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A high resolution, hard x-ray bio-imaging facility at SSRL

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The old saying that seeing is believing has particular resonance for studying biological cells and tissues. Since 1677, when Anton van Leeuwenhoek used a simple light microscope to discover single cell organisms, scientists have relied on structural information obtained from microscopes with improving capabilities to advance the understanding of how biological systems work. Optical and electron microscopes are essential for many of these important discoveries and have been widely employed in biomedical research laboratories. However, various limitations exist in these microscopy techniques. We describe below how the new x-ray imaging facility at the Stanford Synchrotron Radiation Laboratory (SSRL), based on an Xradia nano-XCT full-field transmission x-ray microscope (TXM), can provide complementary and unique capabilities to the current microscopy methods for studying complex biological systems.

The TXM, developed at the 54 pole wiggler beam line 6-2 (BL6-2) at SSRL, is based on zone plate optics using absorption contrast over a wide energy range from 5-14 keV and Zernike phase contrast at 8 keV[1]. The instrument offers complementary capabilities to many imaging tools that are widely deployed in biomedical research. For example, the capability of high

resolution imaging of thick biological specimens is of importance to the study of complex biological systems because the biology of higher living organisms relies on the cooperation of cells. This is evident in the study of development and differentiation and of intra-cellular communication, not only through synapses, but also via the interplay of cells organized into organs and the development of cancer. Even bacterial plaques, so resistant to medical intervention, involve cooperation on the multi-cell level. While these systems are routinely studied by visible light and electron microscopy, visible light microscopy is limited in resolution, and electron microscopy inevitably requires sectioning, thereby losing a great deal of information on the three dimensional architecture. The high-resolution “virtual sectioning” capability of this instrument enables imaging at 40 nm resolution of any number of pre-selected regions within a visible light microscope specimen or even a cylindrical specimen such as obtained with a needle biopsy.

Overall, the instrument offers several unique and complementary capabilities that are expected to have a significant impact for biologists studying complex biological structures. These include:

- High resolution of 40 nm (or better with newer zone plates)
- Imaging of tissues without cross sectioning
- High penetration (~1-20 μm) for nondestructive imaging of relatively thick biological specimens
- High quality images using single exposure times of 0.5 seconds
- Rich contrast mechanisms (Zernike phase contrast at 8 keV, and soon at 5 keV) for imaging biological and marker materials
- Absorption contrast in the 5-14 keV range, and X-ray absorption near edge spectroscopy (XANES), to reveal chemistry
- 3D tomographic reconstruction
- Complimentary x-ray fluorescence analysis

Beam line and microscope overview

The general layout of the x-ray microscope is schematically shown in Figure 1. It operates with the same principle as a visible light microscope, consisting of a source providing the radiation (wiggler through slit, S3), a condenser (focusing mirror, C) relaying the radiation onto an object (sample), an objective (micro zone plate, MZP) forming a magnified image, and a detector (CCD) receiving the image.

BL6-2 has been designed to accept the full vertical emittance of the wiggler and 1.2 mrad in the horizontal, limited by a slit located 10 m from the source. The beam is then collimated by a vertically reflecting Pt coated mirror (M_0) located 13.86 m from the source and followed by the liquid nitrogen (LN) cooled, double crystal monochromator. The LN cooled monochromator is equipped with a set of Si(111) crystals giving an energy resolution of $\Delta E/E = 5 \times 10^{-4}$ which meets the requirements of the zone plate objective of the TXM.

Although this monochromator is not specifically designed to have a fixed exit beam height for different photon energies, the vertical beam motion is only a few microns over an energy range of 100 eV, which is the typical tuning range for near edge spectroscopy applications. This performance is a result of the post-monochromator toroidal M1 mirror (Figure 1), which is configured to focus the beam onto the S3 slit and thus reduces the vertical translation of the focused beam significantly. In addition, a mirror pitch-feedback system also serves to reduce the magnitude of these beam translations. The S3 slit, located 30 m from the wiggler source,

serves as the effective source for the TXM. The slit system is mounted permanently on a support with micron level thermal stability. The slit assemblies consist of both horizontal and vertical blades with 1 micron reproducibility and are remotely operated.

