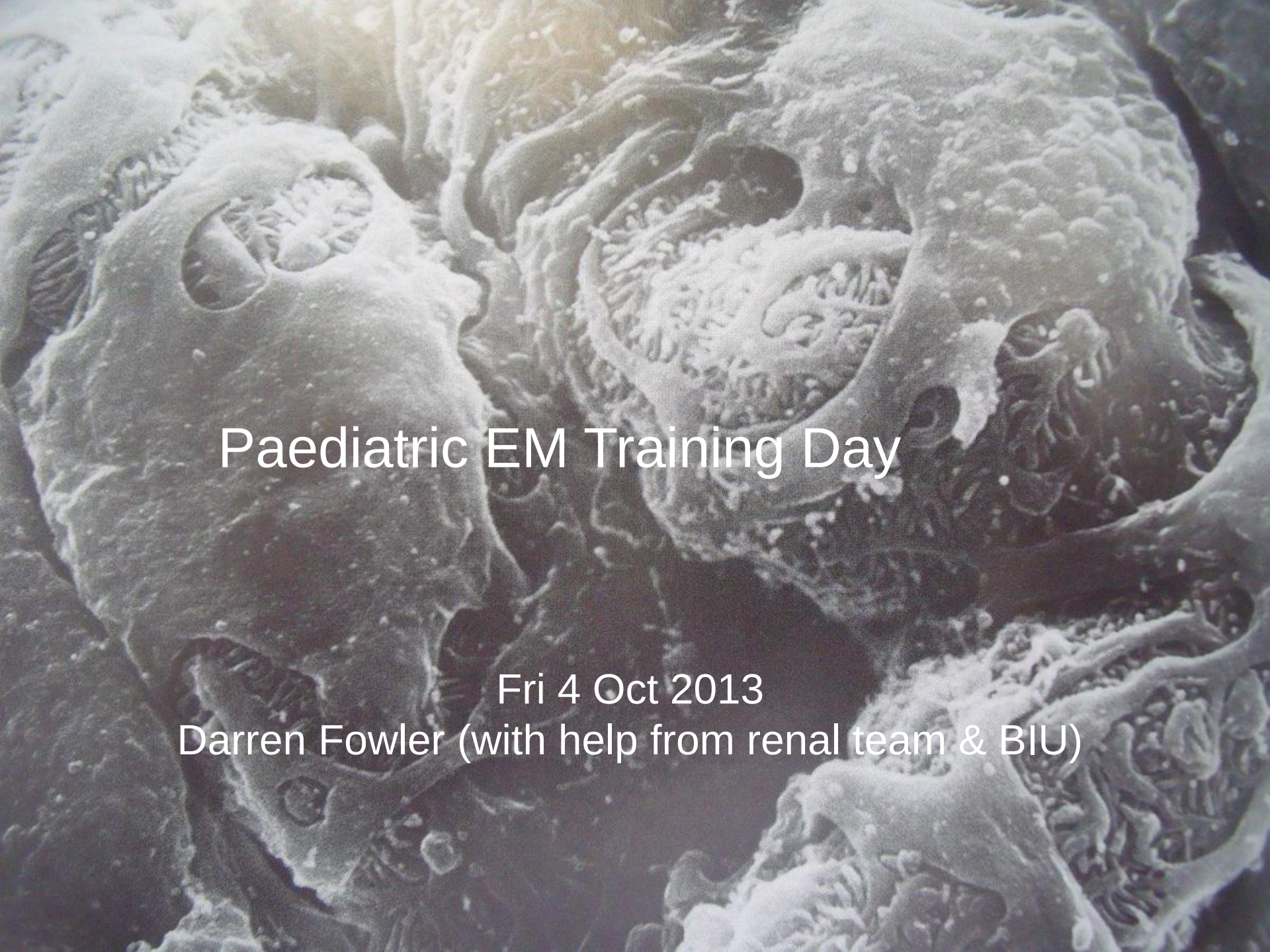
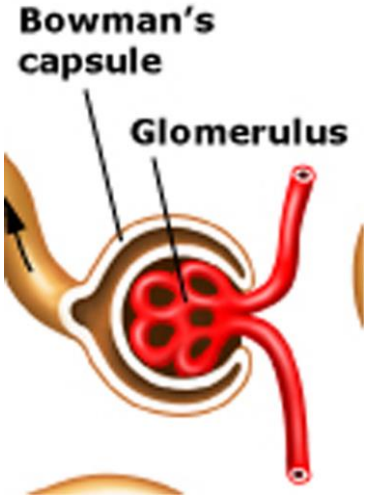


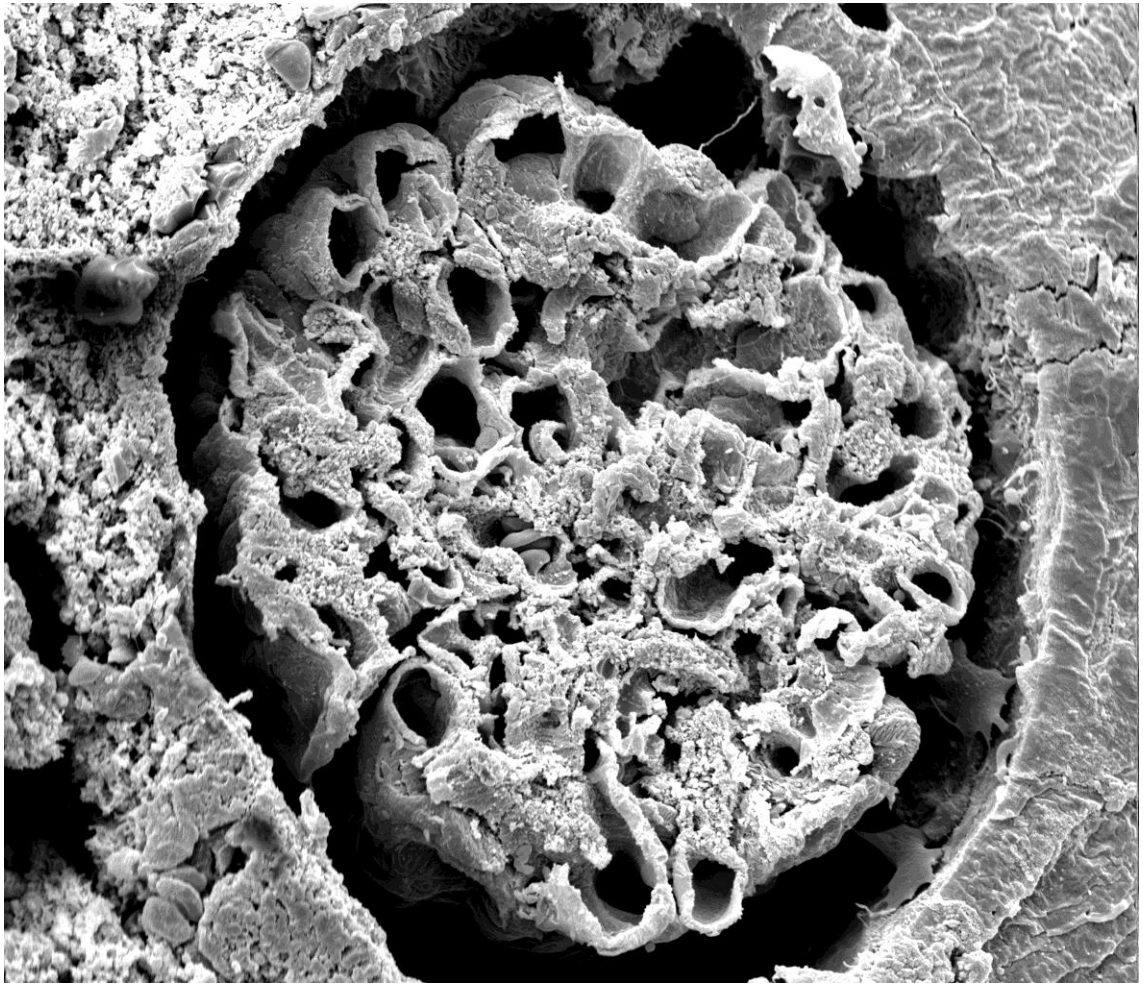
Paediatric EM Training Day

Fri 4 Oct 2013

Darren Fowler (with help from renal team & BIU)

