High-vacuum Optical Platform for cryo-CLEM (HOPE): a New Solution for Non-integrated Multiscale Correlative Light and Electron Microscopy

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ABSTRACT

Cryo-correlative light and electron microscopy (cryo-CLEM) offers a unique way to analyze the high-resolution structural information of cryo-vitrified specimen by cryo-electron microscopy (cryo-EM) with the guide of the search for unique events by cryo-fluorescence microscopy (cryo-FM). To achieve cryo-FM, a trade-off must be made between the temperature and performance of objective lens. The temperature of specimen should be kept below devitrification while the distance between the objective lens and specimen should be short enough for high resolution imaging. Although special objective lens was designed in many current cryo-FM approaches, the unavoided frosting and ice contamination are still affecting the efficiency of cryo-CLEM. In addition, the correlation accuracy between cryo-FM and cryo-EM would be reduced during the current specimen transfer procedure. Here, we report an improved cryo-CLEM technique (high-vacuum optical platform for cryo-CLEM, HOPE) based on a high-vacuum optical stage and a commercial cryo-EM holder. The HOPE stage comprises of a special adapter to suit the cryo-EM holder and a high-vacuum chamber with an anti-contamination system. It provides a clean and enduring environment for cryo specimen, while the normal dry objective lens in room temperature can be used via the optical windows. The ‘touch-free’ specimen transfer via cryo-EM holder allows least specimen deformation and thus maximizes the correlation accuracy between cryo-FM and cryo-EM. Besides, we developed a software to perform semi-automatic cryo-EM acquisition of the target region localized by cryo-FM. Our work provides a new solution for cryo-CLEM and can be adapted for different commercial fluorescence microscope and electron microscope.

FIGURE LEGENDS

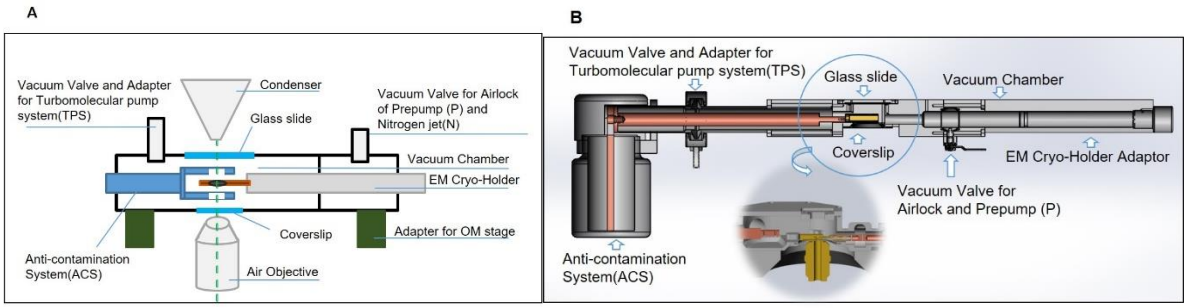

Figure 1. **Design of the HOPE stage**. (**A**) Schematic overview of the HOPE stage design in its operational mode with an inverted fluorescence microscope. (**B**) Cross section view of the HOPE stage design in 3D representation. Each part of HOPE stage is labeled and described.