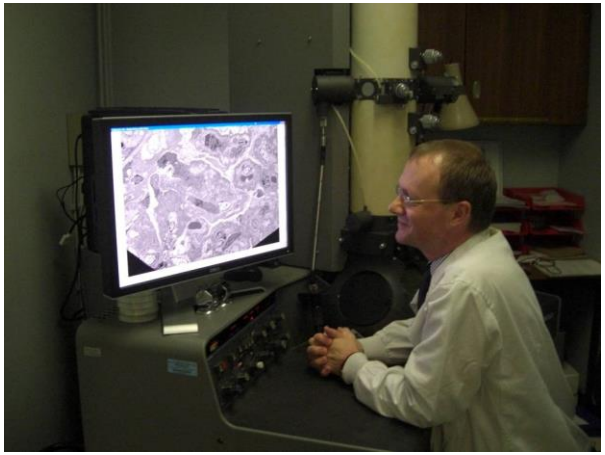
Basic Renal EM workshop

Southampton

September 30th 2011

The Renal Biopsy

Technical Aspects



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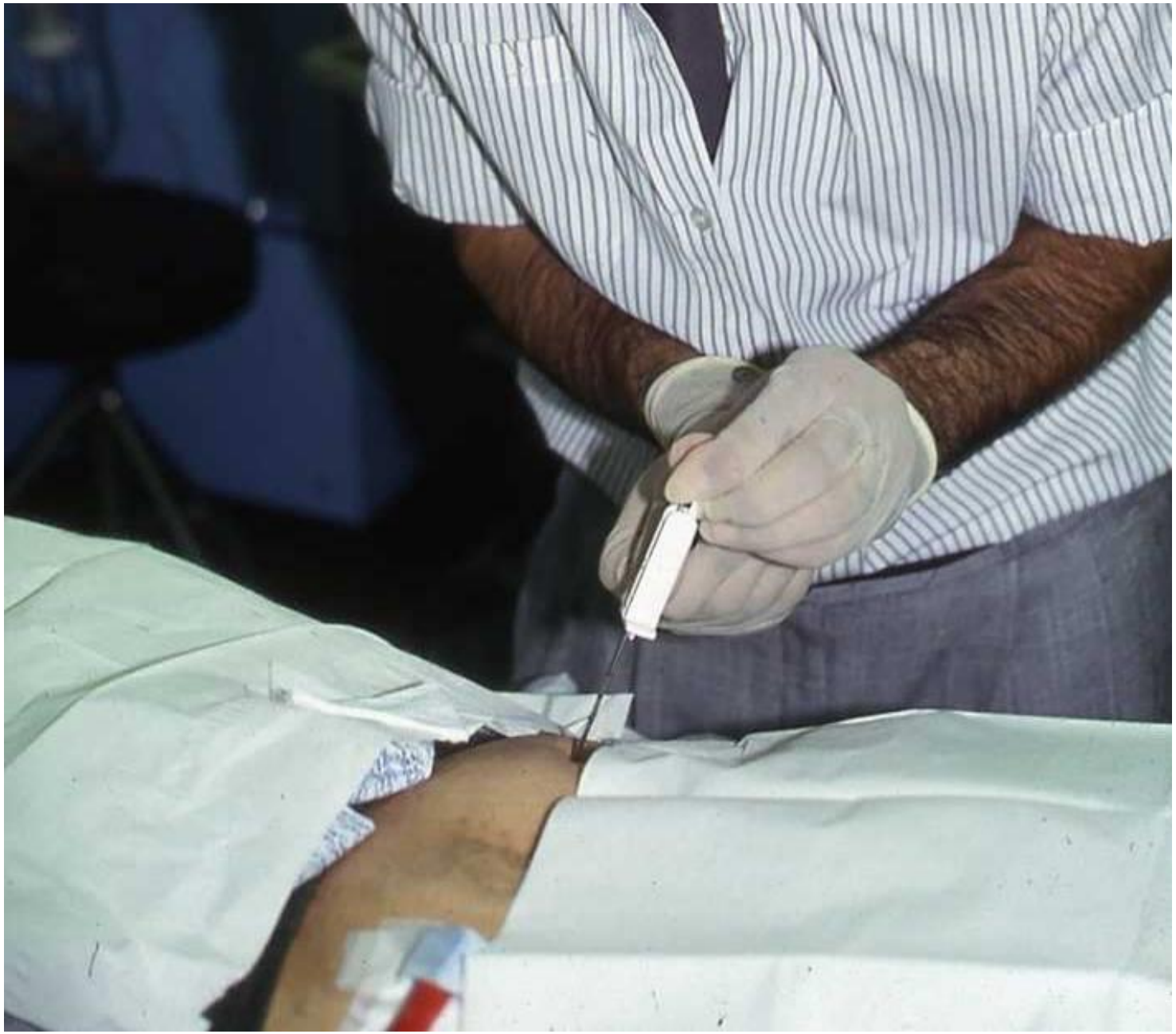
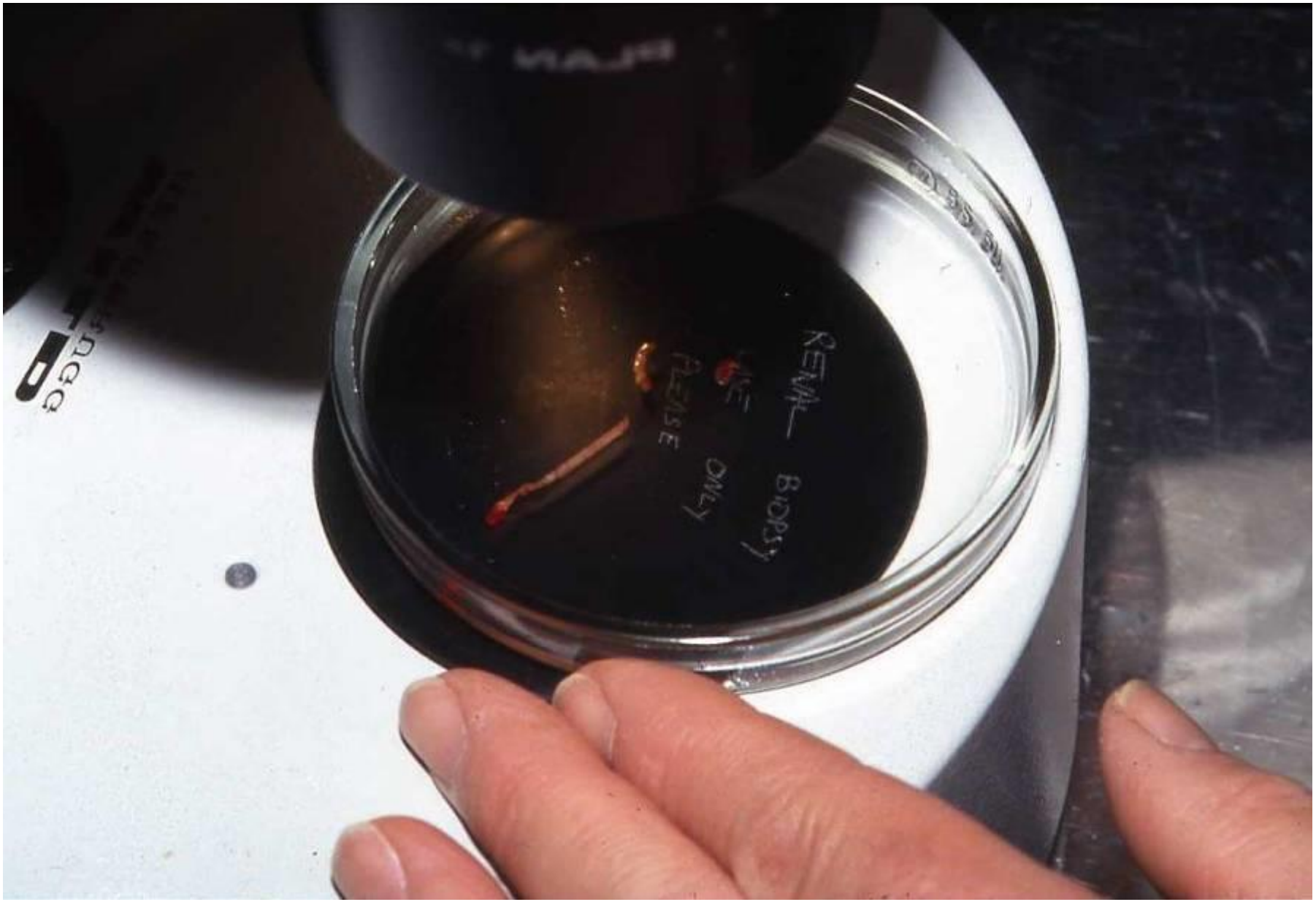


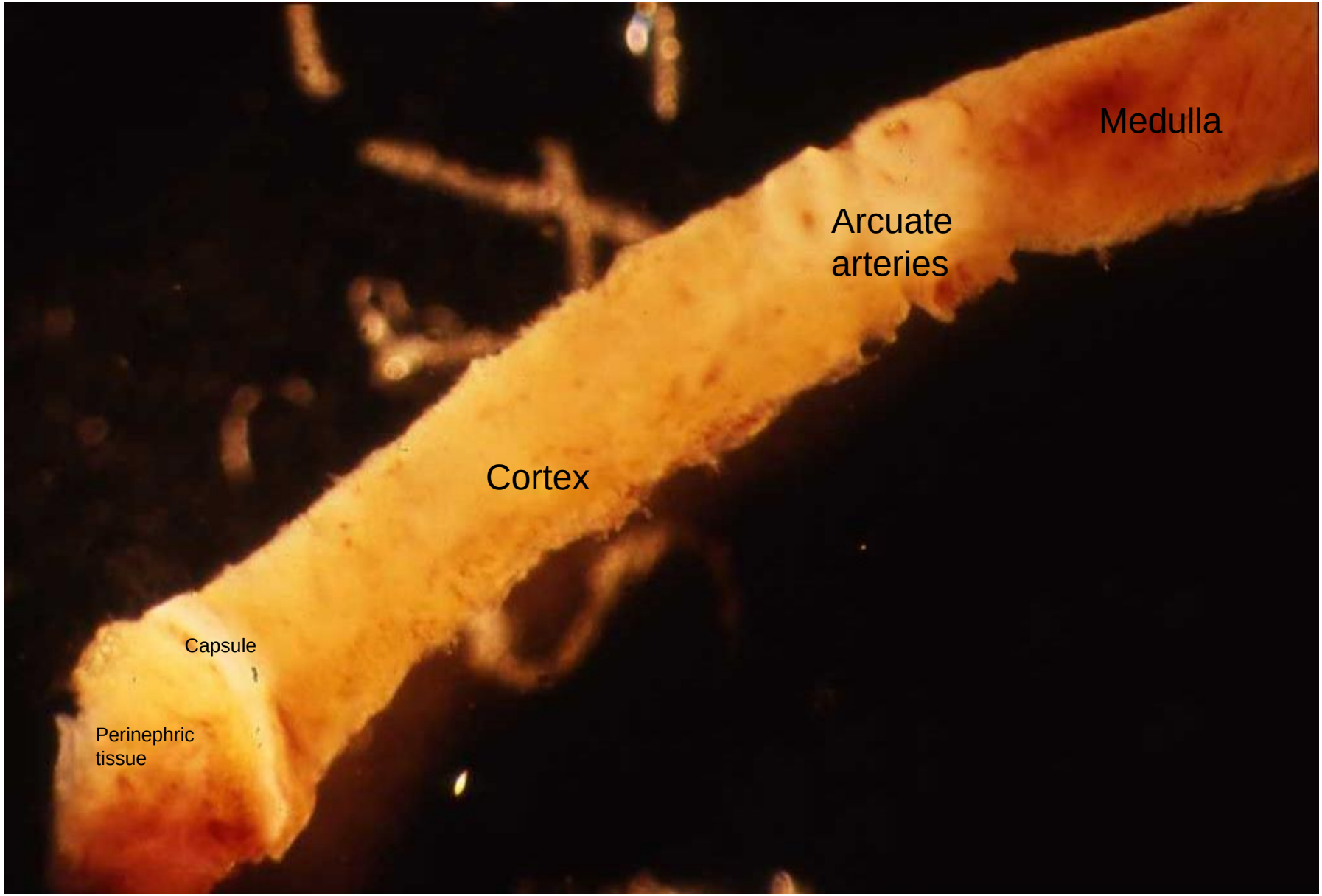
Needle biopsy done with ultrasound guidance under local anaesthesia

Stereo microscope awaiting receipt of renal biopsy for assessment of renal cortex (glomeruli)



Renal biopsy placed immediately in isotonic solution and examined for presence of renal cortical tissue – biopsies can be left in isotonic saline for up to ten minutes without any anoxic damage.

View of renal biopsy core as seen using stereo microscope



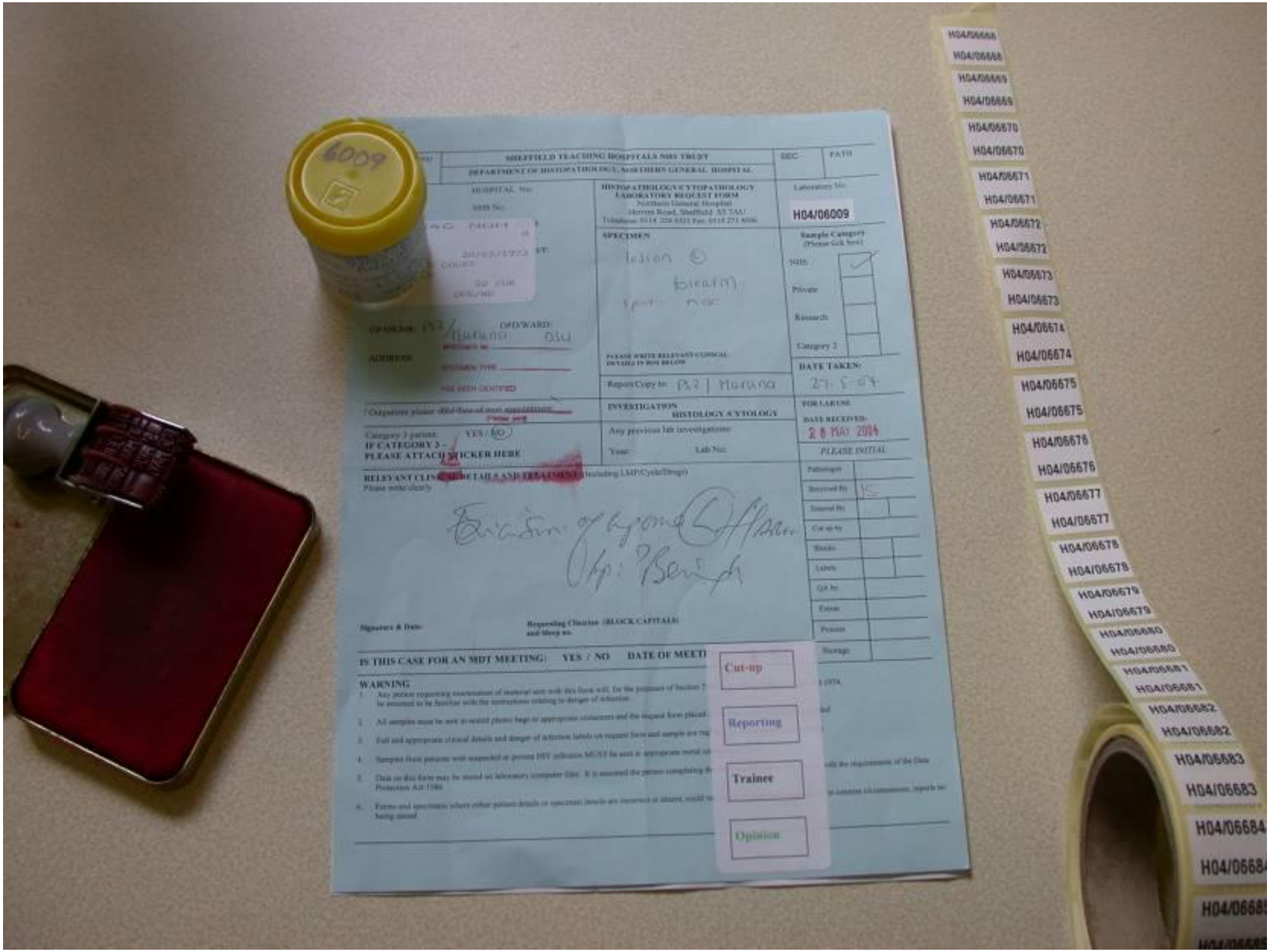
View of renal biopsy core as seen using stereo microscope

Renal biopsy x2
Divided and placed into 3 containers

- Formalin for Histology – Whole of first core
- Glutaraldehyde for Electron Microscopy – Capsule plus 2 – 3 mm of cortex from second core
- Michel’s medium for Immunofluorescence – Remainder of second core

The Renal Biopsy

Histology

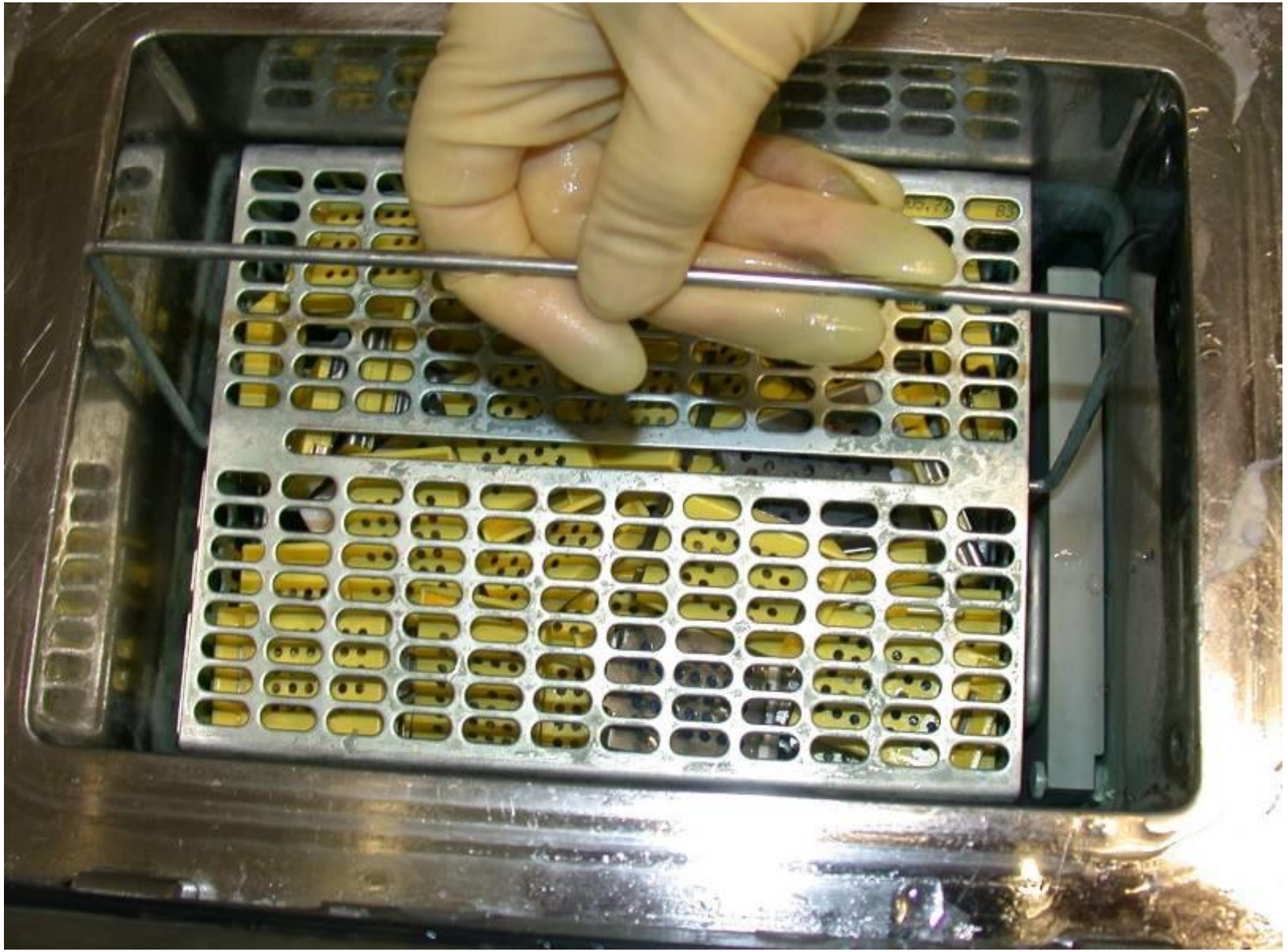


Biopsy placed in formalin - labelled - request form filled in – assigned laboratory number.

Cassettes labelled with laboratory number

Specimens described, dissected, and placed in cassette
– work done on downdraft table to remove formalin fumes

Tissue processing machine



Cassettes with tissue in, placed in wax processing machine.

Reagents pumped into processing chamber

Paraffin processing schedule

- Formalin
- Formalin
- 70% Alcohol
- 95% Alcohol
- 99% Alcohol
- 99% Alcohol
- 99% Alcohol
- 99% Alcohol
- Xylene
- Xylene
- Xylene
- Molten Wax
- Molten Wax
- Molten Wax

Paraffin processing schedule

• Formalin	Fixation
• Formalin	
• 70% Alcohol	
• 95% Alcohol	
• 99% Alcohol	Dehydration
• 99% Alcohol	
• 99% Alcohol	
• 99% Alcohol	
• Xylene	
• Xylene	Transitional solvent
• Xylene	
• Molten Wax	
• Molten Wax	Infiltration
• Molten Wax	

Wax embedding centre

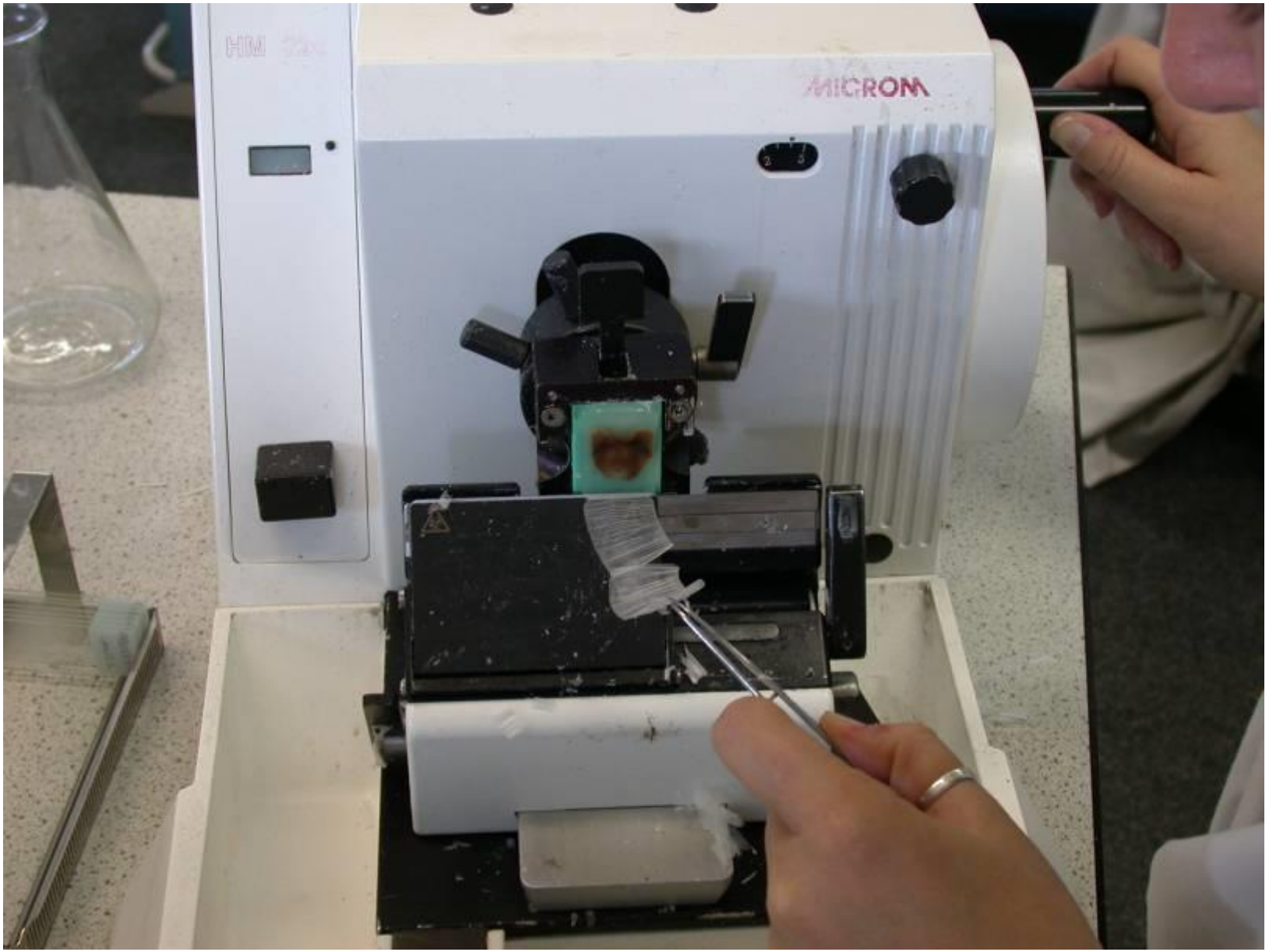
- Tissue removed from cassette.
- Placed in mould
- Filled with molten wax
- Cassette placed on top
- Filled up with wax
- Allowed to set.

