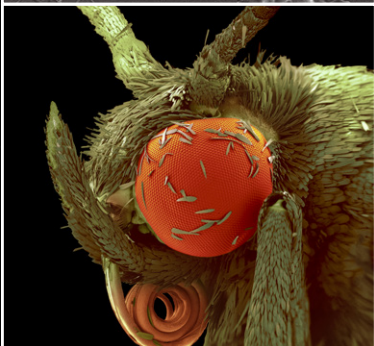
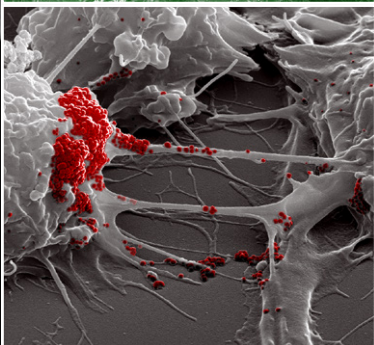
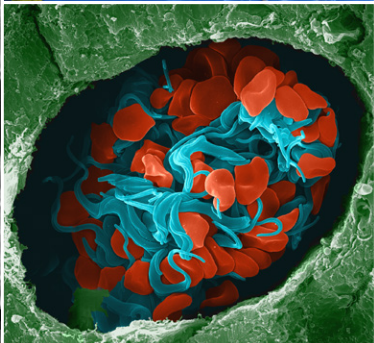
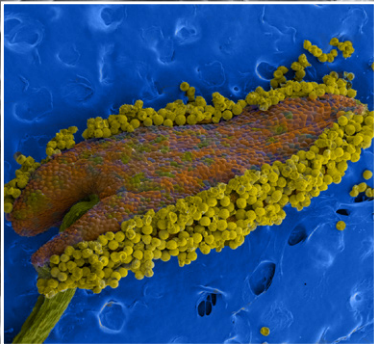


Scanning electron microscopy

for life sciences



Introduction to scanning electron microscopy

A brief history

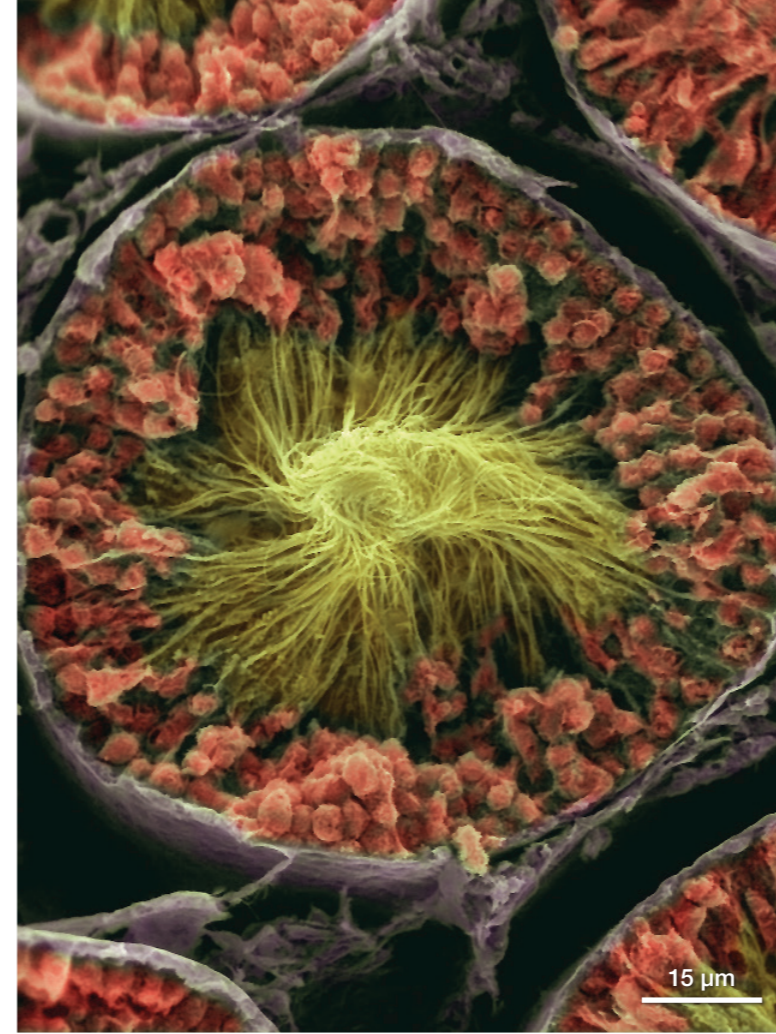
In the 1920s, it was discovered that accelerated electrons behave similarly to light in a vacuum; they travel in straight lines and have wave-like properties, with a much shorter wavelength than visible light. An image can be formed by capturing these electrons as they interact with a sample surface. Figure 1 provides a comparison between light and electron microscopy. This principle, utilizing a focused electron beam, was first described in a 1935 paper by physicist Max Knoll. Manfred von Ardenne also conducted early experiments with scanning electron microscopy (SEM) in 1937. However, it wasn't until 1942 that Zworykin, Hillier, and Snijder described a true SEM with a resolving power of 50 nm. Today, modern SEMs can achieve even higher resolution, below 1 nm.

Structure of a scanning electron microscope

A scanning electron microscope consists of an electron optical column, a vacuum system, electronics, and a computer with display. It uses an electron gun to produce a focused electron beam, which is scanned over a sample surface.

The interactions between the beam and the specimen create various signals, which are measured, stored, and displayed in the resulting image as variations in brightness. The most commonly used signal types are secondary electrons (SE), which are sensitive to the sample surface, and back-scattered electrons (BSE), which show compositional information from a deeper distance into the sample.

