Efficient Workflows for Electron Microscopy Laboratories by Using Automated Specimen Preparation

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Electron microscopy labs are critical bioscience resources providing services that include transmission electron microscopy (TEM), 3D volume EM (vEM: serial block face, focused-ion-beam, array tomography scanning EM), cryo-EM (may include freeze substitution), and EM immunolabeling. Despite the maturity of bio-EM, it continues to revolutionize biomedical and botanical structural biology, especially with the growth of vEM which uniquely provides molecular-resolution structural biology in near-millimeter volumes while enabling correlative light and x-ray microscopy [1-3].

While TEM and vEM microscopes are increasingly automated, EM specimen preparation remains intensely manual in most labs: Specimen prep requires sequential reagent series that include aldehydes, labels, toxic heavy metal stains, solvents, and embedding resins. The manual reagent prep process typically takes 1-3 days for TEM, a week for vEM, and 1-2 days for immunolabeling. This manual process requires extensive hands-on time making it problematic to perform other tasks. While microwaves and auto-processors can improve some TEM workflows, these can't prepare specimens for vEM, immunolabeling, or post-cryo freeze-substitution vEM. Thus, highly trained scientists (81% with MS or PhDs) [4-5] in EM core labs are tied up for hours-days with tedious manual reagent processing.

With the growing demand for vEM, ongoing TEM demand, and decreasing numbers of trained EM scientists and technologists, the labor required by manual specimen prep is a growing burden. This is expected to become more problematic since 44% of highly trained EM scientists and technologists are 51 years or older, thus approaching retirement [4]. Further, the pipeline for new personnel is limited by few formal training programs, and substantial difficulty in attracting new staff at any skill level, with 47% of EM core managers reporting that they have never trained a novice. Further, core management is demanding, typically requiring 55 hours/month on just administrative tasks [5-6].

Since the 2015 introduction of the mPrep™ ASP-1000 (Automated Specimen Processor), dozens of TEM, vEM, and immunolabeling ASP protocols have been published and developed for a wide range of specimen applications including kidney, liver, brain, muscle, nerves, heart, skin, gills, worms, bacteria, and yeast [7-12]. The ASP workflow (Figure 1) uses mPrep/s specimen capsules to provide a range of specimen-handling options for EM scientists and pathologists. These include methods to orient roughly cubic or long specimens for cross-sectioning, and the flexibility to efficiently process just a few specimens, or up to 128 small tissue specimens such as 1-2mm long segments of 18G renal biopsies [7].

Easy-to-operate ASPs provide fast efficient workflows so highly trained staff can focus on knowledge tasks; enabled by automated TEM prep in just 1-3 hours, and vEM prep in 1 day with highly reduced hands-on effort (Figure 2). ASPs improve vEM heavy-metal staining reproducibility while minimizing operator exposure to toxic chemicals [2], and unique mPrep/s capsules enable more efficient freeze-substitution vEM prep [3]. The highly adaptable automated ASP - mPrep capsule workflow frees EM staff from tedious manual tasks for almost all EM applications by providing robotic consistency, speed, documentation, protocol sharing, and other features required in busy EM labs [12].