SEM – Glomerulus

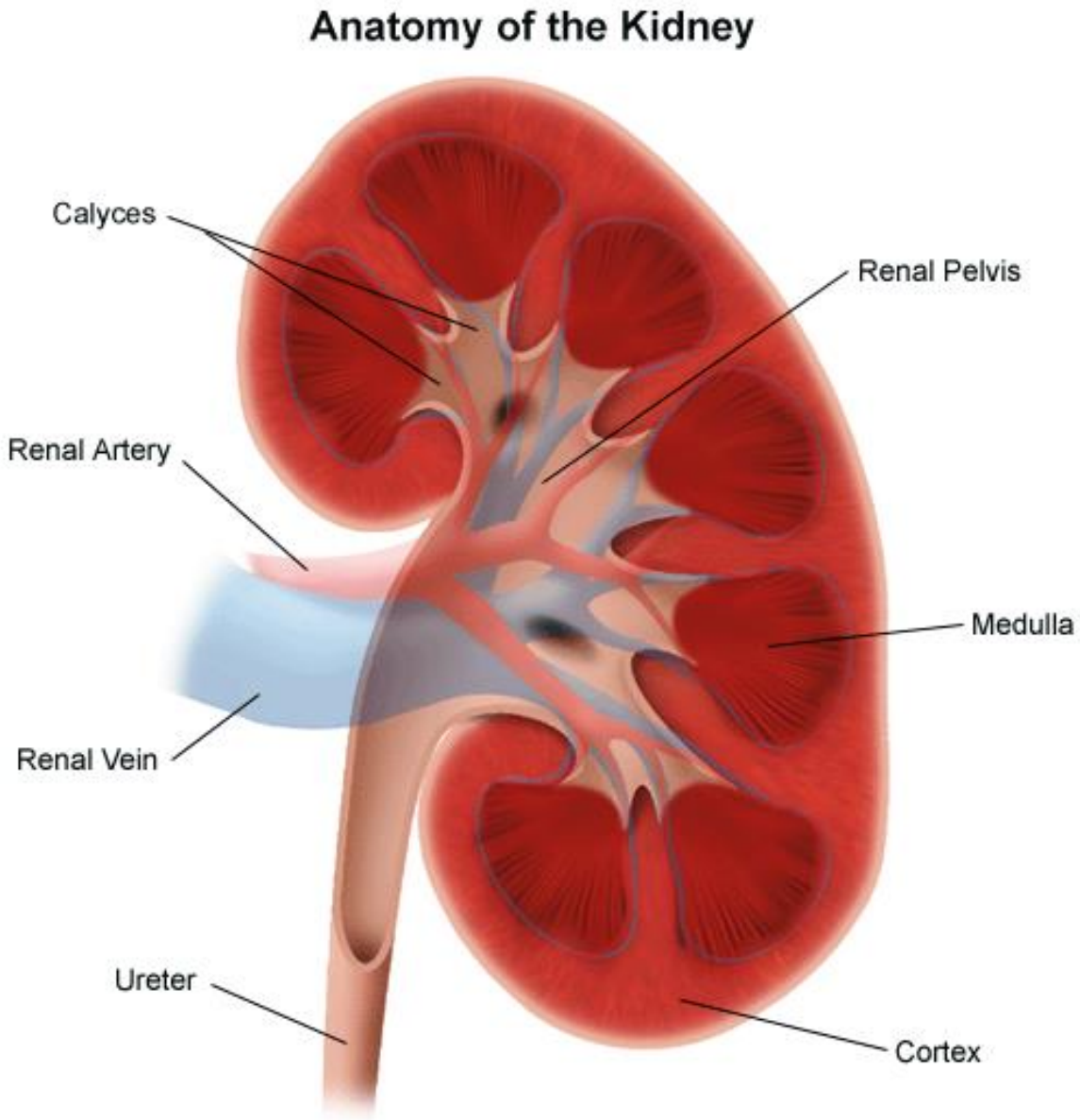




Courtesy of Anton Page

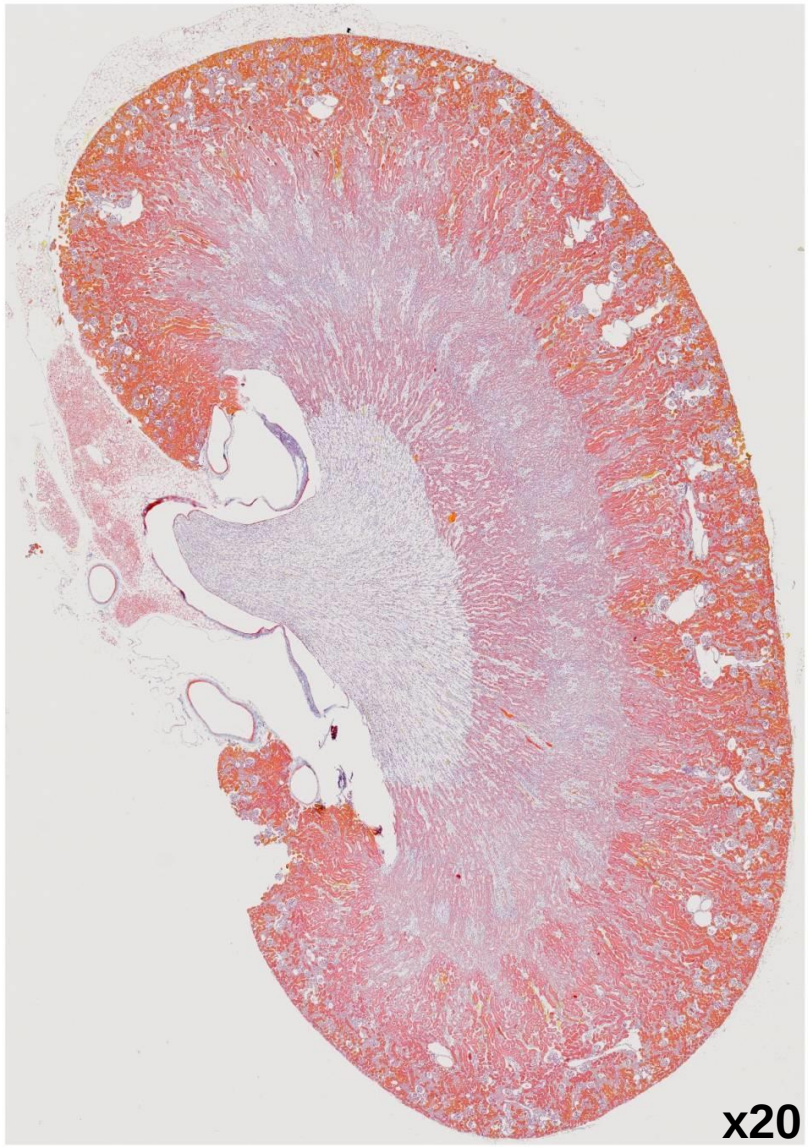
Freeze fractured rodent glomerulus

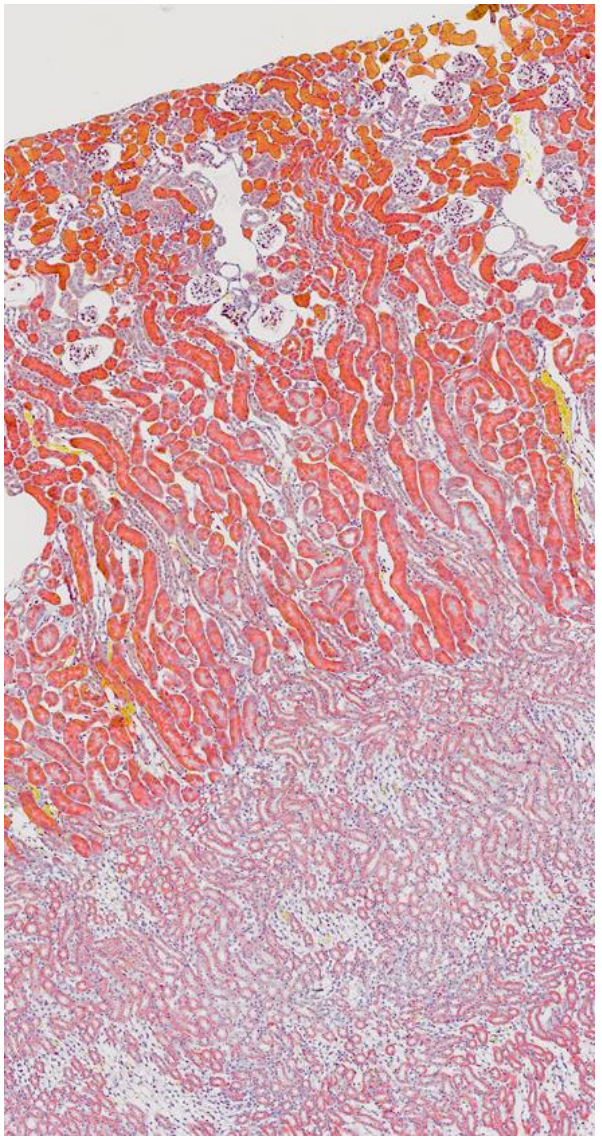
Anatomy of the Kidney



http://www.yalemedicalgroup.org/stw/Page.asp?PageID=STW028980

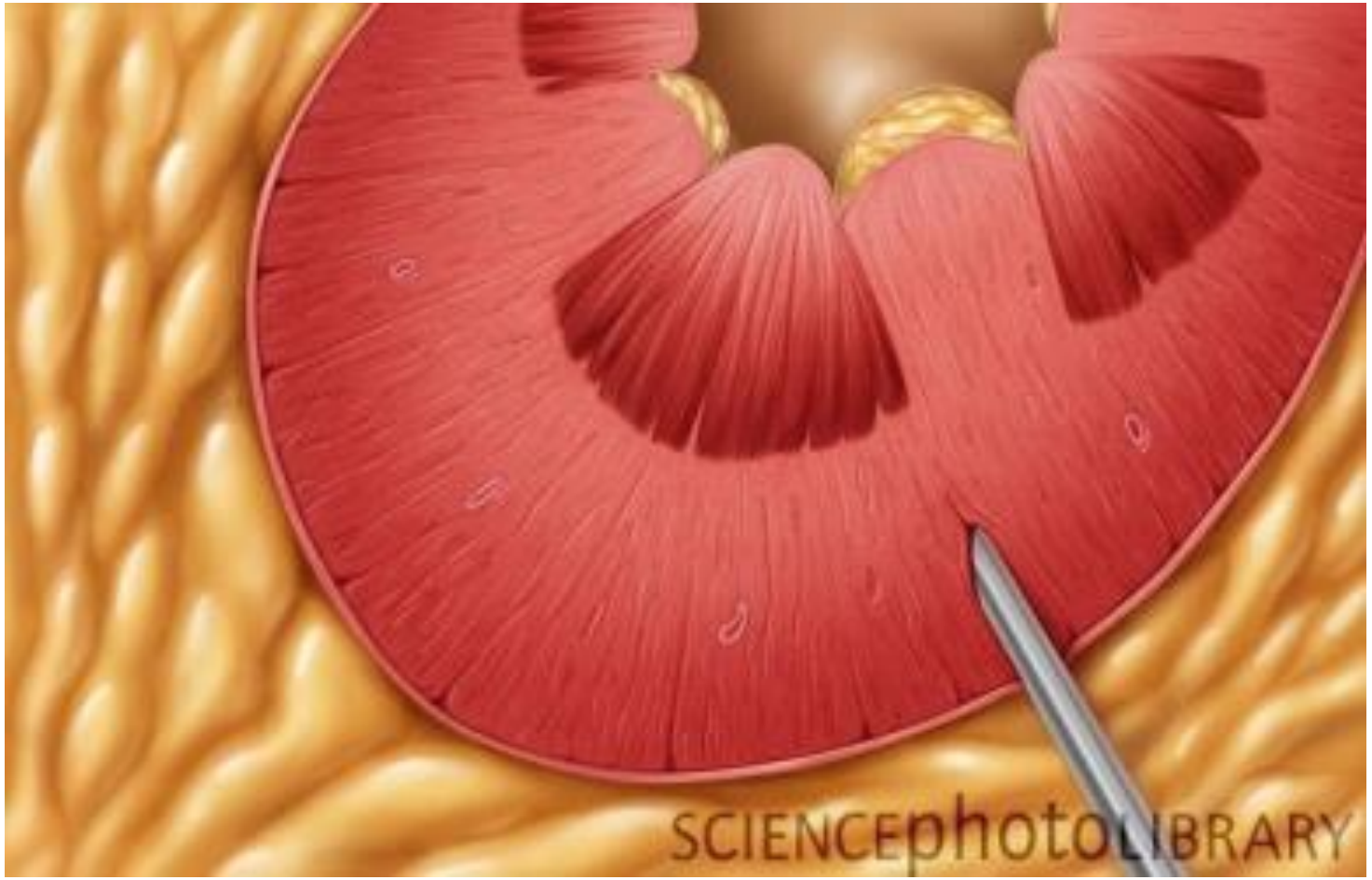
Anatomy of the Kidney



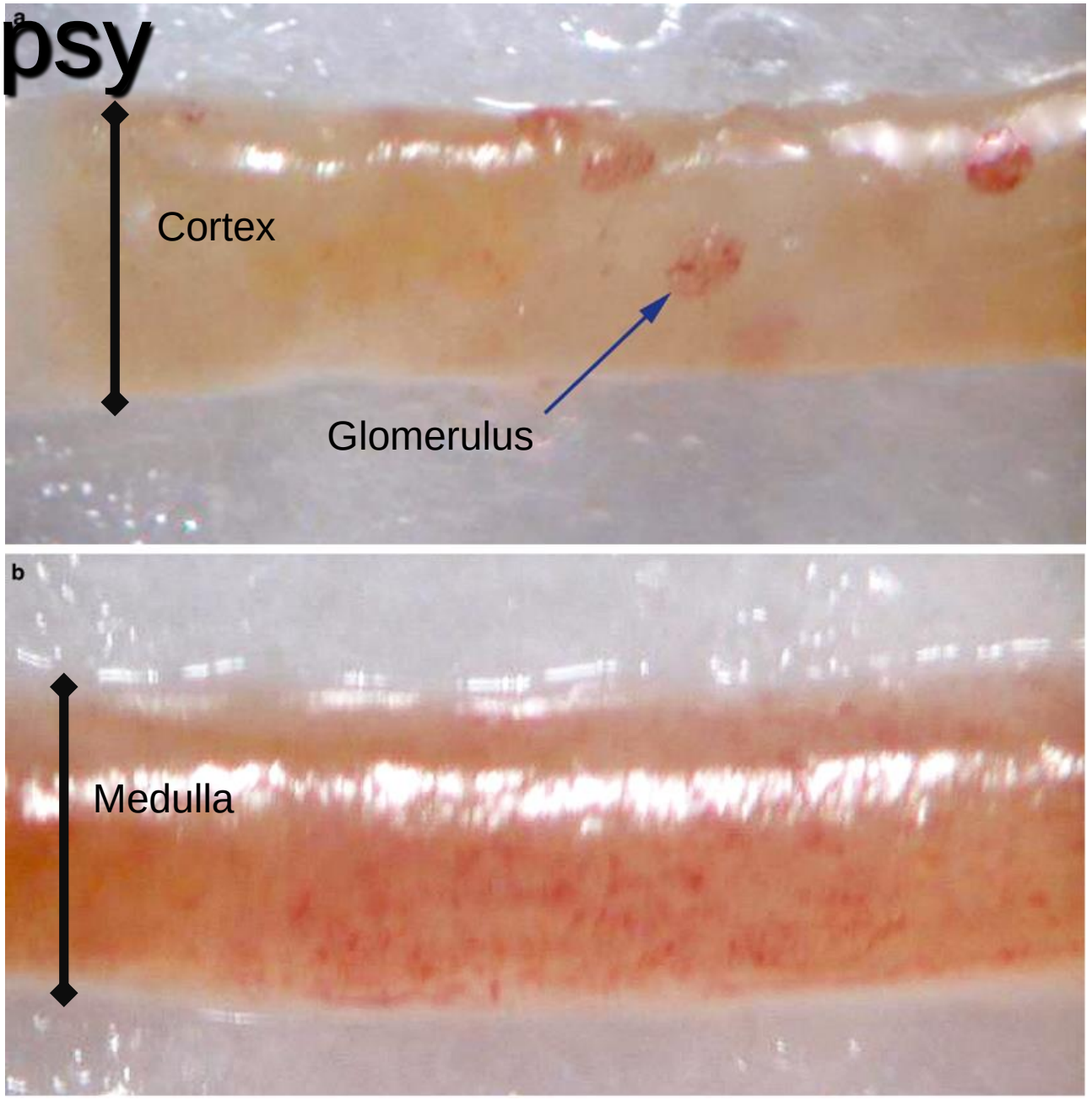


MSB mouse kidney courtesy of Matt Sharp

Needle Biopsy

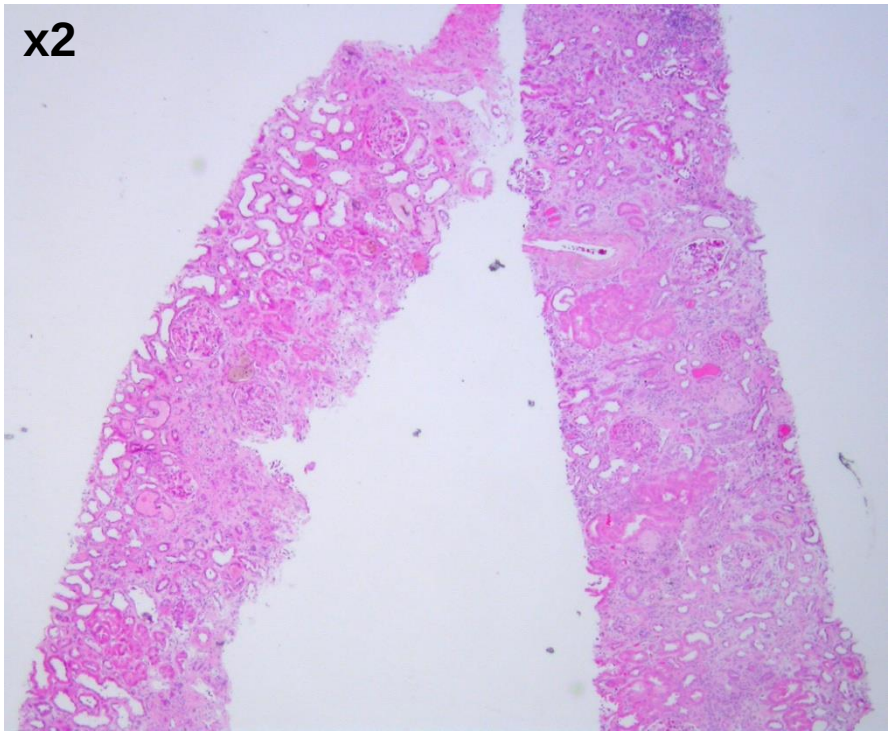


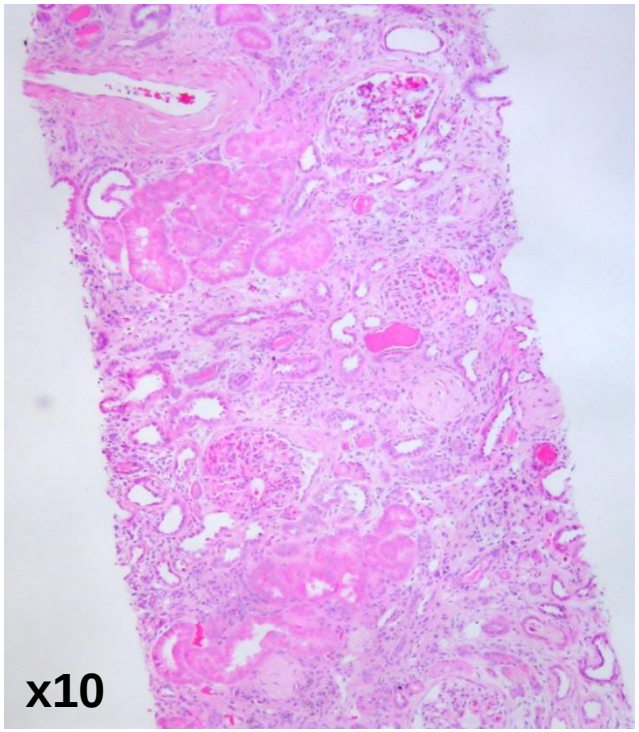
Needle Biopsy

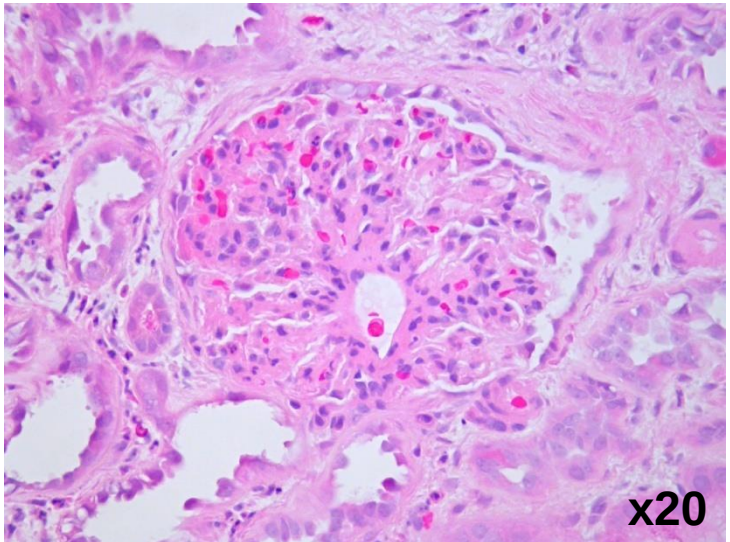


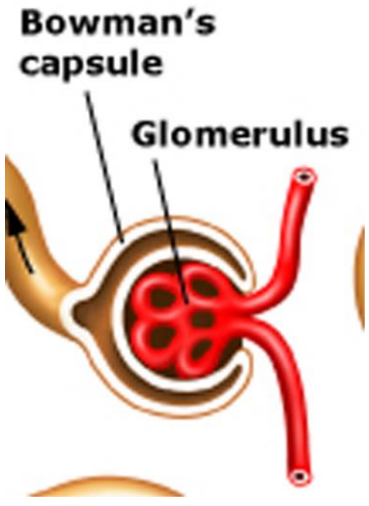
Modern Pathology (2004) 17, 1555–1563

H & E

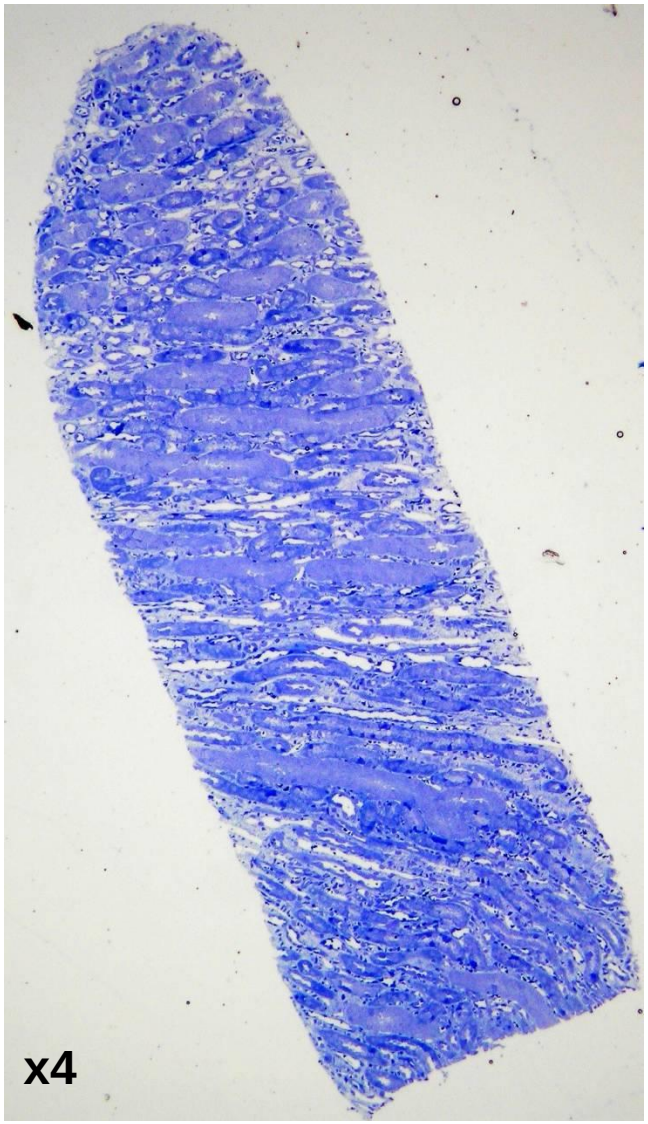


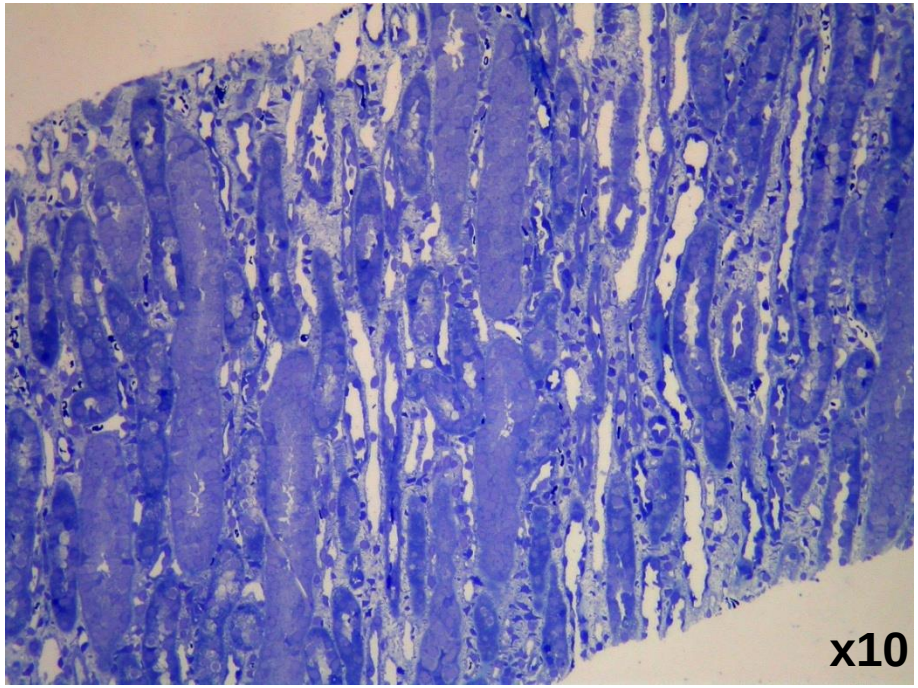




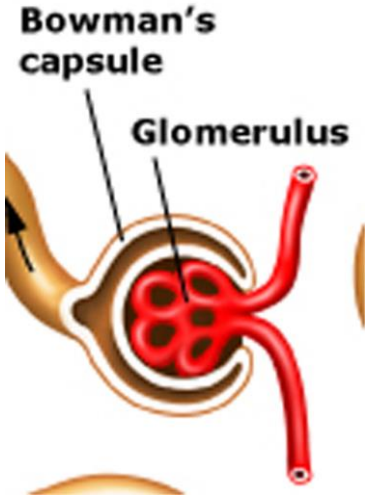


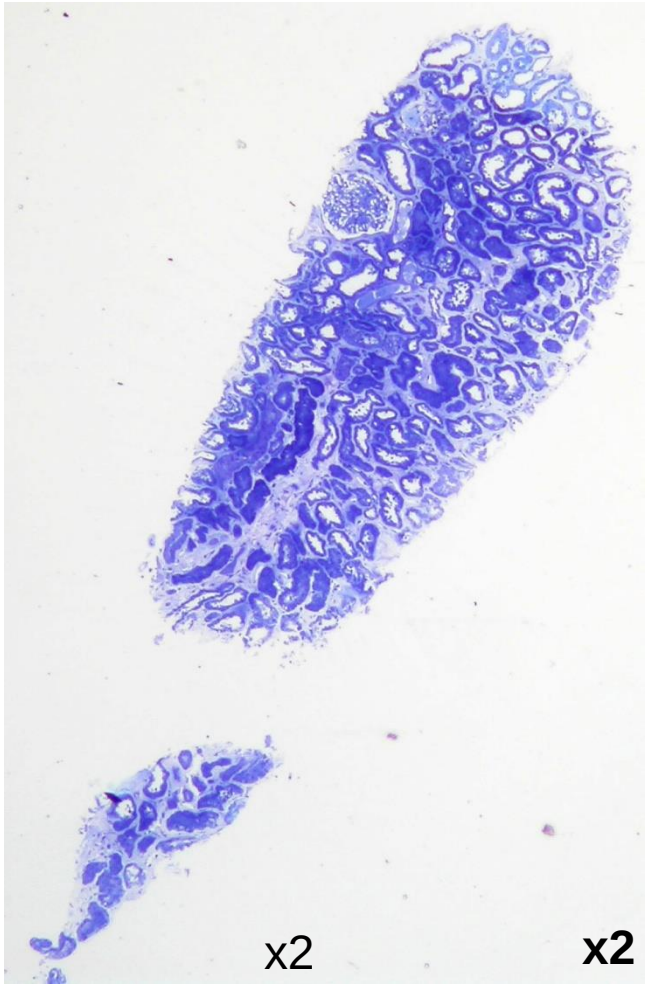
Top and Tail for EM

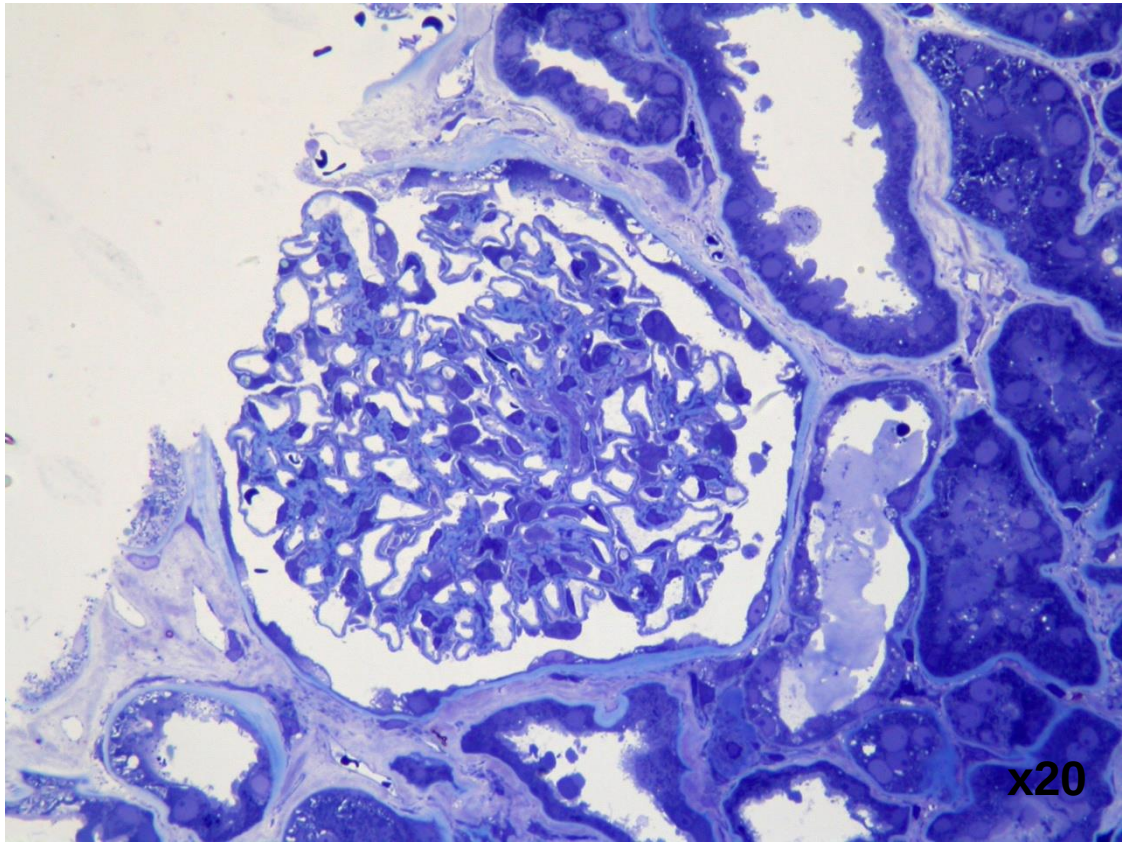


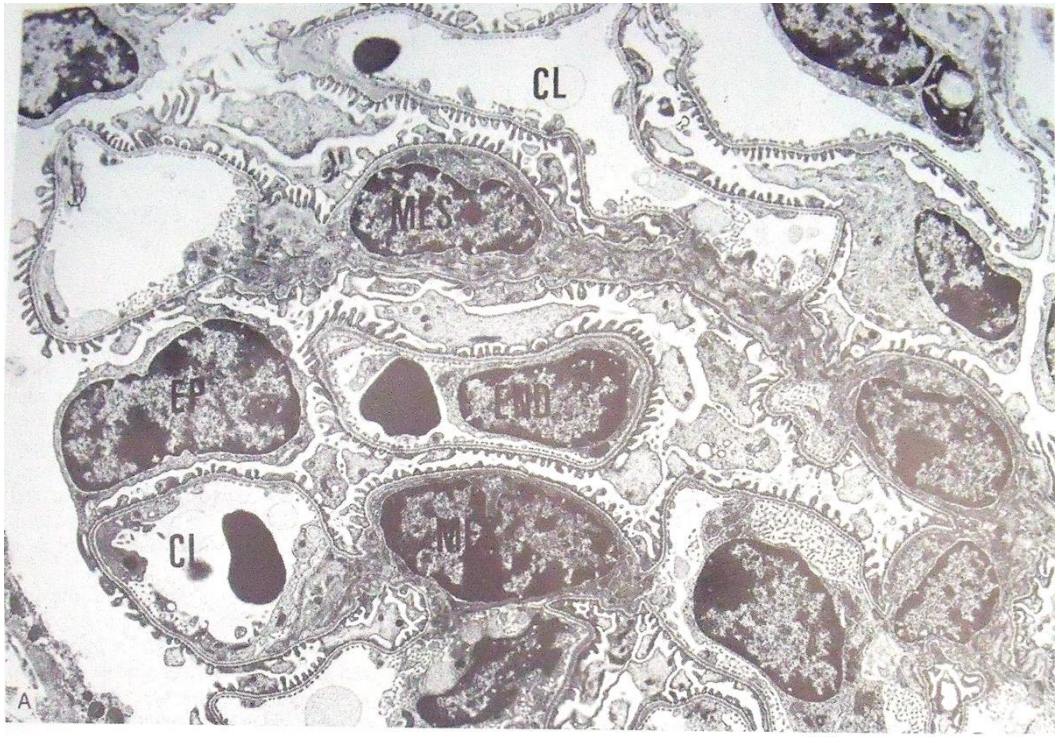


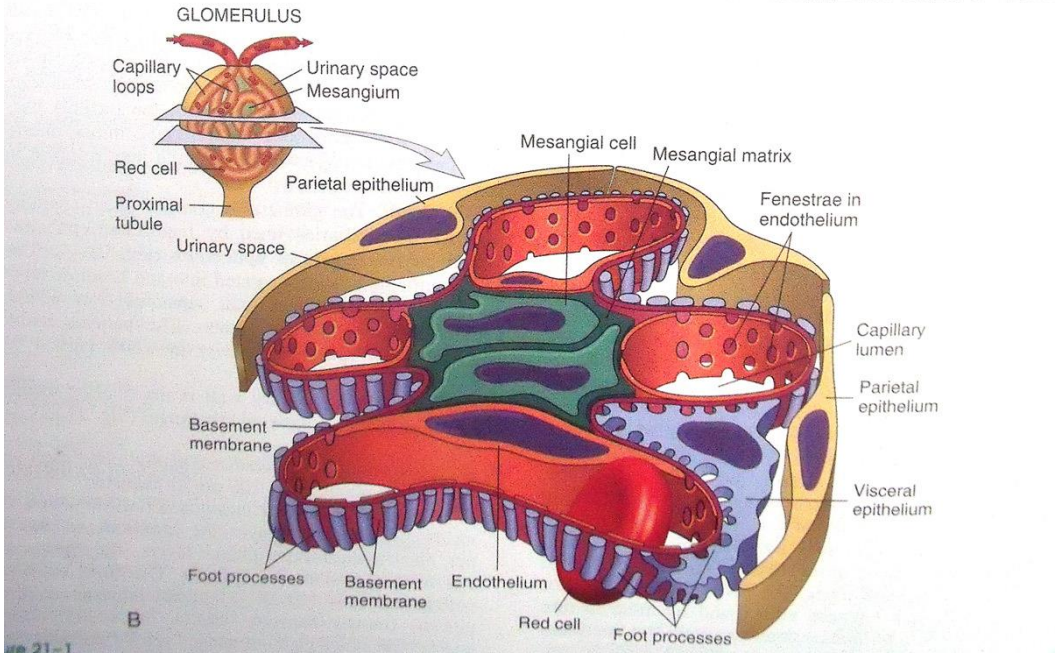
Top and Tail for EM

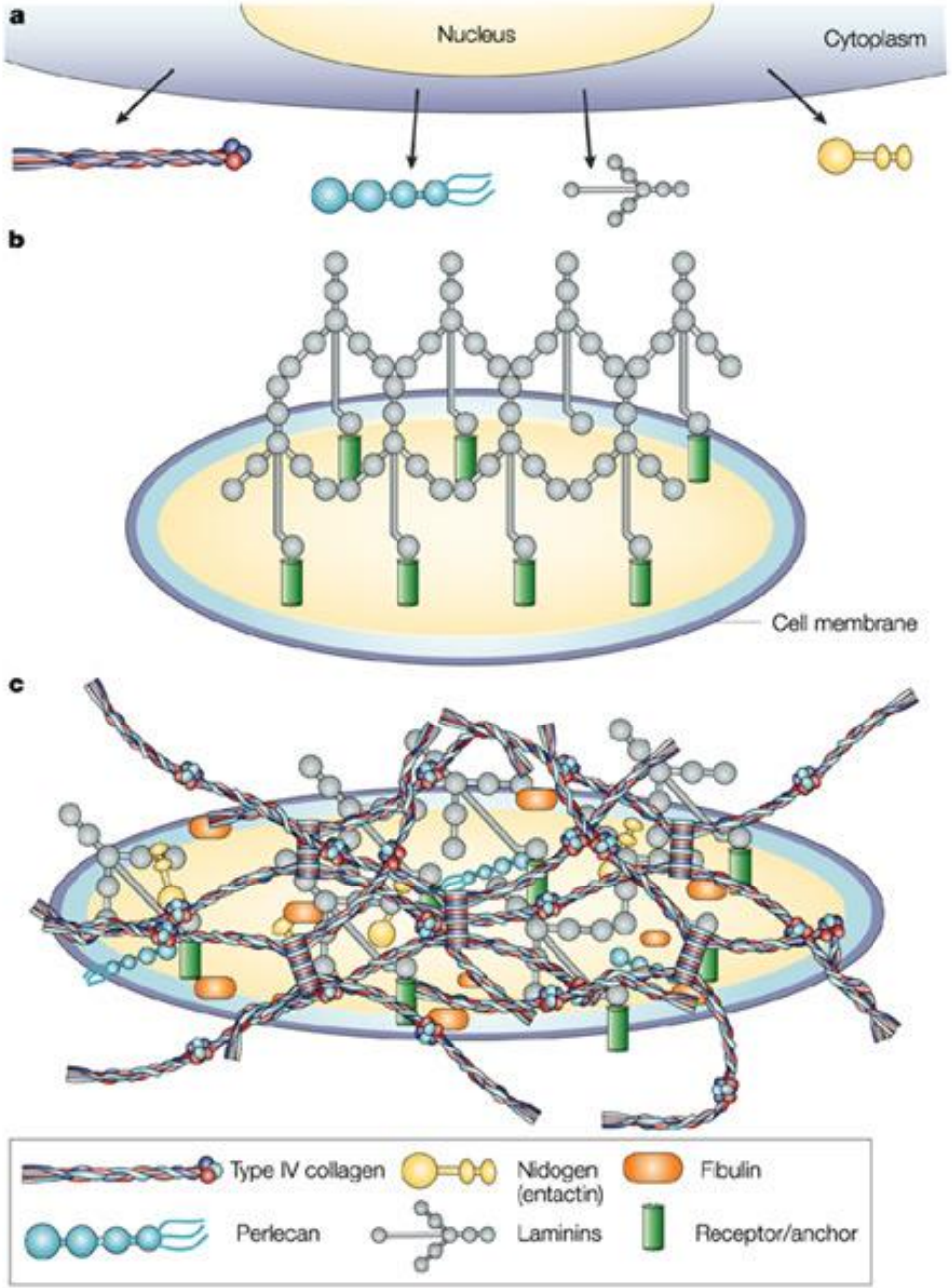




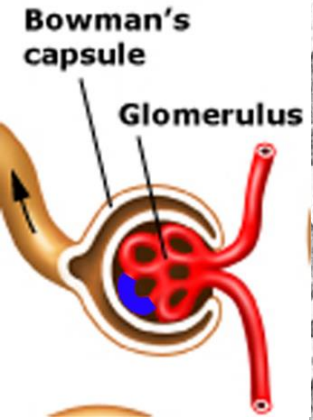


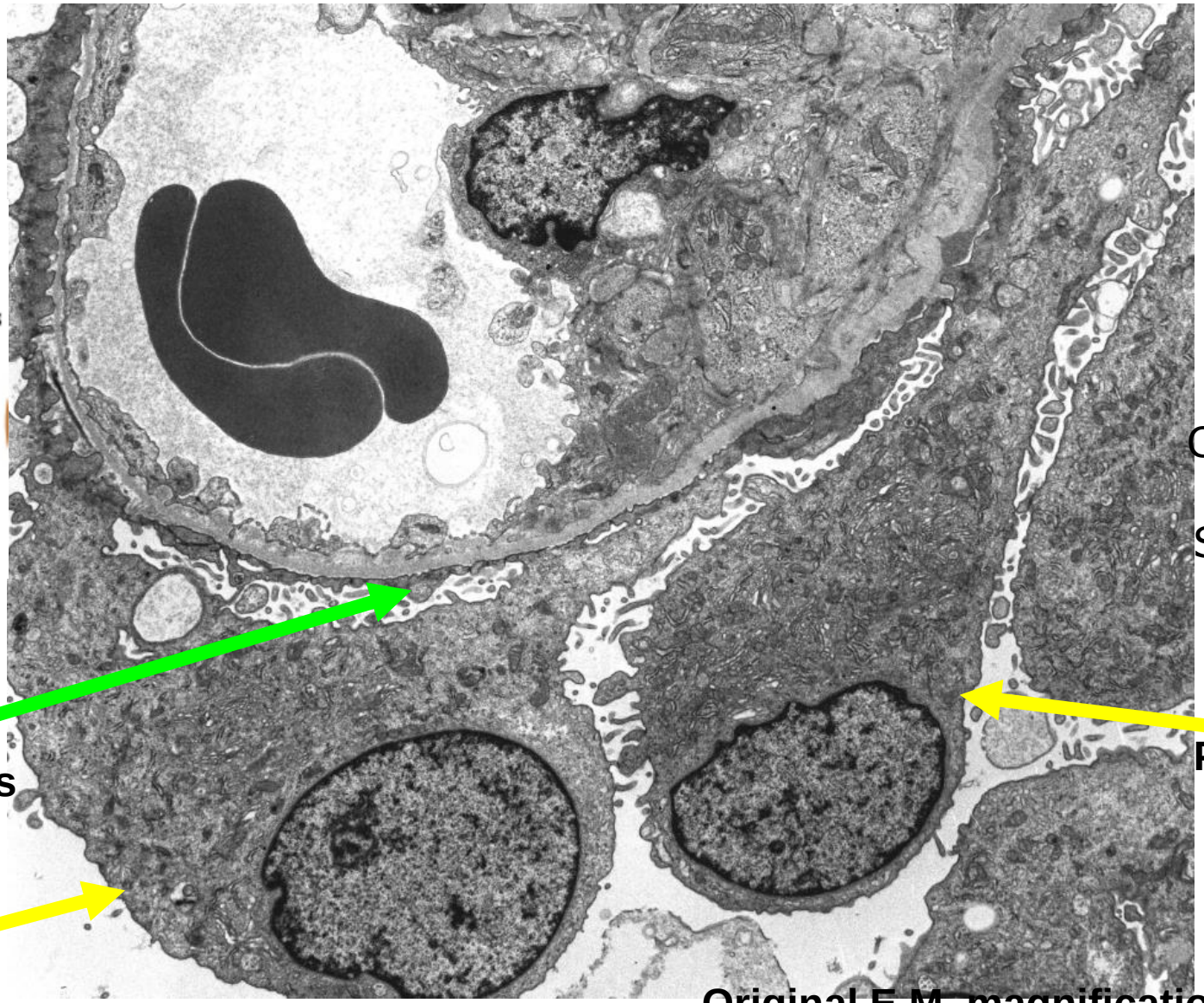

CL
MES
EP
END
CL
A


GLOMERULUS
Capillary loops
Urinary space
Mesangium
Red cell
Proximal tubule
Parietal epithelium
Urinary space
Mesangial cell
Mesangial matrix
Fenestrae in endothelium
Capillary lumen
Parietal epithelium
Basement membrane
Visceral epithelium
Foot processes
Basement membrane
Endothelium
Red cell
Foot processes
B


a
Nucleus
Cytoplasm
b
Cell membrane
c
Type IV collagen
Nidogen (entactin)
Fibulin
Perlecan
Laminins
Receptor/anchor

