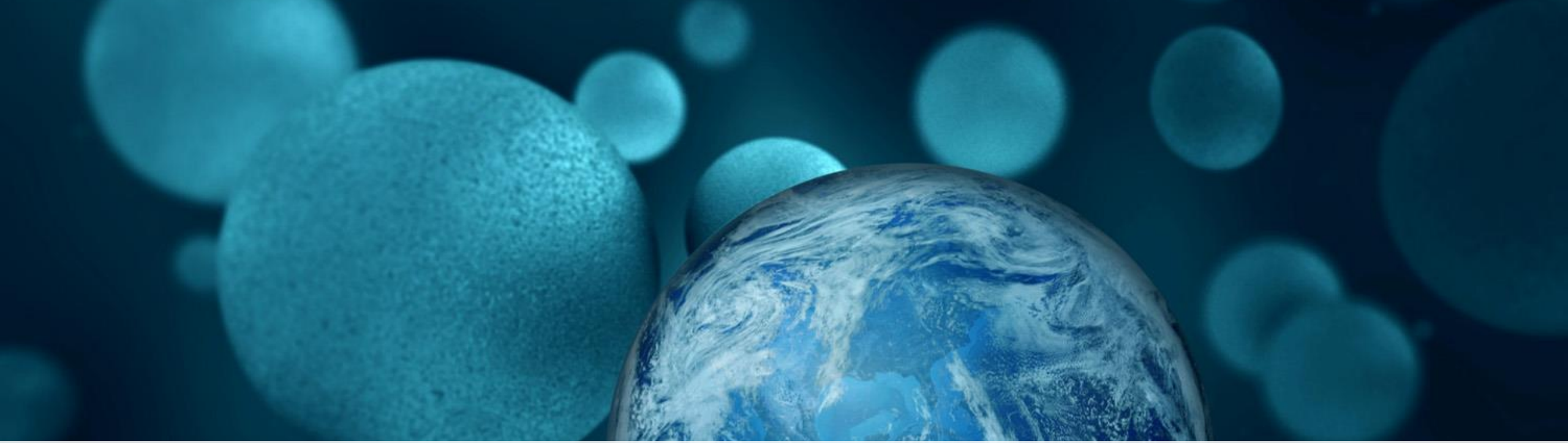


# 生体三次元構造解析のためのクライオ3Dイメージング

2020/3/25

Thermo Fisher Scientific

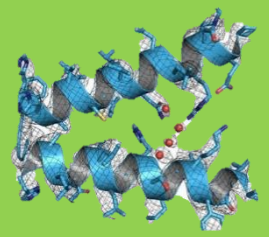
甲斐 翼

# Studying proteins *inside* functional cellular environments

**Single Particl Analysis**
Structure of folded proteins

**Cryo Tomography**
Protein function in cells

**Large Volume Analysis**
Cellular organization in tissue



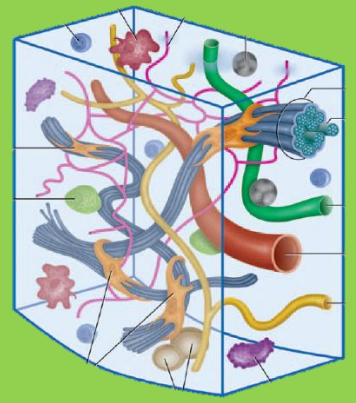


Illustration: Charis Tsevis (flickr)

Cryo-electron tomography fills the gap by visualizing proteins within their functional cellular environments.

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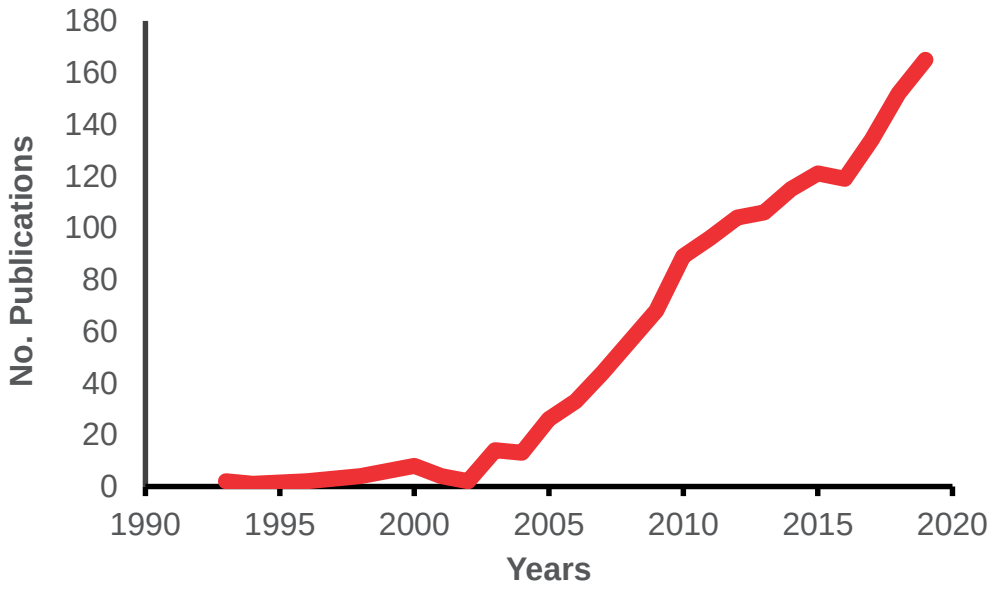
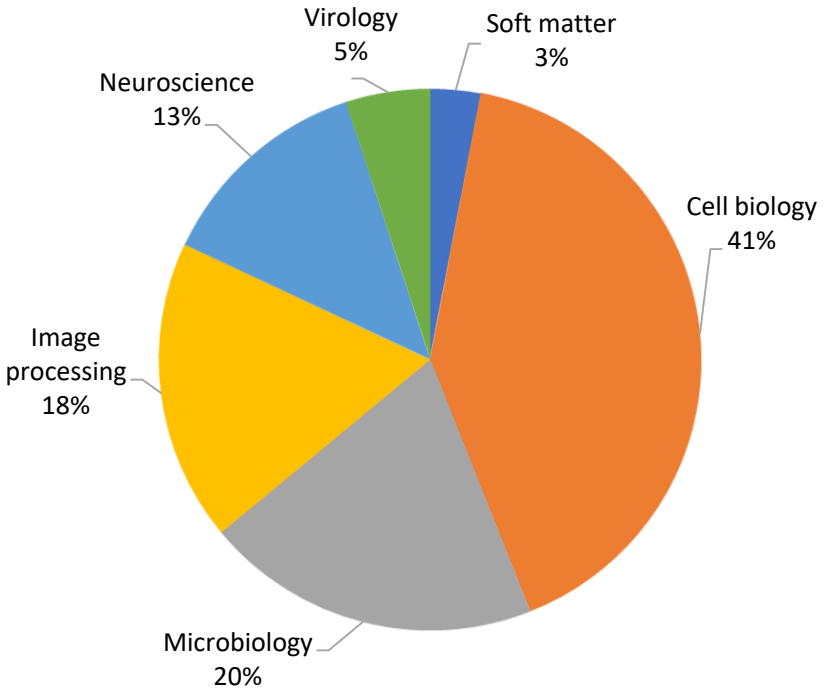
# Cryo-Tomography Articles Published

A significant mean for Cell Biologists is **Cryo-Tomography** in clarifying the cellular interior and visualizes protein complexes within their crowded physiological environments.




