BONE MINERAL DENSITY CALIBRATION

Using Analyze

AnalyzeDirect

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Introduction

Bone mineral density (BMD, mg/cm^3) is an important measure in the evaluation of osteoporosis. The X-ray attenuation coefficients produced in micro-CT are converted to an equivalent BMD value. This conversion is accomplished by using phantoms (liquid K_2HPO_4 or solid resin-embedded hydroxyapatite) of known densities to generate a calibration line[1,2].

Micro-CT provides a non-destructive way of measuring bone mineral density in pre-clinical studies. It has been shown to correlate to the gold standard of ash content measurements when beam-hardening artifacts are minimized[3,4]. The ovariectomized rat is commonly used as an animal model of osteoporosis and osteopenia for preclinical studies[5]. Thormann et al.[6] quantified ossified tissue in the femoral fracture gap of ovariectomized (OVX) and control (SHAM) rats. Decreased BMD has also been observed in ovariectomized sheep[7] and goats[8].

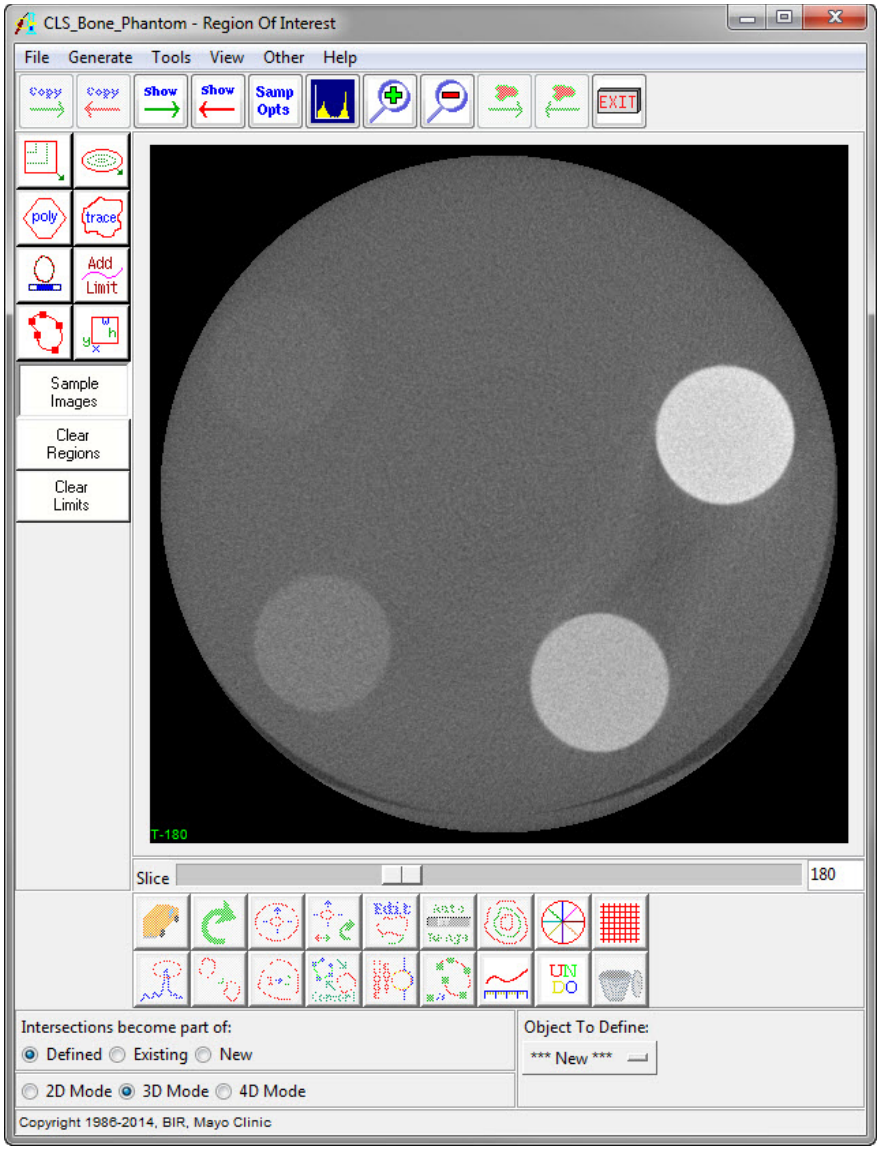
This guide will show how to calibrate micro-CT data to bone mineral density (BMD) values, segment mineralized bone from control and OVX data, and measure bone volume and mean BMD for a volume of interest in Analyze. Sample phantom, OVX, and control data can be downloaded from http://analyzedirect.com/data/.

BMD Calibration

The calibration of bone data to BMD values using a phantom comprises three steps. First, the scan of the phantom inserts is sampled at each phantom density to determine the mean grayscale intensity value of each phantom insert. Next, these grayscale intensity values are graphed against the known phantom densities to form a calibration curve. Last, the bone data is calibrated using the slope and intercept of the linear calibration curve. The phantom should be scanned using the same protocol as was used for the bone data.

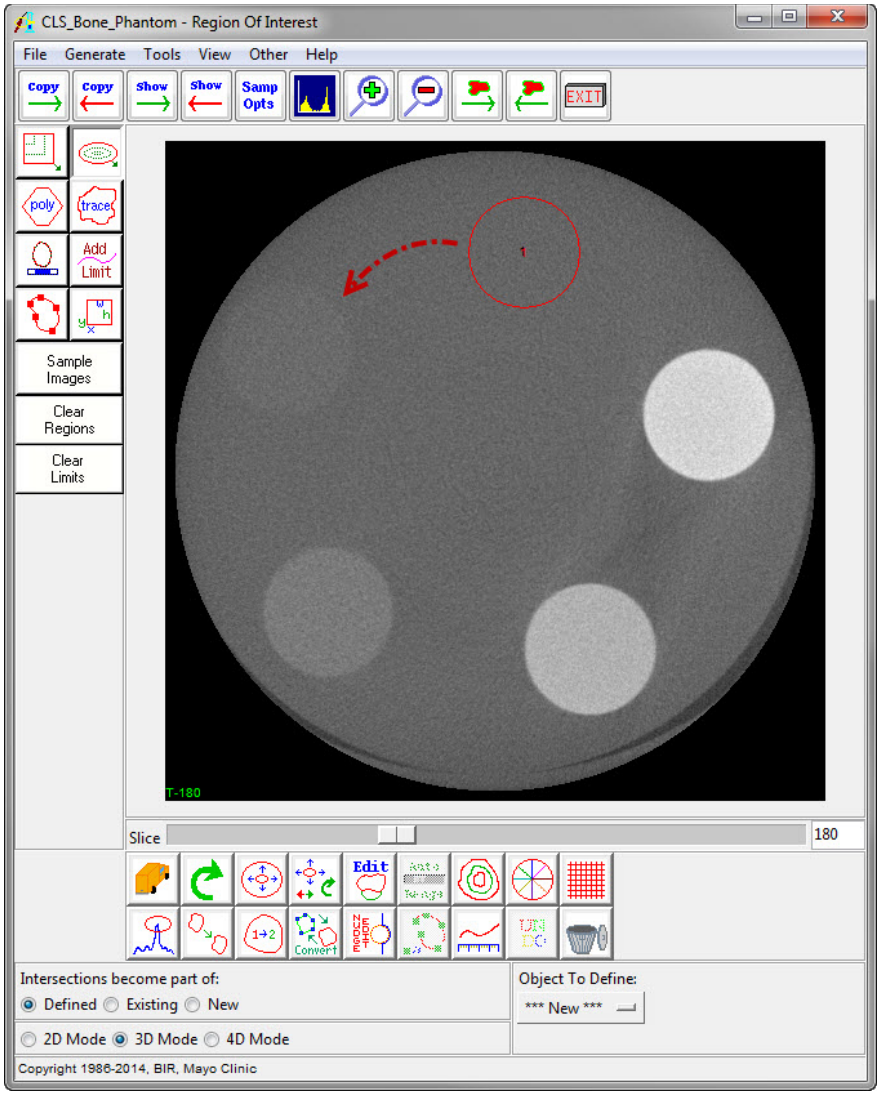
Sampling of Phantom Densities

Load the data set containing the phantom inserts into Analyze using the Load module (**File > Load**). Select the phantom data set in the Analyze workspace and open the Region of Interest module (**Measure > Region of Interest**).



