10 Working With Volumetric Data

10.1 About the 3D Multi-Modality Tools

Use the 3D Multi-Modality tools to:

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

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

Requirements

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

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

Table 10.1 Minimum Graphics Card Specifications

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

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

10.2 Classifying 3D Volumetric Data

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

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

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

Figure 10.1 Histogram Tool Specifies Opacity for Different Voxel Intensities
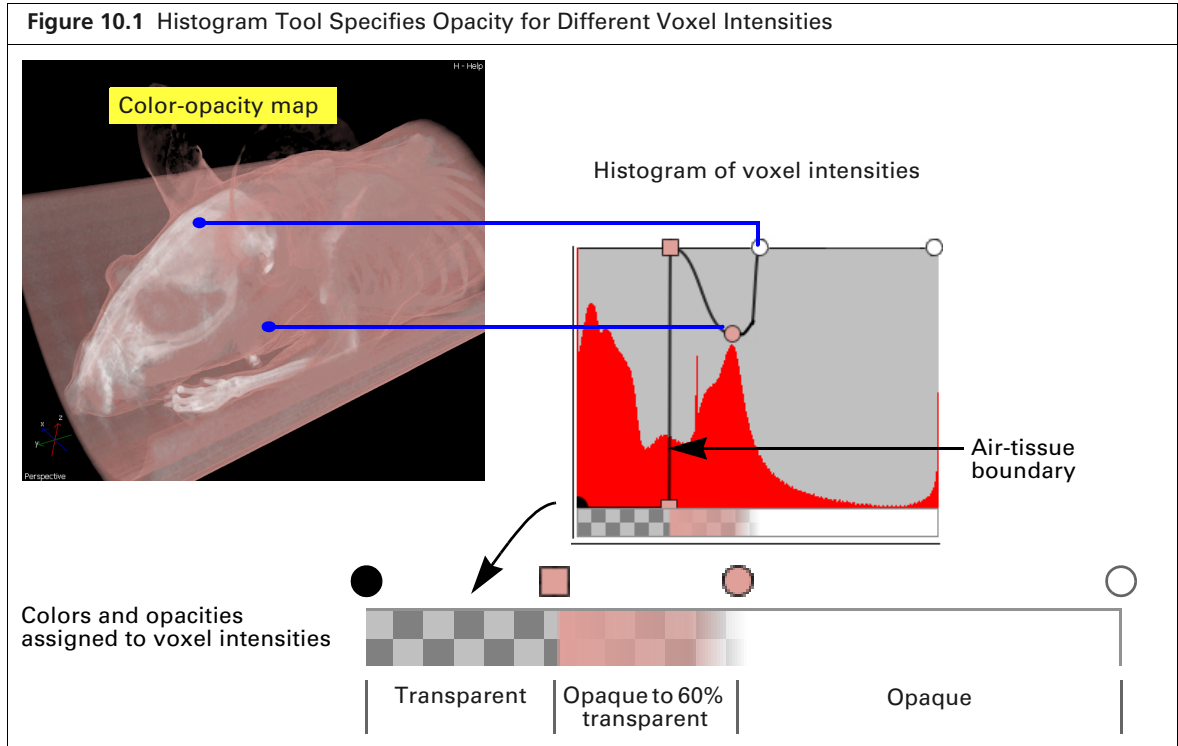
Specifying a Color-Opacity Map

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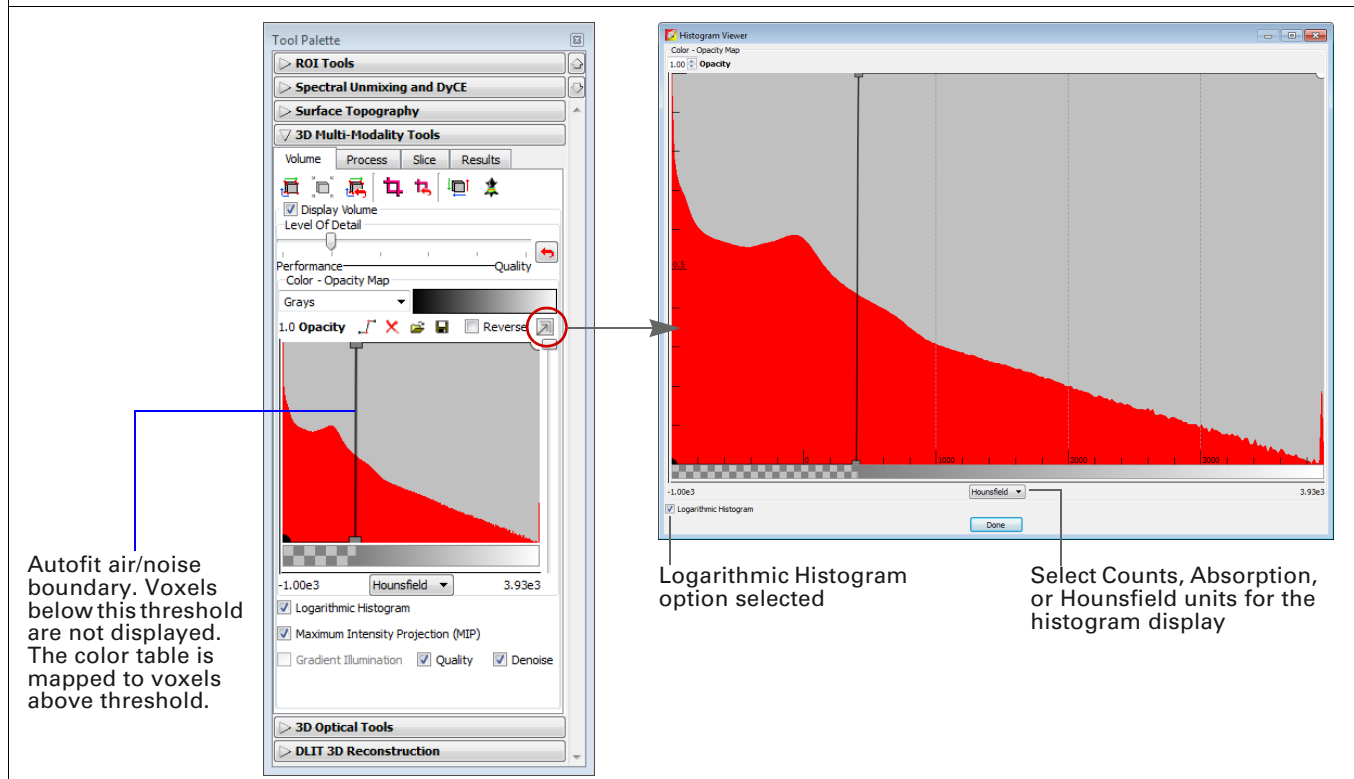
Histogram Display Options

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

Figure 10.2 3D Multi-Modality Tools

Autofit air/noise boundary. Voxels below this threshold are not displayed. The color table is mapped to voxels above threshold.

Logarithmic Histogram option selected

Select Counts, Absorption, or Hounsfield units for the histogram display
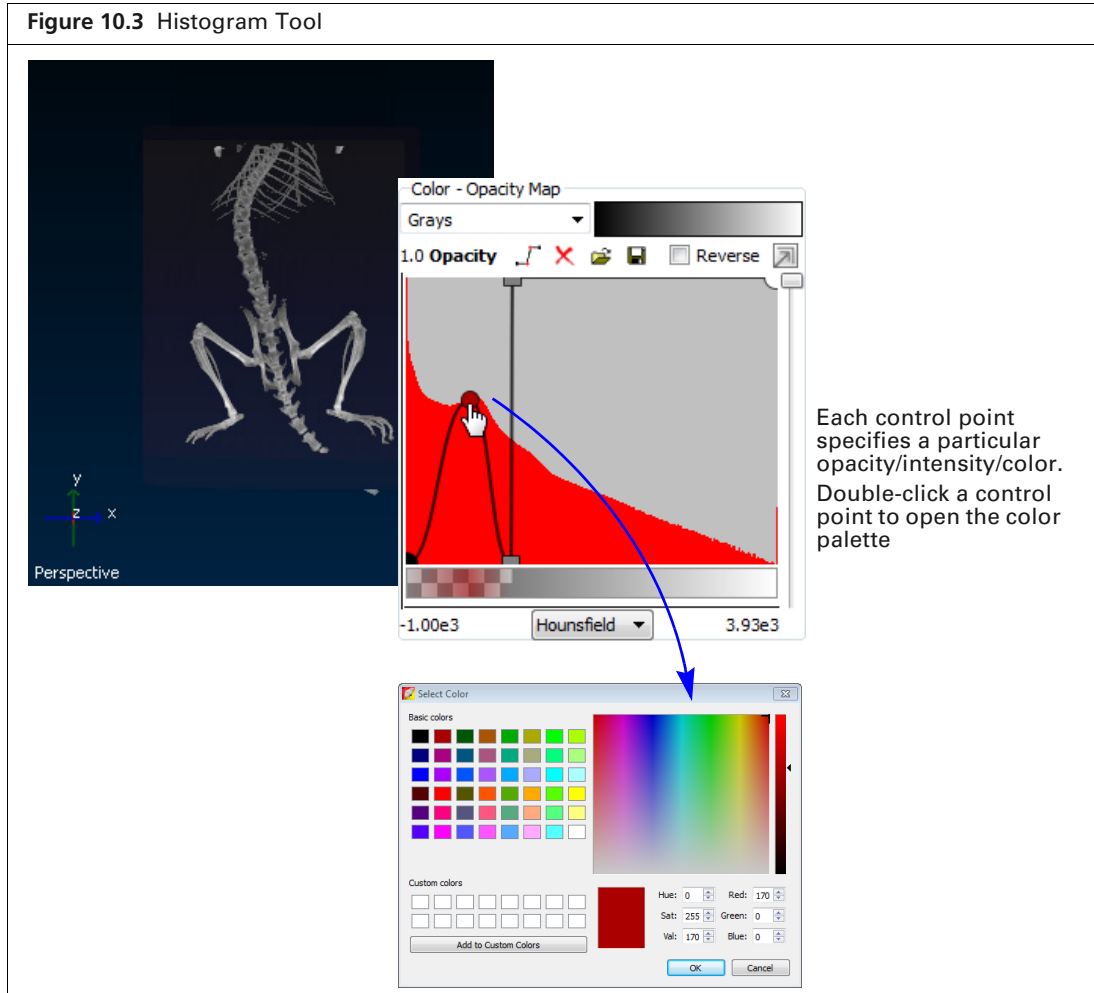
Managing Control Points

