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Fully-automated Immunogold Labeling of Resin Embedded Specimens and On-grid Deposition of Gold Fiducial Particles

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Immunogold labeling (IGL) was introduced in the 1970s as a method for localizing the protein constituents of cells in electron micrographs, through the attachment of antibody-bound colloidal gold particles to antigens on the surfaces of plastic-embedded EM samples. Here we demonstrate use of the Microscopy Innovations mPrep™ System ASP-1000 Automated Specimen Processor to perform unattended, fully-automated IGL grid preparation using an Aurion™ suggested protocol and immuno reagents.

Thin sections of resin-embedded, nematode samples (C. elegans) were placed on pioloform coated nickel slot TEM grids, which were then inserted into mPrep/g™ capsules. The capsules were mounted on the ASP-1000 and processed unattended to accomplish IGL in 7.5 hours. Rinsing reagents were pre-dispensed into 96-well polypropylene microplates and sealed with aluminum film. All necessary immuno-reagents including Glycine, non-specific gold blocker, antibodies (primary and secondary gold conjugate) rinsing buffers and glutaraldehyde were added to the microplate immediately prior to starting the pre-programmed protocol. Samples were automatically agitated by dispensing and immediately re-aspirating 40 µL every 10 minutes during antibody incubations and every 15 seconds during rinsing steps. Upon protocol completion, transmission electron microscopy (TEM) was performed at the UW Medical School EM Facility (Figure 1a). Accurate immunogold labeling using the ASP-1000 was highly reproducible across numerous samples.

Accurate image alignment is essential for Electron Tomography (ET), which is the collection of a tilt series of images from a sample that is rotated under the electron beam. To properly register the 2-D images, colloidal gold particles are adsorbed as evenly as possible across both the top and bottom surfaces of TEM grids, and are used to track and align the image tilt series during software-aided reconstruction. Typically, gold particles in solution are manually applied to the surfaces of the grid, allowed to settle, and then excess is wicked away. This crude method can be inconsistent and lead to an uneven distribution of particles as they move from the liquid to the solid surface.

The ASP-1000 was used to apply gold fiducial particles (10 nm) to 250nm-thick tissue sections collected on TEM grids, by placing the grids in mPrep/g capsules and aspirating and dispensing the solution every 15 seconds for 10 minutes. The gold fiducial particles were adsorbed evenly over the grid surfaces, allowing for highly accurate registration of tilt-series images acquired on a Tecnai TF30 300KV TEM (Figure 1B).

The following Aurion protocol was utilized:

Reagent	Incubation Time (minutes)	Rinsing Time (minutes)	Number of Rinsing Steps
Glycine	15	n/a	n/a
Aurion Blocking Solution	15	5	3
Primary Antibody	120	5	6
Secondary Antibody (gold conjugate)	120	5	9
Glutaraldehyde	5	5	9

