



Version 6.0

Application Guide: 3D Reconstruction

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3D Reconstruction

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Introduction

The **MCID™ Elite** three-dimensional reconstruction module (**M3D™**) is suitable for many applications requiring the reconstruction of a series of consecutive sections as 3D models. Examples of these applications include:

- Light microscopy
- Electron microscopy
- Showing electrode tracks, lesions, infarcts, and other features within brain sections
- Reconstructing PET, CT, or MRI sections
- Showing patterns of receptor binding in 3D

One of the most important features of 3D reconstruction is the conversion of 2D data into a 3D image by a process called “rendering”. **M3D** has a unique and highly efficient rendering algorithm that lets users visualize their data at interactive speeds, using the host central processing unit. A powerful host computer (e.g., a Pentium® III or IV) with at least 256 MB of memory is recommended for 3D reconstruction. Rendering of very large data sets benefits from 512 MB of memory or more.

Images, Image Stacks and Rendering

Scientific images often show the internal structures of opaque objects. This is usually achieved by staining or labeling to reveal anatomical or physiological details. For example, you might want to look at cellular damage caused by an infarct, or glucose uptake in different regions of brain tissue. Because internal structure is not visible from surface views, these structural images must be made from thin sections taken through the specimen. A thin section may show internal structures, functional data (or a combination of both), but it lacks any cues that help you perceive depth and 3 dimensional shape.

In biological sciences, 3D image analysis is usually accomplished using sections of opaque objects (e.g., histological sections, tomographic sections). We can make accurate 2D measurements from sections, and we can use a series of consecutive sections through an object to measure its volume. However, understanding the arrangement of features in three-dimensional structures is not easy using sequential 2D sections. Sectioning interferes with our ability to perceive depth, and it is difficult to deduce and communicate the original 3D structure of a sectioned object.

Three-dimensional reconstruction of thin sections, taken at different levels within an object, re-creates the perception of a 3D object. As a first step in 3D reconstruction, we arrange the serial sections in a “stack.” A stack is a set of concentric sections, lying in a plane defined by the same XY co-ordinates, varying in the depth (Z) dimension. We know the X, Y and Z co-ordinates of each point within the stack, so we can use this information to reconstruct and display a model of the original object.

Three dimensional reconstruction is a two stage process:

1. Constructing a 3D model from a number of consecutive, thin sections called a “stack”.
2. Converting the 3D model into a 2D image.

In a good reconstruction, the final image shows us a realistic 3D representation of the original object, containing features from the stack and clues to depth, shape and orientation.

Note: Some textbooks use the terms rendering, 3 Dimensional rendering, or 3D rendering to describe the reconstruction procedure.

Aligning, Unwarping and Normalizing Images

Instruments that create optical or tomographic sections produce image stacks that are aligned in the X and Y dimensions. For example, a microscope can make optical sections by focusing at different focal planes within a sample. At each focal plane, the images remain aligned with the microscope’s optical axis.

On the other hand, physical sections of tissues are not inherently pre-aligned. Stained sections, autoradiographs, and other physically sectioned specimens need alignment before model construction and subsequent rendering. Through the history of microtechnique, many methods have been proposed to provide internal reference points (or fiducial points) for the alignment of physically sectioned specimens (Barden, 1901, Burston and Thurley 1957, Deverell and Whimster 1989, Dixon and Howarth 1957, Gough 1967, Heard 1931, Heard *et al.*, 1991, Yaegashi, Takahashi and Kawasaki, 1987). Even if intra-specimen reference points exist within a stack, few rendering systems are equipped to use these references for alignment.

M3D has a set of functions for section alignment. This includes moving an image manually until fiducial points or borders map a reference section, or computer-calculated alignment using fiducial points, contours and grayscale information from within the sections.

After the images are aligned, problems can still exist with localized artifacts such as air bubbles, folds or tears. **M3D** image editing functions can remove many of these artifacts. For example, if you wanted to replace an air bubble trapped in a section, you could use the **Copy** function in the **MCID Elite Transform** window to select a group of similar density pixels nearby, then copy these as a patch to cover it.

Warps

Tissue sections often show compression or stretching distortions, sometimes called “warps”. Warps can affect the image in two ways:

1. The simplest warp is a homogeneous alteration in the size or aspect ratio of a section. For example, microtomy results in compression of sections in the plane of the cut. This sort of simple warping is easily corrected using an unwarping algorithm that operates uniformly across the entire section (e.g. Jones, Milthorpe and Howlett, 1994).

2. A more difficult case arises when the warps vary from point to point within and between each 2D image. This is most likely to occur if the sections are stretched as they are mounted on slides. Removing *these* warps requires functions that perform locally variable image stretching and compression. **M3D** includes general and localized methods of warp removal (image registration in the *Transform* operational window).

Normalizing

Images need to be normalized to account for differences in background densities and in calibrations. This is especially true of microscope images taken from serial sections. Variations in thickness may exist between sections, even within a single section the material may not be consistent in different regions. This causes variations in background intensity that will affect reconstruction. **M3D** has routines for normalizing backgrounds and calibrations.

Segmentation

Any stack of images contains features of interest and backgrounds. We want to examine the features and make the backgrounds transparent, or at least, semi-transparent. In 3D rendering, as in other forms of image analysis, we have to segment the stack of images to discriminate between relevant and irrelevant image data. We can segment features from background by tracing around them manually, or by using automatic detection techniques.

M3D provides a set of tools for performing segmentation prior to creating a 3D object from the segmented data. This process of segmentation and 3D object creation is called "geometric modeling". It is similar to the type of modeling seen on standard 3D computer graphics systems; the difference being that anatomical data exist *within* biological specimens and are taken from there. In a standard computer graphics application, the 3D object is created from drawings.

M3D can discriminate features automatically within a 3D dataset using density thresholds. The process of extracting features from a 3D data set is called "volume modeling". The critical difference between geometric and volume modeling is the way that the features are presented. In geometric modeling, we segment features within a 2D dataset by defining their boundaries. In volume modeling, we segment features within a 3D dataset using density information. **M3D** performs both geometric and volume modeling (explained in more detail, below).

3D Modeling

3D modeling describes the interpolation of the stack and it is a critical step in volume rendering. In **M3D**, the 3D dataset is represented as cubic voxels. Each voxel contains two types of information:

1. Density information (the original density)
2. Structural information (the structure to which this voxel belongs)

Figure 1: Interpolation between serial sections. M3D applies a linear intensity interpolation to create the missing pixels between these sections.

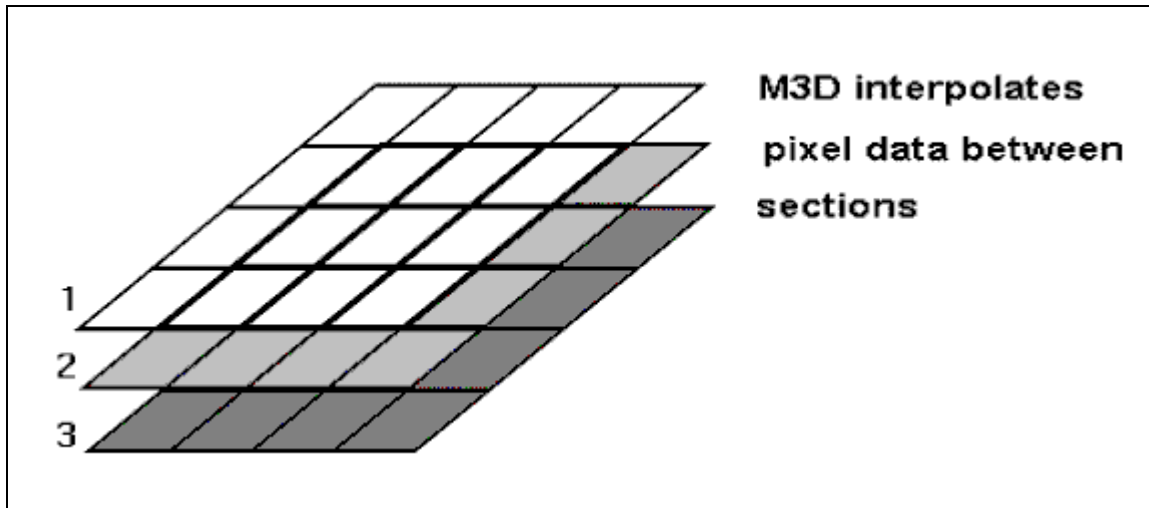
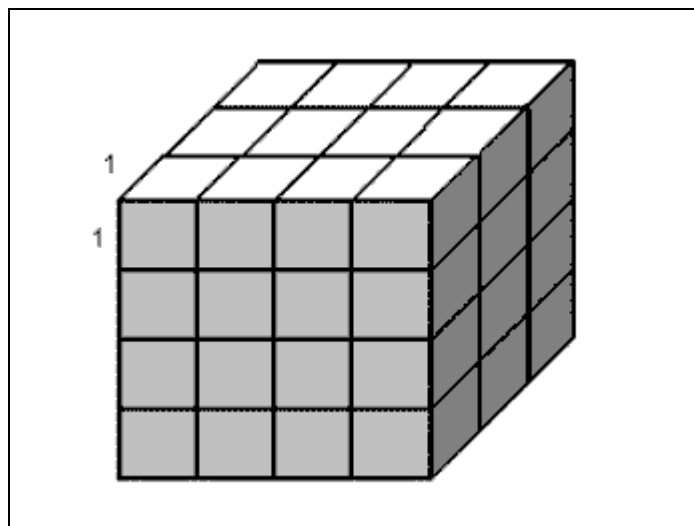


Figure 2: The pixels shown in the sections become cubic voxels in a 3D representation.



M3D calculates voxel density values by linear interpolation of the voxels from adjacent sections. To calculate structural data, **M3D** uses a unique volume interpolation algorithm, the effect of which is illustrated in Figure 3 and 4.

Figure 3: Test images created using MCID Elite graphics tools. This image is interpolated in the next figure to show the effects of the M3D interpolation algorithm.

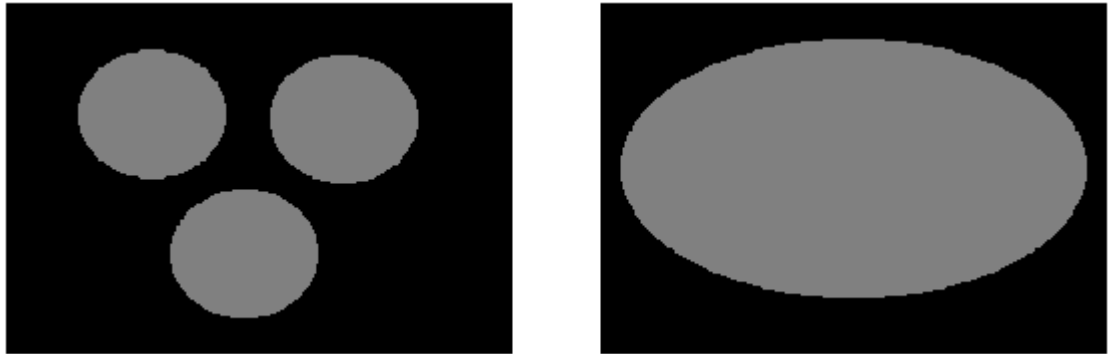
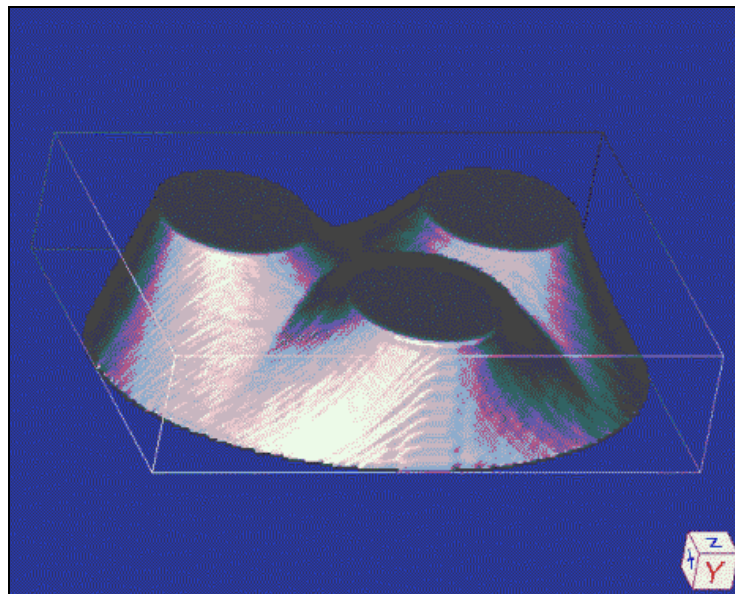


Figure 4: Interpolation of the structures shown in Figure 3. The white skeleton cube shows the outlines of the dataset. Note the Rotation Cube at lower right showing the present orientation of the rendering.



Types of Rendering

M3D has four categories of rendering:

- Contour rendering
- Surface rendering
- Density rendering
- Combined Surface/Density rendering

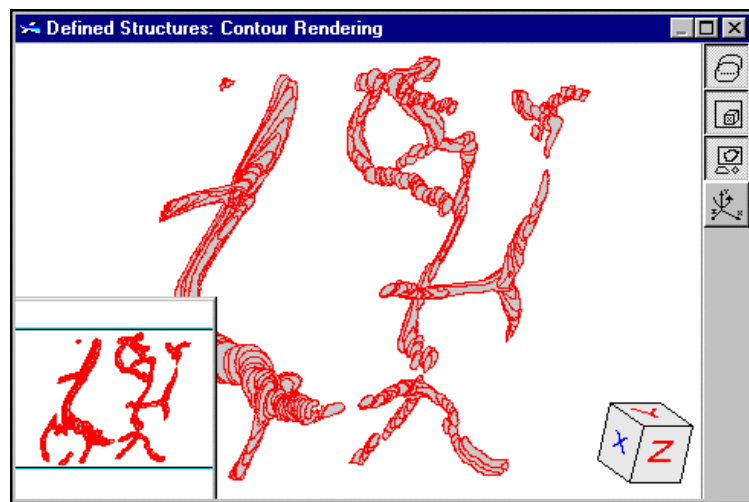
Each method produces different results when applied to a dataset, so the number of possible renderings is very large indeed.

Contour Rendering

On a map, contours define points lying at the same height above sea level. In an image, contours define boundaries of identified structures, or regions, that have the same gray levels. Contour rendering uses defined boundaries for each feature in the 2D data set. The user must define these 2D boundaries, so that the rendering system can use the boundary points to create the 3D object. The simplest type of geometric rendering draws lines between the boundary points within each section, then displays a 3D object that is composed of these lines.

Because the lines lie entirely within the 2D sections, the 3D object does not have a surface. This is known as contour rendering because it creates geometric models composed of lines representing the outer boundaries of features (Figure 5). Contour renderings can be created quickly and they are useful for editing stacks prior to more complex rendering. For example, we might use the contours to show where feature boundaries need editing.

Figure 5: Contour rendering of cerebral microvasculature, made by automatically detecting the vessels in 42 sequential (1 μm) sections of rat cortex.

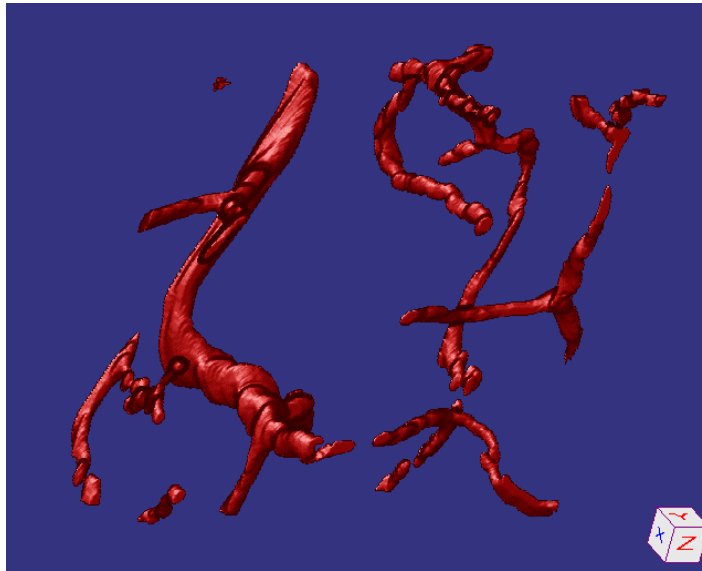


Notice that the vessel boundaries are made up of lines lying within the plane of each section. These planes are not joined in the Z (depth) dimension, so the effect is as if we are viewing a stack of thin sections through the vessels. The vertical (Z) separation of each section from its neighbors corresponds to the sectioning interval.

Surface Rendering

We can create a more realistic view of our 3D object by adding a surface. This procedure is known as “surface rendering”. In **M3D**, the rendered surface is made up from the surfaces of boundary voxels. Applying shading and smoothing to the voxel surface creates quite realistic renderings.

Figure 6: A surface rendering of the cerebral microvasculature of the rat cortex.



The simplest option is to put a surface onto selected voxels by density thresholding. You can apply surface rendering to the structures created as 2D contours or you can just create a structure by density thresholding the 3D density data, then perform a surface rendering on the created “structure”. Dynamic density surface rendering is much less time-consuming, but of course, the features of interest must be differentiated from background.

In contour and surface rendering, feature boundaries need to be explicitly defined. The power of a 3D rendering system comes from the ease with which boundary definition is performed and automatic boundary definition can reduce your work by an order of magnitude. **M3D** has flexible segmentation functions that should make minimal work of boundary definition. The major ways to define boundaries for contour rendering are:

1. Tracing each boundary manually.
2. Using an “Autoscan” tool to point to a feature and automatically define its boundary.
3. Using the “Scan area” function to define multiple boundaries automatically within a section.

Use of the **Autoscan** tools or the **Scan Area** function allows rapid, convenient segmentation if the feature boundaries are distinct from background. If features are not distinct, then boundaries must be defined by manual tracing. We cover automated and manual boundary definition techniques in later sections.

Density Rendering

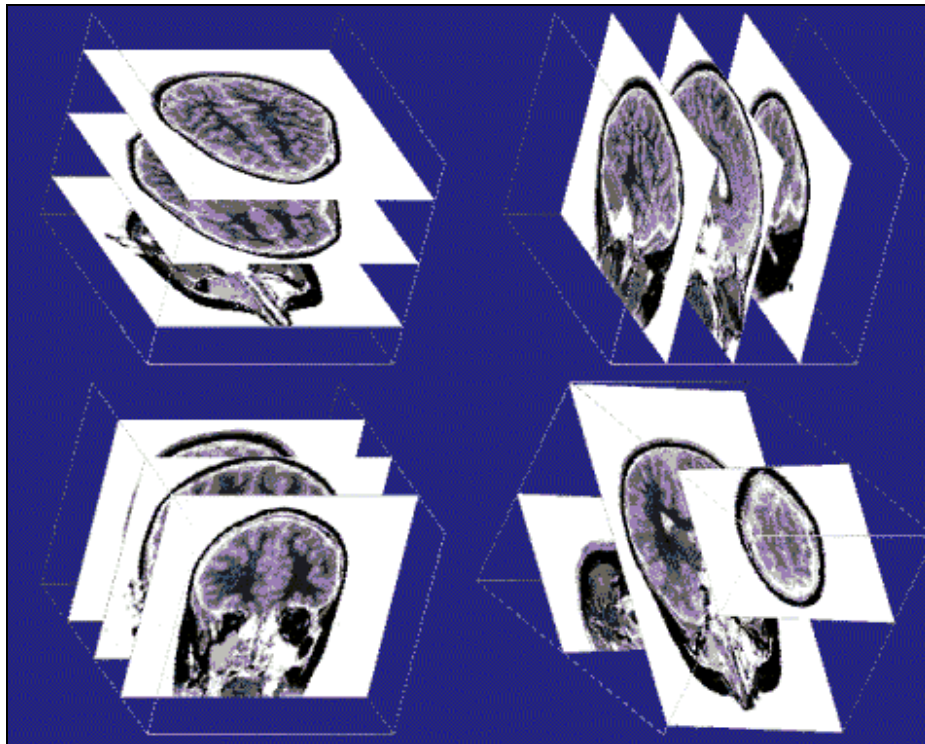
If a 3D dataset contains density information, we can create volume renderings that display the density dimension. **M3D** has rendering types for maximum, minimum, surface density and density with color and variable opacity. This is done by calculating the accumulated voxel density values along the voxel array. The accumulated voxel values are then

projected onto a 2D surface. Accumulated values for maximum and minimum renderings will be the max. or min. voxel densities. For surface density renderings, the value will be that of the first voxel in the density range.

Volume rendering often requires that some densities be shown with high visibility, while other densities are shown as semi-transparent, cloudy shapes. Visibility is manipulated by making some voxels transparent, depending on their density. In showing the 3D view, we may assign high levels of transparency to areas that contain low summed voxel values, and high levels of visibility to areas with high summed voxel values. This creates the familiar appearance of volume-rendered MRI images, where features appear as solid objects surrounded by a cloudy area.

Voxel-based renderings with transparency have the advantage that they represent shapes *and* densities. However, this type of rendering requires very powerful computers, and does not generally produce the most visually appealing views because our objects are not solid, and lack sophisticated lighting and texturing.

Figure 7: A series of magnetic resonance images sections through a human head displayed as cutting planes within a density rendering.



We can display voxels from the matrix in various ways. For example, the cerebrum might be represented by voxels containing density values from 100-200 gray levels, while background contains voxels having values from 0-99 and 201-255 gray levels. We can use the density values to segment the image. In a procedure very similar to 2D thresholding, we set density criteria, and only voxels containing these densities are shown.

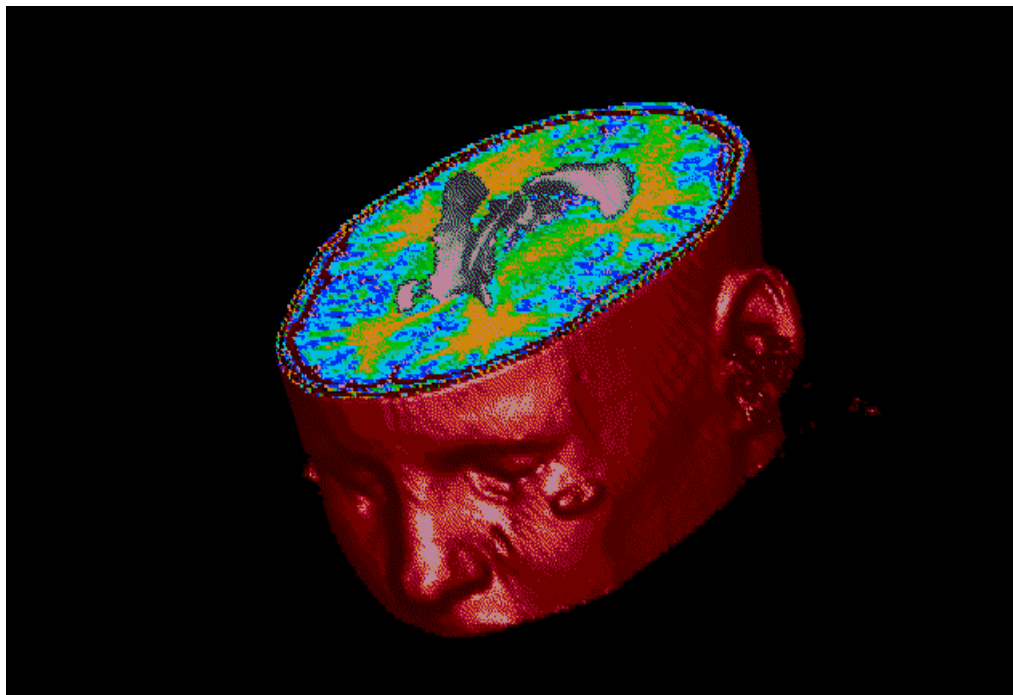
The simplest option is to put a surface onto the selected voxels. For example, the system could be told to show voxels between 100 and 200 gray levels as a surfaced object. This creates an object whose surface is defined by voxels, instead of pixels. However, the appearance of the volume rendering is not particularly different from a surface rendering of the same object.

The brightness of the object surface is not calculated from its original density, but from its spatial relationship with the light source. You might well question the need to perform volume renderings if the results are a surfaced model, but a single thresholding procedure segments the object from the 3D data set. This is much less time consuming than segmenting features in each of the slices.

Surface/Density Rendering

Density renderings on their own do not have enough visual cues to allow perception of three dimensions. You can create a 3D dataset combining density and surface renderings that shows density data with reference to structure.

Figure 8: A Surface/Density rendering of a series of MRI images through a human head showing a density rendering of the cerebral ventricles.



Summary

You will have to choose which method of rendering is most suitable for a specific dataset. If you want to extract simple anatomical structures from a series of sections, then a surfaced volume rendering will often yield the best results. If you want to show the density dimension, volume rendering with transparency is required. **M3D** offers each of these techniques, and the following chapters contain detailed instructions for the creation and optimal display of 3D renderings.

The M3D Tutorial

The **M3D** tutorial is designed to lead you into 3D reconstruction in a structured way. As you move through the lessons, you encounter operations of increasing complexity. We realize that you are probably keen to get started using your own samples right away, but try to resist the temptation to dive into Part 6 now. You will soon become fluent with **M3D** if you learn the basics first.

The tutorial is divided into seven parts:

- Part 1** - Starting **M3D**, loading images, simple rendering of a small stack.
- Part 2** - Defining structures in sections; contour rendering.
- Part 3** - Working with large image stacks.
- Part 4** - The 3D Rendering Control window.
- Part 5** - Presentation; changing the appearance of the rendered image.
- Part 6** - Cine loops; creating dynamic displays of the dataset.
- Part 7** - Making 3D measurements.

This seems like a great deal of work, but you should be able to move through each tutorial session quite quickly. We recommend you work through the tutorial at least twice; first using the sample images provided, and the second time using your own data.

The 3D Sample Images

The **MCID Elite** installation CD-ROM contains a set of sample images to accompany the tutorials.

TO INSTALL THE 3D SAMPLE IMAGES:

1. Select *Start > Settings > Control Panel > Add/Remove Programs*.
2. Select **MCID Elite 6.0** from the list of programs, then press the **[Change/Remove]** button. This launches the *MCID Elite Setup Wizard* (Figure 9)
3. Select **Add/Remove Components**, then press **[Next]**.
4. Select **M3D Tutorial Data** from the list of program components (Figure 10).
Note: DO NOT un-check any other checked components or they will be removed!
5. Insert the **MCID Elite** CD-ROM in your drive, then press **[Next]** to complete the installation.
6. The images are installed in the *\MCID Elite 6.0\Defgeo* folder.

Figure 9: To install the M3D sample images, select the ‘Add/Remove Components’ option...

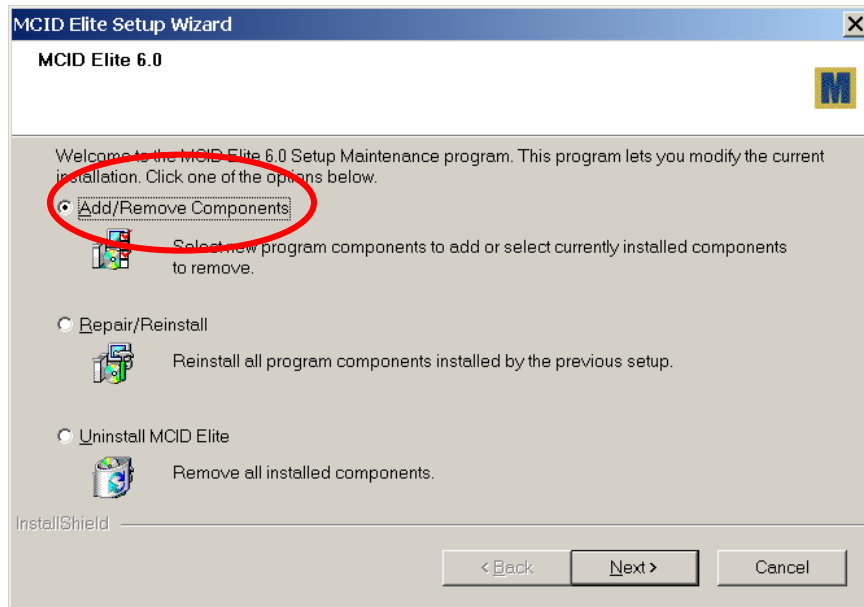
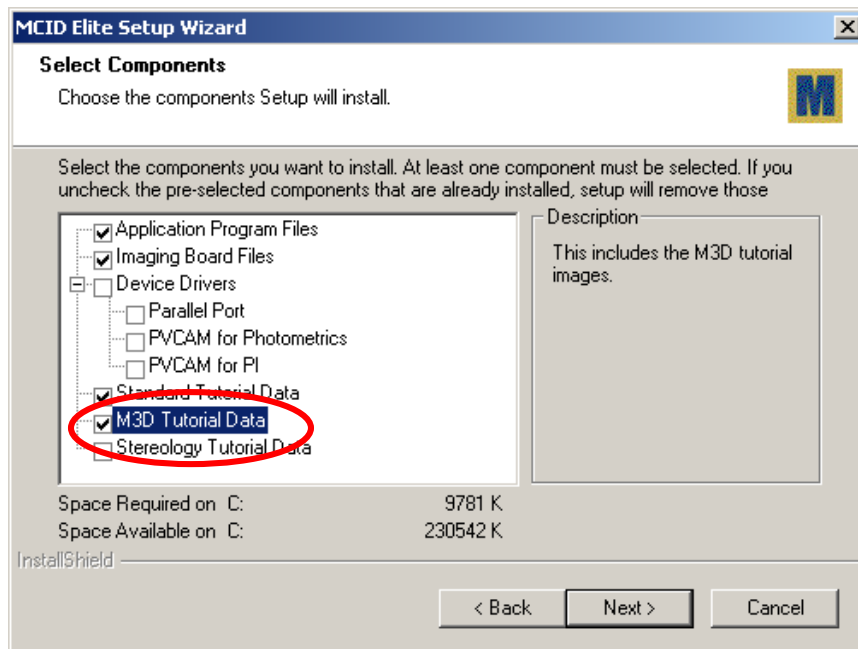


Figure 10: ...then select the ‘M3D Tutorial Data’ option. Do not un-check any other listed components.



