TABLE OF CONTENTS

Introduction .................................................................................................................. 3
  Image Basics .............................................................................................................. 3
  Image Data ................................................................................................................ 4

Display ......................................................................................................................... 5
  Layouts ...................................................................................................................... 6
  Options .................................................................................................................... 7
    Zooming.................................................................................................................... 7
    Ruler/Angle Tools .................................................................................................. 7
    Crosshair Settings .................................................................................................. 8
  Color Tables .............................................................................................................. 8
  Overlays ..................................................................................................................... 9

Regions of Interest ...................................................................................................... 10
  ROI - Multi-slice ...................................................................................................... 11
  ROI - Controls .......................................................................................................... 12
  Mouse Actions ......................................................................................................... 12
    Crosshair ............................................................................................................... 12
    Paint/Edit .............................................................................................................. 13
  Draw ......................................................................................................................... 13
  ROI - Special Tools .................................................................................................... 13
    Seed Growing ........................................................................................................ 14
    Isolate Component ................................................................................................ 14
    Delete Component ................................................................................................ 14
    Dilate/Erode Component ....................................................................................... 14
    Copy Component ................................................................................................... 14
  ROI - Entering/Editing/Synthesizing (3-D) .............................................................. 15
    ROI Logic Calculator ............................................................................................ 15
    Add ROI ................................................................................................................ 15
    Threshold to ROI ................................................................................................ 15
      Range ................................................................................................................... 15
      Shrink Wrap ........................................................................................................ 16
      Convex Hull ........................................................................................................ 16
    ROI Inspector ........................................................................................................ 17
    ROI Morphological Operations ............................................................................. 17
      Erode ...................................................................................................................... 17
      Dilate ..................................................................................................................... 17
      Open/Close .......................................................................................................... 17
      Shrinkwrap .......................................................................................................... 17
      Convex Hull ........................................................................................................ 18
      Propagate ............................................................................................................ 18
      Reflect ................................................................................................................ 18
      Single/All Slices Actions ...................................................................................... 18

Statistics ...................................................................................................................... 19

Graphs .......................................................................................................................... 19
  Histogram ............................................................................................................... 20
  Cross-section .......................................................................................................... 21
Introduction

A great deal of information is needed to effectively analyze tomographic biomedical images acquired using magnetic resonance imaging (MRI), x-ray computed tomography (CT), positron emission tomography (PET), and single photon emission computed tomography (SPECT). Knowledge of anatomy, physiology, of subject(s) health, and imaging modality (MRI, CT, PET, SPECT, etc.) are clearly important, as these are needed to define study objectives and guide analyses. However, an understanding of how medical images are stored, displayed, and analyzed can extend the range of information one can obtain from biomedical images. Many image analysis tools are available, so it is the task of the analyst to select those that best address their needs. As such the user should be aware of the purpose, capabilities and limitations of their image analysis tools. An image analyst should have a clear understanding of their display and analysis methods. In Mango, the focus is on visualization and analysis of tomographic images, so these are used as examples of its display capabilities and analysis procedures. It would be helpful to follow along using Mango with these examples while reading. When Mango is started a Toolbox is displayed with basic menus for opening images, setting options, getting help, selecting windows as well as several special control icons.

Image Basics

A tomographic biomedical image can be thought of as a scale model of a biological object that has a limited field of view spanning a subset of the object (i.e. head, abdomen, knee). These digital 3-D images (also called volumetric images) are divided into small volume elements called voxels (Figure 1). A voxel’s location is designated by its column, row, and slice position. A voxel has two other properties: volume and value. Its volume is equal to the product of its dimensions (mm$^3$). A voxel’s value may be parametric (CT number, T1, T2, cerebral blood flow) or a relative value (MRI signal strength, SPECT counts). Voxel values are stored in image files and organized such that each value can be retrieved using column-row-slice addressing.

It is important to distinguish slice thickness, as acquired by a tomographic imaging system, from slice spacing. For example, an MR image might be acquired using 1x1x5-mm voxels using thick slices (or sections) with a 1-mm gap between. In this case the acquired voxel volume is 1x1x5 mm$^3$ but the dimensions needed for accurate voxel display and spatial

Figure 1. 10X zoom to illustrate voxels in an axial section image of the brain. Voxel at intersection at blue crosshair has image coordinate (95, 93, 75) and world coordinate (-33, 15, 10). Voxel value is 1700 (unitless for this 1-mm isotropic spacing 3-D MR image).
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measurements are 1x1x6 mm³. For this example signals from tissues within the 1-mm gap are missing, which can be problematic for studies when subject scans must be repeated, since the gap will not likely be positioned identically at each imaging session. For tomographic studies with large between-slice spacing compared to within-slice spacing, the precision in spatial measurement is much poorer in the slice direction. *When spatial measurements are the goal (volumes, areas, distances, etc.) images should be acquired using a small isotropic spacing, preferably 1-mm or less, such that accuracy does not depend on orientation.*

An image’s column, row, slice spacing and extent can be modified using a process called ‘reslicing’ (File/Save As...,/Reslice). Voxel spacing in a resliced image is under the control of the user. Voxel values for locations between the original locations are calculated using interpolation. Three interpolation options are provided: 1) Nearest Neighbor, 2) Trilinear, and 3) Sinc. These options are at the Reslice/Transform tab when saving an image. We suggest using nearest neighbor interpolation for studies where the voxel values need to be preserved (i.e. histograms before and after reslicing). *Trilinear interpolation works well with many images and is generally a good option when acquired voxel dimensions are small compared to the imaging system’s spatial resolution (FWHM), such as for fMRI, PET and SPECT.* Sinc interpolation is recommended for use with higher resolution images such as anatomical MRI where the voxel dimensions are approximately equal to the spatial resolution (1-mm or less). For studies where the summed image value must be preserved (i.e. PET or SPECT to preserve total radioactivity) the sum may need to be adjusted after reslicing. This can be done using Mango’s Image Calculator (below).

**Image Data**

Additional data (metadata) about the image are needed to ensure that voxel positions, dimensions, and values accurately represent the object. For example, the association between image directions (col-row-slice) and body directions (x-y-z) and sense must be known. The standard for the human body is that the +x direction is body right, +y direction body anterior, and +z direction body superior; and these are the standards used within Mango. *For nearly symmetric objects, such as left and right brain hemispheres, it is critical that L-R orientation be maintained throughout all steps in image processing.* This orientation is automatically managed within Mango. Additionally, the physical spacing between columns, rows, and slices, and the unit of measure (mm) for each must be known. Without such information, the displayed image shape may be wrong and measurement of ‘physical’ distances, areas, and volumes may not be accurate. Finally, to determine a voxel value we need to know the data type (number of bits 8, 16, 32, 64; integer or float; signed or unsigned and byte order) and the unit of measure.

These critical metadata are documented and recorded at the time the image is acquired on a biomedical imaging system and stored in a portion of the image file called the ’header’.

Most biomedical imaging systems store images using a DICOM file format. Other popular formats include the NIH recommended NIfTI and NIfTI2 and an older format called Analyze.
Data in image files can be organized in different ways, as prescribed in the image headers. To accommodate this Mango supports numerous image file formats, including DICOM files produced by medical imagers.

When an image file is opened, key information from the header is used for processing and display. Mango represents data internally using a 32-bit floating-point format regardless of the data format in the image file. Voxels are indexed using col-row-slice addressing within this floating-point array. The image header summary in Figure 2 shows that the image data were stored in (X, Y, Z) body order where columns (1st dimension) are along X, rows (2nd dimension) along Y, and slices (3rd dimension) along Z. There are 161 columns, 191 rows, and 151 slices. The ‘Data Orientation’ tag gives the sense of image-to-world mapping. In this example increasing the column number is from right to left (toward ‘-x’). Similarly, increasing row number is anterior to posterior (toward ‘-y’) and increasing slice number is superior to inferior (toward ‘-z’). In Mango all images are represented internally using a ‘standard’ orientation. This simplifies image display and processing since internally all images have identical col, row, and slice order.