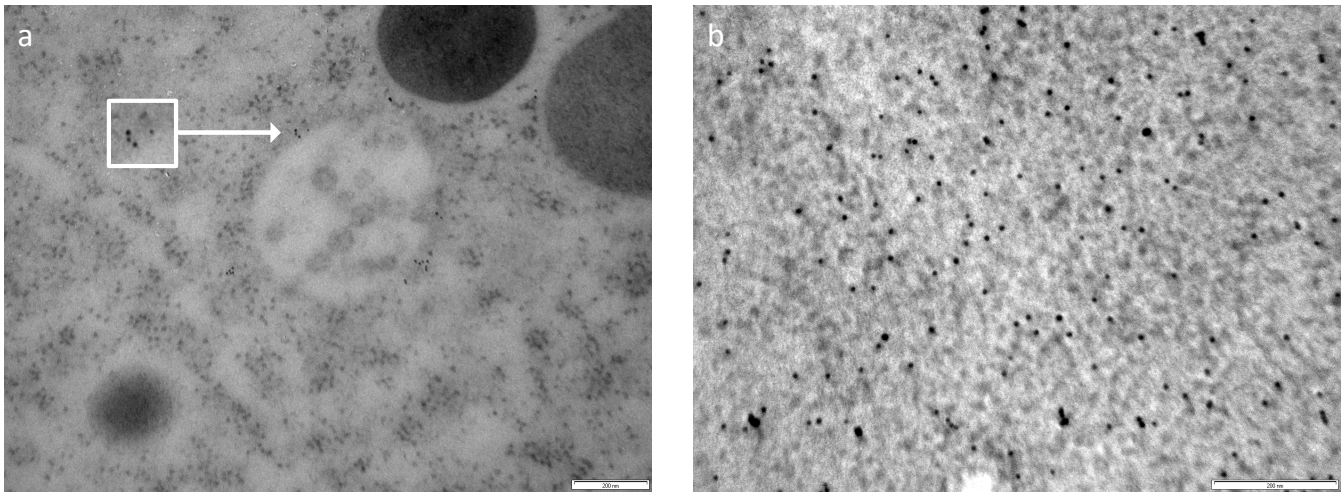

Figure 1. (a) A component of the endosomal sorting complex required for transport (ESCRT) machinery localizes to the limiting membrane of a multivesicular endosome in a C. elegans 1-cell stage embryo, as revealed by immunogold labeling (6 nm Au particles). Scale bar, 200 nm. (b) 10-nm colloidal gold particles are distributed evenly across the surface of a 250-nm thick section prepared for electron tomography. Scale bar, 200 nm.

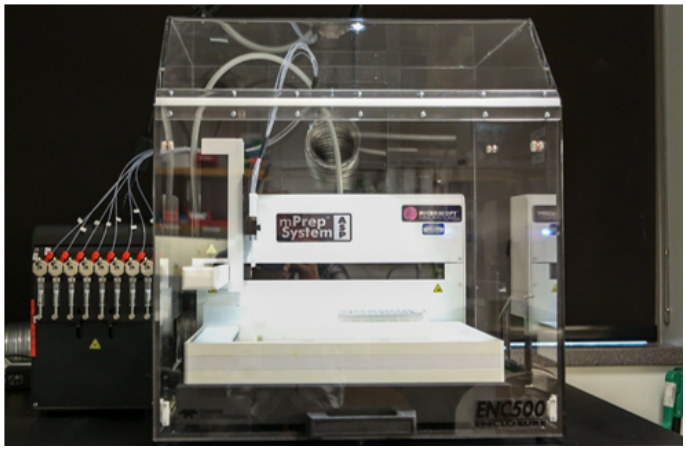

Figure 2. ASP-1000 Automated Sample Processor

Fully Automated Immunogold Labeling of Resin-Embedded Specimens and On-Grid Deposition of Gold Fi

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ABSTRACT

Immunogold labeling (IGL) is a method for localizing the protein constituents of cells in electron micrographs, through the attachment of antibody-bound colloidal gold particles to antigens on the surfaces of plastic-embedded EM samples. Here we demonstrate use of the Microscopy Innovations mPrep™ System ASP-1000 Automated Specimen Processor to perform unattended, fully-automated IGL grid preparation using an Aurion™ suggested protocol and immuno reagents. We also were able to use the ASP-1000 instrument to perform heavy metal staining of EM samples for contrasting purposes, and for depositing colloidal gold particles onto grids for reconstructing 3D tomograms.

METHODS

EM sample preparation for immunogold labeling

Adult *C. elegans* were high pressure frozen using a Balzers HPM 010, freeze-substituted in 0.2% uranyl acetate in acetone at -90˚C for 3 days, ramped up at 4˚C/hr to -20˚C for 12 hours, and then ramped to room temperature at 6˚C/hr. Samples were then exchanged into LR white resin, flat embedded in aclar between two glass slides, and polymerized at 50˚C for 24 hrs. Sectioning of samples was performed at the Medical School Electron Microscopy Facility (www.micro.wisc.edu). Thin sections for 2-D imaging were cut at ~90 nm (determined by interference colors) and placed on pioloform-coated nickel slot TEM grids.

