mesh	If this option is chosen, voxels generated inside the hollow mesh are assigned an intensity so that they are displayed as "tissue" when loaded into visualization software. If no intensity is associated with the voxels, they are considered noise or air and appear hollow.
Hollow mesh	The intensity of pixels inside the surface is set to zero so that the exported surface appears as a hollow empty structure.

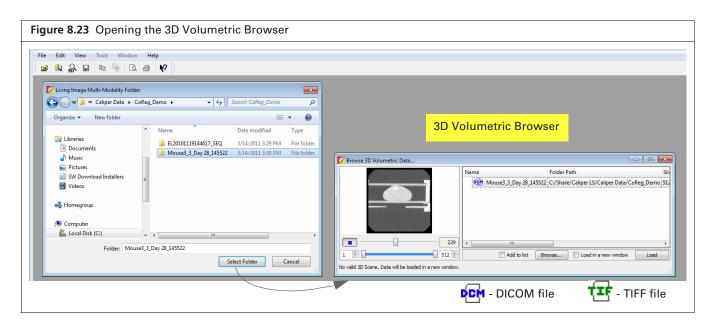
Viewing DICOM Data

3D scenes exported to DICOM can be viewed in the Living Image 3D Browser or a 3D View window.

- **1.** Click the $\frac{1}{30}$ toolbar button. Alternatively, select File \rightarrow Browse 3D Volumetric Data on the menu bar.
- 2. If this is the first time browsing for volumetric data in the session, select a data folder in the dialog box that appears, and click Select Folder.

The Living Image 3D Volumetric Browser appears (Figure 8.24).

NOTE: If the 3D Volumetric Browser was previously opened during the session, clicking the button opens the browser. Click the Browse.. button in the browser, and in the dialog box that appears, select a DICOM data folder.



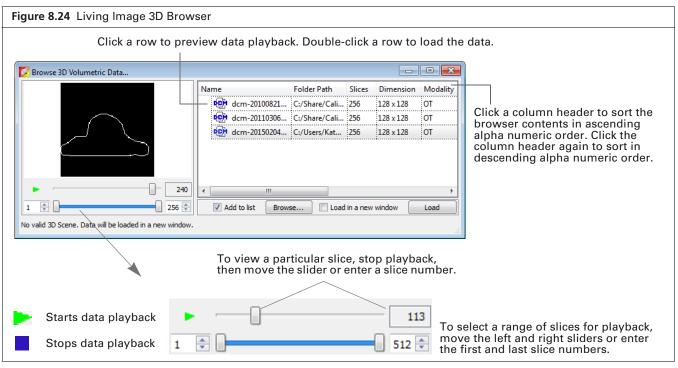


Table 8.7 Living Image 3D Browser DICOM Viewing Controls

Item	Description
Add to list	If this option is chosen, the selected data will be added to the browser. If this option is not chosen, the selected data replaces the contents of the browser, except for the loaded data.
Browse	Opens the dialog box that enables you to select data to display in the browser.
Load in a new window	If this option is selected, DICOM data will be opened in a new 3D View window when you click Load .
	If this option is not selected, DICOM data will be loaded in the active 3D View window.
Load	Opens the DICOM data in a 3D View window.

9 Measuring Signal in 3D Sources

About 3D ROIs
Overview of 3D ROI Tools on page 172

Measuring Sources on page 174

Managing 3D ROIs on page 178

9.1 About 3D ROIs

A 3D *region of interest* (ROI) measures the signal intensity within a user-specified bounding box applied to a:

- 3D reconstruction of a luminescent source.
- 3D reconstruction of a fluorescent source.
- CT volume.



NOTE: CT imaging is not available on the IVIS Spectrum. Living Image software can analyze CT image data acquired on the IVIS Spectrum CT. 3D Multi-Modality tools (see page 181) are required to load IVIS Spectrum CT volumetric data or import volumetric data (PET, MRI, or CT data) from instruments other than the IVIS Spectrum CT).



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 9.2).

If a dataset includes ROIs on both 2D optical and 3D volumetric data, the measurements for the two types of ROIs are displayed in separate tabs of the ROI table (Figure 9.2):

- ROI Measurements tab shows ROI measurements for 2D optical data.
- 3D ROI Measurements tab shows measurements of structural data (for example, CT) or measurements of the 3D optical reconstruction.

Figure 9.2 3D ROI Measuremen	nts Table
Select "3D Volumetric Data" to view measurements of structural data, such as a CT volume Select "Source Voxels" to view measurements of the 3D optical reconstruction.	ROI Measurements Image: SD ROI Measurements RoI Measurements 30 ROI Measurements Data Types: 30 Volumetric Data Data Types: 30 Volumetric Data Sequence Number ROI Voxels Total Counts Average Counts Max Counts BI20111027132749_SEQ ROI 1 39304 5.181e+08 1.318e+04 0.000e+00 5.500e+04
	Copy Select All
	Configure Export Close

9.2 Overview of 3D ROI Tools

Table 9.1 provides a description of the 3D ROI tools. ROI measurements and measurement statistics are available in the ROI Measurements table. The table provides a convenient way to review or export ROI information. See Table 9.2 on page 177 for more details on 3D ROI measurements.

Figure 9	0.3 3D ROI Tools	
Synd Save 3 Name:		
> Surf	ctral Unmixing and DyCE face Topography Multi-Modality Tools Dptical Tools	
	T 3D Reconstruction	Ŧ

Table 9.1 3D ROI Tools

ltem	Description		
Ø	Click to apply a 3D ROI. If multiple datasets are loaded and the "Synchronize 3D ROI" option is selected, a synchronized 3D ROI will be applied to all of the loaded datasets. Synchronized 3D ROIs can be moved and adjusted as a group using the 3D transform tool.		
<u>i</u>	Click to select a 3D ROI from the drop-down list and to turn on the 3D ROI transform tool. See page 175 for instructions on using the tool to move, scale, or rotate a 3D ROI.		
₩ Measure 3D ROIs	Click to display the 3D ROI measurements or compute intensity signal in a 3D ROI. See Table 9.2 on page 177 for more information on 3D ROI measurements.		
X. S All	Click 🗙 for a drop-down list of delete options for 3D ROIs. If "All" is selected when multiple datasets with synchronized 3D ROIs are loaded, the software prompts you to confirm deleting the 3D ROIs from all of the datasets.		
ROI 1 ROI 2	🔽 Living Image® 4.5 (64-bit)		
NOIL	All displayed sequences are currently synchronized. To delete all 3D ROIs in only one sequence, please exit synchronization mode first. Are you sure you want to delete all 3D ROIs in all displayed sequences?		
	<u>Y</u> es <u>N</u> o		
	Note: These commands delete 3D ROIs from a dataset(s). They do not delete 3D ROIs that are saved to the system (3D ROIs which appear in the Name drop-down list, see below).		
Synchronize 3D ROI	If multiple datasets are loaded and the "Synchronize 3D ROI" option is selected, clicking the for button applies a synchronized 3D ROI to all datasets. The transform tool and delete commands are applied simultaneously to synchronized 3D ROIs.		
Save 3D ROIs to the system	Name of a 3D ROI or set of 3D ROIs Save 3D ROIs Name: 3D ROI 3 KSA These ROI tools are only available when a 3D		
	Name: 3D_ROI_3_KSA Inese ROI tools are only available when a 3D reconstruction or volume is loaded.		
	3D ROIs (parameters only such as coordinates and dimensions) can be saved to the system (per user) and used to apply the ROIs to other datasets. These 3D ROIs apper in the Name drop-down list. See page 180 for instructions.		
	Note: 3D ROIs can also be saved with the dataset (the software prompts you to save before closing a dataset). 3D ROIs saved with a dataset do not appear in the Name drop-down list.		

9.3 Measuring Sources

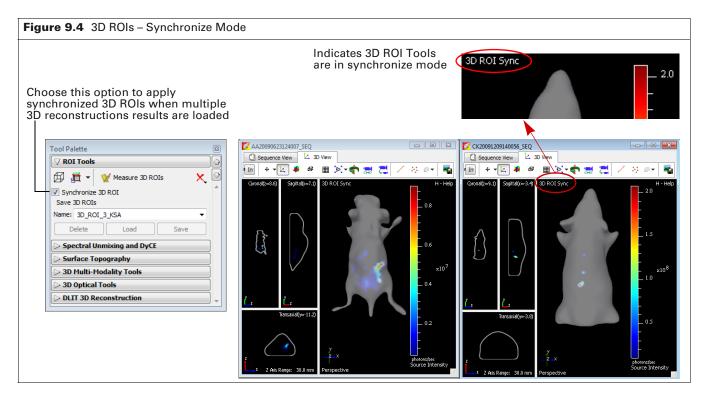
This section explains how to apply 3D ROIs on a 3D reconstruction or CT volume. If multiple datasets are loaded, identical 3D ROIs can be applied to all of the datasets at the same time. These related 3D ROIs are "synchronized" and can be moved or adjusted as a group. Synchronized ROIs provide a convenient way to compare results, for example, results obtained at different time points or from different reporters.

To apply 3D ROIs:

1. Load a 3D reconstruction or CT volume.

If you want to apply synchronized 3D ROIs to multiple datasets:

- Load multiple volumes or 3D reconstructions. Tile the windows (select Window → Tile on the menu bar).
- Choose the "Synchronize 3D ROI" option in the Tool Palette (Figure 9.4).



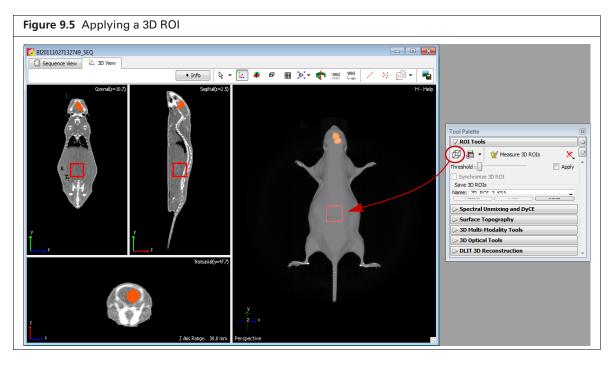
2. Click the 3D ROI button 🗇 in the ROI tools to apply the ROI (Figure 9.5).

NOTE: 3D ROIs applied before selecting the "Synchronize 3D ROI" option or after it is cleared are independent and unrelated to other 3D ROIs.

A red bounding box appears in the 3D View(s). If working with a CT volume and you do not see the red bounding box, do either of the following:

- Select the "Maximum Intensity Projection (MIP)" option in the 3D Multi-Modality tools. OR
- Reduce the volume opacity by adjusting the position of the Air/Noise Boundary in the 3D Multi-Modality tools.

NOTE: See Table 9.1 on page 173 for details on deleting 3D ROIs.



3. It may be helpful to view the surface from different perspectives to check the 3D ROI position and size. To turn and rotate the surface, press and hold the left mouse key, then drag the mouse when the hand $\{n\}$ appears.

If working with synchronized 3D ROIs, it may be helpful to synchronize the 3D views before using the *Synchronizing 3D Views* on page 154 for more details.

- **4.** Adjust 3D ROI position using the transform tools. Adjustments will be applied simultaneously to all synchronized 3D ROIs.
 - a. Click the 3D ROI Transform button ↓ and select the ROI from the drop-down list. The first 3D ROI created during a session is named "ROI 1" by default. A tooltip shows the ROI name when you put the mouse pointer over an ROI.
 - **b.** Click a 3D ROI to begin using the transform tools.

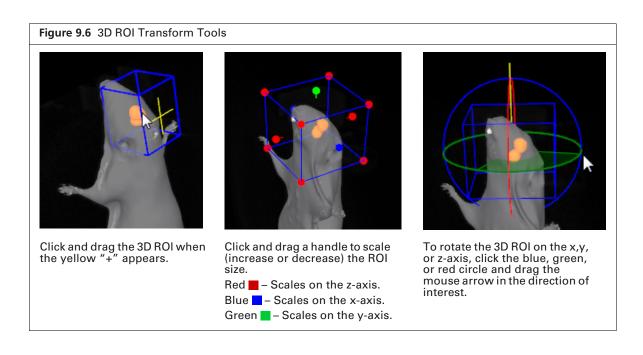
Figure 9.6 explains the tool functions. The ROI position is updated in the slice windowpanes (coronal, sagittal, and transaxial views) after each adjustment.



NOTE: If the 3D ROI disappears from view, right-click in the 3D view to redisplay the 3D ROI at the pointer location.

- c. Press the Tab key to switch between the transformations tools.
- d. Turn off the transform tool when you finish positioning the ROI (click the 3D ROI Transform button, , →).

 $\overline{\mathbb{II}}$



NOTE: The 3D ROI location (x, y, or z-coordinates) and dimensions (width, height, or depth) can be viewed and modified in the 3D ROI Properties dialog box. See page 178 for details.

- 5. Click the [™]Measure 30 ROIs</sup> button in the Tool Palette to view the intensity measurements (Figure 9.7). See Table 9.2 for information about the measurements.
- 6. Click Yes in the prompt when closing the data to save the 3D ROIs with the data. Alternatively, select File → Save on the menu bar.

The 3D ROIs will be displayed the next time the dataset is loaded.

NOTE: 3D ROIs from a dataset can be saved to the system (per user) and applied to other datasets. See *Saving 3D ROIs to the System* on page 180 for details.

Figure 9.7 3D ROI Measurements Table			
Tool Palette	Select the type of data and measurement (see Tal	ole 9.2)	
Measure 3D ROIs X	ROI Measurements SO ROI Measurements		
Synchronize 3D ROI Save 3D ROIs	Data Types: 30 Volumetric Data V Measurement Types: Counts V	Refresh	
Name: 3D_ROI_3_KSA Delete Load Save	Sequence Number ROI Voxels Total Counts Average Counts Min Counts Max Counts bl20111027132749_SEQ ROI1 54015 6.668e+08 1.234e+04 0.000e+00 5.500e+04		
Spectral Unmixing and DyCE Surface Topography			
> surface ropography > 3D Multi-Modality Tools			
> 3D Optical Tools			
		Copy Select All	
	Configure	Close	
	See page 120 and page 122 for information abou custom table configurations and exporting or prin	t creating nting the table.	

Item Description Data Types 3D Volumetric Data - Select this data type to measure the grayscale values of 3D volumetric data such as CT or MRI. Source Voxels - Choose this option to measure the source intensity of the voxels of a 3D optical image. Measurement Types 3D Volumetric Data: Counts - A measurement of a voxel value. The scale is image specific and may not be consistent between images. Absorption – A measurement of the amount of X-rays absorbed by the voxels. Hounsfield - A measurement of voxel grayscale value in Hounsfield units. Note: Absorption and Hounsfield units are only available for IVIS Spectrum CT data. Source Voxels: photons/sec - The total flux of a luminescent source. cells - The number of cells for calibrated sources integrated over the 3D ROI. pmol M⁻¹ cm⁻¹ – Fluorescence yield for uncalibrated sources integrated over the 3D ROI. pmol - The number of picomoles for calibrated sources integrated over the 3D ROI. Sequence Number The identifier of the active image data. ROI Name of the 3D ROI. The number of voxels within the 3D ROI. Voxels 3D Volumetric Data: Counts Total Counts - the sum of all counts for all voxels inside the 3D ROI. measurements (16-bit scale Average Counts - Total Counts/Number of voxels in the 3D ROI with values that change from Min Counts - The smallest number of counts in a voxel within the 3D ROI. image to image) Max Counts - The largest number of counts in a voxel within the 3D ROI. 3D Volumetric Data: Absorption Total Value - The sum of the absorption measurements of all voxels in the Measurements (Fixed 32-bit 3D ROI. scale with values that are Average Value - Total Value/Number of voxels in the 3D ROI. consistent between images.) Stdev Value - Standard deviation of the absorption values for all voxels Note: These measurements are inside the ROI. only available for IVIS Min Value - The smallest absorption value for any single voxel in the 3D Spectrum CT data. ROI. Max Value - The largest absorption value for any single voxel in the 3D ROI. 3D Volumetric Data: Hounsfield Total Hounsfield - The sum of the Hounsfield unit values for all of the measurements (Calibrated CT voxels in the 3D ROI. scale. Fixed from image to Average Hounsfield - Total Hounsfield unit value/Number of voxels in the image.) 3D ROI. Note: These measurements are Stdev Hounsfield – Standard deviation of the Hounsfield unit values for only available for IVIS all voxels inside the ROI. Spectrum CT data. Min Hounsfield - The minimum Hounsfield unit value for any single voxel in the 3D ROI. Max Hounsfield - The maximum Hounsfield unit value for any single voxel in the 3D ROI.

Table 9.2 3D ROI Measurements Table

ltem	Description
Source Voxels: photons/sec measurements	Total Flux [ph/s] – The flux in each voxel summed or integrated over the 3D ROI.
	Average Flux [ph/sec] – Total flux/Number of voxels in the 3D ROI.
	Stdev Flux – Standard deviation of the flux of the voxels inside the ROI.
	Min Flux – The smallest flux value of a voxel.
	Max Flux – The largest flux value of a voxel.
Source Voxels: cells	Total Cells – The number of cells in the 3D ROI.
Note: This measurement type	Average Cells – Total number of cells/Number of voxels in the 3D ROI.
requires a quantification database. See Appendix C on	Stdev Cells – Standard deviation of the number of cells in the 3D ROI.
page 260 for more details.	Min Cell – The smallest number of cells in a voxel included in the 3D ROI.
	Max Cell – The largest number of cells in a voxel included in the 3D ROI.
Source Voxels: pmol M ⁻¹ cm ⁻¹ measurements	Total pmol M ⁻¹ cm ⁻¹ – The fluorescence yield summed or integrated over the 3D ROI.
	Average pmol M ⁻¹ cm ⁻¹ – Total fluorescence yield/Number of voxels in the 3D ROI.
	Stdev pmol M^{-1} cm ⁻¹ – Standard deviation of the fluorescence yield of the voxels in the 3D ROI.
	Min pmol M ⁻¹ cm ⁻¹ – The smallest fluorescence yield in the 3D ROI.
	Max pmol M ⁻¹ cm ⁻¹ – The largest fluorescence yield in the 3D ROI.
Source Voxels: pmol	Total pmol – Total picomoles of fluorescent probe within the 3D ROI.
measurements Note: This measurement type	Average pmol – Total picomoles/Number of voxels.
requires a quantification	Stdev pmol – Standard deviation of the picomole values in the 3D ROI.
database. See Appendix C on	Min pmol – Smallest picomole value in the 3D ROI.
page 260 for more details.	Max pmol – Largest picomole value in the 3D ROI.
Refresh	Updates the ROI Measurements table (for example, after you draw new ROIs, move an ROI, and close or open image data).
Сору	Copies the selected row(s) in the table to the system clipboard.
Select All	Selects all rows in the table .
Configure	Displays the Configure Measurements box that enables you to specify and organize the data categories (column headers) for the table. See for more details.
Export	Opens a dialog box that enables you to export the ROI measurements (.txt or .csv).
Close	Closes the ROI Measurements table.

Table 9.2	3D ROI Measurements Table	(continued)
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9.4 Managing 3D ROIs

ROI Properties

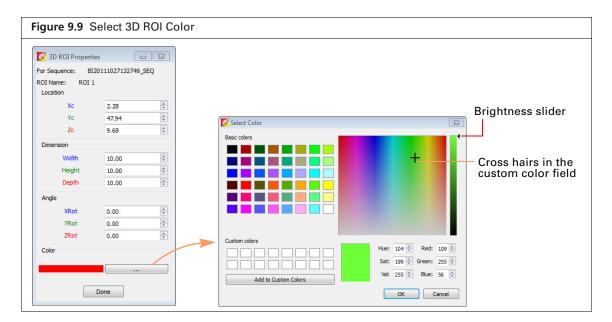
You can view information about the location and dimensions of a 3D ROI and edit these properties.

- **1.** Click the 3D ROI Transform button **☐ -** and select an ROI from the drop-down list.
- **2.** Double-click the 3D ROI.

The 3D ROI Properties dialog box appears.

Figure 9.8 3D ROI Properties			
Image: Sequence View 2, 30 View Image: Image			
Goronal(2=10.7) Sagittal(2=2.5) H - Help	🜠 3D ROI Properti	es 📃	- - X
	For Sequence: B ROI Name: ROI	20111027132749_	SEQ
	Location	3.18	
	Yc	47.96	
	Zc	9.35	
	Dimension		
	Width	12.01	
	Height	14.55	
	Depth	14.72	
Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y	Angle		5
x ¹	XRot	180.89	A
Transavial(y=47.7)	YRot	0.00	
	ZRot	0.00	×
Z Ais Range: 30.0 mm Perspective	Color	Done	

- **3.** Enter new values or use the arrows in the dialog box to modify the location or dimensions of the 3D ROI in the x, y, or z-planes.
- 4. Enter new values or use the arrows in the dialog box to rotate the 3D ROI in the x, y, or z-planes.
- **5.** To change the color of the 3D ROI:
 - a. Click the Browse button _____.
 The Select Color box appears (Figure 9.9).



- **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.

Saving 3D ROIs to the System

Living Image software saves 3D ROIs with a dataset (the software prompts you to save before closing the dataset). 3D ROIs (parameters only) can also be saved to the system (per user) and applied to other datasets. These 3D ROIs appear in the "Name" drop-down list (Figure 9.10). This section explains how to save 3D ROIs to the system.

NOTE: Before closing a dataset, the software prompts you to save 3D ROIs with the dataset. 3D ROIs saved with a dataset do not appear in the "Name" drop-down list.

- 1. After a 3D ROI(s) is applied to a dataset, save it to the system:
 - a. Confirm the default name or enter a new name for the 3D ROI in the Name drop-down list.
 - b. Click Save (Figure 9.10).

Figure 9.10 Name and Save 3D	ROIs to the System
Tool Palette	
∇ ROI Tools	
Measure 3D ROIs	× 🖻
Synchronize 3D ROI	<u>^</u>
Save 3D ROIs	
Name: 3D_ROI_3_KSA	•
Delete Load Save	
17	

- **2.** To apply a 3D ROI to a 3D reconstruction or volume, make a selection from the Name drop-down list and click **Load** (Figure 9.10).
 - **NOTE:** If you load a 3D ROI, then apply (or delete) 3D ROIs, the **Save** button changes to **Overwrite**. If you want to save the 3D ROIs using the existing name, click **Overwrite**. Otherwise, enter a new name and click **Save**.
- **3.** To delete a 3D ROI from the system (per user), select a 3D ROI from the Name drop-down list and click **Delete** (Figure 9.11).

