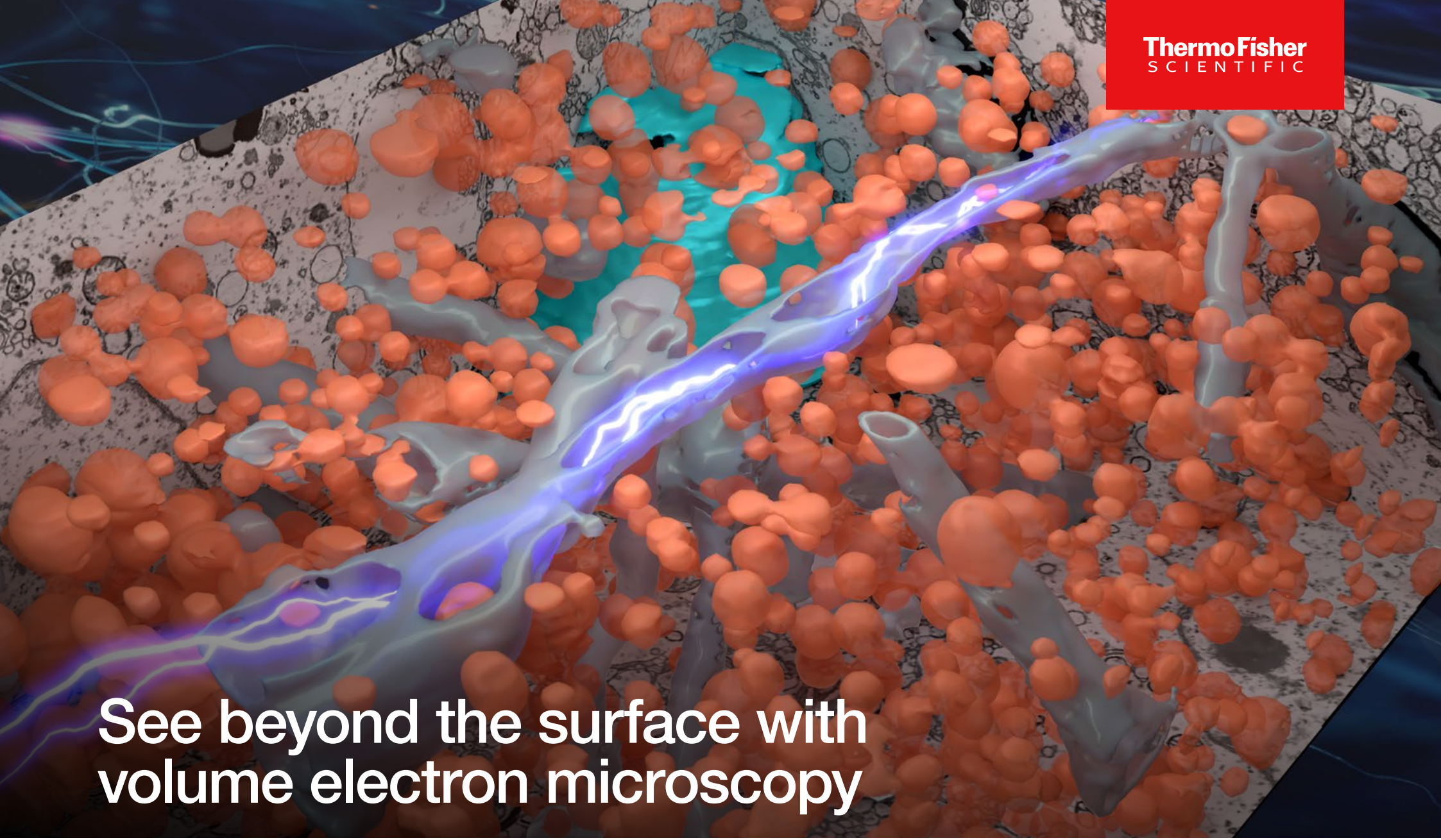
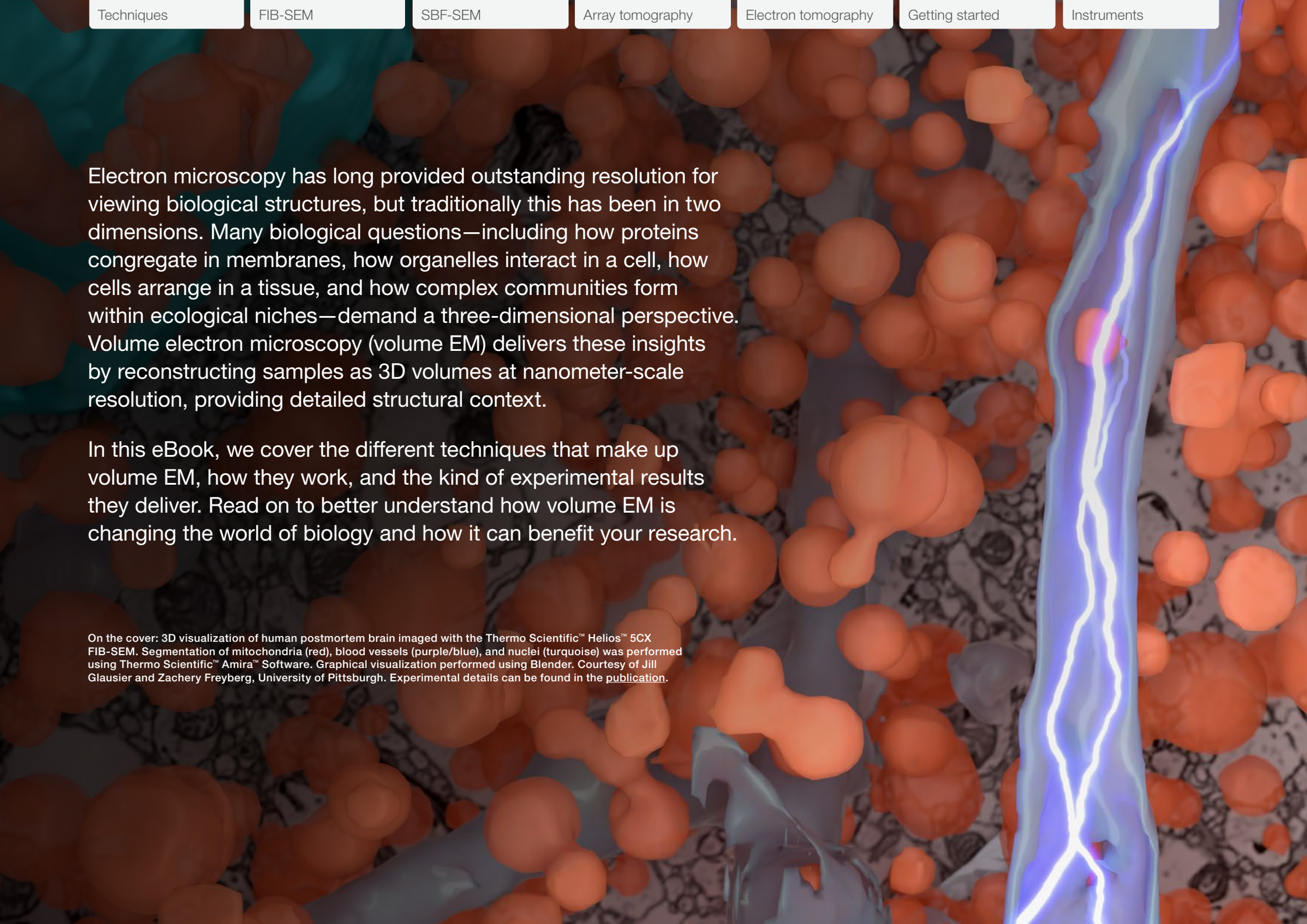


ThermoFisher SCIENTIFIC

See beyond the surface with volume electron microscopy

Explore the depths of biological structure in high-resolution 3D

thermo scientific



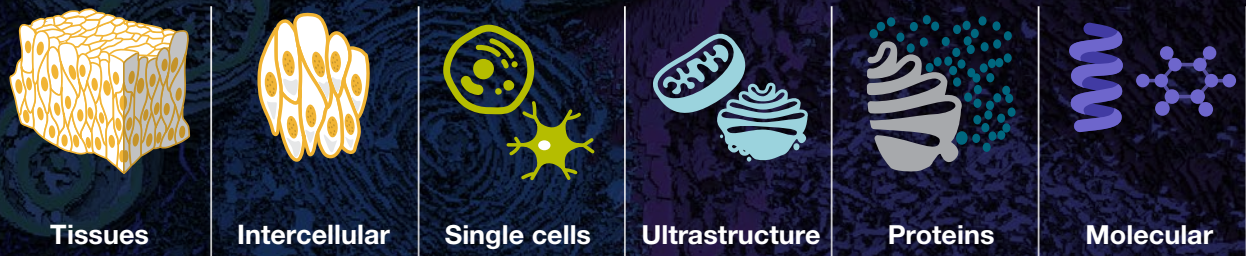
Electron microscopy has long provided outstanding resolution for viewing biological structures, but traditionally this has been in two dimensions. Many biological questions—including how proteins congregate in membranes, how organelles interact in a cell, how cells arrange in a tissue, and how complex communities form within ecological niches—demand a three-dimensional perspective. Volume electron microscopy (volume EM) delivers these insights by reconstructing samples as 3D volumes at nanometer-scale resolution, providing detailed structural context.

In this eBook, we cover the different techniques that make up volume EM, how they work, and the kind of experimental results they deliver. Read on to better understand how volume EM is changing the world of biology and how it can benefit your research.

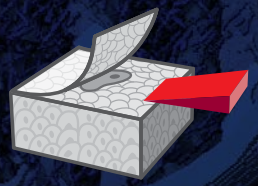
On the cover: 3D visualization of human postmortem brain imaged with the Thermo Scientific™ Helios™ 5CX FIB-SEM. Segmentation of mitochondria (red), blood vessels (purple/blue), and nuclei (turquoise) was performed using Thermo Scientific™ Amira™ Software. Graphical visualization performed using Blender. Courtesy of Jill Glausier and Zachery Freyberg, University of Pittsburgh. Experimental details can be found in the publication.

What are the volume EM techniques?

