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3D X-ray Microscopy: A New High Resolution Tomographic Technology for Biological Specimens

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A new field of 3D X-ray Microscopy (XRM) has emerged bringing dramatic resolution and contrast improvements to X-ray tomographic imaging of biological specimens for correlative studies and hierarchical structure investigations of hard and soft tissue. An X-ray microscope uses an X-ray source rather than a visible light source to view the internal structure of opaque specimens. Analogous to computed tomography (CT) a specimen can be imaged without physical sectioning and a complete 3D view of the object is generated. Yet X-ray microscopes provide superior spatial resolution down to the nanoscale and tunable phase contrast to image nature's vast diversity from cells to entire organisms *ex vivo* up to tens of centimeters in size.

Light microscopy and immunostaining techniques have become essential practice for biological and biomedical research providing functional information on the distribution of gene products such as proteins and ribonucleic acids from primarily 2D images. More recent developments, such as Light Sheet Fluorescence Microscopy (LSFM), have provided an attractive option for developmental biology and other research when observing millimeter-sized live specimens in 3D due to an optical architecture that enables fast acquisition with low phototoxicity [1]. Despite the revolutionary advancements of light microscopy, visible light has physical penetration limits due to specimen thickness or opacity. Micro-computed tomography (microCT), an alternative technique capable of producing full 3D images, uses multiple X-ray projections to reconstruct a 3D representation of an object. MicroCT has been highly useful for large objects such as the human body in vivo and applications requiring resolution no greater than ~5-10 μm.

However, demand is growing for applications that require 3D imaging with high resolution (single micron and below) and high contrast for hard and soft tissue as well as cell-level information. Laboratory XRM, which emerged in the past decade from the foundations of synchrotron-based X-ray imaging technology, produces direct 3D tomographic information from opaque specimens with resolutions well into the sub-micron range, even achieving 10's of nm resolution for certain architectures and applications [2]. This presentation will cover 3D XRM technique, followed by a survey of recent application areas for XRM within the life sciences where it acts as a complementary and correlative bridge between the contrast and resolution standards set by light and electron microscopy.

By integrating an imaging detector with sufficiently small and tunable pixel size coupled to optimized scintillation materials, the standard trade-off of sample size versus resolution found in conventional laboratory microCT are improved with XRM. High total system spatial resolution may be maintained while reducing the dependence on geometric penumbra. This, in turn, reduces the dependence of resolution on geometric magnification, relaxing restrictions on sample placement relative to the source and source spot size [3]. By employing such a geometry, samples of considerable size (tens of centimeters for low density objects) may be imaged in 3D with high resolution. In addition, as a result of small effective detector pixel dimensions and tunable detector and source positions, propagation

phase contrast may be employed to effectively highlight interfaces in samples that exhibit low absorption contrast, such as unstained tissue and other organic specimens.

Research topics currently being explored and facilitated by the use of XRM in the life sciences are diverse, including developmental biology, soft tissue, agriculture, hard-tissue, bio-engineered materials, including 3D scaffold materials, and many others. Bone research has been represented in a number of recent studies, including fatigue loading of human bone and blood-bone interaction in hematopoietic stem cells. Through the use of phase-contrast imaging, additional applications have emerged, in particular the ability to quantify the 3D structure of cartilage, which necessitates the ability to visualize low-density soft tissue (cartilage) alongside higher-density tissue (bone). Also, bone represents an example of a hierarchical material, where it is useful to quantify 3D microstructure across several length scales, as depicted in Figure 1. Bio-mechanical studies utilizing XRM have been explored on bone as well as in the dental field, in particular to explore the interaction of the tooth-bone-periodontal ligament system. Researchers have shed light on this interesting bio-mechanical system and have used XRM as a way to explore the 3D structure under real (and varying) loading environments through the use of various mechanical loading rigs.

XRM has enabled a number of applications in biological research by providing micron and nanoscale information across a wide array of sample sizes. However, no single microscopy modality is capable of imaging structures in 3D across an entire range of length scales and contrast mechanisms. In response to this, efficient correlative microscopy methods have been proposed that use several imaging solutions to analyze a single sample. Non-destructive XRM will contribute greatly to this correlative imaging landscape by providing a bridge between light and electron microscopy in length scale, contrast mechanisms and sample preparation requirements. One active area where XRM is being used in a correlative workflow is in the inspection and identification of known sub-volumes of stained embedded samples (for EM) prior to serial sectioning for 2D or 3D imaging in the SEM, FIB/SEM or TEM. This significantly increases efficiency by navigating to a sub-volume of interest, investing time imaging identified regions of interest with the electron microscope instead of searching for a needle in the haystack. New applications such as this point to a bright future for XRM as a common technique in the research laboratory.

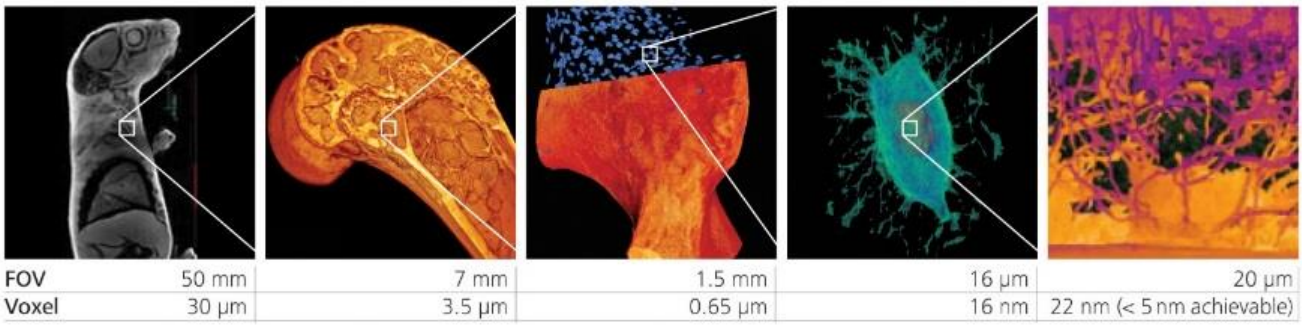

Figure 1 Hierarchical structure in bone with 3D renderings of datasets. Images were collected using ZEISS XRM technology with the exception of ZEISS FIB-SEM dataset (far right).

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