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Automatic Sample Processing for vEM in a Mouse Model of Breast Cancer

Erin S. Stempinski[1], Jessica L. Riesterer[1,2] and Claudia S López[1*]

[1.] Multiscale Microscopy Core Facility, Oregon Health and Science University, Portland, OR, USA.
[2.] Cancer Early Detection Advanced Research-Knight Cancer Institute, Oregon Health and Science University, Portland, OR USA.
* Corresponding author: lopezcl@ohsu.edu

Volume electron microscopy (vEM) for biological samples requires intensive staining protocols to ensure both good contrast and conductivity throughout the entire sample. We have previously shown that the large volume en-bloc staining protocol developed by Hua et al. [1] has been used successfully for human cancer biopsies for both serial block face-scanning electron microscopy (SBF-SEM) and focused ion beam-scanning electron microscopy (FIB-SEM) [2]. However, this protocol requires 2.5 days of bench processing, which can be onerous in a microscopy core facility that may have multiple projects to complete. Additionally, we have previously compared the effectiveness of different bench protocols for vEM on breast cancer tumors [3] and observed that shorter processing time results in inadequate sample contrast and conductivity for SBF-SEM (unpublished data).

In our investigations we observed that the mPrep™ ASP-1000™ automated specimen processor from Microscopy Innovations can reduce the sample preparation time for vEM to roughly 24 hours [4]. We attempt to adapt the bench protocol used in our laboratory for the ASP-1000 and further refine the protocol to use shorter staining times and ethanolic uranyl acetate (UA) as in Thomas et al. [5] to understand morphological changes in a murine breast cancer model [6].

In general, we have observed that ASP-1000 vEM sample automation methods can produce similar results to the bench process in less time, with ethanolic UA increasing membrane contrast. Protocols performed on the ASP-1000 took 4.5 to 5 hours, with an hour of instrument setup and an hour of embedding and cleanup, for a total time of 7 hours of sample preparation time. In contrast, the bench protocol required 20 hours, not including time spent on the overnight steps. Active technician time for the ASP-1000 was 2.5 hours, whereas the bench protocol required over 5.5 hours of technician time. Additional advantages of the ASP-1000 include the reduced exposure of the operator to heavy metals and reproducibility of the automated process.

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