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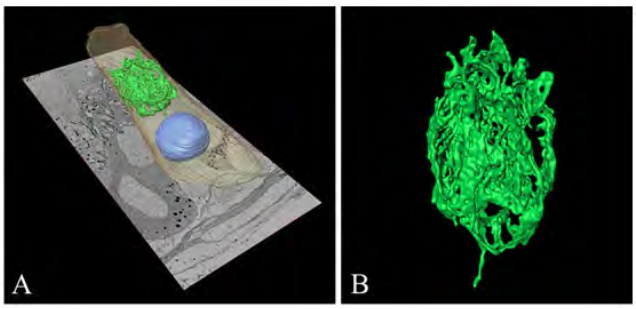
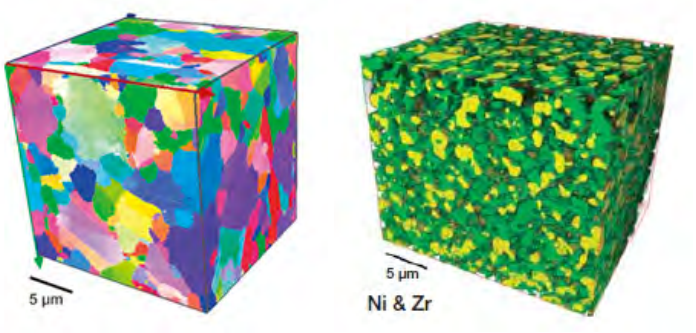
SEM-based Volume Microscopy:
Serial Block Face Tomography,
Array Tomography,
FIB Tomopgraphy





Background

Volume imaging across a wide range of length scales has become critical in order to further our understanding of biological and materials systems. Selecting the most appropriate technique for the materials and the length scales in question is critical, and the use of more than one technique may be necessary to gain a proper understanding of the structures. It may be necessary to observe very wide areas, or very small areas at ultra-high resolution. Additionally, it may be necessary track very fine structures across a large region and combine wide area overviews with high-resolution investigations.

SEM-based volume imaging techniques typically require a large set of sequentially acquired images to be aligned and processed into a 3D data set. In the case of serial block face imaging and serial focused ion beam sectioning, the image is of the resulting exposed bulk surface. In the case of array tomography the images are from multiple thin sections of the original specimen which are laid out on a supporting substrate.

In addition to imaging, it may also be necessary to undertake additional analysis such as energy dispersive X-ray spectrometry (EDS) for elemental information, or electron backscatter diffraction (EBSD) for crystallographic information. Thus the automatic and reliable interlacing of milling, imaging and EDS or EBSDD is important.

Serial block face imaging (SBF) in SEM is undertaken by replacing the SEM stage with a dedicated microtome, or more recently by integrating a microtome on top of the existing stage. SBF is well suited to wide area observations and provides valuable insights into a range of research topics such as cellular ultrastructure, connectomes or the makeup of soft materials like complex functional polymers. The depth resolution can be limited by the beam interaction volume and low voltage imaging is used to avoid oversampling (give an appropriate depth of information for each image), although variation in specimen density means that it is not possible to have an exact and identical sampling depth for each pixel/voxel. It may also be necessary to use methods to mitigate sample charging on the block face since it is usually non-conductive.

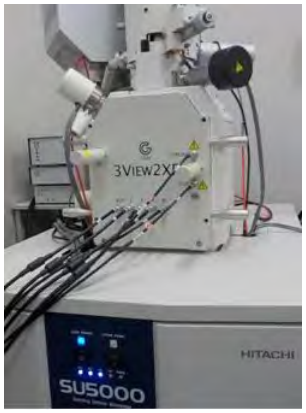
Serial sectioning in FIB also provides imaging of the block face, but is commonly used for smaller regions of interest - making use of the site-specificity of the ion beam compared to a microtome. The ion beam is unable to remove wide areas of material as quickly as the microtome. It is therefore critical to find the region of interest rather than mill huge volumes which can quickly become prohibitively slow.

Array tomography offers an alternative, and on appropriate materials it can provide resolution comparable to TEM tomography. High levels of automation, and improvements in low kV SEM imaging and backscatter detection performance, have recently made the technique more attractive. It also has the advantage that the specimen remains intact for subsequent investigation.

