

# High resolution image in bone biology I. Review of the literature

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## ABSTRACT

Bone microstructure has usually been assessed by obtaining samples invasively and analyzing them with conventional histomorphometric methods. Improvements in high-resolution image acquisition systems have enabled non-invasive assessment of bone morphology and a more precise 3-D evaluation by means of “virtual biopsies”, permitting bone assessment in regeneration or remodeling processes. This review describes the characteristics and limitations of bone assessment using different high-resolution image systems (synchrotron-radiation computed tomography, micro-computed tomography, acoustic scanning microscope; micro-magnetic resonance imaging). Morphometric variables that can be obtained from these images are reported and compared with conventional histomorphometric variables.

**Key words:** *High-resolution image, bone biology, virtual biopsy.*

## RESUMEN

La valoración de la microestructura ósea se ha realizado habitualmente mediante la obtención invasiva de muestras y el procesado y evaluación de las mismas con métodos convencionales histomorfométricos. La mejora de los sistemas de obtención de imágenes con alta resolución permite la valoración no invasiva de la morfología ósea con evaluaciones tridimensionales más precisas, con las denominadas “biopsias virtuales” que permiten realizar la valoración del hueso en procesos de regeneración o remodelación. Este trabajo de revisión describe las características y limitaciones de la evaluación ósea de diferentes sistemas de imagen de alta resolución (Tomografía computerizada mediante radiación sincrotrón; Micro tomografía computerizada; Microscopio de escaneado acústico; Micro imagen por resonancia). También se describen diversas variables morfométricas que pueden ser obtenidas a partir de las imágenes obtenidas y que pueden ser comparadas con las variables histomorfométricas convencionales.

**Palabras clave:** *Imagen alta resolución, biología ósea, biopsia virtual.*

## INTRODUCTION

Various studies have shown that bone resistance cannot be explained by the bone mass index alone (1). Determination of the bone mineral density (BMD) by dual-energy X-ray absorptiometry (DEXA) is inadequate to assess the biomechanical properties of bone. Other factors that appear to influence bone resistance are the trabecular microstructure, bone remodeling index, bone mass distribution, microfissure accumulation, mineral crystal quality, collagen fiber quality, and degree of mineralization (2).

Bone response to local mechanical stimuli was described in 1892 by Wolff (3), who established the hypothesis that "Every change in the form and function of bones, or of their function alone, is followed by certain definite changes in their internal architecture and equally definite secondary alteration in their external conformation, in accordance with mathematical laws". Although the relationship with possible mathematical laws has been questioned, it is not doubted that mechanical changes affect the internal organization of bone as well as its volume. .

Recent advances in high-resolution imaging techniques, micro-magnetic resonance imaging ( $\mu$ MRI) and micro-computed tomography ( $\mu$ CT), permit evaluation of bone microarchitecture in *in vitro* samples (4). Assessment of morphometric values by means of two-dimensional histological images entails error accumulation due to structure overlap and the appearance of artifacts during sample preparation (jump cuts, excessive widths) (5). Development of new equipment for *in vivo* studies opens the way to so-called "virtual biopsies". These will offer more precise three-dimensional (3-D) bone morphometry studies compared with images obtained by invasive acquisition methods with bone biopsy fixation and embedding. Other image acquisition methods (scanning or transmission electron microscope, backscattered electron image, atomic force microscope, infrared spectroscopy, and Raman spectroscopy) have not been proposed for clinical application and were not considered in the present review.

Clinically, bone microarchitecture is assessed to predict bone fracture in patients with osteoporosis and to determine effects of pharmacological treatments (bisphosphonates) that cannot be evaluated by bone densitometry methods (4). *In vitro* images have been used to assess bone microstructure in maxillary bones (6), raising the prospect of using *in vivo* images to predict the prognosis of implantology patients and establish the optimal time for implant loading. We review the different high resolution imaging techniques used for bone biology assessment, describing the main morphometric parameters considered and their function in bone mechanobiology in general and in maxillary bones in particular.

