BONE MINERAL DENSITY CALIBRATION

Using Analyze









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Introduction

Bone mineral density (BMD, mg/cm³) 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_2 HPO₄ or solid resinembedded 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 studies⁵. 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 sheep⁷ and goats⁸.

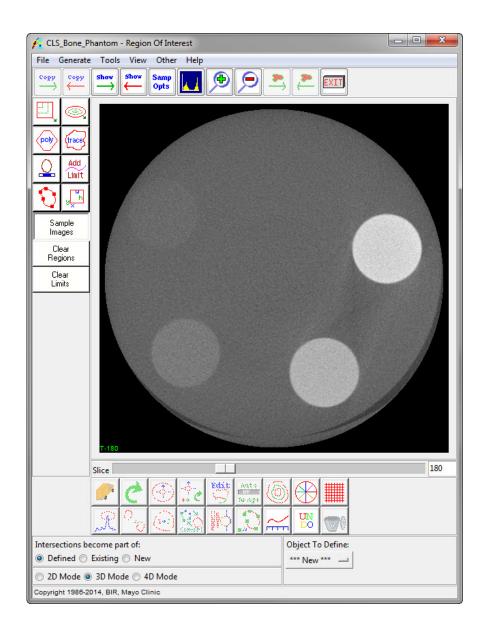
This guide will show how to calibrate micro-CT data to bone mineral density (BMD) values, segment mineralized bone from control and OVX data, and measure bone volume and mean BMD for a volume of interest in Analyze. Sample phantom, OVX, and control data can be downloaded from http://analyzedirect.com/data/.

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.

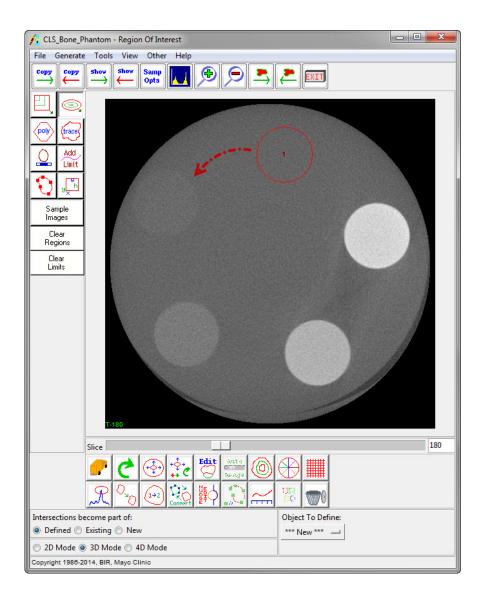
Sampling of Phantom Densities

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 / cm³) will be defined where there is no insert.

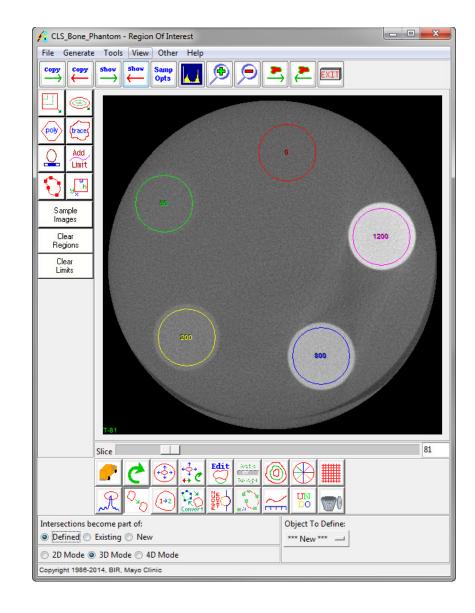
Using the oval draw tool, hold down the Ctrl key, click in the center of the region shown in the figure and drag outward to create a circular object of a size that would encompass most of an insert but not go all the way to the edges. It is easiest to first define the region of negligible bone density,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

Control by 🔘 Object 💿 Attribute	2	Add Object				
Attribute: Name 🔹 Name Field Width: 12 🔹						
#1 Original		Reassign Object(s)				
# 2 0		Remove				
# 3 50		Unused				
# 4 200		Load				
# 5 800		Object(s)				
#6 1200		Load Binary				
		Save Binary				
		Morph Object				
-		Filter Objects				
		Done				



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.

Slice		81
Increment		355
Number		1

Cick 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**).

