Bringing Speed and Automation Together with the ASP-1000.

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There is great motivation in the electron microscopy field to speed up and streamline sample processing, especially for transmission electron microscopy (TEM) which can take days to weeks to complete on the bench. The sample processing microwave has been developed to allow faster protocols, and tissue processors have automated the procedure, but as yet there is no established combination of speed and automation for chemical TEM sample processing. This experiment was designed to test the mPrep capsule system and ASP-1000 robot as a possible alternative to microwaves and tissue processors by taking advantage of rapid continuous agitation to gain both speed and automation.

A new way of streamlining sample preparation for electron microscopy has emerged in the market, the capsule based system called mPrep by Microscopy Innovations [1]. Together with their sample processing robot the ASP-1000, this system is positioned to enable a combination of automation and time reduction in TEM sample processing [2]. The work of Kent McDonald and Rick Webb in the areas of rapid freeze substitution and resin infiltration and embedding have brought about a renewed understanding of the role rapid agitation plays in reducing time for sample processing [3]. In this experiment the ASP-1000 robot was programmed for continuous rapid agitation of the samples for each fluid incubation in order to determine whether similar results to traditional bench processing could be obtained with greatly reduced incubation times coupled with rapid agitation.

Freshwater planarian worm *Schmidtea mediterranea* were processed using the steps from an optimized manual bench protocol as a framework and comparison for this experiment [4]. Since microwaves have already reduced times for TEM processing, timing for established microwave processing protocols for use with the Pelco BioWave Microwave Tissue Processor were combined with rapid agitation to produce the ASP-1000 robot protocol [5,6]. For the pure resin steps, rapid agitation was not possible because the resin's viscosity limited the speed of agitation, so the number of repeats of the agitation program remained the same and only the agitation speed was changed (Table 1). Total time for the robot protocol was 1.5 hours, after which samples were left in a pure resin incubation without agitation for 1.5 hours. Samples were embedded in enclosed flat bottomed capsules in Spurr's resin at 100 C for 5 hours, and 50 nm sections were collected for imaging. Sections of samples from this protocol did not appear significantly different from samples processed using the manual bench protocol (Fig. 1).

In this experiment a new protocol has been designed and tested to process samples for TEM in only 3 hours by taking advantage of robotics and constant rapid agitation. Combined with embedding at high temperature, this protocol has taken 8 hours to produce samples that would have taken 7 days with the previous protocol. As an alternative to other time saving and streamlining sample processing techniques, the ASP-1000 brings speed and automation together, freeing up specialist time. In combination with high temperature embedding, sample blocks can be ready for cutting more quickly, speeding up results.

References:

[1] mPrep/s capsules (Catalog #S0812), Microscopy Innovations LLC, Marshfield, WI, USA.
[2] mPrep ASP-1000 (Catalog #ASP1000), Microscopy Innovations LLC, Marshfield, WI, USA.
[3] K.L McDonald, R.I. Webb, Freeze substitution in 3 hours or less, Journal of Microscopy **243** (2011), p. 227-233.
[4] Melainia McClain, High-throughput Multi-parameter TEM Chemical Processing Protocol Development with the mPrep/s Capsule System *Schmidtea mediterranea*, Microscopy and Microanalysis **20(S3)** (2014) p. 1288-1289.
[5] Giberson, R.T., Austin, R.L., Charlesworth, J. et al, Microwave and digital imaging technology reduce turnaround times for diagnostic electron microscopy, Ultrastruct. Pathol. **27** (2003) p. 187-196
[6] PELCO BioWave® Pro Microwave Tissue Processor (Catalog #36500), Redding, California, USA
[7] Thanks to Tari Parmely for providing *S. mediterranea* samples, Tom Strader and Steven Goodman from Microscopy Innovations for product support, and the Stowers Institute for Medical Research.