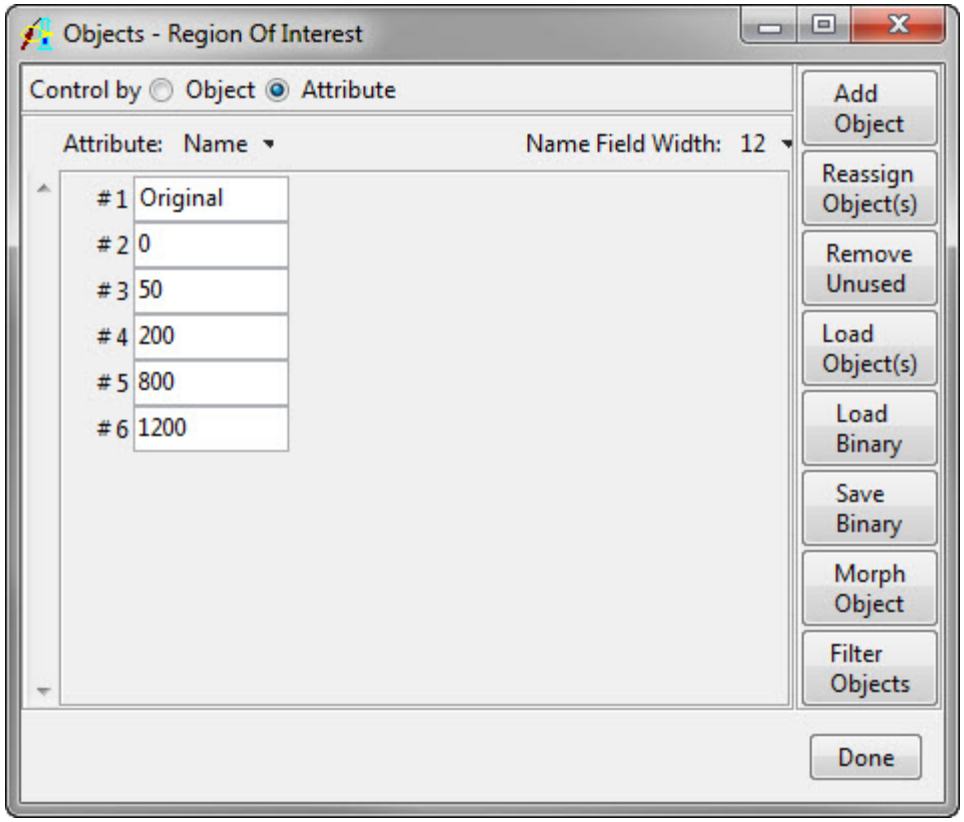
In order to sample the mean density of each phantom insert, we will need to create an object map. Using the slice slider bar, navigate to the first slice where the phantom inserts appear. From this slice, move forward several slices in case of some artifact at the end of the phantom. Note that for this phantom, a region of zero bone mineral density (0 mg HA / cm^3) will be defined where there is no insert.

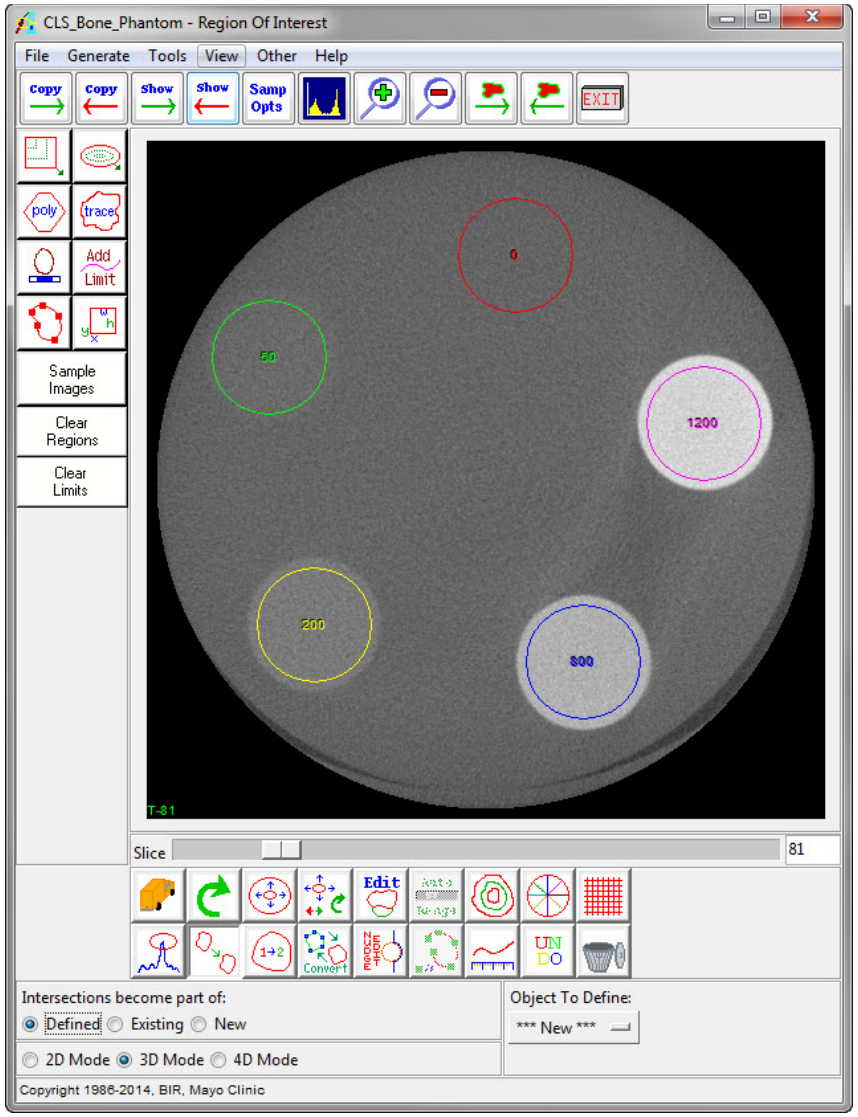
Using the oval draw tool, hold down the Ctrl key, click in the center of the region shown in the figure and drag outward to create a circular object of a size that would encompass most of an insert but not go all the way to the edges. It is easiest to first define the region of negligible bone density, and then define subsequent regions in order of increasing density in a counterclockwise direction, as shown by the red arrow.



To define the other regions, set Object to Define to *** New ***.

Use the Copy Region tool to copy the circular region to the other phantom inserts. To use this tool, click in the first object and drag to place a circle on the next insert. Once all inserts have been defined by circular regions, rename the objects from the objects window (**View > Objects**). Choose to Control by Attribute and change Attribute to Name. Then rename each object with its known density.




CLS_Bone_Phantom - Region Of Interest
File Generate Tools View Other Help
Copy
Copy
Show
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Samp Opts
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Sample Images
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Intersections become part of:
Defined
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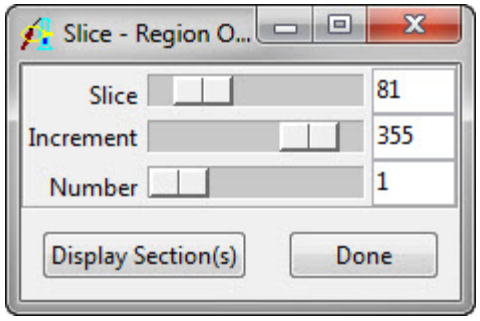
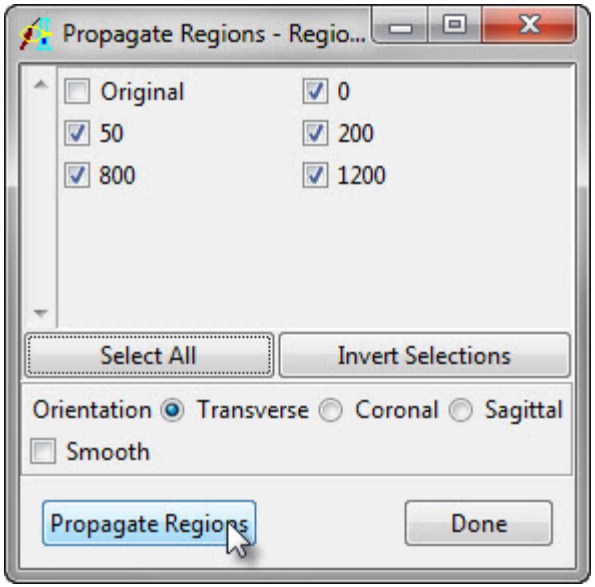
Object To Define:
*** New ***
2D Mode
3D Mode
4D Mode
Copyright 1986-2014, BIR, Mayo Clinic

Next we need to extend these regions throughout the length of the inserts. This can be done using the Propagate Regions tool. To determine the last slice where the objects will be defined, navigate to the last slice where the phantom inserts appear and move back several slices. To determine the increment, subtract the slice number where the objects are first defined from the number of the last slice where the objects will be defined. Open the Slice window (**Generate > Slice**) and set Slice to the first slice where the objects are defined. Set Increment to the value just determined from the subtraction step.

