

A protected inert-gas sample manipulation and transfer environment for cryo electron microscopy and analytics

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The preservation of the native sample state during manipulation and exchange between sample preparation and imaging devices is a prerequisite for artefact and contamination free structure investigation. In particular during cryo-transfer and (re-)mounting of frozen hydrated samples, which often in electron microscopy (EM), have a very large surface area compared to its volume, can be very challenging when samples have to be exchanged or remounted several times. Some of the main challenges are: 1. preventing the sample from warming up to the de-vitrification temperature of water (approximately -137°C), 2. Preventing the sample from being covered with ice or other contaminants condensing from the surrounding atmosphere and 3. Handling these samples without chemical or mechanical alteration of their native state.

Currently the handling and transfer of samples under vacuum and cryo conditions is solved by systems provided from different suppliers. However, the transfer of frozen (vitrified) samples under environmental pressure (e.g. from a high-pressure-machine to a low temperature coater or cryo-EM) is still a challenge and a satisfactory practical solution is missing.

Here, we present an ambient pressure inert-gas sample mounting and exchange chamber (Glove-Box) (Fig. 1 &B) that addresses all the needs for structure and composition preservation during the manipulation, mounting and transfer of mainly frozen or hydration/oxygen sensitive samples. Once mounted these samples can be transferred and exchanged between different cryo-preparation and imaging systems (Fig. 1 E) under controlled inert-gas and temperature conditions. This controlled inert-gas "Glove-Box" includes a LN2 immersed sample mounting area with various interfaces to different inert-gas/cryo transfer systems for RT or low temperature TEM or FIB/SEM applications, Furthermore, monitoring of the environmental parameters (Fig. 1 C) during transfer (humidity, oxygen, carbon dioxide), on-screen imaging to support sample manipulation (Fig. 1 D), heating of tools and recording of all experimental parameters for documentation are included into this dedicated LN2 and inert-gas "Glove-Box". The interface to other sample carrier devices is open and flexible and can be adapted upon individual laboratory needs. Finally, such a "Glove-Box" can play an essential

role in a dedicated modern cryo-EM & analytical environment as sketched in Fig. 1 E.

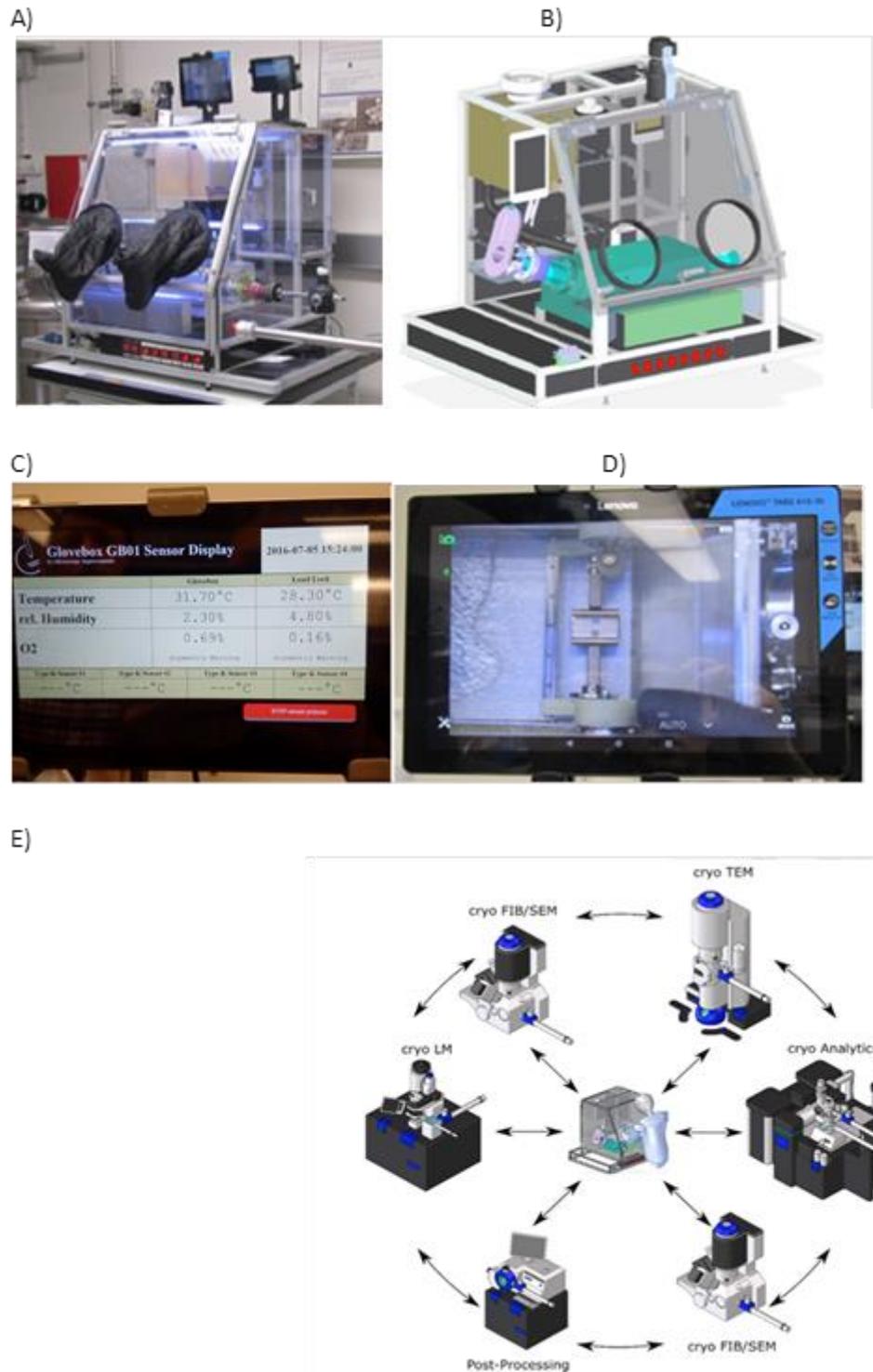


Figure 1 A&B) ambient inert-gas sample manipulation and mounting “Glove-Box” system; C) Display for monitoring of environmental parameters; D) Zoom-in imaging screen of the LN2 sample handling area; E) proposed workflow embedding and interfaces to various preparation and imaging devices