"For resolving structures as they occur in their native cellular context, cryo-ET comes to the fore"

Cryo-ET has provided first insights into the cellular mechanisms underlying neurodegenerative diseases.

# Media coverage of cryo tomography is at a very high level

nature methods

Article | Published: 29 July 2019

## A cryo-FIB lift-out technique enables molecular-resolution cryo-ET within native *Caenorhabditis elegans* tissue

Miroslava Schaffer, Stefan Pfeffer, Julia Mahamid, Stephan Kleindiek, Tim Laugks, Sahradha Albert, Benjamin D. Engel, Andreas Rummel, Andrew J. Smith, Wolfgang Baumeister & Juergen M. Plitzko

Technology Feature | Published: 31 July 2018

## Calling cell biologists to try cryo-ET

Vivien Marx

*Nature Methods* **15**, 575–578(2018) | Cite this article

technology feature

Corrected: Publisher correction

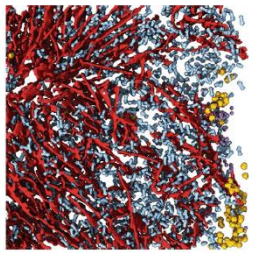
## Calling cell biologists to try cryo-ET

Cryo-electron tomography (cryo-ET) advances can ease the path to 3D renderings of cellular architecture.

Vivien Marx

Plunge-freezing sounds like a summertime jump into a refreshingly cold lake. In the lab, plunge-freezing is a way to vitrify cells in a physiological, hydrated state and avoid staining or chemical fixation. High-pressure freezing is used with tissue or multicellular organisms. In all cases, the process is "the best possible structural preservation that can physically be achieved," says Wolfgang Baumeister, a researcher at the Max Planck Institute of Biochemistry who began working in cryo-electron tomography, or cryo-ET, in the late 1980s. Cryo-ET belongs to the Nobel-Prize-winning family of cryo-electron microscopy (cryo-EM) techniques[1,2].

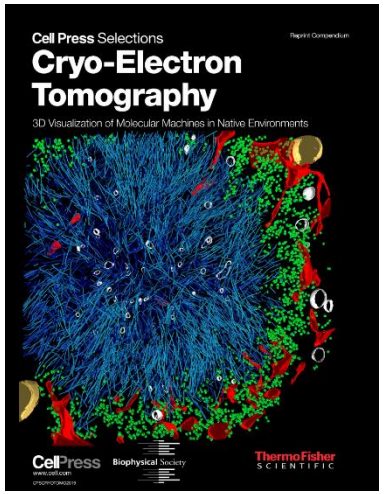
When done well, speedy cooling outpaces the formation of ice crystals in the cell's watery interior. That ice would damage structures and disrupt subsequent imaging in an electron microscope, which is the next step in cryo-ET, says Elizabeth Villa, a researcher at the University of California, San Diego. When she shows the three-dimensional images generated with cryo-ET called tomograms to cell biologists,



Cryo-ET can reveal 'molecular sociology.' This tomogram shows poly-Gly-Ala aggregates (red) and proteasomes (blue) where proteins are degraded. Protein aggregation is a hallmark of neurodegenerative disease. (Credit: Q. Guo, Baumeister Lab, MPI Martinsried.)

Trained as a physicist, Villa's love of cryo-ET arose during a physiology course at the Marine Biological Laboratory and she vowed to never look at isolated molecules again. As a postdoctoral fellow in Baumeister's lab she learned more about how cryo-ET can, in his words, capture the "molecular sociology" of cells, show cellular structures and molecules in the context of interactions and influences that shape function. As she studies when and how proteins talk to their 'friends', Villa says she feels like a "molecular anthropologist." "Proteins are very 'social animals'," she says. At any given time, proteins can belong to a number of different complexes.

Cryo-ET is not easy, quick or cheap, and Villa reminds her students that 'cry' is part of 'cryo.' Experiments often fail, "we fail more," she says. She and other cryo-ET methods developers want to show that the method is more than a challenging path to a pretty picture. Cryo-ET brings to cell biology and structural biology a "wild concept," says Subramaniam: a way to peer inside cells and see proteins in situ at high

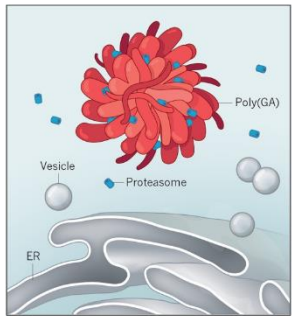


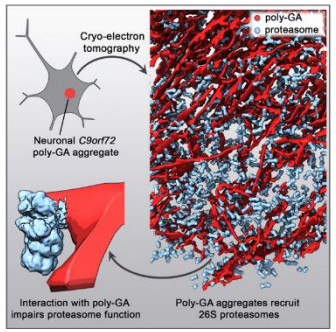


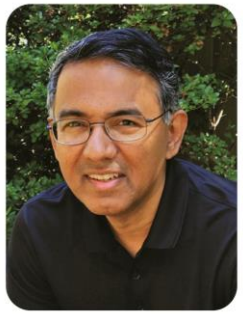
*In Situ* Structure of Neuronal *C9orf72* Poly-GA Aggregates Reveals Proteasome Recruitment

Qiang Guo,[1] Carina Lehmer,[2,3,8] Antonio Martínez-Sánchez,[1,8] Till Rudack,[4,5,8] Florian Beck,[1] Hannelore Hartmann,[2,3] Manuela Pérez-Berlanga,[6] Frédéric Frottin,[6] Mark S. Hipp,[3,6] F. Ulrich Hartl,[3,6] Dieter Edbauer,[2,3,7,*] Wolfgang Baumeister,[1,9,*] and Rubén Fernández-Busnadiego[1,*]

Cell



*Cryo-ET brings to cell biology a way to peer inside cells, see proteins on situ at high resolution and in 3D, says Sriram Subraniam. (NIH)*



