thermoscientific

APPLICATION NOTE

Cryo-Tomography for Cell Biology

Viewing the inner workings of cells

High-resolution cryo-electron tomography (cryo-ET) avoids the alterations caused by conventional preparation techniques such as chemical fixation, allowing for imaging of cellular morphology in fully hydrated conditions. To help researchers understand complex biological mechanisms, proteins structures and complexes are imaged in 3D at nanoscale resolution within a cell while maintaining their context.

Using cryo-ET for sub-cellular imaging

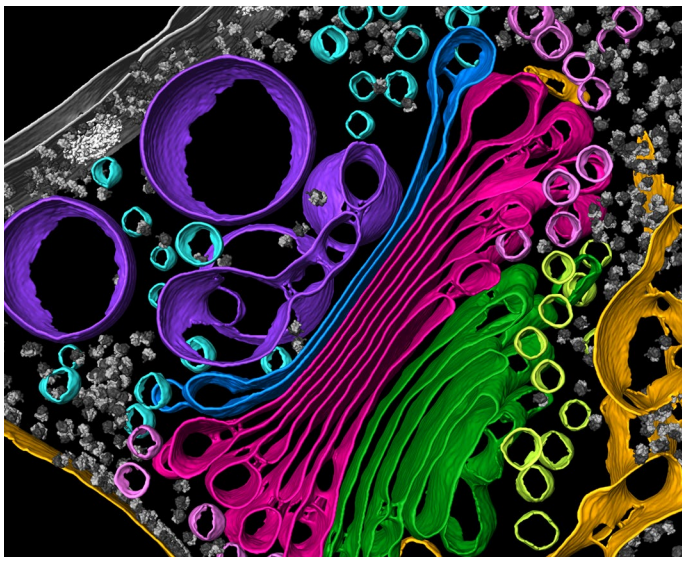
Understanding the structural basis for cellular processes is essential for understanding how cells function. Most cellular structures, proteins and organelles are too small to be resolved by light microscopes, but can be resolved by a cryo-electron microscope (cryo-EM). However, single particle cryo-EM is limited to highly purified and isolated proteins lacking a connection to the cellular context. Cryo-electron tomography expands cryo-EM by visualizing proteins that contribute to a mechanism within their functional cellular environments from a flash-frozen cell—all within a single cryo-ET 3D dataset.

Our integrated cryo-electron tomography workflow provides label-free, fixation-free, nanometer-resolution imaging of targeted regions from light microscopy. Seeing the entire picture on multiple levels, from molecules to organelles, complements existing dynamic techniques for highly accurate data to enable breakthrough discoveries.

Research highlights

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

Close up on COPs. To keep a city running, traffic must flow. 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 that 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 the behaviors of COP1 and COP1-coated vesicles in the cell. Source: Lux Fatimathas, BPoD (10 April 2018)



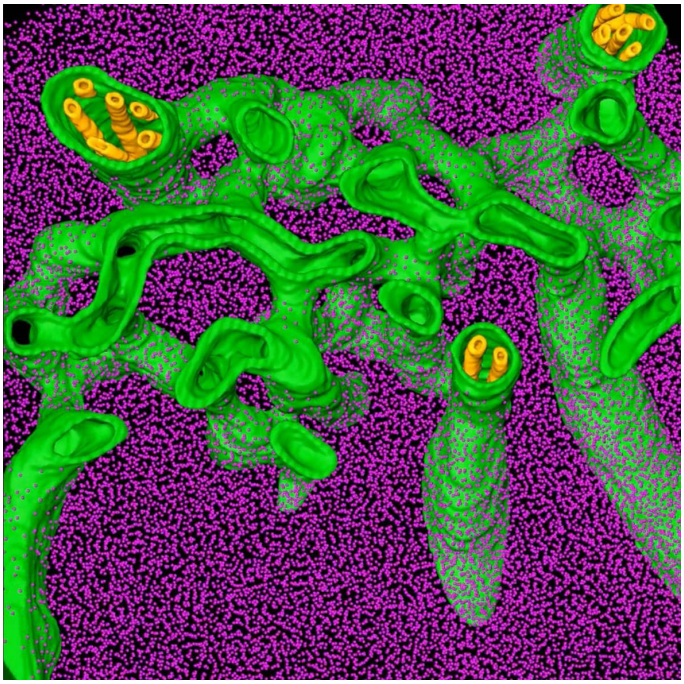
Chlamydomonas Golgi apparatus Courtesy of B. Engel, MPI Biochemistry