TXM Setup

Figure 2 shows a photograph of the TXM inside the hutch. The beam direction as well as the location of the condensers and zone plates are indicated by arrows. After the beam is monochromated and focused onto the virtual source, the beam is then focused onto the sample by a condenser consisting of an elliptically shaped capillary tube [2] located 1 meter from the virtual source. The condenser illuminates a spot on the sample providing a field of view of approximately 14 μm in diameter at 8 keV with a central stop that provides a hollow cone illumination. The sample is placed on a stage with high precision xyz/theta motion to allow for tomography and wider area survey images. An objective lens consisting of a high resolution Fresnel zone plate [3] then projects the image of the sample onto a thin single crystal scintillator, which is optically coupled to a cooled (-70°C) charge-coupled device (CCD) in magnifying geometry yielding an effective pixel size of approximately 0.6 μm on the scintillator screen. In addition, a phase ring can be placed in the back focal plane of the objective zone plate to provide Zernike phase contrast.

The resolution of the image is determined by the outer zone width of the zone plate and the steep hollow cone illumination. The present generation of zone plates has an outermost zone width of 45 nm, but further improvements of the smallest zone width in zone plate fabrication technology are anticipated. A PC workstation controls the motions of all the system optics, sample manipulation, image display, 3D reconstruction and 3D rendering. The 3D reconstruction is performed using a digital signal processor card (DSP) that is optimized for high speed tomographic reconstruction and takes approximately 7 seconds. The vertical divergence of the x-ray beam at the virtual source position is about 0.25 mrad, thus the condenser optics were designed to increase the vertical divergence to match the numerical aperture of the objective zone plate. For applications requiring larger fields of view at the sample position, the capability of translating the sample over many fields of view to obtain a tiled 2D image covering on the order of $100 \times 100 \mu\text{m}^2$ was implemented. Such “mosaic” images are especially useful when trying to identify a promising area for higher resolution or tomographic imaging (see e.g. Figs. 4 and 8).

Figure 3a shows an x-ray phase contrast image taken at 8 keV of a test pattern with features tapering to a 30 nm minimum feature size at the center of the pattern. As can be seen from Figure 3b, which is a blow-up of the of the indicated area on Figure 3a, it is possible to resolve features just beyond the 30 nm tip which can be estimated directly from the figure to be approximately 40 nm. A more accurate analysis using the modulation transfer function confirms this 40 nm estimate. With future zone plates employing smaller zone widths, this resolution will improve accordingly.

Current techniques using the TXM

Some of the various techniques and capabilities of the microscope are outlined below.

Phase contrast

While some specimens of biomedical interest, such as bone, do show amplitude, i.e., absorption, contrast with multi-keV X-rays, most specimens contain only low-Z elements, and are nearly impossible to study in amplitude contrast at these energies. It is well known, however, that phase contrast is very useful under these conditions [4]. The physics behind this difference is that while the imaginary part of the atomic scattering factor f_2 drops rapidly with

energy, the real part, f_1 approaches a constant, equal to the atomic number. It is essential to have a phase contrast mode of operation for bioimaging.

In this setup, the hollow-cone illumination of the specimen is focused to a narrow ring in the back focal plane of the zone plate. A phase-ring at this point is used to both shift the phase, and to attenuate this undiffracted portion of the radiation. Thus modified, this radiation is recombined with the portion that is diffracted by the specimen in the image plane, resulting in a phase-contrast image. A phase shift of odd multiples of $\lambda/4$ results alternately in positive and negative phase contrast. Higher multiples of $\lambda/4$ can be used to better match the strength of the diffracted and undiffracted radiation so as to optimize the phase contrast. Since the exposure required to render a feature visible is inversely proportional to the square of the contrast [5], maximizing the contrast is an important consideration. Images of mouse liver (Fig. 4) and mouse bone (Fig. 5-7) in phase contrast are described later.

