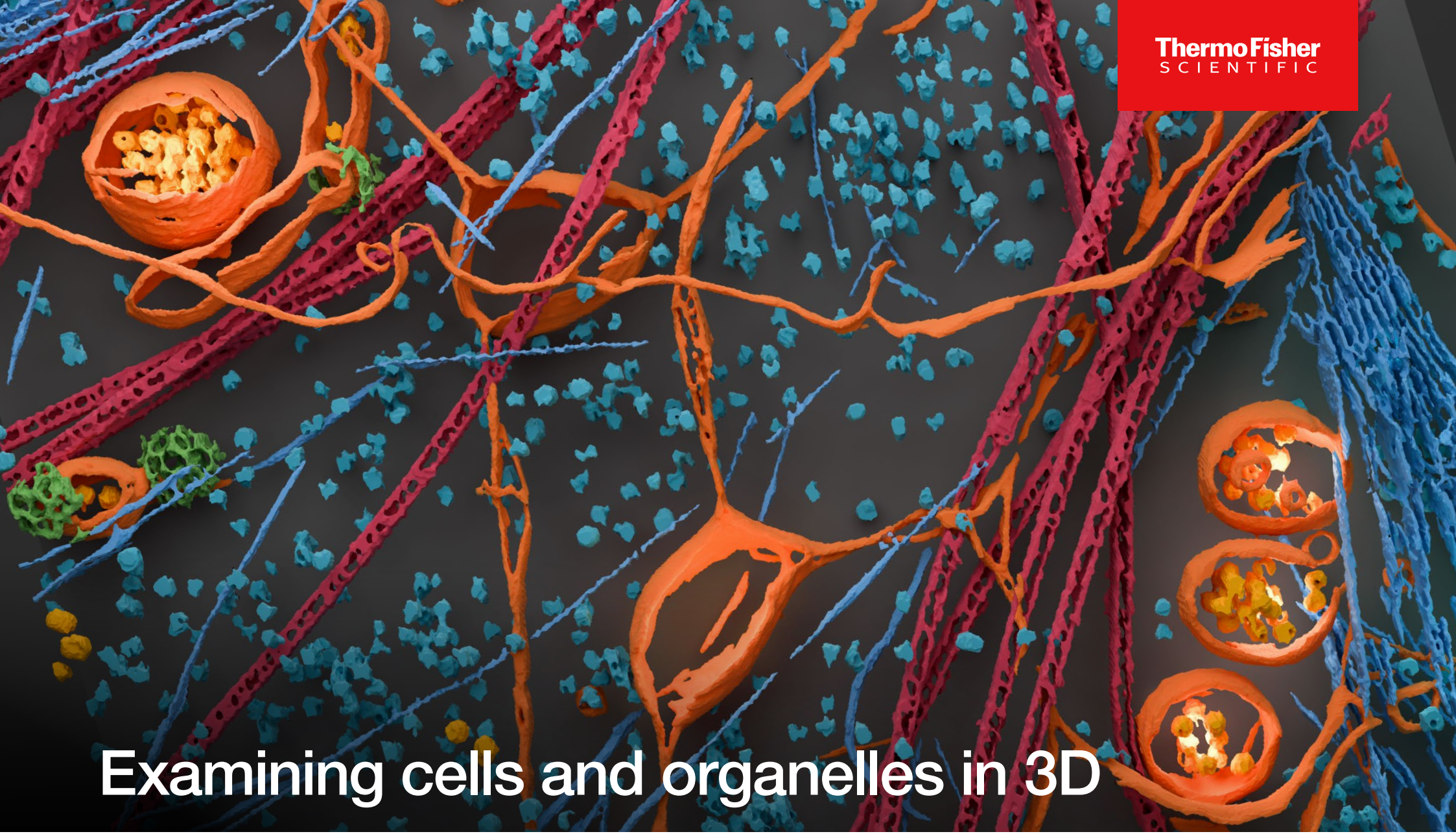


ThermoFisher SCIENTIFIC

Examining cells and organelles in 3D

With the high resolution of cryo-electron tomography

thermo scientific

The importance of cellular context

Cell biologists have long relied on a range of analytical techniques to investigate the inner workings of cells, including fluorescence microscopy for dynamic processes, X-ray crystallography and single particle analysis for molecular structures, as well as electron microscopy imaging for further structural information. Despite continuous improvements in these techniques, it is still challenging to bridge the gap between molecular details and the whole-cell context.

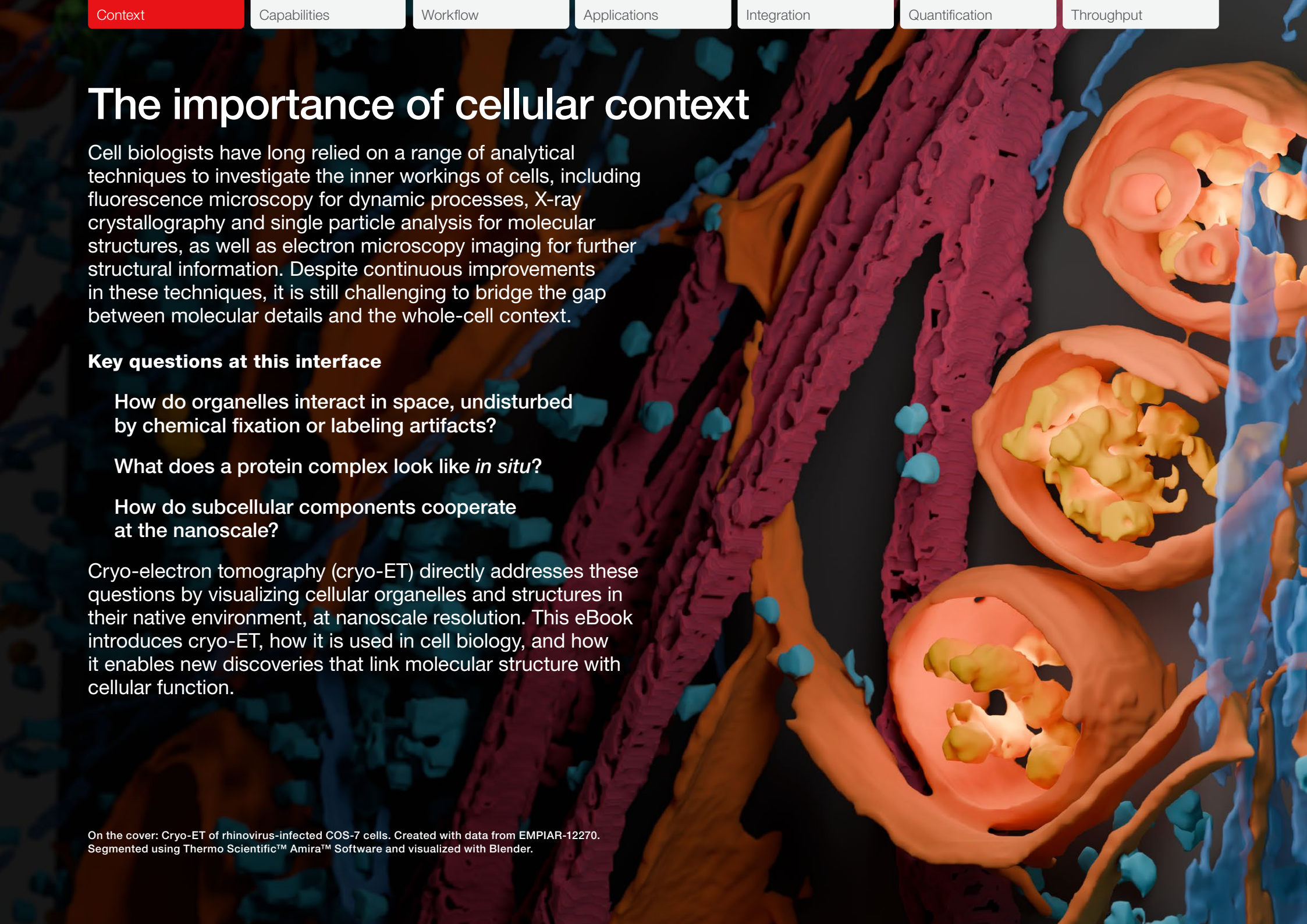
Key questions at this interface

How do organelles interact in space, undisturbed by chemical fixation or labeling artifacts?

What does a protein complex look like *in situ*?

How do subcellular components cooperate at the nanoscale?

Cryo-electron tomography (cryo-ET) directly addresses these questions by visualizing cellular organelles and structures in their native environment, at nanoscale resolution. This eBook introduces cryo-ET, how it is used in cell biology, and how it enables new discoveries that link molecular structure with cellular function.



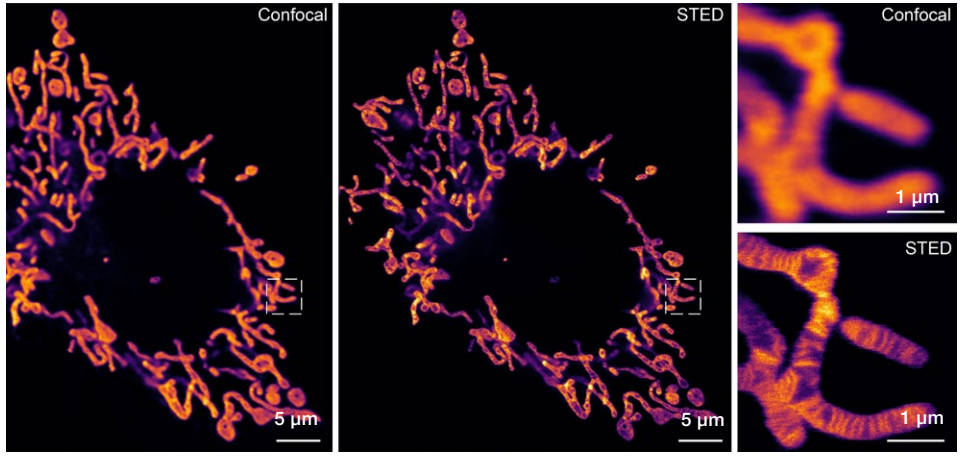
On the cover: Cryo-ET of rhinovirus-infected COS-7 cells. Created with data from EMPIAR-12270. Segmented using Thermo Scientific™ Amira™ Software and visualized with Blender.

