Assessment of Bone Micro-architecture using 3D Computed Microtomography

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The investigation of trabecular bone structure requires a high spatial resolution imaging tool, providing non-destructively 3D images of bone samples. Synchrotron X-ray computed microtomography (CMT) fulfils these requirements and is used to study the evolution of bone structure with aging.

Most bones are composed of a cortical shell of compact bone surrounding an internal part of trabecular bone made of small rods and plates called trabeculae. The proportion of trabecular bone is particularly high in the vertebra, the iliac crest and the os calcis. The importance of the organisation of trabeculae with respect to the fracture risk has been highlighted by different authors. Indeed, the mechanical properties of trabecular bone depend both on the mineral density and on the microarchitecture. Bone mineral density is often estimated *in vivo* using Dual Energy X-ray Absorptiometry (DEXA), which is based on measurements at two different energies and gives an average value of the mineral content of bone (hydroxyapatite). The quantification of bone microarchitecture is mainly based on histology. Histological techniques consist in cutting bone biopsies, generally taken from the iliac crest, in very thin slices which are then imaged by X-ray microradiography or stained and observed using an optical microscope. Image processing techniques are then applied to extract histomorphometric parameters quantifying bone structure in terms of shape and connectivity [1-2]. This technique is destructive and two-dimensional, and only gives an estimate of the properties of the three-dimensional (3D) structure of bone. Moreover, it has been shown that the structure of bone is anisotropic, and requires 3D studies with high spatial resolution since the mean size of the trabeculae is around 100 microns [3]. Non-destructive imaging like X-ray computed tomography (CT) [4-8], or Magnetic Resonance Imaging (MRI) [9] has been developed to fulfil these requirements. X-ray computed tomography and microtomography (CMT) only differ because they have different spatial resolutions. X-ray absorption computed tomography is widely used for medical imaging (scanners), and is performed by reconstructing a 2D or 3D image of a patient from attenuation measurements at different angular positions. The CMT technique is greatly improved by the use of synchrotron radiation. Unlike laboratory X-ray sources, synchrotron radiation offers the possibility to select X-rays with a small energy bandwidth from the wide and continuous energy spectrum, while at the same time keeping the photon fluence rate high enough for efficient imaging. This possibility is of great interest for microtomography since it allows high spatial resolution images to be generated and avoids beam hardening artefacts, which occur with the use of polyenergetic beams for tomographic imaging. Synchrotron X-ray computed microtomography, which provides high resolution 3D images, is well suited for studying porous media in general, [11] and trabecular bone in particular.

Bone is a living tissue under continuous remodelling since the structure is resorbed by cells called osteoclasts and reconstructed by osteoblasts. If the balance between osteoclast and osteoblast activities is disturbed because of a pathology or aging, the architecture of bone deteriorates. Not only the quantity of bone mineral decreases, but bone architecture also becomes weaker due to the structural changes, which may lead to severe vertebral or hip fractures. We are particularly interested in studying the evolution of bone structure with

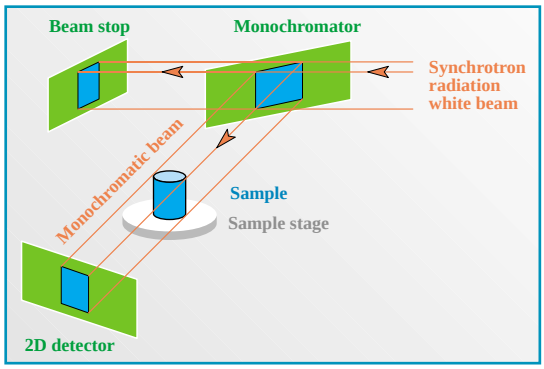

Fig. 1: Scheme of the acquisition set-up.

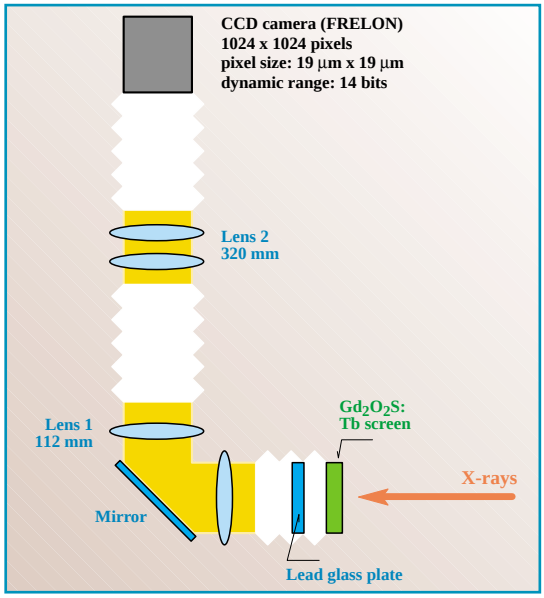

Fig. 2: CCD based 2D detector.

aging. The purpose of this work is to improve the understanding of the changes occurring in bone architecture by finding relevant parameters characterising bone structure and studying their correlation with age.

