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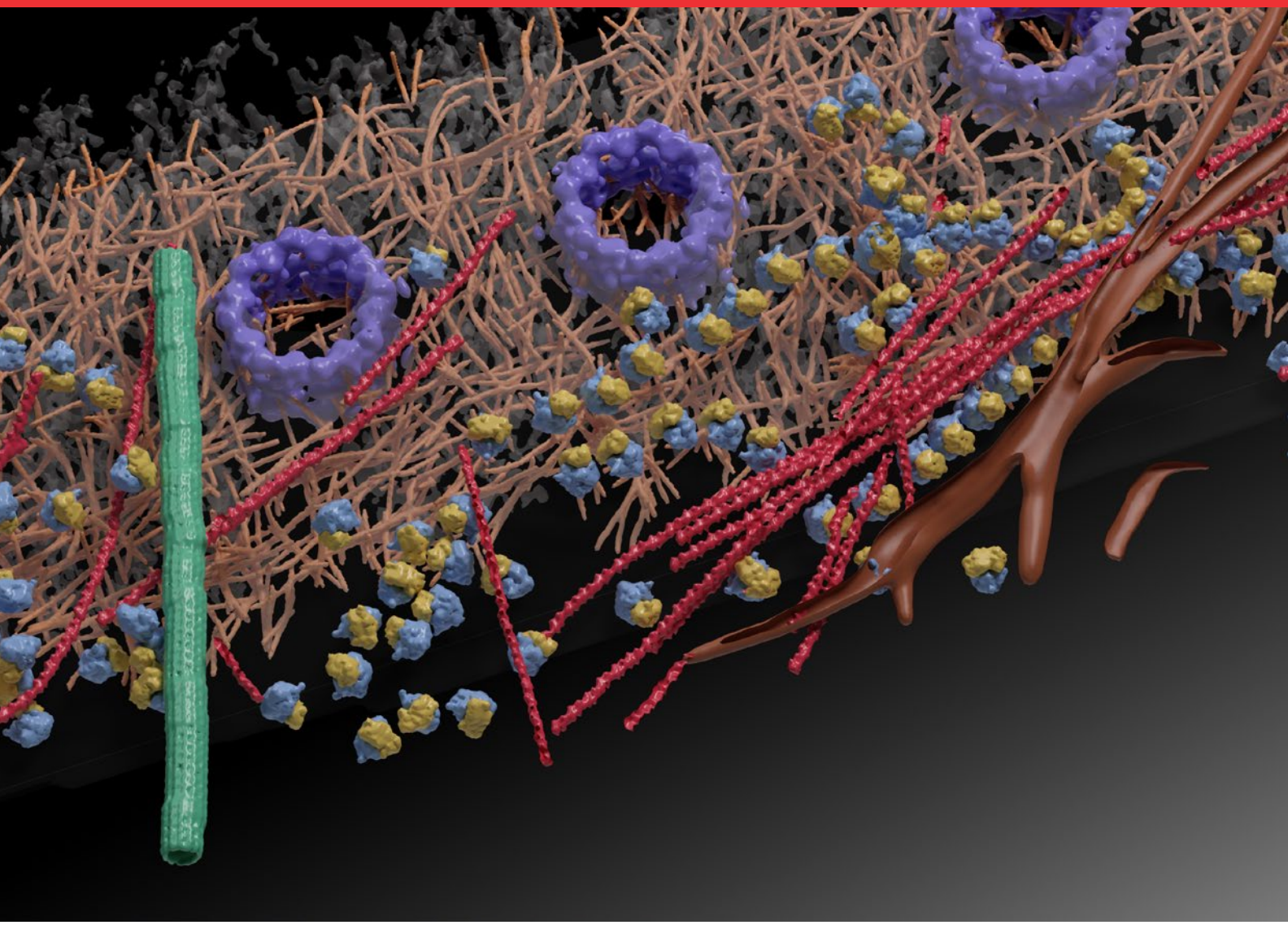


Cryo-Electron Tomography

Selected reference guide

ThermoFisher SCIENTIFIC

About Thermo Fisher Scientific

As the world leader in serving science, our innovative microscopy and application expertise helps customers find meaningful answers to the questions that accelerate breakthrough discoveries, increase productivity, and ultimately change the world.

In 2016, FEI Company, the leader in high-performance transmission and scanning electron and ion microscopy including cryo-electron microscopy (cryo-EM) became a part of Thermo Fisher. Our leading electron microscopy workflows provide images and information at the micro-, nano- and picometer scales.

COVER IMAGE:
Cryo-electron tomography reveals the molecular organization of various components of the HeLa cell in their natural environment. *Data courtesy of Dr. J. Mahamid, Department of Molecular Structural Biology, Max Planck Institute for Biochemistry, Martinsried, Germany.*

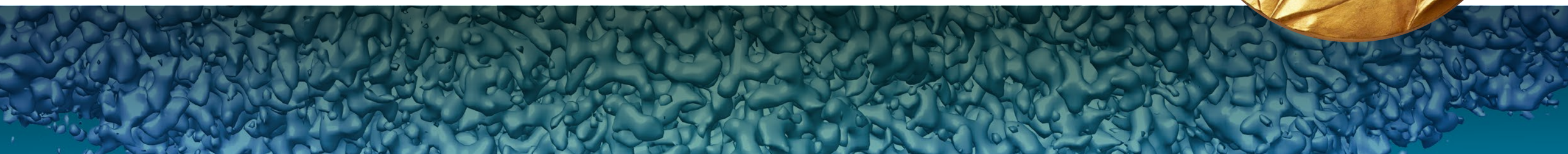
Introduction

The 2017 Nobel Prize in Chemistry recognized the pioneering work of three scientists, Jacques Dubochet, Joachim Frank and Richard Henderson, whose breakthrough developments in cryo-electron microscopy (cryo-EM) have helped to broaden the use of this technology within the structural biology community.

The winners worked with Thermo Scientific™ instruments which allow scientists around the globe to routinely produce highly resolved, three-dimensional images of protein structures. It has taken decades of dedicated work to develop the technological advancements for the structural biology community – hardware, automation, software and detectors – that have made modern cryo-EM possible.

In recent years, single particle cryo-EM has emerged as a mainstream structural biology technique which can determine the 3D structure of proteins and protein complexes at near-atomic resolution. However, single particle cryo-EM is limited to highly purified and isolated proteins that are averaged to determine their 3D structure and lacks a connection to the cellular context.

