

Microstructural Assessment of Cancellous Bone Using 3D Microtomography

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Abstract. Cancellous bones have a porous microstructure and can be modeled as linear elastic solid, heterogeneous and anisotropic. Few studies regarding the morphometric analysis of trabecular bone samples with 3D microtomography have been published so far. The technique has spread worldwide for the characterization of trabecular structures in studies related to bone quality and its relationship with metabolic diseases bone like osteoporosis. In our study cancellous bone samples with cubic and cylindrical geometry were extracted from bovine femur were used to investigate the structural arrangement of bone through high resolution x-ray 3D microtomography (μ CT). Four trabecular microstructural parameters (tissue volume, bone volume, bone volume fraction and tissue surface) were measured by 2D (stereological method) and 3D morphometric analysis using the software CTan Analyser supplied by the manufacturer of the microtomograph (SkyScan, model 1172, Belgium). The measurements were done in three main directions (superior-inferior, medial-lateral and anterior-posterior) to investigate the correlation between the 2D and 3D morphometric analysis. The results show a high correlation between the analysis. The x-ray 3D microtomography technique has a great potential for the assessment of bone quality.

1. Introduction

The assessment of macroscopic properties of biological tissues such as bone as well as the assessment of its microscopic structure is of great interest in the characterization of bone quality. The architecture of bone tissue varies with the region of the skeleton, which may be spongy, named cancellous or trabecular bone, or compact, named cortical bone. Cancellous bone has a porous microstructure with numerous small interconnected trabeculae (100-300 μ m of thickness, 300-1500 μ m spacing between adjacent trabeculae) [1] and a high surface area. It is predominantly found at the ends of long bones protected by a layer of cortical bone. It is also found in the internal structure of the calcaneus, the skull, pelvis and vertebrae of the spine. Approximately 20% of the skeleton is cancellous. The trabeculae tend to be oriented along the direction of principal stress in adaptation to external loading of

the environment. Between 75-95% of the cancellous bone consists of interconnected pores filled with bone marrow. It has the ability of getting deformation prior to fracture when subjected to a load. Trabeculae orientation in a sample of cancellous bone varies with direction, as shown in Figure 1. In these images the zy plane is a preferred orientation of the trabeculae and no preferred orientation can be seen in the planes xy and zx.

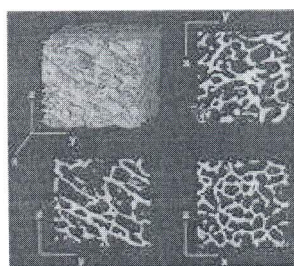


Figure 1: 3D images and cross sections of a cancellous bone sample obtained by 3D microtomography [2].

Trabecular bone density was related to the strength and stiffness [3-7]. Despite the density measurements often lead to reasonable estimates of mechanical properties, they do not explain certain variations in these properties nor are they influenced by the microstructure of cancellous bone, thus representing a scalar measure unable to totally predict the mechanical properties. Two bone samples with volume fractions ($BV / TV = \text{Bone Tissue Volume} / \text{Volume}$) close together, 18% and 19%, respectively, had mechanical properties that ranged from 30% to an order of magnitude, depending on the direction analyzed [5]. X-ray 3D microtomography (μCT) is a non-destructive technique that allows visualization of cross sections (internal cuts or slices) of an object [8], three dimensional reconstruction of the object as well as 2D and 3D morphometric analysis [9, 10]. The μCT technique has made significant contributions to the investigations on bone quality assessment [9-12].

2. Materials and methods

The Eighteen cylindrical samples of cancellous bone (10 mm of diameter and 20 mm of height) were obtained from six bovine femurs using the following methodology carried out at the Biomechanics Laboratory, Institute of Orthopaedics and Traumatology, General Hospital, College of Medicine, University of São Paulo at São Paulo and at the Mechanical Shop of the School of Engineering of Sao Carlos, University of São Paulo at São Carlos:

- a) Freezing the femurs at $-20\text{ }^{\circ}\text{C}$.
- b) Searching at room temperature the main directions of the trabeculae using an x-ray radiograph of each femur.
- c) Removal of a cubic trabecular sample of each femur with the trabeculae oriented in the main directions. The sample dimensions were approximately 30 mm x 30 mm x 30 mm. The removal used an apparatus that allowed angular and longitudinal movement of the femur (Fig. 2) and a circular saw (177.8 mm of radius and 3.2 mm of thickness) attached to a cutting machine working at 5500 rpm.

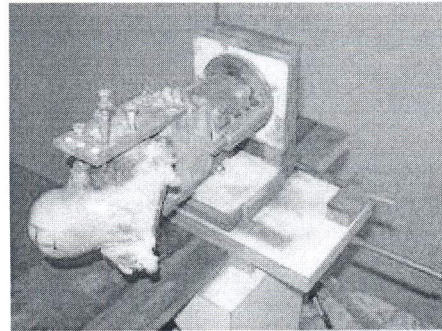


Figure 2: Apparatus for removal of the cubic sample.

