USER MANUAL

Living Image® 4.8.2

Advanced Acquisition & Analysis Tools

IVIS® Spectrum CT

For research use only.
Not for use in diagnostic procedures.

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1 Welcome

1.1 About This Manual

This manual explains how to acquire optical and volumetric image data on the IVIS Spectrum CT 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 CT. Sometimes the screenshots in the manual may not exactly match those displayed on your screen.

1.2 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 User's Manual*	Select **Help** → **User Guide** on the menu bar and select the manual specific for your imaging system.
Living Image technical notes (see Table 1.1 on page 10)	Select **Help** → **Tech Notes** on the menu bar. **Note:** see In Vivo Customer Training for the most recent collection of technical notes.

Table 1.1 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 1.1 Technical Notes

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

Table 1.1 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

1.3 Contact Information

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2 Important Safety Instructions

2.1 Definitions

When you see a caution, warning, or voltage symbol, pay particular attention to the safety information presented.

CAUTION: A caution note indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury and/or mechanical damage. It is also used to alert against unsafe practices. It reminds you that all safety instructions should be read and understood before installation, operation, maintenance, or repair of this instrument.

WARNING! A warning alerts you to an action or condition that can potentially cause serious personal injury or loss of life. Mechanical damage may also result.

VOLTAGE! A voltage warning indicates high voltage or risk of electric shock.

Observance of safety precautions will help avoid actions that could damage or adversely affect the performance of the IVIS Spectrum CT. If the equipment is used in a manner not specified in this manual, the protection provided by the equipment may be impaired.

2.2 Instrument Labels

Pay careful attention to the labels on the instrument. Table 2.1 shows the safety symbols that are found on the IVIS Spectrum CT or in this manual.

Table 2.1 Instrument labels

Label	Description
	WARNING: Caution. Refer to the User's documentation. (ISO 7000-0434B) AVERTISSEMENT: Attention. Se reporter à la documentation de l'utilisateur.
	Risk of puncture injury. (ISO 3864) Risque de blessure par piqûre.
	Hazardous voltage; risk of electric shock. (IEC 60417-6042) Tension dangereuse; risque de blessure par électrocution.
	Caution: X-Rays ON. (ISO 361:1975) ATTENTION: Les rayons x sur.
	CAUTION: X-RAYS. (C.R.C., c. 1370) ATTENTION: RAYONS X.
	CE compliance mark. Marque de conformité CE.
	Korean Certification Mark. Marque coréenne de certification.
	WEEE symbol (EN50419:2005). Do not dispose of as unsorted municipal waste. See the Revvity website (www.Revvity.com) for more information.
	Do not expose to water.
	WARNING: Do NOT operate or service this machine unless you are trained and have read and understand the Operation/Service Manuals and all safety signs. Do NOT operate this machine with safety devices removed. Failure to follow instructions could result in death or serious injury.

2.3 Instructions

WARNING! The IVIS Spectrum CT should only be operated by personnel who have been trained in radiation safety, and the operation and safety instructions contained in this manual. Revvity also recommends that personnel who operate the equipment, or are in close proximity to the equipment, use a radiation film badge or other type of appropriate personal dosimeter.

Read Instructions

Read and understand all the safety and operating instructions before you install, operate, or perform maintenance on this product. Make sure that you fully understand the following safety instructions, warnings, and disclaimers before proceeding to the rest of the manual.

Retain Instructions

Retain the safety and operating instructions for future reference.

Follow Instructions

Follow all operating and handling instructions. Failure to follow operating or handling instructions may void any warranty covering this product.

Heed Warnings

Abide by all warnings on the product and in the operating instructions. Failure to adhere to warnings or safety precautions may void any warranty covering this product.

2.4 X-Ray Safety and Radiation Hazards: Regulations

This equipment produces X-rays when energized. Before operating the equipment, read and understand the specific information in X-ray Safety & Radiation Hazards on page 23.

WARNING! DO NOT operate the IVIS Spectrum CT unless an X-ray safety survey has been performed within the last 12 months. For more information, please contact Revvity technical support (see page 11).

An X-ray safety survey must be performed when the instrument is installed or if abnormal mechanical shocks occurred during movement. A survey is also to be performed when the IVIS Spectrum CT has undergone any form of service in which the safety interlocks have been adjusted or any of the shielding has been removed and re-installed.

After servicing, if the safety interlocks are not operating properly or if the X-ray shielding is not properly re-installed, serious injury can result when operating the system. Conducting an X-ray safety survey is the only way to confirm proper shielding and interlock operation.

WARNING! When performing a radiation survey of the IVIS Spectrum CT, please comply with your own laboratory radiation regulations or contact Revvity technical support for further assistance (see page 11).

Owners and operators of the IVIS Spectrum CT are responsible for complying with all regulations in the country where the equipment is operated. This includes all local, state, and federal regulations. In some states of the US, it may be necessary to register radiation sources with the governing state and/or local public health agencies before operating the instrument. Equipment registration may be required immediately or within 30 days of acquiring the equipment.

Owners and operators of the IVIS Spectrum CT are responsible for contacting the appropriate public health agencies for registration information that pertains to installation of the IVIS Spectrum CT. If you need assistance with this requirement, contact Revvity technical support. For more details and contact information, see *Safe Operating and Emergency Procedures for the Operation of the IVIS SpectrumCT Cabinet X-ray System*. This document was provided with the pre-installation instructions.

WARNING! A Revvity employee will conduct a radiation leakage survey and safety tests when the IVIS Spectrum CT is installed. Revvity employees are trained in radiation safety. However, check with your local radiation control authority to determine the specific radiation survey requirements at your facility. If necessary, have a qualified expert other than a Revvity employee survey the installation before operating the instrument.

2.5 Fluorescence Light Source Safety

WARNING! The IVIS Spectrum CT is equipped with a strong visible fluorescence light source. Do not stare directly into this source.

2.6 Environmental Consideration for System Components

Location for the IVIS Spectrum CT

Before installation, consider the proper environment for the IVIS Spectrum CT components. Install the equipment in an environment where:

- The temperature does not change or fluctuate widely. Choose an environment where the temperature is maintained between 18–24°C (65–75°F).
- The humidity does not exceed 80%.
- No strong electric or magnetic fields exist.
- No vibrations are present.
- No corrosive gases are present.
- High amounts of dust are not present.
- No open flame is present.
- There is sufficient space behind the IVIS Spectrum CT equipment. A minimum space of six inches (15 cm) from the flat surface of the rear panel should be provided behind the IVIS Spectrum CT to provide unobstructed air flow and access to the main power on/off switch.
- The floor is level and structurally sound.

Heat

The system should be situated away from heat sources such as open flames, radiators, heat registers, stoves, and other heat-generating electrical equipment.

Water and Moisture

VOLTAGE! Do not use the IVIS Spectrum CT near water (for example, near a sink or wet room) due to the risk of electric shock, electrical damage, and/or system failure.

Laboratory Space

The IVIS Spectrum CT requires a minimum of 100 square feet (9 square meters) of laboratory space for adequate ventilation. At least of six inches of space is required behind the rear flat panel to provide unobstructed air flow. If you will be using an RAS-4 Rodent Anesthesia System

or other anesthesia system, refer to the anesthesia system manual for additional ventilation requirements to avoid exposure to waste anesthesia gases.

Allocate an area that is at least 36 inches (91.4 cm) wide and 51 inches (129.5 cm) deep for the instrument to allow the door to be fully opened and provide the recommended air space behind the instrument.

An additional area that is 48 inches (121.9 cm) wide should be available, preferably to the right of the instrument, to accommodate a table or cart to hold the computer and provide a work surface.

2.7 Power Considerations

Power Sources

The IVIS Spectrum CT is configured for the voltage requirements of the installation locality that was specified at the time of order. The set voltage is marked on the outside of the product itself, and should only be powered by that voltage. If the system is moved to another area, check to make sure that the same voltage requirements exist at the new location.

An IVIS Spectrum CT that operates on 120VAC requires a dedicated 20 Amp circuit that is not shared with other loads except the computer and the optional RAS-4 Rodent Anesthesia System. Ensure that all equipment is plugged into a properly grounded AC supply.

The computer should share the same circuit as the IVIS Spectrum CT to avoid ground loops. Never use auxiliary power outlets on other equipment to supply the IVIS Spectrum CT. Since the system contains internal surge protection, it must be powered directly from the main electrical supply wall socket. Revvity recommends the use of a surge protector to power the computer, monitor, and any other accessory.

An IVIS Spectrum CT configured for 230 VAC requires a dedicated minimum 10 Amp circuit subject to the same conditions described above. For more details on the operating requirements, see *Electrical Power Requirements* on page 46.

VOLTAGE! The IVIS Spectrum CT can operate at multiple voltages (100, 120, 220, 240 VAC); however, you are not permitted to change the input voltage to any of the system components. The instrument should only be powered by the set voltage which is marked on the outside of the product itself. Several internal modifications are required for voltage change. If the operating voltage must be changed, contact Revvity technical support (see page 11).

Power Cord Protection

Power supply cords should be routed so that they are unlikely to be walked on or pinched by items placed upon or against them. Pay close attention to receptacles and to the points of connection between cords and equipment.

If it becomes necessary to replace the power cord(s) or surge protector for the instrument or any of its accessories, the replacement power cord set and surge protector should be adequately rated for the voltage and current as dictated by the *Power Connection Accessories Table* (Table 2.2 below). Use a power cord set and surge protector that bears the national agency approval marks required for the country of end installation. Revvity offers region-specific power connection kits that include a surge protector and all necessary power cords for the instrument, computer, and monitor (see *Ordering Information on page 396* for available kits).

Facilities should be adequately wired according to local building codes.

Table 2.2 Power Connection Accessories

Item	Connector Type	Minimum Voltage Rating	Minimum Current Rating
120 VAC Power Connections - Type B for North America and Japan			
Instrument Power Cord	NEMA 5-15P x IEC C19	125 V	15 A
Computer Power Cord	NEMA 5-15P x IEC C13	125 V	10 A
Monitor Power Cord	NEMA 5-15P x IEC C13	125 V	10 A
Surge Protector	NEMA 5-15R (Sockets) x NEMA 5-15P (Plug)	120 V	10 A
220 - 240 VAC Power Connections - Type F for Europe and S. Korea			
Instrument Power Cord	CEE 7/7 x IEC C19	250 V	16 A
Computer Power Cord	CEE 7/7 x IEC C13	250 V	10 A
Monitor Power Cord	CEE 7/7 x IEC C13	250 V	10 A
Surge Protector	CEE 7/3 (Sockets) x CEE 7/7 (Plug)	250 V	10 A

Power Outages

If the IVIS Spectrum CT experiences a loss of supply power, turn off the power switch for all components and do not restart the system until reliable power has been restored. For more details on how to restart the system, see *Restarting the System After a Power Outage*, page 65.

Overloading

WARNING! Do not overload wall outlets, extension cords, or integral convenience receptacles as this can result in a risk of fire or electric shock. For electrical power requirements, see page 46.

2.8 Cleaning or Moving the System Components

Cleaning/Liquid Entry

VOLTAGE! Do not use liquid or aerosol cleaners and never spill liquid of any kind on any of the IVIS Spectrum CT components. Sprays and liquids that come into contact with the IVIS Spectrum CT may result in damage to the system or electrocution. For more details on proper care of the system, see *Cleaning the Instrument*, page 380.

Moving the IVIS Spectrum CT

The IVIS Spectrum CT is a sensitive, scientific instrument and should be moved with care. Pay particular attention when rolling the instrument on its casters to avoid toppling the equipment.

The total height of the instrument is 77 inches (195.6 cm). If necessary, the plastic dome can be removed for easier passage through doorways. For more details on removing the dome, see page 386.

If you have any questions about moving the instrument, contact Revvity technical support (see page 11).

2.9 Other Equipment or Chemicals

The use of any equipment other than that recommended by this manual has not been evaluated for safety and, therefore, is the sole responsibility of the user.

No chemicals are required for the operation of the IVIS Spectrum CT. Other user-supplied chemicals or materials may be required as part of your specific biological testing procedures.

Do not modify the IVIS Spectrum CT in ANY manner by making any kind of hole or aperture in the instrument or removing any component of the radiation shielding.

2.10 Servicing

Refer all servicing to Revvity technical support. If the instrument is damaged and requires service, unplug the instrument from the outlet and contact Revvity technical support (see page 11). Servicing by anyone other than those authorized by Revvity voids any warranty covering the instrument.

3 Warnings

3.1 Electrical Safety

WARNING! DO NOT attempt to service the instrument yourself. Contact Revvity technical support for electrical service needs (see page 11). Although there are no voltages in excess of 45V inside the imaging chamber, local line voltages can be present inside the electronics cabinets.

CAUTION! If cleaning is necessary, wipe the exterior surfaces of the IVIS Spectrum CT with a soft, damp cloth only. Do not allow fluids of any kind to enter the system interior under any circumstances. For more details on cleaning the system, see page 380.

This equipment requires a connection to protective earth for safety. To ensure safety, connect the power cord to a properly grounded receptacle.

If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

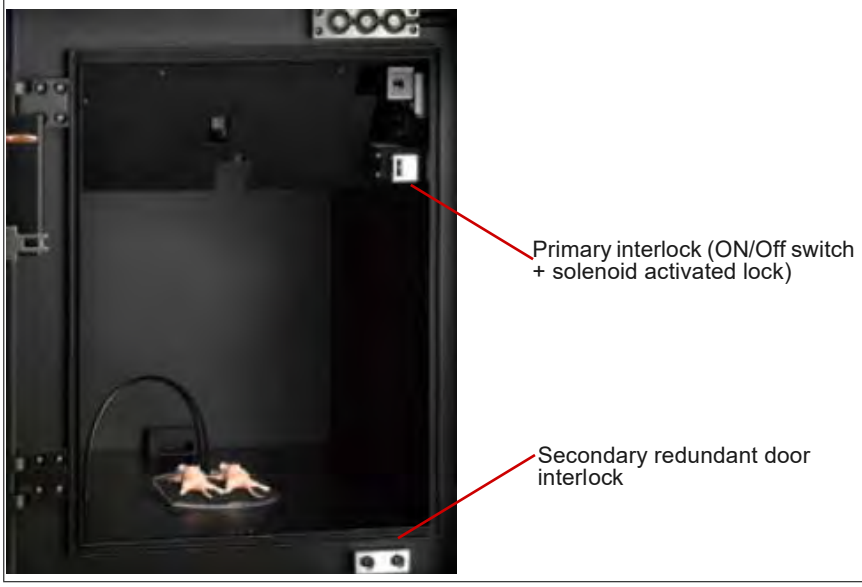
3.2 X-ray Safety

WARNING! This equipment produces X-rays when energized

WARNING! The IVIS Spectrum CT should be operated only by personnel who have been trained in radiation safety, and the operation and safety instructions contained in this manual. Revvity also recommends that personnel who operate the equipment, or are close proximity to the equipment, use a radiation film badge or other type of appropriate personal dosimeter.

The IVIS Spectrum CT has multiple safety interlocks that prevent X-ray generation when the door is open (Figure 3.1). The primary interlock switch prevents any generation of X-rays unless the door is completely closed. A secondary redundant door interlock prevents X-ray generation when the instrument door is opened. See *Safety Interlocks* on page 29 for more details on the primary and secondary safety interlocks.

Figure 3.1 IVIS Spectrum CT safety interlocks



3.3 Mechanical Safety

The instrument has many internal motorized components that can only move when the door is closed and locked. The imaging platform moves frequently during routine use; keep loose objects away from the edges of the platform where they could become jammed.

CAUTION: DO NOT defeat any of the instrument safety interlocks. Do not place anything under the imaging platform.

The imaging platform cannot move unless the door is closed and locked. If the platform moves when the door is unlocked, shut down the system and contact Revvity technical support (see page 11).

The IVIS Spectrum CT contains several delicate optical and mechanical components. Do not touch these components and avoid using sprays or other contaminates that may damage lenses or other optical-mechanical components.

3.4 Chemical and Biological Safety

Normal operation may involve the use of samples that are pathogenic, toxic, or radioactive. It is your responsibility to ensure that all necessary safety precautions are taken before such materials are used.

Dispose of all waste materials according to appropriate environmental health and safety guidelines.

It is your responsibility to decontaminate the IVIS Spectrum CT before requesting service by Revvity technical support (see page 11). Ask your laboratory safety officer to advise you about the level of containment required for your application and the proper decontamination or sterilization procedures.

Handle all infectious samples according to good laboratory procedures and methods to prevent the spread of disease.

3.5 Eye Safety and Burn Hazard

The IVIS Spectrum CT is equipped with a Quartz Halogen light source module and connecting fiber optic cables that are capable of producing intense light that could be damaging to the eyes. The protection means provided by the system prevents access or exposure to the Quartz Halogen light.

3.6 Panels, Covers, and Modules

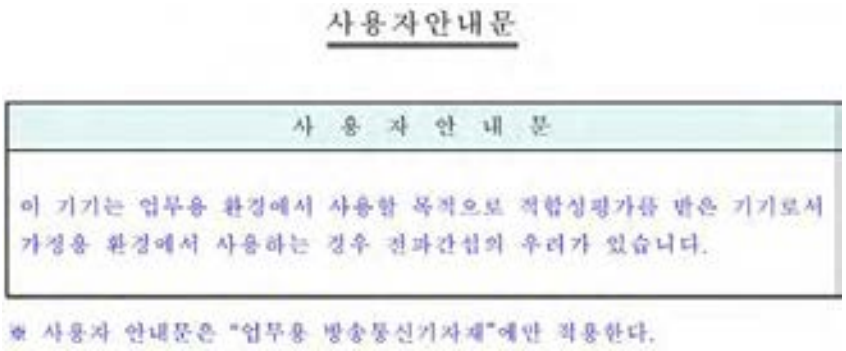
Aside from the imaging chamber door, the filter wheel access panel and the lower rear cart panel are the only user removable panels. The lower rear cart panel allows access to the chiller for coolant filling. There are no user serviceable components in the electronics cabinets of the IVIS Spectrum CT. Do not remove the electronics cabinet covers from the instrument unless instructed by and under the supervision of Revvity technical support (see page 11).

Do not modify the IVIS Spectrum CT in ANY manner by making any kind of hole or aperture in the instrument. Do not remove any component that is part of the radiation shielding. In certain situations, the Emission Filter Wheel Access Panel may be removed (see page 382).

3.7 Korean EMC Registration Statement

EMC Registration is done on this equipment for business use only. The equipment may cause interference if it is used in the home.

This warning statement applies to a product for business use.


사용자안내문

사 용 자 안 내 문

이 기기는 업무용 환경에서 사용할 목적으로 적합성평가를 받은 기기로서 가정용 환경에서 사용하는 경우 전파간섭의 우려가 있습니다.

※ 사용자 안내문은 "업무용 방송통신기자재"에만 적용한다.

4 X-ray Safety and Radiation Hazards

4.1 Introduction

The IVIS Spectrum CT produces X-ray radiation. This radiation is confined to the interior of the imaging chamber. IVIS SpectrumCT will only produce X-rays when the CT function has been armed and energized. The instrument may also be operated in standard bioluminescent or fluorescent mode without X-ray generation.

The IVIS Spectrum CT is defined by most regulatory agencies as a "Cabinet X-Ray System" A cabinet system is one that produces little or no X-ray exposure to the user and is safe to operate with the user in close proximity. Revvity certifies that the IVIS Spectrum CT produces not more than 0.5 milliRoentgen in 1 hour at a distance of 5 cm from the instrument surface. The instrument is also certified to meet all international exposure requirements (typically 0.1 millirem per hour) and other regulations that apply to the user's location. The IVIS Spectrum CT meets all US (FDA) regulations regarding a cabinet X-ray system. For information on international limits for X-ray doses, see page 26.

Product Documentation

Users will receive pre-installation information to help prepare the laboratory for installation of the IVIS Spectrum CT and any accessories purchased with it.

4.2 Radiation Theory and X-ray Generation

Radiation is everywhere. Our bodies are continuously bathed in radiation in the form of sunlight, radio, and television waves as well as radiation produced by the earth's natural background radiation that is produced by radioactivity and cosmic rays. Radiation is distinguished by its ability to ionize chemical bonds and is characterized as either ionizing or non-ionizing.

Ionizing radiation has the ability to affect biological organisms including human beings by interacting with cellular chemistry. Radiation such as sunlight, is usually considered non-ionizing radiation, although there is some overlap when discussing ultra-violet radiation.

X-rays are a form of electromagnetic radiation similar to light; however, X-rays have much shorter wavelengths. X-rays have wavelengths from 10 to 0.01 nanometers, whereas visible light ranges from 700 to 400 nanometers. Non-visible ultraviolet light fills the gap between visible light and X-rays with wavelengths ranging from 400 to 10 nanometers. Non-visible ultraviolet light is also considered an ionizing radiation, but it does not possess the penetrating capability of X-rays. In summary, X-ray radiation is an ionizing form of electromagnetic radiation that has sufficient energy to break chemical bonds.

The instrument uses an X-ray generating tube to produce ionizing radiation, which if left un-shielded could be harmful to people or animals. The IVIS Spectrum CT instrument includes shields that confine the X-rays within the imaging chamber. Consequently, operation of the IVIS Spectrum CT does not expose the operator to unsafe X-ray exposure.

At least two complete radiation surveys are conducted to verify the instrument design, ensure proper manufacturing and installation, and make certain that there is no leakage from the instrument that exceeds the US and international regulations.

X-rays: Ionizing Radiation

As noted above, X-rays are classified as ionizing radiation that is capable of removing or rearranging the electronic bonds of chemical compounds. For this reason they are considered potentially hazardous to living organisms. X-rays can also interact with matter by scattering off of atoms in new directions. Therefore, the IVIS Spectrum CT incorporates shielding that completely blocks scattered radiation in all possible directions, including the primary beam direction.

Ionizing radiation can also be produced by radioactive materials. However, since the IVIS Spectrum CT contains no radioactive materials there is no possibility of transferring a radioactive contaminate from the instrument to the laboratory during a procedure such as cleaning the imaging chamber.

X-rays: Penetrating Radiation

The ability of X-rays to penetrate matter makes them useful in applications such as medical imaging and industrial inspection. The IVIS Spectrum CT uses an X-ray tube source with the minimum energy required to generate X-rays that penetrate and image mice. Even though X-rays are able to penetrate matter such as tissue or plastic, they are not able to make matter radioactive.

How X-rays are Generated

The IVIS Spectrum CT is a computed tomography (CT) imaging system that consists of a fixed X-ray source which produces a cone beam of X-rays. The shelf consists of the main platform and a rotating animal stage. For CT imaging the platform moves down into the bottom of the instrument. During CT Imaging the animal stage rotates about the vertical axis while the fixed horizontal beam of X-rays penetrates the animal. The X-ray source is in the behind the rear of the imaging chamber and the X-ray detector is in the front.

The X-ray source is an X-ray tube (see page 25 for more details on the tube characteristics); its basic function is to generate X-rays of a maximum energy of 50,000 electron volts. The X-rays are produced by the collision of high energy electrons with a tungsten metal target in a vacuum tube. When high voltage is applied between a heated cathode and the tungsten anode, electrons are stripped from the cathode and are accelerated into the anode. The collision of those electrons produces X-rays.

There are two atomic processes capable of producing X-rays from these collisions. One process, known as *bremsstrahlung* (from the German "braking radiation"), generates X-rays by the rapid deceleration of the high energy electrons as they interact with the repulsive electron field of the tungsten target metal.

The second X-ray generating process results from the high energy free electrons interaction with the atomic orbitals of the target metal. The IVIS SpectrumCT X-ray tube generates X-rays using both of these processes. The tube generates a spectrum of X-rays from approximately 10keV to the maximum of 50keV.

Two thin filters, one made of copper and the other of aluminum, can be placed in the X-ray beam to reduce unusable low-energy radiation. A thick filter made from tungsten is placed in front of the beam during source warm-up in order limit the animal's X-ray exposure.

IVIS Spectrum CT X-ray Source Tube

The X-ray generating tube is located behind an interlocked panel on the rear of the IVIS Spectrum CT. The tube is neither accessible nor serviceable by the user. The X-ray tube, high voltage power supply and other electronic circuits are located within the interlocked shielded cabinet. Only Revvity Field Service personnel are allowed to remove the access panel as it requires the use of a special tool to remove the tamper proof screws.

NOTE: The only user-accessible door and panels are the imaging chamber door, the filter access panel (see page 382), and the lower rear cart panel that provides access to the thermoelectric chiller (see page 384). There are no other panels that can be removed or opened by the user.

The tube is rated at 50kV high voltage potential with a maximum beam current of 1milliAmp. Total power is 50.0 watts. The X-ray window is 0.13 mm (0.005 inches) thick beryllium and the X-ray target is tungsten. The X-ray tube controls, including ON/OFF and beam power settings, are carried out by IVIS SpectrumCT software commands which limit the X-ray tube voltage to 50kV maximum.

4.3 Biological Effects of Radiation

X-rays are a form of electromagnetic radiation that has enough penetrating energy to ionize atoms within a cell. Ionization occurs when an X-ray photon interacts with an orbital electron and transfers energy to it, causing the electron to be ejected from the atom. Such ionizations may disrupt molecules such as DNA. The DNA molecule can be broken by the radiation and the cell can be severely damaged resulting in cell death. With enough cell death, tissue and organs can be damaged. Injury to a living organism can also occur in indirect ways such as the creation of free radicals or other ions. The deleterious effects of radiation exposure are classified into two categories: deterministic effects and stochastic effects.

Deterministic Effects

Deterministic effects are those effects in which a clear causal connection can be made between the exposure to radiation and the effect. Deterministic effects are the result of cell killing and tissue damage. This effect is dose related—a radiation dose greater than a certain threshold must occur which produces enough cell death to result in tissue damage. After the dose threshold is exceeded, the severity of the effect is increased by the amount of the dose.

Examples of deterministic effects of radiation due to overexposure of X-rays include: skin changes (reddening, pigmentation changes, blistering, and ulceration), cataract formation, and fetal abnormalities due to exposure *in utero*.

The deterministic effects of radiation can be classified as either acute or delayed. An acute effect such as skin reddening occurs soon after overexposure to radiation whereas a delayed affect such as cataracts may take some time, even years, to develop. For deterministic effects there is a clear connection between the individual exposure to radiation and the biological effect. The biological effect requires a minimum threshold dose, and the severity of the effect increases with increasing dose.

Stochastic Effects

Stochastic effects are biological effects that have a statistical probability of occurring based on the radiation dose. Unlike deterministic effects, stochastic effects have no dose threshold. Even for low radiation doses there is a small probability of a biological effect occurring. The severity of the stochastic biological effect can be unrelated to the magnitude of the dose, but the probability of occurrence increases with increased dose or length of exposure.

Stochastic effects cannot be linked to a specific radiation exposure with certainty. For example, stochastic effects such as cancer can also occur in individuals who have not been exposed to radiation above background levels, therefore it is not possible to determine that the cancer resulted from any specific exposure.

X-ray Dose Limits

The instrument has been tested at maximum operating conditions and Revvity has determined that the local X-ray dose rate at a distance of 5 cm from the surface of the equipment is less than 1.0 µSv/h.

Revvity declares that the IVIS Spectrum CT system conforms to:

- 1996/29/Euratom Directive (Dose rate of 1 µSv/h at 10 cm from any accessible surface under normal operating conditions)
- US CFR21 Part 1020.40 Regulation (Dose rate of milliRoentgen in 1 hour at 5cm outside of the external surface under maximum operating conditions) in accordance with the following standard:

  IEC 61010-1:2001 Standard (Dose limit of 1 µSv/h at 10 cm from the surface of the equipment under maximum operating conditions)

Revvity certifies that the IVIS Spectrum CT has achieved the objectives of the:

- ICRP 60 recommendations of annual public dose limit of 100mrem
- ICRP 103 recommendations of annual public dose limit of 100mrem
- US OSHA workplace annual public dose limits of 100mrem and other international public safety standards and regulations

It is unlikely that properly trained individual using the IVIS Spectrum CT will receive an annual dose that exceeds these public dose limit levels.

4.4 IVIS Spectrum CT Safety Systems

The IVIS Spectrum CT has many different safety features that are intended to keep the operator safe from radiation exposure. Some of these features are discussed in this manual as well as the *Safe Operating and Emergency Procedures for the operation of the* IVIS SpectrumCT *Cabinet X-ray System* (part no. CLS133919),

CAUTION: To prevent the development of unsafe operating conditions in the instrument, do not tamper with the instrument and follow the maintenance procedures in Chapter 10 on page 379.

Radiation Shielding

The IVIS Spectrum CT is considered a cabinet X-ray system because all of the radiation is confined to the inside of the metal structure. This is accomplished by using steel of a sufficient thickness to block X-rays of the energies produced by the X-ray source tube described on page 25. Some cabinet parts incorporate additional shielding to prevent leakage. There are no

ports, apertures, or other openings through which any part of the human body can be placed when X-rays are being generated.

WARNING! Do not modify or remove any of the steel shielding in the IVIS Spectrum CT. If the instrument door does not completely close, do not operate the IVIS Spectrum CT and contact Revvity technical support (see page 11).

The IVIS Spectrum CT Control Panel

The IVIS Spectrum CT is controlled primarily by software through a proprietary Revvity program. Some control functions are located on the main instrument electronics panel in compliance with requirements for cabinet X-ray systems. However, the controls and indicators on the main module are "enablers" only. The initiation and termination of an X-ray imaging session is controlled from the computer through the IVIS SpectrumCT software. Figure 1.1 shows the IVIS Spectrum CT and its main instrument electronics panel.

X-rays can only be generated when the main instrument is armed for X-ray mode. This means that the Emergency Stop switch is in the OUT position, the key switch is in the ON position, and all safety interlocks are working.

X-rays can be terminated from the computer as the result of ordinary operation at the programmed end of image acquisition or from the computer control panel. X-ray generation can be aborted by pushing the Emergency Stop button (IN) or by turning OFF the key switch.

NOTE: The Emergency Stop button will turn off power to the entire system.

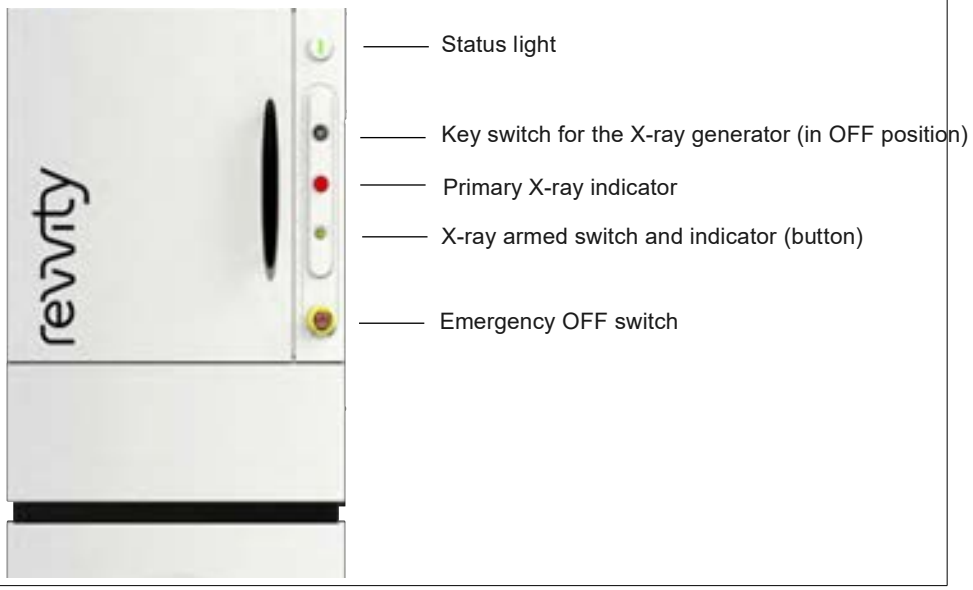
A redundant interlock, as prescribed by the United States FDA, completely disconnects the power to the X-ray source if the door is opened to a gap of 5 mm (0.2 inches). The X-ray leakage seal of the door incorporates an overlap edge design which prevents radiation escape through this gap. Also, during X-ray operation, a solenoid-operated door lock prevents opening of the door. This door lock remains engaged for four seconds after X-ray power has been turned off. This ensures that the door can only open after the X-ray source voltage is turned off and the radiation field has completely collapsed.

Requirements for Turning on X-rays

X-rays can be generated when the following conditions are met.

1. The Emergency Stop button is in the ON (out) position (Figure 4.1).
2. The keyed on/off switch is in the ON position. This keyed switch is the main ON/OFF enabling switch on the instrument control panel.
3. The X-ray Armed push button is on and lighted.
4. The instrument door is closed and locked (engages the primary and redundant safety interlocks).
5. The filter wheel access panel is attached.
6. X-rays are activated from the software.

Figure 4.1 IVIS Spectrum CT instrument control panel



X-ray ON Indicators

The IVIS Spectrum CT is equipped with three indicators that show the status of X-ray generation (Table 4.1).

Table 4.1 X-ray ON indicators

Indicator	Description
	The X-ray indicator light on the main instrument control panel. This indicator is independent of the other two indicators. If it malfunctions, the remaining two indicators are operational.
	The X-ray indicator in the IVIS Acquisition Control Panel in the software.
	A light under the translucent dome at the top of the instrument which is visible from 360 degrees. This figure shows the light with the dome removed. This light illuminates the entire dome when X-rays are being generated

Safety Interlocks

WARNING! Never defeat the primary and the redundant safety interlocks. Defeating the safety interlocks could result in serious injury of death.

The IVIS Spectrum CT has multiple safety interlocks that prevent X-ray generation when the door is open. The primary interlock switch is activated as soon as you close the door. The primary interlock switch is an ON/OFF switch that is combined with a solenoid activated lock. It prevents any generation of X-rays unless the door is completely closed.

An actuator key is inserted into the solenoid-activated ON/OFF switch when the instrument door is closed. This completes the electrical circuit for the X-ray tube. After the X-ray circuit is turned ON, the actuator key is captured by a solenoid lock which prevents the door from opening while X-rays are being generated.

A secondary redundant door interlock prevents X-ray generation when the instrument door is opened.

Figure 4.2 IVIS Spectrum CT safety interlocks

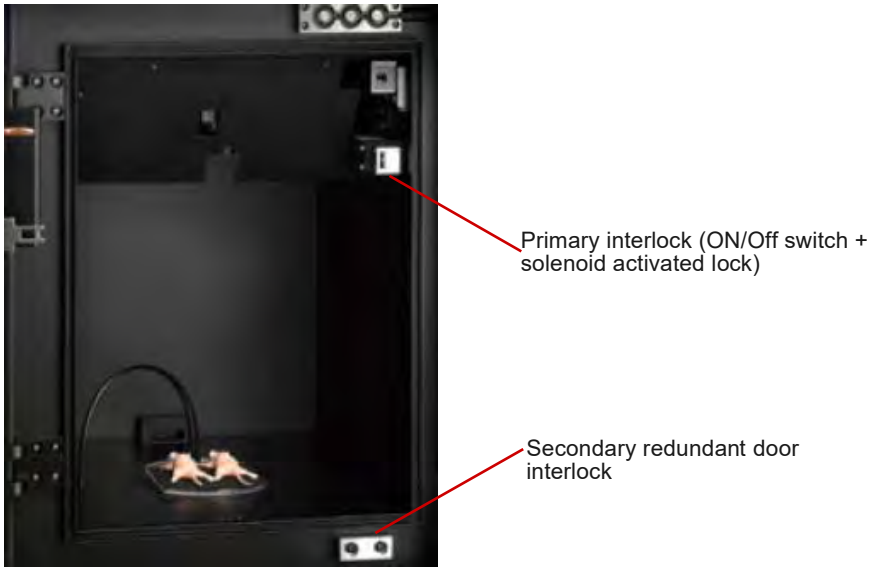
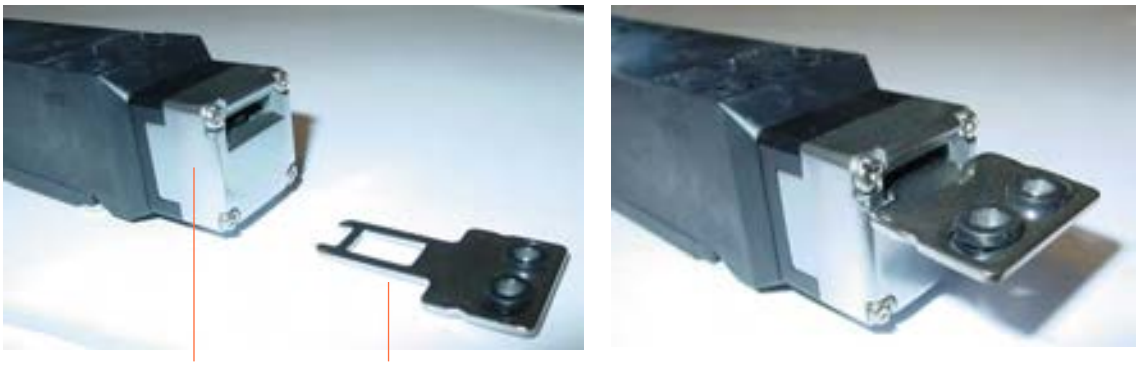


Figure 4.3 shows the primary solenoid lock with its activation key engaged and disengaged to demonstrate its principal of operation. When the solenoid is activated by electrical power, the key is retained by a mechanical lock. The solenoid lock also acts as the primary switch which completes the circuit that allows imaging to begin.

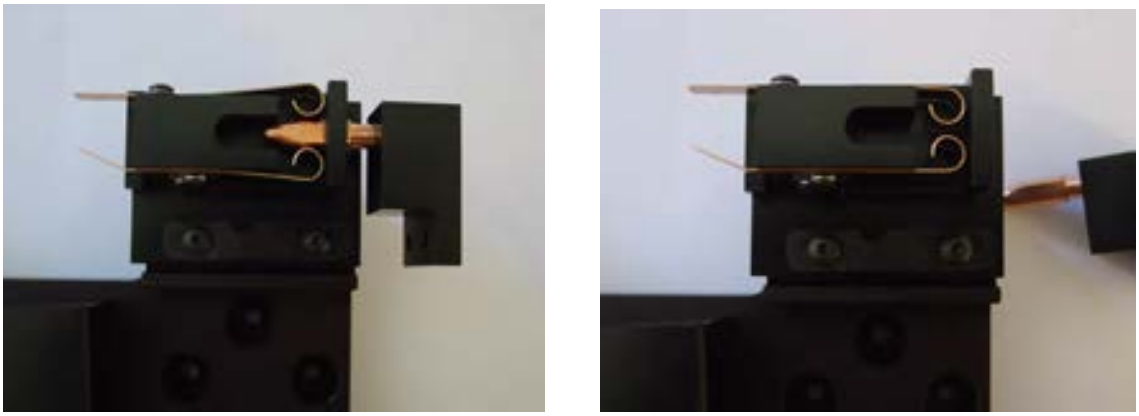
Figure 4.3 Primary door interlock and activation key



A second redundant door interlock prevents X-ray generation by physically removing part of the X-ray electrical circuit when the imaging chamber door is opened.

Figure 4.4 demonstrates the principal of operation for the redundant interlock. A copper rod is permanently mounted on the door. When the door is opened, part of the electrical circuit is removed from the X-ray generating circuit and contact is lost between the two flexible contacts in the switch body. This switch is sometimes referred to as a CDRH switch and meets US FDA requirements as well as some other international regulations.

Figure 4.4 Second redundant safety interlock switch



4.5 Regulatory Compliance and Laboratory X-ray Safety Procedures

Aside from what Revvity has done to make a safe system, there are some things that are required of the user to ensure that the instrument operates safely and within legal authority.

Contact the State or Provincial Radiation Protection Authority

You must contact your state or provincial radiation protection authority before operating the instrument. Chapter 23 provides contact information for United States radiation authorities (page 398) and Canadian Provincial radiation authorities (page 406).

In most cases, you will be required to register the IVIS Spectrum CT with the state or provincial radiation protection authority. This registration may require periodic renewal.

Study Documentation

Before operating the IVIS Spectrum CT, read and understand this manual. Pay particular attention to the safety procedures described in *Safe Operating and Emergency Procedures for the operation of the* IVIS SpectrumCT *Cabinet X-ray System* (PN 133919). This document can help you prepare a radiation safety plan which is described in the next section.

Create a Radiation Safety Plan

Your institution may require that you have a written radiation safety plan. Such plan may already exist at your institution and may also be a requirement of your registration with the state or provincial radiation protection authority. Here are some key points of a radiation safety plan:

- Persons using the IVIS Spectrum CT must read all documentation supplied with the instrument.
- Permit only trained and authorized individuals to operate the IVIS SpectrumCT. Wear personal radiation monitors if required. Revvity recommends wearing them even if they are not required.
- Designate a "master key" person who controls access to the IVIS SpectrumCT.
- If required, install the instrument in a controlled access or restricted access room.
- Post any required "Caution X-ray" signs required by your regulating authority.
- Designate a person responsible for ensuring that the safety and maintenance procedures specified in this manual are performed.
- Frequently verify that all safety procedures are followed, the IVIS Spectrum CT has not been modified, and no safety interlocks have been disabled.
- Follow all guidance supplied by your local, state, or provincial radiation protection authority. If that guidance conflicts with the Revvity supplied information, either written or spoken, contact Revvity technical support so that the conflicts can be resolved (see page 11).
- Keep records of X-ray surveys, instrument repairs, or other data required by the radiation protection authority or your institution. Keep registration certificates, compliance or safety audit reports, instrument inspections, training records, and the list of authorized users. Keep records of any accident or investigation reports, or worker complaints.

Create a Training Plan

If your radiation protection authority or your institution requires a radiation training plan, you may find the following suggestions helpful.

- Use the documentation supplied with instrument for specific training instructions for the instrument. Use supplemental information for more in depth coverage of topics such as radiation safety.
- Identify radiation hazards associated with the use of the IVIS Spectrum CT.
- Characteristics of radiation. Units of dose.
- Discuss the various warning and safety devices incorporated in the IVIS Spectrum CT. Point out the importance of having them in working condition.
- Proper operating procedures for the equipment.
- If the trainee will be conducting radiation surveys, discuss the operation, calibration, and limitations of radiation survey instruments.
- Proper survey techniques (if surveys are conducted by non-Revvity personnel).

- Methods of controlling radiation dose, such as time, distance and shielding. Principles and practice of maintaining X-ray exposure to As Low As Reasonably Achievable (ALARA).
- Personal monitoring.
- Symptoms of acute localized exposure and proper reporting procedure for an actual or suspected exposure.
- Applicable state, provincial, local, and institutional regulation or policies.

5 Imaging Overview

5.1 Example Imaging Workflow

Table 5.1 Example Imaging Workflow

Workflow Step	For More Details:

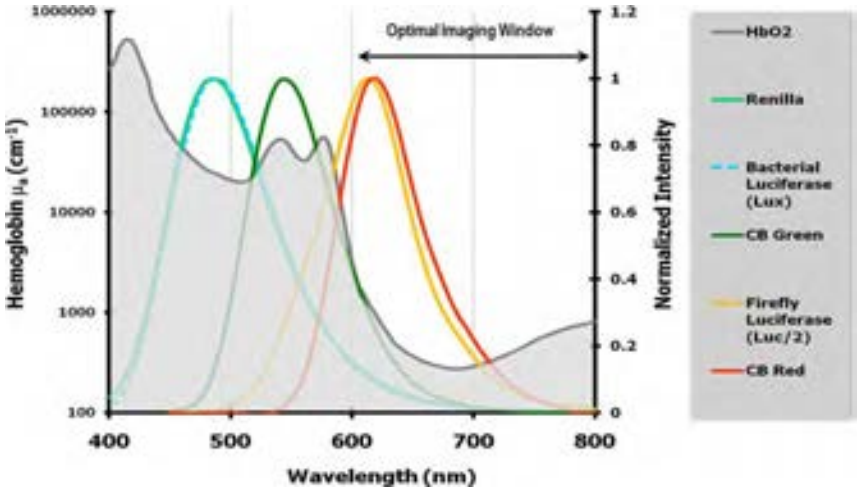
1. Plan the experiment.
▪ Best results are obtained using nude mice. Subjects with black or dark-colored fur and skin are not optimal and may give poor results.
▪ Determine the number of animals required:
  ▪ Always include control animals and replicates (for example, No disease + Probe, Disease + No 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 auto-fluoresces 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.
 	

Table 5.1 Example Imaging Workflow (continued)

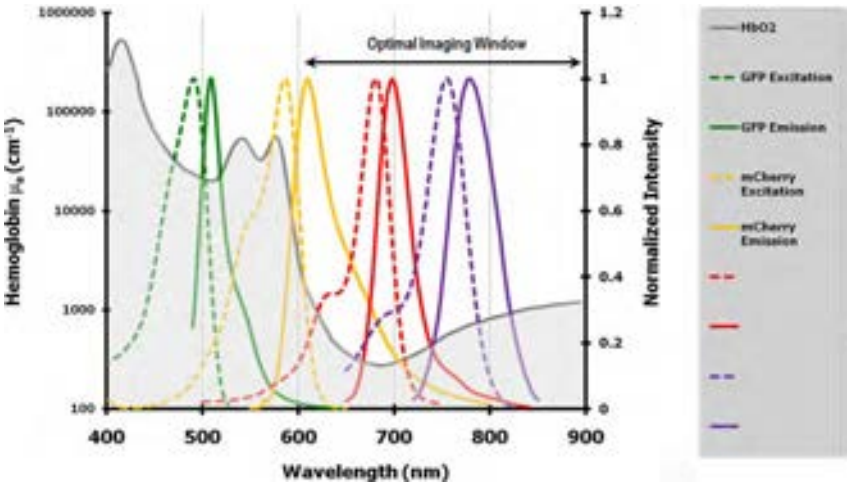
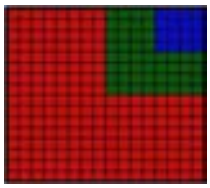
Workflow Step	For More Details:
1. Plan the experiment (continued). Fluorescent signal is usually higher than luminescent signal, but fluorescent imaging has lower sensitivity due to higher noise (instrument background and animal autofluorescence). Optimal fluorescence imaging is from 620 – 900 nm.  	
2. Determine the optimal imaging time post-injection. ▪ Luminescence imaging – Determine a probe kinetic curve for the animal model and cell line. Metabolic rates, which can differ among animal strains, and animal handling procedures affect probe kinetics. Always acquire images during the plateau of the kinetic curve for optimum quantitative results. ▪ Fluorescence imaging – Please see the Technical Data Sheet for the fluorescent imaging agent.	See the technical note *Kinetic Analysis of Bioluminescent Sources* for a protocol to determine a luciferin kinetic curve.
3. Prepare and image the subjects. ▪ If using white or light-colored furred mice, comb the fur before imaging to eliminate any "fluffy" areas that can alter the light emission pattern. It may be necessary to shave the animals or apply a depilatory. ▪ Acquire an image using autoexposure within the optimal time window. If necessary, manually adjust camera settings in the Control Panel (exposure time, binning, F/Stop) to obtain a signal between 600 and 60,000 counts. Signal within this range is above noise, but below saturation. ▪ Exposure time – Shorter exposure times increase throughput, longer exposure times increase signal intensity. If manually setting exposure, the time should be greater than 0.5 seconds and less than 5 minutes for luminescence imaging. ▪ Binning – Applies digital pixel binning to group pixels into one larger “super pixel”.  ▪ Small binning (4 x 4 pixels/super pixel) – Lower sensitivity, higher resolution ▪ Medium binning (8x8 pixels/super pixel) ▪ Large binning (16x16 pixels/super pixel) – Higher sensitivity, lower resolution. ▪ F/Stop – Controls the amount of light the CCD detector receives. Changing the F-Stop, for example from F/1 to F/2, decreases counts by a factor of four. F1 – The lens aperture is wide open for maximum light collection (the default for luminescent imaging). F/8 – The smallest aperture opening. This setting provides the best resolution (default for photograph).	See imaging protocols for Revvity *in vivo* imaging reagents such as ProSense® 680. Also see: Page 36 for an overview of image acquisition. *Image Acquisition on page 83*. IVIS Acquisition Control Panel on page 373.

Table 5.1 Example Imaging Workflow (continued)

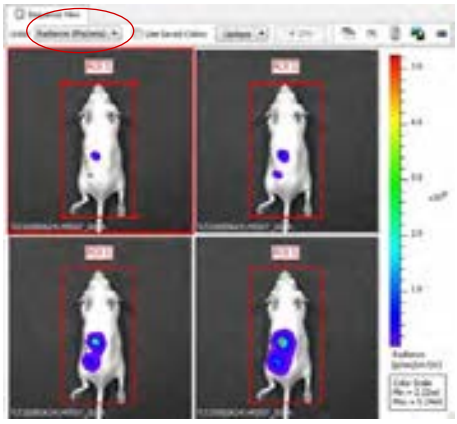
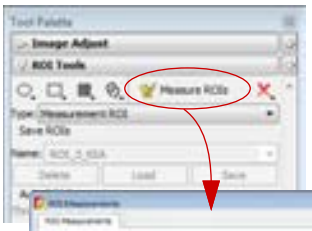
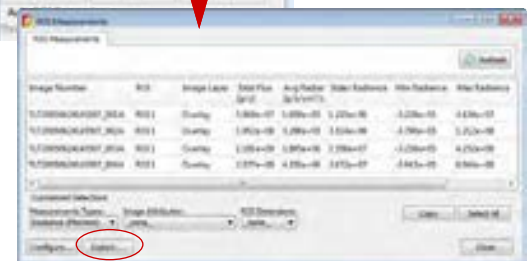
Workflow Step	For More Details:
4. Select images for viewing. ▪ Load the images as a group (select the images in the Living Image Browser and click Load as Group).  ▪ If necessary, adjust the photo brightness, contrast, or opacity using the Image Adjust tools. ▪ Apply the same color scale to all images: ▪ Uncheck the “Individual” option. ▪ Adjust the color scale Min and Max. The changes are simultaneously applied to all images.	See page 123 for more about the Living Image Browser. See page 132 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 on camera settings) and cannot be used to compare signals in different images. ▪ 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 *Concept Tech Note 2 – Image Display and Measurement* for more information on measurement units (select **Help → Tech Notes)**. See page 168 for instructions on measuring signal in optical images.

Table 5.1 Example Imaging Workflow (continued)

Workflow Step	For More Details:
 ▪ View the ROI measurements (click Measure ROIs in the ROI tools).  ▪ Click **Export** to save the ROI measurement data (.txt or .csv) for further analysis in a spreadsheet application.	See Table 13.8 on page 191 for information on ROI measurements.

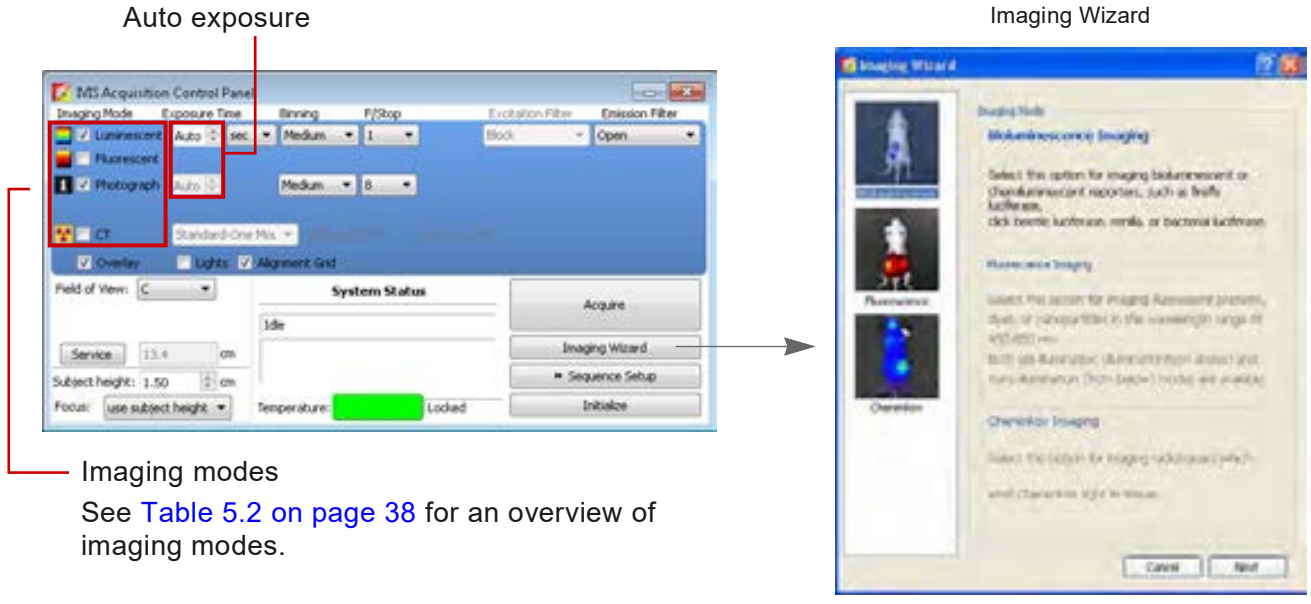
5.2 Overview of Image Acquisition

Control Panel

The control panel provides the image acquisition functions (Figure 5.1). See IVIS Acquisition Control Panel on page 373 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).

Figure 5.1 IVIS Acquisition Control Panel and Imaging Wizard



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 5.1), 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 427 for more details.

Imaging Wizard

The Imaging Wizard provides a convenient option for image or sequence setup (see Figure 11.29 on page 108). 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 11.6 on page 109 shows the types of images or sequences that the Imaging Wizard can set up.

Imaging Modes on IVIS Spectrum CT

Optical imaging detects photons in the visible range of the electromagnetic spectrum. Table 5.2 briefly explains the types of images that can be acquired on the IVIS Spectrum CT.

Table 5.2 Imaging Modes – IVIS Spectrum CT

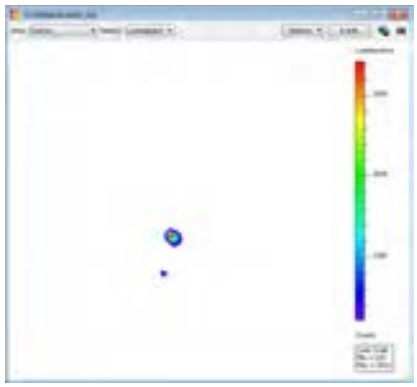
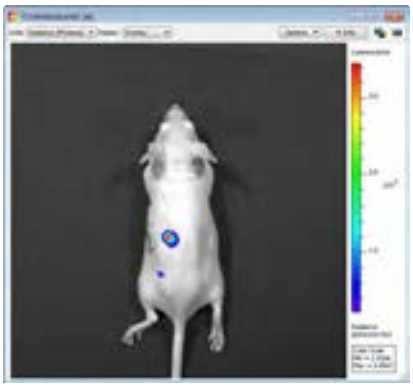
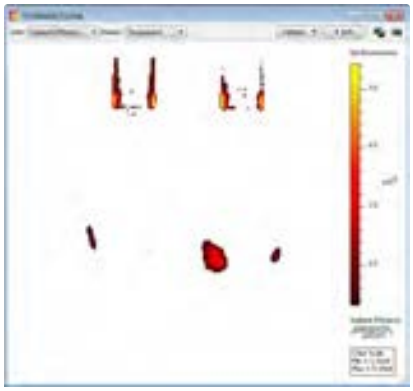
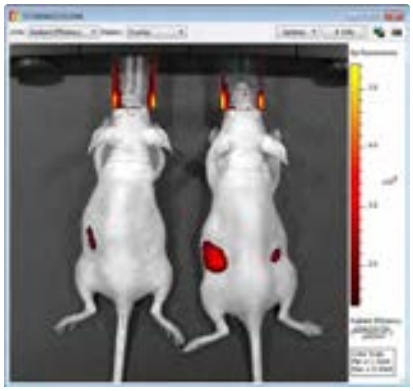
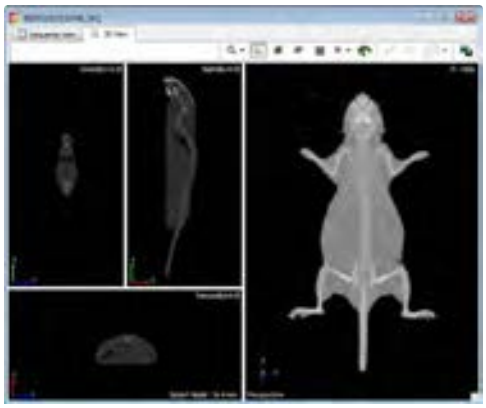
Imaging Mode	Description and Example	
 – Luminescent Luminescent optical imaging detects photons in the visible range of the electromagnetic spectrum	 ▪ Luminescent image – A longer exposure of the subject taken in darkness to capture low level luminescence emission from the surface of the subject. The optical luminescent image data is displayed in pseudocolor that represents intensity.	 ▪ Overlay: Luminescent image on photograph
 – Fluorescent Fluorescent optical imaging detects photons in the visible range of the electromagnetic spectrum	 ▪ Fluorescent image – An exposure of the subject illuminated by filtered light. The light source is located above the imaging stage (epi-illumination). The target fluorophore emission is captured and focused on the CCD camera. ▪ The optical fluorescent image data can be displayed in units of counts or photons (absolute, calibrated), or in terms of efficiency (calibrated, normalized). **Note:** See the concept tech note *Image Display and Measurement* for more on quantifying image data (select **Help** → **Tech Notes** on the menu bar).	 ▪ Overlay: Fluorescent image on photograph

Table 5.2 Imaging Modes – IVIS Spectrum CT (continued)

Imaging Mode	Description and Example
– CT imaging	 CT volume (classified) A series of radiographic exposures of the subject (acquired using the X-ray energy source on the IVIS Spectrum CT) analyzed by computed tomography (CT) to generate a 3D volume.
– Photograph	 A short exposure of the subject illuminated by the lights located in the ceiling of the imaging chamber. The photographic image is displayed as a grayscale image.

5.3 Overview of Living Image Tools and Functions

Living Image tools are organized in the Tool Palette or under "Tools" in the menu bar (Figure 5.2). 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 5.2). Docking can also be set in the general preferences (see Table D.1 on page 424).

Figure 5.2 Living Image Tools are Located in the Menu Bar and Tool Palette

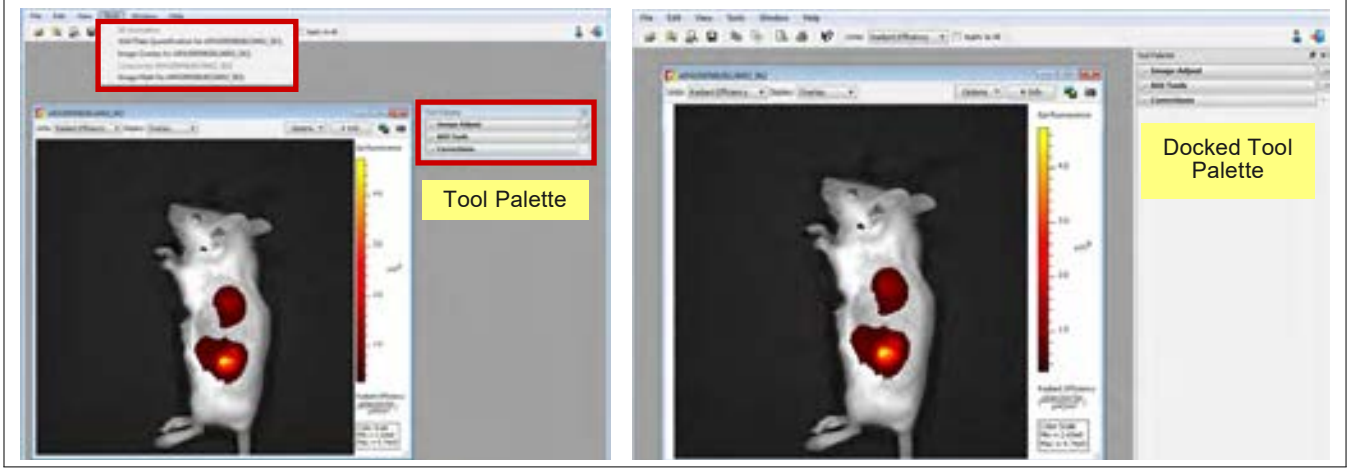


Table 5.3 provides an overview of the tools available for data acquired on the IVIS Spectrum CT.

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

Table 5.3 Living Image Tools Available for Data Acquired on the IVIS Spectrum CT

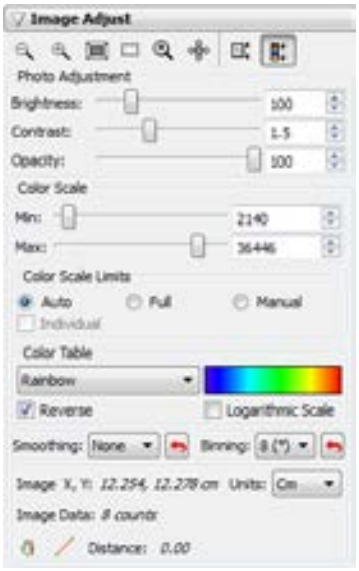
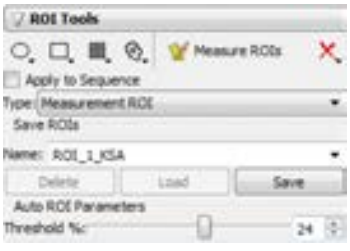
Living Image Tools and Functions	See Page
 **Image Adjust** ▪ Tools for image display (zoom, crop, or pan). ▪ Adjust photo display – Tune the photograph brightness, contrast, or opacity. ▪ Manage the color table for image display. ▪ Apply smoothing or binning to an image. ▪ View optical image data (counts or radiance) at an X,Y location. ▪ Measure distance in an image. ▪ View a line plot of pixel intensities.	123
 **ROI Tools for 2D Image Data** Specify a region of interest (ROI) in an optical image and measure the signal intensity within the ROI.	170

Table 5.3 Living Image Tools Available for Data Acquired on the IVIS Spectrum CT (continued)

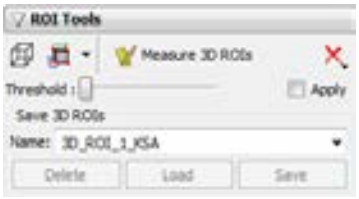
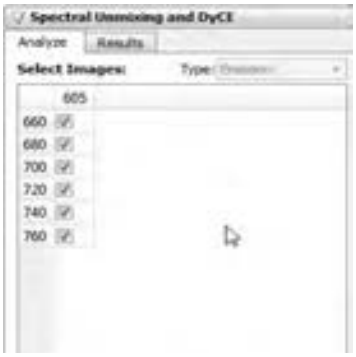
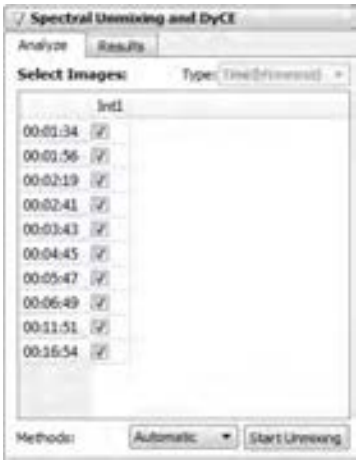
Living Image Tools and Functions	See Page
 **Corrections (2D Image Data)** Corrections for raw data.	409
 **3D ROI Tools (3D Reconstructions or 3D Volumetric Data)** Specify a 3-dimensional region of interest (3D ROI). Measure within the 3D ROI: ▪ Signal intensity of source voxels. ▪ Tissue density in a CT scan or 3D DICOM data.	286
 **Spectral Unmixing** Use spectral unmixing to: ▪ Extract the signal of one or more fluorophores from the tissue autofluorescence. ▪ Analyze luminescent or fluorescent images when more than one reporter is used in the same animal model.	321
 **DyCE (Dynamic Contrast Enhancement)** Use DyCE to: ▪ Determine real-time pharmacokinetic (spatio-temporal biodistribution) of a probe or dye signal. ▪ Extract “temporal spectra” (signal intensity as a function of time) from particular anatomical regions. **Note:** DyCE acquisition and analysis tools require a separate license.	351
 **Surface Topography** Generate a 3D reconstruction of the animal surface (*topography*) derived from the CT 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.	418

Table 5.3 Living Image Tools Available for Data Acquired on the IVIS Spectrum CT (continued)

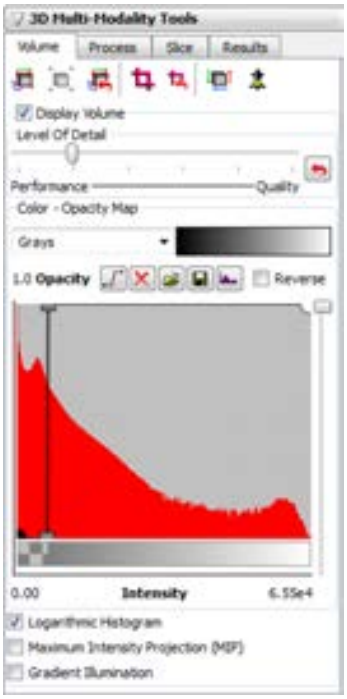
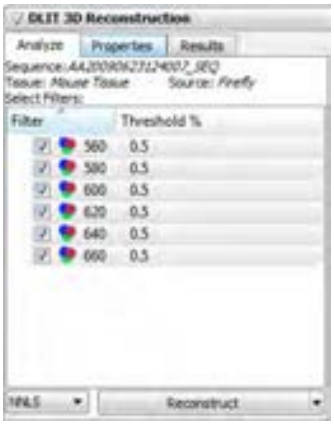
Living Image Tools and Functions	See Page
	**3D Multi-Modality Tools** 295 **Note:** The 3D Multi-Modality tools require a separate license. **Volume Tools** ▪ Set color and opacity values for different intensity ranges of a CT volume so that the color-opacity map shows the volume regions of interest (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. ▪ View slice. ▪ Slice viewing options.
	**3D Optical Tools** Surface tools – Adjust the appearance of the reconstructed animal surface and the photon density maps. 197 Source tools – Adjust the appearance of reconstructed sources, make source measurements, export voxel measurements. 256 Registration tools – Display organs on the reconstructed surface, adjust the location or scale of organs on the surface, import an organ atlas. 268
	**DLIT 3D Reconstruction** 197 *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 *voxels*. If a luminescent calibration database is available, the number of cells per source can be determined in addition to source intensity (photons/ sec).

Table 5.3 Living Image Tools Available for Data Acquired on the IVIS Spectrum CT (continued)

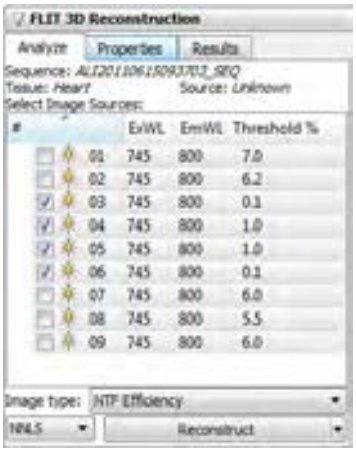
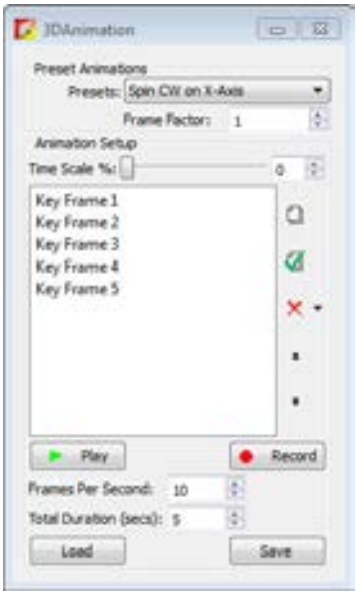
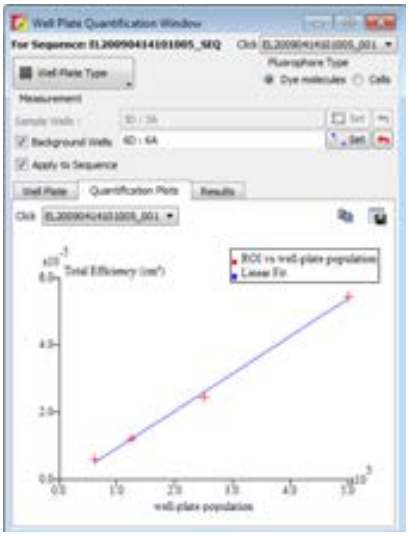
Living Image Tools and Functions	See Page
	**FLIT 3D Reconstruction** 255 *Fluorescent imaging tomography* (FLIT) analysis provides a complete 3D reconstruction of the fluorescent source distribution within the subject. The 3D reconstruction is presented as volume elements called *voxels*. If a fluorescent calibration database is available, the number of fluorophore molecules or cells per source can be determined in addition to the total fluorescence yield.
	**3D Animation Tools** 273 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.
	**Well Plate Quantification** 411 Select **Tools → Well Plate Quantification for <sequence name>** on the menu bar. 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 number of cells in a DLIT source or the number of dye molecules or cells in a FLIT source.

Table 5.3 Living Image Tools Available for Data Acquired on the IVIS Spectrum CT (continued)

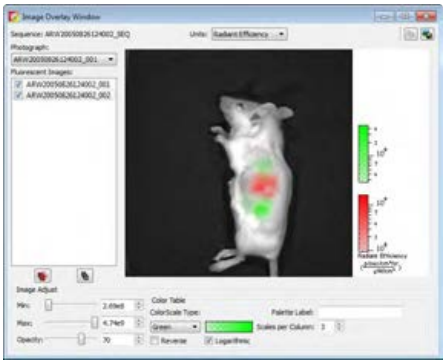
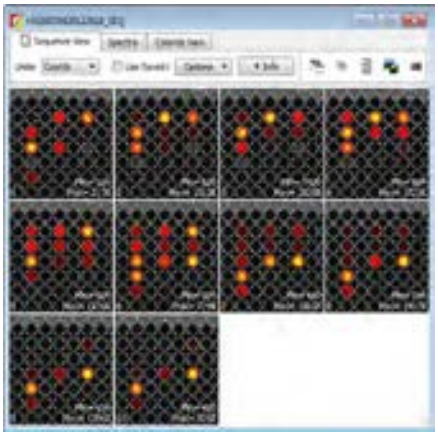
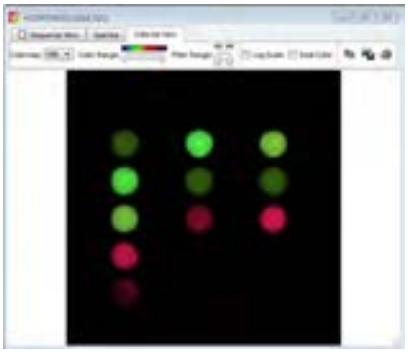
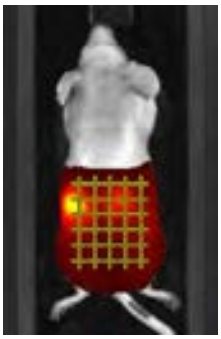
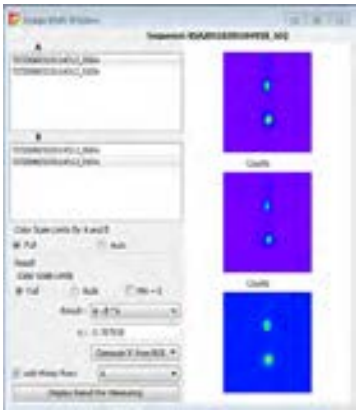
Living Image Tools and Functions	See Page
 **Image Overlay Window** Select **Tools → Image Overlay for <sequence name>** on the menu bar. View multiple fluorescent or luminescent signals in one 2-dimensional image in the Image Overlay window.	123
Colorize View Select **Tools → Colorize for <sequence 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.  Images of Quantum dot nanocrystals (700 or 800 nm) were acquired using different combinations of excitation and emission filters.  Colorize view of the combined images	152
 **Transillumination Overview** Select **Tools → 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.	104

Table 5.3 Living Image Tools Available for Data Acquired on the IVIS Spectrum CT (continued)

Living Image Tools and Functions	See Page
 **Image Math Window** Select **Tools → Image Math for <sequence name>** on the menu bar. Mathematically combine (add, multiply, subtract, or divide) two user-selected images. For example, subtract a blue-shifted background filter image from the primary excitation filter image to remove tissue autofluorescence signal.	144

6 Specifications

6.1 Electrical Power Requirements

Table 6.1 Electrical power requirements

Requirement	Specification
Rated Voltage	100 to 120VAC 50/60Hz 12A or 200 to 240VAC 50/60Hz 6A (Overvoltage Category II for 2500V max transient)
Allowable supply voltage fluctuation	90V to 264VAC
Recommended dedicated circuit	20 A for 100 to 120VAC or 10 A for 200 to 240VAC For more details, *Power Considerations on page 17*.

6.2 CCD Camera (Optical Imaging)

Table 6.2 CCD camera specifications

Item	Description
Sensor Type	Back illuminated
CCD Format	2048 x 2048 pixels
CCD Size	26 x 26 mm
Effective CCD Format	1920 x 1920 pixels
Pixel Dimensions	13.5 x 13.5 µm
Quantum Efficiency	~90% 400-700nm >50% 350-900nm
Readout Noise	<5 electrons RMS

Table 6.2 CCD camera specifications

Item	Description
Dark Current	<100 electrons/s/cm^2 (-90°C)
Minimum Detectable Luminance	<70 photons/s/sr/cm^2
CCD Temperature	Nominal -90°C

6.3 Optics

Table 6.3 Optics specifications

Item	Description
Lens f/stop	f/1 – f/8
Field of view (FOV)	4, 6.5, 13, 19.5, 22.5 cm
Resolution	>60 µm (FOV = 3.9, f/1)

6.4 X-ray Source

The X-ray source is an Oxford Instruments Series 5000 tube with a 0.005 inch thick beryllium window and a tungsten target.

Table 6.4 X-ray source specifications

Item	Description
High voltage	50kV
Max anode current	1mA
Max anode power	50W

6.5 X-ray Detector

Table 6.5 X-ray detector specifications

Item	Description
Sensitive area	229.8 x 64.6 mm
Resolution	3072 x 864
Pixel binning modes	1x1, 2x2, 4x4 pixels
Mean dark current (high saturation mode)	<17 electrons/pixel/second at 40°C
Mean dark current (high sensitivity mode)	<17 electrons/pixel/second at 40°C
MTF	>40% at 3 Lp/mm: >10% at 6 Lp/mm

Table 6.5 X-ray detector specifications

Item	Description
System noise	7.5 analog to digital units
Linearity (high sensitivity mode)	± 4%
Linearity (high saturation mode)	± 3%

6.6 Fluorescent Imaging Components

Table 6.6 Fluorescent imaging components specifications

Item	Description
Excitation filters	12 positions, 25 mm diameter, 10 (ND2) filters supplied standard
Emission filters	22 positions, 60 mm diameter, 18 filters supplied standard
Background (autofluorescence and leakage)	1 part in 107 typical
Lamp	150W quartz halogen 21VDC 3250 Kelvin

6.7 Environmental Temperature and Humidity

Table 6.7 Environmental temperature and humidity specifications

Item	Description
Temperature	18 - 24°C (65-75 °F)
Humidity	0-80% non-condensing
Type of use	Indoor (Pollution Decree 2)
Sound level	61 dB < 500 Hz
Stage temperature	Ambient to 40°C
Altitude rating	<2000 meters (6560 ft.)

6.8 Weight and Dimensions

Table 6.8 Weight and dimension specifications

Item	Description
Weight	334 kg (735 lbs)
Depth	77 cm (30 in.)
Width	65 cm (25.5 in.)
Height	206 cm (77 in.)
Imaging chamber internal dimensions	51 x 51 x 66 cm (D x W x H)

7 Instrument Components

The IVIS Spectrum CT includes the following components:

- Scientific imaging charged coupled device (CCD) camera
- Imaging chamber with specimen warming system
- Thermoelectric camera cooling system
- A pattern illuminator for animal positioning
- System of lenses and filters for bioluminescent image acquisition
- Photographic illumination system
- Integrated fluorescence system for fluorescent image acquisition
- An X-ray generating source, X-ray detector, and rotating animal stage for CT imaging

These components are integrated into one freestanding instrument that is equipped with casters (Figure 7.1).

Figure 7.1 IVIS Spectrum CT Imaging System

A pre-configured Windows-based computer enables you to control the system as well as acquire and analyze images.

IMPORTANT: If you modify the IVIS Imaging System in any way, without prior approval from Revvity, all warranties that cover this product are void. In addition, the computer included with the IVIS Spectrum CT is specifically configured to run all system-related applications. Any modification of existing software or hardware voids all warranties.

If you have any questions, please contact Revvity technical support (see page 11).

7.1 CCD Camera for Optical Imaging

The camera is a scientific grade, thermoelectrically-cooled, back-thinned, back-illuminated, large format CCD manufactured for Revvity. The camera uses a thermoelectric water chiller. Two flexible plastic water lines connect the CCD camera to the thermoelectric chiller unit.

The CCD camera has a heated, vacuum sealing window to prevent condensation that can obscure the image or damage other components.

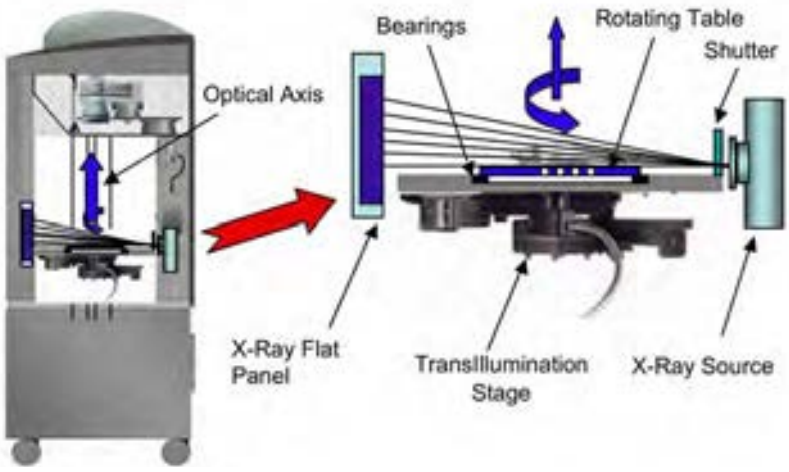
The camera power supply is housed in the base of the instrument and maintains the camera operating temperature when the computer is turned off. It has a 16-bit digitized, low-noise electronic readout for extremely low background images.

7.2 X-ray Source, Detector, and Filters

During CT Imaging, the animal stage moves to the lower compartment of the imaging chamber. The animal stage rotates while X-rays are generated from the X-ray source located at the rear of the IVIS Spectrum CT (Figure 7.2).

A series of images are collected by the detector located at the front of the instrument. During the brief period when the X-ray tube is warming up to its maximum voltage and current, a blocking filter is positioned so that the animal does not receive radiation prior to imaging. Image acquisition begins when the X-ray tube is at full power and a series of images is collected.

Figure 7.2 X-ray source and detector



7.3 X-ray System Control Panel

The front panel located to the right of the imaging chamber door has two switches and two indicator lights that are associated with the instrument X-ray function. The main ON/OFF switch that controls the electrical power to the instrument is on the rear of the instrument. Activating this switch provides power to the instrument, but does not permit the X-ray source to be energized unless the following conditions are met:

1. The imaging chamber door is completely closed and locked by the solenoid lock.
2. All interlocks are closed and panels mounted.
3. The Emergency Stop switch is in the ON (out) position. See the note below.
4. The key selector switch is turned ON.
5. The amber switch has been pushed and the light is ON, indicating that all safety interlocks are functioning properly.

The X-ray source cannot be energized from the Living Image software until these conditions have been fulfilled.

NOTE: The Emergency Stop switch is not intended as a main X-ray source control and should not be used to turn the X-ray function ON or OFF on a routine basis. It should only be used in the unlikely situation where the X-ray source must be immediately turned off. Under normal circumstances, it should be left in the ON position.

7.4 Key Selector Switch and Lost Keys

X-ray safety regulations require controlled access to the instrument. The objective of this requirement is to prevent untrained and unauthorized personnel from operating the X-ray functionality of the instrument. The key-operated switch on the instrument fulfills this requirement when used in conjunction with the user's own written radiation safety procedures. The switch is designed so that the key can only be removed in the OFF position. When an authorized user is finished using the instrument, the key should be removed from the switch.

Two keys are provided with the instrument. A "master key" person usually manages the keys. It is a good practice to store the spare key in a safe location. If the keys are lost, contact Revvity technical support (see page 11).

7.5 Thermoelectric Chiller

The thermoelectric unit is a thermoelectric water chiller (Figure 7.3). Water from the thermoelectrically-cooled CCD camera is pumped to the thermoelectric chiller where the water is cooled and returned to the camera.

Figure 7.3 Thermoelectric Chiller

7.6 Imaging Chamber

The imaging chamber is a highly specialized device consisting of the imaging chamber housing, moveable platform with a rotating CT imaging stage, a lens system with f/stop control, and synchronized filter wheels that control the spectral content of the luminescent or fluorescent images (Figure 7.4).

Figure 7.4 Imaging Chamber

An LED-based illumination system provides the means to acquire photographic information before imaging. The imaging chamber is equipped with gas anesthesia inlet and outlet ports for use with the optional Revvity RAS-4 Rodent Anesthesia System.

The imaging chamber is light tight so that no light penetrates from the outside after the door is locked. The solenoid latch ensures that the door cannot be inadvertently opened during an imaging session.

The interior of the imaging chamber is constructed from non-phosphorescent and non-fluorescent materials to prevent internal light contamination that could compromise sample measurements. The chamber and door are made from steel of sufficient thickness to prevent radiation leakage.

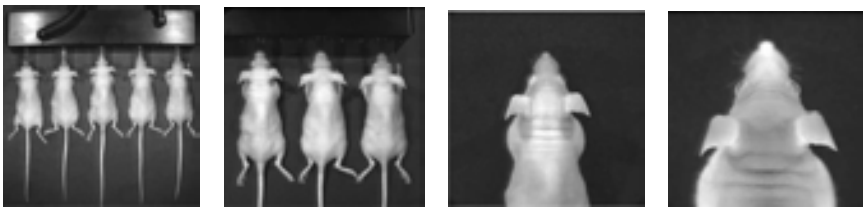
WARNING! Under no circumstances should you attempt to make any mechanical modifications to the imaging chamber, the door, or the interlock switches.

7.7 Optical Components

Imaging System/Lenses

The instrument is equipped with a rotating lens carousel that provides demagnification of 1.5X, 2.5X, 5X, and 8.8X power (Figure 7.6).The filter wheel settings are selected in the Living Image® software.

Figure 7.5 Field of View Examples



Power:	8.8X	5.0X	2.5X	1.5X
FOV:	22.5	13 cm	6.5 cm	3.9 cm
Position:	cm	C	B	A

The automatic shutter and f/stop iris is integrated into the lens system. A window at the top of the imaging chamber protects the lenses and other optical components in the lens compartment from contamination. For more details on the care of this window, see *Cleaning the Lens Protection Window*, page 382.

CAUTION: Do not touch the glass window or permit specimens to come in contact with the window, otherwise image quality may be impaired. If the window is struck by a hard object, it may crack or shatter.

Emission and Excitation Filters

There are two 11-position filter wheels. Filter settings are selected within the Living Image software. For more details on how to access the filter wheels and change filters, see page 382.

For fluorescent studies, the IVIS Spectrum CT Imaging System can illuminate a specimen from the top of the imaging chamber (*epi-illumination)* or from the bottom of the stage (*transillumination*). A 12-position excitation filter wheel module is located in the back of the instrument (not accessible to users). If the filters must be changed or the module requires service, contact Revvity technical support (see page 11). For more details on fluorescent imaging, see page 67.

Figure 7.6 Filter Wheel

7.8 Specimen Warming System

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

The actual surface temperature of the padded, rotating animal stage may be lower than that of the imaging platform.

The imaging platform does not have active cooling. The platform may require up to 20 minutes to passively cool from 37°C to ambient temperature.

7.9 Rotating Stage and Anesthesia Connections

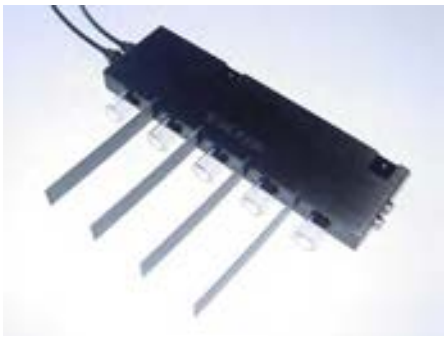
Table 7.1 lists the types of anesthesia connections (manifolds) that are available. Each manifold is equipped with its own gas and exhaust tubes. The ends of the tubes have gendered fittings so that the gas and exhaust tubes cannot be connected incorrectly.

IMPORTANT: Disconnect the manifolds at the gas block so that the gas tube is not inadvertently connected to the exhaust port. Animal death is the likely outcome if the mouse nose cone is connected to the vacuum exhaust.

Table 7.1 Anesthesia Manifolds

Type	Description	For Use With These Imaging Modalities
Single Mouse Manifold	 One anesthesia nose cone is built into a stage which is placed on the imaging platform. Stage Bed (reduces X-ray scattering)	CT Luminescent Fluorescent with epi-illumination Fluorescent with transillumination
Dual Mouse Manifold	 Two anesthesia nose cones are built into a stage which is placed on the imaging platform.  Bed (reduces X-ray scattering) Light baffle (prevents light from one mouse shining on the other)	CT Luminescent Fluorescent with epi-illumination

Table 7.1 Anesthesia Manifolds (continued)

Type	Description	For Use With These Imaging Modalities
Five Mouse Manifold	 Animals are placed directly onto the imaging platform when using the Five Mouse Manifold.	Luminescent Fluorescent with epi-illumination

The IVIS SpectrumCT also accommodates the Mouse Imaging Shuttle (MIS, Revvity part no. 127744). The MIS can be used in CT imaging as well as luminescent or fluorescent (epi-illumination and transillumination) imaging. However, degradation of CT images can be expected due to the plastic walls of the MIS.

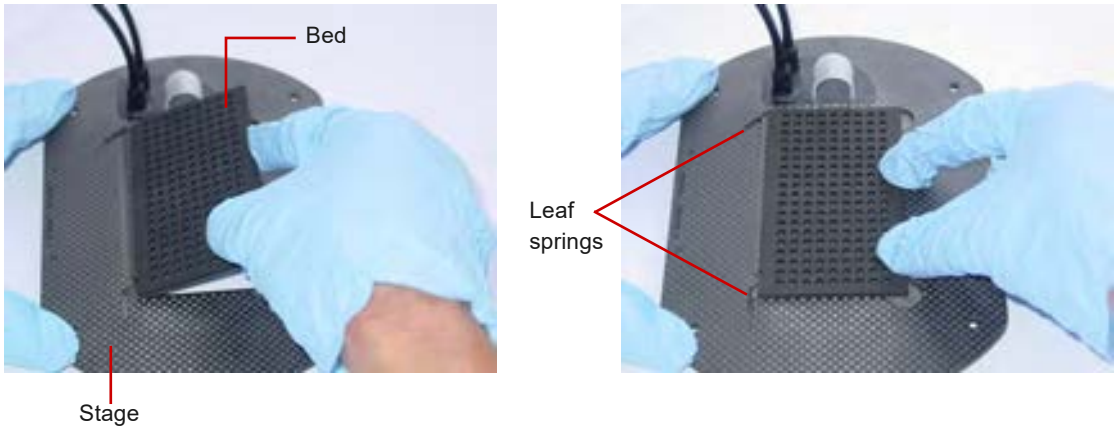
CAUTION: Do not use the Mouse Imaging Shuttle with FOV A. The height of the shuttle could break the glass window at the top of the imaging chamber.

Single Mouse Manifold

The Single Mouse Manifold can be used with all imaging modalities. The manifold is integrated with a stage containing a low density composite foam and carbon fiber bed that reduces X-ray scatter during CT imaging.

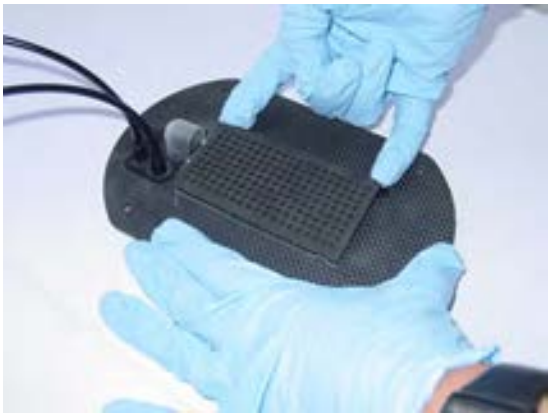
The bed has 171 holes which enable trans-illuminated fluorescence imaging. The bed is a delicate part and should be handled carefully. Figure 7.7 shows how to install the bed into the animal stage.

Figure 7.7 Installing the Bed in the Single Mouse Manifold/Stage



Gently push the left edge of the bed against the edge of the stage cutout, then snap the bed down into place. The plastic leaf springs on the left side of the cutout will secure the bed.

Figure 7.8 Removing the Bed from the Single Mouse Manifold/ Stage



Gently lift up at the corners to remove the bed.

Gas Connections

The distal ends of the gas tubes from the animal stage have gendered Luer fittings which prevent incorrectly connecting the tubes to the GAS and EXHAUST connections on the imaging platform (Figure 7.9).

IMPORTANT: Disconnect the GAS and EXHAUST connections at the outlet port, not at the animal bed.

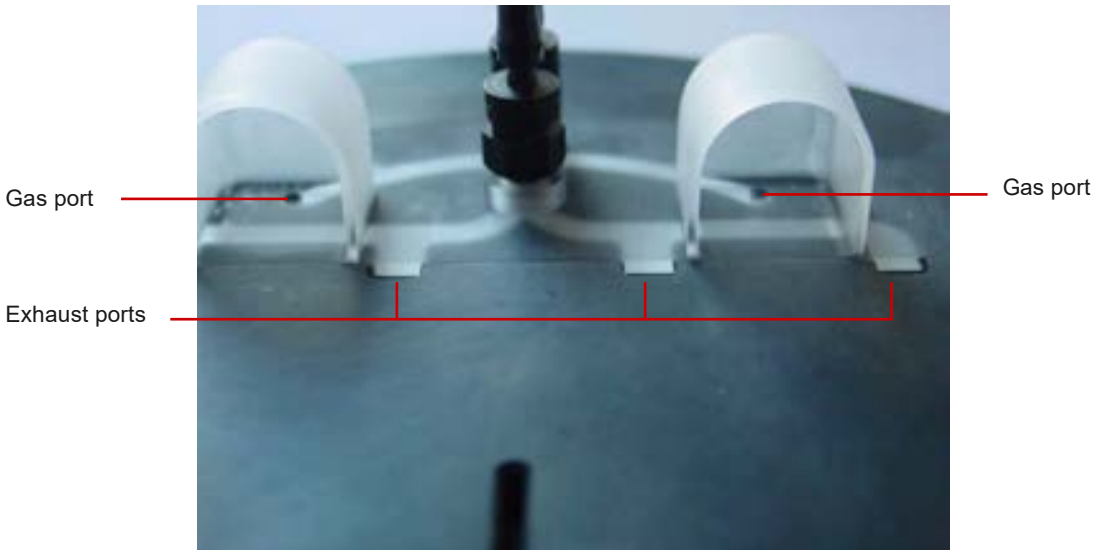
Figure 7.9 Gas and Exhaust Luer Fittings

Cleaning the Anesthesia Manifolds and Stages

NOTE: Do not allow fluids to get into the gas port and, especially, the exhaust port of the stage. Do not allow fluids to get into the interior of the manifold. It is recommended that you cover the ports with masking tape before cleaning or disinfecting the components. If fluid does enter the passages, try blowing it out.

Figure 7.10 Dual Mouse Manifold — Gas and Exhaust Ports

There are two gas ports and four exhaust ports.



To clean the anesthesia manifold and stage:

1. Wipe the components with a cloth dampened with warm detergent.
2. Wipe the components with a cloth dampened with water.
3. To disinfect the manifold, spray with a 5% solution of bleach and distilled water. Do not allow fluids to enter the interior manifold passages.

NOTE: Beds and light baffles are not considered cleanable and should be replaced periodically.

7.10 X-ray Door and Panel Interlocks

A safety interlock prevents unintentional opening of the imaging chamber door (for more details, see Chapter 4, page 29). A solenoid interlock serves two functions, it: 1) acts as a switch which completes the electronic logic so that image acquisition can begin, and 2) mechanically locks the door during imaging so that it cannot be accidentally opened.

These safety interlocks protect the user from X-ray exposure during CT imaging and protect the camera from overexposure to light during optical imaging.

A redundant switch with a removable copper rod cuts all power to the X-ray source should the door be opened and a solenoid switch failure occurs in the electrically ON state.

There are several other interlocks that protect the user from X-ray exposure when one or more of the electronic access panels are removed. The filter wheel access panel is interlocked. The interlock switch will break the X-ray power circuit if the user removes this panel for filter replacement or when using the emergency imaging chamber access procedure described in

Chapter 22, page 393. Two safety interlock switches protect the electronics bay on the right side of the instrument and the upper rear panel which encloses the X-ray source. These panels should only be removed by a Revvity service technician.

7.11 Base Enclosure

IMPORTANT: Do not block the ventilation openings on the base of the IVIS Spectrum CT or position the instrument too close to a wall so that the exhaust flow is obstructed.

The base is equipped with four casters that enable you to move the instrument (Figure 7.11). For more details on how to move the instrument, see page 386.

Figure 7.11 Instrument base with casters

7.12 Acquisition Computer

The IVIS Spectrum CT Imaging System includes a Windows-based computer. The Living Image® software and Microsoft® Office software are installed on the acquisition computer. The Living Image software controls the IVIS Spectrum CT, and displays and analyzes the image data. A 24-inch monitor and a 10/100 Ethernet network adapter card are also included with the imaging system. A printer may be attached to the computer.

7.13 High Reflectance Hemisphere

The optional High Reflectance Hemisphere is used to check for light contamination in the imaging chamber (Figure 7.12). The hemisphere does not emit any photons when it is imaged in a light-tight and contaminant-free imaging chamber. As a result, it is not visible in a luminescent image.

If a light leak or an internal phosphorescent contaminant is present in the imaging chamber, the hemisphere reflects that light and is visible in a luminescent image. If you suspect light contamination in the imaging chamber, see "*Internal light contamination*" in Chapter 22, page 390. A light leak test is also a useful check for radiation leakage. If light can get into the imaging chamber, it is possible that X-rays could escape.

Figure 7.12 High Reflectance Hemisphere

8 Operating the Instrument

The IVIS Spectrum CT is intended for use in bioluminescent, fluorescent, and CT imaging procedures. The system is designed to detect extremely low-level light emissions that are orders of magnitude dimmer than can be detected by the naked eye. The IVIS Spectrum CT allows you to monitor and record cellular and genetic activity within a living organism, in real time. The imaging system captures, quantifies, and images the light emitted by a sample. Additionally, the computed tomography (CT) modality allows structural imaging of the subject.

8.1 Starting the IVIS Spectrum CT

All components of the IVIS Spectrum CT should be left on unless the system will not be used for more than 30 days. 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 camera operation.

Figure 8.1and Table 8.1 show the main instrument controls and indicators.

Figure 8.1 Main instrument controls and indicators

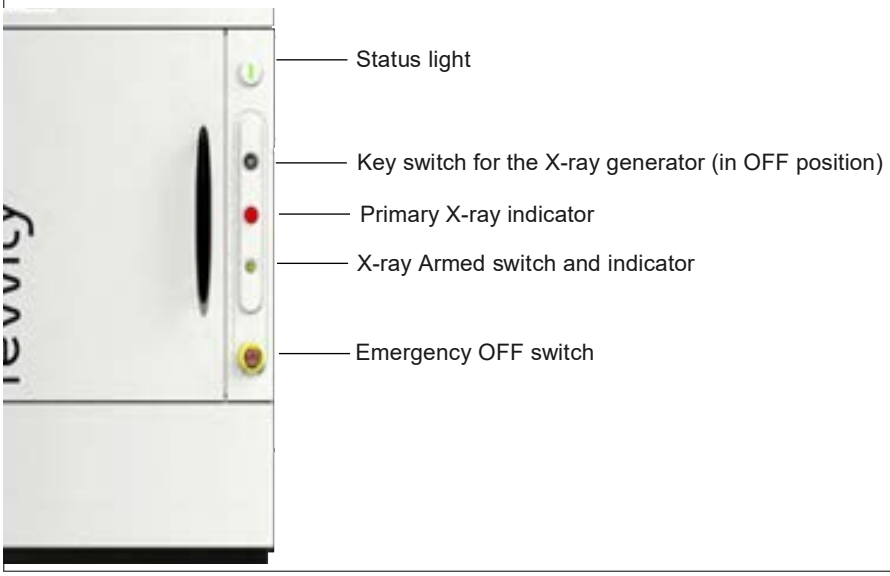


Table 8.1 Status lights and indicators

Item	Location	Description
 Status light	Front panel of the instrument.	Indicates the general status of the IVIS Spectrum CT. Green – The imaging system is ready. Red – The imaging system is not ready.
 X-ray key switch	Front panel of the instrument.	A key-actuated control which ensures that X-rays can only be generated when the key is in the ON (I) position.
 Primary X-ray indicator	Front panel of the instrument.	A red warning light that is illuminated when X-rays are being generated.
 X-ray Armed button	Front panel of the instrument.	This button must be pushed in to enable X-ray generation.
 X-ray lightstack	Under the top dome of the instrument. Only the light is visible, not the lightstack.	A red light which illuminates when X-rays are being generated and is visible from 360 degrees.
	Living Image Acquisition Control Panel	This icon appears in the IVIS Acquisition Control Panel when the instrument generates X-rays.

To start the IVIS Spectrum CT:

1. Turn on the instrument main power switch on the rear of the instrument.
2. Turn the key switch on the front instrument to the ON position.
3. Confirm that the EMERGENCY STOP switch is in the READY (out) position. If necessary, turn the knob clockwise to reset it to the READY (out) position.

NOTE: Pushing in the Emergency Stop switch cuts off power to the entire instrument so all functions stop, including the camera and motor driven components.

4. Push in the X-ray Armed button.

   When the imaging chamber door is shut, X-rays can be generated from the IVIS Acquisition Control Panel within the software on the acquisition computer.

NOTE: For bioluminescent or fluorescent imaging, only the Emergency Stop switch must be in the READY (out) position.

8.2 Restarting the System After a Power Outage

The same procedure is used to restart the system after an intentional shutdown or shutdown due to a power outage.

1. Confirm that the rear panel switch is off, and the power cord is plugged into the instrument and the wall socket.
2. Turn on the main power switch on the rear panel.
3. Turn on the computer and monitor. Start the software.
4. In the Living Image® software, click **Initialize** in the control panel (Figure 8.2).

   The status light is red.

Figure 8.2 Control panel in the Living Image software

5. Wait until the camera has cooled to operating temperature (requires about 10 minutes).

   You can monitor the system temperature in the Living Image software. The status light is green when the system is ready for operation.

8.3 Gas Plumbing

WARNING! Use only isoflurane with the instrument. DO NOT USE FLAMMABLE ANESTHESIA GAS.

CAUTION: Revvity recommends using the RAS-4 Rodent Anesthesia System when imaging small animals. This system supplies a controlled amount of isoflurane to the imaging chamber and continuously reduces the build-up of isoflurane in the chamber. If you plan to use a gas other than the recommended isoflurane/oxygen gas mixture or pure air, contact Revvity technical support (see page 11)

Be careful to use only tubing and other plumbing fixtures that do not fluoresce or phosphoresce (glow) in the imaging chamber. Please contact Revvity technical support for a list of acceptable materials.

The IVIS Spectrum CT is equipped with plumbing connections for supplying isoflurane anesthetic gas to the imaging chamber and exhausting gases out. The flow of gas into the imaging chamber is controlled by the gas valve on the front panel of the RAS-4. The imaging chamber does not include a gas scavenging system. This is provided by suitable auxiliary equipment such as the RAS-4 Rodent Anesthesia System.

Gas ports are located on the rear of the system instrument and are labeled GAS IN and GAS OUT.

- GAS IN (white) – The direction of flow is into this port.
- GAS OUT (yellow) – The flow is exhausted out of this port.

The flow of gas from the rear panel GAS IN port is directed to a distribution block located on the imaging platform where it exits through the port labeled GAS.

Three mouse anesthesia manifolds are provided with the IVIS Spectrum CT. See Table 7.1 on page 56 for more details on these.

CAUTION: Use only the manifolds provided with the IVIS Spectrum CT. Using other manifolds may cause damage to the instrument. Some manifolds for other Revvity imaging systems are taller and will break the lens window if the imaging platform is moved to Field of View A.

