



Method note

Bone mineral density (BMD) calibration in Skyscan CT-analyser

Bone mineral density (BMD) is defined as the volumetric density of calcium hydroxyapatite (CaHA) in terms of g.cm^{-3} . It is calibrated by means of phantoms with known BMD. It can refer to two slightly different measurements: (a) to the density of a defined volume of bone plus soft tissue, as in trabecular (medullary) BMD, or to (b) a density measurement restricted to within the volume of calcified bone tissue, such as cortical (volumetric) BMD.

Hounsfield units are a standard unit of x-ray CT density, in which air and water are ascribed values of -1000 and 0 respectively. It has been found over several decades of CT imaging to be a useful general CT density calibration system.

The Skyscan CT-analyser software provides for an integrated calibration of datasets into these two density scales, HU and BMD. Both require the appropriate calibration phantom scans and measurements. The steps for implementing calibration of BMD are as follows.

It is recommended that you study the method note on HU calibration before reading this note on the BMD calibration.

STEPS FOR IMPLEMENTING HU AND BMD CALIBRATION OF BONE MICRO-CT SCANS.

1. One option for measuring BMD in *ex vivo* scans of bone is to place the bone samples into a tube of water for scanning, and also placing the BMD phantom rods into an identical tube for the calibrating scan. This allows the HU density value to be (optionally) independently calibrated for each individual bone scan. If this approach is used, the water tube should be of the minimum diameter possible. Note: the tube should be plastic, not glass – polypropylene is a suitable low density plastic.

However it is not necessary to scan bones (or other objects) in water for density measurement – they can be scanned “free-standing” in air. However bones scanned for density measurement

should not be dry. Bones for density measurement should be stored either immersed or moistened by physiological saline (0.9%) and during scanning should be wrapped in plastic film to prevent drying. For bones scanned in air, the BMD phantoms should likewise be scanned free-standing (not in a tube of water). In general the x-ray attenuation of the BMD phantoms during scanning should match approximately the attenuation of a bone sample and any surrounding tissue or water.

If the bone scanned for density measurement has been scanned *in vivo*, then a calibrating water tube should be selected with a diameter to match the site that was scanned – for example about 7 mm for the mouse knee, 12 mm for the rat knee.

2. Obtain phantom rods for BMD measurement with a diameter selected to approximately match the crosssectional thickness of calcified bone in the bones you are measuring. For example, the typical mouse sites (distal femur, proximal tibia, lumbar vertebra) are approximated by a solid rod of 2 mm diameter. The same sites from the rat are approximated by a 4 mm rod. (These rods can be purchased from Skyscan.)
3. Scan a typical bone sample, either free-standing or within a tube of water. Then scan two BMD rods (under the same conditions as the bone scan) with BMD values of 0.25 and 0.75 g.cm⁻³ CaHA (refer to figure 1). Note that in both the bone scan and the calibration scan, you should include in the scanned region a part of the water tube containing water only and clear of the bone or BMD rods. This allows the HU calibration to water density. (When selecting a water region for the HU calibration, this region should not be at the periphery of bone or a BMD phantom, and should be a few slices clear of these objects, since slight shadow artefacts close to the denser objects can compromise the water density reference.)

Reconstruct the scan of the bone in the water tube. Select and optimise the reconstruction parameters for this bone scan, taking care to find a good value for beam hardening correction. Also take care with the selection of the lower and upper contrast limits. The lower limit should be zero so that the density scale has a zero origin. The upper limit should be at the top end of the brightness spectrum for the bone (see figure 2). All the reconstruction parameters you select must be applied identically to all bone scans and to the calibration scan of the BMD rods (with the exception of the postalignment correction, the only parameter that should be set uniquely for each scan, which will not compromise comparative measurements).

4. With the scans and reconstructions of the bones and the calibrating phantoms complete, implement the HU and BMD calibration in CT-analyser. (Note of course that all the scan parameters, such as pixel size, rotation step, frame averaging, voltage, filter etc. must be identical for all scans.)