- d) Extraction of three cylindrical trabecular samples (10 mm of diameter and 20 mm of height) from each cube monitoring the temperature of the sample with a infrared digital temperature sensor (MT-350 Brand Minipa, output power below 1 mW, $\pm 0,5$ °C precision, 630-670 nm wavelength) to avoid necrosis of the bone tissue during the drilling process [13]. The extraction is illustrated in Fig. 3. The cylinder in each main direction was taken using a lathe with a radial drill working at low rotation where was attached a carbide cannula trephine with 10 mm internal diameter, 12 mm external diameter and 25 mm of height (Fig. 4).
- e) Immersion of the cylindrical samples in saline solution (0.9% NaCl) until the structural quantification by 3D microtomography using the CT-Analyzer software supplied by mananu.

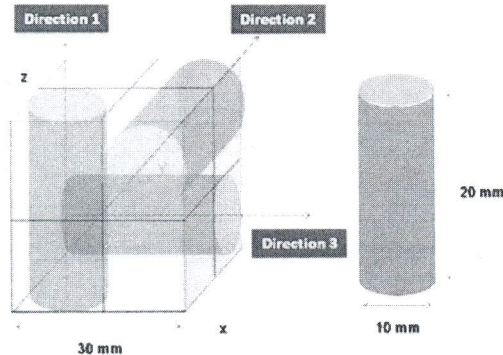


Figure 3: Schematic of the extraction of three cylinders in the three main directions.

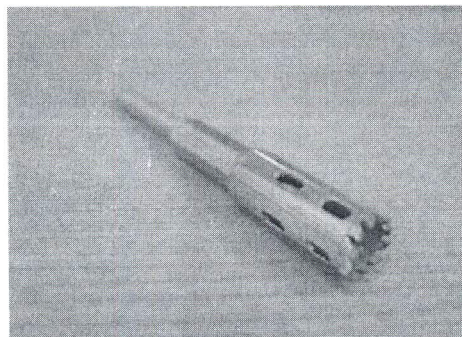


Figure 4: Cannula trephine to extract the 10 mm x 20 mm cylinder.

The high resolution microtomograph (model 1172, SkyScan, Belgium) at EMBRAPA - Agricultural Instrumentation has a micro focus x-ray source operating at high voltage (100kV), a specimen holder with a x, y and z precision movement, and a 10MP (4000 x 2300 pixels) CCD camera detector. A computer host controls the data acquisition. A cluster of computers carry out the tomographic reconstruction. This system can be represented by the diagram in figure 5.

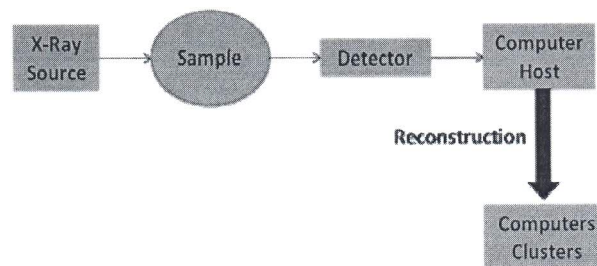


Figure 5: Microtomograph diagram [8]

The microstructural analysis of the cylindrical samples was held at EMBRAPA - Agricultural Instrumentation (Sao Carlos, SP, Brazil) with the following procedure:

- a) Each cylindrical sample was kept in a circular template using "modeling clay" to get a vertical alignment, to avoid undesirable movements and maintain the sample in the visual field of the radiation detector (CCD camera).
- b) The reconstruction of the entire volume of the samples (10mm x 20mm) couldn't be carried out due to problems with the computer memory. A volume of interest (VOI) with 1.4 mm of height (201 cross sections) from the central region of the sample was taken for the 2D and 3D morphometric analysis. The cross sections were TIF/16 bits format images with a resolution of 6.7 μm for each pixel. The software CTan Analyzer version 1.7.0.0 was used in the analysis.

3. Results

Table 1 shows the cubic samples dimensions and the temperature (maximum and minimum) measured during the drilling procedure for getting the cylindrical samples.

