Electron Microscopy

An introduction to Ultrastructure

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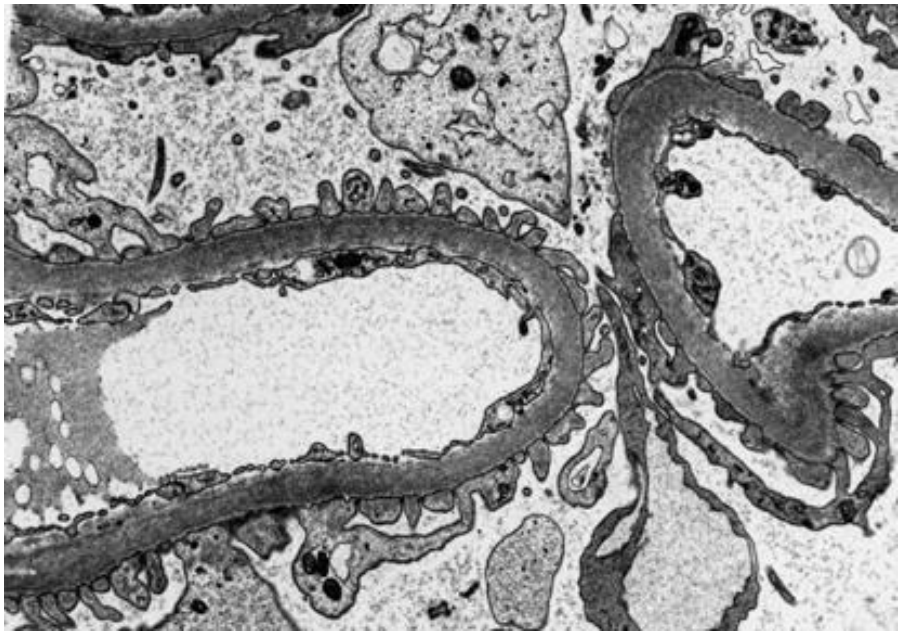


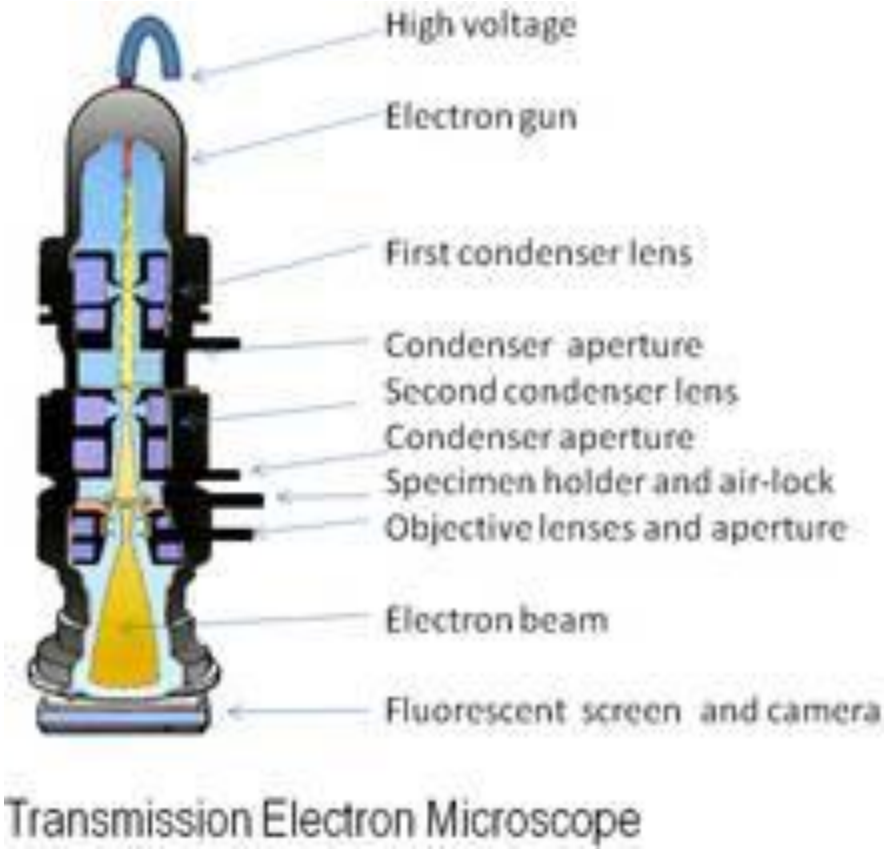
What is Electron Microscopy (EM)?

- TEM – Transmission Electron Microscopy
- 1924 – De Broglie Hypothesis – Wave Particle Duality
- 1926 – 1st Electromagnetic lenses
- 1933 – 1st Electron Microscope that exceeded the magnification of light microscopy
- 1950s – TEM starts to be used in the diagnostic setting – NHS Lothian got its first EM in 1958 and in the 60s, 70s and 80s TEM was at its diagnostic peak
- Magnification range; 500x – 200,000x and beyond

How does it work?

Light

Electrons



Transmission Electron Microscope

NHS Cases in Lothian

2019/20

339 Cases

- 261 Renal biopsies
- 61 Muscle biopsies
- 17 Other (sural nerves, duodenal biopsies, reprocessed blocks)

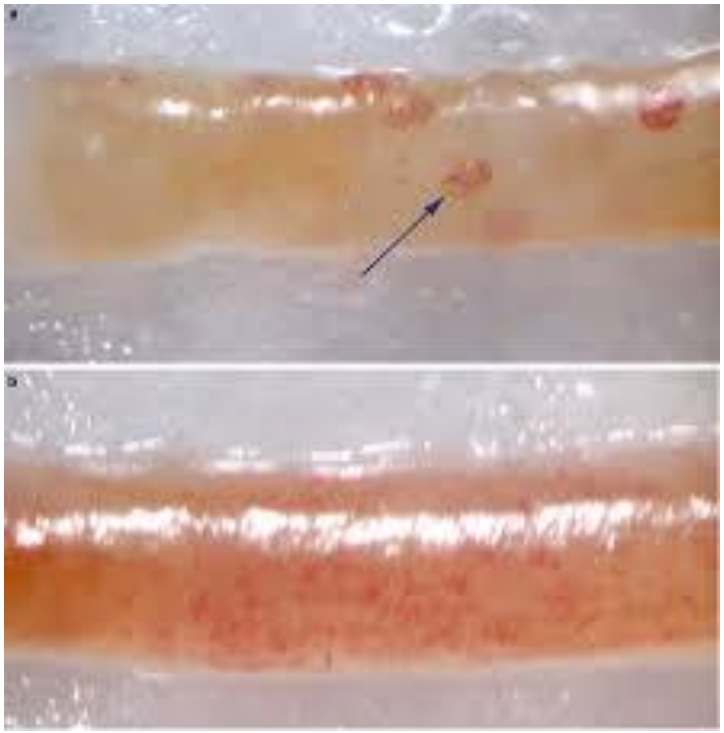
What happens in the EM lab?

1. Tissue selection
2. Processing
3. Cutting and tissue assessment
4. Ultramicrotomy
5. Staining grids
6. Viewing
7. Imaging

So basically the same as processes as the routine paraffin lab – but on a smaller scale and with some very hazardous chemicals

Turnaround from start to finish is at least 5 days, routinely more

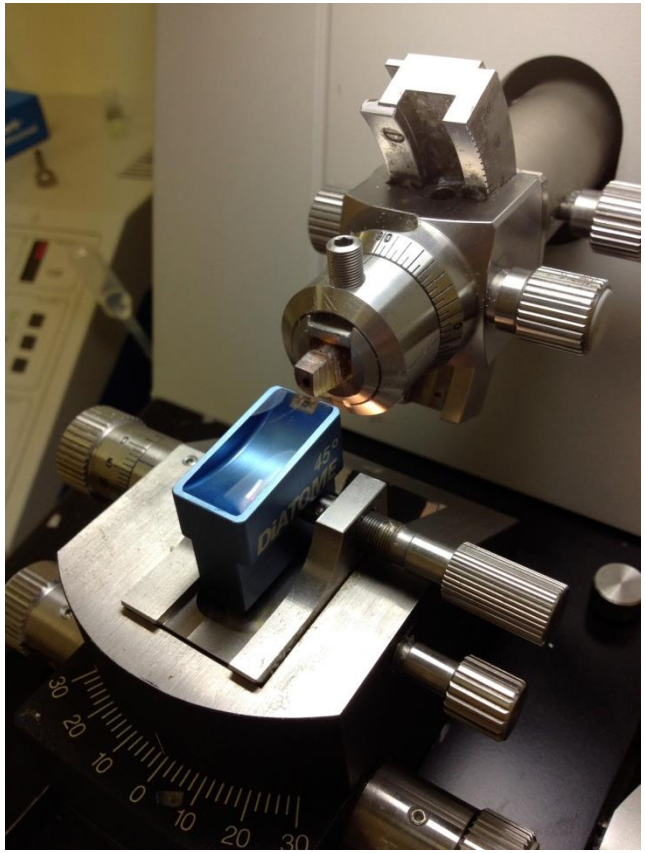
Tissue Selection and Processing



1mm cube size is ideal

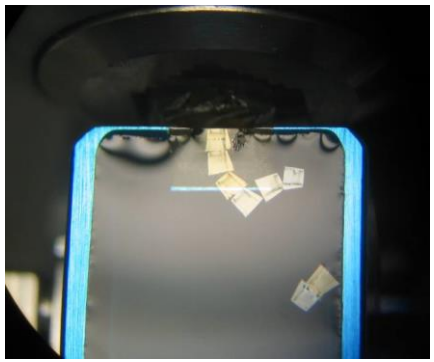
Processing steps
FIX- Glutaraldehyde
2nd FIX – Osmium Tetroxide
DEHYDRATE – Alcohol (or Acetone), followed by Propylene Oxide
IMPREGNATION – Epoxy Resin (Araldite)
EMBEDDING – Curing Resin

