doi:10.1017/S1431927622005013
Microsc. Microanal. 28 (Suppl 1), 2022

Controlled Cryo-EM Sample Preparation Using the VitroJet

Maaike J. G. Schotman[1], Giulia Weissenberger[1], René J. M. Henderikx[1], Frank J. T. Nijpels[1] and Bart W. A. M. M. Beulen[1*]

[1.] Cryosol-World B.V., Weert, the Netherlands.
* Corresponding author: bart.beulen@cryosol-world.com

Cryo-electron microscopy (cryo-EM) has become a powerful tool in determining the structural arrangement of a wide variety of macromolecules in their 'natural' frozen state [1, 2]. This resulted in the Nobel prize in Chemistry of 2017 being awarded for the development of this technique. The quality of the samples is of high importance in regard to the resolution of the resulting structures that can be achieved. The main bottleneck of cryo-EM remains sample preparation, with large inconsistency in grid preparation with the currently used techniques. Manual handling makes grid preparation dependent on the experience of the user [3]. With the introduction of the VitroJet we developed an automated, user-independent sample preparation device.

To provide control over grid preparation, pin printing and jet vitrification techniques are combined in the VitroJet (Figure 1). The pin printing technique uses a sub-nanoliter sample volume, which is deposited on an autogrid creating a thin sample layer containing the macromolecules without any necessary wicking step (Figure 1A). Beforehand, an implemented plasma cleaner allows for the pre-clipped grids (autogrids) to be cleaned in a controlled manner resulting in reproducible wettability. Dewpoint is maintained in a deposition chamber preventing condensation and sample evaporation. An implemented camera allows direct visual feedback of the deposited layer, granting quality check of the deposited layer without the need of a transmission electron microscope. After the sample deposition, jet vitrification in the cryogenic chamber allows rapid cooling of pre-clipped autogrids by activating two ethane jets simultaneously to vitrify the samples, creating a vitreous ice layer (Figure 1B). A total of twelve grids can be prepared and automatically and stored in gridboxes in liquid nitrogen. These can be transferred directly to the microscope or kept in storage. With this method, pre-clipped autogrids can be vitrified in a controlled manner at high cooling rates.

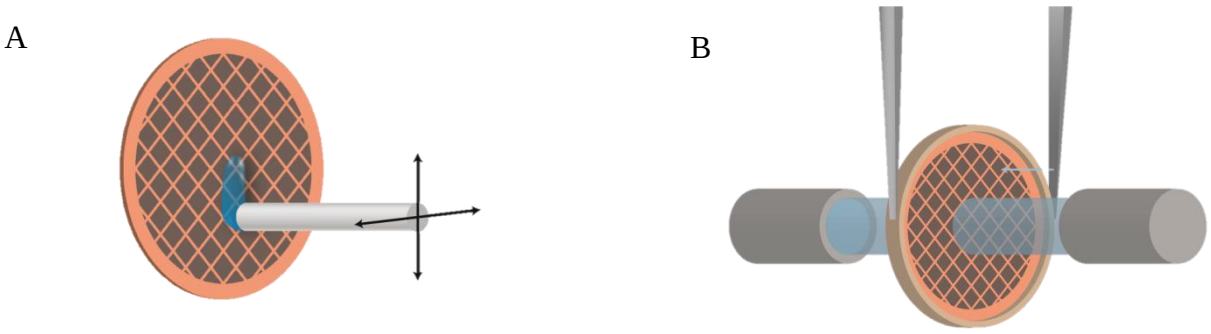

Figure 1. (A) A schematic representation of the pin printing process, in which the pin moves in the x- and y-axis, depositing a thin sample layer on the autogrid. (B) A schematic representation of the jet vitrification process, in which liquid ethane is jetted from both sides to vitrify the grid. Obtained from reference [3].

The VitroJet strives for reproducibility and high-quality sample preparation for cryo-EM. In 2021, a number of commercial VitroJets have been installed in life science labs around the world, and a number of upcoming installations are planned for 2022. We show that the VitroJet is an automated cryo-EM sample preparation device working with pre-clipped autogrids, producing user-independent samples. In-process quality checks by means of live visual feedback saves electron microscopy screening time. Layer deposition on the grid is documented at a magnification similar to cryo-EM atlas and an indication of layer thickness can already be observed, as well as protein aggregation during the preparation process. Latest results and ongoing updates obtained with the VitroJet will be presented. Our vision is to facilitate and relieve the workflow bottleneck of sample preparation, thereby supporting the great potential of cryo-electron microscope technique in life sciences.

References:

[1] Y. Cheng, Science **361** (2018), p. 876-880. doi: 10.1126/science.aat4346
[2] W. Kühlbrandt, Science **343** (2014), p. 1443-1444, doi:10.1126/science.1251652
[3] G. Weissenberger, R. J. M. Henderikx, P. J. Peters, Nature Methods **18** (2021), p. 463-471. doi:10.1038/s41592-021-01130-6