

# Cryo-EM screening on JEOL JEM-1400Flash

## INTRODUCTION

Cryo-EM has enjoyed an enormous ground swell in popularity ever since the advent of more stable and automated electron microscopes, suitable movie-type cameras, and improved acquisition software. Results obtained so far have been nothing short of spectacular as illustrated by several structures in EMDB and EMPIAR solved by cryo-EM to resolutions better than 1.5Å, such as EMD-31314, EMD-33707 and EMD-35984, the latter of which reaching true atomic resolution. This note describes screening of cryo-EM samples on a JEOL JEM-1400Flash with a Gatan ELSA holder before committing to an SPA run on higher-end electron cryo-microscopes, such as the JEOL CRYO ARM (Figs. 1-3).

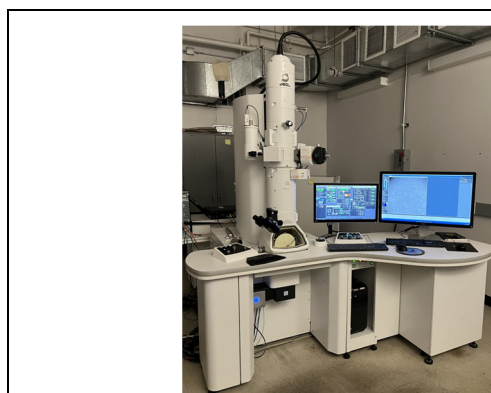


Fig. 1: The JEM-1400Flash at the Donner laboratory of UC Berkeley is a 120 kV LaB6-equipped TEM with a High Contrast pole piece and an integrated cryo box.

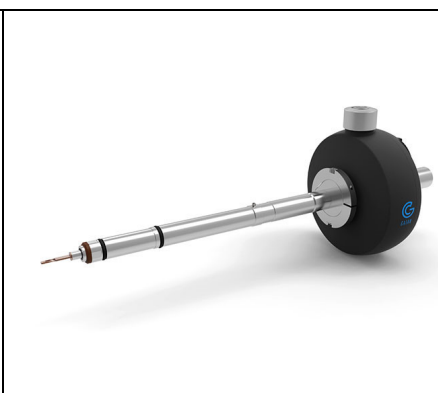


Fig. 2: The Elsa cryo-holder from Gatan/Ametek used for the cryoEM screening.

## RESULTS

Figure 1 shows the JEM-1400Flash at UC Berkeley, which is a turbo-pumped 120 kV electron microscope capable of imaging from 10x to 1.2Mx and diffraction with cameralengths ranging from 15cm to 80m, as well as tomography ( $\pm 80^\circ$ ) and microED. The microscope at UC Berkeley has a High Contrast pole piece (Cs 3.4mm, Cc 3.0mm), an LaB6 filament, a fully integrated cryo box, and is equipped with a Gatan/Ametek OneView camera controlled by DigitalMicrograph and SerialEM. Fig. 2 shows the Elsa cryo-holder, model STP, from Gatan/Ametek used in the experiment which is capable of tilting  $\pm 60^\circ$ .

Not necessarily required, but screening can include preparing negatively stained samples as this gives information on particle size and overall morphology of the molecular complex. Fig. 3 shows a typical micrograph acquired from a sub-100 kDa membrane protein-Fab complex under study, designated here as sample A, prepared on thin C-coated formvar at 60,000x scope magnification.

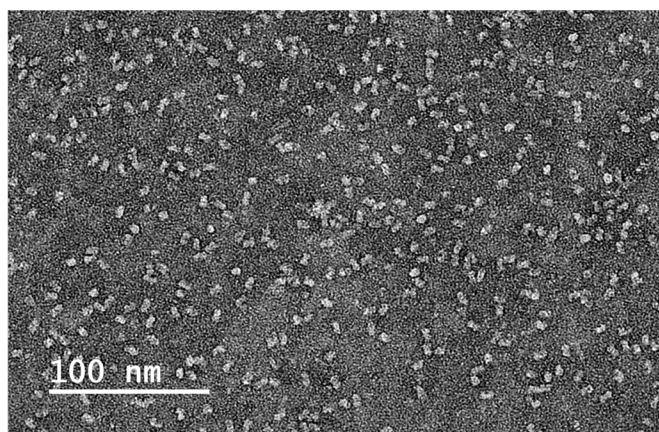


Fig. 3: Sample A imaged at 120 kV at 60,000x in the JEOL JEM-1400Flash equipped with a OneView camera.

Figs. 4 shows images of the vitrified sample at various magnifications, which clearly reveal subtle variations in ice thickness on 1.2/1/3 UltrAuFoil grids, treated by glow-discharging with a Pelco easiGlow.