SBF tomography examples

Fig.11 shows the SBF workflow, from acquiring the serial image set, to aligning them, performing 3D reconstruction and subsequent segmentation. In this example the golgi aparatus in an epithelial principal cell of a rat epididymis has been reconstructed. The golgi aparatus (green) shows complex cisterna resembling the petals of a tulip.

In Fig.2 serial block face imaging has been undertaken at 2kV, using a backscatter electron image, in the Hitachi SU5000 Schottky VP-SEM. A wide area of over 30um field of view is observed, whilst a high pixel resolution of 16k x 16k allows specific structures to be examined or reconstructed after initial data acquisition.



SBF can be performed with a dedicated microtome stage (left) or substage (above)

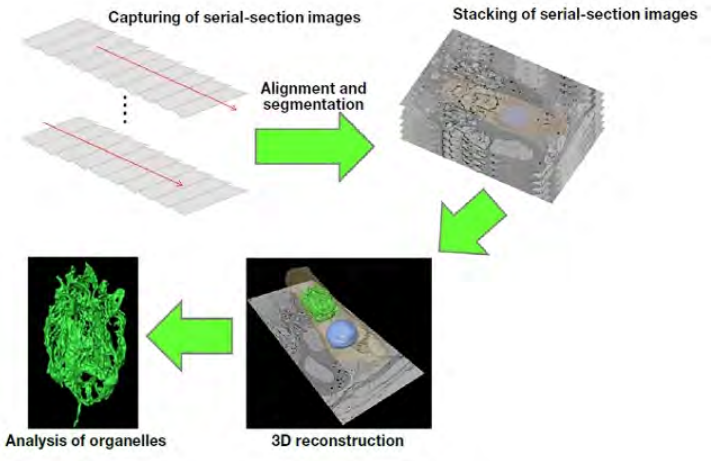
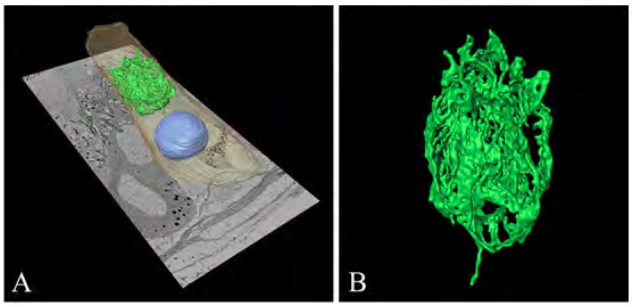
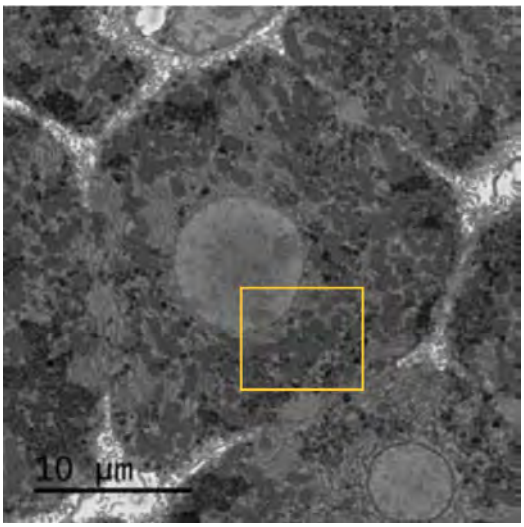


Fig.11SBF reconstruction of Golgi apparatus





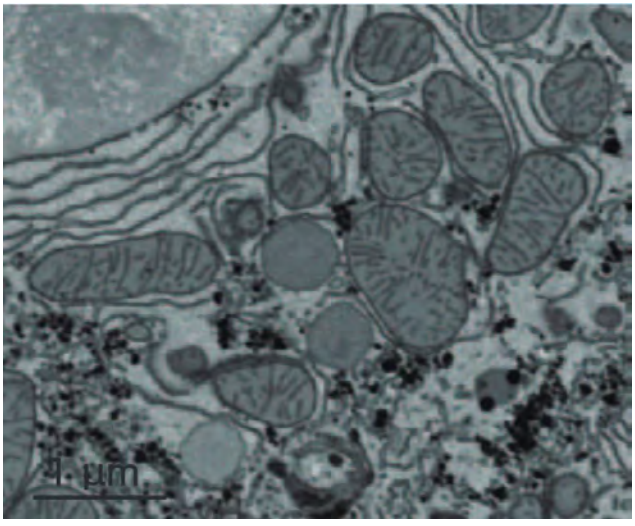


Fig.22 A wide area 16k x 16k pixel acquisition, and subsequent analysis of region of interest in a mouse hepatocyte

Array tomography example

Array tomography initially requires the placement of a set of microtomed sections on a supporting substrate such as a glass slide or silicon wafer. In Fig.3 119 sections (each 1mm x 2mm wide with section thickness 100nm) have been arranged on a glass substrate.

