PerkinElmer®

TrueQuant

Operator Manual

TrueQuant

Operator Manual

PerkinElmer Life and Analytical Sciences
2200 Warrenville Road
Downers Grove, IL 60515
info@perkinelmer.com

Publication No. 8860033 Rev. D

Trademarks

The information contained in this manual is subject to change without notice. PerkinElmer makes no warranty of any kind with regard to this written material. PerkinElmer assumes no responsibility for any errors that may appear in this document.

FMT is a registered trademark of Perkin Elmer, Inc. and TrueQuant, FMT 1500, FMT 2500, FMT 2500 LX, FMT 1000, FMT 2000, FMT 4000, VisEn and the VisEn logo are trademarks of PerkinElmer, Inc.

SQL Server, Excel, Windows, Windows XP, and Windows 7 are registered trademarks of Microsoft Corp.

All other products or name brands are trademarks of their respective holders.

The FMT imaging system is covered under several issued and pending US and international patents.

PerkinElmer Products have been designed for in vivo imaging applications that use fluorescent agents. PerkinElmer Products may be used for a wide range of imaging applications; however, some applications may require a license from a third party. PerkinElmer's sale or other transfer of its Products does not convey any right or license under any third party patent. Therefore, Buyer is advised to consult with counsel to determine whether a license from a third party may be required in order to perform a particular imaging application, for example, imaging applications using genetically modified cells. PerkinElmer disclaims any and all responsibility for any rights or licenses to patents or other intellectual property that may be required from third parties, and/or liability (including, without limitation indemnity), with respect to such third party patents or other intellectual property.

For laboratory research use only. Not suitable or intended for human or animal diagnostic or therapeutic use.

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 680 nm channel or a 750 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 system is also upgradeable to four imaging channels (FMT 4000). The two additional channels excite at 635 nm and 790 nm and emit at 660 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.

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 the software data interface.
- *Chapter 5* describes the scan acquisition process.
- *Chapter 6* describes the analysis process.
- *Chapter 7* describes the agents and agent calibration process.
- *Chapter 8* describes the database management and back-up procedures.
- *Chapter 9* describes installation of TrueQuant on remote desktops.
- *Chapter 10* discusses operating guidelines and troubleshooting.
- *Chapter 11* discusses system maintenance.
- *Chapter 12* provides system warranty and regulatory information.
- *Chapter 13* provides technical services and support information.

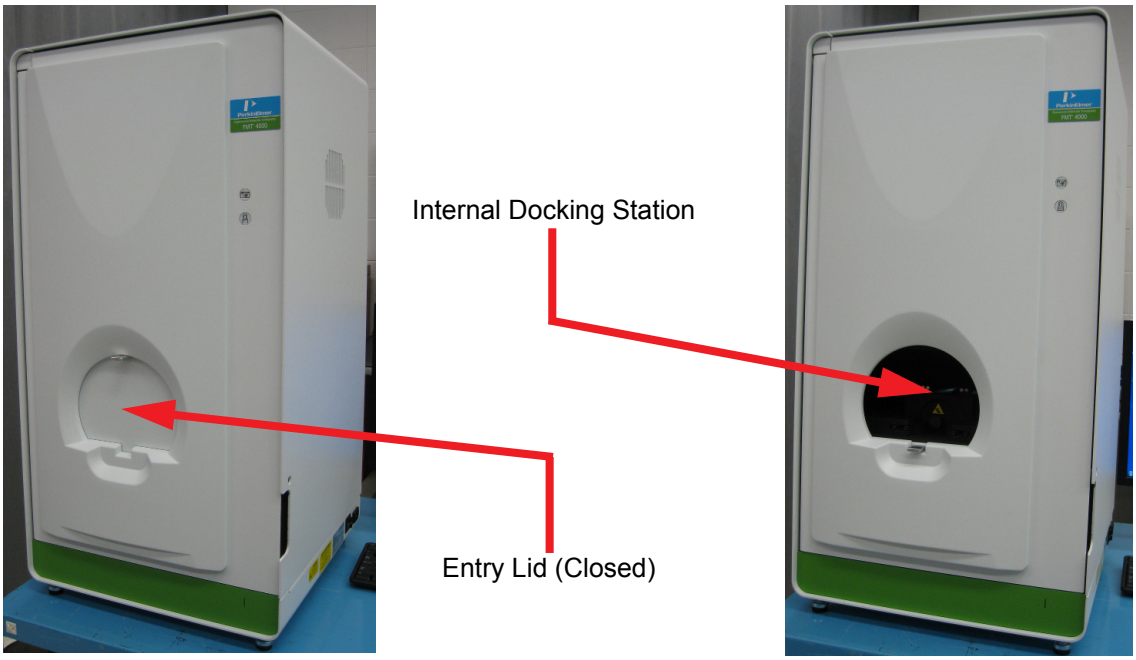


Figure 1-1. FMT system overview.

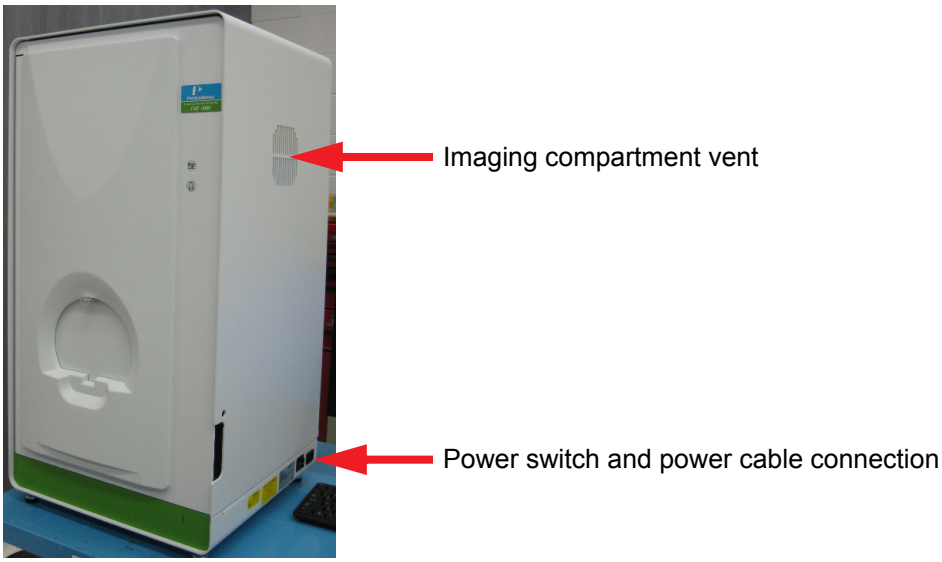


Figure 1-2. Power panel.

Figure 1-3. Imaging cassette.

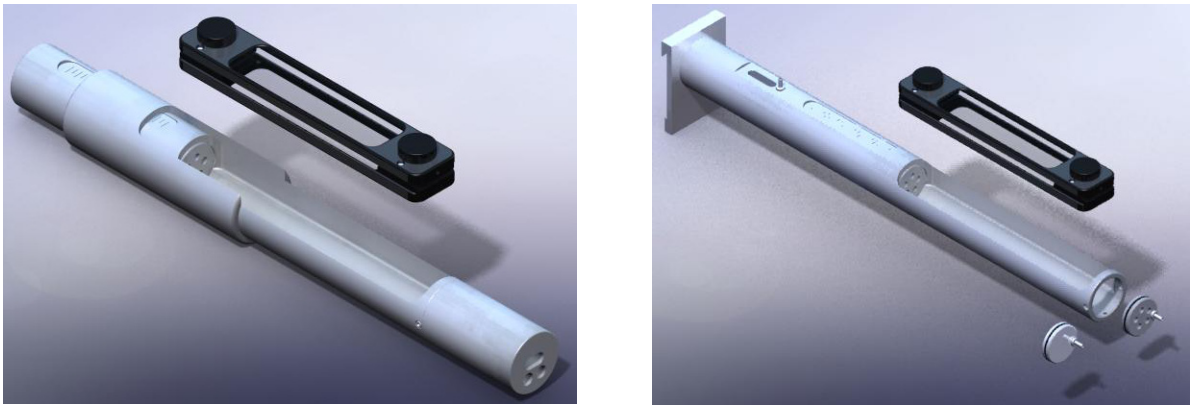


Figure 1-4. Multi-Modality MR adapter (left) and Multi-Modality MicroPET/CT adapter (right).

Referring to the annotated diagram of Figure 1-1 through Figure 1-4, the principal elements of the FMT system are:

1. **Internal Docking Station**: Receptacle for the imaging cassette inside the FMT system. The internal docking station is accessed by opening the specimen entry lid (Figure 1-1).

The docking station includes a laser safety interlock switch that disables the laser as long as the lid remains open, and a gas anesthesia port as well as an integrated warmer to maintain the animal anesthetized and at body temperature (37°C).

2. **Removable Imaging Cassette**: The imaging cassette is designed to hold the anesthetized animal in position during imaging (Figure 1-3). The imaging cassette can be inserted into the internal docking station for imaging or placed in the external docking station for pre-imaging workflow. It can also be placed into a multi-modality adaptor for co-registration imaging (see step 5 below).

3. **Imaging Cassette Top Cover Adjustment Knobs**: The adjustment knobs are used to determine the space between the glass panels of the imaging cassette (Figure 1-3). The knobs are tightened until mild compression of the subject is obtained.

4. **Isoflurane-Based Anesthesia Port**: connect isoflurane-based inhalation anesthesia supply to the port on the side panel, if required.

5. **Multi-Modality Adapters**: The Imaging Cassette may also be transferred for use in other imaging modalities, such as microPET, CT, MRI and SPECT, using the PerkinElmer multi-modality adapters (Figure 1-4). This approach allows 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 the various modalities including CT, PET, MR and Spect.

1.2 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.

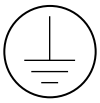
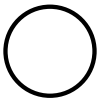
The following are warnings that define precautions that must be observed to avoid injury to personnel. Some of these precautions are specific to particular operator actions. They will appear in the text. Others may be of a “general-purpose” nature, and may not be duplicated in the many places in which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

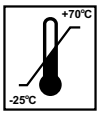
WARNING	Do not attempt to override or modify the interlock system.

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 or procedures other than those specified herein may result in hazardous radiation exposure.
CAUTION	The personal 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 hould not attempt to run the diagnostics software.

1.3 Explanation of Symbols

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
	ON symbol
	OFF symbol
	Fragile
	Store in relative humidity between 20% and 90%

Symbol	Explanation
	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.

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 as well as the switch panel on the right side (see Figure 1-2). The bench or tabletop upon which the system is placed must be capable of supporting the weight of the system. The following are the critical dimensions and weight of the system and the PC:

The following are the critical dimensions and weights of the system and its components:

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)

In order to obtain the best performance from your FMT system:

- Place the FMT system in an environment that is dust-free.
- 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 personal 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 that you plug 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 only 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 front door 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 its host computer already pre-configured with the TrueQuant software. For optimal performance, this computer should be dedicated exclusively for use with the FMT System. In addition, 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.

The host computer specifications are as follows:

- Windows 7 (64-bit only) PC.
- 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 ports and at least 2 RS232 ports; floppy disk drive optional.
- Dell 22" P2210 flat panel monitor or equivalent.

The investigator PC computer minimum requirements are as follows:

- Processor: Dual-core processor.
- Memory: 4GB.
- 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 XP (32-bit only) or Windows 7 (32- or 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, the FMT instrument must be connected to the computer via the USB port and the two serial ports.

2.5.1 Unpacking the Instrument

Examine the cartons and look for any evidence of mishandling in the shipment. Follow institutional procedures for reporting such evidence.

Remove the contents from the shipping cartons. Compare the shipped items with the packing slip and your order. Each VisEn FMT system shipment includes the following items:

- One (1) FMT System—FMT 1000, FMT 2000, or FMT 4000
- One (1) Host Computer—with appropriate country power cord (determined at time of order)
- One (1) Monitor—with appropriate country power cord (determined at time of order)
- Two (2) imaging cassettes
- One (1) agent calibration phantom with holder
- One (1) Keyboard
- One (1) Mouse
- One (1) Operator Manual and CD

Retain the shipping cartons in case of the need for return shipment.

2.5.2 Disposing of any Components from the FMT System

In the highly unlikely case where one may be required to dispose of an FMT system or any of its components, make sure you disinfect and decontaminate the system/components appropriately before disposing of it in accordance with your country's laws for equipment containing electrical and electronic parts so as to avoid contamination 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.

2.5.3 Connecting the Computer Cables

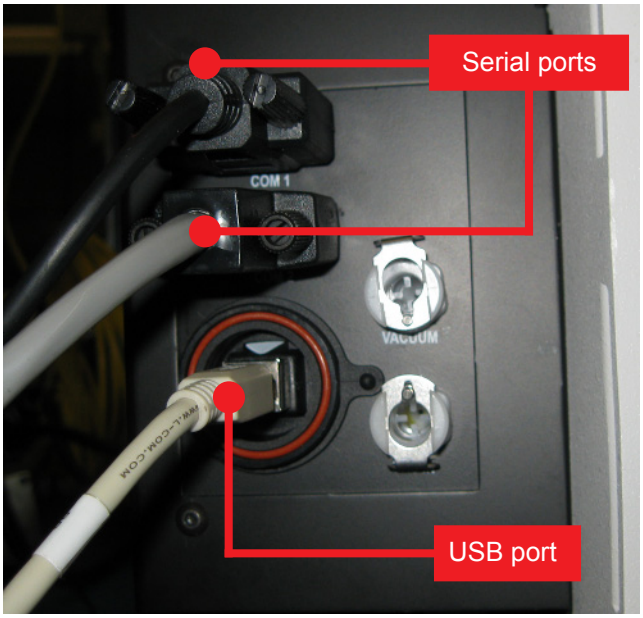


Figure 2-1. Cable connections between FMT and desktop tower PC.

There are three communication cables connections on the PerkinElmer FMT system:

- 1 USB connector
- 2 serial port connectors (COM 1 & 2)

The connections panel is located toward the rear of the left side as you face the instrument. The USB cable must be inserted into the correspondingly labeled USB port in the panel of the FMT system and the desktop tower PC. Similarly, the two serial port connectors must be inserted into the ports on the FMT system panel and into “COM1” and “COM2” serial ports of the desktop tower PC as labeled.

The power cable must be inserted into the receptacle located on the right side of the system (adjacent to the power switch) and the male end plugged into an appropriate power receptacle in the laboratory.

The instrument is now ready to be powered up. The power switch is located on the right-hand side panel as you face the instrument.

NOTE It is advisable to power on to the FMT system first and then wait about 30 seconds before starting TrueQuant.

PerkinElmer recommends you keep the FMT system powered on when not in use unless the system will not be used for extended periods of time. If the system is powered down, you should allow the instrument to warm up for approximately 45 minutes once it is powered on again.

2.5.4 Connecting the Isoflurane-Based Anesthesia Lines

The FMT system may be connected to an isoflurane-based inhalation anesthesia system via the gas inlet port. The adjacent outlet port may be connected to a vacuum line or other appropriate gas discharge handling system. Both ports are located on the side of the instrument (Figure 2-2) and should be connected during installation of the system. The inlet port permits the free flow of anesthesia gas (isoflurane) to move into the system to the internal docking station. Insertion of the imaging cassette into the internal docking station 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. Removal of the imaging cassette automatically closes the gas inlet and the outlet ports and stops the flow of anesthesia gas (Figure 2-3).

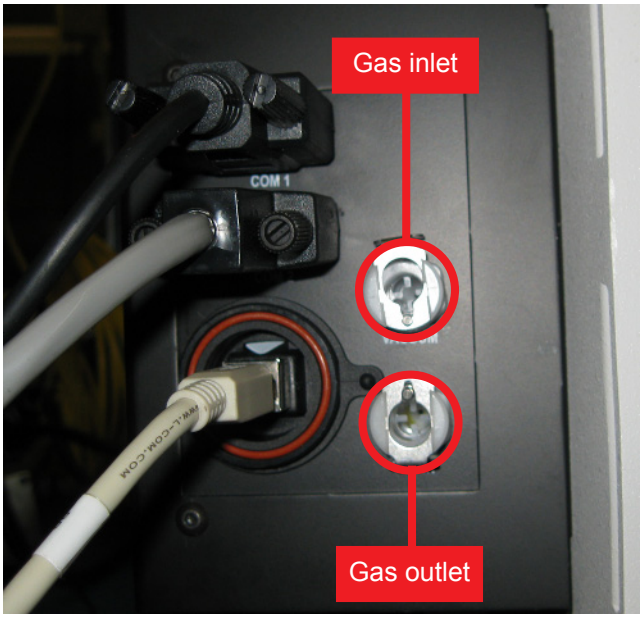


Figure 2-2. Isoflurane-based gas inlet and outlet ports on back of system.

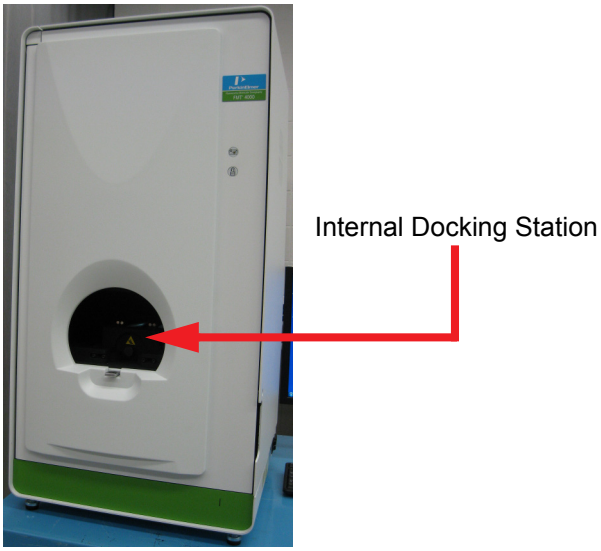


Figure 2-3. The FMT system with the internal docking station visible through the open specimen entry lid.

2.6 List of Abbreviations and Acronyms

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

2.7 TrueQuant Software License Key

TrueQuant software now requires an authorized license key to run. This feature will allow PerkinElmer to more distribute new software versions and features to our customers and to provide flexibility to support multiple system and versions. The Host PC will be pre-configured with a software license key by PerkinElmer, but any additional computers that will be used to run TrueQuant will require a license key to be entered the first time you run TrueQuant. Additional software license keys can be obtained by contacting PerkinElmer.

The following table describes the upgrade options for each of the instrument models.

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

A new license key will be required to upgrade TrueQuant from one system to a higher model number system. After receiving this upgrade license key from PerkinElmer, select **Help** | **Update License** from the menu in TrueQuant, click **Uninstall License**, then enter the new license key and click **OK**. Note, this menu item is unavailable on a Host PC if there are any scans in the reconstruction queue.

3

Typical FMT Imaging Session and Overview of TrueQuant Software

In addition to the FMT imager hardware, the host PC runs the TrueQuant software. This single application integrates the entire imaging sequence from setting up a study and a subject through to performing a scan, executing tomographic reconstruction, performing ROI analysis and exporting results.

3.1 Overview

TrueQuant is designed to have a simple and consistent graphical user interface (GUI). Upon starting the software the user is presented with a window similar to that shown in Figure 3-1. The three tabs at the top of the window labeled **Experiment**, **Scan** and **Analysis** are ordered chronologically from left to right for a typical experimental sequence. A short description of each tab is as follows:

1. **Experiment**: captures experimental protocol and interacts with the database(s).

2. **Scan**: for a given study and subject, acquires reflectance and/or tomographic scan.

3. **Analysis**: displays and analyzes the results of reflectance or tomographic imaging.

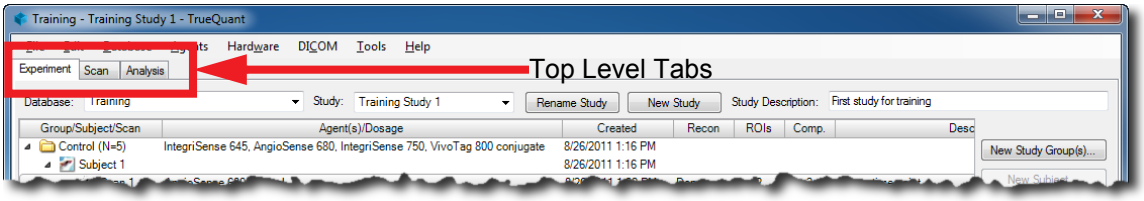


Figure 3-1. TrueQuant top-level window.

3.2 Using the Imaging Cassette and Docking Station

The removable imaging cassette allows for the stable and repeatable placement of the animal within the FMT instrument.

The imaging cassette consists of two parts (Figure 3-2):

- a bottom glass plate
- a top glass plate that includes two height adjustment knobs.

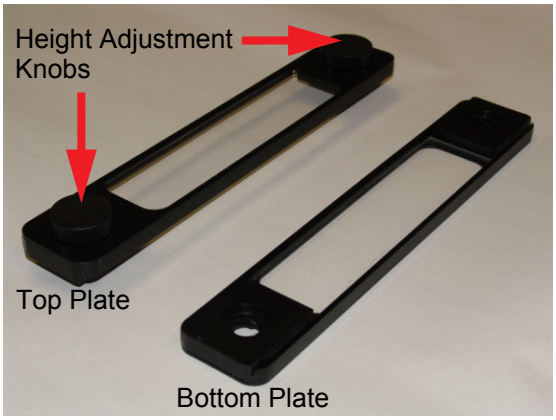


Figure 3-2. Removable imaging cassette (open).

3.2.1 Placing the Animal in the Removable Imaging Cassette

1. Once anesthetized, place the animal face down on the bottom plate of the imaging cassette (Figure 3-3).

2. Make sure the target area to image is located in the center of the glass plate.

3. 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.

4. Place the top plate on top of the subject and start tightening the height adjustment knobs by turning them clockwise.

5. Tighten the height adjustment knobs to obtain mild compression of the subject (Figure 3-4 and Figure 3-5). We recommend tightening the knobs 13 to 15mm.

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

Figure 3-3. Placement of the subject on the bottom plate of the imaging cassette.

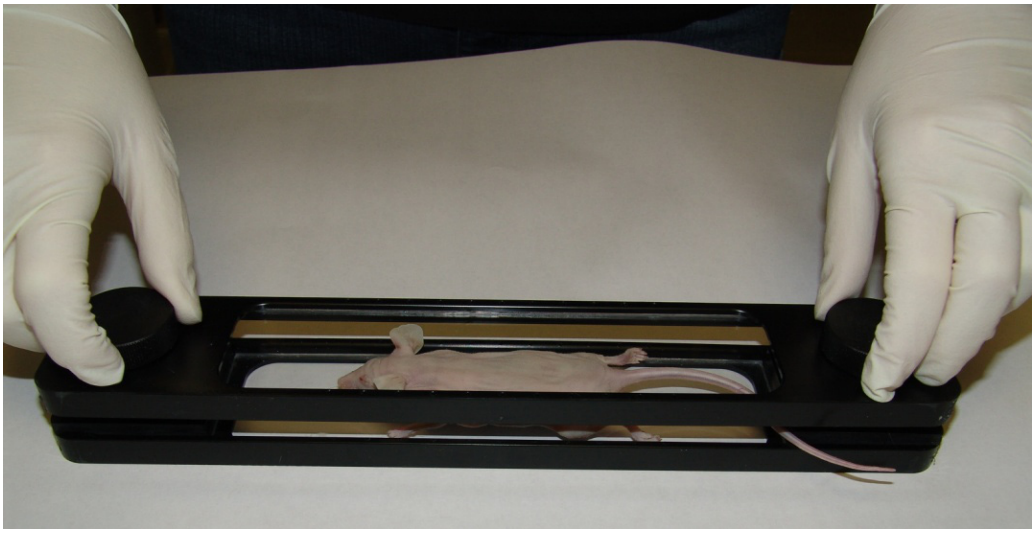


Figure 3-4. Close the imaging cassette using the height adjustment knobs.

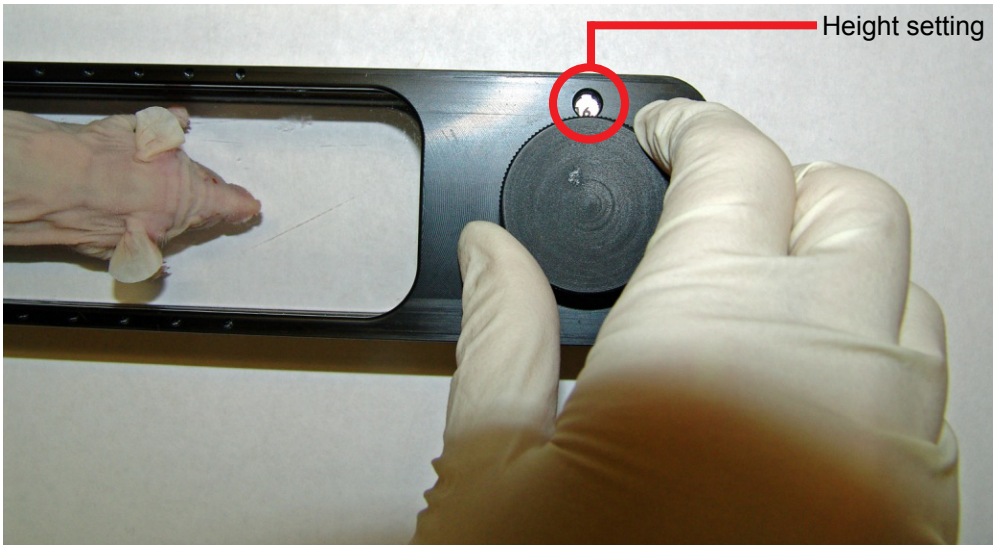


Figure 3-5. Height adjustment (continued).