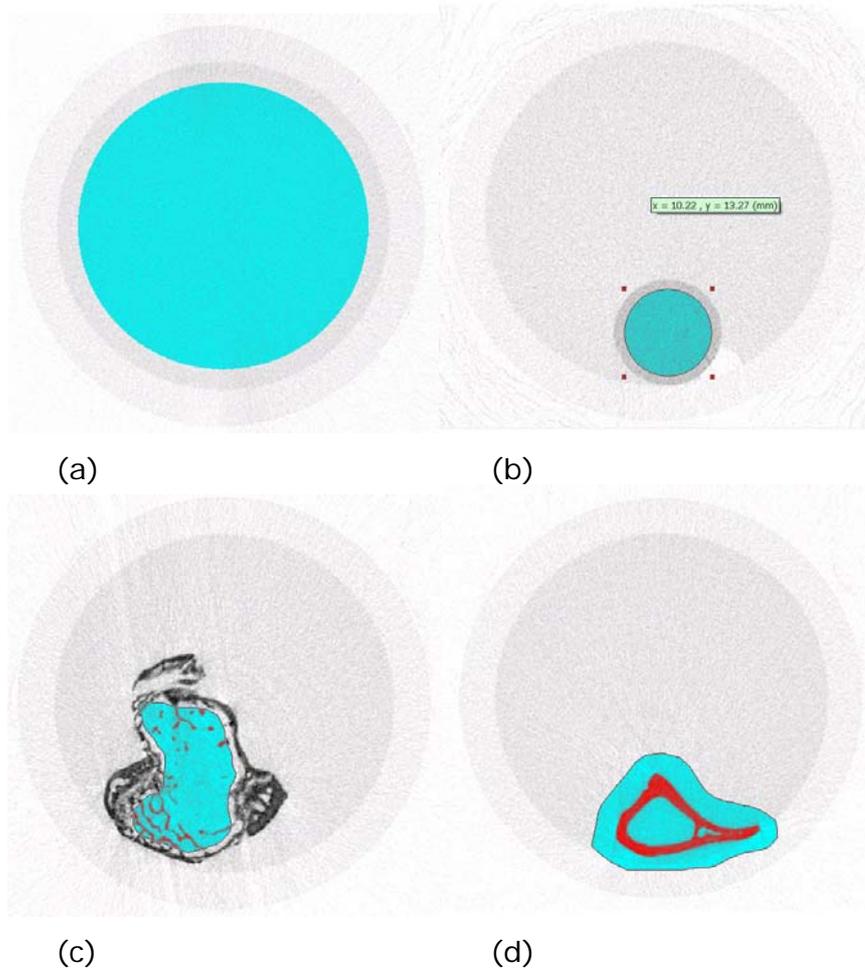


Figure 1. Scanned water tube of appropriate diameter containing (a) a water only region for HU calibration, (b) a BMD calibration rod, (c) bone - mouse tibia – with trabecular region selected, (d) mouse tibia with cortical region selected.

Open the BMD phantom dataset, and select a region of water as shown in figure 1a. Select a volume of interest (VOI) integrating this water cylinder over a number of slices. At the density page in CT-analyser, find the average grey level for this VOI. Note – make sure you select the grey spectrum from “selection” – the second tab – not just from “image” so that density data is integrated over all the VOI levels. The mean grey level value is the first “,mean (total)” value at the end of the spectrum data report, below the histogram. The data will appear as shown in figure 3 below. This value in figure 3 is 22.91402.

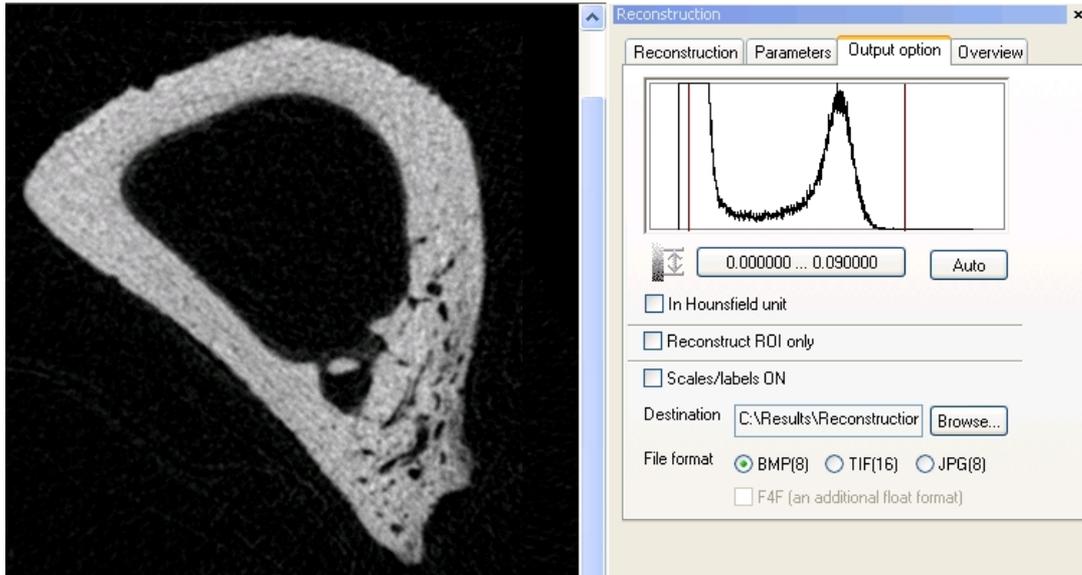


Figure 2. The contrast limits for bone should be set appropriately for density measurement. The lower limit should be zero. The upper limit should be at the upper (right hand) end of the spectrum “tail” representing the highest bone CT density value.