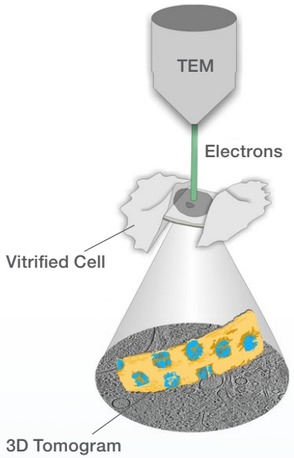
Here, cryo-electron tomography fills the gap by visualizing proteins within their functional cellular environments. This allows observing their relationships and interactions with other cellular components and holds great promise for cell biology, where the ultimate goal is to understand every molecule in the cell – its structure, function, location and interactions.



Cryo-electron tomography

Cryo-ET combines best structure preservation with nanometer-scale resolution. The method acquires 3D snapshots of the cellular interior and visualizes protein complexes within their crowded physiological environments. Such high-resolution 3D images of the interior of cells provide new insights into cellular function and shed light on the arrangement and structure of native protein complexes. The technique can bridge the gap between light microscopy and atomic-resolution techniques like single-particle electron microscopy.

Top 3 reasons Cryo-ET is used to image cells

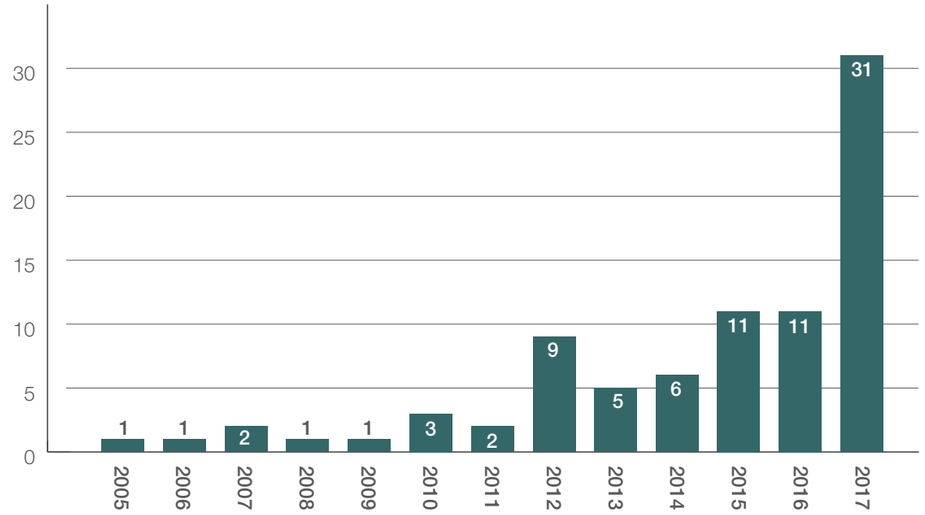


1. Maintains both molecular and structural integrity through the vitrification process
2. Enables the study of proteins at work, thus revealing their functional interactions
3. Provides label-free, fixation-free, 3D nanometer-resolution imaging of cells' inner workings

Before cells can be imaged by cryo-EM, they must be thinned to 150-300 nm with a cryo-focused ion beam (cryo-FIB) microscope to become electron-transparent for TEM tomography.

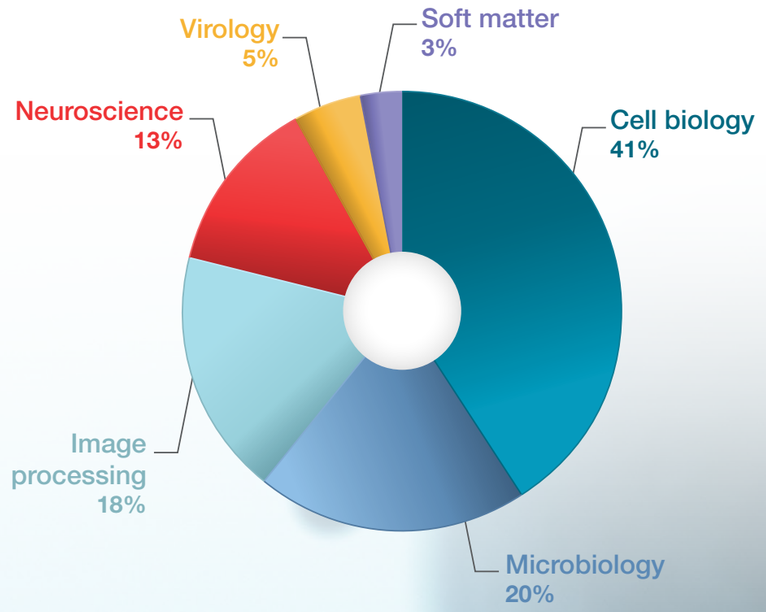
More information on the cryo-tomography workflow on page 16

Cryo-Tomography Articles Published



Since 2015, 32% of cryo-ET articles have appeared in *Cell, Science, Nature* or *PNAS*, and 90% of these articles used Thermo Scientific instruments.

Cryo-tomography articles by application, based on published peer-reviewed articles using Thermo Scientific instruments. Cryo-ET has provided first insights into the cellular mechanisms underlying neurodegenerative diseases such as Huntington's, frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS).



Review Articles

Recommended reviews

Four cryo-ET experts discuss recent advancements to encourage cell biologists to try cryo-ET. This includes not only technology used for sample prep and correlated light & electron microscopy (CLEM), but new cryo-EM access and training centers being set up for learning from Common Fund grants from the National Institutes of Health.

Marx V., 2018 "Calling all cell biologists to try cryo-ET" *Nature Methods* 15: 575–578. doi: 10.1038/s41592-018-0079-y

Cryo-electron tomography (cryo-ET) was featured by Nature as a "Method to Watch" in 2016. Cryo-ET benefits cellular ultrastructure imaging because it can obtain nanometer scale information about macromolecular complexes within frozen samples.

Doerr, A. 2017 "Cryo-electron tomography" *Nature Methods* 14: 34. doi: 10.1038/nmeth.4115

Co-author Grant Jensen likes to quote physicist and Nobel laureate Richard Feynman, who stated in a talk: "It is very easy to answer many of these biological questions, you just look at the thing" (Marx 2018). The authors discuss how cryo-ET has been used to understand questions of cell biology including integration with fluorescence imaging.

Oikonomou C.M., Jensen G.J. 2017 "Cellular electron cryotomography: towards structural biology in situ" *Annual Review of Biochemistry* 86: 873-896. doi: 10.1146/annurev-biochem-061516-044741

Cryo-ET is part of the Nobel prize winning family of cryo-electron microscopy (cryo-EM), from which the latter caused a "resolution revolution" in the structure determination of isolated macromolecular assemblies. The authors discuss how cryo-ET can reveal "molecular sociology" via *in situ* studies, and the potential for discovery by 3D-imaging of important molecular assemblies and machines in unperturbed cellular environments.