3.2.2 Inserting the Imaging Cassette into the Internal Docking Station

Once the animal is secured, you may insert the imaging cassette into the internal docking station using the following steps:

1. Open the specimen entry lid.
2. Lift the docking station door.
3. Slide the imaging cassette inside the docking station (Figure 3-6). The imaging cassette may be inserted up or down, forward or backward. The imaging cassette should be oriented so that the target area faces the camera. For example, if the subject is imaged for lung tumors and the animal is positioned face down, the imaging cassette will be inserted upside down, with the animal's chest upwards, in the instrument.
4. Push the imaging cassette in until you hear it "click" into place.
5. Close the docking station door.
6. Close the specimen entry lid. Closing the lid will disable the laser safety interlock as well as allow isoflurane-based gas to be released within the internal docking station.

Figure 3-6. Inserting the imaging cassette in the internal docking station.

4

The Experiment Tab

4.1 Overview

The information displayed in the **Experiment** tab allows the user to define and record the experimental imaging protocol for the given study. The **Experiment** tab also allows the user 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 any number of local or networked databases. Each database will typically contain multiple studies. A study usually consists of several groups with each group comprised of a number of subjects, typically mice and rats, or other small animals using the Multi-Species Imaging Module (MSIM). A subject may be scanned once or repeatedly, and the results of the scans stored and analyzed. The data hierarchy is therefore as follows:

Database
    Study
        Group
            Subject
                Scan

Start from the upper left corner by selecting a database from the drop-down list of available databases (Figure 4-2). When the application is launched for the first time, a new database will need to be either created or loaded from the network. To create the first database, select **Database** | **Manage** from TrueQuant's menu. The resulting **Manage Databases** dialog is shown in Figure 4-1. Click **New Database** to create a new database.

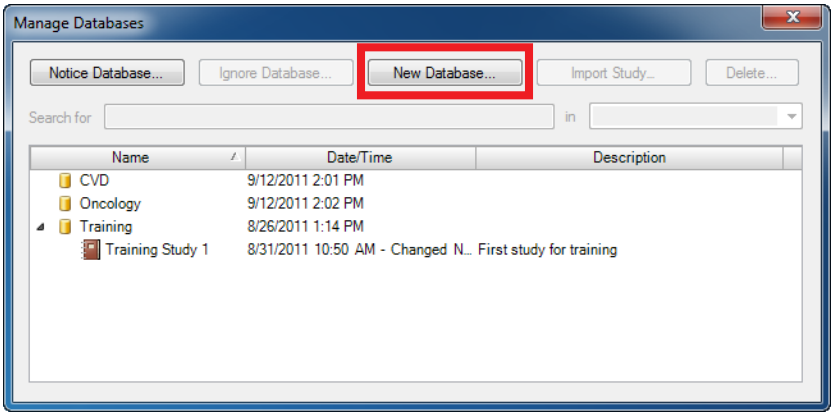


Figure 4-1. Creating a new database from the **Manage Databases** dialog.

You can type any name you like for the new database. Once created, this database becomes the active default database.

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 datbase has expired. You can re-establish remote synchronization for the database by ignoring and then re-noticing the database. See *section 8.1* for more details.

Once a database is selected, you can then create a study by pressing **New Study** to the right of the database (Figure 4-2).

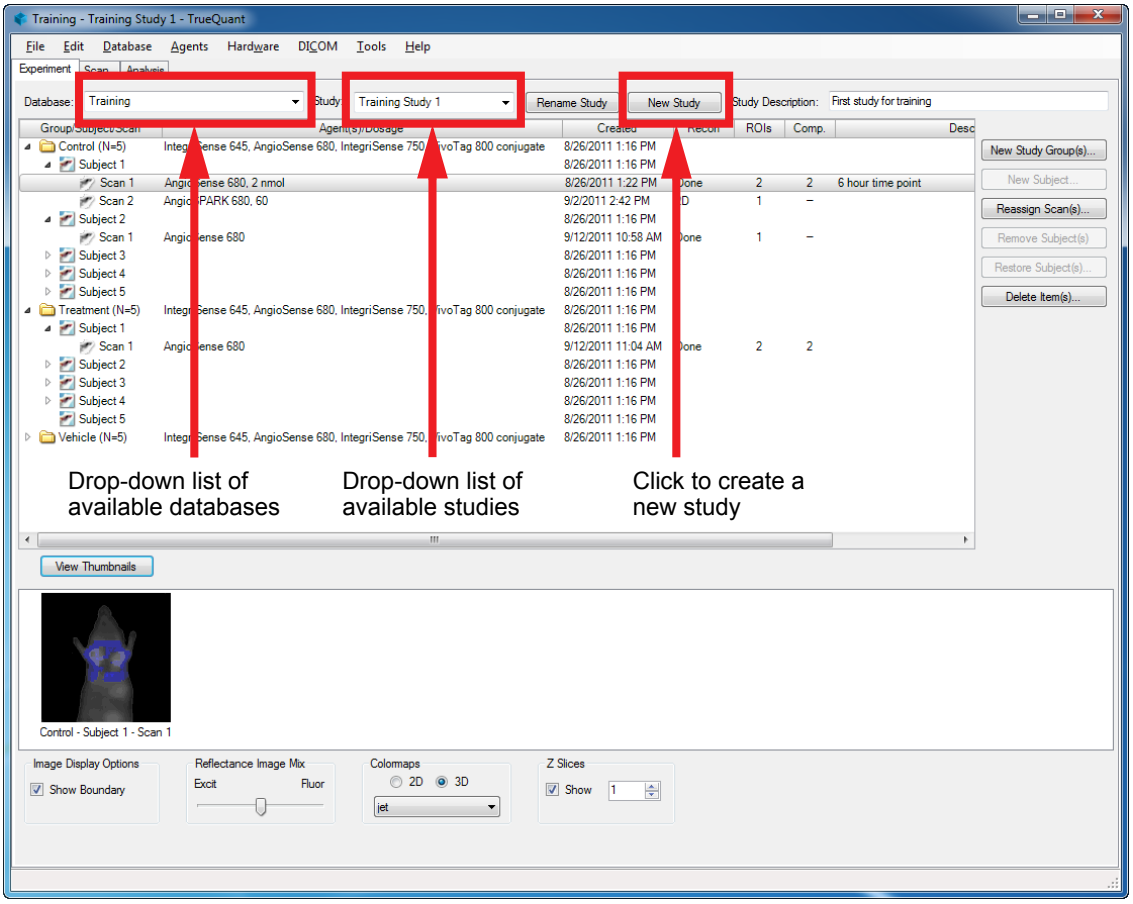


Figure 4-2. **Experiment** tab, database and study drop-down menus and buttons.

A number of studies can be created at any given time, but the main table in the **Experiment** tab only displays the contents of the active study, which can be selected from the **Study** drop-down list.

Once created, a study then needs to be populated with one or more groups of subjects by pressing **New Study Group(s)**. This will open a window that lets you create multiple study groups at once (Figure 4-3). Clicking **New** opens another window (Figure 4-4) to define the properties of a new group.

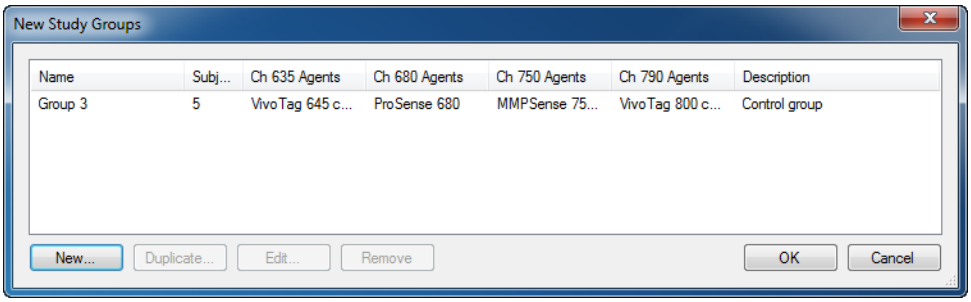


Figure 4-3. Defining new study groups.

Once you define a single group, you can create additional groups with similar properties easily by selecting the first group and pressing **Duplicate**. This will open the same window to define the properties of a group, but with all of the fields filled in using the properties of the selected group. Any of the properties of the defined groups can be changed by pressing **Edit** and changing their properties in the window that opens. Groups can be deleted entirely from the list using **Remove**.

Once the full list of groups has been created, press **OK** to create the groups and return to the **Experiment** tab.

A group will always contain one or more subjects, and you must select an agent or several agents to use in conjunction with this group. Chapter 7, *Imaging Agents and Agent Calibration*, discusses agent-related topics in more detail.

As an illustrative example, Figure 4-5 shows the **Experiment** tab after creation of the study "HT-29" under the "Oncology" database. This study is comprised of three groups, containing 5, 6 and 3 subjects respectively, with the first two groups described as "Experimental" and the third group described as a "Control" group.

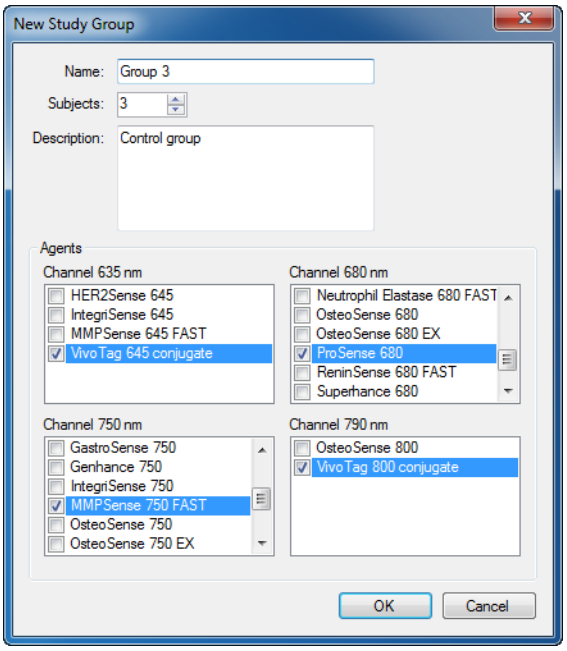


Figure 4-4. Setting the properties of a new study group.

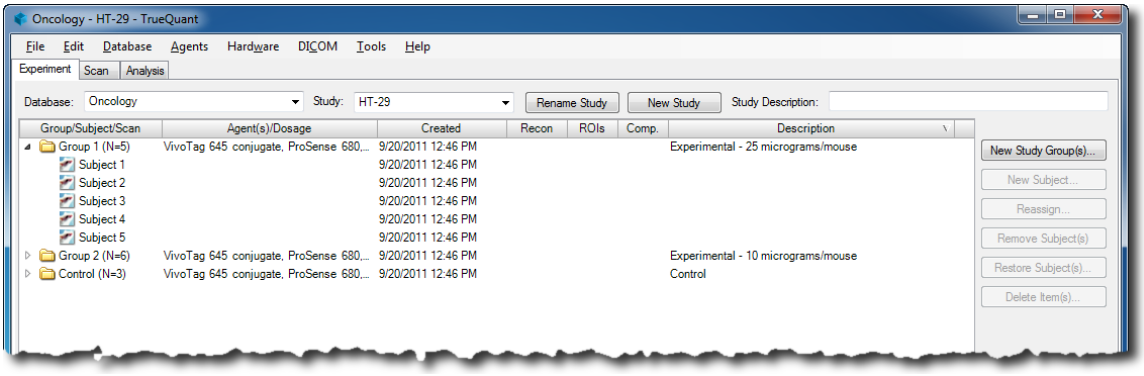


Figure 4-5. Illustrative study with defined groups and subjects.

4.2 Editing Properties

All key properties for database, study, group or subject elements can be edited at a future date.

- To edit the name or description of a database, select **Database** | **Manage** from the main menu. Highlight the database in question, then single-click on either its name or description to edit the corresponding text field (see Figure 4-6).

- To rename a study, press **Rename Study** near the top center of the **Experiment** tab.

- To change a study's description, click in the **Study Description** field and modify the text as desired.

NOTE Study names and descriptions can also be changed in the Manage Databases window using the same method as is used to change those properties for a database.

- A group name or description can be modified similarly to a database: highlight the row corresponding to that group, then single-click on the name or description field.

- A subject's number or description can be modified as for a study group: highlight the row corresponding to that subject, then single-click on the subject number or description field.

- To edit the agent assignment for the group, right click on the group row and select **Properties**, which will bring the **Group Properties** dialog (Figure 4-7). Agent assignments can then be checked or unchecked for each channel.

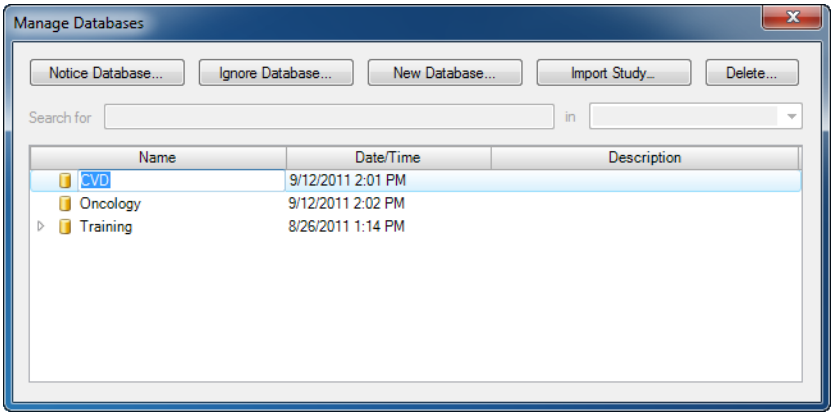


Figure 4-6. Changing the name of a database.

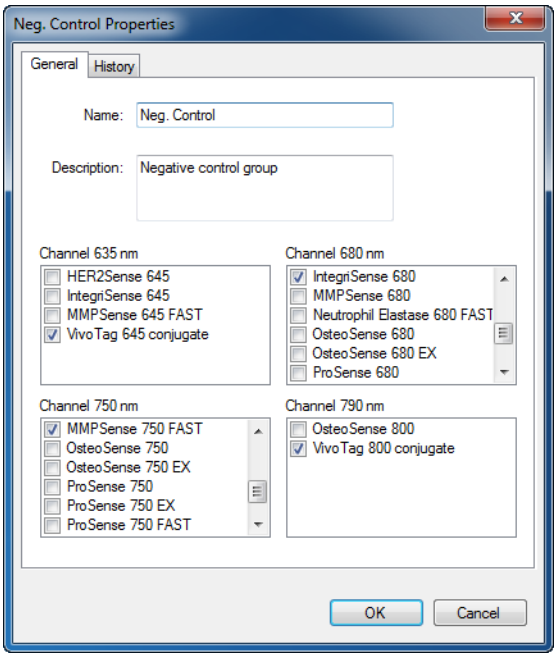


Figure 4-7. Study group properties dialog.

4.3 Adding, Removing and Reassigning Subjects

In addition to editing the name, description and agent assignments for a study group, it is also possible to add, remove, restore or reassign subjects to or from a group.

- Select **New Subject** to open the **New Subject** dialog and add an automatically-numbered subject to the highlighted group (see Figure 4-8). Note, you can specify a number if you like, but it must be unique within the study group in which you are creating the new subject.

- To remove a subject from a group, highlight the subject and select the Remove Subject(s) button. The subject that was removed will be displayed in light gray (see Figure 4-9). Once a subject has been removed, existing scans can still be analyzed but new scans can no longer be added to that subject. As an example, this is useful for indicating subjects that have died during the course of a study.

- If removed inadvertently, a subject can be restored by highlighting the subject and selecting **Restore Subject(s)**. The shading of the subject then changes from light gray back to the normal black.

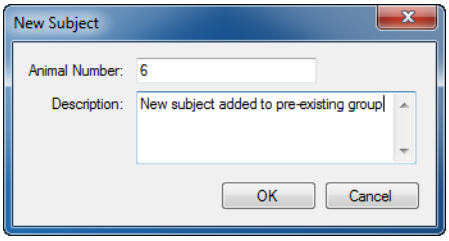


Figure 4-8. Adding a new subject to a predefined group.

- Note that if a subject is deleted by pressing **Delete Item** instead of **Remove Subject**, it is no longer recoverable.

- It is possible to reassign subjects where a clerical error has wrongly assigned a subject to a particular group, and to reassign scans that have been incorrectly assigned to the wrong subject. The error can be corrected by selecting and dragging the scans or subjects and dropping them on the correct subject or study group, or by selecting them and pressing **Reassign**. When using the **Reassign** option, the dialog shown in Figure 4-10 prompts you to select a new group assignment for the subject in question. A similar dialog box is used to reassign scans to a different subject.

Once the reassignment completes, the subjects or scans appear in the destination group or subject. They are also renumbered automatically (Figure 4-11) and the reassignment is recorded in the item's history (Figure 4-12).

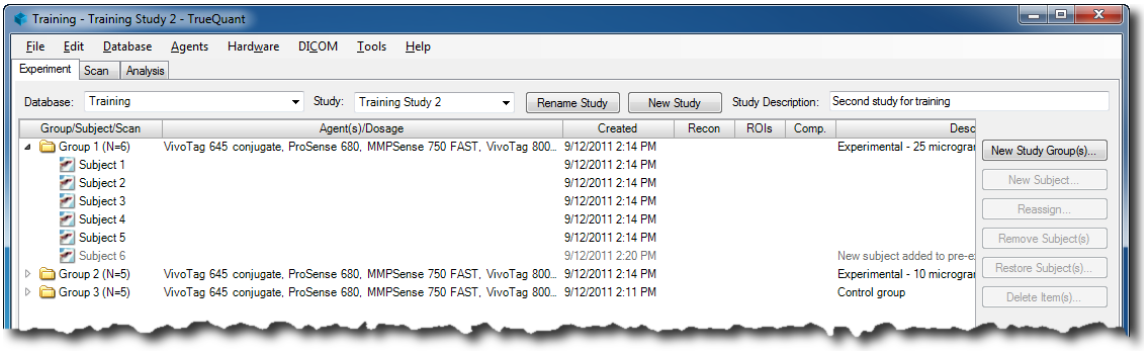


Figure 4-9. Subject 6 was removed from Group 1, and is shown in light gray.

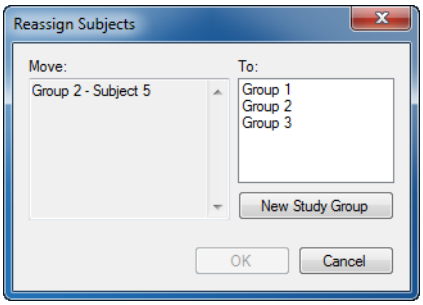


Figure 4-10. Reassigning a previously defined subject to a different group.

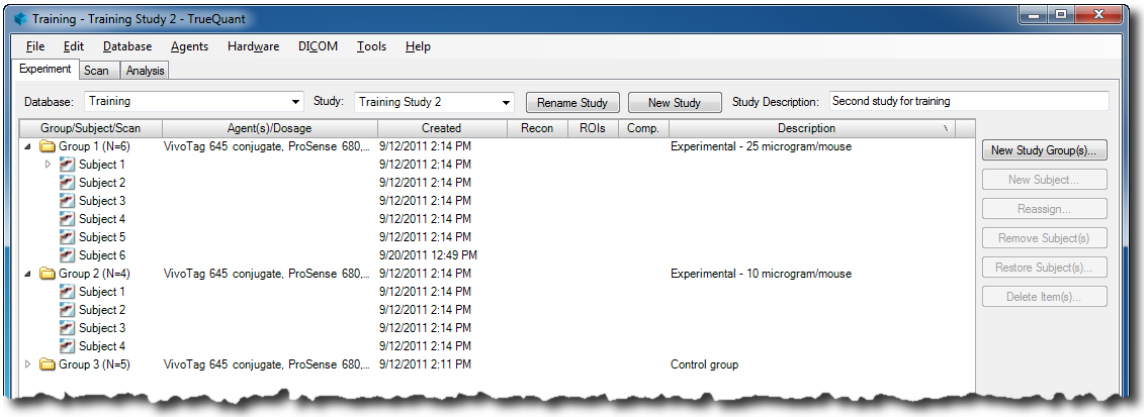


Figure 4-11. The result of reassigning Subject 5 from Group 2 to Group 1.

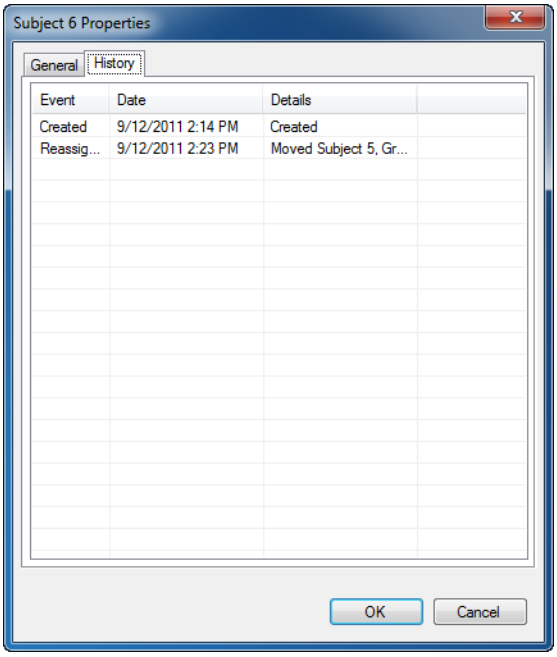


Figure 4-12. The history tab under Subject properties automatically records the reassignment.

4.4 Exporting and Importing Data

Image and data export can be performed from the **Experiment** tab by highlighting a dataset, and selecting **File** | **Export** from the menu(Figure 4-13). Three file format options are provided:

- **ROI Analyses**, using the Comma Separated Value (CSV) file format, which can be read by a spreadsheet program.
- **Scan Data in FMT format**, a format (`.fmt`) to save images.
- **Scan Data in DICOM format**, a format to save images.

The first two of these are detailed in *section 4.5*, while the third is explained in *section 4.6*. For instructions on how to export and import entire studies using TrueQuant's database management functionality, please see *section 8.1.5*.

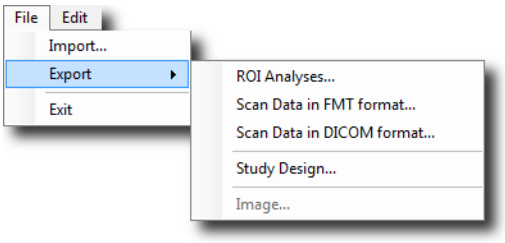


Figure 4-13. The TrueQuant Export menu.

4.5 CSV Data and Image Export

Selecting **File** | **Export** | **ROI Analyses** from the menu prompts you for a name and location for the saved file. Once you provide the required information, **CSV Export Column Selection** dialog opens, allowing you to manually select export items from the database, as shown in Figure 4-14. All database items are selected by default and you can deselect items as desired.

You can export 2D or 3D scan images using the FMT (`.fmt`) format can be used for either 2D or 3D scans.

Scans previously saved in the FMT format can be imported using the **File** | **Import** menu (Figure 4-15). Each imported scan will be 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.

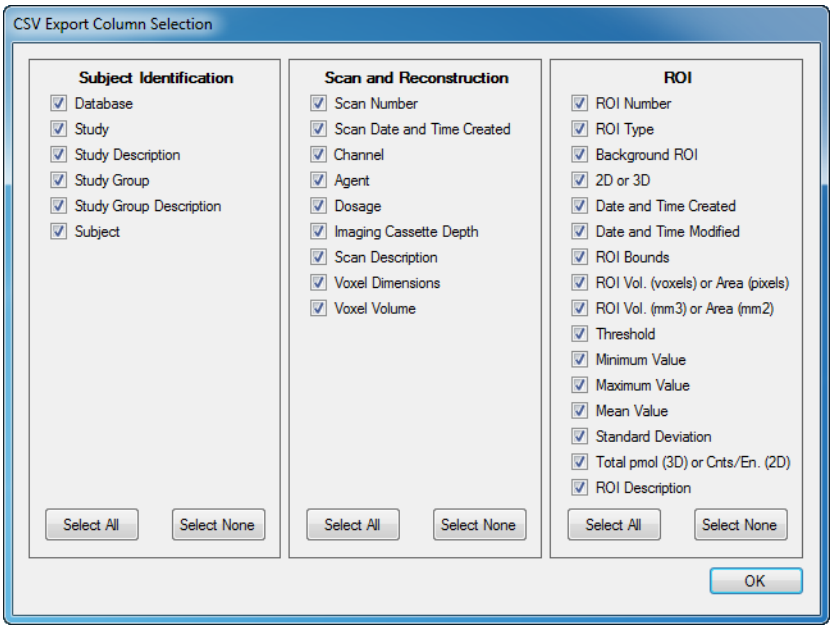


Figure 4-14. Data export dialog box for selection of database records to be exported. All fields are selected for export by default, and can be manually de-selected individually as desired.