EM basement membrane





Courtesy of Sue Cox

Foot process

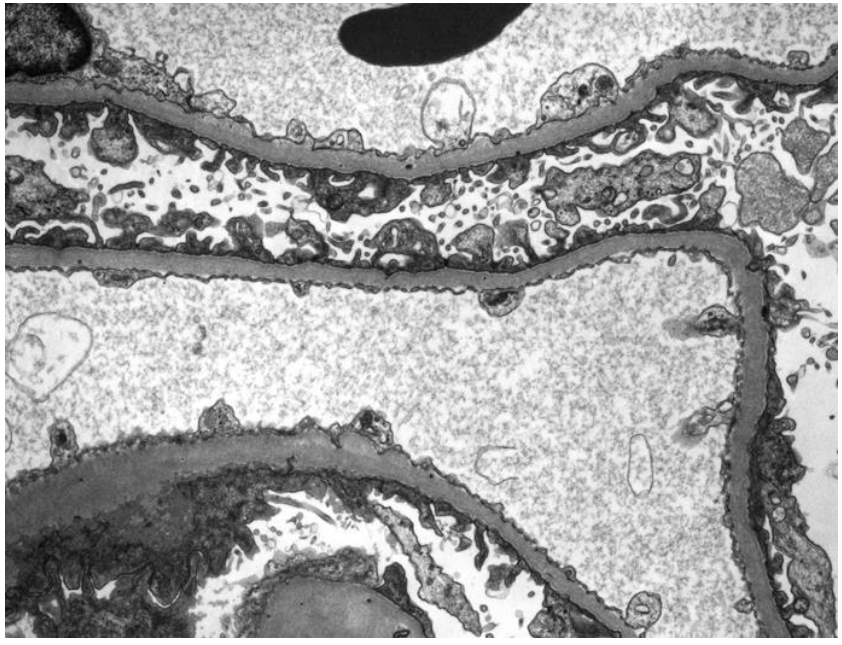
Podocyte

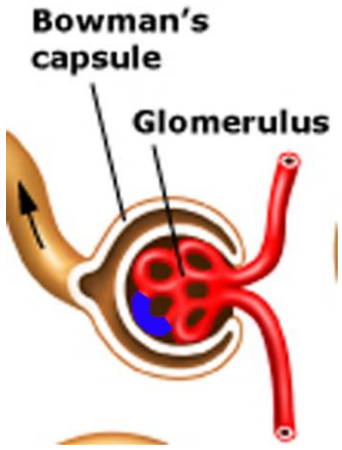
Podocyte

Original E.M. magnification x3,300

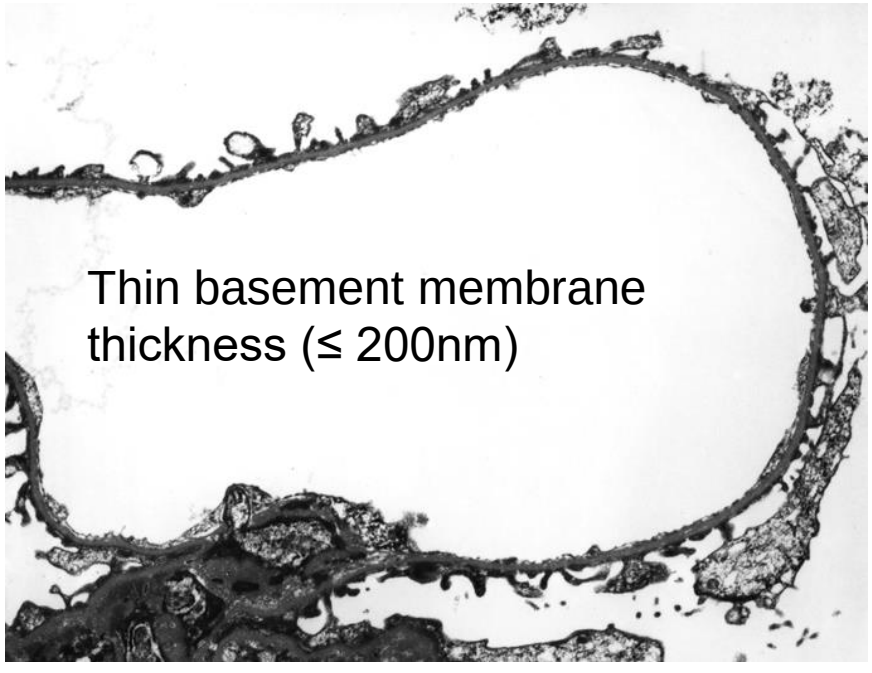
Basement Membrane

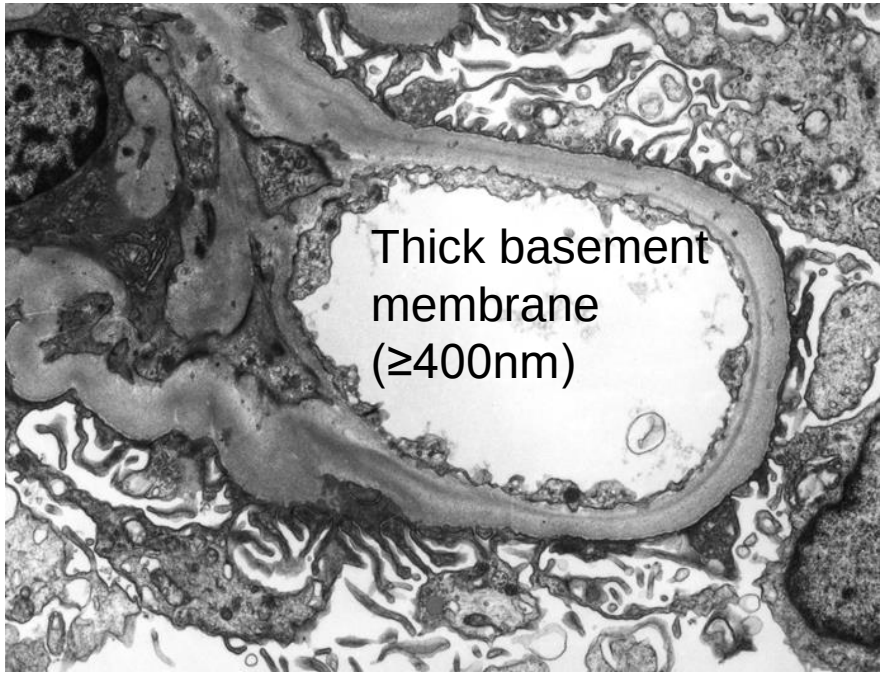
Courtesy of
Sue Cox



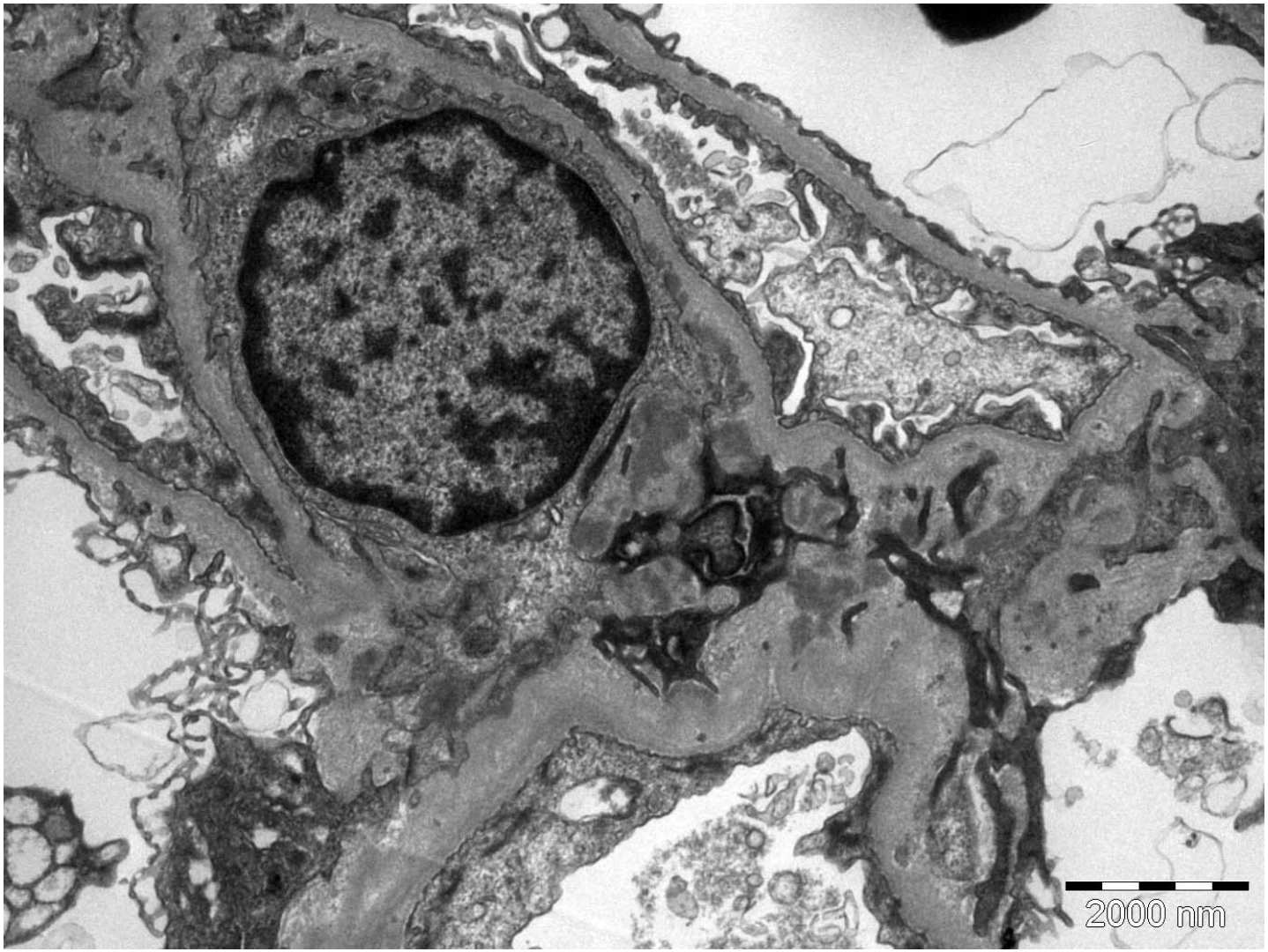


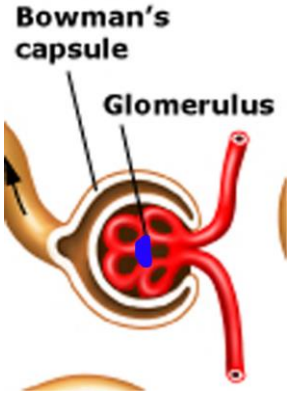
Normal basement membrane thickness (250-350nm)
x 5,000



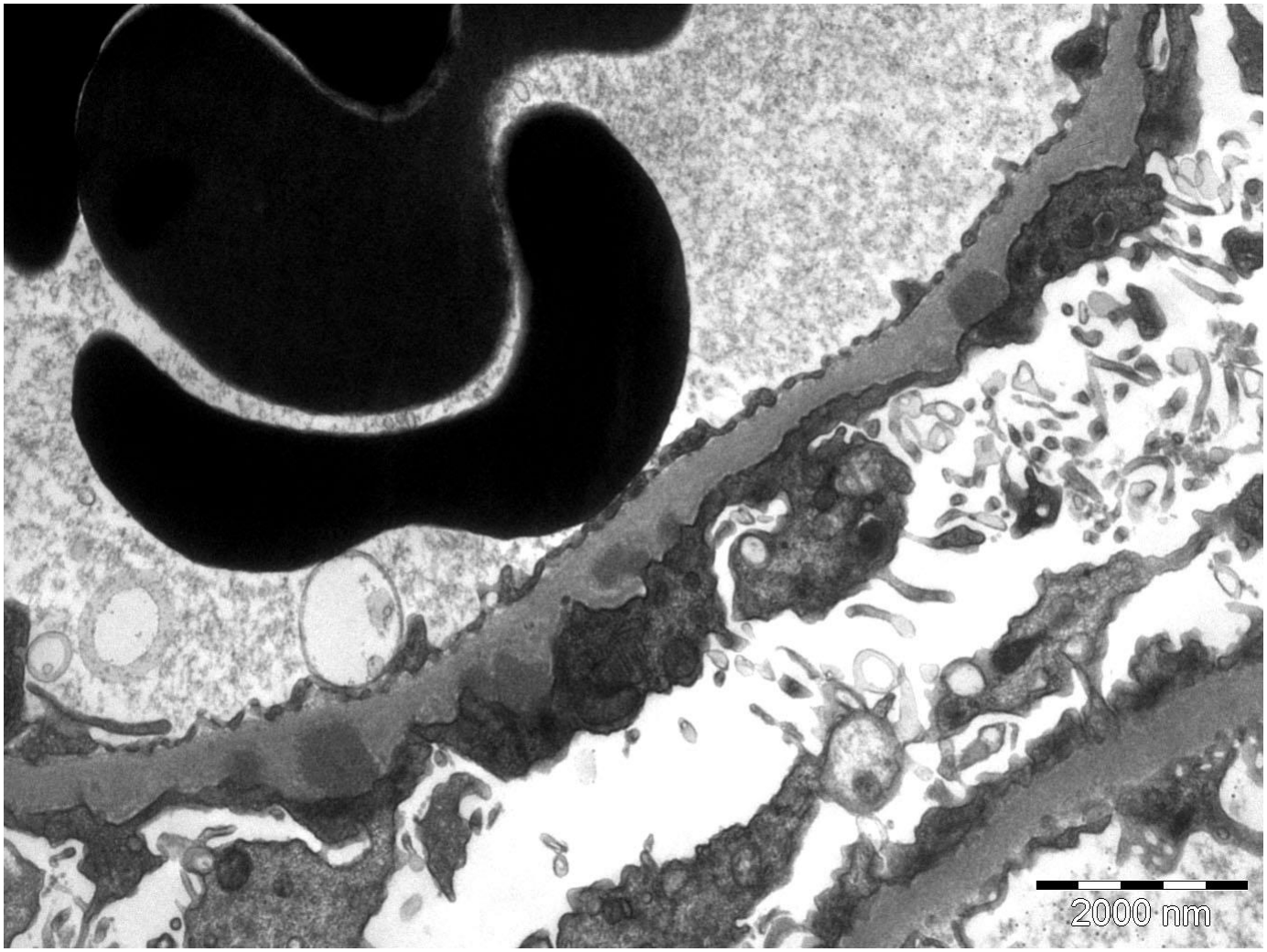


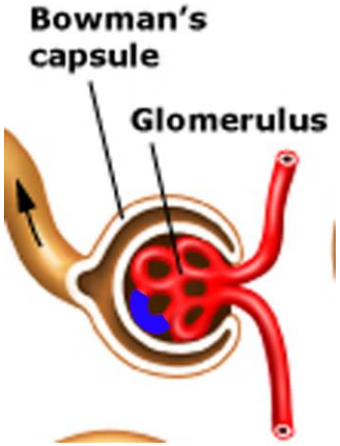
Electron Dense Deposits





Electron Dense Deposits

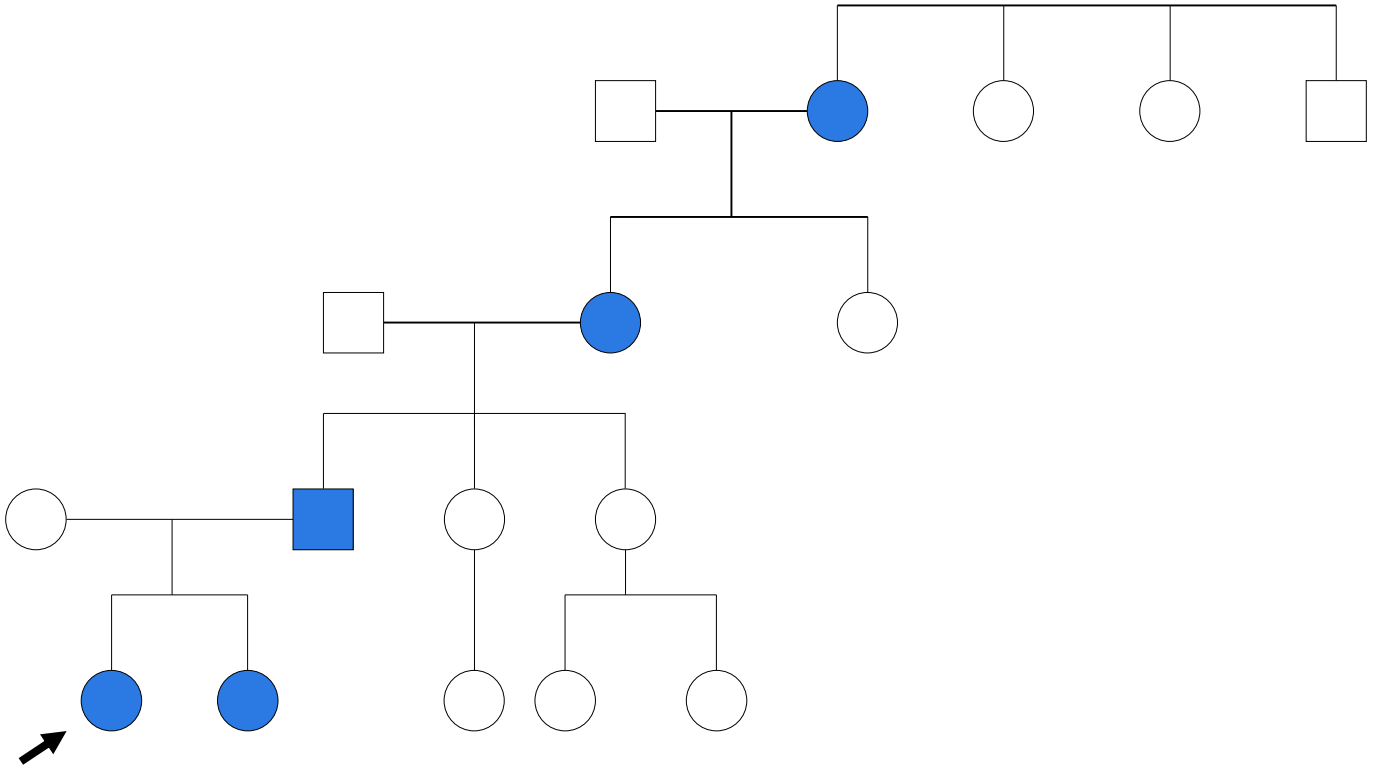




Inside Science ‘Show Us Your Instrument’.

- **From:** Fowler, Darren
  Sent: 19 July 2013 10:54
  To: A.Page@soton.ac.uk; Angus, Elizabeth; Goggin, Patricia; 'Johnston D.A.'; Sharp, Matthew
  Subject: Inside Science
- Dear All, while I was taking the long road (M3) home last night I heard this fascinating piece on R4 "‘Show Us Your Instrument’ all about TEM (live from the basement of UCL). would be interested to hear what you think of it? happy listening. Darren
- http://www.bbc.co.uk/programmes/b036w39v
- the first in our new series ‘Show Us Your Instrument’. Material scientist Mark Miodownik introduces the wonders of the Transmission Electron Microscope,
- http://www.bbc.co.uk/programmes/b036f7w2/episodes/guide

6 year old girl referred for microscopic haematuria



? AD FSGS

- *TRPC6*, *ACTN4* and *INF2*: No abnormalities.

- Exome sequencing: *COL4A5* Gly325Arg
- *NPHS1* (p.N188I, rs145125791) in younger sister
- Father and grandmother's biopsies traced.

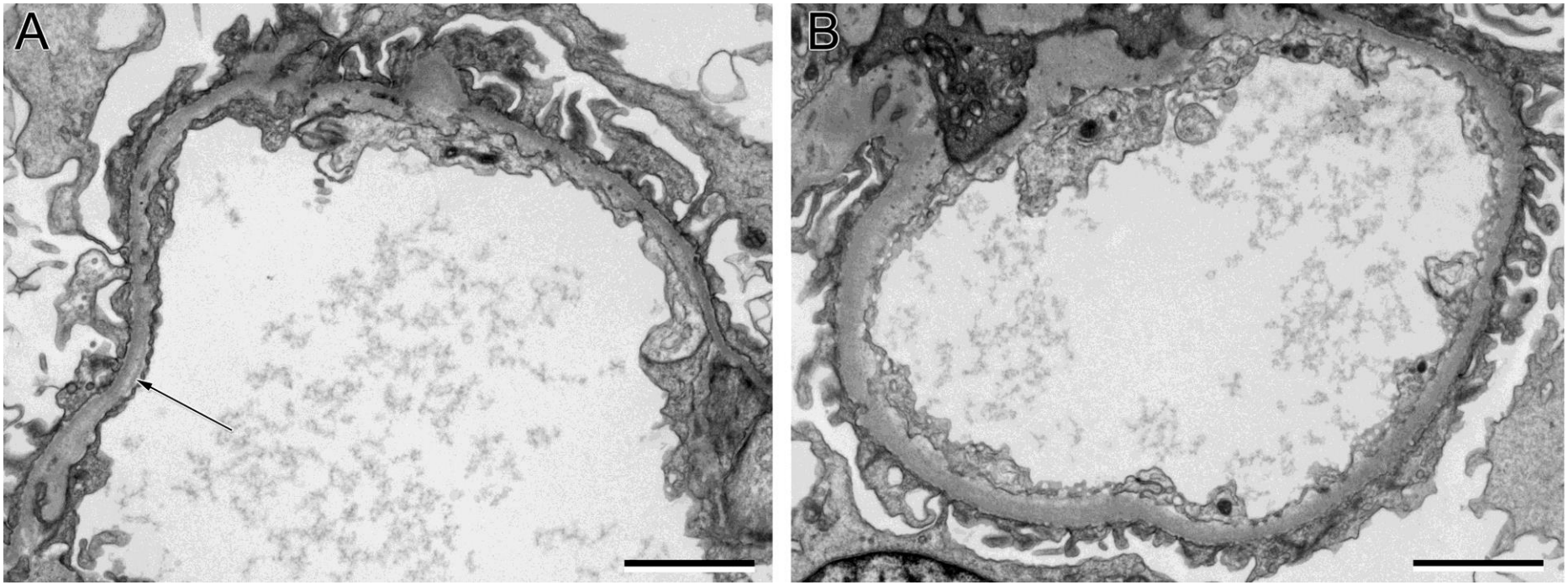
Archived pathology material

Grandmother's renal biopsy diagnosed as FSGS in 1998 (aged 57) thin membrane disease raised as possibility (EM 2013: multiple measurements showed GBM thickness within normal limits.

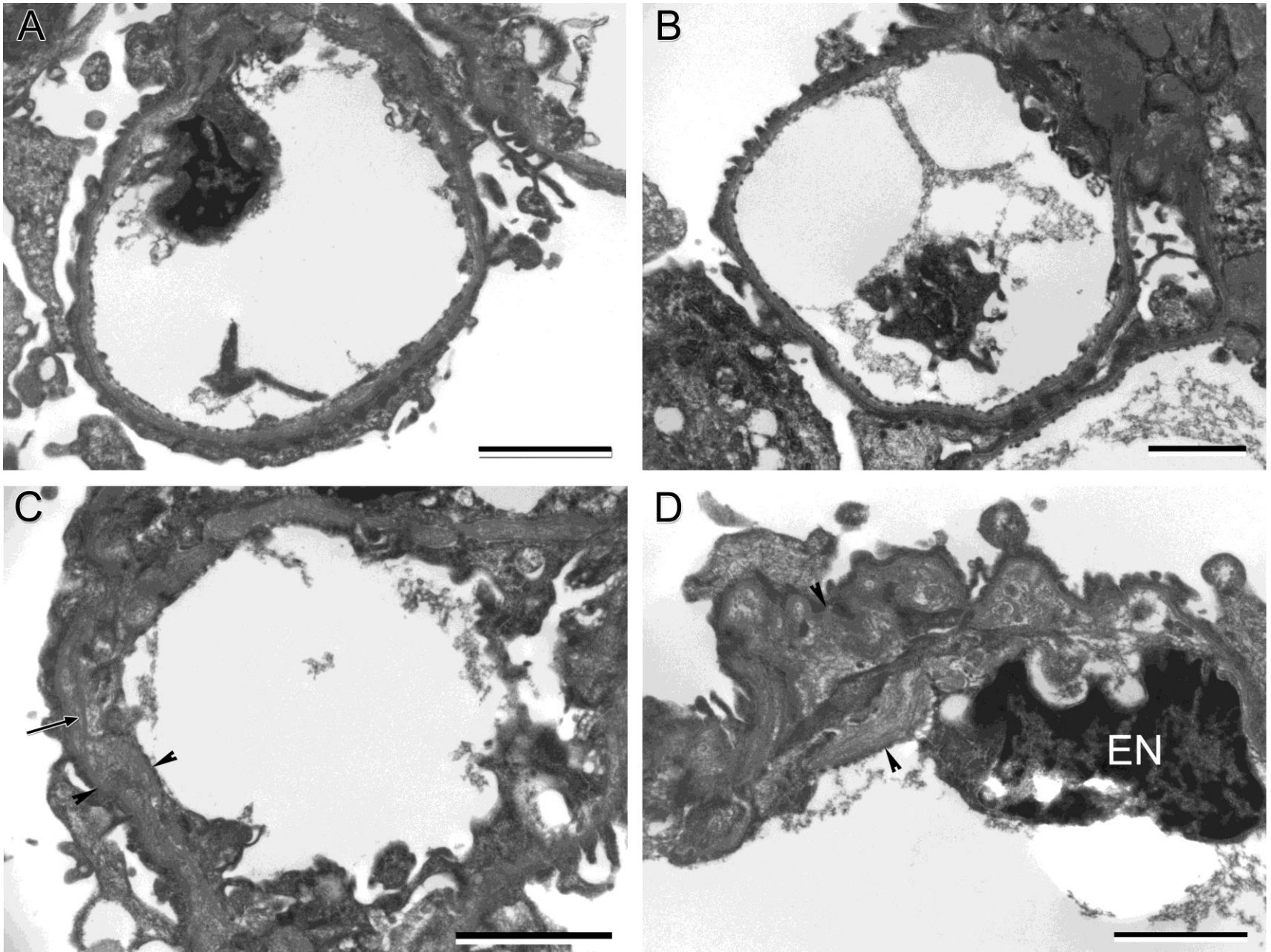
[Normal GBM thickness range (SGH resin)=250-350nm "thick"=400nm plus]

Father's renal biopsy diagnosed as FSGS in 1997 (age 42)

EM grandmother (I-1)



EM father (II-2).



