Invited Speaker

40 Array tomography enables correlative volume electron microscopy and spatial transcriptomics
Mr Georg Kislinger[1,7], Mr Peter Androvic[2], Mr Gunar Fabig[5], Mr Igor Khalin[2], Mr Nikolaus Plesnila[2], Mr Thomas Misgeld[1,3,7], Mr Thomas Müller-Reichert[5], Mr Oz Gokce[2,5], Martina Schifferer[1,3]
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231 Near isotropic, high-resolution multi-beam scanning transmission electron microscopy with iterative milling
Mr. Adrian Wanner[1], Ms. Maria Kormacheva[1], Mr. Arent Kievits[2], Mr Stijn Karacoban[2], Mr Joakim Reuteler[3], Mr Sander den Hoedt[4], Ms Marre Niessen[4], Mr Safe Khan[5], Mr Carles Bosch[5], Mr Jacob Hoogenboom[2], Mr Andreas Schaefer[5]
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Arent Kievits[1], B. H. Peter Duinkerken[2], Ryan Lane[1], Cecilia de Heus[3], Daan van Beijeren Bergen en Henegouwen[2], Tibbe Höppener[1], Anouk H. G. Wolters[2], Nalan Liv[3], Ben N. G. Giepmans[2], Jacob P. Hoogenboom[1]
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718 Developing cryo-Volume Electron Microscopy using the JEOL 4700 cryo-FIBSEM to address biological ultrastructural questions
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[1]King's College London, London, United Kingdom
882 Deconvolution of SBF-SEM images improves quality of data in volume electron microscopy
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[1]Institute of Anatomy, University of Bern, Bern, Switzerland, [2]Scientific Volume Imaging, Hilversum, The Netherlands, [3]Microscopy Imaging Center, University of Bern, Bern, Switzerland
939 Combining synchrotron-based microCT with plasma FIB-SEM for targeted Volume EM
Dr Rachel Templin[1], Dr Denis Korneev[1], Dr Sergey Gorelick[1], Minyu Chan[1], A/prof Georg Ramm[1]
[1]Monash Ramaciotti Centre for Cryo EM, Clayton, Australia

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100 Enhanced imaging for serial Cryo-FIB-SEM microscopy of biological samples with fluorescence navigation
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[1]Central European Institute of Technology, Masaryk University, Brno, Czech Republic
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Mr Lu Thon Beng[1], Mr Victor Racine[2], Mrs Anne Beghin[3], Dr Isabelle Guerin-Bonne[1,2,3]

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518 Optimising a modern high performance FE-SEM for multimodal vEM

Dr Eudri Venter[1], Dr. Sonja Gürster[2]

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631 Preparation of biological samples for cryo-electron microscopy using the HPF "Waffle" method

Mrs. Jana Moravcová[1], Petra Reznickova[2], Martin Polak[1], Jiri Novacek[1]

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733 Cross-sectioning of adherent cells on thin plastic substrate for serial block-face imaging

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744 Cryo-FIB as a preparation tool for soft X-Ray Tomography: Analysis beyond EM

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1101 SoftGrids: towards disease modelling in CryoEM

Abstract Submission Asena Oelschläger[1], Prof. Dr. Stefan Raunser[1], Dr. Sebastian Tacke[1]

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Late Poster Presentation

1278 Correlative-Cryo Microscopy to Characterise Bacteria-Nanopillar Interactions: Achievements and Challenges

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1281 Nanoscale X-ray imaging to Study Bacteria-Nanopillar Interactions

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Array tomography enables correlative volume electron microscopy and spatial transcriptomics

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IM-12, Lecture Theater 5, august 26, 2024, 10:30 - 12:30

Volume Electron Microscopy (vEM) provides high-resolution data of target structures and resolves three-dimensional representations of otherwise ambiguous biological geometries. While EM provides high spatial resolution, search processes in volumes of several hundreds of square microns is tedious. Multimodal methods like correlated light and electron microscopy (CLEM) allow to bridge these scales. Among the available vEM techniques, array tomography methods like automated tape collecting ultramicrotomy (ATUM) have proven particularly powerful for targeting specific or rare biological structures as needed for correlation as they enable repetitive and large field of view imaging. Previously, we have applied diverse ATUM-CLEM approaches to reveal ultrastructural correlates of neurodegenerative pathologies. These classic correlation techniques annotate ultrastructural data with one or a few molecular targets. With the advent of spatial transcriptomics (ST), the localization of cells with specific expression profiles covering several thousands of transcripts has become accessible. So far, diverging sample preparation techniques have hindered correlated ultrastructural investigation. Here, we developed STcEM, a method that links spatially-resolved gene expression of single cells with their ultrastructural morphology by integrating ST and ATUM on adjacent tissue sections. With this method we successfully mapped microglial classes according to their transcription profile and ultrastructural morphology in a mouse model of white matter lesion. Our results offer a comprehensive view of the spatial, ultrastructural, and transcriptional reorganization of single cells after brain injury.

Keywords:

array tomography, volume EM, CLEM

Reference:

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Near isotropic, high-resolution multi-beam scanning transmission electron microscopy with iterative milling

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IM-12, Lecture Theater 5, august 26, 2024, 10:30 - 12:30

Volume electron microscopy is the only technique to date that provides both sufficient resolution (<20 nm) and sufficient field of view (>100 µm) for the dense reconstruction of neuronal wiring diagrams. Currently, there exist two systems that have already delivered mm³-sized synaptic resolution electron microscopy stacks: Multi-beam scanning electron microscopy [1, 2] (mSEM) and Gridtape-based automated transmission electron microscopy [3, 4] (Gridtape-TEM). Both techniques currently rely on collecting and imaging thousands of ultrathin serial sections (30 - 40 nm) being cut with a diamond knife on an ultramicrotome and collected on a tape, silicon wafer or gridtape. However, serial collection of ultrathin sections is delicate and inherently prone to failures and artefacts such as section loss, folds, cracks or knife marks. More than 50% of the errors of today's state-of-the-art automated neuron segmentation algorithms can be attributed to missing information due to serial sectioning. Consequently, more than 40 hours of manual segmentation proofreading by human experts are currently required to reconstruct a single cortical pyramidal cell accurately.

Gas Cluster Ion Beam Scanning Electron Microscopy (GCIB-SEM) [5] and Broad Ion Beam Scanning Electron Microscopy (BIB-SEM) [6] methods have been proposed to address the section loss issue. These methods involve the collection of thicker sections, in a range of 100-10000 nm in thickness, onto a silicon wafer, offering a more robust approach for section collection. The collected volume is then iteratively sub-milled using either GCIB or BIB techniques to match the desired resolution in the Z-axis and then imaged using an SEM.

Here, we adopt the same principle of collecting semi-thin sections with subsequent sub-milling and, to have an imaging time advantage, we propose using a commercially available multi-beam scanning transmission electron microscope - mSTEM (FAST-EM Delmic, Netherlands), and use BIB for iterative sub-milling. We will refer to the combination of the two as BIB-mSTEM. In contrast to mSEM, mSTEM forms the image from high-energy transmitted electrons that are much less sensitive to local electromagnetic fields and milling-induced irregular surface topography than low-energy secondary electrons. Additionally, mSTEM offers the advantage of being more cost-effective compared to mSEM, making it an attractive option for large-scale imaging projects.

As proof of the method, we first collected serial sections of 250 nm thickness directly onto a scintillator plate using the commercially available ultramicrotome (Leica, Germany). Subsequently, we iteratively imaged the sections with FAST-EM and sub-milled the volume using BIB, producing a series of iteratively milled TEM projection images for each section. We then used those projections to computationally reconstruct a high-resolution 3D stack of each section. We used a deconvolution procedure to reconstruct or deconvolve virtual reslices (Fig. 1B+D) from the series of projection images (Fig. 1A+C). The data shows that the proposed method increases the Z-resolution sufficiently

to capture small changes in the ultrastructural appearance of neurites, mitochondria and synaptic vesicles (Fig. 1D) [7].

Fig. 1: Data from an iteratively BIB-milled and FAST-EM imaged 250 nm thick section. A STEM projection images of the iteratively milled section. B Corresponding deconvolved/reconstructed slices. C, D Zoom-in onto a dendrite in A and B, respectively. Scale bar = 1 µm.