•Tissue blocks cooled on ice tray

•Sectioned on microtome using disposal blades – most biopsies at 4 microns, renal biopsies at 2 microns

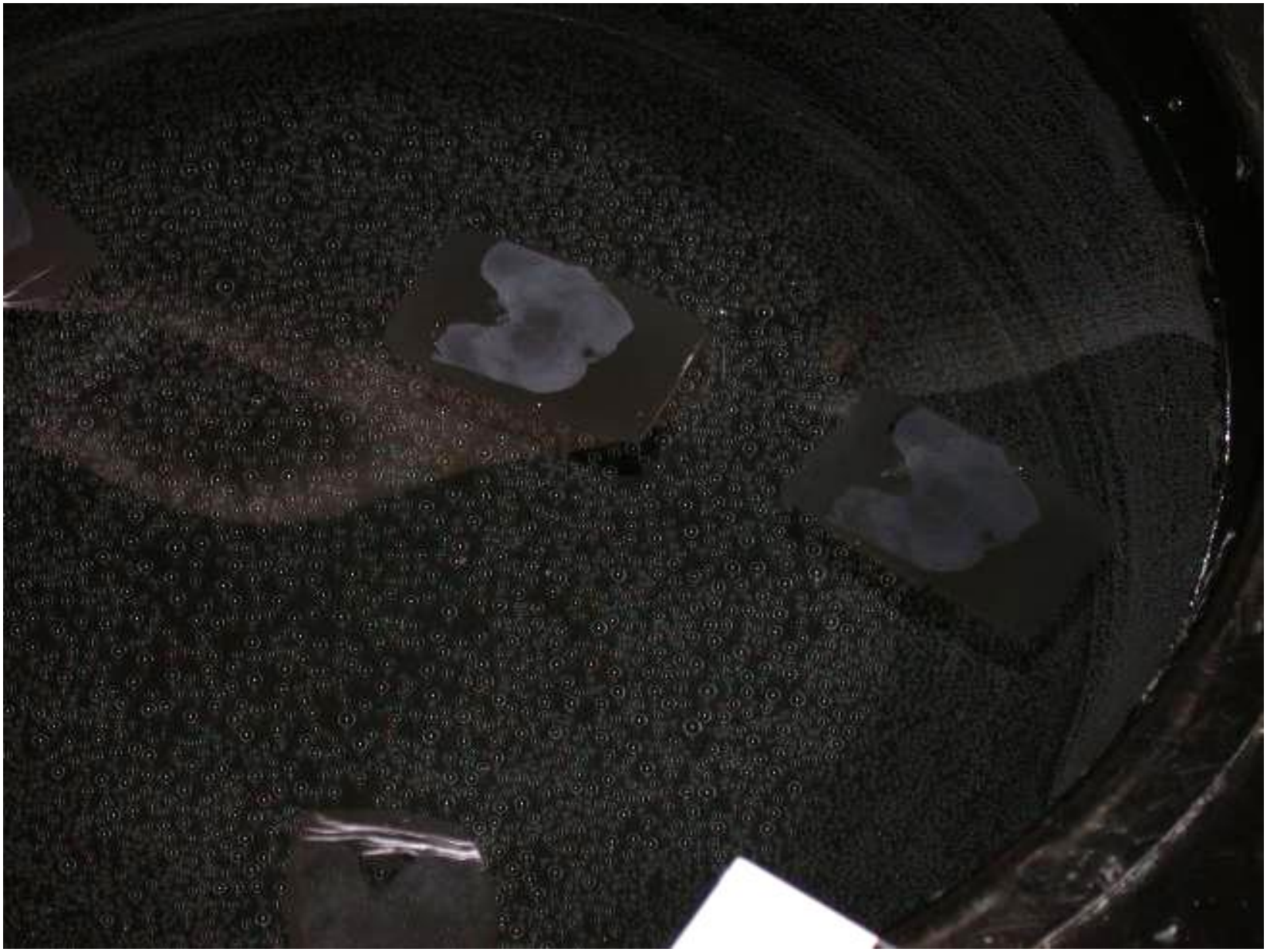
•Sections floated out on warm water bath – just below melting point of wax

•Sections picked up on labelled glass slide



Paraffin sections being cut on rotary microtome

Paraffin sections being cut on rotary microtome – using disposable blade



Paraffin sections floating on water at 56 degrees C

Section taken off water bath and picked up on labelled glass slide

Automated Haematoxylin and Eosin (H&E) staining machine

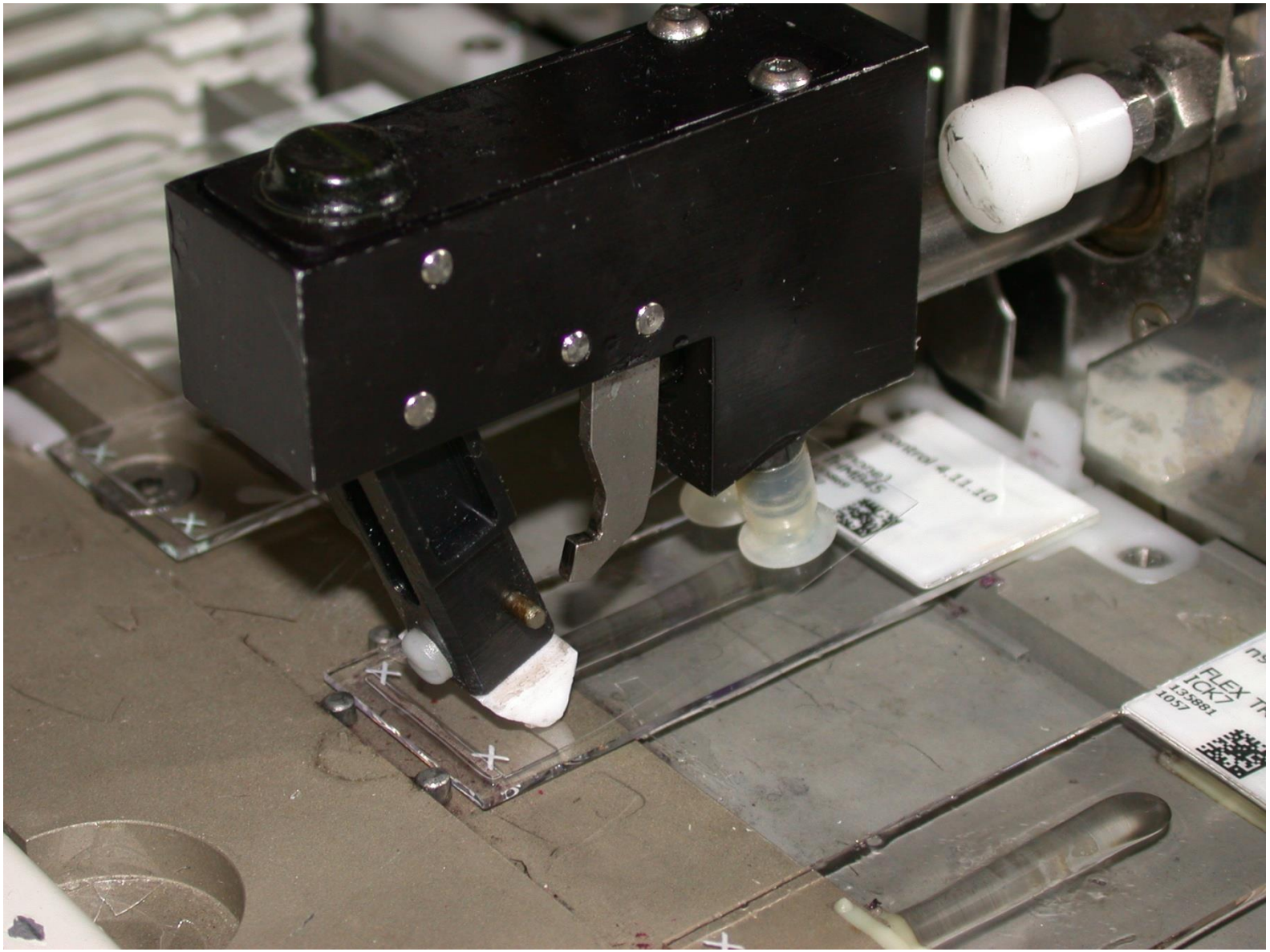
H&E staining

• Heat: to melt wax and stick section onto glass slide.	4.0 min
• Xylene: to remove wax from section.	1.5 min
• Alcohol: to remove xylene from section.	1.5 min
• Water: to remove alcohol.	0.5 min
• Haematoxylin: to stain nuclei.	3.0 min
• Water: to remove excess stain.	0.5 min
• Acid/alcohol to remove stain from cytoplasm.	0.5 min
• Water: to remove acid/alcohol and blue nuclei.	2.0 min
• Eosin: to stain cytoplasm.	1.0 min
• Water: to remove excess stain.	0.5 min
• Alcohol: to remove water.	2.5 min
• Xylene: to remove alcohol	standing

Sections progressing through reagents

Automated coverslipping machine

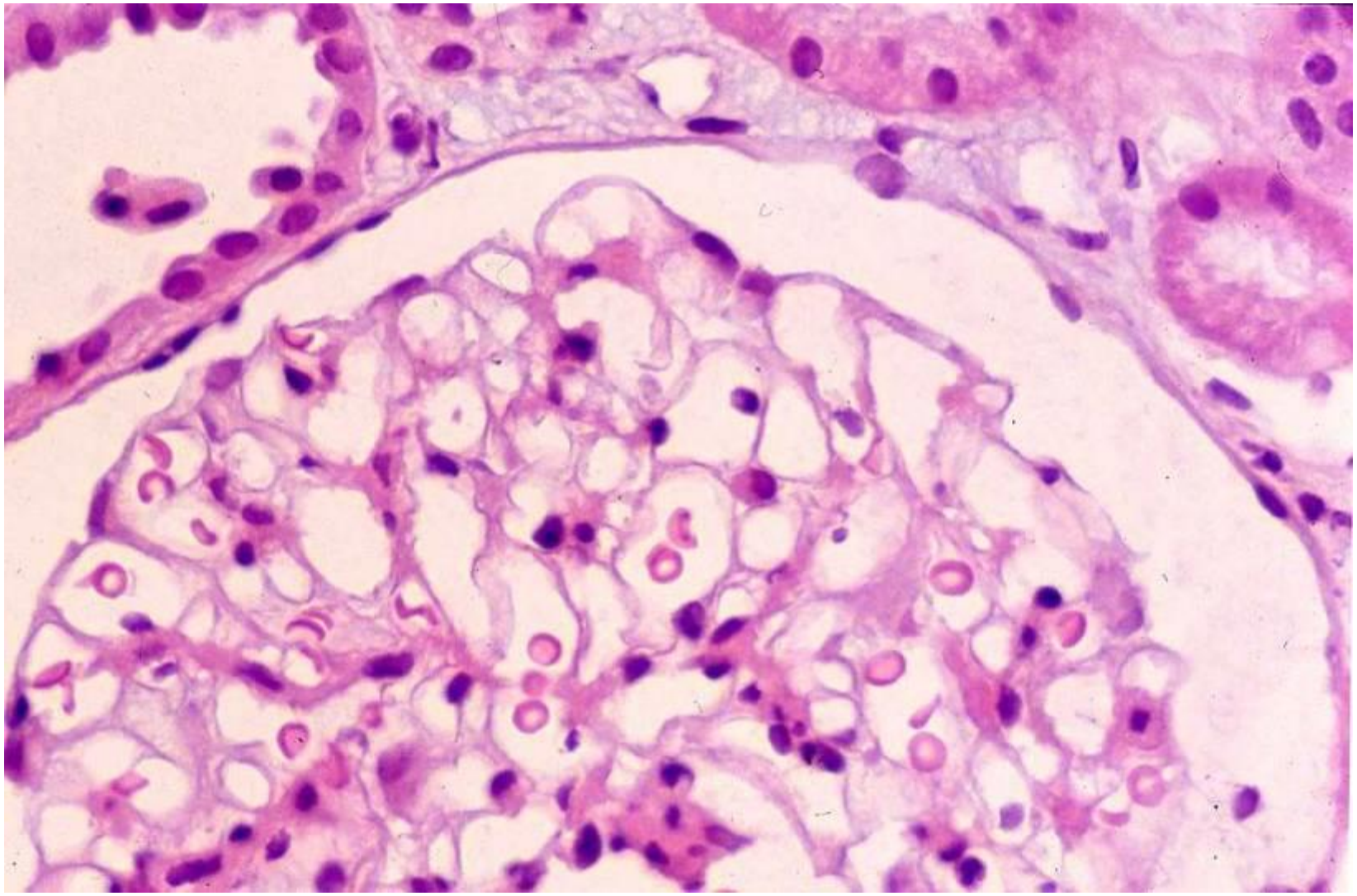
Coverslip placed on mountant (DPX) on slide



DPX is a mixture of Distyrene, a Plasticizer, and Xylene

Stained sections married up with original request form

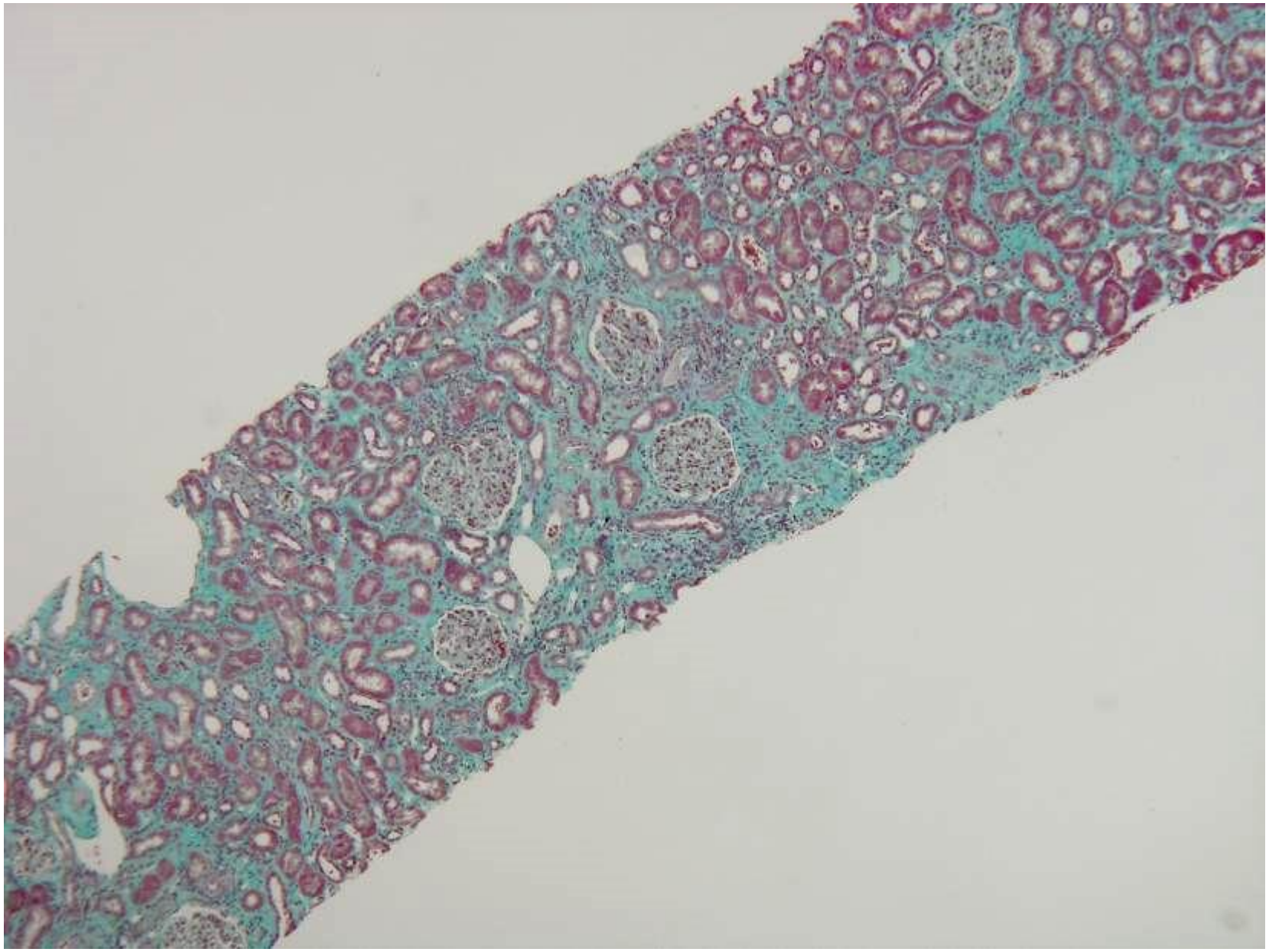
Checked for quality.



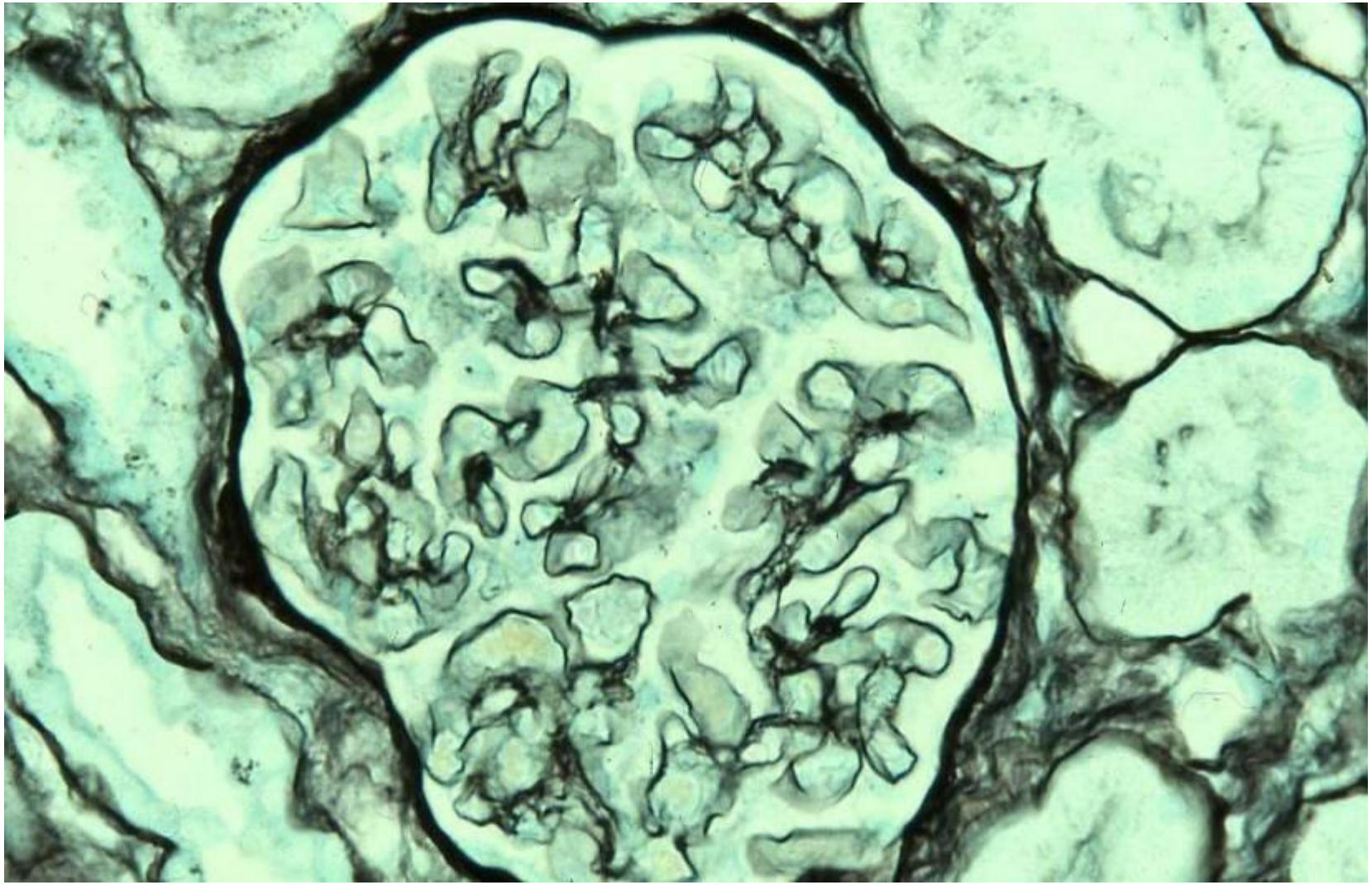
H&E of part of a normal looking glomerulus

Other histochemical stains routinely used for renal histology

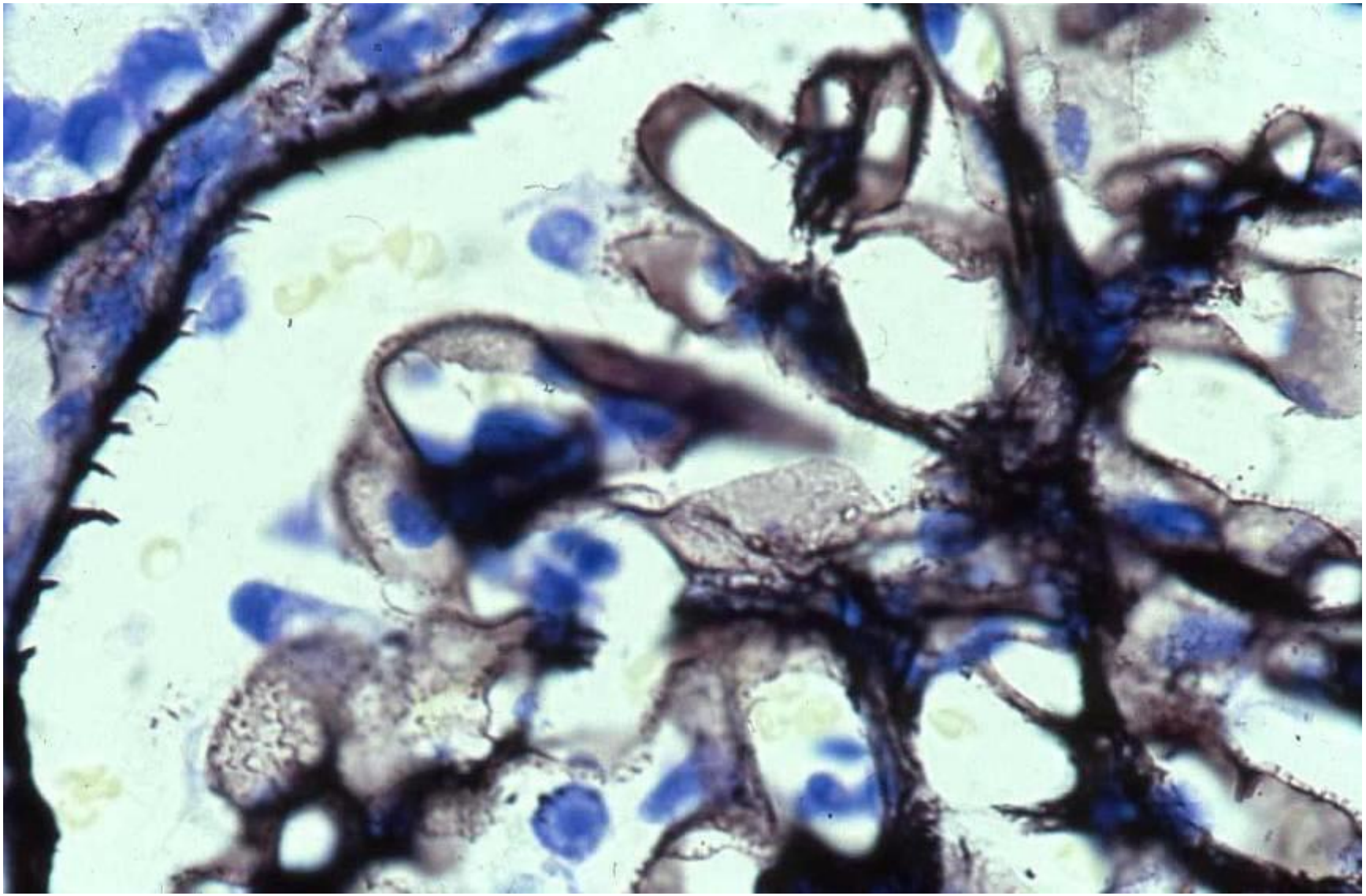
- Masson Trichrome – assesment of interstitial fibrosis
- Methenamine Silver Technique (Grocott-Gomori) – assessment of glomerular basement membrane
- Elastic Stain – assessment of arterial vasculopathy
- Congo or Sirius Red – demonstration of amyloid
- Periodic Acid Schiff's (PAS) stain – demonstration of arteriolar hyalinosis, and basement membranes



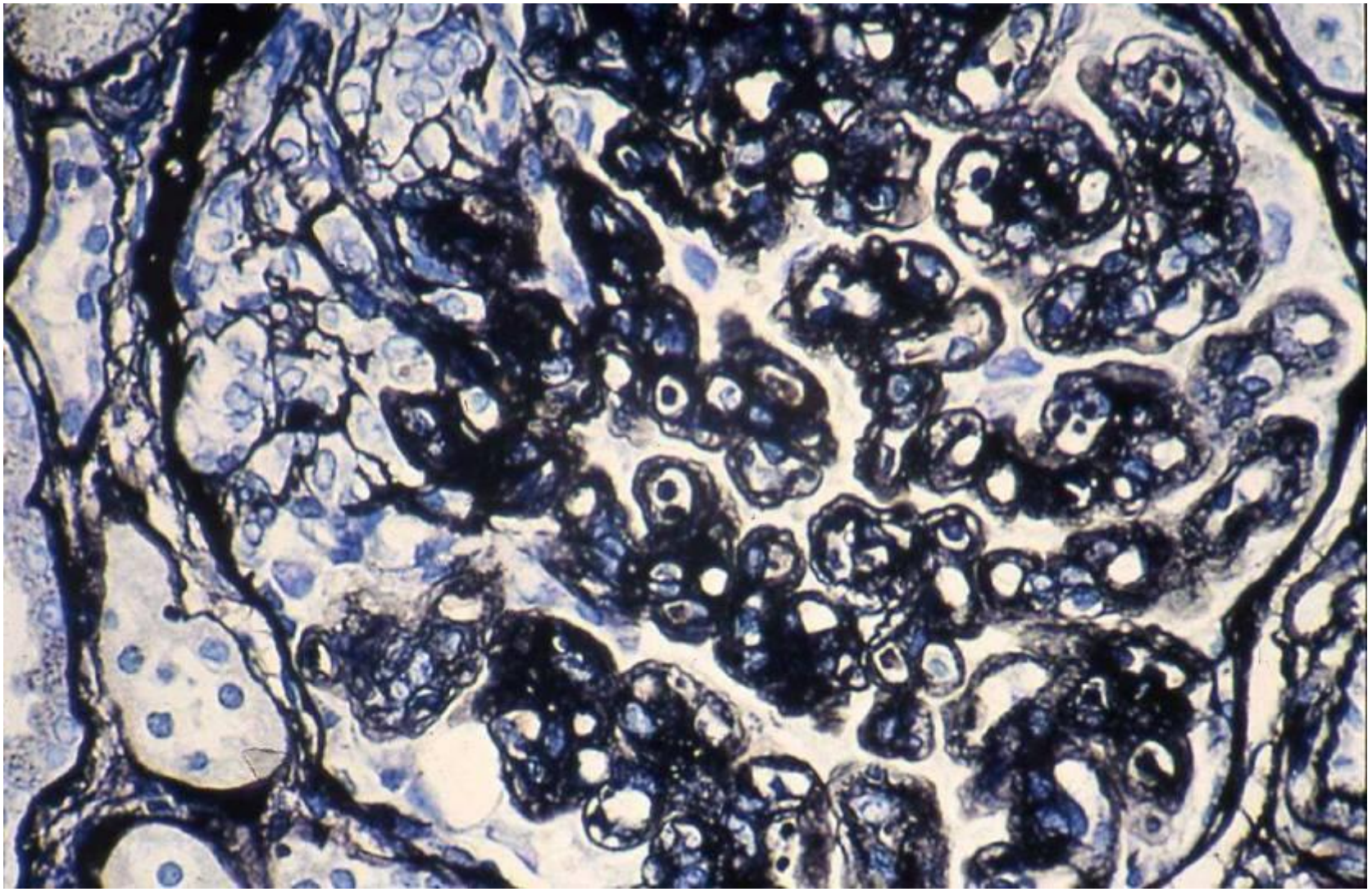
Masson trichrome – interstitium stained green



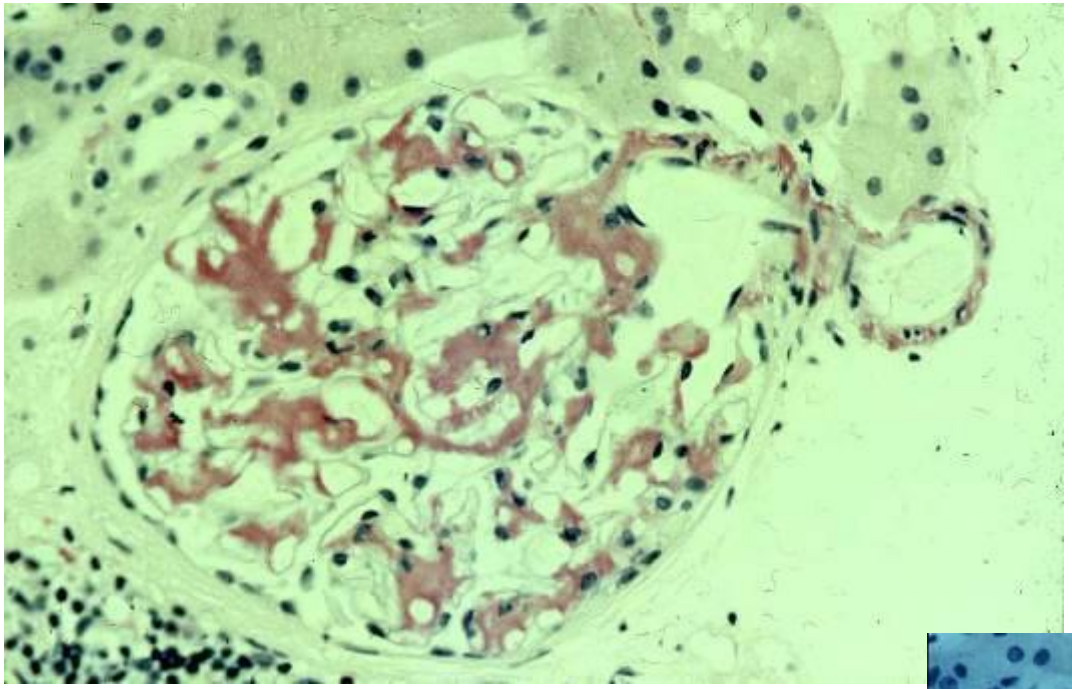
Silver stain – normal glomerulus



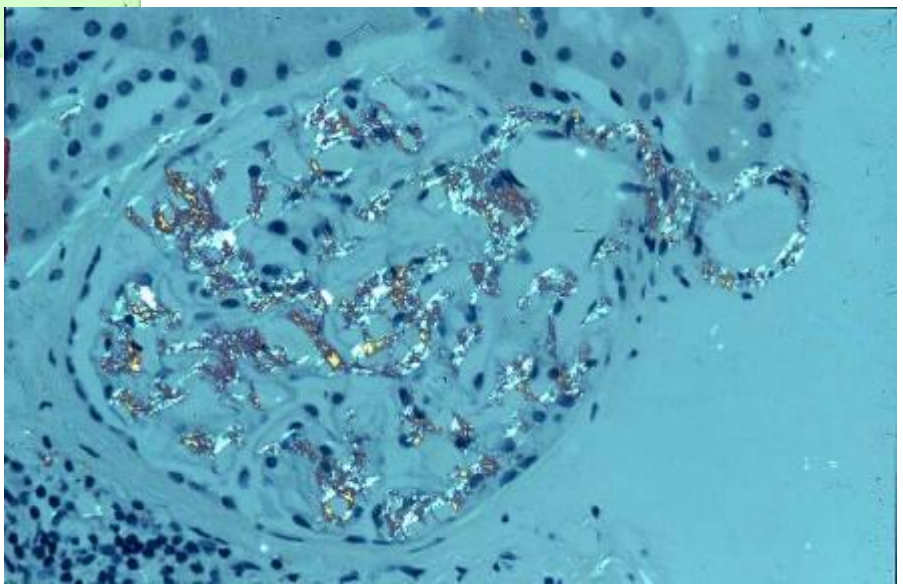
Silver stain – spikes (in between subepithelial deposits)