The manifold port labeled GAS connects to the GAS port on the platform distribution block. The anesthesia manifold also has a port labeled EXH that should be connected to the EXH port on the platform distribution block. The vacuum or exhaust circuit connects to the GAS OUT port on the rear of the system instrument. No gases are exhausted from the imaging chamber unless gas scavenging equipment such as the Revvity RAS-4 Rodent Anesthesia System is used.

8.4 Door Operation and Interlocks

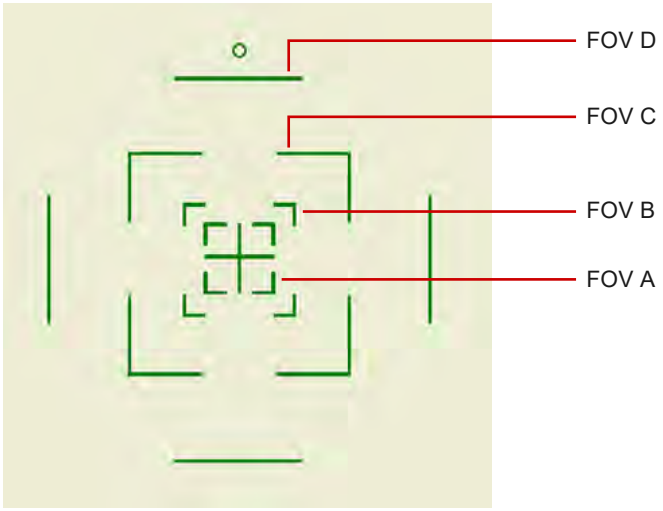
WARNING! Never try to defeat the door interlock or force the door open during image acquisition when the status light is red or X-rays are being generated. Such action could expose the user to a severe pinch hazard from the rapidly moving imaging platform or dangerous levels of X-rays.

After the system has been initialized, the door of the imaging chamber cannot be opened when the system status light is red. The door is equipped with a solenoid lock to prevent accidental opening during an imaging session. The solenoid lock serves two purposes: 1) locks the door during imaging including X-ray operation, and 2) acts as a primary interlock switch for X-ray operation. A second redundant interlock prevents X-ray generation when the door is opened or ajar. This redundant interlock operates by removing part of the X-ray circuit when the door is open. The copper rod attached to the door is the part that completes the circuit.

8.5 Centering Subjects in the Field of View

A green illuminated pattern is projected onto the imaging platform when the instrument door is opened. The pattern of nested lines represents the field of view (FOV) A, B, C and D. It provides a helpful guide for centering subjects in the field of view.

Figure 8.3 Pattern projected onto the imaging platform



8.6 Optical Imaging Basics

The instrument has both bioluminescent and fluorescent imaging capabilities. Optical imaging measures the light emitted by light-producing luminescent or fluorescent reporters such as luciferase or fluorescent proteins.

In the luminescent imaging mode, no excitation or emission filters are used. Selecting the "Auto" Exposure option in the control panel is recommended in most cases.

To change from luminescent to fluorescent operation, select fluorescent mode in the IVIS Acquisition Control Panel and select an appropriate excitation and emission filter (Figure 8.4).

Figure 8.4 Control panel in the Living Image® software

Luminescent imaging mode

Fluorescent imaging mode

Fluorescent Imaging

A 150 Watt quartz tungsten halogen lamp with a dichroic reflector provides the fluorescence excitation light. The Living Image® software controls the lamp intensity level. The fluorescence light source is a module located behind the rear panel and is not user serviceable.

The lamp output is delivered to the excitation filter wheel assembly located in the rear compartment, where it is collimated and transmitted through a fluorescence excitation filter. The 12 excitation filter wheel locations provide 10 fluorescence filters. One filter slot contains a light block that is used during bioluminescent imaging to prevent external light from entering the

imaging chamber. The 12-position excitation filter wheel is motorized and controlled through the Living Image software.

Upon leaving the excitation filter, the light enters an optical switch that directs the light to either the top of the imaging chamber (epi-illumination) or to the bottom of the stage (transillumination). When epi-illumination is selected, the light is split equally to four diffusers to provide uniform stage illumination. When bottom or transillumination is selected, the excitation light is directed to one of the holes in the aperture plate located in the stage surface. This removable plate has a 9 x 19 array of 3 mm diameter holes which provide discrete illumination locations that are user-selectable.

NOTE: Only the Single Mouse manifold can be used with transillumination.

Revvity provides optional accessories for alternative illumination use with the instrument.

- Blank plate – For use with the 5 mouse manifold (compatible with epi-illuminated fluorescent imaging or luminescent imaging). The blank plate fits into a recess on the rotating turntable.
- Mouse Imaging Shuttle – Provides a means of transferring a single mouse between other imaging instruments and the IVIS Spectrum CT without disrupting the subject position. The shuttle also provides a small amount of compression to the mouse in order to enhance the optical signal levels.

Selecting Emission and Excitation Filters

The IVIS Spectrum CT includes a factory-installed filter set. The filters are mounted on a rotating filter wheels. See IVIS Acquisition Control Panel on page 373 for details.

Emission filters can be removed or replaced with filters with other spectral characteristics. For information on how to replace a filter, see *Changing the Emission Filters for Fluorescence Imaging*, page 382.

The excitation filters are housed in a filter wheel module that is not accessible to the user. Unlike the emission filters, the user cannot replace these filters. Contact Revvity technical support if an excitation filter must be replaced (see page 11).

Black Paper

Although the platform is black anodized, it is recommended that you image items on a high quality black paper, especially biological specimens. Revvity has surveyed many types of paper and recommends Swarthmore, Artagain, Black, part no. 445-109, size 8.5 inch x 11 inch (Revvity part no. 117837). This paper prevents illumination reflections and helps keep the stage clean. It can be cut with scissors to make non-luminescent background of custom shapes.

Low Fluorescence Mat

When operating the system in the fluorescent mode, use the Low Fluorescence Imaging Mat (part no. 119000, set of 10) to reduce background fluorescence. The mat is made from a low reflectance textured plastic that is easily cleaned. The plastic mat can be cut with scissors to make non-fluorescing background of custom shapes.

Glowing Materials

Always keep in mind that nearly everything glows (that is, has the potential to phosphoresce and contaminate the image). Most plastics, almost all tape, plants, paint, rodent food (mostly plants), mouse urine, and animal bedding have been found to glow.

Use caution when introducing materials into the IVIS Spectrum CT. It is advisable to prescreen all items by imaging them alone, before imaging them with samples under study. Revvity recommends using non-powdered gloves when working with IVIS Spectrum CT equipment.

8.7 CT Imaging Basics

Computed Tomography imaging is an X-ray based imaging modality that is used to generate a 3D process. During image acquisition, projection images (planar X-ray images) are acquired from a series of perspectives. For the IVIS SpectrumCT, the orientation for each projection represents the angle of the animal with respect to the center line from the X-ray source (at the back of instrument) to the X-ray detector (at the front of the instrument). This stack of project images is then reconstructed to generate a 3D image of the physical object.

The image reconstruction process assumes that the animal does not move during acquisition. As a result, the motion caused by breathing and heartbeat causes inherent degradation of the image resolution which is seen as blur.

The X-ray based imaging process can also cause artifacts. X-rays also attenuate in a variety of materials in proportion to the linear attenuation coefficient and the amount of material in the X-ray path. The linear attenuation coefficient (μ) is related to the atomic number (Z) and is often normalized to density (ρ) and then represented as the mass attenuation coefficient (μ/ρ). Therefore, materials which are large and dense can result in artifacts. Furthermore, materials with a high atomic number and/or high density (for example, stainless steel) can also create artifacts. To minimize all of these potential pitfalls:

- Stabilize the animal as much as possible.
- Image only biological tissues.
- Avoid metal, including ear tags.

Select CT mode in the IVIS Acquisition Control Panel to perform CT imaging or include CT imaging with luminescent or fluorescent imaging (Figure 8.4).

Figure 8.5 Control panel in the Living Image® software

CT imaging mode

Luminescent and CT imaging mode

8.8 Normal System Shut Down Procedure

Revvity does not recommend power cycling the instrument (turning the system components off and on) on a daily basis. Leaving the system on keeps the camera cold and the electronics systems stable. The software is preconfigured to run automatic background accumulation and

self-diagnostics overnight. The platform temperature and the computer monitor may be turned off.

If it is necessary to shut down the system for any reason, such as long term storage (more than 30 days), it is important to follow the procedure below.

1. Close the Living Image® software and save any information of interest at the prompt.
2. Turn off the computer.
3. Turn off any gas to the imaging chamber and disconnect the gas supply if necessary. If you are using the RAS-4 Rodent Anesthesia system, follow the shutdown procedure in the *RAS-4 Rodent Anesthesia System User Manual*.
4. Turn off the main circuit breaker switch located on the rear of the IVIS Spectrum CT and disconnect the power cord from the wall socket.
5. Cover the gas inlet and outlet ports with masking tape to prevent entry of dust.
6. Remove any loose objects from the imaging chamber and close the door. If you plan to move the system, see important information on page 386. If you have any problems during the shutdown procedure, please contact Revvity technical support (see page 11).

8.9 Emergency System Shutdown Procedure

In the event of an emergency where the IVIS Spectrum CT must be shut off immediately, push in the red mushroom-shaped Emergency Stop switch located on the main instrument control panel (Figure 8.6).

Figure 8.6 Emergency Stop button

To enable the system for restarting, turn the switch in a clockwise direction, and allow the knob to pop out. Restart the IVIS Spectrum CT following the procedure in *Restarting the System After a Power Outage* on page 65.

9 Getting Started

9.1 Starting Living Image Software

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

Table 9.1 shows the default software installation locations.

Table 9.1 Living Image Software Installation Locations

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

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

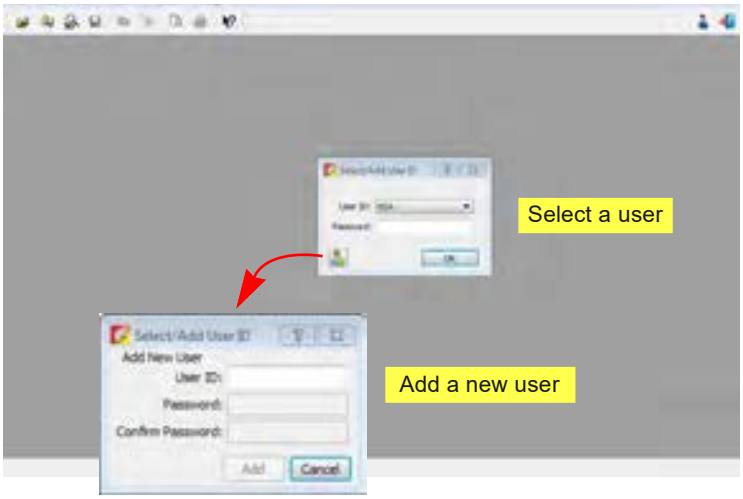
To start the software:

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

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

   The main window appears (Figure 9.1).

Figure 9.1 Living Image Main Window at Startup



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.

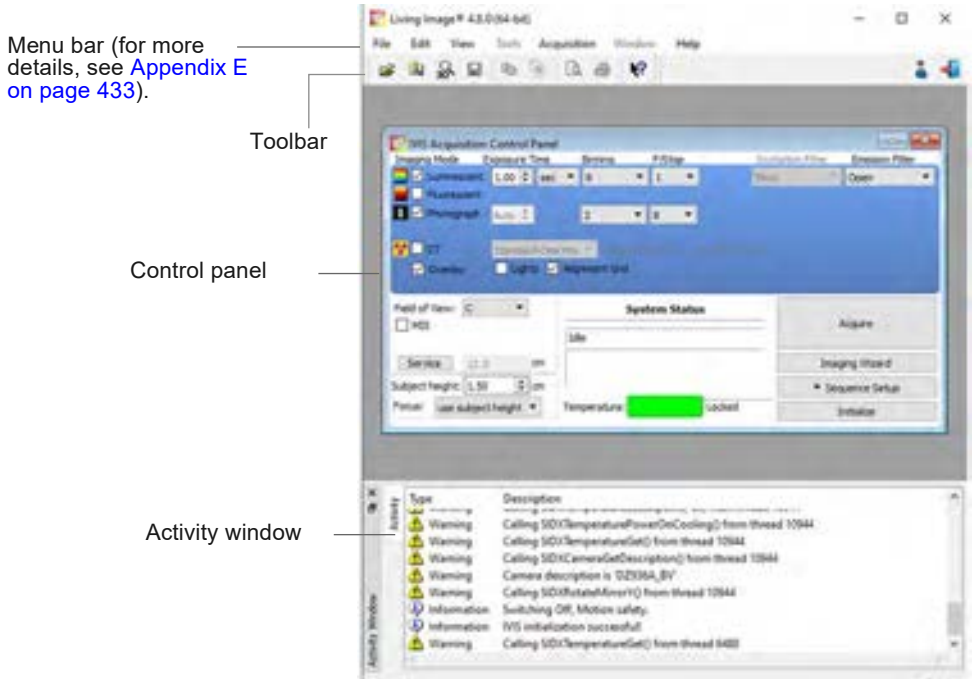
   c. Enter and confirm a password. This is optional.

   d. Click **Add** and **OK**.

   The control panel appears if the workstation controls the IVIS Spectrum CT (Figure 9.2). For more details on the control panel, see Appendix 20 on page 373.

NOTE: Living Image software has optional password protection for user accounts. See page 76 for more details.

Figure 9.2 Living Image Main Window and IVIS Acquisition Control Panel



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.

9.2 Initializing the Imaging System and Checking Temperature

The IVIS Spectrum CT 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 9.3).

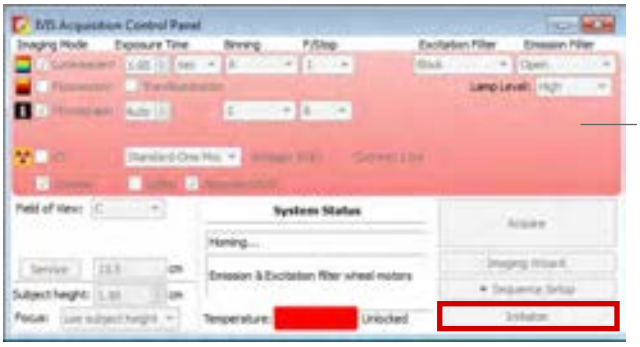
NOTE: The IVIS acquisition control panel is only available on the PC workstation that controls the IVIS Imaging System. The options available in the control panel depend on the imaging system, selected imaging mode (Image Setup or Sequence Setup), and the filter wheel or lens option that are installed

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.

Initializing the IVIS Spectrum CT

1. Start the Living Image software (double-click the icon on the desktop).
2. Click **Initialize** in the control panel that appears (Figure 9.3).
   After several seconds you will hear the instrument motors move.

Figure 9.3 IVIS® Acquisition Control Panel During Initialization



NOTE: When Living Image is launched, a windows service cooler is created and initialized. This service is auto run even if LI has been exited. The service uses the camera temperature setpoint to keep the camera at running temperature.

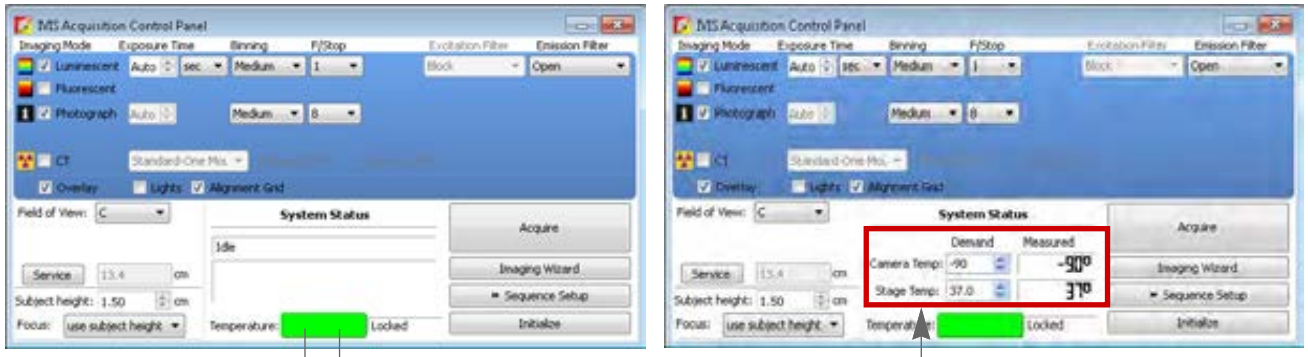
CCD Temperature

The IVIS Acquisition Control Panel indicates the temperature status of the charge coupled device (CCD) camera (see Figure 9.4 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 Revvity Technical Support (see page 11).

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 9.4 Control Panel – Instrument Temperature Status



Temperature box color:

Click the temperature box to display the demand and measured temperatures

White – System is not initialized.

Red – 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°C and is self-monitoring after the system is initialized. The imaging stage may be set to a temperature from 20 - 40°C.

The actual surface temperature of the padded, rotating animal stage may be lower than that of the imaging stage. The stage does not have active cooling. The stage may require up to 20 minutes to passively cool from 37°C to ambient temperature.

Figure 9.5 Set Stage Temperature in the Control Panel

9.3 Managing User Accounts

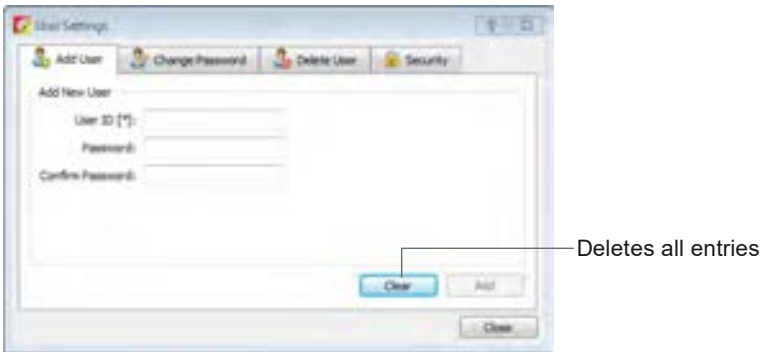
Adding Users

New users can be created in the:

- Main window at startup (see page 72).
- User Settings dialog box (Figure 9.6).

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

Figure 9.6 User Settings – Add User



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

Changing or Adding Passwords

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

Figure 9.7 User Settings – Change Password

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 77 for more details on locking user accounts.

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

Figure 9.8 User Settings – Delete User
In this example, user accounts are locked.

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

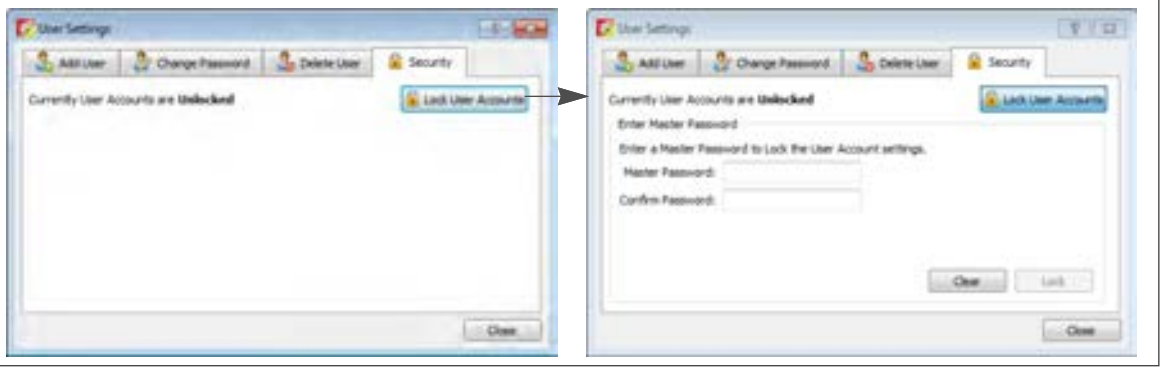
Locking User Accounts

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

To lock user accounts:

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

Figure 9.9 User Settings – Security

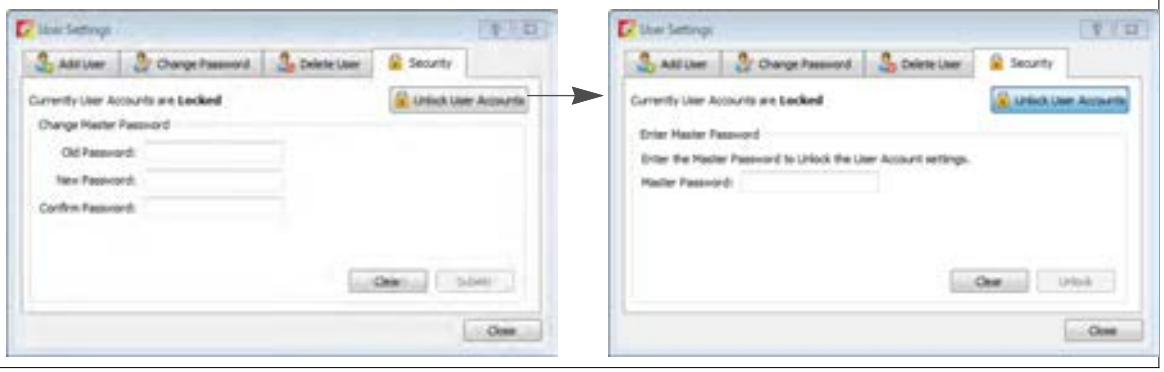


4. Enter and confirm a master password. Click **Close**.
   The master password will be required to delete users.

To unlock user accounts:

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

Figure 9.10 User Settings – Security



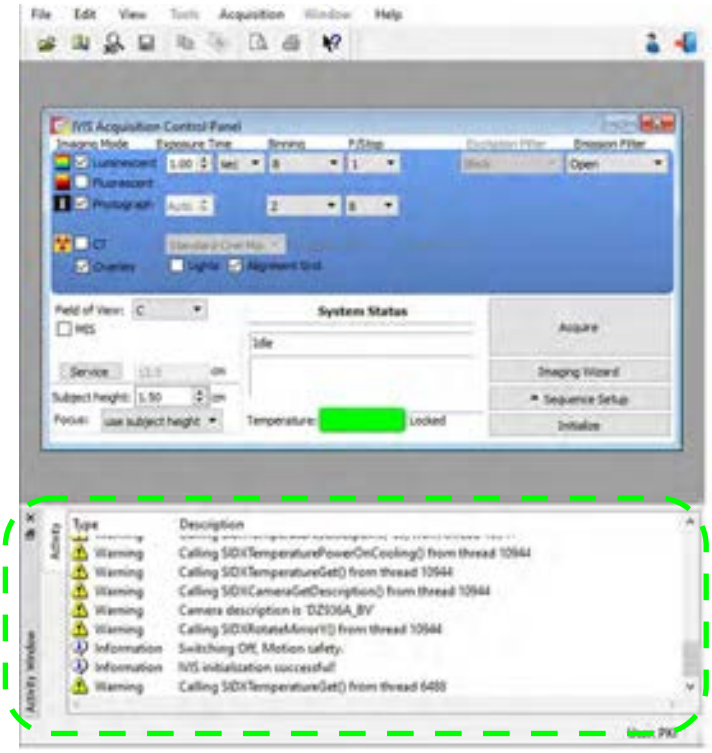
9.4 Tracking System and User Activity

Activity Window

The Activity window shows the imaging system activities (Figure 9.11). The software creates and saves a log of the system activities related to data acquisition. This information may be useful for Revvity 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>_2023.csv) located at C:\Program Files\Caliper Life Sciences\Living Image\Usage).

Figure 9.11 Activity Window



10 High Throughput Imaging

10.1 About High Throughput Imaging

NOTE: This section only applies if your Spectrum CT is configured to accept the Smart Tray. Contact your Revvity Service Engineer if you are uncertain.

The Smart High Throughput Imaging Kit (part no. CLS148874) provides accessories which enable you to:

- Conveniently set up anesthetized subjects (up to five mice) in the Smart Tray.
- Keep subjects warm and anesthetized by placing the Smart Tray on the Benchtop Posing Station.
- Increase throughput by using multiple Smart Trays. While one tray is being imaged, set up another tray and keep it on the Benchtop Posing Station until you are ready to image.

NOTE: See the *Smart High Throughput Imaging Kit* for details on equipment setup and operation.

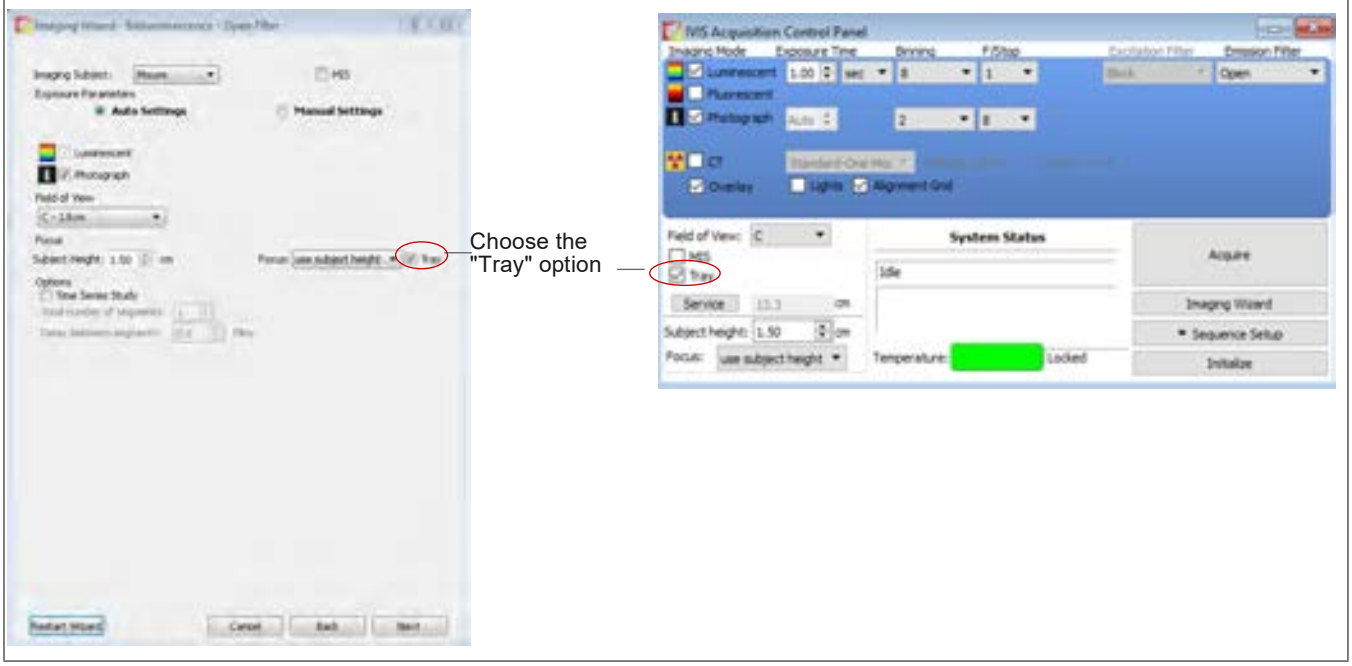
This chapter explains the high throughput imaging workflow.

High Throughput Imaging Workflow	See
1. Confirm that the imaging system is initialized and the temperature is locked. Set up imaging parameters and choose the "Tray" option in the control panel or imaging wizard.	
2. Acquire an image or sequence and auto-generate subject ROIs.	page 80
3. Confirm that the IDs on the screen match the subjects in the Smart Tray.	

10.2 Acquire Images

1. Set the imaging parameters in the Imaging Wizard or control panel. For more details, see:
   - Start the Imaging Wizard and Setup a Sequence on page 108.
   - Acquire the Sequence on page 111.
2. Choose the "Tray" option (Figure 10.1).

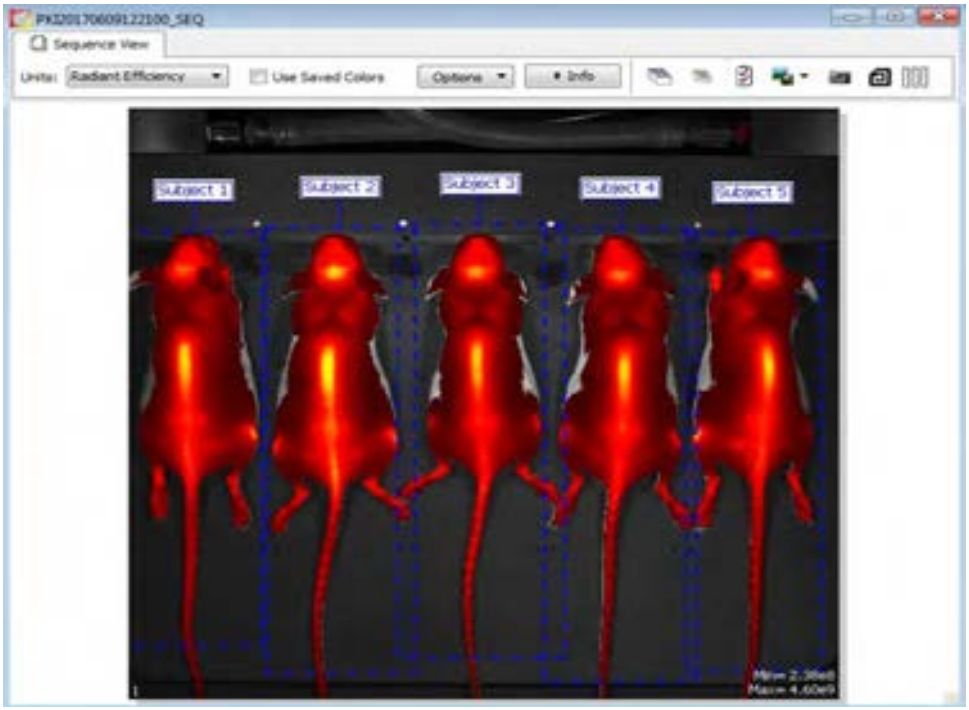
Figure 10.1 Set Imaging Parameters in the Control Panel or Imaging Wizard



3. Acquire the image or sequence.
4. Click the button to automatically apply subject ROIs (Figure 10.2).

NOTE: The button is only available if the "Tray" option was selected during acquisition. This is the preferred way to auto-generate subject ROIs when using the Smart Tray because it creates ROIs based on the tray fiducial marks. This method is quicker and more robust, than the "Auto All" method explained in Measuring Signal on page 172, especially when imaging animals with dark fur.

Figure 10.2 Apply Subject ROIs



11 Image Acquisition

11.1 CT Imaging

Living Image software acquires and reconstructs CT images representing 3D volumes.

NOTE: The IVIS Spectrum CT should be initialized before setting the imaging parameters in the control panel (see page 73 for details).

Acquiring CT Images

1. Place the subjects in the imaging chamber and close the door.
2. Confirm that the IVIS Spectrum CT is ready for X-ray acquisition. Make sure that the:
   a. Key switch on the front of the instrument is turned to the ON position.
   b. EMERGENCY Stop switch is in the READY (out) position. If necessary, turn the knob clockwise to reset it to the READY (out) position.

   NOTE: Pushing in the EMERGENCY Stop switch cuts off power to the entire instrument so that all functions stop, including the camera and motor-driven components.

   c. X-ray Armed button is pushed in. The button is illuminated when engaged.
3. Put a check mark next to **Photograph** in the control panel (optional) (Figure 11.1).
4. Put a check mark next to **CT** and select a CT acquisition mode from the drop-down list.

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

Figure 11.1 Control Panel

Table 11.1 CT Acquisition Modes

CT Acquisition Mode	Voxel Size (μm)	Resolution	FOV LxWxH (cm)	Filter	No. of Views	Binning	Est. Dose (mGv)	Total Time[1] (sec)	File Size (MB)
Standard-One Mouse	150	425	12x12x3	440 Al	720	4	52.8	140	256
Standard-Two Mice	150	425	12x12x3	120 Cu	720	4	23	150	256
Fast	300	850	12x12x3	440 Al	360	4	13.2	90	32
Medium Res	75	225	6x6x3	440 Al	720	2	13.2	210	512
High Res (for imaging the bottom 2 cm of the field of view)	40	150	2.4x2.4x2.0	120 Cu	1440	1	46	300	360
High Res Top (for imaging the top 2 cm of the field of view)	40	150	2.4x2.4x2.0	120 Cu	1440	1	46	300	360

[1]Times are approximate and include overhead, acquisition, and reconstruction time.

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

   If necessary click in the control panel to operate in single image mode. In single image mode, the button appears in the control panel. Use this button to set up optical sequence acquisition (see page 107 for more details).

6. 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 164 for details on adding information to an image after acquisition

Figure 11.2 Enter Information About the 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 11.12 on page 93).

If this is the first image of the session, you are prompted to enable the autosave function (Figure 11.11). When Autosave is enabled, all images acquired during the session are automatically saved to a user-selected location. A different location can be chosen at any time (select **Acquisition** → **Auto-Save** on the menu bar).

7. 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 122 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. The 3D View and Tool Palette appear when acquisition is completed (Figure 11.3).

   See Chapter 17, *Working With Volumetric Data on page 295* for information on viewing and classifying 3D volumetric data.

Figure 11.3 CT Image

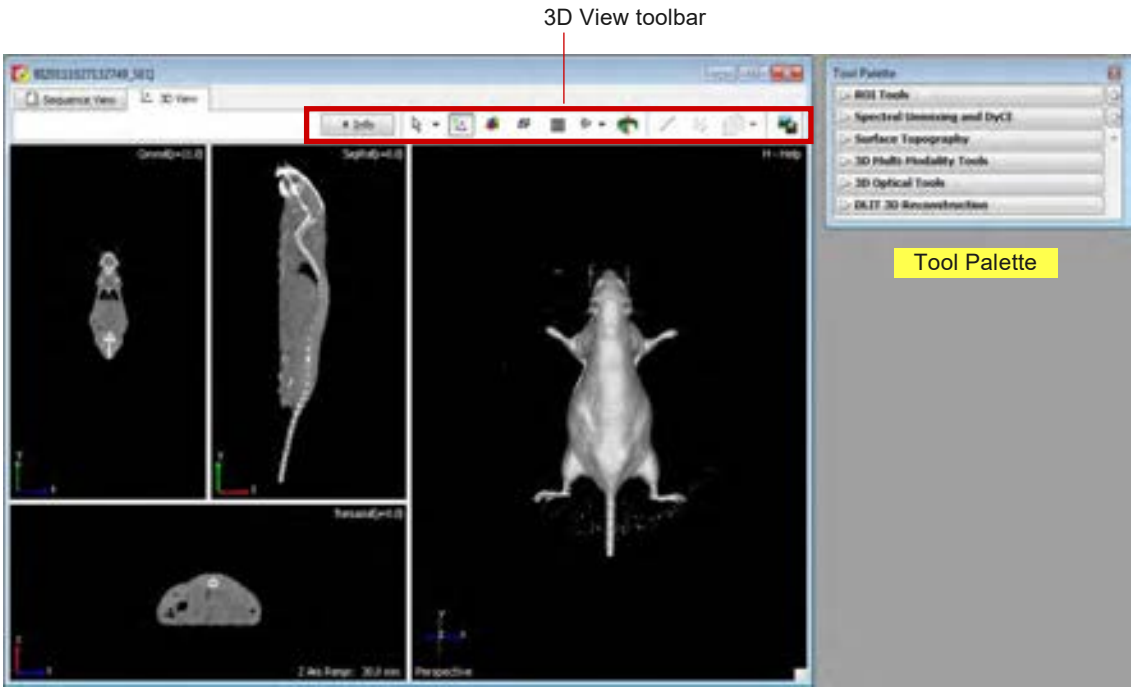


Table 11.2 3D View Toolbar

Item	Description
Info	Click to display or hide the image label. The image label includes information you enter in the Edit Image Labels dialog box (Figure 11.12) and other image information automatically recorded by the software.
Image Tools 	A drop-down list of tools for viewing and working with the surface or DLIT results. or – Rotates or spins the surface in the x, y, or z-axis direction. – Moves the surface in the x or y-axis direction. – Zooms in or out on the image. To zoom in, right-click (Cmd key (apple key) + click for Macintosh users) and drag the toward the bottom of the window. To zoom out, right-click and drag the toward the top of the window.
	Displays the x,y,z-axis display in the 3D view window.
	Displays coronal, sagittal, and transaxial cross-sections through the subject in the 3D view window.

Table 11.2 3D View Toolbar (continued)

Item	Description
	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). For examples of the views, see Figure 15.14 on page 272.
	Select this tool from the drop-down list to display the perspective view.
	Rotates the 3D reconstruction results in the 3D view window (*3D scene*). Click the + or - key to increase or decrease the rotation speed.To stop the rotation, click the 3D scene or the  button.
	Displays measurement tools in the coronal, sagittal, or transaxial views.
	Not available for volumetric data. Reserved for use with DLIT or FLIT reconstruction results (voxels).
	Not available for volumetric data. Reserved for use with DLIT or FLIT reconstruction results (voxels).
	Enables you to save the 3D view to a graphic file (for example, .jpg).

Acquiring Reference Images

During CT imaging, a series of X-ray transmission images is acquired as a specimen rotates on the stage. Each X-ray transmission image is associated with a corresponding angle. Living Image software uses a dark bias (no X-ray) image, X-ray reference image, pixel defect map, and linearization correction to normalize the transmission image on a pixel by pixel basis. The resulting projection images are inputs to the reconstruction algorithm that generates a 3D image of the volume.

New reference images (X-ray and dark bias) must be acquired every five days to ensure that the X-ray transmission images are correctly normalized. The system prompts you acquire reference images. Acquisition cannot proceed until the reference images have been acquired.

To acquire reference images:

1. Remove everything from the imaging chamber, including the animal bed.

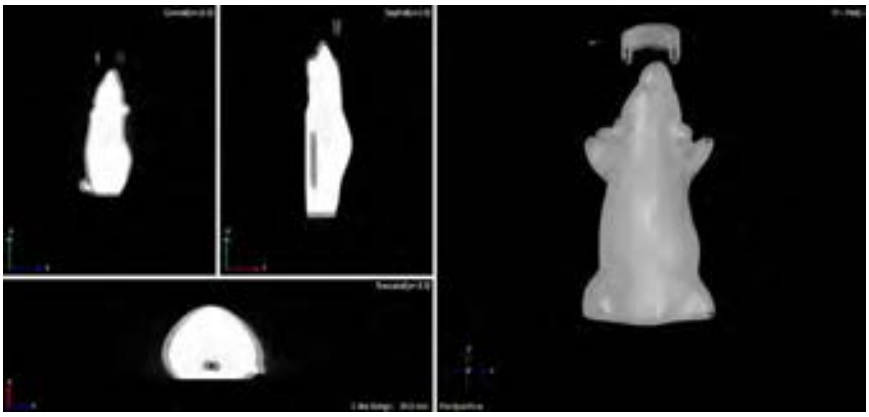
IMPORTANT: If the animal bed is left in the imaging chamber during acquisition, the reference images may result in incorrectly normalized X-ray images.

2. Confirm that the IVIS Spectrum CT is ready to acquire a CT image.
3. Select **Acquisition** → **CT Acquisition** → **Acquire Reference Images** on the menu bar.

Checking Instrument Stage Alignment

On rare occasion, the instrument stage may become misaligned with the X-ray source and detector. If this occurs, CT images may display artifacts (for example, double images as shown in Figure 11.4, blurring, or shadows).

Figure 11.4 Example of Image Artifacts

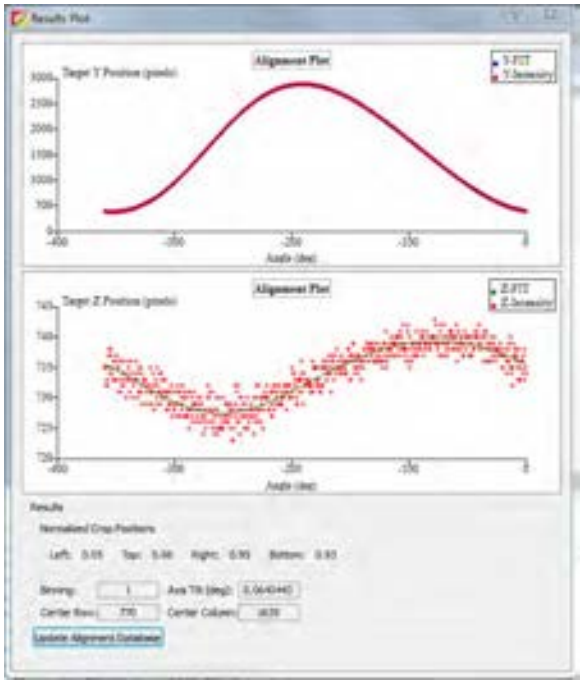


To check stage alignment:

1. Remove all objects from the imaging chamber.
2. Insert the alignment tool into the rotating stage. See the Rotation Stage Alignment Kit instructions for details (PN 134379).
3. Select **Acquisition** → **CT Acquisition** → **Generate Alignment** data on the menu bar.
4. Click **Update Alignment Database** in the dialog box that appears (Figure 11.5).

   The Y-FIT curve (blue) and Y-Intensity curve (red) should be closely superimposed. If not, contact Revvity technical support for assistance with instrument alignment (see page 11).

Figure 11.5 Alignment Results Plot



11.2 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 90.

See page 107 for information on acquiring a luminescent sequence using the Imaging Wizard.

Quick Guide

1. Start Living Image software and initialize the IVIS Spectrum CT (see page 71 for details).
2. Place the anesthetized subjects in the imaging chamber and close the door.
3. In the control panel (Figure 11.6):
   a. Put a check mark next to **Luminescent** and select **Auto** exposure.
   b. Choose **Photograph** (optional, selecting **Photograph** automatically selects **Overlay**).
   c. Select a field of view (FOV) (see Table 11.3 on page 91).
   d. Enter the subject height (cm) and select the **use subject height** focus option.
   e. Click **Acquire**.

Figure 11.6 Control Panel

4. Select a location for the image data when prompted (optional, but strongly recommended).

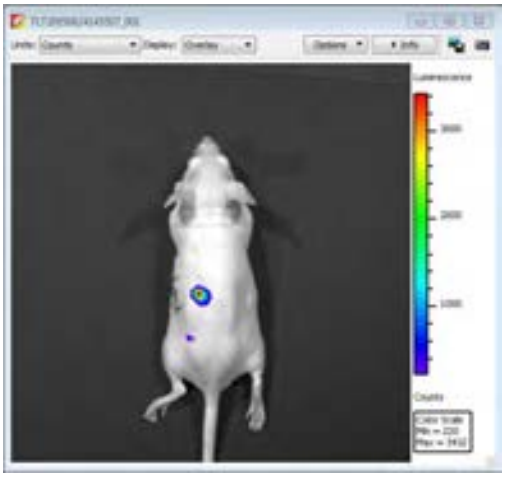
   Image data acquired during the session will be automatically saved to this location.

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 11.7).

Figure 11.7 Quick Guide: Acquire a Luminescent Image

Image Window



See Table 11.4 on page 94 for more details on the image window.

Tool Palette

The Tool Palette includes the:

- Image Adjust Tools (page 132)
- ROI Tools (page 170)
- Corrections Tools (page 409)

Acquire a Luminescent Image

This section provides detailed instructions for luminescent imaging.

NOTE: The IVIS Spectrum CT should be initialized and the temperature locked before setting the imaging parameters in the control panel. See page 73 for more details.

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

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

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

Alternatively, manually set the exposure, binning, and F/Stop. See Appendix 20 on page 373 for details on these parameters.

Figure 11.8 Control Panel

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 11.3).

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

Table 11.3 Field of View (FOV) Settings – IVIS Spectrum CT

FOV Setting	FOV (cm)
A[1]	3.9
B	6.5
C[2]	13
D	22.5

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

4. Select a focus option in the control panel (Figure 11.9).
   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.
   - Enter the height of the animal (cm) and select the **use subject height** focus option.

   OR

- Choose the **manual** focus option and follow the instructions in IVIS Acquisition Control Panel on page 373.

Figure 11.9 Choose a Focus Option in the Control Panel

5. 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.
6. Click **Acquire** when you are ready to capture the image.

NOTE: If necessary click Image Setup in the control panel to operate in single image mode. In single image mode, the Sequence Setup button appears in the control panel. Use this button to set up sequence acquisition (see page 107 for more details on sequence setup using the Imaging Wizard).

7. 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 164 for details on adding information to an image after acquisition.

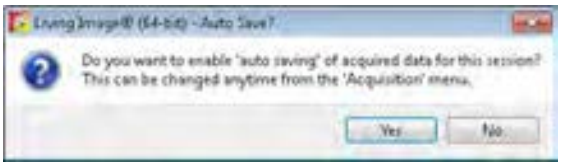
Figure 11.10 Enter Information About the Image

All information entered here will be saved with the image.

However, only the check marked values will appear in the image label (see Figure 11.12 on page 93).

If this is the first image of the session, you are prompted to enable the autosave function (Figure 11.11). When Autosave is enabled, all images acquired during the session are automatically saved to a user-selected location. A different location can be chosen at any time (select **Acquisition** → **Auto-Save** on the menu bar).

Figure 11.11 Autosave Prompt



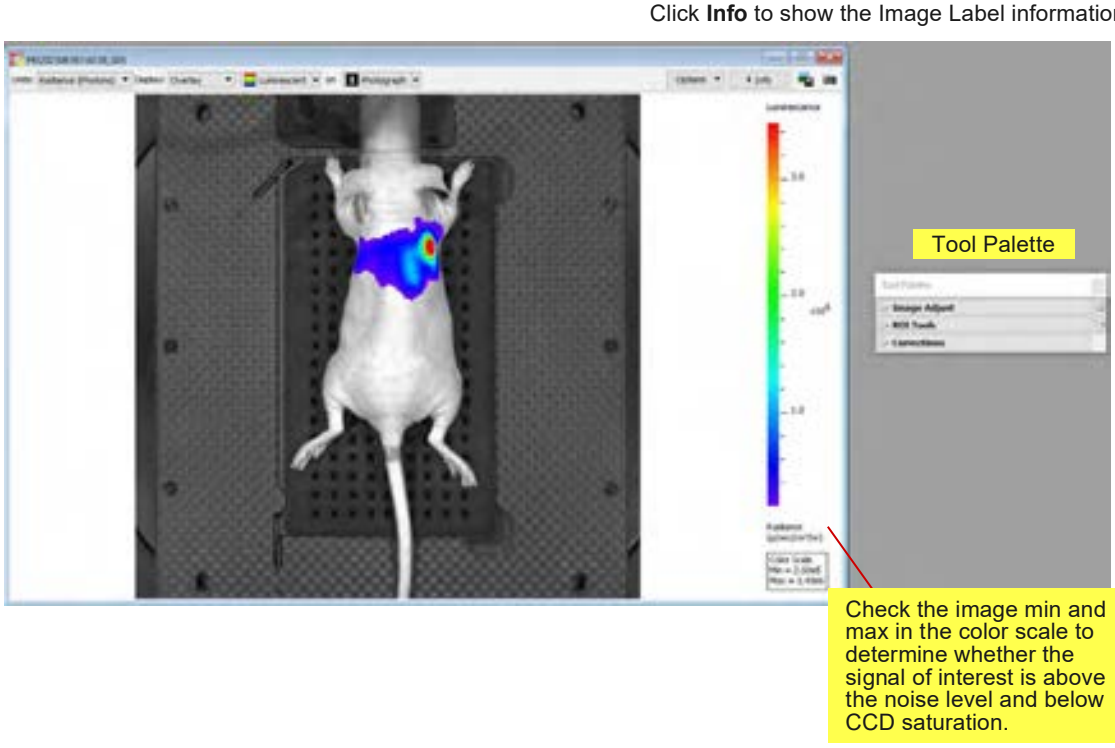
8. Click **Yes** in the prompt to enable autosave, then choose a location in the dialog box that appears. Alternatively, click **No** in the prompt and manually save the image data. See page 122 for details.

   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.

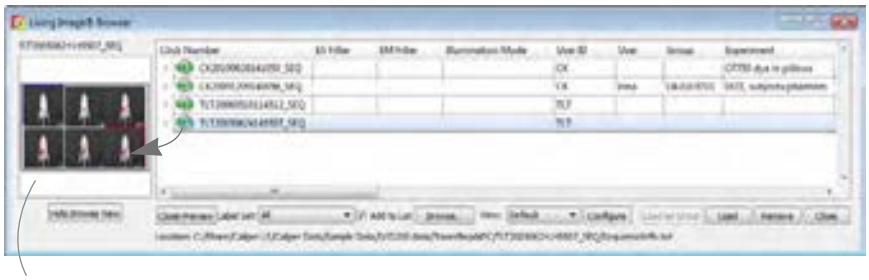
   The control panel returns to blue color when acquisition is finished and the image window appears (Figure 11.12).

Figure 11.12 Overlay (Luminescent Image on Photograph) in the Image Window



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

Table 11.4 Image Window

Item	Description
Units	Select the measurement units for the image display from this drop-down list. The available units depend on the type of image data. See the concept tech note *Image Display and Measurement* for more details (select **Help → Tech Notes** on the menu bar).
Display	A list of image types available for display, for example, overlay. For more details on the different types of image displays, see Table 5.2 on page 38. **Note**: If the acquisition included more than two imaging modes (for example, luminescent, x-ray, and photograph), additional drop-down lists appear so you can conveniently choose any two images to overlay. 
Info	Click to display or hide the image label. The image label includes information you enter in the Edit Image Labels dialog box (Figure 11.12) 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.
	Creates a preview picture (*snapshot*) 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 123.  Preview picture of the data selected in the browser (blue row)
Color Scale	Provides a reference for the pixel intensities in a luminescent or fluorescent image. Pixels less than the color scale minimum do not appear in the image. Pixels greater than the color scale maximum are displayed in the maximum color.

11.3 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 90.

See page 101 for information on fluorescent imaging with transillumination (excitation light source located below the stage). See page 107 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 → Tech Notes** on the menu bar).

Quick Guide

1. Start Living Image software and initialize the IVIS Spectrum CT (page 71).
2. Place the anesthetized subjects in the imaging chamber and close the door.
3. In the control panel (Figure 11.13):
   a. Put a check mark next to **Fluorescent** and select **Auto** exposure.
   b. Select an excitation and emission filter.
   c. Choose **Photograph** (optional, selecting **Photograph** automatically selects **Overlay**).
   d. Select a field of view (FOV) (see Table 11.3 on page 91).
   e. Enter the subject height (cm) and choose the **use subject height** focus option.
   f. Click **Acquire**.

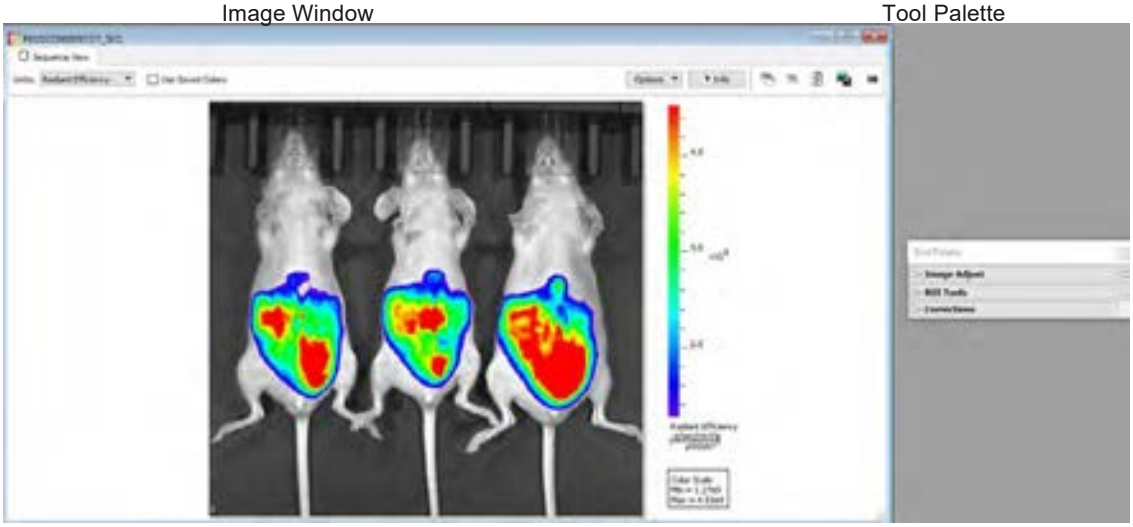
Figure 11.13 Control Panel

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 11.14).

Figure 11.14 Image Window and Tool Palette

Image Window

Tool Palette



See Table 11.4 on page 94 for more details on the image window.

The Tool Palette includes the:

- Image Adjust Tools (page 132)
- Corrections/Filter Tools (page 137)
- ROI Tools (page 170)

Acquire a Fluorescent Image With Epi-Illumination

This section provides detailed instructions for fluorescent imaging.

NOTE: The IVIS Spectrum CT should be initialized and the temperature locked before setting the imaging parameters in the control panel See page 73 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** → **Tech Notes** on the menu bar).

   Alternatively, manually set the exposure, binning, and F/Stop. See Table 20.1 on page 373 for details on these parameters.

2. Put a check mark next to **Photograph**.

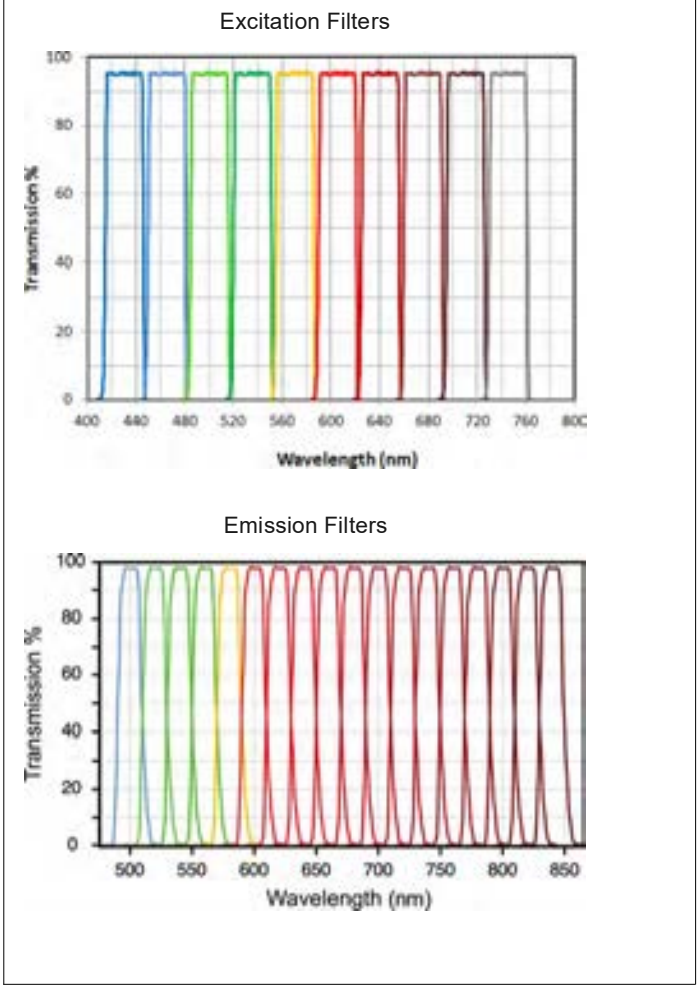
   Selecting **Photograph** automatically selects Overlay, so that an overlay image (registered photograph and luminescent image) is displayed after acquisition.

Figure 11.15 Control Panel

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

   The instrument has 18 narrow band excitation filters that span 490-850nm with a 20nm bandwidth, enabling spectral scanning over the blue to NIR wavelength region (Figure 11.16).

Figure 11.16 IVIS Spectrum CT Excitation and Emission Filters



4. Select a field of view (FOV, size of the stage area to be imaged). See Table 11.3 on page 91 for a list of FOV settings.

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

5. Select a focus option in the control panel (Figure 11.17).

   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.

   - Enter the height of the animal and select the **use subject height** focus option.

     OR

   - Use the **manual** focus option to determine the proper subject height for the area to be imaged. See IVIS Acquisition Control Panel on page 373 for manual focus instructions.

Figure 11.17 Choose a Focus Option in the Control Panel

6. 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.
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 107 for more details on sequence setup).

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

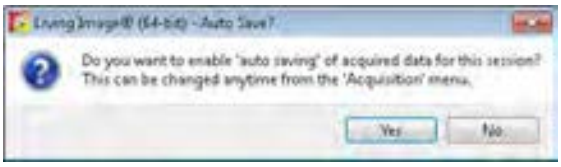
Figure 11.18 Enter Information About the Image

All information entered here will be saved with the image.

However, only the check marked values will appear in the image label (see Figure 11.20 on page 100).

If this is the first image of the session, you are prompted to enable the autosave function (Figure 11.19). When Autosave is enabled, all images acquired during the session are automatically saved to a user-selected location. A different location can be chosen at any time (select **Acquisition** → **Auto-Save** on the menu bar).

Figure 11.19 Autosave Prompt



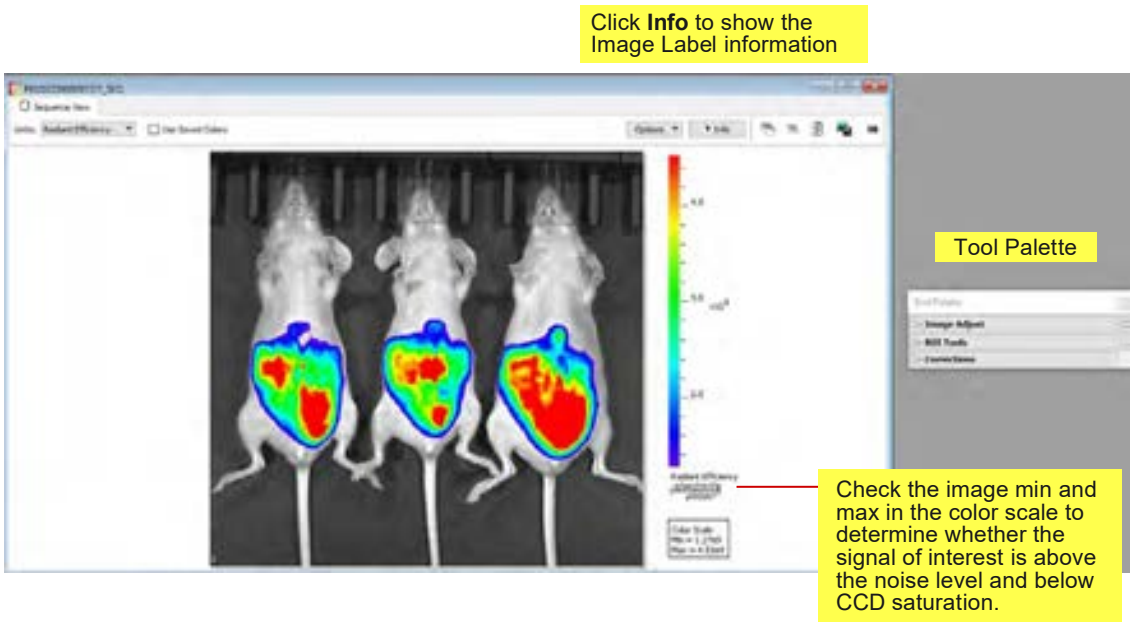
9. Click **Yes** in the prompt to enable autosave, then choose a location in the dialog box that appears. Alternatively, click **No** in the prompt and manually save the image data. See page 122 for details.

   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 11.20). See Table 11.4 on page 94 for details on the image window.

Figure 11.20 Overlay (Fluorescent Image on Photograph) in the Image Window



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

11.4 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 11.21).

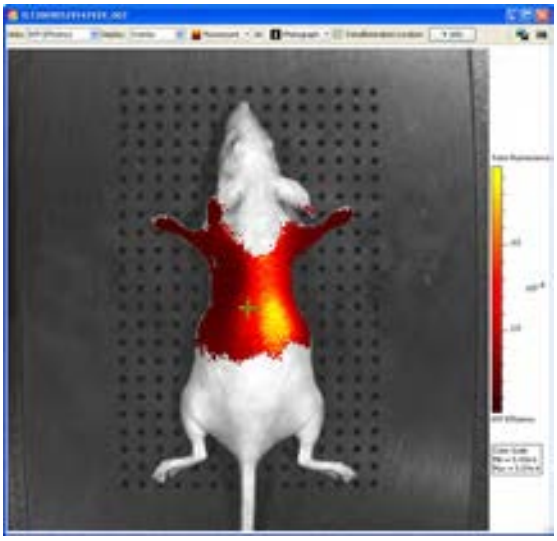
TIP: See these tech notes for helpful information and quick guides (select **Help** → **Tech Notes** on the menu bar):

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

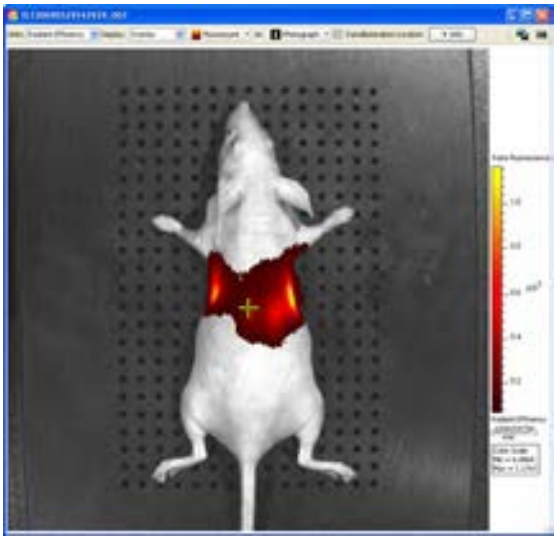
Figure 11.21 Fluorescent Images Acquired with Transillumination

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.

NTF Efficiency Image



Radiant Efficiency Image



This section explains how to acquire a single fluorescent optical image with transillumination. See page 107 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 101 for more information.

Figure 11.22 Control Panel

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 excitation filters that span 490-850nm with a 20nm bandwidth, enabling spectral scanning over the blue to NIR wavelength region (see Figure 11.16 on page 98).
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 11.23).

Figure 11.23 Open the Transillumination Setup Dialog Box

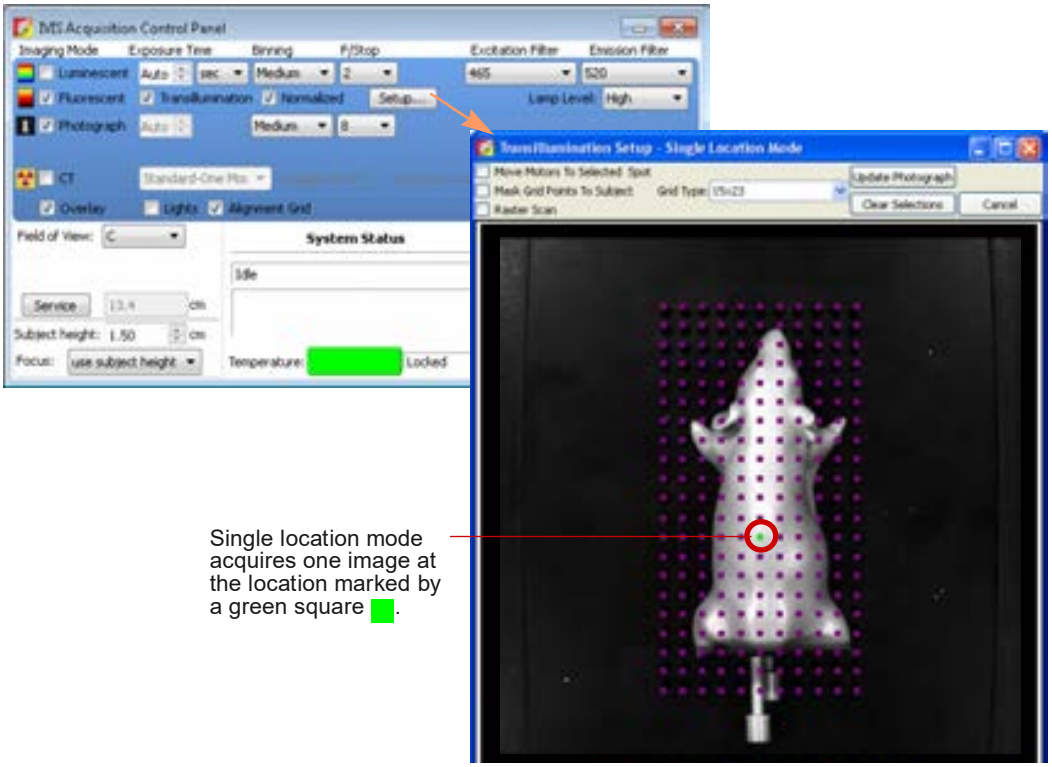


Table 11.5 Transillumination Setup Box

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.

Table 11.5 Transillumination Setup Box (continued)

Item	Description
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** → **Transillumination Overview for <name>_SEQ** 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.

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 11.3 on page 91 for a list of FOV settings.
9. Select a focus option (Figure 11.24).

   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.

   - Enter the height of the animal and select the **use subject height** focus option.

     OR

   - Use the **manual** focus option to determine the proper subject height for the area to be imaged. See IVIS Acquisition Control Panel on page 373 for manual focus instructions.

Figure 11.24 Choose a Focus Option in the Control Panel

10. 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.
11. Click **Acquire** when you are ready to capture the image.

NOTE: If necessary click Image Setup in the control panel to operate in single image mode. In single image mode, the Sequence Setup button appears in the control panel. Use this button to set up sequence acquisition (see page page 107 for more details on sequence setup).

12. 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 164 for details on adding information to an image after acquisition.

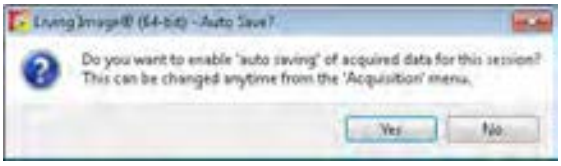
Figure 11.25 Enter Information About the Image

All information entered here will be saved with the image.

However, only the check marked values will appear in the image label (see Figure 11.27 on page 106).

If this is the first image of the session, you are prompted to enable the autosave function (Figure 11.26). When Autosave is enabled, all images acquired during the session are automatically saved to a user-selected location. A different location can be chosen at any time (select **Acquisition** → **Auto-Save** on the menu bar).

Figure 11.26 Autosave Prompt



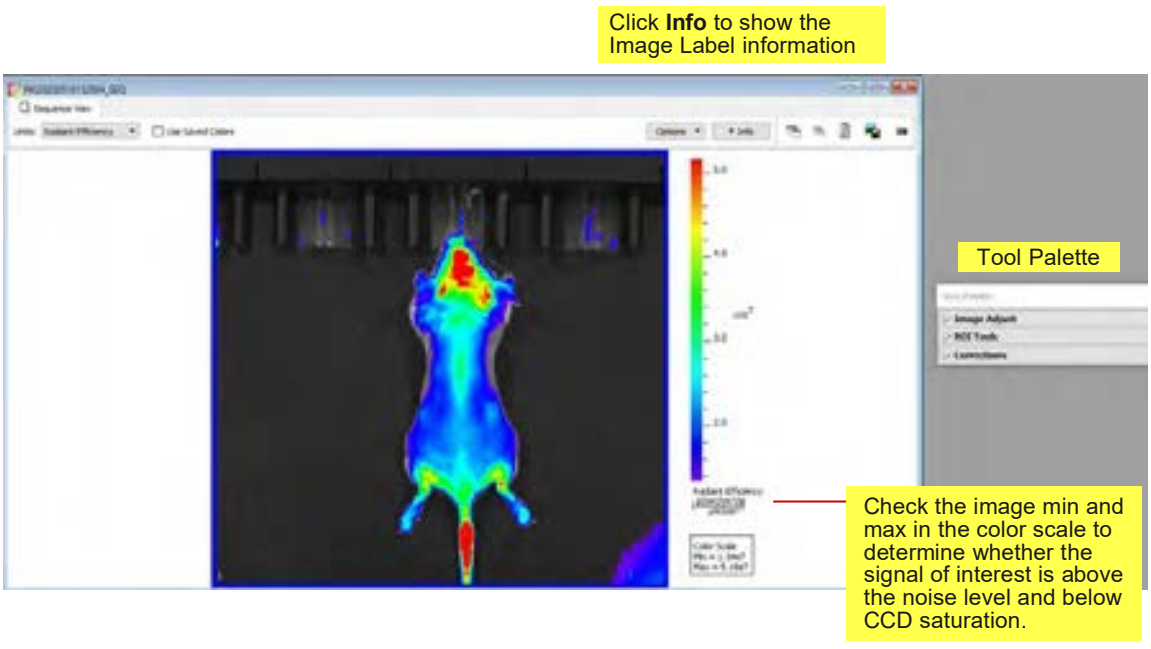
13. Click **Yes** in the prompt to enable autosave, then choose a location in the dialog box that appears. Alternatively, click **No** in the prompt and manually save the image data. See page 122 for details.

    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 11.27). See Table 11.4 on page 94 for details on the image window.

Figure 11.27 Overlay (Fluorescent Image on Photograph) in the Image Window



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

11.5 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 107 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 11.28).

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

   Alternatively, manually set the exposure time, binning, and F/Stop. See IVIS Acquisition Control Panel on page 373 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 427).

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

Figure 11.28 Control Panel

2. Perform Step 2 on page 91 to Step 8 on page 93.

11.6 Acquire a Sequence Using the Imaging Wizard

The acquisition parameters for each image in a sequence must be specified. The Imaging Wizard (Figure 11.29) provides a convenient way to set up a sequence for some imaging applications (see Table 11.6). 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 117 for details).

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

Start the Imaging Wizard and Setup a Sequence

NOTE: The IVIS Spectrum CT should be initialized and the temperature locked before setting imaging parameters. See page 73 for more details.

1. Click **Imaging Wizard** in the control panel (Figure 11.29). If necessary, click **Restart** in the Imaging Wizard to show the first screen of the wizard.

Figure 11.29 Open the Imaging Wizard

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

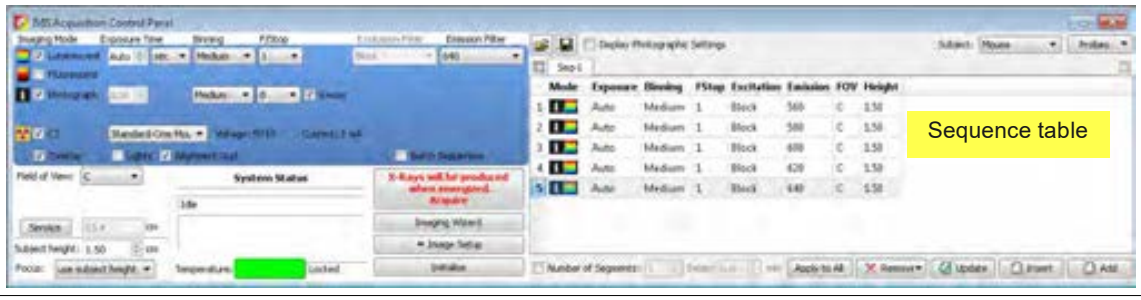
2. Double-click an imaging mode: Bioluminescence  , Fluorescence  , or Cherenkov  .

3. Double-click an imaging option in the next screen (see Table 11.6 on page 109). 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 11.30).

Figure 11.30 Control Panel and Sequence Setup

Each row in the sequence table specifies the acquisition parameters for one image in the sequence. See page 119 for details on the sequence table.



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

   See additional information about:

   - Editing image parameters on page 119.
   - Inserting images in a sequence on page 120.
   - Removing images from a sequence on page 121.

Table 11.6 Imaging Wizard – Imaging Mode Options

Imaging Mode	Options	See Instructions on 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.	322
	DyCE[1] – Acquires a time series of optical images following a bolus injection of probe (radiotracer, bioluminescent, or fluorescent) to track probe biodistribution.	352
	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. **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 *Inserting Images in a Sequence on page 120* for instructions.	200
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.	
	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. ▪ Determine the optimum excitation and emission filter for a probe.	324

Table 11.6 Imaging Wizard – Imaging Mode Options (continued)

Imaging Mode	Options	See Instructions on Page
	DyCE – Acquires a time series of optical images following a bolus injection of probe (radiotracer, bioluminescent, or fluorescent) to track probe biodistribution.	354
	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).	200
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.	357

[1] DyCE analysis tools require a separate license.

Acquire the Sequence

1. Confirm that the IVIS Spectrum CT is initialized and the CCD temperature is locked. (See page 73 for details.)
2. Click **Acquire Sequence** in the control panel when ready to begin acquisition.
3. Enter information about the image in the dialog box that appears (optional, but strongly recommended) (Figure 11.31). 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 164 for details on adding information to an image after acquisition.

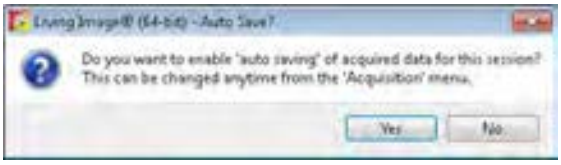
Figure 11.31 Enter Information About the Image

All information entered here will be saved with the image.

However, only the check marked values will appear in the image label (see Figure 11.33 on page 112).

If this is the first image of the session, you are prompted to enable the autosave function (Figure 11.32). When Autosave is enabled, all images acquired during the session are automatically saved to a user-selected location. A different location can be chosen at any time (select **Acquisition** → **Auto-Save** on the menu bar).

Figure 11.32 Autosave Prompt



4. Click **Yes** in the prompt to enable autosave, then choose a location in the dialog box that appears. Alternatively, click **No** in the prompt and manually save the image data. See page 122 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 11.33).

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 CT mode, DLIT data, or FLIT data.

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

Figure 11.33 Image Window and Tool Palette

Table 11.7 Image Window – Sequence View

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

Table 11.7 Image Window – Sequence View (continued)

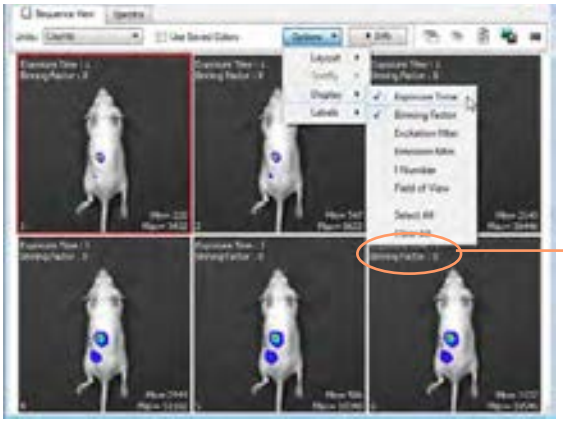
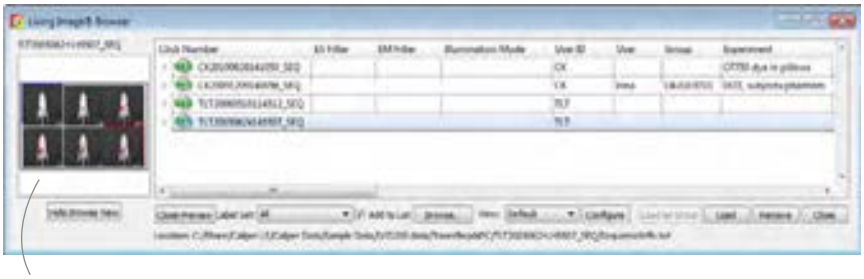
Item	Description
Options	Layout - Choose a display option for the images in a sequence (Default, Dynamic, or Film Strip). For example, here is Film Strip mode: 
	Sort by - Options for ordering images in the sequence window. This option only applies to images that were opened using the “Load as Group” function in the Living Image browser. Default - Order in which the images are stored in the folder. TimeStamp - Ascending order of the image acquisition time. UserID - Ascending alphanumeric order of the user ID
	Display - Choose the types of information to display with each image.  In this example, exposure time and binning factor are displayed on each image
Info	Click to show or hide the image label information (Figure 11.33).
	Opens all of the images in the sequence.
	Closes all open images.
	Opens the Edit Sequence dialog box that enables you to add or remove images from the sequence.

Table 11.7 Image Window – Sequence View (continued)

Item	Description
	Enables you to export the active image as a graphic file (for example, .png, .dcm).
	Creates a preview picture (*snapshot*) of the image or thumbnails that the Living Image Browser displays when the data are selected. See page 123 for more details on the browser.  Preview picture of the selected data

11.7 Acquire Multiple Sequences in Batch Mode

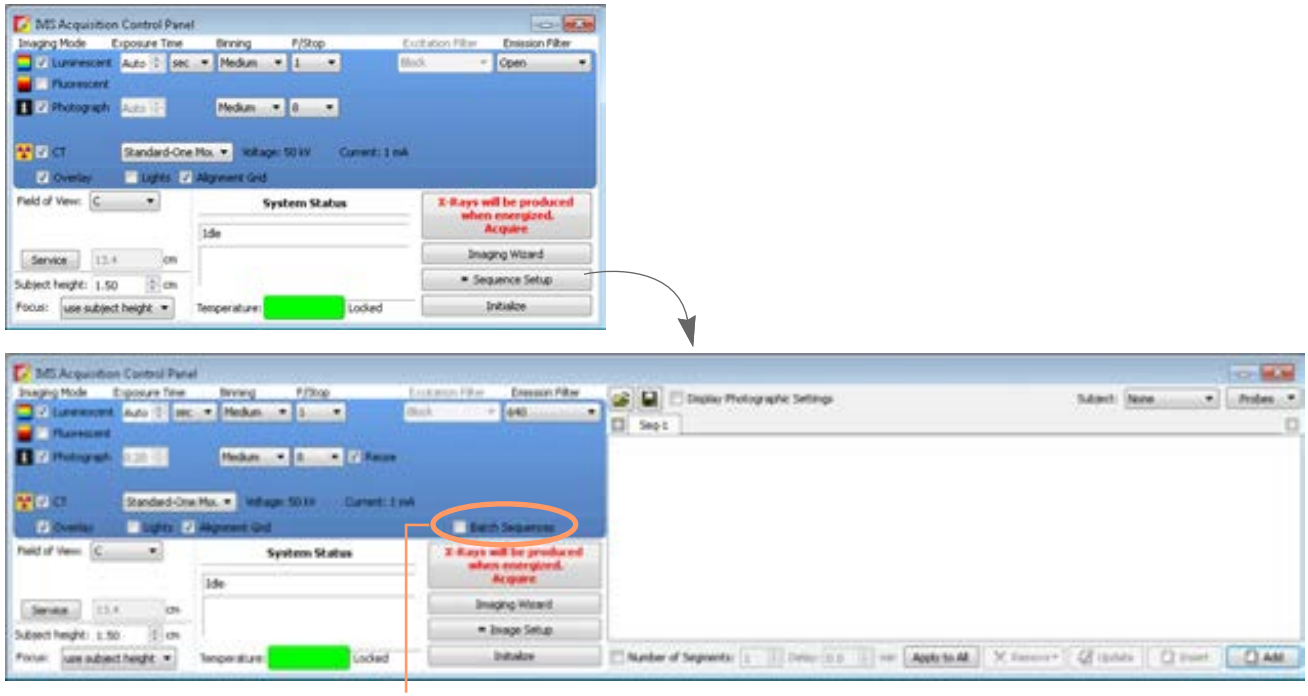
Use batch mode to set up multiple, separate sequences which will be automatically acquired, one after another, without manual intervention.

NOTE: CT image acquisition is not available in batch mode.

To setup and acquire sequences in batch mode:

1. Click **Sequence Setup** in the control panel.
2. Choose the **Batch Sequences** option (Figure 11.34).

Figure 11.34 Control Panel



3. To set up the first sequence, do either of the following:
   - Click **Imaging Wizard** and step through the wizard (see page 107 for details).

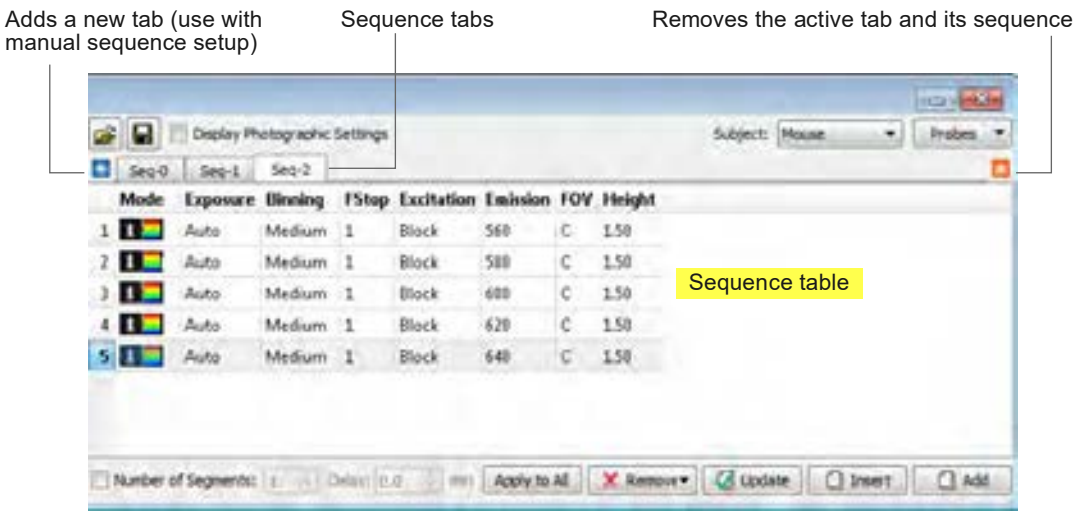
   OR
   - Set up the sequence manually (see page 117 for details).
4. To set up the next sequence:
   - If using the Imaging Wizard, repeat step 3.

     Each sequence is displayed in a separate tab.
   - If setting up the sequence manually, click the button in the sequence table to add a new tab, then proceed with manual setup in the new tab.

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

Figure 11.35 Multiple Sequence Tabs

Three sequences are specified in this example.



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 11.34), 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.

11.8 Manually Set Up a Sequence

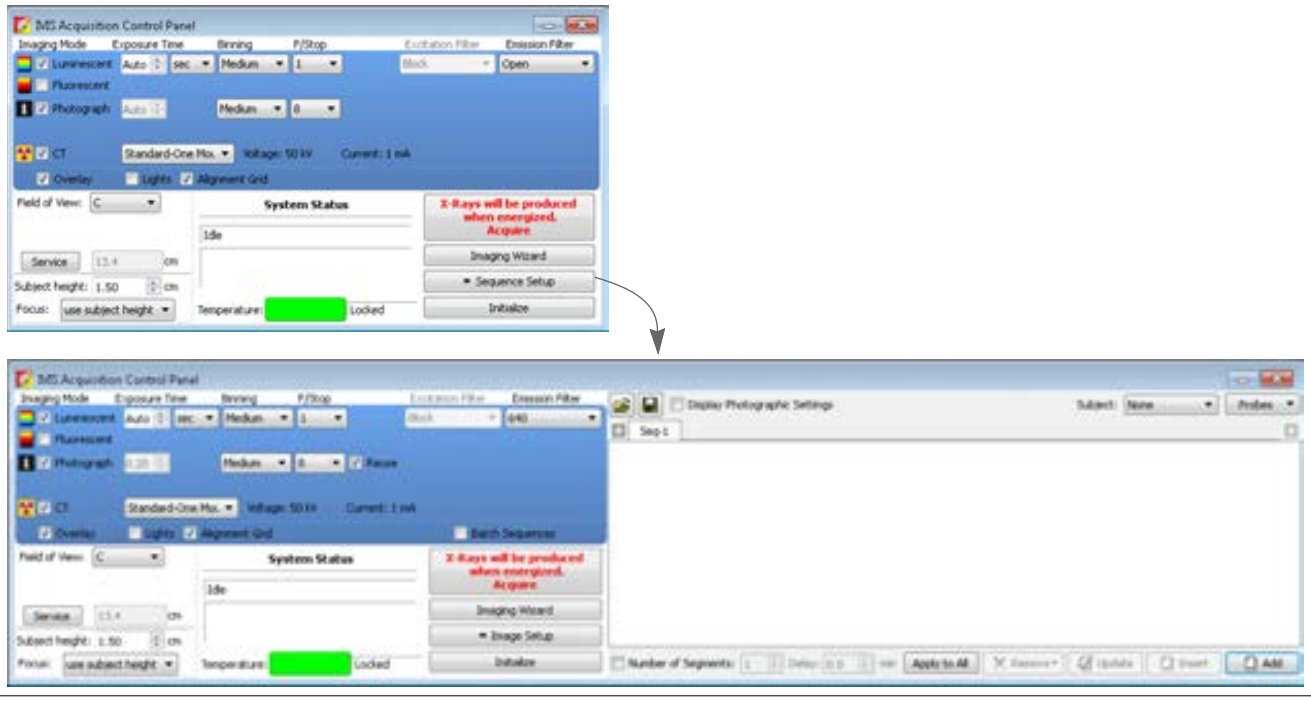
This section explains how to set up an image sequence if you do not use the Imaging Wizard. The sequence parameters in the sequence table can be saved as a Living Image Sequence Setup file (.xsq).

See *Acquire the Sequence on page 111* 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.

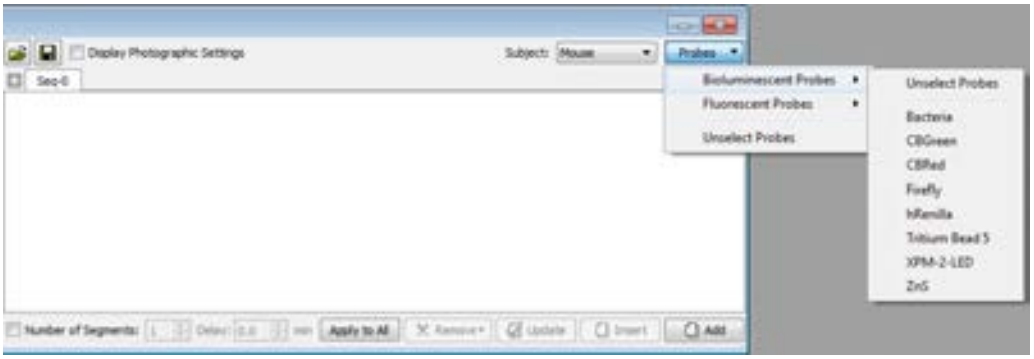
1. Click **Sequence Setup** in the control panel (Figure 11.36).
   The sequence table appears.
2. If necessary, click the **Remove** button Remove and select **All** to clear the sequence table.

Figure 11.36 Open the Sequence Table



3. Choose a subject and probe from the drop-down lists (Figure 11.37)

Figure 11.37 Choose Subject and Probe



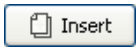
4. Specify the imaging settings for the first image in the sequence. (See IVIS Acquisition Control Panel on page 373 for details on the imaging parameters in the control panel.)

   NOTE: If you selected **Photograph** and the photograph **Reuse** option in the control panel (Figure 11.38), the IVIS Spectrum CT 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 Add.
   The acquisition parameters appear in the sequence table (Figure 11.38).
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 11.38 Control Panel and Sequence Table with Image Settings

Table 11.8 Sequence Table

Item	Description
	Starts the Imaging Wizard.
	Displays a dialog box that enables you to select and open a sequence setup (.xsq), sequenceinfo.txt, or clickinfo.txt file.
	Displays a dialog box that enables you to save the information in the sequence table to a sequence setup file (.xsq).
Display Photographic Settings	Choose this option to include the photograph exposure time, binning, and F/Stop in the sequence table.
	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 *segment*. 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.
	Applies the selected cell value to all cells in the same column.
	Remove Selected - Deletes the selected row from the sequence table. Remove All - Removes all rows from the sequence table.
	Updates the selected row in the sequence table with the acquisition parameters in the control panel.
	Inserts a row above the currently selected row using the information from the control panel.
	Adds a new row at the end of the sequence setup list.

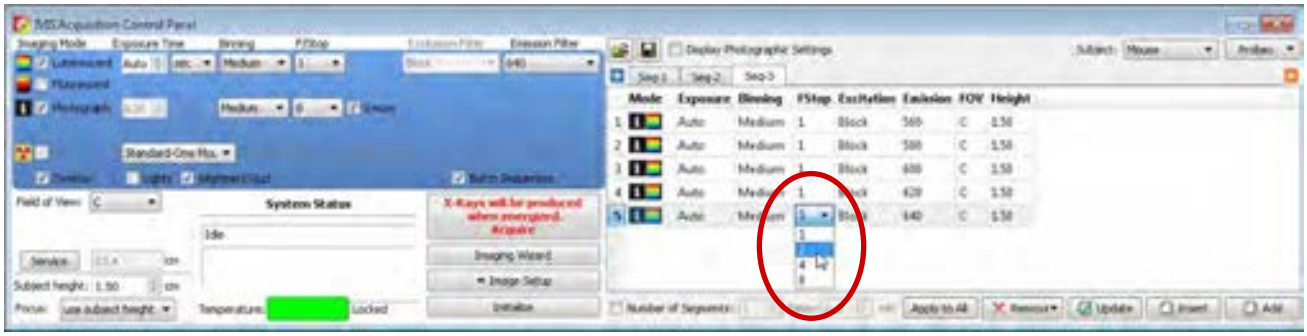
Editing Image Parameters

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

To edit a parameter in the sequence table:

1. Double-click the cell that you want to edit (Figure 11.39).

Figure 11.39 Edit Imaging Parameters



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 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:

Select the rows of interest and right-click the sequence table to view a shortcut menu of edit commands.

Figure 11.40 Sequence Table – Shortcut Menu Edit Commands

Table 11.9 Sequence Table – Shortcut Menu Edit Commands

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

Removing Images From a Sequence

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 11.40).

11.9 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** → **Auto-Save** on the menu bar).

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

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

11.10 Exporting Images

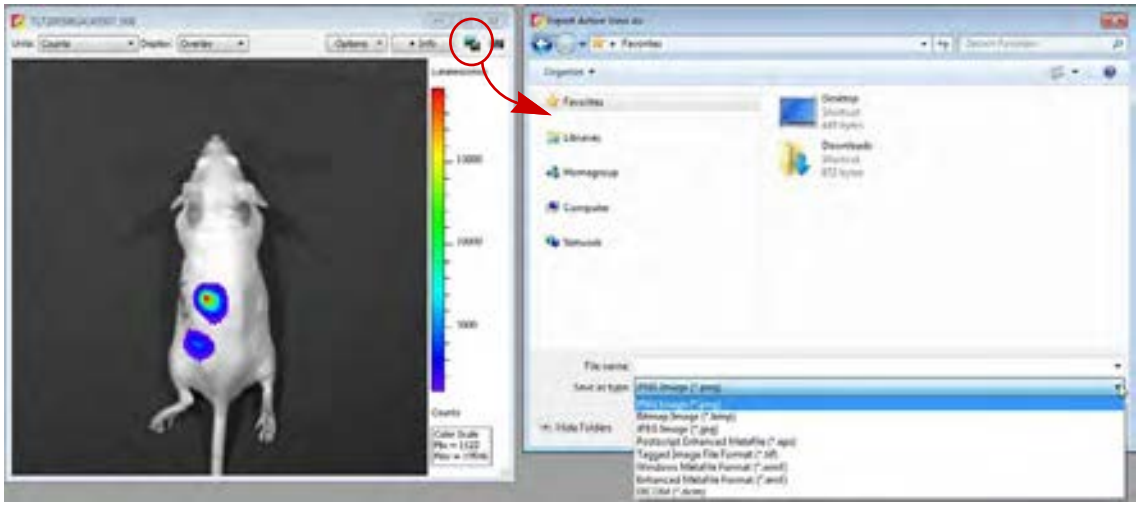
The active image view can be saved in different file formats (for example, .png, .bmp, .dcm).

NOTE: The Image Layout window provides an alternative way to export or print images. See page 156 for more information. See page 158 for information on exporting an image sequence

1. Open an image or sequence.
2. Click the **Export Graphics** button (Figure 11.41).
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 11.41 Export Image to a Graphic File



12 Working With Images

12.1 Loading Image Data

Images can be loaded (opened):

- Using the Living Image Browser (see below).
- From the toolbar or menu bar (page 127),
- 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** → **Recent Files** on the menu bar to view recently opened files.

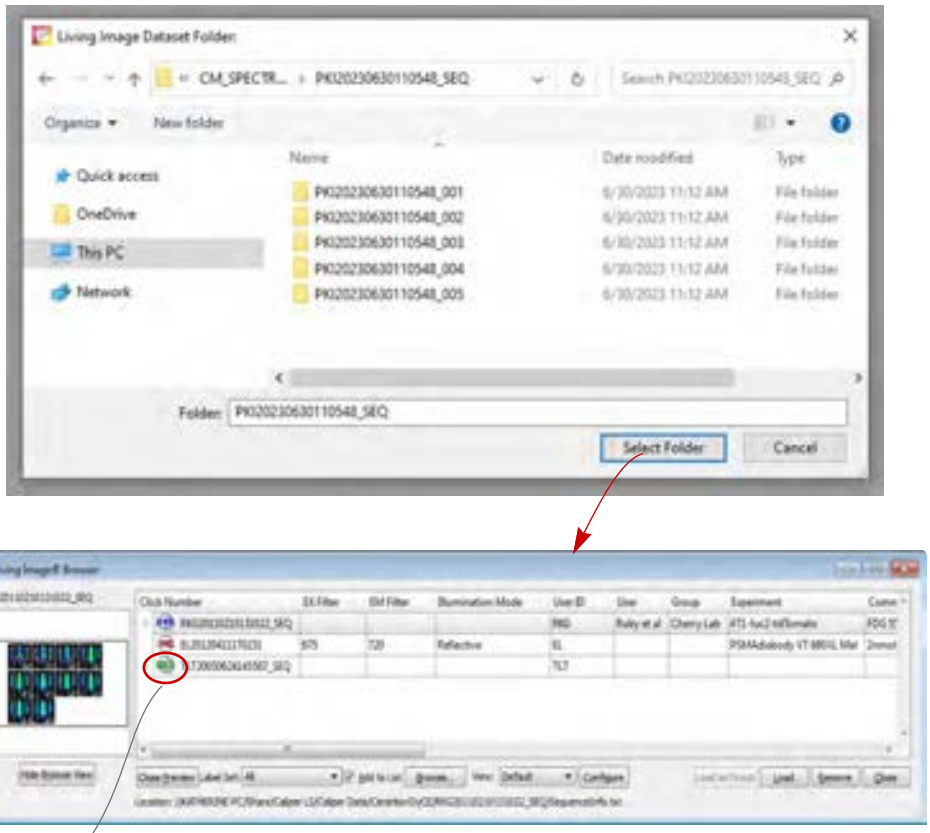
Preview and Load Data Using the Living Image Browser

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 . Alternatively, select **File** → **Browse** on the menu bar.
   b. Select a folder in the dialog box that appears.
      The Living Image Browser appears (Figure 12.1). 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 toolbar button, the software automatically returns to the last folder visited.

Figure 12.1 Opening the Living Image Browser



Living Image Browser

– Image

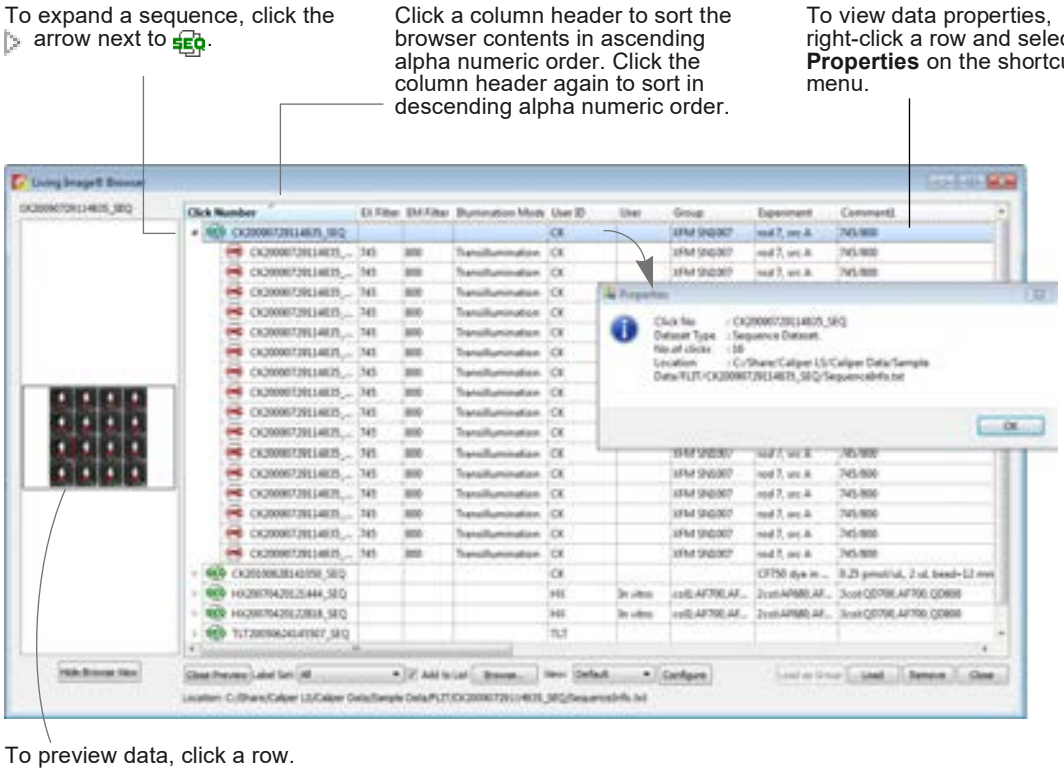
– Image with saturated pixels

– Image sequence

– Image exported as DICOM file

– DyCE or Cherenkov image sequence

Figure 12.2 Living Image Browser



Note: A preview snapshot is automatically taken at the time of image or sequence acquisition. A snapshot can also be captured manually (see page 94 for more details).

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 12.3).

NOTE: Multiple datasets can be loaded.

Figure 12.3 Loaded Image Sequence

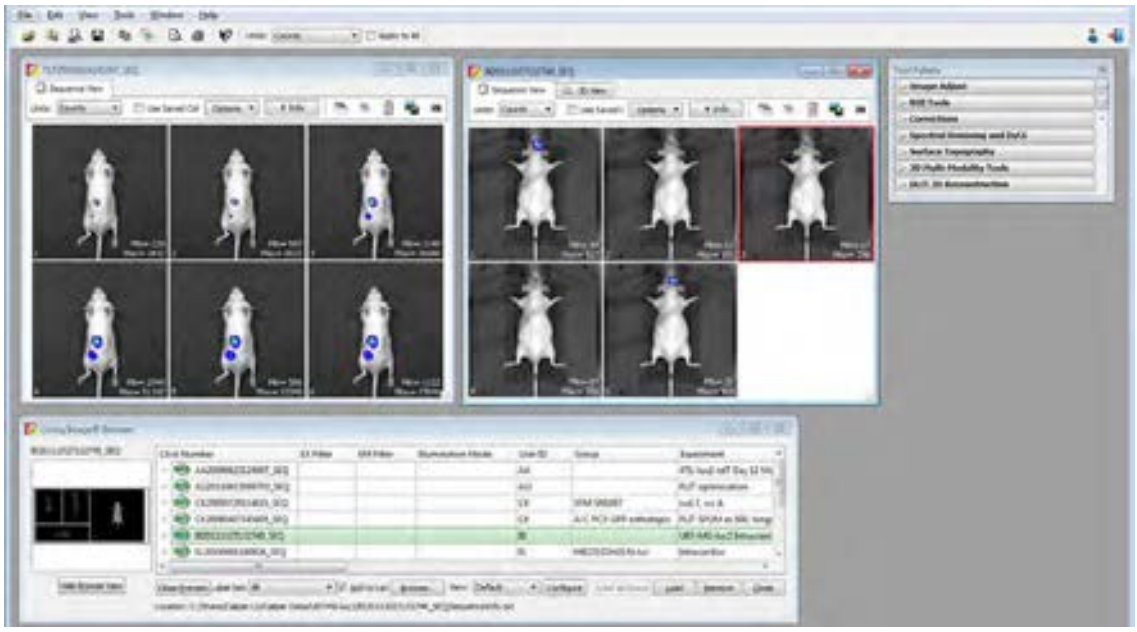


Table 12.1 Living Image Browser

Item	Description
Hide Browse View	Closes the browser table.
Close Preview	Closes the image preview box.
Label Set	A drop-down list of the available label sets which specify 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 12.1 on page 124) 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 12.1 on page 124).
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.

