

Calibration of thickness and BMD measurement in micro-CT imaging of bone

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A. Thickness

In micro-CT measurements, surface finding or segmentation is frequently performed using a global grey level threshold. However, as Ito et al. (1998) pointed out, any structure thickness can be obtained by changing the threshold value. It is therefore necessary to calibrate thickness measurement.

An approach to solving the problem of the dependence of measured thickness on selected global threshold is presented. This consists of:

1. Not using global grey level thresholding. Instead adaptive local thresholding (Canny 1986) is employed which also corrects the thickness biasing and under-reporting of thin structures associated with global grey-level thresholding.
2. Aluminium foils are measured to calibrate the adaptive local thresholding.

Aluminium provides a suitable material for calibration of micro-CT measurement of bone structure thickness. Al has a similar x-ray opacity to cortical bone, and is also materially uniform on a micron scale, unlike hydroxyapatite preparations at densities similar to cortical bone, which is important for precise calibration of thicknesses of microns to tens of microns.

Calibration with 20 and 250 micron thick Al foils showed that both thicknesses could be measured accurately simultaneously. Thickness of aluminium and bone was demonstrated to be measurable by micro-CT where structures are at or above about 3 pixels in width. The thickness calibration with 20 μm thick Al foil was found to be stable over the range of magnifications or $\times 40$ and higher, or pixel sizes 6.8 microns and lower.

B. BMD

The method for bone BMD calibration reported here is a first approach to the problem which after testing and input from users, can be refined and improved.

The calibration of BMD measurement in bone micro-CT scanning will refer here to two measurement scenarios: (a) measuring BMD in a medullary region containing bone trabeculae and marrow (wet or dry) and (b) measuring BMD in cortical bone.

The problem: Object size and geometry and the distribution of x-ray densities in an object will affect the reported CT density within an object. The two most important reasons for this are:

1. Beam hardening (where the x-ray beam is polychromatic), that is, the more rapid removal of soft x-rays than hard x-rays, changing the energy spectrum of the beam passing through the object,
2. Sigmoid surface density gradients causing under-reporting of density of thin objects.

The solution: To create measurement phantoms that closely reproduce the geometry and density distribution of your study objects, so that both the above factors 1 and 2 influencing reported CT density are controlled in the measurement.

Note that an Al filter (1mm) should be used for bone measurements to narrow the x-ray energy spectrum, reducing the low energy component, thus reducing beam hardening.

Consider the example of mouse bone measurement. Scientists employing the mouse (or rat) model most frequently examine certain key bone sites, namely the hindlimb long bone ends at the knee (distal femur and proximal tibia) and the lumbar vertebra. These sites can be approximated as having a trabecular-plus-marrow medullary region about 2 mm in diameter and a surrounding cortical wall of 100-150 micron thick cortical bone. The mouse bone phantom (set of phantoms) consists of a cylinder of 2mm diameter, 2 cm length, composed of a mix of epoxy resin and hydroxyapatite (HA) with a small grain size optimised for micro-CT, produced by CIRS, Almeda, USA, with HA concentration from 0 (soft tissue equivalent) to 250 mg.cm⁻³. Surrounding the epoxy-HA rod is a layer of aluminium foil 100 micron thick, covering one half only (1cm length) of the 2cm long epoxy-HA cylinder. The surrounding aluminium layer simulates the beam hardening effect of the cortical wall surrounding the mouse bone sites.

When measuring the epoxy-HA cylinder one should exclude the 20-30 µm layer adjacent to the Al shell. All scan and reconstruction parameters should be the same as used for calibrated experimental mouse bone samples. To measure medullary BMD, the Al-surrounded part of the phantoms is measured using four HA concentrations: 0, 50, 150, 250 mg.cm⁻³ of Ca-HA. A calibration curve is created by obtaining the grey level distribution and mean grey value from each phantom, and this can be applied to trabecular-marrow medullary volumes imaged in the mouse bones (excluding 20-30 µm adjacent to cortical bone).

Inspection of scans of the epoxy-HA phantoms shows clumping on HA in dark spots. However the clumped HA accounts for only a small part of the density distribution of the phantom which is predominantly normal.

For cortical bone, one should scan and measure the part of the four phantoms uncovered by aluminium, excluding the 10-20 µm surface layer. The calibration curve is applied to cortical bone by extrapolation upward to the measured cortical bone density. Again when selecting cortical bone in the experimental mouse bone samples, the surface layer of 10-15 µm of bone should be excluded.