The sample images consist of a series of 120 sections through a sphere and a hollow cylinder at 1µm intervals. Sections through the sphere are a series of disks of different diameters, and the sections through the cylinder look like a series of equal sized rings. In sections 40 to 79 there is a square inside each disk (Figure 11). Reconstructions of these sections show a sphere and a hollow cylinder (Figure 12). Using different **M3D** rendering

methods and cutting planes reveals a cube inside the sphere or a square at a cut face (Figure 13). These operations are all covered in this tutorial, and in the [Advanced Details](#) section of the manual.

The gray level values of these structures are: sphere 100, square 200, ring 250.

Figure 11: Some of the images in the 3D sample dataset showing sections of the sphere (circles) and the ring. In images 41-79 there is a square inside the circle.

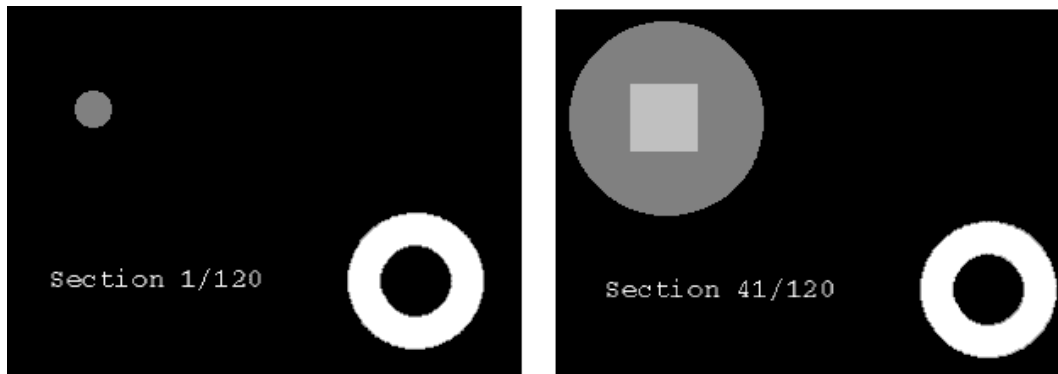


Figure 12: Partial and full reconstructions of the 3D sample dataset.

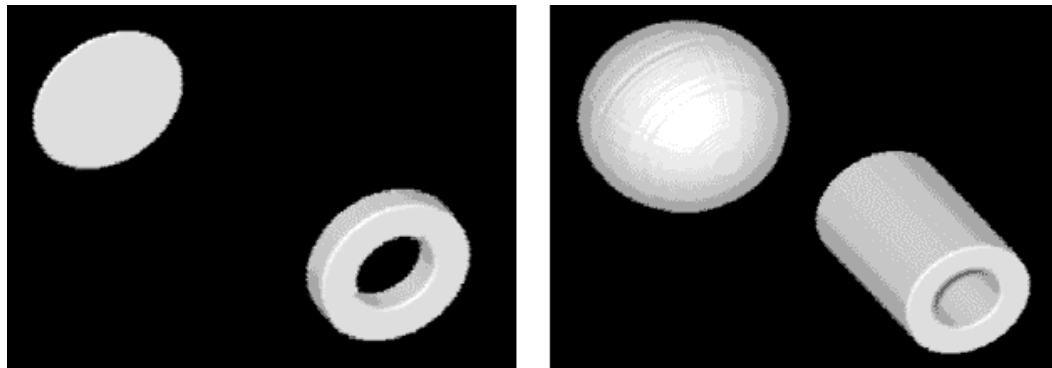
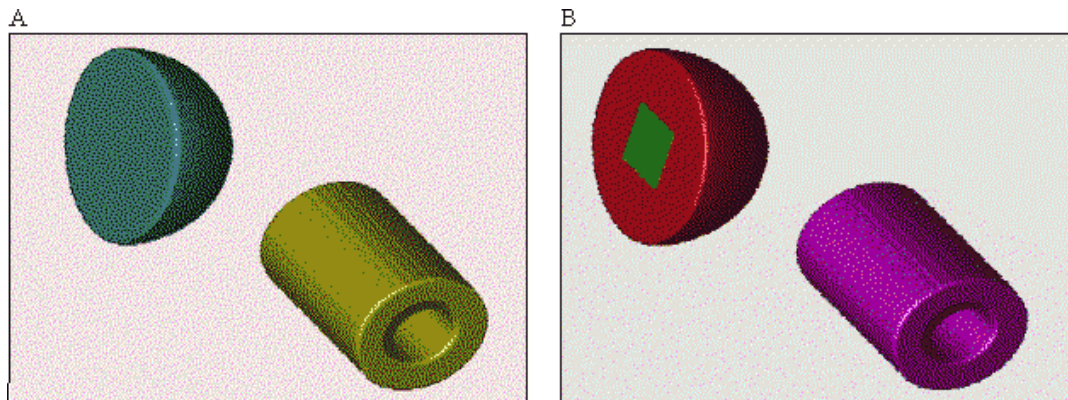


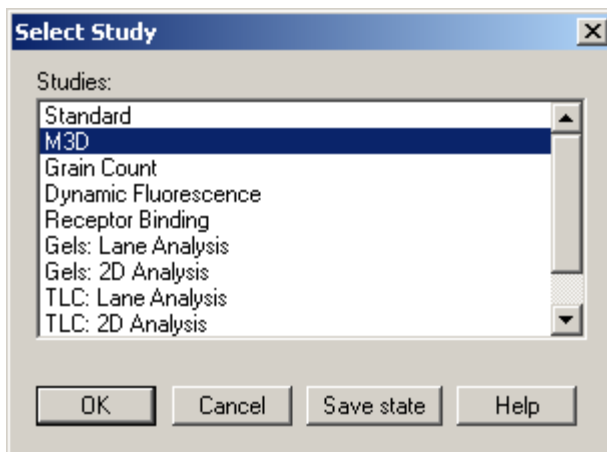
Figure 13: Cutplane applied to a structure rendering (A) and a density rendering (B).



Tutorial Part 1: Starting M3D

Your **M3D** software is supplied as part of a complete, new **MCID Elite** software package. After installing this package you can use the **M3D** option. Open the *Study* menu and choose *Select*. The *Select Study* dialog box appears, choose **M3D**.

Figure 14: To start M3D, select the 'M3D' option from the Select Study dialog box.

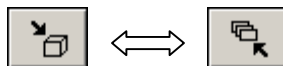


The new version of **MCID** works just like the previous one did and the **M3D** functions do not create any additional complexity in the other **MCID** operations.

M3D Modes

M3D has two modes, **Construction mode** and **Rendering mode**. The **Stack** window is the **M3D** Construction mode and the **3D Rendering Control** window is the **M3D** Rendering mode. Construction mode is the default and allows you to load, edit, align or process 2D images and create a 3D dataset. Rendering mode is used to display 3D datasets, create cine loops and make 3D measurements.

You can switch between these display modes by clicking on the **Rendering mode / Construction mode** icon at the top right of the screen.



A summary of the functions that can be performed in each mode is presented in Table 1, below.

Table 1: A summary of the differences between Construction Mode and Rendering Mode.

Features	Construction Mode	Rendering Mode
Purpose	Preparing datasets for rendering	Rendering prepared datasets
Digitize	Yes	Yes
Stack editing	Yes	No
Contour rendering	Yes	No
Surface rendering	No	Yes
Density rendering	No	Yes
Surface and density rendering	No	Yes
Measurements	2D	2D & 3D

M3D Icons

When you select **M3D** as a study type, new icons appear on the icon bar.

In **Construction mode** you see:



The **Stack** icon. This opens the *Stack* operational window.



The **Rendering mode** icon. This icon switches you from Construction mode into Rendering mode.

In **Rendering mode** you see these icons:



The **Construction mode** icon. When you are in 3D Rendering mode, this icon switches you back to the *Stack* window (Construction mode).



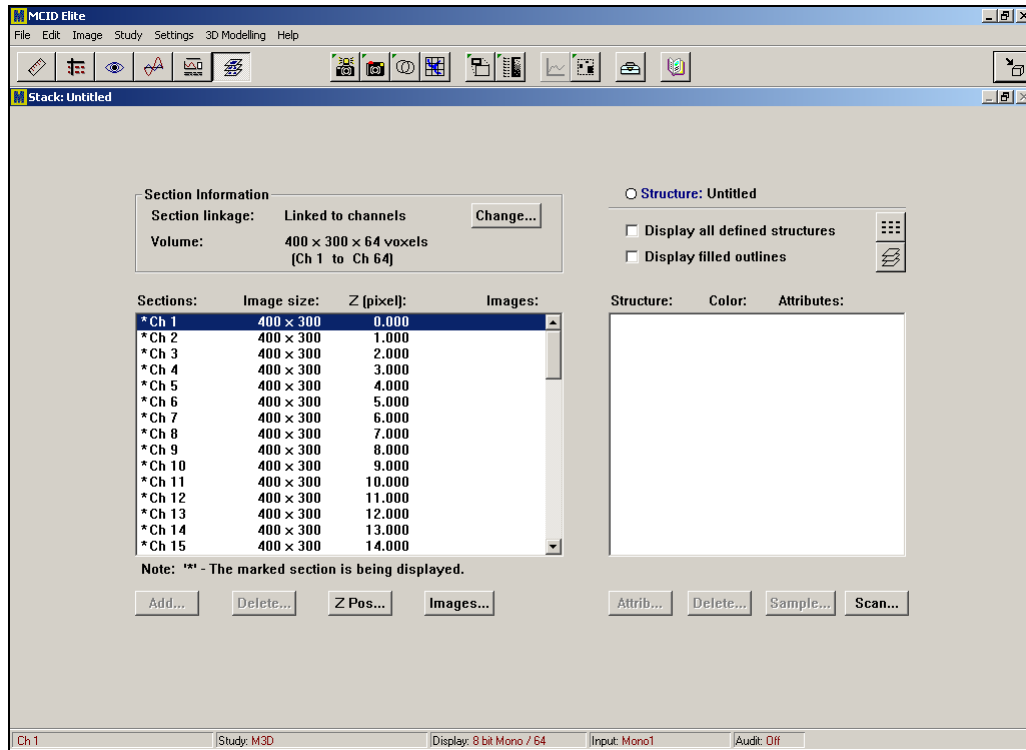
The **3D Rendering Control** window icon. This icon opens and closes the *3D Rendering Control* window.

Loading an Image Set and Creating a Stack

The file handling commands in **M3D** are similar to those in **MCID Elite**, but instead of using individual images you are working with image sets most of the time.

First you must select the number of channels needed to contain the image stack. Click on the *Settings* menu and select a *Display format* that provides 16 channels per page.

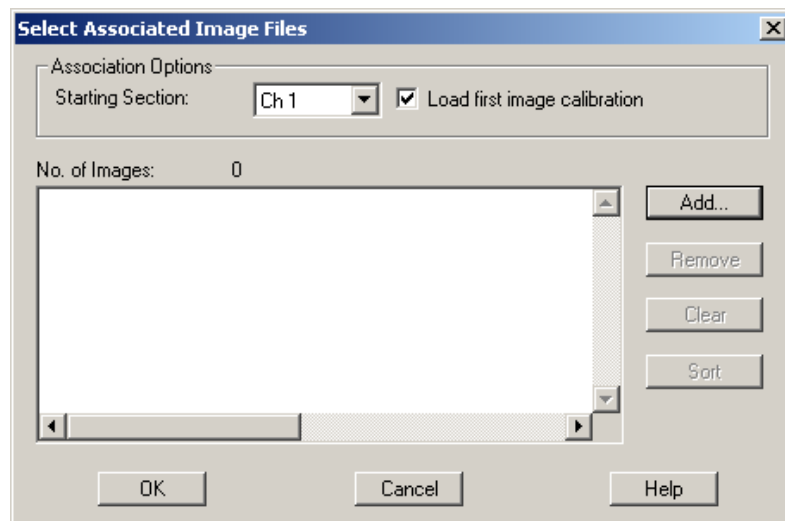
Figure 15: The Stack window now contains a list of 16 channels.



Click on the **[Images]** button at the bottom center of the window. The *Select Associated Images* dialog box appears (Figure 16).

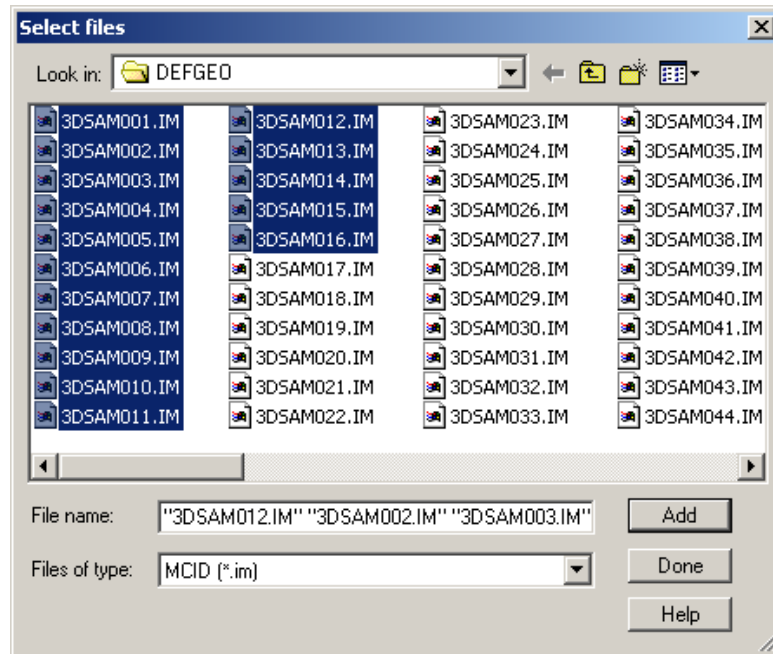
The **M3D** sample image files are saved in a folder named **DEFGEO**. You need to open **DEFGEO** and find the images **3DSAM001.IM** to **3DSAM120.IM**. This is a series of 120 sections from a computer generated image of a sphere and a hollow cylinder. For this part of the tutorial, you will load the first 16 sections into the 16 available channels.

Figure 16: The *Select Associated Image Files* dialog box.



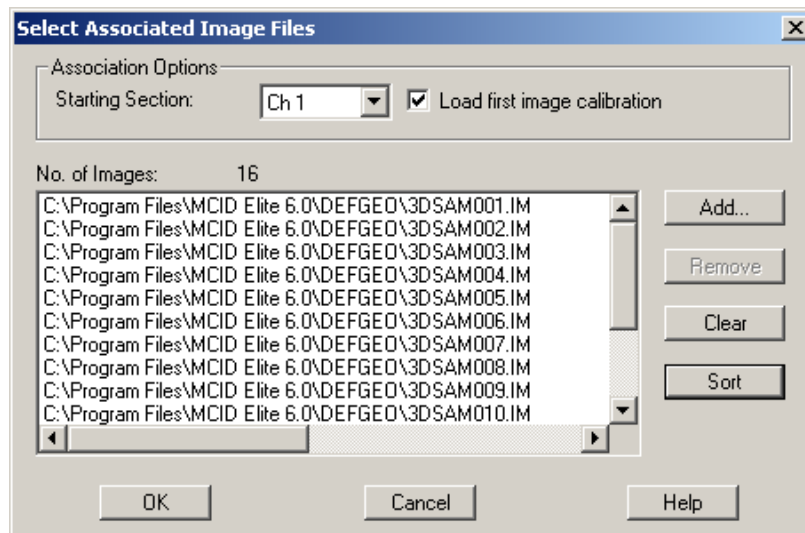
To load the images, click on the **[Add]** button. This will display the *Select Files* dialog box, Figure 17.

Figure 17: *The Select Files Dialog box.*



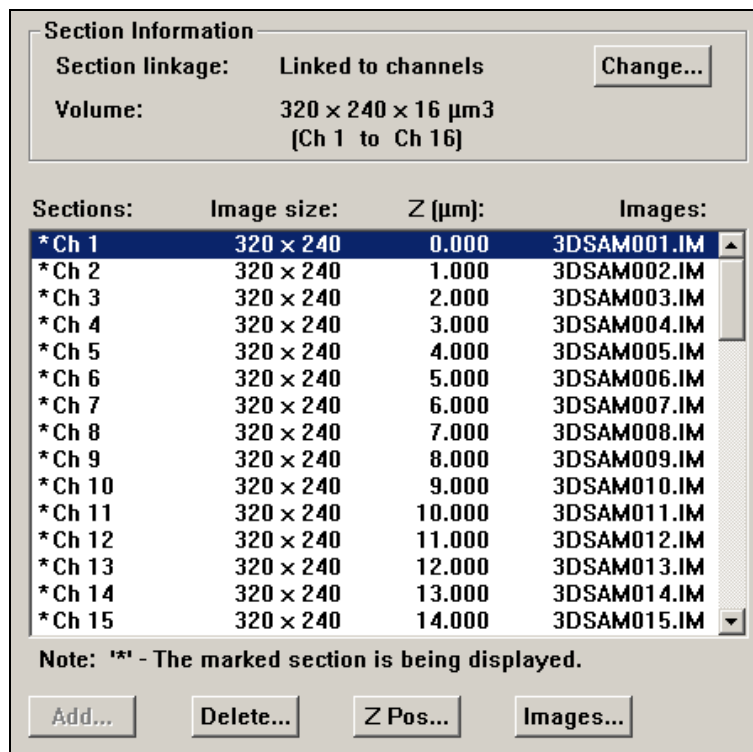
Navigate to the \MCID Elite 6.0\DEFGEO folder. Ctrl-click on the first 16 files in the list (3DSAM001.IM – 3DSAM016.IM), then click on **[Add]** to transfer the selections to the *Select Associated Images* dialog box. Click **[Done]** to return to the *Select Associated Image Files* dialog box (Figure 18). Click the **[Sort]** button to make sure the files are arranged in their proper order. When you click on **[OK]**, the first image loads into channel 1, the second into channel 2 etc. and the last image in channel 16. When loading is complete you have a stack of 16 sections loaded into 16 channels.

Figure 18: *The retrieved files have been sorted and are ready for loading.*



If there is a checkmark in the **Load first image calibration** checkbox, calibration data (including a Z axis calibration) are loaded with the images and displayed in the *Stack* window **Section Information** list (Figure 19).

Figure 19: The Section Information list in the Stack window, showing the first 15 of 16 images. Scroll down to see the image 16.



Images displayed on the image monitor have a star in front of the file name. If this list is loaded into a system with the *Display format* set at 16 channels per page, all the sections are displayed on the image monitor. With the *Display format* set to 4 channels per page, the first four images are displayed on the first page, the next four on the second page, etc.

Making Measurements

You can use any standard **MCID Elite** functions to sample the images in the stack. For example, you can read fluorescence intensity and area from any section. If the images are not pre-aligned, you can use the alignment routines to correct this. Of course, these images *are* pre-aligned, so we will leave a discussion of section alignment until later. You may want to experiment by making measurements and adjusting the image visuals, just to confirm that in Construction mode, **M3D** operates very much like **MCID Elite**.

Creating a 3D Dataset

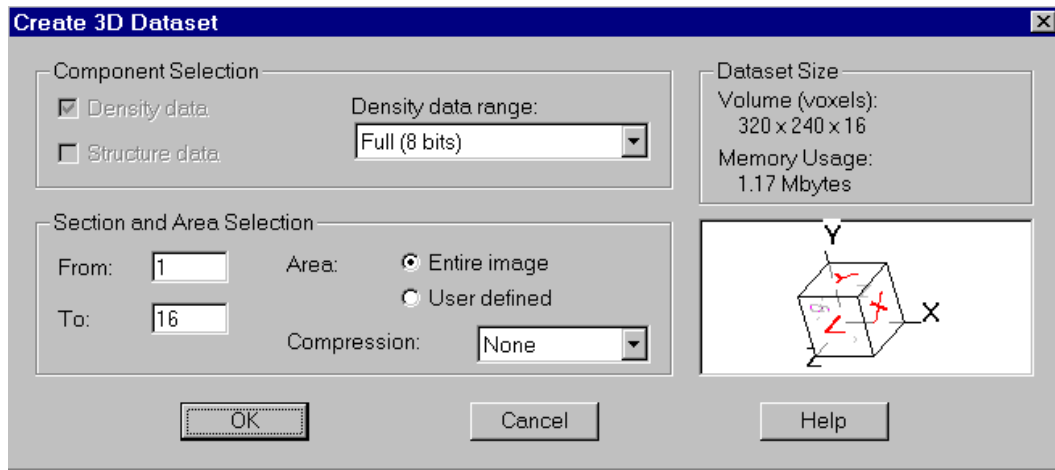
A 3D dataset is prepared by interpolating voxels from a stack of 2D sections. Making the 3D dataset may take some time, because **M3D** must interpolate voxels from the entire stack of images, but once you have created a 3D dataset you can save it and then retrieve it

like any image file. This allows you to retrieve and render 3D datasets without going through the process of loading stacks and interpolating each time.

TO CREATE A 3D DATASET:

1. Click on the *3D Modeling* option in the menu bar.
2. From the *3D Modeling* menu, select *Create 3D dataset*. The *Create 3D Dataset* dialog box appears.
3. Click on [OK] to start the interpolation process.

Figure 20: The *Create 3D Dataset* dialog box.



M3D interpolates the stack to create a dataset. While the computer is performing the interpolation, an information box appears on the screen showing the percentage of data interpolated. The current stack is quite small, so the process will be very fast. When it has finished, another information box appears prompting you to switch to **Rendering** mode. Click on [Yes].



If you answer [Yes], **M3D** switches you into **Rendering Mode**. If for some reason you clicked **No**, you are still in **Construction Mode**. You can switch to **Rendering Mode** by clicking on the mode icon at the top right of the host display.



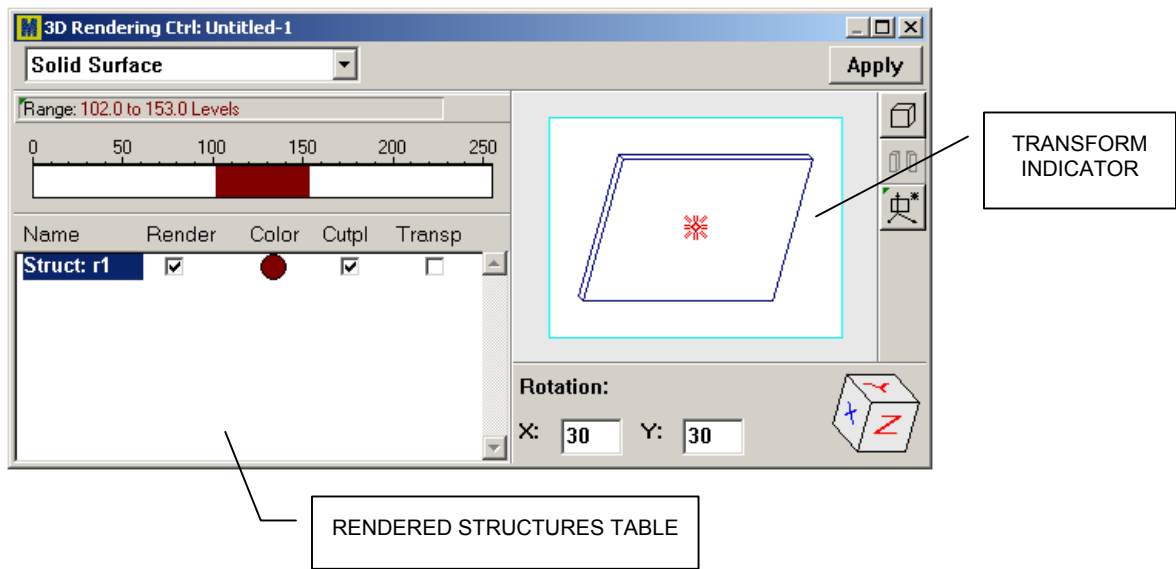
When **M3D** switches to **Rendering Mode**, the 16 images on the display monitor disappear and you see a blank screen. The *3D Rendering Control* window opens on the program monitor.

The *3D Rendering Control* window converts the interpolated stack into a 3D rendering. It allows you to select the type of rendering you want to apply, the gray level ranges to include, the orientation of the rendering and which structures to render.

For example, you can change the angle of illumination of the 3D image, apply different colors, change a structure's transparency or apply a cutting plane to show internal details.

Rendering the Sample Dataset

Figure 21: The 3D Rendering Control window. The transform indicator is thin because you used only 16 sections to create the dataset.

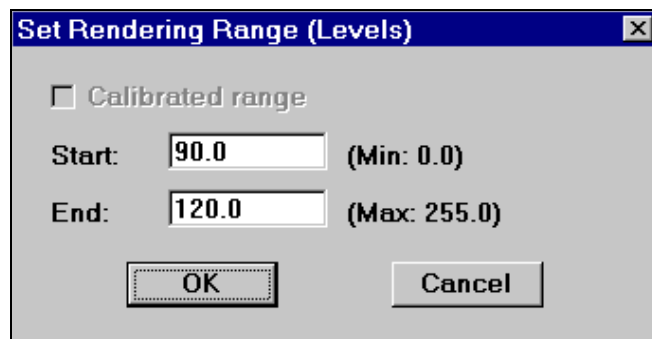


Setting the Rendering Range

With the default gray level range set between 102-153, you would not see a rendering of your dataset because the gray levels of the circle and the ring fall outside these values. You need to adjust the range to include the gray level values of the circle and the ring.

First, adjust the Range bar to change the voxel gray levels forming the image. Double click on **Range** above the bar. This brings up the *Set Rendering Range (Levels)* dialog box (Figure 22).

Figure 22: The *Set Rendering Range (Levels)* dialog box.



Enter 90 gray levels for the **Start** and 120 gray levels for the **End**, (Figure 23A). Click **[OK]** to set the range and then press **[Apply]** to see a solid disk. Double click on **Range** above the bar and enter 200 gray levels for the **Start** and 255 gray levels for the **End**, (Figure 23B). Click **[OK]** to set the range and then press **[Apply]**. This time you see the ring. To see both structures you need to expand the range from a point above 250 to a point below 100.

The left and right ends of the Range slider are adjustable. When you move the mouse cursor onto the left end of the slider, the cursor becomes a double arrow. Hold down the left mouse button and drag the left end of the slider down the scale to a point below 100 (Figure 23C). Click on **[Apply]**. This time you see the disk and the ring.

Figure 23: The Range bar with the slider set to a range of gray levels that displays the disk(A) and the ring (B). Expanding the range (C) shows both structures.

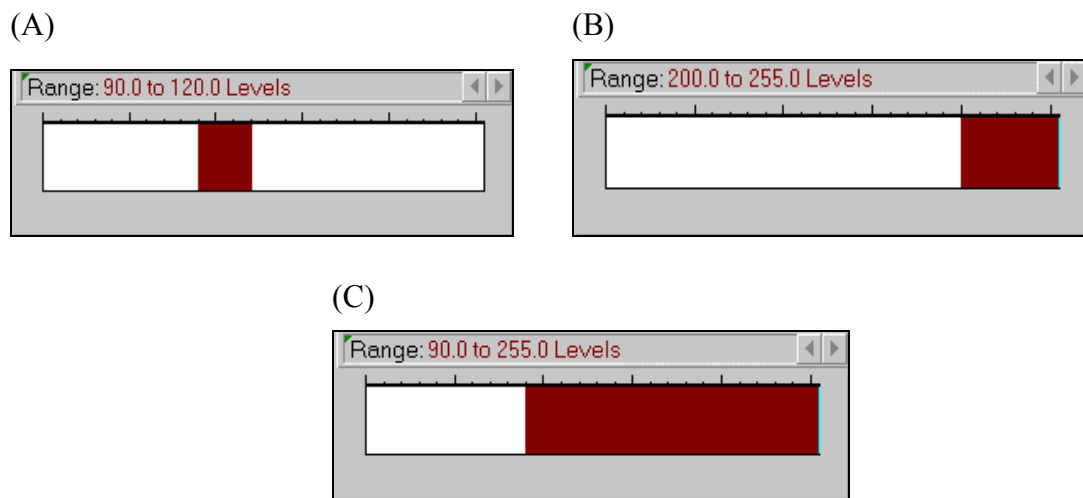
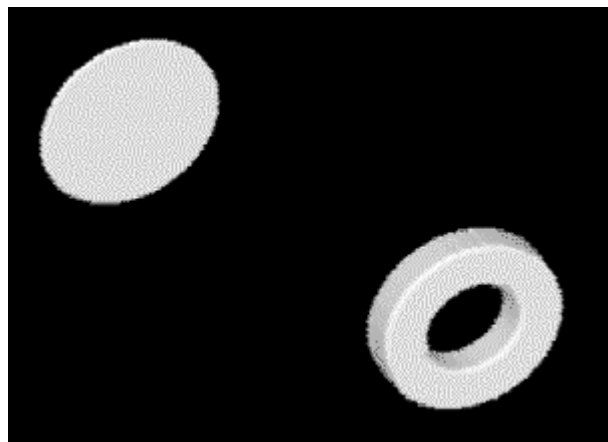


Figure 24: Rendering of the first 16 sample images with a rendering range set between 90 and 255.