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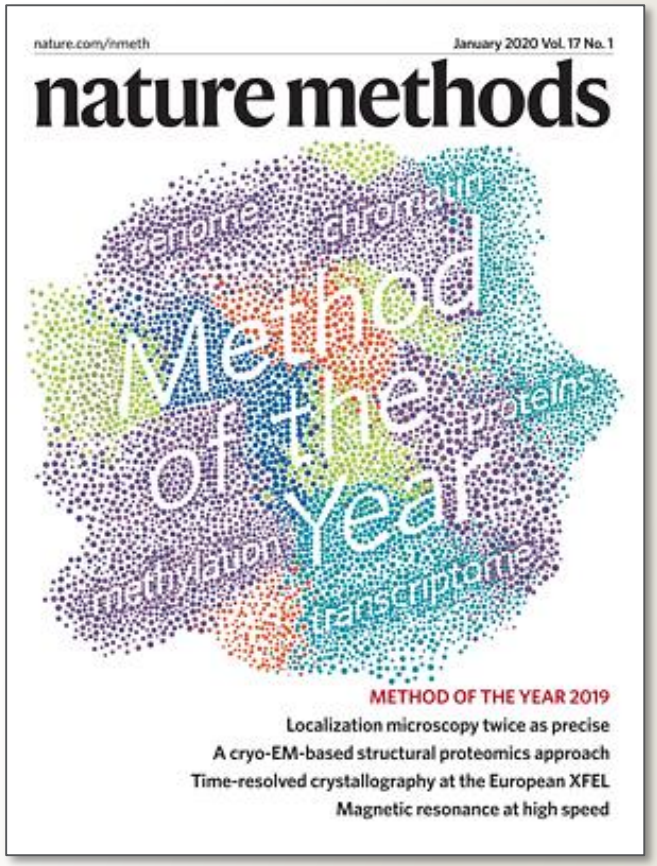
# Cryo-tomography continues to be on the 2020 technology watchlist






## Methods and areas worth watching

### Structures in situ

Cryo-electron tomography reveals the structural biology of native macromolecules.

Single-particle cryo-electron microscopy (cryo-EM) has emerged as a transformative approach for determining high-resolution structures of proteins and nucleic acids. Methods for solving structures by cryo-EM have developed and matured, increasing the uptake of this approach by the broader research community.

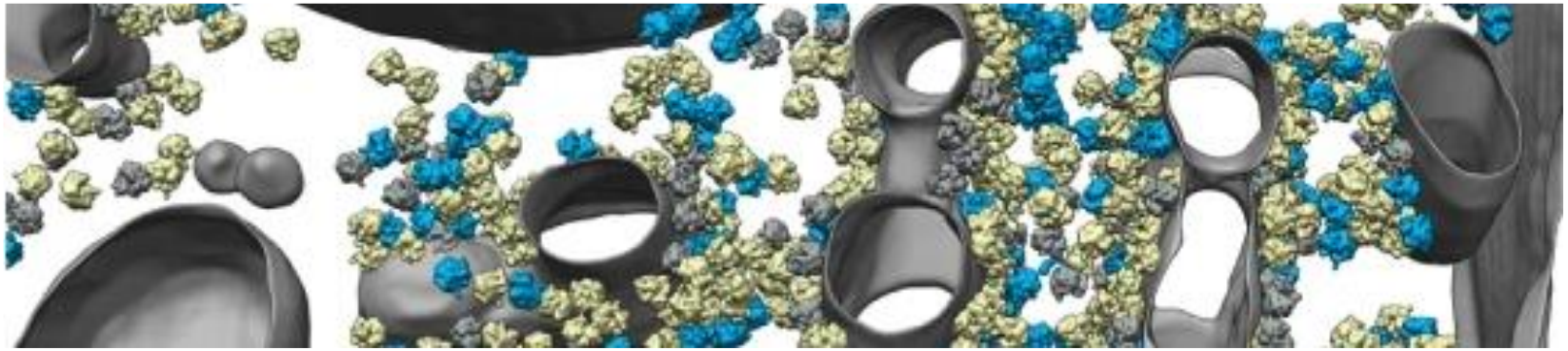
However, the advantages of electron microscopy for structural biology go well beyond single-particle cryo-EM. 634–636, 2015; *Nat. Methods* **16**, 757–762, 2019; *Nat. Methods* https://doi.org/10.1038/s41592-019-0630-5, 2019).

There are also challenges of image analysis. The 'missing wedge' plagues many tomographic approaches and reduces resolution; it arises because images cannot be acquired for all tilt angles. Computational approaches are making exciting headway to address this problem (*J. Struct. Biol.* **206**, 183–192, 2019). Another challenge is annotating particles of interest from the crowded intracellular environment, with computational approaches greatly facilitating the process (*Nat. Methods* **14**, 983–985, 2017). Additional processing and computational workflows further improve cryo-ET (*Nat. Methods* **15**, 955–961, 2018; *Nat. Methods* **16**, 1161–1168, 2019).

We anticipate future methods development will improve throughput, sample preparation, analysis, and achievable resolution of cryo-ET. ❐