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

1. Place a control point on the histogram by clicking anywhere on the histogram between the ● point (represents the lowest intensity in the volume) and ○ point (represents the highest intensity in the volume).

2. Drag any control point up or down to set the opacity level that is associated with the intensity value represented by the point. Drag a user-added control point left or right to change the intensity associated with the opacity specified by the point.

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

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

Figure 10.3 Histogram Tool

Each control point specifies a particular opacity/intensity/color.
Double-click a control point to open the color palette

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

4. To delete a control point, right-click the point. To delete all control points, click the ✖ button.

> **NOTE:** The ● and ○ control points cannot be deleted from the histogram.

Saving a Color-Opacity Map

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

1. Click the Save button 🖫 (Figure 10.4).

2. In the dialog box that appears, select a folder for the file (.tfn) and enter a file name.

3. Click **Save**.

Loading a Color-Opacity Map

1. Click the Open button 🗁 (Figure 10.4).

2. In the dialog box that appears, navigate to the map file (.tfn), and click **Open**.

Figure 10.4 Save or Load a Color-Opacity Map

Click a button to save or load a color-opacity map
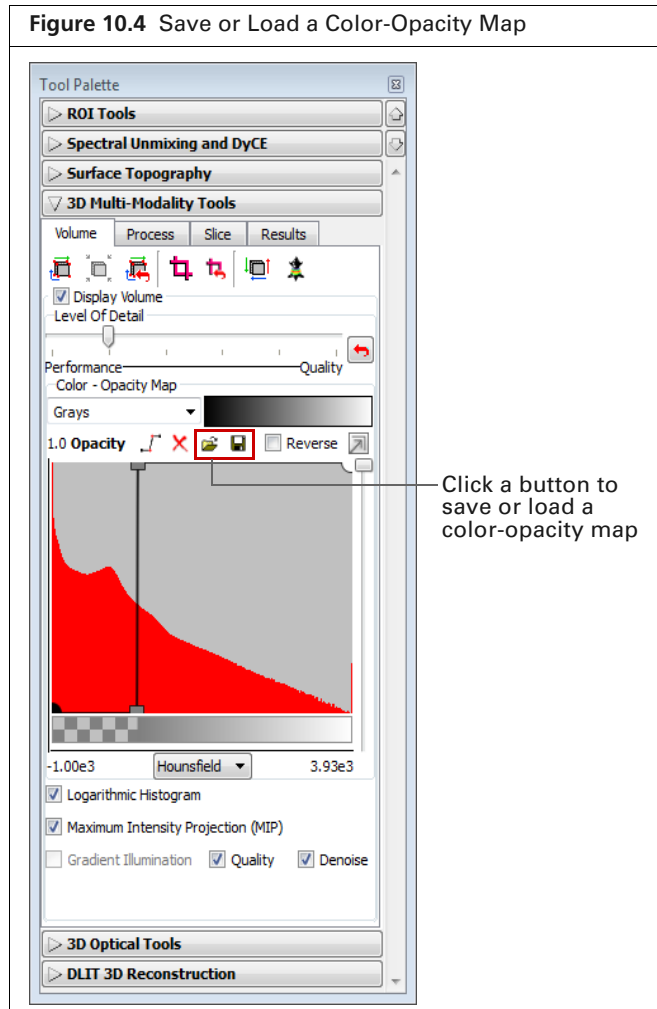
10.3 Volume Display Options

Adjusting Image Quality

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

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

To adjust the image resolution:

1. Move the "Level of Detail Slider" to the left or right (Figure 10.5).

   The color-opacity map is updated.

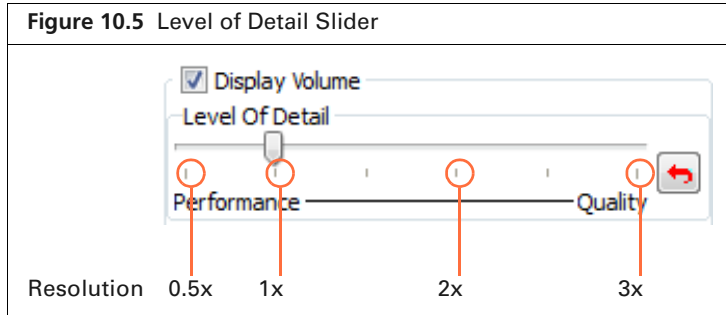2. To return the resolution to 1×, click the Reset button ↰.

Figure 10.5 Level of Detail Slider

Table 10.2 Example Volume With 512 Slices at 1x Resolution

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

Adjusting Volume Opacity

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

Figure 10.6 Adjusting Volume Opacity
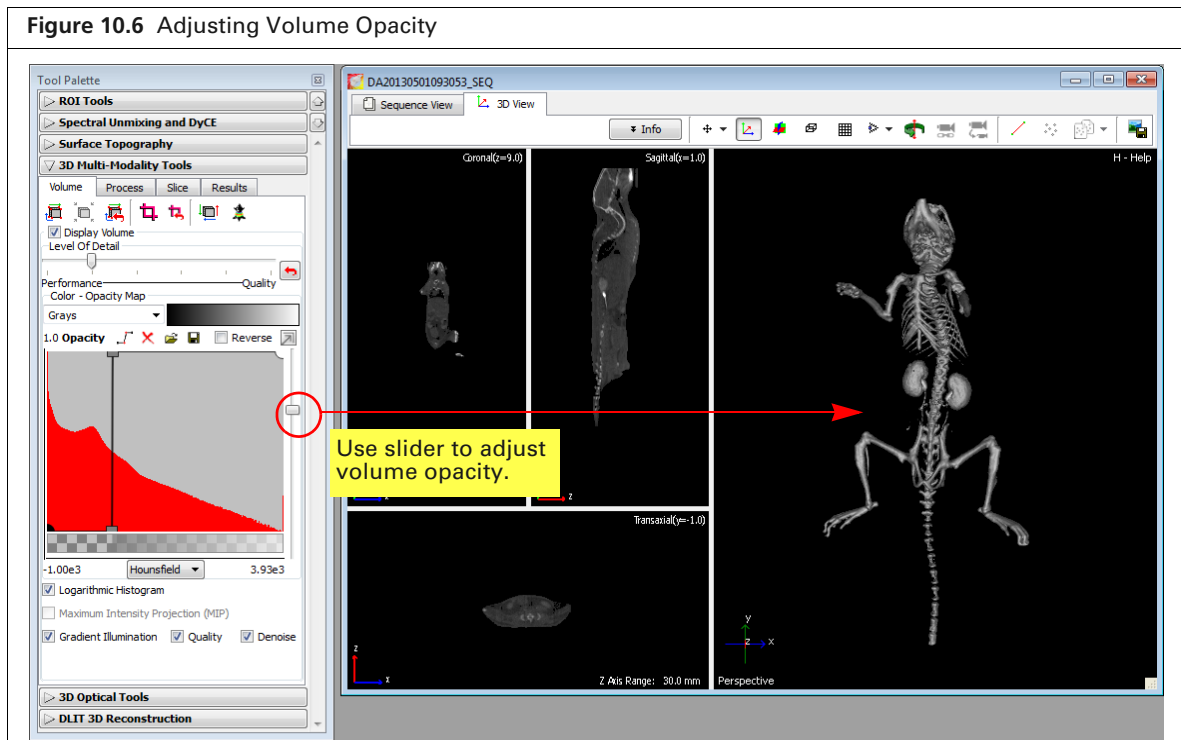
Maximum Intensity Projection

