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Albira CT System/PMOD Software Protocol for Obtaining Bone Mineral Density Units Using a Hydroxyapatite Phantom

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Introduction

Age related diseases such as osteoporosis, bone development and bone development abnormalities, and bone fracture and healing are active areas of investigation in life science research. During the course of these studies, researchers frequently monitor changes in bone mineral density (BMD) as well as various structural bone parameters (1,2). In this application note we highlight a facile method for studying BMD using the Albira CT system. We also compare the BMD measurements made by the Albira CT system and a protocol using 2D X-ray/ Bone Density Software (long bone measurement) in two groups of mice aged 33 days and 80 days.

The Albira CT system is factory calibrated to attenuation units (Hounsfield units (HU)). A calibration curve to convert HUs to BMD units (g/cm³) can be generated using a hydroxyapatite (HA) phantom. The calibration curve can subsequently be used to obtain BMD units for bone specimens imaged with the Albira CT system. This application protocol provides instructions for generating a bone mineral density calibration curve. Guidelines for generating appropriate volumes of interest (VOI) for phantoms and specimen regions are provided.

Protocol

2.1 HA Phantom Imaging

The HA phantom should be imaged using the same parameters that will be used for specimen imaging. We recommend using the Albira CT Best (600 projections) parameters set to both High Dose (400 μ A) and High Voltage (45 keV) for sufficient contrast and resolution. The QRM-MicroCT-HA phantom (QRM GmbH;




Moehrendorf, Germany) was employed in this example. The QRM-MicroCT-HA phantom is 32 mm in diameter and includes 5 cylindrical inserts containing 0, 50, 200, 800, and 1000 mg HA/cm³ with the bone equivalent densities 1.13, 1.16, 1.26, 1.64, and 1.90 g/cm³ respectively. The phantom may be positioned in the rat bed (see figure below) and imaged with an appropriate initial horizontal position and Albira Best CT HD/HV settings. The acquisition may be reconstructed using the standard settings providing a 125 μ m resolution.




Figure 1. The QRM-MicroCT-HA phantom

2.2 Creating a Calibration Curve

To begin, open the HA phantom image in the PMOD software (PMOD Technologies, Zurich, Switzerland) and create a VOI around each HA insert in turn. Place the cross hairs in the center of a single cylinder insert and activate the LOC view. Next select the **VOIs tab**

View **VOIs** **Compare** **Fusion**. Select the CUBE (VOI) from the Apply Predefined VOI pull down menu (see Figure 2). Select the **Cube VOI button** . The Predefined menu will appear. Select **Apply** and a VOI displays over the image. Select Close in the Predefined menu. Drag the corners of the VOI to encompass the cylinder insert to be measured (see Figure 3). Select the **Automatic Isocontour Detection** button . The Enter Threshold menu displays (see Figure 4). Enter a threshold of 75 and select OK. The VOI perimeter should now closely outline the cylinder perimeter. Inspect the image at multiple orthogonal views to verify the Automatic Isocontour detection. Finally, select the **VOI Statistics** button .

Record the Averaged value. This represents the HU unit for the given cylinder insert included in the VOI. A screen recording for the described process can be viewed here: <http://youtu.be/eYEzVD6W0YU>. Repeat this process for each of the cylinder inserts. For the 0 mg HA/cm³ cylinder insert use the **VOI action button**  to move the VOI created for a previous cylinder insert over the position where the 0 cylinder resides to obtain a measurement. Using a statistical/graphing program (e.g. Microsoft Excel) plot the attenuation VOI results versus the bone equivalent densities for each insert. Perform a linear regression (see Figure 5). This formula can be used to derive BMD units from in vivo or ex vivo bone specimens.

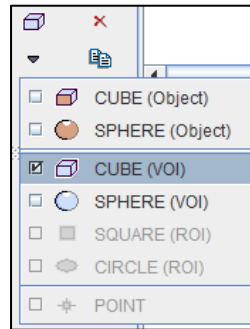


Figure 2. Apply Predefined VOI pull down menu

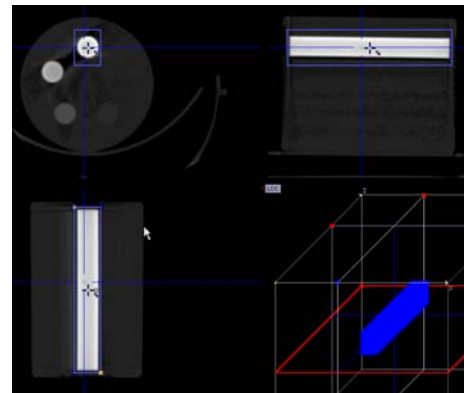


Figure 3. Drag the corners of the VOI to encompass the cylinder insert to be measured