Figure 9.11 Delete 3D ROIs From the System		
Tool Palette		
▼ ROI Tools		
🗗 🔂 v Measure 3D ROIs 🛛 🗙 🕑		
Synchronize 3D ROI		
Save 3D ROIs		
Name: 3D_ROI_2_KSA		
Delete Load Overwrite		

10 Working With Volumetric Data

About the 3D Multi-Modality Tools Classifying 3D Volumetric Data on page 182 Volume Display Options on page 186 Smoothing a Volume on page 191 Viewing and Rendering Slices on page 192 Registering Optical and Volumetric Data on page 195 Volume Information and Results on page 202 Volume Data Viewer on page 203 Viewing RAW Volumetric Data on page 204

10.1 About the 3D Multi-Modality Tools

Use the 3D Multi-Modality tools to:

- Classify volumetric data (reconstructed CT images representing 3D volumes).
- View volume slices.
- Refine the appearance of the volume (volume processing).
- Register optical and imported volumetric data (for example, CT, MRI, or PET data).



NOTE: IVIS Spectrum Imaging System does not acquire volumetric data. However, Living Image software can analyze volumetric data acquired on a different imaging system such as Spectrum CT Imaging System or Quantum microCT Imaging System.

Requirements

The Living Image 3D Multi-Modality tools require a separate license. Additionally, the graphics processing unit (GPU) must meet the minimum specifications shown in Table 10.1.

If the appropriate license is not installed or the GPU does not meet these specifications, the 3D Multi-Modality tools will not appear in the Tool Palette.

Specification	Description	
OpenGL Version Requirement ¹	OpenGL 2.0 and above	
OpenGL Extension Requirement ¹	GL-EXT-texture3D	
Graphics Card Memory	Minimum: 256MB (Dedicated + Shared)	
	Recommended: 1GB (Dedicated)	
Consumer Graphics Cards (Desktop/ Mobile, Windows/Mac)	Supported: ■ NVIDIA [®] GeForce [®] 8 Series and above (8, 9, 100, 200, 300 and 400 series) ■ ATI Radeon [™] HD 4000 Series and above (4000 and 5000 series)	
	Recommended: Desktop - NVIDIA GeForce GT 240 and above Mobile - NVIDIA GeForce GT 230M and above	
Workstation Graphics Cards (Desktop/ Mobile, Windows/Mac)	 Supported: NVIDIA[®] Quadro[®] NVS Series and Above (NVS and FX series) ATI FireGL[™] V5600 and Above (FireGL, FirePro and CrossFire series) Recommended: Desktop - Quadro FX 1800 and above Mobile - Quadro FX 880M and above 	

Table 10.1 Minimum Graphics Card Specifications

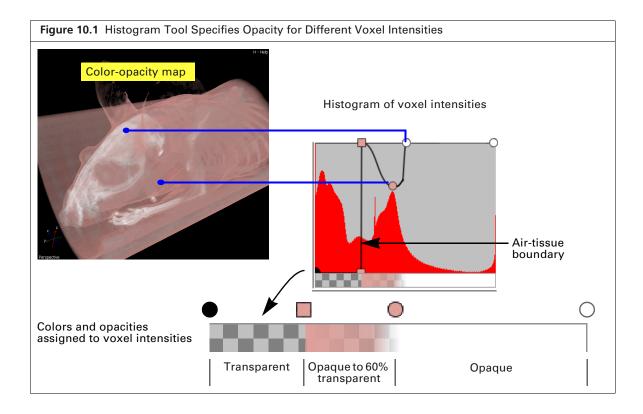
¹ If these specifications are not met, the 3D Multi-Modality tools do not appear in the Tool Palette.

10.2 Classifying 3D Volumetric Data

3D Multi-Modality tools provide a histogram-based method for classifying 3D volumetric data. The histogram represents the distribution of voxel intensities in the 3D volumetric data and their color-opacity values. The goal of classification is to set color and opacity values for different intensity ranges so that the color-opacity map shows the volume regions that you are interested in (opaque in the map) and hides unimportant regions (transparent in the map).

For example, Figure 10.1 shows how the histogram tool designed a color-opacity map that shows both the skin and bone. The histogram tool enables you to easily re-design the color-opacity map to show only the skin or only bone.

3D Multi-Modality tools also enable you to classify the volumetric data by specifying color and opacity values for different intensity ranges so that you can view or hide certain parts of the data as needed. A color-opacity map can be saved.

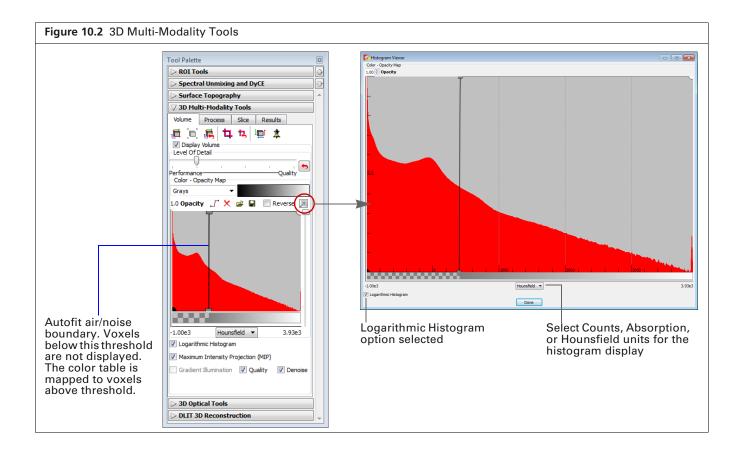


Specifying a Color-Opacity Map

After the surface and volume data are loaded, confirm that the Display Volume option is selected.

Histogram Display Options

- To change the color table for the color-opacity map, make a selection from the Color table-Opacity Map drop-down list. To apply the reverse color table, select the Reverse option.
- To view the histogram in a separate window, click the *button* (Figure 10.2).
- Select units for histogram display (Hounsfield, counts, or absorption). If the histogram intensity range appears narrow or suppressed, choose the Logarithmic Histogram option. This will enhance histogram display by magnifying the smaller regions of interest in the histogram while keeping noise and air-related intensity peaks high. It helps bring out hidden regions of the histogram for easier identification of interesting intensity ranges.



Managing Control Points

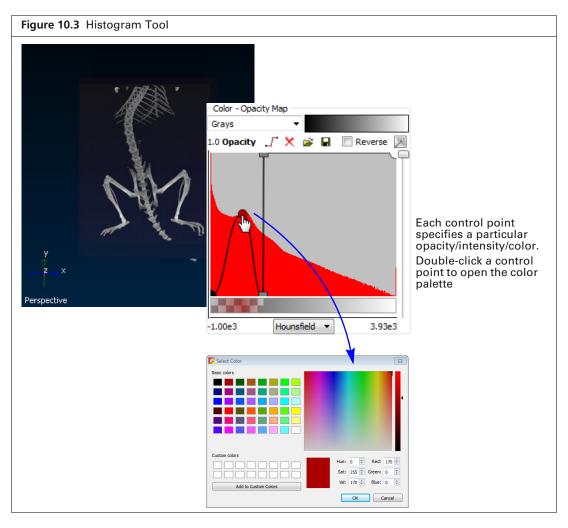
W

Edit the 3D volumetric data color-opacity map using "control points" (Figure 10.3). During volume rendering, the color-opacity map is used to map color and opacity to the corresponding intensity value as well as interpolate color and opacity for all data between adjacent control points.

- 1. Place a control point on the histogram by clicking anywhere on the histogram between the point (represents the lowest intensity in the volume) and O point (represents the highest intensity in the volume).
- **2.** Drag any control point up or down to set the opacity level that is associated with the intensity value represented by the point. Drag a user-added control point left or right to change the intensity associated with the opacity specified by the point.

When you add, delete, or modify a control point, the color-opacity map and the rendering of the volume data are updated in real-time.

NOTE: The minimum and maximum intensity levels associated with the **O** and **O** control points cannot be changed. The opacity level associated with these points can be changed.



- **3.** To select a color for particular data, double-click a control point. In the color palette that appears, choose a color and click **OK**. The software interpolates the color range between adjacent control points.
- 4. To delete a control point, right-click the point. To delete all control points, click the 🔀 button.

NOTE: The **•** and **O** control points cannot be deleted from the histogram.

Saving a Color-Opacity Map

A color-opacity map can be saved and applied to any volumetric dataset.

- **1.** Click the Save button **[]** (Figure 10.4).
- 2. In the dialog box that appears, select a folder for the file (.tfn) and enter a file name.
- 3. Click Save.

Loading a Color-Opacity Map

- **1.** Click the Open button 🚔 (Figure 10.4).
- 2. In the dialog box that appears, navigate to the map file (.tfn), and click Open.

	Figure 10.4 Save or Load a Color-Opacity Map			
Tool Palette ROI Tools Spectral Unmixing and DyCE Surface Topography 3D Multi-Modality Tools Volume Process Slice Results Image: Slice Reverse Image: Slice Slice Image: Slice <	Click a button to save or load a color-opacity map			

10.3 Volume Display Options

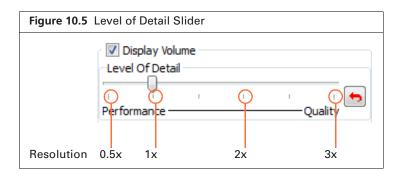
Adjusting Image Quality

By default, the color-opacity map displays the volumetric data at original $(1\times)$ resolution. This means, for example, if the volume comprises 512 slices, then all of the 512 slices are displayed. You can increase or decrease the resolution of the data display from $0.5\times$ to $3.0\times$ resolution (see Table 10.2 for examples).

If the resolution is increased, the software interpolates the data and adds slices to the volume. If the processing performance is impacted at the original resolution, you may want to reduce the resolution to improve performance. Reducing the resolution down-samples the data and fewer slices are displayed.

To adjust the image resolution:

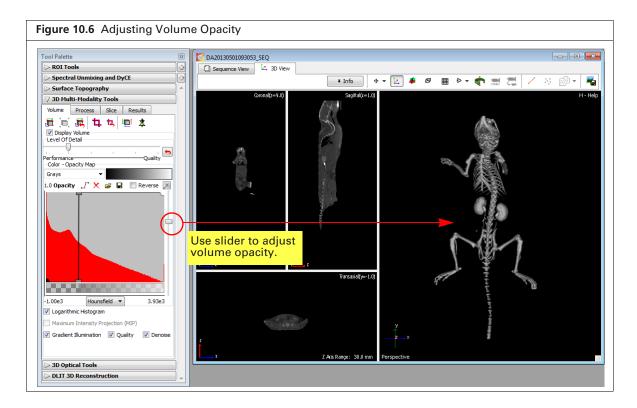
- **1.** Move the "Level of Detail Slider" to the left or right (Figure 10.5). The color-opacity map is updated.
- 2. To return the resolution to 1×, click the Reset button 🏞.



Volume Resolution	No. of Slices Displayed
0.5×	256
$1 \times$ (original resolution)	512
1.5×	768
2×	1024
2.5×	1280
3×	1536

Adjusting Volume Opacity

Adjust the volume opacity using the slider in the 3D Multi-Modality tools.

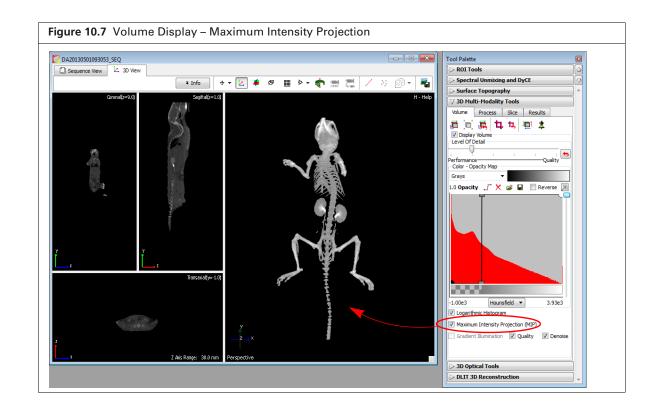


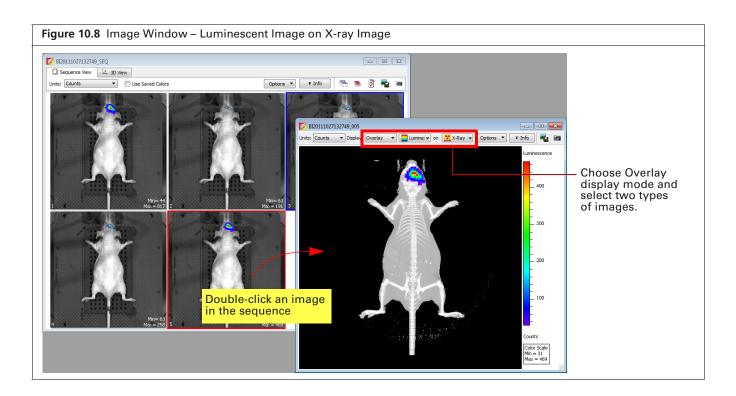
 $\overline{\mathbb{Q}}$

Maximum Intensity Projection

A maximum intensity projection (MIP) projects all maximum intensity voxels in the view along the viewing direction into the viewing plane (Figure 10.7). Living Image automatically extracts a 2D image from the MIP. The extracted image is similar in appearance to an X-ray image and is available in the image window. For example, Figure 10.8 shows an overlay of a luminescent image on an extracted 2D "x-ray" image.

NOTE: If you change the volume opacity (see Figure 10.6 on page 187) and want to extract new 2D images from the MIP, click the ***** button (Figure 10.7).





Gradient Illumination

 $\overline{\mathbb{W}}$

Gradient illumination is based on the idea that light is reflected at boundaries between different voxel intensities, but is not affected when passing through homogeneous regions. Choosing this option illuminates the voxels at boundaries more than voxels within a homogeneous region. The boundaries are based on the gradient magnitude between heterogeneous regions or the change in intensities between neighboring voxels in heterogeneous regions. Using this option enhances the variation in tissue properties and may be helpful for visualizing the boundaries of different tissues.

Two options are available for Gradient Illumination:

• Quality – This option will be automatically selected if your system has an appropriate graphics card. If this option is selected, the volume is displayed with more detail (Figure 10.9).

NOTE: If the system graphics card does not meet the recommended specifications (Table 10.1 on page 182), choosing the Quality option causes slow performance of actions such as rotating the volume.

• Denoise – Filters out image noise in the volume rendering. The raw data are not modified by this filter.

Figure 10.9 Volum	e Display – Gradient	Illumination	
Figure 10.9 Volume		Illumination	Tool Palette Image: Construction of the second se
, z	z Transzaial(y=-1.0) Z Aus Range: 30.0 mm	y Perspective	-1.00e3 Hounsfield 3.93e3 -1.00e3 Hounsfield 3.93e3 U Logarithmic Histogram Maximum Intensity Projection (MIP) Gradent Illumination Quality Denoise 30 Optical Tools DLIT 30 Reconstruction

Modifying Volume Resolution

Changing the pixel or slice spacing modifies the volume resolution. Increasing the pixel or slice spacing reduces resolution, while reducing either increases resolution.

- **1.** In the Volume tab, click the Edit Space and Orientation button \square .
- 2. In the dialog box that appears (Figure 10.10), edit the pixel or slice spacing.

🖉 Volume Information		? 🗙
Slice Information		
Pixel Spacing (Row, Column): (0.1000 🚔, 0.0016 🚔) mm	
Slice Spacing: 0.0016 🚔 mm		
Subject Orientation		
Invert X-Axis	Invert Y-Axis	Invert Z-Axis

10.4 Smoothing a Volume

Smoothing a volume reduces noise in a CT, MRI, or PET image such as excessive variation in voxel grayscale values.

To apply smoothing:

- **1.** Load the volumetric data.
- **2.** Choose the type of smoothing and group size in the Process tab of the 3D Multi-Modality tools (Figure 10.11).
 - Mean Applies the average grayscale value of a group of voxels (for example, a 3x3 group) to the central voxel of the group.
 - Median Applies the median grayscale value of a group of voxels to the central voxel of the group.
 - Gaussian Applies the weighted mean to the central voxel of the group. The weight distribution is similar to a normalized Gaussian shape with the highest value at the central voxel of the group.
- **3.** Click the 🔄 button to remove the smoothing.

						1
ool Palette						
∣> Image /	Adjust					
> Correct	ions / Filte	ering			Ð	
> ROI Too	ols					
> Spectra	l Unmixin	g and Dy	/CE		i l	
> Surface	Topograp	ohy			i l	
⊽ 3D Mult	i-Modality	/ Tools			í l	
Volume	Process	Slice	Results		1	
Volume pro	cessing:					
Smoothing:	None		.	3X3 🔻		
	None					
	Mean Median		2			
	Gaussian		45			
	_					
DLIT 3D	Reconstr	uction				

10.5 Viewing and Rendering Slices

Viewing Slices

View volume slices by double-clicking the Coronal, Sagittal, or Transaxial windowpane (Figure 10.12).

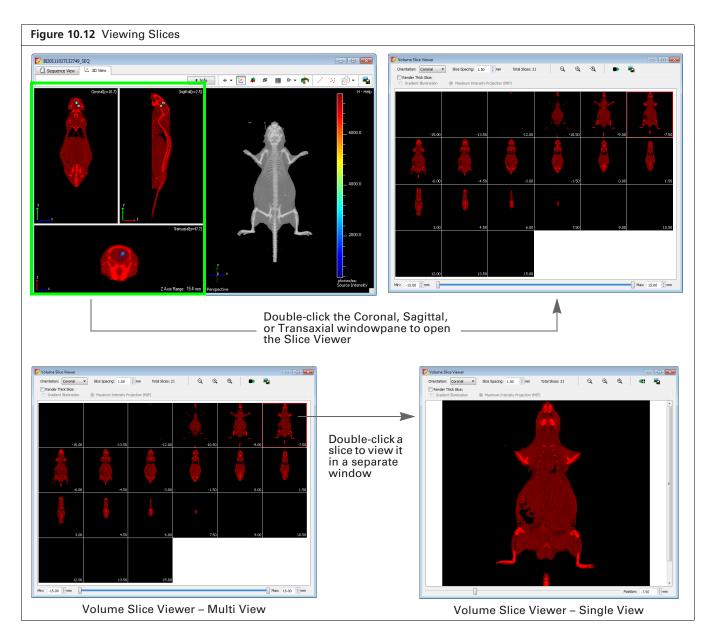


Table 10.3 Volume Slice Viewer

Item	Description
Orientation	Select a slice orientation from the drop-down list.
Slice Spacing	The distance between each slice in the Volume Slice Viewer. Enter a smaller value to increase the number of slices in the viewer or a larger value to decrease the number of slices in the viewer.
Total Slices	The number of slices shown in the viewer.
Render Thick Slice	This option is used to create a sequence of 3D or maximum intensity projection (MIP) renderings from the image stack. When this option is selected, "Slice Spacing" changes to "Slice Thickness". Increasing the slice thickness causes more slices to be extracted from the volume before creating the rendering.
Gradient Illumination	Gradient Illumination is based on the idea that light is reflected at boundaries between different voxel intensities, but is not affected when passing through homogeneous regions. Choosing this option illuminates the voxels at boundaries more than voxels within a homogeneous region. The boundaries are based on the gradient magnitude between heterogeneous regions or the change in intensities between neighboring voxels in heterogeneous regions. Using this option enhances the variation in tissue properties and may be helpful for visualizing the boundaries of different tissues.
Maximum Intensity Projection (MIP)	Projects all maximum intensity voxels in the view along the viewing direction into the viewing plane.
Min: -15.00 x mm	Min – The slice coordinate of the first slice being viewed. Zero is defined as the center plane of the image. Max – The slice coordinate of the last slice being viewed. Specify the position range to include in the viewer using the Min and Max sliders or enter values.
	Click to show the single view of the active slice in the multi view. Alternatively, double-click a slice in the multi view to show the single view.
4 :	Click to show the multi view.
Q	If the single view has been magnified, click this button to zoom out incrementally.
€	Magnifies the single view.
Q	Resets the single view to the default magnification.

Rendering Slices

The Slice tab in the 3D MM tools contains rendering and viewing options for slices.

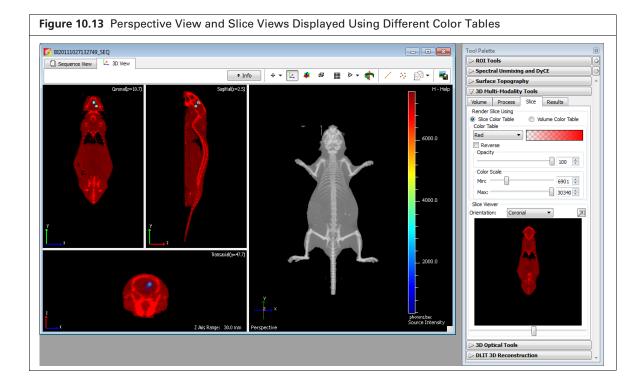


Table 10.4 3D Multi-Modality Tools for Rendering Slices

Item	Description
Slice Color Table	Choose this option to apply the color table selected from the Color Table drop- down list.
Volume Color Table	Choose this option to apply the volume color table of the volume color-opacity map that was selected in the Volume tab.
Color Table Red Red Reverse	Color table options. Choose the Reverse option to apply the inverse color table.
Opacity	Move the slider to adjust the color opacity.
Color Scale	Min – Sets the intensity level associated with the lowest color scale value.
	Max – Sets the intensity level associated with the maximum color scale value.
Slice Viewer Orientation: Coronal	The Tool Palette provides an alternative way to view slices and access the Slice Viewer.
	Choose a slice orientation from the drop-down list. Use the slider to move through the slices. Double-click the slice view or click the slice button to open the Slice Viewer. The selected slice is highlighted in the Slice Viewer.

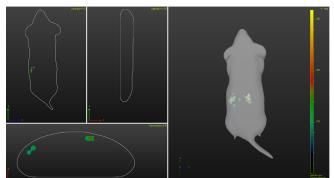
10.6 Registering Optical and Volumetric Data

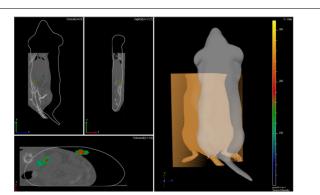
Registering *multi-modal* data (optical and volumetric data) provides an anatomical context for interpreting biological (functional) information. Two registration methods are available:

- Automatic fiducial registration For experiments in which the optical data are acquired on the IVIS Spectrum and the CT data are acquired on the Quantum FX microCT pr Quantum GX microCT. The subject must be contained in the Mouse Imaging Shuttle during both optical and CT imaging, and the CT data must be exported to DICOM format. See page 197 for more details.
- Manual registration Use the 3D Multi-Modality tools to register a 3D surface reconstruction with 3D volumetric data acquired on a third party instrument. See page 199 for more details.

