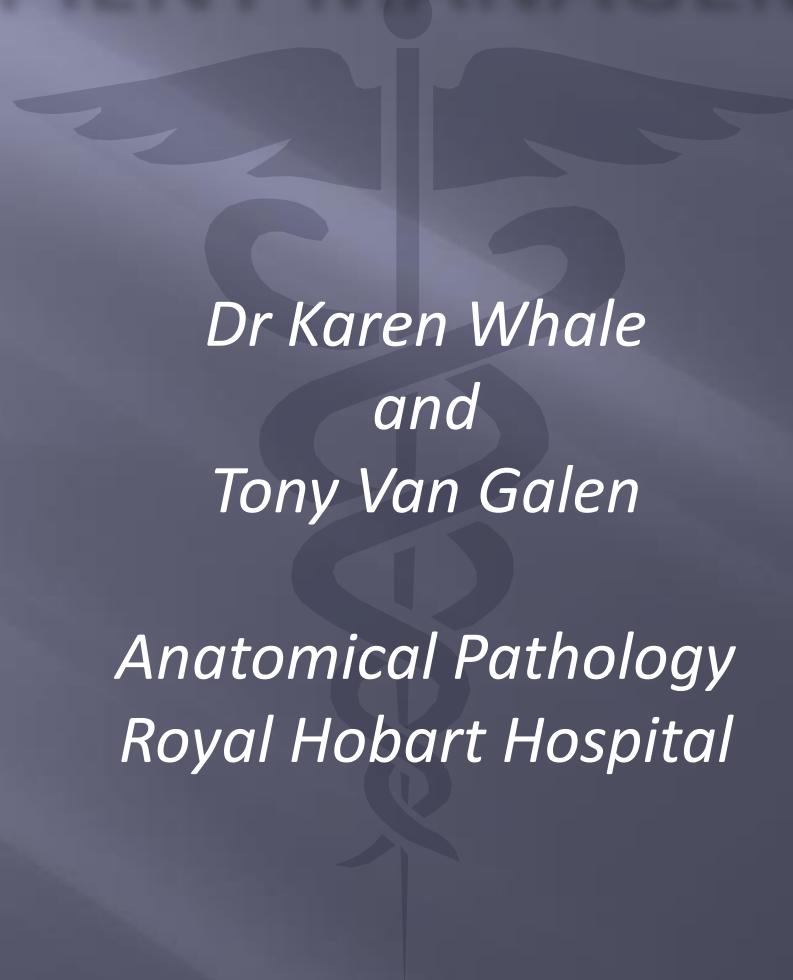


ROLE OF PERCUTANEOUS RENAL BIOPSY IN KIDNEY TRANSPLANT RECIPIENT MANAGEMENT



Dr Karen Whale

and

Tony Van Galen

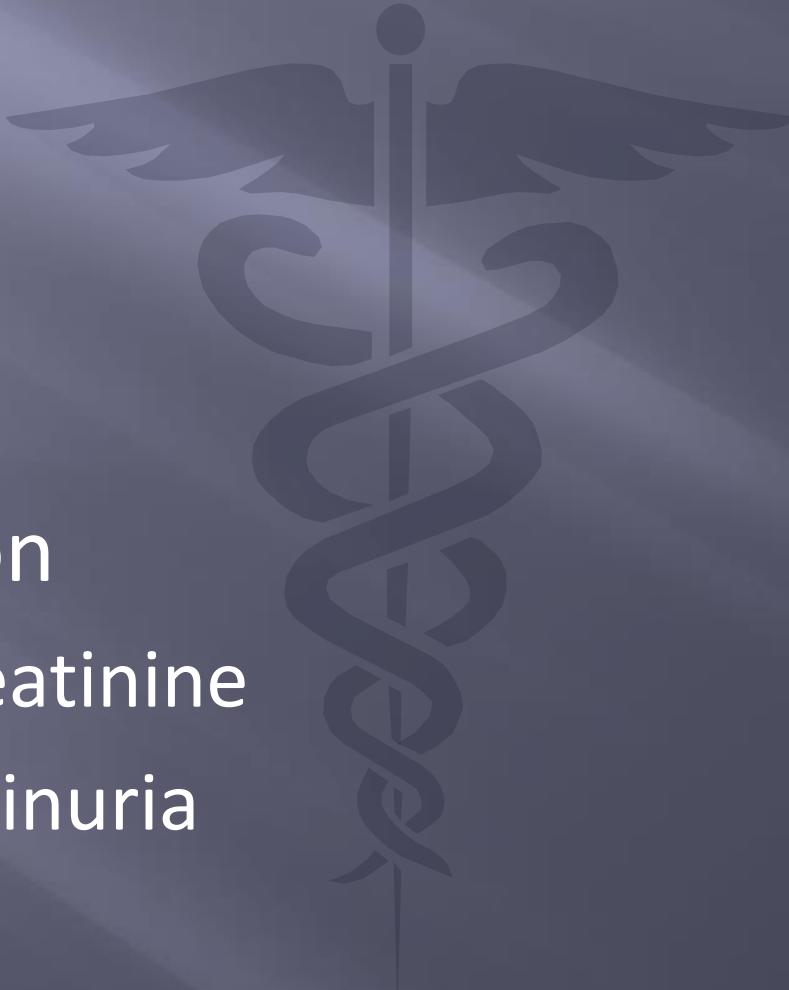
*Anatomical Pathology
Royal Hobart Hospital*

Kidney transplants

- 821 in Australia in 2016
- 266 in Victorian hospitals
- Royal Hobart Hospital
 - State referral lab for renal biopsies
 - 49 transplant renal biopsies in 2016

When is a biopsy performed?

- Implantation
- 3 months
- 12 months
- Clinical indication
 - Rising serum creatinine
 - Increasing proteinuria

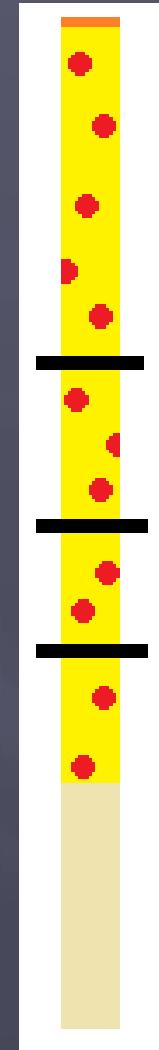
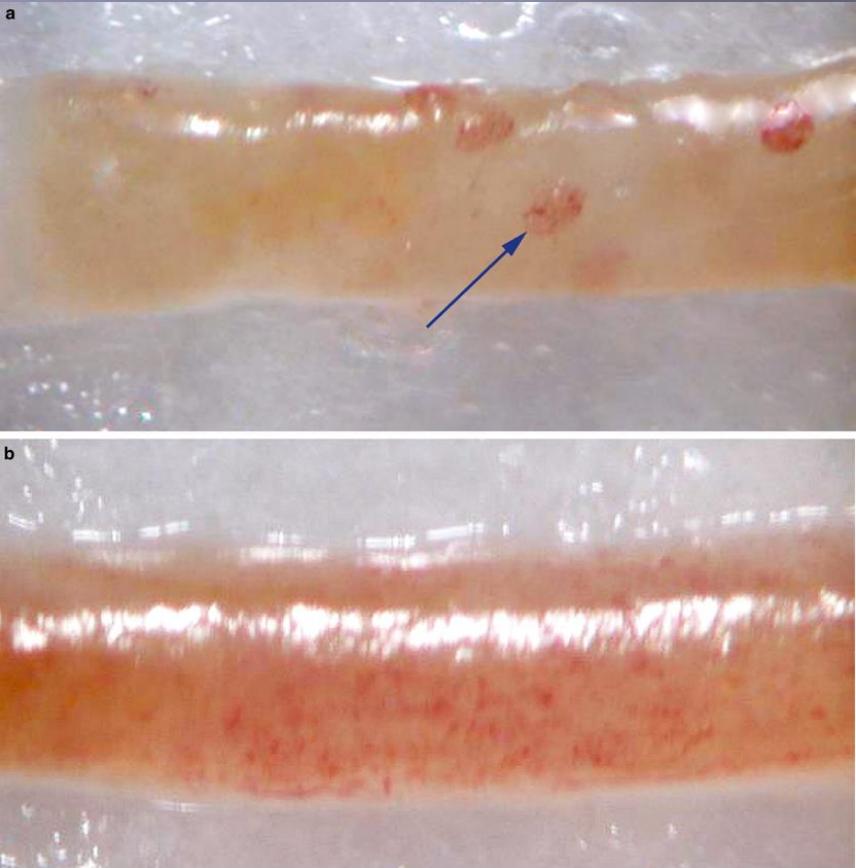


Risks of renal biopsy

- Pain
- Infection
- Bleeding
- Clot obstruction
- Intra-renal AV fistula formation
- Nephrectomy (1/10000)
- Death (1/5000)
- Damage to other organs (bladder, spleen, bowel)
- Failure

Renal biopsy

- 16G core, one core for transplants
- Received fresh and assessed for ancillary studies



Light microscopy

Immunofluorescence

Electron microscopy

Light microscopy

Examination of Renal tissue

- Light Microscopy
- Immunohistochemistry
- Electron Microscopy

Light Microscopy

- Fixation
- Processing
- Sectioning
- Staining



Fixation

- Enhance staining characteristics
- Preserve antigenicity
- Rapid fixation and processing

Fixation

- 10% Neutral Buffered Formalin (pH7.2)
- 60 minutes @ 44°C

2.5hr Processing Cycle

STEP	REAGENT	TEMPERATURE	TIME	P/V
1	70% Alcohol	37°C	10 min.	Ambient
2	95% Alcohol	37°C	10 min.	Ambient
3	Absolute Alcohol	37°C	10 min.	Ambient
4	Absolute Alcohol	37°C	10 min.	Ambient
5	Absolute Alcohol	37°C	10 min.	Ambient
6	Absolute Alcohol	37°C	10 min.	Ambient
7	Xylene	37°C	10 min.	Ambient
8	Xylene	37°C	15 min.	Vacuum
9	Precision Cut Paraffin Wax	62°C	10 min.	Vacuum
10	Precision Cut Paraffin Wax	62°C	10 min.	Vacuum
11	Precision Cut Paraffin Wax	62°C	15 min.	Vacuum

Microtomy Preparation

- Paraffin block edge and surface trimming
- Pre-cool block using ice water, cold plate, fridge freezer & -80°C freezer
- Clean disposable knife blade
- Pre-cool knife holder and blade

Sectioning

- 3 levels of serial sections @ 1.0um H&E
- 3 levels of serial sections @ 0.5um PAS
- 3 levels of serial sections @ 0.5um Silver Methenamine/MT
- 3um Orcein Giemsa
- 6um Congo Red
- 4um SV40 & C4d

Haematoxylin & Eosin

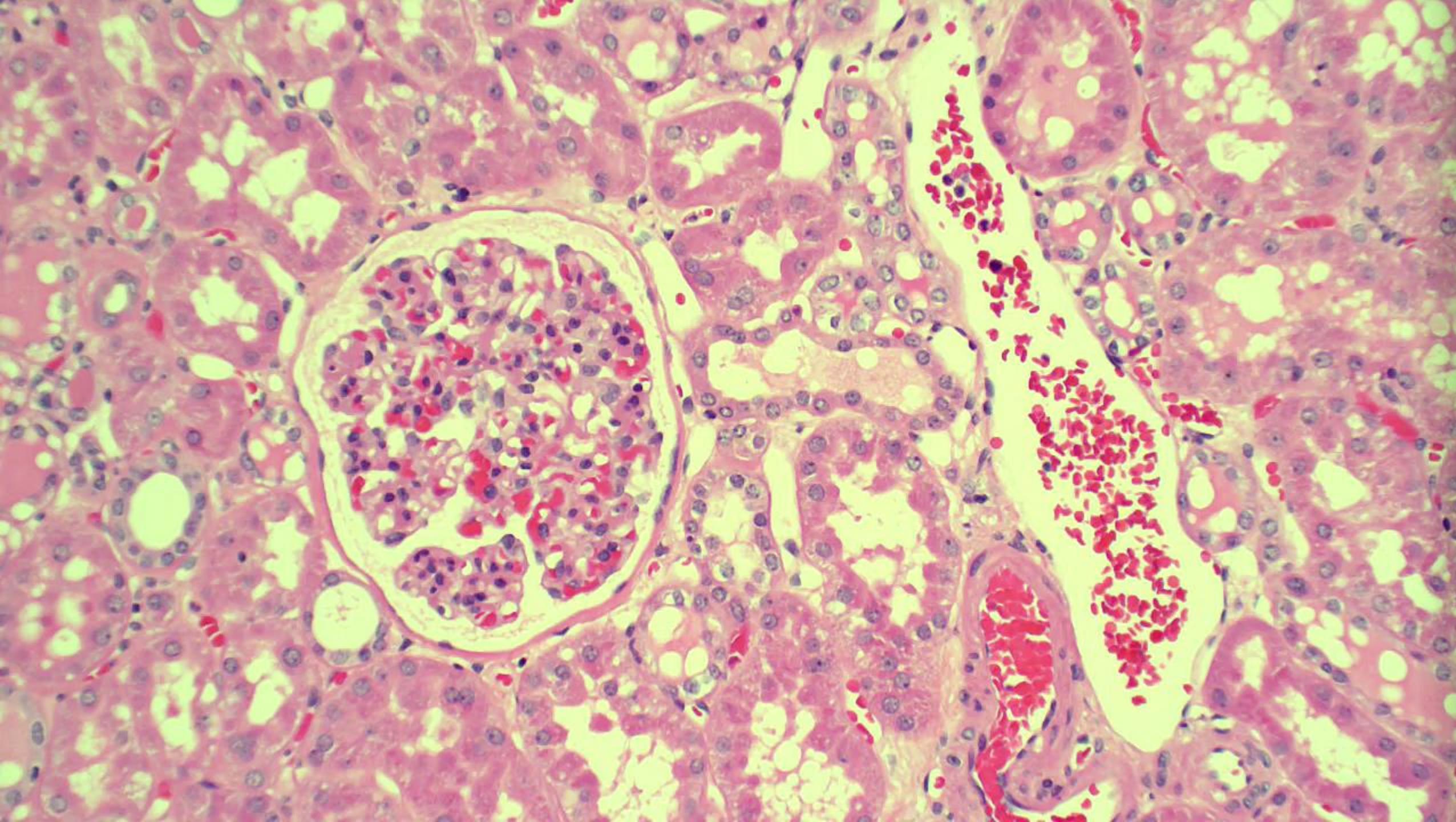
Stains:

- Celestine blue iron mordant
- Eosin Y / Phloxine B

Haematoxylin & Eosin

Results:

- Nuclei, cytoplasmic RNA, some calcium salts, urates, bacteria (weakly) - Blue
- Muscle, coarse elastic fibres, fibrin - Bright Red
- Collagen, reticulin, nerve fibres, amyloid - Pink
- Red blood cells - Orange

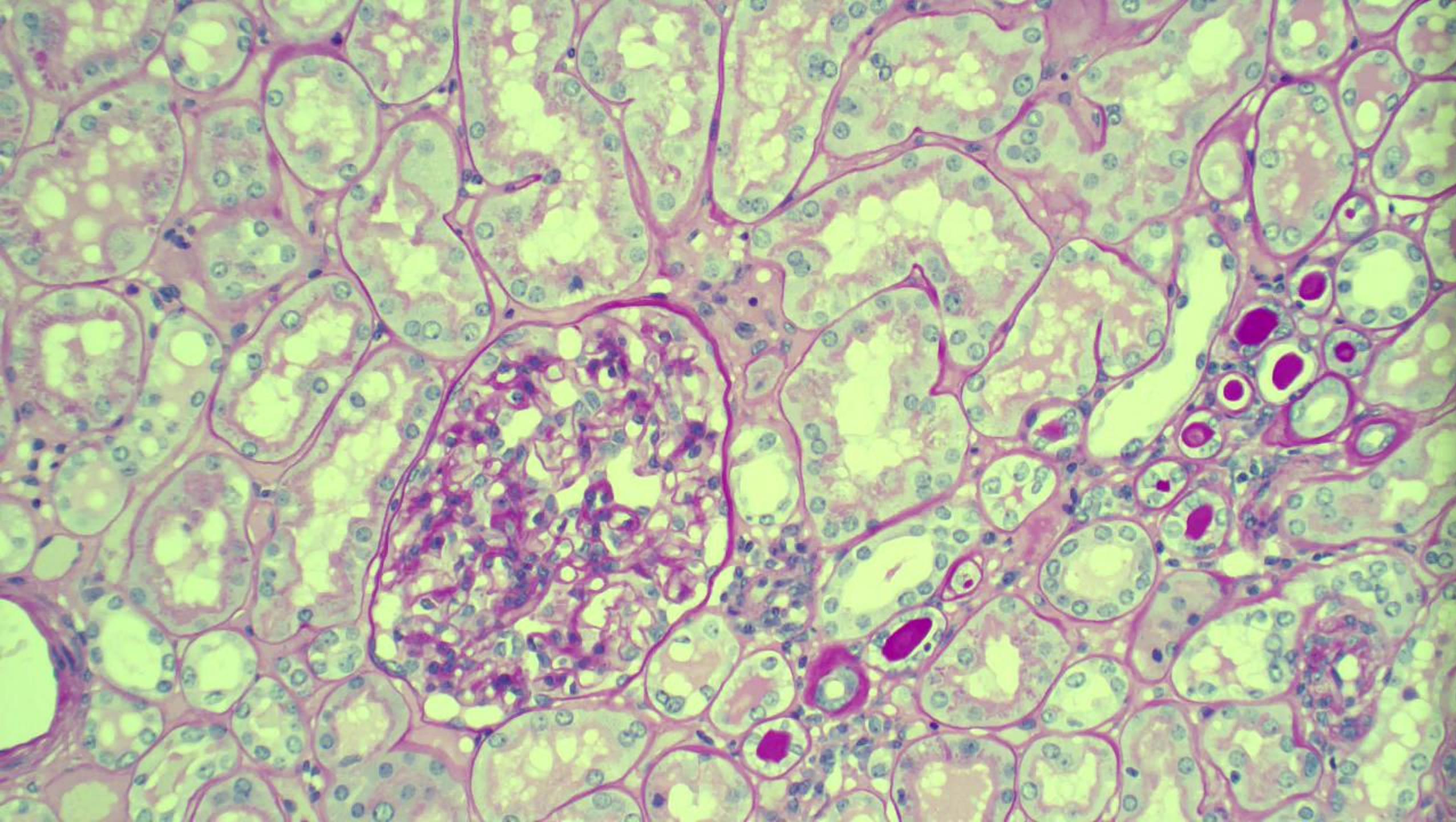


Periodic Acid – Schiff's

Schiff's staining time 30 minutes

Results:

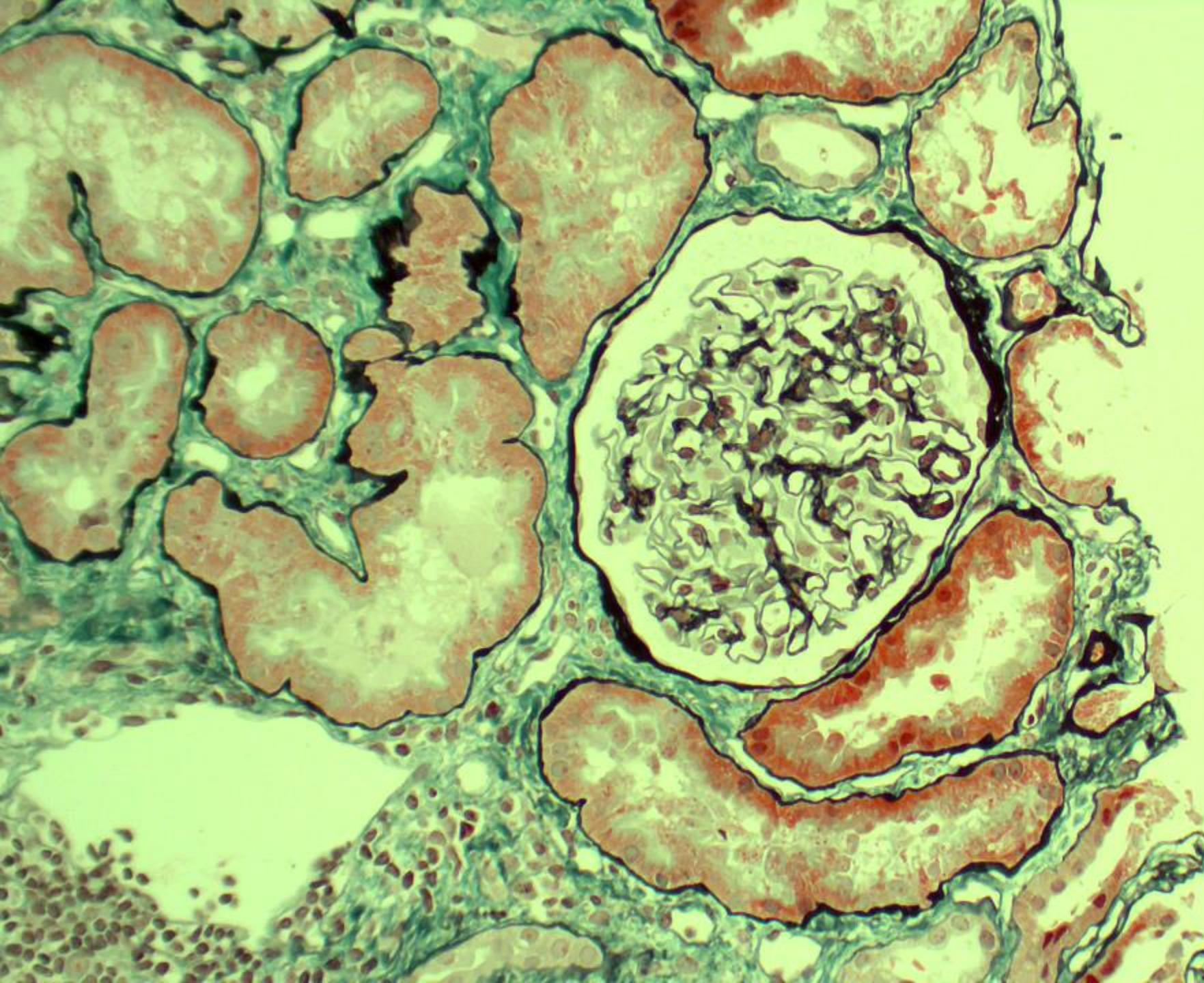
- Nuclei - Blue
- Basement membranes, carbohydrates - Magenta

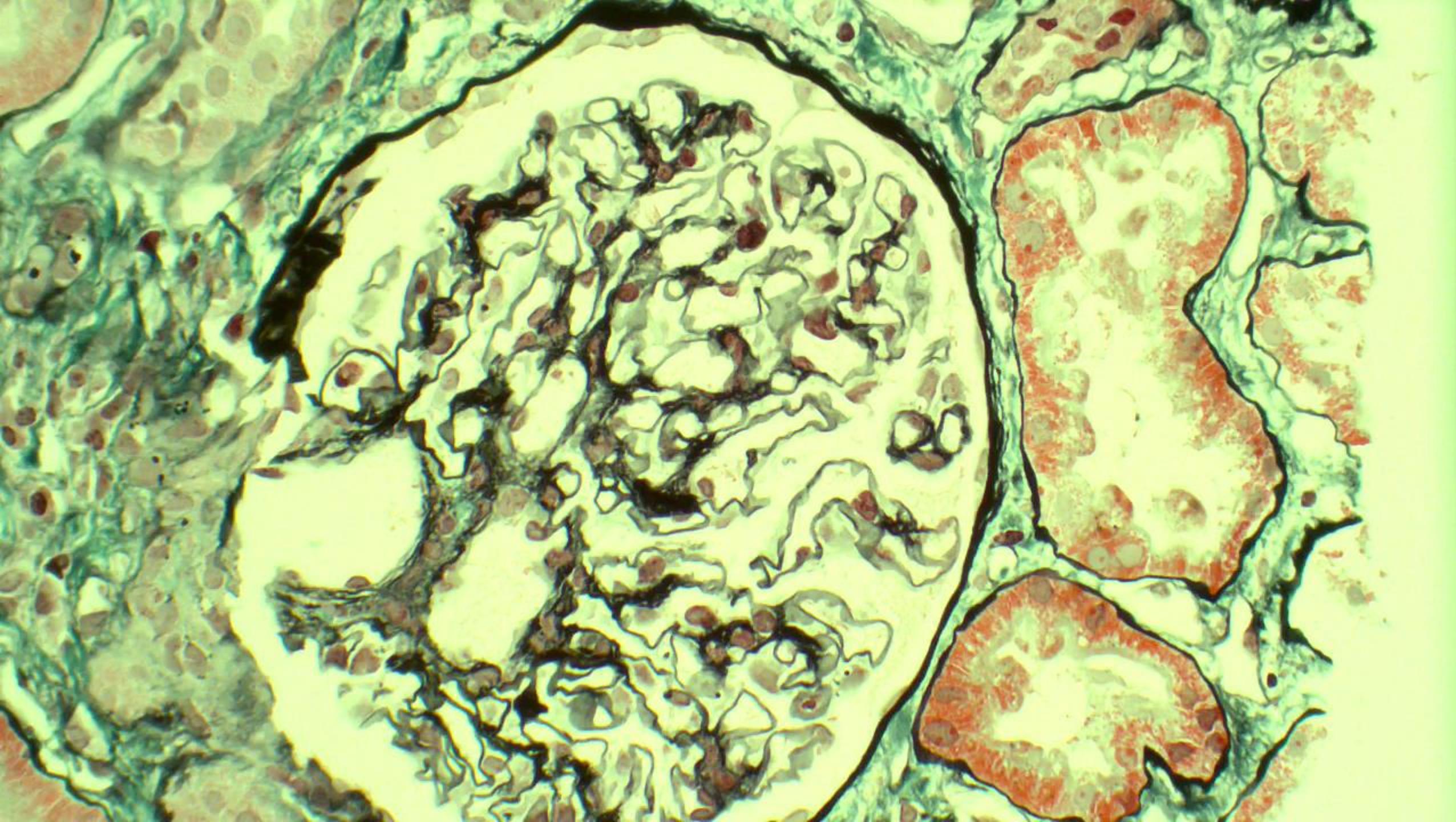


Combined Methenamine Silver / Masson Trichrome

Results:

- Glomerular capillary basement membranes - Black
- Nuclei - Blue/Black
- Muscle, red blood cells, fibrin - Red
- Immunoglobulin - Orange
- Connective tissue - Green





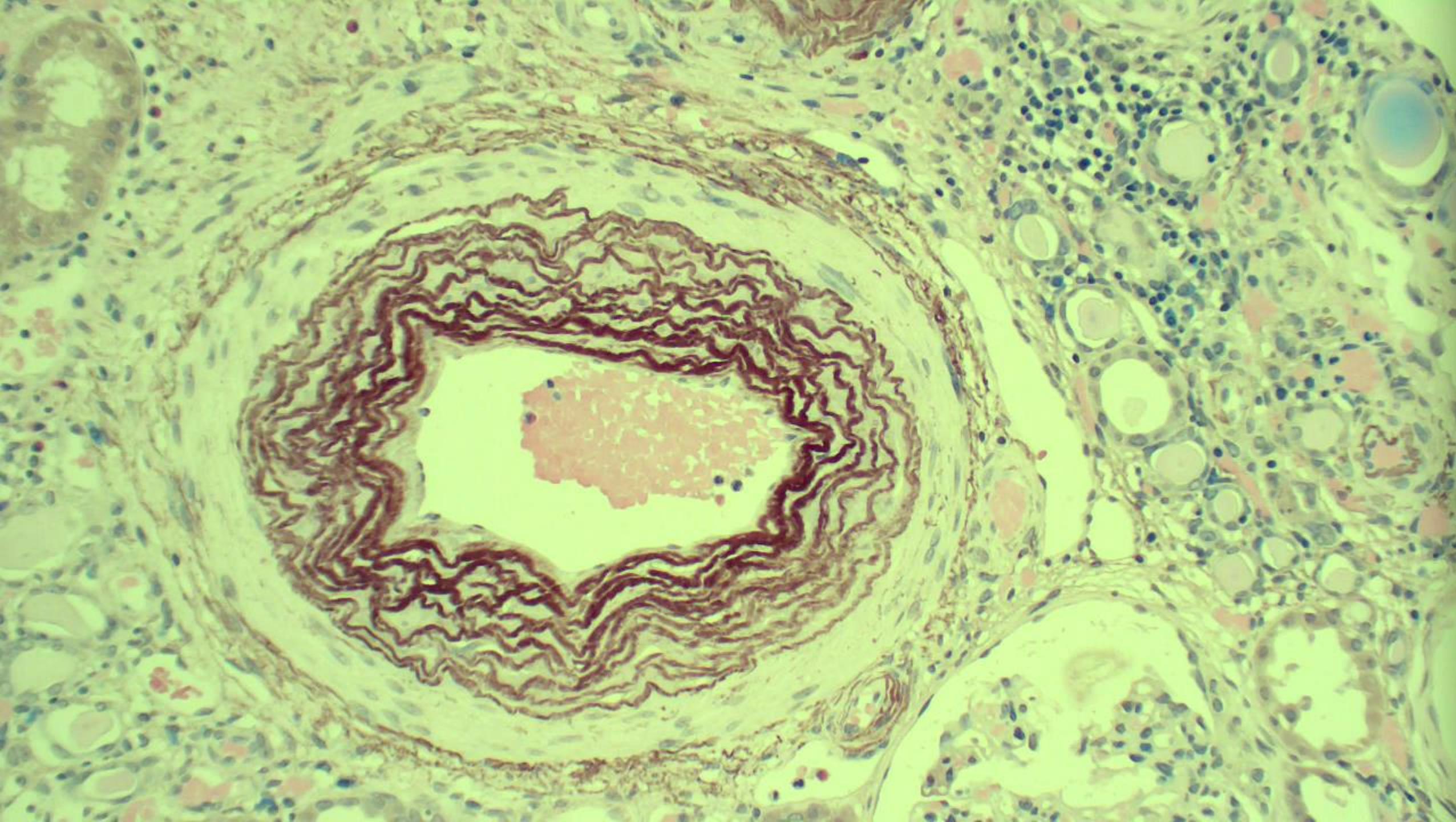
Orcein Giemsa

Simplicity and Reproducibility

Results:

- Elastic fibres - Brown
- Nuclei - Blue
- Background - Pale Pink

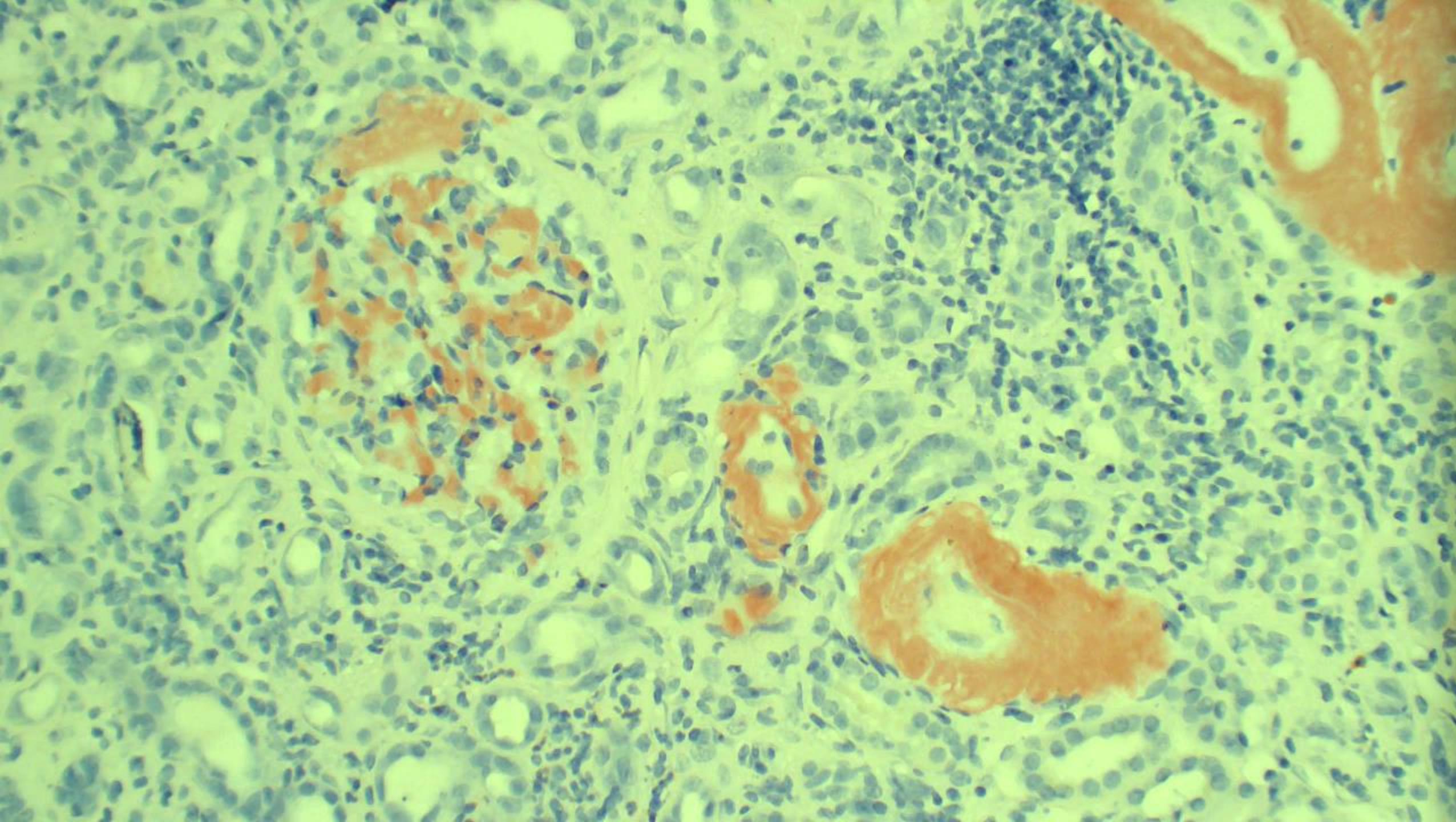




Congo Red

Results:

- Amyloid - Salmon orange
- Nuclei - Blue



Immunohistochemistry

Immunofluorescence (frozen sections)

Pros: Sensitive, simple and rapid.

Cons: Impermanent, fluorescent microscope.

Immunoperoxidase (paraffin wax sections)

Pros: Localisation of immune deposits, permanent.

Cons: More time consuming, costly.

