

EM TECHNICAL EQA RESULTS RUN 1

PETE WELLS-JORDAN

BMS1

LEICESTER ROYAL INFIRMARY

peter.wells-jordan@uhl-tr.nhs.uk

INTRODUCTION

- Pilot Scheme
- Learning experience for all concerned
- 19 Participants
- 3 Assessors
- 1 Co-ordinator
- Semi-thin (survey) sections
- Stained for light microscopy
- Most common tissue type examined
- Questionnaire and method sheet

ADMINISTRATION

- Vicki Martin, EQA Co-ordinator
- Submissions received
- Slides coded and anonymised to eliminate bias
- Anonymised slides examined by assessors alone

MARKING SCHEME

- Subjectively objective
- Section quality
 - Scores
 - Creases
 - Thickness
 - Cover slipped
- Staining quality
 - Over/under stained
 - Contrast
 - Cellular components
 - Tissue structures
- Artefacts
- Possible pathology

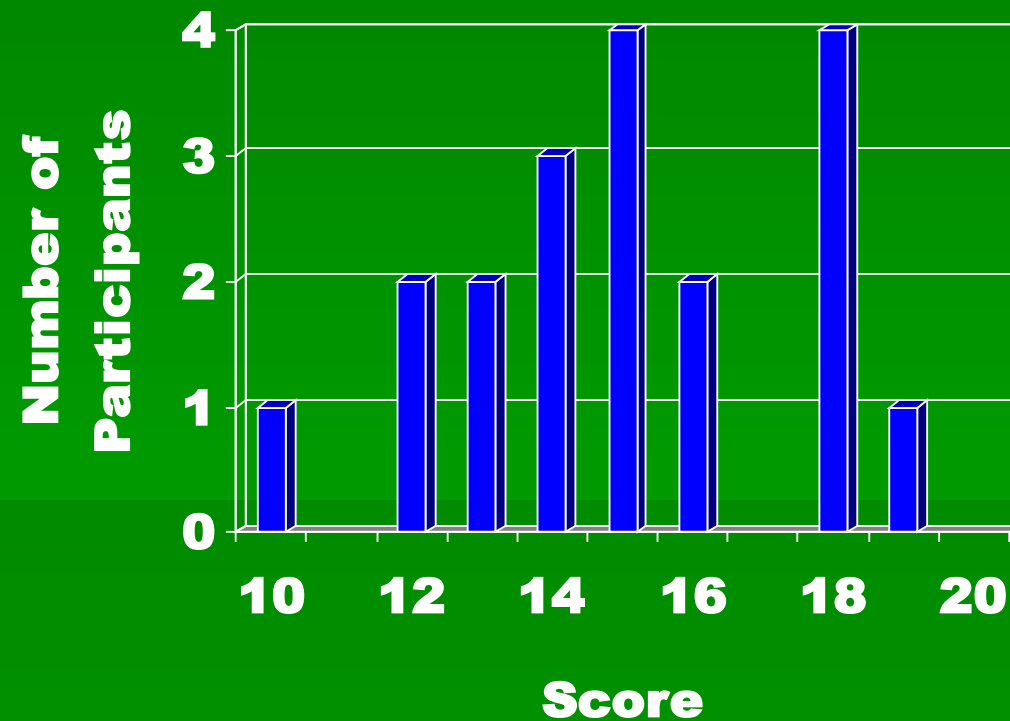
PARTICIPANT SCORES

PARTICIPANT NO.	SCORE
01	14
02	16
03	10
04	13
05	14
06	18
07	12
08	14
09	12
10	16
11	19
12	18
13	13
14	15
15	15
16	14
17	18
18	15
19	18

PASS OR FAIL!?

- Marks given out of 20
- 12+ Pass mark
- 16+ Excellent
- Range from 10 to 19
- Mean = 14.9

