

Saccharomyces pombe visualization using focused ion beam methods

Holmlund, C.¹, Henriksson, S.¹ and Lee, C.C.¹

¹ Umea University, Sweden

Recent development of effective workflow for cryo focused ion beam (cryo-FIB) and cryo-electron tomography has shown great importance for visualization of tissues, cells, subcellular structures and organelles at near native state. At the Core facility for Electron microscopy, at Umeå University, we routinely prepare electron-transparent lamellas from vitrified cells by cryo-FIB milling. Lamellas are subsequently analysed by transmission electron microscopy (TEM), tomography and 3D image reconstruction using the IMOD software package, yielding low contrast but high resolution information. Alternatively, we also use FIB and scanning electron microscopy (FIB-SEM) for 3D cellular imaging by serial sectioning of resin-embedded samples, thus yielding a large reconstructed volume with beautiful intracellular contrast. In this particular study, we observe the morphology and cellular ultrastructure of fission yeast (*Saccharomyces pombe*). *Saccharomyces pombe* is an important model organism, popular for studying basic biology of eukaryotic cells. Both FIB preparation methods resulted in different views of the yeast cells. The cryo-lamella and cryo-electron tomography method gives high resolution information of organelles and molecular complexes but limited information of spatial organization due to thin lamella (200 nm). In contrast, FIB-SEM volume imaging of resin-embedded yeast renders more spatial organization of the organelles and the full cell volume. Combining both techniques enables us to acquire a more complete view and to study eukaryotic cells at full perspective.