The magnification of the displayed image is determined by the ratio of the image size to the scanned area on the sample. Resolution, meanwhile, depends on a number of factors such as the type of signal, the size of the beam spot, the composition of the sample, and the energy of the beam. Generally, at lower voltages, the size of the spot is the main factor affecting image resolution, while at higher voltages, the volume of interaction becomes more important. The best SEMs currently offer resolutions below 1 nm at accelerating voltages as low as 1 kV, allowing for sharp, high-magnification imaging, even of highly sensitive samples.



Sample considerations

An SEM is typically used to gather information about the surface or near-surface region of a specimen. The sample must be able to withstand the chamber vacuum as well as electron beam bombardment. This means that some samples can be placed directly in the chamber, while others may require removal of volatile components or coating with a conducting layer such as iridium, platinum, chromium, or gold. Carbon is also an alternative when X-ray emissions from iridium might interfere with elemental analysis. The layer itself must be thick enough to provide a continuous conductive film, but also thin enough that it doesn't obscure surface details of interest. Typical thicknesses are ~1–10 nm, depending on the sample and application. Special techniques, such as low-voltage SEM, can also be used to avoid charging without the need for conductive coatings. Cryogenic preparation is an additional option for biological or organic materials. Low vacuum mode is also able to dissipate charging by introducing a small quantity of gas molecules, but this does not prevent sample desiccation.

Figure 2. SEM colored image of sperm tails tangled up in a seminiferous tubule.

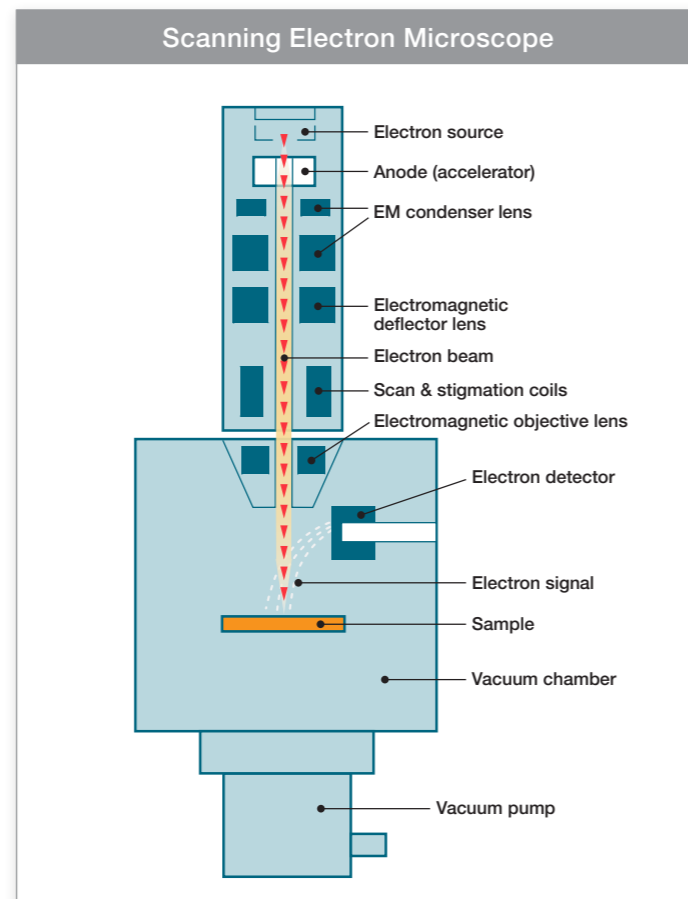
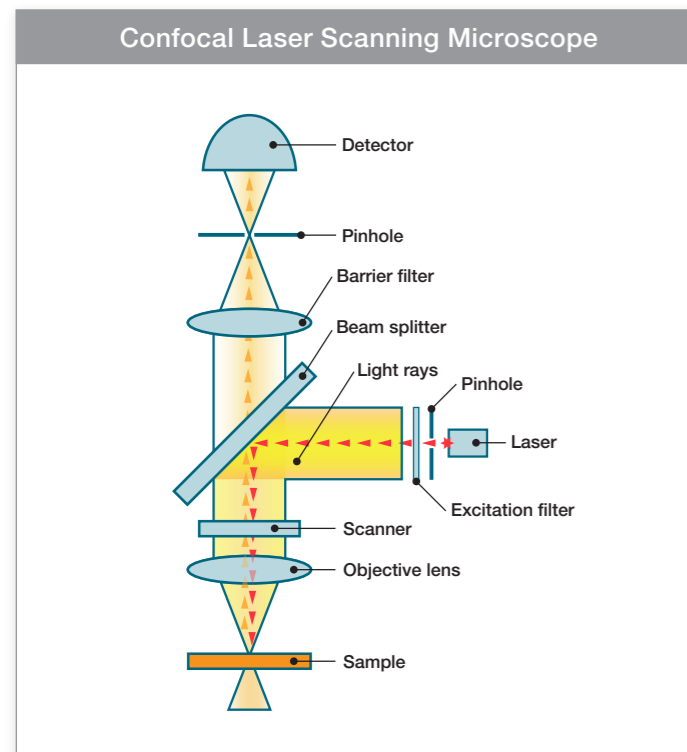


Figure 1 Comparing the structure of a laser scanning confocal microscope and a scanning electron microscope.

Environmental scanning electron microscopy

Environmental scanning electron microscopy (ESEM) is a special technique used to image biological samples in their natural, hydrated state. Unlike traditional SEM, ESEM can examine samples without dehydration. Special environmental chambers maintain a controlled, moist atmosphere. ESEM is valuable for the study of dynamic biological processes, such as the behavior of living cells, tissues, or microorganisms, and provides high-resolution images without extensive sample preparation.

