Walk-away High-throughput preparation of cell pellets and tissue biopsies for TEM in clinical pathology and research using the Prepmaster™ 5100 EM Specimen and Grid Processor

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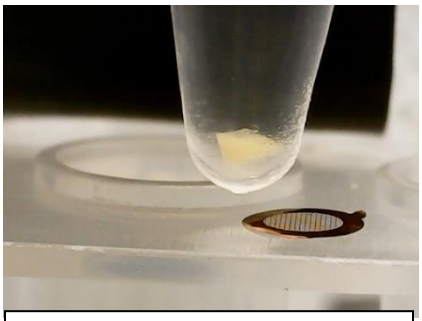
Core electron microscopy laboratories face unique challenges including the need to rapidly prepare many and varied samples of cells and tissues, sometimes derived from complex matrices such as nasal mucus, or miniscule (<0.25mm^3) tissue biopsies for TEM imaging with no margin for loss or error, to assist diagnosis in patients awaiting potentially life-saving treatment. "With this comes great responsibility because the findings of our ultrastructural examinations have the potential to alter diagnoses and patient management."[1]

Various manual and automated techniques have been attempted over the years to make the specimen preparation process for ultrastructural examination of biological tissue for clinical diagnostics and research more reliable and faster. However, one of the biggest obstacles is the risk of loss of these very small specimens. Workflow optimization is an ongoing practice in innovative laboratories, however, like evolution in general, it typically occurs in fits and starts with long periods of little or gradual change interrupted by bursts of rapid change. Validated protocols are expensive and time-consuming to change, evaluate, and re-validate. There is significant reluctance to "mend what isn't broken". The promise of significant improvement in terms of speed and cost while maintaining specimen integrity and image quality must be present to make the effort worthwhile.

Here we present a new paradigm in specimen preparation of all clinical diagnostics specimens and a wide variety of research specimens for TEM imaging. Scientists at the Medical College of Wisconsin (Milwaukee, WI, USA) used a Heartland Biotech Prepmaster 5100 Specimen Preparation Robot (EMS, Philadelphia, PA, USA; www.emsdiasum.com/prepmaster) to prepare large batches (up to 48) of <0.5mm^3 sized tissue biopsies and cell or tissue fragment pellets from fixative rinse through to resin embedding. The Prepmaster's new Agitation Station™ provided previously unavailable highly optimized fluid penetration and saturation conditions to process considerable numbers of specimens simultaneously.



Approximately 1mm^3 renal biopsy next to 3mm dia. grid

Procedure

The specimen preparation process removed the fixative from the specimen, increased electron-density with the application of heavy metals, dehydrated, infiltrated with liquid resin at room temperature, and polymerized the resin at increased temperature into a hard block ready for trimming and sectioning.

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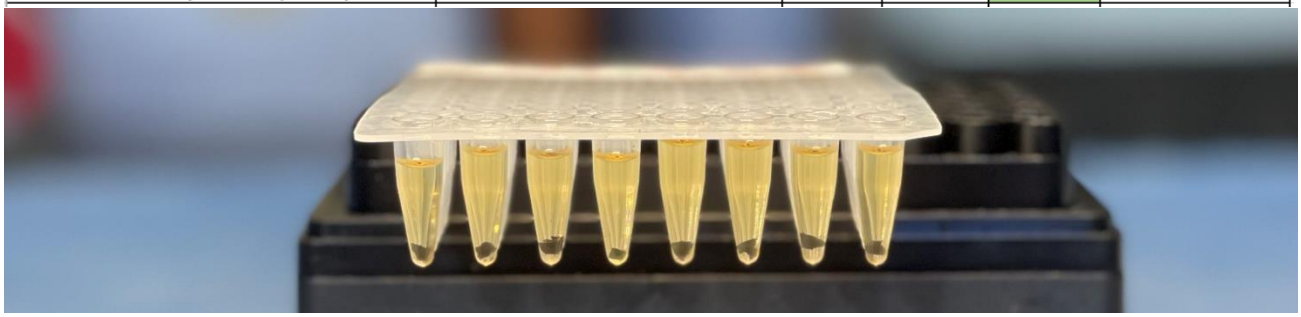
Tissue or cells were received in glutaraldehyde fixative. Cells were pelleted and pellets placed in the processor. Tissue was cut into suitable sized pieces and placed into tubes the processor. The specimens were washed with buffer then Os04 fixed, dehydrated, and then infiltrated with resin while in the processor. Specimens were then transferred to silicone flat molds with new 100% resin for curing, Typically, polymerization was overnight at 70ºC. See table of steps below.