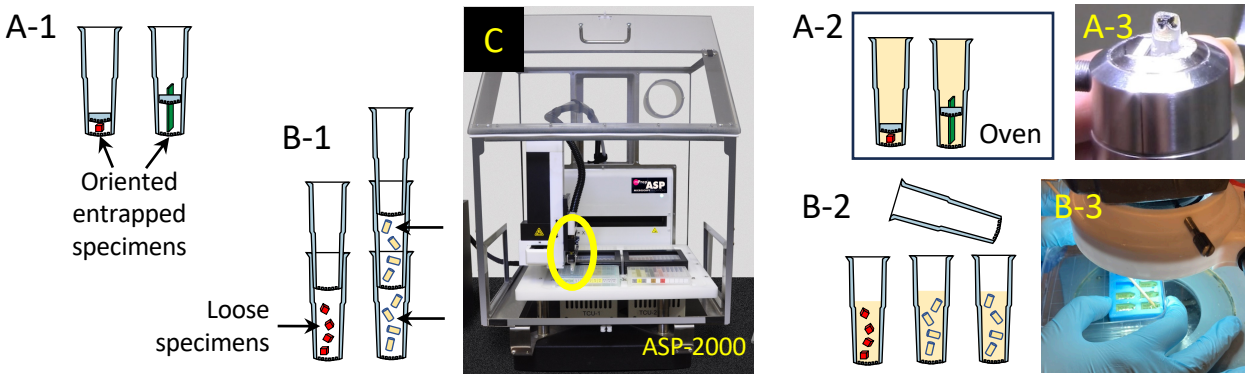

Figure 1. mPrep/s specimen capsules and ASP workflows. Individual specimens can be loaded into mPrep/s capsules with orientation (A-1), or up to 8 small specimens can be loaded into capsules and capped with a second capsule (B-1), with capacity doubled by stacking 2 specimen-filled capsules. Single or stacked capsules are attached to the 8-channel ASP head (C, circled) for simultaneous prep of 8 or 16 capsules (8-128 specimens) from aldehyde to 100% resin. Resin-infiltrated specimens (A-2) may be cured in capsules to maintain orientation for microtomy (A-3). Or loose resin-infiltrated specimens are removed from capsules and embedded in flat molds or other molds (B-3).

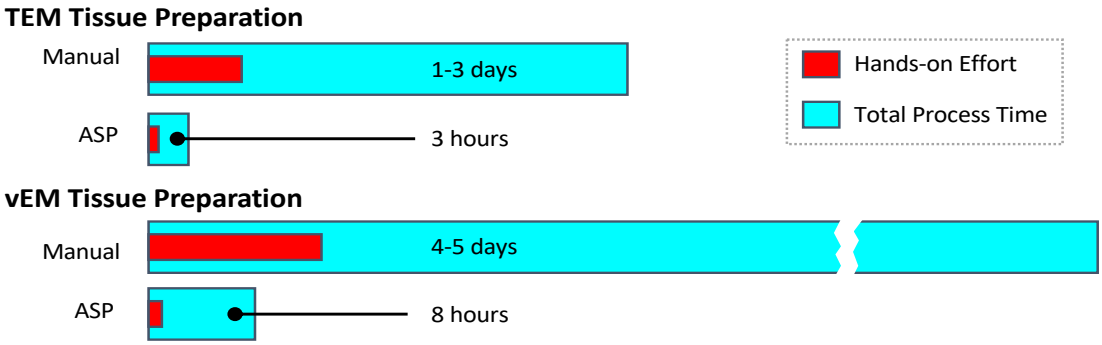

Figure 2. ASP and manual specimen preparation efficiency. ASPs provide faster prep with less cumulative hands-on effort than manual processing. Typical times are shown for both manual and ASP automated preparation for Transmission and Volume Electron Microscopy (TEM, vEM).

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Introduction

Electron microscopy core labs are critical resources for biomedical and botanical structural biology, for clinical research, and often for medical diagnostics. Typical EM labs provide imaging instruments and methods including:

- Transmission electron microscopy (TEM).
- 3D volume EM (vEM), most commonly serial block face & FIB-SEM. vEM is becoming increasingly important as it uniquely provides 3D molecular-resolution in near millimeter volumes.
- Immuno-gold labeling (IGL), which locates molecular structures in TEM and vEM.
- Cryo-EM, which may include freeze substitution.

While EM imaging and image analysis workflows are increasingly automated, EM specimen preparation remains intensely manual in most EM labs: TEM prep takes days and vEM requires most of a week. Cryo freeze substitution incorporates some similar specimen prep as vEM and TEM.

