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Cryo-FIB Lift-out Sample Preparation Using a Novel Cryo-gripper Tool

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In recent years, biological specimen preparation using a cryo-focused ion beam (cryo-FIB) has become a key technique for investigating complex cellular structures *in situ* [1-3]. Cryo-FIB has enabled cryo-electron tomography (cryo-ET) of plunge-frozen hydrated cells, revealing the cellular interior with sufficient resolution and contrast to study membrane-bound macromolecules in their native state [4-7].

In the current standard cryo-FIB preparation technique, lamellas are milled on plunge-frozen TEM grids by completely removing material over tens of micrometers on both sides of the lamella, while the lamella itself remains connected to the bulk material on the grid. Several lamellas can be prepared on a single grid, which is then imaged directly by cryo-ET [8].

However, because this technique can only be applied to biological material that is small enough to vitrify via plunge freezing, larger cells and tissue specimens remain inaccessible. To overcome this limitation, we have developed a cryo-FIB lift-out technique in which the volume of interest is completely extracted from the bulk material and transferred to a separate TEM grid prior to final thinning (Figure 1). Utilizing a newly developed cryo-gripper tool, this extraction and transfer can be performed under the necessary cryo-conditions to avoid devitrification of the sample, and without the need of localized material deposition.

In this work, we demonstrate the successful application of the cryo-FIB lift-out technique to both plunge-frozen and high-pressure-frozen specimens. We show that the lamella quality of the standard preparation technique can be fully reproduced by lift-out.

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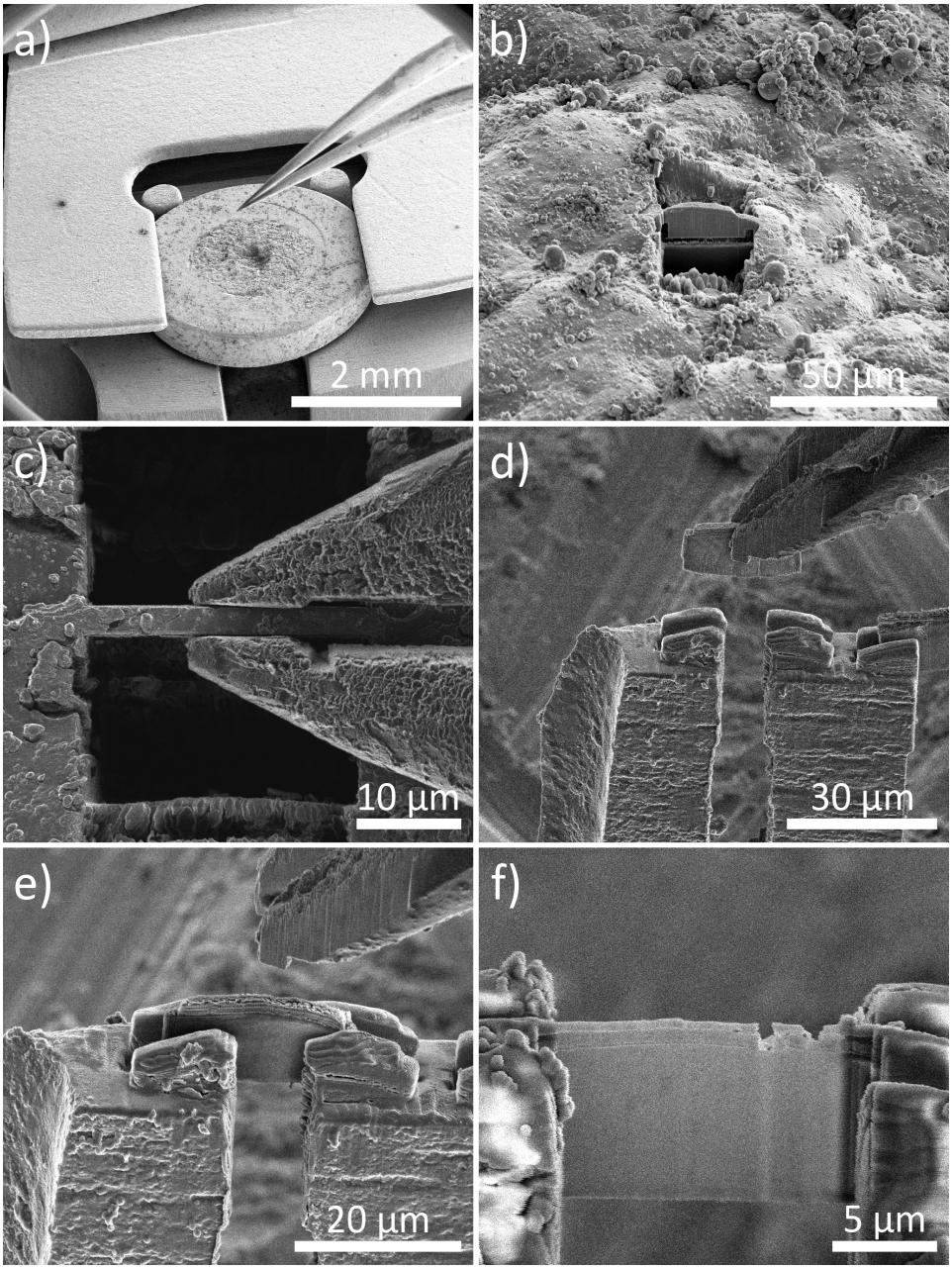

Figure 1. Cryo-FIB lift-out preparation of a leaf of *Arabidopsis thaliana*. a) SEM image of a high-pressure-frozen sample mounted in a cryo-FIB shuttle; b) SEM image of FIB-milled trenches before the lift-out; c) FIB image of the cryo-gripper grabbing the lamella, which is still attached to bulk material; d) SEM image of the lamella before insertion into a slot on the TEM grid; e) SEM image of the transferred lamella before thinning; f) SEM image of the final, thinned lamella.

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