TrueQuant
Operator Manual

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Contents

1 Overview

1.1 System Overview

The FMT® (Fluorescence Molecular Tomography) system is a small-animal fluorescence in vivo imaging system for murine preclinical research use. It is designed to provide calibrated quantitative tomographic images and data of fluorescence signal within biological tissue throughout the depth of the subject. The one channel instrument (FMT 1000) is available with a single near-infrared 635 nm channel, a 680 nm channel, a 750 nm channel, or a 790 nm channel. The two channel instrument (FMT 2000) operates on two near-infrared channels excited at 670 nm and 746 nm, and emitting at 700 nm and 775 nm respectively. The four channel instrument (FMT4000) operates on four channels, which excite at 635 nm, 670 nm, 746 nm, and 790 nm and emit at 660 nm, 700 nm, 775 nm, and 805 nm respectively.

NOTE All four models can perform planar imaging on the full four channels.

In vivo imaging using the FMT system is performed by placing the anesthetized subject into the portable imaging cassette and inserting the cassette into the docking station in the imaging chamber. Scanning, reconstruction, and analysis are achieved using the TrueQuant software. The system is compatible with standard isoflurane-based gas anesthesia systems.

This section contains the following sections:

The remainder of this document is organized as follows:

- *Chapter 2* summarizes system installation and configuration.
- *Chapter 3* describes the typical FMT imaging session and an overview of the TrueQuant software.
- *Chapter 4* describes how to place an animal into the imaging cassette and load the imaging cassette into the instrument.
- *Chapter 5* describes how to set up studies, groups and subjects for imaging and how to import and export data.
- *Chapter 6* describes the scan acquisition process.
- *Chapter 7* describes the analysis process.
- *Chapter 8* describes the agents and agent calibration process.
- *Chapter 9* describes creating and using custom animal strains.
- *Chapter 10* describes the database management and back-up procedures.
- *Chapter 11* discusses operating guidelines and troubleshooting.
- *Chapter 12* discusses system maintenance.
- *Chapter 13* provides system warranty and regulatory information.
- *Chapter 14* provides technical services and support information.

1.2 Hardware Overview

This section describes the FMT system hardware.

1.2.1 FMT Front View

Figure 1-1. FMT System Front View

Camera Indicator: Illuminates when the transillumination scan is in process.

Lock Indicator: Illuminates when the laser cannot turn on because an interlock is tripped (for example, if a door is open).

Entry Lid: Slide down to access the Internal Docking Station. The Laser Safety Interlock Switch disables the laser when the Entry Lid is open.

Figure 1-2. MSIM Internal Docking Station

Internal Docking Station: Receptacle for the imaging cassette inside the FMT system. The internal docking station is accessed by opening the entry lid (Figure 1-1). The docking station includes gas anesthesia ports and vacuum ports to keep the animal anesthetized, and an integrated warmer to maintain the animal at body temperature (37^{o}C).

The instrument can be equipped with either a Mouse Docking Station or an MSIM Docking Station. The Mouse docking station has an inner door that must be lifted up to insert the Mouse cassette and then closed to secure the cassette in place.

Figure 1-3. Mouse Docking Station Door, lift to open

1.2.2 FMT Right Side

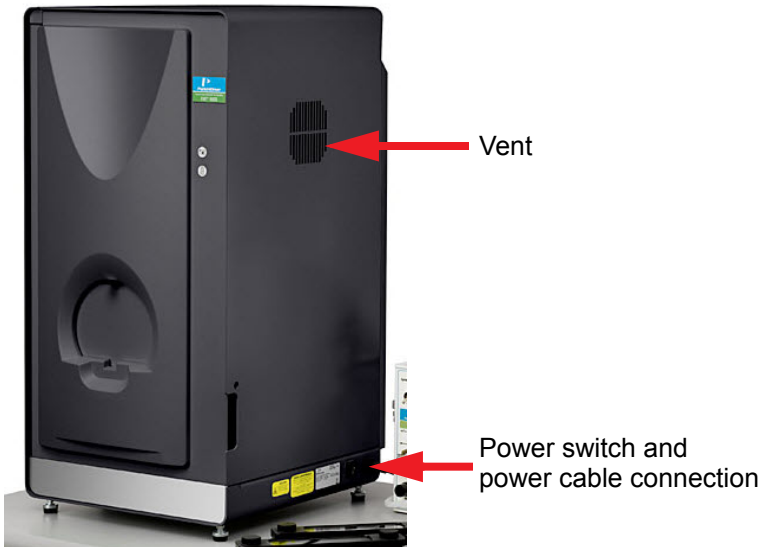


Figure 1-4. Power panel on right side of FMT

Imaging Compartment Vent: Allow at least 2” (5 cm) clearance for proper ventilation.

Power Switch and Power Cable Connection: Supplies power to the FMT.

1.2.3 FMT Left Side

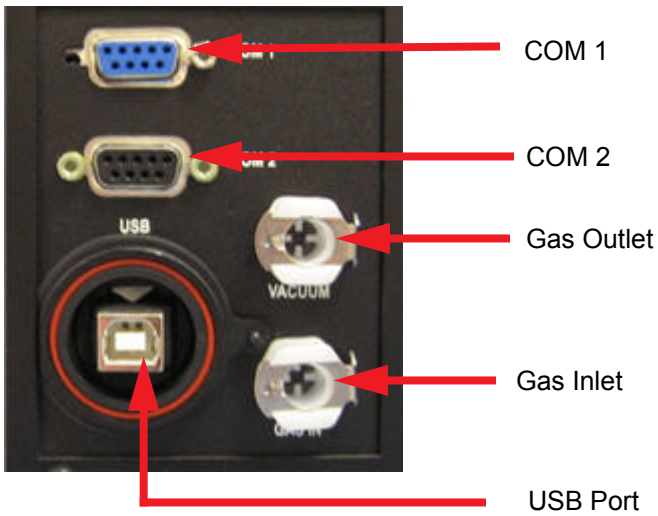


Figure 1-5. Cable and Gas connections on the lower left side of the FMT

USB Port: Connect the USB cable to USB port and to any USB port on the computer. The USB controls the camera.

COM1 and COM2 Serial Ports: Connect the two serial cables to the COM1 and COM2 ports on the instrument and to the COM1 and COM2 ports on the computer. COM 1 controls the main instrument functionality. COM 2 connects to the expansion port on the computer and controls the X-Y stage movement.

Gas Inlet Port: Connect isoflurane-based inhalation anesthesia supply to the Gas Inlet port on the side panel, if required. When the Inlet Valve is open, anesthesia flows through the internal tubing to the imaging cassette.

Gas Outlet Port: Connect to a vacuum line or other appropriate gas discharge handling system. When the Outlet Valve is open, anesthesia is drawn out of the imaging cassette.

Inserting the imaging cassette into the internal docking station and closing the Entry Lid automatically opens the anesthesia inlet and outlet valves and permits the flow of gas into and out of the imaging cassette to maintain anesthesia during scanning. Removing the imaging cassette or opening the Entry Lid automatically closes the gas inlet and outlet ports and stops the flow of anesthesia gas.

1.2.4 Mouse Imaging Cassette

Figure 1-6. Mouse Imaging cassette

Imaging Cassette: The removable imaging cassette holds the anesthetized animal in position during imaging (Figure 1-6). The imaging cassette is inserted into the internal docking station for imaging. If the instrument has an MSIM docking station, a mouse cassette adapter is required. The imaging cassette can also be placed into a multi-modality adapter for co-registration imaging (see *Multi-Modality Adapters* below).

Imaging Cassette Top Cover Adjustment Knobs: The adjustment knobs determine the distance between the glass panels of the imaging cassette (Figure 1-6). The cutout next to each knob displays the distance setting, in mm. Tighten the knobs until mild compression of the subject is obtained.

Multi-Modality Adapters (Optional): The Imaging Cassette can also be used in other imaging modalities, such as microPET, CT, MRI and SPECT, using the PerkinElmer multi-modality adapters (Figure 1-7). The adapters allow for minimal movement of the animal between imaging sessions in the various modalities and provides fiducial markers on the imaging cassettes, for easy co-registration of the FMT and secondary modality data. Adapters are available for a variety of manufacturers and for various modalities including microPET, CT, PET, MRI and SPECT.

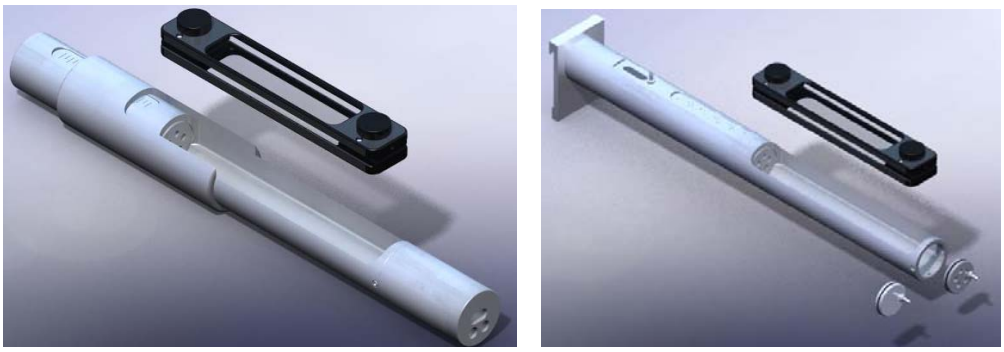


Figure 1-7. Multi-Modality MR adapter (left) and Multi-Modality MicroPET/CT adapter (right).

1.2.5 Multi-Species Imaging Module (MSIM) Cassette (Optional)

Figure 1-8. Multi-Species Imaging Module (MSIM)

MSIM Imaging Cassette (Optional): The removable imaging cassette holds a larger anesthetized animal in position during imaging (Figure 1-8). The imaging cassette is inserted into the MSIM docking station for imaging. (The MSIM imaging cassette is only for use in systems with the MSIM docking station installed and configured.) The MSIM cassette can be inserted in one of three positions in the docking station to image the entire subject. By inserting the cassette head first or feet first, different fields of view of the cassette can be imaged. The three fields of view toward the user as the cassette is inserted into the FMT are the ones that can be imaged. Arrows engraved in the side rails of the cassette indicate the center of each field of view. Up to six images of the subject can be opened in one pane of the Analysis tab to display the entire subject.

The bottom of the MSIM imaging cassette has posts on the corners to secure the top of the cassette. Notches in the bottom post position the top at either 20mm or 35mm.

Fiducial Marks: White marks (screws) on the edges of the MSIM cassette are used to determine the position of the cassette in the docking station. The TrueQuant software uses the MSIM calibration and the position of the fiducial marks in the image to align the images in the Analysis tab and to determine the position and orientation of the cassette as well as the cassette depth during acquisition on the Scan tab.

Head and Tail Markings: Align the animal's head and tail with the head and tail markings on the MSIM cassette so the system can determine the proper cassette and animal orientation.

Height Adjustment: The position of the top determines the distance between the glass panels of the imaging cassette (Figure 1-8). The MSIM cassette can be adjusted to 20, 25, 30 or 35mm. Set the height so that mild compression of the subject is obtained.

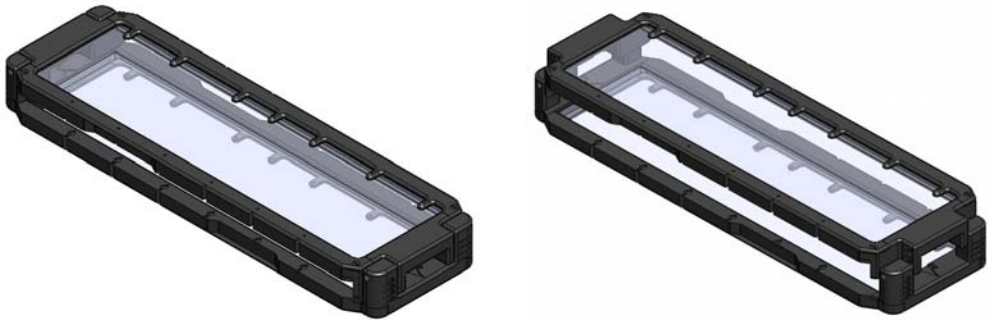


Figure 1-9. MSIM at 20mm and 35mm

1.3 Warnings, Cautions and Notes

The precautions in this manual are grouped into two main categories: warnings and cautions.

In addition, the manual highlights notes of significant information relevant to the monitor display, operator instruction, or operator action being described in the text.

WARNING	Warnings advise against certain actions or situations that could result in personal injury or death.
CAUTION	Cautions advise against actions or situations that could damage equipment, produce inaccurate data, or invalidate a procedure.
NOTE	Notes provide useful information regarding a function or procedure.

The following warnings define precautions that must be observed to avoid injury to personnel. Some of these precautions are specific to particular operator actions and are included in the text. Others are “general-purpose”, and may not be duplicated in the places in which they may be relevant.

WARNING	Do not attempt to override or modify the interlock system.
WARNING	Do not stare into any laser beam. Staring into a laser beam (intrabeam viewing) can cause permanent damage to your eyes.
WARNING	If used in a manner not specified by the manufacturer, the protection provided in the equipment may be impaired.
WARNING	If this equipment is used in a manner not specified by the manufacturer, you may be exposed to hazardous radiation.
WARNING	Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.
CAUTION	The computer supplied with the FMT system must be manually switched to accept either 115V or 230V line voltage. The red voltage selector is located on the rear panel of the desktop tower, as described in the enclosed computer documentation.
CAUTION	Two people are required to lift this equipment.
CAUTION	Only qualified service personnel are to service the equipment or to access areas not defined in this manual as accessible to the operator or appropriate wording to that effect.

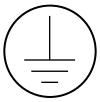
CAUTION Do not use flammable or strong chemical solvents such as isopropyl alcohol, ketones, or hexanes directly on the animal holder as they could damage the glass plates and their anti-reflection coating.

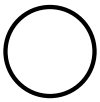
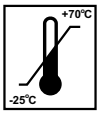
CAUTION Do not use metal utensils, hard tools, or abrasive cloth on the animal holder as they can damage the glass plates and their anti-reflection coating.

CAUTION The system diagnostic features are designed to be used by properly trained service personnel only. Untrained users should not run the diagnostics software.

1.4 Explanation of Symbols

The following symbols may be used in this manual or on the instrument:

Symbol	Explanation
	Finger pinch warning
	Hazardous Voltage
	Refer to Operator Manual
	Protective Ground or Earth
	Date of Manufacture
	Do not discard
	NRTL Approval for the US and Canada, CE Mark
	Do not stack

Symbol	Explanation
	ON symbol
	OFF symbol
	Fragile
	Store in relative humidity between 20% and 90%
	Store in temperatures between -25ºC and +70ºC
	This side up
	Keep from getting wet
	Warning, laser beam
	Radiation of laser apparatus
	Lifting warning: Indicates that two (2) people are required to lift object safely.

1.5 Abbreviations and Acronyms

The following abbreviations and acronyms may be used in this manual:

Abbreviation or Acronym	Explanation
A	Ampere
cm	Centimeter (10^{-2} meter)
Em.	Emission
Ex. or Exc.	Excitation
FMT	Fluorescence Molecular Tomography
Hz	Hertz
IEC	International Electrotechnical Commission
LED	Light-Emitting Diode
MIP	Maximum Intensity Projection
mm	Millimeter (10^{-3} meter)
MSDS	Material Safety Data Sheet
msec	Millisecond (10^{-3} second)
mW	Milliwatt (10^{-3} Watt)
nM	Nanomolar concentration (10^{-9} moles/liter)
Nm	Nanometer (10^{-9} meter)
recon	Tomographic reconstruction
Ref	Reflectance image (also Reference image)
ROI	Region of Interest
USB	Universal Serial Bus
V	Volt

2

System Installation and Configuration

2.1 Environmental and Site Requirements

The FMT system is designed for indoor use only and can be operated within the temperature and humidity ranges normally encountered in laboratories. For normal operation, these ranges are:

- Temperature 15ºC - 28ºC
- Relative Humidity < 55%, non-condensing
- Altitude < 2000 meters

When installing the system, sufficient space should be provided to allow access to all compartments of the system and the switch panel on the right side (see Figure 1-4). The bench or tabletop must be capable of supporting the weight of the system.

The dimensions and weights of the system components are shown in the table below:

Device	Width	Depth	Height	Weight
FMT Instrument	46 cm (18 in.)	48 cm (19 in.)	89 cm (35 in.)	1 or 2 channels: 72.6 kg (160 lbs) 4 channels: 75.3 kg (166 lbs)
Host computer	16.5 cm (6.5 in.)	46 cm (18 in.)	44.5 cm (17.5 in.)	34 kg (75 lbs)
Monitor	44.5 cm (17.5 in.)	20 cm (8 in.)	43 cm (17 in.)	2 kg (5 lbs)

For the best performance from the FMT system:

- Place the FMT system in a dust-free environment.
- Make sure that the bench top is free from vibrations or mechanical shocks.
- Do not place the FMT system or the PC directly against room heating or cooling equipment, ducts, or water pipes.
- Do not place the FMT system or the PC in direct sunlight.
- Leave at least 5 cm (2 in.) between the sides, rear and top of the instrument and between any vertical obstructions (walls, partitions, or other equipment) to allow for adequate ventilation and to easily access the power/AC and communication cables on the right and rear of the instrument.
- The area near the PC must be free of strong magnetic fields.

2.2 Electrical Requirements

The PerkinElmer FMT system operates on power supplies of 115/230 VAC, 50Hz/60Hz. The line supply must be within 10% of the nominal voltage.

CAUTION The computer supplied with the FMT system must be manually switched to accept either 115V or 230V line voltage. The red voltage selector is located on the rear panel of the desktop tower, as described in the enclosed computer documentation.

The rated power of the FMT System is 115/230 VAC, 5/2.5 A, 50/60 Hz.

The rated power of the PC is 115/230 VAC, 6/3 A, 60/50 Hz.

The rated power of the monitor is 100-240 VAC, 2A Max, 60/50 Hz.

PerkinElmer recommends plugging the FMT system, the PC, and the monitor into a surge-protected power strip rather than directly into a wall outlet.

Installation Category	II
Pollution Degree	2
Class	I

2.3 General System Safety and Laser Safety

The PerkinElmer FMT system has been designed and tested in accordance with the safety requirements of the International Electrotechnical Commission (IEC). The System conforms to IEC publication 61010-1 (“Safety requirements for electrical equipment for measurement, control and laboratory use”) as it applies to IEC Class 1 (earthed) appliances, and therefore meets the requirements of EC Low Voltage directive 73/23/EEC, amended by 93/68/EEC.

Any adjustment, maintenance, or repair of the FMT system must be performed by a PerkinElmer person or authorized agent. Any unauthorized repairs, changes, or modifications are deemed to be unsafe and will void the warranty.

The PerkinElmer FMT system is a CDRH Class I, EN 60825-1/IEC 60825-1 Class 1 laser product. The optical train contains two Class IIIb laser diodes emitting continuous wave radiation at wavelengths of 670 nm and 746 nm with a maximum power of 80 mW. The system may also be upgraded to contain two additional lasers at wavelengths of 635 nm and 790 nm with a maximum power of 80mW. Laser radiation is automatically interrupted when either the entry lid is open or the imaging cassette is not inserted.

The PerkinElmer FMT system complies with the following laser safety regulations:

1. 21 CFR Chapter 1, Subchapter J, “Radiological Health”, Part 1040.10, administered by the Center for Devices and Radiological Health, U.S. Department of Health and Human Services.

2. EN 60825-1:1994 and Amendment 1 and Amendment 2 “Radiation safety of laser products, equipment classification, requirements and user’s guide”. EN 60825-1 implements CENELEC European Normalization document EN 60825-1.
3. IEC 60825-1:2001 “Safety of laser products–Part 1: Equipment classification, requirements and user’s guide”.

WARNING Do not attempt to override or modify the interlock system.

WARNING Do not stare into any laser beam. Staring into a laser beam (intrabeam viewing) can cause permanent damage to your eyes.

2.4 Computer Specifications

The PerkinElmer FMT System is shipped with the host computer pre-configured with the TrueQuant software. For optimal performance, this computer should be dedicated exclusively for use with the FMT System. The FMT System should be the only USB device connected to the computer’s USB port. We recommend not installing any additional software applications on this computer.

Host computer specifications:

- Windows 7 (64-bit only) operating system
- Two 6-Core Xeon Processors X5650 2.66GHz or faster
- 24GB of 1333MHz DDR 3RAM or faster
- Stand-alone video card with 1GB of dedicated RAM and full support for OpenGL 2.1, including shaders
- Two TB SATA 7200RPM hard drive or better
- Standard Gb Ethernet NIC, DVD burner/reader
- USB 2.0 port and at least (2) RS232 ports
- Floppy disk drive optional
- Dell 22" P2210 flat panel monitor or equivalent

Additional Investigator computers can be used for data analysis and data export. Investigator computers do not control the FMT instrument. Contact PerkinElmer for installation and proper configuration of TrueQuant on machines other than the FMT system Host PC.

Investigator computer minimum requirements:

- Processor: Dual-core processor
- Memory: 8GB for FMT data only or 16GB if analyzing CT data
- Hard Drive: 50 GB
- Graphics: Stand-alone graphics card with full support for OpenGL 2.1, including shaders. 256MB video RAM recommended
- Network connection to the Host PC: Gigabit Ethernet or faster
- Operating System: Windows 7 (64-bit)
- DVD drive for installation disc

2.5 Installing the FMT System

The FMT imaging instrument is shipped already pre-assembled and configured. Before powering up the system for the first time, connect the FMT instrument to the computer via the USB port and the two serial ports.

2.5.1 Unpacking the System

1. Examine the cartons and look for any evidence of mishandling in the shipment. Follow institutional procedures for reporting such evidence.
2. Remove the contents from the shipping cartons. Compare the shipped items with the packing slip and your order. Each FMT system shipment includes the following items:
   - (1) FMT System—FMT 1000, FMT 2000, or FMT 4000
   - (1) Host Computer—with appropriate country power cord (determined at time of order)
   - (1) Monitor—with appropriate country power cord (determined at time of order)
   - (2) imaging cassettes
   - (1) agent calibration phantom with holder
   - (1) Keyboard
   - (1) Mouse
   - (1) Software CD
3. Retain the shipping cartons in case of the need for return shipment.

2.5.2 Connecting the Computer Cables

To connect the FMT instrument and the computer:

1. Place the FMT on the workbench.
2. Set up the computer next to the FMT, following the instructions included with the computer.
3. Turn off the computer and the FMT power switch.
4. Connect the USB cable to the USB port on the left side of the FMT and to a USB port on the computer.

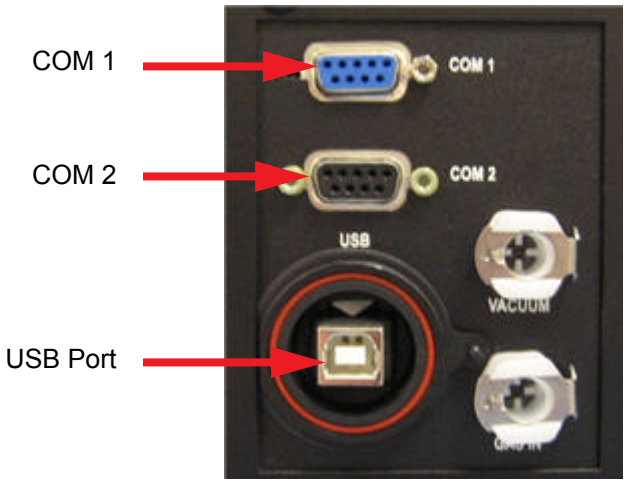


Figure 2-1. Cable connections between FMT and desktop tower PC.

5. Connect one com cable to the COM1 connector on the FMT and to COM1 on the computer.
6. Connect the second com cable to the COM2 connector on the FMT and to COM2 on the computer.
7. Connect the FMT power cable to the power receptacle on the right side of the FMT, next to the power switch, and to a surge-protected power strip or an appropriate power outlet.

2.5.3 Connecting the Isoflurane-Based Anesthesia Lines

The Gas Inlet and Outlet ports are located on the left side of the instrument (Figure 2-2) and should be connected during installation of the system.

1. Connect the tubing from the anesthesia system to the Gas Inlet port.

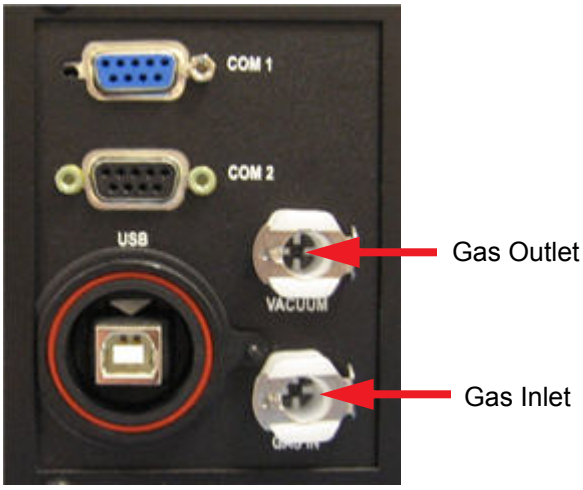


Figure 2-2. Isoflurane-based gas inlet and outlet ports on back of system.

2. Connect the Vacuum or gas discharge handling system tubing to the Gas Outlet port.

2.6 Turning On the FMT

To start up the FMT system:

1. Switch the FMT power switch on the right side of the FMT to the **On** (|) position.
2. Wait approximately **30 seconds** for the FMT instrument to initialize.
3. Turn on the computer and wait for Windows to start.
4. To start the TrueQuant software: Double-click the TrueQuant icon on the Windows desktop, or select **Start** >**All Programs** > **PerkinElmer** > **TrueQuant** > **TrueQuant**.
5. If the system has been off for a long period of time, allow the FMT to warm up for approximately 45 minutes before use. This allows the camera to cool down and the docking station warmer to warm up.

Keep the FMT system powered on when not in use unless the system is not going to be used for an extended period of time.

2.7 TrueQuant Software License Key

TrueQuant software requires an authorized license key to run. The license key controls which features are available in the software and which instrument models are controlled by the TrueQuant software. The Host Computer is pre-configured with a license key by PerkinElmer. Any additional computers used to run TrueQuant require a license key to be entered the first time you run TrueQuant. Contact PerkinElmer to obtain additional software license keys.

2.7.1 Upgrading the Software License

A new software license key is required when upgrading the FMT. The table below describes the upgrade options for the FMT models. Each model is also available with the standard Mouse imaging cassette and docking station, or can be upgraded to the MSIM imaging cassette and docking station.

Instrument Model	Can be upgraded to
FMT 1000	FMT 2000 or FMT 4000
FMT 2000	FMT 4000
FMT 4000	N/A

After receiving the upgrade license key from PerkinElmer:

1. Select **Help** | **Update License** from the menu in TrueQuant. Note, this menu item is unavailable on a Host PC if there are any scans in the reconstruction queue.
2. Click **Uninstall License**.
3. Type the new license key.
4. Click **OK**.

3

Typical FMT Imaging Session and Overview of TrueQuant Software

This section describes a typical FMT Imaging session and links to specific sections for more details on each step in the imaging process.

3.1 Overview

The Host computer runs the TrueQuant software to control the FMT hardware and to analyze the scan data acquired by the FMT. The TrueQuant software controls the entire imaging sequence: setting up a study and a subject, performing a scan, executing tomographic reconstruction, performing ROI analysis, and exporting the results.

Starting the TrueQuant software opens the TrueQuant window as shown in Figure 3-1.

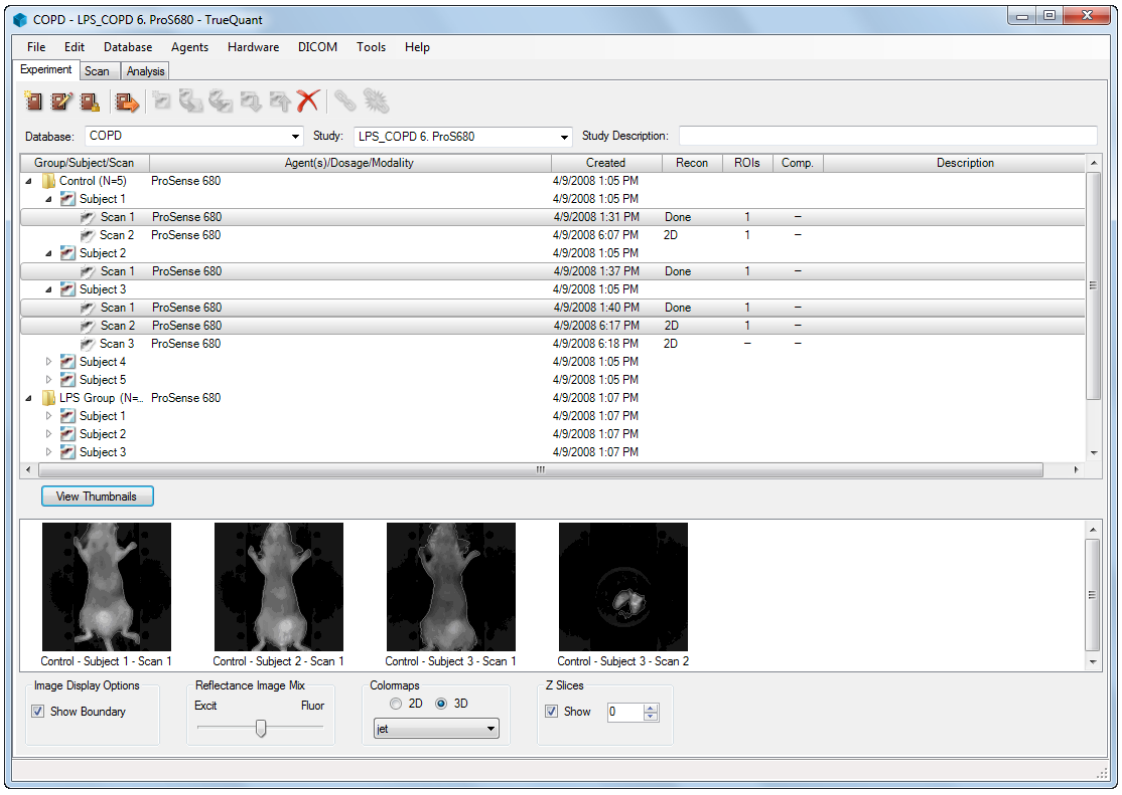


Figure 3-1. TrueQuant Window

The tabs at the top of the window, **Experiment**, **Scan** and **Analysis,** are ordered chronologically from left to right for a typical experimental sequence. A short description of each tab follows:

- **Experiment**: Specifies the imaging protocol and the database where the protocol and imaging data are stored. Also used to import and export data. See “The Experiment Tab” on page 29.
- **Scan**: Acquires and displays reflectance and/or tomographic scans for a specific study and subject. See “The Scan Tab” on page 53.
- **Analysis**: Displays and analyzes the results of reflectance or tomographic imaging. See “The Analysis Tab” on page 63.

3.2 Typical Imaging Session

The steps below describe a simple imaging sequence, where a subject is scanned and then an image is exported. The TrueQuant software offers many more advanced imaging and analysis options than are described in the steps below. Use the “Contents” on page 3 or “Index” on page 129 to locate procedures not listed below.

To perform a simple scan:

1. Turn on the instrument and start the TrueQuant software (see “Turning On the FMT” on page 22). Wait for the instrument to warm up. Turn on the anesthesia as directed in the instructions included with the anesthesia system.
2. Create a new study (see page 32) or open an existing study (see page 39).
3. Prepare the subject and load into the imaging cassette (see “Using the Imaging Cassette” on page 26).
4. Select the scan settings and scan the subject (see “Performing a Scan” on page 54).
5. If desired, view the scan images in the Scan tab (see “Display Controls” on page 58).
6. If necessary, see “Reconstruction Queue” on page 60 to add the scan to the reconstruction queue.
7. Repeat the steps above to scan all the desired subjects.
8. Open the desired scan(s) in the Analysis tab (see “Opening Datasets in the Analysis Tab” on page 63.)
9. Create the desired ROIs on the images (see “Drawing ROIs” on page 66).
10. Export data or images as desired (see “Exporting Images” on page 93).

4

Using the Imaging Cassette

This section describes how to use the Mouse imaging cassette to hold an animal during imaging. The imaging cassette ensures stable and reproducable positioning of the animal for imaging. The imaging cassette is also used to hold the Calibration Phantom during system calibration. Two Mouse imaging cassettes are included with the FMT system.

4.1 Placing the Animal in the Imaging Cassette

1. Anesthetize the animal.
2. Open the imaging cassette by turning the Height Adjustment Knobs counter-clockwise until the top plate releases from the bottom plate.

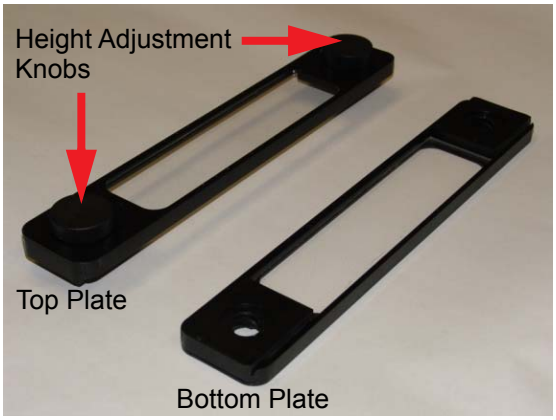


Figure 4-1. Imaging cassette (open)

3. Place the animal on the bottom plate of the imaging cassette in the desired position (see Figure 4-2). Make sure the target area to image is located in the center of the glass plate.

Figure 4-2. Place the subject on the bottom plate of the imaging cassette

4. Extend the animal's legs outward so they are not tucked under the body, and curl the tail so it remains inside the imaging cassette.

5. Place the top plate on top of the subject and start evenly tightening the height adjustment knobs by turning them clockwise (see Figure 4-3).

Figure 4-3. Close the imaging cassette using the height adjustment knobs

6. Tighten the height adjustment knobs to obtain mild compression of the subject (see Figure 4-4). For average mice, tighten the knobs 13 to 15mm.

NOTE Verify that both knobs are on the same setting before inserting the imaging cassette into the docking station.

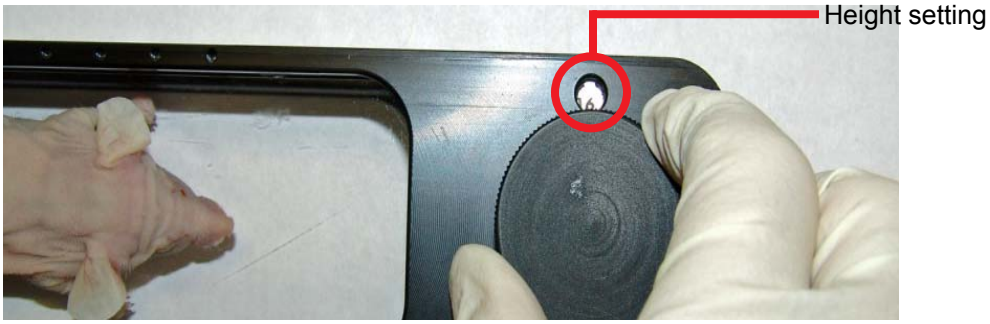


Figure 4-4. Height setting

4.2 Inserting the Mouse Imaging Cassette into the Internal Docking Station

To insert the mouse imaging cassette into the internal docking station:

1. Open the entry lid (see Figure 1-1 on page 8).
2. Lift the mouse docking station door (see Figure 1-3 on page 9). (Only for the mouse docking station. The MSIM docking station, and the mouse cassette adapter for the MSIM docking station do not have doors.)
3. Slide the mouse imaging cassette into the docking station (Figure 4-5). The imaging cassette can be inserted up or down, forward or backward. Orient the imaging cassette so that the target area is toward the camera at the top of the imaging chamber. For example, if the subject is being imaged for lung tumors and the animal is positioned on its stomach in the imaging cassette, insert the imaging cassette upside down, with the animal's chest upwards.

