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WITHDRAWN - Next generation vitrification robot

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Cryo-electron microscopy (cryo-EM) has been established as a very successful structural technique and gains continuously growing scientific interest in the fields of structural, molecular and cell biology due to its increasing capabilities, tremendous throughput improvement and simplification of data acquisition. Protein structure can be resolved at resolutions previously thought to be possible only via X-ray diffraction and de novo atomic models can be built routinely. In some instances, it is even possible to resolve the protein structure at the atomic resolution by using single particle analysis (SPA) [1] or MicroED [2] methods. Using cryo-FIB lamella tomography [3], it is possible to visualize and reconstruct the 3D structure of proteins in the context of their native environment within the cell at a sub-nanometer resolution. All three cryo-EM methods have in common that the specimen is flash-frozen in their native, hydrated state by a vitrification step as described by Nobel-laureate Jacques Dubochet [4]. In this step, the sample is applied on a supporting grid carrier, followed by thinning of sample –liquid film to sub-100 nm thickness [5] and subsequent rapid cooling by plunging in liquid ethane. We have developed a new and highly flexible multi-purpose vitrification robot. The robot has ease of use, success rate and throughput significantly improved relative to previous generations of vitrification robots. The robot can prepare frozen-hydrated samples of aqueous (colloidal) suspensions of protein complexes or micro crystals, or monolayer cell cultures for cryo-electron microscopy. Two modes of sample vitrification are implemented – either plunge-freezing of EM grids or jet-freezing of pre-clipped AutoGrids to facilitate preparation of a wide variety of samples for all cryo-EM workflows. The freezing mode can be selected by the on-system display and following operation is guided by the system software. Direct vitrification of pre-clipped AutoGrids particularly improves ease of use and success rate by reducing the number of manual handling steps in the sample preparation workflow. The sample can be applied via the left or right pipetting port of the machine directly on a loaded grid. Mounting a grid with pre-incubated sample is also supported. Formation of a thin layer of the sample across the grid area is performed by a newly designed single sided blotting mechanism with accurate force control. With this mechanism, the required sample thickness on the bare EM-grid as well as on the pre-clipped AutoGrid can be reproducibly achieved. In combination with rotation of the shooting rod, many vitrification protocols are available as presets or can also be programmed via the on-system display. These protocols facilitate easy preparation of samples for novice users, whereas user-defined protocols enable repeatable settings of optimized sample preparation parameters for specific biological samples. Some protocols might require special steps, for example pre-priming of the grid front surface, multiple blotting actions or priming of the back surface of the grids to enable backside-blotting commonly used for cell-tomography. These special steps are also available in the pre-set and custom-made protocols. The jet-freezing functionality is implemented via two synchronously activated jets of liquid ethane that spray the pre-clipped grid perpendicularly onto both the front and backside surface. Even thick samples like cells are vitrified efficiently, while we observe no damage on the most delicate thin SPA specimen or crystals deposited on the grid foil. The large diameter of the jet also guarantees rapid cooling of the AutoGrid assembly and thus avoids affecting the vitrification process of the actual specimen. Furthermore, automatic preparation and refilling of the liquid ethane reservoir, as the source for the ethane jets and accurate control of the liquid ethane temperature, further improves simplicity of usage of the new vitrification robot. Lastly, digital records of prepared vitrified samples can be managed via automated storing and uploading of critical vitrification parameters into the DMP/Athena database. The digital management of all sample-related information during the vitrification task is streamlined to the SPA, MED and Tomography workflows and adds

to the repetitive production, tracking and comparison of experiments, leading to high quality, high resolution cryo-EM data.