Table 1 - Values of the parameters (cubic samples)

Cubic Sample	Dimension	Cylindrical Sample	Temperature (° C)	
			Minimum	Maximum
(Cube 1) 1st Pair right femur	23 x 26 x 25	1	18,0 ± 0,5	26,0 ± 0,5
		2	21,0 ± 0,5	33,0 ± 0,5
		3	20,0 ± 0,5	26,0 ± 0,5
(Cube 2) 1st Pair left femur	25 x 26 x 26	1	19,0 ± 0,5	42,0 ± 0,5
		2	20,0 ± 0,5	28,0 ± 0,5
		3	20,5 ± 0,5	24,0 ± 0,5
(Cube 3) 2nd Pair right femur	21 x 21 x 23	1	18,0 ± 0,5	27,0 ± 0,5
		2	18,0 ± 0,5	23,0 ± 0,5
		3	19,0 ± 0,5	21,0 ± 0,5
(Cube 4) 2nd Pair left femur	24 x 26 x 27	1	18,5 ± 0,5	26,0 ± 0,5
		2	19,0 ± 0,5	32,5 ± 0,5
		3	18,0 ± 0,5	23,0 ± 0,5
(Cube 5) 3rd Pair right femur	25 x 25 x 23	1	19,0 ± 0,5	25,0 ± 0,5
		2	20,5 ± 0,5	27,0 ± 0,5
		3	20,0 ± 0,5	23,0 ± 0,5
(Cube 6) 3rd Pair left femur	23 x 25 x 22	1	20,0 ± 0,5	26,0 ± 0,5
		2	19,0 ± 0,5	21,0 ± 0,5
		3	19,5 ± 0,5	21,5 ± 0,5

Among the eighteen samples nine were damaged in the drilling process. Two samples (Fig. 6 and 7), which couldn't be scanned in the same region of microtomography analysis were discarded.

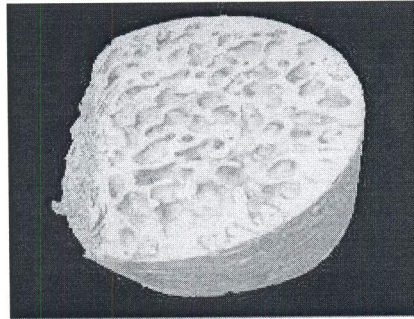


Figure 6: Sample 4, 3D reconstruction.

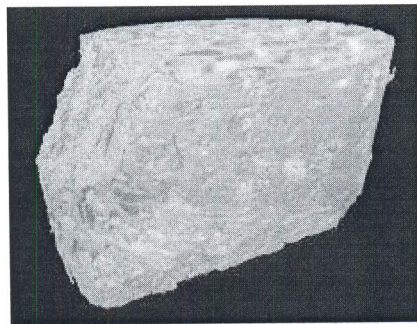


Figure 7: Sample 11, 3D reconstruction.

Tables 2, 3 and 4 show the averaged values for each structural parameter from the 2D and 3D morphometric analysis in the superior-inferior, lateral-medial and anterior-posterior main direction.

Table 2 - Average values of the microstructural parameters from the 2D and 3D morphometric analysis in the superior-inferior direction (n = 6).

Parameters	Abbreviation	Unit	2D	3D
Tissue Volume	TV	mm ³	111,67	111,94
Bone Volume	BV	mm ³	54,69	54,39
Bone Volume Fraction	BV/TV	%	49,05	48,64
Tissue Surface	TS	mm ²	212,23	212,59

Table 3 - Average values of the microstructural parameters from the 2D and 3D morphometric analysis in the lateral - medial direction (n = 5).

Parameters	Abbreviation	Unit	2D	3D
Tissue Volume	TV	mm ³	108,37	108,92
Bone Volume	BV	mm ³	51,10	51,17
Bone Volume Fraction	BV/TV	%	47,15	46,98
Tissue Surface	TS	mm ²	207,88	208,70

Table 4 - Average values of the microstructural parameters from the 2D and 3D morphometric analysis in the anterior-posterior direction (n = 5).

Parameters	Abbreviation	Unit	2D	3D
Tissue Volume	TV	mm ³	110,00	110,42
Bone Volume	BV	mm ³	57,90	58,18
Bone Volume Fraction	BV/TV	%	52,53	52,68
Tissue Surface	TS	mm ²	210,82	211,26

The correlation between the structural values from the 2D and 3D morphometric analysis can be seen in figures 8 to 11. The correlation was calculated by the Statistica Software (version 8.0).

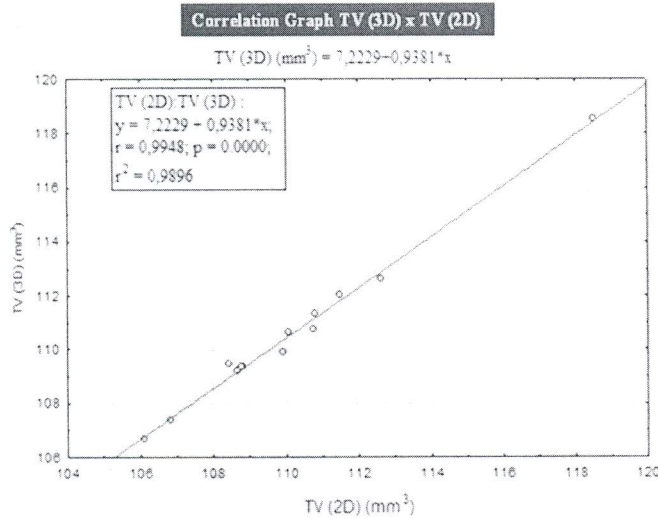


Figure 8: Correlation between bone tissue volume (TV) values from the 2D and 3D morphometric analysis.