Beck M., Baumeister W. 2016 "Cryo-Electron Tomography: can it reveal themolecular sociology of cells in atomic detail?" *Trends in Cell Biology* 26(11): 825-837. doi: 10.1016/j.tcb.2016.08.006

Sample preparation for cryo-ET requires samples to be thinned for imaging in the transmission electron microscope (TEM). Narayan and Subramaniam discuss that "a quiet revolution is underway in technologies used for nanoscale cellular imaging," namely through focused ion beam (FIB) milling. FIB milling technology was developed from the materials sciences and semiconductor fields, but now applied to ultrastructural imaging. This in-depth primer reviews practical aspects of using FIB milling as well as selected examples of imaging subcellular architecture and probing cellular mechanisms underlying host-pathogen interactions.

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Burbaum L., Schaffer M., Engel B.D., Mahamid J., Albert S., Danev R., Baumeister W., Plitzko J.M. 2017 "Charting molecular landscapes using cryo-electron tomography" *Microscopy Today* 25(3): 26-31. doi: 10.1017/S1551929517000384

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Collado J., Fernandez-Busnadiego R. 2017 "Deciphering the molecular architecture of membrane contact sites by cryo-electron tomography" *BBA – Molecular Cell Research* 1864(9): 1507-1512. doi: 10.1016/j.bbamcr.2017.03.009

Dubrovsky A., Sorrentino S., Harapin J., Sapra K.T., Medalia O. 2015 "Developments in cryo-electron tomography for *in situ* structural analysis" *Archives of Biochemistry and Biophysics* 581: 78-85. doi: 10.1016/j.abb.2015.04.006

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Kizilyaprak C., Daraspe J., Humbel B.M. 2014 "Focused ion beam scanning electron microscopy in biology" *J Microscopy* 254(3): 109-114. doi: 10.1111/jmi.12127

Medeiros J.M., Bock D., Weiss G.L., Kooger R., Wepf R.A., and Pilhofer M. 2018 "Robust workflow and instrumentation for cryo-focused ion beam milling of samples for electron cryotomography" *Ultramicroscopy* 190, 1–11. doi: 10.1016/j.ultramic.2018.04.002

Lučić V., Rigort A., Baumeister W. 2013 "Cryo-electron tomography: the challenge of doing structural biology *in situ*" *J. Cell Biol.* 202, 407–419. doi: 10.1083/jcb.201304193

Oikonomou C.M., Jensen G.J. 2016 "A new view into the prokaryotic cell biology from electron cryotomography" *Nature Reviews Microbiology* 14: 205-220. doi: 10.1038/nrmicro.2016.7

Orlov I., Myasnikov A.G., Andronov L., Natchiar S.K., Khatter H., Beinsteiner B., Menetret J.F., Hazemann I., Mohideen K., Tazibt K., Tabaroni R., Kratzat H., Djabeur N., Bruxelles T., Raivoniaina F., de Pompeo L., Torchy M., Billas I., Urzhumtsev A., Klaholz B.P. 2017 "The integrative role of cryo electron microscopy in molecular and cellular structural biology" *Biol Cell* 109: 81-93. doi: 10.1111/boc.201600042

Rigort A., Plitzko J.M. 2015 "Cryo-focused-ion-beam applications in structural biology" *Archives of Biochemistry and Biophysics* 581: 122-130. doi: 10.1016/j.abb.2015.02.009

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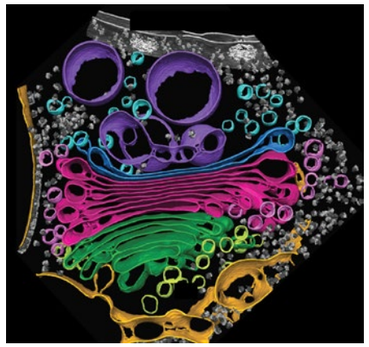
Cell Biology

High-resolution cryo-electron tomography avoids the alterations caused by conventional preparation techniques like chemical fixation to allow imaging of cellular morphology in fully hydrated conditions. To understand complex biological mechanisms, proteins structures and complexes are imaged in 3D at nanoscale resolution within a cell while maintaining their context. Using a correlative light and electron (CLEM) approach allows targeting of tagged proteins by fluorescence microscopy before subsequent higher resolution imaging with cryo-EM.



In situ cryo-electron tomogram of the native *Chlamydomonas* Golgi. *Image courtesy of Ben Engel, MPI Biochemistry*

Close-up on COPs

To keep a city running, traffic must keep flowing. The same goes for cells. Cargo is packaged up in vesicles in a structure called the Golgi and transported throughout the cell. Some vesicles are coated in a protein called COP1, which controls vesicle traffic through the Golgi. The structure of the mammalian version of COP1 was uncovered from experiments conducted outside the cell. Researchers have now investigated the structure of COP1 in its native habitat by studying COP1 in *Chlamydomonas reinhardtii* algae using cryo-electron tomography. They found it looks similar to COP1 in mammals. They also noticed that as the vesicles (pictured, light pink/light blue/light green) moved through different parts of the Golgi (green/magenta/blue/purple), they changed size, membrane thickness and cargo, but the structure of their COP1 coating remained the same. This approach provides a clearer picture of what COP1 and COP1-coated vesicles get up to in the cell.

Source: Lux Fatimathas, BPoD (10 April 2018)

Bykov Y.S., Schaffer M., Dodonova S.O., Albert S., Plitzko J.M., Baumeister W., Engel B.D., Briggs J.A.G. 2017 "The structure of the COPI coat determined within the cell" *Elife* 6: e32493. doi: 10.7554/eLife.32493

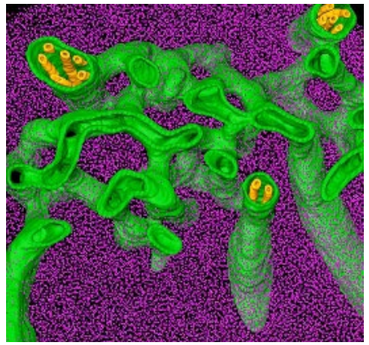


Image courtesy of Ben Engel, MPI Biochemistry

Green algae could hold clues for engineering faster-growing crops

Princeton-led studies provide a detailed look at an essential part of algae's growth machinery, with the eventual goal of applying this knowledge to improving the growth of crops. In this image, the researchers used a technique called cryo-electron tomography to image an algal structure called the pyrenoid, which concentrates carbon dioxide to make it more readily available for photosynthetic enzymes (purple). The yellow tubules inside the green tubes are thought to bring carbon and other materials into the pyrenoid.

