Scanning Electron Microscopy Project Portfolio

Jing Karchin
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ESF
State University of New York
College of Environmental Science and Forestry
Part 1

A portfolio of micrographs demonstrating the following techniques:

• Critical Point Drying or TMS drying
• Depth of Field
• Backscatter
• Low voltage image of uncoated sample
• High Magnification (>50,000)
• Stereo Pair
• Cryofracture
Fig. 1 Mouse kidney visceral epithelial cells (podocytes) were plated on 12 mm x 12 mm coverslips in complete Dulbecco’s Modified Eagle Medium (DMEM) at 37 °C for 14 days. Samples were fixed for 1 h at room temperature with 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Samples were then rinsed 3 times in 0.1 M cacodylate buffer (pH 7.4), followed by secondary fixation in 1 % OsO₄ in 0.1 M cacodylate buffer (pH 7.4) in the dark on ice for 1 h. Samples were then washed 3 times in distilled H₂O. Dehydration was performed with graded ethanol (EtOH) in water. Progressive EtOH steps, 15 min each, were 25 %, 50 %, 75 %, 80 %, 95 % and three steps of 100 % EtOH. Specimens were critical point dried with liquid carbon dioxide (CO₂) to remove EtOH. Dried samples were sputter coated with Au and Pd, and analyzed using SEM. Working distance: 14 mm, accelerating voltage: 5 kV, spot size: 16, objective aperture: 2. Scale bar: 10 μm.
Fig. 2. Mouse kidney tissue were fixed for 3 hrs at room temperature with 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Samples were then rinsed 3 times in 0.1 M cacodylate buffer (pH 7.4), followed by secondary fixation in 1 % OsO₄ in 0.1 M cacodylate buffer (pH 7.4) in the dark on ice for 1 h. Samples were then washed 3 times in distilled H₂O. Dehydration was performed with graded ethanol (EtOH) in water. Progressive EtOH steps, 15 min each, were 25 %, 50 %, 75 %, 80 %, 95 % and three steps of 100 % EtOH. Specimens were critical point dried with liquid carbon dioxide (CO₂) to remove EtOH. Dried samples were sputter coated with Platinum, and analyzed using SEM. Working distance: 15 mm (Figure 2A) and 38 mm (Figure 2B), accelerating voltage: 15 kV, spot size: 10, objective aperture: 2. Scale bar: 50 μm.
Mouse kidney tissue were fixed for 3 hrs at room temperature with 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Samples were then rinsed 3 times in 0.1 M cacodylate buffer (pH 7.4), followed by secondary fixation in 1 % OsO₄ in 0.1 M cacodylate buffer (pH 7.4) in the dark on ice for 1 h. Samples were then washed 3 times in distilled H₂O. Dehydration was performed with graded ethanol (EtOH) in water. Progressive EtOH steps, 15 min each, were 25 %, 50 %, 75 %, 80 %, 95 % and three steps of 100 % EtOH. Specimens were critical point dried with liquid carbon dioxide (CO₂) to remove EtOH. Dried samples were sputter coated with Platinum, and analyzed using SEM. Working distance: 21 mm (Figure 3A and 3B), accelerating voltage: 15 kV, spot size: 10, objective aperture: 2. Scale bar: 20 μm.
Low voltage of uncoated PVDF membrane

Fig. 4. PVDF membrane was mounted on carbon paint, uncoated. Image was taken under accelerating voltage 1.0 kV, objective aperture 2, spot size 8, and working distance 8 mm. Scale bar: 50 µm.
High Magnification image of *C. elegans*

Fig. 5. *C. elegans* were fixed in 2% Glutaldehyde in 0.1 M Cacodylate buffer (pH 7.4) at room temperature for 1 hour. The samples were washed 3 times in 0.1 M Cacodylate buffer (pH 7.4) for 5 min each wash, followed by fixing in 1% OsO₄ in 0.1 M Cacodalyte buffer (pH 7.4) on ice for 1 hour. The samples then underwent dehydration in 25 %, 50 %, 70 %, 95 %, and 100 % EtOH, followed by critical point drying using liquid CO₂. The samples were then sputter coated with Platinum. Accelerating voltage 30 kV, objective aperture 1, spot size 8, working distance 11 mm. Scale bar: 200 nm.
Stereo Pair

Fig. 6. *Periplaneta americana* (also known as American cockroach). The roach was dried in the oven at 50 °C overnight, followed by sputter coating with Platinum. The SEM images were taken at tilt angle 0° and 11°. The images were processed and overlaid using Photoshop, as well as the brightness and contrast adjustment. Working distance: 45 mm; Accelerating voltage: 15 kV; Spot size: 13; Objective aperture: 2. Scale bar: 500 μm.
Cryofracture