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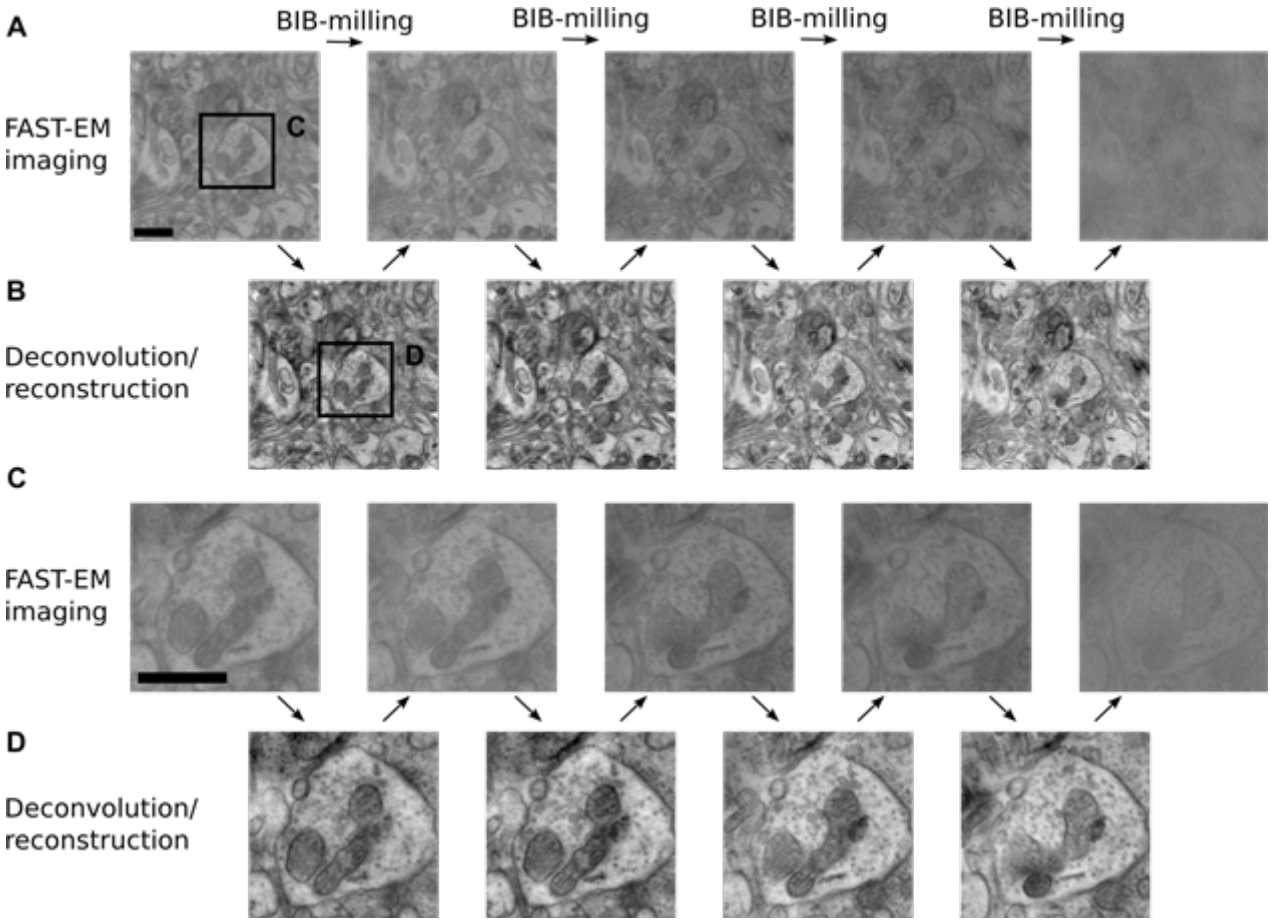

Keywords:

volume electron microscopy, connectomics

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FAST-EM array tomography: a workflow for multibeam volume electron microscopy

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IM-12, Lecture Theater 5, august 26, 2024, 10:30 - 12:30

Background
Emerging volume electron microscopy (vEM) techniques are providing unprecedented insights into the nanoscale 3D structure of biological specimens. However, the limited sustained throughput of electron microscopes hinders large sample handling (1). Multiple approaches, including camera-array transmission EM, beam-deflection transmission EM and multibeam scanning EM, have been developed to improve throughput and are now delivering first results (2, 3). We present a workflow for multibeam volume microscopy, FAST-EM array tomography. FAST-EM is a commercial multibeam scanning transmission electron microscope which speeds up acquisition by scanning the sample with 64 beams in parallel (4).

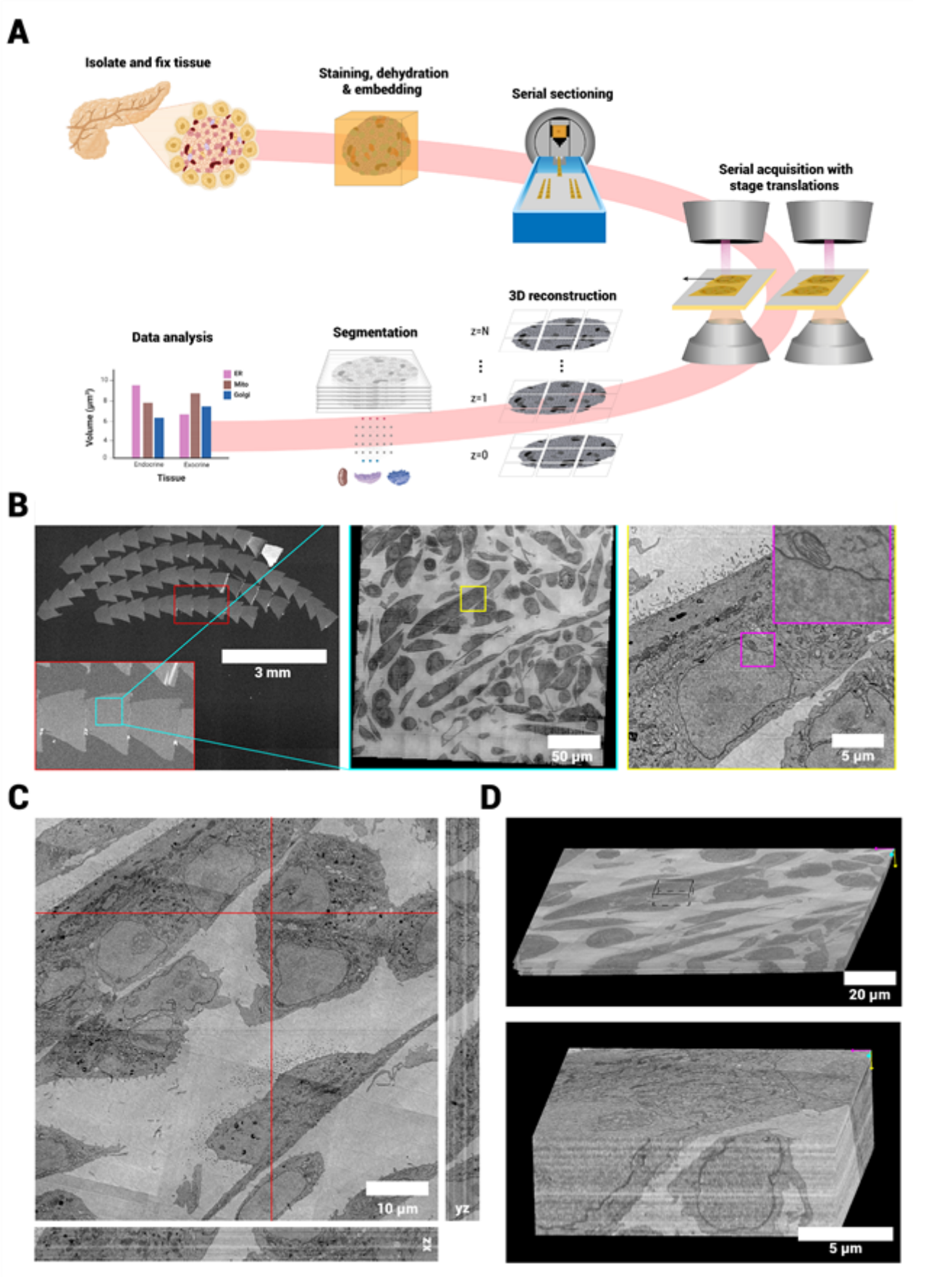
Methods
In FAST-EM array tomography, biological samples are prepared by chemical fixation, heavy metal staining, dehydration and resin embedding (Figure 1A). Serial sections are produced by ultramicrotomy and deposited on a cerium-doped yttrium aluminum garnet (ce:YAG) scintillator crystal, coated with molybdenum. FAST-EM employs optical scanning transmission electron detection (OSTEM), where transmitted electrons are converted into photons by the scintillator, which are then collected and descanned onto a detector array. The sections are imaged sequentially using stage translations. The volume is reconstructed from 2D images by finding point-pair correspondences in the overlap region between individual images. The 3D reconstructed data is then segmented using manual or automatic techniques, after which data analysis is conducted.

Results
Several biological samples, including cultured cells, organoids, as well as tissue samples were imaged with FAST-EM. To demonstrate the workflow, a 265.000 um3 volume of ~70 cultured MCF-7 cells was reconstructed from 72 100nm thin serial sections resolving the mitochondrial cristae and membrane structures (Figure 1B). The data quality and alignment is consistent throughout the volume (Figure 1C-D). To further demonstrate the applicability of analysis tools developed for other electron detection techniques to FAST-EM and OSTEM detection data, all mitochondria were automatically segmented using MitoNet, a convolutional neural network for 3D segmentation (5). MitoNet applied to FAST-EM datasets demonstrated performance metrics similar to benchmark datasets obtained using other vEM modalities.

Conclusion
We implemented a workflow for multibeam volume transmission electron microscopy capable of imaging large regions of interest with feasible acquisition times, while providing images with high resolution and contrast. FAST-EM potentially expedites EM for comprehensive sample scanning and statistical analysis across multiple conditions. This creates opportunities for novel applications, such

as examining the inter-cellular variability in a large population of cells. Further increase of throughput is possible by optimizing the signal generation and collection, reducing overhead and increasing system autonomy.

FIGURE LEGEND:
Figure 1: Volume EM reconstruction of cell culture with FAST-EM array tomography. A: FAST-EM array tomography workflow. B: Overview images of sections, showing a zoom in on a single section, a single multibeam field-of-view and a single beam image respectively. C: Aligned volume reconstruction from 54 100nm thin serial sections showing the orthogonal reslices through the red lines (xz and yz). D: Volume rendering of the full (continuous) stack. Inset shows smaller sub volume at 8nm/pixel resolution.