METHODS

In conventional CT scanners, the sample is usually fixed while the X-ray source and the detector rotate around it. This geometry is of course not possible with a synchrotron radiation source, and the rotation must be applied to the sample. Therefore, the sample to be imaged is mounted on a translation/rotation stage allowing precise alignment in the beam. A 2D detector records projections of the sample for different angular positions as shown in Figure 1. A typical scan includes 900 projections of the sample over 180 degrees. The detector is the most critical part of the set-up since it determines the spatial resolution in the image, contrary to first generation tomography, where the spatial resolution depends on the beam size or the sampling distance. The detector we used is based on the FRELON CCD (Charge Coupled Device) camera developed by the ESRF Detector Group [10]. It consists of a 2D CCD array with 1024 x 1024 elements, each 19 x 19 μm^2 and offers a dynamic range of 14 bits. A thin scintillating Gd2O2S:Tb layer deposited on glass converts X-rays to visible light. Light optics then magnify the image of the screen and project it onto the CCD. The CCD camera is mounted perpendicularly to the X-ray beam in order to protect it and avoid direct interactions which cause noise in the recorded images. Different lenses and scintillators with different thicknesses can be used to adjust the pixel size to the sample size. For bone structure studies, we used an optical magnification of 2.86 resulting in a pixel size of 6.65 microns and a field of view of 6.8 mm x 6.8 mm. A schematic drawing of the detector is presented in Figure 2.

A 3D filtered back projection algorithm, implemented in C language, is used to reconstruct a 3D image of the sample from the series of 2D projections. Given the large quantity of data to be handled (about 2 Gigabytes per sample), the reconstruction process is quite time-consuming. Therefore the reconstruction program was parallelised on the ESRF Networked Interactive Computing Environment (NICE) using PVM (Parallel Virtual Machine), leading to considerable time saving (4 hours on 9 workstations instead of several days on a single workstation to reconstruct a $(512)^3$ volume).

RESULTS

A CMT experiment was performed on the Topography and High Resolution Diffraction beamline (ID19). A series of ten bone samples (4 mm x 4 mm x 4 mm) cut from human vertebrae embedded in lucite were imaged using 20 keV X-rays. The images presented in Figures 3 to 5 show volume rendering views of samples from women at three different ages. Ray tracing was used to obtain these views. The structural change (bone loss, decrease in connectivity) with age is obvious.

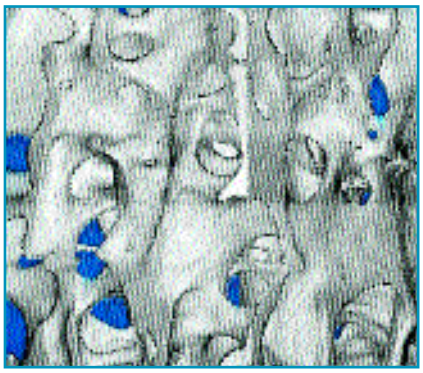

Fig. 3: 3D tomographic reconstruction of a vertebra sample from a 33 year old woman. The field of view is 4 mm x 4 mm.

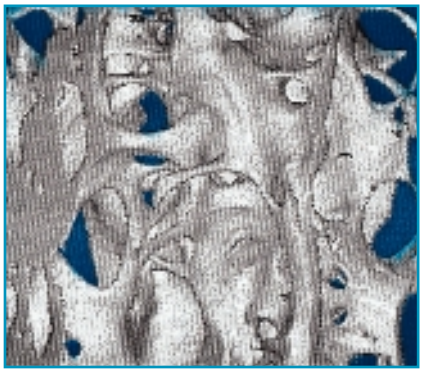

Fig. 4: Same as Figure 3, 55 year old woman.