ASP-1000 Robot Protocol			Manual Bench Protocol	
Time Each Step	Temperature	Steps	Temperature	Time Each Step
40 seconds	RT	4X Buffer	4 C	15 minutes
22 minutes	RT	1% OsO4	4 C	2 hours
40 seconds	RT	4X Buffer	4 C	15 minutes
40 seconds	RT	3X Water	4 C	5 minutes
3 minutes	RT	0.5% Aqueous UA	4 C	Overnight
40 seconds	RT	3X Water	4 C	5 minutes
40 seconds	RT	25%, 50%, 75% Acetone	4 C	20 minutes
40 seconds	RT	4X 100% Acetone	4 C	20 minutes
5 minutes	RT	25%, 50%, 75% Spurr's in Acetone	RT	1 hour to Overnight
10 minutes	RT	3X 100% Spurr's	RT	2 changes per day
1.5 hours	RT	1X 100% Spurr's	RT	2 changes per day
5 hours	100 C	Resin Embedding in Spurr's	60 C	64 hours

Table 1. Sample processing protocols used in comparison. ASP-1000 was not used for embedding.

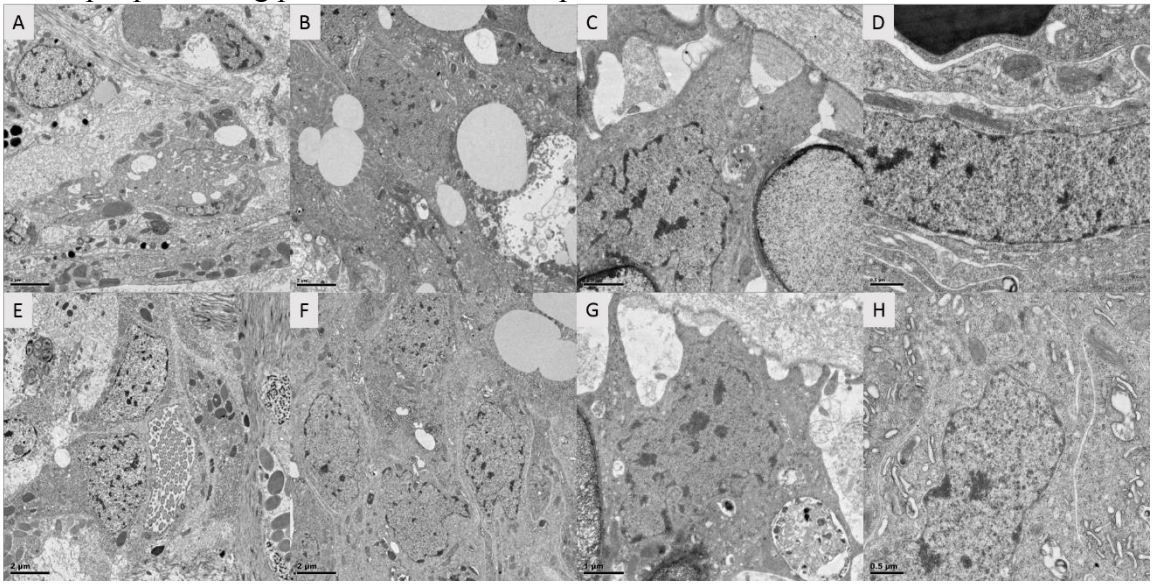

Figure 1. Image comparisons between the protocols. A through D represent the manual bench protocol, E through H the ASP-1000 Robot protocol. A, B, E, F, scale bar 2 um, C and G scale bar is 1 um, D and H scale bar is 0.5 um.

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Introduction

There is great motivation in the electron microscopy field to speed up and streamline sample processing, especially for transmission electron microscopy (TEM) which can take days to weeks to complete on the bench. The sample processing microwave has been developed to allow faster protocols, and automated tissue processors have automated the procedure, but as yet there is no established combination of fast automation for chemical TEM sample processing. This experiment was designed to test the mPrep capsule system and ASP1000 robot as a possible alternative to microwaves and tissue processors by taking advantage of automation and rapid continuous agitation.

