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3D MICROSCOPIC IMAGING BY SYNCHROTRON RADIATION MICRO/NANO-CT

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ABSTRACT

Biological microscopic imaging is receiving increasing interest with the development of new modalities. In this context Synchrotron Radiation (SR) Nano-CT demonstrates a high potential in opening new horizons. We recall the principle of SR micro-CT and its advantages over standard X-ray micro-CT in terms of accuracy and signal to noise ratio. We briefly present data acquisition and reconstruction in absorption and phase CT. While in the first case image reconstruction is based simply on the standard filtered back projection algorithm, in the later case it involves a preliminary step of phase retrieval. Applications of this technique in bone research in conjunction with specific image analysis developments are shown. At the micrometer scale, trabecular and cortical bone have been analyzed in human samples and mice. At the nanometer scale, we present recent data on the osteocyte network which has never before been investigated in 3D. Open problems for the segmentation and quantification of the complex canalicular network are highlighted.

***Index Terms*—** X-ray CT, micro-CT, nano-CT, synchrotron radiation, bone micro-architecture, osteocyte network

1. INTRODUCTION

Biological microscopic imaging is receiving increasing interest with many new emerging modalities. However, the techniques for investigating biological samples in 3D at a micrometer or nanometer scale remain limited. Confocal imaging which is the most widespread 3D technique suffers from anisotropic spatial resolution and limitation in penetration depth. In this paper, we show that advances in Synchrotron Radiation (SR) micro/nano-CT open new opportunities for 3D imaging of biological tissue at the micro and nanometer scales.

X-ray micro-CT relies on the same principle as medical X-ray CT but at a much higher spatial resolution. During the last ten years this technique has moved from laboratory prototypes to commercial systems widely available. The development of this technique has been strongly motivated by the investigation of trabecular bone micro-architecture which required spatial resolution higher than 100µm [5]. Conversely to histology, X-ray micro-CT allows truly 3D non destructive evaluation of core samples. Its applications are now spread out to many other fields in pre-clinical imaging, biology and material science. This technique covers a wide range of spatial resolution between 100µm down to sub-micrometer.

Coupling X-ray micro-CT to synchrotron sources presents a number of advantages in terms of image quality [16]. In this case, a high flux monochromatic X-ray beam is extracted from the synchrotron light. Thanks to brilliance several orders of magnitude higher than that of conventional X-ray sources, the signal to noise ratio (SNR) of the image is considerably improved. This is even more important at very high spatial resolution since for a given SNR, the X-ray dose varies as the fourth power of the pixel size. In addition, the use of a monochromatic beam enables to work with the assumptions of the Beer-Lambert law fulfilled and to avoid bean hardening artifacts occurring in standard CT.

X-ray and SR micro-CT have been extensively used in bone research for the quantitative assessment of trabecular and cortical bone microstructure in humans or in animal models. SR micro-CT is a quantitative imaging technique in the sense that it provides a map of the X-ray linear attenuation coefficient at a given energy. For bone samples, this has the advantage over standard X-ray CT that it gives information simultaneously on microstructure and mineralization. However up to now, the attention has been mainly focused on scales of a few micrometers.

In this paper, we highlight the perspectives offered by SR micro-CT to investigate samples down to nanometric resolutions. In sections 2 and 3 we describe image formation in absorption and phase SR micro-CT. In section 4 we review applications of this technique and associated image analysis to study bone microstructure. Finally we discuss the perspectives in imaging bone cellular network at the nanometer scale and in 3D, together with the developments required in terms of image analysis.

2. DATA ACQUISITION

2.1. Absorption SR micro/nano-CT

Data acquisition in absorption SR micro-CT consists in recording a sequence of radiographs of a sample irradiated

by a monochromatic X-ray beam extracted from synchrotron light. Since the exit of the beam is fixed, the sample is mounted on a rotation stage and is sequentially rotated around an axis. A 3D SR micro-CT setup has been developed on beamline ID19 at the ESRF [16] [19]. The monochromatic beam is obtained by using either a double reflection SI crystal or a multi-layer monochromator. Radiographs are acquired on a 2D detector composed of a scintillator, converting X-ray photons into visible light, light microscope optics for magnification and the 2048x2048 FReLoN CCD camera developed at the ESRF. The energy of the beam can be optimized for each series of samples in a range between 10 to 80 keV. Thanks to different optical systems, the pixel size on the detector can be set between 30 µm down to 170nm.

