

## 900 MHz-acoustic microscopy : nanostructural characteristics of cortical bone determine elasticity at the micron scale

Mathilde Mouchet<sup>1,5</sup>, Aurélien Gourrier<sup>2,4,5</sup>, Fabienne Rupin<sup>1,5</sup>, Kay Raum<sup>3</sup>, Françoise Peyrin<sup>4,5,6</sup>, Amana Saïed<sup>1,5</sup>, Pascal Laugier<sup>\*1,5</sup>. (<sup>1</sup>Univ Pierre et Marie Curie, Paris France; <sup>2</sup>Univ Paris Sud, Paris France; <sup>3</sup>Julius Wolff Intitut & Berlin-Brandebourg School of Regenerative Therapies, Charité Universitätmedizin, Berlin Germany ; <sup>4</sup>ESRF, Grenoble, France ; <sup>5</sup>CNRS ; <sup>6</sup>INSERM)

### 1. Introduction

The osteon is one of the fundamental functional unit of human cortical bone. Each osteon consists of concentric lamellae, made of a composite material of mineral platelets (hydroxyapatite), with variable size and orientation distributions, embedded in an organic matrix of collagen fibers. The elastic properties of lamellae of human cortical bone have been investigated using scanning nanoindentation (1) and acoustic microscopy (2). Both the nanoindentation modulus and acoustic impedance showed a characteristic bimodal lamellar pattern of alternating high and low values. Previous reports suggested that the lamellar modulation of microelastic properties is related to the lamellar level modulation of the mineral content (1) or to variations of the lamellar orientation (2). Both assumptions are plausible, however, the orientation of mineral platelets and collagen fibers were not directly assessed for a face-to-face confrontation with microelastic measurements. Furthermore, the influence of mineral platelets thickness on tissue level elasticity has not been investigated so far. The goal of this study was to evaluate the osteon level variation of acoustic impedance and to assess its relationship with respect with mineral platelet orientation and size with a micrometre resolution.

### 2. Methods

Data were acquired on the same locations of a cross-section of a human femoral mid-shaft using 900-MHz scanning acoustic microscopy, synchrotron radiation micro-computed tomography (SR- $\mu$ CT) and small angle X-ray scattering (SAXS). The local variations of mineral content were determined from SR- $\mu$ CT and the variations of orientation of mineral platelets were obtained from the integrated SAXS intensity. Moreover, the mean thickness of mineral platelets in bone was deduced from the analysis of the SAXS patterns (3).

### 3. Results

The well-known lamellar level modulation was observed in SAM (Fig.1) and SAXS (Fig.2) images, but not in the SR- $\mu$ CT images (Fig. 3). While the absence of modulation on SR- $\mu$ CT images indicates a constant level of mineral in the explored region, the SAM and SAXS images indicate a modulation of microelasticity, orientation and thickness (not shown here) of the osteonal mineral platelets. The local acoustic impedance showed a strong positive correlation with the SAXS intensity ( $R^2=0.91$ ,  $p<0.0001$ ) (Fig.4) and a much lower correlation with the platelets thickness ( $R^2=0.35$ ,  $p<0.05$ ).

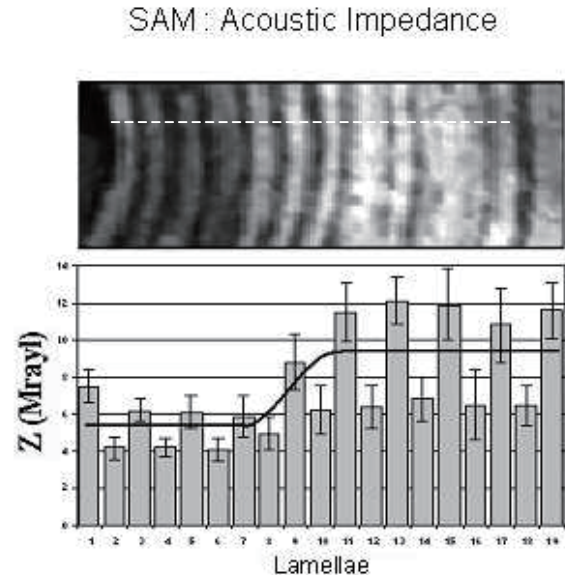


Figure 1: Lamellar modulation of acoustic impedance observed on the image (top) and the distribution across the osteon (bottom).