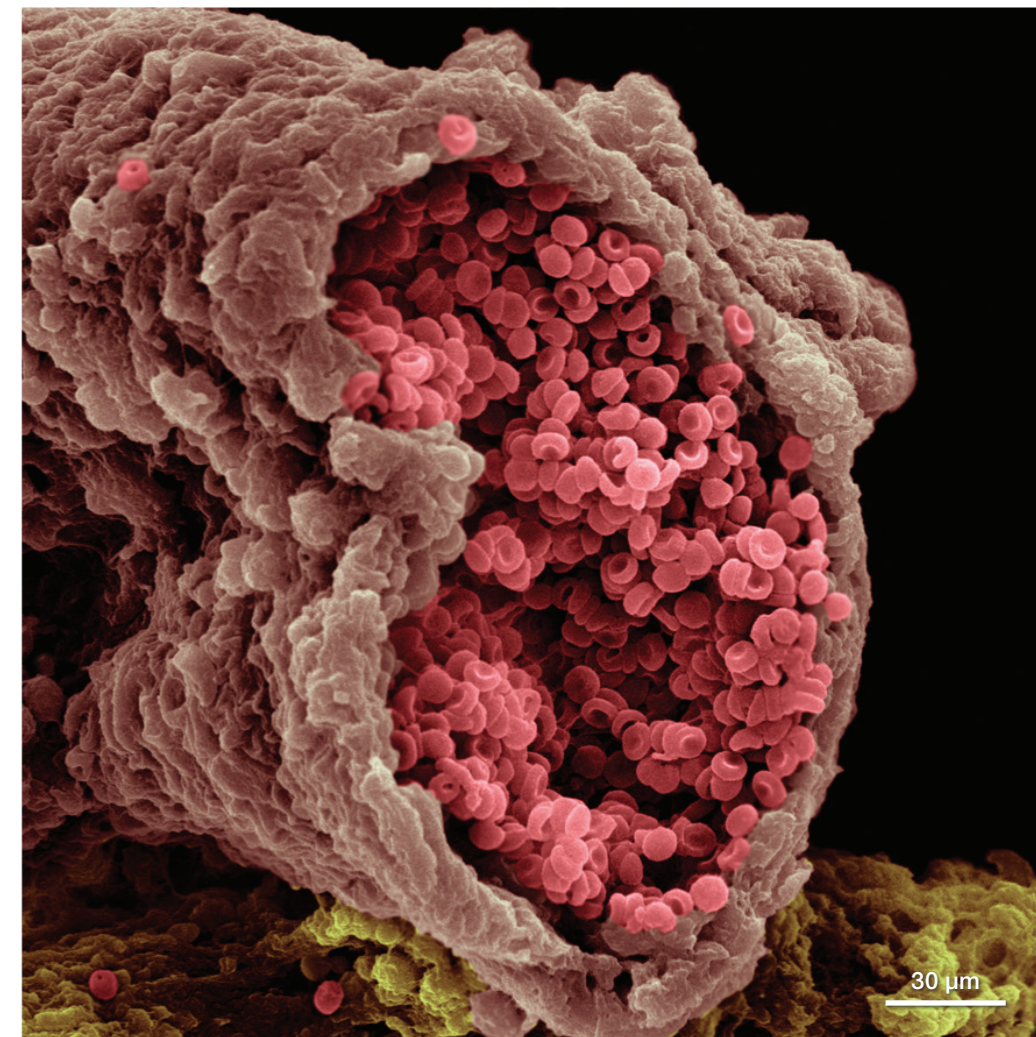
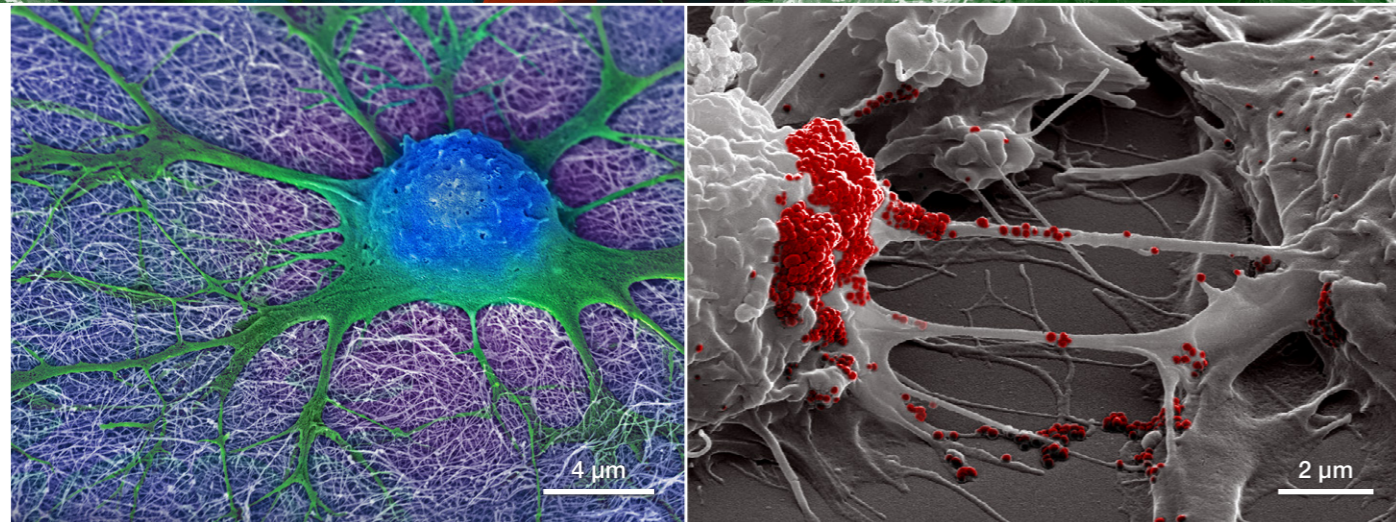