A	Propagate Regions -	Regio					
^	Original	V 0					
	▼ 50	200					
	800	1200					
•	Select All	Invert Selections					
0	Orientation Transverse Coronal Sagittal Smooth						
	Propagate Regions	Done					

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

🕂 Sample Options -	Region Of Interest	• X					
Sample Type Selected Region Object(s) Individual Voxels	 ◇ Original ◇ 0 ◇ 50 ◇ 200 ◇ 800 ◇ 1200 						
	Select All Invert Se	lections					
	Combine Objects: 💿 No 🔘 Yes						
Minimum	Sample Max/Min	Maximum					
0	0 2592						
Ra	Range 🖲 Volume 🔘 DataType						
V	olume 💿 Primary 🔘 Related						
Summing () On () Auto Reset () On ()	Reset Sum						
Sample ()	All Slices Slices Specified in Slice	Menu					
Sequence Display 🔘	On Off						
Stat Type 💿 I	ntensity 🔘 2D Shape 🔘 3D Shape (Fractal					
© E	Boundary Coordinates 🔘 Region Pixe	els					
Decimal Places 4							
Log Stats 💿 On 🔘	Off Configure Log Stats						
Sample Images		Done					

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.

🔏 ROI S	itats - Region Of Interest 😐 💷 🗙							
Plot Log								
File = CLS_Bone_Phantom								
Object Map = CLS_Bone_Phantom_Andre								
	Vol. No. = 1							
	Slice = 180							
	Region Name = Original							
	Maximum = 0.							
	at (1, 1, 1)							
	Minimum = 0.							
	at (1, 1, 1)							
	Mean = 0.							
	St. Dev. = 0.							
	Sum = 0.							
	Entropy = 0.							
	Number Of Voxels = 0							
	Area = 0. mm2							
	Volume = 0. mm3							
	< 0 = 0							
	> 2592 = 0							
	>= 0 & <= 2592 = 0							
	Mean In Range = 0.							
	St. Dev. In Range = 0.							
	Sum In Range = 0.							
	Area In Range = 0. mm2							
	Volume In Range = 0. mm3							
	BAP = 0.							
Log	Stats Done							

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 rightclick and select Save Log or Save Log As to save the log as a .stats file.

				.3:48:20 CST 2015				
				CLS_Bone_Phantom				
	-			2592 SampleMin= 0				
1	Voxe	lW	idth=	0.049799999999999999	<pre>VoxelHeight</pre>	= 0.04979999	99999999997	VoxelDepth=
ŧ								
1	Vol	#	Slice	Name	Mean	Std.Dev.	Voxels	
ŧ	-							
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		1	512	50 sum	1077.3235	55.2368	2106452	
		1	512	200 sum	1243.0352	57.2836	2106452	
		1	512		1795.3200	61.3816	2106452	
		1		1200 sum				
		-						
. [-							
1		_						r
Г	C -	ve						Done

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 dropdown 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.

Text Import Wizard - Step 1 of 3	? X
The Text Wizard has determined that your data is Fixed Width.	
If this is correct, choose Next, or choose the data type that best describes your data.	
Original data type	
Choose the file type that best describes your data:	
 Delimited - Characters such as commas or tabs separate each field. 	
Fixed width - Fields are aligned in columns with spaces between each field.	
Start import at <u>r</u> ow: 1 File <u>o</u> rigin: 437 : OEM United States	•
1 # Tue Jan 13 10:14:39 CST 2015 2 # VolumeFile= CLS_Bone_Phantom 3 # SampleMax= 2592 SampleMin= 0 4 # VoxelWidth= 0.04979999999999997 VoxelHeight= 0.049799999999999 5 #	9997 Voxe
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Cancel < Back <u>Next > </u>	Einish

