Fast, Walk-away, Automated Processing of Mammalian Tissue for LM and TEM

Nicholas R Stewart[1,2], Benjamin K August[1], Thomas E Strader[2], Steven L Goodman[2]

[1.] University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI USA
[2.] Microscopy Innovations, LLC, Marshfield, WI USA

Four types of mammalian tissue; kidney, liver, skeletal and cardiac muscle, were processed for transmission electron microscopy (TEM) using the mPrep ASP-1000 automated specimen processor (ASP). For comparison, tissue samples were also prepared using conventional manual reagent exchanges in vials with rotator agitation. Both ASP and manual processing produced well-prepared tissues.

Mammalian tissues were perfusion-fixed on site with buffered 4% paraformaldehyde and 1% glutaraldehyde. Then, ~2 mm tissue samples were excised and oriented in mPrep/s capsules in the electron microscopy lab. Thereafter, each tissue sample remained in its own labeled capsule throughout all subsequent processing. The capsules, in sets of eight, were attached onto the ASP robotic arm (Figure 1). The ASP robotic arm then automatically moved capsule-loaded specimens to each sequential reagent arrayed in standard trough plates (Figure 1). The ASP then aspirated (pipetted) each reagent into the capsules with rapid agitation provided by aspiration and dispense cycles as quick as 2 seconds per reagent exchange. Tissue processing typically required 130 minutes after attaching capsules to the ASP until epoxy infiltration was complete. Processing time was further reduced with some tissues to only 45 minutes. Resin curing was performed by transferring epoxy filled mPrep/s capsules to the curing oven. Sectioning was accomplished by directly mounting the mPrep/s capsules into the microtome chuck, thus preserving orientation and specimen labeling.

Once inserted into mPrep/s capsules and loaded onto the ASP, muscle samples were washed in cacodylate buffer, while liver and kidney samples were washed in phosphate buffer. All samples were then post-fixed in 1% osmium tetroxide in the corresponding buffer. Samples were then washed in distilled water and dehydrated using graded ethanols, transitioned into acetone and embedded into Embed 812. Once infiltrated, the mPrep/s capsules (with tissue specimens) were removed from the ASP and cured overnight at 60°C. One micron sections were obtained for light microscopy and 70 nm sections were obtained for TEM. Sections were negative stained with 8% uranyl acetate in 50% ethanol and Reynolds lead citrate. OsO_4 post-fixation, dehydration, solvent transition, and resin infiltration were confirmed to be complete using light microscopy of toluidine blue stained 1 μm sections (not shown). No artifacts due to incomplete processing were observed. A Philips CM120 Electron Microscope with a BioSprint camera running AMT Capture Engine V700 was then used to image the 70 nm sections.

Summary: Ultrastructural preservation was publication quality with both ASP prepared and manually prepared comparison samples. With the comparison samples prepared in scintillation vials, only one sample could be processed at a time to achieve a reagent exchange rate that could approach the speed of the ASP. All samples were cured and sectioned using the same methods. ASP prepared kidney, liver,

and skeletal muscle illustrate excellent preparation quality (Figures 2-4), as did cardiac muscle (not shown due to space limits.) The ASP sample preparation was much easier than conventional processing because the process was automated and since specimens were never removed from the mPrep/s capsules, thus eliminating messy and error-prone handling. Additionally, reagent processing was achieved in ~2 hours or less, compared to typical day-long conventional reagent exchange protocols.