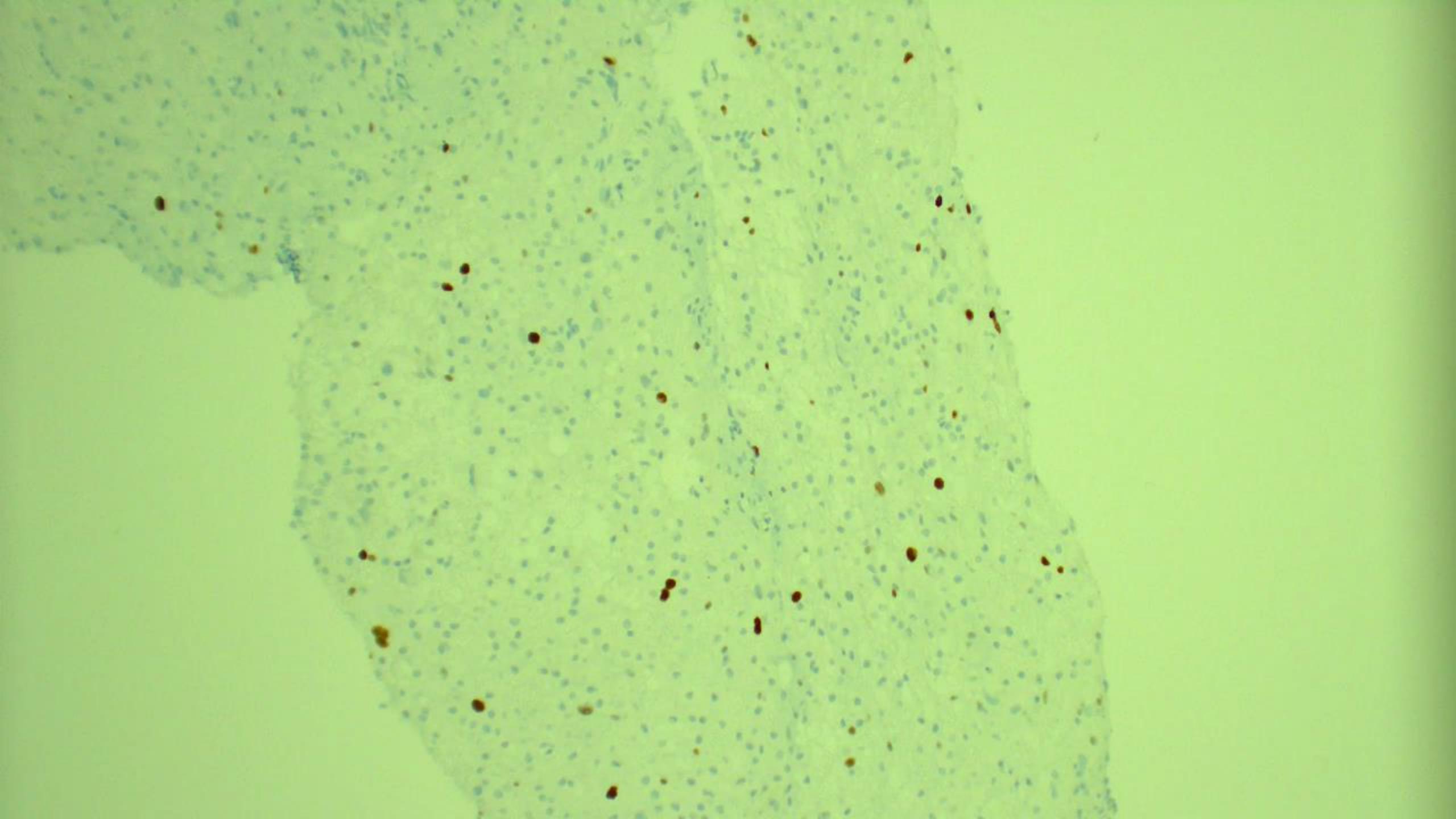
Immunoperoxidase

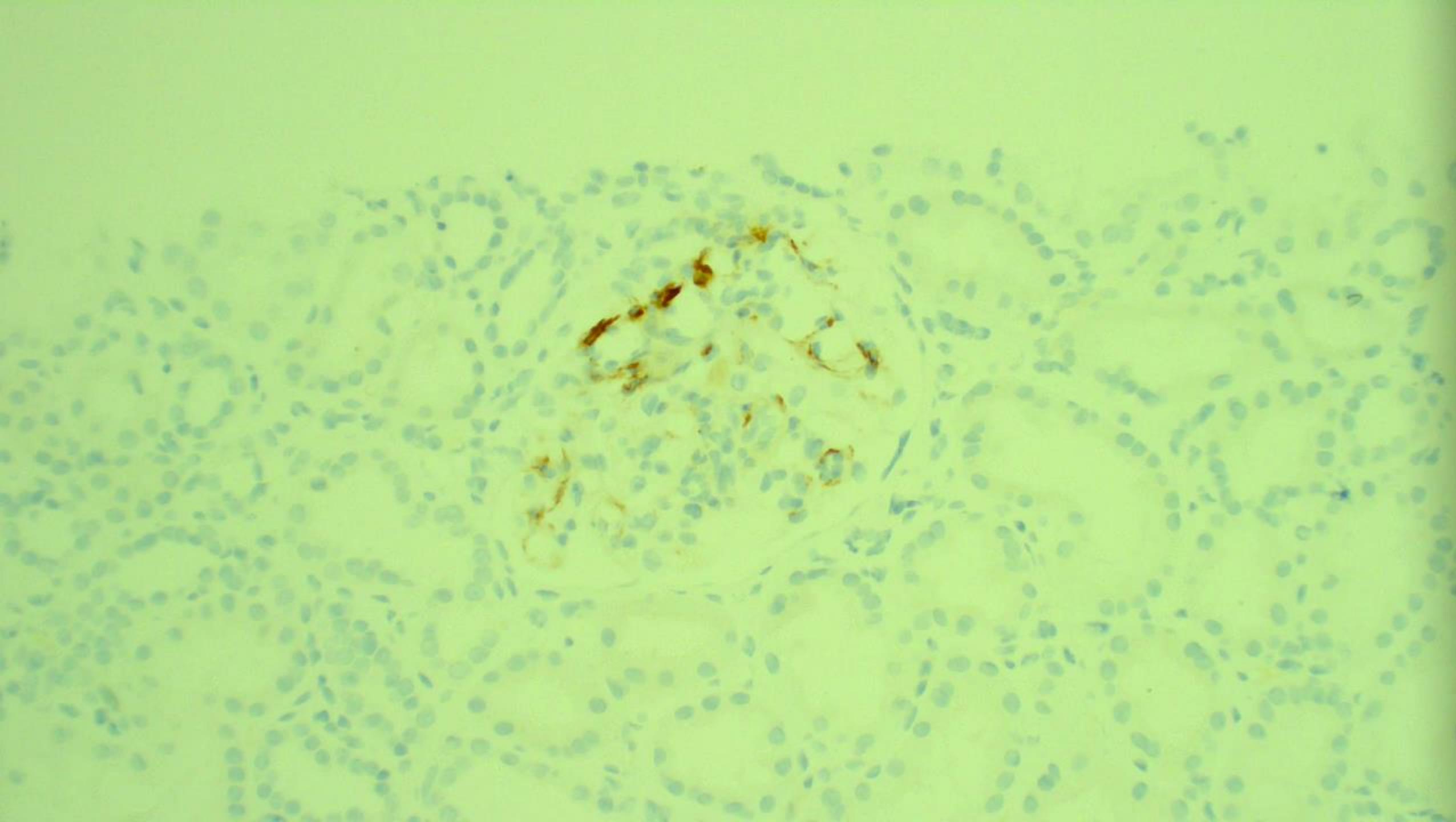
SV40 staining

- Cell Marque SV40 (MRQ-4)
- Nuclear localisation
- Simian virus 40 (polyomavirus)

C4d staining

- Roche C4d (SP91) rabbit monoclonal
- Membranous localisation
- A complement system protein