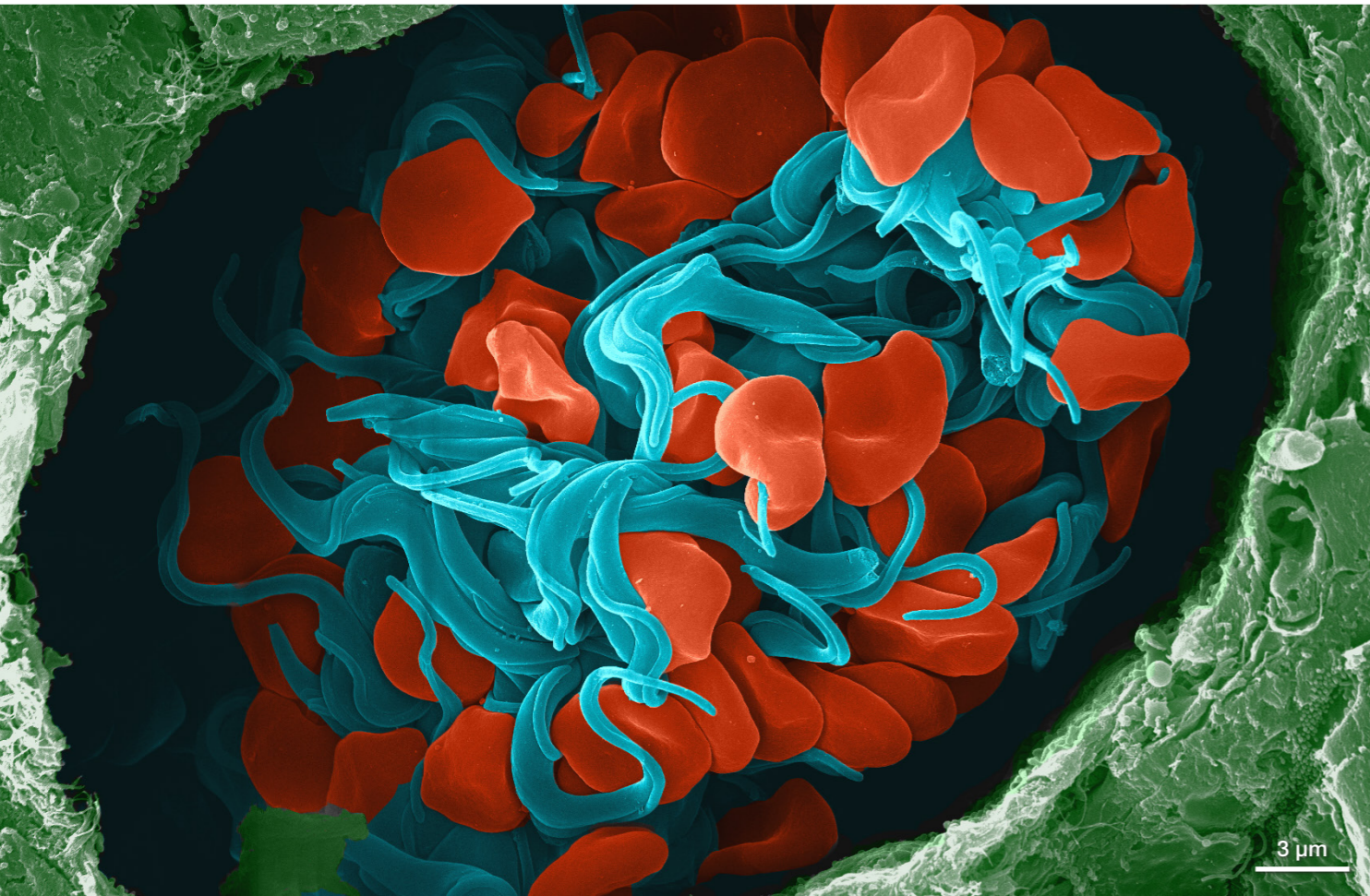