Freeman Rosenzweig E.S., Xu B., Kuhn Cuellar L., Engel B.D., Mackinder L.C.M., Konikas M.C. 2017 The eukaryotic CO_2-concentrating organelle is liquid-like and exhibits dynamic reorganization. Cell 171: 148-163. doi: 10.1016/j.cell.2017.08.008

ThermoFisher SCIENTIFIC

thermoscientific

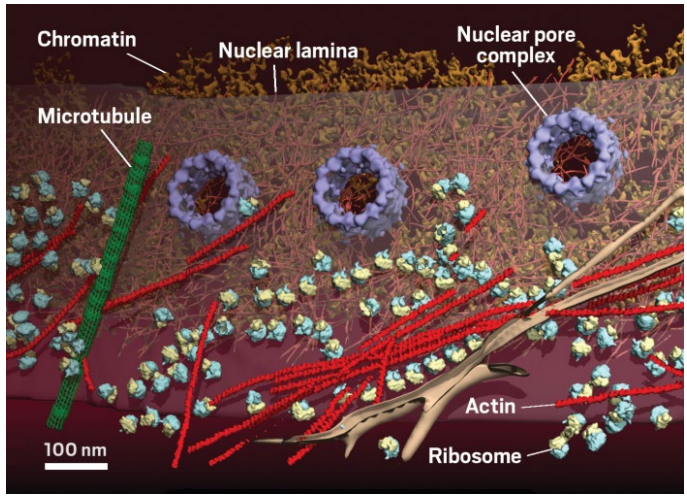
Green algae could hold clues for engineering faster-growing crops. Princeton-led studies provide a detailed look at an essential part of algae's growth machinery, with the eventual goal of applying this knowledge to improving the growth of crops. In this image below, the researchers used cryo-electron 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)



Cryo-electron tomogram of pyrenoid algal structure. Courtesy of B. Engel, MPI Biochemistry

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

Cryo-electron tomography provides first view of a cell's nucleus in its natural, undisturbed environment. This technique shows that protein filaments make the nucleus the stiffest organelle in the region. 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)



Nuclear periphery of a HeLa cell. Data courtesy of J. Mahamid, currently at EMBL.

Further Reading

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

Albert S., Schaffer M., Beck F., et al. 2017 Proteasomes tether to two distinct sites at the nuclear pore complex. PNAS 114, 13726–13731. doi: 10.1073/PNAS.1716305114

Delarue M., Brittingham G.P., Pfeffer S., et al. 2018 mTORC1 controls phase separation and the biophysical properties of the cytoplasm by tuning crowding. Cell 174: 338-349.e320. doi: 10.1016/j.cell.2018.05.042

Hagen C., Dent K.C., Zeev-Ben-Mordehai T., et al. 2015 Structural basis of vesicle formation at the inner nuclear membrane. Cell 163: 1692-1701. doi: 10.1016/j.cell.2015.11.029

Mosalaganti S., Kosinski J., Albert S., et al. 2018 In situ architecture of the algal nuclear pore complex. Nature Communications, 9, 2361. doi: 10.1038/s41467-018-04739-y

Swulius M.T., Nguyen L.T., Ladinsky M.S., et al. 2018 Structure of the fission yeast actomyosin ring during constriction. PNAS 115, E1455–E1464. doi: 10.1073/pnas.1711218115