Rita Strack

Published online: 6 January 2020
https://doi.org/10.1038/s41592-019-0704-4





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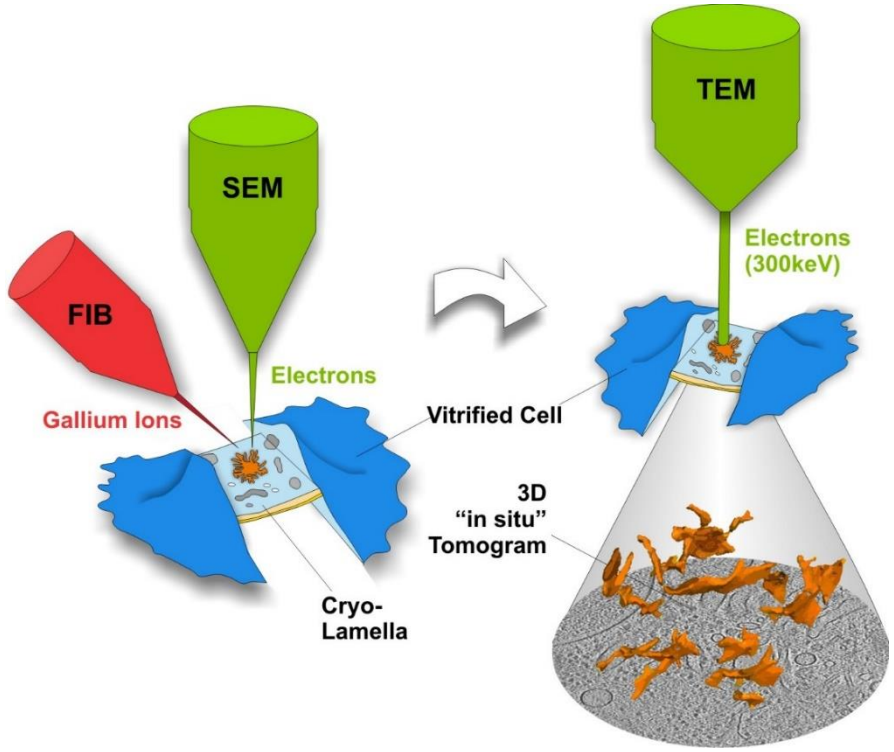
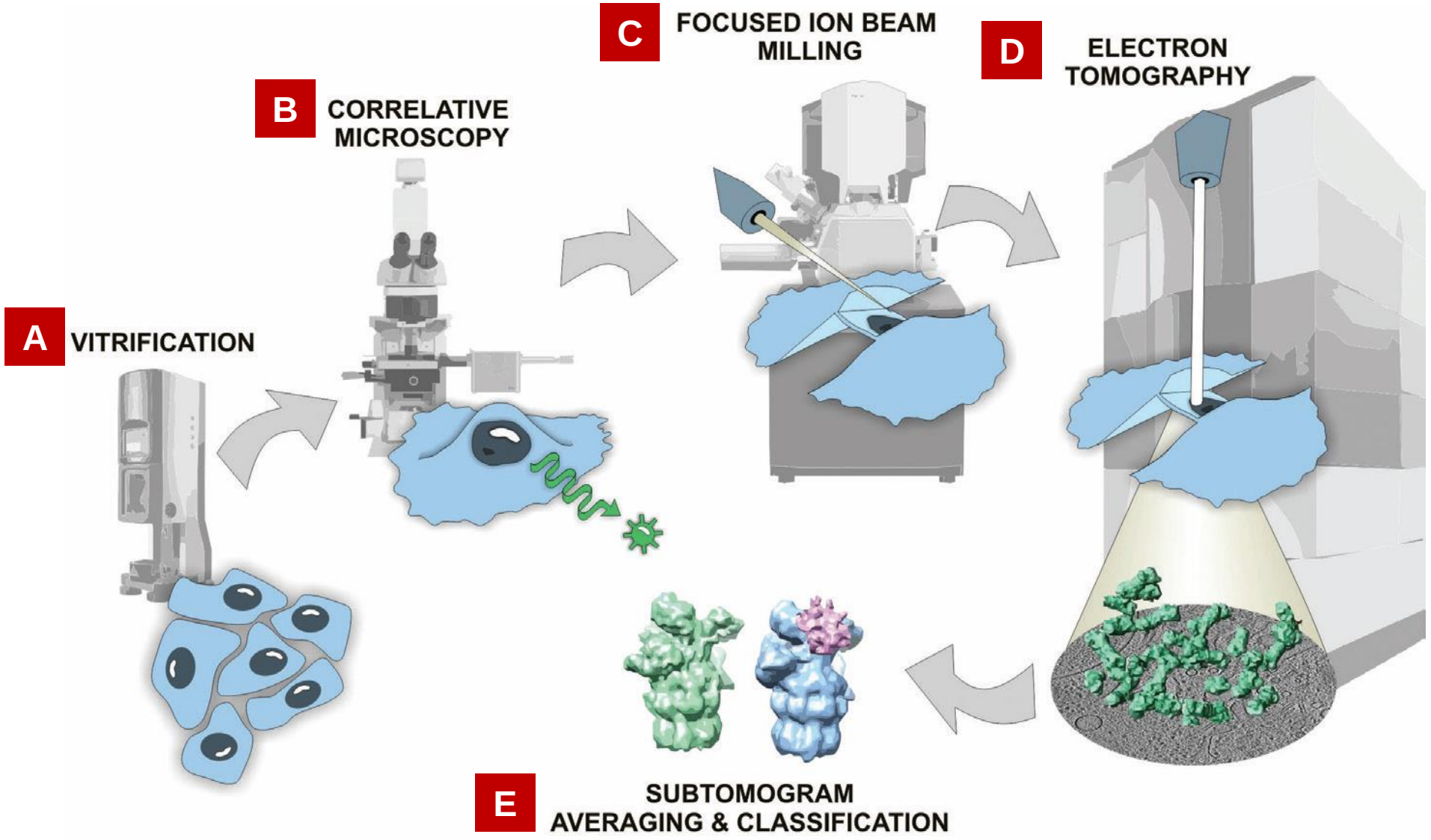
# Top 3 reasons Cryo-ET is used to image cells

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

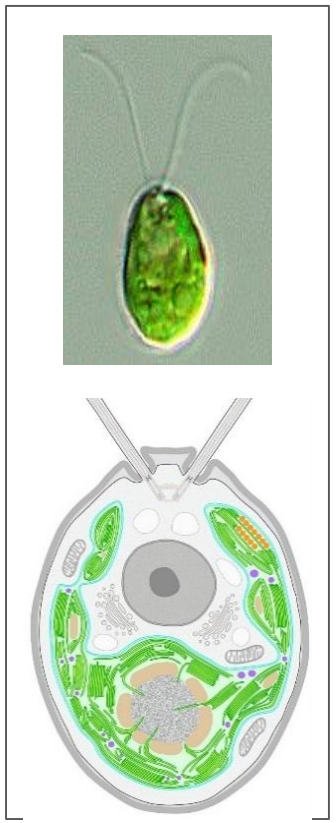
Cryo-electron tomography allows researchers to study proteins in their functional cellular environments and resolve supramolecular structures which cannot be readily purified.