Figure 10.14 shows an overview of the steps to register these types of multi-modal data. After registration, classify the 3D volumetric data to help identify and separate objects (see page 182).

Figure 10.14 Registering Multi-Modal Data

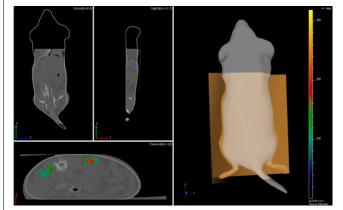




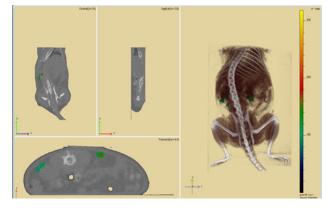
2. Load 3D volumetric data (CT or MRI) (page 196).

1. Load the optical data:

- Bioluminescence or fluorescence image sequence and structured light surface
- 3D source reconstruction (DLIT or FLIT results)



- **3.** Register the 3D source reconstruction and the 3D volumetric data by performing either:
 - Automatic fiducial registration–Available for data acquired on the Quantum FX µCT instrument using the Mouse Imaging Shuttle (page 197)
 - or
 - Manual registration–Match animal surface representations using the Manual Registration tool (page 199)



- **4.** Classify the 3D volumetric data to help identify and separate objects (page 182). Save the color-opacity map (optional).
- **5.** Save the registered 3D multi-modality results (page 202).

Loading Data for Registration

- 1. Load a DLIT or FLIT image sequence and the 3D reconstruction results.
- **NOTE:** The 3D Multi-Modality tools appear in the Tool Palette after you load optical image data. If the 3D Multi-Modality tools do not appear in the Tool Palette, confirm that the 3D Multi-Modality Tools license is installed and that the workstation graphics card meets the specifications in Table 10.1 on page 182.
- 2. Select the DICOM or TIFF volumetric data.
 - **a.** Click the $\frac{1}{30}$ toolbar button. Alternatively, select File \rightarrow Browse 3D Volumetric Data on the menu bar.
 - **b.** If this is the first time browsing for volumetric data in the session, select a data folder in the dialog box that appears, and click **Select Folder** (Figure 10.15).
 - The Living Image 3D Volumetric Browser appears.

NOTE: If the 3D Volumetric Browser was previously opened during the session, clicking the button opens the browser. Click the Browse.. button in the browser, and in the dialog box that appears, select a data folder. Only DICOM or TIFF data can be added to the 3D Volumetric browser. For details on loading other data types (.raw or .vox files) see page 202.

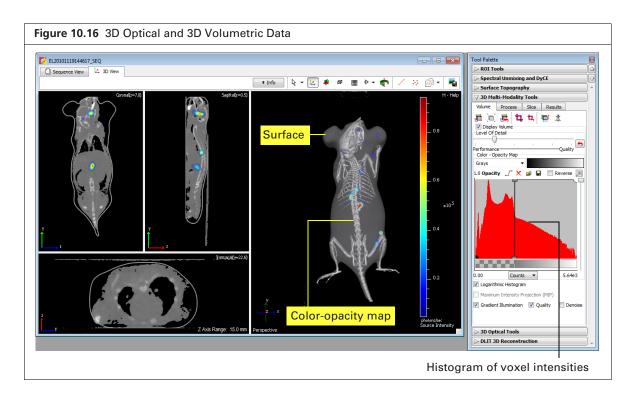
e Edit View Tools Windo	ing the 3D Volumetric Browser			
Living Image Multi-Modality Foli Composition Caliper Data > Organize - New folder	der: CoReg_Demo • • • 49 Search CoReg_Demo	₽ ₽	3D Volumetric Browser	
Ibraries Ib Occuments Image: Music Image: Pictures Image: SW Download Installers Image: Videos Image: Non-State Image: Non-State	Name Date modified Type ▲ EL2010119144617_SEQ 3/14/2011 3:29 PM File fo ▲ Mouse2.3_Day 28_145522 3/14/2011 3:30 PM File fo	older	Name Folder Path	r LS/Caliper Data/CoReg_Demo 512
P Computer	v < m uuse3_3_Døy 28_145522 Select Folder Cancel	No vald 30 Scene. Data will be loaded in		ad in a new window Load

The 3D Volumetric Browser automatically previews a playback of the data along with other information about the data (Figure 10.15). See Figure 8.24 on page 169 for more details on the 3D Volumetric Browser.

- **3.** Load the volumetric data with the optical data.
 - **a.** Confirm that the "Load in a new window" option is not selected. (If this option is selected, the volumetric data are loaded in a new window.)
 - b. Double-click the data row in the browser. Alternatively, select the data row and click Load.

The 3D volumetric data appears in the 3D View window of the optical data (Figure 10.16). The software converts loaded volumetric data into an 8-bit representation to reduce memory overhead and for easier color mapping. The 3D Multi-Modality tools provide an 8-bit color-opacity map for volume visualization which maps each voxel to an RGB color, or a color and opacity value.

A histogram of voxel intensities appears in the Multi-Modality tools and the software sets a default air/noise boundary.



Registering Multi-Modal Data

Automatic Fiducial Registration

About the Mouse Imaging Shuttle

The Mouse Imaging Shuttle (PN 127744) contains the subject during imaging and enables the subject to be transferred between an IVIS[®] Imaging System and the Quantum FX μ CT instrument without disrupting the subject's position.

The Mouse Imaging Shuttle must be correctly docked to the docking station in the IVIS Imaging System and the Quantum FX μ CT instrument. The docking station in the Quantum FX μ CT system is marked with a triangle-shaped fiducial pattern under the plane where the Mouse Imaging Shuttle docks. Automatic fiducial registration is available if both sides of the triangle fiducial pattern are included in the CT images. For more details on using the Mouse Imaging Shuttle, see the *Mouse Imaging Shuttle Instructions* (PN 127820).

To perform automatic fiducial registration:

- **1.** Load the data that you want to register (see page 196).
- 2. Click the Fiducial Registration button .

The multi-modal data are automatically registered and cropped (Figure 10.17).

3. To undo the registration, click the Reset Registration button 🛺

- **4.** To save the registration information:
 - **a.** Confirm the default name or enter a name for the results in the Results tab.
 - b. Click Save.



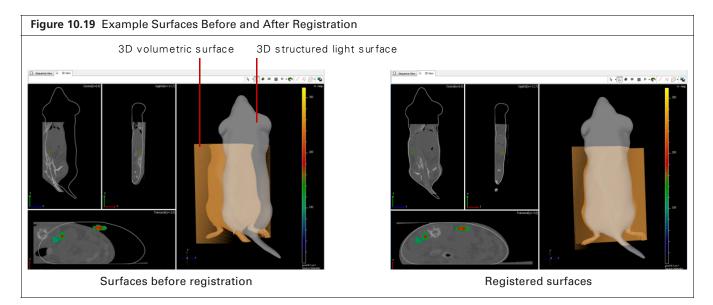
igure 10.18	3D Multi-Modality Tools – Results
⊽ 3D Multi-Moda	lity Tools
Volume Slice	Results
Volume Information:	MULTIMODALITY_6 (Loaded)
Key	Value
StudyDate	20101119
NameOfVolume	Mouse3_3_Day 28_145522_000
PatientsName	Mouse3
Modality	СТ
Manufacturer	2
BitsAllocated	
SamplesPerPixel	
Rows	256
	256
NumberOfFram PixelSpacing	
SliceThickness	
	30.208000\30.208000\60.4160
ImageOrientati	
PatientsBirthDate	
•	4
Save Results	
Name: MULTIMOD	ALITY_7 🗸
Delete	Load Save

 \square

NOTE: Registration information is saved with the results for the volumetric data and is specific for a particular optical dataset.

Manual Registration

To manually register data, use the 3D Multi-Modality tools to translate, scale, or rotate the 3D volumetric surface so that features common to both surfaces are matched and aligned in the x, y, and z planes. Examine the matched surfaces in the 3D slice views to help you fine tune the registration.

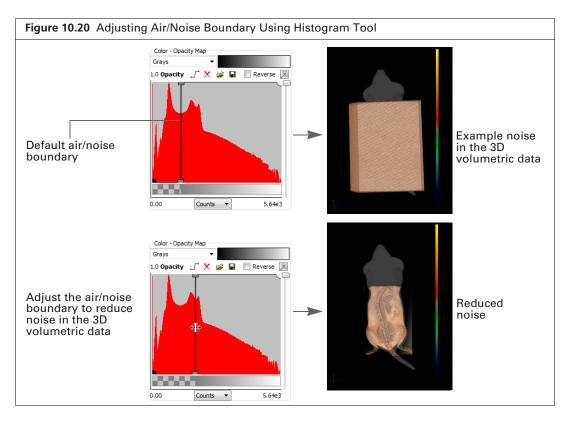


To manually register data:

1. Load the data that you want to register (see page 196 for more details).

The software determines a default air/noise boundary for the 3D volumetric data (Figure 10.20).

2. If you need to remove noise from the 3D volumetric data, move the air/noise boundary to the right in the histogram tool.

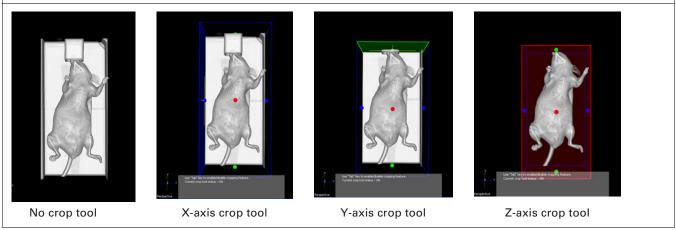


- **3.** If the volumetric data needs cropping (for example, to remove structures such as the stage from the CT view), follow step a to step c below. If cropping is not needed, proceed to step 4. To crop the data:
 - **a.** Click the crop tool button \Box .

The crop tool appears and has six control points:

- Crops the data along the x-axis.
- Crops data along the y-axis.
- Crops data along the z-axis.

Figure 10.21 Crop Data Along X,Y, or Z-Axis



b. Click and hold a control point while you move the crop plane. As you move the crop plane, the slice views are updated. Release the mouse button to crop the data.

- c. To reset the crop planes, click the 🔽 button. When finished cropping, press the Tab key to turn off the crop tool.
- **4.** Click the Manual Registration button **[**].

The transformation tool appears (Figure 10.22). The tool has three modes that enable you to translate, scale, or rotate the 3D volumetric data (press the Tab key to change the tool mode). The slice views are automatically updated when you use the tool.

Figure 10.22 Manual Registration Tool – Transformation ModesImage: Signal of the second sec

NOTE: Make sure that you click the transformation tool so that it is highlighted before you use it. Otherwise the dragging operation is applied to the optical data (structured light surface).

- To return the 3D volumetric data to the default position and size, click the Reset Registration button
- 6. Save the registration information (see page 198).

NOTE: Registration information is saved with the results for the volumetric data and is specific for a particular optical dataset.

10.7 Volume Information and Results

The Results tab displays information about the loaded data taken from the DICOM file header (Figure 10.23).

ROI Tools				
Spectral Unmi	xing and D	yCE		i.
Surface Topog	raphy			
3D Multi-Moda	lity Tools			j
olume Proces	s Slice	Results		
ume Information:	(Unsaved)			
ey	Value			
udyDate	20111027			
ameOfVolume	BI2011102	7132749.dc	m	
atientsName	DevpPhas	e		
lodality	OT			
	Caliper Li	fe Sciences		
tsAllocated	16			
amplesPerPixel	1 100			
olumns	400			
umberOfFram				
xelSpacing	0.300000\	0.300000		
nagePositionP	6.0000\0.8	400\-6.000)	
nageOrientati				
atientsBirthDate	20111027			
			÷.	
ave Results				
ame: MULTIMOD	ALITY_1		•	
Delete	Load	Over	write	
3D Optical Too	s			h l
DLIT 3D Recor				2

Saving the registered and classified data provides a convenient way to share data. The software saves the following:

- Level of detail setting
- Color tables for the opacity map and slices
- Histogram tool control settings and the resulting color-opacity map
- Multi-modal registration settings
- Crop settings

Managing Results

Saving Registered Results

- 1. In the Results tab, confirm the default name in the Name drop-down list or enter a name.
- 2. Click Save.

The registered 3D volumetric data, along with the color-opacity settings, appear in the 3D View window.



NOTE: The results are saved in XML format in the XML folder within the sequence folder. The results can only be accessed from the same optical dataset.

Loading Results

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

Deleting Results

- 1. Select the results from the Name drop-down list.
- 2. Click Delete.
- **3.** Click **Yes** in the confirmation message that appears.<\$startrange>Tool Palette:3D Multi-Modality tools:Results

10.8 Volume Data Viewer

The Living Image software provides a viewer for volumetric data. The 3D Multi-Modality tools are not required to view DICOM or TIFF data.

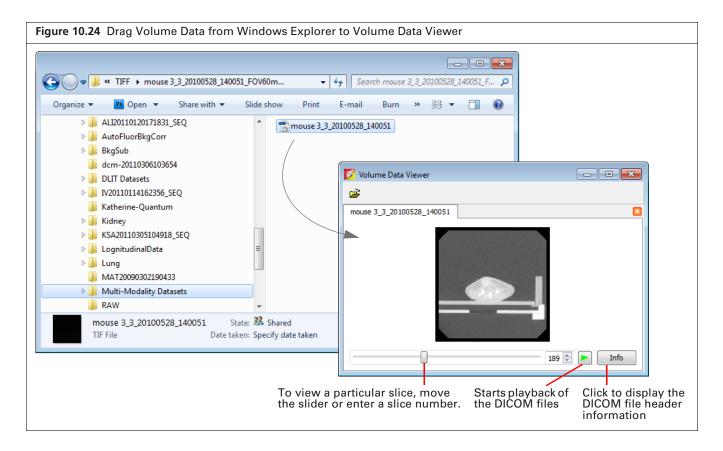
1. Select $View \rightarrow Volume Data Viewer on the menu bar.$

The Volume Data Viewer appears.

- 2. Select volume data by doing either of the following:
 - Drag the data file (DICOM, TIFF) from Windows Explorer to the Volume Data Viewer window

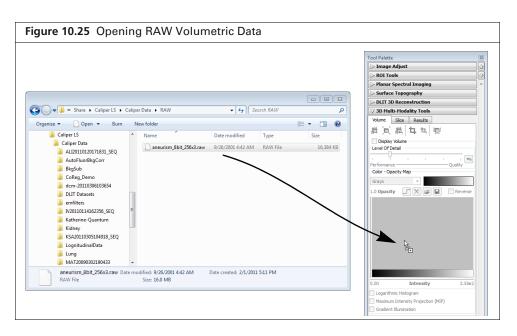
or

- In the Volume Data Viewer, click the Open button $\overrightarrow{}$, and in the dialog box that appears, select a DICOM or TIFF file, and click **Open**.
- **3.** To clear the Volume Data Viewer, click the 🔀 button.



10.9 Viewing RAW Volumetric Data

- 1. Drag a single RAW file (*.raw or *.vox) from Windows Explorer to the 3D Multi-Modality tools (Figure 10.25).
- **NOTE:** Only single *.raw or *.vox files consisting of multiple slices of a 3D volume can be loaded into Living Image.



- 2. In the Volume Information dialog box that appears (Figure 10.26), enter the:
 - Data width, height, and the number of slices.
 - Slice row, column pixel size, and the slice spacing in millimeters.

Figure 10.26 Volume Information	
Volume Information Volume Information File: C:/Share//aneurism_Bbit_256x3.raw Data Type: @-bit Signed Width: 1 pixels Height: Header Offset: 0 Dytes Number Of Silces/Images: Silce Information Pixel Spacing (Row, Column): Pixel Spacing (Row, Column): (0.1000 🔄, 0.1000 🔄) mm Silce Resolution Low: Down-samples volume data. It conserves memory and improves performance. Full Memory Requirement Status Good OK	Select a data type. Enter the: • Width, height, and number of slices • Slice row, column pixel spacing, and the slice spacing in millimeters

3. If loading the data will cause low memory, you are prompted to down-sample the data to improve memory and performance (Figure 10.27). Decrease the slice resolution by moving the Slice Resolution slider to the left until the Memory Requirement Status is "Good".

igure 10.27	Down-sa	mple 3D V	olumetric	: Data	
🖉 Volume Infor	mation				? ×
-Slice Information -					
Pixel Spacing (Rov	v, Column): (0.1	.000 📫, 0.100	0 🕂) mm		
Slice Spacing: 0.1	000 🕂 mm				
-Slice Resolution					
	oles volume data	. It conserves me	mory and improv	es performance.	
		lata using the orig			\frown
Low					
Memory Requirem	ant Status				\sim
Low	encotatus				
LOW					
					Cancel

Changing the Orientation of RAW Volumetric Data

Occasionally, RAW files (*.raw or *.vox) may be loaded with the orientation "flipped" or reversed along the x, y, or z-axis. As a result, the slice views (transaxial, coronal, sagittal) may be flipped or rotated so that the actual view that is displayed does not match the 3D View windowpane name (for example, the Sagittal windowpane does not display a sagittal slice), or the data appears flipped with respect to the surface derived from the IVIS[®] Spectrum.

In such cases, you can:

- Invert the data along the x, y, or z-axis
- Manually rotate the data using the Transformation tool (see page 201 for more details).

To invert the subject orientation:

- **1.** Click the Edit Spacing & Orientation button
- 2. In the dialog box that appears, choose a "Subject Orientation" option and click OK.

Figure 10.28 Volume Int	formation	
Volume Information Slice Information Pixel Spacing (Row, Column): (C Slice Spacing: 0.0016 mm Subject Orientation	0.1000 🔹, 0.0016 🚖) mm	? *
Invert X-Axis	Invert Y-Axis	Invert Z-Axis
		OK Cancel

11 Spectral Unmixing

About Spectral Unmixing Acquire a Sequence for Spectral Unmixing Spectral Unmixing Methods on page 215 Correcting Spectra on page 226 Spectral Unmixing Results on page 228

11.1 About Spectral Unmixing

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) or transillumination (excitation light below the stage).
- Analyze luminescent or fluorescent images when more than one reporter is used in the same animal model.

11.2 Acquire a Sequence for Spectral Unmixing

Set up an image sequence for spectral unmixing using the Imaging Wizard.



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

Choose an imaging mode in the wizard based on the type of probes.

Probe Type	Follow the Instructions for:	
Luminescent	Bioluminescence Imaging on page 207	
Fluorescent	Fluorescence Imaging on page 209	
Radio-isotope	Cherenkov Imaging on page 212	

If you are not using the Imaging Wizard to set up the image sequence, acquire a sequence using several filters which sample the emission or excitation spectra of all probes in the study at multiple points across the entire range. Include tissue autofluorescence for fluorescent spectral unmixing.

Make sure that the band gap between the excitation and emission filters is sufficiently large so that the excitation light does not leak through the emission filter where it can be detected by the CCD.

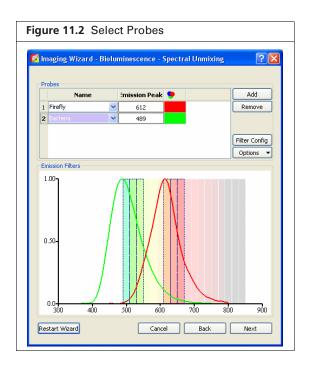
If a dataset includes multiple excitation and emission filter scans, the software automatically unmixes signal according to the filter type with the most entries. For example, a dataset acquired using three excitation filters and four emission filters will be unmixed by emission wavelength.

Bioluminescence Imaging

- **NOTE:** The IVIS Spectrum should be initialized and the temperature locked before setting the imaging parameters. See page 19 for more details.
- **1.** Start the Imaging Wizard. See page 44 for instructions.
- **2.** Double-click the Bioluminescence option in the wizard. Double-click the Spectral Unmixing option in the next screen (Figure 11.1).

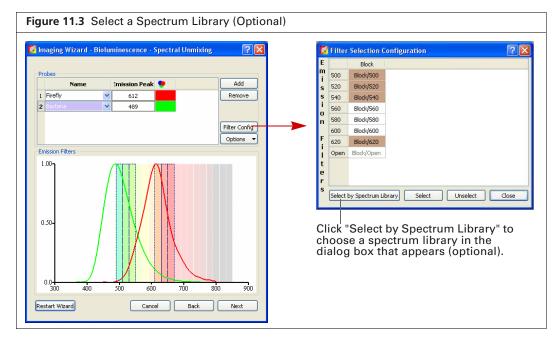
Figure 11.1 Imaging Wizard – Cl	hoose Bioluminescence and Spectral Un	nmixing Options
If this screen does not appear when the wizard starts, click Restart Wizard Restart Wizard (on the wizard screen) to restart the wizard.	ging Wizard Imaging Mode Finance construction Bioluminescence Imaging sectors Select this option for imaging bioluminescent or chem/unmisscent reporters, such as firefly uurferase, chick beetle ludferase, it is option for imaging fluorescent proteins, dyes, or nanoparticles in the wavelength range of 450-850 nm. Belect this option for imaging fluorescent proteins, dyes, or nanoparticles in the wavelength range of 450-850 nm. Both epi-Ilumination (furninationfrom above) and trans-ilumination (from below) modes are available. Cherenkov Imaging Select this option for imaging radotracers which emit Cherenkov light in tissue.	Imaging Wizard - Bioluminescence Imaging Wizard - Bioluminescence Open Filter Bioluminescence - Spectral Unmixing Open Filter Spectral unmixing uses a mathematical operation to allow users to separate the signal from two or more lallow users to separate the signal from two or more landow users to separate the signal from two or more landow users of separate the signal from two or more landow users of separate the signal from two or more landow users of separate the signal from two or more landow users of separate the signal from two or more landow users separate the signal from two or more landow users for the signal from two or more

- **3.** Select a probe from the Name drop-down list (Figure 11.2).
- **4.** Click **Add** and select another probe from the Name drop-down list. Repeat until all of the probes are added.



5. Optional: If you generated a spectrum library (a set of reference spectra), you can select it in the Imaging Wizard. Click **Filter Config**, then click **Select by Spectrum Library** in the dialog box that appears (Figure 11.3).

See Guided Method on page 215 for instructions on creating a spectrum library.