Fig. 7. Tree leaves were fixed for 1 hr at room temperature with 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Samples were then rinsed 3 times in 0.1 M cacodylate buffer (pH 7.4), followed by secondary fixation in 1 % OsO₄ in 0.1 M cacodylate buffer (pH 7.4) in the dark on ice for 1 hr. Samples were then washed 3 times in distilled H₂O. Dehydration was performed with graded ethanol (EtOH) in water. Progressive EtOH steps, 15 min each, were 25 %, 50 %, 75 %, 80 %, 95 % and three steps of 100 % EtOH, followed by breaking in liquid nitrogen (N₂). Specimens were then transferred back to 100 % EtOH and proceeded to critical point drying with liquid carbon dioxide (CO₂) to remove EtOH. Dried samples were sputter coated with Platinum, and analyzed using SEM. Working distance: 12 mm, accelerating voltage: 5 kV, spot size: 10, objective aperture: 2. Tilt: 26.8°. Scale bar: 10 μm.
Part 2
Biological Sample: mouse kidney tissue

Preparation

- Mouse kidney tissue were fixed for 3 hrs at room temperature with 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Samples were then rinsed 3 times in 0.1 M cacodylate buffer (pH 7.4), followed by secondary fixation in 1 % OsO₄ in 0.1 M cacodylate buffer (pH 7.4) in the dark on ice for 1 h. Samples were then washed 3 times in distilled H₂O. Dehydration was performed with graded ethanol (EtOH) in water. Progressive EtOH steps, 15 min each, were 25 %, 50 %, 75 %, 80 %, 95 % and three steps of 100 % EtOH. Some samples were proceeded cryofracture in liquid nitrogen (N₂) in addition to critical point drying with liquid carbon dioxide (CO₂) to remove EtOH. Dried samples were sputter coated with Platinum, and analyzed using SEM.

Imaging process

- Brightness and contrast adjustment using Photoshop
Kidney tubules (SEI)

Working distance: 20 mm
Accelerating voltage: 15 kV
Spot size: 10
Objective aperture: 2
Scale bar: 20 μm
Fig. A. Kidney tubules. SEI

Working distance: 21 mm
Accelerating voltage: 15 kV
Spot size: 10
Objective aperture: 2
Scale bar: 20 μm

Fig. B. Kidney tubules. BEI (Compo)
Podocytes cell bodies (SEI)

Working distance: 15 mm
Accelerating voltage: 15 kV
Spot size: 10
Objective aperture: 2
Scale bar: 50 µm
Podocytes cell bodies – SEI Cryofracture

Working distance: 11 mm
Accelerating voltage: 15 kV
Spot size: 8
Objective aperture: 2
Scale bar: 20 μm
Kidney tubules and glomerulus – SEI

Working distance: 20 mm
Accelerating voltage: 15 kV
Spot size: 10
Objective aperture: 2
Scale bar: 50 μm
Glomerulus – SEI

Working distance: 13 mm
Accelerating voltage: 15 kV
Spot size: 7
Objective aperture: 2
Scale bar: 10 μm

Nilius et al. (2006) *Physiological Reviews*, DOI: 10.1152/PHYSREV.00021.2006
Podocytes foot processes - SEI

Working distance: 11 mm
Accelerating voltage: 30
Objective aperture: 1
Spot size: 8
Scale bar: 1 μm

Nilius et al. (2006) *Physiological Reviews*, DOI: 10.1152/PHYSREV.00021.2006
Part 2
Non-Biological Sample: gelatin powder derived from porcine skin

Composition
• Gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen extracted from the skin, bones, and connective tissues of animal.

Preparation
• Gelatin powder was immobilized on carbon tape. Samples were then sputter coated with platinum, and analyzed using SEM.

Imaging process
• Brightness and contrast adjustment using Photoshop
• Stereo pair overlay using Photoshop
Gelatin powder – SEI

Working distance: 19 mm
Accelerating voltage: 15 kV
Spot size: 8
Objective aperture: 2
Scale bar: 50 μm
Gelatin powder – SEI Stereo pair

Working distance: 19 mm
Accelerating voltage: 15 kV
Spot size: 8
Objective aperture: 2
Scale bar: 50 μm
Tilt: 10.0°
Fig. A. Gelatin powder – SEI

Fig. B. Gelatin powder – BEI (Compo)

Working distance: 11 mm
Accelerating voltage: 15 kV
Spot size: 8
Objective aperture: 2
Scale bar: 5 μm
Gelatin powder – SEI

Working distance: 18 mm
Accelerating voltage: 30 kV
Spot size: 10
Objective aperture: 1
Scale bar: 0.5 μm
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