Manipulating the Image

At the right side of the dialog box, there is an outline block with a red star at its center. This block (called the **Transform indicator**) represents the orientations of the 3D dataset. The red star shows the position of the light source. Move the mouse cursor to the red star, hold down the left mouse button and use the mouse to drag the light source to a new location. Click on **[Apply]** to see the effect of changing the direction of illumination on the image.

The **Rotation Cube** lets you change the orientations of the dataset. To use it, move the mouse cursor to either a cube face, edge or corner and hold down the left mouse button. Drag the cube to change its orientation and then release the left mouse button. Click on **[Apply]**. The rendered image is redrawn in the new orientation. To restore the default orientation, click on the default icon.



At the moment, you are working with a solid surface rendering, as shown in the rendering type drop down menu (just under the title bar of the window). **M3D** can perform many different types of rendering. These are discussed later in the tutorial.

Summary

During this part of the tutorial, you learned how to start **M3D**, how to load sets of 2D images and how to create a 3D dataset. You learned how to switch between Construction mode and Rendering mode and how to perform a simple surface rendering of a density data set. Later in the tutorial you will use the same sample images, but this time rendering the entire 120 section set.

Tutorial Part 2: Defining Structures, Contour Rendering

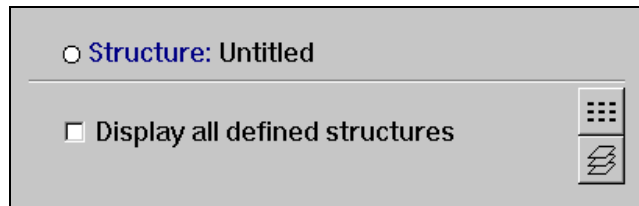
For Part 2 of the tutorial you will be working with the same set of images you used in Part 1. You need to move back to the *Stack* window. Click on the **Construction mode** icon at the top right of the screen.



Defining Structures

Near the top right of the *Stack* window there are two icons, a check box and a labeling control (Figure 25). These control several related operations. They allow you to create a list of labels and display the current one, display the ranges of defined structures, display one or more structures within the image set and perform contour rendering using the structures.

Figure 25: The Structures area of the Stack Window.



Clicking on **Structure:** calls up the Structures list (Figure 26). This lets you define labels for structures and select which structure is the current one. Type in the name of the anatomical structure you want to define, then press **[Add]** to add it to the Structures list. If you want to keep the list of labels, open the *File* menu at the top left of the list and save it using *File > Save*. To load a previously saved label file, use the *File > Open* command. Files are saved with the .LBL file extension. The *Edit* command lets you rename structure labels in the list, or delete them.

Enter the labels “Ring” and “Circle” as described above and delete the “Untitled” label.

Figure 26: The Structures list with the 3D 3dsamp.lbl label file. We called the structures circle and ring, because that’s what you see in the channels. You can use any names you like.





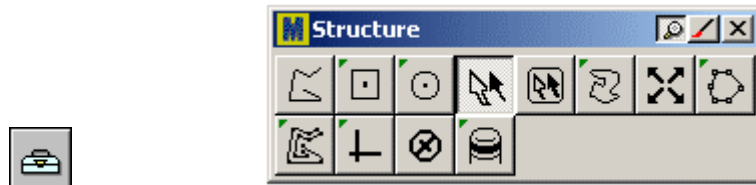
This icon calls up the **Defined Structures: Ranges** window (Figure 31).



This icon calls up the **Contour Rendering** window (Figure 32).

When you have created the labels, you can identify the structures in the images. Click on the **Tools** icon and the **Structure** toolbox appears. Select the **Autoscan A** tool.

Figure 27: The Tools icon and Structure toolbox with Autoscan A tool selected.



Select **Circle** from the Structures list, move the **Autoscan** tool to Channel 1 and click on a region of background. Now move the tool into the circle and click again. The circle is outlined in red. Move to the Channel 2, and repeat the process. Continue until you have defined the circle in all 16 sections.

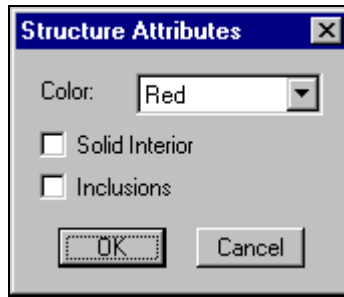
Figure 28: The Defined structures list in the stack window showing circle and ring defined. The Solid interior shows that the solid option was chosen in the Attributes dialog box.

Defined structs.:	Colors:	Interior:
Circle	Red	Solid
Ring	Green	Solid

Attrib... Delete... Sample... Scan...

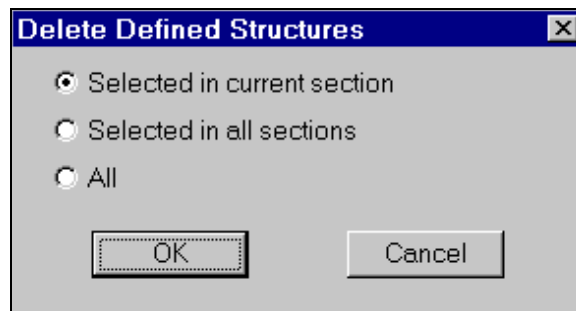
Select **Ring** from the Structures list, move the cursor back to the image display and define the ring. To change the color of the ring, click on **[Attrib]**. The *Structure Attributes* dialog box appears. If there is a mark in the **Display all defined structures** checkbox, all the structures you define are displayed on the images. If the box is unchecked, **M3D** shows only the current highlighted structure.

Figure 29: The Structure Attributes dialog box.



To choose a second color, click on the down arrow to the right of the **Color:** field. If there is a mark in the **Solid Interior** checkbox, you can use the **Hidden** icon in the contour window to “fill” the circles. If you make a mistake defining structures you can delete the error by clicking on the **[Delete]** button beneath the *Defined Structures* list (Figure 30). This calls up the *Delete Defined Structures* dialog box, that lets you delete the selected structure from the current section, all sections or all structures in all sections.

Figure 30: The Delete Defined Structures dialog box.



You can also remove structures individually by selecting the **Delete** tool in the toolbox.

To see a table of the defined structures and the range of sections in which they occur, click on the **Ranges** icon to call up the *Defined Structures: Ranges* window.

Figure 31: The Defined Structures: Ranges window showing the circle and ring defined on images in 16 channels.

Structures:	From:	To:
Ring	Ch 1	Ch 16
Circle	Ch 1	Ch 16

After you have defined one or more structures, you can proceed to interpolate and render them just as if you were using entire images. Very often, complex datasets are easier to understand if you use a “building blocks” approach and begin by working with a few structures from the images rather than attempting the entire set in one step.

Note: Each boundary detection function works within one section at a time, so you must define all the structure boundaries in one section before moving to the next one.

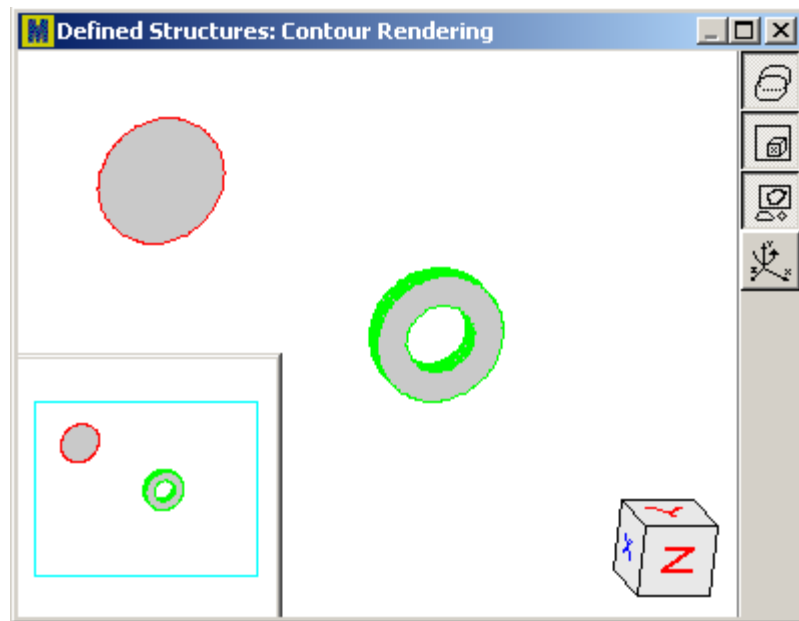
Contour Rendering

Contour rendering produces reconstructions of the defined structures and is often a useful first step to get an idea of how a rendering will appear.

Click on the **Contour Rendering** icon. The *Defined Structures: Contour Rendering* window appears.



Figure 32: The *Defined Structures: Contour Rendering* window showing 16 sections of the circle and ring. Selecting the **Hidden** icon creates the gray fill, and the **Viewport** icon calls up the **Viewport** in the lower left corner.



The *Contour Rendering* window has a **Rotation Cube** and four icons on the right hand side.

Contour Rendering Window Icons



Hidden

The **Hidden** icon controls contour display. Contours are shown as a series of overlapping outlines (often called a “wire-frame” display). This is often very difficult to interpret, especially with more than 3 contours on the display. Clicking on **Hidden** adds a gray fill to each slice and obscures overlapping and confusing internal structures.

**Show rotation cube**

The **Show rotation cube** icon turns the rotation cube on and off. The rotation cube is used to control the orientation of the contour rendering (see below).

**Default rotation**

The **Default rotation** icon resets the orientations (X, Y, Z) of the rotation cube and the contours to those of the original image data (see below).

**Viewport**

The **Viewport** icon displays a “Viewport” in the lower left corner of the window. This contains a light-blue, adjustable frame. Move the mouse cursor onto the frame border. The cursor changes into a double-headed arrow. Hold down the left mouse button and drag the border to change the frame size. Releasing the mouse button redraws the image at a higher magnification.

Move the mouse cursor inside the frame. The cursor changes into a white, 4 headed arrow. Hold down the left mouse button then drag the frame to a different area of the image. Releasing the mouse button redraws the image showing the new features.

Rotation Cube

The **Rotation Cube** lets you redraw the contoured image by changing the position of the X, Y and Z axes. To use it, point to either a cube face, edge or corner and hold down the left mouse button. Drag the cube to change the orientation and then release the left mouse button. The contoured image is redrawn in the new orientation. To restore the original orientation, click the **Default rotation** icon.

Saving the Stack

Defining several structures in a large stack of images takes time and can be rather tedious. You can save the stack by using the *File > Save* command in the *Stack* operational window. Stack files are saved with a *.dg file extension. These files save the screen contents and contain the following information:

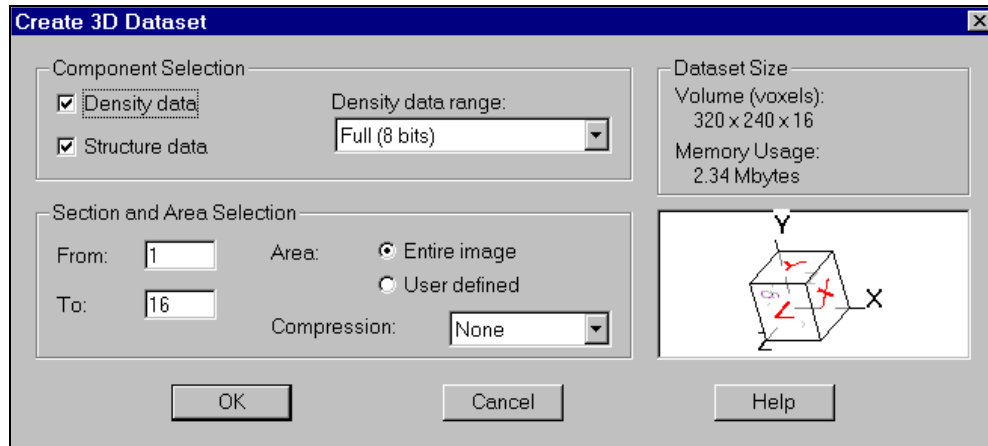
- Section list
- Location of associated images (filenames and file types)
- Density and spatial calibrations
- Defined structures.

When you want to load the saved stack file, use the *File > Open* command in the *Stack* window.

Creating a 3D Dataset from Defined Structures

Creating a 3D dataset using structures is similar to the process you carried out in Part 1. This time, be sure to check the **Structure data** checkbox as well as the **Density data** checkbox, as shown in Figure 33.

Figure 33: The Create 3D dataset dialog box with Structure data selected.



TO CREATE A 3D DATASET:

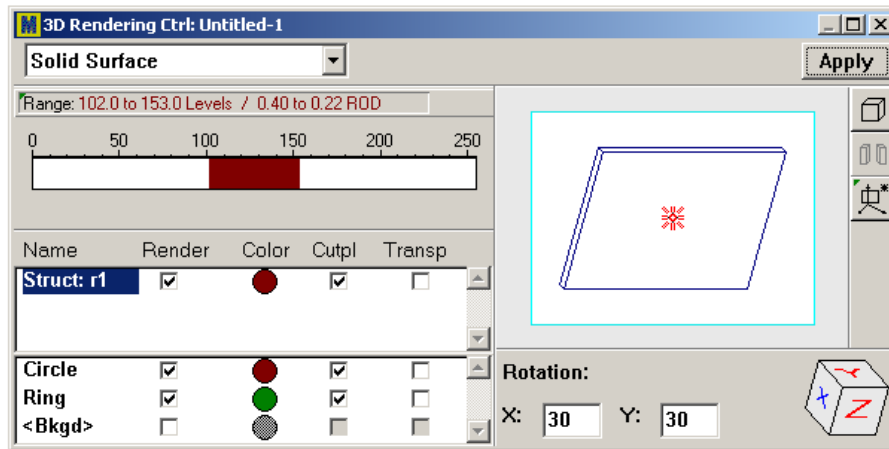
1. Open the *3D Modeling* menu and select *Create 3D dataset*.
2. In the *Create 3D Dataset* dialog box, place checkmarks in the **Structure data** and in the **Density data** checkboxes.
3. Click on [OK] to start the interpolation process.

This time, **M3D** interpolates the defined structures in the stack to create a dataset. While the computer is performing the interpolation, an information box appears on the screen showing the percentage of data interpolated. When it has finished, another information box appears prompting you to switch to **Rendering** mode. Click on [Yes].

Rendering the Dataset

Choose the defined structures you wish to render by clicking on the appropriate checkbox in the **Rendered structures** table. Click on [Apply] to see the current rendering.

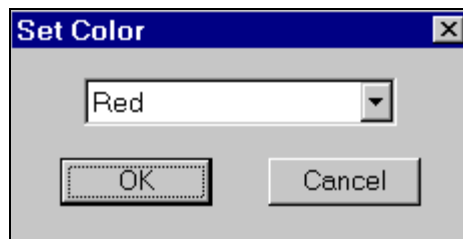
Figure 34: The 3D Rendering Control dialog box. Checkmarks in the Render checkboxes show that the circle and ring defined structures are both part of the current solid surface rendering.



Changing Colors

If you want to show a defined structure in a particular color, click on the **Color** dot in the rendered structures table. The *Set Color* dialog box appears. Click on the down arrow to see a list of color choices. Click on **[OK]** to close the dialog box. Click on **[Apply]** to see the change take effect.

Figure 35: The Set Color dialog box.



Summary

In Part 2 of the tutorial you learned how to define two structures within a series of sections and interpolate the structures to create a contour rendering. Then you used the defined structures to create a 3D rendering and applied some simple presentation changes to the rendering.

Tutorial Part 3: Working with Large Image Stacks

For Part 3 of the tutorial you will be working with the same set of images you used in Parts 1 and 2. You need to move back to the *Stack* window. Click on the **Construction mode** icon at the top right of the screen.



Section Linking

In the previous parts of this tutorial, you worked with 16 images from a set of 120 sections. **M3D** had 16 available channels, so each image fitted neatly into one of those channels. Most 3D renderings, however, use far more than 16 sections. For example, MRI images may contain over 100 sections, and each section must be used for the 3D rendering to appear realistic. In this part of the tutorial, we introduce the idea of linking sections to image files. Unlike linking to physical channels, this is not restricted by the system configuration and lets **M3D** create stacks containing any number of sections.

Linking to Image Files

You should be familiar with the *Settings > Display format* menu that sets the display mode to show a single image on a page (single channel mode), or more than one image on a page (multi-channel mode). Normally, each channel contains one image so the number of displayed images cannot exceed the number of available channels.

Obviously, channel limitation is a serious problem. For example, an image set might contain 100 sections, each measuring 512 x 512 pixels. This would exceed even the channel capacity of **MCID Elite**, unless we compressed the images. There must be a method for handling any number of sections at any resolution. **M3D** uses 'image file linking' as a solution to the problem.

A channel is an **M3D location** for an image. Some of the channels use image memory and are displayed. As you move up or down in the image list selecting a new series of sections for display, **M3D** loads the associated image files. Image files do not use memory resources *until they are displayed*, so any number of sections can be incorporated into a stack.

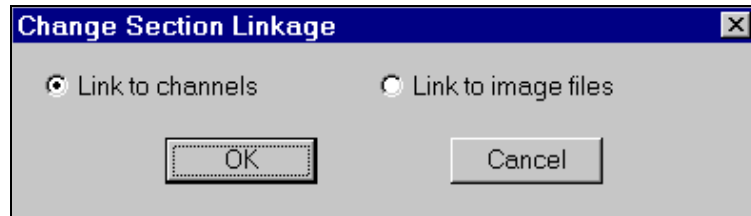
For example, you might load 100 images into an **M3D**. The first four are loaded into channels 1-4, and these are visible on the image monitor. You can see the images in channels 1-4, because they are in image memory. The next four images are associated with image files 5-8, and are not displayed until you select them for display. These images are held in host memory, or on disk, until you choose to view them. At this point **M3D** automatically retrieves the files, and displays sections 5-8 with their associated images.

If you are working with images linked to channels, all **MCID Elite** image processing functions work within the channel. If you are working with images linked to files you must either overwrite the original image, or save the processed images in new files. This is because the file linkage mode works with temporary copies of the images that are replaced when the next images are loaded.

Loading Linked Image Files

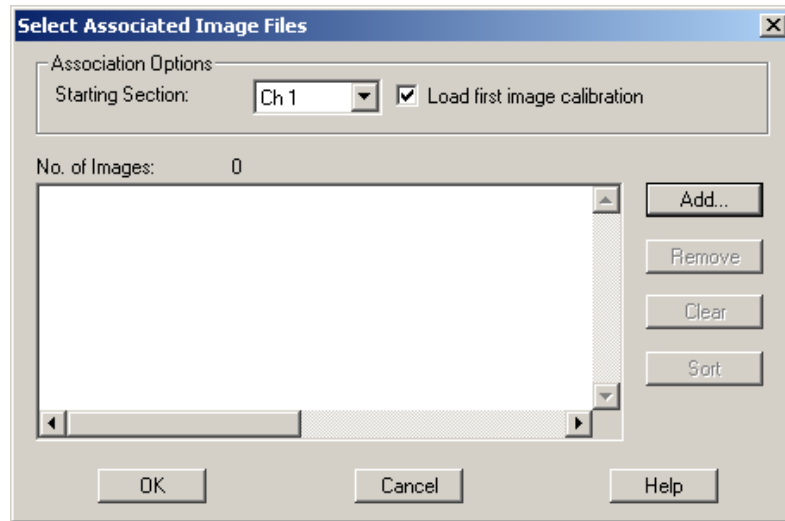
1. Open the *Settings* menu and select *Display format*.
2. Select the **16 channels/ page** display mode to display sixteen images on the monitor.
3. Click **[OK]** to exit the dialog box..
4. Click on the **[Change]** button in the **Section Information** area of the *Stack* window. The *Change Section Linkage* dialog box appears.

Figure 36: The *Change Section Linkage* dialog box.



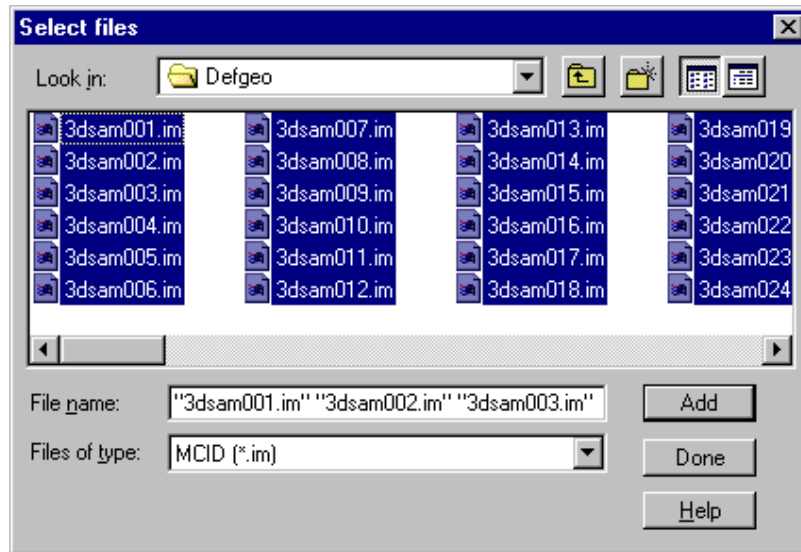
5. Select the **Link to Image Files** radio button.
6. Click **[OK]** to exit the dialog box.
7. Click on **[Images]** button in the *Stack* window. The *Select Associated Image Files* dialog box appears.

Figure 37: The *Select Associated Image Files* dialog box.



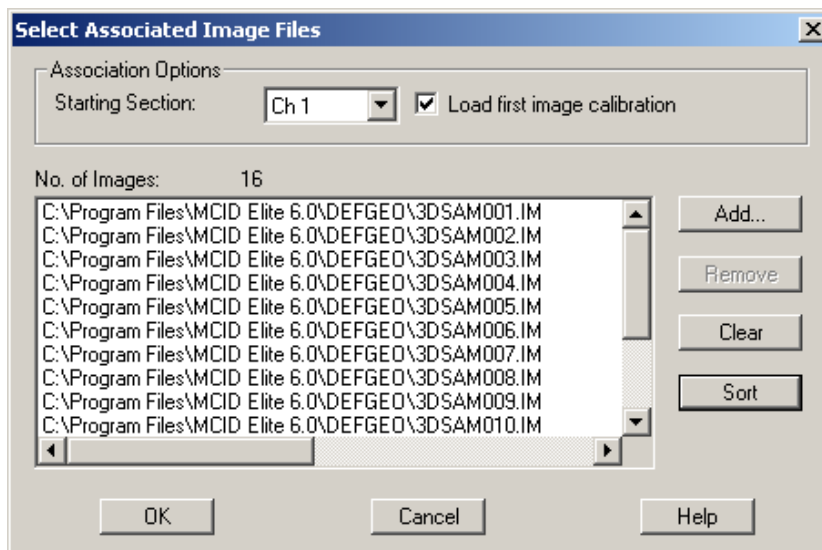
8. Click on **[Add]** to display the *Select Files* dialog box.

Figure 38: The Select files dialog box with all the files highlighted, ready for retrieval.



9. Navigate to the *MCID Elite 6.0\DEFGEO* folder and highlight all 120 files (click on the first file, then Shift-click on the last file).
10. Click **[Add]** to transfer the list to the *Select Associated Images* dialog box.
11. Click **[Done]** to exit the *Select Files* dialog box.

Figure 39: The Select Associated Image Files dialog box now contains 120 images ready for loading.



12. Click **[Sort]** to make sure the images begin with 3dsam001.im through to 3dsam120.im.
13. Click **[OK]**. The image filenames are displayed in the **Section Information** list, and the first 16 sections (Sc1-Sc16) are displayed on the image monitor.

Controlling the Image Display

When you click on **[OK]**, the first set of 16 images/sections are loaded into Channels 1-16. The first image loads into Channel 1, the second in Channel 2, etc. The last image is linked to image file 120. Only the first sixteen channels are displayed on the monitor. To move to sections 17-32, just highlight section 17 in the Section information list.

Images displayed on the image monitor have a star in front of the file name. If this list is loaded into a system with the *Display format* set at 16 channels per page, all the sections are displayed on the image monitor. With the *Display format* set to 4 channels per page, the first four images are displayed on the first page, the next four on the second page, etc.

Defining Structures

You can define the circle, ring and box as separate structures in the entire stack, then create the dataset for a combined surface and density rendering.

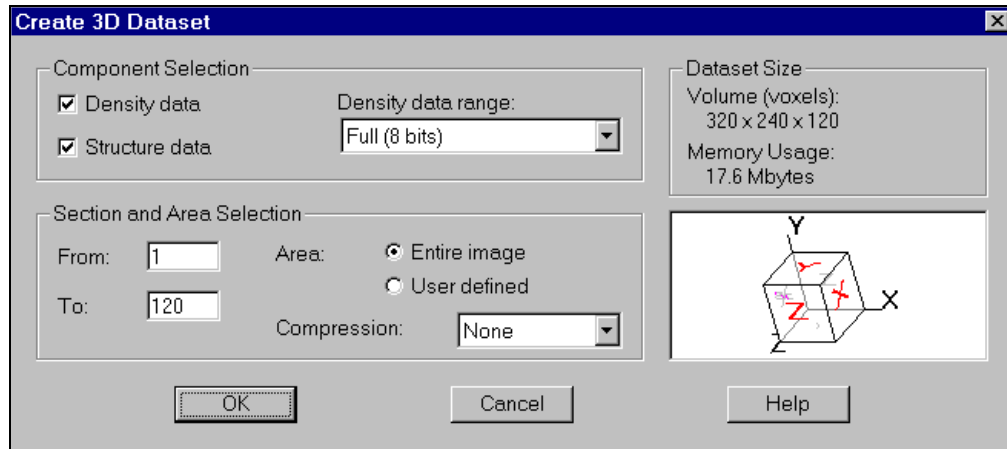
1. Click on the **Tools** icon to display the **Structure** toolbox. Select the **Autoscan A** tool.
2. Select **Circle** from the Structures list, move the Autoscan tool to Channel 1 and click on a region of background. Now move the tool into the circle and click again. The circle is outlined in red. Move to the Channel 2, and repeat the process. Continue until you have defined the circle in each of the 120 sections.
3. Select **Ring** from the structures list, move the cursor back to the image display and define the ring.
4. Add **Box** to the structures list, move the cursor back to the image display and define the box within the circle.

Note: Each boundary detection function works within one section at a time, so you must define the circle and ring structures in all sections. Defining several structures in a stack of 120 images takes time and can be rather tedious. If you wish to stop the structure definition process and return to it later, save the stack by using the *File > Save* command in the *Stack* operational window. Stack files are saved with a *.dg file extension.

Creating a 3D Dataset

You can now interpolate the full set of images and produce a 3D dataset in the same way as you did in parts 1& 2 of the tutorial. This time place a mark in the density and structure checkboxes to create a combined density/structure rendering.

Figure 40: The Create 3D dataset dialog box for combined density and structure rendering.



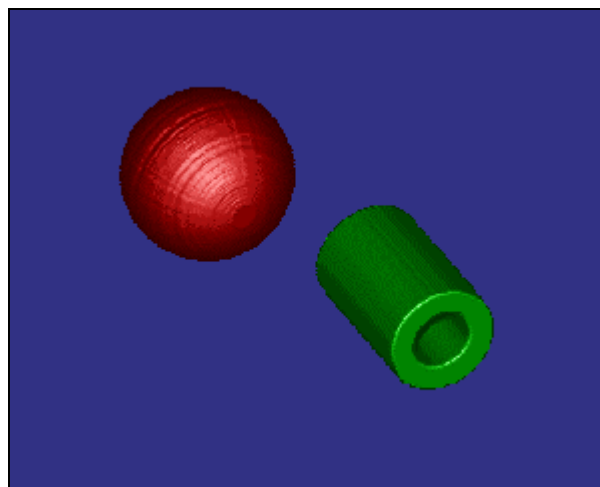
1. Open the *3D Modeling* menu and select *Create 3D dataset*.
2. In the *Create 3D Dataset* dialog box, place checkmarks in the **Structures** and **Density** checkboxes.
3. Click on [OK] to start the interpolation process.

This time, **M3D** interpolates the defined structures and density in the entire stack to create the dataset. While the computer is performing the interpolation, an information box appears on the screen showing the percentage of data interpolated. When it has finished, another information box appears prompting you to switch to **Rendering** mode. Click on [Yes].