The Problems

1) An increasing shortage of biological EM Scientists and Technologists. Current highly-trained EM scientists (81% MS or PhDs) are approaching retirement, with 44% aged 51 years or older. 47% of core lab staff report that they have never trained a novice [1-2]. Many report extreme difficulty in attracting new core staff at any skill level.
2) Specimen preparation for TEM and vEM ties up staff for hours or days with tedious manual reagent processing.
3) Core management is demanding, typically requiring 55 hours per month on administrative tasks [2-3].

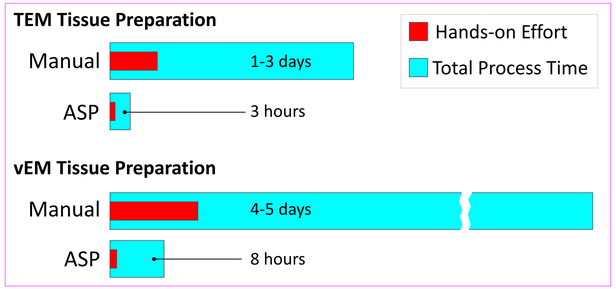
Highly trained EM management and core staff are too few and have too much work.

The Solution

Efficient automated workflows to enables experienced staff to focus on knowledge tasks, rather than manual lab work.

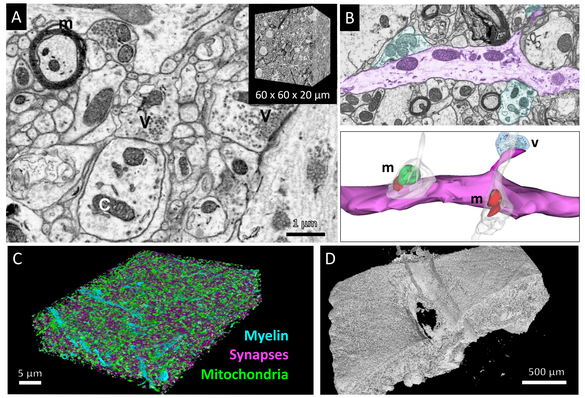
- mPrep™ System automation is adaptable for nearly any type of specimen or TEM grid preparation task.
- ASP™-1000 & ASP™-2000 Automated Specimen Processors autonomously perform nearly any protocol.
- ASPs require minimal training. Even new staff can produce consistent high-quality results.
- Younger personnel are comfortable with 96-well microplates, multi-channel pipettes and lab automation.
- Automation that reduces lab tedium & toxic reagent handling should improve hiring.
- Faster specimen prep can improve turnaround & revenue.
- Lower reagent consumption save money & cuts waste.
- Automated ASP documentation eases admin tasks.

Automated Preparation Workflows with ASP™-1000 or -2000 provides flexibility and reduce personnel effort: A) Specimens are loaded into mPrep/s capsules. Can orient in individual capsules, or load 1-8 specimens per capsule for high capacity. Stacking capsules multiplies capacity. B) Capsules attached to 8-channel ASP™-1000 head (circled). Preparation reagents in 4 sealed microwell plates (arrows) & 2 open plates on 6-plate deck. C) ASP™-2000 with fume enclosure, pump module & laptop controller. ASP-2000 adds 0-100C reagent temperature-control (arrows). Reagents can also be in open microplates. D) ASP Dashboard enables virtually any protocol. Top control bar shows status and timing. Plate map shows reagent locations in 6 plates. E) ASP-processed specimens can be embedded and sectioned in mPrep/s capsules to reduce handling and provide specimen orientation, or F) specimens can be embedded in conventional molds.