Glomerulonephritis (GN) patterns

- “Primary”
- (minimal change disease)
- Mesangial proliferative GN
- Focal segmental glomerulosclerosis
- Membranous GN
- Post infectious GN
- Crescentic GN (=rapidly progressive GN)
- Membranoproliferative GN

- “Secondary” lupus diabetes, amyloid, light chain disease, cryoglobulinemia,

Nephrotic vs nephritic

- Nephrotic
- minimal change disease, focal and segmental, membranous
- Nephritic
- Post-infectious (eg mesangial proliferative (eg. IgA disease/HSP), post-Streptococcal), membranoproliferative)

2000 nm

Happy Easter

fun facts

- Human kidney filters 1700 litres blood daily resulting in 1 litre urine / day
- Both kidneys account for 0.5% body weight but receive 25% of cardiac output
- The cortex is richly vascularised receiving 90% of total renal circulation

Reporting a renal biopsy

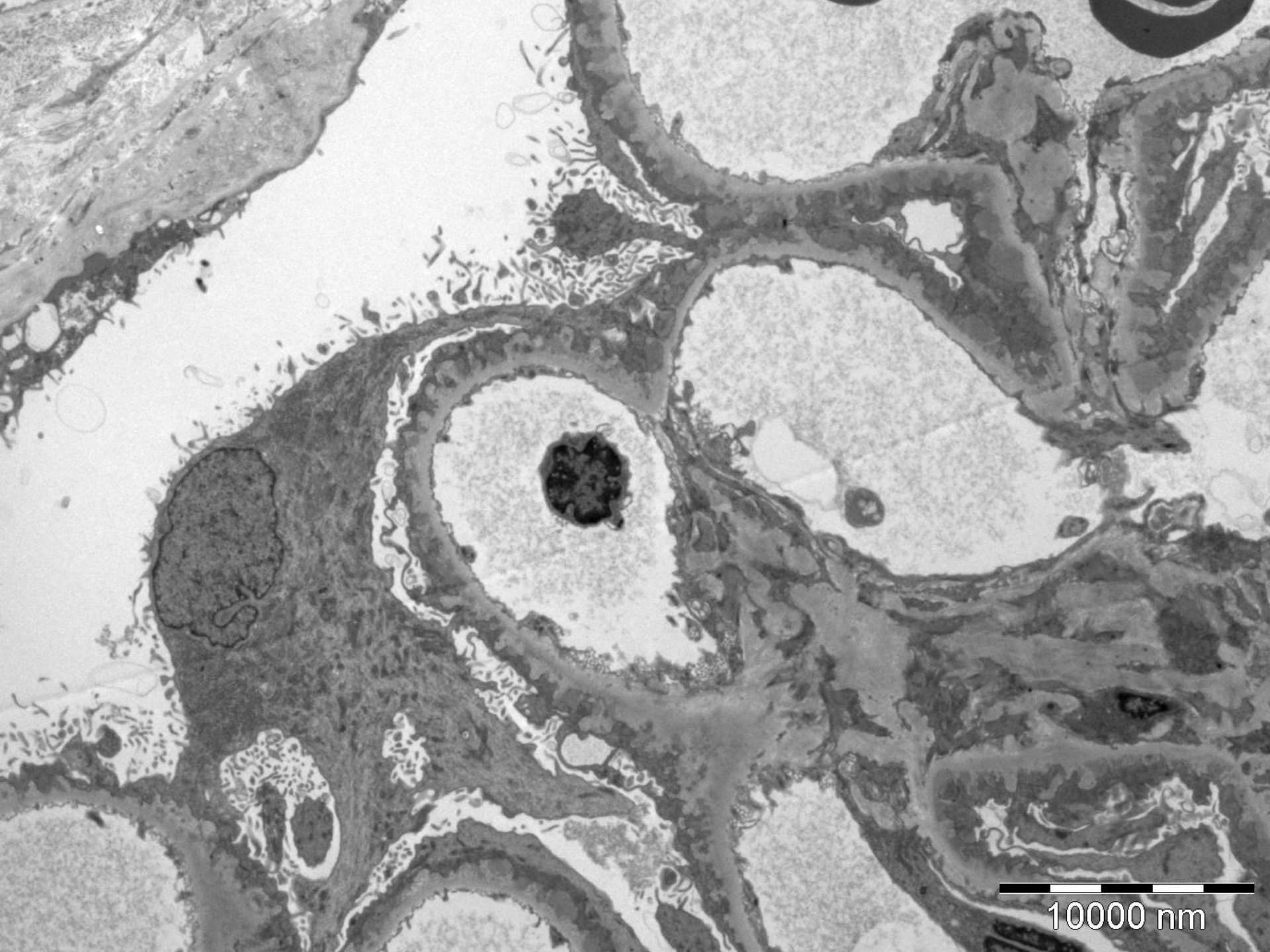
- Light microscopy:
- Glomeruli
- consider architecture, is there chronic change (interstitial fibrosis / tubular atrophy) count glomeruli, look for glomerular abnormality (reduplication, spikes, crescents)
- Tubulointerstitium
- Vessels
- Immunohistochemistry
- Electron microscopy
- Discussion
- Conclusion
- Multidisciplinary meeting, more discussion, clinicopathological correlation, supplementary report

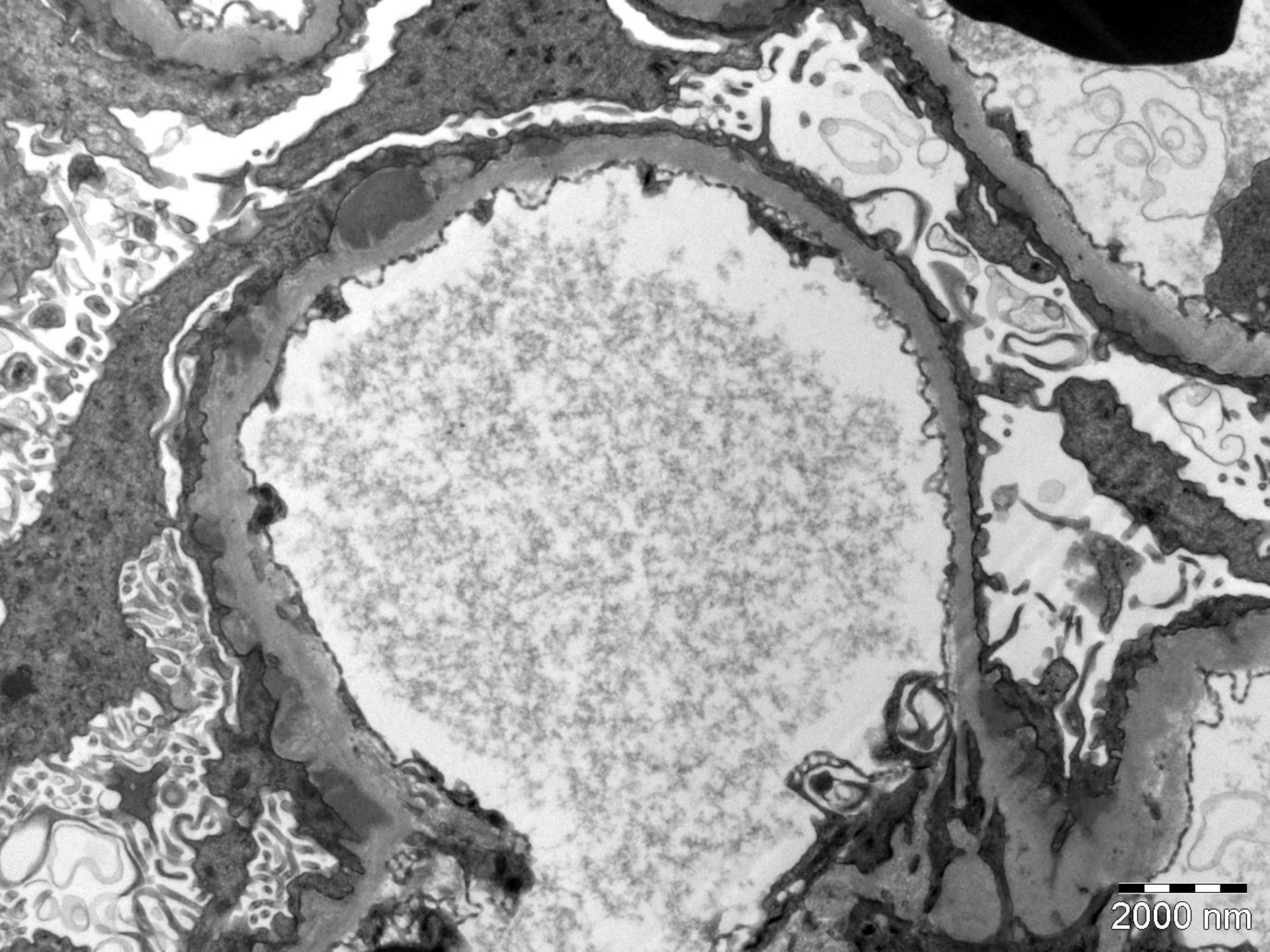
Clinical cases

- IgA / HSP
- Post infectious
- MPGN type 2 (DDD)
- Membranous GN
- BK nephropathy in transplant

Clinical stories

- 73-year old man
- New-onset nephrotic syndrome. Hypercholesterolemia. Diet-controlled diabetic. Immunology screen negative.
- Microscopy: diffuse thickening of capillary walls but no proliferation.
- Immunohistochem: There is diffuse global granular capillary wall positivity for IgG, C3 and C1q. IgA and IgM show no specific positivity.


10000 nm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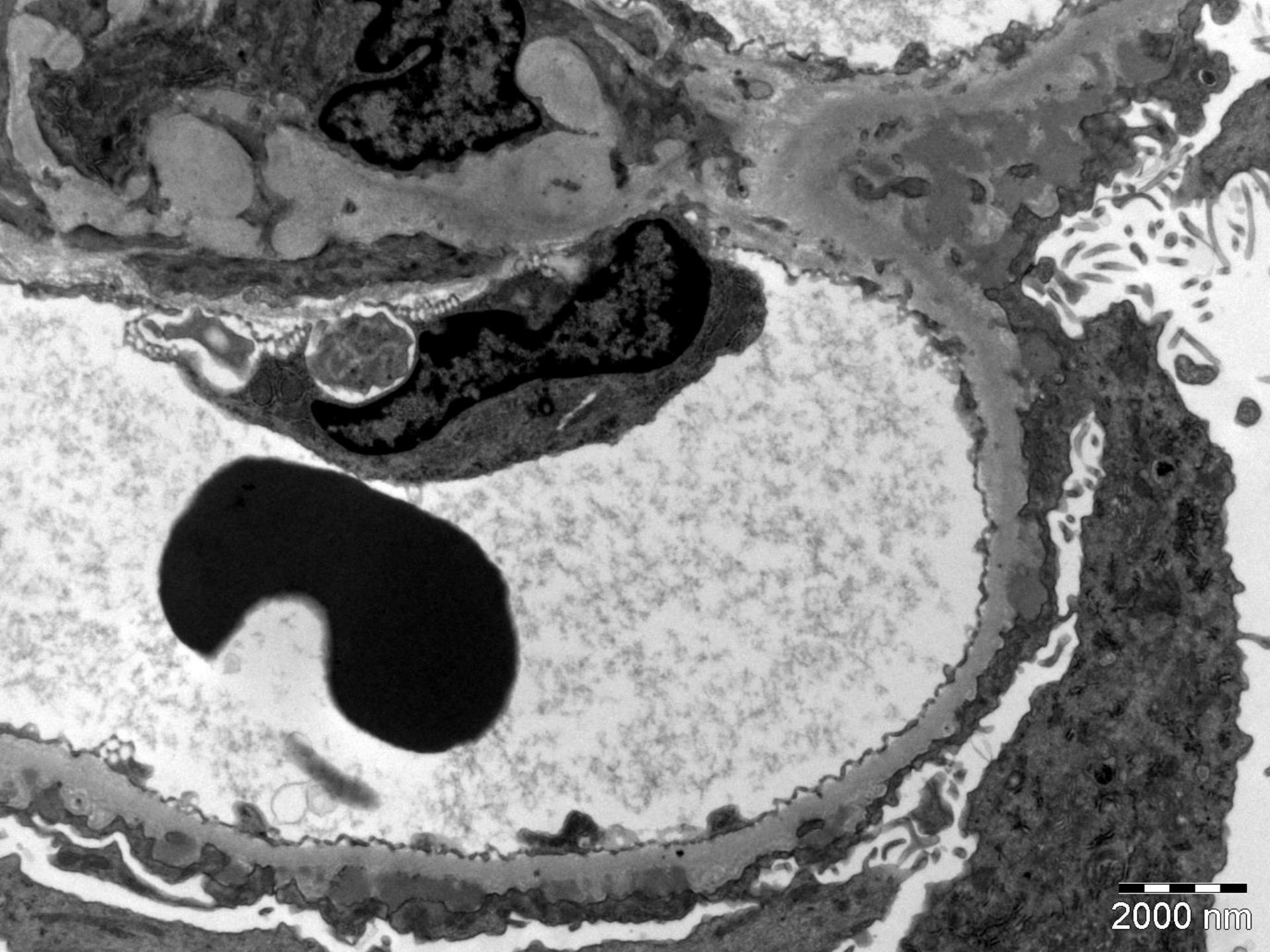
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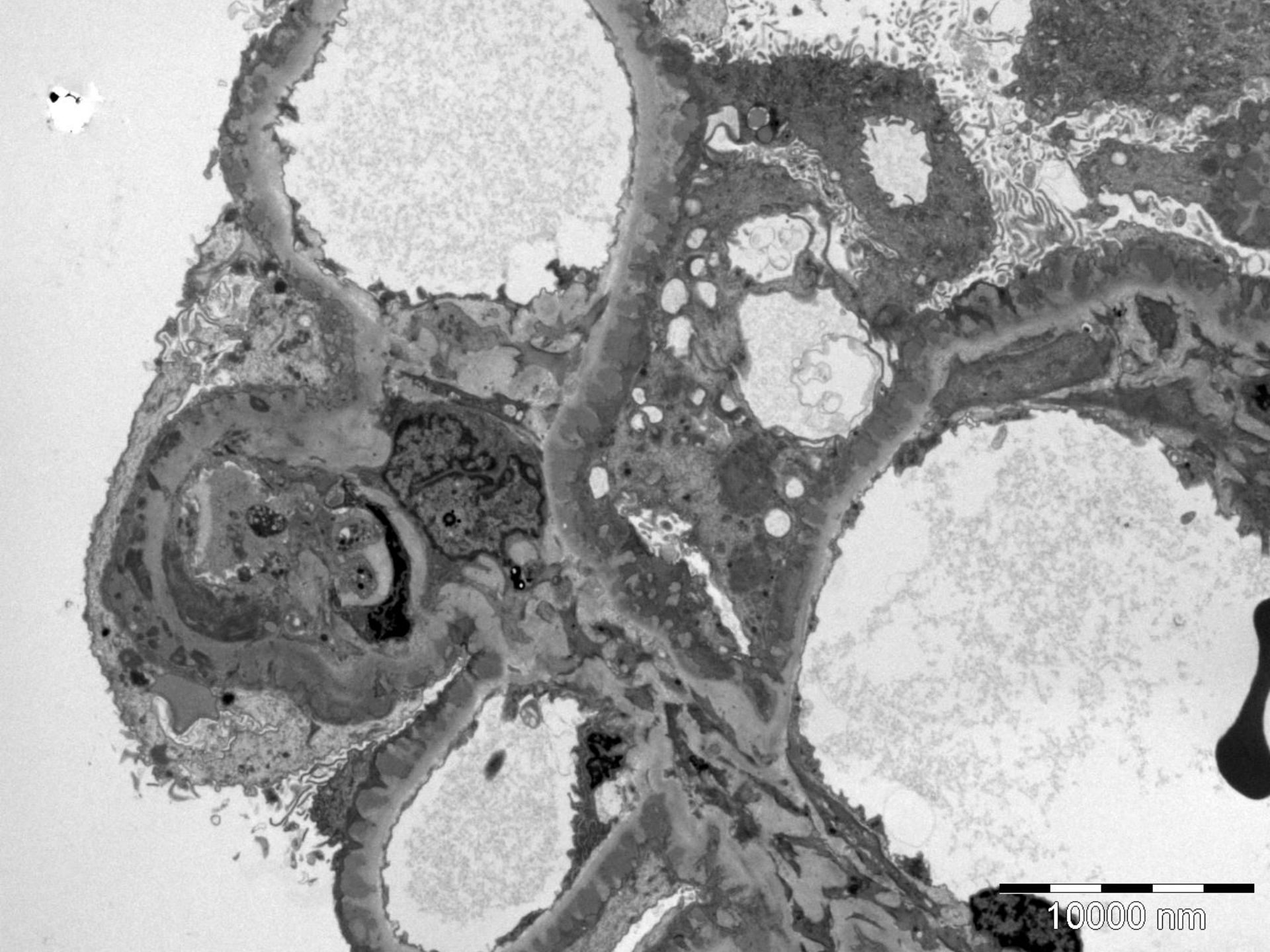
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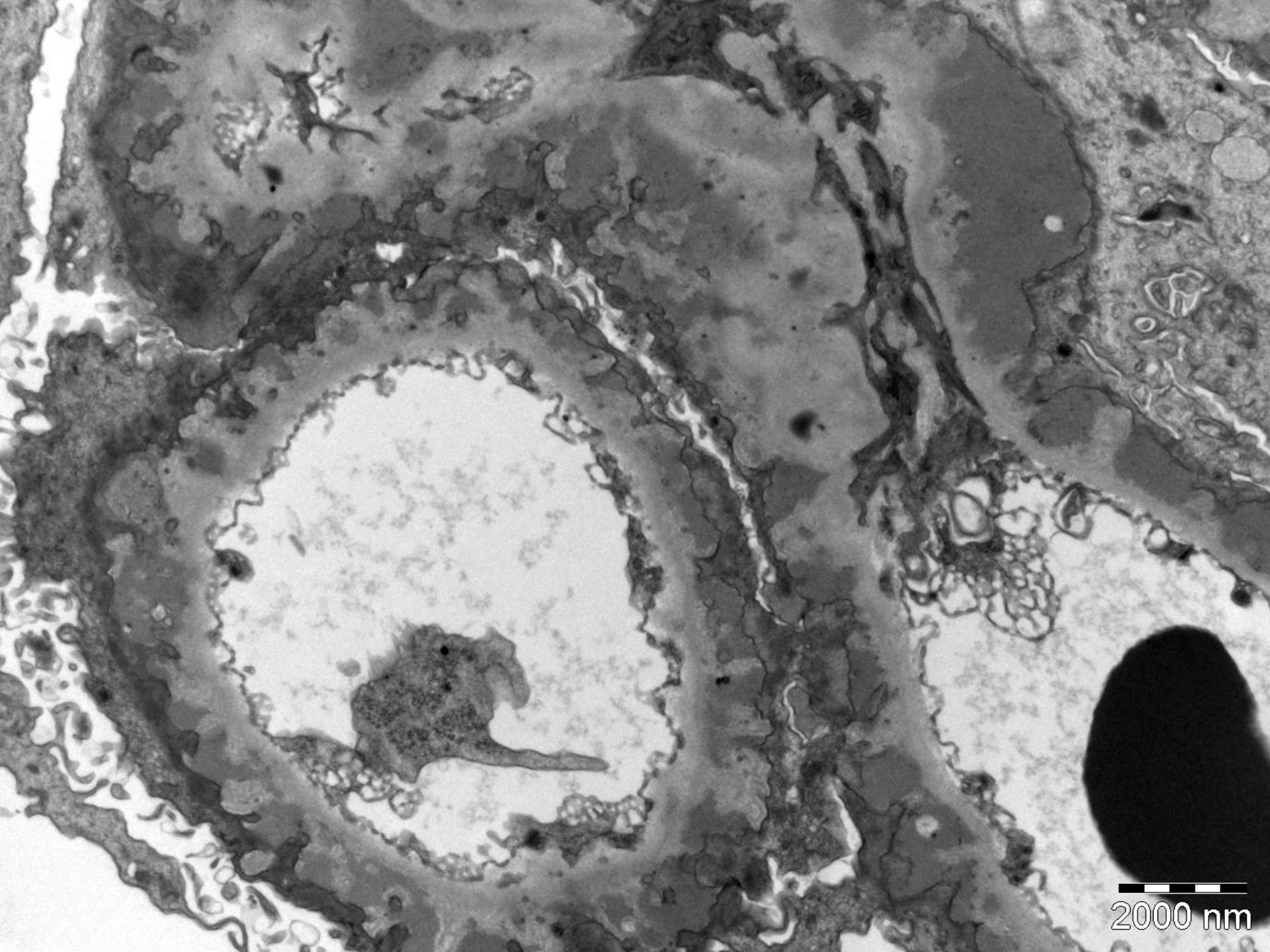
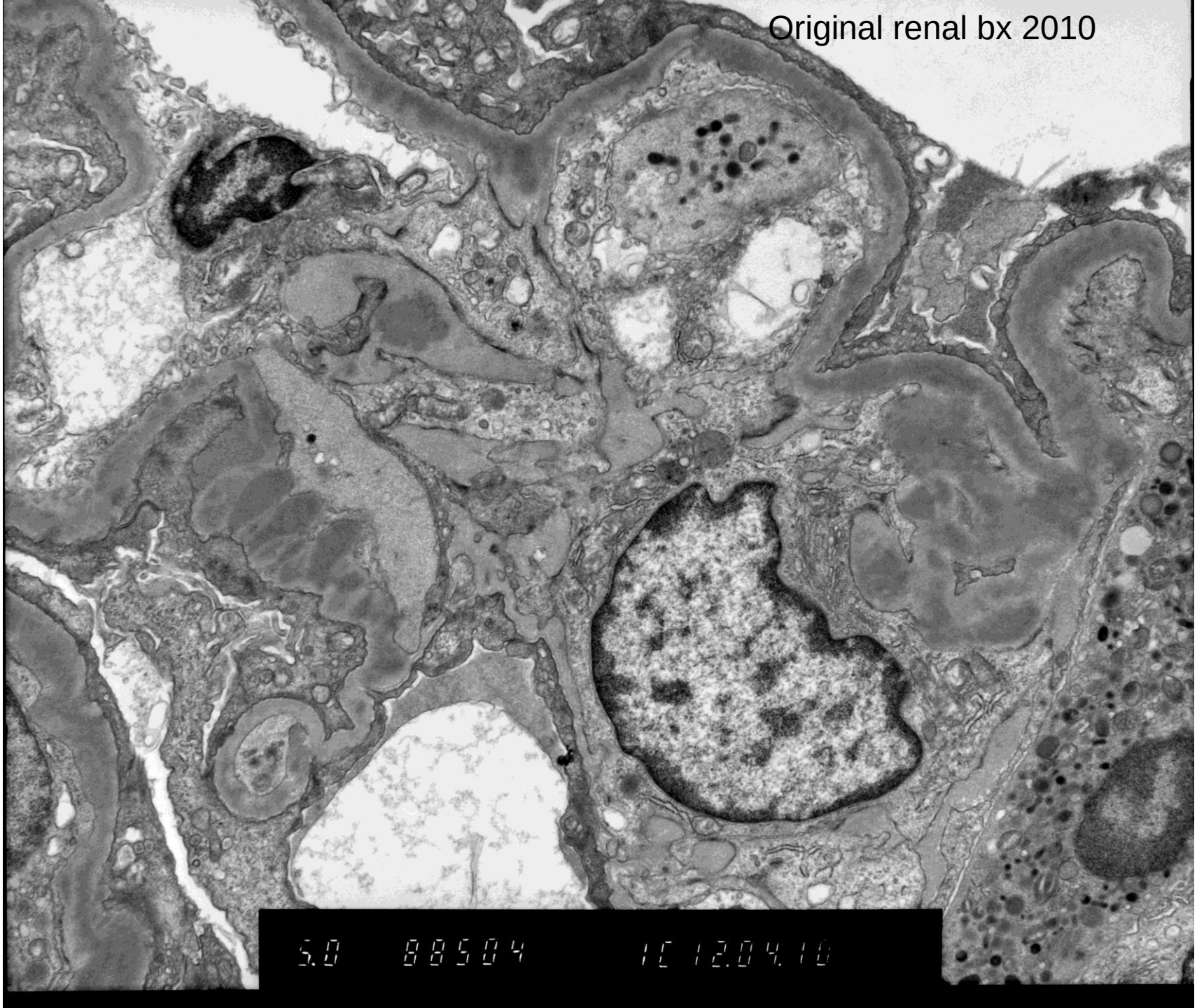

