X-ray microtomography of the vascular canal network in permineralized *Triceratops* bone

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Introduction

Bone has a complex adaptive internal structure with different strategies for rapid growth, for healing of micro- and macrofractures, and for the sensing of and the remodeling response to strains [1]. The hierarchical vascular system of bone spans submicron to meter length scales and is a key player in all bone adaptation. One of the main impediments to understanding the functional interactions of the systems involved is the opacity of the tissue, allowing examination by light and electron microscopy only on exposed surfaces or through thin sections. Hence, prior knowledge of the three-dimensional structures of the complex vascular systems of bone is largely inferential.

We are performing detailed direct x-ray microtomography (XMT) investigations of the three-dimensional bone histology of recent and extinct vertebrate animals. We have demonstrated the effectiveness of XMT for mapping the three-dimensional vascular canal network of bone down to micron length scales in a variety of genera.

Methods and Materials

This experiment was performed at sector 20-ID of the Pacific Northwest Consortium (PNC) beamlines at the Advanced Photon Source. The area detector of our prototype tomography apparatus follows the general considerations of Koch, *et al.* [2], with the exception that an inexpensive eight-bit room-temperature CCD camera was used as an initial cost-saving measure. A cooled CCD camera will be used in the final apparatus. The tomography sample stage consisted of two miniature linear translators for centering mounted atop an Aerotech ART-50 rotary stage. The rotary stage was itself mounted atop a homemade two-axis motorized tilt stage. These two degrees of freedom were used to ensure that the rotation axis was simultaneously perpendicular to the beam direction and to the effective CCD line scan direction with a precision of 10^{-5} radians. A standard filtered backprojection algorithm was used to reconstruct the tomographs from the rotational sequence of radiograms.

The sample studied here was a cranial fragment from a T*riceratops*, with an estimated age 65 million years [3], which had been permineralized (i.e. the vascular canals have been filled with a mineral, most likely calcium carbonate). A 50 µm diameter aluminum wire was glued along one vertical face of the sample as a fiducial orientation and absorption reference.

Results

We show in Figure 1 an XMT slice from the three-dimensional tomogram for this sample. This XMT slice is taken normal to the natural plane of two-dimensional tomographs reconstructed from each pixel row on the CCD, hence suppressing reconstruction artifacts.

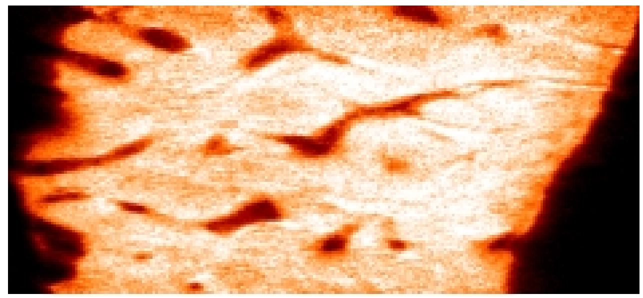

Figure 1: An XMT slice of a T*riceratops* skull fragment. The color scale has been enhanced to demonstrate that the small density differences between the permineralized vascular canals and the fossilized bone tissue can be resolved.

Discussion

Our preliminary work demonstrates that microdensity differences between the mineral fillings of vascular canals and the surrounding bone can be differentiated by XMT in fossil bone tissue, allowing quantitative tracing of changes in the three-dimensional vascular systems through extinct lineages over geologic time.

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