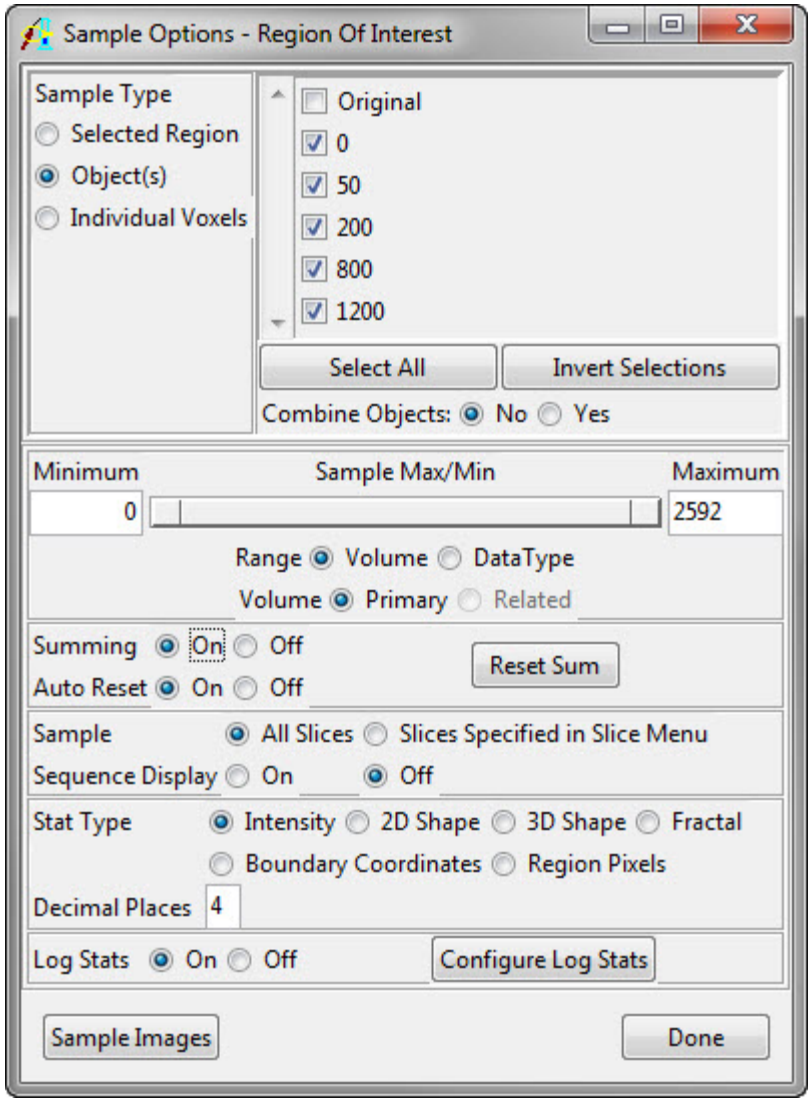


Click the Copy Regions Forward button to copy the regions to the chosen slice. Now the regions are defined on the first and last slice, and the Propagate Regions tool will connect those regions throughout the slices in between. Open the Propagate Regions tool (**Tools > Propagate Regions**). Click Select All to select all the objects, and then click the Propagate Regions button. Use the slice slider to check that the regions have correctly been propagated through the inserts. Save the object map (**File > Save Object Map**).

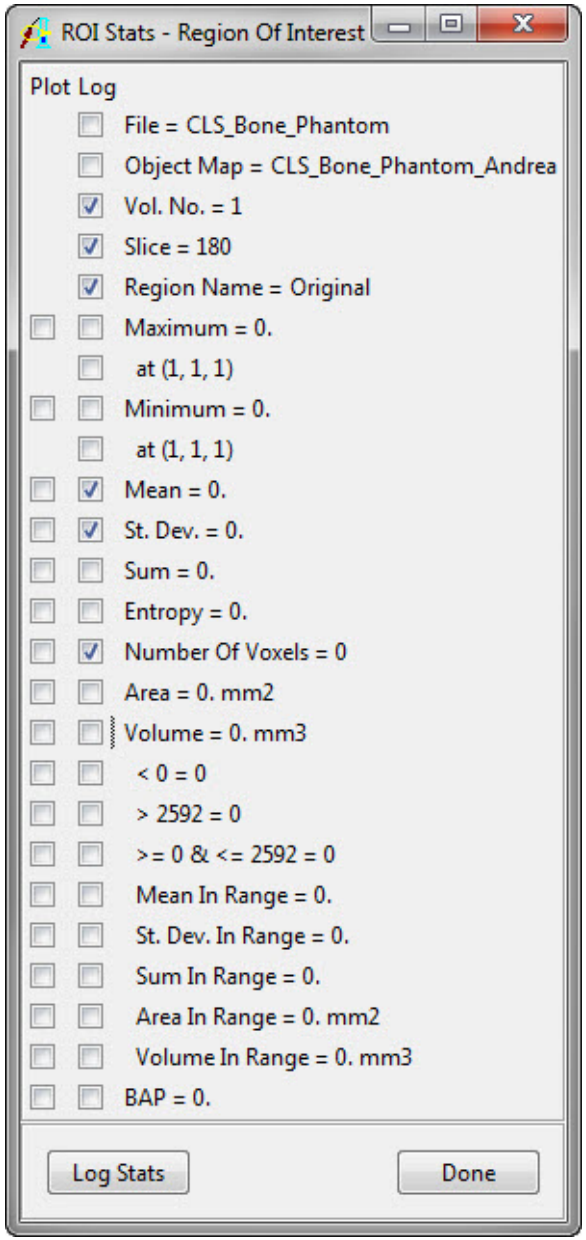


The next step is to sample the phantom objects to determine the mean grayscale intensity value of each. Open the Sample Options window (**Generate > Sample Options**) and set the following parameters:

- **Sample Type** to Object(s)
- Select All Objects **except** Original
- **Summing** to **On**
- **Sample** to **All Slices**
- **Sequence Display** to **Off**
- **Decimal Places** to **4**
- **Log Stats** to **On**

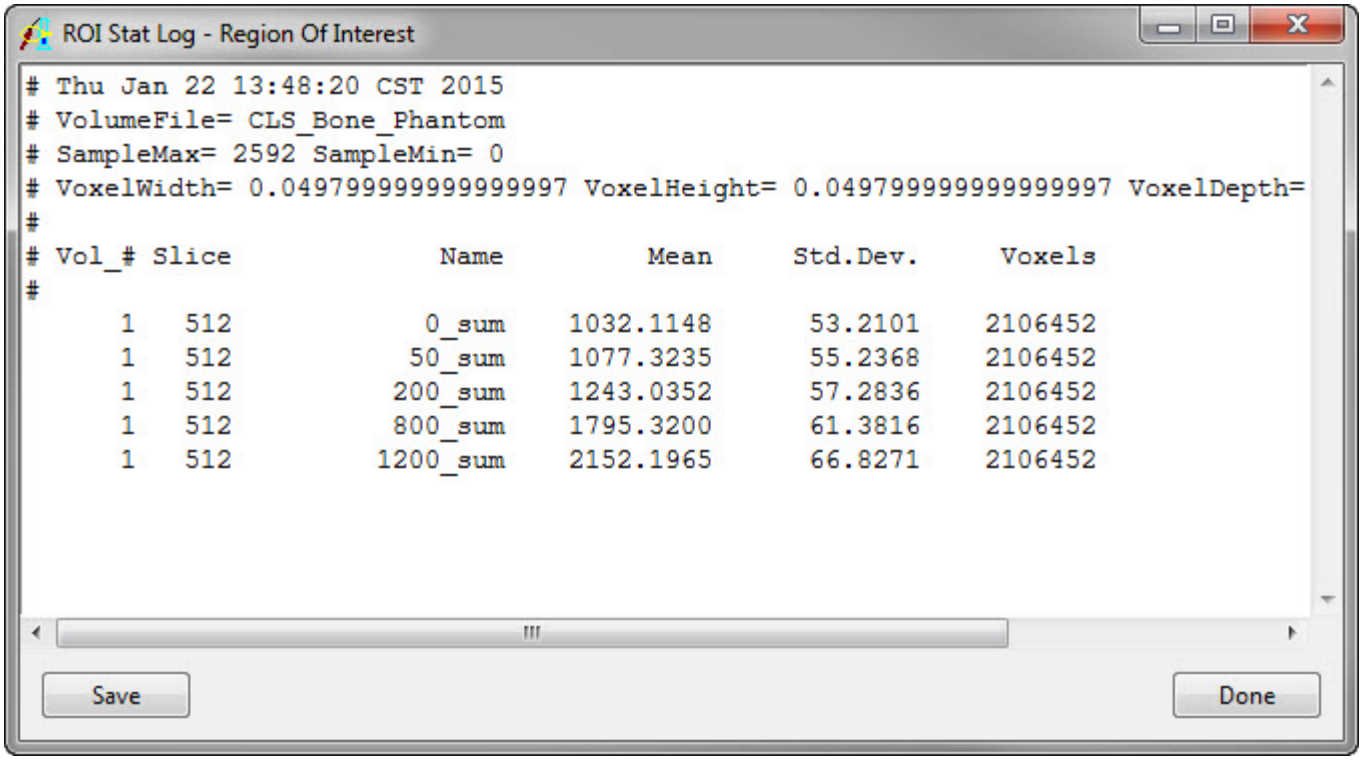


Click the Configure Log Stats button, and deselect Area and Volume, as the mean intensity value is the only measurement of interest. Click Done to close the ROI Stats window.