Figure 4-5. Inserting the imaging cassette in the internal docking station

4. Push the mouse imaging cassette in until it "clicks" into place. The gas valves open to allow the isoflurane-based gas to flow through the internal docking station.
5. Close the mouse docking station door. (Mouse docking station only.)
6. Close the entry lid. Closing the lid disables the laser safety interlock.

5

The Experiment Tab

5.1 Overview

Use the **Experiment** tab to define and record the imaging protocol for a study. The **Experiment** tab is also used to read from and to write to local or remote (networked) databases, to create and retrieve studies, and to assign groups and individual subjects to a study. TrueQuant can create, publish and interact with local or networked databases. Each database typically contains multiple studies. A study usually consists of several groups, with each group comprised of a number of subjects, typically mice, rats, or other small animals. A subject can be scanned once or repeatedly, and the results of the scans stored and analyzed. The data hierarchy is:

Database
Study
Group
Subject
Scan

The following sections explain how to create and edit databases, studies, and subjects:

- “Creating a New Study” on page 32
- “Adding, Removing and Reassigning Subjects” on page 34
- “Opening an Existing Study” on page 39
- “Editing Properties” on page 39

The following sections explain viewing, exporting, and importing data on the Experiment tab:

- “Viewing Scan Thumbnails in the Experiment Tab” on page 41
- “Reassigning Scans” on page 42
- “Exporting and Importing Data” on page 46
- “Exporting ROI Data” on page 47
- “DICOM Import” on page 48
- “DICOM Export” on page 50
- “Exporting a Study Design” on page 52

Use the Experiment tab to define and record the imaging protocol for a study.

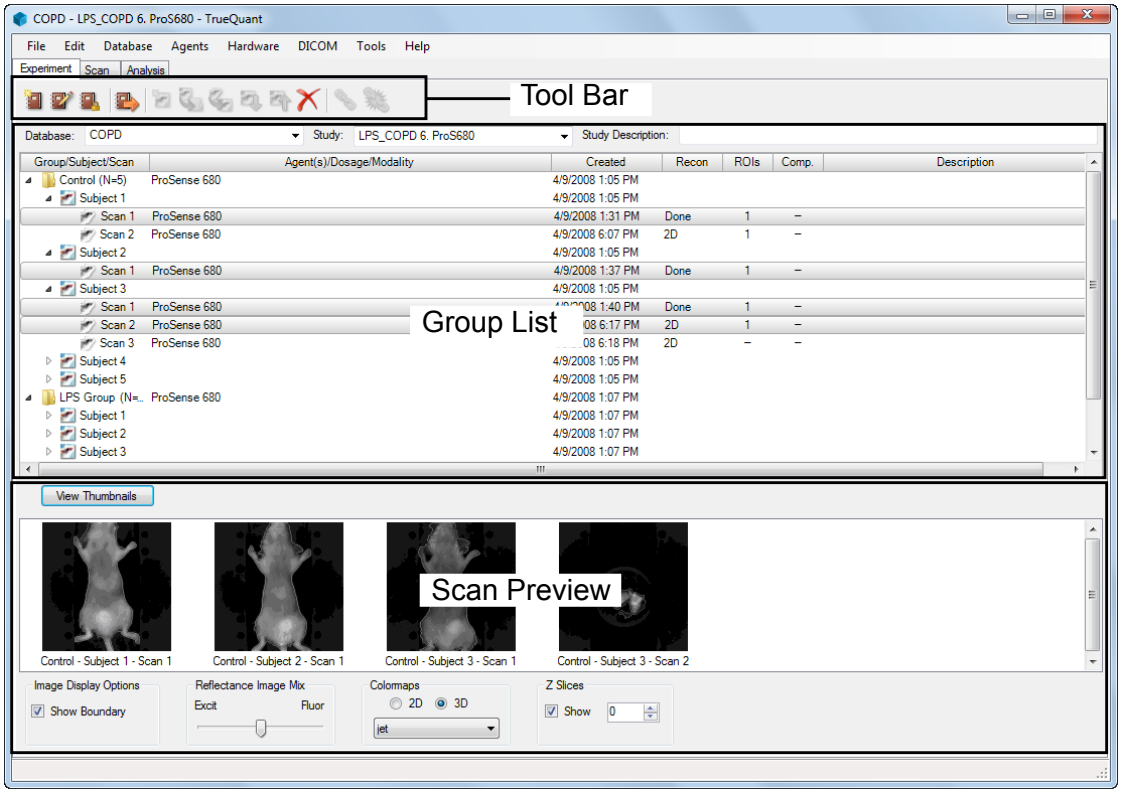


Figure 5-1. Experiment Tab

Experiment Tab Tool Bar - Used to create and edit studies and subjects. See Creating a New Study, Adding, Removing and Reassigning Subjects, and Editing Properties.

Group List - Displays the groups, subjects, and scans in the selected study. See Adding, Removing and Reassigning Subjects, and Reassigning Scans.

Scan Preview - Displays thumbnail previews of the selected scans. See Viewing Scan Thumbnails in the Experiment Tab.

5.1.1 Experiment Tab Tool Bar

The Experiment Tab Tool Bar contains the buttons described below.

Icon	Button Name	Description
	New Study	Opens the New Study window to create a new study.
	Edit Study	Opens the Edit Study Setup window to edit the currently open study.
	Lock/Unlock Study	Locks the currently open study or unlocks a locked study. An unlocked study is permanently protected from some changes and cannot include any new scans. See “Locking and Unlocking Studies” on page 44 for details.
	Export Study Design	Exports the design of the active study to a text of CSV file. See “Exporting a Study Design” on page 52 for details.
	New Subject	Opens the New Subject window to add a new subject to the selected group. See “Adding, Removing and Reassigning Subjects” on page 34 for details.
	Reassign Subject	Opens the Reassign Subjects window to assign the selected subjects to a different group. See “Adding, Removing and Reassigning Subjects” on page 34 for details.
	Reassign Scan	Opens the Reassign Scans window to assign the selected scans to a different subject.
	Remove Subject	Removes the selected subjects from the group. The subjects are not permanently deleted. See “Adding, Removing and Reassigning Subjects” on page 34 for details.
	Restore Subject	Restores a subject that has been removed from a group.
	Delete Item(s)	Permanently deletes the selected scans, subjects, or groups from the database.
	Link and Co-Register Datasets	Links an anatomical dataset with FMT scans, and co-registers the FMT scans with the anatomical dataset, see “Link and Co-Register Datasets” on page 37.
	Remove Dataset Links	Unlinks an anatomical dataset from FMT scans.

5.2 Creating a New Study

The TrueQuant Study contains the set of study groups, the animal subjects, metadata associated with the study (study description, IACUC protocol #, etc.), and metadata associated with the study groups (animal strain, age, agents used, etc.). The events associated with a study, such as dates and times of disease milestones, treatments, and agent injections are also important metadata stored with the study.

The studies created in TrueQuant are saved in SQL databases. If the desired database does not exist, create the new database on the local computer or on a network server (see “Creating Databases” on page 109). For details on setting up the Host and Investigator computers and for Windows Security Group requirements, see the *TrueQuant IT Setup Guide*.

To create a new study:

1. Select the desired database in the **Database** drop-down list on the Experiment tab (see Figure 5-2). If the desired database does not exist, create the new database on the local computer or on a network server (see “Creating Databases” on page 109).

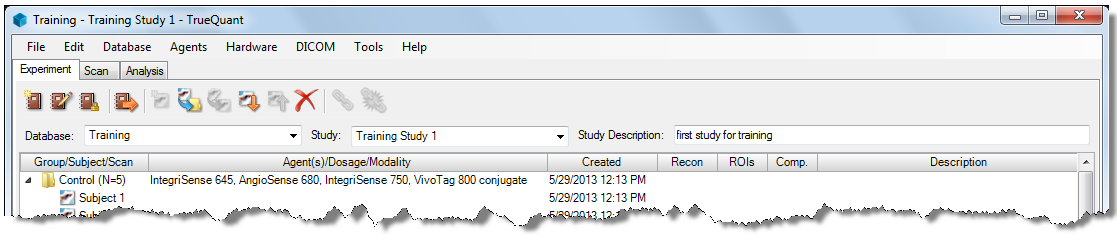


Figure 5-2. Experiment Tab

2. Click the **New Study** button at the top of the Experiment tab. The **New Study** window opens as shown in Figure 5-3.

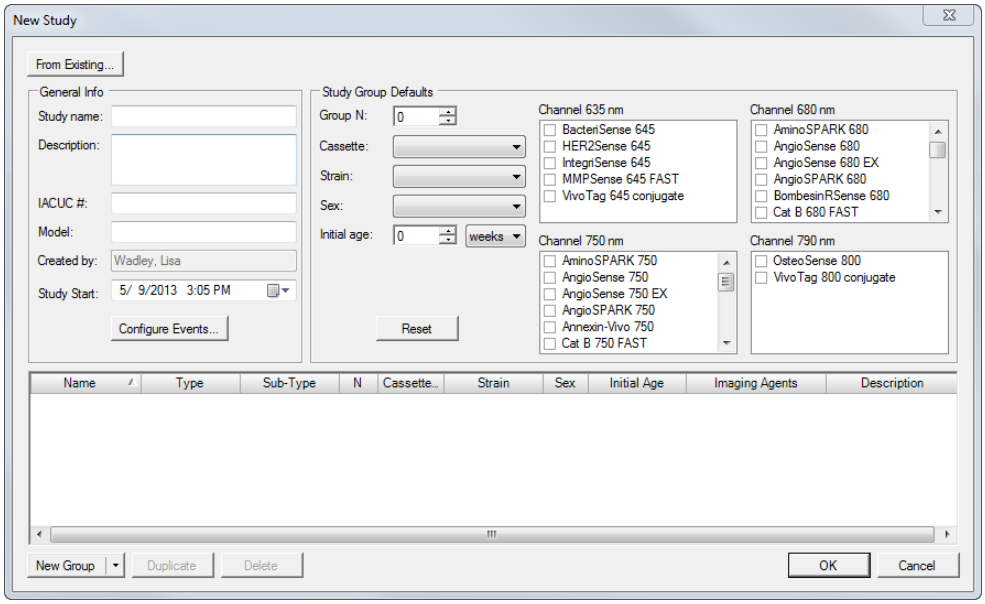


Figure 5-3. New Study Window

3. Type the desired name in the **Study Name** text box
4. Type a description of the study in the **Description** text box, if desired. The Description displays in the Experiment tab and in the Manage Databases window and can be used to identify the contents of the study.
5. Type the facility IACUC number in the **IACUC#** text box, if desired.
6. Type the animal model in the **Model** text box, if desired.
7. If necessary, change the **Study Start** date and time by either editing the date and time in the text box or clicking the calendar drop-down arrow and selecting the desired date.
8. To add events to the study, click the **Configure Events** button. See “Adding Events to the Study” on page 43 for more information.
9. See “Setting the Study Group Defaults” on page 33.

The Group List in the **Experiment** tab only displays the groups in the study selected in the **Study** drop-down list.

5.2.1 Setting the Study Group Defaults

The Study Group Defaults in the New Study window (see Figure 5-3) are used as the default values for each new group that is created in the study. The individual group settings can be changed after a group is created, but since most groups use the same, or very similar, settings, selecting the Study Group Defaults saves time when creating multiple groups in a study.

To specify the Study Group Defaults:

1. Enter the number of subjects in each group in the **Group N** text box. Either type the desired number in the text box or use the up and down arrow buttons to set the number of subjects.
2. Select the type of cassette that will be used to hold the subjects in the **Cassette** list, either Mouse or MSIM (Multi-Species Imaging Module).
3. Select the **Strain** of the subject. The available strains depend on the Cassette type selected. For more information, see “Animal Strains” on page 106.
4. Select the **Sex** of the subjects: Male, Female, or Mixed.
5. Select the **Initial Age** of the subjects, in either Days or Weeks. Type the desired value or use the up and down arrow buttons to select the age.
6. Select one or more Agents to detect in the **Channel** list boxes. The Channels that display depend on the license key and instrument model. See “Imaging Agents and Agent Calibration” on page 96 for more information on agents. Each group must have at least one imaging agent assigned to the group.

The settings are used when Creating Groups in the New Study Window.

If necessary, click the **Reset** button to clear the selections in the Study Group Defaults.

5.2.2 Creating Groups in the New Study Window

Groups in the study can divide subjects into the following types: Positive Control, Negative Control, Experimental, or Treatment. New groups are created in the New Study window (see Figure 5-3). Existing groups are edited in the Edit Study Group window.

To create a new group in the New Study window:

Use one of the methods below:

- Click the **New Group** button to create a new Positive Control Group.
- Click the down-arrow on the New Group button to display the available Group Types and then click Positive Control, Negative Control, Experimental, or Treatment.
- To create a copy of a group, click on the group to select it (highlighted in blue), and then click the **Duplicate** button.

To select the properties for a group in the New Study window:

1. Click on the group in the group list at the bottom of the window to select the group (highlighted in blue).
2. Click on the property to be changed. For the Name, Sub-Type, and Description properties, type the desired text in the text box. For imaging agents, click the "**...**" button and then select the desired agents in the window that opens. For the remaining properties, either type the desired value or select the value from the drop-down list.

To remove groups from the New Study window:

- Click on the group to select it (highlighted in blue), and then click the **Delete** button.

When all of the desired groups have been created, click **OK** to close the New Study window and display the groups in the **Experiment** tab.

To add subjects to the groups, see "Adding, Removing and Reassigning Subjects" on page 34.

5.3 Adding, Removing and Reassigning Subjects

Subjects can be added, removed, restored or reassigned to or from a group. This section contains the following:

5.3.1 Adding a New Subject to a Group

After a group is created in the New Study window, additional subjects can be added to an existing group.

To add a new subject:

1. Open the study in the TrueQuant window.
2. Click on the group name to highlight the group to add the subject to.
3. Click the **New Subject** button on the toolbar. The New Subject window opens (see Figure 5-4) and displays the next subject number.

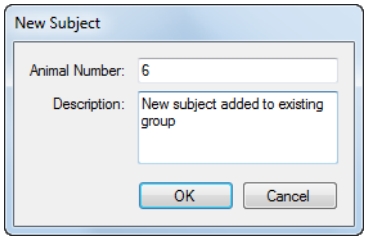


Figure 5-4. Adding a new subject to a predefined group.

4. If desired, change the Animal Number. (The animal number must be unique in each study group.)
5. Type a description if desired.
6. Click **OK** to add the new subject to the group.

5.3.2 Removing a Subject from a Group

A subject can be removed from a group during a study, for example, if a subject dies during a study. Existing scans of that animal can still be analyzed, but no new scans can be added to the subject.

To remove a subject:

1. Open the Study in the TrueQuant window.
2. Expand the Group that contains the study.
3. Click on the name of the subject to be removed.
4. Click the **Remove Subject** button on the toolbar.
5. Click **OK** in the confirmation dialog to remove the subject from the group. The removed subject displays in gray text as shown in Figure 5-5.

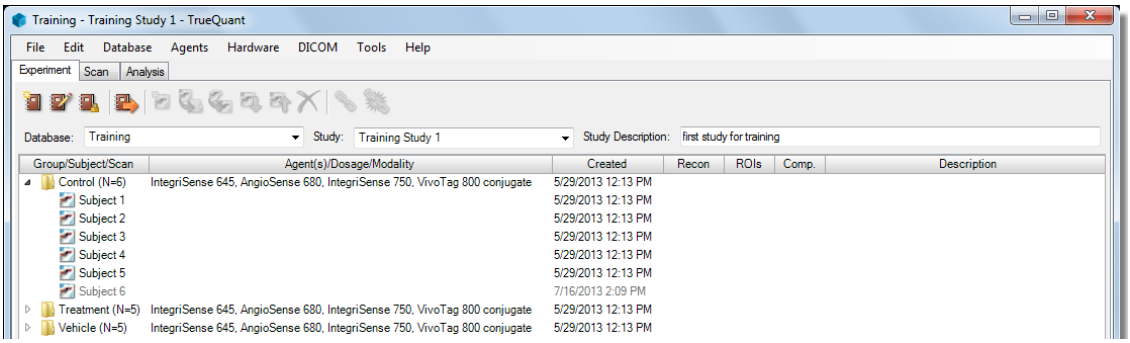


Figure 5-5. Subject 6 was removed from Group 1, and is shown in light gray

5.3.3 Restoring a Subject to a Group

If a subject was removed from a group using the Remove Subject button and needs to be restored, click on the subject and click the **Restore Subject** button on the tool bar.

Note: Subjects that were removed using the **Delete Item** button cannot be restored.

5.3.4 Deleting a Subject

To permanently delete a subject from a group, click on the subject and then click the Delete Item button. The selected items (subjects or groups) are permanently deleted. Any scans of the deleted subject and analyses of those scans are also deleted. To remove the subject from a group and keep existing scans, see “Removing a Subject from a Group” on page 35.

5.3.5 Assigning a Subject to a Different Group

If a subject is assigned to the wrong group, it can be reassigned to a different group.

To assign a subject to a different group:

1. On the TrueQuant window, click on the Subject name to highlight the row.
2. Click the **Reassign Subject** button on the toolbar or right-click on the subject and select **Reassign**. The Reassign Subjects window opens as shown in Figure 5-6.

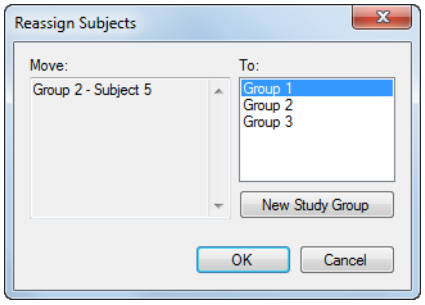


Figure 5-6. Reassign Subjects Window

3. Click on the name of the group. If the Group does not exist, click the **New Study Group** button and see “Creating a New Study” on page 32.
4. Click **OK**.
   - If a subject with the same number already exists in the group, the subject number is changed to the next higher number and the dialog shown in Figure 5-7 displays the new subject number.

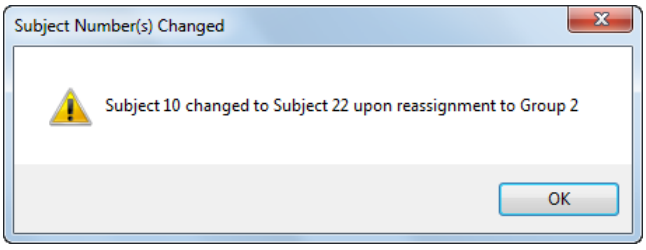


Figure 5-7. Subject Number Changed Window

- If the subject number is unique in the new group the subject number remains the same.

5. The subject displays in the new group and the reassignment is recorded in the item history.

Subjects can also be dragged and dropped onto a different group. A confirmation window prompts you to verify the reassignment. Clicking Yes moves the subject to the new group. Clicking No cancels the reassignment. If the subject is renumbered, the Subject Number Changed Window displays as shown in Figure 5-7.

5.3.6 Link and Co-Register Datasets

5.3.6.1 Linking Datasets in the Experiment Tab

Linking datasets enables the user to link an Anatomical dataset with an FMT image to create depth of the subject. When linking datasets, the datasets co-register automatically.

To link datasets:

1. Scan the animal in the CT instrument, using the appropriate FMT cassette adapter. Orient the animal the same as the orientation in the FMT and verify that the cassette is fitted properly in the cassette adapter.
2. Export the CT scan data in DICOM format to a location that is accessible to the TrueQuant software. Use a name that identifies the scan data, such as <studyname>_<groupname>_<subject#>_date_time.
3. Move the animal to the FMT. (The FMT scan can be done before the CT scan, if desired.)
4. Open the desired study and scan the animal.
5. Reconstruct the image, either manually or as part of the scan.
6. Import the DICOM dataset from the CT scan:
   a. Select the subject in the Experiment tab, right-click on the subject, and select **Import Non-FMT DICOM Series**.
      OR
      Select **File | Import | Non-FMT DICOM Series** and select the subject in the **Select Subject for Import** window.
   b. Navigate to the data folder that contains the exported CT DICOM data files. There may be multiple files.
   c. Select one of the DICOM (*.dcm) files.
   d. Click **Open** and wait until the CT scan data is imported. The Anatomical Data Properties window opens.

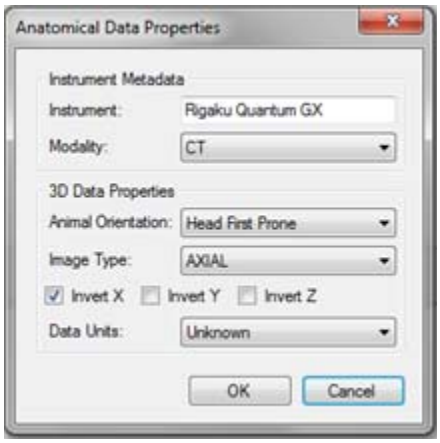


Figure 5-8. Anatomical Data Properties Window

e. Select the Modality, Animal Orientation, and Image Type settings and click the **OK** button.

- Modality: Modality is the CT or FMT type of image.
- Animal Orientation: Animal Orientation consists of eight options, Head-First Supine, Head-First Prone, Feet-First Supine, Feet-First Prone, Head-First Decubitus Left, Head-First Decubitus Right, Feet-First Decubitus Left, and Feet-First Decubitus Right.
- Image Type: Axial, Sagittal or Coronal. The Image Type is determined from the DICOM header.

NOTE The Processing Datasets window will be displayed while CT Segmentation is performed. Wait for segmentation to complete.

NOTE For feet-first scans acquired on the Quantum GX, the **Invert X** and **Invert Y** check boxes will both need to be selected when importing CT data that will be linked to an FMT scan.

f. The CT scan is added to the selected subject, but the datasets are not yet linked.

7. Select both the FMT scan and CT scan in the Experiment tab.

8. Right-click on one of the highlighted scans and select **Link Datasets** or click the **Link** button in the Experiment tab toolbar. (You can also drag one scan and drop onto the other scan to link them.)

NOTE Datasets with animals in different orientations cannot be linked together.

NOTE If co-registration fails, the scans cannot be linked. Verify the correct files were selected, are oriented properly, and stitched correctly.

9. When processing is complete, the datasets are linked and co-registered. The CT data is segmented into Body, Bones, Lungs, and Heart. When one of the datasets is selected, all linked datasets display in italic text to indicate they are linked.

5.3.6.2 Unlinking Datasets in the Experiment Tab

Unlinking datasets deletes the co-registration information from the datasets, and separates the files. Unlinking can be done two ways.

1. Right-Click on one of the linked scans in the Experiment Tab.
2. Click **Unlink Datasets** from the drop-down menu.
3. The Remove Links window opens to confirm the unlinking.

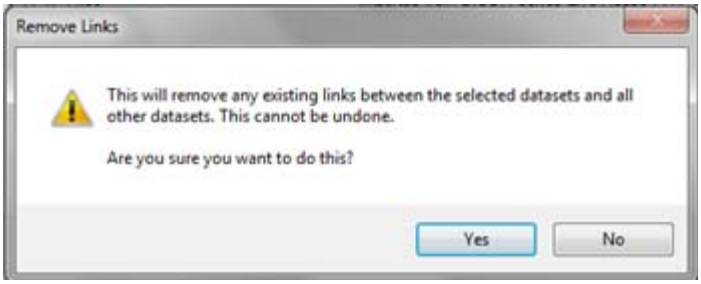


Figure 5-9. Remove Links Window

OR

1. Select one of the linked scans in the Experiment tab.
2. Click the **unlink** button on the tool bar.
3. The Remove Links window to confirm unlinking opens (see Figure 5-9).

5.4 Opening an Existing Study

To open an existing study:

1. On the Experiment tab, select the name of the database that contains the study in the **Database** drop-down list.
2. Select the name of the study to open in the **Study** drop-down list. The Groups in the study display in the Group table.

5.5 Editing Properties

Properties for a database, study, group or subject can be edited after it has been created.

5.5.1 Database Properties

- To edit a database name or description, select **Database** | **Manage** from the main menu. Click the desired database, then click on either the name or description to edit the corresponding text field (see Figure 5-10).

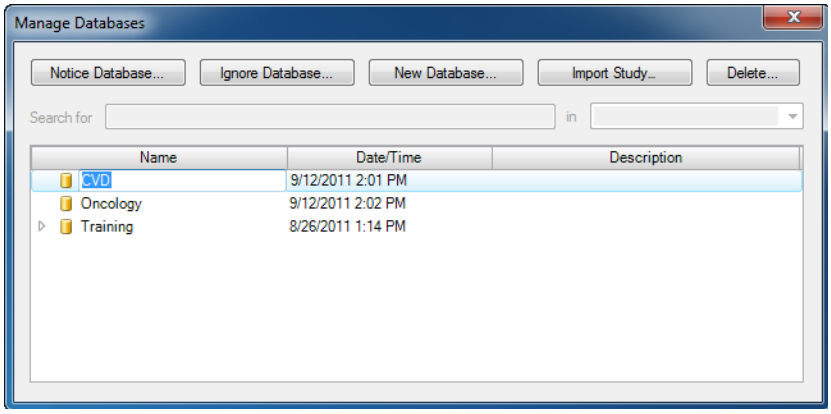


Figure 5-10. Changing the name of a database

5.5.2 Study Properties

- To rename a study, click the **Edit Study** button on the **Experiment** tab tool bar to open the Edit Study Setup window. Change the Study Name and click the OK button.
- To change a study's description, click in the **Study Description** field at the top of the TrueQuant window and modify the text as desired. (The Study Description can also be changed on the Edit Study Setup window.)

NOTE Study names and descriptions can also be changed in the Manage Databases window by clicking on the text field.

- To change study data such as the IACUC number, animal model, study start time, or study events, click the **Edit Study** button on the **Experiment** tab tool bar to open the Edit Study Setup window.

5.5.3 Group Properties

- To change a group name or description in the Group list on the Experiment tab, click on the group row and then click on the name or description field.
- To change group properties, click the **Edit Study** button on the **Experiment** tab tool bar to open the Edit Study Setup window. (To change the number of animals in a group, use the Remove Subject or Delete Items button on the Experiment tab tool bar.
- To edit the agent assignment for the group, right click on the group row and select **Properties**. The **Group Properties** window (Figure 5-11) opens. Click the Browse (...) button next to the Imaging Agents text box. The Select Imaging Agents window (Figure 5-12) opens. Select or clear the desired Agents for each channel and click the OK button.

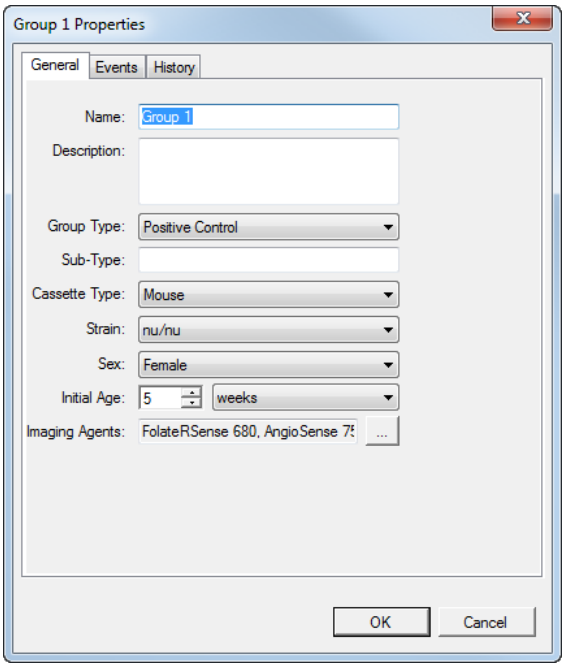


Figure 5-11. Study Group Properties Window

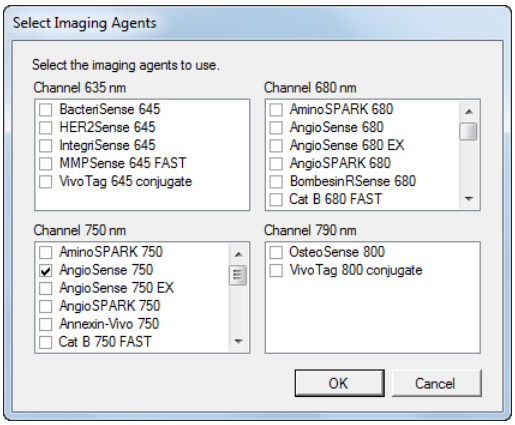


Figure 5-12. Select Imaging Agents Window

5.5.4 Subject Properties

- To change a subject's number or description in the Group list on the Experiment tab, click on the subject row and then click on the subject number or description field.

5.6 Viewing Scan Thumbnails in the Experiment Tab

The Experiment tab displays a thumbnail image of selected scans in the Thumbnail View below the Group list. Open thumbnail images to quickly view multiple scans side-by-side to visually identify the scans without opening the scans in the Analysis tab. If multiple thumbnails are open, the view settings apply to all of the open thumbnails.

To view scan thumbnails:

1. Select the scans for which to display thumbnails:
   - To select a single scan, expand the group (either click the arrow next to the group name or double-click on the group name), expand the subject (either click the arrow next to the subject name or double-click on the subject name), and then click on the scan name.
   - To select multiple scans, CTRL + click the scan names.
   - To select a contiguous block of scans, click the first scan name and then Shift + click the last scan name.
2. Click the **View Thumbnails** button. Thumbnails of the selected scans display in the Thumbnail View. (The thumbnails are static and only change when the View Thumbnails button is clicked.)
3. Select or clear the **Show Boundary** check box to show or hide the subject boundary.
4. Move the Reflectance **Image Mix** slider to the desired transparency setting.
5. Select either 2D or 3D to apply the selected color map to the fluorescence reflectance image (2D) or the Z slices of the reconstruction (3D) under ColorMaps, and then choose the desired color scheme.
6. Select or clear the Z Slices check box to show or hide the slices of the reconstruction. If selected, select the desired slice number, in the Z direction, to view in the thumbnail. Slices are numbered from zero, which is the slice farthest from the camera. Increasing numbers display sequential slices closer to the camera.

5.7 Reassigning Scans

Completed scans can be reassigned to a different subject. either by using the Reassign Scans window or by drag-and-drop. See the instructions below.

To assign scans to a different subject using the Reassign Scans Window:

1. On the Experiment tab, select one or more scans and click the **Reassign Scan** button. (CTRL + click or Shift +click to select multiple scans.) The Reassign Scans window opens as shown in Figure 5-14.

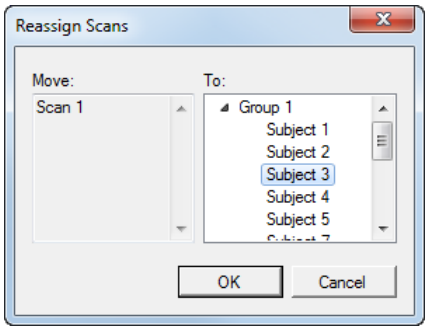


Figure 5-13. Reassign Scans Window

2. Expand the desired group.

3. Click on the subject to assign the scan to and click the **OK** button. The listed scans are reassigned to the selected subject.

To assign scans to a different subject using drag-and-drop:

1. On the Experiment tab, select one or more scans. (CTRL + click or Shift +click to select multiple scans.)
2. Drag and drop the scans onto the desired subject. To expand a collapsed group or subject, hover over the group or subject name.

When the reassignment is complete, the scans display in the destination group or subject. The scans are renumbered automatically if the scan number already exists in the destination subject, and the reassignment is recorded in the Subject Properties on the History tab (Figure 5-14).

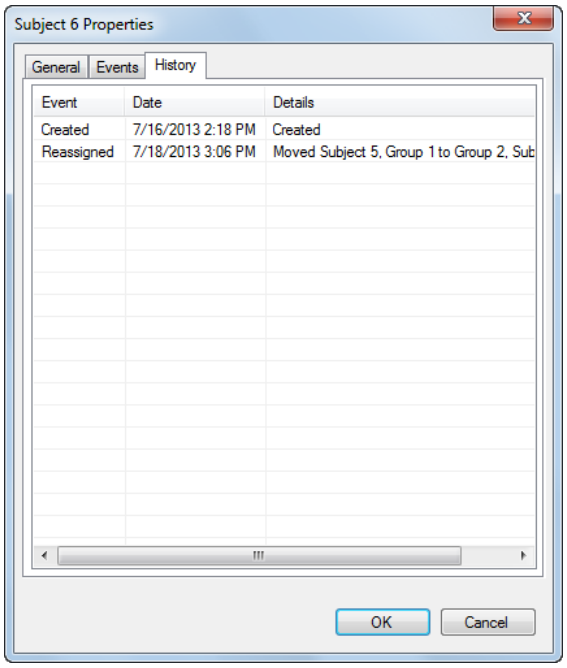


Figure 5-14. Subject Properties Window, History Tab

5.8 Adding Events to the Study

Events can be added to a study to mark the date and time of specific events, such as agent injections, treatments, disease onset, etc. When events are included in the study, the exported ROI Analysis table includes a column for each event. The column displays the elapsed time between the Scan date and time and the Event date and time.

To add an event to a study:

1. On the Experiment tab, open the database and study.
2. Click the **Edit Study** button to open the Edit Study Setup window.
3. Click the **Configure Events** button. The Configure Events window opens as shown in Figure 5-15.

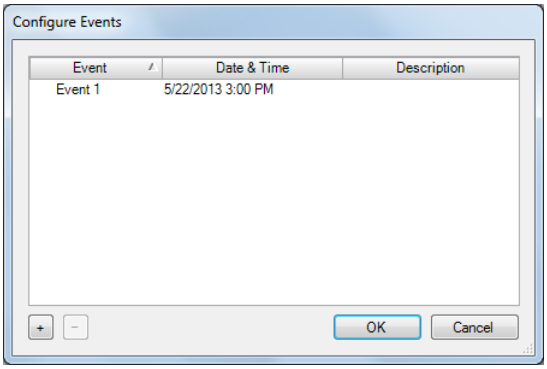


Figure 5-15. Configure Events window

4. Click the **Add** (+) button to add a new event to the study.
5. Select the row and then click in the **Event** column to edit the name of the event.
6. Select the row and then click in the **Date and Time** column to set the time of the event.
7. If desired, type a **Description** of the event.
8. Add additional events as desired.
9. Click **OK** to add the event to the study.

To remove an event from the study, select the event in the Configure Events window and click the **Remove** (-) button.

5.9 Locking and Unlocking Studies

Studies can be locked to prevent changes to the study. Studies can be unlocked to allow specific modifications, but some settings remain permanently locked even after the study is unlocked.

Locking Restrictions

- A study cannot be locked if the study or any items in the study have been in use in online mode for less than 4 hours or in offline mode for less time than the parent database's retention period, roughly 14 days. If the study or any items in the study have been in use in either scenario for longer than the specified time, the user can choose whether or not to lock the study after being presented with a warning message about the study usage.

5.9.1 Locking Studies

The following restrictions apply to locked studies:

- The study name and description cannot be changed.
- The study design cannot be changed.
- Items and item properties in either the Experiment tab or the Analysis tab cannot be modified or deleted.
- The study cannot be moved to a different database.
- The study cannot be deleted.
- The study's parent database cannot be deleted.
- New scans cannot be acquired.
- Scans cannot be imported into the study.
- Scans cannot be added to the reconstruction queue.

To lock a study:

1. Open the study in the **Experiment** tab.
2. Click the **Lock Study** button on the tool bar. The Lock Study Confirmation window opens.
3. Click **Yes** to permanently lock the study or click **No** to cancel locking the study.

5.9.2 Unlocking Studies

The following restrictions apply to unlocked studies:

- The study name and description cannot be changed.
- The study design cannot be changed.
- Items and item properties in either the Experiment tab or the Analysis tab cannot be modified or deleted if those items were created prior to the most recent time the study was unlocked.
- ROIs that existed prior to the “unlock” event cannot be modified in any way.
- The study cannot be deleted.
- The study's parent database cannot be deleted.
- New scans cannot be acquired.
- Scans cannot be imported into the study.

The following actions are permitted in unlocked studies:

- ROIs can be added to existing scans.
- New computed datasets can be created from existing scans.
- ROIs that were added after the most recent “unlock” event can be modified.
- Existing scans can be added to the reconstruction queue.
- Items created after the most recent unlock event can be modified or deleted.
- The study can be moved to a different database.
- The imaging cassette version and animal orientation of any scan can be changed during DICOM export.

