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Automated Cryo-plunging Robot to Prepare Samples for Single Particle Analysis (SPA), Cryo-EM, Cryo-ET, Cryo-fluorescence and Cryo-CLEM

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KEYWORDS: Single Particle Analysis (SPA), protein molecule structure, cryo-EM, cryo-ET, vitrified biological samples, cryo-Correlative Light and Electron Microscopy (cryo-CLEM), cryo-fluorescence microscopy (cryo-FM), cryo-TEM, X-ray crystallography (XRC)

Cryo-imaging of biological samples embedded in vitrified ice has unique benefits and the development and implementation of cryo-EM and SPA has led to the Nobel Prize in 2017 [1]. One advantage is that the vitrification preserves proteins, cellular assemblies and cells in a fully hydrated and near native state. Also vitrified samples are compatible with the vacuum conditions required for EM and ultra-structural preservation under cryo-condition is considered the optimum possible. More and more protein structures are solved with SPA, which complements or even partly replaces the established XRC and crucially, does not require the step of crystallization. There are already more than 10000 protein structures solved with SPA deposited in PDB and EMDB and it is anticipated that SPA overtakes the established XRC method in this decade.

An additional benefit of cryo-preparation and cryo-FM is the significantly reduced photo-bleaching in cryo-fluorescence and the ability to correlate EM data with versatile fluorescence markers in a correlative process. Such a CLEM workflow [2] allows to map and target regions of interest without EM beam damage and by using complementing labelling techniques. Reduced bleaching and improved photon statistics also aid the localization precision for super-resolution techniques.

A key challenge for SPA, cryo-ET and cryo-FM is, however, the consistent and repeatable preparation of vitrified samples and mastering cryo-specific sample handling protocols. To make such workflows for SPA, thin cells or lamella cutting procedures in FIB-SEM more widely accessible, Linkam and LUMC have together developed an automated robotic plunge freezer.

In this device the paper-blotting step, a typical feature of current cryo-plunger devices, has been replaced with a programmable controlled suction method which allows real-time optically monitoring by a built-in microscope and the control of the liquid film thickness prior to plunging. Because the grid handling, glow discharge, sample application, plunging and grid storage are all automated in the robotic system (see Fig.1) the preparation of cryo-samples is simplified and more repeatable.

The typical sequence of preparation steps in the plunging robot is shown in figure 2. All handling steps can be programmed by software and parameters adjusted. The sample can be monitored in real-time via the built-in optical microscope and image data from this pre-screening allows to select or reject samples for further processing in the EM.

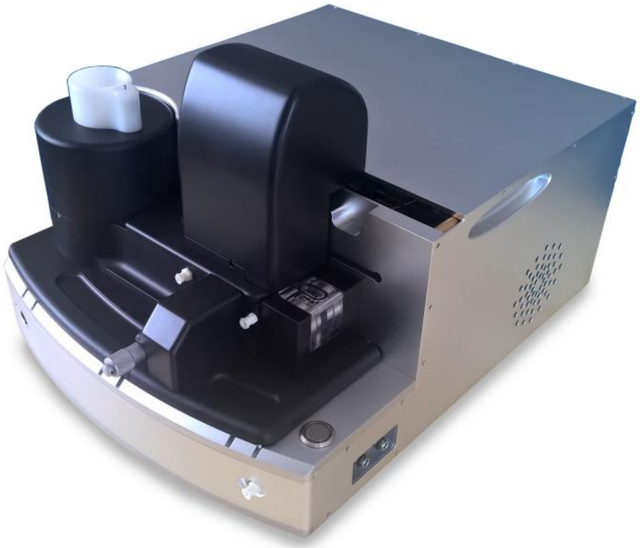

Figure 1. Plunging robot with integrated glow-discharge, humidity area, ethane plunging compartment and built-in microscope.

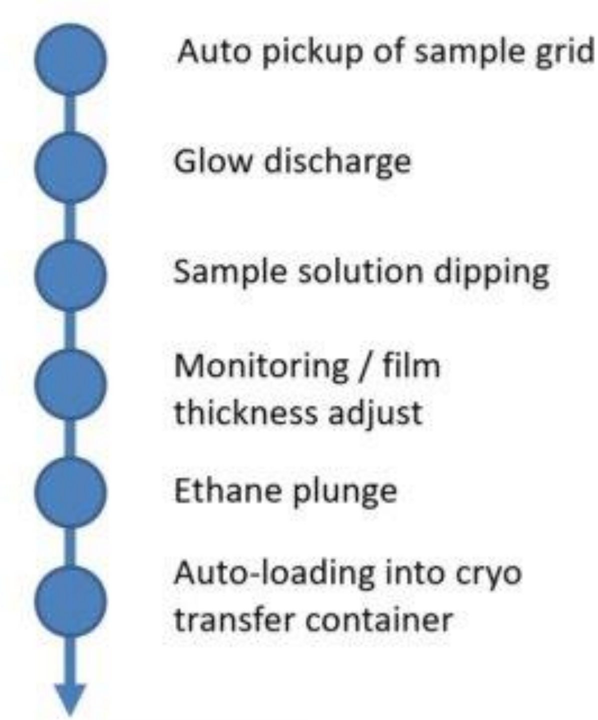

Figure 2. Typical automated sequence for the preparation of cryo-samples on an EM grid in the automated plunger system.

References
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