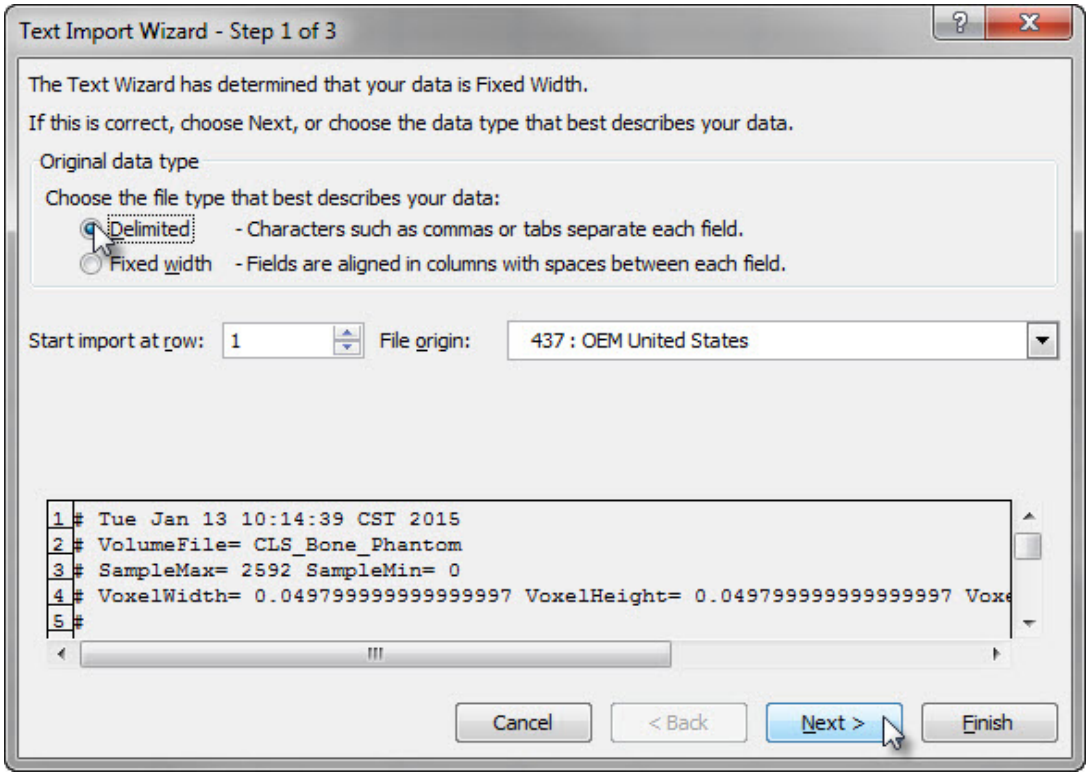
In the Sample Options window, click Sample Images to begin the measurement. The results will appear in the ROI Stat Log window.

Click the Save button, or right-click and select Save Log or Save Log As to save the log as a .stats file.

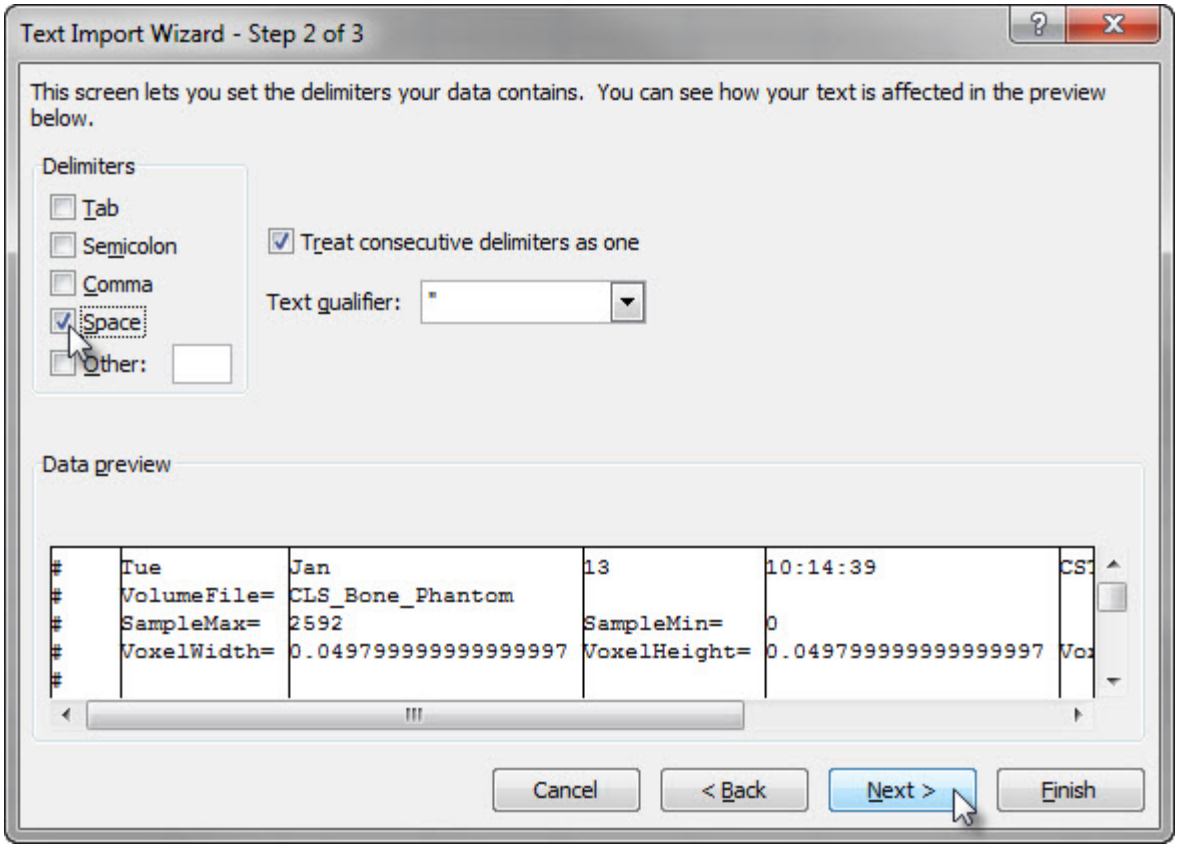


Determination of Calibration Curve

Now that we have the mean intensity value for each phantom of known density, we can generate a calibration curve. Open Microsoft Excel or another spreadsheet software and import the .stats file saved in the previous step as a space-delimited file. In Excel, go to **File > Open**, and set the drop-down menu to All Files instead of All Excel Files. Open the .stats file, and the Text Import Wizard will be launched. In the wizard, set the data type to Delimited and click Next.