### 3. Discussion and conclusion

This is the first study that combines SAXS, SR- $\mu$ CT and SAM imaging in order to elucidate the impact of mineral platelets orientation and mean thickness on microelasticity. The alternating pattern of high and low impedance values across a human

osteon was found in spite of a homogeneous distribution of mineral quantity. Our results suggest that the main factor contributing to these impedance variations is the platelet orientation reflected in modulations of the integrated SAXS intensity and that the mean platelet thickness contributes only to a small extent to the variations in acoustic impedance.

SAXS : Orientation of mineral

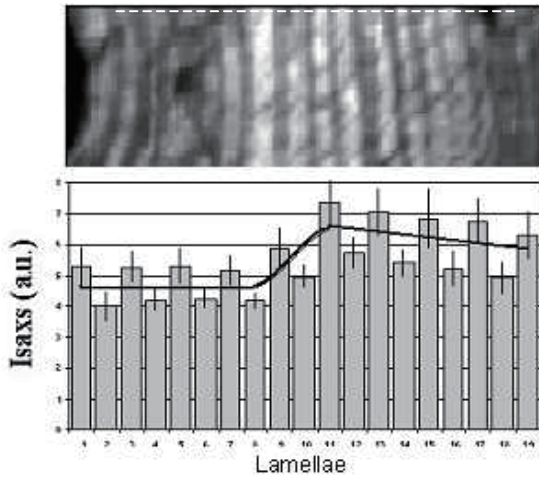


Figure 2: Lamellar modulation of SAXS intensity (reflecting the orientation of hydroxyapatite platelets) observed on the image (top) and the distribution across the osteon (bottom).

SR-μCT : degree of mineralization

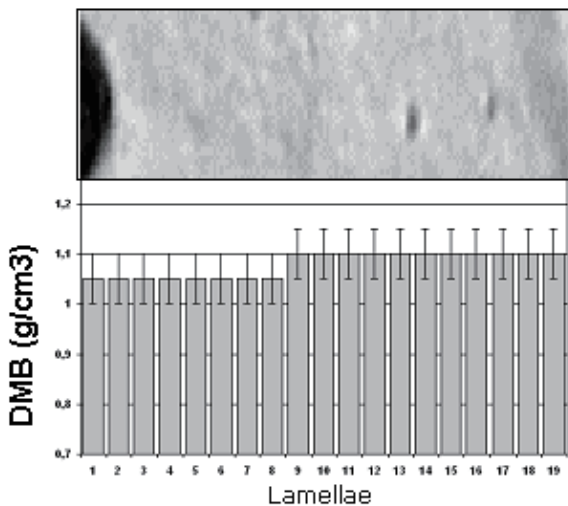


Figure 3: The quasi constant mineralization is illustrated on SR-CT image (top) and distribution across the osteon.

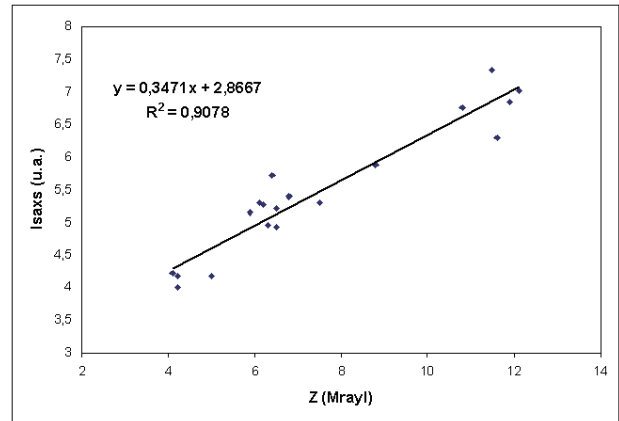


Figure 4: Correlation between acoustic impedance and SAXS intensity

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### References

1. H.S. Gupta, U. Stachewicz and W. Wagermaier. *J Mater Res.* **21** (2006) 1913.
2. T. Hofmann, F. Heyroth, H. Meinhard, W. Fränzel and K Raum. *J Biomech.* **39**(2006) 2282.
3. W. Wagermaier, H.S. Gupta, A. Gourrier, O. Paris, P. Roschger, M. Burghammer, C. Riekel and Fratzl P. *J Applied Crystallography* **40** (2007) 115