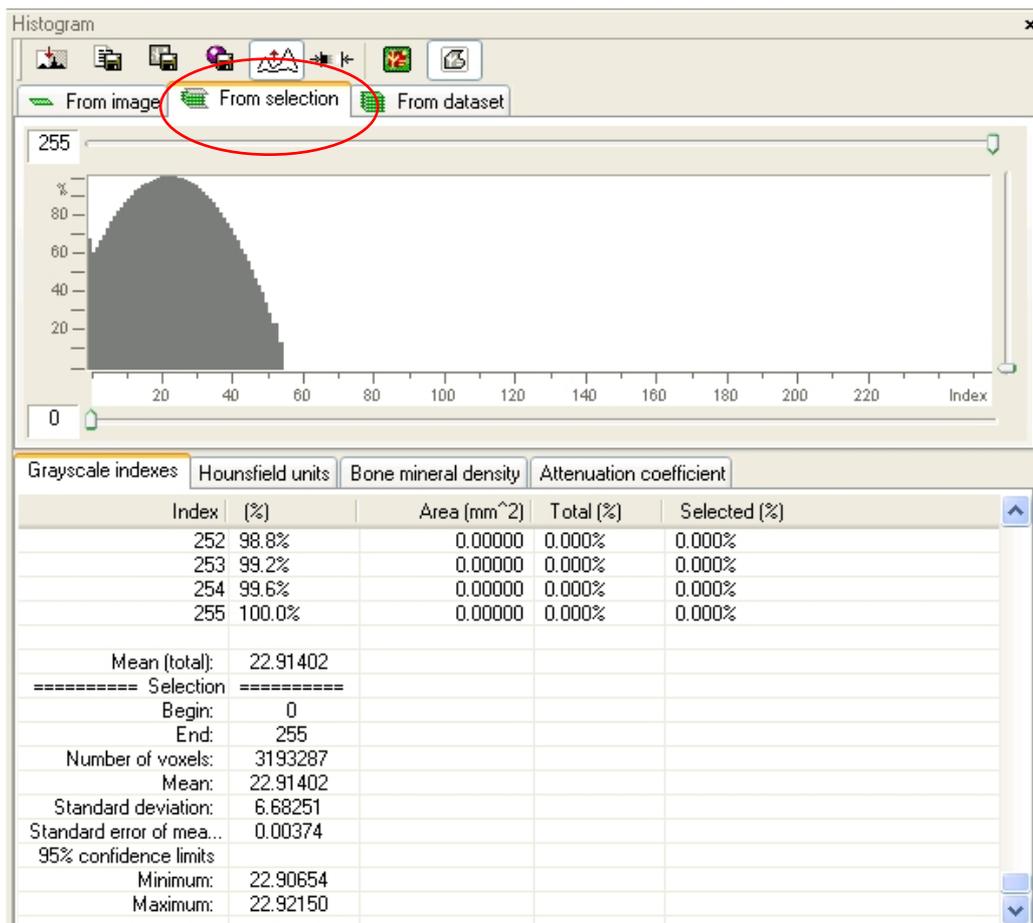


Figure 3. The density data from a selected water region in CT-analyser. *Please note* – above the histogram pane make sure that the tab named “from selection” is set, not “from image”.

- Take the mean grey level value for water – in the above case this is 22.91402 (it is important to use all the decimal places to optimise the accuracy of the calibration). Now click on the HU calibration button  - above the grey spectrum. In the dialog box link the grey (“index”) and HU values appropriately – grey of 0 corresponds to HU of -1000, and grey of 22.91402 corresponds to HU of 0. Click on OK to implement this calibration. Note that the next time you open the HU calibration window, the values shown will appear different – as shown below in figure 4 (right). *Don't panic!* The calibration you have just entered is re-normalised as the equivalent HU values representing the minimum and maximum (black and white) grey values of 0 and 255. The HU value corresponding to the maximum density grey value of 255 – here 10129 – is the single number that you should record to save the HU calibration.

(Both attenuation values can be set to zero while entering the water calibration for HU. If necessary you can reapply the attenuation calibration afterwards, using reconstruction attenuation limits from the dataset log text file.)