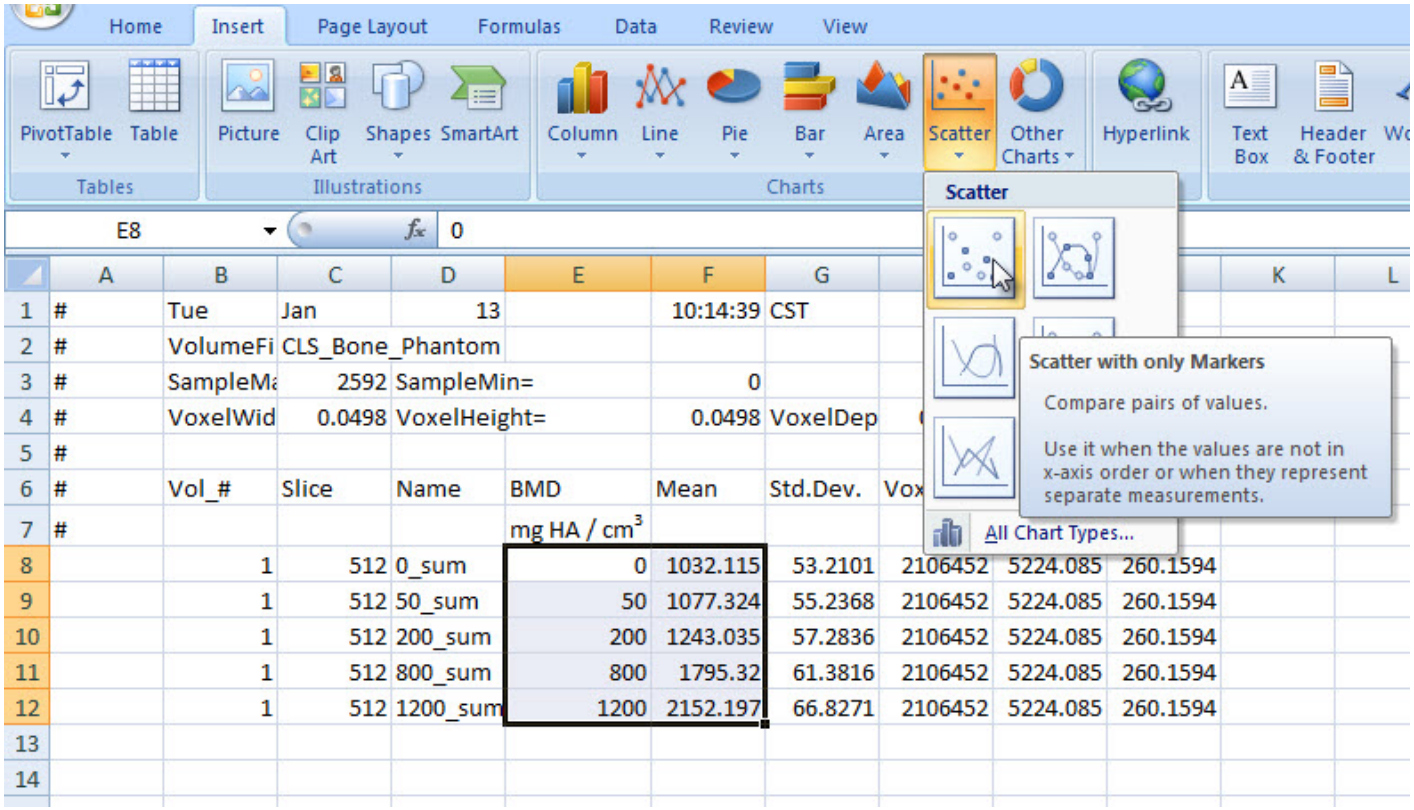


In Step 2 of the Text Import Wizard, select Space as the only delimiter, then click Next.

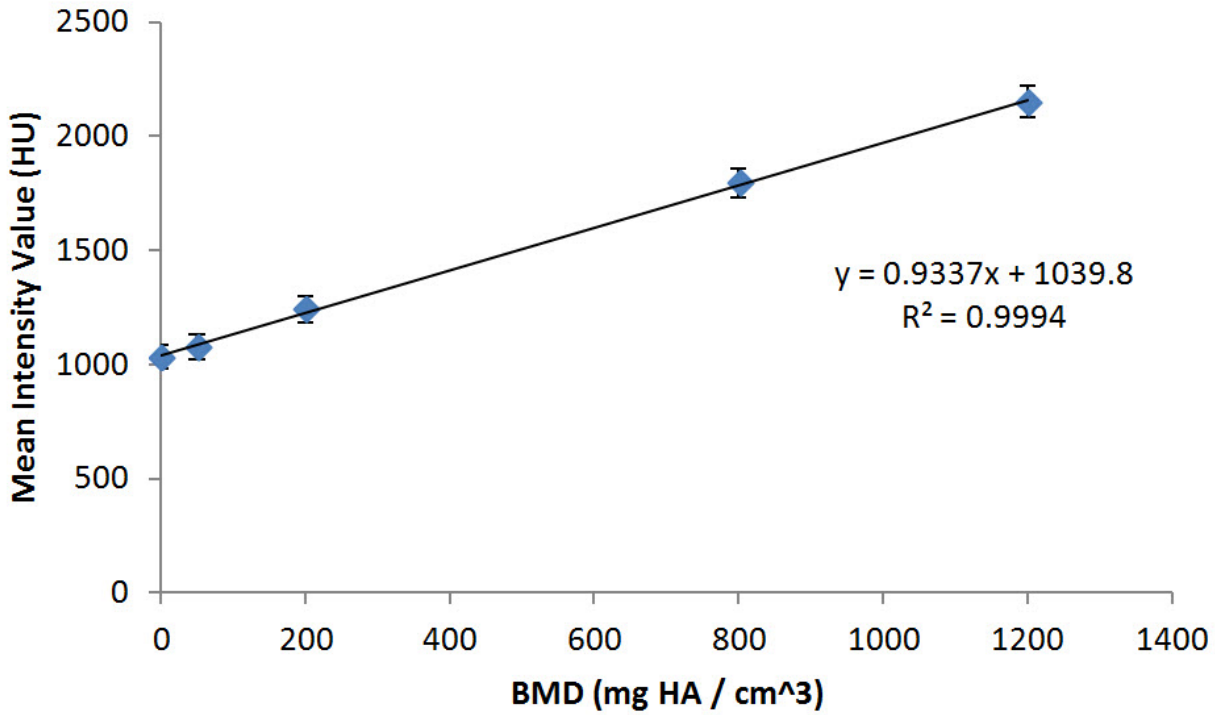


In Step 3 of the Text Import Wizard, click Finish to import the .stats file.

Next we will generate the calibration curve. Right-click on the column containing the mean and insert a new column. Name the new column BMD and label the units mg HA / cm^3. Enter the known insert densities into this column. Select the values in the BMD and Mean columns and create a scatter plot with only markers (**Insert > Charts > Scatter > Scatter with only Markers**).



Right-click on the data series and select Add Trendline. In the Format Trendline window, check the boxes for Display Equation on chart and Display R-squared value on chart. The default trendline is linear, so the regression type does not have to be changed. The error bars in the calibration curve shown here represent the standard deviation of the mean intensity value of each phantom insert, as returned by the measurement algorithm.

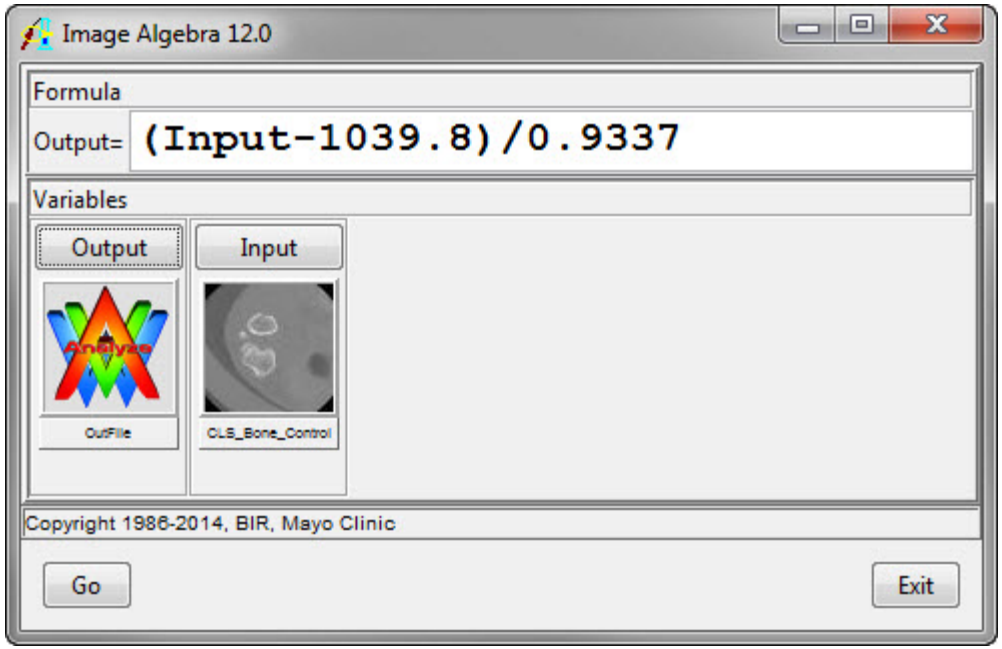


Calibration of Bone Data

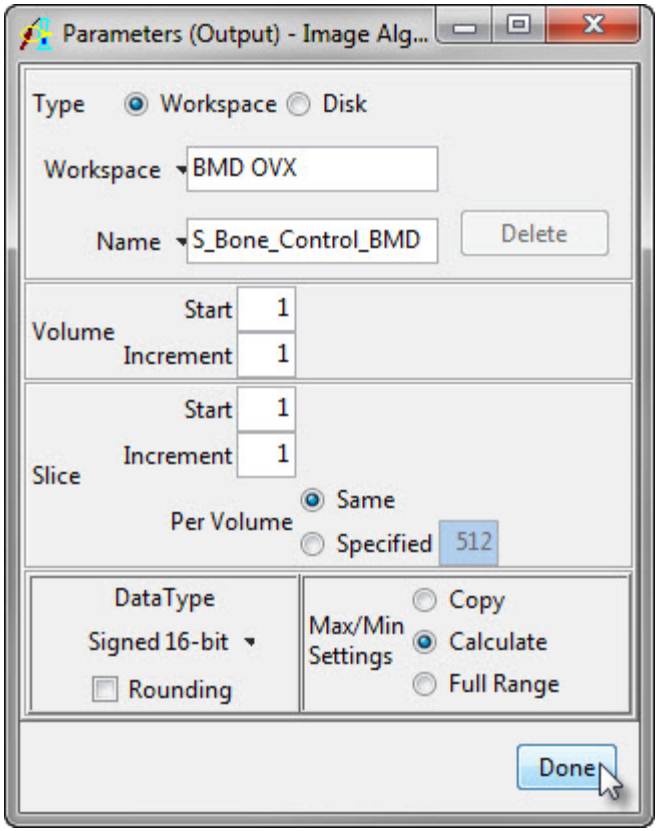
Note that the bone data consists of grayscale intensity values in Hounsfield units (HU), which are represented by the y-values in the linear calibration curve. In order to convert to BMD values, we will need to solve for x in the regression equation. Doing so results in the following:

$$BMD = \frac{Input - Intercept}{Slope}$$

This equation will be applied to the bone data using the Image Algebra module. Open the Image Algebra module **(Process > Image Algebra)** and type the equation for BMD into the Output= box. Drag and drop the control bone data set from the Analyze workspace into the input port.

