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Automated Preparation of Core Needle Biopsy Specimens for TEM Imaging

Thomas E. Strader[1*] and Benjamin K. August[2]

[1.] Microscopy Innovations, LLC, Marshfield, WI, USA.
[2.] University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, USA.
* Corresponding author: tom.strader@microscopyinnovations.com

Rapid processing of biopsies is critical to both clinicians and patients for timely diagnosis and treatment of disease. Renal pathologists require clear, artefact-free TEM images of multiple glomeruli to make fully informed decisions for evaluation of kidney disease and transplant failure. A critical challenge when preparing core needle biopsies for imaging by TEM is their small size and the need to guard against specimen loss/damage.

Researchers at the University of Wisconsin-Madison and Microscopy Innovations have developed a method suitable for rapid, walk-away processing of core needle biopsies for TEM that is substantially faster than typical 1-2 day protocols. Rapid walk-away TEM preparation is accomplished in 2 hours using mPrep/s capsules and the ASP-1000 Automated Specimen Processor.

A batch of eight perfusion-fixed rodent kidney specimens was collected using a 1mm diameter core sampling tool (EMS, Hatfield, PA, USA part # 69039-10) to approximate an 18-gauge core biopsy (1.02mm diameter). The 1 mm diameter specimens were then trimmed and embedded in low melting point agarose (Sigma Agarose Type I low EEO; CAS Number 9012-36-6) using a reagent trough as a mold. The agar embedded specimens were then cut into several 3mm to 5mm long specimens (Figures 1&2).

For each specimen, an mPrep/s capsule screen was positioned in the mPrep/s Workstation and an embedded biopsy precisely placed vertically on the screen to ensure proper orientation for maximum depth for sectioning (Figure 3). A barcode labeled mPrep/s capsule was then placed over the screen/specimen to securely entrap the specimen for processing and polymerization (Figure 4). After these steps, the capsule with specimen was removed from the Workstation.

The eight mPrep/s capsules containing specimens were then attached to the ASP-1000 for a 2-hour automated processing protocol of buffer, 1% phosphate buffered OsO_4, water, en-bloc staining in 1% aqueous uranyl acetate, graded ethanols, acetone, and Embed 812 (Figure 6). Following automated processing through 100% resin, the capsules were removed from the ASP-1000 and polymerized overnight at 60°C. Barcode labeled mPrep capsules were directly mounted in the microtome chuck for facing and sectioning without post staining.

Electron micrographs were obtained with a Phillips CM120 TEM at 80 keV and recorded to a BioSprint camera. TEM images showing excellent contrast and clearly identifiable glomeruli were generated (Figure5).