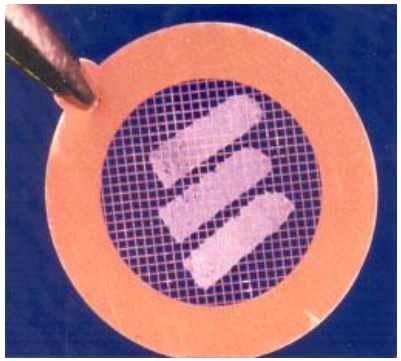
Assessing and cutting tissue



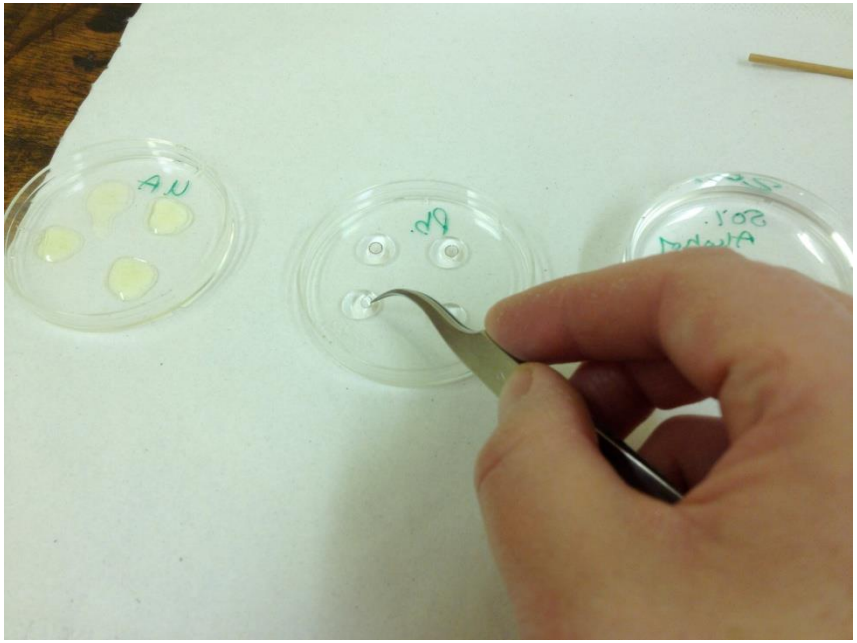
Ultramicrotome with diamond knife

- 1st trim into the block and cut a 1µ section onto a glass slide
- Stain section with Toluidine Blue
- If the area you want is there then you can cut an ultrathin section. Otherwise trim in deeper
- Ultrathin sections are cut at around 90nm
- They float out onto the water bath of the diamond knife and can be picked up
- Ultrathin sections are collected onto a copper grid with a fine mesh





Staining Grids



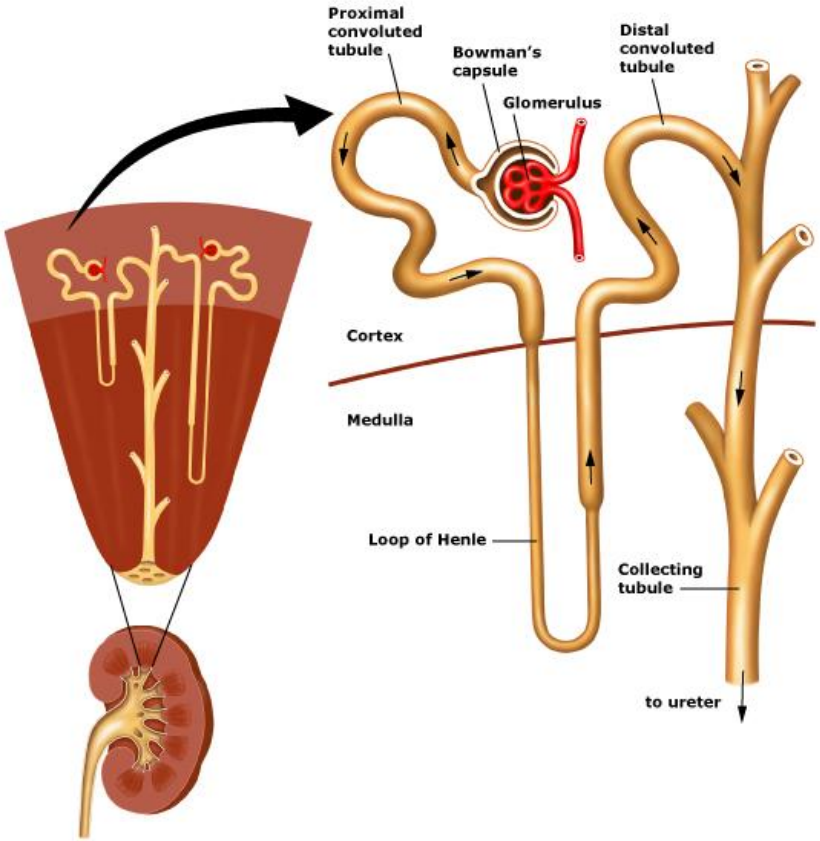
- By hand
- Use heavy metals not coloured dyes
- **Uranyl Acetate** – radioactive (depleted uranium), stains proteins, nucleic acids and phospholipids
- **Lead Citrate** – increases the contrast after uranyl actetate step

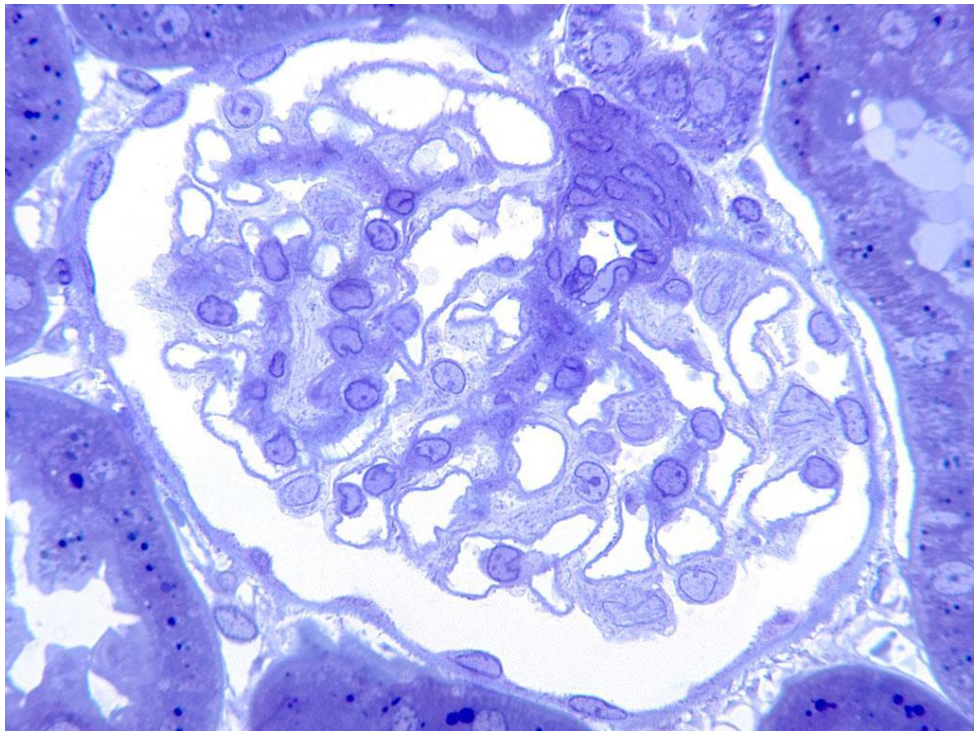
Viewing and Image Transfer

- Stained specimen grids inserted into the electron beam and the electrons ‘transmitted’ through the section excite a phosphorous screen
- In ‘ye olde days’ film photographs were taken, negatives processed and a print produced.
- Digital cameras these days make it easier to upload images onto a shared secure server to be accessed remotely by pathologist.

