In Memoriam of Lena Kourkoutis: The Development of Cryo EXLO

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Meeting-report

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Co-author LAG originally met graduate student Lena Kourkoutis at Cornell during focused ion beam (FIB) training of an FEI Strata 400S in ~ 2007. During training, we used one of Lena's samples and were able to make a low energy polished FIB specimen which she immediately inserted into the scanning transmission electron microscope (STEM). It was instantly apparent to LAG that in addition to Lena's bright and happy disposition, she possessed extraordinary technical skills confirmed by a subsequent Nature publication [1]. While Lena's interest grew in cryo microscopy, LAG sought her guidance and referral letters for grant proposals. EXpressLO received a DOE grant in early 2020 as COVID-19 was shutting everything down which changed our initial proposed plans. LAG placed a call to Lena, and she graciously provided access to her lab and personnel for our equipment and team. This development work would not have been realized without Lena's friendship and generosity.

With Lena's help, we previously showed that cryogenic *ex situ* lift out (cryo EXLO) could be used to manipulate plunge frozen yeast thinned by cryo FIB specimen preparation and subsequently observed via cryogenic transmission electron microscopy (cryo TEM) [2]. Heat transfer modeling also showed theoretical cryo EXLO methods are possible, retaining the vitreous phase necessary for accurate cryo TEM [3].

Cryo FIB methods for cryo EXLO of high pressure frozen (HPF) prepared cells require that we closely follow the methods previously presented using the waffle method [4], with obvious differences specific to EXLO specimens [2, 5]. The methods incorporated here prevent three common problems associated with cryo FIB of HPF samples: (i) specimen breakage due to curtaining artifacts as the specimen thins due to FIB milling on a rough surface, (ii) warping of cryo specimens as they thin during FIB milling and (iii) charging artifacts and specimen loss during cryo FIB milling. In lieu of waffled HPF samples, we added a half-grid on top of the HPF planchet to reduce charging and removed surface ice and smoothed the HPF surface using glancing angle high current FIB imaging. In addition, we employed (i) sputter coating, (ii) copious amounts of cryo Pt deposition, and (iii) stress relief FIB mill cuts.

Fig. 1a shows a schematic diagram of the stage back tilted to its maximum available at -3° for the FEI Strata 400S equipped with a Quorum cryo stage. FIB imaging at low magnification and high beam current (e.g., 21 nA) removed ice formed during storage and smoothed the surface yielding more reproducible results. Fig. 1b is a low magnification scanning electron microscopy (SEM) imaging showing a conductive copper half grid clipped on top of the HPF sample to reduce charging. The FIB trench locations are positioned near the grid edge (< 200 µm) to reduce charging problems. Additionally, cryo Pt deposition is cured up to and on top of the grid within an area of ~ 500 µm x 500 µm.

Fig. 2a shows an SEM image of a final FIB milled specimen ready for cryo EXLO. Fig. 2b shows a specimen manipulated via cryo EXLO to a C-Flat grid ready for cryo TEM. We expect that cryo EXLO will increase throughput and allow for more routine cryo FIB lift out analyses [6].

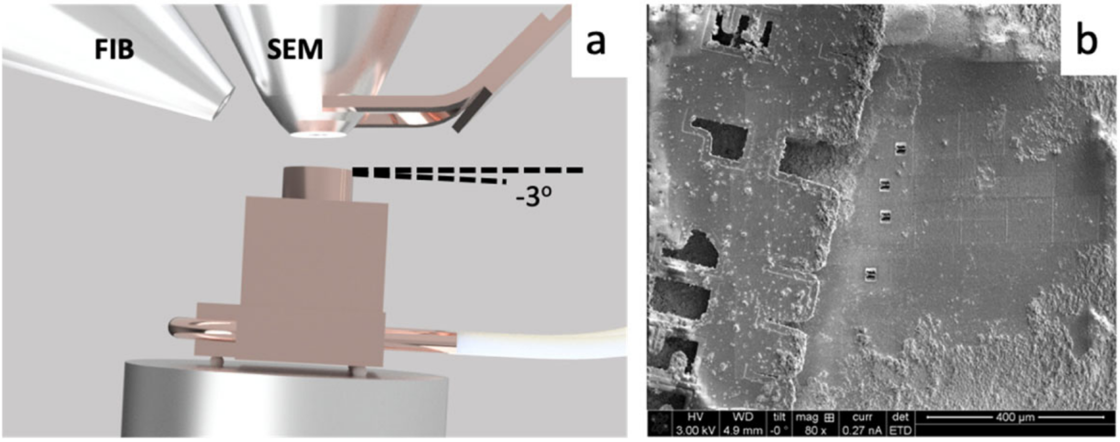

Fig. 1. (a) Schematic diagram showing stage back tilted for FIB ice removal and sample smoothing. (b) SEM image showing the proximity of EXLO specimens to a copper half grid on top of an HPF sample.

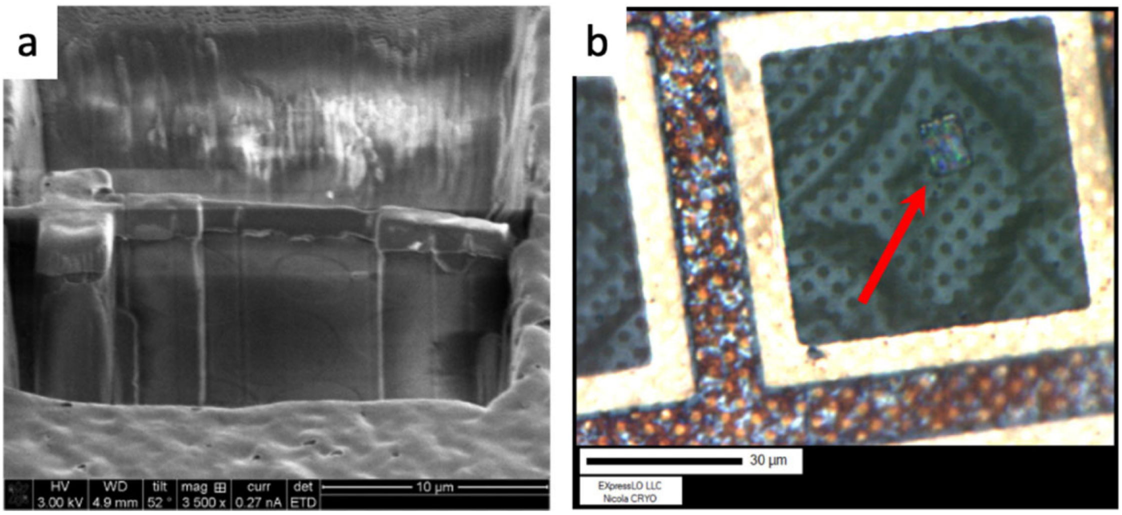

Fig. 2. (a) SEM image of cryo FIB milled specimen ready for cryo EXLO. (b) Cryo EXLO specimen manipulated to a C-Flat grid.

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