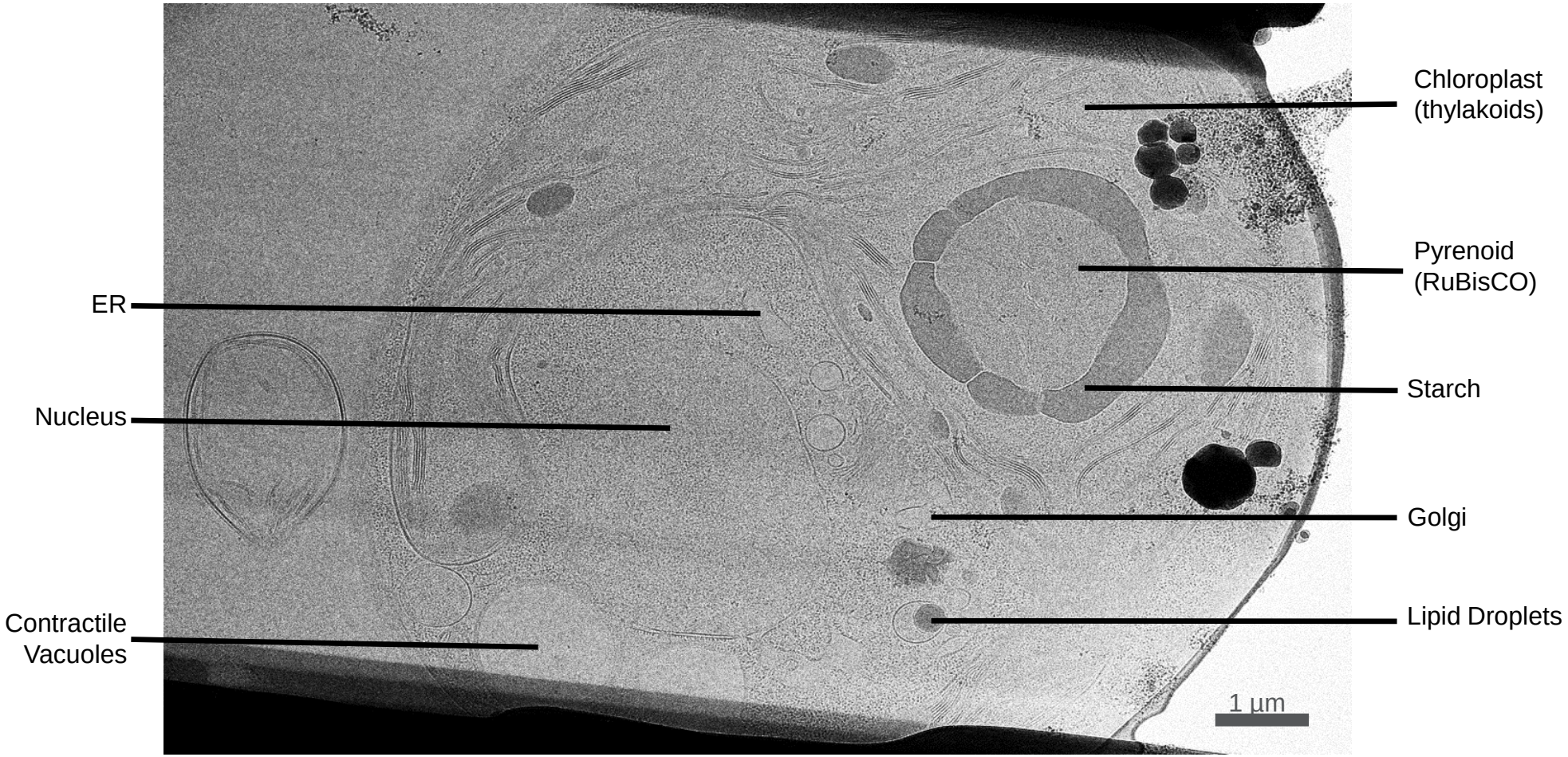


# Cryo-Tomography Workflow



# Cryo-TEM overview image of a *in-situ* cryo-FIB lamella from a *Chlamydomonas* cell

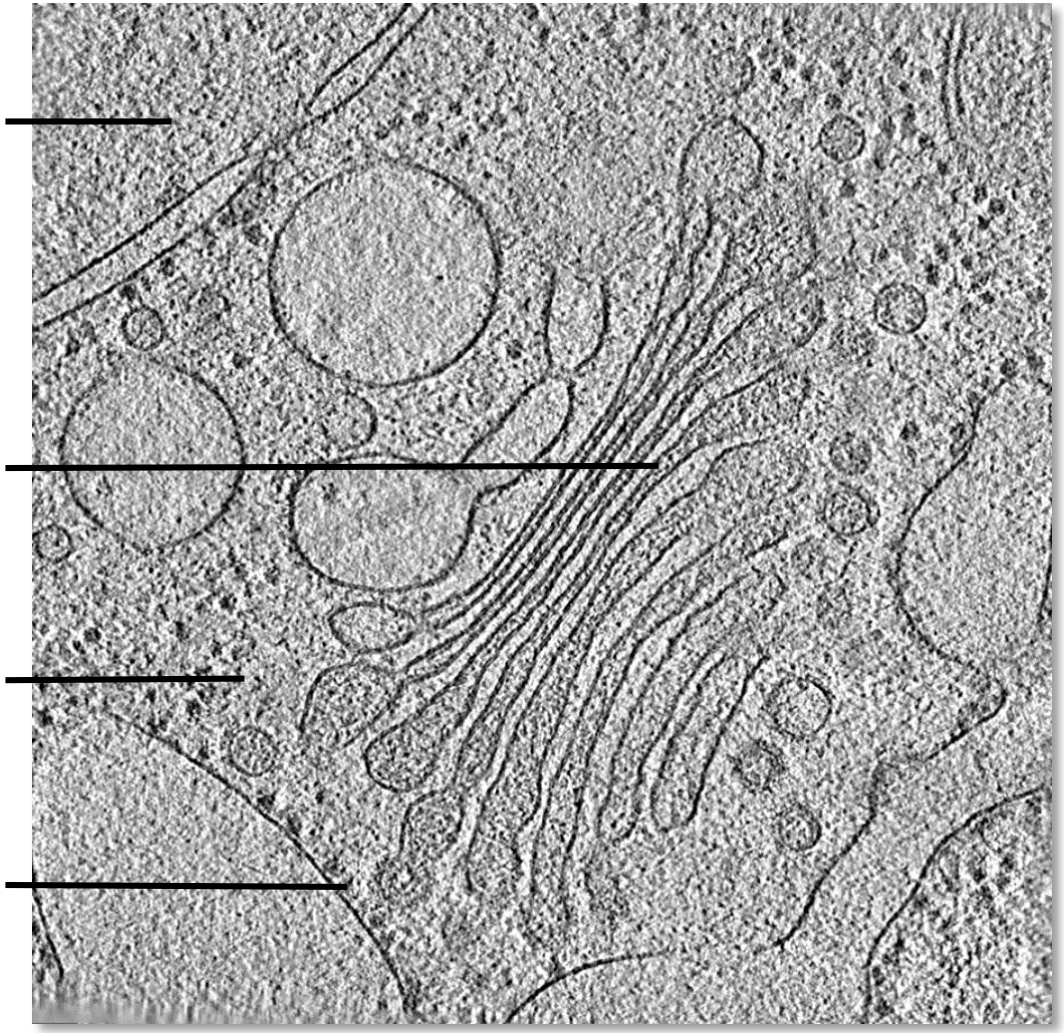