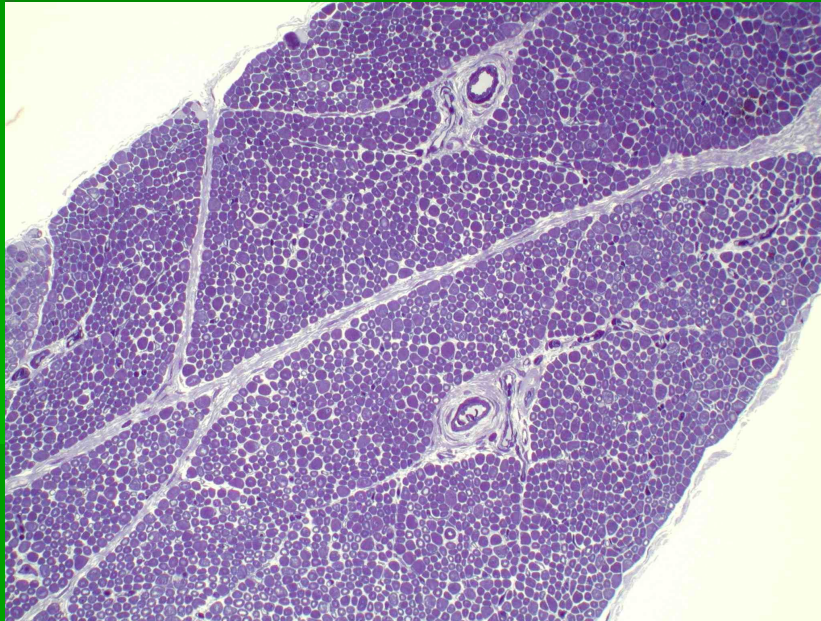
BAR CHART OF SCORES



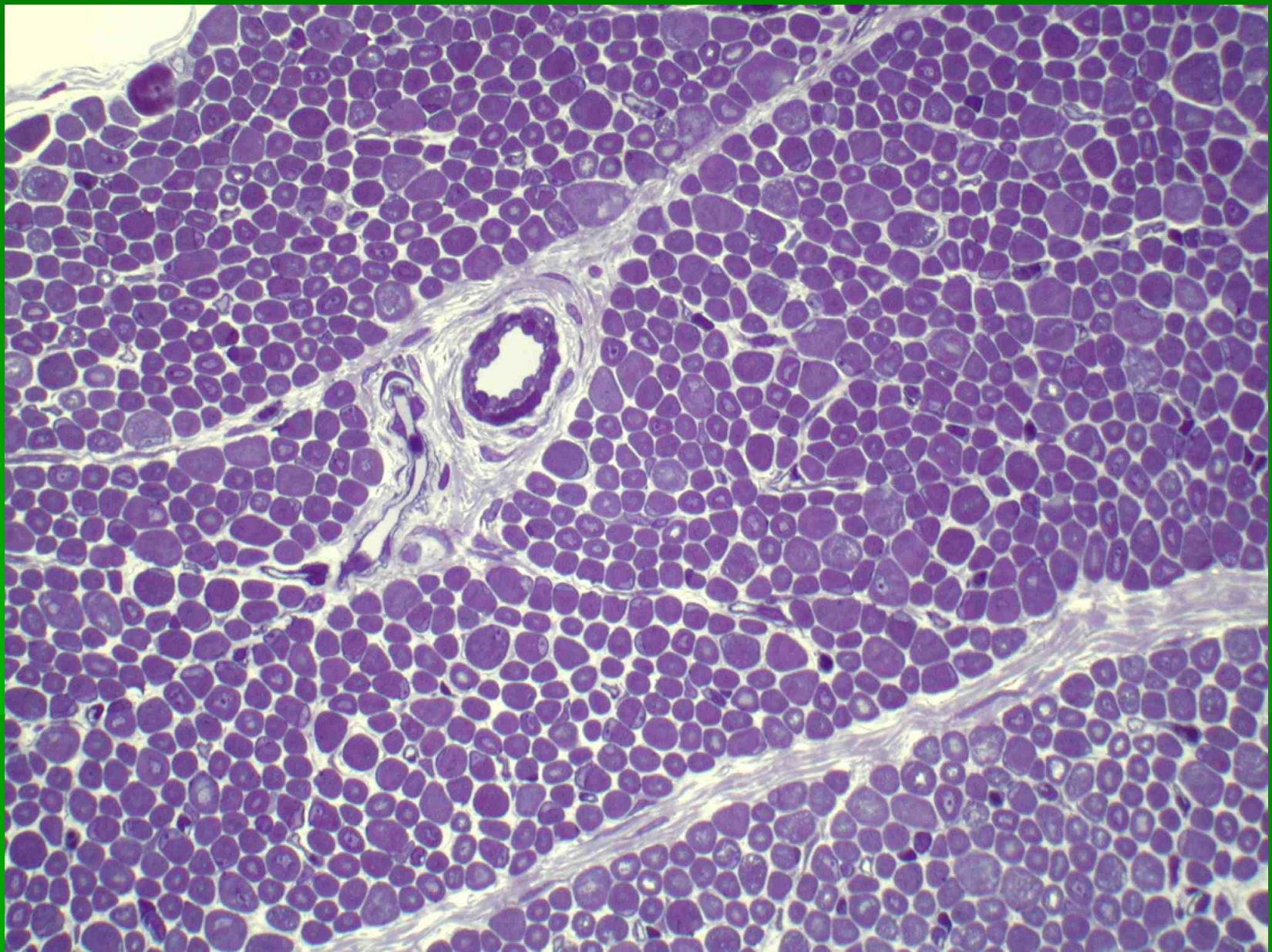
EXAMPLES

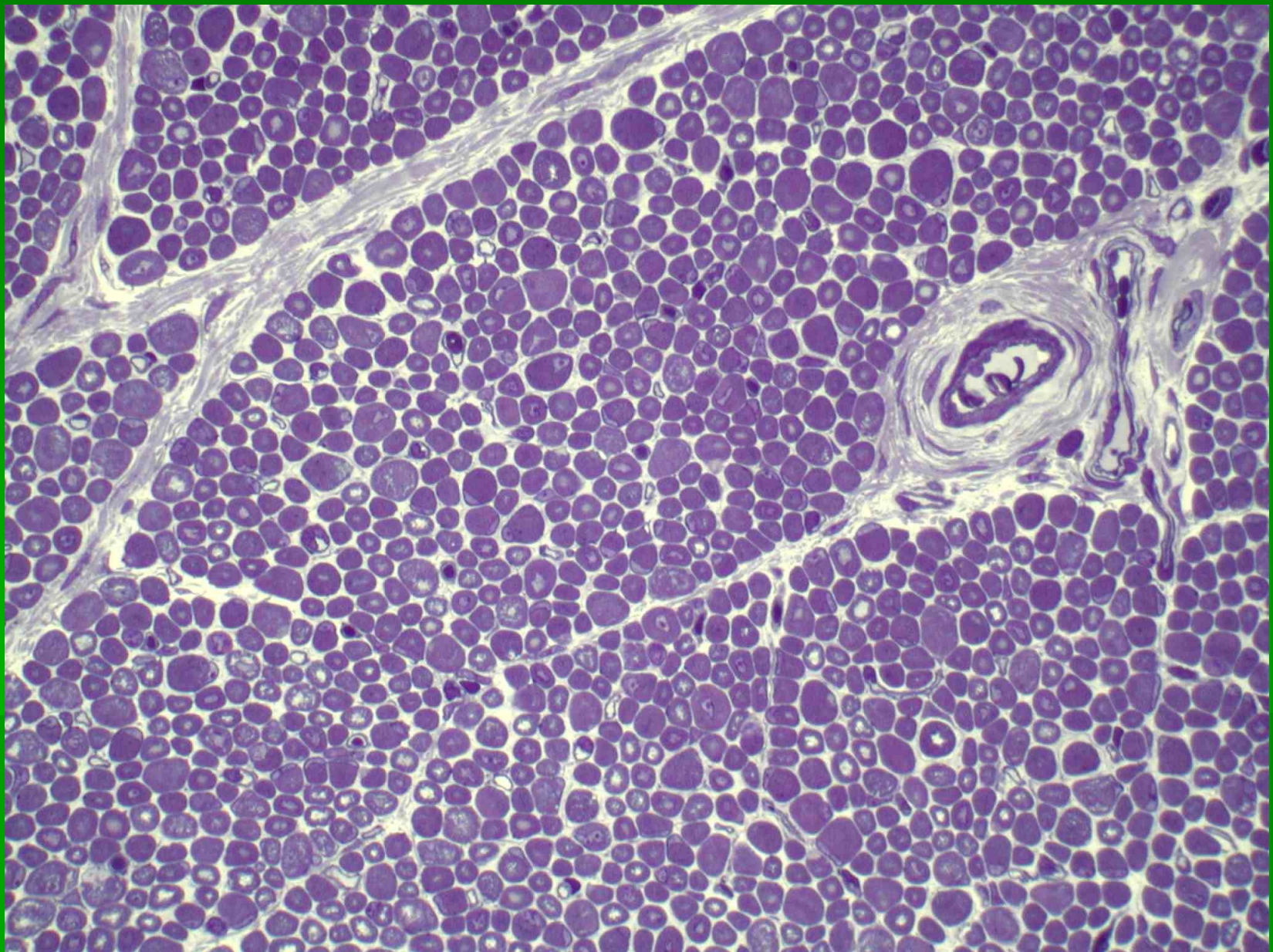
- Best
- Worst
- Different staining methods
- Artefacts
- Staining of tissue components
- Coverslipping

THE BEST

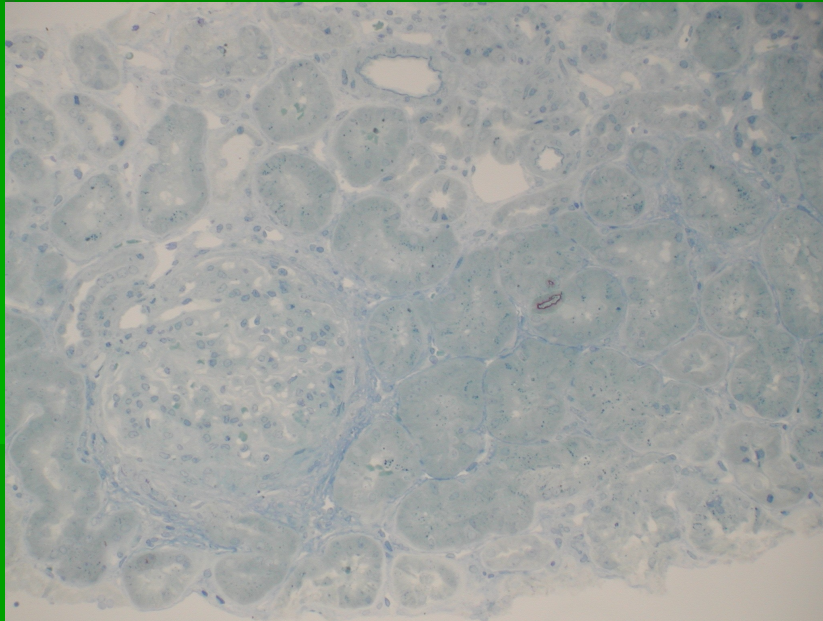


- T/S Muscle
- Flat section
- Good clarity
- Good contrast
- Excellent demo. of components
- Score 19/20
- Alc. Thionin