Keywords:

vEM, SEM, FAST-EM, STEM, OSTEM

Reference:

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Developing cryo-Volume Electron Microscopy using the JEOL 4700 cryo-FIBSEM to address biological ultrastructural questions

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IM-12, Lecture Theater 5, august 26, 2024, 10:30 - 12:30

The Focused Ion Beam Scanning Electron Microscope (FIBSEM) is well suited for site-specific sectioning where the FIB milling exposes a surface that can be imaged either with the FIB or SEM to examine the microstructure within each layer and the interfacial structure between layers. By collecting ultrastructural data from a large volume, to be interrogated in silico; novel understandings of the interconnectivity between many individual cell types can be deduced. These volume Electron Microscopy studies (vEM) overcome two principal limitations of EM: the loss of 3D understanding of ultrastructure and the possibility of "missing" rare ultrastructural events through the limited volume of tissue examined. However, to date, this has only been successfully applied to chemically processed tissue, with their associated artefacts. Non-chemical fixation using low temperature allows cells and tissues to be studied close to their native biological state and has been applied to cryo electron tomography (cryoET) of thin sections of cells and tissues. With cryo lamellae produced from vitrified material by controlled milling using focussed ion beam milling in a dual beam scanning electron microscope at cryogenic temperatures (cryoFIBSEM).

Here we develop cryo vEM (cvEM) to generate larger 3D volumes than possible with cryoET by a sequence of sectioning in a cryo FIBSEM at high resolution and close to the biological native state. By successfully overcoming the high beam sensitivity of vitrified material, lack of heavy metal contrast and significant challenges to the generation of sufficient signal to noise it has been possible to demonstrate the potential of cvEM as a complementary approach to cryo ET capable of generating larger volumes (e.g., an entire cell), and at the same time retaining resolution sufficient to clearly define ultrastructural details at the level of the membrane bilayer (Figure 1). We have incorporated novel scan approaches and evaluated in-painting techniques to manage charge and improve signal to noise (1,2). We also show the workflow can be successfully extended to multicellular volumes by increasing vitrification depth with high pressure freezing, the "waffle" method. By integrating a cryo correlative capability (cryoCLEM) into the workflow, we are able to target regions of interest for cvEM. Data detailing differences between two Euglenoids at the subcellular level has been generated. Clear differences between Eulgena longa and Euglena gracilis Paramylon storage granules and key organelle were observed.

Future development aim to incorporate lamella lift out with cryo CLEM, cvEM to a predetermined region of interest with cryoET of recovered lamella. A complete and flexible full cryo FIBSEM workflow, the circle would be completed for cryogenic samples for complex biological studies.

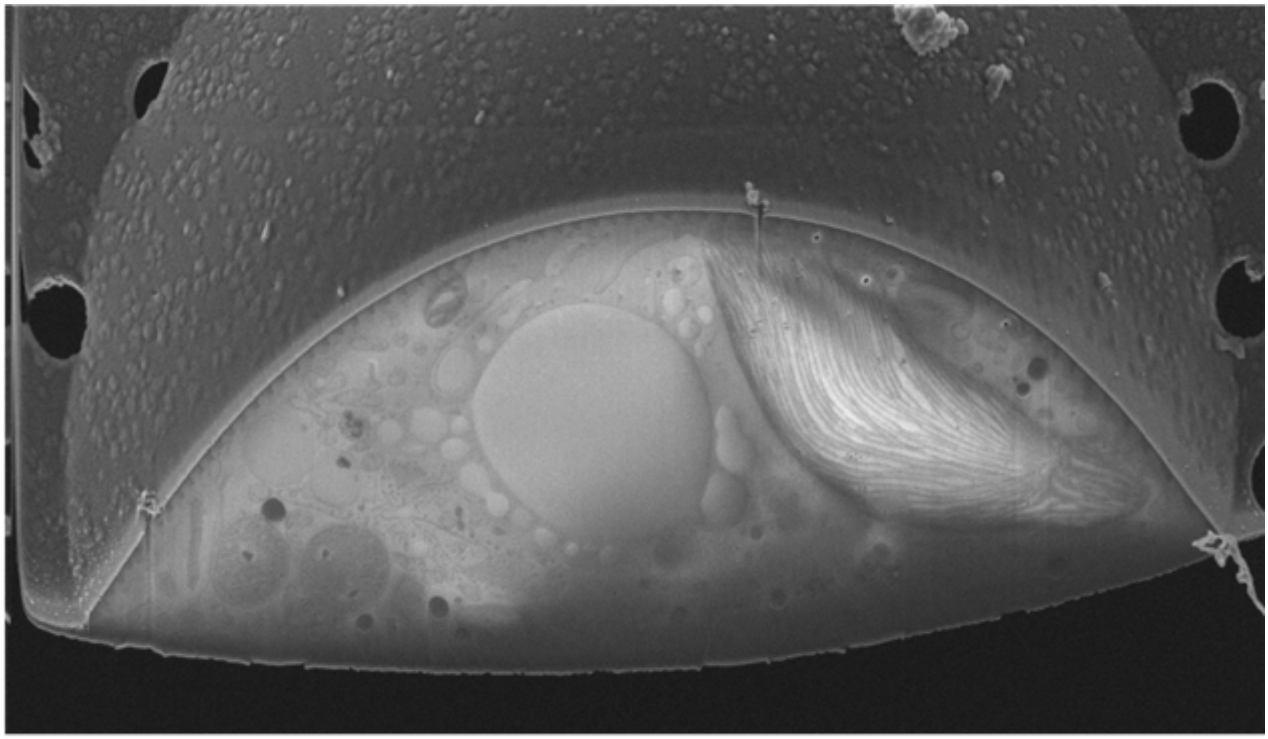

Figure 1. High resolution Image acquired by novel JEOL UHD Scan Interface of *Euglena gracilis* (CCAP 1224/5Z). The intercellular structure is clearly visible with well resolved membrane detail. The image has even contrast and charge is balanced across the entire vitrified sample.

Keywords:

cryo FIBSEM, vEM

Reference:

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Deconvolution of SBF-SEM images improves quality of data in volume electron microscopy

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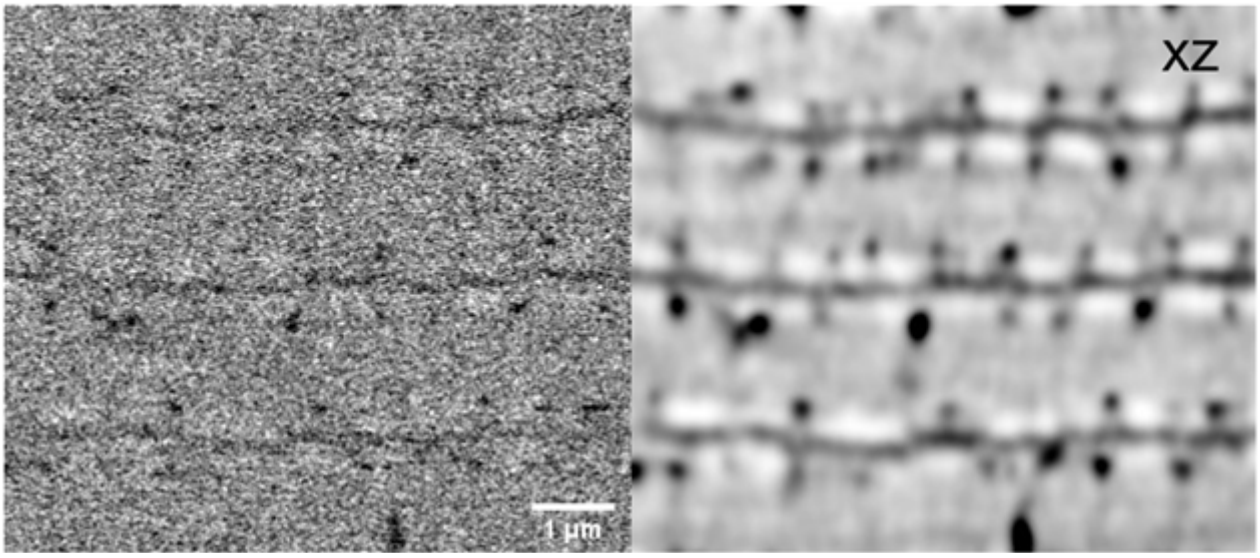
IM-12, Lecture Theater 5, august 26, 2024, 10:30 - 12:30

Introduction
The serial block face scanning electron microscopy (SBF-SEM) is a state-of-the-art method for volume imaging of biological objects. Despite many advantages, it suffers from an unavoidable trade-off between acquisition time and image quality: faster acquisition gives noisier, and consequently less resolved images. This problem is known in light microscopy and solved there by applying different image processing methods such as denoising and deconvolution with classical and AI algorithms. We transfer this concept to electron microscopy by designing SBF-SEM point spread function (PSF) and using it for deconvolving the images with the aim of getting less noisy images with higher resolution.
Methods
First, we model 3D SBF-SEM PSF with Monte Carlo simulations of the electron penetration and scattering in an EPON resin with CASINO software for beam energies ranging from 1kV to 15kV. Second, we image the sample with FEI Quanta 250 FEG scanning electron microscope equipped with a 3View2.XP in situ ultramicrotome using the beam energy within simulated values. Finally, we deconvolve acquired images with Huygens Professional software (SVI, the Netherlands) using simulated PSF and custom-made script with 3D CMLE algorithm and corresponding rescaling of SBF-SEM PSF to light microscopy range.
Results
The simulated SBF-SEM PSF has a characteristic pear-shaped form with additional difference to light microscopy, that it only exists in the lower half-space due to the nature of electron scattering in the sample. We applied simulated SBF-SEM PSF in deconvolution of biological specimens. Deconvolved images have higher quality and consequently reveal finer structures, for example allowing for an improved definition of z-lines in skeletal muscle of a mouse, see picture. As the quality of the image is significantly improved after deconvolution, developed methodology can be applied for improving images with lower beam energy and higher imaging speed.
Conclusions
Transfer of the 3D deconvolution concept from light microscopy to SBF-SEM allows for improvement in resolution and speed of imaging in volume electron microscopy in life sciences.