The cryo-ET revolution in cell biology: from detail to discovery

Cryo-ET is transforming how we study cell biology by connecting molecular structure to cellular context. This technique captures the biomolecular architecture within intact cells, revealing how molecular assemblies organize and interact in their native environment. By combining high-resolution structural data with preserved cellular context, cryo-ET provides insights that were previously out of reach for other imaging modalities. The example below illustrates how observations from fluorescence and scanning electron microscopy complement those from cryo-ET to build a complete view of mitochondrial structure.

Confocal and super-resolution fluorescence microscopy

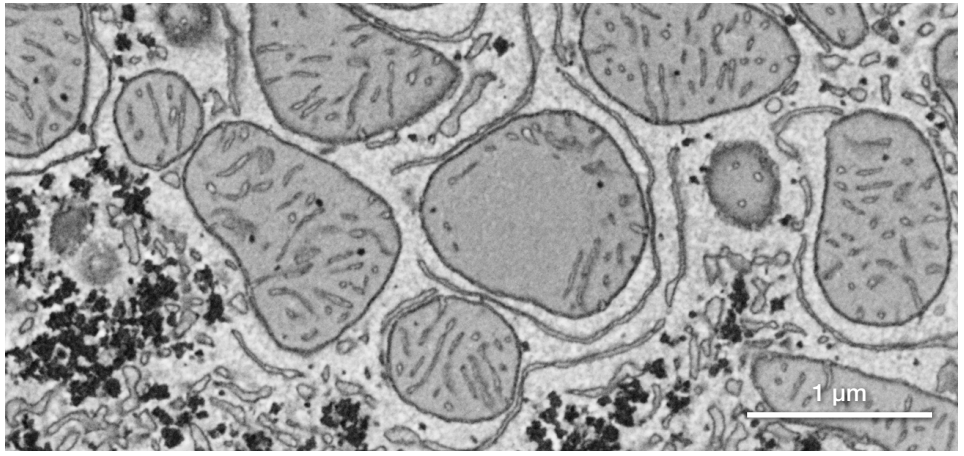
Reveals mitochondrial location and protein distribution through fluorescence labeling, and can track dynamics in living cells, but provides only limited structural information about internal membranes.



Mitochondria of a living COS-7 cell, labeled with HBmito Crimson and imaged using confocal and STED fluorescence microscopy. Figure reproduced from Ge *et al.* under CC BY 4.0.

Scanning electron microscopy

Provides high-resolution images of mitochondrial surfaces and membrane topology. The addition of chemical fixation and heavy-metal staining, however, risks altering native structures. Additionally, SEM cannot reveal internal molecular detail.

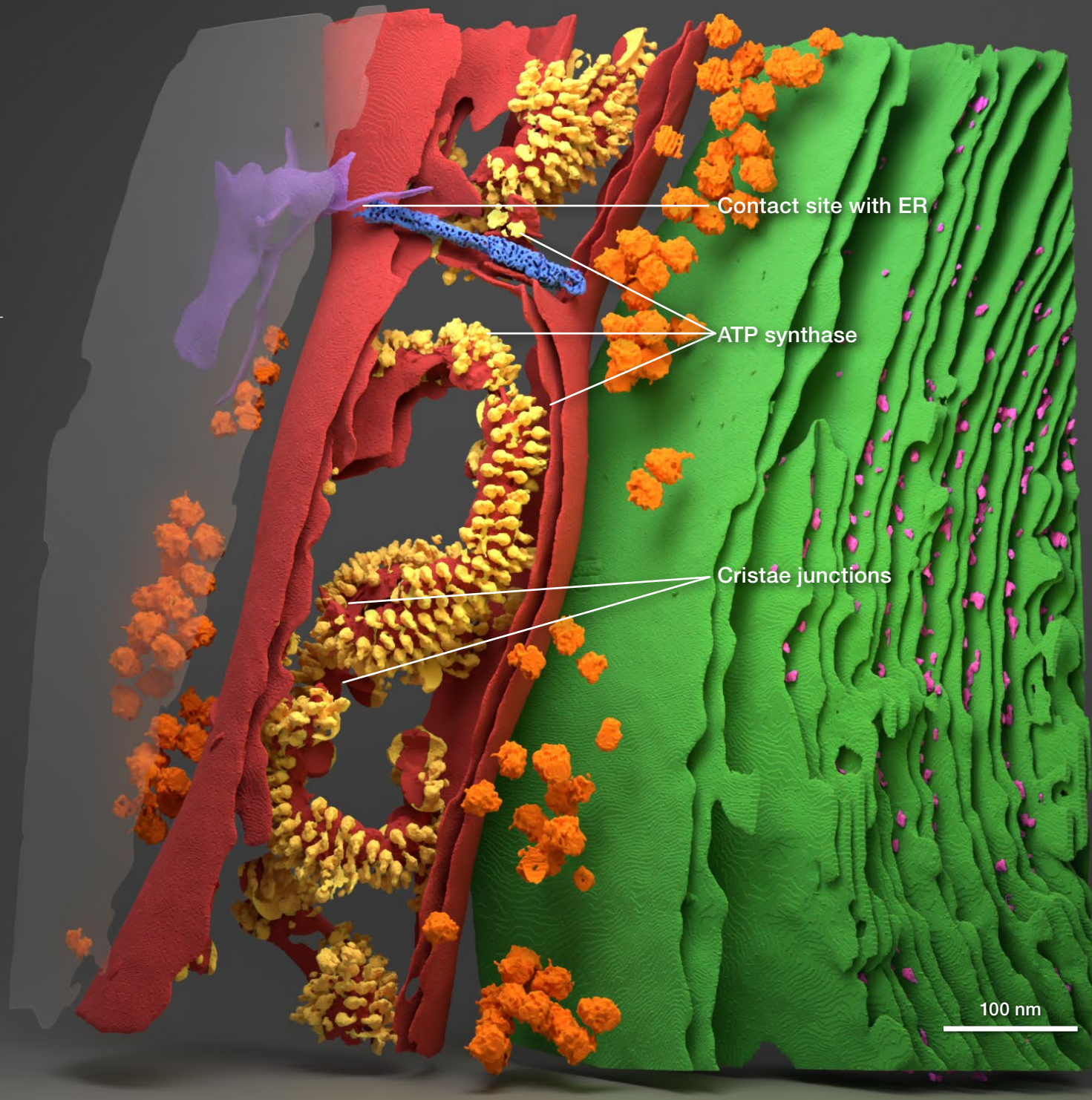


SEM imaging of mitochondria in brain tissue, obtained with the Thermo Scientific™ Apreo™ 2 SEM. The sample was fixed, embedded, and stained with heavy metal for improved contrast.

Cryo-ET

Visualizes mitochondria in a vitrified, near-native state, revealing cristae junctions, respiratory chain proteins such as ATP synthase, and contacts with the ER, *in situ* and in 3D.

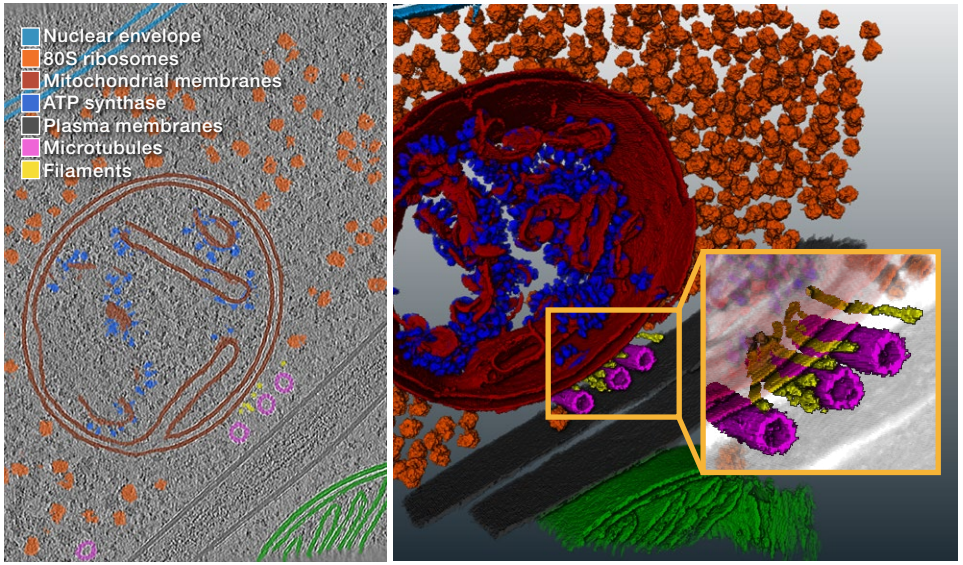
At such length scales, cryo-ET clearly bridges the gap between structural and cellular biology.



Cryo-ET image of *Chlamydomonas reinhardtii,* segmented and rendered in 3D to show mitochondria (red), a fission site containing a filamentous structure (blue), and the endoplasmic reticulum (purple). Figure adapted from Kelley *et al.* under CC BY 4.0.

Novel insights

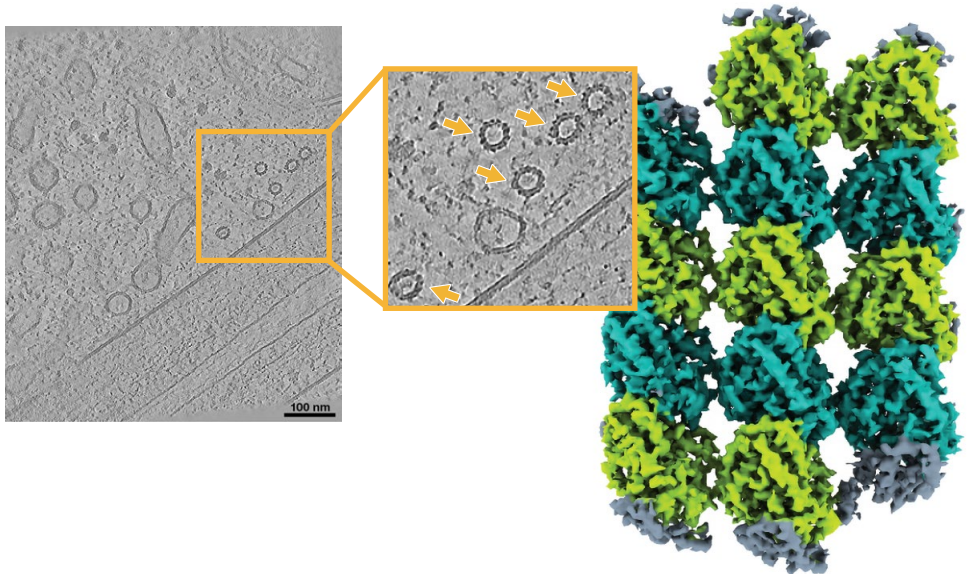
Cryo-ET is expanding what cell biologists can see, revealing new organelles, membrane contact sites, and macromolecular assemblies that were previously hidden in traditional imaging. Observing these features in their native environment provides direct insight into how cells are organized and how their internal structures change during different biological processes.