Table 12.1 Living Image Browser (continued)

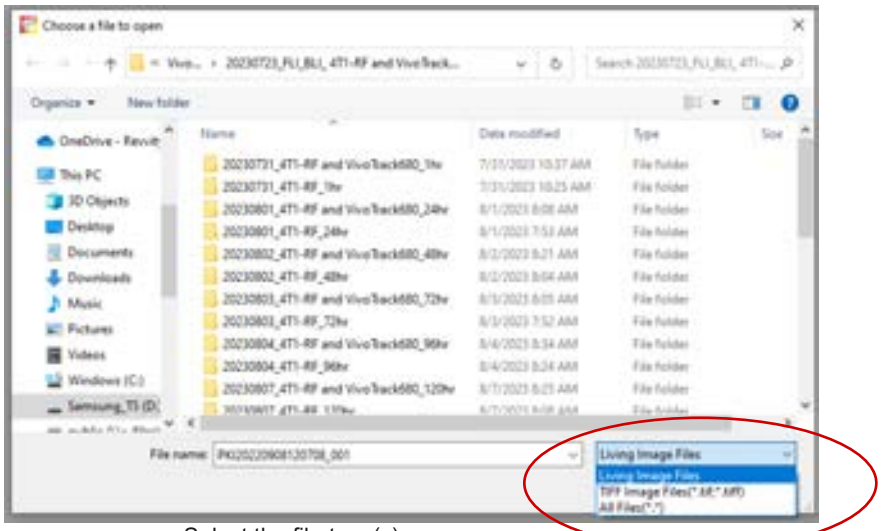
Item	Description
Load as Group	Enables you to select particular images that you want to view as a sequence. The images may be acquired during different sessions. To select adjacent images in the browser, press and hold the **Shift** key while you click the first and last file in the selection. To select 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** → **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.

Load Data From the Menu Bar or Toolbar

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

1. Click the **Open** button on the toolbar. Alternatively, select **File** → **Open** on the menu bar.
2. Choose a file type filter from the drop-down list in the box that appears (Figure 12.4).
   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 12.2 on page 128).

Figure 12.4 Opening Data From the Toolbar or Menu Bar



Select the file type(s)

Table 12.2 File Filters

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

3. Navigate to the file and 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 12.5). Multiple image windows can be open at the same time.

Figure 12.5 Image Windows – Sequence View and Single Image

The options available in the image window depend on the type of active image data.

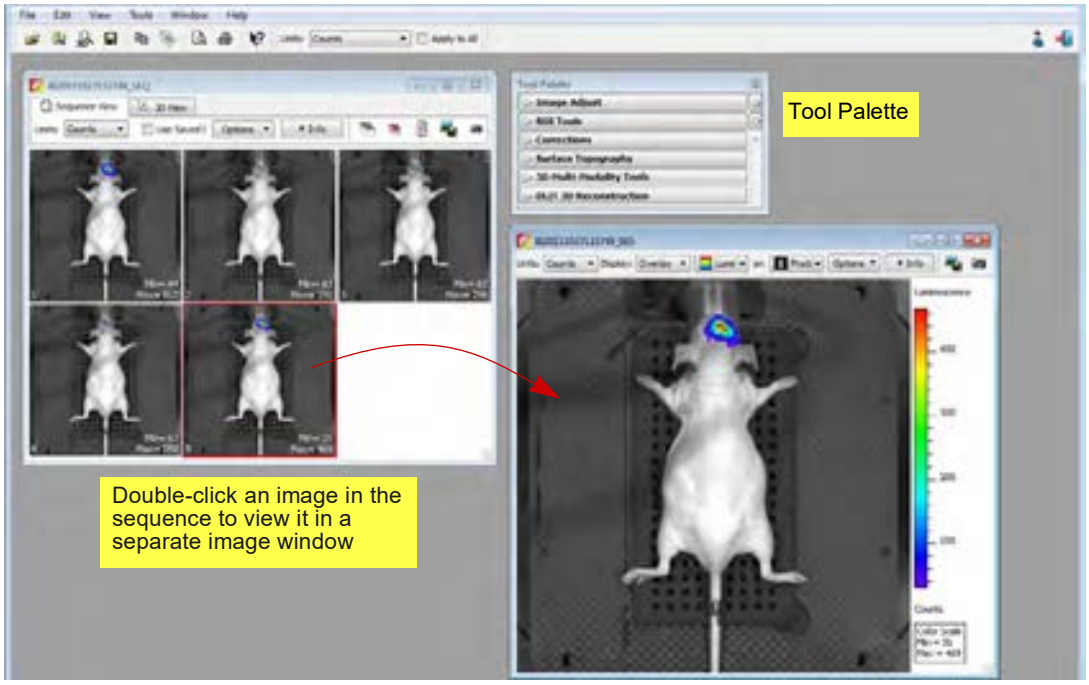


Table 12.3 Image Window

Item	Description
Units	Select the measurement units for the image display from this drop-down list. The available units depend on the type of image data. See the concept tech note *Image Display and Measurement* for more details on measurement units (select **Help → 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.

Table 12.3 Image Window (continued)

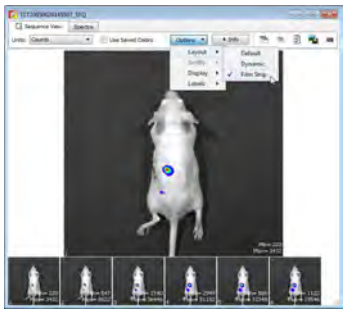
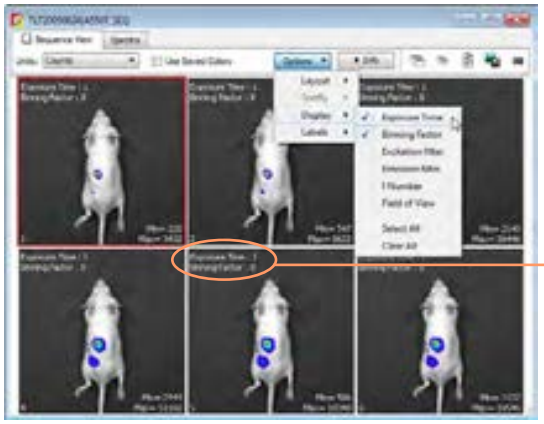
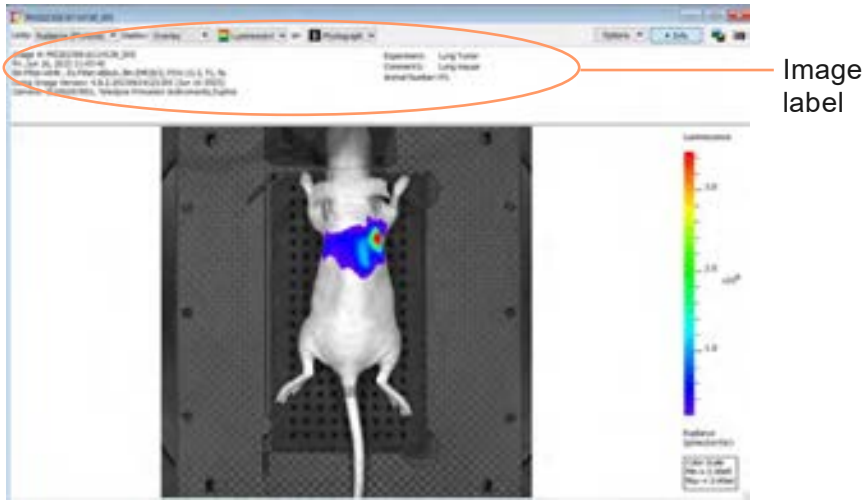
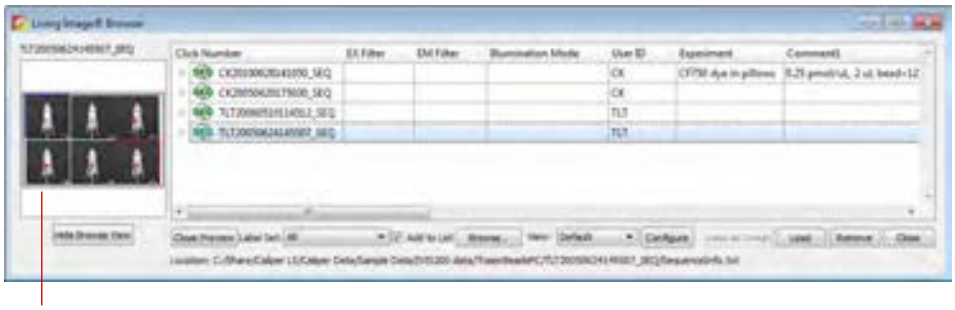
Item	Description
Use Saved Colors (image sequence)	Choose this option to display the image data using the color table that was specified in the Preferences at the time of acquisition. If this option is not selected, image data are displayed using the color table currently specified in the Preferences.
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.
	Display - Choose the types of information to display with each image.  In this example, exposure time and binning factor are displayed on each image

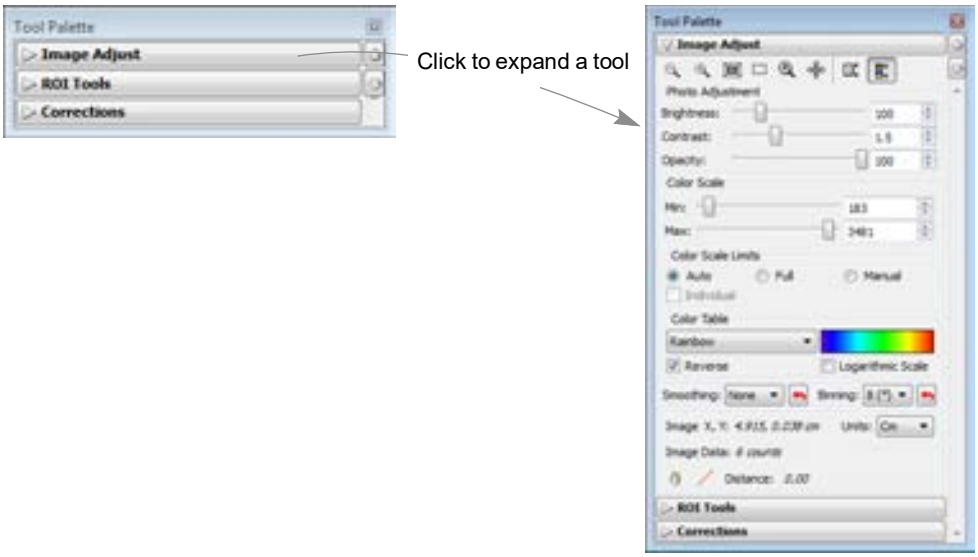
Table 12.3 Image Window (continued)

Item	Description
	Labels – Enables you to select the information to include in the image label. 
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 85) and other information automatically recorded by the software.
	Opens all of the images in a sequence.
	Closes all open images of a sequence.
	Opens the Edit Sequence dialog box that enables you to add or remove images from the sequence display.
	Opens a dialog box that enables you to export the active view as a graphic file.
	Takes a “snapshot” that is displayed with the data in the Living Image Browser. See page 123 for more details on the browser.  Snapshots of an image sequence

The Tool Palette appears when an image or sequence is loaded (Figure 12.6). 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.

Figure 12.6 Tool Palette

See page 39 for an overview of Living Image tools.



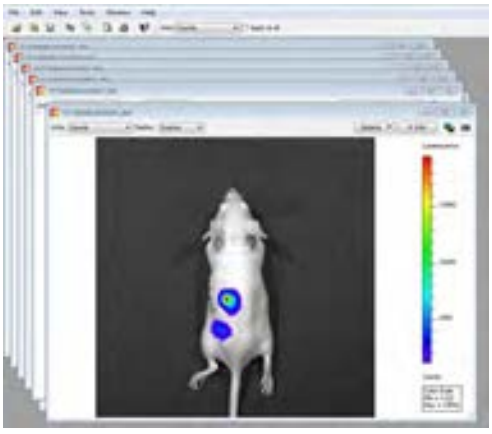
Organizing Images

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

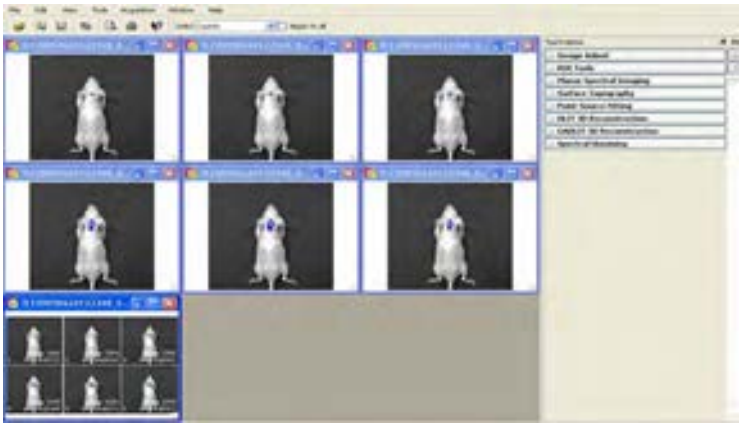
Choose **Window** → **Cascade** or **Window**→ **Tile** on the menu bar.

Figure 12.7 Image Windows

Cascade



Tiled



12.2 Adjusting Image Appearance

Use the Image Adjust tools to adjust image display (Figure 12.8). 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.

Figure 12.8 Tool Palette – Image Adjust Tools

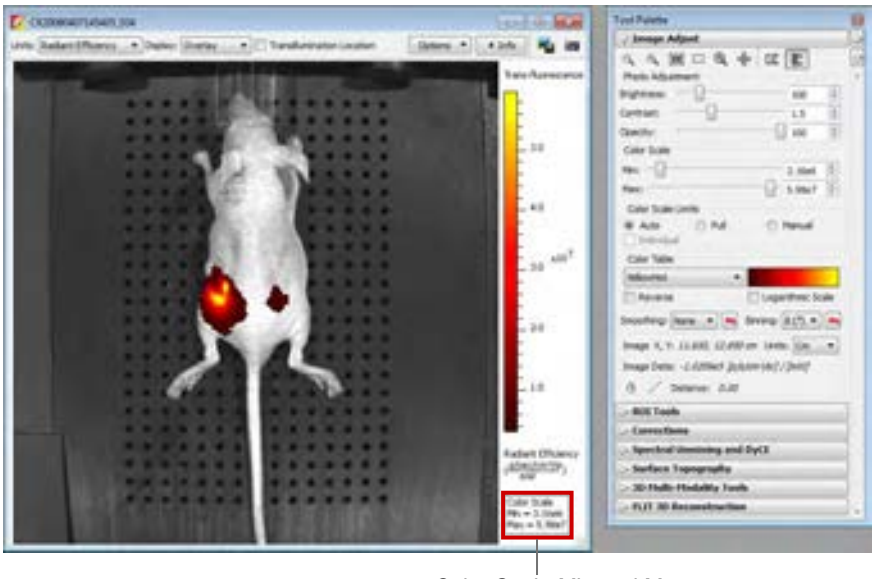


Table 12.4 Image Adjust Tools

Item	Description
	Click this button to incrementally zoom out on the image (reduces the image dimensions in the image window). Note: The zoom tools are also available in the shortcut menu when you right-click the image (**Cmd**-click for Macintosh users).
	Click this button to incrementally zoom in on the image (incrementally magnifies the image in the image window).
	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 *Zooming or Panning on page 135* for more information.
	Click this button to draw a box on an image that can be used to: ▪ Make measurements (see page 141) ▪ Select an area of the image to copy to the system clipboard.
	Click this button to return the image to the default display magnification.
	Click this button to move a magnified image (*pan*) in the image window. For more details, see page 135.
	Click this button to hide or display the image min/max information in the image window

Table 12.4 Image Adjust Tools (continued)

Item	Description
	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 *gamma* 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	 Click the drop-down arrow to select a color table for the image data. See the concept tech note *Image Display and Measurement* for more details on color table (select **Help → 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.
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 12.9). 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.

Table 12.4 Image Adjust Tools (continued)

Item	Description
Binning	Specifies the number of pixels in the image data that are grouped together to form a larger pixel (called *soft* binning). Binning changes the pixel size in the image (see Figure 12.9). Binning can be applied to an image or a sequence. See the tech note *Detection Sensitivity* for more details on binning (select **Help** → **Tech Notes** on the menu bar).  Click this button to return binning to the previous setting and update the image.
Image 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.
	Click this button to display a line profile (see page 139.) This tool is only available when an image is active. **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 141). **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  or  button. Alternatively, right-click the image and select **Zoom In** or **Zoom Out** on the shortcut menu.

To magnify a selected area in an image:

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

   The selected area is magnified when you release the mouse button.

To reset the magnification (remove magnification):

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

To pan the image window:

NOTE: Panning helps you view different areas of a magnified image. Panning is only available if the image has been magnified.

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

Smoothing and Binning

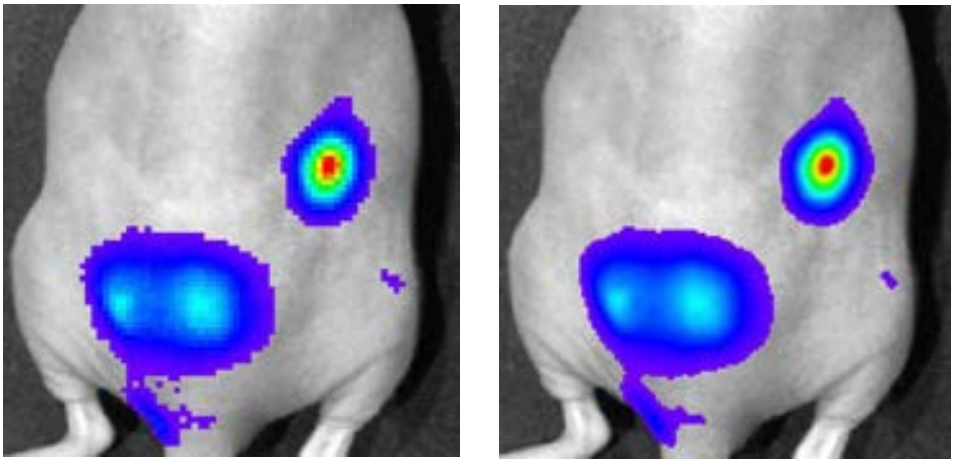
TIP: See the technical note *Detection Sensitivity* for helpful information about binning and smoothing (select **Help → Tech Notes** on the menu bar).

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 (Figure 12.9). 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 12.9). 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.

Figure 12.9 Example of Binning and Smoothing Image Data



Binning at acquisition = 8, no smoothing

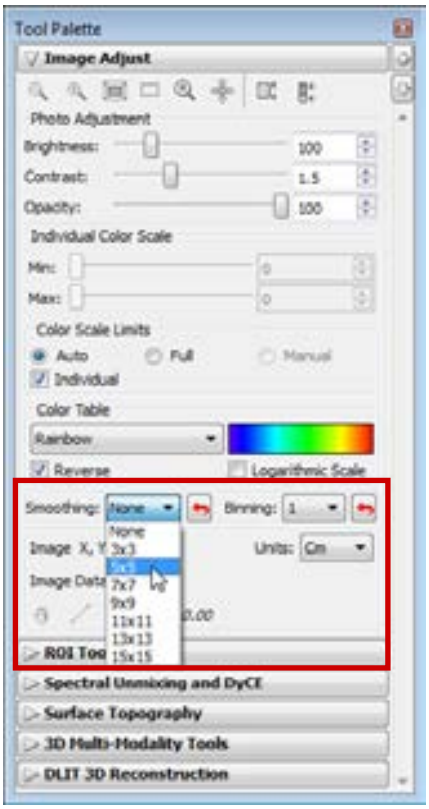
Binning = 2, smoothing = 5x5

To set smoothing and/or binning:

1. Load an image or a sequence.
2. Make a selection from the Smoothing and/or Binning drop-down lists in the Image Adjust tools (Figure 12.10).

   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 12.10 Select Smoothing and/or Binning Levels



12.3 Viewing Intensity Data

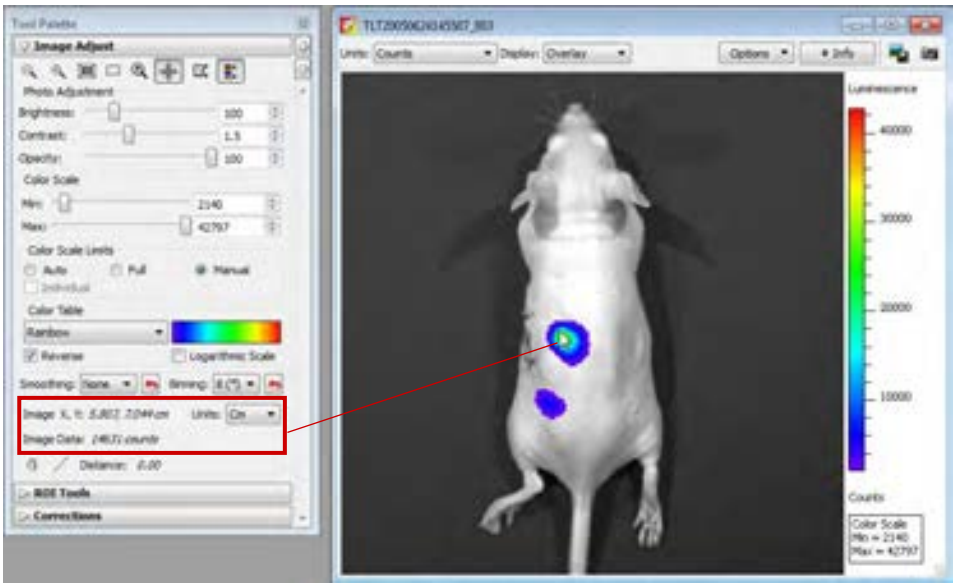
You can view intensity data:

- At a particular x,y location.
- Along a line drawn on the image.
- Within a user-selected region of interest (ROI). See Chapter 13 on page 168 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 12.11).
   - Intensity at the pixel location of the mouse pointer. The intensity is represented in the units currently selected for the image.

Figure 12.11 X,Y Coordinates and Intensity Data at the Mouse Pointer Location



Line Profile

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

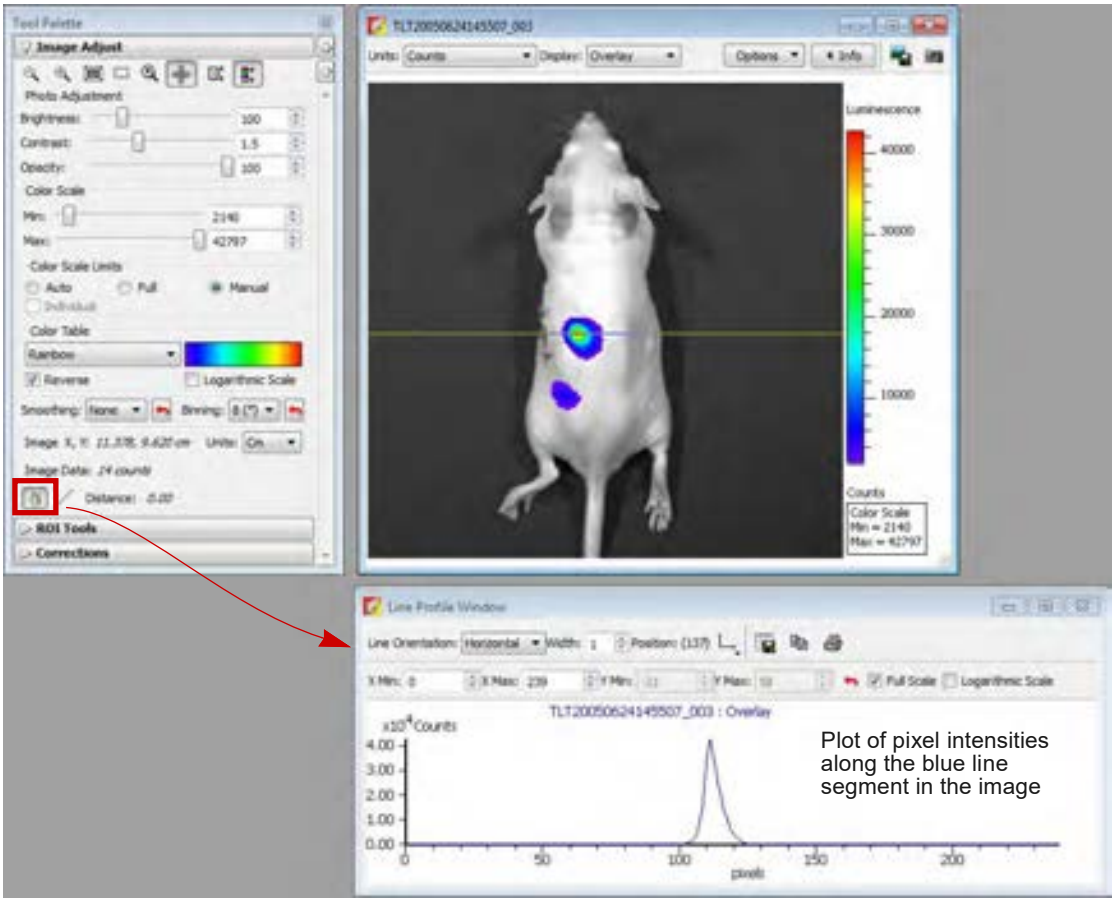
NOTE: In the Overlay display mode, the line profile plots the luminescent data. To obtain a histogram of the photograph, select Photograph from the Display drop-down list.

To display a line profile:

1. Open an image and click the **Line Profile** button in the Image Adjust tools (Figure 12.12).

   A line appears on the image and the Line Profile window opens. See Table 12.5 on page 139 for details on the Line Profile window.

Figure 12.12 View a Line Profile of Pixel Intensities



2. 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.

Table 12.5 Line Profile Window

Item	Description
Line Orientation	Choose Vertical, Horizontal, or Free Hand from the drop-down list to set the orientation of the line in the image window. The Free Hand orientation enables you to drag each line segment endpoint to a user- selected position.
Width	Sets the line width. The Line Profile window displays the average of the pixel values included in the line width.
Position	Line position (pixels).
	Enables you to choose the grid line pattern to display in the line profile window.
	Exports the line profile data to a .csv or .txt file.

Table 12.5 Line Profile Window (continued)

Item	Description
	Copies the line profile graph to the system clipboard.
	Opens the Print dialog box.
X Min X Max	Displays the minimum and maximum value of the x-axis. Use the arrows to change the x-axis min or max. If a calibrated unit such as “radiance” is selected in the image window, the x-axis units = cm. If “counts” is selected in the image window, the x-axis units = pixels. To display the range available for the Min or Max, place the mouse pointer over the Min or Max edit box.
Y Min Y Max	Displays the minimum and maximum value of the y-axis. Use the arrows to change the y-axis min or max. To display the range available for the Y Min or Y Max, place the mouse pointer over the Min or Max edit box.
	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.

12.4 Measuring Distance

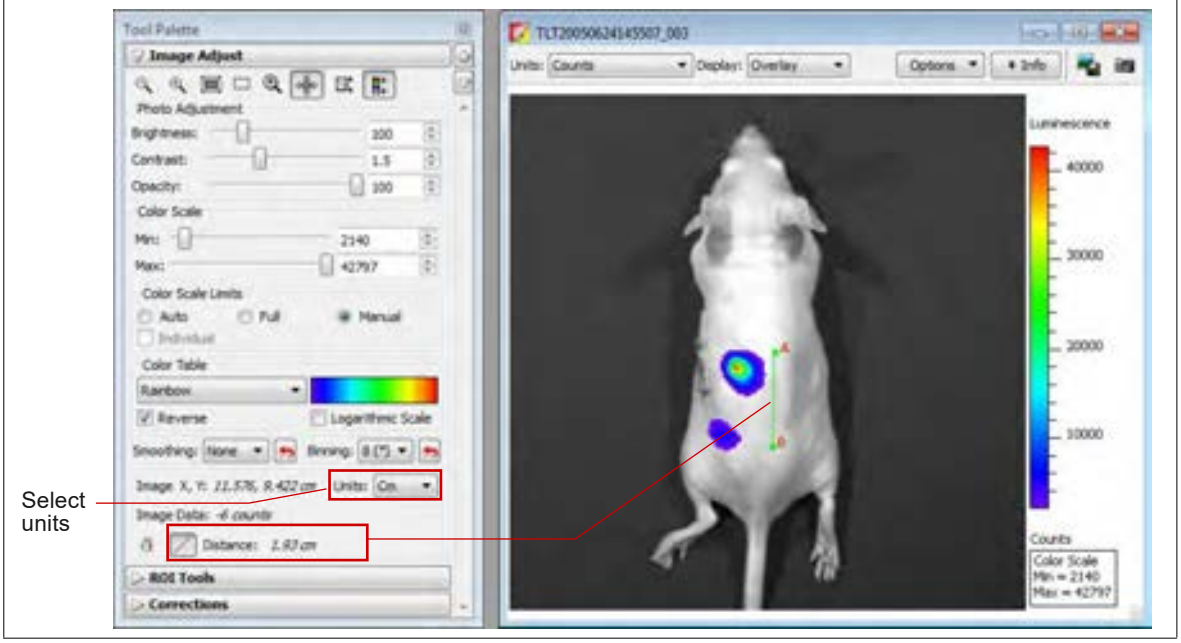
Measure distance in an image using the distance measurement tool or image crop box.

Distance Measurement Tool

1. 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 ( ) appears on the image (Figure 12.13). The Tool Palette displays the length of the cursor.

Figure 12.13 Measuring Distance Using the Measurement Tool

The Tool Palette displays the measurement tool position and length.



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 button to hide the cursor.

Image Crop Box

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 12.14).

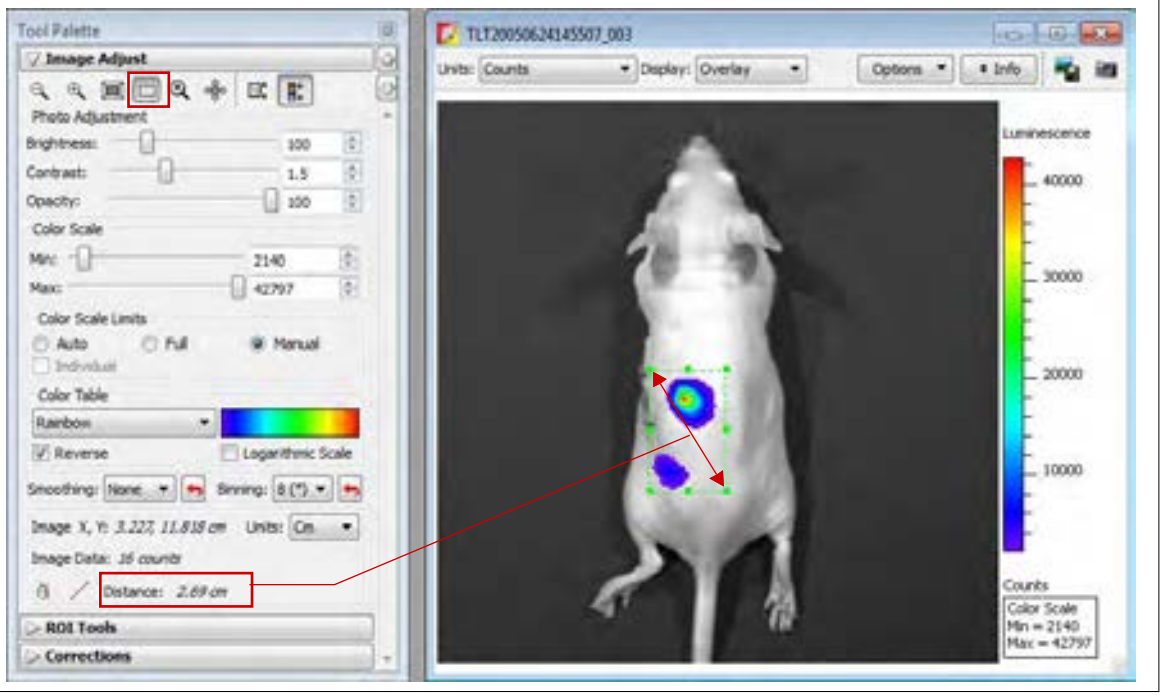
   The mouse pointer changes a "+".
3. Draw a rectangle on the image using the mouse pointer.

   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 button.

5. Click the button to hide the crop box.

Figure 12.14 Measuring Distance Using a Crop Box



12.5 Measuring Area

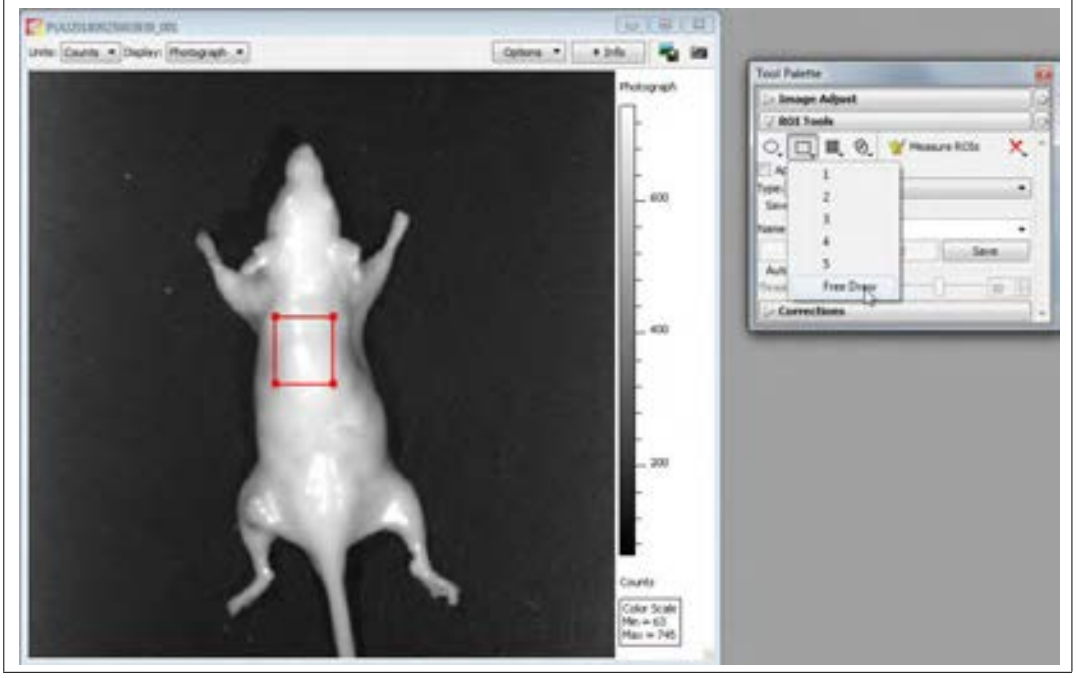
You can measure area in a photograph or photograph sequence using an ROI (region of interest).

1. Acquire a photograph or a sequence of photographs only.

NOTE: Area measurements cannot be made in a photograph from a data set which includes intensity data.

2. Apply an ROI on the area of the photograph that you want to measure. In the ROI Tools:
   a. Click an ROI shape (Circle, Square, or Contour) and select "Free Draw" from the drop-down list (Figure 12.15). If working with a photograph sequence and you want to apply the ROI to all of the photographs, select the "Apply to Sequence" option.
   b. To apply the ROI:
      - Circle or Square ROI – Drag the pointer (+) to draw and size the ROI. To adjust ROI size, click the ROI and put the mouse pointer over an ROI handle. Drag the handle when the pointer changes to .
      - 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.

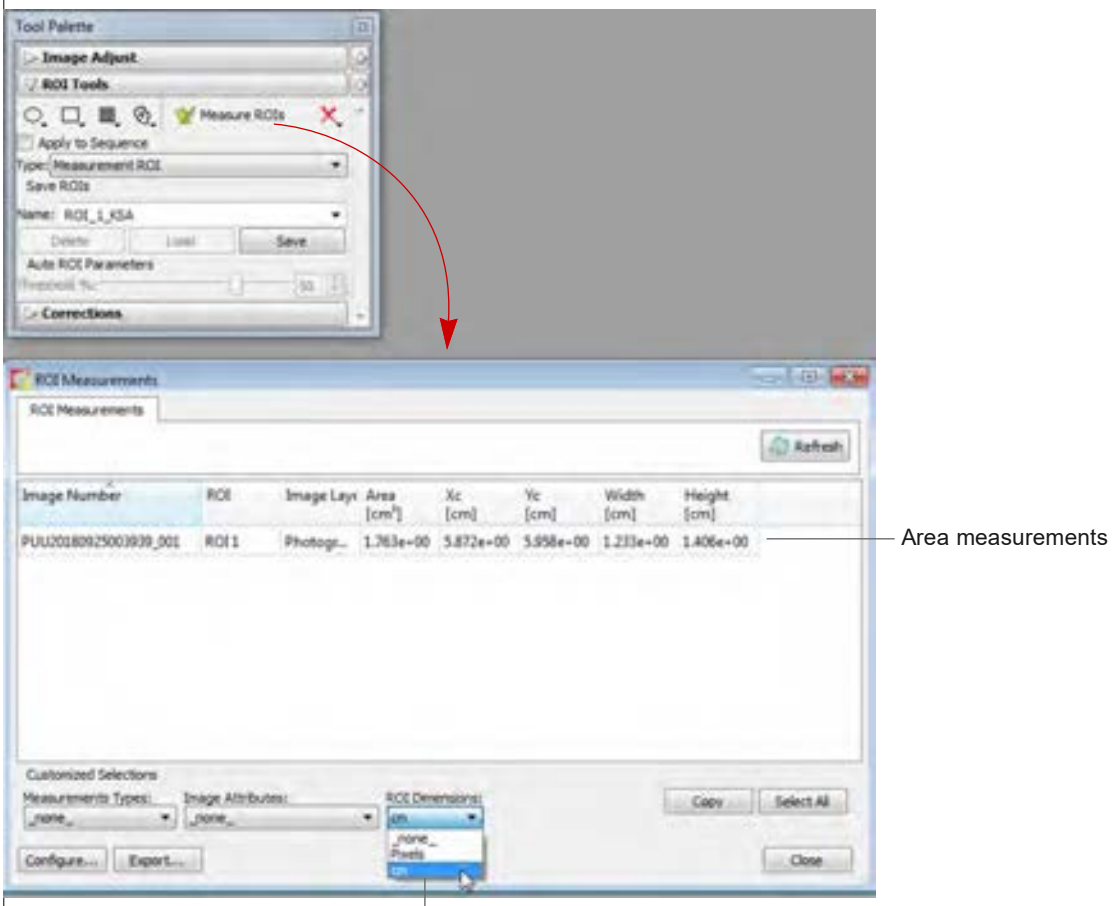
Figure 12.15 Tool Palette – ROI Tools



c. Select **Measurement ROI** from the Type drop-down list.
d. Click the **Measure** button Measure ROIs in the ROI tools.
   The ROI measurements table appears (Figure 12.16).

3. Select "Pixels" or "Cm" from the ROI Dimensions drop-down list (Figure 12.16).

Figure 12.16 Area Measurements



12.6 Combining Images Using Image Math

The Image Math tool mathematically combines two images to create a new image and it's primary use is for subtracting 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 166 for more details on creating a sequence from individual images.

TIP: See the tech note *Image Math* for a quick guide (select **Help → 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) × k, where:

A = primary image (acquired using the excitation filter)

B = background image (acquired using the background filter)

k = (primary signal/background signal)

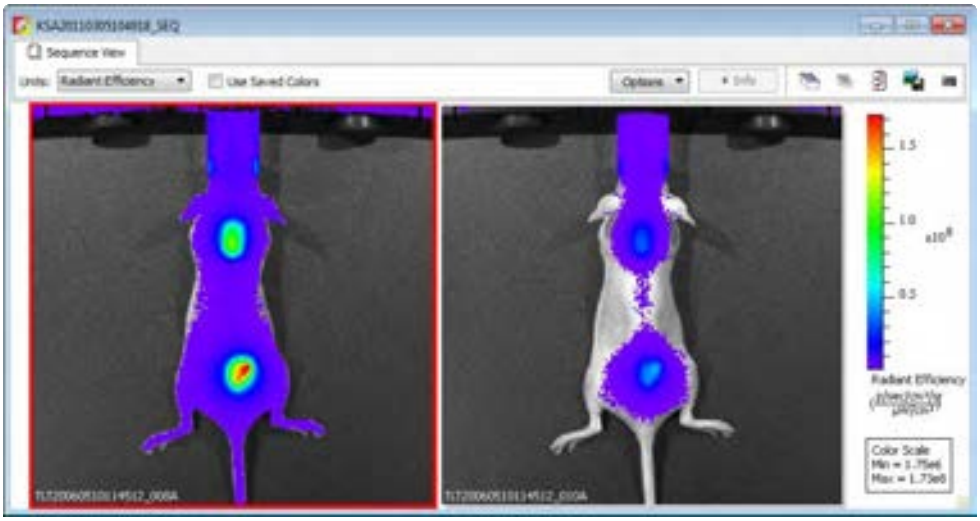
The background signal is obtained from a measurement ROI that is located in an area where no fluorophore signal is present. The scale factor k accounts for different levels of tissue autofluorescence due to different excitation wavelengths and filter transmission characteristics.

After you acquire an image sequence that includes a primary and background image, use the image math tool to subtract tissue autofluorescence. (See page 107 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.

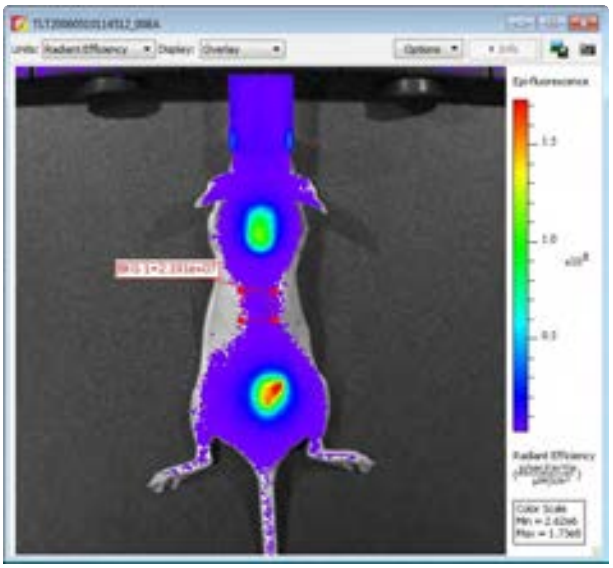
Figure 12.17 Image Sequence



2. Open either the primary or background image and:
   a. Optimize the image display using the color scale Min and Max sliders in the Image Adjust tools.
   b. Draw a measurement ROI on an area of the animal that represents background signal (area where no fluorophore signal is present, Figure 12.18).

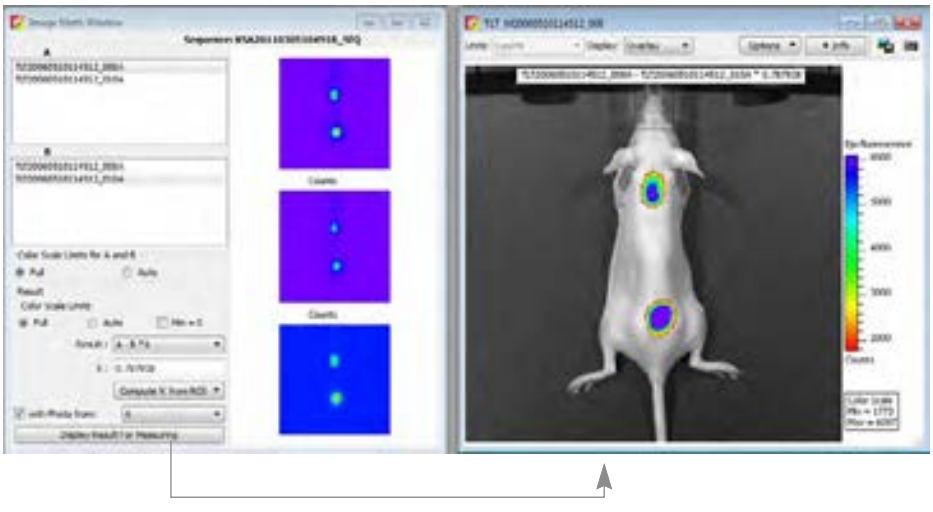
NOTE: You only need to draw the ROI on one of the images. The software copies the ROI to the other image.

Figure 12.18 Draw Measurement ROI on an Area Representative of Background Signal



3. Select **Tools** → **Image Math for <name>_SEQ** on the menu bar.
4. In the Image Math window that appears, select the primary image in box A. Select the background image in box B (Figure 12.19).
   Table 12.6 on page 149 for more details on the Image Math window.
5. Select the math function 'A-B*k' in the Result drop-down list.

Figure 12.19 Select a Math Function and View the Mathematical Result



6. Click Compute 'k' from ROI and select the ROI (created in step 2) from the drop-down list.
   The background-corrected signal is displayed.
7. To view the mathematical result (overlay mode) in a separate image window, click **Display Result For Measuring**.
   If necessary, use the Color Scale Min and Max sliders in the Image Adjust tools to adjust the image display.
8. To save the new image:

a. Click the **Save** button . Alternatively, select **File** → **Save** on the menu bar.
b. Select a directory in the dialog box that appears and click **Save**.

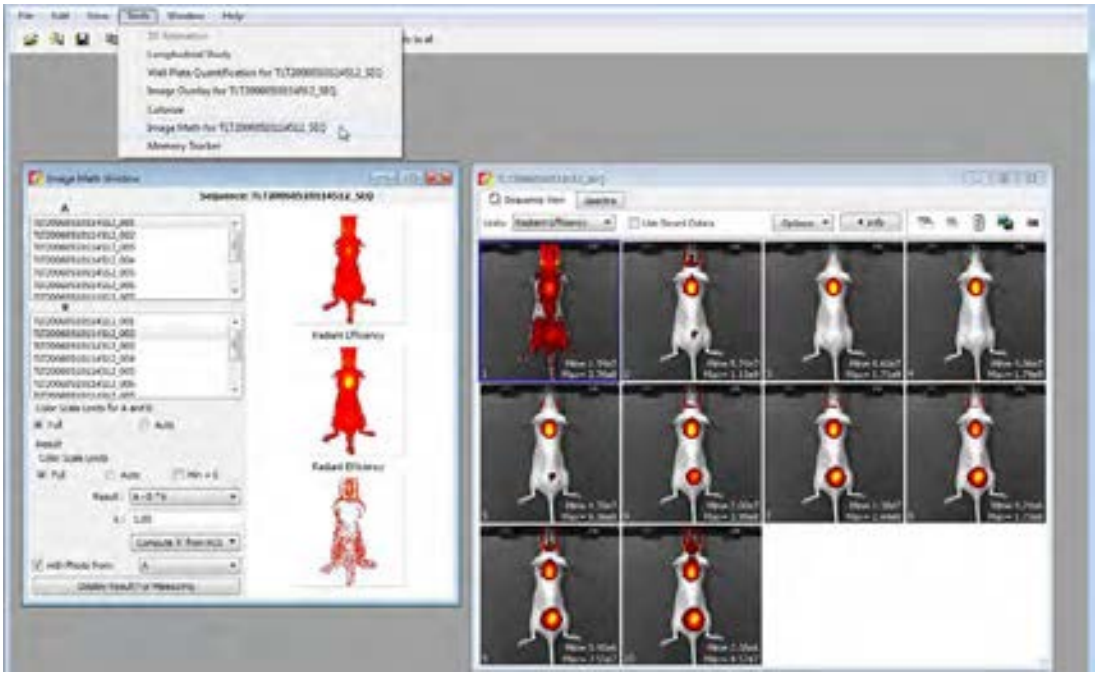
A folder of data is saved to the selected location (AnalyzedClickInfo.txt, ClickInfo.txt, luminescent and photographic TIF images).

9. To export the new image to a graphic file:
   a. Click the **Export** button .
   b. Select a directory in the dialog box that appears, enter a file name, and select the file type from the "Save as type" drop-down list.
   c. Click **Save**.

Creating a New Image

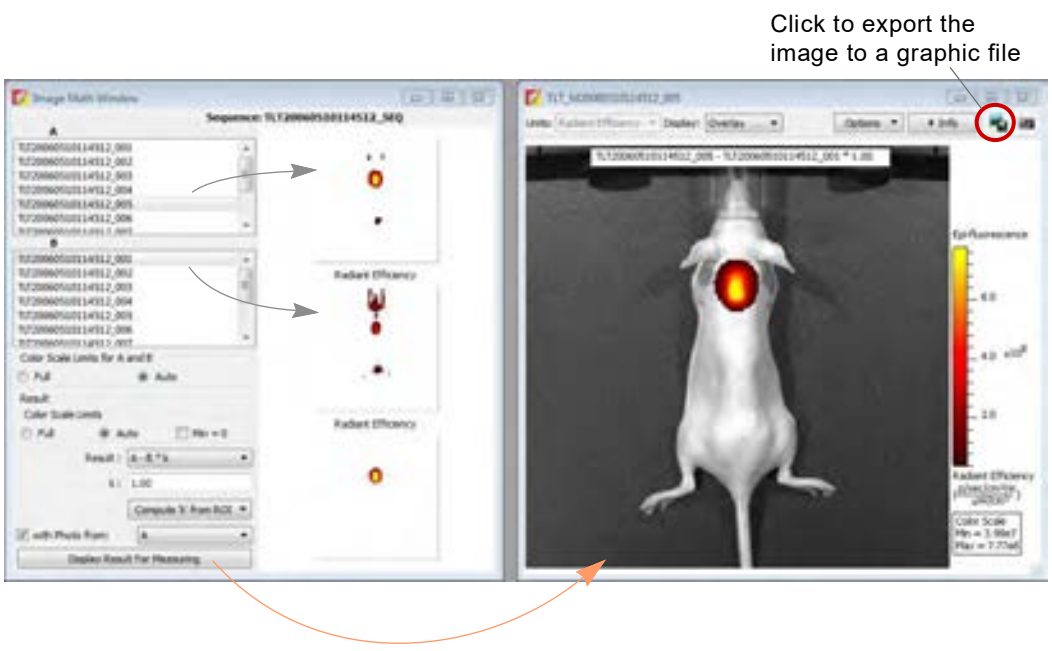
1. Load an image sequence.
2. Select **Tools** → **Image Math for <name>_SEQ** on the menu bar.

Figure 12.20 Opening the Image Math Window



3. In the Image Math window that appears, select an image from box A and from box B (Figure 12.21).
   The Image Math window shows a thumbnail of image A, image B, and the new image.

Figure 12.21 Image Math Window and New Image



NOTE: For more details on items in the Image Math window, see Table 12.6, page 149

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. Alternatively, select **File** → **Save** on the menu bar.
   b. Select a directory in the dialog box that appears and click **Save**.
      A folder of data is saved to the selected location (AnalyzedClickInfo.txt, ClickInfo.txt, luminescent and photographic TIF images).
8. To export the image to a graphic file:
   a. Click the **Export** button (Figure 12.21).
   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 12.6 Image Math Window

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

12.7 Overlaying Multiple Images

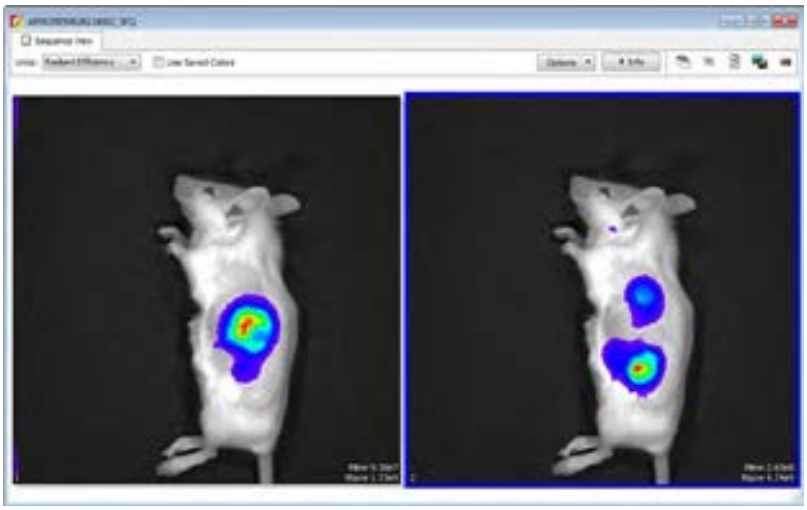
The image overlay tool provides a convenient way to view multiple reporters in one image. You can use the image overlay tool to display multiple luminescence or fluorescence images on one photographic image.

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

To 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 166.)
2. Load the image sequence.

Figure 12.22 Image Sequence



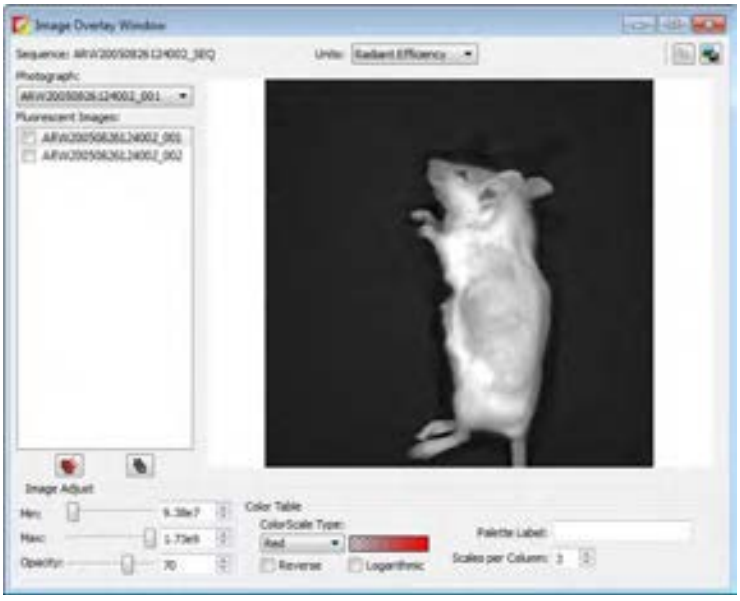
3. Open one of the images and optimize the image display using the color scale Min and Max sliders in the Image Adjust tools.

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

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

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

Figure 12.23 Image Overlay Window



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.

Figure 12.24 Generated Overlay

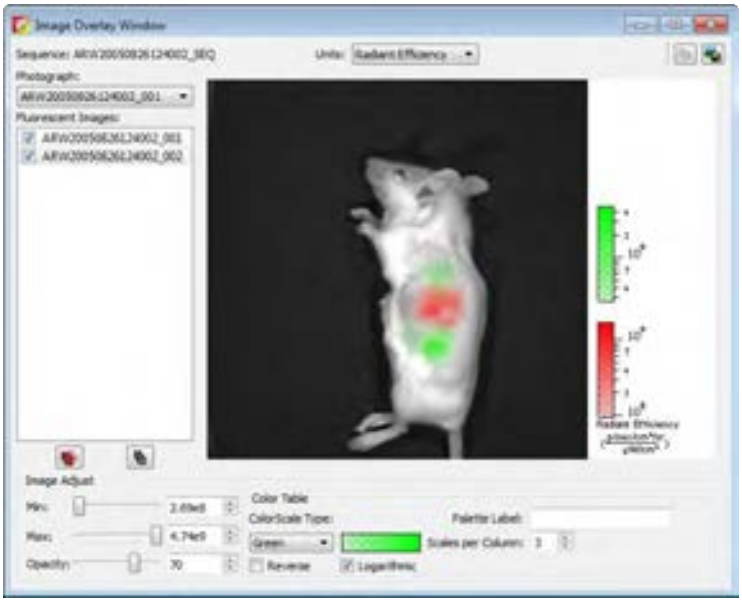


Table 12.7 Image Overlay Window

Item	Description
Units	Choose the type of units for displaying the fluorescent or luminescent image. See the concept tech note *Image Display and Measurement* 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.
	Copies the overlay to the system clipboard.
	Click to export the overlay to a graphic file.
	Click to include all fluorescent or luminescent images in the overlay.
	Click to remove all fluorescent or luminescent images from the photograph.
Image Adjust	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.

Table 12.7 Image Overlay Window (continued)

Item	Description
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.  Click the drop-down arrow to select a color table for the image data. See the concept tech note *Image Display and Measurement* for more details on color tables (select **Help → 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.

12.8 Rendering Intensity Data in Color

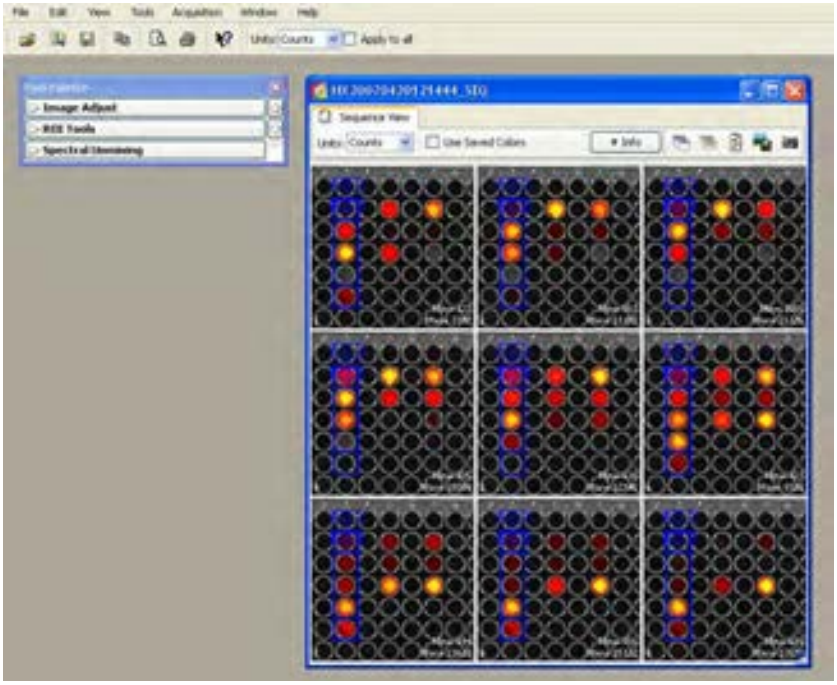
The colorize tool renders luminescence or fluorescence data in color, enabling you to see both intensity and spectral information in a single view. The tool provides a useful way to visualize multiple probes or scale probe signals that are not in the visible range.

To view colorized intensity data:

1. Load an image sequence.

Figure 12.25 Microplate Images

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



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

   The software renders each luminescent or fluorescent image in color and combines them into a single image (Figure 12.26).

Figure 12.26 Colorize View

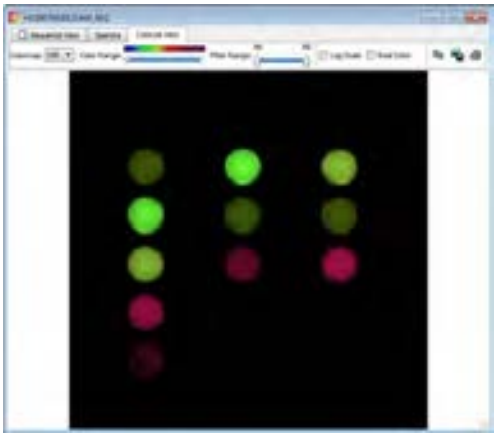


Table 12.8 Colorize Tools

Item	Description
Colorize View	

Table 12.8 Colorize Tools (continued)

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

12.9 Annotating or Tagging Images

You can add notes and tags to images to customize the image.

Adding Comments

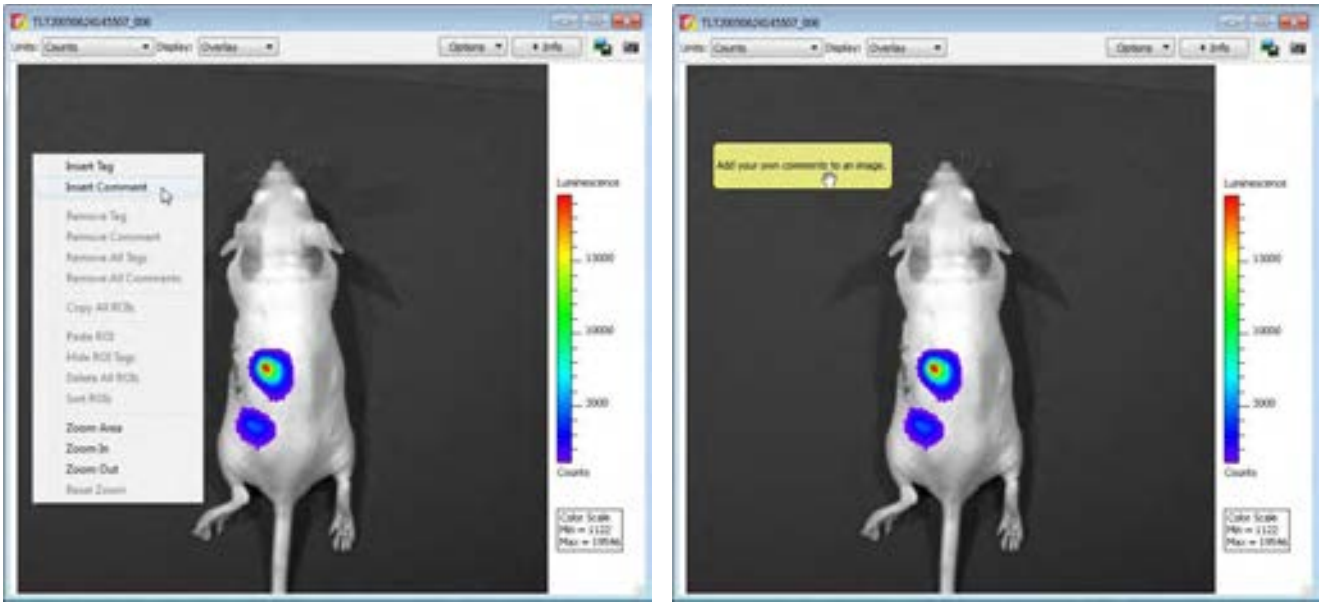
Comments can be added to an image and saved with the image.

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 12.27).

3. To move a comment in an image:
   a. Position the mouse pointer over the comment.
   b. When the hand tool appears, 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.

Figure 12.27 Adding Comments



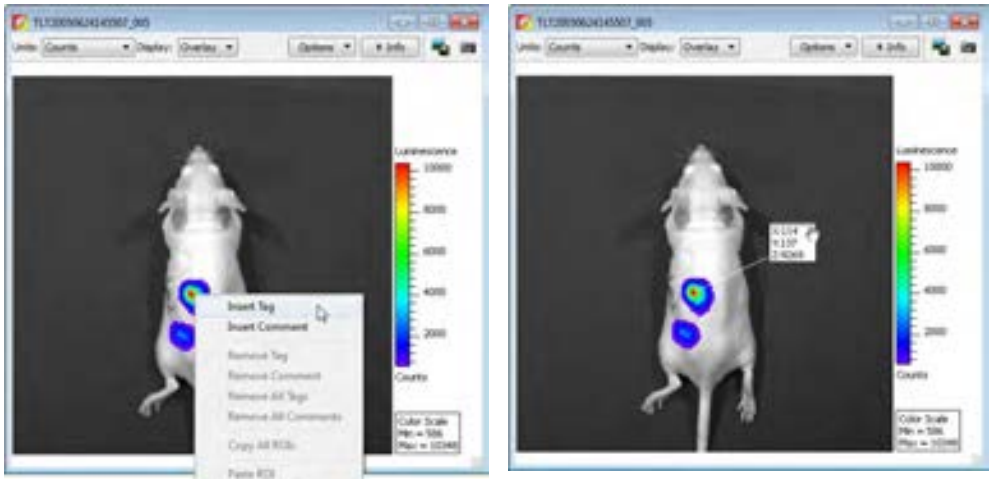
Applying Tags

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.

Figure 12.28 Tag an Image (left), Move the Tag Label (right)



3. To move a tag:
   a. Position the mouse pointer over the tag.
   b. When the hand tool appears , 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.

12.10 Image Export

- Export a single image – See below.
- Export an image sequence – See page 158.

Export a Single Image

The Image Layout window (Figure 12.29) provides an alternative way to:

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

1. 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 at a corner of the image to resize the image.
4. Drag the image to reposition it in the window.

Figure 12.29 Image Layout Window

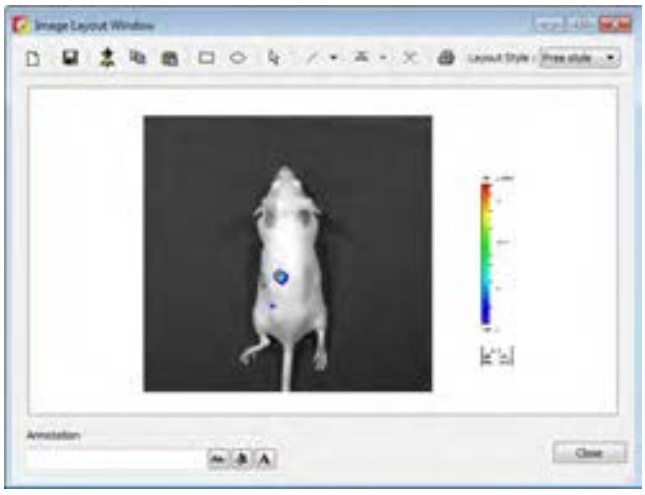


Table 12.9 Image Layout Window

Item	Description
	Clears the Image Layout window. **Note:** If you do not clear the layout (click the button) before you close the Image Layout window, the same window contents are displayed the next time the window is opened
	Opens a dialog box that enables you to save the Image Layout window contents to a graphic file.
	Pastes an image of the active data in the Image Layout window.
	Copies the contents of the Image Layout window to the system clipboard.
	Pastes the contents of the system clipboard to the Image Layout window.
	Rectangle drawing tool
	Ellipse drawing tool
	Pointer tool
	Arrow and line drawing tool

Table 12.9 Image Layout Window (continued)

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

Export an Image Sequence

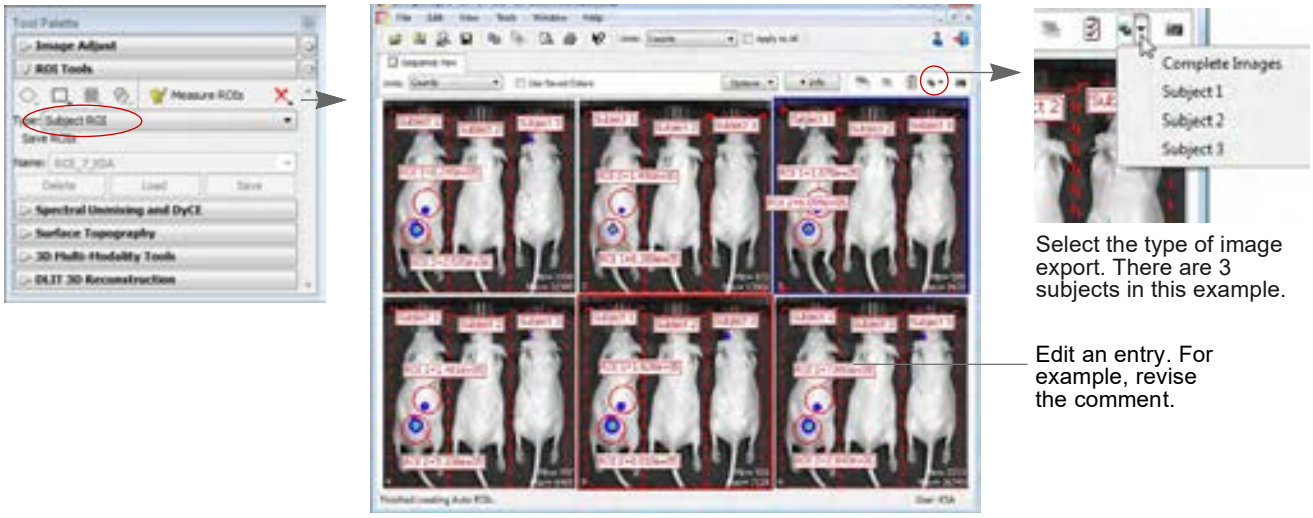
You can export sequence images or images of a user-selected subject from a sequence to a graphic file.

Prepare 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 12.30).
   a. Choose "Subject ROI" from the Type drop-down menu in the ROI tools
   b. Click the button and select **Auto All**.

Figure 12.30 Identify Each Subject With a Subject ROI



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 12.31 on page 160). See below for instructions.
   - Subject "N" – Images of the selected subject can be exported to either:
     - One file (Figure 12.34 on page 161). See page 160 for instructions.

       or
     - Separate files (Figure 12.37 on page 163). See page 162 for instructions.

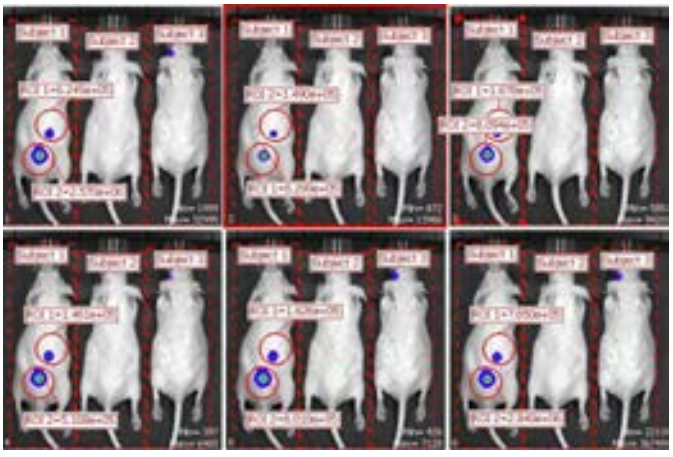
Export All Images

All images of the sequence will be exported to one file (Figure 12.31).

NOTE: Be sure to apply subject ROIs to the images before proceeding with the export. See *Prepare for Export on page 158* for instructions.

1. Click the Export Graphics button down arrow and choose "Complete Images" from the drop-down list (Figure 12.30).
2. Select the file location, file type, and enter a file name in the dialog box that appears. Click **Save**. Figure 12.31 shows an example of the exported image.

Figure 12.31 Example "Complete Images" Export



Export Images of a Subject

NOTE: Be sure to apply subject ROIs to the images before proceeding with the export. See *Prepare for Export on page 158* 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 side-by-side in a single row in the order in which they were acquired (Figure 12.34 on page 161).
- One image per file – Each image of the selected subject will be cropped to the subject ROI and exported to separate graphic file (Figure 12.37 on page 163).

Exporting the Images to a Single File

1. Click the Export Graphics button down arrow and select a subject from the drop-down list.

   The Export Graphics Options dialog box appears (Figure 12.32).

Figure 12.32 Export Graphics Option

2. Choose the "Entire sequence in one file" option, select a graphic file type, and click **OK** (Figure 12.32).
3. Select a location and enter a name for the file in the next dialog box that appears (Figure 12.33). Click **Select Folder**.

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

Figure 12.33 Select a Folder for the Export

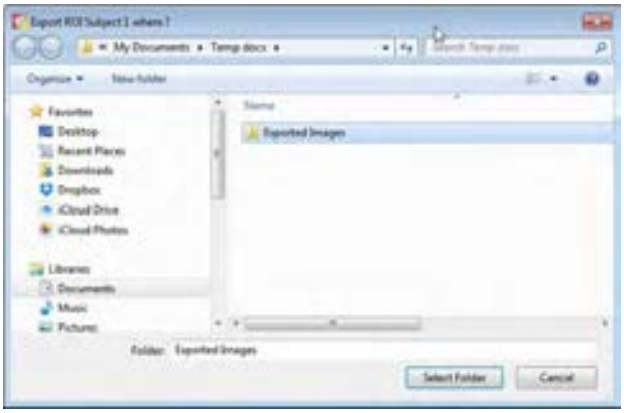
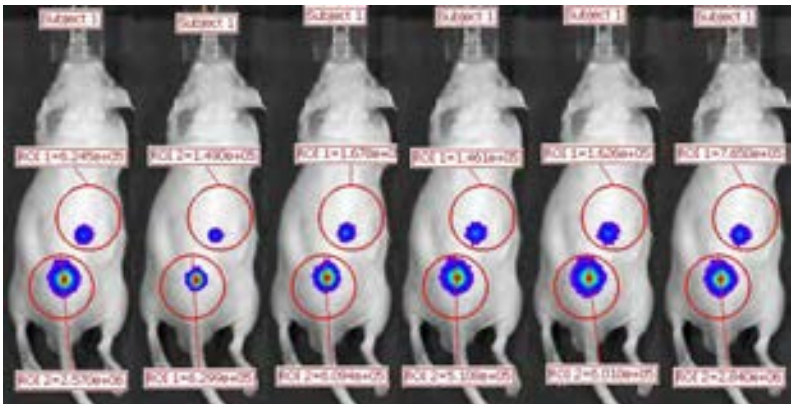


Figure 12.34 Subject Images Exported to One Graphic File



Exporting Each Image to a Separate File

1. Click the Export Graphics button down arrow and select a subject from the drop-down list.

   The Export Graphics Options dialog box appears (Figure 12.35).

Figure 12.35 Export Graphics Option

2. Choose the "One file per image" option, select a graphic file type, and click **OK** (Figure 12.35).
3. Select a folder location in the next dialog box that appears. Click **Select Folder** (Figure 12.36).

   The software will export each image to a subfolder (named with the sequence identifier "xxx_SEQ") in the selected folder (Figure 12.36).

Figure 12.36 Select a Folder for the Export

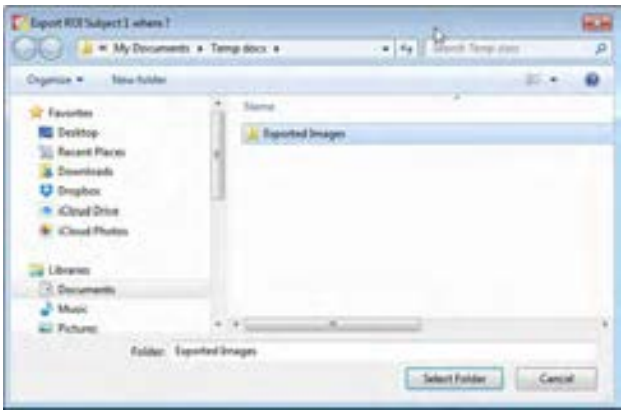
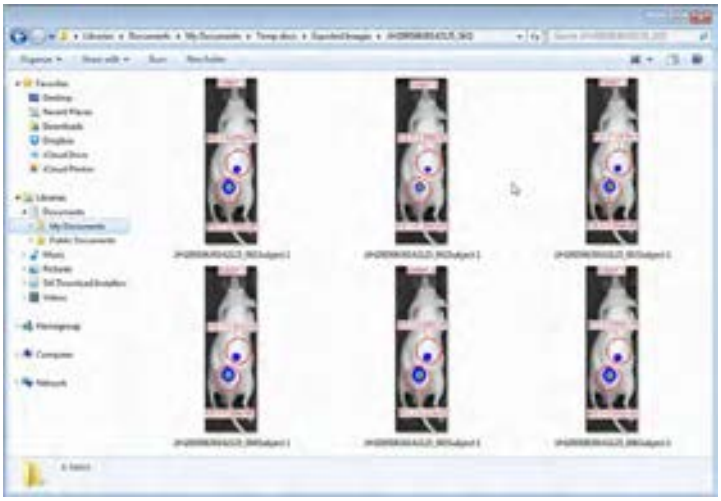


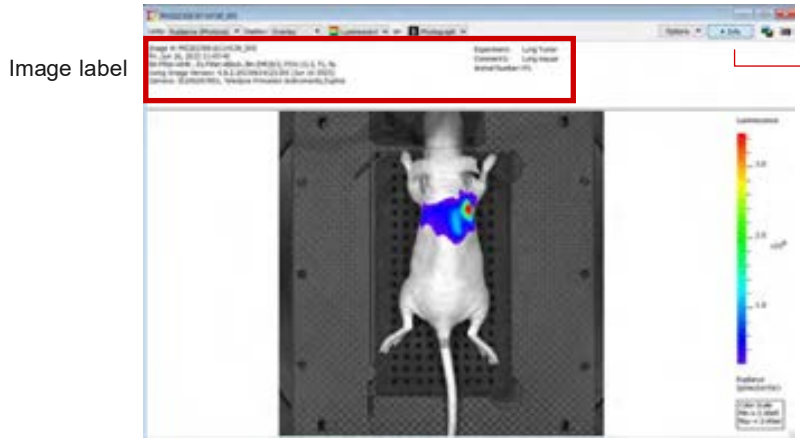
Figure 12.37 Subject Images Exported to Separate Graphic Files



12.11 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 12.38).

Figure 12.38 Image Label Information



Viewing Image Information

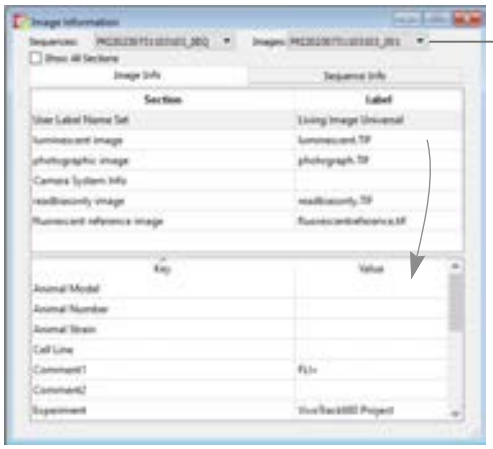
Detailed information about images is available in the View menu.

1. Open an image or sequence.
2. Select **View** → **Image Information** on the menu bar.
   The Image Information window appears.
3. Choose an image by making a selection from the Sequences drop-down list and the Images drop-down list (Figure 12.39).

Figure 12.39 Viewing Image Information

Drop-down list of open sequences. Choose **Individual Images** from the list to show the open single images in the Images drop-down list.

Choose the **Show All Sections** option to display all categories of image information.



Drop-down list of images in the selected sequence. Or a list of single images if “Individual Images” is selected in the Sequences drop-down list.

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.

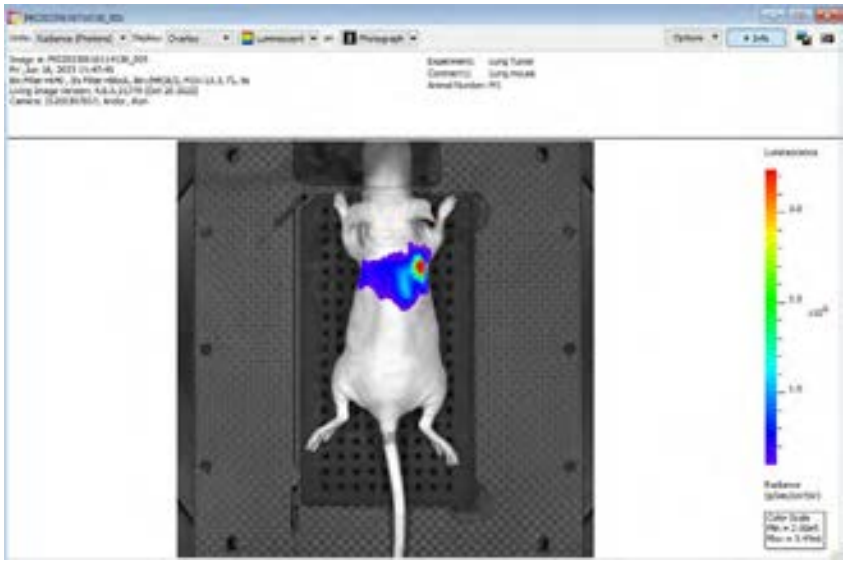
Editing the Image Label

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 12.40).

Figure 12.40 Image Information



3. Edit the label information.

To add information to the image label:

1. Click the toolbar button. Alternatively, select **Edit** → **Image Labels** on the menu bar.
2. Select information and/or enter a comment in the Edit Image Labels box that appears (Figure 12.41).

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 12.41 Edit Image Labels

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

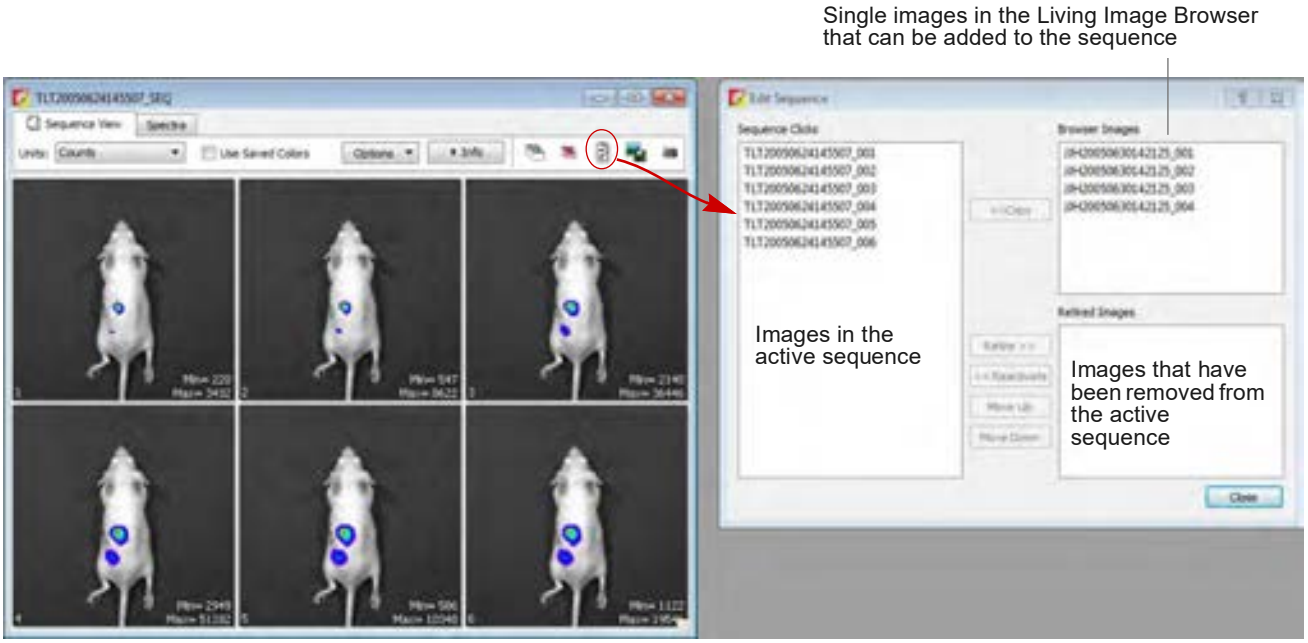
12.12 Managing Image Sequences

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 123 for more details on browsing.)
3. Click the **Edit** button in the image window (Figure 12.42).

Figure 12.42 Editing a Sequence



4. Choose the image(s) to add or remove (*retire*) from the sequence in the Edit Sequence box that appears (Figure 12.42).

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

5. To restore a retired image to the sequence, select the retired image and click **Reactivate**.

6. To reorder the sequence, select an image and click **Move Up** or **Move Down**.

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

7. Click **Close** when you are finished editing the sequence.

   The updated image sequence is displayed.

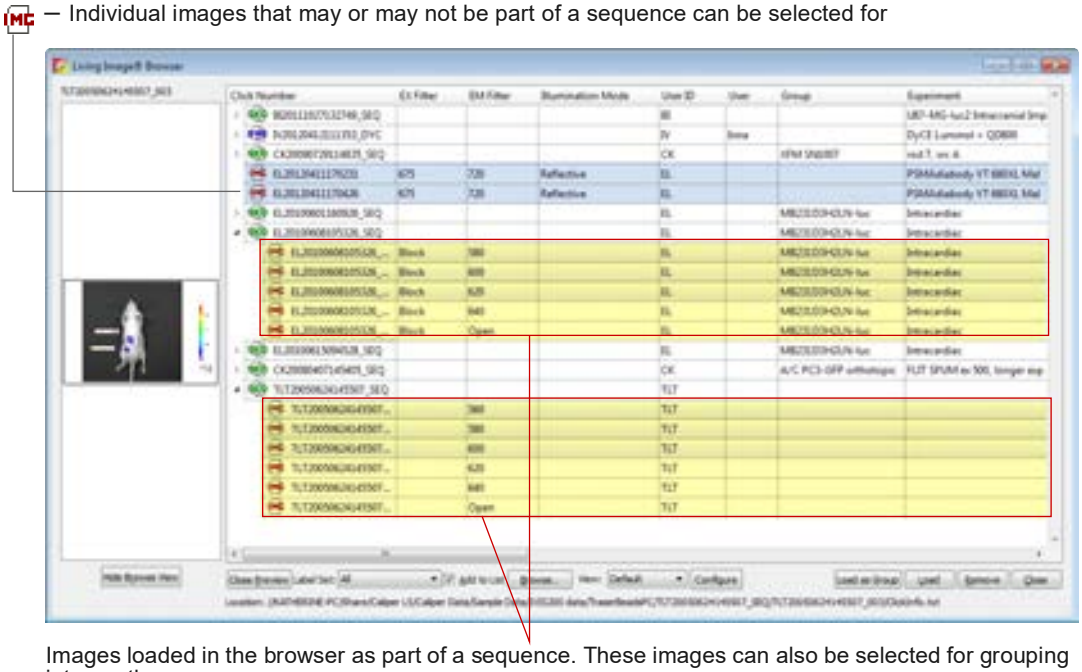
Creating a Sequence From Individual Images

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

1. Browse for the images of interest in the Living Image Browser. (See page 123 for more details on browsing.)

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

Figure 12.43 Living Image Browser



2. In the browser, select the images that you want to group together (Figure 12.43).

   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. Alternatively, select **File** → **Save** on the menu bar.

   b. Select a folder in the dialog box that appears and click **OK**.

13 Measuring Signal in 2D Image Data

13.1 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 13.1).

Table 13.1 Types of ROIs

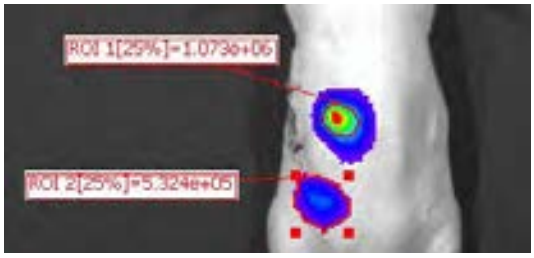
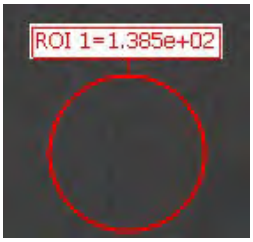
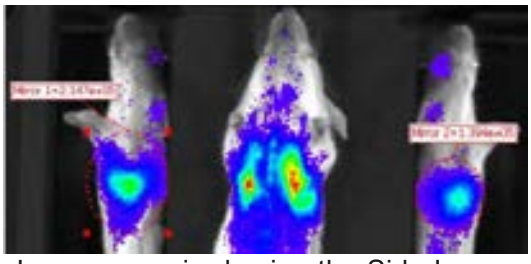
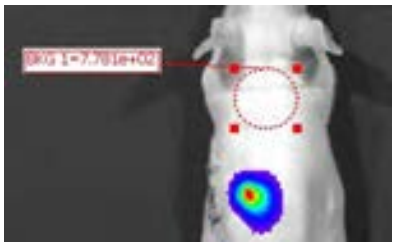
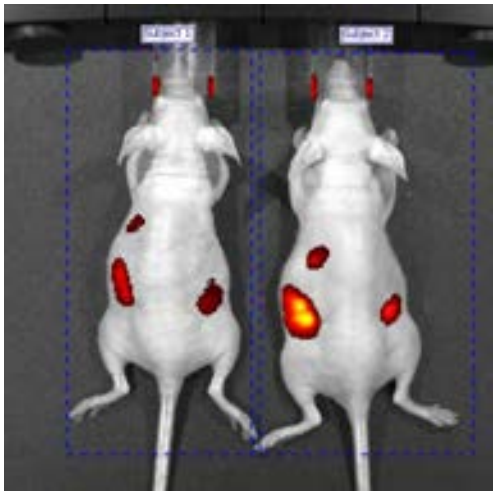
ROI Name	Description	ROI Line and Available Shapes	See Page
Measurement ROI	Measures the signal (surface intensity) in an area of an image. 	 Circle, square, grid, or contour	172
Mirror ROI	 Images acquired using the Side Imager have three views: left, right, and center. ▪ Left or right images – Measure signal using a mirror ROI. ▪ Center image (non-reflected view) – Measure signal using a measurement ROI.	 Circle or square	181

Table 13.1 Types of ROIs (continued)

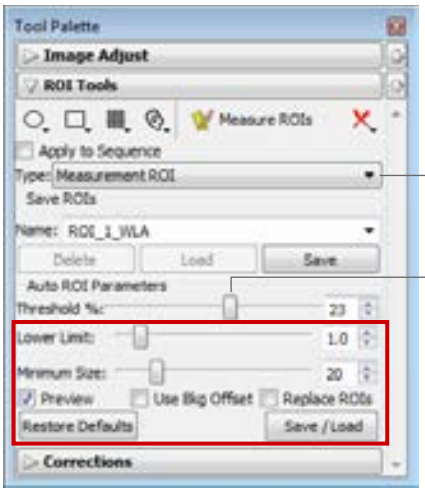
ROI Name	Description	ROI Line and Available Shapes	See Page
Average Background ROI	Measures the average signal (surface intensity) in a user-specified area of an image that is considered background. Only available in image view. **Note:** Using this type of ROI is optional. If the animal has significant 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	177
Subject ROI	Identifies a subject in an image. **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. 	 Square	179

13.2 Overview of ROI Tools

The ROI tools that appear in the Tool Palette depend on the type of ROI selected from the Type drop-down list, and whether an image or sequence is active. Table 13.2 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 191* for more information.

Figure 13.1 ROI Tools



Type of ROI

Adjustment for “Auto” ROIs

These Auto ROI parameters are available if “Show Advanced Options” is selected in the General Preferences. See Appendix D on page 424 for more details on setting Preferences.

Table 13.2 ROI Tools

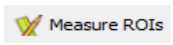
Item	Description
	Click to apply circle ROIs to an image or sequence.
	Click to apply square ROIs to an image or sequence.
	Click to specify the grid ROI to an image or sequence. This tool is useful for an image of a multi-well culture plate or microplate.
	Click to apply contour ROIs to an image or 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 13.3 on page 175 for more details on using the auto ROI features.
	Click to display the ROI Measurements table or compute intensity signal in an ROI. See *ROI Measurements on page 191* for more information.

Table 13.2 ROI Tools (continued)

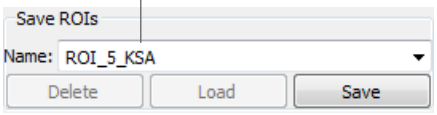
Item	Description
	Click to display a drop-down list of ROI delete options for the active image data. These commands delete ROIs from image data. If an image is active and the "Apply to Sequence" option is selected, the delete operation is applied to all images in the sequence. **Note:** These commands do not delete named ROIs that are saved to the system (ROIs in the Name drop-down list).
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 13.3 on page 175 for more information about related ROIs. ▪ Deleting an ROI in the image will delete related ROIs from the other images of the sequence.
Type	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 system	Name of an ROI or a set of ROIs  These ROI tools are only 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 184 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	Parameters that specify how the auto ROI tool draws an ROI. **Threshold %** – If the Auto All or Auto 1 method is selected, the Threshold % specifies the minimum percent of peak pixel intensity that a pixel must have to be included in an ROI identified by the software. After ROIs are drawn on an image, if you modify the Threshold% (move the slider or enter a new value), the software automatically updates the ROIs.  **Note:** These Auto ROI parameters are only available if “Show Advanced Options” is selected in the general preferences. See Appendix D on page 424 for details on setting preferences.

Table 13.2 ROI Tools (continued)

Item	Description
	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 177 for more details. **Replace ROIs** – If this option is chosen, all auto ROIs are replaced when new ROI(s) are created.
Auto ROI Parameters	**Restore Defaults** – Restores the factory-set defaults for the auto ROI 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.

13.3 Measuring Signal

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

1. Open an image or image sequence.

   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.

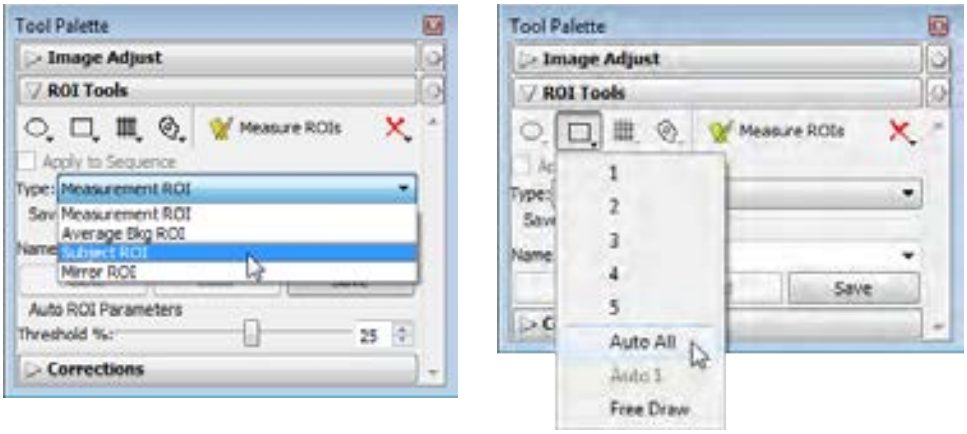
   NOTE: Ensure that the ROI table configuration includes "Subject" and/or "Subject Label" (if the subject ROI label was renamed). See *Creating a Custom ROI Table Configuration on page 194* for instructions.

2. If there are multiple subjects in an image, apply subject ROIs. If not, skip to step 3.

   a. Select **Subject ROI** from the Type drop-down list (Figure 13.2).

b. Click the square ROI shape and select **Auto All** from the drop-down list.

Figure 13.2 ROI Tools – Apply Subject ROIs



3. Select **Measurement ROI** from the Type drop-down list
4. Click an ROI shape (Circle , Square , Grid , or Contour) 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 13.3).

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 176 for instructions on resizing ROIs). Measurement ROIs not completely within a subject ROI will not be counted as part of the subject.

Figure 13.3 Example ROIs

ROI shortcut menu

Right-click an ROI or image (Ctrl-click for Macintosh users) to view a shortcut menu for easy access to many functions for managing ROIs and viewing ROI properties.

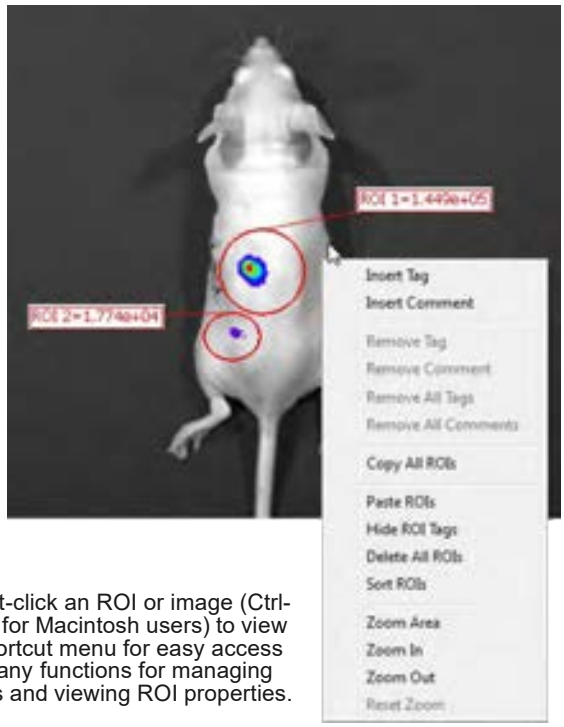


Image shortcut menu

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 13.3 ROI Drawing Tools

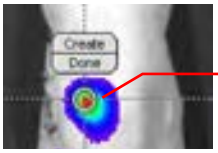
Item	Description
	Select the number of ROIs to apply. The software places up to five ROIs on an image or each image of a sequence, whichever is the active view. ROI position and size will need manual adjustment. See Table 13.4 on page 176 for instructions on adjusting ROIs. Using this method in sequence 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. 
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.  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 *Managing ROIs on page 184* 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.

Table 13.3 ROI Drawing Tools (continued)

Item	Description
Free Draw	Only available in image view. To draw a: ▪ Circle or square 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.

5. Adjust ROI size or position if necessary (see Table 13.4).

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 13.12 on page 186).
- Image view – Right-click the ROI and select unlock options from the shortcut menu.

Table 13.4 Select and Adjust ROIs

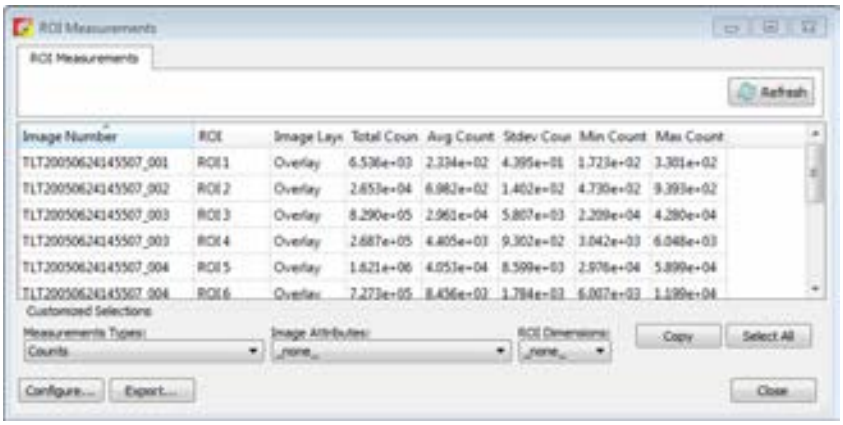
Operation	Single ROI	Multiple ROIs	Related ROIs Applied in Sequence View
Select	Click the ROI border. This will clear a previous ROI selection in the image.	Shift-click the border of each ROI. This does not clear a previous ROI selection in the image.	Press and hold the Control key while you click an ROI border.
Move	Put the mouse pointer over the ROI border. Drag the ROI when the pointer changes to . **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.	Select multiple ROIs and drag them when the pointer changes to . **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.	Press and hold the Control key while you move a selected ROI.
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.

6. Click the **Measure** button Measure ROIs in the ROI tools to show the ROI Measurements table (Figure 13.4).

   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 191* for more details.

Figure 13.4 ROI Measurements Table



7. Click **Yes** in the prompt when closing the data to save the ROIs with the image 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 *Saving ROIs to the System on page 190* for instructions.

13.4 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.

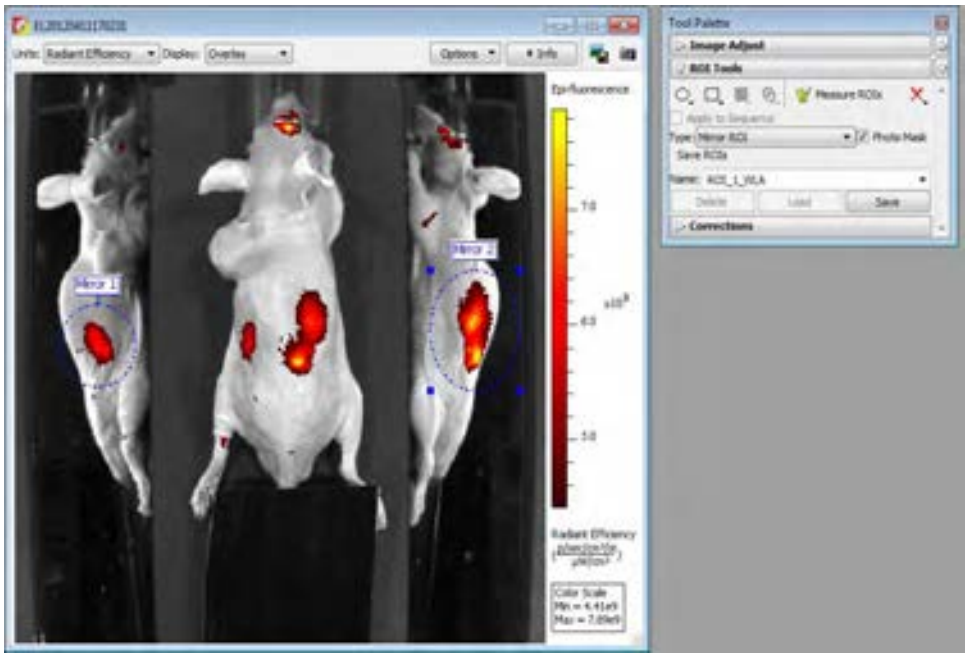
The Image Adjust tools and zoom feature are helpful for selecting an appropriate area for an ROI. By setting the image minimum close to zero and zooming in on a background area in the image, you can determine where naturally occurring background luminescence or autofluorescence is present. For more details on the Image Adjust tools and the zoom feature, see *Viewing Intensity Data on page 137* and *Zooming or Panning on page 135*.

To measure background-corrected signal:

1. Open an image and draw one or more measurement ROIs on the subject (see page 172 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 13.4 on page 176 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 13.5).

