From Autophagy to Pollen Tubes – Harnessing the Full Potential of *In-Situ* Cryo-ET

Dr. Philipp S. ERDMANN
Max-Planck Institute of Biochemistry, Germany

In situ cryo-electron tomography - i.e. cryo-focused ion beam (FIB) milling in conjunction with cryo-electron tomography (cryo-ET) - has changed the way biological systems can be analyzed and modeled. Using this approach, intact cells and even tissue can now be imaged in a native state and at high resolution, allowing both the quantitative analysis of cellular constituents ('visual proteomics') as well as modeling of dynamic cellular processes. Some of these might be localized to specific sites within a cell (e.g. the endoplasmatic reticulum, the cell membrane, etc.) and therefore need to be specifically targeted, to not be missed during the milling process. We therefore use a 3D-correlative approach, combining confocal cryo-fluorescence microscopy (FLM) and FIB milling to study such rare biological events.
Our research covers a broad range of topics – from autophagy in S. cerevisiae, to ribosome biogenesis in C. reinhardtii and to germinating pollen tubes of flowering plants. We here present a snapshot of what cryo-focused ion beam milling together with template-matching and subtomogram averaging can accomplish today and how we are seeking to push the current limitations toward reaching sub-nanometer subtomogram averaging in situ.