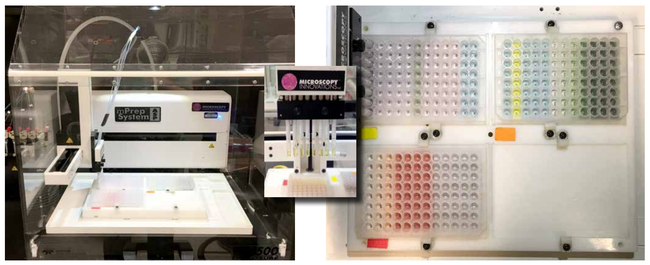

Figure 1. ASP1000 robot with enclosure, capsules attached to the robot ready to be removed after the last resin step, and 96 well plates on the robot deck with filling guides for reagents.

A new way of automation and streamlining for preparing samples for electron microscopy has emerged in the market, the capsule based system called mPrep by Microscopy Innovations [1]. Together with their sample processing robot the ASP1000, this system is positioned to enable a combination of automation and time reduction in transmission electron microscopy (TEM) sample processing. The work of Kent McDonald and Rick Webb in the areas of rapid freeze substitution and resin infiltration and embedding have brought about a renewed understanding of the role rapid agitation plays in reducing time for sample processing [1]. In this experiment the ASP1000 robot was programmed for continuous rapid agitation of the samples for each fluid incubation in order to determine whether similar results to traditional bench processing could be obtained with greatly reduced incubation times coupled with rapid agitation.

Experiment

Freshwater planarian worm Schmidtea mediterranea were processed using an optimized protocol as a framework and comparison for this experiment [2]. Using established microwave processing protocols from Ted Pella for use with the Pelco BioWave Microwave Tissue Processor, the timing for each reagent was adjusted to times appropriate for the sample size [4,5]. For the pure resin steps, rapid agitation was not possible since the resin's viscosity limited the speed of agitation, so the number of repeats of the agitation program remained the same and only the agitation speed was changed, resulting in longer pure resin infiltration steps than what is called for in the microwave protocol (Fig.3). Total time for the robot protocol was 1.5 hours. Samples were left in a fourth pure resin incubation without agitation for 1.5 hours, embedded in enclosed flat bottomed capsules in Spurr's resin at 100C for 5 hours, and 50 nm sections were collected on formvar/carbon coated slot grids for TEM imaging. Sections from this protocol did not look significantly different from samples processed using the former protocol (Fig. 2). In a second run with the same robot program and polymerization at 60C for 2 days instead of 100C for 5 hours, the samples also did not look significantly different (Fig. 2).