Figure 3. Artery with red blood cells (colored image), captured with environmental scanning electron microscopy.

Image any sample in any environment

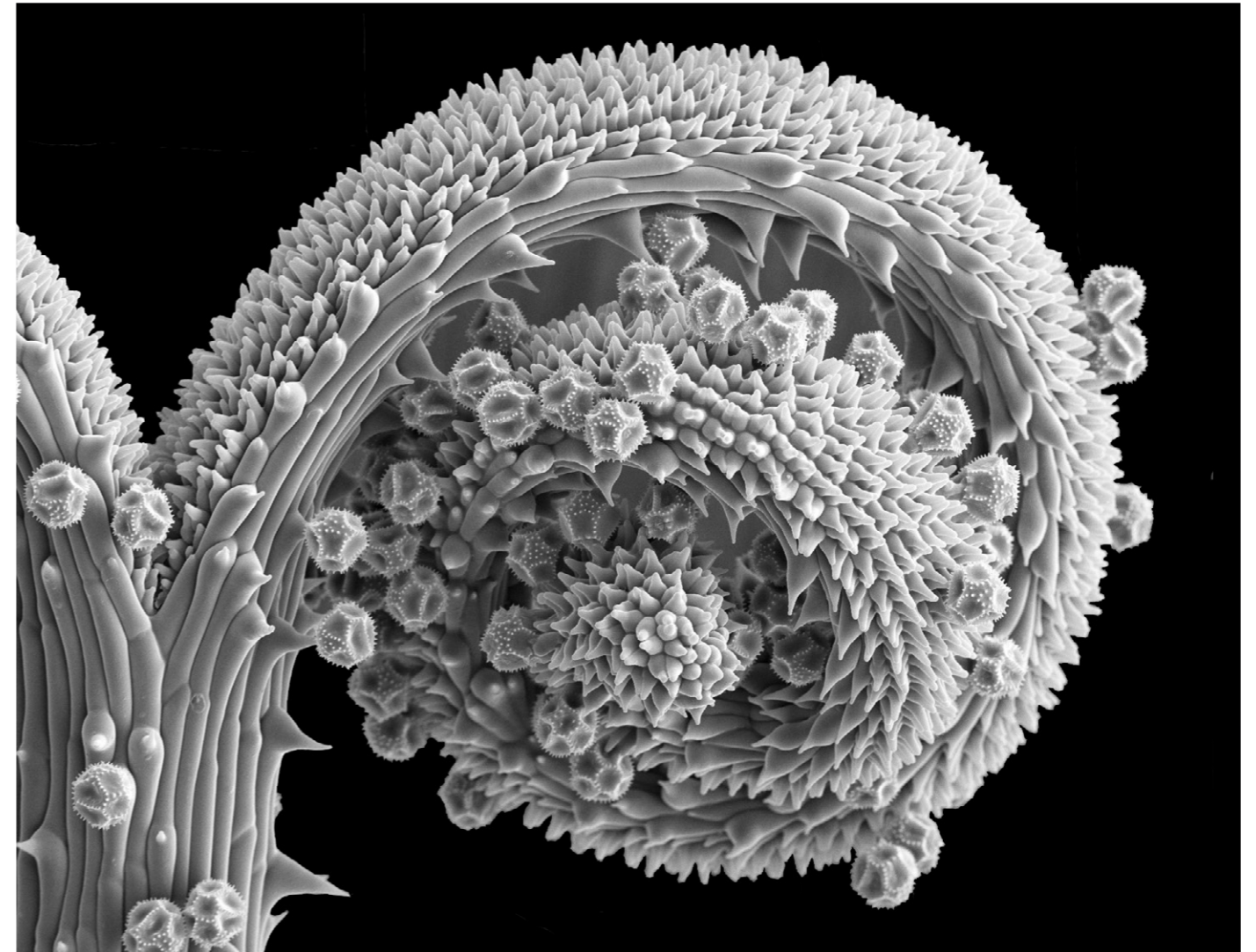


Cells prepared for high-vacuum imaging

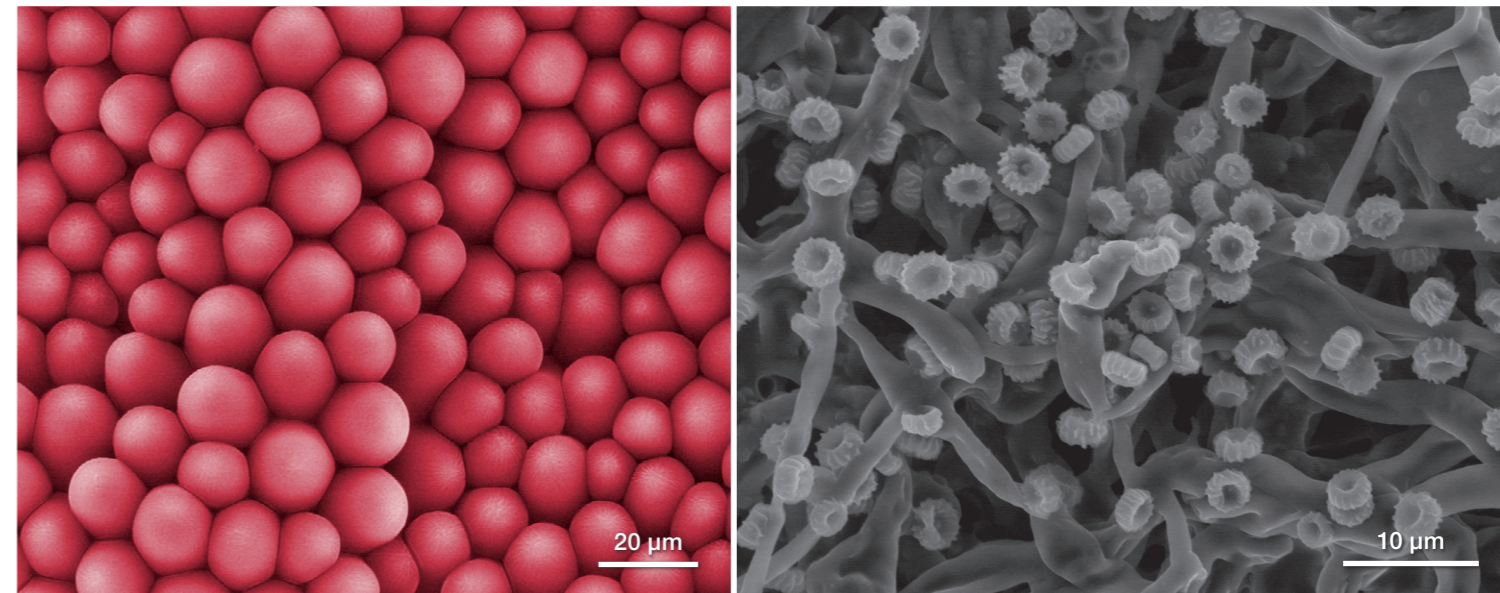
TOP: Image of *Trypanosoma brucei gambiense* (colored blue) intertwined with red blood cells (colored red), in a blood capillary (green). Image courtesy of David Pérez-Morga, Université Libre de Bruxelles.

BOTTOM LEFT: Colored image of an embryonic neural cell (blue/green) derived from a mouse spinal cord and cultured on a synthetic nanofiber gel (purple). The neurites of the cell (green) are extending throughout the nanofibers to re-establish a neural network with other cells. Image courtesy of Dr. Mark McClendon, Northwestern University.

BOTTOM RIGHT: Mouse macrophages communicate through bridges known as tunneling nanotubes, active sites involved in the endocytosis of nanoparticles and their transport between cells. This image was taken at a 52° tilt and the nanoparticles were colored red. Image courtesy of Dr. Rita Serda, University of New Mexico.