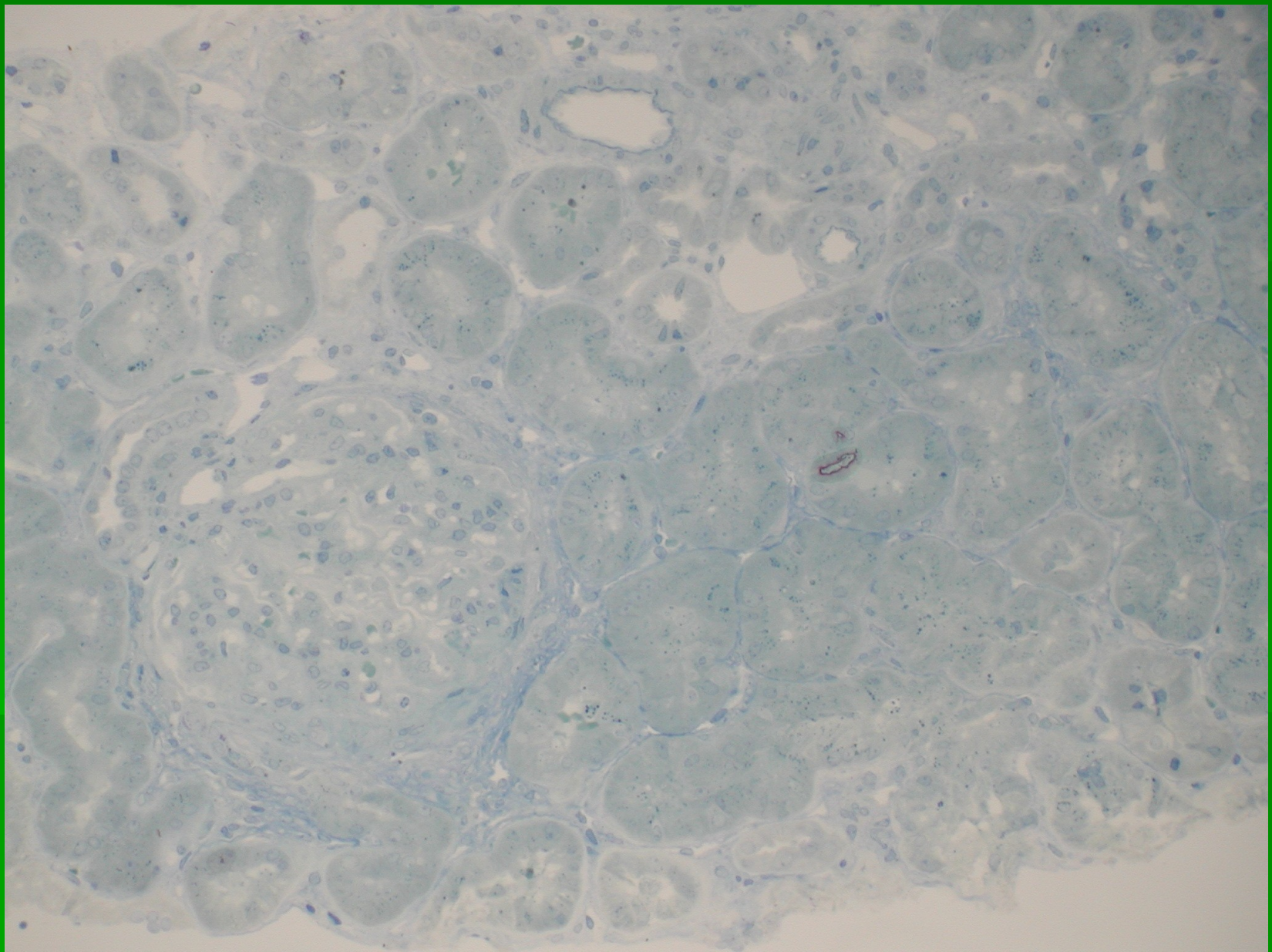


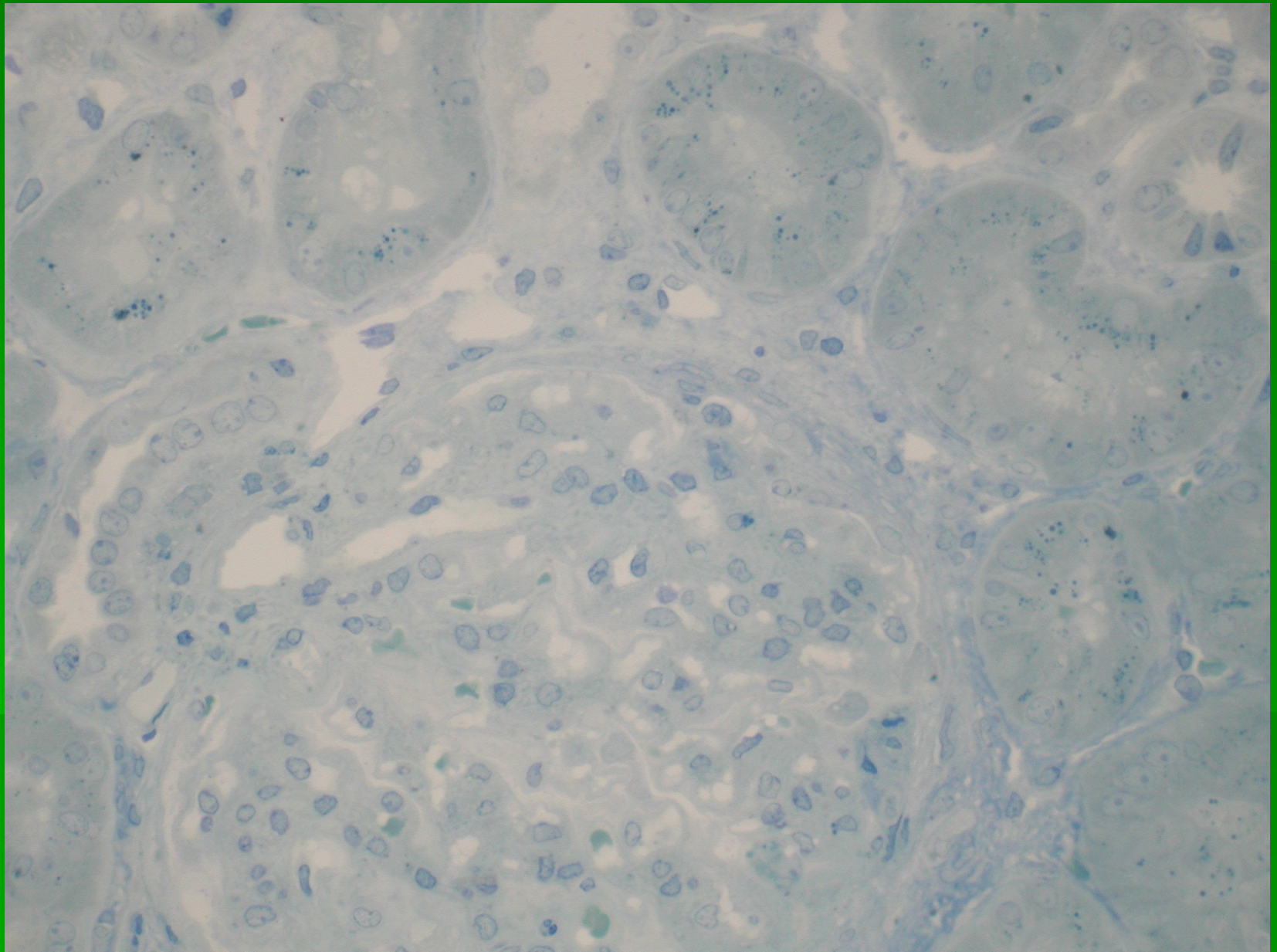


Lowest Scoring

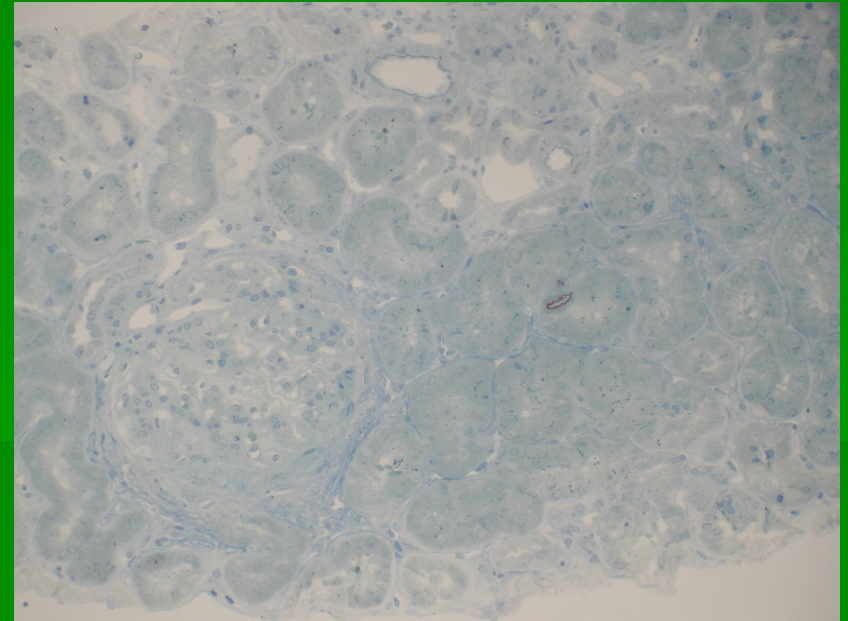
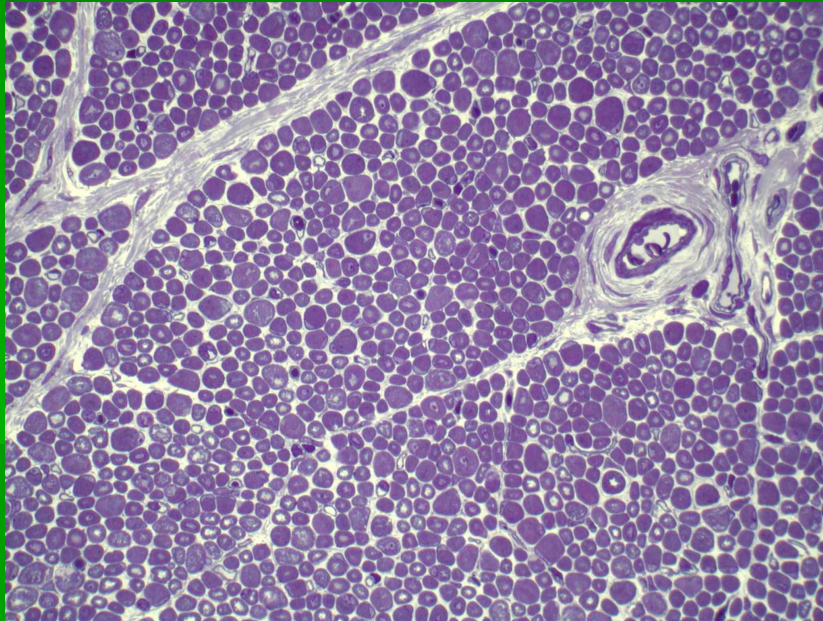


