

Direct Local Measures of Mineral Apposition Rate in 3D

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Bone remodeling, at the level of individual remodeling events, is traditionally studied using dynamic bone histomorphometry. Dynamic histomorphometry in 2D is a specialized application of stereology. Like other stereology methods, 2D dynamic histomorphometry can be highly accurate, but often lacks precision (i.e. large variance measures within a group). Dynamic histomorphometry performed in 2D is also incapable of measuring individual remodeling event size, number, or variability among individual sites. As 3D measures of trabecular microarchitecture show improved precision over 2D approaches, a 3D approach to dynamic histomorphometry has the potential to provide improved precision. A 3D approach would also allow for measures of individual remodeling event size and number. We have developed a high resolution (0.7x0.7x5 $\mu\text{m}/\text{voxel}$) serial block face imaging technique capable of acquiring 3D images of cancellous bone and fluorescent bone formation labels. Here we demonstrate this technique using a common animal model of postmenopausal osteoporosis. Sprague-Dawley rats (N=5 Sham, N=5 OVX, 6-months) were subjected to double fluorochrome labeling. Four months after surgery, the fourth lumbar vertebrae were excised and imaged using serial block face imaging. Images of bone and two different bone formation labels were obtained and processed to achieve 3D images. Image processing was validated through comparison to manual tracing. Mineral apposition rates at individual formation events and variability in mineral apposition rates among formation sites were measured. Average mineral apposition rate within each specimen was also determined. The techniques described here are the first automated direct 3D measures of mineral apposition in cancellous bone.