

Introduction of high throughput and high flexible workflows of cryo TEM

Naoki Hosogi¹

¹JEOL Ltd.,

A Cryo transmission electron microscopy (TEM) provides high resolution structural information of a specimen close to its natural state without any disturbance during the process of sample preparation. Recently, cryo TEM can achieve very exciting biological results in combination with the methods of single particle analysis (SPA) and electron tomography (ET). And these methods require large number of micrographs for obtaining good reconstruction. Therefore, these images should be collected by automatic acquisition system.

We introduce the SPA workflow of the high throughput data acquisition with several features such as “Quick collection” and “ZERO fringe system” of our dedicated cryo TEM named as “CRYO ARM™ 300 II”

(Fig. 1). “Quick collection” realized high-speed data acquisition by combining beam shift with image shift. The excellent beam control of this system realized no beam tilt accompanied by beam shift, resulting in coma-free FOV (field of view) shift. Hence, the high throughput is realized with high image quality. “ZERO fringe system” is a unique “Koehler mode” illumination. In this illumination mode, almost no interference fringe exists in the illumination area and thus can make the illumination area almost same as the detector size. It allows users to acquire more images from a verified sample area. In combination with these features, CRYO ARM™ 300 II could achieve high speed data acquisition such as over a thousand micrographs per an hour.

Quite recently, cryo-ET is widely known as a well-established technique for imaging and assessment of 3D structural details of subcellular macromolecular complexes and organelles in their nearly natural context in the cell. We also introduce the workflow and some application data of cryo-ET. Cryo-ET usually handle with thicker samples than those for SPA because of its target. Energy filters are important hardware components for cryo-ET data collection. This technology enables the removal of inelastically scattered electrons which introduce the background noise in micrographs. CRYO ARM™ 300 II equips an incolumn-type energy filter named as omega filter. The omega filter intercepts energy-loss spectra (electrons) and uses only zero-loss electrons (without energy loss) to form an image. Since this zero-loss image reduces noise, the image provides high contrast.

Keywords: Cryo electron microscopy; Single particle analysis; Cryo electron tomography



Fig. 1 appearance of CRYO ARM™ 300 II
This instrument can take SPA data set with high throughput and tomographic dataset with high contrast.