

Automated Specimen Preparation for Volume Electron Microscopy

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Key Techniques: Automated specimen preparation for vEM. Correlative vEM, XRM and LM. Encapsulated specimen handling for automated & manual prep including freeze-substitution.

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Manual volume electron microscopy (vEM) specimen preparation using chemical fixation, staining, and embedding typically takes 4-5 days with one or more days of active “hands-on” effort that requires considerable handling of toxic reagents. This slows progress and takes time away from imaging, interpretation, analysis, and other more interesting work. Further, the tedium and complexity of manual preparation can lead to process inconsistencies which can negatively affect the reproducibility of results, including automated image segmentation.

We illustrate how several vEM labs use ASP-1000 and ASP-2000 Automated Specimen Processors and mPrep capsules to prepare biological specimens for serial block face-, array tomography- and FIB-SEM. Labs using ASPs report “hands-off” specimen prep as quick as 6 hours (prior to resin curing), with results equivalent or superior to manual prep, while providing the staining reproducibility needed for automated segmentation of mitochondria, myelin, synapses, and nuclei [1-4] with the safety of reduced reagent handling. ASP applications include automated prep of vertebrate and invertebrate tissues [1-4] and cell pellets [4-5]. Other labs use mPrep/s specimen capsules, without (or potentially with) ASP automation to improve freeze-substitution prep reliability by cutting loss and keeping specimens immersed [6]. We will also demonstrate how mPrep-encapsulated specimens can streamline correlative vEM with light and X-ray microscopy, and enable automated immunolabeling [7-8]. We gratefully acknowledge the contributions of ASP and mPrep capsule collaborators and customers

References:

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