2000 nm

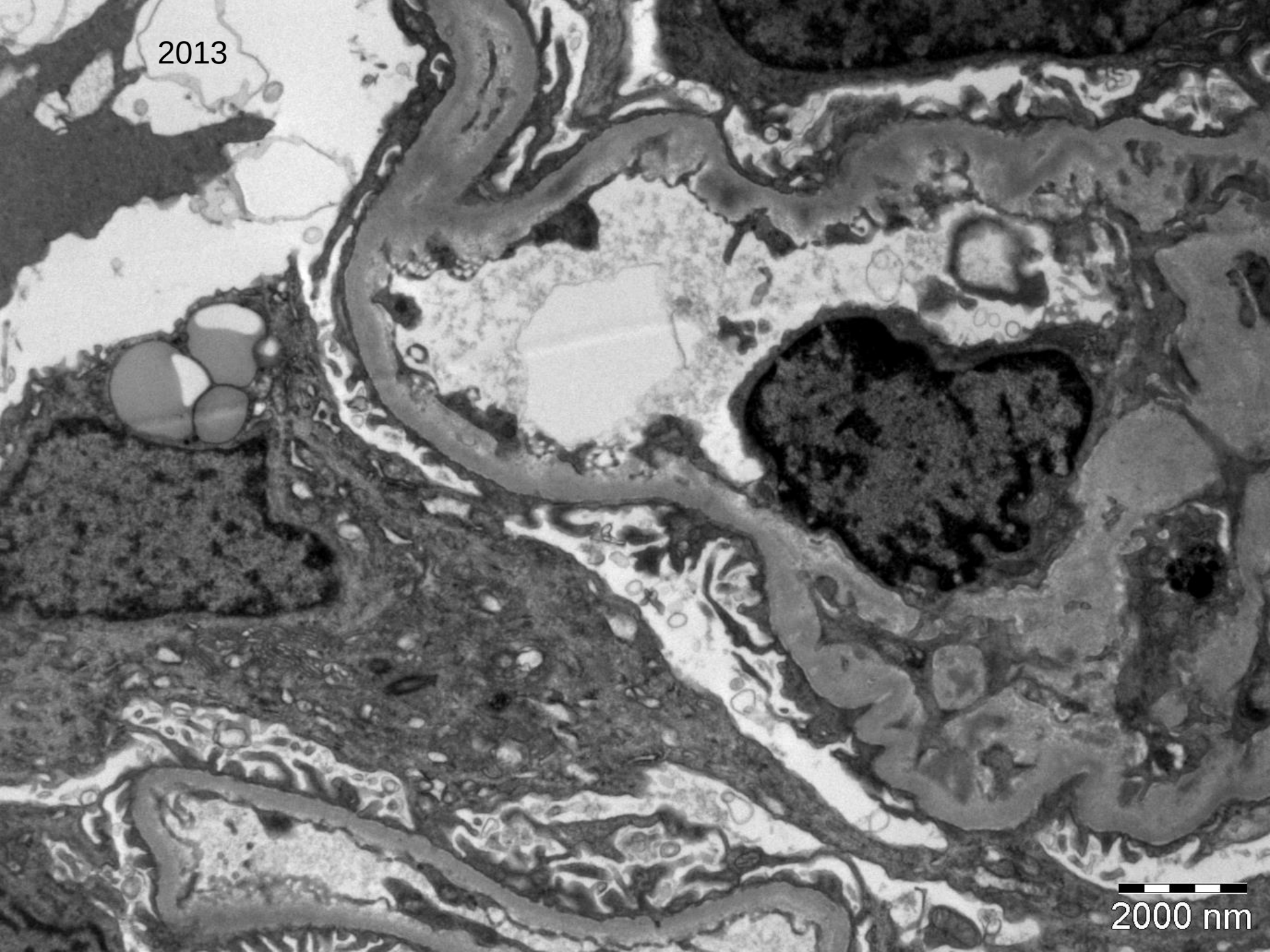
Membranous nephropathy

- Electron microscopy shows widespread subepithelial deposits with associated membrane spikes (stage 2) and diffuse effacement of podocyte foot processes. The changes confirm the LM diagnosis of membranous nephropathy.

transplant

- 12-year old boy, transplant December 2011 (live-related adult donor), heavy proteinura
- Glomeruli: at least one glomerulus /17 on initial H&E) that shows proliferating epithelial cells focally in the urinary space likely representing an early fresh crescent.
- There are foci of interstitial inflammatory infiltrate which appear to be predominantly in the interstitium rather than tubules. No definate tubulitis seen. EM shows small nodular and linear electron dense deposits within the GBM in several areas in all three glomeruli examined.
- External opinion from colleagues in Portsmouth and St Thomas/Guys


Original renal bx 2010
5.0 88504 1C 12.04.10


2013
2000 nm

transplant

- recurrence of dense deposit disease in the transplant

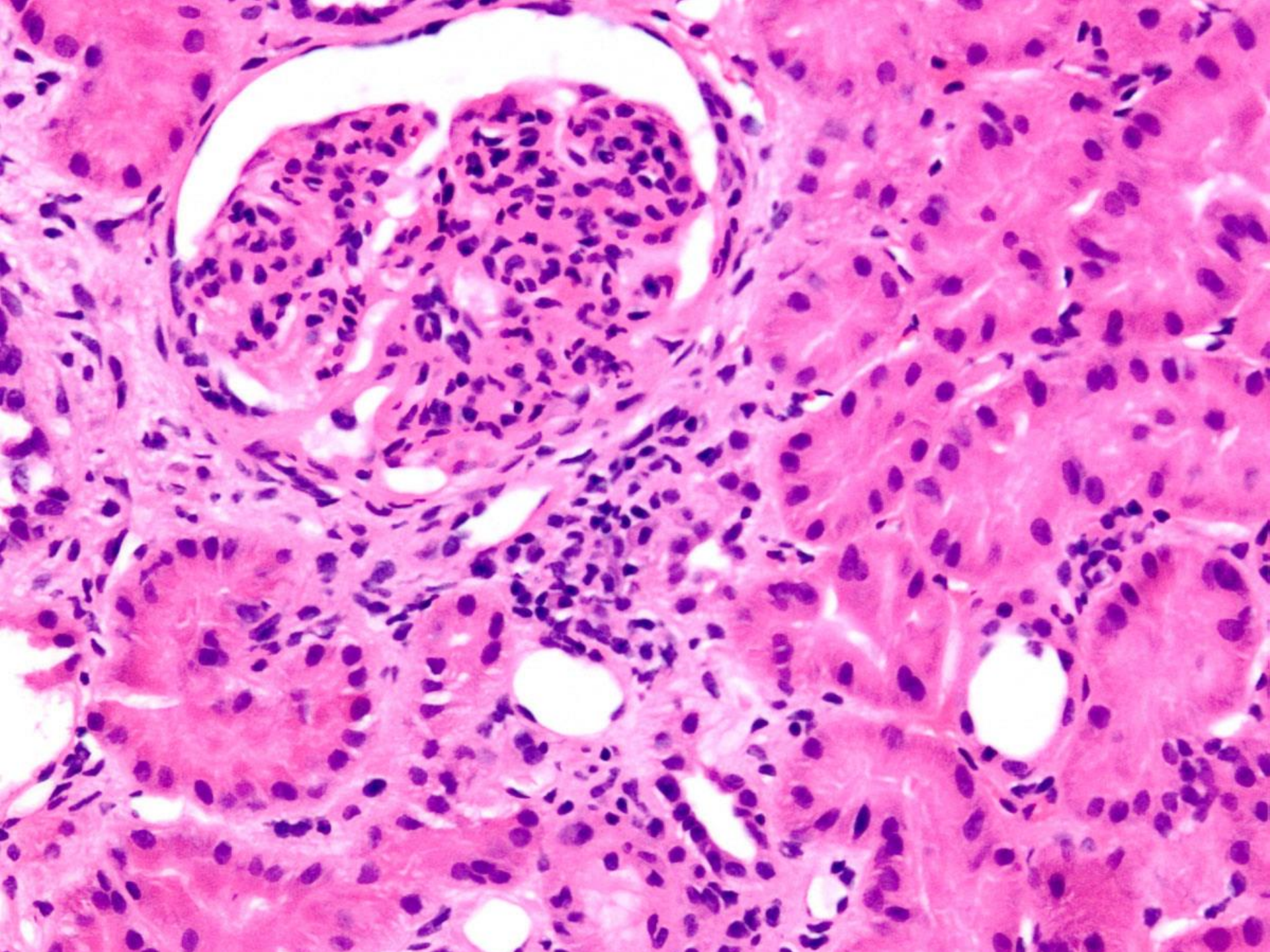
- **Indications for EM in transplants**

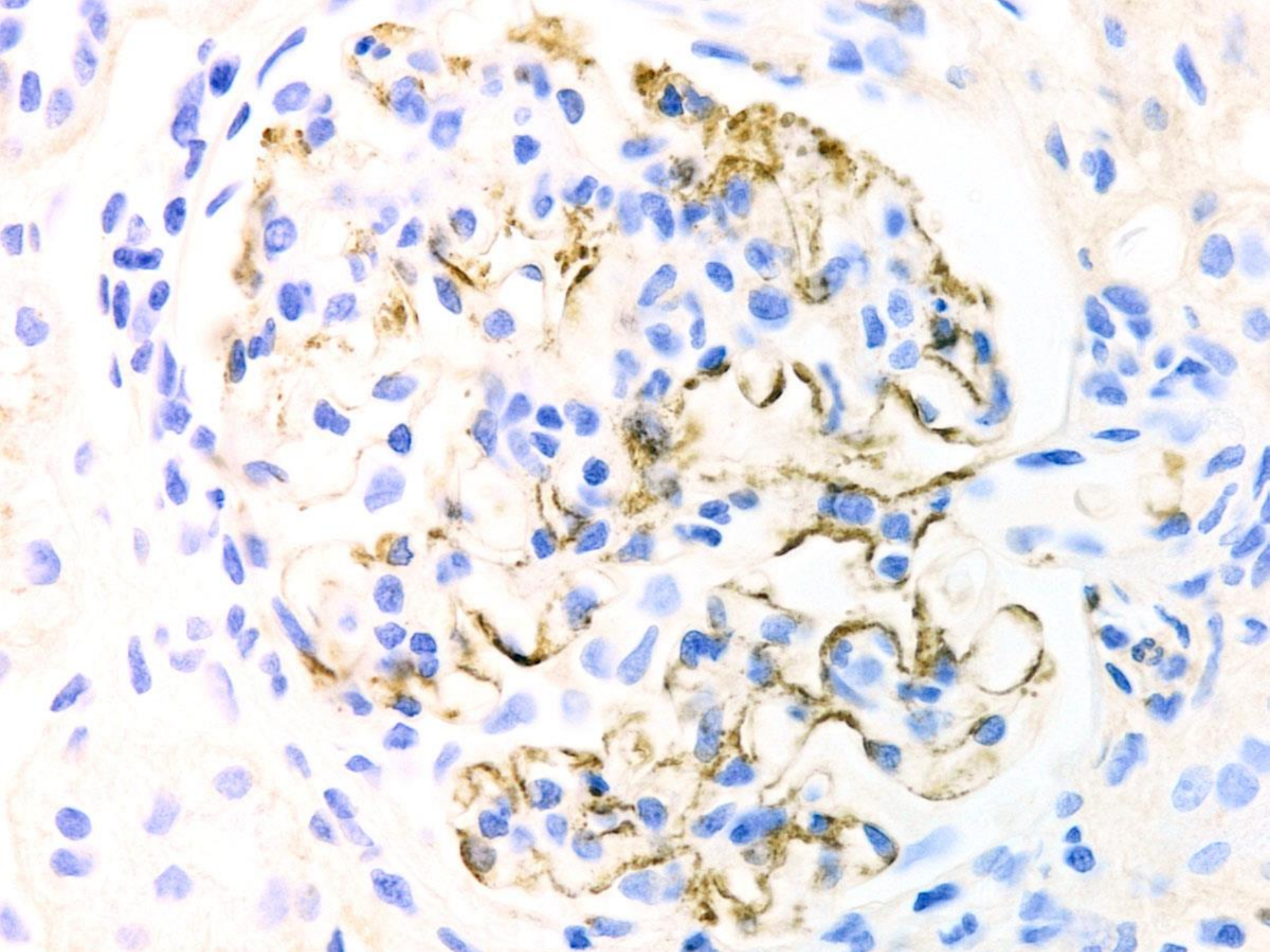
(Bart Wagner 09/07/12)

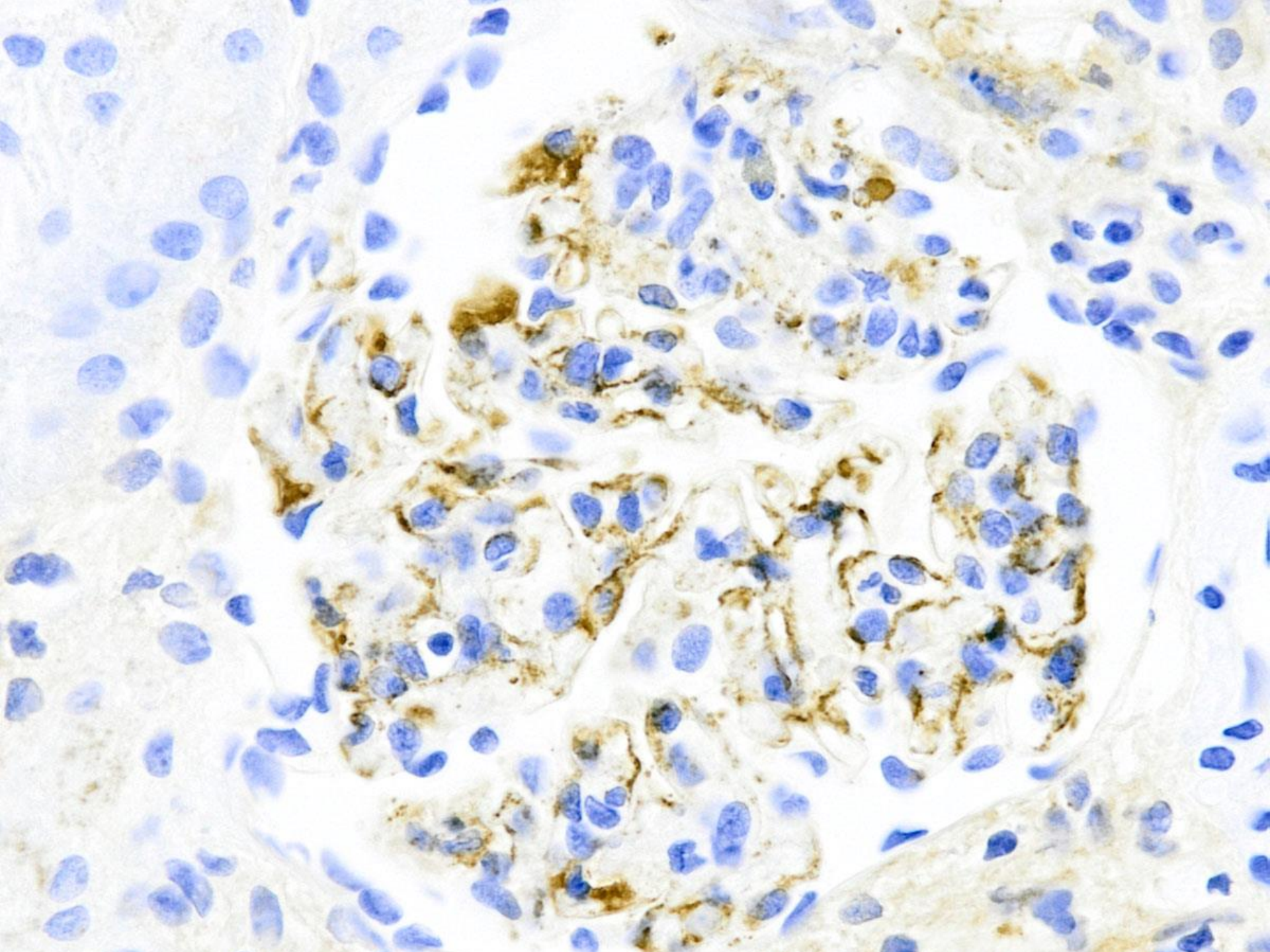
- humoral rejection (whether acute or chronic)
- any recurrent or de novo glomerulopathy
- possible viral nephropathy.
- NO tissue is put into glutaraldehyde if the kidney has only been in for a few weeks or months and in which there is no proteinuria ie if the nephrologists suspect delayed graft recovery or simple cellular rejection.
- Bart does Tol Blues, and describes ALL biopsies because this adds to the sample for light microscopy.
- PathSoc 2013

11-year old girl with nephritic syndrome (MA)

- 11-year old girl
- nephritic syndrome
- Henoch-Schonlein purpura

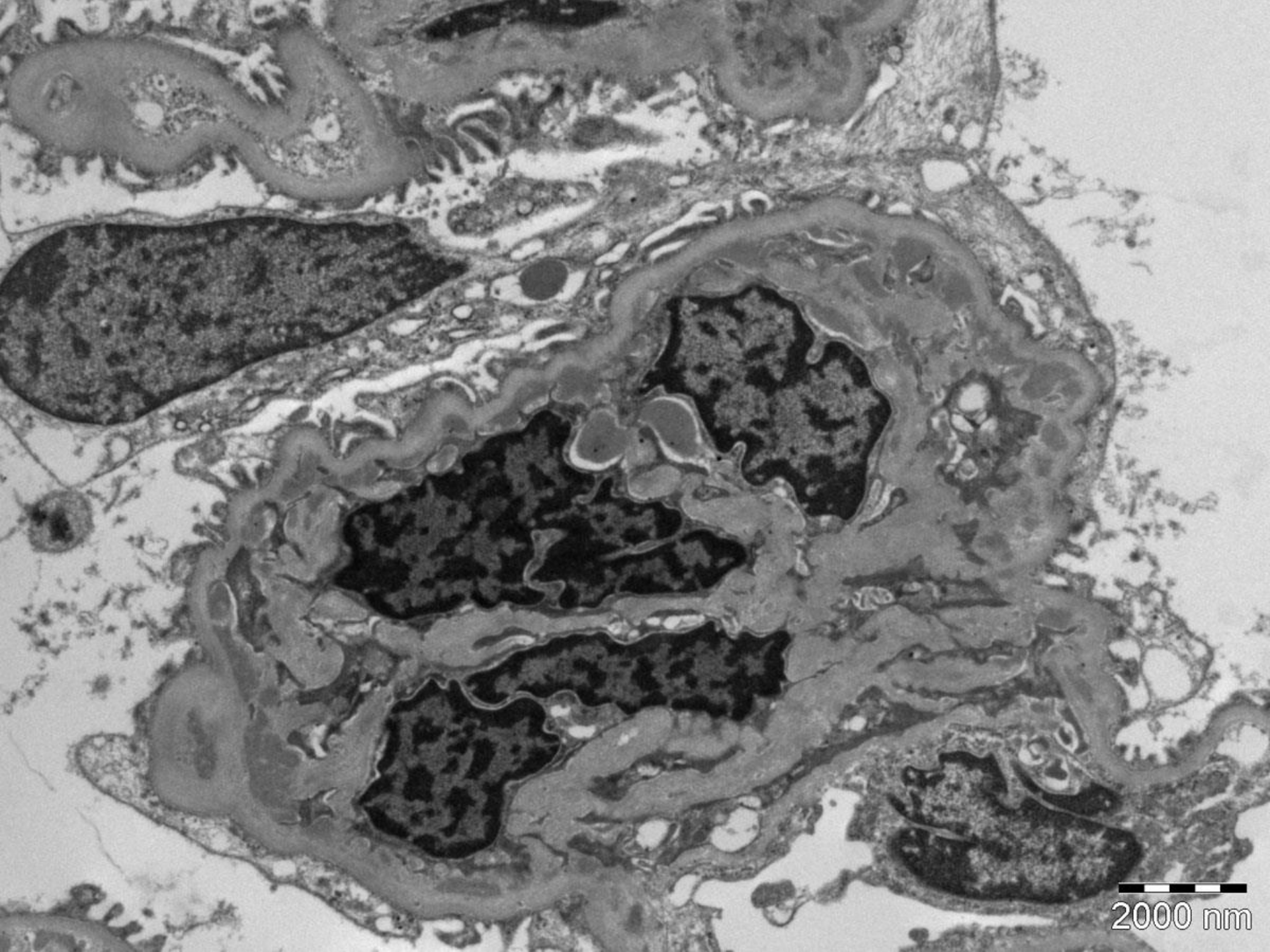






Light Microscopy

- Glomeruli: There are 11 glomeruli none of which are globally sclerosed. Most glomeruli show a lobular appearance with segments showing mesangial matrix expansion and mesangial hypercellularity. There are also capsular adhesions. No crescents, spikes or duplications are identified.
- Immunohistochemistry: IgA immunostaining shows a granular appearance in capillary loops.


2000 nm

EM

- Three glomeruli were imaged that show widespread electron dense deposits in subendothelial and mesangial / paramesangial areas.

- Final Diagnosis=Renal biopsy: IgA nephropathy

4-year old boy, mixed nephritic-nephrotic (RB)

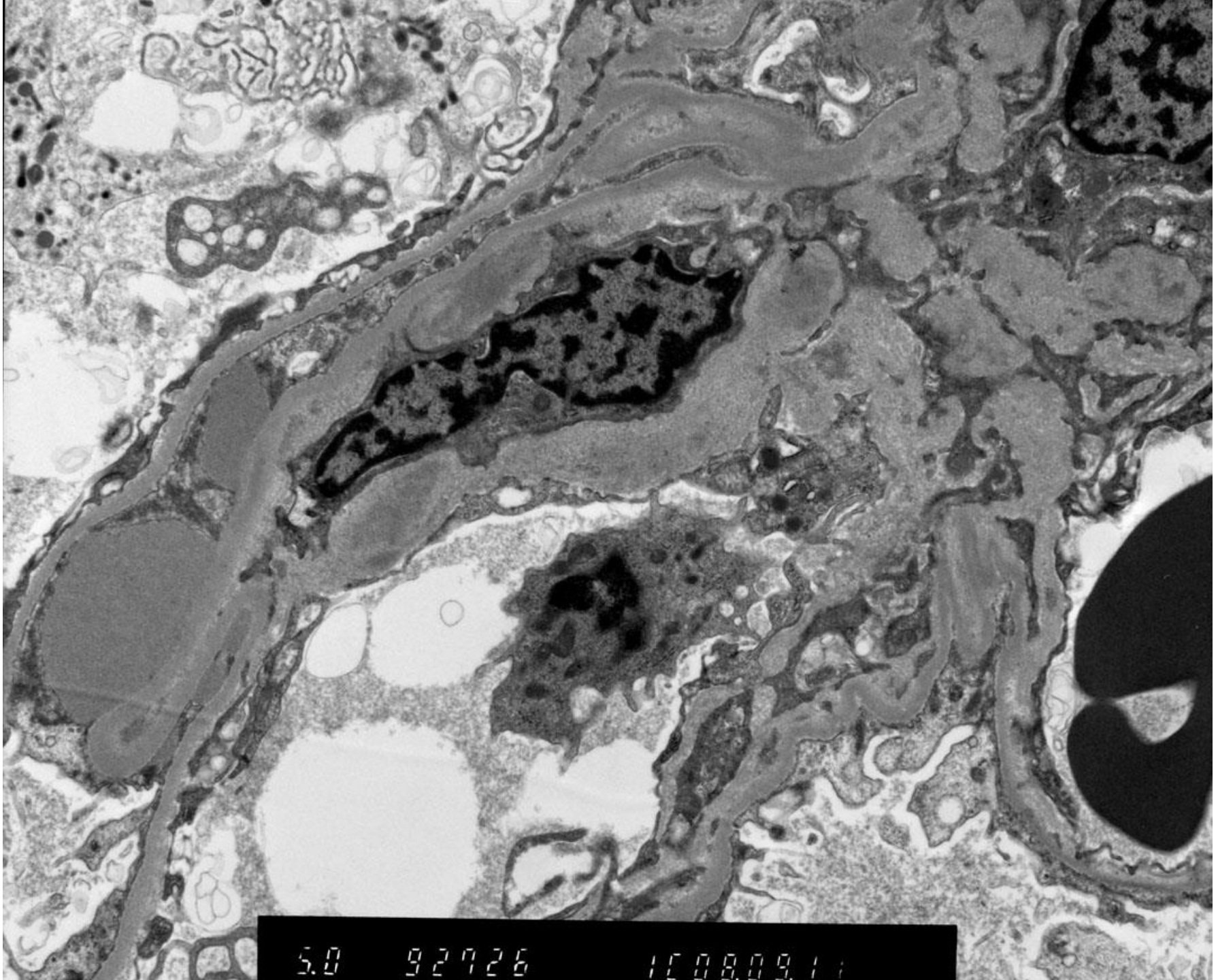
- 4-year old boy, mixed nephritic-nephrotic syndrome. Low C3. (C3 Nephritic factor negative)
- Glomeruli: There are 32 glomeruli all of which appear cellular and show some degree of abnormality; there are numerous crescents present which have a range of appearances, some appear fresh cellular and others more fibrocellular, indicating an evolving process.

Tubulo-Interstitium:

- A patchy acute and chronic inflammatory cell infiltrate including numerous neutrophils and eosinophils. There are no atrophic tubules identified. No interstitial fibrosis is seen.

Semithin preparation:

- Semi-thin sections show three glomeruli and confirm the findings on H&E. There is obliteration of the glomerular capillary loops by endocapillary cell proliferation. Subepithelial deposits ("humps") are present. The basement membrane shows no definite thickening or double contour formation. There is an inflammatory cell infiltrate within the interstitium.


5.0 92926 1C08091

EM

- Electron Microscopy: Electron microscopy shows numerous subepithelial humps as well as subendothelial electron dense deposits.
- COMMENT:

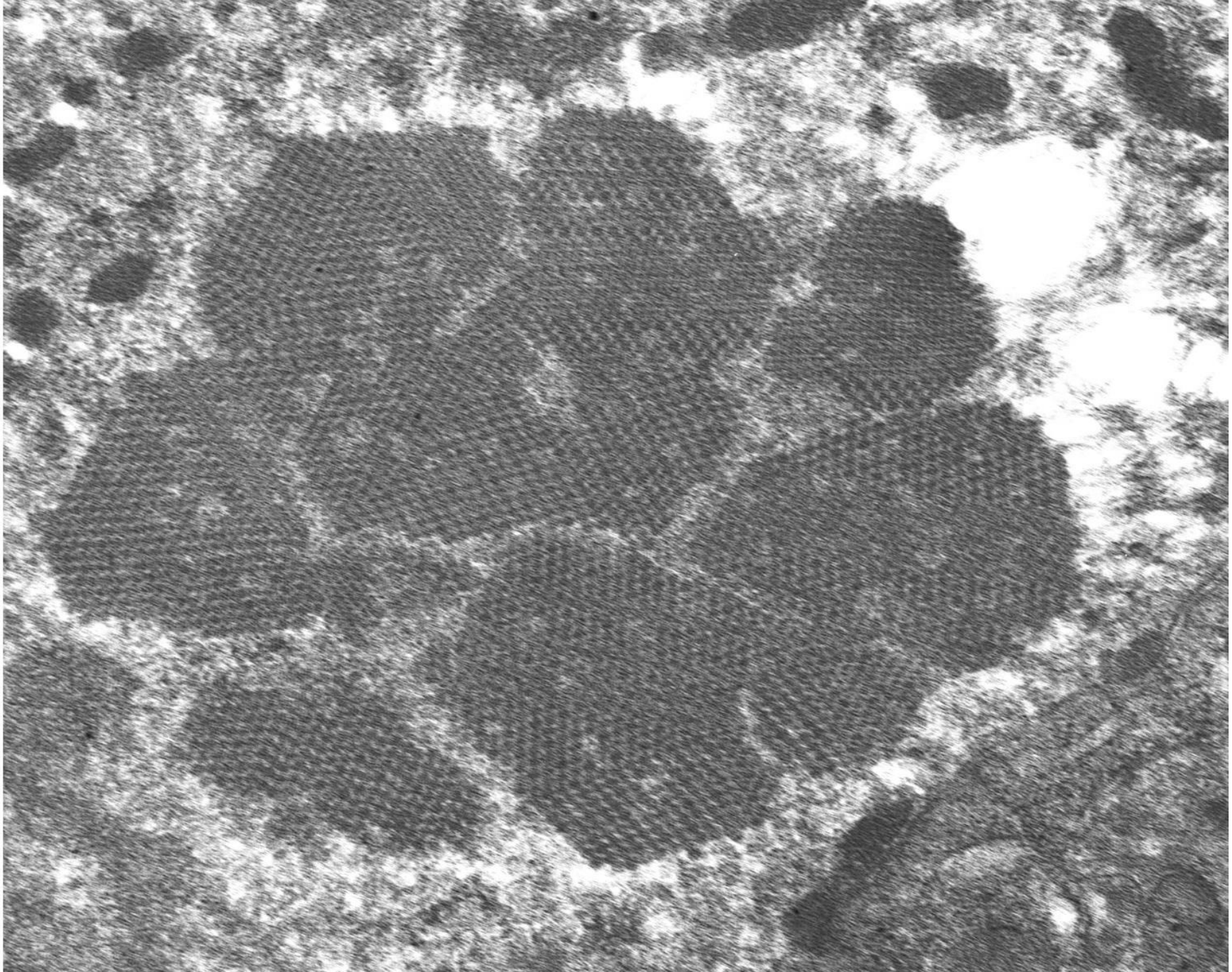
The histological features are those of a diffuse endocapillary proliferative glomerulonephritis with cellular and fibrocellular crescents. There is an acute inflammatory cell infiltrate, including neutrophils within the tubulo-interstitium. Immunohistochemistry shows C3 and IgG deposition in capillary walls. Electron microscopy confirms the presence of subepithelial electron dense deposits.