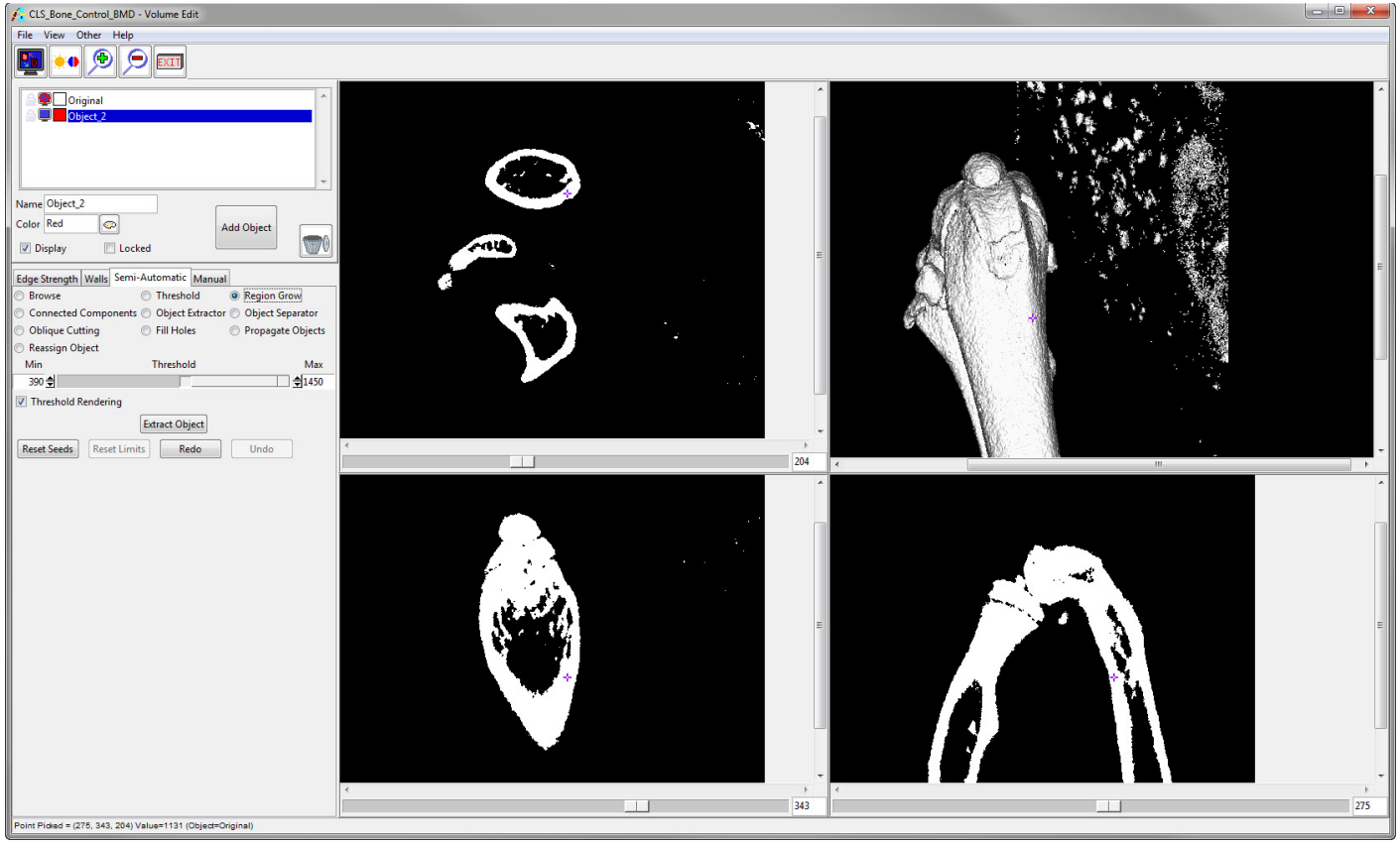


Click the Output button and name the Output data set. Change the Data Type to Signed 16-bit.



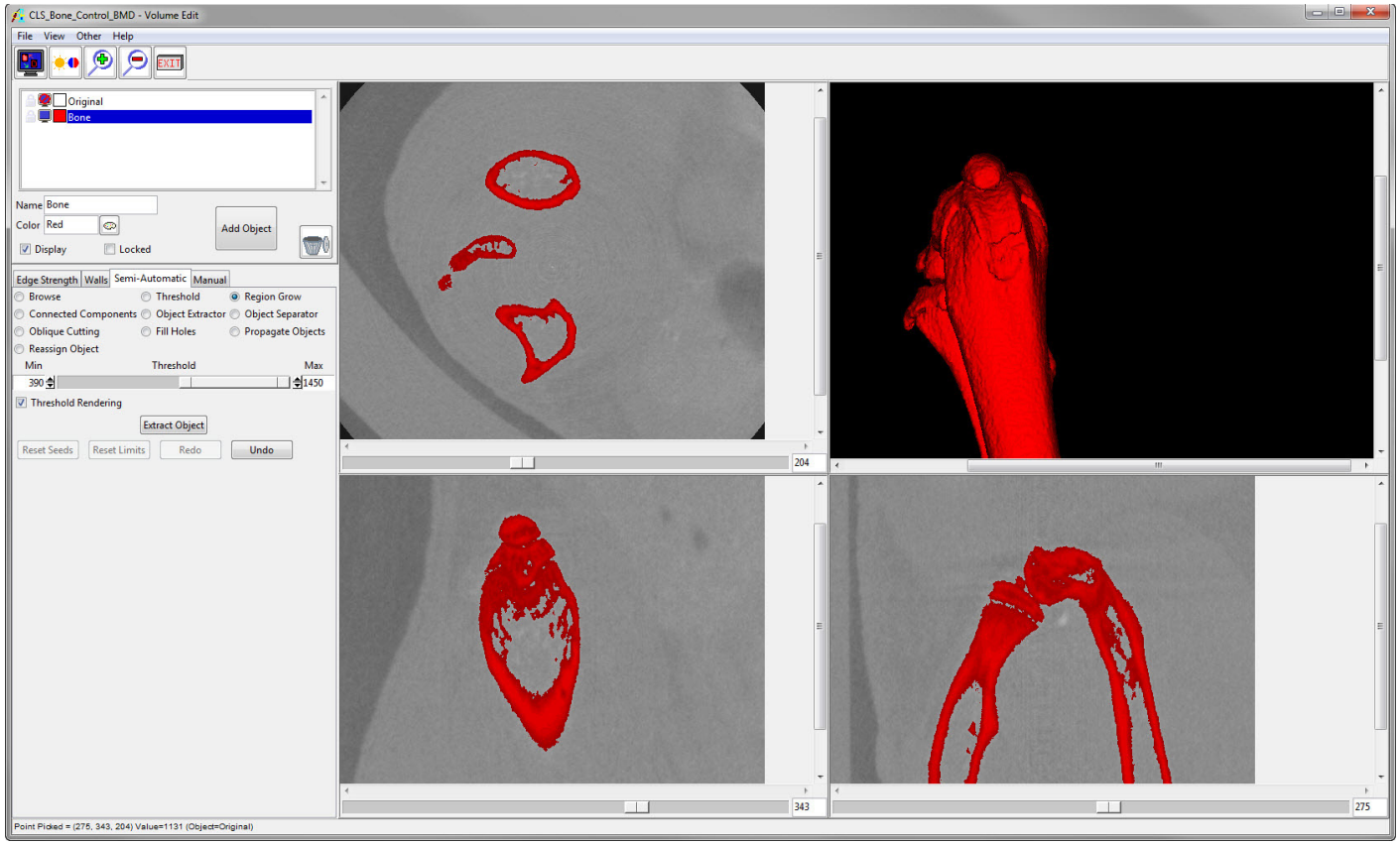
Click the Go button in the Image Algebra module to begin the processing step. To calibrate the OVX bone data set, select the data set and drag and drop it from the Analyze workspace into the Input port of the Image Algebra module. Click the Output button to name this output data set. Leave the Data Type as Signed 16-bit, and click Go in the Image Algebra module to process the new data set. Once processing of all bone data is complete, click Exit to close the Image Algebra module.

