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IT-7-P-2483 High resolution cryo-CLEM: from cryo-light microscopy to cryo-TEM, through cryo-milling

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Correlative light and electron microscopy (CLEM) aims at combining the large field of view and chemical specificity of fluorescence microscopy with the high resolution ultra-structural details revealed by electron microscopy. CLEM can be extremely powerful in extending electron microscopy analysis to rare events that are impossible to target based on EM morphology alone. If CLEM is done on frozen hydrated samples there is also the opportunity to perform structural studies of complexes in situ.

Here is presented an innovative design for a cryo-light microscopy stage, developed to acquire data in cryo-light microscopy maximizing the resolution and minimizing the contaminations typically deposited on the sample during acquisition. The proposed design is extremely simple, where the stage is immobile and an inverted microscope is moved underneath. This allows the sample to be stored at cryogenic temperature, while the microscope and the objective are kept at room temperature in order to optimize the image quality.

Once the sample has been imaged in the light microscope, if suitable, it can directly go into the TEM for cryo electron tomography or single particle data acquisition. Considering the minimal amount of contamination accumulated during imaging it can easily be used for structural studies. In case the sample is too thick to be inspected in the TEM then a thinning procedure can be performed in a cryo dual-beam (Rigort A. et al JSB 2010; Rigort A. et al PNAS 2012). Relocation of a feature of interest identified in the light microscope and the dual beam is trivial thanks to the use of a cryo-shuttle which can be hosted in predefined orientation in both the light microscope and the dual-beam, as well as the use of a dedicated software framework.