Final diagnosis

- Renal biopsy: crescent formation and subepithelial electron dense deposits.
- Features in keeping with post-infectious glomerulonephritis.

4-year old boy

- FSGS, renal transplant 16/10/12, borderline acute cellular rejection 22/1/13, improvement in creatinine with steroids, subsequent creatinine rise, suboptimal tacrolimus level.
- Polyoma (BK) virus immunostain shows two tubules with crisp nuclear positivity in occasional tubular epithelial cells.



C)

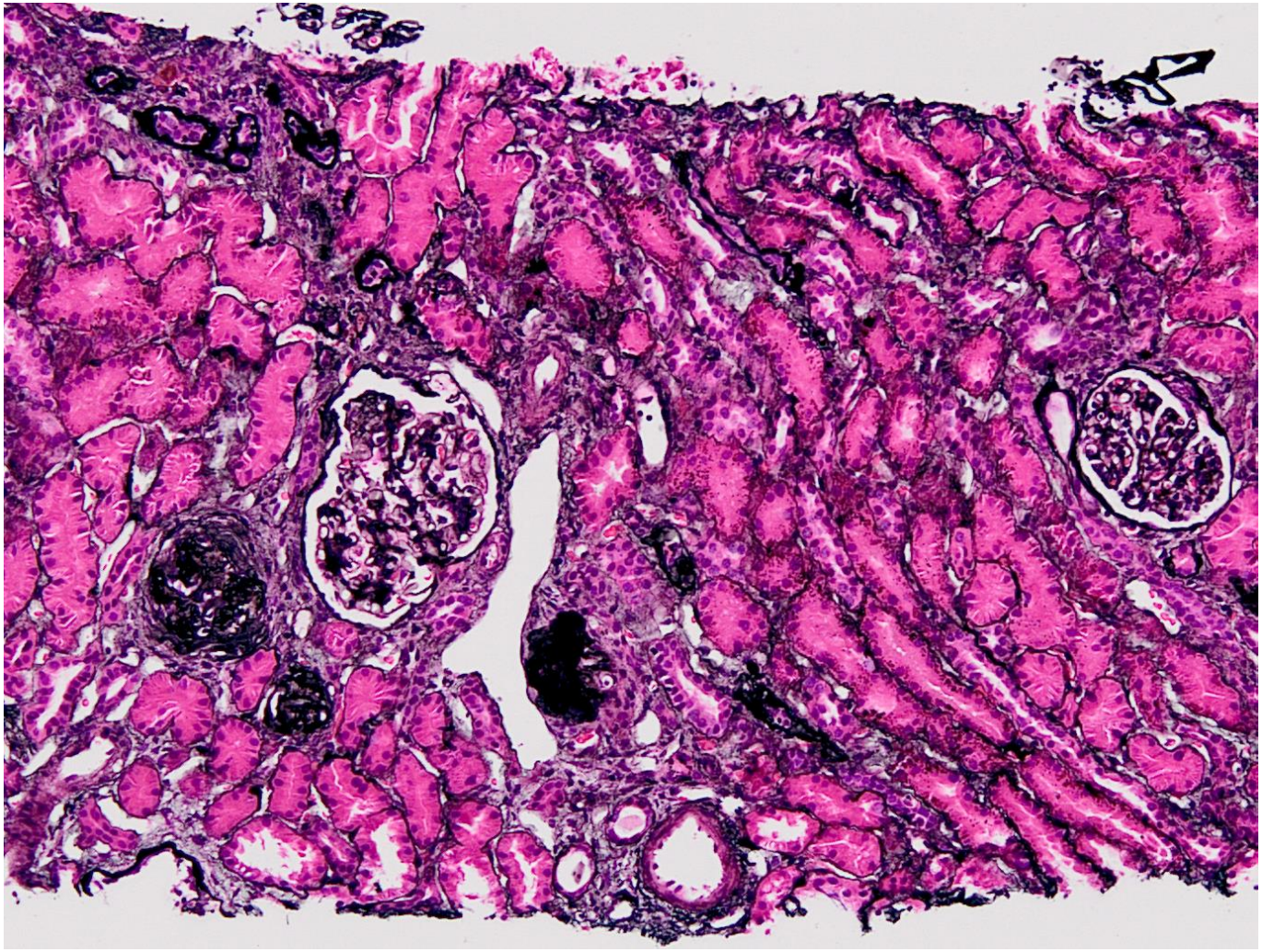
27-month old boy
Neuroblastoma neck

- Chemo: cisplatin and carboplatin
- ? Cisplatin-induced AKI
- Film: Fragmented RBC

Light and electron microscopic findings in haemolytic uraemic syndrome

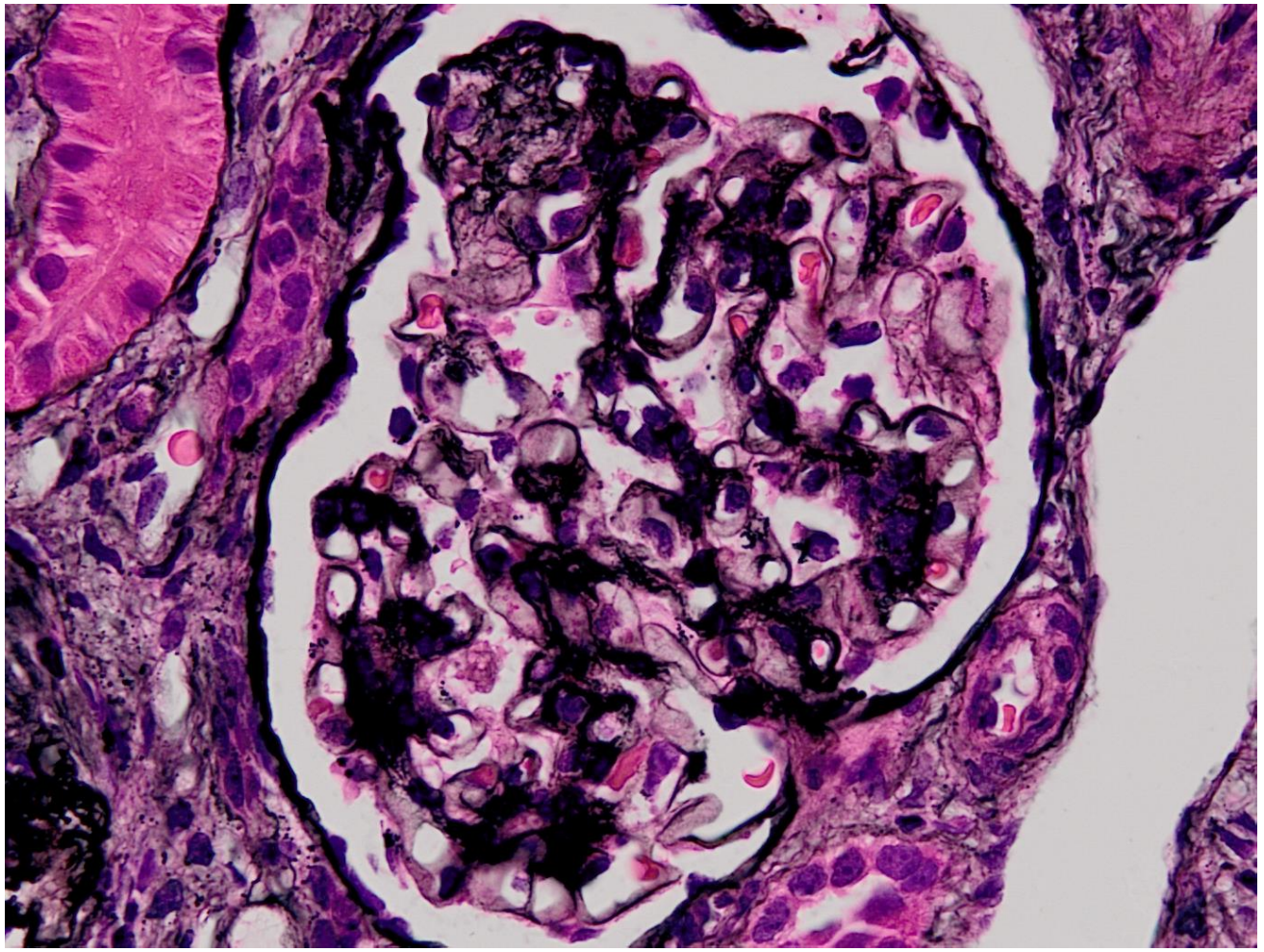
- LM: Glomeruli: increased mesangial matrix expansion and segmental sclerosis, thickened capillary loops, focal tubular atrophy around damaged glomeruli.
- EM: GBM shows widened subendothelial space with “fluffy” electron lucent material.

LM



Jones Silver Stain

LM



Jones Silver Stain

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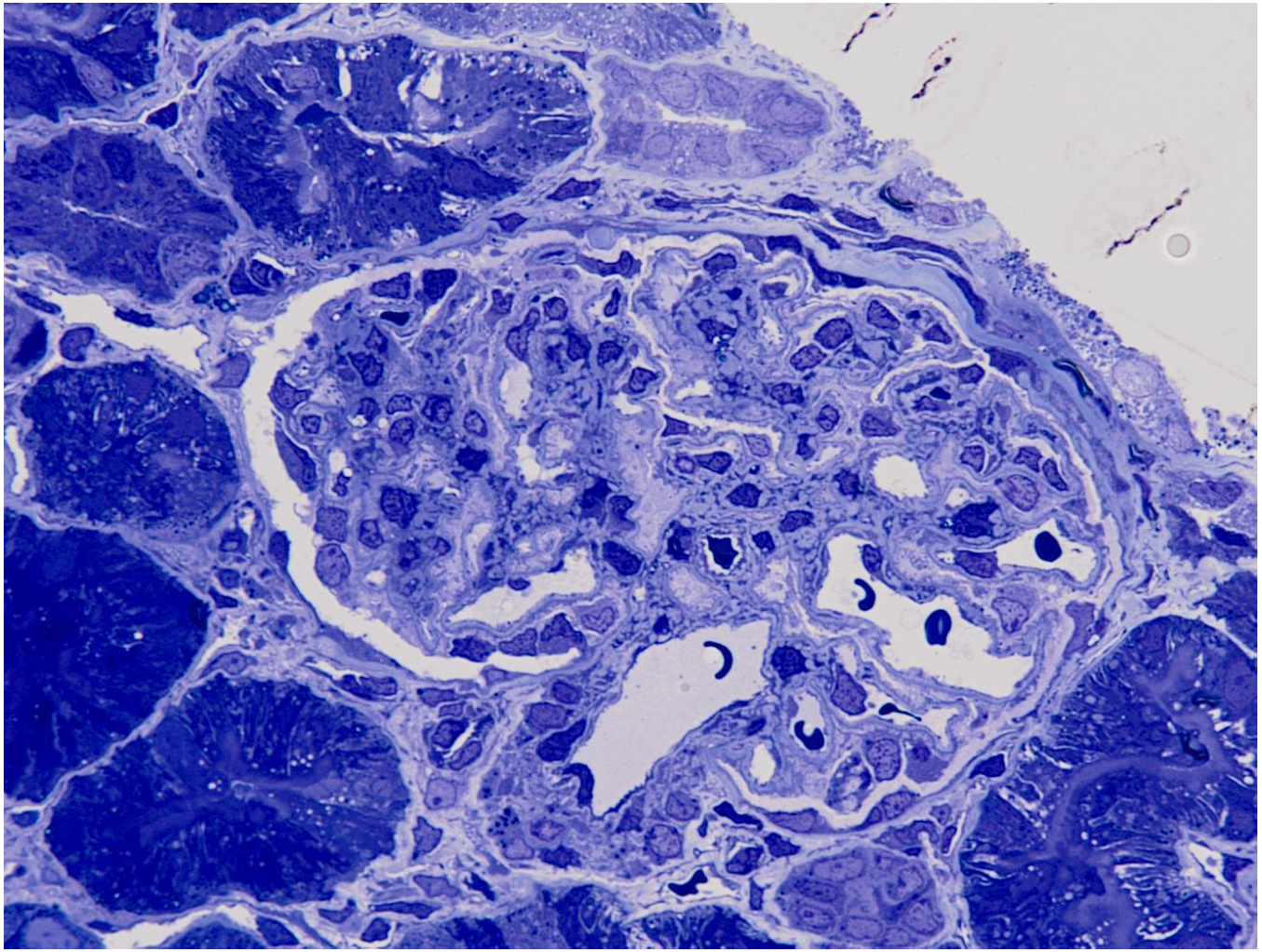
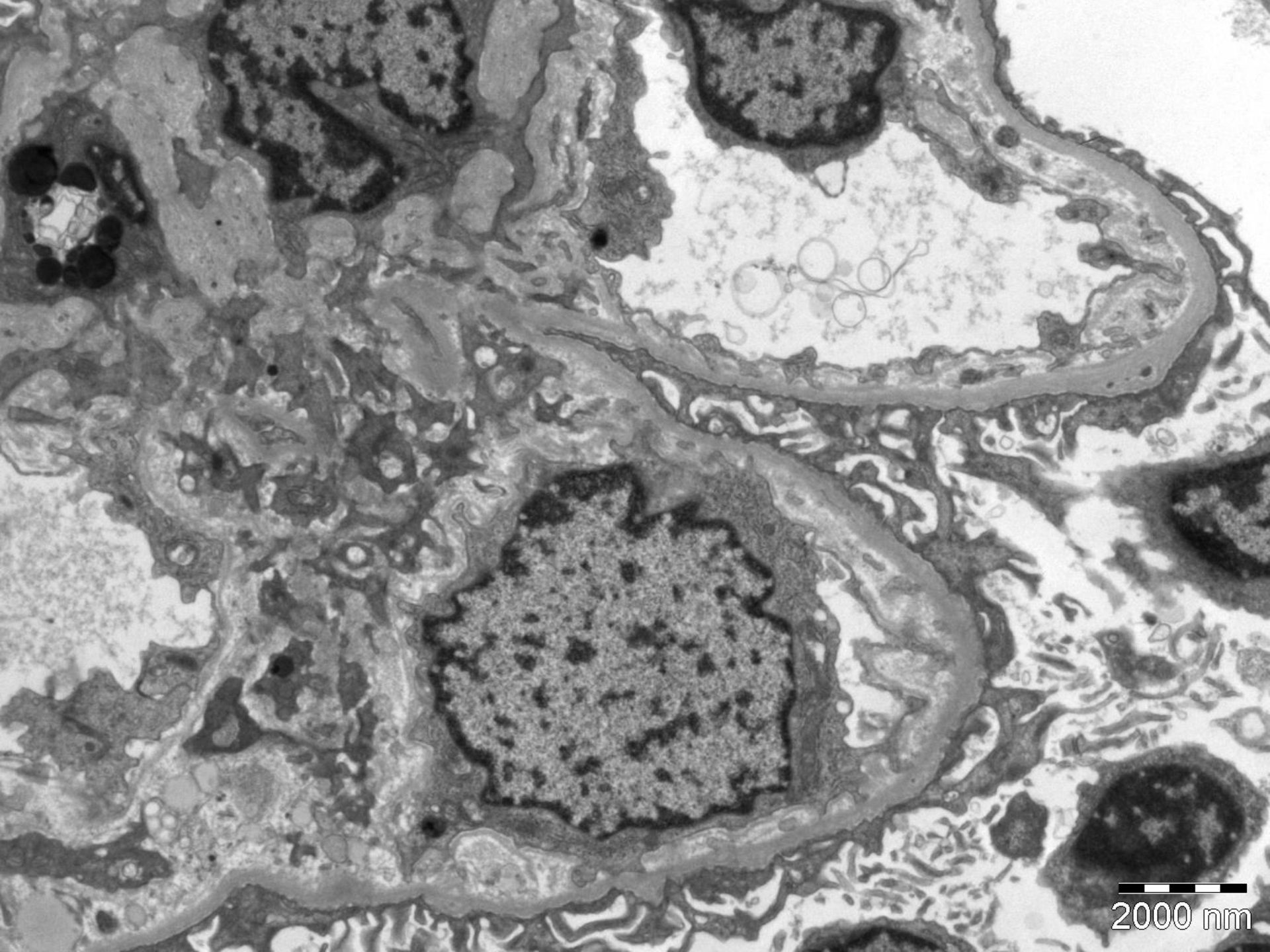
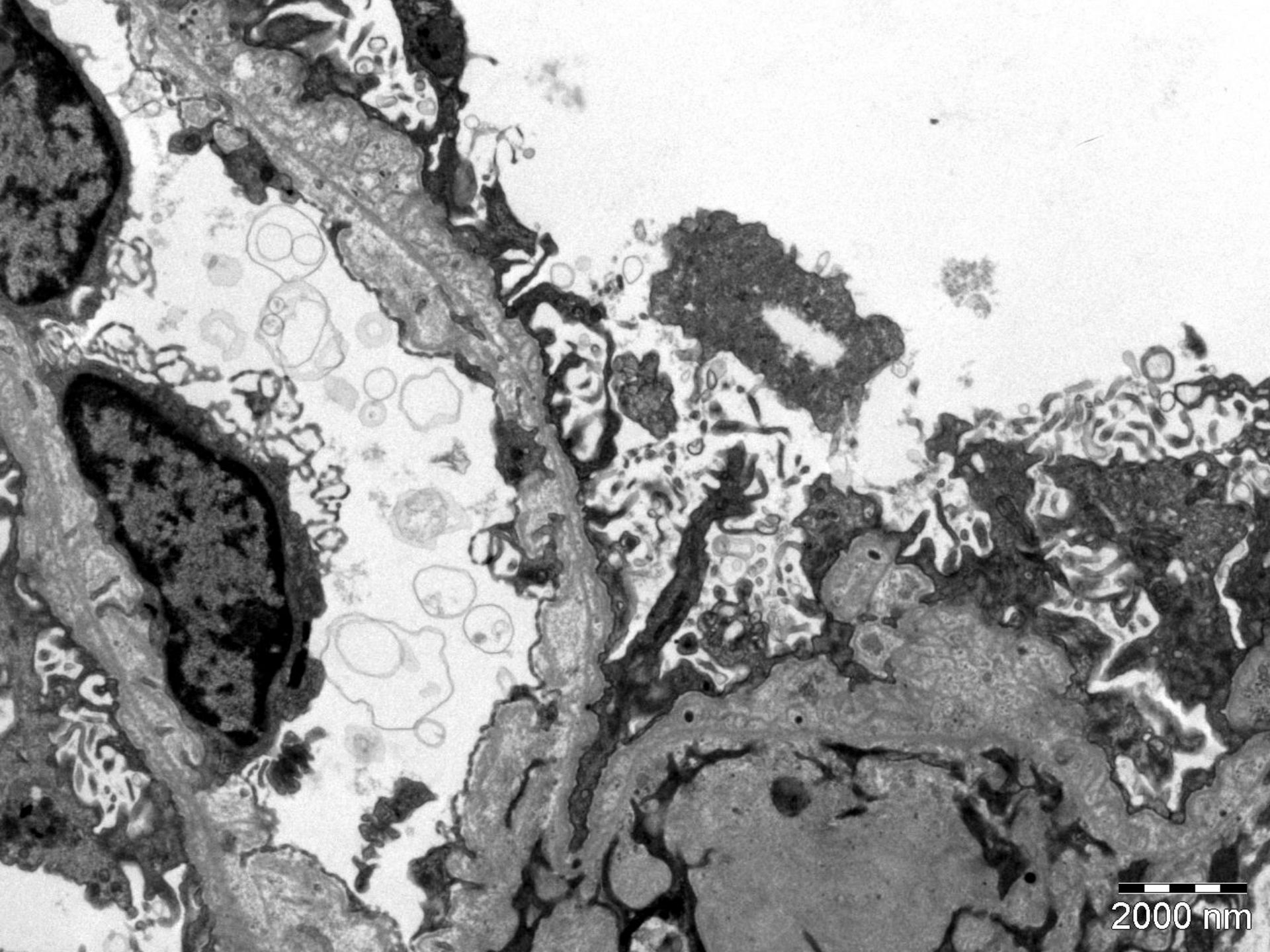


Toludine blue semi-thin


2000 nm


2000 nm

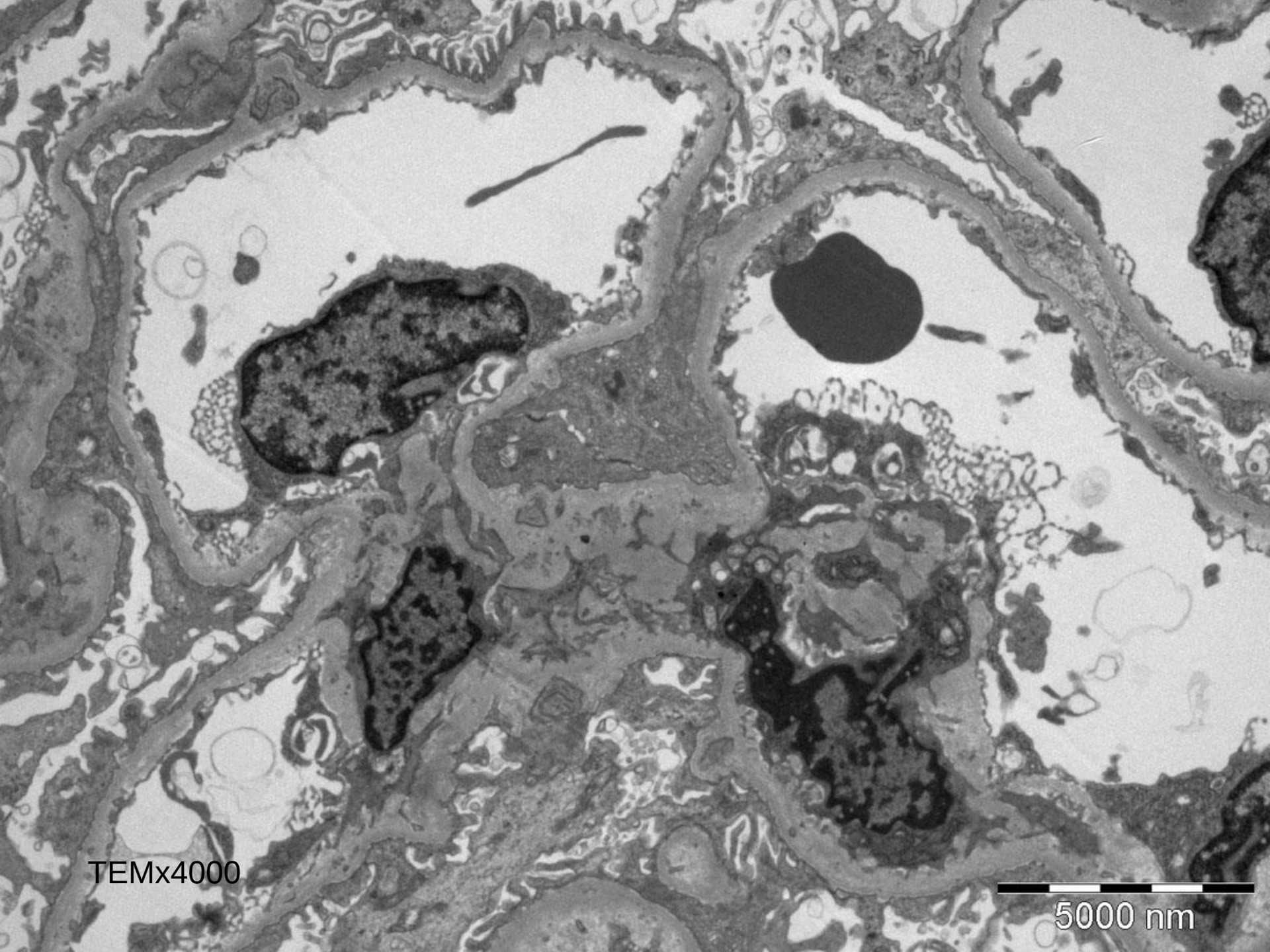
Diagnosis=aHUS

- Sequencing of *CD46* (MCP) gene:
  - Heterozygous c.1027+5G>T in intron 10
    - Not previously described
    - Not present in 188 normals; present in 1 other aHUS patient
    - *In silico* analysis: may produce splice variant
    - Reduced surface expression of CD46 on neutrophils

Treatment: Eculizumab (anti-C5 antibody which prevents the assembly of MAC)

slide courtesy of Dr Rodney Gilbert


Glomeruli=flowers


TEMx4000
5000 nm

Clematis sp

- The genus name is from Ancient Greek *clématis,* a climbing plant
- genus of about 300 species
- buttercup family Ranunculaceae.
- mainly of Chinese and Japanese origin. Most species are known as *clematis* in English, while some are also known as **traveller's joy**, a name invented for the sole British native, *C. vitalba*,