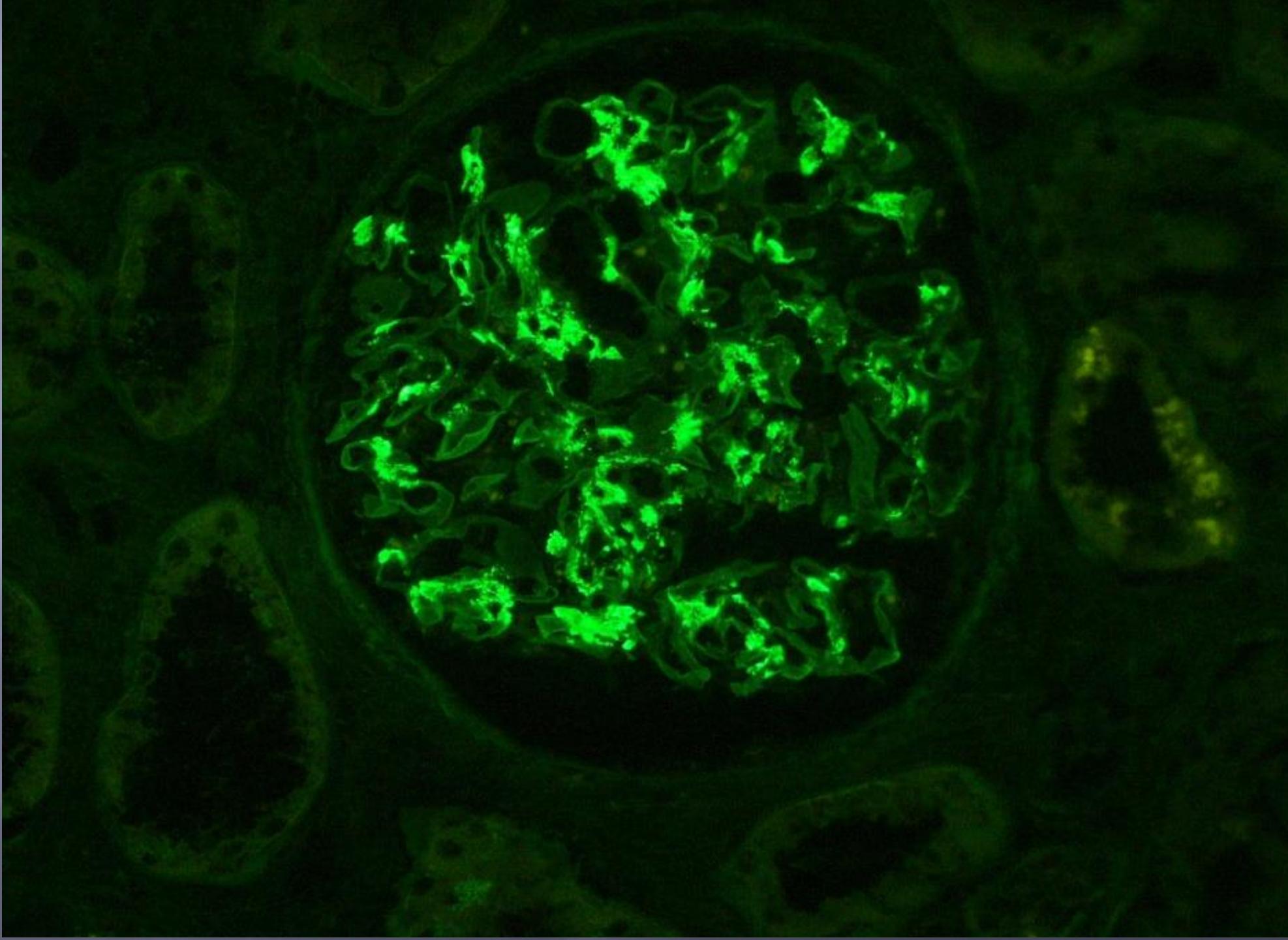


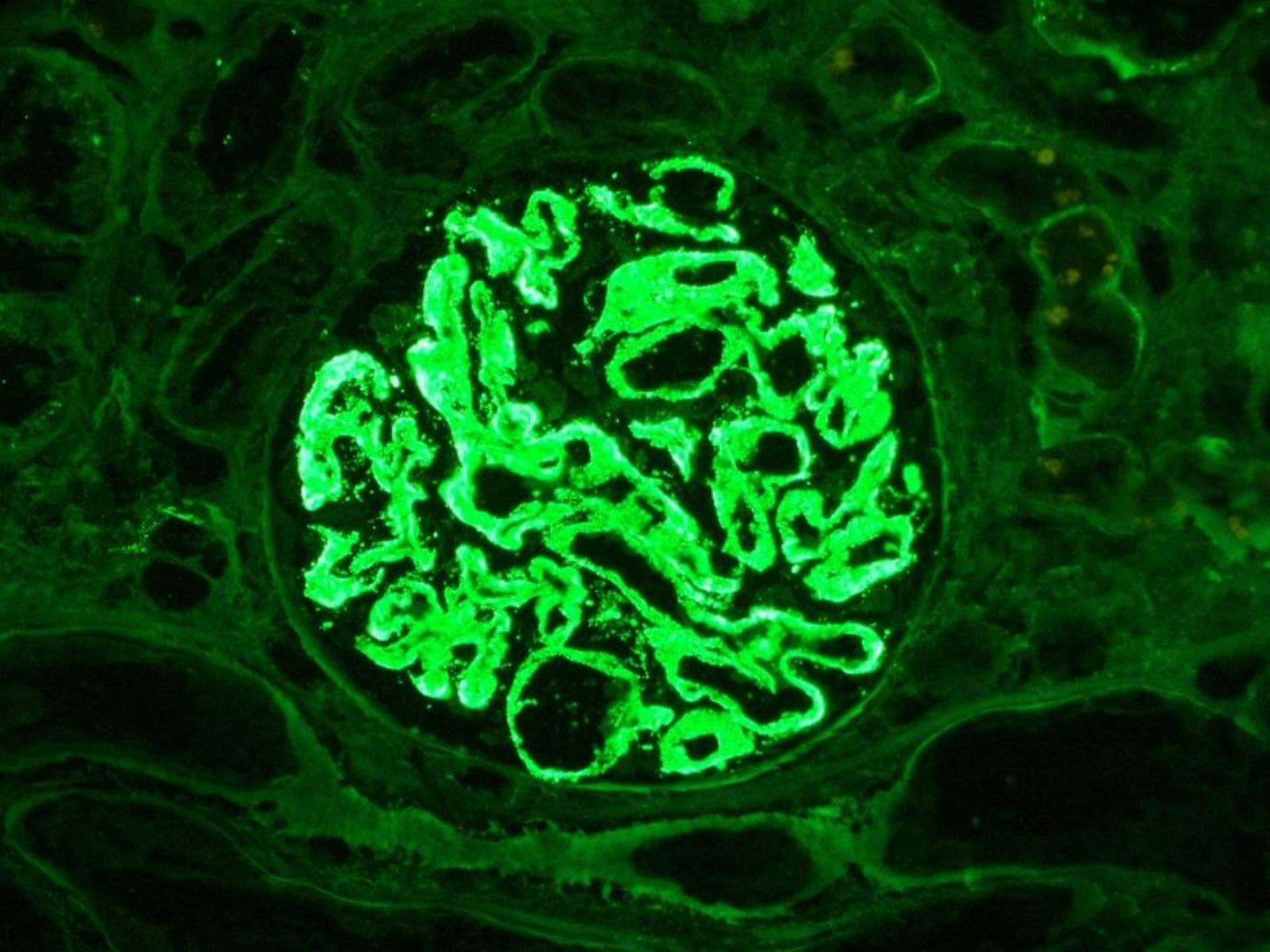


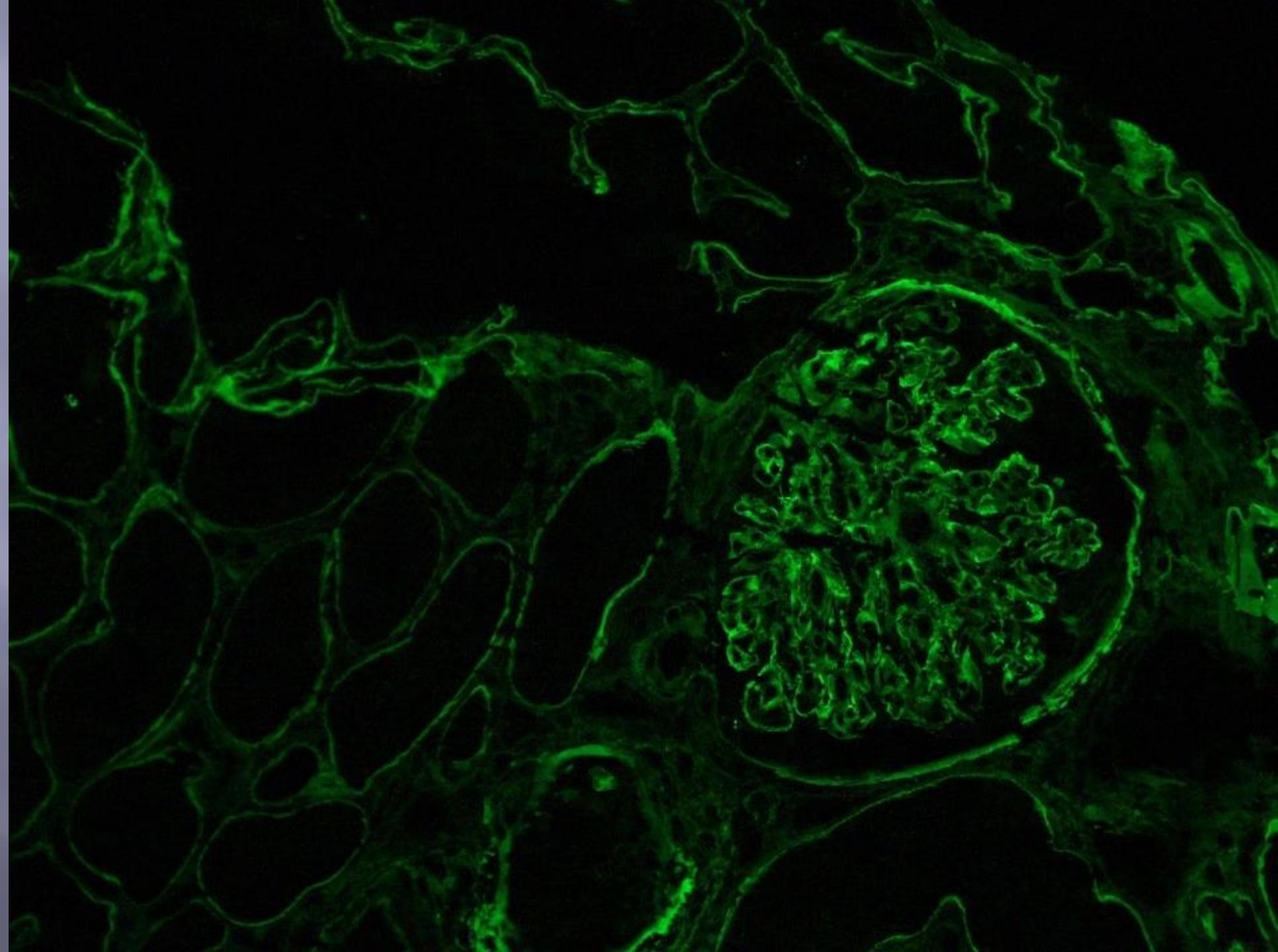
Immunofluorescence

Direct IF method for detection of peritubular capillary deposition of

- Immunoglobulins IgG, IgA & IgM
- Complement conjugates C3 & C1q
- Fibrinogen (fibrin) & Albumin
- Kappa & Lambda light chains







Electron Microscopy

- Fixed in 2.5% Glutaraldehyde in PIPES buffer @ 4°C for 24-48hrs.
- Washed in PIPES buffer.
- Sent to RCH together with LM/IF report.
- Processed, photographed and reported.
- Digital images saved on DVD & IT server.

Biopsy evaluation

- Rejection – Banff classification
- Recurrent/de novo disease
- Infection
- Drug effect

- Can have multiple pathologies
- Comparison to previous biopsies important

Rejection

Banff classification

- Normal
- Antibody mediated rejection
- Borderline or suspicious for acute cellular rejection
- T cell mediated (acute cellular) rejection
- Interstitial fibrosis and tubular atrophy

Banff scoring categories

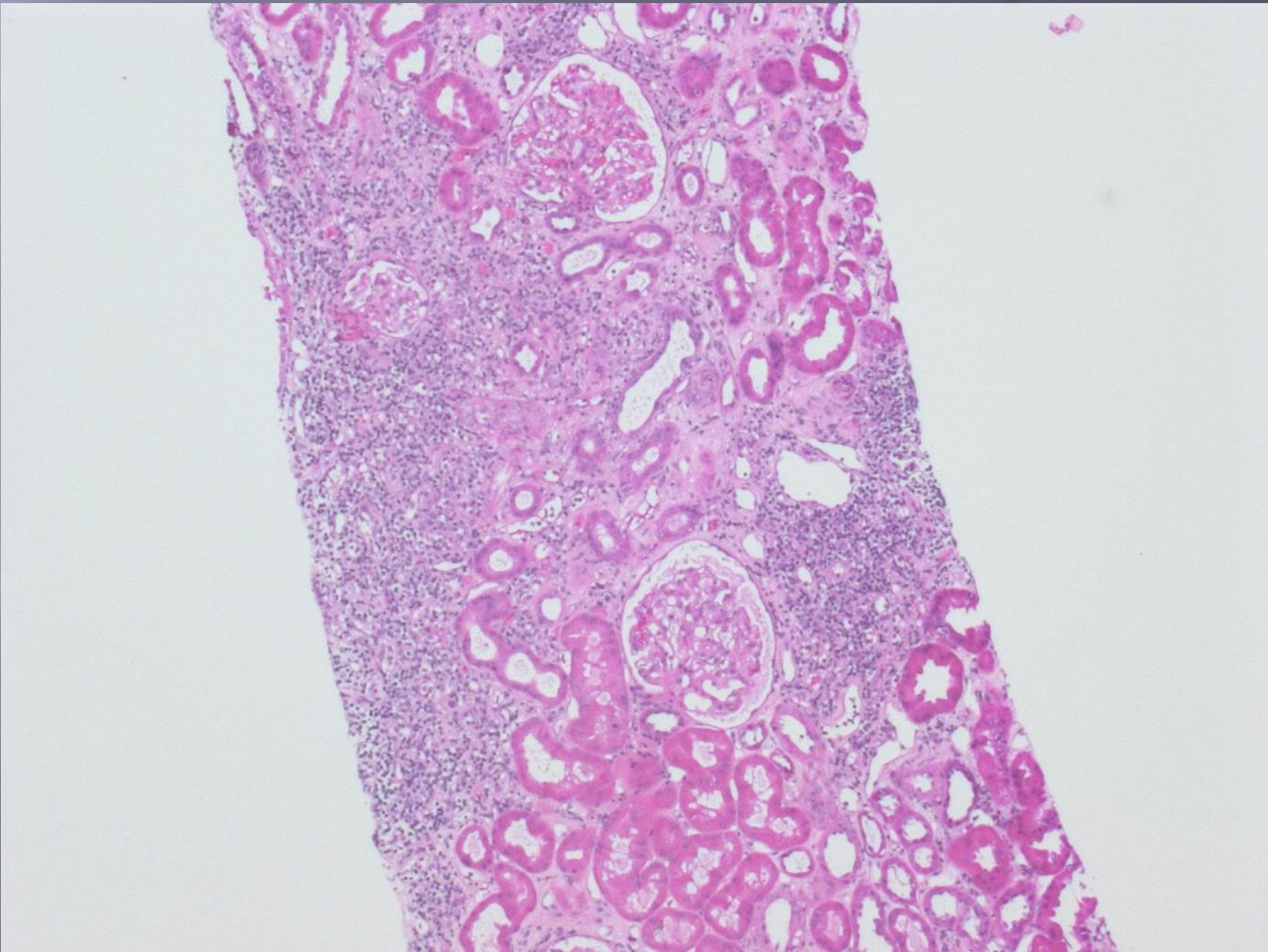
- Interstitial inflammation
- Tubulitis
- Vascular inflammation
- Glomerulitis
- Interstitial fibrosis
- Tubular atrophy
- Arterial fibrointimal thickening
- Transplant glomerulopathy
- Mesangial matrix increase
- Arteriolar hyalinosis
- Peritubular capillary inflammation
- C4d
- Total inflammation

Banff scoring categories

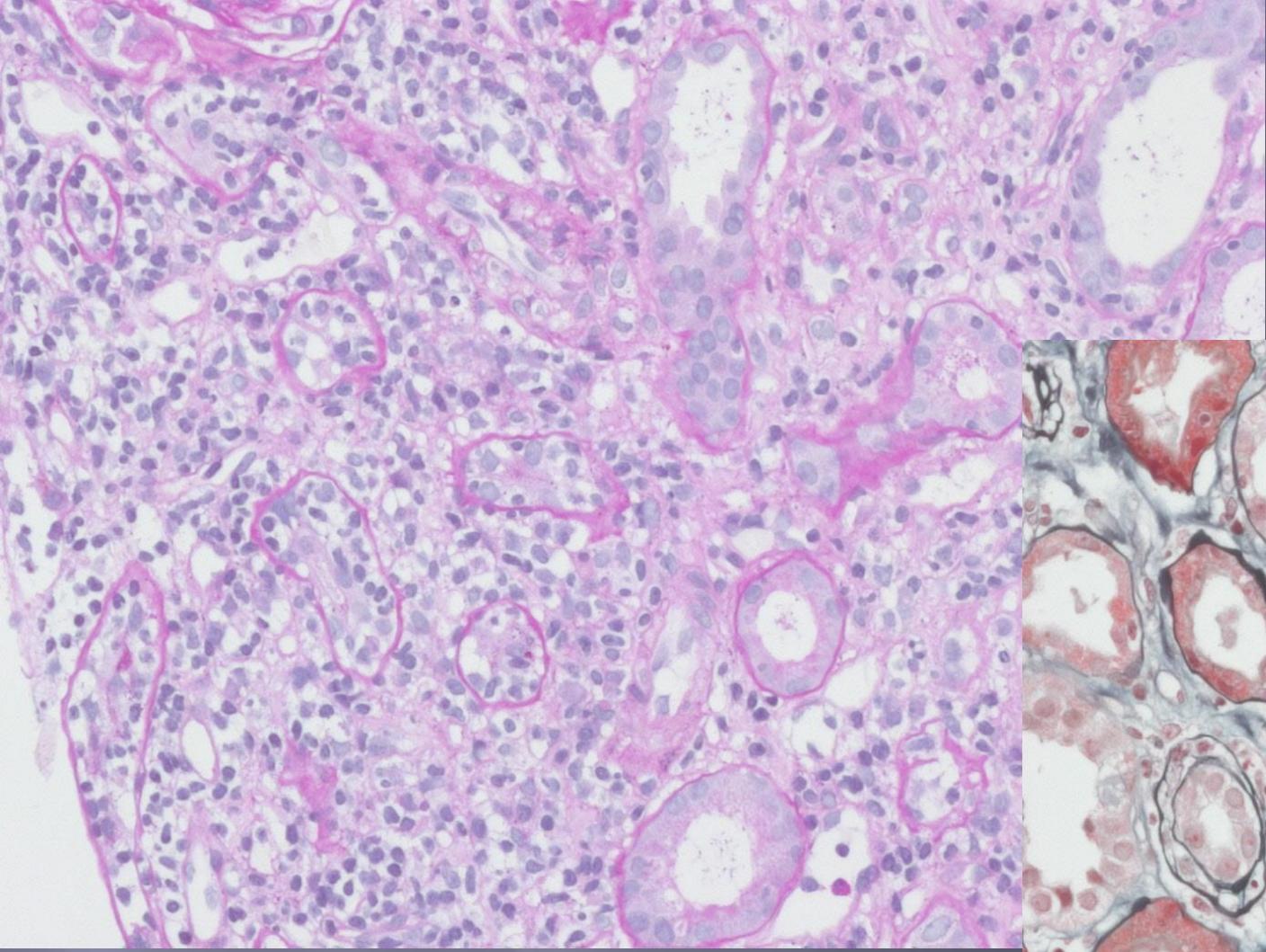
For example:

- Interstitial inflammation
 - i0: <10% of non fibrotic cortex shows mononuclear inflammation
 - i1: 10-25%
 - i2: 26-50%
 - i3: >50%
- > i1 (interstitial inflammation) + > t1 (tubulitis) or > v0 (vascular inflammation) = T cell mediated rejection

