Phase-Contrast Cryo-Electron Tomography of Primary Cultured Neuronal Cells

Yoshiyuki Fukuda¹, Radostin Danev¹, Shoh Asano¹, Miroslava Schaffer¹, Florian Beck¹, Vladan Lučić¹, Wolfgang Baumeister¹.

¹Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, Germany.

To understand synaptic function, unveiling molecular architecture of synapse is one of the important issues. TEM observation of plastic embedded specimens stained with heavy metals has fundamentally contributed to our knowledge about basic structural features of neuronal synapses. However, structural investigations of synapse at the close-to-physiological state are required for a further understanding of synaptic architecture and function.

Cryo-electron tomography (CET) of vitrified, frozen-hydrated cells is a rapidly developing method for 3D visualization of cellular structures preserved in their natural, cellular environment [1]. In the TEM observation, bulk specimens such as cultured cells requires relatively large amount of defocus to enhance image contrast even it is known that large amount of defocus attenuates high frequency components in images.

In previous studies, Zernike type phase plate for TEM has been developed [2] and applied to frozen hydrated biological specimens [3]. Application of Zernike type phase plate was not only to purified specimens but also to cultured cells [4, 5]. Although Zernike type phase plate is possible to enhance image contrast but it also generates fringes around structures which make interpretation of structure difficult. Recently, new type of phase plate for TEM was developed. In this study, we applied CET with new phase plate to frozen hydrated primary cultured neuronal cells for visualizing details of molecular architecture at synapse.

First, we tried to record images of frozen hydrated primary cultured neuronal cells by 200kV TEM with new phase plate to evaluate effectiveness of new phase plate. As specimens, primary hippocampal neuronal cells were cultivated on EM specimen grids for 17-18 DIV and plunge-frozen by a rapid immersion into liquid ethane/propane mixture. In comparison with images recorded by defocus contrast method, images recorded with new phase plate showed improved contrast and without generating fringes around structures.

Recently, direct electron detector device (DDD) was developed. Because of the high sensitivity to electron, DDD has been applied for cryo-TEM observation to acquire high signal to noise ratio images. Therefore, we recorded tilt-series images of frozen hydrated primary cultured neuronal cells by DDD installed in 300kV TEM with new phase plate. In reconstructed tomograms, cellular organelles were clearly visualized. Furthermore, new phase plate enable us to visualize macro protein complexes seems to be a 26S proteasome in reconstructed tomograms. To confirm whether these particles are 26S proteasome, we attempted template matching using a 3D model of double-capped 26S proteasome reconstructed by single particle analysis of purified 26S proteasome as a template. As a result, 3D model reconstructed from picked particles showed specific feature of 26S proteasome such as Rpn1 subunit.

By the combination of new phase plate and DDD, it became possible to visualize fine structures of organelles and macro protein complex in reconstructed tomograms. However, application of CET is
typically restricted to peripheral cellular regions up to approximately 500 nm in thickness. Therefore, it is difficult for CET to observe synapse directly. To overcome the specimen thickness limitation of CET, we applied focused ion beam milling (FIB) [6] to frozen hydrated primary cultured neuronal cells. Then we recorded tilt-series images of FIB milled primary cultured neuronal cells. In the reconstructed tomogram, large presynaptic terminal and vesicles fusing with presynaptic membrane were visualized.

Thus, CET with new phase plate provides effective means for visualizing cellular structure. We expect that the combined application of new phase plate, CET and FIB to vitrified, frozen hydrated neuronal cells will contribute to investigations of synaptic architecture in intact cells at the close-to-physiological state for further understanding of synaptic function.

References


Figure 1. Cryo-electron tomography of frozen hydrated primary cultured neuronal cells with new phase plate. Left: low mag image of frozen hydrated primary cultured neuronal cell. Center: tilt image at 0° corresponding to red square in left image. Right: 4.25 nm thickness slice image from reconstructed tomogram. Scale bars, left 5 µm, center and right 500 nm.