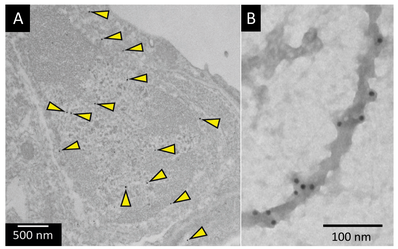


Electron Microscopy Preparation: Elapsed Times & Hands-On Effort

Preparation	Reagent steps	Manual	Carousel processors	Microwave processors	ASP-1000 & ASP-2000
TEM tissue	19	1-3 days prep 6-8 hrs effort	1-1.5 days 1-3 hrs effort	2-4 hrs prep 2-3 hrs effort	1-4 hrs autoprep 40 min effort
IGL TEM grids	27	1 day prep 8 hrs effort	*Not capable*	*Not capable*	5-8 hrs autoprep 30 min effort
IGL Tissue	46 IGL + 19 TEM tissue	3-5 days prep 16 hrs effort	*Not capable*	*Not capable*	12 hrs autoprep 45 min effort
Volume EM tissue	63	4-5 days prep 13 hrs effort	*Not capable*	*Not capable*	8 hrs autoprep 45 min effort



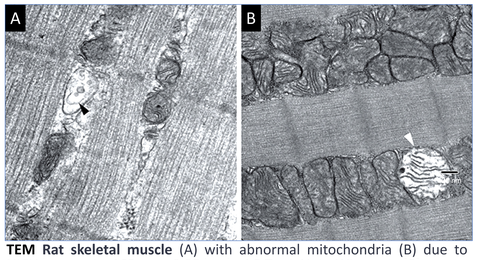
Volume EM rat brain, ASP-prep 8 hrs by E. Benson & G. Kidd, Cleveland Clinic [4-5]: A) Single plane from volume image (inset), mitochondria (m), cristae (c), synaptic vesicles (v). B) Dendrite (magenta) single plane image shows synapses (red & green), and in 3D volume image. C) AI identification & quantification enabled by robotically uniform preparation. D) X-ray microscope images large volumes in same prepared specimens.



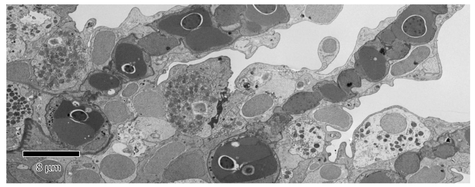
Immunogold TEM murine brain and bacteria

A) Murine brain section with ultra-small immunogold labeled HA-tag overexpressed SIRT1. Automatically labeled with ASP in 18hr, compared to "nearly a whole week" of intermittent manual effort [6].

B) Bacteria whole mount labeled with anti-flagella 10 nm immuno-gold automatically in 6 hrs 45 minutes. ASP "allows overnight operation which is more conveniently integrated in a busy work schedule." Marques P, Lillehoj EP, R Hsia, Univ. Maryland – Baltimore [6-7].



TEM Rat skeletal muscle (A) with abnormal mitochondria (B) due to NICU hyperoxia. ASP-prep 2.25 hrs by LG Tetri, Univ. Wisconsin [8].

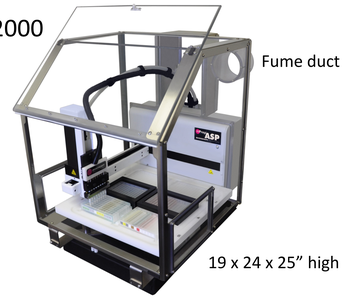


TEM zebrafish gill. ASP-preparation in 3.5 hrs by L. E. Dye, NIH-NICHD.

Automation for EM Core Labs

Easy to operate mPrep™ Automated Specimen Processors (ASP™-1000 & ASP™-2000) meets workflow needs of EM core labs of any size. Enables highly trained staff to focus on knowledge tasks. Provides:

- Fully automated walk-away preparation for TEM, vEM, and even freeze-substitution steps (warmer than 0C).
- Any protocol: 72 steps with 576 reagents, or more.
- Automate your protocols or use protocols shared with other labs.
- Prepare just 1 or up to 128 specimens at a time.
- Minimal technician training.
- Faster preparation (5-10x) reduces turn-around time.
- Easy efficient set-up. Clean up in 5 minutes.
- Minimizes reagent consumption.
- Minimizes hazardous reagent exposure.
- Reduces specimen handling, damage and loss.
- Automated documentation for billing, etc.



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