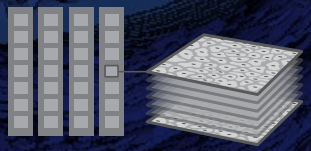
Volume EM datasets can be captured using diverse techniques, with each providing benefits for different sample types. Choose your solution to explore cellular ultrastructure, tissues, and small modal organisms in 3D, at micron to millimeter volume scale, at nanometer-level resolutions, and even in hydrated, native state under cryogenic conditions.



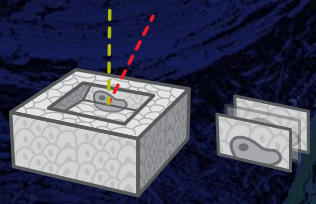
Tissues | Intercellular | Single cells | Ultrastructure | Proteins | Molecular



Serial block-face imaging



Array tomography



Serial FIB and plasma-FIB imaging

TEM tomography and cryo-tomography

Cryogenic conditions

Room temperature

Selecting the right acquisition instrument depends on multiple factors, including the size of the sample, the volume needed, the resolution necessary to resolve the structures of interest, the storage required for the resulting dataset, and the time it takes to complete the acquisition.

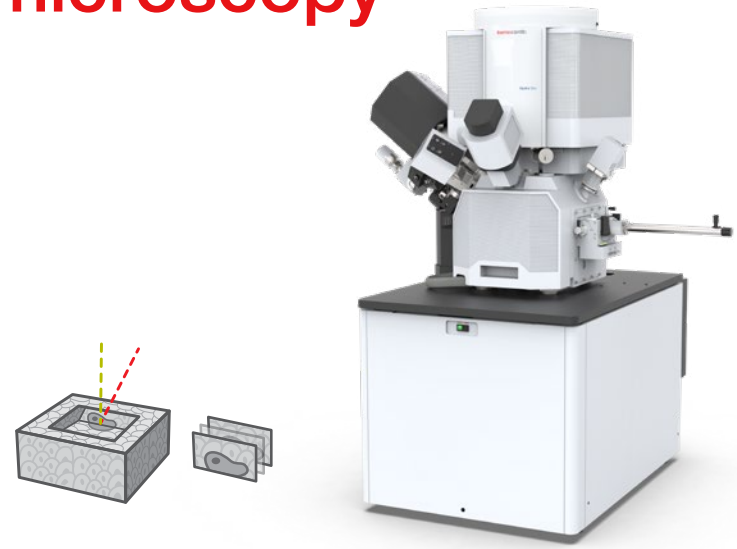
Please contact us to discuss the specific needs of your experiment so we can help you select the most appropriate approach.

Focused ion beam scanning electron microscopy

Focused ion bean scanning electron microscopy (FIB-SEM) uses an ion beam to sequentially mill away thin layers of the sample, revealing a fresh surface for SEM imaging. Gallium was traditionally the ion beam of choice, but newer plasma-FIB systems remove material faster, making it possible to access volumes hundreds of micrometers in size while still observing nanoscale structures in both resin-embedded and cryo-preserved specimens.

What is it used for?

FIB-SEM is an ideal solution for exploring volumes of tissue at high resolution. It allows acquisition of images at nanometer scale with isotropic voxels (typically 4 to 10 nm) and imaging volumes in the order of tens to hundreds of μm^3. Parameters such as field of view, resolution, and slice thickness can be adjusted to ensure acceptable acquisition times and data sizes, as well as sample specificity.



How FIB-SEM captures a volume

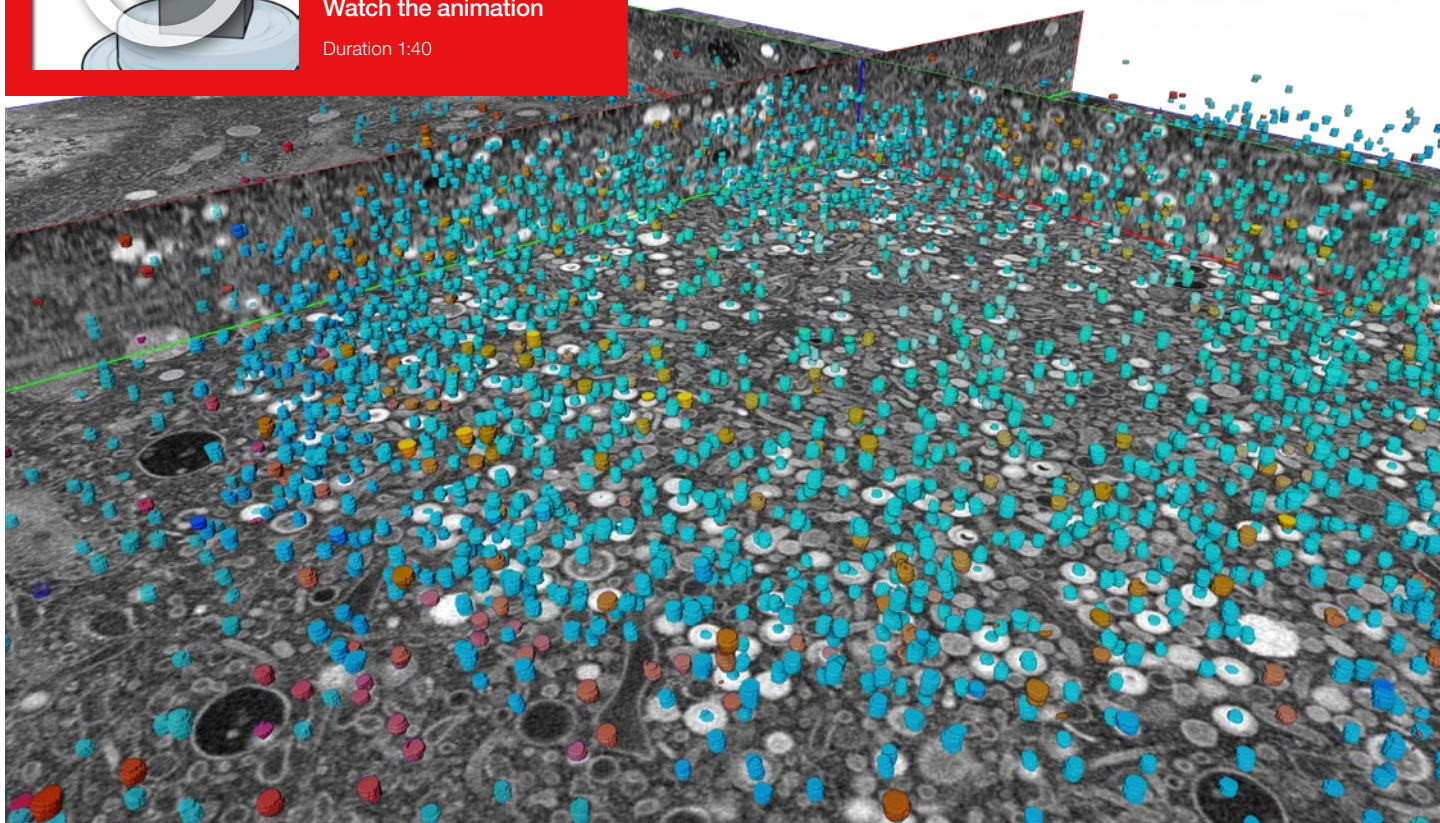
Watch the animation

Duration 0:19

Reveal large surface areas with the Spin Mill Method

Watch the animation

Duration 1:40



Right: 3D reconstruction of fibroblast monolayer infected with human cytomegalovirus (HCMV) embedded in EPON epoxy resin and imaged with the Hydra Bio Plasma-FIB. Segmentation and classification of the viral particles were performed using AI deep-learning-based segmentation in Amira Software. Sample courtesy of Clarissa Read, Central Facility of Electron Microscopy at Ulm University, and Jens von Einem, Institute of Virology, Ulm University Medical Center.

Hydra Bio Plasma-FIB

Your premium volume EM solution for high-resolution applications

Increase throughput by up to 50% with AI-guided workflows

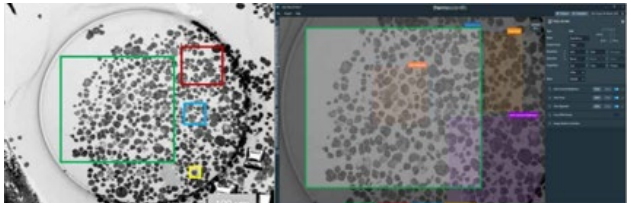
Acquisition time is one of the biggest limitations for FIB-SEM volume EM, with many acquisitions taking days or weeks to complete. Exclusive AI-guided workflows for the Thermo Scientific™ Hydra™ Bio Plasma-FIB reduce the total acquisition time for volume EM data by up to 50% by selectively imaging at high resolution only your structures of interest.

Gain more insights by accessing large sample volumes

To access large tissue volumes and streamline sample preparation, the collimated plasma beam provides rapid milling capabilities by delivering currents higher than a gallium FIB—up to 2.5 µA.

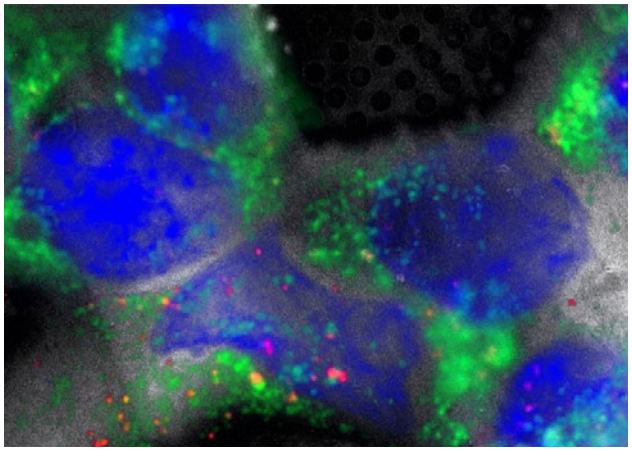
Expose regions up to 1 mm in diameter for imaging

Choosing high-resolution FIB-SEM no longer means compromising the size of the region you can image. The Spin Mill Bio Method allows you to expose large regions up to 1 mm in diameter for imaging, which provides a geometry similar to microtome-based serial blockface imaging but with a slice thickness as small as 5 nm.