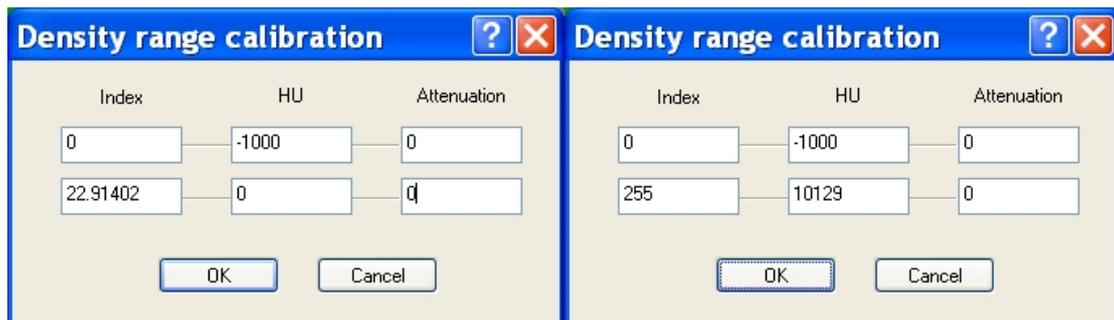


Figure 4. Enter the measured grey value for water (here 22.91402) in the dialog box for HU calibration using all 5 decimal places. This implements the HU calibration. Subsequently re-opening the HU calibration box, you will see the calibration data recalculated as an HU value for the 255 (maximum density) grey value – here 10129.

- Now that HU is calibrated, you can proceed to calibrate BMD. In the BMD phantom dataset select a volume of interest within each BMD phantom as shown in figure 1b – integrating over several image levels. (Keep the selected VOI clear of boundaries of the phantom). Record the calibrated HU density value for each phantom.

In the example mouse tibia illustrated in figure 1, the two BMD phantoms had the following HU density values: 1205.86243 and 3643.45005 for the 0.25 and 0.75 g.cm⁻³ rods respectively. These two calibrated HU values are the basis for implementing the BMD calibration.

- Go to the File / Preferences menu. Select the "histogram" tab. You will see the dialog box as shown below in figure 5 (lower). Click on "calibrate". Enter the BMD and HU values for the two measured phantoms as shown (figure 5 upper). Then clicking on "OK" will implement the BMD calibration.