With appropriate automation software, such as Hitachi's ACAT package, and high performance low kV imaging from Hitachi's cold field emission SEMs (right) high resolution tomography can be performed quickly and easily. Images can be fully automatically acquired from specific regions in each section. The software features auto correlation to handle bent ribbons and orphan sections.

Fig.4 is of a central nervous system node of a rat's optical nerve. The results showed that astrocyte (pink) which expected to cover the CNS nodes in textbook fashion accounted for no more than 13% of coverage in this study, and nodes were approximately 76% surrounded by extracellular matrix (ECM, yellow). The green represents oligodendrocyte.

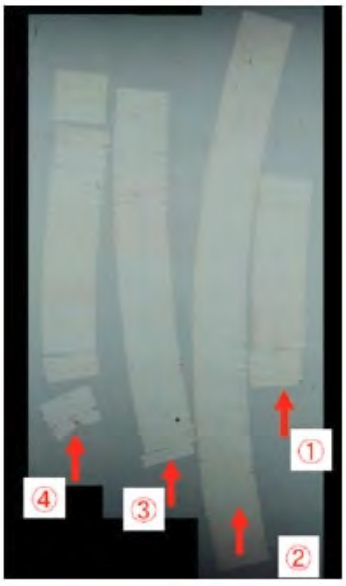


Fig.3 Glass slide holding 119 serial sections. One section 1 mm × 2 mm. Section thickness 100 nm

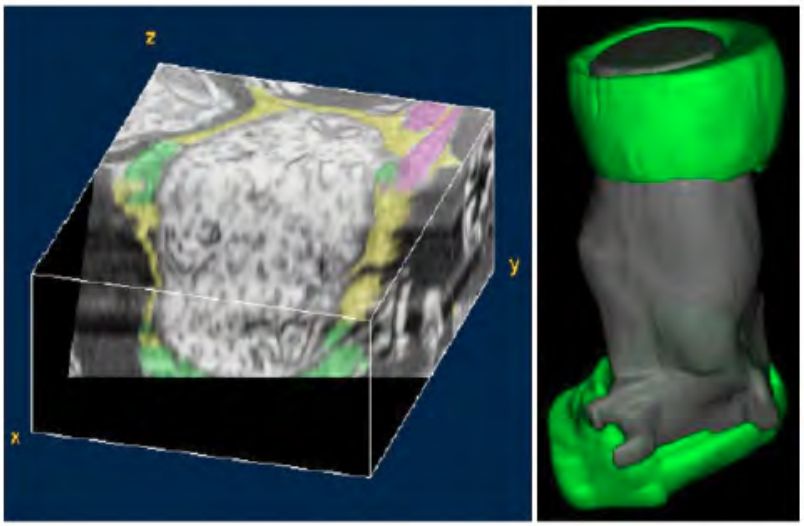


Fig.4 Array tomography of central nervous system nodes (CNS) of a rat optical nerve

FIB-SEM tomography examples

FIB-SEM tomography is now a routine application and can be readily undertaken in FIB-SEMs such as Hitachi's NX5000 and NX9000, including with elemental analysis (EDS) or with crystallographic analysis (EBSD).

Key considerations for good practical performance include high FIB current, as well as the characteristics of the probe under high current conditions. The probe should be well controlled, with optimum shape, even under high current conditions.

Another critical consideration is the relative geometry of the SEM, FIB, sample and detectors. Fig.5 below compares a conventional FIB geometry (left) with that of an orthogonally arrange geometry (right) in the analysis of mouse brain neurons. The orthogonally arranged geometry offers a number of district advantages for serial tomography. The orthogonal arrangement eliminates foreshortening and aspect-ratio deformation in the images and results in cubic voxels offering highly precise 3D structural analysis.

The orthogonal arrangement also offers ideal geometry for serial EBSD (Fig .6, right) , with no stage movement required between milling and EBSD acquisition.