Acute T cell mediated rejection

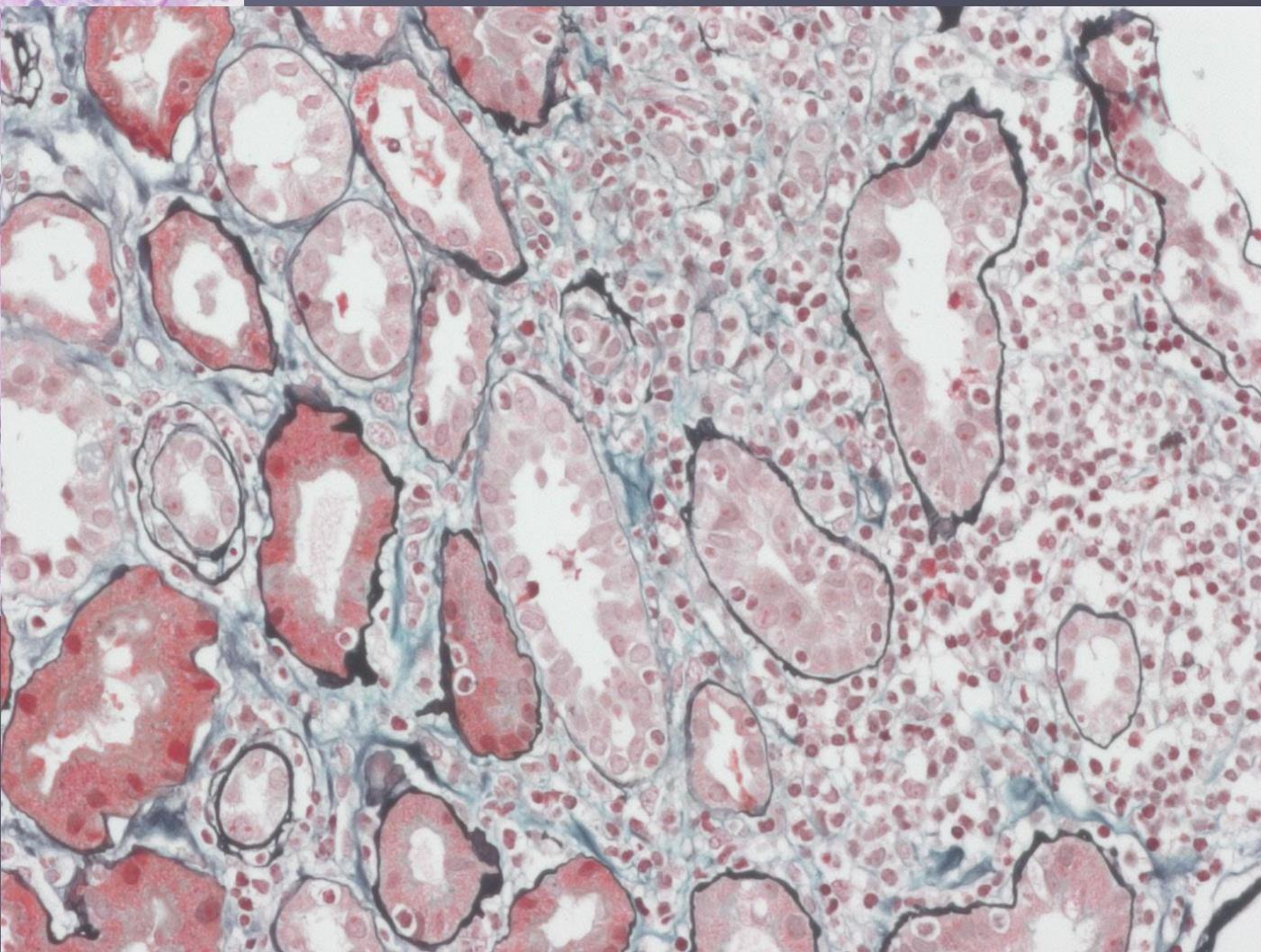


H&E 50x

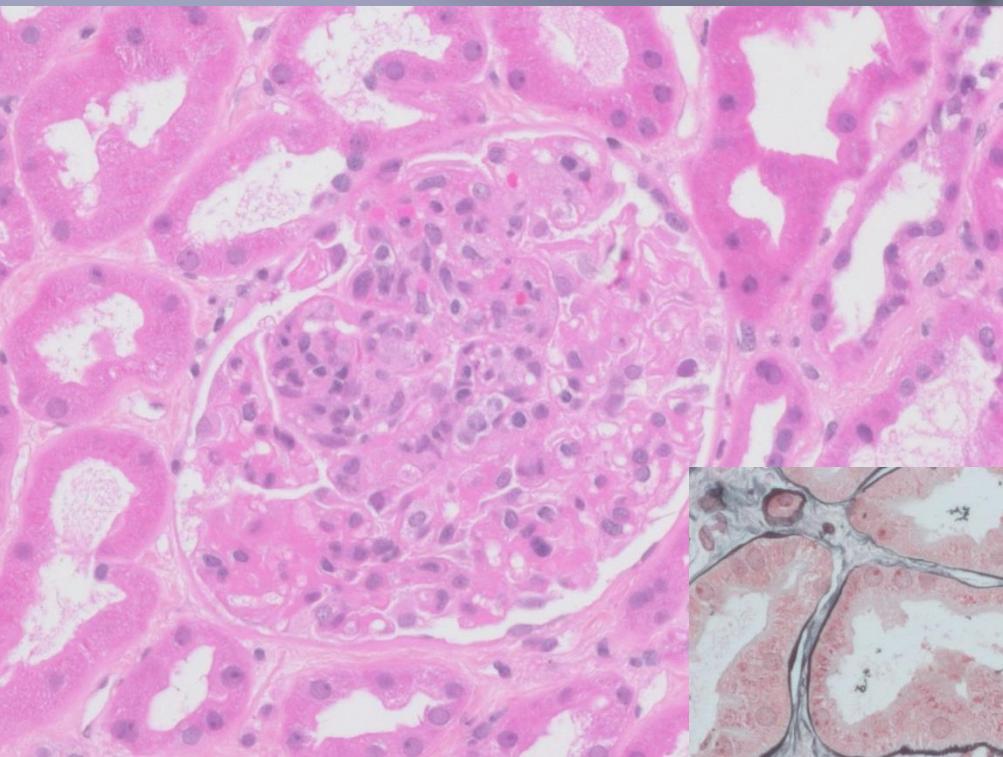


PAS 200x

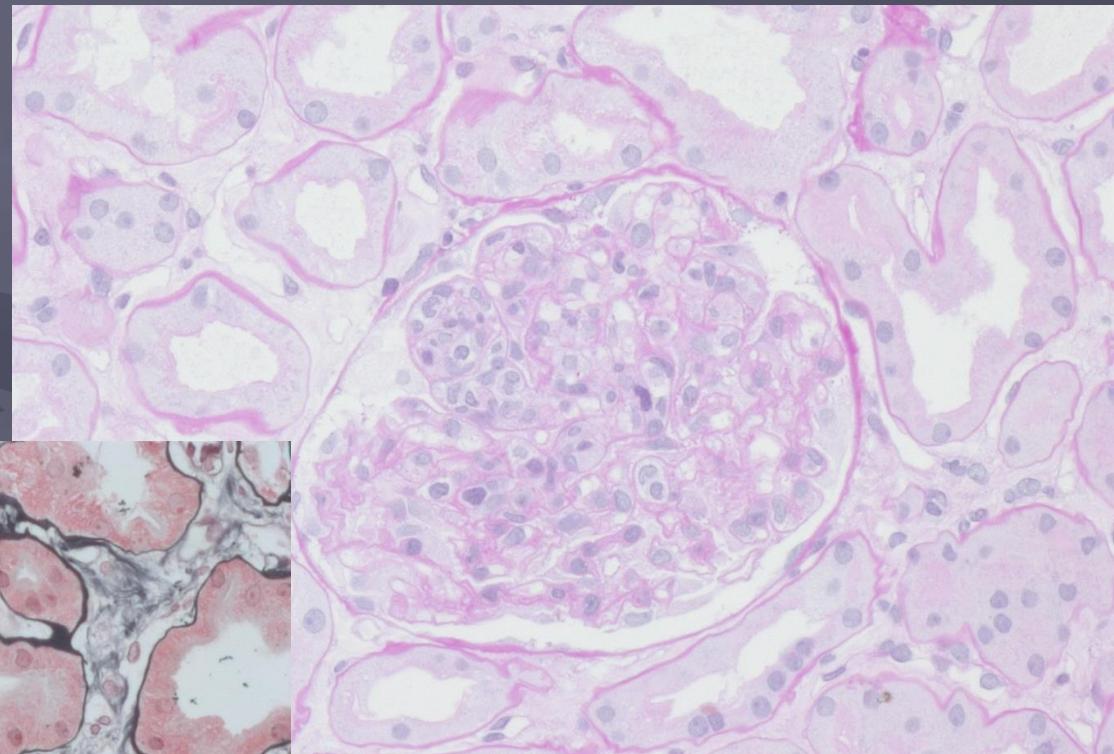
Methenamine Silver / Masson Trichrome 200x