Bone Segmentation



Select the control bone data set that has been calibrated to BMD and open the Volume Edit module (**Segment > Volume Edit**). Select the Semi-Automatic tab and choose the Region Grow radio button. Move to a transverse slice where the cortical bone is well-defined, and click in the cortical bone to set a seed point. Adjust the threshold range so that it defines the bone. The maximum threshold value should be set to the highest possible value, and the minimum threshold value should be set by sliding the lower part of the threshold bar until the binary image shown describes the bone. If there is noise due to artifacts that are not spatially connected to the bone, it will not be extracted by the region grow algorithm and can be included in this binary image used to select the segmentation threshold range. Make a note of the threshold minimum chosen, as this value will be applied to subsequent data sets.

Click the Extract Object button to run the Region Grow algorithm and extract the bone.



Object_2 can be renamed by clicking in the Name box and typing a new name. When you are satisfied with the segmentation, save the object map (**File > Save Object Map**) and close the Volume Edit module.

To segment the OVX bone data set, repeat the segmentation steps from this section, using the same threshold range as for the control data set.

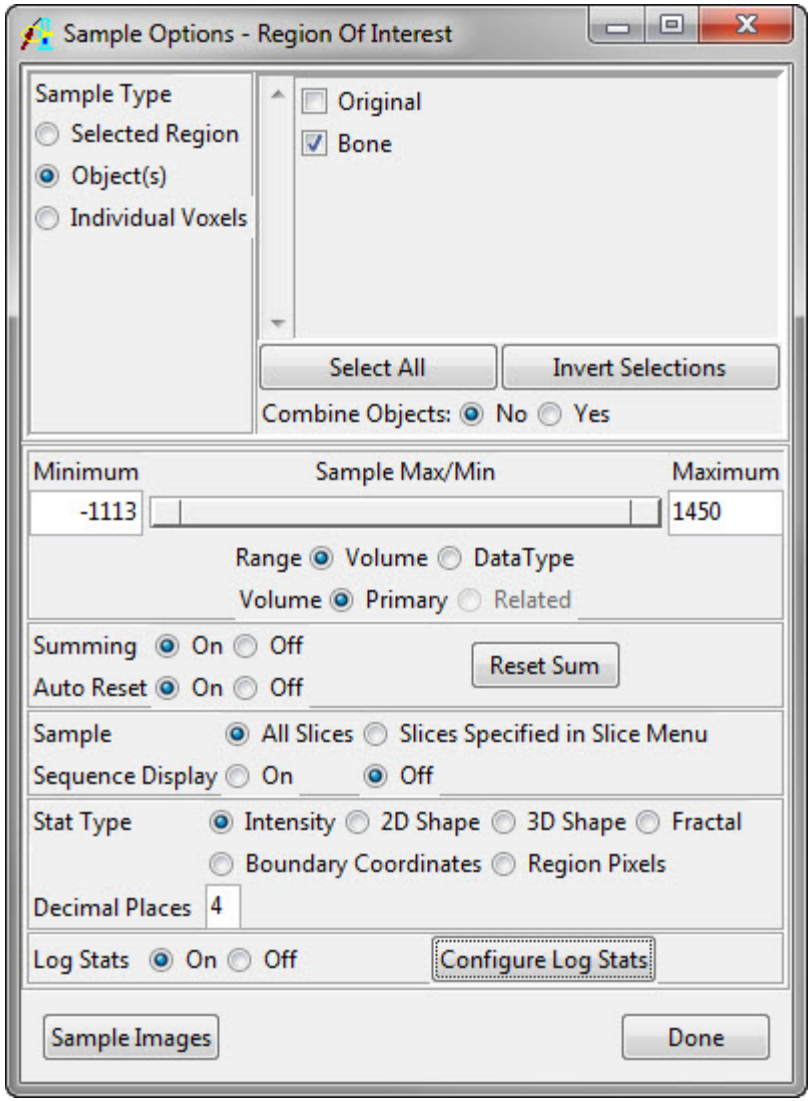
Bone Volume and BMD Measurement

The last step consists of measuring the volume of bone and the mean BMD. This is done in the Region of Interest module. Select the control BMD-calibrated bone data set in the Analyze workspace and open the Region of Interest module (**Measure > Region of Interest**). Load the object map from Volume Edit in the segmentation step.

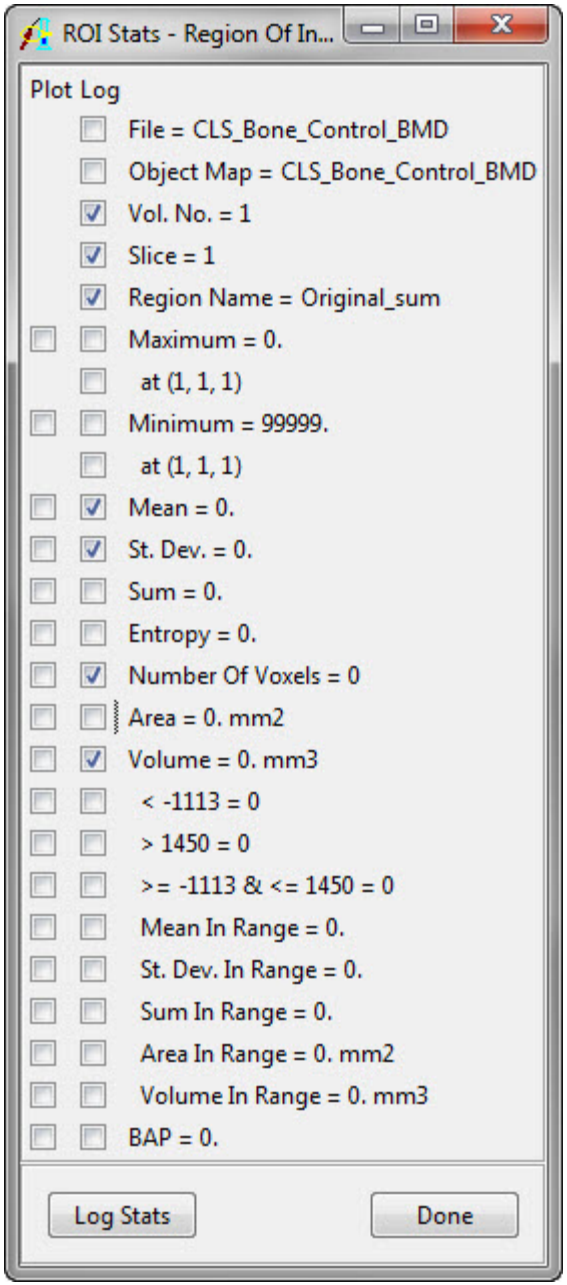
Note: If the object map was saved in the same folder as the corresponding data set and with the same name, it will load automatically.

Open the Sample Options window to set the measurement parameters (**Generate > Sample Options**).