There are several options in the image viewer’s Edit menu that may be helpful when a new image is opened. The Flip Orientation… option can be used to correct the orientation if it was incorrectly specified or not in the image header. The Origin… option can be used to set a world space origin if the origin is incorrect or missing. The Voxel… option can be used to assign a voxel value at the crosshair’s voxel location. This is helpful for imbedding landmarks that track with the image when a coordinate transform is applied. The Notes… option can be used to include a text note. Saving the image updates this information in the image file header.

When Mango opens a tomographic image, it displays the image with body right on the right side of the image (Figure 3), a Neurological style display. This can be changed to a Radiological style with body right on the left in the Toolbox Options-Preferences… menu. Note: menu options ending with ‘...’ open submenus.

**Display**
When an image file is opened a viewer window automatically displays the image along with menus at the top (Figures 3 & 4). Multiple image files can be opened, each with a dedicated viewer. By default, Mango displays images using a linear grey scale. Keyboard options are provided to adjust brightness (`cmd>mouse up/down`) and contrast (`cmd>mouse left/right`). A shortcut is to use right mouse up/down (brightness) and left/right (contrast). Other color tables can be selected (Greyscale inverted, Spectrum, etc.) using the color table menu button (leftmost on the toolbox). This menu button mimics the current color table. Color tables such as the ‘red-to-yellow’ that range between two colors are useful to display overlays placed on a greyscale image.

**Viewer Layouts**

Mango supports several image layouts within a viewer. The default layout for tomographic images has three orthogonal 2-D section images, one a large view and the other two as small views (Figure 3). By default, the large view displays an axial section (x-y) and the two small views display coronal (x-z) and sagittal (y-z) views. The blue crosshair in each view indicates the intersection point of the three section images. The crosshair position is changed by pointing and clicking the mouse or by dragging the mouse. The mouse pointer within the images is a small red open-center crosshair. *The central portion of this pointer is open so that users can better see the image where it is positioned.* The pointer’s voxel location is displayed in the Toolbox window as (col, row, slice) or (x,y,z location in mm) depending on the coordinate mode (image or world) along with the voxel value.

The larger section view is for most user interactions, for example to add regions of interest (ROIs) as points, lines, or 2-D ROIs. Pressing the space bar swaps a different section into the large view, so the user can choose the most appropriate section to work with. Anatomical directions are indicated in the large view (Orientation Option in View Menu can turn these off). Two orthogonal viewer formats are available: ‘wide’ with smaller views to the right of the larger view and

![Figure 3. Viewer layout for orthogonal viewing (Tall format).](image1)

![Figure 4. Viewer layout for single section image view.](image2)
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‘tall’ as in Figure 3. These formats are provided to help the user selected the best match for space available on their monitor. This setting can be wide for one viewer window and tall for another, i.e. it is viewer specific.

A third viewer layout option is for a single slice (section image) in one view (Figure 4). This is the default layout for a 2-D image, such as an optical image or digital x-ray. When the slice viewer is used with tomographic images, the space bar will toggle viewing axial, coronal or sagittal sections. Since there is only one view it can be made large to aid in tasks such as manually tracing a region of interest. Slice, tall, and wide view settings are in each viewer’s ‘View’ menu.

Options

Zooming
The image viewer can be enlarged (cmd +/=) or reduced (cmd-/) to provide a preferred size. There is also an option in the viewer’s ‘Window’ menu to increase size to the maximum supported by the monitor. Mango also provides zoom control within the views without changing the viewer window size, zooming images in and out using (alt_move_mouse up/down) and panning using (shift_alt_drag mouse) [Figure 5]. When enlarging and/or zooming images additional display pixel values are interpolated. This Interpolation is only for display purposes and does not affect image data. Interpolation uses either bilinear interpolation or nearest neighbor interpolation, user selectable preferences in Mango’s Toolbox. Bilinear interpolation is recommended for better display quality so that transitions between voxels appear smooth and therefore more natural (Figure 5A). Nearest neighbor interpolation is provided for older slower computers or if smoothing is not desired (Figure 5B).

Ruler/Angle Tools
Ruler and Angle tools are available in a viewer’s ‘View’ menu to dynamically measure distances and angles. These tools toggle on (when checked) and are displayed in the large slice view. The tools are independently managed in the three orthogonal section views. The ruler tool has small handles at each end that can be dragged to desired locations. The
ruler tool reports distances between its endpoints in units of mm with fractional mm precision. The angle tool is composed of two lines with a common vertex. Similar to the ruler, the endpoints and vertex of the angled lines have handles that can be dynamically moved. The angle tool reports angles in degrees with fractional degree precision. All measurements with these tools are based on the voxels where the tool ends are positioned. These tools can be used in combination with an image transform (Image Menu) to make measurements on oblique planes.

**Crosshair Settings**
The 'View' menu also has options for turning the main and/or smaller view blue crosshairs on and off. The crosshairs are helpful during analysis, but some users prefer to record pictures with these off. The 'View' menu also provides ROI display options of 'Edge only', 'Mask only', or both. When both are checked the edge is opaque but the mask (inside of the ROI) is made 50% transparent to show the image below. The 'View' menu has an option to display ROI stats for the current slice (when checked). This is helpful for reviewing slice-by-slice ROI stats. Slice stats include ROI area, mean value, standard deviation, center and centroid. Spatial units are voxels (image mode) or mm (world mode).

**Color Tables**
Color tables determine how image values are displayed. Color table options and associated controls are at the color menu button in Mango’s Toolbox.

**Understanding Color Tables.** All color tables have settings for red, green, and blue ranging from full off (0) to full on (255), indexed with 256 levels. Image values are mapped into this range of indices (Figure 6). Image values less than or equal to Display Min are set to index 0 and those greater than or equal to Display Max are set to index 255. In this table values between the display min and max image values are linearly mapped to indices from 0-255. This 256-index range is adequate for biomedical images where the dynamic range (signal max/signal noise) is often less than 100, and sufficient to avoid grayscale contouring in most biomedical images. The linear grayscale RGB table changes linearly from a display level of 0 at index 0 to a display level of 255 at index 255 (Figure 6). Image values associated with this figure range from a minimum of -175 to a maximum of 4760, and the user set Display Min to 200 and Display Max to 3000. Image values between Display Min and Display Max follow the grayscale

![Image](image.png)

Figure 6. An example of how 8-bit color tables are implemented for a linear grayscale table.
Table. Those above Display Max are set to index 255 and those below Display Min are set to index 0.

Different color tables have different R-, G-, and B-values depending on needs. When images are displayed using non-grayscale color tables (i.e. spectrum color table) the resulting colors are called pseudo-colors. Color tables provided with Mango include those starting with a pure color and ending as white (red to white, etc.), changing from one color to another (red to yellow, etc.), and combinations of colors (gold, spectrum). Special color tables are used for logical operations, such as overlapping of two regions of interest as ROI A (AND) ROI B. Logical color tables do not vary by index, i.e. they are a single color.

The recommended approach to create a new color table is to edit an existing table (Options/Color Table Manager...). As with many new tasks color table editing requires practice, but special editing tools are provided to simplify the task.

**Overlays**

Overlaying one image (source) onto another image (target) is robustly supported in Mango. Up to eight source images can be overlaid onto a single target image, and these can be from different imaging modalities. The source image is interpolated to match dimension and spacing of the target image when overlaying. Mango’s coordinate mode determines how source and target images are registered for overlaying (Figure 7). When using ‘image’ coordinate mode (3rd Toolbox button menu) the origin of the source image is positioned to align with that of the target image (col, row, slice=0,0,0) (Figure 7, Left). This works well if the objects within these images are oriented the same and positioned the same distance from this origin, which is often not done, as is the case for Figure 7, left.