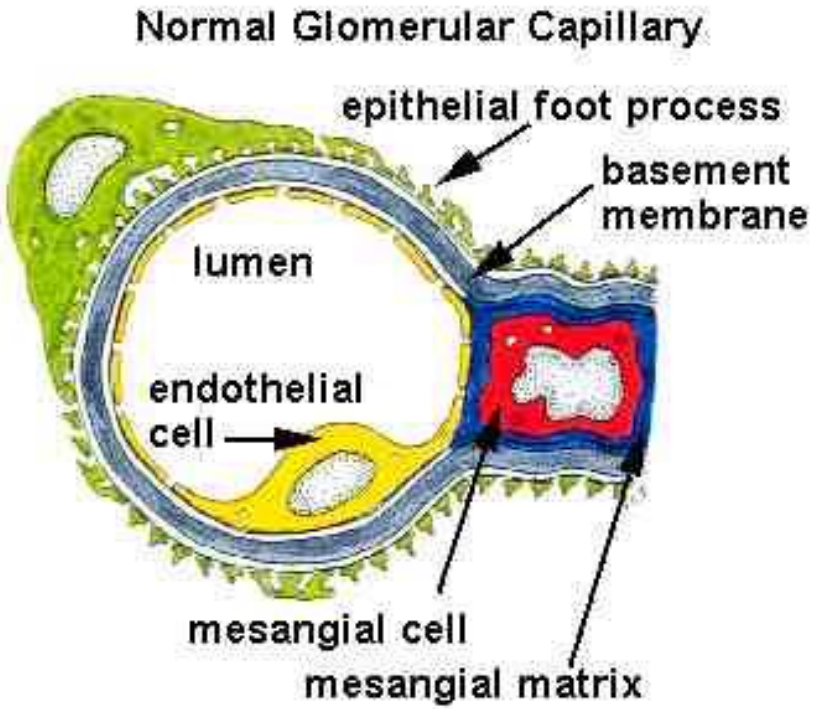
Renal Biopsy

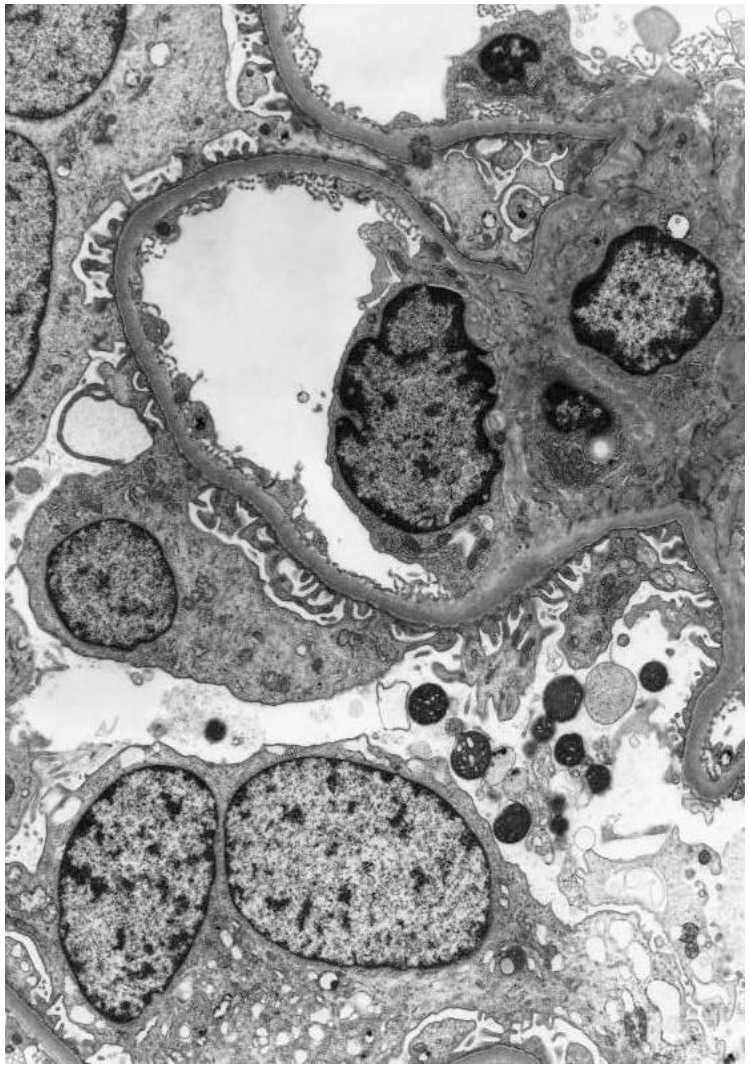
Renal structure - glomerulus



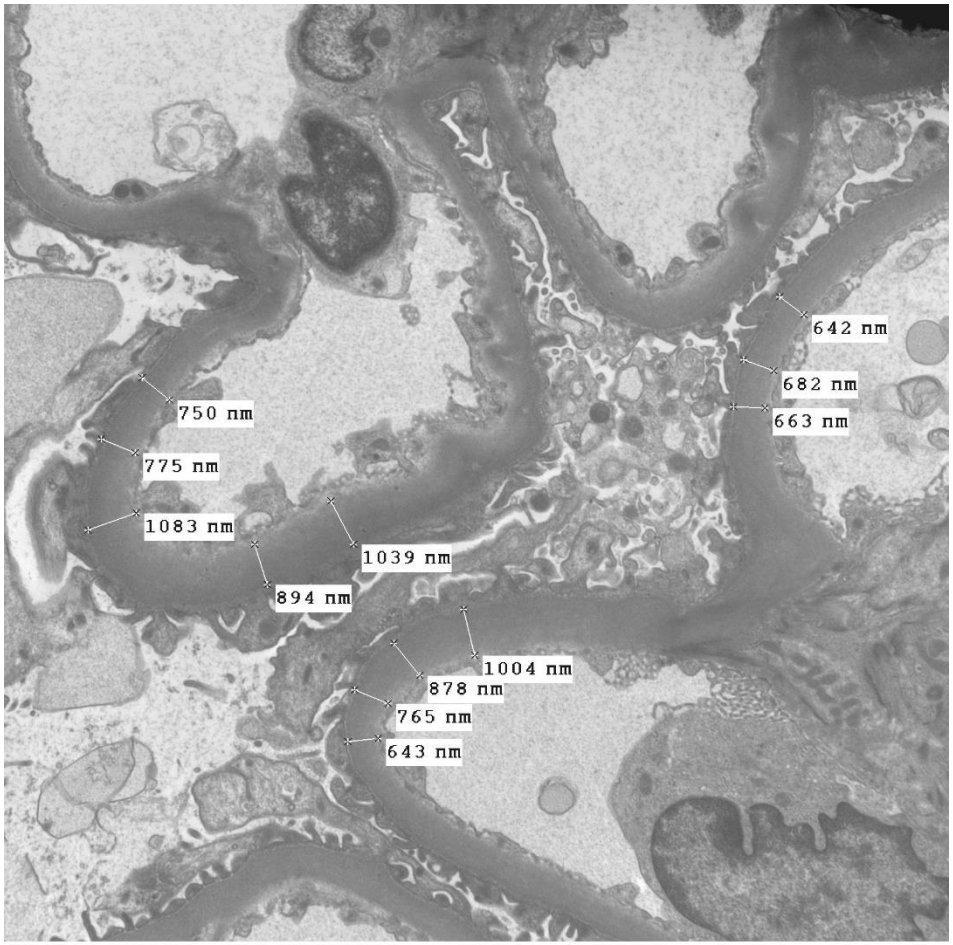


Normal renal – GBM 350nm





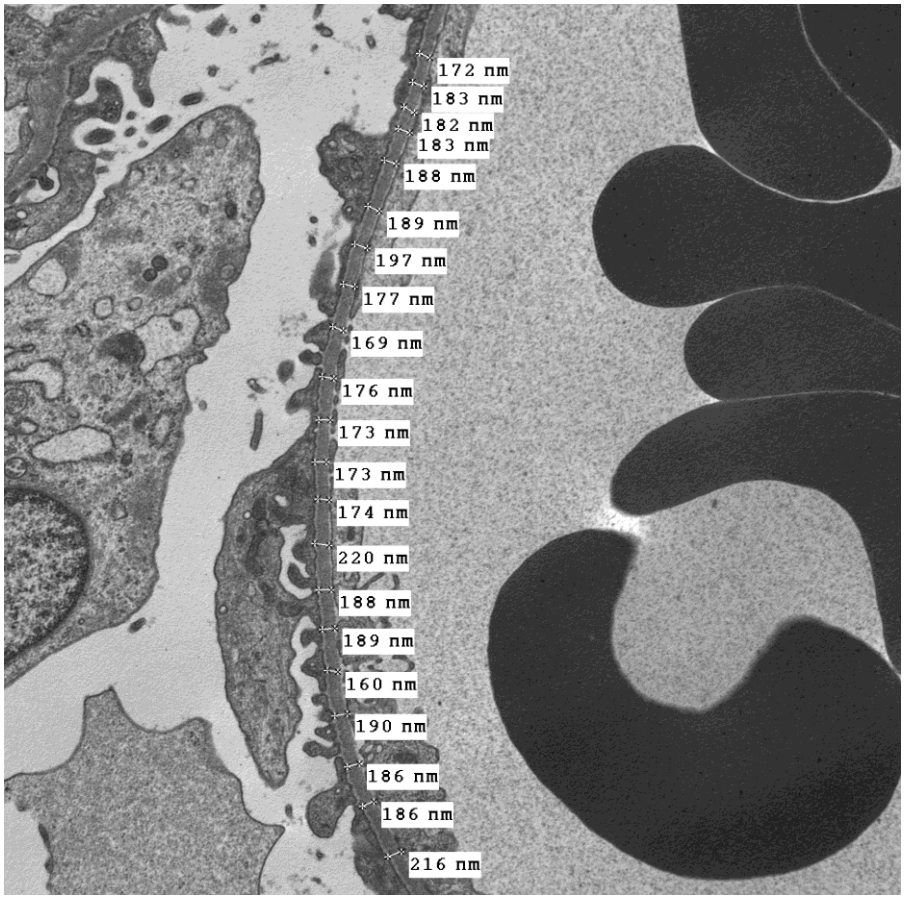
Diabetic Glomerulus



Thickened Glomerular Basement Membrane

Up to over 1000nm

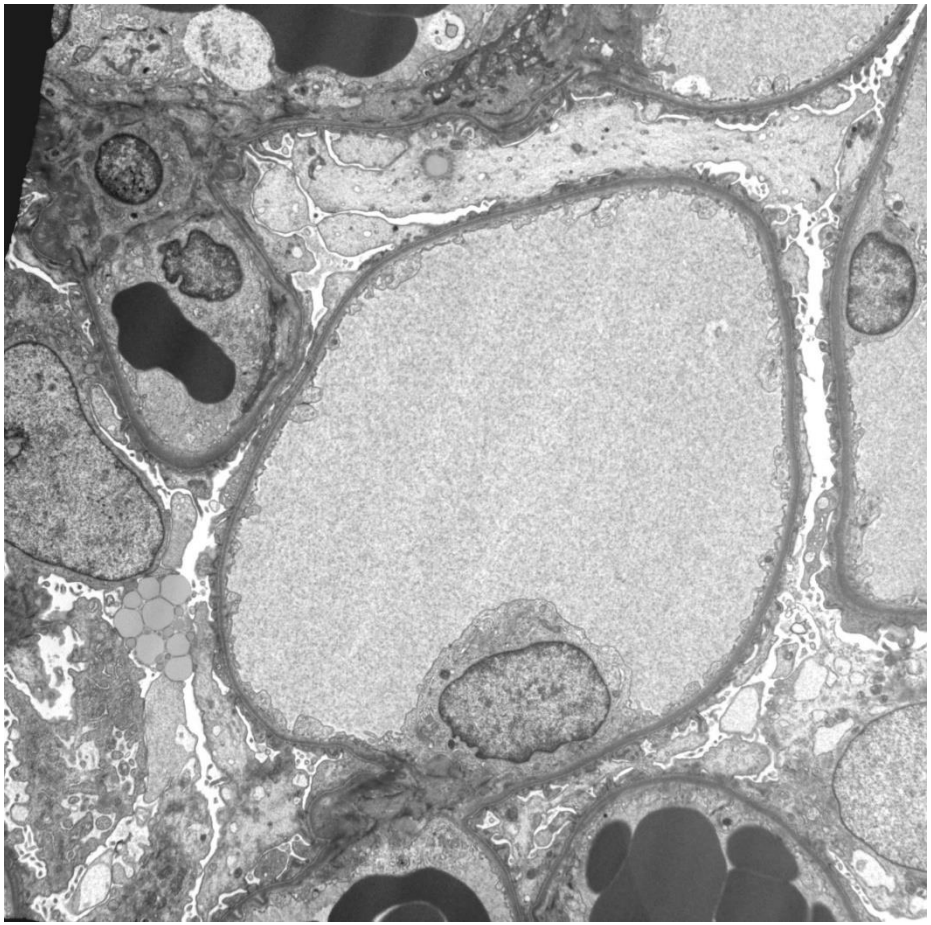
Thin Glomerular Basement Membrane



