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Meeting-report

Biological Specimen Preparation Workflows in EM Laboratories

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Electron microscopy is essential for the biosciences, clinical pathology, and many other fields. EM labs may provide TEM, SEM, and increasingly one or more types of 3D volume EM (vEM), such as serial block face, array tomography, or focused-ion-beam SEM [1-3]. In 2023 vEM, which provides TEM-like molecular resolution but of near-millimeter 3D volumes, was described as "a quiet revolution" [2] and was the subject of a *Methods in Cell Biology* volume [3]. Some core EM labs also may provide correlative light and electron microscopy (CLEM), immuno-gold labeling (IGL), and various types of cryogenic EM.

Biological EM began about 50 years ago with the development of methods to stabilize and provide atomic number contrast of biospecimens by a sequential process of aldehyde fixation, heavy metal osmium tetroxide staining, resin embedding, and microtomy, as recognized with a 1972 Nobel Prize [4]. This chemical prep methodology (Fig. 1), has proven extremely robust, remaining today the most common methodology for biological TEM, and more recently for vEM. Many bio-SEM specimens are prepared using a similar chemical sequence but typically conclude with critical point drying after dehydration.

Although there have been tremendous advances in TEM and vEM instrumentation including automated operation, chemical fixation biospecimen prep in most EM labs is still done manually (Fig. 1). This manual process requires great care for reproducible and quality results. The process chemicals are also toxic, especially for the more intense staining required for vEM. This includes aldehyde fixatives, osmium tetroxide, reactive compounds including thiocarbohydrazide, other heavy metals including Ur, Pb, and Fe stains, and the solvents and embedding resins. Manual processing is time-consuming, with TEM specimen prep typically taking 2–3 days and vEM typically taking 4–5 days, with both requiring tedious manual reagent exchanges every few minutes to hours. While microwave and carousel processors can accelerate or automate some TEM workflows, these are not suitable for all specimens and are not capable of vEM preparation, immunolabeling, or post-cryo freeze-substitution vEM [1, 3].

The long and intense hands-on labor for manual reagent specimen processing ties up highly trained EM scientists (81% with MS or PhD degrees) [5] and prevents them from doing knowledge work, e.g. advising clients, drafting reports, EM imaging, and image interpretation. Long process times also reduce throughput, increase labor costs, and decrease income in fee-for-service labs.

Automated specimen preparation can free lab personnel from the burden of manual reagent processing, but only if automation can perform the necessary wide range of complex and custom preparation protocols used in today's EM labs. In 2015, Microscopy Innovations LLC introduced the mPrep™ ASP-1000 Automated Specimen Processor, and in 2022 the ASP-2000 to add programable reagent temperature control and introduce a new control Dashboard to enhance the capabilities of both ASP models [1,6]. ASPs meet the needs of life science EM labs by cutting labor and enabling nearly any TEM, vEM, and SEM specimen prep tasks. ASPs are efficient, preparing 1 to 128 tissue pieces for TEM in 1-4 hrs, or 1 to 64 vEM specimens in 6-8 hrs, while consuming ~5 ml of each reagent at each protocol step for up to 128 specimens, thus minimizing reagent purchase costs, waste, and user exposure.

Figure 2 diagrams typical times to perform TEM specimen prep, TEM immuno-gold labeling of specimens and TEM grids, and vEM specimen prep. Also shown are cumulative hands-on times. The hands-on effort for manual processing is intermittent over several days, nearly non-stop for microwave oven processing over 1-3 hrs, while hands-on effort for carousel auto-processors and ASPs occurs at the beginning and end of specimen preparation.

ASP specimen preparation protocols are user-shareable, with dozens created for different specimens and applications including TEM, vEM, IGL, and others. ASP preparation reduces hands-on labor from days to 1 hour or less [1, 3, 6] enabling highly trained EM staff to focus on imaging, analysis, and other knowledge activities while reagent processing is performed automatically, and in a fraction of the time compared to manual processing (Fig. 2). Some types of CLEM are also enabled using mPrep/s specimen processing capsules; clear mPrep/s capsules enable light microscope imaging of living specimens cultured within the capsules, and other assays [7], which can be followed by processing encapsulated specimens as demonstrated for SEM and histology [7], and potentially for TEM and vEM.

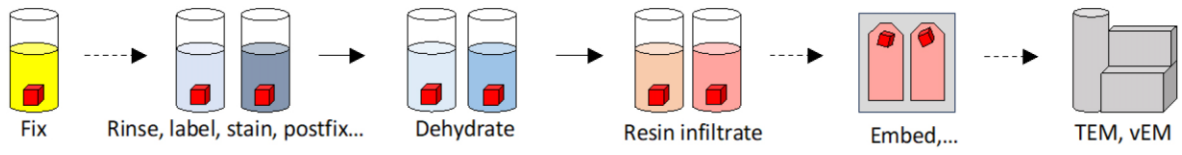

Fig. 1. Typical bio-specimen chemical reagent preparation processing sequence workflow.

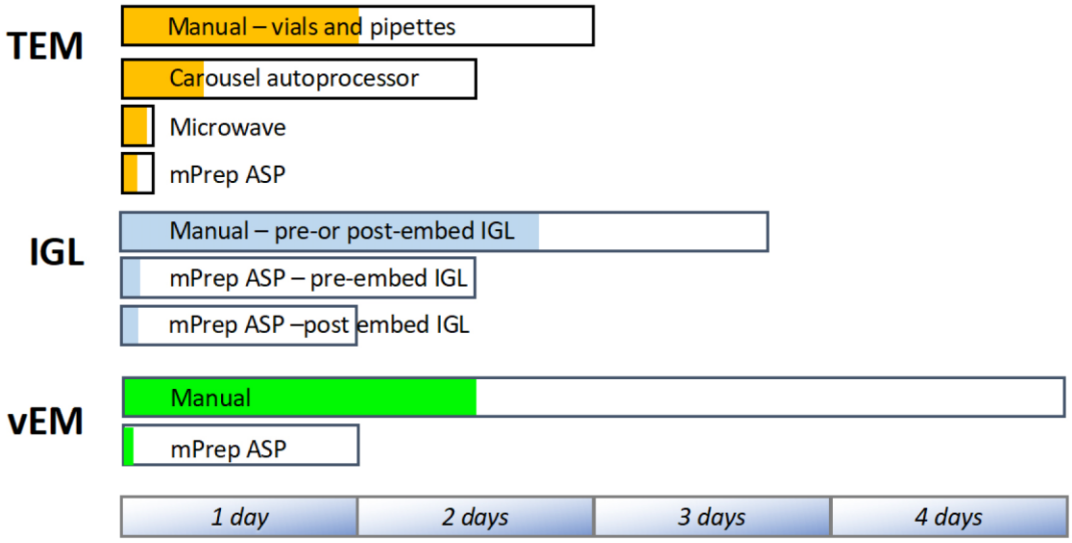

Fig. 2. Process workflow efficiencies for TEM, immunogold TEM, and vEM with different methods. Open bars show typical process times after initial aldehyde fixation until ready for resin curing. Shaded bars show approximate cumulative hands-on effort.

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