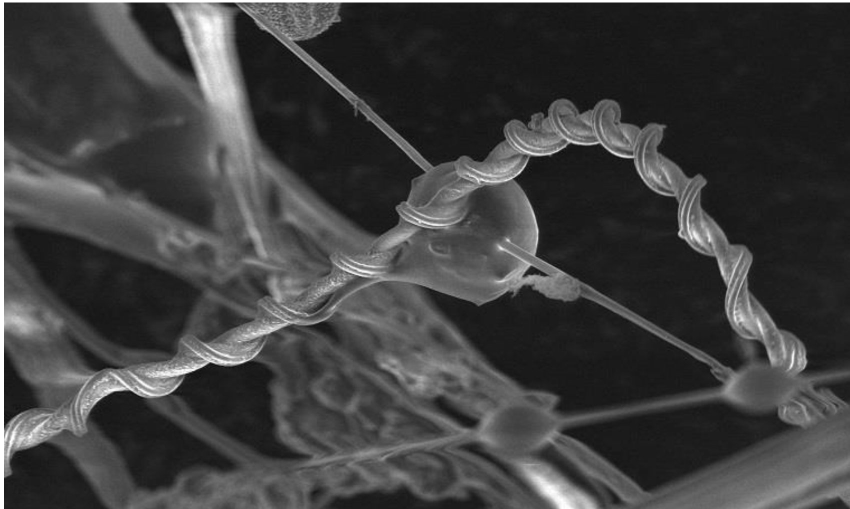
MNTC Electron Microscopy Tips & Tricks



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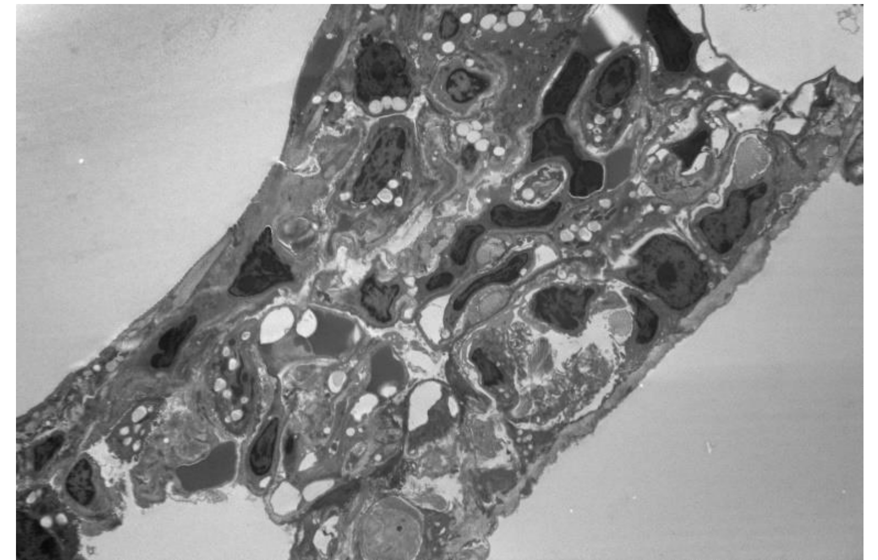


