Cryo-STEM Tomography of Eukaryotic and Prokaryotic Cells

Wolf, S.[1]

[1] Weizmann Institute of Science, Israel

Cryo-electron tomography (CET) allows for macromolecular structure elucidation within the context of the cell milieu, and recent availability of technical advances such as hole-free phase plates and direct electron detectors are providing unprecedented detailed results. The thickness limitation for CET studies (<= 300 nm, close to the mean free path for inelastic scattering) is due to the dependence on phase contrast from elastically scattered electrons. Using energy filters, inelastically scattered electrons can be blocked off, but this reduces signal at higher tilts while still depositing damaging energy into the sample. Thus, imaging is limited to the thin areas of cells, or the cells must be thinned by either cryo-microtomy or focused ion beam milling, thus removing the surrounding cellular context.

Cryo-scanning transmission electron tomography (CSTET) provides an ability to study samples up to three times thicker (the realm of the mean free path for elastic scattering), since electrons are detected (counted) incoherently and the image is formed by recording intensities as a function of position of the scanning beam. Brightfield and darkfield detectors provide independent and complementary information on vitrified specimens. The Brightfield tomogram provides morphology based on mass/thickness contrast, and the darkfield tomogram provides information on chemical content, based on the dependence of atomic number on scattering intensity.

Energy dispersive Xray spectroscopy can provide on-the-spot chemical analysis of deposits or other sufficiently large stores of ions, metals or other species.

Examples from recent studies will be presented, including human and bacterial cells.

References:

SG Wolf, L Houben & M Elbaum, *Nature Methods* **11(4)**, 423-428 (2014).

SG Wolf, P Rez & M Elbaum, *J. Microsc*. **260(2)**, 227-233 (2015).

P Rez, T Larsen & M Elbaum, *J. Struct.Biol.* **196(3)** 466-478 (2016).

M Elbaum, SG Wolf & L Houben, *MRS Bulletin* **41(7)**, 542-548 (2016).

SG Wolf, Y Mutsafi, T Dadosh, T Ilani, B Horowitz, S Rubin, M Elbaum & D Fass, *eLIFE* **6**:e29929 (2017).

SG Wolf, E Shimoni, M Elbaum & L Houben, Chpt 2 in *Cellular Imaging,* Ed. EG Hanssen, Springer (2018).