When overlaying in ‘world’ coordinate mode (3rd Toolbox button menu) the world-space origins (x,y,z=0,0,0) are aligned (Figure 7, Right). This approach is especially useful for overlaying brain images where a standard origin has been adopted, for example the anterior commissure in the mid-sagittal plane of the brain. However, any two objects can be overlaid in world coordinate mode if the origin in each is set to an identical landmark. This assumes that objects to be overlaid are also oriented the same.

When multiple overlays are used each is
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automatically assigned a different color. Color tables can be changed if needed. Color tables for overlays are different from that of the target (underlying) image. An overlay image is not displayed below Display Min, but is displayed according to the color table between Display Min and Display Max. Within this range the overlay can obscure the underlying target image, and to deal with this overlay transparency can be adjusted to show both source and target images (Figure 7). Importantly, overlaying can be done in the ‘world’ coordinate mode even when image dimensions and voxel spacing are much different. For example, in Figure 7, right: target image array is 240x320x192 at 0.8x0.8x0.9 mm spacing; red overlay 256x256x256 at 1-mm isotropic spacing; green overlay 220x320x208 at 0.8 mm isotropic spacing. Note: The brain images in Figure 7 were not spatially normalized but did have the same general orientation and the same world coordinate origin (anterior commissure). Overlays can be hidden or removed using their color table button. *Hiding them is helpful when several overlays are present, and you want to view them individually.*

**Regions of Interest**

Mango supports point, line, single-slice (2-D) and multi-slice (3-D) ROIs. A point ROI is indicated in the viewer as a small colored circle (Fig 8, red). When selected for analysis a white ring appears around the circle. A line ROI is defined as a single or multiple line segments of the same color (Fig 8, green). When selected a white ring appears around its end points. A 2-D ROI can be displayed as an outline (edge), filled (mask) or both (Fig 8, blue). When both options are selected the mask transparency defaults to 50%. The ROI color is determined by the color of the ROI button in Mango’s toolbox. Pressing the button brings up a list of ROI colors. Also, ROI labels can be entered using this button.

To obtain statistics for an ROI, highlight it and select the viewer’s menu option *Analysis/ROI Statistics*. A shortcut for ROI stats clicking the right mouse button with the mouse pointer over the ROI. Summary statistics are posted in the ‘All Results’ window (Figure 8) for

Figure 8. Slice, Point, line and area ROIs with statistics detailed in Results tables.
selected ROIs. ROI Stats can be calculated for the current slice, all slices, or over the ROIs entire volume (slice range). The All Results widow has two tabs, one for Global stats (overall) and the other for Point stats (key locations). Global stats provide mean, sum, standard deviation, size (line[mm] & volume[mm$^3$]), and count (number of voxels). *If the area of a 2-D ROI is desired the user can divide the reported volume by the slice spacing or assess using the voxel count and the area of the voxel face.*

Point stats are provided for the maximum, minimum, and centroid locations of each entry in the global table. The large crosshair can be positioned at the point location in the image by selecting a row in the Points table. In Figure 8 the blue crosshair is positioned at the red ROI for Point 2, and the Point-2 row in the Points Stats table is highlighted. An ROIs centroid may not fall within the ROI, and in Figure 8 it is outside both the green line and blue ROI. Figure 8 also includes data for whole slice stats. *Note that the blue ROI in Figure 8 was treated as a single ROI though it had two non-connected components, one in left hemisphere and one in right.*

**Multi-slice ROI**

A *multi-slice ROI of the same color* is a 3-D ROI (also called a volume of interest or VOI). Figure 9 illustrates the multi-slice nature of Mango’s 3-D ROIs in the orthogonal slice viewer. The surface viewer (Figure 9, lower right) reveals the 3-D nature of the surface of the lateral ventricle formed from its ROI. A 3-D ROI need not be connected between or within slices, so it can contain multiple non-connected components as long as they are the same color.

In the surface display of Figure 9 the 3-D ROI was converted to a surface model using small polygons (triangles) spanning the surface in a mesh-like manner. Mango provides two statistical measures for surface meshes, surface area and volume. Surface area is calculated by summing areas of the small triangles. Volume is calculated using a method based on Gauss’s Divergence Theorem.

*Figure 9. Orthogonal section view of ROI spanning the Lateral Ventricle and 3-D surface view to show the 3-D nature of the ROI.*
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(Eberle et al., 1991, Lancaster et al., 1992). Volumes calculated using surface meshes or from image voxels differ by only 1-2%, so either can be used. Surface area depends on spatial resolution and the nature of the surface, so it is best if all images used in a study of surface area have the same spatial resolution. Simple thresholding can lead to a surface other than that desired, along with unintended volume and area stats. For example, the external surface of a brain is best isolated using the ROI/shrinkwrap thresholding option, which avoids internal brain surfaces. When thresholding is used to define an ROI boundary it is important to follow a standard procedure and one that can be readily reproduced.

ROI – Controls
Delineating a region in an image for analysis is the main purpose of ROIs. Mango provides a wide range of ROI entering, editing, and analysis tools. ROI tool actions are for the current ROI color selected using the color icon in the Toolbox. If multiple ROIs are present, selecting a different color brings that ROI to the front, and action is directed to the selected ROI color. ROI manipulation (move, resize, delete, statistics, etc.) requires that it be selected. To select a single ROI using the mouse it must be in pointer mode. Pointing within and double clicking selects the ROI. Also, in pointer mode shift-dragging will open a dashed selection box that you can use to surround and select an ROI. Once selected the ROI is bounded by a control box that can be used for resizing (Figures 8 & 9). When the mouse pointer is positioned inside this box its icon changes to a small hand, and dragging will move the ROI. Double clicking outside a selected ROI deselects it. The ‘Select All’ and ‘Deselect All’ options in the viewer’s Edit menu can be used to select or deselect all ROIs.

Mouse Actions
The icon-style mouse-action menu in the Toolbox has several categories for changing how the mouse is used (Figure 10). Mouse actions are for the current ROI color.

Crosshair
When selecting options the mouse pointer is a standard small up-left arrow (standard system pointer); however, the mouse pointer changes to a small red open-center crosshair when inside a viewer (a targeting crosshair). The location and voxel value (displayed in Toolbox) changes as the crosshair pointer is moved around using the mouse. This pointer can be used to navigate through the images by clicking and/or dragging.

When other mouse actions are selected, the pointer changes to a shape appropriate for the selected action (Figure 10). These actions are only for use in the larger image view. To navigate to a different slice in the large view click or drag the mouse pointer in one of the smaller views or use the keyboard navigation keys.

Jack L. Lancaster & Michael Martinez 12
A ‘Quick Toggle’ setting in Options/Preferences/Shortcuts provides a way to switch between two mouse actions by pressing the keyboard ‘f’ key. We recommend setting the Crosshair pointer as one option (this is the default) to easily get back to the standard pointer mode from other mouse action modes.

**Paint/Edit**

In the Paint/Edit mouse action mode the mouse becomes a tool for creating and editing ROIs. This category has a submenu where the user can select an elliptical or rectangular shaped tool with three actions: painting, erasing, and editing. Dragging the mouse paints or erases. Painting adds voxels under the tool to the ROI, and erasing removes voxels under the tool from the ROI. The editing option will paint when the tool is dragged within an ROI (used to push border outward) and erase when dragged outside the ROI (used to push border inward). Tool size can be increased (+) or decreased (-) as needed. A specific range of image values for painting can be set, helpful to only paint voxels within a desired value range. These tool actions can be extended from 2-D to 3-D if necessary using the ‘Set Tool Size/Set Tool Slice Range’ option. The mouse-action icon is automatically set to that of the last tool selected.

