AnalyzeDirect

Analyze 15.0
Bone Microarchitecture Analysis Add-On

User's Manual
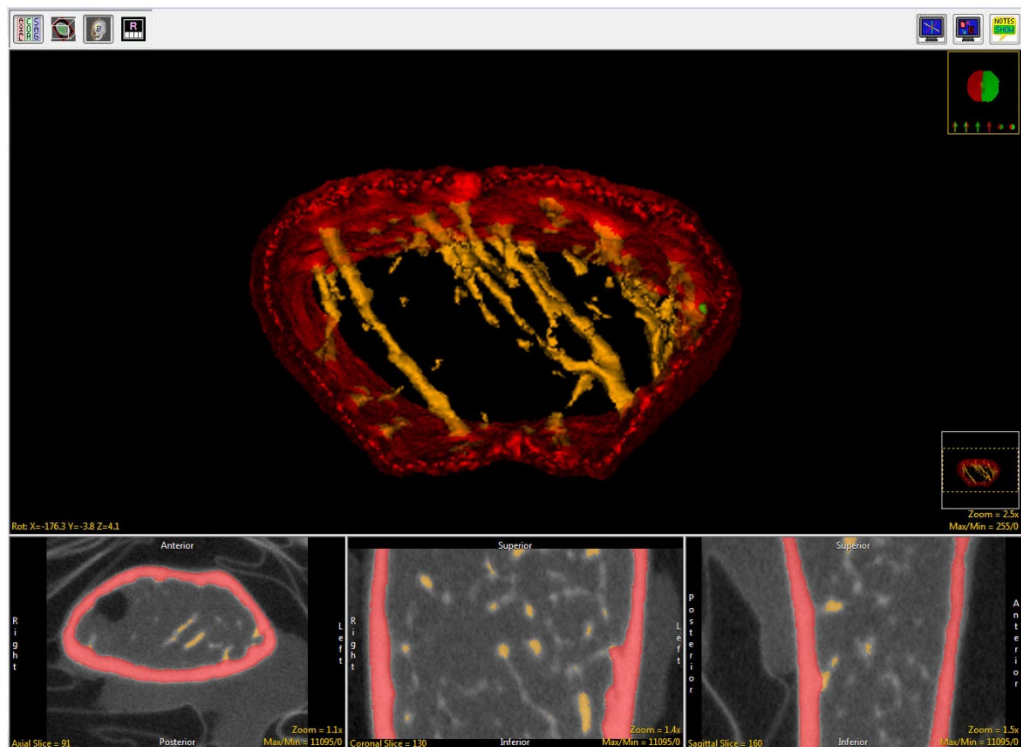
Contents

1. Introduction to the Bone Microarchitecture Analysis Add-On

The Bone Microarchitecture Analysis (BMA) Add-On module for Analyze is a powerful pre-clinical research application designed for the evaluation of 3-D microCT image data. The module provides researchers the ability to process single long bone specimens and isolate 3-D samples of trabecular tissue. It generates an extensive set of bone morphometric indices as described by Bouxsein *et al*.

The BMA Add-On provides automatic and user guided isolation of bone from non-bone tissue by means of threshold-based segmentation and a novel segmentation process. This process also provides for the separation of the cortical and trabecular bone in whole bone specimens. BMA uses the segmented regions to automatically drive the calculation of bone mineral density measurements (Table 1, Figure 1.1) and common bone morphometric indices that provide researchers with a quantitative description of bone microarchitecture. The microarchitectural characteristics of the trabecular and cortical bone are listed below (Tables 2 and 3). For further information about each bone measurement calculated please refer to section 3) Bone Microarchitecture Analysis Measurements.

| Table 1. Bone Mineral Density Measurements		
Measurement	**Description**	**Unit**
BoneMean	Mean BMD of bone	(mg/cm^3)
BoneStd	Standard deviation of bone	(mg/cm^3)
BoneVolume	Volume of bone	(mm^3)
CortexMean	Mean BMD of cortex	(mg/cm^3)
CortexStd	Standard deviation of cortex	(mg/cm^3)
CortexVolume	Volume of cortex	(mm^3)
IntraTrabecularMean	Mean BMD of intratrabecular	(mg/cm^3)
IntraTrabecularStd	Standard deviation of intratrabecular	(mg/cm^3)
IntraTrabecularVolume	Volume of intratrabecular	(mm^3)
TrabecularMean	Mean BMD of trabecular	(mg/cm^3)
TrabecularStd	Standard deviation of trabecular	(mg/cm^3)
TrabecularVolume	Volume of trabecular	(mm^3)
TrabecularTissueMean	Mean BMD of trabecular tissue	(mg/cm^3)
TrabecularTissueStd	Standard deviation of trabecular tissue	(mg/cm^3)
TrabecularTissueVolume	Volume of trabecular tissue	(mm^3)
WholeMean	Mean BMD of whole ROI	(mg/cm^3)
WholeStd	Standard deviation of whole ROI	(mg/cm^3)
WholeVolume	Volume of whole ROI	(mm^3)
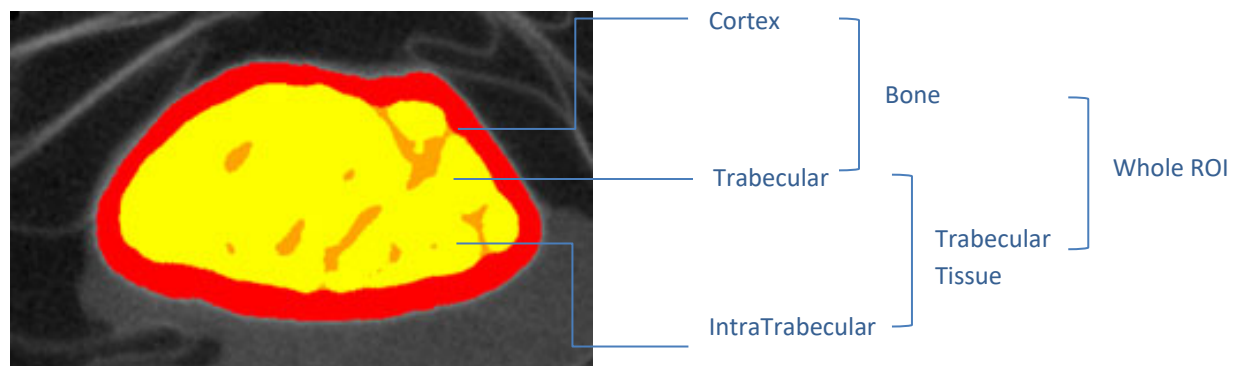
Figure 1.1. BMD Measurement Structure Reference

*Bouxsein, M. L., Boyd, S. K., Christiansen, B. A., Guldberg, R. E., Jepsen, K. J., & Muller, R. Guidelines for Assessment of Bone Microstructure in Rodents Using Micro–Computed Tomography. Journal of Bone and Mineral Research, 25, 1468-1486.

Table 2. Trabecular Bone Morphometric Indices		
Abbreviation	**Description**	**Unit**
TV	Total Volume	mm^3
BV	Bone Volume	mm^3
BS	Bone Surface	mm^2
BV/TV	Bone Volume Fraction	%
BS/TV	Bone Surface Density	mm^2/mm^3
BS/BV	Specific Bone Surface	mm^2/mm^3
SMI	Structure Model Index	
Conn.D	Connectivity Density	1/mm^3
Tb.N	Trabecular Number	1/mm^3
Tb.Th	Trabecular Thickness	mm
Tb.Sp	Trabecular Separation	mm
Tb.Th.SD	Standard Deviation of Trabecular Thickness	mm
Tb.Sp.SD	Standard Deviation of Trabecular Separation	mm
DA	Degree of Anisotropy	A
MIL	Mean Intercept Length	a

Table 3. Cortical Bone Morphometric Indices		
Measurement	**Description**	**Unit**
Tt.Ar	Total Cross-Sectional Area	mm^2
Ct.Ar	Cortical Bone Area	mm^2
Ma.Ar	Medullary Area	mm^2
Ct.Ar/Tt.Ar	Cortical Area Fraction	%
Ct.Th	Average Cortical Thickness	mm
Ps.Pm	Periosteal Perimeter	mm
Ec.Pm	Endocortical Perimeter	mm
/ap	Moment of Inertia Anteroposterior Axis	mm^4
/ml	Moment of Inertia Mediolateral Axis	mm^4
/max	Maximum Moment of Inertia	mm^4
/min	Minimum Moment of Inertia	mm^4
J	Polar Moment of Inertia	mm^4
Ct.Po	Cortical Porosity	%
Po.N	Pore Number	n
Po.V	Total Pore Volume	mm^3
AvgPo.V	Average Pore Volume	mm^3
Po.V.SD	Standard Deviation of Pore Volume	mm^3
Po.Dn	Pore Density	mm^{-3}

1.1 Bone Microarchitecture Analysis Workflow

The BMA workflow in Analyze is divided into two sections: Data Preprocessing and Bone Microarchitecture Analysis. Data preprocessing is a prerequisite for analysis of data via the BMA Add-On. An overview of the entire workflow is provided in Figure 1.2.

1.1.1 Data Preprocessing Overview

The BMA Add-On module requires that input bone specimens have isotropic voxels. Anisotropic data can be resampled on load or via Transform in Analyze. If the image data contains multiple bones, the bone of interest must be isolated using the tools provided in the Segment module and orientated into the correct anatomical orientation using the Transform module. Data preprocessing also includes optional data sub-regioning (cropping) and filtering steps, to reduce data size and enhance initial object segmentation.
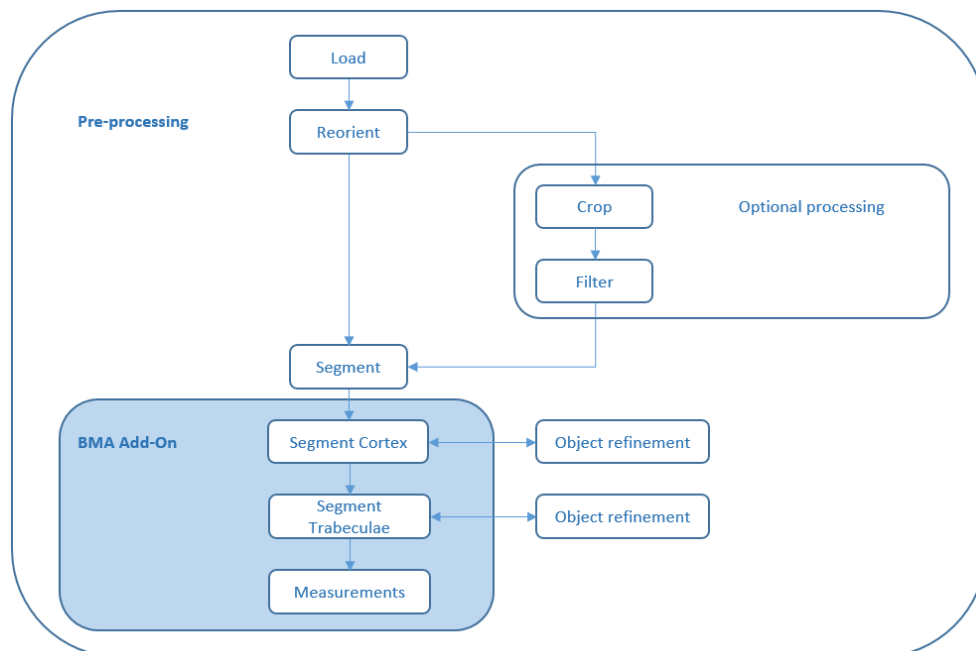
Figure 1.2 Diagram of the BMA Workflow

1.1.2 Bone Microarchitecture Analysis Overview

The second part of the Bone Microarchitecture Analysis workflow is conducted within the BMA Add-On and is separated into three steps:

1. **Cortical Bone Segmentation:** Once the image data is loaded, the BMA Add-On will conduct a preliminary segmentation which will allow the module to identify the cortical shell, cortical pores and trabecular tissue regions.

2. **Trabecular Bone Segmentation:** Upon user approval the module will complete the segmentation process by identifying the trabecular bone in the trabecular region. Two new regions, the trabecular bone and intra-trabecular space, are derived from the trabecular tissue region.

3. **Calculation of Bone Morphometric Indices:** Measurements are automatically calculated for the trabecular and cortical bone and output in several comma-separated value (.csv) files. Files are saved to a user-defined directory.

1.1.3 Processing Single Bone Specimens

Image volumes that contain only a single bone do not require a pre-segmentation step to isolate the bone of interest, but still may need to be reoriented. The workflow is summarized in Figure 1.3.
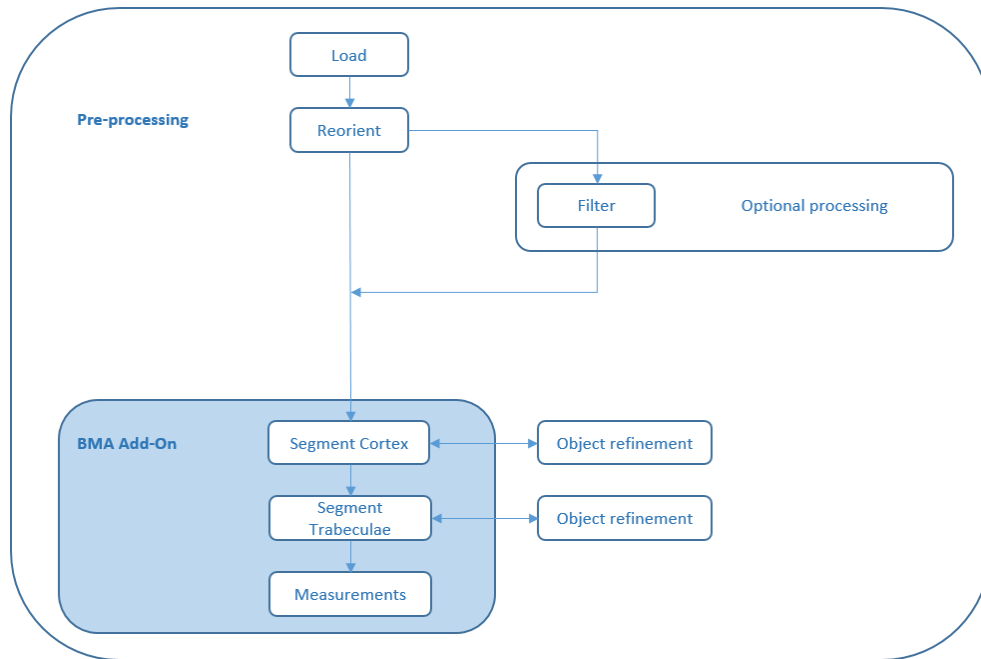
Figure 1.3. Diagram of BMA Workflow for single bone specimens

2. The Bone Microarchitecture Analysis Add-On Interface Overview

The BMA Add-On is started from the Analyze Apps area (Figure 2.1). Please note that if the Add-On is not enabled it can be enabled by right-clicking in the module selection area and then selecting the Apps > Show All menu option (Figure 2.2).
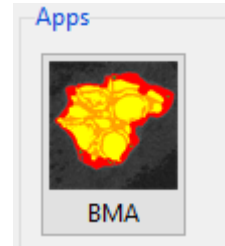
Figure 2.1. Starting the BMA Add-On
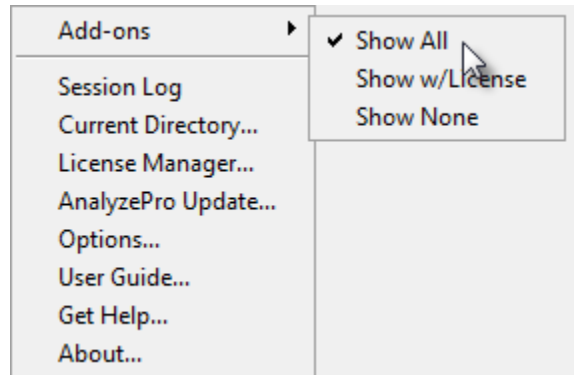
Figure 2.3. Enabling the Analyze Add-Ons

Please note, the BMA Add-On requires an additional license to run, if you do not have a license to access the add-on a validation error will be returned. Please follow the instruction on the message and contact sales@analyzedirect.com for more information about accessing the Add-On.

To load a data set, select the data from the Analyze workspace and open the Add-On (Figure 2.3). Alternatively, data can be dragged from the workspace onto the Add-On.
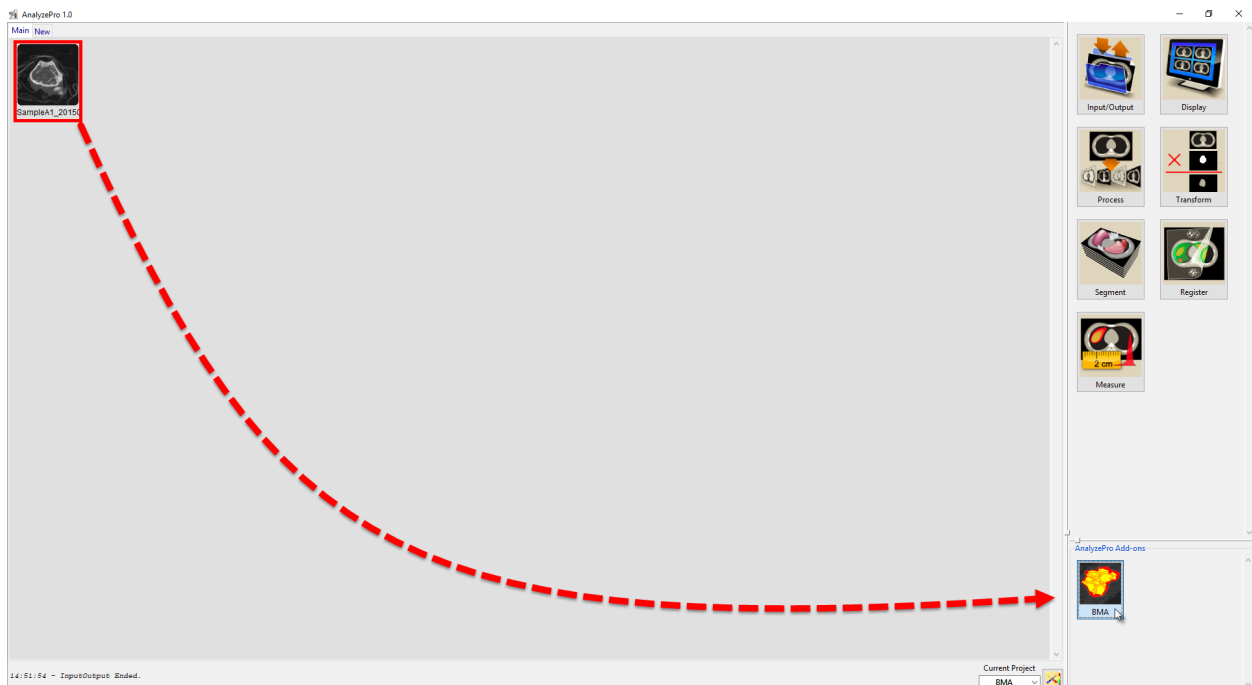
Figure 2.3. Loading Data into the BMA Add-On Module

2.1 The BMA Program Window

The BMA program window (Figure 2.4) provides access to all functions and tools in the Add-On.
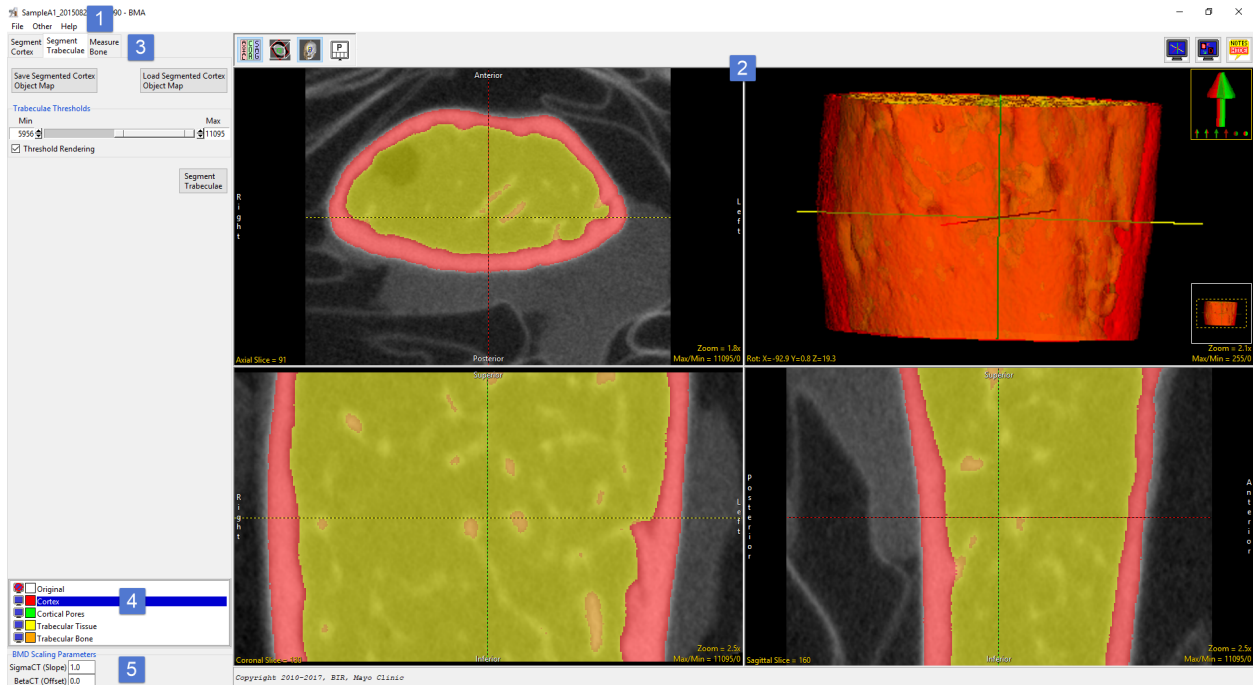
Figure 2.4. The BMA Program Window; 1) Menu, 2) Display Window, 3) BMA Workflow tabs, 4) Objects Control Window, and 5) BMD Scaling Parameters.