Silver stain – reduplication of glomerular basement membrane

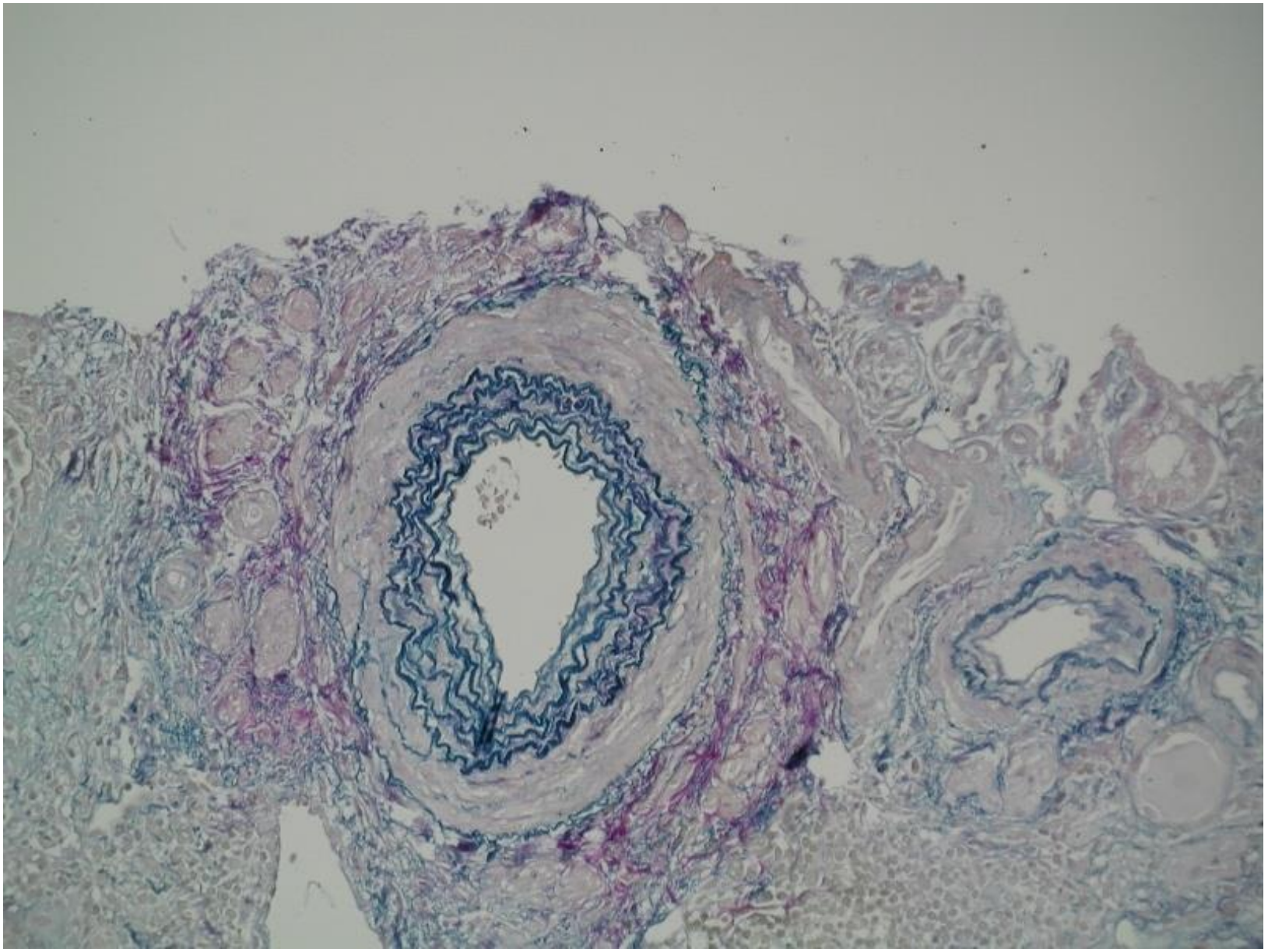


Congo Red – amyloid stain



Congo Red with crossed polarising filters

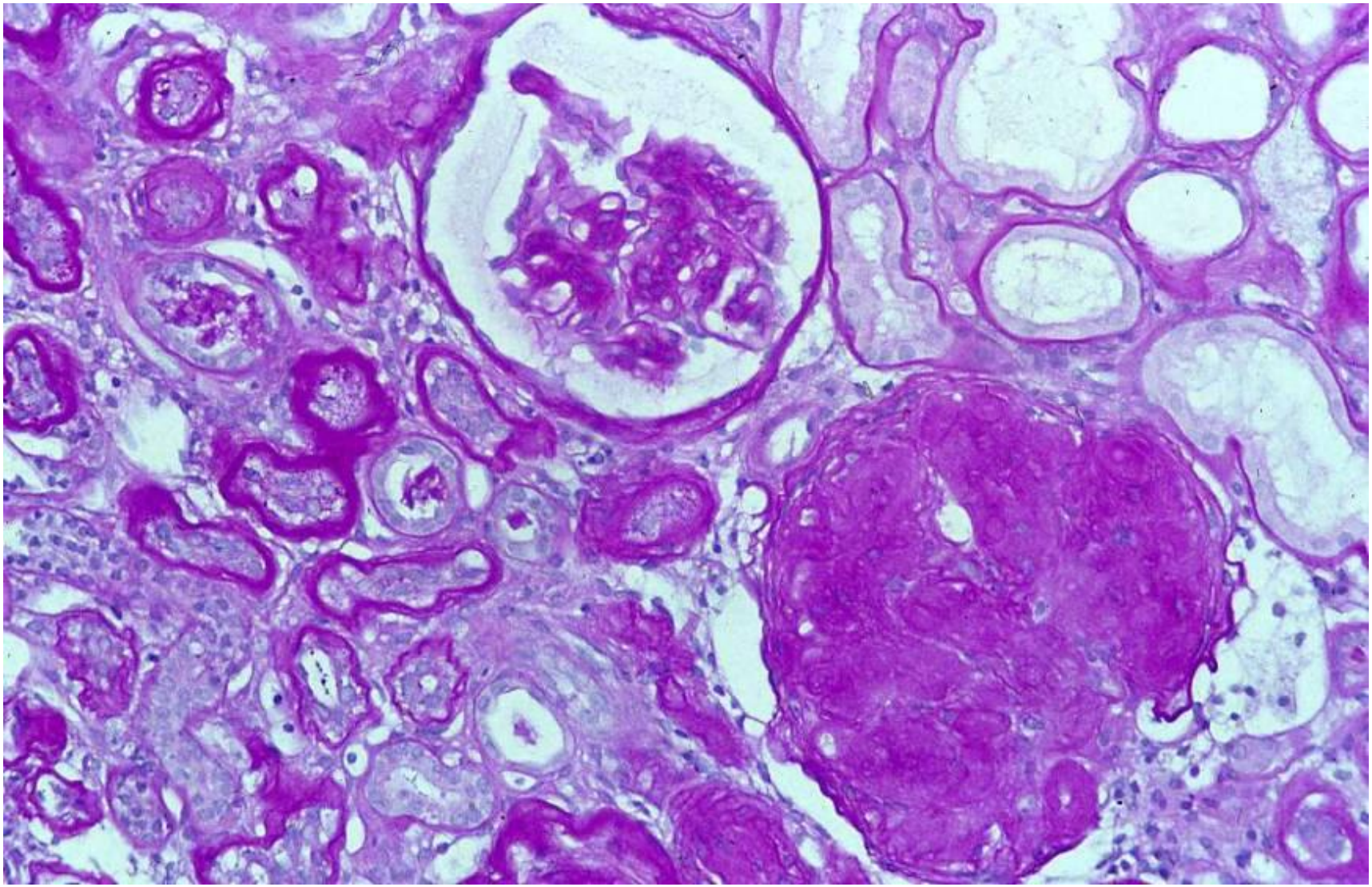
Dichroic birefringence



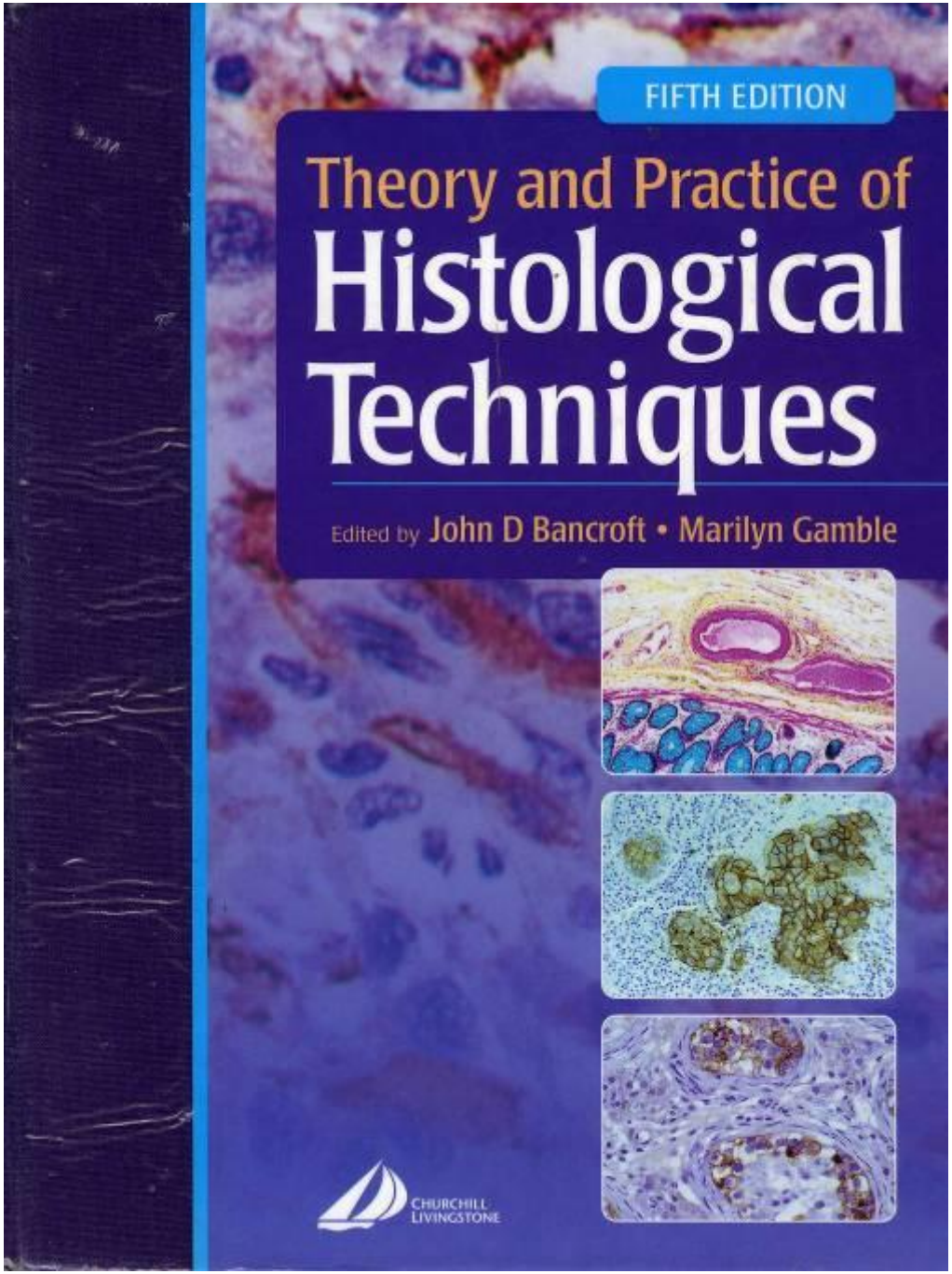
Elastic Van Gieson - Vascular elastic duplication

Periodic Acid Schiff's (PAS) stain

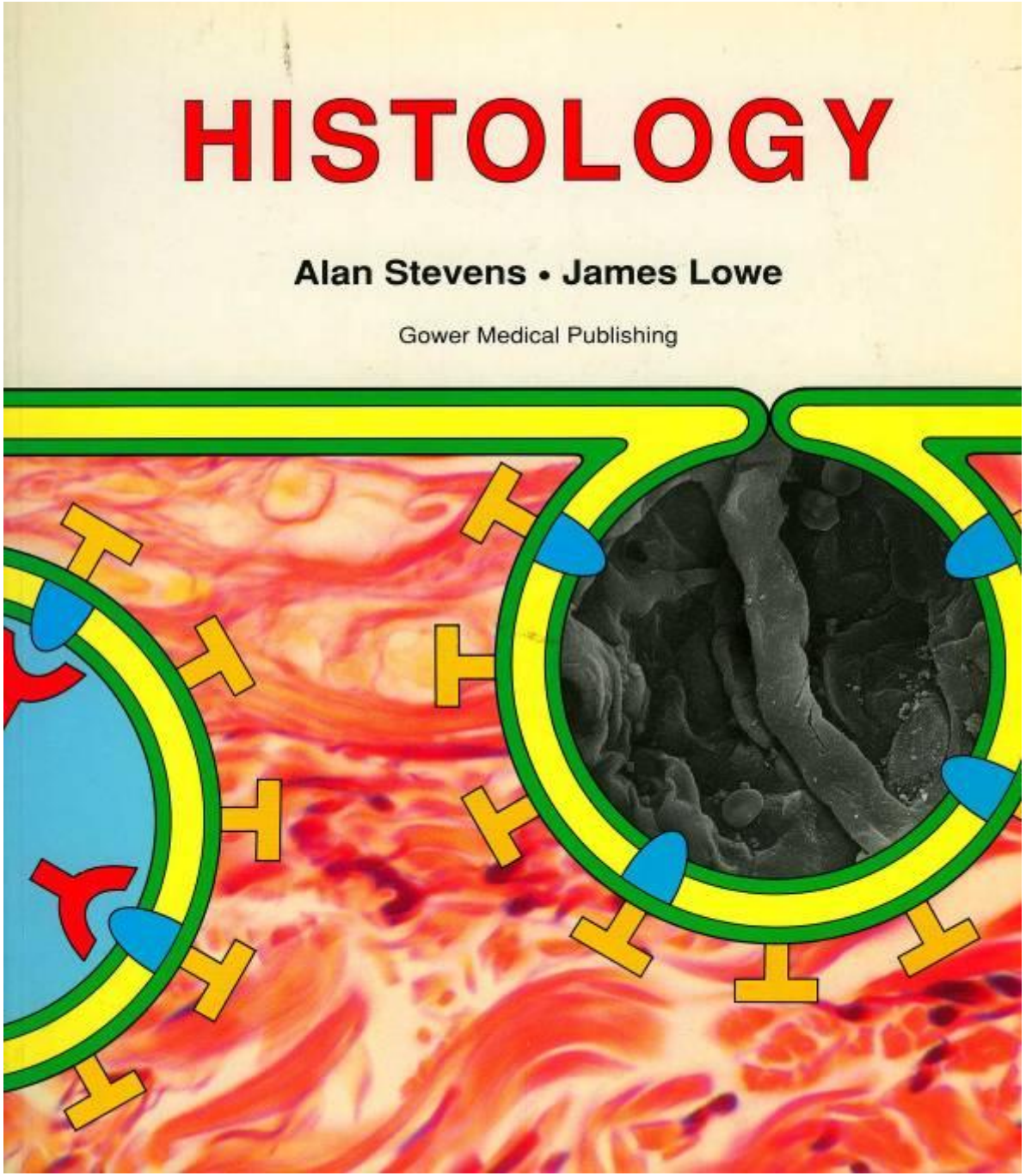
Normal tubules



Atrophic tubules, obsolete glomerulus


FIFTH EDITION
Theory and Practice of
Histological
Techniques
Edited by John D Bancroft • Marilyn Gamble
CHURCHILL
LIVINGSTONE

2002


HISTOLOGY
Alan Stevens • James Lowe
Gower Medical Publishing

1992

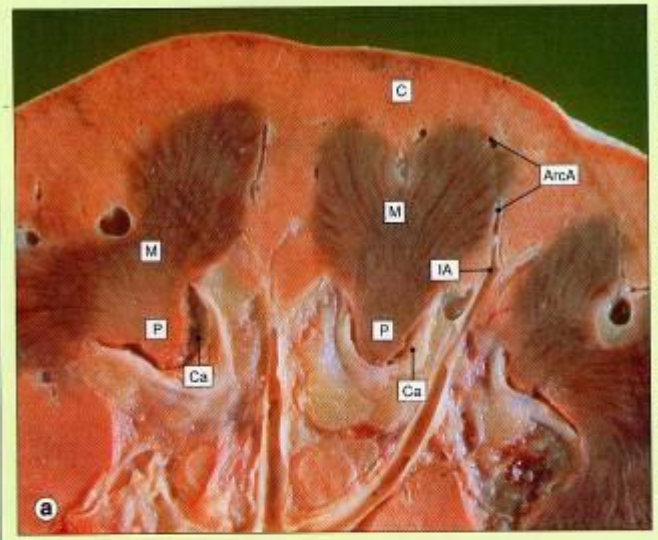


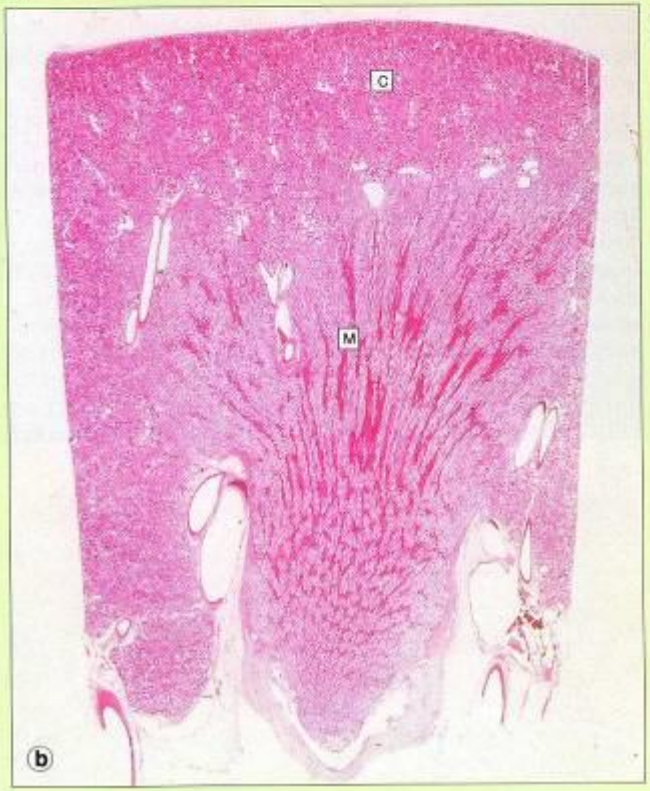
Fig. 16.30 Anatomy of adult kidney.

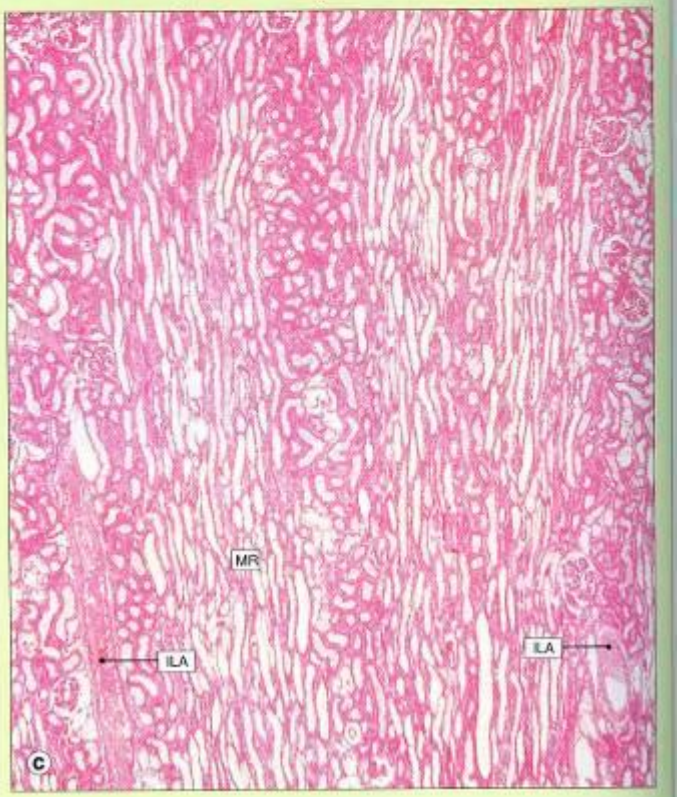
a Photograph of sectioned adult kidney, which has been fixed in formalin and the near natural colour restored in alcohol.Note the cortex (C), the medullary pyramid (M) culminating in the papillary tip (P), which protrudes into the lumen of a calyx (Ca). Interlobar arteries (IA) and arcuate arteries (ArcA) can also be seen. Little detail of cortical structure is visible with the naked eye, but the vertical linearity of the components of the medulla is highlighted by clusters of prominent blood vessels (vasa recta).

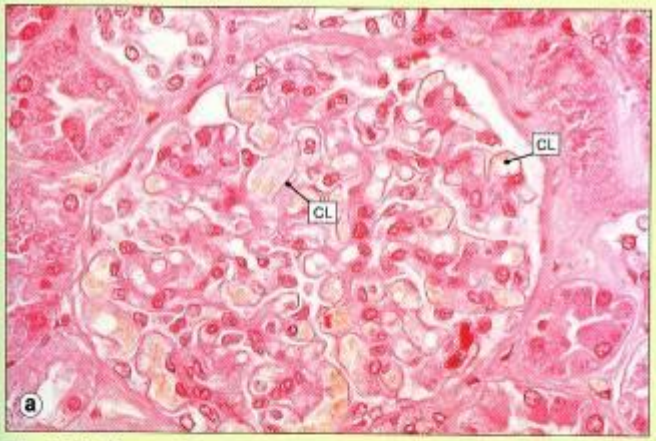
b In this H&E stained paraffin section prepared from the tissue block shown in **a**; the distinction between cortex (C) and medulla (M) can be easily seen. This section also shows the vertical linearity of the components of the medulla, both tubules and vessels.

At this low magnification, glomeruli can be seen as small dots in the cortex. Note that some areas of the cortex are free of glomeruli, but contain vertically running duct systems; these areas are known as medullary rays and represent the sites where cortical tubules drain into the collecting ducts.

c In this micrograph of cortex at a higher magnification than in **b** it can be seen that the medullary ray (MR) area is devoid of glomeruli and that the interlobular arteries (ILA) run in the glomeruli-rich area.







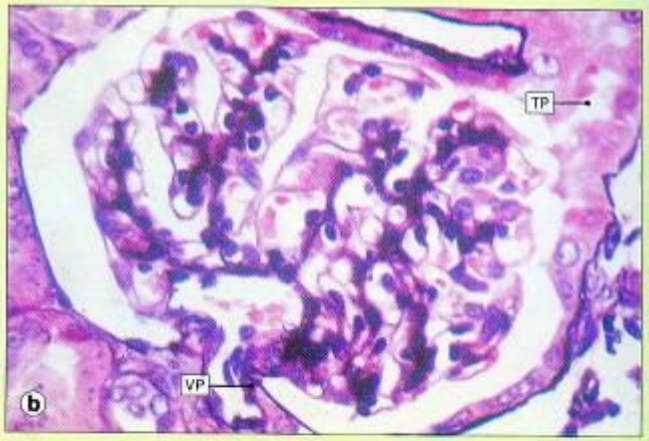


Fig. 16.31 Glomerulus.
a The details of the structure of the glomerular tuft are not easily seen in routine paraffin sections without the assistance of special stains to delineate capillary basement membranes. In this high power micrograph occasional capillary lumina (CL) can be seen, but it is difficult to distinguish clearly between endothelial, mesangial and epithelial podocyte cells.

b A glomerulus stained by the Jones methenamine silver method to show the mesangium and capillary basement membranes. Clear delineation of the capillary basement membrane permits the recognition of endothelial cells (inside the membrane) and epithelial podocytes (outside the membrane). Note that this fortuitous section shows both the vascular (VP) and tubular (TP) poles.

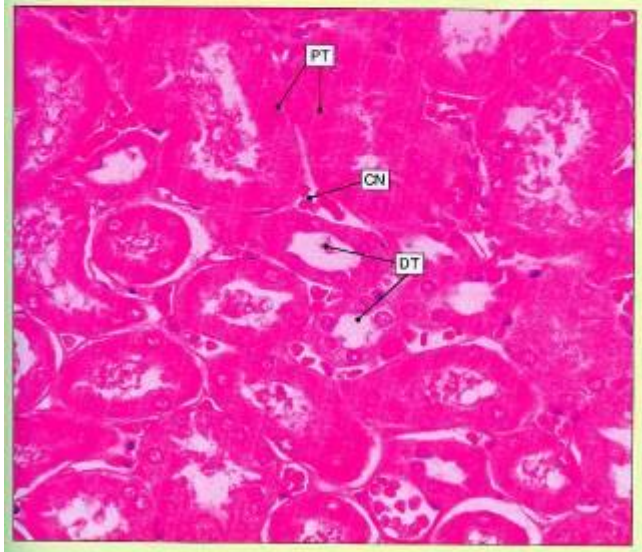


Fig. 16.32 Cortical tubules.
In this high power micrograph of cortical tubules, the proximal tubules (PT) are most numerous and prominent, having tall epithelium and small lumina. Distal tubules (DT) are smaller, have a cuboidal epithelium and proportionately larger lumina. Note the intimate capillary network (CN). Collecting ducts on their way to the medullary ray, and thick and thin loops of Henle are also visible.