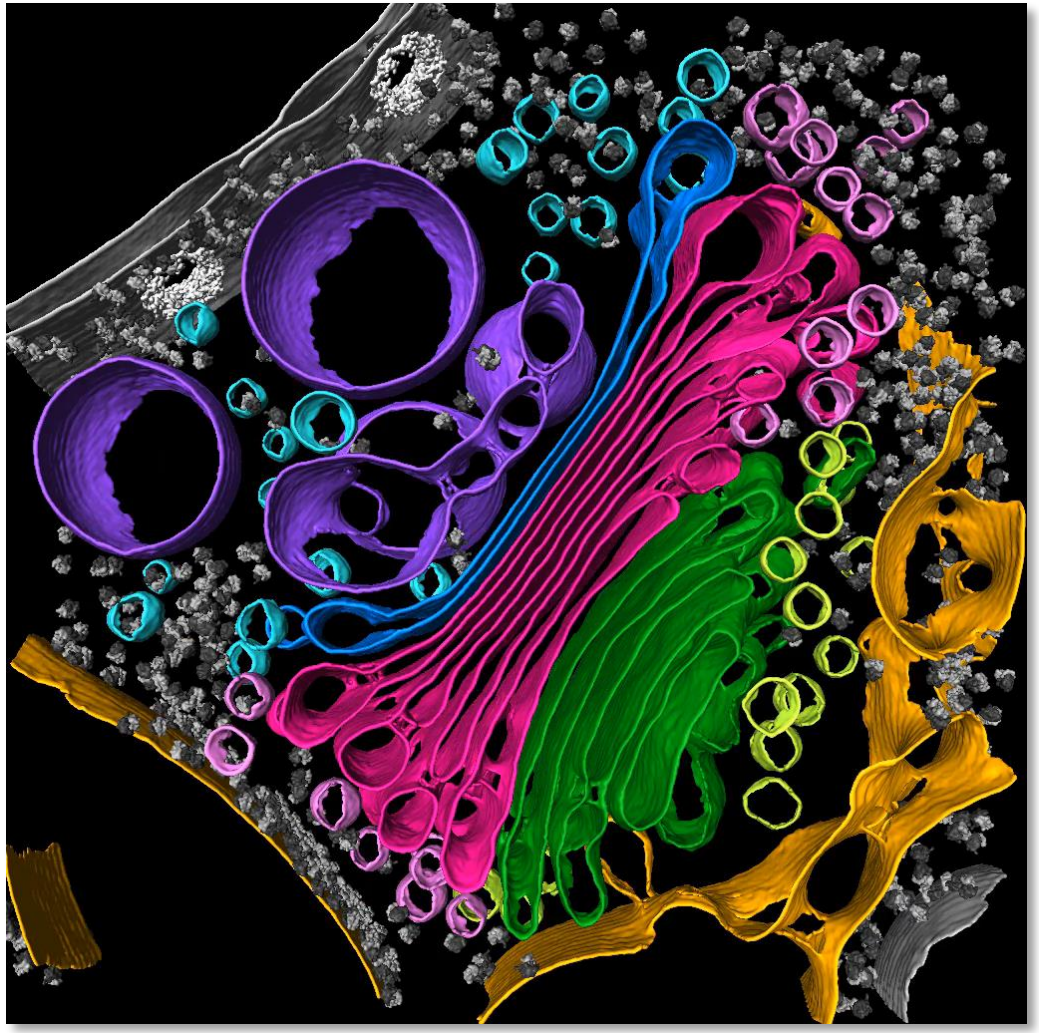

Cryo-TEM overview image of the cryo-lamella, *Chlamydomonas reinhardtii*

Data Courtesy Max Planck Institute of Biochemistry | *Miroslava Schaffer and Ben Engel*