Figure 13.5 Draw a Background ROI on Each Image View Acquired Using the Side Imager



3. Associate each background ROI with a measurement ROI(s) or mirror ROI(s) using one of the methods in Table 13.5.

Table 13.5 Methods for Associating Measurement or Mirror ROIs With a Background ROI

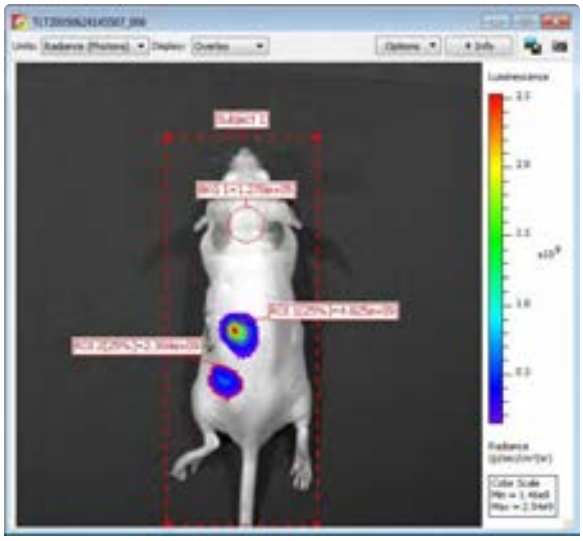
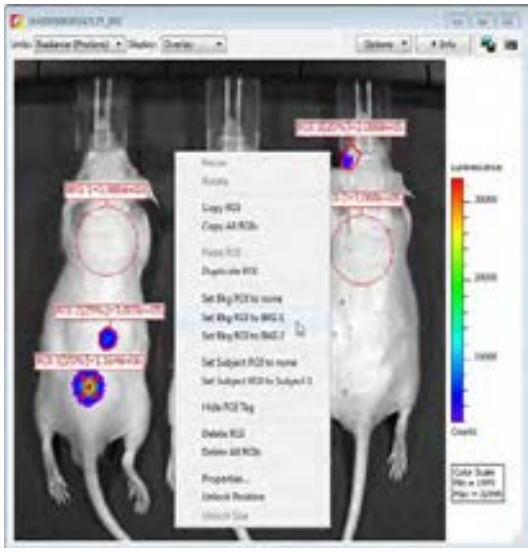
Methods for Associating ROIs	Example
Use a subject ROI to identify a subject in an image and automatically associate a measurement and average background ROI for background-corrected ROI measurements. To draw a subject ROI: **1.** Select “Subject ROI” from the Type drop-down list in the ROI tools. **2.** To apply ROIs: Automatic – Click the button and select Auto All. Manual – Click the button and select “1”. Position the subject ROI so that it includes the measurement ROI(s) and the associated average background ROI.	
Right-click a measurement ROI and select an average background ROI from the shortcut menu.	

Table 13.5 Methods for Associating Measurement or Mirror ROIs With a Background ROI (continued)

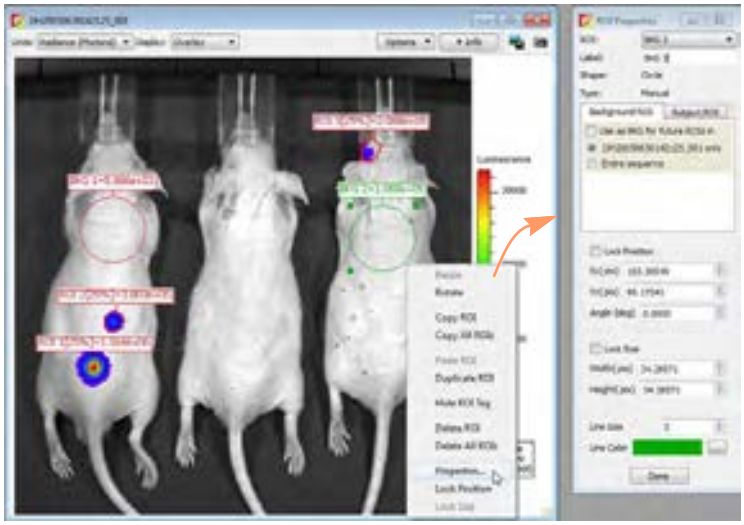
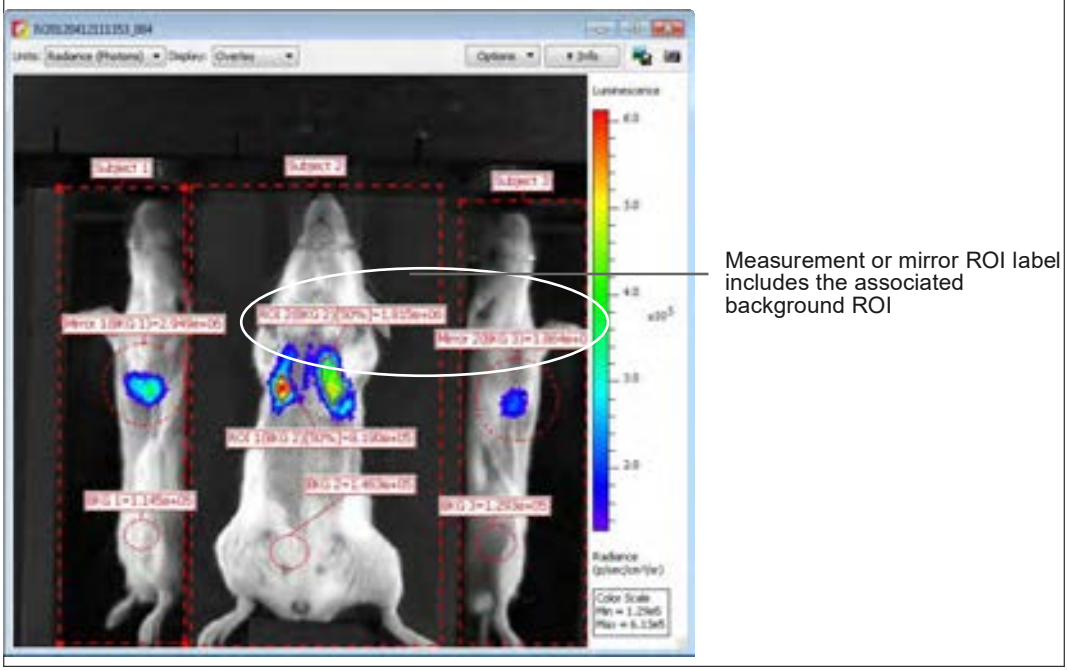
Methods for Associating ROIs	Example
1. Right-click a background ROI and select **Properties** on the shortcut menu. 2. In the ROI Properties box that appears, click the Background ROI tab and put a check mark next to **Use as BKG for future ROIs in**. 3. Choose the image name or the **Entire sequence** option.	

Figure 13.6 Measurement ROIs Successfully Associated with a Background ROI



13.5 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 172 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 13.7). If analyzing a fluorescent image, choose the Photo Mask option.

Figure 13.7 ROI Tools

3. Select the ROI shape:
   a. Click the **Circle**, or **Square**, button.
   b. Select the number of ROIs to add to the image on the drop-down list that appears.
      - If analyzing a reflectance/epi-illumination fluorescent image, go to step 4; otherwise, go to step 5.
4. For reflectance/epi-illumination fluorescent images only:
   a. Confirm the purple data mask in the dialog box that appears (Figure 13.8).

      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 13.6).
   b. Click **OK**.

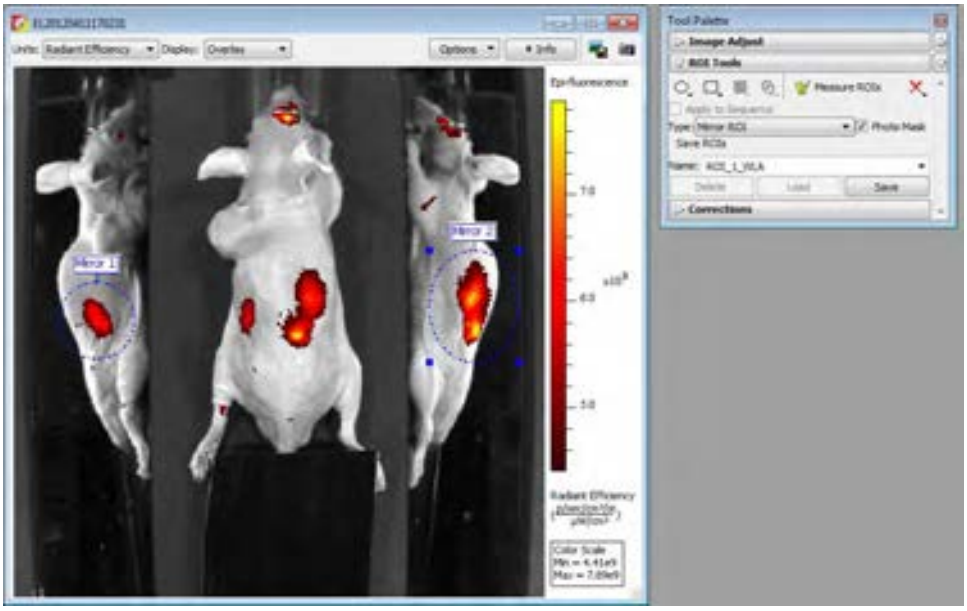
      The mirror ROIs and intensity measurements appear on the image (Figure 13.9 on page 183). Right-click an ROI to view a shortcut menu of ROI commands (Ctrl-click for Macintosh users). The shortcut menu provides easy access to many functions for managing ROIs and viewing ROI properties.

Figure 13.8 Excitation Projection Setup Dialog Box (For fluorescent images only.)

Table 13.6 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.
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.

Figure 13.9 Mirror ROIs on Fluorescent Image Acquired with Side Imager



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 13.4 on page 176,
6. Click the **Measure** button Measure ROIs.

The ROI measurements and table appear. See *ROI Measurements on page 191* for more details.

7. 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 page 190 for instructions.

13.6 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 13.12).
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 13.10).

Figure 13.10 Viewing ROI Properties

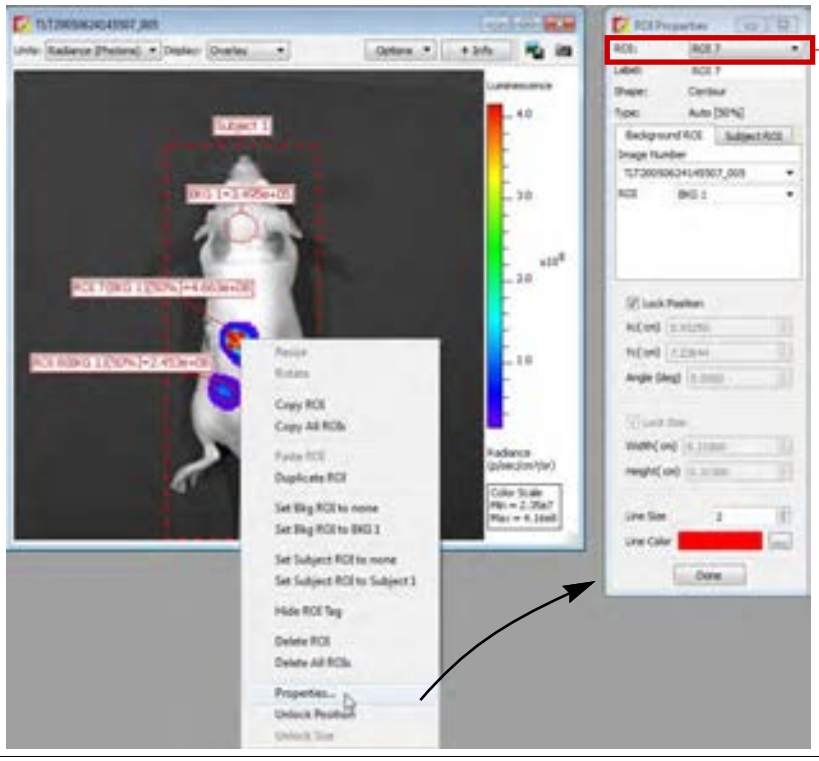


Figure 13.11 ROI Properties – Background ROI Tab

The items in the ROI Properties box depend on the type of ROI selected in the image. See Table 13.7, page 186 for more details.

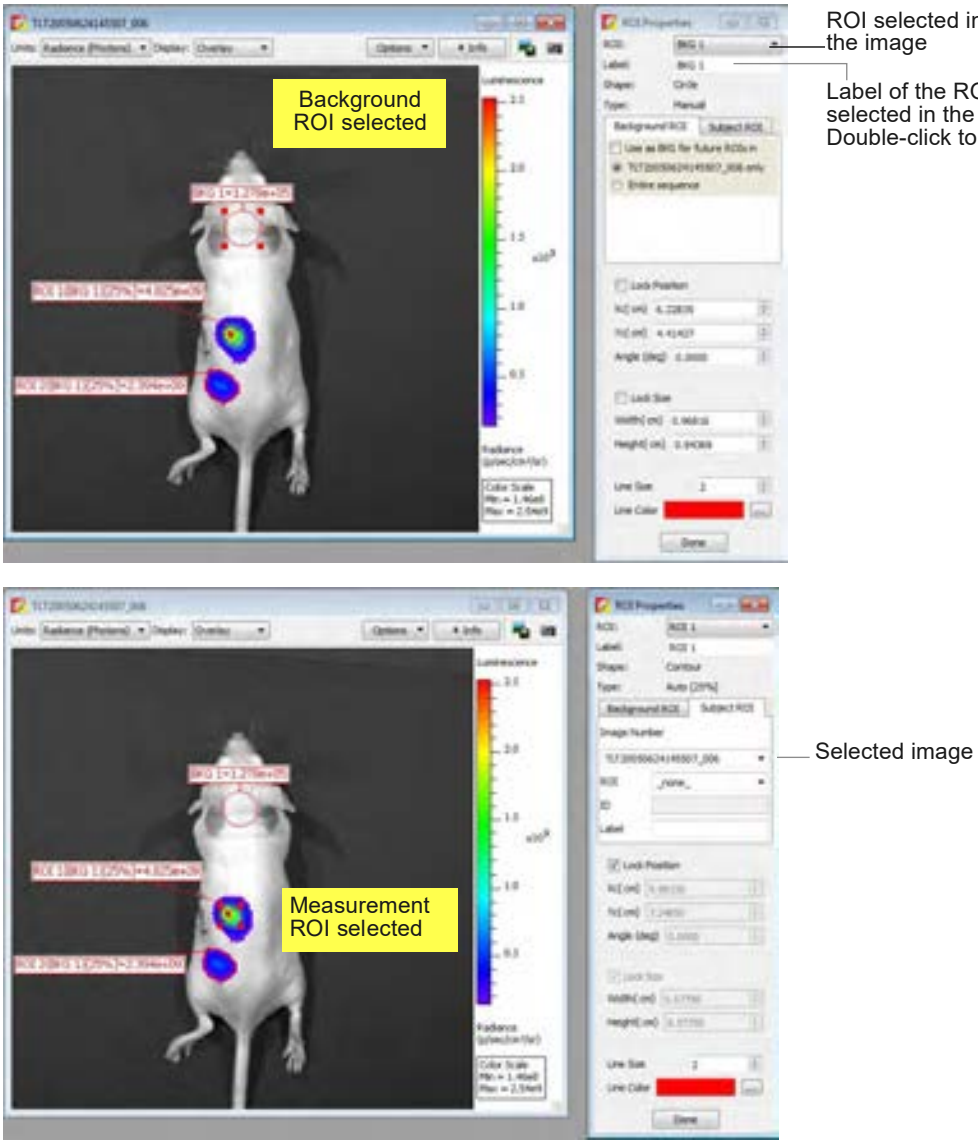


Figure 13.12 ROI Properties – Subject Tab

The items in the ROI Properties box depend on the type of ROI selected in the image. See Table 13.7 for more details.

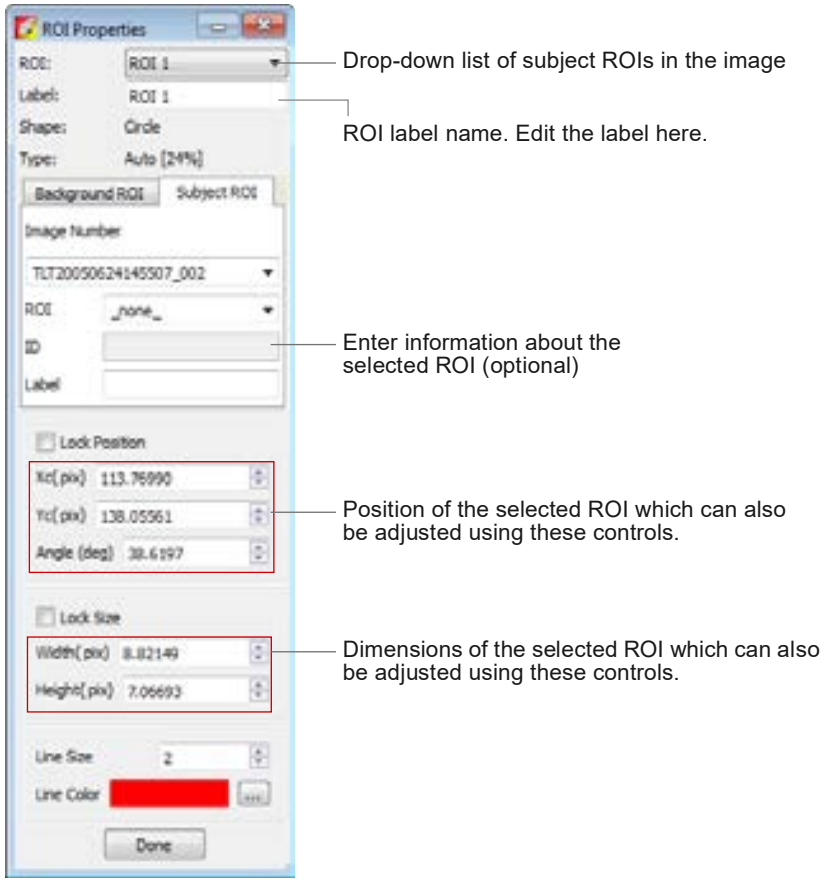


Table 13.7 ROI Properties

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

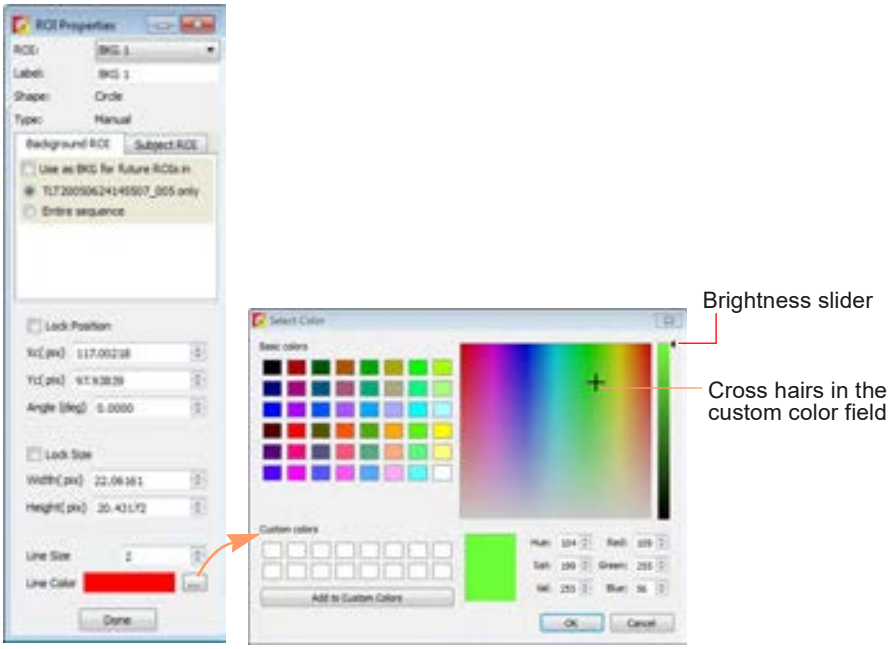
Table 13.7 ROI Properties (continued)

Item	Description
Subj ROI	The Subject ROI tab shows a drop-down list of all subject ROIs in the image number selected above that can be linked to a user-specified measurement ROI or average background ROI (selected from the drop-down list at the top of the dialog box). The Background ROI tab shows a drop-down list of all average background ROIs in the click number selected above that can be linked to a user-specified measurement ROI or subject ROI (selected from the drop-down list at the top of the dialog box).
ID	User-entered information about a subject ROI.
Label	Label name of the selected subject ROI.
Lock Position	Choose this option to lock the position of the ROI selected in the image.
Xc	x-axis coordinate at the center of the ROI selected in the image.
Yc	y-axis coordinate at the center of the ROI selected in the image.
Lock Size	Choose this option to lock the dimensions of the ROI selected in the image.
Width	Width (pixels or cm) of the ROI selected in the image (see *ROI Dimensions on page 192* for more details on setting the units). Edit this value to resize an ROI, except for ROIs applied using the “Auto All” 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 All” 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 .
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 13.5 on page 179 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 13.13).

Figure 13.13 Editing ROI Properties



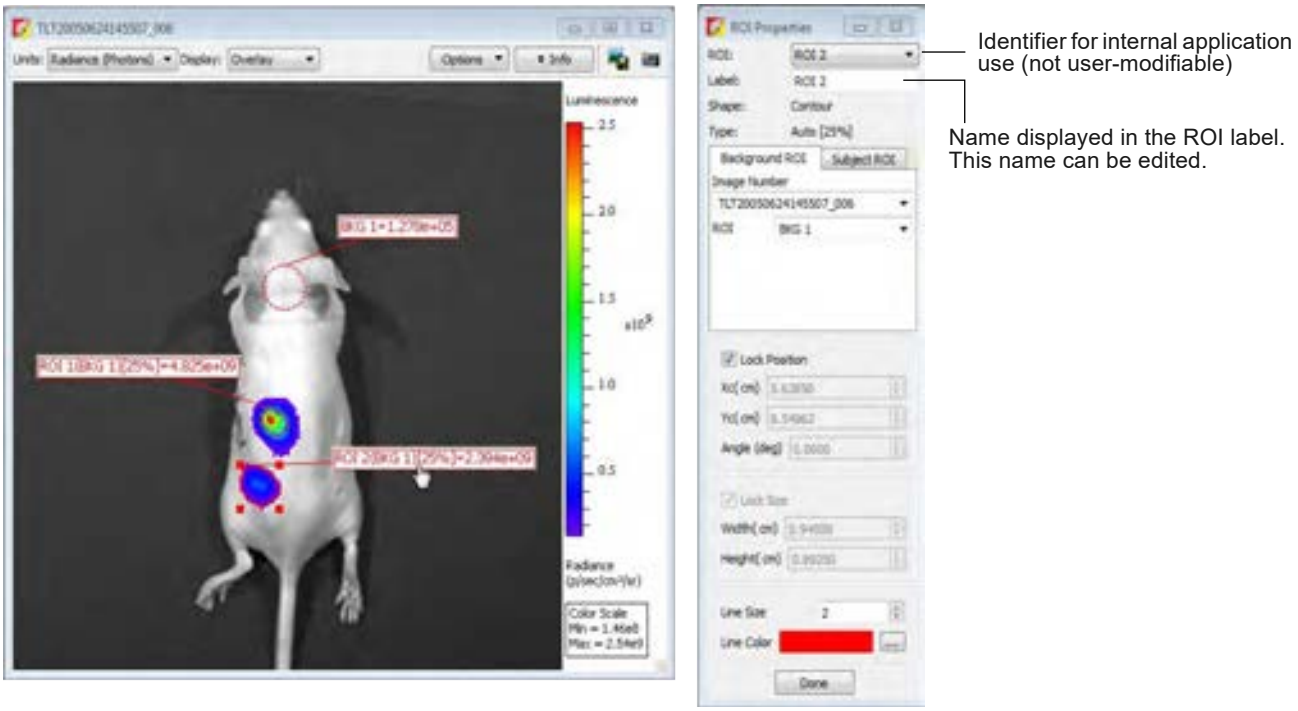
2. To edit the ROI line thickness, enter a new value in the Line Size box. Alternatively, click the arrows.
3. To change the ROI line color:
   a. Click the **Browse** button .
      The Select Color box appears.
   b. Select a basic color or create a custom color for the ROI line:
      - Select a basic 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.
2. When the pointer becomes a , drag the label, then click to release the label at the new location (Figure 13.14).

Figure 13.14 Move or Edit the ROI Label



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 13.14).

Saving ROIs to the System

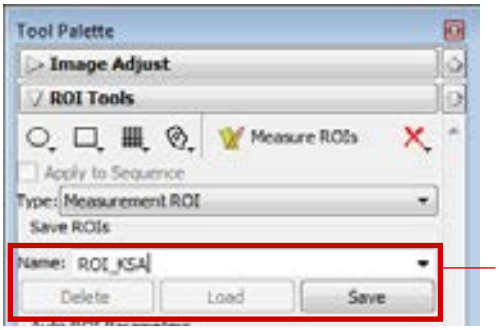
Living Image software saves ROIs with an image (the software prompts you to save before closing the image). ROI measurements are saved in the AnalyzedClickInfo.txt file associated with the image.

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 13.15). 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 13.15).

Figure 13.15 Name and Save ROIs to the System



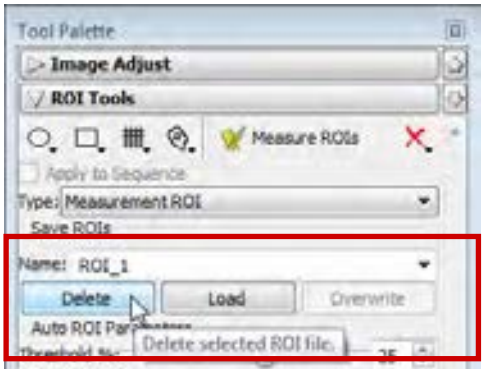
"Named" ROIs specify ROI parameter values that are used to apply ROIs to an image.

2. To apply a "named" ROI to an image, make a selection from the Name drop-down list and click **Load (**Figure 13.15).

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 13.16).

Figure 13.16 Delete ROIs From the System



13.7 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 for luminescence image data.
- Efficiency for fluorescence image data acquired using epi-illumination or NTF efficiency for fluorescence image data acquired using trans-illumination.

Viewing the ROI Measurements

1. Load an image or sequence that includes ROIs.
2. Click the Measure ROIs button in the ROI tools to display the ROI measurement table (Figure 13.17). Alternatively, select **View** → **ROI Measurements** on the menu bar.

Figure 13.17 ROI Measurements Table

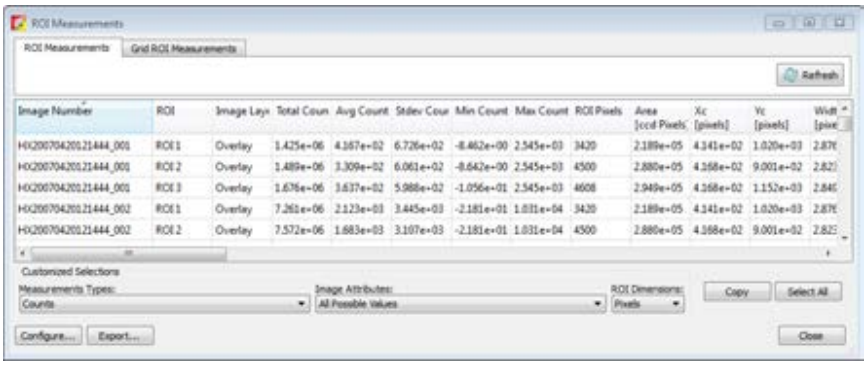


Table 13.8 ROI Measurements Table

Item	Description
Measurement Types	Make a selection from the this drop-down list to select the type of image unit for the ROI measurements in the table. Custom ROI table configurations also appear in this drop-down list. See *Configuring the ROI Measurements Table on page 194* for instructions on creating a custom table.
None	Excludes ROI measurements from the table.

Table 13.8 ROI Measurements Table (continued)

Item	Description
Counts (luminescence and fluorescence)	Includes Total Counts, Avg Counts, Stdev Counts, Min Counts, and Max Counts in the table. Total Counts = the sum of all counts for all pixels inside the ROI. Avg Counts = Total Counts/Number of pixels or super pixels. Stdev Counts = standard deviation of the pixel counts inside the ROI Min Counts = lowest number of counts in a pixel inside the ROI. Max Counts = highest number of counts in a pixel inside the ROI. **Note:** These numbers are displayed if the units selected in the ROI Measurements table and the image are the same. Otherwise, N/A appears in each column. **Tip:** See the tech note Image *Display and Measurement* for more details on count units (select **Help → Tech Notes** on the menu bar).
Radiance (Photons) (fluorescence and luminescence)	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. Average Radiance = the sum of the radiance from each pixel inside the ROI/number of pixels or super pixels (photons/sec/cm^2/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 *Image Display and Measurement* for more details on photon units (select **Help → Tech Notes** on the menu bar).
Radiant Efficiency (fluorescence)	Epi-fluorescence - Fluorescence emission radiance per incident excitation intensity: p/sec/cm^2/sr/μW/cm^2
Efficiency (epi-fluorescence)	Fluorescent emission yield normalized to the incident excitation intensity (radiance of the subject/illumination intensity)
Image Attributes	Make a selection from the drop-down list to specify the click number (image file) information to include in the table. Click attributes include label name settings and camera settings.
None	Excludes image attributes from the table.
All Possible Values	Includes all of the image attributes (for example, label name settings and camera settings) in the table.
All Populated Values	Includes only the image attributes with values in the table.
Living Image Universal	Includes all Living Image Universal label name settings in the table.
ROI Dimensions	Make a selection from the drop-down list to specify the ROI dimensions to include in the table.
None	Excludes the ROI area, x,y-coordinates, and dimensions from the table.
Pixels	Includes ROI area, x,y-coordinates, and dimensions (in pixels) in the table.

Table 13.8 ROI Measurements Table (continued)

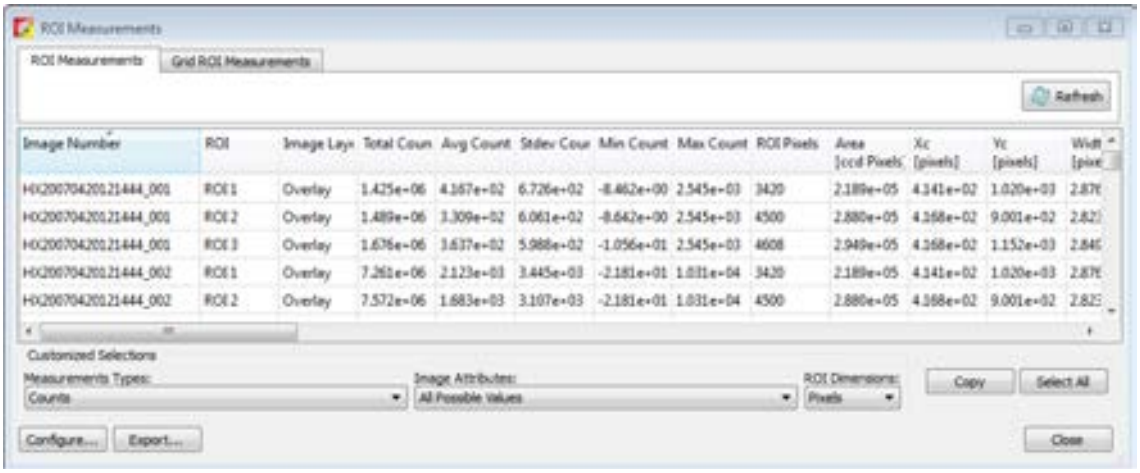
Item	Description
cm	Includes ROI area, x,y-coordinates, and dimensions (in cm) in the table.
Copy	Copies the selected row(s) in the table to the system clipboard.
Select All	Copies all rows in the table to the system clipboard.
Refresh	Updates the ROI Measurements table (for example, after you draw new ROIs, move an ROI, and close or open image data).
Configure	Displays the Configure Measurements box that enables you to specify and organize the data categories (column headers) for the table.
Export	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.

Configuring the ROI Measurements Table

You can customize the data and information (column headers) in the ROI Measurements table (Figure 13.18). 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.

Figure 13.18 ROI Measurements Table



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.

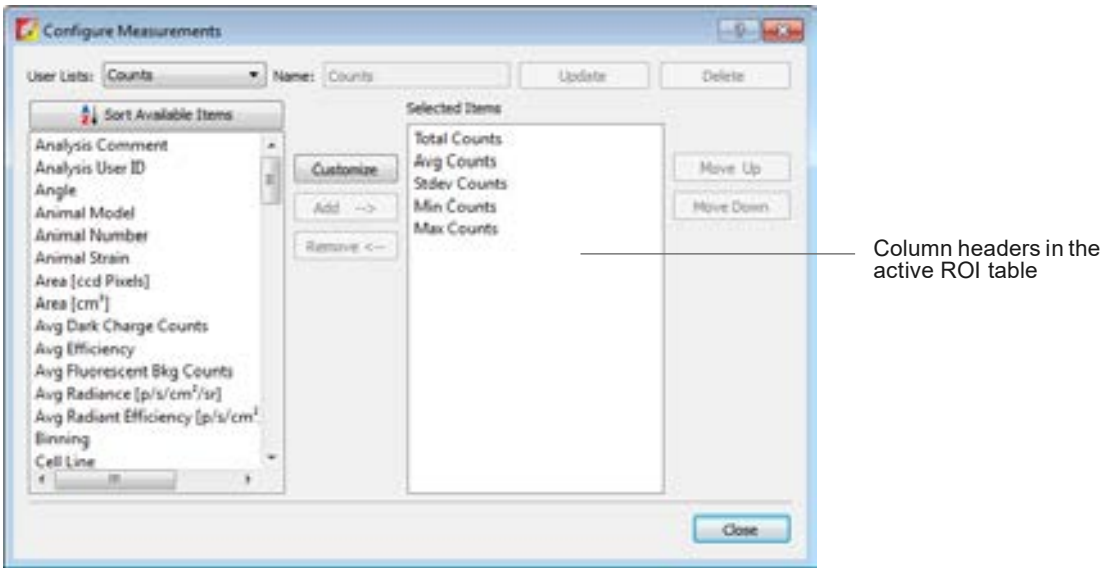
Creating a Custom ROI Table Configuration

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 13.18). 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 13.19).

Figure 13.19 Configure Measurements Dialog Box



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 13.18). 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**.

NOTE: Preset table configurations cannot be deleted.

Copying or Exporting ROI Measurements

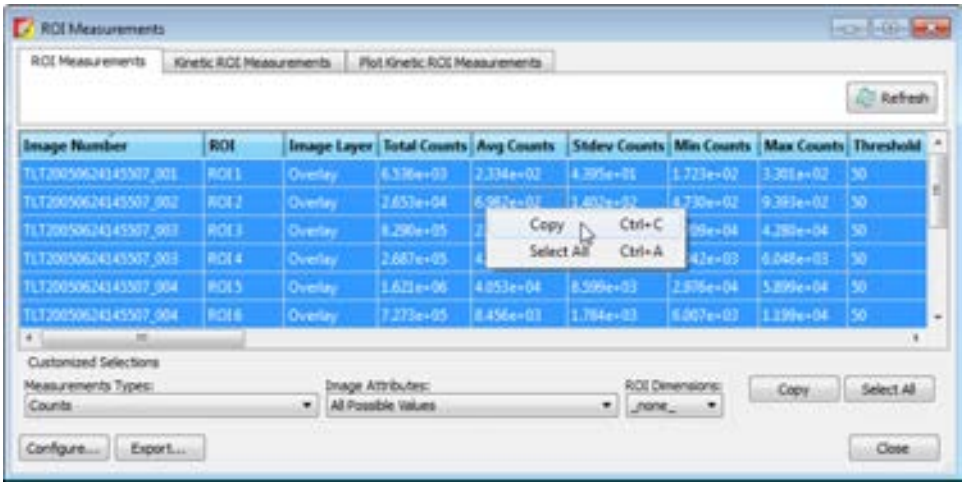
To export the table:

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 13.20).
- 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.

Figure 13.20 Copy All Rows in the ROI Measurements Table to the System Clipboard



14 3D Reconstruction of Sources

14.1 Overview of Reconstructing Sources

Living Image software provides algorithms which analyze 2-dimensional optical image data to reconstruct 3-dimensional (3D) luminescent or fluorescent sources located inside an animal (*tomographic analysis*). Figure 14.1 shows an overview of the 3D reconstruction workflow.

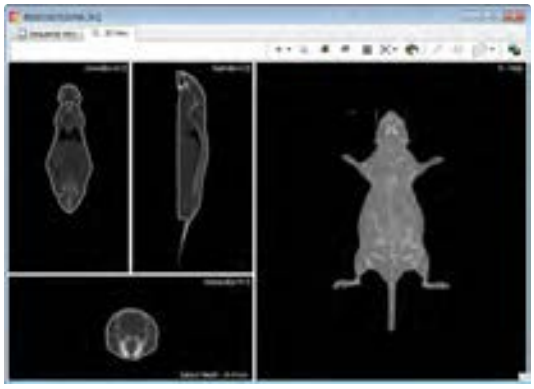
TIP: See the technical note *DLIT and FLIT Reconstruction of Sources* for more details on the DLIT or FLIT algorithm (select **Help** → **Tech Notes** on the menu bar).

3D Reconstruction Algorithm	Description	See Page
Diffuse Tomography (DLIT)	DLIT provides a complete 3D reconstruction of the luminescent source distribution within the 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).	208
Fluorescent Tomography (FLIT)	FLIT provides a complete 3D reconstruction of the fluorescent source distribution within the 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.	213

Figure 14.1 Basic 3D Reconstruction Workflow

1. Set up a DLIT or FLIT sequence using the Imaging Wizard (see page 201). Acquire and load the sequence.

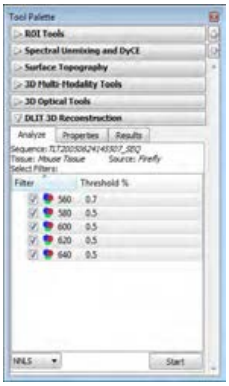
2. Generate or load a surface using the Surface Topography tools. See Appendix C, *Surface Topography on page 418* for more details.



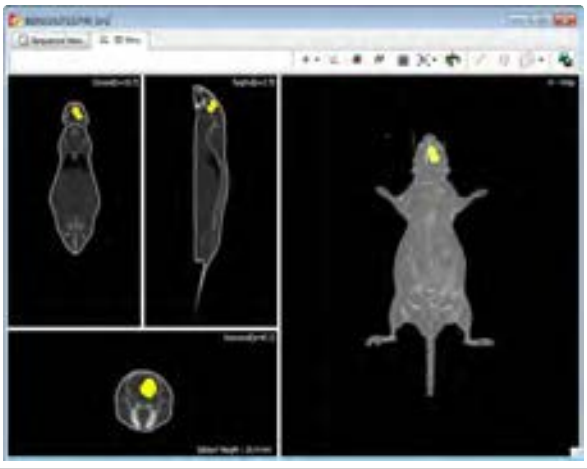
3. In the DLIT or FLIT 3D Reconstruction tools, select the:
   - Wavelengths or excitation point images to analyze
   - Tissue and source properties
4. Reconstruct sources.

   See page 208 for detailed DLIT steps.

   See page 213 for detailed FLIT steps.



5. View source measurements (see page 222).



General Considerations

Animal Requirements

The best surface topography reconstruction is obtained from nude mice. 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. 3D reconstructions using mice with black or dark-colored fur will give poor results.

Luminescent Exposure vs. Luciferin Kinetic Profile

It is important to consider the luciferin kinetic profile when you plan the image sequence acquisition. The DLIT algorithm currently 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.

Reconstruction Inputs

NOTE: Use the Imaging Wizard to set up the DLIT or FLIT image sequence. See *Acquire a Sequence on page 201* for more details.

DLIT

Input data to the DLIT algorithm for a 3D reconstruction of luminescent light sources includes:

- A surface topography of the subject (generated from a a structured light image).
- A sequence of two or more images of the light emission from the subject surface that is acquired at different filter bandpasses (Table 14.1).

Table 14.1 IVIS Spectrum CT Filters for Luminescence or Fluorescence Tomography

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

FLIT

Input data to the FLIT algorithm for 3D reconstruction of fluorescent light sources includes:

- A surface topography of the subject (generated from a structured light image).
- A sequence of images acquired at different transillumination excitation source positions using the same excitation and emission filter at each position (Table 14.1).

Quantification Database (Optional)

If a quantification database is available, it is possible to determine the number of cells in a DLIT source or the number of cells or dye molecules in a FLIT source. The database is derived from an analysis of images of known serial dilutions of luminescent or fluorescent cells, or dye molecules in a well plate.

See Appendix B, *Quantification Database on page 411* for more details on generating a database. Using a quantification database is optional.

14.2 Acquire a Sequence

Use the Imaging Wizard to set up a DLIT (see below) or FLIT (see page 203) sequence. See *Manual Sequence Setup on page 206* if not using the wizard.

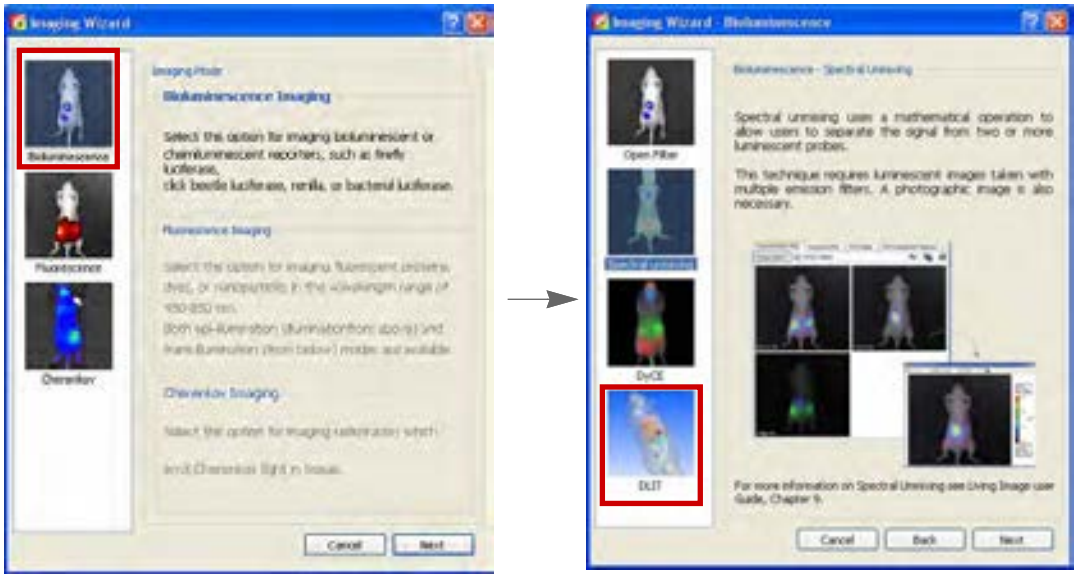
Bioluminescence Imaging

NOTE: The IVIS Spectrum CT should be initialized and the temperature locked before setting the imaging parameters. See page 73 for more details.

1. Start the Imaging Wizard. See *Start the Imaging Wizard and Setup a Sequence on page 108* for instructions.
2. Double-click the Bioluminescence option. Double-click the DLIT option in the next screen (Figure 14.2).

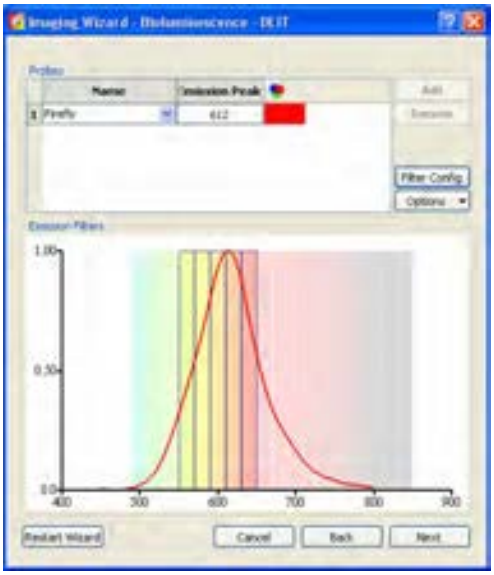
Figure 14.2 Imaging Wizard – Choose Bioluminescence and DLIT Options

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



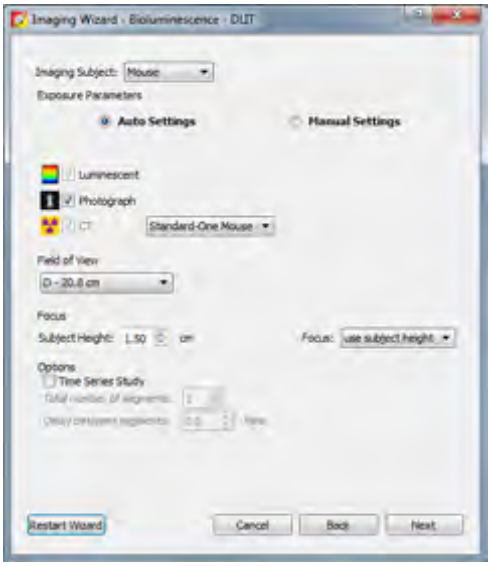
3. Select a probe from the Name drop-down list and click **Next** (Figure 14.3).

Figure 14.3 Select a Probe



4. In the next screen (Figure 14.4):
   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 14.4 Imaging Wizard – Bioluminescence DLIT



5. Set the focus by doing either of the following:
   - Enter a subject height and choose the “use subject height” focus option.

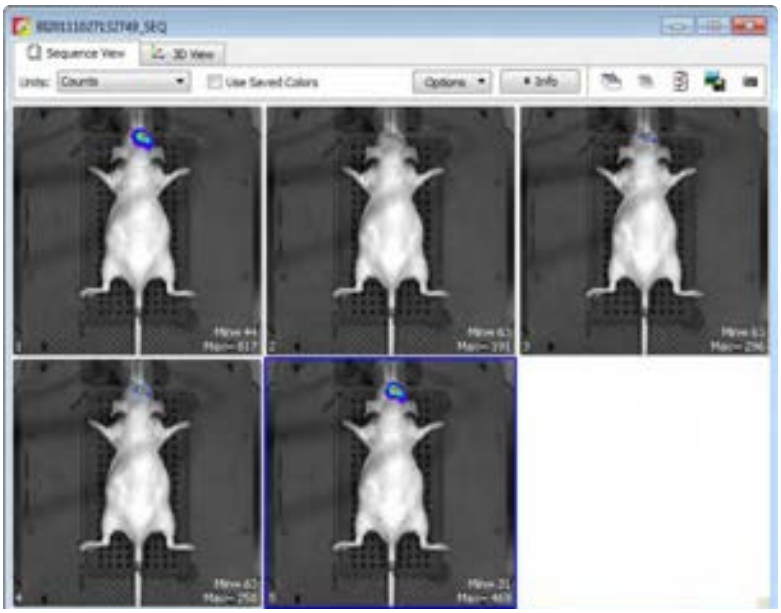
   OR
   - Choose the “manual focus” option from the Focus drop-down list and set the focus parameters in the Manual Focus Window that appears.

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 14.5).

Figure 14.5 Sequence Setup Complete

8. Acquire the sequence following the instructions on page 111.
9. The image window appears when acquisition is completed (Figure 14.6). See Table 11.4 on page 94 for more details on the Image window.

Figure 14.6 Image Window – Sequence View



Fluorescence Imaging

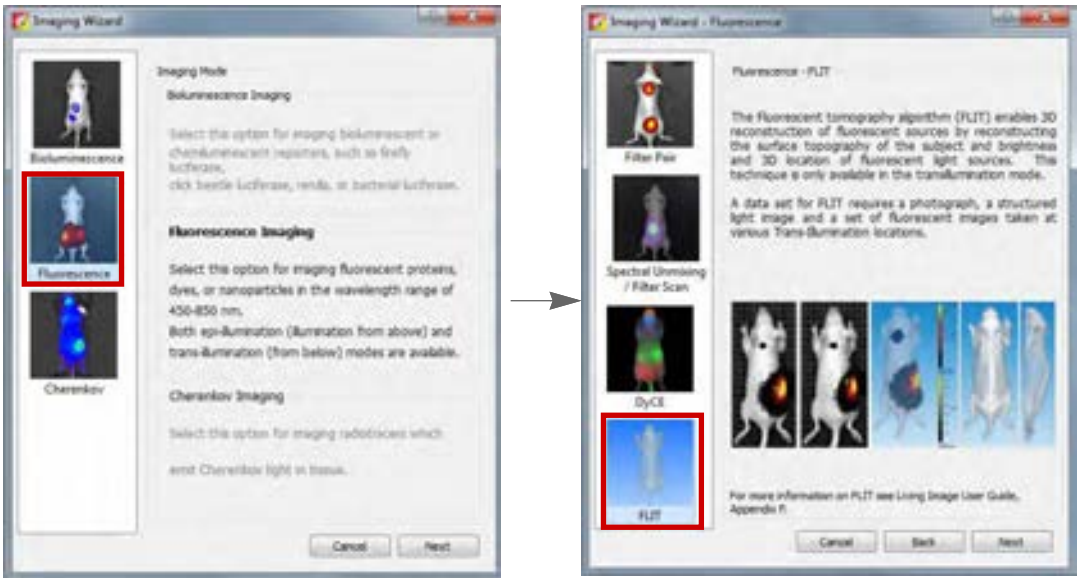
NOTE: The IVIS Spectrum CT should be initialized and the temperature locked before setting the imaging parameters. See page 73 for more details.

1. Start the Imaging Wizard. See *Start the Imaging Wizard and Setup a Sequence on page 108* for instructions.

2. Double-click the Fluorescence option. Double-click the FLIT option in the next screen (Figure 14.7).

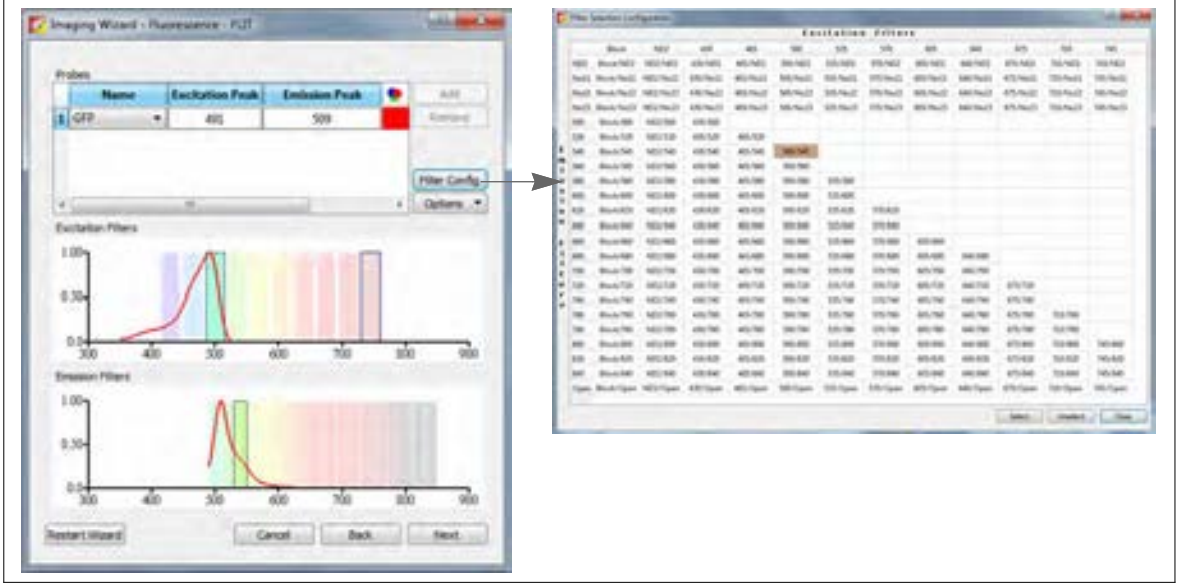
Figure 14.7 Imaging Wizard – Choose Fluorescence and FLIT Options

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



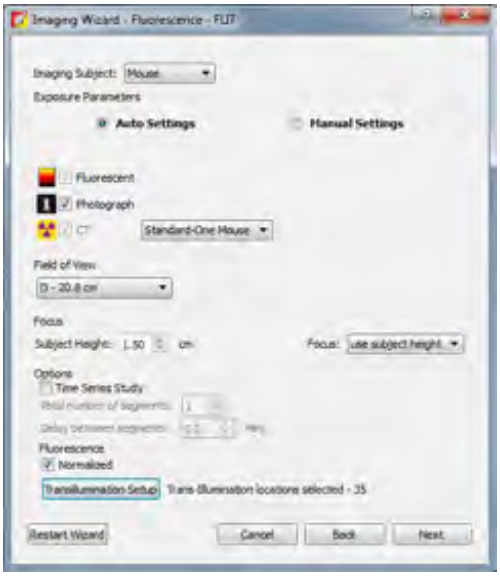
3. Select a probe from the Name drop-down list and click **Next** (Figure 14.8).

Figure 14.8 Select a Probe



4. In the next screen (Figure 14.4):
   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 14.9 Imaging Wizard – Fluorescence FLIT

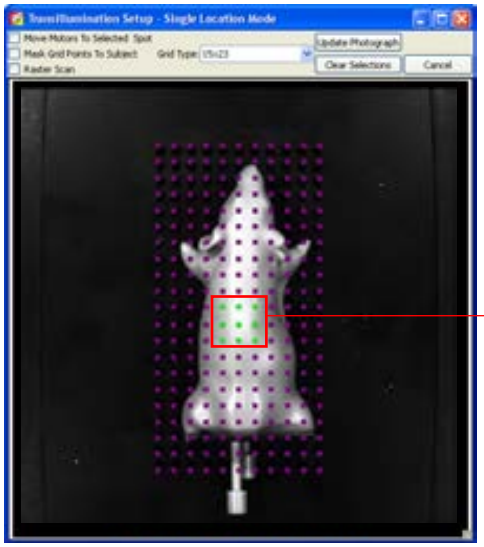


5. Set the focus by doing either of the following:
   - Enter a subject height and choose the “use subject height” focus option.

   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 14.10).

      See Table 11.5 on page 103 for more details on Transillumination Setup.

Figure 14.10 Transillumination Setup Box



8. Click **Next**.
   The specified sequence appears in the sequence table (Figure 14.5).

Figure 14.11 Sequence Setup Complete

9. Acquire the sequence following the instructions on page 111.
   The image window appears when acquisition is completed (Figure 14.6 on page 203). See Table 11.4 on page 94 for more details on the Image window.

Manual Sequence Setup

This sections provides sequence requirements if you will not be using the Imaging Wizard and plan to manually set up the sequence.

Table 14.2 IVIS Spectrum CT Filters for Luminescence or Fluorescence Tomography

Filters	Range	Bandwidth
10 excitation filters	415-760 nm	30 nm

Table 14.2 IVIS Spectrum CT Filters for Luminescence or Fluorescence Tomography

Filters	Range	Bandwidth
18 emission filters	490-850 nm	20 nm

DLIT Sequence Requirements

A sequence must include:

- 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 14.3 shows the recommended optical image sequence.

Table 14.3 Manual Sequence Setup – Recommended DLIT Optical Image Sequence)

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

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

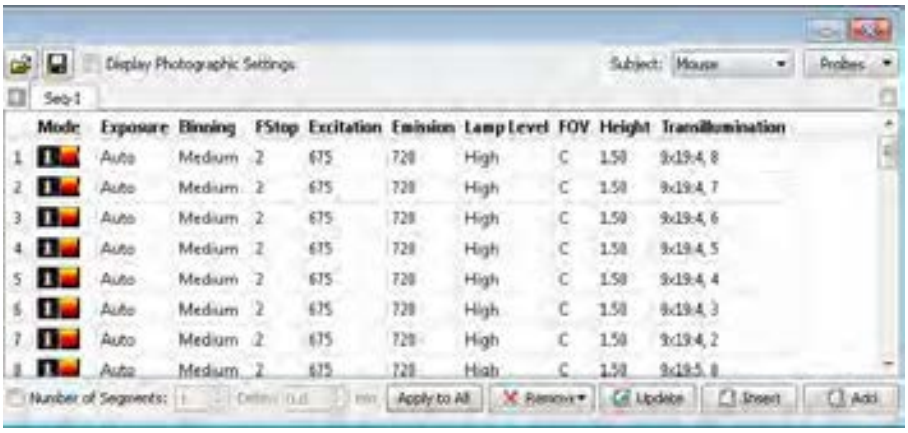
FLIT Sequence Requirements

Use transillumination on the IVIS Spectrum CT and the same excitation and emission filters from at least four source locations that 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 14.12 shows an example image sequence.

Figure 14.12 Example FLIT Sequence Setup

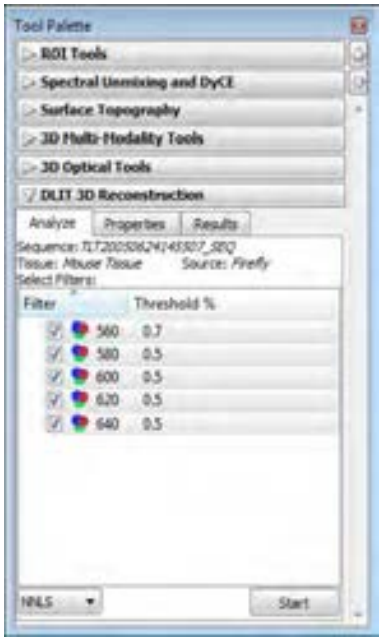


14.3 Steps to Reconstruct Luminescent Sources Using DLIT

1. Load a DLIT image sequence.
2. Generate or load a surface using the Surface Topography tools. For details on generating the surface, see Appendix C, *Surface Topography on page 418*.
3. In the Tool Palette, choose **DLIT 3D Reconstruction**.

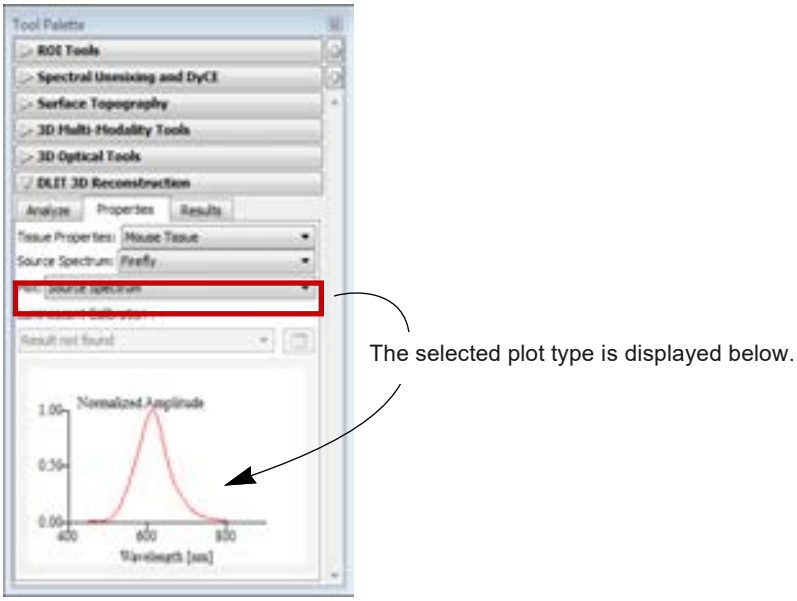
   The Analyze tab shows the data that the algorithm automatically selects for the reconstruction (Figure 14.13). For more details about the Threshold %, see page 215.

Figure 14.13 Analyze Tab



4. In the Properties tab, make a selection from the “Tissue Properties” and “Source Spectrum” drop-down lists (Figure 14.14).

Figure 14.14 Properties Tab

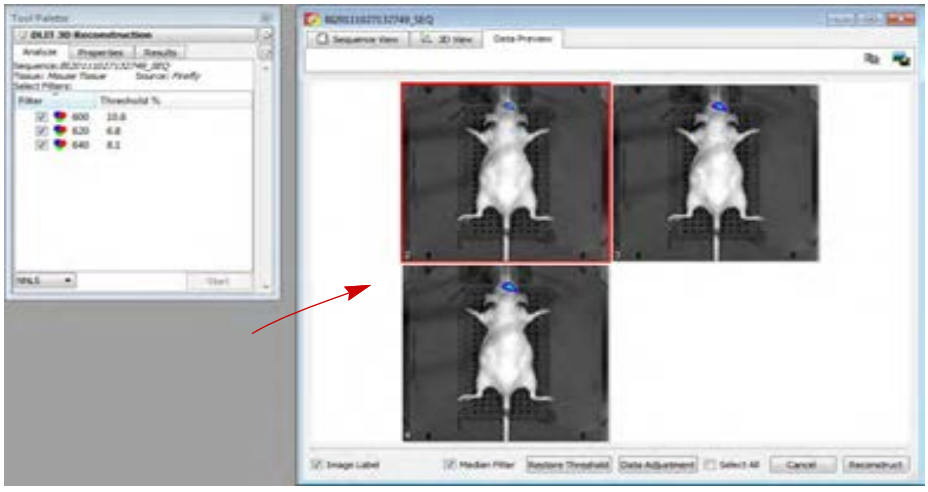


5. To view the tissue properties (μ_a, μ_{eff}, μ'_s) for the tissue and source you selected, make a selection from the Plot drop-down.
6. Select a luminescent quantification database to compute the number of cells per source (optional).

   For details on generating a luminescent quantification database, see page 411.
7. In the Analyze tab, click **Start**.

   The Data Preview window appears and displays the image data that will be included in the reconstruction. Usually, no data adjustment is required. However, it is possible to exclude or include user-selected pixel data from the analysis. See *Including or Excluding Data for 3D Reconstruction on page 215* for more details.

Figure 14.15 Data Preview Window



Data Preview window

8. In the Data Preview window, click **Reconstruct**.

The reconstruction usually requires less than one minute, depending on the reconstruction volume, parameter settings, and computer performance. When the analysis is finished:

- The 3D View window displays the animal surface and the reconstructed sources.
- In the Tool Palette, the Results tab displays the results data and the algorithm parameter values. See page 219 for details on managing results (for example, save, load, or delete).
- The 3D Optical Tools appear after a reconstruction is generated or loaded. Use these tools to modify the source display parameters. See Table 14.4 on page 210 for an overview of the tools; more details are available on page 235 to page 248.

Table 14.4 3D Optical Tools

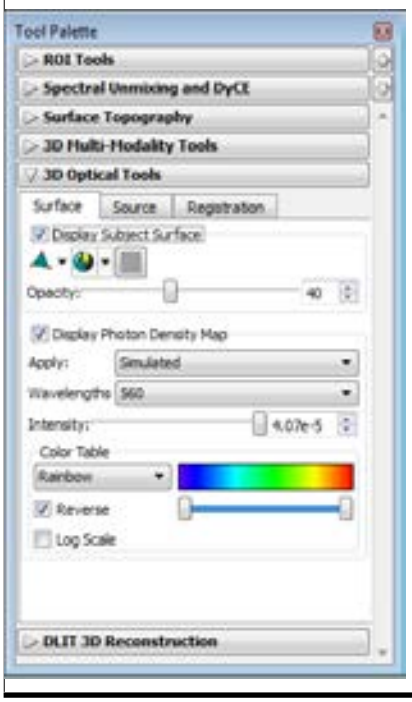
Tool Palette	3D Optical Tools	Functions	See Page
	Surface Tools	Adjust the appearance of the reconstructed animal surface and photon density maps	235
	Source Tools	Adjust the appearance of reconstructed sources, make source measurements, export voxel measurements	238
	Registration Tools	Display organs on the reconstructed surface, adjust the location or scale of organs on the surface, import an organ atlas	241
	Animate Tools Select **Tools → 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.	248

Figure 14.16 DLIT Reconstruction Results

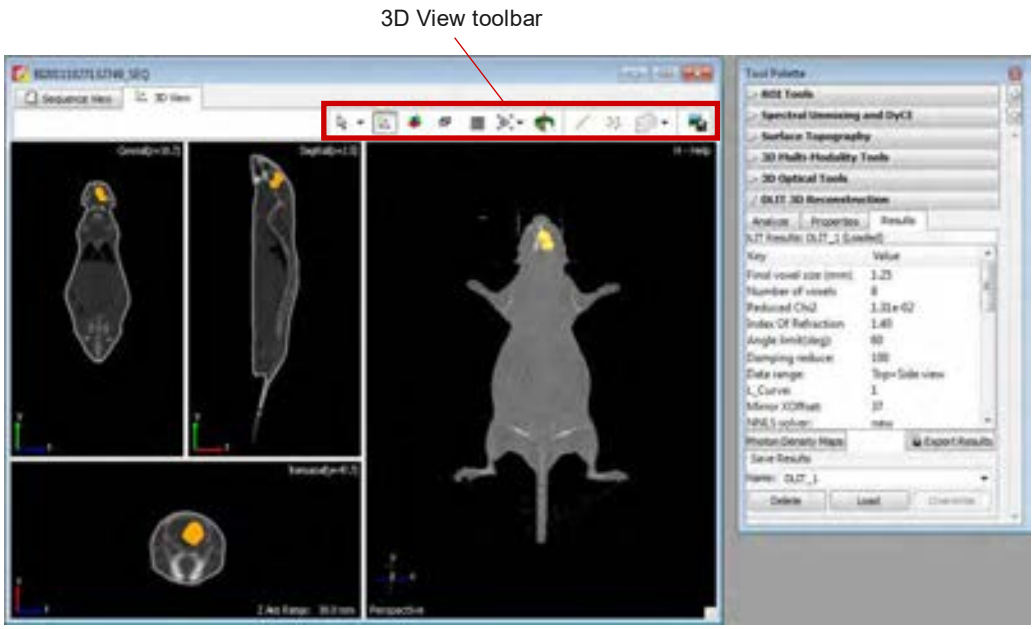


Figure 14.17 3D View Toolbar

Table 14.5 3D View Tools

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 11.18 on page 99) and other information automatically recorded by the software.
Image Tools 	A drop-down list of tools for viewing and working with the surface or DLIT results. or - Rotates or spins the surface in the x, y, or z-axis direction. - Moves the surface in the x or y-axis direction. - Zooms in or out on the image. To zoom in, right-click (Cmd key (apple key) +click for Macintosh users) and drag the toward the bottom of the window. To zoom out, right-click and drag the toward the top of the window.
	Displays the x,y,z-axis display in the 3D view window.
	Displays coronal, sagittal, and transaxial cross-sections through the subject in the 3D view window.

Table 14.5 3D View Tools (continued)

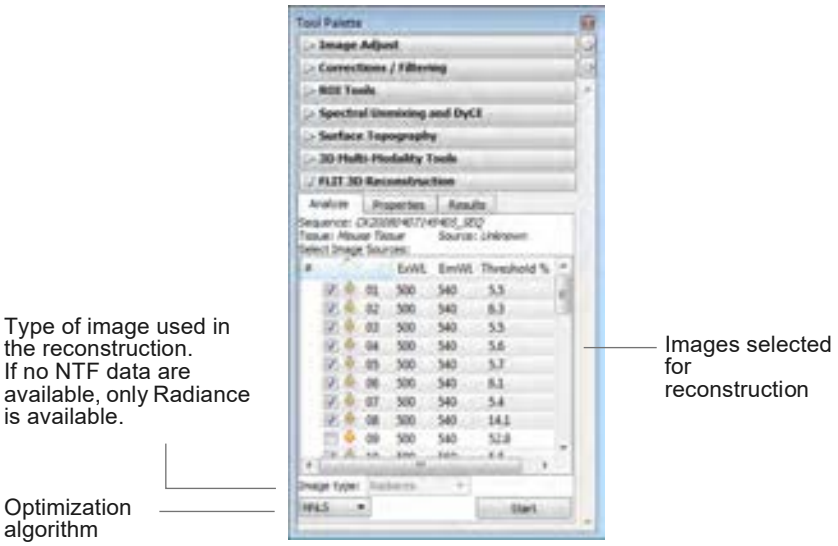
Tool	Description
	Displays a bounding box around the subject.
	Displays a grid under the subject.
	Select this tool from the drop-down list to change the view perspective (top, bottom, left, right, front, back, or perspective view). For examples of the views, see Figure 14.46, page 246.
	Select this tool from the drop-down list to display the perspective view.
	Rotates the 3D reconstruction results in the 3D view window (*3D scene*). Click the + or - key to increase or decrease the rotation speed.To stop the rotation, click the 3D scene or the  button.
	Displays measurement cursors in the coronal, sagittal, or transaxial views.
	Click this button, then select a source or a point in a source to obtain source measurements (total flux, volume, center of mass, host organ) in the 3D Optical Tools (Source tab). For more details, see page 222.
	Copies or pastes voxels or a source surface so that DLIT and FLIT reconstructions can be displayed on one surface. For more details, see page 227.
	Enables you to save the 3D view to a graphic file (for example, .jpg).

14.4 Steps to Reconstruct Fluorescent Sources Using FLIT

1. Load a FLIT image sequence.
2. Generate or load a surface in the Surface Topography tools. For details on generating the surface, see Appendix C, *Surface Topography on page 418*.
3. In the Tool Palette, choose **FLIT 3D Reconstruction**.

   The Analyze tab shows the images that the algorithm automatically selects for the reconstruction based on an appropriate signal level (Figure 14.13). For more details about the Threshold %, see page 215.

Figure 14.18 FLIT 3D Reconstruction Tools – Analyze Tab



4. Select the type of image used in the reconstruction: Radiance or NTF Efficiency (Figure 14.18).

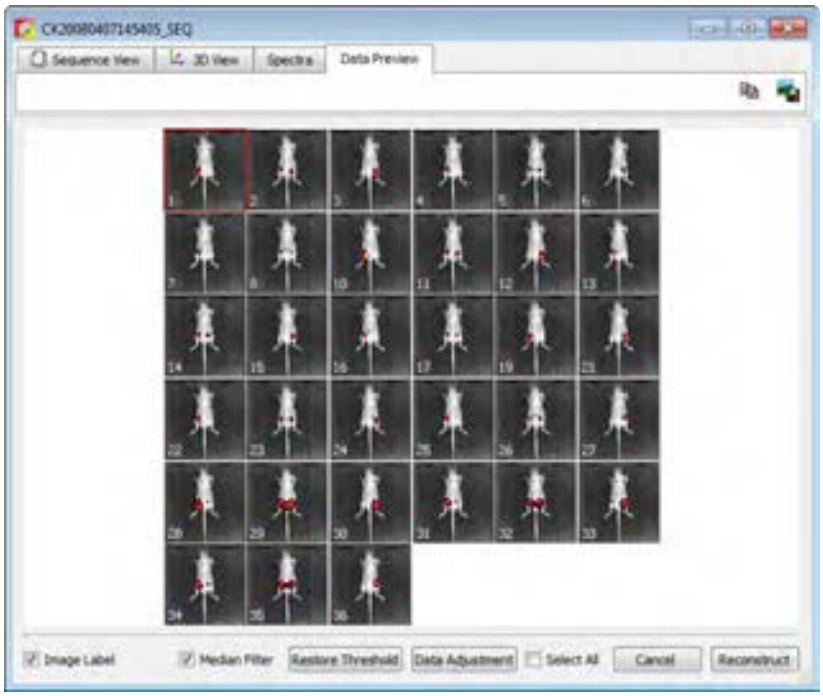
   NTF Efficiency data is the default because it affords higher sensitivity to the embedded fluorescence sources.
5. Make a selection from the “Tissue Properties” list in the Properties tab (Figure 14.14).
6. To view the tissue properties (μ_a, μ_{eff}, μ'_s) for the tissue you selected, make a selection from the Plot drop-down.
7. Optional: Select a fluorescent quantification database to include the number of fluorescent molecules/source in the results.

   For details on generating a fluorescent quantification database, see page 411.
8. In the Analyze tab, click **Start**.
9. The Data Preview window appears and displays the image data that will be included in the reconstruction.

   Usually, no data adjustment is required. However, it is possible to exclude or include user-selected pixel data from the analysis. See page 215 for more details.

You can also include or exclude image data by adding or removing the check mark next to the images listed in the Analyze tab (Figure 14.18).

Figure 14.19 Data Preview Window



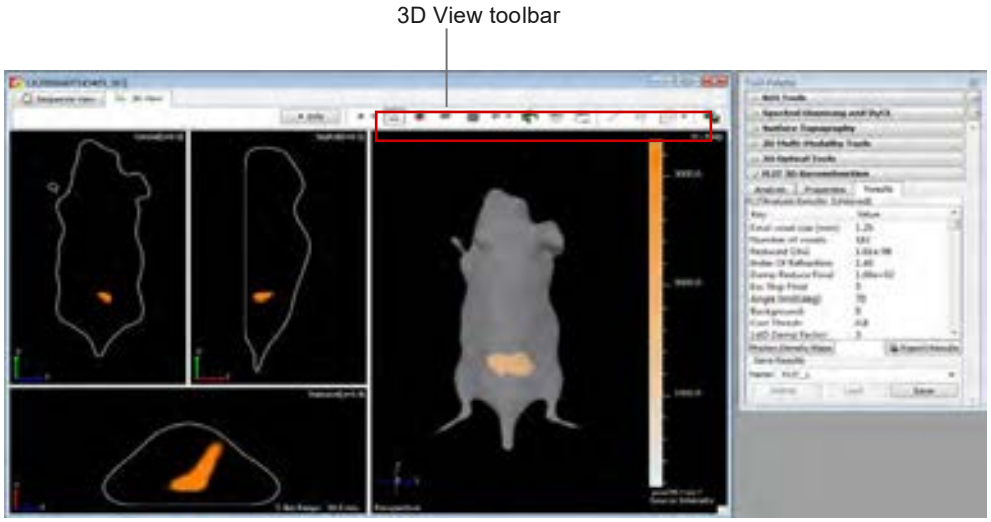
10. Click **Reconstruct**.

    The reconstruction normally requires less than one minute, depending on the reconstruction volume, parameter settings, and computer performance. When the analysis is finished:

    - The 3D View window displays the surface and the reconstructed sources.
    - In the Tool Palette, the Results tab displays the results data and the algorithm parameter values (Figure 14.23). See page 219 for details on managing results (for example, save, load, or delete).
    - The 3D Optical Tools appear in the Tool Palette. Use these tools to modify the source display parameters. See Table 14.4 on page 210 for an overview of the tools; more details are available on page 235 to page 248.

Figure 14.20 FLIT results: 3D View Window and Results Tab

For details on the 3D View toolbar, see Table 14.5, page 211.



14.5 Including or Excluding Data for 3D Reconstruction

The Data Preview window shows the image data that are automatically selected for reconstruction (Figure 14.21). In special cases, you may want to include or exclude particular data from this default selection. There are two ways to do this:

- Change the Threshold % value (see below) – 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. 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.
- Region selection (see page 216) – 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. You can also use this method to focus on particular sources to reconstruct and ignore others.

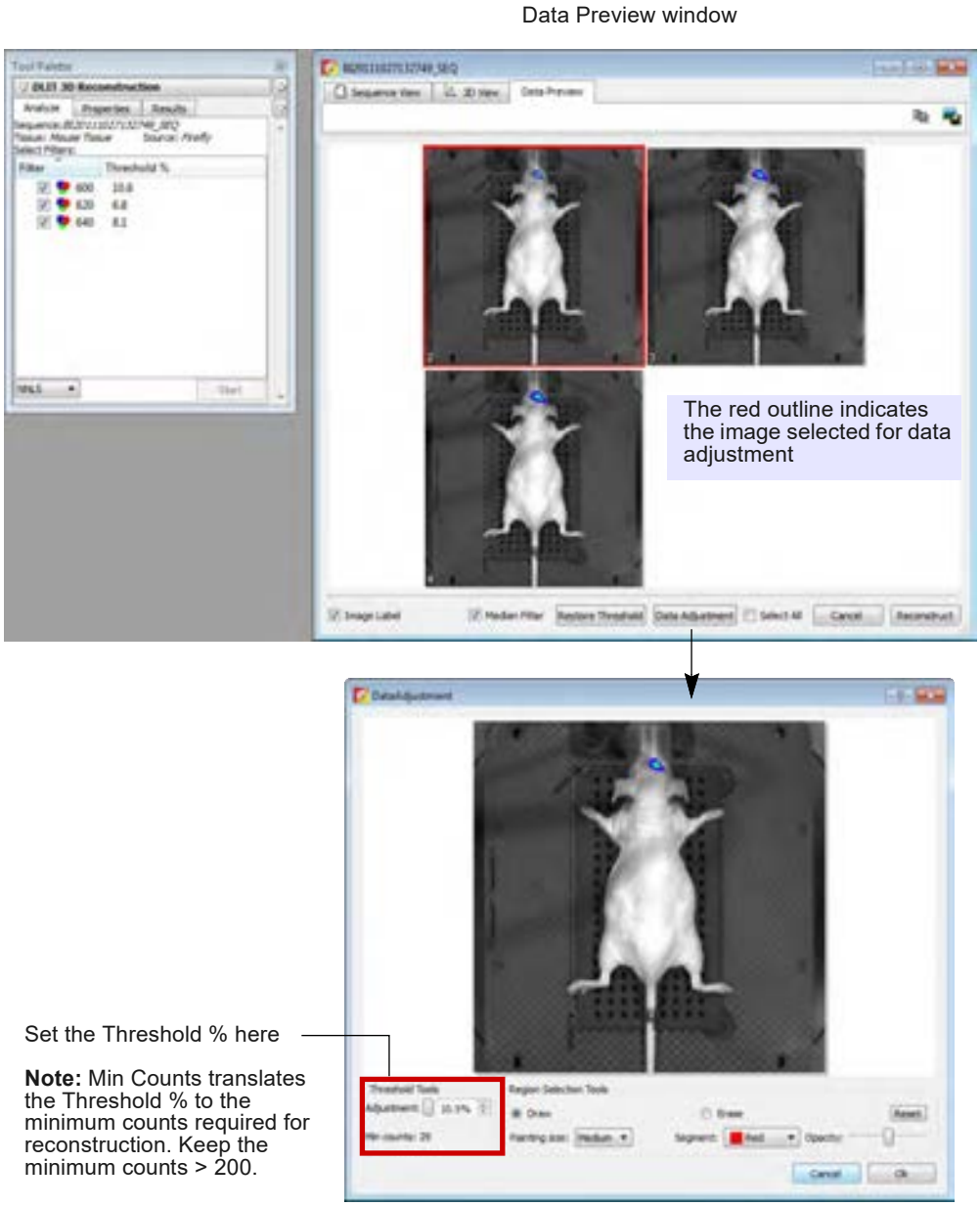
To change the Threshold % for a selected image:

1. Click **Start** in the Analyze tab (Figure 14.21).
   The Data Preview window appears.
2. Click an image in the Data Preview window.

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

3. Click **Data Adjustment**.
4. In the window that appears, enter a new Threshold % value.
   The new Threshold % appears in the Analyze tab.
5. To reset the Threshold % to the default value (for the selected images), click **Restore Threshold**.

Figure 14.21 Adjusting the Threshold %



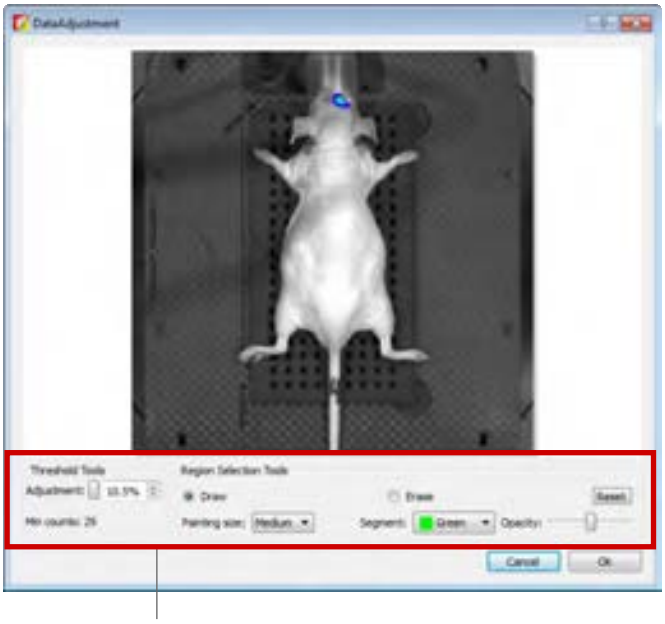
To select particular regions for reconstruction:

1. Open the Data Preview window as shown in Figure 14.21.
2. Click **Data Adjustment**.
3. In the window that appears, choose the **Draw** option and put the mouse pointer over the image so that the pencil tool appears.
4. To automatically select all pixels in a source, right-click with the region with the pencil tool.

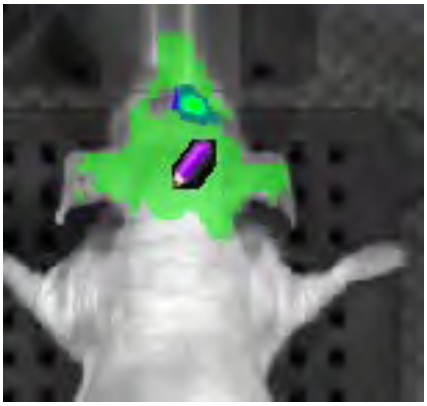
   Alternatively, put the pencil over the image and click the mouse key or press and hold the mouse key while moving the pencil over an area of the image.

NOTE: If the pencil tool markings are applied to the image, only the marked pixels are included in the analysis.

Figure 14.22 Selecting Regions to Include in Reconstruction



Use these tools to select particular image data to include in the analysis. See Table 14.6 for details on the tools.



Choose the "Draw" option then mark the area to include in the reconstruction using the pencil tool. In this example, the green area marked with the pencil tool is will be reconstructed.

Table 14.6 Region Selection Tools

Item	Description
Draw	Choose this option to display the pencil tool. 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.

14.6 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** → **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 14.23 Example DLIT 3D Reconstruction Results

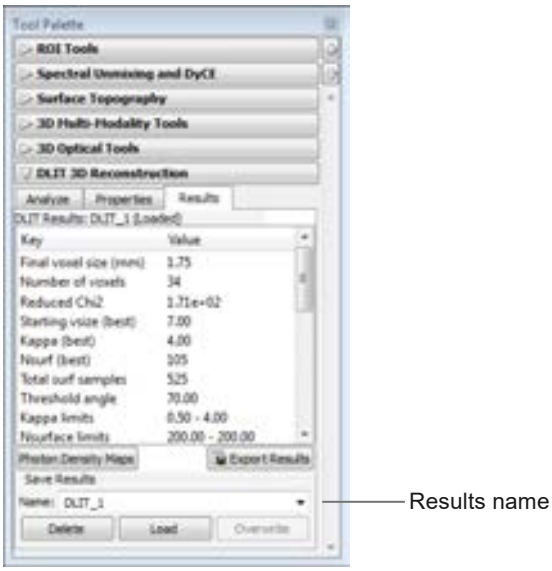


Table 14.7 DLIT or FLIT 3D Reconstruction Results

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.

Table 14.7 DLIT or FLIT 3D Reconstruction Results

Item	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

Managing 3D Reconstruction Results

Table 14.8 Reconstruction results

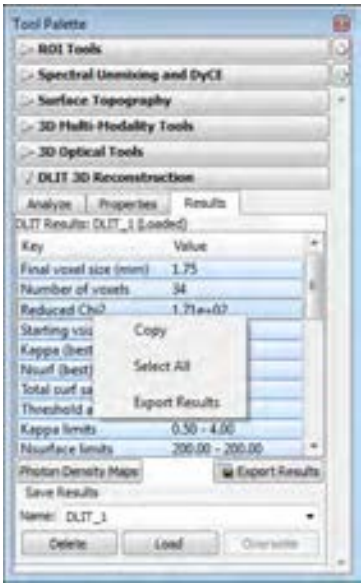
Item	Description
Name	The name for the active DLIT or FLIT results. Select results from this drop-down list.
Delete	Deletes the selected DLIT or FLIT results.
Load	Opens the selected reconstruction results in the 3D View.
Save	Saves the active DLIT or FLIT results to the selected name. The results are 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.

Figure 14.24 Select and Copy Results



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

14.7 Checking the Reconstruction Quality

Comparing the measured and simulated photon density plots is a useful way to check the quality of a 3D reconstruction.

The 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 data set.

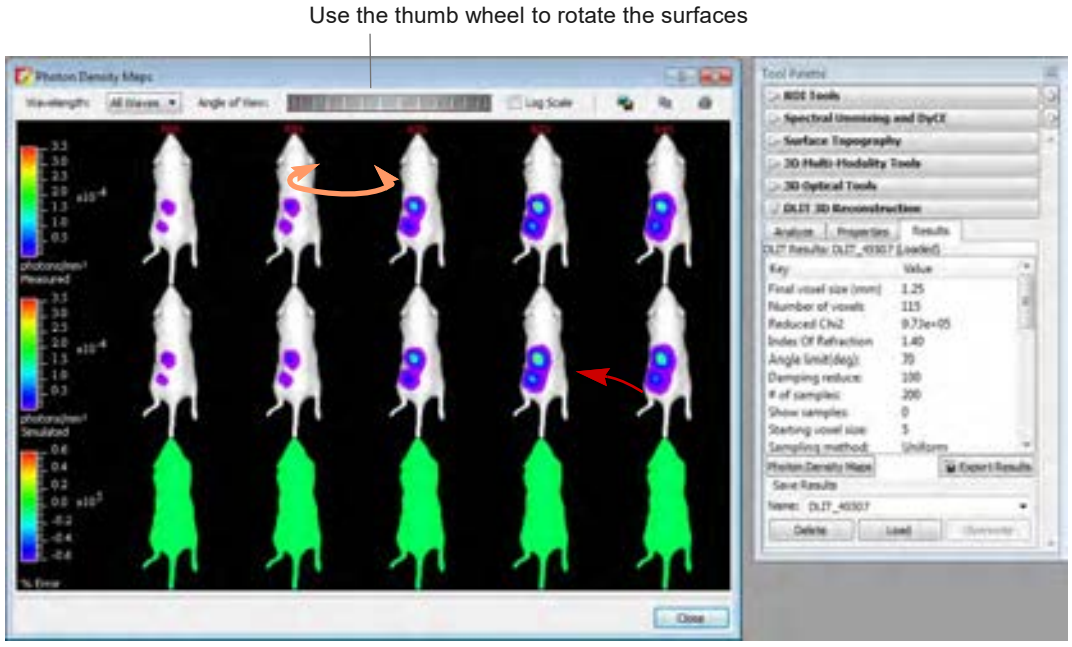
Viewing Photon Density Maps or NTF Efficiency

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

   The photon density maps or NTF Efficiency maps for all image data are displayed (Figure 14.25).

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

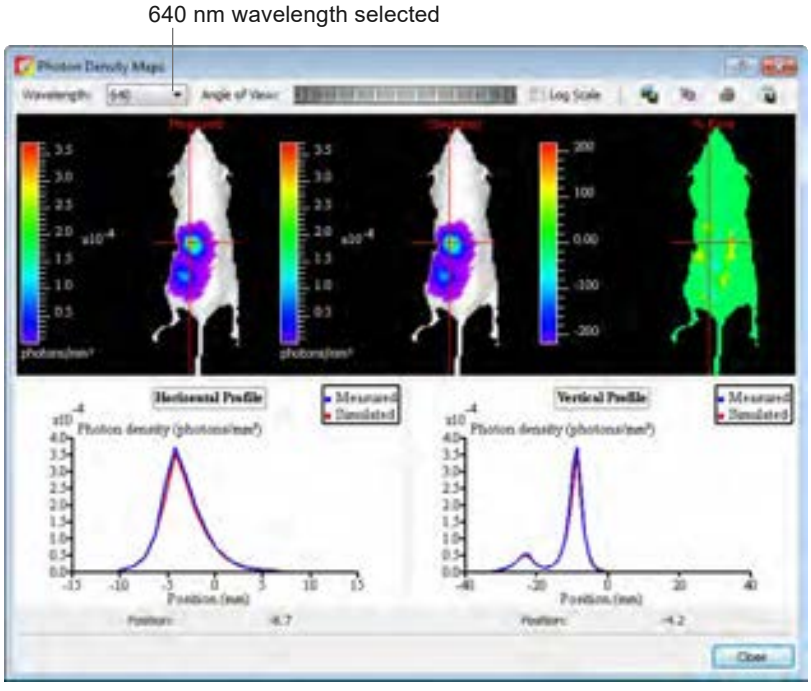
Figure 14.25 Photon Density Maps (or NTF Efficiency Maps for Fluorescence)



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).

Figure 14.26 Simulated (Red) and Measured (Blue) Photon Density (or NTF Efficiency) Plots



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 14.9 Photon Density Maps Window

Item	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.
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).

14.8 Measuring Sources

This section presents a convenient way to measure the source (voxels): total flux or total florescence yield, or if calibrated, the abundance in cells or picomoles after the reconstruction is finished or results are loaded.

The volume, center of mass, and depth at the center of mass are also reported in the 3D Optical Tools, Source tab.

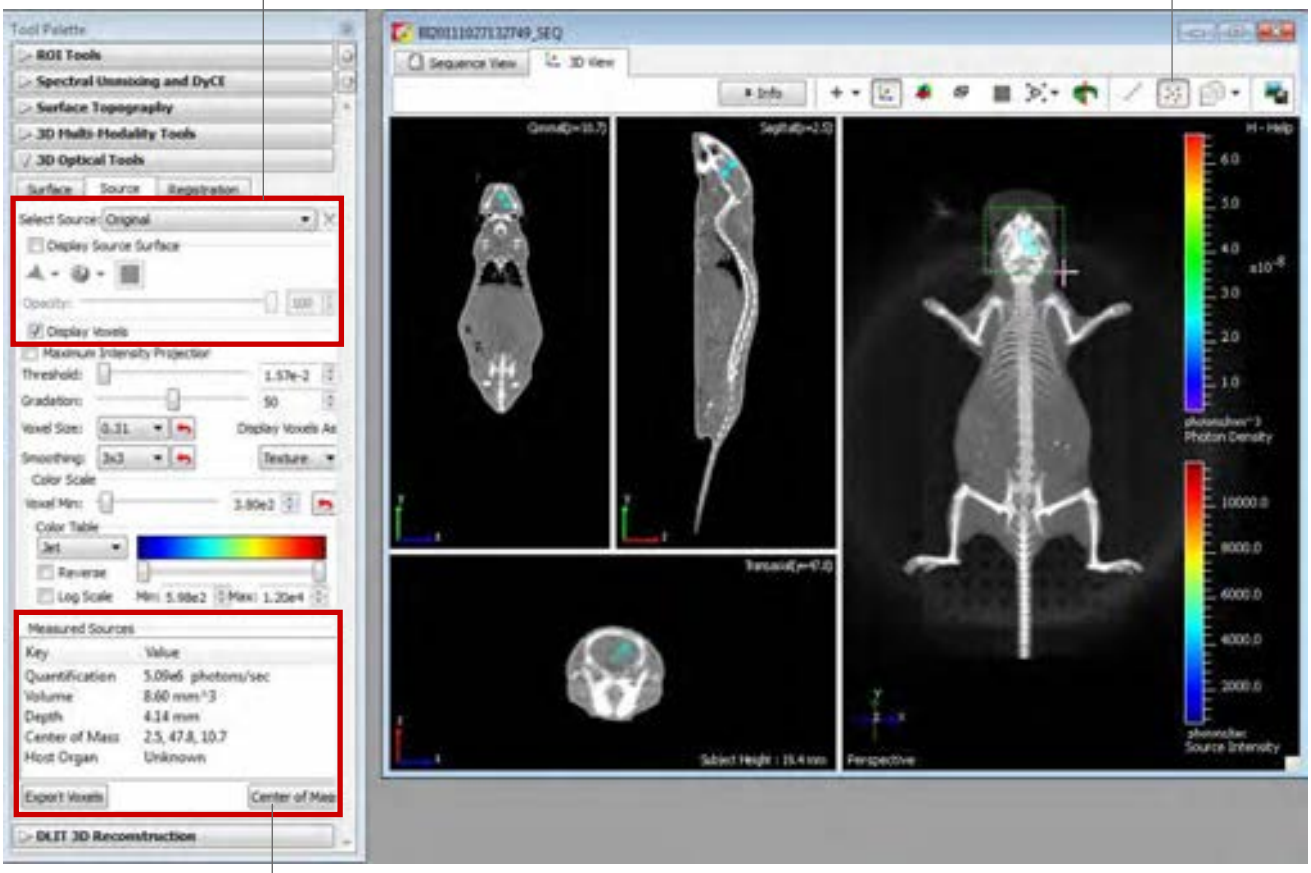
NOTE: If the surface contains voxels pasted from other reconstruction results, choose a source in the 3D Source tools (Figure 14.27). For more details on pasting voxels, see page 227.

Determining the Source Center of Mass

Follow the steps in Figure 14.27 after reconstruction is finished or results are loaded to determine the source center of mass. Alternatively, use the 3D ROI tool for more precise measurements. See page 284 for more details on 3D ROIs.

Figure 14.27 Select and Measure Source Voxels in the 3D View Window

1. If the surface includes voxels pasted from other results, select a source from the drop-down list.
2. Confirm that “Display Voxels” is selected, not “Display Source Surface”.
3. Click the Measure Source button, then draw a box around the source.



4. Click **Center of Mass** to obtain the measured source information.

   Note: The coronal, sagittal, and transaxial planes intersect at the center of mass of the selected source (see Figure 14.28 on page 224).

Table 14.10 Source Measurements

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. For more details on displaying organs, see *3D Optical Registration Tools on page 241*.

Measuring Source Depth

Follow the steps below after reconstruction is finished or results are loaded to measure source depth.

1. If the surface includes voxels pasted from other results, select a source from the drop-down list.
2. Confirm that “Display Voxels” is selected, not “Display Source Surface”.
3. Click the Measurement Cursor toolbar 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.
4. Click the button to display slice planes through the center of mass. See page 226 for more information on planes.

Figure 14.28 Slice Planes

This example shows slice planes through a selected source center of mass and distance measurements from the source center of mass to the surface.

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 14.29). 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.

Figure 14.29 Viewing Y,Z Coordinates in the Sagittal Plane

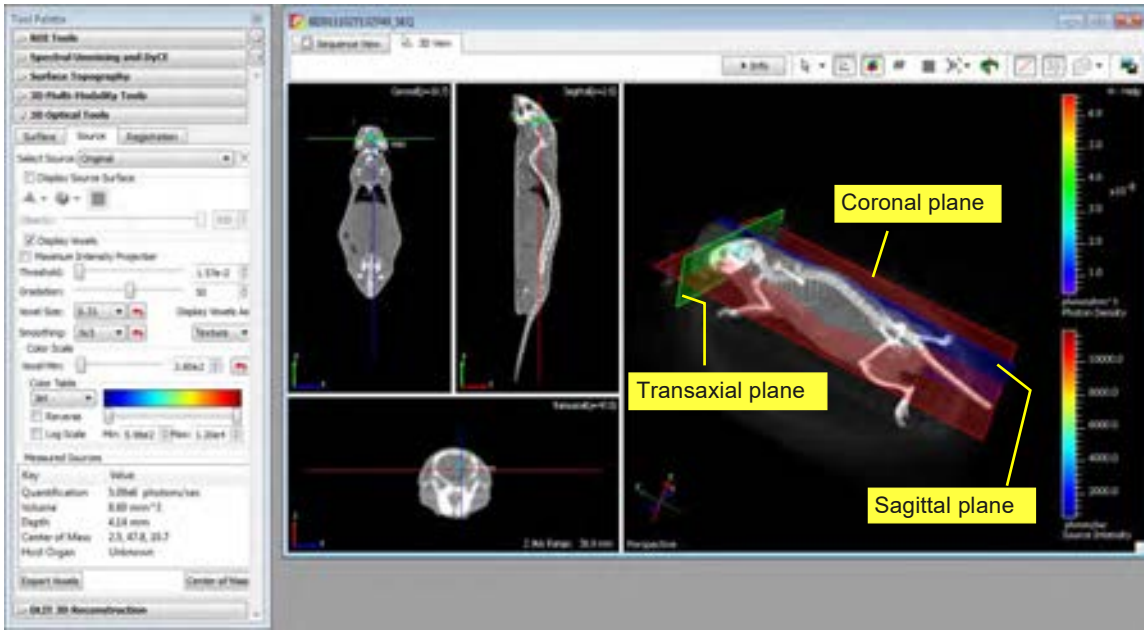
Figure 14.30

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.

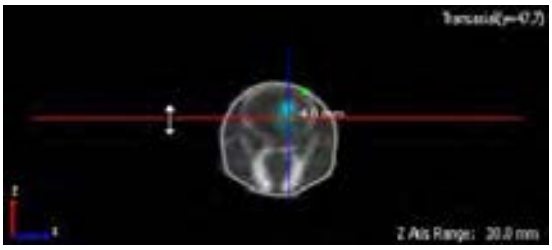
Figure 14.31 Planes Cutting a Reconstruction



3. 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.

Figure 14.32 Moving the Transaxial Plane



14.9 Viewing Luminescent and Fluorescent Sources in One Surface

When an experiment includes luminescent and fluorescent reporters, DLIT and FLIT reconstructions can be displayed in 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 a DLIT reconstruction and a FLIT reconstruction.
2. Choose one of the reconstructions, click the button and select **Copy source voxels**.
3. In the other reconstruction, click the button and choose **Paste source voxels**.

NOTE: Pasted voxels can be measured. For more details on measuring sources, see page 222.

14.10 Comparing Reconstruction Results

Multiple DLIT or FLIT reconstruction results can be viewed side-by-side in the Longitudinal Study window. Voxel intensity within the entire surface or a user-selected area can be measured in all results in the Longitudinal Study window.

The Longitudinal Study window provides a convenient way to compare different results, for example, results obtained at different time points or results from different types of reporters.

Viewing Results in the Longitudinal Study Window

Multiple DLIT or FLIT reconstruction results can be viewed side-by-side in the Longitudinal Study window. Voxel intensity within the entire surface or a user-selected area can be measured in all results in the Longitudinal Study window.

The Longitudinal Study window provides a convenient way to compare different results, for example, results obtained at different time points or results from different types of reporters.

NOTE: The FLIT results selected for display in the Longitudinal Study window must have the same type of units. The DLIT results selected for display in the Longitudinal Study window must have the same type of units.

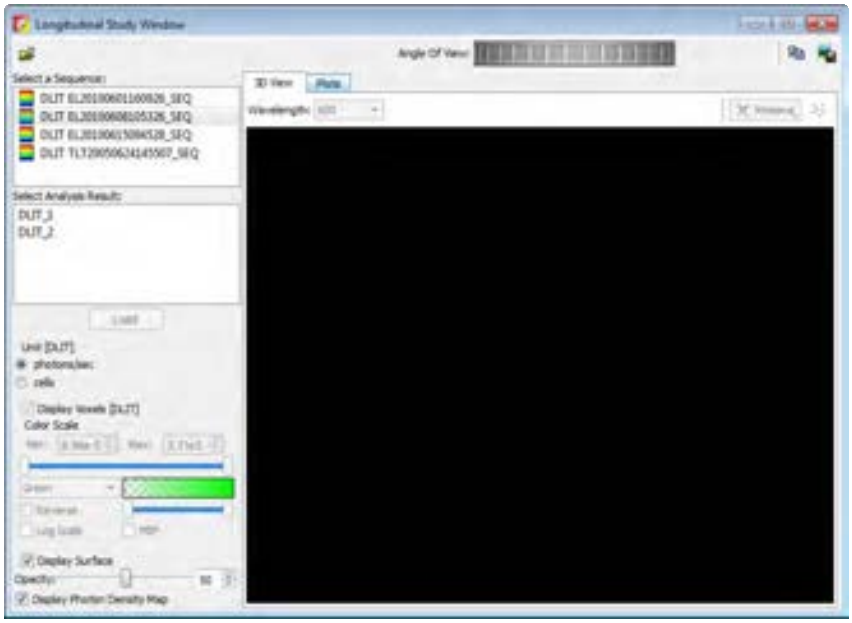
1. Load the DLIT or FLIT sequences with the results that you want to display. Select **Tools** → **Longitudinal Study** on the menu bar.

   The Longitudinal Study window appears.

Figure 14.33 Longitudinal Study Window (no results displayed)

Loaded DLIT or FLIT sequences

Results saved with the sequence selected in the upper box. Select the results to display from this list.



NOTE: After the Longitudinal Study window is open, more sequences can be added to the window by clicking the Open button and selecting sequenceinfo.txt files (found in the sequence data folder).

2. To show particular results:
   a. Select a sequence in the upper box.
   b. Select one or more analysis results in the lower box. To choose multiple adjacent results, press and hold the Shift key while you click the first and last result. To choose non-adjacent results, press and hold the Ctrl key while you click the results.
   c. Click **Load**.
3. To show more results, repeat step step 2
4. To remove results from the Longitudinal Study window, right-click a surface and select **Remove** on the shortcut menu. Alternatively, select a surface, click the Remove button Remove and choose **Selected Result**.
   To remove all results, click the Remove button Remove and choose **All Results**.
5. To view a particular image in a sequence:
   a. Click the surface.
   b. For DLIT results, make a selection from the Wavelength drop-down list. For FLIT results, make a selection from the Image drop-down list.