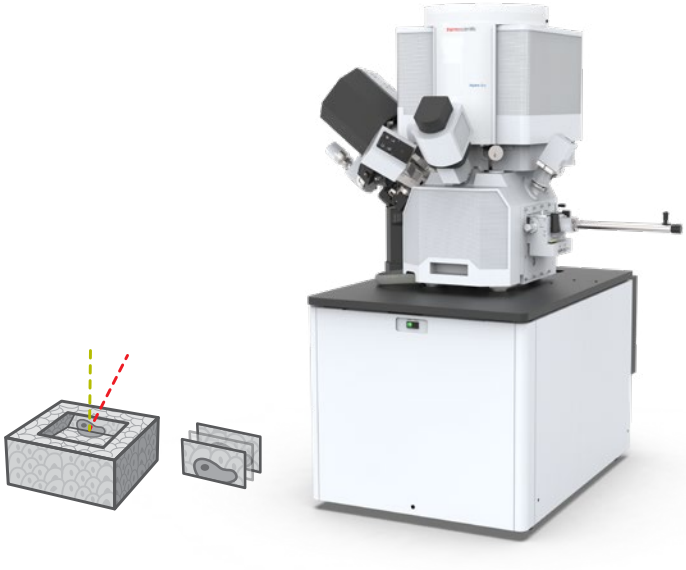
Precisely guide your volume EM acquisitions using fluorescence

Capturing the right volume of your specimen is essential to generating the insights you need. The integrated fluorescence light microscope (iFLM) provides precise localization of fluorescent markers within your imaging tool to help you capture precisely the right region in X, Y, and Z.



Focus on scientific questions rather than sample preparation

To get the most out of every acquisition, it's important to optimize imaging conditions. With access to four different plasma sources—oxygen, nitrogen, xenon, and hydrogen—you can select the optimal ion and milling conditions to suit your sample. The ability to swiftly switch between these ions and even combine them increases flexibility and can further enhance milling precision and image quality.

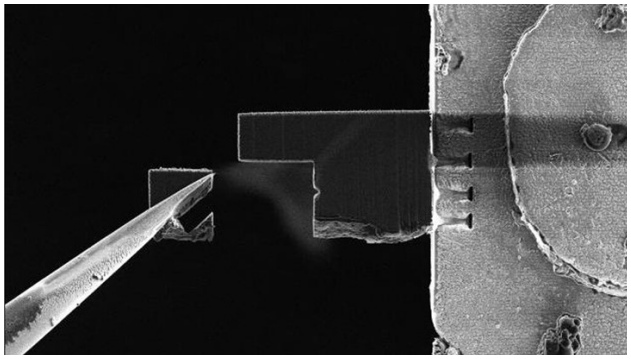


Elevate your research by integrating both room-temperature and cryo workflows

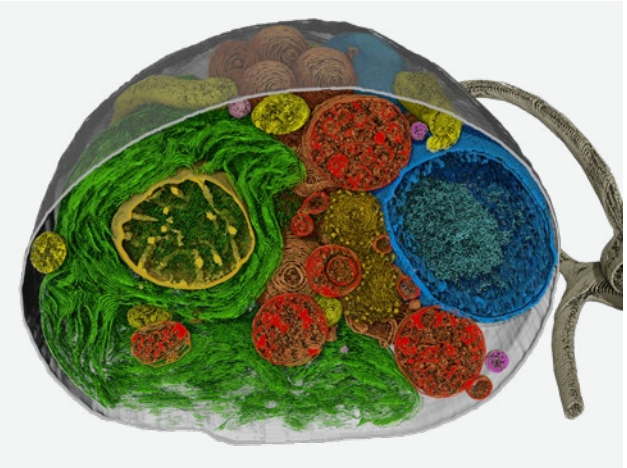
Quickly and easily move between cryogenically frozen specimens and resin-embedded samples to bridge scales and complexity.

Biological imaging across length scales

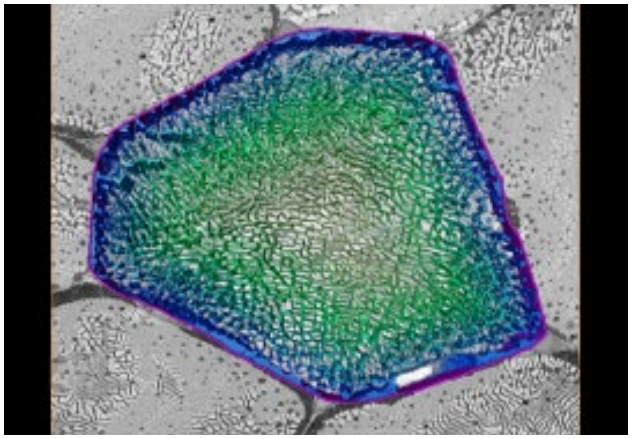
The Hydra Bio Plasma-FIB offers multiscale imaging in biology, accommodating both lamella preparation for *in situ* protein analysis using cryo-electron tomography and large tissue volume acquisition, all within a single platform.



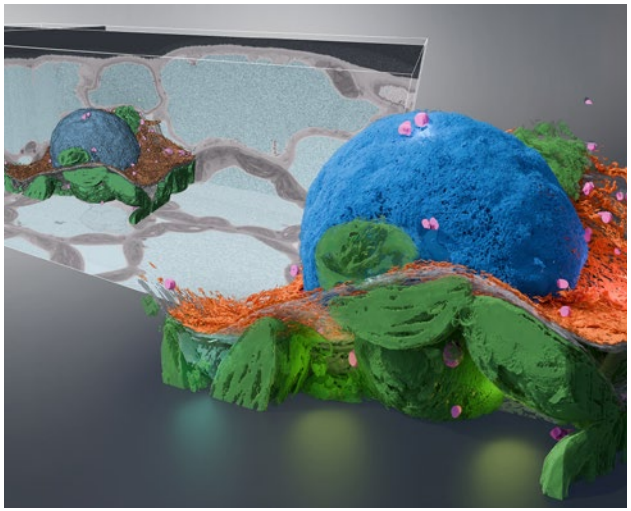
Hydra Bio Plasma-FIB



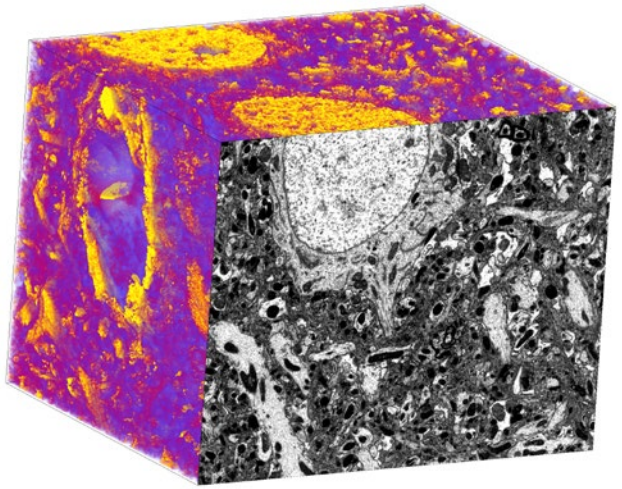
3D visualization of unstained *Chlamydomonas reinhardtii* imaged in cryo conditions with the Hydra Bio Plasma-FIB and segmented with Amira Software.



Mouse skeletal muscle fiber imaged with the Hydra Bio Plasma-FIB using the Spin Mill Method. Segmentation performed using Amira Software. Sample courtesy of Brian Glancy and EM Core, NHLBI/NIH.



Volume reconstruction of a *Nicotiana benthamiana* (tobacco) epidermal cell acquired using the adaptive scanning application on the Hydra Bio Plasma-FIB. Stack alignment, post processing, and segmentation done with Amira Software. Horizontal field of view is 61.25 µm. Sample courtesy of Tessa Burch-Smith, Kirk Czymmek, and Lolita Rotkina, Donald Danforth Plant Science Center.



3D reconstruction of a mouse brain sample embedded in LR White resin and imaged with the Hydra Bio Plasma-FIB. Oxygen was used for milling to prevent charging and curtaining artifacts that are often experienced when using gallium for milling such soft resins. Total volume = 23.4 µm x 18.2 µm x 20.0 µm.

"We are increasingly being challenged to understand how to improve crop resilience to environmental stress and disease. The remarkable capabilities of our new Thermo Scientific Hydra Bio Plasma-FIB will allow us the unprecedented ability to reconstruct entire plant cells and tissue with exquisite detail. This technology will allow us to 'freeze' organisms in time and space and build intricate 3D models that will help us solve our critical food security challenges."

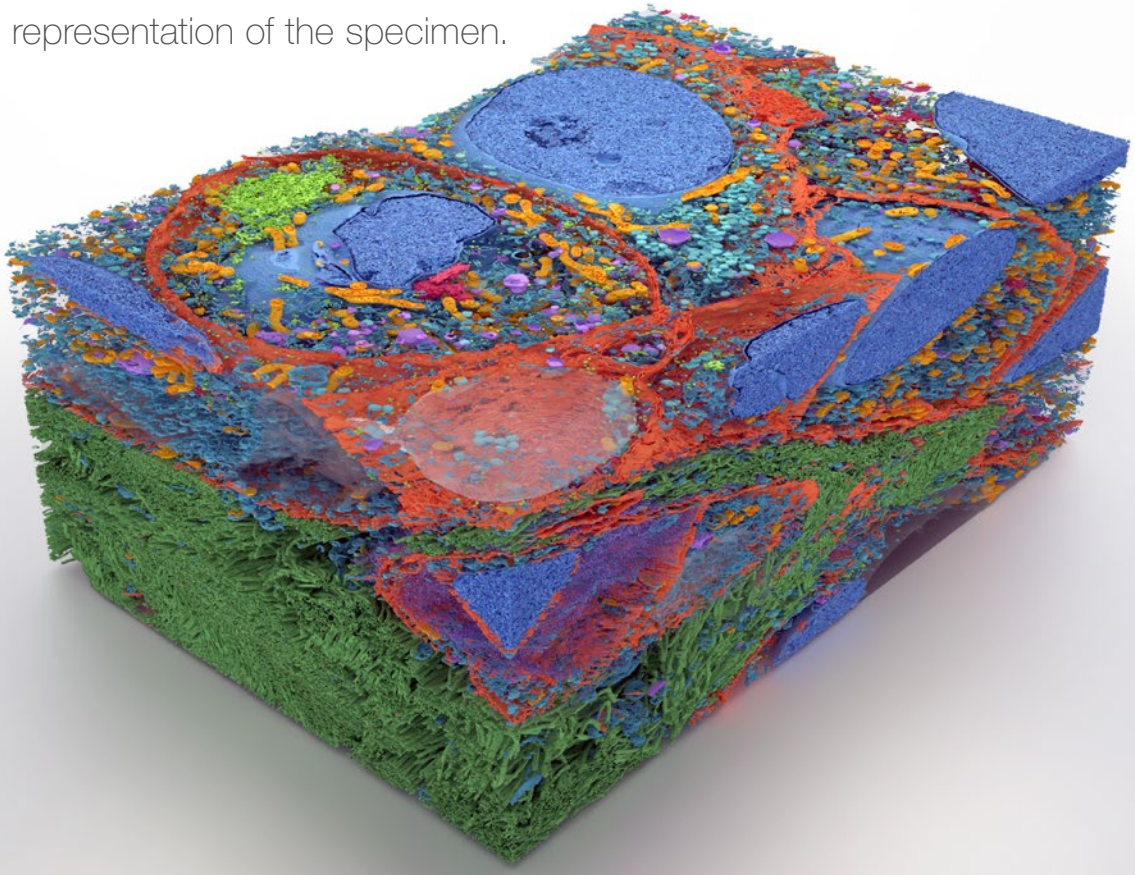
-Kirk Czymmek,
Principal Investigator and Director,
Advanced Bioimaging Laboratory,
Donald Danforth Plant Science Center