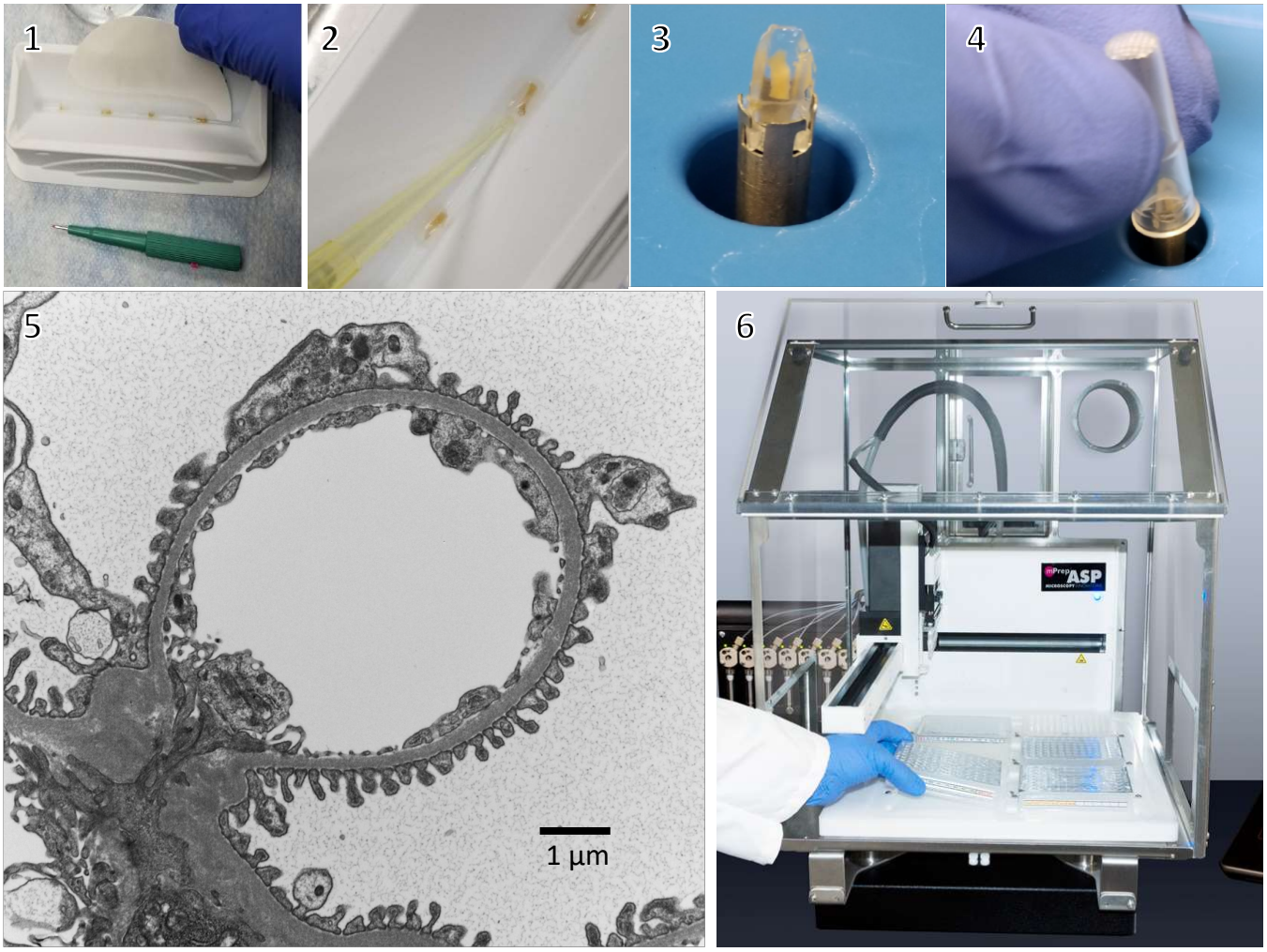

Figure 1. Biopsies are cored with green core sampling tool. Filter paper removes residual buffer.
Figure 2. Warm 50°C agar is pipetted on top of biopsy.
Figure 3. The biopsy, in agar, held upright mPrep/s Workstation to ensure exact orientation.
Figure 4. mPrep/s capsule placed over biopsy to entrap for processing. Barcode label removed to enable viewing the specimen.
Figure 5. TEM image of kidney biopsy tissue
Figure 6. ASP-1000 Automated Specimen Processor

Automated Preparation of 18-gauge Biopsy Specimens for TEM Imaging

Benjamin K August [1] and Thomas E Strader [2]

[1.] University of Wisconsin-Madison. School of Medicine and Public Health, Electron Microscopy Facility, Madison, WI USA

[2.] Microscopy Innovations, LLC. Marshfield, WI USA

Introduction

Fast processing of biopsies is critical to pathologists and researchers who need rapid, accurate test results to make a diagnosis or discovery. A significant challenge when preparing biopsies for imaging by TEM is dealing with their small size. Researchers at the University of Wisconsin-Madison and Microscopy Innovations have developed a method to process 18-gauge (1.02mm diameter) needle biopsies for rapid, walk-away sample processing using the mPrep/s capsule and the ASP-1000 Automated Specimen Processor.