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

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

Figure 10.7 Volume Display – Maximum Intensity Projection
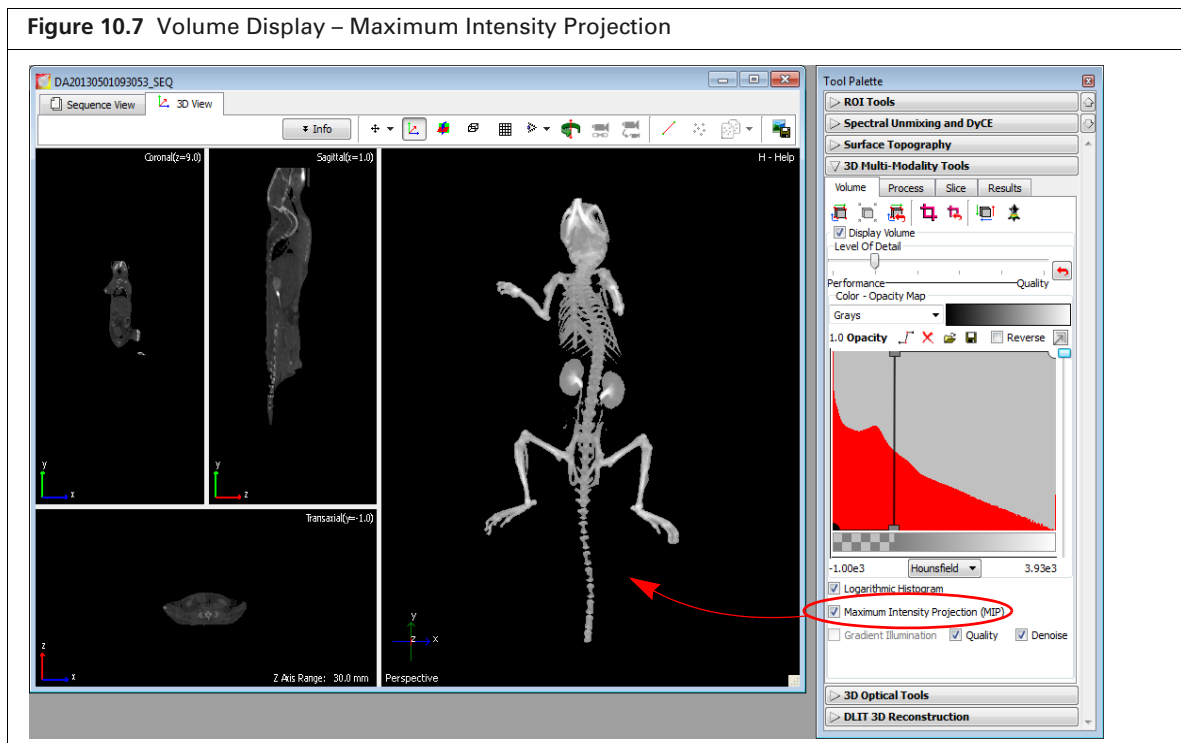
Figure 10.8 Image Window – Luminescent Image on X-ray Image
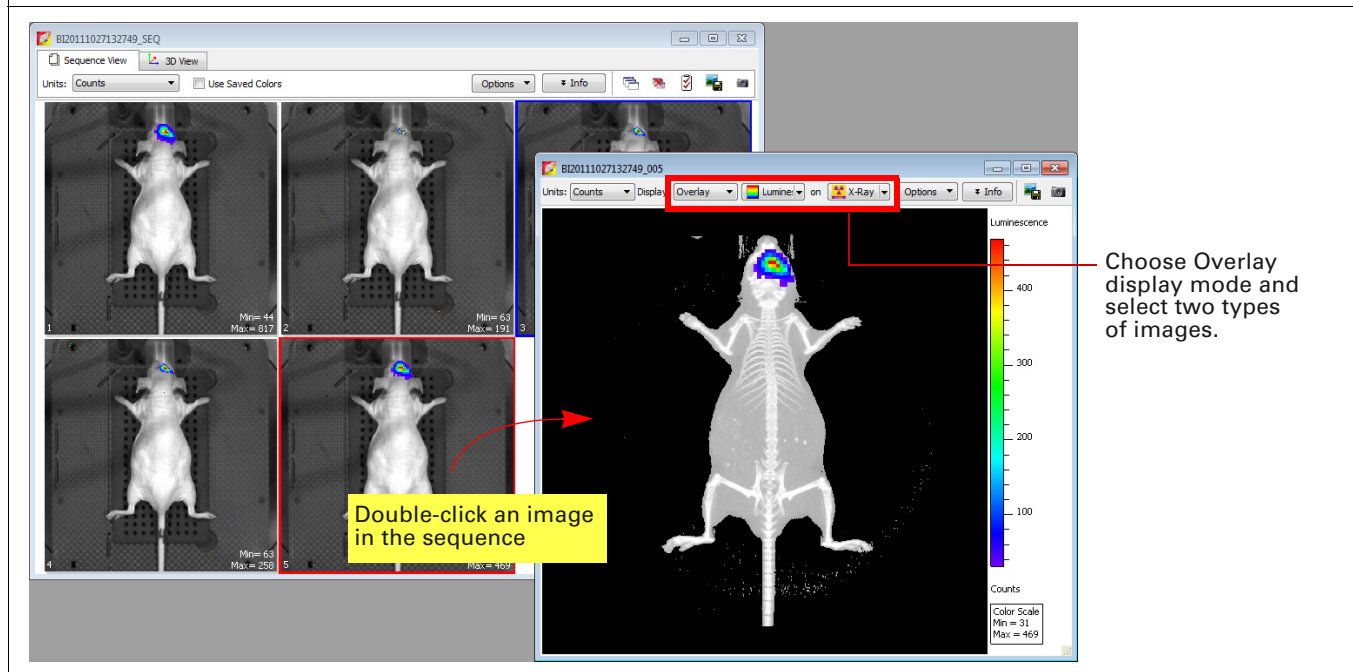
Choose Overlay display mode and select two types of images.

Double-click an image in the sequence

Gradient Illumination

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

Two options are available for Gradient Illumination:

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

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

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

Figure 10.9 Volume Display – Gradient Illumination
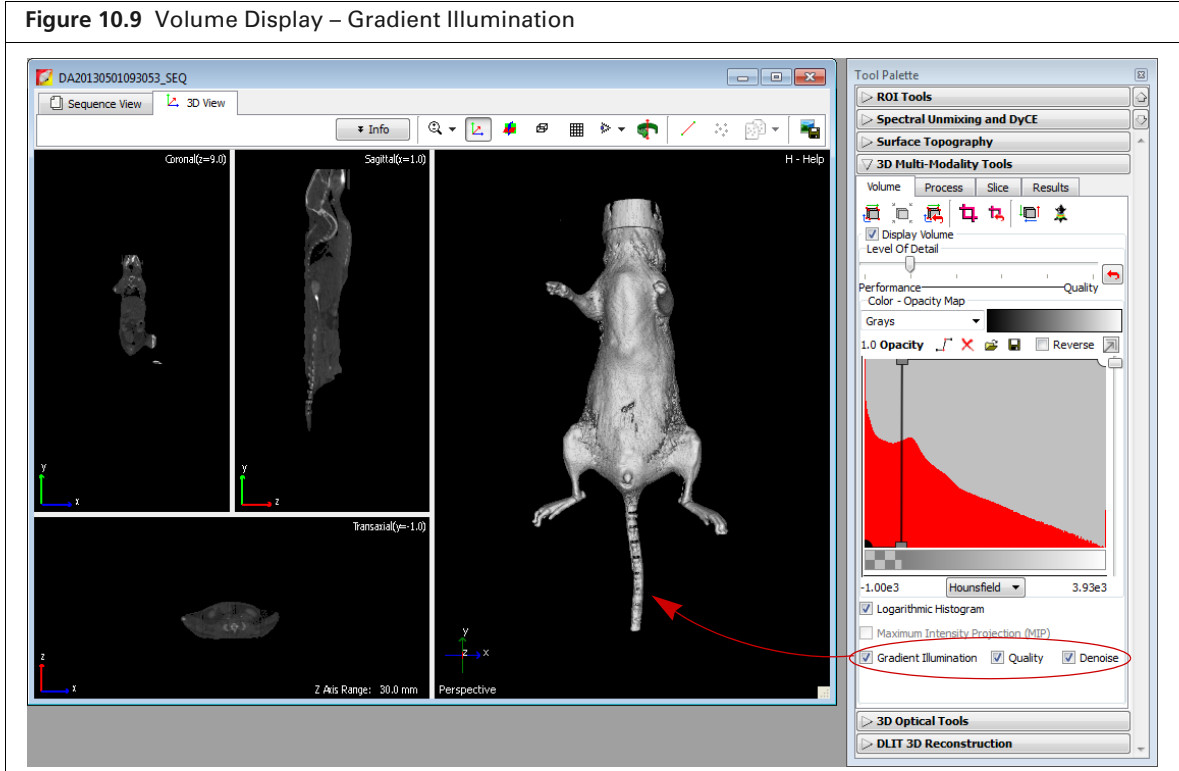
Modifying Volume Resolution

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

1. In the Volume tab, click the Edit Space and Orientation button.

2. In the dialog box that appears (Figure 10.10), edit the pixel or slice spacing.

Figure 10.10 Volume Information Dialog Box
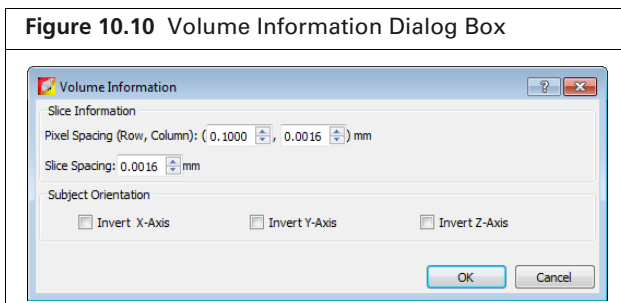
10.4 Smoothing a Volume

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

To apply smoothing:

1. Load the volumetric data.

2. Choose the type of smoothing and group size in the Process tab of the 3D Multi-Modality tools (Figure 10.11).

   - Mean – Applies the average grayscale value of a group of voxels (for example, a 3x3 group) to the central voxel of the group.