To unlock a study:

1. Open the study in the **Experiment** tab.
2. Click the **Unlock Study** button on the tool bar. The Unlock Study Confirmation window opens.
3. Click **Yes** to unlock the study or click **No** to leave the study locked.

5.10 Exporting and Importing Data

After scans are completed, data can be exported from the **Experiment** tab by highlighting a scan, and selecting **File** | **Export** from the menu (Figure 5-16). Three data export options are provided:

- **ROI Analyses**, using the Comma Separated Value (CSV) file format (.csv), which can be read by a spreadsheet program. (See Exporting ROI Data.)
- **Scan Data in FMT format**, a format (.fmt) to save images. (See “Exporting and Importing Scan Data” on page 47.)
- **Scan Data in DICOM format**, a format to save images. (See “DICOM Export” on page 50.)

To export Study Designs, see “Exporting a Study Design” on page 52. To export images, see “Exporting Images” on page 93. To export and import entire studies using TrueQuant database management, see “Exporting Studies” on page 111.

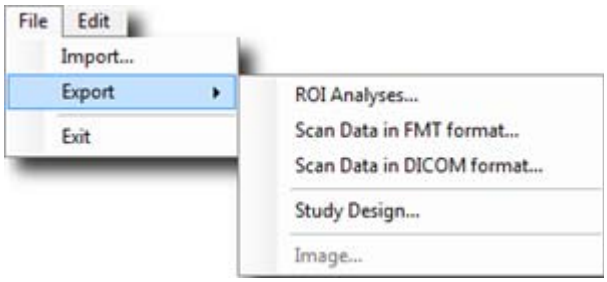


Figure 5-16. The TrueQuant Export menu.

5.10.1 Exporting ROI Data

Exporting ROI data to a CSV file exports all associated data for each ROI in the selected scans.

1. In the Experiment tab, select the scans that contain the ROIs to export.
2. Select **File** | **Export** | **ROI Analyses** from the menu
3. Type the desired file name and navigate to the desired location for the saved file.
4. Click the **Save** button to open the **CSV Export Column Selection** window (see Figure 5-17).

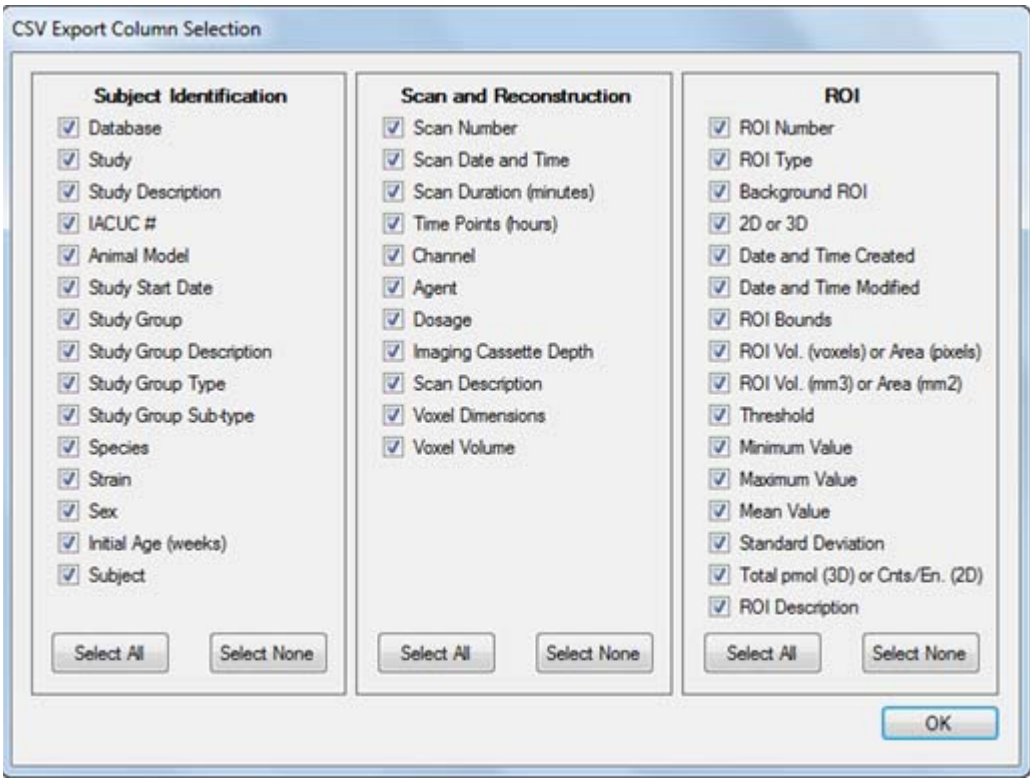


Figure 5-17. CSV Export Column Selection

5. Select the data to export. All database items are selected by default. Deselect items as desired.
6. Click **OK** to create the exported .csv file.

5.10.2 Exporting and Importing Scan Data

5.10.2.1 Importing Scan Data

Scans previously saved in the FMT format can be imported using the **File** | **Import** menu (Figure 5-18). Each imported scan is placed in the active study under a study group with the same name and a subject with the same number it had in its original study. You can also import FMT format exports from previous versions of TrueQuant that were saved as `.zip` files.

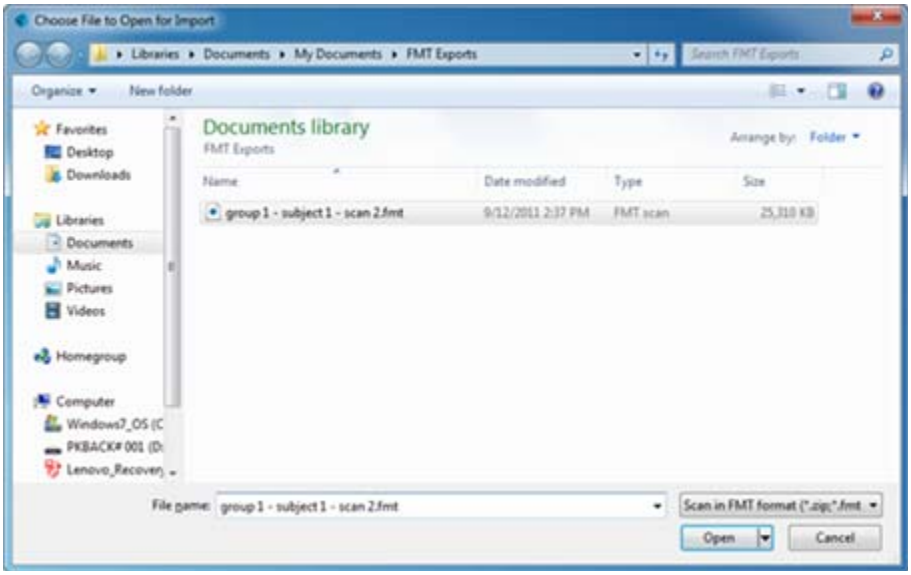


Figure 5-18. Importing a scan

5.10.2.2 Exporting Scan Data

You can export 2D or 3D scan images and data using the FMT (.fmt) format.

1. Select the scan or scans to export in the Experiment tab.
2. Select **File** | **Export** | **Scan Data in FMT Format** from the menu and specify the desired name and location for the saved file.
3. Click the **Save** button to create the .fmt file.

5.10.3 DICOM Import

1. Select **File** | **Import** in the Experiment tab.
2. The Anatomical Data Properties window opens.

NOTE When importing data in the GX DICOM format, the image needs to be rotated or flipped. For GX data, select the **Invert X** check box. For all GX data in the feet-first position, select the **Invert X** and **Invert Y** check boxes in the Anatomical Data Properties window during Import.

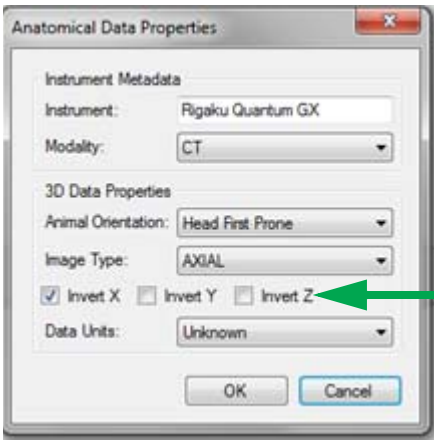


Figure 5-19. Anatomical Data Properties Window

3. The Processing Datasets window opens while the CT scan is being segmented.

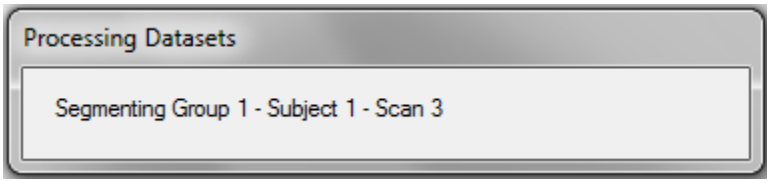


Figure 5-20. Processing Datasets Window

a. If the segmentation fails, the Segmentation Failed window opens, details about the failure will be written to the TrueQuant logs.

NOTE Situations that can cause segmentation failure include poor stitching of sub-scans if the Quantum bed is out of alignment, use of a CT contrast agent, and other rarer cases.

b. Click the **OK** Button.

c. Open the log file to view the error.

d. The scan is imported on the Experiment tab.

e. When opened in the Analysis Tab. Select the **Complete data** set radio button.

NOTE The volume rendering and Isosurface will function. The show animal only and segment features will not function.

5.10.4 DICOM Export

Scans and their associated reconstructions can be exported as DICOM series both as plain files and as a transmission directly to a PACS (image management) server. The exported DICOM series conform to the DICOM specification for “other” modalities not otherwise defined by DICOM. A number of popular DICOM viewers can be used with these series. The following DICOM viewers are supported:

Viewer Name	Platform	Multi-modality Fusion	Notes
AMIDE	All	Yes	16-bit only
Anatomist	All	Yes	16-bit only
DicomWorks	Windows	No	
ImageJ	All	No	
IrfanView	Windows	No	16-bit only
OsiriX	Mac	Yes	
Slicer	All	Yes	
XMedCon	All	No	

Note, these DICOM viewers are verified as compatible with TrueQuant data; other viewers may be functional.

To preserve the maximum possible dynamic range of the reconstructed data, DICOM export saves the data in the reconstruction series as 32-bits per pixel. Some DICOM viewers, including some of those listed in the table above, are limited to only reading 16-bit data. To use a viewer that does not support 32-bit images, change the output format to 16-Bits by selecting **Tools** | **User Options** to open the **Options** window, clicking the **DICOM** tab, and then selecting the desired bit depth.

NOTE The bit depth setting for output of DICOM series is saved on a per-user basis rather than for all users of the computer as was the practice with versions of TrueQuant prior to V3.0. If the bit depth was set using versions of TrueQuant prior to V3.0, the previous setting overrides the current version’s default setting. However, any change made to the bit depth setting after upgrading to the current version of TrueQuant only changes the setting for the current user.

5.10.4.1 Exporting DICOM Files

To export individual scans and their reconstructions as DICOM series:

1. Select **File** | **Export** | **Scan Data in DICOM format**. The Export window opens.
2. Type the desired file name and click **Save** in the **Export** window. The **Animal Orientation** window (Figure 5-21) opens.

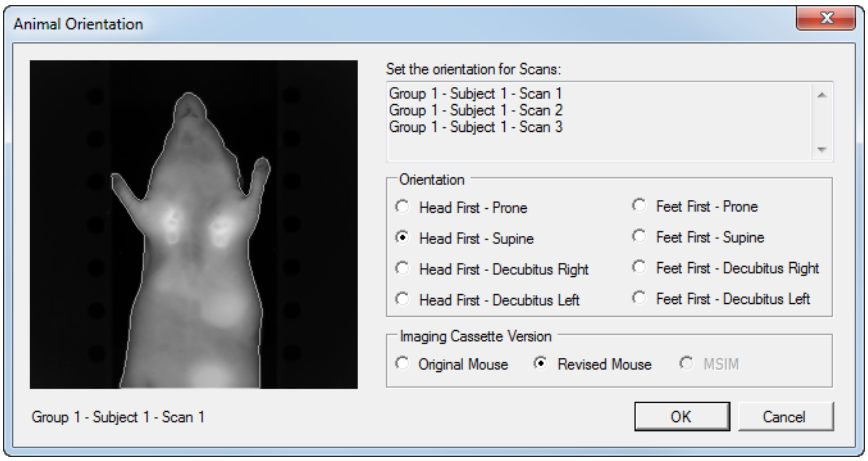


Figure 5-21. Animal Orientation window

3. Select the orientation of the animal in the exported scan or scans. This orientation is saved to the DICOM header and can be used to aid in co-registration of FMT reconstructions with data from other imaging modalities. Note that while the preview for the first scan in the batch is shown, the same orientation is used for all the files being sent so be careful to select only scans where the animal was oriented the same way. The orientations of animals in a large number of scans can be easily verified prior to DICOM transmission using **View Thumbnails** on the **Experiment** tab.

4. If necessary, select the version of the imaging cassette used during scan creation. For scans acquired with TrueQuant V2.1 or higher, the cassette version is saved when the scan is acquired. If the scan was previously exported as a DICOM series, the cassette version that was chosen for the previous export is selected by default.

   You cannot choose MSIM for the cassette type when exporting a scan as a DICOM series unless the cassette calibration is completed on the host machine. In addition, exports for scans acquired using the MSIM cassette only include fiducial marks when exported from the host PC. Correct positioning of the fiducial marks on the exported images requires the calibration information for the cassette, which only exists on the host machine.

5. Click **OK** to export the scan as a DICOM series. This creates a folder with the name specified in the **Export** window. The folder contains three DICOM series: one for the reflectance images, one for the transillumination images, and one for the reconstruction. DICOM exports of 2D scans (those where a transillumination scan was not performed) only contain the DICOM series for the reflectance images.

DICOM series exported from TrueQuant V4.0 or higher can be imported back into TrueQuant as FMT scans. DICOM series exported from versions prior to TrueQuant V4.0 cannot be imported into TrueQuant.

5.10.4.2 Send To PACS

You can also send DICOM exports directly to a PACS server. Before you can do this, both the local computer and the PACS server must be configured as DICOM nodes in TrueQuant. This setup step should be done by an administrator who is familiar with the local PACS configuration.

To configure local and remote DICOM nodes, select **DICOM** | **Configure AETs** from the main menu to open the **Set Up DICOM Nodes** window shown in Figure 5-22. Enter the name of your computer and the port to use for sending DICOM transmissions. Configure a new PACS server by clicking **New**, or edit the properties of an existing PACS server by clicking **Edit**, and fill in the fields in the **Remote DICOM node settings** window (also shown in Figure 5-22). You can choose the **Use DNS** option if you know the name of the PACS computer but not its IP address.

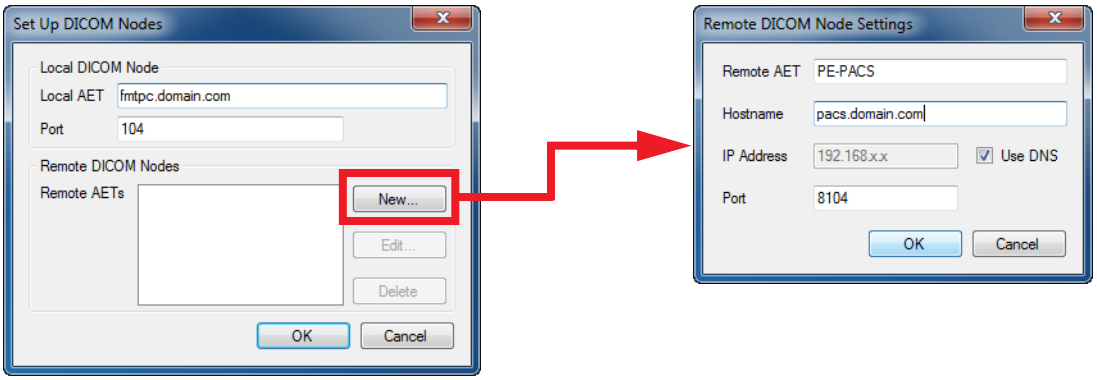


Figure 5-22. Configuring DICOM (AET) nodes.

Once the local and remote DICOM nodes are properly configured, imaging scans can be transmitted to remote nodes by selecting the scans in the **Experiment** tab and then selecting **DICOM** | **Send to PACS** | **Remote Node Name**, where *Remote Node Name* is the remote AET name that you configured above. The **Animal Orientation** window, shown in Figure 5-21, opens so that you can specify the correct animal orientation and cassette version. Choose the appropriate orientation and cassette version and click **OK** to add the scans to the selected node's DICOM transmission queue.

Scans are transmitted one at a time in the order listed in the queue. The queue functions identically to the reconstruction queue (see "Reconstruction Queue" on page 60) except that the active item cannot be removed from the queue.

5.11 Exporting a Study Design

To facilitate report generation, presentations, and other forms of communication, you can export the experimental design of the active study, as captured in the **Experiment** tab, into a text or CSV file.

Click the **Experiment** tab. Select **File** | **Export** | **Study Design** from the main menu. Specify a location and file name and select text or CSV output format. The list of study groups, subjects and scans, and other relevant information about them, is exported to the specified file.

6

The Scan Tab

Use the Scan tab to select the scan settings, scan the subject, and view the resulting images.

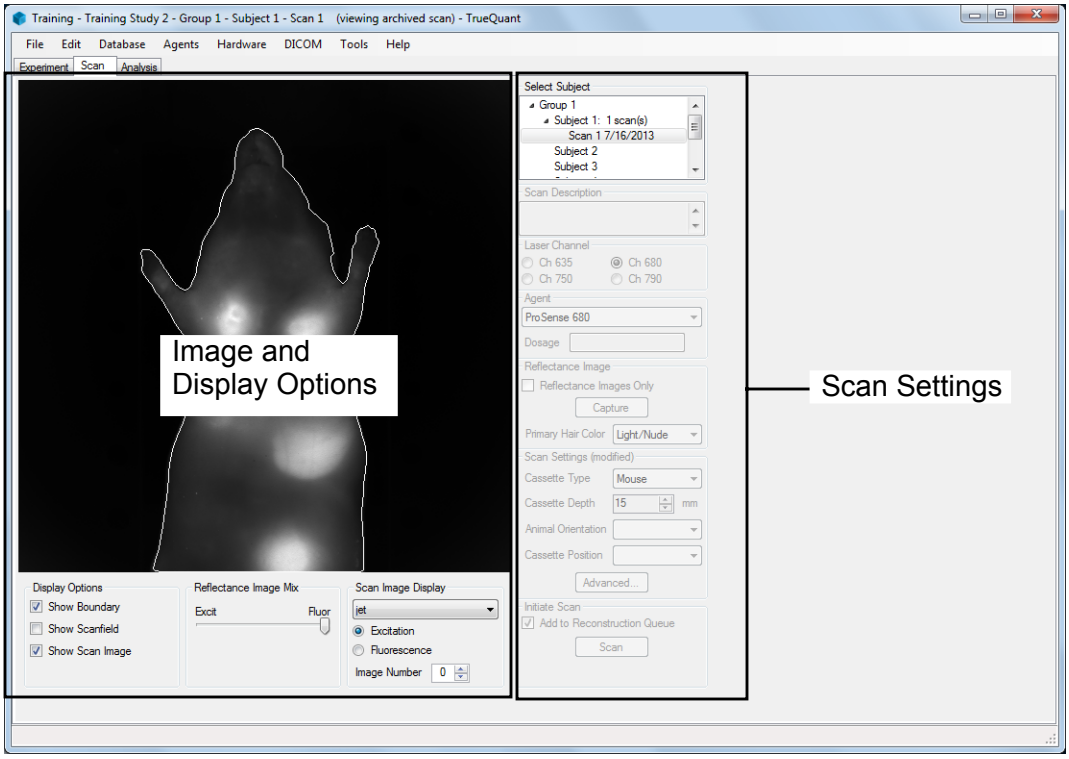


Figure 6-1. Scan Tab

6.1 Performing a Scan

The **Scan** tab controls the imaging of an animal. The currently loaded study is shown in the **Select Subject** list.

To scan a subject:

1. Click the **Scan** tab. The image area is blank and the only option available is the **Select Subject** list as shown in Figure 6-2.

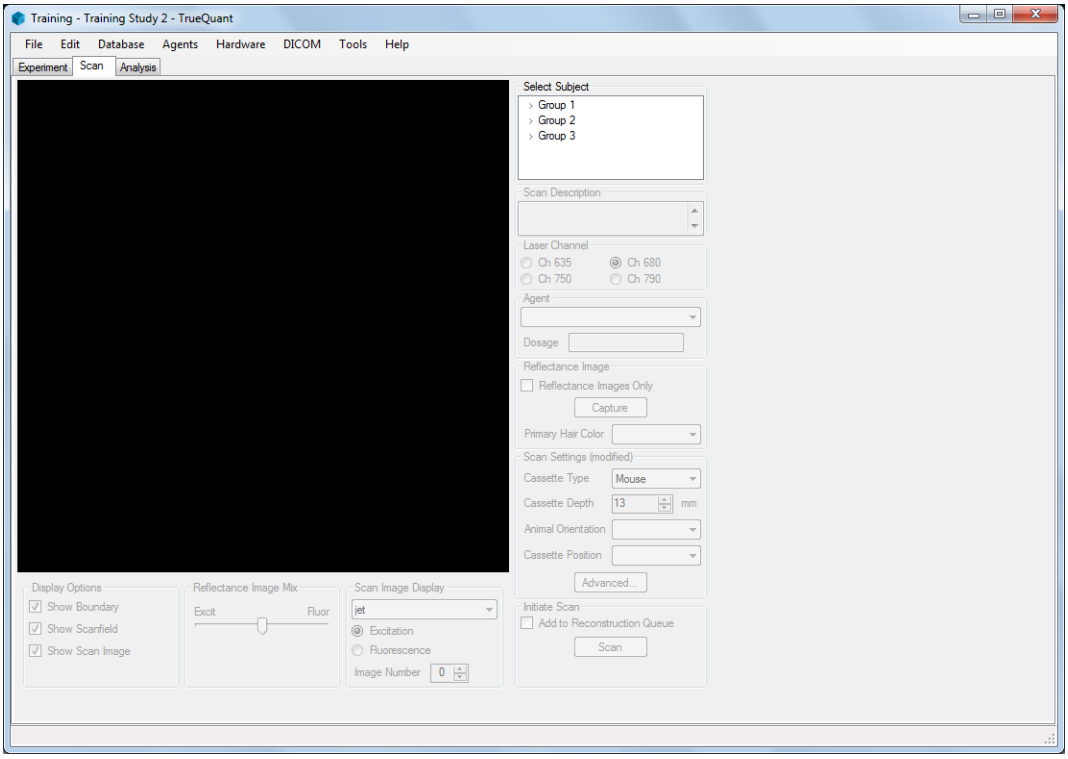


Figure 6-2. Initial view of the **Scan** tab

2. Select the subject to image from the **Select Subject** list. The Groups, Subjects, and Scans in the current study display in the Select Subject list.
3. Type a description of the scan in the **Scan Description** text box, if desired.
4. Select the **Laser Channel**, depending on the fluorescent agent injected into the subject.
5. Select the **Agent** to image from the drop-down list. The selection defaults to the agent selected for the group when the group was created (see Figure 5-3).
6. Type a description of the agent dosage in the **Dosage** text box, if desired.
7. To only acquire a reflectance image, select the **Reflectance Image Only** check box.
8. If desired, change the **Primary Hair Color** of the specimen. (The hair color is used to determine the boundary.) The hair color defaults to the color specified in the animal strain selected for the group.

9. Click the **Capture** button to acquire a Reflectance image.

NOTE If the excitation reflectance image is underexposed, TrueQuant will be unable to reliably compute a subject boundary. If this happens, you can save the captured images as a reflectance only scan, but a full scan will not be possible, since the subject boundary is required for a full scan and reconstruction.

10. If the **Reflectance Image Only** option is selected, the acquisition is complete. Click the **Analysis** tab and see "The Analysis Tab" on page 63.
    If the **Reflectance Image Only** option is not selected, the preliminary scan field displays on the reflectance image. Continue with the steps below.

11. Change the Cassette Type, Cassette Depth, Animal Orientation, and Cassette Position as necessary.
    - These options are set automatically based on automated image processing of the reflectance images. It may be necessary to override the pre-set values.
    - If Mouse cassette type is selected, the Cassette Position is not adjustable.

12. Click inside the **scan field** (see Figure 6-3) on the reflectance image and drag the scan field to the desired location. Note that as you move the scan field over the subject, the sides of the scan field automatically adjust to fall within the boundaries of the animal to avoid direct exposure of the detector to the laser.

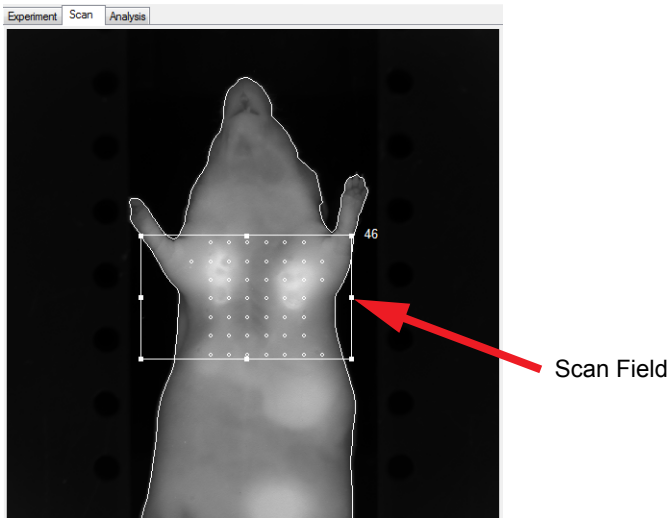


Figure 6-3. The **Scan** tab prior to initiating the laser scan

13. Resize the scan field to enclose the desired area to be scanned.
    - Click and drag one of the sizing handles on the sides or corners of the scan field to increase or decrease the size of the scan field.
    - As the scan field size or location is adjusted, the number of source locations displays near the upper right corner of the scan field (see Figure 6-3).
    - To achieve good localization of depth, a scan field width and height of approximately twice the size of a typical lesion, such as a tumor, represents a good rule of thumb.
    - For optimal results, keep the number of source locations in the range of 35-75 sources. Due to memory limitations, the number of sources should not exceed 120 total sources.
14. If desired, click the **Advanced** button to adjust the Source Density, Sensitivity, and Illumination. See “Advanced Scan Settings” on page 57.

NOTE On FMT 1000, 1500, and 2000 models, if the classic reconstruction module is installed, an estimate of the tomographic reconstruction time displays. For systems with Fast Recon, no time estimate is displayed.
The reconstruction time display is only a rough estimate; the exact reconstruction time is dataset-dependent, depending on the details of the boundary in the vicinity of the scan field. If the reconstruction time estimate exceeds 60 minutes, the font color of the time estimate switches from white to red to alert the user.

15. Select the **Add to Reconstruction Queue** check box to begin the tomographic reconstruction as a background process as soon as the scan completes. If not selected, reconstruction can be performed at a later time by manually adding the scan to the reconstruction queue (see *section 6.4*).
16. When the scan field is sized and positioned as desired, click the **Scan** button.

If an image saturates during the fluorescence half of the laser scan, the corresponding scan point is marked as invalid and excluded from the reconstruction. This is done to maintain a fully quantitative reconstruction while also allowing the scan quality to remain high even in the presence of missing scan points.

6.2 Advanced Scan Settings

The Advanced Settings window (Figure 6-4) overrides the system default settings for the following scan parameters:

- **Source density**: specifies the spatial density of the laser source positions during a tomographic scan. You can choose **Coarse** (5mm spacing between adjacent source locations), **Medium** (3mm, the default setting), **Fine** (2mm) or supply a custom setting.
- **Sensitivity**: adjusts the instrument's ability to detect weak fluorescence sources. Adjusting the **Sensitivity** setting to **High** or **Very High** detects progressively fainter sources of fluorescence but increases the chance of saturation from very bright sources.
- **Illumination levels (counts/pixel)**: adjusts the settings for the auto-exposure scheme in controlling laser power and exposure times to obtain optimal results. The default settings are 20,000 minimum and 50,000 maximum. If exposure times during the transillumination scan are very long or if individual scan points are frequently discarded during the excitation scan due to insufficient signal-to-noise (most often in areas of dense tissue, such as the heart), the minimum counts can be reduced to decrease the chances of discarding scan points. This may decrease the sensitivity to faint sources of fluorescence. A minimum setting below 5000 counts is not recommended.

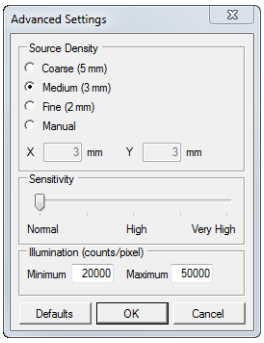


Figure 6-4. Advanced Scan Settings Window

6.3 Display Controls

After a scan is complete, use the controls below the main image panel on the Scan tab to adjust the display:

- **Show Boundary:** Show or hide the animal boundary as automatically extracted from the reflectance image.
- **Show Scan Field:** Show or hide the outline of the scan field and the scan point locations.
- **Show Scan Image:** If selected, displays the transillumination image taken at each scan point in the scan field. In the image number text box, specify the image to display. The corresponding scan point displays in red on the scan field image. No scan image displays if 0 is selected.
- **Reflectance Image Mix:** Adjust the balance between the excitation-wavelength and fluorescence-wavelength reflectance image overlays.
- **Scan Image Display:** Display the raw transillumination scan images for each laser location at either excitation or fluorescence wavelengths, in the selected color map.

An example is shown in Figure 6-5. The **Select Subject** list displays a scan entry for the subject.

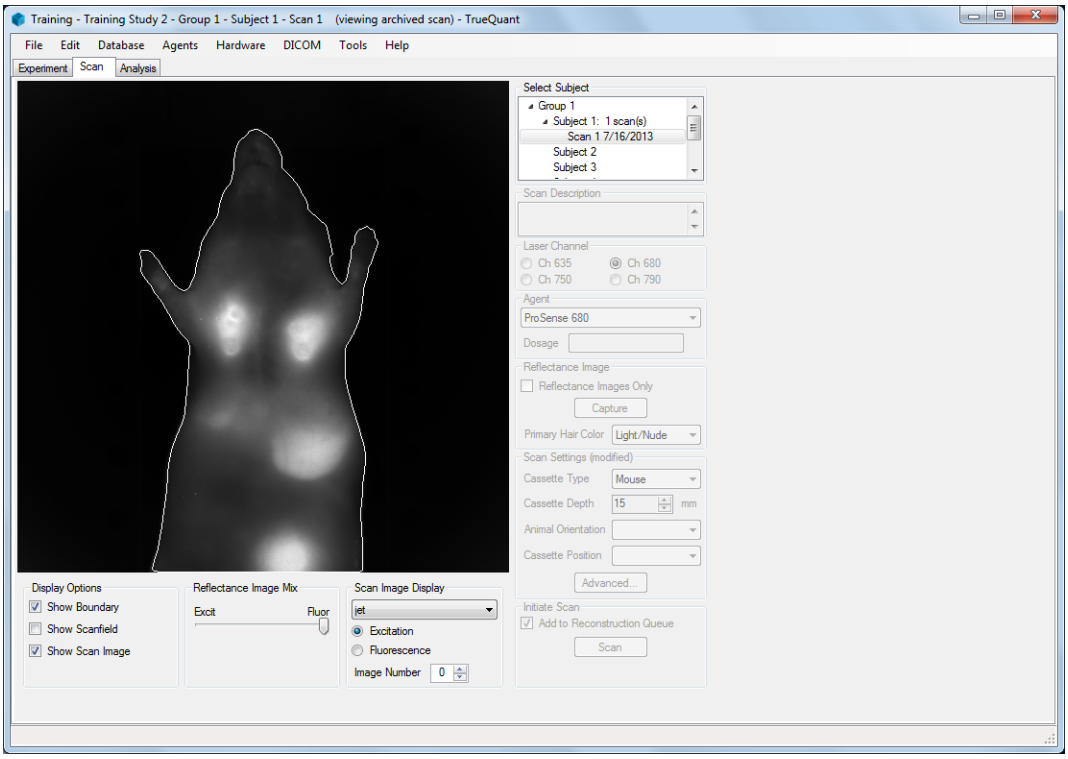


Figure 6-5. Display options on Scan tab

After a scan is complete, the **Experiment** tab shows the scan entry, the reconstruction status, and an optional scan and reconstruction preview below the main panel (see Figure 6-6).

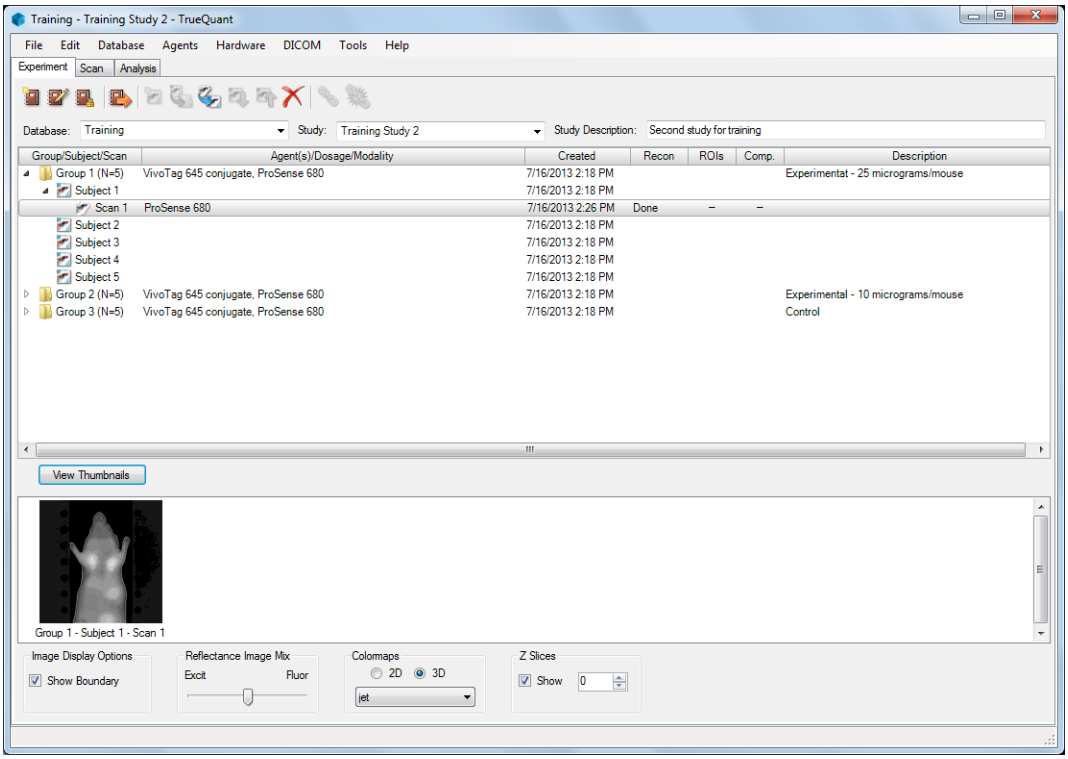


Figure 6-6. Experiment tab after scanning Group 1 Subject 1, Scan 1

6.4 Reconstruction Queue

Upon completion of a scan, a coarse reconstruction for quick preview is performed automatically in the background, using 2mm voxel mesh. The results of this reconstruction can be displayed in the **Analysis** tab, detailed in *Chapter 6*. The preview reconstructions are non-quantitative, so the 3D ROI analysis tools are not available until a full reconstruction has been completed. You can disable the quick preview reconstruction option by selecting **Tools** | **Options** | **Scan**.

If the **Add to Reconstruction Queue** option is selected prior to completing the scan (Figure 6-3), a full-resolution tomographic reconstruction is started as a background process when the scan completes. Otherwise, you can manually add a reconstruction to the queue at any time in the **Reconstruction Queue Manager**.

To manually add a scan to the reconstruction queue:

1. Double-click the **Reconstruction Queue** icon in the Windows tray, at the bottom right of the screen, as shown in Figure 6-7. The **Reconstruction Queue Manager** window opens and initially shows an empty queue (Figure 6-8).