Source: Yasemin Saplakoglu, Princeton University (21 Sep 2017)

Freeman Rosenzweig E.S., Xu B., Kuhn Cuellar L., Engel B.D., Mackinder L.C.M., Konikas M.C. 2017 "The eukaryotic CO2-concentrating organelle is liquid-like and exhibits dynamic reorganization" *Cell* 171: 148-163. doi: 10.1016/j.cell.2017.08.008

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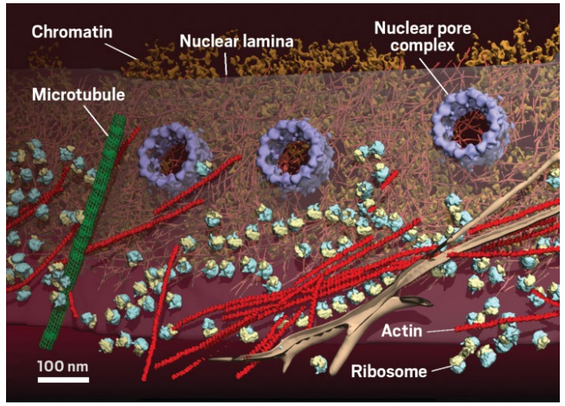


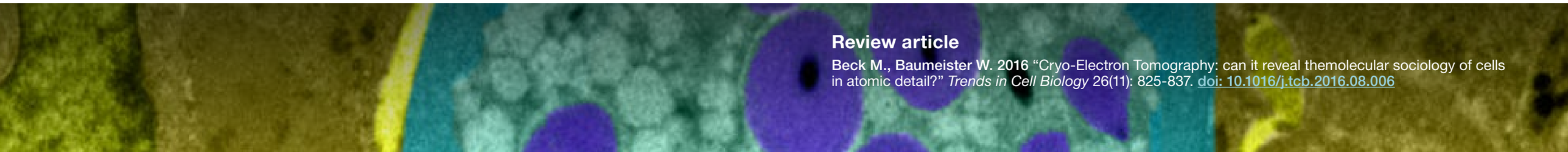
Data courtesy of Dr. J. Mahamid, Department of Molecular Structural Biology, Max Planck Institute for Biochemistry, Martinsried, Germany.

Cryo-electron tomography provides first view of a cell's nucleus in its natural, undisturbed environment

Technique shows that protein filaments make the nucleus the stiffest organelle around. Cryo-electron tomography reveals the molecular organization of various components of the HeLa cell in their natural environment.

Source: Sarah Everts, C&EN (29 Feb 2016 Vol. 94 Issue 9)

Mahamid J., Pfeffer S., Schaffer M., Villa E., Danev R., Cueller L.K., Forster F., Hyman A.A., Plitzko J.M., Baumeister W. 2016 "Visualizing the molecular sociology at the HeLa cell nuclear periphery" *Science* 351: 969–972. doi: 10.1126/science.aad8857

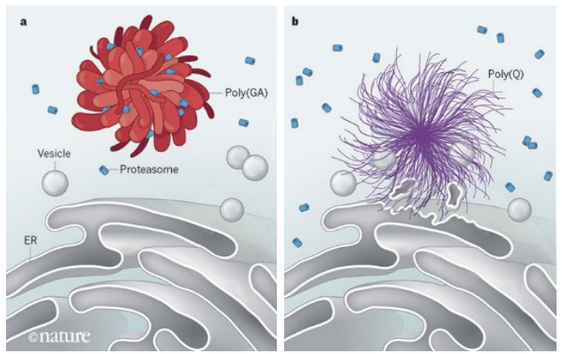


Review article

Beck M., Baumeister W. 2016 "Cryo-Electron Tomography: can it reveal themolecular sociology of cells in atomic detail?" *Trends in Cell Biology* 26(11): 825-837. doi: 10.1016/j.tcb.2016.08.006

Neuroscience

How do molecules regulate neuronal behavior? The answer lies in the neuron and the molecular mechanisms of neuronal communication. While single particle analysis (SPA) solves the structure of highly purified proteins, cryo-electron tomography (cryo-ET) opens windows into cells, allowing a molecular view of functions of proteins in their native environment.



Ilustration showing contrasting mechanisms of Poly(GA) and Poly(Q) aggregate toxicity. *Image © 2018 Elsevier Inc.*

Protein aggregates caught stalling

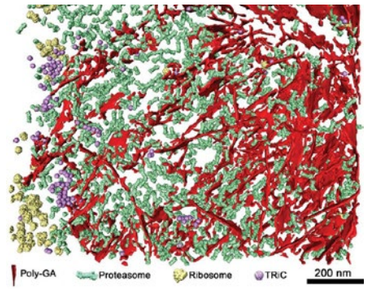
Protein aggregates involved in motor neuron disease (or ALS) have been captured stalling the molecular machines needed for normal protein degradation.

Protein aggregates are a hallmark of neurodegeneration. High-resolution snapshots of the structure of one such aggregate offer an unprecedented view of how these proteins disrupt crucial cellular functions.

Details of contrasting mechanisms of Poly(GA) and Poly(Q) aggregates are detailed in the publications of Guo et al. (Cell, 2018) and Bauerlein (Cell, 2017), respectively, and highlighted in the next section.

Gruber A., Hornburg D., Antonin M., Krahmer N., Collado J., Schaffer M., Zubaite G., Luchtenborg C., Sachsenheimer T., Brugger B., Mann M., Baumeister W., Hartl U. F., Hipp M. S., Fernéandez-Busnadiego R. 2018 "Molecular and structural architecture of polyQ aggregates in yeast" *PNAS* 115, E3446-E3453. doi: 10.1073/pnas.1717978115

Shahmoradian S.H., Genoud C., Graff-Meyer A., Hench J., Moors T., Schweighauser G., Wang J., Goldie K.N., Suetterlin R., Castano-Diez D., et al. 2017 "Lewy pathology in Parkinson's disease consists of a crowded organellar membranous medley" *BioRxiv.* doi: 10.1101/137976



3D rendering of a an aggregate within a neuron: poly-GA ribbons (red), 26S proteasomes (green), ribosomes (yellow), TRiC/CCT chaperonins (purple). *Image © 2018 Elsevier Inc.*

Are ALS Dipeptide Repeat Ribbons Entangling Proteasomes?

In neurons, ALS/FTD poly-Gly-Ala peptides aggregate into a dense network of twisted ribbons.The ribbons sequester a large fraction of the cells' proteasomes. Many trapped proteasomes are frozen in a catalytic transition state.

