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A new method for vitrifying samples for cryoEM

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Almost every aspect of cryo electron microscopy (cryoEM) has been automated over the last few decades [1]. One of the challenges that remains to be addressed is the robust and reliable preparation of vitrified specimens of suitable ice thickness. We will present results from the next generation of a new device for preparing vitrified samples [2]. The success of the device is coupled to a new "self-blotting" grid that we have developed to provide a method for spreading sample to a thin film without the use of externally applied filter paper. The figure below shows: (a) an SEM image of a self-blotting grid that has copper nanowires growing on a supporting copper mesh. A lacey carbon (or gold) support substrate is attached to a smooth rhodium (or palladium or gold) surface on the opposite side to the copper nanowires. In (b) is an atlas of a Spotiton prepared grid showing a strip of vitreous ice created by delivering 50pL drops of sample onto the nanowire grid as it flies past en route to vitrification. This new approach has the advantage that it uses very small amounts of protein material, and results in large areas of ice of a consistent thickness and single particles that are evenly and well distributed within the ice. We believe that these methods will in the future result in a system for vitrifying grids that is almost completely automated and that can be used to explore and optimize more ideal conditions for vitreous ice grid preparation.

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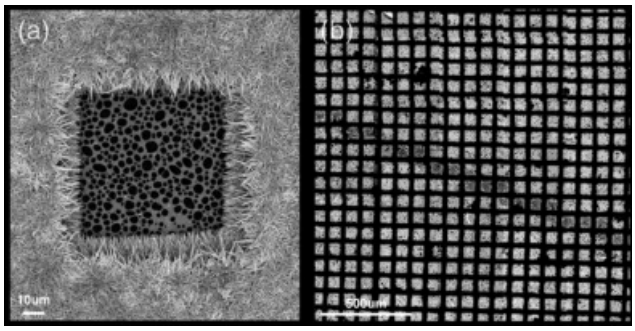

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