Step	Reagent/conditions	Duration (minutes)	Repeats	Step time (mins)	Comments
Primary fixation (samples arrived fixed)	2.5% Glutaraldehyde				
Rinse to remove fixative	0.1M Cacoodylate	2	1	2	On Prepmaster
Secondary fixation	1% Osmium Tetroxide	15	2	30	On Prepmaster
Rinse to remove osmium	Ultrapure H20	1	5	5	On Prepmaster
Dehydration	70% ETOH	2	1	2	On Prepmaster
Dehydration	100% ETOH	2	2	4	On Prepmaster
Dehydration	ACN	5	2	10	On Prepmaster
Infiltration of resin	50% ACN/50% resin	30	1	30	On Prepmaster
Embedding	100% Hard-Plus Resin-812	120	1	120	On Prepmaster
Total time on Prepmaster (hours)				3.4	



Specimens embedded at RT in 100% resin ready to be transferred to a flat mold for curing.

Downstream workflow consumed another 12-14 hours for:

Curing (8 Hours @ 70°C)
Thick section
Stain thick sections
Block selection
Trim blocks
Thin section
Stain grids
Imaging on TEM

Fig. 1. Cured blocks ready for microtomy.

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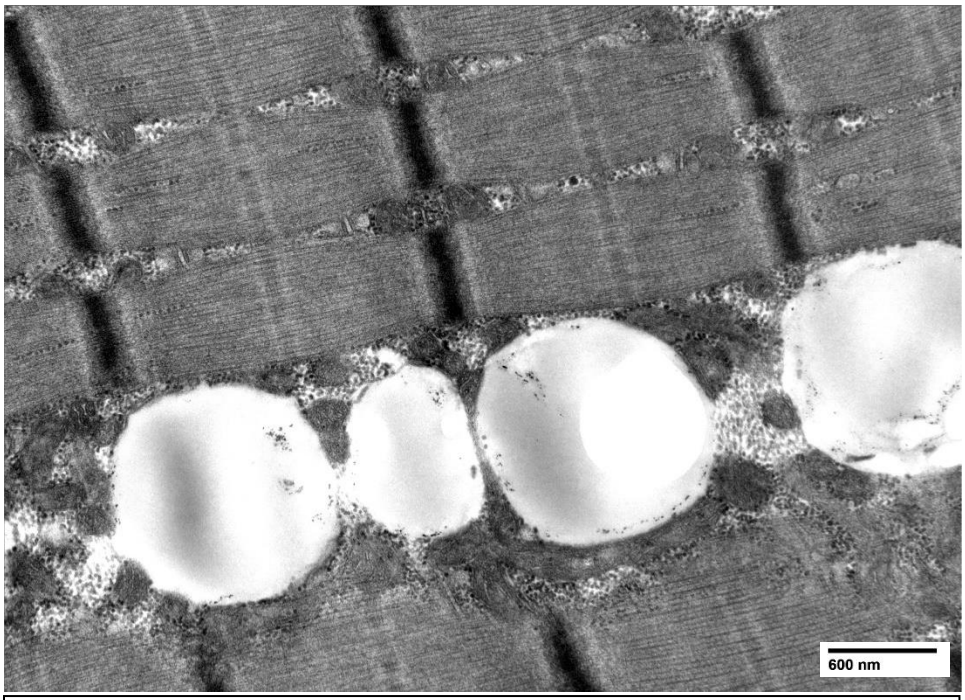

