Need for Speed

Imaging Biological Ultrastructure with the 64-beams FAST-EM

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Meeting-report

Need for Speed: Imaging Biological Ultrastructure with the 64-beams FAST-EM

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Large-scale and volume electron microscopy (EM) has revolutionized the understanding of biological ultrastructure across different spatial scales, but the low throughput of EM acquisition is limiting [1]. To increase throughput of EM, as much signal as possible must be collected in the least amount of time. Optimization of current detection conditions in scanning electron microscopy (SEM) yields up to 20-fold faster imaging [2]. In multibeam SEM (MB-SEM), the sample is scanned in parallel by an array of beams, which increases image acquisition speeds [3, 4]. We pioneered imaging with a commercial multibeam SEM, FAST-EM. FAST-EM employs optical scanning transmission EM (optical STEM): ultrathin (serial) sections are mounted on a scintillator which converts the impinging electrons to photons (Fig. 1). The optical signals are then collected with an objective, descanned and projected onto a detector array [5].

The workflow for reconstructing biological ultrastructure in 3D with FAST-EM has been optimized, including sample preparation, acquisition parameters and image post-processing. Large-scale and 3D imaging on a variety of samples, prepared from multiple cell types, tissues and organisms will be shown (example in Fig. 2). Multibeam array tomography is demonstrated by reconstructing serial ultrathin sections. Our results show that FAST-EM offers great potential to reduce acquisition times in large-scale and volume EM. Ultrastructural information for biological interpretation is obtained within feasible acquisition times of large regions of interest. We follow up to continue to improve maximizing throughput by optimization of signal generation and collection, but also further automation of acquisition and increasing system autonomy. Thus, our approach will help to revolutionize EM microscopy from snapshot images to complete sample scanning for digital analysis like has been introduced for histology in 'digital pathology' during the last decade [6].

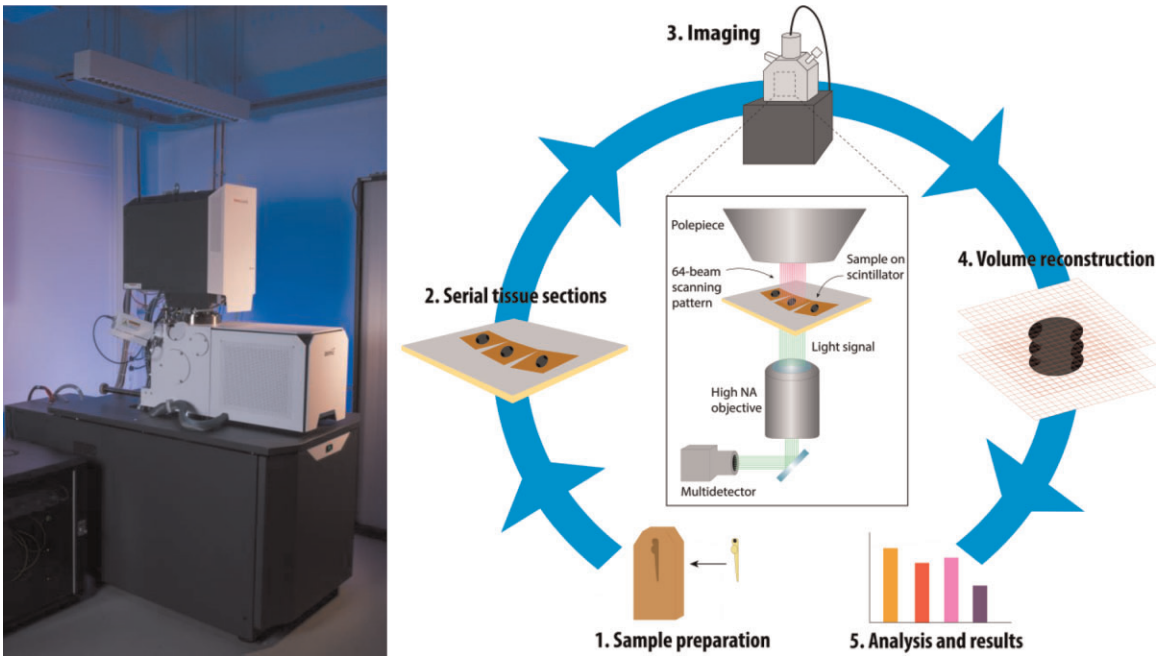

Fig. 1. FAST-EM and workflow for reconstruction of biological samples in 3D (volume EM). 1. Samples are fixed, stained, dehydrated and resin embedded. 2. Ultrathin sections are cut and placed on molybdenum-coated cerium-doped yttrium aluminium garnet (Ce:YAG). 3. High-throughput multibeam imaging using optical STEM. 4. Images are stiched and aligned in 3D. 5. Quantitative analysis can be conducted.