- 6. Click Next and in the screen that appears (Figure 11.4):
 - **a.** Select the type of subject.
 - **b.** Select a field of view.
 - **c.** Set the focus options.

Imaging Subject: Mouse	•			
Exposure Parameters • Auto Settings		lanual Settings		
Luminescent				
Field of View				
C - 12.8 cm 💌				
Focus Subject Height: 1.50 📚 cm	Fo	ocus: use subject h	eight 💌	
Options Time Series Study Total number of segments: Delay between segments: 0,0	Mins			
Delay between segments. 0.0	■ Philis			

- **7.** To acquire a time series of images:
 - **a.** Choose the Time Series Study option (Figure 11.4).
 - **b.** Enter the number of segments and the delay between segments.
- 8. Click Next.

The specified sequence appears in the sequence table (Figure 11.5).

Figure 11.5 Sequence Setup Compl	ete	
Imaging Wizard - Bioluminescence - Spectral Unmixing Topograph States Course Proventess Auto Settings Unminescent Topograph Topograph Course of these	🖉 TVIS Acquisition Control Panel	
Nuts Construction Construction	Imaging Mode Exposure Time Binning F/Stop Excitation Filter	Enciden Filter S40 Store - Source - Store -
Leeg serveen seguens.	Structure V Overlay Ughts V Algoment Gid Batch	2 3 Auto Median 1 Block 6400 No C 1.50 3 1 Auto Median 1 Block 6600 No C 1.50 4 Auto Median 1 Block 6600 No C 1.50
	MIS Ide	e Sequence 5 1 Auto Medum 1 Block 520 No C 1.50 6 1 Auto Medum 1 Block 540 No C 1.50
Restart Woard Cancel Back Next	Subject height: 1.50 Cm	ango Yoard ango Sotup ABalae Number of Segments: 1 0 Delay: 0.0 0 min (Apply to Al) X Remove - ((Update) (Incert) (Add

9. Acquire the sequence following the instructions on page 46.

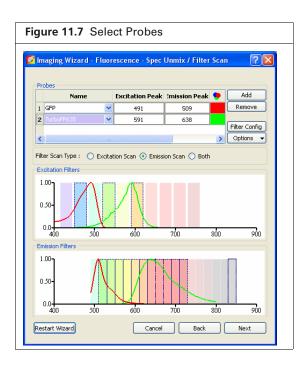
The image window appears when acquisition is completed (Figure 11.17). See Table 4.2 on page 30 for more details on the Image window.

Fluorescence Imaging

- 1. Start the Imaging Wizard. See page 44 for instructions.
- 2. Double-click the Fluorescence option.
- **3.** Select Spectral Unmixing in the next screen (Figure 11.6).
- 4. Select the type of illumination and click Next.
 - Epi-Illumination Light source above the stage.
 - Trans-Illumination Light source below the stage.

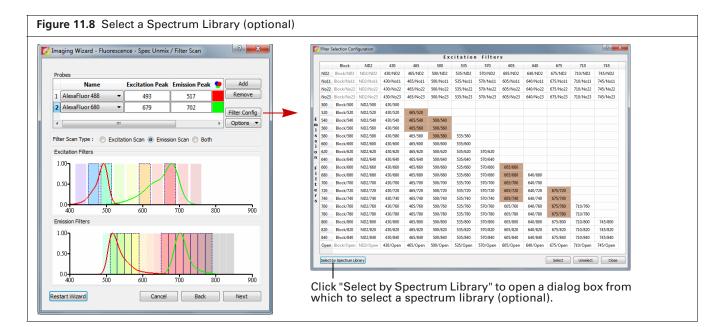
Figure 11.6 Imaging Wizard – Cho	ose Fluores	cence and Spectral Unmixin	ng Options	
If this screen does not appear when the wizard starts, click Restart Wizard [Restart Wizard] (on the wizard screen) to restart the wizard.	Reluminescence Reluminescence Exercision Exercision Cherenkov	Imaging Mode Bioluminescent Imaging Select this option for imaging bioluminescent or chemitknimescent reporters, such as frefly Lucferase, click beetle kufferase, renills, or bacterial kufferase. Fluorescence Imaging Select this option for imaging fluorescent proteins, dress, or nanoparticles in the wavelength range of 450-450 nm. Both epi-llumination (flumination from above) and trans-flumination (flumination flumination flumination flumination (flumination flumination fluminat	✓ Inaging Wizar Filter Pair Spectral Limitsing DycE Filt	Cancel

- 5. Select a probe from the Name drop-down list in the next screen (Figure 11.7).
- 6. Click Add and select another probe from the Name drop-down list. Repeat until all of the probes are added.



7. Optional: If a spectrum library (a set of reference spectra) is available, you can select it in the Imaging Wizard. Click **Filter Config**, then click **Select by Spectrum Library** in the dialog box that appears (Figure 11.8).

See Guided Method on page 215 for instructions on creating a spectrum library.



- 8. Click Next and in the screen that appears (Figure 11.9):
 - **a.** Select the type of subject.
 - **b.** Select a field of view.
 - **c.** Set the focus options.

Imaging Subject: Mo	use 🔽	ec Unmix / Filter S	can 🤶	
Exposure Parameters Auto	Settings	🔘 Manual Sett	ings	
Fluorescent				
Field of View	~			
Focus Subject Height: 1.5) 🗘 cm	Focus: use su	ubject height 🔽	
Options Time Series Study Total number of seg Delay between segr	ments: 1	ns		
Fluorescence	Raster Scan			
-	tup No Trans - Illumina	tion locations selected		

- 9. To acquire a time series of images:
 - a. Choose the Time Series Study option (Figure 11.9).
 - **b.** Enter the number of segments and the delay between segments.
- **10.** If using transillumination, select the transillumination locations.
 - a. Click Transillumination Setup.
 - **b.** Choose the transillumination locations in the Transillumination Setup box that appears (Figure 11.10).

See Table 4.3 on page 40 for more details on Transillumination Setup.

Figure 11.10 Transillumination Setup Box	
Hask Grid Points To Subject Grid Type: [15:23 Clear Selections Cancel	Selected transillumination locations are marked by a green square .

11. Click Next.

The specified sequence appears in the sequence table (Figure 11.11).

igure 11.11 Sequence Setup	Complete							
ging Wizard - Fluorescence - Spec Unmix / Filter Scan 🛛 💽 🔀								
ging Subject: Mouse								
osure Parameters								
Auto Settings Manual Settings								
✓ Fluorescent								
V Photograph								
X-Ray								
of View	🚺 IVIS Acquisition Control Panel							
s	Imaging Mode Exposure Time Binning F/Stop Excitation Filter Em	mission Filter 🛛 🖂 🗖 🗖	Display Photographic Set	inar		Sub	ect: Mouse	V Prob
ect Height: 1.50 🗢 cm Focus: use subject height 👻	Luminescent Auto 🗘 sec 🕶 Medium 💌 2 💌 605 🖤 720		Display Procographic Sec	ungs		500,	House	
6	Evel:	High V Seq-1	function Distance FC	an Frickabler Frida		and Charlestone 1	OU Uninha	
ns Time Series Study	Fluorescent Transilumination Lamp Level: Photograph 0.20 Medium V 8 V Reuse	High Mode	Exposure Binning FS					
ns ine Series Study In uniber of segments:	V Photograph 0.20 C Medium V 8 V Reuse	High Mode	Auto Medium 2	465 520	High	No	1.50	
ns ine Series Study In uniber of segments:	I ♥ Photograph 0.20 0 Medum ♥ 8 ♥ ♥ Reuse	High Mode	Auto Medium 2 Auto Medium 2	465 520 465 540	High High	No No	1.50 1.50	
ns ine Series Study In uniber of segments:	V Photograph 0.20 C Medium V 8 V Reuse	High Mode 1 1 2 1 1 2 1 1 2 1 1 2 1 1 2 1 1 2 1 1 2 1 1 2 1 1 1 2 1 1 1 2 1	Auto Medium 2 Auto Medium 2 Auto Medium 2	465 520 465 540 465 560	High High High	No No No	1.50 1.50 1.50	
ns ime Series Study Il number of segments:	Photograph 0.20 : Medium v 8 v Peuse Souchre Dowelay Lights v Alignment Grid Sech Seq. Feld of Veer. C v System Status	uences 4	Auto Medium 2 Auto Medium 2 Auto Medium 2	465 520 465 540	High High	No N	1.50 1.50	
6	Photograph 0.20 Medium V 8 V Pause Swuture Overlay Lights V Alignment Grid System Status Acgure Se	uences 4	Auto Medium 2 Auto Medium 2 Auto Medium 2 Auto Medium 2	465 520 465 540 465 560 465 580	High High High High	No N	1.50 1.50 1.50 1.50	
ns ime Series Study Il number of segments:		High Mode 1 L 2 L 3 L 4 L 5 L 6 L 7 L 1 L 1 L 2 L 2 L 2 L 2 L 2 L 2 L 2 L 2	Auto Medium 2 Auto Medium 2 Auto Medium 2 Auto Medium 2 Auto Medium 2	465 520 465 540 465 560 465 580 535 600	High High High High High	No N	1.50 1.50 1.50 1.50 1.50	
ns ine Series Study In uniber of segments:	Photograph 0.20 Medium V 8 V Pause Swuture Overlay Lights V Alignment Grid System Status Acgure Se	High Mode 1 Car 2 Car 4 Car 5 Car 6 Car 7 Kard 8 Car	Auto Medium 2 Auto Medium 2	465 520 465 540 465 560 465 580 535 600 535 620	High High High High High High	No N	1.50 1.50 1.50 1.50 1.50 1.50 1.50	

12. Acquire the sequence following the instructions on page 46.

The image window appears when acquisition is completed (Figure 11.17). See Table 4.2 on page 30 for more details on the Image window.

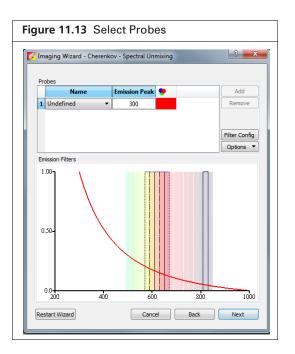
Cherenkov Imaging

NOTE: The IVIS Spectrum should be initialized and the temperature locked before setting the imaging parameters. See page 19 for more details.

- 1. Start the Imaging Wizard. See page 44 for instructions.
- **2.** Double-click the Cherenkov option. Double-click the Spectral Unmixing option in the next screen (Figure 11.12).

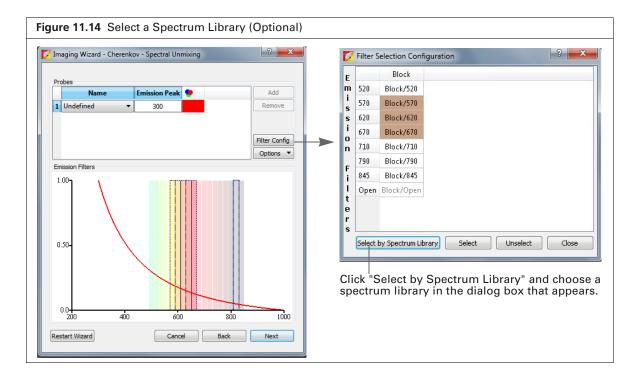
Figure 11.12 Imaging Wizard – Cl	noose Cherenkov and Spectral Un	mixing Options	
If this screen does not appear when the wizard starts, click Restart Wizard Restart Wizard (on the wizard screen) to restart the wizard.	Imaging Mode Bioluminescence Imaging Select this option for imaging bioluminescent or chemiluminescent reporters, such as firefly luciferase, click beetle luciferase, renila, or bacterial luciferase. Fluorescence Imaging Select this option for imaging fluorescent proteins, dyes, or nanoparticles in the wavelength range of 450-850 nm. Both epi-flumination (fluminationfrom above) and trans-flumination (from below) modes are available. Cherenkov Imaging Select this option for imaging radiotracers which emit Cherenkov light in tissue. Cancel Next	Imaging Wizard - Cherenkov Imaging Wizard - Cherenkov Imaging Wizard - Cherenkov Open Filter Imaging Spectral unmixing Spectral unmixing Spectral unmixing DyCE For more information on Spectral Unmixing Cancel	inal from two or more tent images taken with tographic image is also

- **3.** Select a probe from the Name drop-down list (Figure 11.13).
- **4.** Click **Add** and select another probe from the Name drop-down list. Repeat until all of the probes are added.



5. Optional: If you generated a spectrum library (a set of reference spectra), you can select it in the Imaging Wizard. Click **Filter Config**, then click **Select by Spectrum Library** in the dialog box that appears (Figure 11.14).

See *Guided Method* on page 215 for instructions on creating a spectrum library.



- 6. Click Next and in the screen that appears (Figure 11.15):
 - **c.** Select the type of subject.
 - **d.** Select a field of view.
 - **e.** Set the focus options.

Subject Height: 1.50 🕆 cm Focus: Luse subject height 💌	aging Subject: Mouse	• O Manu	al Settings	
C - 13.5 cm - Focus Subject Height: 1.50 + cm Focus: use subject height - Options	Luminescent 5.00	Min • 16 • 1	vop:	
Focus Subject Height: 1.50 (m Focus: use subject height Options				
Options	cus	Focus: (use subject height 🔻	
Total number of segments: 1 + Delay between segments: 0.0 + Mins	tions Time Series Study otal number of segments:			

- 7. To acquire a time series of images:
 - a. Choose the Time Series Study option (Figure 11.15).
 - **b.** Enter the number of segments and the delay between segments.
- 8. Click Next.

The specified sequence appears in the sequence table (Figure 11.16).

Figure 11.16 Sequence Setup Com	plete						
Imaging Wizard - Cherenkov - Spectral Unmixing Imaging Subject: Honae Deposer Parameters Construction Power Son Construc		xclaton Filter Existen Filter cd. v 660 •	Sooil	Photographic Settings	_	_	Subject: Mouse V Probe V
Options The factors Study Total number of segments: 1 Delay between segments: 0.0	V Thotograph 0.2012 Medum A V Reuse V Reuse Vortee Vorteee Vorteee Vorteeeee	Batch Sequences Acquire Sequence Imaging Wzard	Mode Exposur 1 300 2 300 3 300 3 300 4 300 5 300	Medium 1 Medium 1	Excitation Emis Block 580 Block 600 Block 620 Block 640 Block 660	sion FOV Height C 1.50 C 1.50 C 1.50 C 1.50 C 1.50	
Restart Wizard Cancel Back Next	Subject height: 1.50	Image Setup Initialize	Number of Segment	: 1 × Delay: 0	.0 📩 min 🗛	oly to All 🔀 Remo	vev 🕢 Update 🗋 Insert 🗋 Add

9. Acquire the sequence following the instructions on page 46.

The image window appears when acquisition is completed (Figure 11.17). See Table 4.2 on page 30 for more details on the Image window.

11.3 Spectral Unmixing Methods

Living Image software provides four spectral unmixing methods (Table 11.1).

 Table 11.1
 Spectral Unmixing Methods

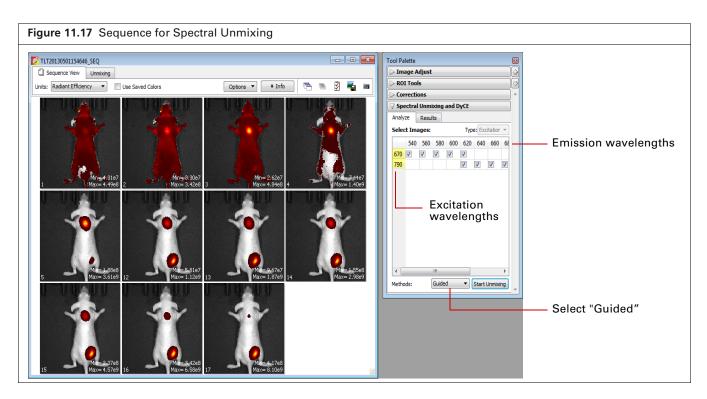
Method	Description	See Page
Guided	Use this method when: Probe locations are known.	215
	 Probe signals are mixed with background signal, but not other probe signals. 	
	Note: This method is not recommended if probe signals are overlapping.	
	Use this method to generate a spectrum library (a set of reference spectra) for probes with known spectra and known locations.	
Library	This method requires a user-generated spectrum library. The library method identifies pixels in the data with spectral characteristics that match the spectrum library.	218
	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 dataset 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.	
Automatic	Use this method when: Probe locations are unknown. Probes are included in the spectrum library.	220
Manual	 Use this method to: Unmix and create libraries for probe signals that overlap. Perform a manual analysis after an automatic analysis, if necessary, to identify additional probe locations. Unmix tissue autofluorescence. Generate a spectrum library. 	223

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.

The fluorophores are Alexa Fluor 680 and Alexa Fluor 750 in Figure 11.17. Images were acquired using 680 and 790 nm excitation filters, and emission filters from 540 to 720 nm in 20 nm increments.



- 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 11.18).

Figure 11.18 Unmixing Windo	w	
	TLT20130501154646_SEQ	
See Table 11.4 on page 229 for more details	No data available	Image cube shows a pseudo color image composite of colorized sequence images.
on the toolbar buttons.	Spectrum List:	
List of the spectral components to unmix. If the Imaging wizard was used to set up the sequence, the list includes the probes selected in the	Background Name Color Pick 1 √ TissueAF Ime ↓ 2 UMX2 red ↓ 3 UMX3 blue ↓ Pseudo Color	
wizard. The default list also includes a tissue autofluorescence background component. ——	Image Cube Viewer Ø Overview 1 Next Cancel	a

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.

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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.

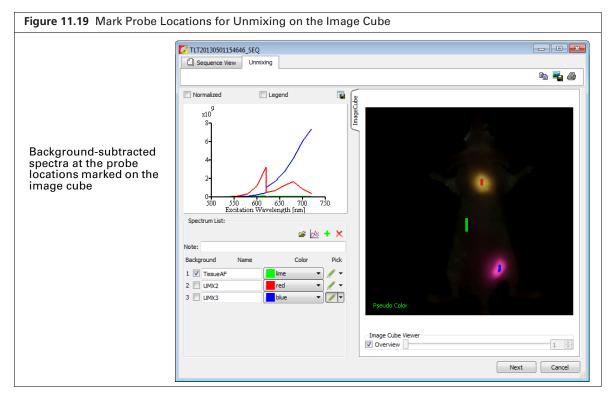
4. Move the mouse pointer over the image cube to see the spectrum at a particular location.

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 *I* 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 11.19).

NOTE: For "Tissue AF", draw a mark on an area of the image cube where no probe signal is present.

- **c.** If necessary, right-click the image cube to erase the mark.
- 6. Repeat step 5 to specify other probe locations.



7. 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 11.20). See *Spectral Unmixing Results* on page 228 for information about the results.

- **8.** To save the results as a spectrum library:
 - **a.** Click the **H** button in the Spectrum List toolbar (Figure 11.20).
 - **b.** Enter a file name in the dialog box that appears and click **Save**.



Library Method

The library method uses a user-generated spectrum library to analyze a dataset. 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.

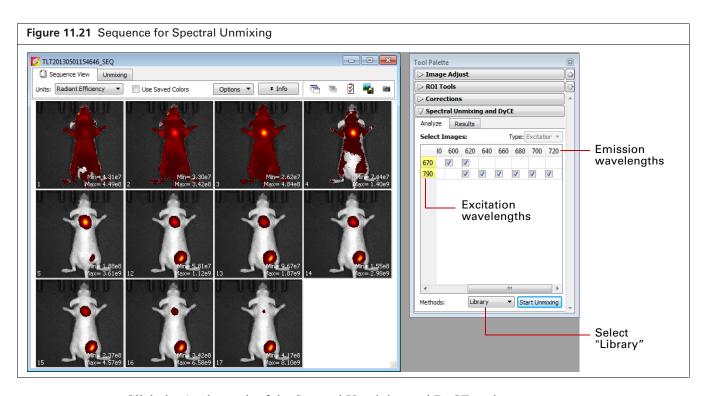
The probe depth in the dataset being analyzed and the spectrum library dataset should be similar for optimum analysis results. For example, do not use a spectrum library generated from in vivo data to analyze in vitro data.

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NOTE: Use the guided or manual method to generate a spectrum library of known probes with known locations (see page 215 for guided method or page 223 for manual method).

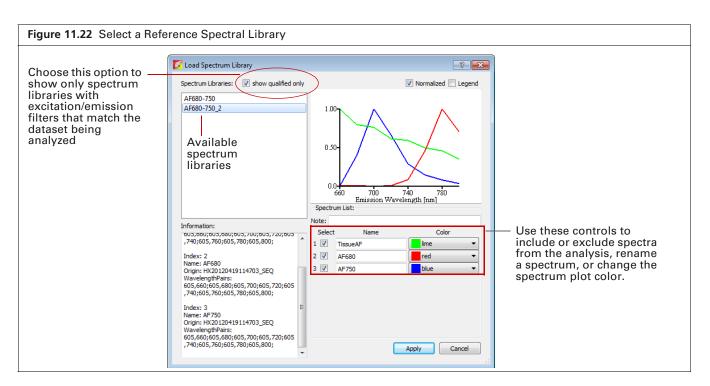
1. Load the image sequence.

In Figure 11.21, the fluorophores are Alexa Fluor 680 and Alexa Fluor 750. Images were acquired using 670 and 790 nm excitation filters, and emission filters from 540 to 720 nm in 20 nm increments.



- 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 11.22). 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 11.20 on page 218).

See Spectral Unmixing Results on page 228 for information about the results.

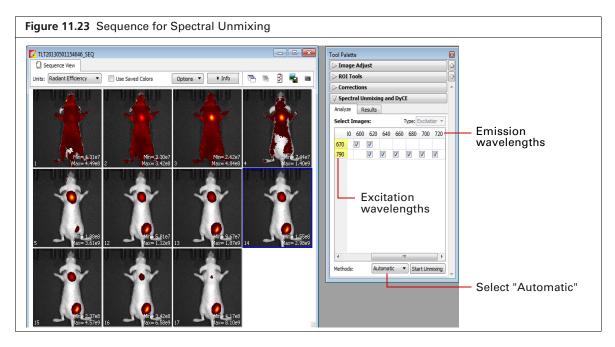


Automatic Method

Use the automatic method to analyze data when the probe locations are unknown and the probe is included in the spectrum library.

1. Load the image sequence.

In Figure 11.23, the fluorophores are Alexa Fluor 680 and Alexa Fluor 750. Images were acquired using 670 and 790 nm excitation filters, and emission filters from 540 to 720 nm in 20 nm increments.



- 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 (Figure 11.24). The purple data mask shows the data that will be included in the analysis (the entire subject is included by default).