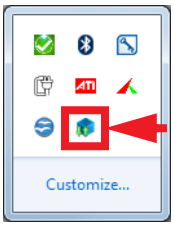


Figure 6-7. Reconstruction Queue icon in the Windows Tray.

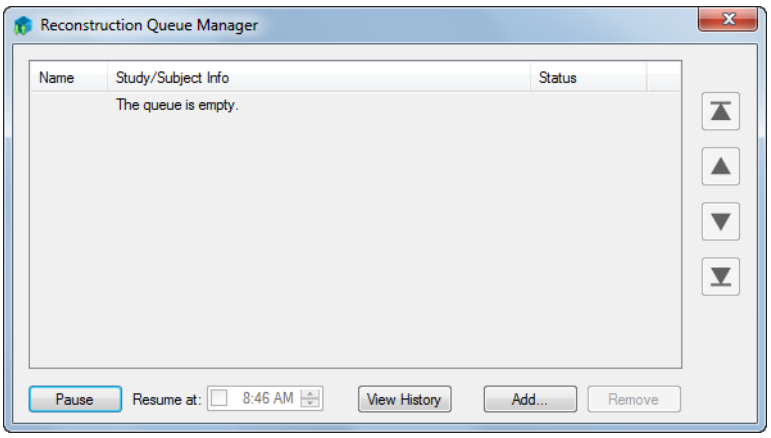


Figure 6-8. Reconstruction Queue Manager window

2. Click the **Add** button to open the **Add Dataset to Queue** window (Figure 6-9) and display all studies, groups, subjects, and scans in the active database.

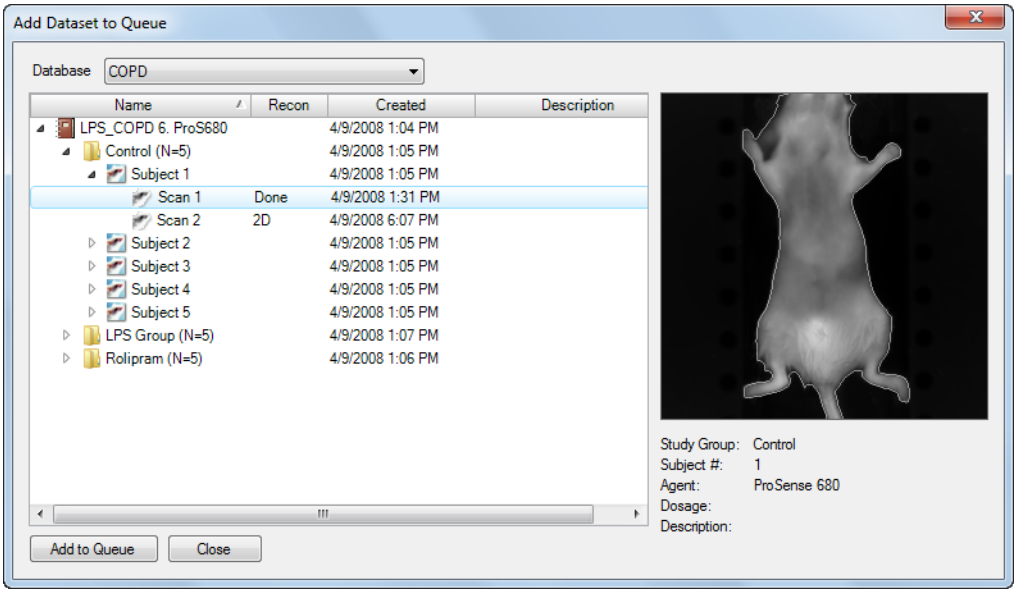


Figure 6-9. Add Dataset to Queue window

3. To select a scan from a different database, select the desired database in the **Database** drop-down list.

4. Select the scans to add to the reconstruction queue and click the **Add to Queue** button. The selected scans display in the Reconstruction Queue Manager window. Scans with previously completed reconstructions cannot be added to the reconstruction queue.

   Note: If a new version of the Reconstruction Module has been installed, and the new version is compatible with the version that produced the previously completed reconstruction, the scan can be added to the queue. Reconstruction will create a duplicate copy of the scan and add the copy to the queue. When complete, both the old and new reconstructions will be available.

To remove a scan from the reconstruction queue, select the scan in the Reconstruction Queue Manager window and click the **Remove** button.

To pause the entire queue, click the **Pause** button on the Reconstruction Queue Manager window. The active reconstruction is not paused, but the next reconstruction in the queue does not start after the active reconstruction finishes.

To restart processing queue items manually, click the **Resume** button.

To restart processing at a specific time, pause the queue, select the **Resume At** check box, and set the desired time for the queue to restart. (The button should still display Resume.) The queue will automatically restart at the selected time.

To change the order of a scan in the queue, select one scan and use the up, down, top, and bottom arrows to move the scan to the desired position.

To view the past reconstructed scans, click the **History** button to open the Reconstruction History Viewer (Figure 6-10).

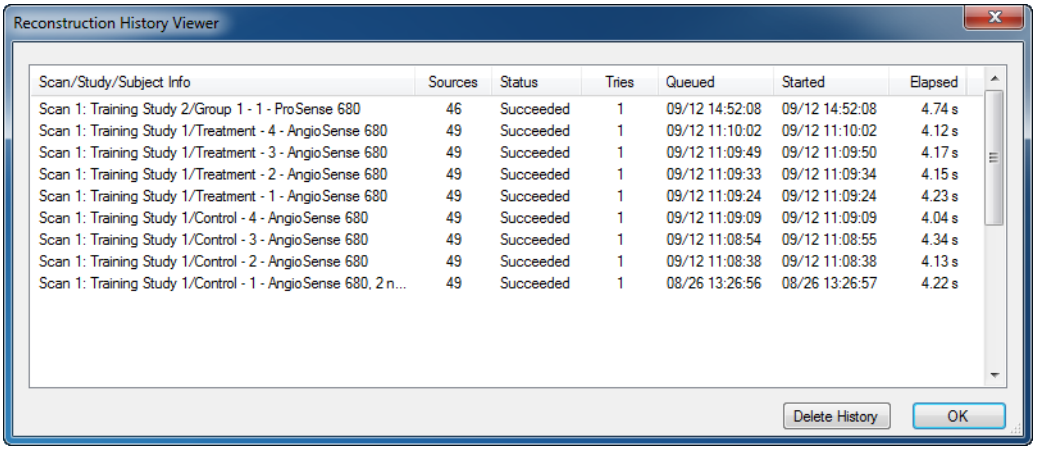


Figure 6-10. Reconstruction History Viewer

7

The Analysis Tab

7.1 Overview

The **Analysis** tab (see Figure 7-1) displays imaging results, displays region of interest (ROI) analyses, and exports images and data to other applications. These operations can be performed on either 2D reflectance images, or on full 3D tomographic reconstructions.

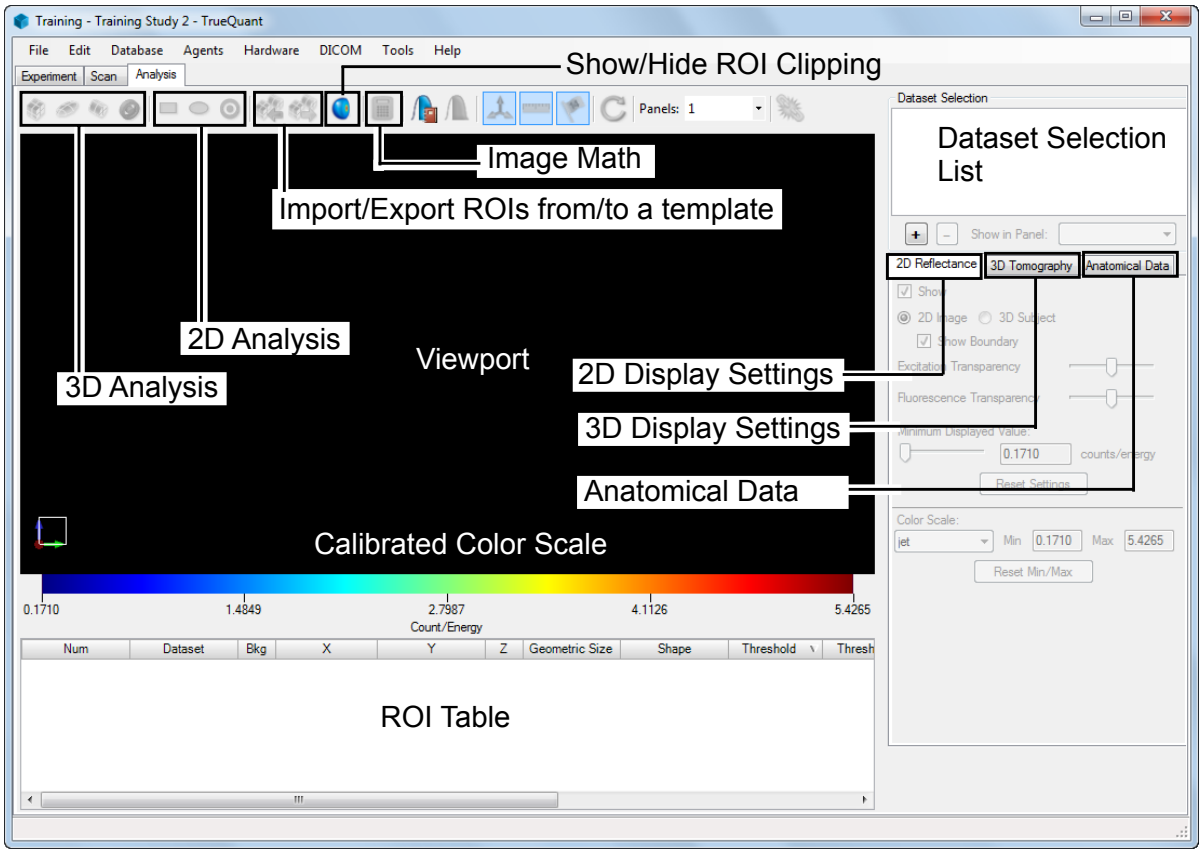


Figure 7-1. Initial view of the **Analysis** tab

7.2 Opening Datasets in the Analysis Tab

Use one of the methods below to load data into the Analysis tab.

- On the **Analysis** tab, click the **Add** button (+) under the **Dataset Selection** list. The **Load Dataset** window, shown in Figure 7-2, opens. Highlight the desired scan or select multiple scans using Ctrl or Shift. Click the **Load** button to open the selected scans. Any scans that were previously open in the Analysis tab remain open.

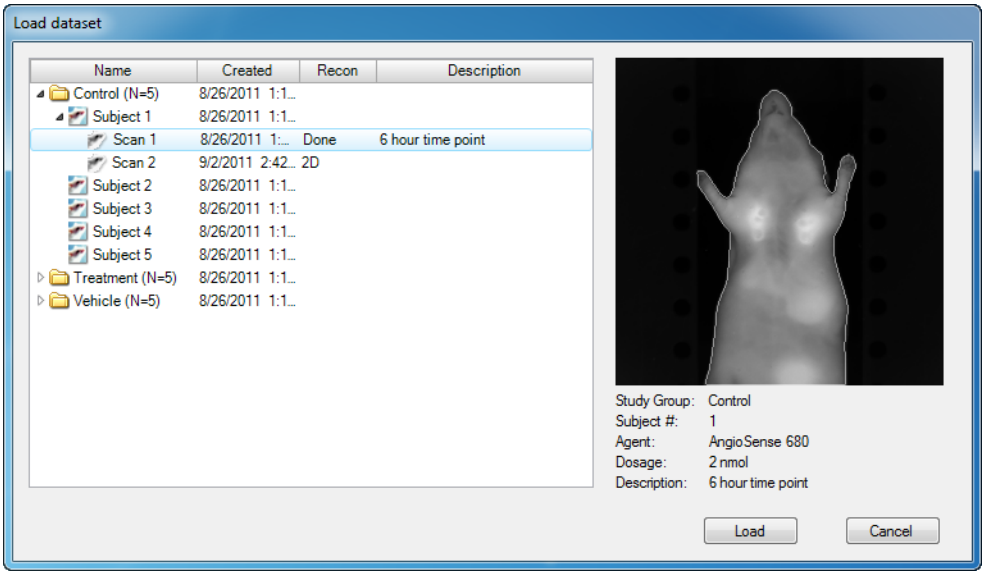


Figure 7-2. Load Dataset Window

- On the **Experiment** tab, select the desired dataset. if desired, use Ctrl or Shift to select multiple datasets. Right-click on a selected dataset and select **View Analyses** from the shortcut menu. The display automatically switches to the **Analysis** tab with the selected dataset loaded and displayed (Figure 7-3). Any scans that were previously open in the Analysis tab are closed.
- On the **Experiment** tab, double-click a scan in the **Experiment** tab to open only the selected scan. Any scans that were previously open in the Analysis tab are closed.

If datasets that were acquired with the Multi-Species Imaging Module (MSIM) in different cassette positions and animal orientations are loaded into the same panel, the scans are displayed offset from one another to display as a single continuous view of the animal. Feet-first scans are rotated so they appear head-first in the **Analysis** tab. This can allow up to six scans of the same animal to appear stitched together to form a continuous image. The scans are still treated as individual datasets for purposes of the ROI analysis tools described below; the stitching together of the scans is purely for visualization purposes.

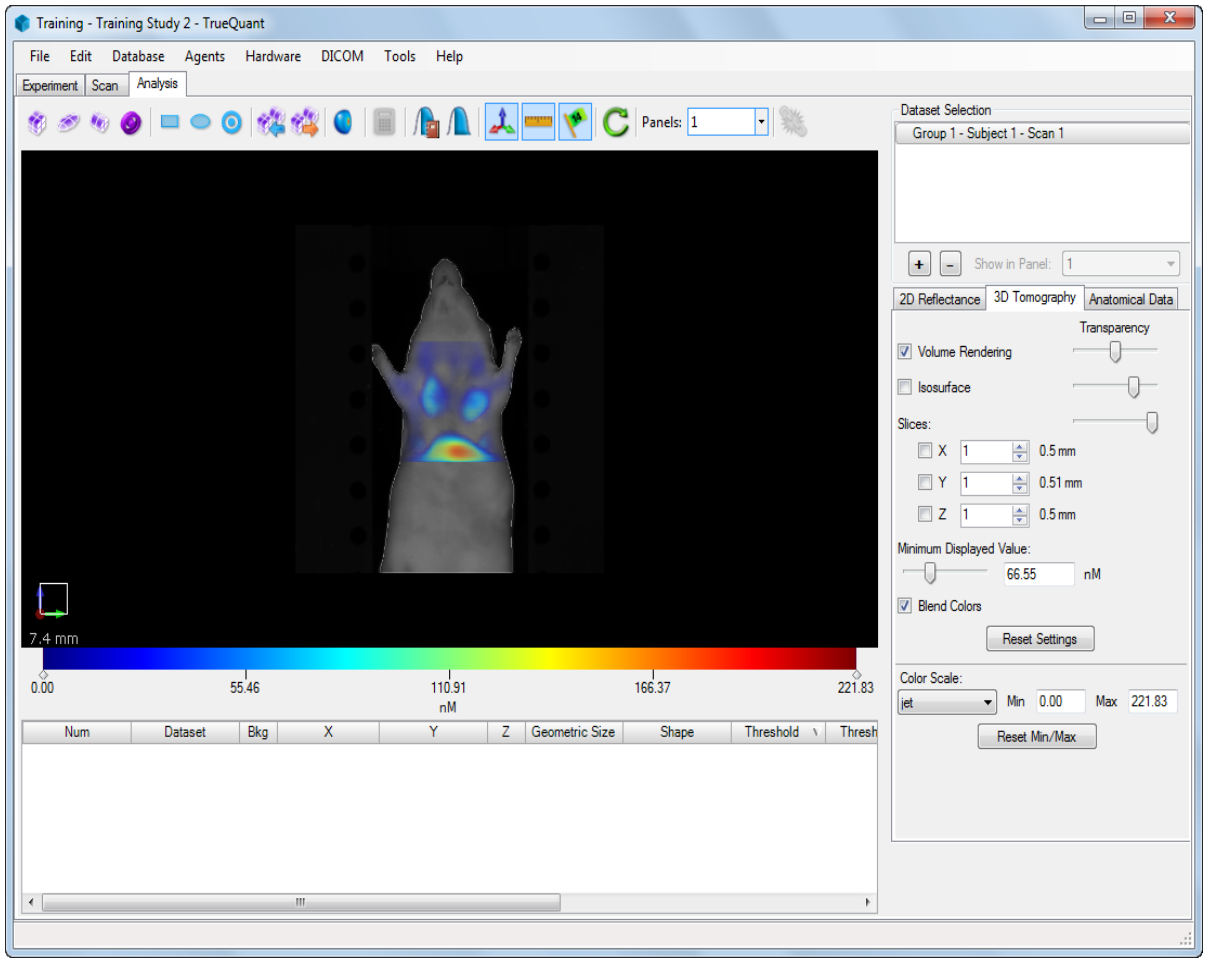


Figure 7-3. The **Analysis** tab after loading a scanned and reconstructed tomographic dataset.

Click the **Remove** button (–) in the **Dataset Selection** list to unload the currently highlighted dataset and remove it from the viewport.

7.3 Changing the View in the Analysis Tab

When viewing images in the **Analysis** tab, change the view as desired:

- **Rotate** the view: Middle-button or right-click and drag
- **Rotate about a single axis**: Ctrl + Shift + right-click and drag across the axis of rotation
- **Pan** the view: Ctrl + middle-click and drag, or Ctrl + right-click and drag
- **Zoom in/out**: Use the scroll wheel, or Shift + middle- or right-click and drag

Click the **Reset View** icon ( ) to return to the default view of the subject.

Axis Orientation

The colored coordinate axes in the lower left corner displays the current orientation. To show or hide the coordinate axes, click the **Show/Hide Axis** icon ( ) above the image.

Size Scale

Coupled to the coordinate axes is a wireframe cube that indicates the physical scale of the data. Below the cube is a size scale that indicates the length of one edge of the cube in millimeters (mm). To show or hide the cube and size scale, click the **Show/Hide Scale** icon ( ) above the image.

Color Scale

The scale for the color bar underneath the viewport defaults to the minimum and maximum concentration values (nM) for 3D reconstructions, minimum and maximum pixel intensities (Count/Energy) for 2D reflectance images, or minimum and maximum units for Anatomical data. To change the Color Scale Minimum and Maximum values, either type the desired values in the text boxes under **Color Scale** or drag the diamond-shaped handles below the color scale.

7.4 Drawing ROIs

This section explains how to add 2D or 3D ROIs to the image.

The purple 3D and blue 2D ROI buttons (see Figure 7-4) display at the top left of the toolbar on the Analysis tab:

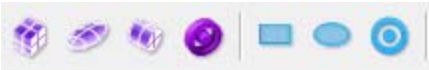


Figure 7-4. ROI buttons

The ROI buttons represent, from left to right:

- a rectangular prism, or parallelepiped ROI
- an ellipsoid ROI
- a cylinder ROI
- an isosurface ROI (see “Adding a 3D Isosurface ROI” on page 70)
- a rectangle ROI
- an ellipse ROI
- an isocontour ROI (see “Adding a 2D Isocontour ROI” on page 71)

To insert a Rectangular Prism, Ellipsoid, Cylinder, Rectangle, or Ellipse ROI:

1. Click the desired ROI button. The ROI displays on the image and a row for the ROI is added to the ROI table below the image.
2. If the ROI is a 3D cylinder, click the **Shape** column for the ROI in the ROI table and choose the desired orientation, **Cylinder X**, **Cylinder Y**, or **Cylinder Z**, from the drop-down list.
3. As needed, click the **Enable/Disable ROI Clipping** button on the toolbar to show or hide the fluorescence outside the ROI.
4. Move the ROI over the desired location on the image (see “Moving an ROI” on page 67).

5. Resize the ROI as desired (see “Resizing an ROI” on page 69).

For example, clicking the Rectangular Prism tool displays a purple, rectangular prism on the fluorescent region and displays the ROI statistics numerically in the ROI table below the image (Figure 7-5).

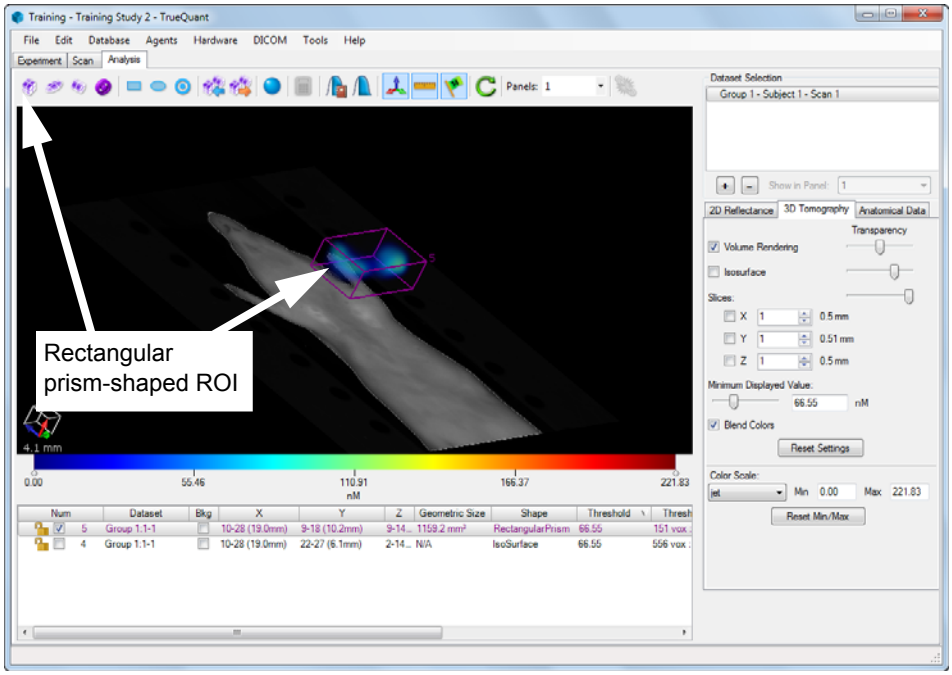


Figure 7-5. Adding a rectangular prism ROI

Select or clear the ROI check box in the ROI table below the image to show or hide an ROI’s wireframe.

7.5 Moving an ROI

ROIs move on only one axis at a time, either X or Y for 2D ROIs, or X, Y, or Z for 3D ROIs.

7.5.1 Moving a 2D ROI

1. Hold the cursor over the center of one side of the ROI until the edge is highlighted in pink. To move the ROI up or down, highlight the top or bottom of the ROI. To move the ROI left or right, highlight the left or right side of the shape.

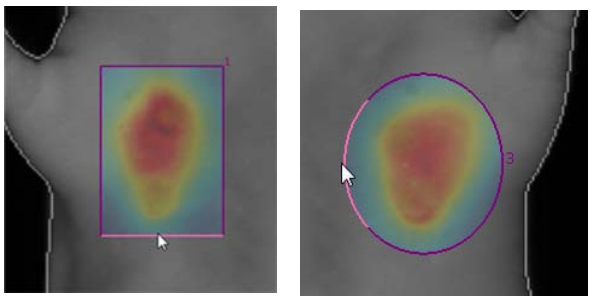


Figure 7-6. Moving 2D ROIs

2. Hold the **Ctrl** key, click on the edge of the shape and drag in the desired direction. The ROI table updates dynamically.

7.5.2 Moving a 3D ROI

1. Hold the cursor over the center of one side of the ROI until the side is highlighted in pink. To move the ROI up or down, highlight the top or bottom of the ROI. To move the ROI left or right, highlight the left or right side of the shape.

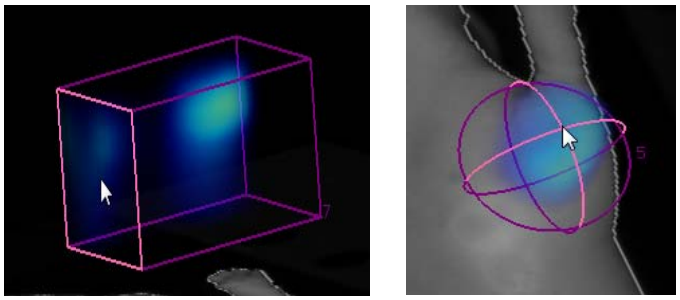


Figure 7-7. Moving 3D ROIs

2. Hold the **Ctrl** key, click on the side of the shape and drag in the desired direction. The ROI table updates dynamically.

7.5.3 Moving a 2D Isocontour ROI

1. Select the 2D Isocontour in the ROI Table.
2. Right-click the selected 2D Isocontour ROI.
3. Click **Pick Isocontour Segment** in the drop-down menu.

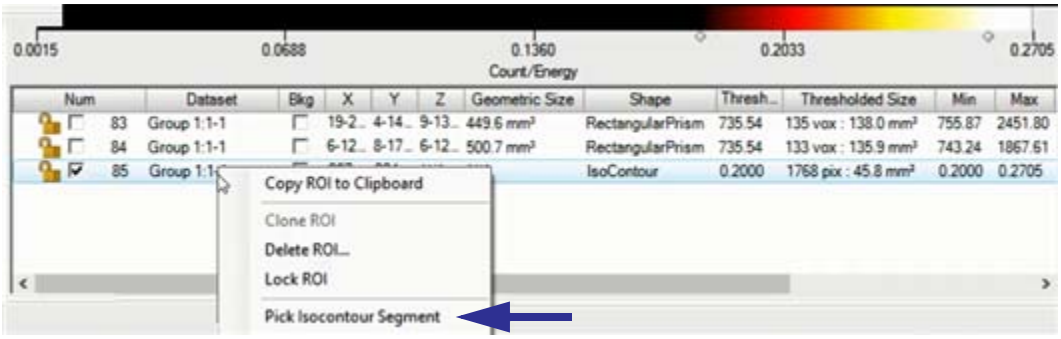


Figure 7-8. Drop-Down Menu - Pick Isocontour Segment

4. Fluorescence within the selected values on the calibrated color scale will show.

NOTE The values in the color bar can be changed to show different fluorescence values on the animal.

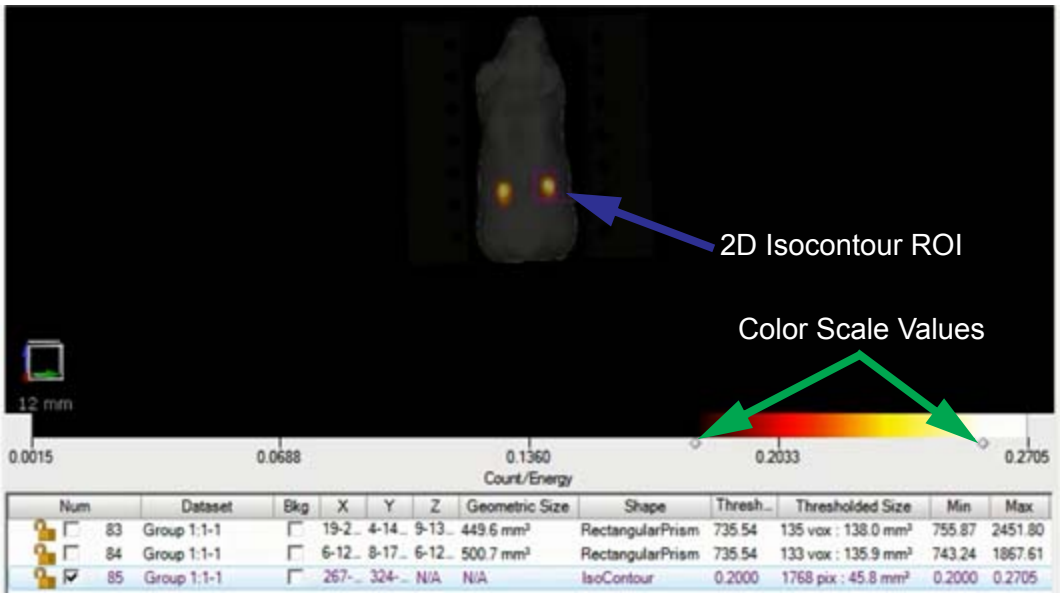


Figure 7-9. Color Scale Values

5. Click on the animal to move the 2D Isocontour ROI to that location.

7.6 Resizing an ROI

To resize a 2D ROI, click on the center of one side of the ROI and drag it to the desired position. The active side is highlighted in pink to indicate it is being moved as shown in Figure 7-10. The pixel coordinates of the ROI in the ROI table below the image update as the ROI is resized. (To create a square or circle, hold the Shift key while clicking and dragging a side.)

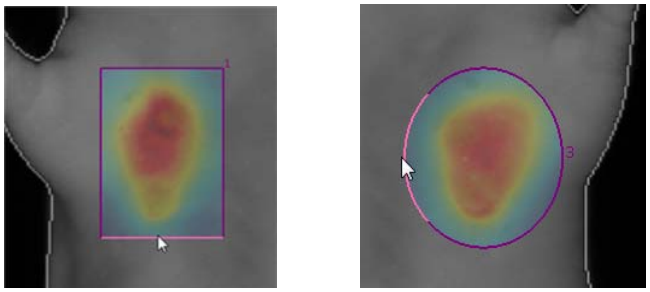


Figure 7-10. Resizing a 2D ROI

To resize a 3D ROI, click on the center of one face of the ROI and drag it to the desired position. The active face is highlighted in pink to indicate it is being moved (Figure 7-11). The voxel coordinates of the ROI in the ROI table below the image change as the ROI is resized. Figure 7-11 shows the result of shrinking the ROI down to the size of the tumor being analyzed. (To create a cube or sphere, hold the Shift key while clicking and dragging a side.)

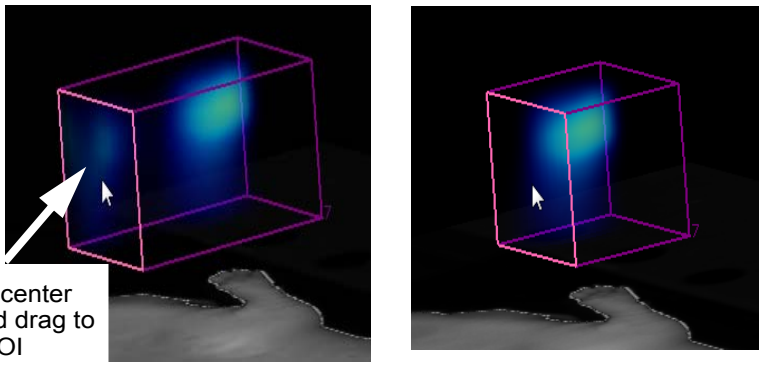


Figure 7-11. Resizing a 3D ROI

7.7 Adding a 3D Isosurface ROI

To add a 3D Isosurface ROI:

1. Adjust the **Minimum Displayed Value** slider until the desired isosurface is isolated from other fluorescence in the image.
2. Click the **Isosurface ROI** button on the toolbar above the image.
3. Click on the desired isosurface. A rectangular prism encloses the area of the isosurface as shown in Figure 7-12 and the ROI table displays the ROI statistics.

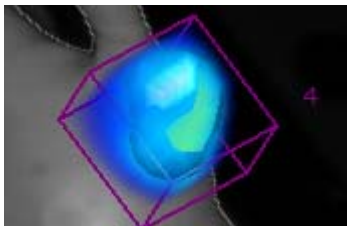


Figure 7-12. Isosurface ROI

To change the ROI Threshold:

1. Click the Threshold column for the ROI in the ROI table. The column expands to display a text box and a slider as shown in Figure 7-13.

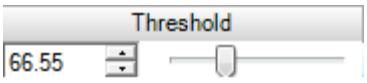


Figure 7-13. ROI Threshold Slider

2. Move the slider to increase or decrease the ROI threshold. The ROI automatically resizes to include only the area above the threshold and the display changes to view only voxels above the threshold.
3. If there are multiple disconnected isosurfaces after increasing the threshold, the ROI encloses the brightest isosurface. (To center the ROI on an isosurface that is not the brightest section of the isosurface, right-click and select **Pick Isosurface Segment**.)

7.8 Adding a 2D Isocontour ROI

A 2D Isocontour ROI can be placed to show the region of interest without changing the data. The 2D Isocontour displays the region based on the selected threshold.

1. Click the Isocontour ROI button on the toolbar.

Figure 7-14. 2D Isocontour ROI

2. Click the area of the animal where you want to place the ROI.
3. Adjust the Threshold slider to the appropriate setting.
4. To move the ROI, see “Moving an ROI” on page 67.

7.9 ROI Table

The ROI Table is shown in Figure 7-15:

Num	Dataset	Bkg	X	Y	Z	Geometric Size	Shape	Thresh...	Thresholded Size	Min	Max	Mean	Std. Dev	Total	Description
1	Control:1-1	☑	5-13 (9.0...	1-7 (...	1-14...	896.8 mm³	RectangularPrism	30.00	17 vox : 17.3 mm³	30.39	58.23	36.54	8.25	0.63 pmol	
2	Control:1-1	☐	8-30 (23.0...	21-2...	1-14...	N/A	IsoSurface	30.00	961 vox : 977.2 mm³	30.29	311.17	106.03	55.44	103.61 pmol	
3	Control:1-1	☐	10-17 (8.0...	8-18...	10-1...	447.4 mm³	RectangularPrism	31.12	206 vox : 209.5 mm³	31.13	117.72	58.04	21.76	12.16 pmol	
4	Control:1-1	☑	19-27 (9.0...	1-7 (...	1-14...	896.8 mm³	RectangularPrism	30.00	20 vox : 20.3 mm³	30.40	55.05	36.74	7.45	0.75 pmol	
5	Control:1-1	☐	20-28 (9.0...	10-1...	10-1...	457.6 mm³	RectangularPrism	31.12	187 vox : 190.1 mm³	31.88	116.49	63.37	21.18	12.05 pmol	
6	Control:1-1	☐	1-31 (31.0...	7-15...	1-14...	3971.7 mm³	RectangularPrism	93.35	25 vox : 25.4 mm³	93.98	117.72	104.09	6.83	2.65 pmol	

Figure 7-15. Typical ROI statistics

The ROI Table includes the following columns from left to right:

- **Num** - A number, an open/closed padlock symbol, and a check box. The number is the unique identifier for the ROI. The number is assigned at runtime, so the ROI number can differ from one analysis session to another. The padlock icon indicates whether the ROI is locked to prevent further editing. The checkbox indicates whether the wireframe of the ROI is visible in the 3D viewer.

  The unique identifier is also displayed at the upper right corner of that ROI’s wireframe in the main image panel. You can show or hide the labels in the viewport by clicking the **Show Labels** icon ( ) in the toolbar above the viewport.
- **Dataset** - Indicates which group, subject number, and scan number the ROI was derived from.
- **Bkg -** Indicates that the selected ROI is a region of background.
- **X, Y, Z Ranges** - Provides the slice numbers and physical distances in the sagittal (X), transaxial (Y), and coronal (Z) orientations included in the ROI. For example, an ROI may span slices number 9 through 29 (21 mm) in the sagittal (X) direction. For 2D ROIs, the pixel ranges and physical distance in X and Y are given.
- **Geometric Size** - Provides the volume (in mm^3 for 3D) or area (in mm^2 for 2D) of the ROI in the absence of any thresholding.

- **Shape** - Indicates the regular solid geometry associated with the selected ROI. For cylindrical ROIs, select the row in the table and then click on the shape name to change the orientation of the cylinder to each of the three axes.
- **Threshold** - Specifies the threshold value below which pixels or voxels are not included in the Thresholded Size. Concentration units are nM (nanomolar or 10^{-9} moles/liter) for 3D ROIs or counts/input energy for 2D ROIs. Change the **Threshold** value by moving the slider control in the ROI table or typing the desired value in the text box.
- **Thresholded Size** - Calculates the volume enclosed within the ROI above the chosen threshold, in units of voxel counts and in mm^3. For 2D ROIs, units are pixels counts and mm^2.
- **Min, Max, Mean, Standard Deviation** - The statistical distribution of fluorochrome concentration in nM (nanomolar or 10^{-9} moles/liter) for all the voxels in the 3D ROI. For 2D ROIs, units are counts/input energy.
- **Total** - The amount of agent in the ROI. For 3D ROIs, units are pmol (picomoles or 10^{-12} moles). For 2D ROIs, units are counts×pixels/input energy.
- **Description** - An optional user-supplied text description. Modify this field by selecting the row in the table and clicking the description to open the description text box. Note, it is considered best practice to include a meaningful description for each ROI. Descriptions are especially important when dealing with many ROIs or very large datasets.

