# Cryo Super Resolution both ways: Advances in cryo light microscopy and electron tomography

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Cryo correlative light and electron microscopy (cryoCLEM) is an emerging technique combining high-resolution structural information of electron microscopy (EM) with functional information provided by fluorescence microscopy (FM). However, precisely locating specific events of interest inside a frozen-hydrated cell has remained extremely challenging. Here we report on our efforts in optimizing both subtomogram averaging and cryoFM towards nanometer precision. We implemented a cryoCLEM pipeline that enables a correlation accuracy in the range of 60 nm by introducing (i) single wavelength markers for precisely correlating FM and EM images, (ii) multi-wavelength markers to correct for shifts between different fluorescent channels, and (iii) individual correlation estimation for each object of interest. However, this approach is limited to objects smaller than the FM resolution. To take it a step further, we developed a fluorescent microscope setup to record single molecule localization under cryo conditions using standard fluorescent proteins. We achieved an average localization accuracy of 30 nm in cryo super-resolution light microscopy and a resolution increased by 3-5 times over wide-field cryoFM. In parallel, developments in subtomogram averaging performed on cryoET data acquired with direct detector cameras, allows us to reconstruct 3D volumes of functional macromolecules with improving resolution. Taken together, these methods provide valuable tools for characterizing rare events inside vitrified cells that are otherwise extremely challenging and time-consuming to find.