- Renal Cortex
- Green / yellow tone
- Flat / pale
- Can't see tissue components
- Some wrinkling of resin
- Score 10/20
- 1% Tol. Blue in 1% Borax





Comparison of Best with Worst



Staining Methods

- Most popular was Tol. Blue in Borax
 - 1% Tol. Blue in 1% Borax. 9 submitted
 - 1% Tol. Blue in 3% Borax. 1 submitted
 - 1% Tol. Blue in 0.5% Borax. 1 submitted
 - 0.5% Tol. Blue in 1% Borax. 1 submitted

Other Tol. Blue Based Stains

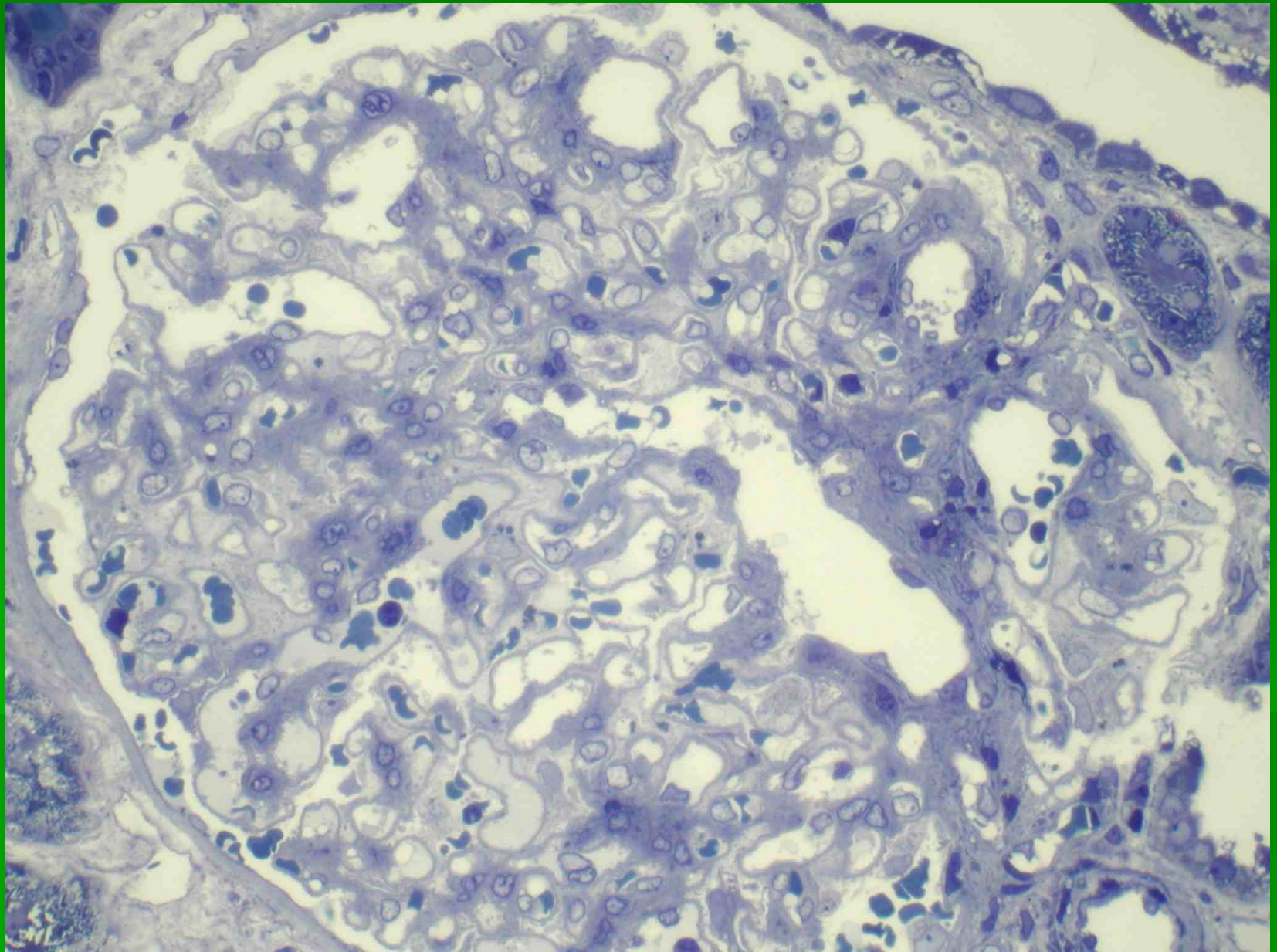
- Tol. Blue. 2 submitted
- 1% Tol. Blue in 1% Borax with 0.2% Pyronin Y. 1 submitted
- 0.2% Tol. Blue in 2.5% Sodium Carbonate. 1 submitted

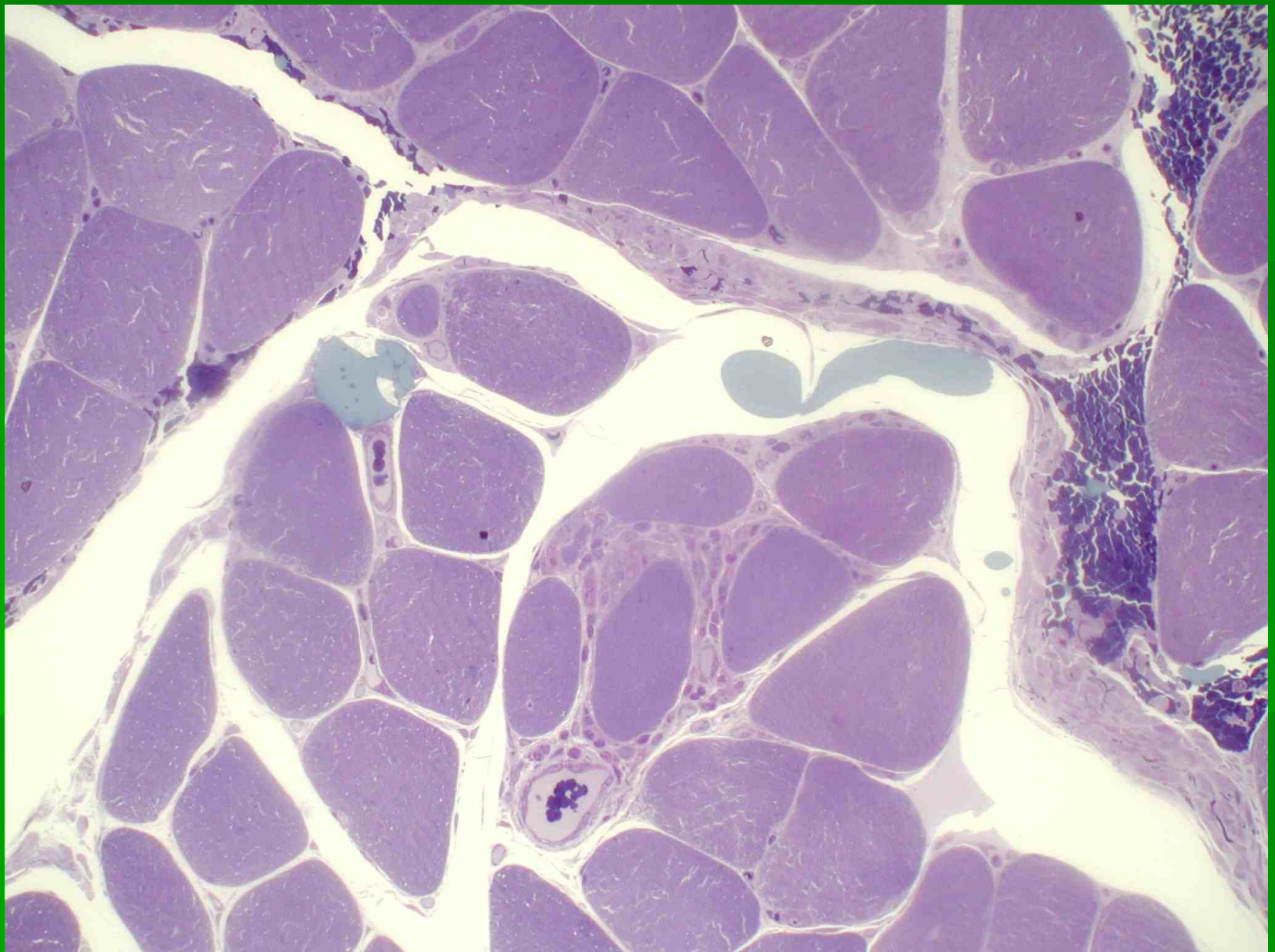
Staining Methods (other than Tol. Blue)