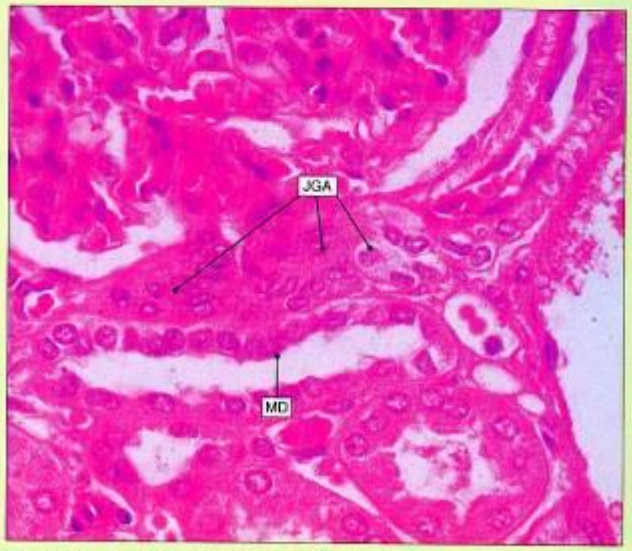
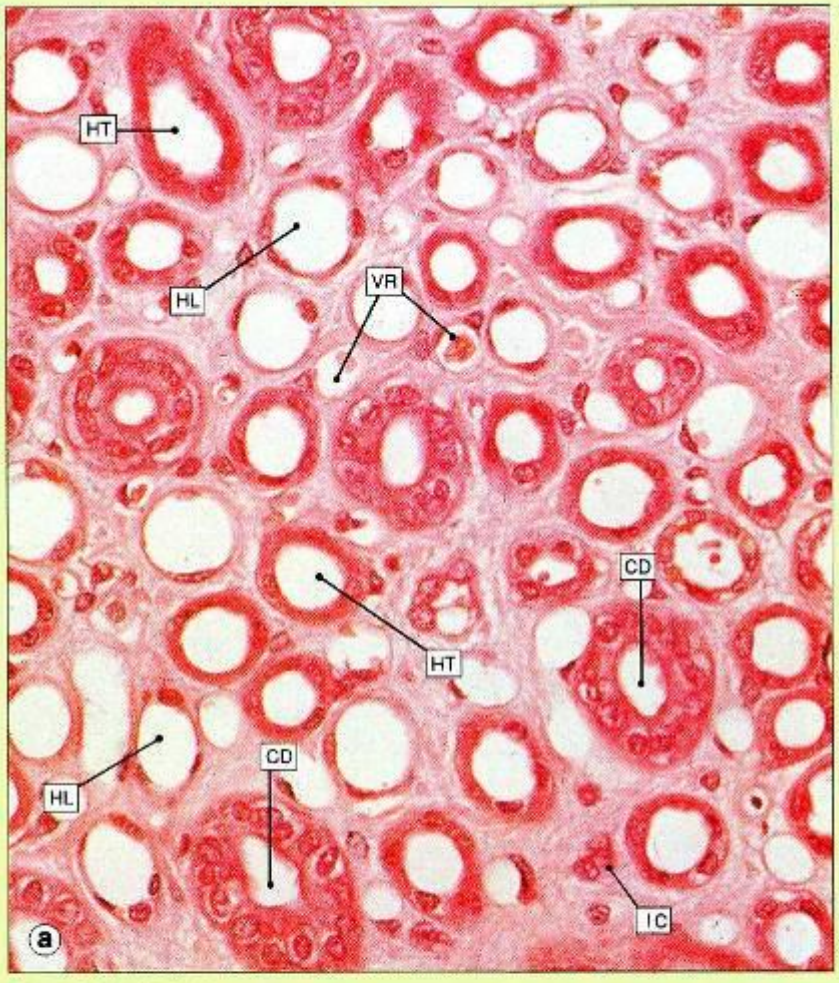


Fig. 16.33 Juxtaglomerular apparatus.
Any glomerulus sectioned through the vascular hilum may show part of the juxtaglomerular apparatus (JGA), though the detailed structure is rarely apparent. The most easily seen component in a paraffin section is the macula densa (MD), and the afferent and efferent arterioles are sometimes visible. Without the assistance of special stains, the juxtaglomerular and lacis cells cannot be specifically identified



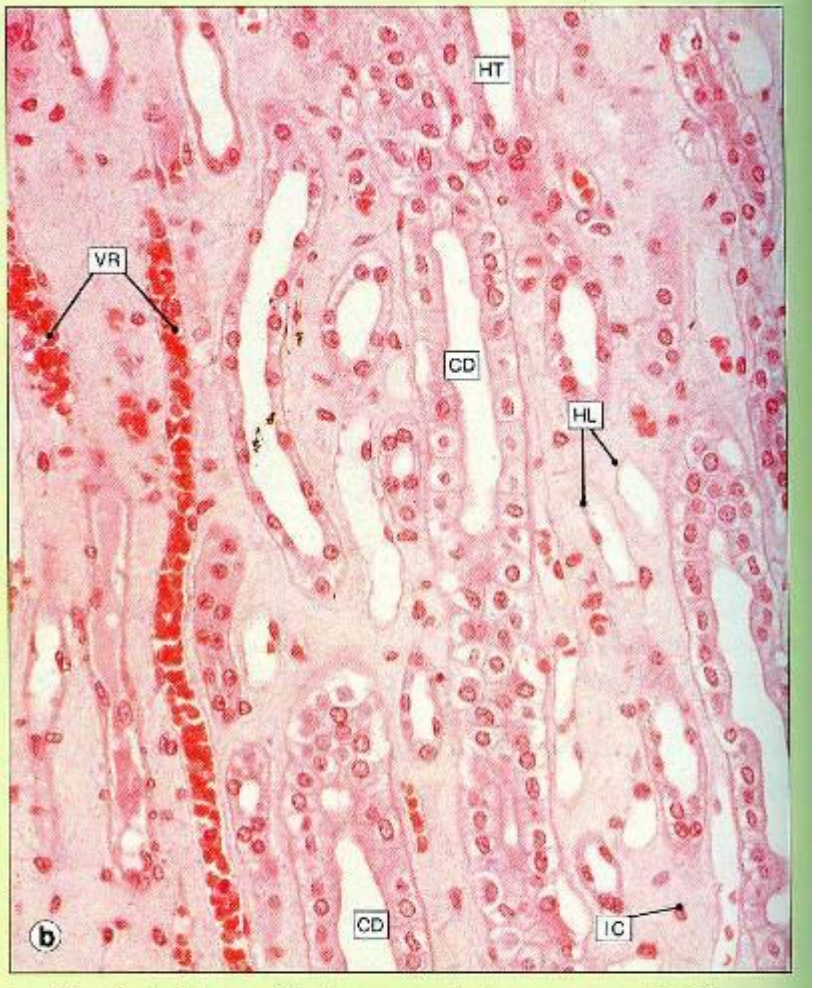
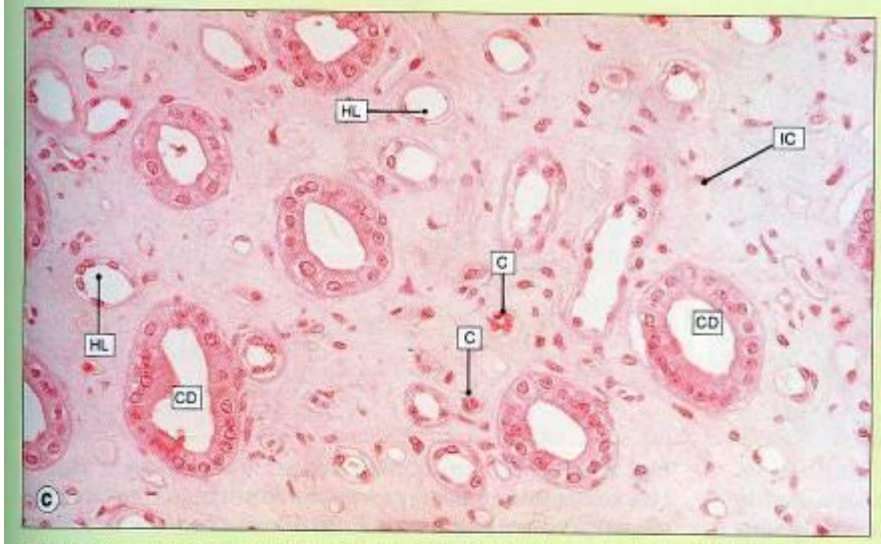


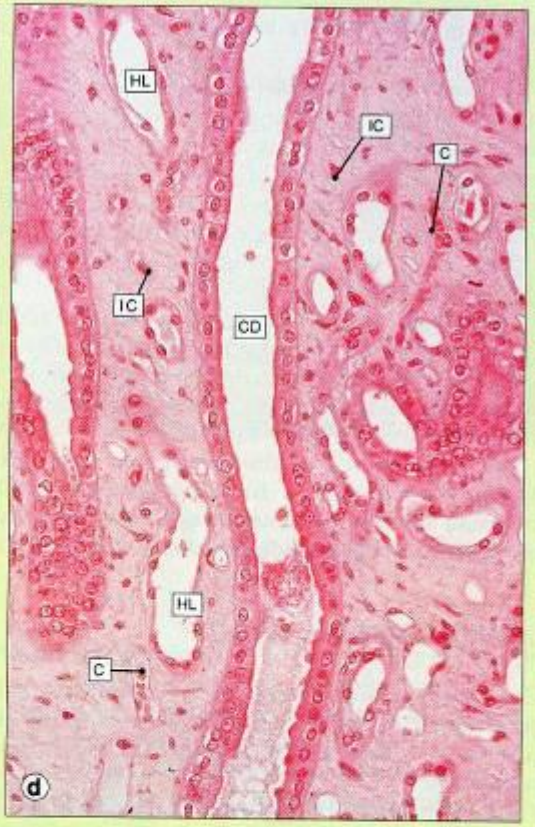
Fig. 16.35 Medulla.
In the medulla, all the various tubules, ducts and vessels run in the same direction towards the papillary tip. The appearance on histological examination depends on whether the section has been cut longitudinally to the axis of the tubules (in which case the tubules and ducts are cut in longitudinal section), or transversely. In most randomly selected tissue blocks, the section is usually oblique to the longitudinal plane of the medulla to a greater or lesser extent.
a In this micrograph of outer medulla, just below the cortico-medullary junction, the tubules and ducts are seen in transverse section. The outer medulla contains a mixture of thick descending and ascending portions of Henle loops (HT), which are histologically very similar to proximal and distal convoluted tubule, thin loops of Henle (HL), small collecting ducts (CD) and vasa recta (VR). In this region there is a small amount of interstitium in which a few interstitial cells (IC) can be seen.
b Micrograph of the same area of outer medulla shown in **a** sectioned almost longitudinally.



c In this micrograph of lower medulla note the difference in the content of tubules and ducts to that of outer medulla shown in **a** and **b**. There are now no thick portions of Henle loops, but thin Henle loops (HL) are numerous as are thin-walled capillaries (C).

The collecting ducts (CD) are larger and lined by distinct clear-celled cuboidal epithelium. The pale-staining interstitium now forms a substantial part of the bulk of the tissue, and scattered small stellate and spindle-shaped interstitial cells (IC) are numerous. In this micrograph the Henle loops, vessels and collecting ducts are in transverse section.

d Micrograph of same area of lower medulla shown in **c** sectioned longitudinally. Note the prominent straight collecting ducts running down towards the papilla; the nearer the tip of the papilla, the larger the ducts become.



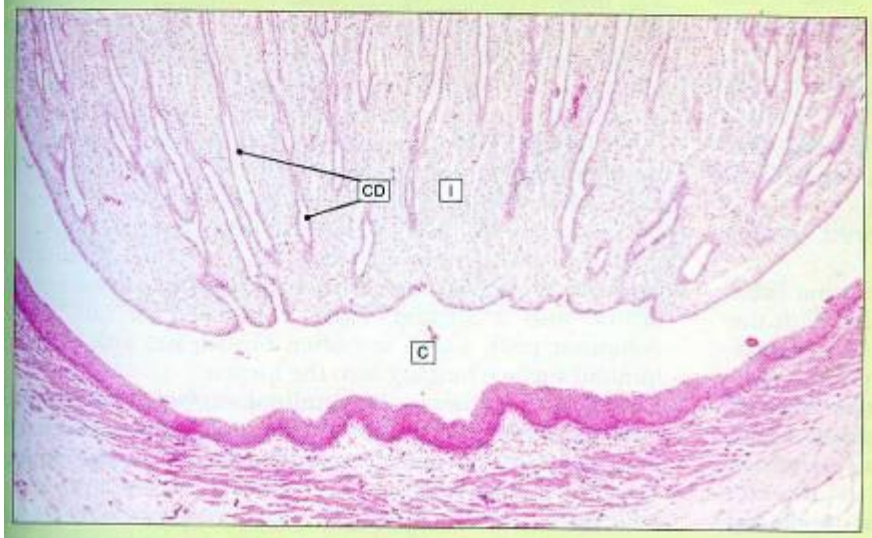
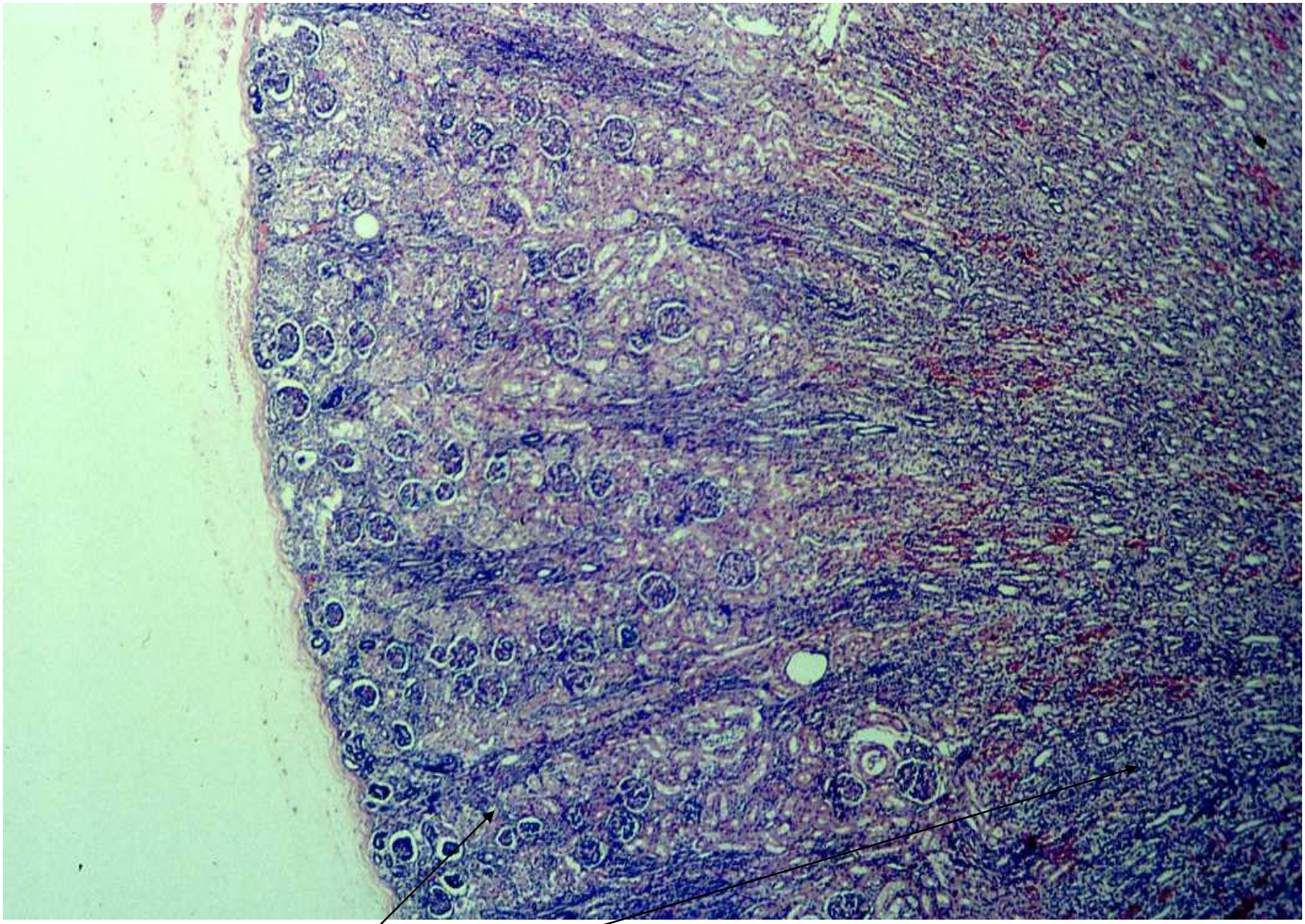


Fig. 16.36 Papilla.
The large collecting ducts (CD), are few in number as a result of fusion, and open into the calyx (C) at the papillary tip. At the papilla, the distal medulla consists almost entirely of large collecting ducts embedded in bulky interstitium (I), with very few thin Henle loops, and a number of vasa recta vessels. Interstitial cells are numerous.



Neonatal kidney – Cortex and medulla - H&E

The Renal Biopsy

Immunofluorescence

Immunofluorescence

- Tissue preserved in Michel’s medium
- Tissue rapidly frozen onto a holder
- Sections cut using a cryostat and picked up on glass slides
- Sections covered with fluorescent labelled antisera
- Sections examined using a fluorescent microscope

Immunofluorescence Antibodies used

- **Immunoglobulins**
- IgG
- IgM
- IgA
- Light chains (K & L)

- **Lymphoctytes**
- CD8
- CD4
- CD3

- **Complements**
- C3
- C1q
- C4D

Lymphocyte markers and C4D only used on transplant kidney biopsies

Serum amyloid component P used to demonstrate glomeruli and if in excess used to assess for presence of fibrillar amyloid

Cryostat (microtome within freezer) for production of frozen sections

Lung nodule - ?cancer

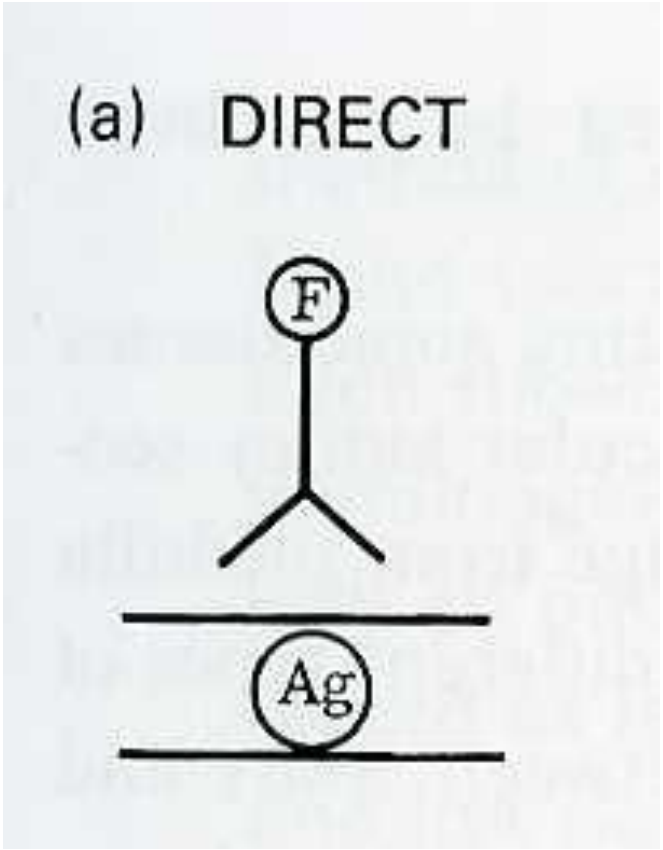
Same principle applies to renal biopsy frozen section production