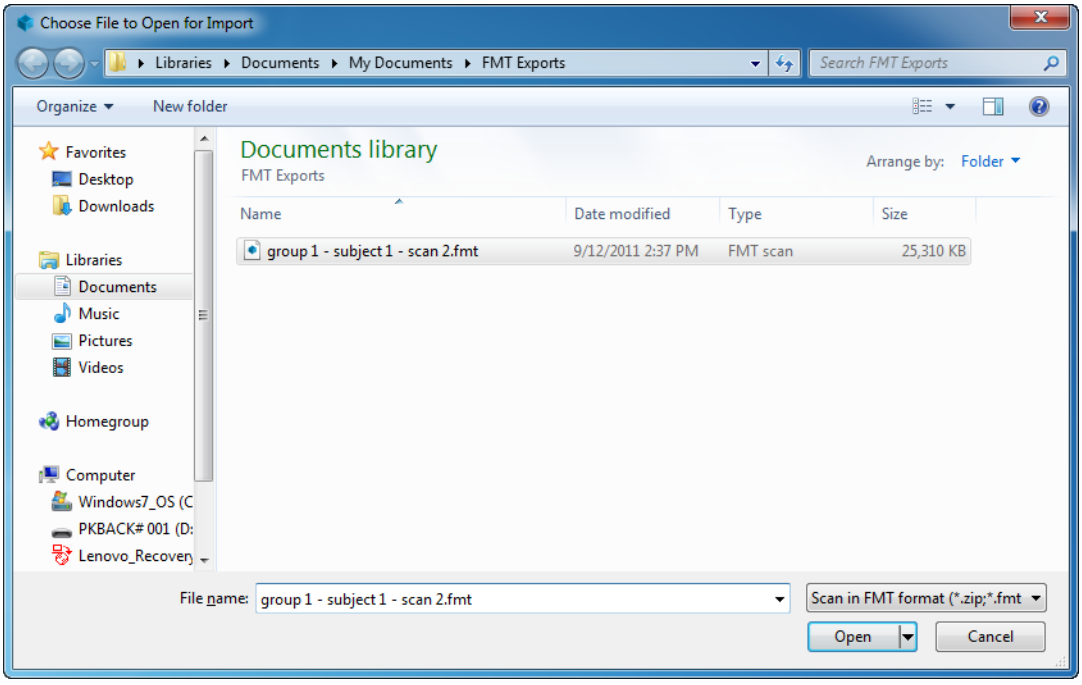


Figure 4-15. Importing a scan.

4.6 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. Supported DICOM viewers are listed in the following table:

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 as well.

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. If you intend to use a viewer that does not support 32-bit images, you can change the output format to 16-Bits by opening the **Options** window using the **Tools** | **Options** menu, then selecting the desired bit depth on that window's DICOM tab.

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 previous versions of TrueQuant. If the bit depth was set using previous versions of TrueQuant, the previous setting will override the current version's default setting. However, any change made to the bit depth setting after upgrading to the current version of TrueQuant will only the current user.

4.6.1 Export DICOM

Individual scans and their reconstructions can be saved as DICOM series using the **File** | **Export** | **Scan Data in DICOM format** menu. This will create a folder with the name provided in the **Export** dialog. The folder will contain three DICOM series: one for the reflectance images, one for the transillumination images, and one for the reconstruction.

Once you enter a file name and click Save on the **Export** dialog, the **Animal Orientation** dialog (Figure 4-16) opens. This requires you to choose 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 will be 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.

After you specify the orientation, select the version of the imaging cassette used during scan creation. If the scan was previously exported as a DICOM series, the cassette version that was chosen for the previous export is selected by default. If this is the first time this scan is being exported, the currently installed version for the appropriate animal type is selected.

Finally, click **OK** to export the scan as a DICOM series.

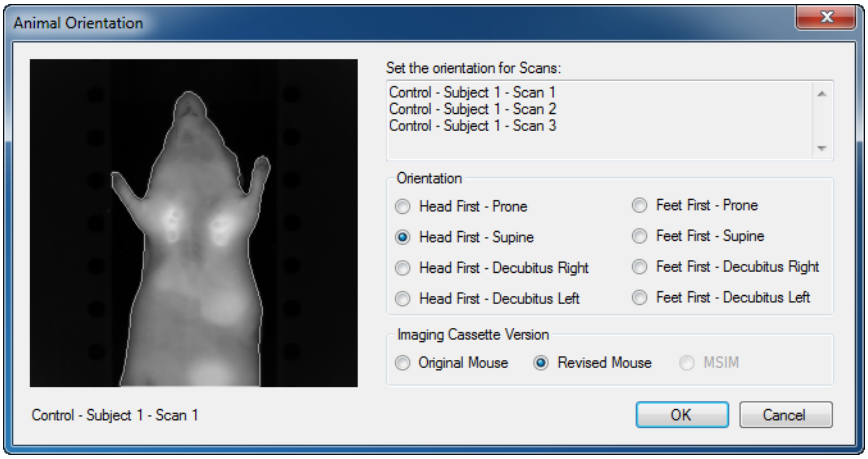


Figure 4-16. Setting the animal orientation for a batch of scans.

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. Correctly positioning of the fiducial marks on the exported requires the calibration information for the cassette, and this calibration information only exists on the the host machine.

4.6.2 Send To PACS

You can also send DICOM exports directly to a PACS server. Before you can do this, however, 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** dialog shown in Figure 4-17. 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** dialog (also shown in Figure 4-17). You can choose the **Use DNS** option if you know the name of the PACS computer but not its IP address.

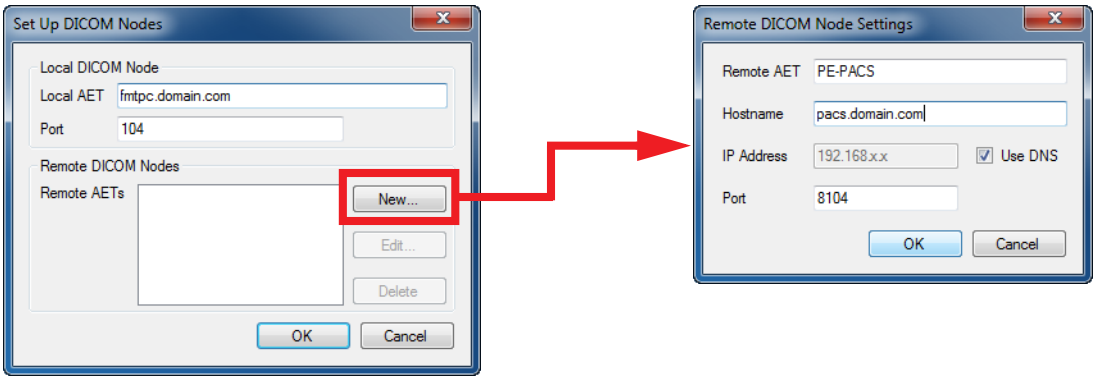


Figure 4-17. Configuring DICOM (AET) nodes.

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 the menu item **DICOM** | **Send to PACS** | **Remote Node Name**, where *Remote Node Name* is the remote AET name that you configured above. The **Animal Orientation** dialog, shown in Figure 4-16, 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 *section 5.4*) except that the active item cannot be removed from the queue.

4.7 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.

While viewing 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, along with other relevant information about them, is exported to the specified file.

5

The Scan Tab

5.1 Performing a Scan

The **Scan** tab controls the experimental workflow of imaging an animal. When you click the **Scan** tab, a screen similar to the one shown in Figure 5-1 displays. Note, the currently loaded study is shown in the **Select Subject** tree.

Proceeding from top to bottom, the typical imaging sequence is as follows:

1. Select the subject you want to image from the **Select Subject** tree.
    - You can enter a text description of the scan if you desire.
2. Select the laser channel, depending on the fluorescent agent injected into the subject.
3. Select the agent you want to image from the drop-down list—the selection defaults to the agent selected for the subject's group at the time of the group's creation (see Figure 4-3).
    - You can enter a text description of the agent dosage if you desire.
4. Acquire a Reflectance image.
    - If the **Reflectance Image Only** option is selected, the acquisition sequence is now complete and you may proceed to the **Analysis** tab (see *section 6* for more information).

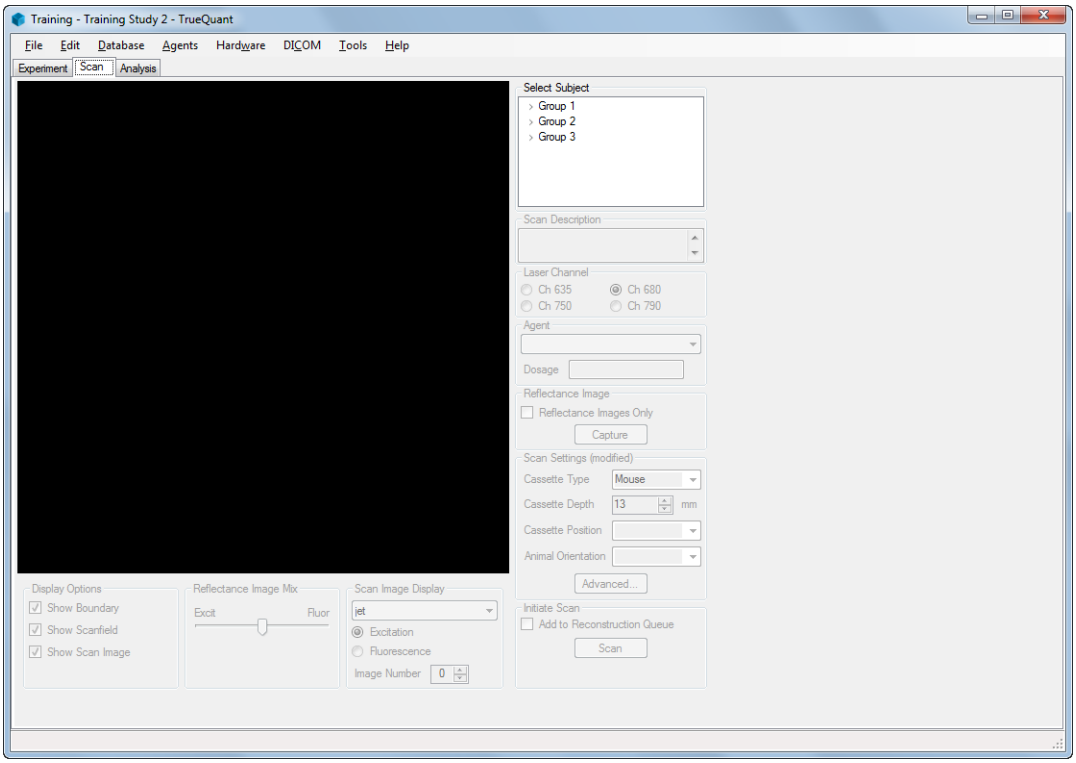


Figure 5-1. Initial screen for the **Scan** tab.

5. If the Multi-Species Imaging Module (MSIM) is installed, select the cassette type, cassette position, and animal orientation as necessary, and manually adjust the cassette depth if required.

   - These options are set automatically based on automated image processing of the reflectance images. However, it may be necessary to override the pre-set values, depending on your needs.
   - If you selected the Mouse cassette type, the position and orientation are not adjustable.

6. If you want a tomographic scan, click the scan field on the reflectance image of the subject and drag the scan field into the desired location. When you have positioned the scan field, press **Scan** (see Figure 5-2).

   - As the size of the scan field is adjusted, the number of source locations is displayed near the upper right-hand corner of the scan field (see Figure 5-2).

NOTE On FMT 1000, 1500, and 2000 models, if the classic reconstruction module is installed, an estimate of the tomographic reconstruction time also displays. For systems with Fast Recon, no time estimate is displayed.

- 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 never exceed 120 total sources.
- The reconstruction time display is only a rough estimate; the exact reconstruction time is data-set-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.
- If the **Add to Reconstruction Queue** option is selected, a tomographic reconstruction will begin as a background process as soon as the scan completes. If not, you can perform a reconstruction at a later time by adding the scan to the reconstruction queue manually (see *section 5.4*).

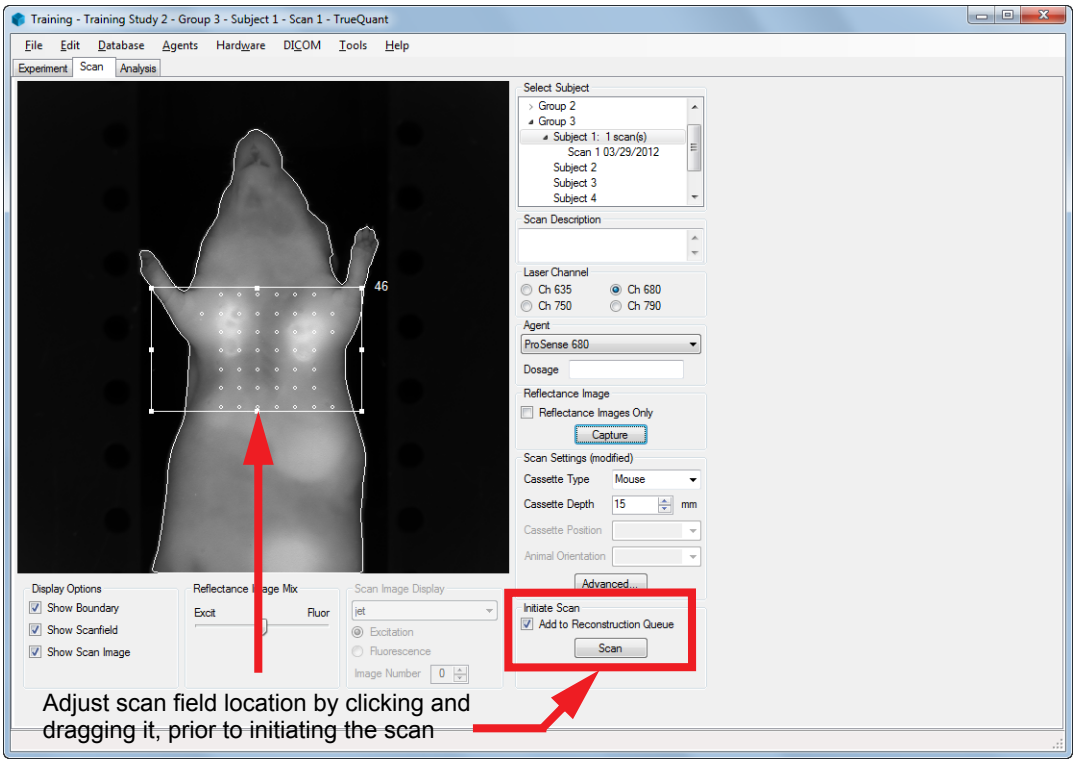


Figure 5-2. The **Scan** tab display prior to initiating the laser scan.

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 detector exposure to the laser.

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.

5.2 Advanced Scan Settings

The advanced scan settings allow you to override the system default settings for the following scan parameters (Figure 5-3):

- **Source density**: this refers to the spatial density of 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 to suit your purposes.
- **Sensitivity**: this slider provides a way for you to adjust the instrument's ability to detect weak flourescence sources. Adjusting the **Sensitivity** setting to **High** or **Very High** allows you to detect progressively fainter sources of flourescence but increases the chance of saturation from very bright sources.
- **Illumination levels (counts/pixel)**: these values adjust the settings for the auto-exposure scheme in controlling laser power and exposure times to obtain optimal results. The default settings are 5000 minimum and 50,000 maximum.

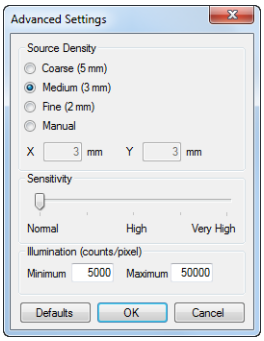


Figure 5-3. Advanced scan settings dialog box.

5.3 Display Controls

Once a scan completes, the display controls below the main image panel allow you to examine a number of display features, including:

- An outline of the scan field and the scan point locations.
- The animal boundary as automatically extracted from the reflectance image.
- An adjustable balance between the excitation-wavelength and fluorescence-wavelength reflectance image overlays.
- The raw transillumination scan images for each laser location at both excitation and fluorescence wavelengths, in a number of different color maps.

An illustrative example is provided in Figure 5-4.

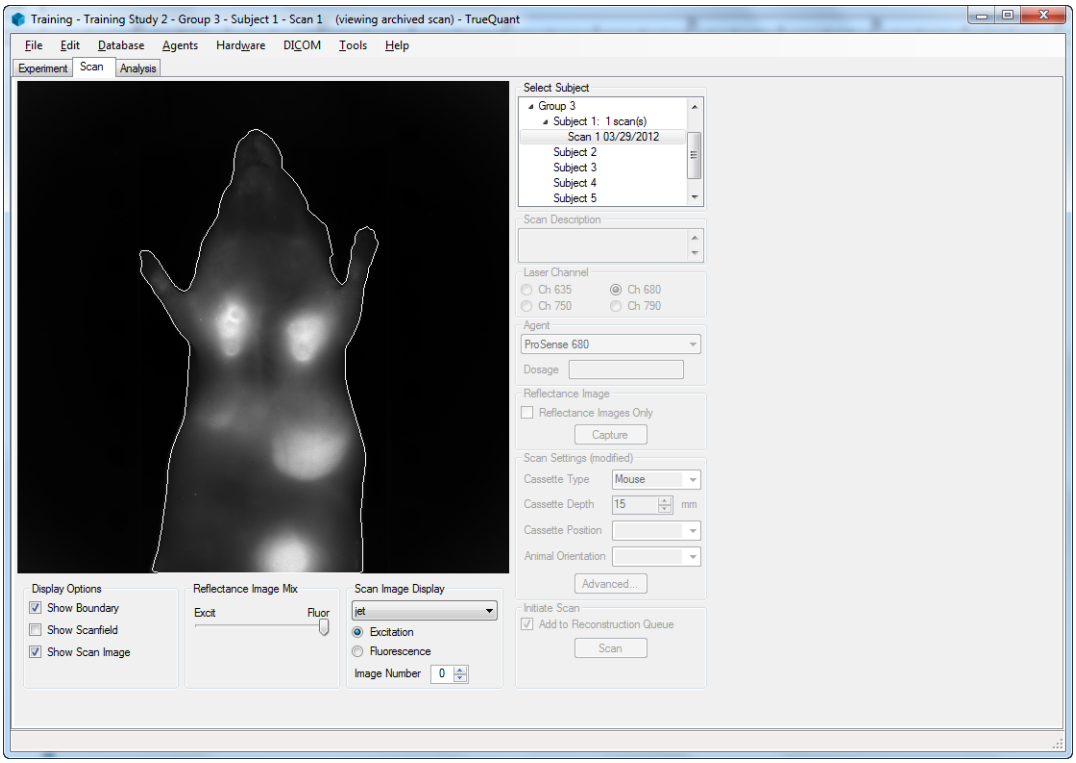


Figure 5-4. Illustrative display options.

Note the **Select Subject** tree now displays a scan entry for this specific subject. This is also available from the **Experiment** tab, which shows the scan entry, the status of the reconstruction, as well as an optional scan and reconstruction preview below the main panel (Figure 5-5).

5.4 Reconstruction Queue

Upon completion of a scan, a coarse reconstruction happens automatically in the background, using 2mm voxel mesh, for quick preview purposes. The results of this reconstruction can be displayed in the **Analysis** tab, detailed in Chapter 5. You can disable the quick preview reconstruction option by selecting **Tools** | **Options** | **Scan**.

If you selected the **Add to Reconstruction Queue** option prior to completion of the scan (Figure 5-2), a full-resolution tomographic reconstruction is started as a background process when the scan completes. Otherwise, you can add a reconstruction to the queue at any later time by launching the **Reconstruction Queue Manager**.

To do so, double-click the **Reconstruction Queue** icon in the Windows tray, at the bottom right of the screen, as shown in Figure 5-6. This opens the **Reconstruction Queue Manager** window, which initially shows an empty queue (Figure 5-7).

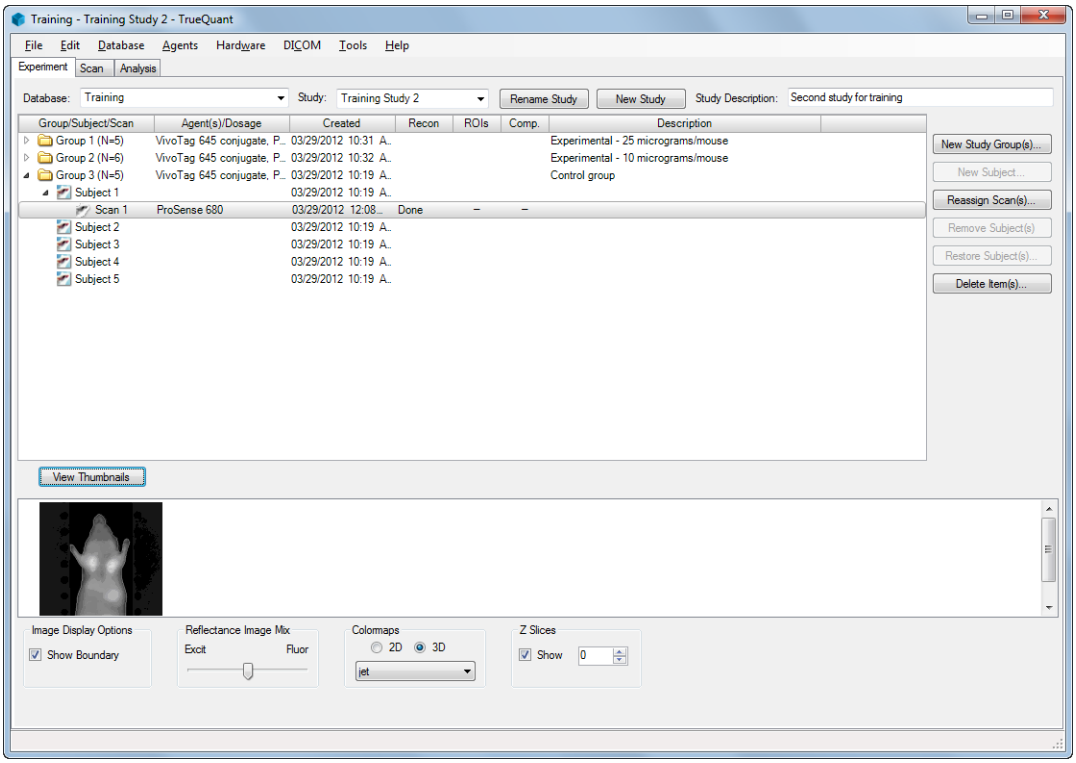


Figure 5-5. Experiment tab tree display after scanning Subject 1, Group 3.

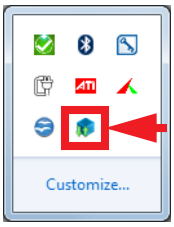


Figure 5-6. Reconstruction Queue icon in the Windows Tray.

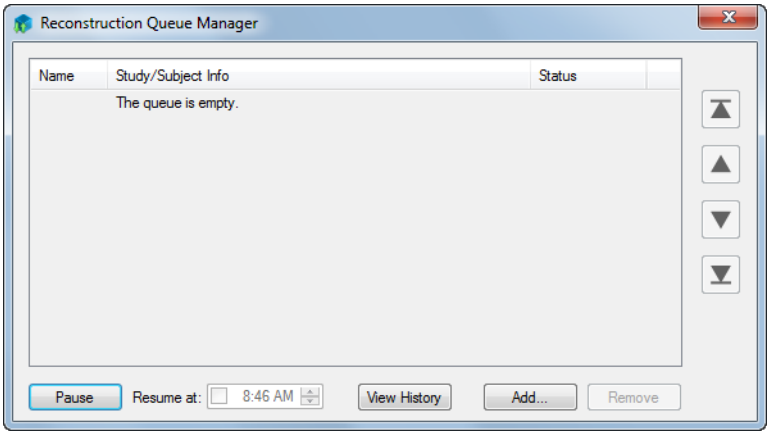


Figure 5-7. The Reconstruction Queue Manager window.

Pressing **Add** opens a data tree for the active database, allowing you to select one or more scans for reconstruction (Figure 5-8). Note, you can add scans from any study in the database to the queue from this dialog. It is not restricted to the active TrueQuant study. Scans with previously completed reconstructions cannot be added to the reconstruction queue again. You can cancel any active reconstruction by selecting it and clicking **Remove**.

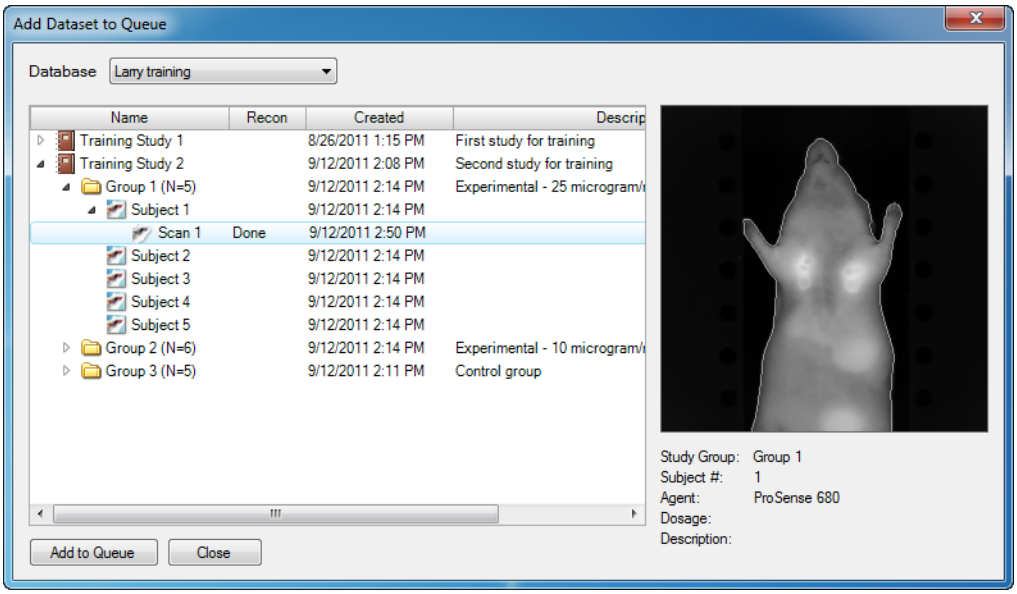


Figure 5-8. Selecting scans to add to the reconstruction queue.

You can also pause the entire queue. This will not pause the active reconstruction, but will prevent the next reconstruction in the queue from starting once the active reconstruction finishes. You can restart processing queue items manually or set it to restart at a predetermined time.

If you want to reorder scans in the queue, simply select one or more items and use the up and down arrows to move the selections into the desired position.

The reconstruction queue manager also features a **History** button that displays the record of reconstructed scans (Figure 5-9).