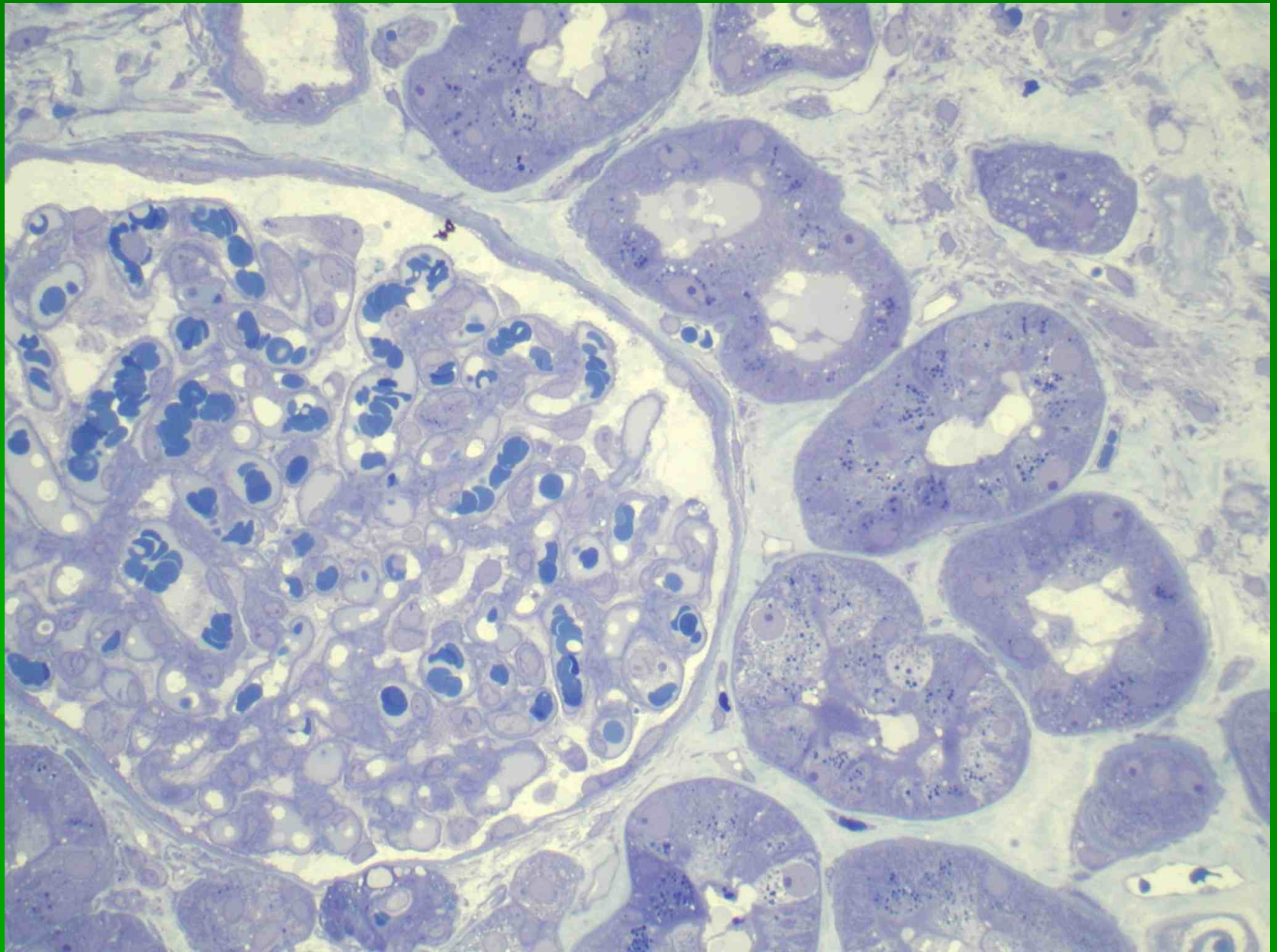
- Alkaline 0.1% Thionin in 50% absolute alcohol. 1 submitted
- 1% Azur II & 1% Methylene Blue in 0.5% Borax. 1 submitted
- Unknown. 1 submitted

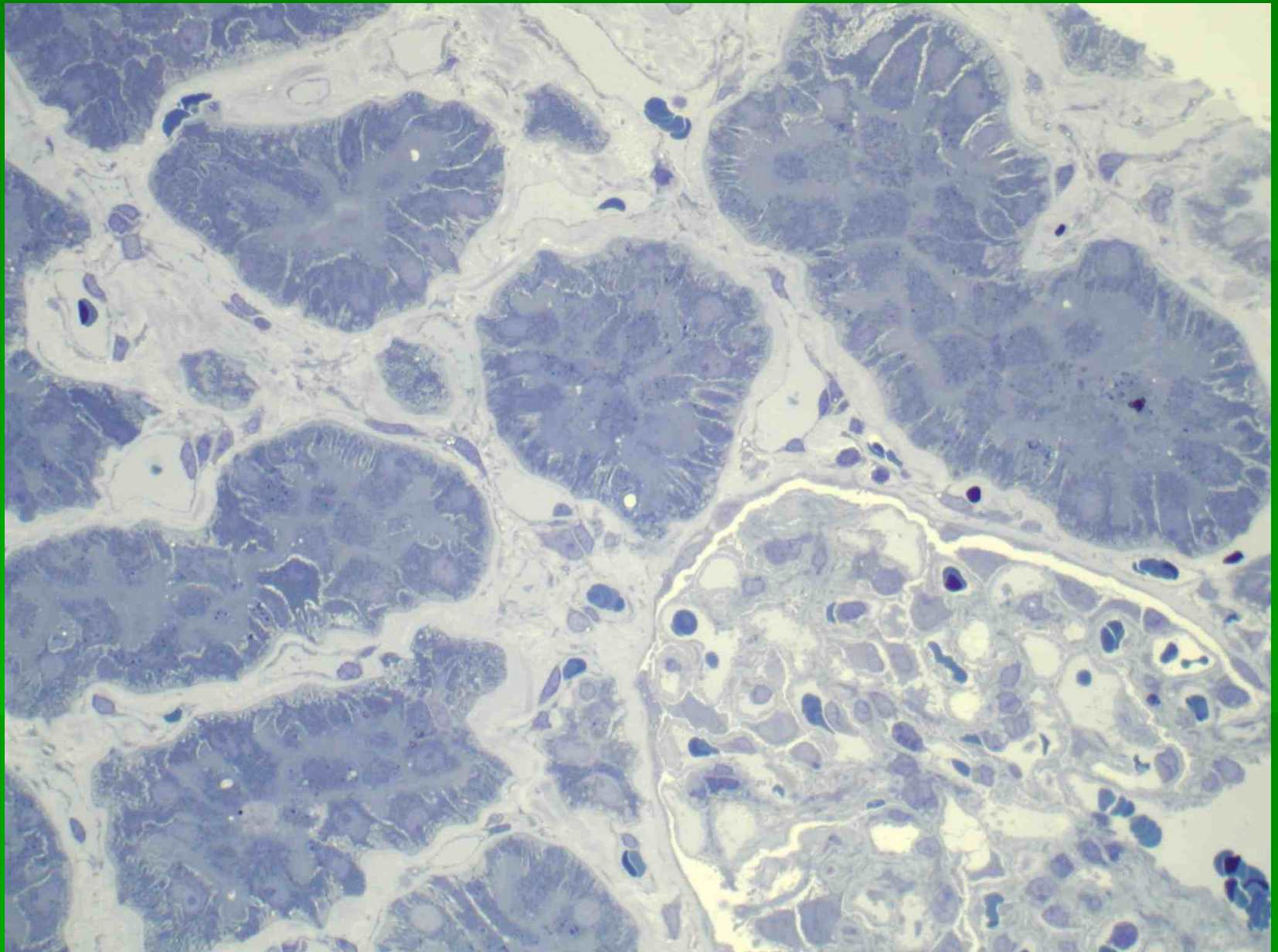
Tol. Blue Staining

- 16 submissions Tol. Blue based
- 6 out of 7 excellent scores were Alkaline Tol. Blue based
- But also lowest score was Tol Blue
- Other factors involved in achieving good staining
 - Fixation
 - Processing
 - Staining time
 - Heat etc.