2.2 Menu

The BMA menu provides access to the File, Other, and Help menus.

2.2.1 File

The File menu provides access to the Input/Output Ports and Exit functions.

- **Input/Output Ports**: Selecting the File > Input/Output Ports option displays an area at the bottom of the Bone Microarchitecture Analysis window, this area facilitates the loading of image data via drag and drop from the Analyze window (Figure 2.5). Image data can also be dragged and dropped directly on the BMA Display window to facilitate data loading into the module.
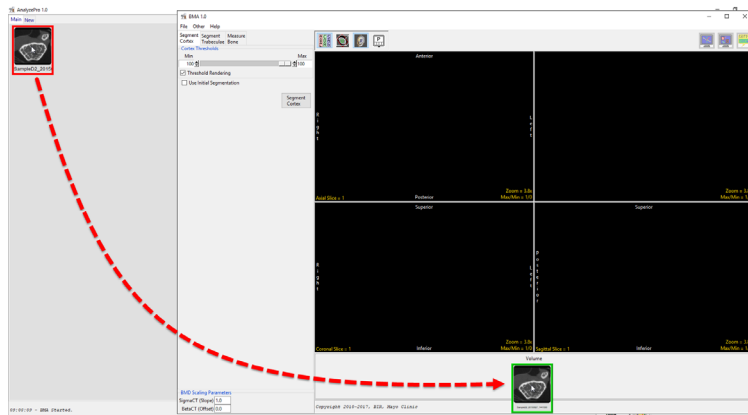

Figure 2.5. Loading Data Via Input/Output Ports

- **Exit:** Selecting the Exit option will close the Bone Microarchitecture Analysis Add-On module.

2.2.2 Other

The Other menu provides access to the module's Options window and PowerBar Editor tool.

- **Options**: When selected opens the Options window (Figure 2.6). This window allows users to configure attributes for the module, which include the following:

  - **Auto Note Update:** When the Notes tool is enabled (see the Set Notes State tool for more information) this option allows users to specify whether to permit interactive update of notes if notes are edited or added to the data in other modules.

  - **Auto Cursor Link:** When enabled, if the layout is set to a single orthogonal view, the Cursor Link is automatically disabled.

  - **Remember Layout:** Remembers the modules current layout configuration between sessions. Uncheck this option to start the module with the default layout each time it is opened.

  - **Threads:** The Threads options allows users to specify the number of processing cores the multithreaded code can utilize. By default, the threads option is set to the maximum number of processors on the system.
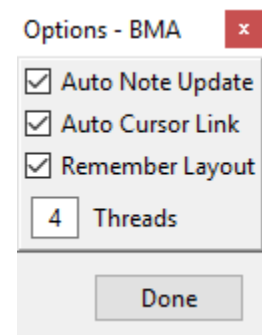
Figure 2.6. The Options Window

- **PowerBar Editor:** Opens the PowerBar Editor window, this window allows users to add or remove buttons from the Toolbar in the upper right-hand corner of the BMA module.

2.2.3 Help

- **About**: The About option opens the Analyze Bone Microarchitecture Analysis About window. The About window provides detailed information about the system and the Analyze software. Selecting Done will close the About window.

- **User's Guide**: The User's Guide option opens help documentation.

2.3. Display Window

The display window is divided into several areas: 1) display controls; 2) tools; 3) axial slice display, 4) coronal slice display, 5) sagittal slice display, and 6) rendering window (Figure 2.7).
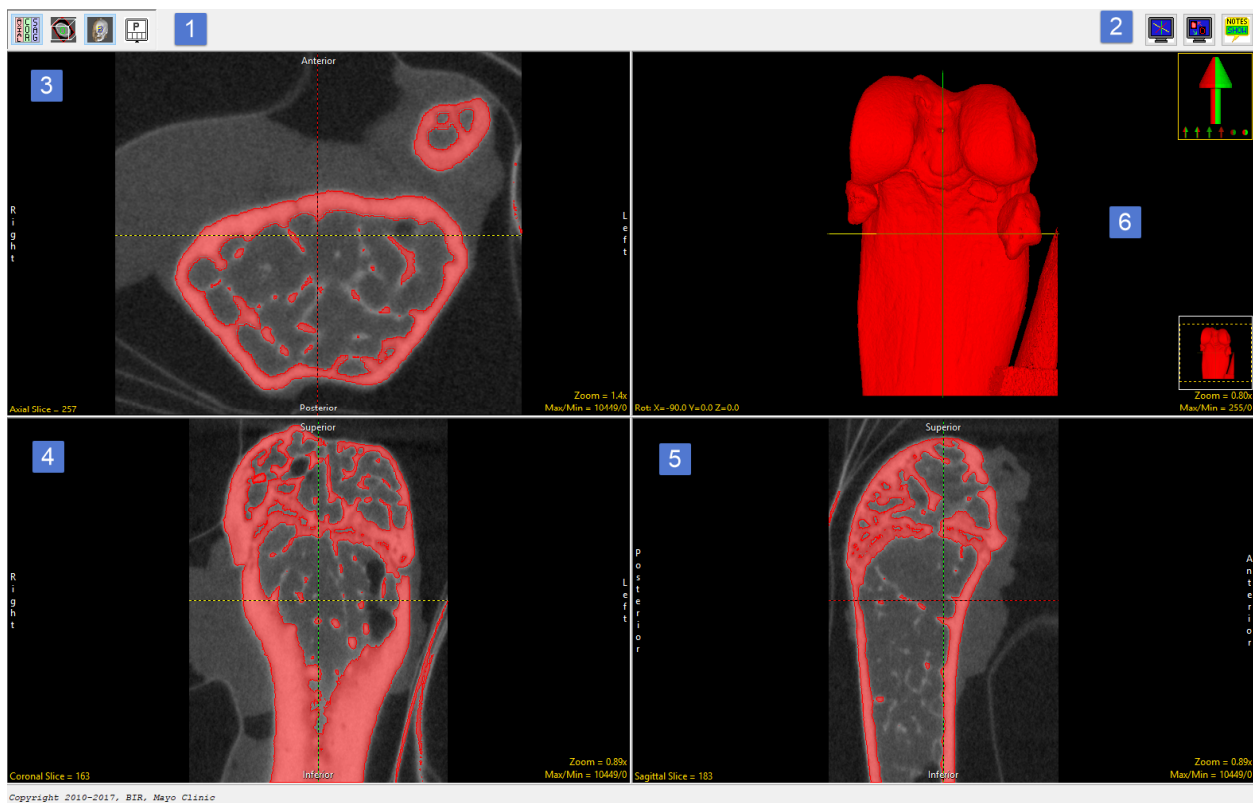
Figure 2.7. The Display Window. 1) Display Controls, 2) Tools, 3) Axial Slice Display, 4) Coronal Slice Display, 5) Sagittal Slice Display, 6) Rendering window.

2.3.1 Display Controls

The Display Controls (Figure 2.8) allow users to control and customize the layout of the BMA display window.

Toggle All Orientations   Toggle Oblique   Toggle Rendering   Controls Layout
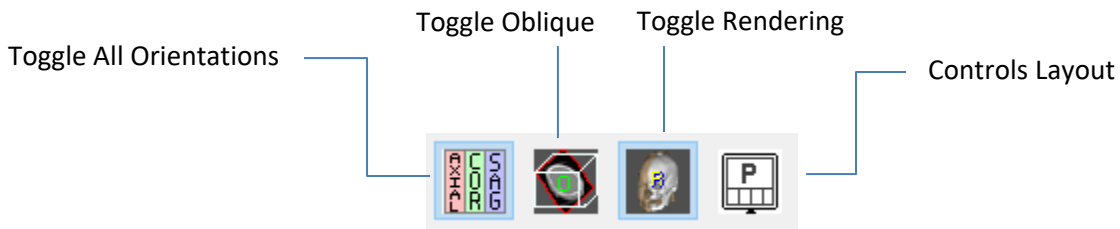
Figure 2.8. Display Control Tools

- **Toggle All Orientations:** Allows users to switch between the display of all three orthogonal displays (the axial, coronal and sagittal) to a single orientation display.

- **Toggle Oblique:** Allows users to switch the oblique display window on and off.

- **Toggle Rendering:** Allows users to switch the Render window on and off.

- **Controls Layout:** This option allows users to control the layout of the display window. Choose to set the Axial as Primary (Figure 2.9), Coronal as Primary, Sagittal as Primary, Render as Primary or specify No Primary image (default option).
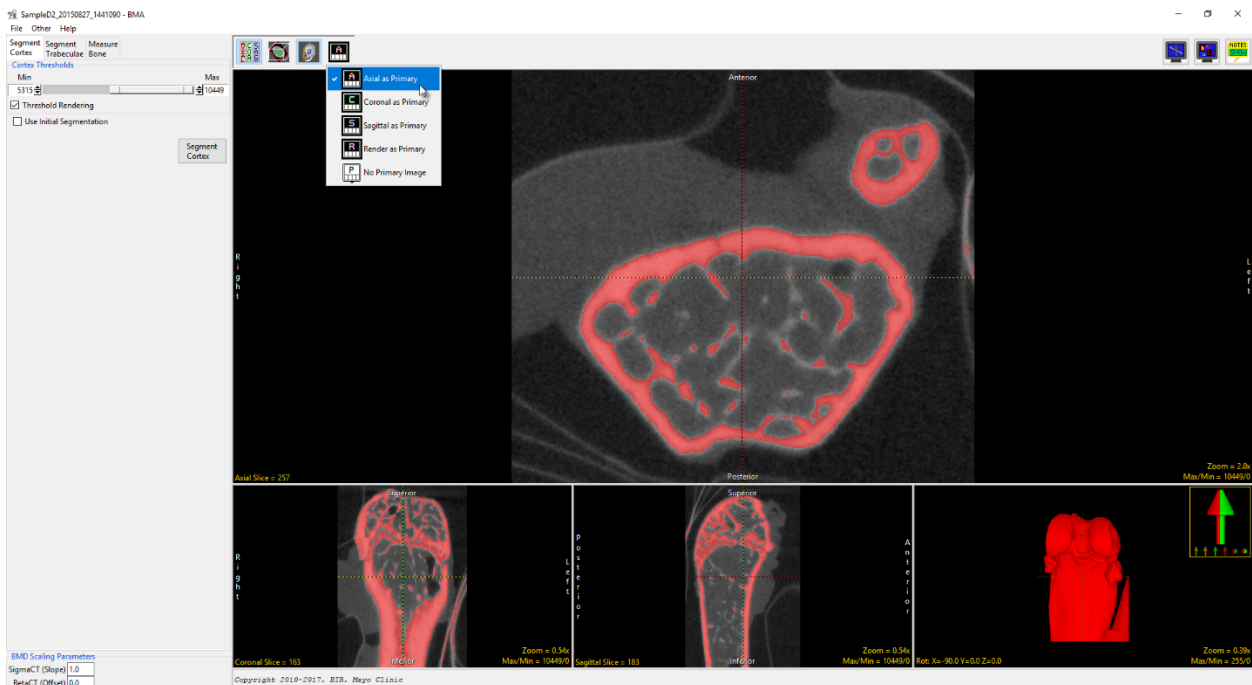
Figure 2.9. BMA with the Axial set as the Primary View

2.3.2 Tools

The Tools options (Figure 2.10) in the upper left-hand corner of the Display Window allow users to control tools for image navigation, display, and notation.

Toggle Region Display

Toggle Cursor Link

Set Note State
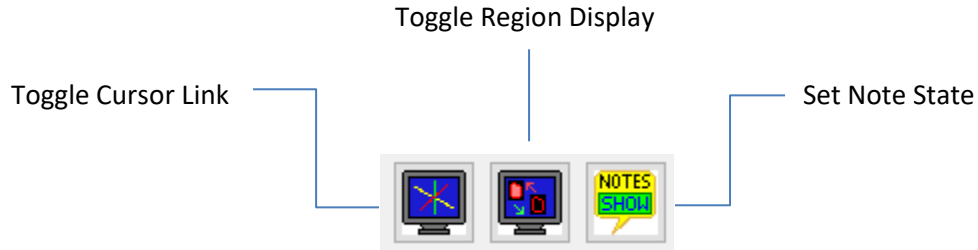
Figure 2.10. Navigation, Region Display, and Notes Tools.

- **Toggle Cursor Link:** Allows users to enable and disable the Cursor Link tool. The Cursor Link tool (Figure 2.11) allows users to interactively navigate through the image data. Moving the Cursor Link in any of the displays will interactively update the other displays. See the Cursor Link tool in the Slice Display and Render Window sections for additional information.
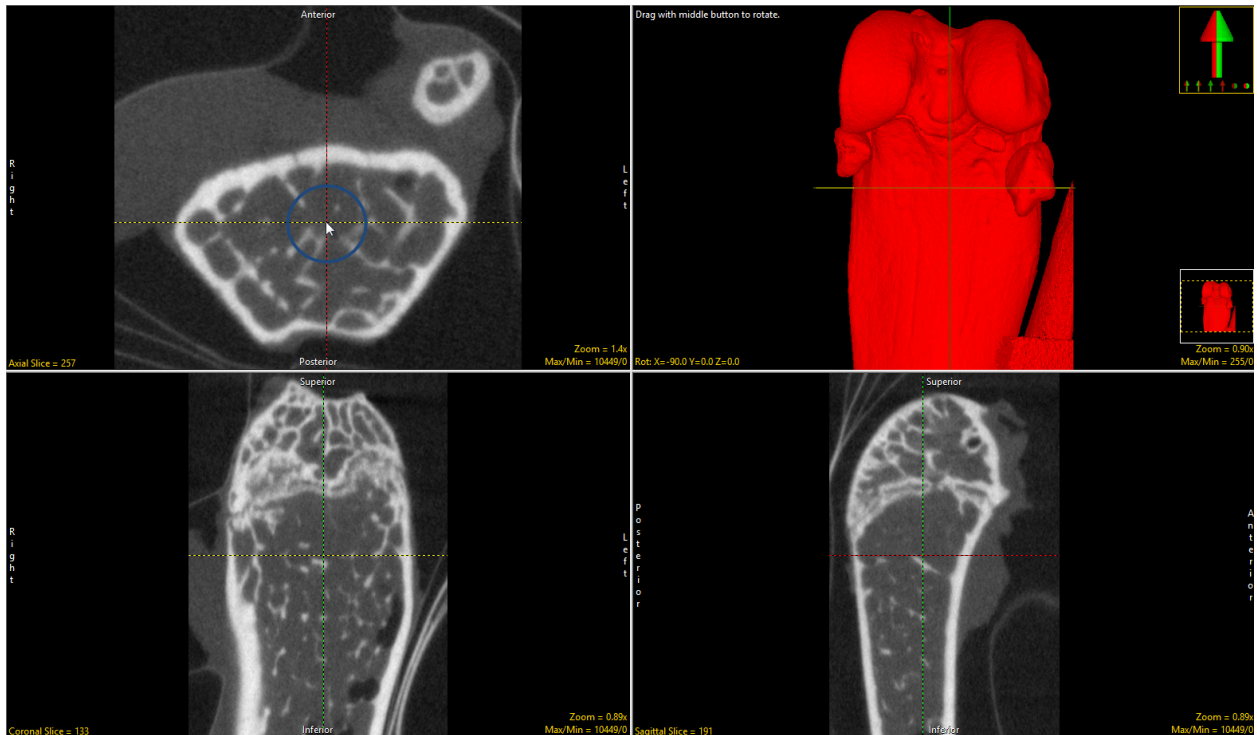
Figure 2.11. The Cursor Link Tool

- **Toggle Region Display:** The Toggle Region Display option allows users to quickly switch between the display of the overlaying regions on the slice displays. The display options are; Filled Regions, Region Edges, Both, and Regions Off (Figure 2.12). Note the keyboard shortcut to toggle regions displays is the t key.
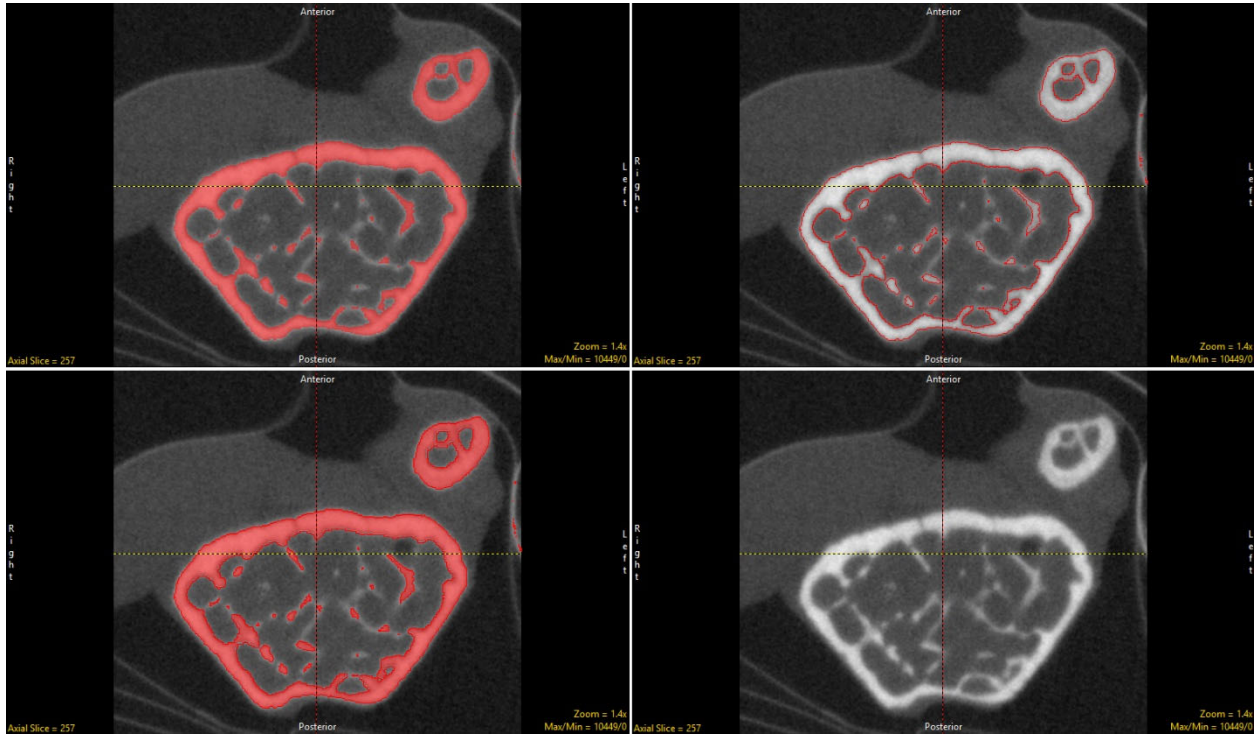
Figure 2.12. Slice Display Showing the 4 Region Display Options; Filled Regions, Region Edges, Both, and Regions Off

- **Set Note State:** Allows users to enable the Notes tool in BMA. The Notes tool allows users to annotate the 2D slice data or 3D rendering. Notes are automatically associated with the data set and can be displayed in other modules. Notes can be hidden or deleted at any time.