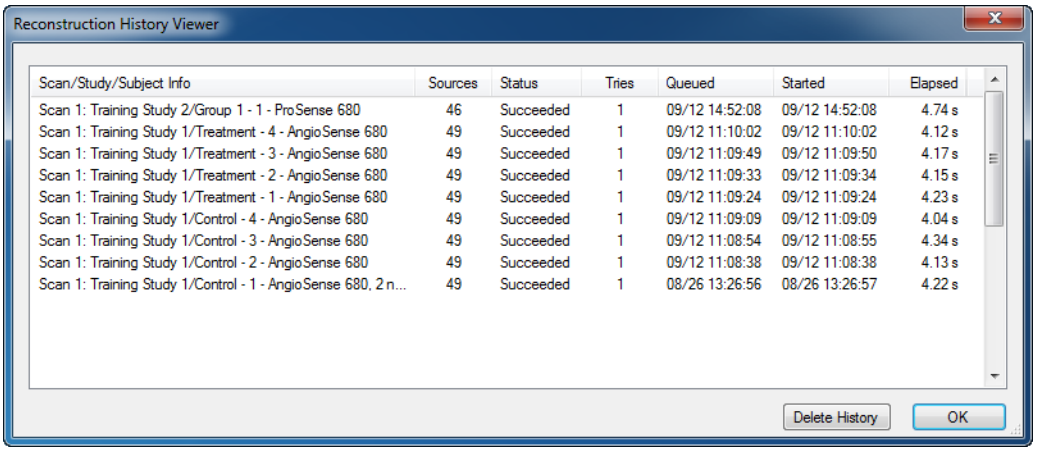


Figure 5-9. Reconstruction history viewer.

6

The Analysis Tab

6.1 Overview

The **Analysis** tab enables the user to view imaging results, perform region of interest (ROI) analyses, and export images and data to other applications. These operations can be performed on either 2D reflectance images, or on full 3D tomographic reconstructions. Figure 6-1 provides an example of the **Analysis** tab.

There are two ways to load data into the **Analysis** tab. The first is by clicking the **Add** button (+) in the **Dataset Selection** control and highlighting the scan or scans to be analyzed. This brings up the **Load Dataset** dialog box, shown in Figure 6-2, enabling the user to select and load the required dataset or datasets.

An alternate means of loading a dataset for analysis is to go to the **Experiment** tab, select the dataset, right-click it and select **View Analyses** from the context menu (Figure 6-3) or simply double-click a scan in the **Experment** tab. This automatically switches the display to the **Analysis** tab with the selected dataset loaded and displayed (Figure 6-4).

It is possible to load multiple datasets simultaneously by following either of the two methods described above.

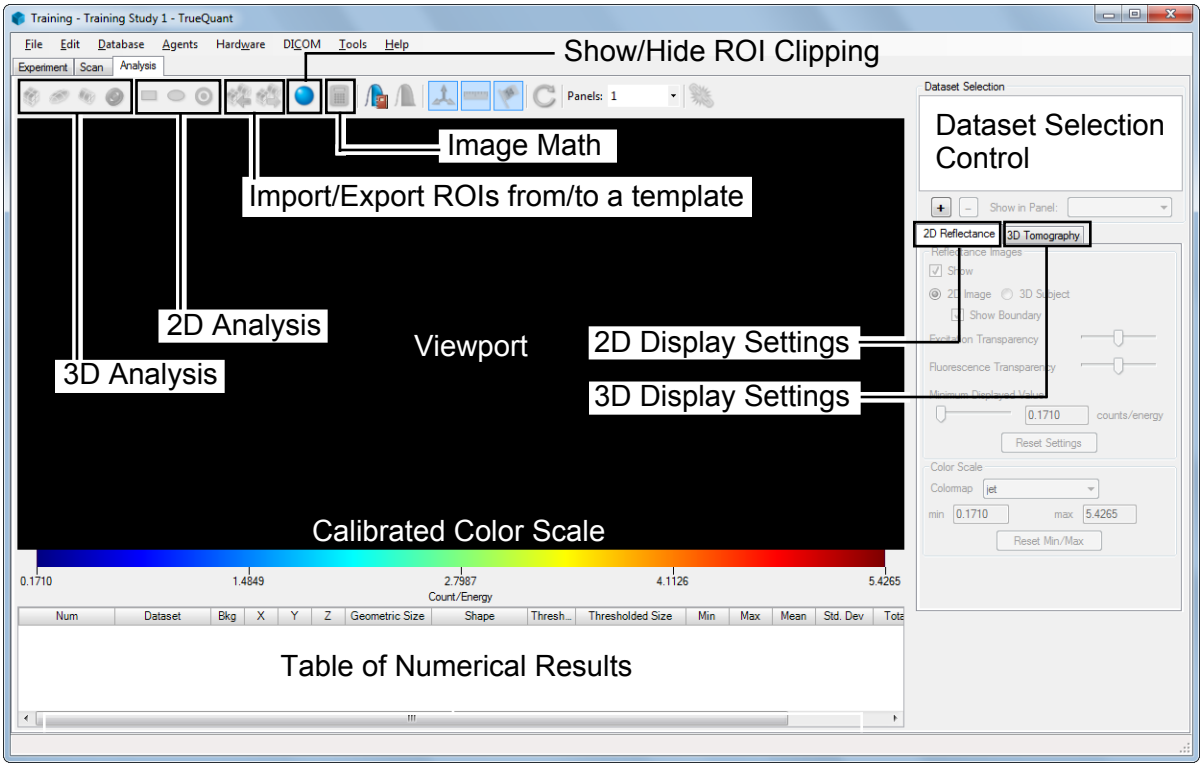


Figure 6-1. Initial view of the **Analysis** tab.

If data are loaded into the same panel that were acquired with the Multi-Species Imaging Module (MSIM) in different cassette positions and animal orientations, those scans will be displayed offset from one another so as to be displayed as a single continuous view of the animal. Feet-first scans will also be 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. These 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.

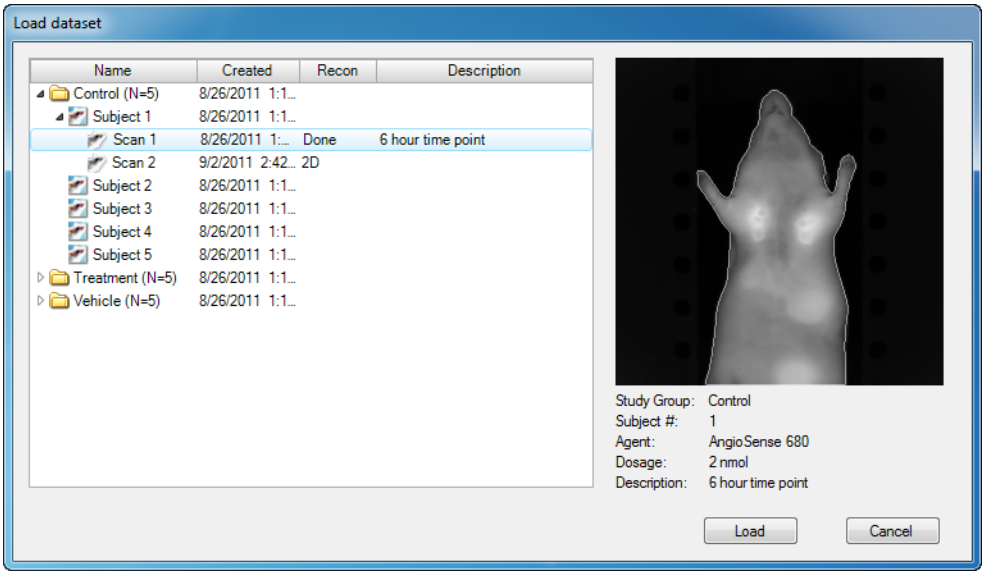


Figure 6-2. Loading datasets into the **Analysis** tab with the dataset selection control.

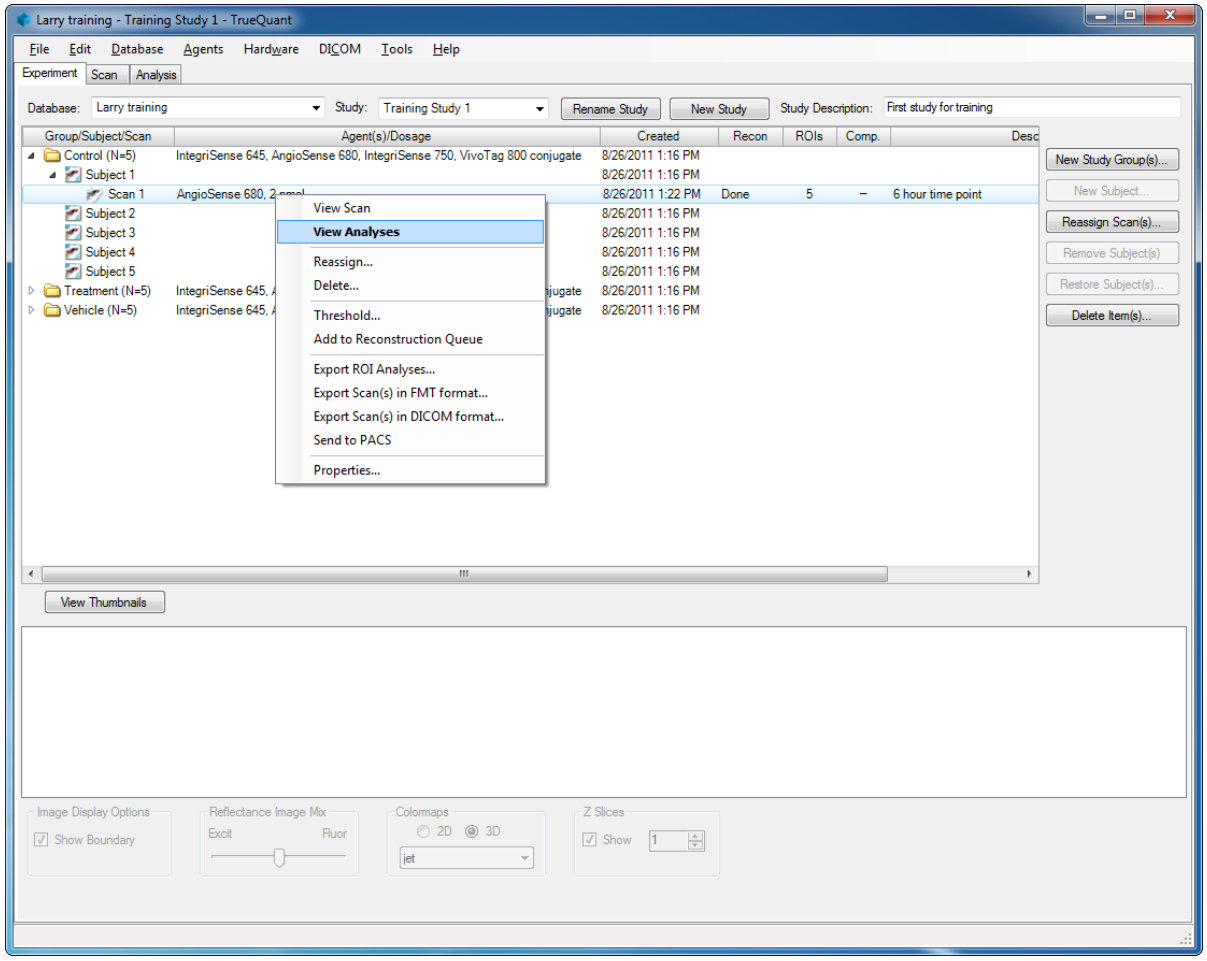


Figure 6-3. Loading a dataset for analysis from the **Experiment** tab.

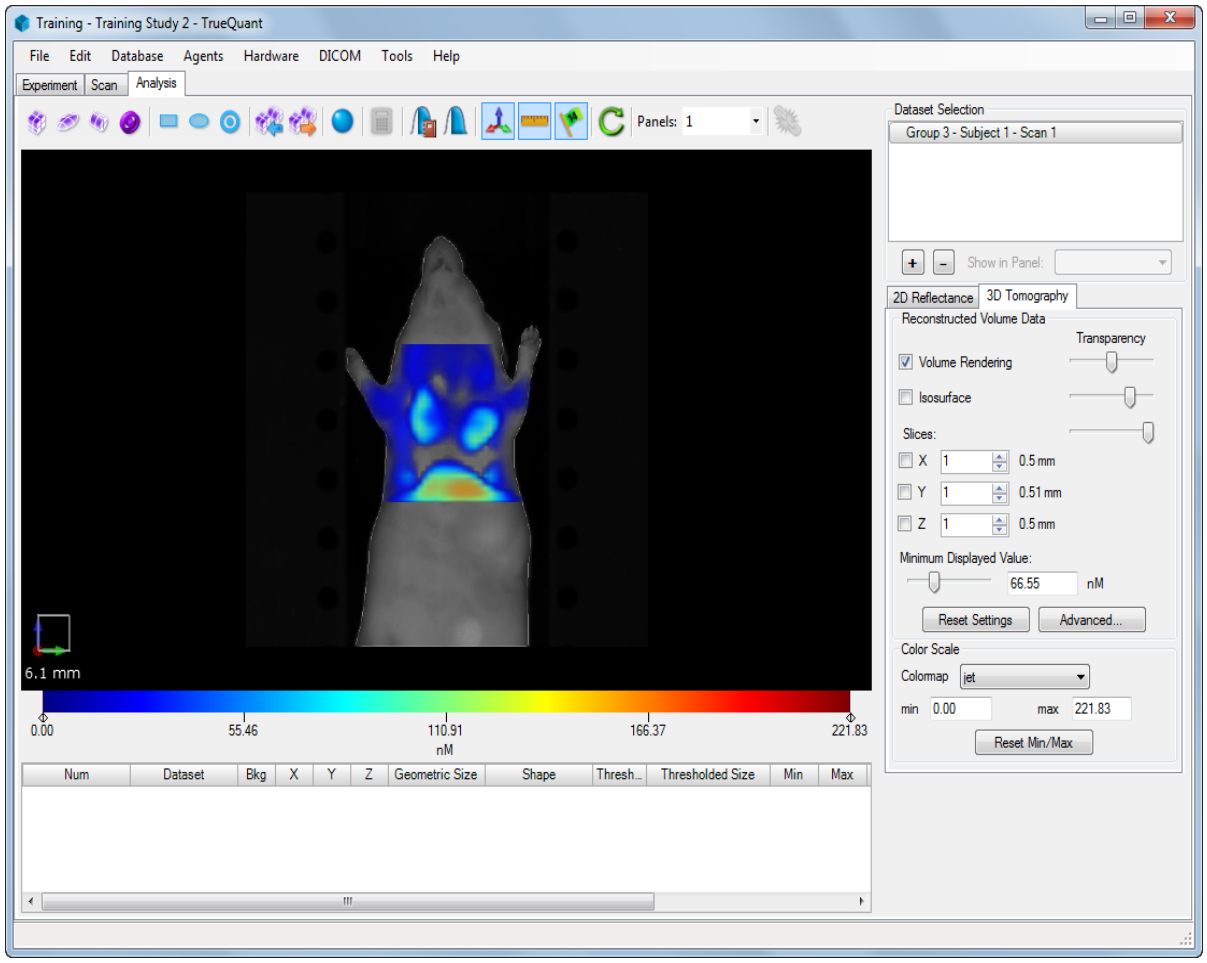


Figure 6-4. The **Analysis** tab after loading a scanned and reconstructed tomographic dataset.

Clicking the **Remove** button (–) in the **Dataset Selection** control will unload the currently highlighted dataset and remove it from the viewport.

When manipulating images in the **Analysis** tab, the following functions are always available using the computer's mouse:

- Rotate the view of the data: Middle-button or Right-button click and drag
- Pan over the data: Control-middle-click or control-right-click and drag
- Zoom in/out: Use the scroll wheel or hold down the shift key while using the middle or right mouse button

The colored coordinate axes in the lower left-hand corner of the display help in visualizing the current orientation. The coordinate axes can be shown or hidden at any time by clicking the **Show/Hide Axis** icon ( ) above the viewport. It is also possible at any time to press the **Reset View** icon ( ) to return to the default view of the subject.

Coupled to the coordinate axes is a wireframe cube that indicates the physical scale of the data being displayed. Below the cube is a size scale in millimeters (mm) that indicates the length of one edge of the cube. The cube and physical scale indicator can be hidden or shown at any time by clicking the **Show/Hide Scale** icon ( ) above the viewport.

The scale for the color bar underneath the viewport defaults to the minimum and maximum concentration values for 3D reconstructions (or minimum and maximum pixel intensities for 2D reflectance images). It is possible to type in user-defined values in the respective entry fields in the Color Scale region to the right of the viewport. You can also adjust the color scale minimum and maximum by dragging the diamond-shaped handles below the color scale.

The 3D purple-colored ROI creation tools are displayed at the top left of the toolbar as shown in Figure 6-5:

Figure 6-5. 3D ROI creation tools.

These ROI creation tools represent, from left to right:

- a rectangular prism, or parallelepiped
- an ellipsoid
- a cylinder
- a contour-based selection tool

For example, by selecting the parallelepiped tool, a purple, rectangular prism is superimposed on the fluorescent region and its statistics are displayed numerically in the table below the viewport (Figure 6-6).

The ROI selection check box in the table below the viewport is used to show or hide an ROI's wireframe.

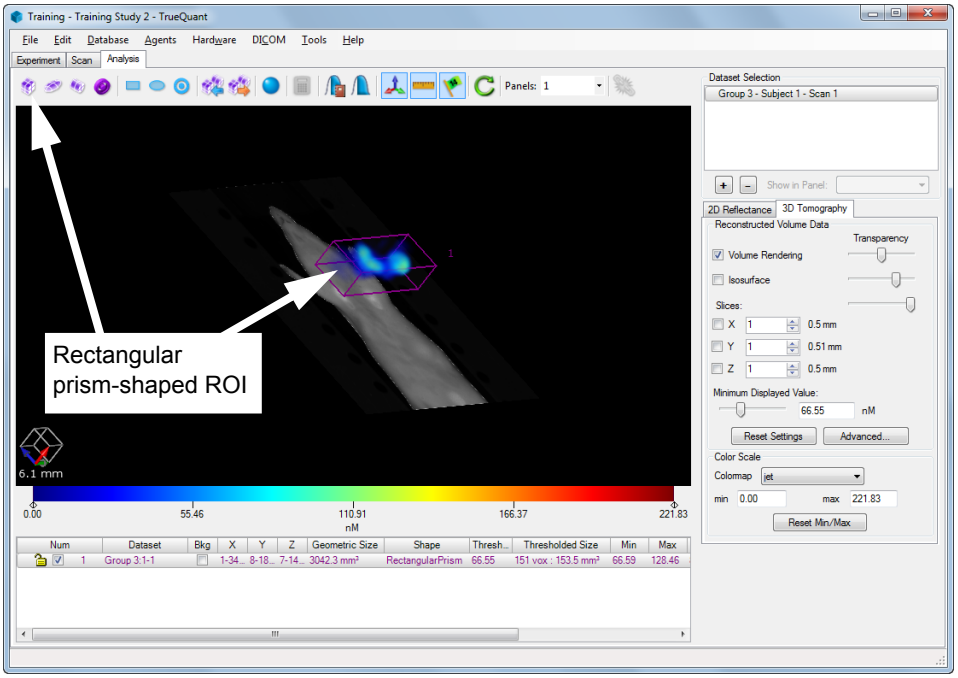
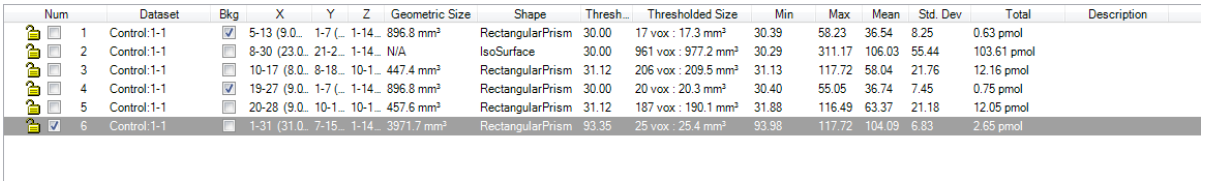


Figure 6-6. Selecting a rectangular prism region of interest.

A closer look at a tabular ROI display is shown in Figure 6-7:



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 6-7. Typical ROI statistics.

The table columns, from left to right, contain the following entries:

Num
A number, together with an open/closed padlock symbol and a check box. The number is the unique identifier for the ROI, the padlock icon indicates whether it is locked to prevent further editing (see *section 6.1*), and the checkbox indicates whether the wireframe of the ROI is being shown in the 3D viewer. TrueQuant assigns these numbers at runtime, so they can differ from one analysis session to the next.

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 at any time by clicking the **Show Labels** icon ( ) in the toolbar above the viewport.

Dataset
Indicates which group, subject number, and scan number this ROI was derived from.

Bkg
Allows you to specify that the selected ROI is a region of background.

X, Y, Z ranges
Provides the slice numbers and physical sizes in the sagittal, transaxial and coronal orientations respectively included in the ROI. For this example, the ROI spans slices number 9 through 29 (21 mm) in the sagittal direction, and so on. For 2D ROIs, only the pixel ranges 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, selecting the row in the table and then clicking on the shape name lets you change the orientation of the cylinder to each of the three possible axes.

Threshold
Given in concentration units of nM (nanomolar or 10^{-9} moles/liter) for 3D ROIs. You can change the **Threshold** value by adjusting the slider control in the **Isosurface Settings** region (see *section 6.3*). For 2D ROIs, this is given in units of counts/input energy.

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, these are given in units of 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 ROI. For 2D ROIs, this is given in units of counts/input energy.

Total

The amount of agent in pmol (picomoles or 10^{-12} moles) in the ROI. For 2D ROIs, this is given in units of counts×pixels/input energy.

Description

An optional user-supplied text description. You can modify this field by selecting the row in the table and clicking the description to open the description text edit box. Note, it is considered best practice to include a meaningful description for the ROIs you create. Doing so is especially important when dealing with many ROIs or very large datasets.

The isosurface-based selection tool allows you to select a particular isosurface by clicking it. The tool then automatically generates the statistics associated with that isosurface at the chosen threshold settings, and highlights the isosurface with a wireframe (Figure 6-8).

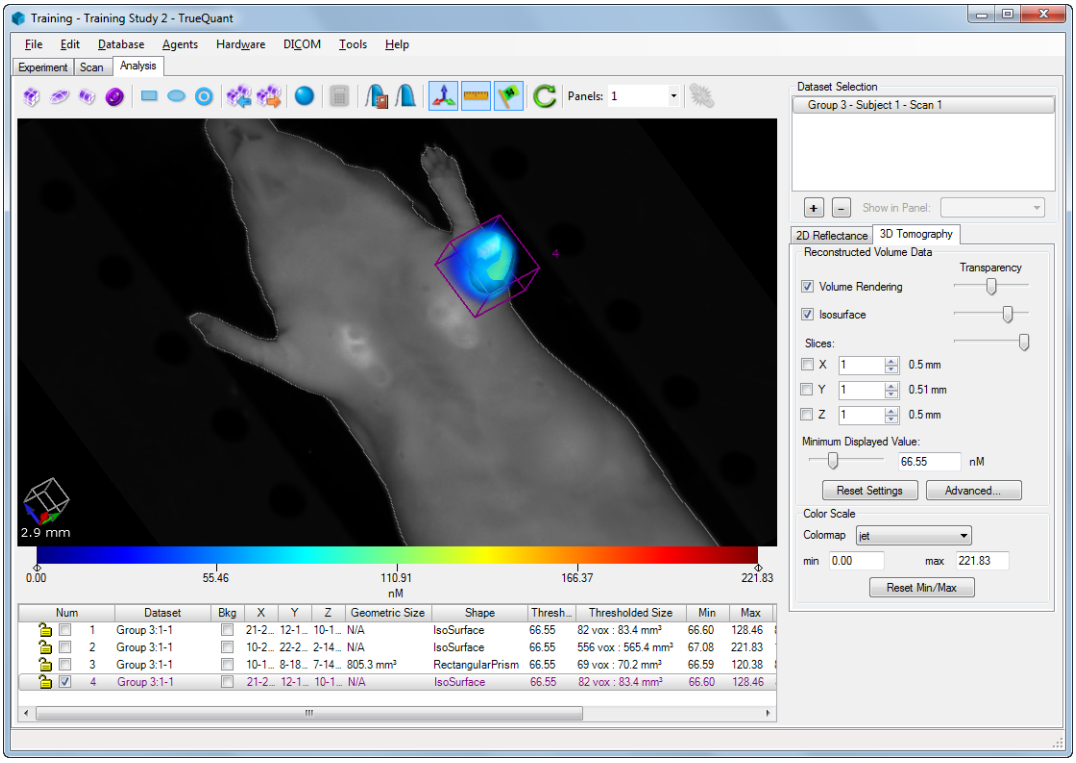


Figure 6-8. The result of creating an isosurface ROI.

TrueQuant also provides the ability for you to import and export ROIs to and from your projects. This allows you to clone ROIs across studies and even 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 will be saved into the ROI template.

6.2 Using The ROI Table

When you select and right-click an ROI row in the numerical table, you can perform several actions, as shown in Figure 6-9.

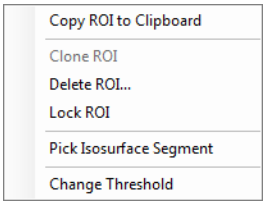


Figure 6-9. ROI Table contextual menu.

The following section describes each of these options in greater detail.

Copy ROI to Clipboard

Copies the table row to the Windows clipboard as an alphanumeric string.

Clone ROI

This 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 that the cloned ROI will be associated 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.

