X-Ray Projection Microscopy with a CCD Detector

Application Note

Researchers at the Commonwealth Scientific and Insustrial Research Organisation (CSIRO) in Australia have developed new X-ray projection microscopy techniques that use phase-contrast information to increase the resolution and information content of images. This work has been funded by XRT Limited, and is currently being commercialised by them. This technique utilises a scanning electron microscope (SEM), as a microfocus X-ray source, together with an optimised sample/detector geometry to optimise the system for 'phase-contrast' imaging. X-ray microscopy has advantages over optical techniques, offering a much greater depth of field, higher resolution, and the ability to image relatively thick, optically opaque and scattering samples.

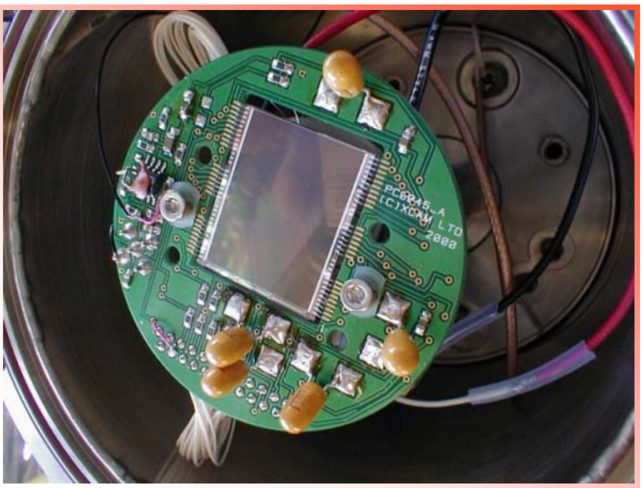

Fig. 1 A prototype custom CCD and headboard developed by Xcam Ltd for this work

X-ray projection microscopy

X-ray projection microscopy is a technique that requires no focusing optics and is capable of giving very high sample magnification over a large X-ray energy range. The technique involves using an X-ray microsource, with a spot size of less than 1 μm to illuminate the sample, forming an X-ray projection image of the sample at the charge coupled device (CCD) detector. The feature resolution size is linked to the source spot size, so that an SEM source of size around 100 nm, will result in a much better resolution than an X-ray microfocus source with a spot size of a few μm. The ability of a CCD to directly detect X-rays with high spatial resolution, high X-ray quantum efficiency in the 1-8 keV range and ability to resolve the incident X-ray energies by pulse height analysis, make the CCD a perfectly suited detector for this application. Figure 1 shows a photograph of an Xcam supplied CCD and surrounding headboard electronics that has been used in such a system.

Phase-contrast imaging

Phase-contrast imaging [1-2] offers greatly improved image information in the high-spatial frequency domain, in particular, features such as edges, corners and boundaries of objects. It also allows the imaging of objects that are weakly-absorbing, which would not be possible by absorption contrast methods alone. Phase shifts in the X-ray wavefront occur as the X-rays interact with the sample, producing Fresnel diffraction fringes in the recorded image. Mathematical phase-retrieval algorithms can then be used on the acquired 'phase-contrast' image to indirectly measure or reconstruct the phase shifts imposed on the X-rays by the sample.

System resolution and the CCD detector

The imaging resolution of an X-ray projection microscopy system is primarily limited by the size of the X-ray source, the source size being determined by the electron interaction volume in the source material. For a given SEM acceleration voltage, the interaction volume is smaller for denser target materials, resulting in high-Z materials being preferable for small source, high efficiency imaging. An additional limitation to the imaging resolution of the system is the detector resolution. Using a CCD as the detector, the detector resolution is simply the CCD pixel size. The true resolution may be slightly larger due to charge spreading in the CCD from individual incident photons, or slightly smaller if software is used for signal interpolation. Typical image configurations use full frame operation with a 1000 × 1000 pixel CCD with 13 × 13 μm pixels.