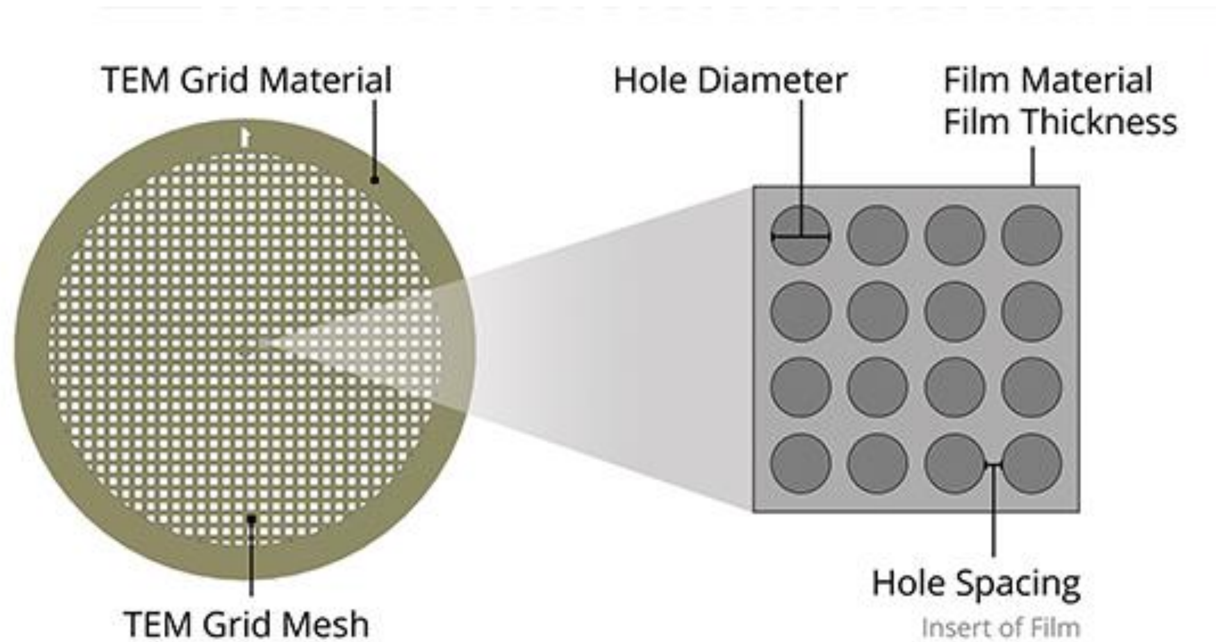
Staining

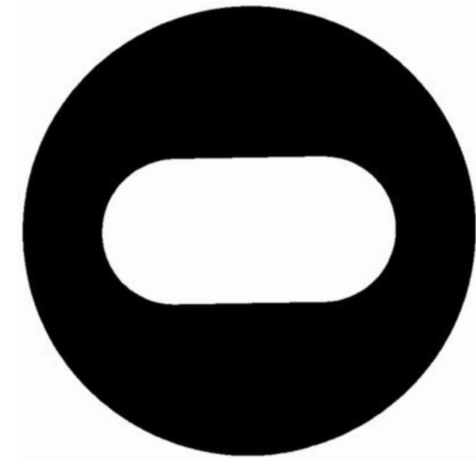
Osmium Tetroxide: stain for lipids in membranous structures and vesicles
https://www.emsdiasum.com/microscopy/products/sem/QX102_pdf/OsmiumTetroxideStainingFcells.pdf

Uranyl Acetate: heavy metal stain that binds to nucleic acids, proteins and membranes.
https://www.emsdiasum.com/microscopy/products/sem/QX102_pdf/mildUranylAcetateStainingcells.pdf#page=1

Lead Citrate: enhances a wide range of cellular structures such as ribosomes, lipid membranes, cytoskleleton and other compartments of the cytoplasm.
https://www.leica-microsystems.com/science-lab/brief-introduction-to-contrasting-for-em-sample-preparation/

TEM Grids





Slot Grids

TEM Sample Preparation

Cultured Cells

1. Prepare fixative: 4% Paraformaldehyde + 2.5% Glutaraldehyde in 0.1M Phosphate Buffer
2. Trypsinize cells from culture
3. Centrifuge at 1,000 rpm for 10 minutes
4. Replace supernatant with fixative
5. Centrifuge at 1,000 rpm for 10 minutes
6. Place pellet in 4°C for 48 hours
7. Wash pellet with 0.1 M Phosphate Buffer
8. Stain pellet with Osmium Tetroxide (OsO_4)
9. Wash pellet with 0.1 M Phosphate Buffer
10. Dehydrate pellet with increasing percentages of ethanol
11. Embed pellet with Durcupan Resin: 24 hours vacuum + 48 hours curing
12. Ultramicrotome sections down to 80 nm and place onto grids
13. Uranyl acetate stain the grids
14. Lead Citrate stain the grids
15. Image in TEM

TEM Sample Preparation

<u>Extracellular Vesicles</u>

1. Prepare fixative: 4% Paraformaldehyde + 2.5% Glutaraldehyde in 0.1M Phosphate Buffer
2. Researcher centrifuges at their preferred schedule
3. Replace supernatant with fixative
4. Keep sample in fixative at 4°C for a minimum of 2 hours
5. Pipette part of pellet onto grids and let sit for 10 minutes – face up on parafilm
6. Blot away excess fixative – face up on parafilm
7. Lightly wash grid with DI & blot - face up on parafilm
8. Stain pellet with water based 4% Uranyl Acetate for 10 min – face up on parafilm
9. Lightly wash grid with DI & blot
10. Image in TEM

TEM Sample Preparation

Tissue

1. Prepare fixative: 4% Paraformaldehyde + 2.5% Glutaraldehyde in 0.1M Phosphate Buffer
2. Perform whole body perfusion (can be performed by the RRC)
3. Section tissue into 1 mm cubes
4. Leave tissue in fixative for 48 hours at 4°C
5. Wash tissue with 0.1 M Phosphate Buffer
6. Stain tissue with Osmium Tetroxide (OsO_4)
7. Wash tissue with 0.1 M Phosphate Buffer
8. Dehydrate tissue with increasing percentages of ethanol
9. Embed tissue with Durcupan Resin: 24 hours vacuum + 48 hours curing
10. Ultramicrotome sections down to 80 nm and place onto slot grids
11. Uranyl acetate (UA) stain the grids for one hour
12. Lead Citrate stain the grids
13. Image in TEM

TEM Sample Preparation

Formalin Fixed Paraffin Embedded (FFPE) Samples

1. Retrieve section of tissue from sample
2. Stain tissue with Osmium Tetroxide (OsO_4)
3. Wash tissue with 0.1 M Phosphate Buffer
4. Dehydrate tissue with increasing percentages of ethanol
5. Embed tissue with Durcupan Resin: 24 hours vacuum + 48 hours curing
6. Ultramicrotome sections down to 80 nm and place onto grids
7. Uranyl acetate (UA) stain the grids
8. Lead Citrate stain the grids
9. Image in TEM

SEM Sample Preparation

Cultured Cells

1. Culture cells on glass slides or Aclar
2. Prepare fixative: 4% Paraformaldehyde + 2.5% Glutaraldehyde in 0.1M Phosphate Buffer
3. Leave sample in fixative for 48 hours at 4°C
4. Wash sample with 0.1M Phosphate Buffer
5. Dehydrate sample with increasing percentages of ethanol and HMDS
6. Image in SEM in low vacuum mode

Notes:
Collagen: Soak in 1% tannic acid at 4°C for 24 hours followed by osmium tetroxide stain and ethanol dehydration.

Perform critical point drying for matted fibrous tissue to separate fibers.

If samples are difficult to see sputter coat as needed.