Keywords:

SBF-SEM, deconvolution, PSF

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Combining synchrotron-based microCT with plasma FIB-SEM for targeted Volume EM

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IM-12, Lecture Theater 5, august 26, 2024, 10:30 - 12:30

Background
Although offering the highest resolution for volume EM, conventional FIB-SEM imaging has a limited field of view, provides only surface-level information, and is unable to access information non-destructively from within the sample volume to pinpoint areas of interest. Hence, integrating an additional imaging modality capable of non-destructive volume imaging becomes necessary to accurately locate and target regions of interest within complex multilayered specimens. Micro-Computed Tomography (MicroCT) offers a larger 3D overview but at lower resolution lacks many subcellular details. For volume EM, the combination of the two methods offers precise targeting of regions of interest that can be pre-identified in the microCT volume.

Methods
We used the new microCT beamline on the 3 GeV Australian Synchrotron, which has an X-ray energy range from 8 to 40 keV. We imaged Epon resin embedded tissue blocks processed for slice and view imaging by FIB-SEM. We initially tested both monochromatic and white beams as well as different detector distances, but found no major differences, so choose to continue with the white beam to take advantage of the much shorter imaging times. Blocks were subsequently pre-trimmed by ultramicrotomy, transfer to a Helios Hydra plasma FIB-SEM and block cross-sections were aligned with the microCT volume. Final trimming was done using oxygen plasma milling on the plasma FIB before starting the slice and view run for volume EM.

Results
We here show examples where we targeted specific brain regions in zebrafish embryos by microCT and volume EM and the correlation of the two volumes.

Conclusion
Synchrotron based microCT is substantially faster than microCT on lab-based instruments. As plasma FIB allows milling of larger volumes more rapidly than conventional FIB-SEM it is possible to perform the final fine targeting on the FIB-SEM. In summary, the combination of synchrotron microCT and plasma FIB-SEM offers particular advantages for targeted volume EM.

Keywords:

synchrotron, brain, vEM, zebrafish, CLEM

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Enhanced imaging for serial Cryo-FIB-SEM microscopy of biological samples with fluorescence navigation

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Poster Group 1

Traditional methods for studying biological specimens with volume electron microscopy face two significant hurdles: the complex process of sample preparation, which includes drying and staining with heavy metals and can introduce artifacts, and the difficulty in maintaining fluorescence signals in samples set in resin. In contrast, cryo-FIB-SEM keeps specimens nearly unchanged in a frozen state, streamlining the preparation process, reducing structural distortions, and allowing easier observation of fluorescence. Yet, imaging non-contrasted biological specimens in cryo-FIB-SEM can be challenging due to low signal-to-noise ratios and charging artifacts.

Our research shows that by fine-tuning the imaging conditions, electron exposure, plasma milling parameters and the workflow management, we can greatly improve the quality of the data, speed up the rate of data collection, and capture large volumes of samples with outstanding ultrastructural clarity. This allows us to view all cellular organelles in detail, achieving resolutions as fine as 16nm. An additional advantage of cryogenic imaging is the preservation of the fluorescence signal, which helps in locating cells and guiding the initial trenches in the sample.

With the mentioned improvements, we acquired high-quality cryo-images. We demonstrate the effectiveness of our cryo-FIB/SEM method by reconstructing a segment of a pancreatic INS-1E beta cell, showcasing the workflow's capability to produce high-quality imaging of biological samples.

Keywords:

FIB-SEM, volumeEM, cryo, native, cell

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ORGANOIDS : UNVEILING INSIGHTS WITH VOLUME ELECTRON MICROSCOPY

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Poster Group 1

Background:
In recent years, the adoption of three-dimensional (3D) multicellular models, such as organoids, spheroids, and organs-on-chips, has surged in biological research due to their superior physiological relevance compared to traditional cell culture methods. These models, cultivated from stem cells or organ progenitor cells, emulate the intricate structure and functionality of real organs, providing researchers with invaluable insights into organ development, disease mechanisms, and drug responses within a controlled laboratory environment. They offer a versatile platform derived from various tissues, including the brain, liver, kidney, intestine, and pancreas, holding immense promise for advancing biomedical research, personalized medicine, and drug discovery endeavours.
Understanding the complex structure of these 3D multicellular models poses a significant challenge, especially when aiming for high resolution. However, recent advancements in electron microscopy (EM) have introduced techniques enabling comprehensive imaging of these samples. Volume electron microscopy (vEM) has emerged as a powerful tool for analysing their overall structure and potential variations corresponding to different states, offering nanoscale resolution imaging capabilities.
While cells and tissues have been extensively studied in two-dimensional (2D) settings, successful machine learning and deep learning solutions for detection and classification have been developed. However, transitioning to 3D complex structures introduces novel complexities. Developing effective deep learning models is hindered by the limited human capacity to annotate large volumes of 3D data accurately and reliably. Additionally, the availability of 3D algorithms significantly lags behind those for 2D, creating an annotation bottleneck that hampers progress and stifles the development of innovative 3D solutions.

Methods :
This study focuses on cancer cell organoids (HCT116) under different conditions, imaged using a Serial Block Face (SBF) Katana microtome within a Scanning Electron Microscope (SEM). The SBF-SEM technique involves a miniature ultra-microtome attached to the motorized stage of an SEM, where a diamond knife repeatedly removes thin layers from a sample block. After each section is removed, the exposed block surface is imaged with a back-scattered electron detector. This automated in-situ method enables the acquisition of a series of images throughout the depth of a large sample. From this stack of 2D images, 3D morphology can be reconstructed, and various organelles of interest can be segmented using developed deep learning models.

Results:
Our study demonstrates the suitability of SBF-SEM for imaging organoids, allowing for the imaging of large volume samples at a relatively fast acquisition speed. The field of view is comparable to Array Tomography (up to 3 mm) but surpasses that of Focus Ion Beam-SEM (less than 100 µm). With this

technology, we successfully imaged the entire organoid within a reasonable timeframe, with sufficient resolution to segment cells, cellular compartments, and organelles of the organoid. Furthermore, we are utilizing manual annotation and deep learning on the image dataset to identify structural changes in cells based on the conditions of the organoid and to correlate with 3D imaging with others technics, like confocal microscopy.

Conclusion :
However, the utility of these models necessitates tools for imaging and analysing the large quantity of complex 3D images required for comprehensive analysis. Convolutional neural networks trained on this extensive dataset can precisely detect and quantify subcellular and multicellular features, including mitotic and apoptotic events, multicellular structures like rosettes, cells, and organelles. The integration of high-resolution 3D microscopy techniques with machine learning approaches enables quantitative descriptions of organoid morphogenesis correlated with phenotypic characterization, facilitating the deciphering of mechanisms involved in morphogenesis and human physiopathology, as well as responses to extreme conditions.

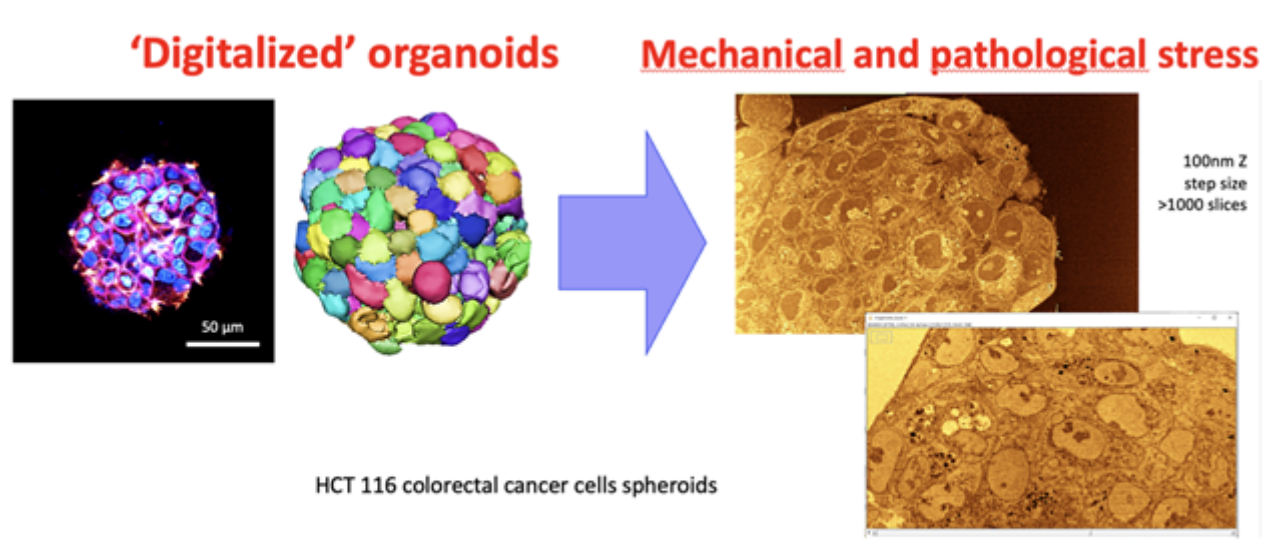

Fig: Streamlining cell culture, 3D imaging and AI based analysis to quantify the homeostasis of organoids

Keywords:

3D, Organoids, volumeEM, Deep learning

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BACKSCATTERED ELECTRON AND X-RAY IMAGING FOR ARRAY TOMOGRAPHY PROVIDES RAPID SPECIMEN CHARACTERISATION AND ROI TARGETING

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Poster Group 1

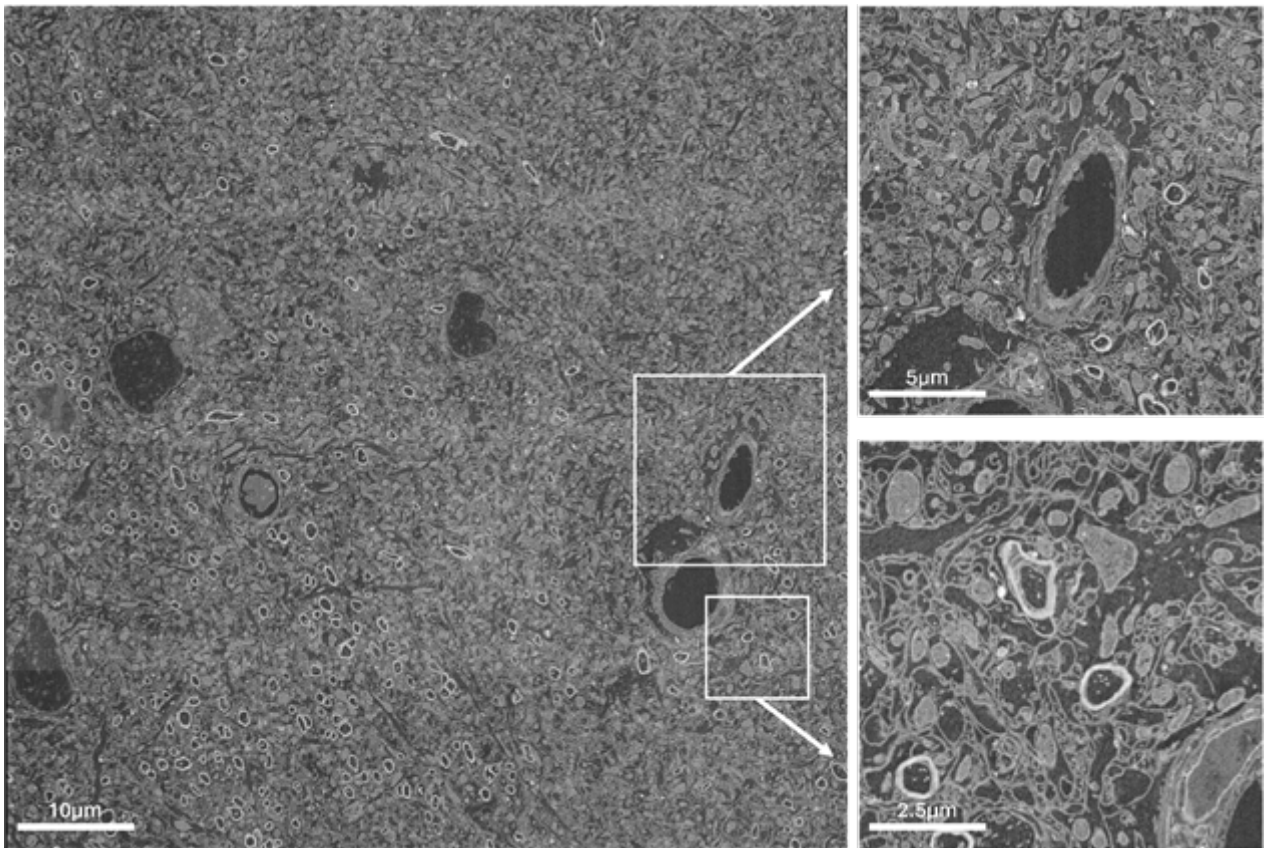
Background: Array tomography is a flexible volume electron microscopy (vEM) workflow due to its non-destructive nature, multi-scale imaging (mm to nm sized imaging regions) and well-established correlative light and electron microscopy (CLEM) compatibility. Sections are collected on a solid, conductive 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.

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.



Keywords:

Array tomography, Backscattered Electron X-ray

Reference:

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Optimising a modern high performance FE-SEM for multimodal vEM

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Poster Group 1

The popularity of volume Electron Microscopy (vEM) has ballooned over the recent past. Noteworthy examples of the increased attention being paid to vEM include: large-scale funding investment initiatives (such as the Chan Zuckerberg Initiative's Imaging program(1)), influential opinion pieces in scientific journals (Seven technologies to watch in 2023 – Nature volume 613(2)) and the growing user base of informative community-run websites and discussion channels (such as the Volume EM community website(3), the Array Tomography community website(4), or the EMofCellsTissuesOrganisms Slack channel(5). One of the main advantages of the increased interest in vEM is the abundance of information to allow prospective vEM users to choose the workflow and methodology that best suits their needs, optimise their experimental approaches and interact with a rapidly expanding user base.

vEM can be accomplished in scanning electron microscopes (SEM), transmission electron microscopes (TEM) or with focused ion beam/ dual beam SEMs (FIB-SEM). This talk will focus on the two main techniques for vEM within a SEM; namely array tomography (AT) and serial block-face SEM (SBF-SEM). Each of these techniques has their own unique pros and cons in terms of the sample preparation requirements, achievable voxel resolution, and re-usability of sample material. Historically, one of the main drawbacks of vEM was that the requisite hardware often limited prospective vEM users to a single instrument choice, or worse yet, to a single vEM technique. Modern advances in hardware and software allows JEOL the ability to offer bespoke solutions for vEM workflows; whether the requirement is to choose a microscope best suited to the scientific questions, the flexibility to have access to both array tomography and serial block-face SEM within a single instrument, or both. The unique software and hardware advances, which allows multimodal vEM to be achieved, will be discussed in this talk.

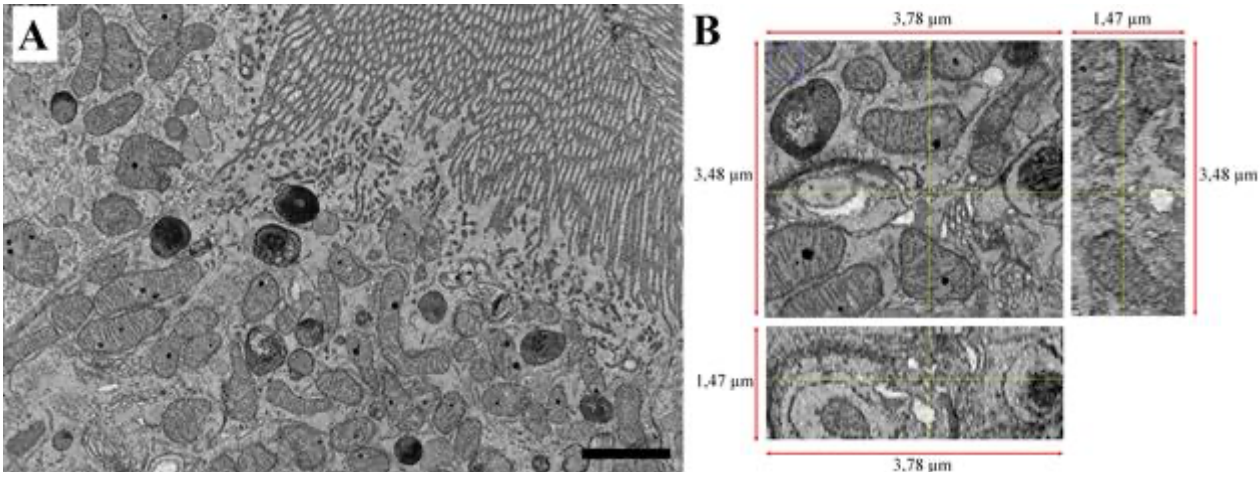
Recent developments in FE gun and SEM column design makes it easy to optimize imaging conditions such as accelerating voltage, probe current, column mode and implementing beam deceleration. Hardware advances to allow this ease-of-use include an in-lens Schottky Plus FEG which integrates the electron gun with a low-aberration condenser lens improving brightness and reducing beam diameter, and an aperture angle control lens (ACL) (located beneath the condenser lens), that continuously suppresses the spread of the incident electrons maintaining the smallest possible probe size even when the probe current is increased.

Other hardware advances include the improved probe-forming capability of a field-free hybrid electromagnetic and electrostatic lens effect along with advancements in column optics for automated control and adjustment of the electron lenses and correction of electron trajectories in real time. Together these allow one to achieve optimized resolution under all imaging conditions. Another key to high quality data acquisition is a new scintillator-type back-scattered detector that is optimised for low kV imaging and allows high-resolution images to be acquired at fast speeds, perfect for the challenges faced by vEM sample preparation.

Transitioning our FEG-SEM from normal imaging mode to SBF-SEM mode is very straightforward through partnership with ConnectomX. All that is required is to vent the chamber, attach the stage-mounted Katana microtome, and pump down again; a process that can be done in just a few minutes. Capturing high quality 3D data has never been easier (Figure 1).

In addition to hardware advances, the evolution of software streamlines and simplifies automated data acquisition. For both AT and SBF-SEM experiments on JEOL microscopes, all of the hardware

control (both microscope and microtome if in use), experiment setup and data acquisition is controlld through SEM Supporter software. Similar guided workflows exist for both techniques, which makes transitioning between the techniques straightforward. For example, the AT workflow complexity is easily condensed into three steps. First a low magnification montage is captured over the entire sample set. Then predictive ROI positioning is used to propagate the ROI over all serial sections. Finally image cross correlation is used to control image shift and scan rotation to keep the successive ROIs well aligned.
Following data acquisition significant advances have also been made to offer software solutions for image alignment, rendering and segmentation, to round out the solution for the whole vEM workflow.
In conclusion, JEOL offers bespoke solutions for different vEM applications, within a single SEM. Optimized solutions for all the steps in the vEM workflow – from high quality data collection, to easy to set up workflows, to intuitive and straightforward means for data processing and analyses, allow vEM to be within reach for researchers of any experience level.