Rendering the Dataset

In the *3D Rendering Control* window, click on [Apply]. This time you see the sphere (circle) and the hollow cylinder (ring).

Figure 41: Rendering of the complete sample dataset.



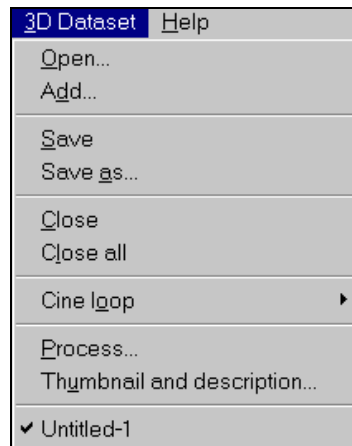
Saving the 3D Dataset

Defining structures in a large stack then waiting for **M3D** to interpolate is rather tedious, and you do not want to do this every time you intend to make a rendering. If you save the 3D dataset, you can recall it at any time without repeating the time-consuming interpolation step. You will need the sample 3D dataset in the remaining parts of the tutorial, so now is an ideal time to save it.

TO SAVE THE DATASET:

1. Open the *3D Dataset* menu (Figure 42).
2. From the *3D Dataset* menu select *Save*. 3D datasets are saved with a *.3st file extension and the saved files contain the following information:
 - Interpolated 3D voxels (density data, structural data or both).
 - Spatial and density calibrations.
 - Settings from the *3D Rendering Control* window (rendering settings, range, color and transform).

Figure 42: The 3D Dataset menu.



It is important to appreciate the relationship between stack files and 3D dataset files. The stack file is an intermediate step between the raw data, which is a series of 2D sections, and the 3D dataset. Saving the stack saves all the relevant information necessary to create many different kinds of renderings from a single image set. Saving the 3D dataset lets you recall it anytime, avoiding the need for tedious and time consuming interpolation.

If you save the stack and 3D dataset files after you have made them, you can save a great deal of time by avoiding the preparation stages. Table 2 shows a comparison of the properties of stack and dataset files.

Table 2: *A comparison of stack files and dataset files.*

Features	Stack file	3D dataset file
Purpose	Saving prepared stacks	Saving rendered datasets
Extension	*.dg	*.3st
Contents	Section list, structures, calibrations, filenames	Interpolated voxels, rendering settings, calibrations, filenames

Summary

In this part of the tutorial you loaded a large image set by linking each section to an image file. As image files are not displayed until the images are loaded, there can be any number of them. You then interpolated the stack of images to create a 3D dataset and saved it.

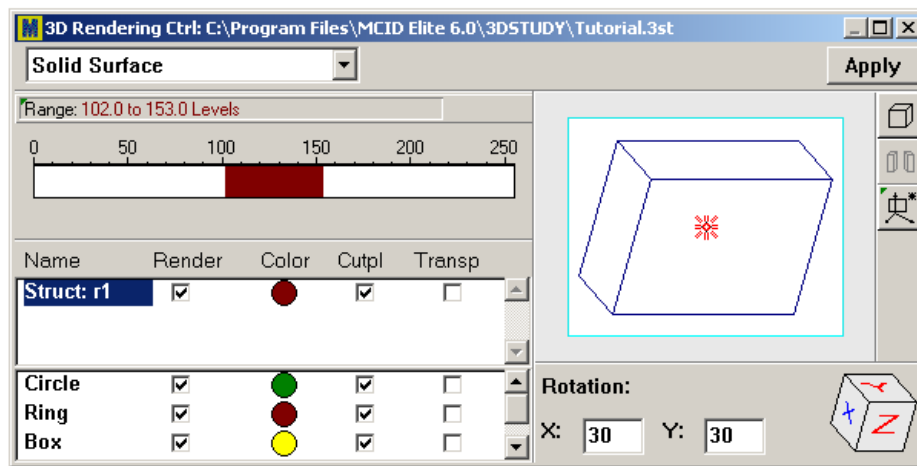
Tutorial Part 4: The 3D Rendering Control Window

For Part 4 of the tutorial you need to be in Rendering mode. If you are in Construction mode, click on the icon at the top right of the display.



The *3D Rendering Control* window controls dataset rendering and the appearance of the rendered image. You have used many of the features in the window already and should be familiar with the operation of the rotation cube, transform and range bar. In Part 4 we describe other areas of the window.

Figure 43: The 3D Rendering Control window.



Density and Structures Data Table

The **Density/Structures** table beneath the range bar is divided into 2 parts with density data shown in the upper part and structure data as defined in the structures list shown below. These labels change to reflect the type of dataset you created from the stack, and the type of rendering you choose. If you created a dataset using only density or structures, the table is not divided into upper and lower parts.

In Figure 43, the density range shows **Struct: r1**. This is abbreviated from Structure: Density Range 1. With more than one structure defined, Range 1 will be the first, Range 2 the second, starting at lower end of the gray level scale. If you choose a density rendering type, this label changes to Density 1, Density, 2 etc.

The checkboxes enable other options. If there is a check in the **Render** box, that data is used in the current rendering and the other checkboxes are active. If the **Cutpl** box is checked, you can apply cutting planes to the rendering to reveal internal details.






Transparency lets you adjust the transparency of the range to show internal structures.

The number of checkboxes, and the legends that describe them, changes according to the rendering type selected. For example, the density rendering types show only a single checkbox, but the Structure: Surf types show four.

Revealing Internal Structures

You can use cutplanes and/or transparency to reveal internal structures in the rendered image. Before starting this part of the tutorial, open the *Settings* menu, choose the *Display format* option, and set the **Display type** to **True Color**.

Figure 44: The Structures table showing 3 rendered structures, circle, ring and box, from the sample dataset. Cut plane applies to all the structures and transparency to none.

Name	Render	Color	Cutpl	Transp
Struct: r1	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Circle	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Ring	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Box	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input type="checkbox"/>
<Bkgd>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>

Cutting Planes

Before you begin, make sure you have checked the appropriate boxes in the rendered structures table. You cannot apply a cutting plane to a structure unless you select the appropriate **Cut planes** checkbox. You can choose from a range of cutting plane functions by clicking on the **Cutting Planes** icon at the top right of the 3D Rendering control window.

Click on the **Cutting Planes** icon.

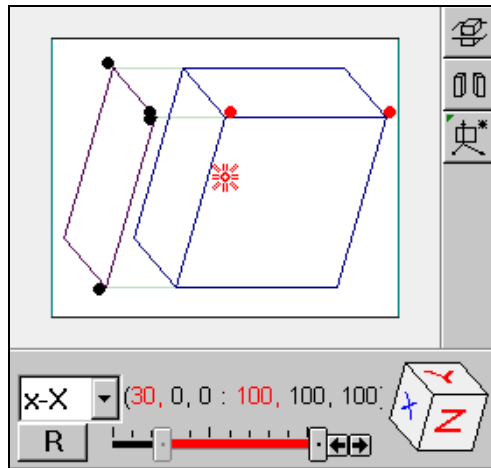


A horizontal icon bar appears. Choose the **Plane-cut** icon.



The transform changes to show the cutting planes applied to each axis and the cutting planes controls.

Figure 45: A cutting plane applied to the rendered sample dataset. The cut is made in the x-X axis and the result is shown in the next figure.

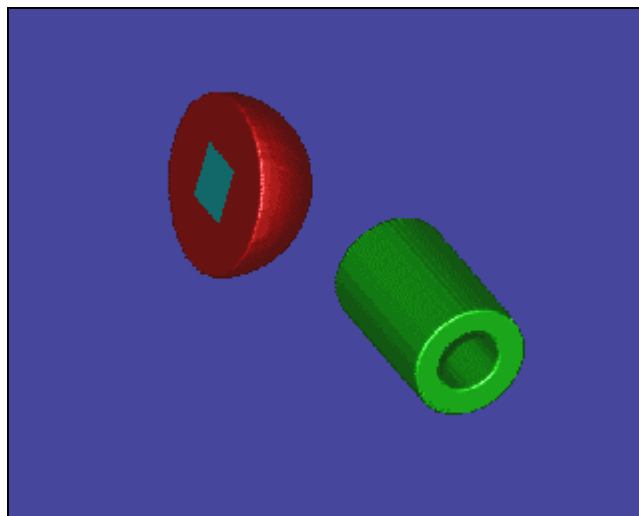


Applying a Cutting Plane to a Rendering

TO APPLY A CUTTING PLANE TO A RENDERING:

1. Click on the down arrow next to the x-X field to see a list of axes options.
2. Put the mouse cursor on one of the sliders, hold down the left mouse button and drag the slider along the scale. As you do this, the transform shows the cutting plane position in blue and the red dots at the corners move along the axis of the cutting plane or planes.
3. Click on [Apply] to see the effects.
4. To reset the sliders to their original position you can either click on the [R] (reset) button or drag the sliders to the ends of the scale.

Figure 46: A cutting plane (x-X axis) intersecting the sphere. The cut face shows a section through the sphere and the cube contained inside.



There are many more cutting planes options and you can experiment with all of them. For more information see the [Advanced Details](#) section.

Transparency

Applying transparency to a density rendering reveals internal structures. Transparency can apply to surface renderings or combined structure and density renderings. Click on the checkbox for any rendered structure that you wish to make transparent. The transparency checkboxes are “grayed” until you check the render box.

Viewport

The Viewport controls the magnification of the rendering. When you place the cursor on any edge of the box the cursor becomes a double arrow. Use this to drag the edge of the box then click on **[Apply]** to change the magnification. To view a different area, place the cursor inside the box, drag the box to a new location, then click on **[Apply]**.

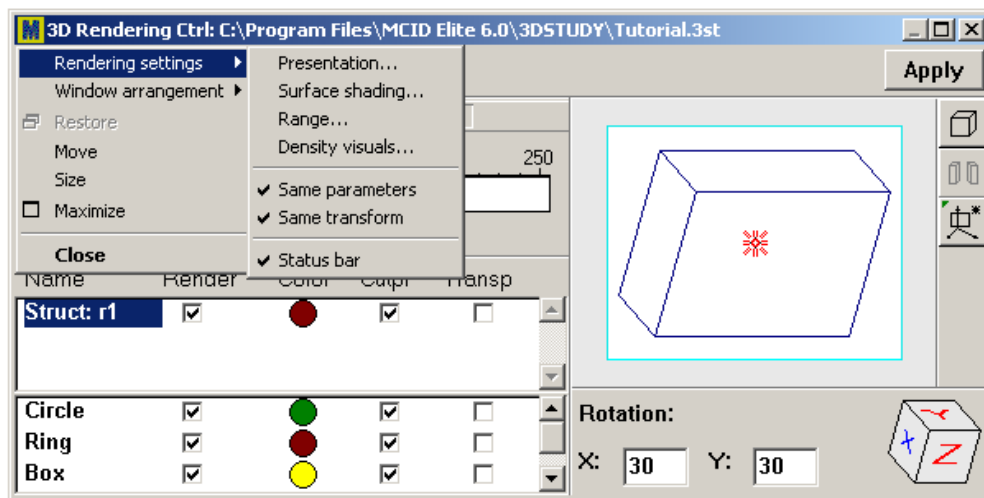
Tutorial Part 5: Presentation

For Part 5 of the tutorial you need to be in **Rendering** mode. If you are in **Construction** mode, click on the icon at the top right of the display.



In the *3D Rendering Control* window, click on the window menu button to see the window menu (Figure 47). This menu contains options for presenting the 3D renderings you create. These display options are interactive and very subtle in effect, so you should be prepared to experiment with different combinations.

Figure 47: The 3D Rendering Control window menu.

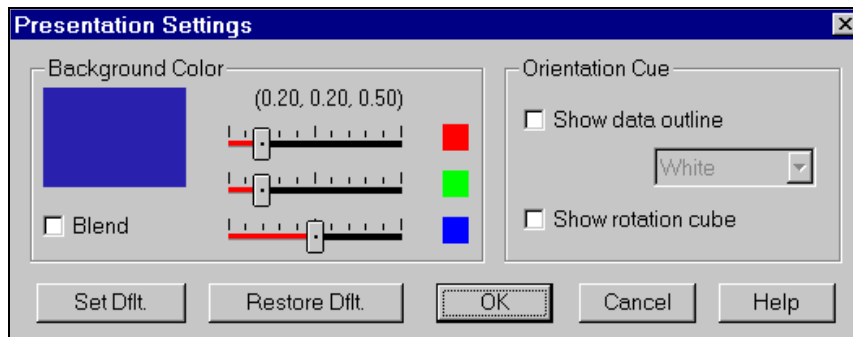


Rendering Settings

Presentation

Click on *Rendering settings* and from the sub-menu choose *Presentation*. The *Presentation Settings* dialog box appears, (Figure 48).

Figure 48: The Presentation Settings dialog box.



Background Color

This dialog box controls the background against which the 3D object is displayed. The default background color is dark blue and this works well with light colored 3D objects, but some very effective images can be created using blue backgrounds and red objects. Other color combinations may or may not work for your samples, but that should not stop you experimenting.

Dragging the red, green and blue sliders along the scale lets you “mix” new background colors. If you want to save the color as the default background, click on the **[Set Dflt]** button.

When you have changed these settings, click on **[Apply]** in the *3D Rendering Control* window to see the effects of your changes.

Orientation Cue

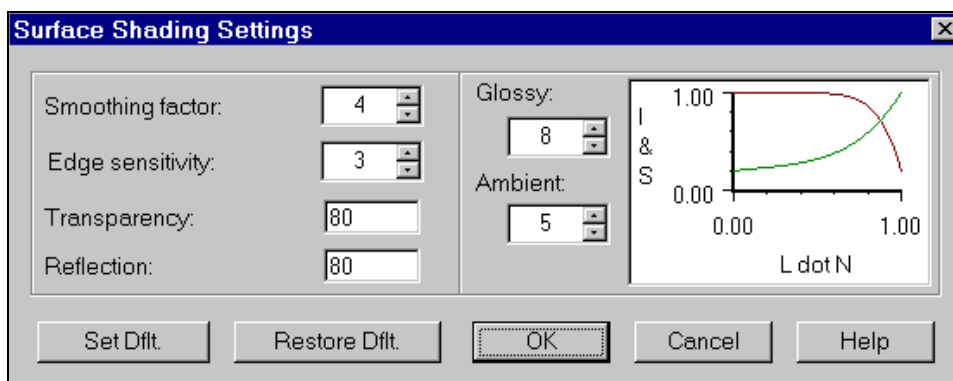
Visual cues are used to orient the object within 3D space. The rotation cube corresponds to the cube in the 3D Rendering Control dialog box and shows the current orientation of the dataset. The data outline shows you the boundaries of the stack of images. Clicking in each checkbox adds these cues to the rendered image display.

When you have changed the orientation, click on **[Apply]** in the *3D Rendering Control* window to see the effects.

Surface Shading

The rendered image is composed of a surfaced set of voxels. The appearance of the surface must be adjusted carefully, to yield a realistic-looking image. Click on *Rendering* settings and from the sub-menu choose *Surface Shading Settings*. The *Surface Shading Settings* dialog box appears. This dialog box gives you control over the surface appearance.

Figure 49: The *Surface Shading Settings* dialog box showing the default settings.



These controls are interactive in their effects and you should experiment with them to appreciate the results.

Smoothing and **Edge sensitivity** tend to counteract each other if both are set high. A low smoothing and no edge sensitivity shows an image with jagged edges, while a high

smoothing on its own removes much of the surface detail. Mid range settings usually produce the most pleasing effects.

Transparency and **Reflection** also tend to be counteractive. Low settings of transparency obviously reveal very few internal structures. Similarly, if both transparency and reflection are set high very little detail may be revealed. Increasing the transparency of a structure while keeping the reflection low reveals internal details. Increasing the reflection makes the transparent structure more visible, but may make an internal structure less visible, especially if the **Glossy** value is set high. Mid to mid-high range settings usually produce the most pleasing effects.

Glossy and **Ambient** apply a metallic sheen to the 3D images. High settings of glossy create this effect while low settings of ambient dim the 3D image.

After you change any of these settings, click on **[Apply]** in the 3D Rendering Control window to see the effects of your changes. Mid range settings usually produce the most pleasing effects. Click on **[OK]** to leave the *Surface Shading Settings* dialog box.

Creating Ranges

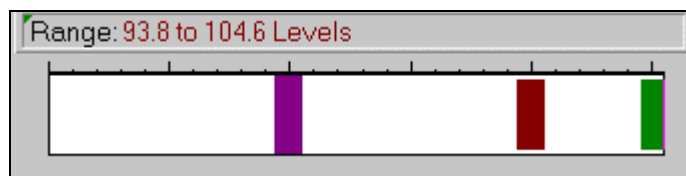
First, create 3 ranges for the 3 gray level values in the dataset. The procedure is:

1. Move the cursor to an empty area in the **Range** bar.
2. Hold down the left mouse button, then drag the pointer to a new position.
3. Release the mouse button.

Create ranges at about 100, 200 and 250 gray levels. Because the dataset contains just 3 levels, the width of each range does not really matter, as long as they do not overlap. If you make a mistake, you can delete the current range by pressing the **[Delete]** key.

The left and right ends of the **Range** sliders are adjustable. When you move the mouse cursor onto one end of the slider, the cursor becomes a double arrow. If you hold down the left mouse button and drag the end of the slider you can increase or decrease the range of gray levels included in the sample dataset. Figure 50 gives an idea of suitable range settings for the sphere, square and ring.

Figure 50: The Range bar with separate ranges defined for each gray level in the sample dataset.



Rendering Types

The number of options in the drop down menu varies, depending on the type of data interpolated in the stack. Table 3 shows a summary of the different rendering types.

Table 3: *Rendering types available with density and structural data interpolations.*

Rendering Type	Structure Data	Density Data	Density and Structure Data
Solid Surface	Yes	Yes	Yes
Solid Surface/Cut Planes	-	Yes	Yes
Density			
Maximum Density	-	Yes	Yes
Minimum Density	-	Yes	Yes
Surface Density	-	Yes	Yes
Density Color Opacity	-	Yes	Yes
Structure:Surface/ Max. Density	-	-	Yes
Structure:Surface/ Min.Density	-	-	Yes
Structure:Surface/ Surface Density	-	-	Yes
Structure:Surf/ Density Color-Opacity	-	-	Yes

The easiest way to understand the difference between each of the **M3D** rendering types is by comparing the effects of each one on the sample dataset. You should be able to compare the different rendering effects side by side, and for this you need to change the *Display format*.

1. Open the *Settings* menu and select *Display format*.
2. Under **Display type**, click on the **True Color** radio button then select 4 channels per page.
3. Click on **[OK]**.
4. Select the *3D Dataset* menu and click on *Open*. Load the dataset you saved at the end of Part 3.
5. Click on **[Apply]**. The dataset appears in Channel 1.

Now you can experiment with different rendering types in channels 2-4. The procedure is very simple:

1. Move the mouse cursor to the image monitor and place it in one of the empty channels.
2. Move back to the *3D Rendering Control* window and select a different rendering type from the drop-down list.

3. Click on **[Apply]**. The sample dataset is displayed in the channel. Any cutting planes or orientations applied to the first rendering apply to all subsequent ones (provided you do not change them), so you should be able to compare the effects of different rendering types directly.

Select each of the different rendering types in turn and apply it to the dataset. You need to create and adjust cutting planes to see some of the effects described in this section.

Surface Rendering

Solid Surface

This displays renderings as surfaces of pre-defined structures or surfaces from selected ranges of densities. You should see a sphere and a hollow cylinder.

Solid Surface/Cut-Plane Density

This displays the rendered surface and the density of the cutting planes. Select this rendering type and place a mark in the **CP dens** checkbox. If you create a cutting plane in the X axis, you should see a hemisphere with a square at the center of the cut face (Figure 46). You may need to reset the transform to its default, and possibly adjust the X axis distance to see exactly the same image. If you move the left cutting plane slider to a point between 18-20 on the scale, you should see something similar. Keep this cutting plane while you experiment with the different rendering types. It will help you understand what each rendering type reveals.

Density Rendering

Max Density

Displays the maximum density in the entire 3D dataset. Make sure that the range is set between 90 and 255 levels. You should see a gray sphere and a white cylinder. The sphere contains a light-gray cube. Create a cutting plane that intersects the sphere in the X axis. You should see a gray hemisphere with a light gray square at the center of the cut face.

Min. Density

Displays the minimum density in the entire 3D dataset. You should see a gray sphere and a white cylinder. If you kept the cutting plane from the previous rendering, you should see a gray hemisphere.

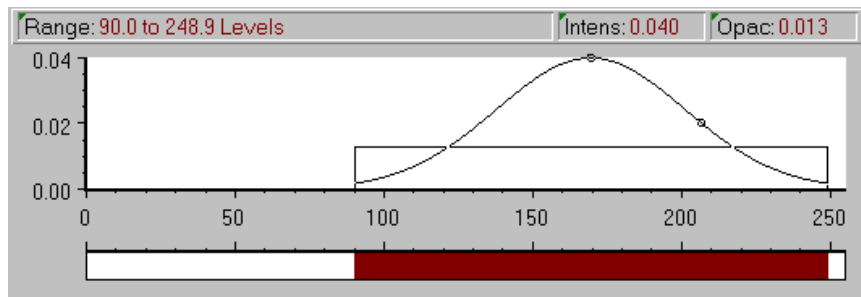
Surface Density

Displays the density at the surface of the 3D dataset. When you apply this rendering type to the sample dataset, the image appears similar to that shown by Minimum Density rendering. This is because the surfaces of the cylinder and sphere both have uniform densities. The “surface” revealed by the cutting plane shows the light-gray square at the center, because the cut face has 2 regions of different densities.

Density Color-Opacity

This lets you adjust the opacity and intensity of the rendered surface to reveal internal details. You need to adjust these settings to very low values (typically ~ 0.05) before you begin to see any effects. Reducing the opacity of the sphere reveals the cube inside. Select 0.04 for the **Intensity** and 0.013 for the **Opacity**. Set the range between 90 and 250. With these settings you will be able to see the cube inside the sphere.

Figure 51: *The Range Indicator when Density Color-Opacity rendering selected.*



1. Point to the **Gaussian** curve and click the left mouse button. Two markers appear, one at the peak and the other at the Full Width, Half Maximum Height point.
2. Move the cursor onto the peak marker then click the left mouse button. The cursor changes into a white double arrow.
3. Hold down the left mouse button and drag the double arrow downwards. This reduces the **Intensity** value in the **Status** bar.
4. Repeat steps 1-3 with the **Square** wave.
5. Click on **[Apply]** to see the effects on the 3D dataset.

Structure/Density Renderings





These rendering types show the surfaces of defined structures and the density information inside. When you choose one of these rendering types, different checkboxes appear in the **Density/Structures** table. Before proceeding further with the tutorial we need to recreate 3 ranges for the 3 gray level values in the dataset.

PROCEDURE:

1. Move the cursor to an empty area in the **Range** bar.
2. Hold down the left mouse button, then drag the pointer to a new position.
3. Release the mouse button.

Create ranges at about 100, 200 and 250 gray levels. Because the dataset contains just 3 levels, the width of each range does not really matter, as long as they do not overlap. If you make a mistake, you can delete the current range by pressing the **[Delete]** key. Select **Structure: Surf/Max Dens.** as the rendering type.

Figure 52: The Density/Structures table when one of the Structure/Surface renderings is selected.

Name	Render	Color	Surface	Transp	Int Dens
Dens: r1	<input checked="" type="checkbox"/>				
Dens: r2	<input checked="" type="checkbox"/>				
Dens: r3	<input checked="" type="checkbox"/>				
Circle	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Ring	<input checked="" type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Box	<input checked="" type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
<Bkgd>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Density Data

The **Density: range #s** in Figure 52 correspond to the three ranges set for the sphere (circle), cube (box) and cylinder (ring). If a **Render** checkbox is empty, density data from this range is not used in the rendering and the solid colored range slider changes to a chequered pattern.

Structure Data

If the **Render** checkbox is empty, the **Surface, Transparency** and **Internal Density** checkboxes are “grayed”. If the **Render** checkbox is marked, some of the boxes become active. There is a linkage between the availability of each checkbox as shown in Table 4.

Table 4: The relationship between Structure checkboxes and their effects on the sample dataset.

Rendering	Surface	Transparency	Internal Density	Effects on 3D datasets
No checkmark	Disabled	Disabled	Disabled	No rendering
Checked	Unchecked	Unchecked	Disabled	No rendering
Checked	Checked	Unchecked	Disabled	Structure Surface Rendering
Checked	Checked	Checked	Unchecked	Structure Surface + Transparency
Checked	Checked	Checked	Checked	Structure Surface, Trans + Int Density
Checked	Unchecked	Disabled	Checked	Internal Density

For this part of the tutorial, turn off the **Ring** structure by removing the checkmark from the **Render** checkbox. Turn off the density **r1, r2** and **r3** densities as well. When you

have seen the effects of these rendering types on the sphere, apply them again to the sphere and the ring.

Structure Rendering of the Sample Dataset

Structure: Surf/Max Density

Displays the surface of a defined structure and the maximum density inside if density data from this structure is used in the current rendering. If you apply this to the sample dataset, you should see the following:

Sphere only -- A solid sphere.

Sphere +Density:r1 -- A translucent sphere.

Sphere +Density:r1+r2 -- A translucent sphere with a white cube at the center.

Structure: Surf/Min. Density

Displays the surface of a defined structure and the minimum density inside if density data from this structure is used in the current rendering. If you apply this to the sample dataset you should see the following:

Sphere only -- A solid sphere.

Sphere +Density:r1 -- A translucent sphere.

Sphere +Density:r1+r2 -- A translucent sphere.

You do not see the cube in this rendering because the minimum density value was rendered and that is 100 gray levels, the value created for the sphere.

Structure: Surf/Surf Density

Displays the surface densities of defined structures. If you apply this to the sample dataset you should see the following:

Sphere only -- A solid sphere.

Sphere +Density:r1 -- A translucent sphere.

Sphere +Density:r1+r2 -- A translucent sphere.

The results of this rendering look identical to the previous type. This is because the surface density data for the sphere is uniform at 100 gray levels, the value used to create the sphere.

Structure: Surf/Density CO

This lets you adjust the opacity and intensity of each defined structure. If you apply this to the sample dataset you should see the following:

Sphere only -- A solid sphere.

Sphere +Density:r1 -- A bright translucent sphere.

Sphere +Density:r1+r2 -- A bright translucent sphere.

The surface color of the sphere changes as you include density data. The reason behind this change is the effect of overlaying one partially transparent surface color onto density data of a different color. The end result is a color addition of both.

Summary

In this part of the tutorial you learned how to change the appearance of a 3D rendered image and experimented with the different types of density and structure renderings in **M3D**.

Tutorial Part 6: Cine loops

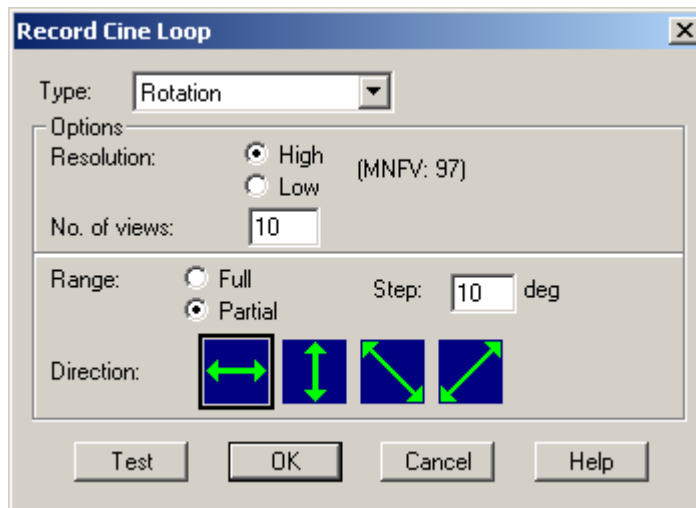
For Part 6 of the tutorial you need to be in **Rendering** mode. If you are in **Construction** mode, click on the icon at the top right of the display.



A cine loop shows the 3D image as a dynamic display. The moving display usually gives a much better appreciation of the relationships between 3D structures. To make a cine loop, you create a number of views of the rendering, each one from a different angle. When you play the cine loop you get the effect of movement of the 3D object or the light source, etc., depending on the type of loop you make.

1. From the *3D Dataset* menu select *Open* and load the dataset that you saved at the end of Part 3.
2. Click on **[Apply]** to display the rendered image on the monitor.
3. Open on the *3D Dataset* menu and select *Cine loop > Record*.
4. The *Record Cine Loop* dialog box appears (Figure 53).

Figure 53: The Record Cine Loop dialog box.



5. Click on the **Partial** radio button. For now, just accept the defaults and create 10 different views of the rendering at 10 degree intervals.
6. Click on the **Horizontal** direction icon.