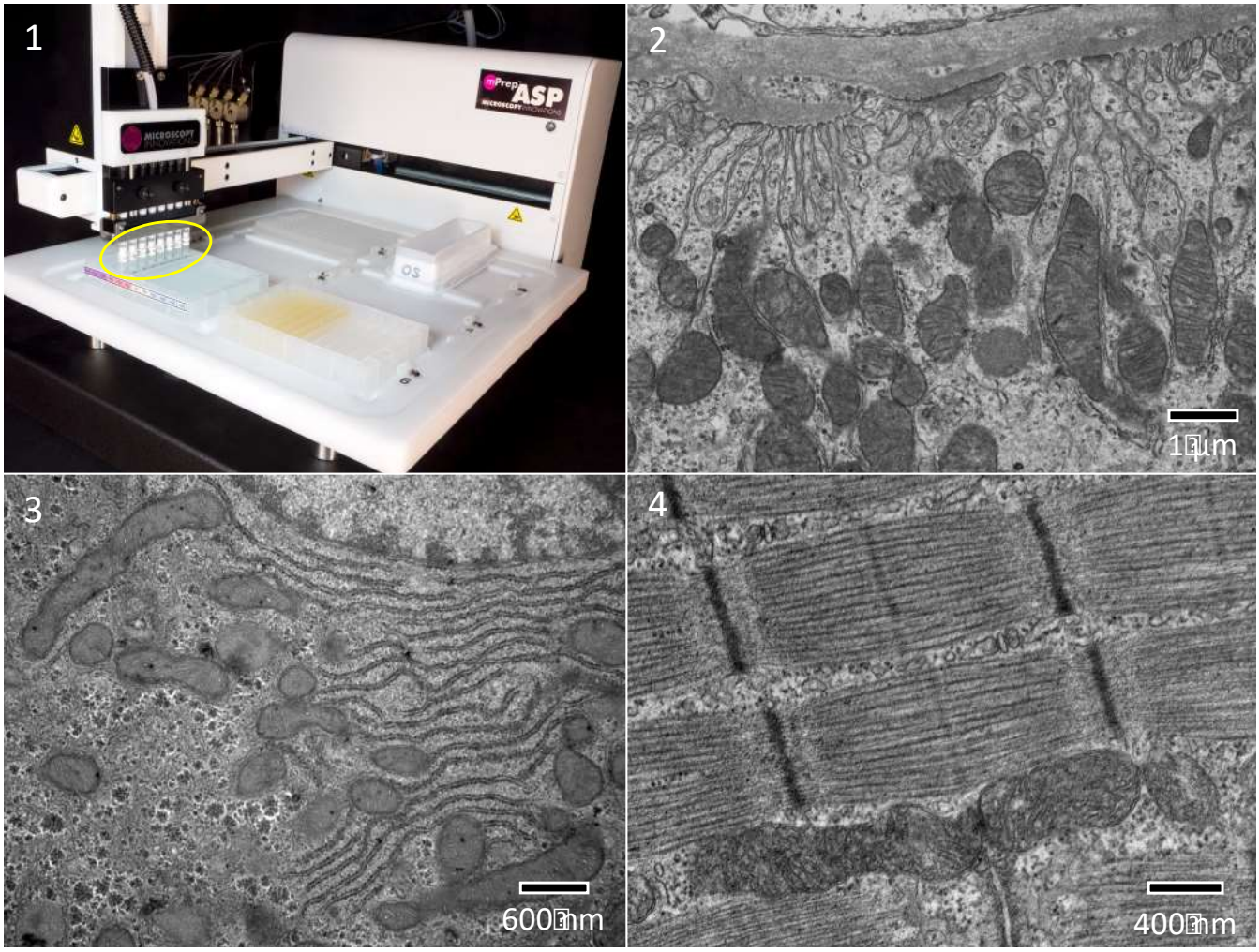


Figure 1: ASP-1000 automated processor with tissue specimens in mPrep capsules on robotic arm (circled). Reagents are drawn into capsules from 12-row trough plates and reservoirs on the platform.

Figure 2: Proximal convoluted tubule cell in rat kidney along the cells basolateral side shows invaginating membrane folds with many long mitochondria. Note fine mitochondrial detail including outer mitochondrial membranes and cristae.