Delete ROI

Removes the ROI from the display and the database. Choosing this option opens a confirmation dialog box. If you choose to proceed with deletion, TrueQuant permanently removes the ROI from the image display and the database.

NOTE Take care when using the delete function, as a delete operation cannot be undone.

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

This option allows you to reassign a configured isosurface ROI to a different segment in the image. After choosing this option, simply select a new segment in the image and the ROI updates in the table and the viewport automatically.

Change Threshold

This option 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** dialog 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 ROIs dataset.

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

6.3 Modifying an ROI Interactively

To modify the size and aspect ratio of an ROI, click the mouse button on the center of one face of the ROI and drag it to the desired position—the active face will be highlighted in pink to indicate it is being moved (Figure 6-10). The voxel coordinates of the ROI in the tabular region below the main image panel change dynamically as you edit the ROI. Figure 6-11 shows the result of shrinking the ROI in Figure 6-10 down to the size of the tumor being analyzed.

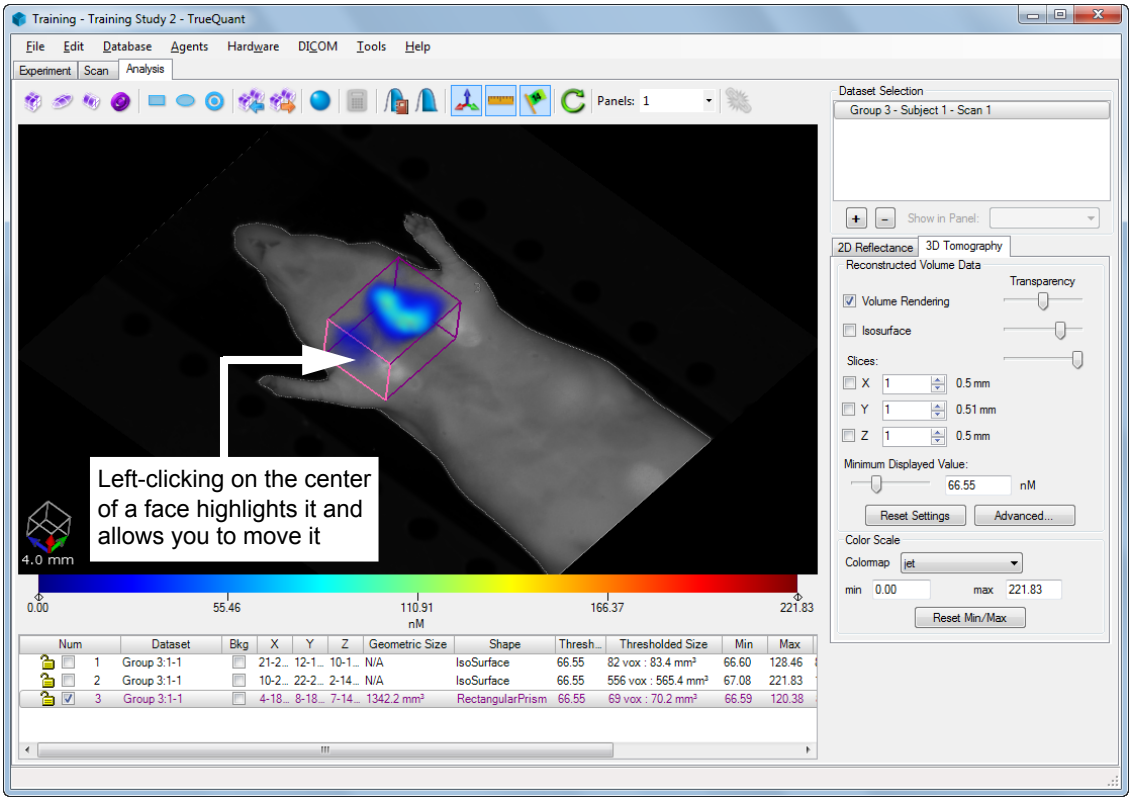


Figure 6-10. Editing an ROI interactively.

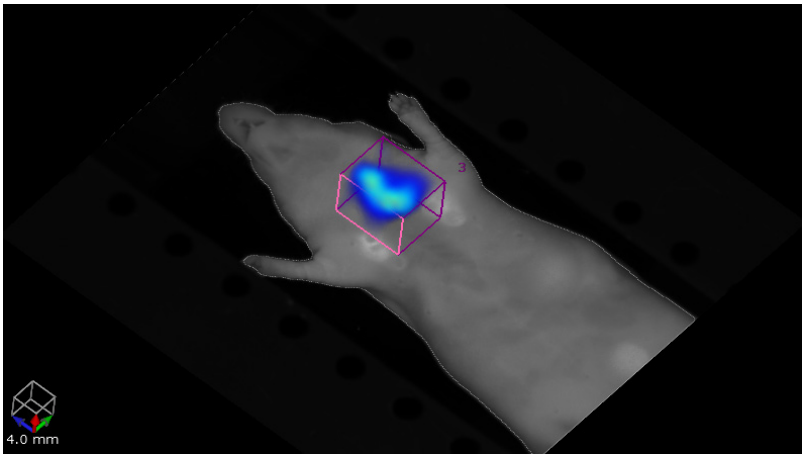


Figure 6-11. The result of editing an ROI interactively.

A 3D ROI can be moved to a new location in x, y or z by selecting the ROI, pressing **Ctrl+m**, and clicking and dragging one side of the ROI to the desired location. While in this mode, a yellow double-arrow move indicator appears in the upper left region of the viewport as a visual cue, as shown in Figure 6-12:

Figure 6-12. ROI movement active indicator

Clicking and dragging on the ROI is done one axis at a time (x, y, or z), with the selected axis being highlighted in pink.

Pressing **Ctrl+m** once more toggles the viewport back to normal mode.

6.4 2D and 3D Image Settings Region

The image settings region 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 affect the actual data, only the manner in which the data is being displayed. Any changes made with these controls will affect only the datasets and ROI that are selected (highlighted in blue) in the dataset selection control. An entire dataset can be temporarily hidden from view by selecting that dataset and un-checking each of the check boxes for that dataset's image settings.

The display controls for the 2D and 3D image settings (see *section 6.3*) apply to whichever dataset or datasets are highlighted in the dataset selection control.

Sliders can be dragged manually to adjust:

- Transparency settings for either excitation or fluorescence reflectance images: Figure 6-13 and Figure 6-14 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, you must 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 6-15 and Figure 6-16). Note, the software adjusts the corresponding numerical unit (counts/energy for 2D and nM for 3D) automatically as you move the slider. Alternatively, you can enter the desired number directly in the text field adjacent to the slider.

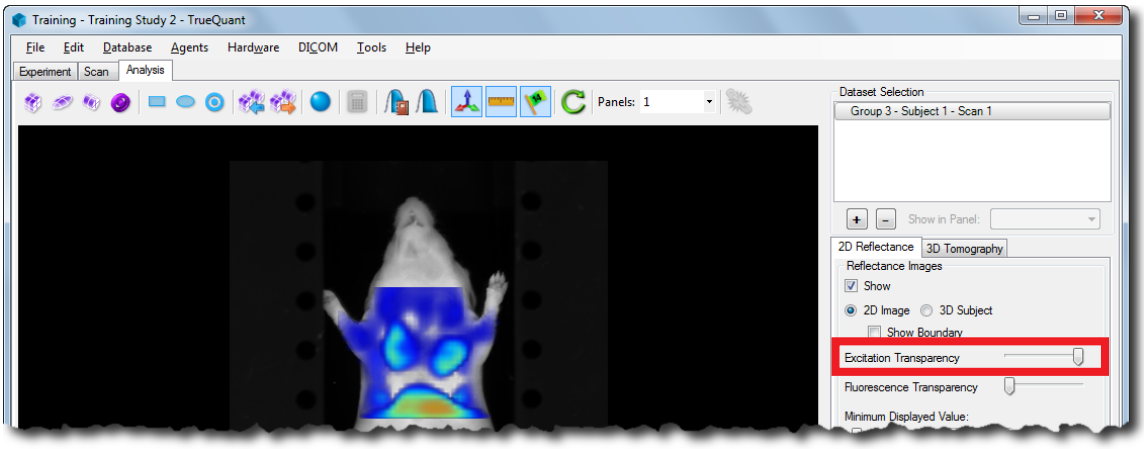


Figure 6-13. Setting the slider to show the excitation reflectance image at a maximum and fluorescence reflectance image at a minimum.

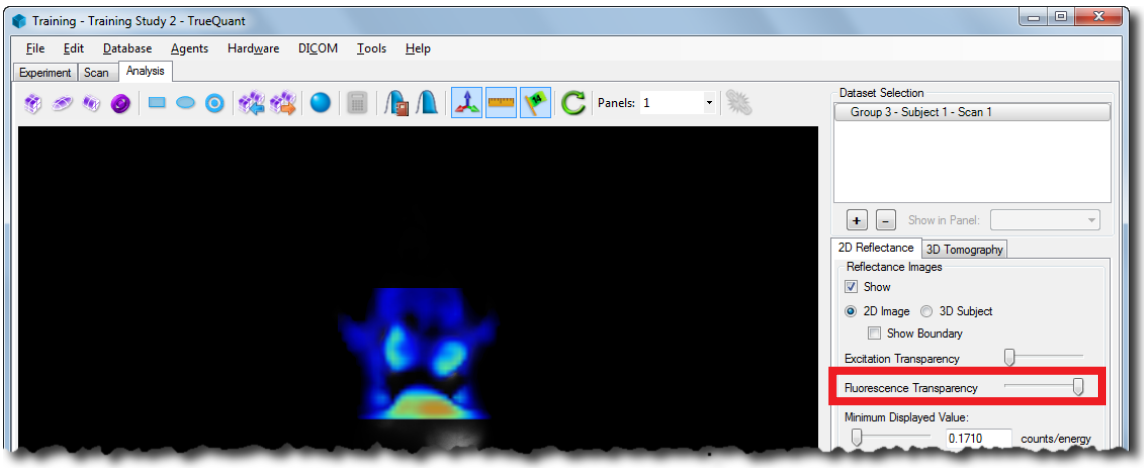


Figure 6-14. Setting the slider to show the fluorescence reflectance image at a maximum and the excitation reflectance image at a minimum.

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. The **Advanced** button can be used to turn off this blending and view the voxels as solid colored cubes.
- The **Reset Settings** button will return all the above settings to their 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 their 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 at any time to match the minimum and maximum values in the data, using the **Reset Color Scale** button.

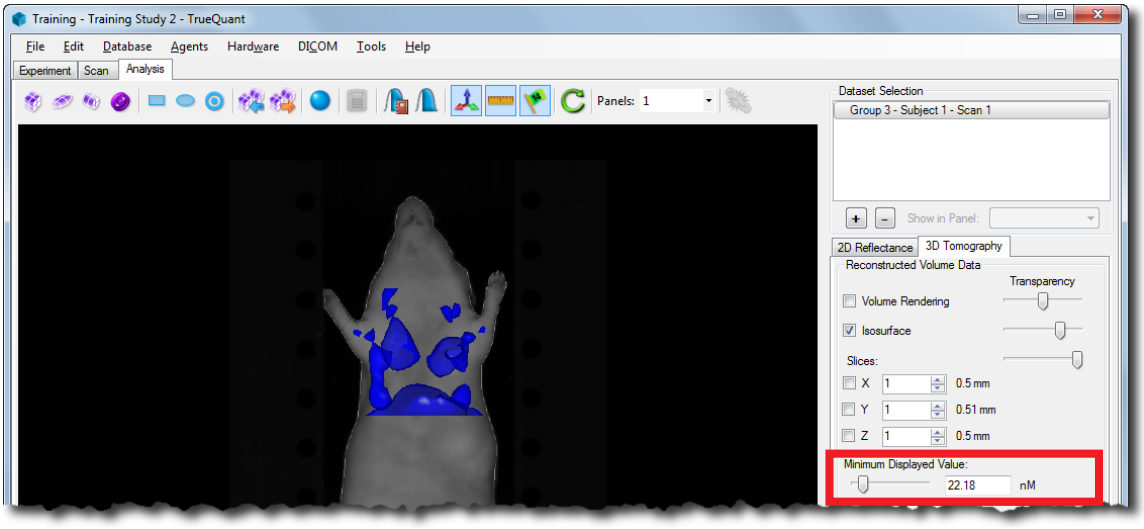


Figure 6-15. Selecting a low value for the 3D isosurface.

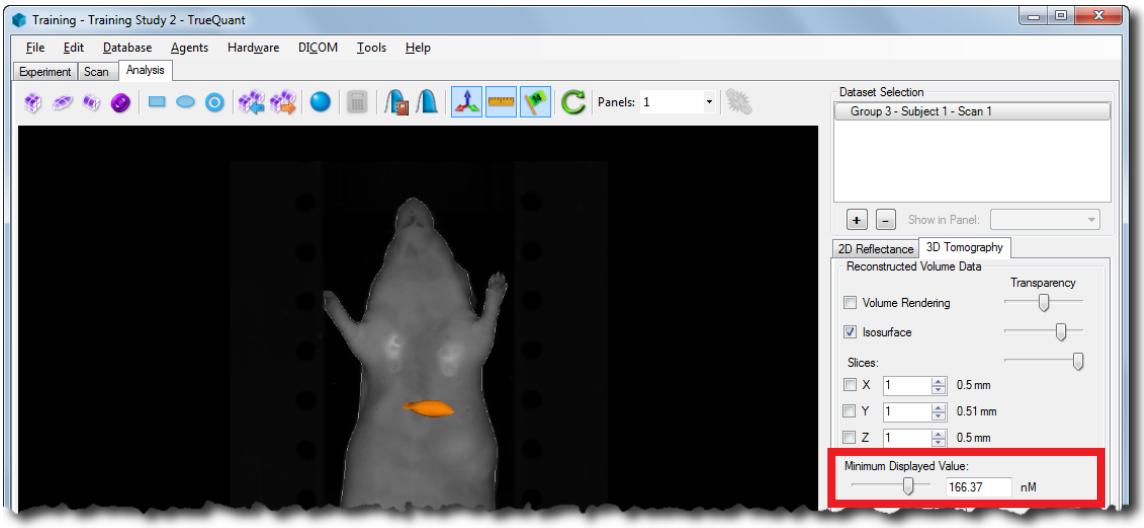


Figure 6-16. Selecting a higher value for the same isosurface.

6.5 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 6-17). The viewport can be split horizontally into 1, 2, or 3 panels, while a second row makes it possible to have 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 their new panel number using the **Show in Panel** drop-down selector below the list of datasets (Figure 6-17). 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.

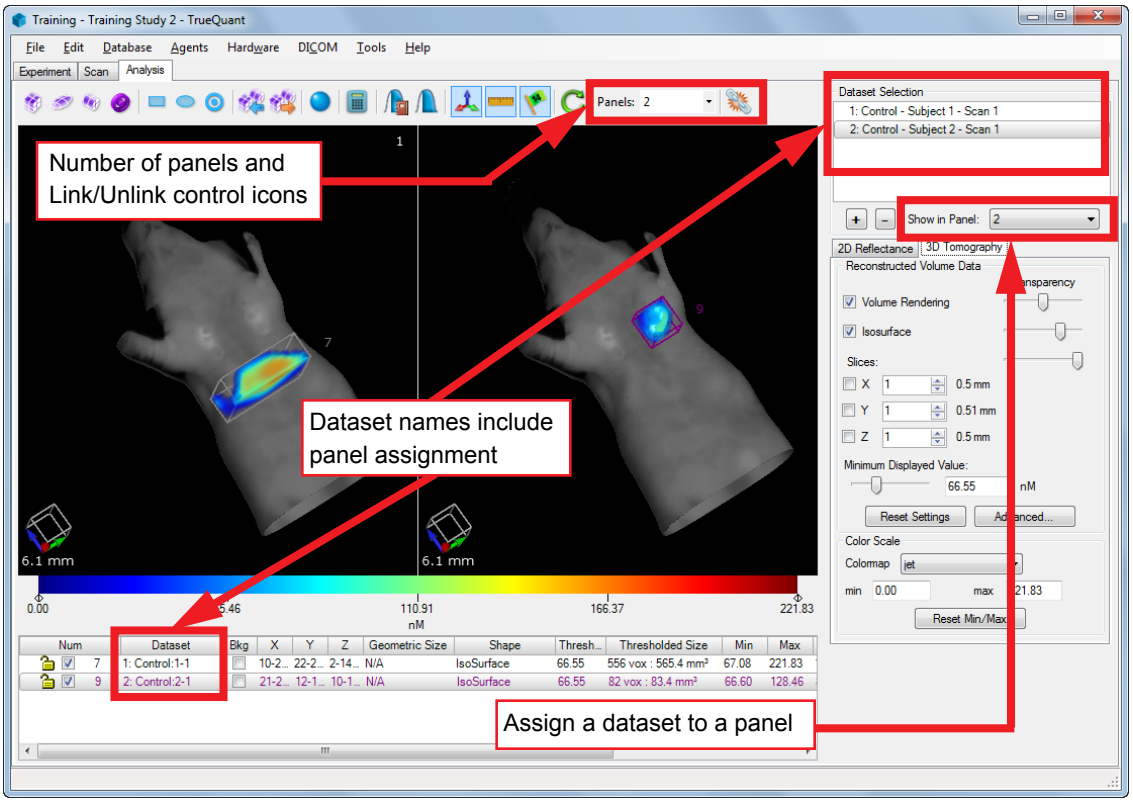


Figure 6-17. Controls for using multiple panels in the **Analysis** tab.

When visualizing 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 6-17). If operating independently, any rotate, zoom and pan operations will apply to the panel under the mouse pointer when the operation starts. In this case, 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 immediately change to match those of the last panel whose view was changed. Clicking the **Unlink** icon ( ) removes the synchronization of the viewports, allowing you to rotate, zoom, and pan each image independently once again.

Each panel has a label at its top right corner to indicate which panel is which. Various labeling schemes are available: numbers, lower- or upper-case letters, and lower- or upper-case Roman numerals. You can set the numbering scheme by choosing a **Label Style** on the **Analysis** tab of the **Options** window, which is accessed through the **Tools** | **Options** menu item (Figure 6-18). The panel labels are given as prefixes to the dataset names in both the dataset selection control and the ROI table below the viewport.

These 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 **Return** to apply the new custom label or **Esc** to keep the previous label.

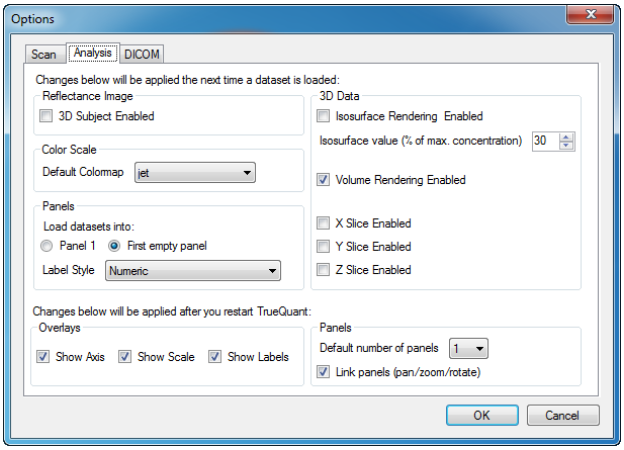


Figure 6-18. The **Analysis** tab of the **Options** window.

Multiple panels are quite useful for simultaneously viewing multiple datasets, but their greatest power comes from the ability to manipulate multiple ROIs at the same time. As an 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 control, 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 by using **Ctrl+m** to activate move mode and then dragging the ROIs to their final locations. 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.

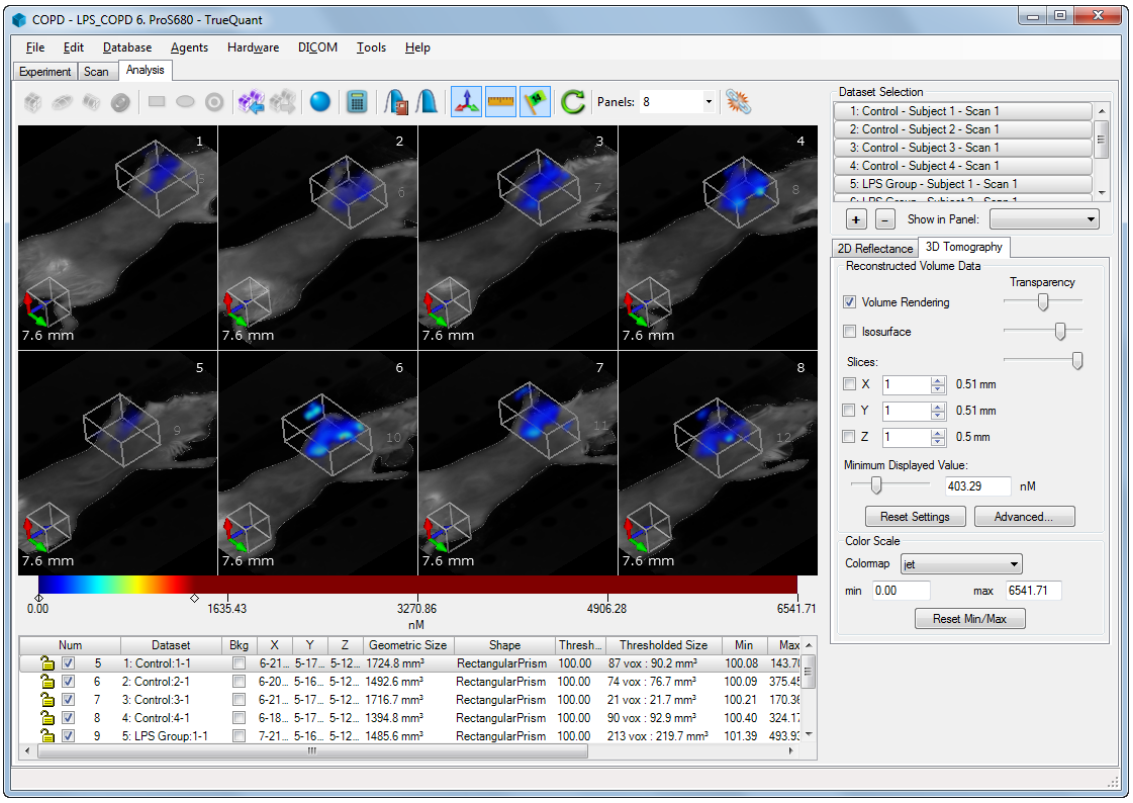


Figure 6-19. Simultaneous analysis of eight datasets.

6.6 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 6-20). This tool includes controls for entering a mathematical or logical equation and specifying computational parameters, as well as a preview window to see the results of your computation before saving them.

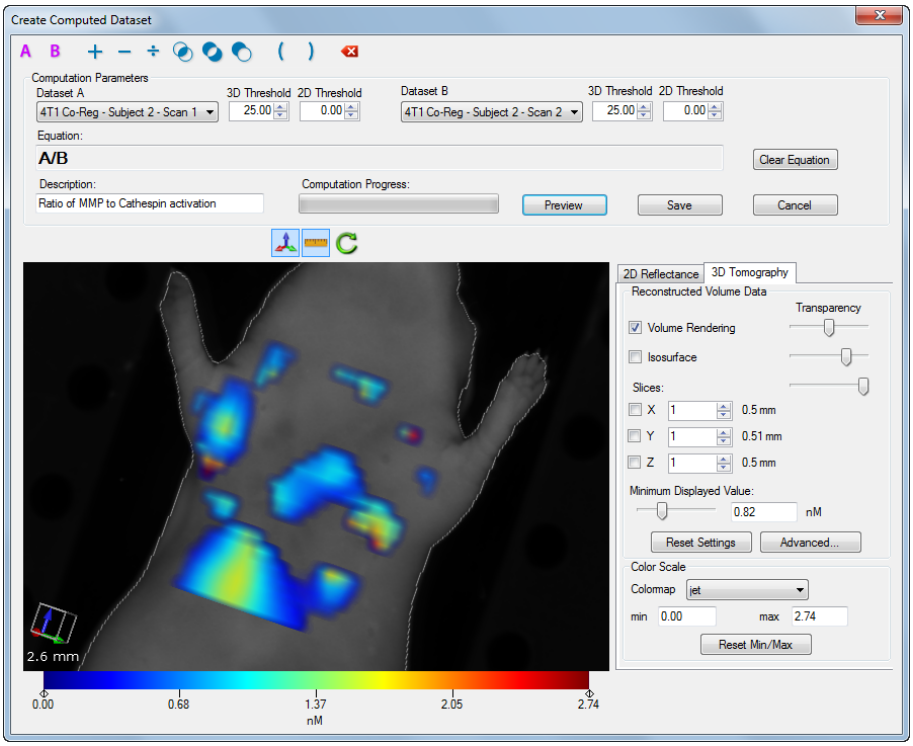


Figure 6-20. The **Create Computed Dataset** window.

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 6-21).

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.

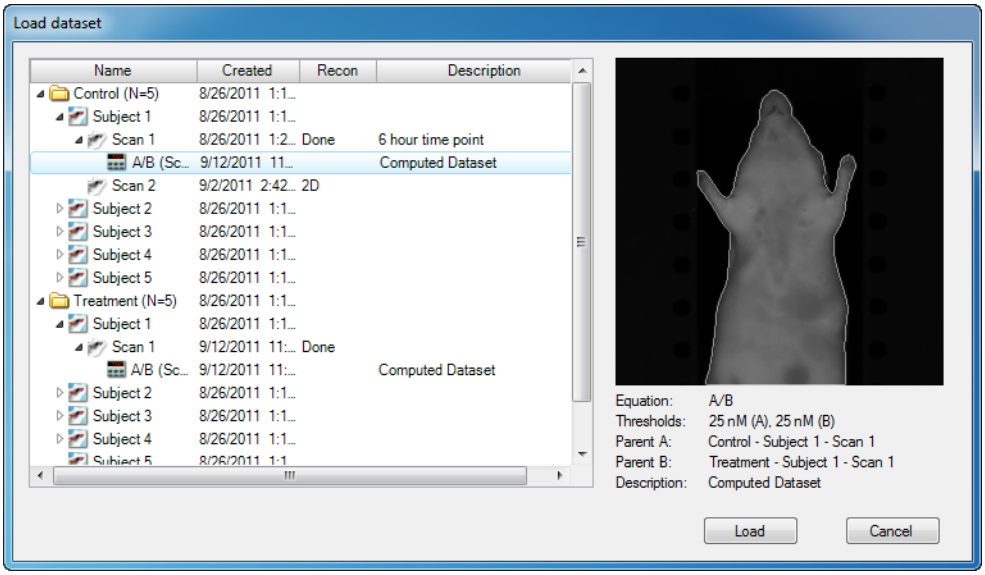


Figure 6-21. Loading a computed dataset into the **Analysis** tab.