Cryogenic samples Cryo-SEM image of *Ixeris chinensis* flower in pollination at 500x magnification. Image courtesy of Dr. Wann-Neng Jane, Academia Sinica.

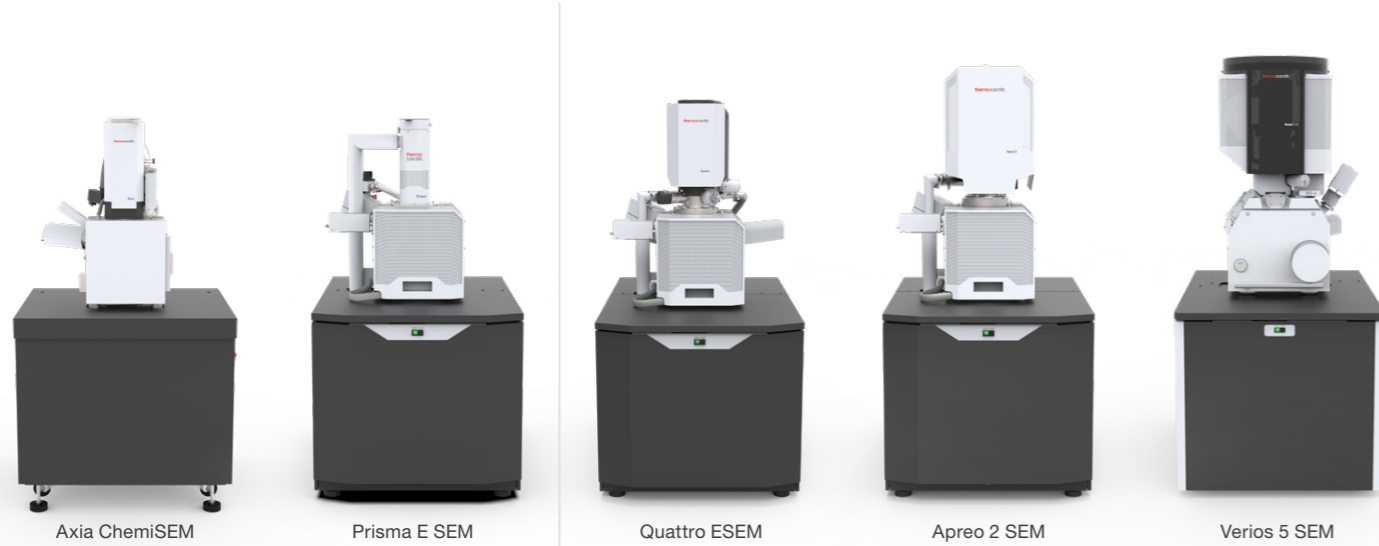


Environmental scanning electron microscopy

LEFT: Papillae on the upper surface of a rose flower petal captured in their hydrated, natural state. ESEM has preserved the sample, preventing any wrinkling or displacement of the papillae. Image courtesy of Mr. Marcos Rosado, Institut Català de Nanociència i Nanotecnologia.

RIGHT: Low-vacuum ESEM image of mold formed on bread.

Unveil biological details



Thermal Emission
(Tungsten)

Field Emission
(Schottky or Cold Cathode)

Attainable
Resolution

3 nm

2 nm

1 nm

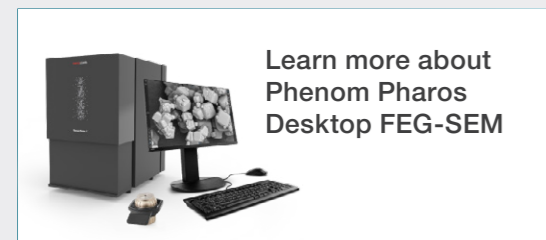
0.6 nm

Thermo Scientific™ Axia™ ChemiSEM

- Live quantitative elemental mapping
- High-fidelity scanning electron microscopy imaging
- Flexible and easy to use, even for novice users
- Easy maintenance

Thermo Scientific Prisma E SEM

- Entry-level SEM with excellent image quality
- Easy and quick sample loading and navigation for multiple samples
- Compatible with a wide range of materials thanks to dedicated vacuum modes



Thermo Scientific Quattro ESEM

- Ultra-versatile high-resolution FEG SEM with unique environmental capability (ESEM)
- Simultaneous SE and BSE imaging in every mode of operation