Figure 14.34 DLIT and FLIT Results in the Longitudinal Study Window

Table 14.11 Longitudinal Study Window

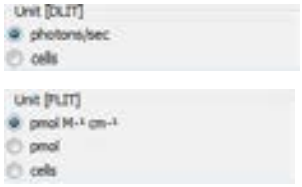
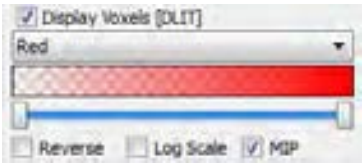
Item	Description
	DLIT – Select photons/sec or cells (results calibrated using a quantification database). FLIT – Select pmole M^{-1} cm^{-1} or pmoles (results calibrated using a quantification database). Only DLIT results of the selected unit will be enabled for loading in the Longitudinal Window.

Table 14.11 Longitudinal Study Window (continued)

Item	Description
	Voxel display controls: **Display Voxels** – Choose this option to show voxels within the surface. From the drop-down list, select a color scheme for the color scale. Move the sliders to adjust the color scale minimum and maximum values. **Reverse** – Choose this option to apply the colors of the selected color table in reverse order to the photon density scale. For example, the Red color table represents the source intensity (photons/sec) from low to high using a color scale from transparent to red. If **Reverse** is chosen, the source intensity (photons/sec) from low to high is represented using the color scale from red to transparent. **Log Scale** – Applies a log scale to the color scale. **MIP** – When this option is chosen, all maximum intensity voxels in the view are projected along the viewing direction into the viewing plane.
	Copies the 3D View tab in the Longitudinal Study window to the system clipboard.
	Opens a dialog box that enables you to export the 3D View tab to a graphic file (for example, .png).
	Enables you to select voxels for measurement. Measurements are displayed in the Plots tab.

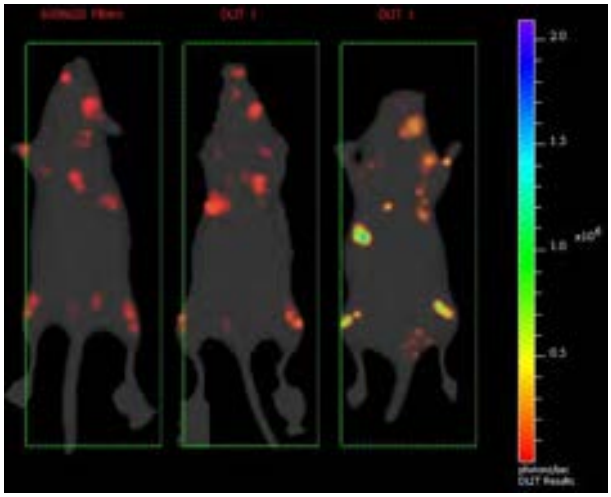
Measuring Intensity

1. Load 3D reconstruction results and click the  button.

   By default, a selection box appears around each surface (Figure 14.35). This means that measurements for the entire surface will be computed.
2. To select a particular region of the surface for measurements, draw a box (by clicking and dragging the mouse) around the area.

   The same box is applied to the other surfaces in the Longitudinal Study window.
3. To clear boxes, click the  button again.

Figure 14.35 Selection Boxes Around Each Surface



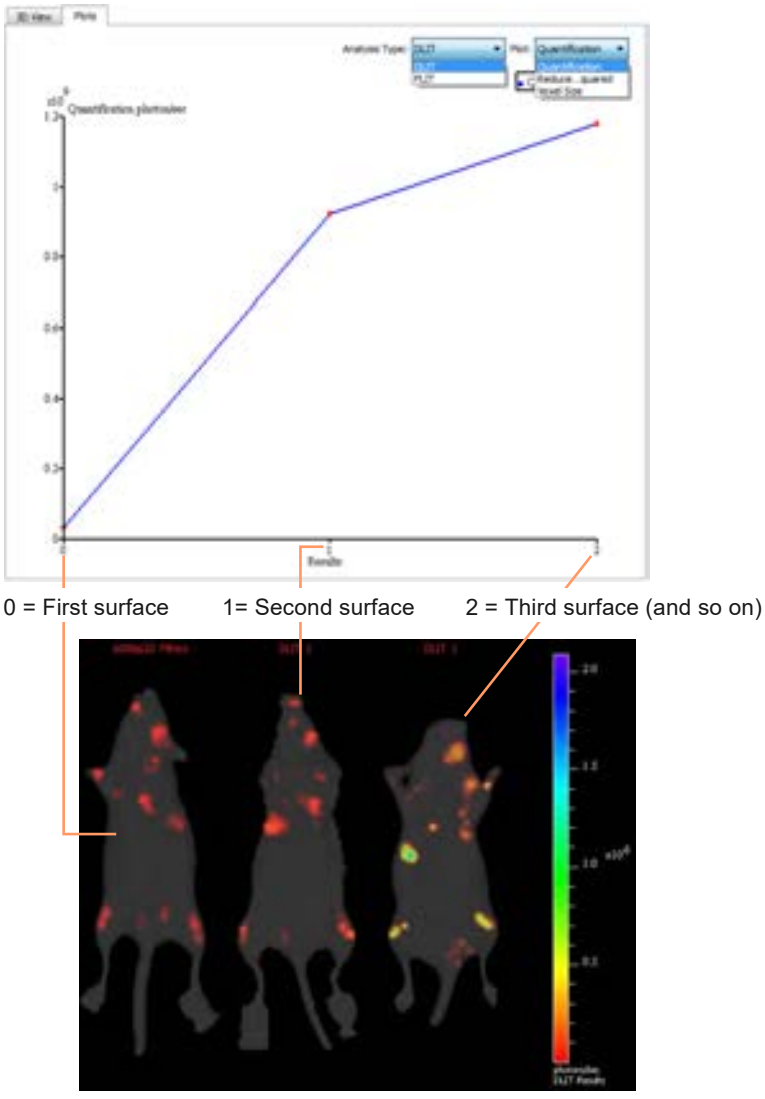
Viewing Plots

To view a graph, make a selection from the Analysis Type and Plot drop-down lists in the Plots tab (Figure 14.36).

The following graphs are available in the Plots tab:

Plot Type	Description
Quantification Profile	Plots the measured intensity within the user-selected area on the surface. If no box was drawn on the surface, measures the total intensity for the entire surface.
Reduced Chi-Squared Profile	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.
Voxel Size	Plots the voxel size at the start of the 3D reconstruction and at the end of the 3D reconstruction.

Figure 14.36 Example Quantification Plot



14.11 Exporting a 3D Scene as DICOM

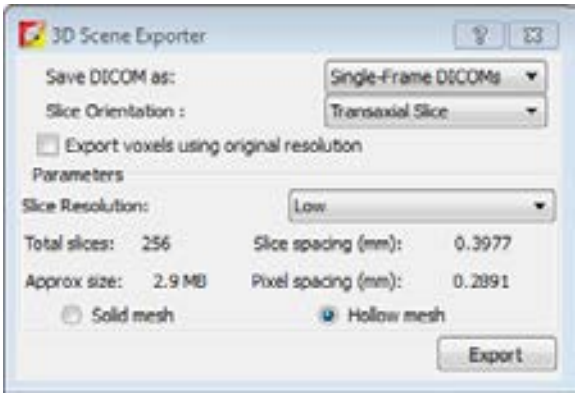
The items in the perspective 3D View are called a *3D scene*. For example, the 3D scene in Figure 14.37 includes a surface and voxels. The 3D scene can be exported to DICOM format and viewed in the Living Image DICOM Viewer or third party software.

Figure 14.37 3D Scene

To export the 3D scene:

1. Load the results that you want to export.
2. Select **File** → **Export** → **3D Scene as DICOM** on the menu bar.
3. In the dialog box that appears, set the export options, and click **Export**.
   For more details on the 3D Scene Exporter, see Table 14.12.

Figure 14.38 3D Scene Exporter Dialog Box



4. In the Browse For Folder dialog box that appears, choose a folder for the DICOM files and click **OK**.
   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 14.12 3D Scene Exporter Dialog Box

Item	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 Slice spacing Pixel spacing	Parameters that determine the number and resolution of the slices to export.
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

The 3D scenes exported to DICOM can be viewed in the Living Image 3D Browser.

1. Select **File** → **Browse 3D Volumetric Data** on the menu bar.
2. In the dialog box that appears, select the DICOM data (.dcm or .dc3) and click **Open**. The 3D Browser window appears.

Figure 14.39 Living Image 3D Browser

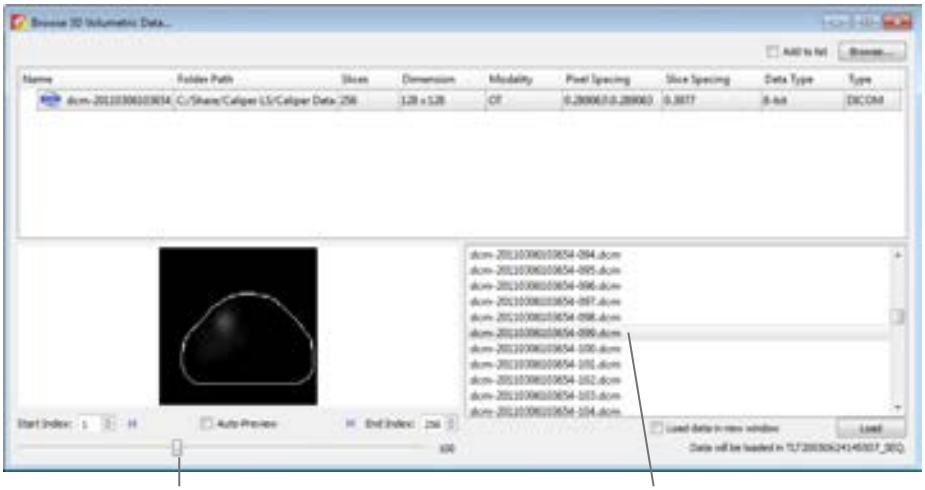


Table 14.13 Living Image 3D Browser DICOM Viewing Controls

Item	Description
Start Index	Specifies the first image (slice) for viewing.
Auto Preview	Select this option to automatically play back the images.
End Index	Specifies the last image (slice) for viewing.
Load	Opens the DICOM data in a 3D View window.
Load data in new window	If this option is selected, DICOM data are opened in a new 3D View window when you click **Load**. If this option is not selected, DICOM data are loaded in the active 3D View window.

14.12 3D Optical Surface Tools

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

Figure 14.40 Surface Tools and Example DLIT Reconstruction with Photon density or NTF Efficiency Maps

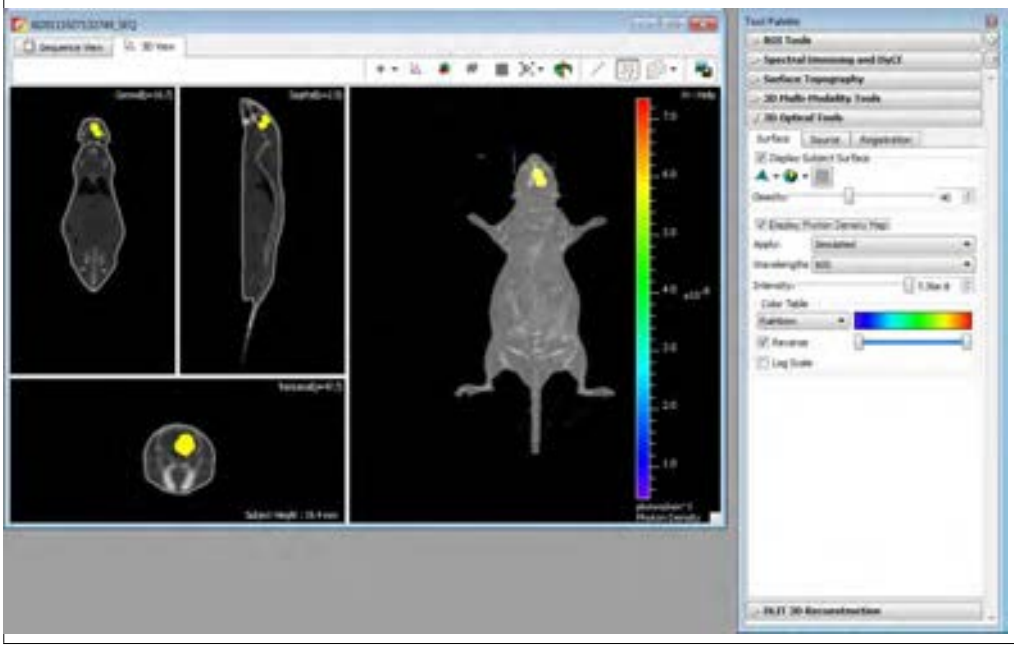


Table 14.14 3D Surface Tools

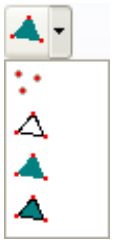
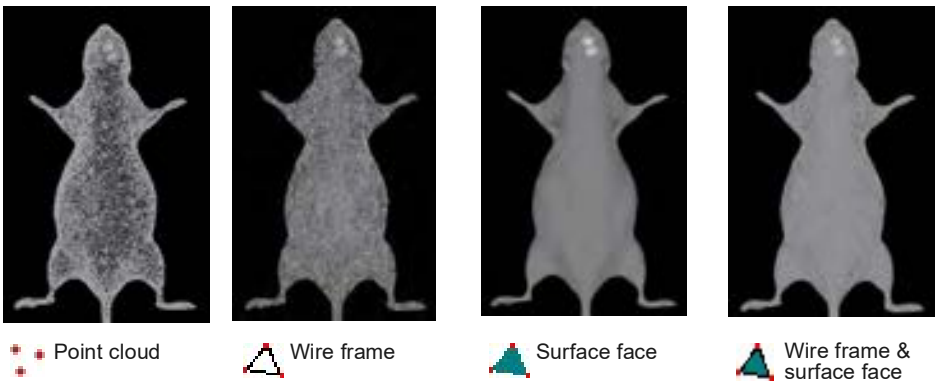
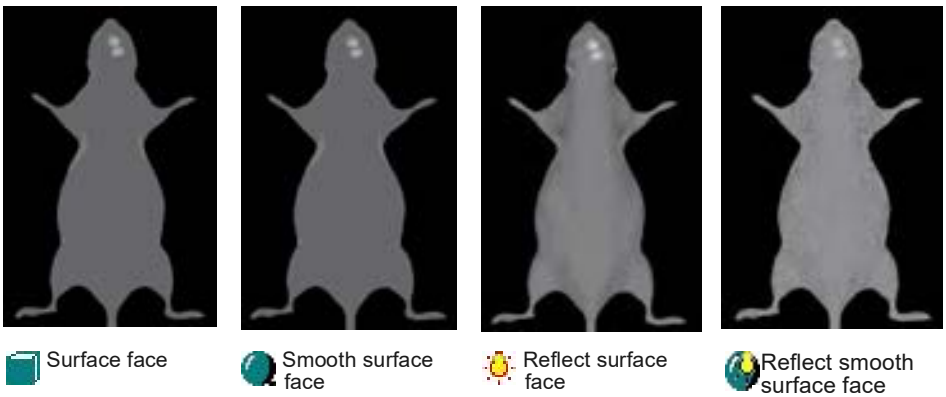
Item	Description
Display Subject Surface	Choose this option to display the surface in the 3D View window.
	Drawing styles for the surface. 
	Shading styles for the surface. 
	Click to open the color palette from which you can select a display color for the surface and the cross section views.
Opacity	Adjusts the surface opacity.
Display Photon Density or NTF Efficiency Map	Choose this option to display the photon density or NTF Efficiency on the surface.
Apply	Choose measured or simulated photon density or NTF Efficiency maps for display.

Table 14.14 3D Surface Tools (continued)

Item	Description
Wavelengths (DLIT) Images (FLIT)	Choose the data to display in the photon density or NTF Efficiency map.
Intensity	Set the maximum intensity of the photon density or NTF Efficiency map using the slider or by entering a value.
Color Table	Color scheme for the photon density or NTF Efficiency map.
Reverse	Choose this option to apply the colors of the selected color table in reverse order. For example, the Red color table represents the mapped intensity from low to high using a color scale from transparent to red. If **Reverse** is chosen, the mapped 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 photon density or NTF Efficiency scale.

14.13 3D Optical Source Tools

Use the Source tools to:

- Adjust the appearance of sources in DLIT or FLIT reconstructions
- Make source measurements (page 222)
- Export voxel measurements (.csv)

Figure 14.41 Source Tools and Example DLIT Reconstruction

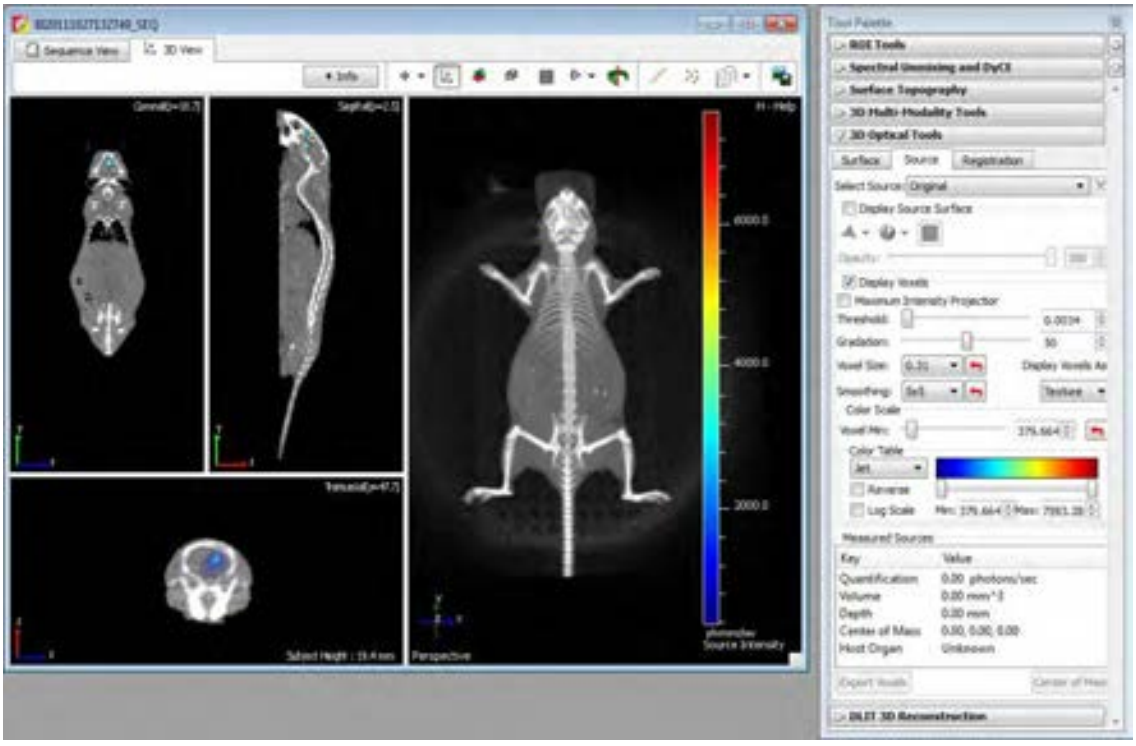


Table 14.15 3D Source Tools

Item	Description
Select Source	A drop-down list of available sources. Original – Results saved with the data. <sequence name...SourceVoxels> – Pasted voxels. (Click the button to remove pasted voxels from the surface.) See *Viewing Luminescent and Fluorescent Sources in One Surface on page 227* for more details on copying and pasting sources from one sequence to another.
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.
	Drawing styles for the source surface (see *Display Source Surface on page 239*).
	Shading styles for the source surface (see "*Display Source Surface on page 239*").
	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.
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 measurement.
Gradation (DLIT or 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.

Table 14.15 3D Source Tools (continued)

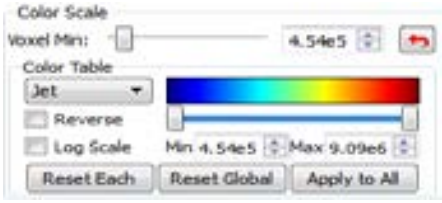
Item	Description
Smoothing	The smoothing box filter size.
Display voxels as	The voxel display mode (cubes, spheres, points, or texture).
Color Scale and Color Table	 Voxel Min: Use the slider, up/down arrows, or enter a value to set the minimum value of the source color scale. Voxels with intensities 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 color table range. 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.
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 222. 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-1cm-1] here for uncalibrated sources. For calibrated sources, this unit will be in either [cells] or [pmol]. For details using this tool, see page 222. Volume – Volume of the selected source (mm3). 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 – The location of the selected source can be referenced to an organ atlas, and the organ from the atlas that is closest to the source will be reported here. This information is available if you select and register an organ atlas with the reconstruction. For more details, see page 246.
Export Voxels	Enables you to export the voxel measurements in their x-, y-, and z-coordinates and source intensities (.csv file).

Table 14.15 3D Source Tools (continued)

Item	Description
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 222.

14.14 3D Optical Registration Tools

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 bioluminescent or fluorescent source anatomical location.

Use the Registration tools to:

- Display organs in the surface (page 242)
- Manually adjust the location or scale of organs in the surface (page 244)
- Check the organ fit (page 245)
- Import an organ atlas (page 246)

You can check the organ fit in the 3D View window (page 245)

Figure 14.42 3D Registration Tools and Surface with Fitted Organs (“Skin” not displayed)

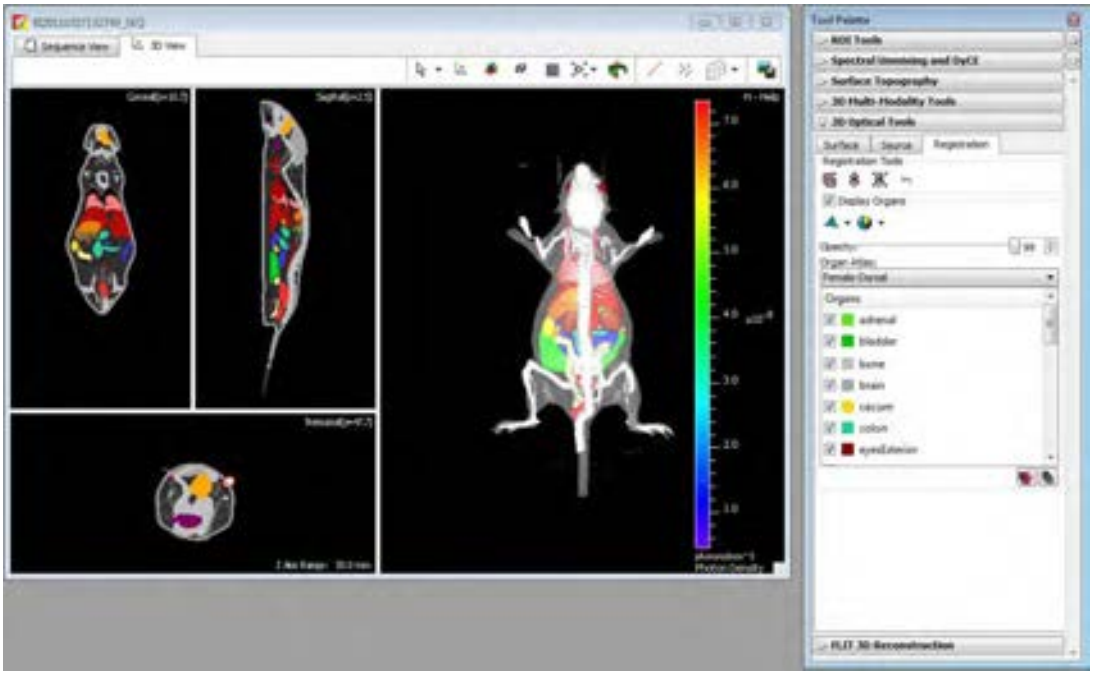
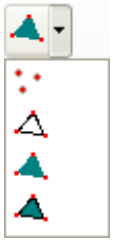


Table 14.16 3D Registration Tools

Item	Description
	Use this tool to manually adjust the scale of location of organs. For more details, see page 244.

Table 14.16 3D Registration Tools (continued)

Item	Description
	Fits the organs to the surface using a linear transformation that keeps the shape of the atlas surface.
	Fits the organs to the surface using linear transformation and volume deformation.
	After fitting organs to the surface using the  or  tool, if necessary, you can 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. For more details, see page 242.
	Drawing styles for the organs (see *Display Organs on page 242*).
	Shading styles for the organs (see *Display Organs on page 242*).
Opacity	Adjusts the opacity of the organ display.
Organ Atlas	Choose a type of organ atlas.
	Click to select all organs in the database and display them on the surface.
	Click to clear the selected organs and remove all organ diagrams from the surface.

Displaying Organs With a Reconstruction

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. In the 3D registration tools, choose the Display Organs option and select an organ atlas.
   The organs in the selected atlas appear on the surface.
3. To fit the organs to the surface, click a registration tool:

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

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 the Scale or Location of Organs on page 244* for more details.)

4. 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.

5. To clear all organs from the surface, click the **Deselect All** button  . To hide a particular organ, remove the check mark next to the organ name.

6. To display a specific organ(s), choose the organ name. To display all organs on the surface, click the **Select All** button  .

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

Manually Adjusting the Scale or Location of 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).

   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, press and hold the left mouse key, then drag the mouse when the hand appears.

2. In the 3D registration tools, choose the Display Organs option and select an organ atlas.

   The organs in the selected atlas appear on the surface. Only “Skin” is selected in Figure 14.43.

3. Click the **Transform tool** button.

   The transform tool appears. Figure 14.44 explains the tool functions.

Figure 14.43 Displaying the Transform Tool

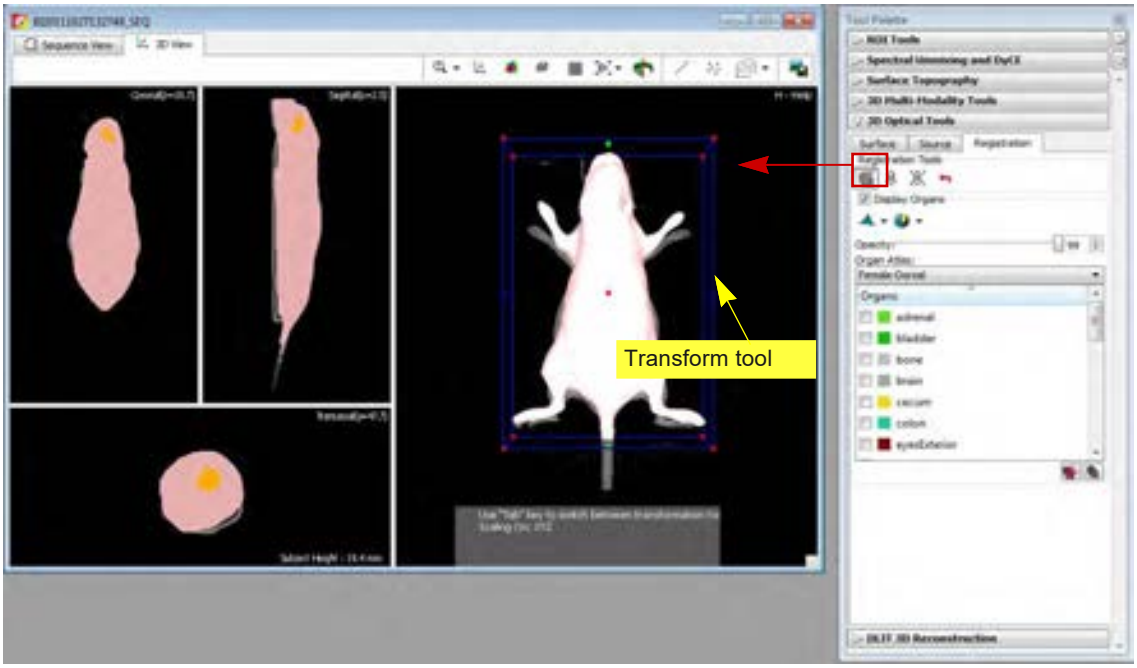
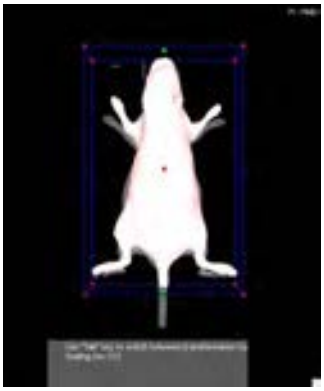


Figure 14.44 Transform Tools

Click and drag the organ(s) when the yellow "+" appears.

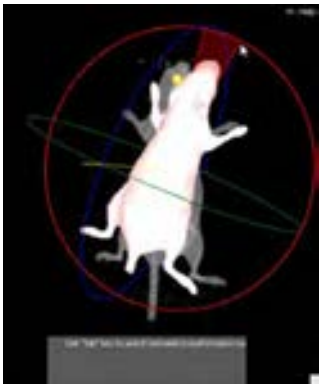


Click and drag a handle to scale (increase or decrease) the size of the organ(s).

Red – Scales on the z-axis.

Blue – Scales on the x-axis.

Green – Scales on the y-axis.



To rotate the organ(s) on the x,y, or z-axis, click the blue, green, or red circle and drag the mouse arrow in the direction of interest.

4. Press the Tab key to switch between the transform tools.

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

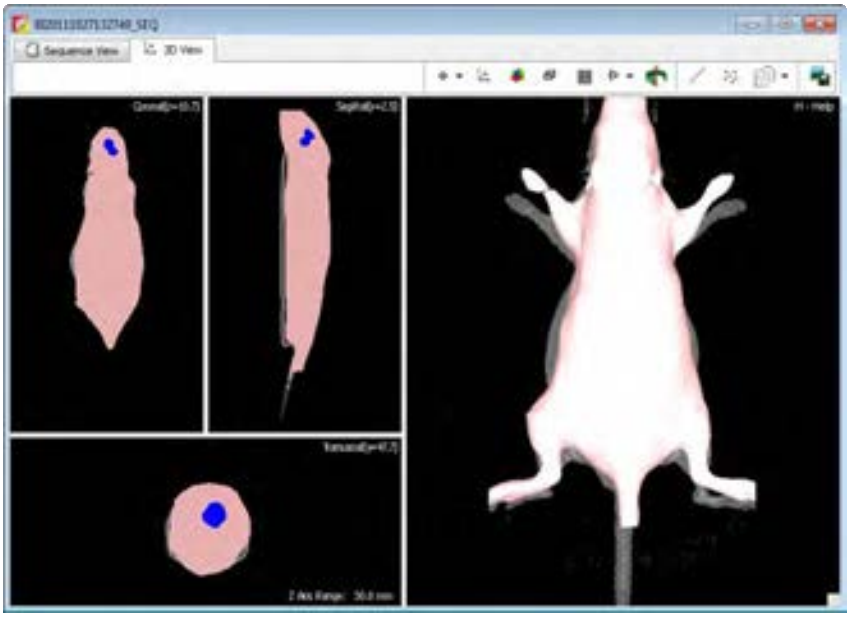
5. Turn off the transform tool when you are done adjusting the position of the organ(s) (click the button).

Checking the Organ Fit

1. Check the fit in the coronal, sagittal, and transaxial windowpanes.
2. Click the **Change view** toolbar button .

   The Top view is displayed.

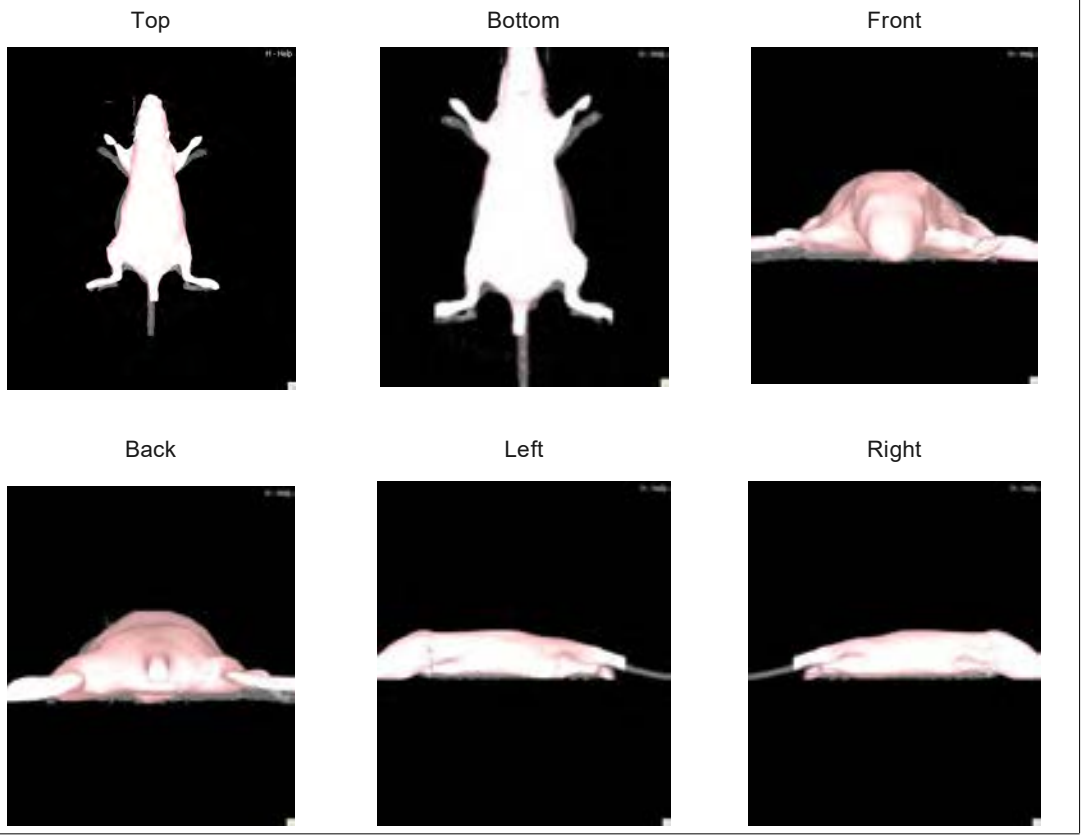
Figure 14.45 Skin (pink) Fitted to Surface (gray)



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

Figure 14.46 Alternative Surface Views

In this example, “skin” is selected from the organ atlas (pink surface). The mouse surface is gray.



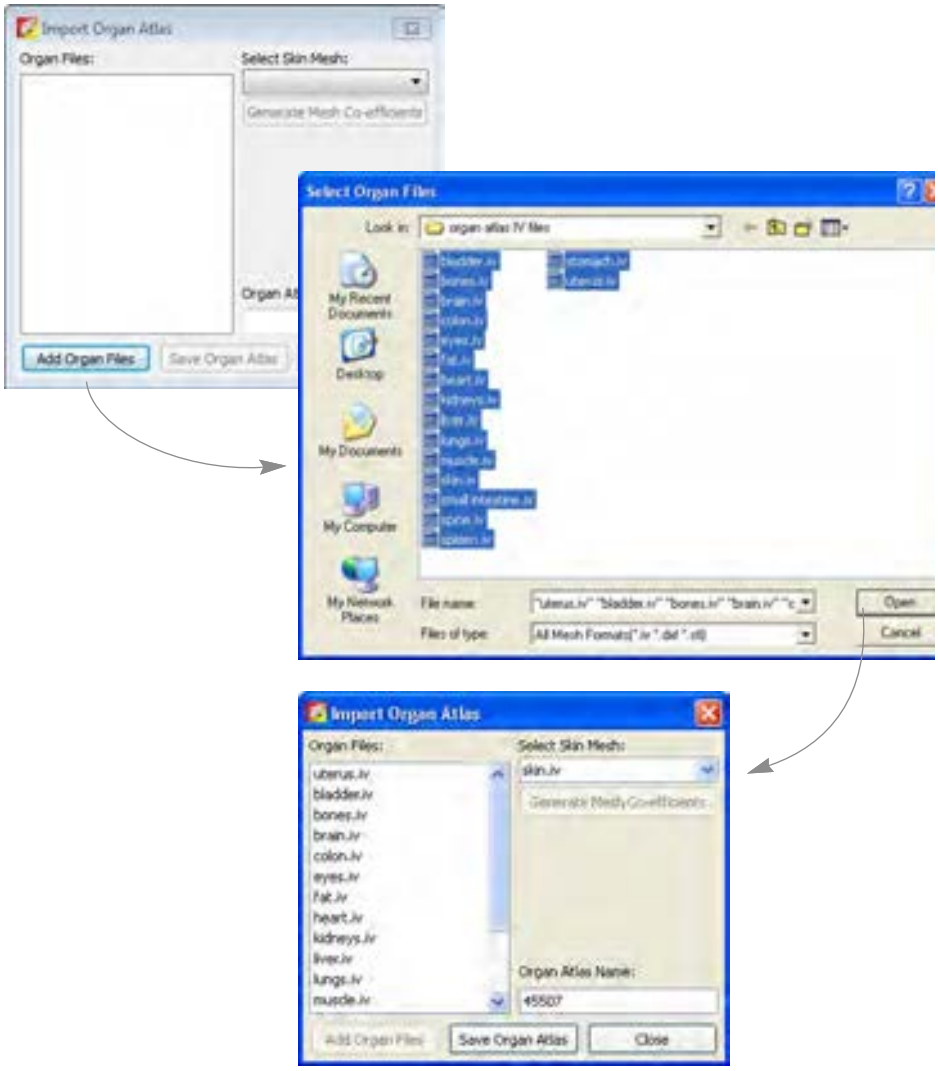
Importing an Organ Atlas

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** → **Import** → **Organ Atlas** on the menu bar.
3. In the dialog box that appears, click **Add Organ Files** (Figure 14.47).

Figure 14.47 Importing an 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**.

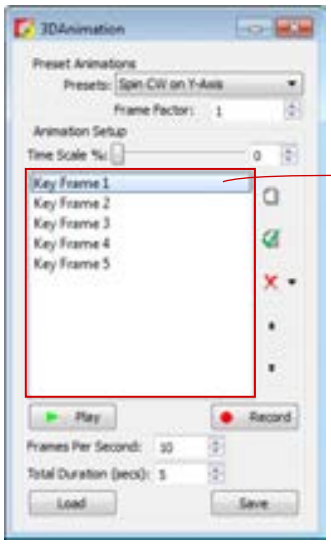
   The organ atlas (.atlas) is created and is added to the Organ Atlas drop-down list (in the 3D Optical Tools, Registration tab).

14.15 3D Animation

The 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 14.48). The animation (series of key frames) can be recorded to a movie file (.mov, .mp4, or .avi). Use the animation tools to:

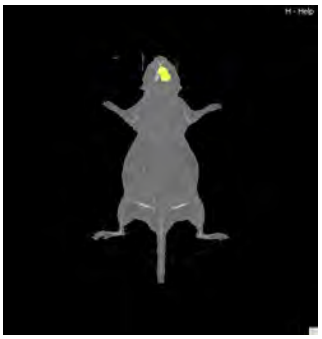
- View a preset animation (generated from a factory-loaded animation setup) (page 250)
- Create a custom animation (created from your custom animation setup) (page 254)
- Save an animation setup (page 252)
- Record an animation to a movie file (page 253)
- Edit an animation setup (page 253)

Figure 14.48 Individual 3D Views (key frames) in the Preset Animation “Spin CW on Y-Axis”

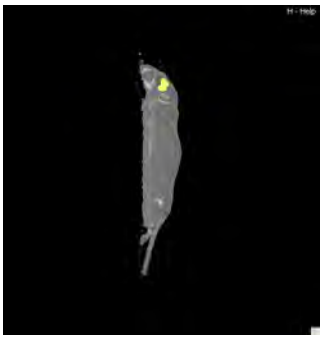


The ☐ box shows the key frames in the current animation setup. Click a key frame in this box to display the associated 3D view and time stamp (position in the time scale (0-100) at which the frame occurs in the animation).

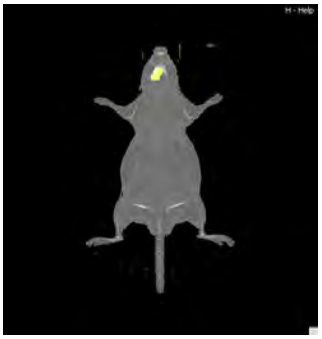
Click Play to view the animation composed of the key frames.



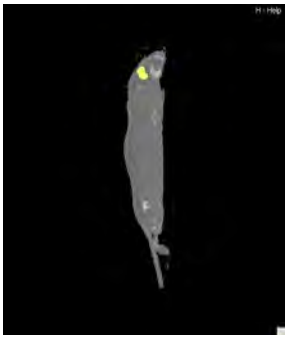
Keyframe 1



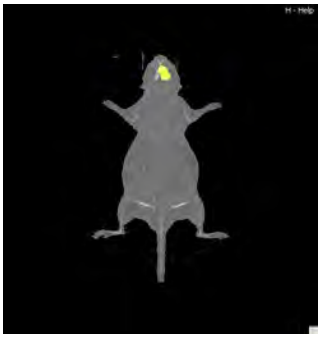
Keyframe 2



Keyframe 3



Keyframe 4



Keyframe 5

Table 14.17 3D Animation Tools

Item	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 x 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.
	Creates a new key frame from the current 3D view.
	Updates the selected key frame to the current 3D view.
	Deletes a selected or all key frames from the key frame box.
	Moves a selected key frame up in the key frame box.
	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.

Table 14.17 3D Animation Tools (continued)

Item	Description
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:

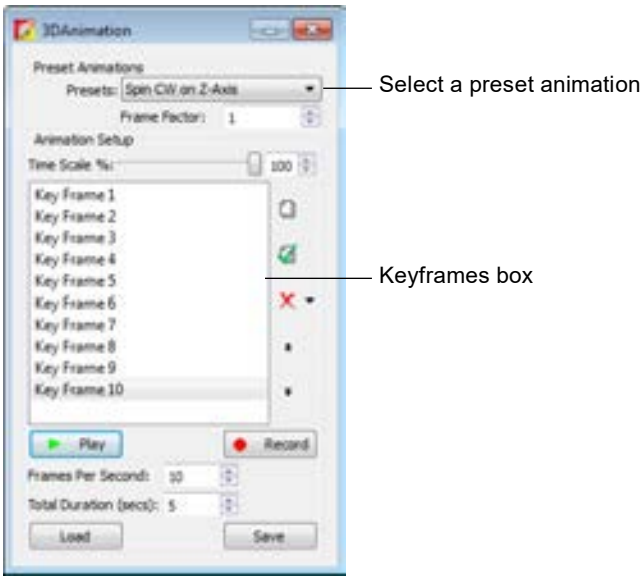
1. Open an image sequence and load 3D reconstruction results.
2. Select properties to display in the 3D View window (for example, organs, sources, surface, or photon density maps).
3. Select **View** → **3D Animation** on the menu bar.
4. In the 3D Animation tools that appear:
   a. Clear the key frame box if necessary (click the ✕ button and select **Delete All**).
   b. Make a selection from the Presets drop-down list. See Table 14.17, page 249 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 14.49 3D Animation Tools\
See Table 14.17, page 249 for details on the animation tools.



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** → **3D Animation** on the menu bar.
   The 3D Animation tools appear (Figure 14.50).
4. Clear the key frame box if necessary (click the ✕ button and select **Delete All**).

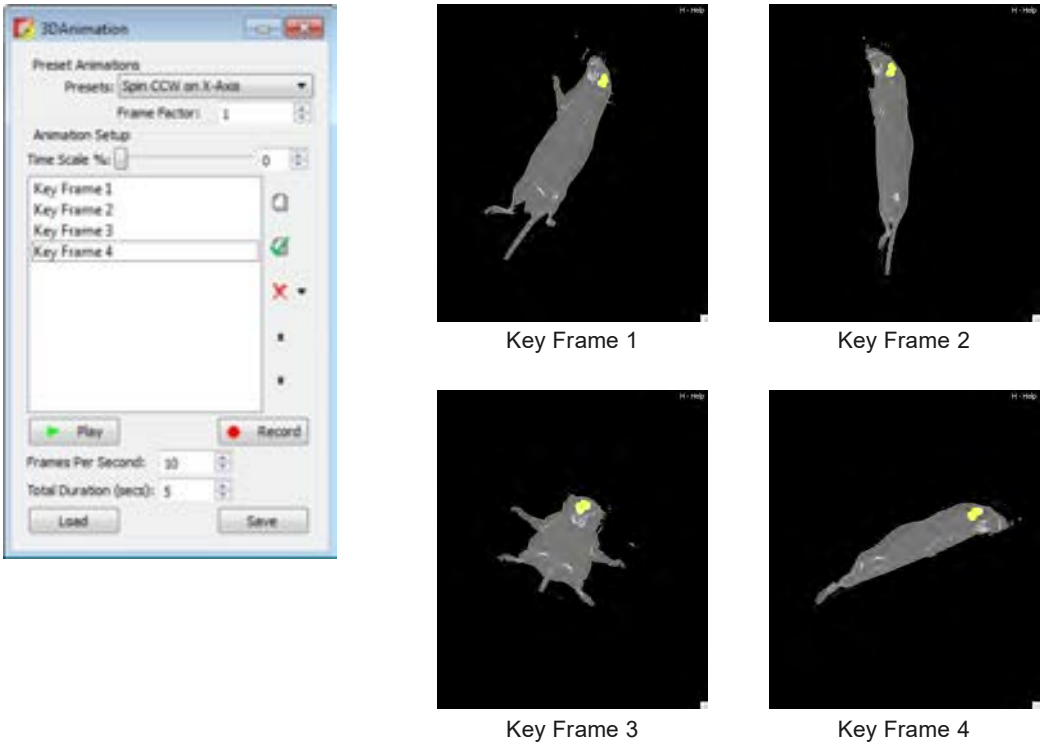
Figure 14.50 Key Frames Cleared

5. To capture the first key frame, click the ⧉ button.

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

6. Adjust the position of the reconstruction in the 3D View using an image tool (for example, , or). For more details on the image tools, see page 211.
7. Click the button.
   The second key frame is added to the key frame box.

Figure 14.51 Example Key Frames for a Custom Animation



8. Repeat step 6 to step 7 until all of the key frames are captured. For details on how to edit the key frame sequence, see page 253.
   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 x Total Duration = No. of frames generated to create the animation. The number of generated frames should be ^{3} 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

To save an animation setup:

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

To record the 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.

To 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.

Figure 14.52 List of Key Frames in the Selected Animation

3. To add a key frame:
   a. Adjust the position of the reconstruction in the 3D view using an image tool (for example, , or). For more details on the image tools, see page 211.
   b. Click the button.
   c. To reorder a key frame in the sequence, select the key frame and click the or arrow.

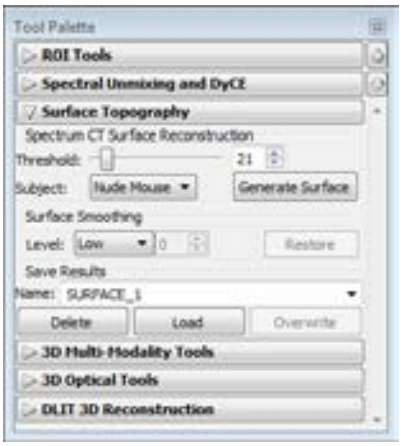
To update a key frame:

   a. Select the key frame and adjust the 3D view.
   b. Click the button.

To delete a key frame:

   a. Select the key frame that you want to remove.
   b. Click the button and select **Delete Current**.

14.16 DLIT/FLIT 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	Metallic objects can scatter the x-rays, resulting in noisy CT images. To avoid the scattered x-ray paths which cause spikes, adjust the threshold that specifies the CT volume data used for surface reconstruction. 
Bad Photon Density of 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.

15 Working With 3D Reconstructions

15.1 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)

Figure 15.1 3D Optical Tools, Source Tab

Table 15.1 3D Optical Tools, Source Tab

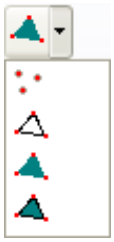
Item	Description
Select Source	A drop-down list of available sources. Original – Results saved with the data. <sequence name...SourceVoxels> – Pasted voxels. (Click the button to remove pasted voxels from the surface.) See *Viewing Luminescent and Fluorescent Sources on One Surface on page 266* for more details on copying and pasting sources from one sequence to another.
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 "*Display Source Surface on page 256*" above).
	Shading styles for the source surface (see "*Display Source Surface on page 256*" 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.

Table 15.1 3D Optical Tools, Source Tab (continued)

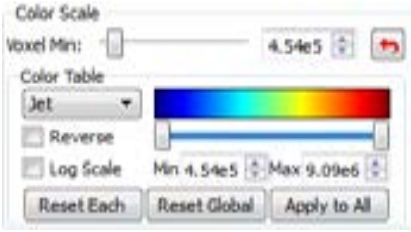
Item	Description
Gradation (DLIT/FLIT)	Use this slider to set a threshold for the percentage voxel intensity above which voxels are opaque and below which voxels will gradually face to transparent. The percentage voxel intensity is the percentage relative to the maximum intensity.
Voxel size	The 3D grid-spacing 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).
Color Scale and Color Table	 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.

Table 15.1 3D Optical Tools, Source Tab (continued)

Item	Description
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 258. 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 258. 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 267 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 258.

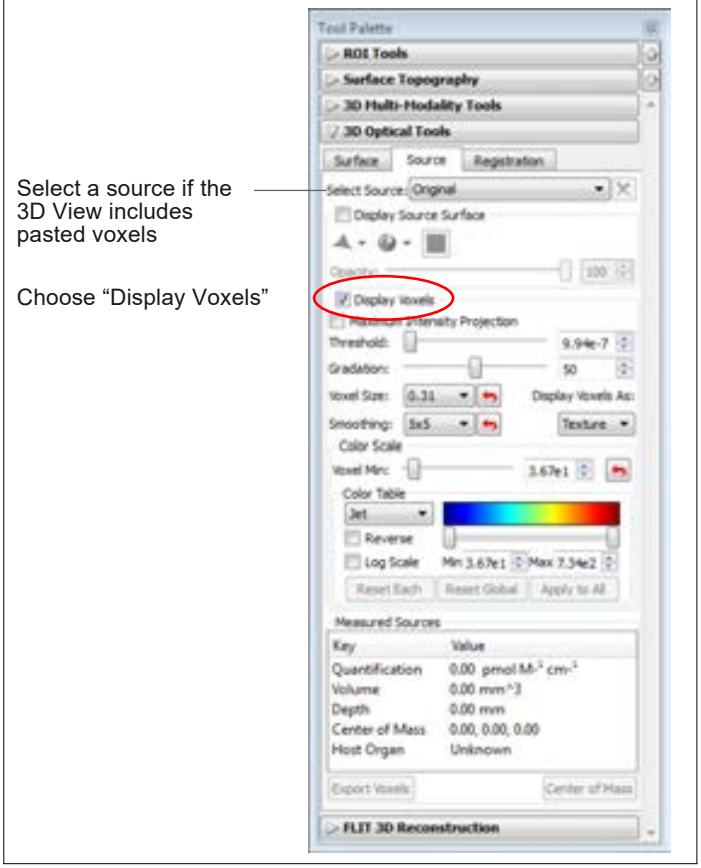
Source Quantitation

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 286 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 15.2).
3. Confirm that "Display Voxels" is selected, not "Display Source Surface".

Figure 15.2 Select and Measure Source Voxels



4. Click the Measure Source button, then draw a box around the source (Figure 15.3).

Figure 15.3 Select Source to Measure

5. Click **Center of Mass** to obtain the measurements (Figure 15.4). See Table 15.2 on page 261 for a description of the measurements.

   The coronal, sagittal, and transaxial planes intersect at the center of mass of the selected source (see Figure 15.5 on page 262).

Figure 15.4 Source Measurements

Table 15.2 Source Measurements

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 *Displaying Organs With a Reconstruction on page 267* for more details.

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  button to display slice planes through the center of mass. See page 263 for more information on planes.

Figure 15.5 Slice Planes

This example shows slice planes through a selected source center of mass and distance measurements from the source center of mass to the surface.

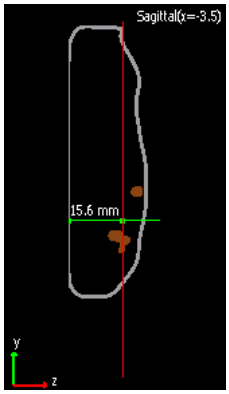
Viewing Location Coordinates

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

The coordinates (mm) of the position are displayed (Figure 15.6). 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.

Figure 15.6 Viewing Y,Z Coordinates – Sagittal Plane

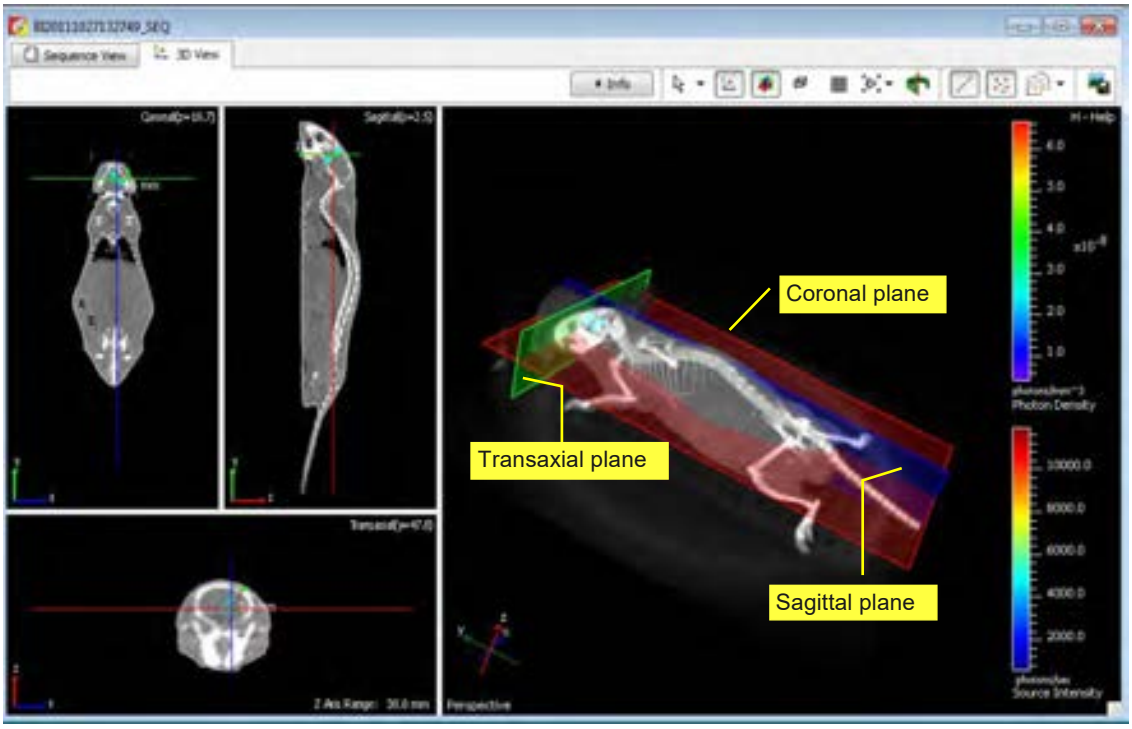


Displaying Slices Through a Reconstruction

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 15.7).

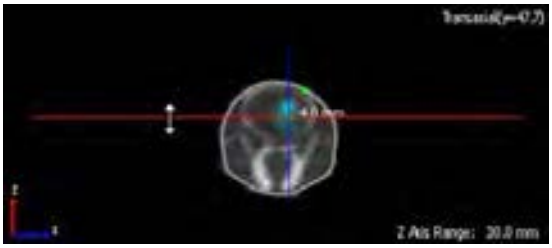
Figure 15.7 Planes Cutting a Reconstruction



3. 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.

Figure 15.8 Moving the Transaxial Plane



15.2 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 286 for more information).

NOTE: The 3D view synchronization feature replaces the Longitudinal Study window found in Living Image Software 4.4 and earlier versions.

1. 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 15.3.

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 15.3 3D View Synchronization Options

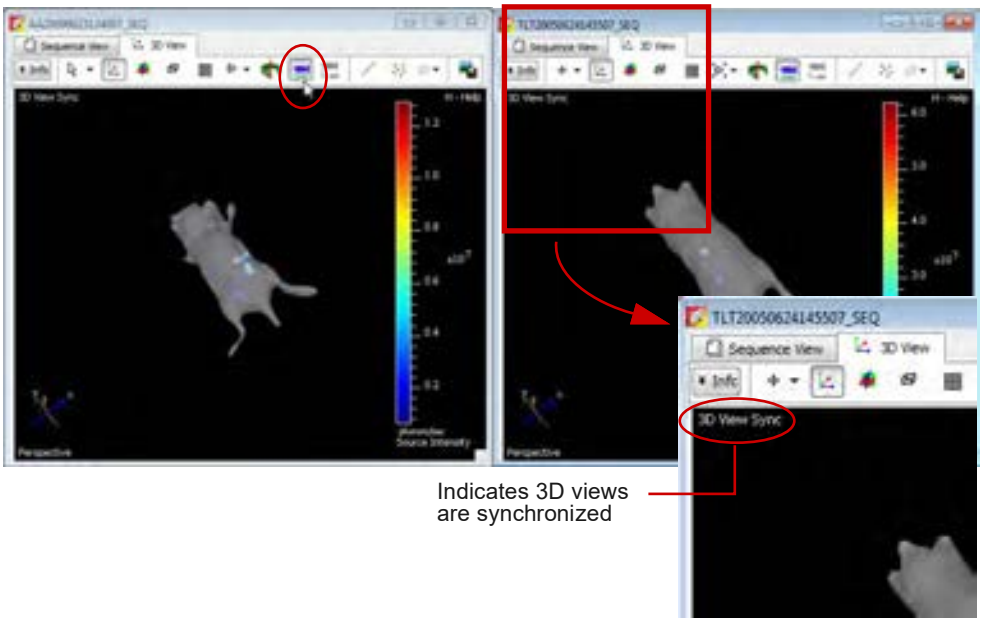
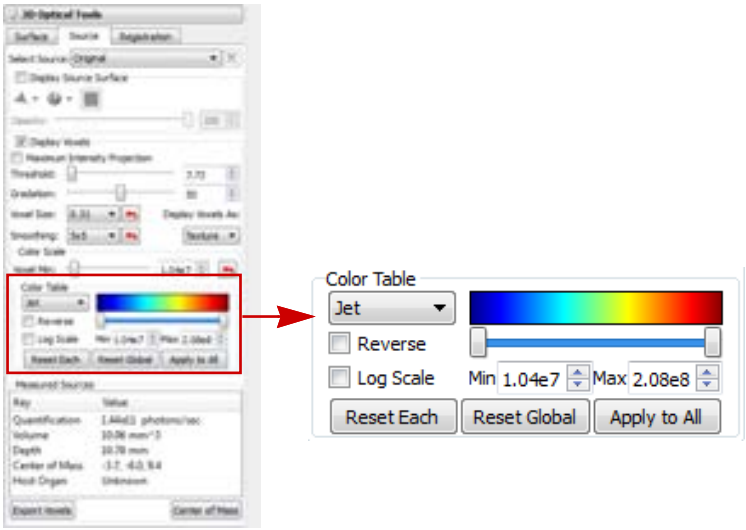
Item	Description
	When multiple 3D reconstructions are loaded, click this button in one of the 3D views to turn on synchronization. All other 3D views will reset to display the same perspective as the active data. Click the button again to turn off synchronization.  If 3D views are synchronized, these tools will be applied to all 3D views at the same time. 
	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.

Table 15.3 3D View Synchronization Options (continued)

Item	Description
Color Table Options in 3D Optical Tools (Source tab)	 **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. See *Color Scale and Color Table on page 257* 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.

15.3 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 button in the 3D View toolbar, and select **Copy source voxels**.
3. In the other reconstruction, click the button and choose **Paste source voxels**.

NOTE: Pasted voxels can be measured. See page 258 for more details on measuring sources.

15.4 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.

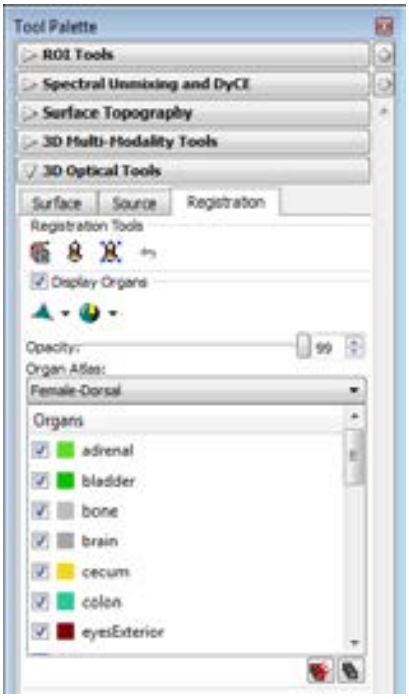
Use the 3D Optical Registration tools to:

- Display organs in the surface (see below)
- Manually adjust the location or scale of organs in the surface (page 269)
- Check the organ fit (page 271)
- Import an organ atlas (page 272)

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 15.9).

Figure 15.9 3D Optical Registration Tools



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 15.10).
4. To fit the organs to the surface, click a registration tool:
   – Rigid registration: Performs linear transformation, but keeps the shape of the atlas surface.

   – 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 269* 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 15.10)
6. To clear all organs from the surface, click the **Deselect All** button. To hide a particular organ, remove the check mark next to the organ name.
7. To display a specific organ(s), choose the organ name. To display all organs on the surface, click the **Select All** button.

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

Figure 15.10 Surface with Fitted Organs (“Skin” not displayed) and 3D Optical Registration Tools

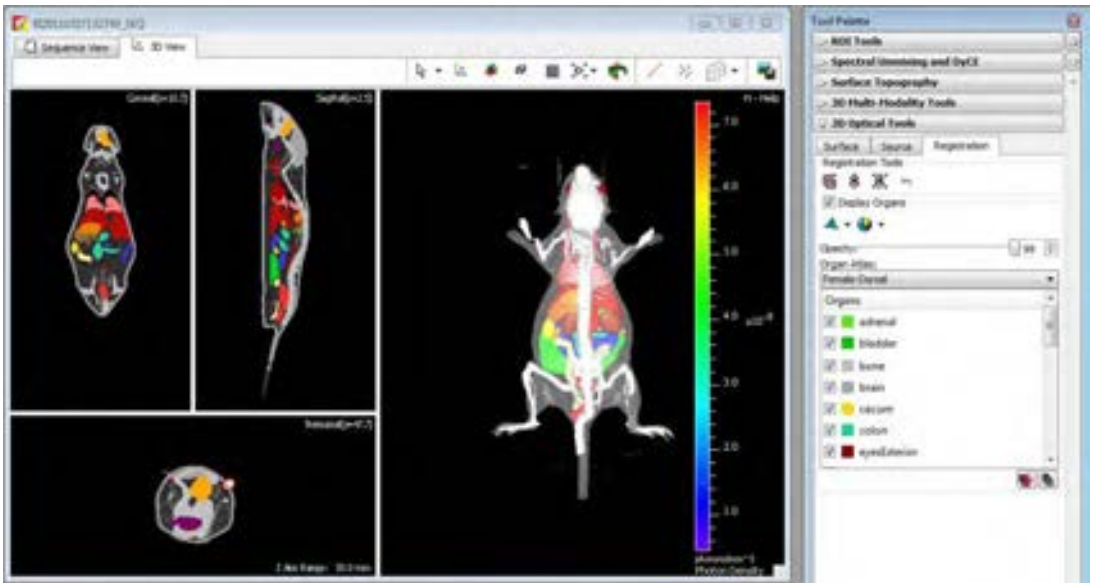
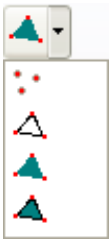


Table 15.4 3D Optical Registration Tools

Item	Description
	Use this tool to manually adjust the scale of location of organs. See page 269 for more details.
	Fits the organs to the surface using a linear transformation that keeps the shape of the atlas surface.
	Fits the organs to the surface using linear transformation and volume deformation.

Table 15.4 3D Optical Registration Tools (continued)

Item	Description
	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.
	Drawing styles for the organs (see “*Display Organs on page 269*” above).
	Shading styles for the organs (see “*Display Organs on page 269*” above).
Opacity	Adjusts the opacity of the organ display.
Organ Atlas	Choose a type of organ atlas.
	Click to select all organs in the database and display them on the surface.
	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 organs (see *Displaying Organs With a Reconstruction on page 267* for instructions).

   The organs in the selected atlas appear on the surface. Only "Skin" is selected in Figure 15.11.In the 3D registration tools, choose the Display Organs option and select an organ atlas.

   The organs in the selected atlas appear on the surface. In Figure 15.11, only “Skin” is selected.
2. Click the **Transform tool** button  .

   The transform tool appears. Figure 15.12 explains the tool functions.

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 appears.

Figure 15.11 Displaying the Transform Tool

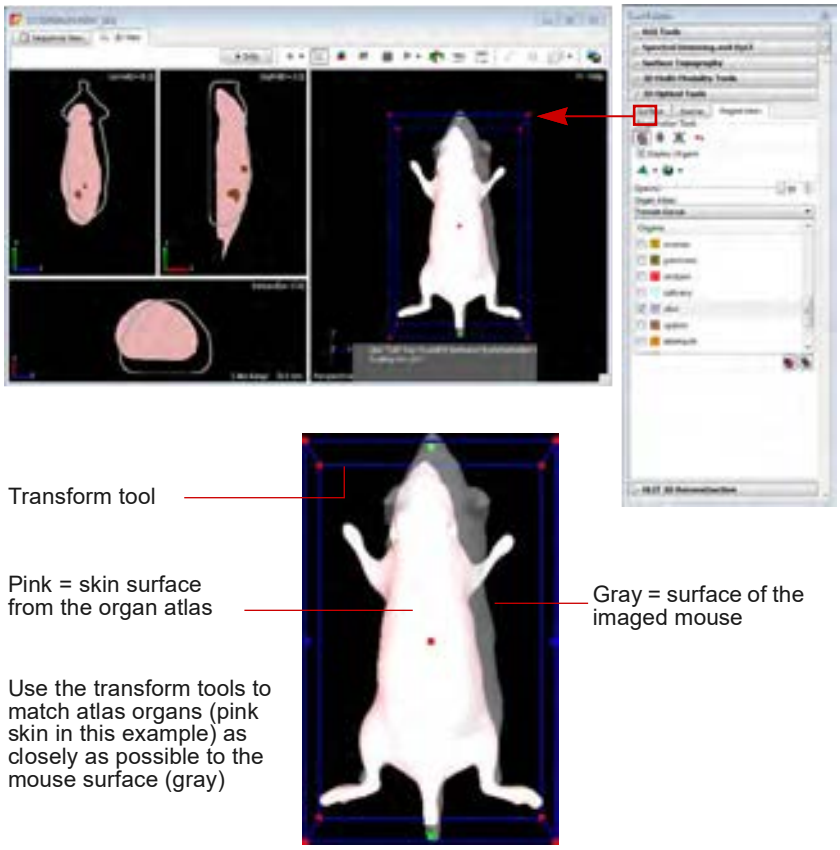
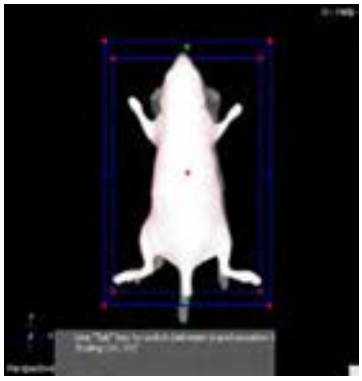


Figure 15.12 Transform Tools

Click and drag the organ(s) when the yellow "+" appears.

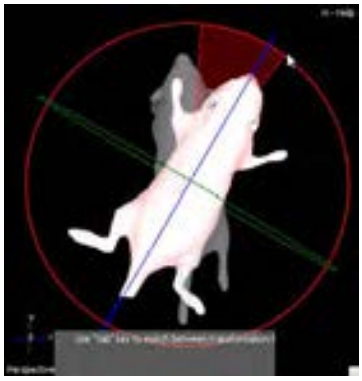


Click and drag a handle to scale (increase or decrease) the size of the organ(s).

Red – Scales on the z-axis.

Blue – Scales on the x-axis.

Green – Scales on the y-axis.



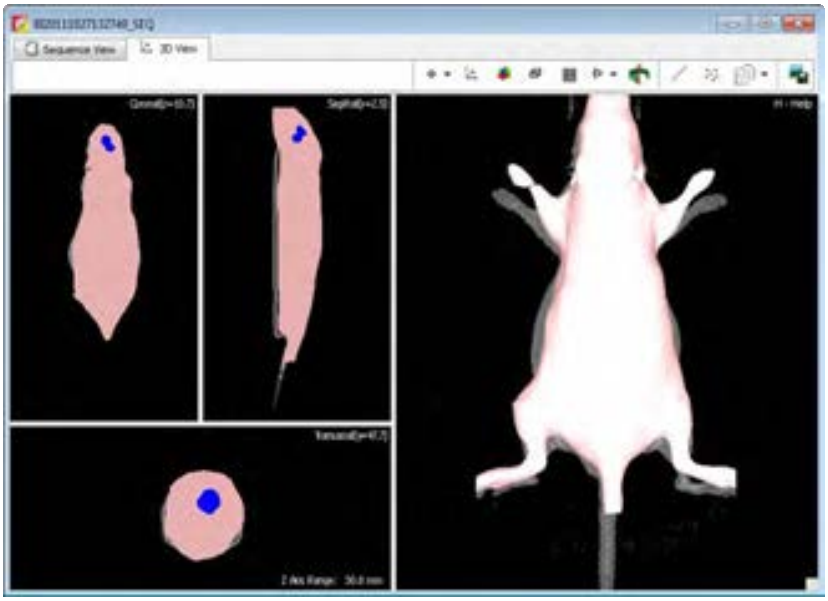
To rotate the organ(s) on the x,y, or z-axis, click the blue, green, or red circle and drag the mouse arrow in the direction of interest.

3. Press the Tab key to switch between the transform tools (Figure 15.12).
   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.
2. Click the **Change view** toolbar button .
   The Top view is displayed (Figure 15.13).

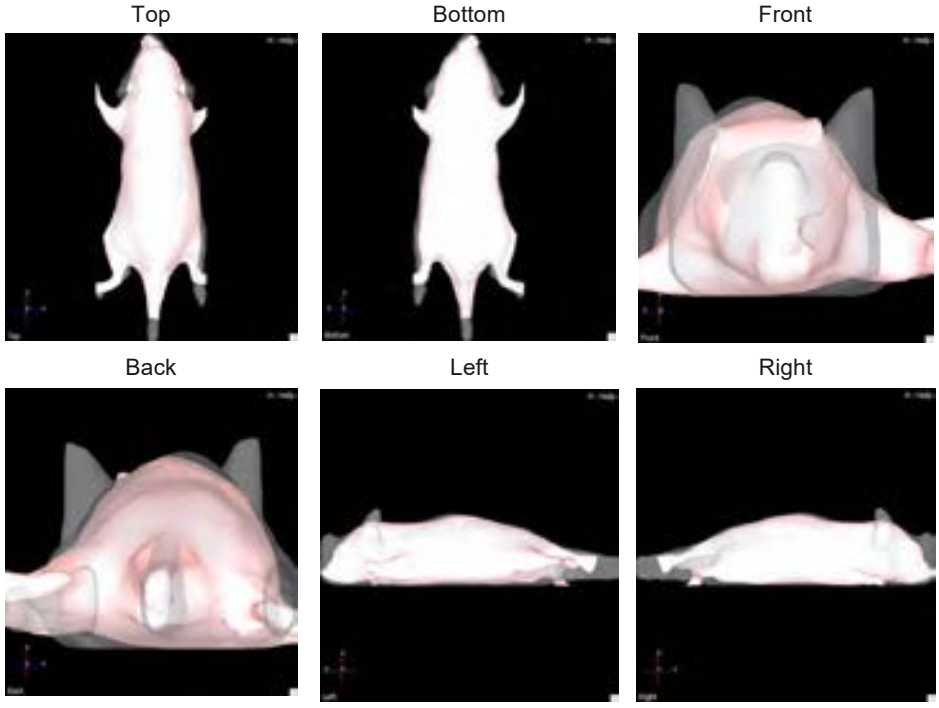
Figure 15.13 Skin (pink) Fitted to Surface (gray)



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

Figure 15.14 Alternative Surface Views

In this example, “skin” is selected from the organ atlas (pink surface). The mouse surface is gray.



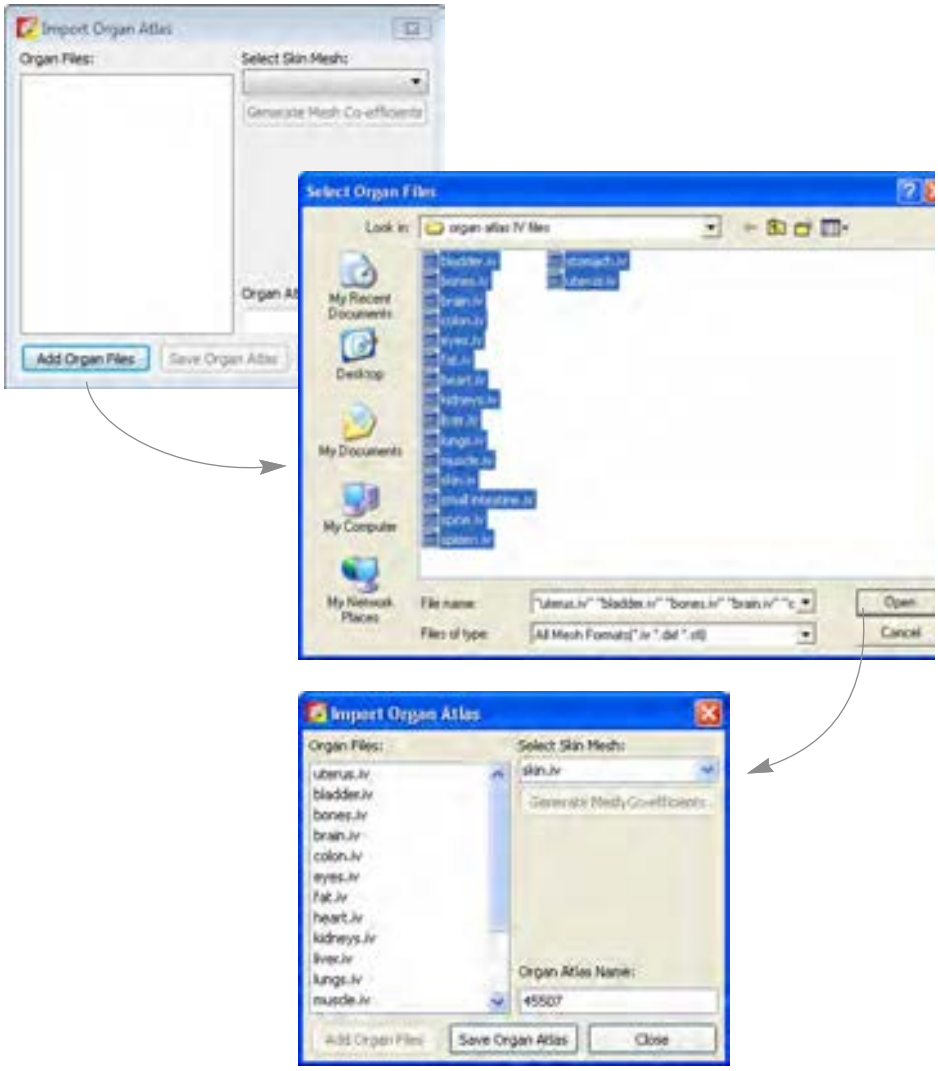
Importing an Organ Atlas

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** → **Import** → **Organ Atlas** on the menu bar.
3. In the dialog box that appears, click **Add Organ Files** (Figure 15.15).

Figure 15.15 Importing an 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).

15.5 3D Animation

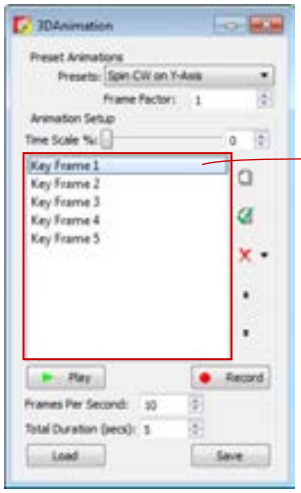
Living Image software can create an animation from a sequence of 3D views (*key frames*). For example, an animation can depict a rotating 3D scene (Figure 15.16). The animation (series of key frames) can be recorded to a movie file (.mov, .mp4, or .avi).

Use the animation tools to:

- View a preset animation (generated from a factory-loaded animation setup, page 275).

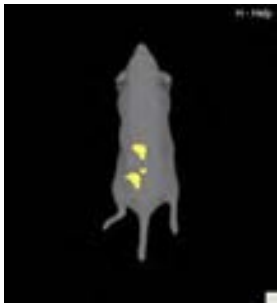
- Create a custom animation (created from your custom animation setup, page 276).
- Save an animation setup (page 278).
- Record an animation to a movie file (page 278).
- Edit an animation setup (page 278).

Figure 15.16 Individual 3D Views (key frames) in the Preset Animation “Spin CW on Y-Axis”



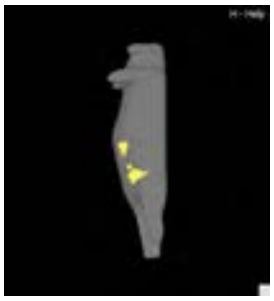
The box shows the key frames in the current animation setup. Click a key frame in this box to display the associated 3D view and time stamp (position in the time scale (0-100) at which the frame occurs in the animation).

Click Play to view the animation composed of the key frames.


Keyframe 1


Keyframe 2


Keyframe 3


Keyframe 4

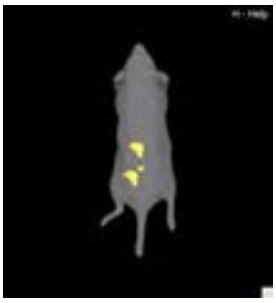

Keyframe 5

Table 15.5 3D Animation Tools

Item	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.

Table 15.5 3D Animation Tools (continued)

Item	Description
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 x 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.
	Creates a new key frame from the current 3D view.
	Updates the selected key frame to the current 3D view.
	Deletes a selected or all key frames from the key frame box.
	Moves a selected key frame up in the key frame box.
	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:

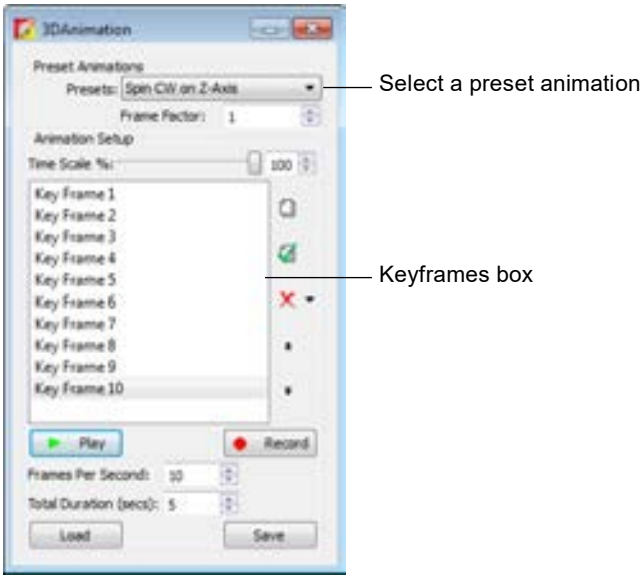
1. Open an image sequence and load 3D reconstruction results.
2. Select properties to display in the 3D View window (for example, organs, sources, surface, or photon density maps).
3. Select **Tools** → **3D Animation** on the menu bar.
4. In the 3D Animation tools that appear:
   a. Clear the key frame box if necessary (click the button and select **Delete All**).
   b. Make a selection from the Presets drop-down list. See Table 15.5 on page 274 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 15.17 3D Animation Tools

See Table 15.5, page 274 for details on the animation tools.



Creating a Custom Animation

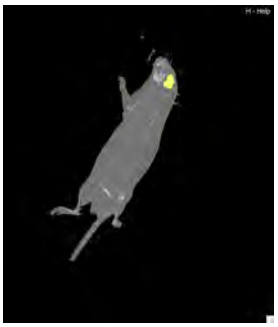
To create an animation, specify a custom animation setup or edit an existing setup.

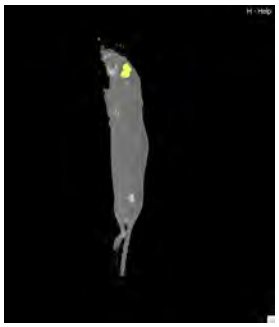
1. Open an image sequence and load 3D reconstruction results.
2. Select properties to display in the 3D View window (for example, organs, sources, surface, or photon density maps).
3. Select **View** → **3D Animation** on the menu bar.
   The 3D Animation tools appear (Figure 15.18).
4. Clear the key frame box if necessary (click the button and select **Delete All**).

Figure 15.18 Key Frames Cleared

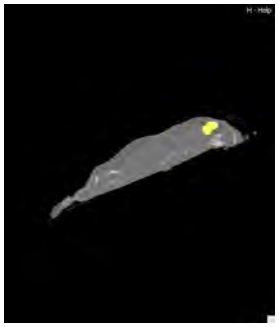
5. To capture the first key frame, click the button.
   The first key frame is added to the key frame box.
6. Adjust the position of the reconstruction in the 3D View using an image tool (for example, , or). See Table 14.5 on page 211 for more details on the image tools.
7. Click the button.
   The second key frame is added to the key frame box.

Figure 15.19 Example Key Frames for a Custom Animation


Key Frame 1


Key Frame 2


Key Frame 3


Key Frame 4

8. Repeat step 6 to step 7 until all of the key frames are captured. See *Edit an Animation Setup on page 278* 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 x Total Duration = No. of frames generated to create the animation. The number of generated frames should be ≥ 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.

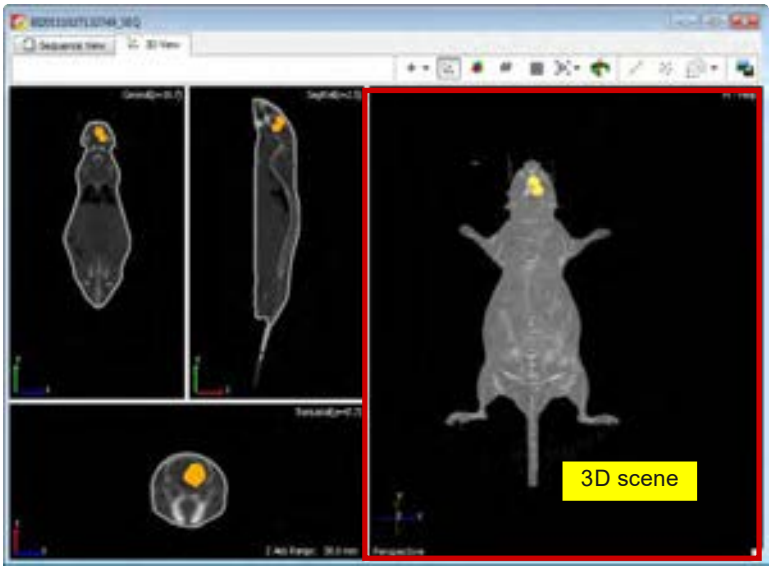
Figure 15.20 List of Key Frames in the Selected Animation

3. To add a key frame:
   a. Adjust the position of the reconstruction in the 3D view using an image tool (for example, , or). See Table 14.5 on page 211 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 or 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 button and select **Delete Current**.

15.6 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 15.21 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.

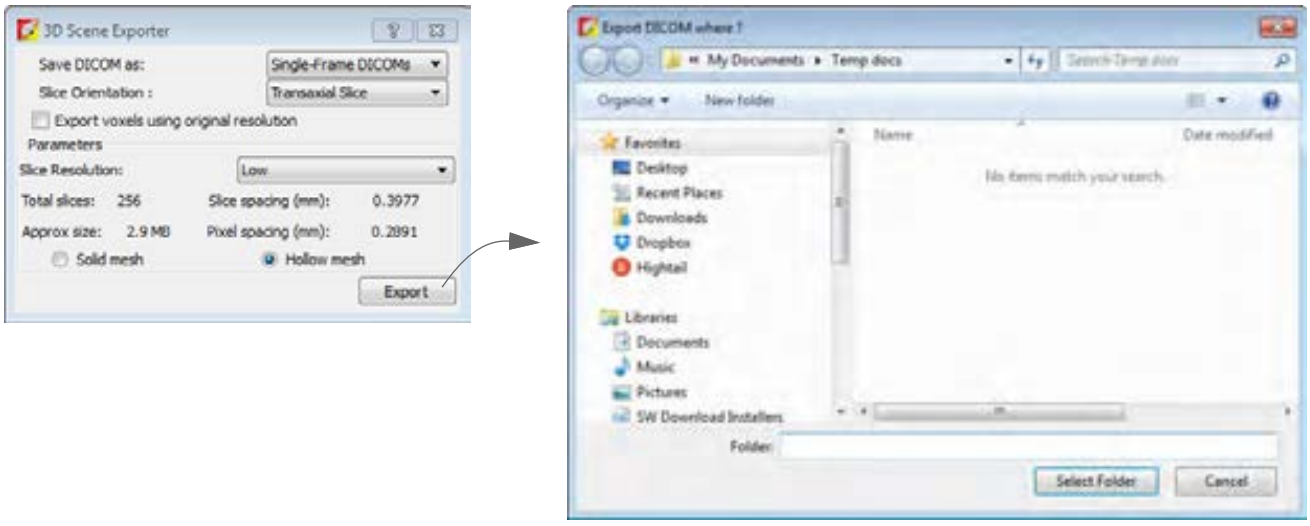
Figure 15.21 3DScene



To export the 3D scene:

1. Load the results that you want to export.
2. Select **File** → **Export** → **3D Scene as DICOM** on the menu bar.
3. In the dialog box that appears, set the export options, and click **Export** (Figure 15.22).
   For more details on the 3D Scene Exporter, see Table 15.6 on page 281.

Figure 15.22 3D Scene Exporter Dialog Box



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 15.6 3D Scene Exporter Dialog Box

Item	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 Slice spacing Pixel spacing	Parameters that determine the number and resolution of the slices to export.
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 3D toolbar button. Alternatively, select **File** → **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 15.24).

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

Figure 15.23 Opening the 3D Volumetric Browser

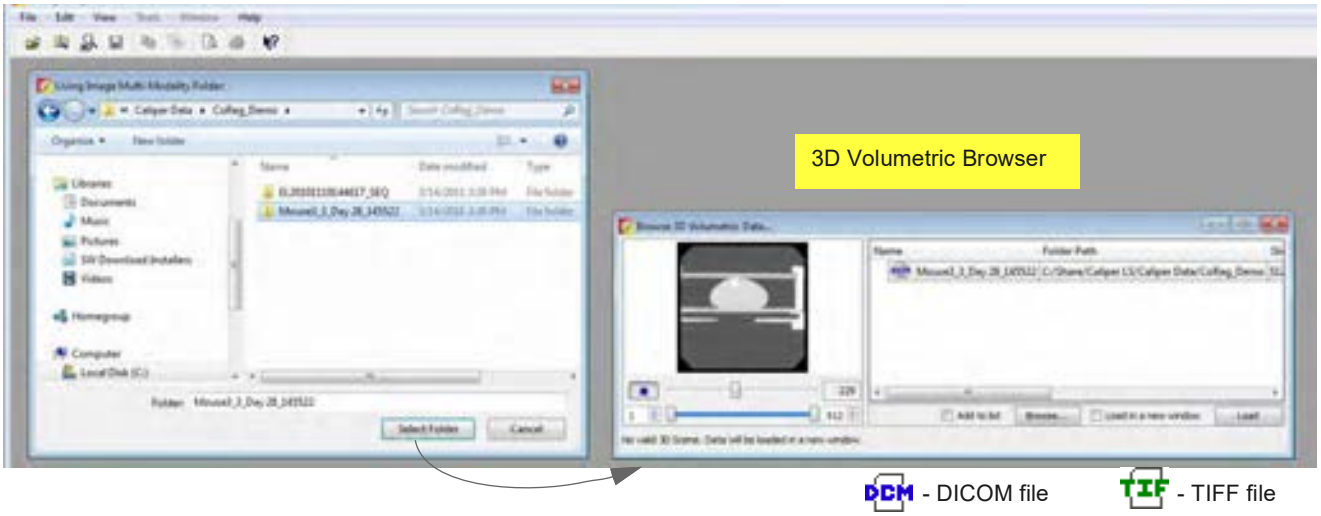


Figure 15.24 Living Image 3D Browser

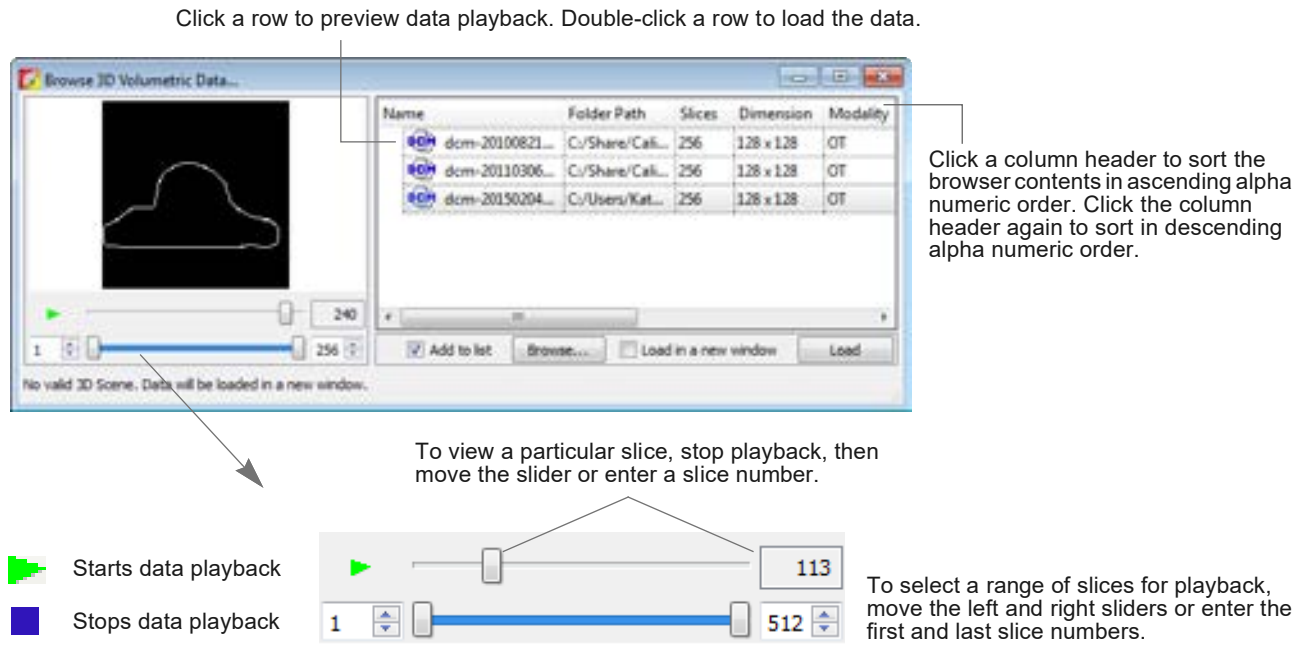


Table 15.7 Living Image 3D Browser DICOM Viewing Controls

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.

16 Measuring Signal in 3D Sources

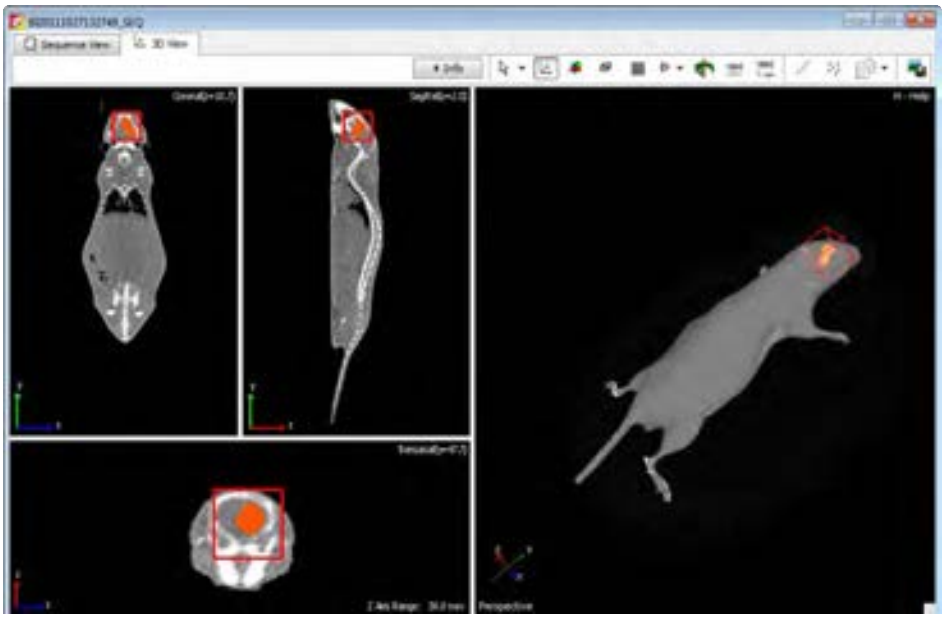
16.1 About 3D ROIs

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: 3D Multi-Modality tools (see page 295) 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).

Figure 16.1 Example 3D ROI



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 16.2).

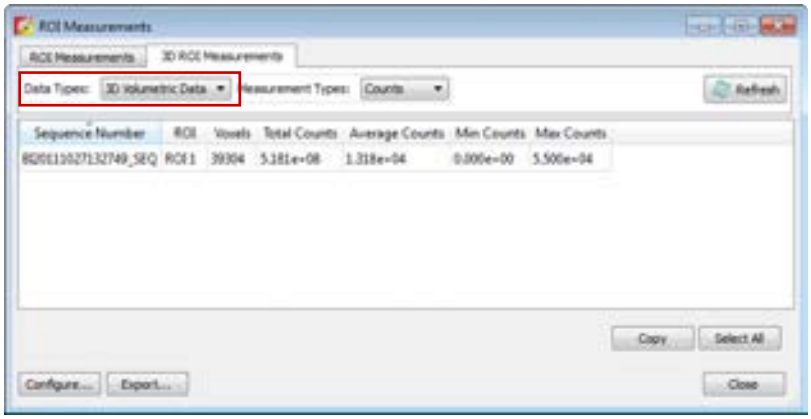
If a dataset includes ROIs on both 2D optical and 3D volumetric data, the measurements for the two types of ROIs are displayed in separate tabs of the ROI table (Figure 16.2):

- 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 16.2 3D ROI Measurements 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.



16.2 Overview of 3D ROI Tools

Table 16.1 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 16.2 on page 290 for more details on 3D ROI measurements.

Figure 16.3 3D ROI Tools

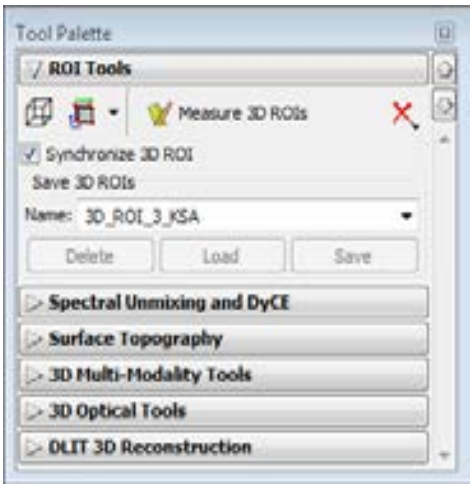
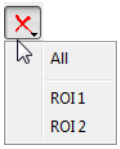
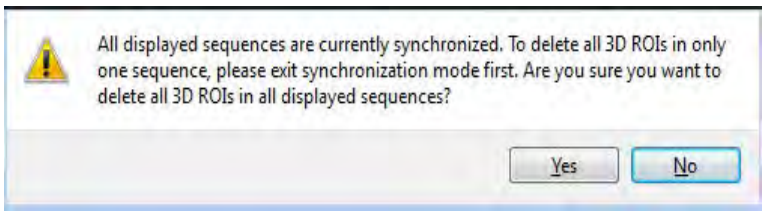
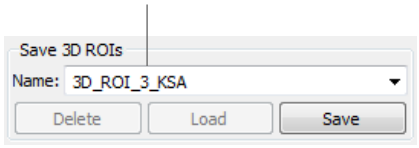


Table 16.1 3D ROI Tools

Item	Description
	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.
	Click to select a 3D ROI from the drop-down list and to turn on the 3D ROI transform tool. See page 288 for instructions on using the tool to move, scale, or rotate a 3D ROI.
	Click to display the 3D ROI measurements or compute intensity signal in a 3D ROI. See Table 16.2 on page 290 for more information on 3D ROI measurements.
	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.  **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  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  These ROI tools are only available 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 appear in the Name drop-down list. See page 293 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.

16.3 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.

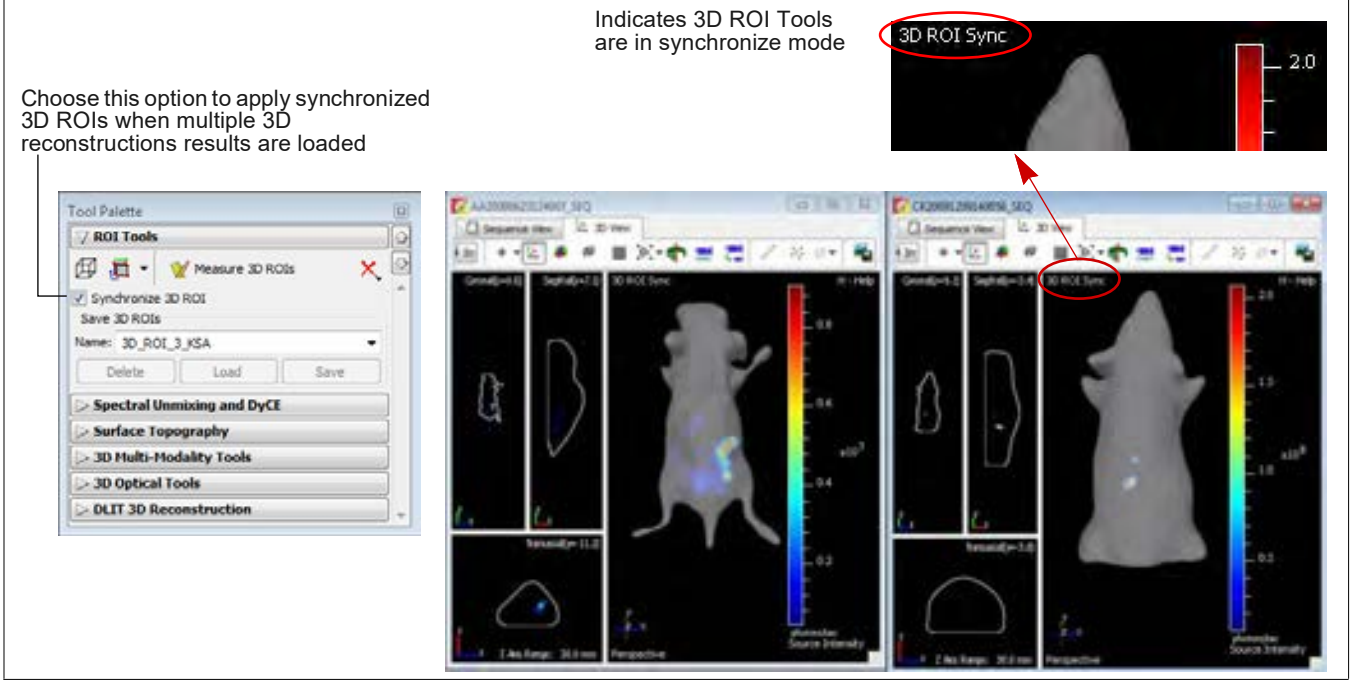
To apply 3D ROIs:

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 16.4).

