Innovation with Integrity

BRUKER MICRO-CT ACADEMY

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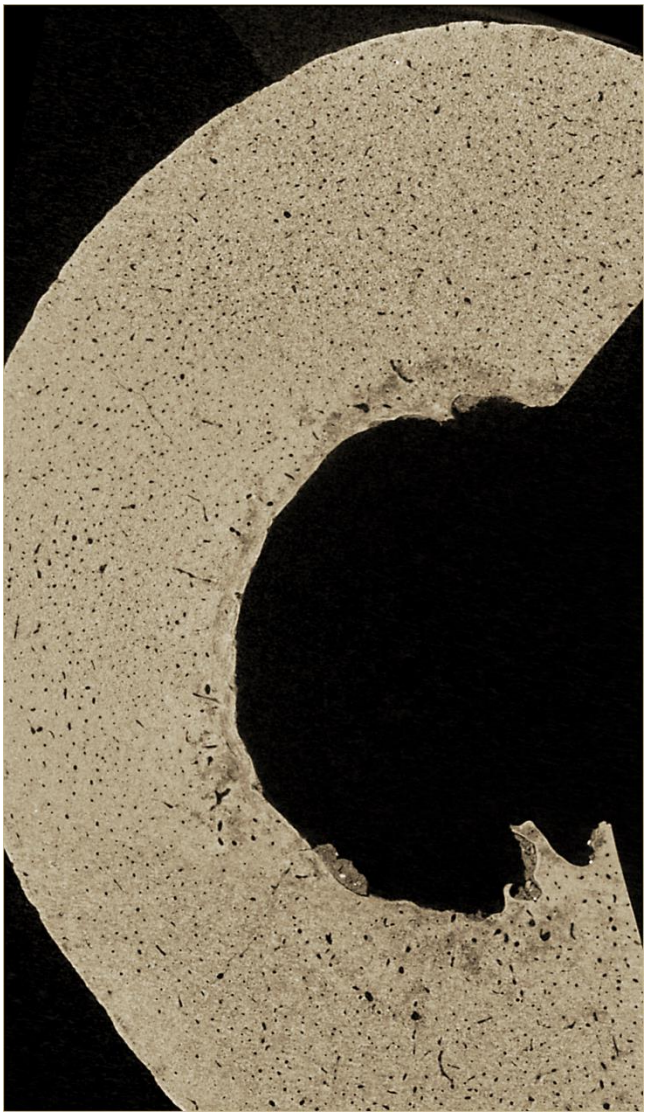
In this issue:

Welcome

What is “density” in microCT? In this newsletter we look how “density” is derived from attenuation of x-rays, introduce method notes on calibration for BMD and Hounsfield units, and discuss context sensitivity and artefacts of densitometry.

Image of the Month:



A microCT cross-section of an archeological sample of human femoral bone (SkyScan1172). The effect on bone mineralisation near surfaces of contact with soil (diagenesis) is visible at the endosteum. Multiscale porosity is also evident.

Density Measurement by MicroCT

Microdensitometry in 3D is important in microCT applications including bone and dental research, geology, material science and others. What does “density” mean for microCT? What it does *not* mean is simply mass density, i.e. the g/cm^3 of the material. “Density” instead means x-ray opacity, the strength of x-ray attenuation within a scanned material. Due to its strong dependence on atomic number “Z” (mass attenuation follows Z^3) x-ray attenuation is sensitive to elemental composition, as well as to physical density, of the scanned material. How much x-ray attenuation happened within each voxel during a CT scan? That is what is expressed by the reconstructed grey scale of each microCT image voxel.

Attenuation coefficient is converted to the image greyscale (8-16 bit) by reference to the intensity window set by the user during reconstruction in NRecon. Reconstructed greyscale can be calibrated by comparison with reference materials, into bone mineral density (BMD) and Hounsfield units (HU).

The principle of density calibration in microCT is *equivalence* of x-ray absorption relative to a reference material. For BMD the material is calcium hydroxyapatite, and for HU air and water. If a bone or tooth volume is found to have a certain BMD value, it means that this is the concentration of calcium hydroxyapatite (g.cm^{-3}) which would give the same x-ray absorption (with the same x-ray settings and in the same scanner) as the measured calcified tissue. If a selected tissue in a mouse or rat scanned *in vivo* is found to have a certain HU value, this expresses how different the attenuation of that tissue is from that of pure water (the HU of water is zero).