Immunogold labeling and staining using the Microscopy Innovations ASP-1000 system

TEM grids containing sections were inserted into mPrep/g™ (Microscopy Innovations) capsules. The capsules were mounted on the ASP-1000 and processed unattended to accomplish IGL in 7.5 hours. Rinsing reagents were pre-dispensed into 96-well polypropylene microplates and sealed with aluminum film. All necessary immuno-reagents including Glycine, non-specific gold blocker, antibodies (primary and secondary gold conjugate) rinsing buffers and glutaraldehyde were added to the microplate immediately prior to starting the pre-programmed protocol. Samples were automatically agitated by dispensing and immediately re-aspirating 40 µL every 10 minutes during antibody incubations and every 15 seconds during rinsing steps. Samples were manually stained in 2% aqueous uranyl acetate for 4 minutes. Upon protocol completion, transmission electron microscopy (TEM) was performed at the UW Medical School EM Facility (Figure 1). Accurate immunogold labeling using the ASP-1000 was highly reproducible across numerous samples.

Deposition of gold particles for tomographic reconstruction and heavy metal staining using the ASP-1000

The ASP-1000 was used to apply 10-nm gold fiducial particles to 250nm-thick tissue sections collected on TEM grids, by placing the grids in mPrep/g capsules and aspirating and dispensing the solution every 15 seconds for 10 minutes. The gold fiducial particles were adsorbed evenly over the grid surfaces, allowing for highly accurate registration of tilt-series images acquired on a Tecnai TF30 300KV TEM.

TEM imaging

Low-magnification thin section images were acquired on a Philips CM120 transmission electron microscope at the Medical School Electron Microscopy Facility, and tomograms were acquired on a Technai TF-30 equipped with an ultrascan CCD camera, using SerialEM. Tomograms were reconstructed and modeled using the free software package Imod (bio3d.colorado.edu/imod/).

ACKNOWLEDGEMENTS

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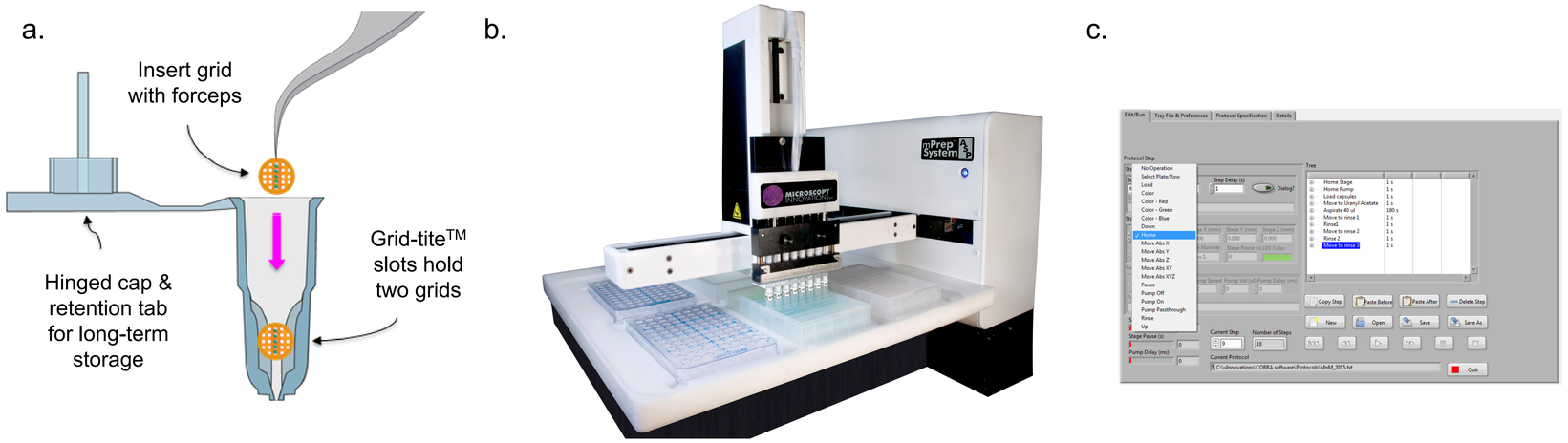
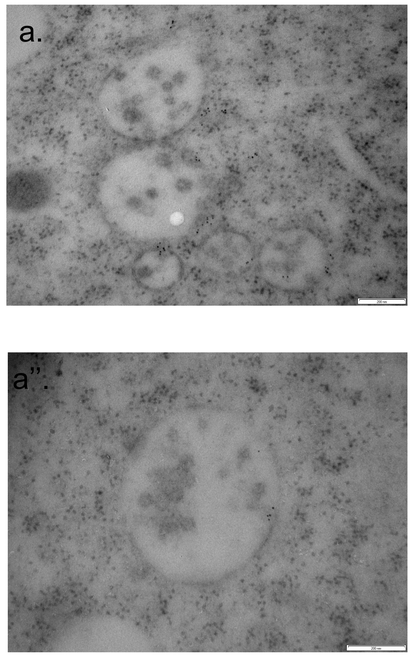


