Implementation Data Processing Pipeline for Cryo FIBSEM Volume Imaging

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There is a growing interest in structural techniques that take advantage of the vitrification of water in biological samples in order to preserve their native conditions. Most of these techniques involve high-resolution methods that aim to solve the structure of purified biological complexes. Nevertheless, these approaches are hindered because the structure is analyzed without its cellular context. For this reason, it is essential to integrate light microscopy techniques in the workflow of the cryo-techniques to localize protein complexes and solve its structure in their native environment.

The Valpuesta group and the cryo-electron microscopy platform at CNB-CSIC are implementing a new facility for cryo-correlative techniques that exploit the advantages of visible light and electron microscopy (cryo-CLEM). In this platform two cryo-CLEM approaches are available. First, the tomographic approach by lamellae preparation allows having a near-atomic resolution by the study of less than 300 nm thin windows opened into the cell (*lamella* FIB milling). Second, serial sectioning allows to obtain a whole cell volume at a resolution of 10 nm. This technique is based on the use of a iterative process of imaging with the SEM and surface milling with the FIB to obtain a stack of images containing the volume of the cell (cryo FIB-SEM tomogram).

Serial sectioning imaging has technique-related artifacts that are unavoidable and hinder the images obtained. To overcome this issue, we are focusing on developing an open-source and user-friendly software package for the processing of cryo FIB-SEM volumes to obtain more accurate insights.

The first operation our package executes is the stripes elimination to get rid of the lines created by the so-called curtain artifact produced by the shielding of the downstream material by a harder one upstream. The second operation is the elimination of the charging artifact generated by the accumulation of electrons in the sample that result in darker and lighter areas in the image. The third part is the stack alignment. Since electrons are accumulated not homogenously in the sample, this also means that these areas deflect differently the electrons creating local deformations in the image, thus making the alignment a non-trivial problem. Last, deep learning segmentation is performed using Cellpose software. This pipeline will reduce significantly the image artifacts within cryo FIB-SEM data allowing the extraction of more reliable information.