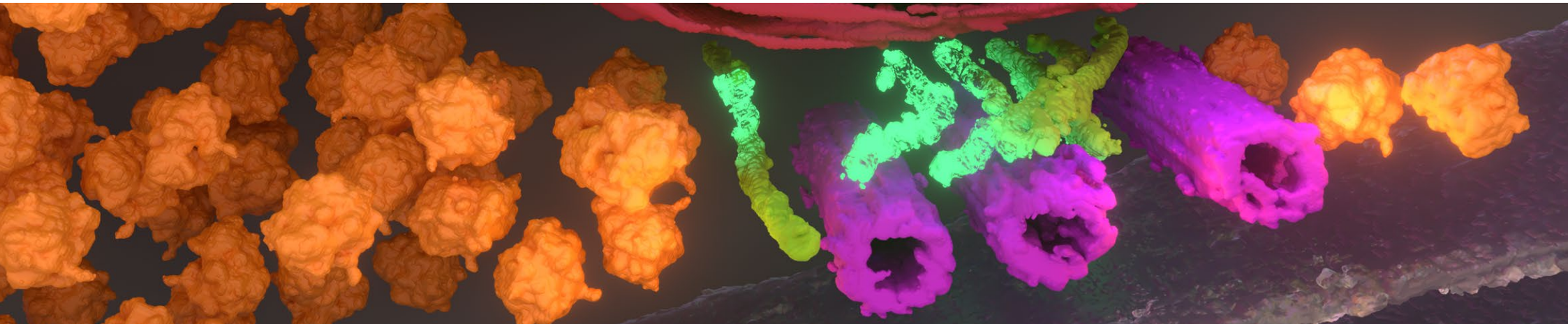
Cryo-ET of *Chlamydomonas reinhardtii*. Left) Example tomogram, annotated for AI segmentation. Right) 3D segmentation using Amira Software showing microtubules (pink) tethered to the mitochondrion via filaments of unknown function (yellow). Figure adapted from Kelley *et al.* under CC BY 4.0.

Previously hidden details

Cryo-ET makes it possible to examine familiar cellular structures, such as microtubules, membranes, and protein complexes, in fine detail. These observations reveal how molecules are arranged within organelles and how this organization relates to cell function. Additionally, improvements in image processing and 3D reconstruction continue to enhance what can be learned from individual tomograms.

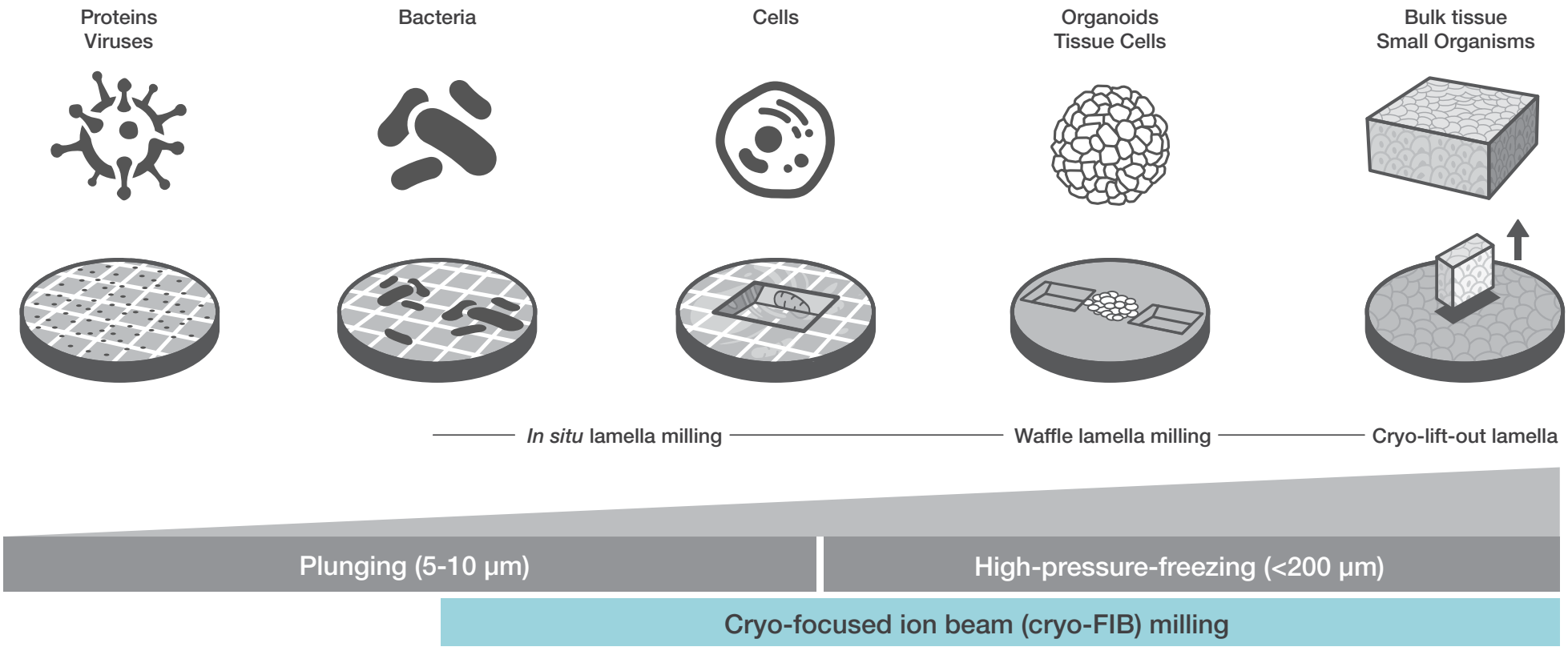


3D reconstruction of *Chlamydomonas reinhardtii*, created from a single sample lamella. Arrows indicate the 2D outline of microtubules. The tomography library generated by Kelley *et al.* can be used to resolve key structures down to biomolecular resolution, including microtubules (right).



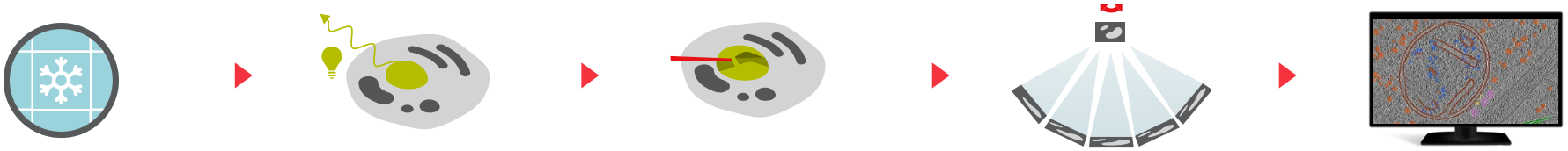
What is cryo-ET and how does it work?

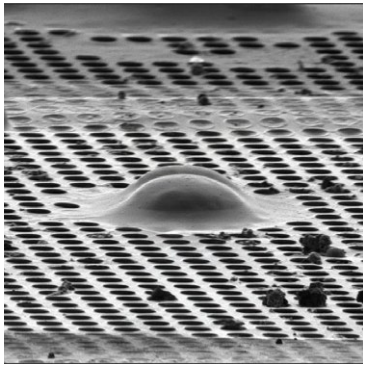
Cryo-ET can visualize the 3D architecture of cells, organelles, and macromolecular structures at sub-nanometer resolution from samples that are in a near-native, frozen-hydrated state, without the need for staining or labeling. Specimens as varied as bacteria, cells, organoids, bulk tissues, and even small organisms can be explored with this technique.



Cryo-ET sample preparation workflow.

Typical cryo-ET workflow for cell biology

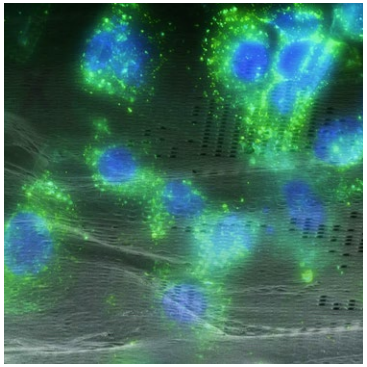




Sample preparation

Samples are vitrified to preserve native structure. Cells and bacteria are plunge-frozen, while thicker specimens are sectioned below 200 µm and high-pressure frozen. Vitrification prevents crystalline ice formation and maintains molecular organization.

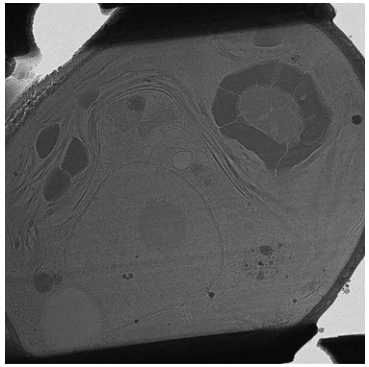
Vitrified cells on a grid, ready for thinning with a FIB-SEM.



Localization by fluorescence

Cryo-fluorescence microscopy identifies regions containing the protein or structure of interest. Integrated fluorescence imaging helps ensure selected areas include the desired target, guiding site selection for preparation and imaging.

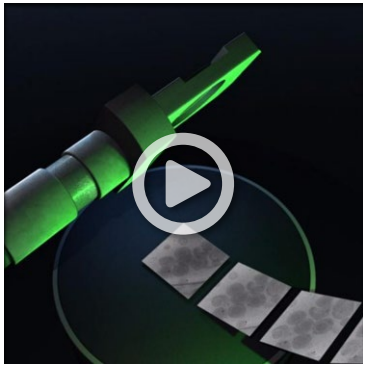
Fluorescence image of cells overlayed with an EM image to enable correlative microscopy.