Source: Daisuke Ito, *ALZ Forum*, 02 Feb 2018

Guo Q., Lehmer, C., Martínez-Sánchez A., Rudack T., Beck F., Hartmann, H., Pérez-Berlanga M., Frottin F., Hipp M.S., Hartl F.U., et al. 2018 "*In Situ* Structure of Neuronal C9orf72 Poly-GA Aggregates Reveals Proteasome Recruitment" *Cell* 172, 696-705.e12. doi: 10.1016/j.cell.2017.12.030

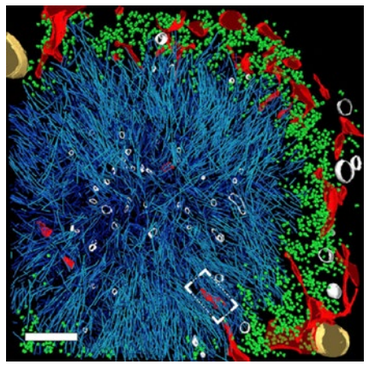


Image © 2017 Elsevier Inc.

Weeds in the brain

A common feature of neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's is the accumulation of toxic protein deposits in the nerve cells of patients. Once these aggregates appear, they begin to proliferate like weeds. If and how these deposits damage nerve cells and lead to their demise remains largely unexplained. A detailed insight into the three-dimensional structure of the protein aggregates should help researchers to solve this puzzle.

Source: *MPI of Biochemistry*, 07 Sept 2017

Bäuerlein F.J.B., Saha I., Mishra A., Kalemanov M., Martínez-Sánchez A., Klein R., Dudanova I., Hipp M.S., Hartl F.U., Baumeister W., et al. 2017 "*In Situ* Architecture and Cellular Interactions of PolyQ Inclusions" *Cell* 171, 179-187.e10. doi: 10.1016/j.cell.2017.08.009

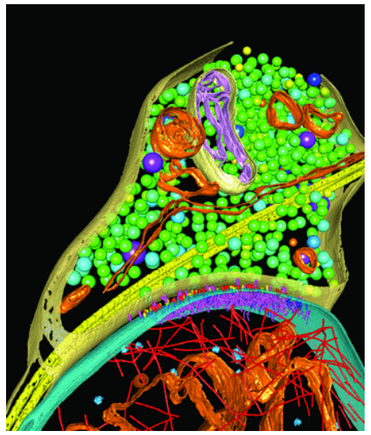


Image © 2018 CC BY 4.0

In situ analysis of ultrastructural organization underlying distinct synaptic functions

This image shows a 3D view of an excitatory synapse between cultured hippocampal neurons revealed by cryo-electron tomography. Segmented structures are rendered as colored surfaces to facilitate visualization of synaptic structures, including presynaptic (light yellow) and postsynaptic (cyan) membranes, ER and endosomes (orange), mitochondrion (outer: gold and light pink), microtubules (yellow), ribosomes (cyan), actin filaments (red), putative presynaptic (magenta) and postsynaptic (yellow) adhesion molecules, putative glutamate receptors (red), postsynaptic density filaments either attached to (blue) or away from (purple) the postsynaptic membrane, as well as dense core vesicles (purple) and synaptic vesicles of various sizes.

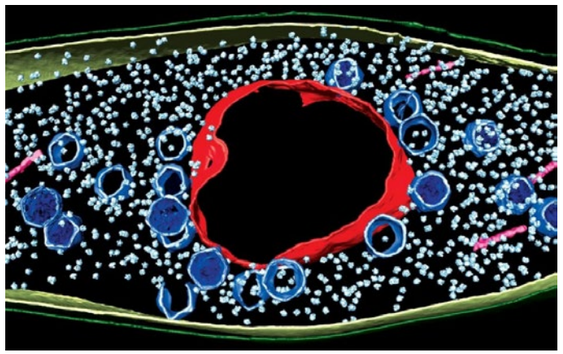
Tao C.-L., Liu Y.-T., Sun R., Zhang B., Qi L., Shivakoti S., Tian C.-L., Zhang P., Lau P.M., Zhou Z.H., et al. 2018 "Differentiation and characterization of excitatory and inhibitory synapses by cryo-electron tomography and correlative microscopy" *JNeurosci* 1548-17. doi: 10.1523/JNEUROSCI.1548-17.2017

Review article

Vaites L.P., Harper, J.W. 2018 "Protein aggregates caught stalling" *Nature* 555, 449-451. doi: 10.1038/d41586-018-03000-2

Microbiology

How do we fight against antimicrobial resistance? What tricks do bacterial invaders play to infect and survive inside their host? Microbiology incorporates many interdisciplinary aspects from cell biology and immunology to genetics and evolution. Cryo-electron tomography (cryo-ET) has enabled the *in situ* visualization of many aspects of prokaryotic cell evolution from development of cellular membrane and "cytoskeleton" to subcellular organization, flagellar rotor complexes for motility and mechanisms of pathogenesis.



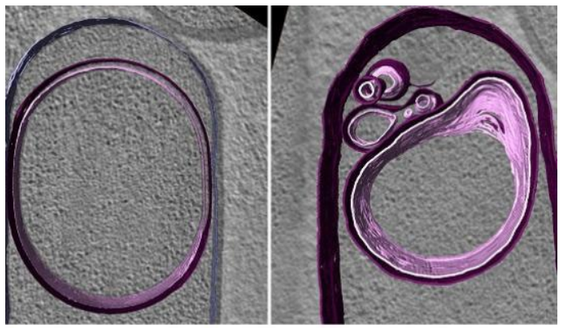
Cryo-electron tomography shows how the bacterial cell is reorganized to resemble a more complicated plant or animal cell with a red nucleus-like compartment and ribosomes, the smaller light blue structures. The reproducing viruses appear with dark blue heads and pink tails. *Image by Vorrapon Chaikeeratisak, Kanika Khanna, Axel Brilot, Katrina Nguyen.*

Biologists discover how viruses hijack cell's machinery

Biologists have documented for the first time how very large viruses reprogram the cellular machinery of bacteria during infection to more closely resemble an animal or human cell – a process that allows these alien invaders to trick cells into producing hundreds of new viruses, which eventually explode from and kill the cells they infect.

Source: UCSD *ScienceDaily*, 12 January 2017.

Chaikeeratisak V., Nguyen K., Khanna K., Brilot A.F., Erb M.L., Coker J.K.C., Vavilina A., Newton G.L., Buschauer R., Pogliano K., Villa E., Agard D.A., Pogliano, J. 2017 "Assembly of a nucleus-like structure during viral replication in bacteria" *Science* 355: 194-197. doi: 10.1126/science.aal2130



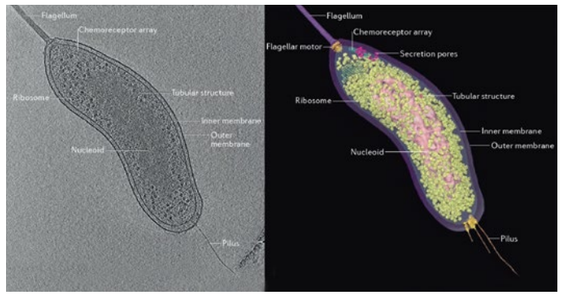
Cryo-electron images of a forespore inflated by DNA (left) and of a deflated forespore in the absence of DNA. *Image © 2018 Elsevier Inc.*

Surprise Finding Points to DNA's Role in Shaping Cells

Like air inflating a balloon, DNA plays an unexpected role in cell architecture. DNA is best known for being the molecule with genetic information but it's becoming more and more obvious that it does other things that are not related to that. This research could have relevance in human cells in terms of how they are generated and shaped, as well as aid explanations of basic mechanical processes and the structure of the nucleus and mitochondria. The results could also allow scientists to have a glimpse in to the origins of cellular life itself.