Thin GBM Disease

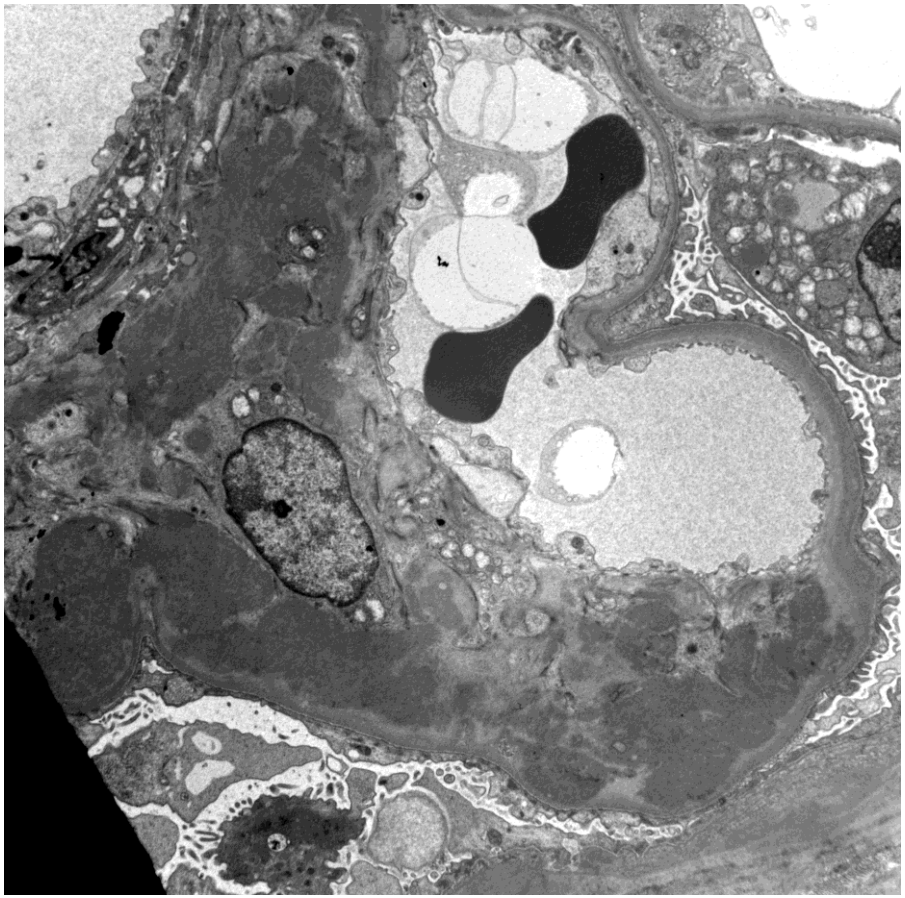
Down to 160nm

Minimal change disease



Podocytes from the epithelial cells have ‘flattened’ off - effacement

Deposits - IgA

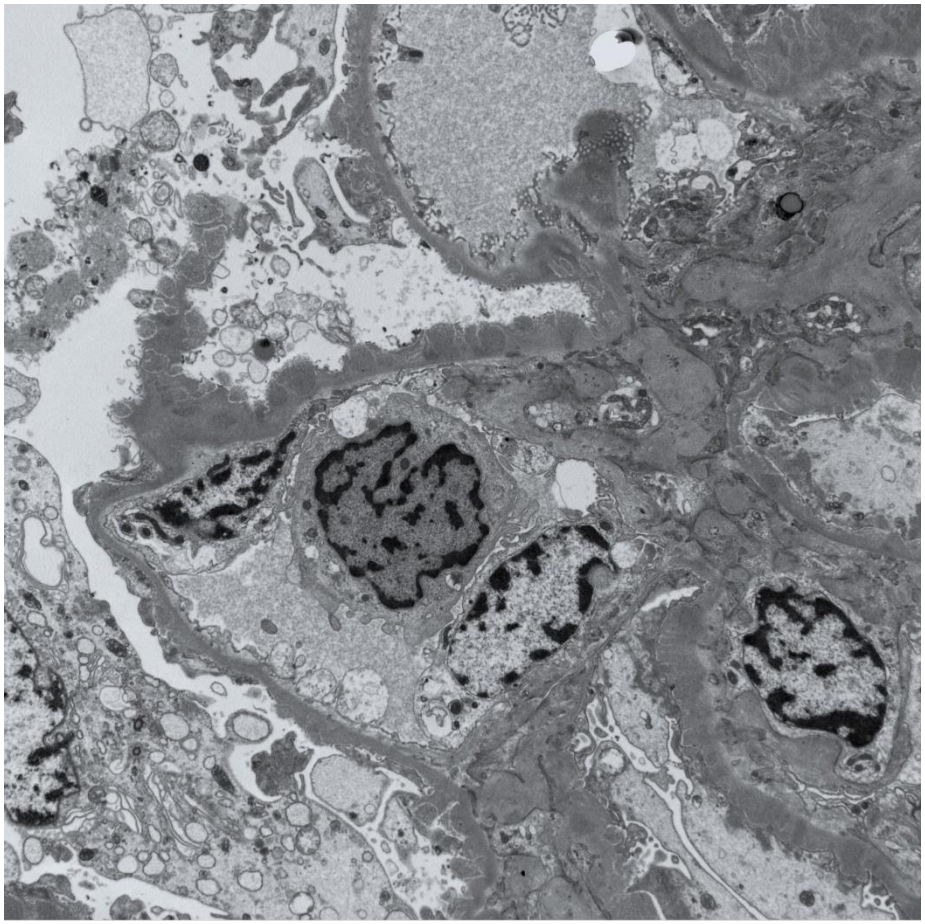


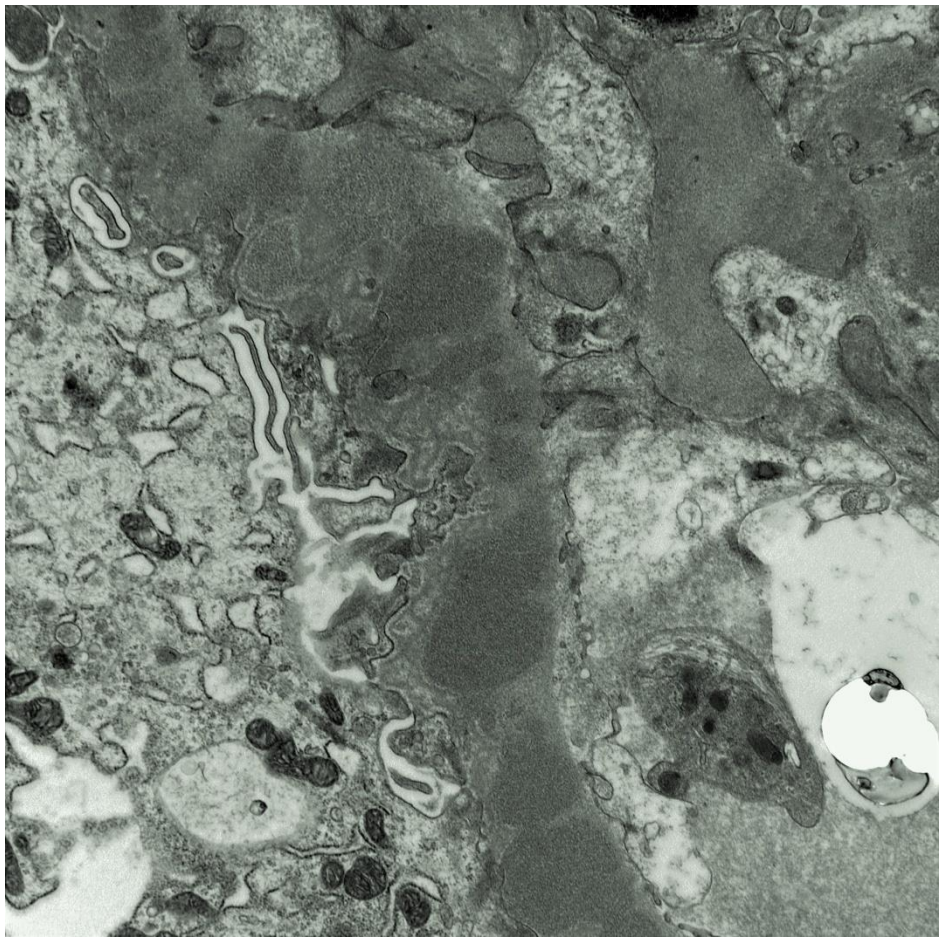
IgA will have been seen on the immunofluorescent slide

But the pathologist might not be able to see exactly where in the glomerulus it is