Absorption Contrast and XANES

Absorption contrast and X-ray Absorption Near Edge Spectroscopy (XANES) both provide information on the chemical state of components in a sample. By taking an image above and below the absorption edge of an element, differences in intensity (i.e. darker images above the edge due to absorption by the element) can indicate presence of the element. This is demonstrated at the Fe K-edge of a meteorite fragment in Fig. 11 below. We also hope to do this above and below the calcium edge, for example, when working with bone, to distinguish calcium-containing tissue. This capability can also be used to provide better contrast with the use of heavy metal stains.

By gathering images at various x-ray energies, we can also construct x-ray absorption near-edge spectra (XANES) of a certain portion of the sample; for example the portions which show the greatest difference above and below the absorption edge for a particular element. The zone plate must be adjusted as the energy changes, but this can be automated, facilitating easy collection of data. By comparison of XANES spectra with those of known compounds we can determine the speciation, i.e. oxidation state and ligands, of the absorbing element. Usable XANES spectra require sufficient signal to noise, and thus either the concentration of the element needs to be sufficiently high, or the data must be carefully pooled, for example using principle component analysis.

Tomographic 3D imaging

In addition to 2D imaging with high spatial resolution, the TXM can also be employed for tomographic 3D imaging. This technique requires that the sample be mounted on a rotation stage as previously mentioned, in order to record many 2D images with a CCD detector at various angular positions of the sample with respect to the x-ray beam. The local 3D linear absorption coefficient is then obtained via computer reconstruction algorithms, which reveals the arrangement of internal structures.

Tomographic reconstruction techniques typically require that a projection contain values from which a line integral of some property of the sample can be derived. For example, in x-ray imaging with absorption contrast, each projection contains a superposition of absorption images of slices of the sample at different depths. This is, however, not true for Zernike phase contrast imaging, which represents an interference pattern of the scattered beam and the direct beam. In this case a blind reconstruction from the projection images will not yield the true 3D map of the imaged values, although it will give the correct 3D position of the features. The contrast and therefore the reconstructed intensity of small features near the resolution limit will be enhanced while large features will have enhanced edges and a diminished center. For some applications, this is acceptable for understanding the structure of the sample. In general, it may

be necessary to develop new ways of interpreting and understanding the reconstructions. For example, Xradia has developed a more sophisticated reconstruction method that unravels the phase information from the phase contrast images, leading to images with contrast that is proportional to the line integral of the phase shift through the sample. This phase integral can then be reconstructed tomographically to produce the exact three-dimensional phase shift and therefore the material position in the sample.

The high flux of the wiggler source enables us to use exposure times as short as 0.5 seconds for alignment images. High resolution, low noise images are obtained using 2 second exposure times per frame with CCD readout times of less than 1 second. Thus, a tomography set can be obtained in about 15-30 minutes, depending on angular resolution.

Depth of Focus

Along with the spatial resolution δ , the depth of focus (DOF) is a very important parameter for tomographic 3D imaging. The DOF is defined by $\text{DOF} = 1.22 \lambda / (\text{NA})^2 = 3.36 \delta^2 / \lambda$, where λ is the wavelength and NA the numerical aperture of the objective zone plate lens. This expression shows that the DOF increases inversely with the wavelength, and thus linearly with the x-ray energy. Assuming a spatial resolution of 60 nm for first order diffraction, the DOF for a photon energy of 10 keV is about 100 μm .