Source: UCSD Press Release, 8 February 2018

Lopez-Garrido J., Ojkic N., Khanna K., Wagner F.R., Villa E., Endres R.G., and Pogliano K. 2018 "Chromosome Translocation Inflates Bacillus Forespores and Impacts Cellular Morphology" *Cell* 172, 758-770.e14. doi: 10.1016/j.cell.2018.01.027



An intact *Bdellovibrio bacteriovorus* cell in standard media was plunge-frozen and imaged by electron cryo-tomography. The resulting tilt-series of images was reconstructed into a 3D tomogram. A slice through the reconstruction is shown (left panel), as well as a segmentation of visible cellular structures (right panel). *Image © 2016 Elsevier Inc.*

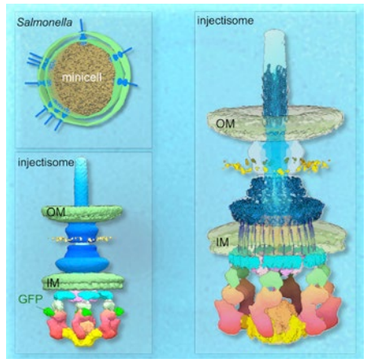
Bock D., Medeiros J.M., Tsao H.-F., Penz T., Weiss G.L., Aistleitner K., Horn M., and Pilhofer M. 2017 "*In situ* architecture, function, and evolution of a contractile injection system" *Science* 357, 713–717. doi: 10.1126/science.aan7904

Boedeker C., Schuler M., Reintjes G., Jeske O., Teeseling M.C.F. van., Jogler M., Rast P., Borchert D., Devos D.P., Kucklick M., Schaffer M., Kolter R., Niftrik L van, Engelmann S., Amann R., Rohde M., Engelhardt H., Jogler C. 2017 "Determining the bacterial cell biology of Planctomycetes" *Nature Communications* 8: 14853. doi: 10.1038/ncomms14853

Chetrit D., Hu B., Christie P.J., Roy C.R., and Liu J. 2018. "A unique cytoplasmic ATPase complex defines the Legionella pneumophila type IV secretion channel" *Nature Microbiology* 3, 678–686. doi: 10.1038/s41564-018-0165-z

Cohen E.J., Ferreira J.L., Ladinsky M.S., Beeby M., and Hughes K.T. 2017 "Nanoscale-length control of the flagellar driveshaft requires hitting the tethered outer membrane" *Science* 356, 197–200. doi: 10.1126/science.aam6512

Wang J., Brackmann M., Castaño-Díez D., Kudryashev M., Goldie K.N., Maier T., Stahlberg H., and Basler M. 2017 "Cryo-EM structure of the extended type VI secretion system sheath–tube complex" *Nature Microbiology 2*, 1507–1512. doi: 10.1016/j.cell.2015.01.037



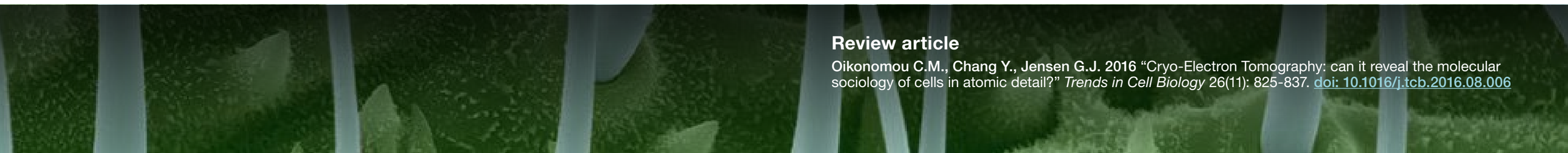
Complete structure of the Salmonella type III secretion machinery explains how bacteria deliver proteins into eukaryotic cells. *Image © 2017 Elsevier Inc.*

Bacterial delivery of proteins

Salmonella and many other bacterial pathogens use a nano syringe-like device to deliver toxic proteins into target human cells. [...] "The device is like a stinger and injects ready-made bacterial proteins into mammalian cells to commandeer them for the benefit of the pathogen," said Jorge Galan, the Lucille P. Markey Professor of Microbial Pathogenesis and co-senior author of the paper. Knowledge of the structure could help researchers devise new anti-infective strategies against a variety of bacterial pathogens such as *Salmonella, Pseudomonas, Escherichia coli, Yersinia pestis,* and *Chlamydia*.

Source: Yale University Reference: Hu et al., *Cell* 168, 1065, 2017.

Hu B., Lara-Tejero M., Kong Q., Galán J.E., and Liu J. (2017) "*In Situ* Molecular Architecture of the Salmonella Type III Secretion Machine" *Cell* 168, 1065-1074.e10. doi: 10.1016/j.cell.2017.02.022

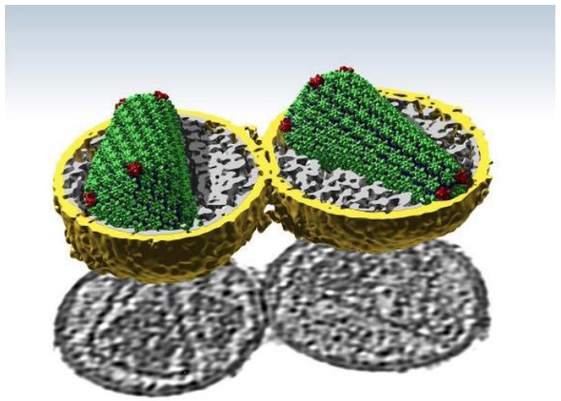


Review article

Oikonomou C.M., Chang Y., Jensen G.J. 2016 "Cryo-Electron Tomography: can it reveal the molecular sociology of cells in atomic detail?" *Trends in Cell Biology* 26(11): 825-837. doi: 10.1016/j.tcb.2016.08.006

Virology

Virus particles are miniature and highly efficient gene delivery machines. Most commonly, fundamental research is focused on the understanding disease and virus evolution with the goal of prevention of viral diseases like Ebola and AIDS. Viruses have also been exploited for beneficial gene therapy to manipulate cells. The most obvious method of virus detection and identification is a direct visualization of the agent. Utilizing cryo-tomography, the morphologies of certain non-cultivatable viruses were resolved to provide sufficient characteristics for classification. Cryo-ET has provided information of structural conformations leading to understanding of new mechanisms for perturbing viral function and therefor to development of future antivirals.