7.10 Deleting ROIs

1. Select the ROI to be deleted in the ROI Table. Multiple ROIs can be selected by holding the Shift key while clicking each ROI.
2. Right-Click on the selected ROI(s).
3. Click **Delete ROI** in the Drop-down menu.
4. The Confirm Delete ROI window opens.

Figure 7-16. Confirm Delete ROI Window

5. Click the **Yes** button to delete the selected ROI(s), or the **No** button to cancel.

7.11 Importing and Exporting ROIs

TrueQuant provides the ability to import and export ROIs to and from projects. This allows you to clone ROIs across studies and across databases.

To import ROIs, make sure the scan or scans you want to import the ROIs into are selected in the **Dataset Selection** list and click the **Import ROIs from a template** icon () on the main menu. This opens a standard Windows **Open** dialog so you can select the desired ROI template file. Likewise, use the **Dataset Selection** list to select the dataset whose ROIs you want to export to a template and click the **Export ROIs to a template** icon () on the main menu. Clicking the icon also opens a standard Window **Open** dialog, allowing you to name and save the ROI template file in the desired location. All the ROIs associated with the selected dataset are saved in the ROI template.

7.12 Using The ROI Table Shortcut Menu

When you select and then right-click an ROI row in the ROI table, the shortcut menu provides the following actions:

- **Copy ROI to Clipboard** - Copies the table row to the Windows clipboard as an alphanumeric string.
- **Clone ROI** - Creates an identical copy of the selected ROI that you can use to analyze a contra-lateral, for example. When cloning an ROI, select the dataset to associate the cloned ROI with, or select **All loaded datasets** to clone it to multiple datasets at once. There are a few restrictions on ROI cloning: isocontour and isosurface ROIs cannot be cloned because they depend on the details of their associated datasets; 3D ROIs cannot be cloned to 2D (reflectance-only) datasets; and 2D ROIs cannot be cloned to computed datasets, whose reflectance images use arbitrary units or units appropriate to the image.
- **Delete ROI** - Removes the ROI from the display and the database. Choosing this option opens a confirmation window. If you choose to proceed with deletion, TrueQuant permanently removes the ROI from the image display and the database.

NOTE Be careful when deleting ROIs. There is no Undo.

- **Lock ROI** - Prevents the ROI from further interactive editing in the image panel, indicated by changing the padlock to a closed padlock icon in the leftmost column of the table. You can change the description of a locked ROI, but not its dimensions and threshold. An ROI that is currently in use by another user on another computer may not be locked.
- **Pick Isosurface Segment** - Reassigns a configured isosurface ROI to a different segment in the image. After choosing this option, select a new segment in the image and the ROI updates in the table and the viewport automatically.
- **Change Threshold** - Allows you to modify the **Threshold** value for the selected row or rows by entering the desired concentration. Selecting this menu item opens the **Change ROI Thresholds** window where you can enter the desired concentration value. Clicking **OK** after entering the value updates the threshold for the selected rows in the table. The view options and the viewport update immediately when you change the threshold value for an ROI. Note, using the **Change Threshold** option on a 3D or 2D ROI automatically adjusts the displayed minimum value setting for that ROI's dataset.

To select multiple rows in the ROI table, hold the **Shift** or **Control** key as you click one or more rows.

7.13 2D and 3D Image Settings

The image settings to the right of the main image panel features 2D (Reflectance Image) and 3D (Isosurface, Volume Rendering and Slices) image controls. These controls do not change the actual data, only the display of the data. Any changes made with these controls only affect the datasets selected (highlighted) in the Dataset Selection list. An entire dataset can be temporarily hidden from view by selecting the dataset and un-checking each of the check boxes for the dataset's image settings.

Sliders can be dragged manually to adjust:

- Transparency settings for either excitation or fluorescence reflectance images: Figure 7-17 and Figure 7-18 show the extreme examples of using maximum settings on the excitation and fluorescence reflectance images respectively.
- Transparency of an isosurface, volume rendering, or set of tomographic slices when overlaid on a reflectance image of a subject. (Note: Select the corresponding **Show** option to display the item.)
- Threshold for the color-mapped display of either a reflectance fluorescent contour, or a 3D isosurface fluorescent contour (Figure 7-19 and Figure 7-20). Note, the software displays a corresponding numerical unit (counts/energy for 2D, nM for 3D FMT data, or the appropriate units for non-FMT data) in the **Minimum Displayed Value** text box as you move the slider. Alternatively, you can enter the desired number directly in the text box next to the slider.

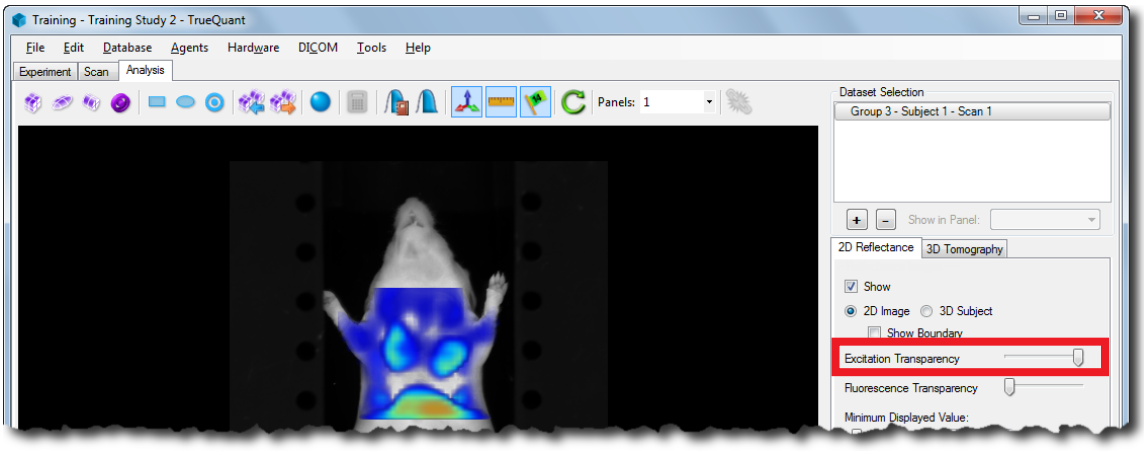


Figure 7-17. Setting the slider to show the excitation reflectance image at a maximum and fluorescence reflectance image at a minimum.

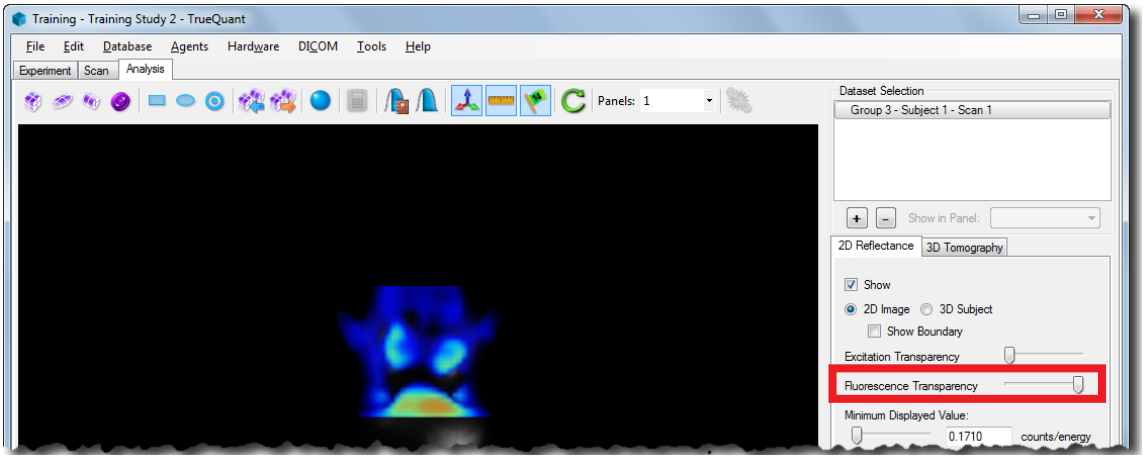


Figure 7-18. Setting the slider to show the fluorescence reflectance image at a maximum and the excitation reflectance image at a minimum.

Other controls include:

- Display the reflectance data as a 2D image or on a simulated “3D subject.” When displayed as a 2D image, the subject boundary can also be displayed or hidden. The fluorescence reflectance image is only visible when being displayed as a 2D image.
- Show or hide the reflectance data, isosurface display, volume rendering, or reconstruction slices.
- By default, the colors in the volume rendering and reconstruction slice displays are blended between voxels to give a smoother display of the 3D data. Use the **Blend Colors** check box to turn off blending and view the voxels as solid colored cubes.
- The **Reset Settings** button returns all the above settings to the defaults.
- Adjust the color scale for the 2D fluorescence reflectance data or the 3D reconstruction. For either scale, the color map can be changed, as can the data values that correspond to the upper and lower limits of the color map. This does not change the data themselves, only the display. The limits can be changed either by dragging the diamond shapes below the colorbar or by entering a number into the text boxes at the bottom right. The color scale can be reset to the minimum and maximum values in the data using the **Reset Min/Max** button.

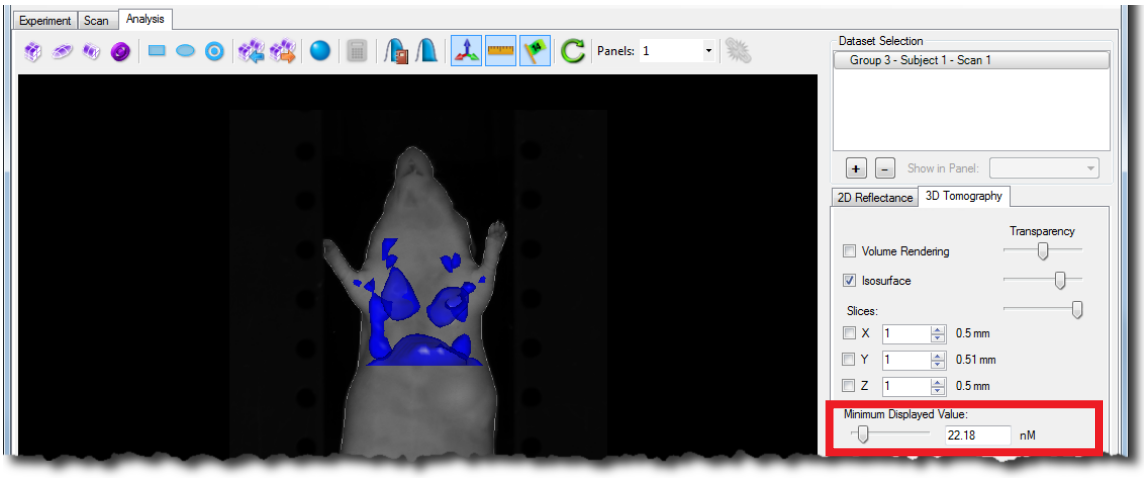


Figure 7-19. Selecting a low value for the 3D isosurface.

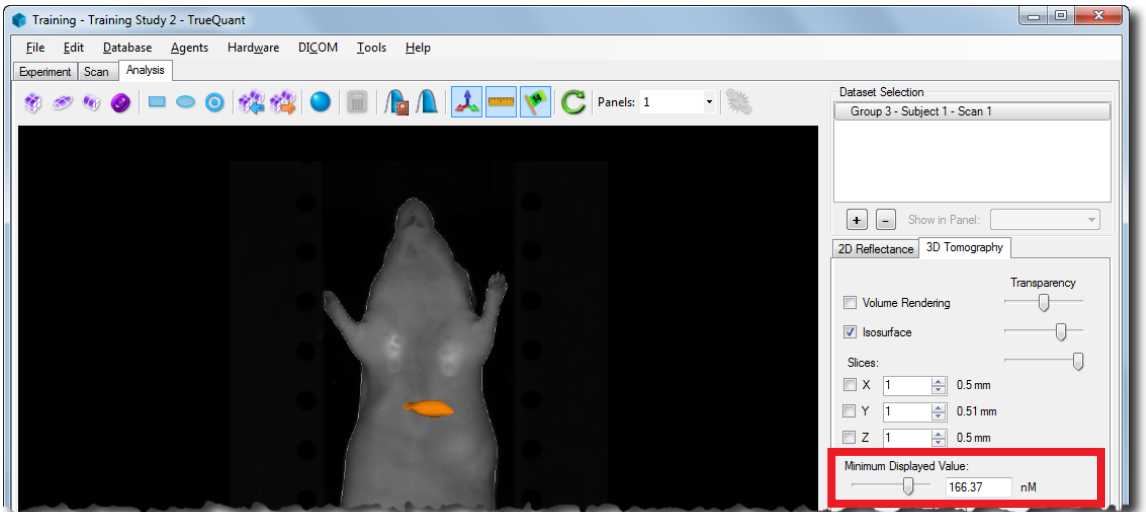


Figure 7-20. Selecting a higher value for the same isosurface.

7.14 Anatomical Data Image Settings

The Anatomical Data tab displays to the right of the main image panel to select image settings for non-FMT datasets. These controls do not change the actual data, only the display of the data. Any changes made with these controls only affect the datasets selected (highlighted) in the Dataset Selection list.

If two datasets are co-registered, for example an FMT scan and a CT scan of the same animal, the two datasets can be opened in the same panel. The datasets are aligned to match up and will pan, zoom, and rotate together when viewed in the same panel. The 2D, 3D, and Anatomical view settings are adjusted independently. To change the display of the 2D and 3D images, select the dataset in the Dataset Selection list and see “2D and 3D Image Settings” on page 74.

The **Show Animal Only** check box is available on the Analysis Tab. When selected, any CT data outside of the animal surface are masked out of the image.

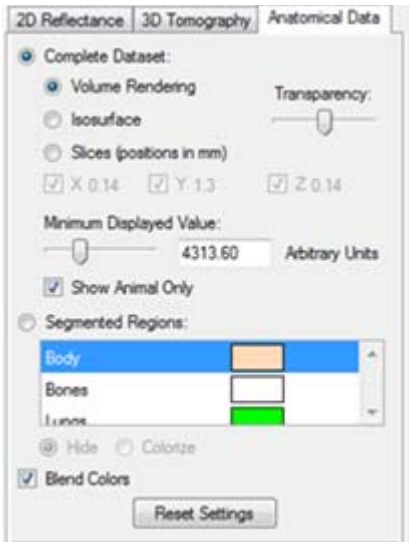


Figure 7-21. Anatomical Data Tab

7.14.1 Viewing Linked Datasets in the Analysis Tab

After datasets are linked, view the images in the Analysis tab.

- When viewing only the FMT dataset in the Analysis tab: The same image settings are available, including Volume Rendering, Isosurface, Slices, and Color Scale. Pan, zoom, and rotate the image the same way as before the image was linked. The same ROI tools are available (ROI type, threshold, etc.).
- When viewing only the CT dataset in the Analysis tab: Image settings on the Anatomical Data tab apply to the CT data. Pan, zoom, and rotate the image as normal. ROI tools are not available for CT images. See the sections below for image settings.
- When viewing the FMT and CT datasets in the same panel in the Analysis tab: Anatomical Data settings apply only to the CT dataset. The 2D and 3D settings apply only to the FMT dataset. Pan, zoom, and rotate apply to both datasets.

NOTE When displaying co-registered FMT and CT scans in the same panel, the Show 2D Image in the 2D Reflectance tab is turned off by default.

7.14.2 CT Volume Controls

1. Open the non-FMT dataset in the Analysis tab. For CT scans, the equipment bore, cassette adapter, cassette, and the animal display.
2. Select **Complete Dataset**.
3. Select **Volume Rendering**.
4. Select the **Show Animal Only** check box.
5. Select the desired Color Scale and adjust the Color Scale Min and Max.
6. Adjust the Transparency.

7.14.3 Isosurface Controls

1. On the Anatomical Data tab, select **Complete Dataset**.
2. Select **Isosurface**.
3. Select the **Show Animal Only** check box.
4. Adjust the Minimum Displayed Value.
5. Select the desired Color Scale and adjust the Color Scale Min and Max.
6. Adjust the Transparency.

7.14.4 Slices Controls

1. On the Anatomical Data tab, select **Complete Dataset**.
2. Select **Slices**.

3. Select the check boxes for the axis (X, Y, or Z) on which to display the slices.

NOTE The X, Y, and Z axis option check boxes are all selected by default. Deselect any axis options that are not necessary.

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4. Adjust the Minimum Displayed Value.
5. Select the desired Color Scale and adjust the Color Scale Min and Max.
6. Adjust the Transparency.
7. To move a slice along an axis, click on the plane and drag along the axis. The outline of the selected plane matches the color on the Axis and Size Scale in the lower left corner of the image display.

NOTE The Show Animal Only check box does not hide the instrument bore, cassette adapter, cassette in the slices.

7.14.5 Show Segmented Region Controls

1. On the Anatomical Data tab, select **Segmented Regions**.
2. Select a segmented region, then select the desired Visualization option:
   - Hide - If selected, the segmented region does not show.
   - Colorize - If selected, the segmented region displays in the selected color.
3. Select the desired colors for each of the regions (Whole Body, Bones, Lungs, Heart) by clicking the color box next to the region and selecting the desired color from the drop-down list.

7.15 Viewing Datasets In Multiple Panels

For consistency, it is generally advisable to perform ROI analysis on multiple datasets from the same study group using the same thresholding, ROI dimensions, etc. for all ROIs. TrueQuant facilitates this process with the use of multiple panels in the **Analysis** tab. These panels make it possible to view and analyze multiple datasets side by side at the same time. In addition, multiple datasets can be loaded into each panel, with the total number of datasets limited only by the computer's available memory.

To use multiple panels, first set the number of panels using the drop-down **Panels** selector to the right above the viewport (Figure 7-22). The viewport can be split horizontally into 1, 2, or 3 panels, and a second row allows the display of 4, 6, or 8 panels.

Once datasets are loaded into the **Analysis** tab, they can be moved from one panel to another by selecting the dataset or datasets to be moved and setting the new panel number using the **Show in Panel** drop-down selector below the list of datasets (Figure 7-22). The number of the panel in which each dataset is displayed is prepended to that dataset's name in the list of datasets and in the **Dataset** column in the table of ROI data. The panel numbers are also displayed in the top right corner of each panel in the viewport.

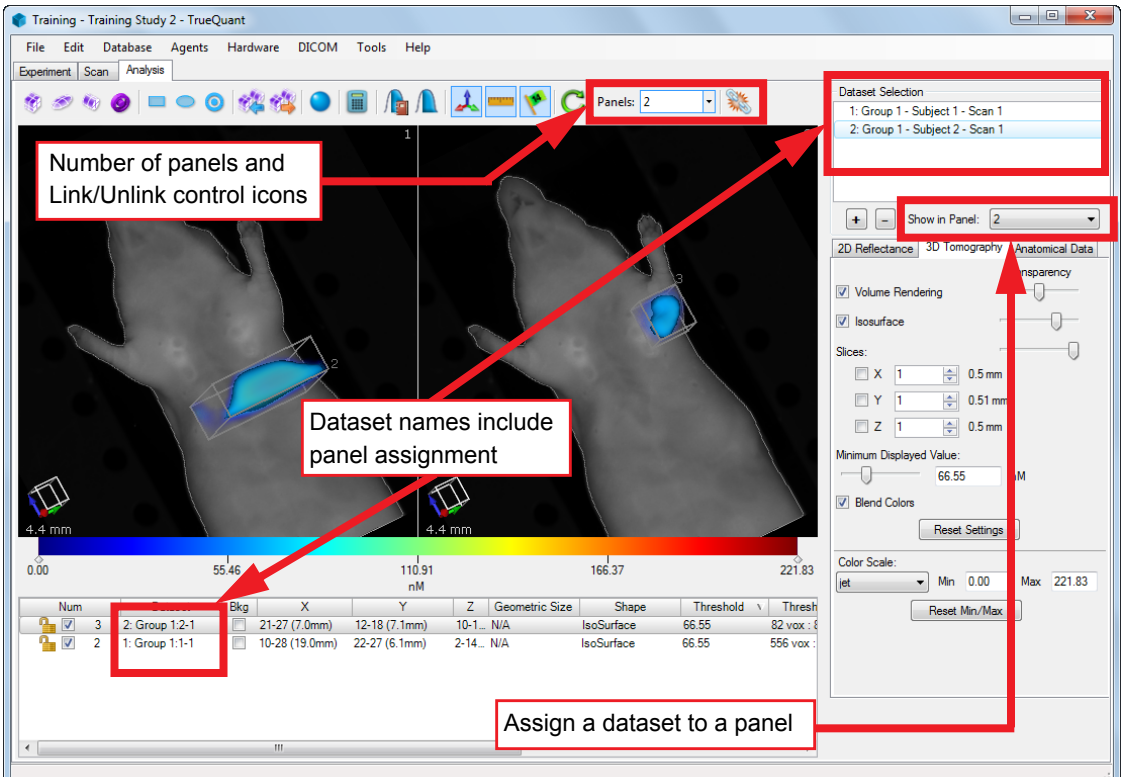

Figure 7-22. Controls for using multiple panels in the **Analysis** tab.

When viewing data in multiple panels, you can rotate, zoom, and pan each panel's view of the data independently, or you can synchronize the view for all the panels using the **Link** icon ( ) above the viewport (Figure 7-22). If operating independently, any rotate, zoom and pan operations apply only to the active panel. For unlinked panels, the **Reset View** icon applies to the last panel where the view was changed. If different panels have different views of the data when you click the **Link** icon, all of the panels' rotate, zoom and pan settings change to match those of the last panel that was changed. Clicking the **Unlink** icon ( ) removes the synchronization of the viewports, allowing each image to rotate, zoom, and pan independently.

Each panel has a label at the top right corner to indicate the panel ID. Various labeling schemes are available: numbers, lower- or upper-case letters, and lower- or upper-case Roman numerals. To set the numbering scheme, select **Tools | User Options** to open the User Options window (Figure 7-23), click the **Analysis tab**, and select the desired **Label Style**. The panel labels are shown as prefixes to the dataset names in both the Dataset Selection list and the ROI table below the viewport.

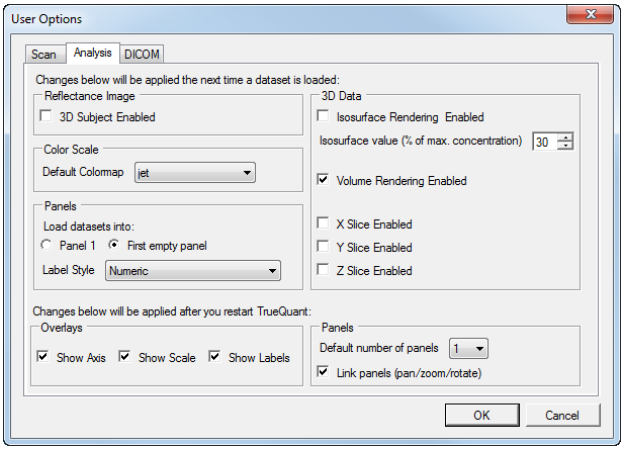

Figure 7-23. The **Analysis** tab of the **User Options** window

The Panel labels can also be customized. Double-click a panel's label to edit it. Enter a custom label of up to 20 characters, then press the **Enter key** to apply the new custom label or press **Esc** to keep the previous label.

Multiple panels are useful for simultaneously viewing multiple datasets, but their greatest power comes from the ability to manipulate multiple ROIs at the same time. For example, consider the analysis of data from a lung inflammation model, where inflammation in four animals from a positive control group is being compared against four animals from a negative control group that has not had any inflammation induced.

First, set the number of panels to eight, then use the + button to load the reconstructed scans of those eight animals into the **Analysis** tab. The software automatically loads the scans into different panels. Next, select the dataset loaded into panel 1 by clicking its name in the Dataset Selection list, create an ROI for that dataset, and resize the ROI appropriately. Now select the ROI (in the table below the viewport) and clone it to **All loaded datasets**. One by one, select each of the cloned ROIs and reposition them as needed. Finally, select all eight ROIs in the table below the viewport, right-click the selection, and choose **Change Threshold** to set a consistent threshold for all of the ROIs.

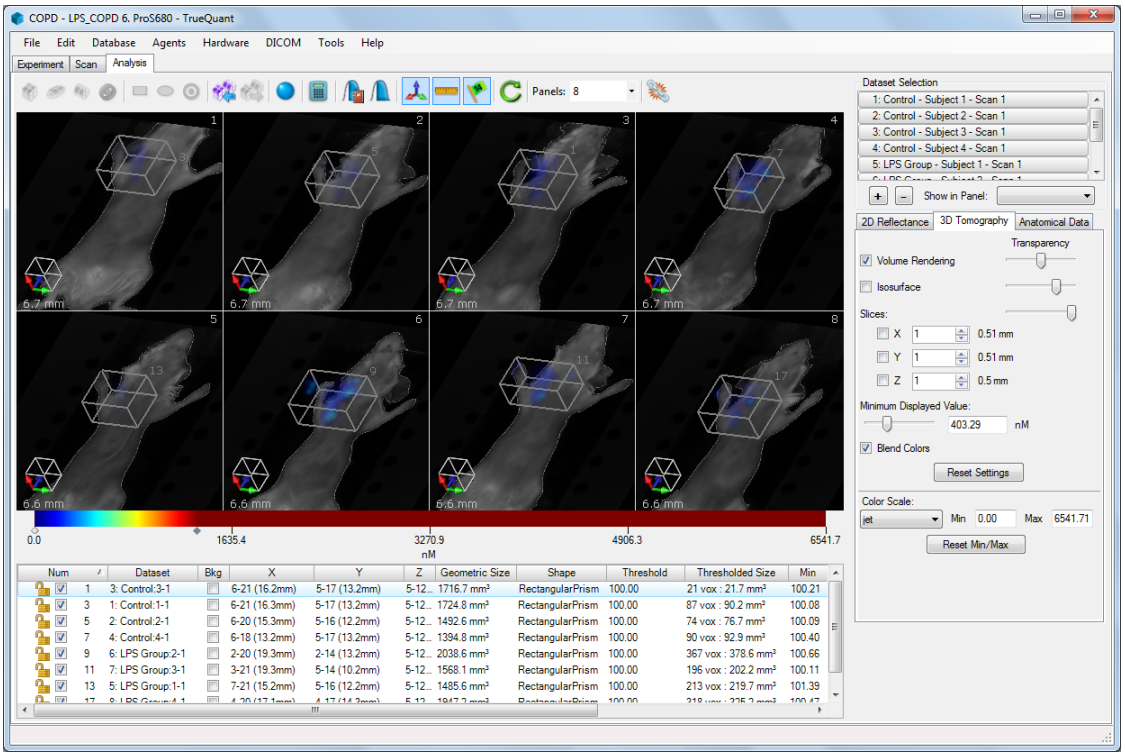

Figure 7-24. Simultaneous analysis of eight datasets

7.16 Math Operations

When multiplexing agents on two or more channels, it is often desirable to compare results between scans of the same animal on different channels. The **Math Operations**, or “computed dataset” tool, located on the main toolbar above the viewport, provides a method for doing this beyond simple ROI analysis. This tool lets you do mathematical and logical operations on the reconstructions of any two scans.

To launch the computed dataset tool, first load the two scans you wish to use into the **Analysis** tab, then click the **Math Operations** icon ( ) on the main toolbar.

This opens the **Create Computed Dataset** window (Figure 7-25). This tool includes controls for entering a mathematical or logical equation and specifying computational parameters, and a preview window to see the results of your computation before saving them.

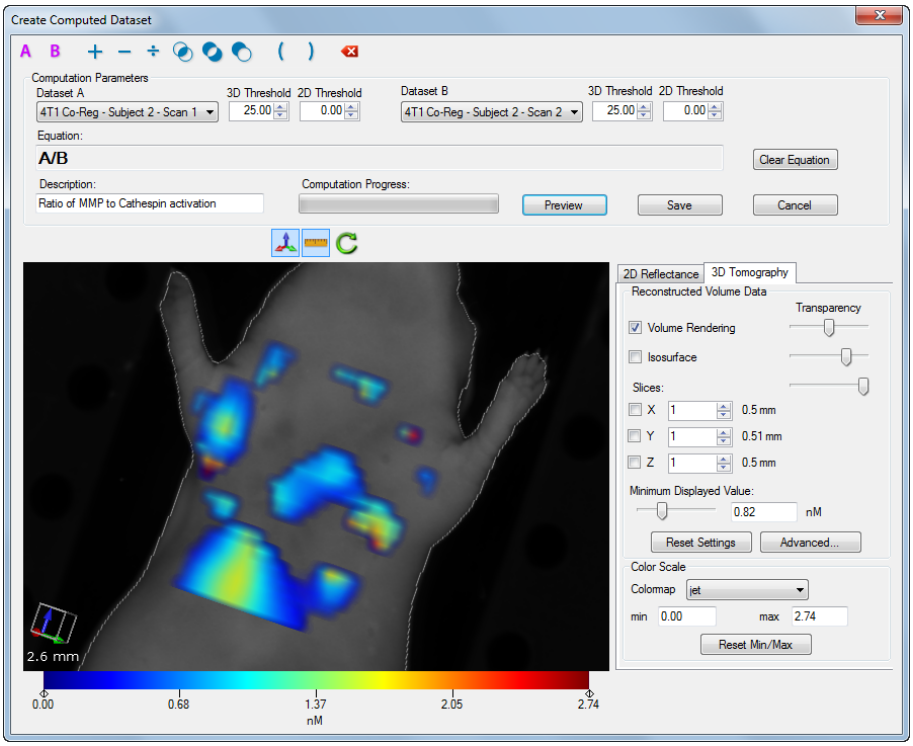

Figure 7-25. The **Create Computed Dataset** window

Begin by selecting the two datasets you wish to use in the computation. One is labeled **Dataset A** and one is **Dataset B**. Next, set the **2D Threshold** and **3D Threshold** values for each dataset. Voxels whose values are below the 3D threshold, and reflectance image pixels whose values are below the 2D threshold, are treated as zeros for purposes of the computation. These voxels and pixels are excluded completely from some operations to avoid dividing by zero.

For a given voxel, thresholds are handled slightly differently depending on the mathematical operation being performed. For addition and subtraction, if either dataset's value is at or above the threshold, the operation is performed on that voxel. For division, both datasets' values must be at or above their respective thresholds for the computation to be performed on that voxel. Any voxels where both datasets' values are below their thresholds are set to zero in the results. The above conditions also apply to pixels in the 2D fluorescence reflectance images.

Once the thresholds are set, enter the **Equation** using the buttons at the top of the window. The **A** and **B** buttons are used to represent the datasets themselves. The next group of buttons are used for mathematical operations (addition, subtraction, and division) and logical operations (intersections, exclusion, and "exclusive or" or "and not"). These operations can be combined into more complicated equations using the parentheses buttons. To delete the last entry in the equation, use the final "backspace" button.

For example, a simple ratio of two reconstructions can be performed using the equation “A/B” with an appropriate threshold to eliminate background fluorescence outside of the area of interest. An example of a more complicated operation is to merge two scans on the same channel with slightly overlapping scan fields. This can be accomplished using the equation “A + (B<AND NOT>A).”

When your equation is complete, optionally enter a **Description**, then click **Preview** to perform the computation and view the result in the preview window. This preview window functions like the **Analysis** tab, with the same controls for changing the display of isosurfaces, volume rendering, color scale, and the like.

After viewing the preview, you may decide to adjust the thresholds or even change the equation. Once this is done, click **Preview** again to view your changes. When you’re satisfied with the results, click **Save** to save the results to the database and load the new computed dataset into the **Analysis** tab. At any point, you can use **Cancel** to close the **Create Computed Dataset** window without saving the results.

At a later time, computed datasets can be reloaded into the **Analysis** tab using the **Load dataset** window. Computed datasets appear beneath both the A and B datasets from which they were derived, and can be loaded from either location (Figure 7-26).

Computed datasets can be analyzed using 3D ROIs like any other scan with a full reconstruction. Because the computed fluorescence reflectance images are scaled to arbitrary units, 2D ROI analysis is not permitted using computed datasets.

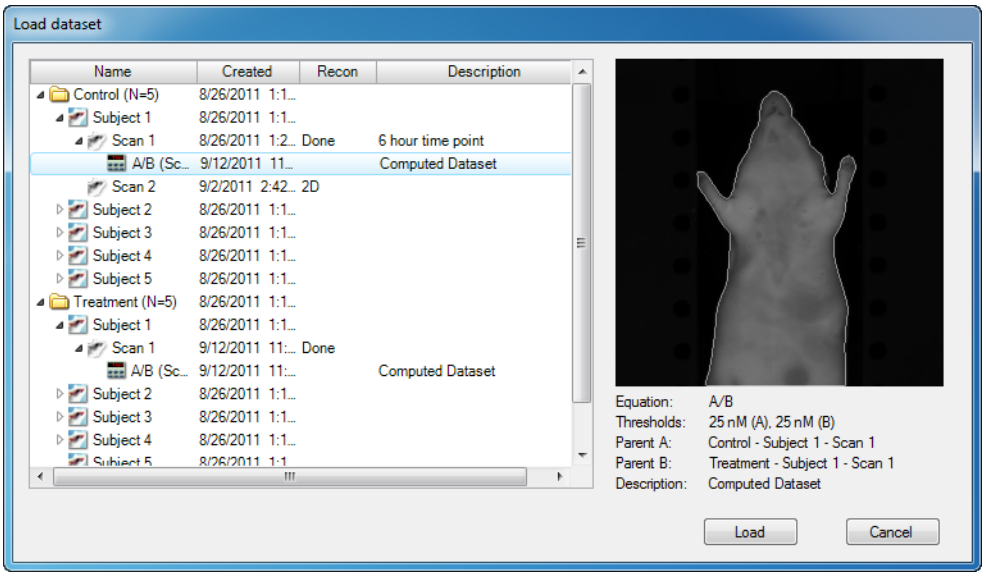

Figure 7-26. Loading a computed dataset into the **Analysis** tab

7.17 Thresholding Advisor

The **Thresholding Advisor** tool finds and sets the threshold for a large number of 3D geometric ROIs simultaneously. This tool also allows you to statistically determine a consistent threshold value from the ROIs you interactively defined in the viewport.

NOTE The Thresholding Advisor is only used with 3D geometric ROIs; it does not work with 2D or isosurface ROIs.

To access the **Thresholding Advisor**, shown in Figure 7-28, select **Tools** | **Thresholding** from the main menu, or use the tool bar buttons at the top of the view port.

When opening the **Thresholding Advisor** window, you can choose to use **All Datasets** in the study or limit the analysis to only the **Loaded Datasets**, as shown in Figure 7-27.