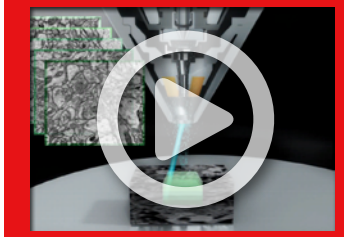
Serial blockface scanning electron microscopy

Serial blockface scanning electron microscopy (SBF-SEM) collects a series of 2D images (sections) from a fixed sample in succession as a microtome removes thin slices from the sample's surface. The sequential images can then be reconstructed into a 3D representation of the specimen.

What is it used for?

SBF-SEM is an ideal solution for exploring larger volumes of tissue. Typical voxel sizes are 10 nm with a slice thickness of 10 nm when imaged with multi-energy deconvolution and sample volumes up to 1 mm^3.





How SBF-SEM captures a volume

Watch the animation

Duration 1:42

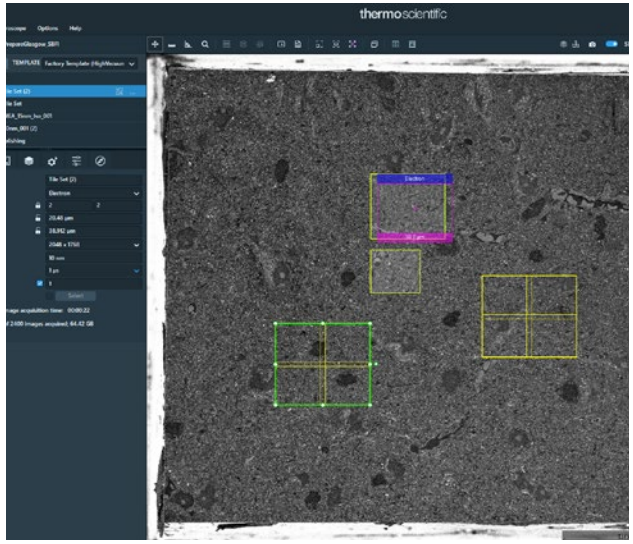
How to mount the Volumescope 2 SEM microtome

Watch the video

Duration 0:19

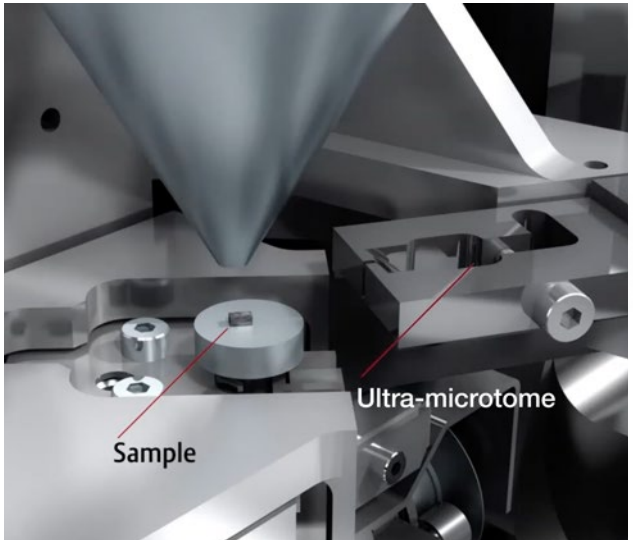
Volumescope 2 SEM

Your workhorse volume EM solution for large volume imaging



Capture multiple locations with different settings

For some specimens, it is not necessary to capture the whole volume of data because regions or events of interest are located in different regions of the volume. With the Thermo Scientific™ Volumescope™ 2 SEM, you can configure multiple regions of interest in your experiment, each with individual settings to optimize for the specific region of interest. Configuration is performed at the beginning of the experiment and, if needed, can be adjusted throughout the experiment to account for any changes in your specimen.



Quickly move from SBF-SEM to other SEM capabilities

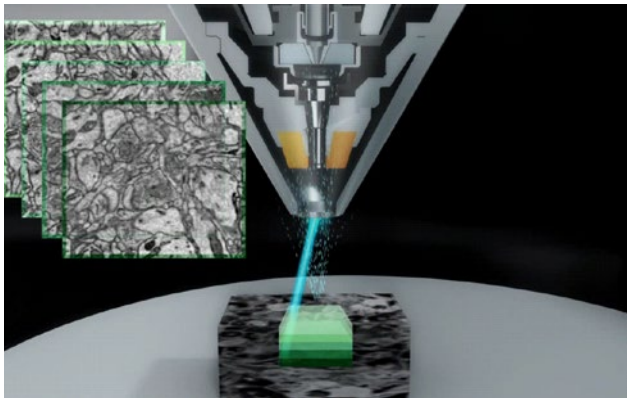
The Volumescope 2 SEM is useful for many different experiments. The microtome unit can be quickly and easily exchanged in and out of the SEM to ensure multiple uses of the system, such as array tomography or energy-dispersive X-ray spectroscopy.

Adjust imaging conditions to suit your sample

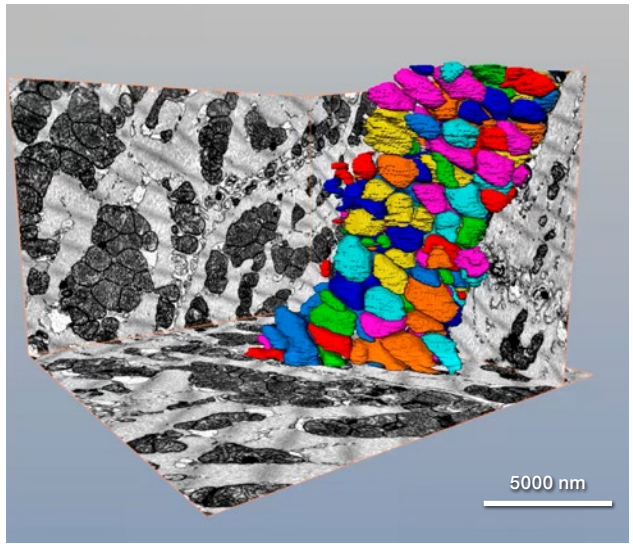
Imaging can be performed in both high and low vacuum, giving you the flexibility to optimize imaging conditions for every sample.

Achieve slice thickness resolution down to 10 nm

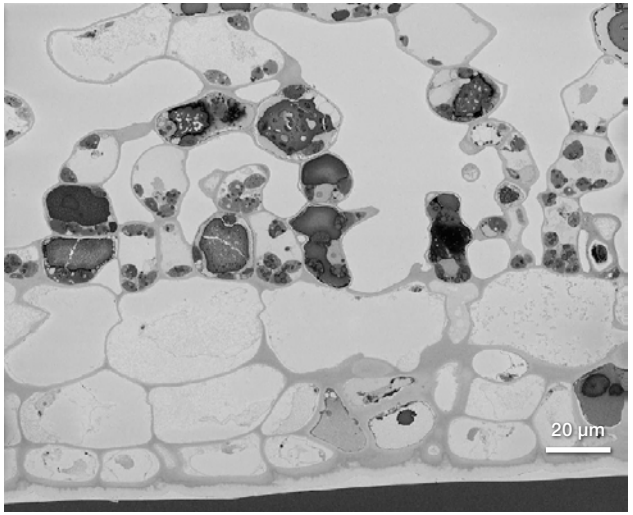
One of the limitations of serial blockface imaging is the minimum thickness of the slice that is removed from the sample using the diamond knife. In typical samples, this minimum is between 25 and 50 nm. Using multi-energy deconvolution removes this physical restriction and can reduce slice thickness to 10 nm, significantly increasing the overall resolution of your dataset.



Volumescope 2 SEM



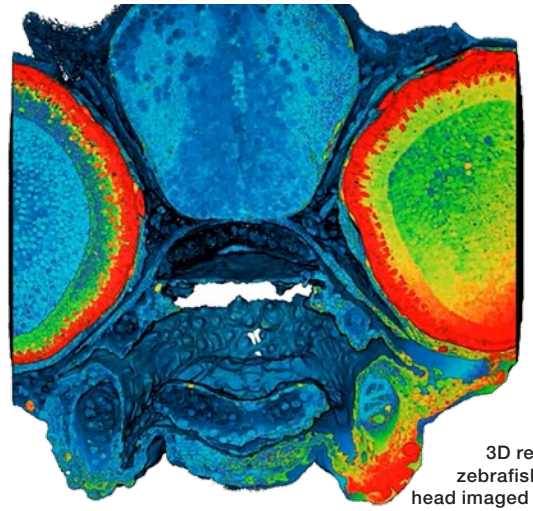
3D render of mouse heart muscle imaged in 3D with the Volumescope 2 SEM with mitochondria segmented. Sample courtesy of Madesh Muniswamy, Temple University.



Single slice from a 3D volume of a leaf's inner ultrastructure imaged with the Volumescope 2 SEM. Courtesy of Jiří Týč, Czech Academy of Sciences, and Jiří Šantrůček, University of South Bohemia.

