

Cryofib Milling of Large Tissue Samples with On-The-fly Localization

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Cryo-electron tomography (cryoET) is a powerful tool for exploring the molecular structure of large organisms. However, technical challenges still limit cryoET applications on large samples. In particular, localization and cutting out objects of interest from a large tissue sample are still difficult steps. I will present a sample thinning strategy and workflow for tissue samples based on cryo-focused ion beam (cryoFIB) milling. This workflow provides a full solution for isolating objects of interest by starting from a millimeter-sized tissue sample and ending with hundred-nanometer-thin lamellae. The workflow involves sample fixation, pre-sectioning, a two-step milling strategy, and localization of the object of interest using cellular secondary electron imaging (CSEI). A furrow-ridge structure with an additional conductive Pt layer to reduce the beam-induced charging is designed and used. CSEI is highlighted in the workflow, which provides on-the-fly localization during cryoFIB milling.