6.7 Thresholding Advisor

TrueQuant provides the **Thresholding Advisor** tool, making it possible for you to threshold 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 useable with 3D geometric ROIs; it will not work with 2D or isosurface ROIs.

To access the **Thresholding Advisor**, shown in Figure 6-23, 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** dialog, you can choose to use **All Datasets** in the study or limit the analysis to only the **Loaded Datasets**, as shown in Figure 6-22.

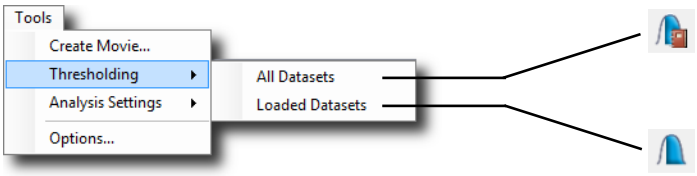


Figure 6-22. Accessing the **Thresholding Advisor** from the main menu and the equivalent toolbar icons.

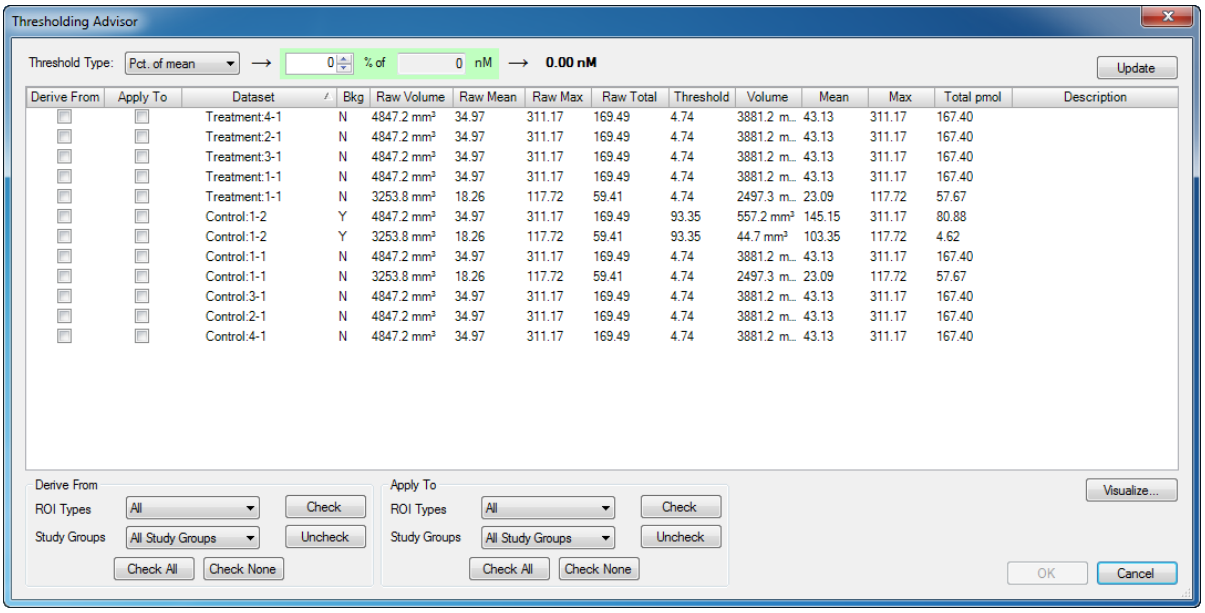


Figure 6-23. The **Thresholding Advisor** dialog.

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. Please note, if a loaded ROI is an Isocontour shape, it will not be used in the statistically derived threshold calculation and the **Derive From** column will be 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. Please note, if the ROI entry in the table is *locked*, as indicated by the locked padlock icon in the **Dataset** column, you cannot apply the statistically derived threshold value to the ROI and the **Apply To** controls are unavailable for that table row. Refer to *section 6.2* 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.

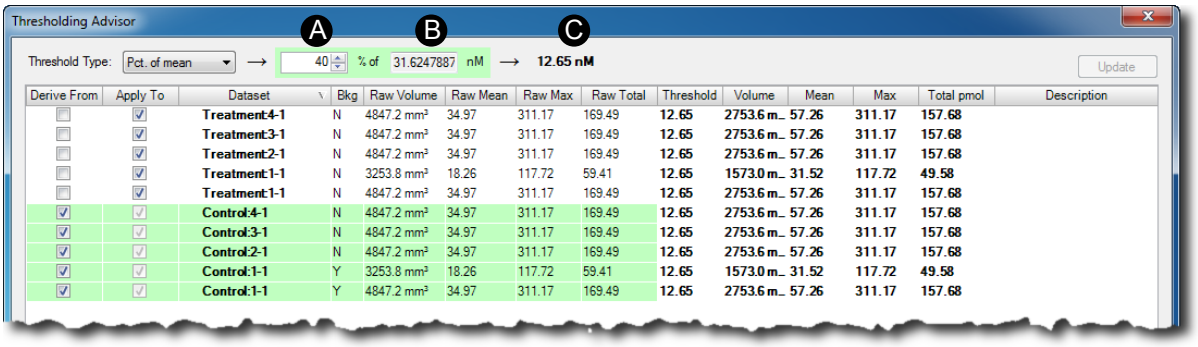
Description
An optional user-supplied text description. Note, meaningful descriptions can be very useful in distinguishing between ROIs in the **Thresholding Advisor**, especially when dealing with many ROIs or very large numbers of datasets.

6.7.1 Threshold Type Option

When using the **Thresholding Advisor**, you have several calculation options from which to choose, including:

Percent of Mean

This option calculates a percentage of the average threshold value over the datasets. 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. With the **Threshold Type** set to **Pct. of Mean**, the **Thresholding Advisor** calculates the mean of the **Raw Mean** values (Figure 6-24, item B) for the ROIs marked as **Derive From**. When you enter the desired percentage into the field (Figure 6-24, item A), the **Thresholding Advisor** calculates the value and updates the display (Figure 6-24, item C). An example of the **Percent of Mean** calculation in the **Thresholding Advisor** is shown in Figure 6-24.



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	12.65	2753.6 m…	57.26	311.17	157.68	
☐	☑	Treatment:3-1	N	4847.2 mm³	34.97	311.17	169.49	12.65	2753.6 m…	57.26	311.17	157.68	
☐	☑	Treatment:2-1	N	4847.2 mm³	34.97	311.17	169.49	12.65	2753.6 m…	57.26	311.17	157.68	
☐	☑	Treatment:1-1	N	3253.8 mm³	18.26	117.72	59.41	12.65	1573.0 m…	31.52	117.72	49.58	
☐	☑	Treatment:1-1	N	4847.2 mm³	34.97	311.17	169.49	12.65	2753.6 m…	57.26	311.17	157.68	
☑	☐	Control:4-1	N	4847.2 mm³	34.97	311.17	169.49	12.65	2753.6 m…	57.26	311.17	157.68	
☑	☐	Control:3-1	N	4847.2 mm³	34.97	311.17	169.49	12.65	2753.6 m…	57.26	311.17	157.68	
☑	☐	Control:2-1	N	4847.2 mm³	34.97	311.17	169.49	12.65	2753.6 m…	57.26	311.17	157.68	
☑	☐	Control:1-1	Y	3253.8 mm³	18.26	117.72	59.41	12.65	1573.0 m…	31.52	117.72	49.58	
☑	☐	Control:1-1	Y	4847.2 mm³	34.97	311.17	169.49	12.65	2753.6 m…	57.26	311.17	157.68	

Figure 6-24. Percent of Mean calculation in the **Thresholding Advisor**.

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

The **Thresholding Advisor** example shown in Figure 6-24 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 6-25. Note, the **Update** button is also no longer active. It will remain inactive until you make some 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 6-25. **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 6-26 provides an example of both button states.

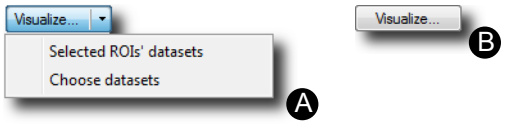


Figure 6-26. Thresholding Advisor button states.

The **Visualize** button without the drop-down menu (Figure 6-26, item B) and the **Visualize** button **Choose datasets** menu option (Figure 6-26, item A) both open the **Choose Datasets to Visualize** window, shown in Figure 6-27.

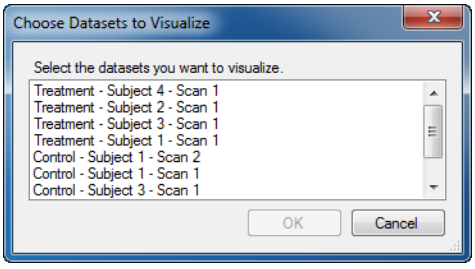


Figure 6-27. Choose Datasets to Visualize window.

Alternately, the **Selected ROIs' datasets** menu option (Figure 6-26, 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 6-28, item B) for the ROIs marked as **Derive From**. When you enter the desired percentage into the field (Figure 6-28, item A), the **Thresholding Advisor** calculates the value and updates the display (Figure 6-28, item C). An example of the **Percent of Max** calculation in the **Thresholding Advisor** is shown in Figure 6-28.

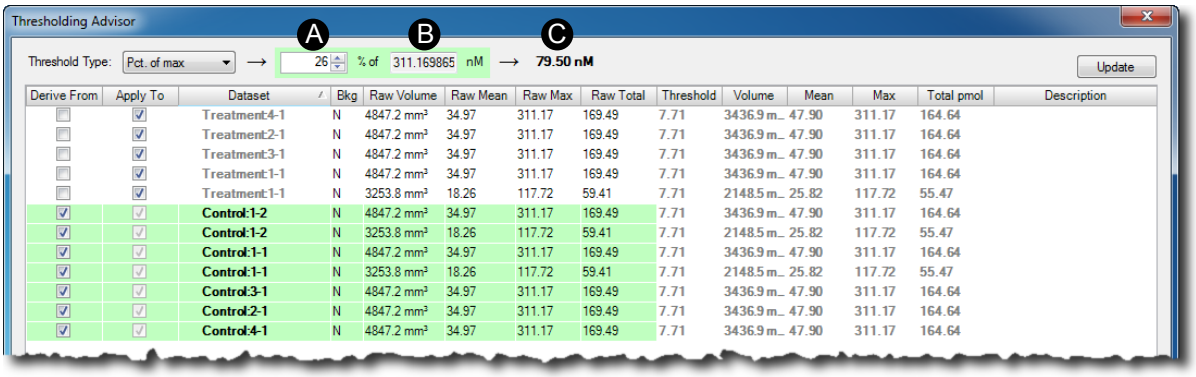


Figure 6-28. Percent of Max calculation in the **Thresholding Advisor**.

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 6-29. Note, the **Derive From** column is ignored when using this threshold type.

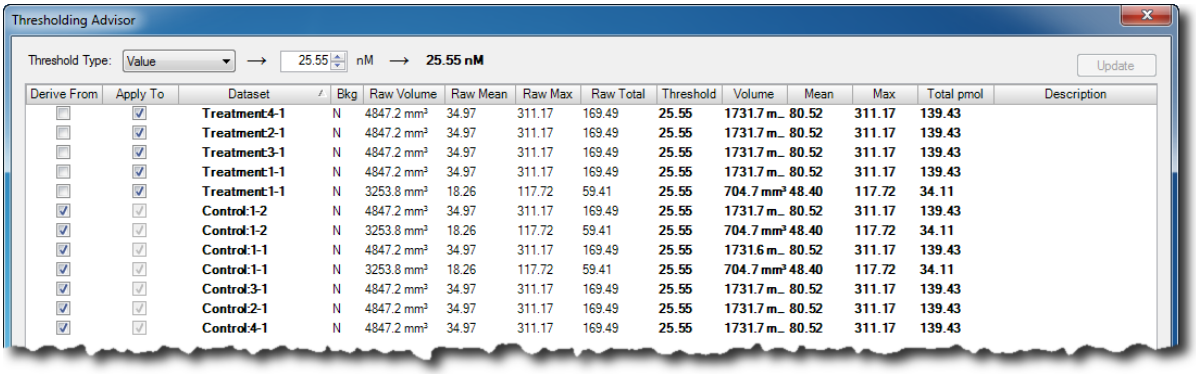
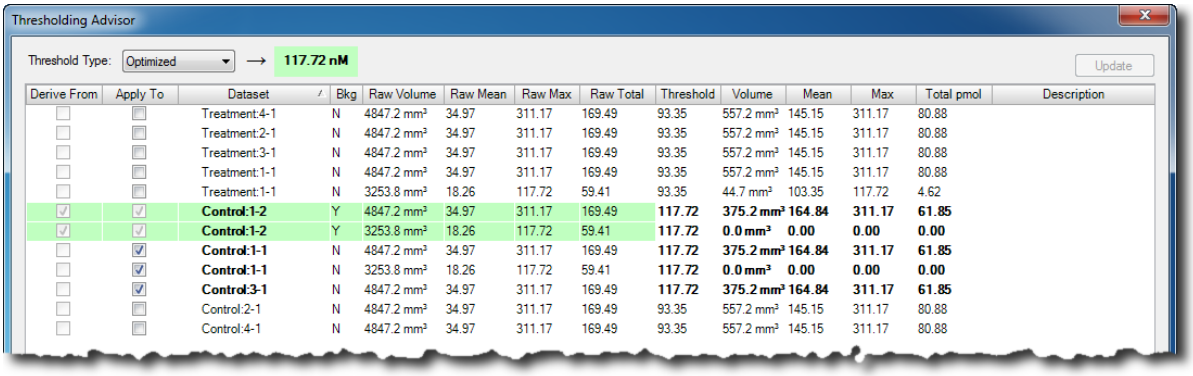


Figure 6-29. Value calculation in the **Thresholding Advisor**.

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 6-30.



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	93.35	557.2 mm³	145.15	311.17	80.88	
☐	☐	Treatment:2-1	N	4847.2 mm³	34.97	311.17	169.49	93.35	557.2 mm³	145.15	311.17	80.88	
☐	☐	Treatment:3-1	N	4847.2 mm³	34.97	311.17	169.49	93.35	557.2 mm³	145.15	311.17	80.88	
☐	☐	Treatment:1-1	N	4847.2 mm³	34.97	311.17	169.49	93.35	557.2 mm³	145.15	311.17	80.88	
☐	☐	Treatment:1-1	N	3253.8 mm³	18.26	117.72	59.41	93.35	44.7 mm³	103.35	117.72	4.62	
☑	☑	**Control:1-2**	Y	4847.2 mm³	34.97	311.17	169.49	**117.72**	**375.2 mm³**	**164.84**	**311.17**	**61.85**	
☑	☑	**Control:1-2**	Y	3253.8 mm³	18.26	117.72	59.41	**117.72**	**0.0 mm³**	**0.00**	**0.00**	**0.00**	
☐	☑	**Control:1-1**	N	4847.2 mm³	34.97	311.17	169.49	**117.72**	**375.2 mm³**	**164.84**	**311.17**	**61.85**	
☐	☑	**Control:1-1**	N	3253.8 mm³	18.26	117.72	59.41	**117.72**	**0.0 mm³**	**0.00**	**0.00**	**0.00**	
☐	☑	**Control:3-1**	N	4847.2 mm³	34.97	311.17	169.49	**117.72**	**375.2 mm³**	**164.84**	**311.17**	**61.85**	
☐	☐	Control:2-1	N	4847.2 mm³	34.97	311.17	169.49	93.35	557.2 mm³	145.15	311.17	80.88	
☐	☐	Control:4-1	N	4847.2 mm³	34.97	311.17	169.49	93.35	557.2 mm³	145.15	311.17	80.88	

Figure 6-30. 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.

6.7.2 Filter Controls

The **Derive From** and **Apply To** controls, at the bottom of the **Thresholding Advisor** window, provide a quick way for you 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.

6.7.2.1 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.

6.7.2.2 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**) as well as **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.

6.8 Creating Movies

You can create and export movies in the Windows Media (.wmv) or Audio Video Interleave (.avi) formats directly from the **Analysis** tab of TrueQuant. Note, MPEG file export, while supported in previous versions of TrueQuant, is no longer available.

The movie clips animate the contents of the viewport as defined by you, and can also include quantitative information from the ROI analysis as desired. These movie files can then be viewed with any standard media player and/or inserted into presentations.

From the **Analysis** tab, load one or more datasets and select **Tools** | **Create Movie** from the menu. This opens the **Create Movie** dialog (Figure 6-31), 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 clip, as explained below. Note, the **Create Movie** menu item is disabled if more than one panel is displayed in the **Analysis** tab.

Note that while the **Create Movie** dialog box is open, the user retains control over all display options to the right of the viewport. However, as long as this dialog box remains open, the **Load**/**Unload** dataset buttons, the buttons to create new ROIs, the advanced view options button and the ability to switch to a different tab are disabled. The show/hide icon for ROI clipping in the **Analysis** tab is saved on a keyframe-by-keyframe basis when creating movies.

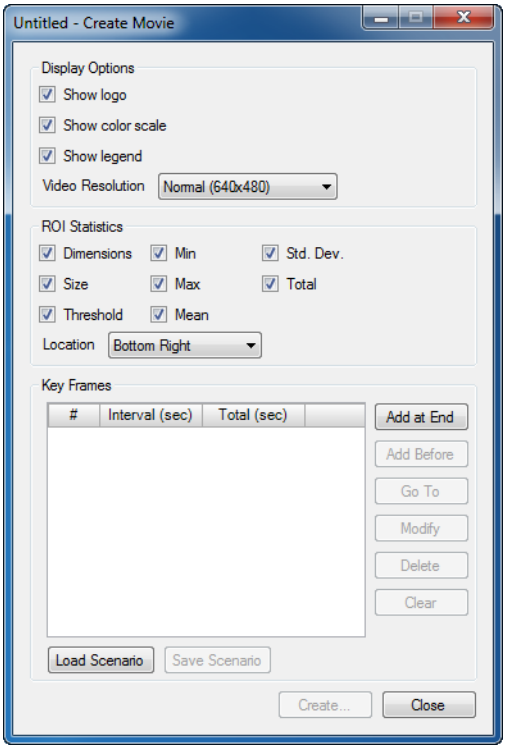


Figure 6-31. The **Create Movie** dialog.

The **Video Resolution** control offers the following options: **Large (1280x960)**, **Normal (640x480)**, or **Small (320x240)** with **Normal (640x480)** being the default resolution. The various options available in the **Display Options** and **ROI Statistics** regions of the dialog control the contents and location of the text and color scale that are superimposed on the movie frames:

- The color scale will be taken from either the 2D or 3D scale, whichever is the active one, with appropriate units. The upper and lower limits of the scale will match their respective counterparts below the viewport. Below the units will be displayed the short name of the dataset (Group name, Subject number, scan number).

- The legend will display the name of the imaging agent(s) used in each of the animated datasets.

- ROI statistics, if applicable, will be overlaid on the movie frames. These are dynamic, in the sense that a change to an ROI during the movie (say a resizing of the ROI) will be mirrored by a change in the numerical information being displayed synchronously. Each ROI statistical quantity will be displayed with the same precision and units as is used in the results table below the viewport. The ROI's description field will be used to label the ROI statistics in the movie clip. Any ROI that is visible in the viewport, i.e. checked in the ROI data table, will have its statistics overlaid on the movie frames.

All the ROI statistics are selected by default, and can be included or excluded as desired. Note that if none of the loaded datasets have any ROIs when attempting to use the **Create Movie** dialog, all the optional settings are disabled by default.

To begin a movie sequence, create a desired starting view using the appropriate controls, then add a key frame by clicking **Add at End**. Create the next view and add another key frame in the same fashion. Once you add the first key frame, the **Add Before** button becomes available to allow you to insert a new key frame before the currently-selected key frame in the **Key frames** list.

Continue until your set of key frames is complete. To review the state at a key frame, select the key frame and click the **Go To** button. To modify the state of a key frame, set the **Analysis** tab to the desired state, then select the key frame and click the **Modify** button. The time between a given key frame and the previous key frame is given by its **Interval** value. You can change this by selecting the key frame, clicking the interval value, and editing the value. If desired, you can delete an individual key frame, and automatically recalculate the camera path, by selecting it and pressing **Delete**. To delete all the key frames in the list, press **Clear** and accept the confirmation presented by the software.

Key frames can include changes to all display controls (Reflectance Image, Isosurface, Volume Rendering, Slices, Color Scales) as well as changes to ROIs. Most commonly, users will pan/zoom/rotate around the 3D reconstruction while creating the movie animation (Figure 6-32). The software will interpolate between successive key frames and create intermediate frames at the rate of 15 frames/second (fps), with the camera path interpolated along a 3D spline. It is recommended to keep rotations between consecutive key frames to less than 90º in order to maintain a well-defined camera path in the final movie.

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 will be 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 pre-existing key frames.

When your key frame list is complete, click **Create** to create a movie file. The intermediate frames between the key frames are automatically interpolated when the movie file is created. Boolean properties, such as **Show isosurface**, will 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. Furthermore, ROI wireframes display in the movie exactly as they appear in the **Analysis** tab. At any time during creation of the output file, you can interrupt the generation of the movie by pressing **Stop** and accepting the resulting confirmation.

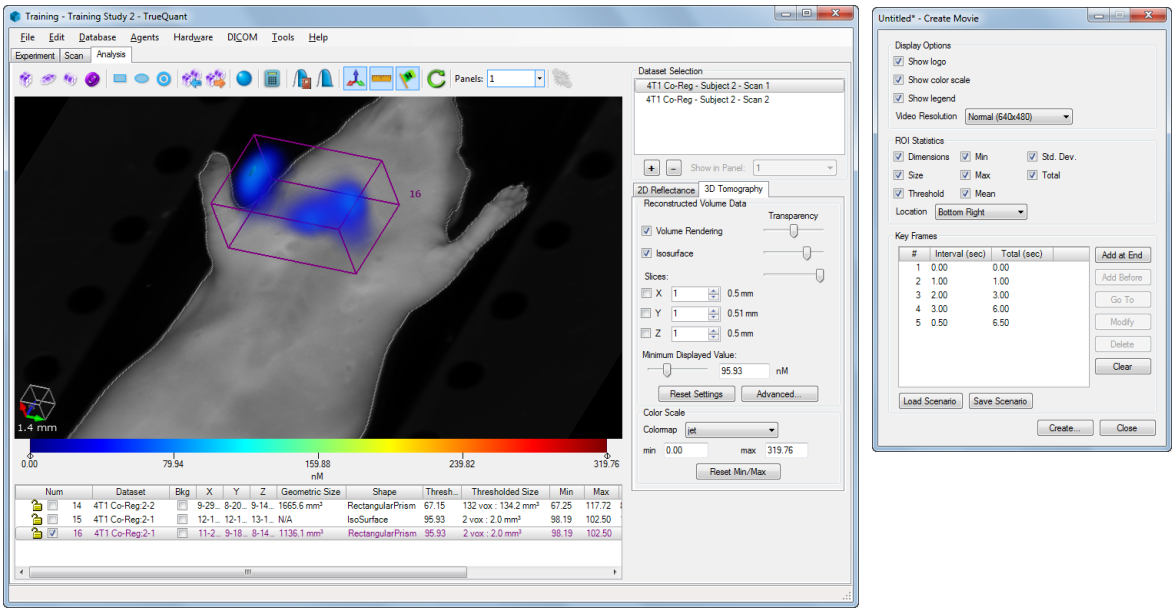


Figure 6-32. Adding key frames to a movie clip while manipulating the viewport.

To cancel creation of a movie, press the **Close** button at any time without saving, which will close the **Create Movie** dialog.

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.

6.9 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 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 to appear as they do in the **Analysis** tab. Figure 6-33 provides an example of the **Image Export Settings** dialog.

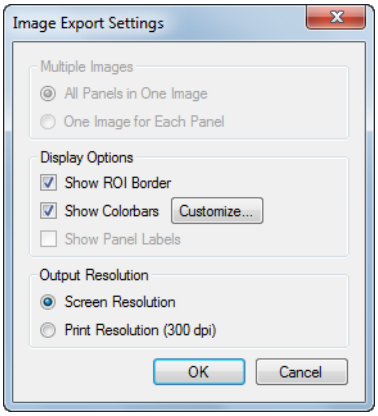


Figure 6-33. Options for image export.

Clicking **Customize** on the **Image Export Settings** window opens the **Customize Color Scales** dialog, shown in Figure 6-34.

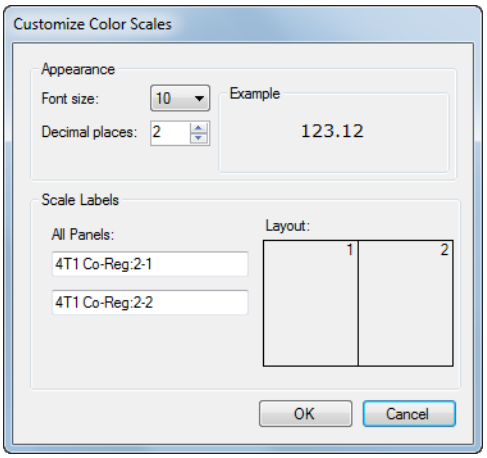


Figure 6-34. Customizing the labels that are printed with the color scales. This example shows an export of one image for each panel with two panels being displayed in the **Analysis** tab.