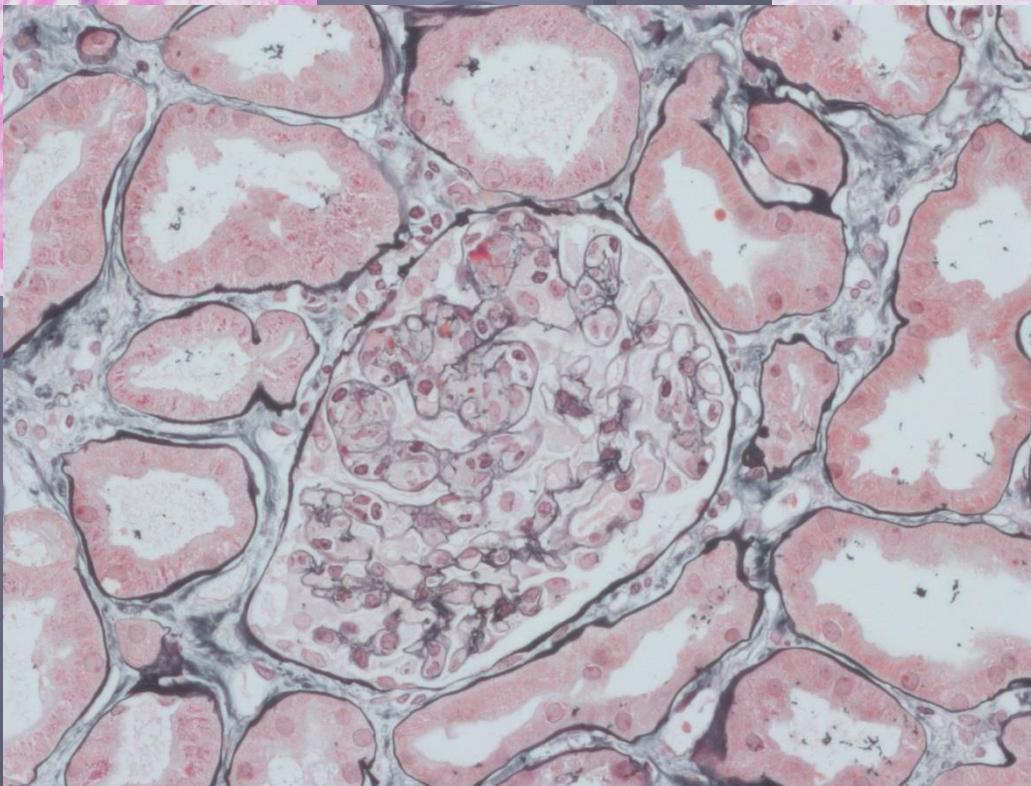
Antibody mediated rejection



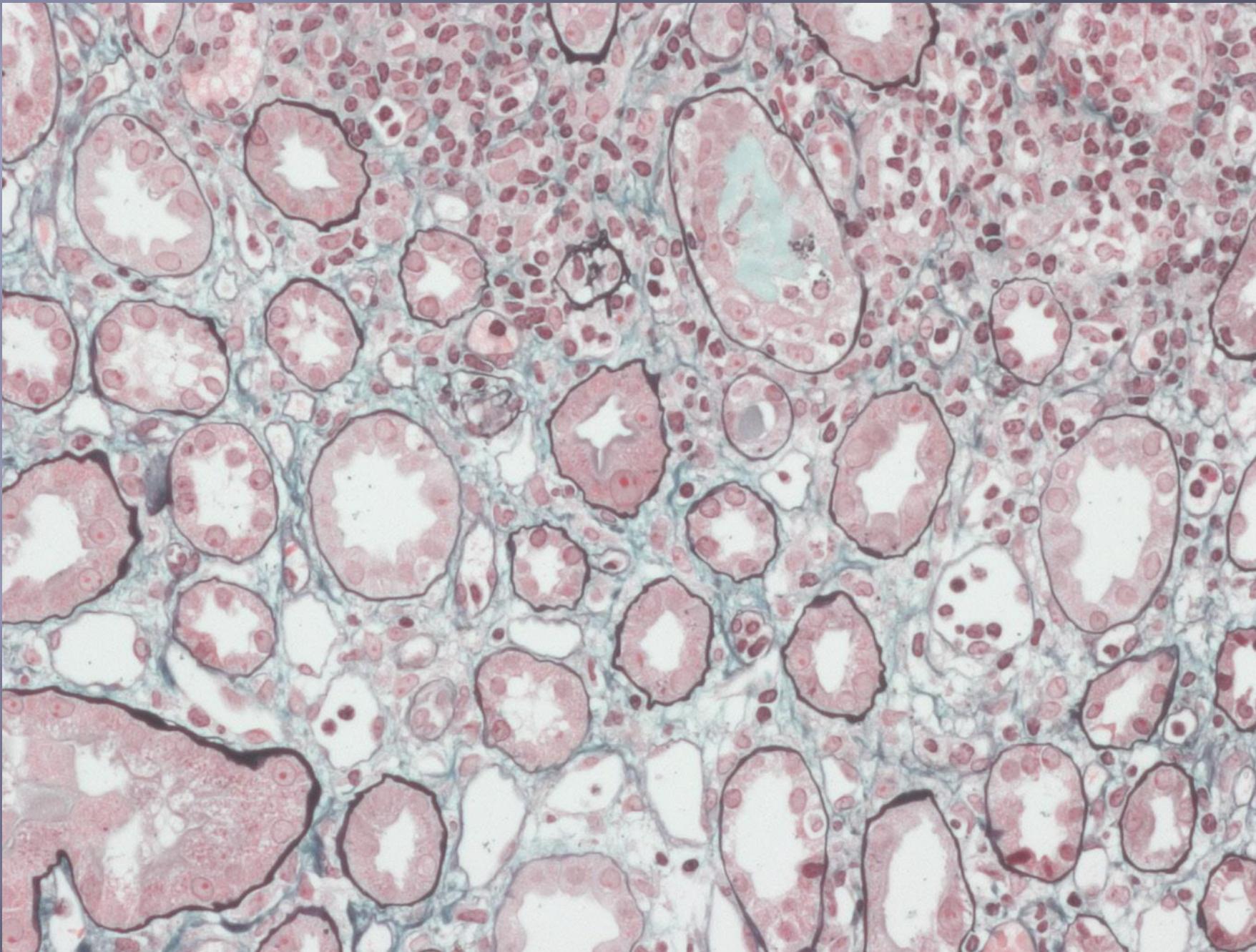
H&E 200x



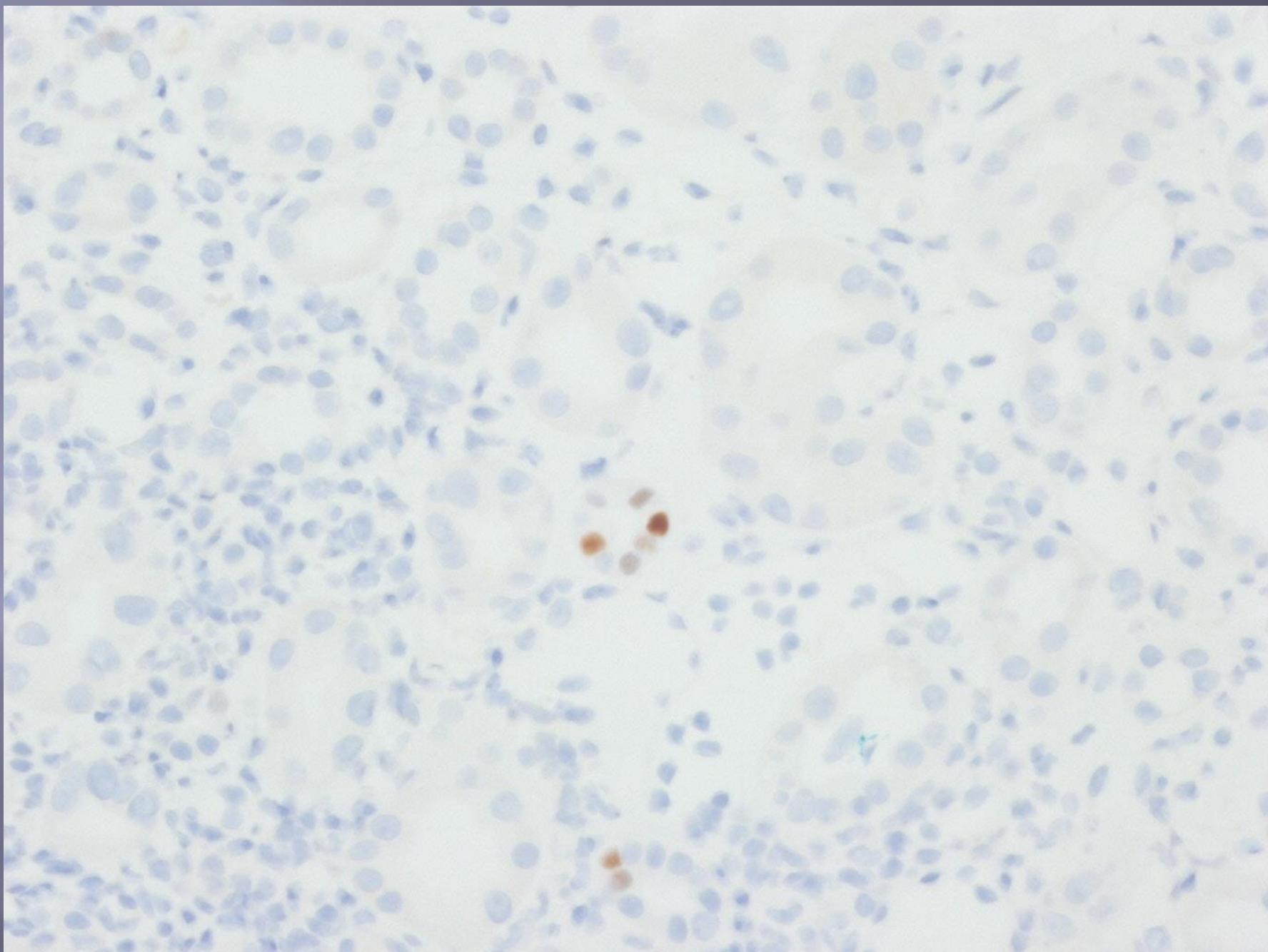
PAS 200x



Methenamine Silver / Masson
Trichrome 200x

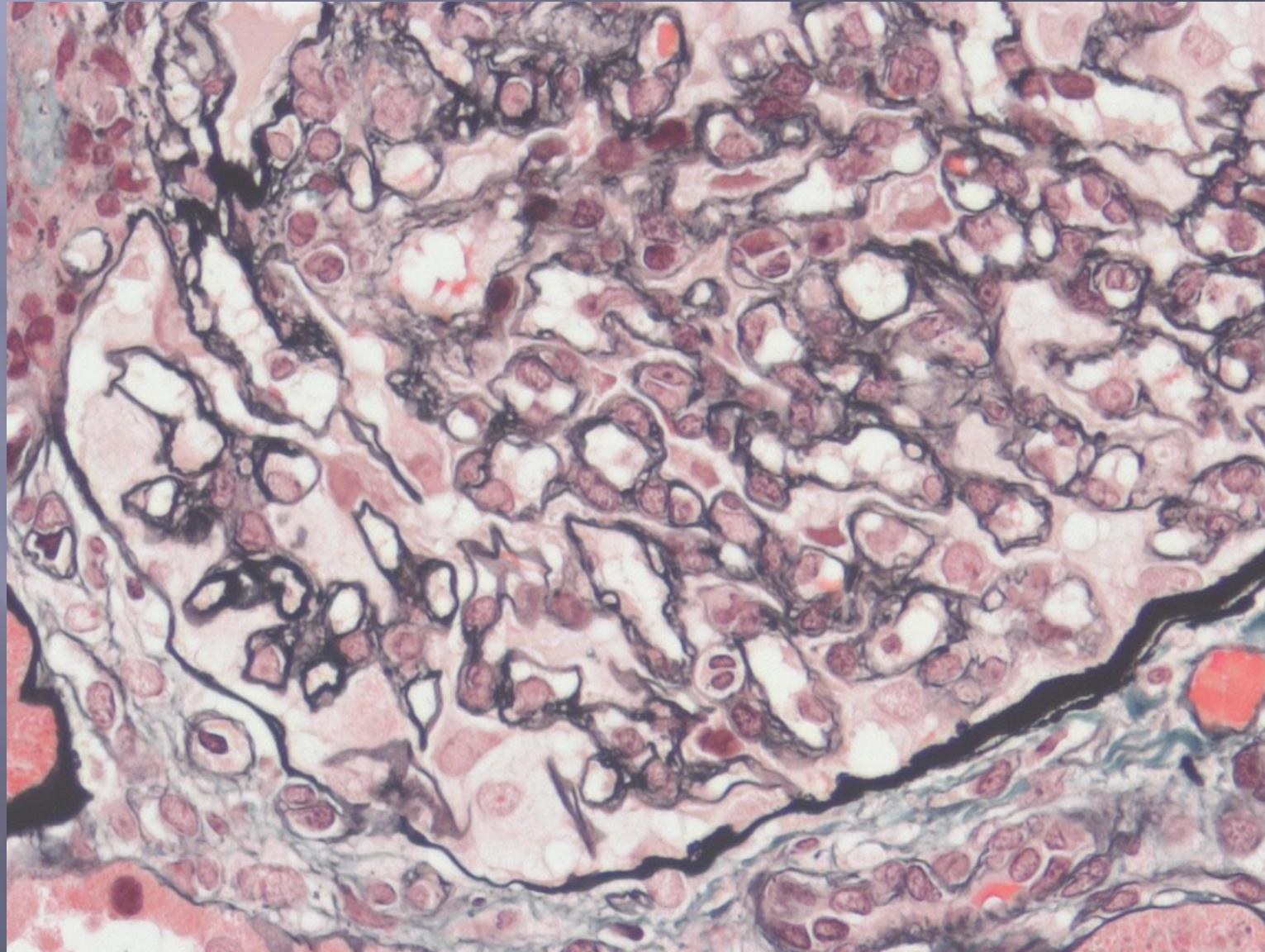


Methenamine Silver / Masson Trichrome 200x

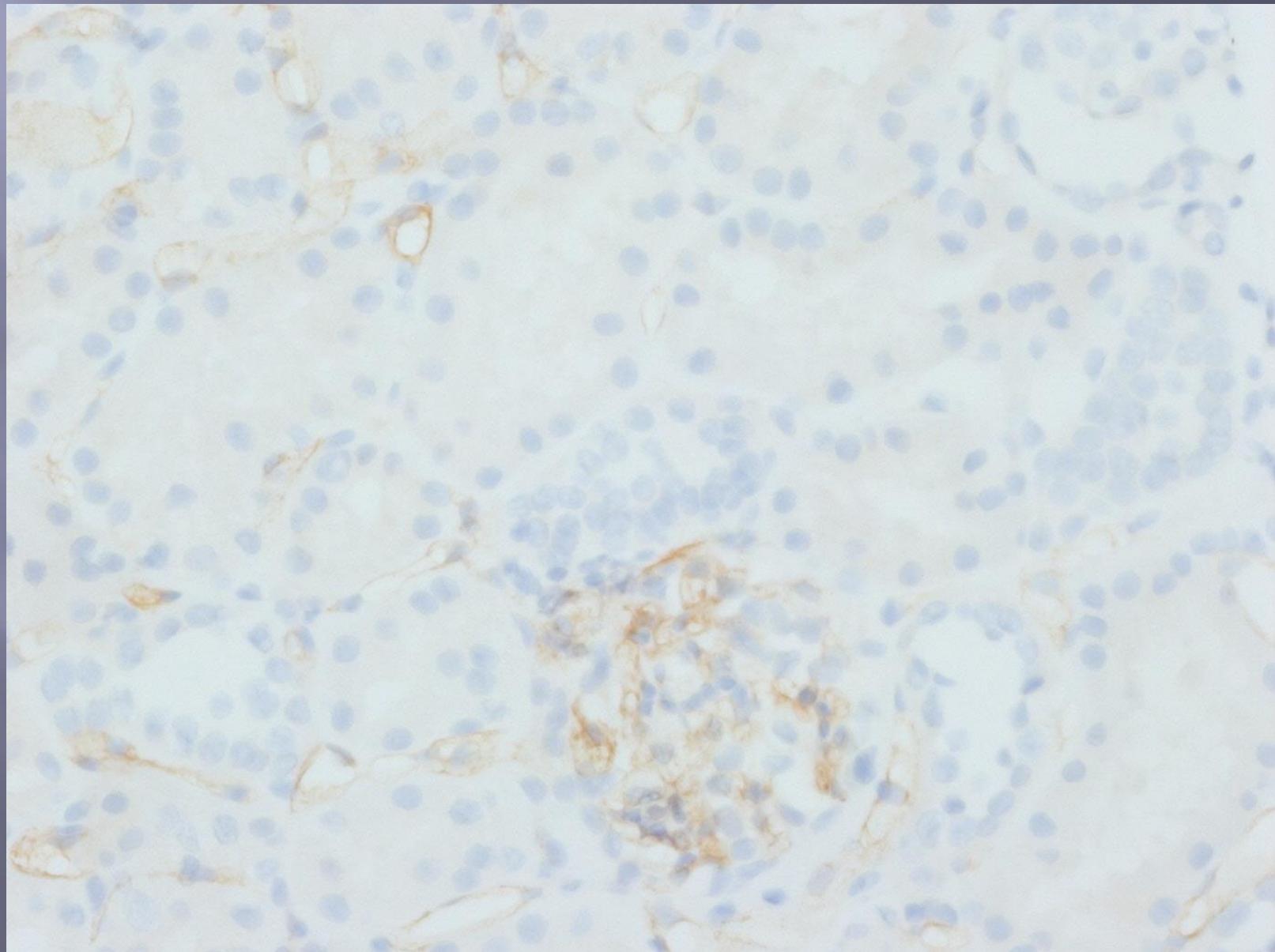


SV-40

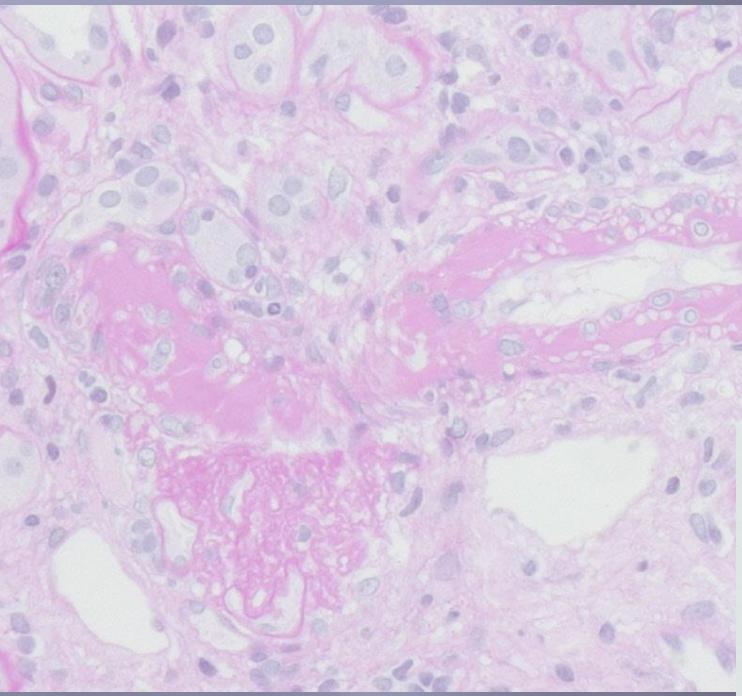
