Bruker microCT

Method Text and References for journal articles: SkyScan microCT bone analysis

Method note

MN-45

1. Introduction

This method note provides text to form the basis of the "materials and methods" section for journal articles involving microCT analysis of bone samples – or indeed other types of sample. The example text sections can be used optionally depending on the method and procedured used.

Sufficient details should be provided to allow other researchers with essential knowledge of microCT scanning to replicate the scan and analysis method, without going into exhaustive and inappropriately detail. It complies with the recommendations given in Mary Bouxsein's 2010 paper "Guidelines for Assessment of Bone Microstructure in Rodents Using Micro–Computed Tomography"1.

2. Sample preparation

For in vivo:

[State ethical committee approval.]

The mouse / rat was mounted for *in-vivo* microCT imaging in the SkyScan 1076 / 1176 / 1276 / 1178 / 1278 scanner in the plastic / carbon fiber bed under isofluorane anaesthesia using the scanner's mask and tubing for both anaesthetic gas supply and scavenging. Body temperature was maintained by thermostat-controlled air heating set to 30° C, and the animals were imaged by optical video camera in real time during scans.

For hindlimb scans:

The right / left hindlimb was carefully placed in a polystyrene / polypropylene tube and held in place by soft dental wax applied to the ankle, while the contralateral left / right hindlimb together with the tail were folded carefully forward and held by tape to keep them out of the scan field of view. Thus only the scanned hindlimb was imaged and irradiated by X-rays. The scans were of [x] minutes duration, delivering

an X-ray dose of [x] mGy as calculated by the Bruker *"CT-ion"* X-ray dose calculator.

For ex vivo:

Following harvesting, the [bone samples] were cleaned of excess soft tissue and fixed in buffered formalin / gluteraldehyde / etc.. / other preparation method.

The [bones] were removed from alcohol / frozen / etc. storage and mounted within a sealed plastic tube sample holder, wrapped in paper tissue moistened in distilled water / saline

OR

The bones were scanned in the same alcohol in which they were stored, in Eppendorf plastic tubes with internal physical anchoring of the bone by sponge foam / dental wax / etc. to prevent movement during scanning.

3. Scan parameters

The [bone samples] were scanned in the [SkyScan model] at a nominal resolution (pixel size) of [x] microns employing an aluminium / a copper / etc. filter [x] mm thick and an applied x-ray tube voltage of [x] kV.

Camera pixel binning of 2x2 / 4x4 was applied

OR camera pixel binning was not applied.

The scan orbit was 180 / 360 degrees with a rotation step of [x] degrees.

Note – frame averaging can optionally be mentioned, it is not essential.

It is better not to mention source current in milli or micro amperes. This parameter does not affect the x-ray energy (only filter and voltage do) and different scanner models have different source power so specifying current is an unnecessary obstacle to replicating research. Likewise it is

not necessary to mention camera exposure time; for other settings being the same this can differ even for different scanners of the same model, and readers attempting to replicate such parameters exactly will again prove unnecessary and counter-productive. X-ray energy characeristics (for most sources with tungsten targets) are determined by filter and voltage alone.

If you wish to mention how the camera signal strength was optimised you could optionally use a sentence such as the following:

"X-ray source current and camera exposure time were adjusted to give an average camera signal intensity, in the raw uncorrected image (no flat field correction) and empty field of view (air only), of 60% of saturation."

4. Reconstruction

Reconstruction was carried out with a modified Feldkamp[2] algorithm using the SkyScan™ NRecon software accelerated by GPU[3].

OR

Reconstruction was carried out with a modified Feldkamp[4] algorithm using the SkyScan™ NRecon software accelerated by hierarchical calculation-reducing algorithm using InstaRecon™ software[5].

Gaussian smoothing, ring artefact reduction and beam hardening correction were applied [as applicable].

Note – numerica values of reconstruction parameters such as smoothing, ring artefact reduction and beam hardening correction do not need to be added as these will apply only to users of SkyScan systems.