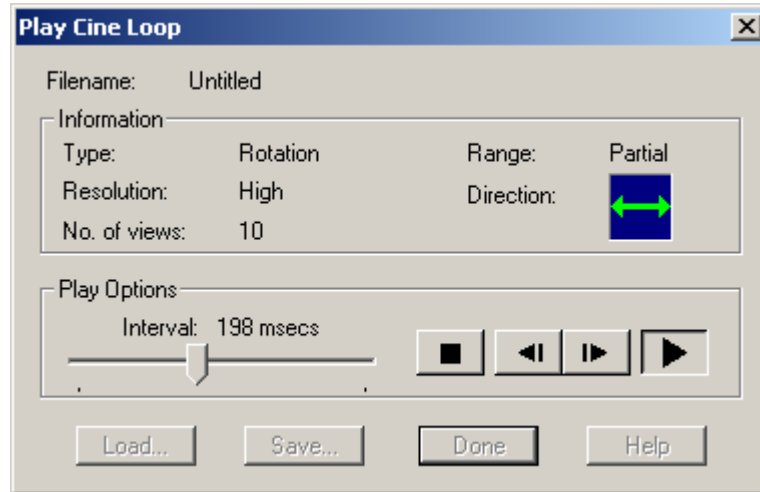
7. Click on **[Test]** to see a “preview” of the cine loop. The transform in the 3D *Rendering Control* window rotates through the series of views that you specified. It is a good idea to use the test function to check the chosen settings *before* going

ahead with a full cine loop, as creating a large number of views of a 3D object takes time. The test function plays the cine loop once, then stops automatically.

8. Click on **[OK]** to create the cine loop.

As **M3D** creates the loop it shows each view on the image monitor, and an information box appears showing you, which step in the loop, **M3D** is creating. When it has finished, the *Play Cine Loop* dialog box appears (Figure 54). **M3D** returns to the first view and begins to run the loop automatically.

Figure 54: The Play Cine Loop dialog box.



The *Play Cine Loop* dialog box contains the name of the cine loop, a description of the loop type and shows loop attributes. The **Play Options** area contains a slider control and 4 buttons. The **Interval** slider changes the time interval between each view and consequently, affects the replay speed of the cine loop. The interval is continuously adjustable between 30 msec and 2.93 sec.



This button starts the loop running in continuous play mode. The loop plays until you stop it.



Steps the loop forward 1 view at a time.



Steps the loop backward 1 view at a time.



Stops the loop.

The **[Load]**, **[Save]** and **[Done]** buttons are not active until you stop the cine loop. If you click on **[Done]** before saving the cine loop, a prompt appears asking if you wish to proceed without saving it. Click on **[Save]** and save the cine loop to disk. Cine loops are saved with a *.cni file extension. Cine loops can also be saved as a collection of component TIFF files for export to other graphics programs. To save a Cine loop as a set of TIFF files, select TIFF from the *Save as type:* drop down menu option.

Now you can create another loop using one of the other direction options. Experiment with the Range control selecting full circles (**Full**), or partial circles (**Partial**) of rotation. If you use 10 views in a Full circle, the motion will be rather jerky (because each view will be taken at an interval of 36 degrees), but the loop runs very quickly. Make a loop using 20 views; image motion becomes smoother but it takes longer to create the loop.

Different types of cine loop are described in more detail in the [Advanced Details](#) section of the manual. To experiment with other types of cine loop, select the **Type:** drop down menu from within the *Record Cine Loop* dialog box.

Summary

In Part 6 you learned how to record and play a cine loop of the rendered dataset.

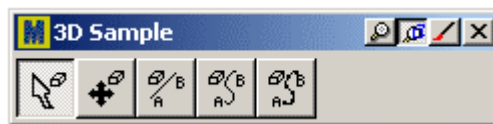
Tutorial Part 7: Making 3D measurements

You can make linear measurements on the rendered image using tools that are similar to those in the Standard study type, except of course they work in 3D. Clicking on the Tools icon while you are in **Rendering** mode opens the **3D Sample** toolbox. Any measurements you make are recorded in the data table.

Note: The **3D Sample** tools do not work with the density rendering types. If you want to make measurements of the sample dataset, apply the Solid Surface rendering type first.

3D Sampling Tools

Figure 55: The 3D Sample toolbox.



The **3D:Pointer**. Move the mouse cursor onto the rendered image and click the left mouse button. The **3D Volume** and **3D Surface** data are added to the table. The default units are 'voxels' for 3D Volume and 'pixels' for 3D Surface.



The **3D:Volume** tool. This tool measures the 3D volume and 3D surface area of all structures of a designated color. Move the mouse cursor onto the rendered image structure and click the left mouse button over the structures you wish to measure.



The **3D:Line** tool. This draws a line between two points and records the 3D distance. Move the mouse cursor onto the rendered image and hold down the left mouse button. Move the mouse and draw a straight line between two features of interest. Releasing the mouse button completes the measurement and the distance is recorded in the data table. The start and end points of the line must lie on the surface of the rendered object, or an error is recorded instead of a measurement.



The **3D Length:Trace** tool. This is similar to an Outline tool. Anchor one end of it to a feature and then trace along the surface drawing a border around the feature. Releasing the mouse button completes the measurement and the distance is recorded in the data table. All the trace points must lie on the surface of the rendered object, or an error is recorded instead of a measurement.



The **3D Length:Connect** tool. This is similar to the Click-Outline tool. Click the left mouse button to define the starting point of the target outline and then move to the next feature and click again. Subsequent clicks of the mouse button joins together each point with a straight line. A final double click with the left mouse button records the data in the data table. All the connected points must lie on the surface of the rendered object, or an error is recorded instead of a measurement.

Tutorial Summary

Now that you have completed the tutorial, you should have a good working knowledge of the **M3D** basics.

You should be able to load images, define structures, and go from there into contour and surface rendering. You should know how to change the appearance of the 3D rendered image, how to make measurements from appropriate renderings and how to make a cine loop. If you are not sure about any of these procedures, work through the relevant tutorial section again.

Part 1 - Starting **M3D**; loading images, simple rendering of a small stack.

Part 2 - Defining structures in sections; contour rendering.

Part 3 - Working with large stacks of images.

Part 4 - The 3D Rendering Control window.

Part 5 - Presentation; changing the appearance of the rendered image.

Part 6 - Cine loops; creating dynamic displays of the dataset.

Part 7 - Making 3D measurements from rendered images.

We recommend you work through the tutorial again using your own samples this time, and exploring more of the features in **M3D**.

There are many features of the program that just cannot be covered in an introductory tutorial. We encourage you to explore the program and read the [Advanced Details](#) section of this manual.

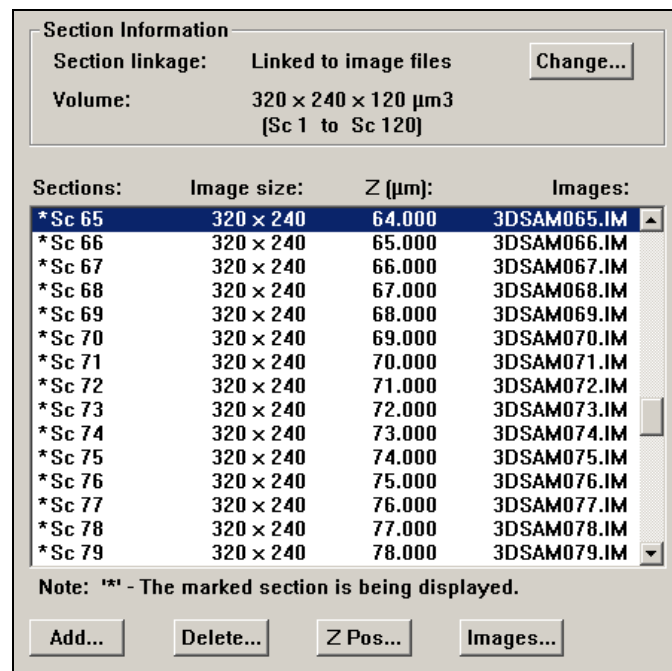
Advanced Details: Stack Window

The **Stack** window is the **M3D** construction mode for loading and displaying images, defining anatomical structures within the images, and making contour renderings of defined structures. It also has menus for aligning sections, normalizing backgrounds and interpolating the stack to create a 3D dataset. You can also save stacks, datasets and lists of defined structures.

Image Management

The area on the left of the Stack window is used to manage the image stack. From here you can view image information, choose which images to display, add or delete individual images, change the Z-axis calibration or position, and/or change the way in which the images are linked (i.e., to channels or files).

Figure 56: The image management area of the Stack window.



Displaying Images

The **Section Information** list lists all sections in the stack, the size of each image (Height x Width), the Z-axis position of each section, and the image filename associated with each section. The list is also used to select which images to display on the image monitor. Images currently displayed on the image monitor have an asterisk in front of the Section label.

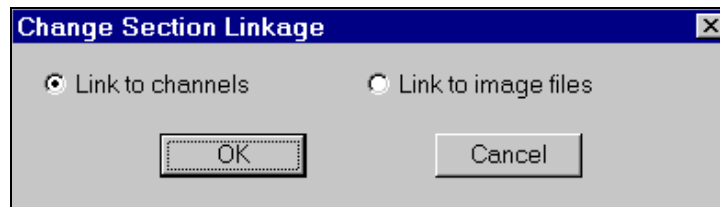
Images are displayed in sets that correspond to the **Channels per page** setting in the current display format (*Settings > Display format*). For example, if the display format is set to 16 channels per page, images are displayed in consecutive sets of 16 images. If you

click on Section 8, **M3D** will display Sections 1-16. If you click on Section 20, **M3D** will display Sections 17-32.

Section Linkage

Images may be linked to channels or to image files. Clicking on the [**Change**] button calls up the *Change Section Linkage* dialog box.

Figure 57: The Change Section Linkage dialog box.



If you select the **Link to Channels** option, every image in the stack is immediately loaded into a discrete channel. The maximum number of images you can load is consequently limited to number of channels physically present on the system. This is acceptable if you are working with small sets that contain only a few sections, but is a serious limitation when working with large image sets. Most 3D reconstructions require large numbers of images.

If you select the **Link to Image Files** option, images are linked to image files that do not use memory until they are needed by **M3D**. That is, the images are not loaded into channels until they are selected for display. You can load any number of images in this mode.

Z-Axis Calibrations and Positions

The X Y spatial calibration is defined in the **Calibration** window under the *Establish > Distance* menu option. The Z-axis position value is also initially defined in the **Distance Calibration** window by entering values for section thickness and section interval. When you create a new stack file, **M3D** uses the section interval data from the calibration settings as default values. When you create a new volume from the stack, the depth of the interpolated cube is defined by the interval between the first and last slice, plus the section thickness.

The [**Z Pos**] button allows you to establish a section interval if the images were not calibrated to begin with. You can also use this feature to change the interval between two individual images (e.g., if a serial section is missing), or to change the Z-axis position of the image in the stack.

Pressing the [**Z Pos**] button calls up the *Set Section Z Positions* dialog box (Figure 58).

TO ESTABLISH OR CHANGE THE SECTION INTERVAL:

1. Select the **Interval** radio button.
2. Check the **Change all** checkbox.

3. Enter the section interval in the entry field (i.e., the distance from the top of one section to the top of the next).
4. Press [**Set All**].

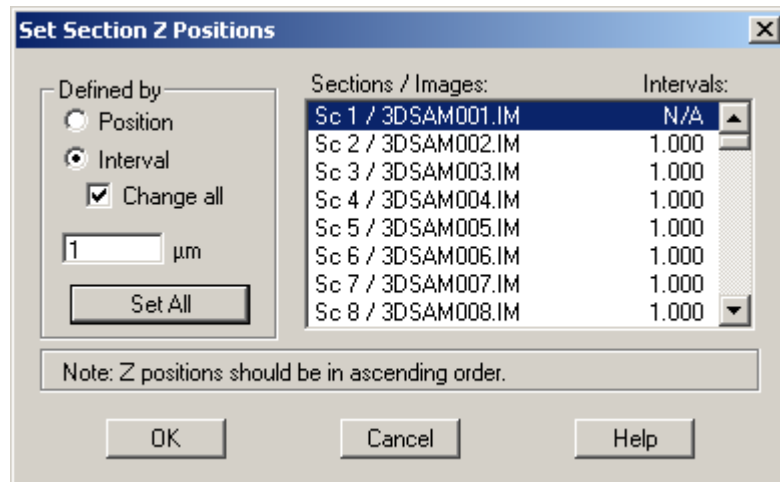
TO CHANGE THE INTERVAL BETWEEN TWO INDIVIDUAL SECTIONS:

1. Select the **Interval** radio button.
2. Un-check the **Change all** checkbox.
3. Select the upper section from the list.
4. Enter the section interval in the entry field (i.e., the distance from the top of the selected section to the top of the next).
5. Press [**Set Selected**].

TO CHANGE THE Z POSITION OF AN IMAGE:

1. Select the **Position** radio button.
2. Select the section from the list.
3. Enter the new Z position (in spatial units relative to the position of the first section in the stack) in the entry field.
4. Press [**Set Selected**].

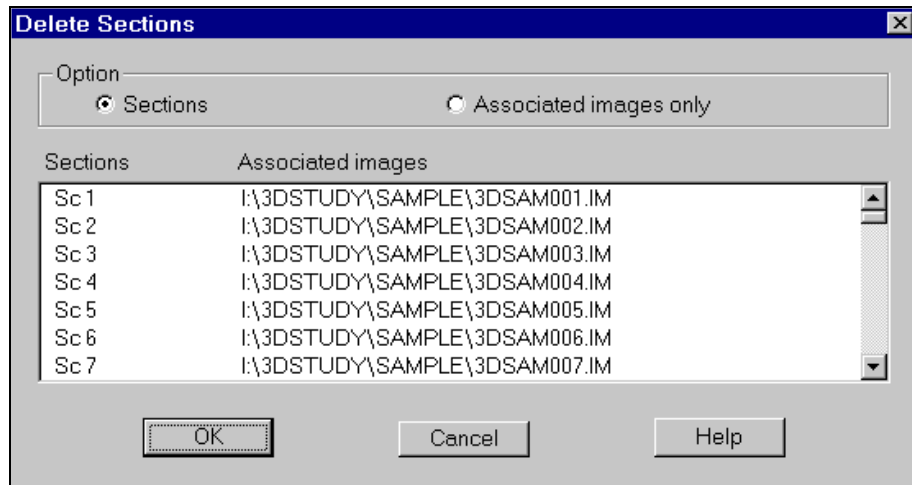
Figure 58: The Set Section Z Positions dialog box.



Deleting Images from the Stack

The [**Delete**] button lets you remove images from the list in the left of the images window. Clicking on the [**Delete**] button calls up the *Delete Sections* dialog box.

Figure 59: The Delete Sections dialog box.



Sections

This radio button is active only when the section linking is set to **Image files**. When you delete a section, the image and any defined structures are removed. To delete one or more sections, highlight the relevant name(s) in the list then click on **[OK]**.

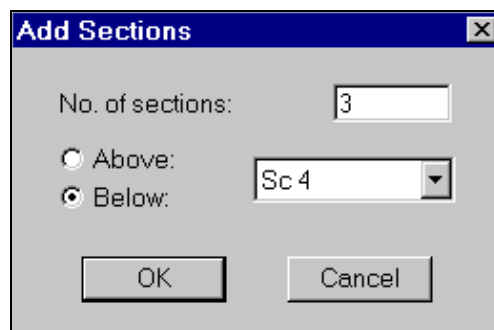
Associated images only

This radio button is active with both **Image files** and **Channel linking**. When you delete the associated image, only the image itself is removed. Any defined structures are kept, and you can load another image into the file if you wish.

Adding Images to the Stack

The **[Add]** button lets you add images to the stack. You define the number of sections you want to add to the stack, and the section above or below which you want to insert them. For example, if you want to insert 3 sections below section 4 you enter this information then click on **[OK]**. The image list will “shuffle” down to create room for these additional image files.

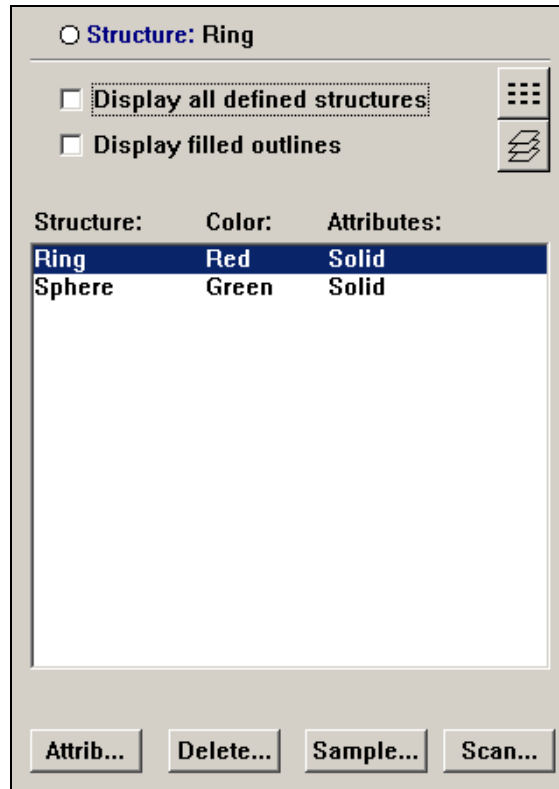
Figure 60: The Add Sections Dialog box.



Defining Structures in Images

The right side of the **Stack** window contains a set of controls for defining structures (e.g., anatomical entities) in individual images (Figure 61). Contour and surface renderings may then be made from any defined structure.

Figure 61: The Structures area of the Stack Window.



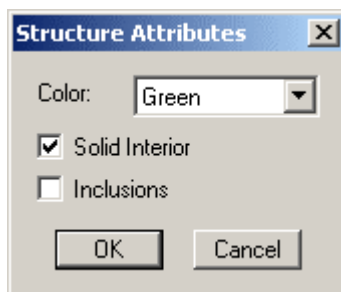
Using these Structure Definition controls you can:

- Create structure labels
- Control the way in which structures are displayed
- Delete defined structures
- Create a contour rendering of defined structures

Creating Structure Labels

Clicking on **Structure:** calls up the *Structures* list. This lets you define labels for structures and select which structure is the current one. Type in the names of the anatomical structures you want to define. If you want to keep the list of labels, open the *File* menu in the dialog box and save it using *File > Save*. Label list files are saved with the *.lbl file extension. To load a previously saved label file, use the *File > Open* command. The *Edit* command lets you rename structure labels in the list, or delete them.

Figure 64: The Structure Attributes dialog box.



To change the color of the structure, click on the down arrow to the right of the **Color:** field.

Solid interior

If you place a mark in the **Solid Interior** checkbox, when you select the **Hidden** icon in the Contour Rendering window a gray “fill” is applied to the contours. If the box is not checked, only surface voxels are created in the 3D dataset, and the 3D structure will be hollow.

Inclusions

M3D creates 3D surface renderings of anatomical structures from a 3D ‘dataset’. The dataset is prepared by interpolating voxels from a stack of 2D sections. The 3D dimensions of any given structure are consequently determined by i) the section interval, and ii) the outline of the anatomical structure (as defined by the user in the individual sections).

While this method provides an accurate 3D visualization of relatively large structures, it may not be suitable for visualizing small, spherical or particulate matter (e.g., vesicles). If the diameter of such structures is less than the section interval, for example, the structures will appear tube-shaped in the rendered image.

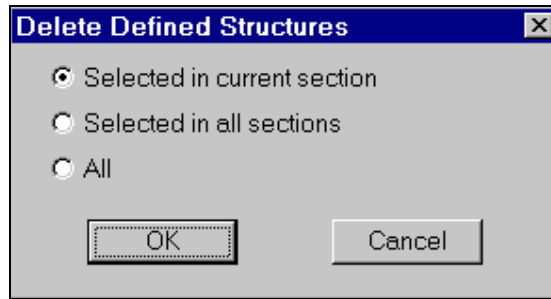
M3D allows the user to designate these kinds of structures as ‘inclusions’. When designated as such, **M3D** will not perform any interpolation. Instead, **M3D** will model and display the rendered structures as discrete spherical-shaped objects. The radius of each sphere is derived from the dimensions of its corresponding 2D outline (mean of its height + width) and each sphere is positioned in the Z-axis so that its center bisects the surface plane of the 2D section.

To designate a defined structure as an inclusion, check the **Inclusions** box. When checked, all objects defined under the given structure name will be rendered as individual spherical-shaped objects.

Deleting Structures

This lets you delete a structure from the list. You can delete a selected structure from the current section only or from every section. If you select **All**, you will be prompted to confirm that you want to delete all defined structures.

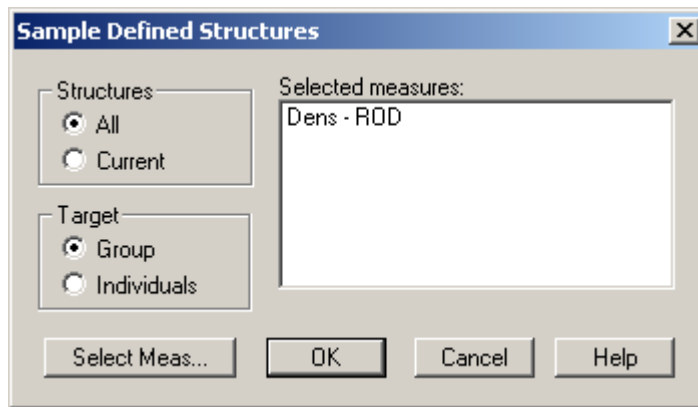
Figure 65: The Delete Defined Structures dialog box.



Gathering Data from Structures

Structure tools do not normally gather data. However, if you wish to gather data from structures as they are defined, press the **[Sample]** button. This opens a dialog box that lets you select measurements and gather data from structures in individual images. Data are subsequently reported in the data table of the **Sample** window.

Figure 66: The Sample Defined Structures dialog box with some selected measures.



Structures

The **All** and **Current** radio buttons select sampling of all the defined structures and targets or only the current one.

Target

Targets refer to the outlines of a defined structure in a series of sections. A defined structure can consist of more than one target. Data can be reported for either Groups or Individual targets.

Group -- shows the mean of all the targets of each structure within each section.

Individual -- reports data from each individual target.

Clicking on **[Select Meas]** opens the *Measures Selection* dialog box. You can choose from several categories of measurements (see *Chapter 4: Selecting Measures* in the online

Reference Manual). When you click [OK], the system processes the selected structures in each section and records the data in the data table.

Note: In M3D, a “region” is another name for a structure.

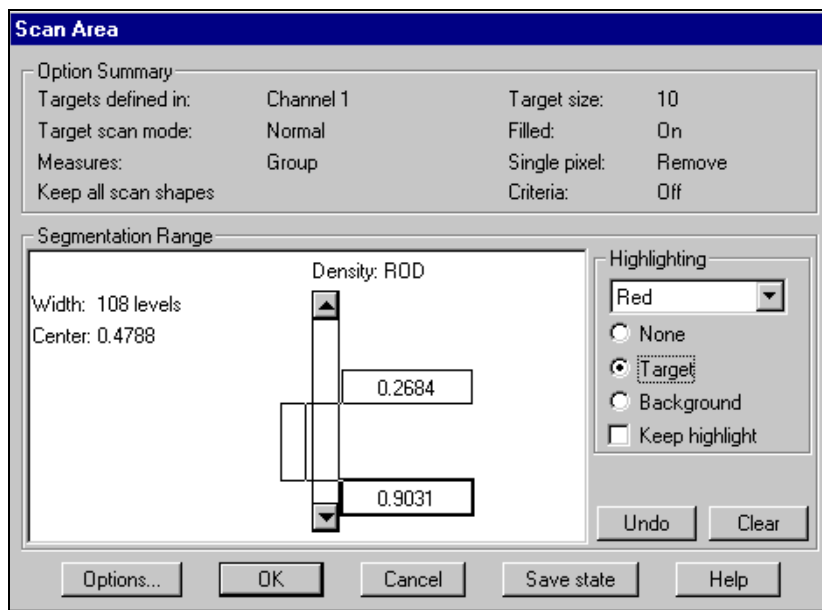
Automatic Structure Definition

MCID Elite’s automatic target detection feature (Scan Area) may be used to automatically define structures in individual images. To use this feature, first click on the **Scan Area** icon:



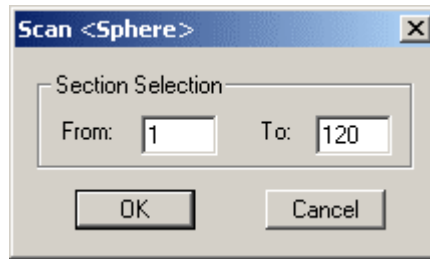
The **Scan Area** dialog box appears, which allows you to establish a set of “target criteria” (i.e., features that distinguish structures of interest from other image features). Target criteria may be based on any combination of density, size, or shape. For complete instructions, see *Chapter 8: Automated Target Detection* in the online *Reference Manual*.

Figure 67: The Scan Area dialog box is used to define target criteria and to access a variety of target detection options and features.



Once you have established a set of target criteria for a given structure, press the [Scan] button. A *Scan <Structure Name>* dialog box appears next, which allows you to specify a range of sections to scan. You can scan the entire stack or a subset of sections. The idea is to select a range of sections in which the structure of interest is actually present. When you exit the dialog box, M3D automatically searches the specified sections and outlines the structures that satisfy all of the target criteria.

Figure 68: The Scan dialog box is used to specify the range of sections in which the structure of interest is present.



Other Controls

Two additional icons are displayed in the Structures area of the **Stack** window.



Ranges

Press the **Ranges** icon to display the *Defined Structures: Ranges* dialog box (Figure 69). This dialog box lists the range of sections/channels in which the structures have been defined. If you see ** alongside an entry in the table, this indicates that the structure is not consecutively defined in the range.

Figure 69: The *Defined Structures: Ranges* window showing structures in sections 1 to 16.

Structures:	From:	To:
Ring	Ch 1	Ch 16
Circle	Ch 1	Ch 16



Contour rendering

Press the **Contour rendering** icon to display a contour rendering (“wire-frame” reconstructions) of selected structures. Contour rendering is described below.

Saving Stacks and Structures

Defining several structures in a large stack of images takes time and can be rather tedious. You can save the stack by using the *File > Save* command in the **Stack** window. Stack files are saved with a *.dg file extension. These files save the screen contents and contain the following information:

- Section list
- Location of associated images (filenames and file types)
- Density and spatial calibrations
- All defined structures.

When you want to load the saved stack file, use the *File > Open* command in the *Stack* window.

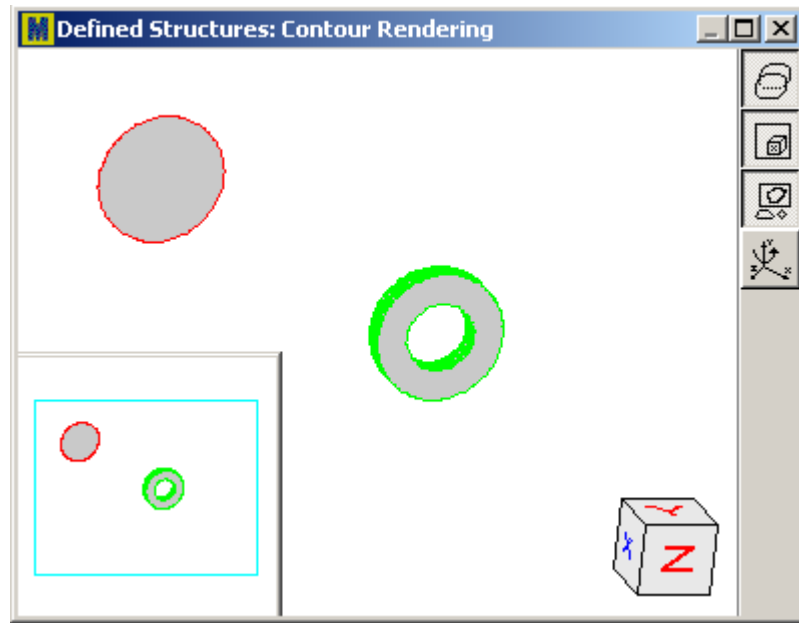
Contour Rendering

Contour rendering produces 3-dimensional “wire-frame” reconstructions of defined structures. To create contour renderings, press the **Contour rendering** icon:



This displays the *Defined Structures: Contour Rendering* window (Figure 70).

Figure 70: The *Defined Structures: Contour Rendering* window.



The *Contour Rendering* window has a **Rotation Cube** and four icons on the right hand side.

Contour Rendering Window Icons



Hidden

The **Hidden** icon controls contour display. Contours are shown as a series of overlapping outlines (often called a “wire-frame” display). This is often very difficult to interpret, especially with more than 3 contours on the display. Clicking on **Hidden** adds a gray fill to each slice and obscures overlapping and confusing internal structures.



Show rotation cube

The **Show rotation cube** icon turns the rotation cube on and off. The rotation cube is used to control the orientation of the contour rendering (see below).



Default rotation

The **Default rotation** icon resets the orientations (X, Y, Z) of the rotation cube and the contours to those of the original image data (see below).



Viewport

The **Viewport** icon displays a “Viewport” in the lower left corner of the window. This contains a light-blue, adjustable frame. Move the mouse cursor onto the frame border. The cursor changes into a double-headed arrow. Hold down the left mouse button and drag the border to change the frame size. Releasing the mouse button redraws the image at a higher magnification.

Move the mouse cursor inside the frame. The cursor changes into a white, 4-headed arrow. Hold down the left mouse button then drag the frame to a different area of the image. Releasing the mouse button redraws the image showing the new features.