2.3.3 Slice Display

The Slice Display windows displays the 2D slice data in each of the three orthogonal orientations. In the default display mode, the upper left window displays the axial orientation (Figure 2.13), the lower left the coronal orientation, and the lower right the sagittal orientation.
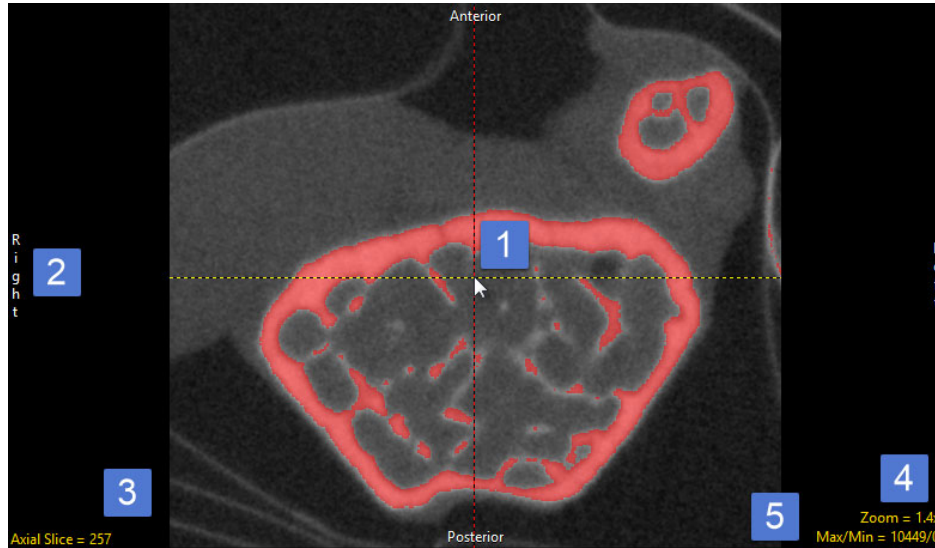
Figure 2.13. The Axial Slice Display window, showing 1) Cursor Link, 2) Orientation labels, 3) Slice tool, 4) Zoom tool, and 5) Max/Min tool.

Note that the configuration of the slice display windows can be modified using the Display Control Tools. See the Display Control Tools section or refer to the Analyze User's Guide for further information.

The Slice Display window includes several tools for viewing and navigating through the image data, these include:

Cursor Link: The cursor link allows users to interactively navigate through the image data. The tool can be controlled from any of the 2D orientations or from the 3D rendering. Moving the tool will interactively update the corresponding slice displays. The linked cursor can be enabled or disabled using the Toggle Cursor Link button.

**Arrow Keys:** The arrow keys can also be used to move the linked cursor. The keys will move the linked cursor in the corresponding direction (up, down, left and right) for the active orientation.

Page Up and Down Keys: The page up and page down keys can be used to move through the slices in the active orientation.

Orientation Labels: Displays helpful orientation labels. The labels can be abbreviated by right-clicking on them.

The Slice Tool: Users can navigate through image slices using the slice tool. Click the yellow Slice text in the lower left-hand corner of the image pane. An up/down slice cursor will appear (Figure 2.14). Hold down the left mouse button and slide the cursor upward to move to a higher slice number and downward to move to a lower slice.
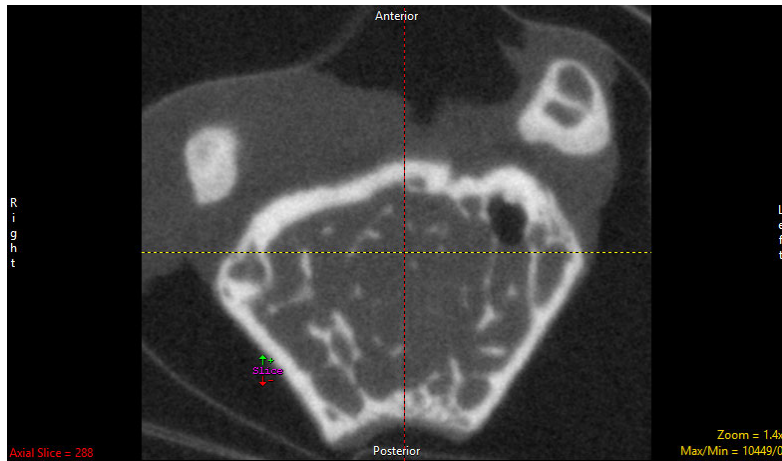
Figure 2.14. The Slice Cursor

Users can quickly navigate to the First, Middle and Last slice by right-clicking the yellow Slice text. A specific slice number can be chosen by double-clicking the yellow Slice text and typing in the desired slice number.

Zoom Tool: Users can increase and decrease the display size of the data using the Zoom tool. Click on the yellow Zoom text in the bottom right corner of the image pane. A zoom cursor will be displayed (Figure 2.15). While clicking and holding the left mouse button, move the mouse upward to zoom in and downward to zoom out. Let go of the left mouse button when the desired zoom factor is reached.
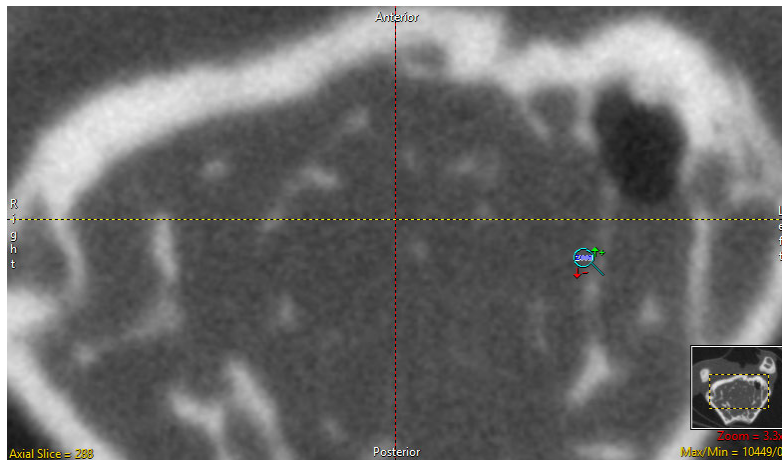
Figure 2.15. The Zoom Cursor

Right-click on the Zoom tool to reveal additional options:

- Zoom Presets — Right-click the yellow Zoom text and the following options will appear:
- Zoom levels — 0.25x, 0.33x, 0.50x, 1.00x, 2.00x, 3.00x, 4.00x
- Fit Window — Uses a zoom factor that allows the data to fill the image pane.
- Fit Width — Uses a zoom factor that allows the data to fit the width of the image pane.
- Fit Height — Uses a zoom factor that allows the data to fit the height of the image pane.
- Interpolation — Allows the interpolation method to be changed for the display of the data. The default is Bicubic (Input Driven). Users can switch to Linear (ID), Nearest Neighbor (Output Driven), Linear (OD), Bicubic and Windowed Sinc.

- Zoom Center - Uses the center of the image to set as the zoom point.
- Configure - Opens additional configuration options - see the main Analyze User's Guide for additional information.

Max/Min tool: The Maximum and Minimum display intensities can be changed by clicking on the yellow Max/Min Tool at the bottom right corner of the image window. A cursor will return (Figure 2.16) that allows the values to be manipulated. Hold down the left mouse button and move the mouse up/down or left/right. See Figure 2.17 to determine how values are changed.
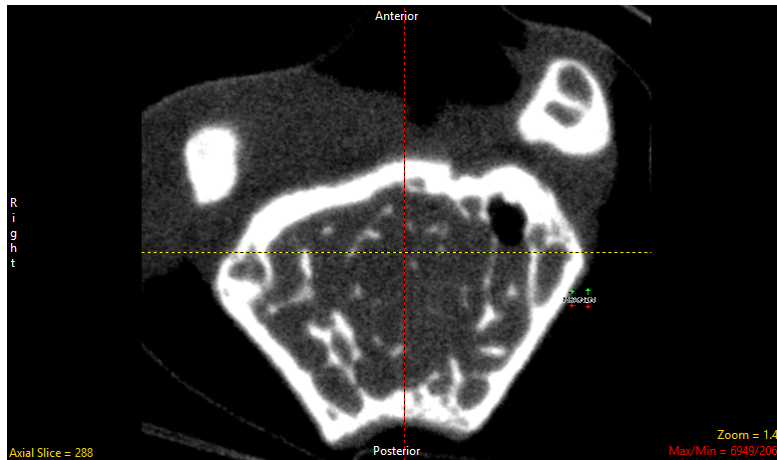
Figure 2.16. The Max/Min Cursor.

To change only the Max value, hold the Shift key while using the Max/Min cursor. The Min values can also be edited independently by holding the Ctrl key while moving the Max/Min cursor. Note [Shift] + Right Mouse Button can be used to adjust Max/Min in any window without having to first select the Max/Min option.

Right-click on the Max/Min tool to reveal Preset Intensity options. See the main Analyze User's Guide for additional information.

- Manual Intensity Input — Users can type in a Max/Min value by double clicking on the yellow Max/Min text. A box that contains editable fields will be returned. Input the desired values and press enter or click outside the box.

Figure 2.17. Max/Min Intensity Adjustment.

Right Menu Options - Right-clicking in the Slice Display window reveals additional options.

Middle Button Action: Allows users to set the action of the middle mouse button. Choose from:

- Change Slice (default) – Allows users to change the slice display by clicking the middle mouse button and moving the mouse forward, to move slice display forward, or backwards, to move the slice display backwards.

- Change Zoom – Allows users to change the zoom display by clicking the middle mouse button and moving the mouse forward, to zoom in, or backwards to zoom out.

Figure 2.18. Right Menu Options for Slice Display windows

- Change Intensity – allows max/min intensity values to be adjusted using the middle mouse button. Holding the middle mouse button and moving the mouse forward or backwards will adjust the maximum intensity value. Moving left and right will adjust the minimum intensity value.

- Default – returns middle mouse button action back to the system default.

- Annotate Changes – Enables and disables the annotations associated with changing slices, zoom, or intensities.

Mouse Wheel Action: Allows users to set the action of the middle mouse wheel. Choose from:

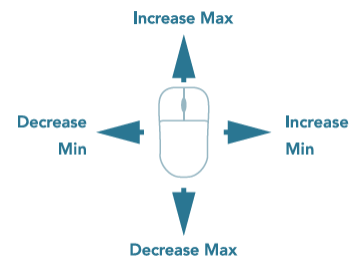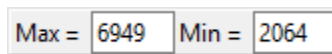- Change Slice (default) – Allows users to change the slice display by scrolling the middle mouse button forwards, to move slice display forward, or backwards, to move the slice display backwards.

- Change Zoom – Allows users to change the zoom display by scrolling the middle mouse button forwards, to zoom in or backwards, to zoom out.

- Change Intensity Minimum – Allows users to adjust the minimum intensity value by scrolling the middle mouse wheel forwards or backwards.

- Change Intensity Maximum - Allows users to adjust the maximum intensity value by scrolling the middle mouse wheel forwards or backwards.

- Choose Middle Button Action – Allows users to scroll through the display tools (Slice, Zoom, or Intensity) for the Middle Button action. Selecting a tool will modify the action of the middle mouse button instantly.

- Default – returns middle mouse button action back to the system default.

- Annotate Changes – Enables and disables the annotations associated with changing slices, zoom, or intensities.
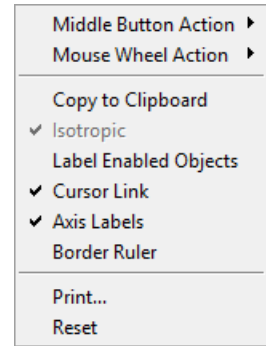
Copy to Clipboard – Copies the current slice display to memory.

Isotropic – Active only for anisotropic data. Allows users to toggle between an isotropic or anisotropic display of the data.

Color Only Enables Objects – Available when Region Display is set to Filled Regions. The option allows users to enable the display of the object color overlay in the current orientation even if the display of the object is switched off in the Object Control Window. Option updates to Traces or Objects depending on the Region Display setting selected.

Set Object Opacity (50%) – Allows users to adjust the alpha blend of the object color overlay on the grayscale image data. When enabled an Opacity slider is enabled below the current image allowing for interactive adjustment.

Label Enabled Objects – Allows users to enable/disable the display of object names on the slice display.

Cursor Link – Toggles the Cursor Link tool Off/On in the current display.

Axis Labels – Allows users to enable or disable the display of the axis labels on the perimeter of the slice display window.

Boarder Ruler – Enables/Disables the boarder ruler tool.

Print – Sends capture of current slice display to printer.

Reset – Resets all options to default.

2.4.2 Rendering Window

In the default view the upper right window is the rendering window (Figure 2.19), here the window displays a 3-D rendering of the image volume.
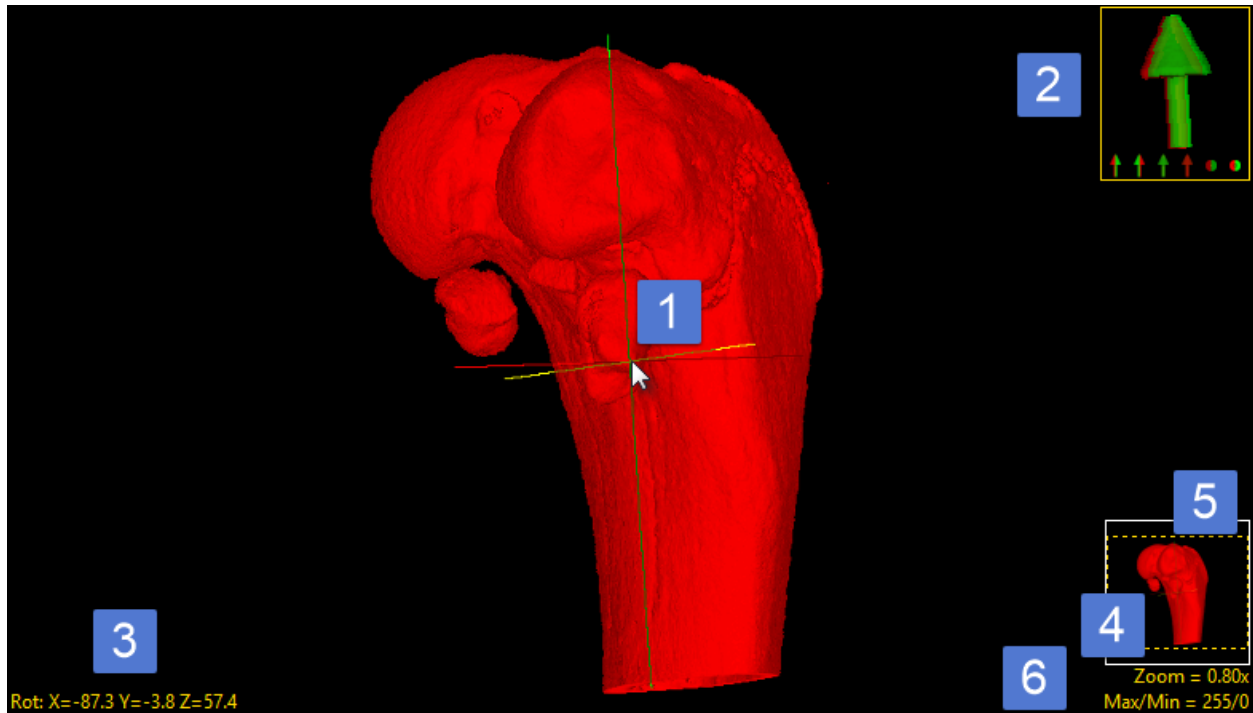
Figure 2.19. The Render Window, showing 1) Cursor Link tool, 2) Navigation Reference Icon tool, 3) Rotation tool, 4) Zoom tool, 5) Pan tool, 6) Max/Min tool.

The Rendering Window includes several tools for viewing and navigating through the image data, these include:

Cursor Link: As described in the Slice Display section above, the cursor link allows users to interactively navigate through the image data. The tool can be controlled from the 3D rendering or from any of the 2D orientations. Moving the tool on the rendering will interactively update the orthogonal slice displays. The cursor linked can be enabled or disabled using the Toggle Cursor Link button.

The Navigation Icon tool: The Navigation Icon is not just a reference for the current orientation of the 3D image data, the tool also allows users to rotate the data or specify a standard view; front, back, left, right, top, bottom. To rotate the data, left click on the Navigation Icon and hold the left mouse button down. The cursor will update to the rotate cursor (Figure 2.20) enabling interactive rotation of the rendering viewpoint. Releasing the left mouse button will dismiss the rotate cursor.
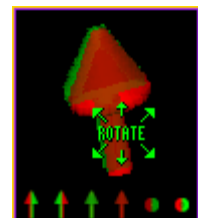
Figure 2.20. Rotate Cursor

The Zoom tool: As with the Zoom tool for the 2D Slice Display, the Zoom tool in the Rendering Window allows users to increase and decrease the display size of the data. For more information refer to the Zoom tool description in the Slice Display section.

The Pan tool: The Pan tool is automatically enabled when the Zoom size of the image is larger than the current window. Clicking in the tool allows users to Pan the zoomed area over the current display.

The Max/Min tool: The Maximum and Minimum display intensities for the rendering can be changed by clicking on the yellow Max/Min Tool at the bottom right corner of the window. A cursor will return that allows the values to be manipulated. Hold down the left mouse button and move the mouse up/down or left/right. See Figure 2.17 to determine how values are changed.

Right Menu Options - Right-clicking in the Rendering Window reveals additional options. Most of these options, if available, are covered in the Slice Display section, however, there are few additional configuration options specific to the Rendering window.

Middle Button Action: Allows users to set the action of the middle mouse button. See the Slice Display section for additional information.

Mouse Wheel Action: Allows users to set the action of the middle mouse wheel. See the Slice Display section for additional information.

Copy to Clipboard: Copies the current rendering display to memory.

Figure 2.21. Right Menu Options for the Render Window.

Interactive Render: Allows users to select between Reduced Quality (useful for interactive rotation of extremely large image volumes) and Full Quality.

Cursor Link – Toggles the Cursor Link tool Off/On in the current display.

Transparency: The Transparency option turns transparent rendering on or off. The transparency rendering mode is useful for visualizing objects contained within objects or objects with views obscured by other objects. Object opacity levels can be set using the Opacity options in the Object Control window.

- **Background Opacity**: The Background Opacity option is only available when Transparency rendering is enabled. The option allows users to specify the background opacity for the transparency rendering.

Background Color: Allows users to set the color of the rendering background. The option opens a Color selection window enabling selection of desired color.

Print – Sends capture of current slice display to printer.

Reset – Resets all options to default.

2.5 Object Control Window

The Object Control Window (Figure 2.22) functionality is limited in BMA to enabling and disabling the display of the selected object. To disable the display of an object, click on the monitor icon next to the object, to enable the display click on the icon again.
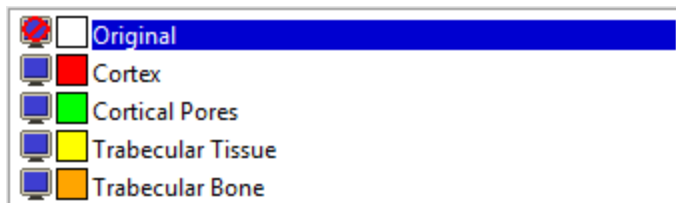
Figure 2.22. The Object Control Window

2.6 BMD Scaling Parameters

A set of input fields has been provided for users to enter specific slope (SigmaCT) and offset (BetaCT) values to convert CT numbers (Hounsfield units) to bone mineral density (BMD) in units of mg/CC (Figure 2.23).

Figure 2.23. BMD Scaling Parameters

Generic values are provided, but users may modify these values as required. Depending upon the vendor and scanning protocol, these values may be available in the header information of the data. For data acquired from the PerkinElmer QuantumFX or QuantumGX or the Rigaku R_mCT2 microCT scanner, these values need to be calculated. For information on how to determine the SigmaCT and BetaCT values for data acquired from these systems, please refer to Section 6) BMD Calibration.

2.7. BMA Workflow Tabs

The BMA workflow tabs provides access to the three processing steps in the BMA workflow: Segment Cortex; Segment Trabeculae; and Measure Bone.
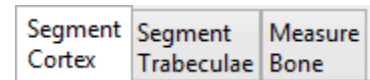
Figure 2.24. BMA Workflow Tabs

2.7.1 Segment Cortex

The first step of the BMA workflow process is to segment the cortex from the trabecular bone and intratrabecular space (Figure 2.25). If the image volume is of sufficient resolution to reveal cortical pores, they will be identified at this stage.