Tissue in mounting media freezing onto holder in cryostat

Frozen section being cut

Frozen section sitting on knife before being picked up on glass slide

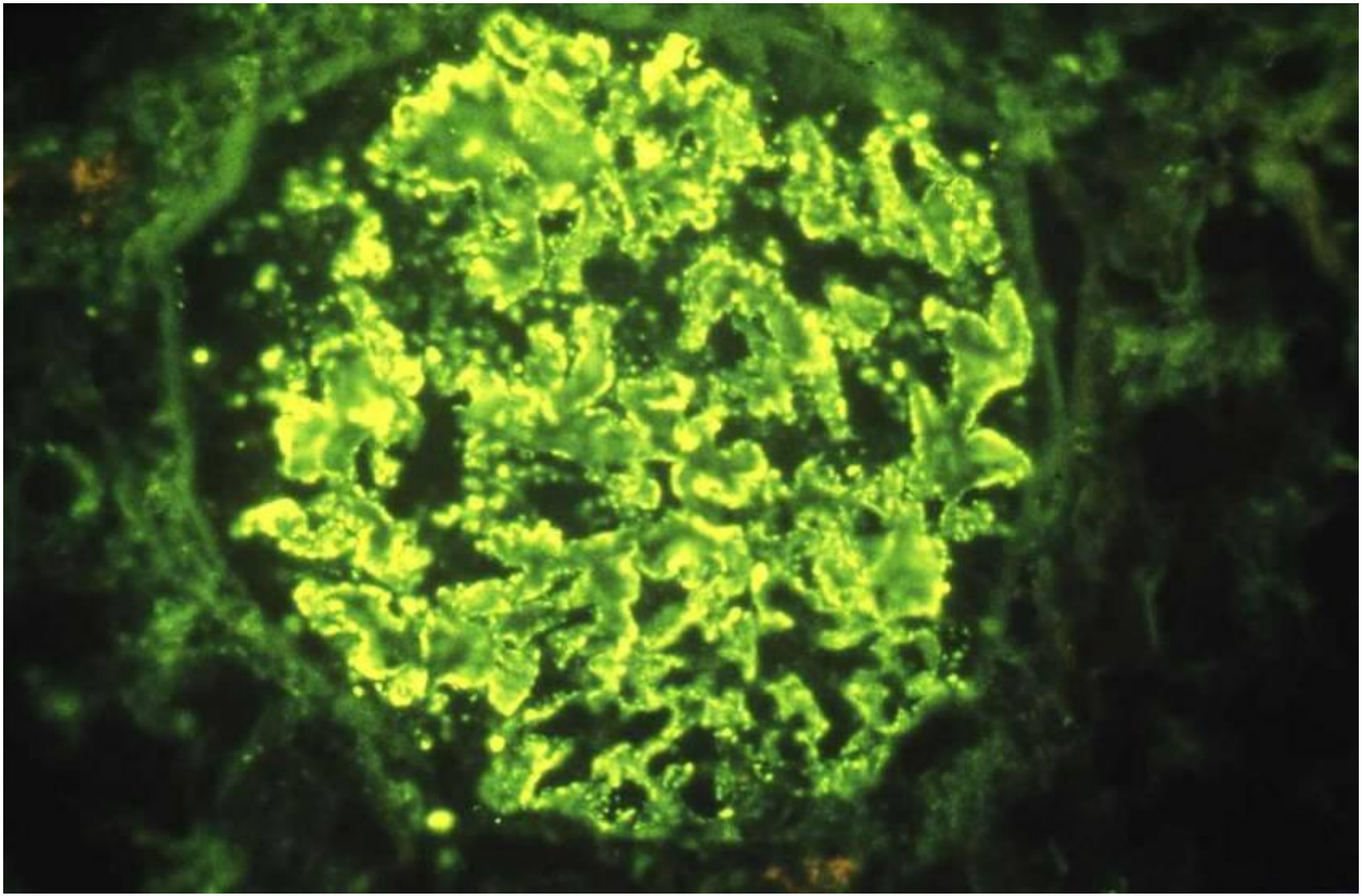
Direct immunofluorescence technique



Fluoresceine label

Antibody

Antigen in section

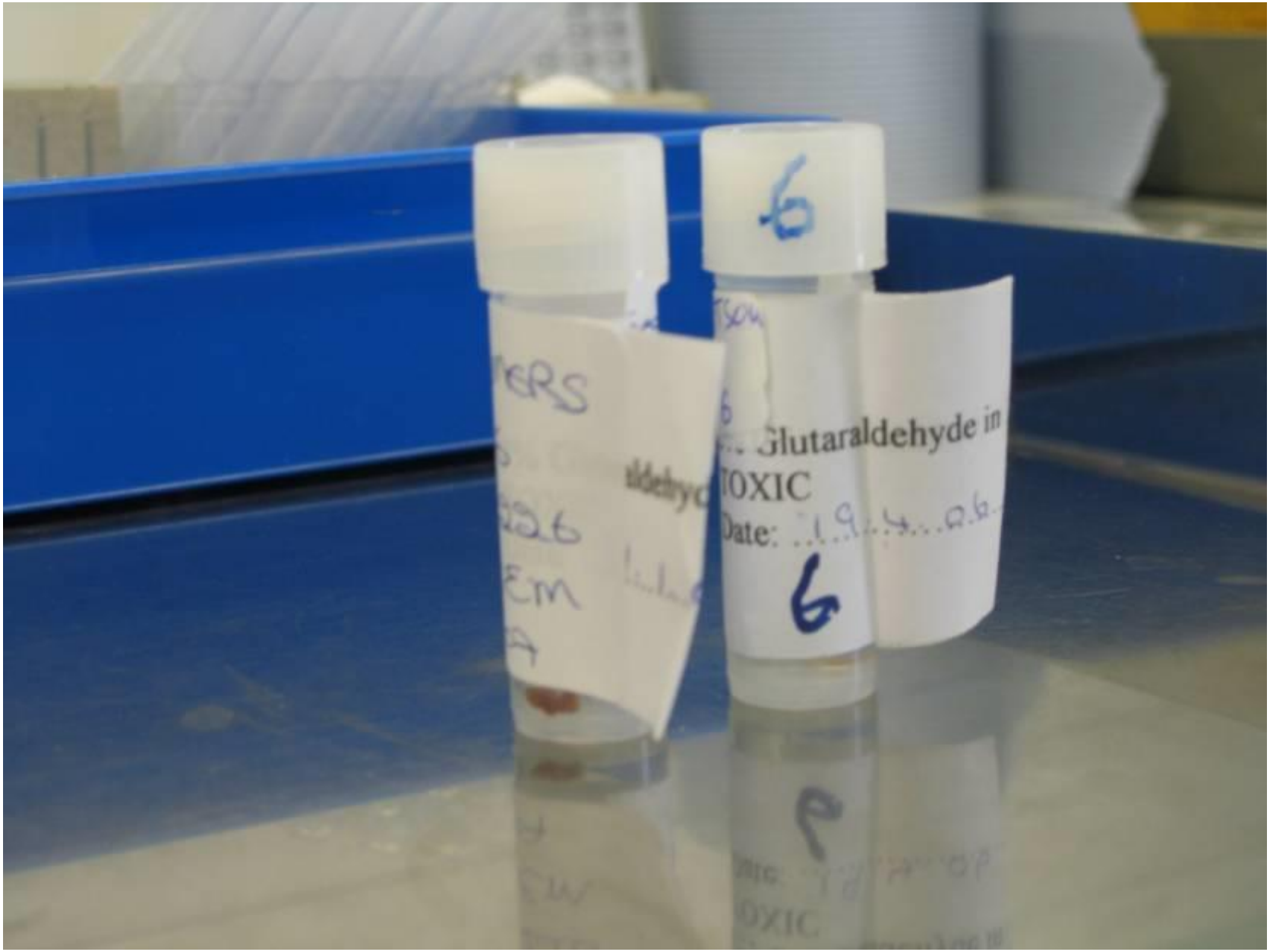


Immunofluorescence

IgG along glomerular basement membrane

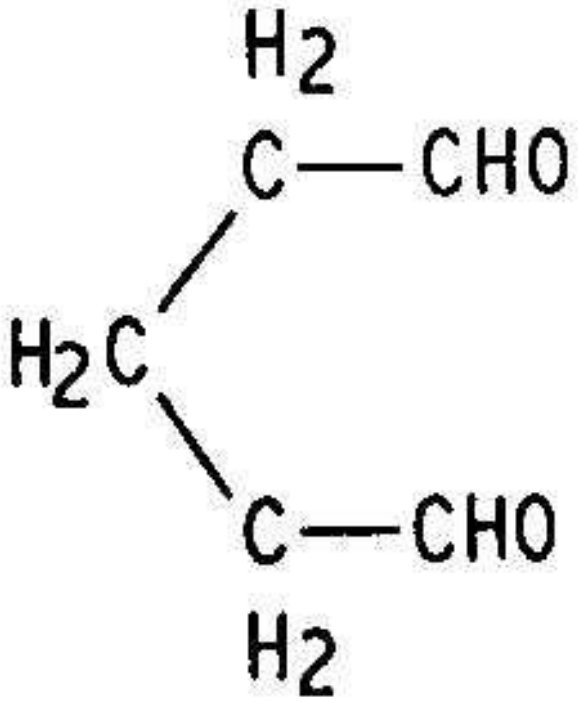
The Renal Biopsy

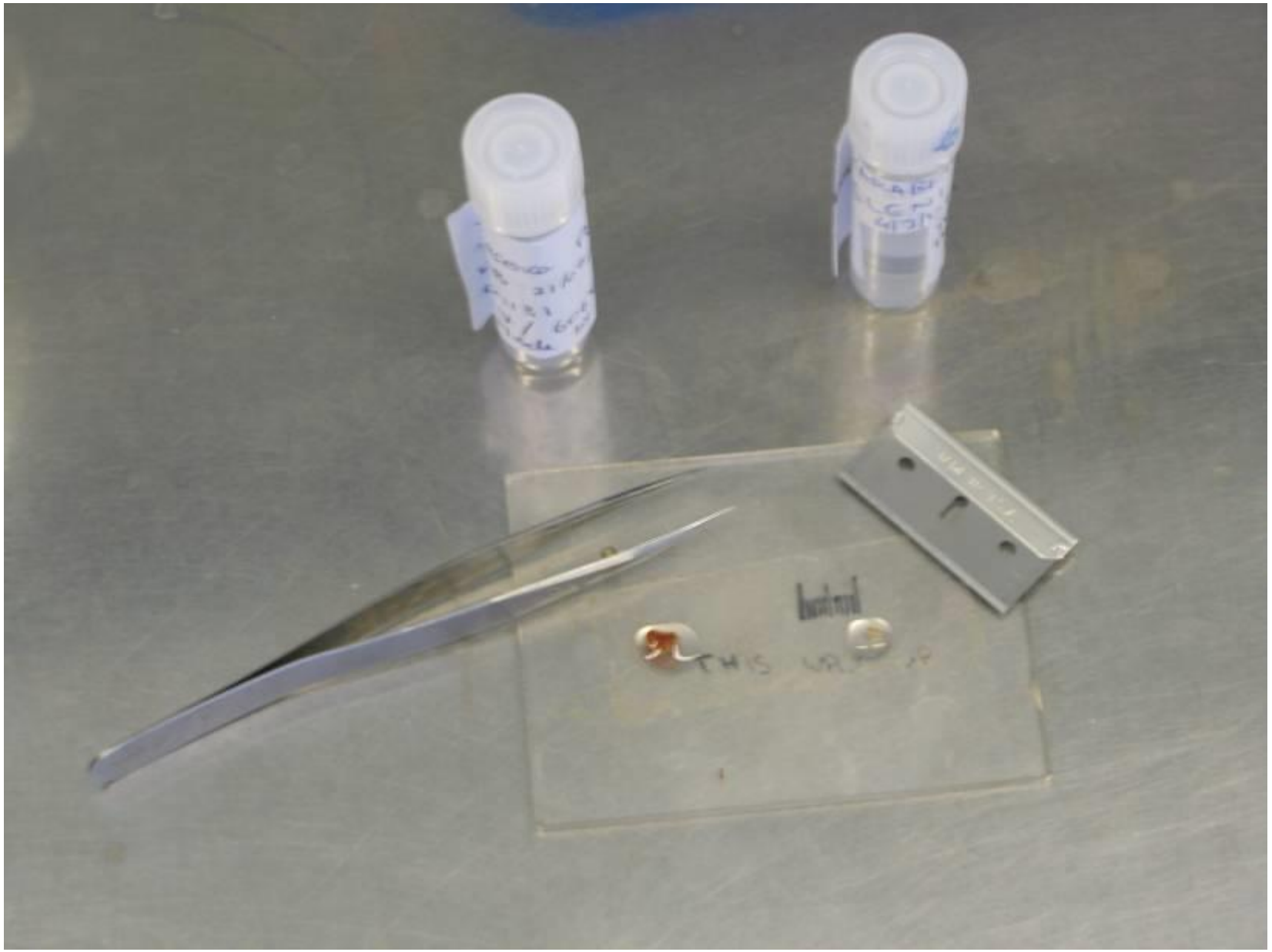
Electron Microscopy



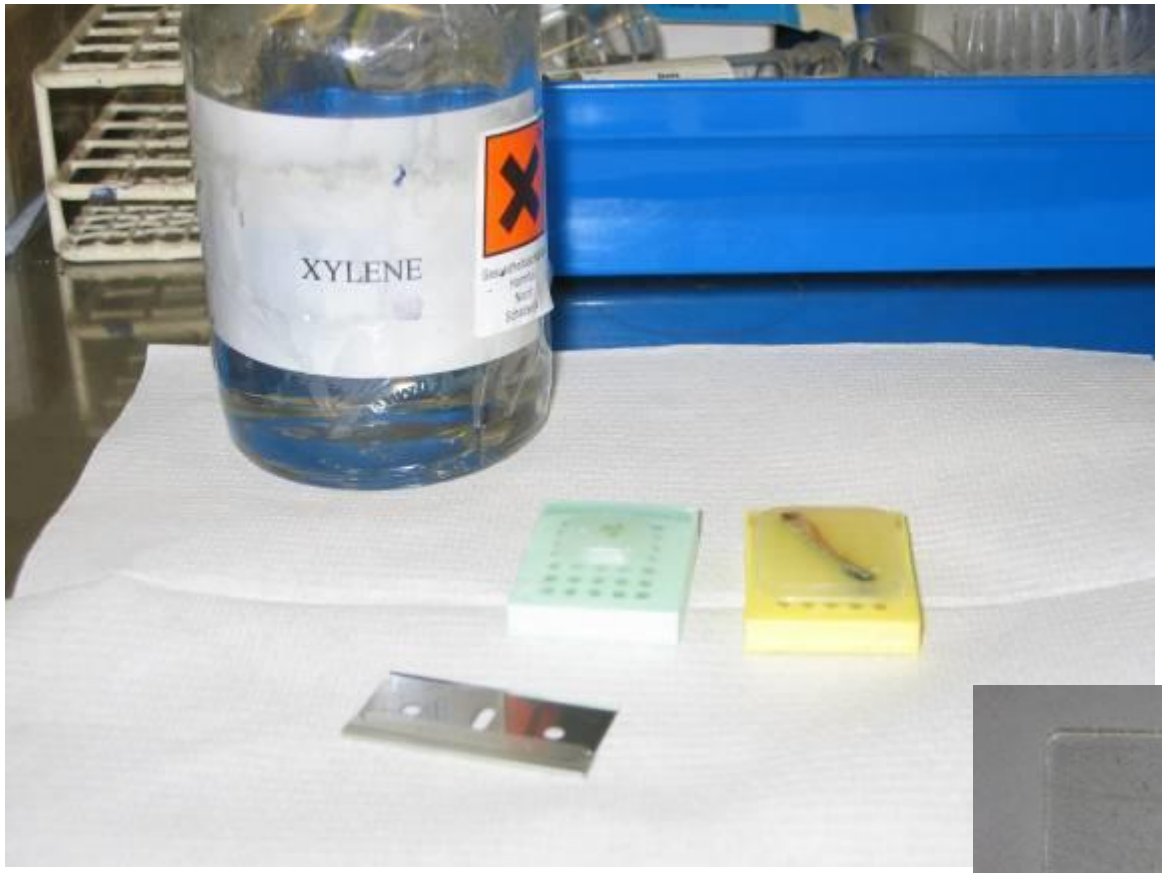
Tissue fixed in buffered glutaraldehyde

Glutaraldehyde

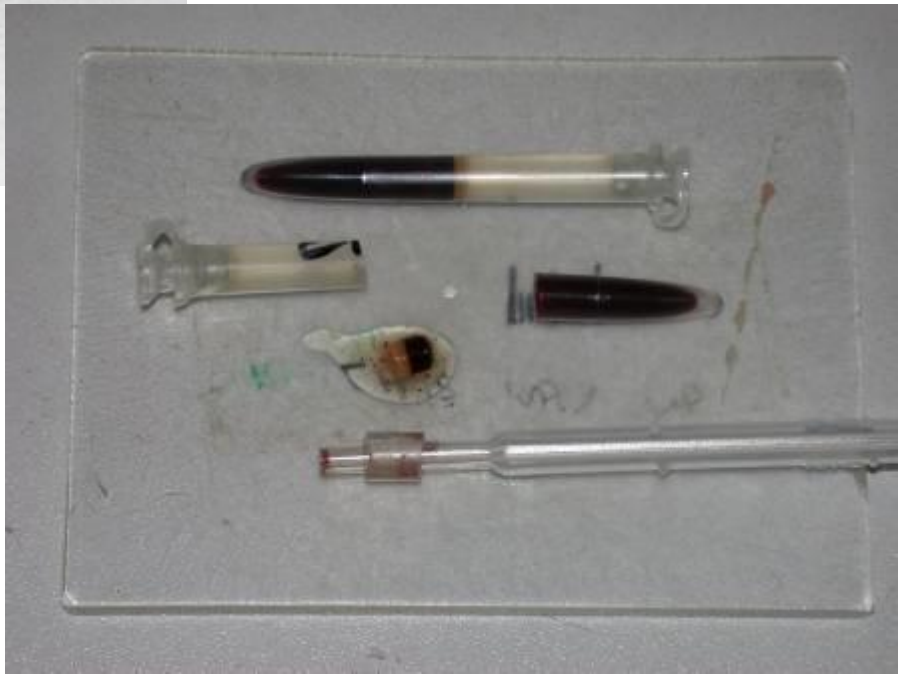




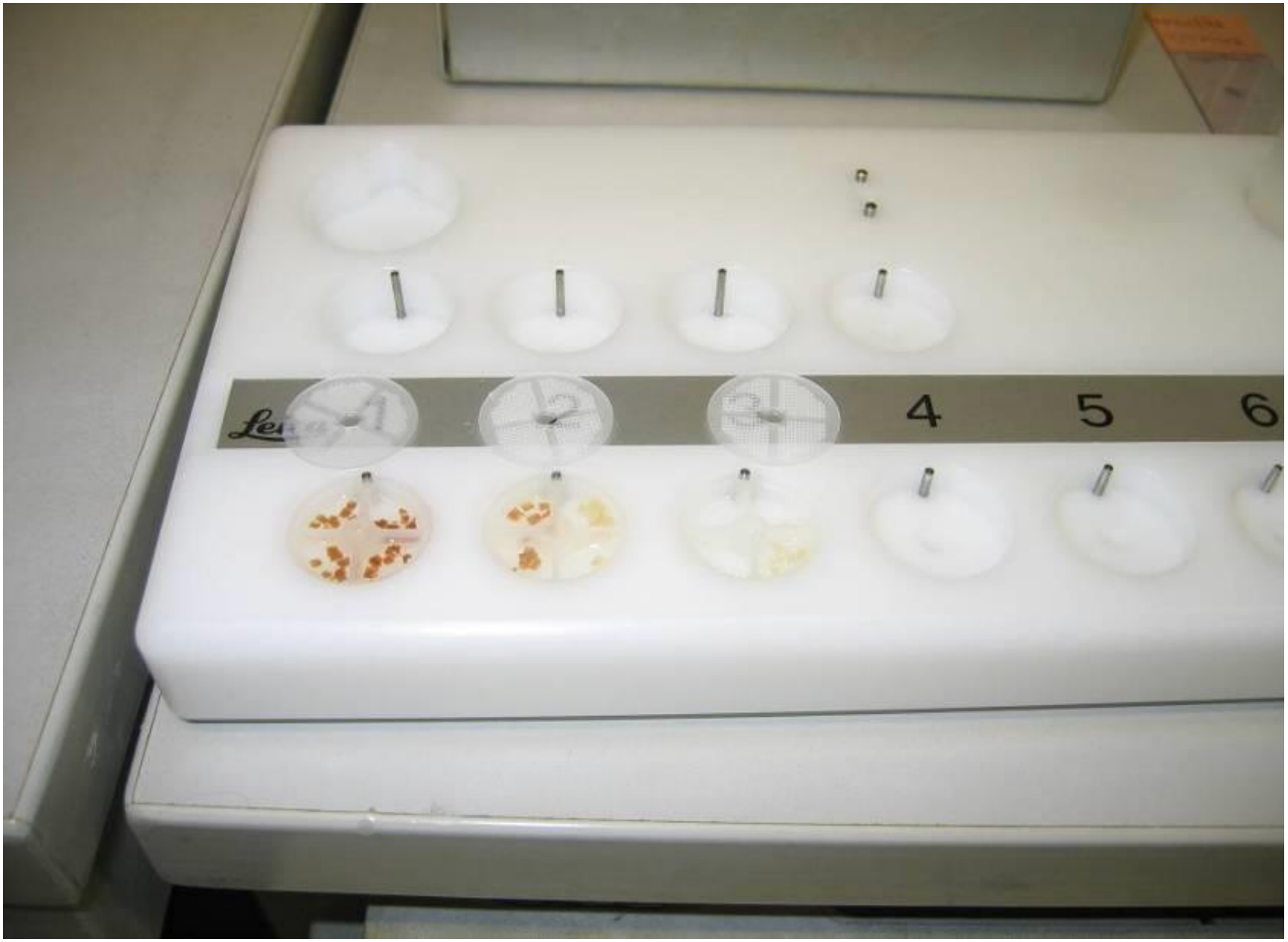
Tissue described and chopped into an appropriate orientation and size



Can process centrifuged, glutaraldehyde fixed blood



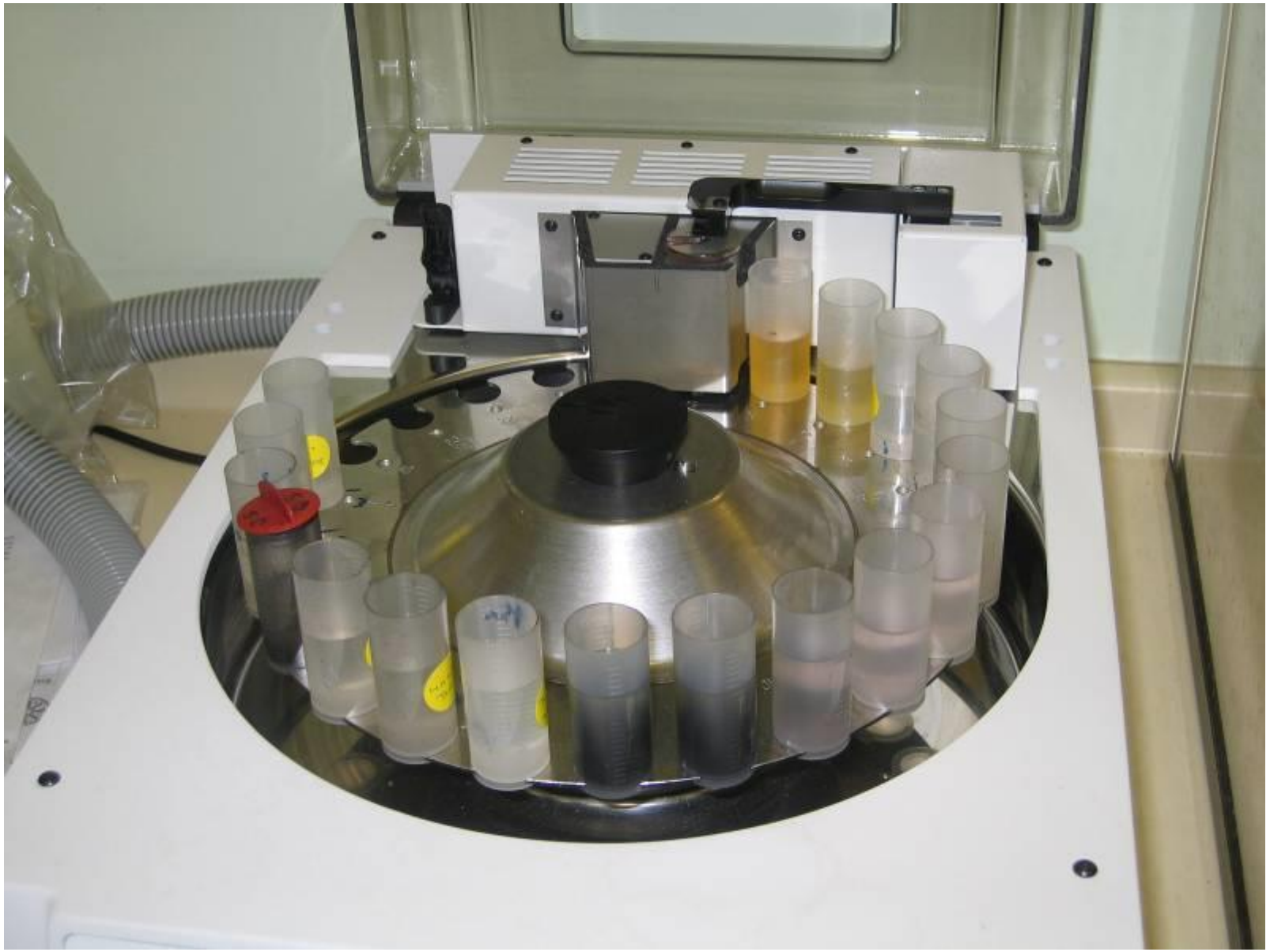
Can retrieve tissue from wax block



Tissue sampled and placed in processing baskets

Tissue placed in small baskets, and attached to processing machine

Resin processing machine

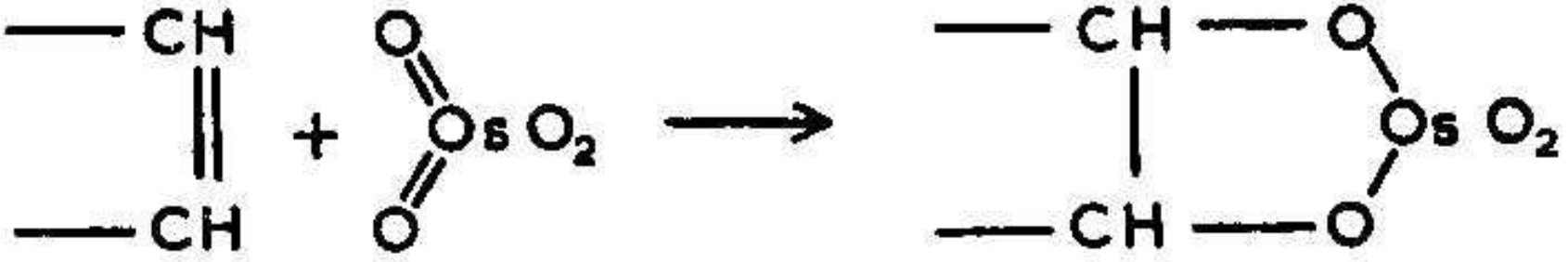


EM tissue processor – at end of run

EM tissue processing schedule

• Buffered Glutaraldehyde	Protein fixative
• Distilled water	Rinse
• Osmium Tetroxide	Unsaturated lipid and phospholipid fixative
• Distilled water	Rinse
• 70% Alcohol	
• 95% Alcohol	
• 99% Alcohol	Dehydration
• Acetone	Transitional solvent
• Resin/acetone mix	
• Pure resin monomer	Infiltration of tissue
• Embedded in fresh resin	Polymerisation at 60 degrees

Osmium tetroxide



(colourless) (black)

I

Different moulds for resin blocks

Ultramicrotome

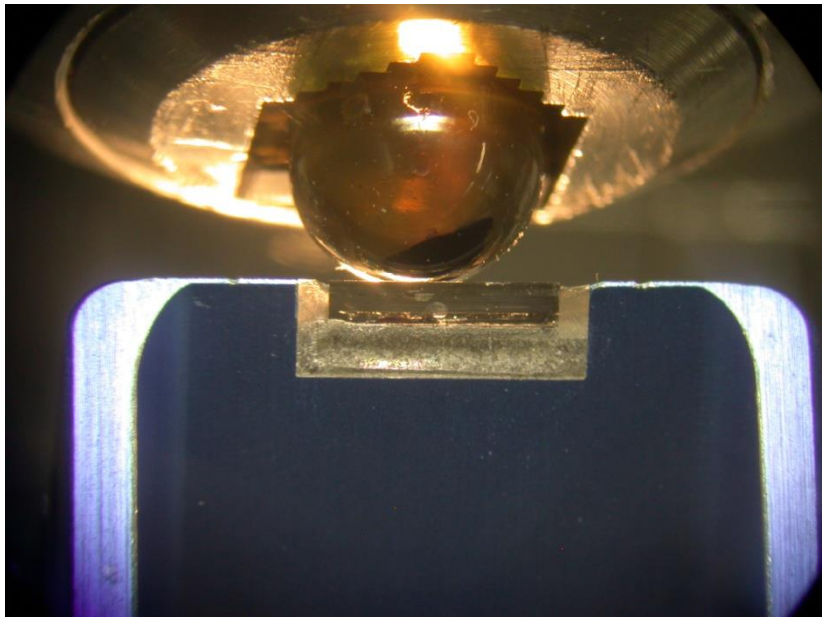
Resin blocks in chucks (holders)

