Minireview

From proteomic inventory to architecture

Wolfgang Baumeister*

Department of Structural Biology, Max-Planck-Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany

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Abstract Electron tomography can provide three-dimensional reconstructions of large pleomorphic structures at molecular resolution. While the principles of electron tomography have been known for decades, its use has gathered momentum only in recent years. Technological advances have made it possible to apply it to ice-embedded biological material (cryotomography), thereby ensuring a close-to-life preservation of the samples. In combination with advanced computational methods, such as molecular identification based on pattern recognition, it is a promising approach to comprehensively map macromolecular architecture inside organelles and cells and to visualize macromolecules at work in their natural environment.

Keywords: Proteomics; Structural biology; Electron tomography; Image analysis

1. Introduction

New approaches are needed to analyze the molecular interaction networks that underlie cellular behavior. Mass-spectrometry-based proteomics provides the indispensable parts lists ideally in a validated and quantitative form [1]. The next task will be to analyze interaction patterns in functional modules ranging from supramolecular assemblies to organelles or even whole cells. Some of these modules are tightly integrated – hence designated as 'complexes' – and robust enough to withstand the isolation and purification procedures that are traditionally used in biochemistry, and they are amenable to detailed studies with the established tools of structural biology [2]. Other supramolecular functional modules exist transiently and interact weakly because they are designed to undergo remodeling in response to specific signals. Here, affinity-based isolation methods in conjunction with mass-spectrometry can provide valuable insights into the composition of such modules, and they can detect interactions, whether they be direct or indirect [3]. However, such approaches are prone to errors and they fall short of revealing how exactly the components interact. As the complexity of functional modules increases, parts lists and low-dimensional interaction data no longer suffice to describe the architecture of networks. Three-dimensional images at molecular resolution, ideally taken in a non-invasive mode to avoid perturbations of the systems under scrutiny, could in principle provide this information. Electron tomography has unique potential for studying large pleomorphic structures and for visualizing macromolecules in a functional cellular context. With the implementation of automated low-dose data-acquisition procedures, it has become possible to study biological samples embedded in vitreous layers of ice, thereby combining the potential of three-dimensional imaging with a close-to-life preservation of structure [4]. Therefore, we are poised now to bridge the (resolution) gap that currently exists between cellular and molecular structural studies. Cryotomograms of organelles or cells with molecular resolution are essentially images of the respective proteomes and, in conjunction with advanced pattern recognition techniques, the tomograms can be used to reveal and interpret molecular architecture. Combining tomographic cellular maps with high-resolution structures of their components should ultimately enable us to generate pseudoatomic maps of large or otherwise elusive functional modules [5].

2. Principles and problems of electron tomography

Owing to the large depth of focus, electron micrographs are essentially two-dimensional projections of the object under study. Features from different levels are superimposed and as a consequence, such images are hard to interpret. Based on a principle first described by Radon [6], tomographic techniques acquire projections of an object as viewed from different angles and then synthesize these projections into a three-dimensional density map. In electron tomography, the specimen holder is tilted incrementally around an axis perpendicular to the electron beam and projection images are taken at each position. Before the three-dimensional density map is calculated – most commonly by a 'weighted back-projection' algorithm – the projection images must be mutually aligned within a common frame of reference (Fig. 1).

A fundamental problem in electron tomography, which for more than two decades stood in the way of its widespread use and restricted its application to radiation-hardened samples, is to reconcile two conflicting requirements. Firstly, to obtain a detailed and minimally distorted reconstruction, it is desirable to cover as wide an angular range as possible in the tilting experiment with increments as small as possible, i.e., one would like to maximize the number of projection images. At the same time, the cumulative electron dose in recording a tilt series that often comprises more than a hundred images must be kept within tolerable limits to prevent radiation

*Fax: +49 89 8578 2641.
E-mail address: baumeist@biochem.mpg.de (W. Baumeister).