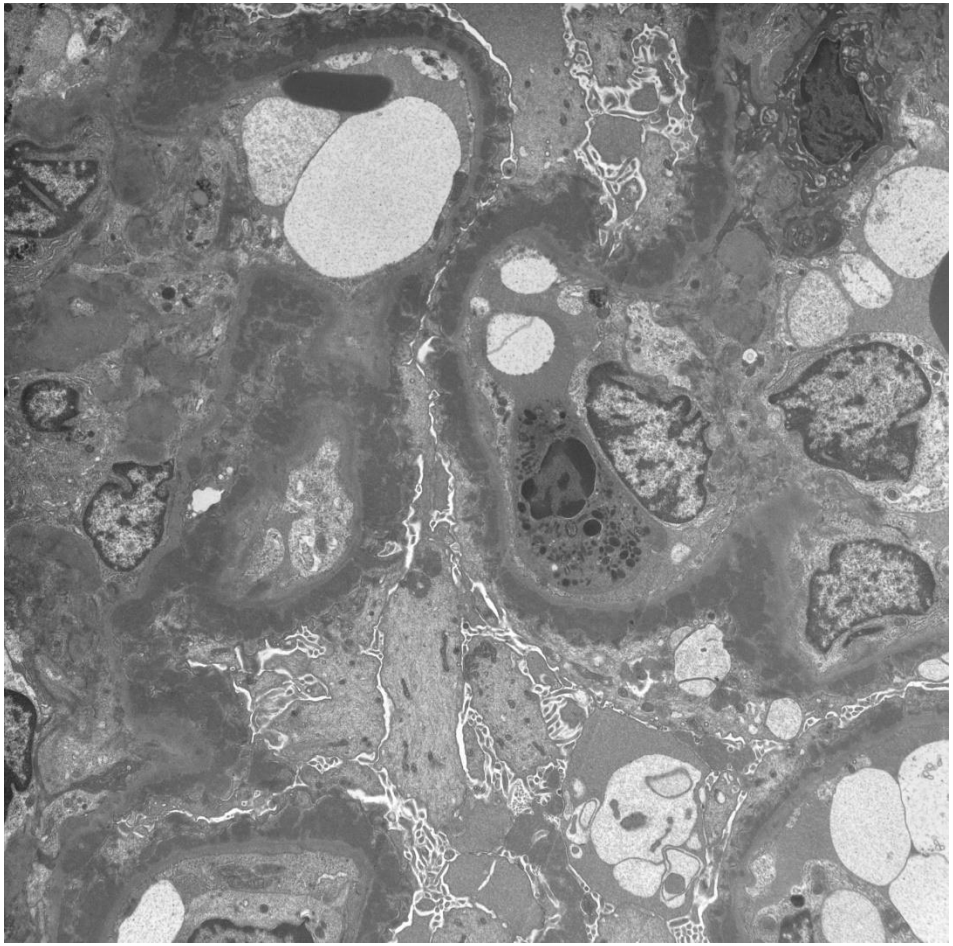
On EM the density and any organisation of the deposits can be more clearly seen

Basement Membrane Thickening and Spikes





Systemic Lupus Erythematosus

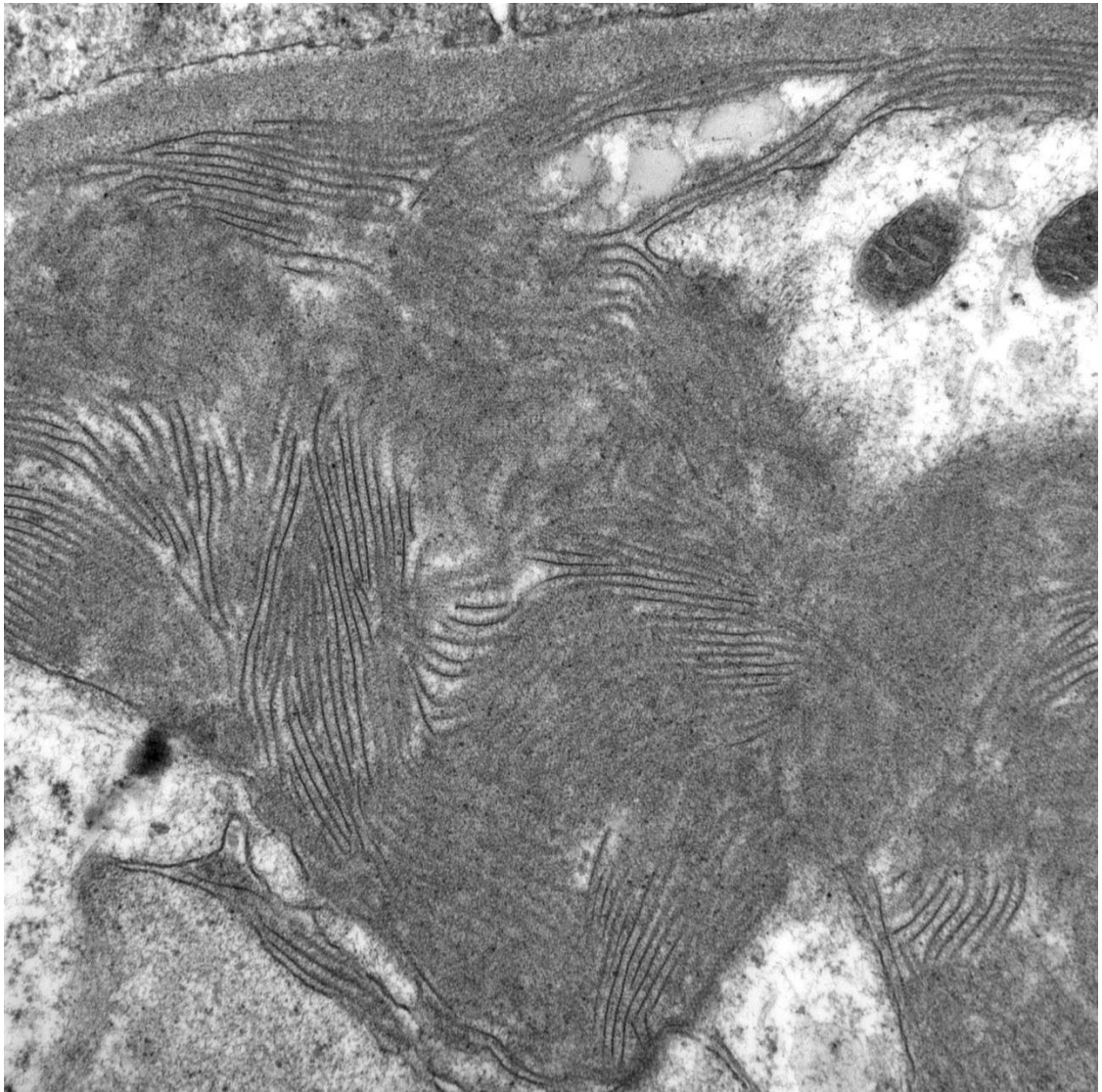


Many of the immunofluorescent slides will have stained positive.

Deposits seen epithelial, mesangial and/or endothelial areas of the Glomerulus

Any organisation of deposits helps staging of the disease

Organised Deposits



Serum showed a monoclonal IgG lambda band

B-Cell related lymphoproliferative

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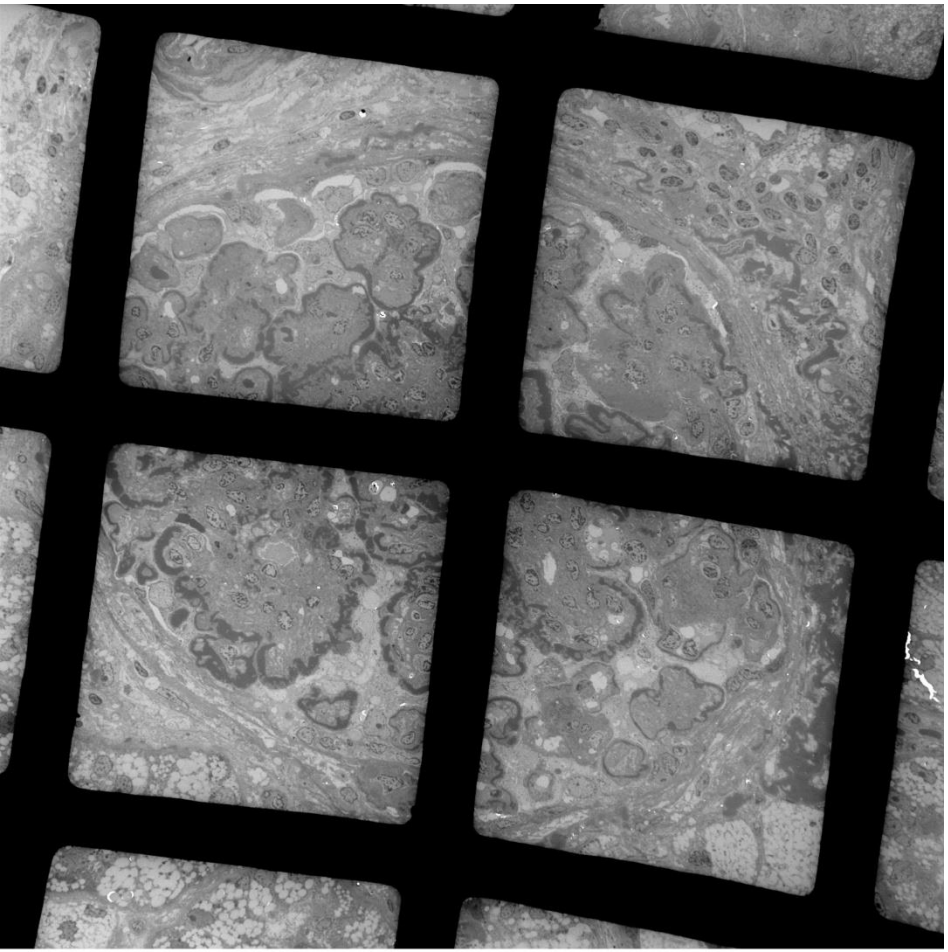
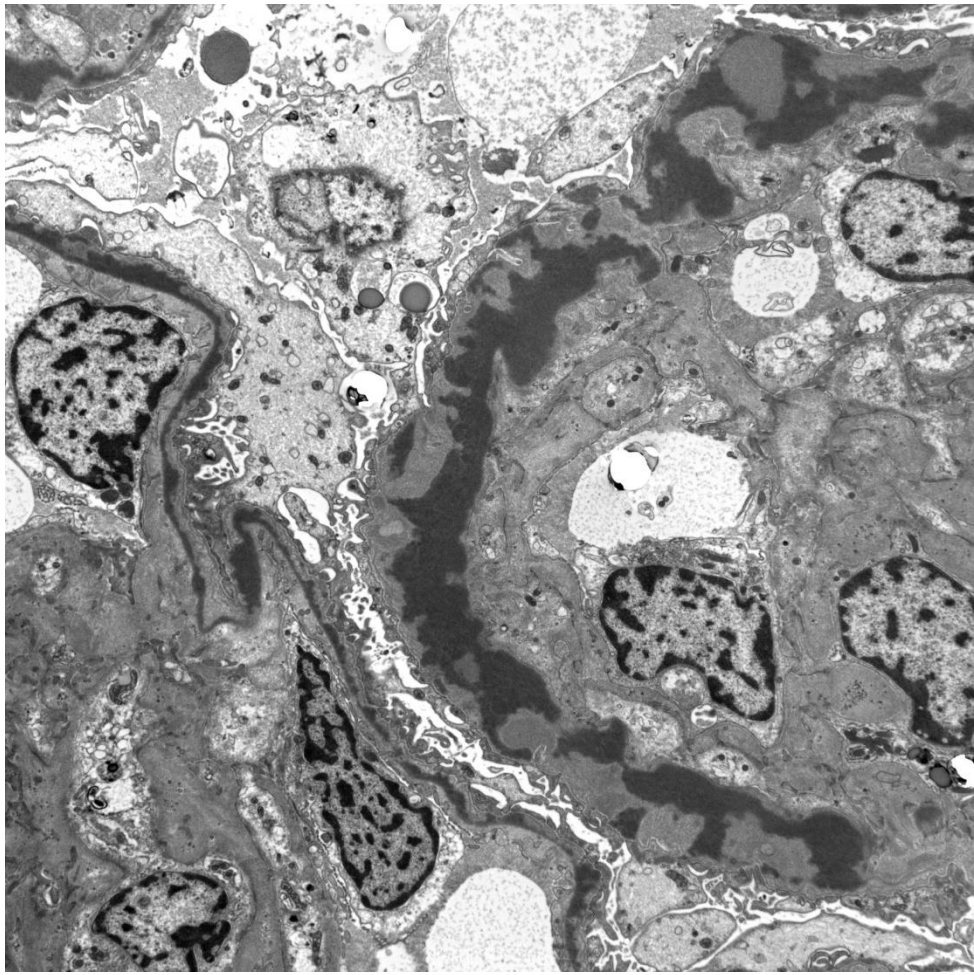
Dense Deposit Disease – C3