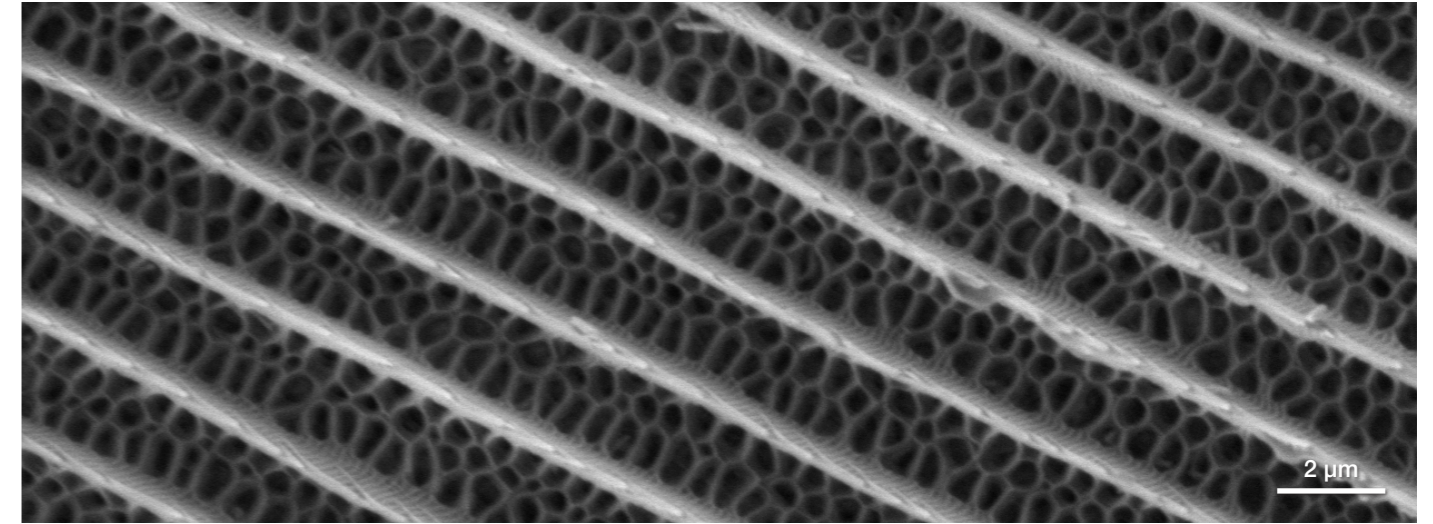
Thermo Scientific Apreo 2 SEM

- Versatile, high-performance SEM for nanometer or sub-nanometer resolution imaging
- In-column T1 backscatter detector for sensitive contrast
- Simple switching between normal SEM use and serial block-face imaging

Thermo Scientific Verios 5 SEM

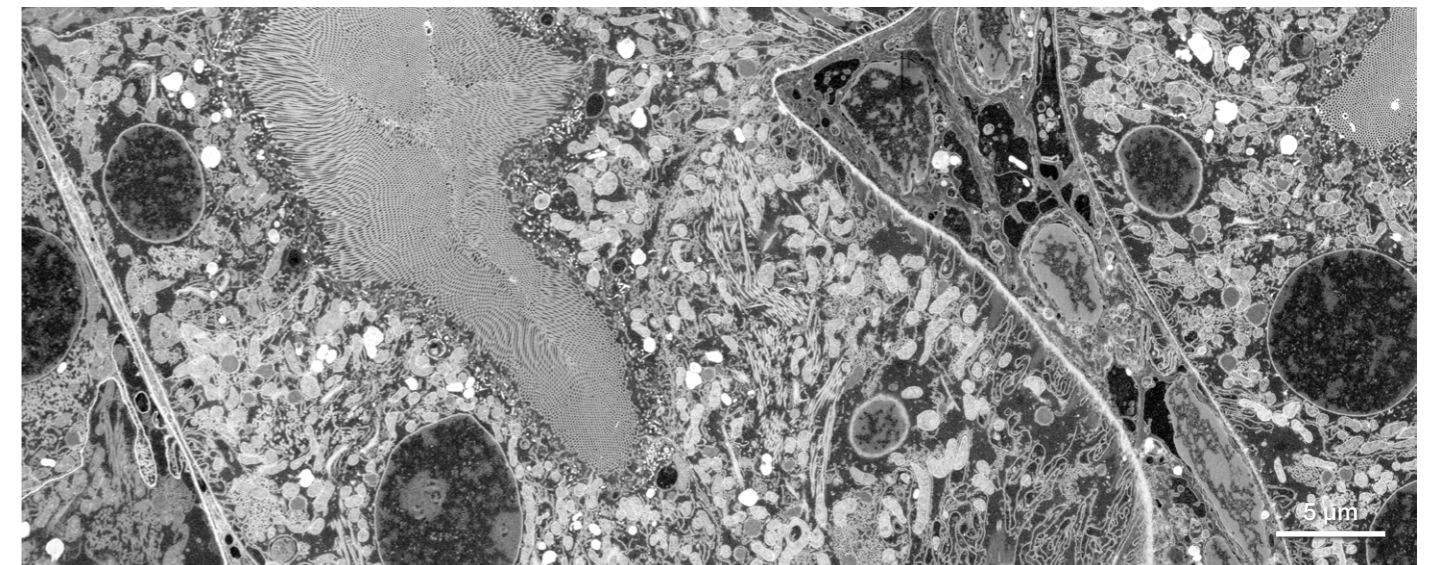
- Monochromated SEM for sub-nanometer resolution over the full energy range (1–30 keV)
- Easy access to beam landing energies as low as 20 eV
- Excellent stability with piezo stage as standard

Quattro ESEM



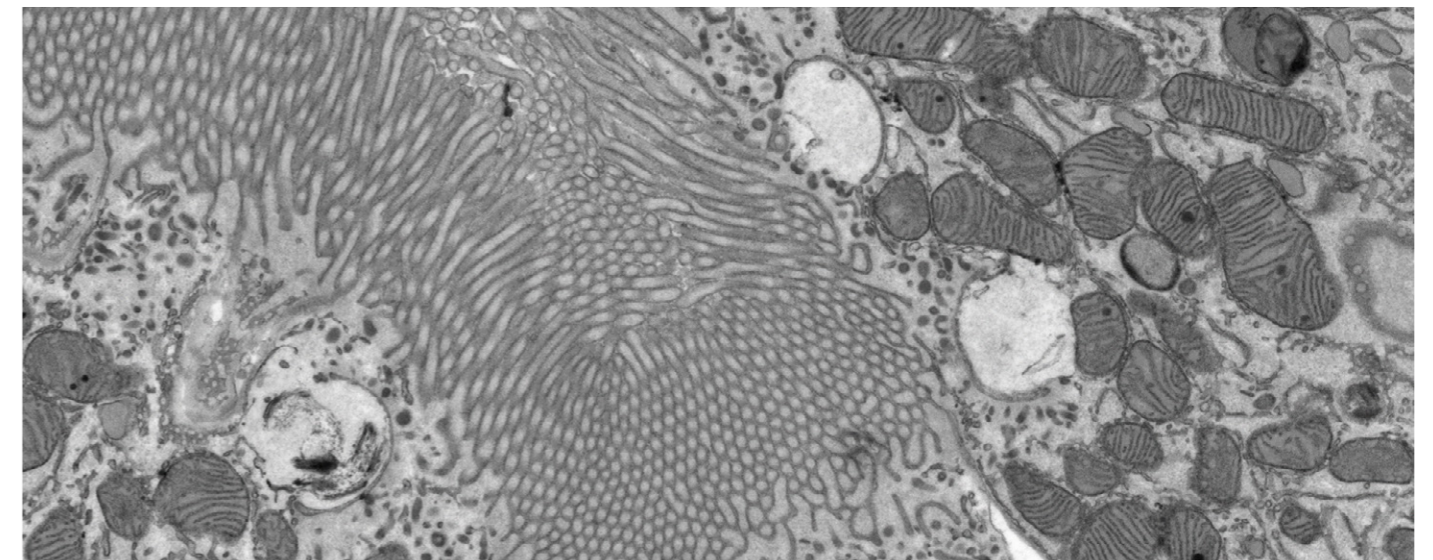
Butterfly wing imaged in a Quattro SEM under low acceleration voltage and low vacuum (80 Pa). Surface details are revealed at low acceleration voltage. Low energy electrons probe top surface rather than penetrating deeper within sample.