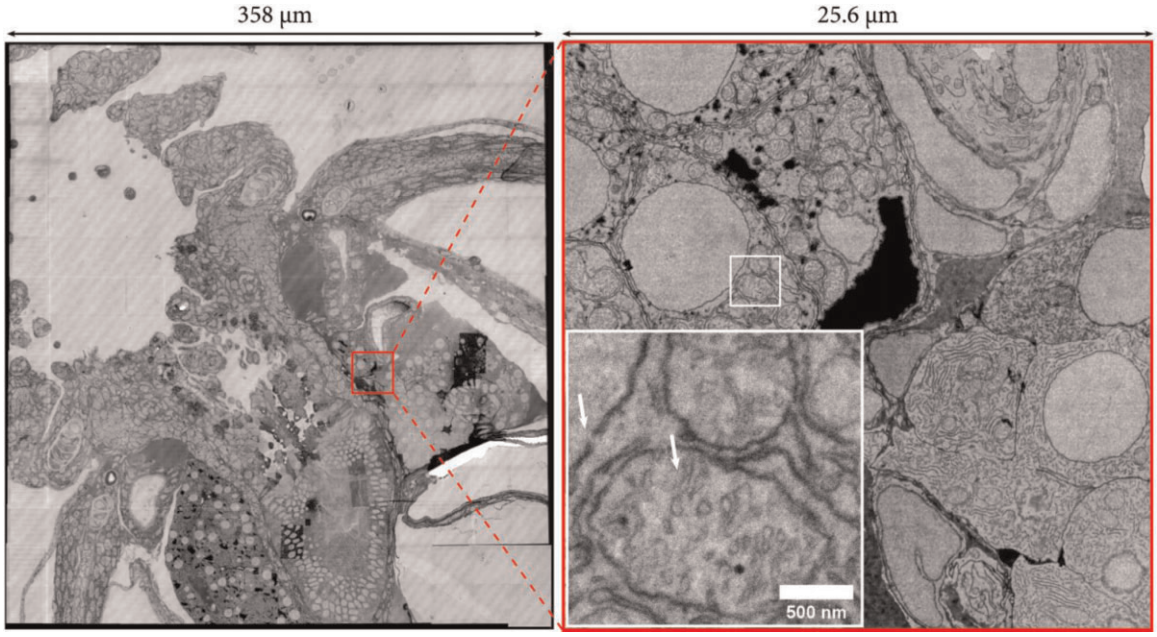

Fig. 2. Example of imaging with FAST-EM. Zebrafish larva were post-fixed with reduced osmium and stained en bloc with 4% neodymium acetate. Acquisition settings: 5 keV landing energy, 0.4 nA beam current, 10 µs dwell time and 4 nm pixel size. Left: full overview of acquisition; right: single image with 64 beams. Inset on right illustrates full resolution with arrows indicating small features such as mitochondrial cristae and nuclear pore. Please visit Nanotomy.org/OA/Kievits2023MMA for full datasets at full resolution.

References

1. Kievits, A.J., *et al.*, How innovations in methodology offer new prospects for volume electron microscopy. *Journal of Microscopy*, 2022. **287**(3): p. 114-137.
2. Lane, R., *et al.*, Optimization of negative stage bias potential for faster imaging in large-scale electron microscopy. *Journal of structural biology: X*, 2021. **5**: p. 100046-100046.
3. Eberle, A.L., *et al.*, High-resolution, high-throughput imaging with a multibeam scanning electron microscope. *Journal of microscopy*, 2015. **259**(2): p. 114–120-114–120.
4. Ren, Y. and P. Kruit, Transmission electron imaging in the Delft multibeam scanning electron microscope 1. Journal of Vacuum Science & Technology B, Nanotechnology and Microelectronics: Materials, Processing, *Measurement, and Phenomena*, 2016. **34**(6): p. 06KF02-06KF02.
5. Fermie, J., *et al.*, High-throughput imaging of biological samples with Delmic's FAST-EM. *Microscopy and Microanalysis*, 2021. **27**(S1): p. 558–560-558–560.
6. de Boer, P. and B.N.G. Giepmans, State-of-the-art microscopy to understand Islets of Langerhans: what to expect next? *Immunology and Cell Biology*, 2021. **99**(5): p. 509–520-509–520.