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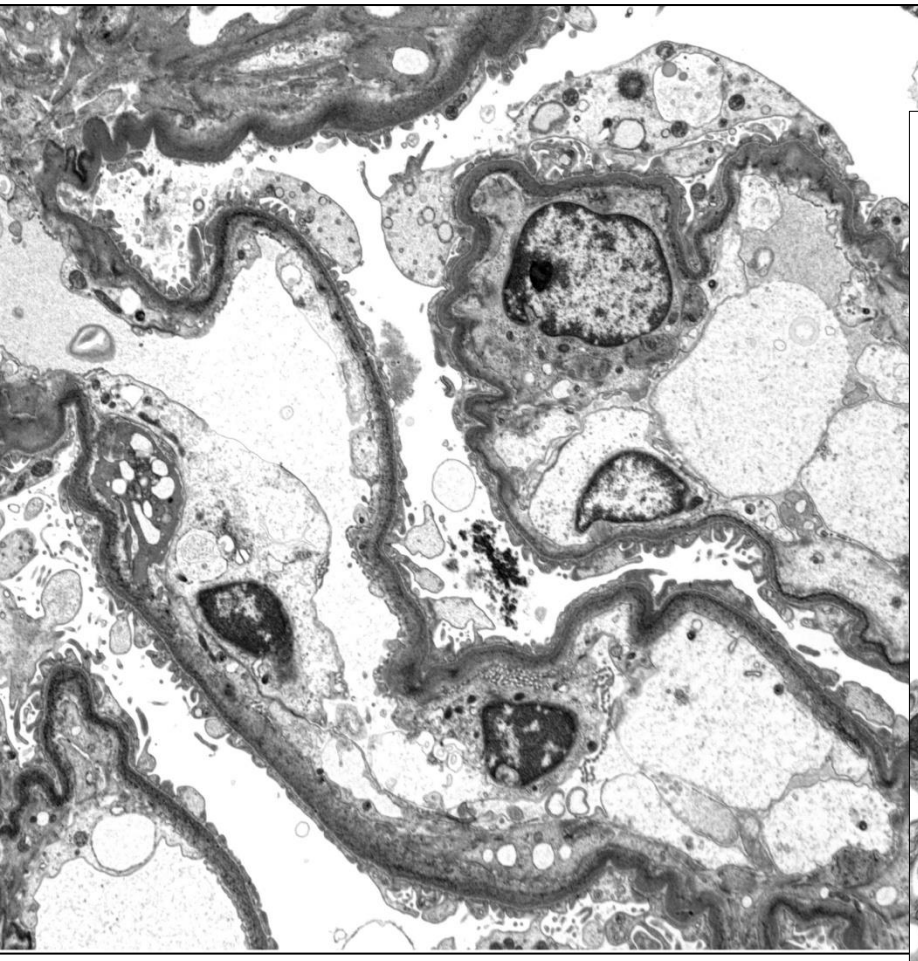
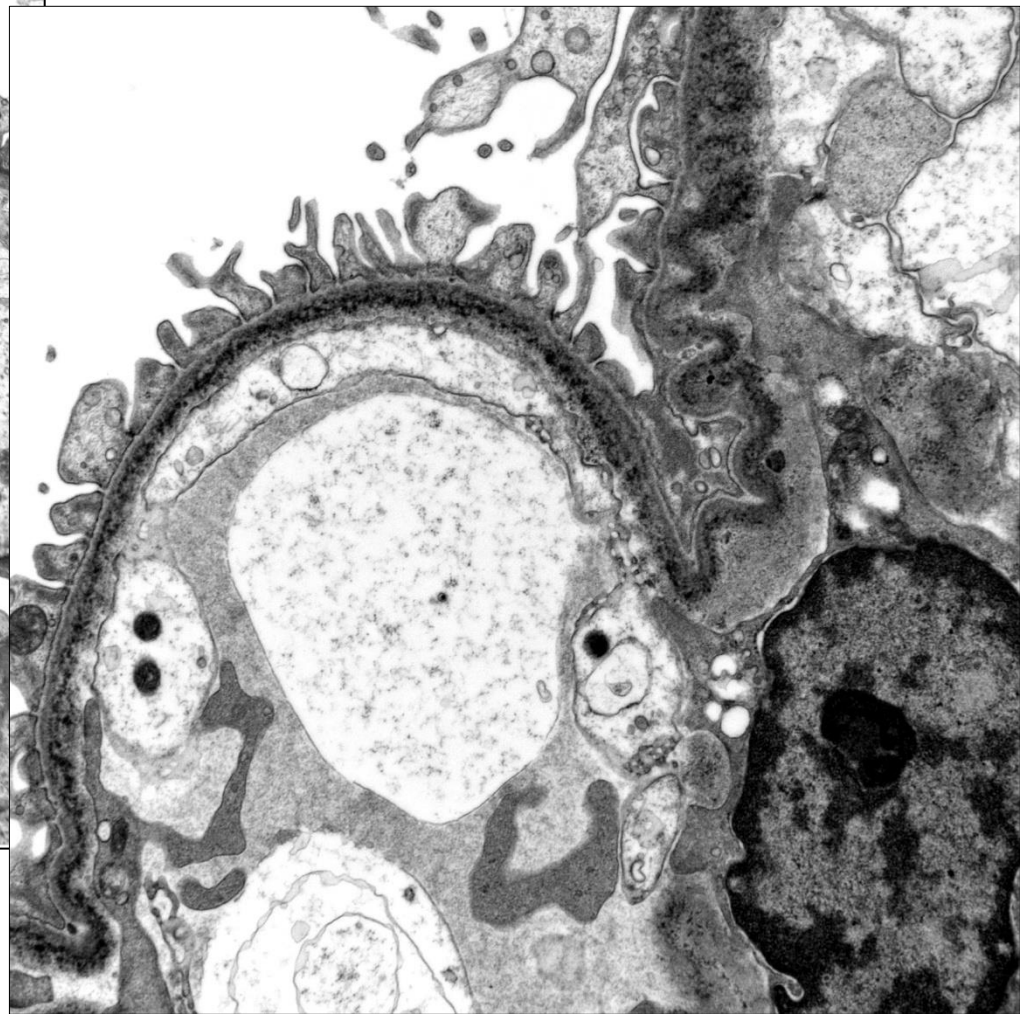
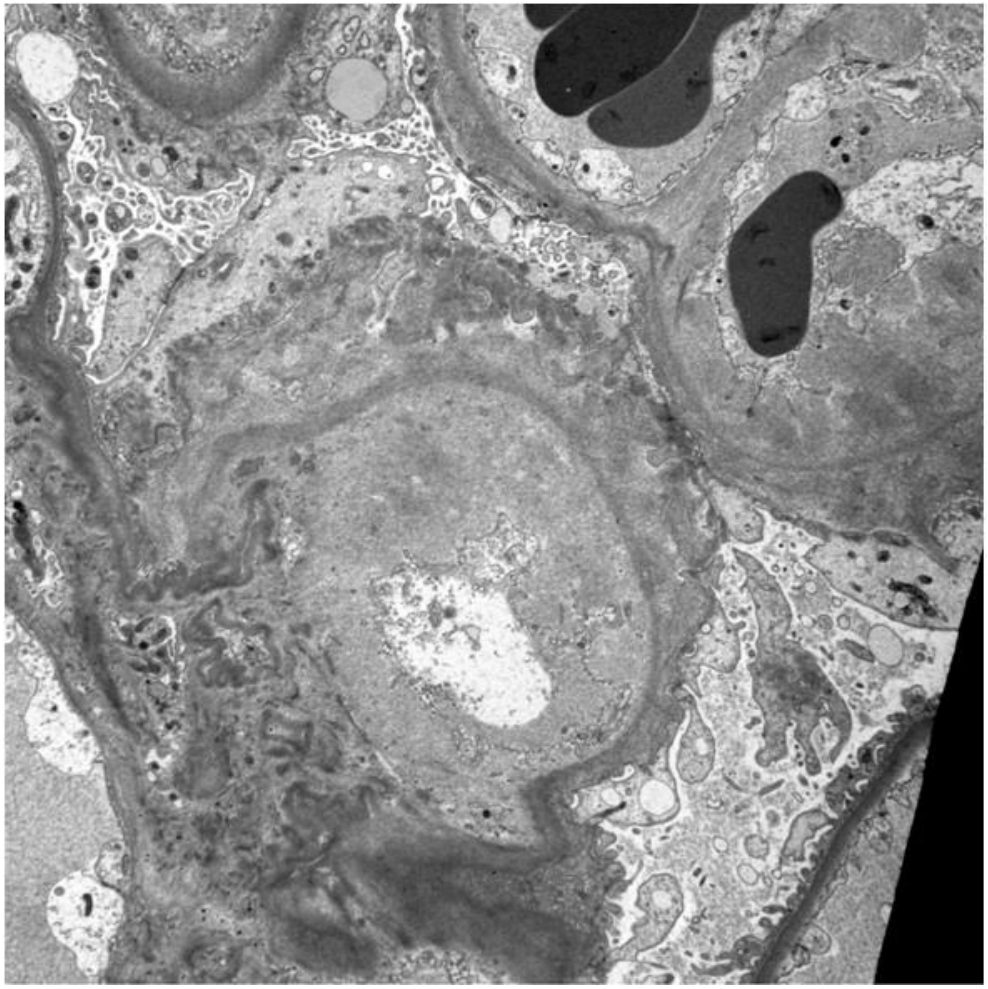
Light Chain Deposition Disease