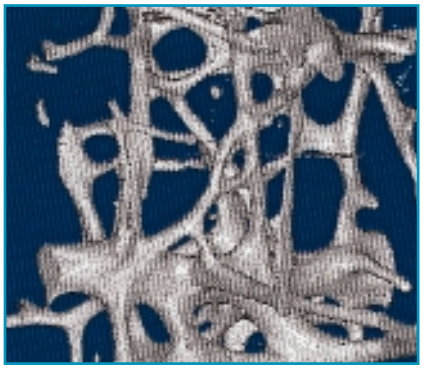

Fig. 5: Same as Figure 3, 72 year old woman.

The first step in the analysis of images is to distinguish bone tissue from the background, here lucite. This operation is called segmentation and provides a binary image. The contrast in the images was high enough to make possible segmentation with a global threshold, equal for all images. Conventional parameters defined in histomorphometry such as trabecular bone volume fraction in the sample (denoted BV/TV), trabecular bone surface-to-volume ratio (BS/BV), and mean trabecular thickness (Tb.Th), were extracted from the binary images and correlated to age. As an example, the age dependency of BV/TV is presented in Figure 6. A significant correlation coefficient (r = 0.75) was found between BV/TV and age.

The measured parameters were compared to histomorphometric measurements performed previously on a histological slice of the same vertebra and were found to be in good agreement. A correlation with r = 0.8 was also established between BV/TV and the ash weight measurement performed on a 1 cm^3 sample of the same vertebra (see Figure 7). Synchrotron radiation microtomo-graphy confirms the results obtained in histology, and overcomes its limits. Firstly the measurements performed using microtomography should be more accurate because they are done on a larger sample than a single isolated slice and thus take into account the variability inside the vertebra, and secondly this technique allows 3D connectivity measurements which can hardly be estimated from 2D slices and justify fully the need for 3D images of trabecular bone. We are currently working on developing such 3D connectivity parameters derived from so called skeletonisation of the 3D images [11].

CONCLUSION

Synchrotron radiation microtomo-graphy is an excellent tool for investigations of trabecular bone because it gives access to the 3D organisation of bone structure at a relevant scale. So far, we have studied alterations of bone structure as a function of age in women, but many other studies are possible in the field of bone research, e.g. the effect of drugs against bone diseases like osteoporosis and foetal vertebrae growth. ■

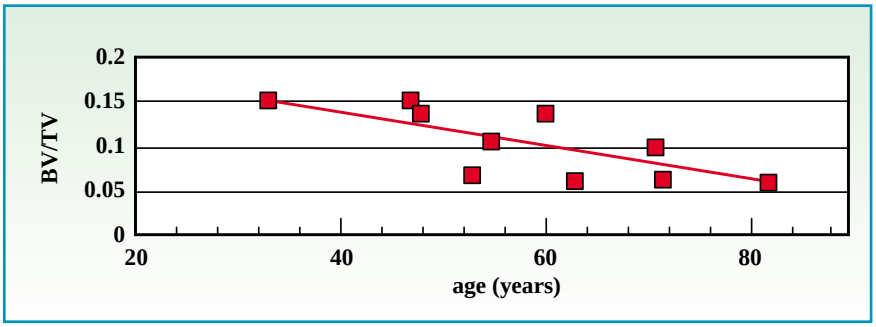

Fig. 6: BV/TV plotted as a function of age.

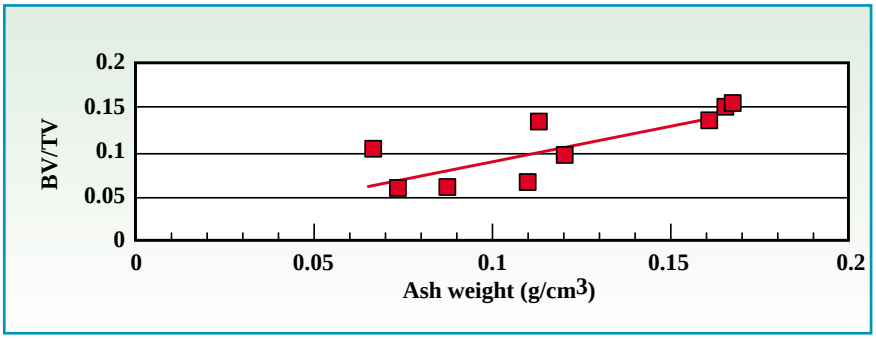

Fig. 7: BV/TV plotted as a function of ash weight measured on a 1 cm^3 sample.

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