Figure 3: Rat liver hepatocyte in the sinusoid shows well preserved layers of rough endoplasmic reticulum, glycogen alpha particles (electron dense rosette-like clusters) mitochondria and a portion of the cell's nucleus. Note well-preserved nuclear envelope, outer mitochondrial membrane and cristae.

Figure 4: Rat gastrocnemius (skeletal) muscle shows a myofibril with sarcomeres along with subsarcolemmal mitochondria and sarcoplasmic reticulum. Clearly seen are the sarcomere Z-lines, M-lines and mitochondrial cristae.

Fast, Walk-away, Automated Processing of Mammalian Tissue for LM and TEM

Nicholas R Stewart [1-2], Benjamin K August [1], Thomas E Strader [2], Steven L Goodman [2]

[1.] University of Wisconsin-Madison. School of Medicine and Public Health, Electron Microscopy Facility, Madison, WI USA

[2.] Microscopy Innovations, LLC. Marshfield, WI USA

School of Medicine and Public Health
UNIVERSITY OF WISCONSIN–MADISON

MICROSCOPY INNOVATIONS LLC

Introduction

Specimen preparation for transmission electron microscopy (TEM) is time consuming and labor-intensive. Orientation in high viscosity resin is messy, samples may be damaged, mix-ups occur and operators are exposed to toxic reagents. These problems are reduced with mPrep automation that eliminates most operator interventions, minimizes sample handling, cuts reagent exposure, and can prepare tissue samples in 1-2 hours.

Summary

Kidney, liver, skeletal and cardiac muscle were prepared using the mPrep ASP-1000 programmed for rapid agitation reagent exchanges. Processing consistency was tested by preparing 24 samples of liver from the same source in 3 batches of 8 specimens. No differences were seen between samples or batches. ASP preparation of muscle was also compared to manual reagent exchanges, with both producing well-prepared tissues.

Experimental

Rats were perfusion-fixed with buffered 4% paraformaldehyde - 2% glutaraldehyde. Kidney, skeletal and cardiac muscle were cut into ~2 mm cubes. To examine reproducibility, 24 liver samples were cut into 2 mm discs from 500 µm sections. All tissues were oriented in mPrep/s capsules (Figure 1A). Samples remained in a labeled capsule for all preparation.

Specimen capsules, in sets of 8, were attached to the ASP (Figure 1B). Reagents were placed in reservoirs. Phosphate buffer was used for liver and kidney, and cacodylate for muscle. The ASP rapid processing protocol exchanges reagents every few seconds (Table below). After ASP infiltration with Embed 812, resin-filled capsules were removed from the ASP and cured overnight at 60°C.

Step	Reagent	Fluid exchanges	Delay (sec)	Time (min)
1	Buffer Fix Rinse 1	60	0	2.3
2	Buffer Fix Rinse 2	60	0	2.3
3	Buffer Fix Rinse 3	60	0	2.3
4	Osmium	600	0.5	35
5	Osmium H20 Rinse 1	60	0	2.3
6	Osmium H20 Rinse 2	60	0	2.3
7	50% Ethanol	60	0	2.3
8	70% Ethanol	60	0	2.3
9	90% Ethanol	60	0	2.3
10	95% Ethanol	60	0	2.3
11	100% Ethanol	120	0	4.6
12	Acetone 1	120	0	4.6
13	Acetone 2	120	0	4.6
14	25% Resin	180	0.5	10.5
15	50% Resin	180	0.5	10.5
16	75% Resin	180	0.5	10.5
17	100% Resin 1	180	0.5	10.5
18	100% Resin 2	180	0.5	10.5
19	100% Resin 3	180	0.5	10.5
			Total time:	132.5

Capsules were mounted into microtome chucks for sectioning, preserving orientation and specimen labeling (Figure 1C). 1 µm sections were stained with toluidine blue for light microscopy. 70 nm sections were stained with 8% UA in 50% ethanol and Reynolds lead citrate. TEM imaging used a Philips CM120 and a BioSprint camera running AMT Capture Engine V700.