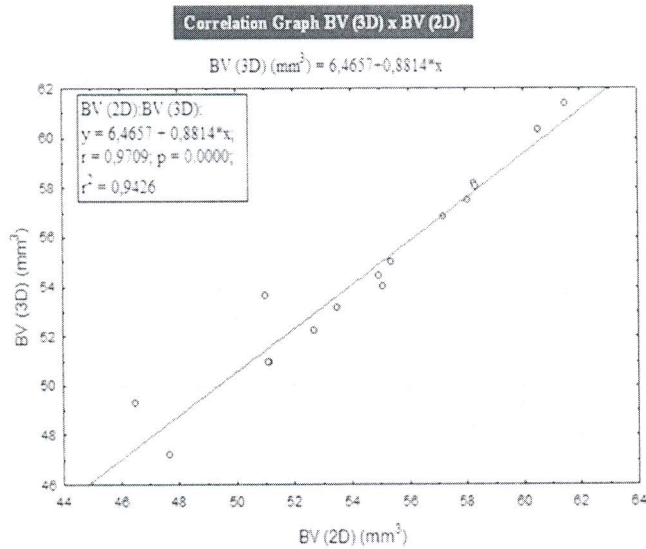


Figure 9: Correlation between bone volume (BV) values from the 2D and 3D morphometric analysis.

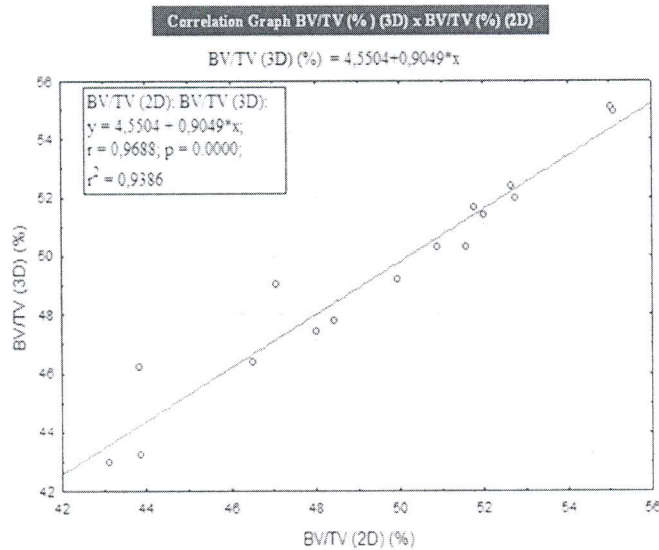


Figure 10: Correlation between bone volume fraction values (BV/TV) from the 2D and 3D morphometric analysis.

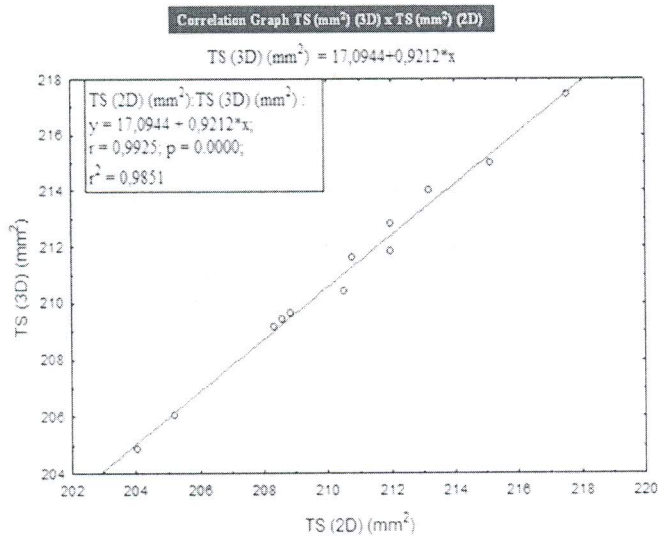


Figure 11: Correlation between bone tissue surface (TS) values from the 2D and 3D morphometric analysis.

4. Conclusion

There is a strong correlation between measurements of the structural parameters tissue volume (TV), bone volume (BV), bone volume fraction (BV/TV) and tissue surface (TS) measured by 2D and 3D morphometric analysis using x-ray 3D microtomography (μ CT) and the CTan Analyser software supplied by the manufacturer of the microtomograph (SkyScan, model 1172, Belgium). The μ CT is an important tool for the quantification of cancellous bone architecture.

Acknowledgments

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