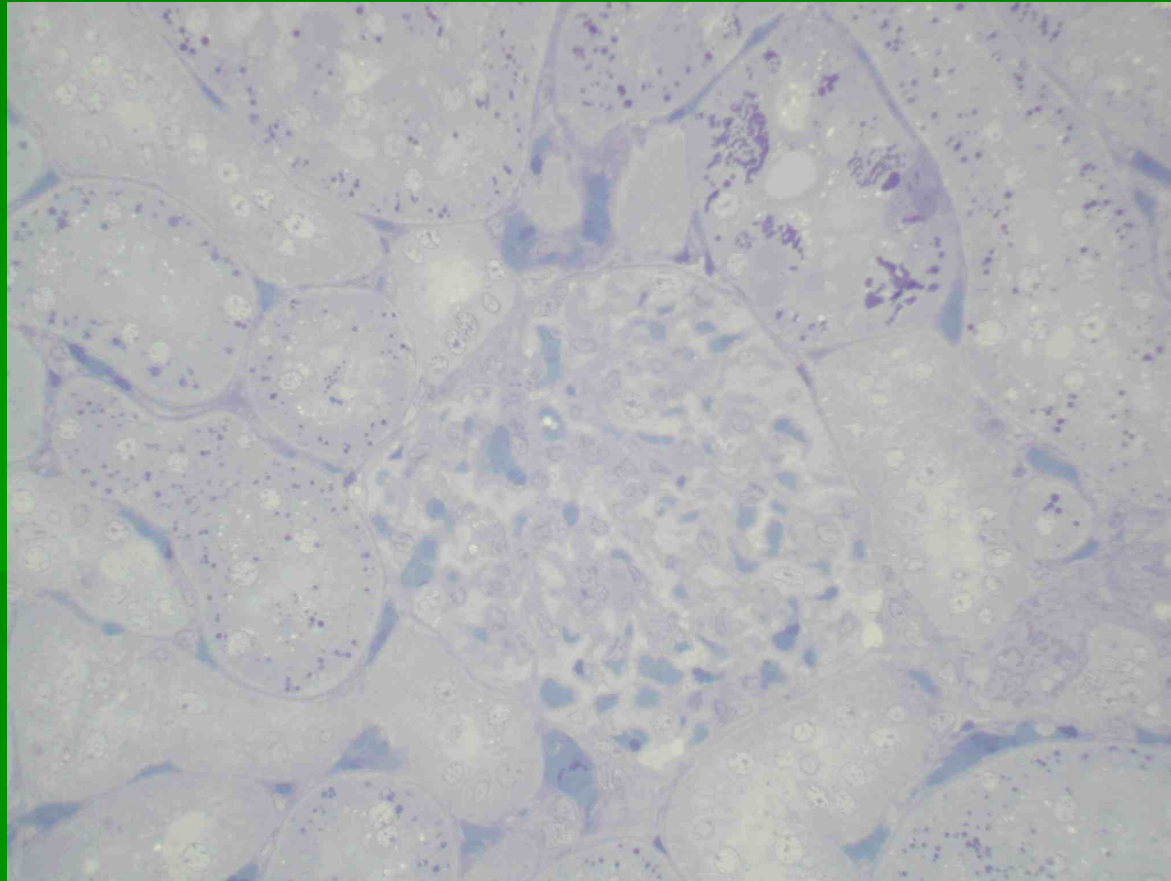




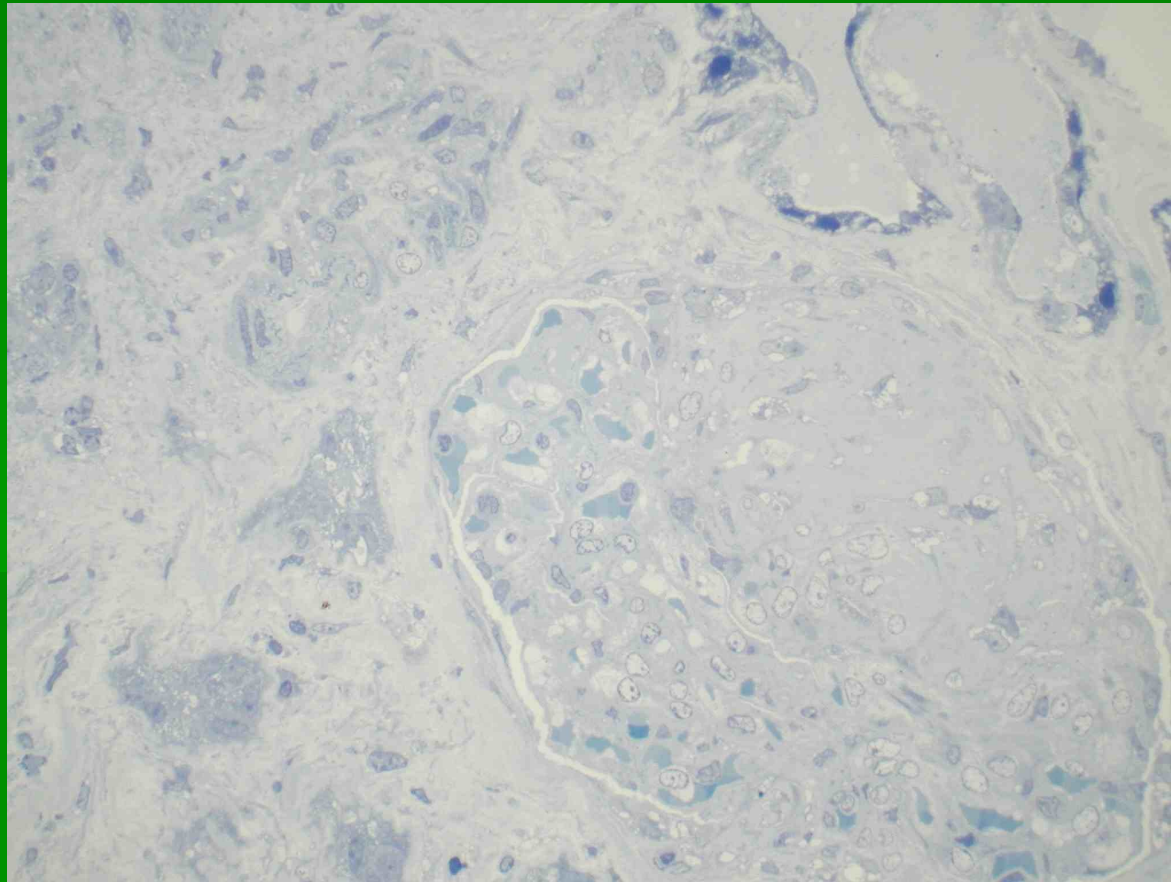




1% Tol. Blue with 1% Pyronin Y



1% Azur II, 1% Methylene Blue in 0.5% Borax

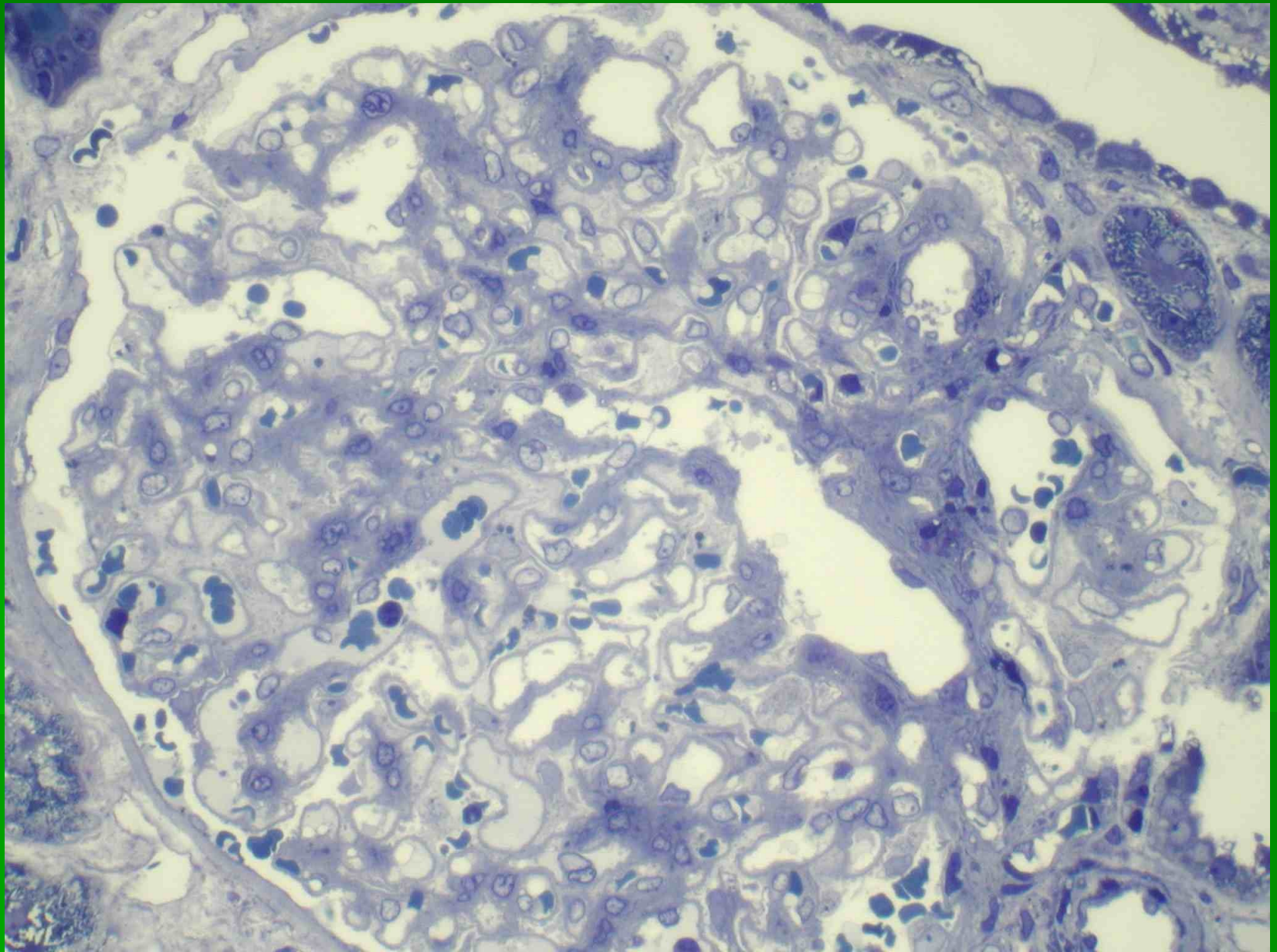


Unknown

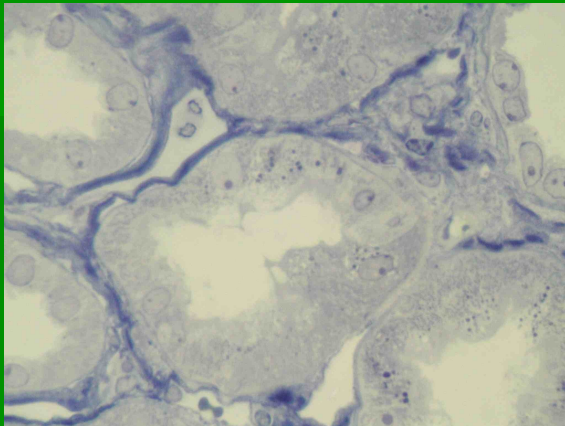