Sample thinning

Since electrons cannot penetrate thick specimens, samples can be thinned with FIB milling to produce 100–300 nm cryo-lamellae. Lift-out provides an alternative approach to isolate a small sample volume for subsequent lamella preparation.

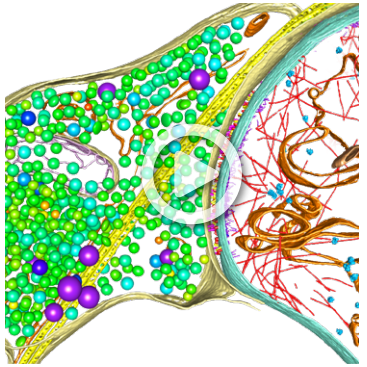
Vitrified lamella ready for cryo-ET imaging, prepared using the Thermo Scientific™ Hydra Bio™ Plasma-FIB.



Imaging and reconstruction

The lamella is transferred into a cryo-transmission electron microscope. A low-dose tilt series of 2D projections is acquired and a detailed 3D tomogram is then computationally reconstructed by back-projection from the 2D images.

Animation illustrating the cryo-ET workflow. Courtesy of the Max Planck Institute of Biochemistry, Department of Molecular Structural Biology.



Visualization and analysis

The final 3D tomogram can be segmented in a variety of ways to enhance its display and/or to allow comparative quantification. Small subsets of data can also be subjected to subtomogram averaging and analyzed further with other image processing methods.

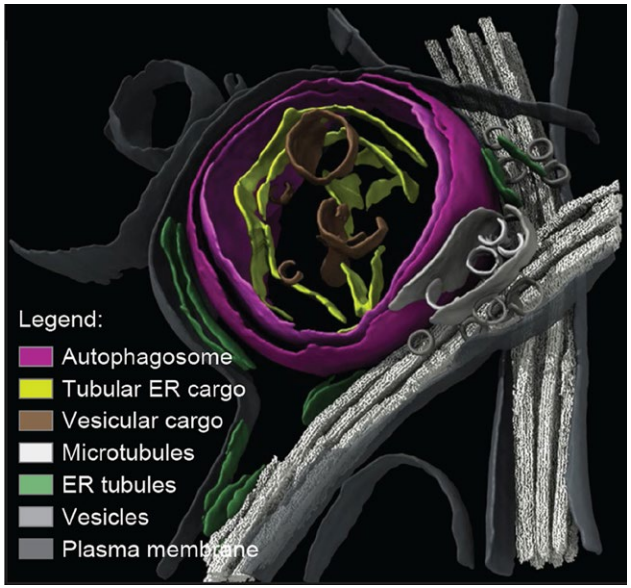
Hippocampal neuron excitatory synapse, imaged with cryo-ET. Segmentation and visualization with Amira Software. Courtesy of Guoqiang Bi and Hong Zhou.

High impact studies: from structures to organelles

Numerous high-impact studies have leveraged the high-resolution cellular insights provided by cryo-ET. These publications highlight growing trends in adoption, driven by increasing access to instrumentation and expertise. Below are just a few examples showcasing how cryo-ET is redefining cell biology.

Endoplasmic reticulum (ER)

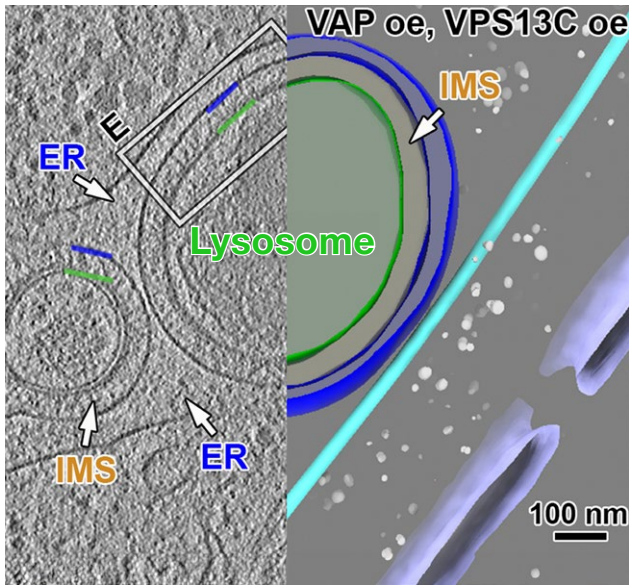
The ER forms a dynamic, interconnected network that supports protein and lipid synthesis, organelle communication, and autophagy. Using cryo-ET, researchers can visualize its fine membrane architecture and capture transient interactions that reveal how the ER reorganizes during cellular remodeling.



Cryo-ET of autophagosomes can visualize the trafficking of ER engulfment receptors and the capture of tubular ER membranes. This provides insights into the dynamic remodeling of the ER during autophagy and enhances our understanding of how cells maintain ER function and quality control under stress conditions. Figure reproduced from Petrovic *et al*. under CC BY 4.0.

Lipid transport

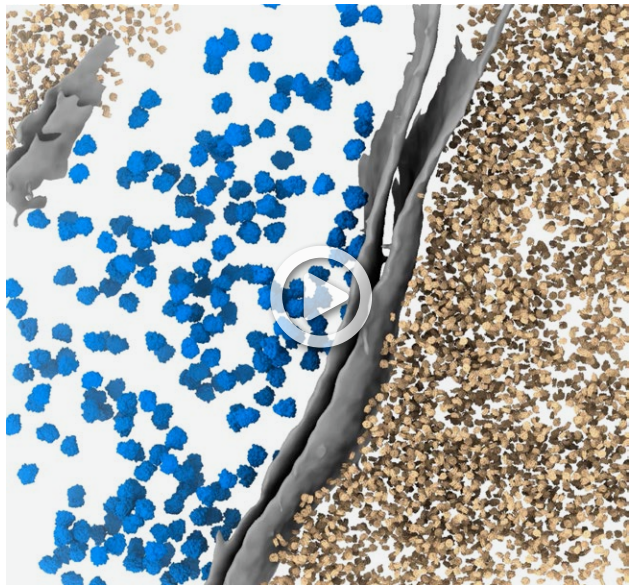
Membrane contact sites are vital for lipid and metabolite exchange between organelles. Cryo-ET visualizes the organization of these junctions in 3D, showing how large transport proteins bridge opposing membranes to coordinate cellular lipid homeostasis.



Visualization of rod-like VPS13C–VAP-B assemblies spanning the ER–lysosome interface demonstrates how lipid transport proteins form physical connections across organelles. The left half is a 2D projection image and the right half shows the 3D reconstruction. IMS = the inner-membrane space between the ER and the lysosome membrane. Figure adapted from Cai *et al.* under CC BY 4.0.

Chromatin organization

Chromatin packing determines how DNA is accessed and regulated within the nucleus. Cryo-ET enables direct visualization of the 3D structure of chromatin fibers in their native environment, revealing the spatial relationships between nucleosomes and higher-order DNA organization within cells.



Cryo-ET of interphase human T-lymphoblasts shows irregular, flexible chromatin fibers rather than uniform 30 nm filaments, challenging long-standing models of nuclear organization. This video is a representative 3D reconstruction taken at the nuclear envelope, where grey = membranes, blue = ribosomes, and gold = nucleosomes. Reproduced from Hou *et al.* under CC BY 4.0.

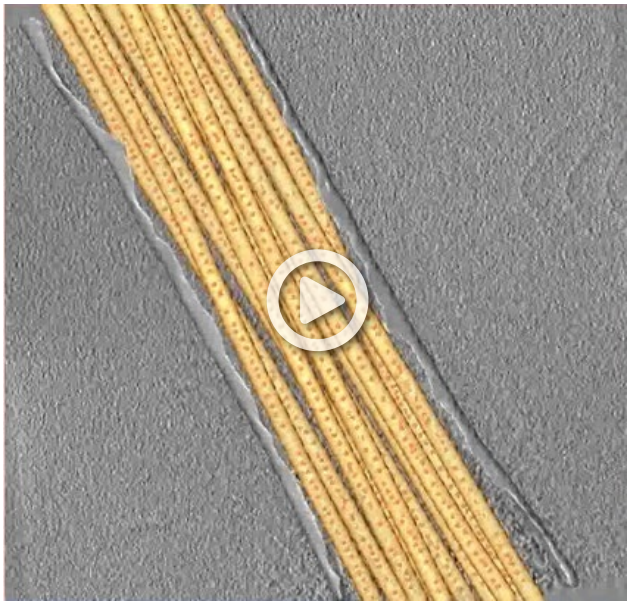
Myosin filaments

The actin–myosin network underpins muscle contraction and force generation. Cryo-ET reveals the molecular arrangement of these filaments *in situ*, linking their organization to mechanisms of contraction, coordination, and muscle regulation within the sarcomere.

3D reconstructions of cardiac sarcomeres maps the interactions between actin, myosin, and MyBP-C, providing insight into how filament organization controls muscle performance. Reproduced from Tamborrini *et al.* under CC BY 4.0.

Microtubules

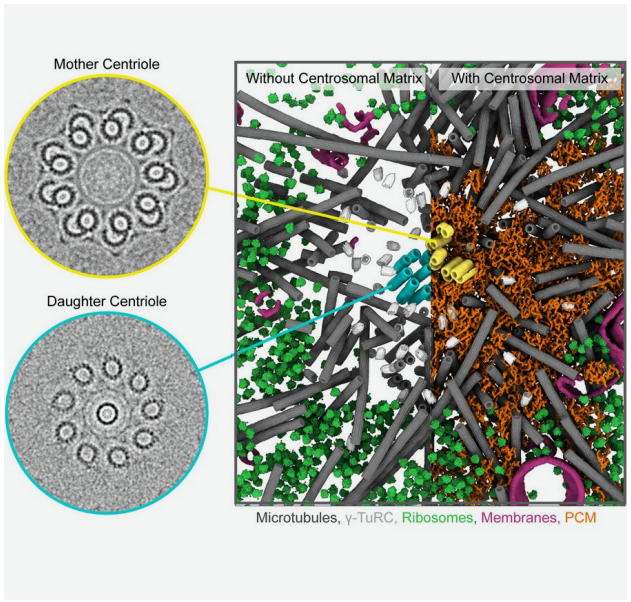
Microtubules form the structural framework of the cytoskeleton, supporting intracellular transport, cell shape, and division. Cryo-ET can visualize microtubules in 3D within cells, revealing their precise organization, interactions with associated proteins, and internal features, such as luminal particles.