To achieve a depth resolution which is comparable to the high transverse resolution, it is desirable that the full field microscope used in tomographic 3D imaging have a DOF that is comparable or longer than the linear dimension of the 3D reconstruction volume to be imaged. This is necessary so that the features within the 3D reconstruction volume can be maintained in focus for all projection angles. Therefore, an x-ray microscope operating at higher energy is preferred for high-resolution tomographic 3D imaging of thick specimens because a substantial number of important applications in biology have linear dimensions in the 1–30 μm range.

Fluorescence

X-ray fluorescence analysis offers significant elemental analysis sensitivities for nearly all the elements in the periodic table. In combination with the high spatial resolution mapping capability, this elemental sensitivity offers the capability of investigating the role of trace elements in the function of biological systems with sub-cellular resolution.

The design of the Xradia TXM allows an easy implementation and integration of a fluorescence microprobe for trace element analysis and mapping without introducing any modifications to the optical configuration of the TXM. This will significantly extend the capabilities of the instrument and will facilitate fluorescence analysis of specific regions on the sample observed in the TXM image. The main idea is that when an interesting feature is observed within the $\sim (10 \times 10) \mu\text{m}^2$ field of view of the TXM, it will be possible to bring the feature into the center of the image and reduce the field of view and measure the x-ray fluorescence in this region. In fact, this can be done concurrently with imaging. The fact that the TXM provides a simultaneous high-resolution image of the area being analyzed by the fluorescence microprobe will give a definite advantage in identifying the features in biological specimens contributing to the fluorescence emission. This works well for thin samples but requires careful consideration for thick samples because the fluorescence signals originate from the whole thickness.

Representative examples of biomedical imaging with TXM

TXM images of mouse liver

A 5×5 mosaic of a 10 μm thick sample of mouse liver section (supplied by M. Ralle of the Oregon Health & Science University, Portland, OR) imaged in phase contrast is shown in Figure 4. A significant amount of detail is revealed. The image also shows residual beam structure (artifacts) as can be evidenced by the round features seen in the center of each of the individual fields of view. This was the result of less than perfect normalization and is one of the areas that we are working to make more consistent. Although the stitching is not perfect between fields of view, the image provides the equivalent of a “low magnification” view of the sample. However, because each individual image has the full resolution of the TXM, once a feature of interest is identified, additional detail is readily observed by blowing up the particular field of view of interest on the computer screen. (This is shown later in Figure 7 where the central part of a mosaic image of mouse bone is magnified, presenting significantly more detail.) It should also be noted that although this mouse liver sample has inherently low absorption contrast at 8 keV, the phase contrast allows a significant amount of detail to be seen.

TXM imaging of the nanostructure of loaded/unloaded hind-limb bone

In collaboration with Eduardo Almeida (NASA Ames) and Marjolein van der Meulen (Cornell University), we have compared the nanostructure in normal and osteoporotic mouse bone. Osteoporosis was induced by hind-limb unloading, a NASA-developed method to simulate microgravity effects in ground experiments. Specifically, mice were suspended by the tail in a pulley system that allows them to ambulate and feed/drink using only their forelimbs.

The study of bone structure and bone material properties is of great importance to the medical community because of the prevalence of osteoporosis in the aged population and also to NASA because it severely afflicts astronauts in microgravity. While the physiology and cellular dynamics of bone remodeling in osteoporosis are relatively well known, current detailed understanding of bone structure is limited to the macro and microscales and is based on conventional light microscopy and x-ray microtomography. Furthermore, although we can model the nano-organization of collagen and calcium hydroxyapatite in bone, the electron microscopic methods we currently use to study this bone nanoarchitecture suffer from many sample preparation artifacts and likely do not accurately depict the true three dimensional organization of the tissue. Our working hypothesis is that the three-dimensional bone nanostructure will be an important predictor of bone material properties that cannot be explained by macro or microstructure. This hypothesis implies that bone fracture risk may vary even if macro and microstructure appear similar when analyzed by conventional macro and micro methods.