**Draw**

The Draw action button provides a submenu with options for drawing rectangular and elliptical ROIs or for tracing an ROI. Standard rectangular and elliptical shaped objects are drawn by clicking to set the starting position and dragging to set size. Tracing is used to manually outline an area of interest. Clicking the mouse places a point, and moving then clicking sets a new point and connects the preceding point to the current point with a line. This is continued until the user clicks near the starting point to complete the ROI (closes the outline). Tracing can be done by dragging the mouse, but dragging creates more complex outlines (many points to track); however, it can help where smooth outlines are needed. Small errors made when outlining an ROI can be corrected using edit mode. Tracing is mostly used to manually outline a structure to make a 2-D ROI.

The Draw mode also has options for point and line ROI entry. Points are entered for each click and lines by clicking two endpoints. Clicking multiple points draws a multi-segmented line, and pressing enter closes the line ROI. In ‘trace-line’ mode, endpoints of the line can be moved if needed. The line can be moved around (up-down & left-right) in standard pointer mode if the line is selected.

Reminder: The mouse actions of painting, drawing, and editing apply to the section image in the large view, so they are specifically 2-D ROI actions, but their actions can be extended to nearby slices using the slice range option as indicated under Paint/Edit. Note: When a single-slice ROI of the same color is entered on multiple slices it becomes a 3-D ROI.

**ROI – Special Tools**

The Special mouse action menu has tools that automate entry and editing of ROIs. The icon for these is similar to the ‘Magic Wand’ used in Photoshop, so we refer to these as Magic-
Wand tools. These tools are for editing 2-D or 3-D ROIs that consist of one or more non-connected components. Some tools are specifically designed for independent editing of such components. The concept of voxel neighbors is important for processing components. In Mango, a neighboring voxel is one that shares a face (a direct neighbor) and a voxel in a 3-D image can only have six direct neighbors. All voxels within the same component of an ROI are neighbors, but none are neighbors of voxels in other components of the ROI.

**Seed Growing**
For the *Add Region in Value Range* option the user clicks a location in the image to set a starting or ‘seed’ voxel for the ROI. A neighbor voxel will be added to the seed ROI if its value falls within the user-designated value range. This process is repeated and the ROI enlarged until no new neighboring voxels are found within the designated range. This process is called *seeded region growing*. The range can be a symmetric range (Relative Range) or a min-max range (Explicit Range) about the seed voxel’s value (less sensitive to where the seed is selected). The processing is semi-automatic since the user selects the seed location and sets up the value range. This tool is useful for making a 3-D ROI for a small region of interest that is clearly distinguished from its surrounds but which has values similar to more distant (non-connected) regions. *One use of this option is to make ROIs for individual structures such as the lateral ventricles in Figure 9, or caudate, putamen, or thalamus in high-resolution MR images.*

**Isolate Component**
The *Preserve Region* option is used to isolate a single component of multi-component ROI. Automated ROI methods sometimes leave many small, undesired non-connected components. These can be removed by clicking in the desired component ‘preserving’ it and removing all others.

**Delete Component**
An alternative is *Remove Region* that deletes a component of an ROI. Clicking within the undesired components removes them. This option is useful to remove a small number of undesired components.

**Dilate/Erode Component**
The *Dilate/Erode Region* option will dilate (click) or erode (shift-click) a component of an ROI using a 3x3x3 tool (See ROI Morphological Operations below). These actions enlarge or shrink the component ROI. These actions are also menu selectable by right-clicking over the ROI.

**Copy Component**
The *Copy Region* will copy a selected component of an ROI that can be pasted using standard keyboard or edit menu options. To do this we recommend changing the ROI color before

Figure 11. Viewer ROI Menu.
pasting to designate it as a different ROI, or alternatively pasting to a different image.

3-D ROIs – Entering/Editing/Synthesizing
Multiple options are available in the viewer’s ROI Menu that extend Mango’s automated entry, analysis, and editing ROI capabilities (Figure 11). These options are primarily for 3-D ROIs. Some actions such as ‘Dilate’ are meaningless for point or line ROIs, and actions that are not applicable for a selected ROI type are greyed out.

ROI Logic Calculator...
The ROI logic calculator provides a means to make new ROIs from existing ones using the logic operators “NOT, AND, XOR, and OR”. An example is provided in Figure 12 where overlapping ROIs of the visual system were made using the “AND” operator. Users can select the colors and assign names to each ROI. Other options include “union” and “intersection”. Parentheses are provided to support mixed and compound operations.

Add ROI...
Add ROI provides options to insert a cubic or spherical 3-D ROI of selected size (mm) centered at the blue crosshair. This is useful to place an ROI at a brain x-y-z coordinate in world coordinate mode. An implied assumption when using world coordinates for brains is that the brain image had been spatially normalized to either the Talairach or the MNI brain space and that the origin was properly defined. Cubic and spherical ROIs can be used to dynamically probe and analyze different parts of an image when combined with ROI Stats.

Threshold to ROI ...
Threshold to ROI is an automated method to define a 3-D ROI. There are multiple thresholding options. The default uses a single threshold and all voxels with values greater than or equal to the threshold value are included within the ROI. This option is suitable when the tissue of interest has the highest range of voxel values, such as bone in x-ray CT images. NOTE: No menu option is selected for this operation.

Range. With this thresholding option the user can select minimum and maximum values for the ROI. Range defaults to the minimum and maximum display settings, but can be changed as needed. This option is useful for images where ranges are necessary to delineate tissue types such as x-ray CT (fat, soft tissue, bone). This option is also useful to
make an ROI for a range of negative values, for example a range of only z-scores in a statistical parametric image, without including positive ones.

Shrink Wrap. This thresholding option forms a closed 3-D ROI (no holes) about an object using a single threshold value (minimum or % of max). This option is used when simple or range thresholding leads to ROIs with unwanted holes. Shrink Wrap is often used to make a whole brain ROI without holes caused by internal regions, such as ventricles, when using simpler thresholding. The Shrink Wrap option first finds a single closed external ROI then inverts it to form an ROI containing only object voxels. The ‘Shrink Wrap’ procedure uses two criteria when forming the ROI, a threshold and connectivity. For 2-D options connectivity is enforced either 1) within axial sections, 2) within coronal sections, or 3) within sagittal sections; ignoring directions orthogonal to the section. The 3-D connectivity option ensures within- and between-slice connectivity. The 2-D and 3-D options yield slightly different ROIs, but the 3-D approach is recommended unless you want to avoid connectivity between sections. The Shrink Wrap ROI procedure can be used to define the exterior boundary of any object when a single threshold is appropriate.

Convex Hull. This threshold ROI option forms the ROI as a convex hull using a single threshold value (minimum or % of max), avoiding concavities.

ROI Inspector
The ROI inspector opens a new window that lets the user quickly move the large crosshair to a specific ROI by clicking its color icon. This is helpful when many ROIs are present in an image or the desired ROI is hard to locate (perhaps a point ROI). In Version 3.0 of Mango the Navigate ROIs feature replaced the older Count ROIs feature, and in Mango Version 4.0 the ROI Inspector replaces Navigate ROIs. ROIs are listed in groups as Points, Lines, and Regions (separated vertically). A color button along with the number of items is indicated alongside point and line color icons. When a color icon is clicked the large crosshair is moved to the indicated ROI. For points and lines with multiple ROIs, repeatedly clicking the icon cycles through them. The 2-D and 3-D ROIs are listed in ‘Regions’ rows by color. There are options to select an ROI when moving the crosshair to it and to deselect others. ROI labels can be entered and/or edited in the inspector’s text field. Note: The number of ROIs will expand by rows of eight if necessary.