Insights into HIV in unprecedented resolution: The green and red CA protein structures form the conical protective envelope of the virus genome. *Image by Simone Mattei, EMBL.*

Structural insights into capsid flexibility

Viral capsids are protein structures that enclose the genetic material of viruses. Previous structural studies of the HIV-1 capsid have relied on recombinant, cross-linked, or mutant capsid proteins. Mattei et al. now report subnanometer-resolution cryo–electron tomography structures of the HIV-1 capsid from intact virions. These structures confirm the hollow cone shape of the capsid and allow for the specific placement of each individual capsid hexamer and pentamer within the lattice structure. The structures also reveal the flexible nature of the capsid, which likely helps it to accommodate interactions with host cell factors.

Source: Mattei, et al. *Science* 254, 1434, 2016

Bharat T.A.M., Kureisaite-Ciziene D., Hardy G.G., Yu E.W., Devant J.M., Hagen W.J.H., Brun Y.V., Briggs J.A.G., and Löwe J. 2017 "Structure of the hexagonal surface layer on Caulobacter crescentus cells" *Nature Microbiology 2*, 17059. doi: 10.1038/nmicrobiol.2017.59

Gallagher J.R., Torian U., McCraw D.M., and Harris A.K. 2017 "Structural studies of influenza virus RNPs by electron microscopy indicate molecular contortions within NP supra-structures" *J. Structural Biology* 197, 294–307. doi: 10.1016/j.jsb.2016.12.007

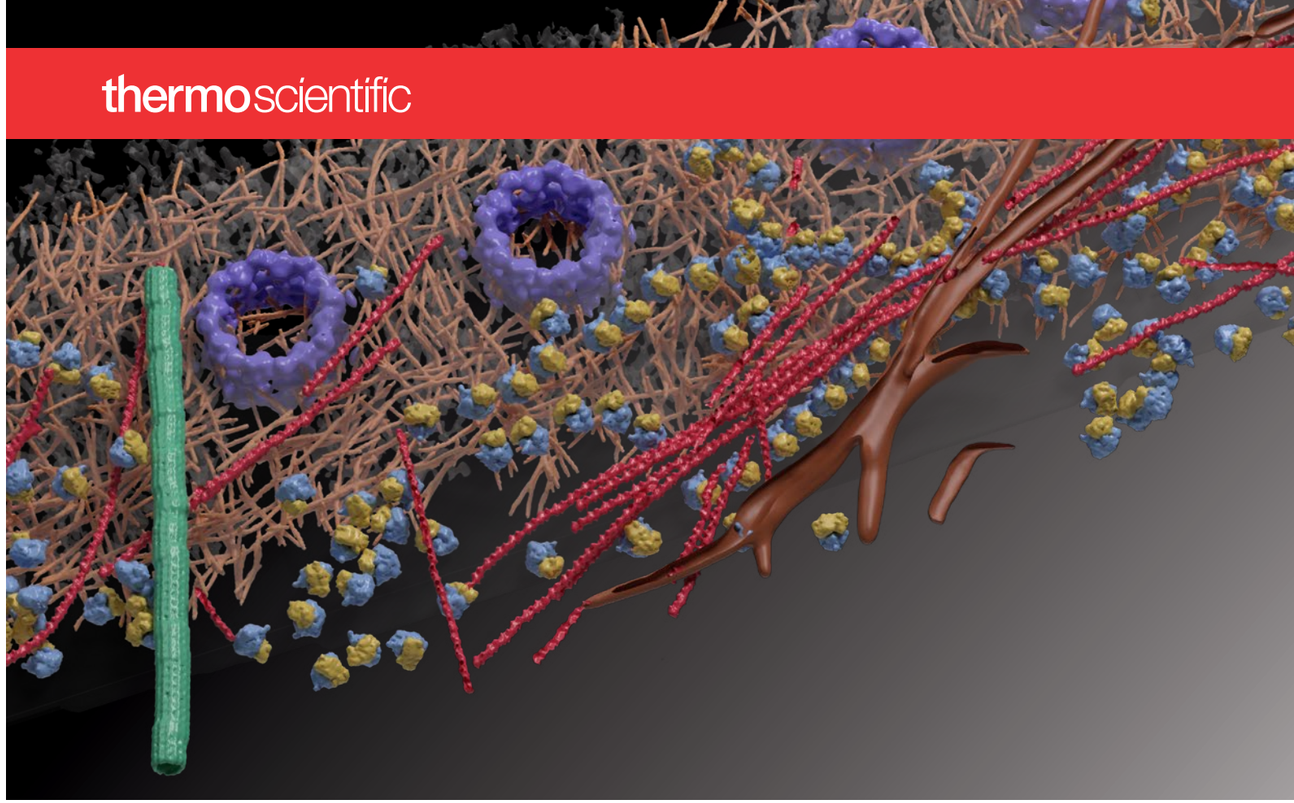
Wan W., Kolesnikova L., Clarke M., Koehler A., Noda T., Becker S., and Briggs J.A.G. 2017 "Structure and assembly of the Ebola virus nucleocapsid" *Nature* 551, 394–397. doi: 10.1038/nature24490

Mattei S., Glass B., Hagen W.J.H., Kräusslich H.-G., and Briggs J.A.G. 2016 "The structure and flexibility of conical HIV-1 capsids determined within intact virions" *Science* 354, 1434–1437. doi: 10.1126/science.aah4972

Highlighted article

Mattei S., Glass, B., Hagen W.J.H., Kräusslich H.G., and Briggs J.A.G. 2016 "The structure and flexibility of conical HIV-1 capsids determined within intact virions" *Science* 354, 1434–1437. doi: 10.1126/science.aah4972

thermoscientific



Cryo-electron tomography reveals the molecular organization of various components of the HeLa cell in their natural environment. Data courtesy of Dr. J. Mahamid, Department of Molecular Structural Biology, Max Planck Institute for Biochemistry, Martinsried, Germany.

Advancing *In Situ* Cell and Structural Biology

Cryo-electron tomography allows researchers to study proteins in their functional cellular environments and resolve supramolecular structures which cannot be readily purified. The Thermo Scientific™ Aquilos™ Cryo-FIB is the first cryo-DualBeam™ (focused ion beam/scanning electron microscope) system dedicated to preparation of frozen, thin lamella samples from biological specimens for high-resolution tomographic imaging in a cryo-transmission electron microscope (cryo-TEM). Imaging cellular ultrastructure at unprecedented resolution in 3D is possible while maintaining structural integrity to accelerate understanding of entire processes inside cells.