Apreo 2 SEM

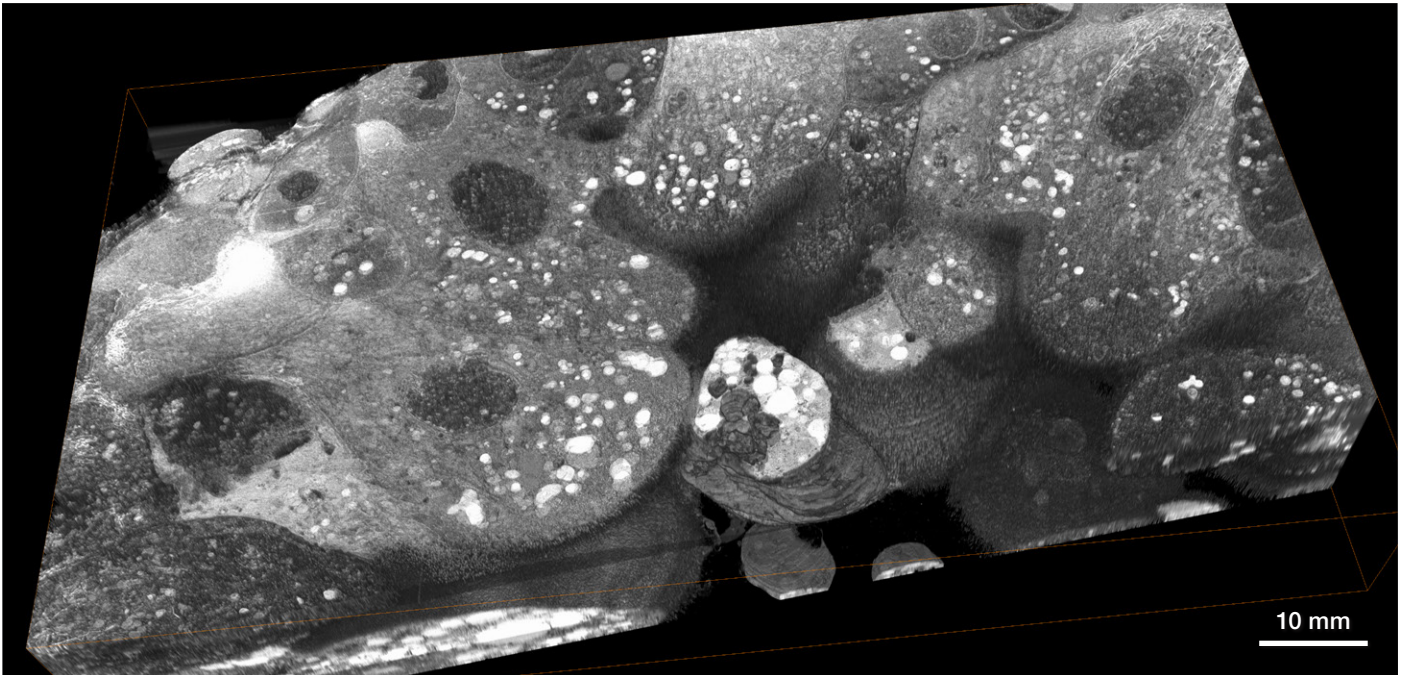


Wide area view of murine kidney imaged by an Apreo 2 SEM and Maps Software for Array Tomography. Image is a single slice taken from a 3D volume stack.

Scanning Transmission Electron Microscopy in SEM



STEM mode in SEM enables imaging of ultrathin resin embedded biological sections resulting in TEM-like images.



Array Tomography in SEM 3D volume rendering of drosophila gut recorded in 80 sections at 3.9 nm resolution. Array tomography can provide rapid localization of cells and their interaction partners in tissues. A sample is sectioned into individual slices and then imaged at nanoscale resolution using SEM.

3D imaging with volume EM

Volume electron microscopy (vEM) was named one of Nature's Technologies to Watch in 2023. The emerging field of volume EM encompasses a variety of imaging approaches and processing techniques that use electron microscopy. Explore below the surface of cellular ultrastructure, tissue, and small model organisms in 3D, at micro- to millimeter volume scales, at nanometer-level resolutions, and even at native state under cryogenic conditions.

Scan to learn more

FRONT COVER:
 (large panel) *Ixeris chinensis* flower in pollination!; (tiles) pollen of *Caesalpinia pulcherrima*, courtesy of Ohoud Alharbi, KAUST; *Trypanosoma brucei gambiense* parasite in a blood capillary; Mouse macrophages; *Ailanthus* webworm common moth (*Atteva aurea*), courtesy of Mark McClendon, Northwestern University.

Learn more at thermofisher.com/sem

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