Backscattered electron and x-ray imaging for array tomography provides rapid specimen characterisation and roi targeting

Pedro Machado[1], George Stonadge[1], Haithem Mansour[1], Alejandra Carbajal[2], Guilherme Neves[3], Juan Burrone[4], Louise Hughes[1], Dr Louise Hughes
[1]Oxford Instruments NanoAnalysis, High Wycombe, United Kingdom, [2]Centre for Ultrastructural Imaging, King's College London, United Kingdom, [3]MRC Centre for Neurodevelopmental Disorders, Institute of Psychiatry, Psychology and Neuroscience, King's College London, United Kingdom, [4]Centre for Developmental Neurobiology, Institute of Psychiatry, Psychology and Neuroscience, King's College London, United Kingdom

Background:
Array tomography is a flexible volume electron microscopy (vEM) workflow due to its non-destructive nature, multi-scale imaging (mm to nm sized imaging regions) and well-established correlative light and electron microscopy (CLEM) compatibility. Sections are collected on a solid, conductive support, and can be imaged multiple times at different resolutions.
There is a constant evolution and improvement of protocols/techniques for array tomography. Acquisition bottlenecks have pushed the development of workflows to tackle imaging efficiency. However, establishing standards for reproducible specimen preparation remains unsolved. There are a wide variety of preparation methods, contrasting techniques, and individual heterogeneity that affect uptake of stains and the visualisation of ultrastructure. This makes it difficult to directly compare experiments and different types of specimens, which can have repercussions on the reconstruction of vEM data. Quantitative compositional analysis, such as energy dispersive x-ray spectrometry (EDS), can help to identify common baselines and standards, positively impacting comparative data analysis and potentially facilitating automated segmentation of volume data.
We present Backscattered Electron and X-ray (BEX) imaging for the simultaneous and combined acquisition of backscattered electron (BSE) and x-ray data in a scanning electron microscope. BEX acquires ultrastructural and composition data simultaneously, providing fast and automated mapping across a large area of sample.

Methods:
We used Unity (a BEX detector, Oxford Instruments, UK) combined with an Ultim 100 (Oxford Instruments, UK) to image array tomography slices of mouse brain tissue prepared with an adapted ROTO protocol. Large areas were collected automatically, using cartography mode, in a Zeiss 460 (Carl Zeiss Microscopy GmbH, Germany) operated at 8kV, 1-2nA probe current, at 7.5mm WD, 10µs dwell time.

Results:
BEX cartography data achieved high resolution and fast mapping of array tomography brain slices. Large areas were imaged with a relatively short beam dwell time (10µs), which reduces beam damage, drift, and resin charging (Figure 1). Elemental information was acquired simultaneously and provided chemical differentiation that can be used to further distinguish between sample features, opening the possibility of improving subsequent segmentation of data. BEX also provided information about strain distribution, which EDS quantified. Being able to measure the amount of stain taken up by the sample improves our ability to make direct comparisons between samples and also enables us to optimise our sample preparation techniques.

Figure 1. Large area 7x8 tiles acquired using cartography mode. Top inset displayed at 50% zoom allows to easily distinguish different cell borders. Bottom inset at 100% zoom with a 10nm pixel size with detailed membranes in mitochondria and small vesicles (15-30nm range).
Conclusions: Creating reference specimens for the vEM community has been previously proposed, as benchmarks to compare microscopes, imaging conditions and image segmentation tools. As a high speed and sensitive imaging technique, BEX opens the way towards controlled reproducibility by complementing array tomography ultrastructural images with chemical information.

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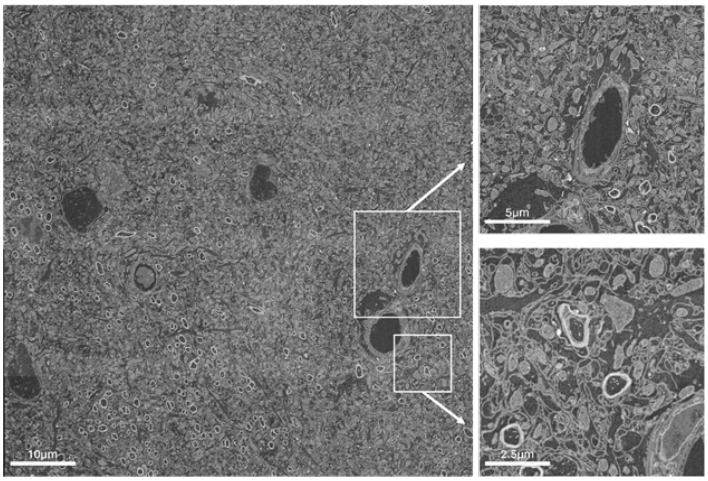
Graphic:



Keywords:

Array tomography, Backscattered Electron X-ray

Reference:

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