"We have worked hard to optimize both sample preparation and acquisition routines for the Volumescope 2 SEM and we are now imaging entire organisms over many weeks at a time, something that is greatly supporting a number of projects at the University."

-Nicole Schieber, Facility Manager,
Centre for Microscopy and Microanalysis,
The University of Queensland, Australia



3D render of a zebrafish embryo head imaged with the Volumescope 2 SEM. Sample courtesy of R. Creton, Brown University.

Rat brain section imaged in 3D using the Volumescope 2 SEM. Total dimensions are 85 x 85 x 123 µm. Segmentation and visualization of the vasculature was performed with Amira Software. Sample courtesy of Graham Knott, EPFL, Lausanne.

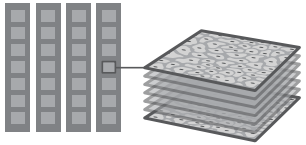
Array tomography

In array tomography, samples are first cut into a series (array) of sections that are then imaged with SEM. The images are aligned and recombined into a 3D reconstruction. This is a non-destructive volume EM approach—you can store sample sections and reimage them at any time.

JOVE: Array tomography workflow

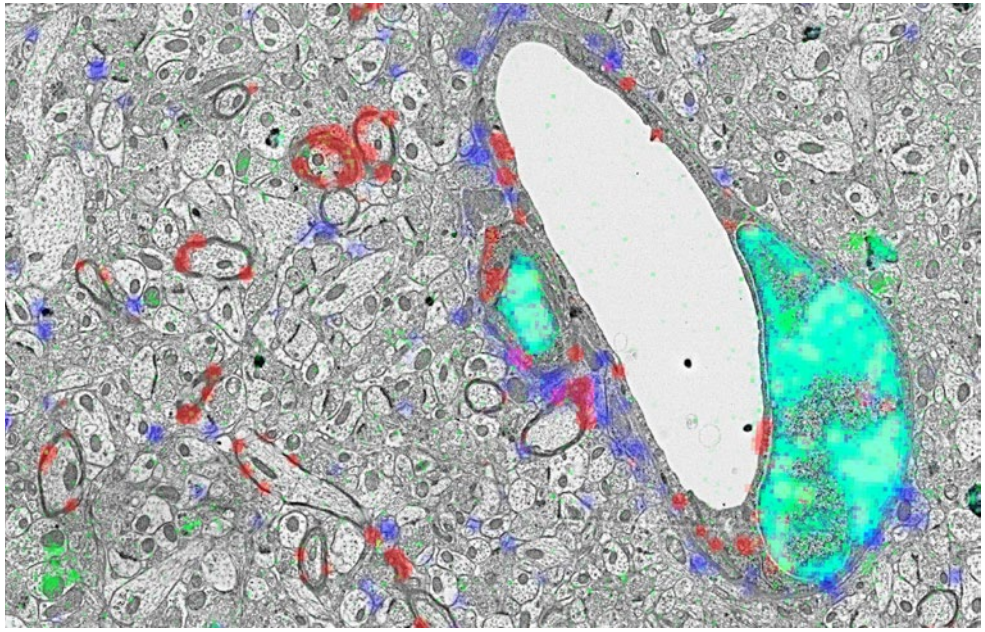
Watch the video

Duration 9:46



What is it used for?

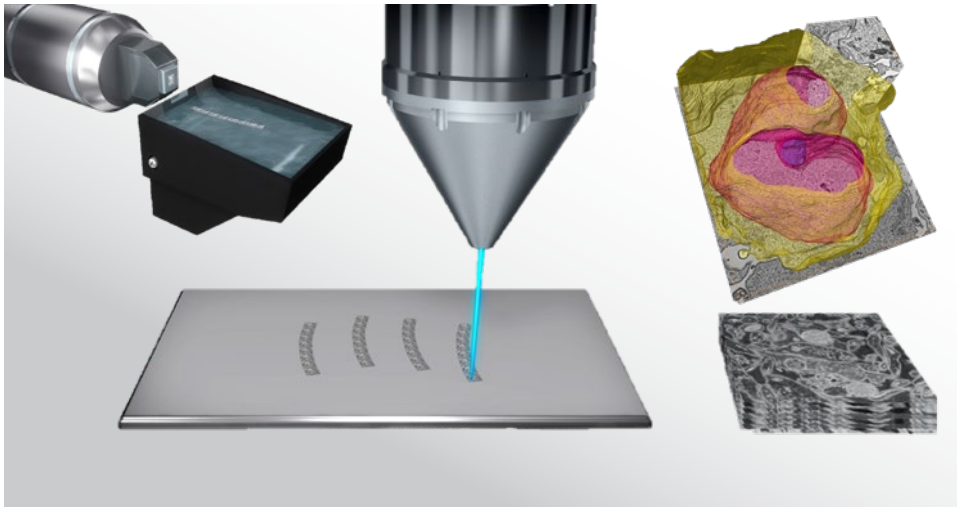
Array tomography is an ideal solution for large specimen volumes, especially in combination with fluorescence. Typical voxel sizes are 10 nm with slice thickness of 30 to 100 nm, depending on sample properties, the microtome, and experimental goals. Sections can be as large as 7 mm, limited by sample preparation, the knife, and the microtome. It is also possible to cut ribbons of sections from smaller blocks, so that hundreds of sections can be collected on a single wafer.



Correlative microscopy of a mouse brain sample. Immuno-flourescence (color) from light microscopy and ultrastructure (gray) from SEM using the Apreo 2 SEM. Maps Software (far left) was used for SEM acquisition and correlation. Sample and light microscopy images courtesy of Kristina Micheva, Stanford University.

Maps Software with array tomography

Easy-to-access volume EM



Overview of the array tomography workflow from microtome to electron imaging to 3D data visualization.

Streamline acquisition with automatic section recognition

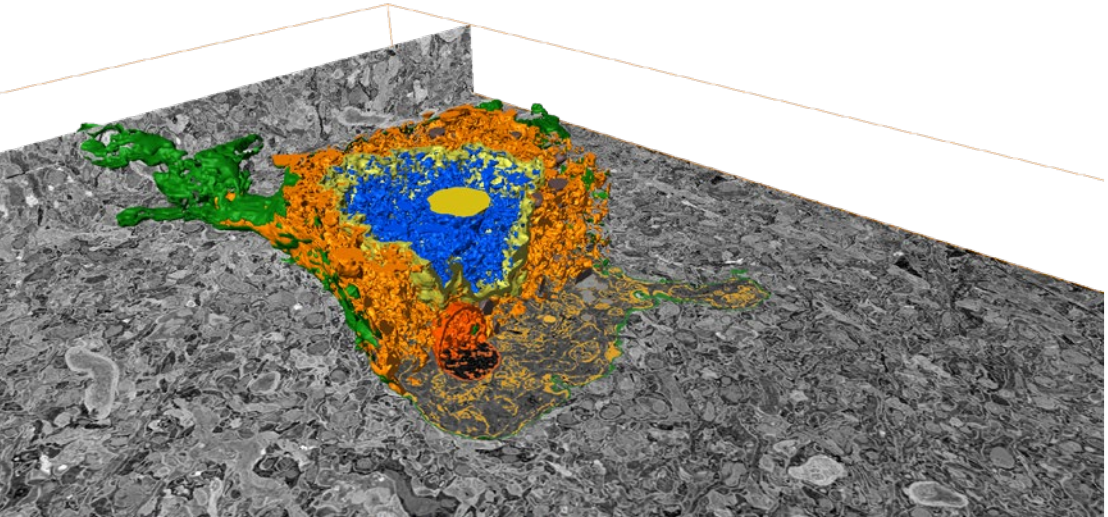
One of the most time-consuming steps in array tomography is identifying individual sections in a ribbon of slices. Thermo Scientific™ Maps Software addresses this with its automatic Section Finder, which quickly and easily identifies sections. You can then define the collection order with just a few clicks to acquire slices in the correct sequence and capture 3D data with minimal configuration time.

Maintain imaging conditions throughout long-term imaging

Successful long-term imaging relies on precise alignments, including brightness, contrast, focus, and stigmation. The auto functions in Maps Software continuously maintain all these alignments. This high success rate significantly reduces the time required for reviewing results and re-imaging failed acquisitions, streamlining the overall imaging process.

Capture only the data you need

In array tomography, consecutive imaging regions can shift slightly due to minor rotational or stretching differences in each slice. Precisely identifying these regions is crucial. Maps Software offers automatic position refinement, which helps you accurately capture smaller regions on each section without the risk of losing target features. This precision keeps consecutive imaging regions consistently positioned in the same relative location across all sections. All you have to do is assign a region of interest for imaging.



3D reconstruction of a mouse brain sample imaged using Maps Software for array tomography and processed and segmented using Amira Software.

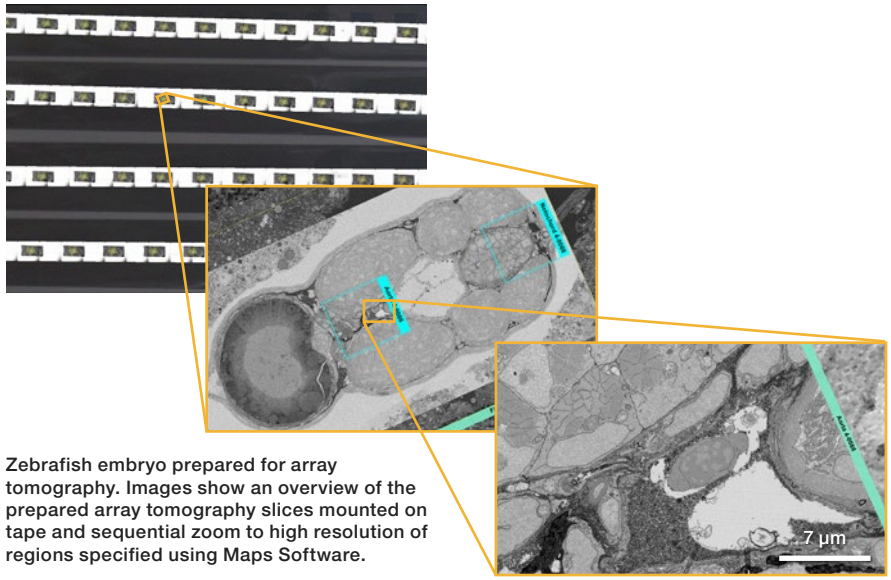
Add volume EM to any SEM or FIB-SEM

Accessing volume EM using dedicated platforms is often a barrier to benefitting from volume acquisition. Maps Software can add array tomography to any Thermo Scientific SEM or FIB-SEM to offer volume EM at an affordable price point, lowering the barrier to entry and allowing you to test and optimize experiments without a large financial outlay.

