doi:10.1017/S1431927610056059
Microsc. Microanal. 16 (Suppl 2), 2010


# X-ray Imaging and Microtomography Integrated into the SEM: XuM Examples from Biology

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The great advantage of SEM-based X-ray microscopy is the ability to image the internal composition and structure of specimens rather than only the surface information obtained by standard SEM imaging. The two techniques, however, combine in the same SEM to provide the maximum amount of structural information.

The SEM-hosted X-ray ultra microscope (XuM) uses projection microscopy and consists of 1) a series of up to 5 metallic targets mounted on a high precision movable positioning arm, which is placed beneath the electron beam to produce a point source of X-rays; 2) a sample holder; and 3) a high sensitivity CCD camera for X-ray detection. These components (Fig. 1) are fully compatible with the SEM working environment and allow switching between electron and X-ray imaging modes without venting the specimen chamber. The desired X-ray energy can be "tuned" to the particular sample by selecting the target material which will provide optimal intensity and contrast in the images. This is particularly useful for low contrast biological or polymer specimens where absorption contrast is weak and phase contrast may be much stronger.

Projection X-ray microscopy allows the sample to be always in focus and great depth of field enables stereo imaging and 3D micro-tomography to be carried out. In general, spatial resolution of 200nm can be achieved for 2D imaging, while tomographic resolution can be about 1um or better. The latter is dependent on the nature of the sample, SEM electron gun type, and stability.

For microtomography a high resolution sample rotation stage capable of a step less than 0.1deg. is used to acquire a projection series. Robust image phase contrast extraction algorithms are available especially for low density sample imaging and 3D reconstruction. Full tomographic data acquisition and reconstruction can be completed in less than 3 hours which makes the XuM a viable addition to most general purpose SEMs. The simple and quick movement of the X-ray targets in the SEM chamber allows normal electron imaging of the sample without having to open the specimen chamber.

Gatan's DigitalMicrograph software platform controls all aspects of the system calibration, image acquisition, specimen motion, 3D tomographic reconstruction and visualization, and virtual slicing of the reconstructed specimen. To provide ultimate ease of use, complete integration of the XuM with the host SEM ranges from SEM specimen stage control to shutting off the HT at the completion of a tomographic run.

For many small biological specimens such as seeds, insects, and tissue engineering samples, among others, the SEM-based X-ray imaging and microtomography offers non-destructive capability. Additional benefits include little or no sample preparation and the possibility of microtomography in a high-water vapor pressure imaging environment.

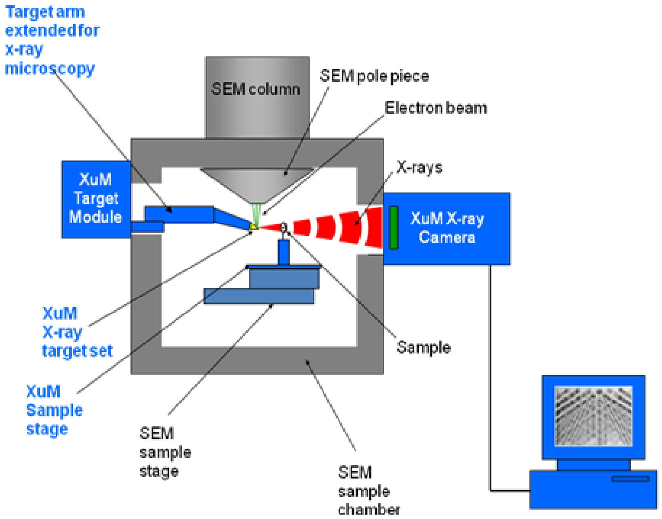


FIG. 1. Schematic drawing of the XuM with components in position for acquisition of X-ray projection images.

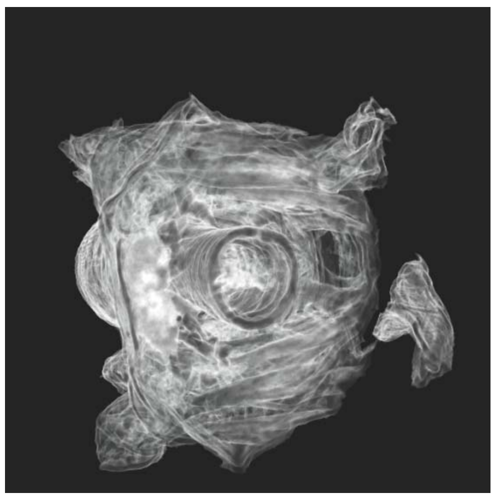

FIG. 2. Oblique virtual section through reconstructed fly specimen showing central gut.

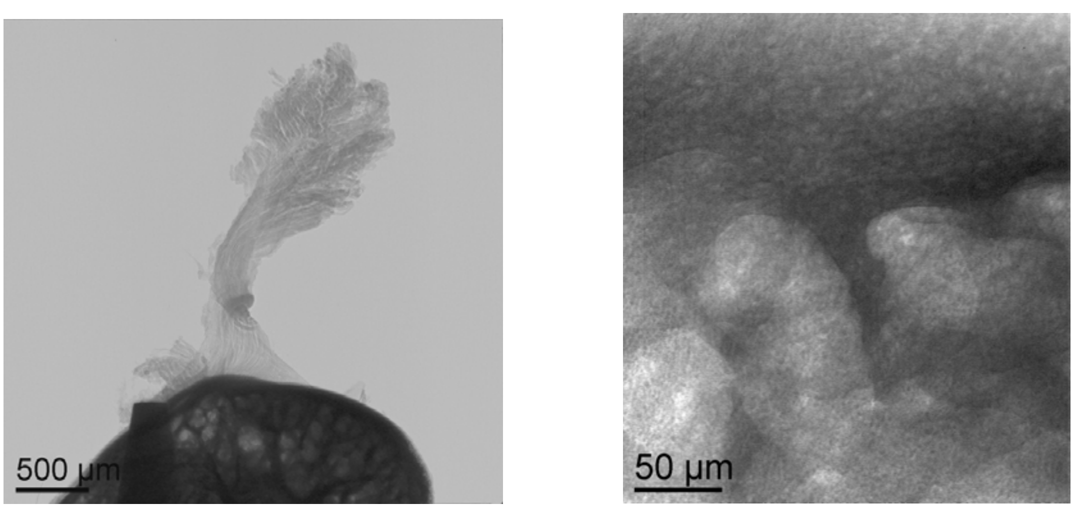


FIG. 3. Left image: 2D image of mouse tendon attached to bone. AgLa radiation.
Right image: 2D image of tendon-bone insertion point. WLa radiation.

https://doi.org/10.1017/S1431927610056059 Published online by Cambridge University Press