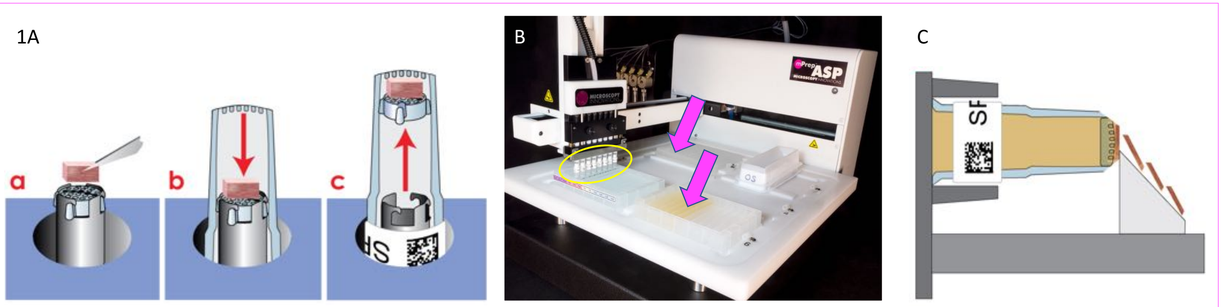


Figure 1: A) Aldehyde fixed tissue is oriented and entrapped in mPrep/s capsules using the mPrep Workstation. B) Eight capsules (circled) are attached to the robotic arm of the ASP-1000 Automated Specimen Processor (ASP). Reagents are placed in microwell plates (arrows). C) Blocks are sectioned by clamping mPrep capsule directly into microtome chuck.

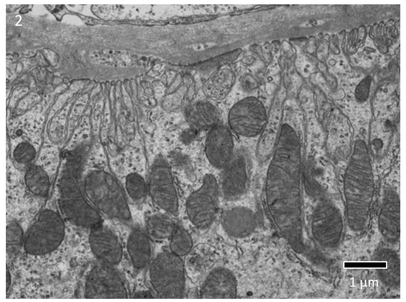


Figure 2: Proximal convoluted tubule cell in rat kidney along basolateral side with invaginating membrane folds and many long mitochondria. Note mitochondrial membranes and cristae.

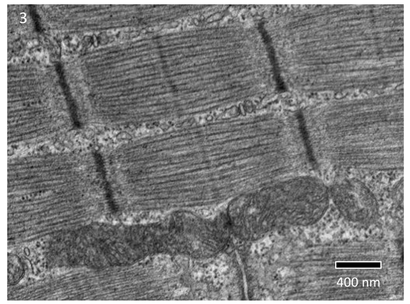


Figure 3: Rat gastrocnemius (skeletal) muscle shows myofibril with sarcomeres, sub-sarcolemmal mitochondria and sarcoplasmic reticulum. Note Z-lines, M-lines, and cristae.

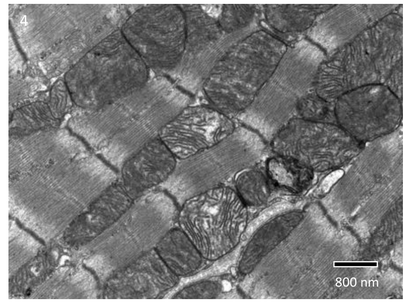
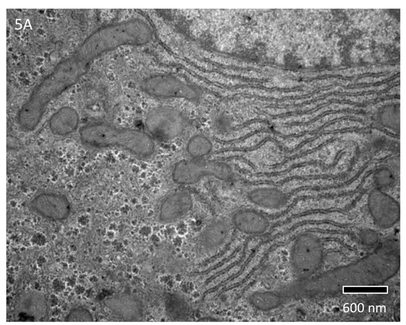


Figure 4: Cardiac muscle shows myofibrils and sarcomeres, mitochondria and sarcoplasmic reticulum. Also note mitochondrial cristae.