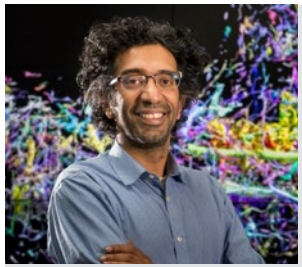
Guided step-by-step workflow

Maps Software contains several workflow panels for each step, from Overview Recording to Array Acquisition. Each panel contains clear instructions and all buttons and tools needed for the current workflow step. This ensures minimal input is needed to set up an acquisition, saving time and minimizing error.

Maps Software with array tomography

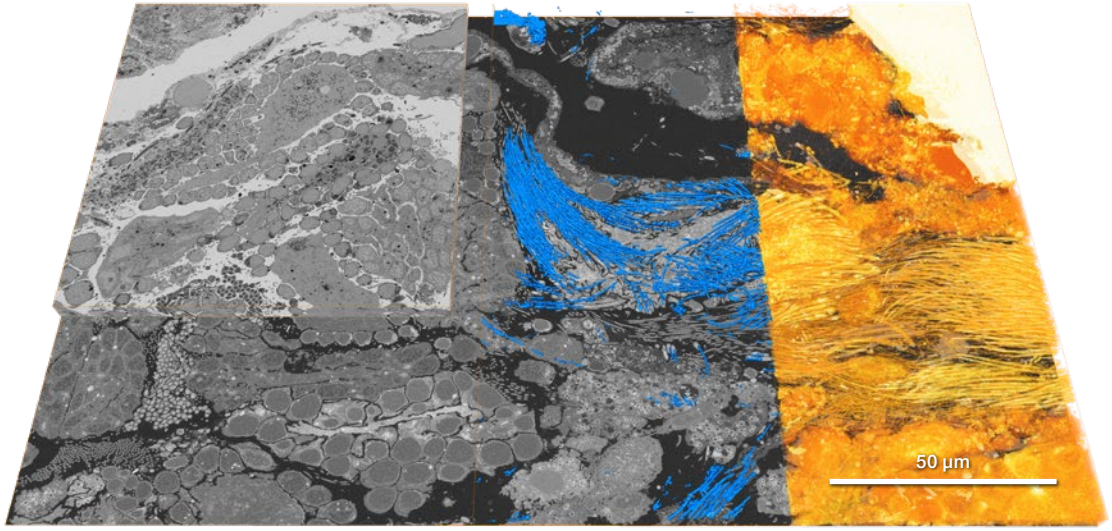


Zebrafish embryo prepared for array tomography. Images show an overview of the prepared array tomography slices mounted on tape and sequential zoom to high resolution of regions specified using Maps Software.

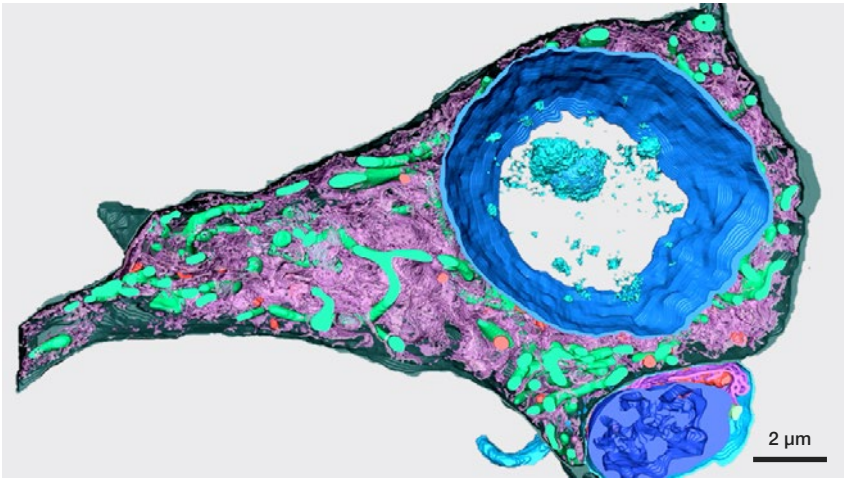


"In Maps Software with array tomography, less than 10 µm section-to-section variability of imaging region placement allows imaging with much less overhead. This variability can be hundreds of microns in other solutions. Imaging with less overhead speeds up time to data by a large factor, especially when imaging small target structures."

-Narayanan 'Bobby' Kasthuri,
Neuroscientist, University of Chicago



Earthworm seminal vesicles. Amira Software was used to create this composite containing orthogonal views, segmented mitochondria, and volume renderings of a large field of view recorded in 48 sections. Data has 26.1 nm resolution. Sample courtesy of Karol Matola, University of Silosia, Poland.



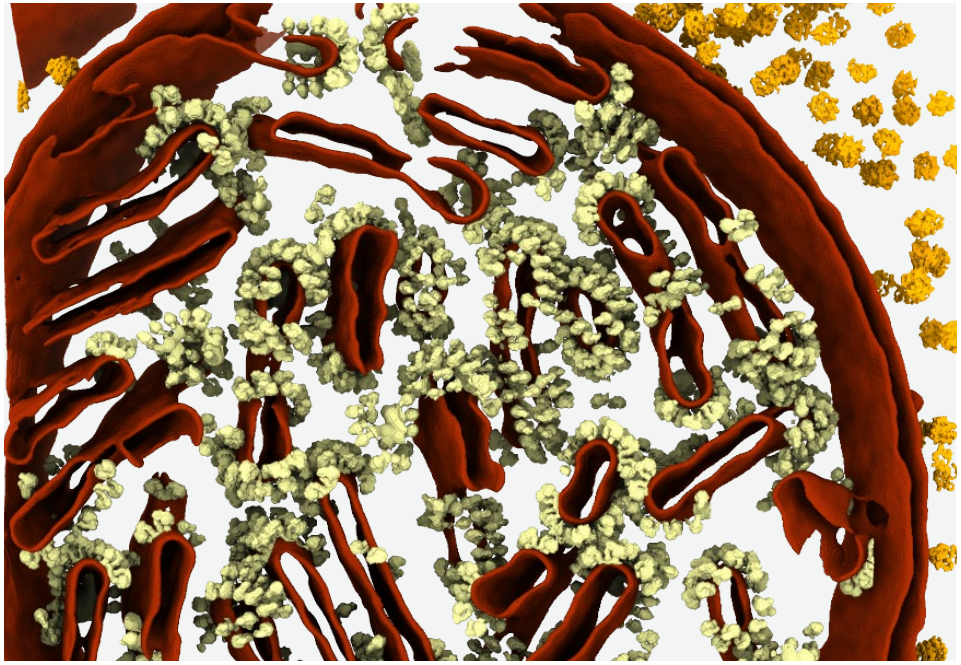
Mouse brain sample recorded with Maps Software for array tomography on the Thermo Scientific™ Apreo 2 SEM. The reconstruction shows mitochondria, the endoplasmic reticulum, telo-lysosomes, and the nucleus in the cell body, along with an apical dendrite of a neuron. Reconstruction consists of 50 mosaics of 3 x 3 images, 19.97 x 13.31 x 3.5 µm in size.

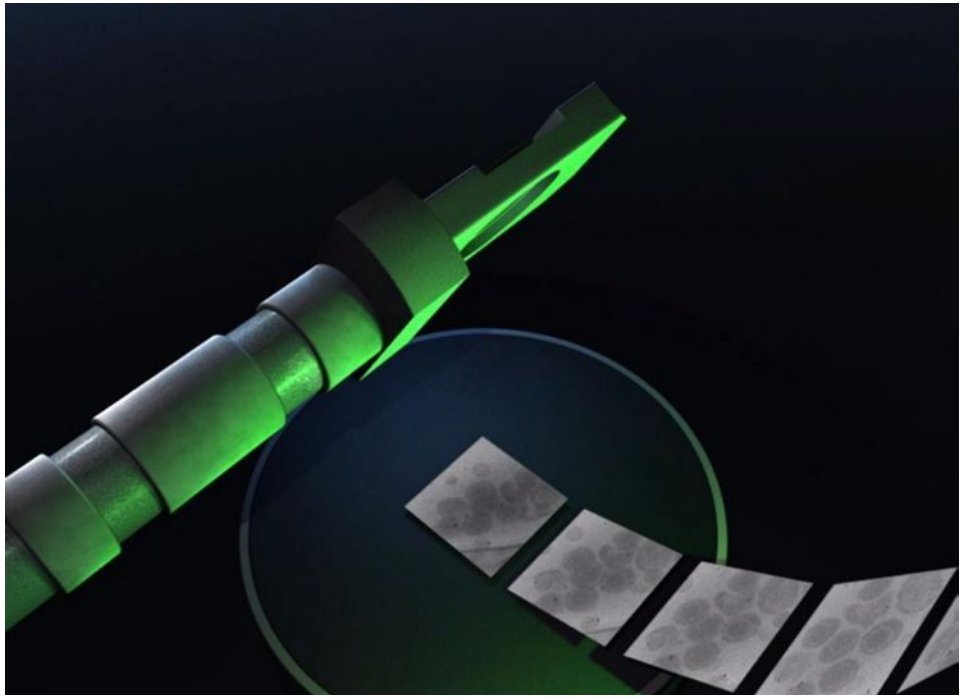
Electron tomography

Tomography with transmission electron microscopy (TEM) collects 2D images of electron-thin samples at a range of different angles (i.e., a tilt series). The results are recombined into a 3D reconstruction of the sample. Cryo-electron tomography (cryo-ET) is a label-free cryogenic variant that provides similar 3D data at nanometer resolution while preserving the sample's physiological context.

What is it used for?

Electron tomography is an ideal solution for exploring subcellular organelles, proteins, biomolecular complexes, and structures within the context of the cellular environment. It delivers sub-nanometer resolution in lamellae prepared typically to 100 to 300 nm in thickness.