Figure 11.24	Auto Unmix Window
	HX20120419114703_SEQ ① Sequence Wew Auto Unmix
Data — mask (purple)	Select Image Mask

4. If you do not want to analyze the entire subject, draw a mask on a particular area (Figure 11.25). For example, it is useful to mask shaved or depilated areas.

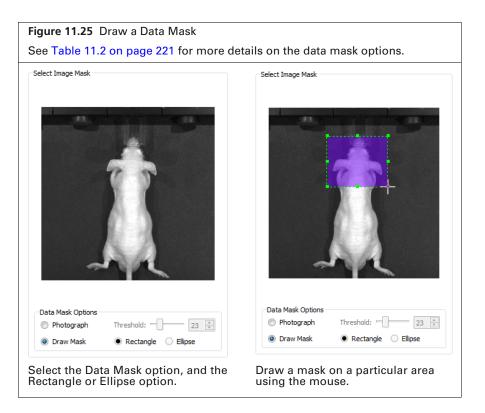
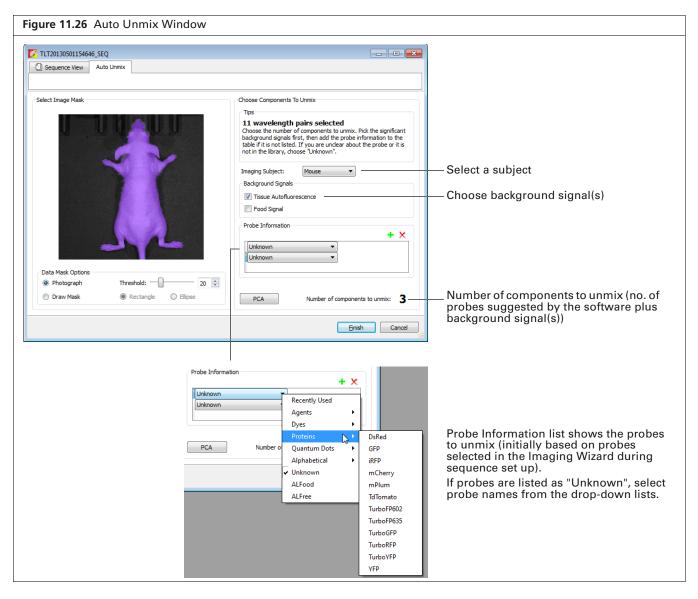


 Table 11.2
 Data Mask Options

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) (Figure 11.26).



6. Click the PCA button.

The Principle Component Analysis window shows the amount of signal explained by the suggested components (Figure 11.27). 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.

	ent Analysis 🛛 🔗 💌	
Suggested # of compo	nents: <i>3</i>	
Explained variance	e (%): 99.5914	
# of Components	Explained Variance(%)	
1	58.7555	
2	92.3562	
3	99.5914	
4	99.8463	
5	99.9128	
6	99.9572	
7	99.9855	
8	100	

7. Click Finish.

The Unmixing window shows the analysis results which include unmixed spectra, unmixed images, and a composite of the unmixed images (Figure 11.20 on page 218). See Spectral Unmixing Results on page 228 for information about the results.

Manual Method

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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 or if the probe signals overlap. The example in this section shows how to manually analyze results from a previous analysis.

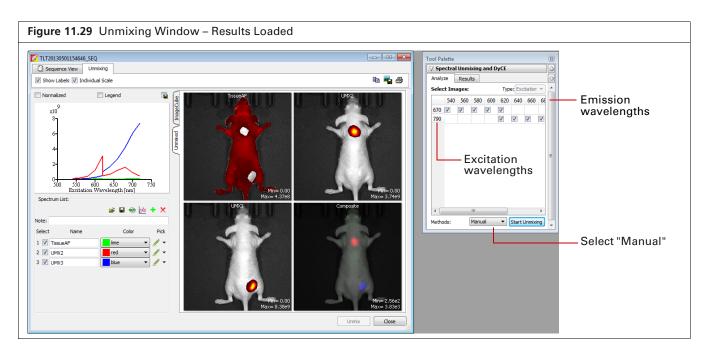
- **1.** Open the image sequence.
- 2. Select the results and click Load.

Figure 11.28 Open a Sequence and Select Results to Load		
✓ TLT20130501154646_SEQ □ Sequence View Units: Radiant Efficiency ▼ □ Use Saved Colors Options ▼ ▼ Info ○	Tool Palette B > Image Adjust > > ROI Tools > > Corrections > > Analyze Results Spectral Unmixing Results: . Item Value	
1 Min-2.667 1 Min-2.667 Max=3.428 3 Min-2.667 Max=4.8483 4 Min-2.467 Max=4.8483 4 Min-2.467 Min-2.677 Min-2.6777 Min-2.6777 Min-2.6777 Min-2.6777 Min-2.6777 Min-2.6777		
2 00-2002 12 02-2002 1	Save Results Name: SPUM_1	Select the results and click Load

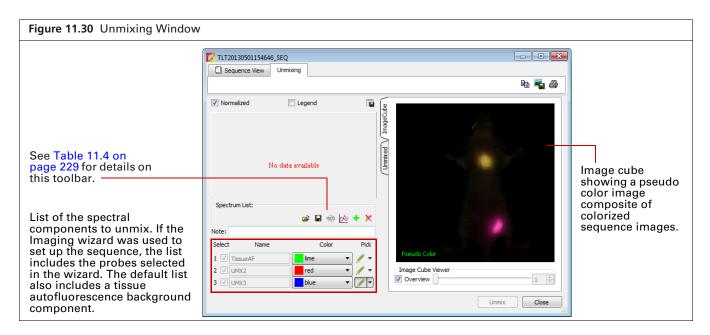
3. Click the Analyze tab of the Spectral Unmixing and DyCE tools.

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

In Figure 11.29, the fluorophores are Alexa Fluor 680 and Alexa Fluor 750. Images were acquired using 670 and 790 nm excitation filters, and emission filters from 540 to 720 nm in 20 nm increments.



4. Select "Manual" from the Methods drop-down list and click **Start Unmixing**. The Unmixing window appears (Figure 11.30).



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 (Figure 11.31).

Figure 11.31 Image Cube – Single	e Image Mode	
lmage c	cube tab	
TLT20130501154646_SEQ		
Normalized No data available Spectrum List: Image: Select Note: Select Name Color 1 Image: TissueAF Image: TissueAF	Adjust image brightness using the slider.	
Uncheck the Overview option to view an individual image. Select an image using the slider or enter a number.		

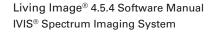
5. Move the mouse pointer over the image cube to see the spectrum at a particular location.

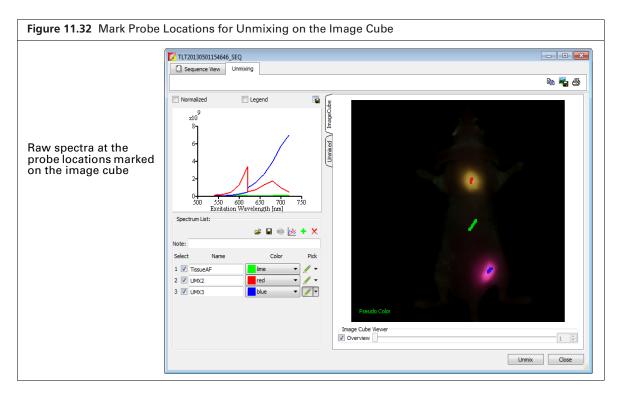
The spectrum at the pointer location is updated as you move the pointer.

- 6. To specify a probe location for unmixing:
 - **a.** Click the */* **v** 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 11.32).

- **NOTE:** Mark a region of tissue autofluorescence only (where no probe signal is present) 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.
- c. If necessary, right-click the image cube to erase the mark.
- **7.** Repeat step <u>6</u> to specify other probe locations.
- **8.** Manually subtract autofluorescence background. See *Correcting Spectra* on page 226 for instructions.





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

The Unmixing window shows the analysis results which include unmixed spectra, unmixed images, and a composite of the unmixed images (Figure 11.20 on page 218). See *Spectral Unmixing Results* on page 228 for information about the results.

11.4 Correcting Spectra

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

- **1.** Click the <u>button</u> in the Unmix window.
- 2. Choose the spectra to subtract in the dialog box that appears. (Figure 11.33).
- **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.

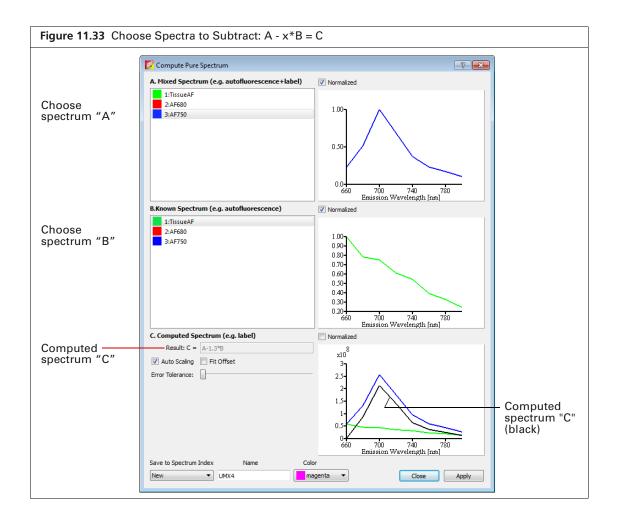
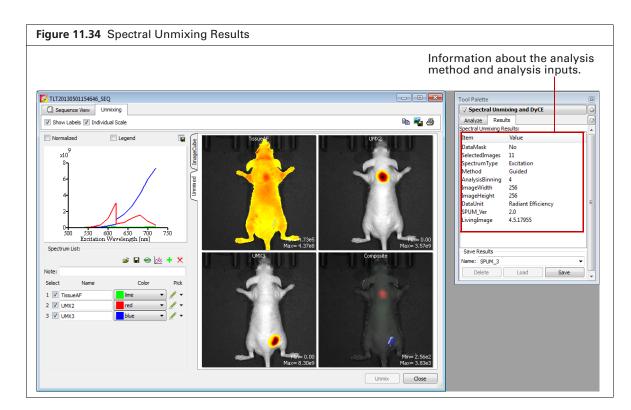


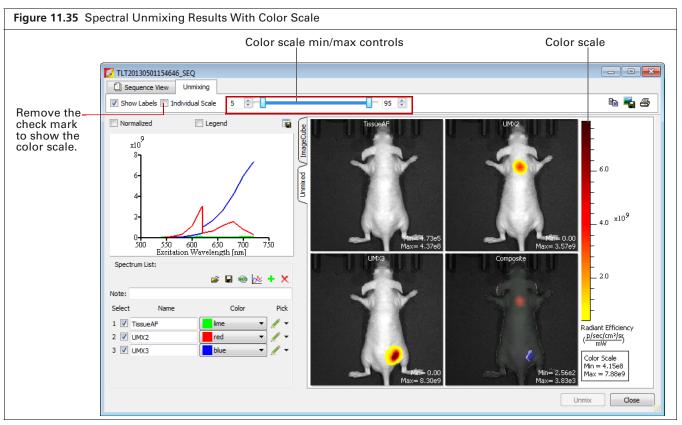
 Table 11.3
 Computed Spectrum

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.
Save to Spectrum Index Name Color New UMX4 1 2 3	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.
	Choose a spectrum number from the drop-down list to overwrite that spectrum with the computed spectrum when you click Apply .

11.5 Spectral Unmixing Results

The results include a signal distribution map of each unmixed result and a composite image of all signals, each signal displayed in a different color. Remove the check mark next to "Individual Scale" to view a signal color scale (Figure 11.35).





Spectra Plot

Figure 11.36 Spectra Plots V Normalized Legend 1.00-0.50-0.0 550 600 650 700 Excitation Wavelength [nm] 750 Spectrum List: 🛎 🖬 🐵 📐 🕇 🗙 Note: Select Name Color Pick 1 🔽 TissueAF lime • / -2 🔽 UMX2 red 🥒 🔻 3 🔽 UMX3 blue

Spectra plots show the unmixed spectra.

Table 11.4 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.
Ê	Opens a dialog box that enables you to select and load a spectrum library.
	Opens a dialog box that enables you so save spectral unmixing results as a reference spectrum library for use with the "library" method of spectral unmixing. See page 218 for more details on the library method.
	Note: Do not save reference spectrum libraries at other locations. The software only looks for reference spectrum libraries in this specific folder.
-	Enables you to view and save the unmixed images as a sequence dataset 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 <i>Correcting Spectra</i> on page 226).
+	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 provide 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 step 6 on page 225 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 11.37).

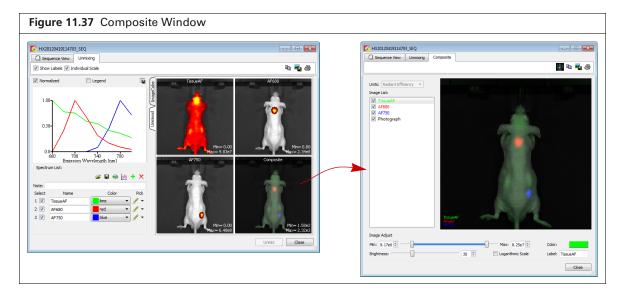


Table 11.5	Composite Window
------------	------------------

ltem	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).

ltem	Description
121	Sends the composite image to the "top" of the image cube. This helps improve the pseudo color visualization of the image cube.
B	Copies the composite image to the system clipboard.
*	Click to export the composite image to a graphic file (for example, .jpg).
4	Opens the Print dialog box.

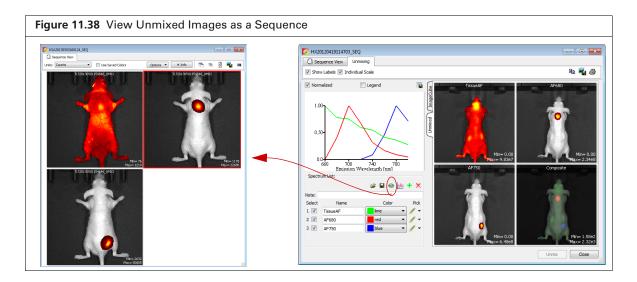
Table 11.5	Composite	Window	(continued)
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Analyzing Images

Do either of the following:

- Click the eight button to view all images as a sequence (Figure 11.38).
- 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.



Managing Spectral Unmixing Results

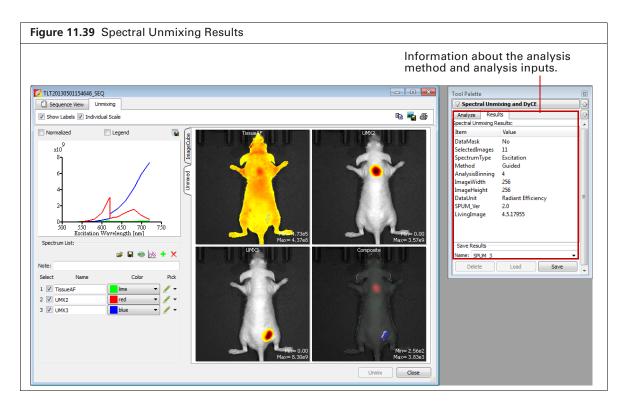


 Table 11.6
 Spectral Unmixing Results

ltem	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.

12 Biodistribution Studies Using DyCE Imaging

About DyCE (Dynamic Contrast Enhancement) Acquire an Image Sequence for DyCE Analysis on page 234 DyCE Analysis on page 241 DyCE Results on page 248

12.1 About DyCE (Dynamic Contrast Enhancement)

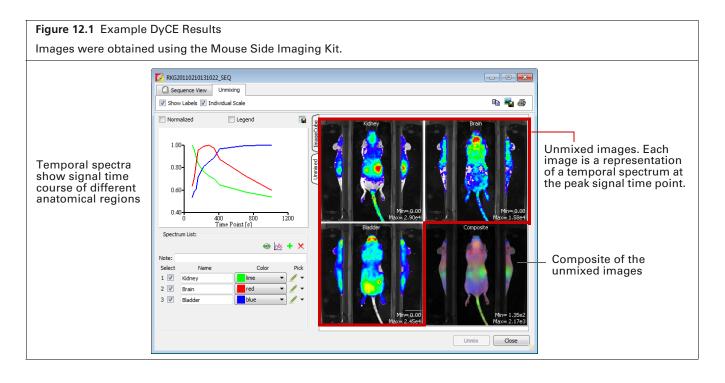
 $\overline{\mathbb{W}}$

NOTE: The DyCE acquisition and analysis features of 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. 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.

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 representing the peak signal time point for a particular temporal spectrum.
- A composite image An overlay of the unmixed images.



12.2 Acquire an Image Sequence for DyCE Analysis

A DyCE sequence is set up using the Imaging Wizard and includes 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).

Choose an imaging mode in the wizard based on your probe type.

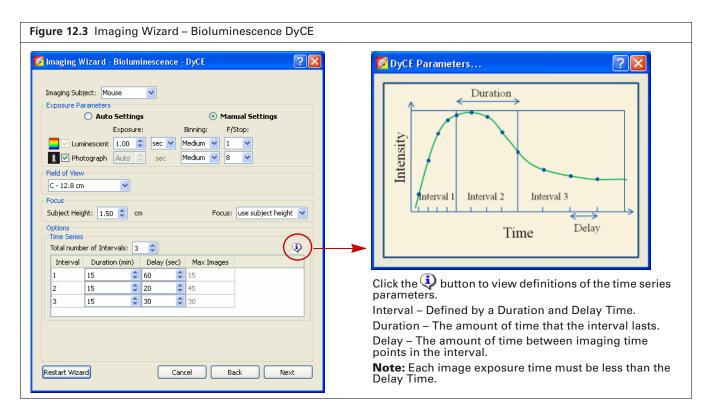
Probe Type	Follow the Instructions for:
Luminescent	Bioluminescence Imaging (below)
Fluorescent or near infrared	Fluorescence Imaging on page 236
Radiotracer	Cherenkov Imaging on page 239

Bioluminescence Imaging

- **NOTE:** The IVIS Spectrum should be initialized and the temperature locked before setting the imaging parameters. See page 19 for more details.
- **1.** Start the Imaging Wizard. See *Start the Imaging Wizard and Setup a Sequence* on page 44 for instructions.
- **2.** Double-click the Bioluminescence option. Double-click the DyCE option in the next screen (Figure 12.2).

Figure 12.2	Imaging Wizard – Choose Biolu	, ,	
Biolurrinescence	Imaging Mode Bioluminescence Imaging Select this option for imaging bioluminescent or chemiuminescent reporters, such as firefly lucificate, click beetle lucificate, renila, or bacterial lucificates. Fluorescence Imaging Select this option for imaging fluorescent proteins, dyes, or nanoparticles in the wavelength range of 450-850 nm. Both epi-flumination (iluminationfirom above) and	Open Filter	Bioluminescence - DyCE DyCE in the luminescent mode is an optical imaging approach that can provide detection of radiotracer distribution in preclinical animal models by tracking the Cerenkov emission from the charged decay products. While the charged particles travel faster than the speed of light in the medium (such as tissue), photons in the visible band are emitted via the Cerenkov effect, and these photons can be detected by optical images acquired following a small bolus injection of a radiotracer. As the radiotracer circulates through the body, each organ corresponds to characteristic pharmacokinetics depending on whether the isotope is accumulating, washing through or being metabolized .
Cherenkov	trans-ilumination (from below) modes are available. Cherenkov Imaging Select this option for imaging radiotracers which emit Cherenkov light in tissue.	Dyce Dur	the second secon
	Cancel Next		Cancel Back Next
	en does not appear when the v Restart Wizard (at the lower left wi		

3. Select the type of imaging subject in the next screen (Figure 12.3).



- 4. Choose "Manual Settings" and set appropriate exposure parameter values for your probe.
- 5. Select a field of view from the drop-down list.
- 6. Set the focus by doing either of the following:
 - Enter a subject height and choose the "use subject height" focus option.

OR

W

- 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 = 2 (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 = 4 (or larger).
- **7.** Specify the time series.
 - A time series can include up to three intervals. Each interval is defined by duration (minutes) and delay between images (seconds) (Figure 12.3).

Maximum number of images = Duration/Exposure if exposure is greater than delay. Maximum number of images = Duration/Delay if exposure is less than delay. A time series can include a maximum of 200 images.

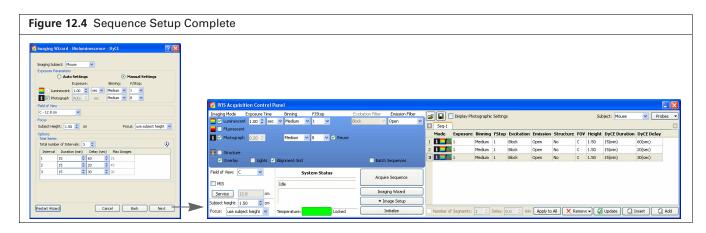
- **a.** Enter the number of intervals.
- **b.** Enter the duration and the delay between images for each interval.

The software computes the number of images to acquire during the interval.

NOTE: The software alerts you if the number of images in the time series exceeds 200. If necessary, adjust the duration or delay between images of one or more intervals to reduce the number of images.

c. Click Next.

The specified sequence appears in the sequence table (Figure 12.4).



8. Acquire the sequence following the instructions on page 46.The image window appears when acquisition is completed (Figure 12.13 on page 241). See Table 4.2 on page 30 for more details on the Image window.

Fluorescence Imaging

- **NOTE:** The IVIS Spectrum should be initialized and the temperature locked before setting the imaging parameters. See page 19 for more details.
- **1.** Start the Imaging Wizard. See *Start the Imaging Wizard and Setup a Sequence* on page 44 for instructions.
- 2. Double-click the Fluorescence option (Figure 12.5).
- **3.** Select DyCE and the type of illumination in the next screen (Figure 12.5):
 - Epi-Illumination Excitation light source above the stage.
 - Trans-Illumination Excitation light source below the stage. If this option is selected, NTF Efficiency images will be produced in which the fluorescent emission image is normalized by the transmission image measured with the same emission filter and open excitation filter.