   - Median – Applies the median grayscale value of a group of voxels to the central voxel of the group.

   - Gaussian – Applies the weighted mean to the central voxel of the group. The weight distribution is similar to a normalized Gaussian shape with the highest value at the central voxel of the group.

3. Click the ↩ button to remove the smoothing.

Figure 10.11 3D Multi-Modality Tools – Process Tab
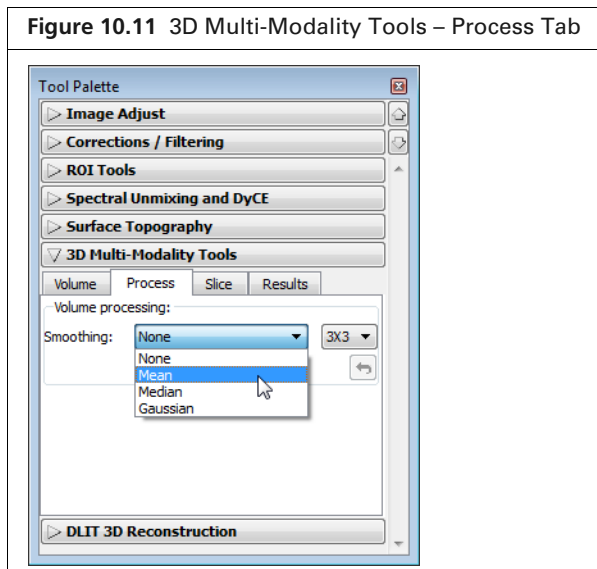
10.5 Viewing and Rendering Slices

Viewing Slices

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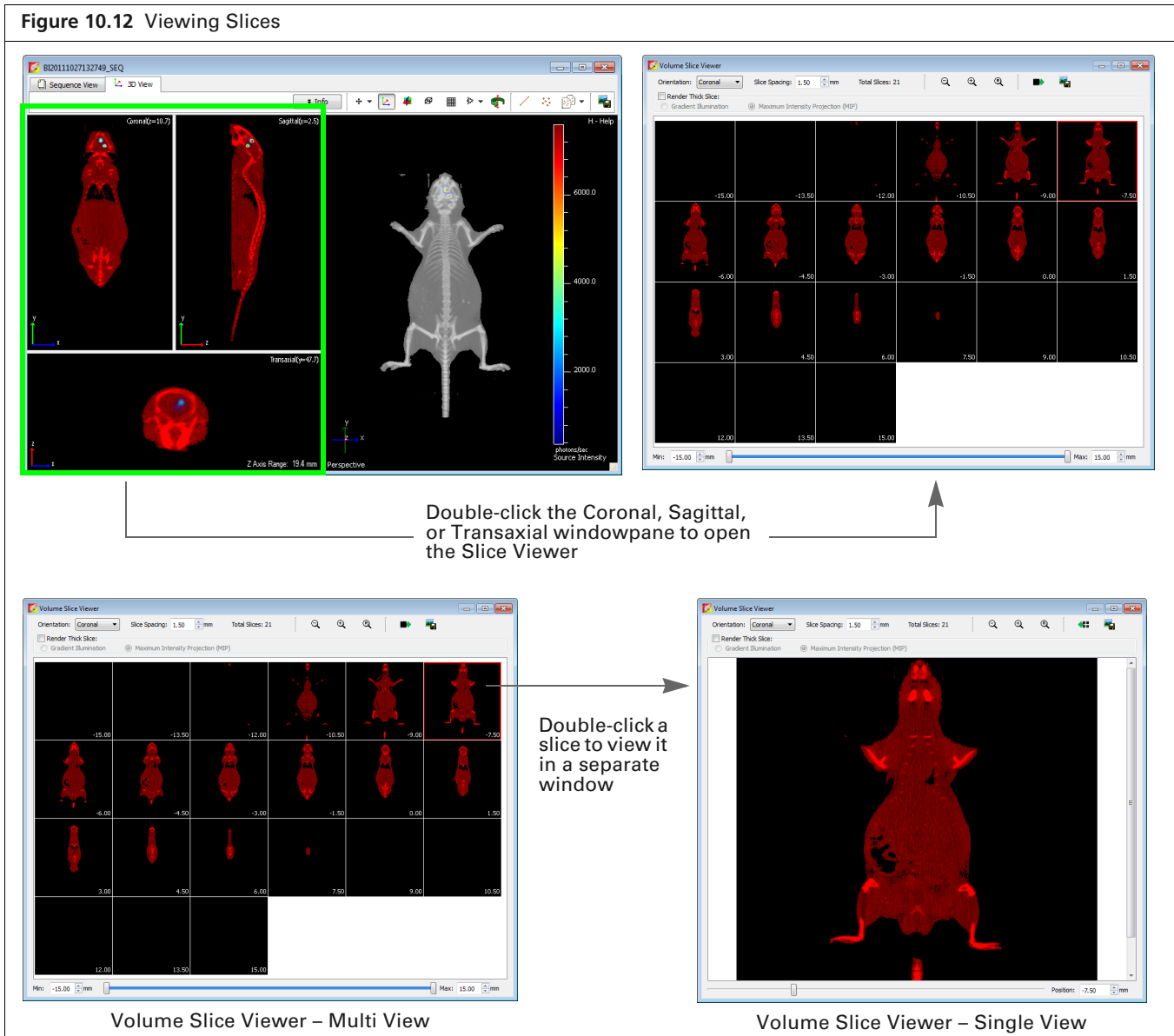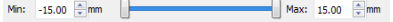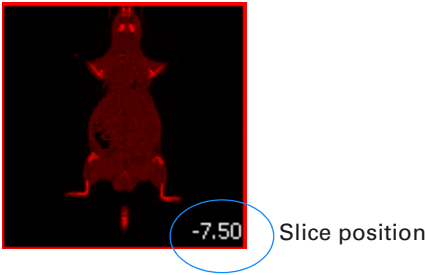
Figure 10.12 Viewing Slices

Double-click the Coronal, Sagittal, or Transaxial windowpane to open the Slice Viewer

Double-click a slice to view it in a separate window

Volume Slice Viewer – Multi View

Volume Slice Viewer – Single View

Table 10.3 Volume Slice Viewer

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

Rendering Slices

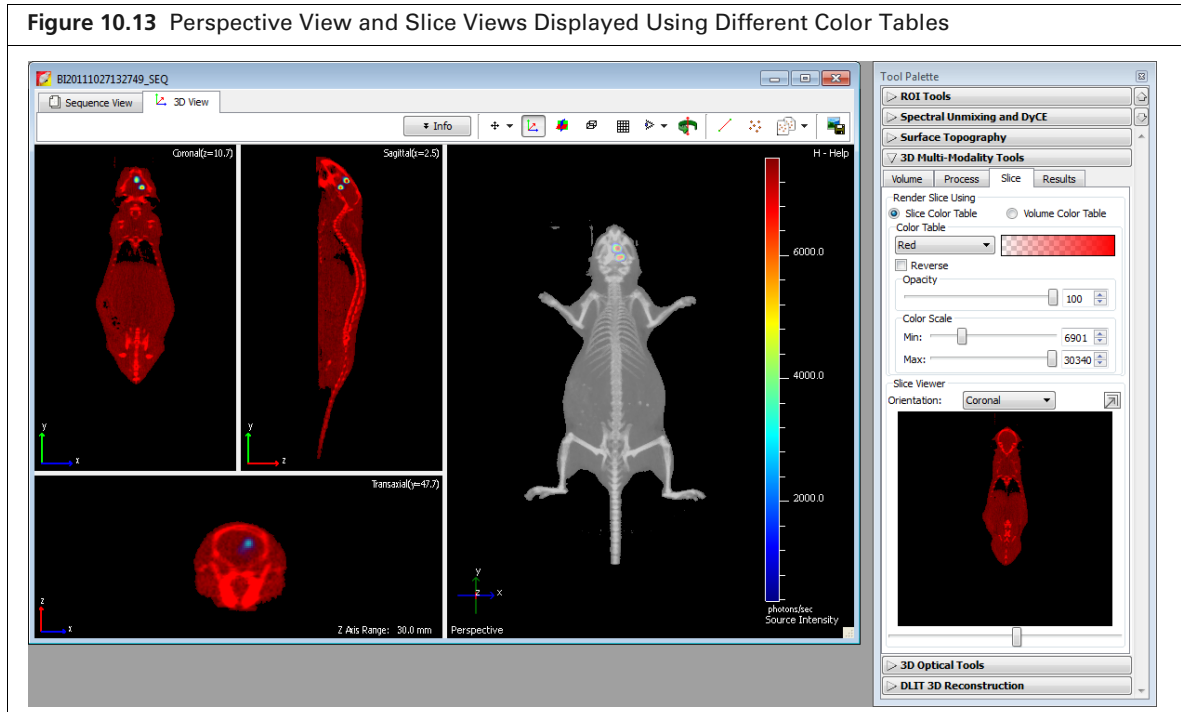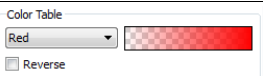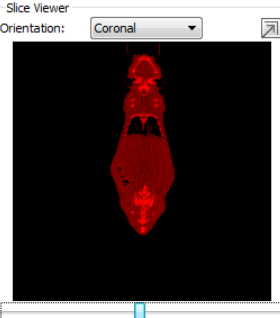The Slice tab in the 3D MM tools contains rendering and viewing options for slices.

