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Automated Cryo-ET: State of the Art and Future Perspectives

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The key problem in electron tomography and especially in cryo-electron tomography on biological samples, which for many years was a big obstacle, is to reconcile two requirements that are in conflict with each other: To obtain a reconstruction that is detailed and largely undistorted, one has to acquire images over as wide a angular range as possible with tilt increments as small as possible [1,2]. At the same time, the electron dose must be minimized, because at a distinct critical dose, the specimen undergoes structural degradation that in the worst scenario, can render a reconstruction meaningless. In principle one could fractionate the dose over as many projections as an optimized tilt geometry might require [3]. However there is a practical limitation to it: The signal-to-noise ratio (SNR) of 2 dimensional (2D) images has to be sufficient enough to permit the subsequent accurate alignment of the recorded projections, either by cross-correlation or by fiducial markers. This problem is further aggravated by the far-from-perfect mechanical accuracy of the tilting device that causes image shifts and changes in focus. Therefore, for each change of tilt angle, the specimen or its image has to be realigned and refocused. It is obvious that doing this manually and with minimal exposure to the electron beam is almost impossible.
In the late 1980s and early 1990s when computer controlled TEMs and large-area CCD cameras became available, the opportunity arose to automate tomographic data acquisition [4-11]. Automation clearly made the recording of of data sets not only less cumbersome and less time consuming, but first and foremost it allowed to keep the cumulative dose within tolerable limits. The fraction of dose that is spent on ‘overhead’ can be kept as low as 3 % of the total applied dose [12,13]. His changed the perspectives of electron tomography in a profound manner, while used from time to time for ultrastructural studies, mostly on plastic embedded biological material, cryo-ET studies on ice embedded biological samples has recently gained momentum.

With the use of automated procedures and user-friendly software, meanwhile, the recording of tilt series and their processing has almost become routine. With smaller structures, like bacteriophages, a resolution of 2.5 nm has been obtained [14] and for whole prokaryotic or thin eucaryotic cells resolutions are in the range of 4-5 nm, but prospects for further improvements are good [15]. Cryotomograms of organelles and cells, even at this level of resolution, contain an imposing amount of information. Essentially they are 3 dimensional (3D) images of the entire proteome, and they should ultimately enable us to map the spatial relationships of the full complement of macromolecules in an unpeturbed cellular context. However, new strategies and innovative image analysis techniques are needed to gain access to this information at the highest possible resolution.
With the new technological advances made in instrumentation, like the introduction of cryo-stages operating at liquid nitrogen temperature as well as at liquid helium temperature, new designs of energy filters with larger entrance apertures and better aberration correcting optics, a improved stability of the electron optical lens perfomance, goniometers with a higher accuracy, multi-specimen and dual-axis-cryo holders, ultra high sensitive and large array CCD detectors etc., it became additionally possible to improve and even speed up the acquisition process for tomographic tilt series and cryo-EM data [16-23].

The common objective is the fast fully automated access to the raw data, which can be a tilt series or a set of 2D images for single particle analysis, and a subsequent fast and fully automated analysis of the data at hand. Still the best strategy is defined by the sample material. However, a combination of different approaches can be powerful if not necessary for an improvement in resolution and in the first instance for a better understanding of the complexity of biological structures.
At the moment we can select different software packages incorporating the classical 'full-tracking', semi-automated, pre-calibrated or 'on-the-fly' prediction schemes. In the course of this paper we will compare different acquisition schemes, accentuate their pros and cons and we will try to identify future perspectives in hard- and software development for automated cryo-electron tomography.

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