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The State of the Art in Cryo-Electron Tomography

Jürgen M. Plitzko, Stephan Nickell, Günter Pfeifer and Wolfgang Baumeister

Max-Planck-Institut für Biochemie, molekulare Strukturbiologie, Am Klopferspitz 18A, 82152 Martinsried, Germany

Electron tomography (ET) has become a major tool for various investigations in life sciences and recently also in materials sciences over the last years. The technique is not really new, but the developments made in instrumentation, automation, acquisition control and in data processing within the last decade have directly led to the breakthroughs of ET for studying the 3-dimensional structure with resolutions in a few nanometer range [1, 2].

Cryo-electron tomography is a non-invasive imaging technique, which, in conjunction with cryo-sample preparation techniques, allows the study of pleiomorphic biological structures, such as supramolecular assemblies, organelles and whole cells in a close-to-native state [3, 4]. However, biological structures are very sensitive to the radiation of the electron beam, because they are build up of very weak scattering, light atoms and they do occur mostly in dimensions of several microns while some of the interesting features are on the molecular level. It is obvious that investigations by means of cryo-ET are pretty restricted, sometimes complicated or even impossible. All these constraints have to be addressed one by one to find a suitable solution or in some instances to find a good compromise. This has been extensively done in the past and the field of electron tomography is virtually booming at the moment. But there is still plenty of room for improvements, in order to make it a tool for a routine use or even for high throughput applications.

There are several approaches to tackle the instrumental constraints and sample related restrictions. Since recently the achievements, which have been made, are now implemented in commercially available instruments. One of these instruments is the Tecnai F30 Helium TEM (‘Polara’, FEI, Eindhoven, NL), equipped with a post column energy filter (GIF 2002, Gatan, Pleasanton, USA) and a 2kx2k CCD Camera. The ‘Polara’ is dedicated to do cryo-electron tomography. It allows investigations not only at liquid nitrogen temperature (l-N_2 ~ 80 K) but also at liquid helium temperature (l-He ~ 10 K). This should enable us to increase the total dose applied to the specimen either by increasing the dose per image or by using a higher sampling rate, i.e. an increased number of images with a finer angular increment. Additionally, the mechanical stability of the stage as well as the cryo-stability of the instrument is improved, which facilitates the investigation enormously.

Our main goal is the investigation of the molecular arrangement, structure and interaction within whole intact cells. Therefore resolutions of about 2 nm are necessary. Meanwhile we can look back on various ET-studies revealing structures in the range between 4 and 6 nm, depending on the sample material under investigation [5, 6, 7]. However, we will present recent results, regarding the still ‘ominous’ cryo-protection factor for studies at l-He temperature and we will compare tomograms of the same sample material, which were acquired at l-He temperature and at l-N_2 temperature [8]. In this regard we will discuss the ‘blessing or curse’ of l-He ET studies.
Additionally we will address the benefits of using double-axis-tilting under cryo-conditions in electron tomography [9, 10]. In single-axis-tilting the tilt range is limited to approximately +/-70°,

due to the arrangement and design of the standard specimen holder. This leads to a ‘missing wedge’ in Fourier space, which results in reconstruction artefacts. Together with Gatan we have already developed and implemented a double-tilt cryo-holder, where the sample can be rotated around more than 90° after the acquisition of a first tilt-series for recording a second set of tilted images perpendicular to the first one. Double-axis-tilting reduces the ‘missing wedge’ to a ‘missing pyramid’, which increases the isotropy and thus the quality of the resulting tomogram.
Furthermore, we will present advances in image processing, which is of equal importance in the analysis of the acquired and reconstructed tilt series. This will mainly include results in the automation of localization and detection of proteins within tomograms of cellular structures [11].

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