ROI Morphological Operations
Morphological operations are provided to assist in modifying 3-D ROIs. These operations are based on a 3-D structure element, with size user selectable. For size = 3 the structure element is a 3x3x3 region. The default size of the structure element is 3, but this can be changed before using the operator. If an even number is entered it is adjusted up to an odd number to ensure that the operation is symmetric about its center. Note: Morphological operations are not provided for point or line ROIs. Also, these operations can be restricted to 2-D by selecting the single vs. multiple slice option in Mango’s toolbox.
**Erode ...**

The Erode operation reduces the boundary of an ROI. To aid with the description of this operation we will assume that voxel values within the ROI = 1 and outside the ROI = 0. During the erode operation the structure element is moved throughout the image testing for boundary ROI voxels. When the structure element is centered over ROI(x,y,z) the erosion action sets its value to the minimum value within the structure element’s range. Away from a boundary where the structure element is either fully outside (all 0’s) or inside the ROI (all 1’s) no action is taken. Near a boundary, where there are both 0’s and 1’s within the structure element’s range, ROI(x,y,z) is set to zero, removing boundary voxels. Erode ROI will remove an isolated component for an ROI that is smaller than the structure element. Eroding action is useful to remove small components outside the main ROI that were inadvertently included when the ROI was made, such as those due to phase noise in MR images. The Preserve action in the Cursor Special menu can be used to remove components that were disconnected by eroding, preserving only the main component.

**Dilate ...**

The Dilate operation extends the boundary of an ROI. The dilate operation is similar to the erode operation except it sets the ROI value at the structure element’s position to the maximum value within the structure elements range, i.e. the numeric value assigned to the ROI. This action adds voxels to the boundary of the ROI. ROI dilation is useful to fill in holes within an ROI that are smaller than the structure element. It can also be used to bridge gaps and enlarge thin connections between different parts of the ROI.

**Open Close ...**

Erosion and dilation can be used in pairs since they can undo each other’s operation. As such two other ROI morphological operators are provided: 1) Close that dilates then erodes and 2) Open that erodes then dilates. These operations are successful as long as desired small regions are not eliminated (erosion) or a desired small hole are not filled in (dilation) by the first operation. Analyst should practice using these morphological operations to gain more experience and determine conditions when they will be useful in editing ROIs.

**Shrink Wrap ...**

The Shrink Wrap... menu option uses a single ROI as the starting point for shrink wrapping the region of interest. The starting ROI is used to establish a working region surrounding the structure of interest, while excluding areas that might inadvertently be included based on the threshold value. For example, it can be used to form a 2-D ROI about the corpus callosum (CC) in the mid-sagittal slice. First, roughly trace an outline ROI about the CC in the mid-sagittal section image. Inspect the histogram from within this ROI to determine a threshold value, then shrink wrap the ROI onto the CC (Figure 9). This option works with both 2-D and 3-D ROIs. Additionally, it performs independent shrink wrapping for each component of multiple component ROIs.
**Convex Hull**

Another option is to convert an ROI to a Convex Hull ROI. A convex hull is a surface that has no concave regions, in this case an ROI’s surface. There are a number of algorithms for this but you can think of it as adding voxels to fill in concavities. The **convex hull** option can be used to make an ROI in a high-resolution brain image that is geometrically similar to one from a low-resolution image (PET, SPECT, fMRI) **both without sulcal regions**. Some investigators have used the convex hull as a means to determine the level of gyrification, comparing a convex hull brain ROI with a corresponding shrink-wrapped brain ROI.

**Propagate ...**

The Propagate... operation extends a 2-D ROI (such as one formed using ROI painting) to adjacent slices, forming a contiguous 3-D ROI. Options are provided for the user to set the desired slice range or all slices. A common use of this operation is to enter an ROI enclosing the data of interest to remove exterior image data, noise or otherwise. To achieve this the user first enters a rectangular ROI that extends across a section image. This ROI is then propagated to enclose the slice range of interest. Finally, the Image Calculator (below) is used to zero all voxel values outside of the ROI.

**Reflect ...**

The Reflect about Vertical and Horizontal option makes a copy of an ROI reflected about the vertical or horizontal position of the large crosshair. **This is useful to compare stats of an ROI formed in the one brain hemisphere with an identical ROI in the other hemisphere by reflecting it about the brain’s midsagittal plane.**

**Single/All Slices Actions**

You can toggle ROI actions between single slice and all slices using the slices button in the Toolbox. The icon for this button has three horizontal bars, representing slices. The default setting is the ‘All Slices’ mode where all three bars are bold. In the ‘Single Slice’ mode only the middle bar is bold. Clicking the button toggles the slice modes. These modes are also referred to as ROI/VOI processing modes. The single-slice mode can be used to apply different ROI thresholds for different slices during entry. The single-slice option can also be used to erode or dilate a single slice of an existing 3-D ROI. **Most ROI entering and editing is best done in the default ‘all slices’ mode.**

**Important:** Many ROI actions in the viewer’s ROI menu can be undone using the ‘Undo’ option in the viewer’s Edit Menu. A buffer stores the prior result providing this undo capability. There is also an option to ‘Redo’ that will reverse the action of Undo. **Only one level of undo/redo is supported, so make sure you are done before beginning other processing.**
Statistics
The most basic image analysis feature in Mango is a statistical summary of an image or its ROIs (mean, sum, standard deviation, size, and voxel count). Image and ROI statistics are options in the viewers Analysis menu. A shortcut for image analysis is to right click within the viewer, which pops up a small menu with an option to Analyze the image. The results of the analysis pops up the ‘All Results’ window which tabulates Global (full image) and Point statistics (Figure 13). When image statistics is highlighted in the viewer's Analysis menu more options are available than provided by the quick popup analysis. Additional options include calculating over the entire 3-D image, the current slice, or slice-by-slice. For 4-D data, options are provided for ‘Entire Series’ or ‘All Volumes’. If multiple ROIs are present, individual stats will be calculated for all that are selected. Tabulated stats can be highlighted and copied into another document (word, excel, etc.) or exported to a comma-separated values (csv) file format for opening in excel. *Note: The slice-by-slice option is useful for comparing stats of a convex hull vs. shrink wrapped ROI of the brain for coronal brain slices.*

The two tabs at the top of the All Results window toggle between global or point statistics. Global Size is in units of mm³ for images and global Count is the number of voxels. The points table lists values and x-y-z location for the maximum, minimum, and centroid of the image. If an atlas is active its four labels will be listed for each point.

Graphs
Mango provides x-y style graphs for three different data types: 1) a frequency distribution of image values (histogram), 2) data along a line (cross-section), and 3) data along a 4th dimension (time-series). *All graph windows are resizable.*
**Histogram**

A histogram is a graph of the number of voxels within small value bins plotted over a range of image values (Figure 14). When the histogram option is selected it opens a new window with a histogram graph and options for inspection and recalculating.

Histogram values are displayed in the graph area using the mouse down action to move a red line across the range (Figure 14). In this figure, the graph’s value range is 0-3000 (x-axis) and the histogram is plotted using 188 bins of size ~ 16. The highlighted bin range is from 1739.44 to 1755.40, and there are 65,957 voxels with values within that bin.

The graph’s y-axis max is automatically set to the max value in the histogram. The x-axis Min & Max values default to those of the image display settings when the histogram window is first opened. These can be changed by entering new values in the Min and Max text boxes. The plotted histogram can be customized by altering: 1) Min & Max (here 0 & 3000) and 2) the number of bins (here 188 and determined automatically). Clicking Calculate updates the histogram. There are two other settings: 1) Exclude Zero (default setting)– done to keep many zeros often found outside the object from setting the max y-axis display too high and 2) Auto Binning that uses an empirical algorithm to estimate a good bin size for the graph. These can be turned off if desired. We suggest setting Min >0 if Exclude Zero is not selected.

