

# Automated Specimen Staining for TEM Using a Liquid Handling Workstation

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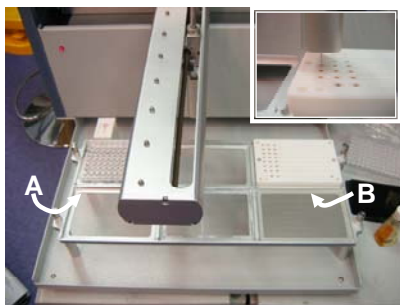
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## Introduction

Automation of data collection with Leginon<sup>1</sup> and robotic loading of grids into the microscope<sup>2</sup> has made it now feasible to perform large scale screening with tens to hundreds of grids. In our laboratory, 96 grids can be imaged automatically without human intervention over a weekend. Applications for these larger scale EM experiments include combinatorial screening for applications in protein purification, crystallization or the evaluation of assembly conditions. Manual preparation of the large number of negatively stained specimen grids to support these experiments can be a tedious process that is not well controlled. To address this, we have prototyped an automated staining system that uses standard micro-titer plate formats.



**Fig. 1.** The Aurora Biomed Microplate VERSA 100 liquid handling system loaded with a microtiter plate (A) and an EM grid tray (B). Inset shows specimen being transferred to a grid by the system.

## Methods

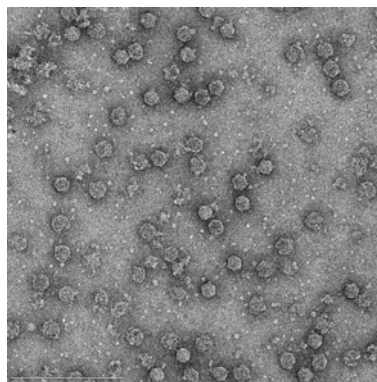
The automated staining system uses a commercial bench top liquid handling system (VERSA 100 nanoliter pipetting workstation, Aurora Biomed, Inc.) equipped with a NanoPipettor head that can dispense or aspirate volumes between 30nl and 350  $\mu$ l (fig. 1). Carbon coated grids are placed in a grid tray that has the same format as a standard 96 well microtiter plate (fig. 2). The grid trays are also compatible with a robotic X,Y and Z arm system that automatically loads grids into the microscope from the grid tray<sup>2</sup>. Since the same grid tray is used for staining and loading, the prepared grids are never handled manually, therefore, minimizing the potential for bending or damaging the grid.

The following staining protocol was tested with the VERSA 100 system: 1.) 0.1-2 $\mu$ l drop of specimen is placed on the grid; 2.) the grid is washed several times with 5  $\mu$ l drops of buffer and then any excess liquid is aspirated from the grid; 3.) a 0.5 $\mu$ l drop of negative stain (0.5% Nano-W) is immediately applied to the grid and the grid is allowed to dry. The process takes ~2.5 min/grid. A more complex protocol was also tested by adding a fixative step where 4ul of glutaraldehyde was added prior aspiration and to washing.

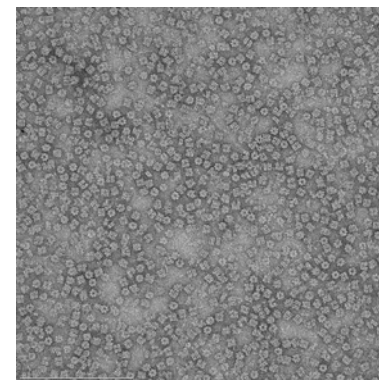
## Results

Representative TEM images for GroEL and cow pea mosaic virus (CPMV) prepared using the VERSA 100 are shown in figs. 3 and 4. The results are comparable to those obtained by manual negative staining methods.

**Fig. 3.** CPMV



**Fig. 3.** GroEL



## Discussion

We have shown the feasibility of using a commercial liquid handling system to prepare negatively stained specimens. There are several advantages to automating the preparation of negatively stained grids for TEM including the reduction of manual labor and the often rough handling that the delicate EM grids sometimes receive. A robotically controlled system can also more precisely control timings of each step and the positioning of the pipette. The system is designed to use very small aliquots of the protein preparations which are often in short supply. The protein can be dispensed onto the grid in small drops and, once the specimen has had time to adhere to the grid, any excess protein can be aspirated and saved for further use. The system is compatible with either 96 well or 384 well micro titer plate formats and the system is fully programmable for almost any protocol. One significant improvement to the system would be to include a mechanism to securely hold the grids in the tray as occasionally the grid is partially lifted out of the tray by the surface tension of the pipette tip.

## References

- <sup>1</sup> Suloway, C., Pulokas, J., Fellmann, D., Cheng, A., Guerra, F., Quispe, J., Stagg, S., Potter, C. S., and Carragher, B. (2005). Automated molecular microscopy: the new Leginon system. *J Struct Biol* 151, 41-60.
- <sup>2</sup> Potter, C. S., Pulokas, J., Smith, P., Suloway, C., and Carragher, B. (2004). Robotic grid loading system for a transmission electron microscope. *J Struct Biol* 146, 431-440.

## Acknowledgements

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