

Meeting-report

# Next Generation Automated Programmable Electron Microscopy Preparation

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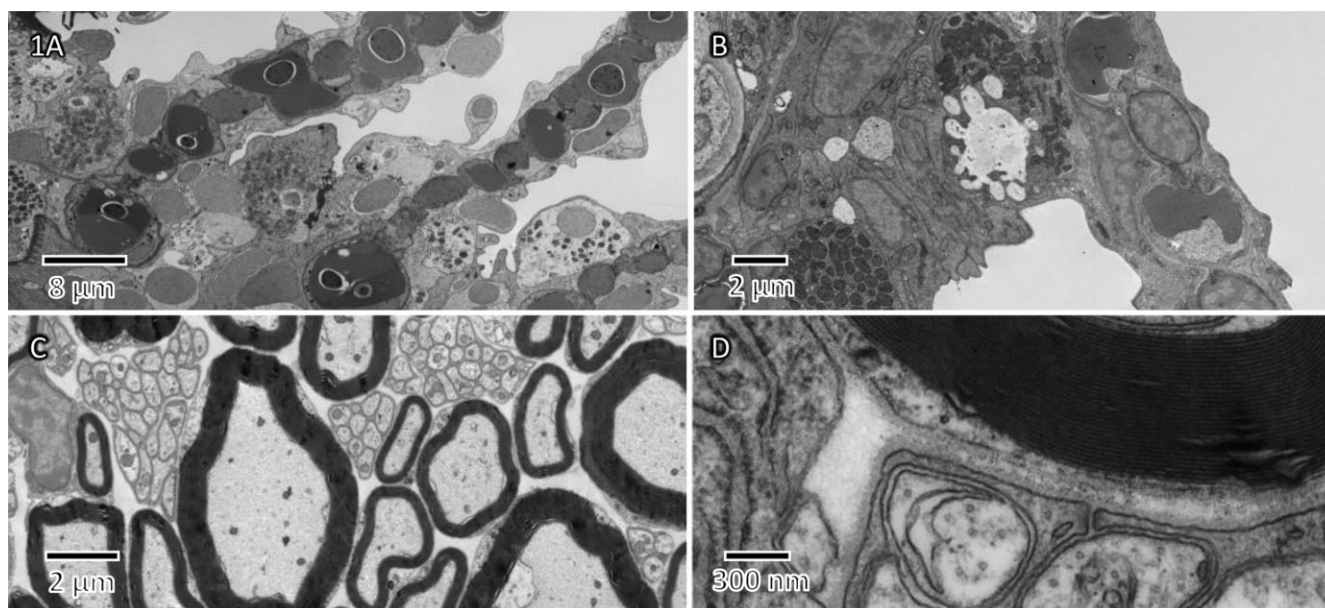
Preparing biological specimens for electron microscopy (EM) requires considerable hands-on time and effort. Tissue preparation for transmission EM entails sequential reagent exchanges that can take one or more days, while 3D volume EM (vEM, including serial block face, array tomography and FIB-SEM) preparation can take nearly a week. Immuno-gold labeling is another long and tedious EM prep process. In 2015, Microscopy Innovations introduced the mPrep™ ASP-1000™ Automated Specimen Processor to ease these tasks in any size lab. The ASP uniquely enables microscopists to run automated specimen or grid preparation protocols of any length or complexity. This frees users from the tedious task of repetitive reagent exchanges while providing robotic reproducibility. In 2021, we reviewed the 6-year history of the ASP-1000 including showing numerous examples of published works demonstrating automated TEM and vEM specimen preparation, as well as automated pre- and post-embedding immuno-gold labeling [1].

Here we introduce the mPrep ASP-2000™. The ASP-2000 adds the ability to control reagent temperatures from 0 to 100C, either within automated protocols, or independently from protocols. Here we also introduce new enhanced control software for both the ASP-1000 and ASP-2000. The new controller improves ease-of-use, provides greater flexibility, facilitates protocol sharing with other ASP users, and provides enhanced integrated help. ASPs can automate virtually any preparation protocol by enabling an effectively unlimited number of protocol steps and reagents. ASPs also provide alerts, including SMS texting, to inform users that, “Operator started a TEM specimen prep”, “Preparation protocol completed”, and that its time to add a fresh or labile reagent: “Protocol paused until you add fresh TCH”.