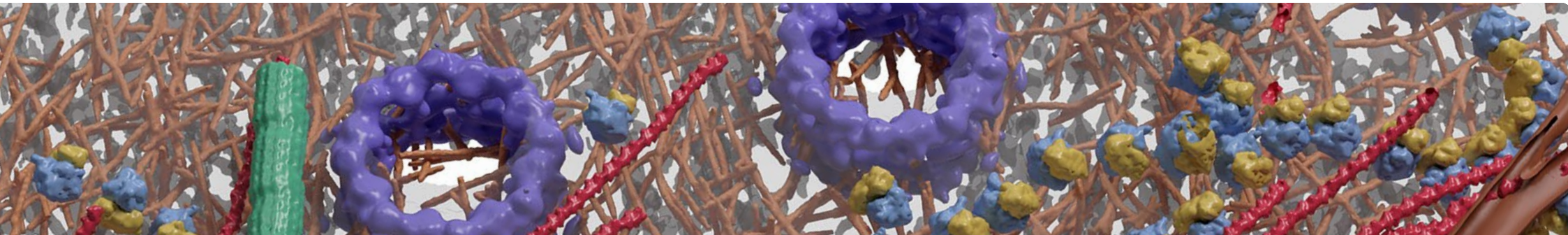
Tomographic slices of neuronal microtubules reveal unexpected structural features, including particles within their lumen, which may play a role in maintaining microtubule stability and quality control. Reproduced from Chakraborty *et al.* under CC BY 4.0.

Centrosome architecture

The centrosome is a conserved organelle that plays a critical role in the organization of microtubules and the formation of mitotic spindles. Cryo-ET provides 3D views of its internal organization and can reveal how centrioles remodel throughout the cell cycle.

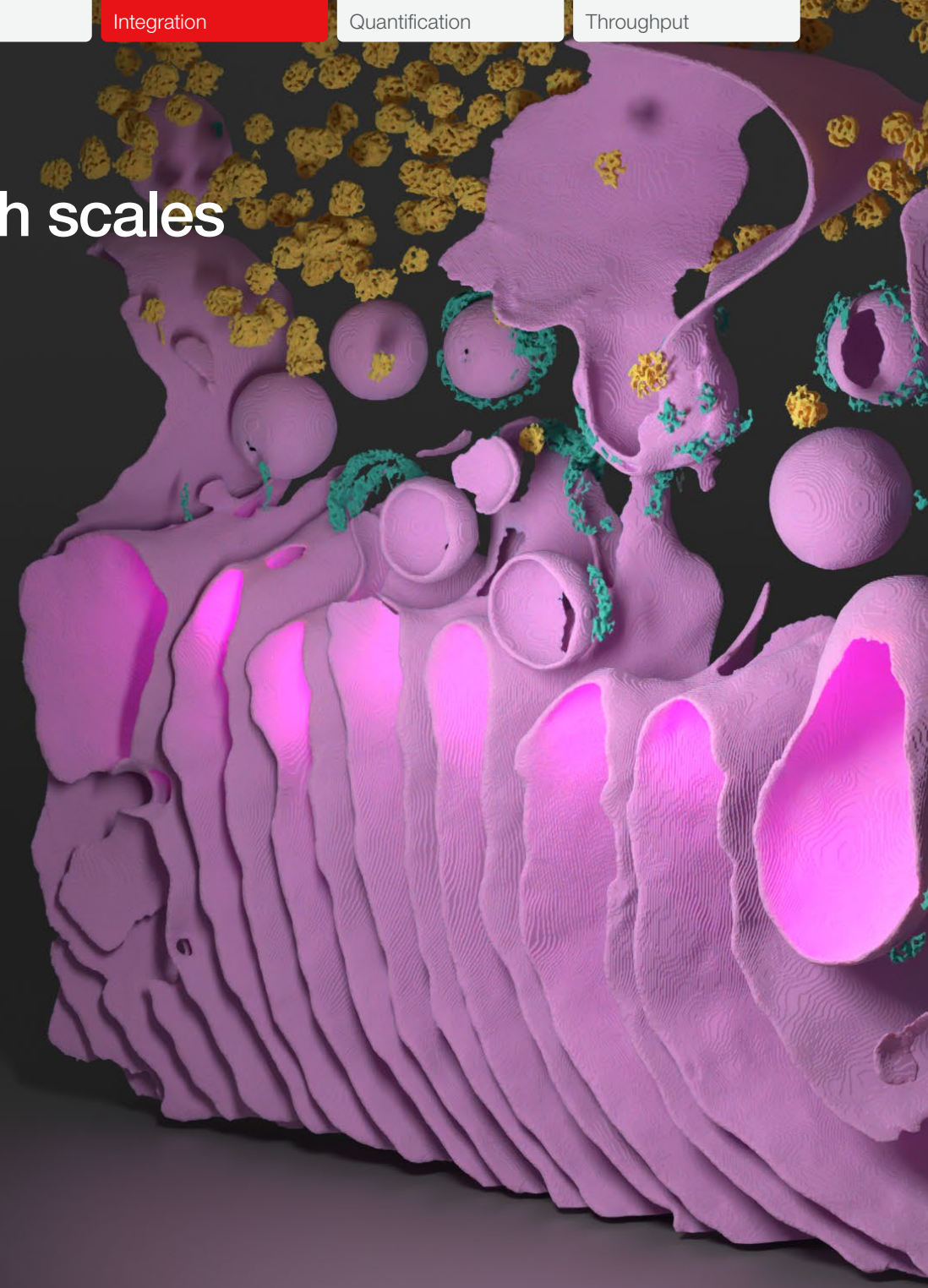


Cryo-ET was used to directly visualize how centrosome structure changes throughout the cell cycle of *C. elegans* embryo cells, offering new insights into how centrosomes assemble and function. Reproduced from Tollervey *et al.* under CC BY 4.0.



Organelles in focus – new perspectives across length scales

Cryo-ET is uniquely suited for the exploration of organelles in their native context due to its high resolution (~3–5 nm) and ability to generate volumetric imaging. It can uncover subtle features and interactions that contribute to cellular function, complementing the insights obtained with other volume electron microscopy techniques, such as FIB-SEM. The same sample type can be imaged with multiple of these 3D methods, providing a rich variety of multiscale insights.



Introduction to FIB-SEM

FIB-SEM is a powerful volume EM technique for high-resolution 3D imaging, combining precise FIB milling and SEM imaging to capture detailed volumetric data of cellular structures. This technique can generate a comprehensive overview of a specimen through large-scale 3D reconstructions, revealing the spatial layout of organelles within the whole cell context.

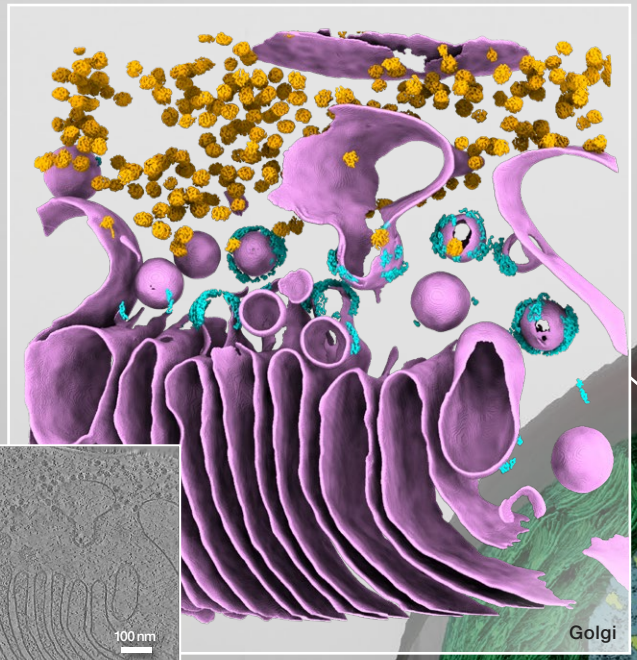
FIB-SEM is particularly useful for studying the interactions and coordination of organelles, offering insights into their function and organization.

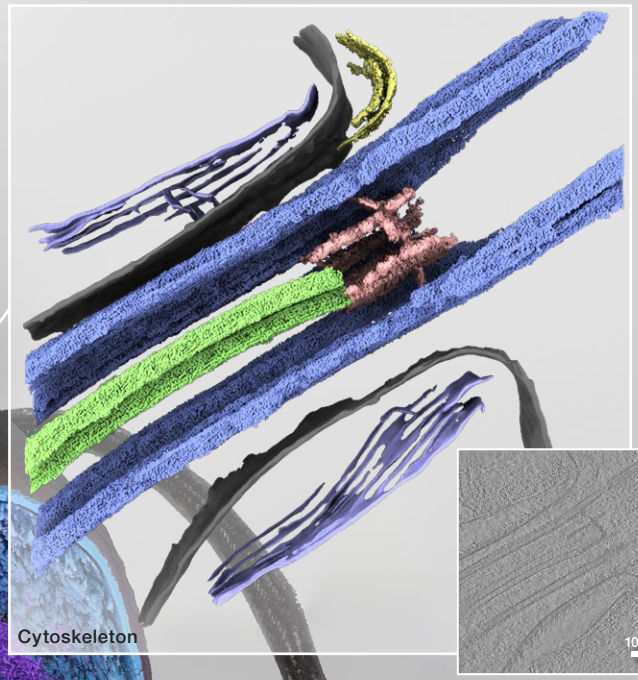
Complementary insights from cryo-ET and FIB-SEM

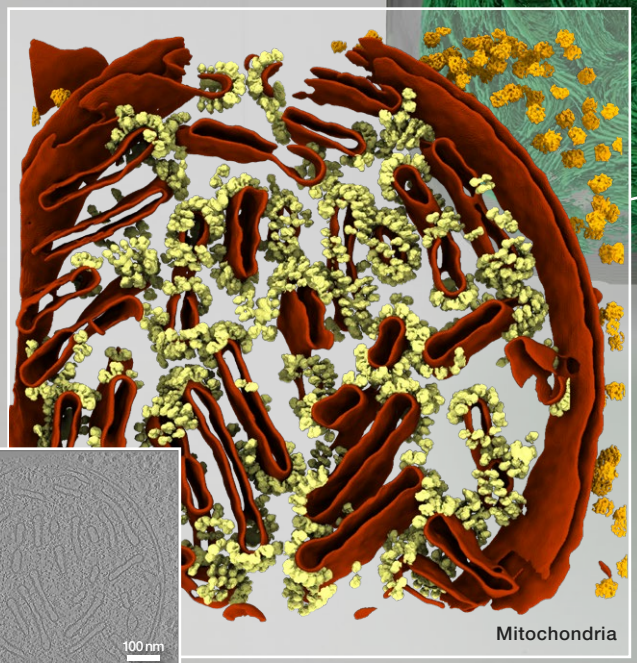
Cryo-ET and FIB-SEM are highly complementary techniques. While cryo-ET excels at high-resolution analysis of organelles and their interactions at the nanoscale, FIB-SEM offers a broader view of the cellular landscape. By combining these methods, researchers can achieve a multiscale understanding of cellular and organelle structure/interaction, from their overall organization down to the intricate, nanoscale details of organelle and cell behavior.