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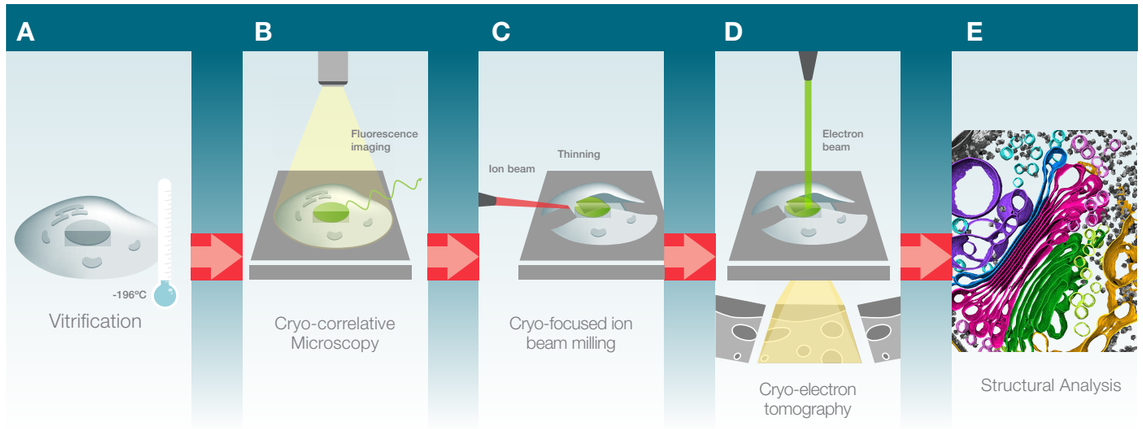
Cryo-Tomography Workflow

Cryo-ET provides label-free, fixation-free, 3D nanometer-resolution imaging of cells' inner workings. Next to this, it allows 3D snapshots of the cellular interior and visualizes protein complexes within their physiological environments. Using a correlative light and electron (CLEM) approach allows to target tagged proteins by fluorescence microscopy before subsequent higher resolution imaging with cryo-EM. Many cells are too thick for electrons, so the vitrified cells must be thinned with a cryo-focused ion beam microscope (cryo-FIB) prior to imaging in transmission electron microscope.



A – Sample preparation by vitrification

Cells prepared by routine culture methods are grown on carbon-coated gold electron microscopy (EM) grids and plunge-frozen in a cryogenically cooled fluid, typically liquid ethane. The water in the sample freezes so rapidly that it does not crystallize, thus avoiding the molecular-scale disruption (by formed ice crystals) that would occur with a normal slow freezing process.

B – Localization by fluorescence

Using, cryo-correlative microscopy, the sample is transferred to a cryo-fluorescence light microscope, where structures of interest are identified. A dedicated cryo-LM stage keeps the sample in its vitrified state during cryo-fluorescence imaging.

C – Thinning by milling

A dedicated cryo-FIB prepares a thin, uniform lamella at the vitreous temperature (approximately -170°C).

D – Imaging by TEM

During cryo-tomography, the sample is tilted in known increments about an axis. The individual projection images from the tomographic tilt series are then combined computationally in a procedure known as back-projection, which creates the 3D tomographic volume.

E – Reconstruction & Visualization

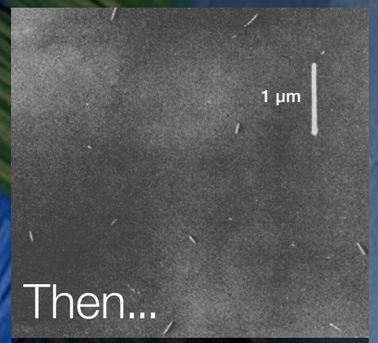
The 3D tomogram featuring cellular structures can be segmented and colored in a variety of ways to enhance its display and presentation. From the tomogram, small subsets of data, containing the structures of interest, can be computationally extracted and subjected to image processing methods.

Recommended literature

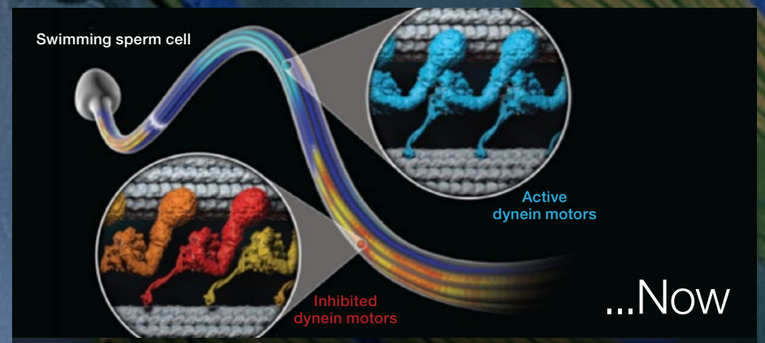
Schaffer M., Engel B.D., Laugks T., Mahamid J., Plitzko J.M., Baumeister W. 2015 "Cryo-focused Ion Beam Sample Preparation for Imaging Vitreous Cells by Cryo-electron Tomography" *Bio Protoc* 5(17) pii: e1575. doi: 10.21769/BioProtoc.1575

Schaffer M., Mahamid J., Engel B.D., Laugks T., Beumeister W., Plitzko W. 2016 "Optimized cryo-focused ion beam sample preparation aimed at in situ structural studies of membrane proteins" *J Struct Biol.* 197(2): 73-82. doi: 10.1016/j.jsb.2016.07.010

Arnold J., Mahamid J., Lucic V., de Marco A., Fernandez J.J., Laugks T., Mayer T., Hyman A., Baumeister W., Plizko J. 2016 "Site-Specific Cryo-Focused Ion Beam Sample Preparation Guided by 3D Correlative Microscopy" *Biophys J* 1 110(4): 860-869. doi: 10.1016/j.bpj.2015.10.053



Electron micrograph of 30S dynein from cilia isolated from *Tetrahymena pyriformis*. Gibbons & Rowe, 1965.



The structure and behavior of these flagella weren't revealed until the advent of electron microscopy. Cryo-ET was used to image the active flagellum of swimming sea urchin sperm cells. *Image © 2018 The Authors, some rights reserved; exclusive licensee AAAS. No claim to original U.S. Government Works.*

Lin J., and Nicastro D. 2018 "Asymmetric distribution and spatial switching of dynein activity generates ciliary motility." *Science 360*, eaar1968. doi: 10.1126/science.aar1968

Notes

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