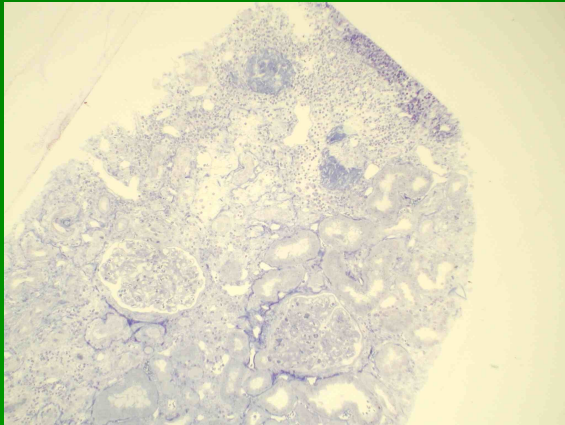


Factors Affecting Staining & Section Quality

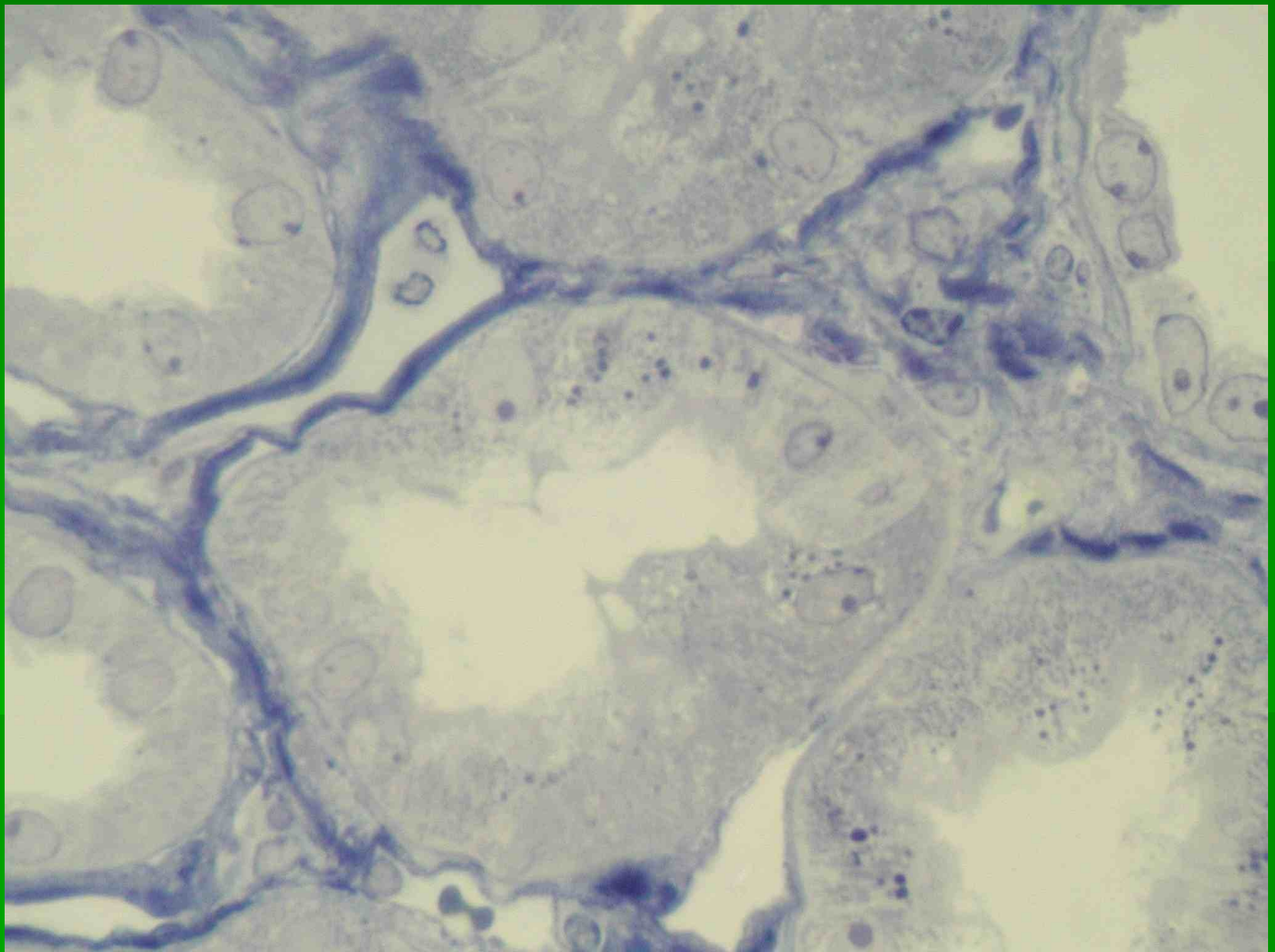
- Differential / preferential staining of cellular / tissue components
- Collagen
- Mitochondria
- Artefacts
- Section Thickness
- Over / under staining

