

# Robotic Specimen Preparation for TEM: Seeding Astrocytes On Coverslips

Astrocytes are star-shaped glial cells which perform a variety of tasks involving neurogenesis, neuron growth, and neuron maintenance. Astrocytes also serve as intercellular structural support within the central nervous system and help transport nutrients to their associated neurons. Being able to routinely differentiate human pluripotent stem cells (hPSCs) into astrocytes allows researchers to examine their physiology. This is often accomplished by seeding onto glass coverslips in 24-well polystyrene culture plates.<sup>1</sup>

## Optimizing the visualization of astrocytes

After chemical fixation for imaging by Transmission Electron Microscopy (TEM), two important questions are beneficial to answer to optimize for consistently high-quality image generation:

- 1) Are there detectable differences between phosphate or cacodylate buffer used during fixation?
- 2) Does post-fixation with osmium tetroxide or osmium tetroxide reduced with potassium ferrocyanide affect contrast with regard to membrane preservation?

It is crucial that initial TEM fixation and subsequent specimen preparation of cultured astrocytes be reliable and consistent to generate timely and reproducible experimental results. Conventional methods often involve numerous labor-intensive exchanges of liquid reagents between various containers and the possibility of damage to glass coverslips from excessive handling.

## Comparing reagents via automated processing

TEM laboratory technicians might universally desire improved sample preparation protocols, but time and resource limitations often lead to the use of previously relied on methods. Head-to-head comparisons of potentially better performing reagents are very difficult using traditional manual techniques. However, simply and easily preparing multiple identical specimens to test various fixation and staining protocols is now possible using automated processing.

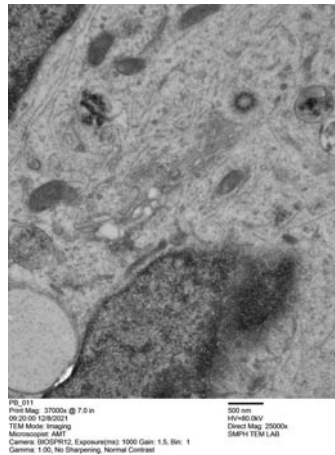
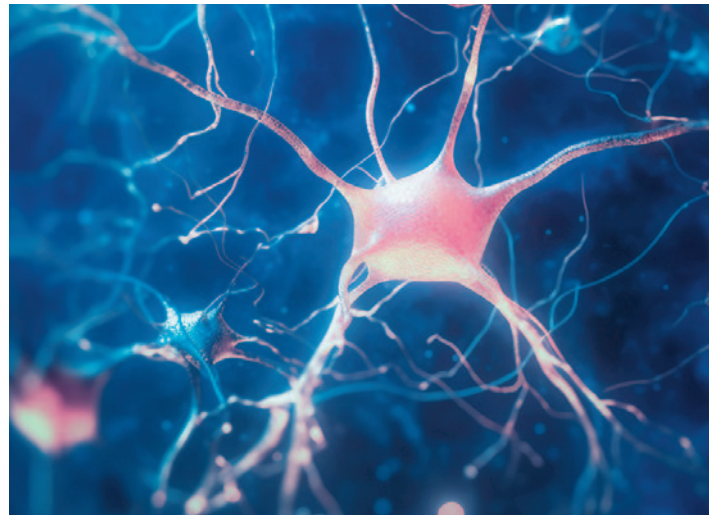
Shown at right are TEM micrographs produced after using the Prepmaster™ 5100 Specimen Preparation Robot to compare various alternatives for preparing cultured cells on glass coverslips in the same polystyrene plate in which they were cultured.

## Results

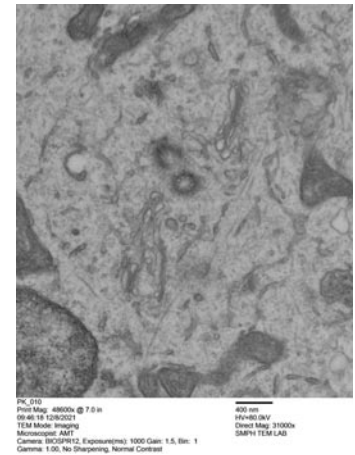
All combinations resulted in generally acceptable images (Images 1, 2, and 3). However, osmium reduced with potassium ferrocyanide, in both cacodylate buffer and phosphate buffer applications, appears to show greater membrane contrast, lower cytoplasmic background, and more easily discernable cytoskeletal elements (Image 4).

Membrane preservation, contrast, and ultrastructural details did not appear to be affected by the choice of phosphate buffer versus cacodylate buffer.

