

Cryo-electron tomography: State of the art and future perspectives

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The long prevailing view of a cell as a membrane-bound reaction compartment filled with freely diffusing and colliding macromolecules can no longer be maintained. There is growing awareness that fundamental cellular functions are carried out by ensembles of macromolecules, protein complexes or 'molecular machines'. And, as in a factory, the operation of these machines must be coordinated to give rise to a stochastically variable supramolecular architecture. On this level of structure, the cell is, by and large, an uncharted territory. None of the existing imaging techniques enables the study of pleiomorphic structures, such as organelles or whole cells with a resolution of a few nanometers, as is required for identifying macromolecules *in situ* and for describing their interaction networks. Therefore, there is a strong incentive to develop methods, ideally non-invasive, to study the supramolecular architecture in a cellular context.

However, cryo-electron tomography (ET) is an imaging technique which allows the three-dimensional (3D) visualisation of cells within 4 to 6 nm resolution. ET is by no means a new imaging technique, but with the advent of computer-controlled electron microscopes and the automation of elaborate image acquisition procedures, it became possible to obtain molecular-resolution tomograms of structures as large and complex as whole prokaryotic cells or thin eukaryotic cells embedded in amorphous ice.

Tomograms of cells at molecular resolution are essentially 3D images of the cell's entire proteome, but with the current resolution, one can address only larger complexes in a cellular context. To widen the scope of cellular electron tomography it will be necessary to improve the resolution. Theoretical considerations and ongoing instrumental improvements, such as liquid helium cooling, improved detector designs and dual-axis tilting, make a resolution near 2 nm a realistic goal. These technical developments together with sophisticated automation and image analysis procedures should help to push the limits of cryo-ET and brighten the prospects to explore the uncharted territory of the molecular architecture of the cytoplasm.

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