2.2. Phase SR micro/nano -CT

While standard micro-CT is based on X-ray attenuation contrast, coherent synchrotron sources give access to another mode of contrast, known as phase contrast. This may be simply obtained experimentally by propagation, i.e; by letting the beam propagate after interaction with the sample. The advantage of phase contrast on absorption is that it has higher sensitivity and thus permits to image low absorbing samples, such as plants and soft tissues or enhance the differences between materials with similar attenuation.

Experimentally data acquisition in phase SR micro-CT consists in recording one or several CT scans at different distances d of propagation. The experimental setup is the same as previously, but it includes a horizontal translation stage for the detector. Phase contrast appears as fringes patterns that increase with the sample-detector distance d.

3. IMAGE RECONSTRUCTION

3.1. Absorption SR micro/nano-CT

Let $\mu(\mathbf{x})$ be the 3D linear attenuation coefficient image for the X-ray beam of wavelength λ where $\mathbf{x}=(x,y,z)$. Its 2D projection $p_\theta(u,v)$ for rotation angle θ around the z-axis can be modeled by the Beer-Lambert law as:

$$p_\theta(u,v) = ln\left(\frac{I^0_\theta(u,v)}{I_\theta(u,v)}\right) = \int_{D_{\theta,u,v}} f(x,y,z)\ ds \qquad (1)$$

where $D_{\theta,u,v}$ is the line describing the X-ray path.

Unlike in most standard X-ray micro-CT where a cone beam X-ray is used, the projection can be considered as parallel due to the long distance between the source and the object. Thus, tomographic reconstruction is exact and can be performed by standard Filtered Back Projection (FBP):

$$\mu(x,y,z) = \int_0^\pi \tilde{p}_\theta(x\cos\theta + y\sin\theta, z)\ d\theta \qquad (2)$$

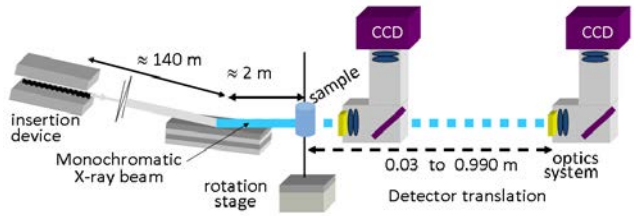

Figure 1: Illustration of data acquisition in SR micro-CT. The detector can be translated to enable beam propagation and phase contrast imaging.

where $\tilde{p}_\theta(u,v)$ is the projection filtered with respect to the first variable with the conventional 1D ramp filter.

3.2. Phase Retrieval in micro/nano CT

The modeling of phase contrast involves the 3D complex refractive index $n(\mathbf{x}) = (1-\delta(\mathbf{x})) + i\lambda\mu(\mathbf{x})/4\pi$ [9]. The transmittance function $T_\theta(u,v)$ corresponding to the incident wave transmitted by the object is defined as:

$$T_\theta(u,v) = exp(-B_\theta(u,v) + i\varphi_\theta(u,v)) \quad with:$$

$$B_\theta(u,v) = \frac{1}{2}\int_{D_{\theta,u,v}} f(\mathbf{x})ds \qquad \varphi_\theta(u,v) = \frac{-2\pi}{\lambda}\int_{D_{\theta,u,v}} \delta(\mathbf{x})ds \qquad (3)$$

By modeling the effect of propagation by the Fresnel transform, the intensity of the recorded phase radiographs for rotation angle θ and distance d is expressed as:

$$I_{\theta,d}(u,v) = \left|(T_\theta * P_d)(u,v)\right|^2$$
$$P_d(u,v) = \frac{-i}{\lambda d} exp\left(\frac{i\pi}{\lambda d}(u^2+v^2)\right) \qquad (4)$$

Since only the intensity is available, the phase term $\varphi_\theta(u,v)$ is encoded in this expression but not directly available. Thus, reconstruction of the 3D refractive index decrement $\delta(\mathbf{x})$ requires first to solve a "phase retrieval" problem to estimate $\varphi_\theta(u,v)$ from images at different propagation distances, and second, to perform tomographic reconstruction from the phase maps for the different rotation angles.