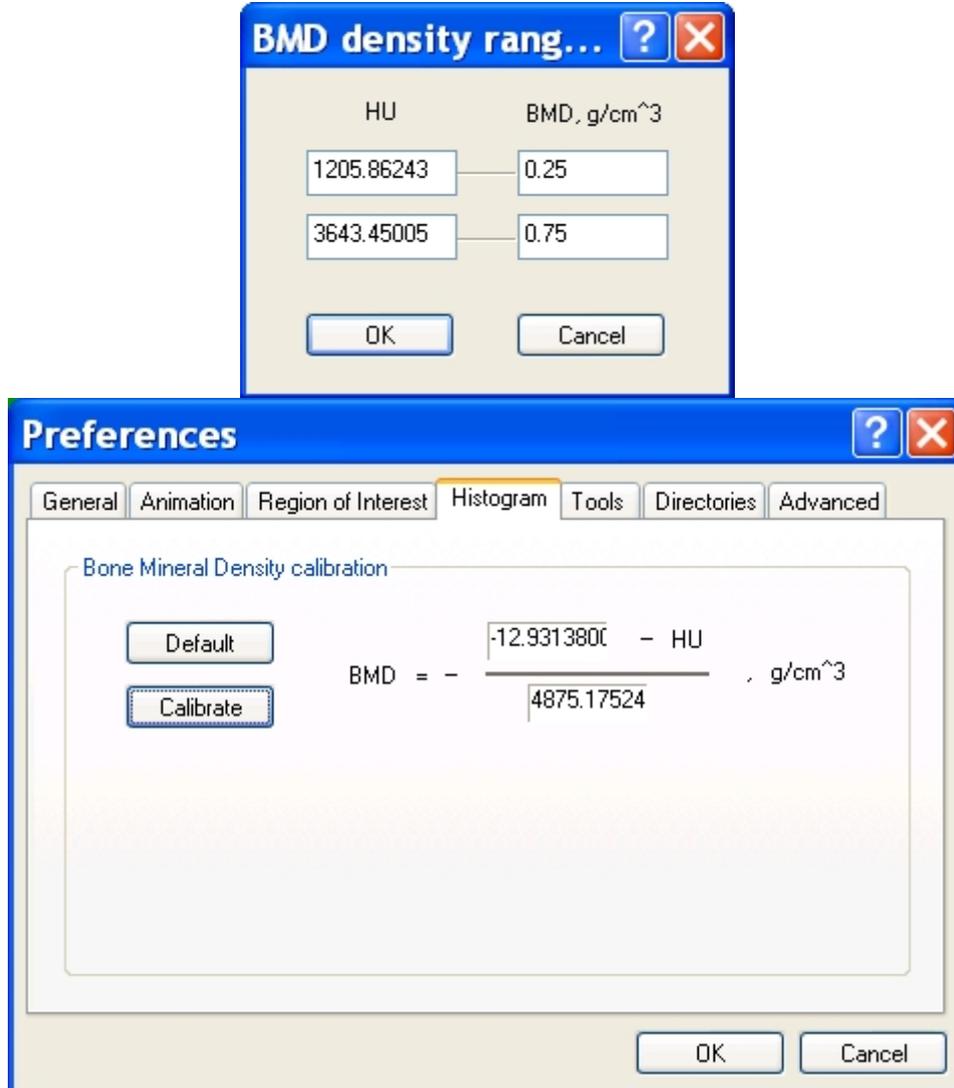


Figure 5. The dialog for calibrating BMD against HU values, in the file / preferences / histogram page. The equation linking HU and BMD is shown. Implement the BMD calibration on the basis of two phantom measurements by clicking on "calibrate" and entering the measured values as shown in the dialog box which opens.

- It is recommended that you save a record of the HU calibration number (see part 5) and the calibrated HU values of the two BMD phantoms: this represents the complete calibration information needed to implement the same calibration for other scanned bones in the same experimental series, scanned in the same conditions (e.g. in the same water tube) as the calibration phantoms.

9. A different reading or interpretation of the density measurement is required for trabecular and cortical bone. For trabecular bone the density you should measure is an average of the trabecular bone and the marrow, for a selected volume of interest inside the bone medulla (see below figures). Volumetric density of thin trabecular structures is generally under-measured due to the partial volume effect, and is thus difficult to measure accurately. So the trabecular BMD measurement is a regional medullary BMD (as in DEXA and pQCT measurements of trabecular BMD).

So to measure BMD within a medullary region containing trabecular bone as well as marrow (but no cortical bone) you need to use the average density from the whole of the grey level spectrum. Refer to the text and figures below, from the end of text files outputted from the CT-analyser binarise page. The first BMD value given in the summary data, is the mean (total) value. This is the mean from the whole spectrum (here 0.34898) and is the value that you should use for trabecular bone. The subsequent data (begin, end) relate to the currently selected limits of the binary selection.

However if you are analysing cortical bone, you should use the second, restricted mean value for density - e.g. 1.24719 in the below example. Cortical bone being thicker is not affected by the partial volume effect that compromises the trabecular density. So for cortical bone, select binarisation limits that binarise the cortical bone and exclude the soft tissue, then take the second, restricted mean value. Note, as always, the same binarisation limits must be applied to all cortical bones from a single study. Also make sure you have a VOI which encloses only cortical bone from an anatomically referenced location.

TRABECULAR

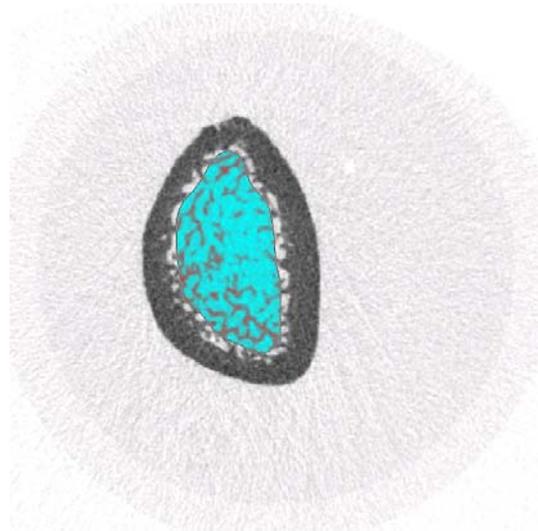
Mean (total): **0.34898**

===== Selection =====

Begin: 0.56741
End: 2.08032
Number of voxels: 1284766
Mean: 0.88456
Standard deviation: 0.19818
Standard error of mean: 0.00017

95% confidence limits

Minimum: 0.88400
Maximum: 0.88491



CORTICAL

Mean (total): 0.49453

===== Selection =====

Begin: 0.56741
End: 2.08032
Number of voxels: 1981273
Mean: **1.24719**
Standard deviation: 0.18150
Standard error of mean: 0.00013

95% confidence limits

Minimum: 1.24693
Maximum: 1.24745