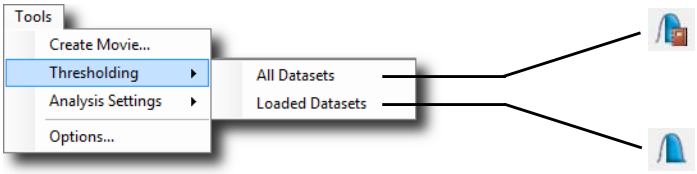

Figure 7-27. Accessing the **Thresholding Advisor** from the main menu and the equivalent toolbar icons

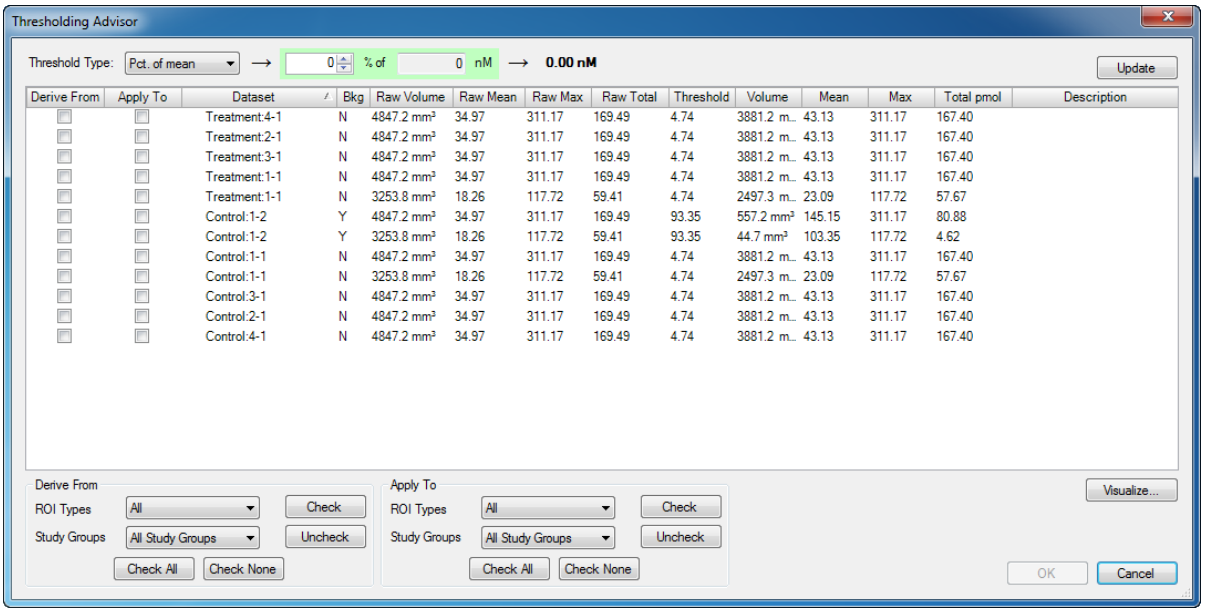

Figure 7-28. The **Thresholding Advisor** window

The table contains the following information:

Derive From

Indicates the selected row is used as part of the statistical analysis to calculate the new threshold value. Selecting the **Derive From** option for a row in the table automatically selects its **Apply To** option as well. Note, if a loaded ROI is an Isocontour shape, it is not used in the statistically derived threshold calculation and the **Derive From** column is unavailable for that table row.

Apply To

Indicates the selected row's threshold value will be updated using the statistically derived threshold value. The **Apply To** option is marked automatically for any row whose **Derive From** value is active. Note, if the ROI entry in the table is *locked*, indicated by a locked padlock icon in the **Dataset** column, you cannot apply the statistically derived threshold value to the ROI and the **Apply To** check box is unavailable for that table row. Refer to *section 7.12* for more information about locking and unlocking ROIs.

Dataset

Indicates which group, subject number, and scan number the selected ROI belongs to.

Bkg

Indicates whether the ROI is a region of background. A value of **Y** means the entry is a background region while a value of **N** identifies a non-background region.

Raw Volume

The ROI's volume, prior to adjustment using the statistically derived threshold value.

Raw Mean

The *mean* fluorochrome concentration in nM (nanomolar or 10^{-9} moles/liter) for all the voxels in the ROI prior to adjustment using the statistically derived threshold value.

Raw Max

The *maximum* fluorochrome concentration in nM (nanomolar or 10^{-9} moles/liter) for all the voxels in the ROI prior to adjustment using the statistically derived threshold value.

Raw Total

The amount of agent in pmol (picomoles or 10^{-12} moles) in the ROI prior to adjustment using the statistically derived threshold value.

Threshold

The new threshold value, statistically calculated using the selected **Threshold Type** option.

Volume

The ROI's volume, after adjustment using the statistically derived threshold value.

Mean

The *mean* fluorochrome concentration in nM (nanomolar or 10^{-9} moles/liter) for all the voxels in the ROI after adjustment using the statistically derived threshold value.

Max

The *maximum* fluorochrome concentration in nM (nanomolar or 10^{-9} moles/liter) for all the voxels in the ROI after adjustment using the statistically derived threshold value.

Total pmol

The amount of agent in pmol (picomoles or 10^{-12} moles) in the ROI after adjustment using the statistically derived threshold value.

Description

An optional user-supplied text description. Note, meaningful descriptions can be very useful in distinguishing between ROIs in the **Thresholding Advisor**, especially when dealing with many ROIs or very large numbers of datasets.

7.17.1 Threshold Type Option

In the **Thresholding Advisor**, choose the desired calculation option:

Percent of Mean

This option calculates a percentage of the average threshold value over the datasets. Type the percentage value into the field, in whole percentage points, or use the up and down arrows, to the right of the input field to adjust the value. With the **Threshold Type** set to **Pct. of Mean**, the **Thresholding Advisor** calculates the mean of the **Raw Mean** values (Figure 7-29, item B) for the ROIs marked as **Derive From**. When you enter the desired percentage into the field (Figure 7-29, item A), the **Thresholding Advisor** calculates the value and updates the display (Figure 7-29, item C). An example of the **Percent of Mean** calculation in the **Thresholding Advisor** is shown in Figure 7-29.

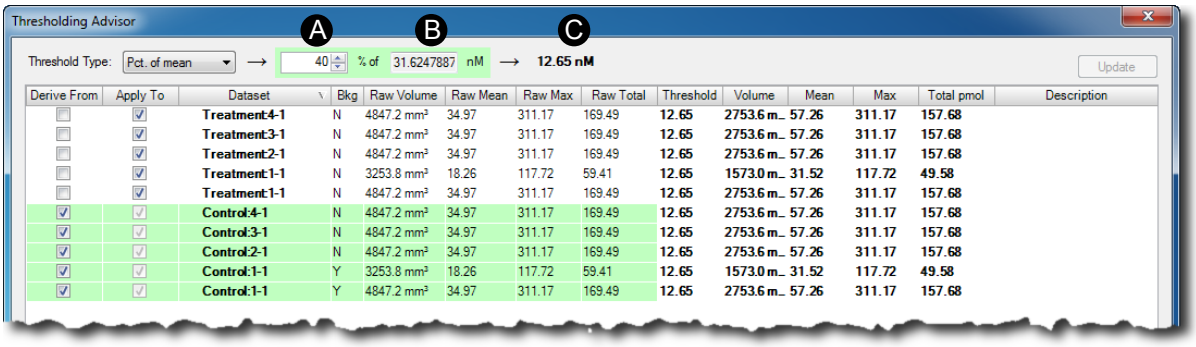

Figure 7-29. Percent of Mean calculation in the **Thresholding Advisor**

For example, if you enter *40* into the percent field in the window shown, the derived threshold value updates to *12.65 nM*.

The **Thresholding Advisor** example shown in Figure 7-29 provides several visual indicators about the operation it is performing. Notice the values used to make the calculation, the percentage and calculated mean threshold values, both at the top of the window, are set on a green background. Likewise, the rows in the table used to calculate the mean threshold value are similarly set on a green background. This lets you see at a glance which of the ROIs in the list contributed to the calculated value.

While the system has already calculated the desired percentage of the mean threshold value, the calculation is not yet applied to the ROIs in the table that are marked as **Apply To**. This is indicated by the values in the table displaying in gray text. Clicking **Update** applies the calculated threshold values to the ROIs in the table. Once updated, the text color for the calculated fields in the table changes from gray to bolded black, as demonstrated in Figure 7-30. Note, the **Update** button is no longer active and remains inactive until you make a change that modifies the calculated value.

Derive From	Apply To	Dataset	Bkg	Raw Volume	Raw Mean	Raw Max	Raw Total	Threshold	Volume	Mean	Max	Total pmol	Description
☐	☑	Treatment:4-1	N	4847.2 mm³	34.97	311.17	169.49	7.71	3436.9 m…	47.90	311.17	164.64	
☐	☑	Treatment:2-1	N	4847.2 mm³	34.97	311.17	169.49	7.71	3436.9 m…	47.90	311.17	164.64	
☐	☑	Treatment:3-1	N	4847.2 mm³	34.97	311.17	169.49	7.71	3436.9 m…	47.90	311.17	164.64	
☐	☑	Treatment:1-1	N	4847.2 mm³	34.97	311.17	169.49	7.71	3436.9 m…	47.90	311.17	164.64	
☐	☑	Treatment:1-1	N	3253.8 mm³	18.26	117.72	59.41	7.71	2148.5 m…	25.82	117.72	55.47	
☑	☐	Control:1-2	N	4847.2 mm³	34.97	311.17	169.49	7.71	3436.9 m…	47.90	311.17	164.64	
☑	☐	Control:1-2	N	3253.8 mm³	18.26	117.72	59.41	7.71	2148.5 m…	25.82	117.72	55.47	
☑	☐	Control:1-1	N	4847.2 mm³	34.97	311.17	169.49	7.71	3436.9 m…	47.90	311.17	164.64	
☑	☐	Control:1-1	N	3253.8 mm³	18.26	117.72	59.41	7.71	2148.5 m…	25.82	117.72	55.47	
☑	☐	Control:3-1	N	4847.2 mm³	34.97	311.17	169.49	7.71	3436.9 m…	47.90	311.17	164.64	
☑	☐	Control:2-1	N	4847.2 mm³	34.97	311.17	169.49	7.71	3436.9 m…	47.90	311.17	164.64	
☑	☐	Control:4-1	N	4847.2 mm³	34.97	311.17	169.49	7.71	3436.9 m…	47.90	311.17	164.64	

Figure 7-30. Thresholding Advisor with updated ROI table

You can also click **Visualize** to view the results of your calculated threshold value without committing the changes to your experimental ROIs. This allows you to experiment with values and calculation types to determine which meet your needs. The **Visualize** button has differing behavior depending on the state of the **Thresholding Advisor** ROI table. If you select one or more rows in the ROI table by clicking them, the **Visualize** button updates to include a drop-down menu component. If no rows are selected, the **Visualize** button remains a standard button. Figure 7-31 provides an example of both button states.

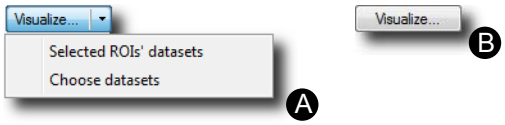

Figure 7-31. Thresholding Advisor button states

The **Visualize** button without the drop-down menu (Figure 7-31, item B) and the **Visualize** button **Choose datasets** menu option (Figure 7-31, item A) both open the **Choose Datasets to Visualize** window, shown in Figure 7-32.

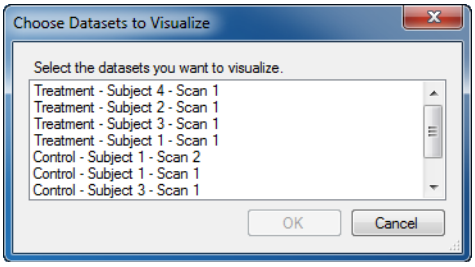

Figure 7-32. Choose Datasets to Visualize window

Alternately, the **Selected ROIs' datasets** menu option (Figure 7-31, item A) opens the **Visualize** window and displays the selected ROI row or rows from the table.

Even though the table in the **Thresholding Advisor** is updated, the threshold values for the ROIs in the **Analysis** tab remain unchanged until you click **OK**. Click **Cancel** to discard any changes you made and close the **Thresholding Advisor** window.

Percent of Max

This option calculates a percentage of the maximum threshold value over the specified ROIs. You type the percentage value into the field, in whole percentage points, or use the up and down arrows, to the right of the input field, to adjust the value. When you use the **Pct. of Max** option, the **Thresholding Advisor** determines the maximum value (Figure 7-33, item B) for the ROIs marked as **Derive From**. When you enter the desired percentage into the field (Figure 7-33, item A), the **Thresholding Advisor** calculates the value and updates the display (Figure 7-33, item C). An example of the **Percent of Max** calculation in the **Thresholding Advisor** is shown in Figure 7-33.

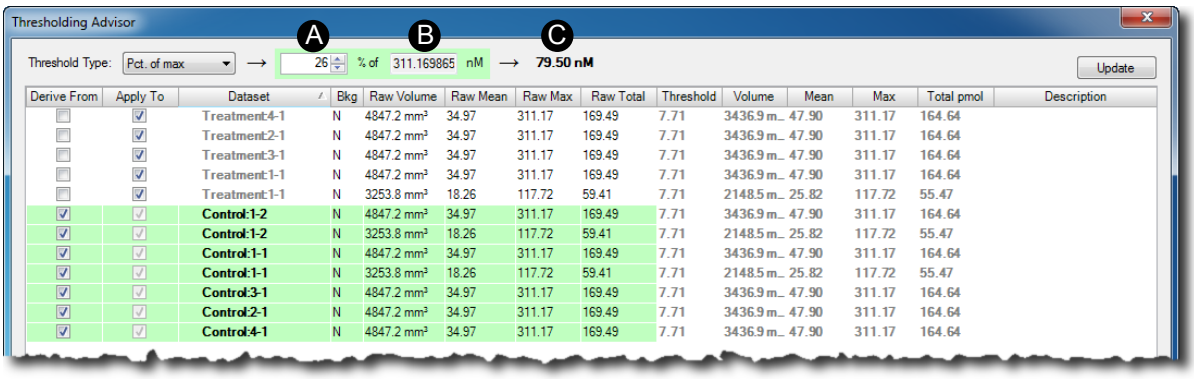

Figure 7-33. Percent of Max calculation in the **Thresholding Advisor**

The visual indicators described regarding the **Pct. of Mean** behave exactly the same way in this situation. Likewise, the **Update**, **Visualize**, **OK**, and **Cancel** controls all perform exactly the same functions here as described previously.

Value

This option assigns a user-provided threshold value to the dataset. You type the concentration value into the field, and the **Thresholding Advisor** updates the threshold value for the items in the dataset that you marked as **Apply To**, as shown in Figure 7-34. Note, the **Derive From** column is ignored when using this threshold type.

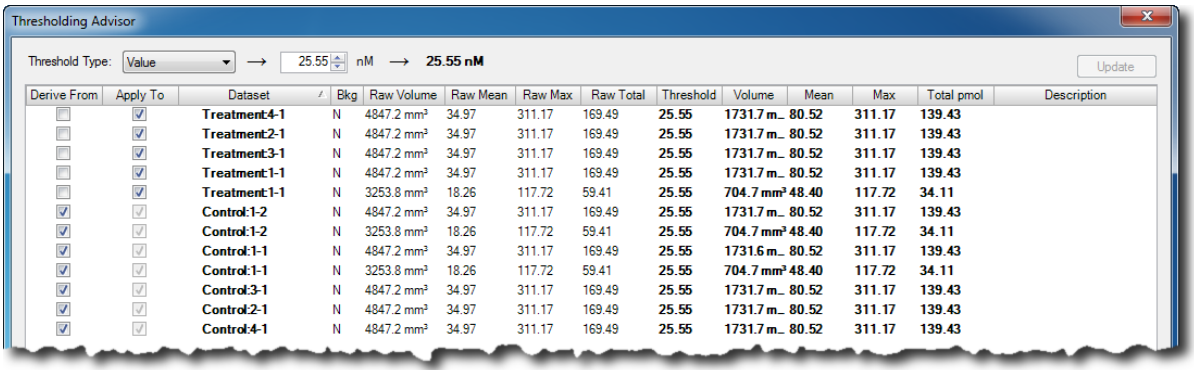

Figure 7-34. Value calculation in the **Thresholding Advisor**

The visual indicators and the **Update**, **Visualize**, **OK**, and **Cancel** controls all perform exactly the same functions here as described previously, except there are no green backgrounds since the **Derive From** column is ignored when using the **Value** threshold type.

Optimized

This option calculates the mean and standard deviation using the raw max value for all background ROIs in the dataset. After determining the standard deviation, the **Thresholding Advisor** determines the smallest maximum value. The dataset must include at least two background ROIs in order to use the **Optimized** calculation option. Furthermore, any ROI in the dataset with a max value outside two standard deviations is considered an outlier and is ignored when determining the smallest maximum value to use as the new threshold value. An example of the **Optimized** calculation in the **Thresholding Advisor** is shown in Figure 7-35.

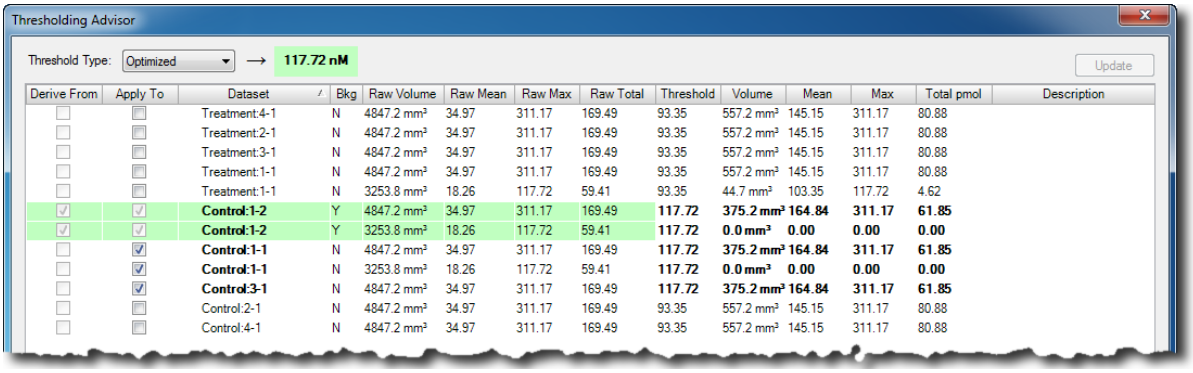

Figure 7-35. Optimized calculation in the **Thresholding Advisor**

Like the previous **Threshold Type** options, the visual indicators described in the discussion of the **Pct. of Mean** option behave exactly the same way in this situation. The **Update**, **Visualize**, **OK**, and **Cancel** controls all perform exactly the same functions here as described previously as well.

7.17.2 Filter Controls

The **Derive From** and **Apply To** controls, at the bottom of the **Thresholding Advisor** window, provide a quick way to select which table values to use when performing the statistical analysis and which ROIs to adjust using the new calculated threshold value. These controls are especially useful when you are analyzing a large number of ROIs.

7.17.2.1 Filtering by the Derived From option

You can filter the list of ROIs in the table and set the **Derived From** options based on ROI types (**All**, **Background**, and **Non-background**) and by **Study Group** (**All Study Groups** or by individual study group name). The **Check** and **Uncheck** buttons use the chosen filters to select or deselect the applicable ROIs' **Derive From** options. The **Check All** and **Check None** buttons set the **Derive From** option for all the ROIs in the table.

7.17.2.2 Filtering by the Apply To option

You can perform the same type of filtering on the **Apply To** option and select ROIs based on type (**All**, **Background**, and **Non-background**) or **Study Group** (**All Study Groups** or by individual study group name). The **Check** and **Uncheck** buttons use the chosen filters to select or deselect the applicable ROIs' **Apply To** options. The **Check All** and **Check None** buttons set the **Apply To** option for all the ROIs in the table.

7.18 Creating Movies

You can create and export movies in the MPEG-4 (.mp4) or Windows Media (.wmv) format directly from the **Analysis** tab of TrueQuant.

NOTE AVI file export is no longer available.

The movie clips animate the contents of the viewport in the selected sequence, and can include ROI analysis information. The movie files can then be viewed with any standard media player and/or inserted into presentations.

The movie is generated by creating *key frames* of the desired views. The intermediate frames between the key frames are automatically interpolated when the movie file is created. Boolean properties, such as **Show isosurface**, take effect at the key frames where they change, while continuous properties, such as thresholds, transparency levels, or ROI boundaries, are interpolated to intermediate values between key frames. ROI wireframes display in the movie exactly as they appear in the **Analysis** tab.

The software interpolates between successive key frames and creates intermediate frames at the rate of 15 frames/second (fps), with the camera path interpolated along a 3D spline. Most commonly, users will pan/zoom/rotate around the 3D reconstruction while creating the movie animation (Figure 7-37). Key frames can include changes to all display controls (Reflectance Image, Isosurface, Volume Rendering, Slices, Color Scales) and changes to ROIs. Rotations between consecutive key frames should be less than 90º in order to maintain a well-defined camera path in the final movie.

To create a movie:

1. Load one or more datasets in the **Analysis** tab.
2. Select **Tools** | **Create Movie**. The **Create Movie** window opens (Figure 7-36), allowing you to define a series of views called *key frames*. The software interpolates the frames between the defined key frames to create a continuous movie. Note, the **Create Movie** menu item is disabled if more than one panel is displayed in the **Analysis** tab.

   While the **Create Movie** window is open, the display options to the right of the viewport can be changed. The following controls are not available while the Create Movie window is open: the **Load**/**Unload** dataset buttons, the buttons to create new ROIs, and the ability to switch to a different tab. The show/hide icon for ROI clipping in the **Analysis** tab is saved on a keyframe-by-keyframe basis when creating movies.
3. Select the desired movie settings in the **Create Movie** window:

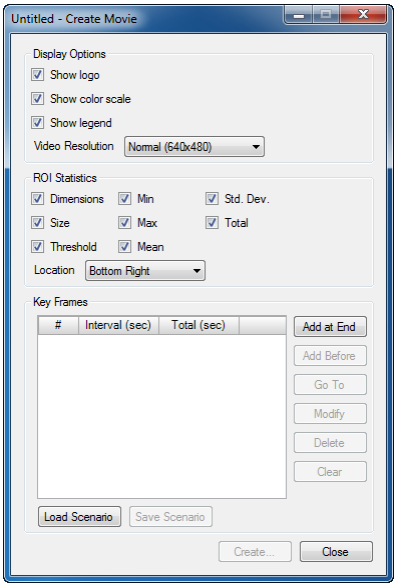

Figure 7-36. Create Movie window

- Select the desired **Video Resolution**: **Large (1280x960)**, **Normal (640x480)**, or **Small (320x240)**. The default resolution is Normal. The **Display Options** and **ROI Statistics** options control the contents and location of the text and color scale that are superimposed on the movie frames:
- If selected, the **Color scale** displays on the right side of the movie. The color scale is taken from either the 2D or 3D scale, whichever is active, using the appropriate units. The upper and lower limits of the scale match the limits displayed below the viewport. The short name of the dataset (Group name, Subject number, Scan number) displays below the units.
- If selected, the **Legend** displays on the left side of the movie. The legend displays the name of the imaging agent(s) used in each of the animated datasets.
- The selected ROI statistics for any ROIs visible in the viewport are overlaid at the bottom of the movie. The statistics are dynamic, and change if the ROI is changed during the movie. For example, resizing the ROI changes the numerical information. Each ROI statistic displays with the same precision and units as is used in the ROI table below the viewport. The ROI's description field is used to label the ROI statistics in the movie clip.

  All ROI statistics are selected by default. If none of the loaded datasets have any ROIs, all ROI statistics are disabled by default.

4. Use the controls in the Analysis tab to set up the desired starting view.
5. Click the **Add at End** button to create the first key frame in the movie.
6. Change the view as desired for the next key frame and add another key frame. After the first key frame is added, the **Add Before** button is available to allow you to insert a new key frame before the currently-selected key frame in the **Key frames** list.
7. Continue adding and adjusting the keyframes as described below until the set of key frames is complete.

8. To review a key frame, select the key frame and click the **Go To** button.
9. To modify a key frame, set the **Analysis** tab to the desired state, then select the key frame and click the **Modify** button.
10. To change the time between a selected key frame and the previous key frame, click the **Interval** value and type the desired interval.
11. To delete an individual key frame and automatically recalculate the camera path, select the key frame and click the **Delete** button.
12. To delete all the key frames in the list, click **Clear** and accept the confirmation.
13. When the key frame list is complete, click **Create** to create a movie file. At any time during creation of the output file, you can interrupt the generation of the movie by clicking **Stop** and accepting the resulting confirmation.

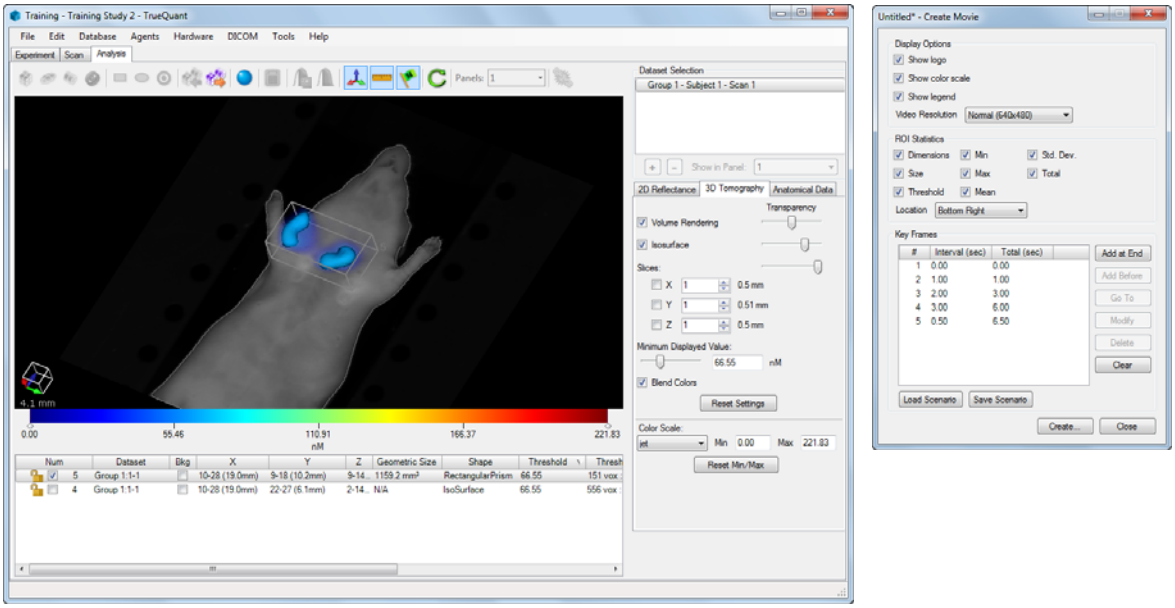

Figure 7-37. Adding key frames to a movie clip

To cancel creation of a movie, click the **Close** button at any time without saving, which closes the **Create Movie** window.

You can save a key frame list as a **Scenario**, which can be loaded at any time. Scenarios can be applied to any dataset, but any information about ROIs in individual key frames is discarded if a scenario is used for a dataset or datasets other than the ones that were loaded when it was created. If you defined any key frames before attempting to load a scenario, the software presents a warning dialog notifying you that the loaded scenario will replace the existing key frames.

NOTE For computer systems with sufficient video cards, exported movies look identical to the viewport representation, including multiple ROI clipping. For systems with less-modern video cards, multiple ROI clipping is not supported in exported movies. Refer to *section 2.4* for a listing of system requirements and recommendations.

7.19 Exporting Images

You can export images from the **Analysis** tab by selecting "**File** | **Export** | **Image**" from the main menu. Images can be saved in a variety of standard image formats, including Windows Bitmap (BMP), Joint Photographic Experts Group (JPEG), Portable Network Graphics (PNG) and Tagged Image File Format (TIFF). Images can also be saved at screen resolution for electronic presentation or at print resolution for publication. Display of the ROI boundaries, ROI Statistics, and color scales can be turned on or off. If multiple panels are being used, the panel labels can be turned on or off, and the panels can be exported individually as separate files or tiled into a single file as displayed in the **Analysis** tab. Figure 7-38 provides an example of the **Image Export Settings** window.

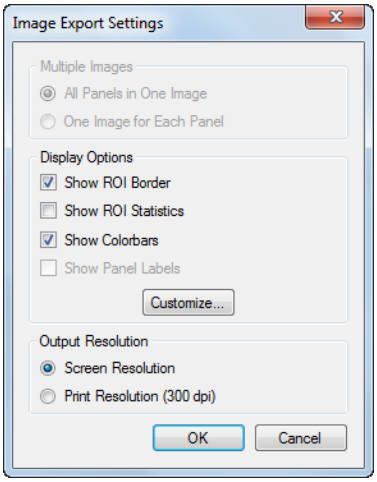

Figure 7-38. Image Export Settings

Clicking **Customize** on the **Image Export Settings** window opens the **Customize Exported Images** window, shown in Figure 7-39.

Select the size of the label font and the number of decimal places displayed. Type the desired labels for the color scales. The color scale label displays below the color scale for the panel. Select or clear the desired ROI Statistics to overlay on the image. Select the location for the ROI Statistics. The example in Figure 7-39 shows an export of one image for each panel with two panels displayed in the **Analysis** tab.

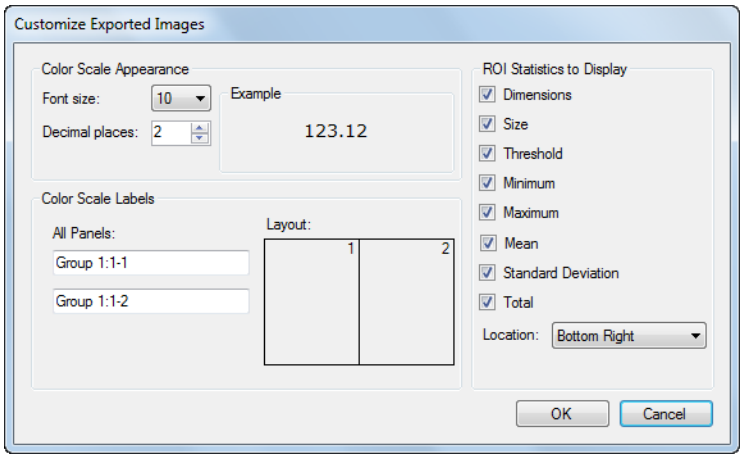

Figure 7-39. Customize Exported Images Window

NOTE The modifications in the **Customize Exported Images** window are saved in the current Windows user profile. As such, the changes are included in all future image exports performed by the current user.

The exported image matches the image display in the **Analysis** tab exactly, including the coordinate axes and any ROIs that are visible. In addition, the exported image includes the optional color scale for each displayed dataset if the **Show colorbars** option was selected during export.

For computer systems with sufficient video cards, the exported images look identical to the viewport representation, including multiple ROI clipping. For systems with less-modern video cards, image exports approximate multiple ROI clipping using image compositing. Refer to *section 2.4* for a listing of system requirements and recommendations.

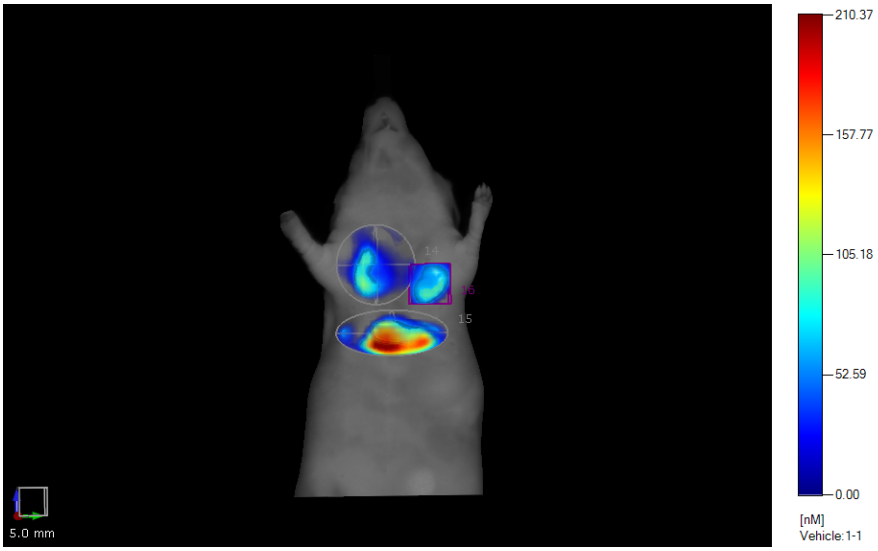

Figure 7-40. Sample exported image with multiple ROIs

7.20 Saving and Loading Analysis Tab Settings

It may sometimes be desirable to consistently apply the same display settings across an entire study, for example when analyzing imaging data within a large study. TrueQuant provides a mechanism for saving display settings in the **Analysis** tab for subsequent application of these settings to any dataset.

Select **Tools** | **Analysis Settings** | **Save** from the menu to save the current settings to an XML file. This saves all the analysis view options to the right of the viewport, the color scale settings, and the pan, zoom, and rotation settings of the viewport. The settings that are saved are taken from the dataset that is selected in the Dataset Selection list. This menu item is disabled if no datasets are selected or if more than one is selected.

NOTE The ROI clipping setting is not included in the saved **Analysis** tab settings.

To apply the display settings to subsequent datasets, load the desired dataset or datasets, select the datasets in the Dataset Selection list, select **Tools** | **Analysis Settings** | **Load** from the menu, and select the desired settings file (Figure 7-41).

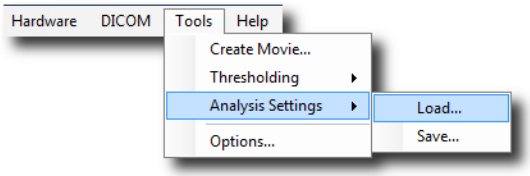

Figure 7-41. Saving and loading the **Analysis** tab settings

7.21 User Preferences

TrueQuant offers the option of overriding a number of default settings with user preferences. Selecting **Tools** | **User Options** opens the User Options window to set the **Scan**, **Analysis**, and **DICOM** settings (Figure 7-23).

Note that these preference settings do not affect the data, but only the way the data is displayed automatically when the application is started. These settings can be changed individually and interactively during a session.

The Scan Tab specifies the default colormap displayed during a scan. Changes to the scan colormap are applied after the software is restarted. In addition, preview reconstructions, which are lower resolution uncalibrated reconstructions, can be disabled. For the Multi-Species Imaging Module, the default animal orientation within the MSIM cassette is set here. The last two preferences take effect the next time a scan is performed in the **Scan** tab, without requiring you to restart TrueQuant.

The Analysis tab displays the default settings for displaying data and images in the **Analysis** tab. Some of these preferences apply the next time a dataset is loaded, while others apply only after you restart TrueQuant. This is indicated above the preferences.

The DICOM tab sets the bit depth at which DICOM series are exported. See *section 5.10.4* for further details.

8

Imaging Agents and Agent Calibration

The FMT system requires a calibration scan for each imaging agent used with the system to enable precise quantification. The calibration process effectively measures the agent's photochemical properties as detected by the instrumentation, and accounts for them in tomographic reconstruction and subsequent analyses. An uncalibrated agent cannot be used in tomographic mode, but the software will allow the acquisition of its reflectance images even when uncalibrated.

The FMT instrument is pre-calibrated in the factory for a number of agents from PerkinElmer on each wavelength channel. The software allows for calibration of new, user-provided agents and other commercial agents. It is also recommended, although not required, to initiate an agent calibration at the onset of every major study.

8.1 Calibrating an Agent

The calibration process is an abbreviated and automated version of a regular in vivo scan, tomographic reconstruction and template-based ROI analysis. Calibration of a single agent takes approximately 10 minutes.

The automated agent calibration process requires using a calibration kit provided by PerkinElmer. To calibrate custom agents, you can follow the dye formulation procedure summarized in *section 8.3*.

From TrueQuant's main menu, select **Agents** | **Calibration** to open the automated Agent Calibration window (Figure 8-1).