Figure 2.25. BMA – Segment Cortex

Cortex Thresholds: Provides access to a threshold slider bar to allow for segmentation of the cortex. Note that the module will automatically compute the minimum threshold value via histogram analysis, however, this may be adjusted by the user before initiating the segmenting of the cortex.

Threshold Rendering: This option turns on and off the interactive 3D rendering display as the threshold slider changes. It is useful to switch off this option if you are working with extremely large data sets and the interactive 3D update is slow.

Use Initial Segmentation: If the image volume is a single bone, this option is not needed. If the image volume contains several bones, it must be pre-segmented by use of an object map to indicate the bone of interest. This pre-segmentation will only be used to avoid segmenting and measuring the other bones in the image. The cortex and trabecular bone will still be identified by the segmentation routines in the program.

Initial Object Map: Opens a file browser to allow the users to navigate to and select the object map for specimens containing multiple bones.

Bone Object: If the object map contains multiple objects, the Bone Object drop down menu allows users to select objects corresponding to the bone of interest.

Segment Cortex: The Segment Cortex button begins the cortical segmentation process. Depending on the input data and the resources of the system, this process could take several minutes. Segmentation progresses by transverse slice and varies in speed by the characteristics of the section. Mid-shaft sections are generally fastest, sections containing significant trabecular tissue and/or articulating surfaces are slower, and sections with cortical defects or traumatic damage can be slowest to resolve.

2.7.2 Segment Trabeculae

The Segment Trabeculae tab is displayed at the end of the Segment Cortex procedure (Figure 2.26). The cortical segmentation algorithm has been designed to perform well on intact long bones, but it may make minor errors on unusually shaped bones or on complicated sections near the metaphysis or on sections containing cortical defects. On this tab the user may save the cortical segmentation as an object map and use any of the manual segmentation tools in Analyze to correct regions believed to be inappropriate. For more information on manual object map refinement please review the BMA exercise in section 5) The Bone Microarchitecture Analysis Add-On: Processing Multi-Bone Samples. Likewise, this tab may be selected when starting the program and a corrected object map loaded to proceed. At this point, the trabecular bone and intratrabecular space are combined as a single object, as this is easier to correct than the detailed object map.

Figure 2.26. BMA – Segment Trabeculae

Save Segmented Cortex Object Map: This option opens a save window, allowing the user to save the segmented cortex object map.

Load Segmented Cortex Object Map: This opens a load window, allowing the user to load a corrected cortex object map.

Trabeculae Thresholds: The minimum threshold value is set from the Cortical Thresholds slider on the Segment Cortex tab. This is usually the best threshold to use, but the user may adjust this slider if a different decision is desired within the trabecular compartment. As the slider is adjusted, the sectional images show the resulting trabecular bone.

Object Map List: The list of objects shows the color associated with each object and whether the object is displayed in the rendering or not. To turn off the display of an object, select the monitor icon next to the object name. To change the color of an object, select the color square next to the object name.

Segment Trabeculae: When pressed the Segment Trabeculae button commits the threshold decision to the object map, populating the Trabecular Bone object.

2.7.3 Measure Bone

The Measure Bone tab is displayed at the end of the Segment Trabeculae procedure (Figure 2.27). The Final segmentation may be saved as an object map if corrections are desired, or the tab can be selected when data is loaded, and a corrected final object map loaded. This tab allows the selection of the specific bone measurements desired (all by default) and initiates the measurement process. Each measurement group is saved to a comma separated values (.csv) file named using the image volume name and an additional extension described below. The first line of each .csv file contains the variable names of the measurements in the following row(s). An additional .csv file named "VolumeName.csv" is also saved containing all the measures calculated in a format including explicit units for all measurements.
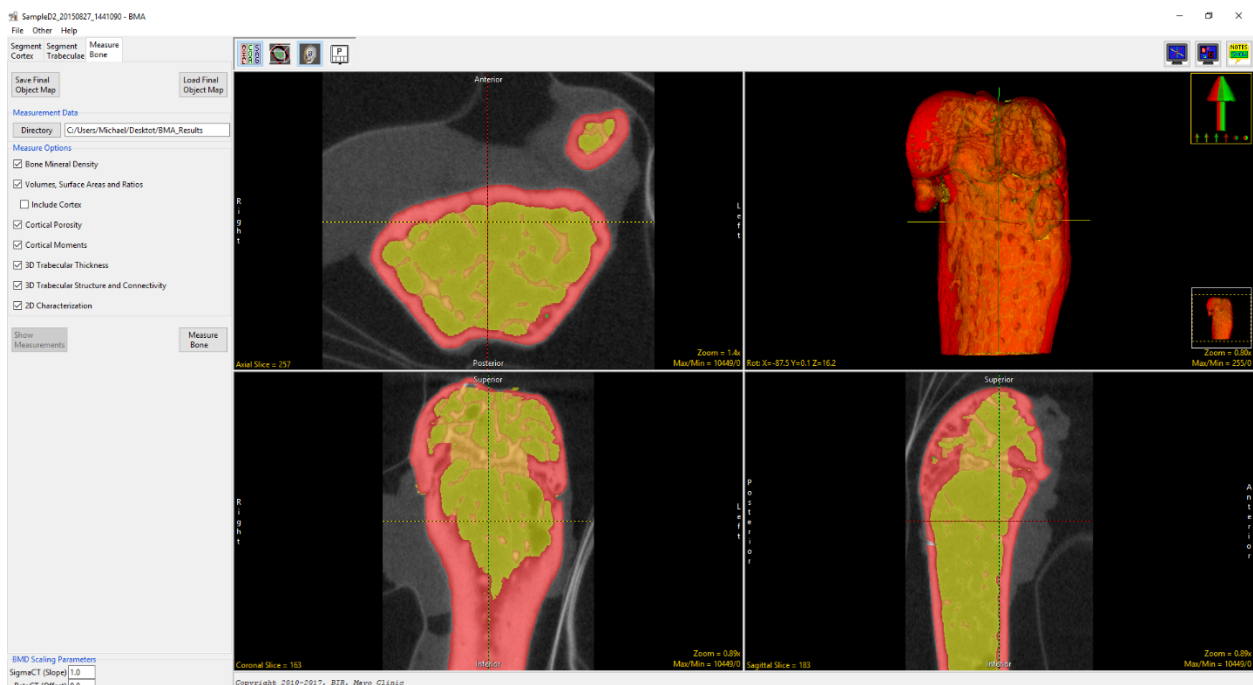
Figure 2.27. BMA – Measure Tab

Save Final Object Map: This option opens a save window, allowing the user to save the final object map.

Load Final Object Map: This opens a load window, allowing the user to load a corrected final object map.

Measurement Data Directory: This is initially set to the Current Directory, this can be modified by right-clicking in the main Analyze window, for more information please refer to the Analyze User's Guide.

Directory: This option allows the user to set the measurement data directory, the location where the .CSV files will be saved to.

Measure Options: Measure Options allows the user to specify groups of bone measurements to sample. Unchecking the checkbox to the left of the group name before sampling excludes the measurement. For detailed information on each of the measurements generated, please refer to section 3) Bone Microarchitecture Analysis

Measurements. For a quick reference summary of the measurement groups, abbreviations, descriptions and which .csv file each measurement is stored in please refer to Table 4.

- **Bone Mineral Density:** Means and standard deviations measured in the grayscale image volume under the various objects and converted by use of the SigmaCT and BetaCT to mg/cc units. These are saved in a single file named "VolumeName_BMD.csv".

- **Volumes, Surface Areas and Ratios:** Global values saved in a file named "VolumeName_VSR.csv". To include the cortical bone with these measurements, check the Include Cortex check box. Please note, most users will not want to check this option, which is unchecked by default.
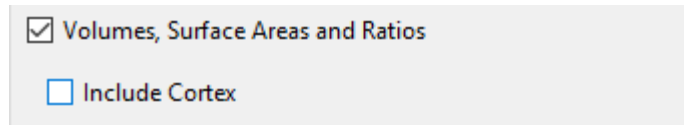

Figure 2.28. Volumes, Surface Areas and Ratios Cortex Option.

Please refer to section 3.2.1 Volumes, Surface Areas, and Ratios for additional information.

- **Cortical Porosity:** Global values saved in a file named "VolumeName_CP.csv".

- **Cortical Moments:** Values per transverse section saved in a file named "VolumeName_M.csv".

- **3D Trabecular Thickness:** This produces histograms of trabecular thickness and intratrabecular space over the entire 3D extent of the bone. The histogram .csv files ("VolumeName_TTH.csv" for trabecular thickness and "VolumeName_TSP.csv" intratrabecular space) declare the range of thickness (in mm) in each bin in the first line, and the values in the following lines are the volume of trabecular bone (or intratrabecular space) (in mm^3) of that thickness. The mean and standard deviation of these measurements are stored in "VolumeName_TrSt.csv".

- **3D Trabecular Structure and Connectivity:** Two global characterizations of trabecular structure that are also stored in "VolumeName_TrSt.csv".

- **2D Characterization:** Multiple values per transverse section in file VolumeName_Ch2D.csv".

Note that detailed descriptions of each measurement generated for each group of bone measurements is provided in the section 3) Bone Microarchitecture Analysis Measurements.

- **Measure Bone**: Begins the measurement process. All the measurement groups selected will be sampled. When complete, the .csv file viewer will be displayed.

- **Show Measurements:** If the .csv file viewer is closed by the user, it may be recalled with this button.

Table 4. Summary of Measurement Groups, Measurements, Descriptions and .csv file Reference			
Group	**Abbreviation**	**Description**	**.csv File Name**
Bone Mineral Density			
	BoneMean	Mean BMD of bone	DataName_BMD.csv
	BoneStd	Standard deviation of bone	
	BoneVolume	Volume of bone	
	CortexMean	Mean BMD of cortex	
	CortexStd	Standard deviation of cortex	
	CortexVolume	Volume of cortex	
	IntraTrabecularMean	Mean BMD of intratrabecular	
	IntraTrabecularStd	Standard deviation of intratrabecular	
	IntraTrabecularVolume	Volume of intratrabecular	
	TrabecularMean	Mean BMD of trabecular	
	TrabecularStd	Standard deviation of trabecular	
	TrabecularVolume	Volume of trabecular	
	TrabecularTissueMean	Mean BMD of trabecular tissue	
	TrabecularTissueStd	Standard deviation of trabecular tissue	
	TrabecularTissueVolume	Volume of trabecular tissue	
	WholeMean	Mean BMD of whole ROI	
	WholeStd	Standard deviation of whole ROI	
	WholeVolume	Volume of whole ROI	
Volumes, Surface Areas and Ratios			
	TV	Total Volume	DataName_VSR.csv
	BV	Bone Volume	
	BS	Bone Surface	
	BV/TV	Bone Volume Fraction	
	BS/TV	Bone Surface Density	
	BS/BV	Specific Bone Surface	
Cortical Porosity			
	Ct.Po	Cortical Porosity	DataName_CP.csv
	Po.N	Pore Number	
	Po.V	Total Pore Volume	
	AvgPo.V	Average Pore Volume	
	Po.V.SD	Standard Deviation of Pore Volume	
	Po.Dn	Pore Density	
Cortical Moments			
	/ap	Moment of Inertia Anteroposterior Axis	DataName_M.csv
	/ml	Moment of Inertia Mediolateral Axis	
	/max	Maximum Moment of Inertia	
	/min	Minimum Moment of Inertia	
	J	Polar Moment of Inertia	
3D Trabecular Thickness			
	Tb.Th	Trabecular Thickness	DataName_TrSt.csv
	Tb.Sp	Trabecular Separation	
	Tb.Th.SD	Standard Deviation of Trabecular Thickness	
	Tb.Sp.SD	Standard Deviation of Trabecular Separation	
3D Trabecular Structure and Connectivity			
	SMI	Structure Model Index	DataName_TrSt.csv
	Conn.D	Connectivity Density	
2D Characterization			
	Tt.Ar	Total Cross-Sectional Area	DataName_Ch2D.csv
	Ct.Ar	Cortical Bone Area	
	Ma.Ar	Medullary Area	
	Ct.Ar/Tt.Ar	Cortical Area Fraction	
	Ct.Th	Average Cortical Thickness	
	Ps.Pm	Periosteal Perimeter	
	Ec.Pm	Endocortical Perimeter	
	Tb.N	Trabecular Thickness	
	DA	Degree of Anisotropy	
	MIL	Mean Intercept Length	

3. Bone Microarchitecture Analysis Measurements

3.1 Bone Mineral Density Measurements

Bone Mineral Density (BMD) measurements are reported for following regions; 1) Whole ROI – Cortex + Trabecular + IntraTrabecular, 2) Bone – Cortex + Trabecular, 3) Cortex, 4) Trabecular Tissue – Trabecular + IntraTrabecular, 5) Trabecular, 6) IntraTrabecular, these regions are illustrated in Figure 3.1.
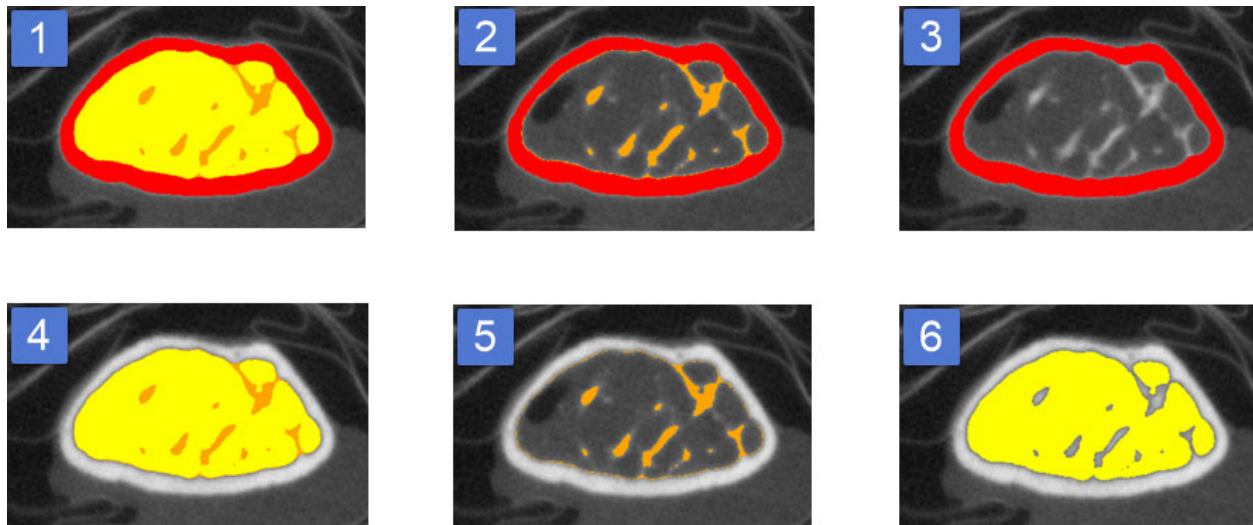

Figure 3.1. BMD Structure Measurement Reference. 1) Whole ROI – Cortex + Trabecular + IntraTrabecular, 2) Bone – Cortex + Trabecular, 3) Cortex, 4) Trabecular Tissue – Trabecular + IntraTrabecular, 5) Trabecular, 6) IntraTrabecular.

All BMD measurements can be found in the DataName_BMD.csv file.

3.1.1 Whole Region Measurements

WholeMean (mg/cm^3) is the mean BMD value reported for the whole region of interest (ROI); the cortex, trabecular and intratrabecular.

WholeStd. is the standard deviation of the sampled voxels for the whole ROI.

WholeVolume (mm^3) is the volume of the whole ROI.

3.1.2 Mineralize Bone Regions Measurements

BoneMean (mg/cm^3) is the mean BMD value reported for the mineralized bone; cortex and trabecular.

BoneStd. is the standard deviation of the sampled voxels for the mineralized bone.

BoneVolume (mm^3) is the volume of the mineralized bone.

3.1.3 Cortex Region Measurements

Cortex Mean (mg/cm³) is the mean BMD value reported for the cortex.

Cortex Std. is the standard deviation of the sampled voxels for the cortex.

Cortex Volume (mm³) is the volume of the cortex.

3.1.4 Trabeculae Tissue Measurements

TrbecularTissueMean (mg/cm³) is the mean BMD value reported for the trabecular tissue; trabecular and intratrabecular.

TrabecularTissueStd. is the standard deviation of the sampled voxels for the trabecular tissue.

TrabecularTissueVolume (mm³) is the volume of the trabecular tissue.

3.1.5 Trabecular Region Measurements

TrabecularMean (mg/cm³) is the mean BMD value reported for the trabecular.

TrabecularStd. is the standard deviation of the sampled voxels for the trabecular.

TrabecularVolume (mm³) is the volume of the trabecular.

3.1.6 InterTrabecular Region Measurements

IntraTrabecularMean (mg/cm³) is the mean BMD value reported for the intratrabecular region.

IntraTrabecularStd. is the standard deviation of the sampled voxels for the intratrabecular region.

IntraTrabecularVolume (mm³) is the volume of the intratrabecular region.

3.2 Trabecular Bone Microarchitecture Measurements

The trabecular bone microarchitecture measurements are organized into four groups of measurements: Volumes, Surface Areas and Ratios; 3D Trabecular Structure and Connectivity; 3D Trabecular Thickness; and 2D Characterization.

3.2.1 Volumes, Surface Areas, and Ratios

Total volume, bone volume, bone surface and their derivatives, bone volume fraction, bone surface density and specific bone surface are trabecular bone measurements. These measurements can be found in the DataName_VSF.csv file. These measurements do not include cortex, however, if the inclusion of cortex is required, an option is available to do this, as described in section 3.2.5.

Total volume (TV, mm³) is the volume of the region of interest. In the BMA module, this volume of interest includes the trabecular tissue that is the trabecular bone and the intratrabecular space. TV is used to calculate the bone volume fraction and bone surface density indices below that allow comparison between bones of different subjects.

Bone volume (BV, mm³) is the volume of the region segmented as trabecular bone. BV is used in the calculation of bone volume fraction as defined below.

Bone surface (BS, mm²) is the surface area of the region segmented as trabecular bone. BS is used to calculate the bone surface density and specific bone surface as defined below.

Bone volume fraction (BV/TV, %) is the ratio of the segmented bone volume to the total volume of the region of interest.

Bone surface density (BS/TV, mm²/mm³) is the ratio of the surface area of trabecular bone to the total volume as defined above.

Specific bone surface (BS/BV, mm²/mm³) is the ratio of the bone surface area to the volume of trabecular bone.

3.2.2 3D Trabecular Structure and Connectivity

The trabecular structure and connectivity measurements can be found in the DataName_TrSt.csv file with the 3D Trabecular Thickness measurements.

Connectivity density (Conn.D, 1/mm³) is a measure of the degree of connectivity of trabeculae, normalized to the total volume.

Structure model index (SMI) is a dimensionless number ranging from 0 to 4 that describes the dominant trabecular structure. Perfect plates are represented by 0, perfect rods by 3, and perfect spheres by 4. Most real trabecular structures will lie somewhere between the ideal plate and ideal rod structure. At very high or low BV/TV values, the SMI may be outside of the defined range.

3.2.3 3D Trabecular Thickness

The 3D trabecular thickness measurements are reported in the DataName_TrSt.csv file with the trabecular structure and connectivity measurements.

Trabecular thickness (Tb.Th, mm) is the mean thickness of trabeculae as determined by a 3D sphere-fitting method.

Trabecular separation (Tb.Sp, mm) is the mean distance between trabeculae as determined by a 3D sphere-fitting method. Tb.Sp is often referred to as trabecular spacing.

Standard deviation of trabecular thickness (Tb.Th.SD, mm) is a measure of the heterogeneity of trabecular thickness (Tb.Th, above).

Standard deviation of trabecular separation (Tb.Sp.SD, mm) is a measure of the heterogeneity of trabecular separation (Tb.Sp, above).

3.2.4 2D Characterization

The trabecular 2D characterization measurements are reported in the DataName_Ch2D.csv with the cortical 2D characterization measurements.

Trabecular number (Tb.N, 1/mm) is the average number of trabeculae per unit length. Tb.N is calculated by finding the mean distance between mid-axes of the trabecular structures, then taking the inverse. This mean distance is determined using a 3D sphere-fitting method.