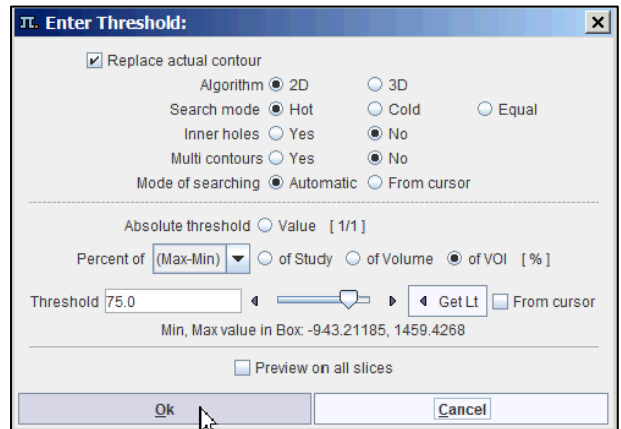


Figure 4. Enter Threshold menu

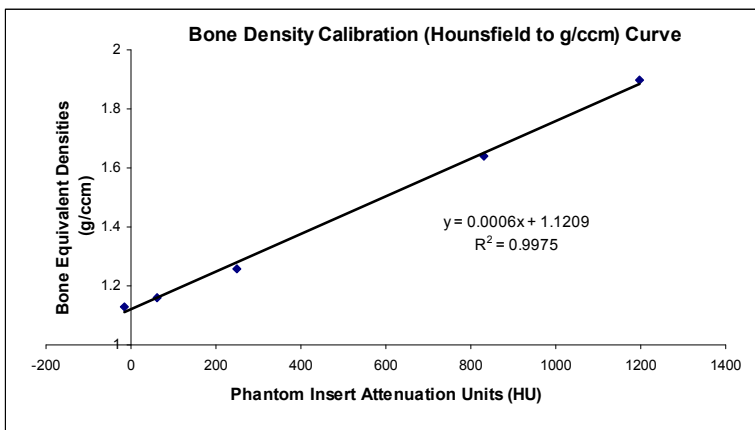
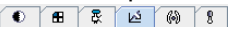
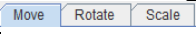



Figure 5. Calibration curve/formula

2.3 Bone Specimen Imaging and Analysis

Specimen imaging should be performed with the same imaging parameters used to image the HA phantom. Whole animal samples may be imaged in the image bed positioned normally. Ex vivo bone specimens should be positioned on a flat surface within the imaging bed. VOIs may be created for bone samples using the method mostly as described above for the HA phantom cylinder inserts. It may be necessary to rotate the in vivo images to generate a VOI neatly over a desired bone specimen. To do this, select the **Reslicing** tab  the **Move** tab,  and using the rotation tool handle  and rotate the image to the desired angle. Create a VOI around a bone volume of interest using the method outlined above. Use the **Automatic Isocontour Detection Threshold of 60** for in vivo bone samples and 50 for ex vivo samples. Convert the obtained attenuation unit average to BMD units using the calibration curve formula created with the HA phantom.

Results and Conclusions

Representative results using this protocol are provided in the figure below. The QRM-MicroCT-HA phantom was imaged and a calibration curve was generated as described above. SKH1 mice aged 33 days (n=3) and 80 days (n=3) were imaged using the Albira CT **Best** HD/HV setting. A whole-femur VOI was generated in each mouse sample image and the Averaged value was recorded. The calibration curve formula was used to convert attenuation unit results to BMD units. A 2D X-ray image was also acquired using the In-Vivo MS FX PRO. The femur long-bone density was analyzed using Imaging Bone Density Software which allows for analytical radiography of planar radiographic images (3). Animals were subsequently sacrificed. Soft tissue was gently removed from the femur. The ex vivo femurs were subsequently imaged using the Albira CT Best HD/HV setting. A whole femur VOI was generated in each ex vivo sample image and the Averaged value was recorded.

The calibration curve formula was used to convert attenuation unit results to BMD units (see Figure 6).

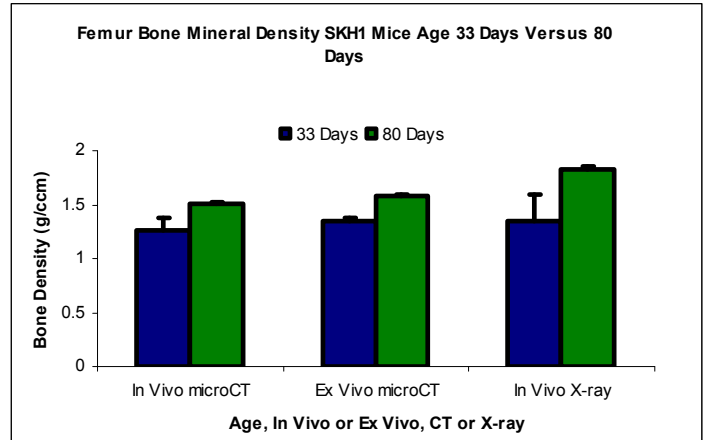


Figure 6. BMD measurements with *in vivo* CT, *ex vivo* CT and *in vivo* X-ray for mice aged 33 days versus mice aged 80 days

While the bone density range for mice is variably dependent on strain, these results are within an expected range consistent with other published reports of mouse femur BMD (4). Mouse bone density increases through the early to mid life. This difference is detected with this Albira CT BMD measurement protocol (in vivo), with the mice aged 33 versus 80 days having a femur BMD of 1.26 g/cm³ and 1.51 g/cm³ respectively (p=0.02). Ex vivo measurements made with these samples using this Albira CT BMD measurement protocol, as well as in vivo measurements using 2D X-ray and the Bone Density Software, provided similar results and statistically significant differences for mice aged 33 vs. 80 days.

Here we have provided details of an Albira CT protocol for obtaining BMD units. Mouse femur BMD values obtained using this protocol are within an expected range and age-related differences in femur BMD was detected. Additionally, the protocol can be employed using in vivo or ex vivo samples.

Furthermore, the results obtained here are consistent with the results obtained using 2D X-ray and the Bone Density Software. While the protocol was validated in a murine model it may be possible to adapt this method for other species and samples including ex vivo rabbit samples.

References:

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