Chronic antibody mediated rejection



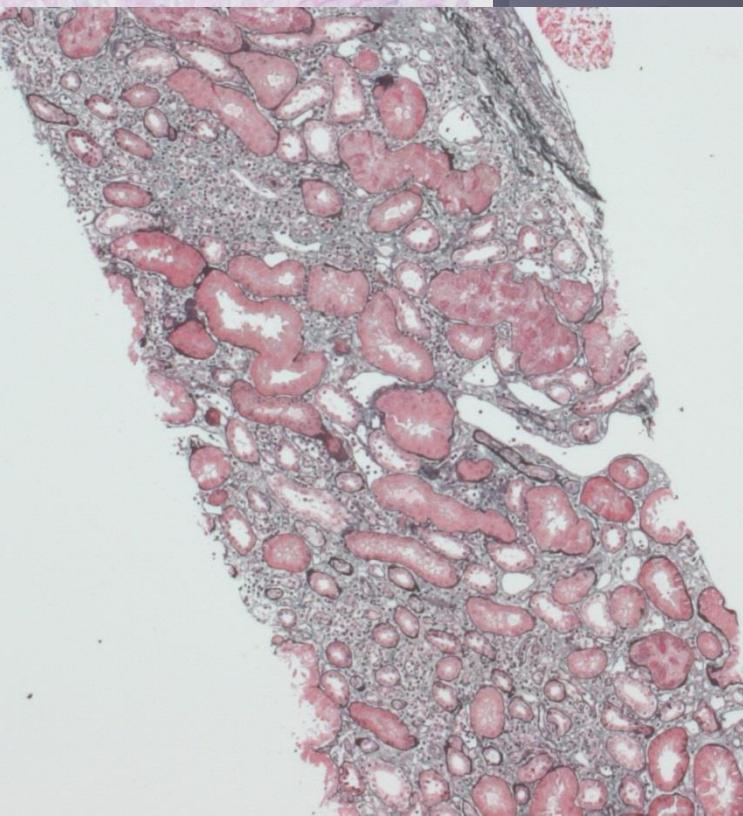
C4d



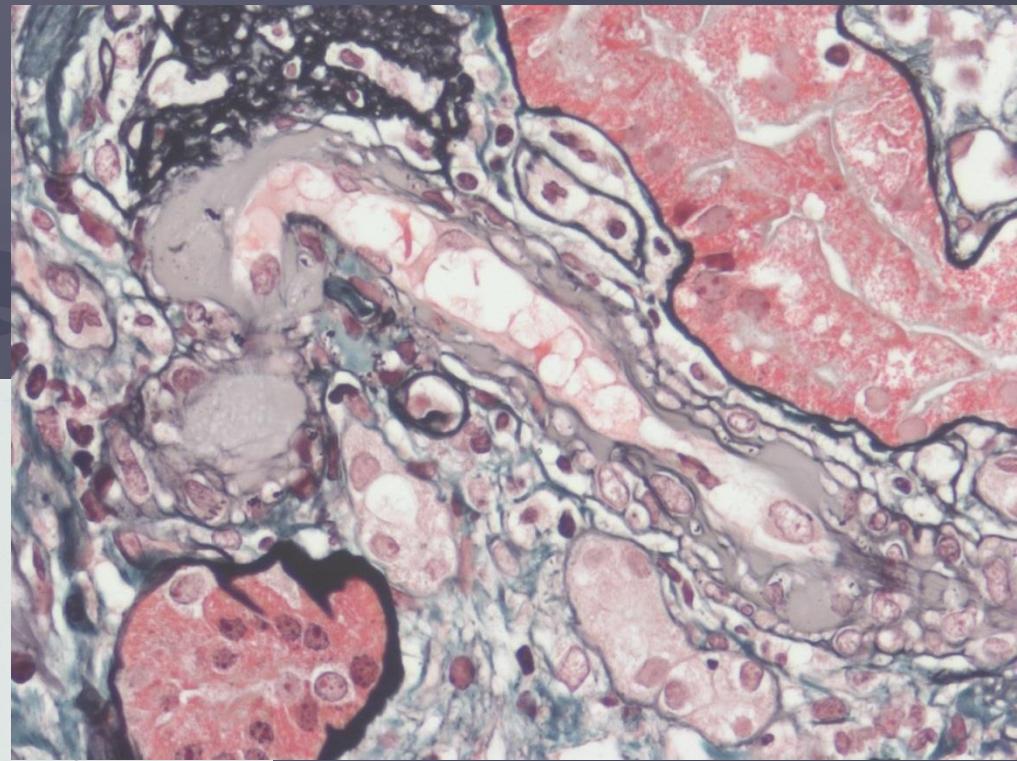
CNI toxicity



PAS 200x

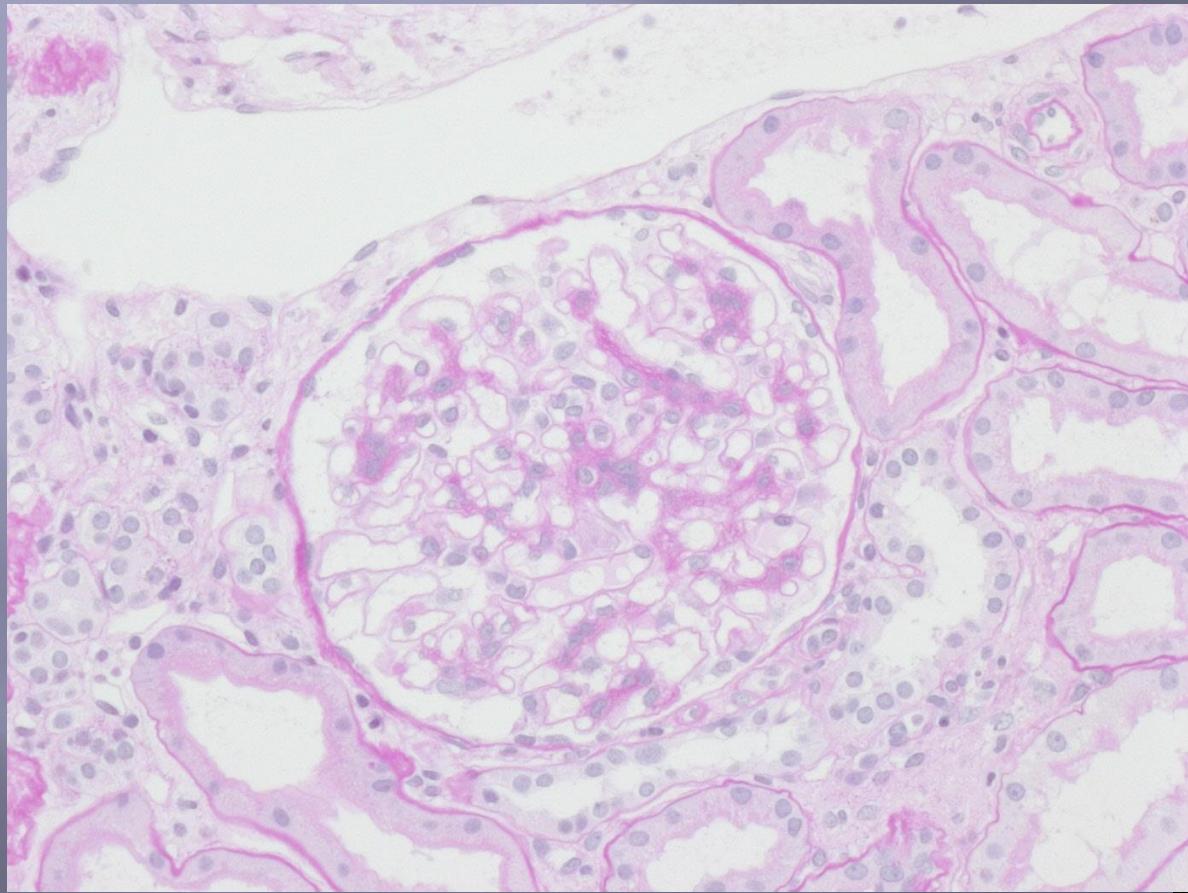


50x

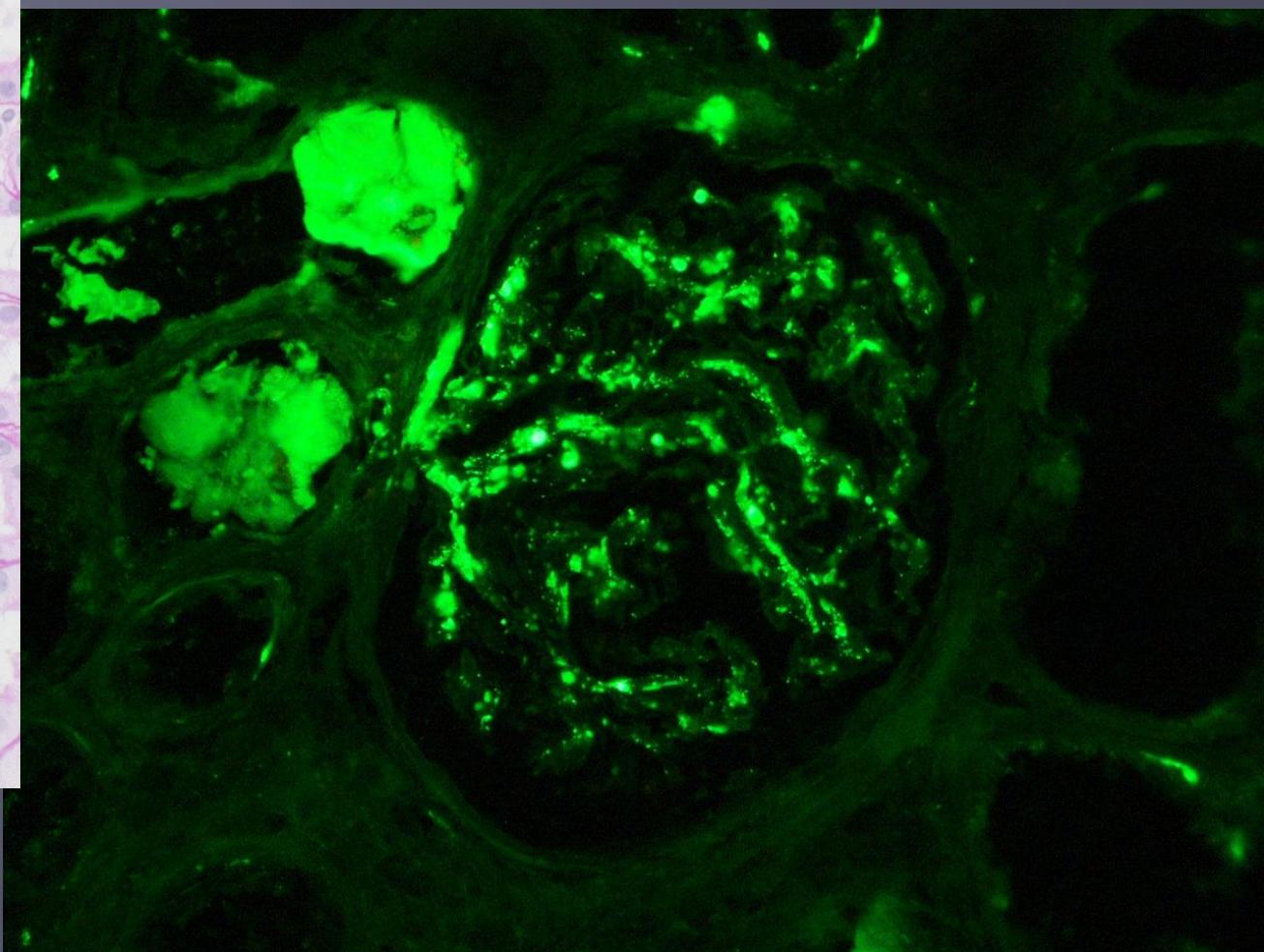


200x
Methenamine Silver / Masson
Trichrome

IgA nephropathy recurrence



PAS 200x



IgA immunofluorescence

Acknowledgements

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