## HIGH RESOLUTION IMAGING TECHNIQUES

Three-dimensional assessment techniques must be adapted to the characteristics of bone microstructure. The thickness of spongy bone trabeculae is usually 100-150  $\mu$ m separated by spaces of 500-1000  $\mu$ m. If the resolution is not adjusted to trabecula size (sections thicker than 500  $\mu$ m), variables

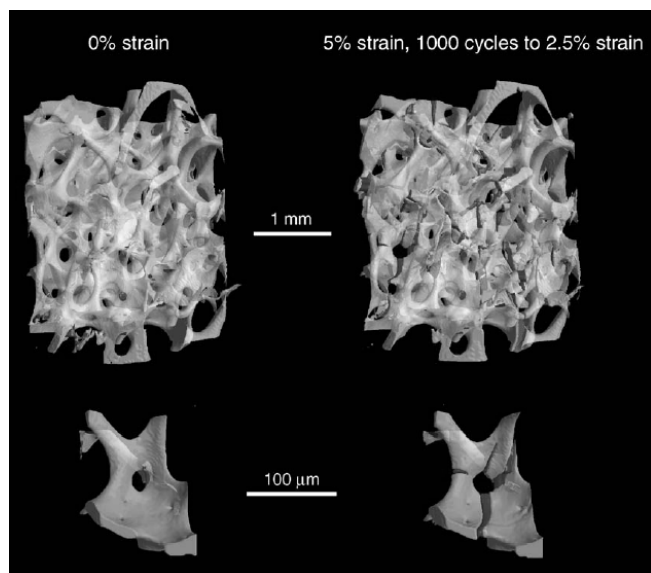
obtained are considered "apparent" or as "texture analysis", since trabeculae can overlap and no direct access to the trabecular structure is possible (4, 6). Thus, conventional tomography methods, with a maximum resolution of 250  $\mu$ m, cannot be used to study the average individual size of a trabecula (7).

Synchrotron-Radiation Micro-Computed Tomography (SR $\mu$ CT) is the reference method for assessing trabecular microarchitecture parameters and can produce resolutions of around 1  $\mu$ m and even below the micrometric scale (nanoCT). Parallel monochromatic radiation (photons with the same energy that do not produce beam hardening) is used, with no geometric distortion and an optimal signal-noise ratio (8). It is based on the acceleration of electrons that generate a high amount of secondary radiation emission. More coherent, monochromatic and parallel light beams are obtained with this method than with conventional X-ray source, which improves the resolution and contrast of the final image. The system requires a high-cost infrastructure. Only equipment for use in *in vitro* studies is currently available. (Fig. 1) (9).

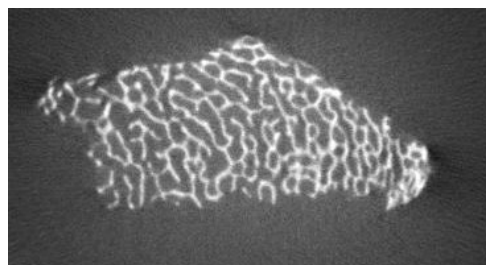
-Micro Computerized Tomography ( $\mu$ CT). Polychromatic radiation is generated (photons with different energy) and the beam diverges conically, which may produce geometric distortion, unlike in SR $\mu$ CT, (8). The resolution obtained ranges from 7 to 20  $\mu$ m depending on the equipment (Fig. 2). One difficulty concerns the appropriate setting of the differentiation threshold between mineral tissue and bone marrow, which can have a major effect on morphometric results. With polychromatic radiation, the center of the sample is more exposed to higher energy X-rays and has a darker appearance versus the periphery. Therefore, the gray level distribution is determined by sample size as well as by the degree of mineralization if a "beam hardening" correction is not included in the image reconstruction process (10).

With 14- $\mu$ m resolution, the percentage difference between histomorphometry and  $\mu$ CT is 2.5% for bone volume/tissue volume (BV/TV) and 6.1% for trabecular separation (Tb.Sp) (11). It is at present an invasive method and can only be performed in small *in vitro* samples, although new equipment is being developed for use in small animals. This procedure has some shortcomings: a) The scintillation crystals that receive radiation contain defects that worsen with use, producing dark points that result in ring artifacts in the 3D reconstruction; b) an aliasing effect is produced by space resolution with a pixel size larger than the structure under study; and c) energy-dependent absorption produces beam hardening, generating refraction phenomena that can be partially corrected by reconstruction algorithms (5).

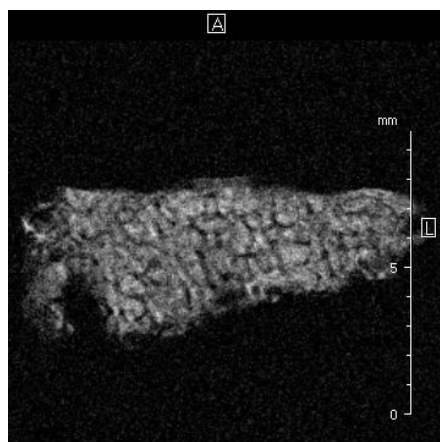
Using systems with lower resolution capacity is sometimes called micro-quantitative computed tomography ( $\mu$ QCT), which allows *in vivo* images to be obtained. It is used to assess bones in peripheral areas of limbs (distal radius, distal tibia or calcaneus) (pQCT). With current tomography equipment, spatial resolutions of about 250  $\mu$ m are obtained, too high for the width of the trabecula (12).