Amyloid

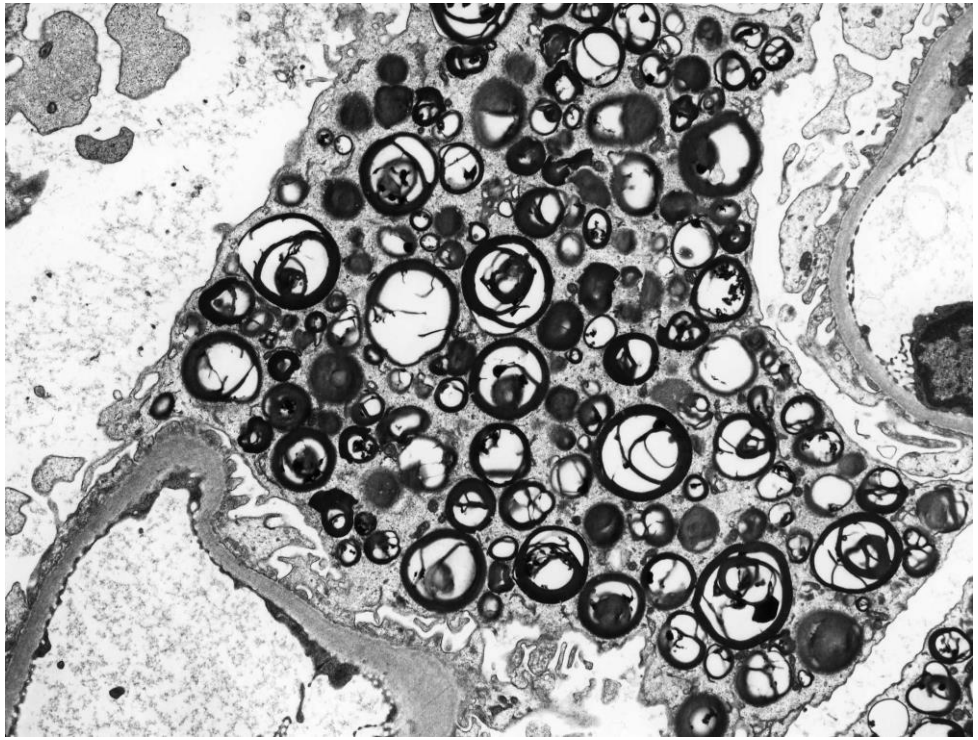


Amyloid fibres are 8-10nm in diameter and give the appearance of being ‘fluffy’ under EM

On an HE the amyloid will have a pink haziness to it, and it might have stained with the Congo Red stain, and seen under polarised light to have apple green birefringence

This capillary loop has lost any functional structure as the amyloid deposits have taken over

Fabry's Disease



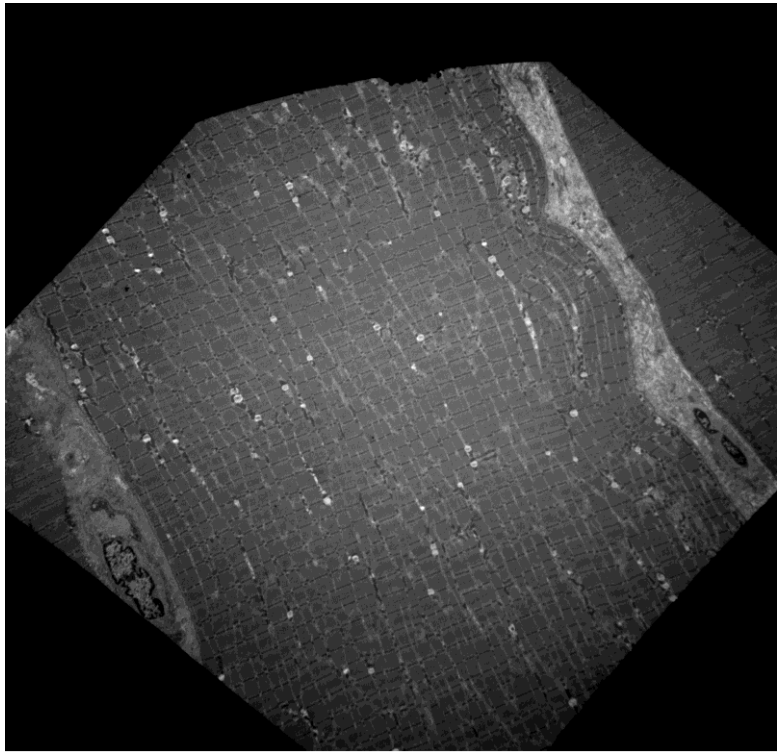
Lysosomal storage disease - inability to process sphingiolipids

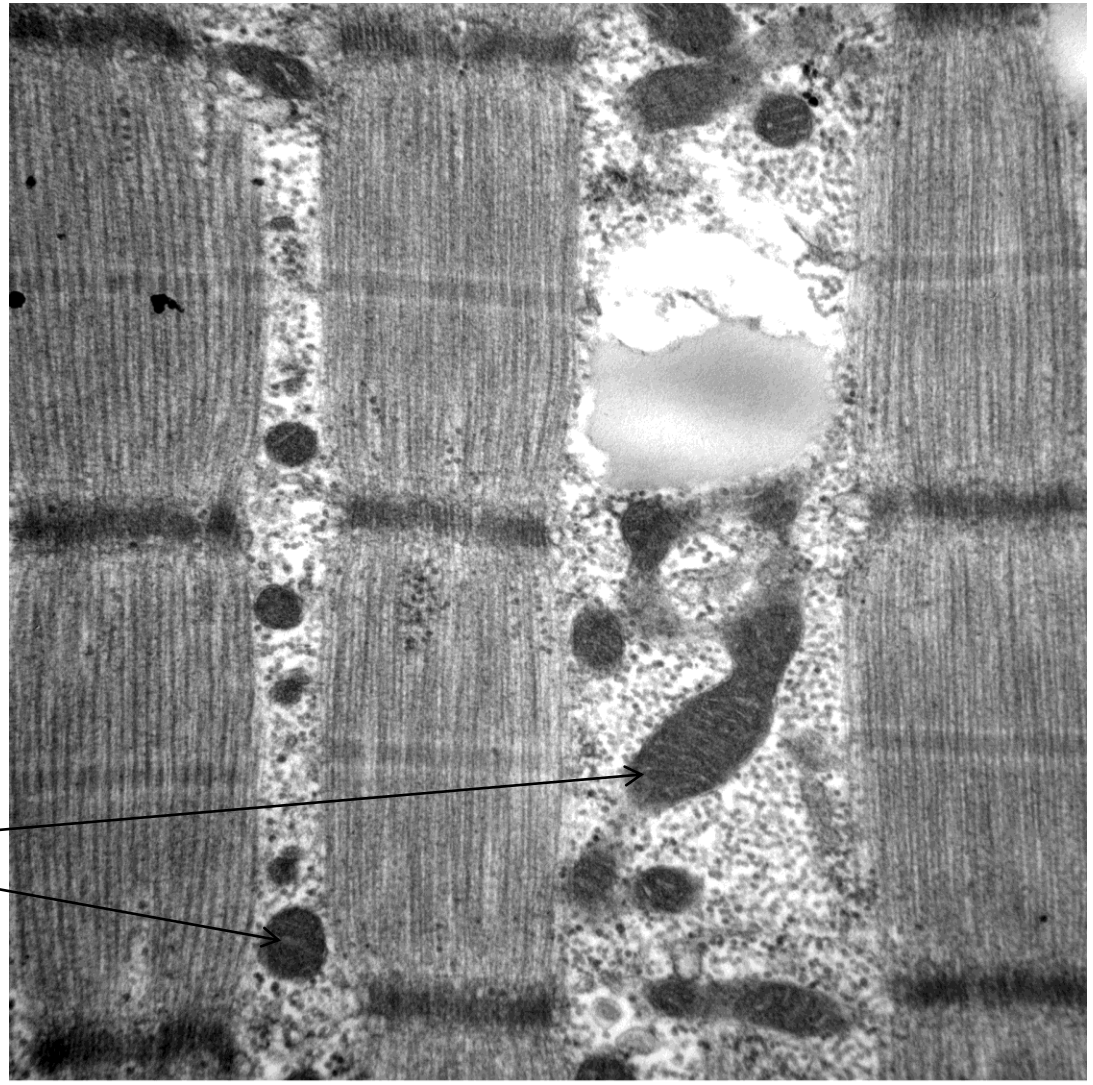
Rare mutation on the X chromosome causes a deficiency of enzyme alpha -galactosidase A