Fig. 2. Muscle tissue imaged on JEOL 1400 TEM kV=60

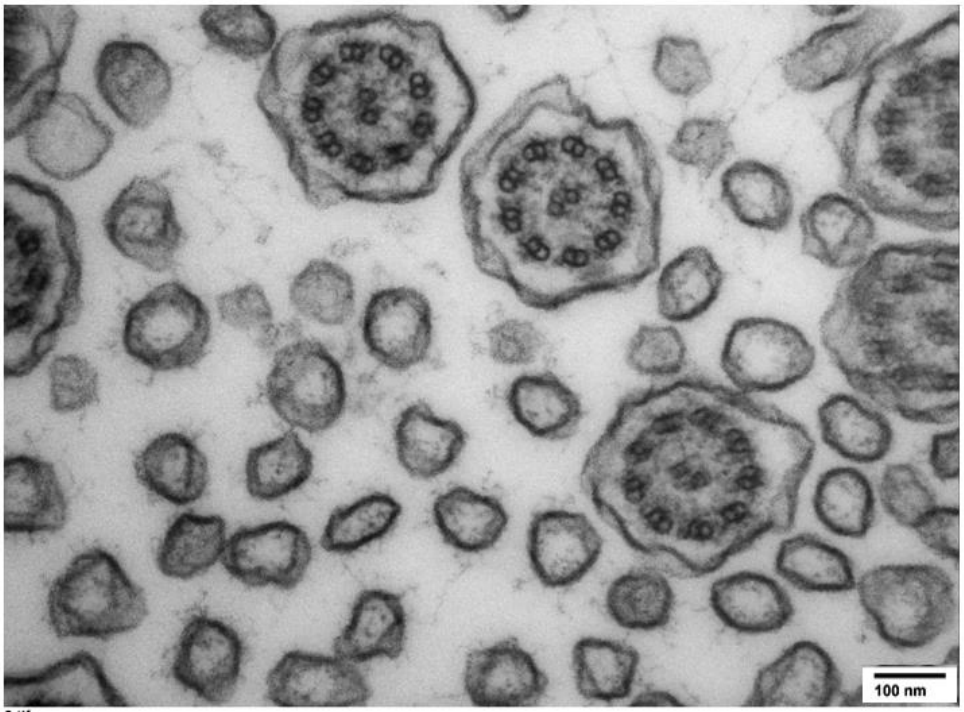

Fig. 3. Cilia from cell pellets imaged on JEOL 1400 TEM kV=60

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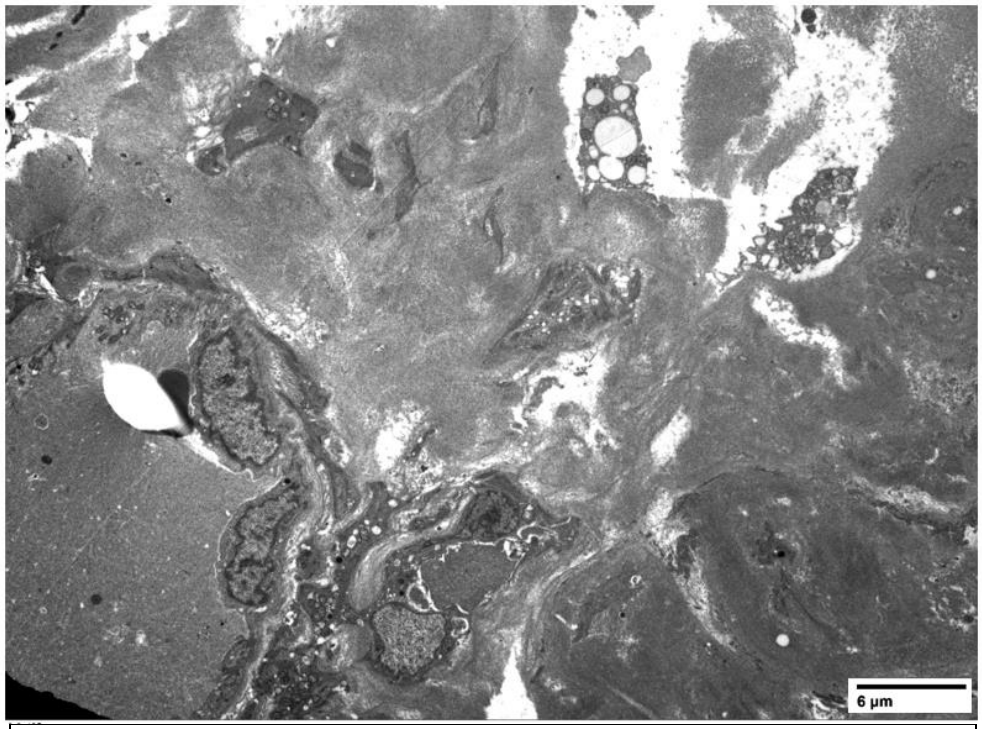