This allows you to customize the size of the label font as well as the number of decimal places displayed. Finally, the color scales in the images can be labeled with user-defined names to enhance data reporting functionality. The labels default to the names of the datasets corresponding to the color scales. Each panel's customized label is displayed above the custom color scale labels associated with that panel.

NOTE The modifications you make on the **Customize Color Scales** dialog are saved in your Windows user profile. As such, your changes are included in all future image exports you perform.

The exported image will match the image display in the **Analysis** tab exactly, including the coordinate axes and any ROIs that are visible. In addition, it will include 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.

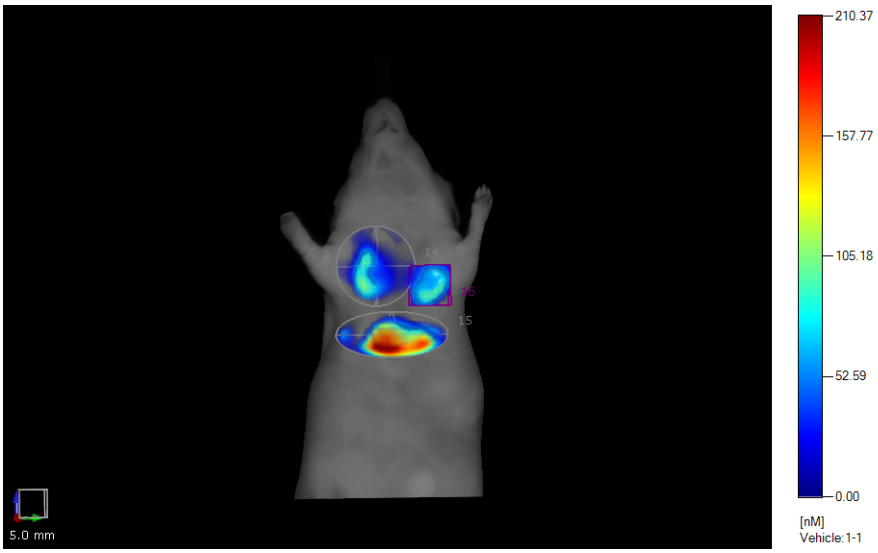


Figure 6-35. Sample exported image with multiple ROIs.

6.10 Saving and Loading Analysis Tab Settings

It may sometimes be desirable, for example when analyzing imaging data within a large study, to consistently apply the same display settings across the entire study. TrueQuant provides a simple 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 will save 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 control. As such, this menu item will be disabled if no datasets are selected or if more than one is selected.

NOTE The selected option for ROI clipping is not saved when saving **Analysis** tab settings.

The button that enables and disables ROI clipping in the **Analysis** tab is not included in the settings that are saved when using the save analysis settings functionality.

To then apply these display settings to subsequent datasets, load the dataset or datasets to which you wish to apply the settings and select those datasets in the dataset selection control, then select **Tools** | **Analysis Settings** | **Load** from the menu and select the desired settings file (Figure 6-36).

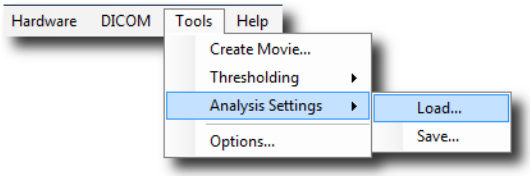


Figure 6-36. Saving and loading the **Analysis** tab settings.

6.11 User Preferences

TrueQuant offers the possibility of overriding a number of default settings with user preferences. These are accessed by selecting the **Tools** | **Options** menu item, which brings up a window with tabs for several **Scan**, **Analysis**, and **DICOM** settings (Figure 6-18).

Note that these preference settings do not affect the data, but only the way the data is displayed automatically when the application is launched. It is also possible to change these settings individually and interactively during a session.

The Scan Preferences allow the user to override the choice of colormap displayed during a scan. Changes to the scan colormap will apply after the software is restarted. In addition, Preview reconstructions, which are lower resolution uncalibrated reconstructions, can be disabled. Finally, for users of the Multi-Species Imaging Module, the default animal orientation within the MSIM cassette can be set here. These last two preferences take effect the next time a scan is performed in the **Scan** tab, without requiring you to restart TrueQuant.

The Analysis preferences allow you to apply your own conventions to displaying data and images in the **Analysis** tab. Some of these preferences will apply the next time a dataset is loaded, while others will apply only after you restart TrueQuant. This is indicated above the preferences themselves.

The DICOM preferences set the bit depth at which DICOM series are exported. See *section 4.6* for further details.

7

Imaging Agents and Agent Calibration

The FMT system requires a calibration scan for each imaging agent used in conjunction with the system in order 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 as well as other commercial agents. It is also recommended, although not required, to initiate an agent calibration at the onset of every major study.

7.1 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 7.3*.

From TrueQuant's main menu, select **Agents** | **Calibration** to bring up the automated agent calibration window (Figure 7-1).

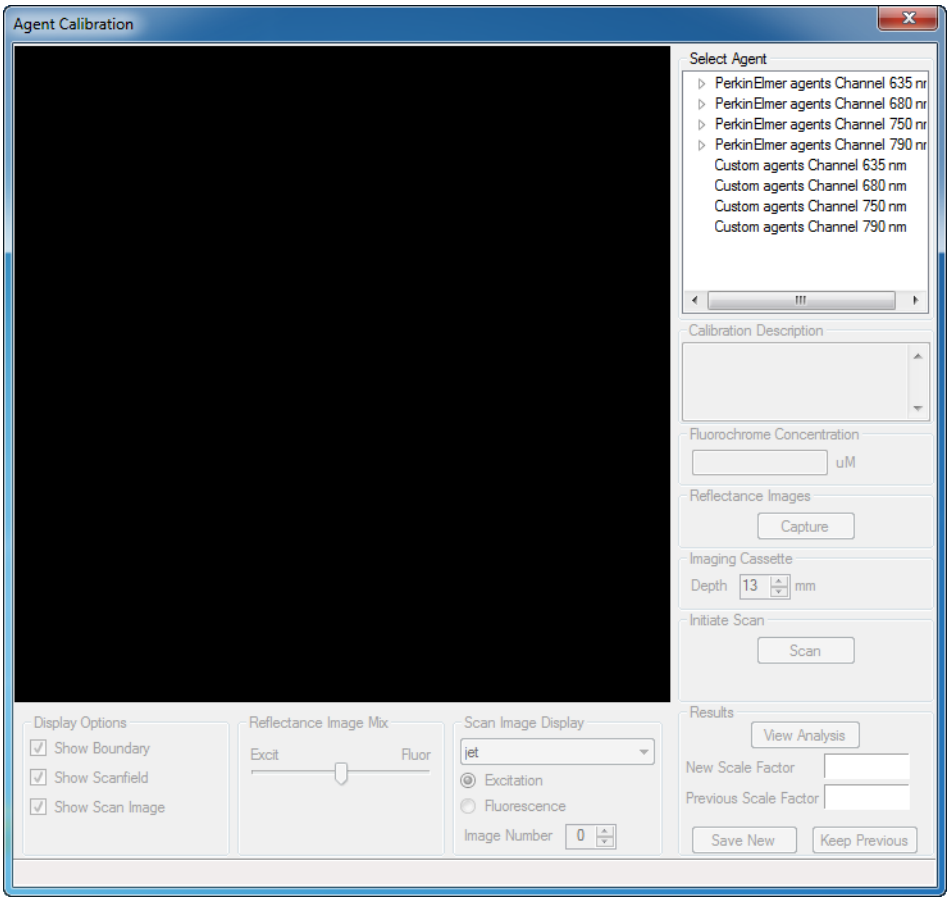


Figure 7-1. The agent calibration window.

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 7-2). 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.

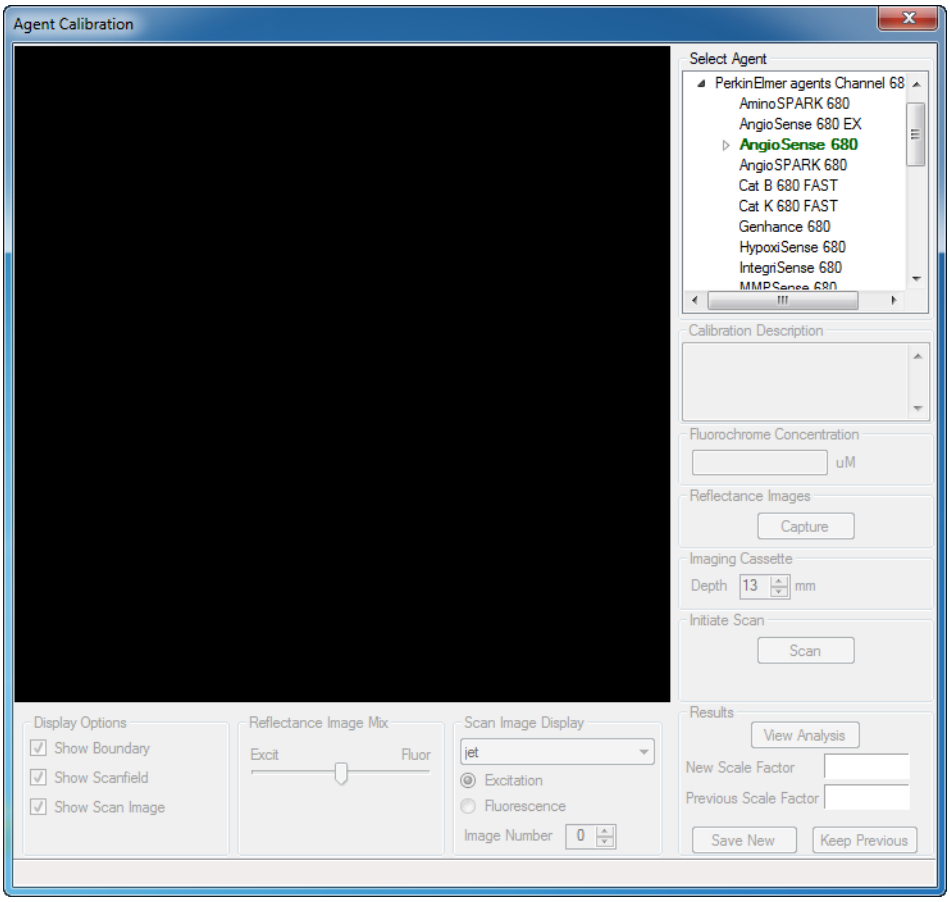


Figure 7-2. Listing of calibrated (green) versus uncalibrated (black) agents.

The steps to calibrate an agent are as follows:

1. Select an agent from the list and enter the 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 7-3).

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.

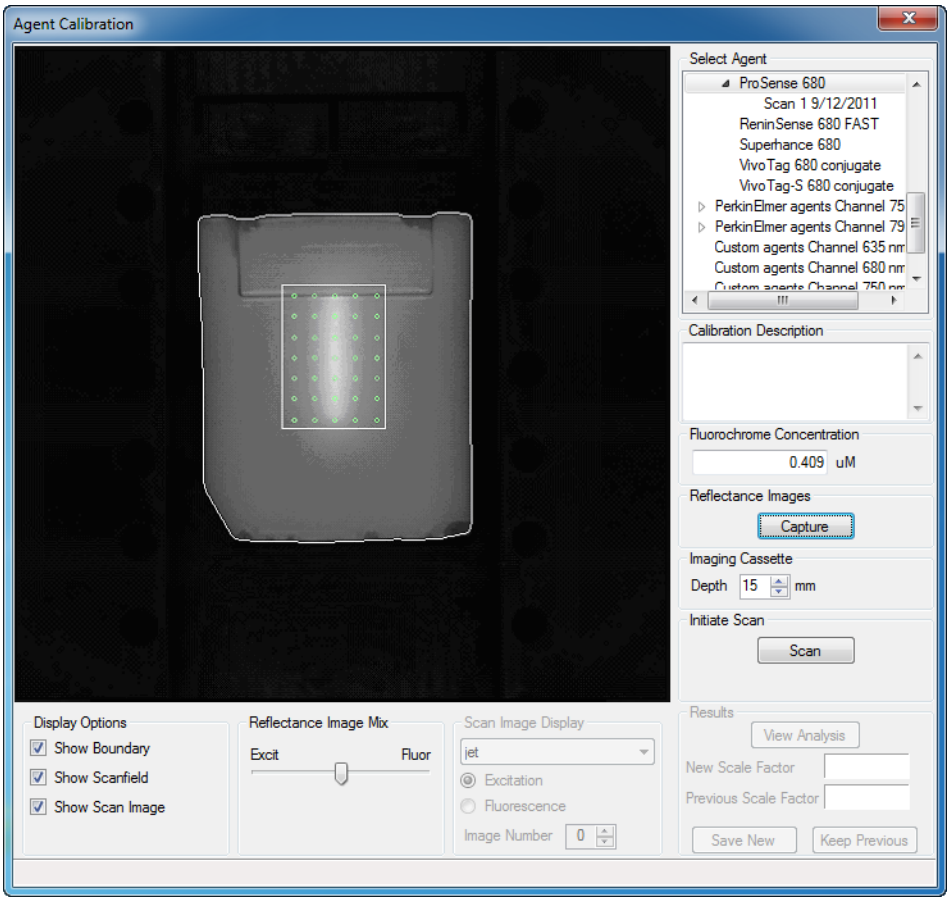


Figure 7-3. Reflectance image capture of the calibration phantom.

Once the scan is completed, it is recommended (although not strictly necessary) to view the results of tomographic reconstruction and analysis by pressing **View Analysis** (Figure 7-4). 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 7-5). Press **Save New** underneath to save the calibration results.

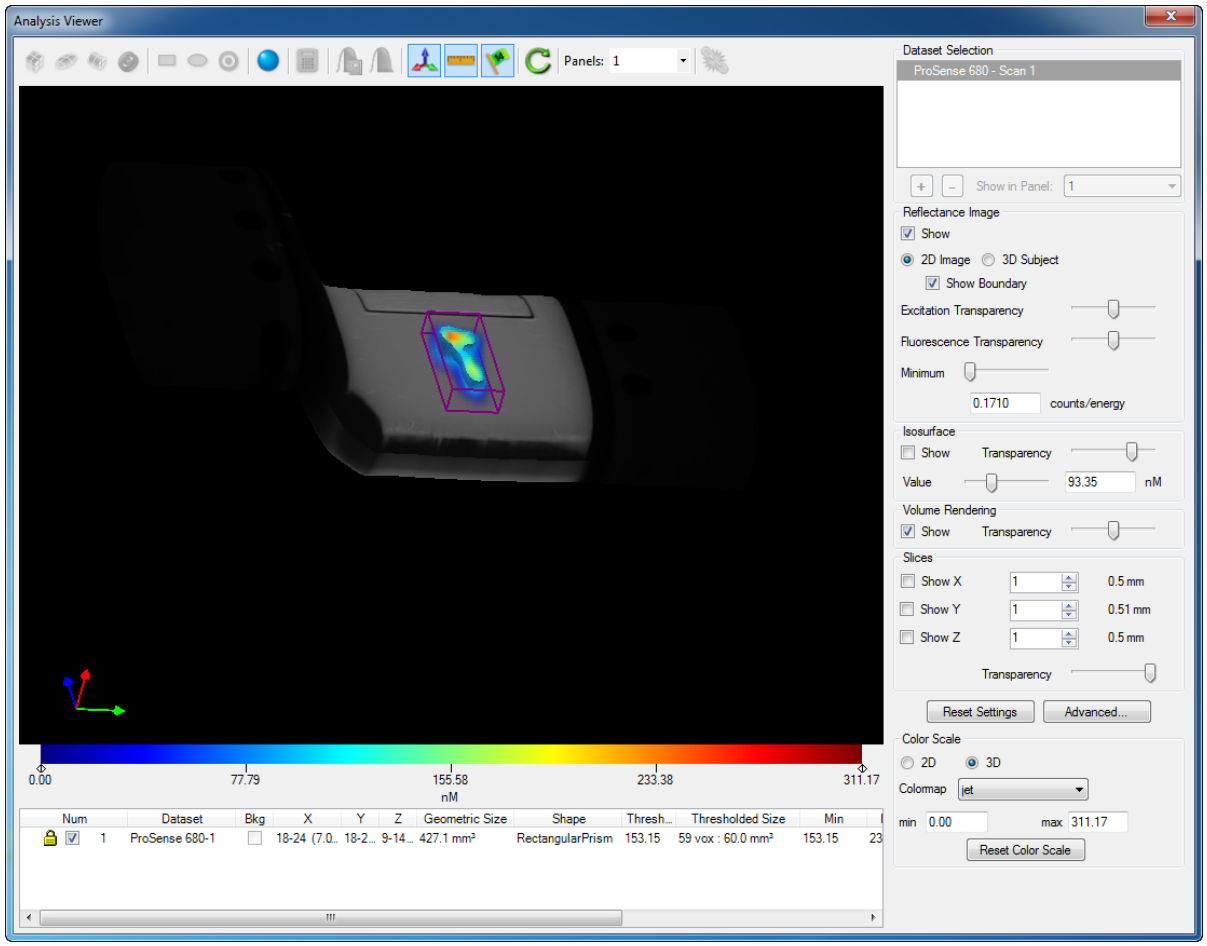


Figure 7-4. Analysis of the calibration scan.

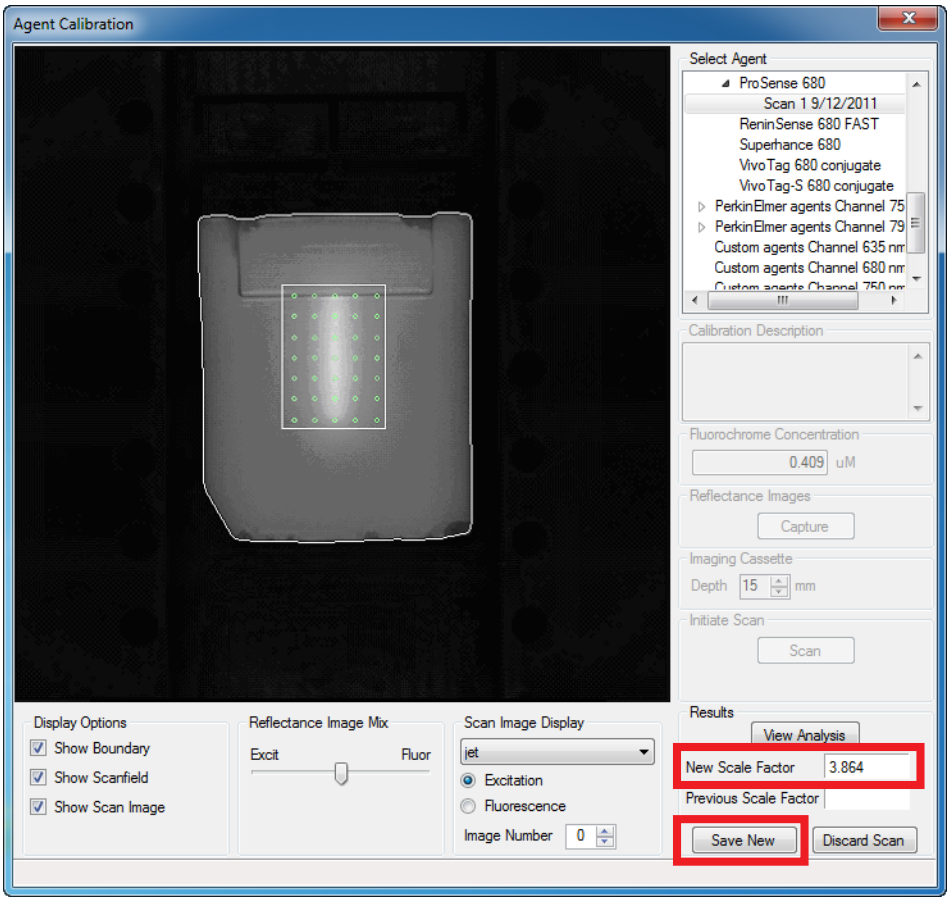


Figure 7-5. Computing and saving the scale factor.

7.2 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 brings up the **Manage Agents** window (Figure 7-6). 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.

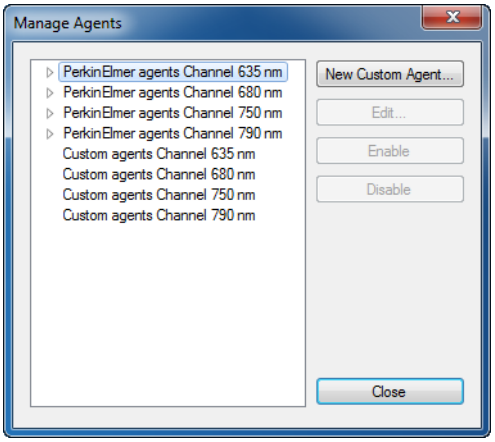


Figure 7-6. The **Manage Agents** window.

To create a user-defined agent, press the **New Custom Agent** button, which brings up the **Add New Custom Agent** window (Figure 7-7). 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 will add 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, it will be necessary to calibrate the agent using the procedure outlined in *section 7.1*.

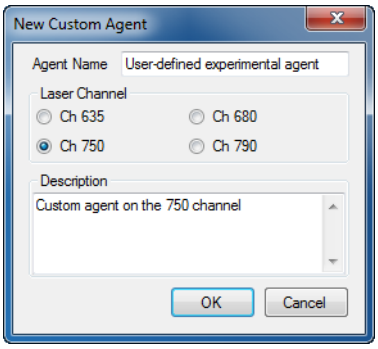


Figure 7-7. Adding a new custom agent.

Now that the new agent has been created, it appears in the list in the **Manage Agents** window (Figure 7-8).

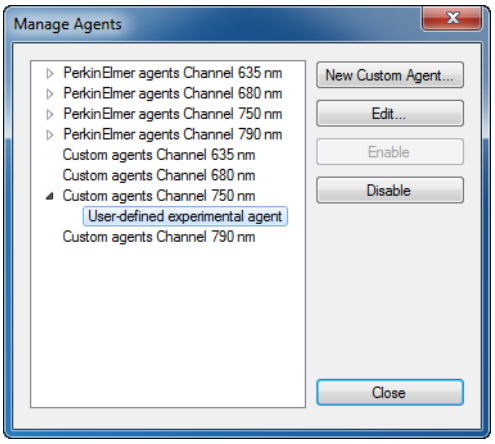


Figure 7-8. The **Manage Agents** window displaying the newly-created agent.

Once a custom agent has been created, its name and description can be edited by selecting the agent and pressing **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.

7.3 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 7.2*. 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.

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

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.

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

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.

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

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*)

---

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 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 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 C, determine the concentration of Solution C (C_C) with the following equation.

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

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.

7.4 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 7-9). 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.

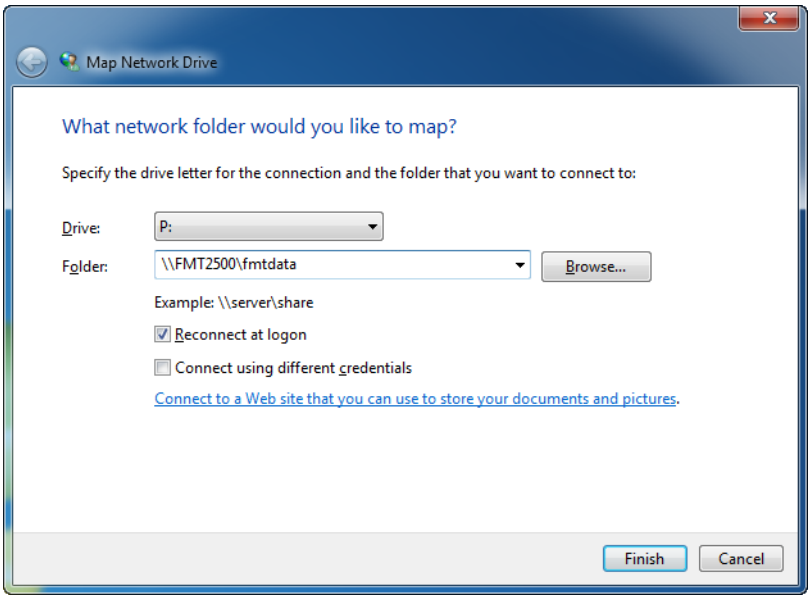


Figure 7-9. Mapping the master agent list to a drive letter in Windows.

8

Database Management and Backups

8.1 Database Management

Creating and deleting databases, as well as subscribing to and unsubscribing from remote databases is all done within TrueQuant. To access any of these functions, select **Database** | **Manage** from the application menu to open the **Manage Databases** window. This window can also be used to move studies from one database to another.

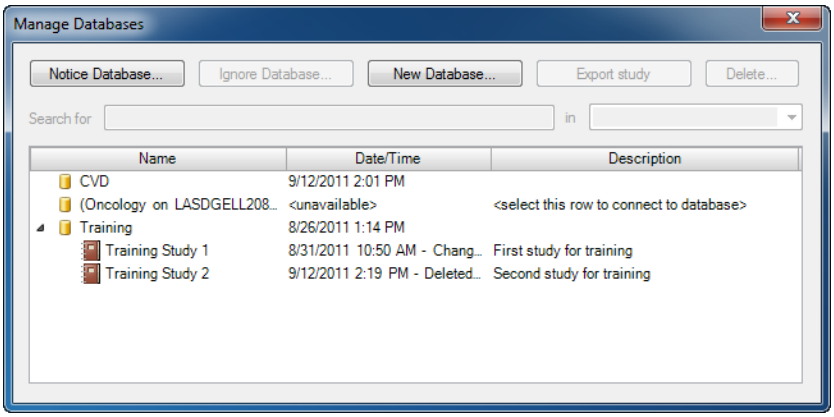


Figure 8-1. The **Manage Databases** window.