Bone samples prepared by sawing 50-100 μm fresh unfixed bone slices were placed in cold phosphate buffered saline (PBS) and imaged with phase contrast at 8 keV. Figure 5 compares Micro-Computed Tomography (micro-CT) (left) and differential interference contrast (DIC) imaging (center), of loaded (control) hind-limb trabeculae with TXM (right), which provides far more detail. Micro-CT imaging of the structure of bone in the micron range (Fig. 5, left; 10 μm isotropic resolution) has been used to understand how bone microarchitecture is dynamically remodeled and changed in response to applied load and to disease states such as osteoporosis. Micro-CT technology has also allowed the determination of bone structure, volume fraction, and density in small animals and human biopsies.

Despite the power of micro-CT to image bone in the 10 μm resolution range, the significance of achieving the equivalent of nano-CT of bone at 40 nm resolution using TXM, as seen in Fig. 5 (far right) enables the opening of a more detailed structural understanding of bone.

Additionally, it may also provide the ability to model and predict material properties of bone and to assess treatment efficacies at the micro and nanoscale.

In the TXM image, the leaf like structures (oval, longitudinal) are footprints of single osteoblasts starting to mineralize the trabecula, and the filament network consists of partially mineralized collagen fibers or canaliculi at the surface. Note that the edges of the bone in this image of control (loaded) mouse bone appear smooth and even.

As mentioned above, the stitching of the mosaic images is not perfect. However, this is not seen as a failing of the instrument because the instrument is designed for high resolution 2D and 3D imaging within a single field of view. The ability to make the mosaic images is an important feature that allows us to better choose a particular area of interest. The observed stitching errors of $\pm 2 \mu\text{m}$ appear to be within the expected repeatability tolerances of the high precision stages used to move the sample. We are continuing to test the accuracy of the stages using on-board high precision encoders in order to improve the situation as much as possible. However, for tomographic imaging and 2D imaging within a given field of view, the stages supply the required resolution.

The first panel of Fig. 6 shows a light microscopy/DIC image of a single mouse bone trabecula with a superimposed Hoechst 33347 fluorescent DNA stain image of cell nuclei. Trabeculae are low-density bone tissue that fill the center cavity of bones. Lacunae (i.e. small spaces) in the trabeculae show stained osteocyte, and possibly osteoblast, nuclei. Osteocytes, the most common bone cells, are connected by microchannels called canaliculi. Canaliculi allow the processes of osteocytes to communicate and are thought to be sensitive to bone strain or loading. The image on the right is the TXM equivalent of the area highlighted in the box, showing a single lacuna and surrounding trabecular tissue with an elaborate fiber network. Fibers along the edges of surfaces are most likely collagen, and the filament-like structures are most likely canaliculi. Imaging of these structures using cast replicas and also lipid fluorescence tracing reveal structures very much like these. One of the advantages of TXM over other techniques is the minimal surface/ sample preparation: no cutting, polishing or sputtering of the tissue is required.

Fig. 7 depicts TXM images of mouse bone that was hind-limb unloaded. In contrast to Fig. 5 of loaded mouse bone, the edges of unloaded mouse bone are distinctly different, with rough, pitted surfaces. On the right is a high-resolution image of a lacuna from the center of the second row of the mosaic. These results serve as a preliminary demonstration that we can distinguish between hind-limb loaded and unloaded bones using TXM. This has implications for studies of osteoporosis, and for astronauts spending time in weightless environments.

TXM absorption contrast of mercury accumulation by *Spartina foliosa*

We are studying mercury accumulation in the cordgrass *Spartina foliosa* as part of a wider effort to understand the biogeochemical cycling of mercury throughout the food web. *S. foliosa* is native to the San Francisco Bay Area, where waterways are contaminated with mercury due to historic mercury mining during the gold rush (mercury was used to amalgamate gold). Methylation and subsequent biomagnification of mercury up the food chain results in levels up to 10 millions times greater in sport fish than in the surrounding waters.

