Developing a multimodal imaging pipeline for molecular biochemical studies with a 3D approach

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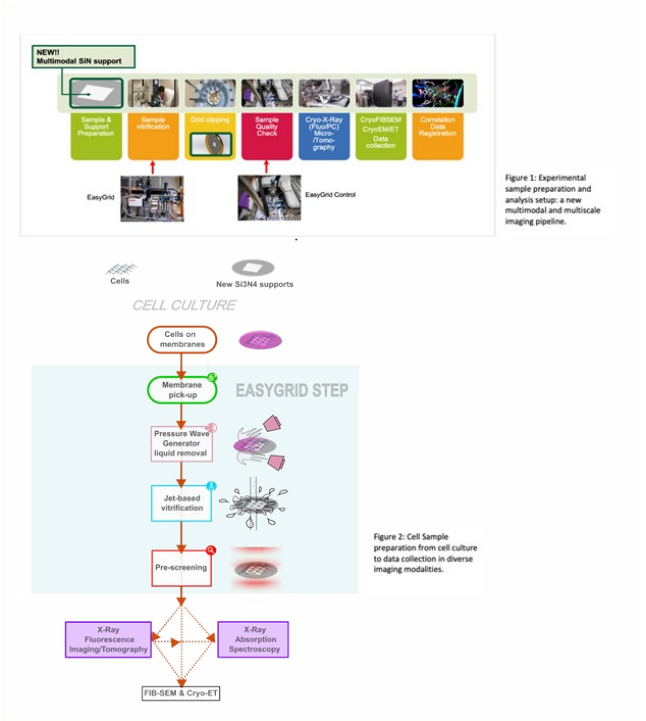
Background incl. aims
It is increasingly important to understand the 3D ultrastructure of cells and tissues particularly in the study of diseases and infection. The study of infection requires a complete understanding of where the virus, parasite or pathogen is located within the cell, how it interferes with the host cell's mechanisms and to follow its development. Several types of imaging are extremely powerful techniques in the study of these processes: XRFM & PC imaging/tomography, XAS & cryoEM/ET. Each of these techniques produces unique information that is crucial in the overall understanding of the problem. However, each technique requires different sample preparation (e.g. ice protective layer thickness) & different supports and holders, which makes it extremely difficult to locate and study the same area of interest in each sample in diverse imaging modalities.

Methods & Results
A complete experimental setup (Fig. 1) accompanied by an efficient workflow, to reach an optimised preparation process allows the easy transfer of the sample from one technique to another. New supports and sample holders are being designed and developed to be compatible with diverse targeted cryo-imaging techniques on near-native state frozen-hydrated samples (Fig. 2). EasyGrid machine [1] is used to automate and validate the sample vitrification. The preliminary results on the dose show that the radiation damage is very limited, suggesting that we can study the same sample by EM after having targeted a region of interest (bio-elemental accumulation/targeted organelles) with XR imaging. The optimization of the sample vitrification with a better amorphous ice quality and a reduction of crystallin ice, ice cracks and a better control of the ice thickness seem to allow a reduction of the flux used for the same image quality. For a multimodal and multiscale sample analysis, a new multimodal imaging pipeline is currently in development with the new design of sample support (collaboration with Silson company) compatible with the above-mentioned imaging and sample preparation instruments. It will allow the study of the same region of the same sample across many scales and tracking down the full process of biochemical mechanisms.

Conclusion

The generation of a new cryo-sample preparation process, suitable for XRFM, PC and Cryo-EM techniques, will make the beamtime (synchrotron/EM) use more efficient, potentially improving the success rate of multimodal imaging projects that will benefit a wide user community. The target community is the historical X-ray imaging users in biological fields who need to overcome difficulties in certain challenging projects, and a rapidly growing new population of users: the non-experts who will benefit directly from a complete multimodal imaging pipeline.

Graphic:



Keywords:

Electron Microscopy/Tomography, X-rayFluorescenceNanoscopie, phase contrast,EasyGrid

Reference:

[1]Gemin et al. EasyGrid. BioRxiv, in review in Nature Methods (2023)