Figure 16.4 3D ROIs – Synchronize Mode



2. Click the 3D ROI button in the ROI tools to apply the ROI (Figure 16.5).

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:

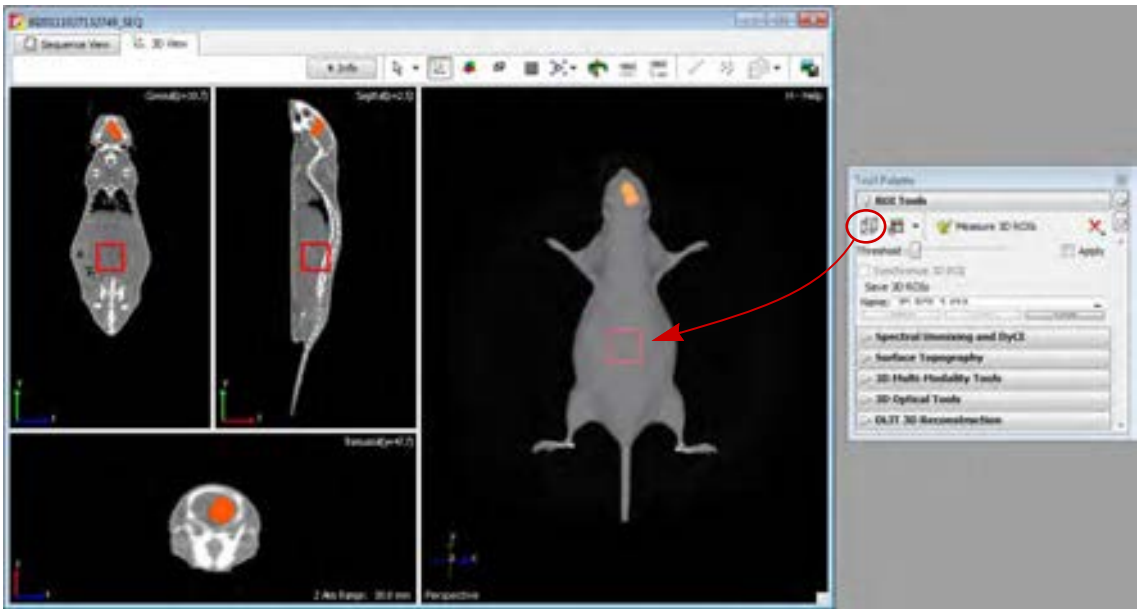
- Select the "Maximum Intensity Projection (MIP)" option in the 3D Multi-Modality tools.

  OR

- Reduce the volume opacity by adjusting the position of the Air/Noise Boundary in the 3D Multi-Modality tools.

NOTE: See Table 16.1 on page 286 for details on deleting 3D ROIs.

Figure 16.5 Applying a 3D ROI



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 appears.

   If working with synchronized 3D ROIs, it may be helpful to synchronize the 3D views before using the tool. See *Synchronizing 3D Views on page 264* 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.

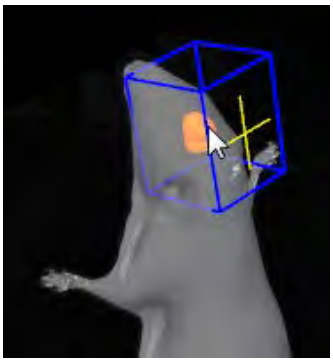
      Figure 16.6 explains the tool functions. The ROI position is updated in the slice windowpanes (coronal, sagittal, and transaxial views) after each adjustment.

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

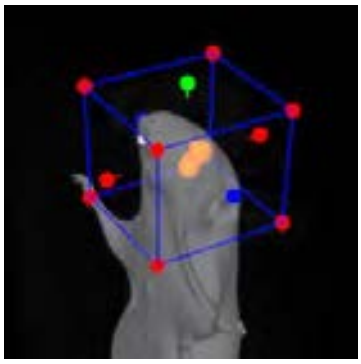
   c. Press the Tab key to switch between the transformations tools.

   d. Turn off the transform tool when you finish positioning the ROI (click the 3D ROI Transform button).

Figure 16.6 3D ROI Transform Tools



Click and drag the 3D ROI when the yellow "+" appears.

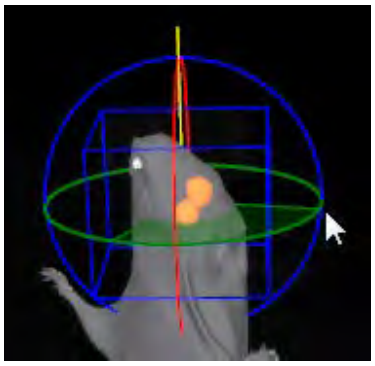


Click and drag a handle to scale (increase or decrease) the ROI size.

Red – Scales on the z-axis.

Blue – Scales on the x-axis.

Green – Scales on the y-axis.



To rotate the 3D ROI on the x,y, or z-axis, click the blue, green, or red circle and drag the mouse arrow in the direction of interest.

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 292 for details.

5. Click the Measure 3D ROIs button in the Tool Palette to view the intensity measurements (Figure 16.7).

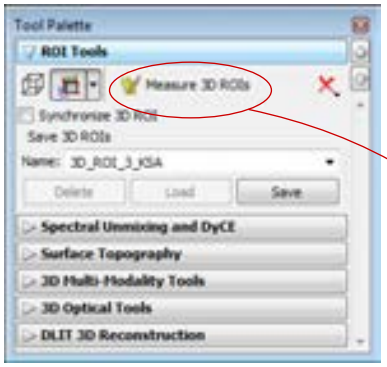
   See Table 16.2 for information about the measurements.

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.

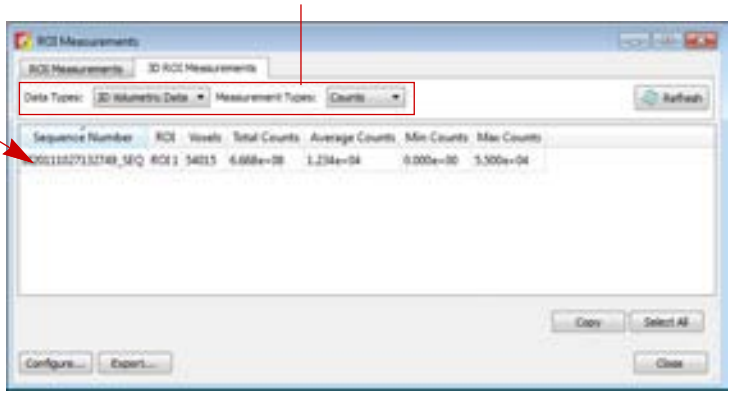
   The 3D ROIs will be displayed the next time the dataset is loaded.

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 293* for details.

Figure 16.7 3D ROI Measurements Table



Select the type of data and measurement (see Table 16.2)



See page 194 and page 196 for information about creating custom table configurations and exporting or printing the table.

Table 16.2 3D ROI Measurements 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.
Voxels	The number of voxels within the 3D ROI.
3D Volumetric Data: Counts measurements (16-bit scale with values that change from image to image)	Total Counts – the sum of all counts for all voxels inside the 3D ROI. Average Counts – Total Counts/Number of voxels in the 3D ROI Min Counts – The smallest number of counts in a voxel within the 3D ROI. Max Counts –The largest number of counts in a voxel within the 3D ROI.
3D Volumetric Data: Absorption Measurements (Fixed 32-bit scale with values that are consistent between images.) **Note:** These measurements are only available for IVIS Spectrum CT data.	Total Value – The sum of the absorption measurements of all voxels in the 3D ROI. Average Value – Total Value/Number of voxels in the 3D ROI. Stdev Value – Standard deviation of the absorption values for all voxels inside the ROI. Min Value – The smallest absorption value for any single voxel in the 3D ROI. Max Value – The largest absorption value for any single voxel in the 3D ROI.

Table 16.2 3D ROI Measurements Table (continued)

Item	Description
3D Volumetric Data: Hounsfield measurements (Calibrated CT scale. Fixed from image to image.) **Note:** These measurements are only available for IVIS Spectrum CT data.	Total Hounsfield – The sum of the Hounsfield unit values for all of the voxels in the 3D ROI. Average Hounsfield – Total Hounsfield unit value/Number of voxels in the 3D ROI. Stdev Hounsfield – Standard deviation of the Hounsfield unit values for all voxels inside the ROI. 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.
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 **Note:** This measurement type requires a quantification database. See Appendix B on page 411 for more details.	Total Cells – The number of cells in the 3D ROI. Average Cells – Total number of cells/Number of voxels in the 3D ROI. Stdev Cells – Standard deviation of the number of cells in the 3D ROI. 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 measurements **Note:** This measurement type requires a quantification database. See Appendix B on page 411 for more details.	Total pmol – Total picomoles of fluorescent probe within the 3D ROI. Average pmol – Total picomoles/Number of voxels. Stdev pmol – Standard deviation of the picomole values in the 3D ROI. Min pmol – Smallest picomole value in the 3D ROI. Max pmol – Largest picomole value in the 3D ROI.

Table 16.2 3D ROI Measurements Table (continued)

Item	Description
Refresh	Updates the ROI Measurements table (for example, after you draw new ROIs, move an ROI, and close or open image data).
Copy	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 page 194 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.

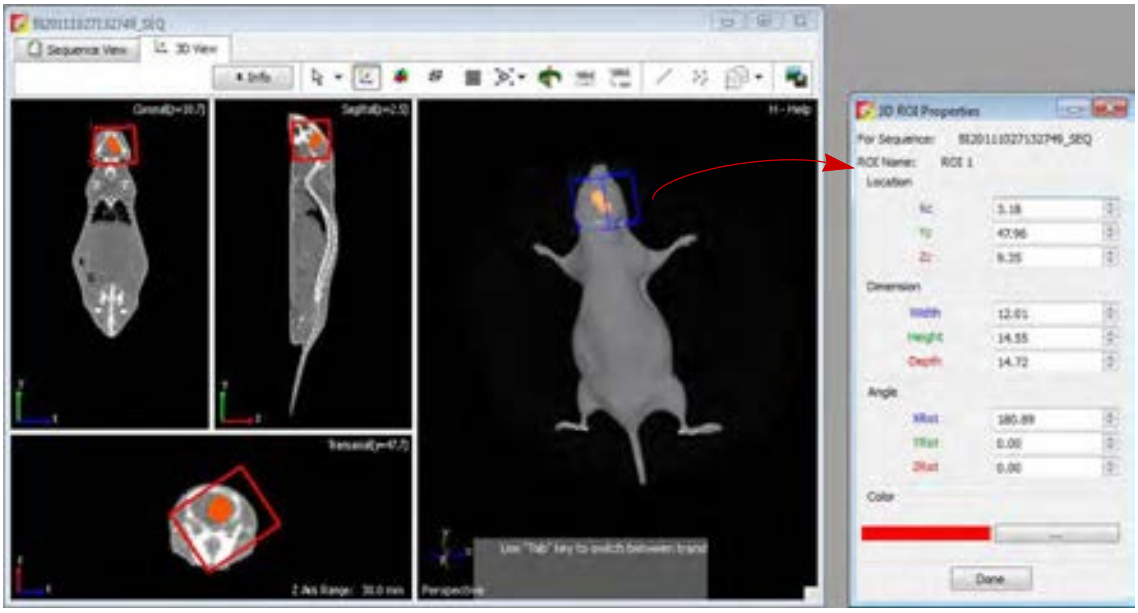
16.4 Managing 3D ROIs

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.

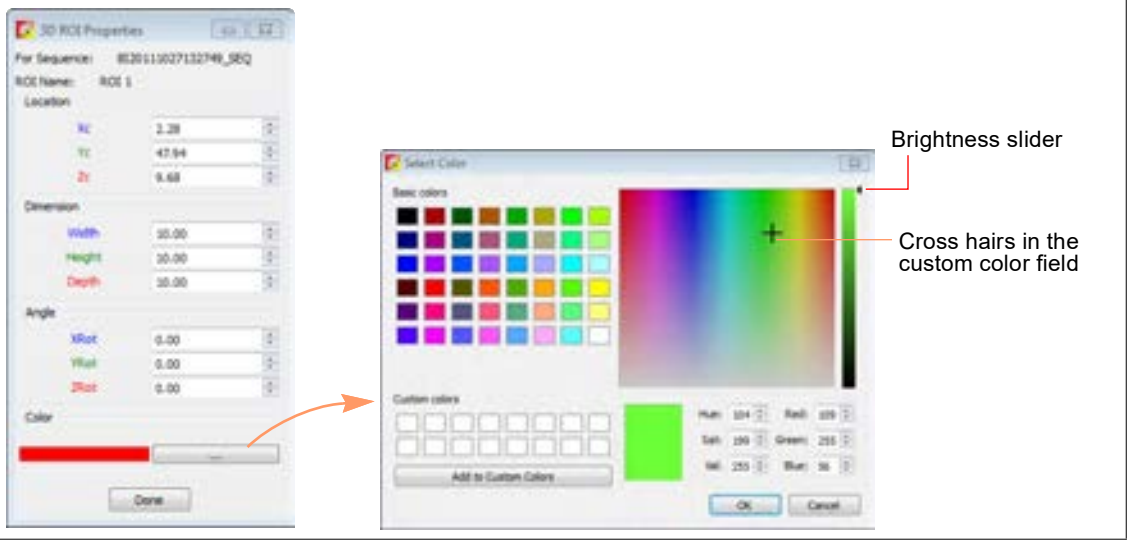
Figure 16.8 3D ROI Properties



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 16.9).

Figure 16.9 Select 3D ROI Color



   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**.

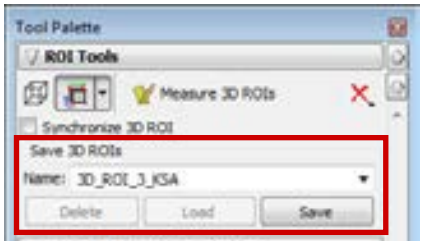
Saving 3D ROIs to the System

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 16.10). 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 16.10).

Figure 16.10 Name and Save 3D ROIs to the System

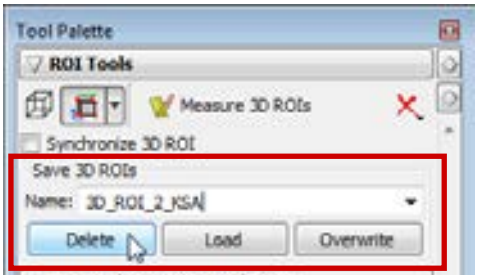


2. To apply a 3D ROI to a 3D reconstruction or volume, make a selection from the Name drop-down list and click **Load** (Figure 16.10).

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 16.11).

Figure 16.11 Delete 3D ROIs From the System



17 Working With Volumetric Data

17.1 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).

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 17.1.

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.

Table 17.1 Minimum Graphics Card Specifications

Specification	Description
OpenGL Version Requirement[1]	OpenGL 2.0 and above
OpenGL Extension Requirement	GL-EXT-texture3D
Graphics Card Memory	Minimum: 256MB (Dedicated + Shared) Recommended: 1GB (Dedicated)

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

If the MIS cassette is being used to acquire data from multiple instruments, refer to the relevant technical notes for detailed usage instructions. Technical notes can be found in C:\Program Files\Caliper Life Sciences\Living Image\Help\Tech Notes.

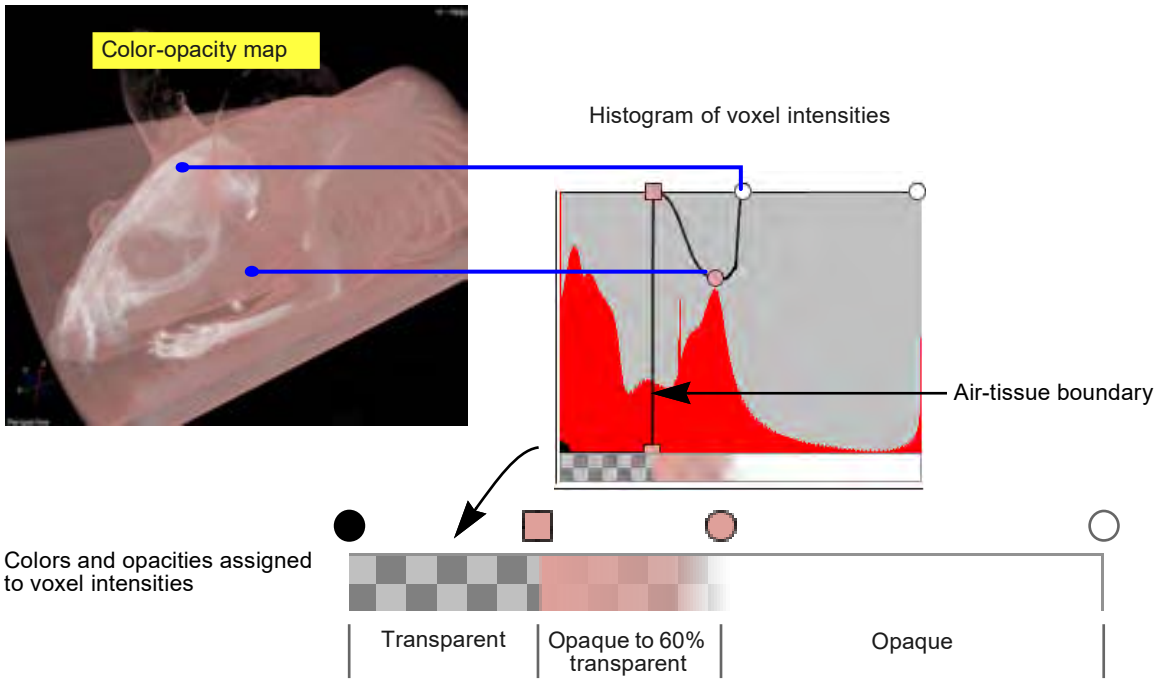
17.2 Classifying 3D Volumetric Data

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 17.1 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.

Figure 17.1 Histogram Tool Specifies Opacity for Different Voxel Intensities



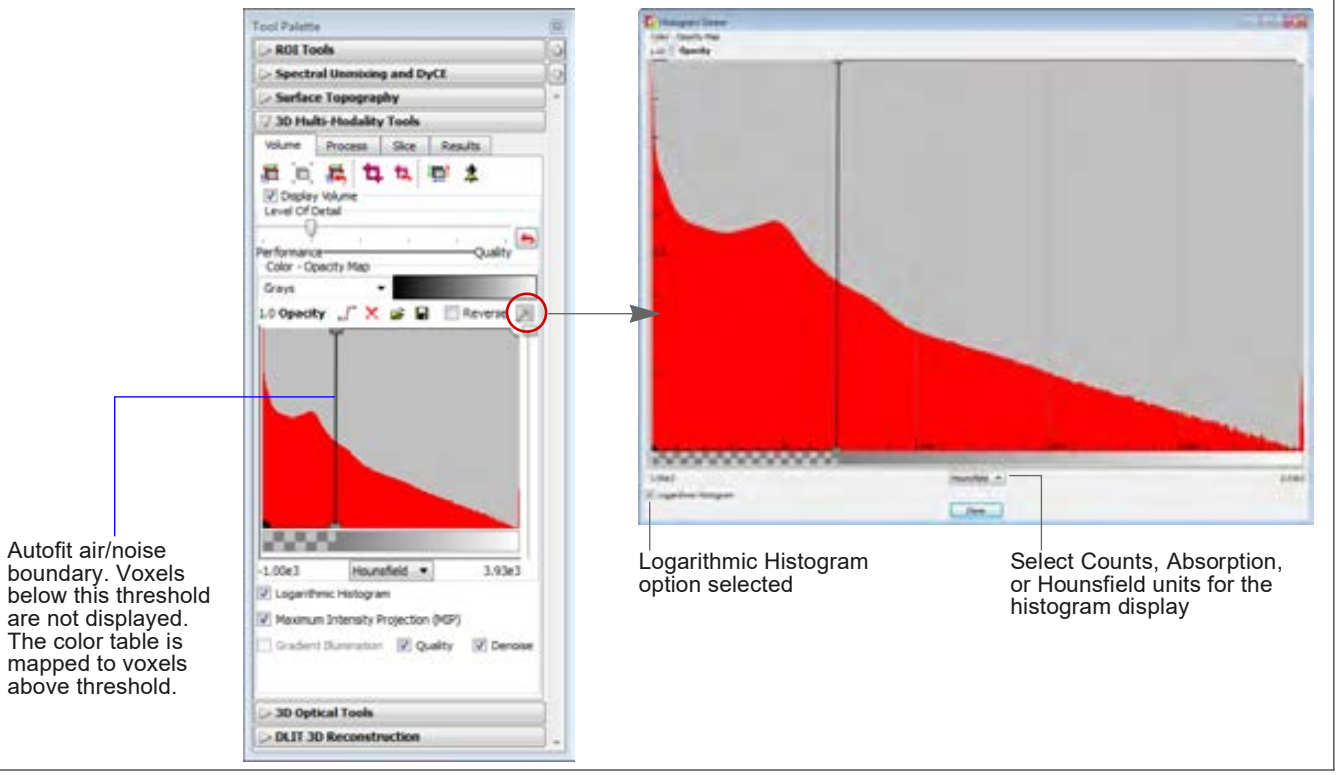
Specifying a Color-Opacity Map

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 17.2).
- 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.

Figure 17.2 3D Multi-Modality Tools



Managing Control Points

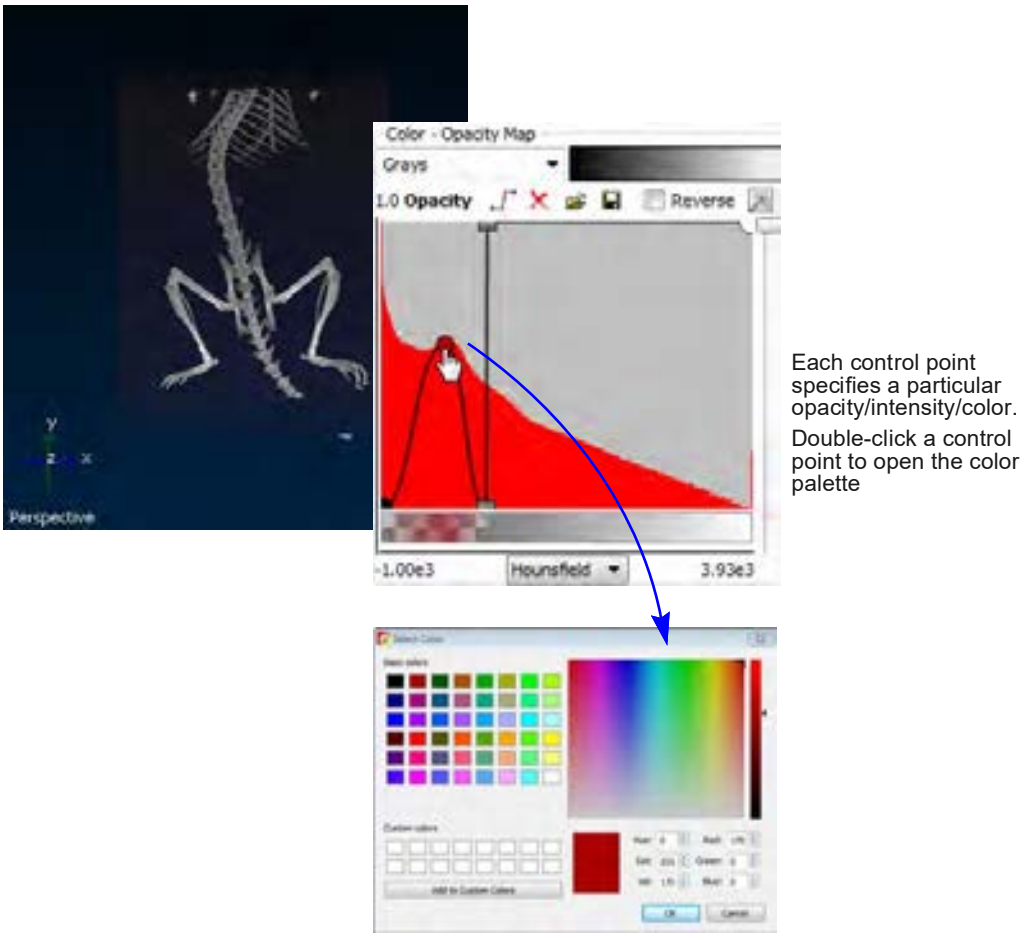
Edit the 3D volumetric data color-opacity map using "control points" (Figure 17.3). During volume rendering, the color-opacity map is used to map color and opacity to the corresponding intensity value as well as interpolate color and opacity for all data between adjacent control points.

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 ○ 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 ● and ○ control points cannot be changed. The opacity level associated with these points can be changed.

Figure 17.3 Histogram Tool



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 ○ control points cannot be deleted from the histogram.

Saving a Color-Opacity Map

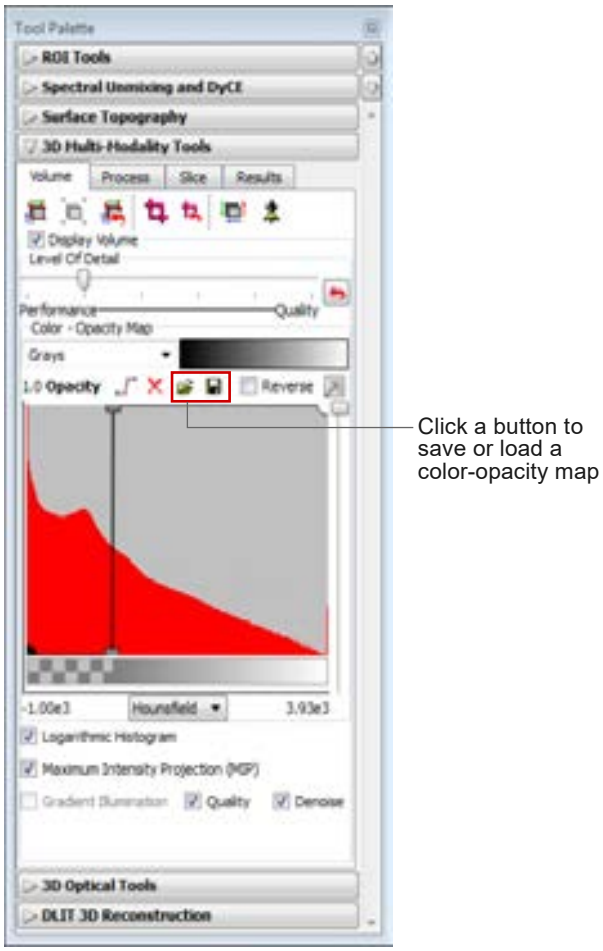
A color-opacity map can be saved and applied to any volumetric dataset.

1. Click the Save button (Figure 17.4).
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 17.4).
2. In the dialog box that appears, navigate to the map file (.tfn), and click **Open**.

Figure 17.4 Save or Load a Color-Opacity Map



17.3 Volume Display Options

Adjusting Image Quality

By default, the color-opacity map displays the volumetric data at original (1×) 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× to 3.0× resolution (see Table 17.2 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 17.5).
   The color-opacity map is updated.

2. To return the resolution to 1×, click the Reset button .

Figure 17.5 Level of Detail Slider

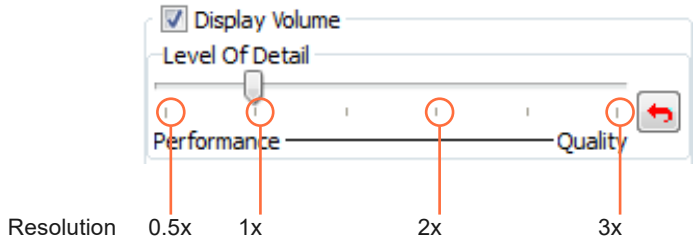


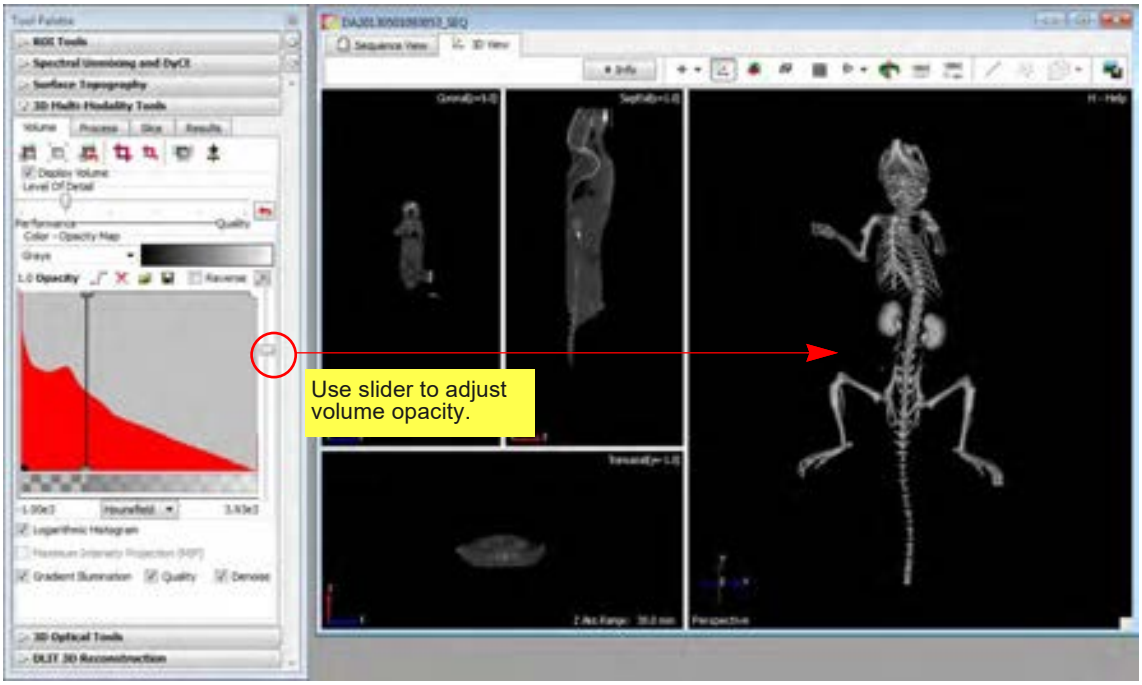
Table 17.2 Example Volume With 512 Slices at 1x Resolution

Volume Resolution	No. of Slices Displayed
0.5×	256
1× (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.

Figure 17.6 Adjusting Volume Opacity



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 17.7). 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 17.8 shows an overlay of a luminescent image on an extracted 2D "x-ray" image.

NOTE: If you change the volume opacity (see Figure 17.6 on page 301) and want to extract new 2D images from the MIP, click the button (Figure 17.7).

Figure 17.7 Volume Display – Maximum Intensity Projection

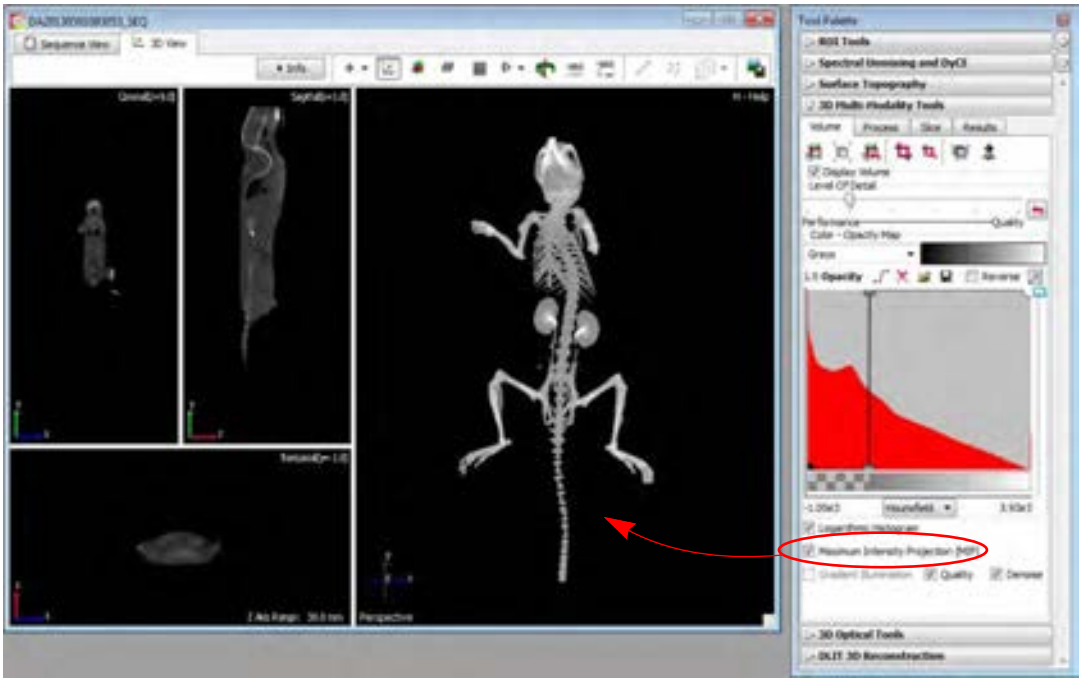
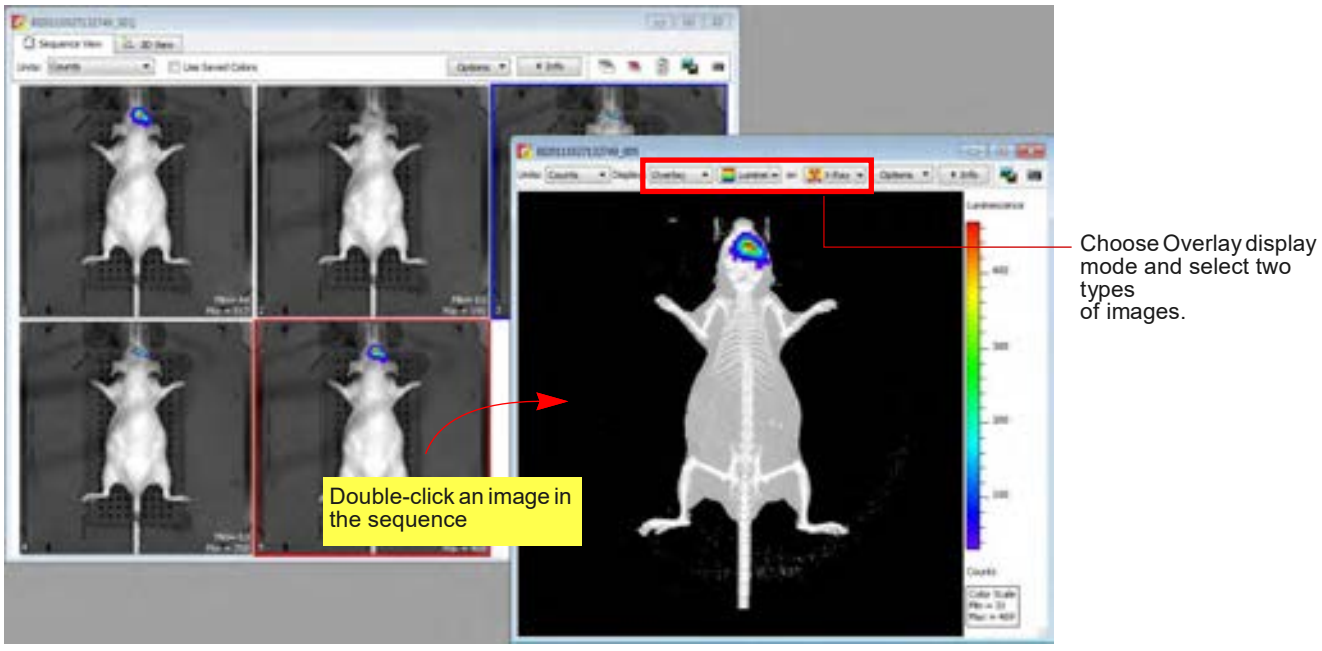


Figure 17.8 Image Window – Luminescent Image on X-ray Image



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.

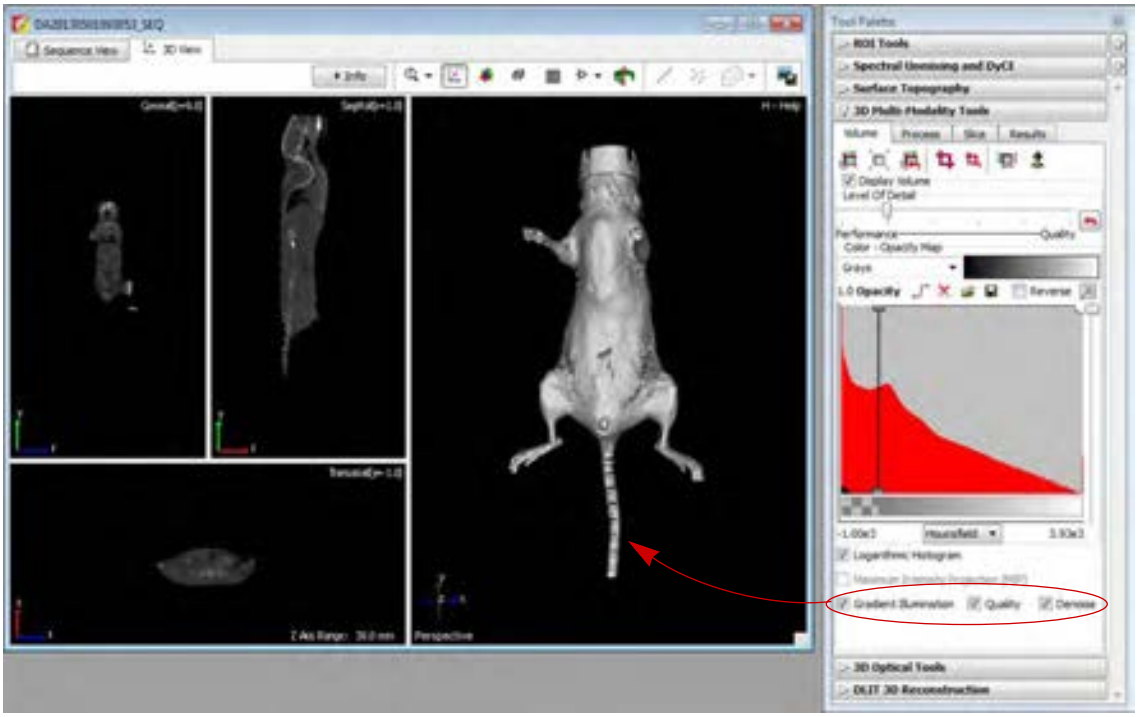
Two options are available for Gradient Illumination:

- 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 17.9).

NOTE: If the system graphics card does not meet the recommended specifications (Table 17.1 on page 296), 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 17.9 Volume Display – Gradient Illumination



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 .
2. In the dialog box that appears (Figure 17.10), edit the pixel or slice spacing.

Figure 17.10 Volume Information Dialog Box

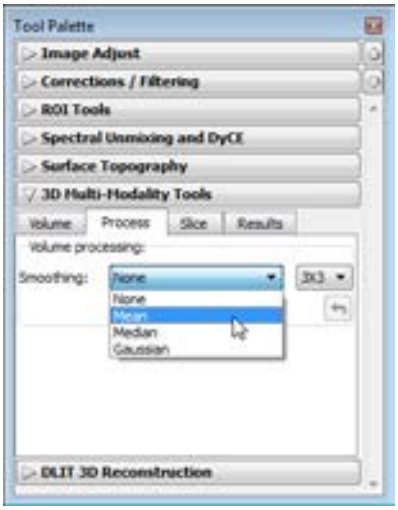
17.4 Smoothing a Volume

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 17.11).
   - 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.

Figure 17.11 3D Multi-Modality Tools – Process Tab



17.5 Viewing and Rendering Slices

Viewing Slices

View volume slices by double-clicking the Coronal, Sagittal, or Transaxial windowpane (Figure 17.12).

Figure 17.12 Viewing Slices

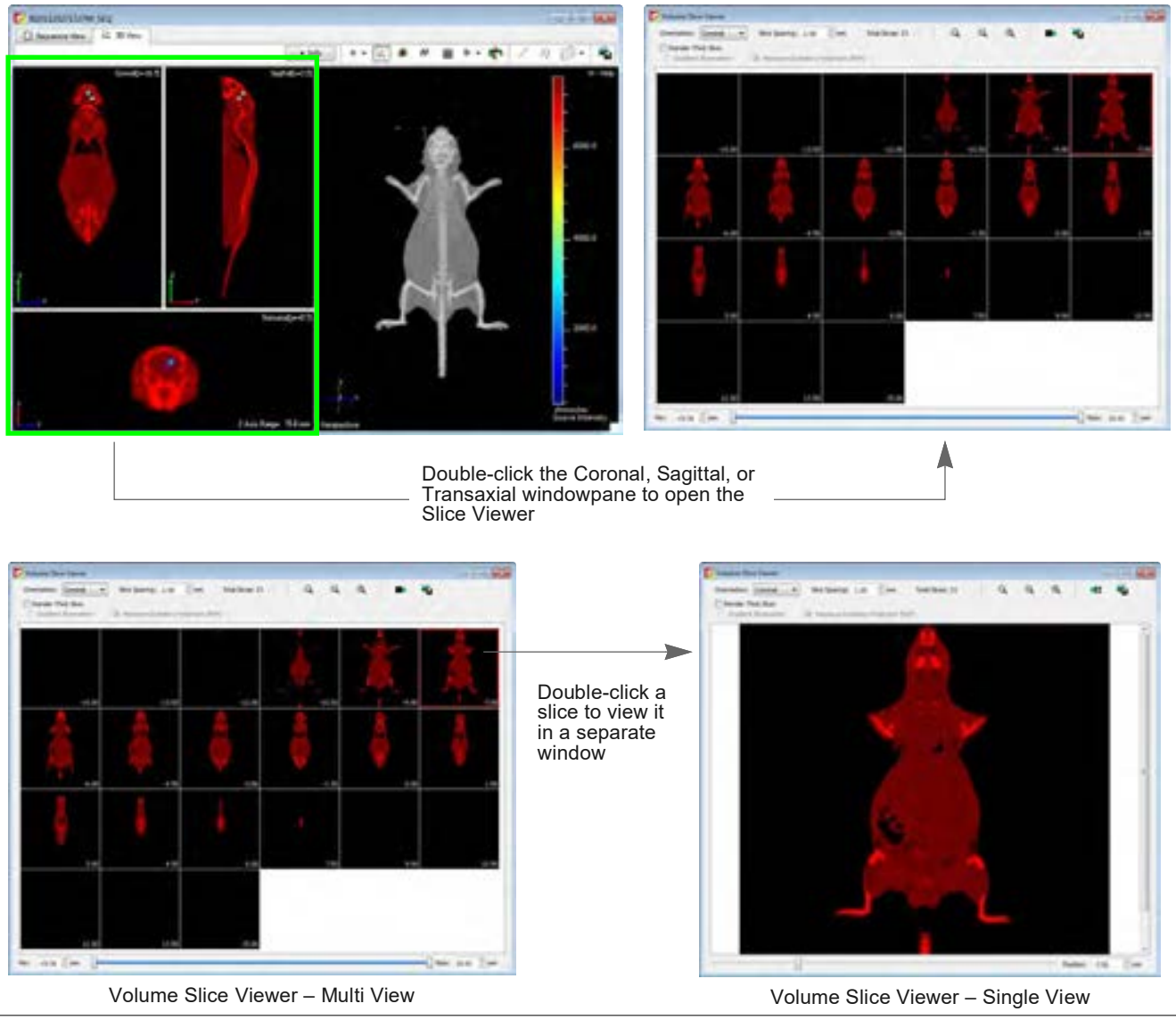


Table 17.3 Volume Slice Viewer

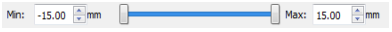
Item	Description
Orientation	Select a slice orientation from the drop-down list.
Slice Spacing	The distance between each slice in the Volume Slice Viewer. Enter a smaller value to increase the number of slices in the viewer or a larger value to decrease the number of slices in the viewer.
Total Slices	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 – 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.

Table 17.3 Volume Slice Viewer (continued)

Item	Description
	Click to show the multi view.
	If the single view has been magnified, click this button to zoom out incrementally.
	Magnifies the single view.
	Resets the single view to the default magnification.
	Click to export the slice view as a graphic file (for example, .bmp)

Rendering Slices

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

Figure 17.13 Perspective View and Slice Views Displayed Using Different Color Tables

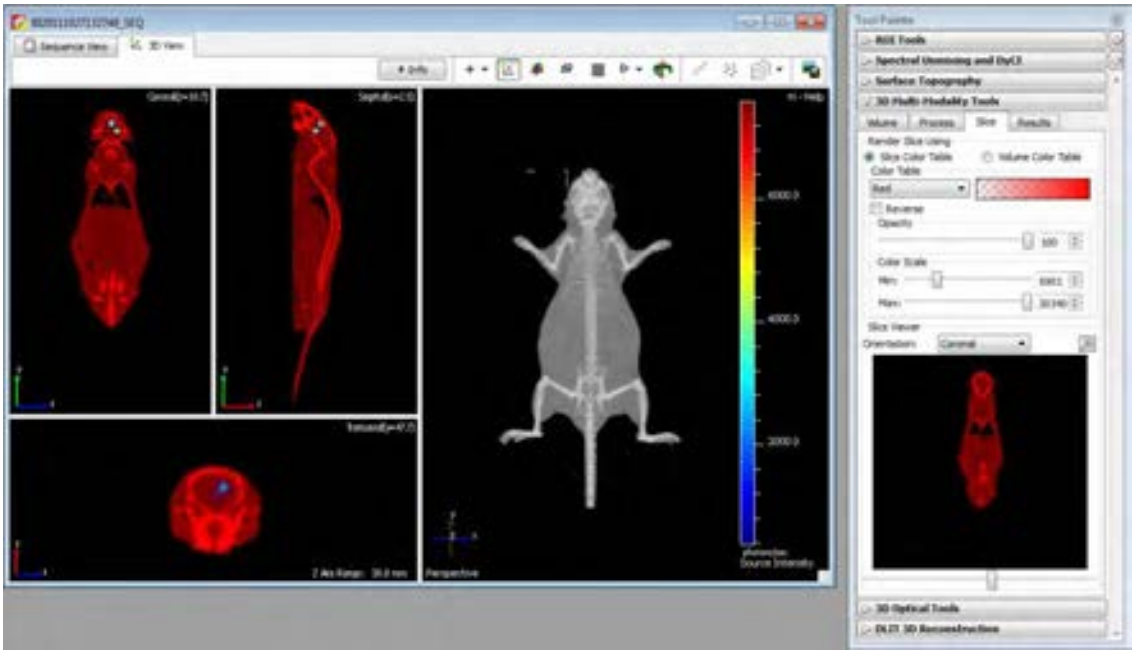


Table 17.4 3D Multi-Modality Tools for Rendering Slices

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 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.

Table 17.4 3D Multi-Modality Tools for Rendering Slices (continued)

Item	Description
	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 button to open the Slice Viewer. The selected slice is highlighted in the Slice Viewer.

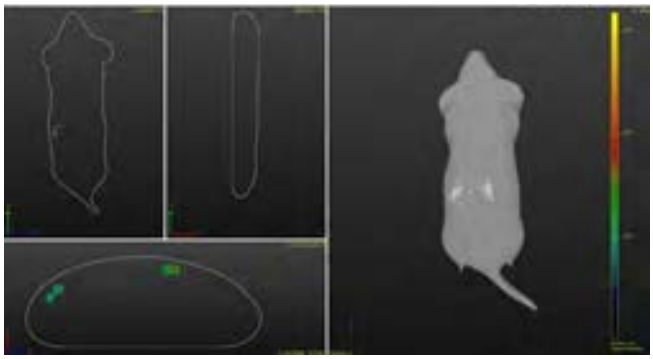
17.6 Registering Optical and Volumetric Data

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

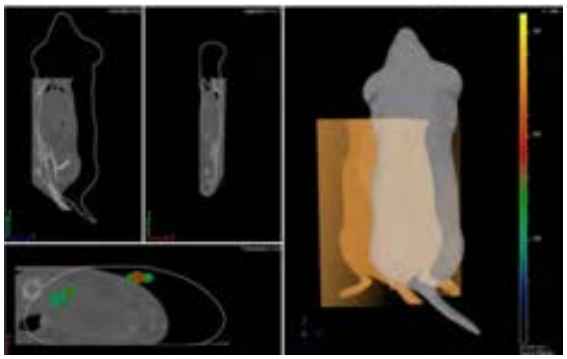
- Automatic fiducial registration – For experiments in which the optical data are acquired on the IVIS Spectrum CT and the CT data are acquired on the Quantum GX microCT series. The subject must be contained in the Mouse Imaging Shuttle during both optical and CT imaging, and the CT data must be exported to DICOM format. See page 312 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 314 for more details.

Figure 17.14 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 296).

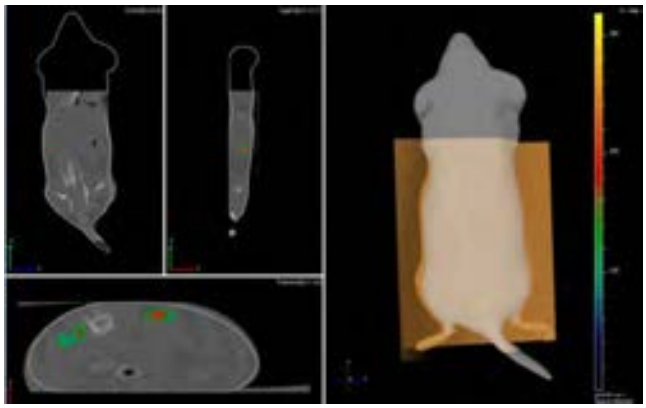
Figure 17.14 Registering Multi-Modal Data



1. Load the optical data:
   - Bioluminescence or fluorescence image sequence and structured light surface
   - 3D source reconstruction (DLIT or FLIT results)

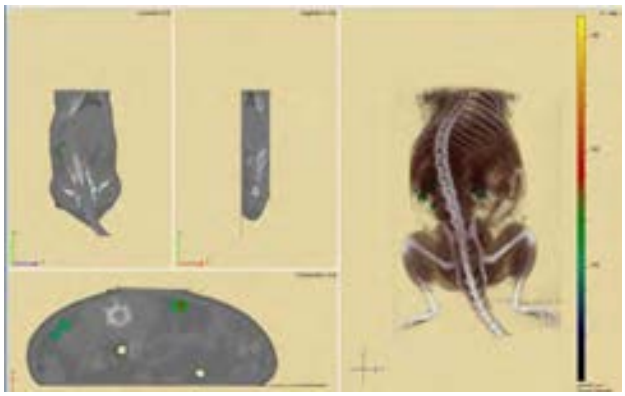


2. Load 3D volumetric data (CT or MRI) (page 310).



3. Register the 3D source reconstruction and the 3D volumetric data by performing either:
   - Automatic fiducial registration–Available for data acquired on the Quantum GX series µCT instrument using the Mouse Imaging Shuttle (page 312)

   or
   - Manual registration–Match animal surface representations using the Manual Registration tool (page 314)



4. Classify the 3D volumetric data to help identify and separate objects (page 296). Save the color-opacity map (optional).
5. Save the registered 3D multi-modality results (page 317).

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 17.1 on page 296.

2. Select the DICOM or TIFF volumetric data.

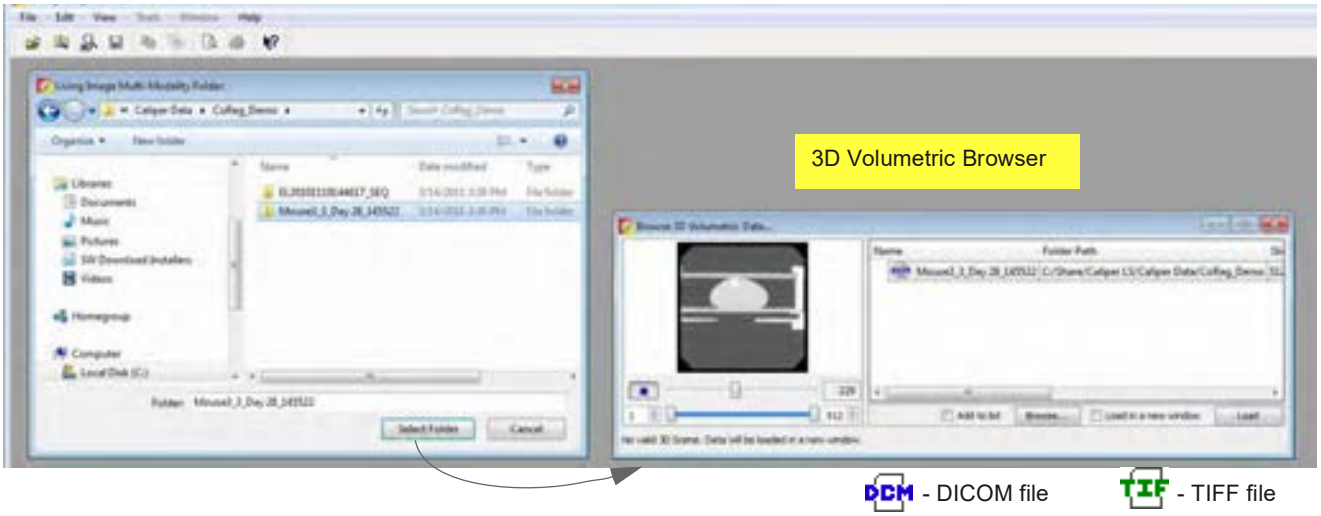
a. Click the 3D toolbar button. Alternatively, select **File** → **Browse 3D Volumetric Data** on the menu bar.

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 17.15).

   The Living Image 3D Volumetric Browser appears.

NOTE: If the 3D Volumetric Browser was previously opened during the session, clicking the 3D 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 317.

Figure 17.15 Opening the 3D Volumetric Browser



The 3D Volumetric Browser automatically previews a playback of the data along with other information about the data Figure 17.15. See Figure 15.24 on page 283 for more details on the 3D Volumetric Browser.

3. Load the volumetric data with the optical data.

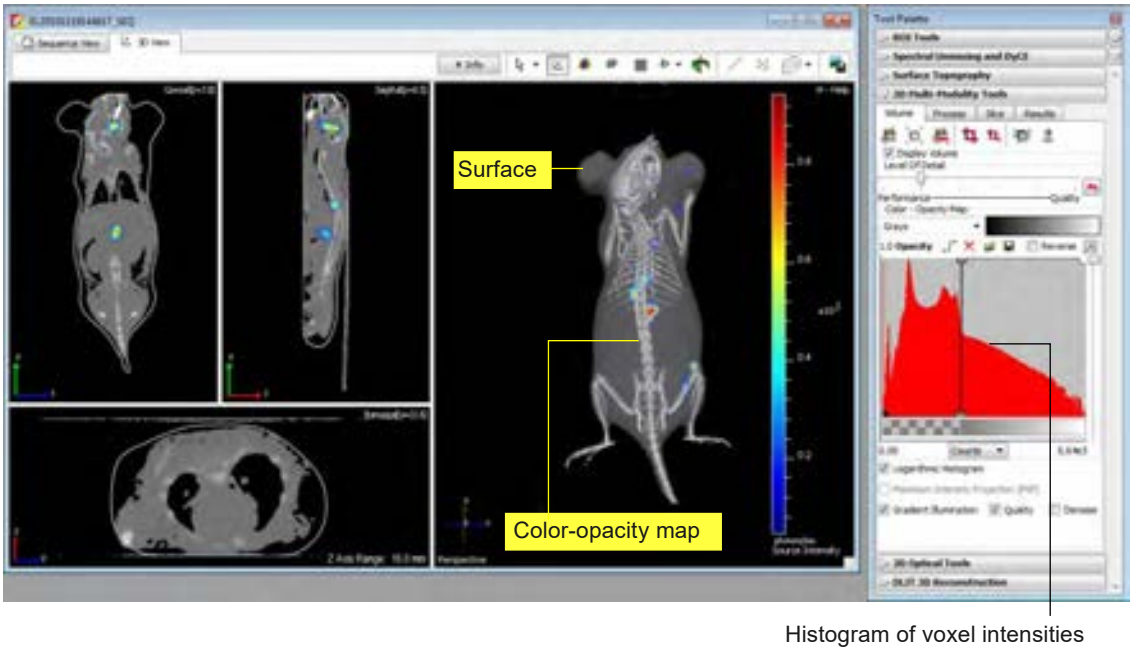
   a. Confirm that the “Load in a new window” option is not selected. (If this option is selected, the volumetric data are loaded in a new window.)

   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 17.16). 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.

Figure 17.16 3D Optical and 3D Volumetric Data



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 µCT instrument without disrupting the subject's position.

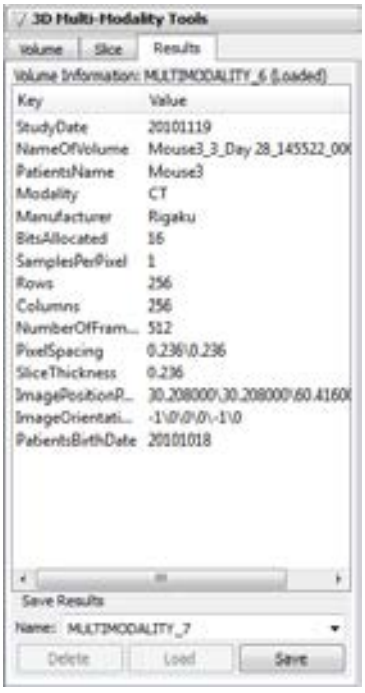
The Mouse Imaging Shuttle must be correctly docked to the docking station in the IVIS Imaging System and the Quantum GX series µCT instrument. The docking station in the Quantum GX series µCT system is marked with a triangle-shaped fiducial pattern under the plane where the Mouse Imaging Shuttle docks. 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 310).
2. Click the Fiducial Registration button  .
   The multi-modal data are automatically registered and cropped (Figure 17.17).
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**.

Figure 17.17 Registered 3D Optical and 3D Volumetric Data

Figure 17.18 3D Multi-Modality Tools – Results

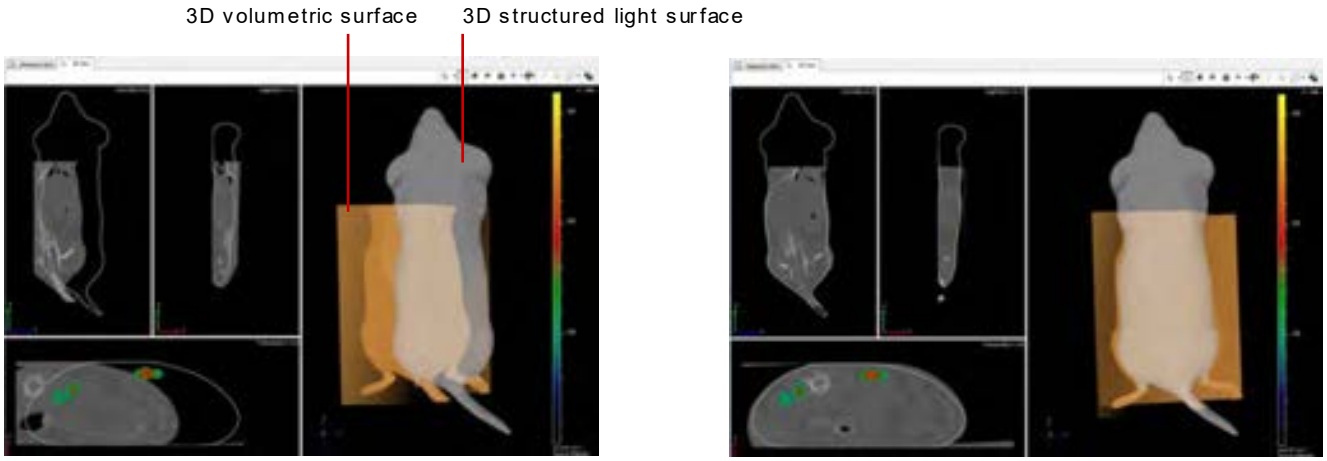


NOTE: Registration information is saved with the results for the volumetric data and is specific for a particular optical dataset.

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.

Figure 17.19 Example Surfaces Before and After Registration



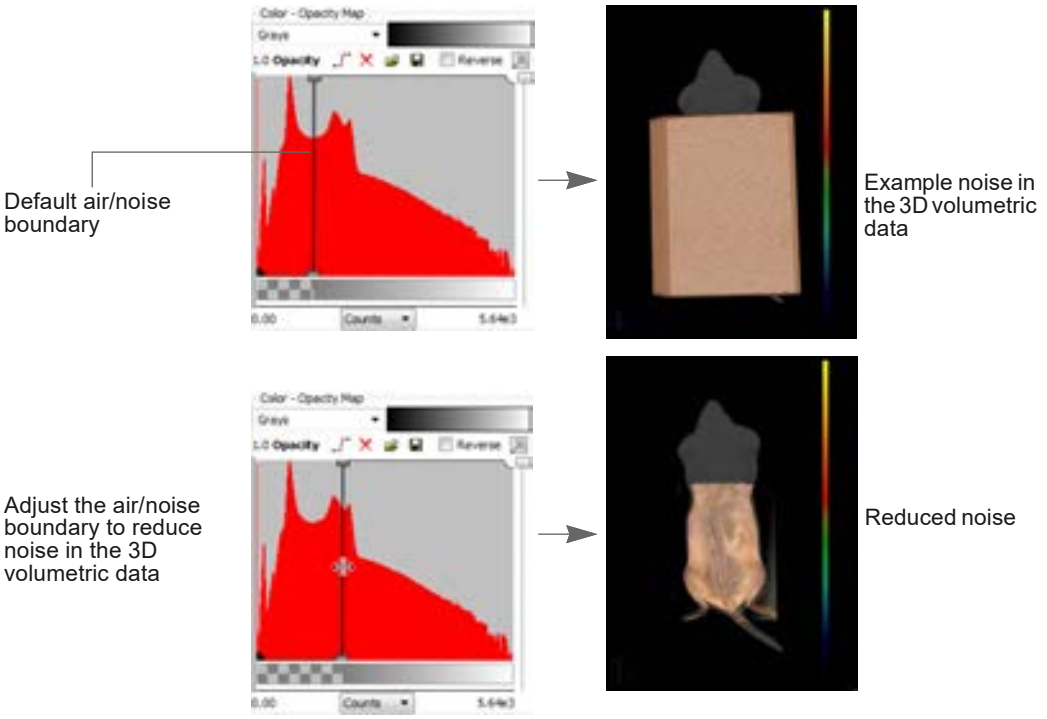
Surfaces before registration

Registered surfaces

To manually register data:

1. Load the data that you want to register (see page 310 for more details).
   The software determines a default air/noise boundary for the 3D volumetric data (Figure 17.20).
2. If you need to remove noise from the 3D volumetric data, move the air/noise boundary to the right in the histogram tool.

Figure 17.20 Adjusting Air/Noise Boundary Using 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 .

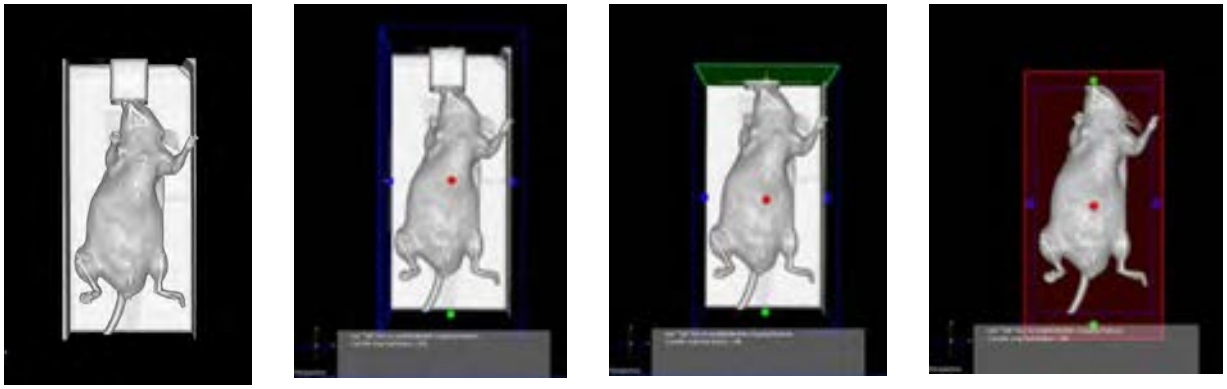
      The crop tool appears and has six control points:

      – Crops the data along the x-axis.

      – Crops data along the y-axis.

      – Crops data along the z-axis.

Figure 17.21 Crop Data Along X,Y, or Z-Axis



No crop tool | X-axis crop tool | Y-axis crop tool | Z-axis crop tool

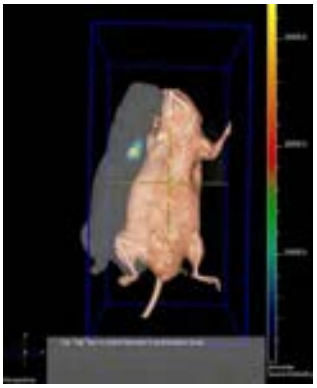
   b. Click and hold a control point while you move the crop plane. As you move the crop plane, the slice views are updated. Release the mouse button to crop the data.

c. To reset the crop planes, click the button. When finished cropping, press the Tab key to turn off the crop tool.

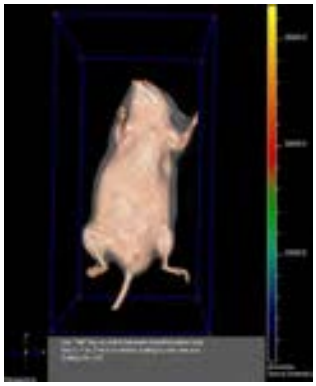
4. Click the Manual Registration button .

   The transformation tool appears (Figure 17.22). 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 17.22 Manual Registration Tool – Transformation Modes



Translate—Moves the volume in the x, y, or z-axis. Drag the tool to adjust the position of the volume.



Scale—Increases or decreases (scale the size of the volume, drag a red cube at a corner of the volume. To restrict scaling to a particular axis, press the X, Y, or Z key, then drag a red cube.

Rotate—To rotate the volume on the x, y, or z-axis, click the blue, green, or red circle and drag the mouse arrow in the direction of interest.

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).

5. To return the 3D volumetric data to the default position and size, click the Reset Registration button .
6. Save the registration information (see page 312).

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

17.7 Volume Information and Results

The Results tab displays information about the loaded data taken from the DICOM file header (Figure 17.23).

Figure 17.23 Volume Information

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

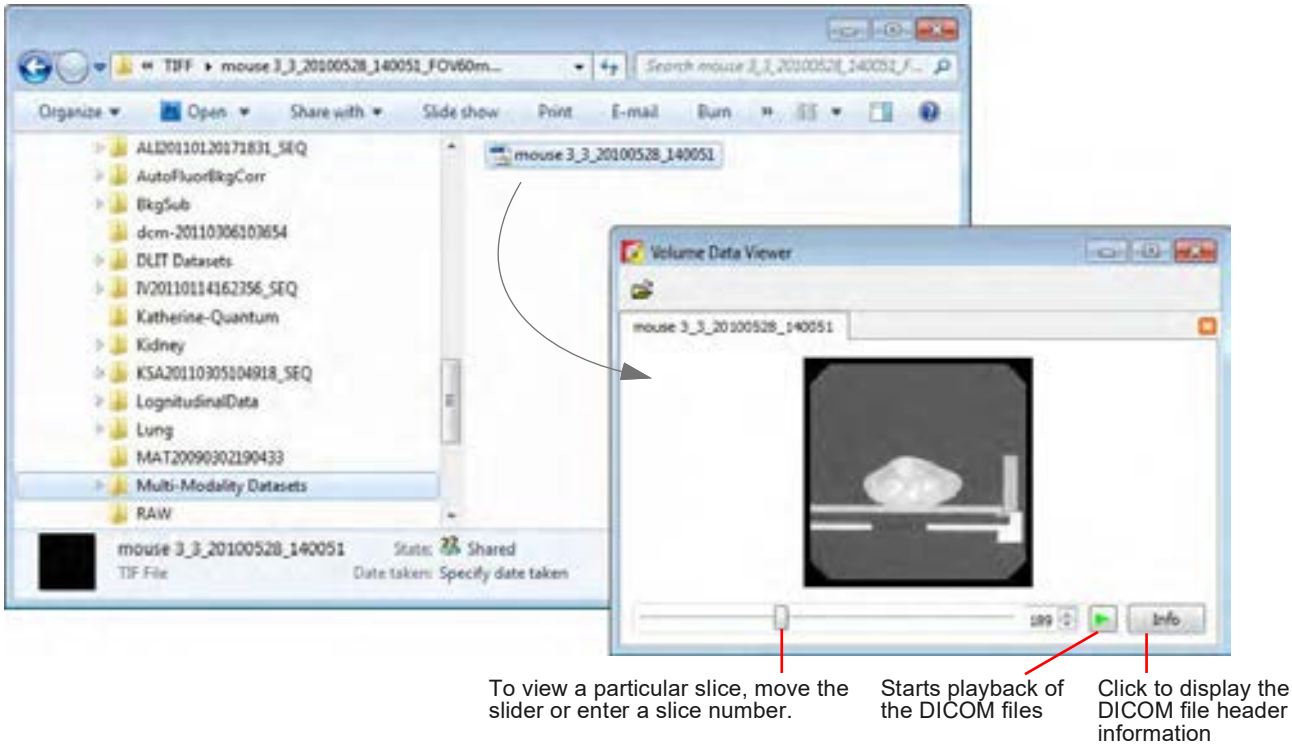
1. Select the results from the Name drop-down list.
2. Click **Delete**.
3. Click **Yes** in the confirmation message that appears.<$startrange>Tool Palette:3D Multi-Modality tools:Results

17.8 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** → **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, 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.

Figure 17.24 Drag Volume Data from Windows Explorer to Volume Data Viewer

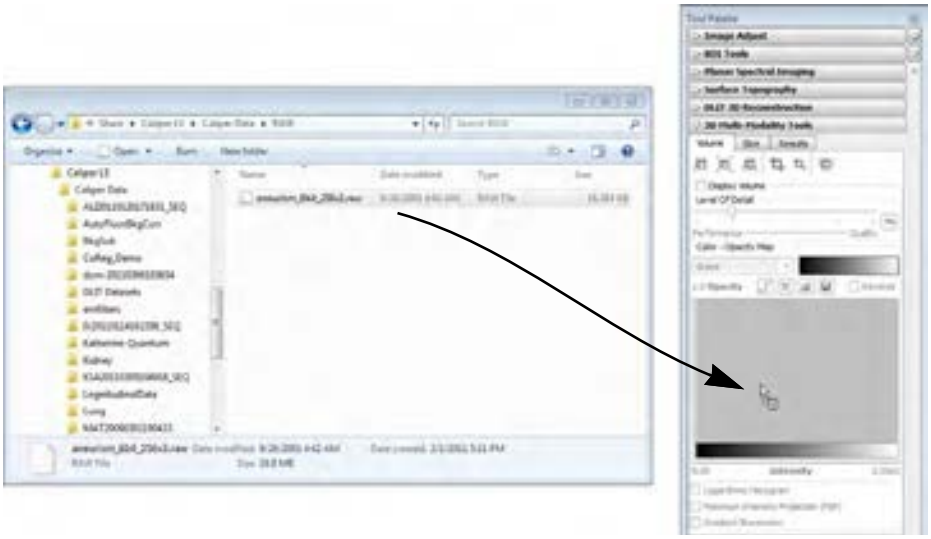


17.9 Viewing RAW Volumetric Data

1. Drag a single RAW file (*.raw or *.vox) from Windows Explorer to the 3D Multi-Modality tools (Figure 17.25).

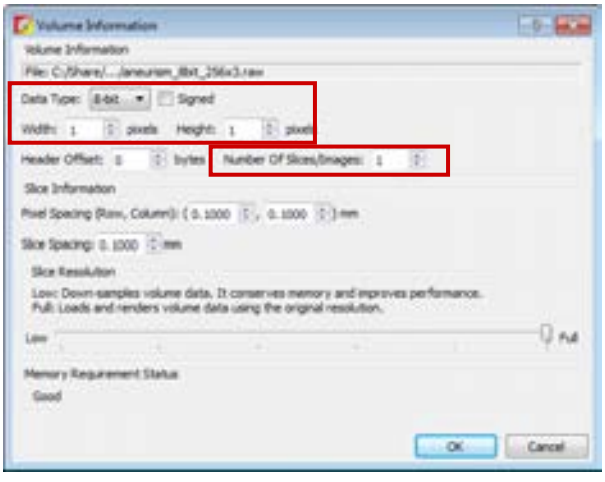
NOTE: Only single *.raw or *.vox files consisting of multiple slices of a 3D volume can be loaded into Living Image.

Figure 17.25 Opening RAW Volumetric Data



2. In the Volume Information dialog box that appears (Figure 17.26), enter the:
   - Data width, height, and the number of slices.
   - Slice row, column pixel size, and the slice spacing in millimeters.

Figure 17.26 Volume Information

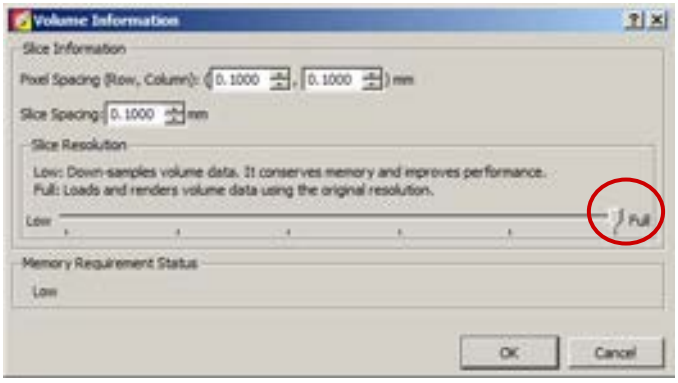


Select a data type.

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 17.27). Decrease the slice resolution by moving the Slice Resolution slider to the left until the Memory Requirement Status is “Good”.

Figure 17.27 Down-sample 3D Volumetric Data



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 CT.

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 316 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 17.28 Volume Information

18 Spectral Unmixing

18.1 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

18.2 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 → 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 322*
Fluorescent	*Fluorescence Imaging on page 324*
Radio-isotope	*Cherenkov Imaging on page 328*

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.

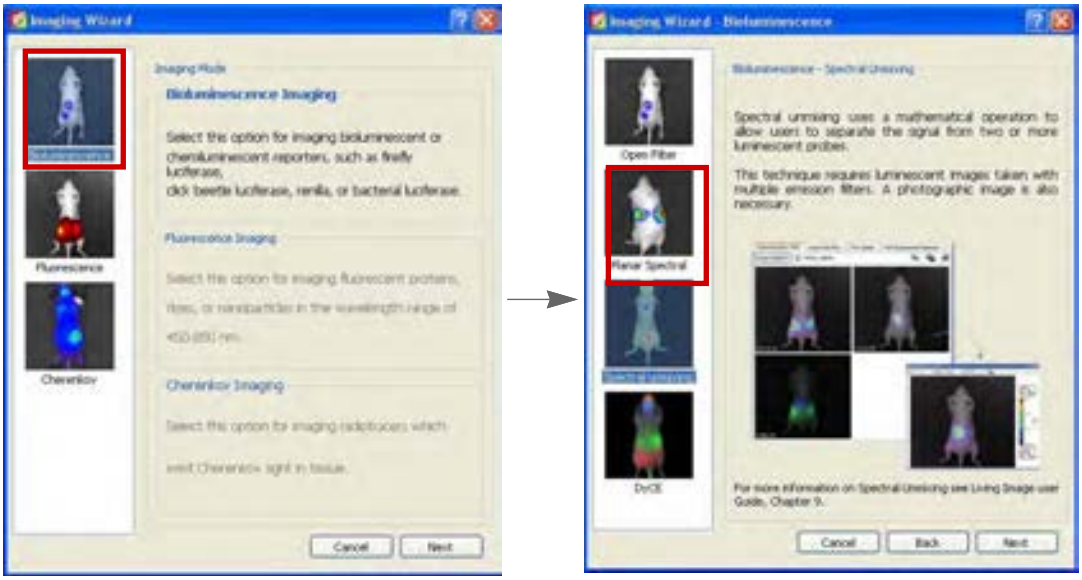
Bioluminescence Imaging

NOTE: The IVIS Spectrum CT should be initialized and the temperature locked before setting the imaging parameters. See page 73 for more details.

1. Start the Imaging Wizard. See page 108 for instructions.
2. Double-click the Bioluminescence option in the wizard. Double-click the Spectral Unmixing option in the next screen (Figure 18.1).

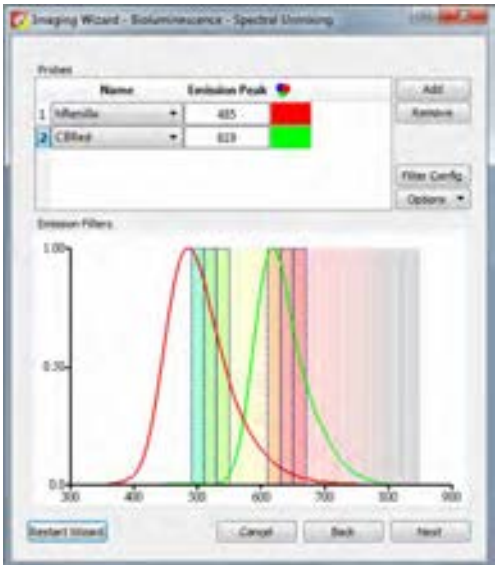
Figure 18.1 Imaging Wizard – Choose Bioluminescence and Spectral Unmixing Options

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



3. Select a probe from the Name drop-down list (Figure 18.2).
4. Click **Add** and select another probe from the Name drop-down list. Repeat until all of the probes are added.

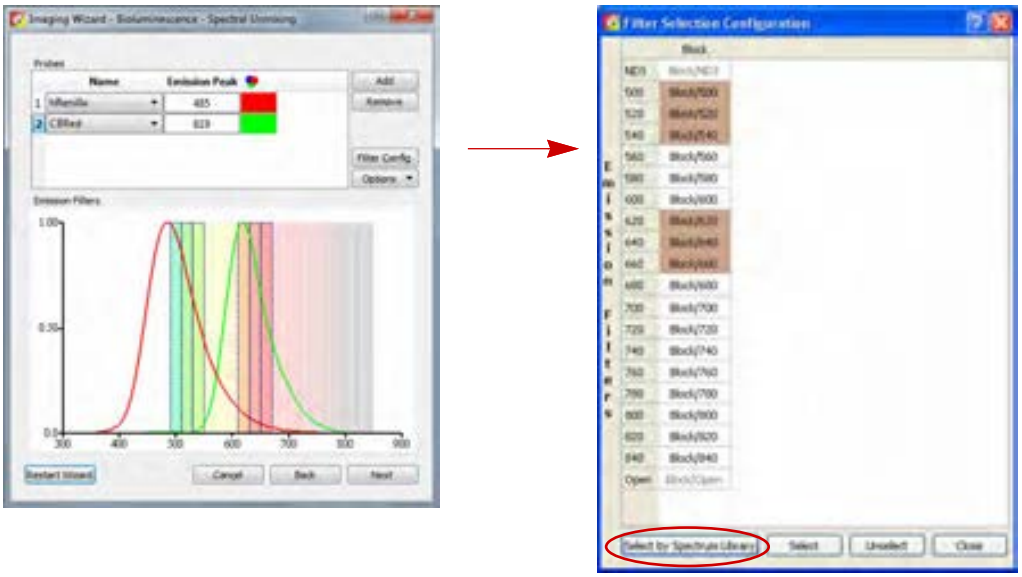
Figure 18.2 Select Probes



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 18.3).

   See *Guided Method on page 331* for instructions on creating a spectrum library.

Figure 18.3 Select a Spectrum Library (Optional)



Click "Select by Spectrum Library" and choose a spectrum library in the dialog box that appears (optional).

6. Click **Next** and in the screen that appears (Figure 18.4):
   a. Select the type of subject.
   b. Select a field of view.
   c. Set the focus options.

Figure 18.4 Select Subject and Set Acquisition Parameters

7. To acquire a time series of images:

a. Choose the Time Series Study option (Figure 18.4).
b. Enter the number of segments and the delay between segments.

8. Click **Next**.
   The specified sequence appears in the sequence table (Figure 18.5).

Figure 18.5 Sequence Setup Complete

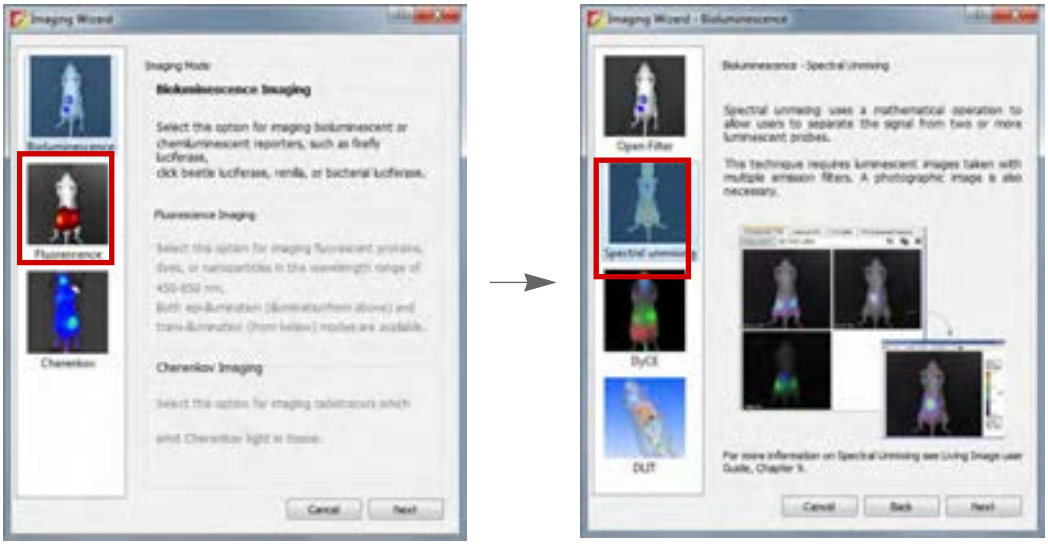
9. Acquire the sequence following the instructions on page 111.
   The image window appears when acquisition is completed (Figure 18.17). See Table 11.4 on page 94 for more details on the Image window.

Fluorescence Imaging

1. Start the Imaging Wizard. See page 108 for instructions.
2. Double-click the Fluorescence option.
3. Select Spectral Unmixing in the next screen (Figure 18.6).
4. Select the type of illumination and click **Next**.
   - Epi-Illumination – Light source above the stage.
   - Trans-Illumination – Light source below the stage.

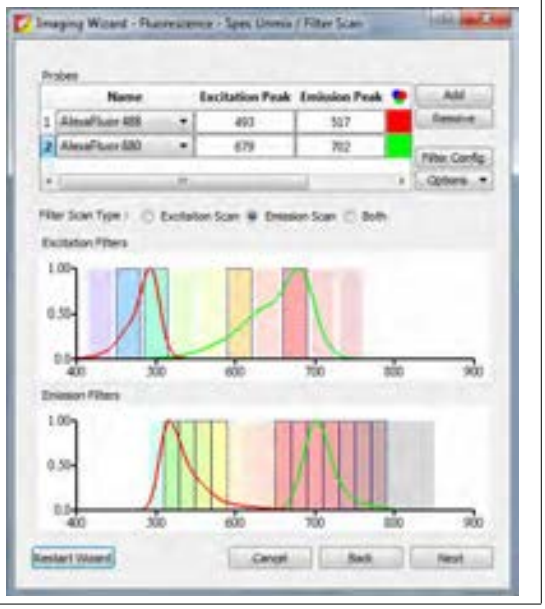
Figure 18.6 Imaging Wizard – Choose Fluorescence and Spectral Unmixing Options

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



5. Select a probe from the Name drop-down list in the next screen (Figure 18.7).
6. Click **Add** and select another probe from the Name drop-down list. Repeat until all of the probes are added.

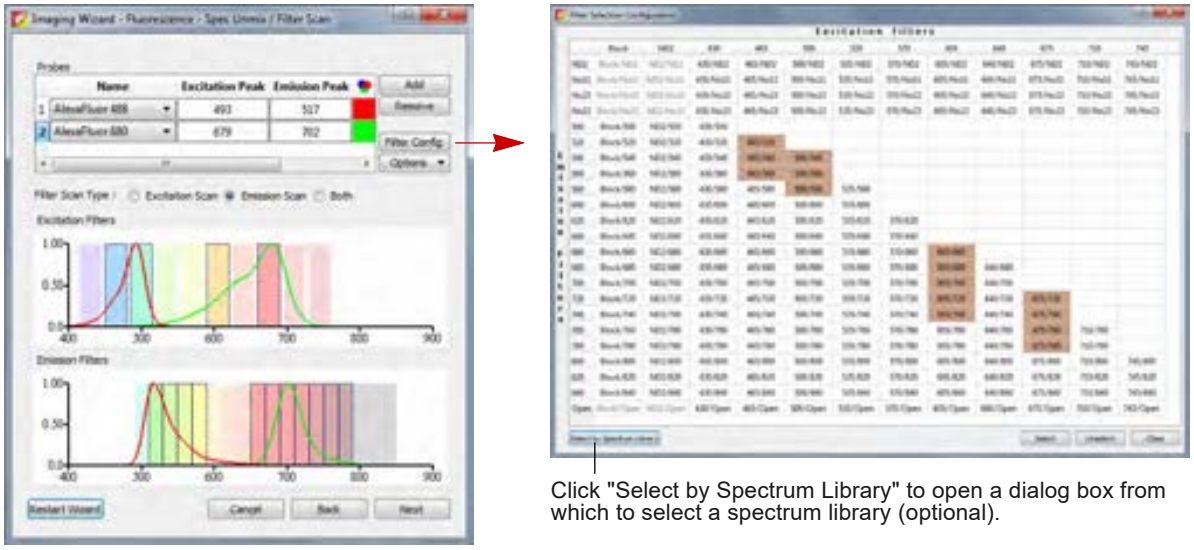
Figure 18.7 Select Probes



7. 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 18.8).

   See *Guided Method on page 331* for instructions on creating a spectrum library.

Figure 18.8 Select a Spectrum Library (optional)



8. Click **Next** and in the screen that appears (Figure 18.9):
   c. Select the type of subject.
   d. Select a field of view.
   e. Set the focus options.

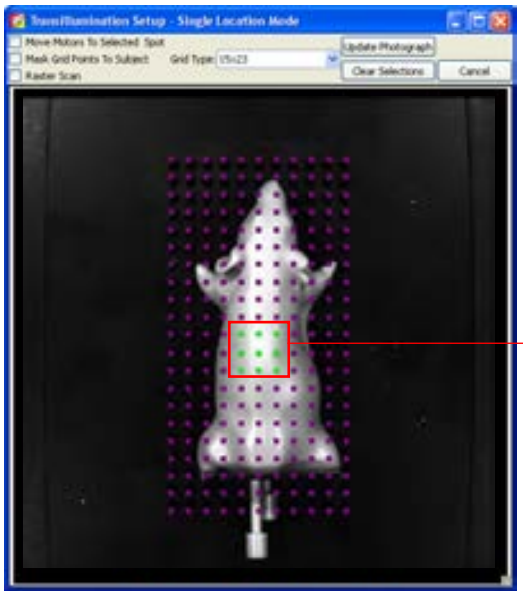
Figure 18.9 Select Subject and Set Acquisition Parameters

9. To acquire a time series of images:
   a. Choose the Time Series Study option (Figure 18.9).
   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 18.10).

See Table 11.5 on page 103 for more details on Transillumination Setup.

Figure 18.10 Transillumination Setup Box



11. Click **Next**.

The specified sequence appears in the sequence table (Figure 18.11).

Figure 18.11 Sequence Setup Complete

12. Acquire the sequence following the instructions on page 111.

The image window appears when acquisition is completed (Figure 18.17). See Table 11.4 on page 94 for more details on the Image window.

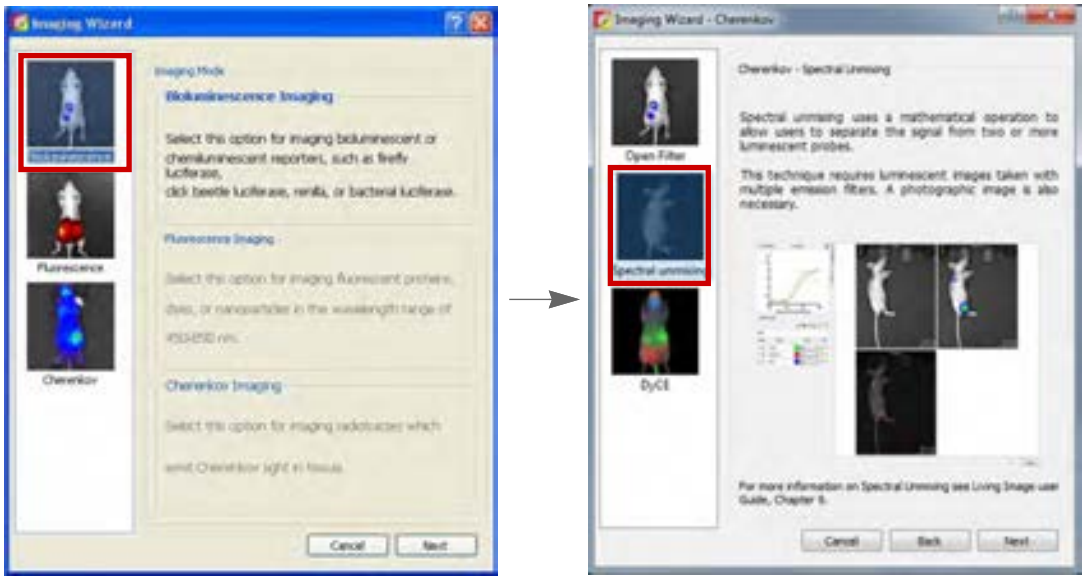
Cherenkov Imaging

NOTE: The IVIS Spectrum CT should be initialized and the temperature locked before setting the imaging parameters. See page 73 for more details.

1. Start the Imaging Wizard. See page 108 for instructions.
2. Double-click the Cherenkov option. Double-click the Spectral Unmixing option in the next screen (Figure 18.12).

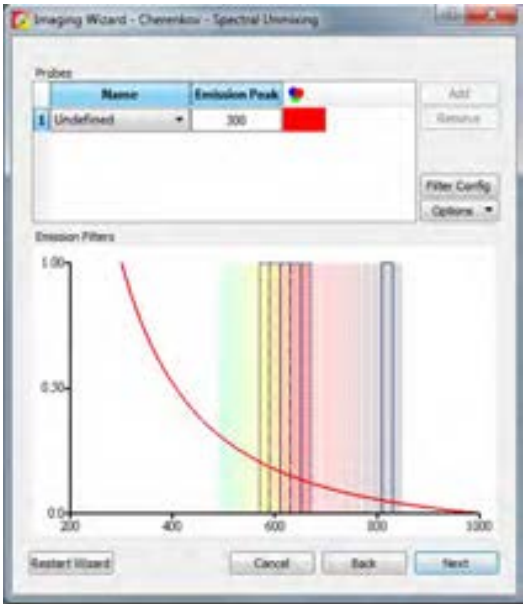
Figure 18.12 Imaging Wizard – Choose Cherenkov and Spectral Unmixing Options

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



3. Select a probe from the Name drop-down list (Figure 18.13).
4. Click **Add** and select another probe from the Name drop-down list. Repeat until all of the probes are added.

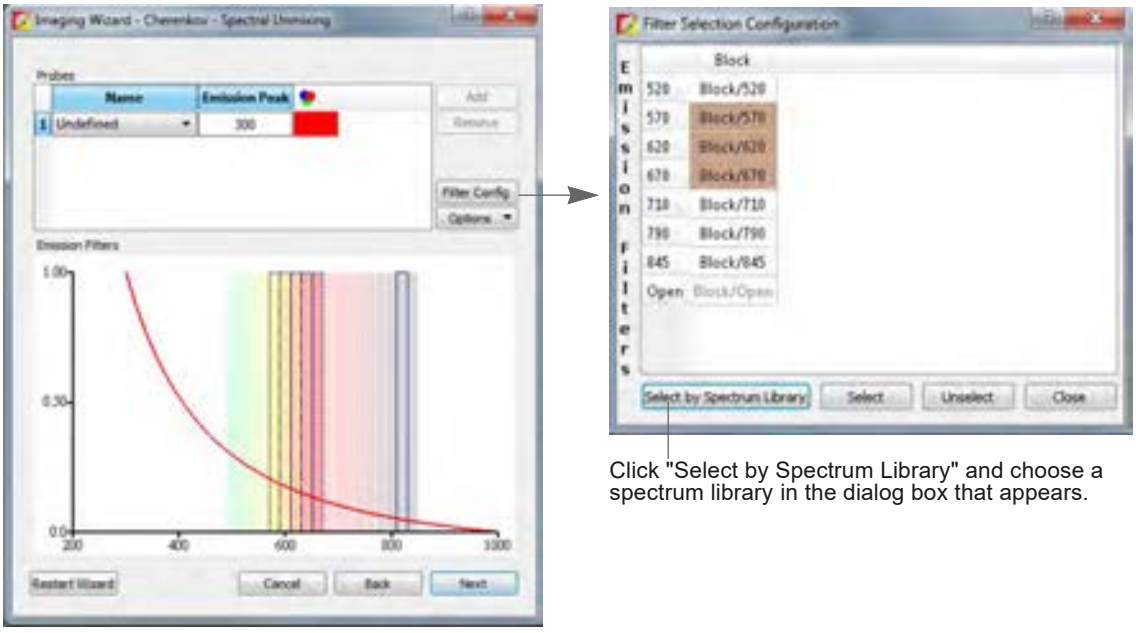
Figure 18.13 Select Probes



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 18.14).

   See *Guided Method on page 331* for instructions on creating a spectrum library.

Figure 18.14 Select a Spectrum Library (Optional)



6. Click **Next** and in the screen that appears (Figure 18.15):
   c. Select the type of subject.
   d. Select a field of view.
   e. Set the focus options.
7. To acquire a time series of images:
   a. Choose the Time Series Study option (Figure 18.15).
   b. Enter the number of segments and the delay between segments.

Figure 18.15 Select Subject and Set Acquisition Parameters

8. Click **Next**.
   The specified sequence appears in the sequence table (Figure 18.16).

Figure 18.16 Sequence Setup Complete

9. Acquire the sequence following the instructions on page 111.
   The image window appears when acquisition is completed (Figure 18.17). See Table 11.4 on page 94 for more details on the Image window.

18.3 Spectral Unmixing Methods

Living Image software provides four spectral unmixing methods (Table 18.1).

Table 18.1 Spectral Unmixing Methods

Method	Description	See Page
Guided	Use this method when: ▪ Probe locations are known. ▪ 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.	331
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. **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 *in vivo* data to analyze *in vitro* data and vice versa.	334
Automatic	Use this method when: ▪ Probe locations are unknown. ▪ Probes are included in the spectrum library.	336
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.	340

Guided Method

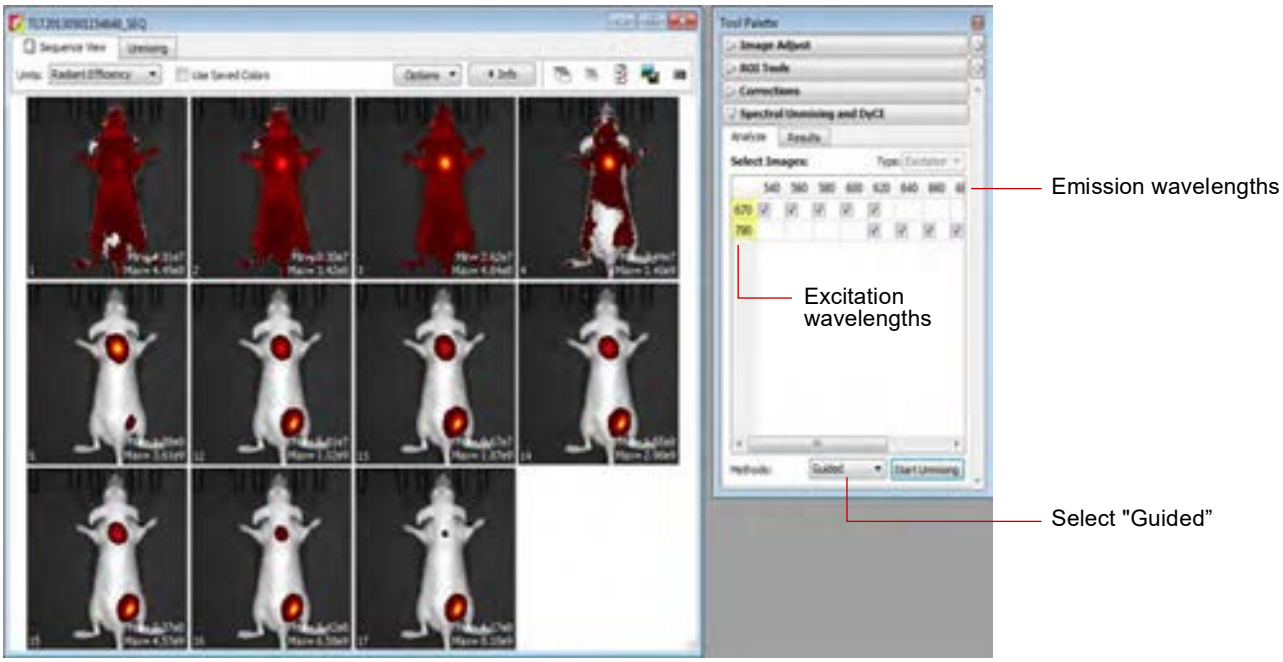
Use the guided method:

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

1. Load the image sequence.

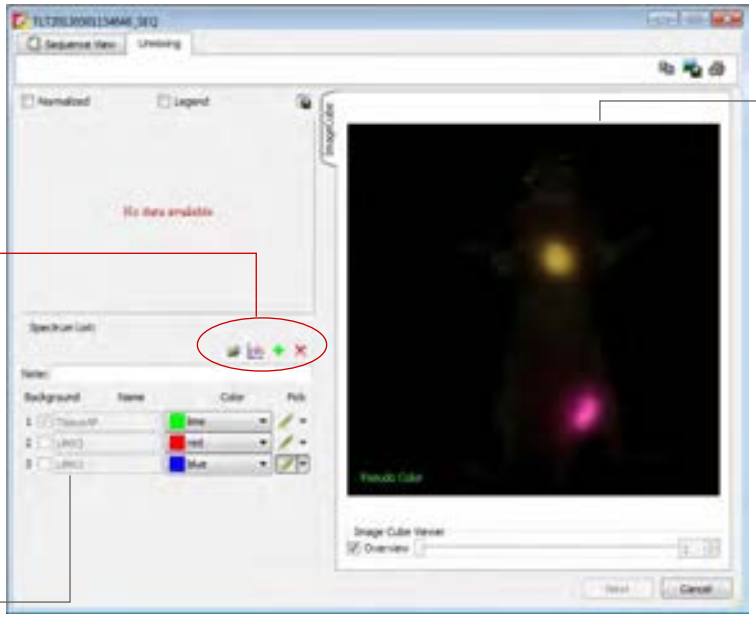
   In Figure 18.17, the fluorophores are Alexa Fluor 680 and Alexa Fluor 750. Images were acquired using 680 and 790 nm excitation filters, and emission filters from 540 to 720 nm in 20 nm increments.

Figure 18.17 Sequence for Spectral Unmixing



2. Click the Analyze tab of the Spectral Unmixing and DyCE tools.
   By default, all wavelengths are included in the analysis. Remove the check mark next to wavelengths that you want to exclude from the analysis.
3. Select "Guided" from the Methods drop-down list and click **Start Unmixing**.
   The Unmixing window appears (Figure 18.18).

Figure 18.18 Unmixing Window



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

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

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

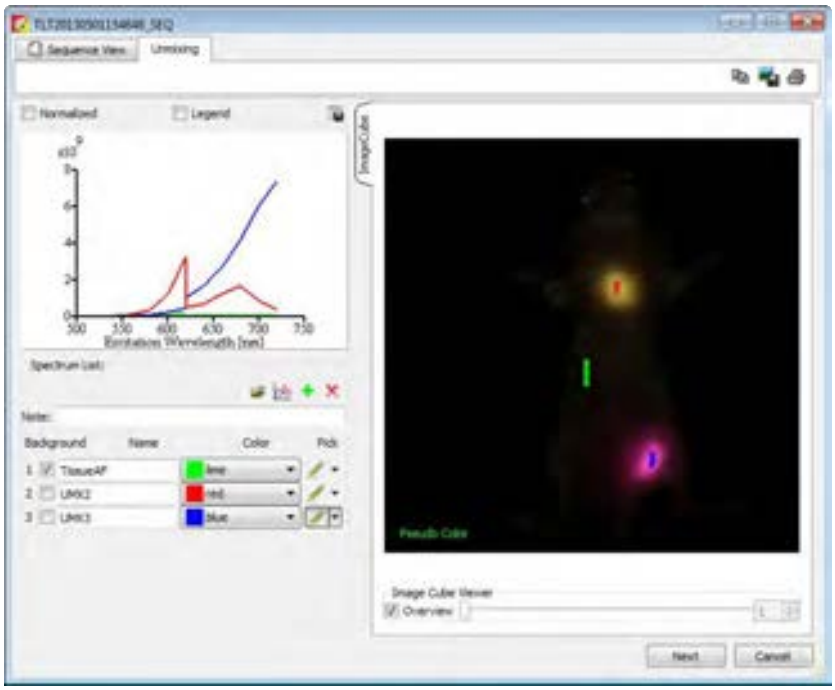
4. Move the mouse pointer over the image cube to see the spectrum at a particular location.
   The raw spectrum at the pointer location is updated as you move the pointer.
5. To specify a probe location for unmixing:
   a. Click the button for a spectrum.
   b. Using the mouse, draw a mark on an area of the image cube which represents the probe signal.
      The software plots a background-subtracted spectrum of the signal (Figure 18.19).