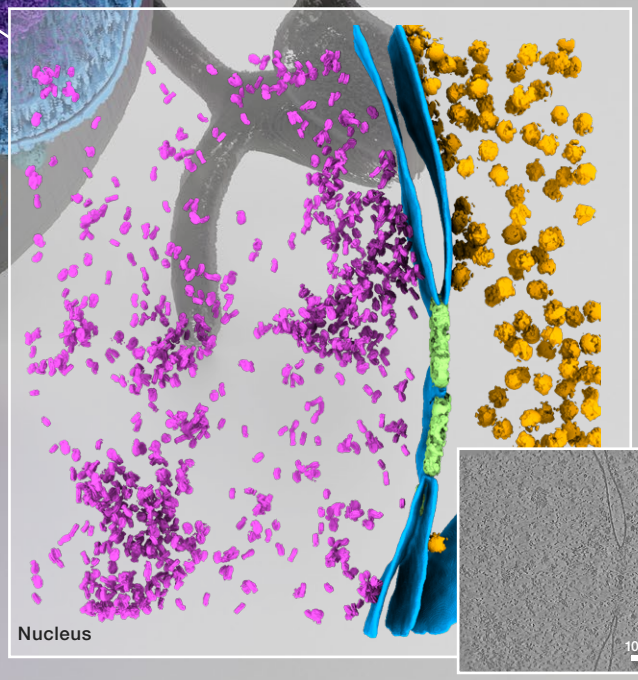
Chlamydomonas reinhardtii visualized with multiple volume EM techniques.

Plasma-FIB was used to capture a complete overview of this cryo-preserved sample; the resulting 3D reconstruction shows an overview of the internal organelles.









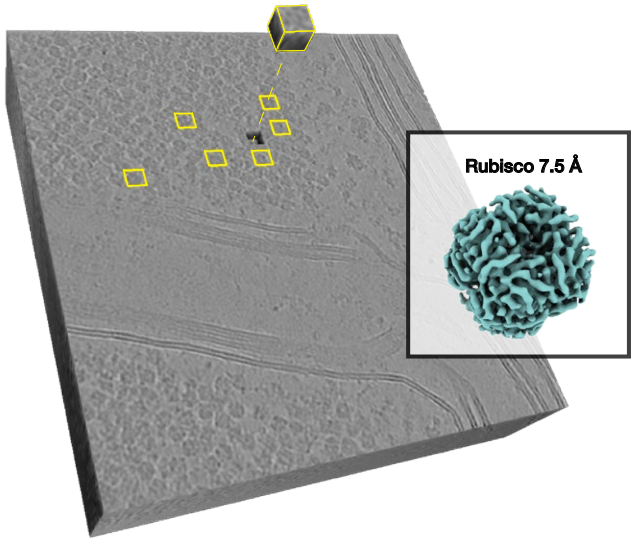
Individual cryo-ET datasets were captured from separate samples. Example 2D projection images, and associated 3D segmentations, are shown for each of the organelles (golgi, cytoskeleton, mitochondrion, and nucleus). Subnanometer details of the cellular constituents and organelles can be seen in each case.

Quantifiable cellular insights from cryo-ET

Cryo-ET data can provide a detailed snapshot of a cell's internal architecture, but this data can also be analyzed further for even greater structural insights.

Sub-tomogram averaging

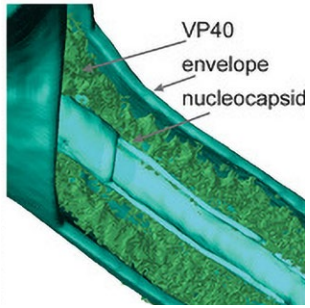
Sub-tomogram averaging can be used to extract and average multiple copies of the same protein or macromolecular complex from different regions of a tomogram. This enhances the signal-to-noise ratio of the data, producing higher resolution structures. This technique is especially powerful when protein-level information is required, as it can reveal detailed structural information about individual proteins and their conformational states.

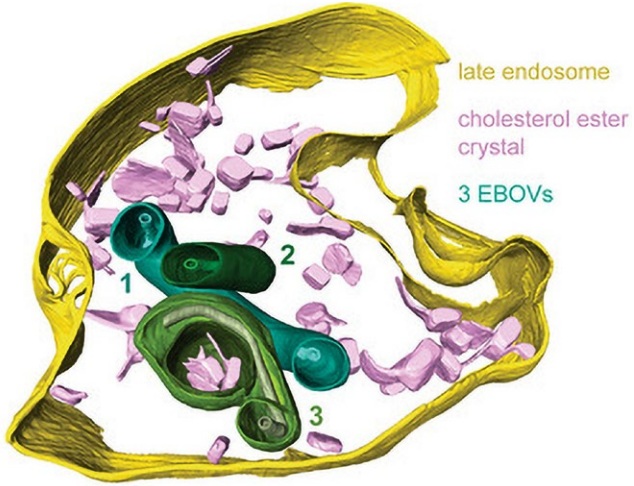


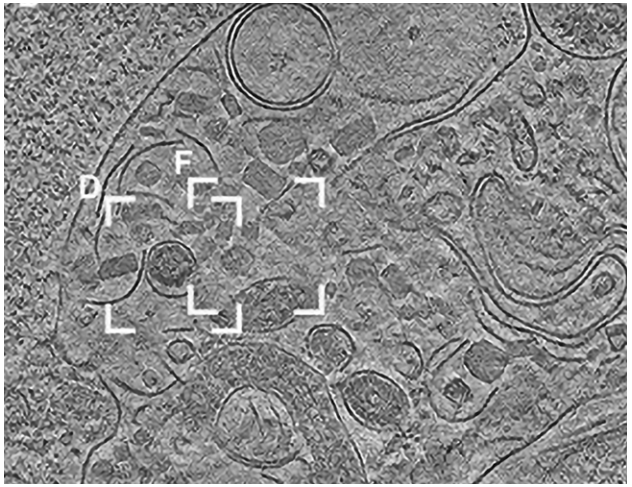
Template-based identification of individual RuBisCo particles in EMD 11830. Particle subtomograms (yellow boxes) were averaged to obtain high-resolution information, including the protein structure of RuBisCo at 7.5 Å resolution.

Segmentation

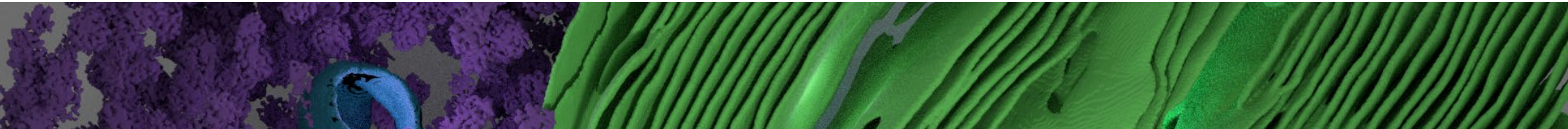
With segmentation, structures of interest are identified and labeled within a tomogram. The raw image data is then transformed into meaningful biological models, distinguishing a range of critical structures and interactions; for instance, viral particles and their host membranes.







Segmentation, performed with Amira Software, clearly distinguishes key components of an Ebola virion and its host membrane. Figure reproduced in part from Winter *et al.* under CC BY 4.0.



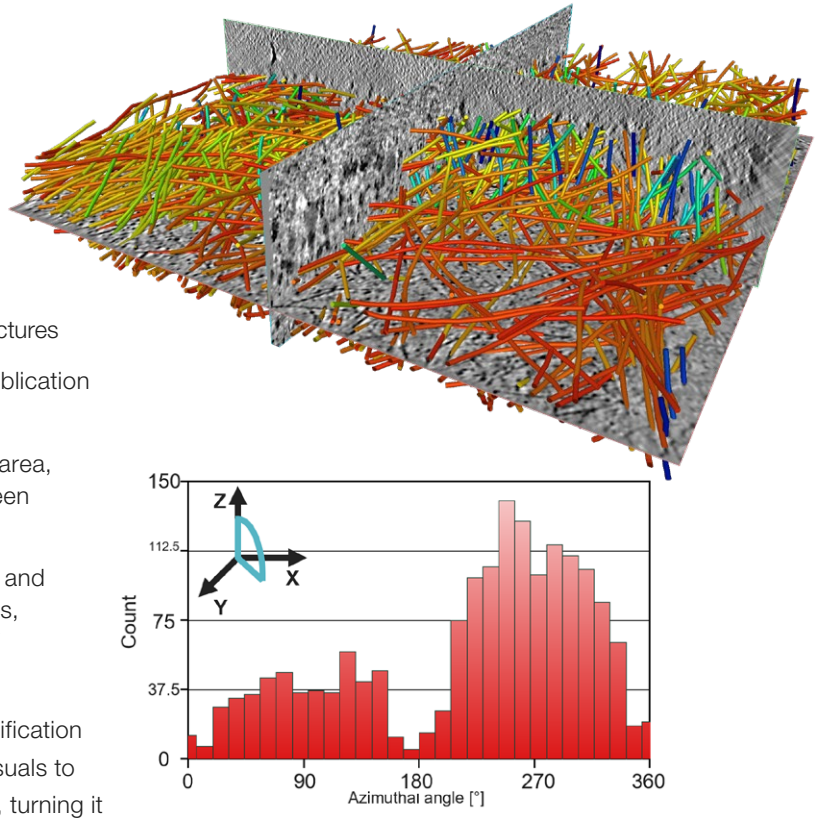
Quantifiable cellular insights from cryo-ET