Clicking or dragging the mouse selects and highlights a single bin in the histogram. The highlighted bin range can be expanded using the small arrows. The highlighted range can be dragged across the graph to inspect different regions of the histogram. In Figure 14 there are three brain tissue ranges with distinct peaks, the rightmost peak is for white matter (~2300), the middle peak for grey matter (~1750), and the third peak for cerebral spinal fluid (~800). Voxels within a highlighted histogram range are also highlighted in the main image viewer using the same color. Histogram highlight colors can be changed using the color icon button in Mango’s Toolbox. Highlighting in the image is dynamically updated as the highlighted range in the histogram is adjusted (width or position). This is helpful for determining a threshold to use to define edges for regions of interest. In fact, if the range is acceptable, clicking the Generate ROI option will make an ROI using the highlighted histogram range. ROI editing tools can be used to edit the ROI if needed. **Histogram data can be exported as a ‘csv’ file for further analysis.** This is done by clicking on the small icon adjacent to the camera icon in the histogram dialog window.
As with many of Mango's analysis features the histogram can be calculated for the entire image or for a ROI. The ROI color icon in Mango’s Toolbox determines which ROI to use for histogram analysis. Clicking the ROI checkbox in the histogram window switches from whole image histogram to ROI histogram mode. This is especially useful in refining a roughly formed ROI, such as one traced outside the corpus callosum (CC). The roughly formed ROI excludes most of the brain but contain all of the CC (like in Figure 9). The histogram calculated using this roughly formed ROI can be used to determine a range for refining the CC's ROI.

Finally, the histogram’s graph can be toggled between Data (the default), Rate, and Cumulative modes. Data and Cumulative modes are the most common. The y-max value of the cumulative mode is the number of voxels within the histogram’s range settings (excluding zeros if that was selected). The median voxel value can be estimated from the cumulative distribution as the x-value on the graph where \( y = \frac{1}{2} y\text{-max} \) (where \( \frac{1}{2} \) of the voxels are below the indicated x-value).

Cross-section

A cross-section is a graph of image values spanned by a line ROI. The graph can be plotted as data (voxel values), rate, or cumulative similar to the histogram graph. The position along the line can be displayed in image (voxels) or physical (mm) units (Figure 15). The average value of the cross-section’s data is indicated adjacent to the y-axis. This graph is useful for inspecting left vs. right side profiles using a line spanning from the left to the right side of the body (especially in the brain).

Line ROIs are drawn at displayed resolution and are redrawn to accurately track as the view or viewer are zoomed. The mm-spacing along a line defaults to the smallest unit in the image, but can be made smaller or larger (in mm-mode) by typing the desired value in the mm text area. Values are interpolated to support that use. Moving the mouse within the graph displays positions and values (Figure 15) and dragging additionally displays a point marker on the line ROI in the image viewer. Double clicking in the left margin of the vertical axis brings up a small dialog window to change the vertical min-max range. As with the histogram graph the cross-section graph can be exported as a ‘csv’ file for further analysis. Note: The cross-section data is exported using the line spacing selected for the graph.

Multiple line cross-sections are supported in either the same or different colors. The current ROI cross-section in the display is highlighted in the image viewer. The Next Line option graphs the lines in the order they were entered. Store Copy makes a copy of a cross-
section graph to compare with a graph from a different line brought up using Next Line. *Clear Copies* removes the copies of graphs.

### 4-D Series

A time-series graph is a plot of ROI statistics for a 4-D image where the x-axis is time (Figure 16). The fourth dimension can be time (therefore the name) or any series of images (such as different components from ICA analyses). Mango’s series graph defaults to a static ROI (fixed point, line, 2-D or 3-D) to create time series, so an implicit assumption is that the object within the ROI is not moving in the time series. Mango provides an option for dynamic ROIs for other series when the object is moving (beating heart) or if images of different objects are present in the series (ICA components). In these latter examples ROIs are used to track spatial rather than temporal changes.

A static ROI works well for nuclear medicine studies to assess organ uptake or clearance of a radiotracer. Static ROIs also work well for resting state fMRIs where time series can be used to calculate region-to-region correlations.

Data for a series graph can be the ROI mean, max, min, standard deviation, sum, or volume. Mean and sum are useful when tracking the change in activity of a radiotracer (PET, SPECT, etc.) or performing region-to-region analyses. Volume is useful when tracking volume changes in time such as the left ventricle for a beating heart. The y-axis range for a series graph is automatically set to maximum and minimum y-values. Double clicking to the left of the y-axis opens a dialog window where these can be changed.

Mouse position is indicated as a small circle on the graph along with time (or series index) and value. Clicking the mouse in the graph sets a vertical marker in the graph and switches the image in the viewer to the corresponding image in the series. Dragging the mouse across the graph window changes images, providing control for reviewing the series images.

As with cross-section graphs, a copy of a graph can be temporarily saved for comparison with other graphs. All graphs data can be exported as a csv file for further analysis.
Image Calculator

The Image Calculator (found in the Viewer’s Image Menu) is used to formulate a mathematical equation to manipulate images, similar to using a scientific calculator (Figure 17). Numbers and simple math operators are entered into an equation from the calculator keypad. Images are entered into the equation using ‘this’ or ‘other’ buttons. Pressing the Calculate button evaluates the equation, performs the indicated actions, and assigns results to a ‘new’ image or ‘this’ image (new in Fig 17). The equation can be formulated using numeric values, built-in functions, and images. Recently used equations can be recalled using the Show History listing.

General. The image where the calculator is opened is referenced in equations as ‘this’. Other open images are referred to as other(n) where ‘n’ is the order in which they were opened. The ‘other’ button brings up a list of images as other(n) along with file names (n = 1 through the number of other images open). When selected, other(n) is entered into the equation. In Figure 17 a new image is calculated as the difference between ‘this’ image and another open image ‘other(1)’. The new image is assigned a name based on this image’s name with ‘-new’ appended.

Processing Control

There are two processing control categories:

1. Region. If one or more ROIs are selected there are options to process 1) all voxels, 2) only inside the ROI, or 3) only outside the ROI. Option 3 is good to zero out everything outside the ROI (e.g. background around the object of interest).

2. Options. If the current image is a 4-D image, there are several alternatives: 1) apply to all series points (default) or 2) apply only to slices with ROIs. If a transform is in use then there is an option to apply it before performing calculations.

Make Statistical Image from a Series …

This option is only available in the viewer’s Image menu if the current image is 4-D. A single statistical parametric image (mean, max, min, standard deviation, or sum) is output based on voxel analysis in the 4th dimension (time for fMRI images). Processing range is
from starting image (or time point) to ending image (or time point). Statistics can be calculated using group size = 1 up to size = full range. For example, if there are 10 images in a series and group size is 2 the result will be a series of 5 grouped images (in 4-D format) with stats calculated for each group in the series. If group size is the same as the range then only one statistical image is calculated (the default).

Using Mixed Images Array Sizes
Some image processing software requires that all images have the same number of rows, columns, slices, and time points (i.e. identical array configurations) for algebraic manipulation. However, Mango supports calculations even when image arrays differ. The processing schema is similar to how Mango performs other multi-image operations such as copying an ROI from one image and pasting it into another. In image coordinate mode images are aligned according to row, col, slice, time = 0,0,0,0. In world coordinate mode (globe icon) images are aligned according to x, y, z, t = 0,0,0,0. Mango manages differences in spacing and data types regardless of image mode. Calculations are only done where all images overlap, which can vary considerably with mixed array configurations. The array configuration of the resulting image is set to match that of the current (this) image. When images with differing array configurations are used in a calculation we recommend using the world coordinate mode and that each image have a consistent ‘origin’.

Mathematical Operations
The Mathematical, Boolean, Statistical, and Miscellaneous menus in the image calculator each provide multiple options.

Mathematical
This menu includes standard trigonometric functions and their inverses along with common mathematical operations such as absolute value. The value of \( \pi \) is included since it is often needed with trigonometric functions. Arguments in trigonometric functions are in radians. For example \( \sin(\pi/2) = 1 \) and \( \text{asin}(1) = \pi/2 = 1.57... \). An example use of the power function is \( 'new = \text{pow}(this, 1.67)' \) which makes a new image by raising each voxel in ‘this’ image to the 1.67 power. As another example, \( 'this = \sin(2 \pi*\text{row}/128)' \) will make a one period sine wave across the rows of an image with 128 rows.