      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.

Figure 18.19 Mark Probe Locations for Unmixing on the Image Cube

Background-subtracted spectra at the probe locations marked on the image cube



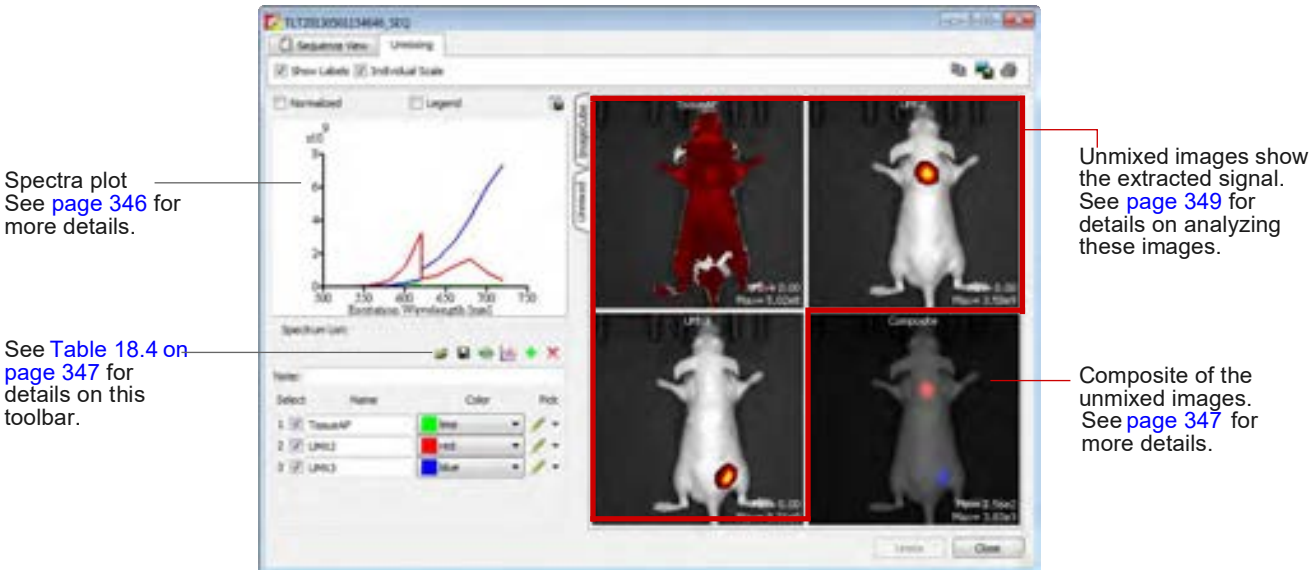
7. Click **Next** after you finish marking the probe locations.

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 18.20).

See *Spectral Unmixing Results on page 345* for information about the results.

8. To save the results as a spectrum library:
   a. Click the button in the Spectrum List toolbar (Figure 18.20).
   b. Enter a file name in the dialog box that appears and click **Save**.

Figure 18.20 Spectral Unmixing Results



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.

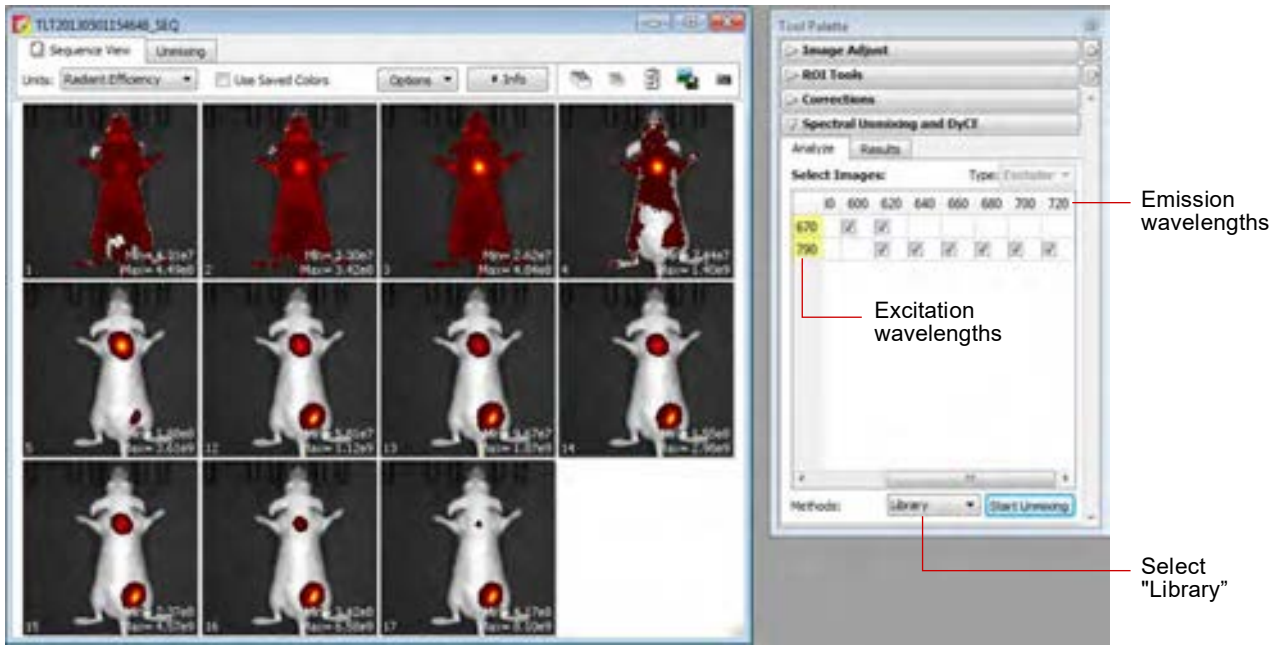
The probe depth in the dataset being analyzed and the spectrum library dataset should be similar for optimum analysis results. For example, do not use a spectrum library generated from in vivo data to analyze in vitro data.

NOTE: Use the guided or manual method to generate a spectrum library of known probes with known locations (see page 331 for guided method or page 340 for manual method).

1. Load the image sequence.

   In Figure 18.21, 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.

Figure 18.21 Sequence for Spectral Unmixing



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

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

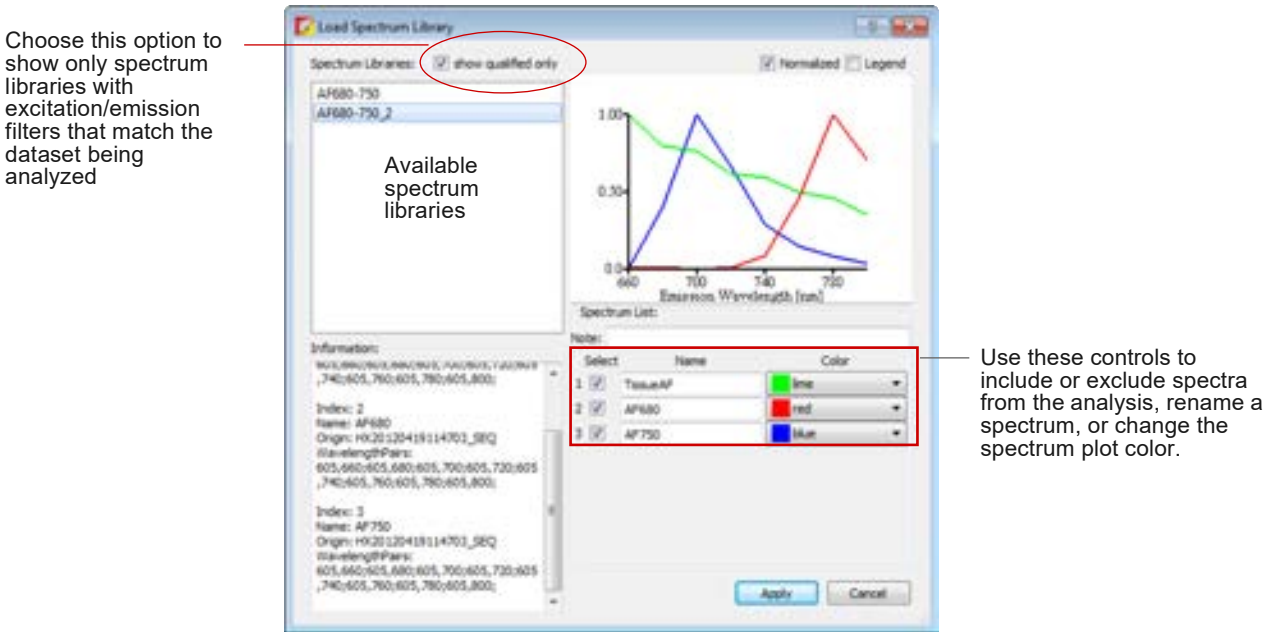
3. Select "Library" from the Methods drop-down list and click **Start Unmixing**.

4. Select a reference spectral library in the dialog box that appears and click **Apply** (Figure 18.22).

   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 18.20 on page 334).

   See *Spectral Unmixing Results on page 345* for information about the results.

Figure 18.22 Select a Reference Spectral Library



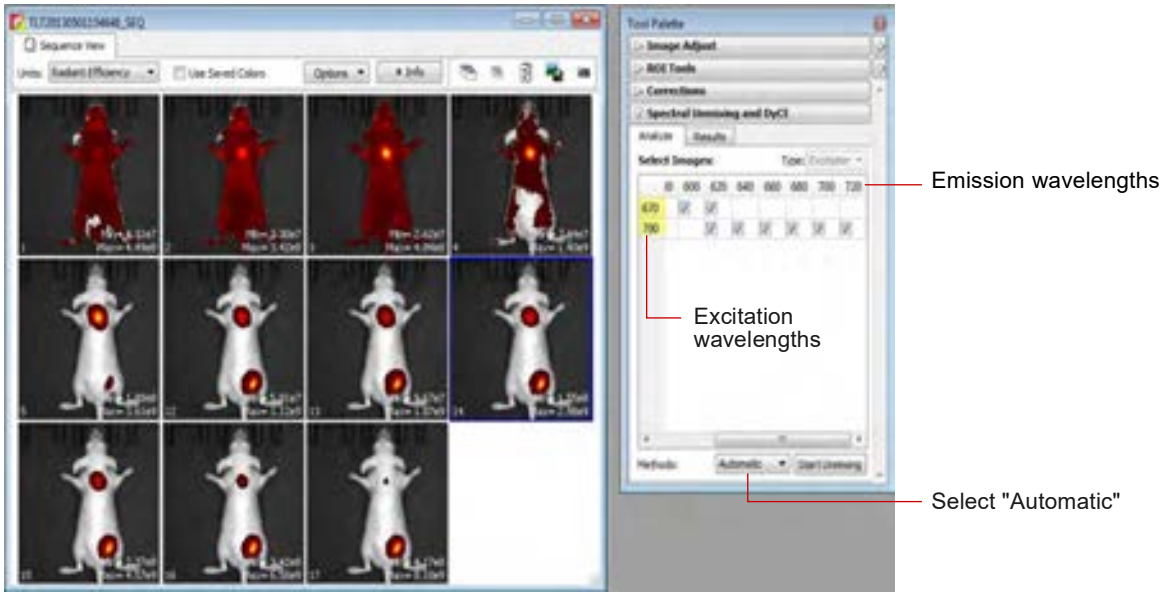
Automatic Method

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 18.23, 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.

Figure 18.23 Sequence for Spectral Unmixing



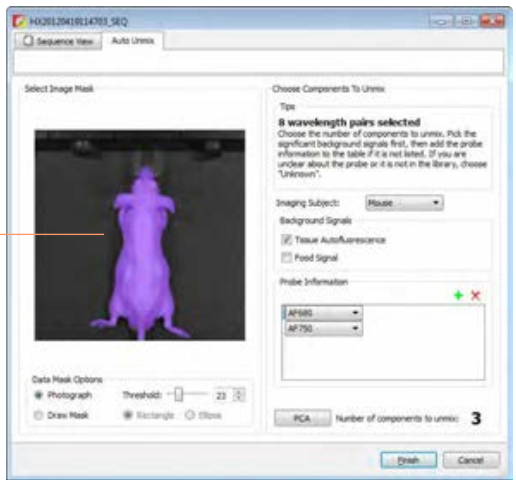
2. Click the Analyze tab of the Spectral Unmixing and DyCE tools.

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

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

   The Auto Unmix window appears (Figure 18.24). The purple data mask shows the data that will be included in the analysis (the entire subject is included by default).

Figure 18.24 Auto Unmix Window



4. If you do not want to analyze the entire subject, draw a mask on a particular area (Figure 18.25).

   For example, it is useful to mask shaved or depilated areas.

Figure 18.25 Draw a Data Mask

See Table 18.2 on page 338 for more details on the data mask options.

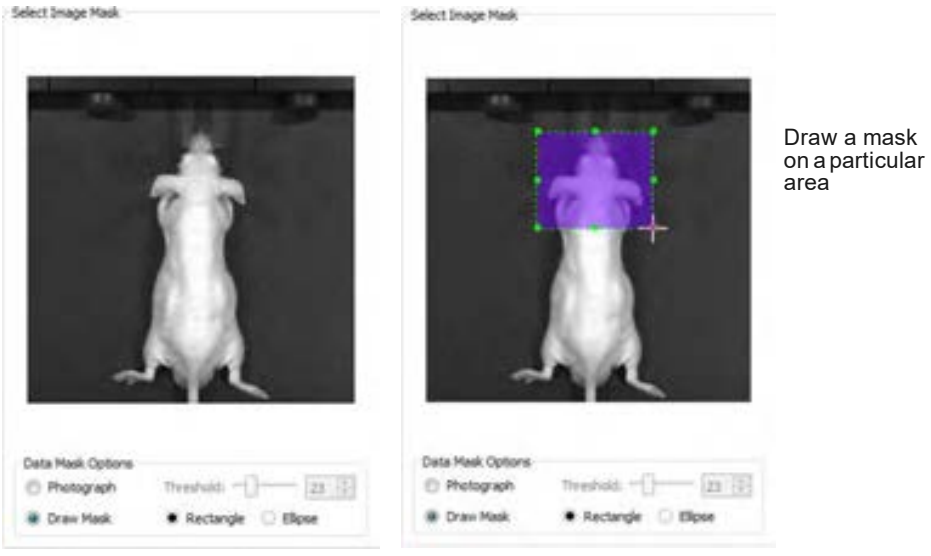
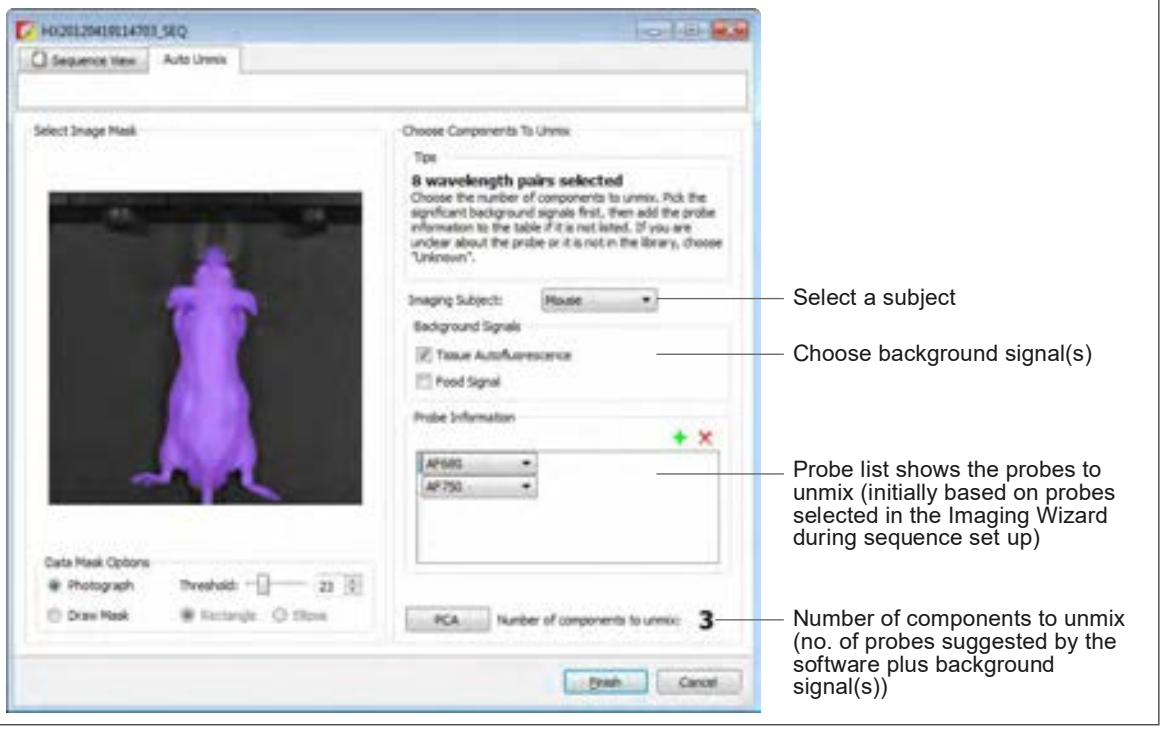


Table 18.2 Data Mask Options

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 18.26 Auto Unmix Window

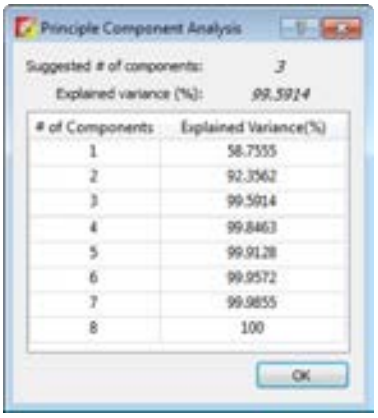


6. Click the **PCA** button.

   The Principle Component Analysis window shows the amount of signal explained by the suggested components (Figure 18.27). 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.

Figure 18.27 Principle Component Analysis



7. Click **Finish**.

   The Unmixing window shows the analysis results which include unmixed spectra, unmixed images, and a composite of the unmixed images (Figure 18.20 on page 334).

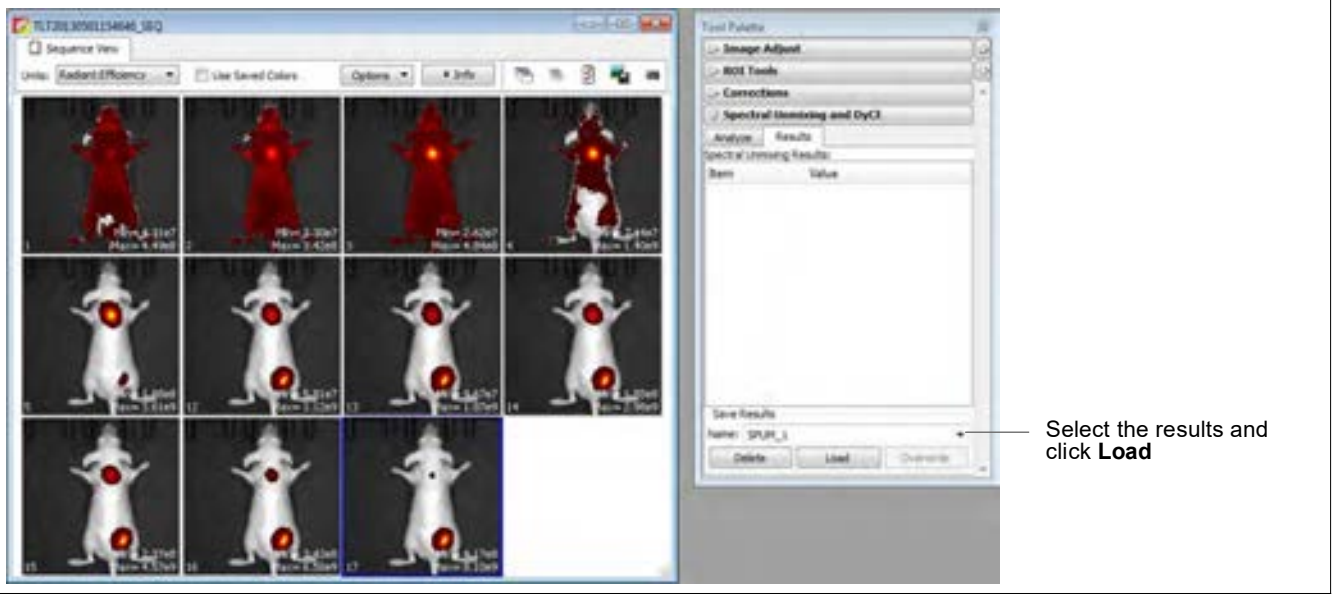
See *Spectral Unmixing Results on page 345* for information about the results.

Manual Method

Sometimes you may want to manually analyze results, for example, if the explained variance of the principle component analysis of an automatic analysis seems low 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 18.28 Open a Sequence and Select Results to Load

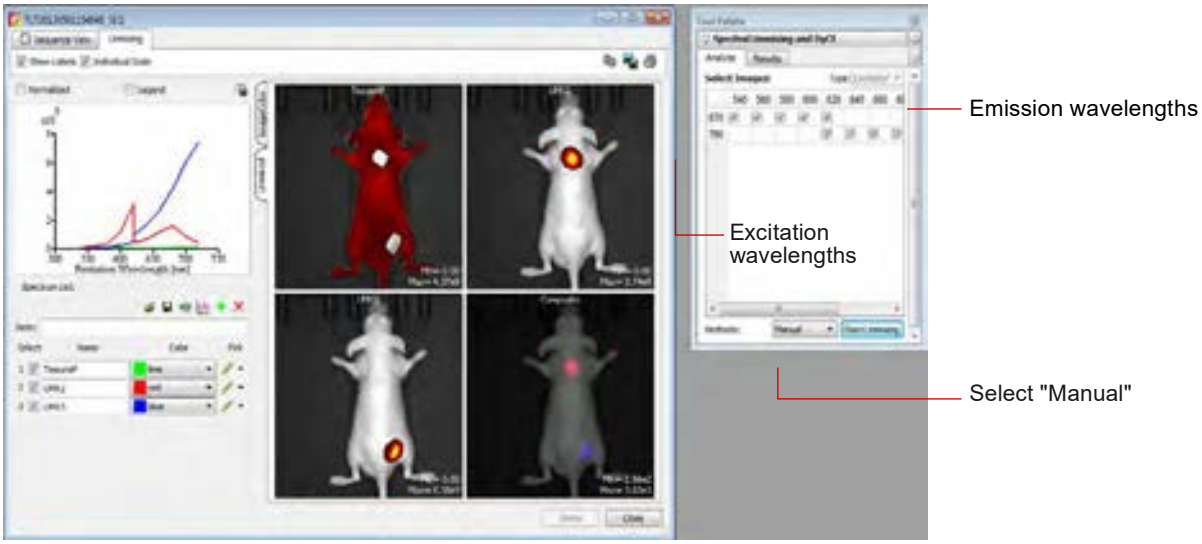


3. Click the Analyze tab of the Spectral Unmixing and DyCE tools.

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

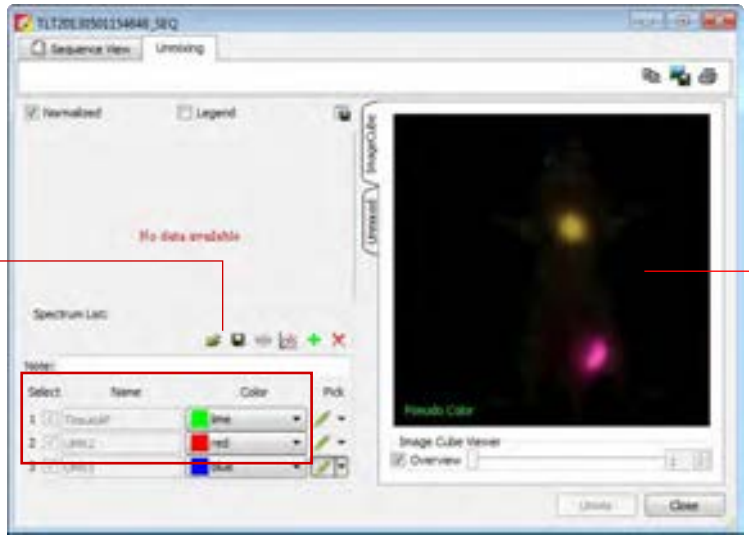
In Figure 18.29, the fluorophores are Alexa Fluor 680 and Alexa Fluor 750. Images were acquired using 670 and 790 nm excitation filters, and emission filters from 540 to 720 nm in 20 nm increments.

Figure 18.29 Unmixing Window – Results Loaded



4. Select "Manual" from the Methods drop-down list and click **Start Unmixing**. The Unmixing window appears (Figure 18.18).

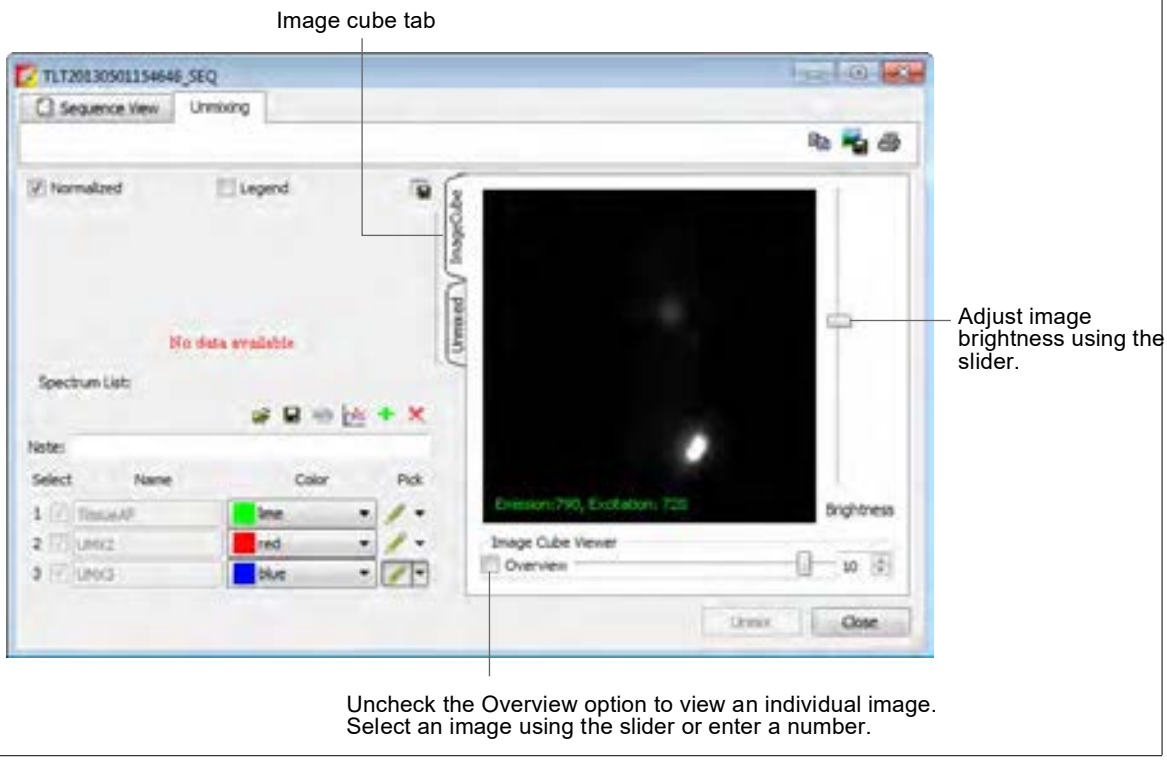
Figure 18.30 Unmixing Window



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

The entire image cube is calibrated and visualized on the same scale. To view a particular image, remove the check mark next to the Overview option and move the slider or enter an image number (Figure 18.31).

Figure 18.31 Image Cube – Single Image Mode



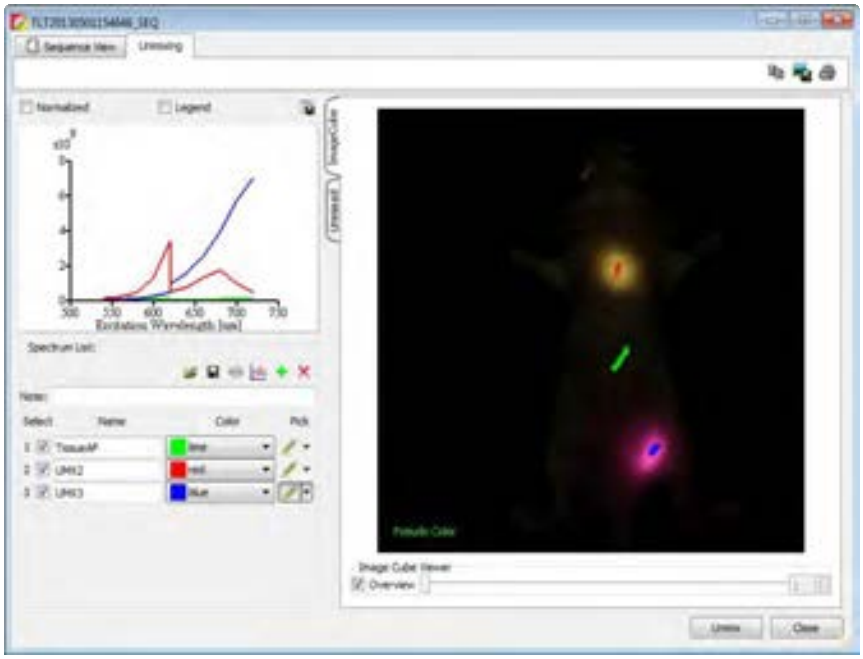
5. Move the mouse pointer over the image cube to see the spectrum at a particular location.
   The spectrum at the pointer location is updated as you move the pointer.
6. To specify a probe location for unmixing:
   a. Click the button for a spectrum.
   b. Using the mouse, draw a mark on an area of the image cube which represents the probe location.
      The software plots a normalized spectrum of the signal (Figure 18.32).

   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 step 6 to specify other probe locations.
8. Manually subtract autofluorescence background. See *Correcting Spectra on page 344* for instructions.

Figure 18.32 Mark Probe Locations for Unmixing on the Image Cube

Raw spectra at the probe locations marked on the image cube



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

   The Unmixing window shows the analysis results which include unmixed spectra, unmixed images, and a composite of the unmixed images (Figure 18.20 on page 334).

   See *Spectral Unmixing Results on page 345* for information about the results.

18.4 Correcting Spectra

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

1. Click the button in the Unmix window.
2. Choose the spectra to subtract in the dialog box that appears. (Figure 18.33).
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.

Figure 18.33 Choose Spectra to Subtract: A - x*B = C

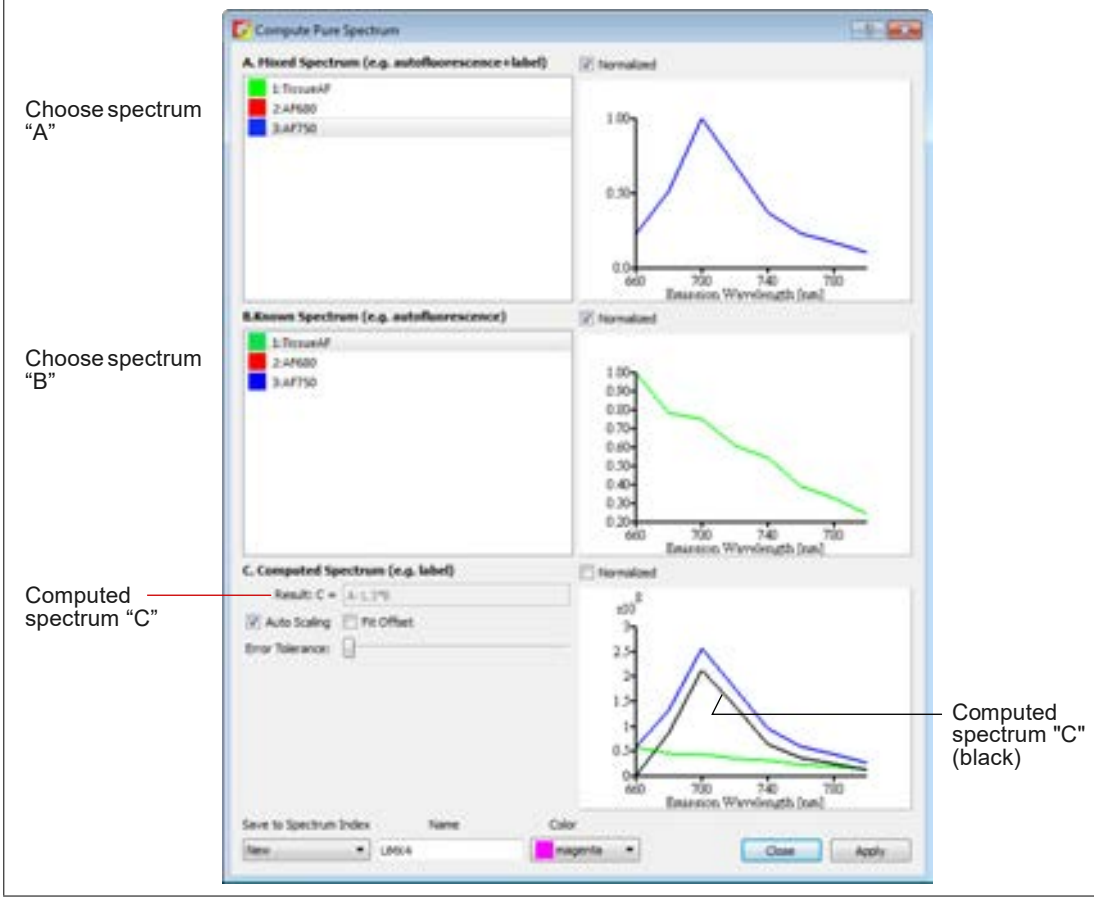
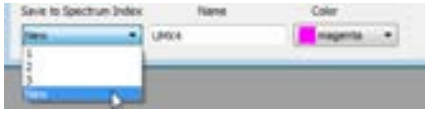


Table 18.3 Computed Spectrum

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.

Table 18.3 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.
	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**.

18.5 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 18.34).

Figure 18.34 Spectral Unmixing Results

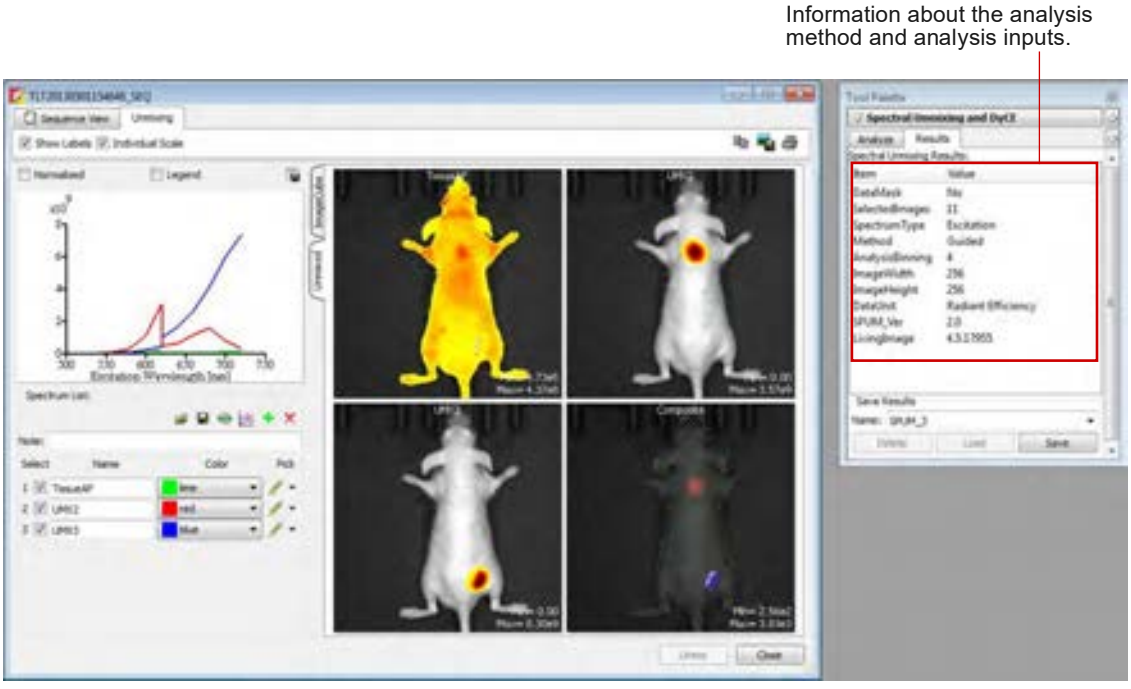
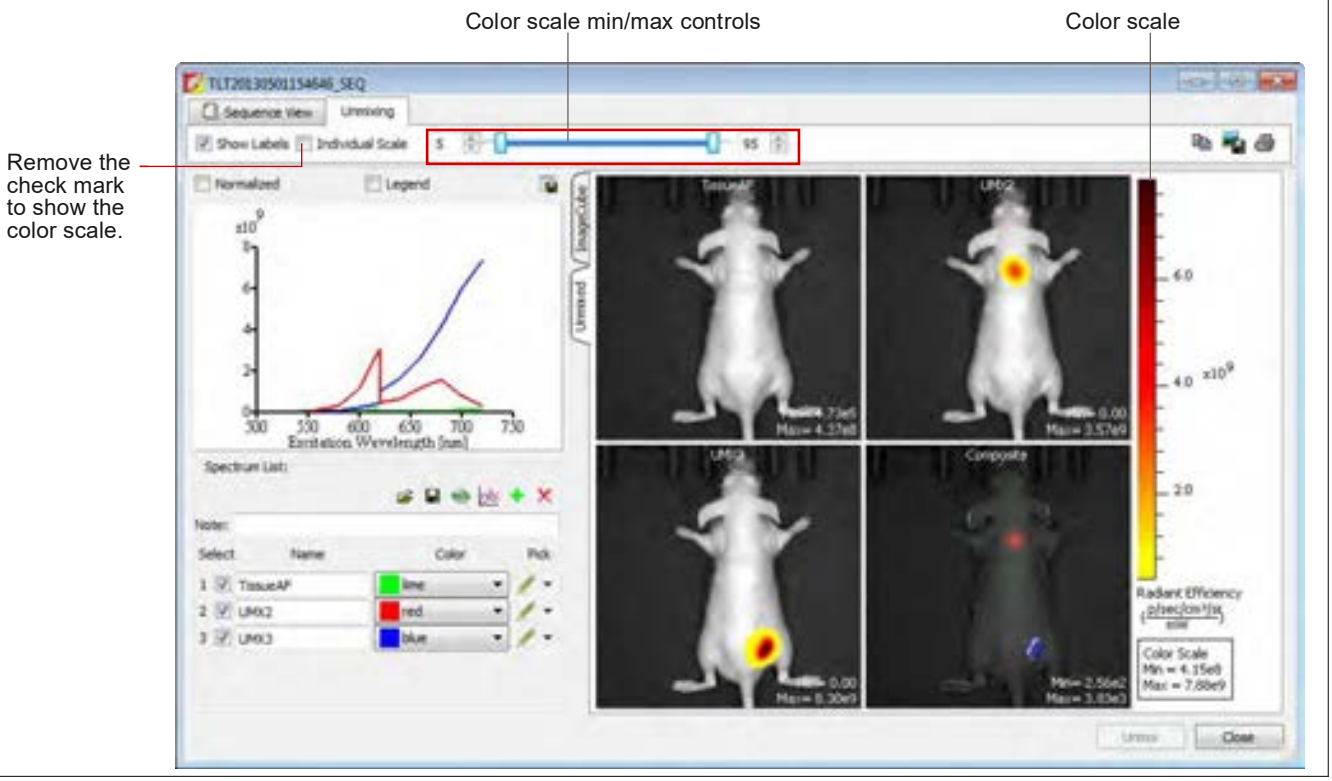


Figure 18.35 Spectral Unmixing Results With Color Scale



Spectra Plot

Spectra plots show the unmixed spectra.

Figure 18.36 Spectra Plots

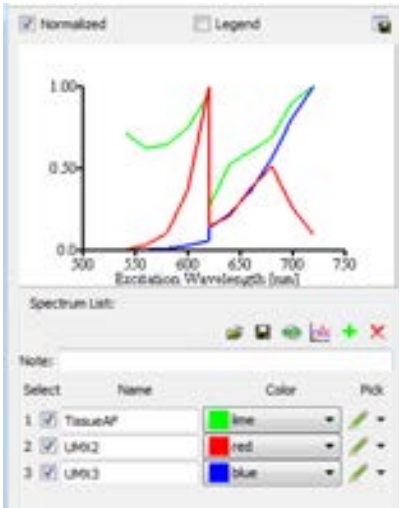


Table 18.4 Spectra Window

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.
	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 334 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 *Correcting Spectra on page 344*).
	Adds a component to the spectrum list.
	Deletes the last spectrum in the spectrum list.

Adding Spectra to the Plot

To Add:	Do This:
A spectrum library	Click the  button and select a spectrum library in the dialog box that appears. **Note:** A spectrum library is a user-created set of reference spectra generated by analyzing probes with known spectra and known locations.
A spectrum from a user-defined region	Add a new spectrum to the list in the Unmix window and identify the region by drawing a mark on the image cube. See Step 6 on page 342 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 18.37).

Figure 18.37 Composite Window

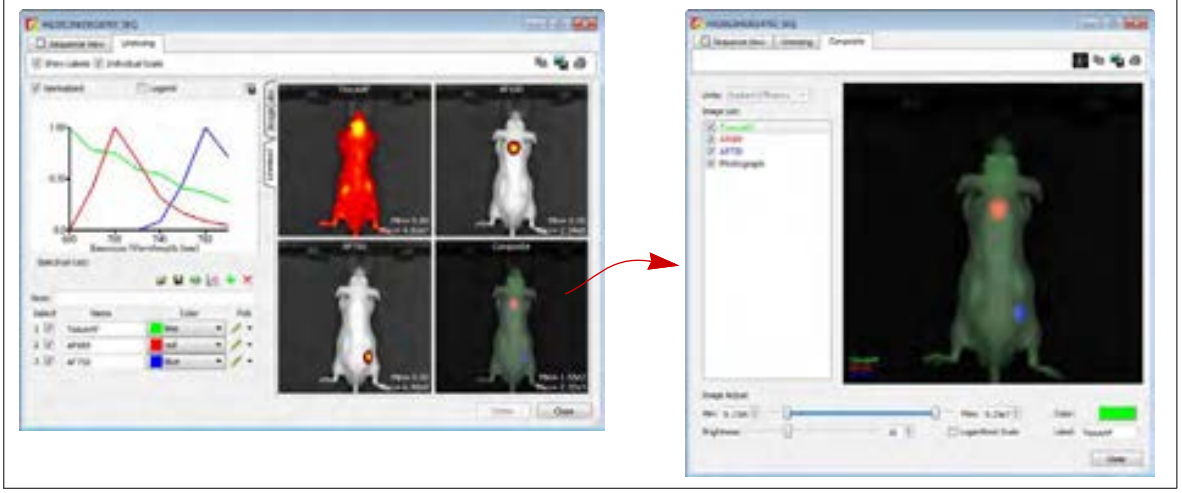


Table 18.5 Composite Window

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

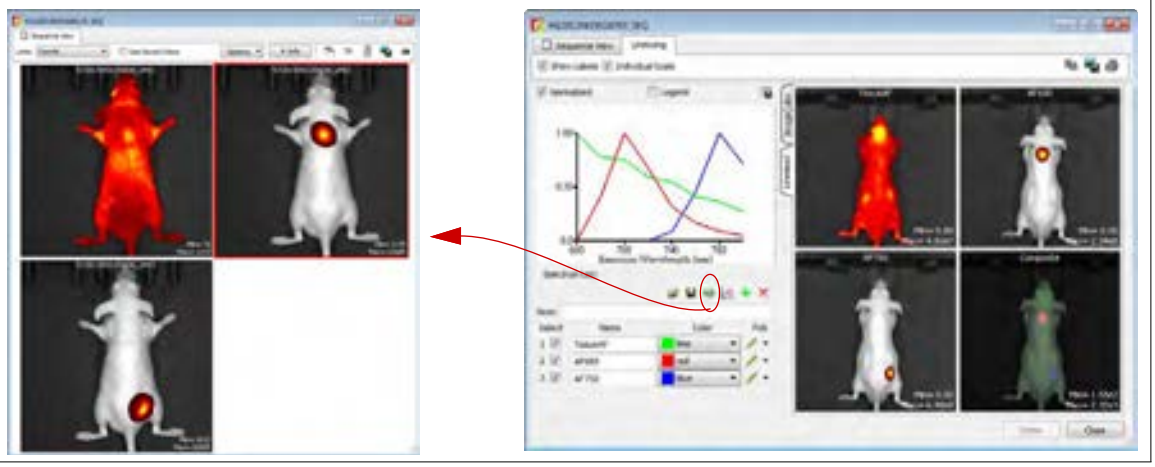
Analyzing Images

Do either of the following:

- Click the button toolbar button to view all images as a sequence (Figure 18.38).
- 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.

Figure 18.38 View Unmixed Images as a Sequence



Managing Spectral Unmixing Results

Figure 18.39 Spectral Unmixing Results

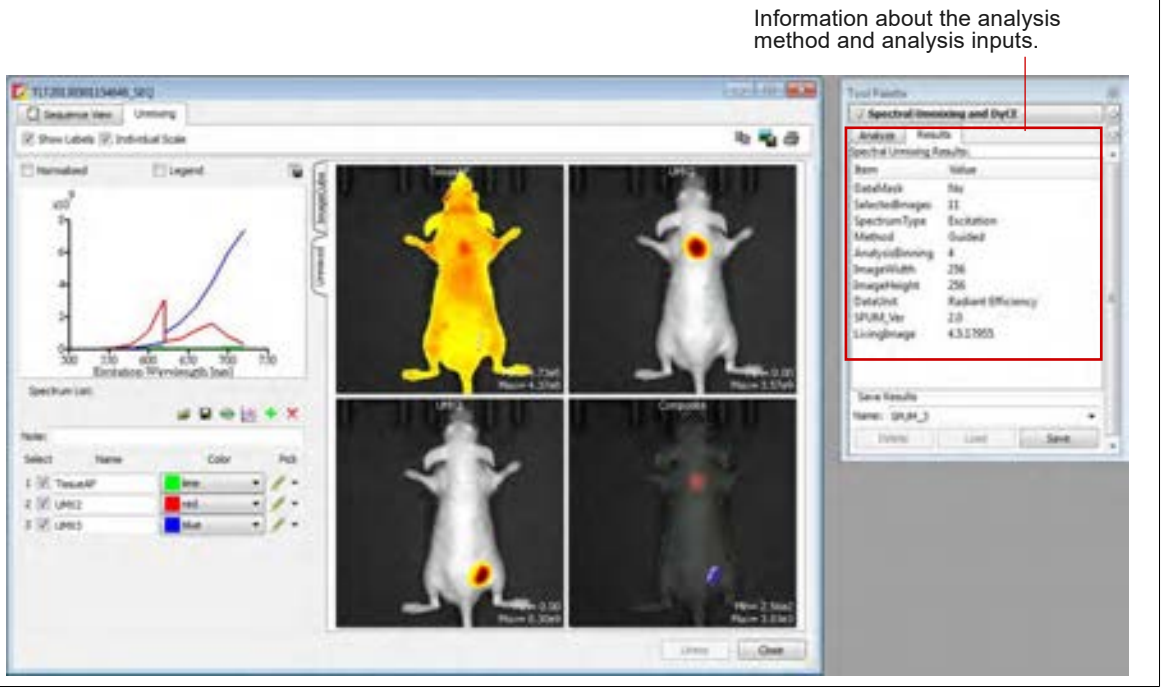


Table 18.6 Spectral Unmixing Results

Item	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.

19 Biodistribution Studies Using DyCE Imaging

19.1 About DyCE (Dynamic Contrast Enhancement)

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

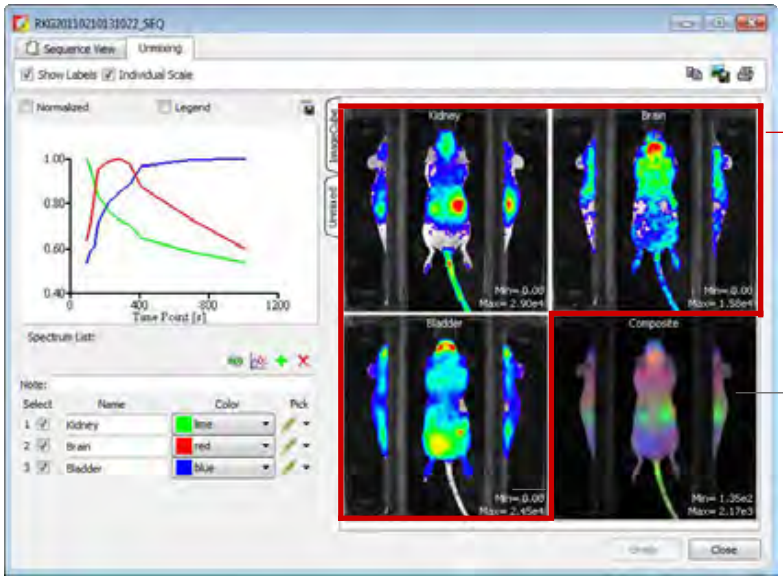
DyCE imaging and analysis is intended for biodistribution studies. DyCE imaging captures a time series of optical images immediately following a bolus injection of a probe or dye. Living Image software temporally unmixes the data on a pixel-by-pixel basis for each image of the time series and determines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

Figure 19.1 Example DyCE Results

Images were obtained using the Mouse Side Imaging Kit.



19.2 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.

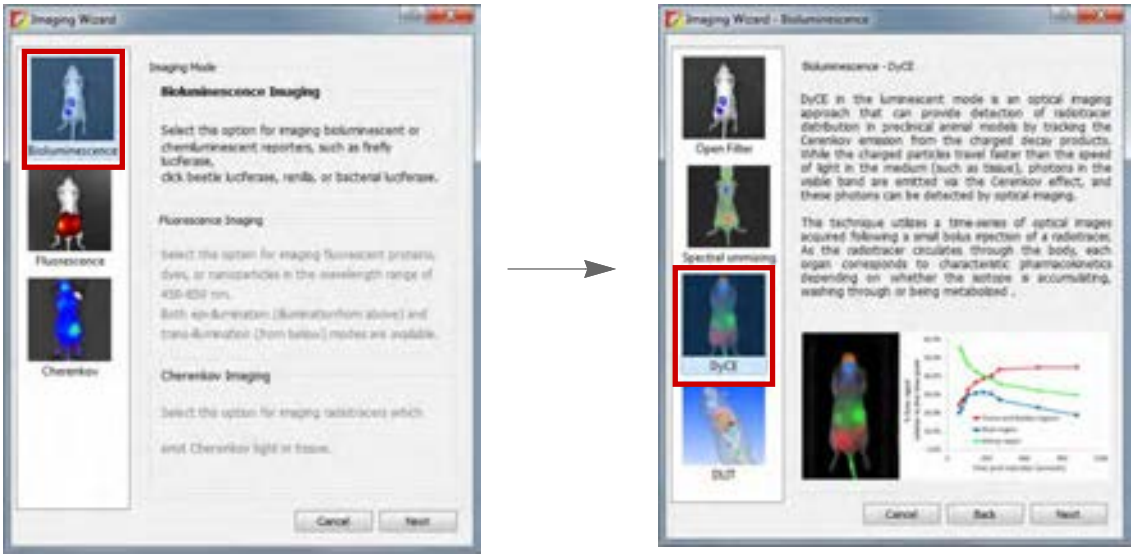
Probe Type	Follow the Instructions for:
Luminescent	*Bioluminescence Imaging on page 352*
Fluorescent or near infrared	*Fluorescence Imaging on page 354*
Radiotracer	*Cherenkov Imaging on page 357*

Bioluminescence Imaging

NOTE: The IVIS Spectrum CT should be initialized and the temperature locked before setting the imaging parameters. See page 73 for more details.

1. Start the Imaging Wizard. See *Start the Imaging Wizard and Setup a Sequence on page 108* for instructions.
2. Double-click the Bioluminescence option. Double-click the DyCE option in the next screen (Figure 19.2).

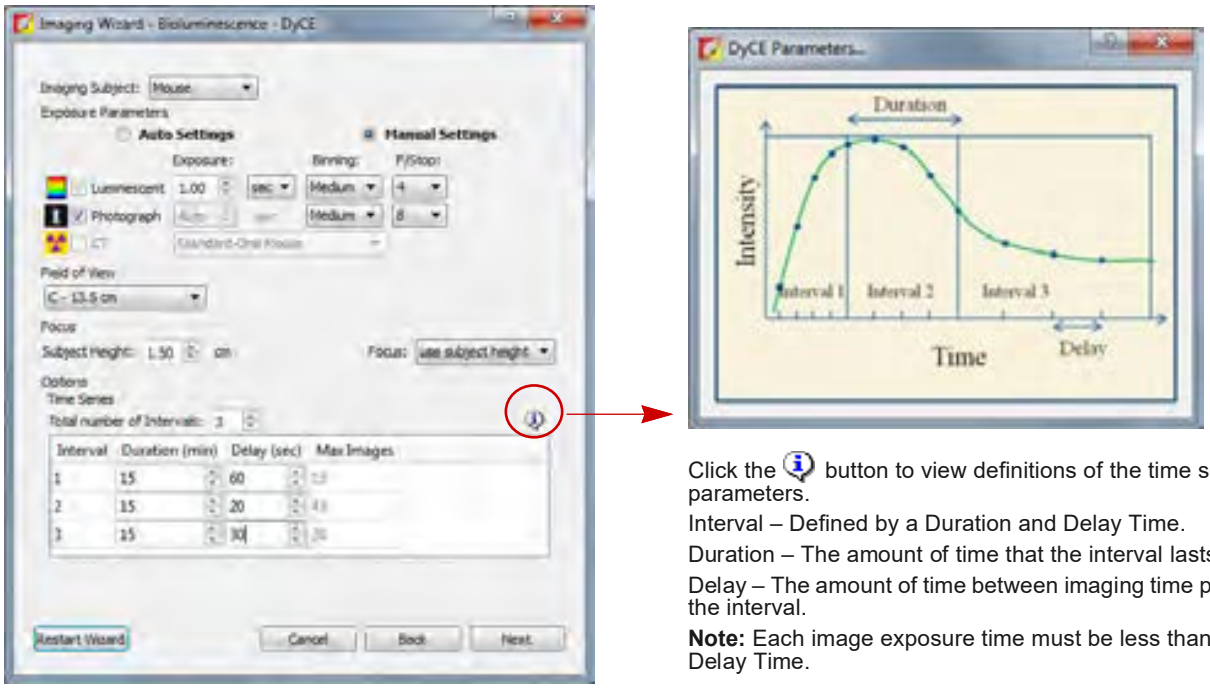
Figure 19.2 Imaging Wizard – Choose Bioluminescence and DyCE Options



If this screen does not appear when the wizard starts, click Restart Wizard (at the lower left wizard screen).

3. Select the type of imaging subject in the next screen (Figure 19.3).

Figure 19.3 Imaging Wizard – Bioluminescence DyCE



Click the ⓘ button to view definitions of the time series parameters.

Interval – Defined by a Duration and Delay Time.

Duration – The amount of time that the interval lasts.

Delay – The amount of time between imaging time points in the interval.

Note: Each image exposure time must be less than the Delay Time.

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

- 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 19.3).
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 19.4).

Figure 19.4 Sequence Setup Complete

8. Acquire the sequence following the instructions on page 111.
The image window appears when acquisition is completed (Figure 19.13 on page 360). See Table 11.4 on page 94 for more details on the Image window.

Fluorescence Imaging

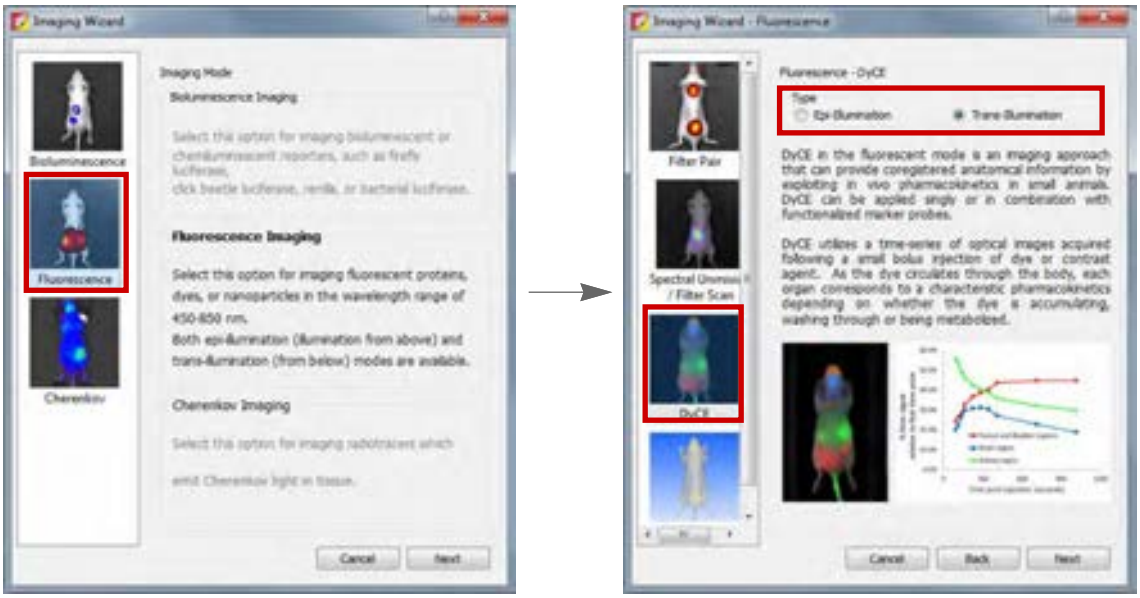
NOTE: The IVIS Spectrum CT should be initialized and the temperature locked before setting the imaging parameters. See page 73 for more details.

1. Start the Imaging Wizard. See *Start the Imaging Wizard and Setup a Sequence on page 108* for instructions.
2. Double-click the Fluorescence option (Figure 19.5).
3. Select DyCE and the type of illumination in the next screen (Figure 19.5):
   - 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** → **Tech Notes** on the menu bar):

- *Transmission Fluorescence*
- *Transmission Fluorescence – Normalized Transmission Fluorescence*

Figure 19.5 Choose the Fluorescence and DyCE Options

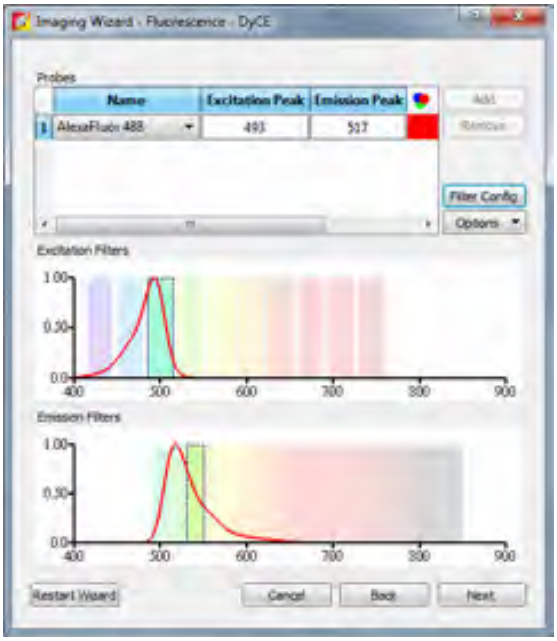


If this screen does not appear when the wizard starts, click Restart Wizard (at the lower left wizard screen).

4. Click **Next**. Select a probe from the Name drop-down list in the next screen (Figure 19.6).

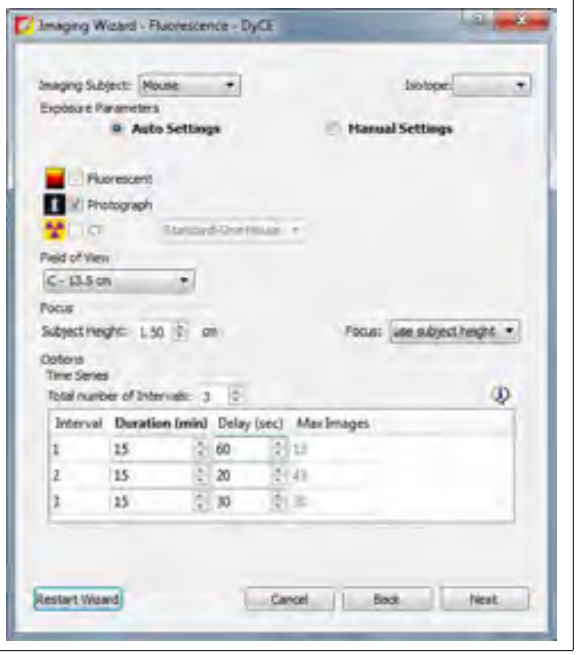
   If your fluorescent probe is not in the list, select “Input” and enter the fluorescence excitation and emission peak wavelengths. Click **Next**.

Figure 19.6 Select a Probe



5. Select the type of imaging subject in the next screen (Figure 19.7).
6. Choose the Auto Settings option.

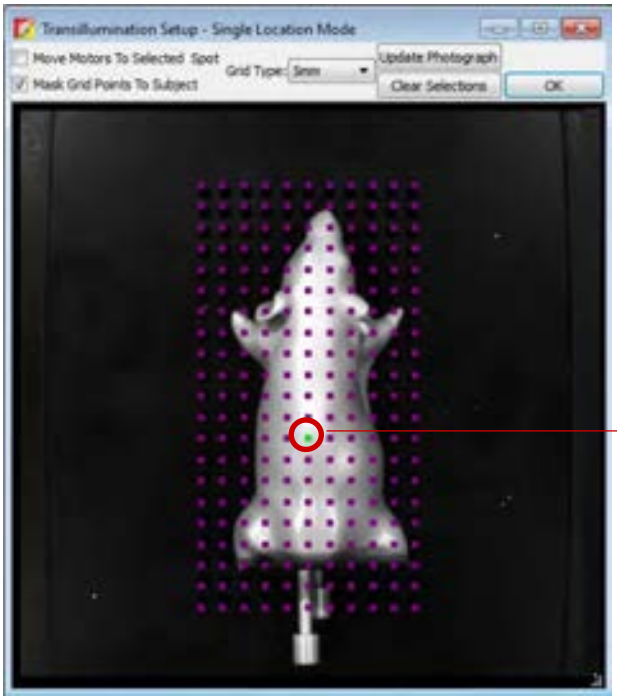
Figure 19.7 Imaging Wizard



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 19.8).

See Table 11.5 on page 103 for more details on the Transillumination Setup.

Figure 19.8 Transillumination Setup Box



8. Perform step 5 to Step 7 on page 354.
   The specified sequence appears in the sequence table (Figure 19.9).

Figure 19.9 Sequence Setup Complete

9. Acquire the sequence following the instructions on page 111.
   The image window appears when acquisition is completed (Figure 19.19 on page 365). See Table 11.4 on page 94 for more details on the image window.

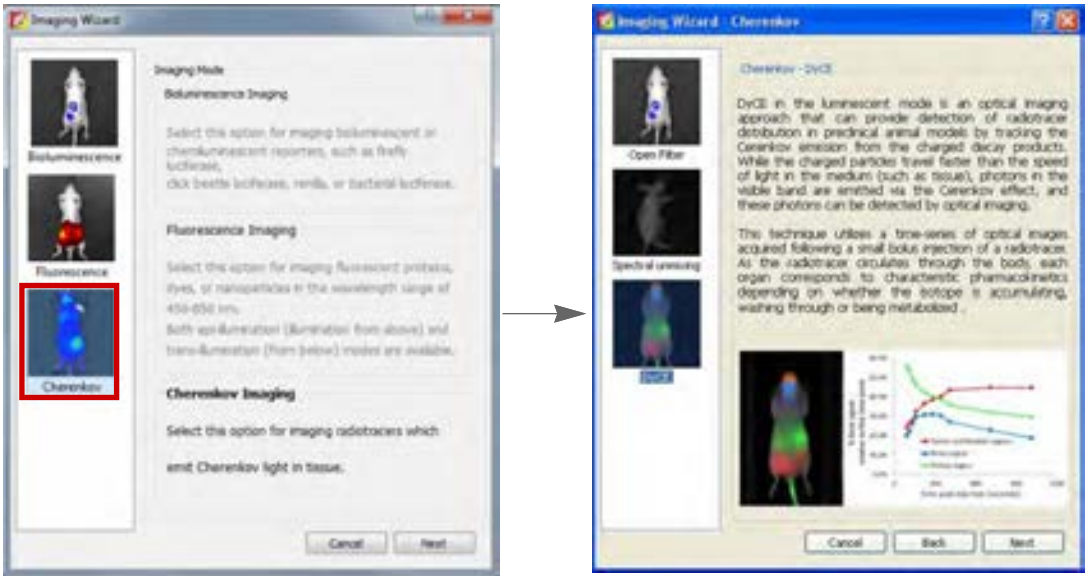
Cherenkov Imaging

NOTE: The IVIS Spectrum CT should be initialized and the temperature locked before setting the imaging parameters. See page 73 for more details.

1. Start the Imaging Wizard. See *Start the Imaging Wizard and Setup a Sequence on page 108* for instructions.
2. Double-click the Cherenkov option (Figure 19.2). Double-click the DyCE option in the next screen (Figure 19.10).

Figure 19.10 Choose Cherenkov and DyCE Options

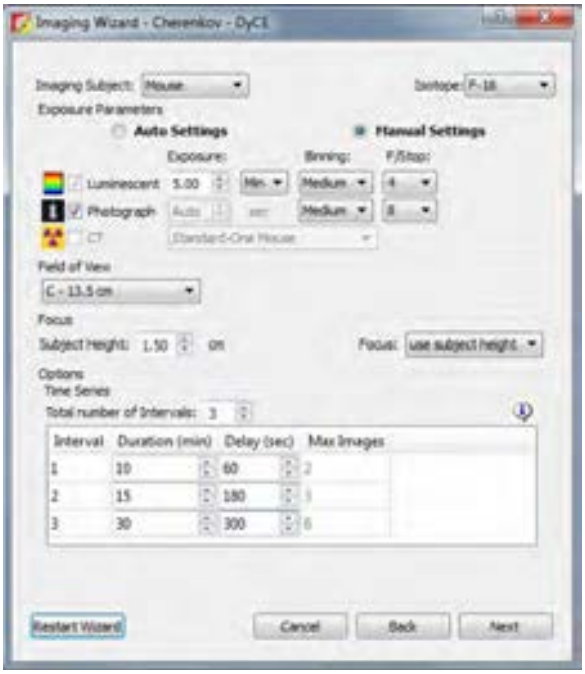
If this screen does not appear when the wizard starts, click Restart Wizard (at the lower left wizard screen).



3. Select the subject type and radio-isotope from the drop-down lists in the next screen (Figure 19.11). 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.

Figure 19.11 Imaging Wizard – Cherenkov DyCE



5. Perform step 5 to Step 7 on page 354.
   The specified sequence appears in the sequence table (Figure 19.12).

Figure 19.12 Sequence Setup Complete

6. Acquire the sequence following the instructions on page 111.
   The image window appears when acquisition is completed (Figure 19.19 on page 365). See Table 11.4 on page 94 for more details on the image window.

19.3 DyCE Analysis

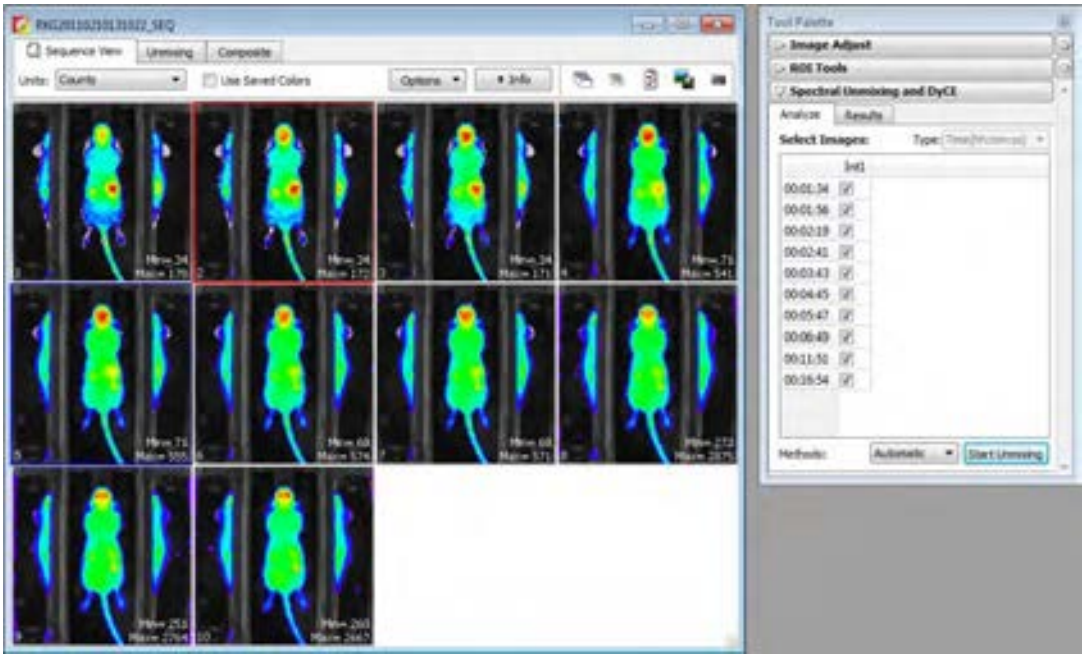
Automatic or manual DyCE analysis is available. Revvity recommends performing an automatic analysis first, followed by manual analysis to identify possible additional temporal components.

Automatic DyCE Analysis

1. Load a DyCE sequence.The DyC 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 136* for details.

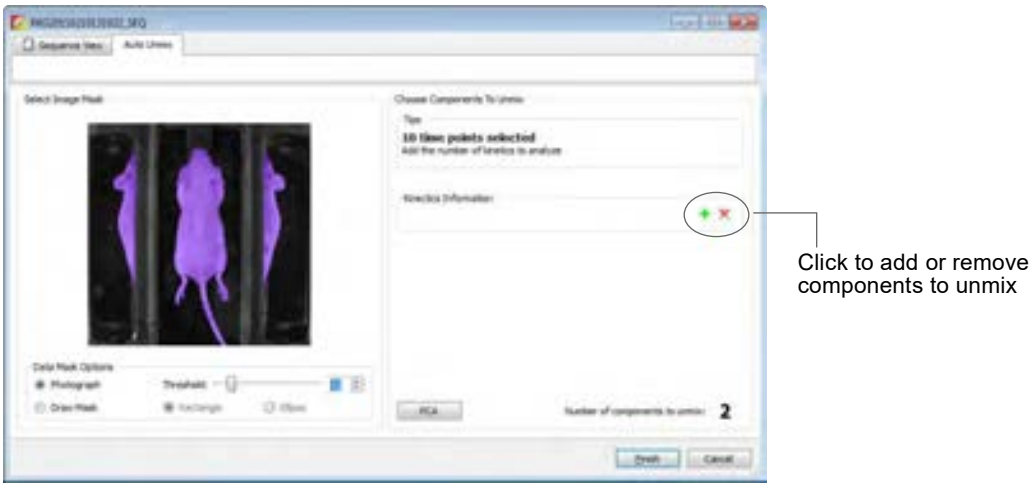
Figure 19.13 Load a DyCE Sequence



2. Click the Analyze tab in the Spectral Unmixing/DyCE tools.
3. Select **Automatic** from the Methods drop-down list and click **Start Unmixing**.

   The Auto Unmix Wizard appears and shows the purple data mask that specifies the analysis area (Figure 19.14). 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 19.14 Auto Unmix Wizard

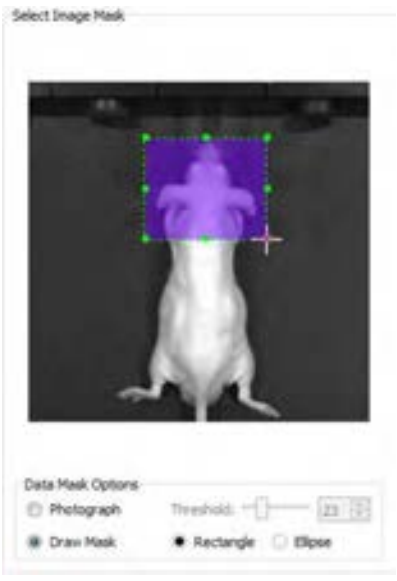


5. If you do not want to analyze the entire subject, draw a data mask on a particular area using the data mask options (Figure 19.15) See Table 19.1 for more details on the options.
   a. Select **Draw Mask** and choose the **Rectangle** or **Ellipse** option.
   b. Draw a mask over an area using the mouse. If necessary, click the mask to discard it, and redraw the mask.

Figure 19.15 Draw a Data Mask

See Table 19.1 for more details on the data mask options.

Select the Data Mask option, and the Rectangle or Ellipse option.



Draw a mask on a particular area using the mouse.

Table 19.1 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 + button to add components to unmix.

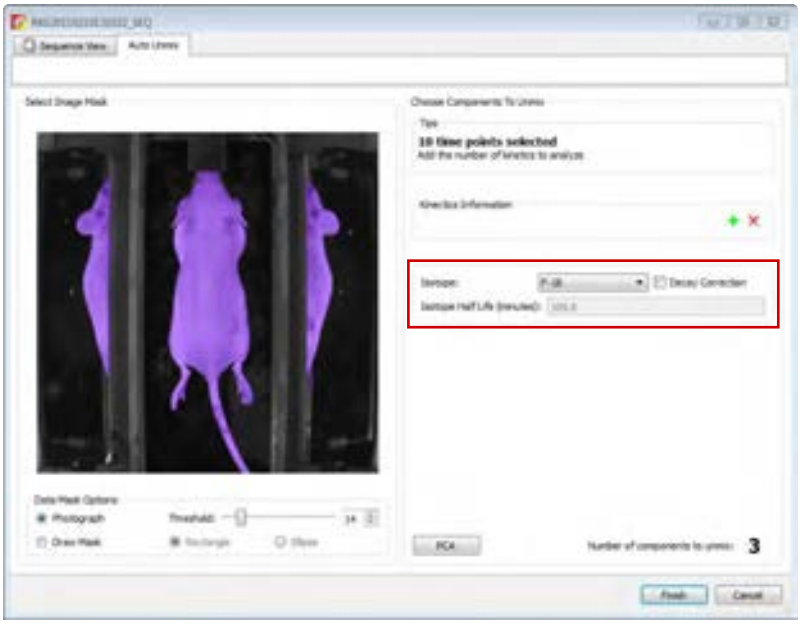
NOTE: Two or three components are recommended for the initial automatic analysis. The DyCE results obtained from the automatic analysis can be manually analyzed to identify possible additional components (see page 364 for details on manual analysis).

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 19.16).

Figure 19.16 Auto Unmix Wizard – Cherenkov Data

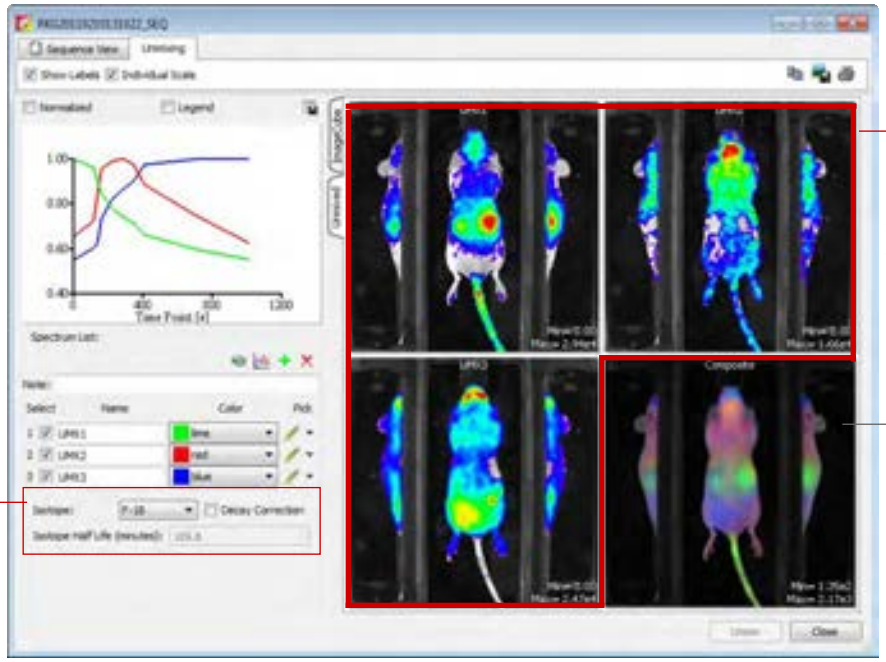


Available for Cherenkov data only

8. Click **Finish**.

   The Unmixing window shows a plot of the temporal spectra, unmixed images, and a composite of the unmixed images (Figure 19.17).

Figure 19.17 DyCE Results – Three Temporal Components



Temporal spectra show signal time course of different anatomical regions

Available for Cherenkov data only

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

.

Composite of the unmixed images

Table 19.2 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.
	Enables you to subtract one spectrum from another (see page 371).
	Adds a temporal component to the spectrum list when performing a manual analysis. See page 364 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 19.18).
   b. Click **Save**.

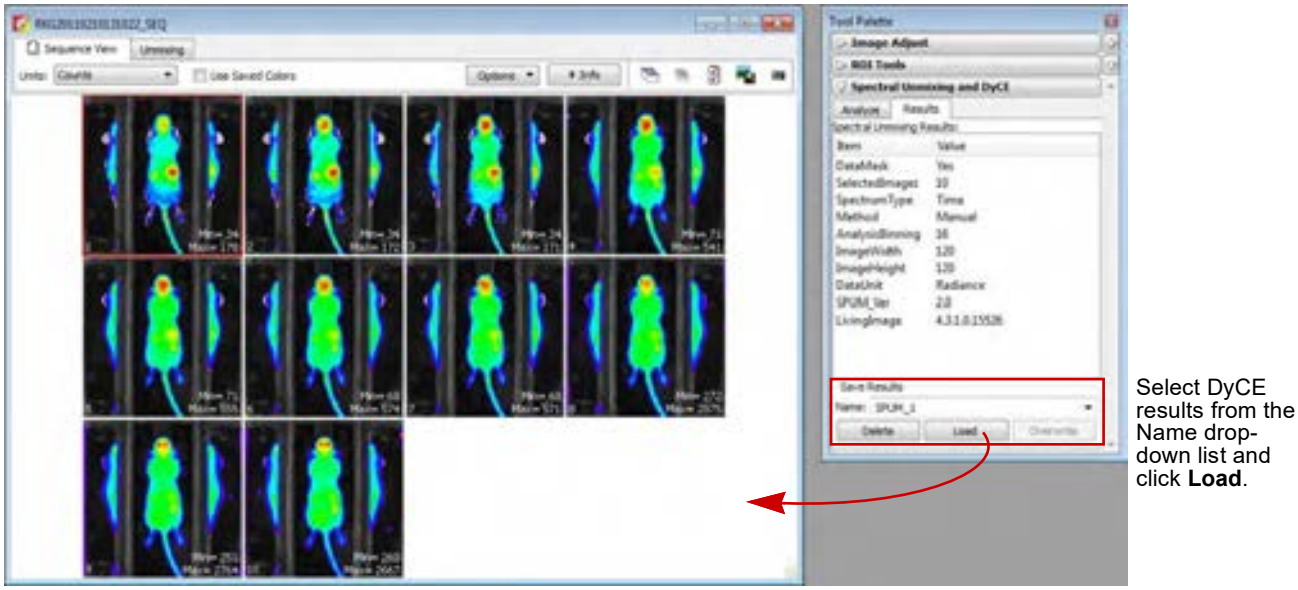
Figure 19.18 DyCE Results

Manual DyCE Analysis

1. Load a DyCE image sequence. Alternatively, load DyCE results obtained from an automatic analysis (Figure 19.19).

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

Figure 19.19 Load DyCE Results



2. Click the Image Cube tab (Figure 19.20).

   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 19.20).

   To view a particular image, remove the check mark next to Overview and move the slider or enter an image number (Figure 19.21).

Figure 19.20 Image Cube – Overview Mode

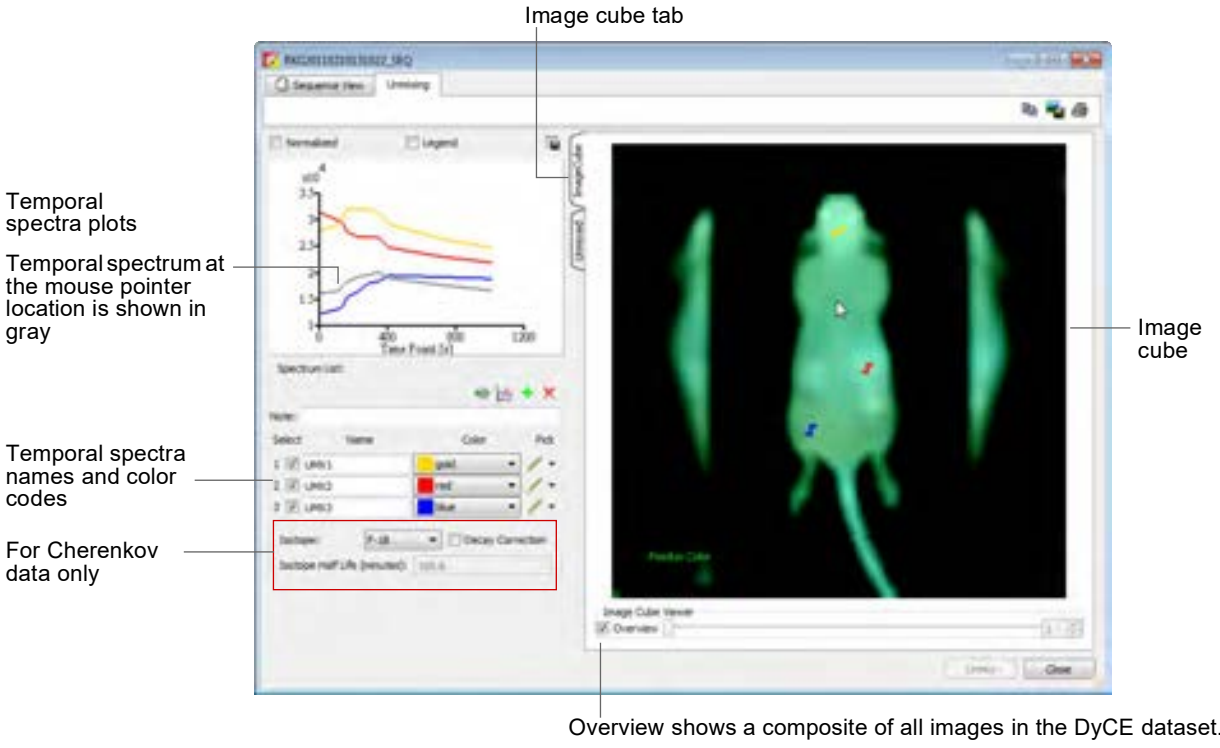
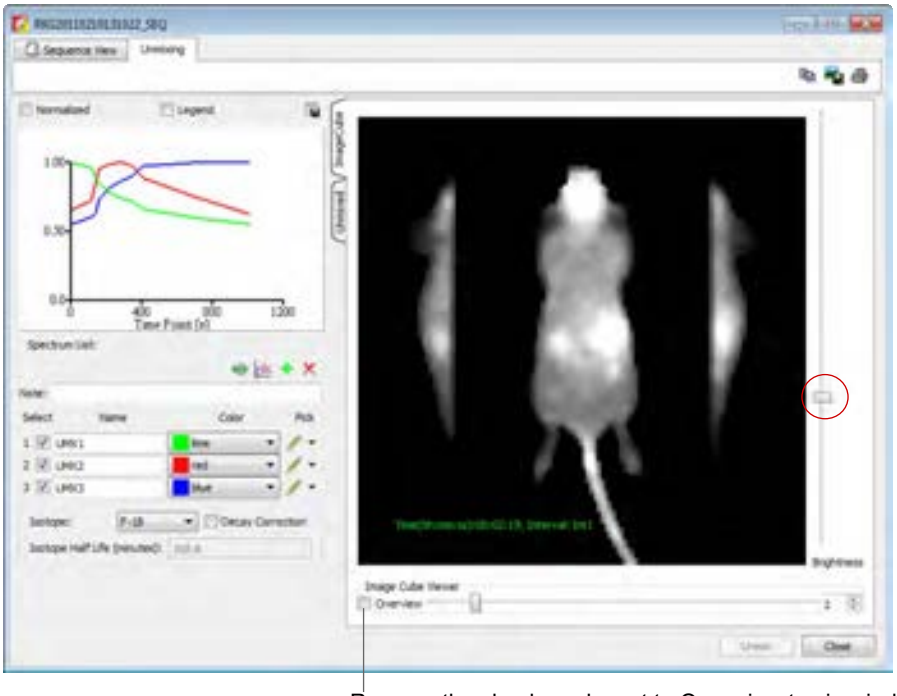


Figure 19.21 Image Cube – Single Image Mode



3. Move the mouse pointer over the image cube to see the temporal spectrum at a particular location.

   The temporal spectrum at the pointer location is updated as you move the pointer.

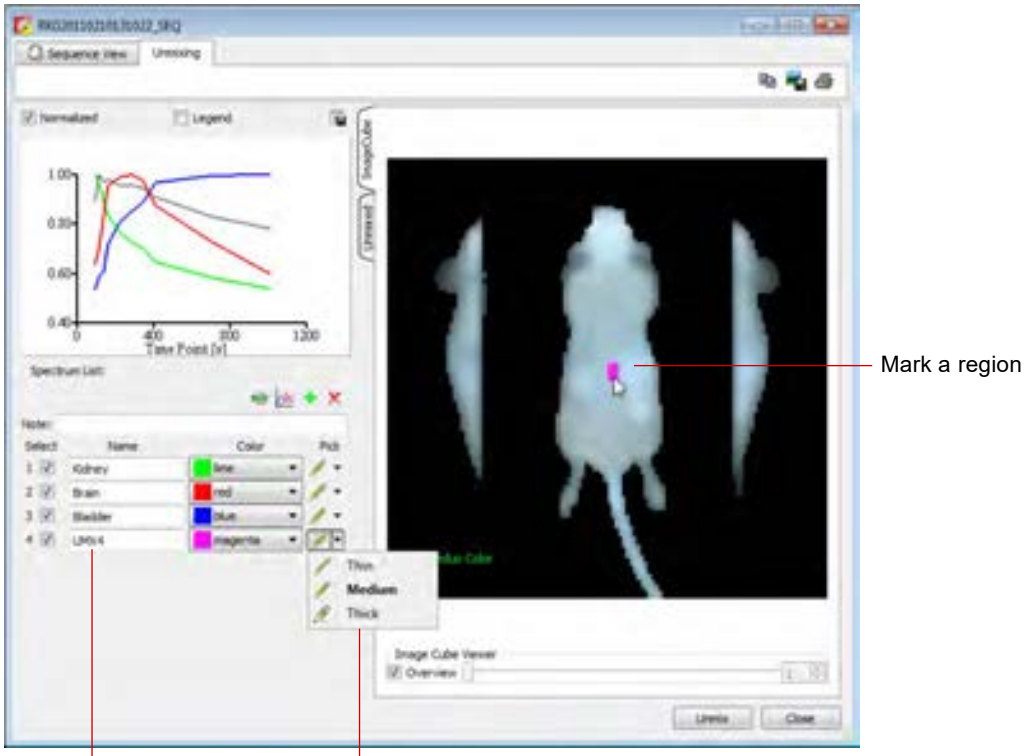
NOTE: If analyzing DyCE results, the Normalized option for the spectrum plot must be checked to see all of the temporal spectra when the mouse pointer is over the image cube.

4. To add another component to unmix:
   a. Click the + button.

      A new name appears in the spectrum list (Figure 19.22)
   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 drop-down list.
   c. If necessary, right-click the image cube to erase the mark.
5. Repeat step 4 to specify additional temporal components.

NOTE: A maximum of 10 components can be unmixed.

Figure 19.22 Mark the Area of a Temporal Component on the Image Cube



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

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

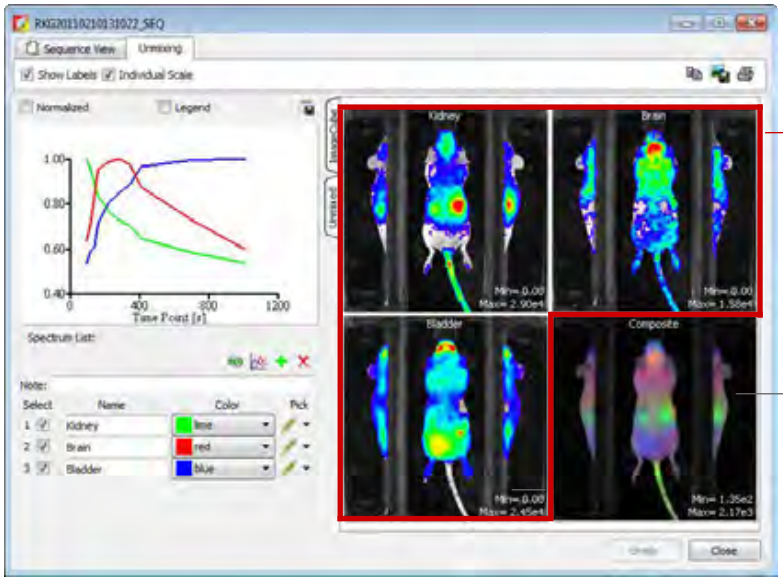
Table 19.3 Spectrum List Toolbar

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.
	Enables you to subtract one spectrum from another (see page 371).
	Adds a component to the spectrum list.
	Deletes the last spectrum in the spectrum list.

19.4 DyCE Results

The Unmixing window shows the DyCE results. The example in Figure 19.23 shows three “temporal spectra” (signal as a function of time).

Figure 19.23 DyCE Results – Three Temporal Spectra

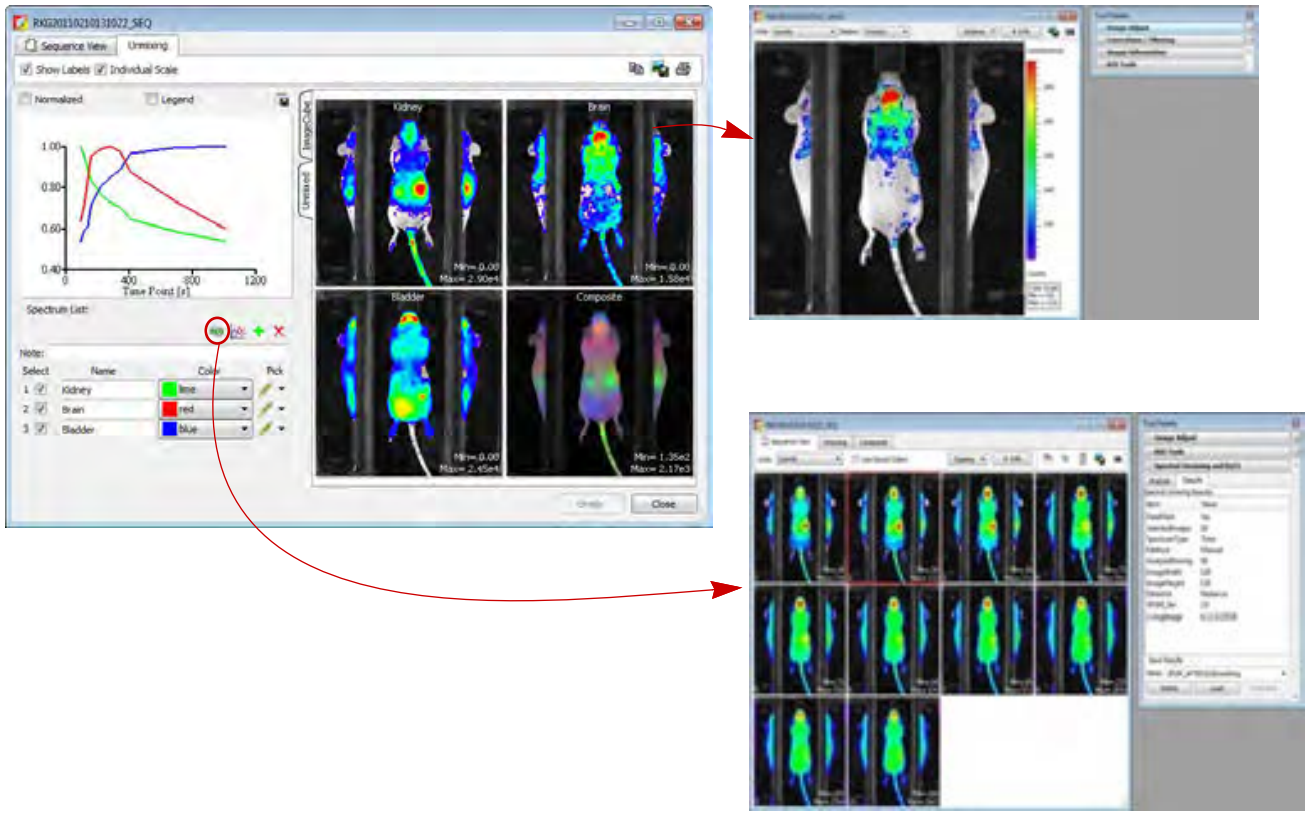


Viewing Unmixed Images

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

- Double-click an unmixed image to view it in an image window (Figure 19.24). The Tool Palette is available for viewing and analyzing the image.
- Click the button to view the unmixed images as a sequence(Figure 19.24). 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.

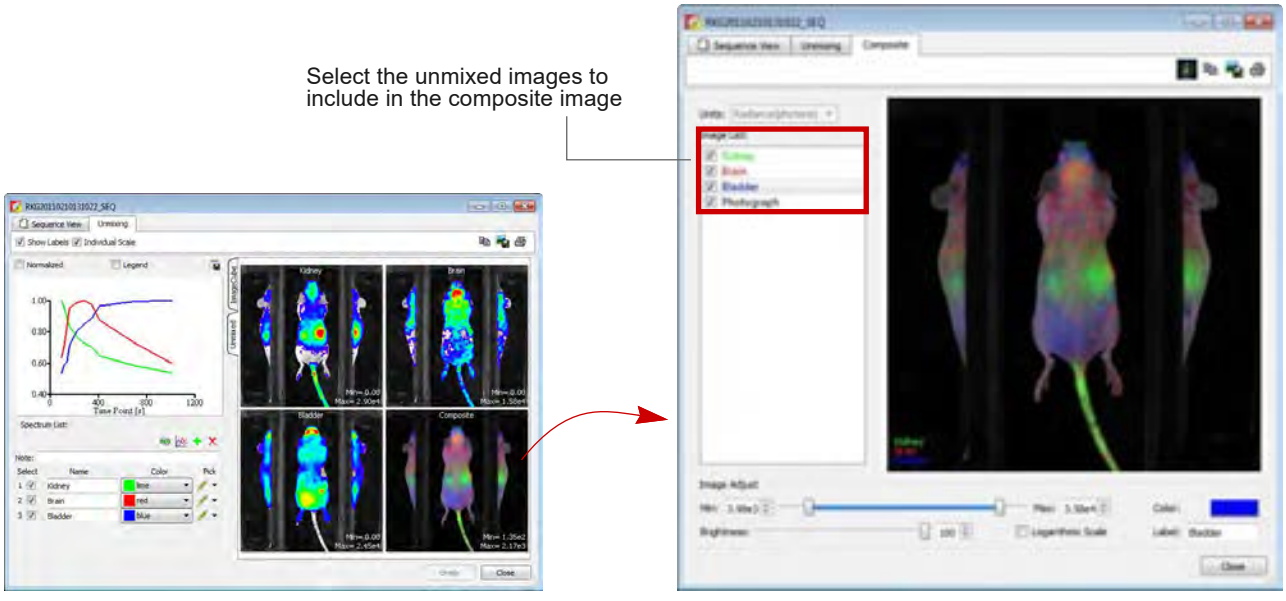
Figure 19.24 View an Unmixed image in an Image Window or View all Unmixed Images as a Sequence



Viewing the Composite Image

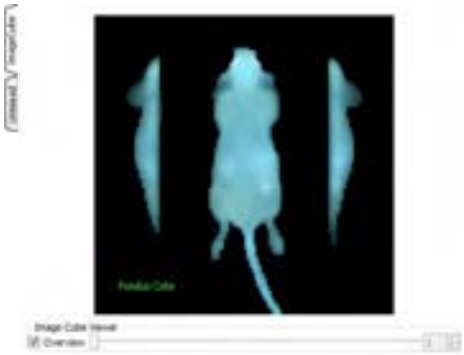
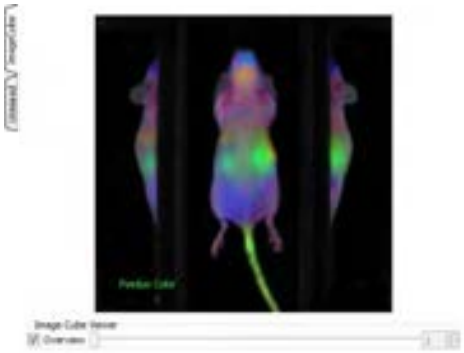
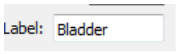
1. Double-click the composite thumbnail.
   The Composite window opens.

Figure 19.25 Open the Composite Window



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

Table 19.4 Composite Window

Item	Description
	Sends the composite image to the “top” of the image cube.  Click the Image Cube tab in the Unmixing window to view the image cube. See Figure 19.20 on page 365 for more details on the image cube.  Composite image displayed on top of the image cube.
	Copies the Composite window to the system clipboard.
	Opens a dialog box that enables you to export the composite image to a graphic file (for example, .png).
	Opens the print dialog box.
	Shows the color of the data for the highlighted image.  Click the color swatch to open the color palette which can be used choose a color for the selected image data.
	Data name for the highlighted image. Double-click the name to edit it.

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 button in the Unmixing window.
2. In the dialog box that appears, choose the spectra to subtract (Figure 19.26).

Figure 19.26 Choose Temporal Spectra to Subtract: A - x*B = C

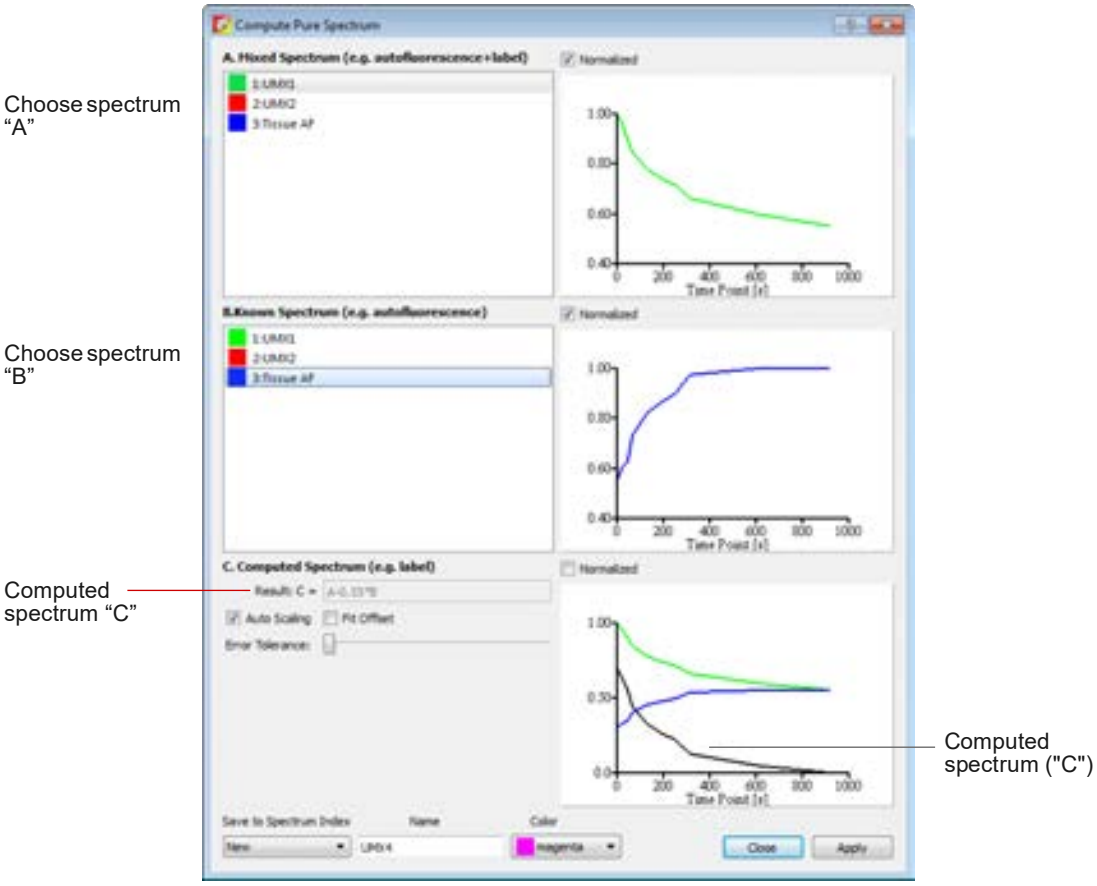


Table 19.5 Computed Spectrum

Item	Description
Normalized	Choose this option to normalize the spectra with respect to time zero.
Result: C = A - x*B	The subtraction performed by the software where "x" is a factor that ensures the residual signal is positive.
Autoscaling	Choose this option to normalize spectra signal on a scale of zero to one.