**Fig. 1.** 3-D visualization using SR $\mu$ CT image of bovine trabecula (spatial resolution of 7 $\mu$ m). Microcracks are visualized (in red) in sample subjected to compression with fatigue (on the right) and in sample subjected to compression without fatigue (on the left). Published by permission of Professor Müller.



**Fig. 2.** Topographic image of bovine vertebra sample by  $\mu$ CT (axial slice). Spatial resolution: 0.059 x 0.059 x 0.059 mm. Acquisition time: 8 min. HQT-Y15D System, SUINSA Medical Systems.



**Fig. 3.** 2-D image of bovine vertebra by T1-weighted spin-echo (high-resolution magnetic resonance imaging). Spatial resolution: 0.059 x 0.059 x 1.00 mm. Acquisition time: 6 min

Although resolutions of around 50  $\mu$  can be obtained with this technique, large X-ray doses (about  $25 \pm 360$  mSv) are required, which limits its use. Although a lower resolution is achieved compared with the above methods,  $\mu$ QCT can be used to obtain in vivo images of peripheral bone areas and for micro-finite element ( $\mu$ FE) studies (4).

- Scanning Acoustic Microscope (SAM). With this approach, the object to be visualized is explored with sound waves and the signal is digitally reconstructed in a single image. By this means, an image of the acoustic (or elastic) properties of a material is obtained, providing better definition of different types of tissue within the bone (mature and immature bone) as a function of their elastic properties. The main advantage of this method over direct biomechanical tests is its non-destructive character. Detected acoustic impedance (Z) can be assessed in 2-D with a resolution proportional to the wavelength used (25  $\mu$ m for 50 MHz, and 2  $\mu$ m for 900 MHz)(13). The main disadvantage versus the SR $\mu$ CT technique is that a specific preparation of the sample is required and 3-D study is not possible. However, it offers quantitative assessment of the elastic properties of the material (13).

- Nanoindentation is a mechanical (not imaging) assessment technique that, like the above imaging technique, visualizes the elasticity properties of structures in the bone. It is based on conventional methods for estimating material hardness, making a mark on the material with a pyramidal diamond indenter (14).

- Micro-Magnetic Resonance Imaging ( $\mu$ MRI). The resonance signal has its origin in transitions between excited and relaxed states of protons in live tissue after being subjected to an electromagnetic field (6). In contrast to CT, images of bone tissue show high grayscale levels (near-black) close to background noise, whereas bone marrow shows low gray levels (near-white). One of the main drawbacks is that the gray level of any air bubble in the sample is similar to that of mineralized tissue (6). Image segmentation is especially difficult under these conditions, and the setting of an inappropriate threshold may lead to an under- or over-estimation of morphometric values.

Spatial resolutions of around 50  $\mu$ m can be obtained but high magnetic fields are required. Development of this method is centered on the improvement of performance (obtaining magnetic fields of >10 tesla). To date, this technique has been limited to small in vitro samples of around 1 cm<sup>3</sup>, although, as with  $\mu$ CT, systems are being developed for in vivo studies of small animals (4).

With this technique, resolutions of 80-200  $\mu$ m and slice thicknesses of 300-700  $\mu$ m are obtained (15). Acquisition of 3-D clinical images in vivo is impossible because of the time required to acquire the signal. However, this technique is useful for in vitro experiments and for the in vivo study of small experiment animals (6). (Fig. 3)

There are two main types of signal acquisition: gradient-echo and spin-echo sequences. The former has a higher probability of artifacts and therefore overestimation. Spin-echo has a better signal-noise ratio (SNR) but requires

longer acquisition times, which can be compensated for by higher resonator power (16). The increase in magnetic field intensity (3 Tesla fields) in clinical equipment implies an increase in the signal-noise ratio, enhancing image quality, reducing acquisition time, and improving spatial resolution (16). In calcaneal cadaver bone, images obtained with a 3-T field showed a higher correlation with  $\mu$ -CT values than did those obtained with a 1.5-T field (16).