Phase retrieval is a non linear ill-posed inverse problem. The usual solutions to this problem are based on linearized approximation of the problem. The classical solutions are known as Transport of Intensity Equation, Contrast Transfer Function or mixed approaches. We have recently compared quantitatively these approaches from simulated and constructed phantoms [9]. In presence of noise, the reconstructions are often corrupted by low frequency artifacts introducing a non constant background. To solve this problem, a regularized method based on an homogeneity prior was recently proposed and showed a significant improvement in various applications [10].

4. APPLICATION IN BONE RESEARCH

4.1. 3D bone microstructure imaging

Bone tissue has a hierarchical organization from the macro to the nano scale. At the microstructural level, one can distinguish two types of bone, a highly porous bone called trabecular bone and a dense bone called cortical bone.

Trabecular bone consists of a complex arrangement of struts or trabeculae having a mean thickness around 100-150µm. Figure 2a) shows a 3D rendering of trabecular bone acquired in SR micro-CT with a voxel size of 10µm. Thanks to the high SNR, the continuity of the network is well preserved even after simple thresholding which is not guaranteed with standard X-ray micro-CT [4]. After segmentation, typical morphometric parameters such as partial bone volume, trabecular thickness, trabecular spacing and bone surface to bone volume can be calculated [7][8][12]. The estimation of such parameters from 2D histological sections is based on stereology and requires model assumptions. The availability of the 3D image permits the computation of model independent parameters which are thus more accurate. Connectivity and topology can also be quantified. In previous work, we proposed a local topological analysis method to label each voxel of the 3D image as a plate or a rod (see Fig 2b)) [2]. Since it is known that young trabecular bone is organized as a mixture of plates and rods which evolves towards a more rod-like structure with ageing, it is of interest to quantify the volume and ratio of plates and rods [15].

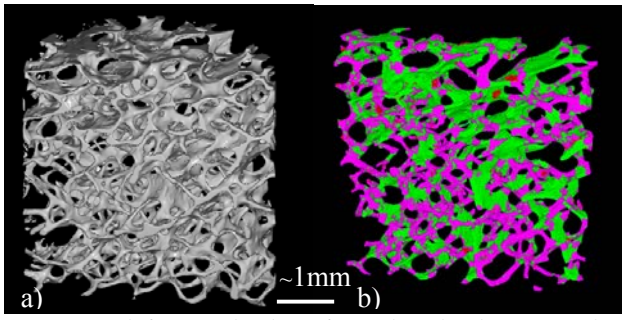

Figure 2 : left : 3D display of a trabecular bone sample in human femoral bone (voxel size : 10µm), right : labelling of plates (green) and rods (pink) structures.

Cortical bone is illustrated in Figure 3a) showing a raw SR micro-CT slice [3]. Due to the quantitative nature of the image which is a faithful map of the linear attenuation coefficient, the gray levels are representative of the local concentration in mineral [13]. According to the remodeling process in bone, darker gray levels (lower mineral concentration) correspond to newly formed bone. Thus conversely to standard micro-CT, SR micro-CT allows to study mineralization simultaneously to microstructure. Nevertheless the contrast of the remodeling regions is weak, and we proposed a specific method to segment them [14].

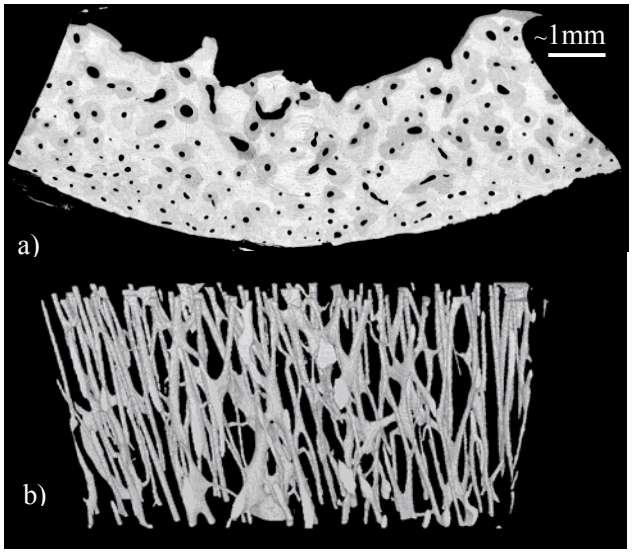

Figure 3 : top : SR micro-CT slice in a cortical bone sample in human femoral bone (voxel size : 10µm), right : labellisation of plates (green) and rods (pink) structures

The organization of the pores visible as black areas in a single slice can be visualized in 3D as illustrated in Figure 3b). The morphometry, anisotropy and connectivity of the pore network can thus be studied. This canal network hosts vessels which were able to be observed after opacification with a contrast agent [10].