Table 19.5 Computed Spectrum (continued)

Item	Description
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.
	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**.

20 IVIS Acquisition Control Panel

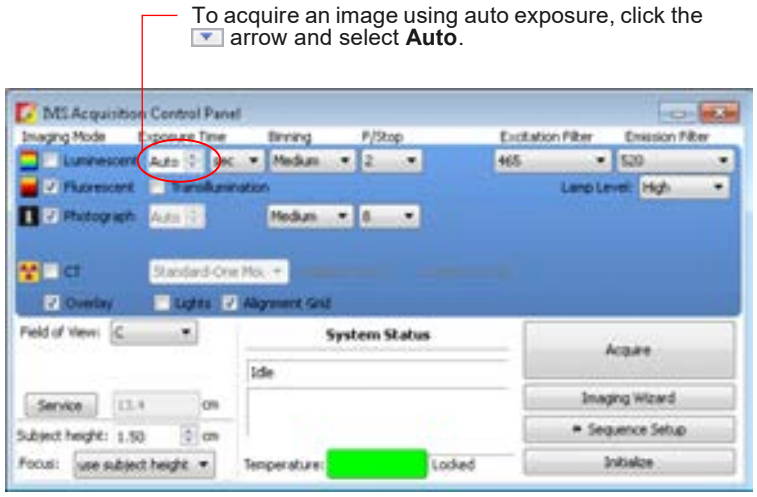
Control Panel Functions

Manually Setting the Focus on page 377

20.1 Control Panel Functions

The control panel provides the image acquisition functions (Figure 20.1).

Figure 20.1 IVIS Acquisition Control Panel – Auto Exposure Selected



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 20.1 IVIS Acquisition Control Panel

Item	Description
Luminescent	Choose this option to acquire a luminescent image.
Fluorescent	Choose this option to acquire a fluorescent image. 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.

Table 20.1 IVIS Acquisition Control Panel (continued)

Item	Description
 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 *Photo Adjustment on page 134*).
CT	Choose this option to acquire a CT image. Select a CT imaging mode from the drop-down list. See Table 11.1 on page 84 for details on the imaging modes.
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 *in vivo* images where light emission is diffuse. For more details on binning, see the reference article *Detection Sensitivity* (select **Help** → **References** on the menu bar). Recommended binning: Small (1-4) for imaging of cells or tissue sections, Medium (4-8) for *in vivo* imaging of subjects, or Large (8-16) for *in vivo* 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 *Detection Sensitivity* (select **Help** → **References** on the menu bar).

Table 20.1 IVIS Acquisition Control Panel (continued)

Item	Description
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.
Transilluminati on	Choose this option to display the transillumination setup window that enables you to select the locations for image acquisition using bottom illumination that originates beneath the stage.
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.
Overlay	If this option is chosen, the system automatically displays the overlay after acquisition is completed (for example, luminescent image on photograph).
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 20.2 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.

Table 20.1 IVIS Acquisition Control Panel (continued)

Item	Description
Mouse Imaging Shuttle	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 instrument 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 377.
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 114 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 74 for more details.
Acquire or 	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.
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 117 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.

Table 20.1 IVIS Acquisition Control Panel (continued)

Item	Description
Image Setup	Click to close the sequence table.
Initialize	Click to initialize the IVIS Spectrum CT. See page 73 for more details on initializing the system.

Table 20.2 Optical Field of View (FOV) Settings

FOV Setting	FOV (cm)
A[1]	3.9
B	6.5
C[2]	13
D	22.5

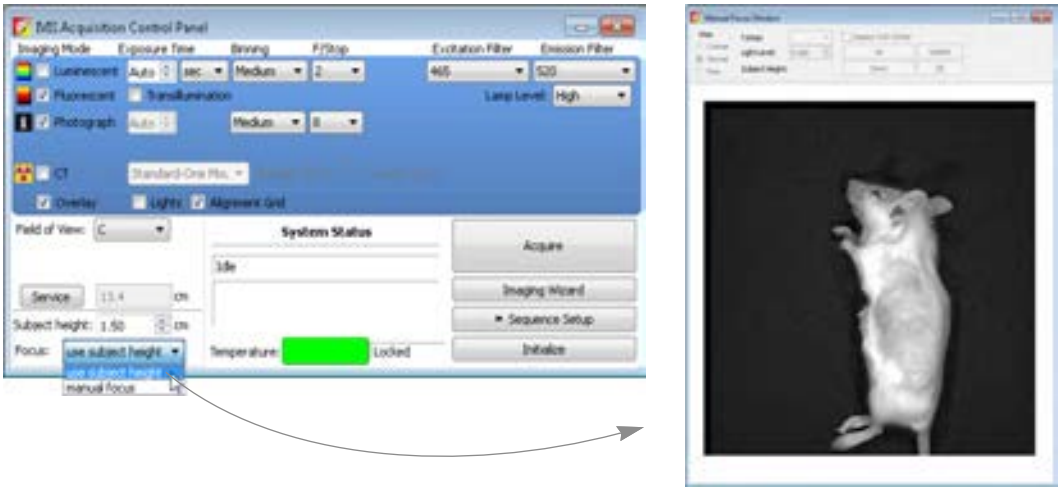
[1] Position A is not recommended for epi-fluorescent imaging because corrections for non-uniform excitation light pattern are not available.
[2] Position C is the default setting.

20.2 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 20.2 Opening the Manual Focus Window



2. To mark the center of the camera in the window, put a check mark next to Display CCD Center.
3. Select the size of the step increment that the stage moves: **Coarse**, **Normal**, or **Fine**.
4. Click **Up** or **Down** to move the stage and change the focus.
5. If necessary, select another F/stop setting from the drop-down list and adjust the light level using the arrows.
6. Click **Update** to apply the settings.

   The resulting focal plane (cm above the stage) is automatically entered in the Subject height box.
7. Click **OK** when the image is focused.

21 Care and Maintenance

21.1 Surveying the IVIS Spectrum CT for Radiation Leakage

Revvity recommends, and some local government agencies may require, that you perform an X-ray leakage safety test under the following conditions:

- Every 12 months
- When the system is installed at a new site requiring truck transport. Moving the IVIS Spectrum CT on its casters should not require a re-survey.
- After Revvity performs maintenance or service.
- After any abnormal condition that could impair any of the safety systems. For example, if the imaging chamber door becomes difficult to open or close.

Conducting the X-Ray Radiation Survey

A radiation leakage test is a complicated matter requiring sensitive and expensive equipment. Some states or localities may require special training and certification to perform the test. Contact Revvity Technical Support (page 11) for information regarding these tests or for scheduling a Revvity-trained technician to conduct the survey as part of an overall safety check.

21.2 Maintenance and Safety Checks

Daily Safety Checks

The following safety checks should be performed on a daily basis.

1. Verify that the door interlocks discussed in *Safety Interlocks* on page 29 are in good repair.
2. Verify that the key switch functions properly.
3. Verify that the following "X-ray ON" indicators are functioning properly when the X-ray modality is used. These include the:
   - Red light on the front control panel
   - Red light under the translucent dome at the top of the instrument
   - "X-ray ON" indicator  in the IVIS Acquisition Control Panel in the Living Image software
4. Verify that the amber "X-ray Armed" indicator light is working.
5. Light leak check (see page "*External light contamination*", page 390).

Weekly Safety Checks

The following safety checks should be performed on a weekly basis.

1. All of the daily safety checks.
2. Inspect the solenoid lock and the redundant interlock assembly and make sure that it has not loosened. Inspect the screws holding the two mating keys.
3. Inspect the metal knife edges on the door and the light box for damage such as bending. The knife edges keep X-rays inside the light box and prevent light from entering.

Monthly Safety Checks

The following safety checks should be performed every month.

1. All daily safety checks.
2. All weekly safety checks.
3. Activate the "Emergency Stop" switch to verify operation.
   All indication of X-ray generation should cease when the switch is pushed in.

NOTE: This will cut all power. X-rays will need to be generated to perform this test.

4. Reset the Emergency Stop switch by turning the red knob clockwise. The knob should pop out.
5. Initialize the system.
6. Re-arm the X-ray source and restart X-ray generation from the software.

Annual Safety Checks

The following safety checks should be performed every 12 months.

1. All safety checks performed on a daily, weekly, and monthly basis.
2. A full radiation survey performed by a qualified person.

21.3 Cleaning the Instrument

If necessary, wipe the exterior surface of the instrument instrument with a soft cloth. If the system requires more aggressive cleaning or sterilization, contact Revvity Technical Support (see page 11).

DANGER! DO NOT use sprays or fluids to clean the exterior or interior of the module. Do not allow fluids of any kind to penetrate the electronics cabinet under any circumstances. Sprays and liquids that come into contact with the IVIS Spectrum CT may result in damage to the system or electrocution.

WARNING! Do not use fluids or moistened towels to clean any part of the instrument where electrical or fiber optic cables make connections. Do not use fluids of any kind near the filter wheel assembly. Turn off the electrical power to the IVIS Spectrum CT by turning off the rear panel switch before engaging in cleaning operations that use fluids. The imaging chamber power switch is located on the rear of the instrument.

Approved Cleaning Solutions

The compounds shown in Table 21.1 do not damage the internal finish of the IVIS Spectrum CT imaging chamber and are suitable as cleaners, if required. Do not use any solution not included in this list. In particular, avoid strong bases, bleach, or acids that may potentially damage the unit and compromise its operation.

IMPORTANT: Do not spray cleaning solutions inside the imaging chamber.

Table 21.1 Approved cleaning solutions for the imaging chamber

Cleaning Solution	Manufacturer
Cidexplus® Solution (3.4% glutaraldehyde)	Johnson & Johnson Medical
Sporicidin® Sterilizing Solution (1.56% phenol)	Contec Inc
Clidox-s® Disinfectant	Pharmacal Research Laboratories, Inc.
70% methyl alcohol/30% deionized water solution	
70% ethyl alcohol/30% deionized water solution	

NOTE: Revvity makes no claims as to the sterility of the imaging chamber after cleaning with the solutions in Table 21.1. Please refer to the manufacturer's literature for information as to the applicability of the compound for the organism of interest. Consider dedicating an imaging system for immunodeficient animals. This will reduce the risk of cross contamination.

Cleaning the Imaging Platform

It is recommended that you use a lint-free wipe, such as Scott Pure® wipe or a Kaydry EX-L® wipe to minimize the presence of particulate matter in the imaging chamber.

After saturating a lint-free wipe, clean the internal surfaces using a gentle circular motion. Do not pour or spray the solution directly onto internal surfaces. Rinse surfaces using a wipe saturated with sterile deionized water. Do not allow puddles of water to remain on the surfaces.

Avoid cleaning the ceiling of the image chamber except when necessary. When cleaning the chamber ceiling, use extreme care to avoid damaging the lens protection window and the light diffusers in this area.

To avoid any phosphorescence from the cleaner, make sure that the surfaces are dry before using the imaging chamber.

Cleaning the Rotating Stage

It is particularly important not to allow any liquids to drip into the rotating stage assembly. The stage is mounted on a precision bearing that can become corroded over time if it is exposed to water or other cleaning liquids.

Cleaning the Lens Protection Window

Please contact Revvity Technical Support (page 11) for information about cleaning or sterilizing any of the optical components or replacing the optical filters. A clear window at the top of the imaging chamber protects the lenses and other optical components from contamination. Do not touch or allow subjects to touch this window, otherwise image quality may be impaired. Further, the glass window could be cracked or broken if struck by a hard object. The lens protection window should be cleaned periodically.

To clean the surface of the lens protection window:

1. Blow off any accumulated dust using a Dust-Off® compressed gas duster.
2. Dampen a lint-free cloth with isopropyl alcohol and gently swab the window surface to remove any smudges or streaks.
3. Repeat step 2 using a clean area of the cloth or a new cloth until the window is clean.
4. Take an image to confirm that the image quality is restored.

21.4 Changing the Emission Filters for Fluorescence Imaging

This section explains how to change one or more of the optional emission filters used for fluorescence imaging in the instrument.

NOTE: After changing a filter or a filter position, you must also change the corresponding filter label in the software. For more details, see Options on page 426.

1. Before you begin, gather the following tools:
   - 5/32" hex key
   - #2 Phillips head screw driver
   - One pair of lint free, powderless gloves

Figure 21.1 Tools required for deactivating the solenoid lock

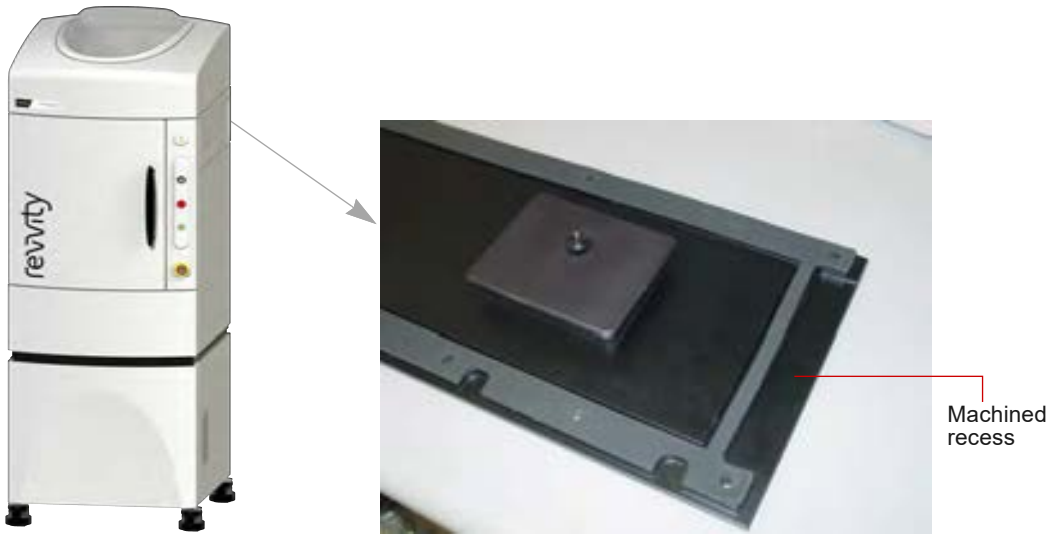
2. In the software, save any important data, then exit the program.
3. Turn off the main power at the rear of the instrument.
4. Using the 5/32" hex key, remove the eight screws that hold the filter access panel in place on the upper right side of the instrument (Figure 21.2).

   The panel is held in place by four pins until it is pulled forward.

CAUTION: Be careful not to damage the light-sealing gasket when removing the panel.

NOTE: The panel has a machined recess on the inner left-hand side (towards the front of the instrument). Noting this orientation is helpful for reinstalling the panel.

Figure 21.2 Filter wheel access panel



NOTE: Starting with step 5, wear lint-free, powderless gloves to prevent smudging the filters.

5. Notice that the 1/2" shaft on the right side of the filter wheel compartment has a carriage clamp lock with a handle. Turn the handle counter-clockwise to loosen the clamp so that it can slide freely over the shaft.
6. Grip the filter wheel by the metal rim and pull the filter wheel carriage forward until it stops. Be careful not to touch the glass filters or to dislodge the two drive belts.

   Since the carriage slides out about eight inches, only two or three of the filters will be visible. The filter wheel holds the filters numbered 1-11.
7. To remove a filter from the filter wheel:
   a. Slowly rotate the filter wheel until the filter of interest is exposed.
   b. Use a #2 Phillips head screw driver to loosen the flat head screw ONE TURN.

      The screw has a captured o-ring that retains the glass emission filter.
   c. Use two gloved fingers to remove the filter. Alternatively, gently push the filter up with a gloved finger.
8. To clean a filter, use Dust-Off® compressed gas to apply an air stream at an angle to the filter surface; otherwise dust particles could be driven into the filter coating.

CAUTION: Do not blow air onto a filter in the filter wheel as dust could be spread to other optical components

9. To install a filter, insert the filter into the filter wheel recess and tighten the O-ring screw until it bottoms out (about one turn).

NOTE: Make sure that the filter retaining screw and o-ring are fully seated to prevent interference or rubbing against components above them. The top of the filter metal ring should be nearly flush with the wheel. The filter encapsulating metal ring has one side that is thinner than the other. The thin part of the ring should face downward in the wheel recess so that the arrow that is printed on the side of the filter points up.

10. Push the filter wheel carriage back into the instrument by placing one finger on the rim of the upper filter wheel.

11. While holding the carriage in the closed position, turn the carriage-locking clamp in the clock-wise direction. Be careful not to dislodge any drive belts off of their pulleys when locking the clamp.
12. Replace the access panel by positioning it onto the four pins. The inner machined out recess should be on your left side.
13. Loosely install the eight screws, then lightly tighten the four central screws and in a crisscross pattern. Lightly tighten the four outer screws in a crisscross pattern.

NOTE: Do not over tighten the screws. The panel is designed to seal out light without large clamping force.

14. Restart the system following the procedure on on page 65.

21.5 Servicing the Chiller Unit

The coolant level in the chiller unit may diminish over time and require replenishing. It is recommended that you check the coolant level in the chiller unit every six months. This section explains how to prepare and add coolant to the chiller unit.

Required Items

The following tools and items are required, but not provided.

- Allen Wrench (hex head wrench): 3/32" measured across the flats (AF).
- Koolance® LIQ-705 Coolant Fluid.
- Paper towels or cloth for clean up.

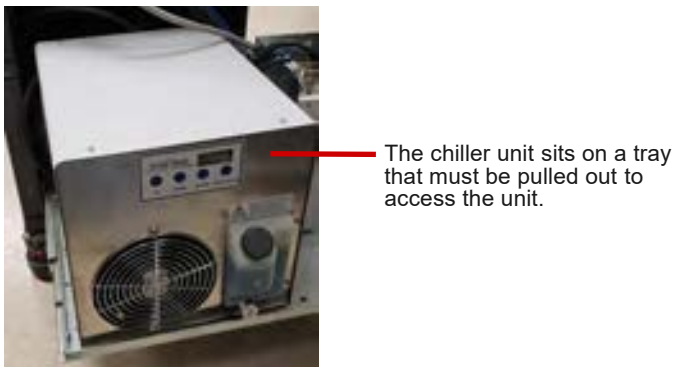
Coolant Replenishment Procedure

VOLTAGE! Do not remove any panels from the instrument until you turn off the IVIS Imaging System and disconnect the power cord. Only trained personnel should perform the following procedures. Serious risk of electric shock is possible if instructions are not followed completely.

1. Turn off electric power to the main instrument:
   a. Turn off the main power switch on the rear panel and disconnect the instrument power cord from both the wall socket and the instrument.
   b. Move any cables away from the lowest rear panel of the instrument so that the panel can be removed. (No other cables need to be removed.)
2. Remove the instrument rear panel:
   a. Use a 3/32" hex head wrench to remove the screws that secure the bottom rear (fan) panel (Figure 21.3).
   b. Carefully remove the panel and lean it against the side of the instrument.

CAUTION: Be careful not to pull too hard on the rear fan panel, otherwise its electrical connector may become disconnected. If this happens, contact Revvity Technical Support (see page 11).

Figure 21.3 Remove the lower rear panel using a hex head wrench to expose the chiller unit



3. To access the chiller:
   a. Move any cables or tubes out of the way so that the tray with the chiller can be pulled out freely.
   b. Pull out the tray with the chiller.

NOTE: The chiller unit has its own power switch located at the rear of the unit. The power switch is not visible until the unit is pulled out. Make sure that the power switch is not accidentally turned off when you slide the tray out or back into the instrument

4. Check the coolant level from the translucent reservoir. If it is below the "FILL" line:
   a. Unscrew the black cap and feed the straw from the Koolance® squirt bottle through the opening of the reservoir. Ensure that the straw is fed several inches into the reservoir (Figure 21.4).

Figure 21.4 Filling the chiller with coolant

   b. Squeeze the Koolance® squirt bottle to add the coolant until the "FILL" line is reached.

CAUTION: DO NOT OVERFILL the reservoir. Over filling the reservoir can cause the fluid to run down the chiller walls and contact the power plug.

c. Carefully remove the straw, wiping excess coolant off the straw as it is removed.
d. Wipe away any liquid from the chiller and the instrument.
e. Close the chiller cap.

5. Slide the tray back into the instrument while holding hoses and cables out of the way to prevent snagging.
6. After the chiller is mounted in position, confirm that:
   - The chiller power switch is on and no tools or other hardware remain inside the instrument.
   - The cap is secure.
   - No fluids have spilled onto the chiller or the IVIS SpectrumCT pull out shelf.
7. If any fluid is present, wipe it off before proceeding to the next step.Replace the lower rear panel using a 3/32" hex head wrench to tighten the screws (Figure 21.3 on page 385).
8. Restart the IVIS Spectrum CT:
   a. Confirm that the instrument rear panel power switch is OFF.
   b. Reconnect the power cord first to the instrument and then to the wall socket.
   c. Confirm that all other cables are properly connected.
   d. Turn on the computer.
   e. Turn the rear panel power switch ON.
   f. Start the Living Image software and initialize the system.

      The status light is red and turns green when the system is initialized.

      Wait until the camera has cooled to the demand temperature before you begin an imaging session (approximately 10 - 20 minutes).

21.6 Moving the System

Removing the Dome

It may be necessary to remove the plastic camera dome when you move the instrument though a doorway or other low overhang.

To remove the dome, wrap a flat blade screw driver with a thin cloth or paper towel and use the screw driver to gently pry the dome off at the rear of the instrument.

Moving the System

If the instrument is only repositioned a short distance within the range of the power cord and cables, the power need not be turned off. However, be careful not to accidentally turn off the power when gripping the system at the right rear.

When possible, two people should move the IVIS Spectrum CT. The instrument is tall and it is difficult to see potential obstacles when pushing the instrument from behind. Make sure that the path is clear of items that could jolt the casters and cause the instrument to tip. Move the instrument slowly so that you are always in control of its motion.

To move the system:

1. Turn off the main power switch on the rear panel of the instrument, and disconnect the power cord from the wall socket and the instrument.
2. Remove all cables from the rear panel of the instrument.

   CAUTION: Any cables that dangle from the instrument could cause the system to topple if caught by a caster.
3. Unlock the four casters.
4. Grip the instrument at a convenient location, but do not use the door handle as a grip. Be careful when gripping the right rear of the instrument so that you do not damage the connectors or fan grills.
5. After the system is positioned at the new location, lock the casters.
6. Reconnect the cables and power cord.
7. If necessary, reinstall the dome.
8. Restart the system:
   a. Confirm that the rear panel power switch is off and all cables are reconnected.
   b. Confirm that the power cord is plugged into the instrument and the wall socket.
   c. Turn on the rear panel main power switch.
   d. Turn on the computer and start the software.
   e. Initialize the system from the software.

      The status light is red.

      The status light turns green when the system is initialized. Wait until the camera has cooled to the demand temperature before you begin an imaging session (about 10 minutes).

22 Troubleshooting

22.1 Measured Temperature is not Equal to the Demand Temperature

At start up, the software programs the CCD camera to maintain the CCD temperature at -90°C.

To check the temperature of the CCD, click the Temperature square (red or green) in the Camera Control Panel.

Figure 22.1 IVIS Acquisition Control Panel

Click the temperature box to view the demand and measured temperatures of the CCD camera and stage

Temperature box color indicates:

- System not initialized
- System is initialized, but CCD camera temperature is out of range and not ready for imaging
- System is initialized and CCD camera is at or within acceptable range of the demand temperature and locked. The system is ready for imaging.

22.2 Photographic Image is Unacceptable

Default camera controls are programmed during the initialization of the software. Changes to these settings can greatly affect the photographic image. Confirm that the f/stop and binning levels are set to the default conditions.

If you have questions about the photographic settings, contact Revvity Technical Support (see page 11).

See Chapter 11, *Image Acquisition on page 83* for details.

Table 22.1 Troubleshooting photographic images

Problem	Possible Cause	Corrective Action
Image is streaked.	Subject moved during the exposure.	Check to see if the subject may have moved. If the subject is not on the sample stage, it is probably on the floor of the imaging chamber. If the sample has moved, locate and re-anesthetize it. If gas anesthesia is being used, confirm that the anesthesia is turned on and the flow rate is appropriate.
Image is blurry.	Subject height is not correctly entered in the software.	If you are using the "Sample Height" setting to determine the focus, confirm that the correct height of the sample is entered. If you are using the "Manual" setting to determine the focus, confirm that the focus has been manually adjusted. The f/stop for photographs should be set to f/8. An f/stop smaller than 8 reduces the depth of field in the photograph.
A white spot appears in the center of the field of view.	An excessively moist environment in the imaging chamber can result in condensation on the CCD window (Figure 22.2).	Remove excess moisture in the imaging chamber and allow it to thoroughly dry. Place a desiccant in the imaging chamber to decrease the drying time. Do not touch or let specimens touch the window at the top of the imaging chamber. For more details on the care of this window, see page 382. If the problem persists, contact Revvity Technical Support for assistance (see page 11).

Figure 22.2 Example photograph showing the result of condensation on the CCD window

22.3 Luminescent Image is Unacceptable

Binning, f/stop, and exposure time affect the appearance of a luminescent image. See Chapter 11, *Image Acquisition on page 83* for instructions on setting binning, exposure time, and f/stop values.

In order to function properly and reduce camera noise, the CCD camera must be cooled to the demand temperature before acquiring an image. If the camera is not cooled to the demand temperature, imaging may result in false positive signals.

Table 22.2 Troubleshooting luminescent images

Problem	Corrective Action
Camera noise	Verify that the camera is cooled to the demand temperature. 1. Check the measured temperature in the Camera Control panel to ensure that it is locked. If the camera temperature is locked, the camera temperature box is green. 2. If the camera temperature box is red, click the red box to display the actual temperature. See *Measured Temperature is not Equal to the Demand Temperature on page 388*.
Internal light contamination	Check to see that there are no extraneous light sources inside the imaging chamber. Many substances phosphoresce when exposed to light. Be especially cautious of plastics and substances that contain pigment. Be sure to prescreen any substance or material before performing actual experiments.
External light contamination	A 2" diameter High Reflectance Hemisphere (Figure 7.11 on page 61) is available from Revvity to help check for light leaks (XRH-1, Revvity part no. 118937). To check for light leaks: 3. Place the high reflectance hemisphere in the imaging chamber on the stage using a subject height of 3.5 cm at field of view D. 4. Take a luminescent image of the hemisphere using the luminescent settings: f/stop = 1, Binning = Large (high sensitivity), and exposure time = 5 minutes. If the hemisphere can be easily seen, there is a light leak. Contact Revvity Technical Support for assistance (see page 11).

WARNING! If there is a light leak in the imaging chamber based on a test using the High Reflectance Hemisphere, there is also a strong possibility that X-rays may be leaking from the chamber. For this reason, do not use the IVIS Spectrum CT in CT mode until a radiation survey has been completed.

22.4 No Optical Image is Produced

If no optical image is produced, there may be an error in the software, a problem with the physical connections to the camera, or a hardware failure. To troubleshoot the problem:

1. Close the software and restart the computer.
2. Restart the software and try to acquire an image.

If no image was produced after restarting the computer, contact Revvity Technical Support (see page 11).

22.5 No CT Image is Produced

Verify that the X-rays were "armed".

Verify that nothing has been left on the shelf that could block X-rays (for example the 5-port manifold), then perform the steps listed above in *No Optical Image is Produced*.

22.6 Hardware Problems

Most of the components in the IVIS Spectrum CT are not user-serviceable due to the integrated design of the instrument.


VOLTAGE! Do not remove any panel from the IVIS Spectrum CT, except for the filter wheel access panel or the lower rear cart panel. Removing a panel could expose the user to hazardous voltages.

The procedure for removing the filter wheel access panel is described on page 382. Removing the lower rear cart panel for access to the chiller is described on page 384.

If you suspect a problem with the instrument hardware, contact Revvity Technical Support (see page 11).

Circuit Breaker (Power Entry Module)

Under normal operation, the circuit breaker that is incorporated into the rear panel power entry module (main power switch) should not trip. Frequent tripping of this circuit breaker may indicate an underlying electrical problem. If this occurs, unplug the system and contact Revvity Technical Support (see page 11).

If the circuit breaker is occasionally tripped, perform the following steps.

1. Confirm that the rear panel switch is off, and the power cord is plugged into the instrument and the wall socket.
2. Turn on the rear panel main power switch.
3. Turn on the computer and, if necessary, start the software.
4. Initialize the system (click **Initialize** in the control panel).

   The status light is red and turns green when the system is initialized. Wait until the camera has cooled to the demand temperature before acquiring images.

Computer and Camera Connections

Never disconnect or wiggle the rear panel computer or camera connections when the IVIS Spectrum CT is powered on. If you suspect that any of the rear panel computer connections are loose, follow these steps:

1. Close the software and shut down the computer.
2. Turn off the X-ray key switch.
3. Turn off the rear panel circuit breaker switch.
4. Check the connections between the computer and the rear panel of the IVIS Spectrum CT. Tighten any loose connection.
5. Turn on the rear panel main power switch.

   The status light is red.
6. Turn on the computer and start software.
7. Initialize the system (click **Initialize** in the control panel).

   The status light is red and turns green when initialization is complete. Wait until the camera has cooled to the demand temperature before acquiring images.

Pattern Illuminator

The pattern illuminator is not user-serviceable. If you notice any changes to the alignment grid (for example, change in the brightness or position of the grid), contact Revvity Technical Support (see page 11).

Lamp Replacement

The quartz halogen lamp that provides the fluorescent light source typically operates for thousands of hours, depending on the lamp level (low or high). This light source is housed in a compartment that contains hazardous voltages and is not user accessible or serviceable.


VOLTAGE! DO NOT attempt to service the quartz halogen lamp.

If the lamp brightness is decreased or if lamp replacement is required, contact Revvity Technical Support (see page 11).

22.7 Accessing the Area Under the Imaging Platform

During normal operation, the imaging platform always returns to the loading position before the door can be opened. This helps ensure user safety and convenience when loading subjects. Occasionally you may need access to the area beneath the platform for cleaning or to retrieve a subject. Since the platform moves rapidly and produces strong forces, the area beneath the platform is not accessible unless the normal operation mode is overridden.

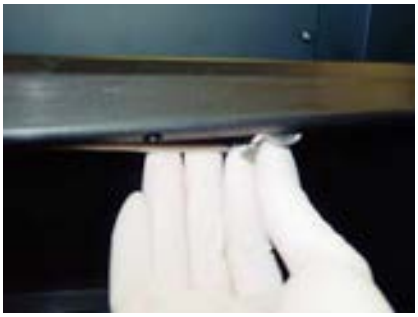
To access the space below the imaging platform:

1. Remove all items from the imaging stage that are taller than 2 cm and close the door.
2. In the IVIS Acquisition Control panel, click **Service** button.
   The platform moves to the service position and the door can be opened.
3. Place the stage lock down bar in position (Figure 22.3).
   Be careful not to disturb the platform heater cable or the gas tubing located in this area.

To return the platform to the operating position:

1. Return the lock down safety bar to the stowed position under the front edge of the imaging platform and close the door.
2. In the IVIS Acquisition Control panel, click the **Load** button.
   The imaging platform returns to the normal operating position.

Figure 22.3 Stage lock down bar



The stage lock down bar is located at the bottom front edge of the imaging platform (stowed position).

Pull the bar down from the stowed position and place it as shown above. Return the bar to its stow position before returning the imaging platform to the operating position.

22.8 Imaging Chamber Emergency Access

There is a remote possibility that the solenoid door lock could fail in the locked position which would prevent you from opening the imaging chamber door after an imaging session. If this occurs, the solenoid can be manually deactivated using a special key provided by Revvity.

CAUTION: If the solenoid door lock fails, do not use the IVIS Spectrum CT after the solenoid is mechanically deactivated. Contact Revvity Technical Support for a solenoid replacement (page 11).

Initial Steps

If you are unable to open the imaging chamber door, first try to disengage the lock by cycling through a few brief imaging sessions while pushing and pulling on the door handle. If this fails, turn the power on and off several times at the main power switch on the rear of the instrument.

If these attempts do not disarm the solenoid, follow the steps below to mechanically deactivate the solenoid.

Mechanically Deactivating the Solenoid

This procedure requires the Emergency Access Kit (part no. 133881) which includes a:

- 5/32" Hex driver (part no. 133880) (Figure 22.4)
- Solenoid unlocking key (part no. 133533)

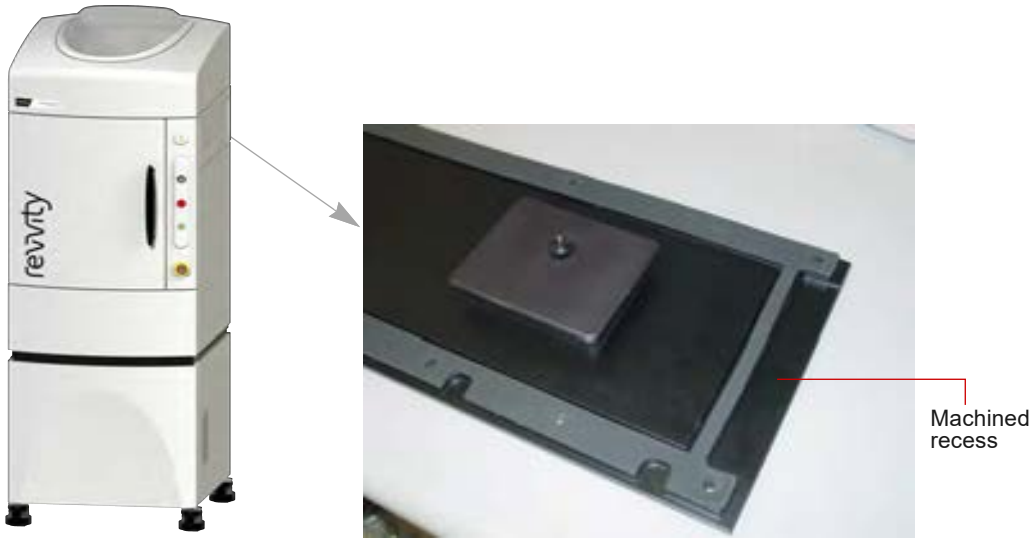
Figure 22.4 Tools required for deactivating the solenoid lock

1. Save any data within the software, then exit the program.
2. Turn off the main power at the rear of the system instrument.
3. Using the 5/32" hex driver, remove the eight screws that hold the filter access panel in place on the upper right side of the instrument (Figure 22.5).

   The panel is held in place by four pins until it is pulled forward.

   Be careful not to damage the light-sealing gasket when removing the panel.

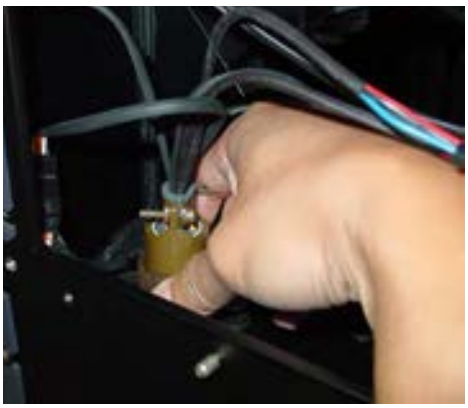
Figure 22.5 Filter wheel access panel



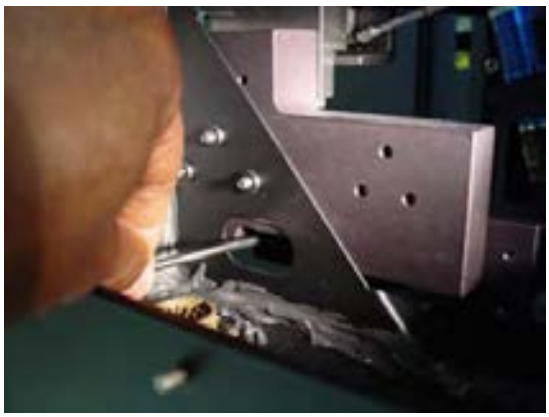
   The filter access panel has a machined recess on the inner left-hand side (towards the front of the instrument). Noting this orientation is helpful when you reinstall the panel (Figure 22.5).
4. Disconnect the electrical connector towards the front of the instrument by rotating the knurled ring counter clockwise (Figure 22.6).
5. After the electrical connector is removed, locate the triangle-shaped rotating key receptacle.

   One vertex of the triangle has a small dot that points to LOCK (Figure 22.7).

Figure 22.6 Disconnecting the electrical connector and inserting the unlocking key into the key receptacle



Disconnect the electrical connector to access the key receptacle.



Insert the unlocking key through the aperture in the sheet metal.

Figure 22.7 Key receptacle and solenoid unlocking key

Dot on the key receptacle

Key receptacle in the LOCK position

Unlocking key inserted into the key receptacle

6. Insert the unlocking key into the triangular receptacle and turn the key counter-clockwise to so that the dot on the key receptacle points to the UNLOCK position (Figure 22.7).
   The solenoid will mechanically disengage.
7. Open the door and remove the subject(s) from the imaging chamber.
8. Using the key, return the solenoid to the LOCK position by fully turning the key in the clockwise direction.
9. Reinstall the electrical connector. Make sure that it is fully engaged.
10. Replace the filter wheel access panel.

CAUTION: Do not use the IVIS Spectrum CT after mechanically deactivating the solenoid lock. Contact Revvity Technical Support for a solenoid replacement (page 11).

23 Contact and Ordering Information

23.1 Revvity Contact Information

Technical Support

Telephone 800-762-4000 (US Toll Free)
+1 203-925-4602 (Worldwide)

E-mail support@revvity.com

Fax +1.203.944-4904

23.2 Ordering Information

Table 23.1 Ordering information

Item	Part No.
Kit, Power Connections, Type B, 120 V, North America and Japan	CLS153478
Kit, Power Connections, Type F, 250 V, Europe	CLS153479
Kit, Power Connections, Type F, 250 V, S. Korea	CLS153484
Black Paper, Swarthmore, Artagain, 9x12" 24 Sheet pad	117837
Kit, Foam Animal Beds, Single Mouse, 3mm holes (set of 10)	134371
Kit, Foam Animal Beds and Light Baffles, Dual Mouse Manifold (set of 10)	134372
Kit, Animal Bed Substrate (2mm holes) with Lamination Instructions	134377
Light Baffle, Foam, Dual Mouse Manifold	134151
Kit, Tools for Emergency Access to IVIS Spectrum CT Imaging Chamber	133881
Low Fluorescence Imaging Mats, 11.6" square (set of 10)	119000
Mouse Inhalation Nose Cones for 5 Port Manifold (set of 10)	119001
Light Baffle, 0.5” High (single)	117151
Light Baffle, 0.7” High (single)	117152
XRS-10 Rubber Stoppers for 5 Mouse Manifold (set of 10)	119006
XNC-LP Rat Nose Cones, 5 Port Manifold (For FOV B, C, and D only)	119025

Table 23.1 Ordering information (continued)

Item	Part No.
Optical Lens Cleaner	123495
Wipe, Lint Free (50/package)	126291
Phantom Mouse, XFM-2 CT-FLIT	133803
XRM-5, Phantom, Mouse, XRM-5X-Ray (simulates fat, inner bone, cortical bone, solid water)	133793
Assembly, Phantom, Mouse, XPM-2 CT-DLIT	133805
XLS-4 Calibrated Light Source	118897
XRH-1 High Reflectance Hemisphere with Container	118937
RAS-4 Rodent Anesthesia System	CLS146737
XPM-2 Bioluminescent Phantom Mouse	118993
IWB-48 Workbench (48" wide)	CLS149577
IWB-60 Workbench (60" wide)	CLS149578
Kit, Mouse Imaging Shuttle, IVIS Spectrum CT	134366
Kit, Animal Bed Lamination Fixture and Instructions	134375
Kit, Rotation Stage Alignment (measures shelf, pitch and yaw alignment) and Instructions	CLS135992
Kit, High Throughput	CLS148874

23.3 US Radiation Authorities

This contact information is provided for your convenience but it may not be the most current.

ALABAMA
Office of Radiation Control
State Dept. of Public Health
201 Monroe St/PO Box 303017
Montgomery, AL 36130-3017
334-206-5391

ALASKA
Radiologic Health Program
Department of Health and State Services
5455 Dr. Martin Luther King, Jr. Ave
Anchorage, AK 99507-1270
907-334-2107

ARIZONA
Arizona Radiation Regulatory Agency
4814 South 40th St.
Phoenix, AZ 85040-2940
602-255-4845 Ext. 222

ARKANSAS
Arkansas Department of Health
Radiation Control Section
4815 W. Markham St., Slot H-30
Little Rock, AR 72205-3867
Phone: 501-661-2301

CALIFORNIA
California Department of Public Health
Division of Radiation Safety and Environmental Management
PO Box 997377, MS-7610
1500 Capitol Ave
Sacramento, CA 95899-7377
916- 440-7899

COLORADO
X-Ray and Mammography Compliance
Registration and Certification
4300 Cherry Creek Drive South
Denver, CO 80246-1530
303-692-3446

CONNECTICUT
Dept. of Public Health
Division of Radiation
79 Elm Street
Hartford, CT 06106-5127
860-424-3029

DELAWARE
Office of Radiation Control
Division of Public Health
417 Federal Street
Dover, DE 19903
302-744-4546

DISTRICT OF COLUMBIA
Department of Health
HRLA/Radiation Protection Div.
717 14th Street NW, Room 639
Washington, DC 20005
202-724-8800

FLORIDA
Dept. of Health/Bureau of Radiation Control
4052 Bald Cypress Way, Bin C21
Tallahassee, FL 32399-1741
850-245-4266
Radiologic Technology Program
4052 Bald Cypress Way
Tallahassee, FL 32399-1741
850-245-4540

GEORGIA
Department of Community Health
2 Martin Luther King Jr. Drive SE, East Tower
Atlanta, GA 30334
404-657-5700

HAWAII
Indoor Radiological Health Branch
Department of Health
591 Ala Moana Blvd.
Honolulu, HI 96813
808-586-4700

IDAHO
Dept. of Health and Welfare
Idaho Bureau of Laboratories
2220 Old Penitentiary Rd.
Boise, ID 83712-8299
208-334-2235

ILLINOIS
IL Emergency Management Agency
Division of Nuclear & Radiation Safety
2200 South Dirksen Parkway
Springfield, IL 62703
217-782-2700
Registration and Certification Section
217-524-3504 / Fax 217-782-1328

INDIANA
State Department of Health
Epidemiology Resource Center/
Indoor and Radiological Health
2525 North Shadeland Avenue, E3
Indianapolis, IN 46219
317-351-7190, Ext. 257

IOWA
Bureau of Radiological Health
Lucas State Office Bldg., 5th Fl
321 E. 12th St.
Des Moines, IA 50309
515-281-3478

KANSAS
Radiation Section
1000 SW Jackson St, Suite 310
Topeka, KS 66612-1366
785-296-1565

KENTUCKY
Radiation Health Branch
Radiation Producing Machines Program
Cabinet for Health and Family Services
275 East Main Street, HS1C-A
Frankfort, KY 40621
502-564-3700

LOUISIANA
Department of Environmental Quality
Emergency and Radiologic Services Division
PO Box 4312
Baton Rouge, LA 70821
225-219-3041

MAINE
Division of Environmental Health
Radiation Control Program
286 Water Street, 4th Floor
Augusta, ME 04333
Telephone: 207-287-5677

MARYLAND
Radiologic Health Program
Maryland Dept of the Environment
1800 Washington Blvd., Suite 750
Baltimore, MD 21230-1724
410-537-3300

MASSACHUSETTS
Radiation Control Program
Department of Public Health
Schrafft Center, Suite 1M2A
529 Main Street
Charlestown, MA 02129
617-242-3035

MICHIGAN
MIOSHA/Radiation Safety Section
Michigan Department of Labor and Economic Opportunity
525 West Allegan Street
PO Box 30643
Lansing, MI 48933
517-284-7820

MINNESOTA
Section of Indoor Environments and Radiation
Division of Environmental Health
Department of Health
625 Robert Street N.
P.O. Box 64975
St. Paul, MN 55164-0975
651-201-4602

MISSISSIPPI
Division of Radiological Health
State Department of Health
3150 Lawson Street
Jackson, MS 39215-1700
601-987-6893

MISSOURI
Radiation Control Program
Department of Health and Senior Services
912 Wildwood
PO Box 570
Jefferson City, MO 65102
573-751-6400

MONTANA
Radiological Health Program
MT Dept. of Public Health and Human Services
Licensure Bureau
P. O. Box 202953
Helena, MT 59620-2953
406-444-2868

NEBRASKA
Division of Public Health/Office of Radiological Health
Dept. of Health and Human Services
P. O. Box 95026
Lincoln, NE 68509
402-471-2168

NEVADA
Radiation Control Programs
Nevada Division of Public and Behavioral Health
675 Fairview Drive, Suite 218
Carson City, NV 89701
775-687-7550

NEW HAMPSHIRE
Radiological Health Section
Division of Public Health Services
Dept. of Health and Human Services
29 Hazen Drive
Concord, NH 03301
603-271-4585

NEW JERSEY
Bureau of X-ray Compliance/Radiation Protection Programs
Dept. of Environmental Protection
25 Arctic Parkway
Ewing, NJ 08638
609-984-5463

NEW MEXICO
Radiation Control Bureau
New Mexico Environment Department
P.O.Box 5469
Santa Fe, NM 87502-5469
505-476-8600

NEW YORK
Center for Environmental Health
New York State Department of Health
Empire State Plaza-Corning Tower
Albany, NY 12237
518-402-7550

NORTH CAROLINA
North Carolina Radiation Protection Section
Department of Health and Human Services
1645 Mail Service Center
Raleigh, NC 27699
919-814-2250

NORTH DAKOTA
Radiation Control
Department of Environmental Quality
4201 Normandy Street
Bismarck, ND 58503-1324
701-328-5166

OHIO
Bureau of Radiation Protection
Ohio Dept. of Health
246 North High Street
Columbus, OH 43215
614-466-1390

OKLAHOMA
Consumer Protection Services
State Department of Health
1000 Northeast Tenth Street
Oklahoma City, OK 73117-1299
405-271-5243

OREGON
Radiation Protection Services
Oregon Health Services,
Department of Human Services
800 NE Oregon Street, Suite 640
Portland, OR 97232-2162
971-673-0499

PENNSYLVANIA
Bureau of Radiation Protection
Department of Environmental Protection
Rachel Carson State Office Bldg.
400 Market Street
Harrisburg, PA 17101
717-787-2480

PUERTO RICO
Radiological Health Division
Department of Health
P. O. Box 70184
San Juan, PR 00936-8184
787-274-7802

RHODE ISLAND
Radiation Control Program
Department of Health
3 Capitol Hill, Room 206
Providence, RI 02908
401-222-5960

SOUTH CAROLINA
Bureau of Radiological Health
Dept. of Health and Environmental Control
2600 Bull Street
Columbia, SC 29201
803-898-3432

SOUTH DAKOTA
Office of Health Care Facilities
Licensure and Certification
615 East 4th St.
Pierre, SD 57501-1700
605-773-3356

TENNESSEE
Division of Radiological Health
Department of Environment and Conservation
William R. Snodgrass Tennessee Tower
312 Rosa L. Parks Avenue, 15th Floor
Nashville, TN 37243
615-532-0364

TEXAS
Bureau of Radiation Control
Dept of State Health Services
PO Box 149347
Austin, TX 78714-9347
512-834-6688

UTAH
Division of Waste Management and Radiation Control
Department of Environmental Quality
Multi Agency State Office Building
195 North 1950 West, DEQ Second Floor
Salt Lake City, UT 84116
801-536-0200

VERMONT
Radiological Health Program
Department of Health
108 Cherry Street
Burlington, VT 05402
802-863-7220

VIRGINIA
Division of Radiological Health
Department of Health
Madison Bldg.
109 Governor Street
Richmond, VA 23218
804-864-8150

WASHINGTON
Office of Radiation Protection
Department of Health
PO Box 47827
Olympia, WA 98504-7827
360-236-3300

WEST VIRGINIA

Radiological Health Program
WV Department of Health and Human Services
Office of Environmental Health Services
350 Capitol Street, Room 313
Charleston, WV 25301-3713
304-558-2981

WISCONSIN

Radiation Protection Section
Dept. of Health Services
PO Box 2659
Madison, WI 53701-2659
608-267-4797

WYOMING

Office of Healthcare Licensing
6101 Yellowstone Road, Suite #400
Cheyenne, WY 82002
(307) 777-7124

23.4 Canadian Radiation Authorities

This contact information is provided for your convenience but it may not be the most current.

Alberta
Radiation Health and Safety Specialist
Alberta Human Resources and Employment
10808-99th Ave, 8th Floor
Edmonton, AB T5K 0G5
Tel: (780) 415-0612
Fax: (780) 422-0014

British Columbia
Radiation Protection Services
BC Centre for Disease Control
655 - 12th Avenue West
Vancouver BC V5Z 4R4
Tel: (604) 660-6630
Fax: (604) 660-6628

Department of National Defence
Director General Nuclear Safety
Department of National Defence
Rm 1702 Standard Life Building
280 Slater Street
Ottawa, ON K1A 0K2
Tel: (613) 995-8253
Fax: (613) 992-5537

Federal Health Canada
H.P. (Harri) Maharaj
Physicist and Head,
Nonmedical X-Rays, CCRPB
Health Canada
775 Brookfield Rd
Postal Locator 6301A
Ottawa, ON K1A 1C1
Tel: (613) 954-0318
Fax: (613) 941-1734
Email: H_P_Maharaj@hc-sc.gc.ca

Manitoba
Head of Radiation Protection
Medical Physics Division
Cancer Care Manitoba
675 McDermot Ave
Winnipeg, MB R3E 0V9
Tel: (204) 787-2213
Fax: (204) 775-1684

New Brunswick
Health Protection Branch
Department of Health
PO Box 5100
Fredericton, NB E3B 5G8
Tel: (506) 453-2424
Fax: (506) 453-8702

Newfoundland and Labrador
Department of Government Services
West Block, 4th Fl, Confederation Bldg
PO Box 8700
St. John's, NL A1B 4J6
Tel: (709) 729-0218
Fax: (709) 729-3445

Northwest Territories
Workers' Safety and Compensation Commission
Northwest Territories and Nunavut
PO Box 8888
Yellowknife, NT X1A 2R3
Tel: (867) 669-4407 or 1-800-661-0792
Fax: (867) 873-0262

Nova Scotia
Occupational Health and Safety Division
Department of Labour and Workforce Development
PO Box 697.
Halifax, NS B3J 2T8
Tel: (902) 424-7115
Fax: (902)424-5640

Ontario
Radiation Protection Service
Occupational Health and Safety
Ontario Ministry of Labour
81A Resources Road
Weston, ON M9P 3T1
Tel: (416) 235-5765
Fax: (416) 235-5926

Prince Edward Island
Environmental Health
Dept. of Health and Social Services
16 Garfield Street
PO Box 2000 Charlottetown,
PE C1A 2N8
Tel: (902) 368-4792
Fax: (902 368-6468

Quebec
CSST Quebec
Direction de la prevention-Inspection
Commission de la sante et de la securite du travail
524, rue Bourdages, local 250
CP 1200, succursale Terminus
Quebec, QC, G1K 7E2
Contact: Mrs. Candide Fournier
Tél: (418) 266-4699 ext. 2005

Saskatchewan
Radiation Safety Unit
Department of Labour
400 - 1870 Albert St
Regina, SK S4P 4W1
Tel: (306) 787-4538
Fax: (306) 787-2208

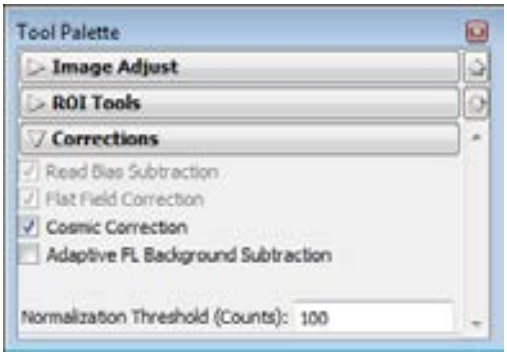
Yukon
Workers' Compensation Health and Safety
401 Strickland St.
Whitehorse, YT Y1A 5N8
Tel: (867) 667-5376
Fax: (867) 393-6279

Appendix A Optical Image Data Corrections

The Corrections tools apply corrections or subtract adaptive fluorescence background from optical image data.

TIP: See these technical notes for helpful information (select **Help → Tech Notes** on the menu bar).
- *Luminescent Background Sources and Corrections.*
- *Fluorescent Imaging* for more about fluorescent background.
- Adaptive Fluorescence Background Subtraction.

Figure A.1 Tool Palette – Corrections Tools



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 A.1 Optical Image Data Corrections

Item	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 *Luminescent Background Sources and Corrections* (select **Help → Tech Notes** on the menu bar).

Table A.1 Optical Image Data Corrections (continued)

Item	Description
Flat Field Correction	Select this check box to apply flat field correction to the image data. **Note:** In photons mode, flat field correction is a mandatory default. In counts mode, the check box can be cleared.
Cosmic Correction	Select this check box to correct image data for cosmic rays or other ionizing radiation that interact with the CCD. See the tech note *Image Display and Measurement* for more about cosmic correction (select **Help** → **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 *Adaptive Fluorescence Background Subtraction* (select **Help** → **Tech Notes** on the menu bar).
Normalization Threshold (Counts)	The minimum number of counts required to perform normalization.

Appendix B Quantification Database

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.

B.1 Preparing and Imaging Samples

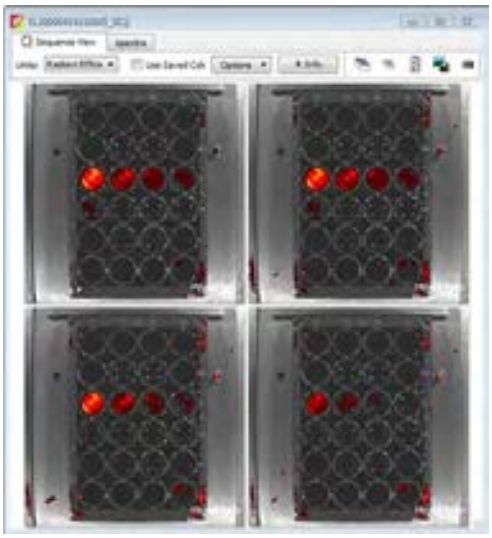
1. Prepare a well plate (4 x 6, 6 x 4, 8 x 12, or 12 x 8 well format) that contains a dilution series of luminescent cells or fluorescent dye at four or more concentrations.
2. Include at least four background wells that contain diluent only.
3. Place the well plate on the IVIS stage, positioning it so that it is centered and square in the field of view.

NOTE: All of the wells must be within view in the image. For wells containing fluorophores, FOV D is recommended to reduce shadows from well walls and ensure more uniform excitation of the wells.

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 B.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.

Figure B.1 Well Plate Data



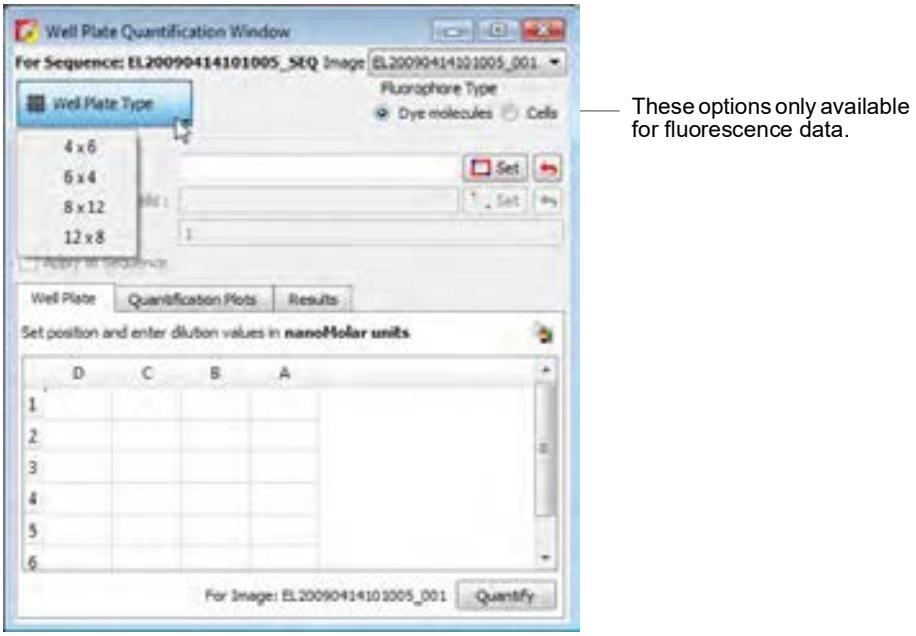
B.2 Creating a Database

1. Load the well plate image sequence.
2. Select **Tools** → **Well Plate Quantification for "<name>_SEQ"** on the menu bar.

The Well Plate Quantification window appears.

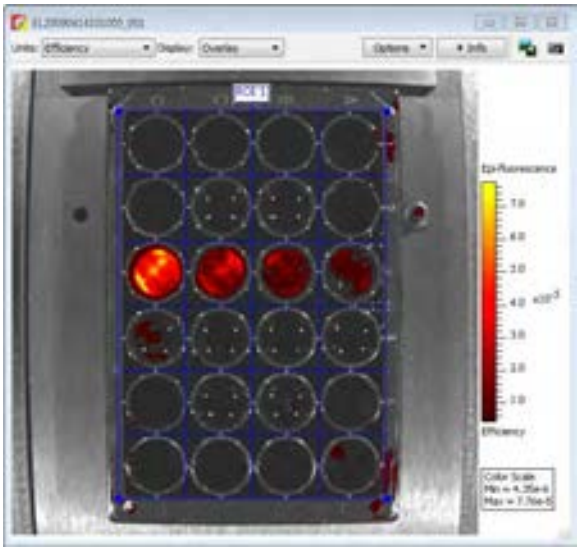
3. For fluorescent samples, choose the Dye molecules or Cells option.

Figure B.2 Well Plate Quantification Window



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.

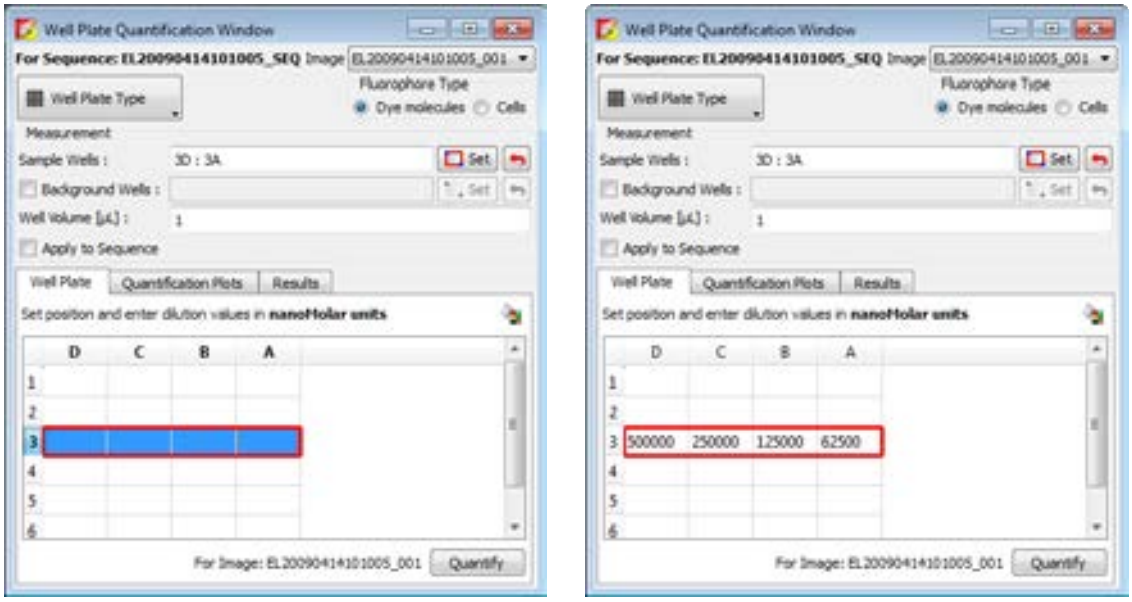
Figure B.3 Well Plate Image With Grid ROI



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 B.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 button. Alternatively, right-click the selected table cells and choose Background Color on the shortcut menu.
   b. Choose a color from the color palette that appears.

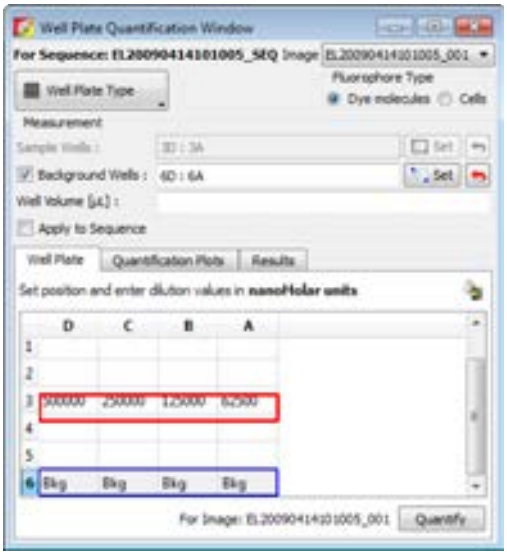
Figure B.4 Select Sample Wells and Enter Cell Numbers or Molecule Concentrations



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**.

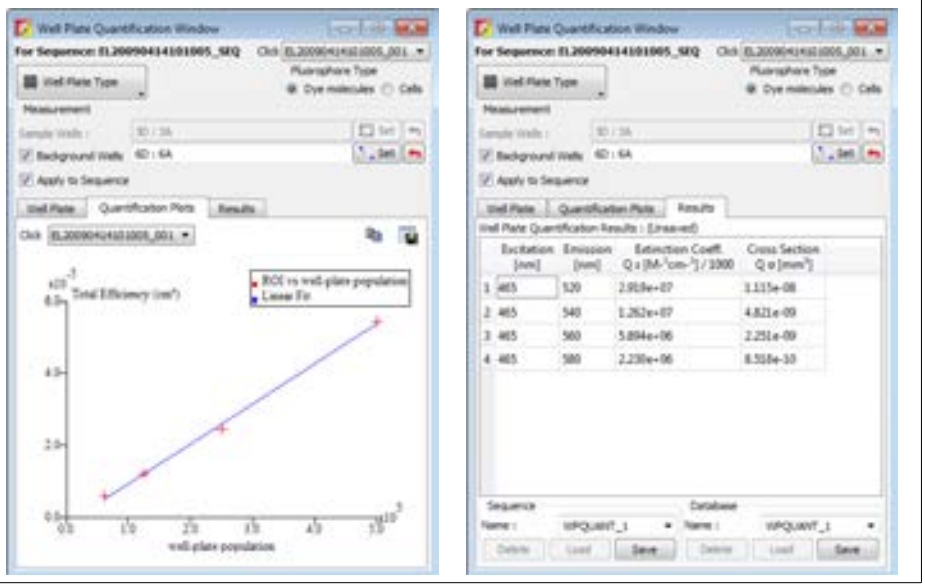
    Clicking a row or column header selects the entire row or column. To remove the “background” well designations, click the button.

Figure B.5 Set Background Wells



15. Click **Quantify**.
    The results are displayed

Figure B.6 Example Fluorescence Quantification Plot and Results



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 B.1 Quantification Results

Item	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.

B.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

Figure B.7 Saving Quantification Results

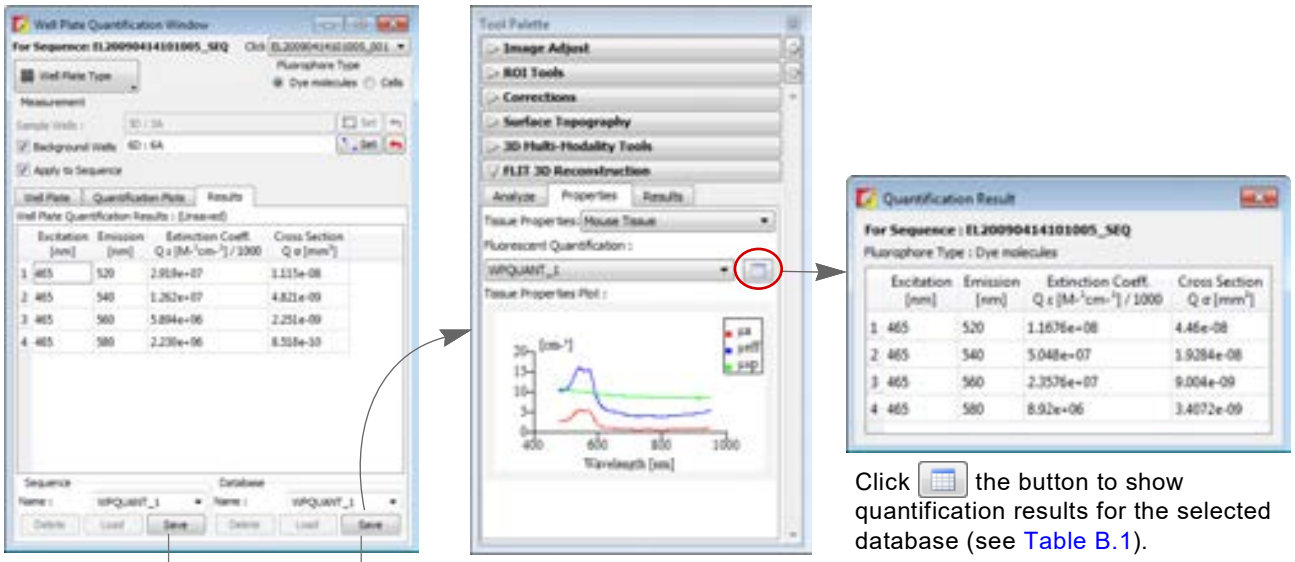


Table B.2 Managing Quantification Results

Item	Description
	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.
	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

Figure B.8 Well Plate Quantification Results

Appendix C Surface Topography

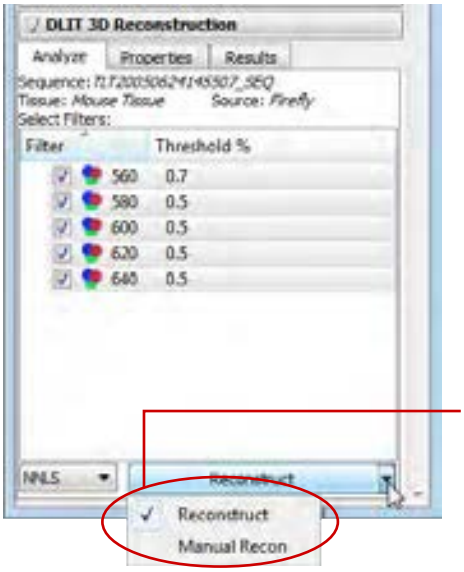
C.1 About Surfaces

A surface is a 3D reconstruction of the animal surface (topography) derived from a CT image Figure C.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.

Figure C.1 Example Surface

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 C.2). This appendix explains how to create and select a surface using the Surface Topography tools.

Figure C.2



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.

C.2 Generating a Surface

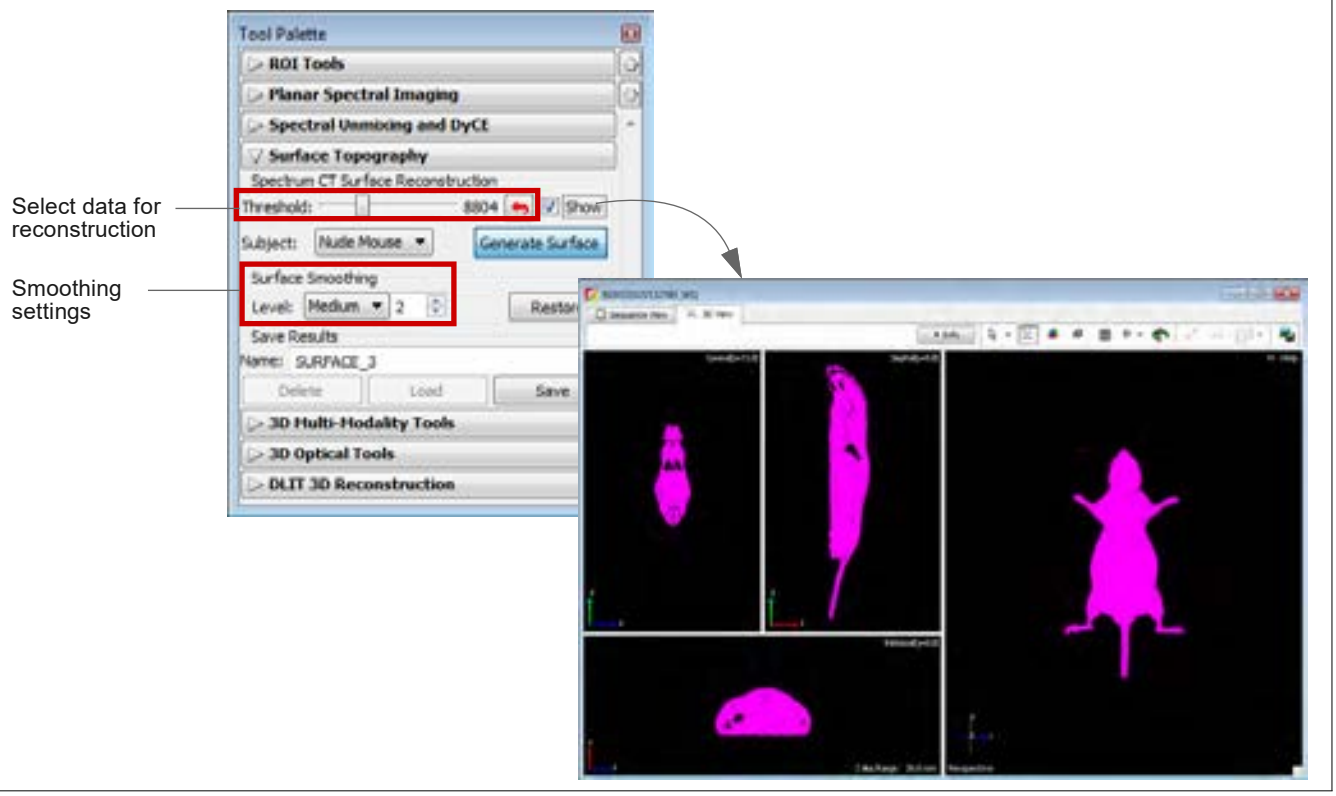
1. Load the image sequence for the reconstruction, for example, a sequence acquired for DLIT analysis or a sequence acquired for FLIT analysis.

   NOTE: If the image sequence includes multiple subjects, manual 3D reconstruction will require a separate surface for each subject. Use the 3D Multi-Modality crop tool to select a particular subject for surface topography. See Step 3 on page 315 for instructions.

2. Select a subject (Nude Mouse or Phantom).
3. Select the data for surface reconstruction by moving the "Threshold" slider (selected data are shown in pink when you move the slider) (Figure C.3). Click the button to return to the default threshold setting.

   Clicking the Show option also displays the pink selected data. The Surface Topography tool usually provides a surface which very closely resembles a surface that is "shrink-wrapped" around the pink mask.

Figure C.3 Data Selected for Surface Reconstruction (pink)



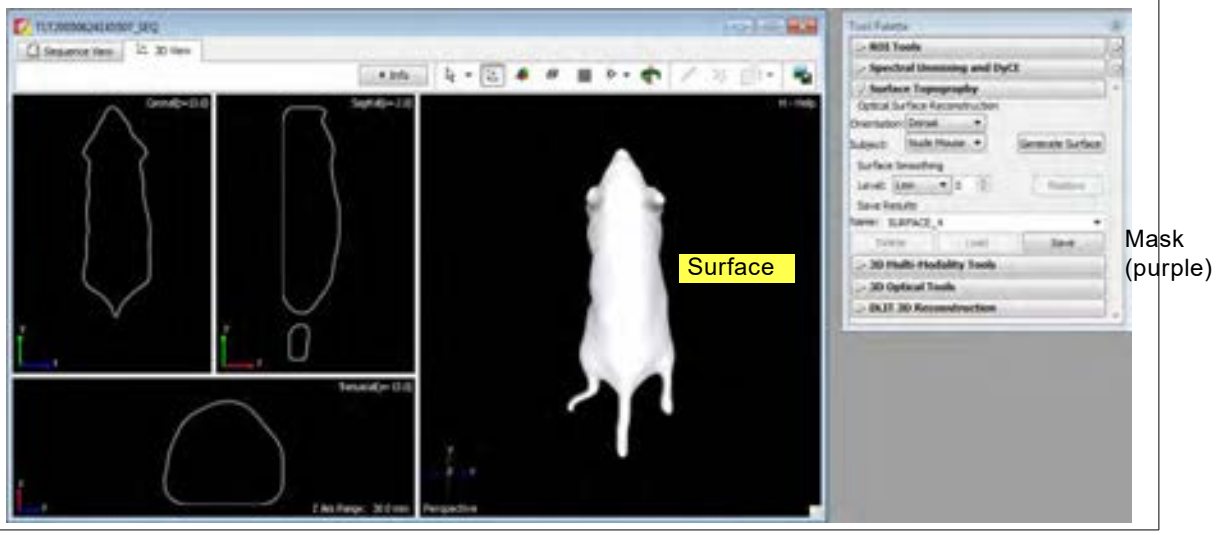
4. To apply surface smoothing (optional), select a smoothing level (low, medium, or high) and enter the number of times to apply the smoothing operation (Figure C.3).
5. Click **Restore** to reset smoothing to the default settings.

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.

6. Click **Generate Surface**.
   The surface appears in the 3D View and the 3D Optical Tools appear in the Tool Palette. See *3D Optical Tools, Source Tab on page 256* for more details on the 3D Optical Tools.

Figure C.4 3D view and 3D Tools in the Toolbar and Tool Palette

The 3D View displays the surface (white) overlaid on the CT data (gray).



Changing the View Perspective

You can drag the surface to view it from different perspectives. Alternatively, do one of the following:

- Select to change the view (Figure C.5).
- Click the surface in the 3D View window, then press the **V** key to cycle through the different views of the surface.

Figure C.6 shows examples of the available views.

Figure C.5 Surface – Perspective View

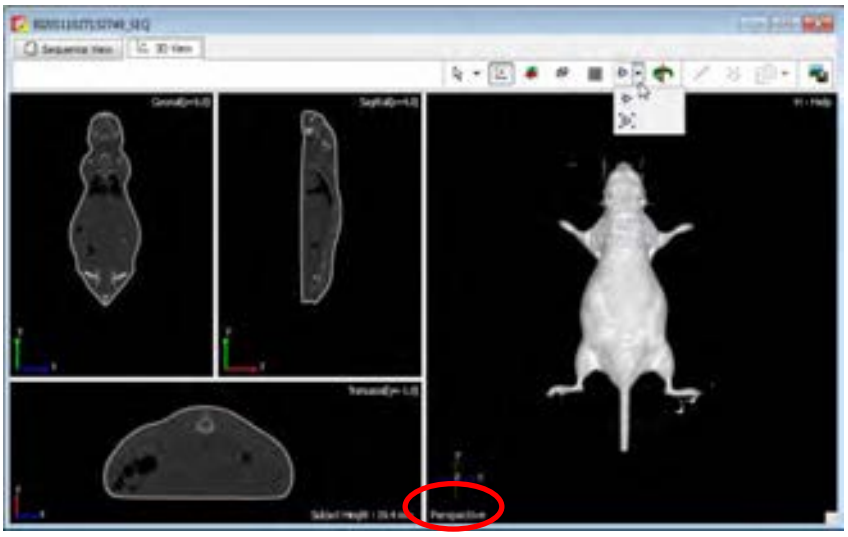
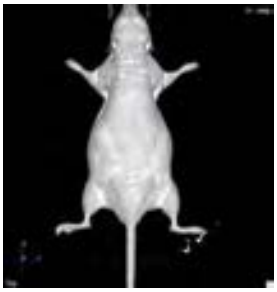
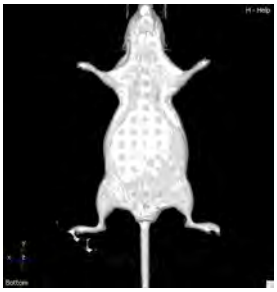


Figure C.6 Alternative Surface Views

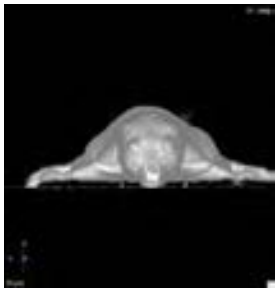
Click the surface, then press the “V” key to change the view.



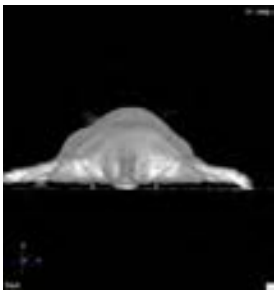
Top



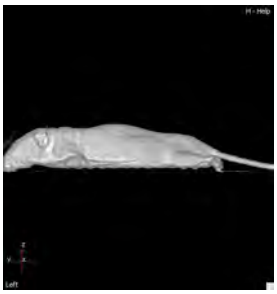
Bottom



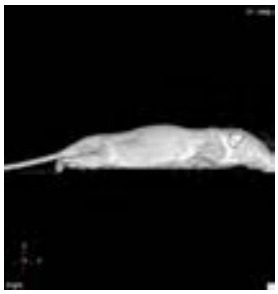
Front



Back



Left



Right

C.3 Managing Surfaces

After the surface is saved, it can be shared by the DLIT or FLIT tools.

Figure C.7 Tool Palette – Surface Topography Tools

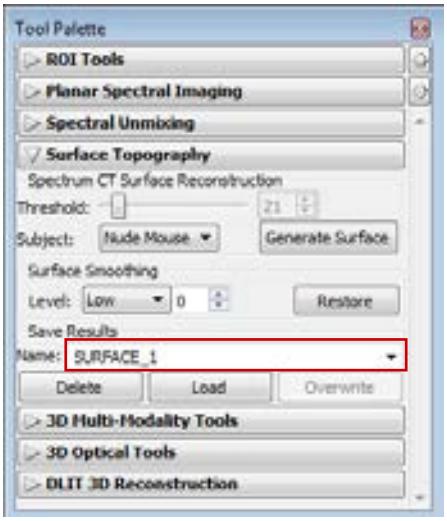


Table C.1 Surface Topography Tools – Managing Surfaces

Item	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.

NOTE: Surface import capability is only available if “Show Advanced Options” is selected in the general preferences (see page 424).

1. Load a surface.
2. Select **File** → **Export (or Import)** → **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 C.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 272 for more details.

Table C.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 D Preferences

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

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

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

D.1 General Preferences

Figure D.1 General Preferences

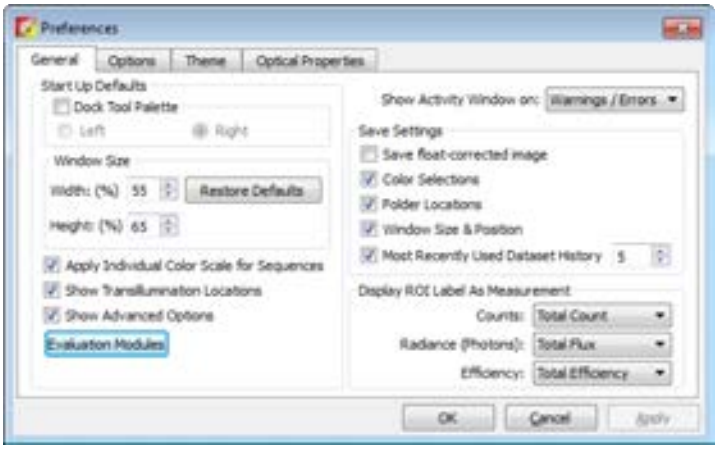


Table D.1 General Preferences

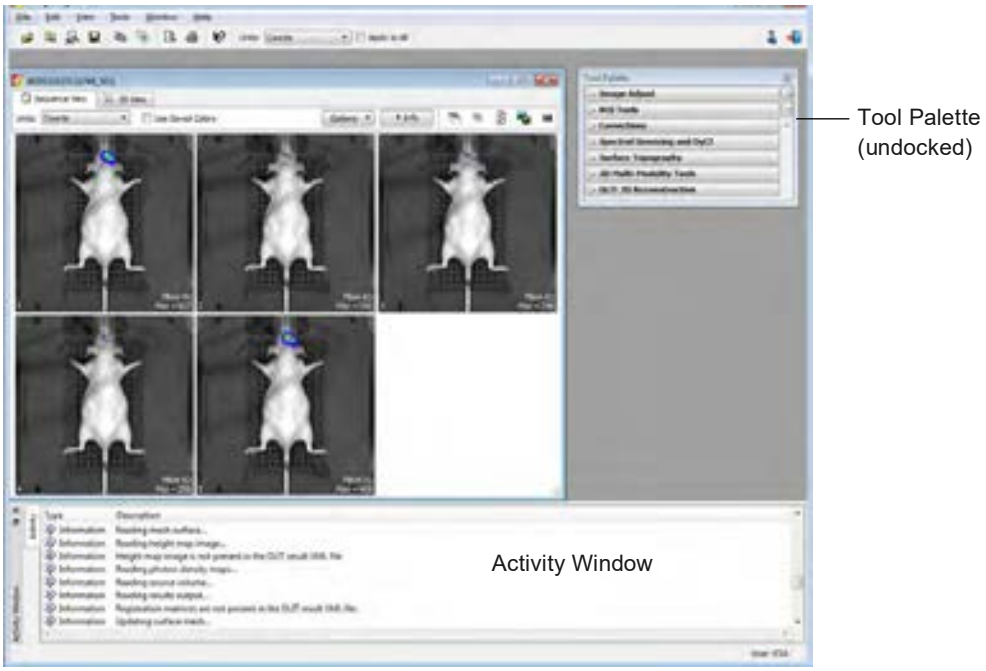
Item	Description
Start Up Defaults	Dock Tool Palette - Choose this option to set the position of the Tool Palette in the application window. Choose left or right. **Note:** To undock the Tool Palette, click on the palette title bar and drag it a distance greater than its width.
Window Size	Specifies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

Table D.1 General Preferences (continued)

Item	Description
Apply Individual Color Scale for Sequences	Choose this option to apply a separate color scale to each thumbnail of a sequence. If this option is not chosen, all of the thumbnails are displayed using the same color scale.
Show 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: ▪ Additional 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. ▪ CT Target Type option is available in the 3D View preferences. See page 430 for more information.
Show Activity Window on:	A drop-down list of options for when to display the activity log (Figure D.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 D.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** → **Recent Files** → **Menu**.
Display ROI Label As Measurement	Sets the type of measurement in counts, radiance (photons), or efficiency to show in the ROI label

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

Figure D.2 Main Application Window



D.2 Options

Figure D.3 Preferences – Options

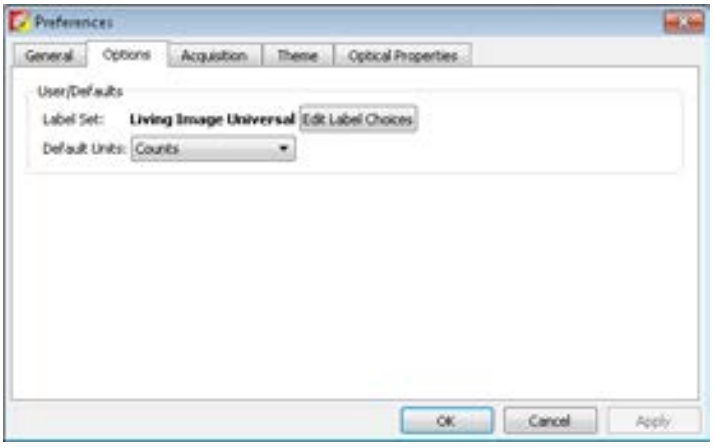


Table D.2 Preferences – Options

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

D.3 Acquisition

Figure D.4 Acquisition Preferences – Auto Exposure

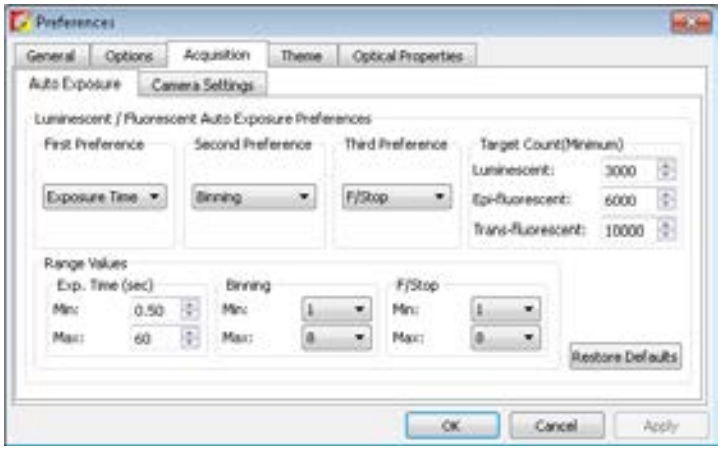


Table D.3 Auto Exposure Settings

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 D.5 Acquisition Preferences – Camera Settings

Table D.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.

D.4 Theme

Figure D.6 Image View Preferences

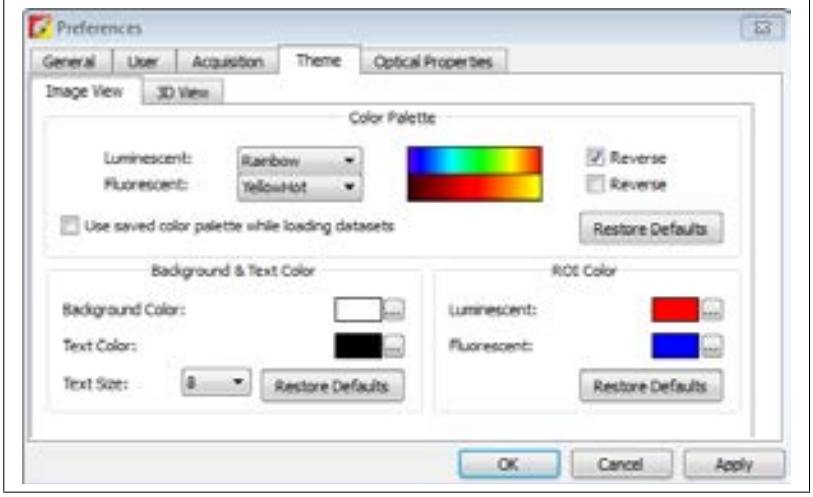


Table D.5 Image View Preferences

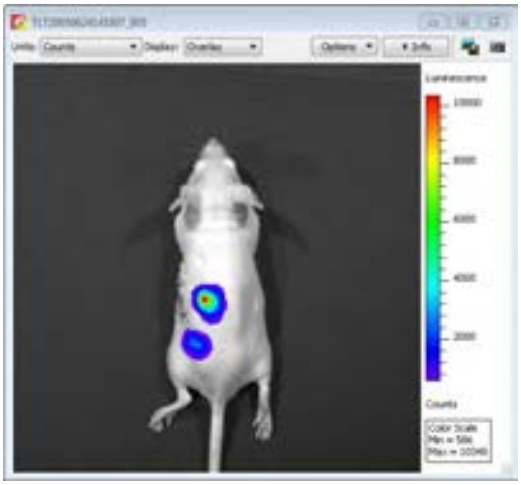
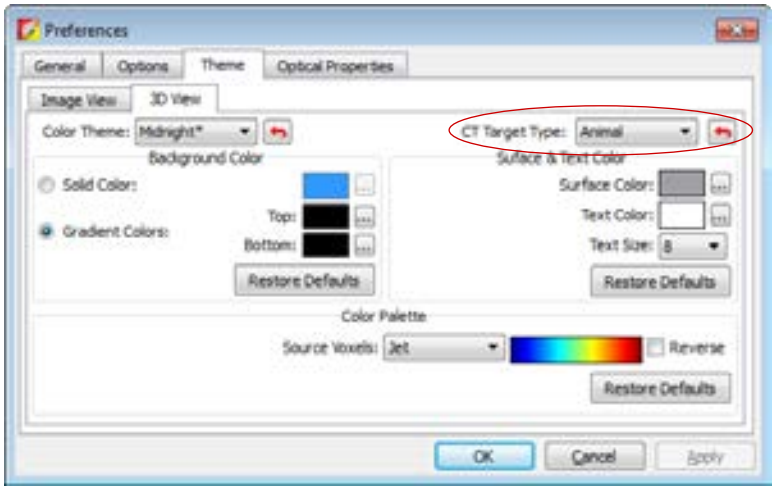
Item	Description
Color Palette	Use these controls to select a color table for luminescent and fluorescent image data. Choose the Reverse option to reverse the min/max colors of the selected color table.
Use saved color palette while loading datasets	If this option is chosen, data are displayed using a user-specified color palette. For example, after you load data, specify a color table in the Image Adjust tools, and save the data. The user-specified color table is automatically applied whenever the data are loaded.
Background & Text Color	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.

Figure D.7 3D View Preferences



This option is available if "Show Advanced Options" is selected in the General Preferences.

Table D.6 3D View Preferences

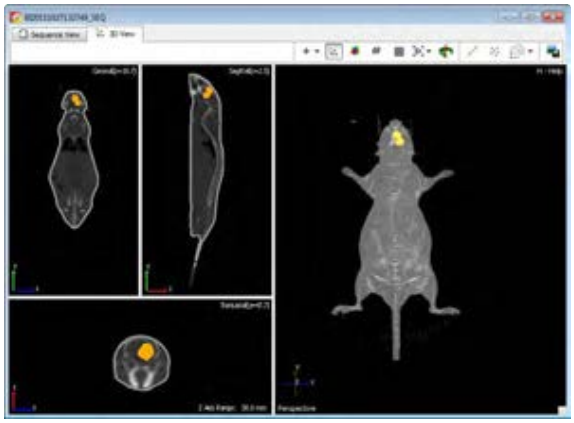
Item	Description
Color Theme	Predefined color schemes available for the 3D View window shown here. Click the ↩ button to restore the defaults for the selected color theme. 
CT Target Type	This option is available if "Show Advanced Options" is selected in the General Preferences. Two target types are available for CT imaging: ▪ "Animal" – Use this setting for best CT image quality when imaging animals. ▪ "Metal" – Use this setting only when imaging phantom targets that contain metal. Do not use this setting when imaging animals as it will produce poor CT image quality for animals.

Table D.6 3D View Preferences (continued)

Item	Description
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.

D.5 Optical Properties

Figure D.8 Set Default Optical Properties Preferences (left) for the Properties Tab in the Planar Spectral Imaging, DLIT, or FLIT Tools

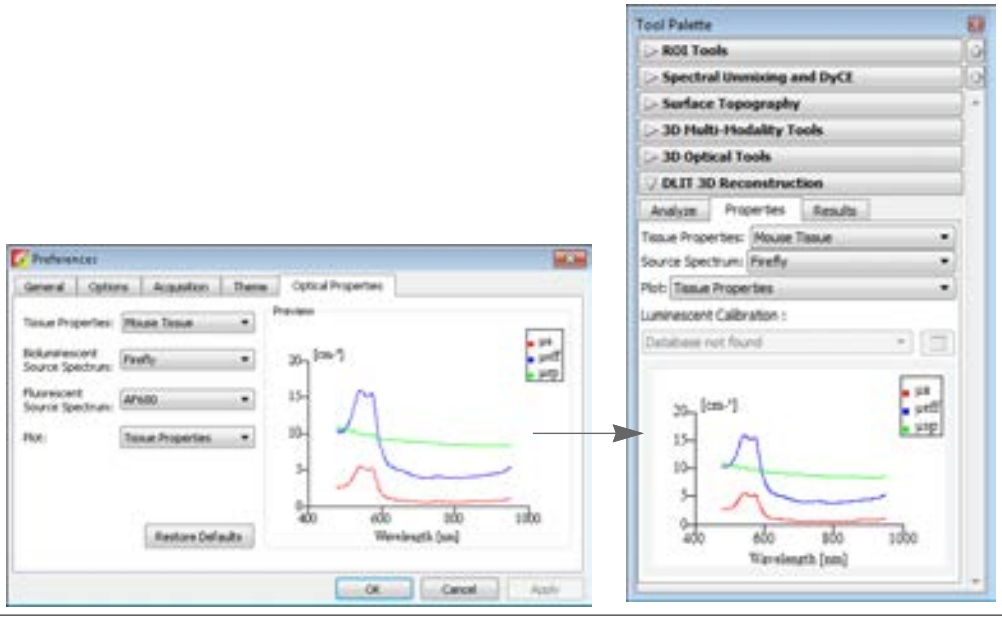


Table D.7 Preferences – Optical Properties

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

Table D.7 Preferences – Optical Properties

Item	Description
Source Spectrum	Choose the default luminescent source spectrum. This Source Spectrum will be used 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 μsp). 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 E Menu Commands, Toolbars, and Shortcuts

Figure E.1 Living Image Toolbar

Table E.1 Menu bar commands and toolbar buttons

Menu Bar Command	Toolbar Button	Description
File → Open		Displays the Open box so that you can select and open an image data file. Double-click a SequenceInfo.txt file or ClickInfo.txt file to open the image data file (see page 127).
File → Browse		Displays the Browse For Folder box so that you can select and an image data folder. The selected folder is displayed in the Living Image Browser.
File → Browse 3D Volumetric Data		Displays the Browse For Folder box so that you can select a volumetric data folder (for example, DICOM format, TIF data). The selected folder is displayed in the 3D Browser.
File → Save		Saves (overwrites) the AnalyzedClickInfo text file to update the analysis parameters, but the original image data files are not altered.
File → Save As		Displays the Browse For Folder box so that you can specify a folder in which to save the image data. The original data is not overwritten.
File → Import → 3D Surface		Opens a dialog box that enables you to import a surface. **Note:** This menu item is only available if “Show Advanced Options” is selected in the Preferences (see page 424).
File → Import → 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 424).
File → Import → Atlas		Opens a dialog box that enables you to import an organ atlas (.iv, .dxf, .stl).
File → Export → Image/ Sequence as DICOM		Opens the Browse for Folder dialog box that enables you to export the active image data to DICOM format (.dcm).
File → Export → 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 → Export → 3D Voxels		Opens a dialog box that enables you to save the voxel information from the active data.

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

Menu Bar Command	Toolbar Button	Description
File → Export → 3D Scene as DICOM		Opens a dialog box that enables you to save a 3D reconstruction and/or surface in DICOM format. The Multi-Frame DICOM option supports 3D CT reconstruction in third party software.
File → Print		Displays the Print box.
File → Print Preview		Displays the Print Preview box that shows what will be printed.
File → Recent Files		Shows recently opened datasets. **Note:** The number of files displayed can be set in the Preferences box (select **Edit → Preferences** and click the General tab).
File → Logout		Opens the Select/Add User ID dialog box so that another user can logon or a new user ID can be added to the system.
File → Exit		Closes the Living Image software.
Edit → Copy		Copies the active image window to the system clipboard.
Edit → Image Labels		Opens the Edit Image Labels dialog box that enables you to edit the label set information for the active data (see page Figure 12.41 on page 165).
Edit → Preferences		Opens the Preferences box (see page 424).
View → Tool Bar		Choose this option to display the toolbar.
View → Status Bar		Choose this option to display the status bar at the bottom of the main window.
View → Tool Palette		Choose this option to display the Tool Palette.
View → Activity Window		Displays the Activity window at the bottom of the main application window. The Activity window shows a log of the system activity.
View → Image Information		Displays the Image Information box that shows the label set and image acquisition information for the active data.
View → ROI Properties		Displays the ROI Properties dialog box (see page 184).
View → 3D ROI Properties		Displays the 3D ROI Properties dialog box (see page 292).
View → ROI Measurements		Displays the ROI Measurements table.
View → 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.

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

Menu Bar Command	Toolbar Button	Description
Tools → 3D Animation		Opens the 3D Animation window that enables you to view a preset animation or create an animation.
Tools → Longitudinal Study		Opens the Longitudinal Study window for side-by-side comparisons of DLIT or FLIT results.
Tools → Well Plate Quantification for ...		Opens the Well Plate Quantification window.
Tools → Image Overlay for...		Opens the Image Overlay window for the active data.
Tools → Colorize		Opens the Colorized View tab for the active sequence.
Tools → Image Math for...		Opens the Image Math window for the active data.
Acquisition → Background → Measure Dark Charge		Opens a dialog box that enables you to acquire a dark charge measurement. **Note:** The imaging system automatically acquires a dark charge measurement every day at a set time, usually during overnight hours. Living Image will automatically shut down after taking the dark charge measurement.
Acquisition → Background → Add or Replace Dark Charge		Opens a dialog box that enables you to select an instrument luminescent background. This background measurement is subtracted from luminescent images.
Acquisition → Background → Measure and Replace Dark Charge		Measures the dark charge under the same conditions as the currently selected image. When the measurement is complete, the newly acquired dark charge image will be included in the dataset of the current image, replacing any existing dark charge image that may be present in the dataset.
Acquisition → Background → View Available Dark Charge		Opens a dialog box that enables you to view the dark charge measurements for the system.
Acquisition → Background → Clear Available Dark Charge		Clears all dark charge images from the system.
Acquisition → Background → Auto Background Setup		Opens a dialog box that enables you to acquire background images, or schedule or disable automatic background acquisition.
Acquisition → Fluorescent Background → Measure Fluorescent Background		Starts a measurement of the instrument fluorescent background.
Acquisition → Fluorescent Background → Add or Replace Fluorescent Background		Opens a dialog box that enables you to select an instrument fluorescent background measurement for the active image data. If the “Fluorescent Background” Subtraction option is chosen in the Corrections/Filtering Tool Palette, the background measurement is subtracted from the image data.

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

Menu Bar Command	Toolbar Button	Description
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 → Auto-Save		If Auto-Save is selected, all images are automatically saved to a user-selected folder.
Acquisition → CT → Generate Alignment data		Acquires images of the Rotation Stage Alignment tool that are used to generate alignment data for the IVIS Spectrum CT.
Acquisition → CT → Acquire Reference images		Acquires dark and bright reference images that are used to determine corrections that are applied to the raw projection images during the CT reconstruction process.
Acquisition → Auto-Save To		Opens a dialog box that enables you to select a folder where images will be saved to automatically.
Window → Close		Closes the active image window.
Window → Close All		Closes all image windows.
Window → Cascade		Organizes the open image windows in a cascade arrangement (see Figure 12.7 on page 132).
Window → Tile		Organizes the open image windows in a tiled arrangement (see Figure 12.7 on page 132).
Window → 1. <Image or Sequence name> **Window → 2. <Image or Sequence name>** **Window → etc.**		A list of the open image windows. Click a window in the list to make it the active window (indicated by a check mark).
Window → Other Windows → <window name>		Lists other windows that are open. For example, If the Living Image Browser is open, use these commands to make the browser the active window and display it on top of all other open windows.
Help → User Guide		Displays a list of IVIS manuals

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

Menu Bar Command	Toolbar Button	Description
Help → Tech Notes		Displays a folder of technical notes. **Note:** For the most recent collection of technical notes, see In Vivo Customer Training.
Help → License information		Displays the license information.
Help → Plug-in Information		Displays a list of tool plug-ins and Tool Palette plug-ins.
Help → IVIS Reagents		Opens the Revvity web page for In Vivo Imaging Reagents.
Help → About Living Image		Displays information about the Living Image software and Revvity technical support contact information.
		Click this button, then click an item in the user interface to display information about the item.

Table E.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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