

## Optimization of Automated Immuno EM for Both Pre- and Post-Embedding Labeling

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Immuno electron microscopy (IEM) enables the study of the interrelationship between the cellular content of biomolecules and their proposed function at high resolution. It concurrently provides sensitive antigen detection and detailed cellular structure information. However, IEM is considered one of the most challenging techniques in cell biology [1]. In order to ensure high signal/noise ratio, optimization of incubation/washing regimen and antibody dilutions are critical. Furthermore, IEM experimental procedures are labor-intensive and involve frequent maneuvering of fragile grids or tiny specimens at frequent 5 to 15 minute intervals. The standard post-embedding protocols performed manually in the Electron Microscopy Core Imaging Facility (EMCIF) at the University of Maryland include nearly 50 liquid exchange steps. As a result, experimental outcomes are prone to variation.

An automated post-embedding immunogold labeling procedure using a newly developed automated specimen processor ASP-1000 (Microscopy Innovations, WI, USA) was recently reported [2]. ASP1000 holds tissue specimens or grids in specially designed mPrep capsules [3]. An 8-channel fluid handling system and a three-dimensional robotic platform are used to perform all liquid exchanges and mixing. Researchers only need to handle grids or specimens at the initial loading and final unloading steps. Solution changes and mixing are pre-programed and performed automatically, thus increasing the reproducibility of the labeling outcome. With strategic planning, labeling can be set up as an overnight run leaving the instrument free for other usage during the day. Labile reagents such as silver enhancement solution can be appropriately timed and added during a brief pause in the reaction.

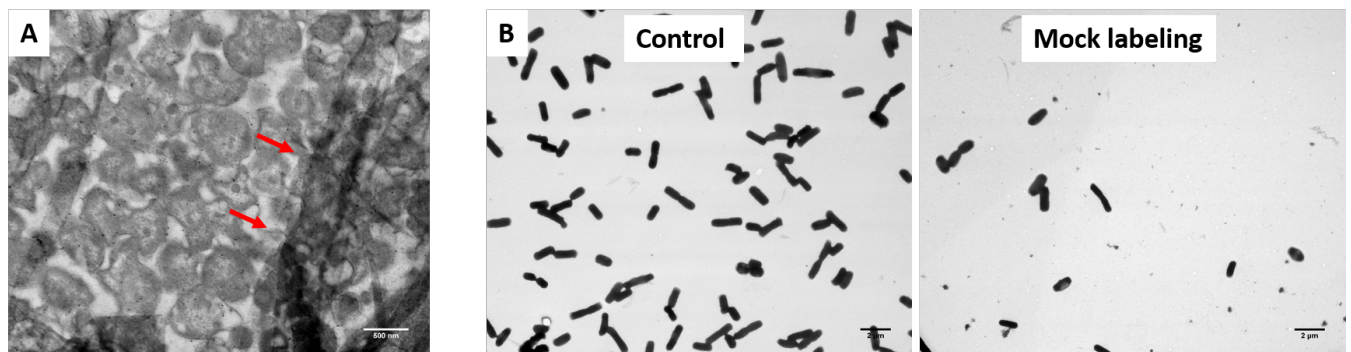
We report here an enhanced procedure for automated post-embedding immunogold labeling in which the pumping speed and frequency of mixing were reduced in order to minimize peeling and folding of resin sections and loss of particulate specimens (Figure 1). Furthermore, the volume of each washing solution was increased by adopting deeper multi-well plates. These changes improved the consistency and the quality of the grids and increased the signal-to-noise ratio (Figure 2). Figure 2 illustrates the detection of bacterial flagellar antigen of *Pseudomonas aeruginosa* freshly applied to EM grids (Figure 2A), of a major chlamydial surface protein on infected Hela cells embedded in unicryl (Figure 2B). A pre-embedding labeling protocol was also developed using mPrepS capsules [3] to hold tissue pieces for labeling and subsequent embedding. We have compared the labeling outcomes of manual and automated labeling. Although both methods resulted in similar labeling efficiency, the automated labeling method consistently yielded lower background noise.

In summary, we have developed automated IEM methods for both post- and pre-embedding labelling using the ASP1000 automated specimen processor. This has not only increased the reproducibility of the immuno labelling results, but also drastically reduced effort and dexterity required to conduct these challenging techniques. The modern demand for efficiency and fast throughput has led to instrument-

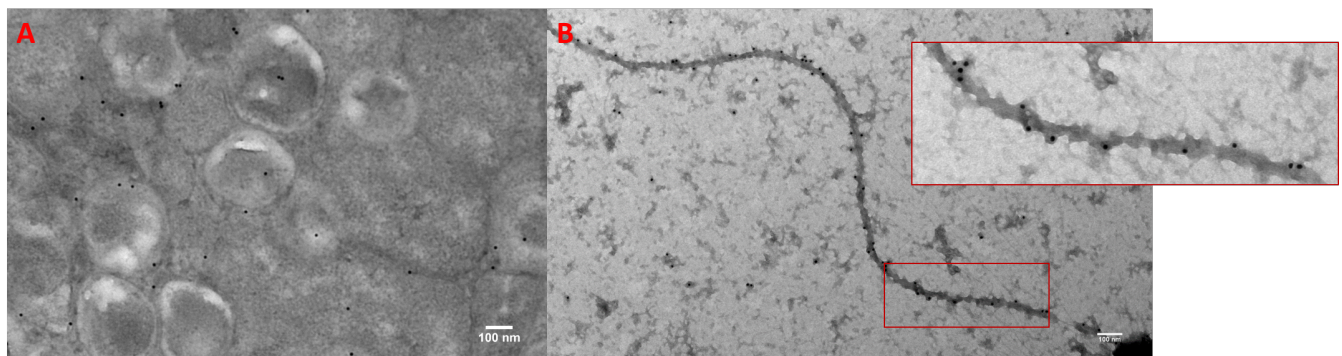
assisted automation being increasingly adopted by EM facilities. The ASP-1000 is a versatile instrument that can be programmed to perform EM specimen processing, staining and immunolabelling. The current model is limited to processing 8 specimens at one time and there is no temperature control. However, a new deck design with heating and cooling module is being developed. We are currently developing additional protocols to accommodate diversified specimens so as to broaden the range of application for this instrument [4].

#### References:

- [1] R Melo *et al*, *Nat Protoc* **9** (2014), p. 2382.  
 [2] E Frankel *et al*, *Microsc. Microanal.* **22** (3) (2016), p. 1010.  
 [3] S Goodman *et al*, *Microscopy Today* **23** (2015), p. 30.  
 [4] The authors wish to acknowledge that the ASP-1000 purchase was funded via Defense University Research Instrumentation Program (Proposal number 70183-LSRIP), Department of Defense.



**Figure 1.** Rigorous mixing of the automated labeling can result in curling and folding (red arrow) of sections (A) and loss of particulate sample (B)



**Figure 2.** Immunogold labeling results using a modified ASP-1000 program for the detection of a major chlamydial surface protein in infected Hela cells embedded in unicryl (A), and a flagellum specific antigen of *Pseudomonas* (B). *Scale bar* = 100 nm.