## MORPHOMETRIC PARAMETERS WITH HIGH RESOLUTION IMAGES

Histomorphometry and stereology are the conventional methods to assess bone microstructure. With these techniques, the sample is observed under light microscope with a resolution of around  $1\mu\text{m}$ . In this case, 3-D measurements are made by inference, with the resulting errors derived from the anisotropic nature of trabecular bone. Reducing the number of errors in 3-D estimation requires serial sections and a higher sample volume, and a biopsy must be obtained (4). In contrast, conventional radiology can achieve a resolution of  $40\text{-}\mu\text{m}$  thickness, and digital methods permit 2-D texture analyses but not 3-D evaluations.

Histomorphometric variables were originally designed for calculation from 2-D images acquired after the processing of bone tissue samples (17). Many variables used for conventional histomorphometry can be used with high resolution images, which offer greater precision for 3-D variables compared with conventional stereology techniques. Regardless of the 3-D reconstruction method used, the result is an image in grayscale. The information must be reduced in such a way that each voxel has a "bone" value or "marrow" value in the computer segmentation process (8).

- Bone Volume (Bone Volume/ Tissue Volume [BV/TV]): This 3-D parameter relates bone volume to total tissue volume. The value is obtained by adding together the volume of voxels within the triangles into which the 3-D surface is divided. The number of voxels with bone is then divided by the total number of voxels. When this measurement is taken from a 2-D image, the bone area is obtained (Bone Area/Tissue Area [B.Ar/T.Ar]) (6). This value is below 100% in trabecular bone but close to 100% in cortical bone (7). The BMD (Bone Mineral Density) can be obtained the voxels that contain bone by means of the different gray levels and mineralization ( $\text{g}/\text{cm}^3$ ), which represents the proportion of hydroxyapatite crystals (18).

- Bone Surface (Bone Surface/Bone Volume [BS/BV]). This 3-D parameter relates the bone surface to total bone volume and is obtained by triangulation of the trabecular surface. It increases with a decrease in the number of trabeculae. In patients with osteoporosis, the value of this ratio increases with progression of the disease (12, 19).

- Number of trabeculae (Trabecular Number [Tb.N]),. This 2-D parameter can be calculated by using the Mean Intercept Length (MIL) method developed by Whitehouse (20). A grid of parallel lines is superimposed on the image. A ratio is obtained between the intersection points of the lines and the bone-marrow interface of the trabeculae, in

relation to the total length of the grid-lines. This method can also be used to obtain trabecular separation (Tb.Sp) and trabecular thickness (Tb.Th) parameters, but it is not valid for 3-D assessment. These three variables can be obtained directly from a 3-D image by measurements in each voxel or with the system by the localization of spheres within and among trabeculae (21).

- Index of the structure of the model (Structure Model Index [SMI]). This variable indicates the prevalence of the rod-like or plate-like structures of the trabecular structure. It is obtained by differential analysis of the triangulated surface of a 3-D structure using a mathematical model. It is quantified from level 0 (plate-like structure) to level 3 (rod-like structure) (22). Osteoporosis is characterized by a shift from plate-like to rod-like structures of trabeculae.

- Degree of Anisotropy (DA). The concept of isotropy refers to the completely regular or irregular spatial orientation of a structure. Thus, bone that usually presents completely disoriented structures (trabeculae) combined with oriented structures (depending on the load) is considered anisotropic material (8). The DA can also be obtained from a 3-D image, as the ratio of the maximum to minimum radius of an ellipsoid created by the MIL method using a grid on all dimensions of the volume of interest. The DA increases with a decrease in the biomechanical resistance of bone and is reduced by the trabecular orientation caused by loads (isotropic orientation) (12, 23). It can also be obtained using the Volume Orientation (VO) method described by Odgaard (24).

- Bone Mineralization Ratio-Volume (BMR-V). This index of bone mineralization as a function of volume requires very high spatial resolution levels to distinguish between the different degrees of mineralization of bone and can be obtained by synchrotron radiation imaging. It is calculated by dividing the number of voxels with high mineralization by the number with low mineralization (19). This parameter has also been demonstrated to correlate with conventional histomorphometric bone turnover values (25).

- Number of Havers Canals/Cortical Bone Area (N.Ca/Ar). This variable is used to assess the porosity of cortical bone alone. A resolution of at least  $20\mu\text{m}$  are required for its assessment, mainly by SAM or SR $\mu$ CT (13).

## CONCLUSIONS

- High-resolution image acquisition methods are not yet available in the clinical setting, and their use is limited to experiments with small animals or to the study of in vitro samples.

-The resolution obtained with magnetic resonance imaging does not achieve the results obtained with CT in bone. The development of higher power resonators could avoid CT radiation in in vivo studies of bone architecture.



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