Shedding New Light on Ultrastructure

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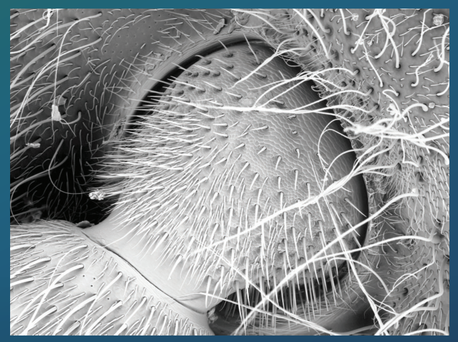
A SILENT REVOLUTION IN 3D EM

Life takes place in three dimensions. Although many scientists think of scanning electron microscopy (SEM) as producing captivating topographical images – see the bee image below – showing surface structures in fine detail, it also offers opportunities to expose internal cellular architecture in 3D. Intricate ultrastructural information can now be delivered by improved SEM technologies and methods, known collectively as volume EM (vEM). Importantly, these volumetric SEM methods keep getting easier to use and more available.

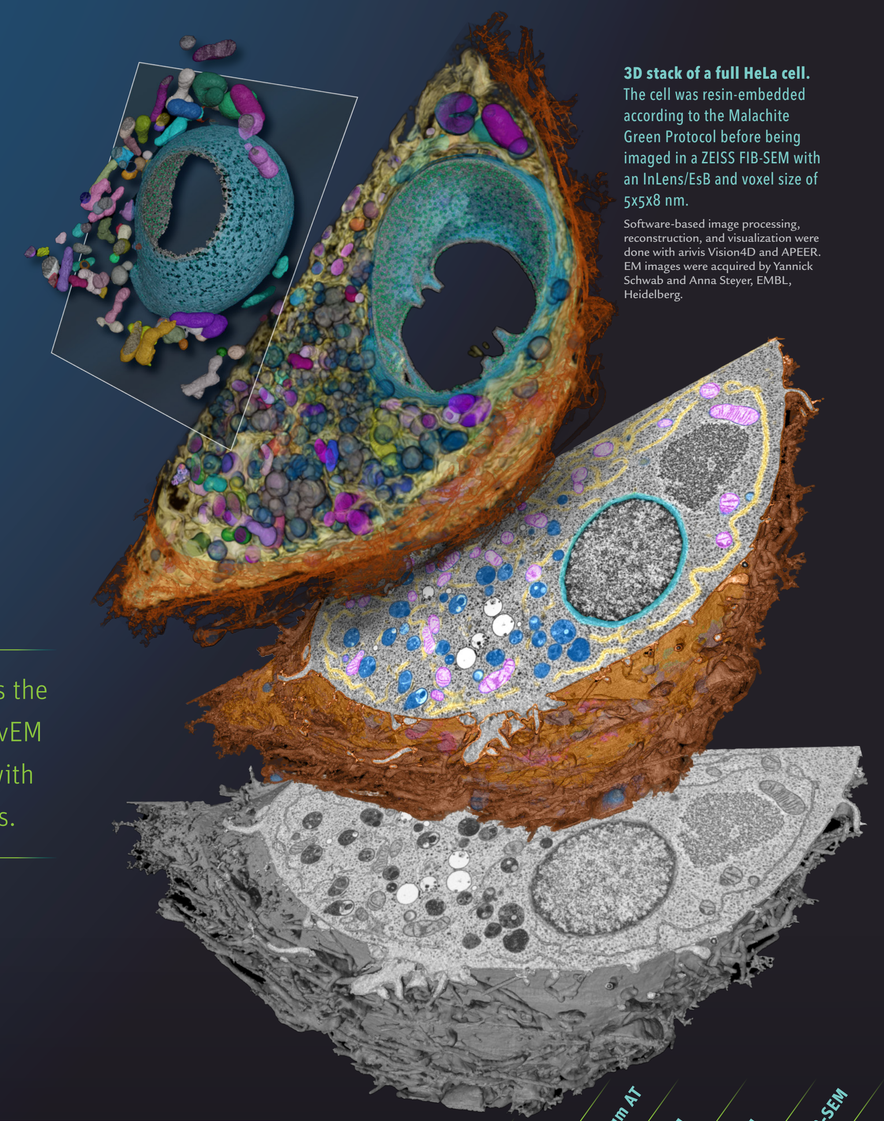


Base of a bee antenna acquired with topographic scanning electron microscopy.

vEM has the promise to drive research in a range of fields, including connectomics in neurobiology and foundational studies in cancer research, developmental biology, plant science, and more. Still, many biologists remain unaware of this silent revolution in 3D EM.

All vEM methods include three key steps: prepare a sample for EM, collect a stack of 2D images from a series of sections, and computationally reconstruct the images in 3D. As these advanced methods of vEM become easier to use, more and more scientists have the opportunity to reveal parts and processes of biology previously beyond the reach of any imaging modalities. Moreover, the evolution continues. Advances in artificial intelligence (e.g., deep learning) and big-data handling open up new possibilities for image acquisition, visualization, and analysis.