Quantification

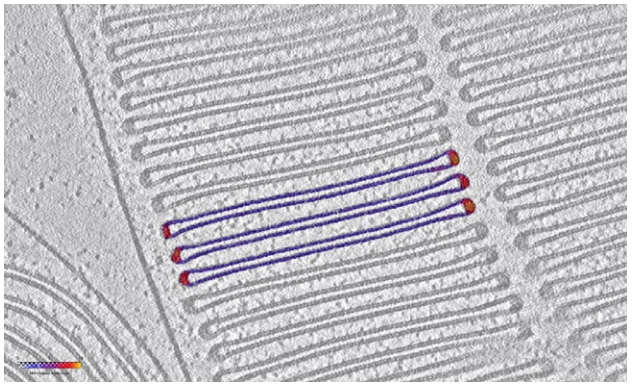
Quantification builds upon segmentation, enabling statistical analysis of features such as filament structure and membrane thickness. This characterization is powered by visualization and analysis software such as Thermo Scientific™ Amira™ Software, which helps researchers;

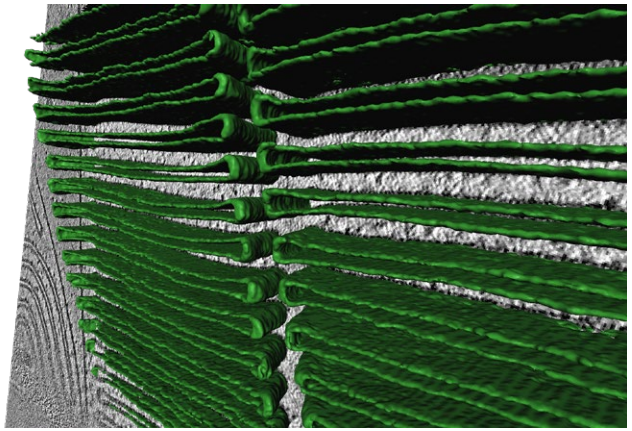
- Interactively segment complex cellular structures
- Create 3D renders and animations for publication or communication
- Quantify spatial metrics such as surface area, shape descriptors, or the distance between organelles
- Perform image denoising, segmentation, and filament analysis with AI-based algorithms, enhancing the accuracy and efficiency of cryo-ET data analysis

The combination of segmentation and quantification takes cryo-ET data beyond its impressive visuals to measurable, reproducible biological insights, turning it into a valuable quantitative tool for modern cell biology.

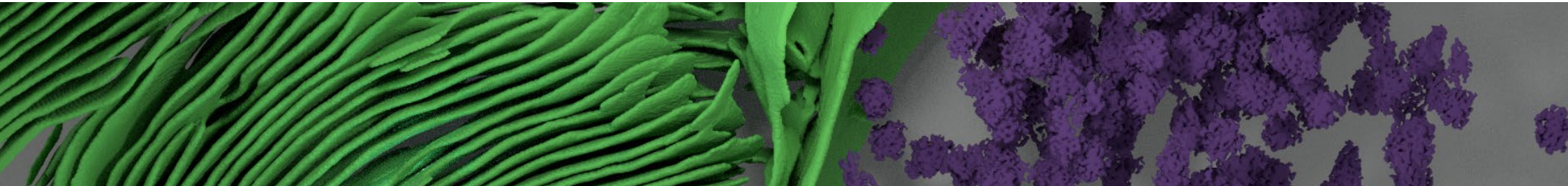


Cryo-ET of a ***D. discoideum*** cell, showing a propagating actin wave; the actin filaments were detected using the fiber analysis module in Amira Software. The direction of the fibers has been computed and is represented with a color code, which is also quantified in the histogram. Data courtesy of Dr. Marion Jasnin, Department of Molecular Structural Biology, Max Planck Institute for Biochemistry, Martinsried, Germany.





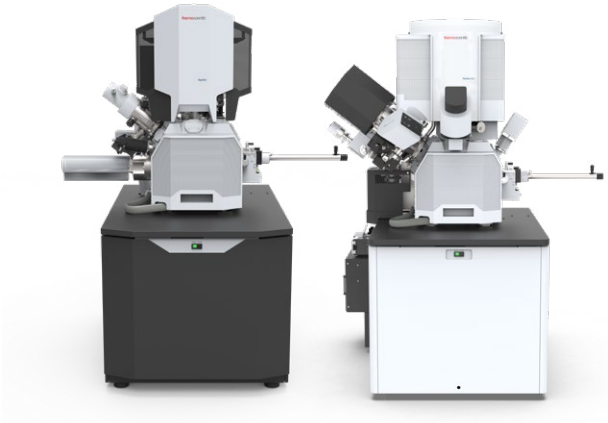
The outer segment of mouse rod photoreceptor cells, imaged under cryogenic conditions with the Krios Cryo-TEM. 3D reconstruction performed using Amira Software with data from EMPIAR-10772. Results originally published by Pöge *et al.*



Scaling cryo-ET experiments for statistically powerful data

Until recently, cryo-ET was limited by throughput, and it was challenging to acquire enough high-quality lamellae and tomograms for statistically meaningful comparisons. This posed a major challenge for cell biology, where biological variability necessitates multiple analyses to reveal consistent patterns and functional insights. Cryo-ET solutions from Thermo Fisher Scientific address this challenge by providing enhanced workflows with high-throughput from sample preparation to data acquisition and analysis.

1. Optimize lamella preparation



Thermo Scientific™ Aquilos™ 2 Cryo-FIB and Hydra Bio Plasma-FIB

Optimize lamella preparation parameters to meet the needs of your experiments.

2. Scale lamella preparation

Arctis Cryo-Plasma-FIB

Scale up sample preparation to reliably generate tens to hundreds of high-quality lamellae from multiple specimens in the same experiment.

3. Transfer clean lamella to TEM

Vacuum Capsule

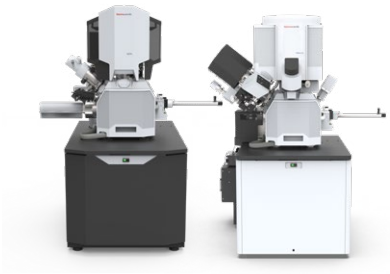
Combined with the Autoloader, the Vacuum Capsule can move high-quality lamellae from the Thermo Scientific™ Arctis™ Cryo-Plasma-FIB to the Thermo Scientific™ Krios™ 5 Cryo-TEM without the risk of ice contamination.

4. Perform high-throughput tomography

Krios 5 Cryo-TEM

Load multiple samples at once and use automated acquisition with a multi-shot workflow to capture and reconstruct tens of tomograms per day.