To achieve a resolution of 0.2 μm, the source size should be no greater than 0.1 μm, requiring a magnification of 130 to match this resolution to the CCD pixel size. The actual magnification used in the system which took the images below, shown in Figure 2, was 260 to allow for over sampling, which corresponds to a field of view at the sample of 50 × 50 μm, or around 500 × 500 pixels. Magnification was achieved by moving the sample closer to the X-ray source. The spatial feature size for which maximum phase-contrast occurs can be optimised by consideration of the X-ray energy used and by some refining of the source/sample, sample/detector distances. The ultimate practical limitation to the resolution is the minimum feature size that can be distinguished from the noise in the system e.g. the noise given by photon statistics if photon-counting. Typical exposures are 1-2 minutes, and several images are summed to get a total exposure time of around 10-15 minutes for a high quality, high resolution X-ray microscopy image, such as those shown in Figure 2; the summing of multiple images improving the signal to noise ratio. Once the X-ray image has been taken by the CCD software, image processing can be used to correct for any detector artefacts.

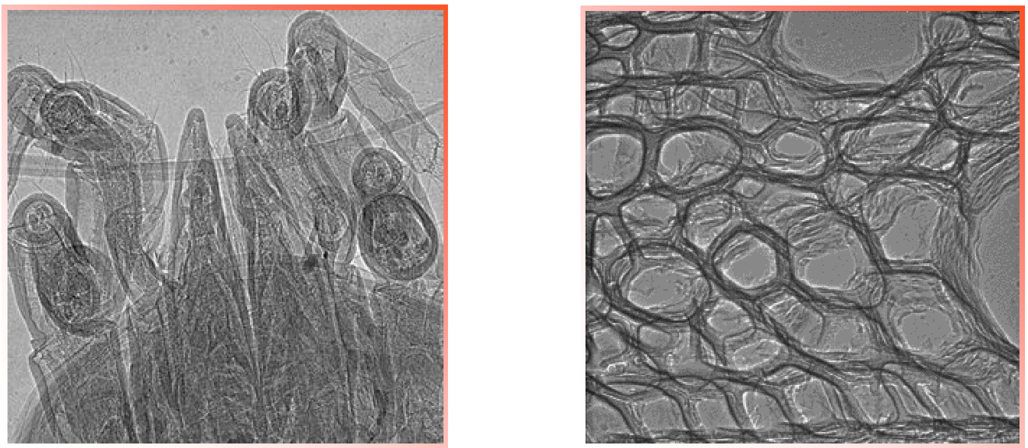

Fig. 2 Image taken using the Xcam system, of part of a mite (left) and the microstructure of a section of wood (right) [3]

Multi-spectral imaging and multi-energy phase retrieval

The linear nature of the size of the charge signal generated in the depletion layer of a CCD, and its relationship to the incident X-ray photon energy can be used to measure the energy of incident photons to within ~200 eV, dependent upon the X-ray energy. This allows relatively monochromatic images formed by X-rays of different energies to be accumulated. A sufficiently low X-ray flux is used to generate only isolated pixel events in the CCD, all split events being rejected by image analysis software [4]. The histogram of recorded events corresponds to the incident X-ray spectrum, and is used to calibrate the X-ray energy from the known X-ray source spectrum. Once calibrated, images formed at different energies can be reconstructed, and obtained simultaneously. This technique of multi-spectral imaging avoids any alignment problems that would occur if such images were acquired separately. Phase retrieval algorithms can then be used to extract the density distribution of the sample from the multi-spectral image. Within the detectable X-ray energy range of the CCD a number of different X-ray sources can be used, including silver, tantalum, gold and titanium. Figure 3 shows three reconstructed images of 9 μm latex spheres at X-ray energies of 3.3 keV (gold, Au-M), 1.7 keV (tantalum, Ta-M) and 5.0 keV (titanium, Ti-M) and the resulting density distribution obtained by phase retrieval.