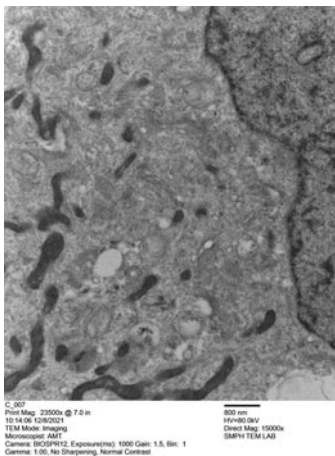
The Prepmaster 5100 enabled easy and precise testing of 4 variables in one simple experiment using computer-controlled automated liquid handling. A complicated array of reagents was delivered to each individual sample properly and precisely. Human inaccuracy was eliminated. This revolutionary increase in performance is due to the Prepmaster 5100 having zero possibility of decision-making errors, plus liquid handling accuracy and precision unattainable by a human being.



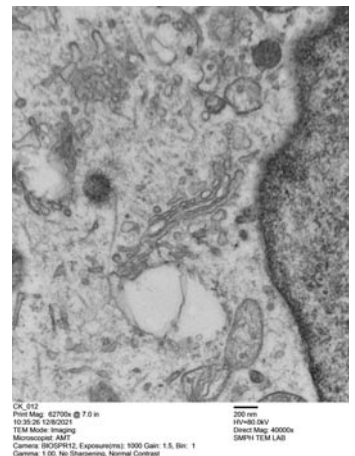
**Image 1.** (Phosphate buffer/OsO<sub>4</sub>) Phosphate buffer combined with unreduced osmium tetroxide produces clearly identifiable Golgi and centrosome.



**Image 2.** (Phosphate buffer/OsO<sub>4</sub>-KFC) Phosphate buffer with reduced osmium tetroxide gives low cytoplasmic background with well-defined centrosome and Golgi apparatus, including clear, well contrasted membrane preservation of the microtubules in the centriole.



**Image 3.** (Cacodylate Buffer/OsO<sub>4</sub>) Cacodylate buffer with unreduced osmium tetroxide shows Golgi apparatus however lack of clear and easily discernable membranes makes it difficult to resolve against the dense background cytoplasm.



**Image 4.** (Cacodylate buffer/OsO<sub>4</sub>-KFC) Cacodylate buffer with reduced osmium tetroxide shows beautifully preserved Golgi apparatus membranes, low cytoplasmic background, and easily visible microtubules.

## Robotic Specimen Preparation for TEM: Seeding Astrocytes On Coverslips *(continued)*

### Equipment

#### Prepmaster™ 5100 Specimen Preparation Robot

Cells seeded on delicate and fragile coverslips are particularly difficult to prepare due to fear of breakage. The Prepmaster 5100 effortlessly prepares them quickly and safely for you so you can spend your valuable research time more effectively.

### Features

- Heated (RT–60°C) Agitation Station™ specimen dock provides gentle, constant shaking movement for rapid and thorough post-fixation and rinsing.
- Heated (RT–60°C) reagent reservoir for enhanced post-fixation with hot heavy metals or other reagents.
- Cooled (5°C–RT) reagent reservoir for cold dehydration or cold reduced osmium in the Ellisman rOTO protocol for example.
- Windows® laptop computer control for easy creation, modification, and storage of unlimited protocols.
- UV light protected ventilated enclosure keeps noxious fumes contained and vented. Small (60 x 60cm) footprint enables convenient in-hood option with enclosure removed.

### Benefits

- Easy to set up and clean up.
- Versatile — can process most biological samples.
- Can effortlessly prepare up to 24 coverslips in less than 2 hours. Can prepare 8 kidney specimens in less than an hour or 96 kidney specimens in less than 2 hours.
- Effortlessly prepares coverslips for TEM or SEM in the same 24-well polystyrene microplate in which the cells were seeded and maintained. No transferring glass coverslips and fear of breakage.
- Excellent choice to run Ellisman rOTO protocol for vEM specimen prep.
- Up to 24 unique reagents or rinses.
- Reliable unattended overnight operation.
- High quality, consistent processing.

### References

1. <https://www.waisman.wisc.edu/stem-cell-research-program/zhang-lab/>



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Robot...

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#### Prepmaster consumables include:

EMS Cat. No.	Description
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51000-20	Reagent Reservoir, 1.2ml Square Plate, 10 Pack
51000-25	Bulk Reagent Reservoirs, 48 Pack
51000-30	Pipette Tips, 100 Pack

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