In this study, in collaboration with Cynthia Patty of SSRL, *S. foliosa* plants exposed to 1 ppm Hg (as HgCl_2) for 7 days were washed, frozen, and mounted in windows anchored by Kapton tape for TXM analysis. Figure 8 is a 6×6 mosaic image of the edge of a thin piece of root (approximately 200 μm in diameter), imaged with absorption contrast (no phase ring) at 9 keV. Dense spherical particles, mostly less than 1 micron in diameter, can be seen along the outside of the root, and the fibrous material is probably fine root hairs, on the order of one micron

thick. The spherical particles are very dark due to absorption of Hg. (Hg has significant absorption at 8 keV, comparable to that at the mercury L3-edge at 12.3 keV.) Fig. 9 is a more detailed image of the particles and root hairs, taken at 8 keV in absorption contrast. It is apparent from a 3D tomographic reconstruction (Fig. 10) that the particles are at or near the surface of the root. In an additional tomographic image (not shown) it is apparent that the larger dark particles contain mercury just at the surface, indicating that they may be microorganisms rather than dense Hg nanoparticles.

Demonstration of Spectroscopic Imaging

As mentioned in the previous section, it is possible to vary the photon energy of the TXM to perform spectroscopic imaging, i.e., to obtain images across an absorption edge that can either indicate the presence of a particular atomic species; or even to take images at closely spaced photon energies to determine the chemical state of a particular species. This technique is known as X-ray Absorption Near Edge Spectroscopy, or XANES. In order to perform these measurements, it is necessary to refocus the TXM at each photon energy by moving the zone plate along the beam direction. Fortunately, the optical system is precise and reproducible so that we have not had any problems in doing this. We have been able to calibrate the focus as a function of photon energy and have found that it is only necessary to move the zone plate 3.4 μm per 1 eV change in photon energy at 7 keV. In addition, Xradia has developed a scripting language so that we can scan the beam line monochromator through an SSRL supplied interface as well as adjust the microscope focus for a series of images across any given absorption edge in the range of the TXM.

To demonstrate this capability, we imaged a meteoritic spallation fragment below and above the Fe absorption edge showing iron-rich crystallites in an olivine matrix. The images below and above the Fe absorption edge at 7101 and 7140 eV, respectively, are shown in Figure 11. In the image at 7140 eV (right), the iron containing crystallites are apparent as dark features because the Fe absorbs the x-rays strongly above its absorption edge. On the other hand, below the absorption edge, Fe does not absorb and the contrast from the iron crystallites is lost in the image taken at 7101 eV (left). The dark areas in the 7101 eV image are features containing elements with high absorption at 7 keV. Although not identified in these absorption spectra, these features could be easily identified with the x-ray fluorescence detector that will be added to the TXM later this year. Although not shown here, images were also collected in 1 eV increments across the absorption edge and are presently being analyzed using a principal components technique that will highlight the different chemical species of iron that might be present. This will be an important capability for bioimaging applications when elements of interest are present at sufficient concentrations.

Summary and future work

In summary, the transmission x-ray microscope imaging facility at SSRL is significant to biomedical research because of its capabilities of phase contrast, absorption contrast, spectroscopic imaging (XANES), and *in-situ* 3D imaging of a $(10\mu\text{m})^3$ volume with 40nm resolution. In addition, function specific imaging can also be developed by making use of the different x-ray optical properties of many labeling agents, such as gold.

The microscope will see further improvement with the addition of higher resolution zone plates and phase contrast capability at 5 keV to improve contrast in biomaterials. A scanning microprobe capability is integral to the system thus allowing elemental mapping and fluorescence yield XANES to be performed with a spatial resolution in the micron range without introducing any changes to the optical configuration of the instrument. Principle component analysis will be implemented to pool data from similar sites on a sample, improving data quality to provide chemical information on more dilute samples.