Degree of anisotropy (DA) is a dimensionless variable defined as the length of the longest mean intercept length vector divided by the length of the shortest mean intercept length vector. For an isotropic (perfectly oriented) system, DA is equal to 1. As the system becomes more anisotropic (less well-oriented), the DA increases to some value greater than 1.

Mean intercept length (MIL) is a measure that is part of the algorithm used to determine the degree of anisotropy (DA, above).

3.2.5 Including Cortical Bone in the Trabecular Bone Microarchitecture Measurement's

By default, the trabecular bone microarchitecture measurements do not include cortex. However, an option is available below Volumes, Surface Areas, and Ratios that allows users to include the cortex when calculating these measurements. When checked the total volume, bone volume, bone surface and their derivatives, bone volume fraction, bone surface density and specific bone surface measurements will include the cortex. The option is disabled by default as most users will not include the cortex in the calculation of these measurements. See Volumes, Surface Areas and Ratios in section 2.7.3 for more information.

3.3 Cortical Bone Morphology Measurements

Cortical bone morphology measurements are grouped into three sets of measurements; 2D characterization, cortical moments and cortical porosity.

3.3.1 2D Characterization

The cortical 2D Characterization measurements are reported in the DataName_Ch2D.csv with the trabecular 2D characterization measurements.

Total cross-sectional area inside the periosteal envelope (Tt.Ar, mm^2) is measured for each slice in a volume

Cortical bone area (Ct.Ar, mm^2) is calculated as the cortical volume (Ct.V) divided by the product of the number of slices times the thickness of each slice. The Ct.Ar is a separate measurement for each slice.

Medullary (or marrow) area (Ma.Ar, mm^2) is the cross-sectional area of the medullary part of the bone on any given slice.

Cortical area fraction (Ct.Ar/Tt.Ar, %) is the ratio of cortical cross-sectional area to total cross-sectional area within a given slice.

Average cortical thickness (Ct.Th, mm) is the average thickness of the cortex of each slice.

Periosteal perimeter (Ps.Pm, mm) is the perimeter of the periosteum (the membrane around the outside of the cortex) and is calculated for each slice.

Endocortical perimeter (Ec.Pm, mm) is the perimeter of the endosteum (the membrane that forms the boundary between the cortex and medulla) and is calculated for each slice.

3.3.2 Cortical Moments

The cortical moments measurements are reported in the DataName_M.csv file.

Moment of inertia about the anteroposterior axis (I_{ap}, mm^4) is a measure of how material is distributed about the anatomical anteroposterior (front to back) axis through the area centroid of each slice. Area moments of inertia are also known as second moments of area, or SMAs, and characterize resistance to bending around a given axis.

Moment of inertia about the mediolateral axis (I_{ml}, mm^4) is a measure of how material is distributed about the anatomical mediolateral (left to right, or right to left) axis through the centroid of each slice. Area moments of inertia are also known as second moments of area, or SMAs, and characterize resistance to bending around a given axis.

Maximum moment of inertia (I_{max}, mm^4) is a measure of how material is distributed about the major centroidal axis, which is orthogonal to the minor centroidal axis.

Minimum moment of inertia (I_{min}, mm^4) is a measure of how material is distributed about the minor centroidal axis, which is orthogonal to the major centroidal axis.

Polar moment of inertia (J, mm^4) is the sum of any two perpendicular area moments of inertia ($I_{ap} + I_{ml}$ or $I_{max} + I_{min}$) and characterizes resistance to torsion.

3.3.3 Cortical Porosity

Cortical porosity measurements are reported in the DataName_CP.csv file.

Cortical porosity (Ct.Po, %) is calculated as the pore volume Po.V divided by the total cortical compartment volume Ct.V.

Pore number (Po.N, n) is a count of the number of cortical pores in a given volume.

Total pore volume (Po.V, mm³) is the total volume occupied by cortical pores in the volume of interest.

Average pore volume (AvgPo.V, mm³) is calculated as the total pore volume Po.V divided by the pore number Po.N.

Standard deviation of pore volume (Po.V.SD, mm³) is a measure of the heterogeneity in size of cortical pores in the volume of interest.

Pore density (Po.Dn, 1/mm³) is calculated as the pore number Po.N divided by the total cortical compartment volume Ct.V, giving the number of pores per unit volume in the cortex.

4. The Bone Microarchitecture Analysis Add-On: Processing Single Bone Samples.

This exercise will help guide users through the steps to process a single bone sample using the Analyze BMA Add-On. Note, that in this example the image data used is isovolumetric, orientated correctly, and as it is a single bone sample does not require any pre-segmentation steps to identify the target bone, thus this exercise will not cover any of the pre-processing steps. After completing this exercise please review section 5 for information about how to pre-process images in preparation for BMA analysis.

4.1 Data Load

1. Download data set BMA_Single_Bone.AVW from www.analyzedirect.com/data.

2. Open Analyze and then open Input/Output.

3. In Input/Output navigate to and select the BMA_Single_Bone.AVW data set (Figure 4.1).
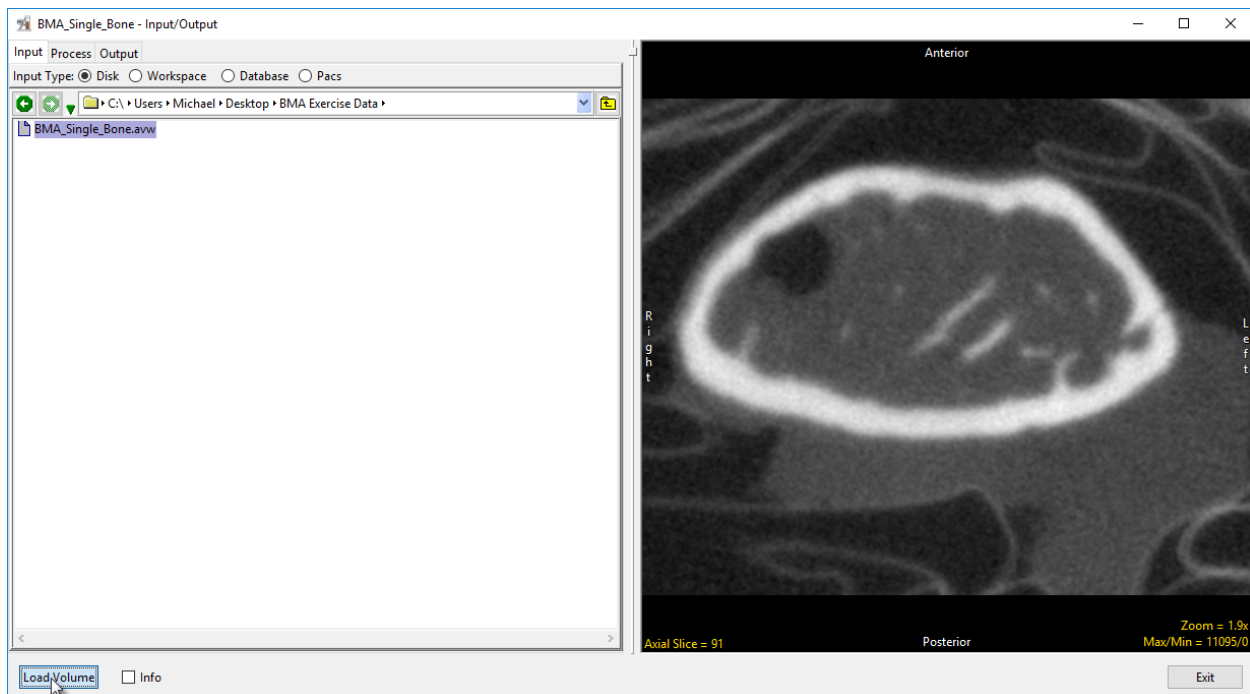
Figure 4.1. Input/Output Module.

4. Click the Load Volume button to load the data set into Analyze.

4.2 BMA Processing

Once data load is complete the image data is ready for processing with the Bone Microarchitecture Analysis Add-On module.

4.2.1 Segment Cortex

The first step in the BMA process is the segmentation of the cortex.

5. Select the BMA_Single_Bone.AVW data set and then open the BMA Add-On module from Apps (Figure 4.2). Note, if you do not have access to the Add-Ons see Section 2.2. Enabling the Analyze Add-On.

6. When BMA opens the data will be automatically loaded. Once loaded BMA immediately analyzes the image histogram and calculates a threshold range to segment the mineralized bone. (Figure 4.3). It is necessary to specify a threshold range that correctly identifies the bone for the cortex segmentation algorithm to function. If necessary, the Cortex Threshold slider to adjust the calculated min/max range.
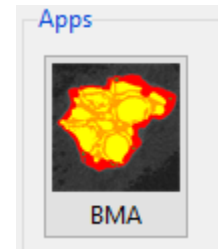
Figure 4.2. Starting the BMA Add-On
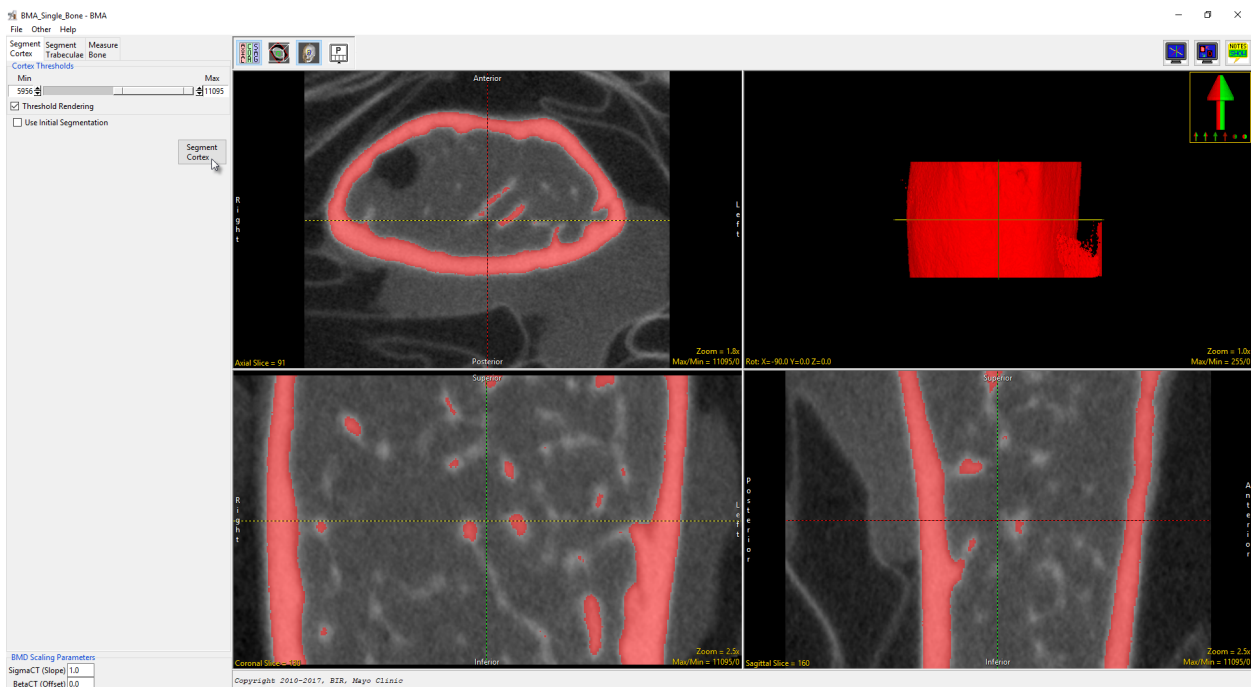
Figure 4.3. The BMA Module – Segment Cortex.

7. To initiate the cortical segmentation process, select the Segment Cortex button. The algorithm will conduct a preliminary segmentation segregating the sample into three regions; the cortical shell, cortical pores and trabecular tissue. Note that the cortex segmentation algorithm is a computationally intense process and could take several minutes to complete.

8. Once processing is complete the module will automatically move to the Segment Trabeculae tab.

4.2.2 Segment Trabeculae

The second step of the BMA process provides for segmentation of the Trabecular region into Trabecular Bone and Intra-Trabecular Space. This segmentation is achieved via threshold-based segmentation.

9. To segment the Trabecular region into the Trabecular Bone and Intra-Trabecular Space objects, set a threshold range using the Trabeculae Thresholds slider. The threshold min/max values here are the same as those used to define the initial mineralize bone region in the Segment Cortex step. However, the

Cortex object is now locked and cannot be modified in the Segment Trabeculae tab, so adjusting the minimum or maximum threshold range to identify the trabecular bone will not modify the cortex. Adjust the minimum threshold to 5500 (Figure 4.4).
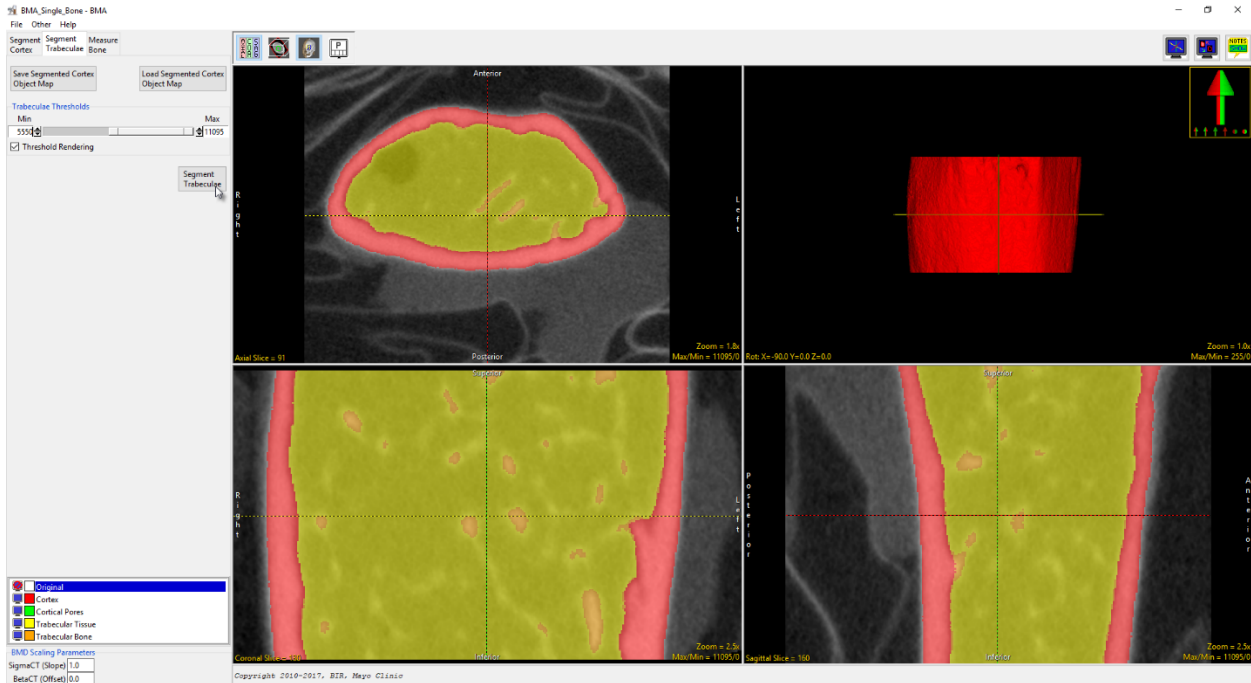

Figure 4.4. The BMA Module – Segment Trabeculae.

10. Once the threshold has been specified click the Segment Trabeculae button. The voxels specified by the threshold range will be assigned to the Trabecular Bone object. The module will automatically proceed to the Measure Bone tab.

4.2.3 Measure Bone

The third and final step of the BMA processing workflow is the generation of the bone morphometric indices.

11. At this point it is a good idea to save the object map. To do this, select the Save Final Object Map button and name the object map Final.obj.

12. Specify the directory where all of the measurement data spreadsheets (.csv's) will be saved (Figure 4.5).
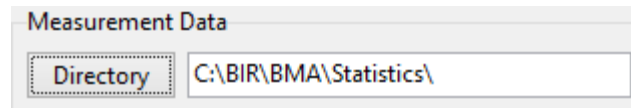
Figure 4.5. Directory Selector for Specification of Output Files.

13. By default, all of the Measure Options will be checked (with the exception of the Include Cortex option). Uncheck any option you do not wish to generate statistics for. Note that this data set does not have sufficient resolution to derive cortical porosity measurements, therefore the Cortical Porosity option should be unchecked.

14. Click the Measure Bone button to start the measurement process. Note that it could take several minutes to calculate all the bone morphometric indices. Once the measurements have been generated, they will be displayed in a new tabbed window below the display of the image data (Figure 4.6). Each of the tabs provides access to a different set of measurements.

Figure 4.6. The BMA Module – Measure Bone.

15. When the process is complete, several .csv files will be saved to the location specified in step 12. The .csv files can be opened in MS Excel or any other stats analysis package.

5. The Bone Microarchitecture Analysis Add-On: Processing Multi-Bone Samples.

This exercise will help guide users through the steps to load and prepare image data for processing multi-bone samples with the Analyze BMA Add-On.

5.1 Data Preprocessing

5.1.1 Data Load

The BMA Add-On module requires that input bone specimens have isotropic voxels. To ensure your image data is loaded with isotropic voxels in Analyze use the Process > Resize option in Input/Output to resample anisotropic data when loading.

1. Download data set BMA_PE_micro_CT.AVW from www.analyzedirect.com/data.

2. Open Analyze and select Input/Output (Figure 5.4).

3. In Input/Output navigate to and select the BMA_PE_micro_CT.AVW data set downloaded in step 1 and click Load Volume to load the data into Analyze (Figure 5.5).
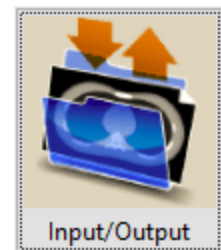
Figure 5.2. Input/Output.
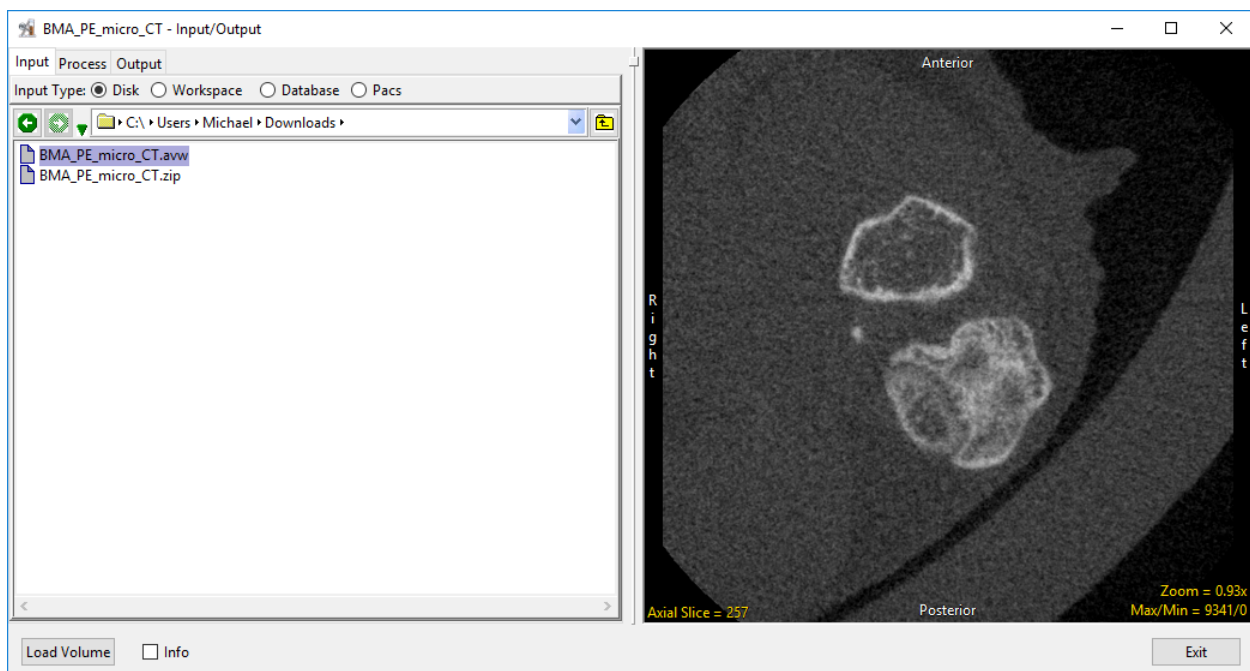
Figure 5.3. Input/Output Data Load.