Summary

Eight rodent kidney specimens were collected with a core sampling tool then trimmed and embedded in low melting point agar for fast processing in mPrep/s capsules on the ASP-1000 Automated Specimen Processor. The resulting images show excellent quality, facilitating fast and accurate analysis for diagnosis and/or publication.

Experimental

Perfusion-fixed rodent kidney specimens were collected using a 1mm diameter core sampling tool (EMS, Hatfield, PA, USA part # 69039-10). The 1mm diameter cored biopsy specimens were then trimmed and embedded in low melting point agarose (Sigma Agarose Type I low EEO; CAS Number 9012-36-6). Specimen length was between 3mm and 5mm. (Figures 1&2). Specimens were precisely held in the upright position on the mPrep Workstation (Microscopy Innovations, part # 42100). Reagents were placed in reservoirs. The ASP-1000 was programmed to perform the reagent exchanges (see table below).

Step	Reagent	Fluid exchanges	Cycle time (secs)	Time (min)
1	Fix Rinse 1 (buffer)	75	2	2.5
2	Fix Rinse 2 (buffer)	60	2	2.0
3	Fix Rinse 3 (buffer)	60	2	2.0
4	Osmium	750	2	25.0
5	Osmium Rinse 1 (water)	75	2	2.5
6	Osmium Rinse 2 (water)	60	2	2.0
7	Osmium Rinse 3 (water)	60	2	2.0
8	Uranyl Acetate	750	2	25.0
9	UA rinse 1 (water)	75	2	2.5
10	UA rinse 2 (water)	60	2	2.0
11	UA rinse 3 (water)	60	2	2.0
12	50% Acetone	100	2	3.3
13	70% Acetone	100	2	3.3
14	80% Acetone	100	2	3.3
15	95% Acetone	100	2	3.3
16	100% Acetone	120	2	4.0
17	100% Acetone	120	2	4.0
18	100% Acetone	120	2	4.0
19	25% Resin	30	5	2.5
20	50% Resin	30	5	2.5
21	75% Resin	30	5	2.5
22	90% Resin	60	5	5.0
23	100% Resin 1	60	5	5.0
24	100% Resin 2	60	5	5.0
25	100% Resin 3	10	5	0.8
		Total time:		118.2

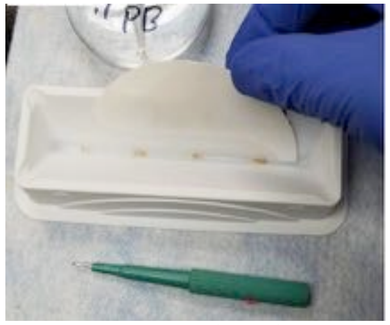

Figure 1: Biopsies are cored using the green coring tool.

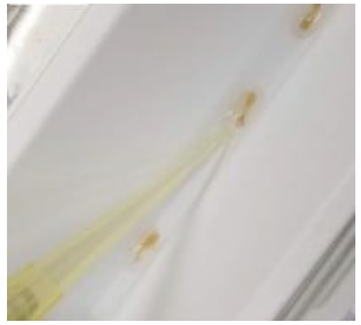

Figure 2: Warm agar is gently pipetted on top of the biopsy.

Figure 3: The agar-embedded biopsy is held in the Workstation in the upright orientation.

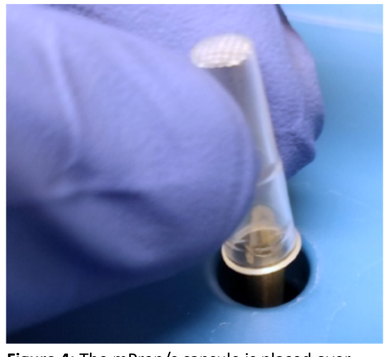

Figure 4: The mPrep/s capsule is placed over the specimen to securely hold the biopsy for processing.

Figure 5: ASP-1000 Automated Specimen Processor.

