Cryo-electron Tomography of Whole Cells

Three-dimensional Mapping of Intact Cellular Architecture

Cryo-electron tomography was used for studying the molecular organization of the cytoplasm in intact cells. Flat areas of cells grown on EM grids and preserved by rapid freezing within milliseconds were imaged in 3D. In the resulting maps the selected morphological and structural features were analyzed at unprecedented detail, and frequently correlated to light-microscopy images. We portray here host cell-virus interactions, the morphology of neurons and malaria parasites.

Analysis of Cellular Structures and Processes

Cryo-electron tomography (cryo-ET) recently emerged as a powerful method for studying complex biological specimens. Unordered and ordered assemblies, such as pleiomorphic viruses [1] and axonemal microtubules [2] respectively, are captured by rapid freezing in intact structural and functional states, and are then subjected to tomographic imaging. Resulting three-dimensional datasets provide unprecedented information. When applying cryo-ET to intact cells preserved by rapid freezing, one would expect to visualize a true representation of the cellular cytoplasm [3]. Repeatable structures, as well as the objects with conserved symmetry, can be further refined by the averaging of sub-tomograms, similarly to the ‘single particle' and crystallographic approaches that are routinely used in EM data processing [4]. And notably, the cellular details that are commonly resolved at 4-6 nm in the tomographic datasets can be directly correlated to the transmitted light and (video)-fluorescence images recorded at the light microscope level. This combined approach bridges the gap between light- and electron-microscopy imaging and leads to a comprehensive analysis of cellular structures and processes. We use cryo-ET to study the morphology and structural organization within several eukaryotic cells: *Plasmodium* sporozoites, the form of malaria parasite transmitted by mosquitoes to vertebrate hosts, poxvirus-infected cells, and neuronal processes. In the resulting tomograms we expect resolving molecular details at a resolution of approximately 4-6 nm, providing the regions of cells under investigation are thinner than 0.5-1 µm.
**Vaccinia Virus Infected Cells**

To study the structure of vaccinia virus in early stages of infection we cultivated fibroblast cells on EM grids, infected them with the intracellular mature virus, and preserved them by rapid freezing.

![OLYMPUS](image)

The structural changes in the virus core were observed at various time intervals prior to and after internalization into the cell, resulting in a time resolved sequence depicting fine details of the controlled virus disassembly. As a structural reference we used the tomographic maps of intact, isolated virions (fig. 1a) recorded and computed in the same conditions as in the whole cell approach [1]. We surprisingly found that the major changes to the vaccinia structure happen right before the internalization into the host cell cytoplasm [5]. This is the stage when the virus interacts with receptors exposed on the host cell plasma membrane. These rearrangements appear to be prerequisites for successful virus infection and proliferation, and were previously believed to happen in the reducing environment of the host cell cytoplasm. The changes include the release of the outer viral membrane compartment, an expansion of the core volume by a factor of two, and most notably, the de-condensation of DNA within the core, which is then delivered to the cytoplasm as an entity (fig. 1b). Pore like openings detected in the cores of intact virions were indeed appearing intact throughout the entire process of infection, up to the point of proteolytic core rupture.

**Neuronal Cells - Processes**

Cultivated neuronal cells are well-established models for studying neuronal function, and when grown on EM grids (fig. 2a) and investigated in the cryo-electron microscope they reveal diverse specialized features (fig. 2b). Prior to electron tomographic examination the living cells were usually imaged in the light
microscope, in order to potentially relate their function to their structures at molecular resolution. In the cryo-tomograms of intact neurons we primarily concentrated on cytoskeletal elements. In the lumen of microtubules we detected a particulate material of about 200kD that appeared regularly distributed and followed the pattern of tubulin monomers [6] (fig. 2c and d). We expect to gain a better insight in the structure and function of these luminal microtubule associated proteins after isolating and purifying them from the CNS microtubules. We are currently characterizing the cytoskeletal organization and examining actin arrays within the shafts (fig. 2c) and growth cones of neuronal axons and dendrites. Actin and very likely also microtubules play a central role in establishing neuronal cell polarization in young embryo hippocampal neurons. Cryo-ET enables a detailed inspection of these cytoskeletal elements in different parts of cells, thus contributing to important issues related to neuronal cell development.