5. Region/Volume of interest selection for trabecular and cortical bone

Region / volume of interest selection, segmentation to binary and morphometric analysis were all performed using SkyScan CT-Analyser ("CTAn") software. Volumes of interest (VOI) for both trabecular (metaphyseal) and cortical (metaphyseal-diaphyseal) were selected with reference to the growth plate. A crossectional slice was selected as a growth plate reference slice by finding the first visible bridging of the chondrocyte seam across the medulla while moving axially slice-by-slice toward the growth plate from the metaphysis/diaphysis. Trabecular and cortical regions were defined as segments along the long axis of the femur relative to the growth plate reference level. The trabecular region commenced about [x] mm ([x] image slices) from the growth plate level in the direction of the metaphysis, and extended from this position for a further [x] mm ([x] image slices) in a diaphyseal direction. Within the trabecular VOI, separation of the trabecular from cortical bone was done using a freehand drawing tool, the boundaries of the selected trabecular VOI running parallel and close to the endocortical boundary – but excluding peripheral vestiges of the growth plate and associated primary spongiosa.

The cortical VOI commenced about [x] mm ([x] image slices) from the growth plate level in the direction of the diaphysis, and extended from this position for a further [x] mm ([x] image slices) in a diaphyseal direction.

[Note – if the sample is a different bone site or an object other than bone, it is still necessary to explain the essential principal and criteria by which the VOI was selected for analysis.]

6. Image segmentation to binary

Global thresholds were selected

- by visual matching with greyscale images

OR

- by the Otsu algorithm[6].

The same global threshold values were applied to all measured [bone samples] corresponding to bone mineral density (BMD) values of [x] and [x] g.cm^{-3} calcium hydroxyapatite (CaHA), calibrated by reference phantoms (Bruker-microCT, Kontich, Belgium) containing 0.25 and 0.75 g.cm^{-3} CaHA evenly mixed in epoxy resin rods which were of similar diameter to the scanned bones to minimise beam hardening error.

OR

Adaptive thresholding was applied based on localised analysis of density, to minimise partial volume effect and thickness biasing.

[Note – for non-bone samples the threshold values should be expressed as attenuation coefficient (AC). Values of AC obtained from NRecon and CTAn should be multiplied by 2 x ∏. For non-mineralised biological tissues Hounsfield units (HU) can be used as the x-ray opacity unit. Both AC and HU are also available in the CTAn software.]

7. Morphometric analysis

3D and 2D morphometric parameters were calculated for the trabecular and cortical selected VOIs.

Noise objects were removed from the binarised image by the despeckle and / or open and close morphological operations in CTAn.

OR

The signal to noise ratios of the reconstructed images and segmented binary images did not necessitate any subsequent image processing.

Morphometric parameters in 3D were based on analysis of a Marching Cubes[7] type model with a rendered surface.

Calculation all of 2D areas and perimeters was based on the Pratt[8] algorithm of sub-pixel perimeter interpolation.

Morphometric parameters measured by CT-analyser have been validated on both virtual objects and aluminium foil and wire phantoms[9]. Structure thickness in 3D was calculated using the local thickness or "sphere-fitting" (double distance transform) method[10, 11, 12], and structure model index (an indicator of the relative prevalence of plates and rods) was derived according to the method of Hildebrand and Ruegsegger[13]. Degree of anisotropy was calculated by the mean intercept method[14]. Euler connectivity was calculated using the "Conneulor" method[15] and fractal dimension was calculated in 3D by the Kolmogorov box counting method[16],[17] . The definitions, symbols and units for bone morphometric parameters follow the ASBMR standardised nomenclature[18].

8. 3D Model construction

Surface-rendered 3D models were constructed for 3D viewing of trabecular and cortical analysed regions, using SkyScan CTVolume ("CTVox") software. Model construction was by the "Double time cubes" method[19], a modification of the Marching cubes method[5].

OR

Volume rendered 3D images were generated using an RGBA transfer function[20] in SkyScan CT-Voxel ("CTVox") software.

9. References

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