Keywords:

Multimodal vEM, SEM, SBF, AT

Reference:

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Preparation of biological samples for cryo-electron microscopy using the HPF "Waffle" method

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Poster Group 1

Background incl. aims
Cryo-electron microscopy (cryo-EM) has emerged as a pivotal technique in structural biology, offering unparalleled insights into the architecture of macromolecules at near-atomic resolution.
A crucial requirement for acquisition and collection of high quality data is properly vitrified and highly concentrated specimen. However, sample preparation still presents challenges in thicker specimens as bigger cells or cellular clusters, those are frozen by conventional plunge freezing method and may suffer with improper vitrification. Another problems could be low concentration or inadequate distribution of sample on electron microscopy grid or preferred orientation of the specific sample [1]. Here, we focus on the recently introduced "Waffle" method [1] and show it potential for preparation of various types of sample used in cryo-EM.
Methods
The waffle method is based on sample vitrification within the thickness of the TEM grid bars and it combines plunge freezing on the electron microscopy grid with a technique of high pressure freezing, that provides an advantage of proper vitrification of specimens thicker than 15um.
Thus, a 20-30um thick layer is prepared which needs to be further processed by cryo-focused ion beam micromaching (cryo-FIBM) to final thickness ~200nm before cryo-EM imaging.
Results and conclusions
We show benefits and limitations of the waffle method for vitrification of purified proteins, protein crystals, bacterial cell suspensions and eukaryotic cells.

Keywords:

waffle, vitrification, electron microscopy

Reference:

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Cross-sectioning of adherent cells on thin plastic substrate for serial block-face imaging

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Poster Group 1

Background

The single-celled enteric parasite Giardia lambia (Figure 1A) uses a ventral disc [1], consisting of a spiral-formed microtubule apparatus, for adhesion to their host duodenal epithelium. For analyzing the phenotype of various mutations of the ventral disc proteins, we started with conventional thin sectioning of the parasites cultivated on thin plastic bottom of plastic culture dishes (Figure 1B). The complexity of the observed phenotypes prompted us to consider volume imaging for a more comprehensive analysis of the ventral disc morphology. Instead of performing an additional cultivation and processing for volume imaging, we tested, if the already available sample blocks, prepared with our standard protocol for thin sectioning (osmium tetroxide, tannic acid, uranyl acetate/UA-Zero, Epon) [2], were suitable for serial block-face (SBF) scanning electron microscopy (SEM) at sufficient resolution. To our surprise, the resulting data sets were of sufficient quality (Fig. 1C) to reconstruct the entire ventral disc microtubule apparatus of the parasite in 3D (Figure 1D), but limited in resolution. SBF SEM usually requires a high-contrast en bloc staining of the sample with various heavy metals and reduction of the charging by improving the electrical conductivity (e.g. conductive filler) or by charge compensation (e.g. gas injection). As a consequence, the time of sample preparation usually is much longer for SBF imaging than for standard thin section imaging. Moreover, the introduction of more heavy metals changes the ultrastructural appearance of the sample. With our study, we wanted to find a generic sample preparation workflow for adherent cells which provides sufficient contrast and image quality in SBF SEM.

Methods

For the experiments we used adherent HeLa cell cultures grown in plastic dishes which were equipped with small silicone inserts on their plastic bottom (ibid µ-dishes, with microinserts). To stepwise improve the image contrast and quality, we introduced various simple modifications to our standard embedding protocol: minimal resin embedding, addition of silver colloids, higher processing temperature during heavy metal treatment, higher concentration of osmium tetroxide, additional heavy metal incubation steps (osmium tetroxide, lead aspartate). Cells were embedded in situ on their substrate in Epon, extracted with a hot scalpel and mounted on an aluminum stub using conductive epoxy glue. After trimming to a size of 1x0.25x0.25 mm, SBF SEM was performed with a Teneo Volumescope at high and low vacuum conditions.

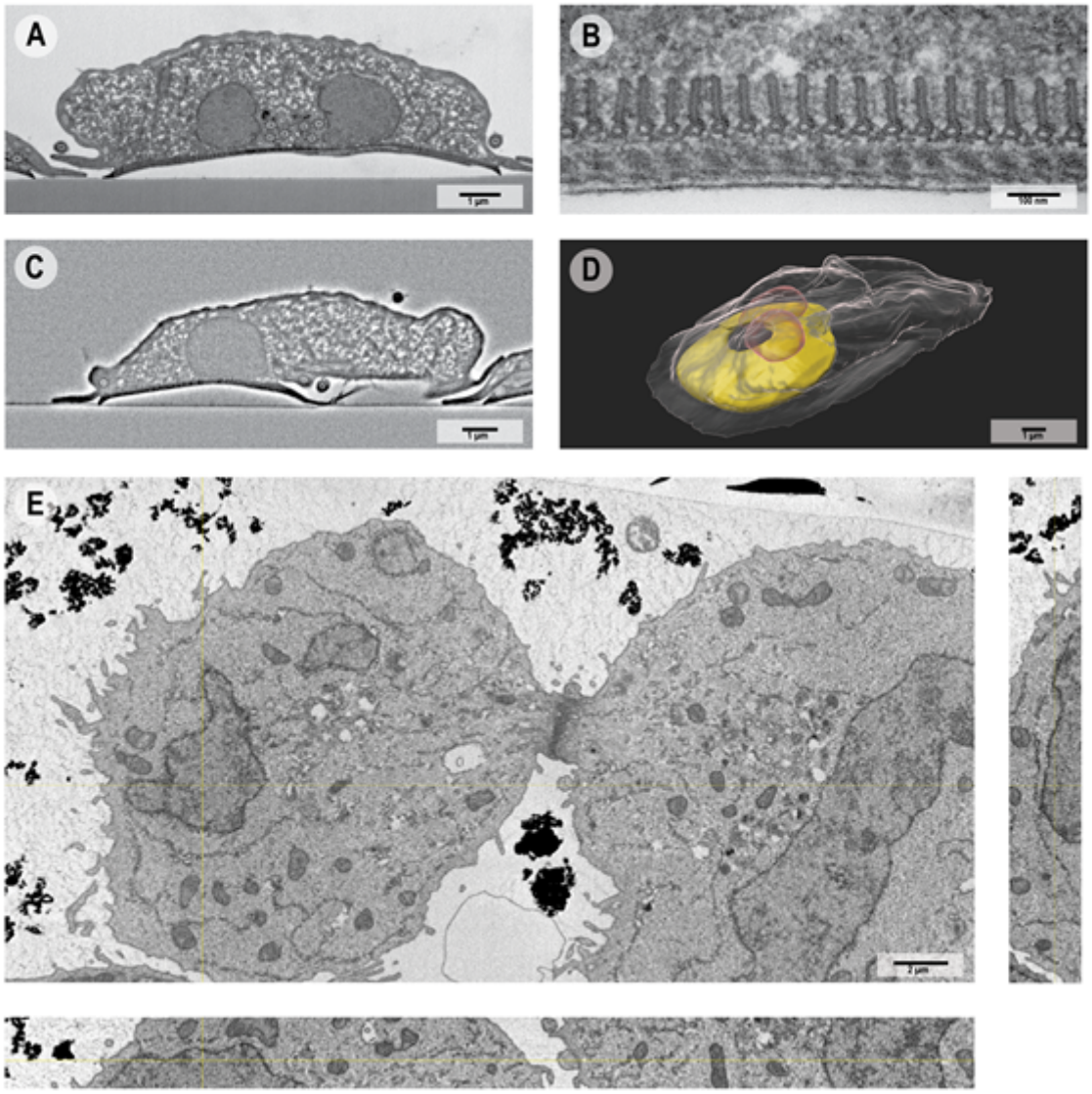
Results

The addition of further incubation steps with heavy metals to our standard sample preparation protocol resulted in improved resolution and image quality in block-face imaging. The contrast of the samples which were prepared with a single osmium tetroxide incubation and an additional en bloc treatment with lead aspartate (Figure 1E) allowed a proper discrimination of the main organelles and cytoplasmic structures already at low magnification and was very similar to the appearance of cells in thin sections taken from samples prepared by our standard protocol after on-section staining. The increased heavy metal load introduced by an additional osmium tetroxide incubation provided

slightly higher lateral resolution at the cost of a rather unusual contrast. SBF imaging of adherent cells at low vacuum resulted in more stable image series at higher resolution in all directions than at high vacuum, regardless of the heavy metal load. The addition of silver colloid improved only the imaging at high vacuum with less significance at highest heavy metal load tested.

Conclusion

Our cross-sectioning workflow for SBF SEM of adherent cells on plastic substrate offers the possibility of direct investigation of cell-substrate interactions. The upright mounting of the sample allows us to collect complete volumes of many cells and large total sample volume. Finally, we could find a protocol which provides comparable ultrastructural appearance than in conventional thin section EM and with only limited additional effort compared to our standard embedding protocol.