Plasmodium Sporozoites

*Plasmodium* sporozoites are less than 1 µm thick and thus among the thinnest eukaryotic cells known. The cytoplasmic architecture of these highly motile parasites is amenable for cryo-ET studies in a whole-cell-approach without the need of sectioning, and allows examining thicker areas of a cell, such as those containing the nucleus, that would not be accessible in larger cells to conventional transmission electron microscopy. The whole cell, when examined *in toto* and close to its living state, reveals exceptional details of the cytoskeleton, organelles and membrane compartments, which have hitherto been unresolved using classical preservation methods. The apical end of a sporozoite that defines the phylum Apicomplexa hosts several organelles, which are important during host cell invasion (micronemes, rhoptries, dense granules). So called subpelicular microtubules reach out to the nucleus at about 2/3 of the sporozoite length and form a cage that encloses these organelles (fig. 3a). A detailed investigation of both the arrangement and structure of these microtubules exhibited several new features [7]. For instance it showed the unsymmetrical radial distribution of the microtubules in 3D and a flexible, side-on interaction with the polar ring, a structure of unknown composition and function that seems to hold the microtubule cage together. Additionally, a detailed 3D analysis of microtubules revealed unexpected periodic densities that are tightly coupled to the microtubule inner surface. These densities were also found in the related parasite toxoplasma and could thus constitute a factor that endows the microtubules of these parasites with their known unusual stability (fig. 3b).

**Future Prospects**
The powers of light-fluorescence microscopy can be proficiently combined with those of cryo-electron microscopy by correlative studies. This combination of microscopy techniques relates the dynamic records gathered by various light microscopy approaches with the underlying structural players (fig. 4). The correlative microscopy approach is suited, for instance, for the structural study of sporozoites, whose response to various drugs is reflected in different patterns of cell movements that can be monitored with fluorescence video-microscopy, whereas the underlying molecular machineries are approached by cryo-ET. Furthermore, cryo-light microscopy is a mode of imaging recently developed to facilitate the correlation of dynamic processes highlighted by fluorescent labelling with cryo-electron microscopy examinations.

When a fluorescently labelled cell, such as a sporozoite, is plunge-frozen directly after recording its physiology and movements on an EM grid with fluorescence video-microscopy, it is imaged again with cryo-light microscopy to determine its exact position on the EM grid and ensure both its preservation and physiological state prior to imaging with cryo-EM and cryo-ET. For this purpose, a special holder was designed that allows fluorescent light imaging of the frozen-hydrated sample at cryogenic temperature while protecting it from ice contamination [8]. The use of marker grids allows the unambiguous identification of specific cells and the targeting of fluorescently labelled areas within the same cells in both cryo-imaging schemes, thus minimizing pre-irradiation of the sample during search in cryo-EM. The forthcoming investigation in the electron microscope is limited by specimen thickness, so that most cells are inaccessible for whole-mount imaging. The recent progress in the field of cryo electron microscopy of vitreous sections (CEMOVIS) offers an excellent solution to this problem. In CEMOVIS the cells or tissues are instantly frozen at high pressure and then cut into thin sections that are examined in cryo-EM [9]. The areas of cells that were too thick for EM imaging as a rule, such as the nucleus and its surrounding, are now accessible for investigation in the native, fully hydrated state, avoiding the known artifacts of classical EM sample preservation (fig. 5a). Accordingly, the three dimensional investigation of vitreous sections by cryo-ET emerges now as a powerful tool for studying structural details of molecules and assemblies within the intact cytoplasm of any cell or tissue (fig. 5b).

A combination of these techniques opens new ways of elucidating the structure-function relationship on a routine basis.

References:
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