Cryo-electron microscope developed for simultaneous STEM, SEM imaging and its application to biological samples

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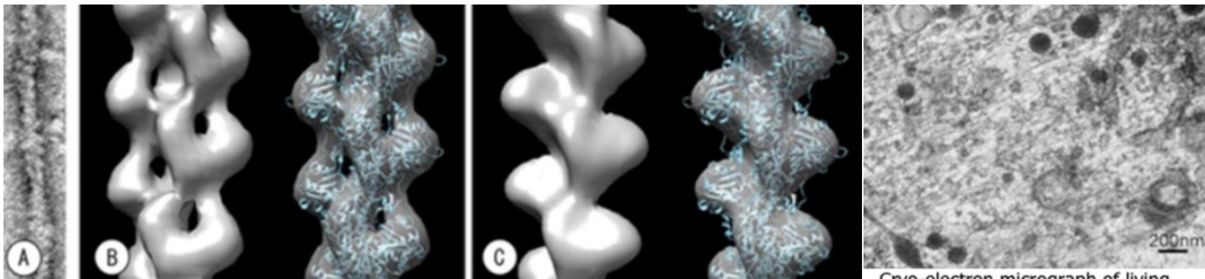
Cryo-electron microscopy (cryo-EM), which has become gradually popular in recent years, is used exclusively herein for single particle analysis of purified proteins and viruses in their native state. Meanwhile, cryo-EM is not only useful for single particle analysis, but is also an extremely powerful tool for conventional structural observations. In cryo-EM, living cells are quickly frozen and observed without any further treatment, that allows even biological samples to be observed at practical EM resolution. This is a crucial point, because conventional EM preparation techniques such as freeze-etching replicas or ultra-thin sections incur a reduction of resolution during pre-treatment. We aim to develop a cryo-EM that everybody can use easily in a wide field of sciences with an economical budget. In particular, a low-voltage cryo-EM (STEM) was developed based on in-lens SEM (Hitachi SU9000) equipped with a STEM detector to capture SEM and STEM images simultaneously. For this purpose, new cryo-transfer holder and anti-contamination trap are developed, in which are equipped with a vacuum system to release the gas from liquid nitrogen for convergence to slush nitrogen. In this way, we succeeded ultimately in reaching temperatures as low as -190°C. Practical observation of samples was carried out while maintaining the specimen holder at -180°C. We used unroofing method instead of cryo-sectioning to observe membrane cytoskeleton panoramically. Because soluble components in a cell are washed away upon unroofing, the cytoskeleton could be observed with sufficient contrast on focus. Surprisingly, ribosomes and endoplasmic reticulum remained partially intact after unroofing. Another important finding was derived from the single particle analysis using the cryo-EM developed in this study. The objective lens is not directly involved in image formation in STEM optics, which seems to be optimal for single-particle analysis. This advantage is particularly prominent in our developed low-voltage cryo-EM installed with a cold-field emission electron gun, which generates a very focused electron beam (0.34 nm in diameter). Indeed, molecular structure of actin filaments calculated from only 20 images obtained by our 30 kV STEM exhibited a much higher resolution than that obtained by conventional 100 kV TEM. Because the images were obtained without CTF correction and with high contrast on focus, the molecular structure was determined more precisely than in the conventional way by single particle analysis using a small number of images. This fact suggests the possibility of *in situ* single particle analysis revealing the real molecular structure in a cell, but not purified molecules.



Three dimensional reconstructed actin filaments in 30kV STEM (B) show better fitting with molecular model (Oda et al, Nature 2009) than those in conventional 100kV TEM(C).

Cryo-electron micrograph of living cells (unroofed) showing actin filaments, microtubules, ER and ribosomes at high contrast.