Scaling cryo-ET experiments for statistically powerful data

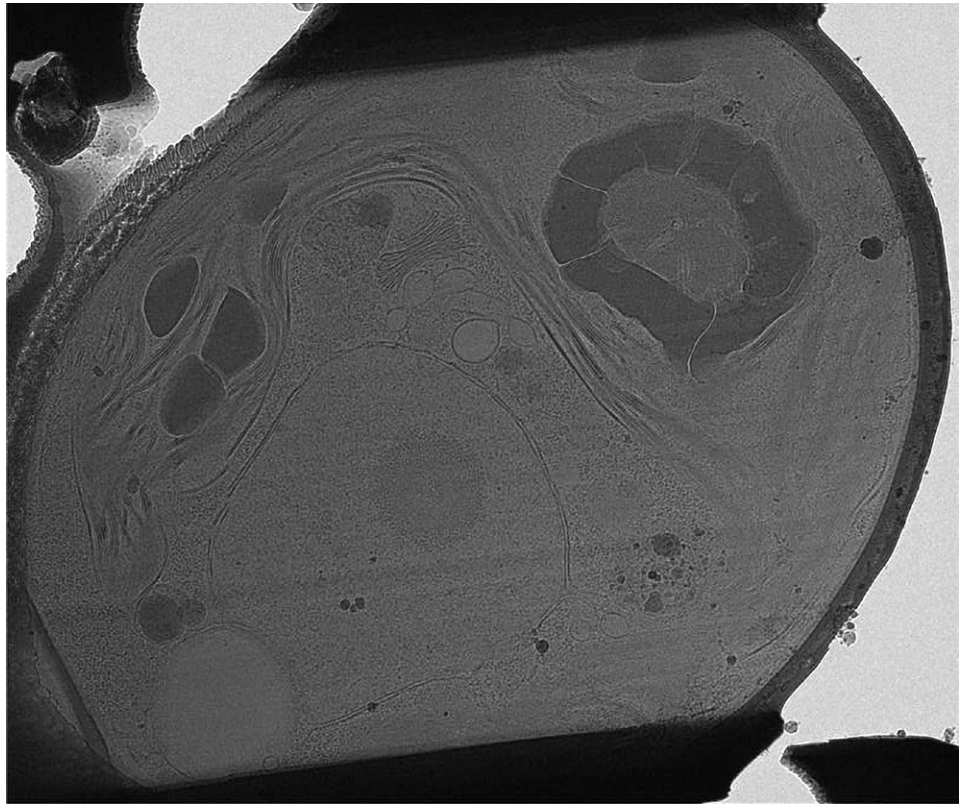


1. Optimize lamella preparation

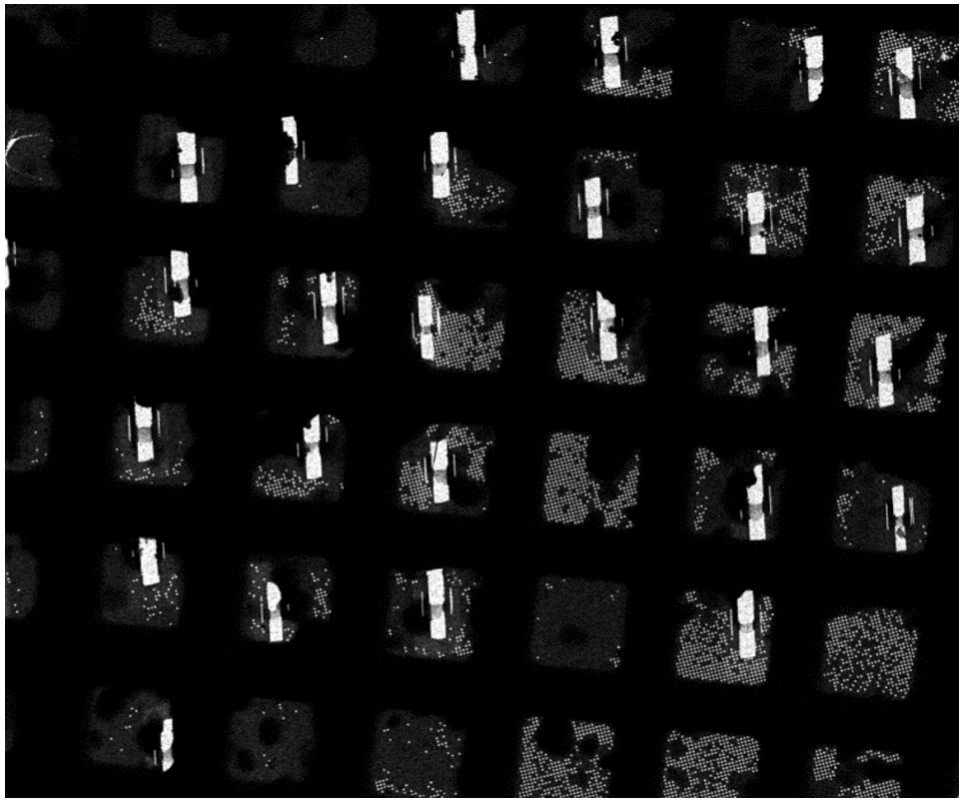
The Hydra Bio Plasma-FIB and Aquilos 2 Cryo-FIB provide complementary solutions for optimized lamella preparation across diverse biological samples. The Hydra Bio PFIB offers multiple plasma ion species for fast, flexible milling, while both systems feature guided workflows and integrated cryo-lift-out for consistent, high-quality lamellae from cells to tissues.

2. Scale lamella preparation

Once optimized, those workflows can be scaled up using the Arctis Cryo-Plasma-FIB, which automates high-throughput lamella preparation, enabling tens of lamellae to be configured and created from multiple specimens in a single session.



A cryo-lamella prepared with an optimized protocol on the Hydra Bio Plasma-FIB, ready for tomographic analysis.

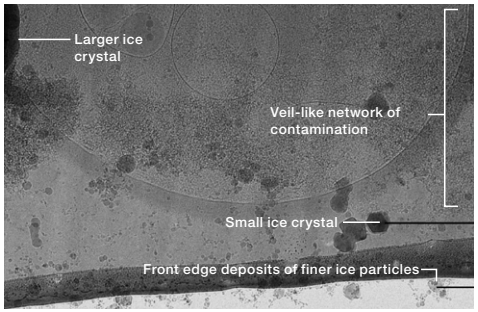


Multiple lamellae prepared from a single sample using automated protocols on the Arctis Cryo-Plasma-FIB.

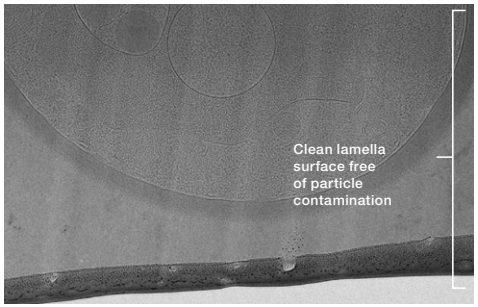
Scaling cryo-ET experiments for statistically powerful data

3. Transfer clean lamella to TEM

The safe transfer of multiple prepared lamellae to a cryo-TEM is a critical step, as any ice contamination caused by this process can negatively impact the resulting tomographic data. The Thermo Scientific Vacuum Capsule protects the lamellae from any contamination that would be caused by contact with liquid nitrogen, thereby maintaining the high quality of the lamellae produced by the cryo-FIB.



After liquid nitrogen exposure



With Vacuum Capsule

The Vacuum Capsule minimizes the contamination that is otherwise common during lamellae transfer between the cryo-FIB and the cryo-TEM.

4. Perform high-throughput tomography

During tomogram collection, multigrid, multishot, and multisite acquisition on the Krios 5 Cryo-TEM automatically captures multiple tomograms per lamella and multiple lamellae per experiment, dramatically increasing sampling efficiency.

Together, these workflow advances make it feasible to scale cryo-ET experiments, enabling quantitative, statistically significant studies that can resolve subtle yet critical differences in cellular organization; a major step forward for cell biology research.

How to get started with cryo-ET

Cryo-ET is reshaping how we see cells, one tomogram at a time.

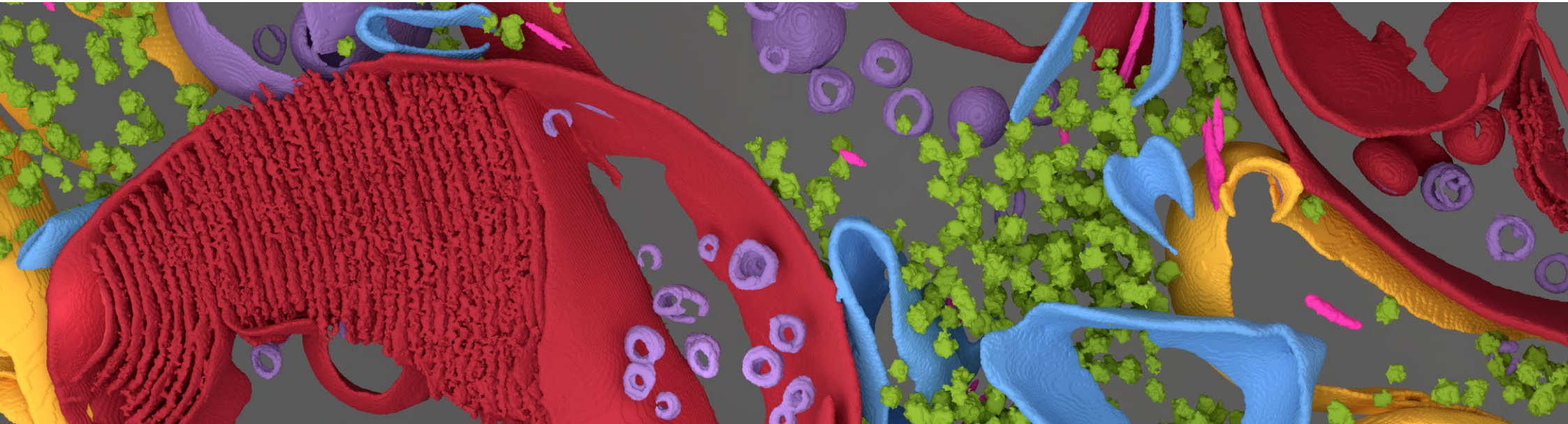
Cryo-electron tomography presents cell biologists with novel opportunities to explore structural details ranging from membrane events, to organelle interactions, to structural changes during disease, and more.

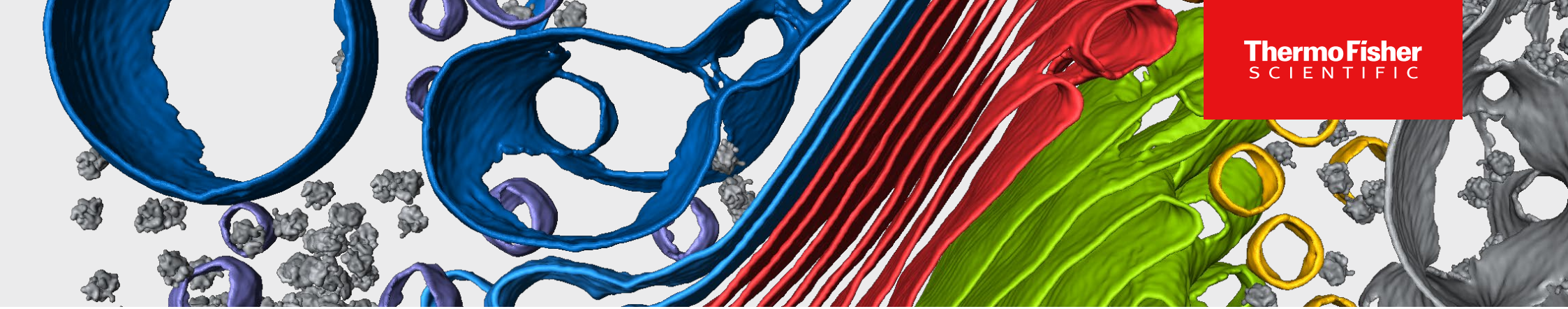
Cryo-ET workflows from Thermo Fisher Scientific, optimized through collaboration with the microscopy community, have allowed this technique to become an approachable method for the investigation of cellular phenomena.

Sample preparation has been significantly streamlined, and the entire workflow seamlessly combines instrumentation, hardware, and software to generate the statistically relevant data needed to answer cell biology questions. Many core imaging facilities now have the ability to perform these complete workflows, making the technique highly accessible.

Cryo-ET has evolved from a niche technique to a veritable necessity in cell biology research. Many researchers begin their journey through shared facilities, collaborative projects, or access programs. Please **contact us** to explore your options and discuss your experimental needs.

Learn more at **thermofisher.com/cryo-tomography**





The following resources provide valuable information, tools, and community support to help you explore the potential of cryo-ET.

Visit the Thermo Fisher Scientific website

Looking for more information on cryo-ET technology, including applications across the life sciences and the latest advancements? Visit our website to learn about the instruments and software solutions available to support your experiments.

Explore cryo-ET

Join the CCP-EM mailing list

The CCP-EM (Collaborative Computational Project for Electron cryo-Microscopy) mailing list lets you stay up to date with the latest research, events, and discussions in the cryo-EM community. Ask questions, share insights, and connect with other researchers.

Join the CCE-EM Mailing List

Get started with Cryo EM 101

Cryo-EM 101 provides clear, practical guidance on sample preparation, imaging, and data analysis, with step-by-step tutorials, troubleshooting advice, and best practices for producing high-quality results.

Visit Cryo EM 101

Sign up with the Cryo-ET Portal

Access a wide range of resources, including tutorials, webinars, and a community of experts. Stay informed about the latest developments in cryo-ET and enhance your research capabilities.

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Learn more at **thermofisher.com/cryo-tomography**

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