Method of density calibration and measurement by microCT

CT-Analyzer ("CTAn", Bruker-microCT) provides calibration of greyscale or "density" into BMD and HU. The full methods for measurement of BMD and of HU are described in the following two method notes:

MN009 BMD calibration in CTAn,
MN039 HU calibration in CTAn

Once the appropriate calibration has been performed, the method for density measurement is similar to any other quantitative measurement in that it begins with the selection of the appropriate region or volume of interest (VOI). With this done, the mean density value of all voxels within the VOI can be reported in units of greyscale, attenuation coefficient, HU or BMD. As always in microCT measurement, the meaning of the measured value is determined by the VOI. For instance, if the VOI is a bone medulla containing trabecular bone and marrow, then the measured value is called trabecular "BMD". However if a binarised image of cortical bone is set as the VOI, excluding soft tissue, then the measured value should instead be called "TMD" meaning tissue mineral density. Distinguishing "BMD" (bone and soft tissue averaged density) from "TMD" (density of mineralized tissue only) is a useful distinction introduced by Bouxsein et al. (JBMR 25(7): 1468-86, 2010).

Context sensitivity of microCT density due to beam hardening and other artefacts

It would be conveniently simple if the greyscale of every voxel was in direct proportion to the composition and consequent attenuation of the material in that voxel, as expected in theory. However three artefacts can break this direct proportionality – these are (a) the partial volume effect, (b) beam hardening and (c) truncation. Understanding these artefacts helps both to plan and to interpret microCT imaging studies.

(a) The partial volume effect: voxel attenuation near an object surface follows a sigmoid gradient from low to high intensity (normal to the surface). With thin objects near to the resolution limit, all voxels are near a surface and thus can have their density artificially reduced. Figure 1 shows a scan of four aluminum foils of different thickness, all with the same actual material density. The density profile across all four foils at high resolution shows similar density for all foils; however in the lower resolution scan images the density of the thinnest foil is artificially reduced. This is a demonstration of the partial volume effect.

Practical implication: an object should have a thickness of about 10 pixels or more for density measurement free of partial volume effect.

(b) Beam hardening: Laboratory microCT x-ray sources are polychromatic – they emit a wide range of x-ray energies. Passing through scanned objects, low energy x-rays are attenuated faster than high energy photons. So more photons are absorbed near outer surfaces than deeper in the scanned volume. This causes an artefact of apparently elevated attenuation at outer surfaces of scanned objects (where real density is in fact uniform).

Software correction of beam hardening is available in Bruker-microCT NRecon software. This can effectively remove the artificial surface-to-depth density gradient from beam hardening provided that most of the x-ray absorption is from material of a similar x-ray attenuation (opacity).

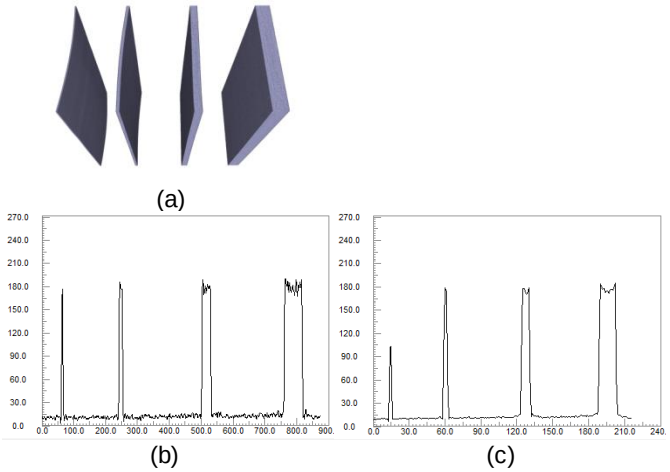


Figure 1. *(a) Thick and thin aluminum foils. (b) The attenuation profile across the foils from a high resolution scan. X axis is distance; y axis is attenuation as greyscale. (c) The attenuation profile across the foils from a lower resolution scan. The thinnest foil has its attenuation artificially reduced as all voxels are affected by the surface intensity gradient.*

However if a scanned object is surrounded by a lower density medium, such as liquid around a sample or – in an *in vivo* scan – soft tissue around bone, the software beam hardening correction is much less effective, since it becomes a more complex two-material scenario.

Figure 2 shows that, with no software beam hardening correction, surrounding medium such as water does reduce the beam hardening gradient; however it prevents further software correction. By contrast in air, software correction is much more effective and can remove beam hardening gradients entirely. Practical implication: When scanning bone *ex vivo*, reduce the thickness of surrounding material to a thin plastic or moist paper layer. Immersing samples in liquid can make beam hardening uncorrectable. Bear this in mind also when interpreting *in vivo* scans with surrounding tissue. BMD calibration phantoms should approximately match the diameter of the calibrated bone with surrounding tissue "simulated" by scanning the phantoms in a water-filled plastic tube.