This poster explores the key features of six vEM methods – each with specific strengths.



3D stack of a full HeLa cell. The cell was resin-embedded according to the Malachite Green Protocol before being imaged in a ZEISS FIB-SEM with an InLens/EsB and voxel size of 5x5x8 nm.

Software-based image processing, reconstruction, and visualization were done with arivis Vision4D and APEER. EM images were acquired by Yannick Schwab and Anna Steyer, EMBL, Heidelberg.

Volume EM (vEM) Techniques

Array Tomography (AT)

Easy access to nondestructive ultrastructural imaging

A lab with an SEM can easily explore vEM with array tomography. In AT, ideally 30–70 nm thick serial sections are cut from a resin-embedded sample and attached consecutively to a sample carrier in the order they were cut, representing depth of the sample volume in the z dimension. After the serial sections are imaged with SEM, the image stack is reconstructed to create a 3D dataset. AT is particularly suitable for correlative workflows, such as when proteins of interest are first localized using fluorescence microscopy. A huge advantage of AT is that it does not destroy the sections, which can be archived for future use.

Multibeam Array Tomography

Fast acquisition of ultrastructural details in large volumes

Conventional SEM scans a sample with a single electron beam, but multibeam SEM uses up to 91 electron beams simultaneously, which significantly accelerates image acquisition. Multibeam AT can quickly image volumes larger than a cubic millimeter at nanometer resolution. The rapid acquisition speed and ability to capture ultrastructural details in large volumes favors research fields like connectomics, which require more contextual information. In the future, multibeam AT could even be used to comprehensively map all of the neuronal connections in an entire mouse brain.

Serial Block-Face SEM (SBF-SEM)

Volume-data acquisition through automated sectioning and imaging

In SBF-SEM, an ultramicrotome inside the EM chamber cuts 15–30 nm thick sections from a resin-embedded sample block. The exposed face is imaged, then sections are cut and a newly exposed block-face is imaged until the desired or entire stack is acquired in the z direction of the sample. The series of images are reconstructed to create a 3D dataset. This SEM-imaging method's key attributes include easy sample preparation, a highly automated imaging process, and a higher resolution in the z dimension of a sample than is possible with AT.

Focused Ion Beam SEM (FIB-SEM)

High-resolution ultrastructural details in accurate proportions

In FIB-SEM, a focused ion beam mills a trench in a resin-embedded sample to expose a targeted feature that can be imaged with SEM. Then, the beam mills as little as 3–10 nm deeper to expose a new layer for imaging. Repeating this process along the z direction provides images of the sample volume. The very small step size between layers (ideally equal to the resolution in the x-y plane of a section) enables isotropic 3D data, which makes this a good method for reconstructing ultrastructural details – such as subcellular features or the connections of neurons – in accurate 3D proportions without missing any information due to limited resolution in all 3D directions.

Cryogenic FIB-SEM

Imaging ultrastructure in its near-to-native state

Chemical fixation of a cell or tissue sample can lead to undesirable changes in its ultrastructure. Instead, vitrifying the sample at very low temperatures, about –196°C, preserves structures in their near-to-native state and can even lock in cellular dynamics at a stage of interest. Kept vitrified with dedicated cryo equipment during the entire imaging process, such a sample can be investigated with FIB-SEM, excavating and imaging its subcellular reality without artifacts. Cryogenic FIB-SEM can be combined with other cryo methods using light and confocal microscopes as well as TEM.

Serial Section Transmission Electron Microscopy (ssTEM)

Volume information from a well-established TEM method

Unlike the SEM-based methods of vEM described here, ssTEM is a representative example of a method based on electron transmission that combines full-field acquisition with nondestructive imaging. Before image acquisition, an ultramicrotome cuts the sample into transparent 25–100 nm serial sections that are usually placed on grids supported by a polymer film. The ultrathin sections assure perfect imaging conditions and good resolution along the z-axis of a sample, which enables the complexity of subcellular structures to be clearly visualized. However, compared to SEM-based methods, the ssTEM limits the size of the sample, and consequently, the volume that can be imaged.

	AT	Multibeam AT	SBF-SEM	FIB-SEM	Cryo FIB-SEM	ssTEM
Volume size						
Resolution (x,y)						
Resolution (z)						
Acquisition speed[1]						
Ease of use[2]						
Instrument accessibility						
Image processing[3]						
Nondestructive[4]	✔	✔				✔

KEY: worst ⟷ best

FOOTNOTES: 1: Assumption: comparison based on the same volume size; 2: Complete workflow, including sample handling; 3: Includes big data handling; 4: Sections can be stored and reused after imaging.

Comparisons of vEM Techniques

The values in this table provide general guidance for comparing the methods along different dimensions. Depending on the user's implementation and application, the comparison values may vary.