# *In situ* cryo-electron tomogram of the native *Chlamydomonas* Golgi.




**TOMOGRAM**

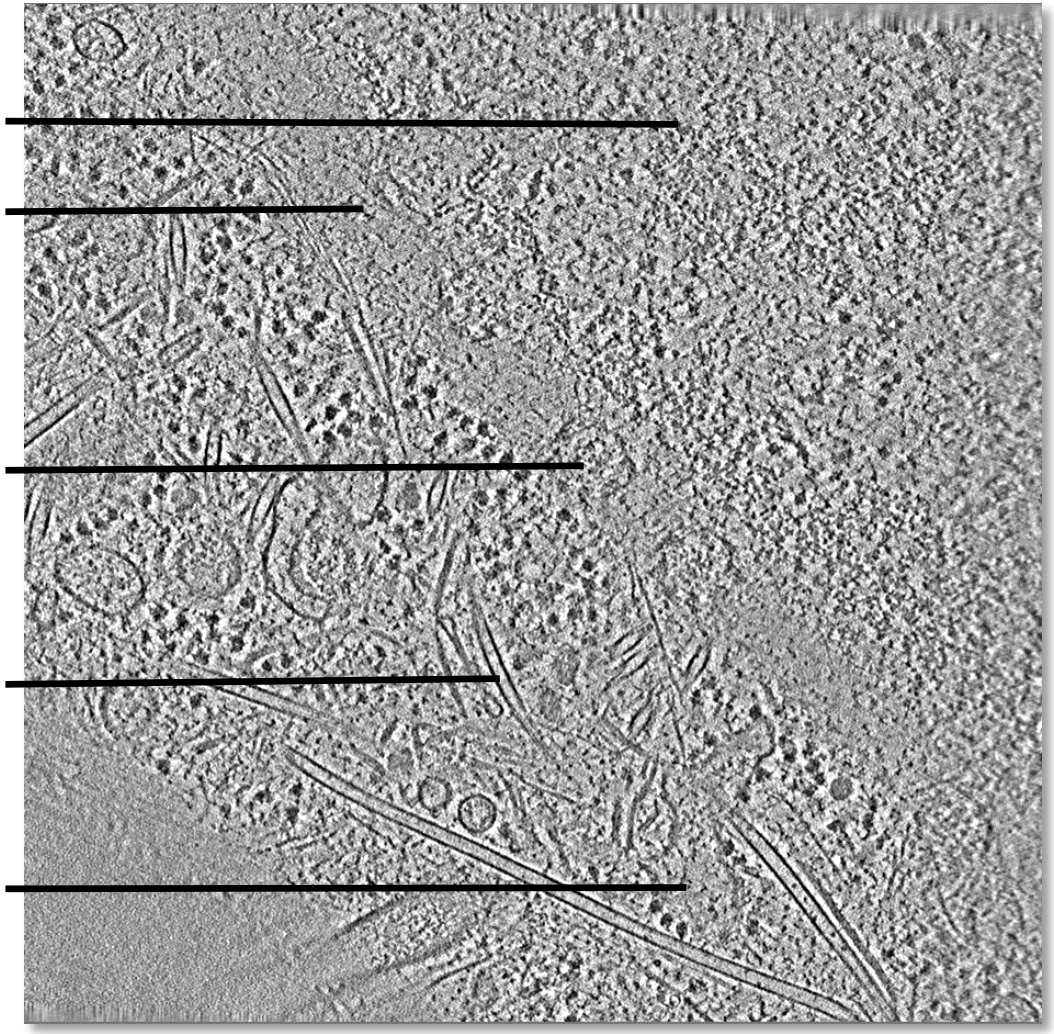


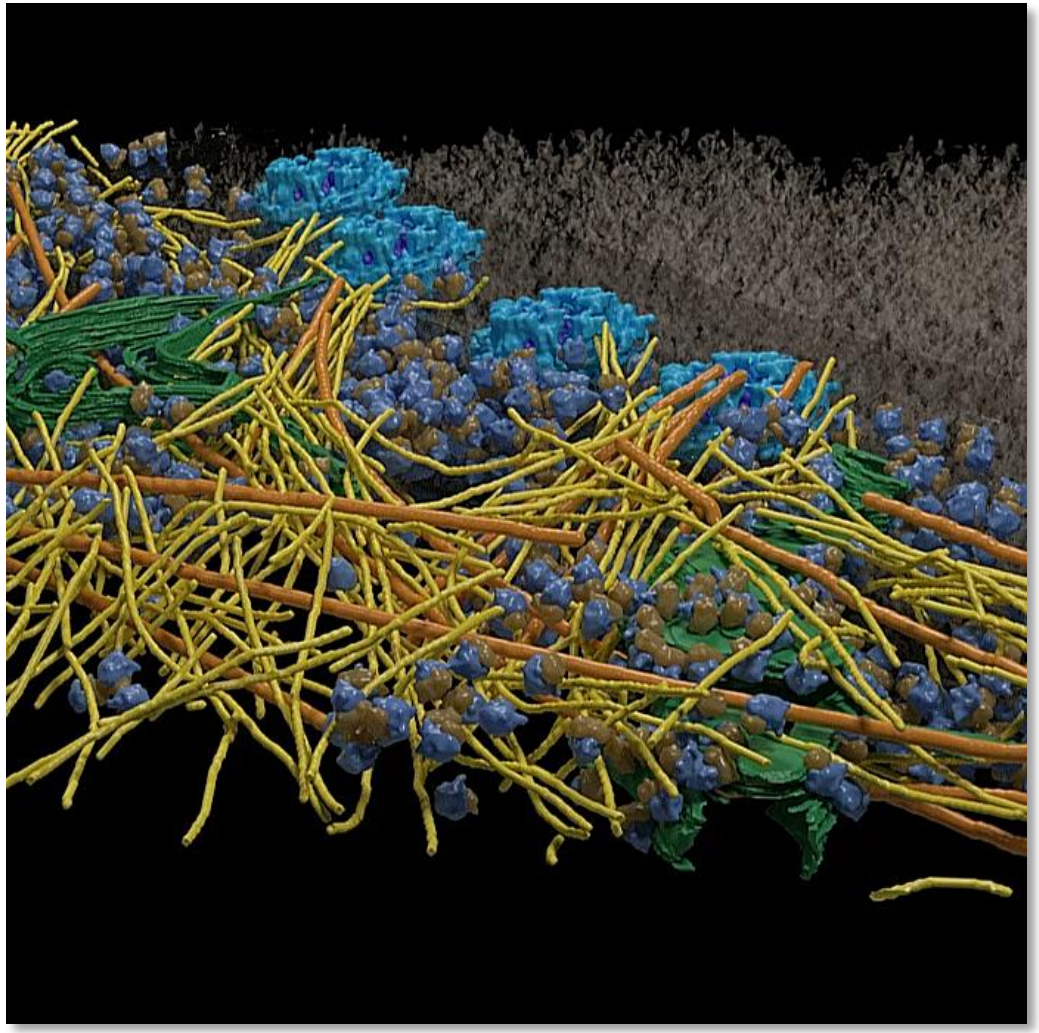
**VISUALIZATION**



Data Courtesy Max Planck Institute of Biochemistry | *Bykov et al., eLife 2017*

# Cryo-tomography of cryo-lamella, the molecular organization of the HeLa cell



**TOMOGRAM**

**VISUALIZATION**



Cryo-Tomography of Hela Cell, Data Courtesy Max Planck Institute of Biochemistry | *Mahamid et al., Science 2016*

# Cryo-3D Imaging Seamless workflow | Cryo-Tomography and Cryo-Slice&View

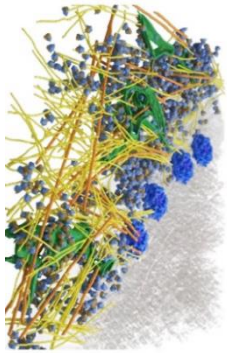
**CRYO-FIB MILLING**

**Aquilos 2**

Cryo-lamella

**CRYO-TEM TOMOGRAPHY**

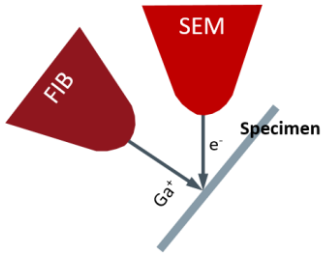
**Krios G4**



## Cryo-Tomography

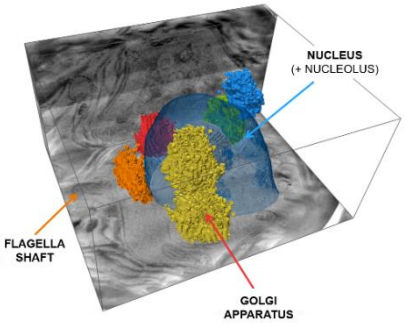
Before cells can be imaged by cryo-EM, they must be thinned to 150-300 nm with a cryo-FIB microscope to become electron-transparent for TEM tomography.

Slice & View




**CRYO-SLICE&VIEW**

**Auto Slice & View™**




## Cryo-Slice&View

Sequentially removing frozen material with the ion beam and imaging the milled block faces with the electron beam to obtain 3D data.