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

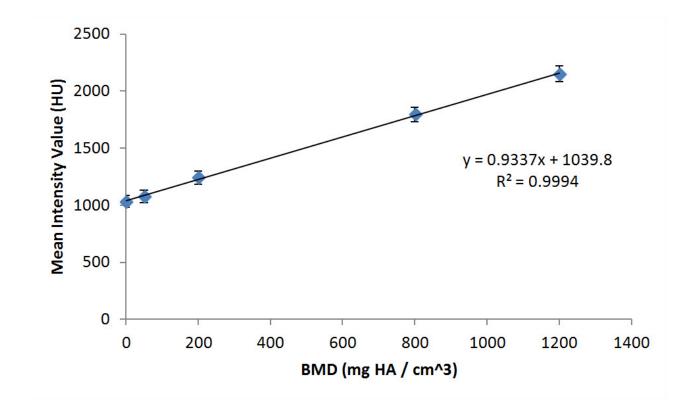
Text Impo	ort Wizard - Ste	p 2 of 3			? X			
This scree below.	en lets you set th	e delimiters your data contains	. You can see how	your text is affected in t	ne preview			
Delimiter	-							
E <u>T</u> ab] Treat consecutive delimiters a	as one					
	Comma Text gualifier:							
Spa Dth								
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ŧ	Tue	Jan	13	10:14:39	cst 🔺			
ŧ		CLS_Bone_Phantom	10	10.14.05				
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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 / cm³. 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).

0	Home	Insert	Page La	yout Fo	rmulas Data	a Reviev	w View				
1	otTable Tab	le Picture	Art	apes SmartA	urt Column L	ine Pie	-	rea	Scatter * Other Charts * Hy	Q yperlink	Text Header Wo Box & Footer
	Tables		Illustrati				Charts		Scatter		
	E8	-	(f _≭ 0					0.0 9-9		5
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3	#	SampleMa	2592	SampleMi	in=	0				e pairs of v	
4	#	VoxelWid	0.0498	VoxelHei	ght=	0.0498	VoxelDep				
5	#							1			ues are not in n they represent
6	#	Vol_#	Slice	Name	BMD	Mean	Std.Dev.	Vox		measurem	
7	#				mg HA / cm ³				All Chart Types		
8	<u>(</u>	1	512	0_sum	0	1032.115	53.2101	21	06452 5224.085	260.1594	
9		1	512	50_sum	50	1077.324	55.2368	21	.06452 5224.085	260.1594	
10	1	1	512	200_sum	200	1243.035	57.2836	21	.06452 5224.085	260.1594	
11		1	512	800_sum	800	1795.32	61.3816	21	.06452 5224.085	260.1594	
12		1	512	1200_sum	1200	2152.197	66.8271	21	.06452 5224.085	260.1594	
13											
14											

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.

👔 Image Algebra 12.0						
Formula						
Output= (Input-1039.8)/0.9337						
Variables						
Output Input OutFile CLS_Bone_Control						
Copyright 1986-2014, BIR, Mayo Clinic						
Go	Exit					

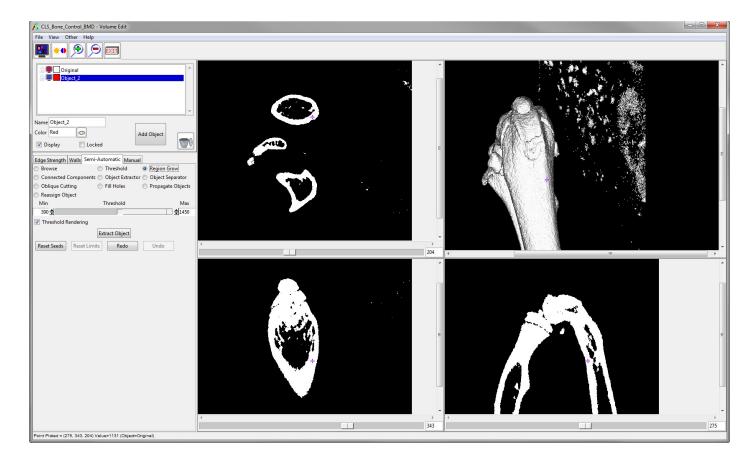
Click the Output button and name the Output data set. Change the Data Type to Signed 16-bit.

🜈 Parameters (Output) - Image Alg 💶 💷 🗮 🎫									
Type 🔘 Workspace 🔘 Disk									
Workspace BMD OVX	Workspace BMD OVX								
Name VS_Bone_Co	ontrol_BMD Delete								
Start 1									
Increment 1									
Start 1	~								
Increment 1									
Per Volume	Per Volume Same								
	Specified 512								
DataType	🔘 Сору								
Signed 16-bit 🤻	Max/Min Settings © Calculate								
Rounding	🔘 Full Range								
	Done								

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.

