EasyGrid: a new automated cryo-multimodal sample preparation

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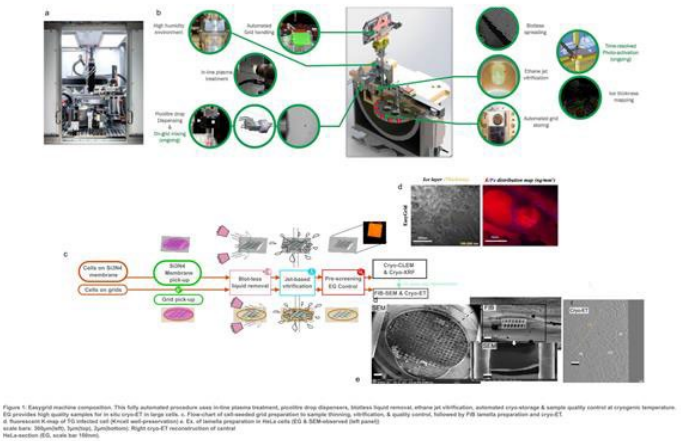
Background incl. aims
In structural biology, Cryo-EM is one of the major imaging technologies. Even if the limits of the EM towards higher resolutions and faster data-collection have been really improved, the sample preparation step still remains poorly automated and difficult to manage for untrained and trained users (reproducibility issues). Many developments (e.g., Vitrojet1, Chameleon2) exist to make cryo-EM sample grid preparation easier, faster, and more reliable, and some of them also offer time-resolved module than remains a less user-friendly solutions (TrEM3, Joachim Franck's mixing/spraying method4). However, no machine currently offers a universal and easy solution. Several relative issues also exist in the field of X-ray imaging where there is no unique, reproducible sample preparation. This is the main reason why the instrumentation team of EMBL-Grenoble began the development of the EasyGrid5 device in 2017 that aims to provide a versatile tool that addresses most of the current difficulties encountered in the cryogenic preparation of diverse sample supports.

Methods & Results
This fully automated procedure (Fig.1a,b) uses in-line plasma treatment, picolitre drop dispensers, blotless liquid removal, ethane jet vitrification, automated cryo-storage and sample quality control at cryogenic temperature. The in-depth vitrification step is crucial to study native sample conditions for cells. Here we report initial results in two main domains; thin film sample vitrification for SPA experiments (cryo-EM), and whole-cell vitrification for cryo-ET/-XRF deep in large cells (Fig 1c,d,e).

Conclusion
EasyGrid proposes a versatile and robust multimodal sample preparation framework that can be easily adapted to a large variety of sample preparation protocols: cell freezing, classical SPA sample grid preparation, light triggered time resolved freezing, on-grid mixing, and this for diverse imaging modalities requiring different supports and conditions. The adherent cell vitrification

protocol has been adapted for XRF using Si3N4 membranes (Silson), however, other supports can also be envisaged. The blotless liquid removal process allows for control of the ice layer thickness, a crucial parameter to optimise, to limit radiation damage on the sample during data-collection and maximise signal and contrast. The fully automated sample preparation procedure, leveraging precise robotics and an axis control system with sub-millisecond time-base, is highly reproducible. Hence, process optimization for different samples becomes accessible, and the screening of each process parameter can be reliably executed.

Graphic:



Keywords:

ElectronMicroscopy, X-rayFluorescence (XRF), Single-particleAnalysis, Cryo-samplePreparation

Reference:

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