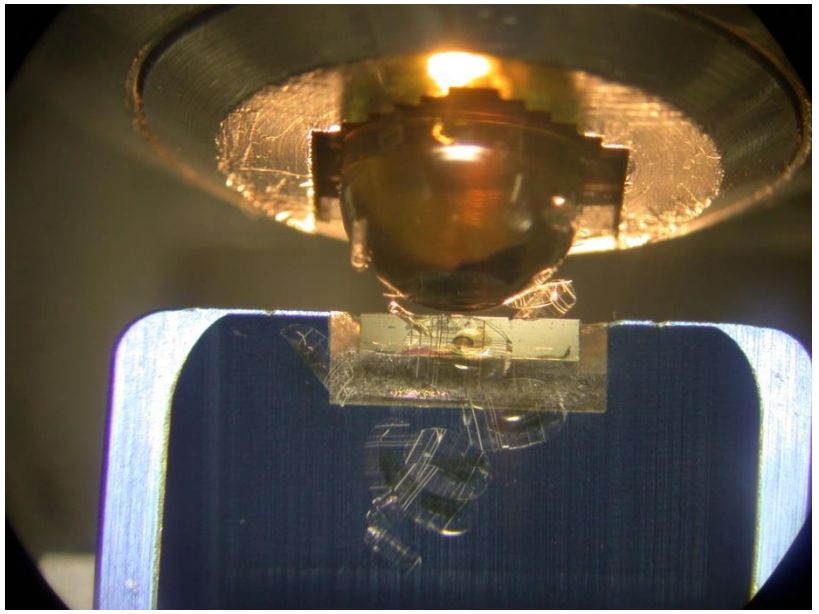
Ultramicrotome in use by BE Wagner

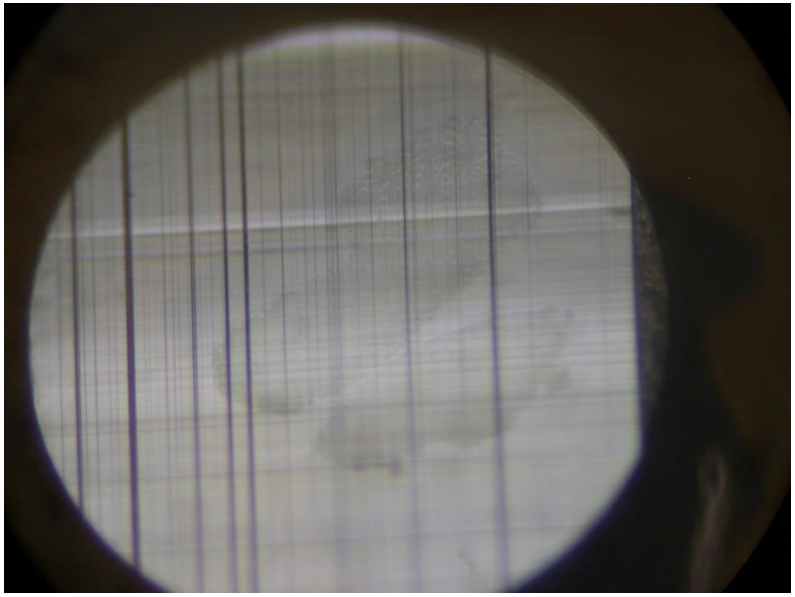
Glass knife maker

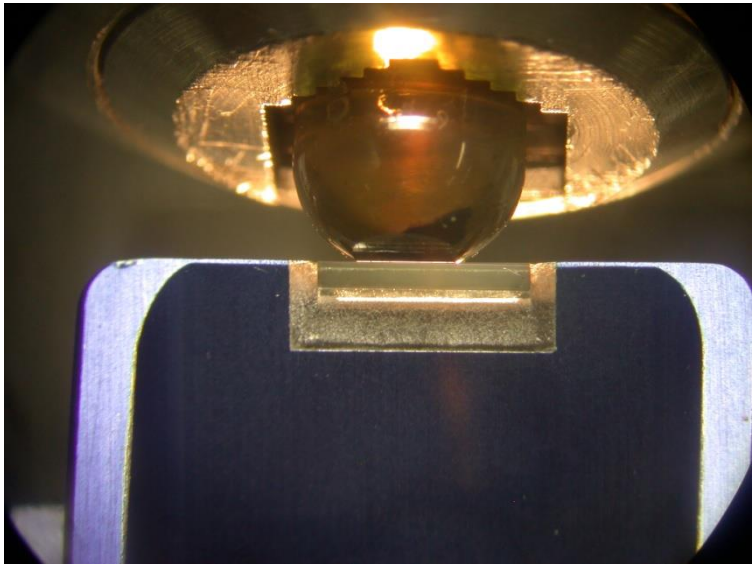
Diamond knife

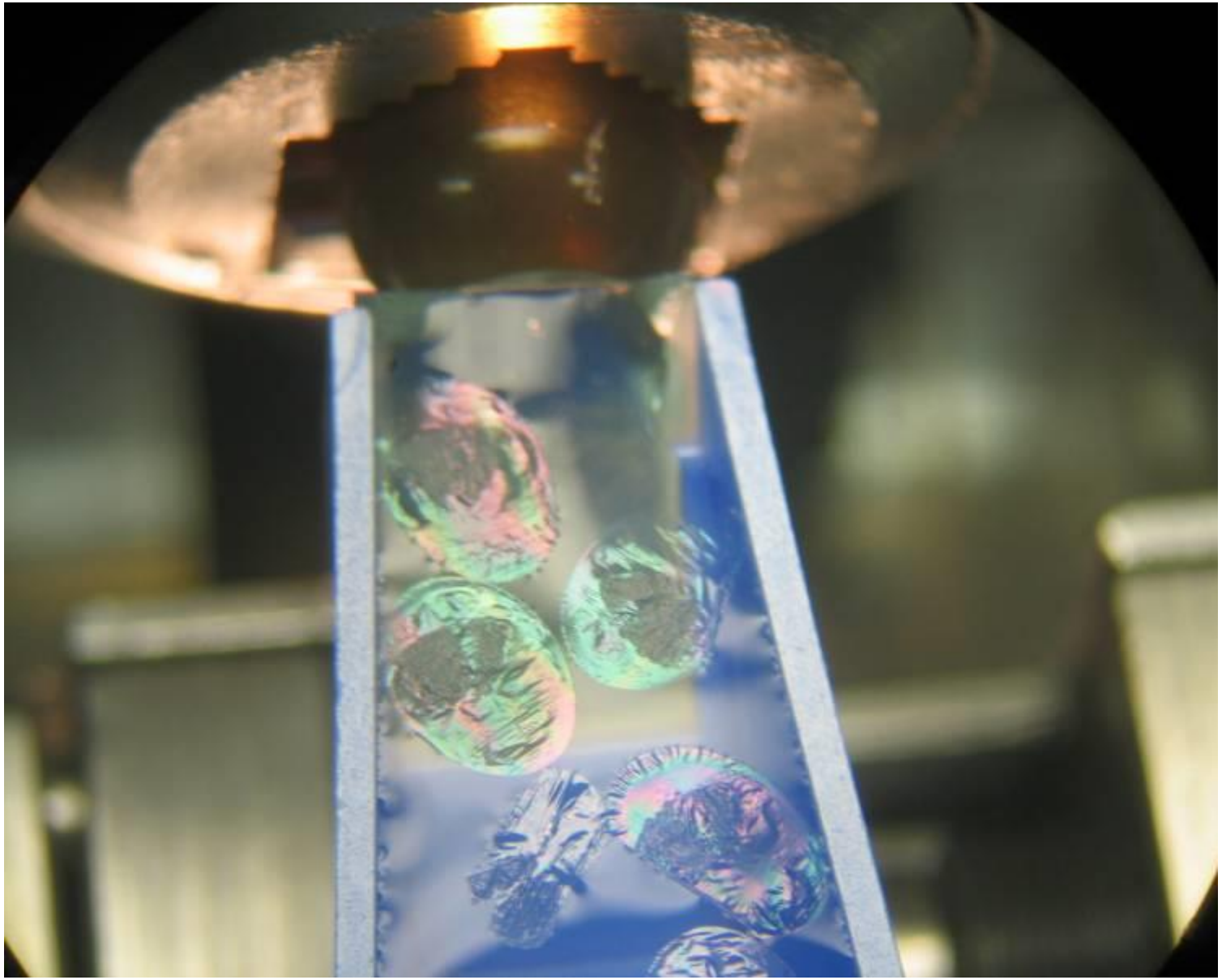
Glass knifes - with trough and without





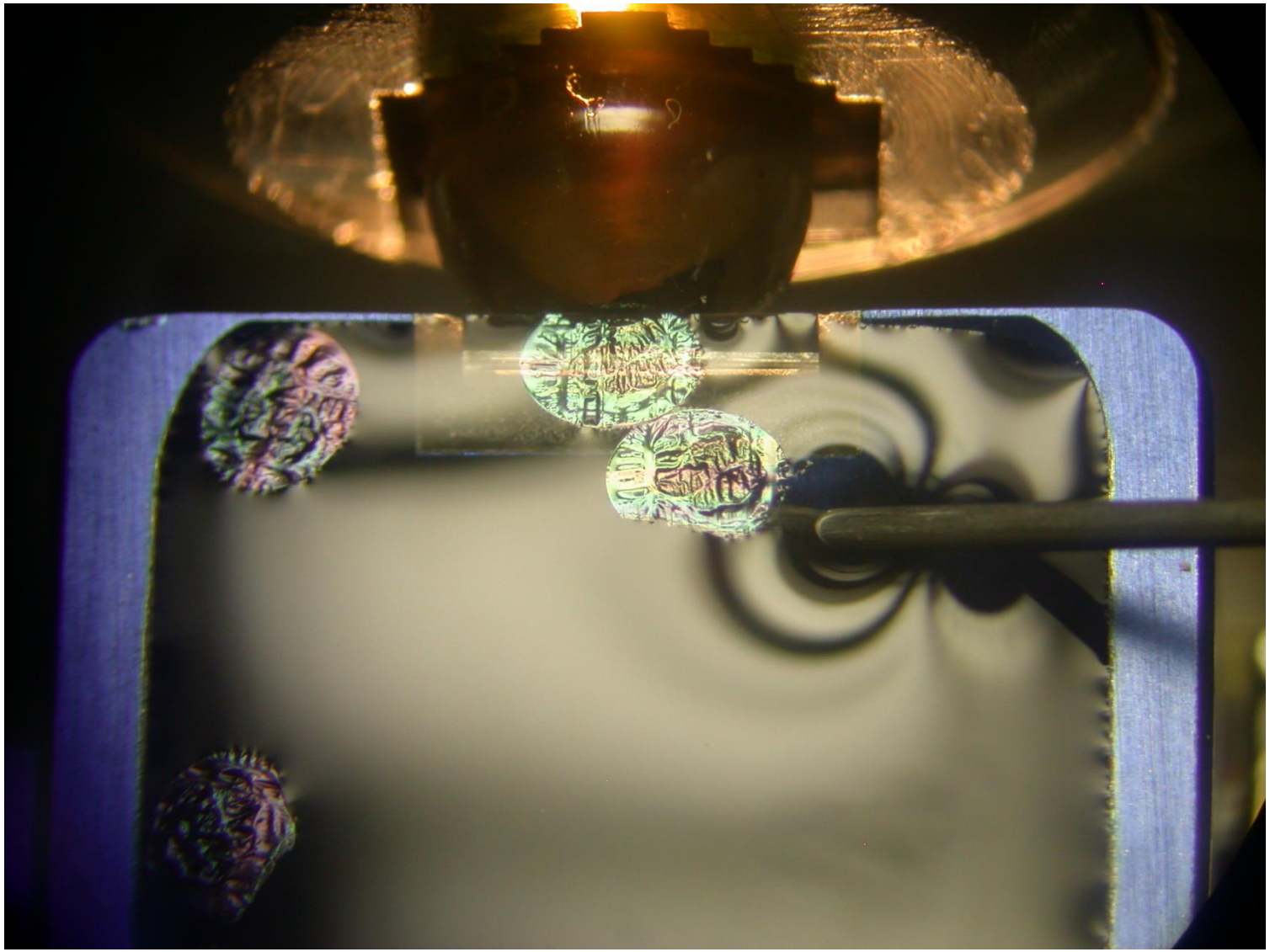




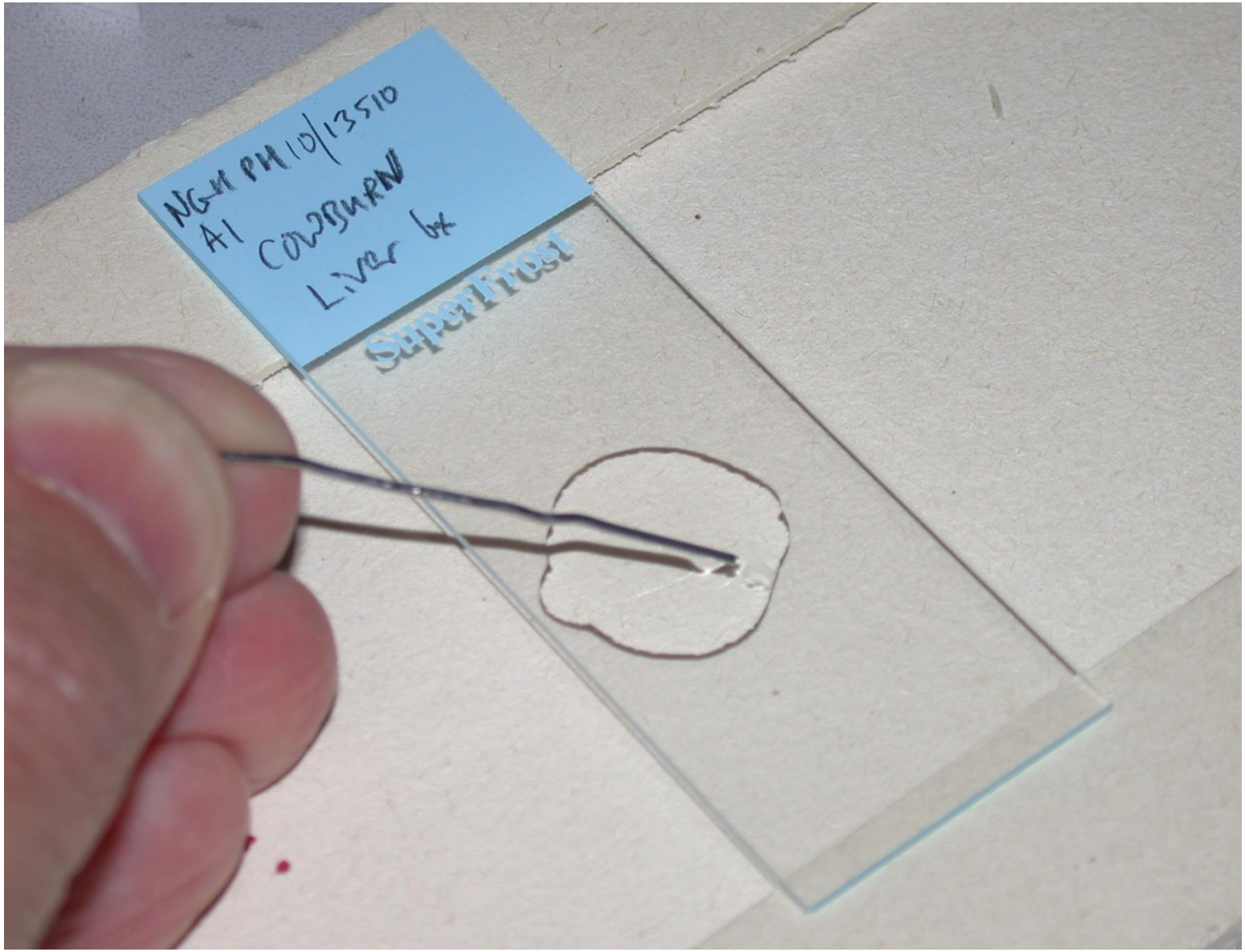


Sections at 0.6 microns being cut on glass knife – sections picked up and placed on glass slide

Sections cut using diamond histoknife



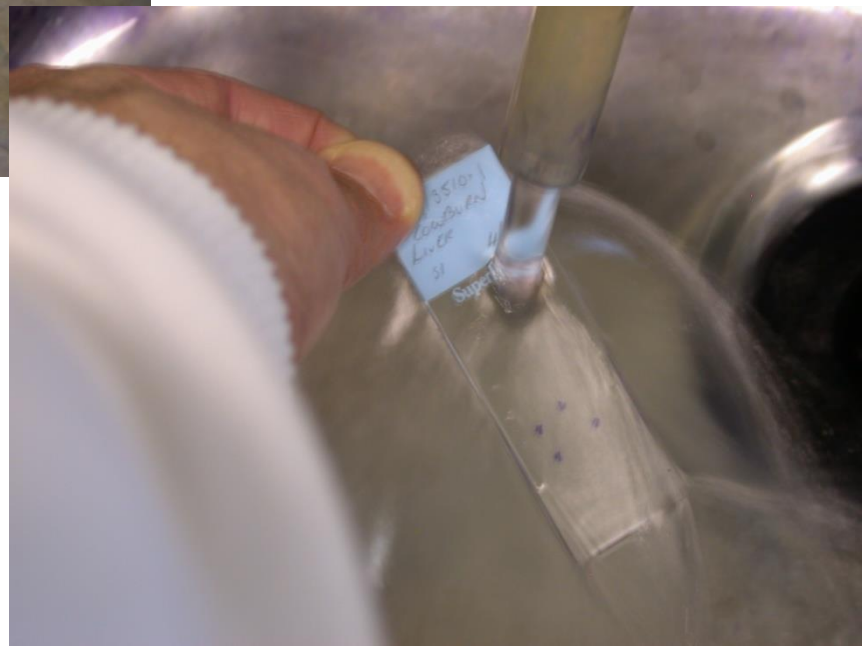
Sections picked up off surface of water



Section transferred to drop of water on glass slide

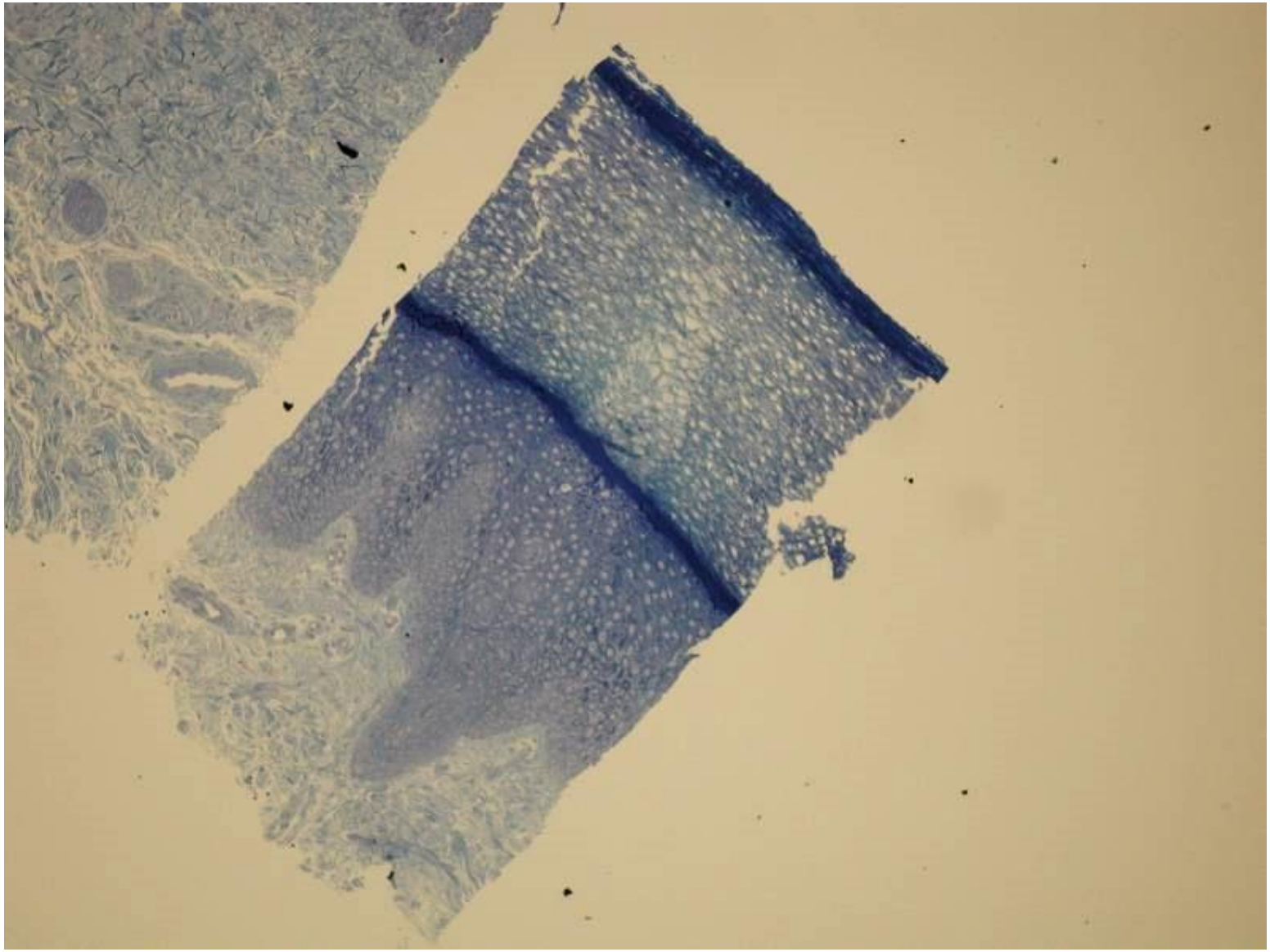
Section flattened using heat

Sections stained with toluidine blue on hot plate



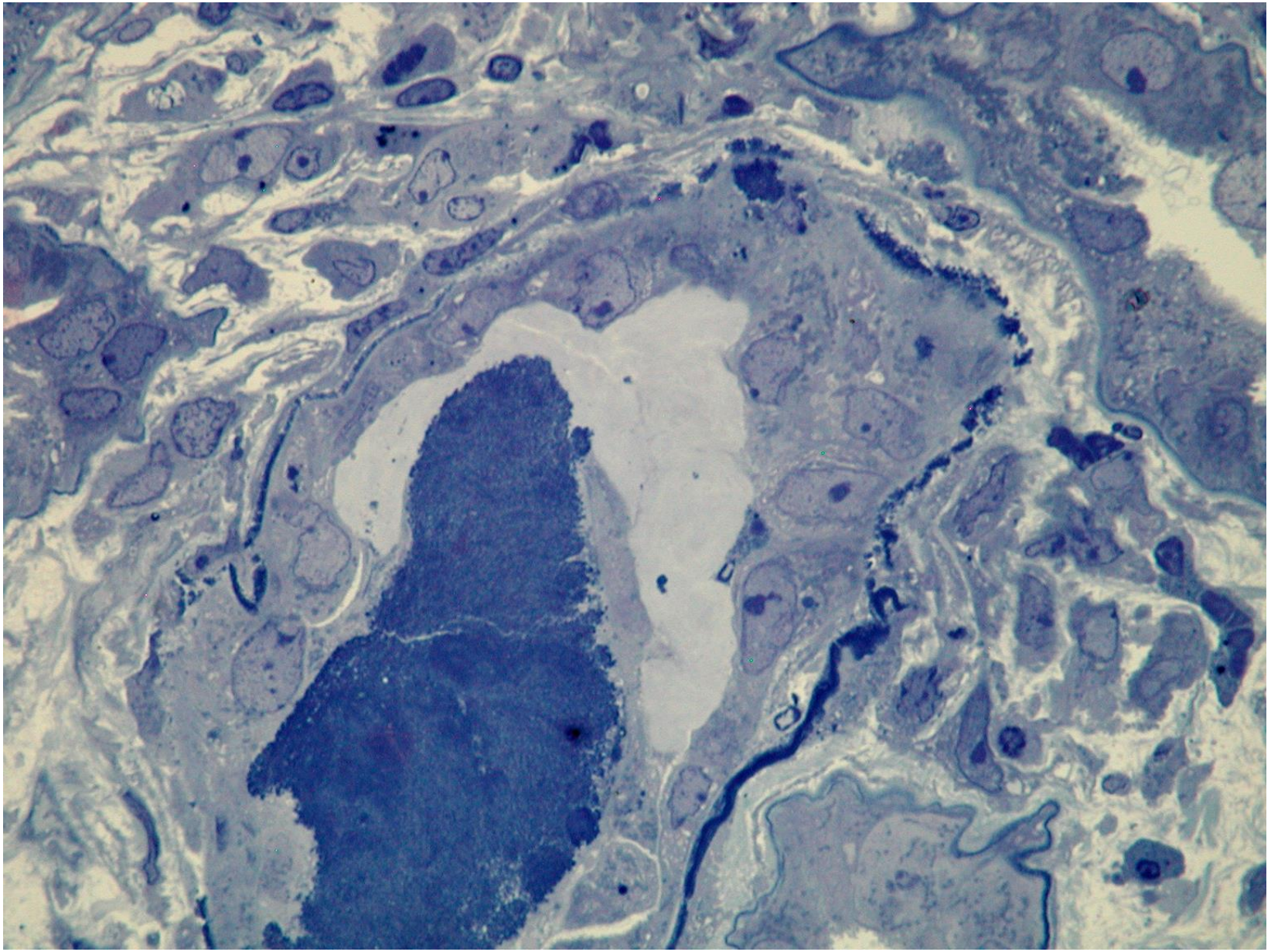
Toluidine blue stain washed off slide using hot tap water

Skin biopsy

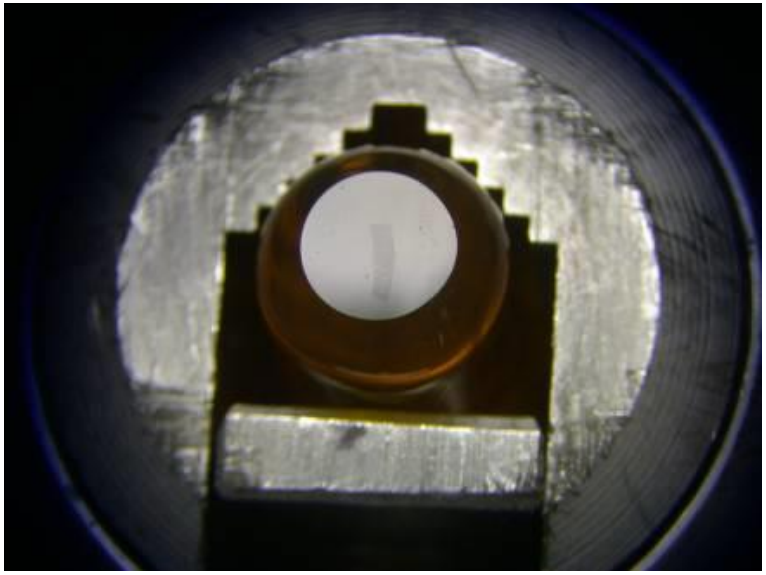


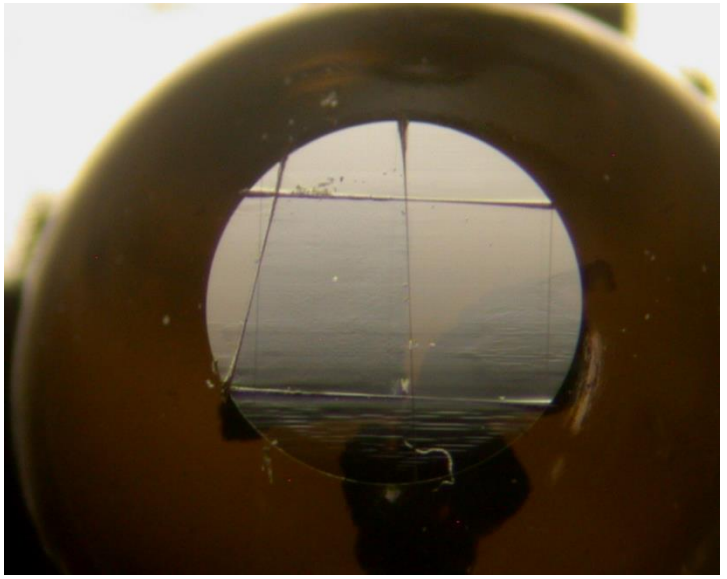
Toluidine blue stained section – sections referred to as tol blues, thicks, or semi-thins

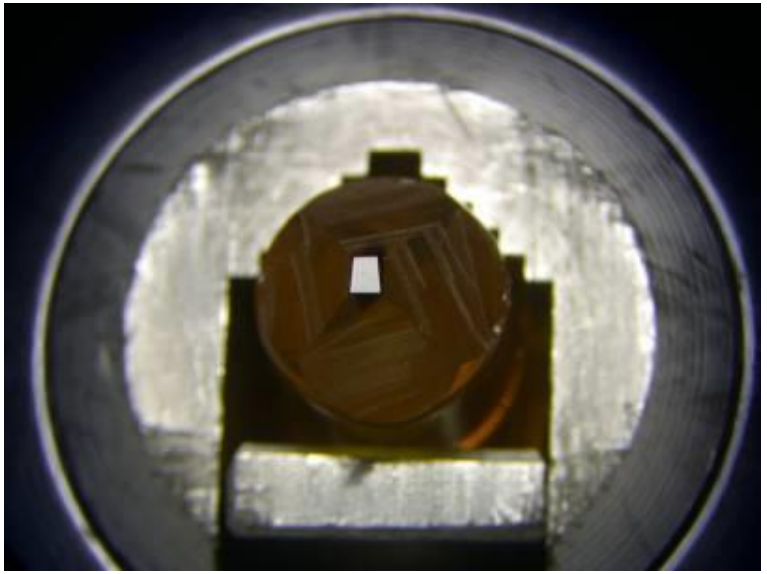
Best block selected, then trimmed down smaller for thin-sectioning.

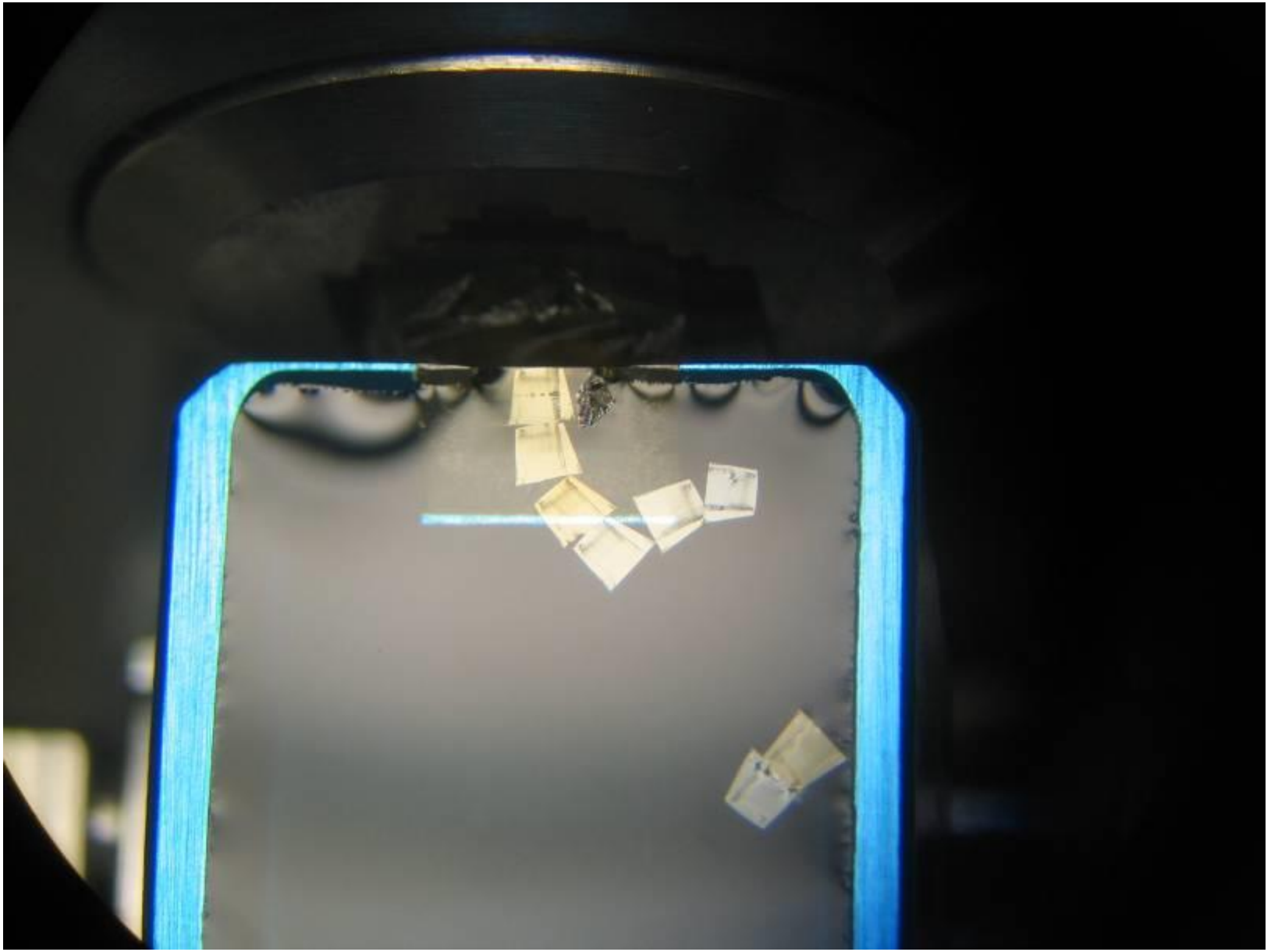


Toluidine blue stained section of renal biopsy tubules



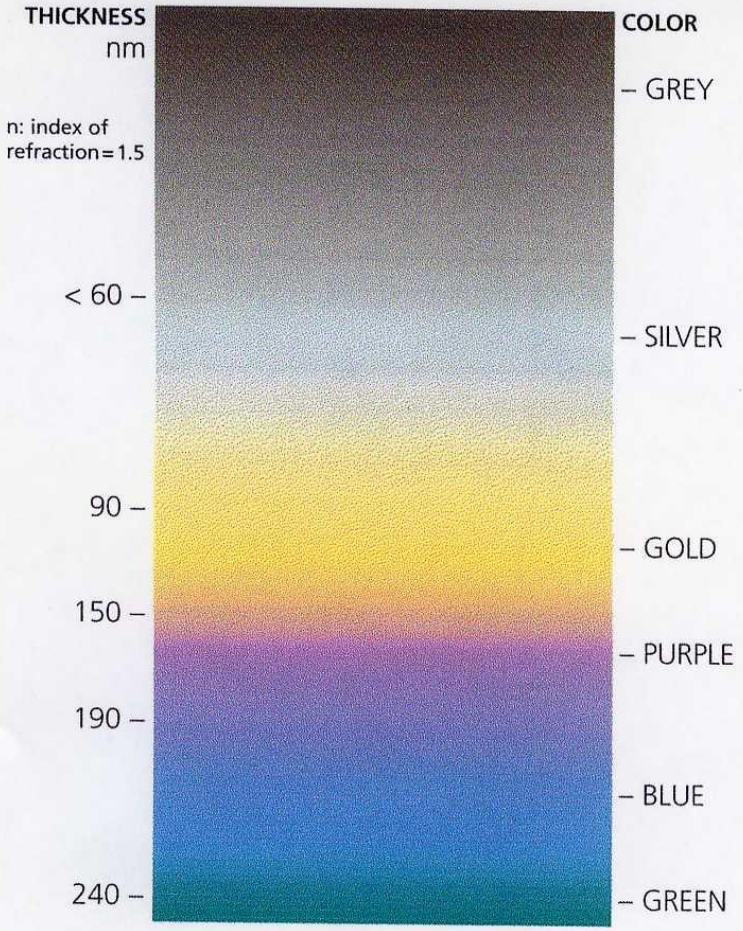






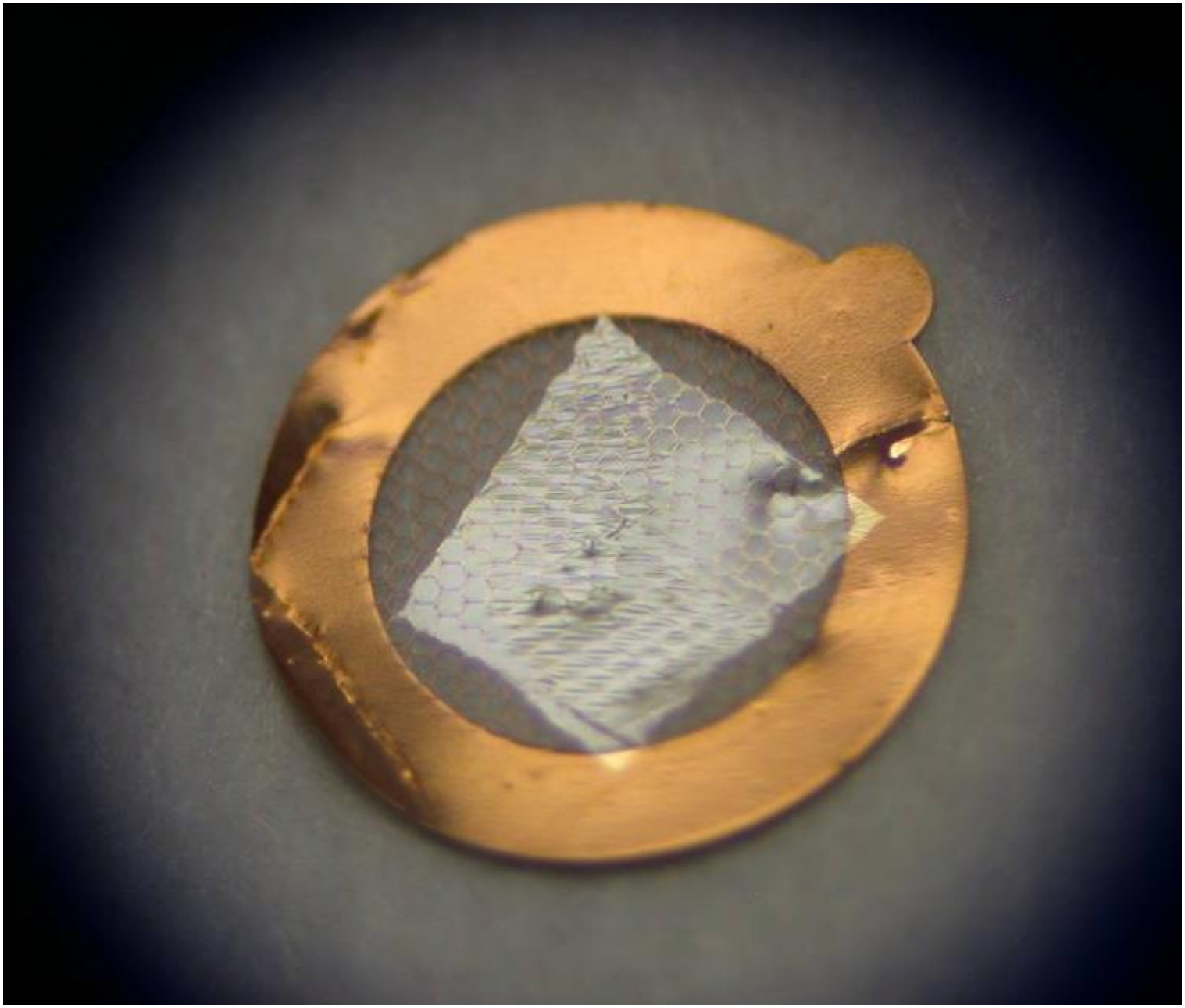
Thin sections cut on diamond knife – cut at interference colour gold (85nm)

Ultramicrotome Section Color Reference Chart

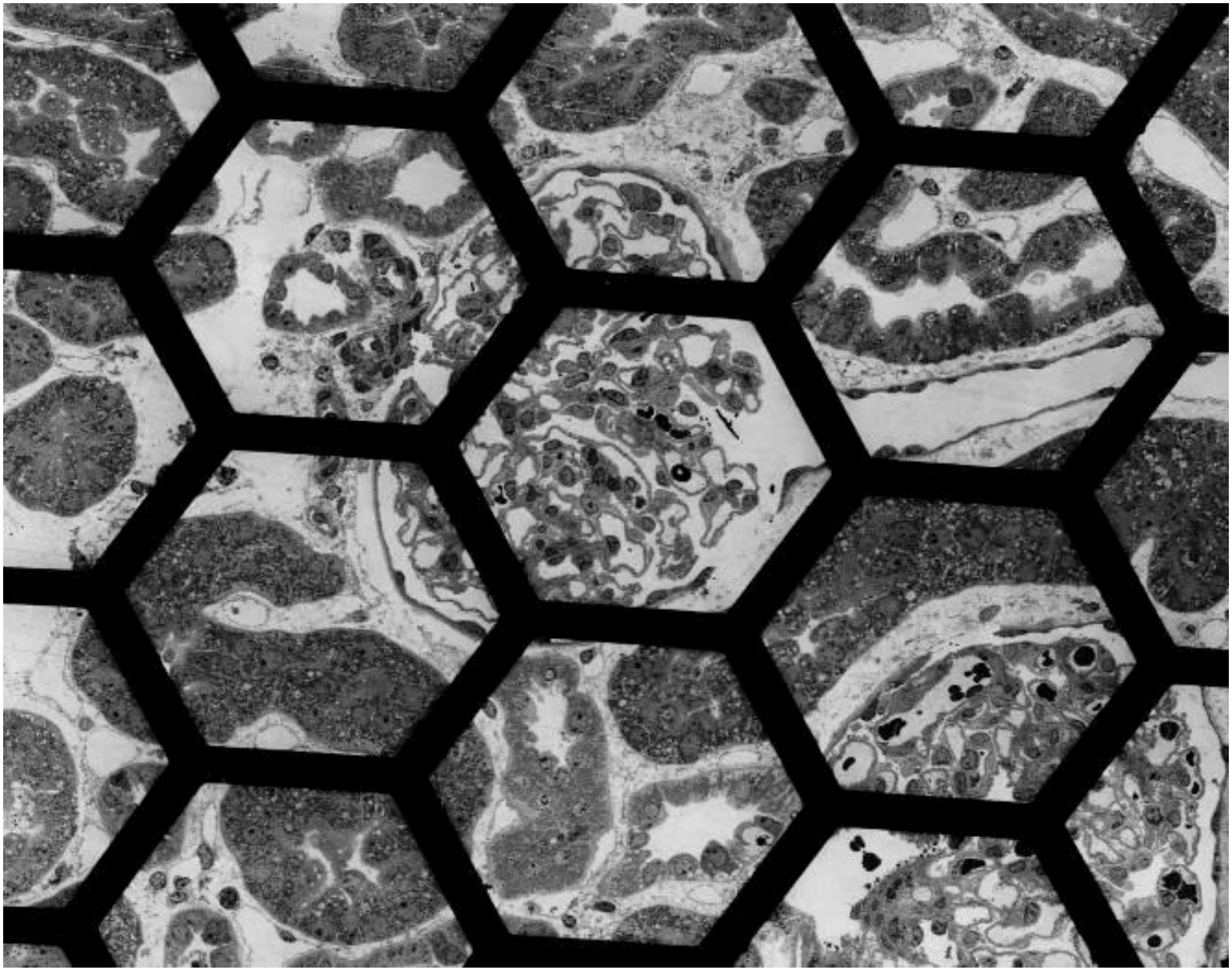


The thickness of sections used for electron microscopy may be estimated within 10 or 20 nm using this scale by noting the colors of the sections as they float in the trough. This scale is applicable to any embedding material having a refractive index close to 1.5 (methacrylates, epoxy resins, etc.).