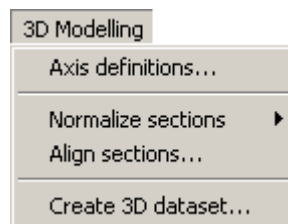
Rotation Cube

The **Rotation Cube** lets you redraw the contoured image by changing the position of the X, Y and Z axes. To use it, point to either a cube face, edge or corner and hold down the left mouse button. Drag the cube to change the orientation and then release the left mouse button. The contoured image is redrawn in the new orientation. To restore the original orientation, click the **Default rotation** icon.

3D Modeling Menu

The *3D Modeling* menu includes functions that you might want to use before interpolation, such as axes definition, alignment and background normalization.

Figure 71: The 3D Modeling Menu.



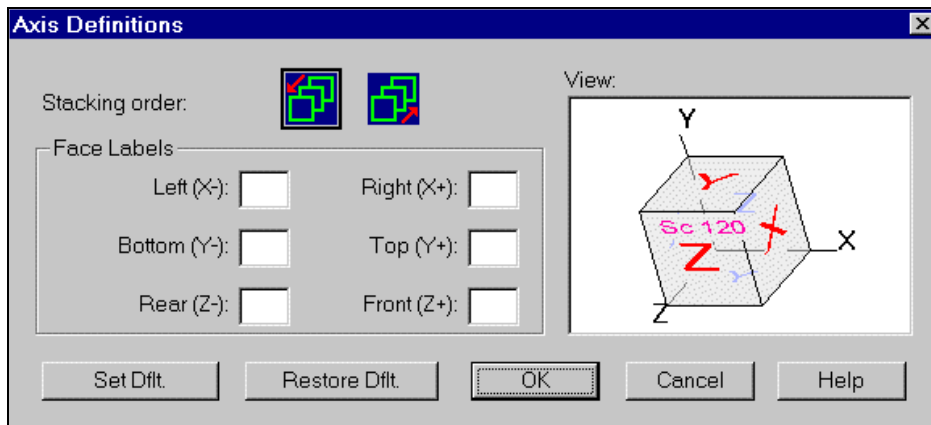
Each of these operations is described below.

Axis Definitions

Sequential images collected on a microscope, or by an MRI scanner follow the accepted Cartesian convention in describing orientation and axes (i.e., X, Y and Z). The **Axis Definition** feature allows the user to identify axes of the stack by assigning anatomical labels to them.

Select *3D Modeling > Axis definitions* to display the *Axis Definitions* dialog box (Figure 72).

Figure 72: The Axis Definitions dialog box. Ch 16 on the Z face of the orientation cube shows that sections are stacked in descending order.



Stacking Order

You can arrange sections in ascending or descending order by clicking on either of the **Stacking order** fields. The default is to have the sections arranged in ascending order, as this is consistent with the orientation of subsequent renderings.

Face Labels

The cube faces are labeled X, Y and Z by default, but any appropriate labels can be entered using the **Face Labels** entry fields. You might want to define them as “Dorsal-Ventral”, “Left-Right” or “Anterior-Posterior”. Only one character is allowed for each face, however, so in the above example you could use “D-V”, “L-R” and “A-P” respectively.

View

The area shows a skeleton cube and the horizontal, vertical and orthogonal axes. The cube faces show the axes labels. X, Y and Z are the default labels and the red letters indicate the faces that you can see in this particular orientation. Faces you cannot see are labeled with pale blue letters.

Set Dflt

You can set any combination of axes labels as the default.

Restore Dflt

This button restores the default conditions, if you have made temporary adjustments.

Normalizing Sections

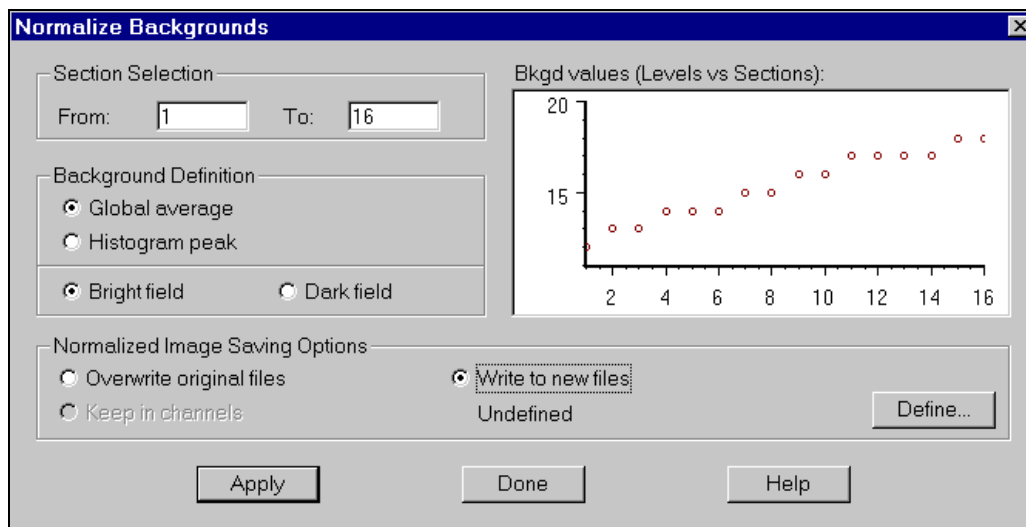
Images need to be normalized to account for differences in background densities and calibrations. This is especially true of microscope images taken from serial sections. The sectioning process may cause variations in thickness between sections, and the material may not be consistent in different regions within a single section.

To normalize a set of sections, select *3D Modeling > Normalize*. You can normalize *Background* densities (in uncalibrated images) or *Calibrated* densities (in calibrated images).

Normalizing Backgrounds

If the stack images have no density calibrations, select *3D Modeling > Normalize > Backgrounds*. The *Normalize Backgrounds* dialog box (Figure 73) and the **Background** toolbox (Figure 74) appear.

Figure 73: The *Normalize Backgrounds* dialog box. The graph shows background pixel values as density levels (0-255) on the Y axis plotted against section numbers on the X axis.



Section Selection

The default settings will normalize all the sections in the current stack, but you can use a smaller number of sections if you wish. Type in the first and last sections you want to use in the entry fields at top left of the dialog box.

Background Definition

Background density/intensity can be defined in two ways. The **Global average** method calculates the background value as the average gray level value of the entire image or in a user-defined area. The **Histogram peak** method calculates the background value from the gray level at the histogram peak of the entire image or in a user-defined area.

If you wish to base the normalization on a user-defined area, select a suitable **Background** tool then transfer the cursor to the image. Move the target tool to a region of typical background density and click the left mouse button. A target appears on the display and background values within this target are recorded. Draw a target on each section in the stack.

Indicate whether you are working with **Brightfield** specimens (i.e., dark objects on a light background) or **Darkfield** specimens (i.e., bright objects on a dark background). This determines which algorithms **M3D** will use for normalization.

Figure 74: The Background Target Selection toolbox.



Normalized Image Saving Options

You can overwrite the existing image files, or save the normalized data in new files.

Overwrite Original Files -- The normalized images overwrite the original data.

Write to New Files -- This option lets you define new filenames to save the normalized images. Press **[Define]** to specify the new filenames.

Keep in Channels -- You may decide to work with the normalized images but not to save them. If this is the case, click on this radio button.

Note: The **Keep in Channels** option is not available if you are working with images linked to channels. In this case, you must decide whether to overwrite the original files or store the normalized images as new ones. Use *File > Save* (or *Save As*) to save the stack file after performing a normalization.

Starting the Normalization Process

Click on **[Apply]** to start the background normalization process. The graph at the top right of the dialog shows the background values for the selected sections. When the normalizing process has finished, the graph updates to show the normalized background values. To close this dialog box, click on **[Done]**.

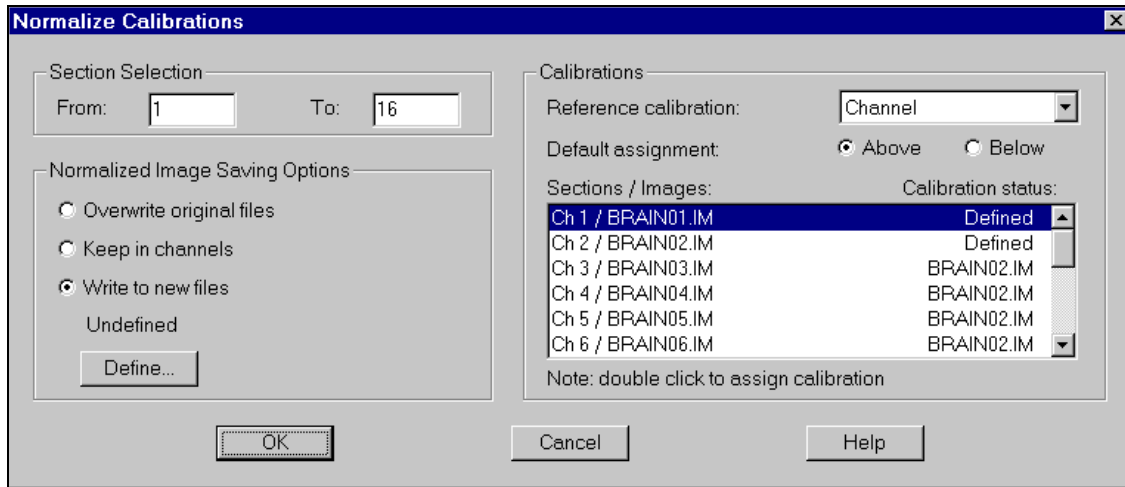
Normalizing Calibrations

Calibrations need to be normalized to account for variations in density values arising as a result of experimental procedures. For example, suppose you had a series of isotopically labeled brain sections exposed on different pieces of film. The calibration for any section will not apply to other sections because the exposure will not be the same for each piece of film.

If the sections you are using are calibrated by relative calibration (e.g., as part of a consecutive series of images), a warning appears advising you that Normalize Calibrations is not available.

Selecting *3D Modeling > Normalize > Calibrations* calls up the *Normalize Calibrations* dialog box (Figure 75).

Figure 75: The Normalize Calibrations dialog box. The images in channels 3 to 6 do not include calibration data. These are assigned a calibration status from section 1 in the stack. M3D normalizes calibrations for these sections using the assigned values.



Section Selection

You can define a range of sections to use in normalizing calibrations. This might be all the sections in the stack, or only those which have calibration data. Any section that does not have intrinsic calibration data can be assigned data from a neighboring section, as shown in Figure 74. Calibration status shows which image calibration is assigned for the section.

Calibrations

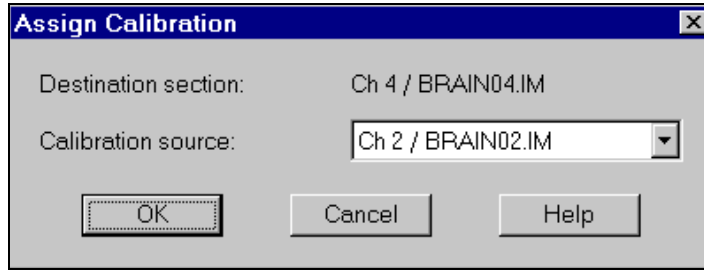
Reference Calibration -- Clicking on the down arrow shows a list of sections that include calibration information. Any of these sections may be used as the calibration reference. Images that do not have calibrations do not appear in the list.

Default Assignment -- Sets the default condition for calibration assignment. If the **Above** radio button is selected, any section lacking calibrations uses the values from the section immediately above it in the list. With **Below** selected, the next section in the list is assigned for calibrations.

Sections/Images -- Shows the list of channels, the filename of the image in each channel and its calibration status.

Calibration Status -- Shows whether a section has its own calibration or is assigned a calibration from another. If the default calibration is not correct, double clicking on a section in the list calls up the *Assign Calibration* dialog box (Figure 76).

Figure 76: The Assign Calibrations dialog box. This selection overrides the default assignment.



The **Destination** section is the one that you double clicked on to open this dialog box. The **Calibration** source is the section currently assigned to it. Clicking on the down arrow shows a list of images that include calibrations. Select one you want to assign to the **Destination** section, then click **[OK]**.

Normalized Image Saving Options

You can overwrite the existing image files, or save the normalized data in new files.

Overwrite Original Files -- The normalized images overwrite the original data.

Write to New Files -- This option lets you define new filenames to save the normalized images. Press **[Define]** to specify the new filenames.

Keep in Channels -- You may decide to work with the normalized images but not to save them. If this is the case, click on this radio button.

Note: The **Keep in Channels** option is not available if you are working with images linked to channels. In this case, you must decide whether to overwrite the original files or store the normalized images as new ones. Use *File > Save (or Save As)* to save the stack file after performing a normalization.

Aligning Sections

Image sets created by tomographic scans or optical sectioning are inherently pre-aligned. The section alignment functions are used to compensate for shifts in the relative positions of anatomical structures that often occur during physical sectioning of tissues and organs. If sections are not pre-aligned, this procedure is essential for correct 3D model construction and rendering.

Select *3D Modeling > Align sections*. The **Selection** toolbox and the *Align Sections* dialog box appear.

Figure 77: The Selection toolbox.



The **Selection** toolbox contains 3 tools:



The **Box** tool is used for defining image texture areas on the reference section.



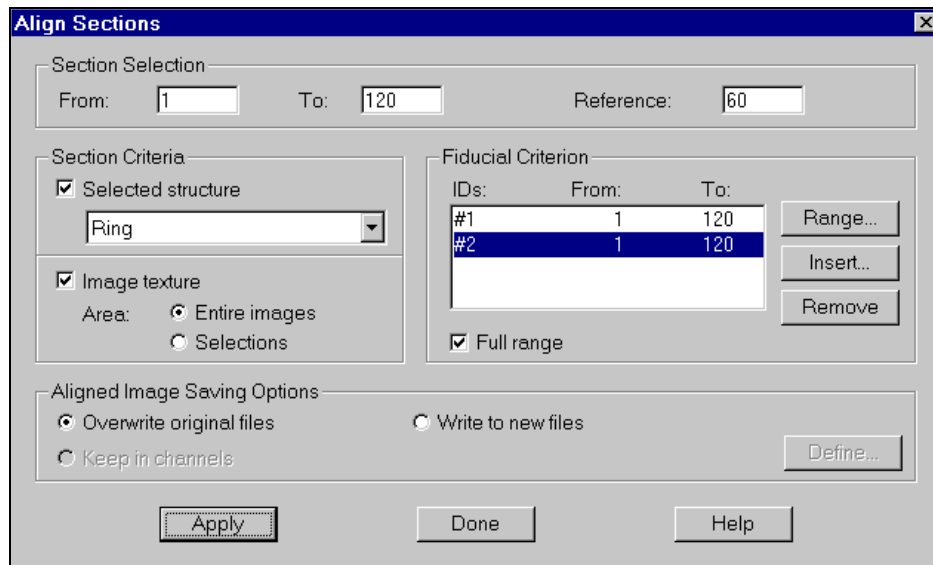
The **Fiducial** tool is used to place fiducial points on images.



The **Delete** tool is used to remove box targets and fiducial points.

Note: The **Box** tool is active only when you choose **Area: Selections** as your section alignment criteria.

Figure 78: The Align Sections dialog box showing selected structure and fiducial points. Notice that 'Keep in channels' is grayed because M3D is using 120 sections linked to image files.



Section Selection

The default settings will use all the sections in the current stack, but you can align a smaller number of sections if you wish. Type in the first and last sections you want to use in the **From: To:** fields at top left of the dialog box. You may also change the reference section if you wish.

Section Criteria

You can use any combination of section criteria for alignment. You can use entire images, selected regions of images, a defined structure or a combination of these parameters.

Selected Structure

If you defined one or more distinct structures in the stack, you can use one for image alignment. Click on the checkbox and the drop down menu becomes active. Click on the down arrow to see the complete list of defined structures. There is a constraint in using

defined structures for alignment. You can have only one outline per section for the selected range.

Image Texture

Click in this checkbox to make the **Area** options active.

If you select the **Area: Entire Images** radio button, the alignment uses entire sections.

If you select the **Area: Selections** radio button then press the middle mouse button to move the **Box** tool to the image monitor. Re-size the box if necessary and move it to the **Reference** section. When you click the left mouse button, a target box is drawn on the reference image. You can define targets on the reference section only, it is not possible to draw targets on the other sections. There is no limit to the number of targets you can define, but you should try to use similar features that are present in each section. When you click on [**Apply**], the alignment proceeds using the selected area on all sections.

To remove the box target from the reference section, select the **Delete** tool place it inside the box then click the left mouse button.

Fiducial Criteria

Fiducial points are markers that can be accepted as a fixed basis for reference or comparison. In a series of sequential images fiducials may be clearly defined anatomical structures, or they may be artificially inserted markers. You can designate any point as a fiducial if it is present in all the sections you want to align, and at the same relative position in each one.

Select the **Fiducial** tool from the **Selection** toolbox, then click the middle mouse button to transfer the cursor to the image display. The **Fiducial** tool (a red cross-hair) appears on one of the images. Move it to a suitable fiducial point (e.g., an anatomical landmark that is visible in all images to be aligned) and click the left mouse button. A red cross and the fiducial point number appear at the same XY coordinates on each of the displayed images. Move to the next point and click the left mouse button to define the second fiducial point. The most recently defined point is shown in the highlight color (green), previous ones in the current color (red). Accurate alignment requires at least 2 fiducial points, but 3 or more is recommended.

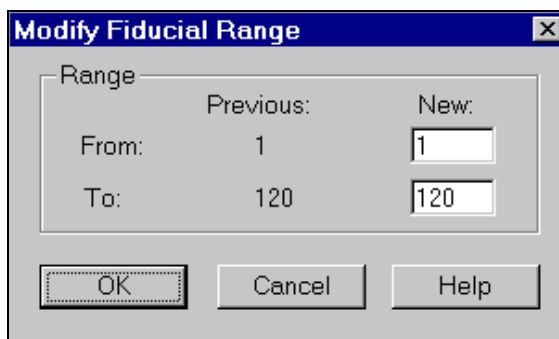
The idea is to now move the fiducial points in the other images so that they are aligned with the same *anatomical* landmarks as the reference image. To move a fiducial point, place the **Fiducial** tool over the numbered fiducial point. The **Fiducial** tool changes into a hand with the index finger extended. The fiducial point marker and label change from red to green. By moving the finger onto the fiducial point and pressing the left mouse button you can “nudge” the point to a new location. This lets you fine-tune the alignment of the fiducial points with the features of interest. Minute adjustments are much easier at higher magnification. Press <Ctrl - Z> to zoom your image.

To perform the alignment operation, press [**Apply**]. **M3D** rotates and/or shifts the images so that the anatomical landmarks are in the same XY coordinates as the reference image.

Fiducial points are saved with an image series if you save the image stack using *File > Save* command after closing the alignment dialog box. When you re-open the file, however, the fiducial points are not visible until you select *Align Sections* from the *3D Modeling* menu.

To delete a fiducial point from a section, select the **Delete** tool, move the tool onto a point and click the left mouse button.

Figure 79: The *Modify Fiducial Range* dialog box.



Range

The **[Range]** button lets you define or modify the range of selected fiducial points. Some of the fiducial points may not extend through the entire range of sections. For example, in a range of 40 images, fiducial number 3 may be present only in sections 1-23. In this case you would select it for this limited range, then create another fiducial for sections 23-40. Notice that you need an overlap between the two fiducial ranges.

If the **Full Range** checkbox is marked, fiducials are defined on each section in the stack. For example, in our sample dataset we have 120 images, so this will be the full range. If the **Full Range** checkbox is not selected, and you define a fiducial point, a dialog box appears prompting you to enter the range for this fiducial point.

Insert

The **[Insert]** button lets you insert an additional fiducial point into an existing series of points. For example, if you were using the delete tool and you deleted fiducial point number 1 from section number 10 by mistake. Pressing **[Insert]** lets you correct the error by re-defining the missing fiducial point.

Remove

This lets you remove a fiducial point from all sections simultaneously.

Aligned Image Saving Options

You can overwrite the existing image files, or save the aligned images in new files.

Overwrite Original Files -- The aligned images overwrite the original image files.

Write to New Files -- This option lets you define new filenames to save the aligned images. Press **[Define]** to specify the new filenames.

Keep in Channels -- You may decide to work with the aligned images but not to save them. If this is the case, click on this radio button.

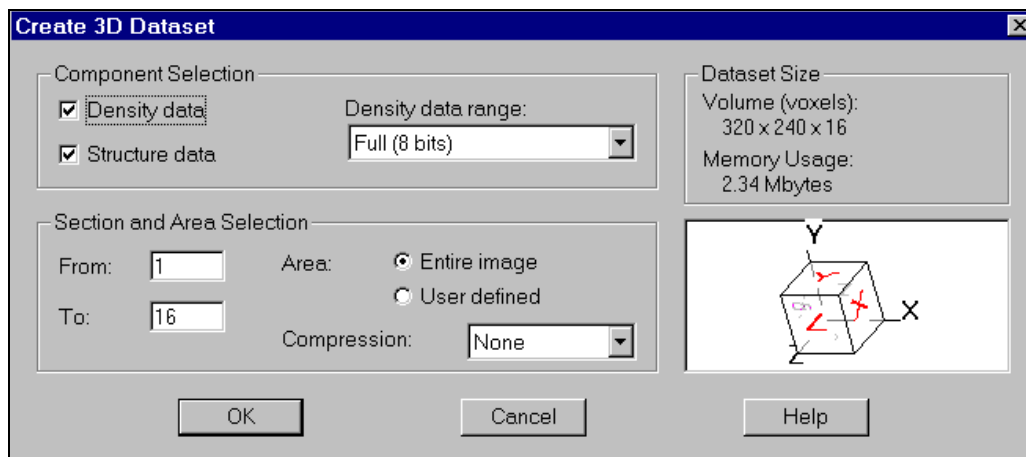
Note: The **Keep in Channels** option is not available if you are working with images linked to channels. In this case, you must decide whether to overwrite the original files or store the aligned images as new ones. Use *File > Save* (or *Save As*) to save the stack file after performing an alignment.

When you want to leave this dialog box, click **[Done]**.

Creating a 3D Dataset

The *3D Modeling > Create 3D dataset* option interpolates the images in the stack. When you click on **[OK]**, the interpolation process begins and when it is complete you are prompted to move into the **Rendering** window.

Figure 80: The Create 3D Dataset dialog box.



Component Selection

If you defined one or more structures when you were in the **Stack** window, the next two items are active, otherwise they are “grayed out.” Selecting the **Density** and/or **Structures** checkboxes controls the types of renderings available in the Rendering types list (Table 5).

Density Data -- This option creates a 3D dataset containing density information.

Structure Data -- This option creates a 3D dataset containing structural information (i.e., based on defined structures).

If you base your rendering on **Density data**, you can choose between the full range of image data (e.g. 8, 10, 12 bit) or use a compressed range. Compression reduces the bytes:voxel ratio from 2:1 to 1:1 and speeds up the rendering process at a cost of sacrificing density resolution.

Table 5: *The full range of M3D rendering types.*

Rendering Type	Structure Data	Density Data	Density and Structure Data
Solid Surface	Yes	Yes	Yes
Solid Surface/Cut Planes Density	-	Yes	Yes
Maximum Density	-	Yes	Yes
Minimum Density	-	Yes	Yes
Surface Density	-	Yes	Yes
Density Color Opacity	-	Yes	Yes
Structure:Surface/ Max Density	-	-	Yes
Structure:Surface/ Min.Density	-	-	Yes
Structure:Surface/ Surface Density	-	-	Yes
Structure:Surf/ Density Color-Opacity	-	-	Yes

Section and Area Selection

You may decide to use only part of the stack to create a 3D dataset. Enter the first and last sections you wish to use in the **From: To:** entry fields.

You may decide to use **Entire images**, or only some areas within sections to create the 3D dataset. If you select the **User defined** radio button then press the middle mouse button, a box tool appears on the image monitor. Move the box to one of the channels and re-size it if necessary. When you click the left mouse button, the box is drawn on each of the displayed channels. If you do not want all of the sections to contribute to the 3D dataset, select which ones to include in the **From: To:** fields. When you click on **[OK]** the interpolation proceeds, but includes only the user defined regions of interest in the specified sections.

The **Compression** option lets you choose from no image compression (**None**), up to **8x8x8** image compression. Rendering of large datasets is much faster with compressed images, and these do not use as much disk space or memory. Of course, increasing the rendering speed sacrifices some of the rendering quality.

The **Dataset Size** section of the dialog box shows the volume size in voxels and the amount of memory needed to create the dataset. If you selected **User defined**, the field displays an **Invalid** message until you define an area on the sections.

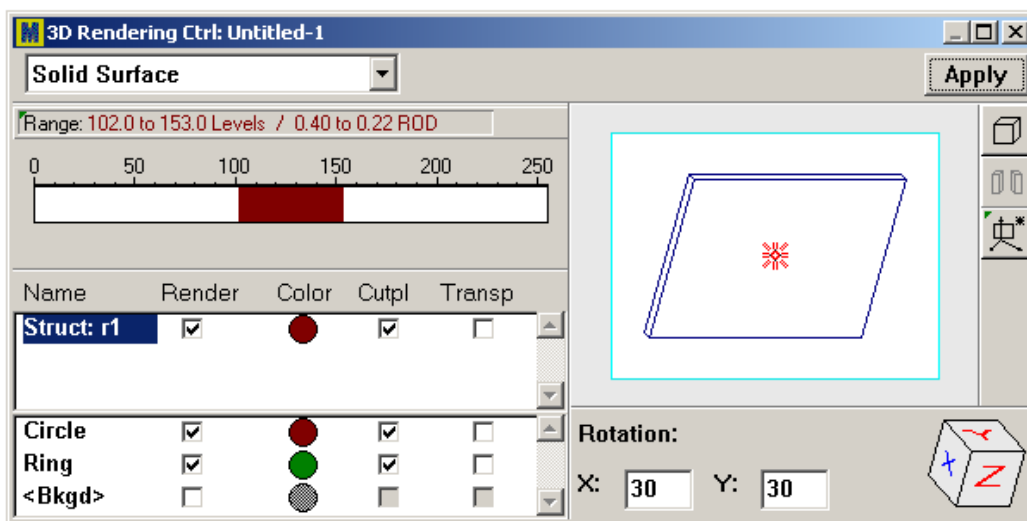
Advanced Details: Rendering Control Window

The **3D Rendering Control** window controls the rendering of 3D datasets created from a stack of images. When you interpolate a 3D dataset in the **Stack** window, the system prompts you to switch to rendering mode and into this window. If you want to work with a previously saved 3D dataset, you can open the window by clicking on this icon.



Note: Until you interpolate a stack to create a 3D dataset or open a saved dataset file, the **Rendering types** list, **Range Bar** and **Density/Structures** table are empty. The **Transform indicator** is set to its default, and the **[Apply]** button is grayed out until you open or interpolate data.

Figure 81: The Rendering Control window.

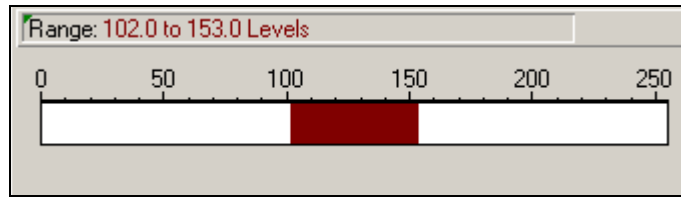


The *3D Rendering Control* window contains functions that affect the way in which an image is displayed. The best way to appreciate these functions, and their subtle interactions, is to create a 3D rendering and then experiment with different rendered ranges, types of shading, angle of illumination, transparency, etc. Very small changes in these parameters can often have a very noticeable effect on an image.

The Range Bar and Density/Structures List

The **Range** bar shows the entire density range of the dataset and the chosen density range for any combination of defined structures and rendering type. If the images are not calibrated, it displays the range as gray levels. If a density calibration was established, it displays the range in Calibrated Units/Levels (or Levels/Calibrated Units).

Figure 82: The Range bar when the Solid Surface rendering type is applied.



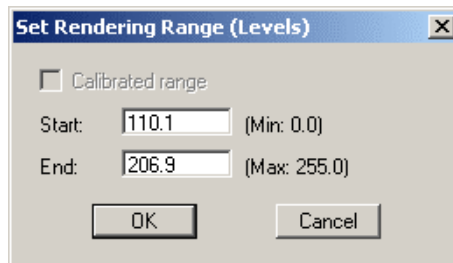
The **Range** bar allows you to render any image object that falls within the range of density values shown. To change the density range, simply drag the ends of the **Range** bar, then press **[Apply]** to create a rendering. To create multiple density ranges, place the cursor in an empty area of the **Range** bar and drag.

Figure 83: The Status bar.



The status bar has a green flash in the top left hand corner. Double clicking on the green flash or double clicking on any of the sliders in the range bar calls up a *Set Rendering Range* dialog box. This is useful if you want to enter very precise values for the range settings and find the sliders difficult to adjust accurately.

Figure 84: The Set Rendering Range dialog box with Calibrated range selected. This shows the start and end of the rendering range in the calibrated units of the current dataset.