Statistical
This menu lists functions that return statistics calculated for this image. Two general categories are provided: 1) ROI stats (selected by index) or 2) Time Series stats (range designated as start and end).

The ROI stats functions return a single value. Separate functions are provided for mean, minimum, maximum, standard deviation, and sum. For example, \( 'new = \text{bin}(this>\text{meanROI}(0))' \) will make a new binary image based on the condition that this image is greater than the mean value of ROI ‘0’ in this image. If more than one ROI is present the argument can be set to the desired ROI number.
Time series stats returns an image based on the selected stats function and range within the image series. For example, ‘new = meanTime(5,20)’ will return an image that is the mean value of images 5-20 in this series image.

Boolean
This menu lists Boolean operators useful for formulating logical statements (the ‘AND’ operator was used in a previous example). Characteristics of Boolean operators are documented in many texts as well as a variety of websites. A particularly useful logical function is if(condition, value else value). For example, ‘new = if(this>=other(1), this else 0)’ makes a new image with values that are from this image if they are greater than or equal to those in the other(1) image and equal to zero if not. This effectively uses the other(1) image as a variable threshold for this image.

Miscellaneous
This menu has several special features that do not fall within Mango’s other math categories. There are four special variables that automatically increment across rows, columns, slices, or time. For example, ‘new = rows’ creates a new image where each row is the row number, resulting in a ramp-like image. These variables can be used in arguments of other functions.

The volume(value) function is used when this image is a series. It returns a copy of a series image volume with index = value. For example ‘new = volume(13)’ will copy the image (here called a volume) at index 13 to a new image.

The max(value, value) function returns the maximum value of the two arguments. This function can be used to calculate an image that is formed as the maximum value of two images. For example ‘new = max(this, other(2))’ will make a new image where each voxel is the maximum of that found either in this or the other(2) image. The min(value, value) and remainder(value, value) operations function similarly.

The bin(condition) function returns a binary valued image based on testing a logical condition in the argument (returns 1 if condition is true and 0 if it is false). For example ‘new = bin((this >100) and (other(1)<100))’ will set voxels in the new image = 1 where voxels in this image are greater than 100 and those in the other(1) image are less than 100. This function is helpful for forming a binary mask for use with other image processing tasks.

The round(value), ceil(value), and floor(value) functions are used to convert a floating-point value to an integer. For example, if this image has fractional values then ‘new = round(this)’ will make a new integer image using the 4-5 rounding rule. Similarly, floor rounds down (negative direction) and ceiling rounds up (positive direction).
Image Transforms

This option is in the Viewer's Image Menu. When selected it opens a new dialog window with controls to apply a 4x4 affine transform to the viewer’s image (Figure 18). There are 12 transform parameters that can be adjusted (3- translate, 3- rotate, 3- scale, & 3- skew). These parameters can be adjusted independently and their effect on the current image is immediately seen in the viewer. The transform remains active when the transformed image is overlaid onto another image, and can be used to interactively align two images. This is particularly useful if landmarks to guide registration are not present (functional vs anatomical images) or if automated registration fails.

Load.../Save...

Transforms can be saved and loaded later to apply to the same image. The 4x4 affine transform matrix is displayed by default (Show Matrix), but you can switch this to display the parameters of the transform (Show Parameters) (Figure 18). Note: The Toolbox transform icon with the curved arrow can be used to turn the transform on (black arrow) and off (grey arrow) to see the effect of the transform on the image. Transform parameters differ depending on world or image modes and the user can select which version to display.

Options ...

Predefined Transforms

Two predefined transforms are provided: 1) MNI to Talairach and 2) Talairach to MNI. These transforms assume that the image has been spatially normalized to fit the MNI brain space (option 1) or the Talairach brain space (option 2). These transforms manage differences in position (origins), orientation and size, but the origin may need to be adjusted to that of the AC, since the origin in Talairach space differs slightly from that in MNI space.

Center of Rotation

Several options are provided to change the center of rotation when applying transforms: 1) image origin, 2) image center (the default), or 3) current coordinate. Option 1 ensures that image origins remain aligned. Option 2 is appropriate when the image center was used as the center of rotation to formulate a transform (common practice). The keyboard ‘c’ moves crosshair to the image center to check out this option. Option 3 provides a way to rotate about a particular anatomical landmark.
**Forward/Inverse**

This button switches from a forward **(the default)** to the inverse version of the transform matrix. *This option is helpful when the loaded transform was designed to adjust image A to match image B and you need it to adjust image B to match image A.*

**Concatenate**

This button is used to extend the range of an existing transform using concatenation (multiplying the current 4x4 transform matrix by another 4x4 transform matrix to make a net transform). When the Concatenate button is clicked the user can enter additional translation, rotation, scale, and skew to the previous transform. The changes will be seen immediately in the displayed transform and transformed image. There is no limit on the number of concatenation stages, but more than two seem unreasonable. *This option is helpful when the range of the transform needed is greater that provided by the sliders for a single transform.*

**Undo**

Following concatenations, the undo button reverses this action removing the last concatenation each time it is pressed. For example, if you used two additional concatenated transforms these can be removed by clicking undo twice. Undo can also be used to remove a single transform (clears all settings).

**Image Filters**

This option is in the Viewer’s Image Menu. The user can select from a list of built-in and user-defined filters. Filters can be 1-D (temporal), 2-D (slice) or 3-D (multi-slice). Temporal and spatial filtering can both be applied to a 4-D image. Most filters act immediately when selected, but the Rank Filter opens a dialog window with options for size, type (min, max, median), and extent (slice, image volume, time), providing customization based on needs. **NOTE: Filtering operations cannot be undone**, but reload will reset the image to its unfiltered state. The built-in filters should be adequate for normal use, but those with knowledge and experience in filter design should enjoy making filters customized for their applications using the ‘Add/Edit Filter...’ option from the Toolbox Options menu.

**Surface Rendered Images**

![Surface display with zoomed version of triangles from a small region on the surface.](image1)

![Brain surface with orientation and axes (blue).](image2)
Mango provides viewing and analysis capabilities for surface images synthesized from voxel-based images (Figure 19). Surface images can be dynamically moved and rotated for viewing an object's surface from any direction. Surface-based analysis tools are provided for measuring distances (straight line and along a surface), angles, volumes and surface areas. ROIs and overlays from the voxel-based image can be formulated as secondary surfaces (called shapes).

**Synthesis**

A user selected threshold value is needed to distinguish surface voxels in the voxel-based image from those external or internal to an object. This approach is similar to that done when using thresholds to define an ROI. A shrinkwrap ROI is used by default to record coordinates along an external surface. The 'Marching Cubes' algorithm uses coordinates of the surface voxels as input to assemble a large number of connected triangles tessellating the objects surface in a mesh-like manner (Figure 19, right).

**Viewing**

A *shading* model, where light reflected from the surfaces of triangles varies in brightness based on orientation, provides a realistic three-dimensional appearance for the surfaces (Figures 19-21). By default, surfaces facing the screen will be bright and those oriented perpendicular will be dark, enhancing visualization of the surface’s landscape. Mango uses smooth shading such that brightness varies smoothly across the surface of triangles (Fig 20). The mouse pointer in the surface display window is similar to that in the viewer, where the pointer’s surface x-y-z location is indicated in the Toolbox window. Dragging this pointer across the screen changes the object’s orientation, allowing users to view the object’s entire surface. Zooming and panning controls are similar to those in the image viewer, and additionally the scroll wheel can be used to zoom. The large crosshair in the surface display (blue) is synchronized with that in the image viewer (also blue). To position the large crosshair at the surface, *double-click* with the mouse pointer over the desired position.