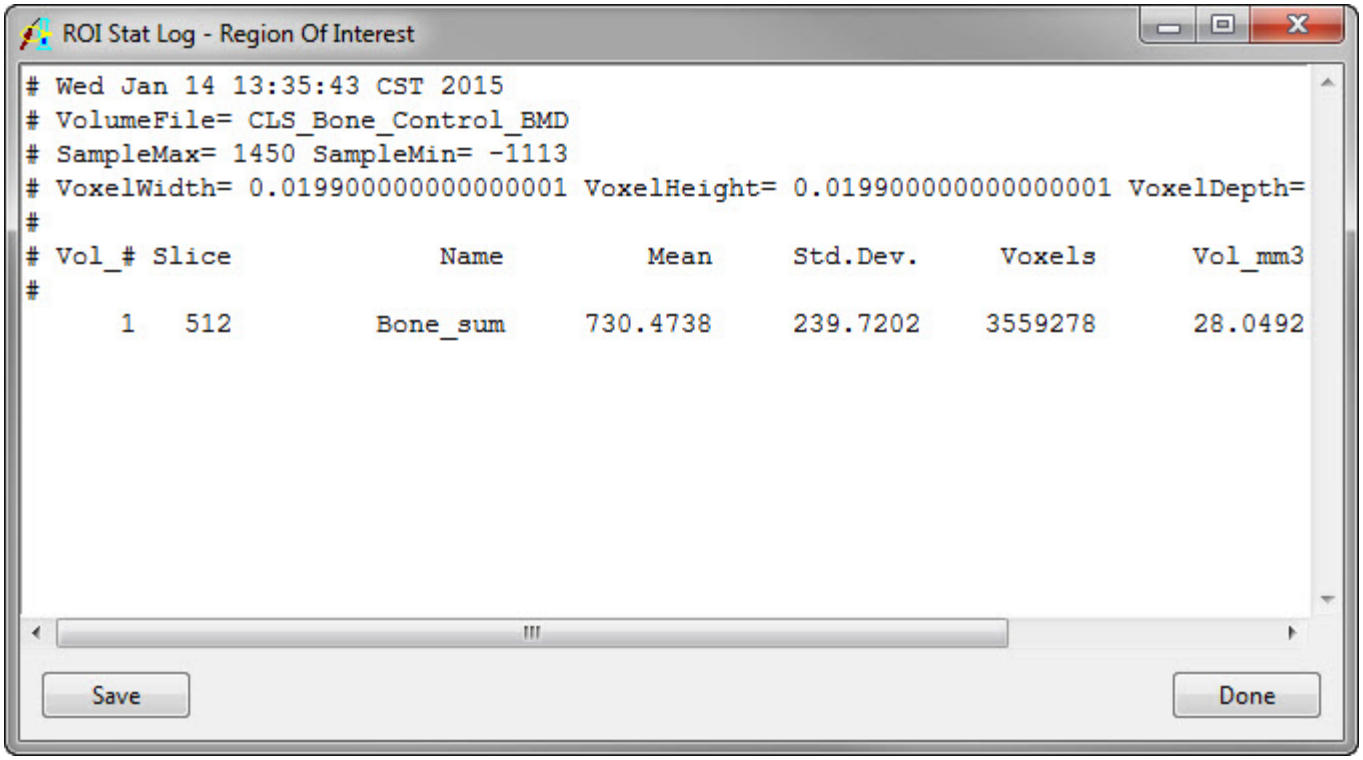
- **Sample Type** to Object(s)
- Select **only** the Bone Object
- **Summing** to **On**
- **Sample** to **All Slices**
- **Sequence Display** to **Off**
- **Decimal Places** to **4**
- **Log Stats** to **On**



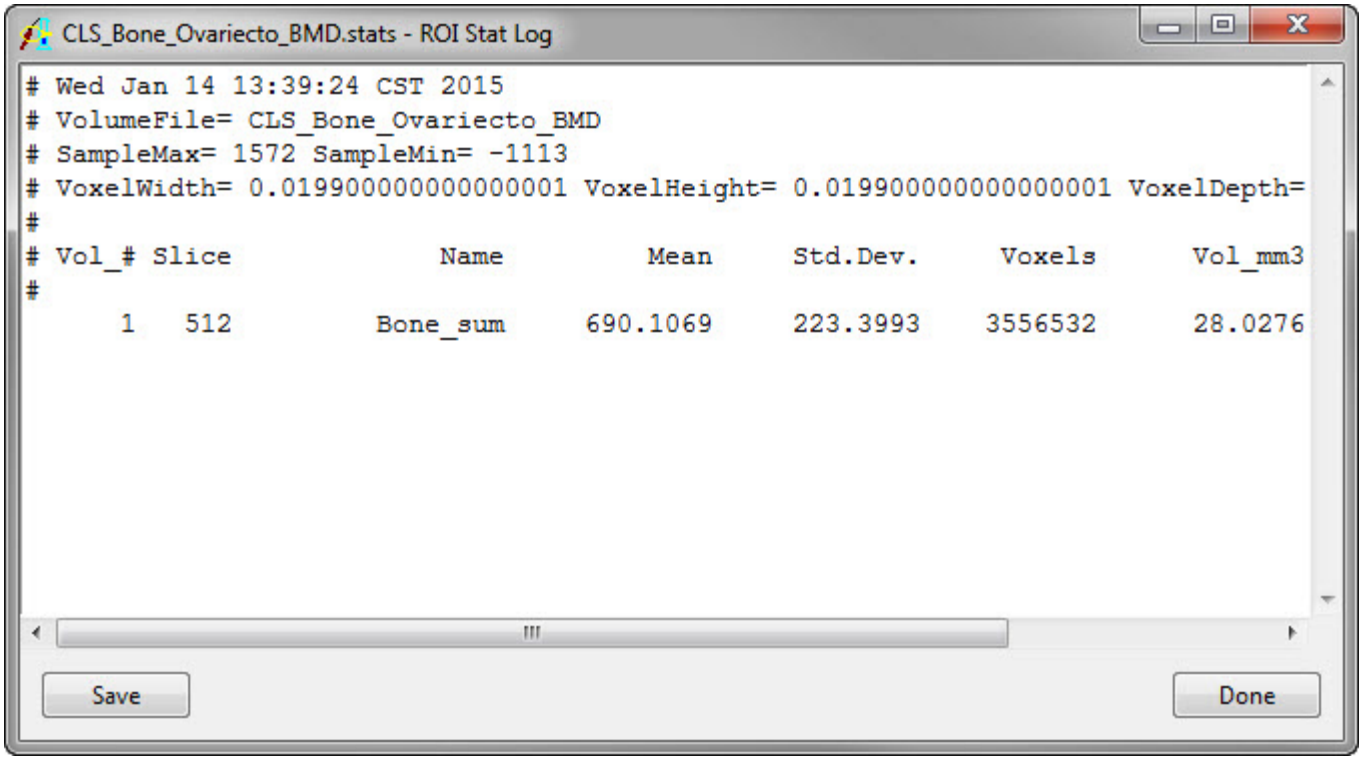
Click the Configure Log Stats button and uncheck the Area measurement, then click Done to close the ROI Stats window.



In the Sample Options window, click the Sample Images button to initiate the measurement algorithm. The results will appear in the ROI Stat Log window and can be saved to disk as a .stats file by right-clicking in the window and choosing Save Log or Save Log As, or by clicking the Save button.



Repeat the measurement steps for the OVX bone data set.



Note that the Mean value represents the mean BMD of the bone object, as the data set was calibrated to BMD. The results for the single OVX and control data sets measured here are a mean BMD of 690 mg/cm^3 for the OVX condition and a mean BMD of 730 mg/cm^3 for the control. The bone volume has not changed significantly. The .stats files generated from the Region of Interest module can be imported into Excel or other spreadsheet software as space-delimited files, as shown in the BMD Calibration section of this guide.

If you wish to make more complex measurements, such as separately measuring the BMD of trabecular and cortical bone, you may be interested in the Bone Microarchitecture Analysis Add-On. This Add-On is capable of segmenting cortical and trabecular bone and measuring common bone morphometric indices. Beyond BMD, the Bone Microarchitecture Analysis Add-On measures bone volume fraction, bone surface density, connectivity density, structure model index, trabecular number, thickness, and separation, second moments of inertia, various measurements of cortical porosity and more.

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8. Siu, W. S., Qin, L., Cheung, W. H. & Leung, K. S. A study of trabecular bones in ovariectomized goats with micro-computed tomography and peripheral quantitative computed tomography. *Bone* **35**, 21–6 (2004).

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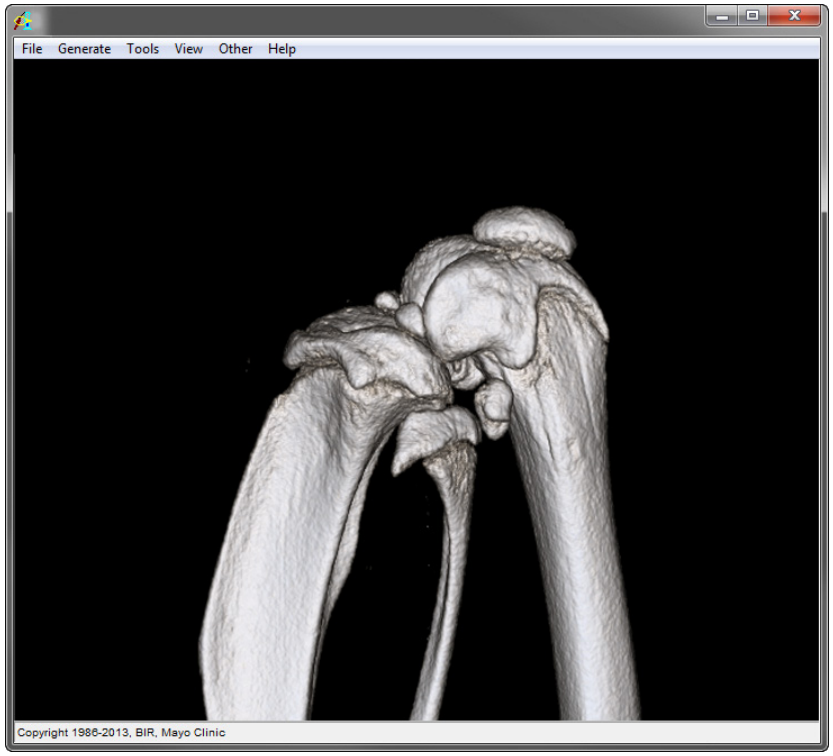

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