In addition, a cryostage will be added to minimize radiation induced structural changes in a biological specimen during x-ray imaging. The issue of radiation damage will become especially crucial for imaging at the highest resolutions which require increased doses to achieve the desired signal to noise ratio. Therefore, radiation damage can impose a limit on the resolution achievable. By implementing a cryostage, we will be able to examine cells that have been rapidly frozen and viewed in a state more closely approximating that seen in living cells. To avoid ice crystal damage, high pressure freezing techniques will be employed.

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Figure 1.

Schematic view of beam line 6-2 coupled to the Xradia TXM. The source of the radiation is a 54-pole wiggler. The x-rays are first vertically collimated by a bent flat mirror (M_0), they pass through a LN-cooled, double crystal monochromator, and are then focused onto a slit (S3) at 25 m by a toroidal mirror (M_1). The full field microscope refocuses the beam formed at S3 with its Condenser lens (C) onto the sample and then magnifies this image onto a CCD with a micro zone plate (MZP).

Figure 2. Photograph of the TXM inside the hutch of BL6-2. The beam direction, location of the condensers, zone plates, beam monitor and alignment camera are indicated by the arrows. The CCD (not shown) is located to the right.

Figure 3.

(a) Phase contrast image taken at 8 keV of an alignment target with features tapering to 30 nm at the center. The field of view is 14 μm and the scale bar is 1 μm ; (b) Blow-up of the indicated region showing the resolved features which can be estimated from the image to be 40 nm. Analysis of the modulation transfer function confirms a resolution of 40 nm.

Figure 4.

Image of a 10 μm thick section of mouse liver taken at 8 keV in phase contrast. The bar at the bottom of the image is 4 μm long. The image consists of a 5×5 array of 14 μm fields of view taken in a raster pattern. Residual beam normalization effects are evident in each field of view. The boundaries between rows can be seen in the image and are primarily due to beam normalization differences between each row rather than stitching errors. However, note that within a row, the different fields of view merge almost seamlessly.

Figure 5. Mouse tibia trabecular region in microCT at 10 μm resolution (left), followed by 20X objective differential interference contrast picture of a single thin trabeculae, at $\sim 0.75 \mu\text{m}$ resolution (center), followed by TXM mosaic composite with phase contrast at 8 keV, with 40 nm resolution (right). Each frame is 13.9 μm square. Background removal was improved using Adobe Photoshop.

Figure 6.

Light microscopy/DIC image of a single mouse bone trabecula with a superimposed Hoechst 33347 fluorescent DNA stain image of cell nuclei (left). TXM equivalent of the area highlighted in the box (right), showing a single lacuna and surrounding trabecular tissue with an elaborate fiber network. Background removal was improved using Adobe Photoshop.

Figure 7.
(Left) TXM 5×5 mosaic composite of unloaded mouse bone, with phase contrast at 8 keV. Each frame is 14 microns square. (Right) Averaged (20 1-sec scans) high-resolution image of lacuna from mosaic on the left.

Figure 8. TXM 6×6 mosaic image of *S. foliosa* root at 9 keV. Each frame is 13.9 μm square. Tomography of the semitransparent round objects has revealed that mercury is mainly at the surface, not the center.

Figure 9.
TXM averaged image of *S. foliosa* root (20 1-sec scans) at 8 keV in absorption contrast. Absorption in the image is mainly due to mercury. Bar on lower left is 2.5 microns.

Figure 10.
2D projection of tomographic reconstruction of Hg-containing particles from *S. foliosa* root in phase contrast at 8 keV.

Figure 11.

Meteoritic spallation fragment imaged below (7101 eV) and above (7140 eV) the Fe absorption edge showing iron rich crystallites in an olivine matrix, demonstrating the ability of the TXM to perform high resolution chemical mapping. The dark areas in the image below the Fe absorption edge may represent regions with other elements that are strongly absorbing at 7101 eV such as Ni. Images are large 9×9 mosaics taken with a uniform beam that resulted in excellent normalization.