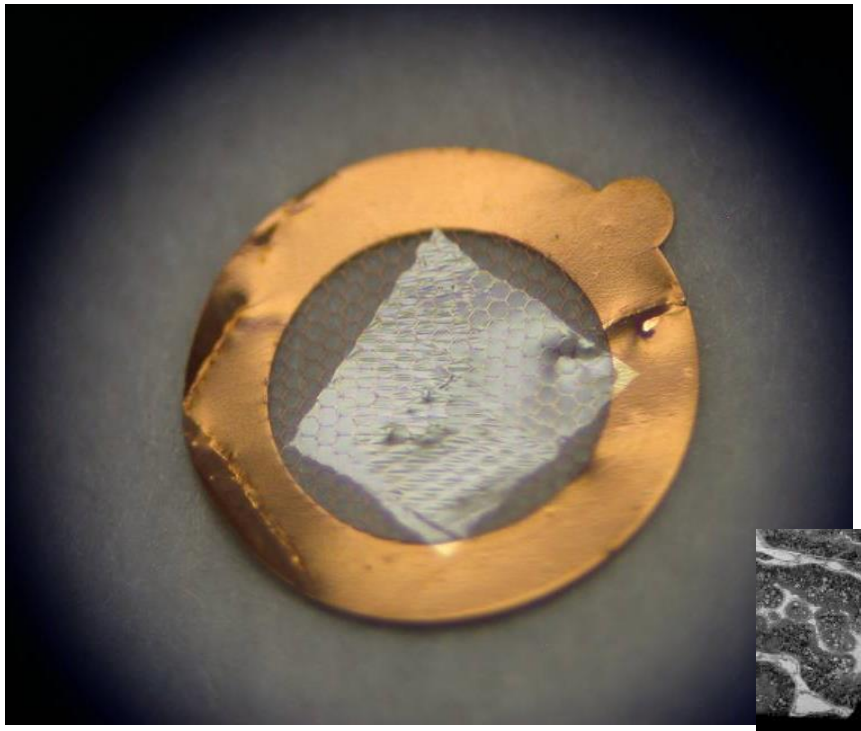
Thin sections picked up on copper grids (held using jewellers forceps) , sections manipulated with eyebrow hair fixed at end of black metal rod. All manipulation done whilst looking down stereo microscope.



Thin-section mounted on copper grid

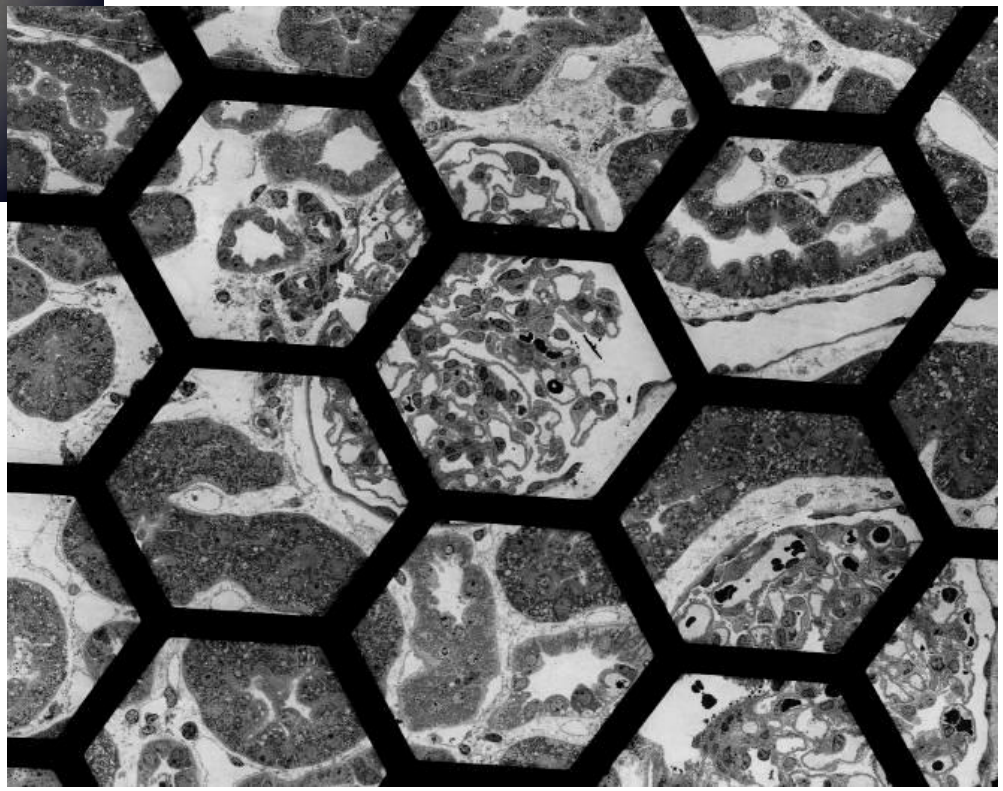


Low magnification image including grid bars



Section on grid
Not to scale

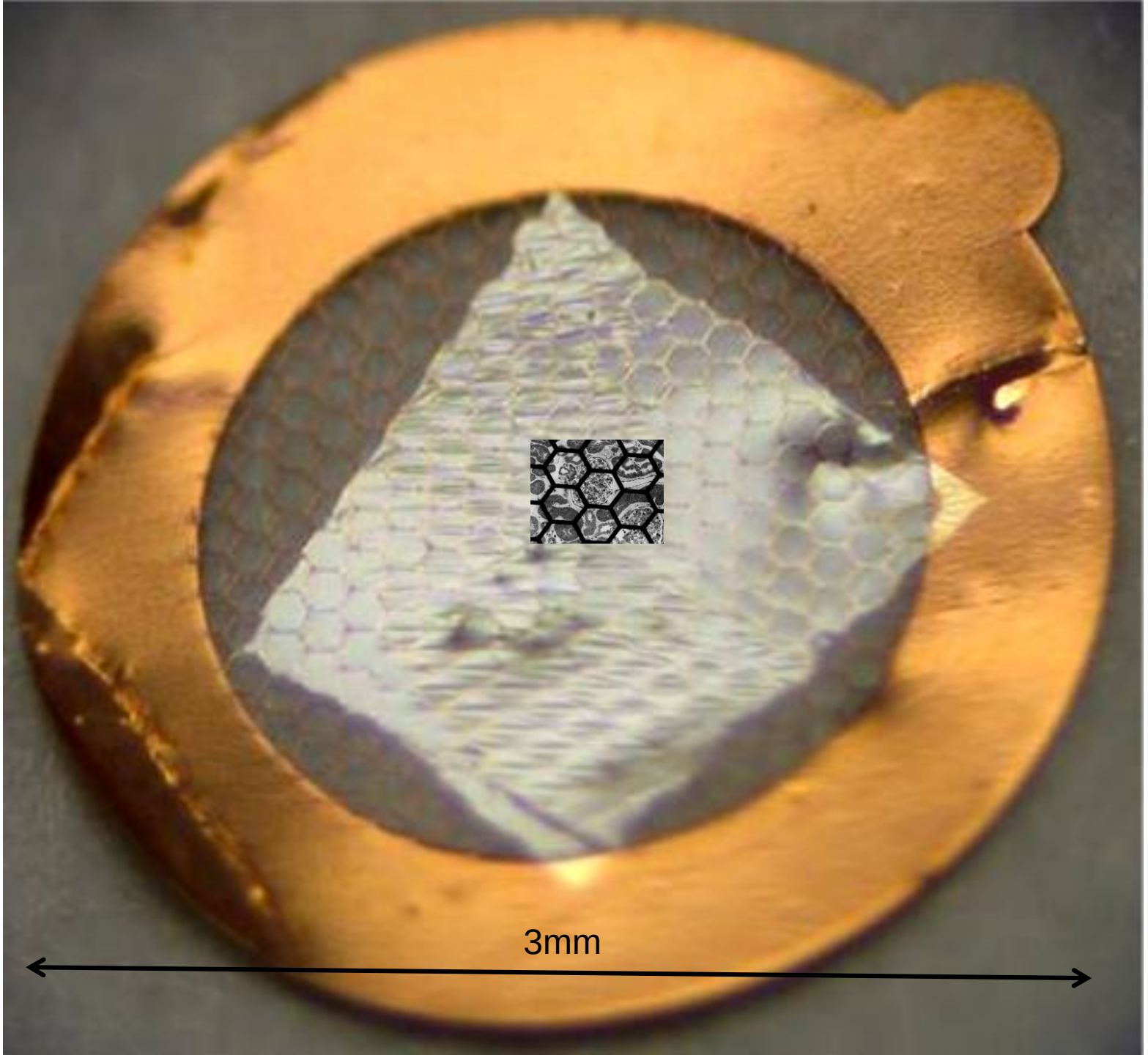
Stereo
microscopy



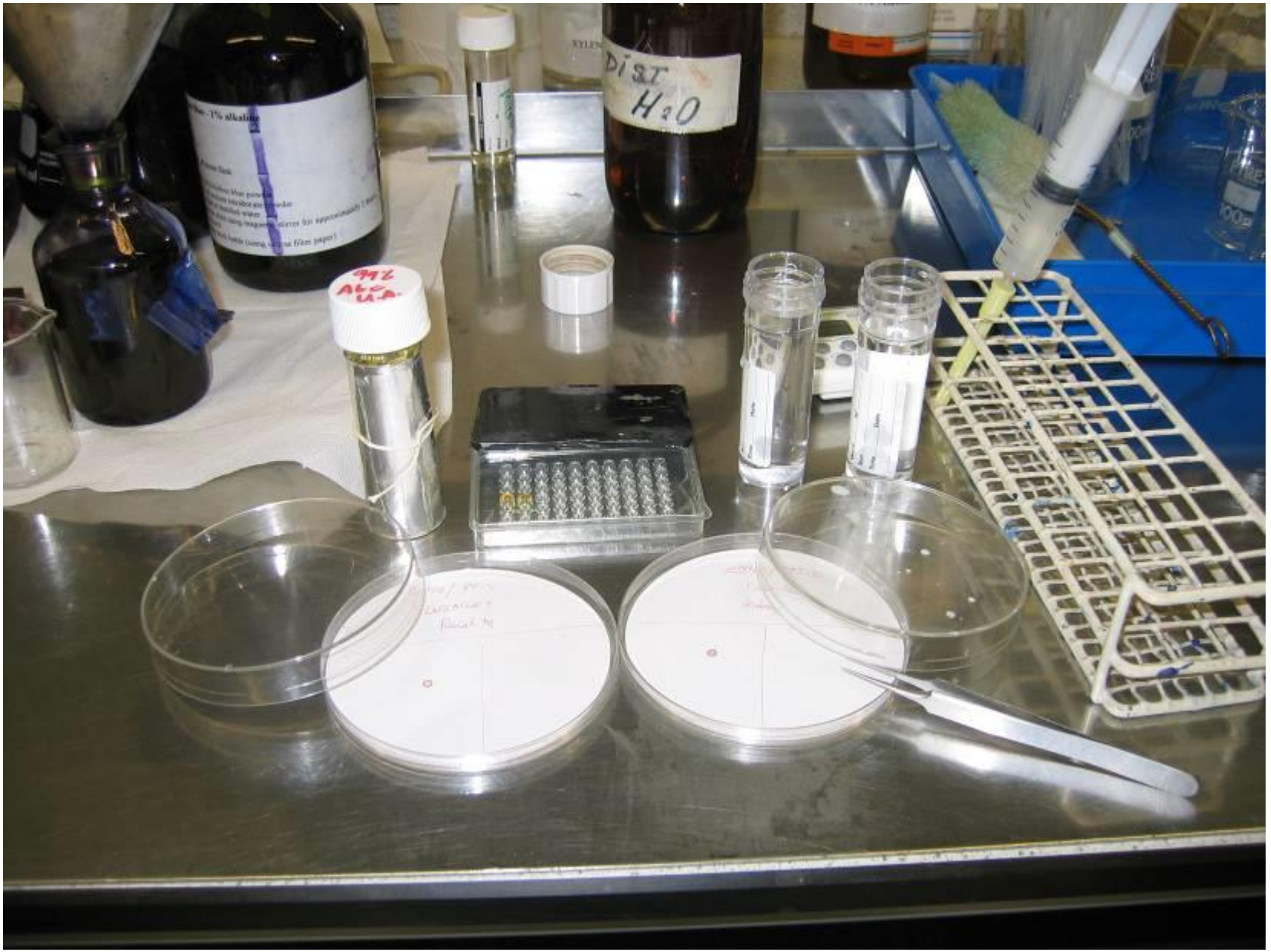
Transmission electron
microscopy

Section on grid.

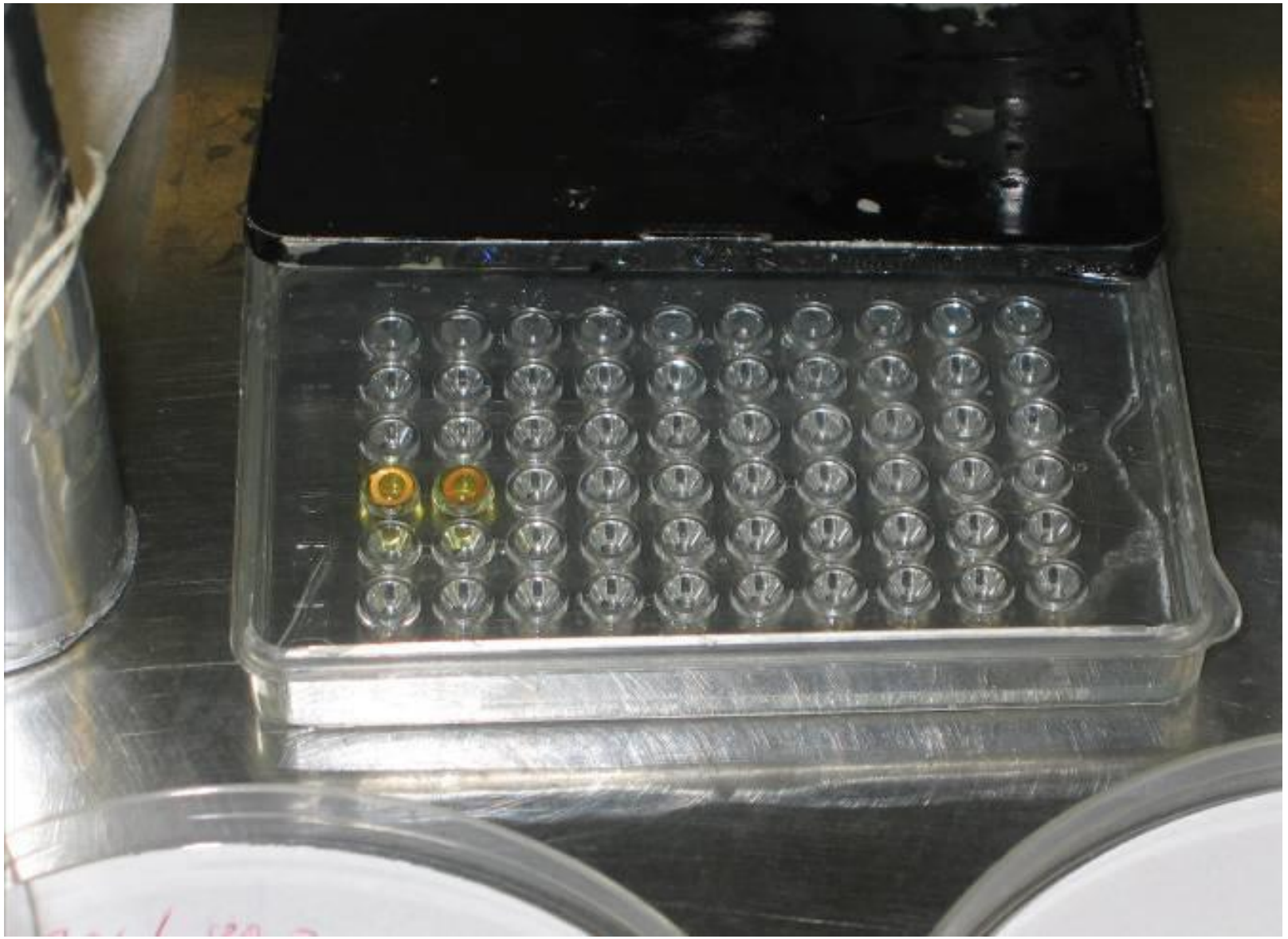
To same scale.



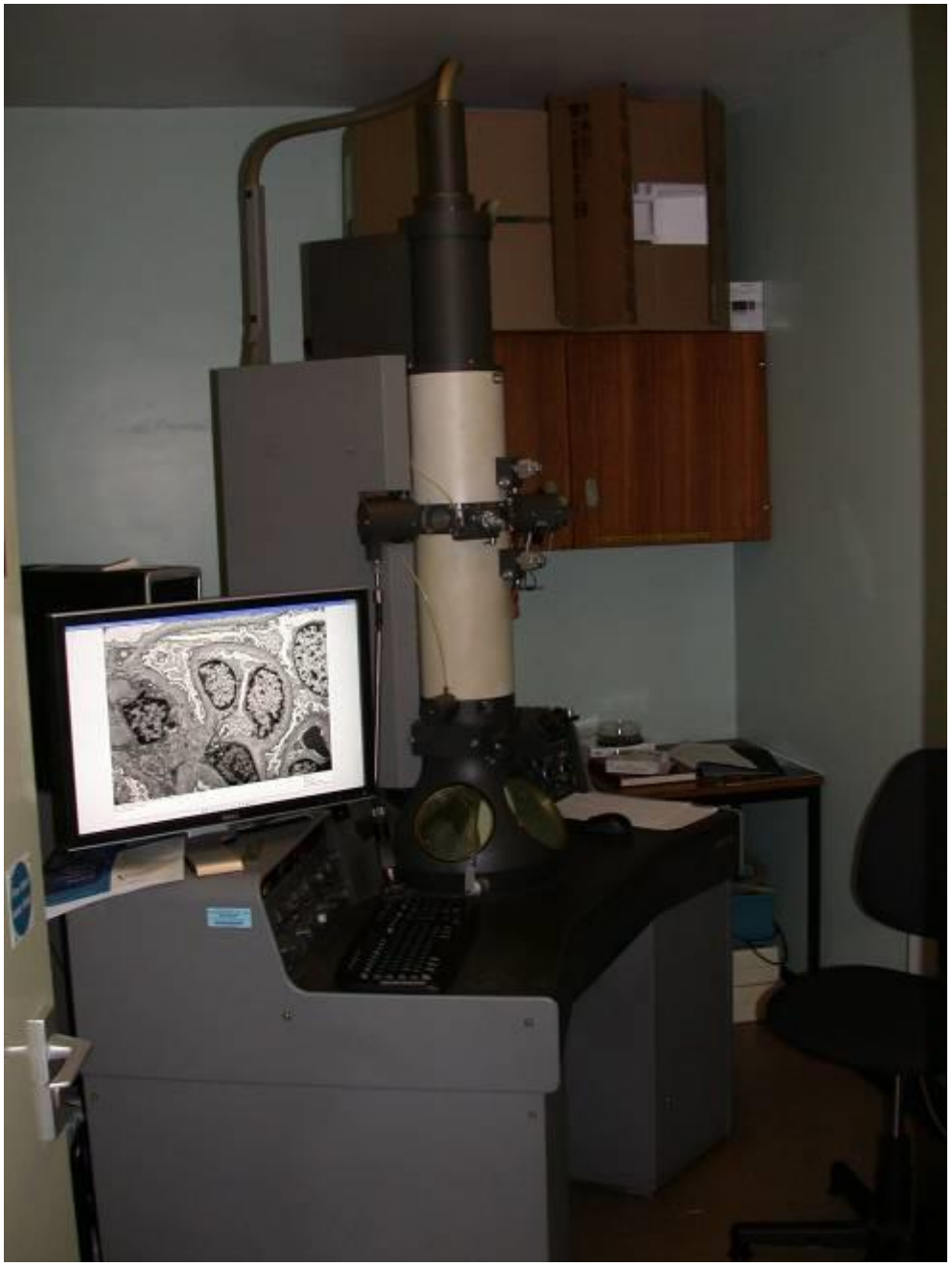
Toluidine blue stained section on glass slide and thin section on copper grid



Grids stained in Uranyl acetate and Lead citrate

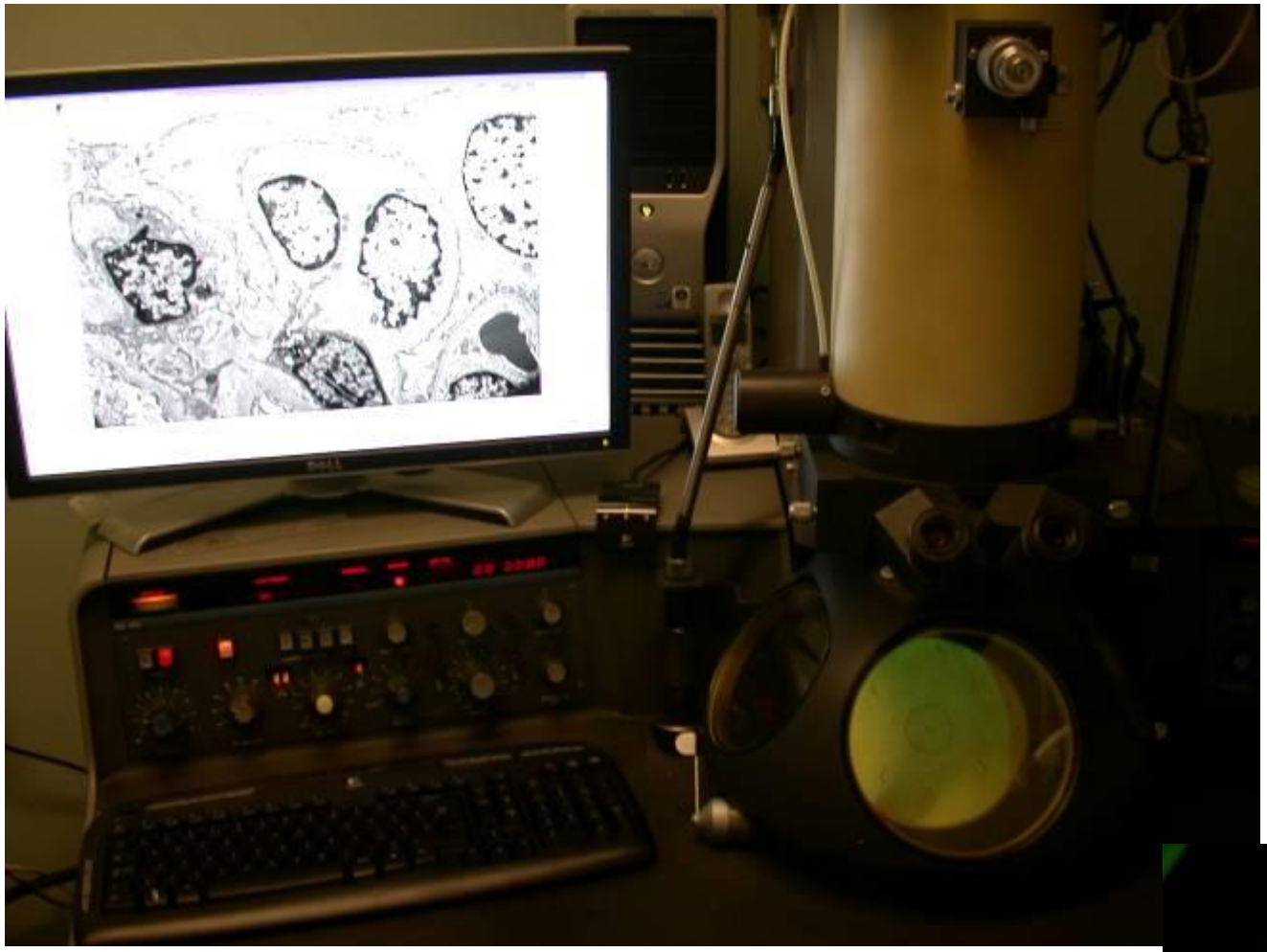


Grids in alcoholic uranyl acetate

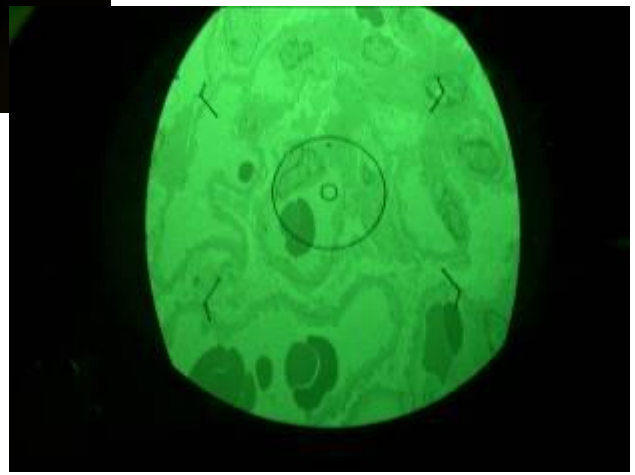


Phillips 400 transmission electron microscope.