Fig. 4a Renal biopsy containing amyloid imaged on JEOL 1400 TEM kV=60

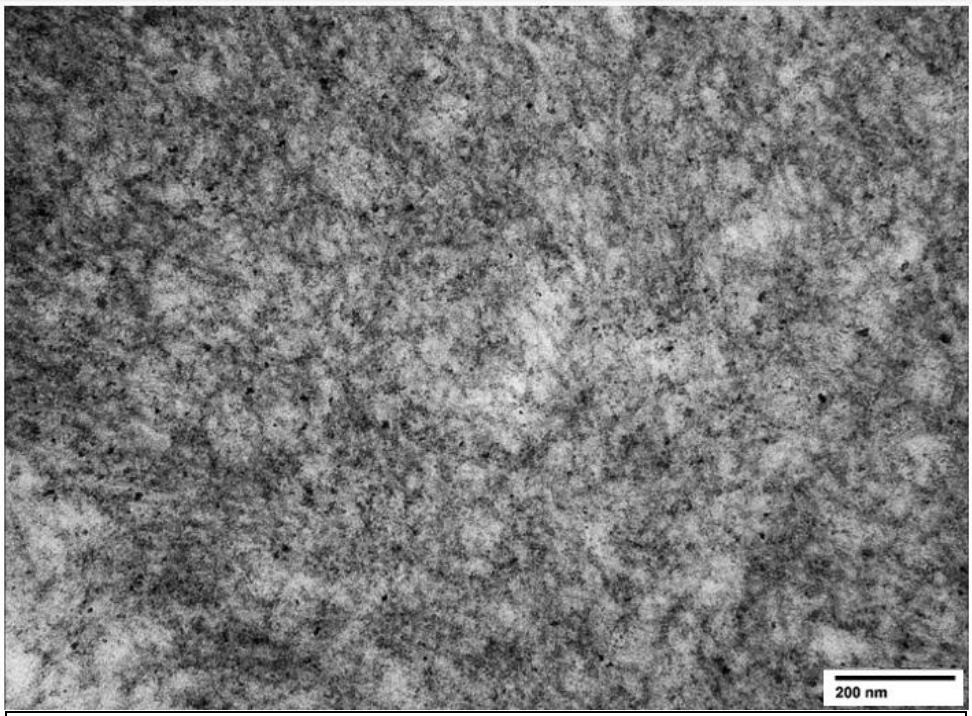

Fig. 4b Amyloid in renal biopsy imaged on JEOL 1400 TEM kV=60

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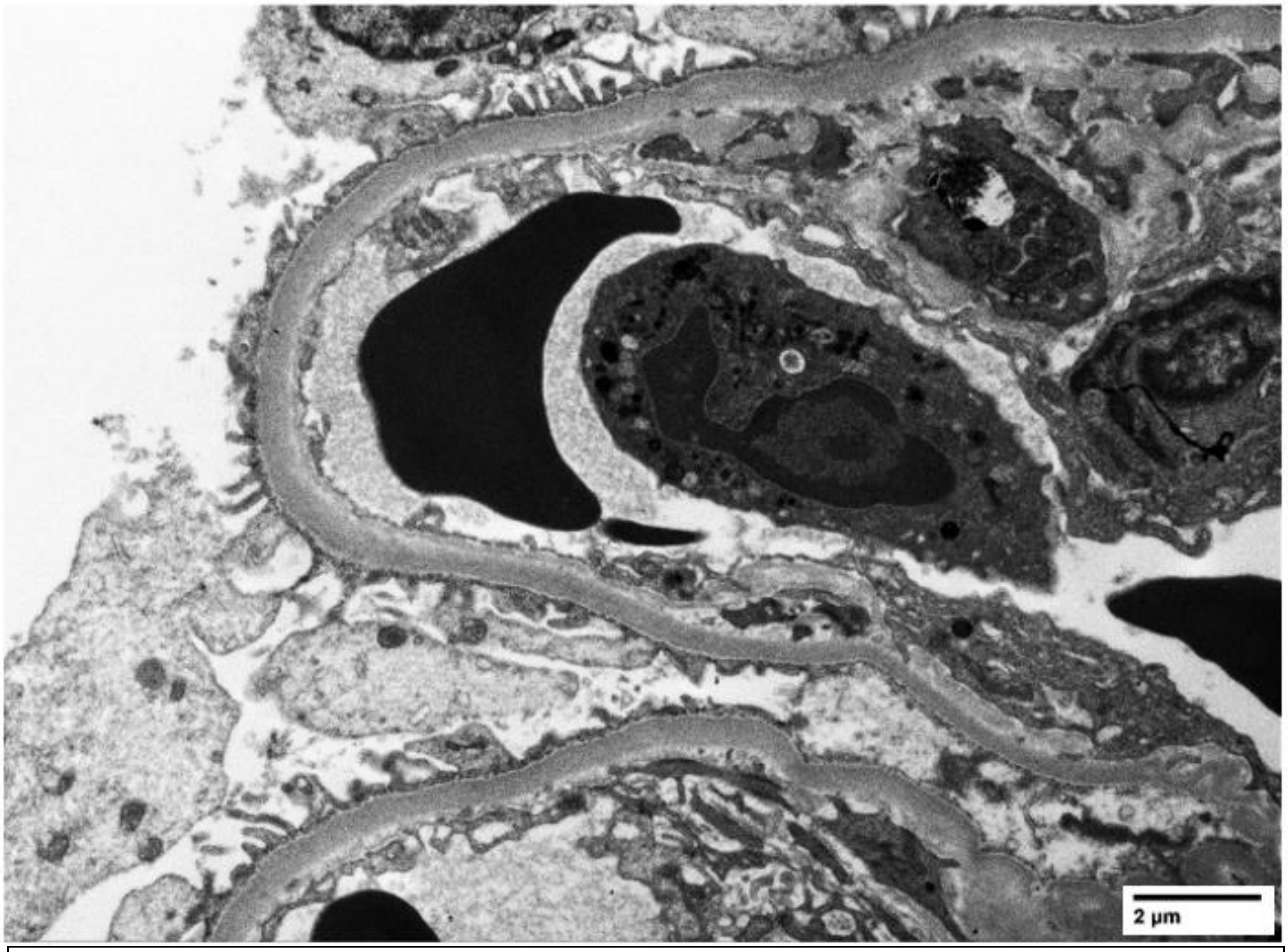

Fig. 5 Capillary loop in renal glomerulus imaged on JEOL 1400 TEM kV=60

Conclusion

The Medical College of Wisconsin Core electron microscopy lab has derived significant benefit from using the Prepmaster 5100 due to its advanced features and capabilities. Very small samples were processed alongside much larger and robust samples with no loss or degradation of either sample. Processing of samples for both diagnostic and research work was streamlined, and the sample preparation was more efficient with technologists freed to perform other tasks such as trimming and sectioning blocks. The Prepmaster 5100 delivered time savings, consistent results, cost-effectiveness, and enhanced reproducibility in sample preparation. Its automation capabilities enable clinical professionals and scientists to be confident in the sample preparation ensuring reliable, reproducible quality of sample preparation and efficient diagnostic processes that ultimately contribute to improved patient care.

References:

1. John Brealey, President Society for Ultrastructural Pathology. www.ultrapath.org