

Novel Use of EXLO for Cryo-Manipulation of FIB Specimens

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The use of cryogenic focused ion beam (cryo-FIB) specimen preparation for cryogenic transmission electron microscopy (cryo-TEM) is growing in both life sciences and physical sciences applications [1–11]. Manipulating cryo-FIB specimens with *in situ* lift out (cryo-INLO) has become the norm despite its cumbersome and lengthy procedures and often poor reproducibility [11].

Conversely, it is well known that ambient *ex situ* lift out (EXLO) is fast and reproducible capable of successful manipulation of > 20 specimens per hour [12]. Thus, we present a natural evolution and combination of conventional EXLO methods applied to cryo-FIB specimens for the development of cryo-EXLO equipment, procedures, and methods. We present a novel use of cryo-EXLO to manipulate specimens prepared using cryo-FIB milling methods, including a polymer specimen and a plunge-frozen yeast specimen. As will be shown, both the polymer specimen and plunge-frozen yeast specimen were cryo-EXLO manipulated in minutes, improving time to results over cryo-INLO methods. In addition, cryo-TEM results show that both specimens were cryo-EXLO manipulated with minimal ice contamination and the vitreous phase was retained in the yeast specimen. Thermal heat transfer modeling of the cryo-FIB processes is consistent with, and corroborate, our cryo-EXLO experimental methods and results [13].

References:

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