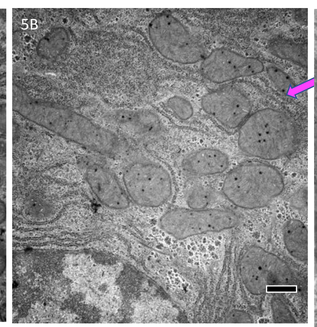


Figure 5: A) Rat liver hepatocyte in sinusoidal region shows well-preserved rough endoplasmic reticulum, glycogen alpha particles (electron dense rosettes) mitochondria and a portion of nucleus. Note nuclear envelope, mitochondrial membranes and cristae. B) A second liver specimen from reproducibility study also shows similar well preserved structures. This region is shown in Figure 5C (arrow).

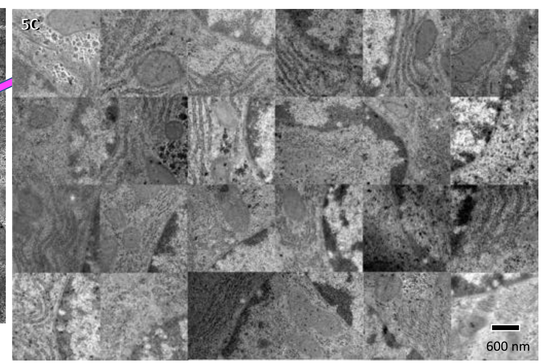


Figure 5C: Reproducibility study of 24 rat liver samples processed in 3 separate batches of 8 capsules over the course of a day. Note consistently well preserved and fully embedded tissue.

Results and Discussion

The ASP produced well-prepared kidney, skeletal muscle, cardiac muscle and liver (Figures 2-5). Mitochondrial and muscle ultrastructure were well preserved (Figure 2-4). All 24 liver samples, prepared in 3 ASP processing runs, produced consistent well-prepared tissue (Figure 5). Complete fixation and infiltration of kidney and liver was also confirmed by light microscopy (Figure 6). Manual vial processing provided comparable results, but only one sample could be processed at a time to even approach the ASP reagent exchange rate.

- Complete reagent processing was accomplished with the ASP in only 133 minutes from postfix rinse through 100% resin infiltration for all specimens. Kidney was also processed successfully in only 45 minutes.
- ASP processing provided microwave-like speed but without the need for manual reagent exchanges every few minutes.
- Handling of resin-infiltrated specimens and transfer to embedding molds was eliminated.
- Specimens were directly handled only once - to place them in labeled mPrep/s capsules.
- Automated processing reduced operator reagent exposure and potential operator error.

Conclusions

Using the mPrep ASP-1000 Automated Specimen Processor for tissue provided:

- Fast, walk away, processing of mammalian tissue for LM and TEM.
- Consistent publication quality preparation in ~2 hours or less, compared to typical day-long conventional reagent exchange protocols.
- Elimination of all direct specimen handling once specimens were placed in labeled capsules, including messy transfers in embedding resin.
- Always traceable specimens since they were never removed from labeled mPrep/s capsules.

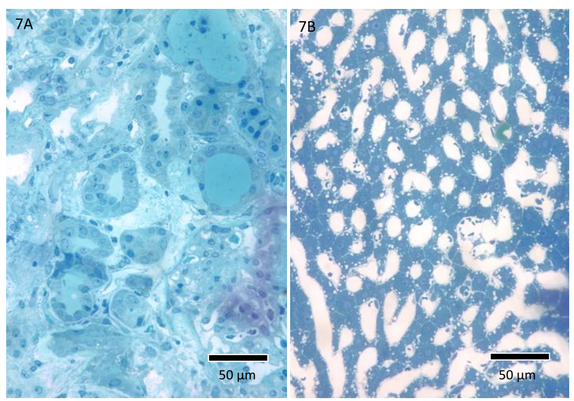


Figure 6: Toluidine blue light microscope images of rat kidney (A) and rat liver hepatocyte (B) confirming complete embedding.