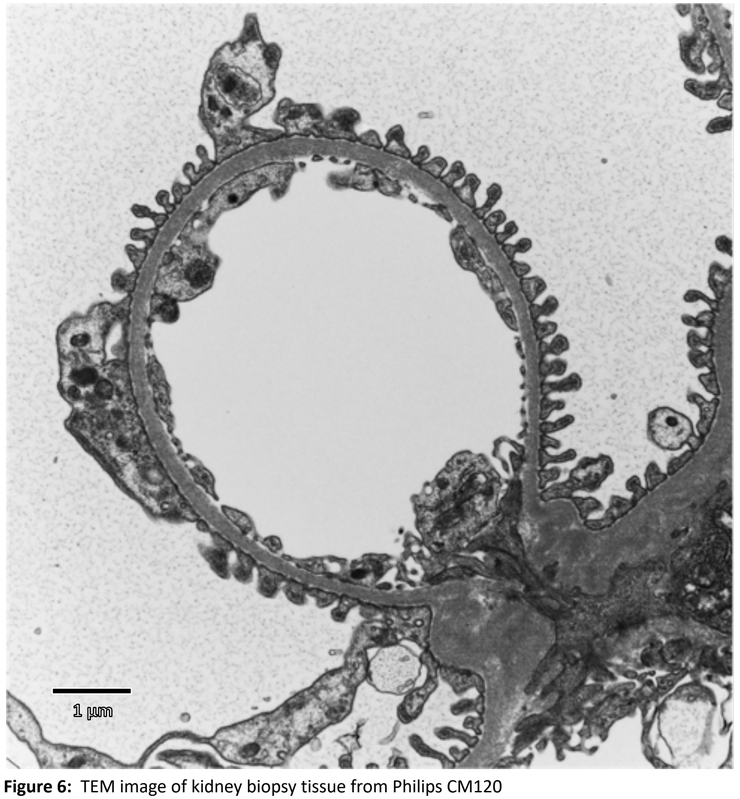

Figure 6: TEM image of kidney biopsy tissue from Philips CM120

Experimental (continued)

For each specimen, an mPrep/s capsule screen was mounted to the post of the mPrep/s Workstation and an agar embedded biopsy precisely placed on the screen to ensure proper orientation for cross-sectioning the biopsy (Figure 3). Alternatively, one could orient the specimen horizontally, however that leaves only 1mm of depth for sectioning. A bar-code labeled mPrep/s capsule was then gently placed on the screen/specimen, securely holding the potentially irreplaceable specimen for processing and polymerization (Figure 4).

The mPrep capsules were then attached to the ASP-1000 for 2-hour automated processing including en-bloc uranyl acetate staining (Figure 5). Following robotic processing, the capsules were polymerized overnight at 60°C, trimmed, and sectioned; all while still in the barcode labelled mPrep capsule. No post-staining was performed.

Capsules were mounted into microtome chucks for sectioning; preserving orientation and specimen labeling. Images from 70 nm sections were collected on a Philips CM120 TEM with a BioSprint camera running AMT Capture Engine V700.

Results and Discussion

- The ASP-1000 provided a robust and easy-to-use option for fast, walk-away processing of kidney biopsies with unparalleled traceability and safety.
- Automated processor performed thousands of liquid handling steps autonomously freeing up technician time for other, more important, tasks.
- Specimens were directly handled by a human only once - to place them in labeled mPrep/s capsules.
- Automated processing reduced operator reagent exposure and potential operator error.

Conclusions

Using the mPrep ASP-1000 Automated Specimen Processor for automated preparation of 18-gauge kidney biopsy punch tissue provided:

- Easy, secure handling of tiny, delicate biopsies plus automated processing for reduced overall lab workload.
- Fast, walk-away processing of kidney biopsies with en-bloc UA staining for TEM in just 2 hours!
- Elimination of all direct specimen handling once specimens were placed in labeled capsules, including messy transfers in embedding resin.
- Positive ID and intact chain-of custody since specimens were never removed from labeled capsules.
- Safe working conditions for technicians.

Corresponding author contact info:
tom.strader@microscopyinnovations.com