Figure 10.13 Perspective View and Slice Views Displayed Using Different Color Tables

Table 10.4 3D Multi-Modality Tools for Rendering Slices

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

10.6 Registering Optical and Volumetric Data

Registering *multi-modal* data (optical and volumetric data) provides an anatomical context for interpreting biological (functional) information. Two registration methods are available:
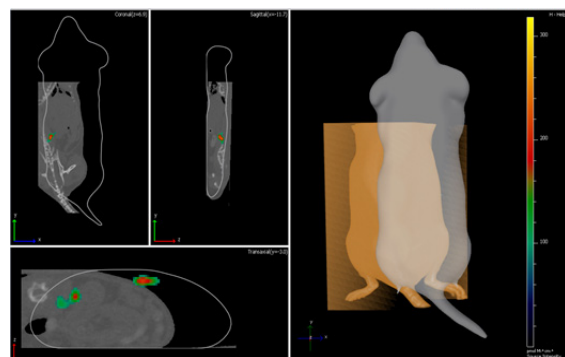
- Automatic fiducial registration – For experiments in which the optical data are acquired on the IVIS Spectrum and the CT data are acquired on the Quantum FX microCT pr Quantum GX microCT. The subject must be contained in the Mouse Imaging Shuttle during both optical and CT imaging, and the CT data must be exported to DICOM format. See page 197 for more details.

- Manual registration – Use the 3D Multi-Modality tools to register a 3D surface reconstruction with 3D volumetric data acquired on a third party instrument. See page 199 for more details.

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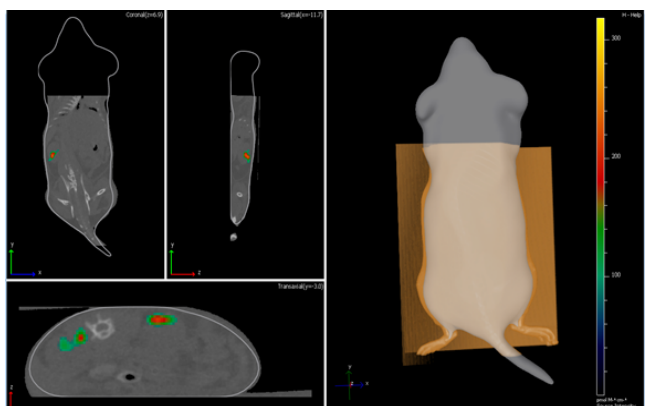

**Figure 10.14** Registering Multi-Modal Data
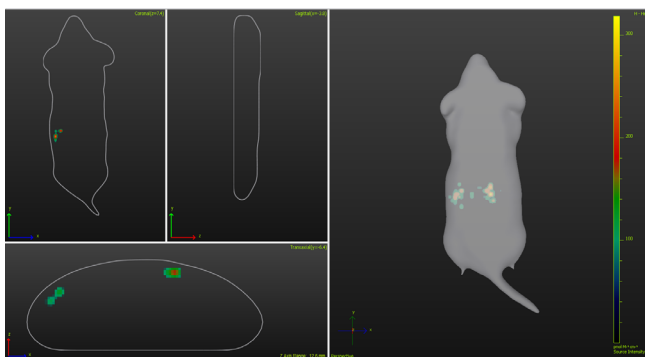
1. Load the optical data:
   - Bioluminescence or fluorescence image sequence and structured light surface
   - 3D source reconstruction (DLIT or FLIT results)

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

3. Register the 3D source reconstruction and the 3D volumetric data by performing either:
   - Automatic fiducial registration–Available for data acquired on the Quantum FX µCT instrument using the Mouse Imaging Shuttle (page 197)

   or

   - Manual registration–Match animal surface representations using the Manual Registration tool (page 199)
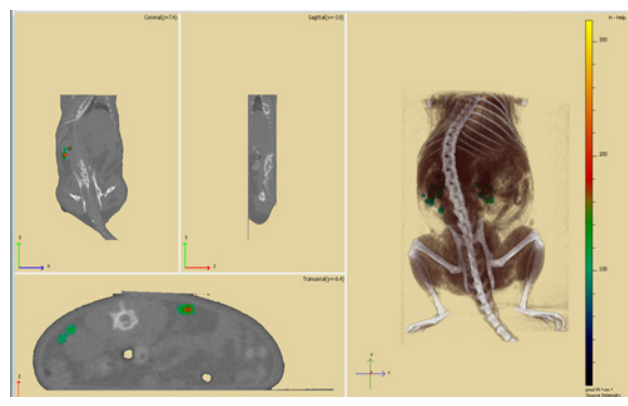
4. Classify the 3D volumetric data to help identify and separate objects (page 182). Save the color-opacity map (optional).

5. Save the registered 3D multi-modality results (page 202).

Loading Data for Registration

1. Load a DLIT or FLIT image sequence and the 3D reconstruction results.

> **NOTE:** The 3D Multi-Modality tools appear in the Tool Palette after you load optical image data. If the 3D Multi-Modality tools do not appear in the Tool Palette, confirm that the 3D Multi-Modality Tools license is installed and that the workstation graphics card meets the specifications in Table 10.1 on page 182.

2. Select the DICOM or TIFF volumetric data.

    a. Click the [icon] toolbar button. Alternatively, select **File → Browse 3D Volumetric Data** on the menu bar.

    b. If this is the first time browsing for volumetric data in the session, select a data folder in the dialog box that appears, and click **Select Folder** (Figure 10.15).

    The Living Image 3D Volumetric Browser appears.

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

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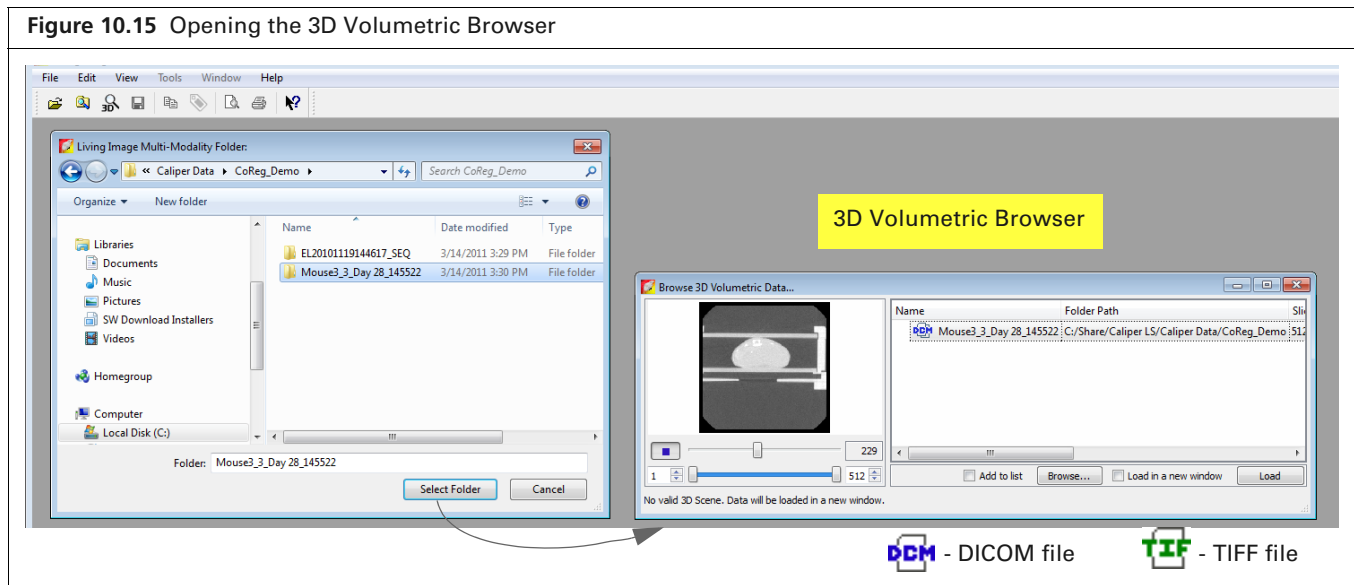
The 3D Volumetric Browser automatically previews a playback of the data along with other information about the data (Figure 10.15). See Figure 8.24 on page 169 for more details on the 3D Volumetric Browser.

3. Load the volumetric data with the optical data.

    a. Confirm that the "Load in a new window" option is not selected. (If this option is selected, the volumetric data are loaded in a new window.)

    b. Double-click the data row in the browser. Alternatively, select the data row and click **Load**.