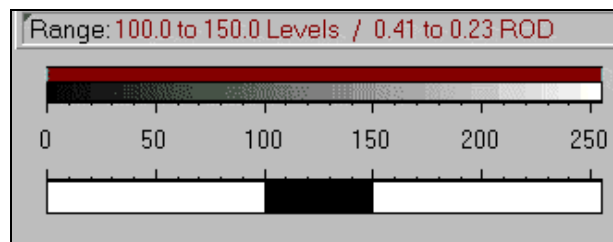


Selecting other rendering types changes the appearance of the **Range** bar (see below).

Maximum, Minimum and Surface Density Rendering

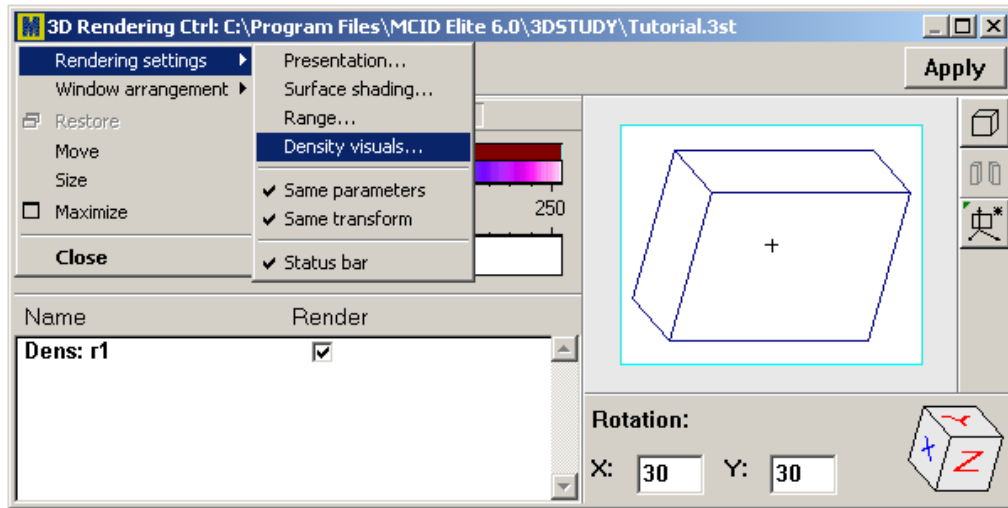
Density renderings can display the Maximum, Minimum or Surface densities of the 3D dataset.

Figure 85: The Range Indicator when Maximum, Minimum or Surface Density is selected.



The density range indicator is now split into two parts, a **Range** bar and a set of **Visuals** controls. The **Range** bar is used to specify the range of density values to be rendered (see section above). The **Density Visuals** controls (displayed above the **Range** bar) are used to modify the shades of gray (or colors) to which the density values are mapped. They work much like the LUT contrast controls in the *Visuals* operational window. To compress the LUT to a smaller range of density values, drag the ends of the control bar above the gray scale/color bar. To select another LUT (*.vis file), open the *3D Rendering Control* window menu and select the *Rendering settings > Density visuals* option.

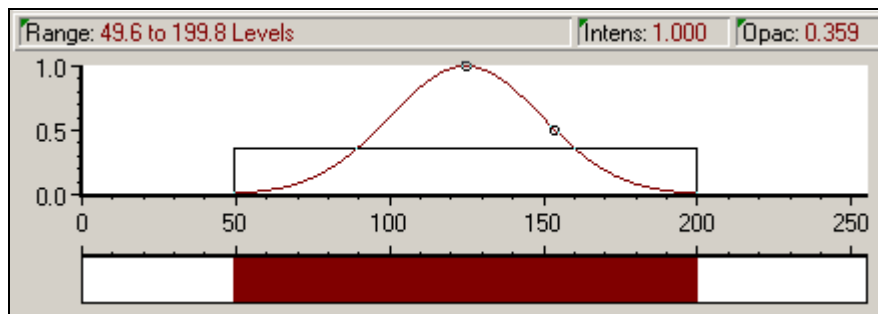
Figure 86: Changing the gray scale/color map used for density renderings.



Density Color and Opacity Rendering

When you choose either of the color-opacity rendering types you see a graph of the distribution of density values displayed above the range of gray levels. The default mode has a Gaussian curve applied to Density, and a Square wave applied to Opacity. Other curve types are available for both parameters.

Figure 87: The Range Indicator when Density Color-Opacity rendering selected. The display is similar with Structure: Surface Density-CO selected.



Clicking on the Gaussian curve displays two markers, one at the peak and the other at the Full Width, Half Maximum Height point. Move the cursor onto the peak marker and drag

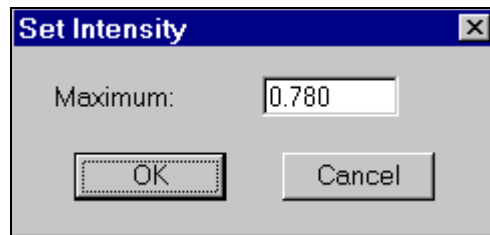
it downwards. This reduces the height of the curve and the **Intensity** value in the Status bar changes. Click on **[Apply]** to see the effects on the 3D dataset. Other curves contain similar adjustable markers.

Click on the Square wave to reveal the marker in the center of the horizontal part of this curve. Hold down the left mouse button and drag the double arrow downwards. This changes the **Opacity** value. Click on **[Apply]** to see the effects on the 3d dataset.

The graphic scale of **Intensity/Opacity** values is auto-sizing. If you continue to drag the curve markers downwards, the scale and the curves are redrawn to new values.

The **Intensity** and **Opacity** buttons in the status bar both have green corner tabs. Clicking on one of these buttons calls up a dialog box that lets you enter **Intensity/Opacity** values to 3 decimal places.

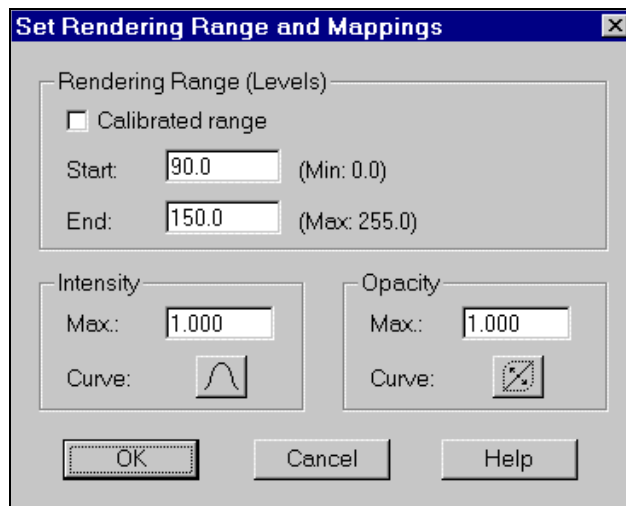
Figure 88: The Set Intensity dialog box. The Set Opacity dialog box is similar.









Rendering Range and Mappings

Double clicking on the **Range** bar calls up the *Set Rendering Range* dialog box. This dialog will differ according to the current rendering type; with maximim, minimum or surface density renderings it shows the rendering range only.

Figure 89: The Set Rendering Range and Mappings dialog box as it appears for color-opacity renderings, showing the range displayed in gray levels and the intensity/opacity curves.

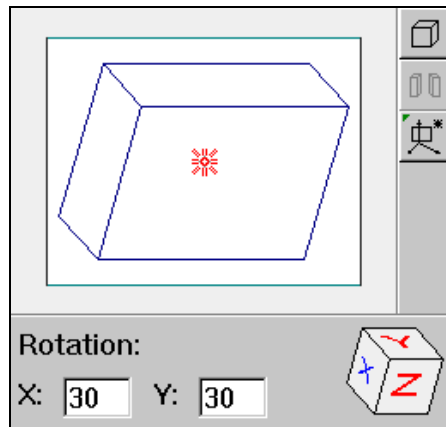


The dialog box shows the extent of the Rendering Range and includes a **Calibrated range** checkbox. Placing a mark in this box changes the scale to ROD units. The dialog has fields for modifying **Intensity** and **Opacity** values. Each field contains a numerical value box (**Max:**) and an icon showing the curve currently applied to this rendering range. Click on either icon to see a fly-out bar with other possible curve types.

-  Applies a Square Wave to the rendered range.
-  Applies a Gaussian to the rendered range.
-  Applies a Left Gaussian to the rendered range.
-  Applies a Right Gaussian to the rendered range.
-  Applies a left Hyperbola to the rendered range.
-  Applies a right Hyperbola to the rendered range.

The Transform Indicator

Figure 90: The Transform Indicator.



The red star at the center of the frame marks the position of the light source that is illuminating the 3D object. Move the mouse cursor onto the star and drag it to a new location. When you click on **[Apply]**, the rendering is re-created showing the effect of the changed position of the light source. You can place the light source anywhere inside the **Transform Indicator**, it is not restricted to the **Viewport**.

The light blue frame is the **Viewport**. Move the mouse cursor onto one of its edges or corners, the cursor becomes a double arrow. Hold down the left mouse button and drag the frame edge to change the frame size. When you release the mouse button and click on **[Apply]**, the rendering changes to shown the area inside the frame at a different magnification.

If you move the mouse cursor into the frame, the cursor becomes a 4-headed arrow. Hold down the left mouse button and drag the frame to a new position. When you release the

mouse button and click on [**Apply**], the rendering changes to shown the new area inside the frame.

Icons

There are three icons located at the right hand side of the window.



Cutting Plane Modes

This icon allows you to select different ‘cutting plane’ modes (i.e., different ways of sectioning a rendered object in order to view internal surfaces). The default mode is to apply no cutting planes to the rendering.



Default Transform

This resets the Transform Indicator to its default. The orientation of the 3D rendering resets when you click on [**Apply**].



Mirror Complement

This produces the mirror image of the cut plane surfaces. The icon is active with the **Plane-cut**, **Corner-cut** and **Corner-keep** cutting plane modes (see below).

Cutting Plane Modes

Cutting planes are used to reveal internal structures in rendered images. Five cutting plane modes are available:



No-cut



Plane-cut



Corner-cut



Corner-keep



Slice

Only one of these icons is visible at any time (default = **No-cut**). Click on it to see the others.

Selecting a cutting plane icon enables the cutting planes controls (Figure 91). There are three ways to define cutting planes:

1. Move the mouse cursor to the slider, press the left mouse button and drag the slider along the scale.

2. Point and click on the left and right pointing arrows. This increases or decreases the axes values one step at a time.
3. Move the mouse cursor onto one of the red dots at the corners of the transform indicator. The cursor changes into a 4-headed arrow. Press the left mouse button and drag the red dot along the axis. If you have only one axis active, the other axes are marked by black dots. Click on one of the black dots, it changes into a red dot to show that this axis is now adjustable.

You can adjust each axis independently, or all of them simultaneously. When you are adjusting the axes, the transform indicator is immediately redrawn to reflect the changes. The scale above the sliders is also updated, indicating the position of the cutting plane. For example, if you selected only the X axis, the values for that axis are shown in red while the non-adjusted axes values are shown in black. Reading from the left, the scale shows values for cutting planes in the X, Y and Z axes.

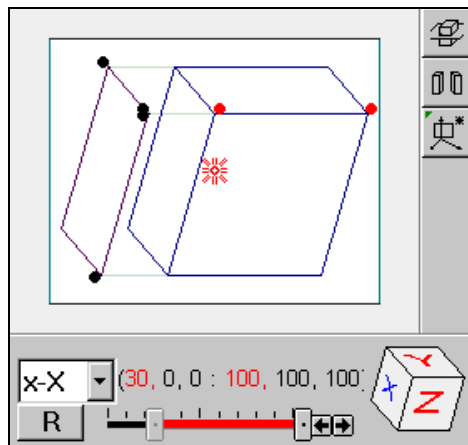
To see the effects of cutting planes on the 3D image, click on **[Apply]**.

To restore the axes to the default condition, click on the **[Reset]** button (labeled **R**). The transform indicator resets immediately, the image is re-drawn when you click on **[Apply]**.

Plane-cut

The **Plane-cut** icon lets you intersect the image using X, Y or Z cutting planes and display the cut surface to show internal structures. You can combine the cutting planes of all three axes, or use each one separately.

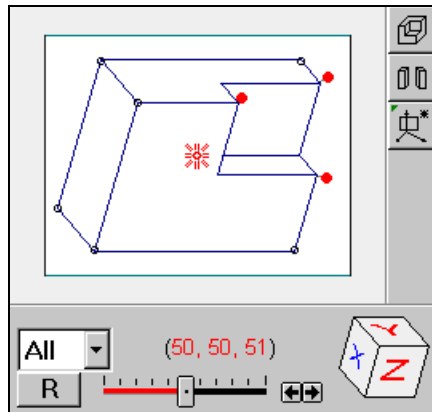
Figure 91: The Cutting Planes controls with the Plane-cut icon selected and X axis adjusted.



Corner-cut

The **Corner-cut** icon lets you remove a corner from the 3D dataset. The outer surfaces of the cut planes are displayed when the corner is removed. Clicking the **Complement** icon lets you display the inner surfaces of the removed part.

Figure 92: The Cutting Planes controls with the Corner-cut icon selected and X, Y and Z axes adjusted simultaneously.

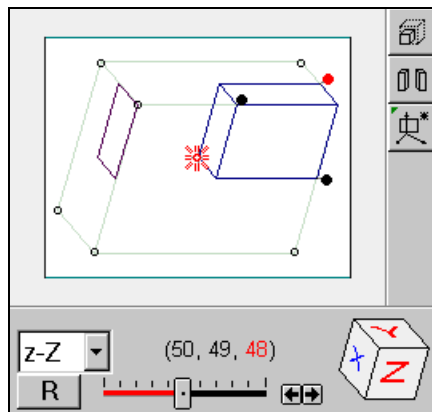


Each of the corners in the transform is marked with a small circle. You can remove any corner by clicking on one of these circles.

Corner-keep

The **Corner-keep** icon lets you display a corner defined by X, Y and Z axes. If you do not change the axes settings you used with the Corner-cut icon, the corner will be the one that was removed by that set of parameters. In other words, Corner-cut and Corner-keep are complement to each other.

Figure 93: The Cutting Planes controls with the Corner-keep icon selected and Z axis adjusted.



There are small, hollow circular markers at each corner of the transform. Clicking on one of these markers selects that corner as the origin of the cutting plane(s). Click on **Apply** to see the effect of the cutting plane on the 3D dataset.

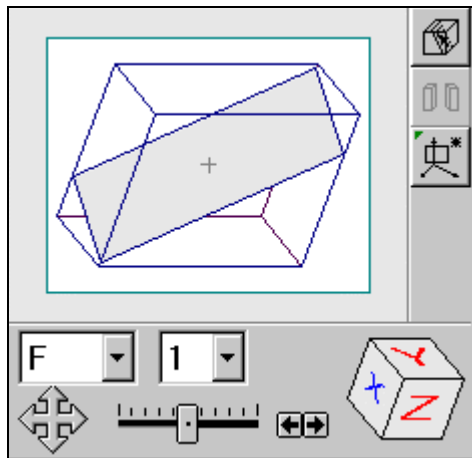
Slice

The **Slice** icon is included in the fly-out icon bar when you select the **Surface Density** rendering type. It lets you create and display slices through the 3D image at one or more levels. One slice is the default, the maximum number of slices you can create is 15.

There are 5 different **Slice** cutting planes: **Free**, **Perpendicular**, **X-axis**, **Y-axis** and **Z-axis**. These are identified by **F**, **P**, **x-X**, **y-Y** and **z-Z** and in the field at the left of the transform indicator. To select these **Slice** cutting planes, click on the arrow next to the field then choose from the drop down list. To select the number of slices, click on the arrow next to the number field. To see the effects on the 3D dataset, click on **[Apply]**.

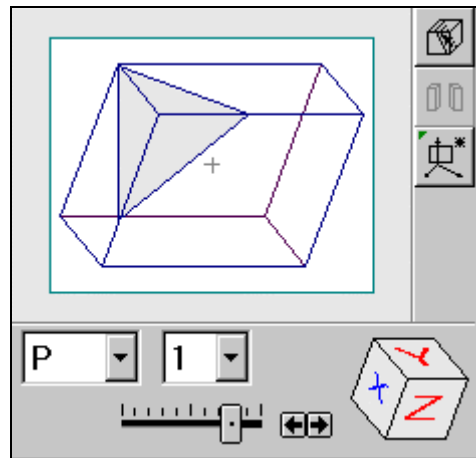
Figure 94: *Slice cutting planes.*

a. Free cutting plane



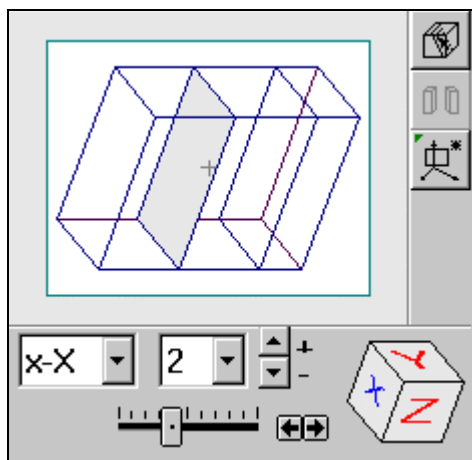
A slice created in the X-axis is tilted by clicking on one head of the 4-headed arrow. The slice may have any orientation.

b. Perpendicular cutting plane



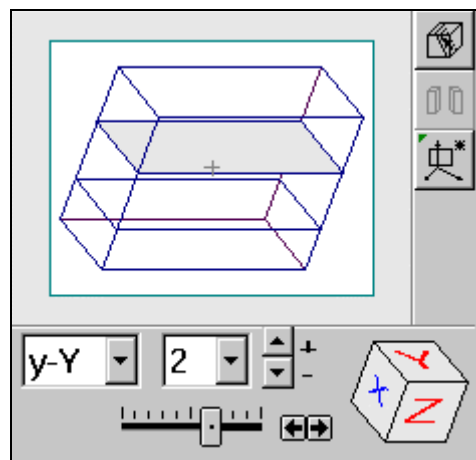
A slice created at the intersection of the X, Y and Z axes. The slider controls the distance along all axes simultaneously, always perpendicular to the viewing axis.

c. X axis planes



Two slices created perpendicular to the X-axis of the dataset. Moving the slider controls the positions of the slices. The +/- arrows control the distance between each slice.

d. Y axis planes



Two slices created perpendicular to the Y-axis of the dataset. Moving the slider controls the positions of the slices. The +/- arrows control the distance between each slice.

Mirroring Complement



This option displays a mirror image of the cut face when either the **Plane-cut**, **Corner-cut** or **Corner-keep** cutting planes are applied. A good analogy for this procedure might be removing a segment when you prepare an orange. When you remove a segment from the orange, you reveal the internal surfaces that separate the segment and its neighbors. The segment’s internal surfaces (which are now exposed) are the complement to the surfaces of neighboring segments. Some pre-conditions need to be met before you can apply the mirroring effect, and **M3D** prompts you to change parameters, if the current conditions are not appropriate for mirroring.

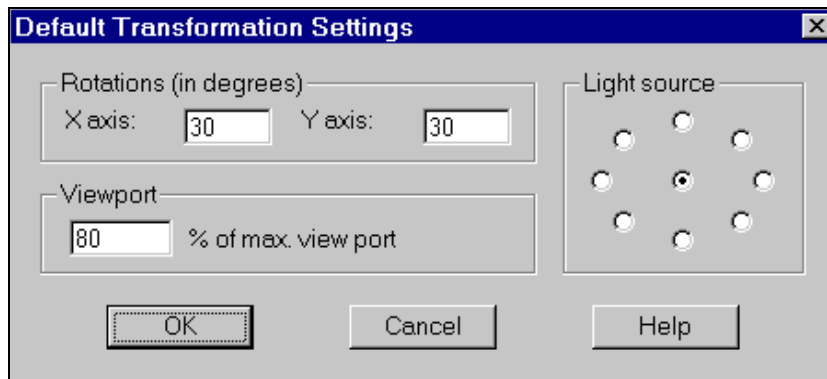
Click on the **Complement** icon and the transform indicator is redrawn to show the mirror image of the intersections of any cutting planes. To see the mirror image on the 3D dataset, click on **[Apply]**. **M3D** draws the mirror image showing the internal faces of the removed segment. The **Complement** icon acts as a toggle; to see the original rendering again, click on the icon, then click on **[Apply]**.

Default Transform



You can edit the default values by clicking on the Default Transform icon while holding down the **<Ctrl>** key. This calls up the *Default Transformation Settings* dialog box.

Figure 95: The Default Transformation Settings dialog box.



Rotations

Sets the default angles for the transform X and Y axes.

Viewport

This is a zoom function that increases or decreases the magnification of the rendered image.

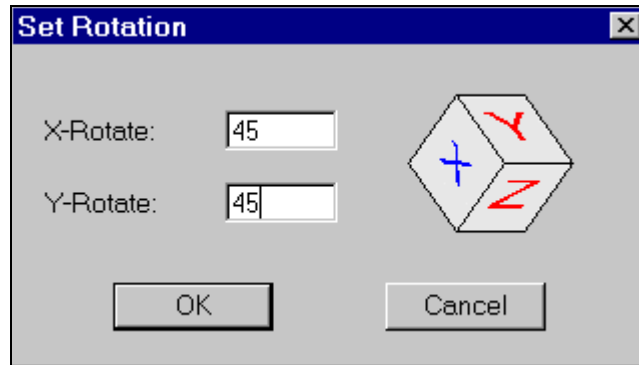
Light Source

The radio buttons select the default angle for the light source.

Rotation Cube

Double clicking on the Rotation Cube calls up the **Set Rotation** dialog box (Figure 96). Type in the X and Y default values for the rotation cube, then click on **OK**.

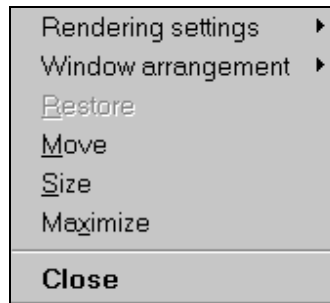
Figure 96: The Set Rotation dialog box.



The Window Menu

The window menu opens when you click on the button at the top left hand corner of the *3D Rendering Control* window.

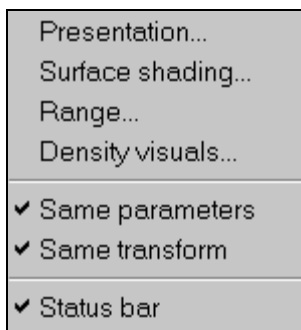
Figure 97: The 3D Rendering Control window menu.



Rendering Settings

Clicking the *Rendering settings* menu option opens a sub-menu.

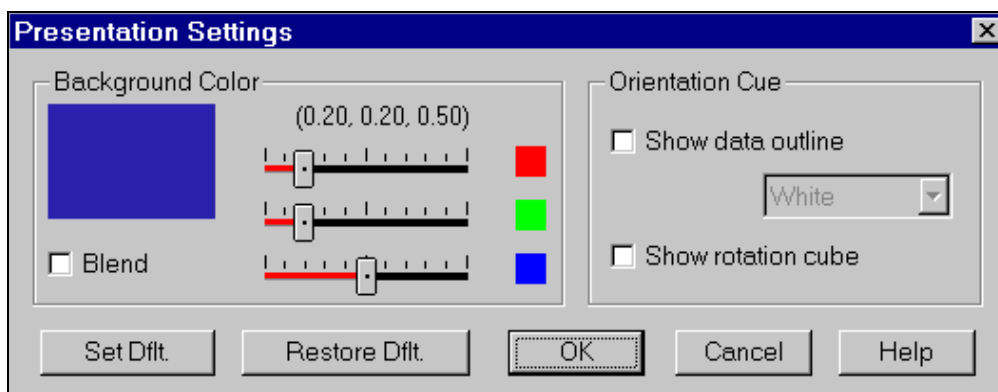
Figure 98: The Rendering Settings menu.



Rendering Settings: Presentation

The *Presentation Settings* dialog box controls how the 3D object is displayed against the background. The default background color is black and this works well with light 3D objects. You will have to experiment to find the best combination of colors for your samples.

Figure 99: The Presentation Settings dialog box.



Background Color

Moving the sliders adjusts the amount of red, green and blue in the background color. If each of the sliders is set to the far left of the scale, the background color will be black. If they are set to the far right, the background color will be white. Moving the sliders lets you “mix” a new background color. In the *3D Rendering Control* window, click on **[Apply]** to display the new background color. Click on **[OK]** to close the dialog box.

If **Blend** is selected, it makes the background transparent and blends it into the rendered objects, but you will see the effects only if rendered structures are transparent. If it is not selected, the background color applies only to the area surrounding the rendered object.

Orientation Cue

Show data outline shows the 3D dataset outline as well as the rendered image. The outline is displayed as a skeleton cube in your choice of color.

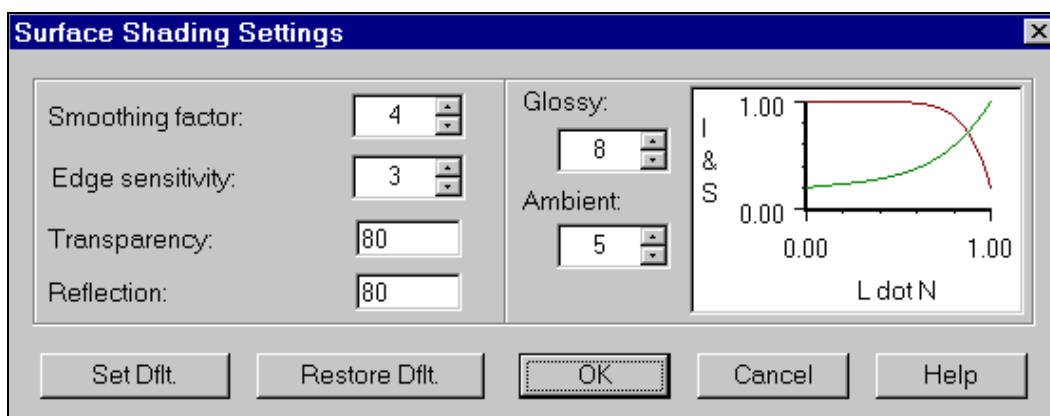
Show rotation cube displays the rotation cube with the rendered image. If you have rendered a complex image, (a large neuron for example) it is helpful to know the original orientation of the data.

The **[Set Dflt]** button lets you save any combination of background colors and parameters as default settings. Press **[Restore Dflt]** to restore default conditions after adjusting the presentation settings. After changing any of these parameters, click on **[OK]** to leave the dialog box then click on **[Apply]** to see the changes take effect.

Rendering Settings: Surface Shading

The *Surface Shading Settings* dialog box (Figure 100) controls the appearance of the rendered surface.

Figure 100: The Surface Shading Settings dialog box.



Smoothing factor

Smoothing is applied to the 3D data set. This has the effect of minimizing the “voxellated” effect that makes the images look “blocky”. The degree of smoothing is variable from 1 to 4.

Edge Sensitivity

Edge sensitivity refers to the preservation of edge information during the smoothing process. It should be set low if images are inherently smooth. It can be set high if images contain a lot of detail that must be shown. The degree of edge sharpening is variable between 0 and 10.

Transparency

To display structures inside 3D renderings, the rendered surface needs to be transparent. Transparency is expressed as a percentage between 0 and 100%. The higher the percentage, the greater the degree of transparency and the easier it is to see internal features. To change the transparency, enter a new value then click on **Apply** to see the effect. You may find it necessary to make several adjustments to the transparency before you are satisfied with the result.

Reflection

This affects the brightness of a transparent structure and is variable between 0 and 100%.

Glossy

3D images often look best if the surface has a metallic sheen. High settings of the glossy parameter create this effect. The setting is variable between 0 and 10.

Ambient

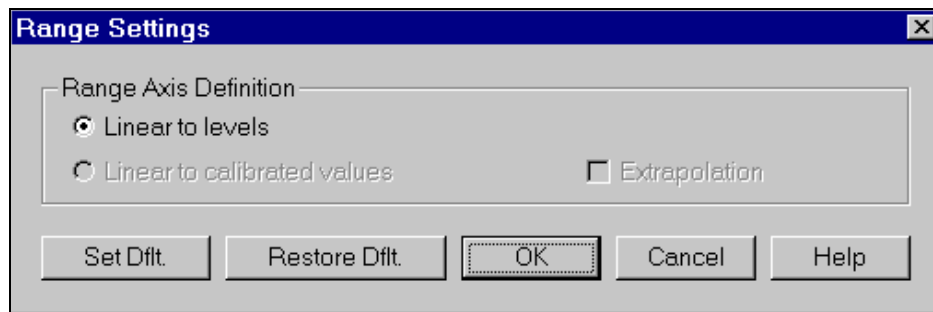
Brightens and dims the 3D image representing the effect of ambient light reflected from that part of the object that is parallel to the light source. The setting is variable between 0 and 10.

The [Set Dflt] button lets you save any combination of surface shading parameters as default settings. Press [Restore Dflt] to restore default conditions after adjusting the settings. After changing any of these parameters, click on [OK] to leave the dialog box then click on [Apply] to see the changes take effect.

Presentation Settings: Range

Selecting *Range* calls up the *Range Settings* dialog box. This interacts with the rendering range settings and affects the display of density values and units in the range bar.

Figure 101: The Range Settings dialog box.