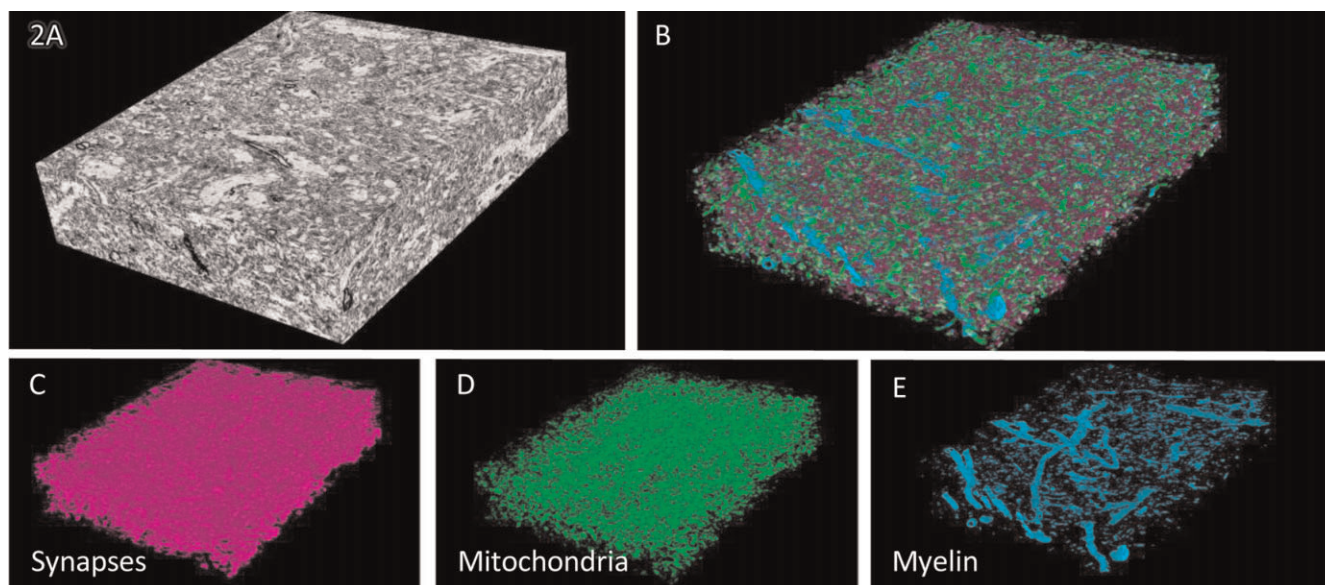
The new ASP operator control interface simplifies record keeping and billing by documenting each preparation run with the operator’s name, user notes, the preparation protocol, and the start, end, and elapsed times. The controller simultaneously displays all settings, including: 1) reagent locations, 2) reagent and agitation steps, 3) time to complete the current reagent step, 4) time to complete the entire protocol, 5) the operator’s name and project ID, and 6) the reagent temperatures (ASP-2000 only). To aid users that wish to create/modify protocols, best practice assistance is provided for both specimen and grid preparations. This is further eased by now enabling protocol development on office PCs and Macs, and by utilizing the ever-expanding library created by other ASP users.

ASP protocols are available for a very wide range of research and clinical specimen types. These include protocols that reduce TEM tissue prep to just 1-3 hours [1-2] and vEM prep to just 1 day [1, 3]. There are also protocols for pre- and post-embedding immuno-gold labeling [4]. ASPs can efficiently prepare just a single specimen, or simultaneously prepare up to 8 or 24 specimens, or 8-32 grids. Examples of TEM and vEM specimens prepared using the new controller operator interface are shown in Figures 1 and 2.

In summary, the mPrep Automated Specimen Processor product line has been improved with a major control system update, and with the introduction of the ASP-2000 which adds integrated reagent temperature control. With either ASP model, tedious manual reagent processing is replaced with automated efficiency and consistency.



**Fig. 1.** TEM specimens prepared with ASP-2000 from aldehyde fixative rinse-out to 100% resin infiltration: A-B) Zebrafish gill prepared in ~3.5 hrs. C-D) Mouse sciatic nerve prepared in ~5 hrs. Grids section stained with Uranylless and lead citrate. Courtesy L. E. Dye, NIH-NICHD.



**Fig. 2.** Rat brain cortex serial block face vEM 28x32x7μm image from larger dataset. ASP-1000 prepared in ~8 hrs: aldehyde fix rinse-out-FeOs-TCH-Os-Ur-Pb-100% resin infiltration. Robotic uniformity provides standardized contrast for trained AI networks (e.g., U-Net) for image segmentation and quantification. A) 3D volume image. B) Composite of AI segmented synapses (C), mitochondria (D), and myelin (E). Courtesy E. Benson & G. Kidd, Cleveland Clinic Foundation.

## References

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