Keywords:

conductivity, cell culture, sample mounting

Reference:

[1] Hagen, K. D., Nosala, C., Hilton, N., Mueller, A., Holthaus, D., Laue, M., Klotz, C., Aebischer, A., & Dawson, S. C., “The domed architecture of Giardias ventral disc is necessary for attachment and host pathogenesis,”in bioRxiv : the preprint server for biology, Jul. 2023, https://doi.org/10.1101/2023.07.02.547441

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Cryo-FIB as a preparation tool for soft X-Ray Tomography: Analysis beyond EM

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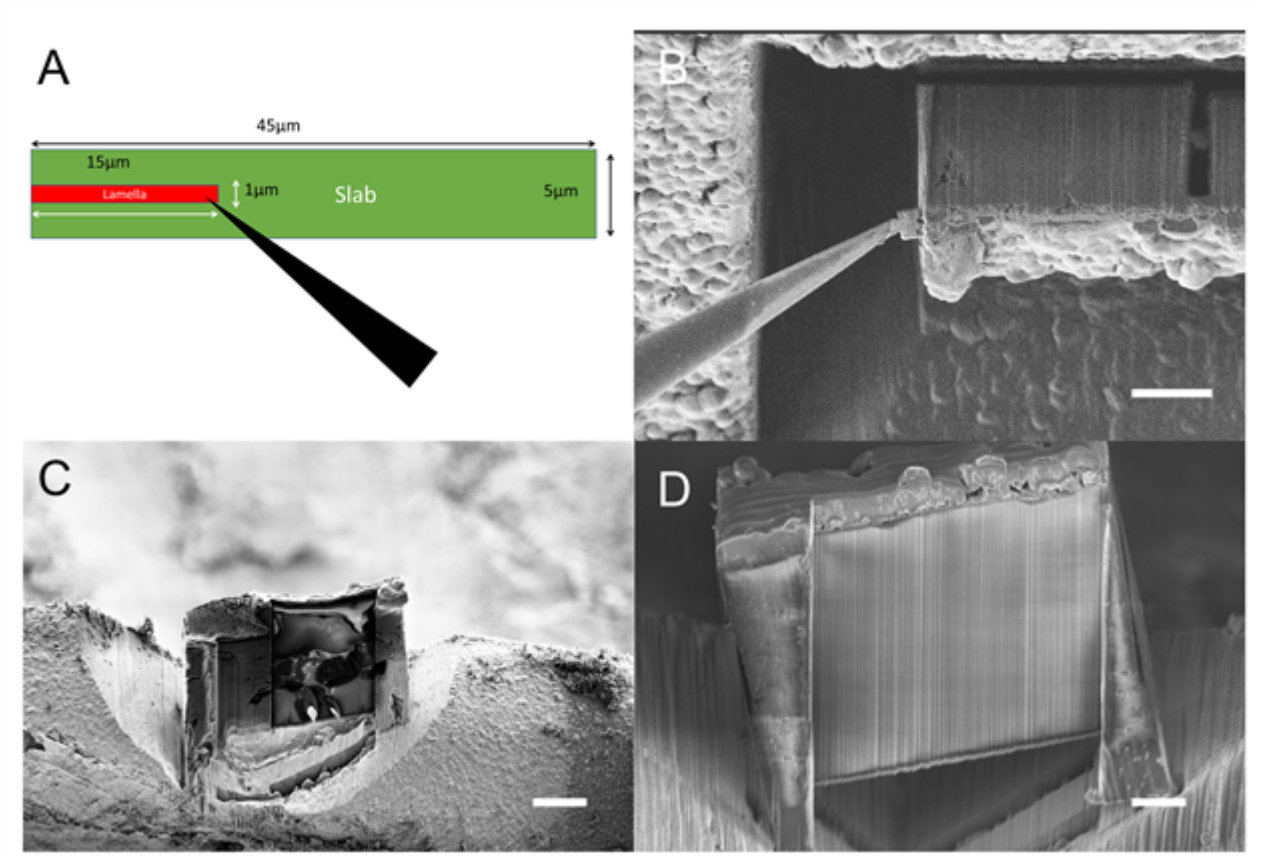
Poster Group 1

Background incl. aims
Focused Ion Beam Scanning Electron Microscopes (FIB-SEM) used at Cryogenic (cryo) conditions have been used as a preparation tool for soft-matter samples since 2006 with a recent rapid expansion of their use in the case of the 'on-grid' thinning approach for cryo-electron tomography (cryo-ET). Less well developed is the site-specific cryo-FIB preparation of samples that cannot be grown (or deposited) and thinned on a TEM grid, such as tissue or other soft matter samples e.g. battery materials. Site-specific FIB preparation followed by extraction (lift-out) is a route to achieving the thickness requirements of the TEM prior to transfer. By adapting this approach to meet the dimensions and requirements of other analytical equipment it is possible to expand beyond the limit of electron microscopy.
Methods
This work utilised a Crossbeam 550 FIB-SEM (Carl Zeiss) equipped with a Quorum 3010 (Quorum Technologies) cryo-system. The micromanipulator was an Omniprobe 200-cryo (Oxford Instruments). Samples were frozen by slushy nitrogen, metal mirror (MM) freezing or high pressure freezing (HPF) before transfer to the cryo-system. Prior to FIB-SEM the samples were platinum sputter coated in the preparation chamber and subsequently by the platinum organometallic precursor of the gas injection system (GIS) of the FIB. Gallium FIB preparation was performed at 30kV accelerating voltage currents of between 15nA (initial larger volume milling) and 50 pA (final polishing of samples). SEM of the samples was performed at 2-5 kV. Cryo-redeposition milling was used to attach samples to the micromanipulator and support substrates.
Results
Building on pre-existing protocols for the preparation of TEM compliant samples (lamellae) we increased the dimensions of the volumes prepared to meet the requirements of a soft-x-ray tomography (sRT) microscope which to approx. 45µm and a thickness of 5µm which we term 'slabs' (fig 1a). The sXT can penetrate a sample up to 10 µm thick however, we accounted for the sample needing to be tilted in the X-Ray beam (+/- 55°). These slabs were transferred to specially modified copper support structures (fig 1 b-d).

Fig 1. (A) Schematic showing the relative dimensions of the lamella and slab, the tip of a micromanipulator is included for scale. (B) FIB image showing the micromanipulator with attached slab during slab extraction. (C&D) Micrographs of the slabs following deposition onto the support structure and subsequent polishing using the FIB. Scale bars 10 µm (B&C) 4 µm (D).

Conclusion
Cryogenic FIB preparation combined with lift-out using a cryogenically cooled in situ micromanipulator is demonstrated as a viable preparation tool for the preparation of soft-matter sample (~45x5 µm) slabs for sXT. We have presented recent efforts to use Cryogenic Focused Ion Beam Scanning Electron Microscopes (cryo-FIB-SEM) as a preparation tool for cryogenic analysis beyond the FIB itself, embracing emerging themes in multi-length-scale analysis and correlative microscopy.



Keywords:

Cryo-FIB, soft-X-Ray Tomography, correlative microscopy

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Large-volume cryoEM sample preparation for the investigation of the plant-microbiome interaction

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Poster Group 1

Background incl. aims

In recent years, cryo-electron tomography (cryoET) has proven to be a powerful tool for studying the organization and complexity of cellular ultrastructures in their near-natural state. Nevertheless, its applicability was primarily restricted to single cells and monolayers due to the limited capabilities of volume vitrification techniques. Advances in cryo-electron microscopy (cryoEM) aim to overcome these limitations, which has led to the development of methods that can vitrify larger volumes. Despite these advances, further thinning of samples remained a challenge until focused plasma ion beam (PFIB) microscopy and the lift-out technique were introduced. By combining these improved technologies, we have succeeded in pushing the boundaries of sample preparation for cryoET. In particular, we set out to develop and optimize methods for the preparation of large-volume samples, focusing on samples from fungi and plants to facilitate the study of plant-microbiome interactions at the cellular, and ultrastructural level, and we present our results in this context.

Methods

Samples were collected from an arbuscular mycorrhizal (AMF) fiber network, known for its symbiotic relationship with approximately 80% of plant species. Additionally, samples of plant roots were obtained from both Arabidopsis thaliana seedlings and Ri T-DNA-transformed plant root organs cultivated on a standard MSR medium. Cryogenic preservation by high-pressure freezing (HPF) was performed in 3 mm planchettes and the waffle method on grids. Using hexadecane and phosphate buffer solution as filler to prevent the formation of gas bubbles. Prior to vitrification, all samples were fluorescence stained with Nile Red and Calcofluor to enhance visualization of lipid-rich structures and cell walls, respectively. Following, grids were evaluated and processed in Arctis cryo-PFIB, while planchettes were processed in Helios Hydra V a PFIB dual beam scanning electron microscope. Trenches were prepared, enabling both 3D large-volume imaging and preparation for cryo-lift-out on planchettes. Lamellae were prepared on grids and from lift-out samples and subsequently transferred to Titan Krios for data collection.

Results

This study demonstrates the successful vitrification of AMF, enabling the visualization of the cellular organization in a near-native state. Fluorescence staining with Nile Red and Calcofluor white facilitated the differentiation of cellular structures, including the distinction between filled and empty AMF hypha. In Addition, 3D volume images of both, the AMF network and plant root tissue, were generated, providing insights into their respective structures. In particular, plant organs provided better results compared to other plant tissue used in this study. Furthermore, the used methodology enabled the imaging of plant tissue in a near-native state without additional fixation steps after HPF, which is a remarkable advance over previous approaches. The combination of methods offers a

promising approach for studying plant-microbe interactions on a structural level and provides valuable insights into complex biological systems.