Range Axis Definition

The levels are displayed in the range bar area of the window. Levels may be included or excluded by changing the width of the sliding bar or moving the slider along the range. Both gray levels or gray levels/calibrated values are shown in the status bar.

Linear to levels -- uses gray level values. This is active for any image and the status bar shows either gray levels (if the images are not calibrated) or gray levels followed by calibrated values if a calibration applies.

Linear to Calibrated values -- uses calibrated values and the status bar shows calibrated values followed by gray levels. This radio button is “grayed” if the dataset does not include calibrated values.

Extrapolation -- allows you to use values outside the calibrated range. The checkbox toggles off/on between the established calibration curve and the extrapolation. The checkbox is “grayed” if cannot be extrapolated.

The [**Set Dflt**] button lets you save any combination of *Range Settings* parameters as default settings. Press [**Restore Dflt**] to restore default conditions after adjusting the settings. After changing any of these parameters, click on [**OK**] to leave the dialog box then click on [**Apply**] to see the changes take effect.

Presentation Settings: Density Visuals

Selecting a new visuals file affects rendering methods that display densities. Click on [**Load**] to see a list of visuals files (i.e., look-up tables). Select the one you want then click on [**OK**] to close the dialog box. Click on [**Apply**] to see the visual applied to your image.

Figure 102: The Visuals Settings dialog box.



Any visuals file can be set as the default by clicking on the [**Set Dflt.**] button.

Presentation Settings: Same Parameters

If this menu option is selected, the same range settings apply when you choose different rendering types. This makes comparisons between different renderings much easier to understand.

Presentation Settings: Same Transform

If this menu option is selected, the same transform applies if you choose different rendering types. You can switch between the transform you created and the default.

Presentation Settings: Status Bar

This turns the status bar above the range indicator on or off.

Window Arrangement

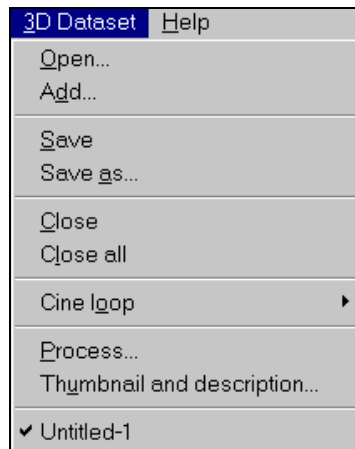
The *3D Rendering Control* window may be displayed in **Vertical** or **Horizontal** format. Vertical format lets you fit the *3D Rendering Control* window inside the *Sample* window without obscuring the data table.

The 3D Dataset Menu

The *3D Dataset* menu contains options for opening, saving, and processing 3D datasets. It also lets you record then play cine loops, and create thumbnail sketches. Some of the

menu options are not available until you interpolate a stack, or open a saved 3D dataset. The 3D dataset is saved with a *.3st file extension.

Figure 103: The 3D Dataset menu showing the current dataset.



Menu Options

Open – Opens a saved 3D dataset. It is easier to open a saved dataset rather than repeat the interpolation of the stack. To see the opened dataset, click on **Apply** in the 3D Rendering Control Window

Add – Appends a 3D dataset to the system list. A list of opened datasets appears at the bottom of the 3D Dataset menu, and the current dataset has a checkmark beside it. To change the current dataset, just click on another one in the list.

Save – Saves the current 3D datasets.

Save As – Saves a file with a different filename so previously saved data are not overwritten.

Close – Closes the current 3D dataset. If you do not have any datasets open, this menu option is “grayed”.

Close All – Closes all opened 3D dataset files. If you do not have any datasets open, this menu option is “grayed”.

Cine loop – Options for creating and playing movie-like sequences of a rendered image (e.g., rotating back and forth). See below for details.

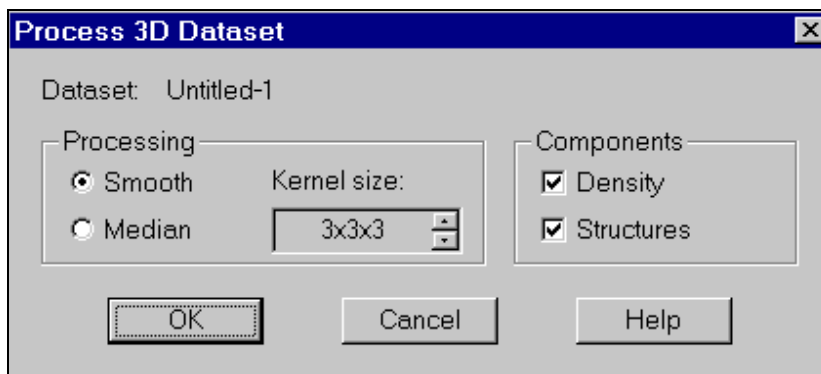
Process – Applies an image processing filter to the 3D dataset. See below for details.

Thumbnail and description – Create a compressed image of a rendered 3D dataset. The image is displayed in the *Open/Add 3D Dataset* dialog box. See below for details.

Processing 3D Datasets

This opens a dialog box that contains processing filters that you can apply to density or structure data. The *Process* menu option is grayed out until you open a dataset.

Figure 104: The Process 3D Dataset dialog box showing a 3x3x3 Smoothing kernel.



Smooth -- Smoothing applies a low pass filter to the 3D dataset. High frequency components are attenuated but low frequencies are not affected. Larger kernels have a coarser effect.

Median -- This replaces each pixel with the median value of surrounding pixels. A median filter is similar to a smoothing filter, but feature edges are not as smooth.

Kernel size -- Four pre-set sizes of kernel exist from 3x3x3 to 9x9x9. Click on the Up/Down arrows to step through the list. You cannot edit these kernels.

Components -- You have to choose either or both of the following components for processing:

Density -- Applies kernels to the density data.

Structure -- Applies kernels to defined structure data.

Click on **[OK]** to start the process. Click on **[Apply]** in the *3D Rendering Control* window to see the result. Save the 3D dataset, if you want to keep the processed data.

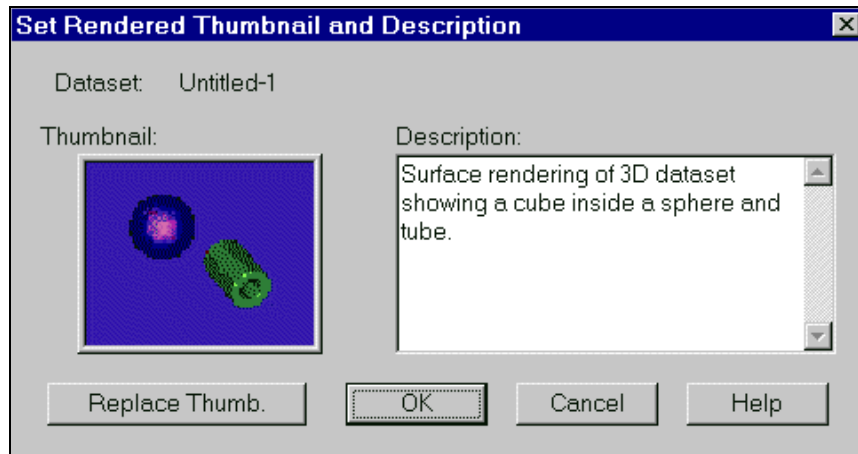
Thumbnail and Description

This menu option lets you create a “thumbnail sketch” and a description of the current 3D dataset. Thumbnails are compressed images that allow you to see the contents of a dataset without loading the entire file into memory. You may find the addition of the thumbnail useful for quick identification of datasets.

To create a thumbnail, click on the **[Set Thumb]** button. A compressed version of the current rendered image appears in the **Thumbnail** box. Place the cursor in the **Description** box and type a brief note to describe the image. Click on **[OK]** then open the *3D Dataset* menu and *Save* the thumbnail and description with the 3D dataset file.

When you have created the thumbnail, the **[Set Thumb]** button becomes a **[Replace Thumb]** button. Suppose you save the thumbnail, then create another rendering and want to replace the original image. Click on **[Replace Thumb]** and a thumbnail of the new rendered dataset appears in the dialog box. If you want to keep the new thumbnail, you have to *Save* it again. The *Thumbnail* option is “grayed” until you create a 3D dataset from a stack, or open a saved file.

Figure 105: The Set Rendered Thumbnail and Description dialog box.



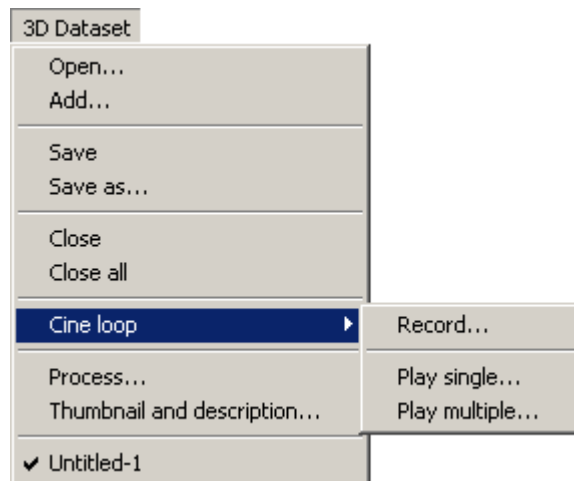
Cine Loops

Cine loops display the rendered image in a dynamic way, which often gives a much better perception of the 3D structure. You can create many possible types of cine loops to show the effects of different degrees of rotation, position of the light source, cutting planes and density ranges. In some cases, you can combine different types of loop, for example light source and rotation, and if none of these types is exactly what you want, you can create your own loop interactively with the **User define** option and the *3D Rendering Control* window.

To create or replay loops, select *3D Dataset > Cine Loop*. The *Cine Loop* sub-menu appears (Figure 106).

Note: The *Record* and *Play multiple* options will be disabled if you do not have a 3D dataset open. To play multiple loops, you must select the 4 channels per page Display Format.

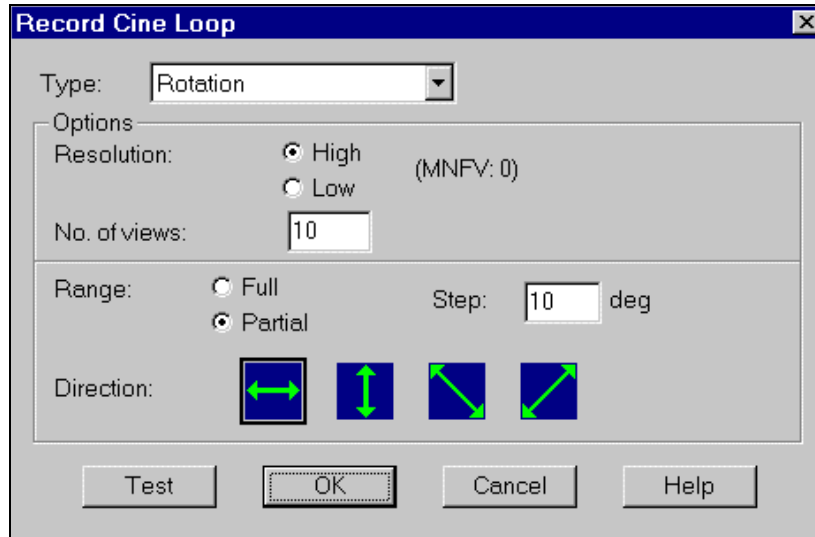
Figure 106: The Cine Loop sub-menu.



Recording Cine Loops

To create a cine loop, chose *Record* from the *Cine Loop* sub-menu. The *Record Cine Loop* dialog box appears (Figure 107).

Figure 107: The *Record Cine Loop* dialog box. *Rotation* is the default type of cine loop.



Types of Cine Loops

In most cases you create a cine loop that rotates the 3D object, but other options are available. Click on the down arrow at the right of the **Type** box to see the list. The list will change depending on the type of rendering you choose and whether any cutting planes are active.

We suggest that you use the [**T**est] button to try out a new cine loop, especially while you are learning. Clicking [**T**est] will let you see how the loop applies to the transform indicator in the *3D Rendering Control* window before you apply it to your dataset.

Rotation Cine Loops

The object rotates through an arc defined by the user. Rotation may be full (360 degrees) or partial (<360 degrees). The smoothness of motion depends on the number of views and the step size. You can define the number of views in the loop and the size of the step between each one. Many views and small steps create a smooth loop, but these conditions are very demanding of system memory and may take some time to create.

Cutting Planes Cine Loops

Cutting planes loops show the effects of applying cutting planes to the 3D rendering dynamically. The cutting plane is applied at intervals determined by the step size and the image accumulates as the loop runs. For example, if you are using 10 views to create a cutting plane loop in the X axis, each view will be 10% of the X axis dimension. The effect is one of a series of slices being added together to produce the complete rendered

object. You can define the axis of a cutting plane individually, (e.g. create a loop cutting in X) or all axes simultaneously (cutting planes in X, Y and Z).

Note: **Cutting plane** loops are not an option in the cine loop menu unless a **Cutting Planes** icon is selected.

Light Source Cine Loops

The light source moves with respect to the object. If the **Rotation** box is checked, the object rotates at 3° intervals in the Y axis as the light source moves.

Density Range Cine Loops

This option creates a loop by mapping the density ranges in the 3D dataset. It is easiest to understand if you use the test function first to see what happens when you create a density range cine loop. Keep the number of views set at 10. The steps between views are now measured in levels rather than in degrees. Set the range slider to mid range values, say 100-150. Click on test and the slider steps through the range at intervals of 15 levels.

If there is a check mark in the Rotation box, you can create combined density and rotation loops. The object rotates at 3° intervals in the Y axis.

Resolution Options

Resolution refers to the size of the rendered images.

High -- High resolution means a quarter screen display, 640x512 pixels.

Low -- the image size is 320x256 pixels.

MNFV -- Maximum Number of Fast Views is the storage capacity of the frame buffer on the imaging board and is linked to the **Resolution** setting. If there are more views in the loop than the capacity of the buffer, the excess views are stored in host memory. This makes the cine loop playback slower.

No. of Views

The number of “still” frames linked together to produce the cine loop. The number of views must be greater than 1.

Range Options

Full -- Creates a cine loop using the full range defined by the number of possible views. For example, a full range rotation loop shows movement through 360°.

Rotation	Cutting Planes	Light Source	Density Range
360°	100%	120°/360°	Full dynamic range

Partial -- Creates a cine loop defined by the number of views and the step size (interval) between each view. The product of number of views *x* step size must be less than the full

range or warnings appear on the display. If this happens, change either the number of views or the step size.

Step Options

Sets the interval between each view in a partial range loop. Step sizes are indicated in degrees for **Rotation** and **Light source** cine loops, in percentage for **Cutting Planes** loops and in levels for **Density** loops. With **Full** range selected, the number in the **Step:** entry field is grayed. You cannot enter any values, but the grayed numbers change as you change the number of views.

Direction Options

The number of **Direction** field icons in the *Cine Loop* dialog boxes varies according to the type of cine loop chosen. There are no direction options for **Cutting Plane** cine loops.

Rotation Cine Loops



Shows the rendered image rotating in a horizontal plane.



Shows the rendered image rotating in a vertical plane.



Shows the rendered image rotating in a diagonal plane from upper left to lower right.



Shows the rendered image rotating in a diagonal plane from upper right to lower left.

Light Source Cine Loops

The **Light source** cine loop dialog box displays six direction fields. If the light source is located at the center of the transform indicator, only the first four direction fields appear.



Light source rotates around rendered object(s) in a horizontal plane.



Light source rotates around rendered object(s) in a vertical plane.



Light source rotates around rendered object(s) in a diagonal plane from upper left to lower right.



Light source rotates around rendered object(s) in a diagonal plane from upper right to lower left.



The light source rotates around the center of the transform. The + shows the center of rotation and the red square shows the light source.



The light source rocks through an arc, defined by the center of the transform and the current light source position. The limits are defined by the product of number of steps x degrees. The midpoint of the arc is indicated by the position of the + and the red square shows the light source.

For example, if you create a loop of 10 views at 9° each, the light source will rock through an arc 45° either side of the midpoint. Maximum limits of arc are 120° (60° either side of the midpoint for a full loop).

To create an arc, drag the red star away from the center of the transform then click on this icon.

Density Range Cine Loops



Each view in the loop is created by moving the rendered range along the scale of gray levels. Each view is separated by the number of levels in the step.



Each view in the loop is created by moving the lower end of the rendered range along the scale of gray levels. The upper end of the range remains fixed, and each view is separated by the number of levels in the step.



Each view in the loop is created by moving the upper end of the rendered range along the scale of gray levels. The lower end of the range remains fixed, and each view is separated by the number of levels in the step.

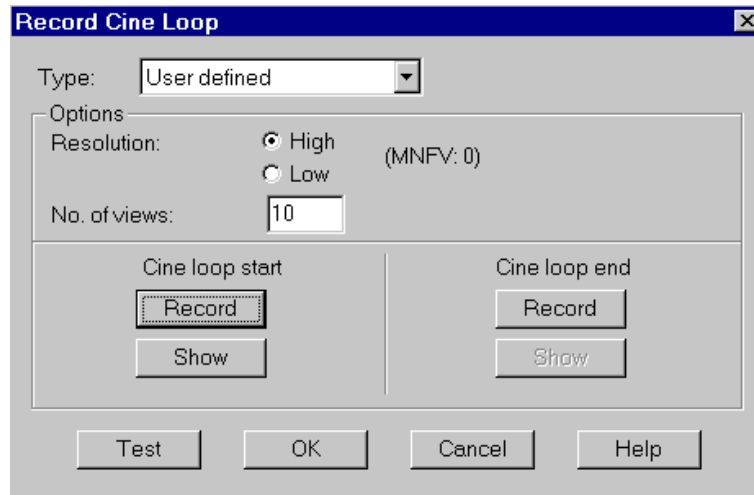


Each view in the loop is created by moving both ends of the rendered range along the scale of gray levels. Each view is separated by the number of levels in the step.

User Defined Cine Loops

This lets you record a cine loop interactively from the *3D Rendering Control* window. You define the settings for the start and end views, then **M3D** uses these parameters to interpolate the $n-2$ intermediate views.

Figure 108: The Create Cine Loop dialog box for User defined cine loops.

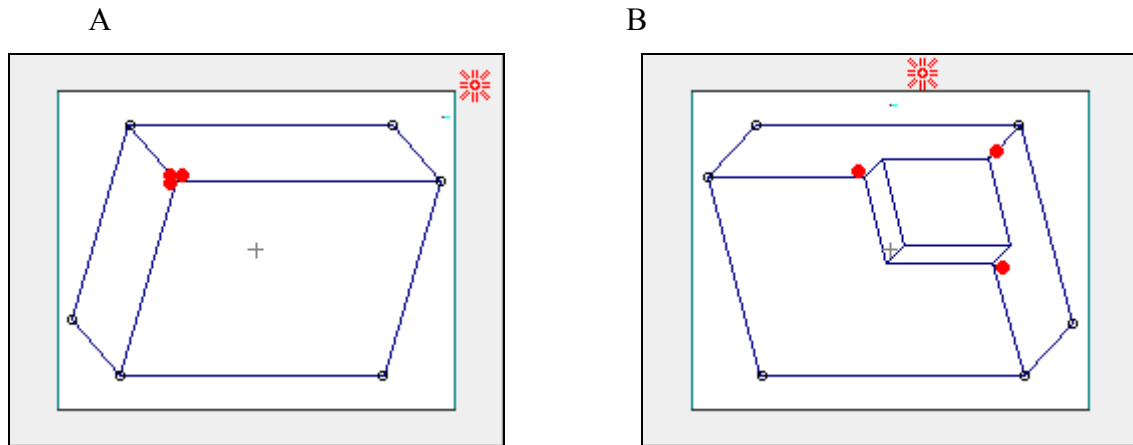


For example, suppose you decided to create a loop that showed the effects of rotation, changing the position of the light source and applying cutting planes, in 10 views.

The procedure for recording the cine loop is very simple:

1. Click on one of the **Cutting plane** icons, then open the *Record* cine loop menu option and select **User define** as the cine loop type.
2. Select the **Resolution** and enter the **Number of views**.
3. Use the **Rotation** cube to set the transform indicator into position for the first view then set the initial positions of the light source and any cutting planes.
4. Move the cursor to **Cine Loop Start** and click on [**Record**].
5. Now adjust the position of the transform indicator, position of light source and cutting planes for the last view.
6. Move the cursor to **Cine Loop End** and click on [**Record**].
7. Click on the [**Show**] buttons in the **Start** and **End** fields to see the parameters set for these views.
8. Click on [**Test**]. The interpolated views between **Start** and **End** are shown in the transform indicator.
9. Click on [**OK**] to create the cine loop.

Figure 109: The start (A) and end (B) views of a user defined cine loop displaying rotation, change in position of the light source, and the application of cutting planes.

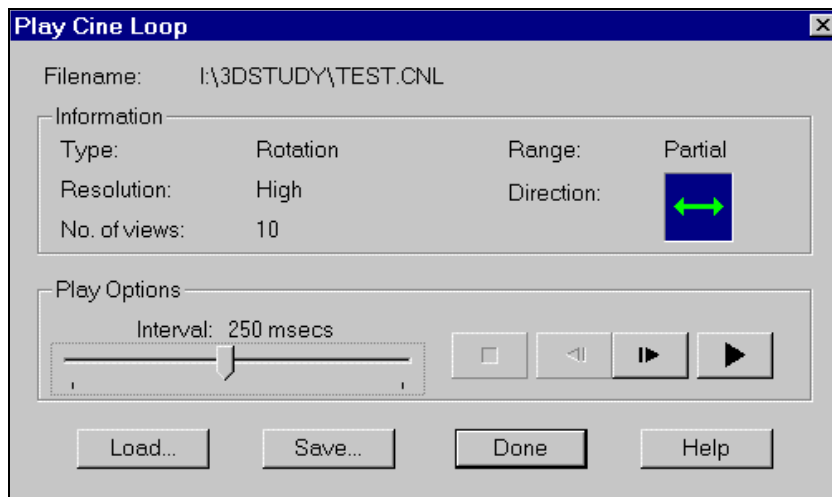


Playing Single Cine Loops

This option plays a single cine-loop in one channel. When you choose *3D Dataset > Cine Loop > Play single*, the *Play Cine Loop* dialog box appears (Figure 110).

The *Play Cine Loop* dialog box contains the name of the loop (if it has been saved), a description of the loop type, and loop attributes such as resolution, number of views, range and loop direction. The **Play Options** area contains a slider control and four buttons that control playback.


Figure 110: The *Play Cine Loop* dialog box.




The **Interval** slider control changes the time interval between each view and consequently, affects the replay speed of the cine loop. The interval between views is adjustable from 30 msec to 2.93 sec.



This button starts the loop running in continuous mode. The loop plays until you stop it.

 Moves the loop forward 1 view at a time.

 Moves the loop backward 1 view at a time.

 Stops the loop.

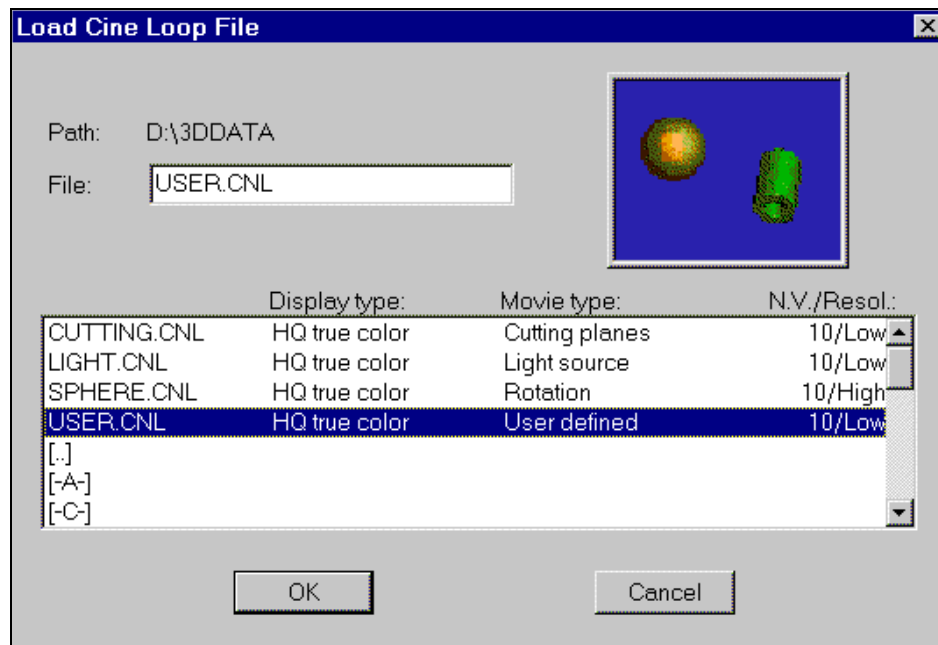
The **[Load]**, **[Save]** and **[Done]** buttons are not active until you stop the cine loop. The **Help** button is active in any mode except continuous play.

Load -- Calls up the *Load Cine Loop File* dialog box (). If you click on **[Load]** before saving the current cine loop, **M3D** prompts you to save it before loading another loop.

Save -- Saves the cine loop. Cine loops are saved with *.cml file extensions.

Done -- Closes the dialog box. If you click on **[Done]** before saving the cine loop, **M3D** prompts you to save it before closing the dialog box.

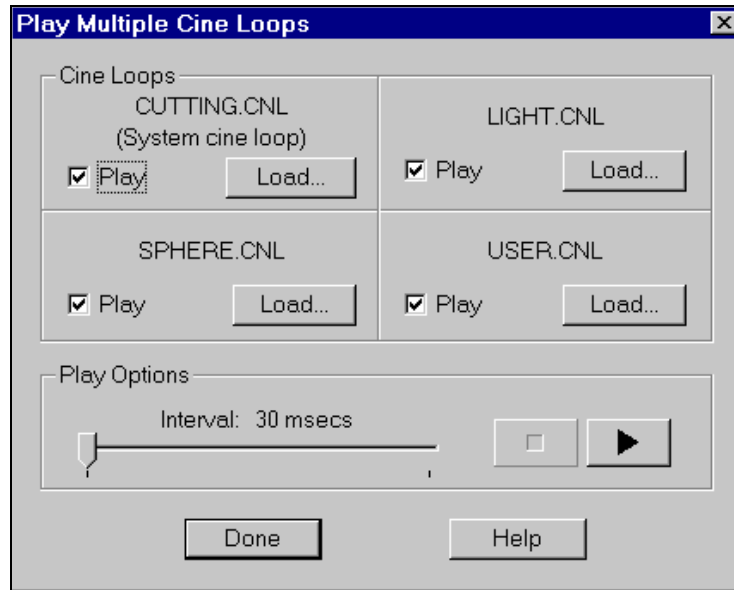
Figure 111: The Load Cine Loop File dialog box. This contains a list of saved cine loops. It shows the display type, loop type, number of views and resolution. When you highlight a filename, a view from the loop appears in the small frame.



Playing Multiple Cine Loops

This option replays up to four different cine loops in four channels. It is not active until you select **4 channels per page** in the *Settings > Display format* menu.

Figure 112: The Play Multiple Cine Loops dialog box with four loops created from the sample dataset. The quadrants in the dialog box correspond to the 4 channels on the image monitor.



The **System Cine Loop** is the loop played by **M3D** during the last cine loop session. It is stored in the system’s memory and overwritten by the loop played in the current session.

Load -- Clicking on [**Load**] calls up the *Load Cine Loop File* dialog box (Figure 111).

Play -- If there is a mark in this checkbox, the selected loop is played in the corresponding quadrant on the image monitor.

Interval -- The Interval slider control changes the time interval between each view and consequently, affects the replay speed of the cine loop. The interval between views is adjustable from 30 msec to 2.93 sec. In multiple loop mode, the slider affects all of the selected loops simultaneously.



This button starts the selected loops running in continuous mode.



Stops the loops.

3D Data Measurement

M3D has a dedicated set of **3D Sample** tools accessible in the *3D Rendering Control* window.

Note: The **3D Sample** tools do not work with the density rendering types. If you want to make measurements of the sample dataset, apply the **Solid Surface** rendering type first.

Figure 113: The 3D Sample toolbox.



3D Sample Tools



3D Pointer

The **3D Pointer** tool measures the volume (**3D Volume**) and surface area (**3D Surface**) of any object in the rendered image. Move the cursor onto the rendered image and click on an object. The **3D Volume** and **3D Surface** data are reported in the data table. If the dataset contains no spatial calibrations, volume is expressed in ‘voxels’ and surface area in ‘pixels’.



3D Full Volume Scan

The **3D Full Volume Scan** tool measures the total volume and surface area of all structures rendered in the same color. To select a structure, click the Full Volume Scan tool on it. The volume and surface area of all the targets are reported in the data table.



3D Line

The **3D Line** tool measures straight-line distances between points on the surface of a single rendered structure. Press the left button and move the mouse to draw a rubber band line. The **3D Length** data are reported in the data table.



3D Length: Trace

The **3D Length: Trace** tool measures length of line between two points on a single object, as drawn. Press the left button and move the mouse to sketch a line. Release the button to sample.



3D Length: Connect

The **3D Length: Connect** tool measures length of line along a series of connected points on a single object as drawn. Press the left button to define points and **M3D** connects the points as you go along. Click the right button to back up a point. Double-click the last point to obtain a measure.

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