Trends in Biochemical Sciences | Technology of the Month

Revisiting focused ion beam scanning electron microcopy

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The need for resolving complex dynamic spatial relationships between and within cells has driven the development of super-resolution microscopic techniques [1–3]. The explosion of computational and data storage capabilities has propelled the evolution of fully automated, high-resolution microscopes capable of rendering fine cellular ultrastructure in 3D with high fidelity. Focused ion beam scanning electron microscopy (FIB-SEM) is a technique with resolution <10 nm in all planes, making it ideal for exploring organelle–organelle interactions such as endoplasmic reticulum–mitochondrial contacts sites or resolving fine synaptic features [2,4]. FIB-SEM works by pairing a focused beam of ions to finely ablate the surface of heavily contrasted, resin-embedded samples with a scanning, low-voltage electron beam and backscatter electron detector for surface imaging [2]. An array of software are available for 3D reconstruction of features of interest (Table 1) [5,6]. For more versatility, FIB-SEM can be paired with cryo-capabilities and secondary detectors (Figure 1) [7,8].

Table 1. Examples of 3D reconstruction analysis software

Open-source or publicly available	Commercially available
Microscopy Image Browser	Thermo Scientific Amira
Reconstruct	Imaris
IMOD	Dragonfly Pro
ilastik	
3D Slicer	
ImageJ/FIJI	
napari	

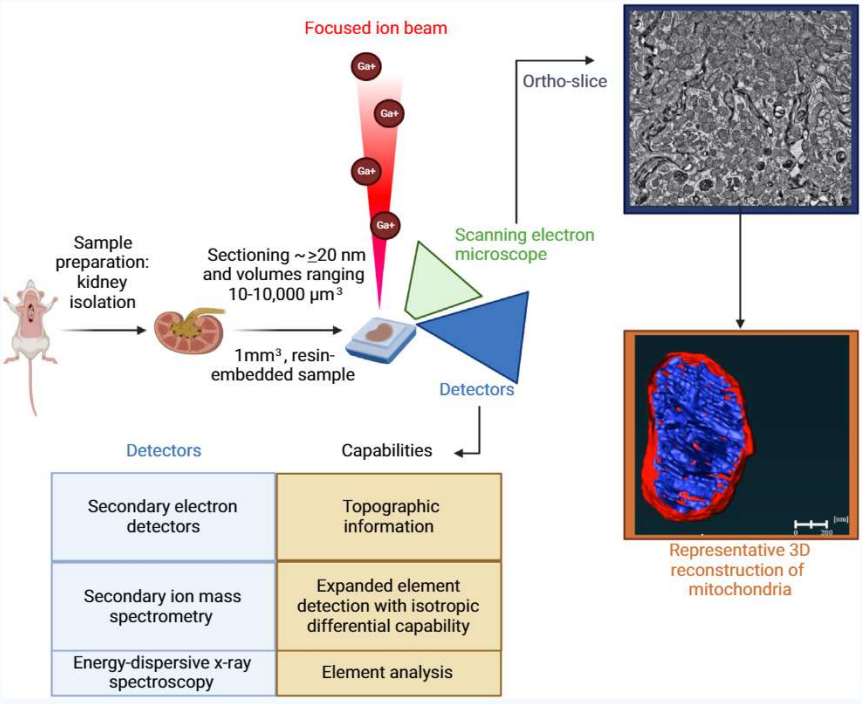

Figure 1. Representative schematic of 3D reconstruction of murine kidney muscle using focused ion beam scanning electron microscopy (FIB-SEM). Following sample collection, FIB-SEM can allow for orthoslices to be obtained as well as 3D reconstruction. Representative image of 3D reconstruction mitochondria (red) with inner-membrane folds of cristae (blue) from murine kidney is shown.

ADVANTAGES:

Precise, fine sample removal along the z axis.

Fine balance of ultra-resolution and volumetric sampling for 3D imaging.

Amenable to pairing with a wide array of techniques that can yield localization, topography, and elemental composition with high spatial resolution.

Easy access to image processing software.

Fully automated.

Adjustable, allowing for beam strength to be adjustable and multiple specimens in a small area to be surveyed to ensure efficiency.

Cryo-capabilities may aid in fluorescence confocal imaging, the imaging of proteins and molecules in their native state, and avoiding potential damage and morphological changes which may occur with fixation and embedding of traditional FIB-SEM techniques.

CHALLENGES:

Unlike transmission electron microscopy (TEM), cannot resolve fine, nanostructural details, such as visualization of viral spike proteins or between intermembrane organellar connections [2,9].

Longer acquisition time and smaller volume sampling capabilities than other volumetric EM techniques.

Sample is consumed during image milling and acquisition.

Needs large RAM and storage (~TB range) for efficient data transfer and processing for volumetric applications.

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Declaration of interests

The authors have no conflicts of interest to declare.

References

1. Glancy, B. (2020) Visualizing mitochondrial form and function within the cell. *Trends Mol. Med.* 26, 58–70
2. Biazik, J. *et al.* (2015) The versatile electron microscope: an ultrastructural overview of autophagy. *Methods* 75, 44–53
3. De Castro, O. *et al.* (2022) Magnetic sector secondary ion mass spectrometry on FIB-SEM instruments for nanoscale chemical imaging. *Anal. Chem.* 94, 10754–10763
4. Xu, C.S. *et al.* (2017) Enhanced FIB-SEM systems for large-volume 3D imaging. *eLife* 6, e25916
5. Garza-Lopez, E. *et al.* (2022) Protocols for generating surfaces and measuring 3D organelle morphology using amira. *Cells* 11, 65
6. Lam, J. *et al.* (2021) A universal approach to analyzing transmission electron microscopy with ImageJ. *Cells* 10, 2177
7. Vidavsky, N. *et al.* (2016) Cryo-FIB-SEM serial milling and block face imaging: large volume structural analysis of biological tissues preserved close to their native state. *J. Struct. Biol.* 196, 487–495
8. Hayles, M.F. and De Winter, D.A.M. (2021) An introduction to cryo-FIB-SEM cross-sectioning of frozen, hydrated Life Science samples. *J. Microsc.* 281, 138–156
9. Baena, V. *et al.* (2021) FIB-SEM as a volume electron microscopy approach to study cellular architectures in SARS-CoV-2 and other viral infections: a practical primer for a virologist. *Viruses* 13, 611

Contamination may occur with gallium beam, affecting physical and electrical attributes.

Learning curve for the analysis software, which varies across different software, may have separate user interfaces, require coding knowledge, or require manual segmentation.

High-cost commitment (instrumentation, facility, maintenance, operating, and training costs) and expertise are required, inhibiting ancillary uses of FIB-SEM in many cases; furthermore, cryo-FIB-SEM requires further extensive specialized techniques and materials.

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