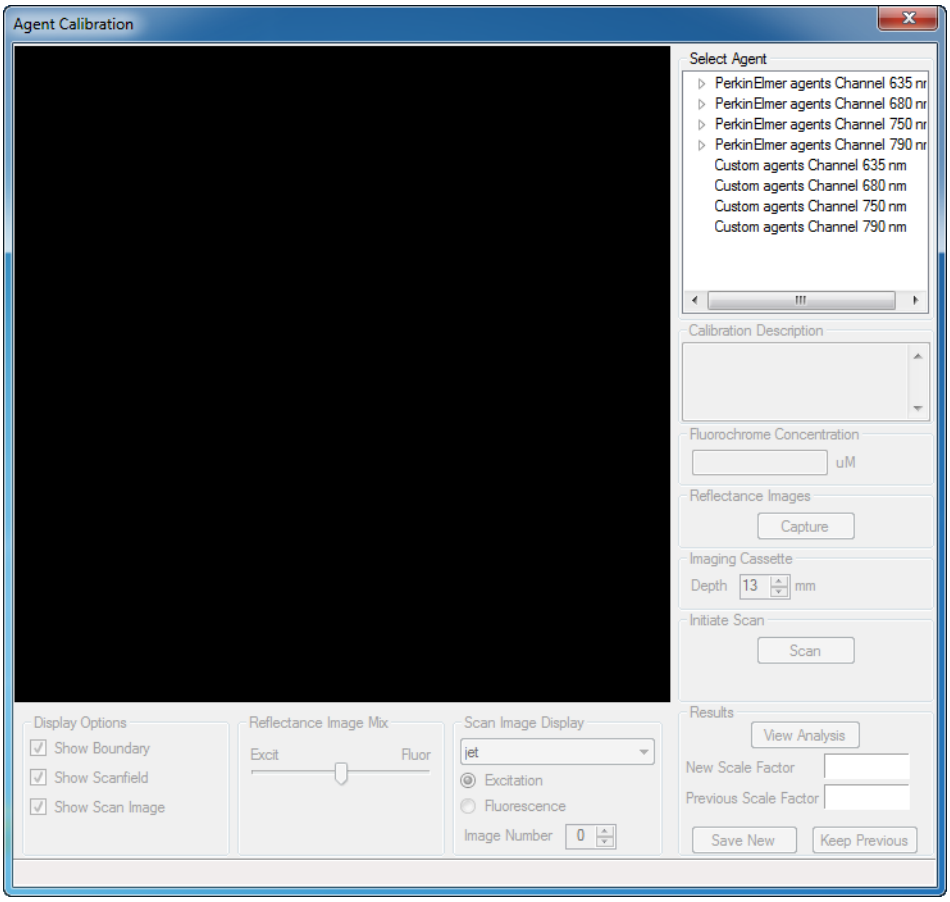

Figure 8-1. Agent Calibration window

Expanding the tree listing of agents on either channel shows the current agents entered into the software, and highlights in a bold green font the agents for which the system has already been calibrated (Figure 8-2). The agents listed in regular black font do not have a calibration associated with them, and can therefore be imaged in reflectance mode only (i.e. not in tomographic mode). Any agent can be re-calibrated on the FMT at any time.

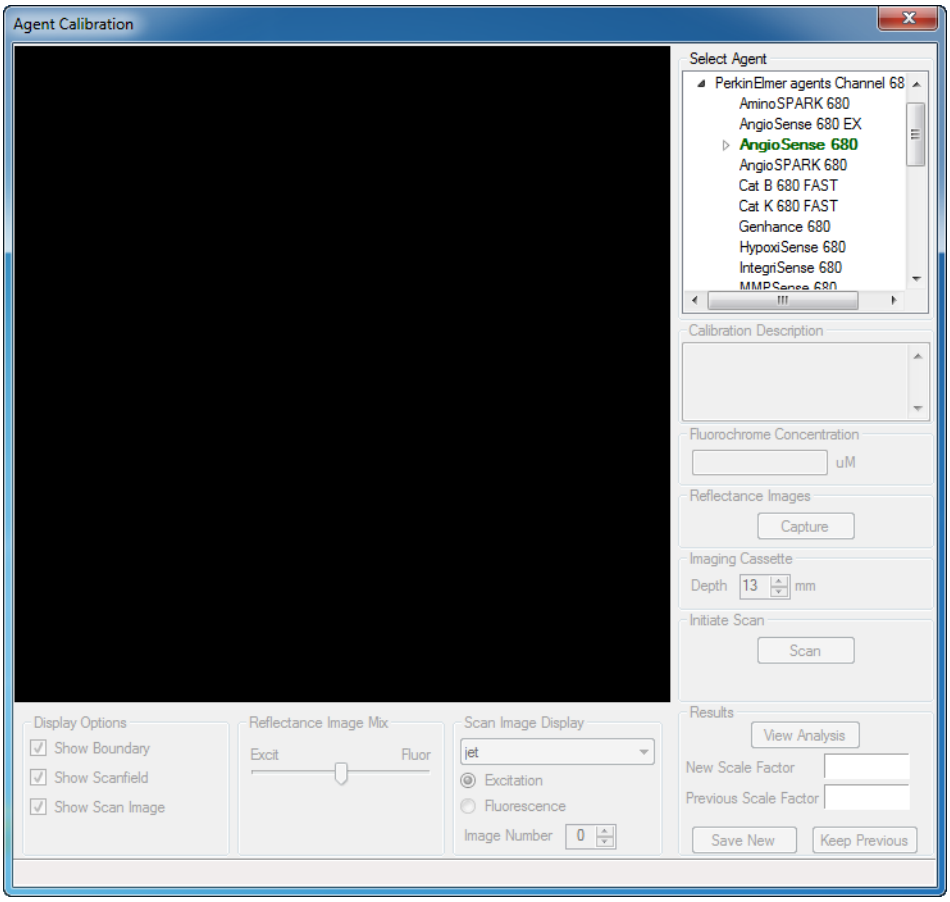

Figure 8-2. Listing of calibrated (green) versus uncalibrated (black) agents.

To calibrate an agent:

1. Select an agent from the list.

1. Type an optional text description in the **Calibration Description** text field, if desired.

2. Inject 100 µL of fluorochrome corresponding to the agent being calibrated into the calibration phantom provided by PerkinElmer. Place the phantom in the imaging cassette and into the imaging chamber.

3. Enter the concentration of the calibration solution, as measured by absorption in a spectrophotometer – in units of micromolar (µM). Recommended range: 0.1 µM to 0.5 µM.

4. Capture a reflectance image (Figure 8-3).

5. Initiate the scan.

It is important, for the accuracy of the calibration, to accurately dispense 100 µL of solution into the phantom, and to provide the exact concentration of that solution in steps 2 and 3 above, respectively. These two numbers are both taken into account in the computation of the resulting scale factor.

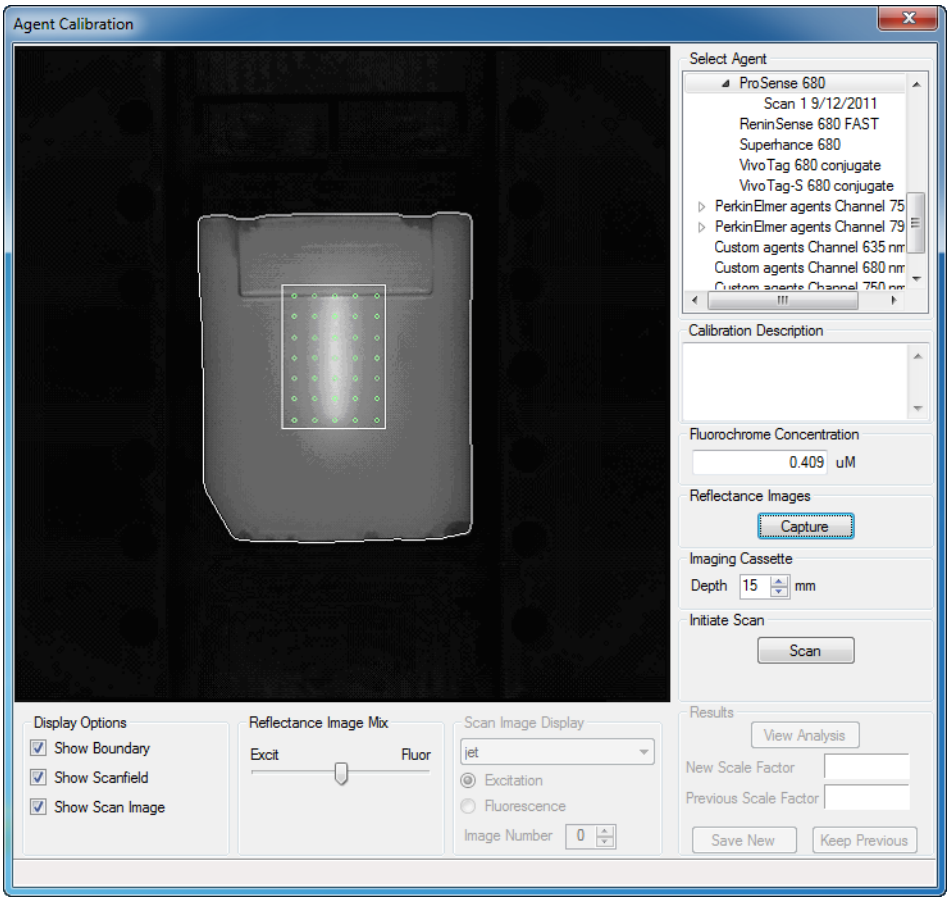

Figure 8-3. Reflectance image capture of the calibration phantom.

Once the scan is completed, it is recommended (although not strictly necessary) to view the results of tomographic reconstruction and analysis by clicking **View Analysis** (Figure 8-4). The result of the calibration is displayed as a numerical scale factor in the **New Scale Factor** field near the bottom of the display (Figure 8-5). Click **Save New** underneath to save the calibration results.

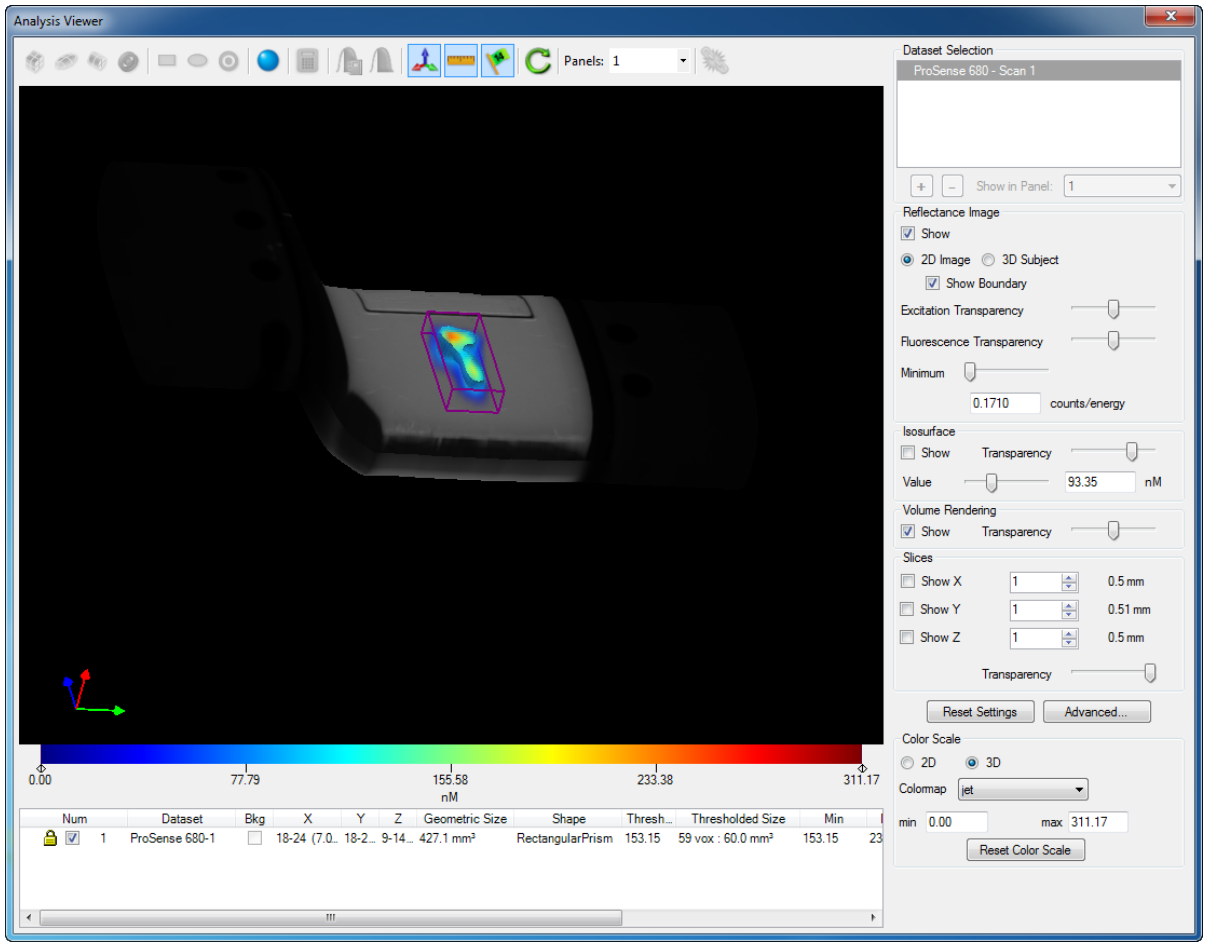

Figure 8-4. Analysis of the calibration scan.

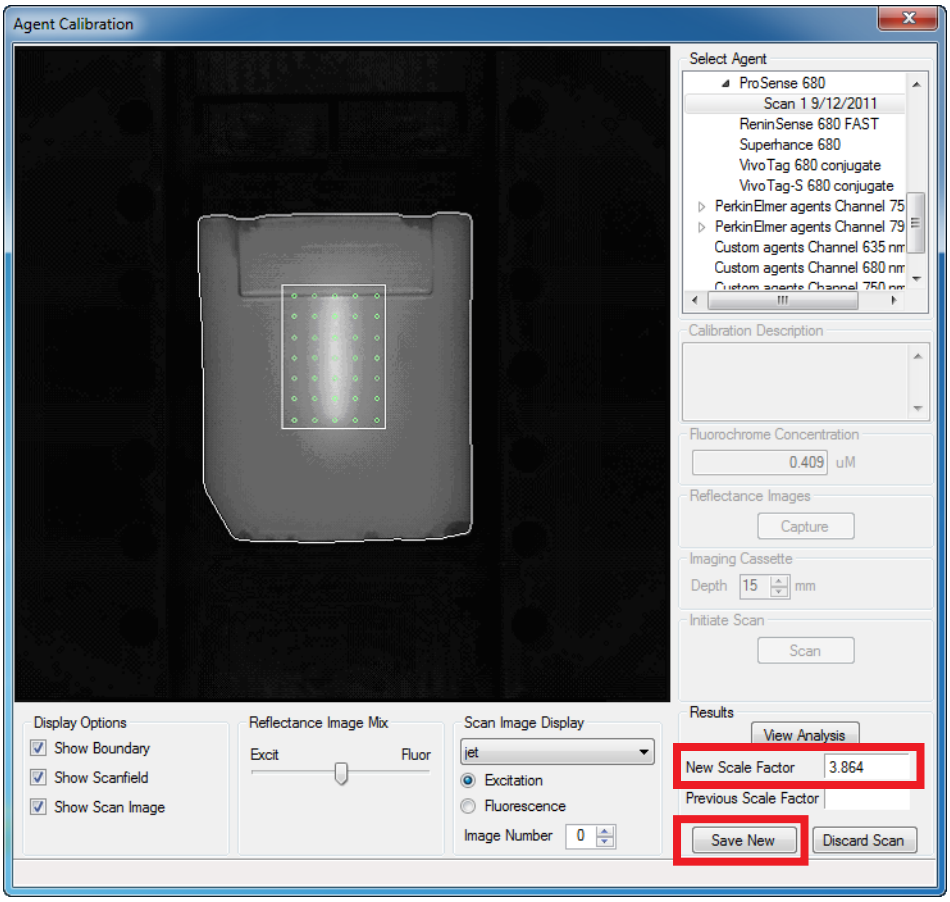

Figure 8-5. Computing and saving the scale factor.

8.2 Managing Custom Agents

In addition to the PerkinElmer agents that come preconfigured for use with TrueQuant, user-defined agents can also be used. To create new custom agents or manage existing ones, select **Agents** | **Manage** from the application menu, which opens the **Manage Agents** window (Figure 8-6). The **Manage Agents** window shows a list of the currently configured PerkinElmer agents and user-defined agents, and has a set of buttons that can be used to perform tasks related to user-defined agents.

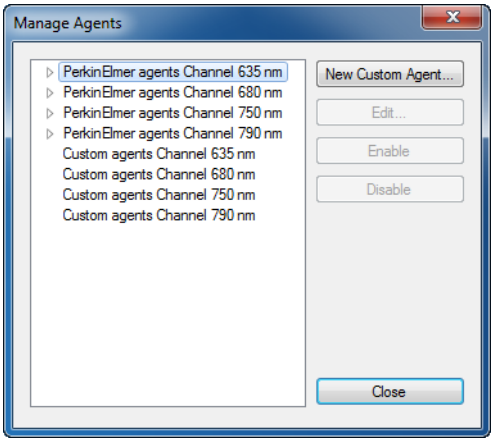

Figure 8-6. The **Manage Agents** window.

To create a user-defined agent, click the **New Custom Agent** button, which opens the **Add New Custom Agent** window (Figure 8-7). Enter a name for the new agent, select the laser channel on which it will be used, and enter an optional description, then click **OK**. This adds the newly created agent to the agent database. The new agent is treated like all other agents—prior to a tomographic scan and reconstruction of a subject injected with this agent, the agent must be calibrated using the procedure in *section 8.1*.

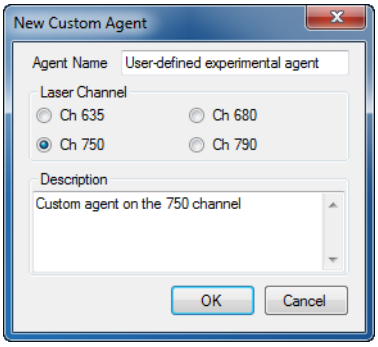

Figure 8-7. Adding a new custom agent.

The new agent name displays in the list in the **Manage Agents** window (Figure 8-8).

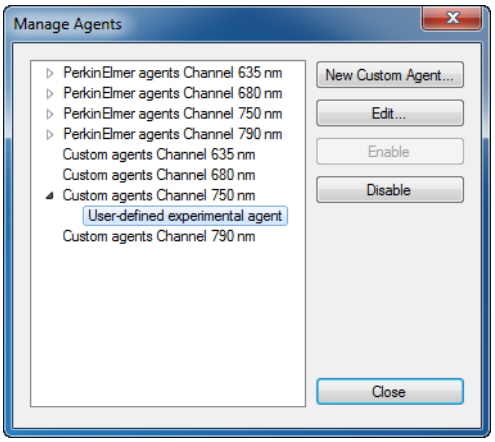

Figure 8-8. The **Manage Agents** window displaying the newly-created agent.

Once a custom agent has been created, its name and description can be edited by selecting the agent and clicking **Edit**. This opens a window identical to the **New Custom Agent** window, but with the fields already filled in with the name, channel, and description of the selected agent.

If the list of custom agents on one channel gets to be fairly long, it can be difficult to manage, e.g. when selecting the agents on that channel for a new study group. Custom agents that are no longer in use can be disabled from the **Manage Agents** window. Disabled agents are displayed in gray in the **Manage Agents** window, and are not included in the list of custom agents anywhere else in TrueQuant. For example, disabled agents are not displayed in the **Agent Calibration** window or in the **Scan** tab. This will in effect shorten the list of custom agents to a more manageable length.

8.3 Titrating a Calibration Solution for a Custom Agent

The FMT system makes it possible for you to define and calibrate new agents, as described in *section 8.2*. It is important to formulate and dispense the calibration solution accurately. This section outlines, for illustrative purposes, the method followed at PerkinElmer to prepare a 400nM calibration solution.

Dye Preparation

1. Remove a 1.0 molar vial of dye from the freezer/refrigerator[1]. Record the lot number, absorptivity value, and absorbance maximum. Allow the vial to warm to room temperature.
2. Add 1.0 mL of water to the vial and vortex to dissolve completely. (*Solution A*)
3. Add 0.2 mL of the dye solution to a new vial and combine with 14.8 mL of water. Vortex to combine. (*Solution B*)

Solution B Concentration Determination

1. Add 1.0 mL of water to a cuvette to serve as a blank. Add 1.0 mL of Solution B to 3 cuvettes.
2. Set a UV-Vis Spectrophotometer to scan the absorbance profile from 400-900nm.
3. Perform a background correction using the blank cuvette. Auto-zero using the blank cuvette. Scan the water cuvette to verify the baseline.
4. Scan all 3 cuvettes containing Solution B and record their maximum absorbance. Determine the mean value of the absorbance's and record the value.
5. Using the absorptivity value (ε) from the dye vial, and the mean absorbance value (Mean Abs) of Solution B, determine the concentration of Solution B (C_B) using the following equation.

$$C_B = \left(\frac{MeanAbs}{\varepsilon}\right) \times (1.0 \times 10^9) \qquad [\text{nM}]$$

1. The exact amount, and the resulting concentration of Solutions A and B, will vary depending on the dye's extinction coefficient. This difference is normalized during the creation of Solution C.

Solution C Preparation

1. Using the concentration of Solution B (CB) calculated in step 2.5, determine the volume of Solution B (V_B) required to prepare a 400 nM solution using the following equation.

$$V_B = \frac{2400}{C_B} \quad [\text{mL}]$$

2. Using the volume of Solution B (V_B) calculated in step 3.1, determine the volume of water (VW) required to prepare a 400 nM solution using the following equation.

$$V_W = 6.0 - V_B \quad [\text{mL}]$$

3. Transfer the volume of water determined in step 3.2 to a new vial. Transfer the volume of Solution B determined in step 3.1 to the vial. Cap and vortex to combine. (*Solution C*)

Solution C Concentration Determination

1. Add 1.0 mL of water to a cuvette to serve as a blank. Add 1.0 mL of Solution C to 3 cuvettes.
2. Set the UV-Vis Spectrophotometer to scan the absorbance profile from 400-900nm.
3. Perform a background correction using the blank cuvette. Auto-zero using the blank cuvette. Scan the water cuvette to verify the baseline.
4. Scan all 3 cuvettes containing Solution C and record their maximum absorbance. Determine the mean value of the absorbency and record the value.
5. Using the absorptivity value (ε) from the dye vial, and the mean absorbance value (Mean Abs) of Solution C, determine the concentration of Solution C (C_C) with the following equation.

$$C_C = \left(\frac{MeanAbs}{\varepsilon}\right) \times (1.0 \times 10^6) \quad [\mu\text{M}]$$

6. Verify that the concentration of Solution C determined is 400 ± 10 nM.

Storage

1. Transfer 2.0 mL of Solution C into a vial and label with the exact concentration.
2. Store the vial at 2-8ºC and protect from light.

8.4 The Master Agent List

The complete list of all PerkinElmer and custom agents that have been configured for use is stored in the master agent list. This list should be stored in a location that is accessible by any remote computers on which TrueQuant is installed. The default location of the master agent list on the FMT system host PC is on its local network as `\\[FMT2500]\fmtdata\Probes`, where `[FMT2500]` is the Windows Computer Name of the Host PC, but any other networked file server will work equally well.

To access the master agent list from a remote PC, the Windows share on which the list resides must be mapped to a drive letter. Using the default location as an example, we first map \\FMT2500\fmtdata to the P: drive. Note, you can choose any unused drive letter in place of P.To do this choose **Windows Start**, right-click **Computer,** and select **Map Network Drive** from the menu. Select drive letter P: and enter \\FMT2500\fmtdata as the folder (see Figure 8-9). Be sure to select the **Reconnect at logon** option so the drive will be correctly mapped the next time you log onto the computer. Once the network drive has been successfully mapped, open TrueQuant and select **Agents** | **Master Agent List Location** from the menu. Enter P:\Probes as the new location, or click **Browse** and navigate to that folder. Click **OK** to set P:\Probes as the new master agent list location.

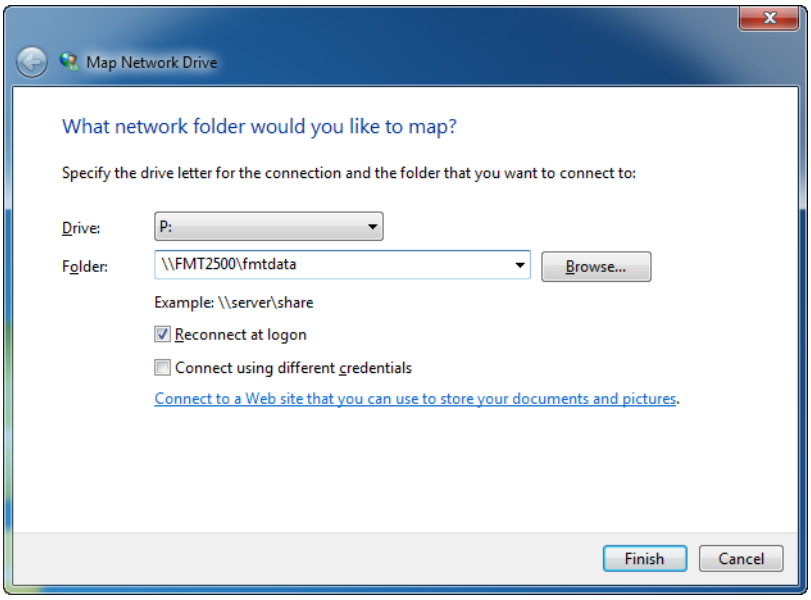

Figure 8-9. Mapping the master agent list to a drive letter in Windows.

9 Animal Strains

The Animal Strain specifies the cassette type used for the animal and the hair color of the animal. The animal hair color is used when determining the animal boundary. The cassette type is applied automatically when setting up the study.

Animal strains that use the MSIM cassette only display if the MSIM option is installed and configured in the software.

9.1 Creating a New Animal Strain

To create a new animal strain:

1. On the TrueQuant main menu, select **Tools** | **Customize Animal Strains** to open the Customize Animal Strain window (Figure 9-1).

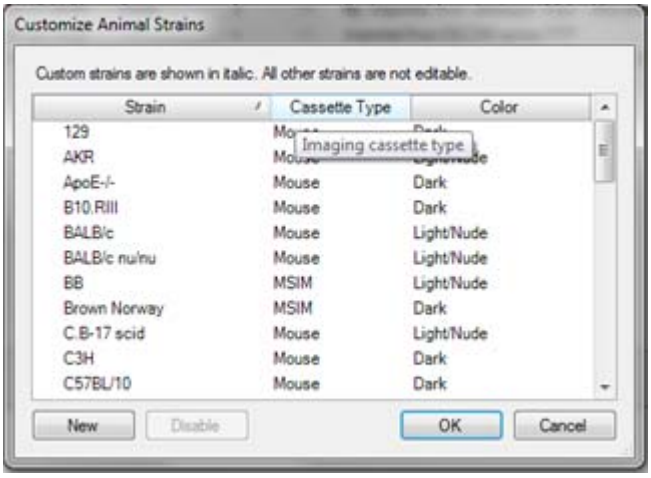

Figure 9-1. Customize Animal Strain window

2. Click the **New** button. A new animal strain displays at the bottom of the window.
3. Click on the **Strain** name and type the desired name for the new strain.
4. Under **Cassette Type**, select either Mouse or MSIM.
5. Under **Color**, select the fur color of the animal.
6. Click **OK** to create the new animal strain and close the window. The new animal strain is available in the New Study window and the Edit Study window.

9.2 Disabling Animal Strains

Animal strains can be disabled to prevent certain strains from displaying in the list of available Strains.

To disable specific animal strains:

1. On the TrueQuant main menu, select **Tools** | **Customize Animal Strains** to open the Customize Animal Strain window (Figure 9-1).
2. Click on an animal strain to hide.
3. Click the **Disable** button. The strain displays in gray text.
4. Repeat steps 2 and 3 for each animal strain to hide.
5. Click the **OK** button to close the Customize Animal Strain window. The disabled animal strains do not display in the New Study window or the Edit Study window.

To display an disabled animal strain, select a disabled animal strain and click the **Enable** button.

9.3 Editing Custom Animal Strains

Custom animal strains can be edited to change the name or the hair color. Only custom animal strains can be edited. The preconfigured strains cannot be edited.

To edit a custom animal strain:

1. On the TrueQuant main menu, select **Tools** | **Customize Animal Strains** to open the Customize Animal Strain window (Figure 9-1).

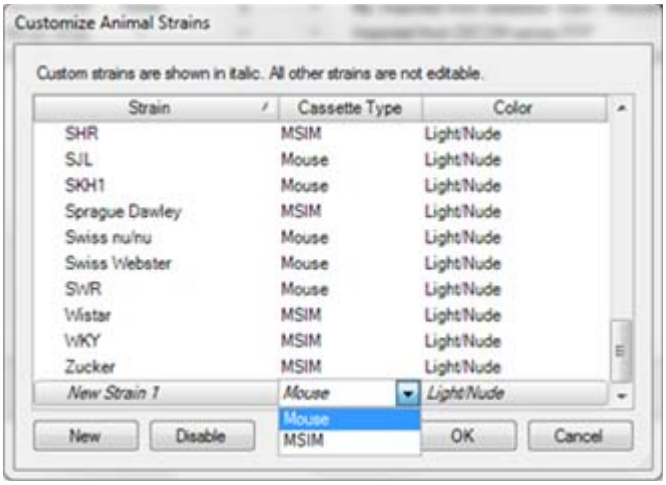

Figure 9-2. Editing a custom animal strain

2. Click on the **Strain** name and type the desired name for the strain.
3. Under **Cassette Type**, select either Mouse or MSIM.
4. Under **Color**, select the fur color of the animal.
5. Click **OK** to change the animal strain and close the window.

10

Database Management and Backups

10.1 Database Management

Creating and deleting databases, and subscribing to and unsubscribing from remote databases is done in TrueQuant. To access any of these functions, select **Database** | **Manage** on the main menu to open the **Manage Databases** window (see Figure 10-1). This window is also used to move studies from one database to another.

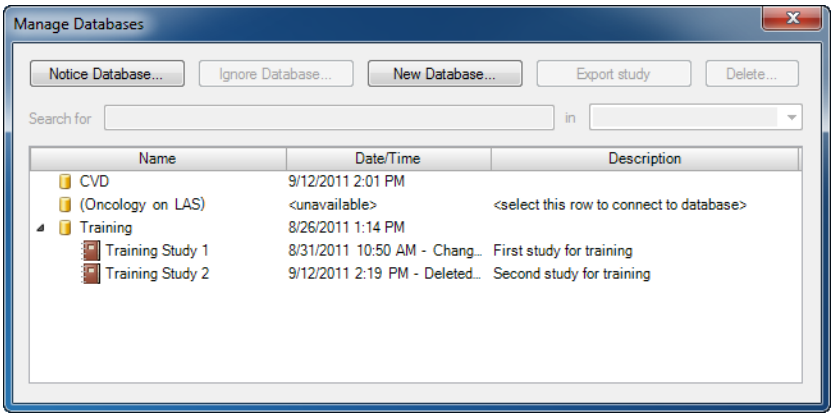

Figure 10-1. Manage Databases window

10.1.1 Creating Databases

To create a new database:

1. Click the **Experiment** tab on the TrueQuant main window.
2. Select **Database** | **Manage** from the menu. The **Manage Databases** window opens as shown in Figure 10-2.

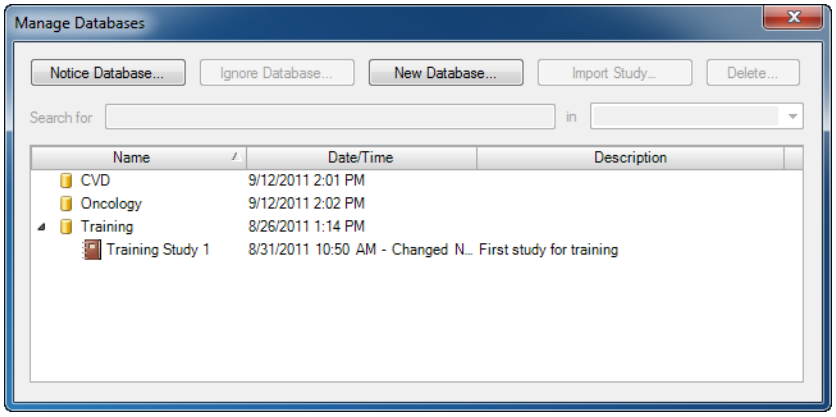

Figure 10-2. Manage Databases Window

3. Click the **New Database** button. The Create Database window opens as shown in Figure 10-3. The Create Database window displays the computers connected to the Local Area Network (LAN). The local computer name displays by default in the Database Server text box.

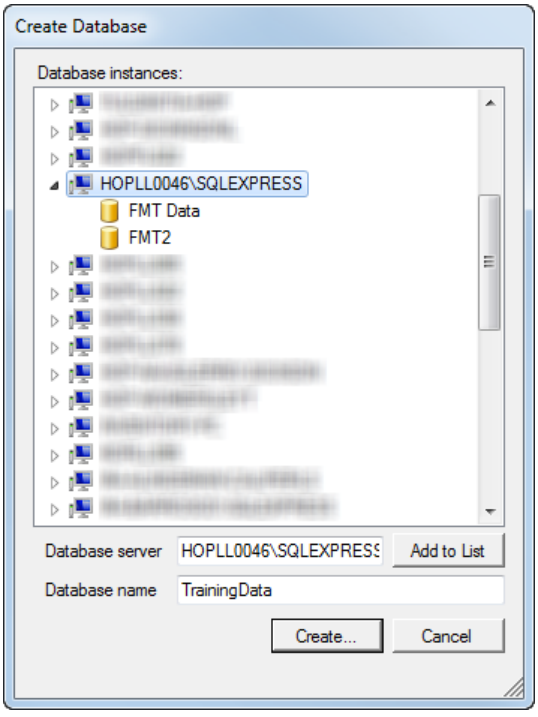

Figure 10-3. Create Database Window

4. If the desired computer is not on the LAN, type the computer name in the **Database Server** text box and click the **Add to List** button.
5. Select the database server where the new database will be saved. The selected computer name displays in the **Database Server** text box.
6. Type the desired name for the new database in the **Database Name** text box.
7. Click the **Create** button. The Select Security Group window opens.

Figure 10-4.Select Security Group window

8. Select the Windows Security Group that will be allowed to access the new database. Each TrueQuant database can be accessed by the user who created it, plus one other Windows user or group that is designated here. If a Windows security group is used, all members of that group are able to view and edit the new database.
9. Click **OK** and wait for the new database to be created, which can take several minutes. The new database is created and is set as the current database in the Experiment tab.

NOTE When using databases hosted on another computer, you must connect to the remote database at least once every 14 days. If you do not, you will not be able to synchronize data between the local and remote computers. Attempting to do so generates an error message informing you the subscription to the database has expired. TrueQuant will attempt to re-establish remote synchronization for the database automatically. See *section 10.1* for more details.

10.1.2 Deleting Databases

To delete a database, select the database from the list in the **Manage Databases** window and click **Delete**. You are asked to confirm that you want to delete the database; clicking **Yes** permanently deletes the selected database.

10.1.3 Noticing an Existing Database

There are several circumstances when a database that you have access to already exists, but you are not yet able to use it until you “notice” it in TrueQuant. The act of noticing a database subscribes your computer to that database, making it available for use in TrueQuant. For example, if another user creates a database, it is not immediately visible to all members of the group that was given access to that database. Similarly, if you create a database on the FMT system Host PC, you are not able to use it on an investigator PC until it is noticed on that second PC.

To notice an existing database, click **Notice Database** in the **Manage Databases** window. This opens a window similar to the **Create Database** window. Click the graphical symbol (plus sign for Windows XP or an arrow for Windows 7) next to the database server that hosts the database you want to notice. This displays a list of databases on that server to which you have access. Select the database to notice, and click **OK**. Alternatively, you can manually type the names of the database server and the database itself in the text fields. If the database is hosted locally on your computer, it is available immediately. If it is hosted on a remote computer, the process of subscribing to that database can take several minutes depending on your network bandwidth.

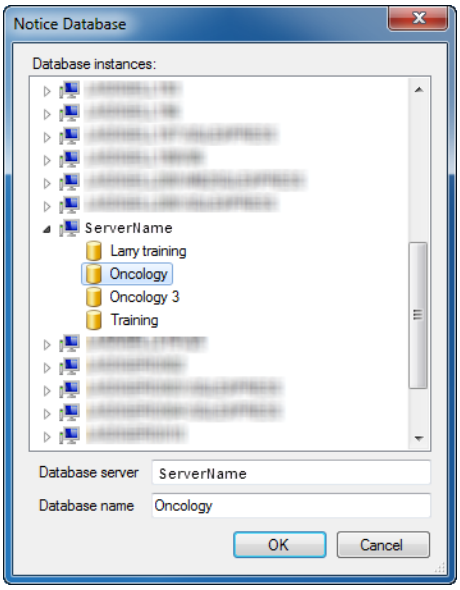

Figure 10-5. The **Notice Database** window.

