Beyond Histology
with 3D X-ray Microscopy

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A new field of 3D X-ray microscopy is emerging, bringing dramatic resolution and contrast improvements to the imaging of biological specimens for correlative studies and hierarchical structure investigations. ZEISS 3D X-ray microscopes enable a diverse range of new studies in the life sciences, from observing unstained soft tissues to analyzing new biomaterials at the highest yet achievable resolutions, from the micron to nanometer scale.

Within a year after Roentgen's discovery of X-rays in 1895, X-ray imaging facilities were established globally and a new medical imaging discipline was born. The field made a critical leap in the 1970s after computers enabled X-ray computed tomography (CT), a technique capable of looking beyond 2D and producing full 3D images of large objects like the human body at macro resolution. About a decade later, micro-computed tomography (micro-CT) was developed to build on CT capabilities for 3D imaging of small objects at resolutions of sub-millimeter to several microns.

X-ray imaging technology has recently evolved again. Advancements in X-ray optics and detectors have led to the emergence of 3D X-ray microscopy, which extends micro-computed tomography capabilities into the submicron and even nanometer scale resolution range.

3D Information for Correlative Microscopy Studies

Entirely new avenues of research in the life sciences have opened in disciplines from plant biology to cellular analysis and biomaterials studies. The non-destructive nature of X-rays allows for multi-length scale or multi-modal imaging of the same sample for analysis of hierarchical structures. Moreover, the most advanced 3D X-ray microscopes employ novel imaging methods to move past contrast limits of low X-ray absorption in soft materials to provide images of unstained soft tissues. ZEISS laboratory 3D X-ray microscopes, Xradia Versa and Xradia Ultra, offer the highest resolution for virtual X-ray histology of submicron to nanometer scale features. In addition to the highest resolution, these solutions provide the highest contrast in soft tissues and cells, without the need for staining.

1. **High Resolution for Rapid 3D Histology**
   ZEISS Xradia Versa achieve submicron resolution and Xradia Ultra achieve down to 20 nanometer pixel (50 nm spatial) resolution for rapid 3D histology including visualization of subcellular structures. The two microscopes comprise a multilength-scale suite to facilitate new correlative studies in biology.

2. **High Contrast**
   X-ray imaging has thus far relied on measuring only the absorption of X-rays. Because the carbon-based materials relevant for life science research appear the same in X-rays with little to no difference in absorption contrast, visualization of such materials has been difficult to impossible. ZEISS enables phase contrast imaging, which uses the refraction of X-rays rather than absorption, providing clear images of unstained plants and soft tissues. The microscope detectors also amplify contrast for high sensitivity to small density gradations.

3. **Non-Destructive – Little to No Sample Preparation**
   X-ray microscopy removes the need for tedious sample preparation and cross sectioning that can introduce artifacts, especially when the materials are hard, brittle, soft, moist, or elastic. As an example, serial sectioning through SEM for organs such as a mouse eye can take up to a year while ZEISS Xradia Versa achieve comparable high resolution results in days or hours.

4. Enables *In Situ* Analysis for BioEngineering

Critical to bioengineering of prosthetics, biomaterials, and implants are *in situ* studies in which the sample is observed close to its natural environment, such as under tensile loading or in hydrated environments, to predict failure and utility. ZEISS provides the largest working distance at highest resolution 3D X-ray imaging and measurements of samples *in situ*.

Xradia Versa and Xradia Ultra

ZEISS 3D X-ray microscopes provide unique advantages for imaging biological samples. Recent investigations using 3D XRM demonstrate a number of benefits for histology research, including the following:

Bone – Osteocyte Lacunae and Canaliculi

Micro-CTs are often used to observe microstructures such as bone trabeculae; ZEISS X-ray microscopes provide multi-length scale 3D observation beyond trabecular length scales to image osteocyte lacunae (5-20nm) and bone nanostructures (50-150nm), such as canaliculi. [Sakdinawat & Atwood 2010]

Teratology

Observed chick embryo development through multiple stages and demonstrated visualization of heart loop, otic vesicles, cranial nerves, and more in unstained and stained embryos. [Mestcher 2009]