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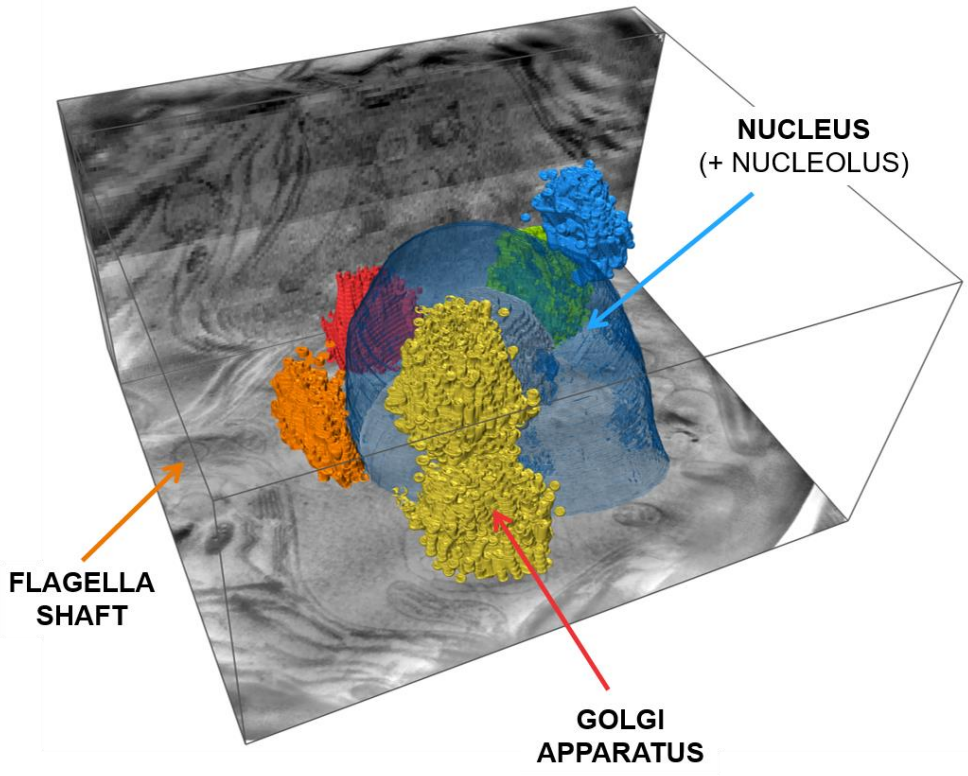
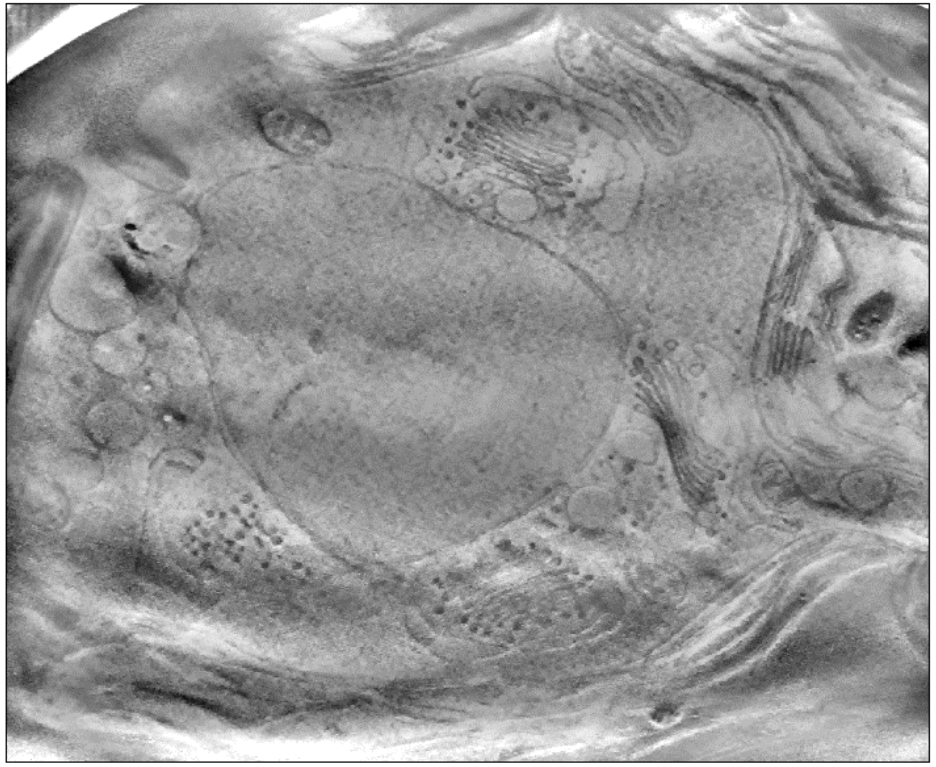
# Cryo-Auto Slice & View | Aquilos 2 in Cryo-3D Volume Imaging



**Cryo ASV** | *Chlamydomonas cell*
Aquilos Cryo-FIB/Cryo-Slice & View

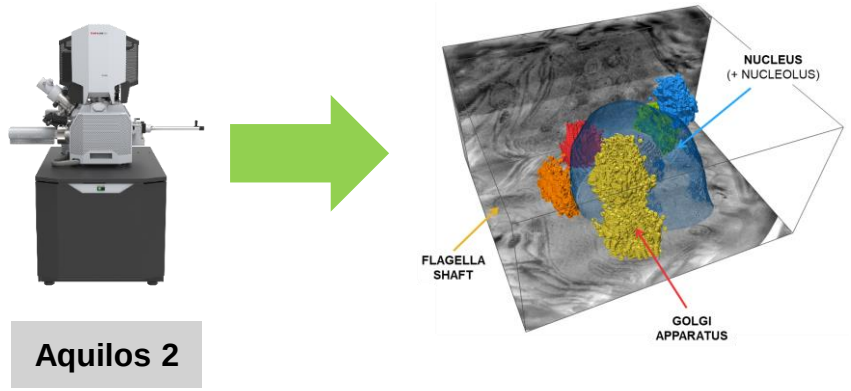
# In situ Cryo-3D imaging

## Cryo Tomography
## High resolution 3D imaging

Aquilos 2 | Krios G4

- Aquilos 2, dedicated Cryo-FIB to prepare lamella of biological specimens and transfer to cryo-TEM for acquiring tomography data.
- Cryo-FIB as a key technology herein, and cryo-TEM are combined within Cryo-Tomography workflow.
- To understand complex biological mechanisms, proteins structures and complexes are imaged in 3D at nanoscale resolution within a cell while maintaining their context.

## Cryo-Slice & View
## Volume 3D imaging




Aquilos 2

- Auto Slice & View™ in Aquilos 2 acquires 3D images under cryogenic conditions by sequentially milling then imaging a cross-sectioned area, such as the interior of a vitrified cell.
- Aquilos is integrated with cryo-stage and adjacent cryo-hardware that protects the frozen-hydrated samples from contamination.
- Cryo-Slice & View allow to obtain volume 3D imaging for understanding Cellular organization in tissue.

# For more information:

Thermo Fisher Scientific
日本エフイー・アイ株式会社

**E-Mail:** JPTOK.sales-jp@thermofisher.com

**Tel:** 03-3740-0970

ライフサイエンス部門

甲斐　翼：　**E-mail:** tsubasa.kai@thermofisher.com

**Mobile:** 080-3579-6241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