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Cryotomography: Low-dose Automated Tomography of Frozen-hydrated Specimens

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Electron tomography is an imaging technique that provides 3D images of a specimen with nanometer scale resolution. The range of specimens that can be investigated with this technique is particularly wide, from large (500–1000 nm) unique variable structures such as whole cells to suspensions of thousands of small identical macromolecules (>200 kDa). When applied to cryofixed frozen-hydrated biological material, the technique is often referred to as cryotomography. In combination with automated low-dose data collection and advanced computational methods, such as molecular identification based on pattern recognition, cryotomography can be used to visualize the architecture of small cells and organelles and/or to map macromolecular structures in their cellular environment. The resolution that can be obtained with cryotomography depends on several fundamental and technical issues related to specimen preparation, microscopy and subsequent image processing steps, but will typically be in the range of 5–10 nm.

1. INTRODUCTION

1.1. Basic Concept

Most objects, either biological or inorganic, have a 3D architecture. The higher the complexity of an object, the less revealing is the 2D image that is obtained with transmission electron microscopy (TEM) due to the superposition of multiple 3D structural features into one 2D projection image. 3D imaging of cellular structures has great impact on how we understand the cellular architecture, and provides a powerful tool to expand upon the 2D images obtained of cell biological structures when studied by conventional 2D TEM during the last decades.

Because of the large depth of focus of the instrument, electron micrographs taken with TEM are essentially 2D projections of the imaged specimen. In electron tomography, 2D images of a specimen are acquired as viewed from different angles and then synthesized into a 3D mass density map, often referred to as a tomogram. The specimen holder is tilted incrementally around an axis perpendicular to the electron beam, e.g. from –65° tilt to +65° tilt with 1° increments, and images are taken at each position. Before computation of the tomogram, the projection images must be mutually aligned within a common frame of reference.

The 3D reconstruction (tomogram) is computed either in real space or via interpolation in Fourier space and subsequent back-transformation. The description in Fourier space helps to explain the requirements for data