Deep views into eukaryotic cells using CryoSTEM tomography

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CryoSTEM tomography (CSTET) is a recently developed technique [1-4] combining cryo-electron tomography (CET) and scanning transmission electron microscopy (STEM). CET of cryogenically preserved prokaryotic and eukaryotic cells has provided rich detail on the in-situ organization of macromolecules and organelles. Energy filtering, and recent phase-plate and direct detector technologies will allow for in-situ structure determination of macromolecules. However, sample thickness is limited to around 300 nm (close to the mean free path for inelastic scattering), due to the dependence on phase contrast from elastically scattered electrons. Trimming biological specimens down to these thicknesses is not trivial for vitrified specimens.

With STEM modality, the signal is acquired incoherently, with contrast provided by variations in mass thickness along the path of the electron, and atomic number. The natural limit for sample thickness is extended to the realm of the mean free path for elastic scattering, which is roughly three times that for inelastic scattering for biological and soft materials specimens consisting of light elements [4]. In addition, the STEM data acquisition naturally allows for dynamic focusing, providing fully focused images even at high tilts. Key elements for successful data collection include careful choice of semi-convergence angle and distribution of electron dose.

While the bright-field STEM data provides excellent morphological information, the simultaneously collected dark field signal provides information on chemical content, due to its sensitivity to atomic number. In addition, Energy-dispersive X-ray spectroscopy (EDX) can provide on-the-spot chemical characterization.

Using these methods, we have obtained tomographic reconstructions of intact vitrified mammalian cells in areas up to one micron in thickness, and have characterized amorphous deposits of calcium phosphate granules in the mitochondrial matrices. These granules may be used as a form of calcium storage in mitochondria.

References

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