How cryo-electron tomography captures a volume

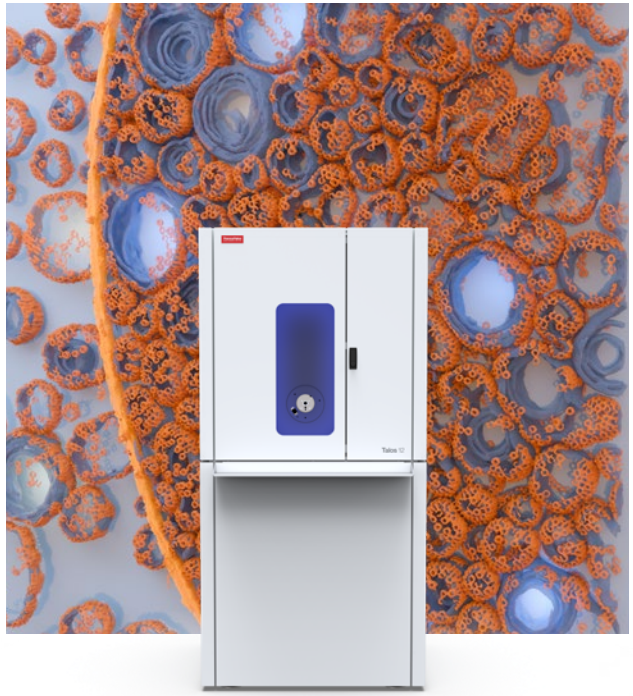
Watch the animation

Duration 1:13

Mitochondron from *Chlamydomonas reinhardtii* imaged with a Krios Cryo-TEM using cryo-ET and segmented using Amira Software.

Thermo Scientific tomography instruments

Volume EM with biomolecular resolution

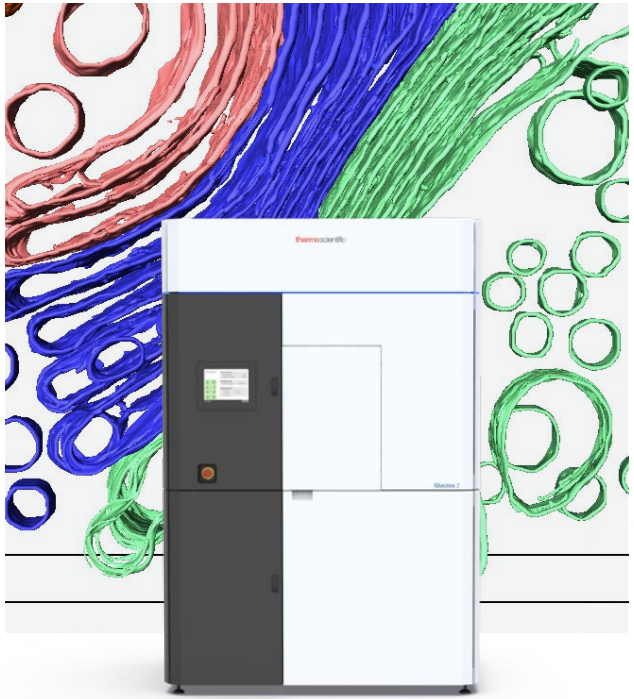


Talos 12 TEM

Versatile 120 kV TEM with cryogenic capability

The Thermo Scientific™ Talos™ 12 TEM offers several low-dose techniques that produce high-quality images even for beam-sensitive specimens. The optional cryo-box facilitates imaging of cryogenically preserved samples, useful for sample screening. It also supports tomography acquisitions for many different samples.

Image: Reconstructed 3D tomogram of cryo-preserved FraC proteoliposomes imaged with the Talos 12 TEM. Fine details of actinoporins within the membrane environment of large unilamelar vesicles (LUVs) are visible.



Glacios 2 Cryo-TEM

200 kV system for wide-ranging biological specimens

The Thermo Scientific™ Glacios™ 2 Cryo-Transmission Electron Microscope (Cryo-TEM) allows you to easily collect near-atomic data from a broad range of biological targets and specimens.

Image: 3D visualization of a Golgi apparatus from the green alga *Chlamydomonas reinhardti*. The sample was prepared with the Thermo Scientific™ Aquilos™ Cryo-FIB and imaged using the Glacios Cryo-TEM. Data segmentation and visualization performed with Amira Software.

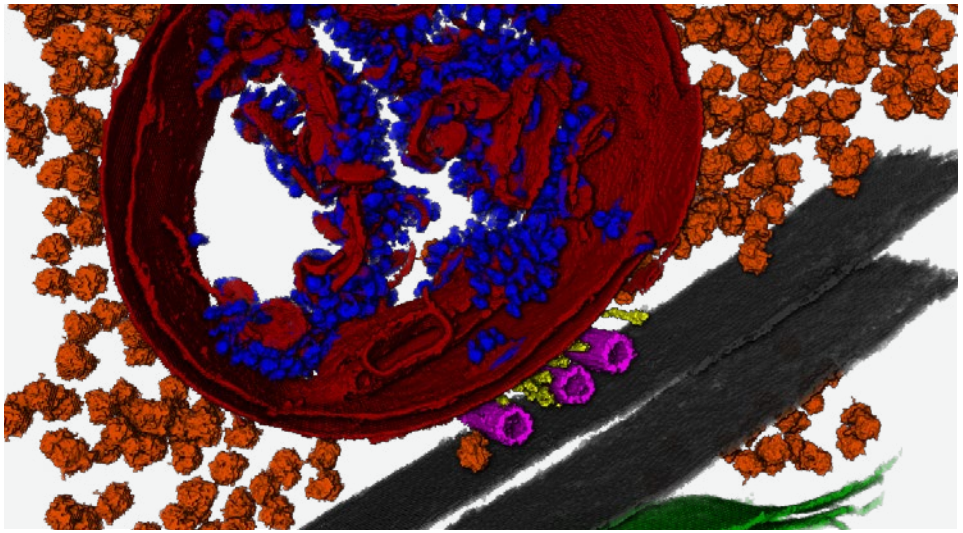
Krios 5 Cryo-TEM

300 kV system for high-resolution, automated acquisitions, even in thick lamellae

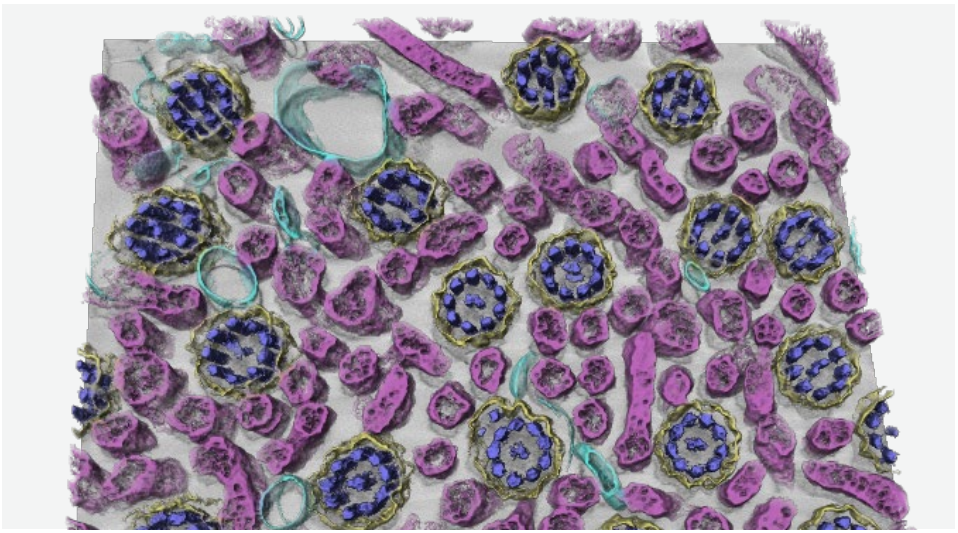
The Thermo Scientific™ Krios™ 5 Cryo-TEM was designed for 3D visualization and localization of proteins and molecular machines. It captures their dynamics within the architecture of the cell, helping you obtain critical biological insights.

Image: Reconstructed tomogram of the excitatory synapse of a hippocampal neuron imaged using the Krios Cryo-TEM. The reconstruction clearly depicts PSD filaments similar to post-synaptic structures, glutamate receptors, and pre-synaptic structures such as vesicles or adhesion proteins. Courtesy of Guoqiang Bi and Hong Zhou of UCLA.

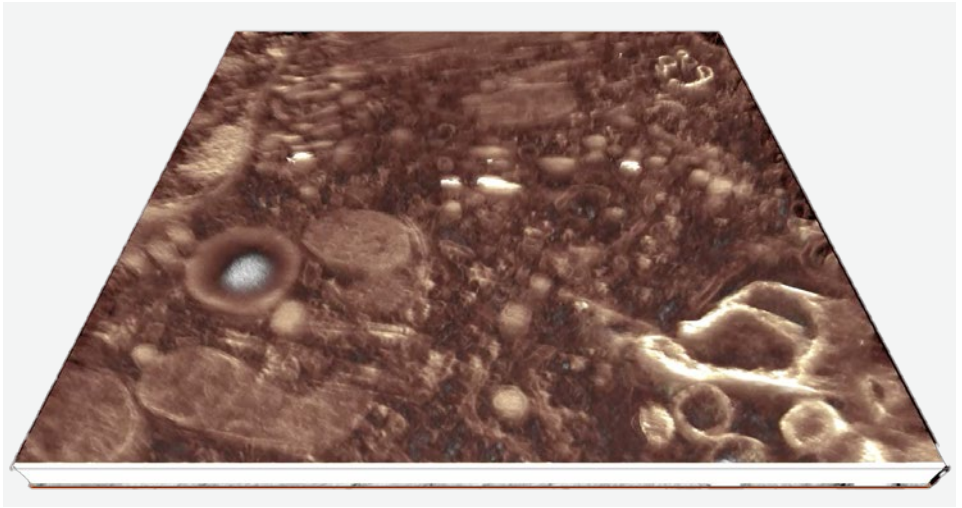
Thermo Scientific tomography instruments



3D reconstruction of a cryo-electron tomogram of *Chlamydomonas reinhardtii* showing mitochondrial membranes (red), ribosomes (orange), ATP synthase (dark blue), nuclear envelope (light blue), plasma membrane (gray), microtubules (pink), filaments (yellow), and thylakoid membrane (green).