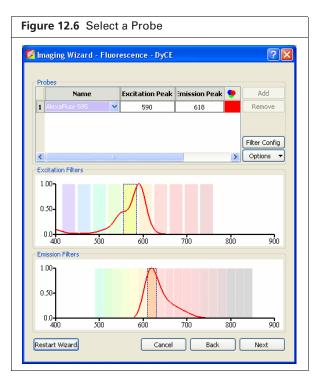


TIP: See these tech notes for helpful information and quick guides (select **Help** \rightarrow **Tech Notes** on the menu bar):

- Transmission Fluorescence
- Transmission Fluorescence Normalized Transmission Fluorescence

Figure 12.5 (Choose the Fluorescence and DyCE Tmaging Mode Bioluminescence Imaging Select this option for imaging bioluminescent or chemitanescence reporters, such as firefly luciferase, click beetle luciferase, renilla, or bacterial luciferase. Fluorescence Imaging Select this option for imaging fluorescent proteins, dyse, or nanoparticles in the wavelength range of 450-950 nm. Both epi-Illumination (Illumination from above) and trans-illumination (from below) modes are available. Cherenkov Imaging Select this option for imaging radiotracers which emit Cherenkov light in tissue. Cancel Next	Options	Cancel Received Rece
	n does not appear when the wiza Restart Wizard (at the lower left wizar		

4. Click **Next**. Select a probe from the Name drop-down list in the next screen (Figure 12.6). If your fluorescent probe is not in the list, select "Input" and enter the fluorescence excitation and emission peak wavelengths. Click **Next**.

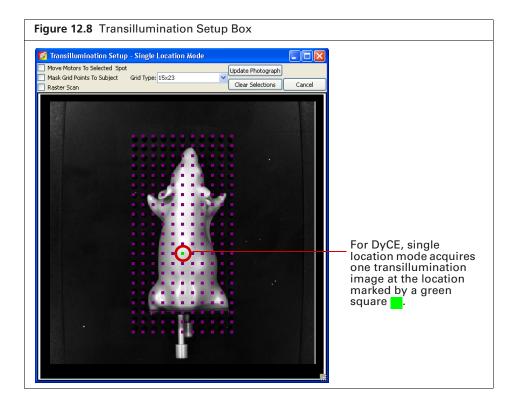


- 5. Select the type of imaging subject in the next screen (Figure 12.7).
- 6. Choose the Auto Settings option.

Imaging Sub Exposure Pa	· · · · · · · · · · · · · · · · · · ·	V		O Manu	al Settings
	V Auto Settin	ys			ai seccings
📕 🗹 Flu	orescent				
Pho	otograph				
Field of Viev	-				
C - 12.8 cn	n 🚩				
Focus					
Subject Height: 1.50 📚 cm Focus: use subject height 💌					
Options	5				
Time Series		3 🗘			(پ
	er of Intervals:	5 👻			
			c)	Max Images	
Total numb		Delay (se	-	Max Images	
Total numb	Duration (min)	Delay (se		-	
Total numb Interval	Duration (min)	Delay (se 60 20	-	15	
Total numb Interval 1 2	Duration (min) 15 15 15	Delay (se 60 20	*	15 45	

- **7.** If using transillumination, select a transillumination location. Only one excitation point location is allowed.
 - a. Click Transillumination Setup.
 - **b.** Choose the location for transillumination by clicking a grid square in the Transillumination Setup box that appears (Figure 12.8).

See Table 4.3 on page 40 for more details on the Transillumination Setup.



8. Perform step 5 on page 235 to step 7 on page 235.

The specified sequence appears in the sequence table (Figure 12.9).

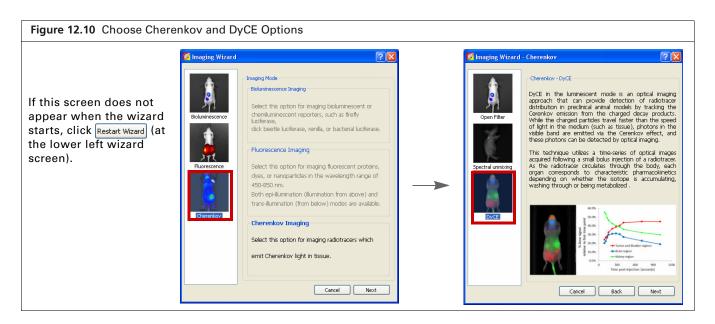
maging Wizard - Fluorescence - DyCE											
maging Subject: Mouse											
Exposure Parameters											
Auto Settings Manual Settings											
Fluorescent											
1 Photograph	(_
Field of Wew	🚺 IVIS Acquisition Control P	Panel									
C - 12.8 cm 👻	Imaging Mode Exposure Time	Binning F/Stop	Excitation Filter Emission Filter	🚽 🕞 🔲 🗆 Displa	w Photographic Sett	pas			Subject: Mo	ice 🗸	Probes
focus	📃 🗌 Luminescent 🛛 Auto 🗘 sec	✓ Medium ✓ 2 ✓	745 🛛 🖌 800 🖍	v	, moog april 2000				Subject 110		110000
Subject Height: 1.50 🗘 cm Focus: use subject height 💙	📕 🗹 Fluorescent 📃 Transilumin	nation	Lamp Level: High 🗸 🗸	Seq-1							
Dptions Time Series	1 V Photograph 0.20 0	Medium 💙 8 💙 🗹 Reuse			sure Binning FSt						
Total number of Intervals: 3 3				1 Auto	Medium 2	745 81		No	C 1.5		60(
Interval Duration (min) Delay (sec) Max Images	Structure			2 Auto	Medium 2	745 81	0 High	No	C 1.5	0 15(min)	20(
1 15 0 60 0 15		Alignment Grid	Batch Sequences	3 Auto	Medium 2	745 8	0 High	No	C 1.5	0 15(min)	30(
2 15 20 45			- Batter Solder roop								
3 15 30 30	Field of View: C	System Status									
	MIS	Idle	Acquire Sequence								
Fluorescence V Normalized	L MIS	Idle	-								
	Service 12.8 Cm		Imaging Wizard								
Transilumination Setup No Trans - Illumination locations selected											

9. Acquire the sequence following the instructions on page 46.

The image window appears when acquisition is completed (Figure 12.13 on page 241). See Table 4.2 on page 30 for more details on the image window.

Cherenkov Imaging

- **NOTE:** The IVIS Spectrum should be initialized and the temperature locked before setting the imaging parameters. See page 19 for more details.
- **1.** Start the Imaging Wizard. See *Start the Imaging Wizard and Setup a Sequence* on page 44 for instructions.
- **2.** Double-click the Cherenkov option. Double-click the DyCE option in the next screen (Figure 12.10).



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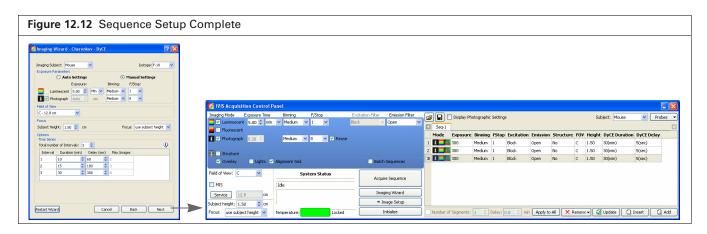
- **3.** Select the subject type and radio-isotope from the drop-down lists in the next screen (Figure 12.11). If your radio-isotope is not available in the list, choose "Undefined".
- **4.** Choose the Manual Settings option and set exposure parameter values that are appropriate for your radiotracer probe.

NOTE: Typical exposures are in the range of minutes because Cherenkov light emission is very low. Beta decays with higher energies allow shorter exposure times than lower energy beta decays.

Exposure Pa	rameters O Auto Setti	nae		Manual Settings
	Expos	-	Binning:	F/Stop:
🗖 🗹 Lum	inescent 5.00	🍣 Min 🗸	Medium 🔽	1 🗸
1 🔽 Pho	tograph Auto	sec	Medium 🔽	8 🗸
Field of View				
C - 12.8 cm	~			
Focus				
Subject Heig	ht: 1.50 😂	cm	Fo	cus: use subject height 💉
Options				
Time Series				
	er of Intervals:	3 🗘		4
Interval	Duration (min)			jes
	10	\$ 60	2	
1				
1 2 3		180 300	3	

5. Perform step 5 on page 235 to step 7 on page 235.

The specified sequence appears in the sequence table (Figure 12.12).



6. Acquire the sequence following the instructions on page 46.

The image window appears when acquisition is completed (Figure 12.13 on page 241). See Table 4.2 on page 30 for more details on the image window.

12.3 DyCE Analysis

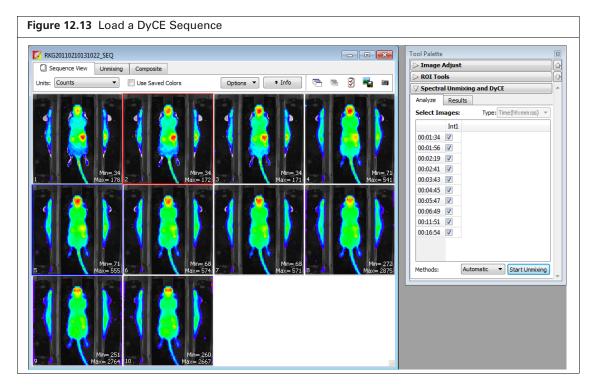
Automatic or manual DyCE analysis is available. PerkinElmer recommends performing an automatic analysis first, followed by manual analysis to identify possible additional temporal components.

NOTE: If working with large datasets (e.g. studies with many subjects), a 64-bit analysis workstation with 16 GB memory capacity is recommended.

Automatic DyCE Analysis

1. Load a DyCE sequence. The reprint icon in the Living Image browser indicates a DyCE sequence.

NOTE: If the data is noisy, as is common for Cherenkov data, smooth all the images in the sequence using the Smoothing tools (under Image Adjust in the Tool Palette). This can be done in sequence view mode. See *Smoothing and Binning* on page 69 for details.



- 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 12.14). The data mask includes the entire subject by default.

4. If necessary, change the threshold level to adjust the purple mask so that it matches the underlying subject photograph as closely as possible without including any area outside the subject image.

Figure 12.14 Auto Unmix Wizard	1	
Sequence View Auto Unmix		
Select Image Mask Select Image Mask Deta Mask Options Photograph Threshold: Draw Mask Rectangle Elected Elected	Choose Components To Uninx Tps 10 time points selected Add the number of finetics to analyze Knectics Information + × Recta Number of components to unnix: 2	Click to add or remove components to unmix
	Einish Cancel	

- **5.** If you do not want to analyze the entire subject, draw a data mask on a particular area using the data mask options (Figure 12.15). See Table 12.1 for more details on the 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.

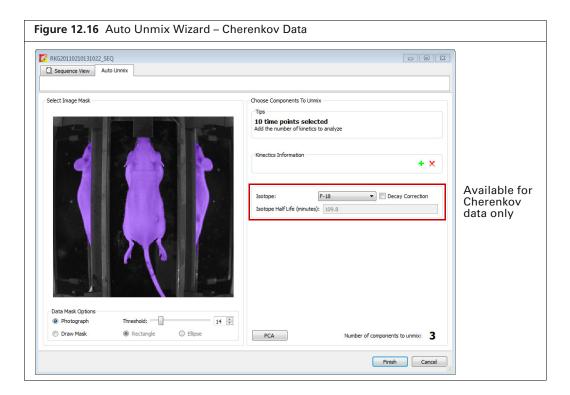
Figure 12.15 Draw a Data Mask	
See Table 12.1 for more details on the da	ta mask options.
Select Image Mask	Select Image Mask
Data Mask Options	Data Mask Options
Photograph Threshold: 23	Photograph Threshold: 23 🐳
Oraw Mask	Oraw Mask Rectangle Ellipse
Select the Data Mask option, and the Rectangle or Ellipse option.	Draw a mask on a particular area using the mouse.

Table 12.1 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.

6. Click the **b**utton 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 245 for details on manual analysis).
- **7.** For Cherenkov data only:
 - Choose the Decay Correction option to apply decay correction to the image data before analysis.
 - **NOTE:** If using Decay Correction, Cherenkov decay correction will be applied to every pixel in image, including pixels where the Cherenkov isotope is not present. Therefore, ensure that the data mask covers only the image region of interest, for example, only the mouse.
 - If the radio-isotope used in the experiment was selected in the Imaging Wizard prior to acquisition, it will be displayed in the Isotope drop-down list. If the incorrect radio-isotope was selected at acquisition, choose a different radio-isotope from the drop-down list. If your radio-isotope is not available in the list, choose "Undefined" and enter the isotope half-life in minutes (Figure 12.16).



8. Click Finish.

The Unmixing window shows a plot of the temporal spectra, unmixed images, and a composite of the unmixed images (Figure 12.17).

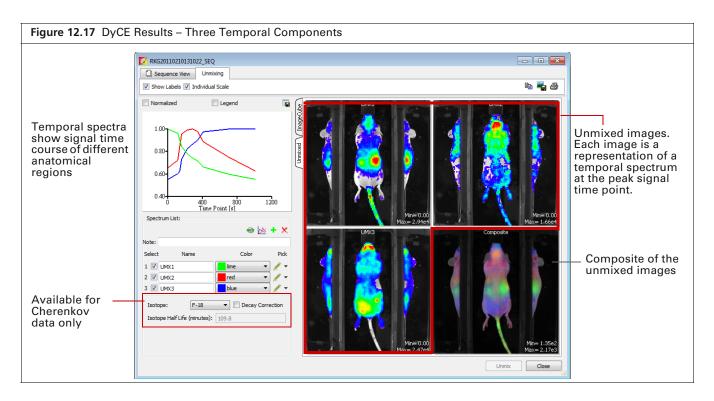


Table 12.2 Spectrum List Toolbar

Item	Description
÷	Enables you to view and save the unmixed images as a sequence dataset. The image adjust, corrections/filtering, image information, or ROI tools are available for the images.
\sim	Enables you to subtract one spectrum from another (see page 251).
•	Adds a temporal component to the spectrum list when performing a manual analysis. See page 245 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 12.18).
 - b. Click Save.

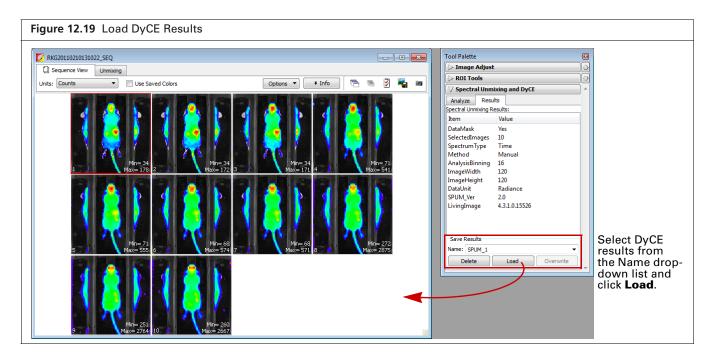
Tool Palette		8	
7 Spectral Unm	ixing and DyCE		
Analyze Resu	lts	⊡	
Spectral Unmixing R	esults:		
Item	Value		
DataMask	Yes		
SelectedImages	10		
SpectrumType	Time		
Method	Automatic		
AnalysisBinning	16		
ImageWidth	120		
ImageHeight	120		
DataUnit	Radiance		
SPUM_Ver	2.0		
LivingImage	4.3.1.0.15621		
Save Results Name: SPUM_4 Delete	Load	▼ Save	—Results name

Manual DyCE Analysis

1. Load a DyCE image sequence. Alternatively, load DyCE results obtained from an automatic analysis (Figure 12.19).



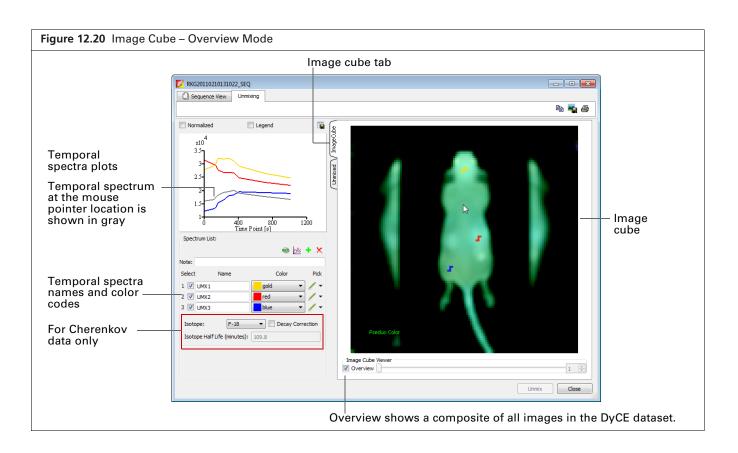
NOTE: This section illustrates manual analysis of DyCE results obtained from an automatic analysis.

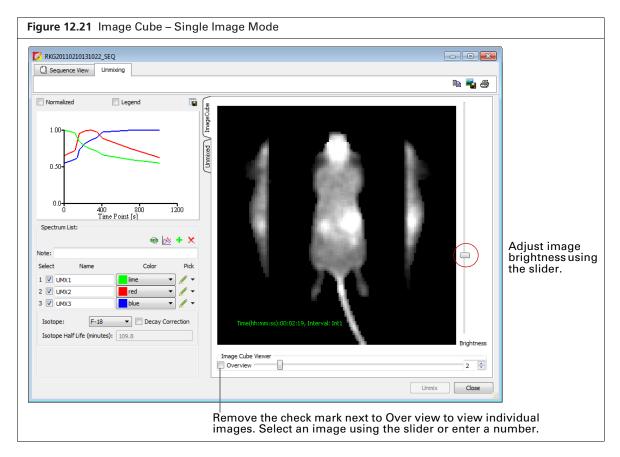


2. Click the Image Cube tab (Figure 12.20).

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 (Figure 12.20).

To view a particular image, remove the check mark next to Overview and move the slider or enter an image number (Figure 12.21).





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.

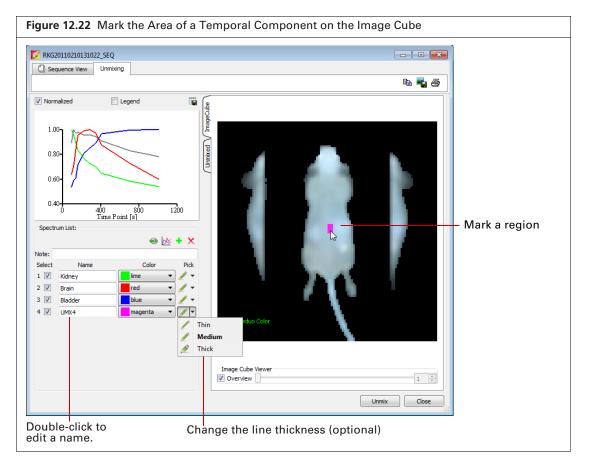
- **4.** To add another component to unmix:
 - **a.** Click the **b**utton.

A new name appears in the spectrum list (Figure 12.22)

- **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 dropdown 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.

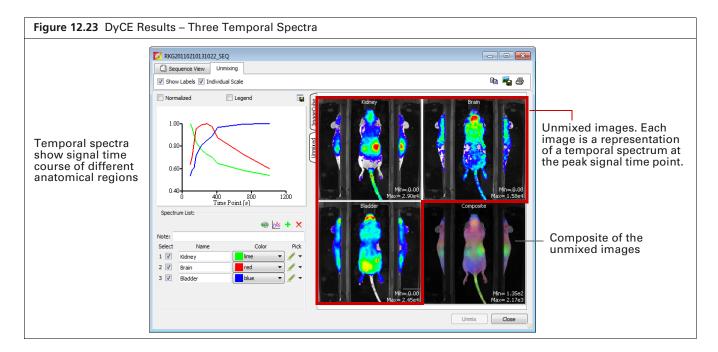
The software generates unmixed images for the new temporal spectra and updates the composite image with these components.

ltem	Description
-	Enables you to view and save the unmixed images as a sequence dataset. The image adjust, corrections/filtering, image information, or ROI tools are available for the images.
\sim	Enables you to subtract one spectrum from another (see page 251).
+	Adds a component to the spectrum list.
×	Deletes the last spectrum in the spectrum list.

Table 12.3 Spectrum List Toolbar

12.4 DyCE Results

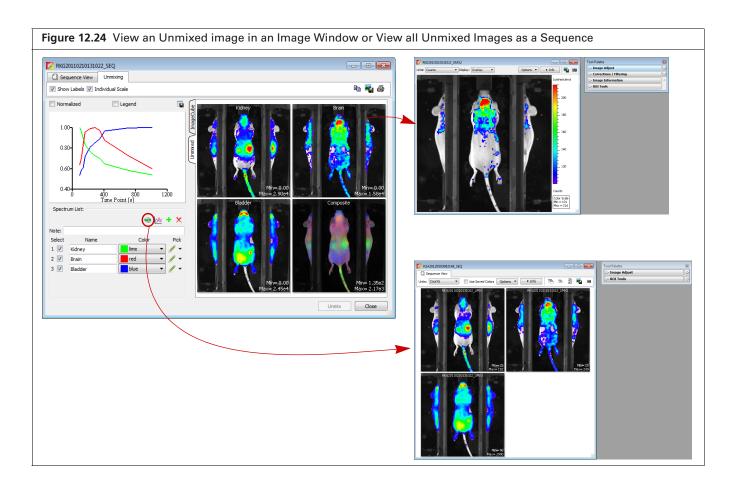
The Unmixing window shows the DyCE results. The example in Figure 12.23 shows three "temporal spectra" (signal as a function of time).



Viewing Unmixed Images

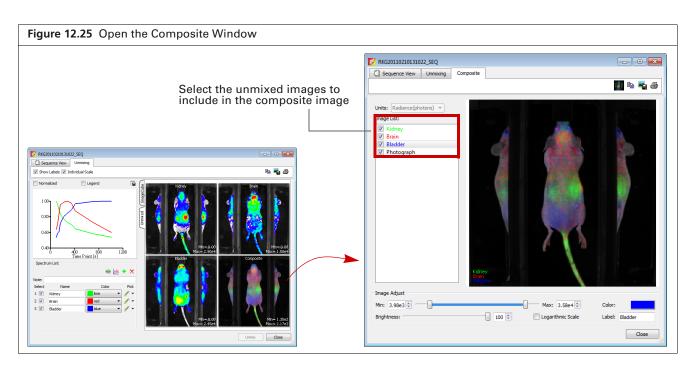
An unmixed image shows the maximum signal of a temporal spectrum.

- Double-click an unmixed image to view it in an image window (Figure 12.24). The Tool Palette is available for viewing and analyzing the image.
- Click the solution to view the unmixed images as a sequence (Figure 12.24). 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.



