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Date: Monday, November 30th, 2015
Time: 11:00 am– 12:00 (noon)
Venue: C016, Lab1, Level C

"Investigation of in situ cellular architecture by Electron Cryo-Tomography with Volta Phase Plate"

Abstract:

Studies of molecular sociology of cells and *in situ* studies of macro molecular assemblies are critical for our understanding of cellular function. Electron cryo-tomography (ECT) of vitrified, frozen-hydrated cells provides a means of studying the three dimensional structure of pleomorphic objects, such as organelles or cells preserved in their natural, cellular environment, with a resolution of 1 to 3 nm range. However, low signal-to-noise ratio in image is a drawback of ECT.

In order to improve the image contrast of frozen-hydrated specimens, several kinds of phase plates have been developed. Among them, Zernike type phase plate has been used for studies of frozen-hydrated biological specimens in previous studies. Although Zernike type phase plate is possible to enhance image contrast but it also generates fringes around structures which make structural interpretation difficult. Recently, new type of phase plate for TEM named Volta phase plate (VPP) which enhances image contrast without generating fringes was developed.

In this study, we applied VPP to ECT of frozen-hydrated cells such as magnetotactic bacteria, *Thermoplasma acidophilum* and primary cultured neuronal cells for investigation of cellular architecture *in situ*. Due to the improvement of visibility provided by VPP, 26S proteasome particles could be visualized in primary cultured neuronal cells. For the *in situ* studies of macro molecular structure, we attempted template matching using a 3D model of single-capped 26S proteasome and sub-tomogram averaging of matched particles. Two of major conformational states “ground state” and “substrate-processing state” are obtained by classification.

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