Reconstructed 3D tomogram of a resin-embedded section of lung cilia imaged with the Talos 12 TEM.



Reconstructed 3D tomogram of a 200 nm resin section of a macrophage with density segmentation performed in Amira Software.

Dr. Danielle Grotjahn on how cryo-ET is advancing our understanding of cellular biology

Duration 2:54

"Cryo-tomography really shines because it's one of the few techniques that allows us to not only look at the details of the individual parts but also all of them holistically together."

-Dr. Danielle Grotjahn,
Department of Integrative Structural and Computational Biology,
Scripps Research Institute

New to volume EM?

Volume EM's rise in popularity correlates with improved automation, enhanced technical implementation, integrated software workflows and advanced computing resources for 3D reconstruction. As researchers increasingly ask multi-scale questions that connect molecular details to whole-cell or tissue-level phenomena, volume EM provides a powerful toolkit for studying biological samples. Coupled with better data handling as well as segmentation and analysis pipelines, volume EM is no longer a niche technique. It's becoming a standard approach in leading imaging facilities worldwide.

How can I get started with volume EM?

At Thermo Fisher Scientific, we aim to make volume EM attainable for more scientists and facilities. Our portfolio of instruments and software at different price points can help you get started regardless of budget or previous experience.

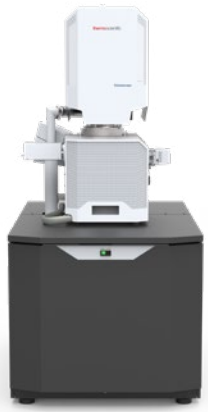
Our instruments are designed with an eye toward ease of use, flexibility, and low cost of ownership. We also offer comprehensive training and onboarding programs, dedicated customer success managers, and site preparation services. No matter your work, we're here to help you succeed.

A suite of volume EM tools

Hydra Bio Plasma-FIB

Premium volume EM instrument for resin- and cryo-preserved samples

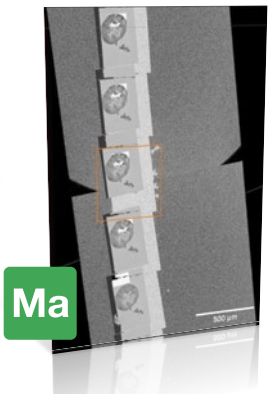
Learn more



Volumescope 2 SEM

Stable companion for long-term acquisition of resin block volumes

Learn more



Maps Software

Streamlined workflow for fast and easy capture of thousands of array tomography sections

Learn more

Talos 12 TEM

Versatile 120 kV TEM with cryogenic capability

Learn more

Glacios 2 Cryo-TEM

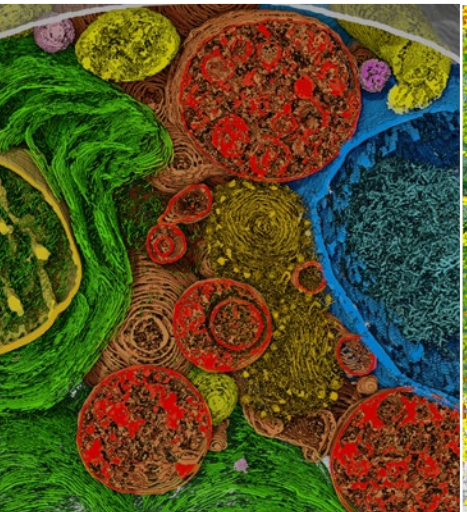
200 kV system for wide-ranging biological specimens

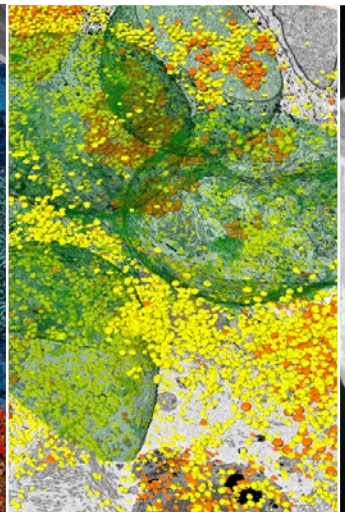
Learn more

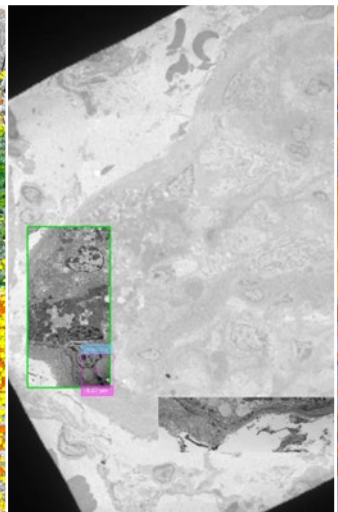
Krios 5 Cryo-TEM

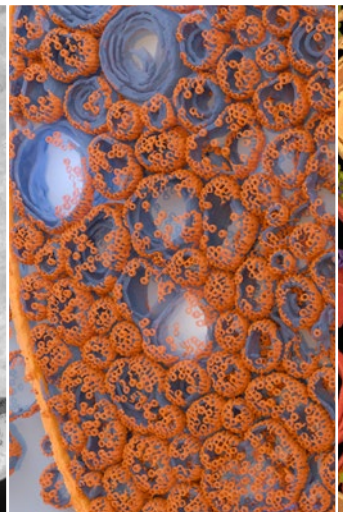
300 kV system for high-resolution, automated acquisitions, even in thick lamellae

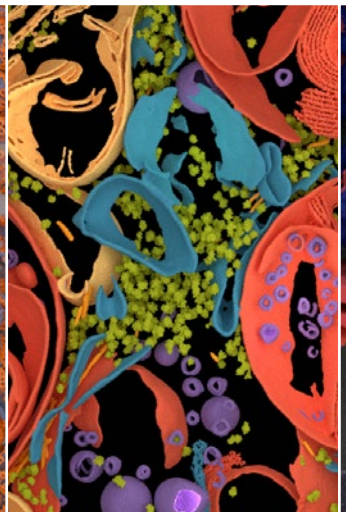
Learn more

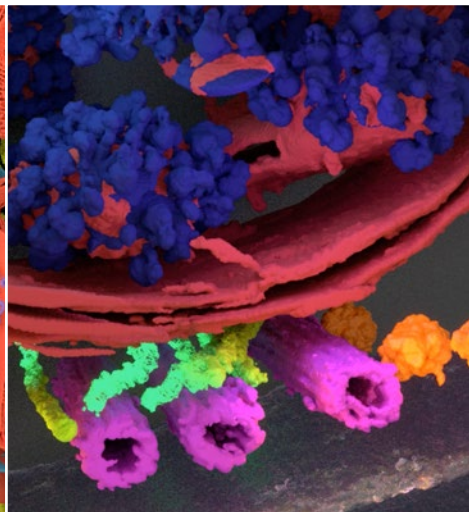


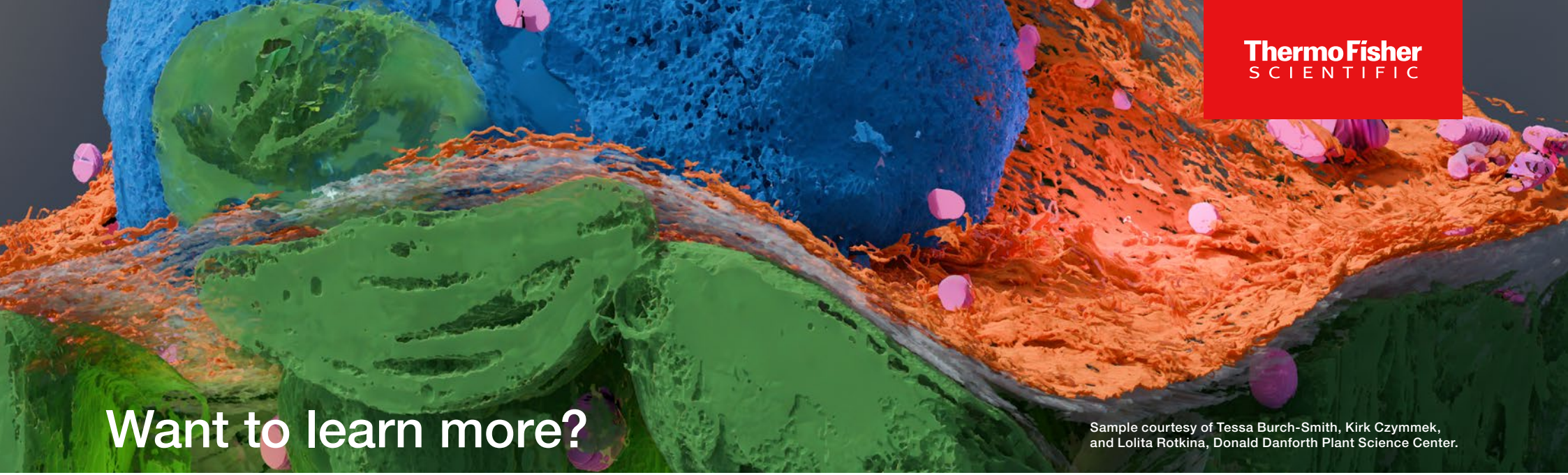












Want to learn more?

Sample courtesy of Tessa Burch-Smith, Kirk Czymmek, and Lolita Rotkina, Donald Danforth Plant Science Center.

Whether you are a beginner looking to understand the basics or an experienced researcher seeking advanced techniques and community support, these resources will help you expand your knowledge and enhance your skills in volume EM.

Volume EM webpage

Discover detailed information about volume EM techniques, practical use cases, and the advanced technologies utilized in the field. Our website provides a wealth of knowledge on the applications and benefits of volume EM to help you deepen your understanding and stay up to date on the latest technology advancements.

Learn more

Volume EM primer

Gain a comprehensive understanding of volume EM techniques, sample preparation, and image generation. This primer offers an in-depth overview of all aspects of volume EM, making it a great starting point for those new to the field as well as a valuable reference for seasoned researchers.

Read the article

Volume EM community

Join the vibrant volume EM community and connect with experts and enthusiasts on the associated Slack channel. This community is a great place to share experiences, learn best practices, and stay updated on the latest developments in the field. Engaging with the community can provide valuable insights and support as you navigate your volume EM journey.

Join the community

Learn more at **thermofisher.com/VolumeEM**

thermo scientific