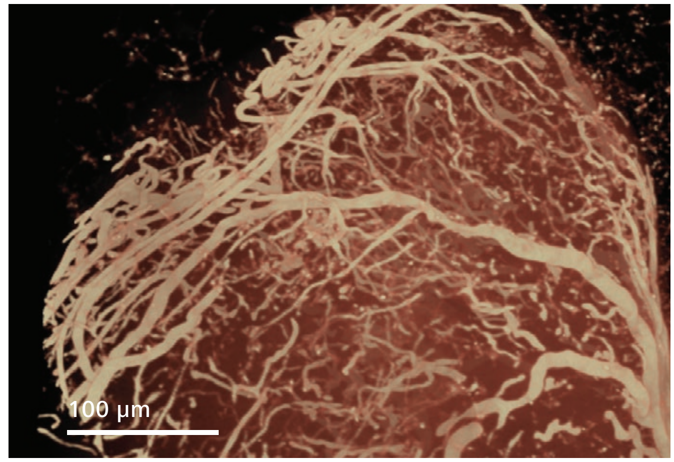

Figure 1: Tumor Vasculature and Angiogenesis

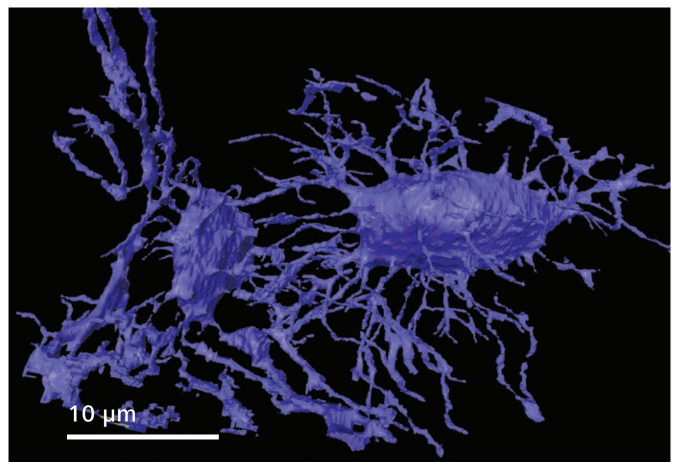

Figure 2: Two Osteocyte Lacunae and Canalicular Network

Preclinical Research and Disease Characterization

Using ZEISS 3D X-ray histology method, where 2D slices can be viewed through software at any point in a 3D volume, provides high resolution views of atherosclerotic plaque development in murine hearts. [Martinez et al. 2010]

Bioscaffolds – Tooth Reattachment

Observe mineralized tissue morphology and test loading strength after progenitor-driven reattachment of tooth roots. [Dangaria et al. 2010]

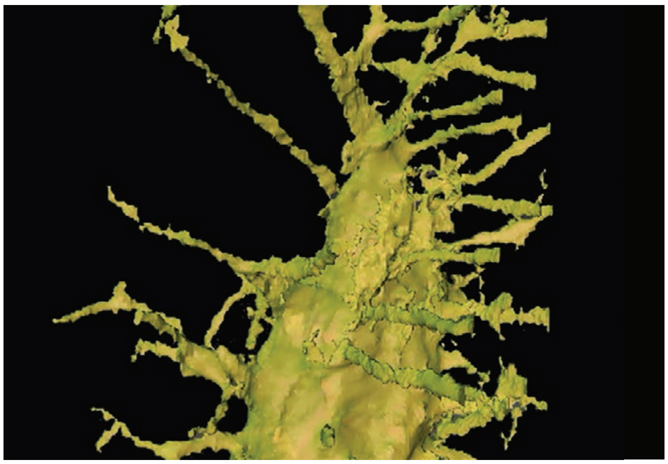

Figure 3: High resolution bone. Osteocyte lacunar-ccanalicular network – Xradia Ultra

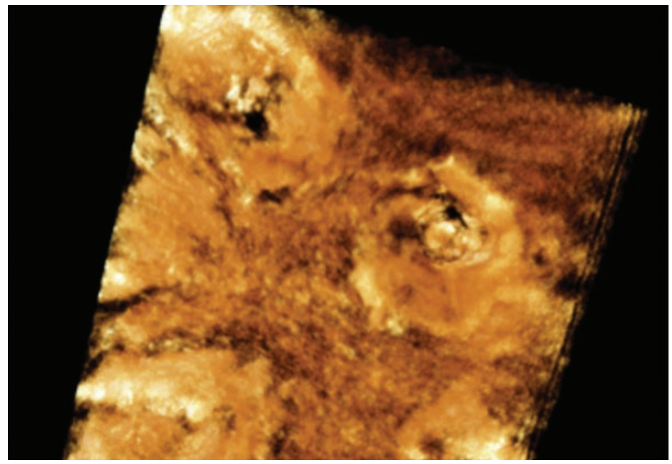

Figure 5: Dental. Dentine tubiles – Xradia Ultra

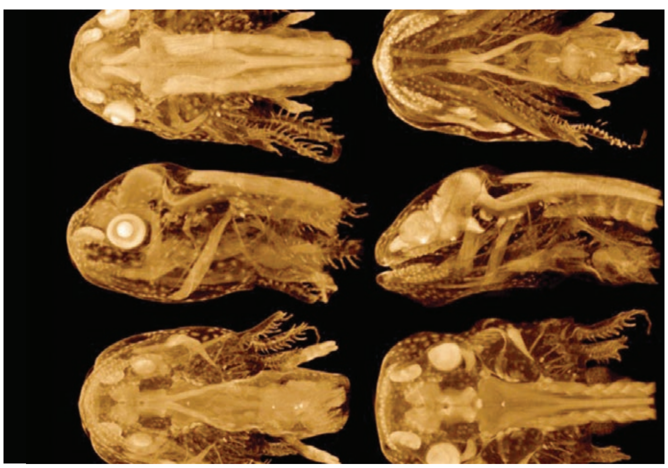

Figure 4: Teratology. Axolotl Larvae – Xradia Versa

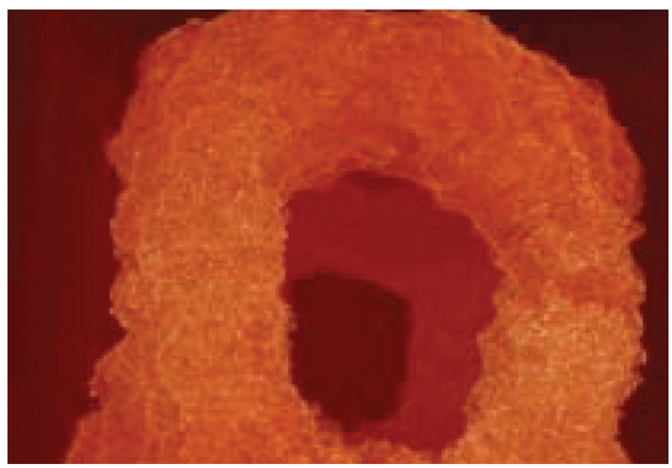

Figure 6: Implants/bioscaffolds. PLGA bioscaffold – Xradia Versa

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