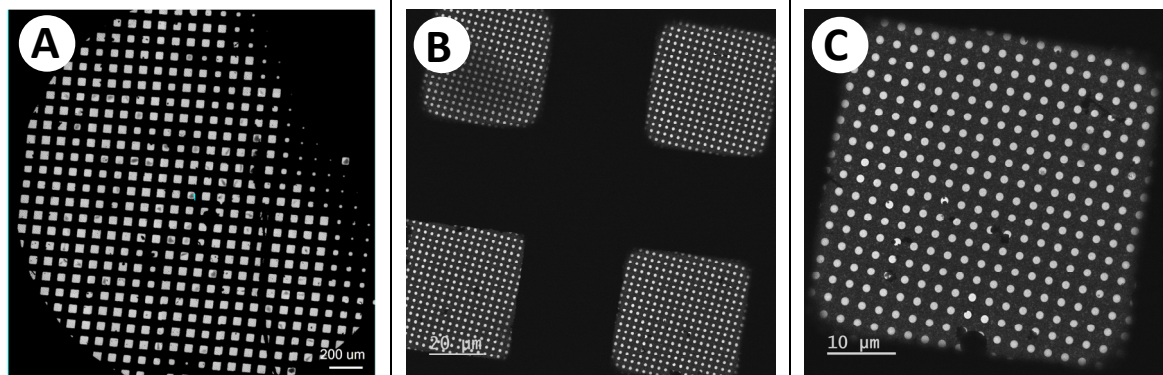
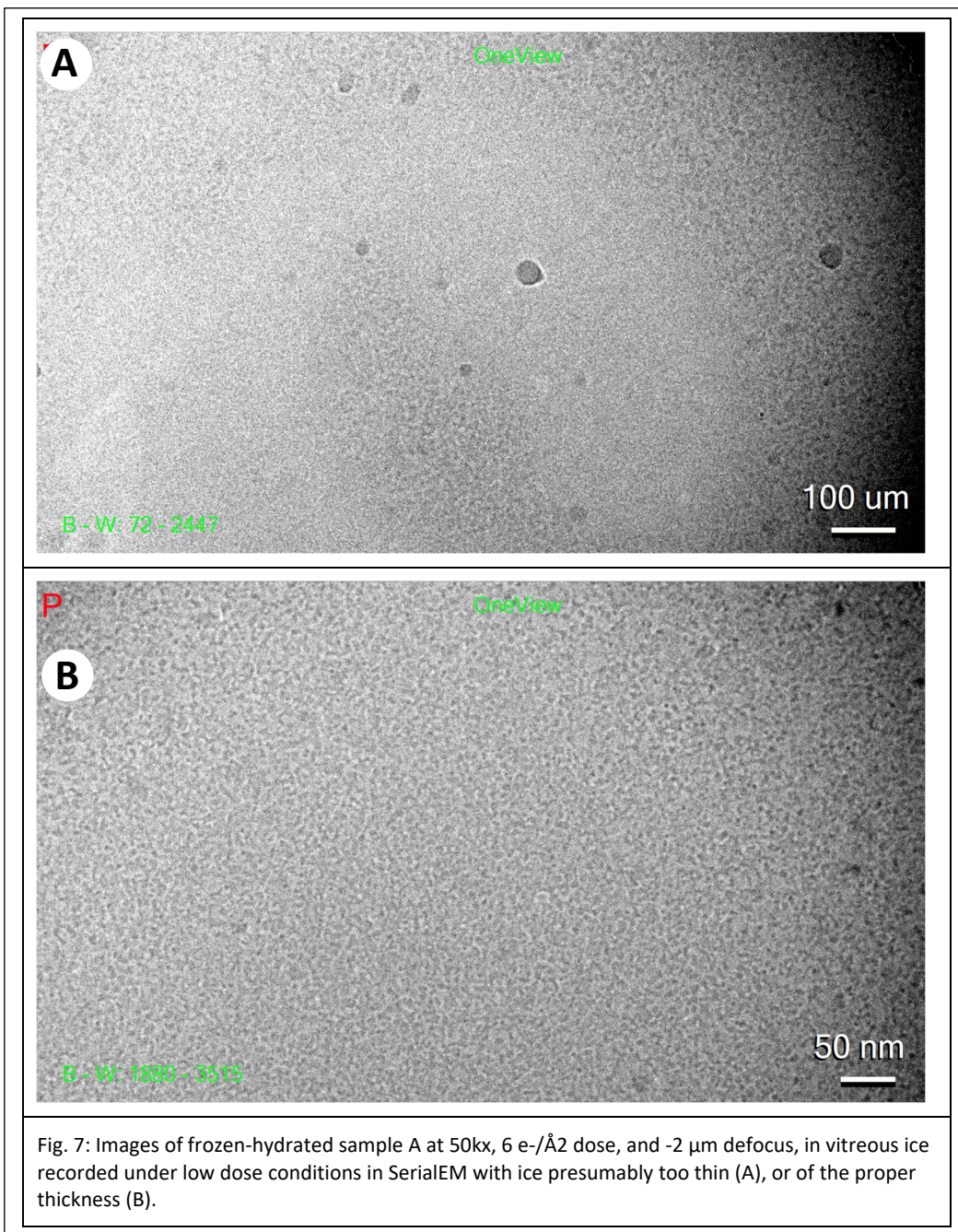


Fig. 4: Atlas of frozen-hydrated sample A imaged at 80x in the JEOL JEM-1400Flash. These 1.2/1.3 UltrAuFoil grids were flash-frozen in liquid ethane using a Vitrobot (A). Same grid, but now imaged at 300x showing 4 grid squares that reveal the subtle variation in ice thickness across these UltrAuFoil grids (B). Single grid square of the same grid imaged at 800x. All the holes of the UltrAuFoil grid are filled with thin ice (C).

Fig. 7 shows two micrographs depicting vitrified sample A in ice that is presumably too thin (Fig. 7A), or in ice of the proper thickness (Fig. 7B).



## CONCLUSION

The JEOL JEM-1400Flash is an excellent choice for screening frozen-hydrated specimens and can easily be used in a mixed environment of high-end, autonomous cryo scopes using appropriate cryo-holders.