Lysosomes in the epithelial cells form whorls and zebra bodies

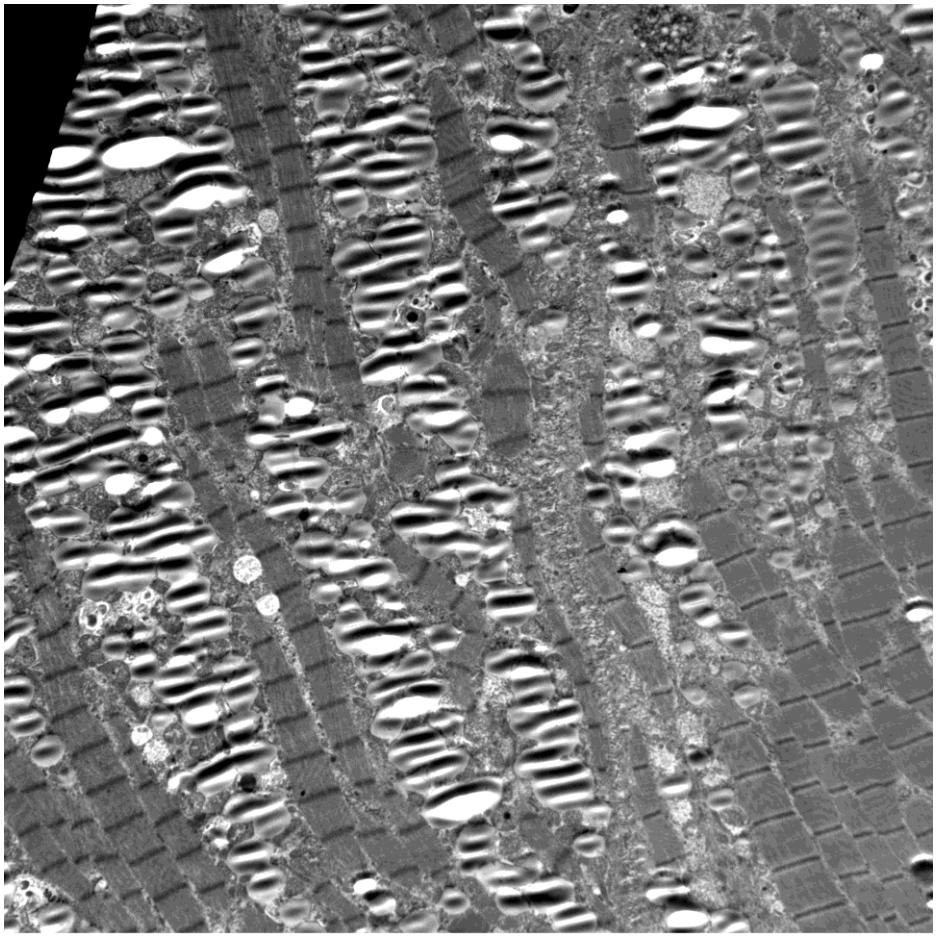
Muscle

Muscle





Lipid Storage Disease

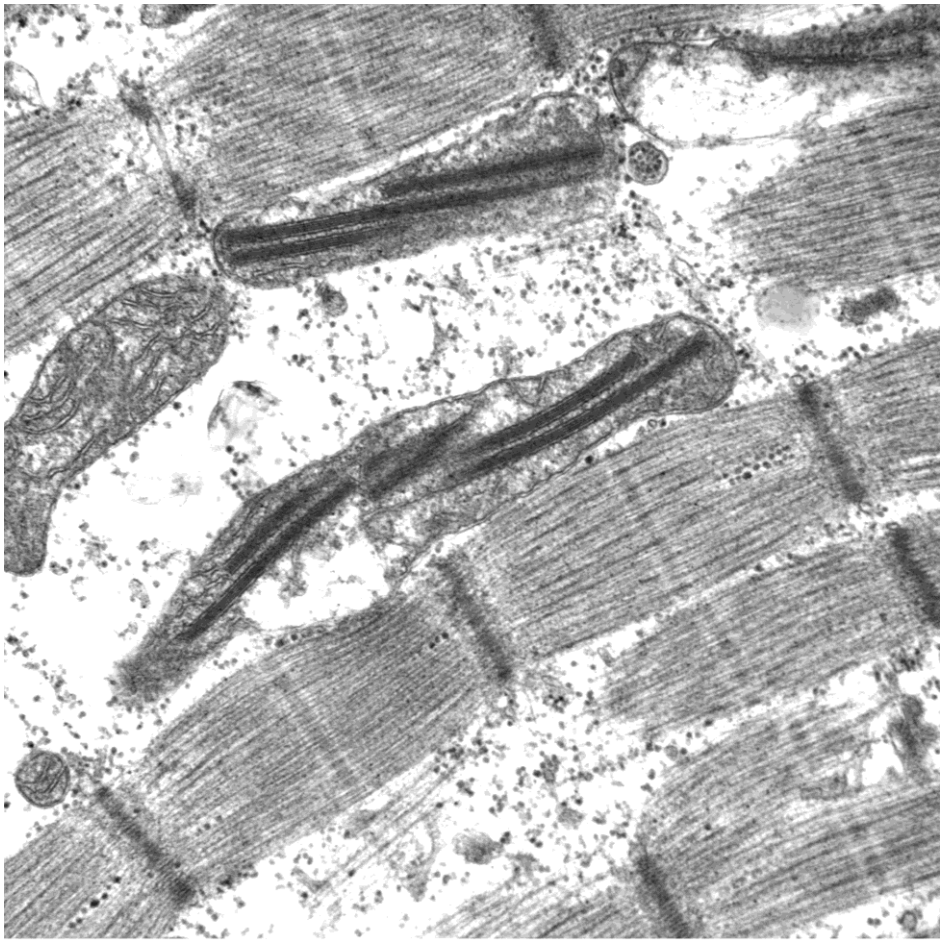


Multiple blobs of lipid displacing the myofibrils

Part of an inherited group of metabolic diseases

Seeing lipid is not a diagnosis of a particular disease but a guidance to which family of disorders to investigate further

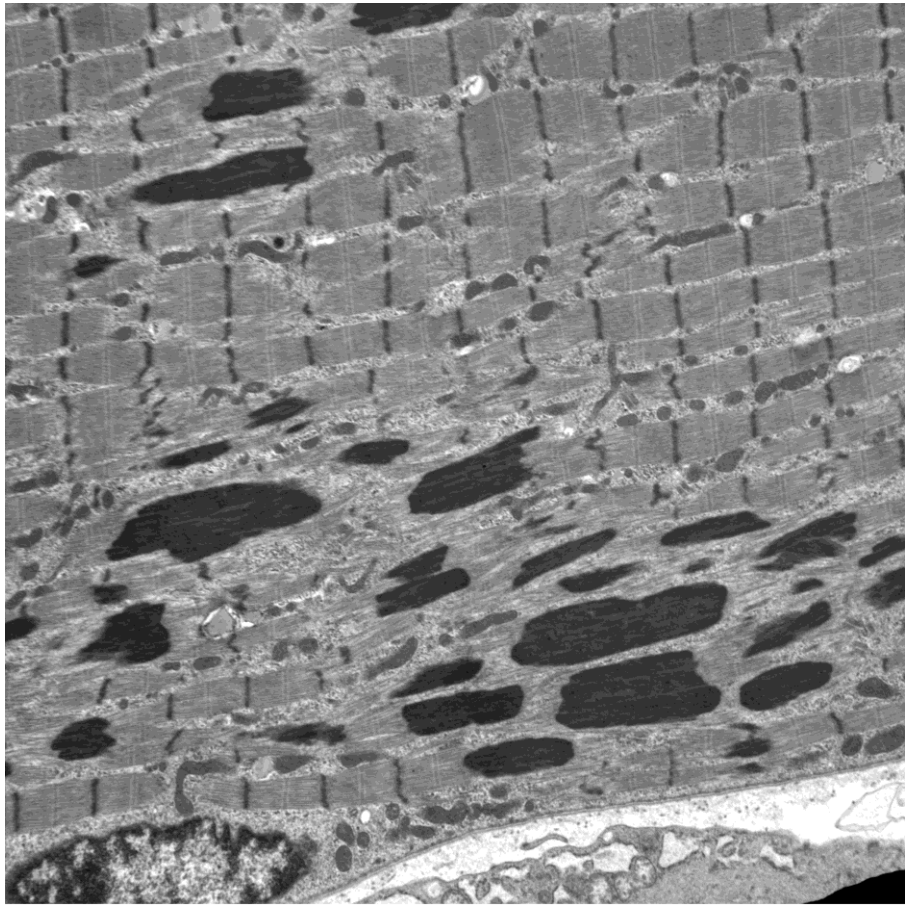
Mitchondrial Inclusions



Crystalline inclusions in the mitochondria.

Part of mitochondrial myopathy family of disorders

Rod myopathy



Z line streaming – actin anchoring points

Another inherited myopathy affecting muscle function

Other stuff

- Sural nerves
- Reprocessed blocks – amyloid, undifferentiated tumours

Research – rare cases

Cilia Dyskenesia

Any questions, drop me a message:

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Specialist Portfolio

Section 7.5b Electron Microscopy - examples

Q1 What fixatives are used in routine EM?
Q2 List the hazardous chemicals used in EM? What are some of the hazards?
Q3 What type of embedding medium is used?
Q4 In renal biopsies, what are you looking for in semi-thin section?

E1 Visit the EM laboratory then detail the specimen journey through the lab from receipt to image upload, paying particular attention to the similarities and differences between paraffin and resin processing.