Pharm / Toxicology

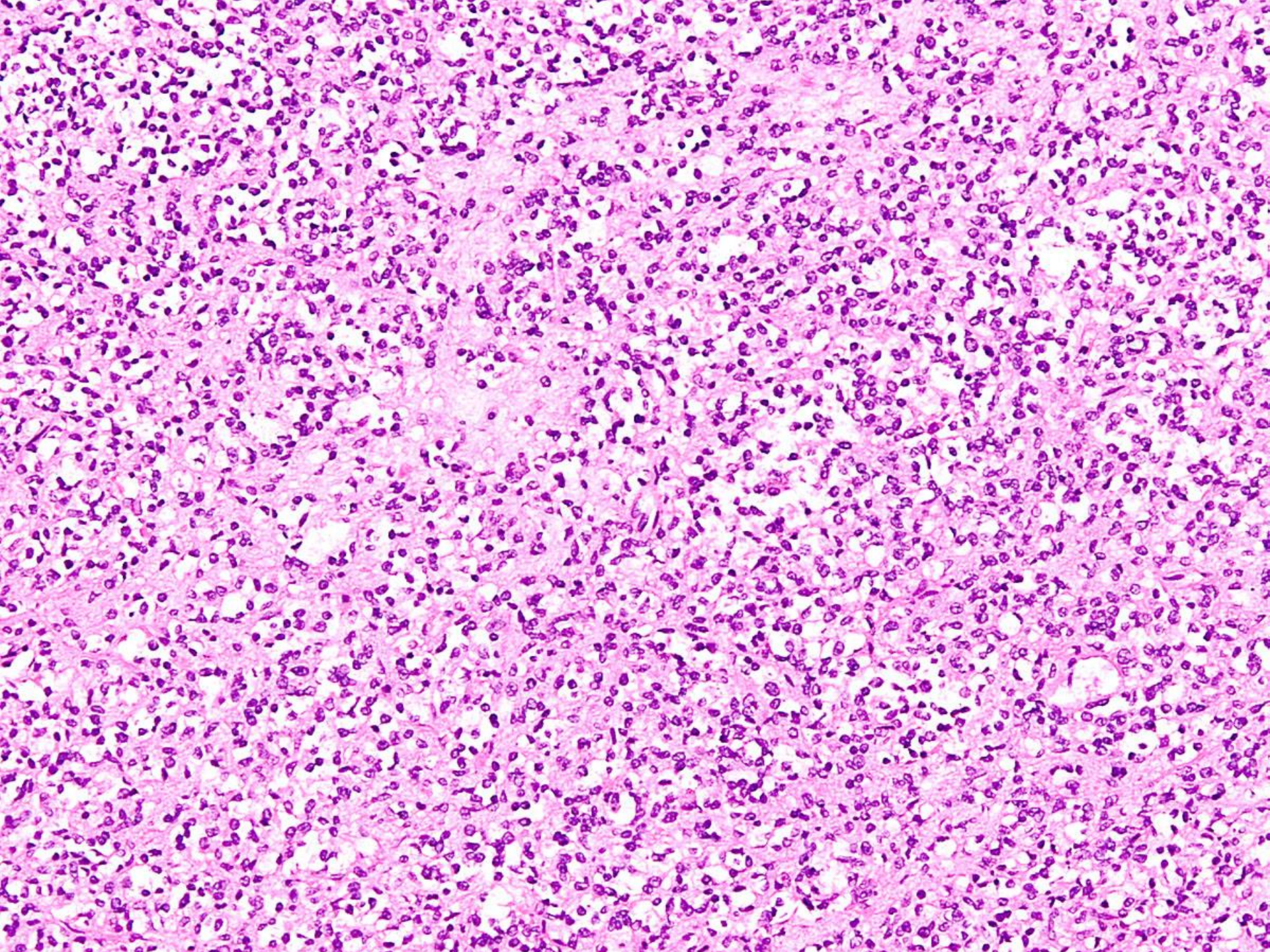
- In the American Old West the Western white clematis, *Clematis ligusticifolia*, was called **pepper vine** by early travelers and pioneers of the American Old West, who took a tip from Spanish colonials and used seeds and the acrid leaves of *yerba de chivato* as a pepper substitute.[40] The entire genus contains essential oils and compounds which are extremely irritating to the skin and mucous membranes. Unlike black pepper or *Capsicum*, however, the compounds in clematis cause internal bleeding of the digestive tract if ingested in large amounts. *C. ligusticifolia* is essentially toxic. When pruning them, it's a good idea to wear gloves.

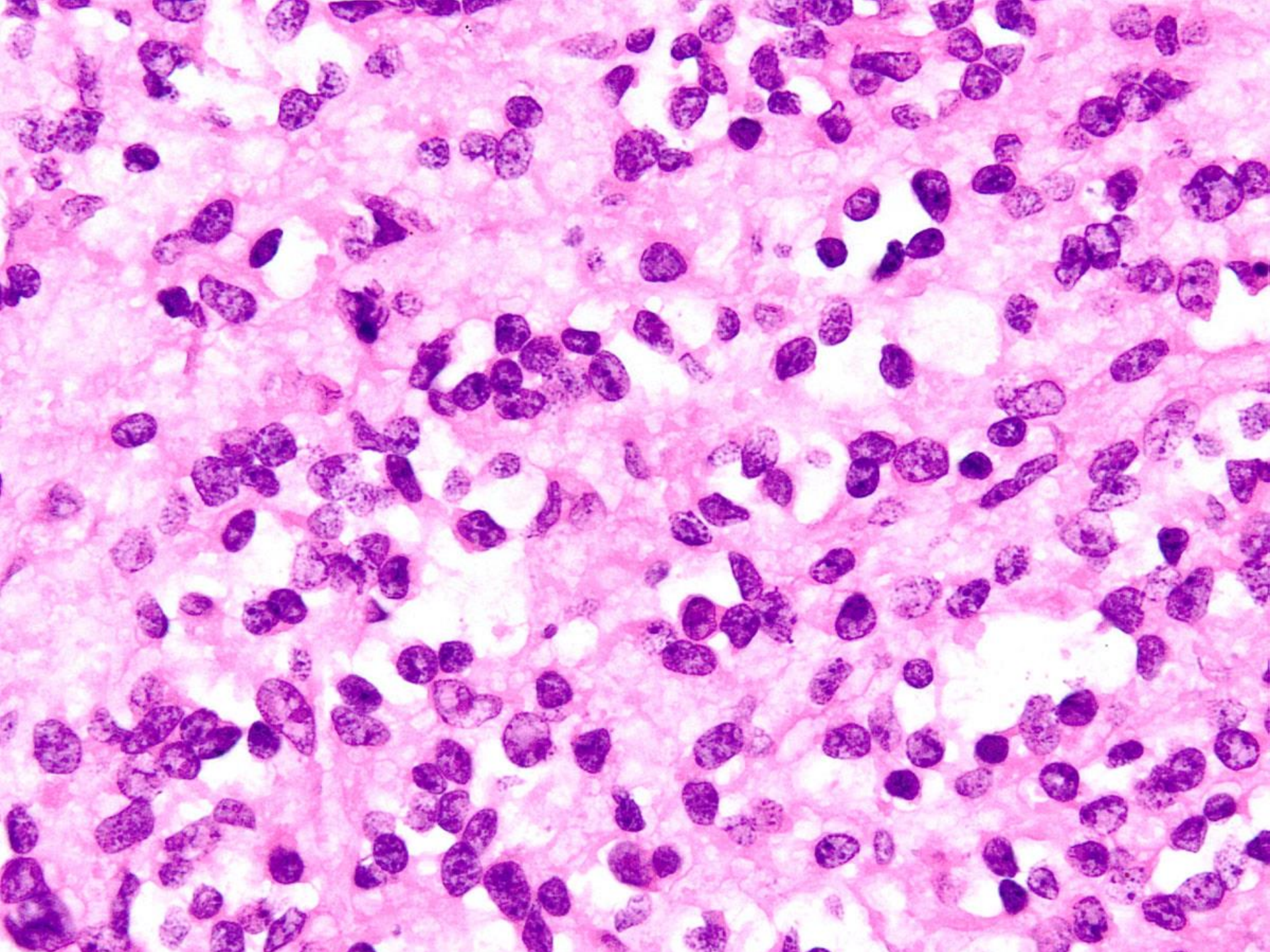
Transplants

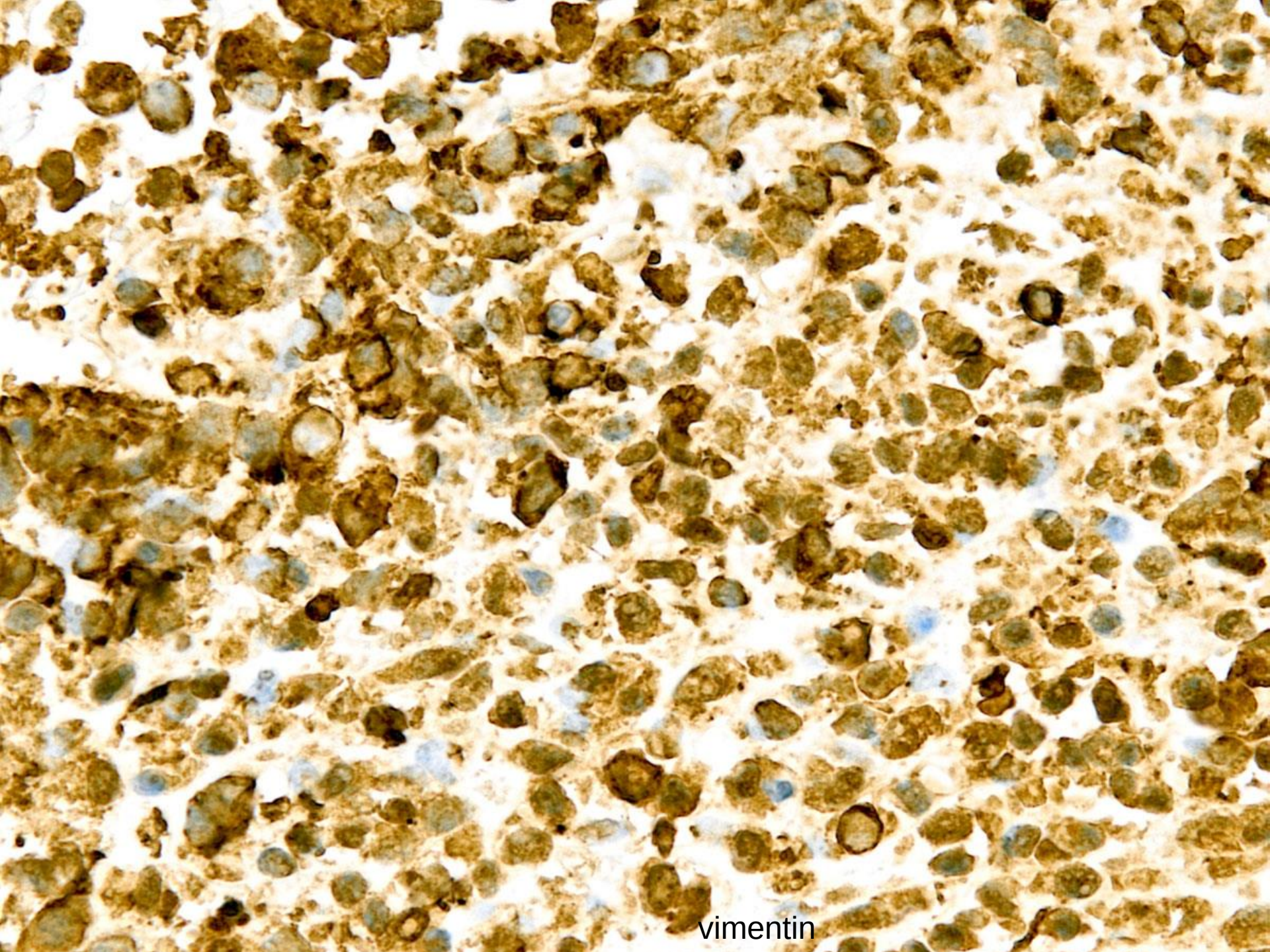
- transplant / organ donation (NHS Register)
- Organ transplants reach record high, NHS figures show http://www.bbc.co.uk/news/health-23707182
- British Transplant Games 2013 begin in Sheffield
- http://www.bbc.co.uk/news/uk-england-south-yorkshire-23711578
- **People in Wales will be presumed to have agreed for their organs to be donated after death from December 2015.**
- **http://www.bbc.co.uk/news/uk-wales-24032031**

Unusual situations

- 5-year old female. Ulna nerve palsy, left side, wasted pectoralis major. Lump in left axilla. ?sarcoma ?PNET
- Frozen Section 27/12/2012 (1A) "Lesional tissue, likely tumour - does not look like necrosis“
- Light Microscopy
- sheets of highly cellular round-ovoid malignant tumour. The tumour cells are vesicular and have prominent nucleoli. Some of the cells appear to have inclusions. There are large areas of necrosis and cells can be seen in apoptosis and mitosis. No recognisable histological structures are identified.

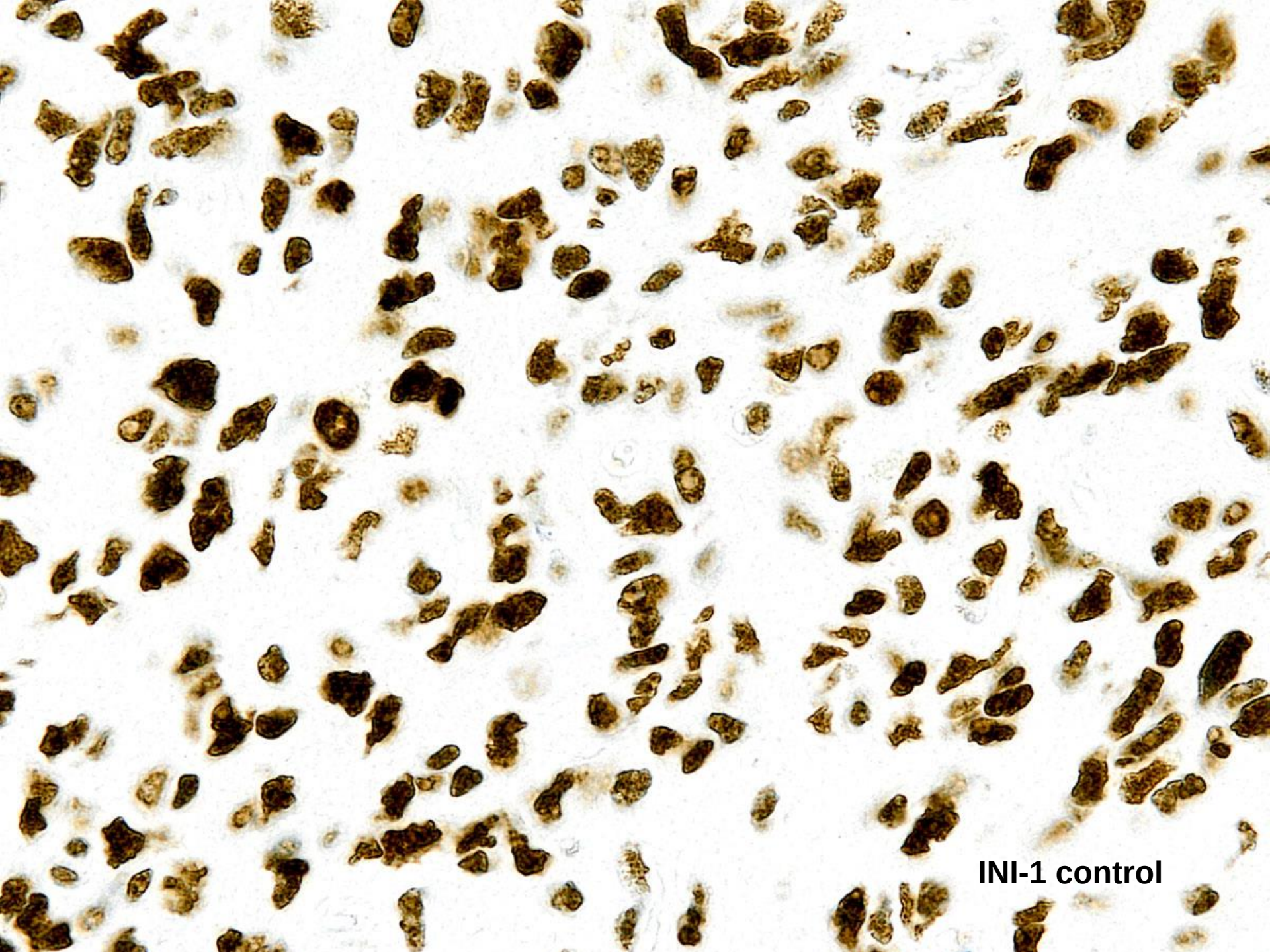


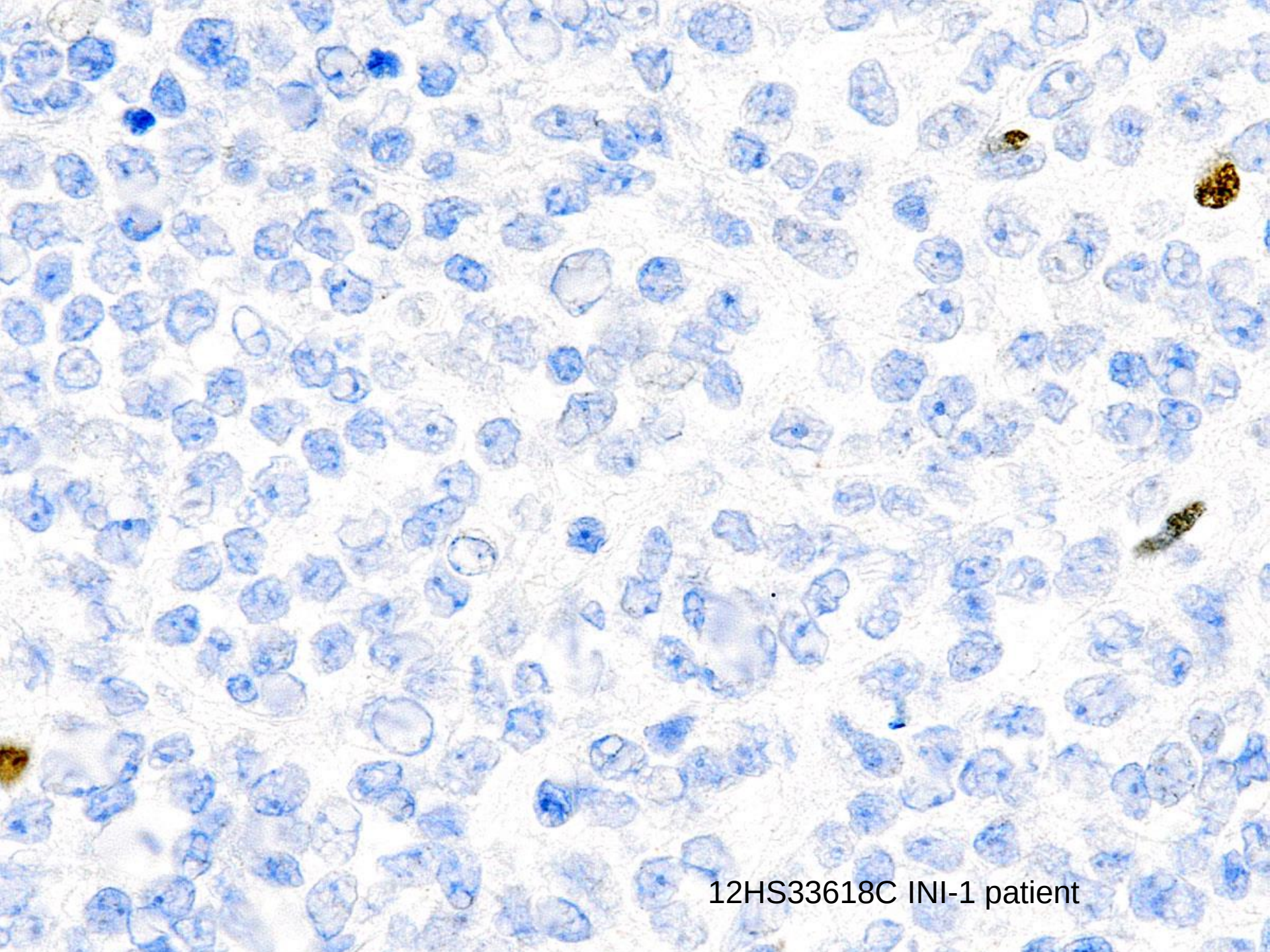



vimentin

Negative results

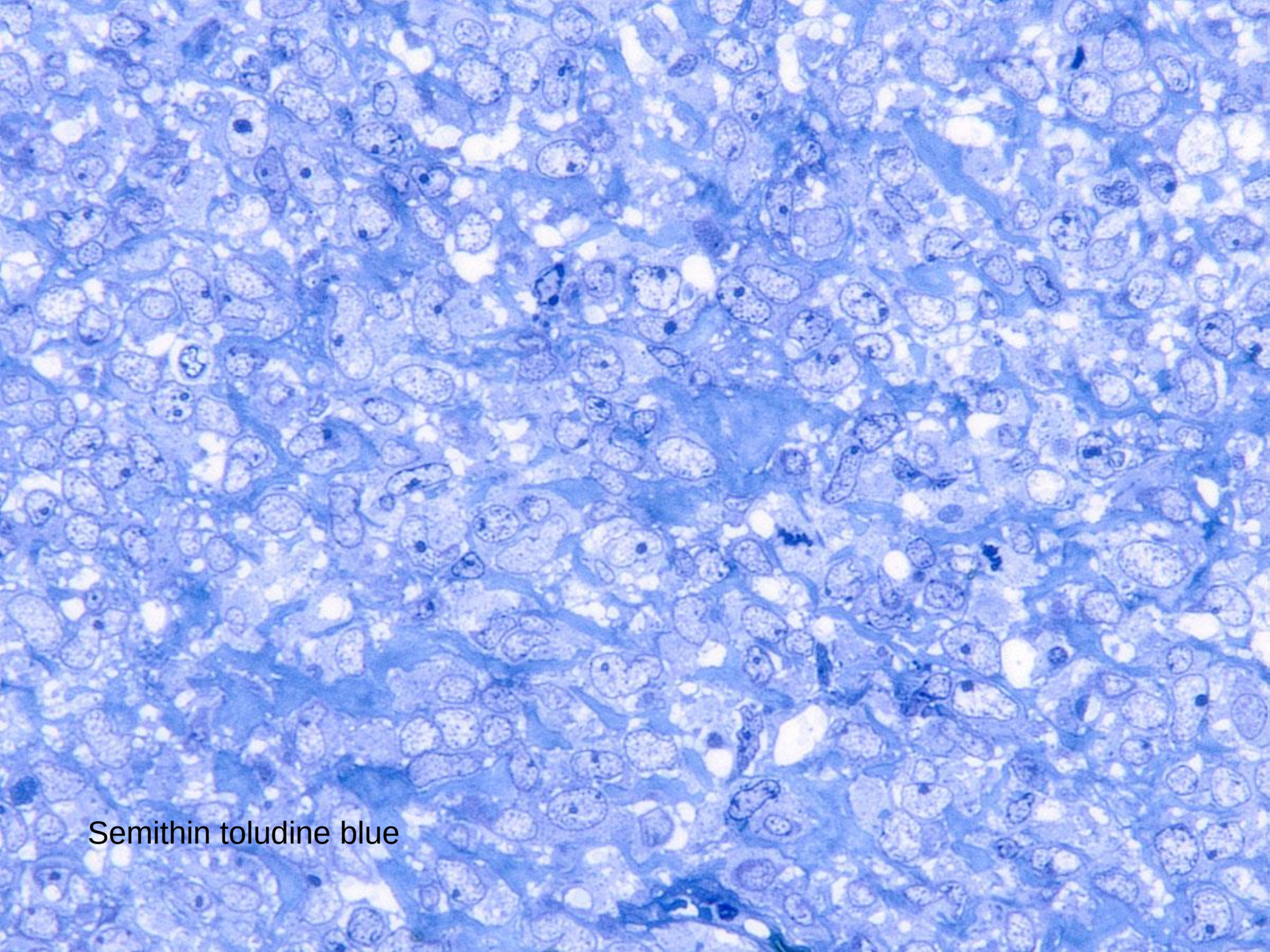
- Immunostains for CD99, MNF116, AE1/3, desmin, myogenin, S100, WT-1, CD45, NB84 and CD34 are negative.
- INI-1 is negative in the presence of a positive control (repeated twice giving the same result)