The material properties of a surface used in shading can be changed including color of reflected light and transparency. Lighting properties can be changed for surface reflection characteristic (specular, diffuse) and for the brightness and position of the light illuminating the surface.

The surface viewer can provide simultaneous display of surfaces and slices where the surface is cut away and slices displayed in place of the cut-away portion. Slices combined with surfaces in this manner are called cut planes (Figure 21).
Surface Analysis

Mango provides four basic measurements for surfaces: distance, angles, surface area and volume. Ruler and Angle tools are selectable on the View menu. These are dynamic tools that can be moved about surfaces to make measurements while viewing and rotating the surface into position as needed. Another distance option is based on points placed on the surface. Point ROIs are placed on the surface using *shift-double-click*. Lines can be drawn between points or between points and the large crosshair location (whether inside or on the surface). Lines can be either a straight-line between points (similar to the Ruler) or a path along the surface between surface points. *The path option is not provided with the Ruler.* Many paths are possible along the surface, so we chose a path that lies in a specific plane passing through the two endpoints, where the plane is oriented midway between their normal vectors. The path is at the intersection of this plane and the surface. Surface points defining the line can be selected using the *shift-double click* action, moved and deposited elsewhere with a *click*. All lines associated with the moved point are then updated. An object’s surface area is calculated as the sum of areas of the triangles covering the surface, and most of these will be very small compared with the surface spanned. For brain surfaces, there are hundreds of thousands of triangles. *Surface-based distances and surface areas are properties only obtainable from the surface image, not possible from the voxel-based image.*

The volume of a surface is calculated using a form of Gauss’s Divergence Theorem (relating surface properties to the volume inside the surface) Eberle et al., 1991, Lancaster et al., 1992. The surface-based volume calculation is faster than counting the corresponding ROIs voxels in the base image. Volumes calculated from surfaces of paired ROIs from voxel-based images are slightly different (1-2%). This is due in part to differences in surface thresholds, differences in connectivity rules used during surface synthesis, and differences due to decimation (reduction of number of triangles) when finalizing the surface model.

Shapes

Mango supports adding additional surface objects as shapes derived from overlays and/or ROIs in the voxel-based image. The color of a shape object is based on the color of the overlay or ROI from which it is made. Transparency of the base object’s surface can be reduced to help visualize an enclosed shape (~50% in Figure 22).

Multiple shapes are supported, one from each overlay and/or ROI. Colors are assigned to distinguish each. Analysis of shapes is the same as for surfaces (distance, angles, surface area, & volume). Viewing of surfaces, shapes, slices, and ROIs can be turned on and off from the surface viewer’s View window. *To place points and lines on shapes you must first turn the surface view off to uncover them.* Points are placed on the first surface encountered, on the outer surface even if the surface is transparent.
Volume Rendered Images
Volume rendering provides a variety of viewing options for voxel-based images using a technique called ray tracing. A ray follows a straight-line path passing through the object perpendicular to the display plane. Each pixel in the display is associated with a single ray. During ray tracing all voxels along the ray’s path are inspected and a ray value assigned to the associated pixel. A common ray value is the maximum voxel value along the ray. Mango supports two other options; median and minimum ray value. The displayed brightness of the volume rendered image is proportional to ray values. This option is found in the viewers Image/Build Projection... menu.

It would be helpful to dynamically change object orientation for viewing volume rendered objects as provided for surface viewing. However, volume rendering is much more computationally demanding than surface rendering, and without special hardware full motion dynamic control of object orientation is not practical. Alternatively, dynamic viewing is possible when the desired views can be precalculated. In Mango, volume rendered projections (views) can be calculated for rotation about one of the three major 3-D axes. The volume rendered views can then be animated for dynamically viewing while the object is rotated about the chosen axis providing full-circle viewing.

Additional Information
Special Features

Find Overlay Clusters...
A thresholded overlay can be made up of multiple non-connected regions. It is possible to collect these into a set of clusters using the ‘Find Overlay Clusters...’ option in the Analysis Menu. For example, this processing is helpful to break up ICA components derived from a resting state fMRI study into separate parts. A dialog window opens when this option is selected and automatically identifies the largest eight non-connected regions or clusters, or fewer if there are less than eight. The statistics window can be opened using the tool icon on the right side of the cluster dialog to review stats of each cluster. Users can choose to adjust the number (n) of clusters to generate. The selected number of clusters can then be converted to separate ROIs using the ‘Create ROIs of clusters’ option in the cluster dialog tool menu. Once converted to ROIs independent analyses are possible.

Create Logical Overlays
When more than one overlay is present it is helpful to color code their overlapping volumes. The ‘Create Overlay Logicals...’ option in the Analysis Menu does this. This option supports more than two overlays (each with a different color table) and the number of combinations is automatically set up and calculated. The statistics window can be opened to review...
results for overlapping regions. Similar to clusters, the logical regions can be converted to ROIs for further analyses.

**Add Parametric Overlay...**
This option is in the Viewer’s File Menu. It is used when the source image for overlaying has both positive and negative values of interest such as z-score images. When selected the file dialog is opened and the user selects the image to overlay onto the current viewer. This option assigns two color tables to the overlay, one for negative values and one for positive values, which can be adjusted independently.

**Load Online Overlay...**
This option is in the Viewer’s File Menu. It is used to load an image as an overlay using a URL for images archived at a web address.

**Load Online ROI...**
This option is the Viewer’s File Menu. It is used to load ROIs using a URL that have been archived at a web address.

**Simple How To...**

**Edit Origin**
The user can change the site to use as the origin for an image. If not defined in the image header the default origin is the center of the image array. If desired, the origin can be changed to match the column-row-slice of a specific landmark within an image. To determine where the origin is currently located press the ‘o’ key and the crosshair will be positioned there. If a different origin is desired use the mouse to navigate the crosshair to the desired location (or ‘esc’ and enter the coordinate) then select the ‘Origin...’ option in the Viewer’s Edit Menu. A small dialog will open with the current col-row-slice origin near the top and the current col-row-slice crosshair position in the x-y-z textbox. Pressing OK sets the origin to the values in the x-y-z textbox. You can type in col-row-slice values for the origin if you know them. Save the image to make changes permanent.

**Edit Voxel Value**
The ‘Voxel...’ option in the Viewer’s Edit Menu provides a means to view a voxel’s value at the location of the crosshair and to change it. We mostly use this option to set points outside an object of interest to track movement when applying a coordinate transform or to track the change in resolution when filters are applied. Save the image to make changes permanent.

**Enter Notes**
The ‘Notes...’ option in the Viewer’s Edit Menu is where comments specific to an image can be entered. Suggestions include project title, subject code, transforms applied, filtering
done, etc. Do not place HIPPA restricted information here. Save the image to make changes permanent.

Update Display to Full Range
The max and min display setting used by others are saved in an image’s header so that the next time the image is opened the user will view the image with the same settings. To set the max-min range to the full range of image values click the ‘Update to Image Range’ option in the Viewer’s Edit Menu. Save the image to make changes permanent.

Review Image Header Information
Image header information is accessed using the ‘Image Info’ option in the Viewer’s File Menu. Optionally, press ‘command and i’ keys. Two levels of information are provided as tabs in the dialog window for this option:

Summary Tab
The summary information is a subset of the image header formatted for easy reading (example in Figure 2). It includes the file name and location as well as key data that are needed to properly display images and make measurements.

Header Tab
The information at the ‘Header’ tab is formatted as text tags and data from the true image header. The most extensive image header is that for DICOM images. For traditional multi-slice DICOM images each slice’s data is in a different file with a different header. Mango has a next button to move to different slices; however, moving the crosshair to the slice of interest will also bring up the corresponding slice header. Since headers contain a great deal of text and data specific tags we provided an option to search within the header. Header data can be edited if incorrect or missing, but be careful because entering incorrect data in key fields (rows, cols, slices, etc.) may make the image unreadable.