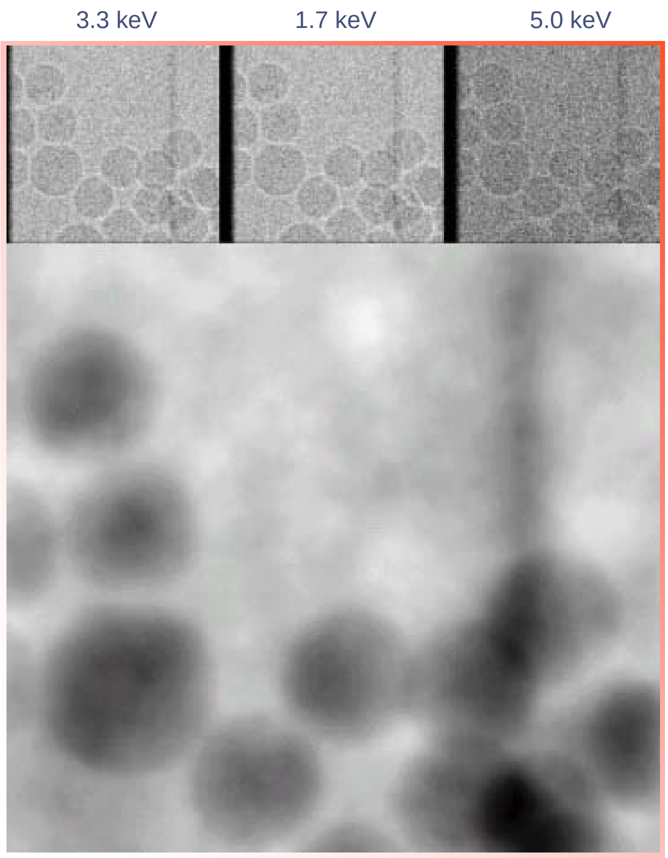

Fig. 3 Three images of 9 μm latex spheres acquired simultaneously using multi-spectral imaging at 3.3, 1.7 and 5.0 keV (upper left-to-right respectively) and (lower) a projected density distribution obtained by phase retrieval from the three images

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Microtomography using phase-contrast imaging

Another imaging technique that can be utilised by the Xcam system is that of 'microtomography'. This technique enables a full three-dimensional representation of an object to be constructed from a series of X-ray images taken with the object at different angles. The sample is rotated in fixed interval steps between each image. The final image resolution is limited by the number of sample images obtained and by the data collection time. The images are reconstructed from phase-contrast data, and, as a result, show phase-contrast related features such as the bright fringes at the external edges of objects. An example microtomography image of a fly leg joint is shown in Figure 4.

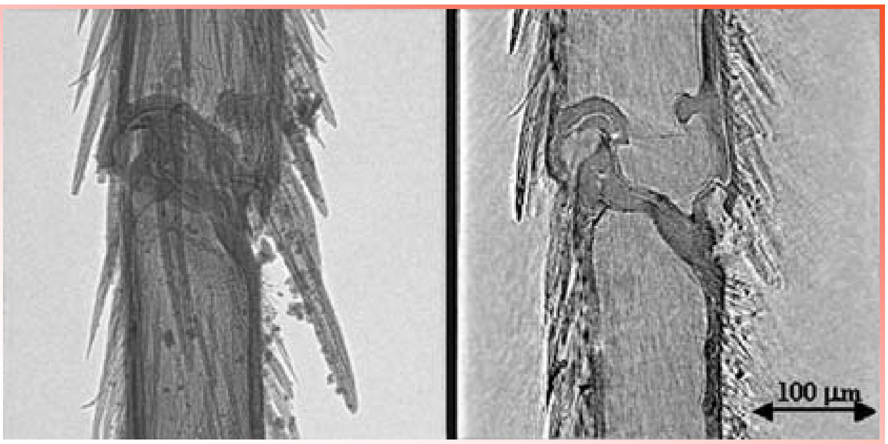

Fig. 4 An image of a fly's leg joint. The collected tomographic dataset image (left) and a longitudinal section through the reconstructed three dimensional volume (right)

Future developments

Further improvements to the imaging capability of the system are possible, including the use of deep-depletion CCDs for imaging thicker samples with higher energy X-rays; open electrode CCDs for imaging low contrast samples; and improved spatial resolution. Further information about the CCD and associated electronics used to take the presented images can be obtained from Xcam.

References

[1] S. C. Mayo et al., “X-ray phase-contrast microscopy and microtomography”, *Optics Express*, vol. **11**, no. 19, (2003), pp. 2289-2302.

[2] S. W. Wilkins, T. E. Gureyev, D. Gao, A. Pogany, A. W. Stevenson, *Nature*, vol. **384**, (1996), pp. 335-338.

[3] S. C.Mayo et al., “Quantitative X-ray projection microscopy: phase-contrast and multi-spectral imaging”, *Journal of Microscopy*, vol. **207**, pt. 2, (2002), pp. 79-96.

[4] D. H. Lumb, A. D. Holland, “Event recognition techniques in CCD X-ray detectors for Astronomy”, *Nuclear Instruments and Methods*, vol. **A273**,(1988), pp. 696-700.

[5] V. E. Cosslett, W. C. Nixon, “X-ray Microscopy”, Cambridge University Press, London, 1960.

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