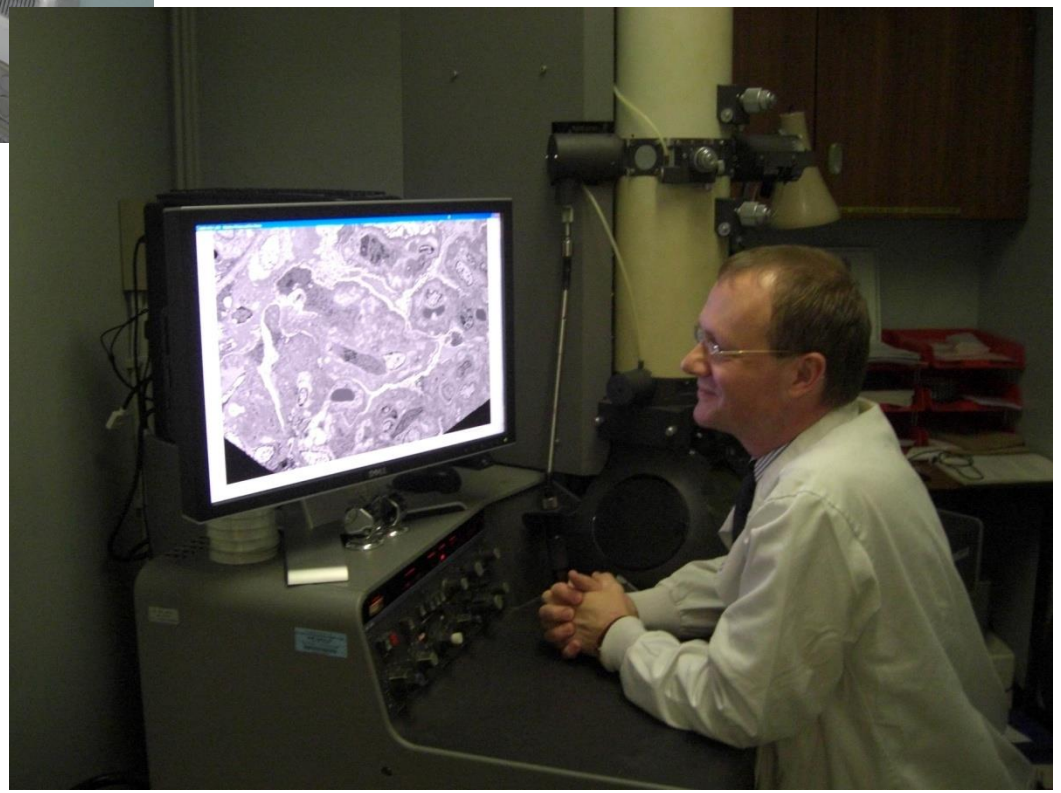
AMT 16 megapixel digital camera

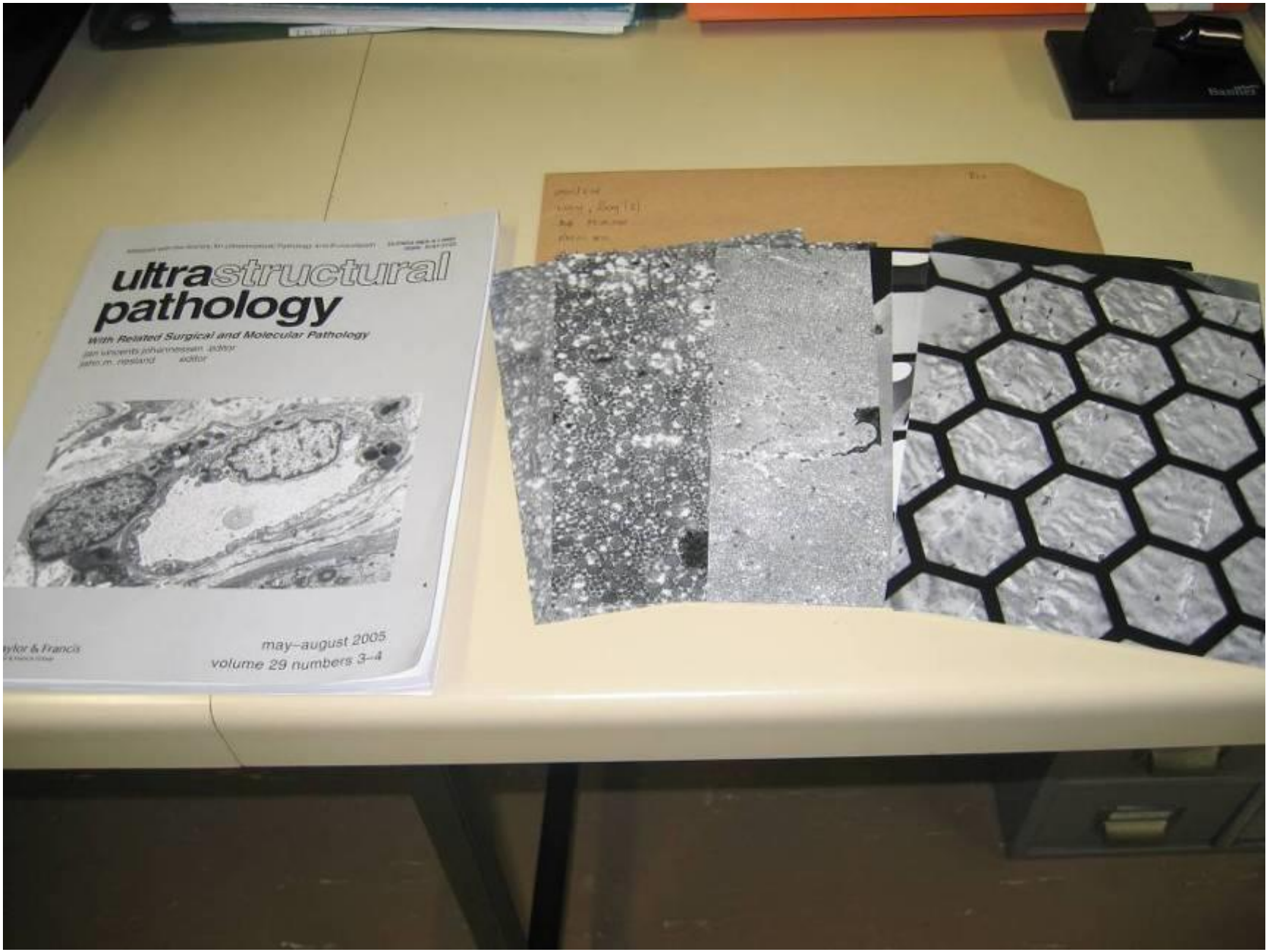


Electrons travel down the microscope column, through the section and then hit either the phosphorescent screen or the digital camera detector



Section imaged using phosphorescent screen and computer monitor





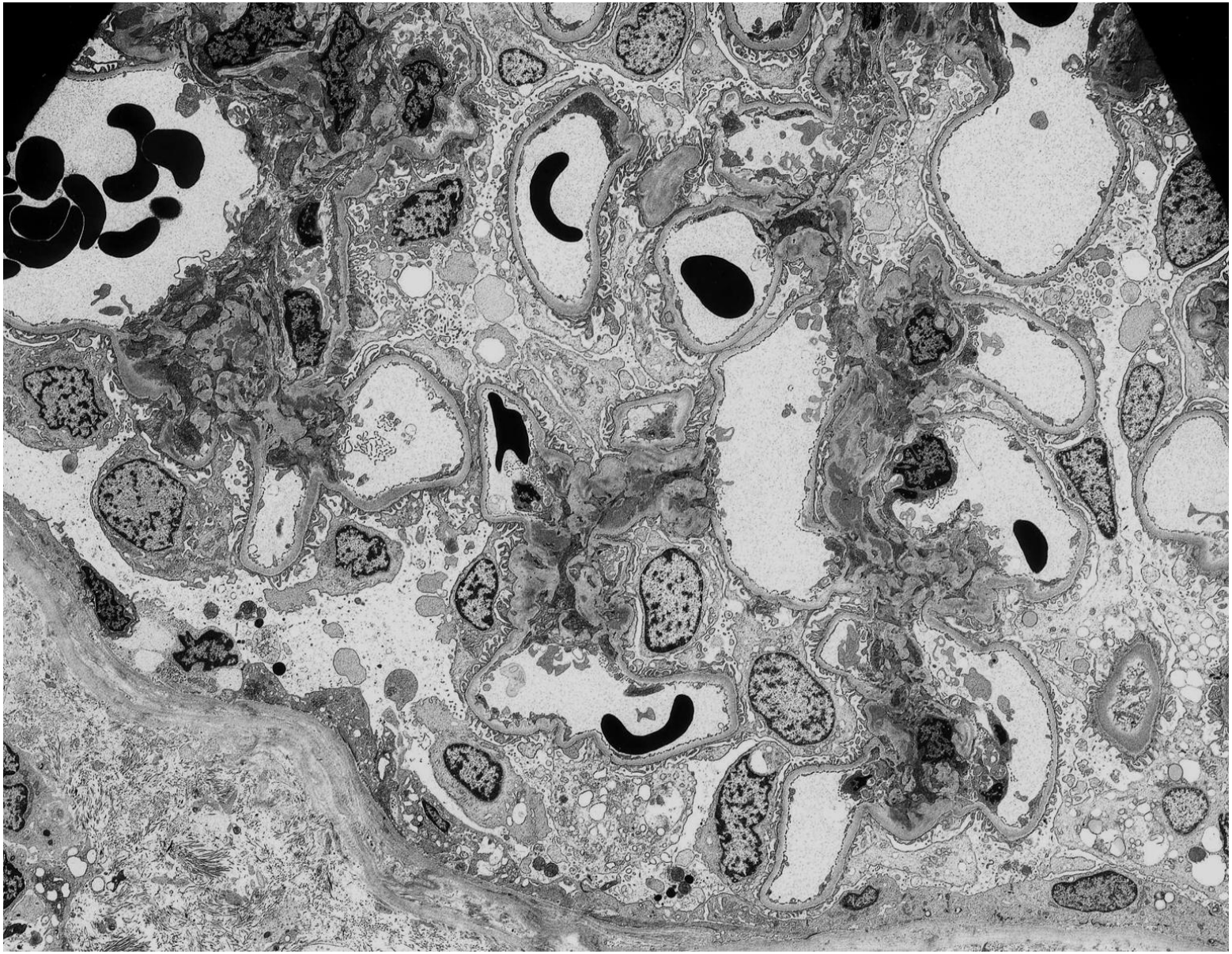
Production of electron micrographs

A selection of electron microscopy and general pathology text books

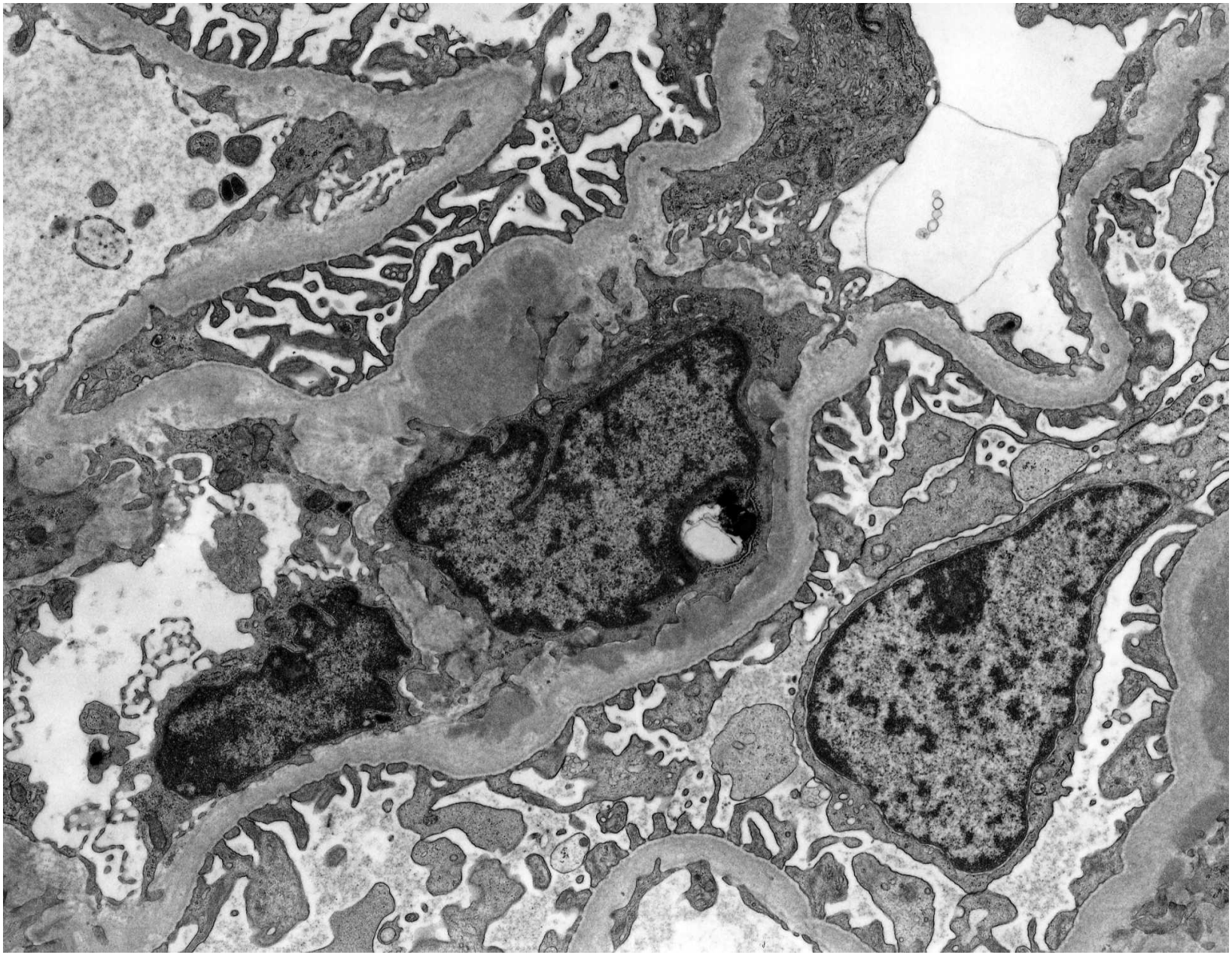
Teaching set of electron micrographs

Negative, block and slide filing system

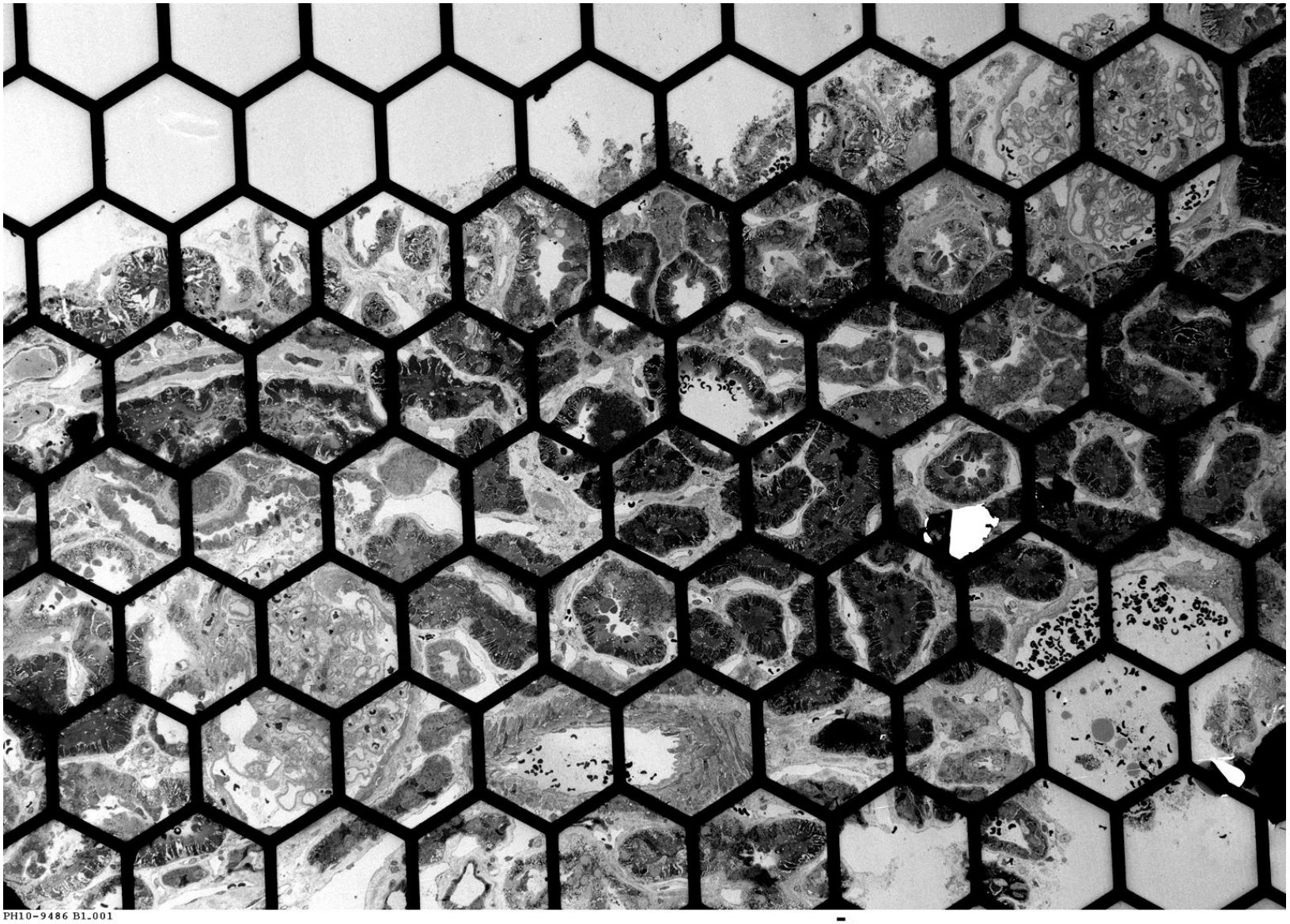
Electron micrograph using large format Kodak negative



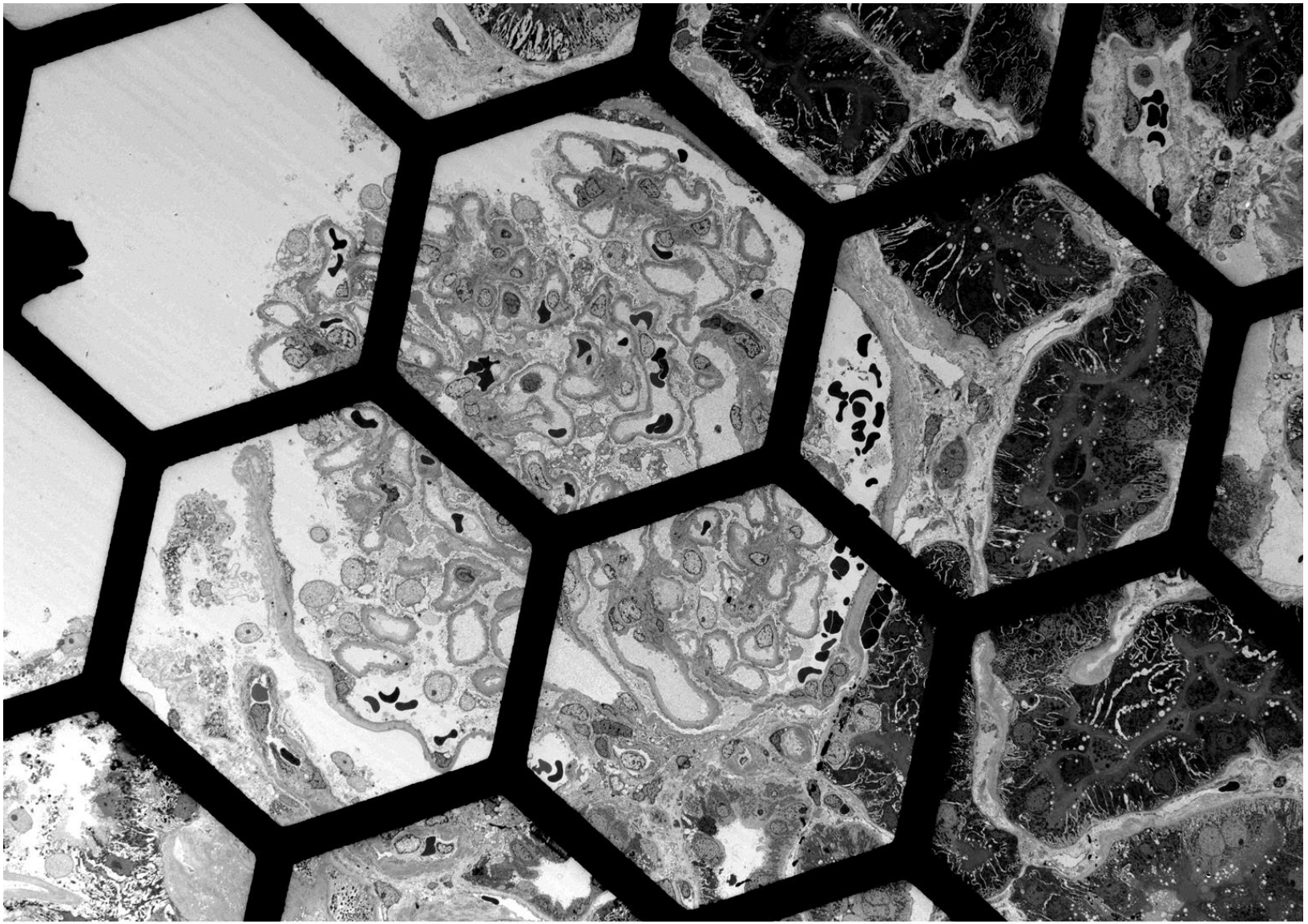
Electron micrograph using large format Kodak negative



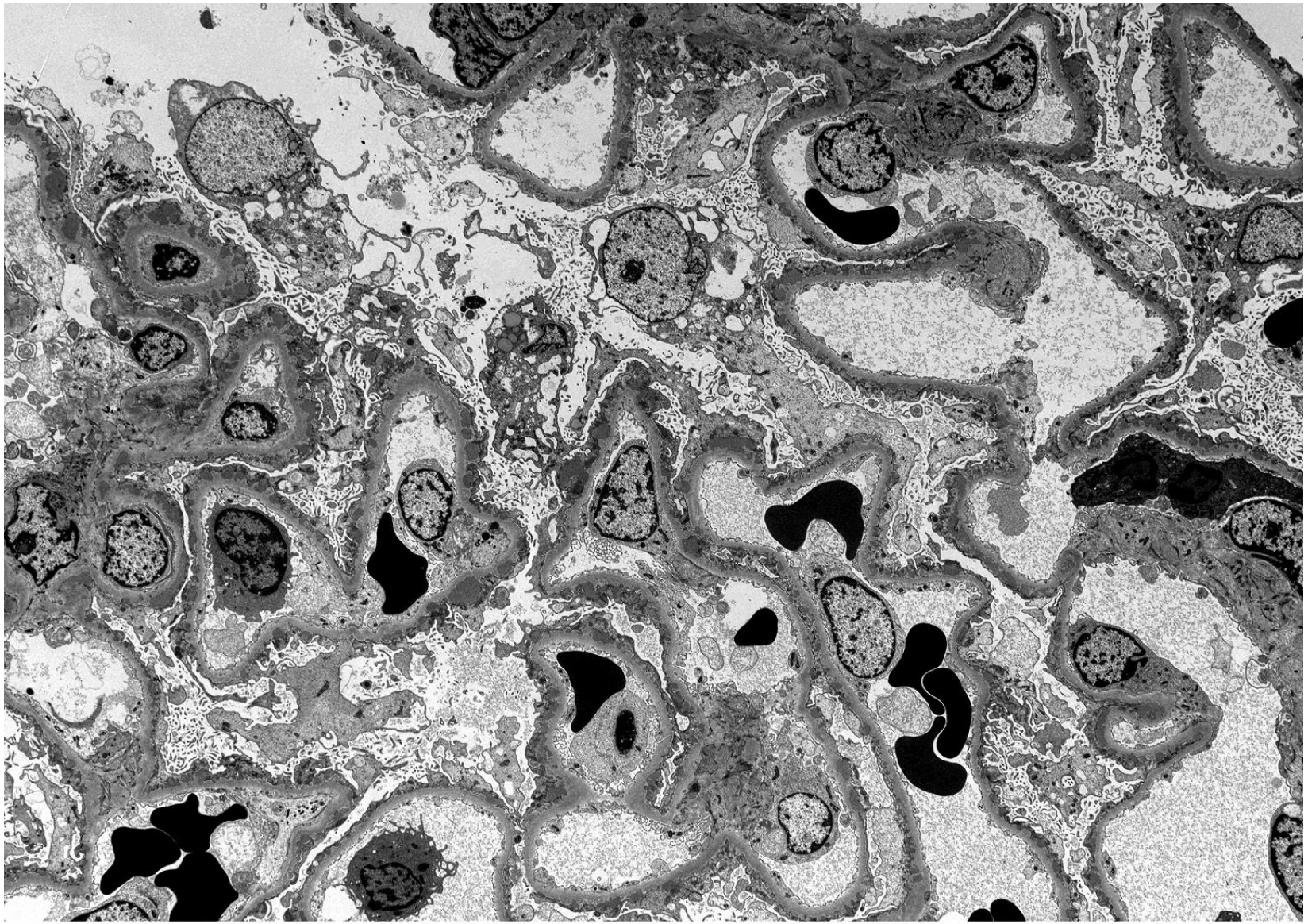
Electron micrograph using 16megapixel AMT digital camera



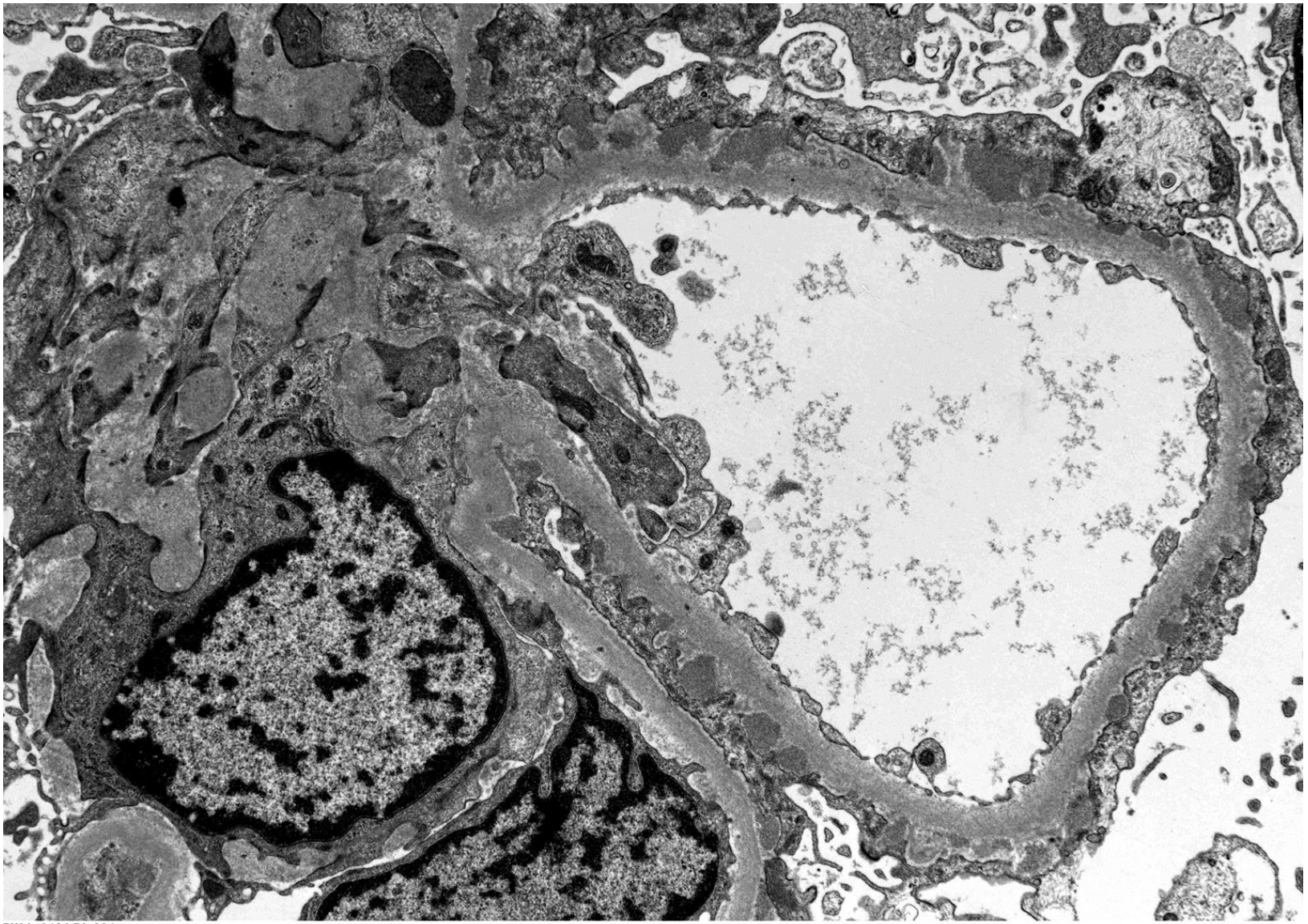
Electron micrograph using 16megapixel AMT digital camera



Electron micrograph using 16megapixel AMT digital camera



Electron micrograph using 16megapixel AMT digital camera



Electron micrograph using 16megapixel AMT digital camera