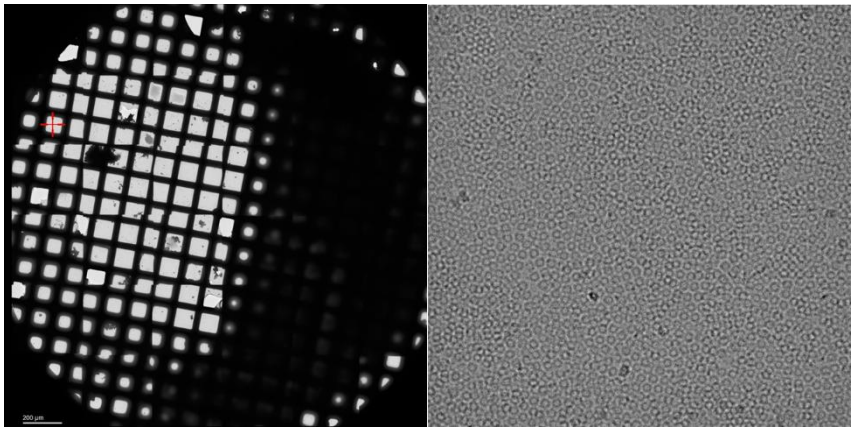

Figure 1. ATLAS overview image of a pre-clipped grid which was frozen using the jet freeze method. The ATLAS shows the large number of useable squares on the grid for high resolution imaging, like the example micrograph of Apoferritin shown on the right.

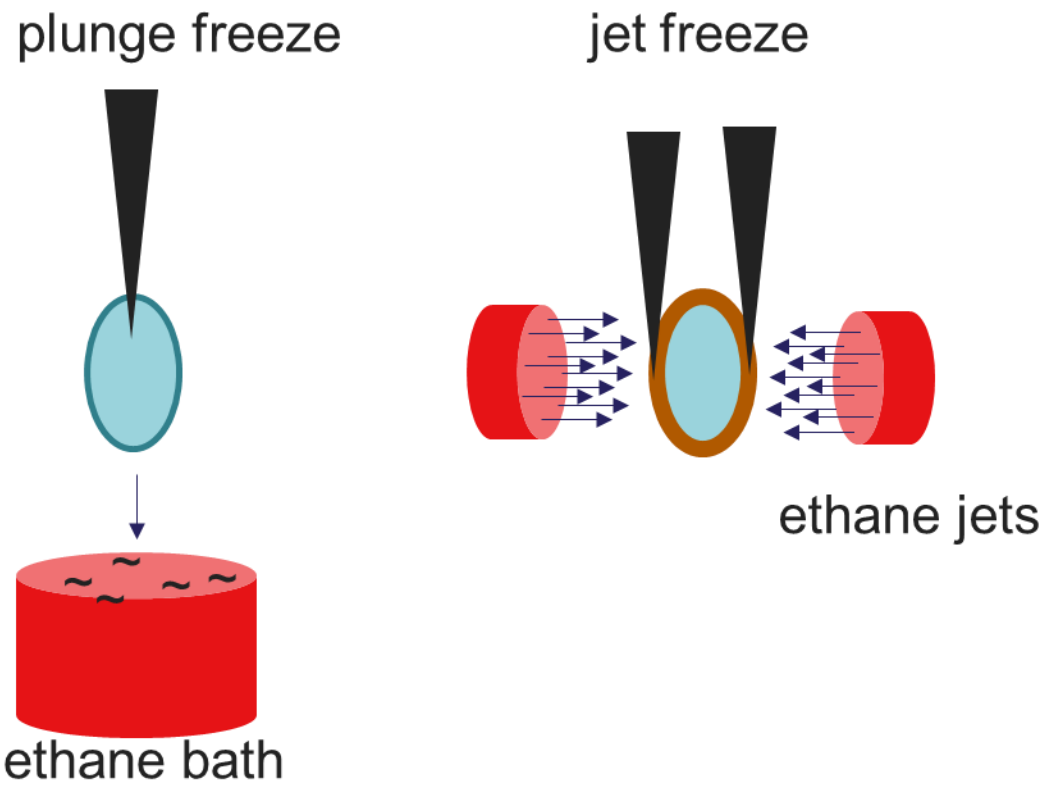

Figure 2. Schematic depiction of the two supported freezing methods: plunge freezing and jet freezing.

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