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Workflow Optimization for Cryo Electron Microscopy using Side Entry Dual Grid Cryo Transfer Holder and Automated Cryogen Auto-Refilling System

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Recent years have seen an explosive growth in the field of cryo electron microscopy (Cryo EM). Its application exhibits huge potential to unlock high impact research in the field of life sciences. High cost cryo electron microscopes [1], often equipped with an autoloader and an automated liquid nitrogen (LN_2) refilling system have been found to be an indispensable tool at many structural biology research centers. However, there are numerous established facilities across the globe that are still equipped with transmission electron microscopes (TEMs) having a side entry goniometer. Here we introduce a suite of research tools that help to optimize the Cryo EM processes for various applications including single particle collection (SPC), tomography and applications that involve beam sensitive materials also requiring protection from the atmosphere and oxidization prior being imaged in the TEM.

SPC as well as batch tomography requires the uninterrupted use of Cryo EM for an extended duration of time. The automated cryogen auto-refilling system is a unique solution that helps to overcome the limitations of traditional TEMs by providing a safe and cost-effective way to periodically refill LN_2 in a cryo specimen holder and also, if required, the TEM anti-contamination device (ACD). The 35 liter system dewar(s) is equipped with a low pressure LN_2 pump(s). The primary user selection includes 'Number of refills' and 'Time between refills'. The system sensor assemblies provide continuous temperature feedback from the dewars and prevent overflow during refilling. A precise stage slides the LN_2 nozzle assembly into position to align them with the refill funnels on the dewars to be filled. The refilling system is compatible with various TEMs and cryo holders. The system can operate without human intervention for up to one week, providing extended duration for data collection. In addition, there is also a provision to control the system remotely. This functionality can be used to initiate actions, such as refilling LN_2 using the TEM data acquisition software. This system is integrated with the TVIPS software EM-Tools [2] and with SerialEM [3, 4].

The dual grid cryo transfer holder complements the cryogen auto-refilling system. This holder provides stable imaging conditions and can be loaded with two TEM grids – either standard grids or ThermoFisher Autoloader grids. The holder provides a long LN_2 holding time (up to 10 hours without refilling), effective cryo protection during cryo transfer and a low tip profile. The holder accessories include a workstation with built-in LED, heater and separate units for temperature readout of tip temperature and activated carbon regeneration. The grid clamping (see Figure 1) as well as the grid selection is optimized for ease of use under LN_2 conditions. The thermally optimized LN_2 dewar helps to maintain low steady state sample temperature and a low deviation from the mean steady state temperature over time (see Figure 2). The axis symmetric design of the dewar helps to provide a stable center of mass of the dewar for a given level of LN_2 when tilting. In addition, the low rate of LN_2 evaporation helps to increase the stability and performance of the holder due to providing a near constant center of mass.

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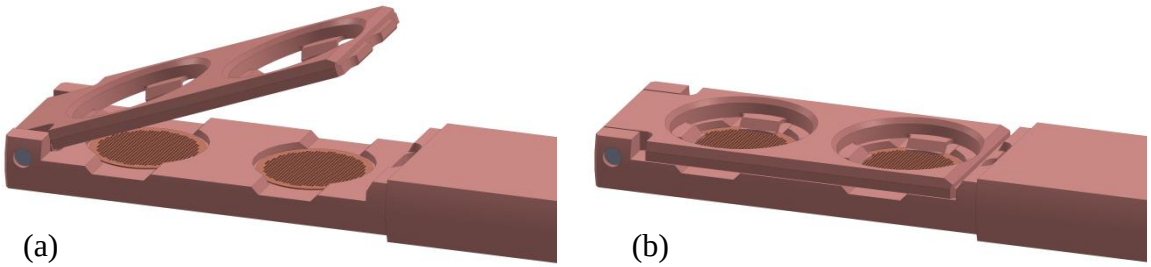
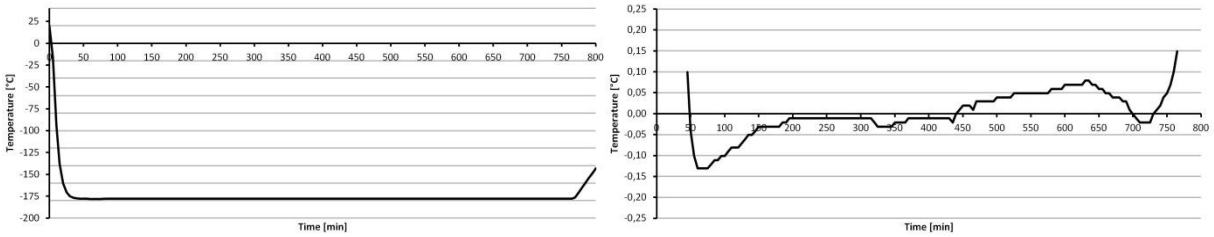
For specialized applications involving materials that need to be protected from the atmosphere and oxidization while being also beam sensitive when exposed to electrons in the TEM, the vacuum/inert gas cryo holder can be used to easily load the sample under a protective vacuum or inert gas environment within a glove box. This holder maintains the protective environment during the transfer to the TEM and can then be subsequently cooled in the TEM for imaging.

Figure 1. Shows the newly designed specimen clamp in an open position (a) and in a closed position (b).



(a) (b)

Figure 2. Shows the tip temperature over time when cooling the cryo holder from room temperature in a turbo pumping station; the high temperature stability of around +/- 0.1°C over a long period of time is essential for low drift rates and acceptable data acquisition in the TEM. (a) shows the tip temperature, (b) shows the deviation of the tip temperature from the mean temperature.



(a) (b)

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