4. If the data is anisotropic (i.e. the voxels are not cubic) then select the Process tab and choose the Resize option. Check the Force Cubic option (Figure 5.6) and then click Load Volume.
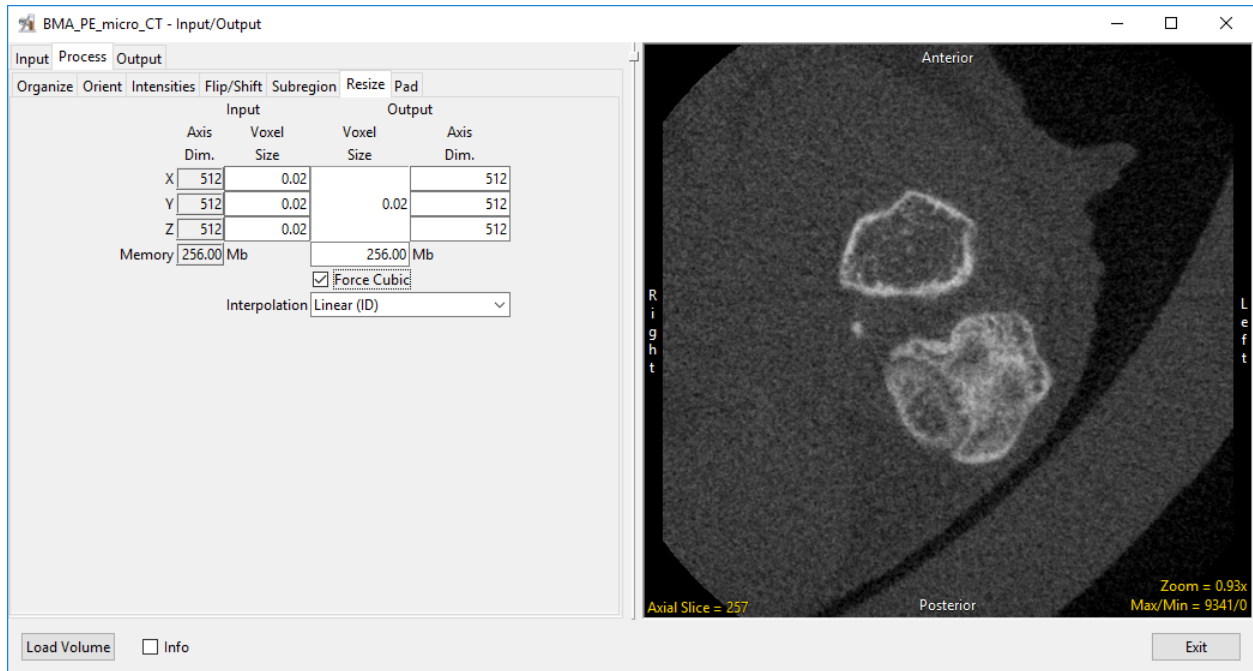
Figure 5.4. Resampling Data to be Cubic with the Input/Output > Process > Resize > Force Cubic Option.

5.1.2 Reorientation

The BMA Add-On requires that the input bone specimens are orientated into the correct anatomical orientation (Figure 5.4). Transform provides the tools necessary to quickly re-orientate image data.
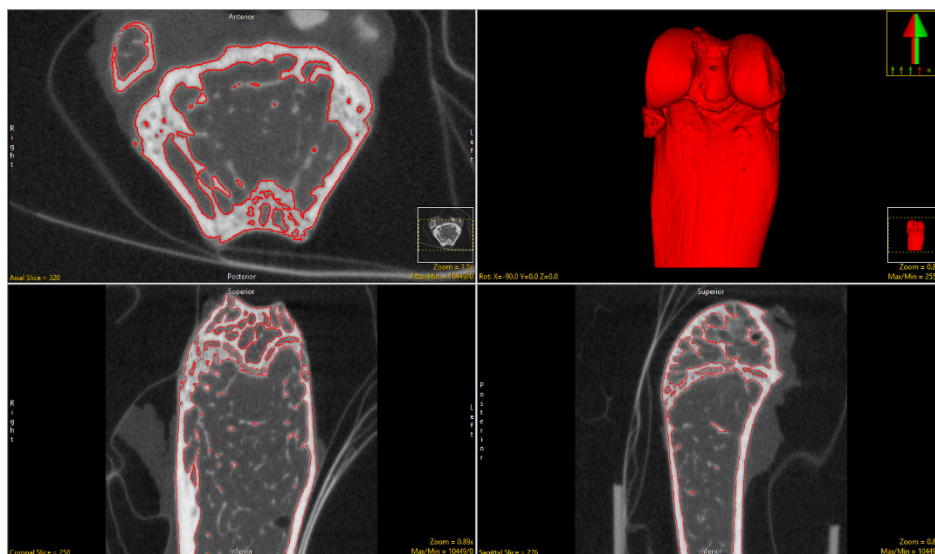

Figure 5.4. Correct Bone Orientation for BMA

5. Select the BMA_PE_micro_CT data set from the workspace and open Transform (Figure 5.5).

6. In Transform select the Spatial Transforms tab (Figure 5.6 - A) and then choose the Apply Matrix option (B).

7. Click the Toggle Oblique button (C) to enable to Oblique window (D).

8. Right-click in the coronal window and select the Perpendicular Axis tool (E).
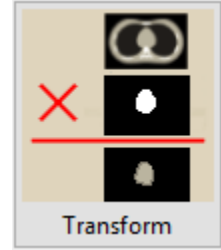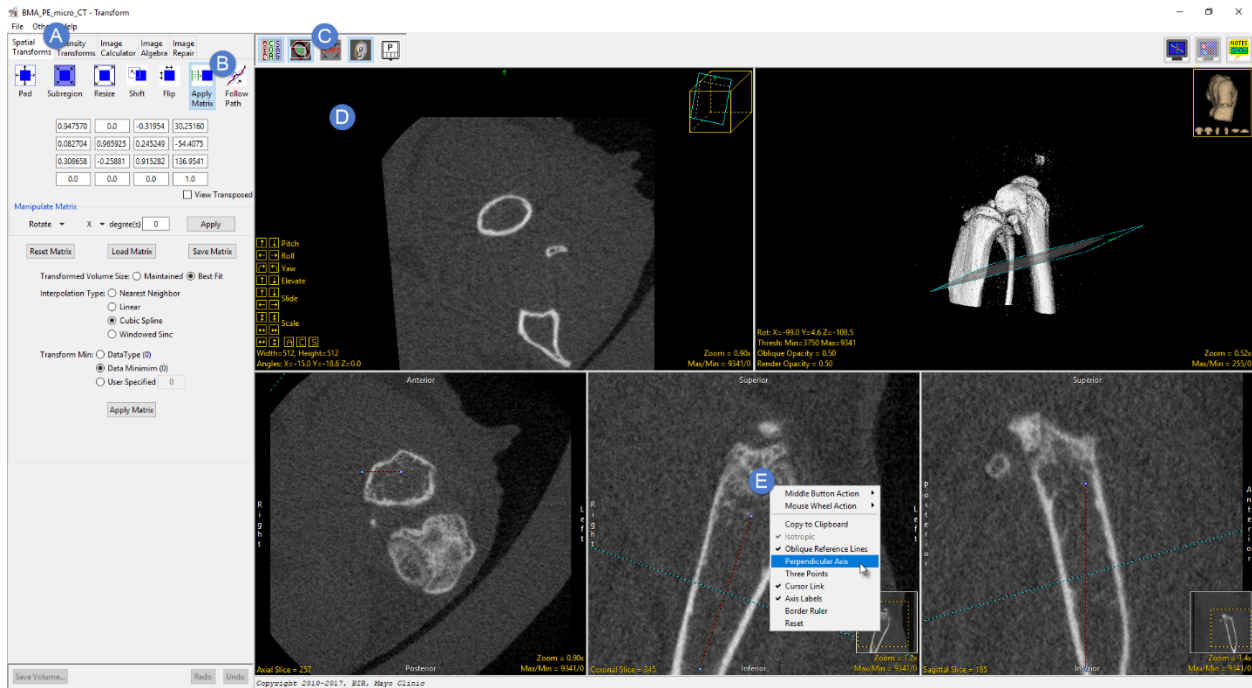

Figure 5.5. Transform.

Figure 5.6. Transform

9. Use the Perpendicular Axis tool (red and black dashed line) to define a line down the center of the bone to be analyzed. Do this by repositioning the ends of the line. In this example this is done on the coronal window (Figure 5.7). An oblique plane (shown by the blue and black dashed line) will be defined perpendicular to the red and black line.

10. The plane will need to be adjusted in the sagittal view. To do this, use the Pitch buttons in the oblique window to manipulate the blue line until it is perpendicular to the long axis of the bone in the sagittal window. Note that in this example the axis (shown in blue and black on the sagittal image) should be pitched slightly upwards (Figure 5.8), this is achieved using the Pitch Up button (Figure 5.9).
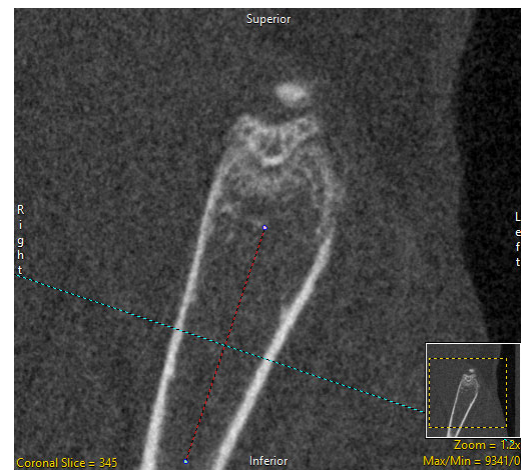

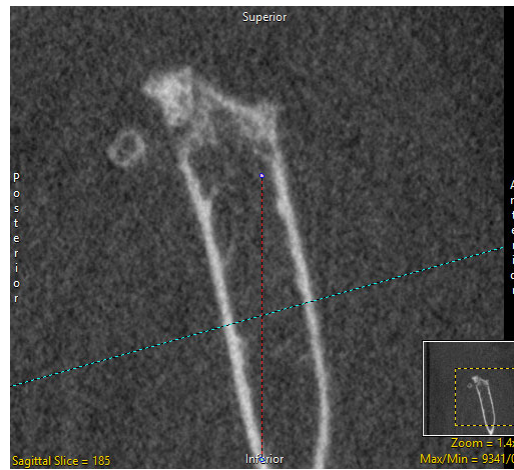*Figure 5.7. Definition of Oblique Plane in Coronal View.*

Figure 5.8. The Oblique Plane Adjusted in the Sagittal View.

Figure 5.9. The Pitch Buttons in the Oblique View.

11. To apply the transformation to the data set click the Apply Matrix button.

12. Once the transformation is complete the data can be saved to the workspace byclicking the Save Volume button.

13. In the Save Tranformed window returned choose the 'Create a new workspace volume' option and then append '_reformatted' to the original file name. Finally click Save Volume. The reformatted data set will be saved to the workspace.

5.1.3 Subregioning

The reformatting of the image data will increase the total volume size. Before exiting Transform, you may crop the image data to remove unwanted data and reduce the overall size of the data set. Note that this is an optional step, however, cropping the data to the bone of interest to reduce the total size of the volume will decrease the subsequent BMA processing time later.

14. Click the Toggle Oblique button (Figure 5.10 - A) to switch off the Oblique window.

15. From the Spatial Transforms tab (B) select the Subregion option (C). Note that a box will surround the image displayed in each orthogonal view.

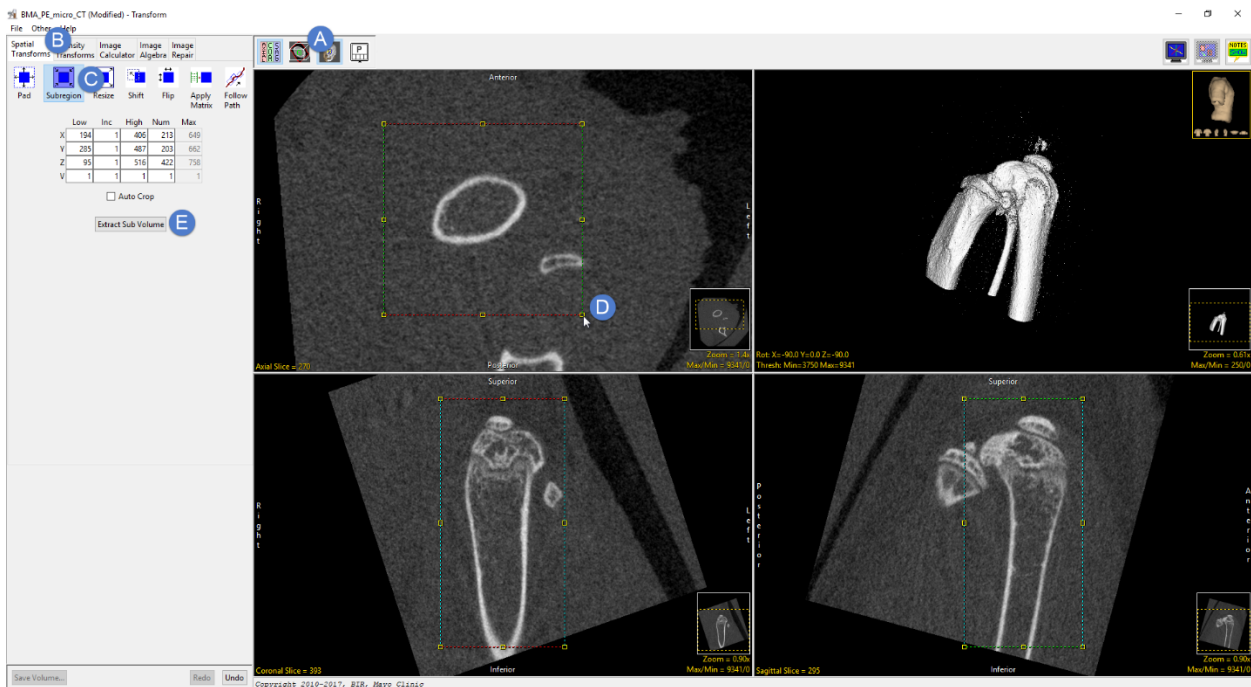16. Use the yellow boxes to reposition the box around the bone of interest in each view (D).

Figure 5.10. Transform > Subregion

17. Once the crop is set in all three orientations click the Extract Sub Volume button (E).

18. Once the sub volume is extracted click the Save Volume button.

19. In the Save Transformed window returned choose the 'Create a new workspace volume' option and then append '_reformatted_cropped' to the original file name, then click Save Volume. The reformatted and cropped data set will be saved to the workspace.

20. Close Transform.

5.1.4 Image Filtering

Filtering the image data is another optional step that can help improve the segmentation of the cortex and is often a necessary step for the segmentation for data acquired on certain micro-CT systems.

21. Select the reformatted and cropped data set, BMA_PE_micro_CT_reformatted_cropped, and then open Process.

22. In Process set the Process Type to Spatial Filter (Figure 5.11 – A).

23. Set the Filter Type to Median (B) and the Kernel Size to 5 by 5 by 5 (C).

24. Note that the filter has been interactively applied to the image data displayed in the Processed column (D).

Figure 5.11. Process > Spatial Filter.

25. To apply the filter to the image data click the Process Volume button (E).

26. Once filtering is complete, save the data using the Save Volume button. In the Save Volume window returned select the 'Create a new workspace volume containing the processed data' option. Note that Med – for Median, will be applied to the end of the file name. Click Save Volume.

27. Close Process.

5.1.5 Presegmentation

As this data set contains multiple bones it is necessary to conduct a preliminary segmentation to isolate the bone of interest. This will be used as an input in the BMA Add-On module to guide the cortical segmentation process.

28. Select the filtered data, BMA_PE_micro_CT_reformatted_cropped_Med, and open Segment.

29. In the Object control panel click the Add Object button and name the object 'Bone' (Figure 5.12).
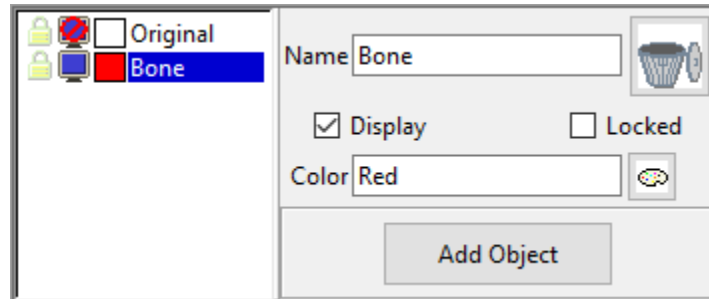

Figure 5.12. Segment Object Control Panel

30. Next select the Semi-Automatic tab and choose the Region Grow option.

31. Click on the bone of interest in any of the 3 orthogonal displays to set a seed (Figure 5.13) and use the threshold slider to set a minimum and maximum threshold criteria that represents bone. Now click Connect Object (Figure 5.14). All voxels that fall within the specified minimum and maximum threshold range that are spatially connected to the seed will be assigned to the Bone object.
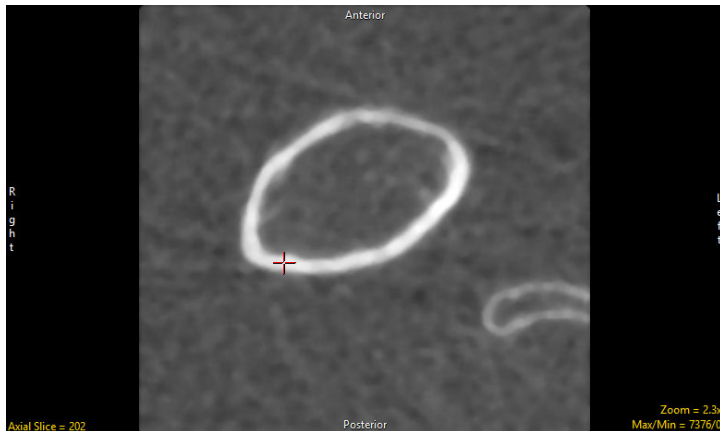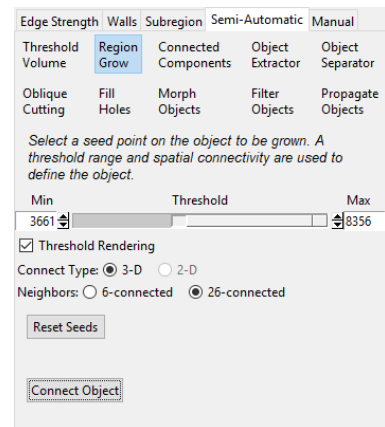

Figure 5.13. Region Grow Seed Point

Figure 5.14. Semi-Automatic Tab

32. In the Object control panel add a new object and name it 'Extra'.

33. From the Semi-Automatic tab select Object Separator. Click on the bone of interest to set the first seed point location. Next click on the bone you wish to disconnect; this will set the second seed point (Figure 5.15).
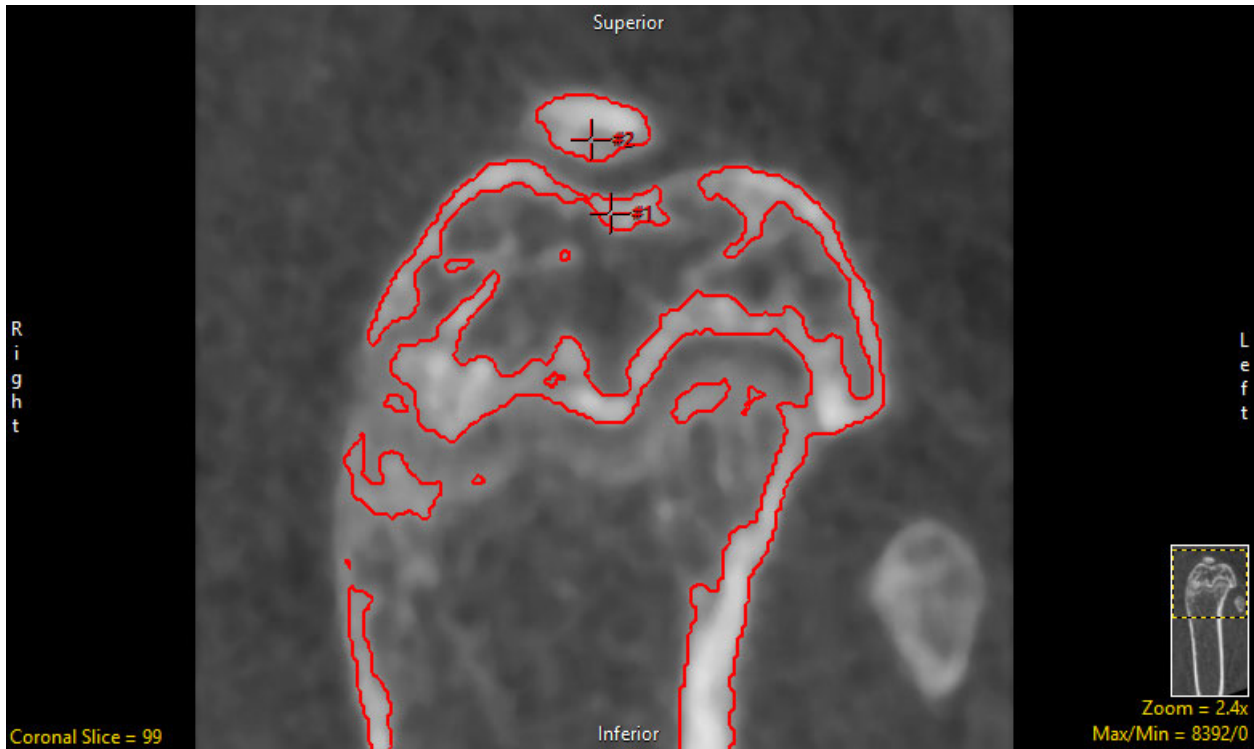
Figure 5.15. Object Separator Seed Points