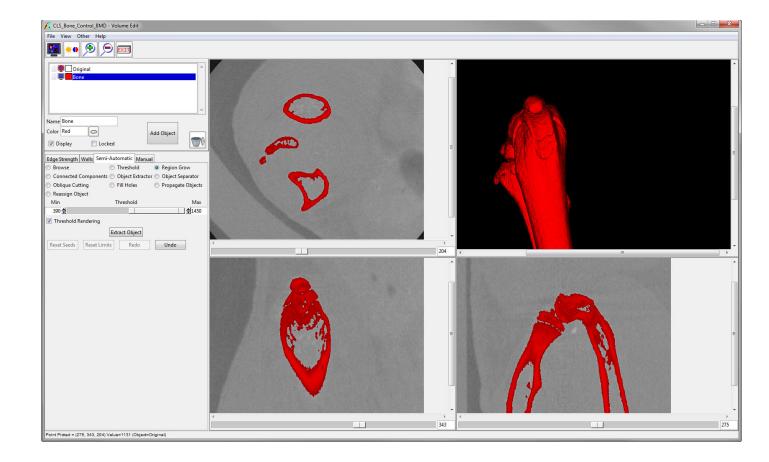
Bone Segmentation

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

🕻 Sample Options - Region Of Interest								
Sample Type Selected Region Object(s) Individual Voxels	Criginal							
	Select All	Invert Selections						
	Combine Objects: 🔘 I	No 🔘 Yes						
Minimum	Sample Max/Min	Maximum						
-1113		1450						
	inge 🔍 Volume 🔘 Da olume 🔍 Primary 🔘 F							
Summing 💿 On 🔘 Auto Reset 💿 On 🔘	F	Reset Sum						
Sample 💿	All Slices 🔘 Slices Spe	cified in Slice Menu						
Sequence Display 🔘	On Off							
Stat Type 🛛 💿 In	ntensity 🔘 2D Shape (🗇 3D Shape 🔘 Fractal						
🔘 B	Boundary Coordinates Region Pixels							
Decimal Places 4								
Log Stats 💿 On 🔘		gure Log Stats						
Sample Images		Done						

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

		itats - Region Of In 🗖 🗉 🔀			
Plot	t Log				
		File = CLS_Bone_Control_BMD			
		Object Map = CLS_Bone_Control_BMD			
	V	Vol. No. = 1			
		Region Name = Original_sum			
		Maximum = 0.			
		at (1, 1, 1)			
		Minimum = 99999.			
		at (1, 1, 1)			
	1	Mean = 0.			
	1	St. Dev. = 0.			
		Sum = 0.			
		Entropy = 0.			
	V	Number Of Voxels = 0			
		Area = 0. mm2			
	V	Volume = 0. mm3			
		< -1113 = 0			
		> 1450 = 0			
		>= -1113 & <= 1450 = 0			
		Mean In Range = 0.			
		St. Dev. In Range = 0.			
		Sum In Range = 0.			
Area In Range = 0. mm2					
	Volume In Range = 0. mm3				
		BAP = 0.			
	Log	Stats Done			

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.

1	ROI	Stat	Log - Re	gion Of Interest				X	
# #	Volu	ume	File=	3:35:43 CST 2015 CLS_Bone_Control_BMD 450 SampleMin= -1113					^
#	-			0.0199000000000000000	VoxelHeight=	0.0199000	000000000000	VoxelDepth=	
#	Vol_	#	Slice	Name	Mean	Std.Dev.	Voxels	Vol_mm3	
		1	512	Bone_sum	730.4738	239.7202	3559278	28.0492	-
•	Sa	ve		III				Done	

26

Repeat the measurement steps for the OVX bone data set.

1	CLS_Bo	one_Ovarie	ecto_BMD.stats - ROI Stat Log				- 0 X		
			13:39:24 CST 2015	1953				^	
#	VolumeFile= CLS_Bone_Ovariecto_BMD SampleMax= 1572 SampleMin= -1113								
#	-		0.01990000000000000000	VoxelHeight=	0.0199000	000000000000000000000000000000000000000	VoxelDepth=		
#	8								
#	Vol_#	Slice	Name	Mean	Std.Dev.	Voxels	Vol_mm3		
#	1	510	Dese	600 1060	000 0000	2556522	00 0076		
	1	512	Bone_sum	690.1069	223.3993	3556532	28.0276		
								-	
1			III				F		
	Save						Done		
	Save	=					Done		

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/cm³ for the OVX condition and a mean BMD of 730 mg/cm³ 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 <u>Bone Microarchitecture Analysis Add-On</u>. 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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