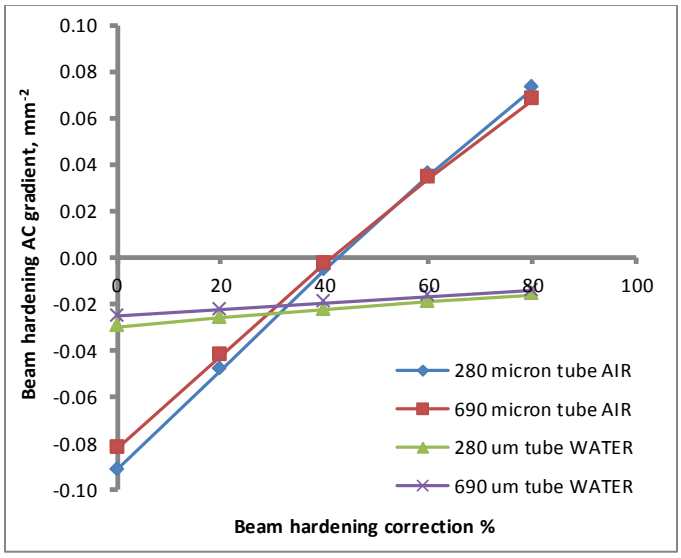


Figure 2. *Beam hardening (BH) gradients in aluminum scanned in air and 20mm of water. The y axis is the surface gradient in attenuation coefficient (AC) from beam hardening, so zero means no – or corrected – beam hardening. In air, beam hardening correction of 40% fully removes the surface gradient artefact. In 20mm water however, software correction becomes much less effective and cannot remove the surface gradient artefact (all lines stay below zero).*

(c) Truncation: This is when the camera field of view (FOV) is too small to contain all the sample or animal width during the scan rotation. Data in figure 3 show the significant, abrupt effect of truncation, which clearly compromises density measurement. Truncation can occur for instance during *in vivo* scans of bone in scanners with a small camera, where the bone stays within the FOV but a varying amount of surrounding soft tissue rotates outside the FOV. The result is a large artificial "quantum" step change in the reconstructed density of the scanned tissue such as bone (fig. 3c). Practical implication: Densitometry requires an FOV wide enough to include the whole sample, ideally with ambient air to the right and left. In highly truncated scans densitometry is compromised. Try to minimize truncation for densitometry.

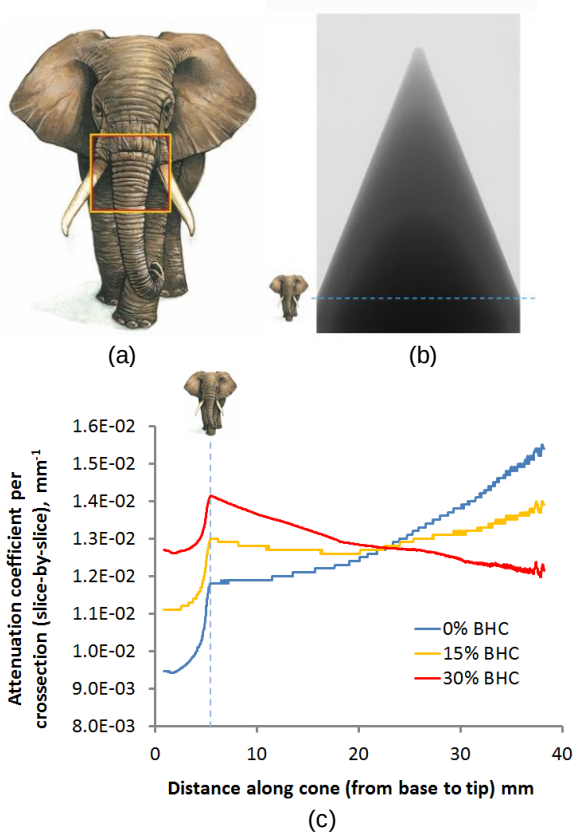


Figure 3. *(a) Truncation means loss of image data outside of the FOV width (not height); (b) A plastic cone was scanned with the base of the cone truncated (dotted blue line); (c) Crossectional attenuation plotted with height in the cone. Truncation grossly alters the reconstructed attenuation.*

Upcoming Events

Bruker microCT will participate with an exhibit in the forthcoming conferences. Please click the link below for more information. We hope to see you there!

ESB	Aug 31- Sep 3,	Liverpool, UK
IMA	Sep 1-5,	Gauteng, South Africa
IMC	Sep 7-12,	Prague, Czech Rep.
ASBMR	Sep 12-15,	Houston, USA
WMIC	Sep 17-20,	Seoul, South Korea
IMPC	Oct 20-24,	Santiago, Chile
XRM	Oct 26-31	Melbourne, Australia