4.3. 3D bone cellular imaging

At the cellular level, the osteocytes play a fundamental role as they have been demonstrated to act as orchestrators of bone remodeling process. The osteocytes are located in small ellipsoidal cavities (a few µm long), called lacunae and they communicate through slender channels (diameter of a few hundreds of nm) called canaliculi. Today, a major concern is the limited methods currently available to assess the lacunar-canalicular system that would ideally require a very high resolution 3D technique providing a sufficiently large field of view.

We recently showed the feasibility of visualizing the 3D organization of this complex network by using SR nano-CT [1]. Figure 4a) represents a 3D phase contrast slice of a cortical bone sample acquired with a voxel size of 280nm. As can be seen on a magnified ROI (Figure 3b)), black ellipses correspond to osteocyte lacunae while very small linear features or spots correspond to the intersection of canaliculi with the sectioning plane. After contrast enhancement by a vesselness filter, it was possible to obtain a 3D display of the lacunar-canalicular network (Figure 3c)). This 3D rendering demonstrates the high complexity of this cellular structure.

SR nano-CT thus appears as a promising tool to observe in 3D cellular structures such as the lacunar-canalicular network in sufficiently large field of views.

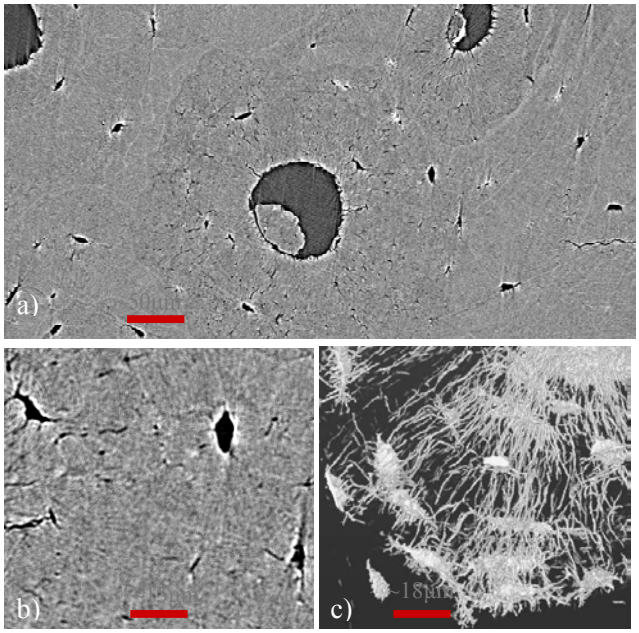

Figure 4: top: SR nano-CT slice in a cortical bone sample in human femoral bone (voxel size: 280 nm), bottom left: zoom, right: 3D display of lacunae and canaliculi.

These unprecedented images are demanding in terms of image segmentation: the automatic detection of canaliculi is challenging due to the size of canaliculi, very close or slightly below the resolution limit, the high canalicular density, and noise unavoidable at this resolution even with the very high flux of synchrotron sources. A truly 3D approach is required and has to be efficient on very large data sets (> 8 Gb) thus adding to the complexity of the task.

5. CONCLUSION

SR micro CT possesses significant advantages for the analysis of biological samples. Thanks to the high SNR and contrast of the image, it enables quantitative analysis of composition and microstructure at a scale of a few micrometers. In addition, it has the capability to image features at the nanometer scale in 3D.

This technique opens remarkable perspectives for the three-dimensional evaluation of biological tissue at the cellular scale. However, at this scale, the image quality is limited and future works will be devoted to improve both reconstruction and image analysis.

In conclusion, SR nano-CT associated to dedicated image analysis is expected to yield new and unique volumetric data on biological systems at the cellular scale.

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