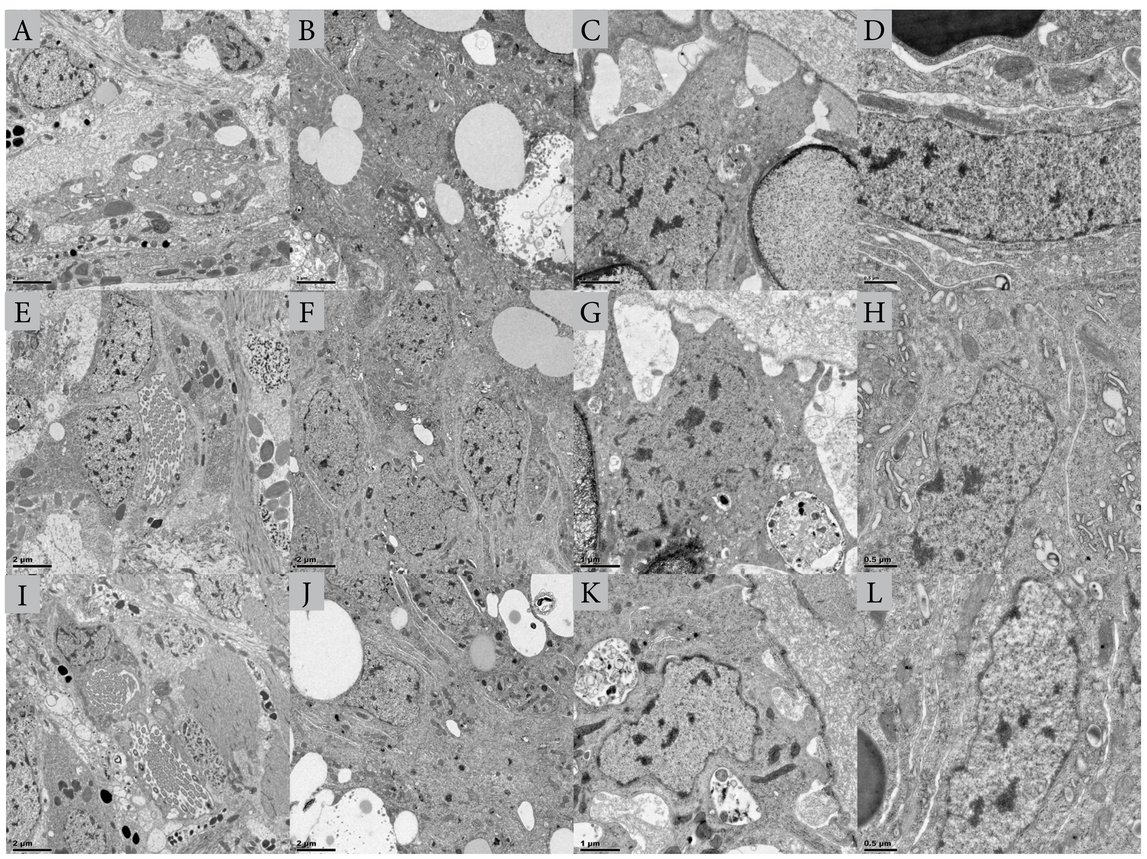

Figure 2. Image comparisons between the protocols. A through D represent the manual bench protocol, E through H the ASP1000 robot protocol, and I through L data from a second run with the same robot protocol. A, B, E, F, I, J scale bar 2 um, C G and K scale bar is 1 um, D H and L scale bar is 0.5 um.

Discussion

In this experiment a new protocol has been designed and tested to process samples for TEM in only 3 hours by taking advantage of robotics and constant rapid agitation. Combined with embedding at high temperature, this protocol has taken 8 hours to produce samples that would have taken 7 days with the previous protocol. As an alternative to other time saving and streamlining sample processing techniques, the ASP1000 brings speed and automation together. For up to 8 samples at a time an experiment can be set up and left running on its own until embedding, freeing up specialist time and producing sample blocks for cutting more quickly at the same time.

ASP-100 Robot Protocol			Manual Bench Protocol	
Time Each Step	Temperature	Steps	Temperature	Time Each Step
40 seconds	RT	4X Buffer	4 C	15 minutes
22 minutes	RT	1% OsO4	4 C	2 hours
40 seconds	RT	4X Buffer	4 C	15 minutes
40 seconds	RT	3X Water	4 C	5 minutes
3 minutes	RT	0.5% Aqueous UA	4 C	Overnight
40 seconds	RT	3X Water	4 C	5 minutes
40 seconds	RT	25%, 50%, 75% Acetone	4 C	20 minutes
40 seconds	RT	4X 100% Acetone	4 C	20 minutes
5 minutes	RT	25%, 50%, 75% Spurr's in Acetone	RT	1 hour to Overnight
10 minutes	RT	3X 100% Spurr's	RT	2 changes per day
1.5 hours	RT	1X 100% Spurr's	RT	2 changes per day
5 hours	100 C	Resin Embedding in Spurr's	60 C	64 hours

Figure 3. Sample processing protocols used in comparison. Embedding not done with ASP1000.

References:

[1] mPrep/s capsules (Catalog #S0812), Microscopy Innovations LLC, Marshfield, WI, USA.

[2] K.L McDonald, R.I. Webb, Freeze substitution in 3 hours or less, Journal of Microscopy 243 (2011), p. 227-233.

[3] mPrep ASP1000 (Catalog #ASP1000), Microscopy Innovations LLC, Marshfield, WI, USA.

[4] Melainia McClain, Microscopy and Microanalysis 20(S3) (2014) p. 1288-1289.

[5] Giberson, R.T., Austin, R.L., Charlesworth, J. et al, Microwave and digital imaging technology reduce turnaround times for diagnostic electron microscopy, Ultrastruct. Pathol. 27 (2003) p. 187-196

[6] PELCO BioWave® Pro Microwave Tissue Processor (Catalog #36500), Redding, California, USA

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