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

Reference:

R. P. Winarski, M. V. Holt, V. Rose, P. Fuesz, D. Carbaugh, C. Benson, D. Shu, D. Kline, B. G. Stephenson, I. McNulty and J. Maser, "A Hard X-Ray Nanoprobe Beamline for Nanoscale Microscopy," *Synchrotron Radiation*, vol. 19, no. 1, pp. 1056-1060, 2012.

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Abstract:

It remains difficult to visualize bone's three-dimensional (3D) structures at sub-micron scales to understand their functions. Lens-based synchrotron X-ray computed tomography (CT) offers much higher resolution (~ tens of nm) as compared to traditional CT. The goal of the present research is to demonstrate a successful method and results allowing imaging the nanostructure of trabecular bone through the use of femtosecond laser ablation and state-of-the-art high resolution synchrotron X-ray CT. Selected locations were micro-machined from bovine trabecular bone in the form of single-edge notched specimens using a femtosecond pulsed laser. The reduced sections after machining were approximately $12 \times 12 \mu\text{m}$ that is transparent to hard x-ray from Synchrotron. 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. For CT, a series of 1801 projections were taken every 0.1° from 0° to 180° . Open source TXM Wizard was used for alignment and reconstruction and ImageJ was used for subsequent image processing and 3D visualization. There was no major filtering on gray-scale. The nano-CT specimens were cut from individual trabeculae within bovine trabecular network of cancellous bone packets. This work focuses on high quality reconstructions (i.e., full series) to discern mineralized collagen fibrils and fibrillar bundles in particular the change in orientation of fibrillar bundles. Some of the samples were arbitrary subjected to bending loading in order to introduce damage to the sample. The damage does not disrupt the entire cross-section and was limited to sub-fracture state. The laser machining was then cut from the damaged section and nano-CT was performed to study how the damage starts appearing in the trabecular bone in comparison with the no-damage samples.

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