Synchrotron X-Ray CT of Wet Cortical Bone's Nanostructure: Technique and Results

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Introduction:

Bone's complex hierarchical structure, from the microstructural patterns of osteons and bone packets (~100-200 μm), to lamellae (~3-7 μm) to mineralized collagen fibrils (~100 nm), results in its remarkable fracture resistance. However, it remains difficult to visualize bone's three-dimensional (3D) structures at sub-micron scales to understand their functions. Further experimental challenges arise due to the wet environment required for reliable bone structure imaging. Lens-based synchrotron X-ray computed tomography (CT) offers much higher resolution (~ tens of nm) as compared to traditional CT and can be performed in a wet environment. The goal of the present research was to demonstrate a successful method allowing to image the nanostructure of wet (i.e., fresh) bone through the use of femtosecond laser ablation and state-of-the-art high resolution synchrotron X-ray CT.

Specimens and Experimental Technique:

Longitudinal 100 μm thin slices of fresh bovine cortical bone specimens were obtained by grinding and polishing (Fig. 1a). Selected locations were then micro-machined in the form of single-edge notched specimens using a femtosecond pulsed laser. The Ti-Sapphire femtosecond laser had a pulse length of 40 fs centered at 800 nm with a repetition rate of 1 kHz and was focused on the specimens with a 10x microscope objective. A coarse machining was first performed with energy of 30 μJ followed by a finer machining at energy of 10 μJ to limit damage to the specimens. The reduced sections after machining were approximately 12 × 12 μm (Fig. 1b).

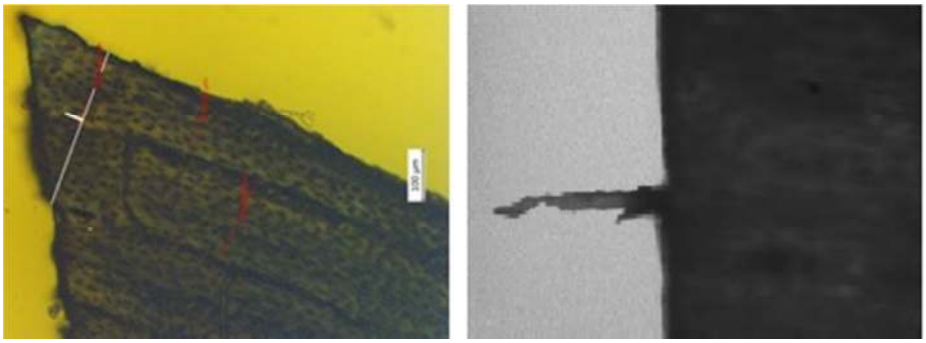

Figure 1 a) Longitudinal 100 μm thin section of bovine fibrolamellar bone (10x). Bone packets are easily visible, b) Laser micro-machined nano-CT specimen with reduced section of about 12 × 12 μm (10x). The location of the laser cut is shown in a.

X-ray nano-CT experiments were carried out at sector 26 of the Advanced Photon Source (Argonne National Laboratory). The hard X-ray nano-probe set-up consists of a capillary condenser which focuses the X-rays onto the specimen and a zone plate which collects and focuses the X-rays on a CCD camera [1]. The system is mainly sensitive to absorption contrast and provided a resolution of 14.74 nm/pixel at 10 kV. In order to properly align the projections and facilitate 3D reconstruction, gold particles (0.5-0.8 μm in diameter) were deposited on the specimens' surfaces.

Wet specimens were mounted inside a cylindrical environmental chamber made of 20 µm thick, X-ray transparent Kapton film. The specimens were partly immersed in PBS solution, not to interfere with the X-ray path. The chamber provided a moist environment for the specimens during the 3 hours required for the tomography scans.

For CT, a series of 1801 projections were taken every 0.1° from 0° to 180°. Due to computational RAM limit, a subset of 900 images (every 0.2º) was selected for the initial reconstructions. TXM Wizard [2] was used for alignment and reconstruction and ImageJ [3] was used for subsequent image processing and 3D visualization. There was no major filtering on gray-scale.

Results and Discussion:

The nano-CT specimens were cut within individual bovine fibrolamellar bone packets (Fig 1a). In the case shown in Fig. 1a, the cut was performed in a lamellar region between an osteocyte lacunae and a blood channel. This location contains many transversally oriented canaliculi (> 1×10^6 canaliculi/mm^3 [4]) linking the osteocyte lacunae to the blood channel. Hence, there was a high probability of observing canaliculi in the reduced section of the specimen that would be imaged with nano-CT (Fig. 1b). Fig. 2 shows the ortho-slices reconstructed tomography of the reduced section (within the drawn black line). A 200 nm diameter canaliculi can be clearly seen (dark channel) running across the section, as expected. Future work will focus on high quality reconstructions (i.e., full series) to discern mineralized collagen fibrils and fibrillar bundles.

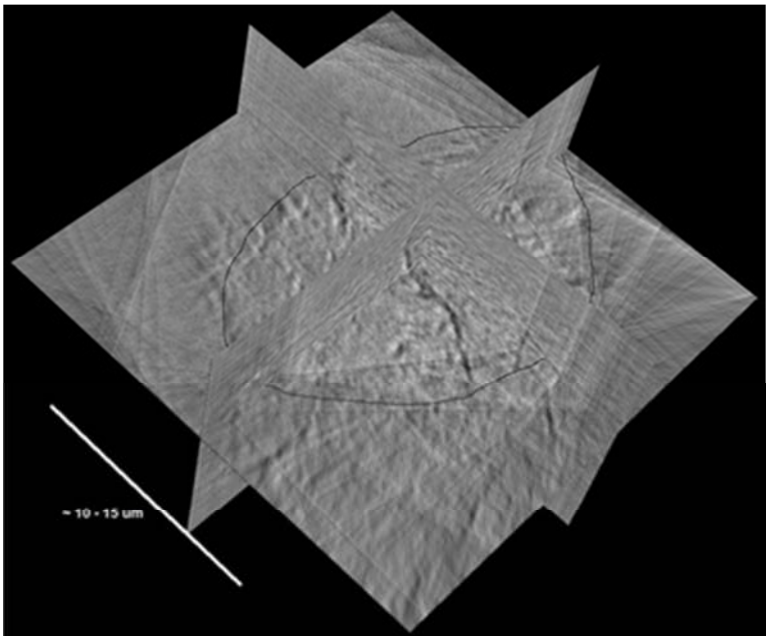

Figure 2 Ortho-slices of the reconstructed nano-tomography showing a 200 nm diameter canaliculi (black channel) running across the reduced section. Longitudinal direction from top-right to bottom-left.

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