Conclusion

The combination of methods used for large-volume sample preparation in cryoET represents a significant advance in the study of plant-microbiome interactions at the structural level. The inclusion of fluorescent staining in the sample preparation facilitated the identification of areas of interest on the PFIB and enabled the precise alignment for lamella milling of different cellular structures. The acquisition of 3D volume images of both AMF networks and plant organs provides comprehensive insights into their structures and interactions. In particular, our approach demonstrates the enhanced vitrification quality of plant organs over other plant tissues. Furthermore, the ability to image plant tissue in a near-native state without additional fixation steps preserves tissue integrity and ensures accurate visualization of cellular ultrastructure. Overall, our methodology offers a promising opportunity to improve our understanding of plant-microbiome interactions. By elucidating complex dynamics at the cellular level, our work contributes to the broader knowledge of complex biological systems. Further refinement and application of our methodology have the potential to unravel the mechanisms underlying the interactions between plants and microbiomes.

Keywords:

large-volume, plant tissue, host-microbe, mycorrhiza

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SoftGrids: towards disease modelling in CryoEM

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Poster Group 1

Background incl. aim
In vitro cells derived from pluripotent stem cells (iPSCs) show some changes in their phenotype compared to their adult counterparts in vivo. Studies have shown that the application of external stimuli to provide a more natural environment has a major impact on many characteristics of cultured cells. Of particular interest is mechanical stimulation with a material whose stiffness is adapted to the tissue in which the cell type naturally occurs. Typical electron microscopy substrates such as EM grids do not offer this possibility. These usually consist of a combination of very hard grid bars and a very soft film and therefore do not offer the optimal conditions for some cell types. The aim of this work is to offer a workflow for cryo electron tomography (CryoET) that closes this gap by coating grids with a material whose mechanical properties can be easily modified.

Methods
In order to show the influence of the stiffness of EM grids coated with substrates of different stiffness, mesenchymal stem cells (MSCs) are seeded onto the grids and then examined for the quantitative expression of specific markers proteins using fluorescence microscopy. The markers selected were those that are usually expressed to a high degree in cells with strongly differentiated natural stiffness domains. They are categorized into neurogenic, myogenic and osteogenic markers. In addition, control cultures are created from progenitor cells that express the specific markers to a particularly high degree. The stiffness of the respective substrates is measured using atomic force microscopy.
For the integration into the CryoET workflow, iPSC-derived neurons and iPSC-derived cardiomyocytes are seeded on the grid and plunged after 7-10 days. With the help of a FIB, thin lamellae of about 200 nm are prepared. In a transmission electron microscope, tilt series are generated by gradually tilting the stage, from which tomograms are then generated.

Results
The measured intensities from the marker expression in MSCs show a maximum for a certain stiffness and a decreasing signal for cultures on grids whose stiffness leads further away from the ideal value.
The generated tomograms show vitrified content. The lamellae can be produced in a comparable time compared to non-coated standard grids. By using stripe grids for the topological stimulation of cardiomyocytes, the cells can be polarized efficiently. This configuration allows a very high slat density per grid.

Conclusion
We have shown that the cultivation of cells on our grids has an effect on the phenotype of certain cell types compared to standard EM grids and that this effect depends on the stiffness of the material. This behavior has been sufficiently proven for non-grid substrates in many studies. With the provided workflow for CryoET, the possibility has been created to perform model-based research with iPSC-derived cells in structural biology. This opens another door, particularly for desease research on neuonal and cardiac model systems.

Keywords:

Cryo electron tomography, Mechanical stimulation

Reference:

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Correlative-Cryo Microscopy to Characterise Bacteria-Nanopillar Interactions: Achievements and Challenges

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Poster Group 1

Background
Nature-inspired nanopillar surfaces have been shown to kill surface-attached bacteria by physical means, providing a novel route to potentially control bacterial colonisation on implant surfaces and thus combat implant-related infections. It is proposed that structural damage to the bacterial cell wall kills bacteria as they attach to the nanopillars[1,2]. However, more than a decade since this concept was introduced, the precise mechanism by which bacteria are killed remains an open question[1-4]. Using fluorescent staining, damage to the cell wall is predicted to happen at a single time point after the interaction. However, light microscopy cannot visualise damage to the cell wall, while traditional electron microscopy (EM) techniques associated with sample processing cannot characterise hydrated samples and may introduce artefacts during processing. This work aims to enable the native interaction between bacteria and nanopillars to be visualised at nanometre resolution and so better understand the bactericidal mechanism by establishing a cryo-correlative workflow to characterise the bacterial cell wall interacting with the surface nanotopography while maintaining a hydrated environment[5]. Here, we discuss our assessment of different vitrification techniques and different cryo-microscopy techniques to characterise bacteria within a hydrated state.

Methods
The project used silicon (Si) and titanium (Ti) as test substrata and Escherichia coli as a model bacterium to develop the workflow. Freshly grown bacteria, diluted into PBS, were incubated for 30 minutes on the test substrata at the room temperature. Samples were then vitrified using different techniques: 1) liquid nitrogen slush freezing; 2) plunge freezing (PF); or 3) high pressure freezing (HPF). Both fluorescently-labelled and non-labelled bacteria were vitrified by PF and imaged by cryo-SEM to identify surface-attached bacteria. Single bacterial cells in HPF samples were identified for further analysis after screening under cryo-fluorescence microscopy. Single bacterial cells were lifted using cryo-FIB/SEM and placed onto TEM grids for cryoET. After polishing the lamella, the presence of bacterial cells was confirmed using cryo-fluorescence microscopy.

Results
Slush frozen samples could allow the visualisation of surface-attached bacteria with minimal sample preparation. Both Si and Ti samples were successfully vitrified for cryoSEM. However, the technique could not be used for further investigations as the workflow was not set up for sample transfer between different microscopes. PF and HPF resulted in vitrification of the water layer on the substrate. Bacterial cells were not visible under cryoSEM when vitrified by HPF or PF as they were buried under the surface of the liquid phase. Therefore, bacteria could only be identified when they were fluorescently labelled. Some of the PF samples showed ice crystals as blotting was not optimal. Vitrification of samples by HPF was more reproducible without ice crystal formation. However, finding and isolating individual bacterial cells for further analysis was a challenge with both

techniques. Therefore, cryo-fluorescence is an essential step in this workflow. For HPF samples, estimation of depth was a challenge as the test substrate surface could not be accurately measured on the micrometer scale under the cryofluorescence microscope. When a bacterium was selected in the XY plane, it was not necessarily attached to the substrate. This could only be verified after lift-out of lamella, followed by cryo-fluorescence imaging.

Conclusions

Nitrogen slush freezing is a rapid way of vitrifying the sample and suitable for cryo-SEM to study the morphology of bacterial cells interacting with nanopillar topography. Both HPF and PF are promising techniques to vitrify samples with bacteria attached to the substrate. However, identification of individual bacteria attached to the substrate is a challenge.

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Keywords:

Mechano-bactericidal action, Nanopillars, VolumeEM, Cryo-microscopy

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Nanoscale X-ray imaging to Study Bacteria-Nanopillar Interactions

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Poster Group 1

Background

Exploitation of nanotopography found in nature with bactericidal properties was reported a decade ago as an alternative method to combat the emerging threat of bacterial antibiotic resistance.[1],[2] However, a consensus has yet to be reached on the mechanism(s) by which bacteria are killed upon contact with pillar-like nanostructures. Without knowing the mechanism by which the cell wall is damaged, it is unlikely that this concept can be further implemented for real-world applications. Using fluorescent staining, damage to the cell wall is envisioned to happen at a single time point after the interaction. However light microscopy cannot visualise the damage to the cell wall, and serial cross-sectioning and imaging in FIB-SEM can create a pseudo-3D image of bacteria, and is a destructive approach. Our FIB-SEM data shows that bacteria cell wall is in contact with nanopillars at random positions and many nanopillars under the bacterium is not touching the cell wall.[2-4]This project aims to investigate the use of lab based X-ray nanotomography to study the 3D volume of bacteria attached on to nanopillar surfaces non-destructively at nanometer resolution.

Methods

The project uses nanostructured Silicon (Si) as test substrate and E.coli as model organism to develop the workflow. Freshly grown bacteria sample diluted into PBS media, incubated for 30 minutes on test substrata and chemically fixed, dehydrated by ethanol series and dried by critical point drying to be compatible for electron microscopy. Then subsamples of 15 µm in size with bacteria centred was isolated and placed on a sharp needle tip using a plasma FIB, as in preparation for nanoCT. These samples are then imaged in Zeiss Xradia 810 nanoCT, with low X-ray energy, with a voxel size of 150 nm.

Results

Micro manipulation of the samples on to a needle top was successfully achieved by plasma FIB. Creating of these sub-samples of 10 µm in size is essential to enable nanoCT characterisation. Ultra-high resolution was not successful, but high-resolution imaging nanoCT data clearly shows the bacterium attached on the top of nanopillars of 50-70 nm in size. As 3D image of bacteria and it's immediate surrounding is visualised by a non-distrructive method, now TEM lamellas can be prepared from the positions where bacterial cell wall is interacting with nanopillars.

Conclusion

Use of plasma FIB/SEM to create subsamples compatible for nanoCT was successful. The analysis of the samples in nanoCT was successful as bacteria and nanopillars could be located using 100 nm voxel resolution. This allows to characterise the overall 3D shape of bacterium when they interact with nanopillars non-destructively. approach to find individual bacteria cells. This approach allows to pinpoint positions where bacterial cell-wall interact with nanopillar surface so that TEM lamellas can be prepared for high-resolution TEM tomography to study the cell-wall damages in detail.

Keywords
nanoCT, FIB/SEM, Mechano bactericidal action, nanopillars, bactericidal surfaces, electron tomography

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Keywords:

nanoCT, FIB/SEM, nanopillars, bactericidal surfaces,

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