34. Use the drop menu to define seed #1 as the Bone and seed #2 as Extra (Figure 5.16).
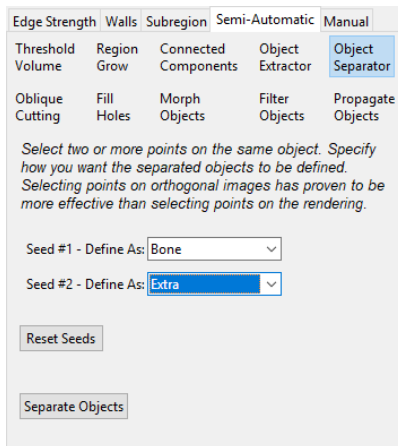35. Click Separate Objects to disconnect the two bone objects (Figure 5.17).
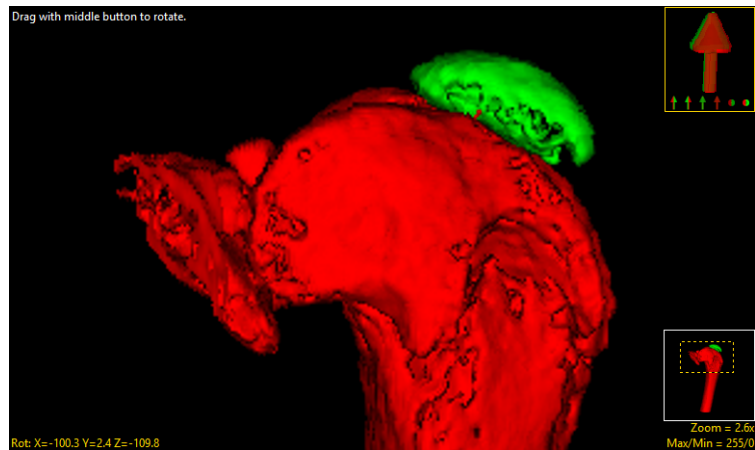

Figure 5.16. Object/Seed Assignment

Figure 5.17. Rendering of the Separated Bone Objects

36. Continue to disconnect any superfluous bones surrounding the bone of interest (Figure 5.18).

Figure 5.18. The Isolated Bone of Interest

37. Once all bones surrounding the bone of interest have been reassigned to the Extra object, delete the object by clicking on the trash can button.

38. Select File > Save Object Map. Name the object map initial.obj.

5.2 BMA Processing

Once data preprocessing is complete the image data is ready for processing with the Bone Microarchitecture Analysis Add-On module.

5.2.1 Segment Cortex

The first step in the BMA Add-On module is the segmentation of the cortex. Once the cortex is segmented, two additional objects will be derived: Cortical Pores and Trabecular Tissue.

39. Select the BMA_PE_micro_reformatted_cropped_Med data set and then open the BMA Add-On module.

40. As noted previously this data set contains multiple bones. To allow the module to distinguish between the bone of interest and all other bones, it is necessary to load the initial segmentation object map created using Segment. To do this check the Use Initial Segmentation option (Figure 5.19) and then load the object map initial.obj saved in step 38.
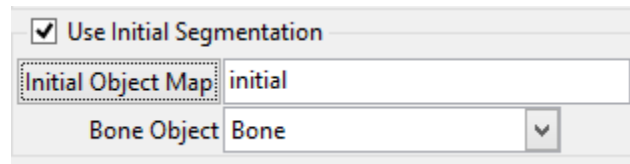

Figure 5.19. Initial Segmentation Option

41. The target bone will be displayed in green. Although an object map has been loaded it is necessary to specify a threshold range that identifies the bone in order for the cortex segmentation algorithm to function. BMA automatically analyzes the image histogram and calculates a threshold range to segment the mineralized bone. If on inspection of the 3D image it appears that the threshold range for segmentation of the bone is not acceptable, use the Cortex Thresholds slider to specify a min/max threshold range to segment the bone (Figure 5.20).

Figure 5.20. BMA Module with Initial Segmentation Object Map Loaded

42. To initiate the cortical segmentation process, select the Segment Cortex button. The algorithm will conduct a preliminary segmentation separating the sample into three regions; the cortical shell, cortical pores and trabecular tissue. Note that the cortex segmentation algorithm is a computationally intense process and could take several minutes to complete.

43. Once processing is complete the module will automatically move to the Segment Trabeculae tab (Figure 5.21).

Figure 5.21. BMA Segment Trabeculae Tab

44. At this point in the workflow the user should visually inspect the segmentation result to determine if the cortex segmentation is acceptable or not. To inspect the result use the Cursor Link to navigate through the image data in all 3 orthogonal slice displays, review both the cortex and trabecular region segmentation results. If the result is acceptable then processing can continue and the user can proceed to section 5.2.3 Segment Trabeculae.

45. If the segmentation result is not acceptible then manual refinement of the object map using Segment will be reqired. Examples of manual refinement that may be needed include the reassignment of incorrectly labelled bone structures and the filling of holes and gaps in the trabecular tissue region. After object refinement is complete the modified object map can be reloaded back into BMA at this point in the workflow using the Load Segmented Cortex Object Map option.

46. To save the object map select the Save Segmented Cortex Object Map button.

47. Minimize the BMA Add-On window.

5.2.2 Manual Refinement of the Cortex Object Map

Segment provides access to all of the segmentation tools in Analyze necessary to modify the cortex object map. Here we will review some common tools that can applied to the manual adjustment of the object map to correct small regions of trabecular bone identified as cortical bone and to fill holes in the trabecular region. Editing the object map will allow for greater accuracy in the measurements generated by BMA.

48. With the BMA_PE_micro_reformatted_cropped_Med data set selected open Segment.

49. Select File > Load Object Map and load the Cortex object map saved in step 47.

5.2.2.1 Reassigning Cortex to Trabecular Tissue

50. In the coronal orientation navigate through the slice data. Note that there are a number of cortical regions (shown in red) that should be assigned to the trabecular tissue object (shown in yellow) (Figure 5.22).
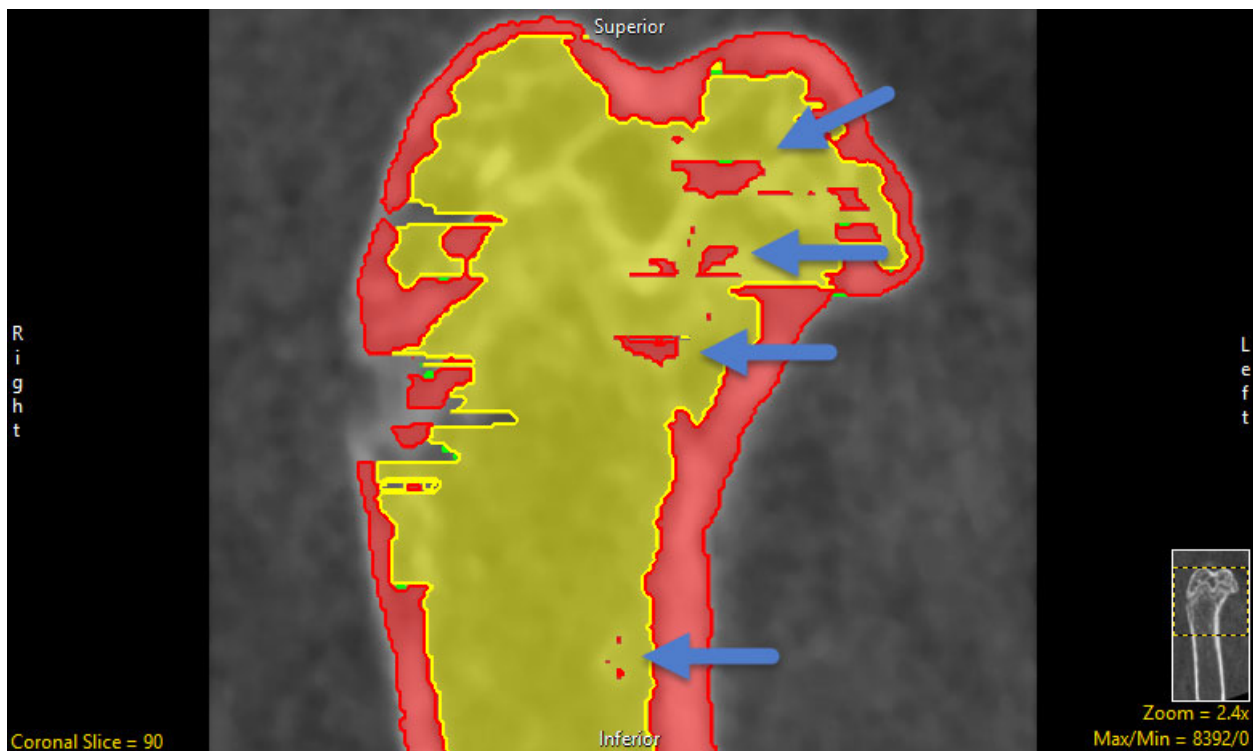

Figure 5.22. Incorrectly Labeled Trabecular Tissue

51. Select the Trabecular Tissue object and then click on the Manual tab then and choose the Draw tool.

52. Next click on the image and draw over the Cortex object to be reassigned (Figure 5.23).
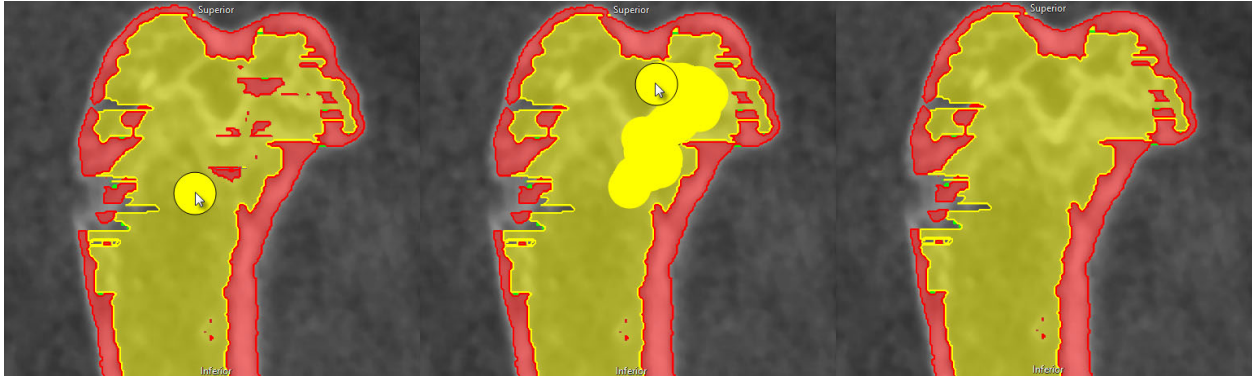
Figure 5.23. Steps to Reassign Incorrectly Labelled Regions Using the Draw Tool

53. Continue moving through the slice data and correcting regions using this method.

5.2.2.2 Filling Holes and Gaps in the Trabecular Tissue

54. Fill Holes can be used to correct for holes in the trabecular tissue. To do this select the Semi-Automatic tab and then choose Fill Holes. Select between the following Fill Types; 3-D, 3-Pass, 2-D All Images, and 2-D Current Image

55. Click on the trabecular tissue object. Holes in the object will be filled (Figure 5.24). To prevent the cortex and cortical pores objects being reassigned to the trabecular tissue object, lock these objects by clicking the lock icon next to each object.
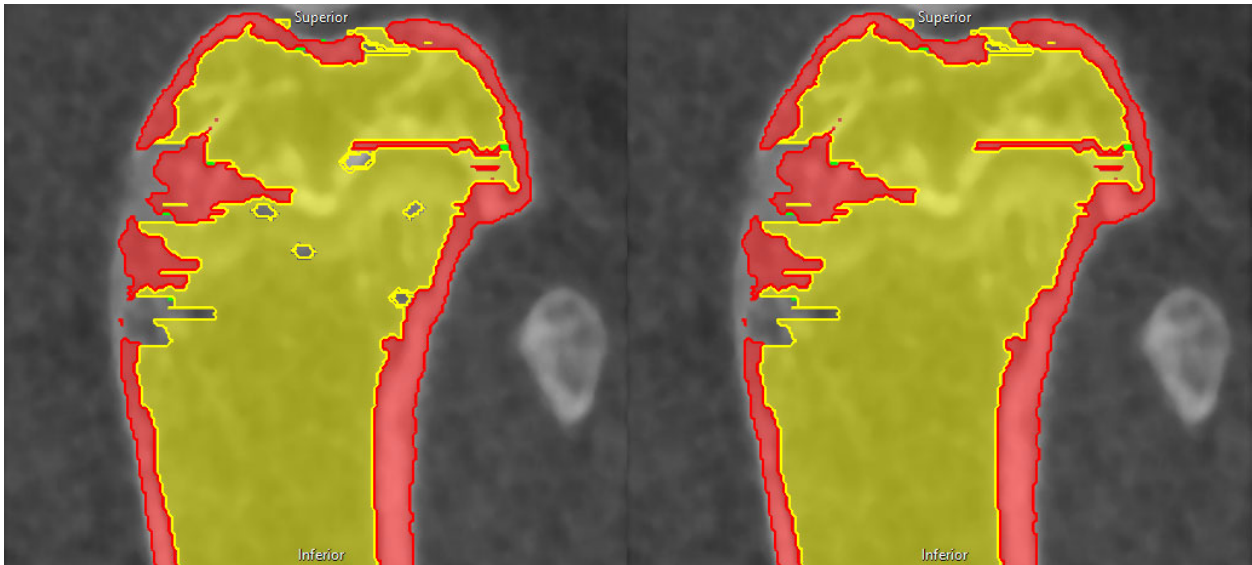

Figure 5.24. Using Fill Holes to Correct Data

56. Once editing is complete use the File > Save Object Map option to save the object map. Append '_edited.obj' to the name and then close Segment.

57. Return to the BMA Add-On and use the Load Segmented Cortex Object Map to load the edited object map saved in step 56.

5.2.3 Segment Trabeculae

The second step of the BMA process provides for segmentation of the Trabecular region into Trabecular Bone and Intra-Trabecular Space. This segmentation is achieved via threshold-based segmentation

58. To segment the Trabecular region into the Trabecular Bone and Intra-Trabecular Space objects set a threshold range using the Trabeculae Thresholds slider (Figure 5.25). The threshold min/max values here are the same as those used to define the initial mineralize bone region in the Segment Cortex step. However, the Cortex object is now locked and cannot be modified in the Segment Trabeculae tab, so adjusting the minimum or maximum threshold range to identify the trabecular bone will not modify the cortex.

Figure 5.25. Segment Trabeculae Tab with Thresholds Set to Segment Trabecular Bone.

59. Once the threshold has been set click the Segment Trabeculae button. The voxels specified by the threshold range will be assigned to the Trabecular Bone object. The module will automatically proceed to the Measure Bone tab (Figure 5.26).

Figure 5.26. The Measure Bone Tab Showing the Segmented Trabecular Bone in Orange

60. At this point in the workflow the user should visually review the segmentation result to determine if the trabecular bone segmentation is acceptable. To inspect the result use the Cursor Link to navigate through the image data in all 3 orthogonal slice displays, review the trabecular bone region segmentation result.

61. If the result is acceptable then processing can continue and the user can proceed to the section 5.2.5 Measure Bone.

62. If the segmentation result requires manual refinement, the user has the option to output and modify the object map using Segment. To do this see section 5.2.4.

63. To save the object map select the Save Final Object Map button. Name the object map Final.obj.

64. Minimize the BMA Add-On window.

5.2.4 Manual Refinement of the Final Object Map

Here we will review manual segmentation tools in Segment. This will facilitate the correction of small regions of trabecular bone identified as cortex. Object map refinement will improve the accuracy of the measurements generated by the BMA Add-On.

65. With the BMA_PE_micro_CT_reformatted_cropped_Med data set selected open Segment.

66. Select File > Load Object Map and load the Final object map saved in step 63.

67. In the Coronal orientation navigate through the slice display. Note that there are several cortical regions (shown in red) that need to be assigned to the trabecular bone (shown in orange).

68. Lock the Trabecular Tissue (yellow) object by clicking the lock icon next to the object name. Select the Trabecular Bone object, click on the Manual tab and select the Manual Trace tool.

69. Left click on an image to begin tracing and move the cursor around the cortical regions to reassign them to the trabecular bone object (Figure 5.27).
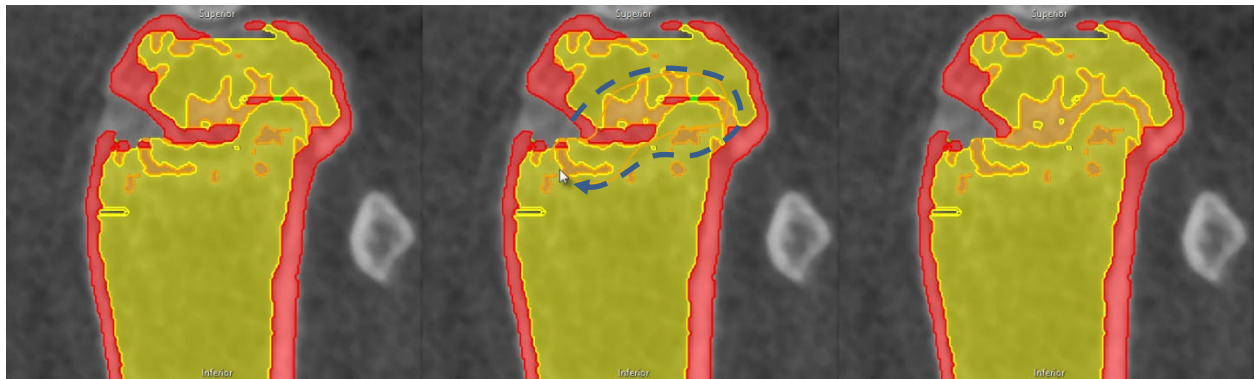

Figure 5.27. Steps to Reassign Incorrectly Assigned Regions to the Trabecular Bone

70. Continue working through the slice data to correct regions in this manner. When completed, select File > Save Object Map and save the object map as Final-edited.obj.

71. Close Segment.

72. In BMA use the Load Final Object Map button to load the Final-edited.obj map saved in step 70.

5.2.5 Measure Bone

The third and final step of the BMA processing workflow is the generation of the bone morphometric indices.