8.1.1 Creating Databases

To create a new database, click **New Database**. This opens the **Create Database** window. Either use the list to select the name of the database server on which to create the new database or type the name of the server into the text field. Enter the name of the database you wish to create, and click **Create**.

A new window will open to let you select the security group that will have access to 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 will be able to view and edit this new database. Click **OK**, to create the new database. To give all users access to a database, select the `Users` group. Refer to the *TrueQuant IT Setup Guide* for details.

The process of creating the new database can take up to several minutes. Once the database is created, it will be listed in the **Manage Databases** window and will become the active database in the **Experiment** tab.

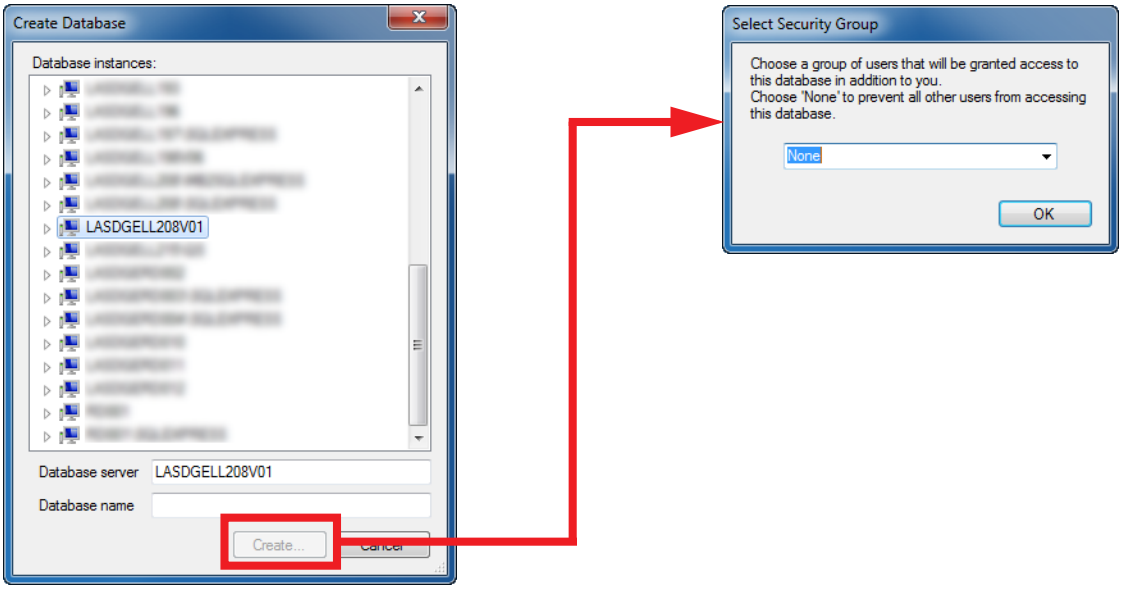


Figure 8-2. The **Create Database** window.

8.1.2 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.

8.1.3 Noticing an Existing Database

There are several circumstances when a database that you have access to will already exist, but you will not yet be 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 will not immediately be 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 will not be 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.

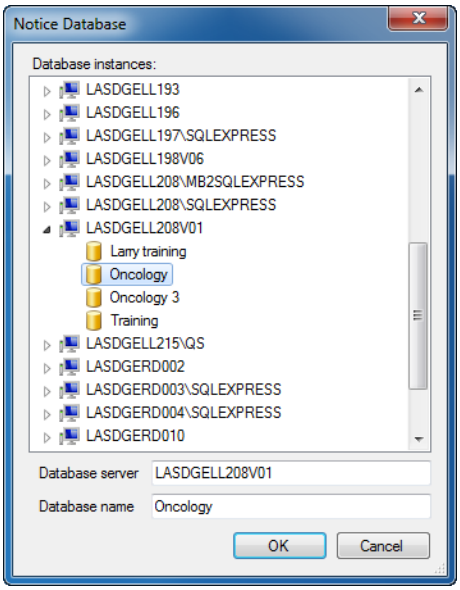


Figure 8-3. The **Notice Database** window.

If a database has not been used in the last 14 days, your computer will not be able to 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 8.1.4*, then re-notice the database.

8.1.4 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 will then prompt you to confirm that you wish to ignore the selected database.

8.1.5 Exporting studies

To export an entire study, select **Database** | **Manage** from the application menu to open the **Manage Databases** window (see Figure 8-4). 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 8-5). Select the top level folder of the study to be imported—the folder that contains the XML files and Scans and Recons folders.

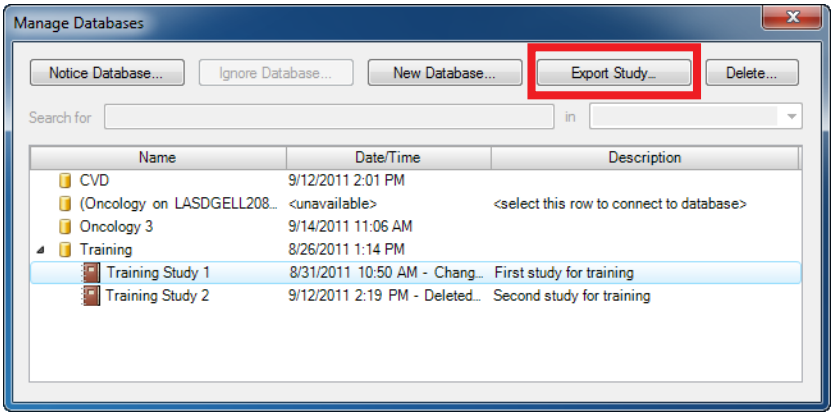


Figure 8-4. Exporting an entire study.

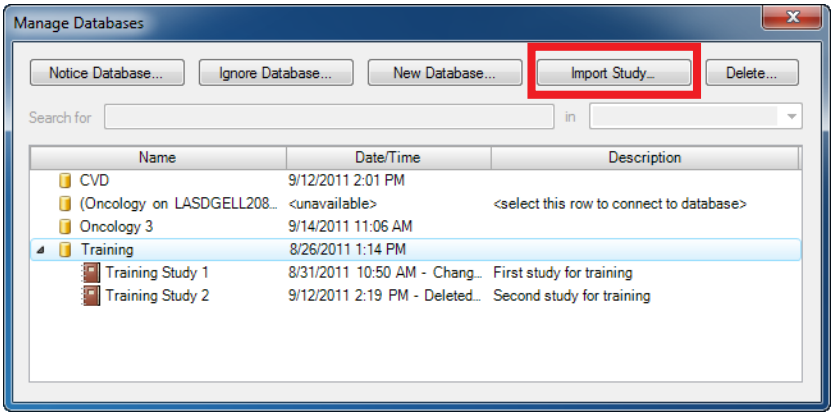


Figure 8-5. Importing a study into a database.

8.2 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 as well as on individual investigator PCs. On these machines, each database will be 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.

8.3 Backups

The FMT system host computer is configured with SQL Server 2005 as well as SQL Server Management Studio.

To backup databases, the following steps should be implemented:

1. Open and start **SQL Server Management Studio** from the **Windows Start** | **All Programs** | **Microsoft SQL Server 2008 R2** 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 8-6).

4. Select **Full** for the **Backup type** option. Under **Destination**, click **Add** (Figure 8-7)

5. Enter the path and file name for the backup file, and click **OK** (Figure 8-8)

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

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

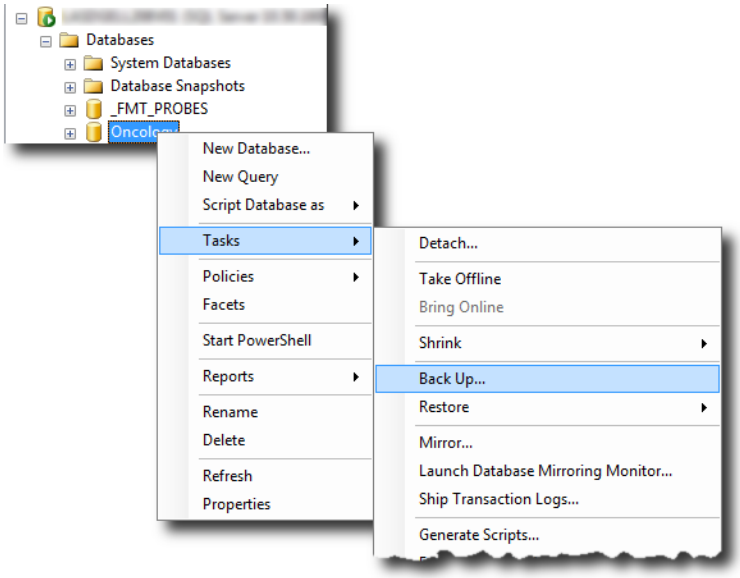


Figure 8-6. Backing up databases from SQL Server Management Studio.

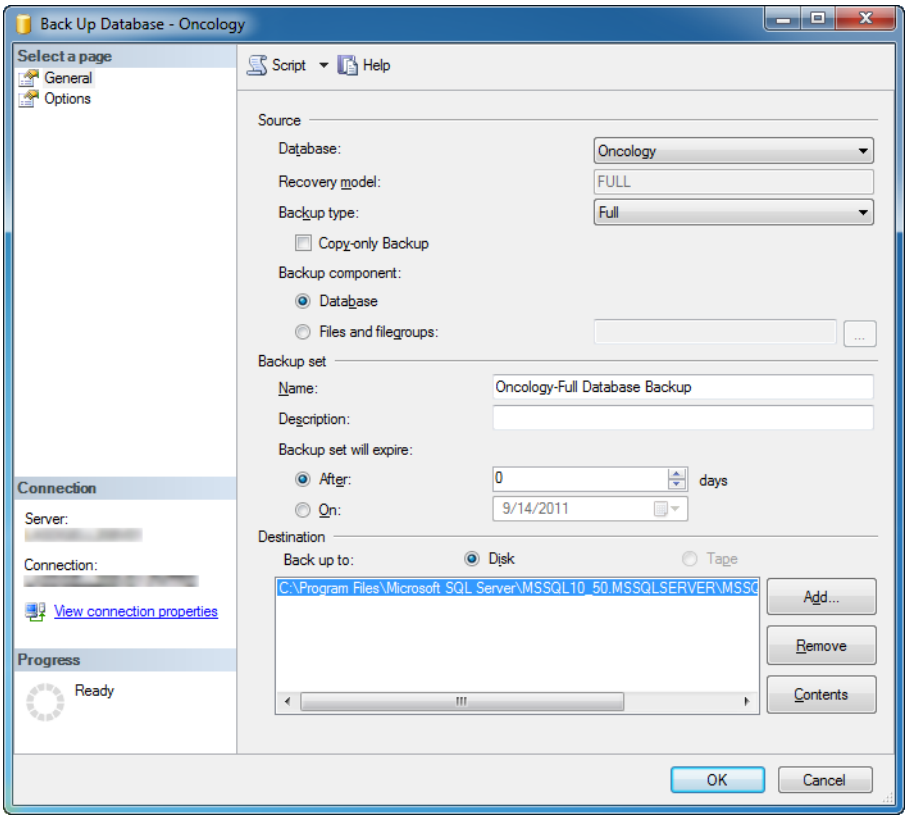


Figure 8-7. Selecting **Full** backup type, and clicking **Add**

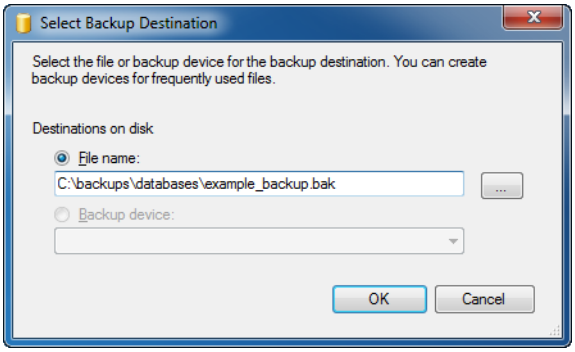


Figure 8-8. Entering a path and file name for the backup file.

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 8-9). 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.

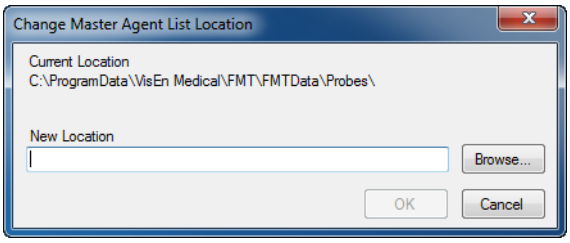


Figure 8-9. Determining the location of the master agent list.

8.4 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 8-10).

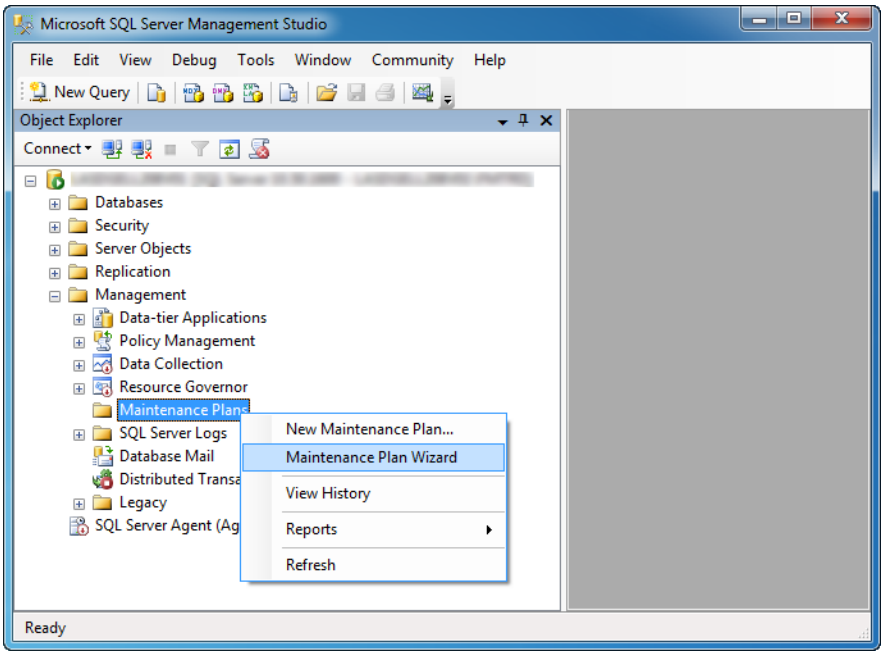


Figure 8-10. Starting the **Maintenance Plan Wizard**.

After the wizard's splash screen, give your backup plan a name, and press the Change button to schedule when the backups will occur. It is recommended that you schedule your backups daily for a time when no one will be using the machine, such as 1:00 AM. A sample plan schedule is shown in Figure 8-11. 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.

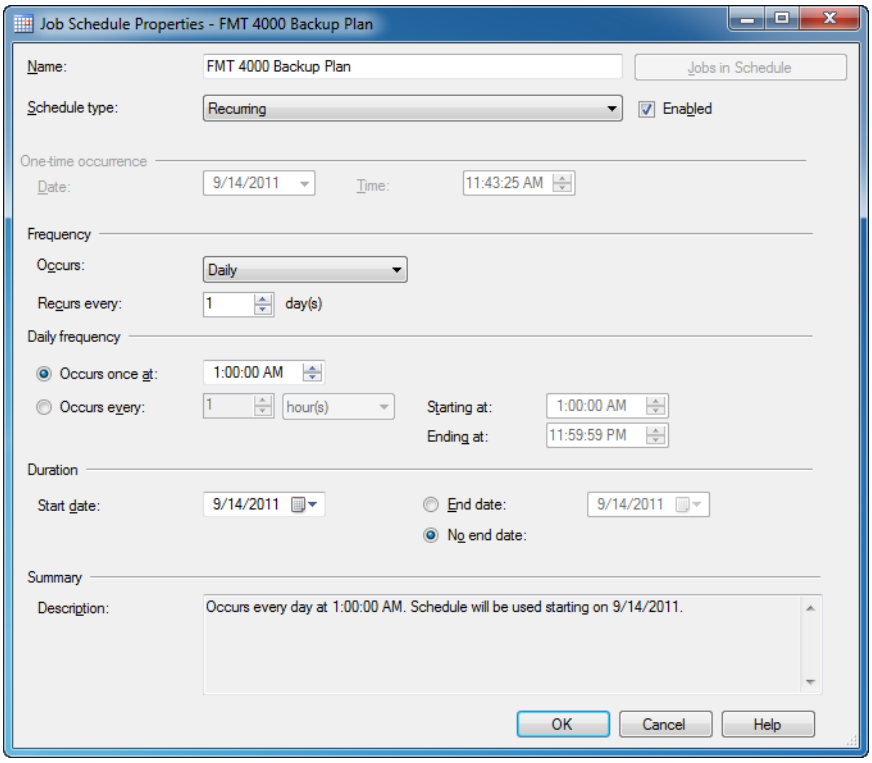


Figure 8-11. A sample maintenance plan schedule.

Next, select the maintenance tasks this plan will perform. For completeness, select all three backup types: **Full**, **Differential**, and **Transaction Log**. The wizard will automatically set these up to execute in the order given above.

The next step is to select which databases to back up for each backup type. So that newly created databases will automatically get 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 will need to set the schedule for this backup task. Repeat this setup process for each of the three backup types. A sample configuration is shown in Figure 8-12.

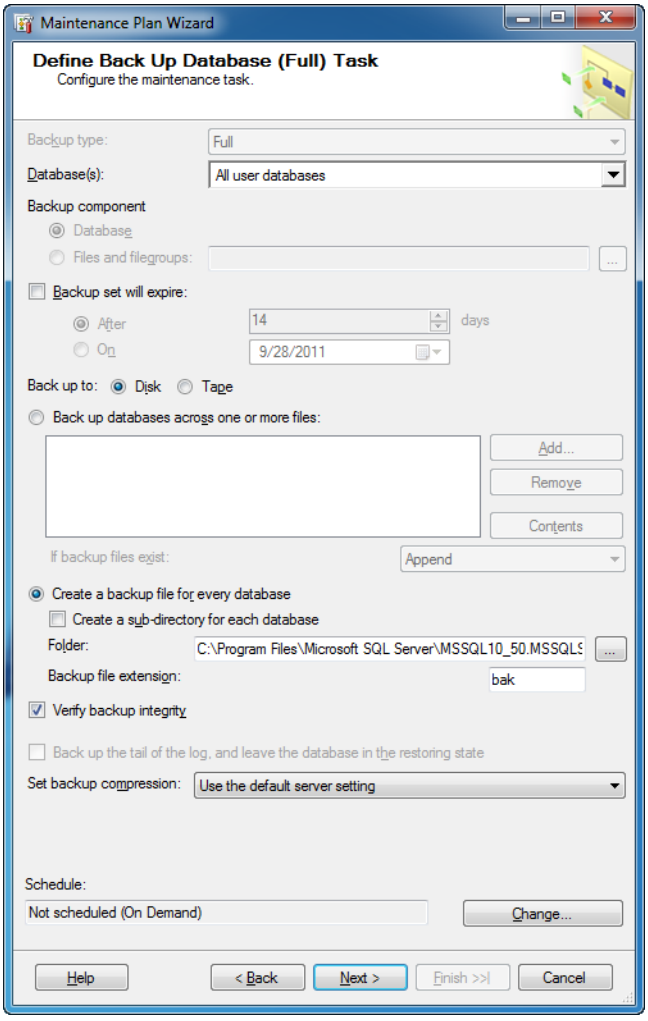


Figure 8-12. Configure the databases to be backed up.

By default, the wizard will configure the backups to write a report to a text file so you can verify that each backup completed successfully. Finally, it will display 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, press **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.

9

Installation and Operation of TrueQuant on Remote Computers

9.1 Software Installation on Additional Machines

TrueQuant can be run on additional computers besides the Host PC. For proper operation of TrueQuant on client computers, please verify the following minimum system requirements:

- Processor: dual-core processor
- Memory: 4 GB
- Hard drive: 50 GB of free space
- Graphics: Full support for OpenGL 2.1, including shaders, on a stand-alone, gaming-quality video card. 256MB video RAM recommended.
- Network connection to the host PC: Gigabit or faster
- Operating System: Windows XP (x86 only) or Windows 7 (x86 or x64)
- DVD drive for installation disc

Please contact PerkinElmer for installation and proper configuration of TrueQuant on machines other than the FMT system Host PC.

9.2 On-line/Off-line modes

For users with laptops, TrueQuant provides the ability to take studies or even 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 will be uploaded to the remote database. At the same time, any changes made on that remote database by other users will be downloaded to the local computer.

After this point, the study will be back "online" and you will 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 will not be able to be 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 9-1), allowing you to select the studies you want to take offline. Select the checkboxes next to the individual studies you want to have accessible when offline and click **OK**. To bring previously offline studies back online, simply deselect the checkboxes 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.

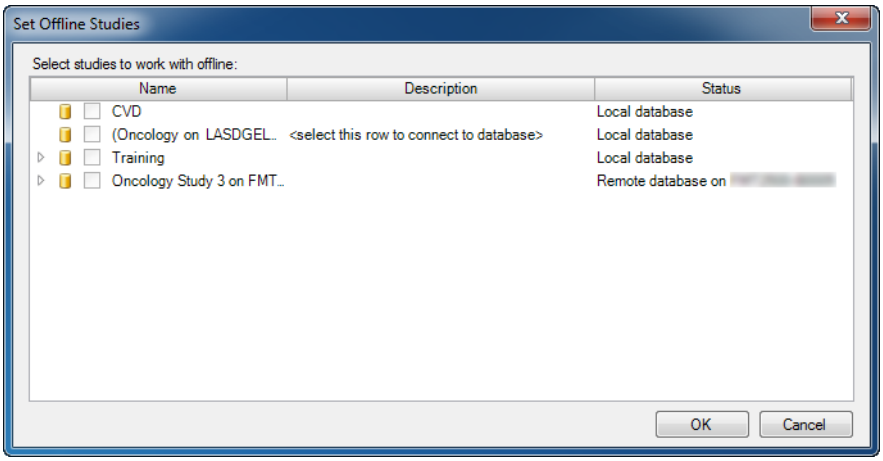


Figure 9-1. The **Set Offline Studies** window.

10

Guidelines and Troubleshooting Tips

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

10.1 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** dialog, shown in Figure 10-1.

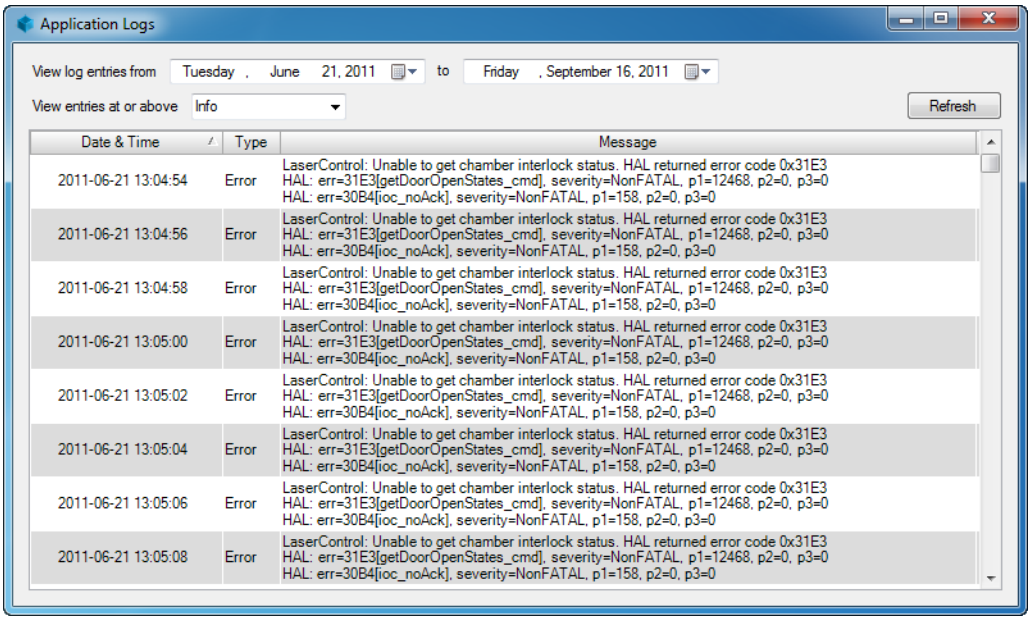


Figure 10-1. TrueQuant Log Viewer dialog.

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, and so on.) 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.

10.2 Troubleshooting Tips

The following is a list of common questions and solutions:

10.2.1 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 your computer's 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 your computer as well as the memory on your graphics card will allow you to have more datasets loaded at once.

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 your computer's video driver, go to your computer manufacturer's web site to download and install the latest video driver for your specific computer model. Many computer manufacturers' web sites will let you enter a serial number or service tag for your computer to find the appropriate drivers. You should make sure you quit all running applications before updating the driver, as a reboot is typically required after installing any Windows driver.

10.2.2 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.

10.2.3 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, do the following:

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 will be taken at the end of that 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.

10.2.4 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, it will only function 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.

11 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. The rest of this section discusses the use of the built-in diagnostics and calibration menus.

11.1 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.

11.2 System Diagnostics

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

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

These diagnostic dialogs allow qualified service personnel to check and set the status of various hardware elements. Please contact PerkinElmer for further technical assistance with the **System Diagnostics** feature.

12

Regulatory Information

12.1 Regulatory Information

12.1.1 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

12.1.2 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

13

Technical Services and Support

13.1 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

13.2 Repackaging the VisEn 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.