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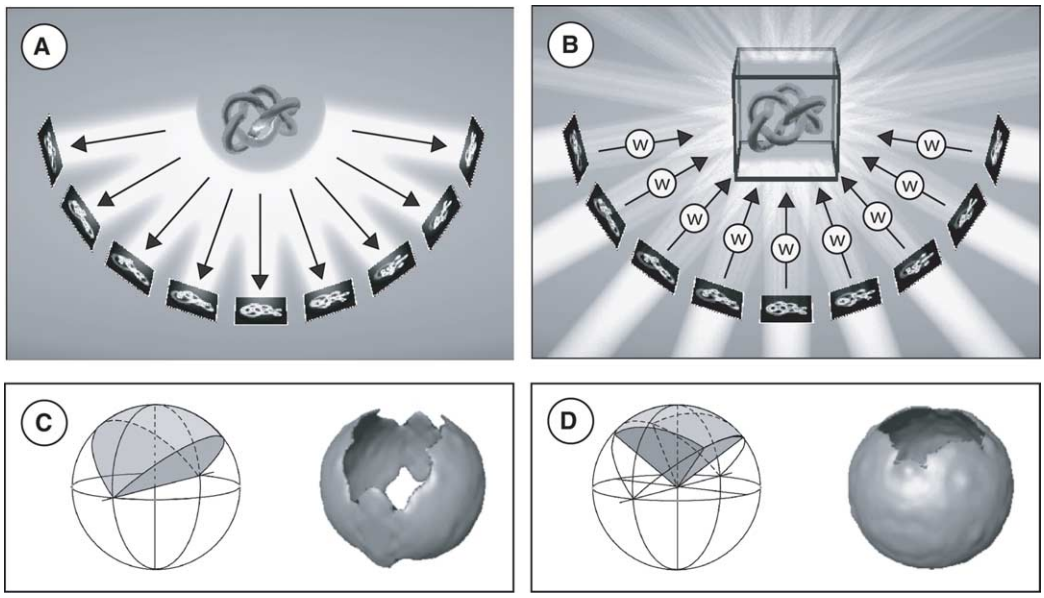

Fig. 1. (A) Single axis tilt tomographic data acquisition. The object is represented by a 'flexible knot' to emphasize the fact that electron tomography can reconstruct structures with unique topologies. A set of projection images is recorded as the object is tilted incrementally. (B) Following alignment of the projection images, the object is reconstructed by weighted backprojection. The sum of all projection bodies represents the three-dimensional density distribution of the object – the tomogram. (C) Owing to the limited tilt range (±60°), a 'missing wedge' remains unsampled resulting in a distorted reconstruction. (D) Dual axis tilting reduces the 'missing wedge' to a missing pyramid and, as a result, the reconstruction is less affected by distortions.

damage from erasing finer details of the structure or in the worst case, from rendering reconstructions meaningless. Biological materials embedded in vitreous ice, in particular, are extremely sensitive to radiation damage; therefore, it is of prime importance to minimize their exposure to the beam.

The successful realization of cryoelectron tomography takes advantage of the 'dose fractionation theorem'. Early theoretical considerations [7] that were essentially corroborated by more recent computer simulations [8] had suggested that, in principle, the electron dose that is needed to visualize structural features close to the resolution limit is the same for two and three-dimensional images containing equivalent information. Thus, according to this theorem, the separation of features in the z-direction is something that is freely available for the taking. In principle, one could distribute the dose over as many projections as the optimized tilt geometry might require, of course at the expense of lowering the signal-to-noise ratio of the individual two-dimensional images. By combining the (partially) redundant information of the projection images, the signal-to-noise ratio once again improves, similar to the improvement obtained by averaging statistically noisy images of repetitive structures. There is, nevertheless, a practical limitation. The signal-to-noise ratio of the two-dimensional images has to be sufficient to permit the accurate alignment that is needed to establish a common framework of coordinates. Alignment is done by cross-correlation, and this can be facilitated by adding high-contrast fiducial markers, usually gold nanoparticles, to the specimen.

3. Automated cryoelectron tomography

With the advent of computer-controlled transmission electron microscopes in the late 1980s, and the availability of large-area charge-coupled device (CCD) cameras, it became possible to develop sophisticated image-acquisition procedures that run in a fully automated manner [9–11]. This has allowed the recording of tomographic data sets with the specimen kept centered and at a uniform level of focus; whilst maintaining the cumulative electron dose within tolerable limits. The fraction of the dose that is spent on overhead (search, recentering, (auto)focusing) can be as low as 3% of the total dose; it would be utterly impossible to achieve such an efficiency with manual operation [12]. This has changed the perspectives of electron tomography in a profound manner. As demonstrated originally with 'phantom cells', i.e., liposomes encapsulating macromolecules [13,14], and more recently with prokaryotic [15,16] and eukaryotic cells [17], it enabled us to combine the potential of three-dimensional imaging with the best possible preservation of biological samples. Vitrification by rapid freezing ensures not only a close-to-life preservation of molecular and cellular structures, but it also allows one to take 'snapshots' of dynamic events [18]. It avoids the risks of artifacts traditionally associated with chemical fixation and staining or with the dehydration of cellular structures. Of equal importance, tomograms of frozen-hydrated structures represent natural density distributions, whereas staining reactions tend to produce intricate mixtures of positive and negative staining. As a consequence, the interpretation of such tomograms in molecular terms may be very problematic [19].

4. Resolution, signal-to-noise ratio and visualization of tomograms

With the use of automated procedures, the recording of low-dose tilt series has become routine, and user-friendly software is available for subsequent processing [20]. It is in fact now less cumbersome and less time-consuming to obtain a cryotomogram than going through the conventional procedures of plastic