INI-1 control

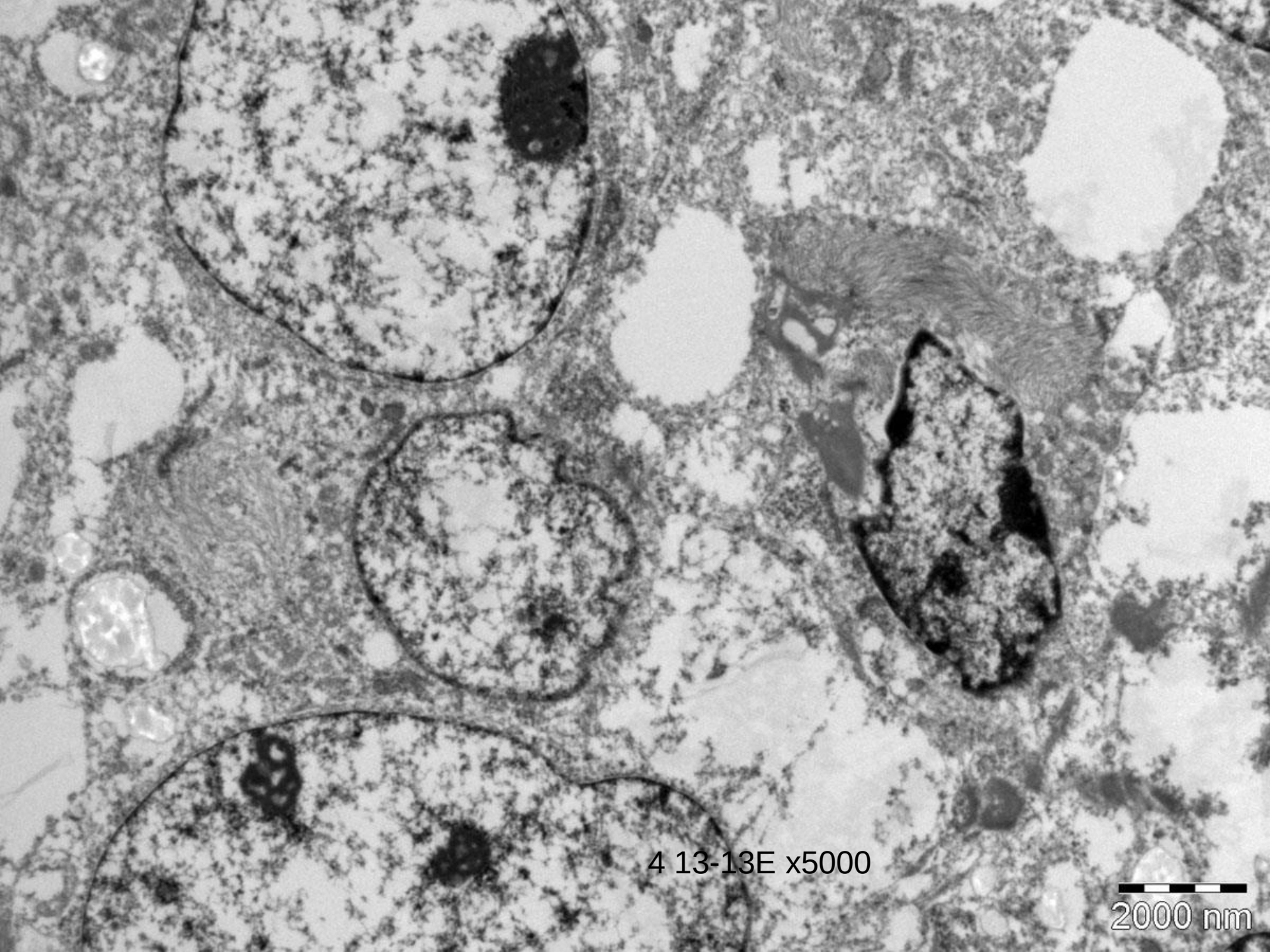

12HS33618C INI-1 patient

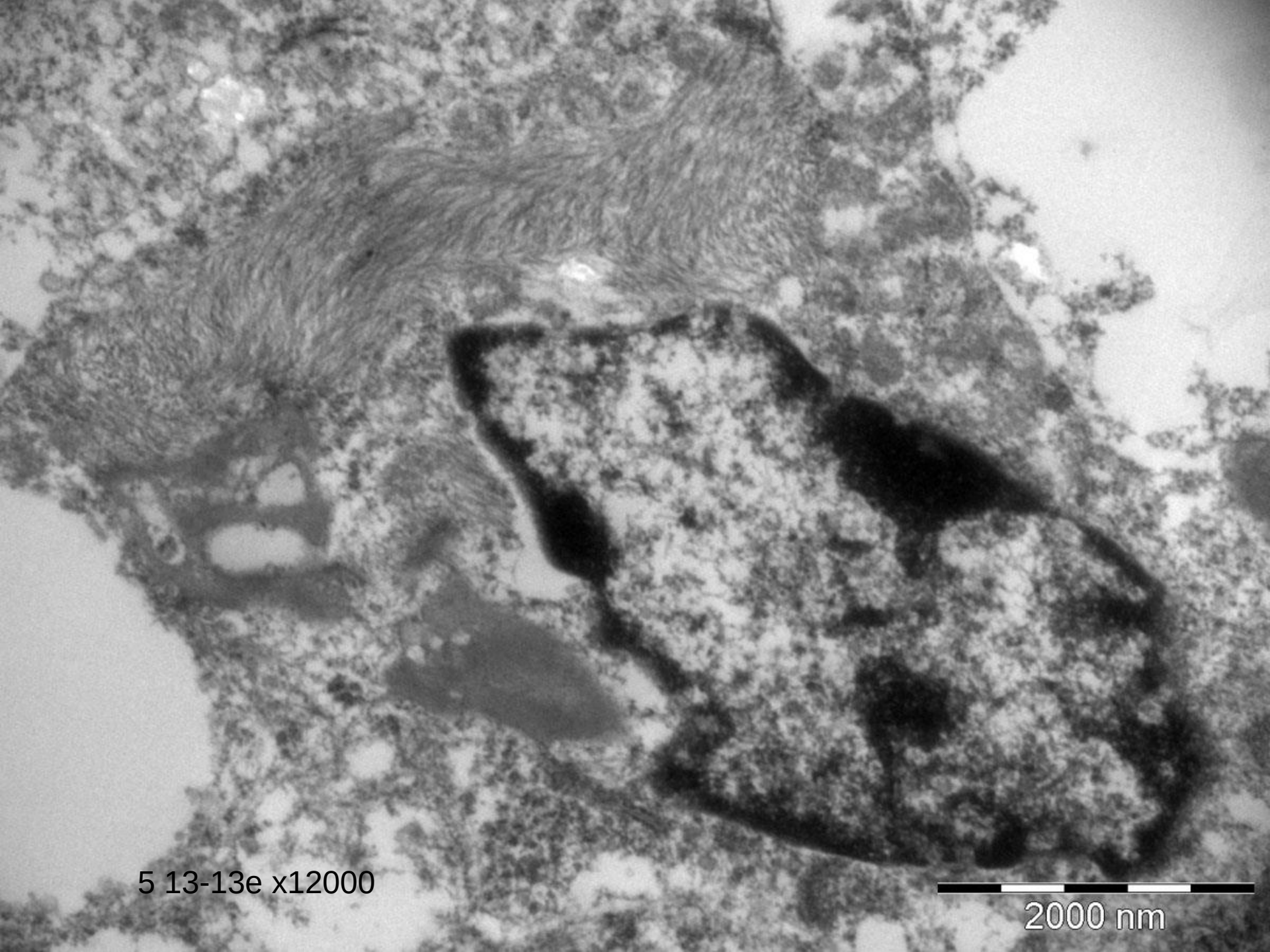
EM to the rescue

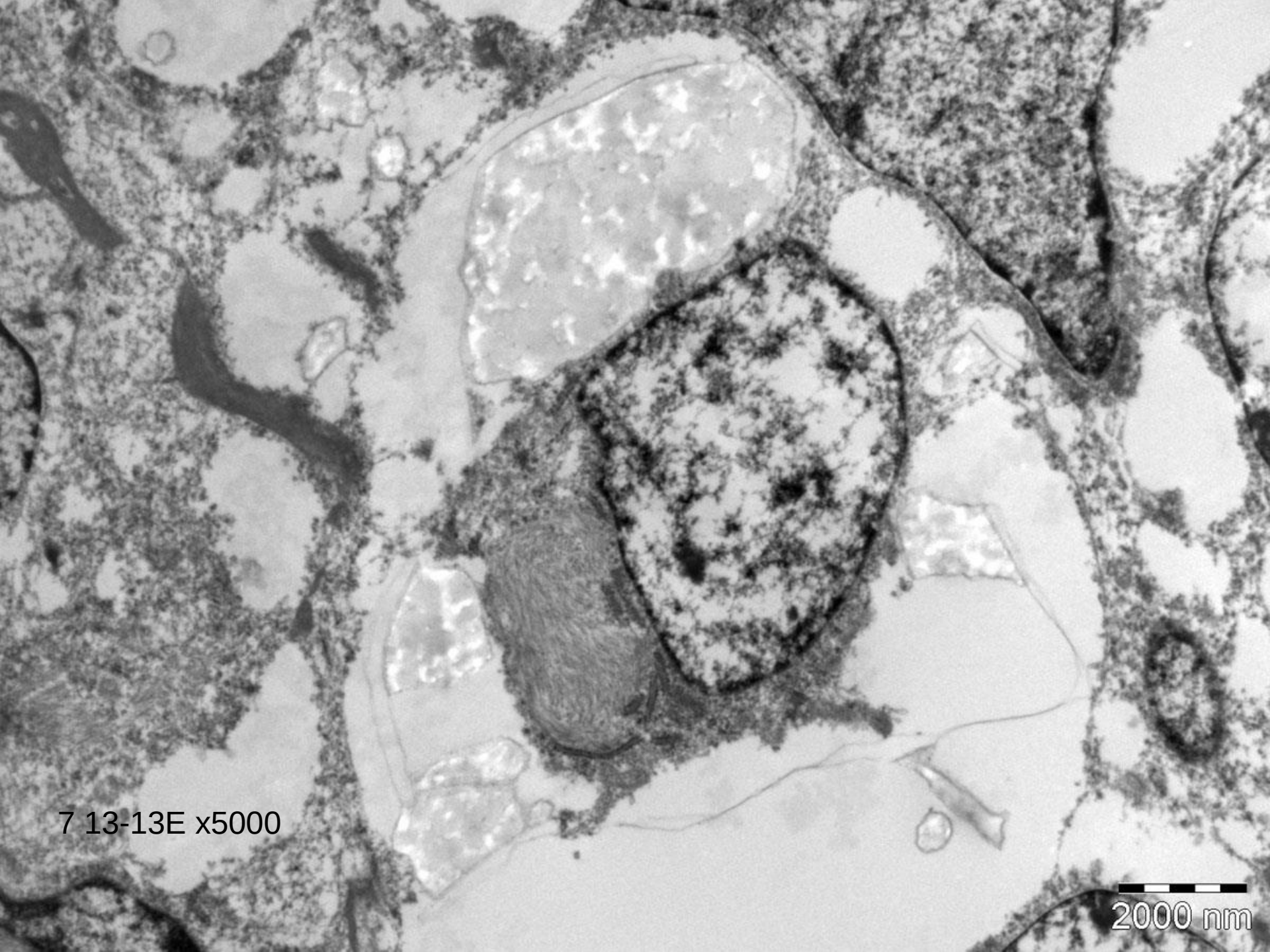
- Suspicion of malignant rhabdoid tumor
- Comment “Because no molecular diagnostic method is in routine clinical use at present we will attempt to use a different method (electron microscopy) to try to confirm this diagnosis” DJF
- 02/01/13

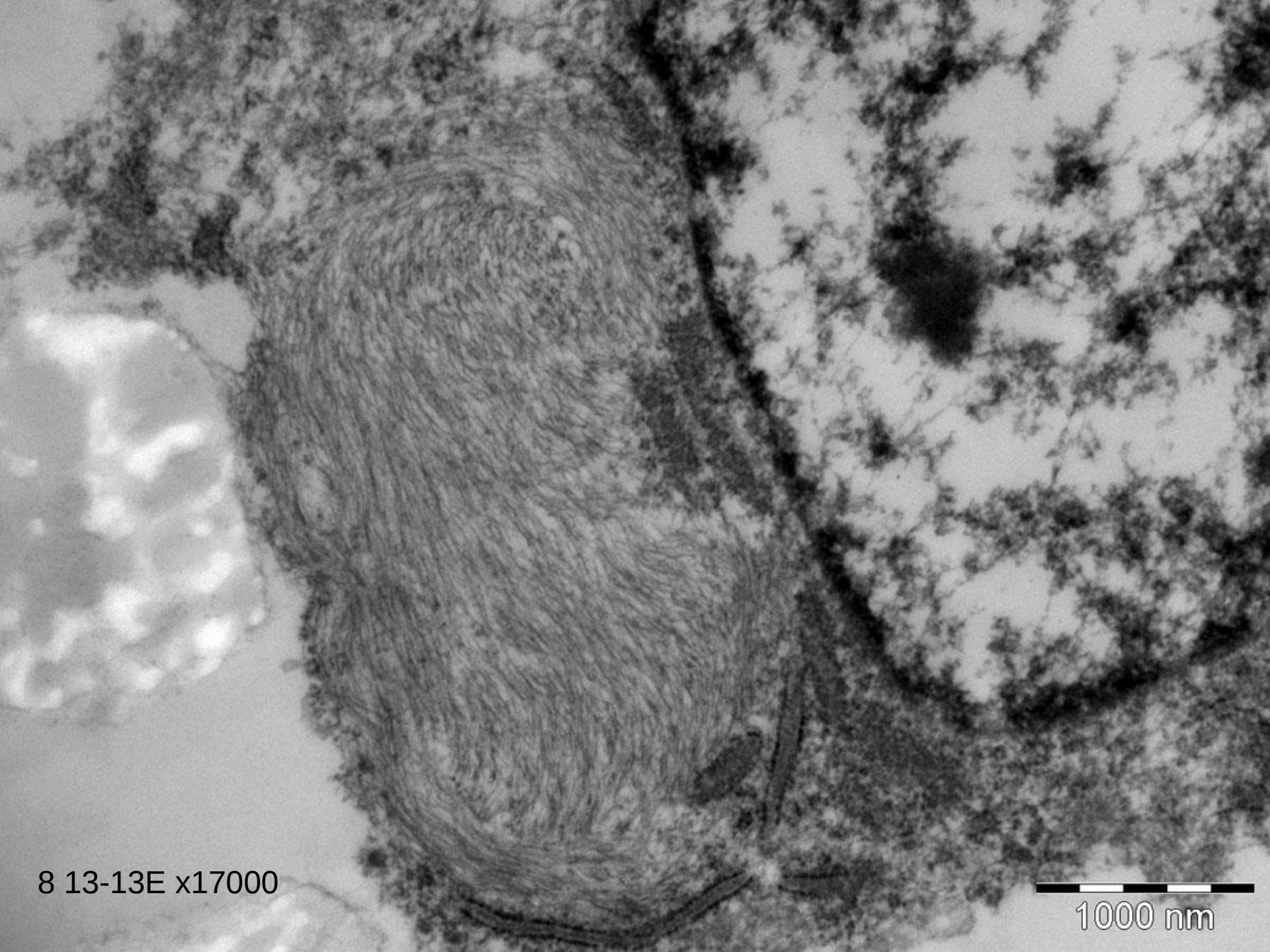


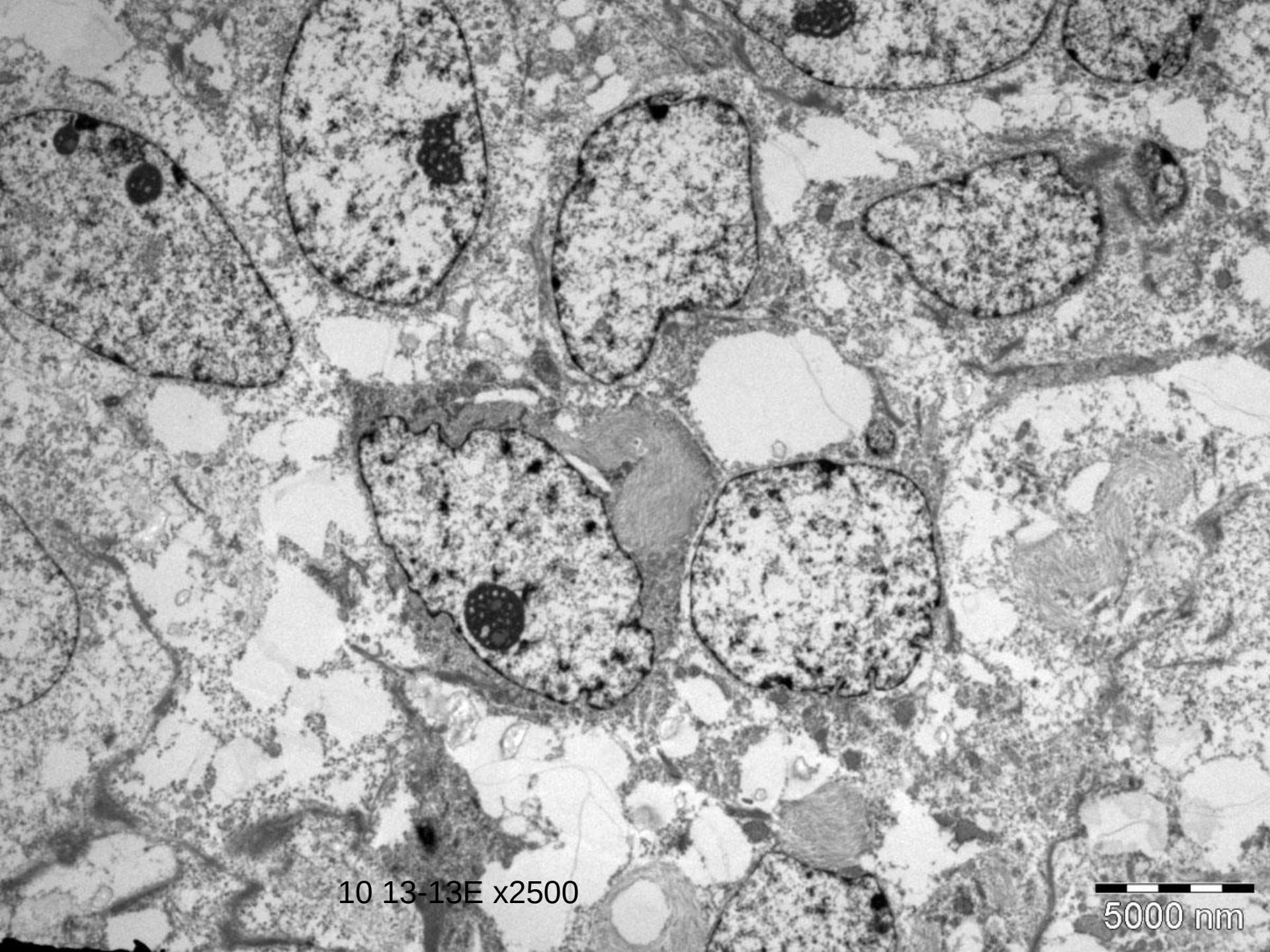
Semithin toludine blue

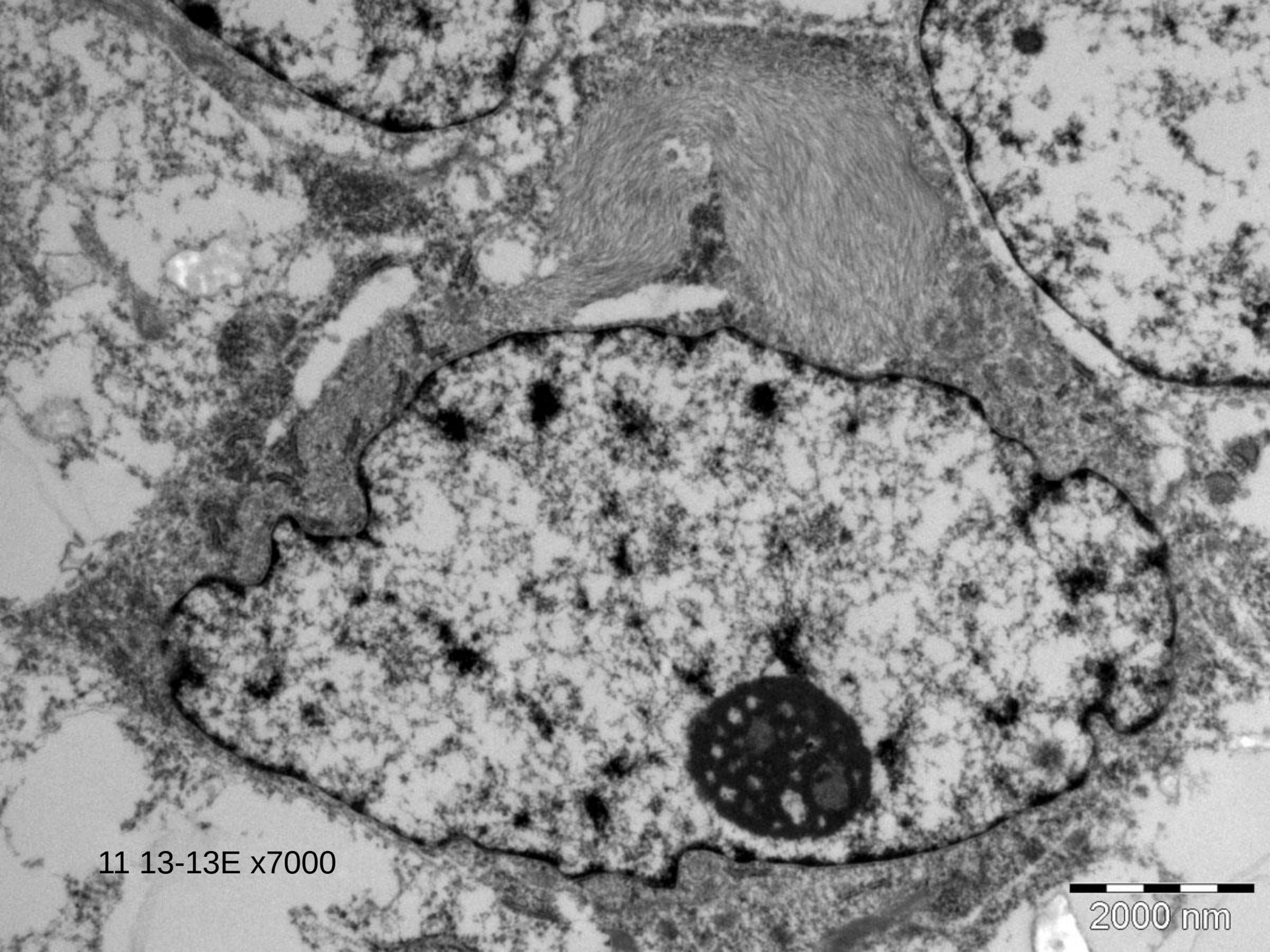

4 13-13E x5000
2000 nm

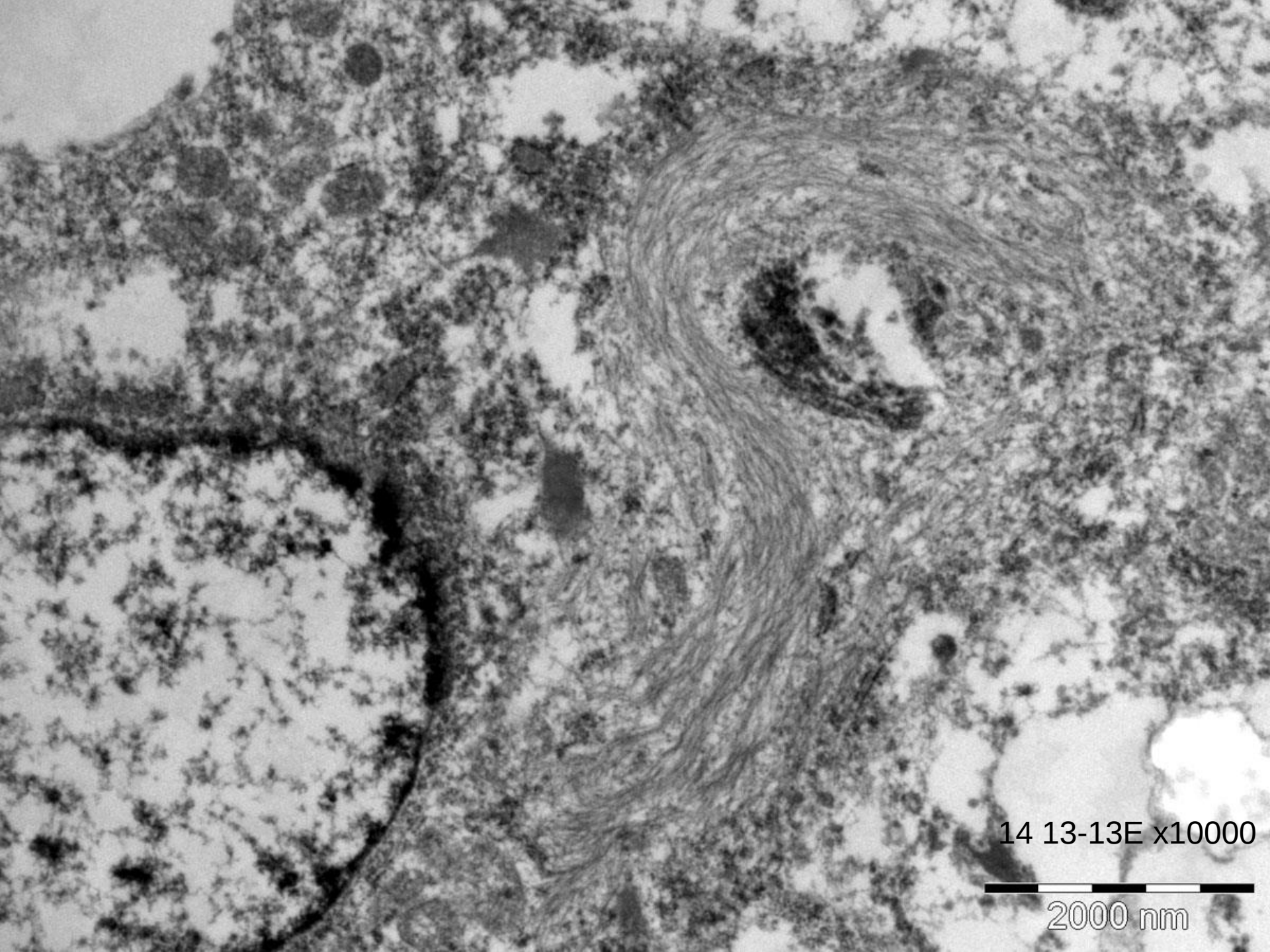

5 13-13e x12000
2000 nm


7 13-13E x5000
2000 nm


8 13-13E x17000
1000 nm


10 13-13E x2500
5000 nm


11 13-13E x7000
2000 nm


14 13-13E x10000
2000 nm

“Mention in dispatches”

- Tissue was retrieved from paraffin blocks (by Lizzie Angus). Multiple EM fields at different magnifications (2500 - 50000x) show paranuclear whorls of intermediate filaments confirming the diagnosis of malignant rhabdoid tumour. 10/01/2013

FFPE tissue sections

- molecular genetics / paediatric malignancy unit GOSH
- No mutations were detected in the SMARCB1 (INI1) gene by direct sequence analysis.

Additional information: The majority of rhabdoid tumours demonstrate SMARCB1 loss and concominant deletions or mutations of the SMARCB1 gene

26/02/13

Further molecular work

- Royal National Orthopaedic Hospital, Stanmore

- Interphase FISH for FUS and SS18 genes rearrangement: Negative.
- Interphase FISH for EWSR1 gene rearrangement: Non-informative.

Final molecular report GOSH

- Bi-allelic loss of the entire SMARCB1 (INI1) gene was detected by MLPA analysis. 03.04.13
- FINAL DIAGNOSIS: malignant rhabdoid tumour

Timeline: 27/12/12 biopsy

02/01/13 LM impression

10/01/13 EM diagnosis

26/02/13 direct sequencing

03/04/13 MLPA, molecular confirmation

Acknowledgements

- Biomedical Imaging Unit / EM (Lizzie Angus, Sue Cox, Katrin Kronenberger, Matt Sharp, Janna Collier, Patricia Goggin, David Johnston, Anton Page, Suzanne)
- Paediatric renal team (Dr Rodney Gilbert, Dr Shuman Haq, Dr Arvind Nagra)
- Dr Paul Bass, Dr Peter Gonda, Dr Nicholas Marley, Prof Terry Cook, Dr Catherine Horsfield,
- Renal patients, parents & families

South Academic (“Pathology”) Corridor