Increasing throughput in cellular cryo-Electron Tomography

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In this lecture I will present two developments that increase the throughput and usability of cryo-ET in cells and tissue.

Cryo-ET in situ enables resolving the protein structures in their native environment. Recent advances in cryo-FIB milling automation enabled imaging of all cellular regions. To apply cryo-FIB milling to large biological tissue, cryo-lift-out was introduced. This method entails the isolation of a region from the bulk to allow TEM imaging. Since the utility of this method is limited by its complexity, we introduced AutoLiftout, machine learning-based software to automate and guide cryo-lift-out across various specimens.

Once samples have been isolated, it is extremely useful to image large fields-of-view while maintaining a high resolution, a procedure that requires tiling. Transmission electron microscopes typically have round beam profiles; therefore, tiling across a large field-of-view is either imperfect or results in uneven exposures, which is a problem on dose-sensitive samples. Here, I will present a square electron beam that can be easily retrofitted in existing microscopes and demonstrate its application showing it can title nearly perfectly and deliver cryo-EM imaging with resolution comparable to conventional setups.