

## Cryo-EXLO for Cryo-TEM of FIB Specimens

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Cryogenic focused ion beam (cryo-FIB) specimen preparation has been successfully used for cryogenic transmission electron microscopy (cryo-TEM) in both the life sciences and physical sciences [1-11]. The use of cryo-FIB *in situ* lift out (cryo-INLO) has become the norm despite its complex procedures and often poor reproducibility [11]. Conversely, it is well known that ambient *ex situ* lift out (EXLO) is fast, easy, reproducible, and capable of successful manipulations of > 20 specimens per hour [12]. Thus, we extended EXLO methods to cryo-FIB specimens and developed cryo-EXLO techniques and instrumentation for cryo-TEM.

Cryo-EXLO was applied to cryo-FIB milled plunge-frozen yeast specimen. Conventional EXLO FIB milling procedures were used and a cryo-holder containing the FIB specimen was transferred to an EXLO station under controlled humidity and environmental conditions. After cryo-EXLO manipulation, the specimen was transferred to a cryo-TEM for analysis. Cryo-TEM results show minimal ice contamination while retaining the vitreous phase during the manipulation and transfer steps. In addition, thermal heat transfer modeling of the cryo-FIB process is consistent with, and supports, our cryo-EXLO experimental methods and results [13].

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