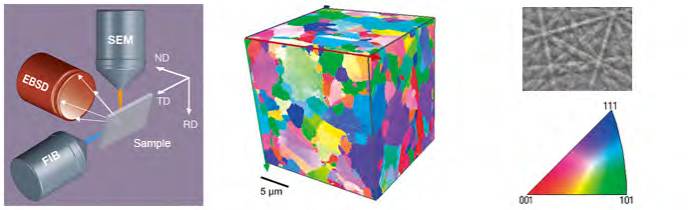


Fig.6 Serial EBSD of Ni in an orthogonally arranged FIB-SEM

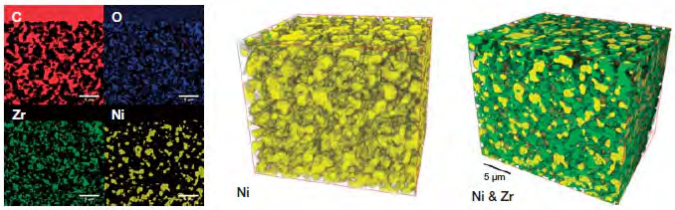


Fig.7 High resolution EDX map and tomogram of a fuel cell electrode

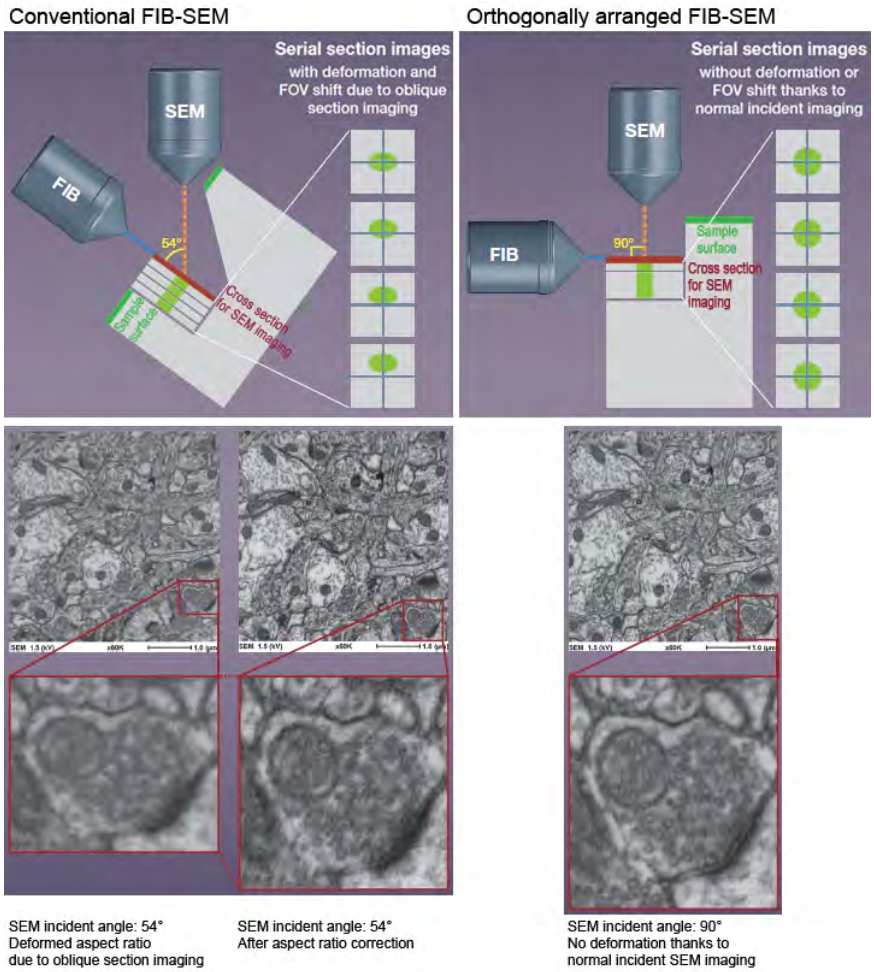


Fig.5 Comparison of conventional and orthogonal geometry for tomography of mouse brain neuron

Acknowledgments

Dr Daisuke Koga, Associate Professor of Department of Microscopic Anatomy and Cell Biology, Asahikawa Medical University
(Fig.1 - golgi)

Dr Koujiro Tohyama, Iwate Medical University, Center for Electron Microscopy and Bio-Imaging Research (EMBIR)
(Fig.3/4 - rat optical nerve)

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(Fig.5 - mouse brain neuron)

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