Viewing the Composite Image

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



- 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.

ltem	Description			
127	Sends the composite image to the "top" of t	he image cube.		
	Trage Cube Never	Click the Image Cube tab in the Unmixing window to view the image cube. See Figure 12.20 on page 246 for more details on the image cube.		
	Tange Cube Viewr	Composite image displayed on top of the image cube.		
Ê≥:	Copies the Composite window to the system	clipboard.		
-	Opens a dialog box that enables you to expor (for example, .png).	Opens a dialog box that enables you to export the composite image to a graphic file (for example, .png).		
8	Opens the print dialog box.	Opens the print dialog box.		
Color:	Shows the color of the data for the highlighted image. Image List: Vidney Vidney Bladder V Bladder Photograph Click the color swatch to open the color palette which can be used choose a color for the selected image data.			
Label: Bladder	Data name for the highlighted image. Double	e-click the name to edit it.		

Table 12.4 Composite Window

Correcting Temporal Spectra

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

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

- **1.** Click the <u>button</u> in the Unmixing window.
- 2. In the dialog box that appears, choose the spectra to subtract (Figure 12.26).

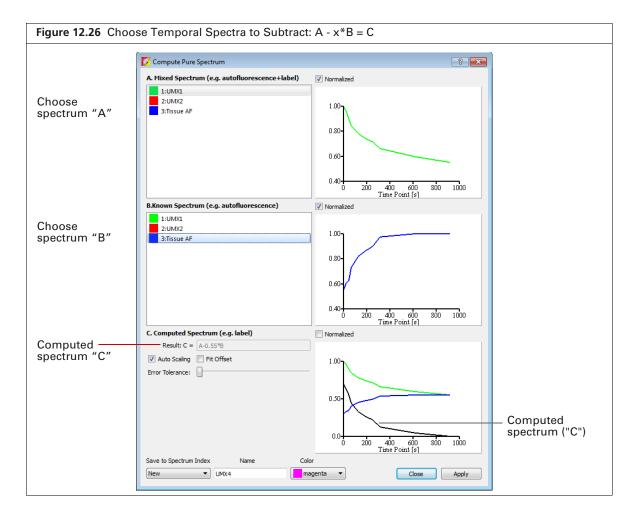


Table 12.5	Computed Spectrum
------------	-------------------

ltem	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 12.5 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.
Save to Spectrum Index Name Color New Color 1 UMX4 2 New New New	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.
	Choose a spectrum number from the drop-down list to overwrite that spectrum with the computed spectrum when you click Apply .

Appendix A IVIS Acquisition Control Panel

Control Panel Functions

Manually Setting the Focus on page 257

A.1 Control Panel Functions

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

Figure A.1 IVIS Acquisition Control Panel – Auto Exposure Selected					
To acquire an image using auto exposure, click the 💌 arrow and select Auto .					
🜠 IVIS Acquisition Control P	Panel				
Imaging Mode Exposure Time	Binning F/Stop	Excitation Filter Emission Filter			
Luminescent Auto 🗘 Jec	V Medium V 1 V	Block 🗸 Open 🗸			
🚺 🗹 Photograph 🛛 0.20 🤤	Medium 🔽 8 🔽				
Verlay Lights V Alignment Grid					
Field of View: C	System Status	Acquire			
MIS	Idle	Acquire			
Service 12.8 cm		Imaging Wizard			
Subject height: 1,50 😂 cm		* Sequence Setup			
Focus: use subject height 🔽	Temperature:	ed Initialize			

NOTE: The control panel is only available on the PC workstation that controls the IVIS Imaging System. The options available in the IVIS acquisition control panel depend on the imaging system, selected imaging mode (Image Setup or Sequence Setup), and the filter wheel or lens option that are installed.

Table A.1	IVIS Acqui	isition (Cor	ntrol Panel
		(

ltem	Description
Luminescent	Choose this option to acquire a luminescent image.
Fluorescent	Choose this option to acquire a fluorescent image.
	If the Fluorescent option is selected, the following options also appear in the control panel:
	Transillumination - Choose this option to acquire a fluorescent image using transillumination (excitation light located below the stage).
	Normalized - This option is selected by default when the Fluorescent and Transillumination options are chosen so that NTF Efficiency images can be produced.
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 <i>Photo Adjustment</i> on page 67.

ltem	Description
Structure	Choose this option to take a structured light image (an image of parallel laser lines scanned across the subject) when you click Acquire. The structured light image is used to reconstruct the surface topography of the subject which is an input to the Diffuse Luminescence Imaging Tomography (DLIT™) algorithm that computes the 3D location and brightness of luminescent sources.
	When this option is chosen, the f/stop and exposure time are automatically set to defaults for the structured light image (f/8 and 0.2 sec, respectively). The spatial resolution of the computed surface depends on the line spacing of the structured light lines. The line spacing and binning are automatically set to the optimal values determined by the FOV (stage position) and are not user-modifiable.
Overlay	If this option is chosen, the system automatically displays the overlay after acquisition is completed (for example, luminescent image on photograph).
Exposure time	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.
	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.
Binning	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 \rightarrow References on the menu bar).
	Recommended binning: Small (1-4) for imaging of cells or tissue sections, Medium (4-8) for <i>in vivo</i> imaging of subjects, or Large (8-16) for <i>in vivo</i> imaging of subjects with very dim sources.
F/stop	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.
	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 \rightarrow References on the menu bar).
Excitation Filter	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. Note: On some models with standard filter sets, the excitation filter selection
	automatically sets the emission filter.
Emission Filter	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.

Table A.1	IVIS Acquisition	Control F	Panel (c	continued)
Table A.T	TVIO Acquisition	CONTROLL		,ontinucu,

Table A 1	IVIS Acquisition Control Panel (contin	uued)
	TVIS Acquisition Control Farler (Contri	iueu)

ltem	Description
Lamp Level	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. 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.
Lights	Turns on the lights located at the top of the imaging chamber.
Alignment Grid	Choose this option to illuminate an alignment grid on the stage when the imaging chamber door is opened. The alignment grid shows the sizes and positions of the possible fields of view. If subject alignment is not completed in two minutes, place a check mark next to Alignment Grid to turn on the grid.
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 A.2 for more details on the calibrated FOV positions.
Service	Moves the stage to a position for cleaning the imaging chamber below the stage.
Load	Moves the stage from the cleaning position back to the home position.
MIS	Choose this option if the subject will be contained in the Mouse Imaging Shuttle during image acquisition.
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. IMPORTANT! The IVIS Imaging System has a protection system to prevent instrument damage, however always pay close attention to subject height. For example, it is possible for a large subject (10 cm ventral-dorsal height) to contact the top of the
	imaging chamber if you set the subject height = 0 and choose a small FOV.
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 257.
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 50 for more details.
Temperature	 The temperature box color indicates the temperature and status of the system: 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 20 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.

Table A.1 IVIS Acquisition Control Panel

ltem	Description						
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 52 for more details on manually setting up an image sequence.						
Imaging Wizard	Click to start the Imaging Wizard						
Sequence Setup	Click to open the sequence table.						
Image Setup	Click to close the sequence table.						
Initialize	Click to initialize the IVIS Spectrum. See page 19 for more details on initializing the system.						

Table A.2 Optical Field of View (FOV) Settings

FOV Setting	FOV (cm)
A ¹	4
В	6.5
C ²	13
D	22.5 (19.5) ³
E	22.5 (26) ²

¹Position A is not recommended for epi-fluorescent imaging because corrections for non-uniform excitation light pattern are not available.

²Position C is the default setting.

³Some IVIS Spectrum Imaging Systems may have the FOV in parentheses. FOV 19.5 and 26 were replaced with FOV 22.5.

A.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.

Figure A.2 Opening the Manual Focus Window	
VIS Acquisition Control Panel Imaging Mode Exposure Time Binning F/Stop Excitation Filter Emission Filter Unimescent Auto © sec Floorescent Floorescent	Manual Focus Window Step Fptop: • Display CCD Center Coarse Light Level: 0.000 Up Light Level: ® Internal Price Subject Height: Down Oc
V Photograph 0.20 Medium 8 V Structure V Overlay Lights V Alignment Grid	
Field of View: System Status Acquire MIS Idle Imaging Wizard Subject height: 15.0 0 cm * Sequence Setup Focus: manual focus Temperature: Locked	

- 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.
- If necessary, select another F/stop setting from the drop-down list and adjust the light level using the sarrows.
- 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 B Optical Image Data Corrections

The Corrections tools apply corrections or subtract adaptive fluorescence background from optical image data.



 $\overline{\mathbb{W}}$

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

- Luminescent Background Sources and Corrections.
- Fluorescent Imaging for more about fluorescent background.
- Adaptive Fluorescence Background Subtraction.

Tool Palette	X
> Image Adjust	÷
> ROI Tools	
	*
✓ Read Bias Subtraction	
✓ Flat Field Correction	
Cosmic Correction	
Adaptive FL Background Subtraction	

NOTE: Read Bias Subtraction and Flat Field Correction are default mandatory corrections in Radiance units mode. These corrections can be cleared in counts mode.

Table B.1	Optical	Image	Data	Corrections
-----------	---------	-------	------	-------------

ltem	Description				
Lens Distortion Correction	Select this option to correct for distortion at the perimeter of an image due to curvature of the CCD lens. Lens distortion correction is available for data acquired by Living Image® software version 4.3 and higher. The correction is particularly important for IVIS Spectrum CT data acquired for DLIT or FLIT.				
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 \rightarrow 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.				

ltem	Description					
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 \rightarrow Tech Notes on the menu bar).					
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 \rightarrow Tech Notes on the menu bar).					
Normalization Threshold (Counts)	The minimum number of counts required to perform normalization.					

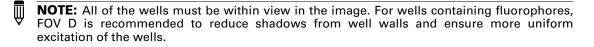
Appendix C Quantification Database

Preparing and Imaging Samples Creating a Database on page 261 Managing Quantification Results on page 265

If a quantification database is available, it is possible to determine the number of cells in a DLIT or FLIT source, or the number of dye molecules in a FLIT source. The database is derived from an analysis of images of known serial dilutions of luminescent cells or fluorescent cells or dye molecules. This chapter explains how to construct a database.

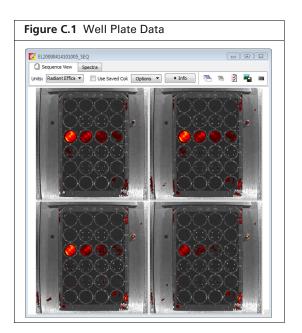
C.1 Preparing and Imaging Samples

- 1. Prepare a well plate $(4 \times 6, 6 \times 4, 8 \times 12, \text{ or } 12 \times 8 \text{ well format})$ that contains a dilution series of luminescent cells or fluorescent dye at four or more concentrations.
- 2. Include at least four background wells that contain diluent only.
- **3.** Place the well plate on the IVIS stage, positioning it so that it is centered and square in the field of view.



- 4. Acquire the images:
 - Bioluminescent samples Acquire one 'Open' filter image of the well plate.
 - Fluorescent samples Acquire reflectance-illumination Filter Scan images using the appropriate excitation and emission bandpass filters.

The well plate in Figure 14.1 contains a dilution series of a sample at four concentrations. The image sequence is a filter scan set of images with the excitation filter centered at 465 nm for all the images, and emission filter images centered at 520 nm, 540 nm, 560 nm, and 580 nm.



C.2 Creating a Database

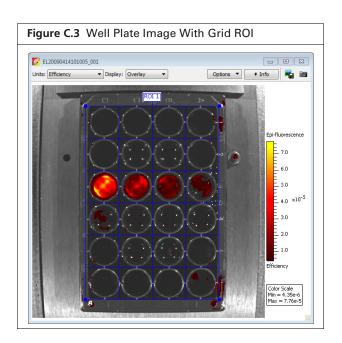
- **1.** Load the well plate image sequence.
- **2.** Select Tools \rightarrow Well Plate Quantification for "<name>_SEQ" on the menu bar.

The Well Plate Quantification window appears.

3. For fluorescent samples, choose the Dye molecules or Cells option.

Figure C.2	Well F	Plate Q	uantifica	tion Window	
## Well Plate 4 x 6 6 x 4 8 x 12 12 x 8 "Apply to S Well Plate	e: EL2009 Type ells : equence Quantifi	1 ication Plot	005_SEQ Im	age EL20090414101005_001 Fluorophore Type Dye molecules Cells Set	—— These options only available for fluorescence data.
D 1 2 3 4 5 6	C	B For Im	A	414101005 001 Quantify	

4. Select the well plate dimensions from the Well Plate Type drop-down list. The first image in the sequence opens and a grid ROI appears on the image.



5. Adjust the grid ROI to closely fit the plate wells.

- **6.** In the well plate table, select the sample cells, and click **Set** (Figure C.4). Clicking a row or column header selects the entire row or column.
- 7. To remove the "sample" designations from table cells, select the cells and click the 🕤 button.
- **8.** To apply a color to table cells:
 - a. Select the table cells and click the selected table cells and choose Background Color on the shortcut menu.
 - **b.** Choose a color from the color palette that appears.

💈 Well Plate	Quantifi	cation Wi	ndow			Well Plat	e Quantif	ication W	indow	
	-			age EL20090414101005			-			nage EL20090414101005_001
Well Plate		0414101	005_5EQ III	Fluorophore Type	[#	Well Plat		9041410	1005_SEQ In	Fluorophore Type
Measurement		•		0 -,-		4easuremen	ıt	•		0 .,
ample Wells :		3D : 3A		🗖 Se	et 📥 Sa	ample Wells	:	3D : 3A		🔲 Set (🖛
Background	Wells :			Se	et (=)	Backgrour	nd Wells :			Set (+
	1:	1				ell Volume [j	uL]:	1		
Apply to Se	-	•				Apply to S	-	-		
Well Plate		cation Plot	s Results	7		Well Plate		fication Plo	ts Results	
	•									
Set position an	d enter di	lution valu	es in nanoM	lar units	Se Se	et position a	ind enter o	dilution val	ues in nanoM	olar units 🛛 🐴
D	С	В	Α		<u>^</u>	D	С	В	Α	
1					1	L				
2					_ 2	2				_
3					- 3	3 500000	250000	125000	62500	=
4					4	1				
5					5	5				
						5				-
6										

- **9.** Enter the concentration values in the table cells in nanomolar units, if calibrating fluorescent dyes. Enter the cell values in dimensionless units if calibrating cells.
- **10.** To delete a concentration or cell value, select the table cell and press the Delete key. Alternatively, right-click a selected value to view a shortcut menu of edit commands (for example, cut, copy, paste).
- **11.** If calibrating fluorescent molecules, enter the fluid volume (microliters) for the highlighted wells. The highlighted well volumes must be equal.
- **12.** Choose the Apply to Sequence option.
- **13.** Choose the Background Wells option.
- 14. In the well plate table, select the background wells and click Set.

Clicking a row or column header selects the entire row or column. To remove the "background" well designations, click the \bigcirc button.

	Well Plat	te Quantif	fication W	/indow			
or Sequence: EL20090414101005_SEQ Image EL20090414101005_001							
I Well Plate Type					Fluorophore		
Ope molecules Cel							
	nple Wells		3D : 3A				Set (*
	-	nd Wells :	6D : 6A				Set 🔸
Vel	ll Volume [j	μ L] :					
Apply to Sequence							
-	Apply to a	sequence					
	/ell Plate	-	fication Plo	ots F	Results		
W	/ell Plate	Quanti				r units	
W	/ell Plate t position a	Quanti and enter o	dilution val	ues in n		r units	4
W Set	/ell Plate	Quanti				r units	4
w Set	/ell Plate t position a	Quanti and enter o	dilution val	ues in n		r units	a
W Set	/ell Plate t position a	Quanti and enter o	dilution val	ues in n		r units	*
w Set	/ell Plate t position a	Quanti and enter o	dilution val	ues in n	anoMola	r units	
W Set 1 2	/ell Plate t position a D	Quanti and enter o	dilution val	ues in n A	anoMola	r units	
W Set 1 2 3	/ell Plate t position a D	Quanti and enter o	dilution val	ues in n A	anoMola	r units	

15. Click Quantify.

The results are displayed

Figure C.6 Example Fluorescence Quantification Plot	and Results
Well Plate Quantification Window For Sequence: EL20090414101005_SEQ Click EL20090414101005_001 ▼ Image: Sequence of the sequ	Well Plate Quantification Window For Sequence: EL20090414101005_SEQ Click EL20090414101005_001 ▼ Image: Well Plate Type Fluorophore Type Image: Well Plate Type Image: Well Plate Type Image: Well Plate Type <t< th=""></t<>
Sample Wells : 3D : 3A	Sample Wells : 3D : 3A Set 🕤
Background Wells 6D : 6A	Background Wells 6D : 6A
Apply to Sequence	Apply to Sequence
Well Plate Quantification Plots Results	Well Plate Quantification Plots Results
Click EL20090414101005_001 ▼	Well Plate Quantification Results : (Unsaved)
	Excitation Emission Extinction Coeff. Cross Section [nm] [nm] Q ε [M- ² cm- ²] / 1000 Q σ [mm ²]
ROI vs well-plate population 6.0 Total Efficiency (cm ²)	1 465 520 2.919e+07 1.115e-08
	2 465 540 1.262e+07 4.821e-09
	3 465 560 5.894e+06 2.251e-09
40-	4 465 580 2.230e+06 8.518e-10
20-	
0.0 × 10 ⁵	Sequence Database
0.0 1.0 2.0 3.0 4.0 5.0 ^{x10³}	Name : WPQUANT_1 Vame : WPQUA
well-plate population	Delete Load Save Delete Load Save

16. Check the linear fit of the data for each image in the quantification plot.

A good fit to the straight line gives confidence to the results values. Large deviations of individual points from a straight line could indicate possible issues with the dilution series or errors when entering sample dilution values.

17. To export the quantification plot values:

- **a.** Click the **button**.
- **b.** In the dialog box that appears, select a folder for the file (.csv) and click **Save**.

18. To copy the quantification plot values to the system clipboard, click the 🖹 button.

Table C.1 C	luantification	Results
-------------	----------------	---------

ltem	Description
Fluorescence	
Excitation (nm) Emission (nm)	The excitation and emission filter wavelengths for the image. 'Excitation' and 'Emission' filters will be specified for fluorescent images, and the 'Open' filter for 'Emission' will be specified for bioluminescent images.
Extinction Coeff	A measure of excitation photon absorption interaction with the well plate samples based on a base-10 logarithmic derivation. The quantum efficiency factor of the conversion of the absorbed photon to the emission wavelength is also included.
Cross Section	A measure of excitation photon absorption interaction with the well plate samples based on a natural logarithmic derivation. The quantum efficiency factor of the conversion of the absorbed photon to the emission wavelength is also included.
Bioluminescence	
Total Flux/cell	A measure of total flux (photon/sec) emitted from a single cell. This number can be used to estimate the number of cells from the total flux in the 3D quantification.

C.3 Managing Quantification Results

Quantification results can be saved with the image sequence and as a calibration database that is available in the DLIT or FLIT 3D reconstruction tools (Properties tab). If a calibration database is selected when defining the properties for performing 3D reconstruction, the 3D reconstruction results will be displayed in calibrated units for cell numbers or molecule quantities in picomole units.

Save, Load, or Delete Results

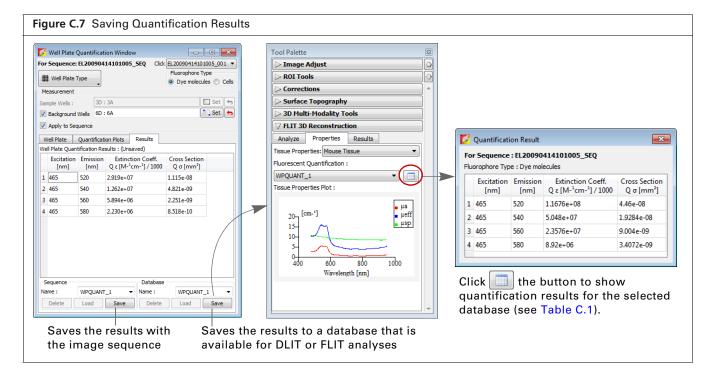


Table C.2 Managing Quantification Results

ltem	Description
Sequence Name : PC3M_GFP V Delete Load Overwrite	 Delete - Removes the active quantification results from the image sequence. Load - Opens quantification results from the sequence path. Save - Saves the quantification results with the selected image sequence. Overwrite - Saves the results with the selected image sequence and overwrites previous results.
Database Name : WPQUANT_1 V Delete Load Overwrite	Delete - Deletes the database from the system. Load - Opens quantification results from the system path. Save - Saves the quantification results to a system database that is available for DLIT or FLIT reconstruction. Overwrite - Saves the results to the selected database name and overwrites previous results.

Exporting Quantification Results

Right-click the results table to view copy and export options.

- Copy Copies the selected rows to the system clipboard
- Select All Selects all rows in the results table
- Export Results Opens a dialog box that enables you to export the selected results to a text file

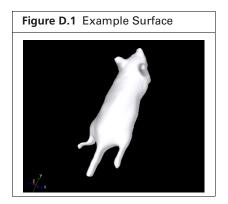
		•	tion Window 114101005 SE0 Click	EL2009041410100	D5 001 -		
## Well Plate Type Fluorophore Type Image: Operating the second se							
San	Measurement Sample Wells : 3D : 3A Set Set A Apply to Sequence						
Well Plate Quantification Plots Results							
Dat	tabase : WPQ	UANT_1					
Dat	tabase : WPQ Excitation [nm]	_	Extinction Coeff. Q ε [M- ¹ cm- ¹] / 1000	Cross Section Q σ [mm²]			
	Excitation	Emission	Q ε [M- ¹ cm- ¹] / 1000				
1	Excitation [nm]	Emission [nm]		Qσ[mm ²]			
1 2	Excitation [nm] 465	Emission [nm] 520	Q E [M- ¹ cm- ¹] / 1000 Copy Select All	Q σ [mm ²] 1.115e-08			
1 2 3	Excitation [nm] 465 465	Emission [nm] 520 540	Q ε [M-¹cm-¹] / 1000 Copy	Qσ[mm ²] 1.115e-08 4.821e-09			
1 2 3 4	Excitation [nm] 465 465 465	Emission [nm] 520 540 560	Q E [M- ¹ cm- ¹] / 1000 Copy Select All Export Results 24259E+00 Database	Q σ [mm ²] 1.115e-08 4.821e-09 2.251e-09			

Appendix D Surface Topography

About Surfaces Generating a Surface on page 268 Managing Surfaces on page 271

D.1 About Surfaces

A *surface* is a 3D reconstruction of the animal surface (topography) derived from a structured light image (Figure D.1). A surface is a required input for 3D reconstruction of luminescent or fluorescent sources displayed as voxels. You can also import a surface or export a surface for viewing in other 3D viewer applications.