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

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

Figure 10.16 3D Optical and 3D Volumetric Data

Histogram of voxel intensities
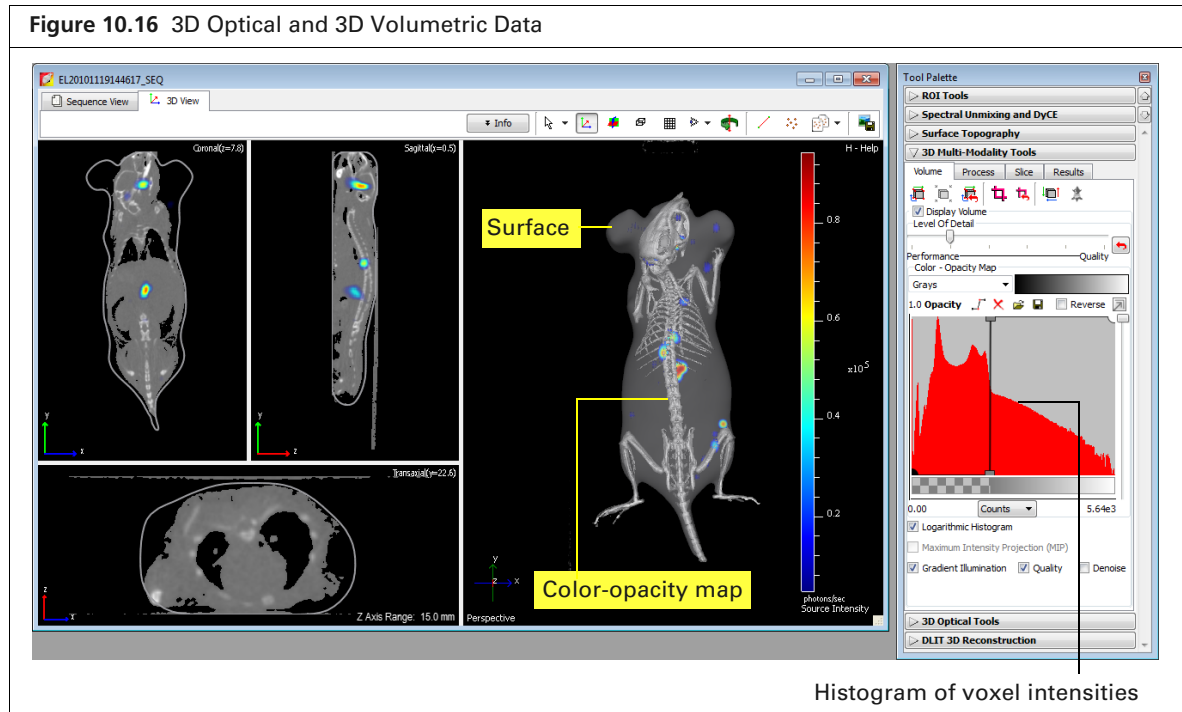
Registering Multi-Modal Data

Automatic Fiducial Registration

About the Mouse Imaging Shuttle

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

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

To perform automatic fiducial registration:

1. Load the data that you want to register (see page 196).
2. Click the Fiducial Registration button ⬚.
    The multi-modal data are automatically registered and cropped (Figure 10.17).
3. To undo the registration, click the Reset Registration button ⬚.

4. To save the registration information:

   a. Confirm the default name or enter a name for the results in the Results tab.

   b. Click **Save**.

Figure 10.17 Registered 3D Optical and 3D Volumetric Data

**Figure 10.18** 3D Multi-Modality Tools – Results
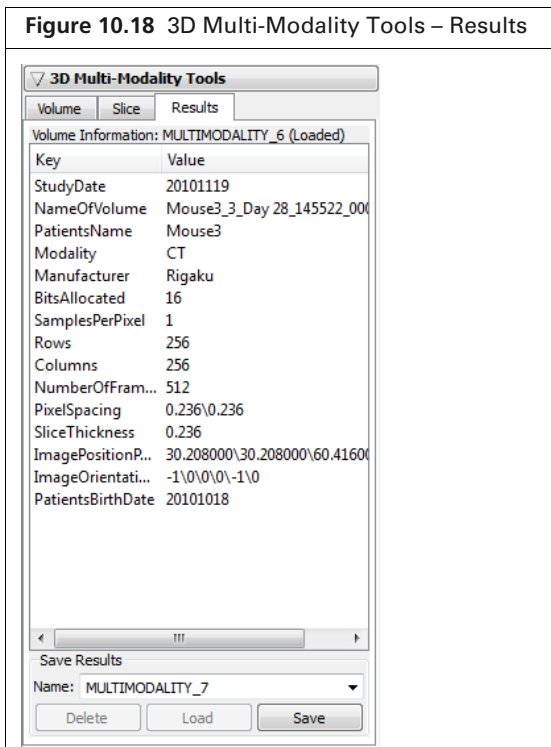
NOTE: Registration information is saved with the results for the volumetric data and is specific for a particular optical dataset.
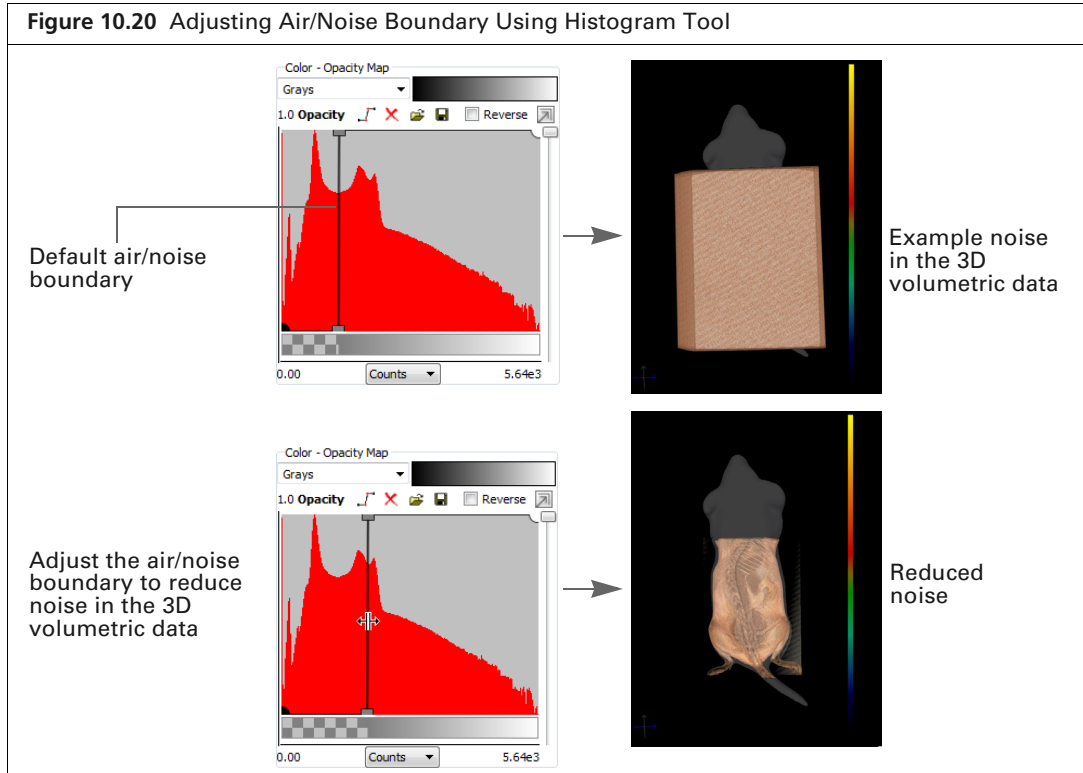
Manual Registration

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

Figure 10.19 Example Surfaces Before and After Registration

3D volumetric surface   3D structured light surface

Surfaces before registration   Registered surfaces
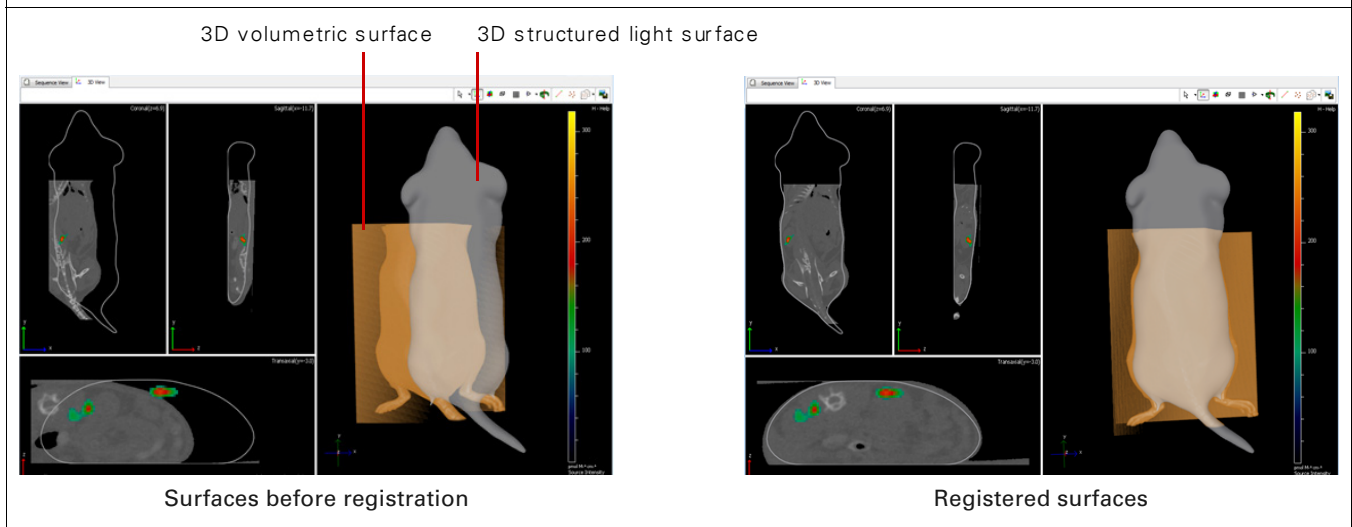
To manually register data:

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

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

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

Figure 10.20 Adjusting Air/Noise Boundary Using Histogram Tool

Default air/noise boundary

Example noise in the 3D volumetric data

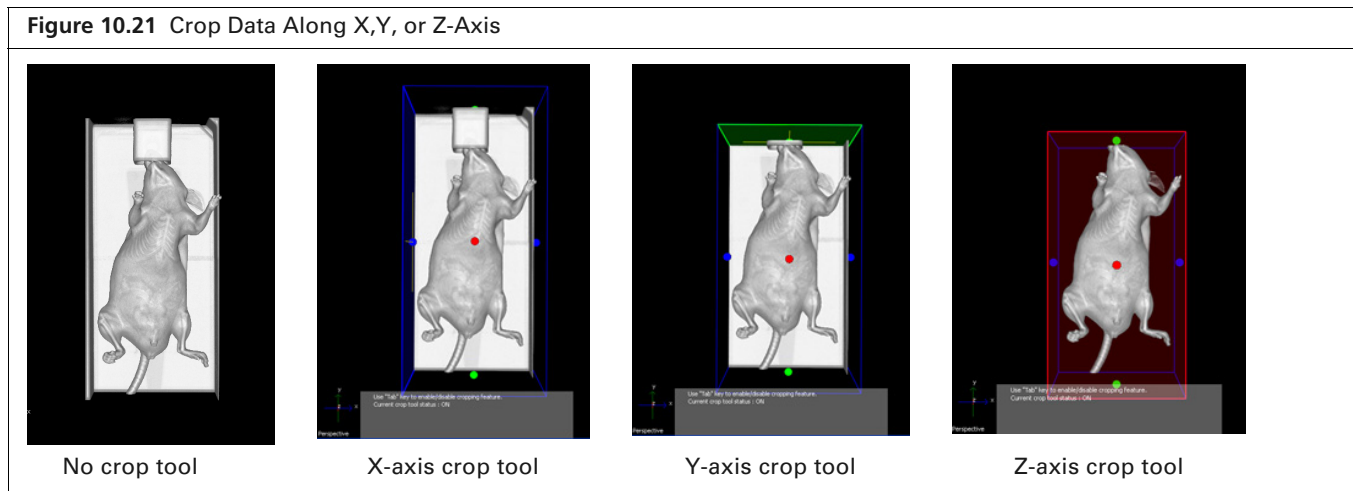Adjust the air/noise boundary to reduce noise in the 3D volumetric data

Reduced noise

3. If the volumetric data needs cropping (for example, to remove structures such as the stage from the CT view), follow step a to step c below. If cropping is not needed, proceed to step 4.

To crop the data:

a. Click the crop tool button ⬚.

The crop tool appears and has six control points:

🔵 – Crops the data along the x-axis.

🟢 – Crops data along the y-axis.

🔴 – Crops data along the z-axis.

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

No crop tool   X-axis crop tool   Y-axis crop tool   Z-axis crop tool

b. Click and hold a control point while you move the crop plane. As you move the crop plane, the slice views are updated. Release the mouse button to crop the data.
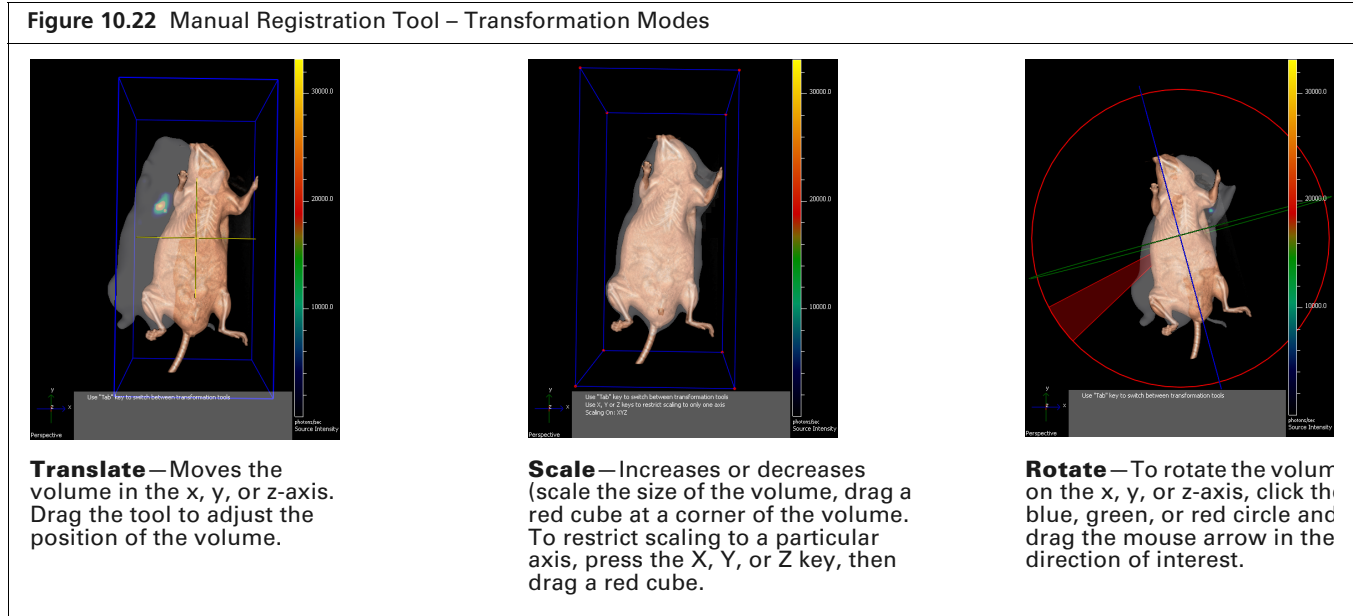
    c. To reset the crop planes, click the ⬛ button. When finished cropping, press the Tab key to turn off the crop tool.

4. Click the Manual Registration button ⬛.

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

Figure 10.22 Manual Registration Tool – Transformation Modes

Translate—Moves the volume in the x, y, or z-axis. Drag the tool to adjust the position of the volume.

Scale—Increases or decreases (scale the size of the volume, drag a red cube at a corner of the volume. To restrict scaling to a particular axis, press the X, Y, or Z key, then drag a red cube.

Rotate—To rotate the volume on the x, y, or z-axis, click the blue, green, or red circle and drag the mouse arrow in the direction of interest.


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

5. To return the 3D volumetric data to the default position and size, click the Reset Registration button ⬛.

6. Save the registration information (see page 198).

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

10.7 Volume Information and Results

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

Figure 10.23 Volume Information
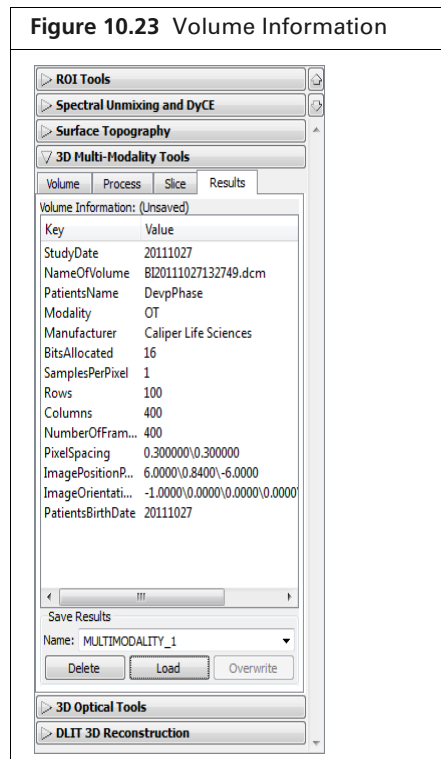
Saving the registered and classified data provides a convenient way to share data. The software saves the following:

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

Managing Results

Saving Registered Results

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

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

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

Loading Results

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

Deleting Results

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

10.8 Volume Data Viewer

The Living Image software provides a viewer for volumetric data. The 3D Multi-Modality tools are not required to view DICOM or TIFF data.
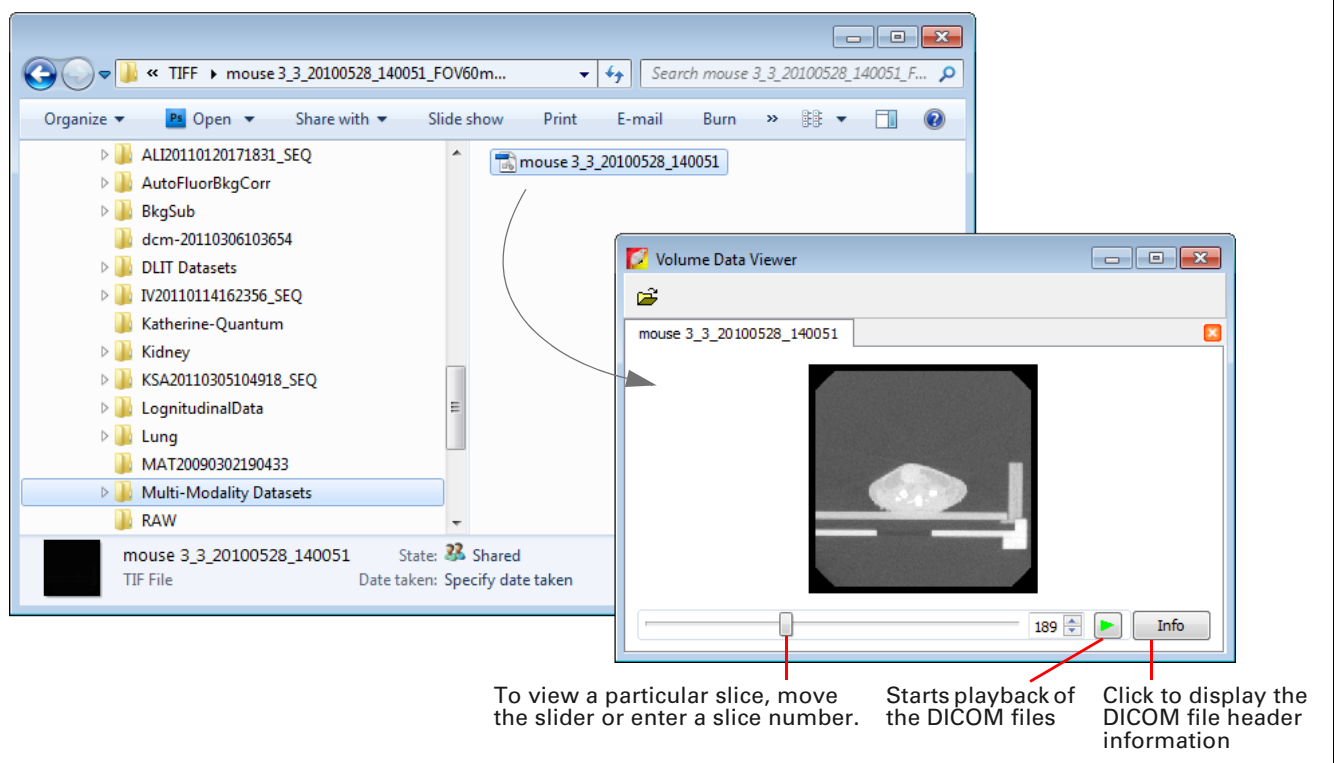
1. Select **View → Volume Data Viewer** on the menu bar.

    The Volume Data Viewer appears.

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

    or

    - In the Volume Data Viewer, click the Open button 📂, and in the dialog box that appears, select a DICOM or TIFF file, and click **Open**.

3. To clear the Volume Data Viewer, click the 🟧 button.

Figure 10.24 Drag Volume Data from Windows Explorer to Volume Data Viewer

To view a particular slice, move the slider or enter a slice number.

Starts playback of the DICOM files

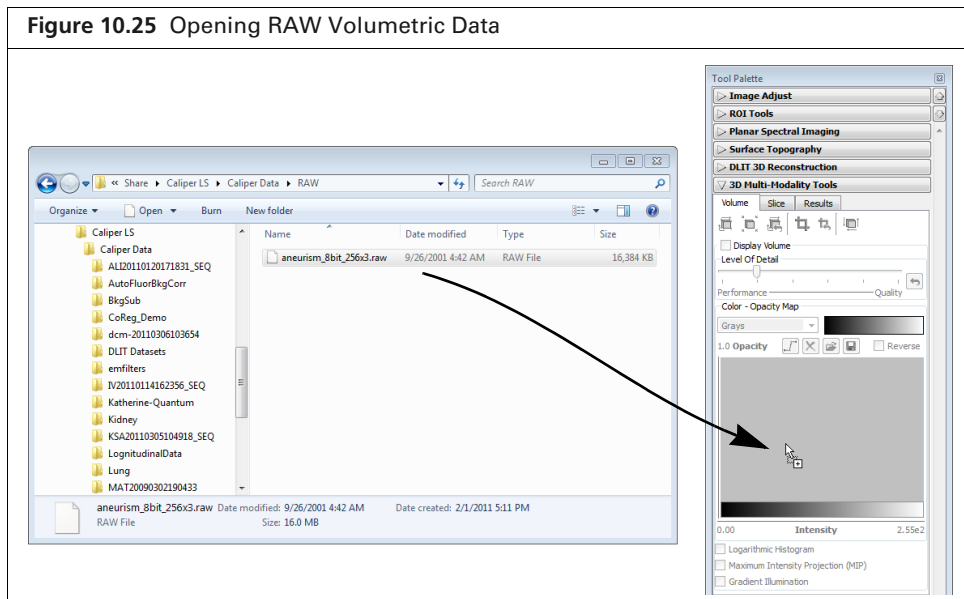Click to display the DICOM file header information

10.9 Viewing RAW Volumetric Data

**1.** Drag a single RAW file (*.raw or *.vox) from Windows Explorer to the 3D Multi-Modality tools (Figure 10.25).

> ✎ **NOTE:** Only single *.raw or *.vox files consisting of multiple slices of a 3D volume can be loaded into Living Image.

Figure 10.25 Opening RAW Volumetric Data

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

Figure 10.26 Volume Information

Select a data type.

Enter the:
- Width, height, and number of slices
- Slice row, column pixel spacing, and the slice spacing in millimeters
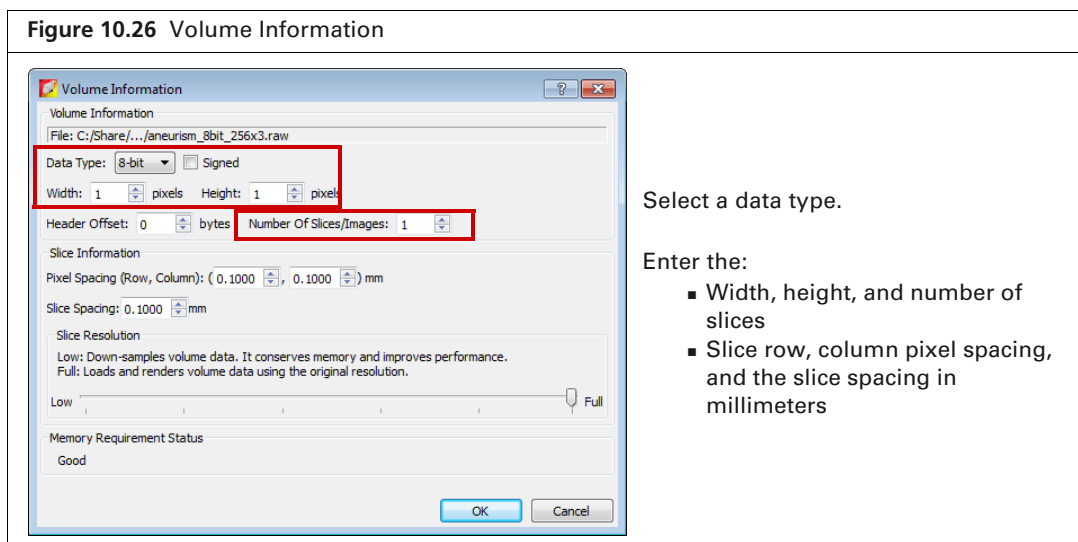
3. If loading the data will cause low memory, you are prompted to down-sample the data to improve memory and performance (Figure 10.27). Decrease the slice resolution by moving the Slice Resolution slider to the left until the Memory Requirement Status is "Good".

Figure 10.27 Down-sample 3D Volumetric Data
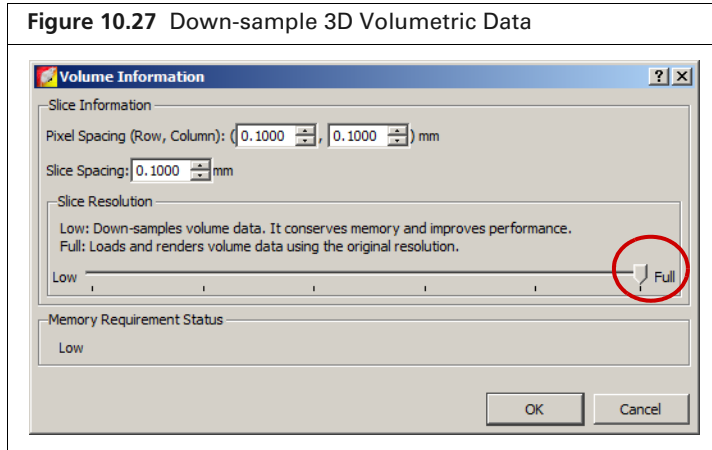
Changing the Orientation of RAW Volumetric Data

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

In such cases, you can:

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

To invert the subject orientation:

1. Click the Edit Spacing & Orientation button.

2. In the dialog box that appears, choose a "Subject Orientation" option and click **OK**.

Figure 10.28 Volume Information