73. Specify the directory where all the measurement data spreadsheets will be saved (Figure 5.28).
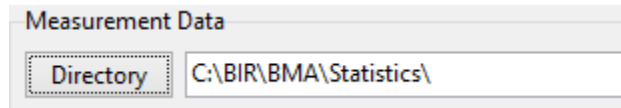

Figure 5.28. Directory Selector for Specification of Output Directory

74. By default, all of the Measure Options will be checked (with the exception of the Include Cortex option). Uncheck any option not required for measurement. Note that this data set does not have sufficient resolution to derive cortical porosity measurements, therefore the Cortical Porosity option should be unchecked

75. Click the Measure Bone button to start the measurement process. Note that it could take several minutes to calculate all the bone morphometric indices. Once the measurements have been generated, they will be displayed in a new tabbed window below the display of the image data (Figure 5.29). Each of the tabs provides access to a different set of measurements.
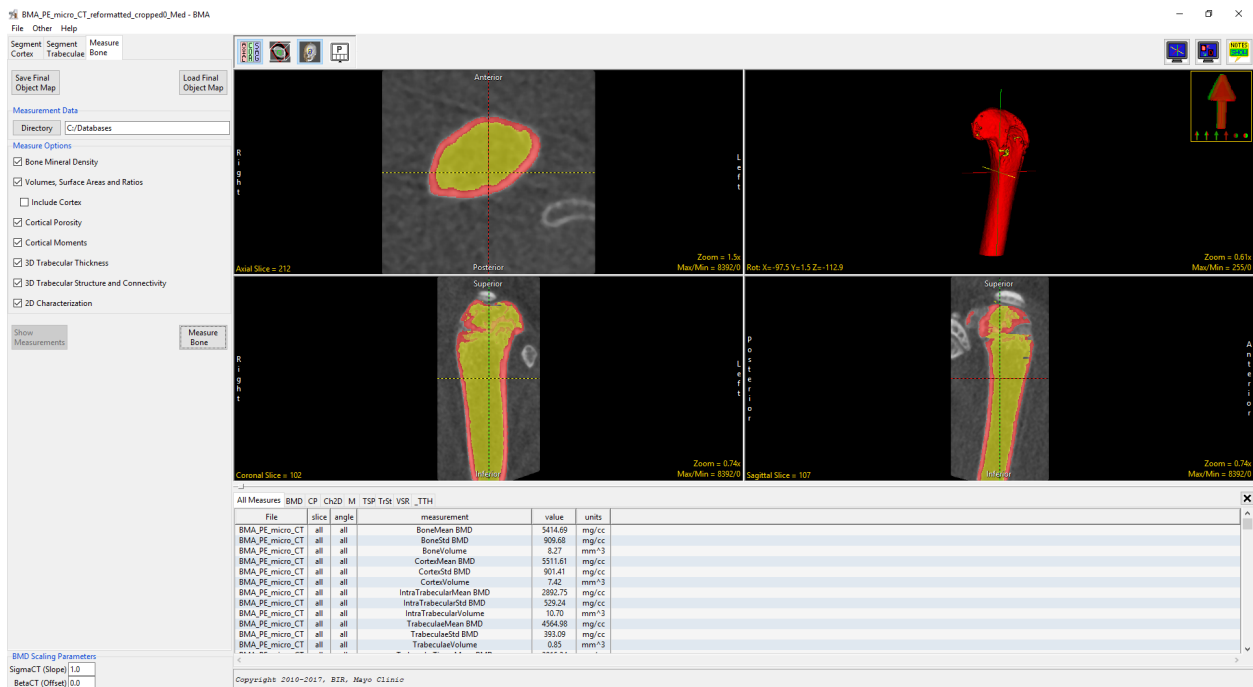

Figure 5.29. The BMA Module – Measure Bone.

76. When the process is complete, several .csv files will be saved to the location specified in step 73. The .csv files can be opened in MS Excel or any other stats analysis package.

6. Bone Mineral Density (BMD) Calibration

For certain vendor data it is necessary to calculate BMD scaling parameters for proper conversion of CT or grayscale numbers to BMD units (mg/CC). This requires that a BMD phantom be scanned with the image data using the same acquisition protocol. This calibration exercise has been developed for microCT data acquired on the PerkinElmer QuantumFX/QuantumGX and the Rigaku R_mCT2 microCT scanners, however, the exercise methodology can be applied to microCT image data acquired using other microCT systems.

6.1 Data Analysis

1. Scan both the bone samples and the BMD phantom with an identical appropriate acquisition protocol.

2. Load the image data for the phantom into Analyze using Input/Output.

3. To reduce noise in the phantom image data set, filter the image data. To do this, select the image and open Process.

4. Set the Process Type to Spatial Filters. Choose the Median filter option and set the Kernel Size to 5x5x5 (Figure 6.1). Observe the images in the "Processed" column in the center panel. If there is still noise present in the images, increase the Kernel Size to 7x7x7, 9x9x9 or 11x11x11 until the noise is reduced.
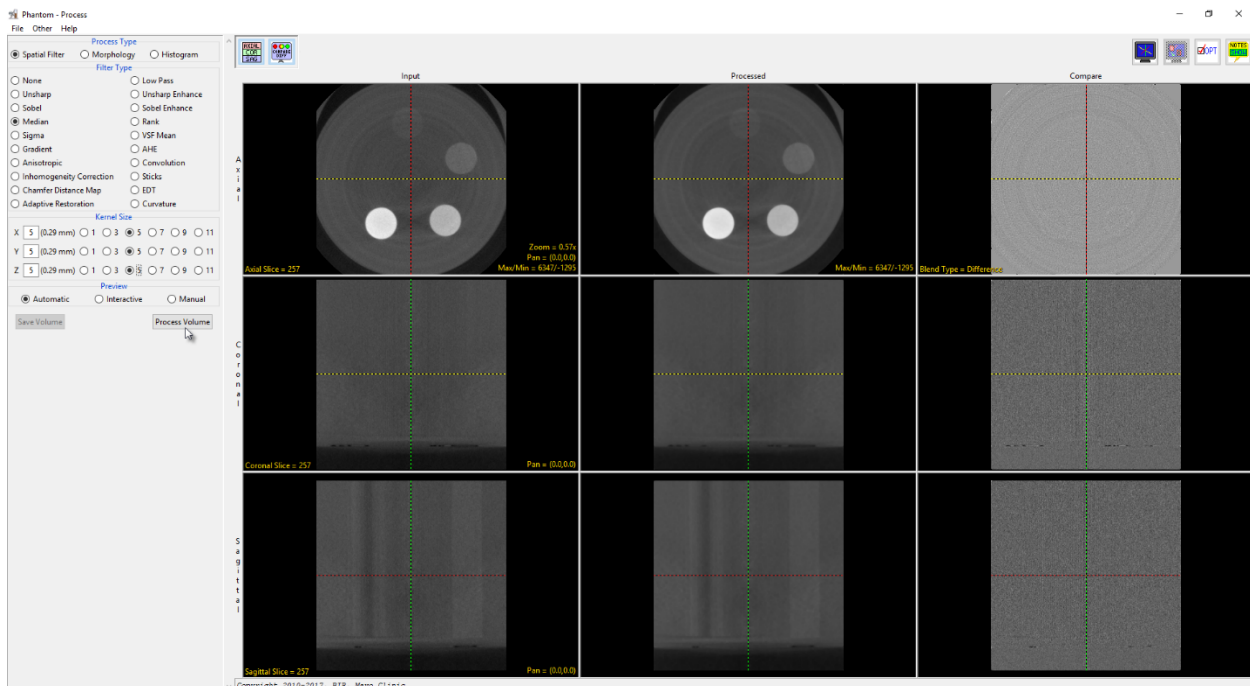

Figure 6.1. Analyze 14.0 Process Module – Image Filtering.

5. Select Process Volume. The filter will be applied to the data set.

6. Once filtering is complete select Save Volume. In the Save Volume window returned choose the 'Create a new workspace volume containing the processed data' option. Note that '_Med' will be appended to the end of the name of the filtered data set. Select Save Volume (Figure 6.2).
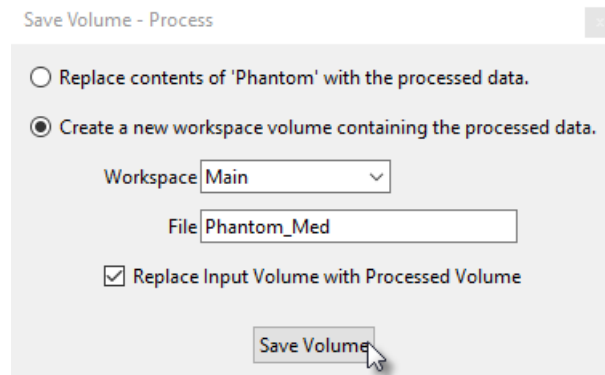

Figure 6.2. Save Volume Window.

7. Close the Process module. From the Analyze workspace, select the filtered BMD phantom data and open Segment.

8. In the Segment module, select the Manual tab and then choose the Fabricate Shape(s) tool (Figure 6.3).

9. Under Object to Create, set the Dimension to 3-D and then set the shape to Cylinder.

10. Using the Radius option, adjust the radius of the displayed circle in the axial orientation to be a little smaller that the size of the phantom insert.

11. Now use the Center X and Center Y options to place the circle over the 0 mg/cm^3 insert.

12. Adjust the Center Z and Length so that the cylinder object spans most of the insert, making sure to avoid the lower and upper ends to the insert.

13. Click Apply.

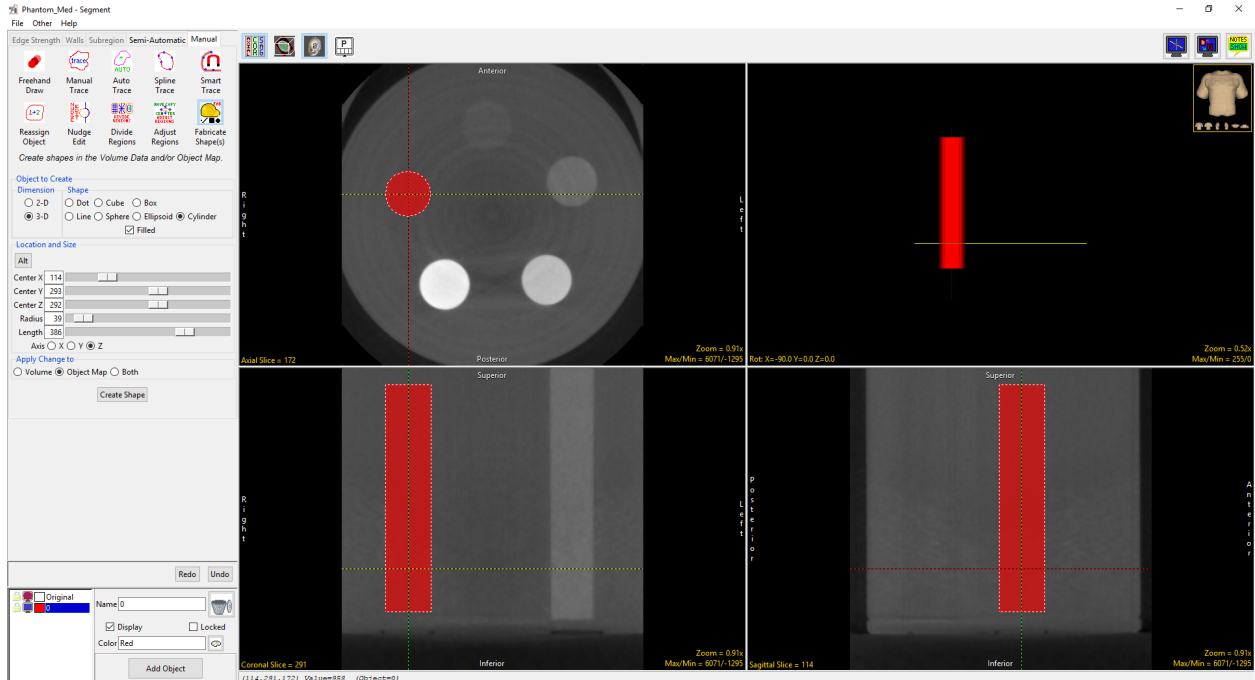14. In the Objects Display panel, change the name of the object to 0.

Figure 6.3. Analyze 14.0 Segment Module.

15. Add 4 additional objects by clicking the Add Object button 4 times. Select the next object and update the name to 50. Drag the circle in the axial view onto the 50 mg/cm^3 insert. Note it may be necessary to adjust the Z center. Click Apply.

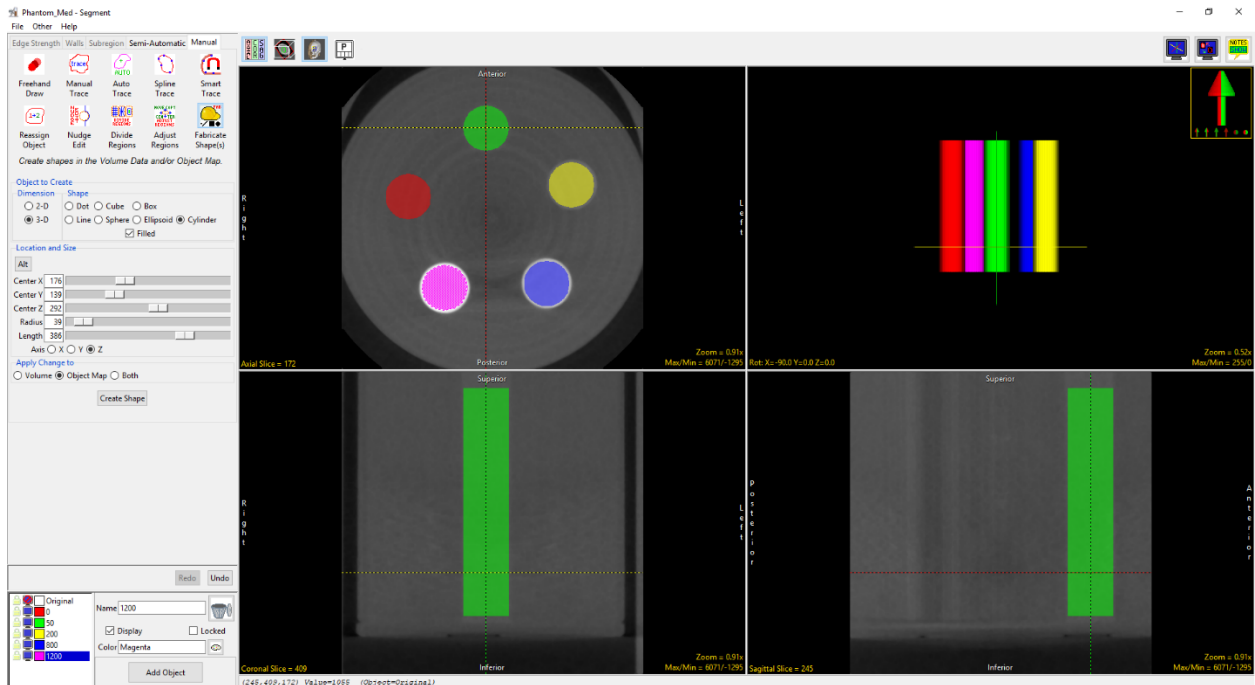16. Repeat for the 200, 800 and 1200 mg/cm^3 inserts (Figure 6.4).

Figure 6.4. Analyze 14.0 Segment Module – Phantom Insert Segmentation.

17. Select File > Save Object Map to save your work. After saving, close Segment.

18. Select the filtered BMD phantom data from the Analyze workspace and open Measure.

19. When Measure opens, the previously saved object map will automatically load. If not, use the File > Load Object Map option to load the Object Map saved in step 17.

20. Once the object map is loaded set the Sample Type option to 3D (Figure 6.5 - A).

21. Next choose the Enabled Objects option directly below (B).

22. Select the Size Intensity tab (C) and uncheck the Volume option (D).

23. Check the Auto Log Stats Option (E).

24. To sample the mean grayscale value of each of the cylinder insert objects, select Sample Enabled Objects (F).
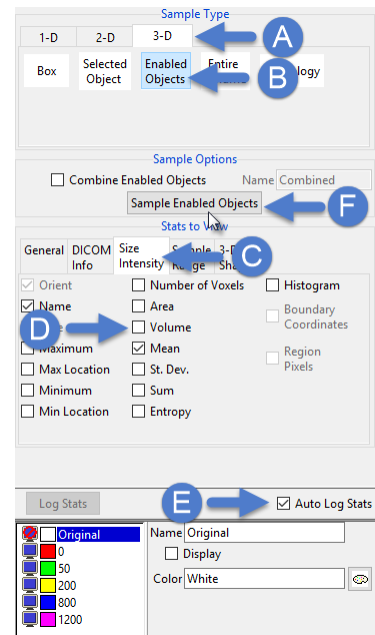
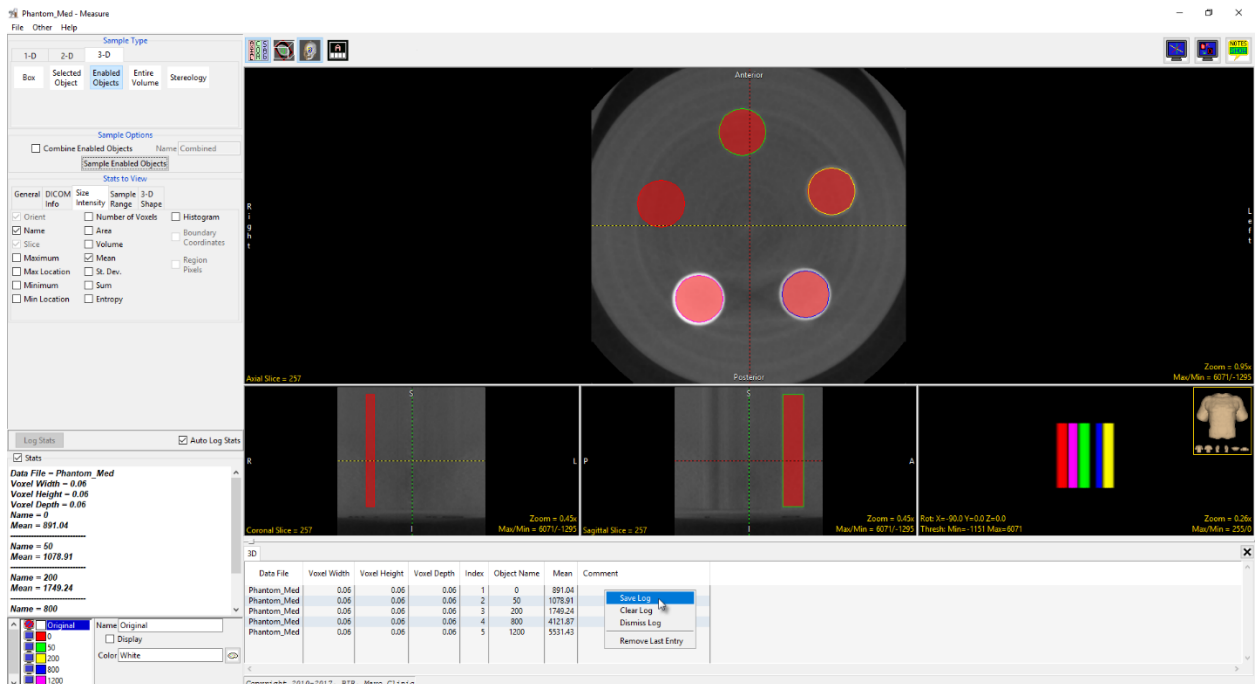*Figure 6.5. Measure – Sample Type Configuration.*

Figure 6.6. Analyze 14.0 Measure Module – Phantom Sampling.

25. The mean greyscale value for each insert will be reported in the log below the display window (Figure 6.6). To save the measurements as a .csv file, right-click in the log and select Save Log.

6.2 Calculation of SigmaCT and BetaCT

1. Open the .csv file with Microsoft Excel.

2. Plot the grayscale value versus mineral density of the phantom.

3. Create a linear regression of mean grayscale value versus phantom mineral density (known concentration of HA in mg/cm^3).

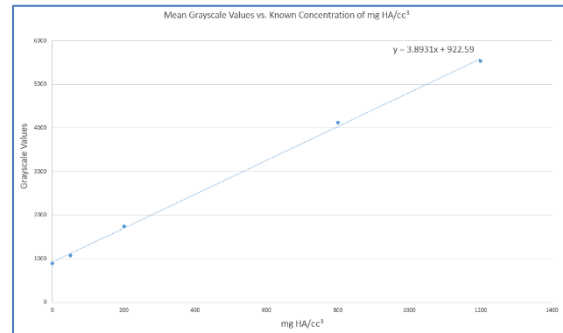4. Record the slope (SigmaCT) and intercept (BetaCT) values.

Figure 6.7. Mean Grayscale vs. Known Concentration of mg HA/CC^3.

6.3 Conversion of CT numbers to Bone Mineral Density

1. Select the bone image data from the Analyze workspace and open the BMA Add-On module

2. To calibrate the image data to BMD, enter the slope (SigmaCT) and offset (BetaCT) values in the BMD Scaling Parameters in the lower left-hand corner of the BMA module interface (Figure 6.8). Note that these values can be entered at any time in the BMA process prior to calculation of bone measurements. For data sets previously sampled and not calibrated to BMD, it is possible to resample the data if BMD calibration parameters were derived after initial sampling, if a final object map was saved.

Figure 6.8. BMA Module Window with BMD Scaling Parameters Populated.