Living Image software (version 4.5 and higher) automatically generates or selects a surface during reconstruction. You can also manually create or select a particular surface (Figure D.2). This appendix explains how to create and select a surface using the Surface Topography tools.

Figure D.2	
✓ DLIT 3D Reconstruction	
Analyze Properties Results Sequence: TLT20050624145507_SEQ Tissue: Mouse Tissue Source: Firefly Select Filters:	
Filter Threshold %	
🔽 🜻 560 0.7	
V 🕈 580 0.5	
V 🕈 600 0.5	
0.5	
V 🗣 640 0.5	
NNLS Reconstruct Reconstruct Manual Recon	 Reconstruct – Choose this option for "Single-Click" reconstruction. Living Image software will automatically generate or select a surface during reconstruction. Manual Recon – Choose this option to manually create and/or select a surface using the Surface Topography tools.

Animal Requirements

The best surface topography reconstruction is obtained from nude mice. Furred mice are not recommended for DLIT or FLIT. The Surface Topography tool can appropriately generate the surface of a furred mouse. However, the optical data pattern can be grossly shifted by the fur. 3D reconstructions using mice with black or dark-colored fur will give poor results.

It is possible to perform 3D imaging on white or light-colored furred mice if the fur is reasonably smooth over the mouse surface. Therefore it is recommended that you comb the fur before imaging to eliminate any "fluffy" areas that may alter the light emission pattern and/or trigger artifacts during the surface topography reconstruction. In this case, it is recommended that you shave the animals or apply a depilatory.

D.2 Generating a Surface

- **1.** Load the image sequence for the reconstruction, for example, a sequence acquired for DLIT analysis or a sequence acquired for FLIT analysis.
- 2. Select an orientation (dorsal or ventral) and subject in the surface topography tools.
- **3.** Select a smoothing level.



NOTE: The default "Low" smoothing level is sufficient in most cases, but it may be necessary to modify this if there are tufts of hair on the animal which disrupt the surface smoothness.

4. Click Generate Surface.

The entire subject is selected for reconstruction by default in the Single View Surface Tomography window that appears (Figure D.3).

igure D.3 Data Selected for Su	Irface Reconstruction
Tool Palette	Single View Surface Topography Analysis
▷ Image Adjust ▷ ROI Tools	Crop image
Spectral Unmixing and DyCE	
✓ Surface Topography	Crop image.
Optical Surface Reconstruction	
Drientation: Dorsal	(Draw a region of interest
Subject: Nude Mouse 🔻 Generate Surface	rectangle to proceed)
Surface Smoothing	
Level: Low 0 Restore	
Save Results	
lame: SURFACE_1	
Delete Load Overwrite	
> 3D Multi-Modality Tools	
> DLIT 3D Reconstruction	
	TLT20050624145507_001
	<pre>< Prev Next > Valid crop region.</pre>
	< Back Next > Cancel

5. If you want to reconstruct only a particular region of the subject, resize the rectangle (drag a green handle) so that it includes only the area of interest.



NOTE: If the image sequence includes multiple subjects, manual 3D reconstruction will require a separate surface for each subject. Adjust the rectangle to select a particular subject for surface topography.

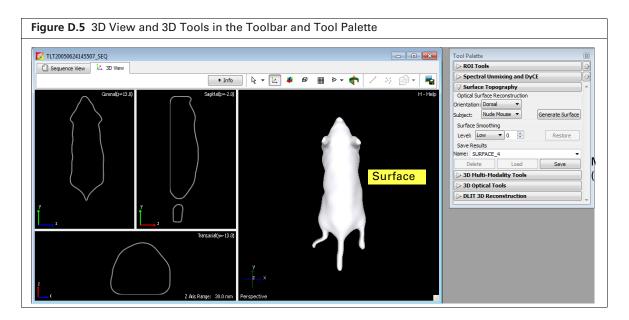
6. Click Next.

The purple data mask appears. The mask is an overlay on the subject image that defines the area of interest for the surface topography reconstruction. The mask should match the underlying photograph of the subject as closely as possible without including any area outside the subject image.

Figure D.4 Data Mask (purple)
তি Single View Surface Topography Analysis
Threshold image
Threshold image. (Use left and right arrow keys to nudge the threshold value to generate a mask image) TLT20050624122348_001
< Prev Next > Threshold: 22 +
Enish Cancel

- **7.** If it is necessary, adjust the threshold value so that the mask fits the subject image as closely as possible. To change the threshold, do one of the following:
 - Press the left or right arrow keys on the keyboard.
 - Move the Threshold slider left or right.
 - Click the 🚔 arrows or enter a new value in the box.
- 8. Click Finish.

The surface appears in the 3D View and the 3D Optical Tools appear in the Tool Palette. See Table 7.1 on page 128 for more details on the 3D Optical Tools.

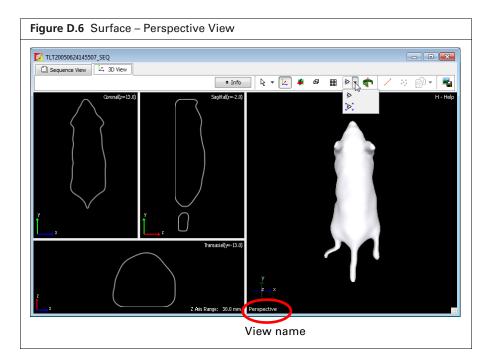


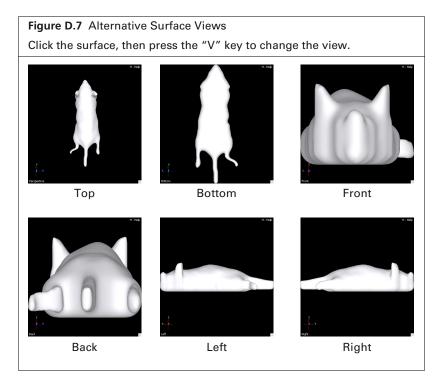
Changing the View Perspective

You can click and drag the surface to view it from different perspectives. Alternatively, do one of the following:

- Select to change the view (Figure D.6)
- Click the surface in the 3D View window, then press the V key to cycle through the different views of the surface.

Figure D.7 shows examples of the available views.





D.3 Managing Surfaces

After the surface is saved, it can be shared by the DLIT or FLIT tools.

igure D.8 Tool Palette – Surface Tope	ogra	aphy Tools
Tool Palette Tool Palette ROI Tools Spectral Unmixing and DyCE Surface Topography Optical Surface Reconstruction Orientation: Dorsal Subject: Nude Mouse Generate Surface	ogra	aphy Tools
Surface Smoothing Level: Low Carter O Carte		
Name: SURFACE_1 Delete Load Overwrite		Surface name
 > 3D Multi-Modality Tools > 3D Optical Tools > DLIT 3D Reconstruction]	

Table D.1 Surface Topography Tools - Managing Surfaces

ltem	Description			
Name	 If the loaded dataset has not been analyzed, the "Name" field shows the default name "SURFACE_1 (new)". 			
	 If the dataset has been analyzed and the results saved, the "Name" field shows the name of the surface saved with the .sequence. 			
Delete	Removes the selected surface from the system.			
Load	Opens the selected surface.			
Save	Saves a surface to the default name (e.g. "SURFACE_2") or a user-specified name.			
Overwrite	Saves the surface and overwrites the previous surface results.			

Export or Import a Surface

A surface can be shared with other users or viewed in other 3D viewer applications.

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NOTE: Surface import capability is only available if "Show Advanced Options" is selected in the general preferences (see page 274).

- **1.** Load a surface.
- **2.** Select File \rightarrow Export (or Import) \rightarrow 3D Surface on the menu bar.
- **3.** In the dialog box that appears, select a folder, enter a file name, and select a file type (see Table D.2).

NOTE: Importing a surface by this method is for viewing purposes only, not for registration with optical reconstructions in Living Image software. To import a surface or other organs for registration purposes, import an organ atlas. See page 161 for more details.

Table D.2 Surface File Types

Export Option	Description	Export	Import
Surface mesh (.xmh)	A native file format of the Living Image software that is used to exchange 3D surface information between Living Image software and other third party analysis tools. It is based on a basic indexed face set format which stores all of the vertex information first, then stores the triangle information in terms of indexes into the vertex list.	yes	yes
AutoCAD DXF (.dxf)	Drawing exchange format that is compatible with most DXF file viewers.	yes	yes
VRML 1.0 (.wrl)	VRML 1.0 (.wrl) - Virtual reality modeling language format that is compatible with most VRML viewers.	yes	no
Open Inventor (.iv)	The ASCII version of the IV file format which is supported by all IV viewers.	yes	yes
STL (.stl or ASCII format)	Stereo lithography binary format compatible with most STL viewers.	yes (binary)	yes

Appendix E Preferences

General Preferences Options on page 275 Acquisition on page 276 Theme on page 277 Optical Properties on page 280

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 $Edit \rightarrow Preferences$ 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.

E.1 General Preferences

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Figure E.1 General Preferences	
Preferences	
General Options Theme Optical Proper	rties
Start Up Defaults	Show Activity Window on: Warnings / Errors 💌
🔘 Left 🛛 🞯 Right	Save Settings
Window Size	Save float-corrected image
Width: (%) 55 🚔 Restore Defaults	Color Selections
	Folder Locations
Height: (%) 65 ≑	Window Size & Position
Apply Individual Color Scale for Sequences	Most Recently Used Dataset History 5
Show Transillumination Locations	Display ROI Label As Measurement
Show Advanced Options	Counts: Total Count
Evaluation Modules	Radiance (Photons): Total Flux
	Efficiency: Total Efficiency 🔻
	OK <u>C</u> ancel Apply

Table E.1 General Preferences

ltem	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 E.1	General	Preferences	(continued)
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ltem	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 Transillumination Locations	Choose this option to display a cross hair at each transillumination location when you load transillumination data. When you mouse over a cross hair, a tool tip displays the transillumination coordinates. If this option is not chosen, you can choose the Transillumination Location option in the sequence view window to display the transillumination locations.
Show Advanced Options	If this option is selected, advanced features are available in the menu bar and Tool Palette, including: Additional ROI functionality for Auto ROI parameters.
	 Additional export and import option for 3D surfaces and voxels.
	 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 E.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 E.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 datasets to remember and display when you select File \rightarrow Recent Files \rightarrow 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.

File Edit View Tools Window Help	to All	2 4
	Image Adjust Image Adjust	Tool Palett (undocked
Ype Description Johannamic Losding pUT Analysis Difformation Losding analysis version: Living Image® 4.2 (Mar 1 2011, 12:25:56) Information Reading heads underscale Johannamic Reading heads Johannamic Re	sion:4.2.0.13060 Activity Window	

E.2 Options

Figure E.3 Preferences – Options
Preferences General Options Acquisition Theme Optical Properties User/Defaults Label Set: Living Image Universal Edit Label Choices Default Units: Counts
OK Cancel Apply

Table E.2Preferences – Options

ltem	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.	

E.3 Acquisition

Preferences General Options Acquisition	Theme Optical Propertie	-	×
General Options Acquisition Auto Exposure Camera Settings	Theme Optical Propertie	s	
-Luminescent / Fluorescent Auto Exp			
First Preference Second Pre	eference - Third Preference -	Target Count(Minin	
		Luminescent:	3000 🚖
Exposure Time 🔻 Binning	▼ F/Stop ▼	Epi-fluorescent:	6000 🚔
		Trans-fluorescent:	10000 🚖
Range Values			
Exp. Time (sec) Binnir	F/Stop		
Min: 0.50 🚔 Min:	1 🔹 Min:	1 -	
Max: 60 🚔 Max:	8 🔻 Max:	8 🔻	
		Res	tore Defaults
		Cancel	Apply

Table E.3 Auto Exposure Settings

Item	Description
Luminescent/Fluorescent Auto Exposure Preferences	
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 E.5 Acquisition Preferences – Camera Settings			
Vreferences			
General Options Acquisition T	Theme Optical Properties		
Auto Exposure Camera Settings			
Default Image Exposure	Default Image Binning		
Photographic: Auto 🚔		Photographic: Luminescent:	
Luminescent: 1.00 🚔 sec 💌	Standard	Medium 🔻 Medium 💌	
Fluorescent: 1.00 두 sec 🔻	Manual	2 + 8 +	
Auto Save			
Folder:			
		Restore Defaults	
Ľ	ОК	Cancel Apply	

Table E.4 Camera Settings

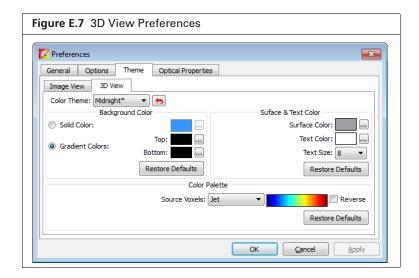
Item	Description
Default Image Exposure	Sets the default exposure settings that appear in the IVIS acquisition control panel.
Default Image Binning	Standard - Binning choices include Small, Medium and Large. These are predetermined, factory-loaded binning values that depend on the imaging system camera.
	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.

E.4 Theme

Figure E.6 Image View Preferences	
Preferences	—
General Options Acquisition Theme Optica	al Properties
Image View 3D View	
Color Palette	e
Luminescent: Rainbow 🔻	Reverse
Use saved color palette while loading datasets	Restore Defaults
Background & Text Color	ROI Color
Background Color:	Luminescent:
Text Color:	Fluorescent:
Text Size: 8 Restore Defaults	Restore Defaults
	OK Cancel Apply

ltem	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	Sets the color of the: • Background in the image window (shown below) • Text for the color bar To change a color, click the button that opens the color palette. ************************************			
ROI Color	Sets the colors for the ROI outline. To change a color, click the button that opens the color palette. Luminescent - Color of the ROI outline on a luminescent image. Fluorescent - Color of the ROI outline on a fluorescent image.			
Restore Defaults	Click to apply the default settings.			

Table E.5 Image View Preferences



ltem	Description				
Color Theme	Predefined color schemes available for the 3D View window shown here. Click the 👈 button to restore the defaults for the selected color theme.				
Background Color	Settings that modify the appearance of the background in the 3D View window				
	Solid Color - Choose this option to apply a non-gradient background color to the 3D view in the image window.				
	Gradient Color - Choose this option to apply a gradient background color to the 3D view in the image window. Top = the color at the top of the window; Bottom = the color at the bottom of the window.				
Surface & Text Color	Settings that modify the display of the surface and text in the 3D View window.				
Color Palette	Source voxels - Choose a color table for voxel display.				
	Reverse - Choose this option to reverse the min/max colors of the selected color table.				
Restore Defaults	Click to apply the default settings.				

Table E.6 3D View Preferences

E.5 Optical Properties

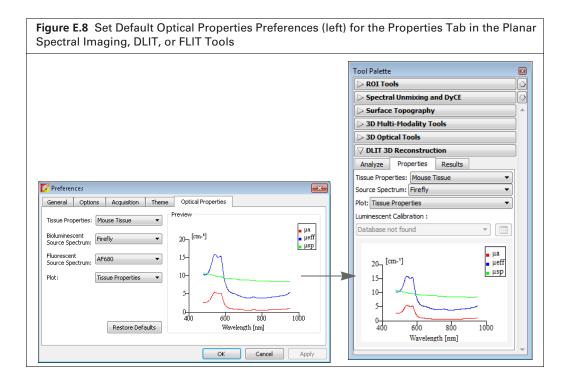


Table E.7 Preferences – Optical Properties

ltem	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.		
Source Spectrum	Choose the default luminescent source spectrum. This Source Spectrum will bused if a Subject Type is not selected in the Imaging Wizard and saved during acquisition for DLIT sequences.		
Plot	Tissue Properties - Choose this option to display a graph of the absorption coefficient (μ_a), effective attenuation coefficient (μ_{eff}), and reduced scattering coefficient (μ'_s or μ sp).		
	Source Spectrum - Choose this option to display the source spectrum for DLIT reconstructions.		
	Bioluminescent Spectrum - Choose this option to display the spectrum of the bioluminescent source (available for DLIT reconstructions only).		
	Fluorescent Spectrum - Choose this option to display the spectrum of the fluorescent source (available for FLIT reconstructions only).		
Restore Defaults	Click to restore the defaults in the Optical Properties tab.		

Appendix F Menu Commands, Toolbars, and Shortcuts

Figure F.1	Living Ima	age Toolbar			
🗃 🙆	_& ⊑	🗈 🄇) d <i>s</i>	N ?	Units: Counts

Menu Bar Command Toolbar Description Button File \rightarrow 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 61). File \rightarrow Browse Displays the Browse For Folder box so that you can select and an image data 0 folder. The selected folder is displayed in the Living Image Browser. File \rightarrow Browse 3D Displays the Browse For Folder box so that you can select a volumetric data 30 **Volumetric Data** folder (for example, DICOM format, TIF data). The selected folder is displayed in the 3D Browser. File \rightarrow Save Saves (overwrites) the AnalyzedClickInfo text file to update the analysis parameters, but the original image data files are not altered. File \rightarrow 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. Opens a dialog box that enables you to import a surface. File \rightarrow Import \rightarrow 3D Surface Note: This menu item is only available if "Show Advanced Options" is selected in the Preferences (see page 273). File \rightarrow Import \rightarrow 3D Voxels Opens a dialog box that enables you to import a source volume. Note: This menu item is only available if "Show Advanced Options" is selected in the Preferences (see page 273). File \rightarrow Import \rightarrow Atlas Opens a dialog box that enables you to import an organ atlas (.iv, .dxf, .stl). File \rightarrow Export \rightarrow Image/ Opens the Browse for Folder dialog box that enables you to export the active Sequence as DICOM image data to DICOM format (.dcm). File \rightarrow Export \rightarrow 3D Surface Opens a dialog box that enables you to save the 3D surface of the active data to a file such as Open Inventor format (.iv). File \rightarrow Export \rightarrow 3D Voxels Opens a dialog box that enables you to save the voxel information from the active data. File \rightarrow Export \rightarrow 3D Scene as Opens a dialog box that enables you to save a 3D reconstruction and/or DICOM surface in DICOM format. The Multi-Frame DICOM option supports 3D CT reconstruction in third party software. File \rightarrow Print Displays the Print box. File \rightarrow Print Preview Displays the Print Preview box that shows what will be printed.

Table F.1 Menu bar commands and toolbar buttons

Table F.1	Menu bar	commands	and toolbar	buttons	(continued)
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Menu Bar Command	Toolbar Button	Description
File \rightarrow Recent Files		Shows recently opened datasets.
		Note: The number of files displayed can be set in the Preferences box (select Edit \rightarrow 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 \rightarrow Exit		Closes the Living Image software.
Edit → Copy	Ð	Copies the active image window to the system clipboard.
Edit \rightarrow Image Labels	\mathbf{N}	Opens the Edit Image Labels dialog box that enables you to edit the label set information for the active data (see Figure 4.6 on page 29).
$\mathbf{Edit} \rightarrow \mathbf{Preferences}$		Opens the Preferences box (see page 273).
View \rightarrow Tool Bar		Choose this option to display the toolbar.
View \rightarrow Status Bar		Choose this option to display the status bar at the bottom of the main window.
View \rightarrow Tool Palette		Choose this option to display the Tool Palette.
View \rightarrow Activity Window		Displays the Activity window at the bottom of the main application window. The Activity window shows a log of the system activity.
View \rightarrow Image Information		Displays the Image Information box that shows the label set and image acquisition information for the active data.
View \rightarrow ROI Properties		Displays the ROI Properties dialog box (see page 112).
View \rightarrow 3D ROI Properties		Displays the 3D ROI Properties dialog box (see page 178).
View \rightarrow ROI Measurements		Displays the ROI Measurements table.
View \rightarrow Volume Data Viewer		Enables you to open and view DICOM data.
View → Image Layout Window		Opens the Image Layout window that enables you to paste an image of the active data in the window.
Tools \rightarrow 3D Animation		Opens the 3D Animation window that enables you to view a preset animation or create an animation.
Tools \rightarrow Longitudinal Study		Opens the Longitudinal Study window for side-by-side comparisons of DLIT or FLIT results.
Tools \rightarrow Well Plate Quantification for		Opens the Well Plate Quantification window.
Tools \rightarrow Image Overlay for		Opens the Image Overlay window for the active data.
$\textbf{Tools} \rightarrow \textbf{Colorize}$		Opens the Colorized View tab for the active sequence.
Tools \rightarrow Image Math for		Opens the Image Math window for the active data.
Acquisition \rightarrow Background \rightarrow Measure Dark Charge		Opens a dialog box that enables you to acquire a dark charge measurement.
Acquisition \rightarrow Background \rightarrow 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.

Menu Bar Command	Toolbar Button	Description	
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 \rightarrow Background \rightarrow Clear Available Dark Charge		Clears all dark charge images from the system.	
Acquisition \rightarrow Background \rightarrow 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 dataset of the current image, replacing any existing background image that may be present in the dataset.	
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.	
Acquisition \rightarrow Auto-Save		If Auto-Save is selected, all images are automatically saved to a user-selected folder.	
Acquisition \rightarrow Auto-Save To		Opens a dialog box that enables you to select a folder where images will be saved to automatically.	
Window \rightarrow Close		Closes the active image window.	
Window \rightarrow Close All		Closes all image windows.	
Window \rightarrow Cascade		Organizes the open image windows in a cascade arrangement (see Figure 5.7 on page 66).	
Window \rightarrow Tile		Organizes the open image windows in a tiled arrangement (see Figure 5.7 on page 66).	
Window \rightarrow 1. <image name="" or="" sequence=""/>		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 \rightarrow 2. <image name="" or="" sequence=""/>			
Window \rightarrow etc.			

Menu Bar Command	Toolbar Button	Description
Window \rightarrow Other Windows \rightarrow <window name=""></window>		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.
$\textbf{Help} \rightarrow \textbf{User Guide}$		Displays the Living Image Software Manual.
$\mathbf{Help} \rightarrow \mathbf{Tech} \ \mathbf{Notes}$		Displays a folder of technical notes.
		Note: For the most recent collection of technical notes, please see the IVIS University download page.
$\textbf{Help} \rightarrow \textbf{License information}$		Displays the license information.
Help \rightarrow Plug-in Information		Displays a list of tool plug-ins and Tool Palette plug-ins.
Help \rightarrow IVIS Reagents		Opens the PerkinElmer web page for In Vivo Imaging Reagents.
Help \rightarrow About Living Image		Displays information about the Living Image software and PerkinElmer technical support contact information.
	}?	Click this button, then click an item in the user interface to display information about the item.

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

Table F.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.				
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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