Figure 1. EM grids are inserted into mPrep capsules (a) and are loaded onto the ASP-1000 sample processor (b), which is controlled in easily edited software (c).



b.

Reagent	Incubation Time (minutes)	Rinsing Time (minutes)	Number of Rinsing Steps
Glycine	15	n/a	n/a
Aurion Blocking Solution	15	5	3
Primary Antibody	120	5	6
Secondary Antibody (gold conjugate)	120	5	9
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Figure 2. A component of the endosomal sorting complex required for transport (ESCRT) machinery localizes to the limiting membrane of a multivesicular endosome in *C. elegans* 1-cell stage embryos, as revealed by immunogold labeling (6 nm Au particles). Scale bars, 200 nm. The immunogold labeling protocol (b) utilized was adapted from the Aurion conventional postembedding immunogold protocol (http://www.aurion.nl/products/conventional.php)

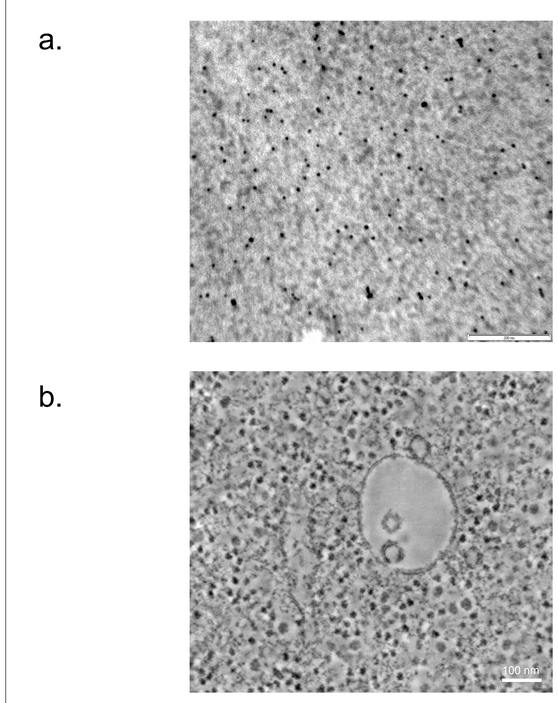

Figure 3. Distribution of well-distributed colloidal 10-nm gold particles for tomographic reconstructions (a), and a slice of a tomogram of a multivesicular endosome reconstructed using gold particles (b).