If a database has not been used in the last 14 days, your computer cannot synchronize the database between your computer and the remote computer. When you try to activate such a database by selecting it in the **Experiment** tab, **Reconstruction Queue Manager**, **Manage Databases** window, or elsewhere, TrueQuant displays an error message stating the database's subscription has expired. To resume synchronizing this database with the remote host, ignore the database as described in *section 10.1.4*, then re-notice the database.

10.1.4 Ignoring a Database

To remove a database from the list of noticed databases in the **Manage Database** window, select the database you wish to ignore and click **Ignore Database**. TrueQuant then prompts you to confirm that you wish to ignore the selected database.

10.1.5 Exporting Studies

To export an entire study, select **Database** | **Manage** from the application menu to open the **Manage Databases** window (see Figure 10-6). Select the desired study, and click **Export Study**.

Choose a location and name for the exported study and click **Save**. An exported study consists of a folder with the specified name, which contains two XML files and two folders of image and reconstruction data.

To import an entire study, select the database you want to import into, then click **Import Study** (see Figure 10-7). Select the top level folder of the study to be imported—the folder that contains the XML files and Scans and Recons folders.

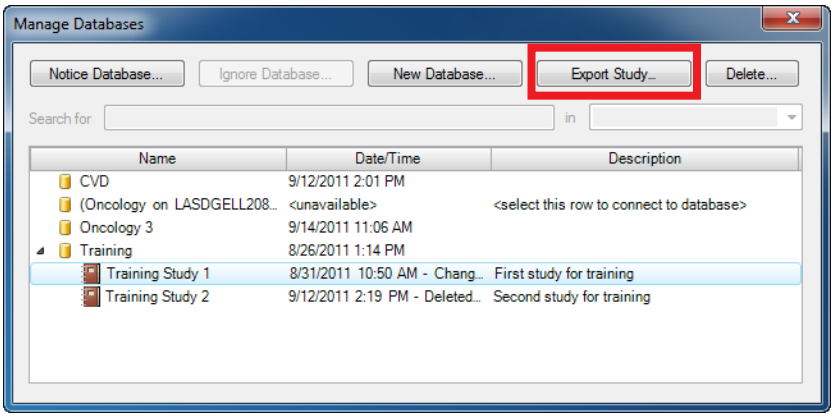

Figure 10-6. Exporting an entire study.

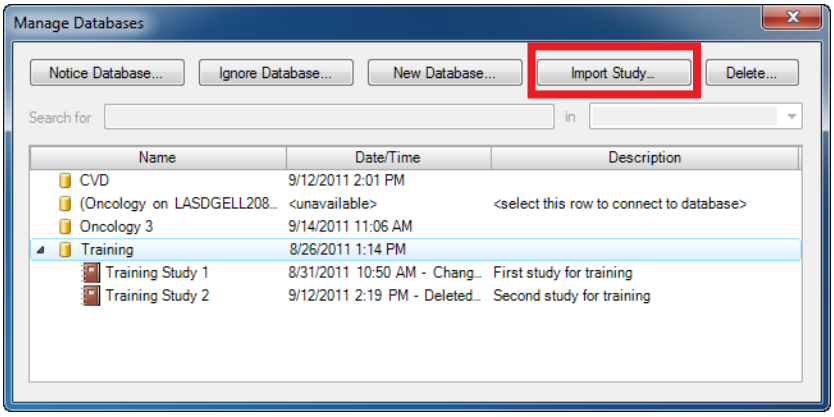

Figure 10-7. Importing a study into a database.

10.2 On-line/Off-line modes

For users with laptops, TrueQuant provides the ability to take studies or entire databases into “offline” mode for use on the road.

- In normal “online” mode, all data analysis is saved directly into the database where the study resides, even if this database is on another computer such as a file server on a local area network.
- When the computer doing the analysis no longer has network access to the remote database, offline mode can be used to store a temporary local copy of that remote database. In offline mode, all analyses are saved as local copies.
- When the computer is back on the network, the study or studies can be brought back into online mode, and any work that was done while offline is uploaded to the remote database. At the same time, any changes made on that remote database by other users are downloaded to the local computer.

After this point, the study is back “online” and you once again save your analysis directly into the remote database.

Any database you noticed from a remote host must be synchronized with the remote host at least once every 14 days, or it will no longer be able to synchronize. This is especially important for databases that have been taken into offline mode. If a database is offline for more than 14 days, any changes made while offline are not synchronized with the remote host. You must bring offline databases back online within 14 days of taking them offline or any changes made while in offline mode will be lost.

Offline functionality is accessed through the **Database** | **Set Offline Studies** menu. This opens the **Set Offline Studies** window (Figure 10-8), allowing you to select the studies you want to take offline. Select the check box next to the individual studies you want to have accessible when offline and click **OK**. To bring previously offline studies back online, deselect the check boxes next to those studies before clicking **OK**.

Please note that only remote studies can be selected for offline operation, as the studies stored on a local database, such as the local hard drive, are by definition always available.

Taking a study offline requires a few minutes to synchronize the data files over the local area network between the local machine and remote database.

Offline studies can be loaded into the Experiment tab from the offline database. When a study is offline, some operations, such as deleting scans, are disabled. Other operations, such as adding a study group or importing data, are allowed.

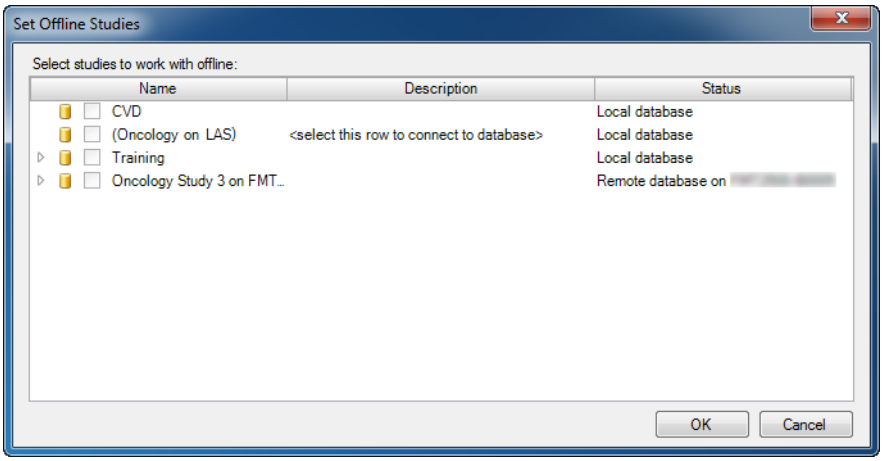

Figure 10-8. The **Set Offline Studies** window.

10.3 Database Schema Upgrades

New major releases of TrueQuant occasionally require changes to the database structure, also known as the database *schema*. This usually results from application-driven needs to capture an increasing amount of information in the database. These schema changes are entirely backward compatible and do not affect prior FMT data that was acquired and/or archived before the upgrade.

The process of upgrading the database schema is handled automatically for local databases on the FMT Host PC and on individual investigator PCs. On these machines, each database is upgraded automatically to the new schema version the first time it is activated in TrueQuant.

Host PCs and Data Storage Servers should be upgraded to the new version of TrueQuant first, and all of their databases should then be upgraded to the new schema version. Data Storage Servers should use the **Database Upgrade Assistant** for performing database schema upgrades. Once this is complete, investigator PCs should be upgraded to the new version of TrueQuant, at which point users of those computers can use their remote databases.

10.4 Backups

The FMT system host computer is configured with SQL Server 2014 and SQL Server Management Studio.

To backup a database:

1. Open and start **SQL Server Management Studio** from the **Windows Start** | **All Programs** | **Microsoft SQL Server 2014** menu.
2. Connect to your SQL Server database.
3. In the left column, expand **Databases**, right-click the database you want to back-up and choose **Tasks** | **Back Up** (Figure 10-9).
4. Select **Full** for the **Backup type** option. Under **Destination**, click **Add** (Figure 10-10)

5. Enter the path and file name for the backup file, and click **OK** (Figure 10-11)

6. Review the settings for the backup, and click **OK** to begin the backup.

These instructions create a complete backup of your database, and are recommended on a regular basis to ensure that all data is backed up.

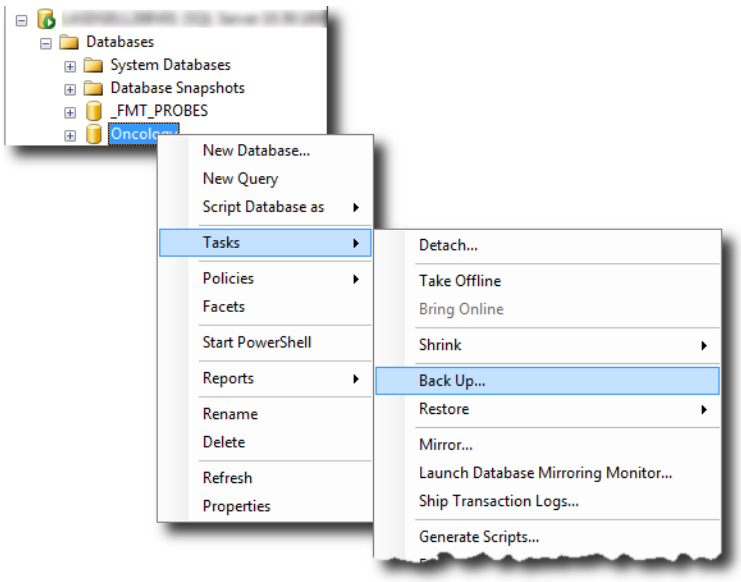

Figure 10-9. Backing up databases from SQL Server Management Studio.

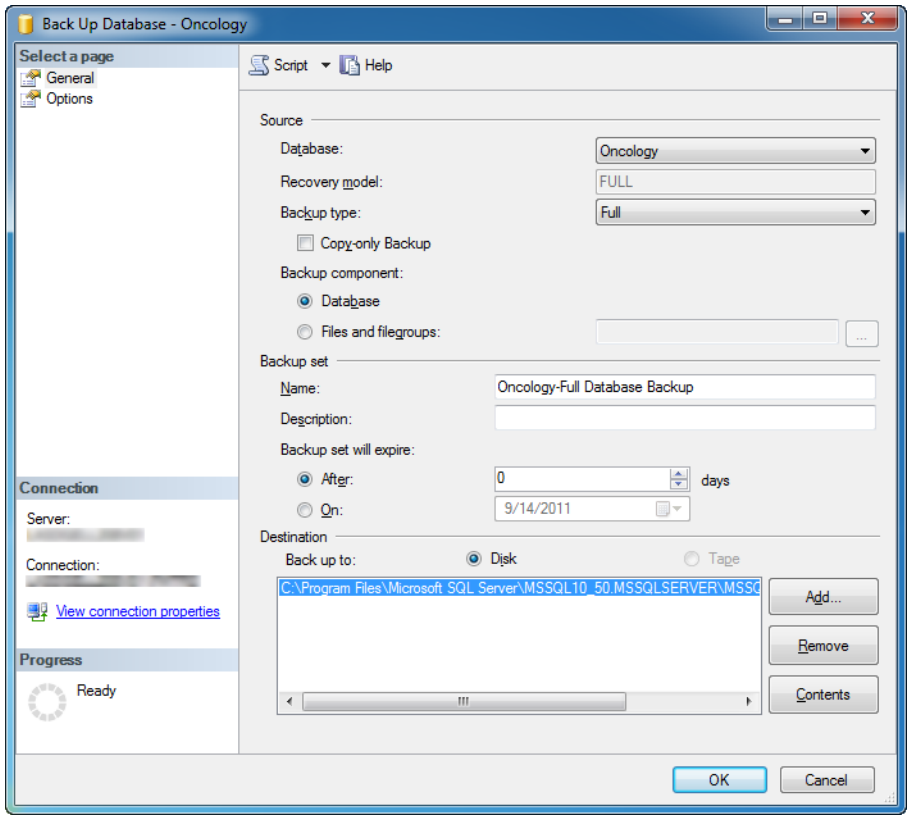

Figure 10-10. Selecting **Full** backup type, and clicking **Add**

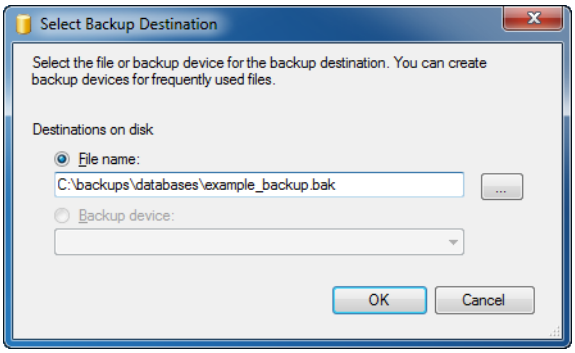

Figure 10-11. Entering a path and file name for the backup file.

In addition to backing up the database, it is recommended that you back up the image data, hardware calibration data, and master agent list on a regular basis.

The image data and hardware calibration information is stored in one of the following locations, depending on your operating system:

Windows XP

C:\Documents and Settings\All Users\Application Data\VisEn Medical\FMT

Windows 7

C:\ProgramData\VisEn Medical\FMT

This folder can be backed up using your usual system for file system backups.

The location of the master agent list can be determined by selecting **Agents** | **Master Agent List Location** from the menu of TrueQuant. The current location of the master agent list is given on the second line of the resulting window (Figure 10-12). It is a good idea to back up the contents of this folder regularly using the same method as the image data and hardware calibration folders.

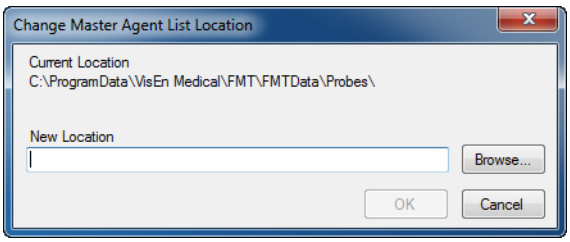

Figure 10-12. Determining the location of the master agent list.

10.5 Automating Backup of SQL Server Databases

The previous section described how to back up both databases and image files produced during imaging on the FMT system. However, the process of backing up Microsoft SQL Server databases that is described there is purely a manual one. This process can also be automated. In combination with a user-provided automated backup client, this makes it possible to perform automated backups of both the databases and image files.

Automated database backups are scheduled by setting up a Maintenance Plan in SQL Server. The easiest way to do this is using the Maintenance Plan Wizard in SQL Server Management Studio (SSMS) on the FMT system Host PC. To start the wizard from SSMS, open the **Management** folder, right-click on **Maintenance Plans**, and select **Maintenance Plan Wizard** (Figure 10-13).

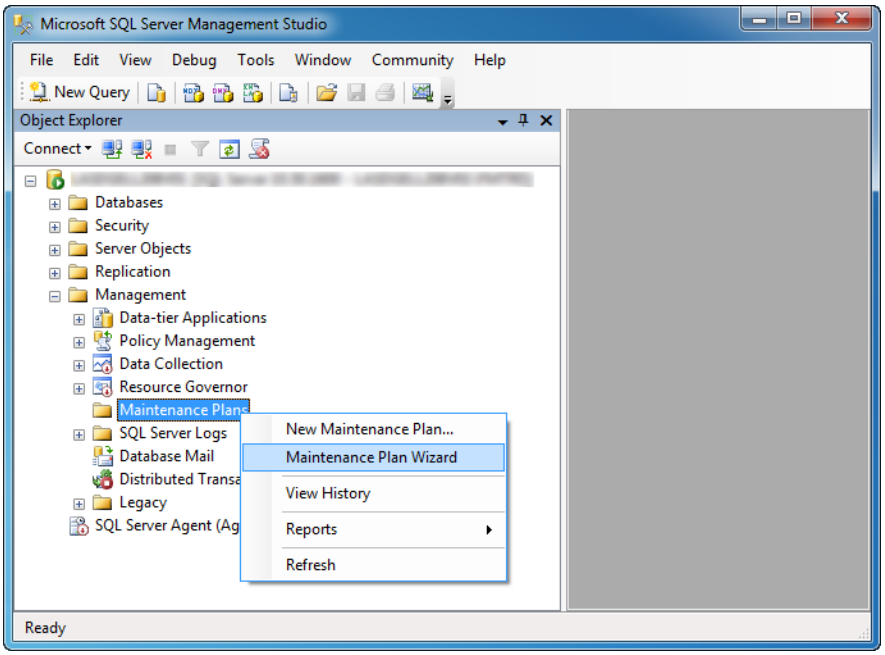

Figure 10-13. Starting the **Maintenance Plan Wizard**.

After the wizard's splash screen, give your backup plan a name, and click the Change button to schedule when the backups occur. It is recommended that you schedule your backups daily for a time when no one is using the machine, such as 1:00 AM. A sample plan schedule is shown in Figure 10-14. Alternatively, you can select **Separate schedules for each task** if, for example, you want to perform full database backups only once a week but want to perform incremental backups nightly.

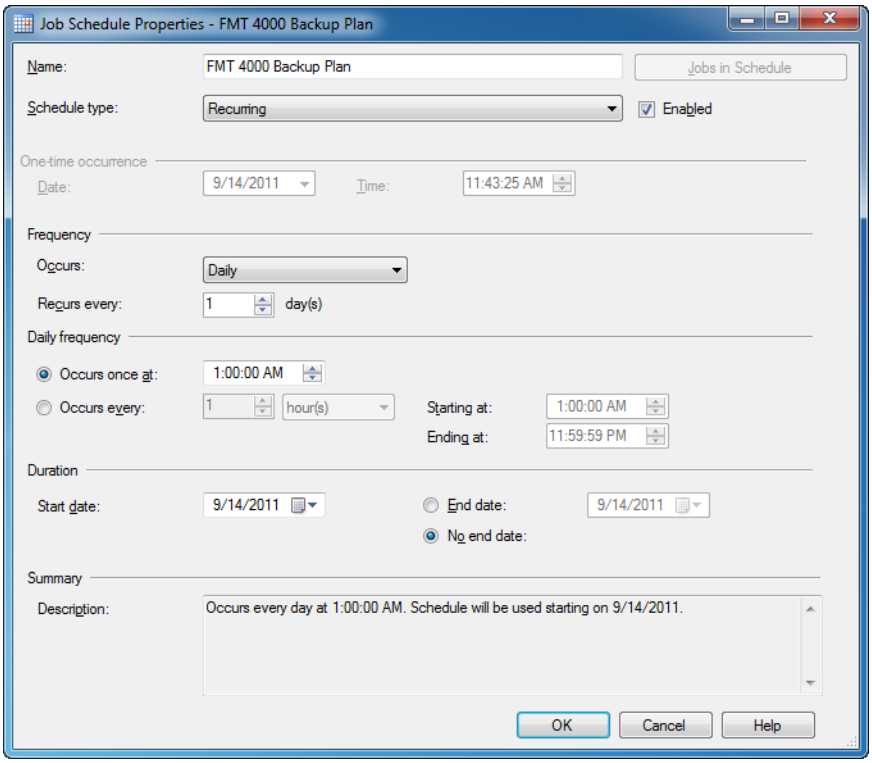

Figure 10-14.A sample maintenance plan schedule.

Next, select the desired maintenance tasks. For completeness, select all three backup types: **Full**, **Differential**, and **Transaction Log**. The wizard automatically sets these up to execute in the order above.

The next step is to select which databases to back up for each backup type. So that newly created databases are automatically added to the list of databases to back up, select **All user databases**. It is easiest to leave the default backup location of `C:\Program Files\Microsoft SQL Server\MSSQL.1\MSSQL\Backup` since this folder is created automatically by SQL Server. In addition, it is recommended that you choose the option **Verify backup integrity** as a safety measure. If you selected **Separate schedules for each task** above, you must set the schedule for this backup task. Repeat the setup process for each of the three backup types. A sample configuration is shown in Figure 10-15.

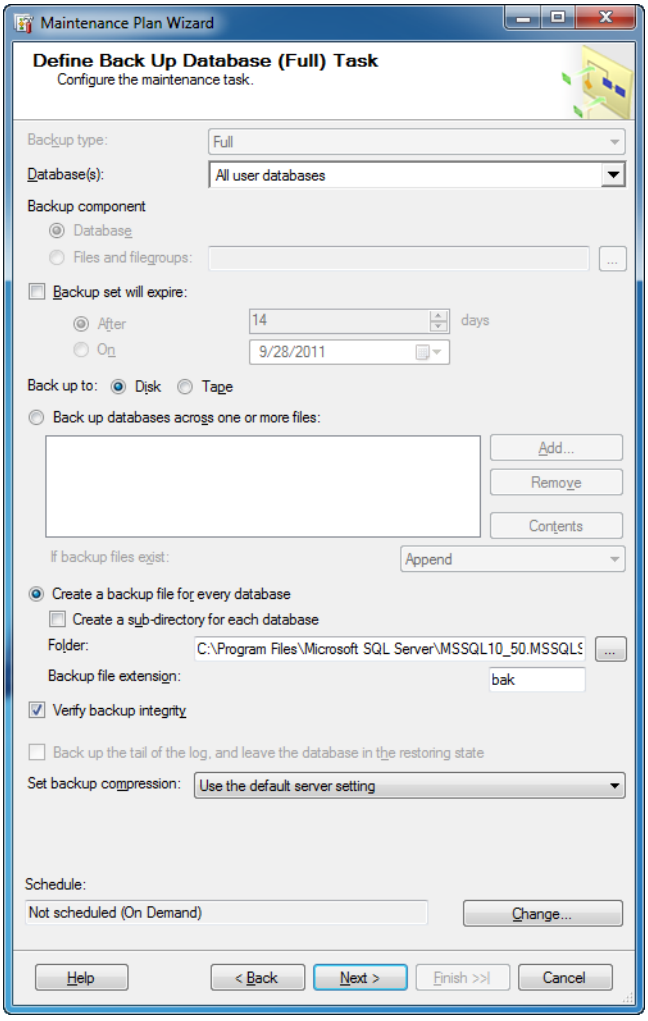

Figure 10-15. Configure the databases to be backed up.

By default, the wizard configures the backups to write a report to a text file so you can verify that each backup completed successfully. Finally, it displays a summary of the configuration options you selected. You can change any of them by using the **Back** button to get to the window where the desired options are set, then use the **Next** button to continue. When you are satisfied with the configuration, click **Finish** to create the maintenance plan.

NOTE To avoid having these backup files fill the available disk space, be sure to archive and clear older backup files regularly. Alternatively, you can set up a second maintenance plan can to clean up these files. Contact your local IT department to help set up this part of the backup plan.

Once you complete the automated backup configurations, automated backup software installed by the customer should be configured to back up the files from the backup location specified above.

11 Guidelines and Troubleshooting Tips

Please contact PerkinElmer for technical support and troubleshooting of other aspects of instrument operation.

11.1 TrueQuant Log Files

TrueQuant provide access to the log files generated by the software during its normal operation. Select **Help** | **Logs** from the main menu to open the **Application Logs** window, shown in Figure 11-1.

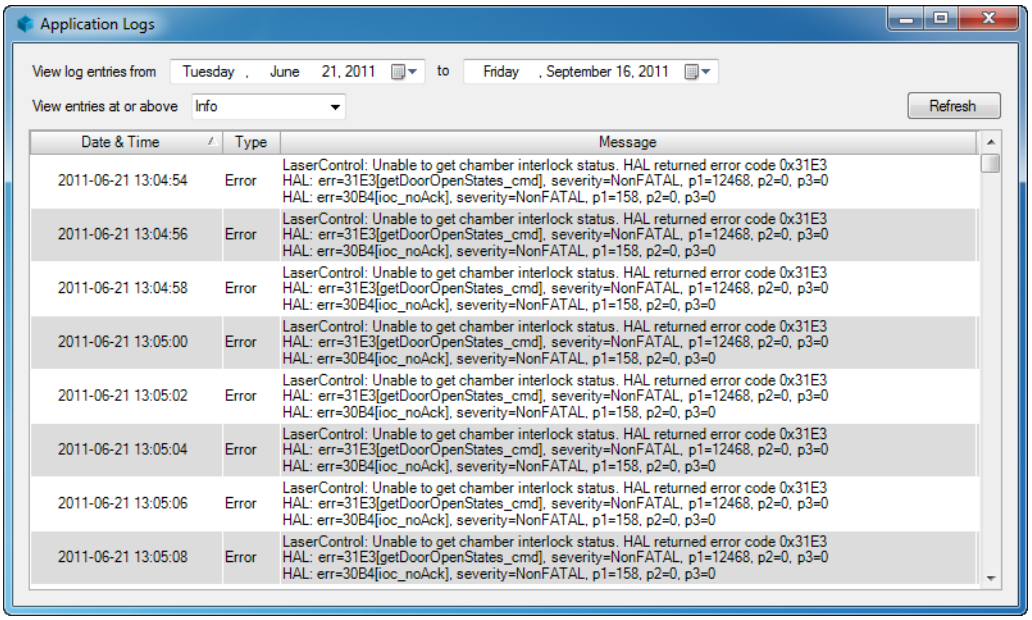

Figure 11-1. TrueQuant Log Viewer window.

The log viewer interface allows you to search, sort, and filter the log files using various criteria such as date or severity of the logged event (information, warning, error, etc.) Using the calendar controls at the top of the window, you can specify a range of dates and view only the events logged during that range.

You can copy individual log messages to the Windows clipboard for use in other software such as spreadsheets and word processors. To copy a log event, select the row in the viewer, right-click on the row, and select **Copy** from the pop-up menu that opens.

You can also access the log files directly. The log files are located in the following locations, depending on which operating system you are using:

Windows XP:

%ALLUSERSPROFILE%\Application Data\VisEn Medical\FMT\Logs

Windows 7:

%ALLUSERSPROFILE%\VisEn Medical\FMT\logs.

NOTE To access log files generated by the TrueQuant Admin, you must log into the Admin program.

11.2 Troubleshooting Tips

The following is a list of common questions and solutions:

11.2.1 Data Analysis

Q: When I lock an ROI, what properties of the ROI are locked?

A: The only property of a locked ROI that can be edited is the description. The threshold and size/location (which are the only other settable parameters of an ROI) are not adjustable once an ROI has been locked.

Q: I locked an ROI, but the next time I load that scan and ROI the color settings are different. What's going on?

A: Color scale and other display parameters are performed on a scan-by-scan basis, not on an ROI-by-ROI basis.

Q: Can I freely rotate a 3D ROI?

A: No, but it's still possible to analyze regions of interest that don't fall conveniently on a rectilinear grid by using the Isosurface ROI tool (see section 6).

Q: I get an "Out of memory" error when I try to export an image at print resolution. How can I prevent this?

A: Exporting print resolution images uses a lot of computer memory. When exporting at print resolution, try to keep the number of datasets loaded in the **Analysis** tab to as few as possible. Increasing the primary memory on the computer and the memory on the graphics card allows you to load more datasets.

Q: Image exports from the **Analysis** tab on my computer look fine as long as I export them at screen resolution. Why do image exports at print resolution look garbled?

A: Some outdated video card drivers, especially on laptops, have incomplete OpenGL support that creates problems with print resolution image export. To update the computer's video driver, go to the computer manufacturer's web site to download and install the latest video driver for the specific computer model. Many computer manufacturers' web sites request a serial number or service tag for the computer to find the appropriate drivers. Quit all running applications before updating the driver, as a reboot is typically required after installing any Windows driver.

11.2.2 Scan and Tomographic Reconstruction

Q: The software is reporting the wrong thickness for the imaging cassette, but I want to do a scan anyway. How can I make sure the correct thickness is used for the full reconstruction?

A: If you notice the discrepancy before you initiate the full scan, you can adjust the imaging cassette depth directly in the **Scan** tab. If you notice it after the scan has started or finished, and before the reconstruction has been performed, you can still ensure that the correct thickness is used for the full recon by using the following procedure:

1. Deselect the **Add to Reconstruction Queue** option above the **Scan** button if the laser scan is still in progress. If the laser scan is complete, and the scan is already in the reconstruction queue, remove the scan from the queue.

2. Find the scan in the **Experiment** tab. Right-click it and select **Properties**.

3. In the **Properties** window, select the **Advanced** tab and scroll to the top. Find the field labeled **ChamberSize**. Type the correct thickness in this field and press **Enter**.

4. Close the **Properties** window and add the scan to the reconstruction queue.

Q: After recalibrating an agent, are my previous scans invalid? Can they still be reconstructed?

A: Recalibration only affects scans performed after the recalibration. Scans done before the recalibration but not yet reconstructed will reconstruct using the scale factor that was current at the time the scan was performed.

Q: I can't add tomographic scans to the reconstruction queue when the scan's cassette depth is set to zero. What do I do?

A: You must first correct the scan's cassette depth using the Advanced tab on the scan's properties window. Once you complete this correction, you can add the scans to the reconstruction queue.

11.2.3 Database

Q: A colleague created a database and set it so that members of group X could access it. I'm a member of group X, but when I try to notice that database, I'm told that I don't have permission to access it.

A: Groups that are set up for FMT access must be Windows Security groups, not Distribution groups. Check the properties of group X and make sure it's not a Distribution group.

Q: Using an investigator PC, I tried to notice a remote database that's on the FMT system Host PC, but got an error saying that "The schema script '`dbmetadata_article_2.sch`' could not be propagated to the subscriber."

A: The permissions on the Host PC's replication data folder are not set properly. To set them correctly:

1. Go to **My Computer** on the Host PC and navigate to `C:\Program Files\Microsoft SQL Server\MSSQL.1\MSSQL` on Windows XP or `C:\Program Files\Microsoft SQL Server\MSSQL10_50.MSSQLSERVER\MSSQL` on Windows 7.

2. Right-click on the `repldata` folder and select **Sharing and Security** from the context menu.

3. On the **Sharing** tab, ensure that this folder is shared and that the name of the share is `repldata`.

4. Click the **Permissions** button, and ensure that the user *Everyone* has Read access.

5. Go to the **Security** tab and make sure that each FMT group has permission to *Read & Execute*, *List Folder Contents*, and *Read*.

Q: A couple of weeks after connecting the FMT system Host PC to our network, I can't remotely connect to any databases from any investigator PC. When I try to connect, I get an error message that the subscription is invalid.

A: It can take a couple of weeks for this problem to appear because the database snapshots that are used for synchronizing between investigator PCs and the Host PC expire after 14 days. Under normal conditions, a new snapshot is taken at the end of the 14 day period before the old snapshot expires. These snapshots are taken by the SQL Agent service, which runs on the Host PC. Network security policies on some networks require that the SQL Agent on the Host PC be run using a domain account in order to have permission to write new database snapshots to disk. By default, the SQL Agent runs as the Local System account. Using the Services control panel, change the login account for the SQL Agent to that of a domain user. You may need to ignore and re-notice your databases from the investigator PCs after changing the SQL Agent setup.

Q: My computer crashed while I was importing a study, and now my database includes an incomplete study with a strange name starting with "tmp". What should I do?

A: That study is the partially imported study, and can safely be deleted in the **Manage Databases** window by selecting that study and clicking **Delete**. You can re-import the study normally.

11.2.4 General

Q: Can I run TrueQuant in Windows on Windows (WoW) mode under Windows XP 64-bit?

A: No, TrueQuant must be run under the 32-bit version of Windows XP, as some of the third-party libraries used by TrueQuant are only available in versions that are not compatible with WoW. However, the 64-bit version of Windows 7 is fully supported by TrueQuant.

Q: I set my display's DPI setting to "Large size (120 DPI)" so my desktop icons' fonts won't be so small, and now TrueQuant is behaving strangely. My scan field appears offset from the mouse and some of the controls don't appear where they should. What's going on?

A: In part because of the imaging libraries that TrueQuant uses, TrueQuant only functions properly when the DPI setting is set to "Normal (96 DPI)." To increase the size of fonts, use the **Font size** setting in the **Appearance** tab of the **Windows Display Properties** control panel.

12 Maintaining the System

The PerkinElmer FMT system requires minimal on-going maintenance and is easy to clean. The instrument's imaging cassette is resistant to mild chemicals and cleaners. This section also contains hardware diagnostics and system disposal information.

12.1 Cleaning the Imaging Cassette

The imaging cassette does not require cleaning after each imaging session. The cleaning frequency of the cassette is user-determined.

Recommended solutions for cleaning the imaging cassette include the following:

- Quatricide in water (for example, Quatricide PV from Pharmacal); dilute as indicated by the

CAUTION Do not use metal utensils, hard tools, or abrasive cloth on the animal holder as they could damage the glass plates and their anti-reflection coating.

CAUTION Do not use flammable or strong chemical solvents such as isopropyl alcohol, ketones, or hexanes directly on the animal holder as they could result in significant material damage to the system.

12.2 Hardware Diagnostics

The **Hardware Diagnostics** window in the main menu is password-protected and intended to be used by Service personnel only.

CAUTION The hardware diagnostics are designed to be used by properly trained service personnel only. Untrained users should not run the diagnostics software.

These Hardware Diagnostics allow qualified service personnel to check and set the status of various hardware elements. Contact PerkinElmer for technical assistance if necessary.

12.3 Disposing of any Components from the FMT System

If disposing of an FMT system or any of its components, disinfect and decontaminate the system/components appropriately before disposing in accordance with the country's laws for equipment containing electrical and electronic parts to avoid contaminating or infecting personnel, the environment or other equipment.

For disposal of parts and accessories, follow local regulations regarding disposal of laboratory waste.

For disposal of lithium batteries, follow local regulations for safe disposal.

13

Regulatory Information

13.1 Regulatory Information

13.1.1 Electromagnetic Compatibility

This product conforms to CENELEC regulations relating to Radio Frequency devices and complies with the requirements of the EMC directive (89/336/EEC). These have been satisfied by testing the product against, and being found to be compliant with:

EN 61326-1: Class A: Electrical equipment for measurement, control and laboratory use – EMC requirements. Including Amendment A1: 1998, Amendment A2: 2001, Amendment A3: 2003

EN 61000-3-2 - Electro-magnetic compatibility (EMC) — Part 3-2: Limits - Limits for harmonic current emissions (equipment input current up to and including 16 A per phase)

EN61000-3-3 - Electro-magnetic compatibility (EMC) — Part 3-3: Limits - Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current ≤ 16 A per phase and not subject to conditional connection

13.1.2 Safety Information

This product shall comply with the requirements of the European Union Low Voltage Directive (73/23/EEC), United States, Canadian, European and International requirements.

United States: UL61010-1 - Safety requirements for electrical equipment for measurement, control, and laboratory use — Part 1: General requirements

Canada: CAN/CSA C22.2 No. 61010-1 - Safety requirements for electrical equipment for measurement, control, and laboratory use — Part 1: General requirements

Europe: EN 61010-1 - Safety requirements for electrical equipment for measurement, control, and laboratory use — Part 1: General requirements

International: IEC 61010-1 - Safety requirements for electrical equipment for measurement, control, and laboratory use — Part 1: General requirements

14

Technical Services and Support

14.1 Obtaining Technical Assistance

To request service, replace a part, or to access support documentation for your FMT system, log into the PerkinElmer imaging service portal:

http://evoportal.perkinelmer.com

14.2 Repackaging the FMT System

Inappropriate packing will void the PerkinElmer FMT warranty. Follow these steps when packing the FMT for shipment:

- Remove any animal or imaging subject from the imaging chamber.
- Clean and decontaminate the unit to meet Federal and State Regulatory and Safety standards.
- Disconnect the power cord and all the computer interface cords. Tie all cords and secure with bubble wrap. Cables DO NOT have to be returned with the system for repair.
- Cradle the camera using the foam padding originally supplied with the unit.
- Fill the imaging chamber with the foam padding originally supplied with the unit.
- Close and tighten imaging chamber access panels and camera access panel.
- Pack the PerkinElmer FMT system in its original crate. If the original crate was discarded, contact PerkinElmer to arrange for delivery of new packing materials.

NOTE All returned units must be decontaminated prior to their return. No returns will be accepted without a Return Material Authorization (RMA) number and proper decontamination documentation.

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