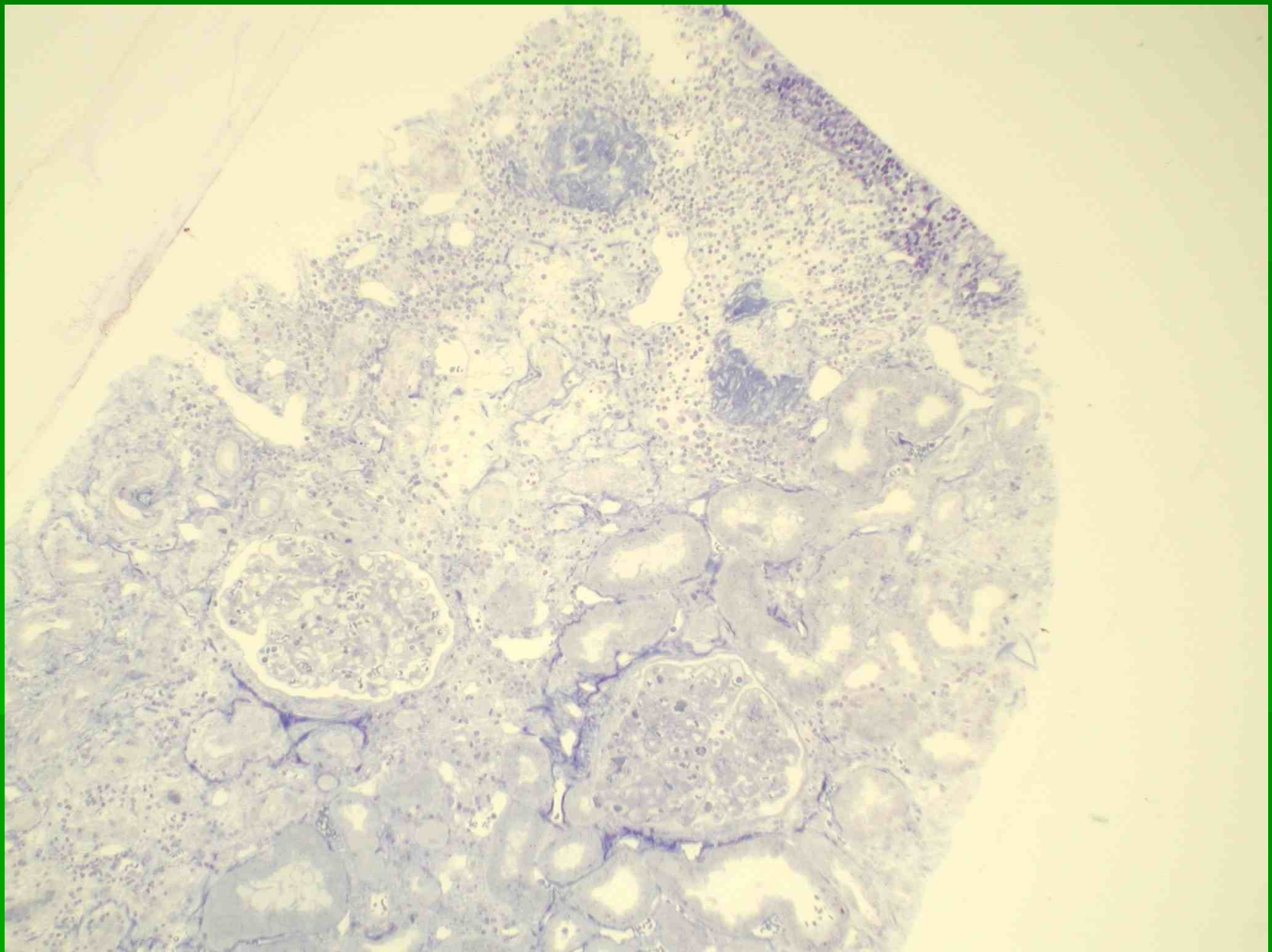


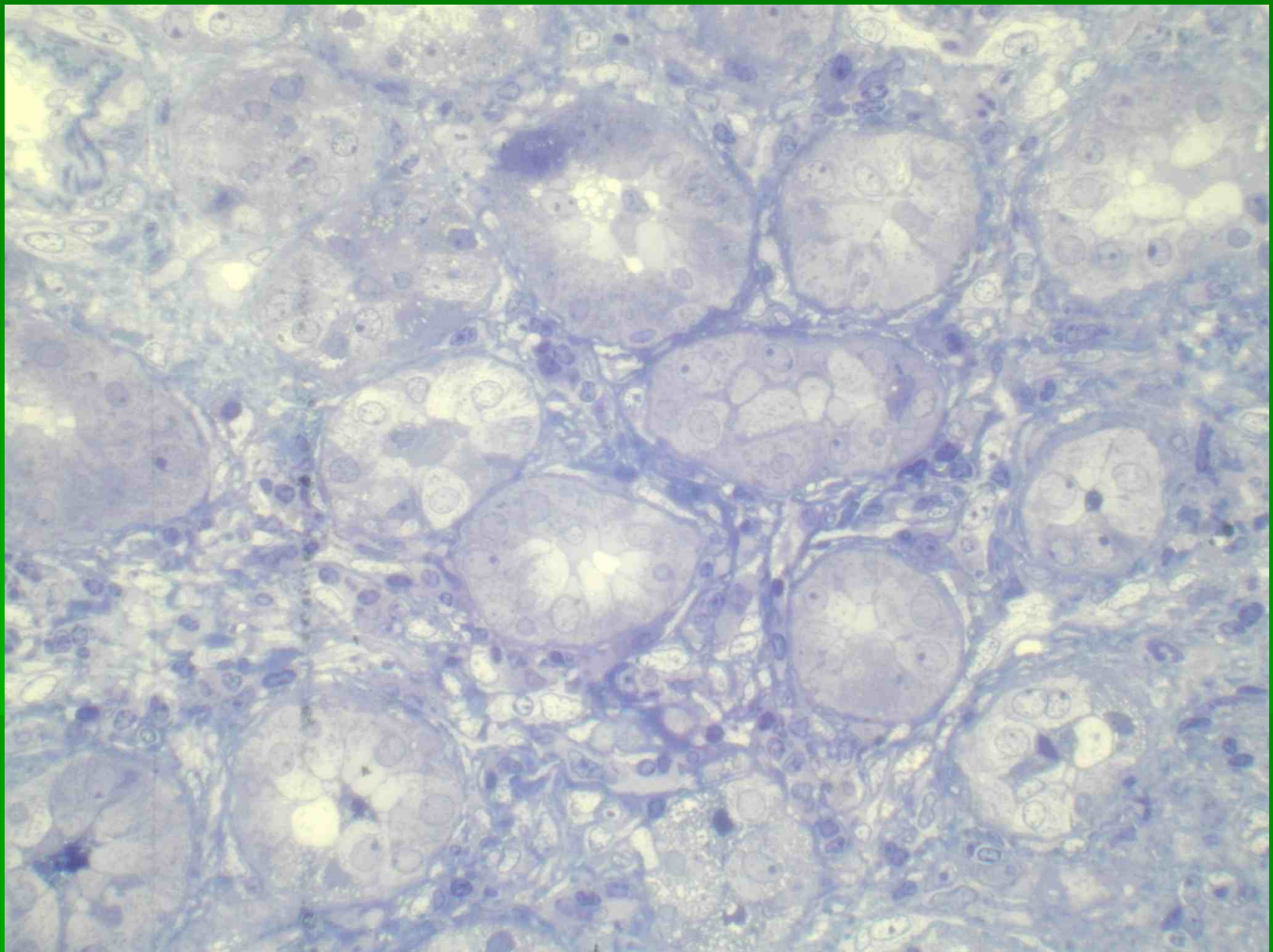
Collagen



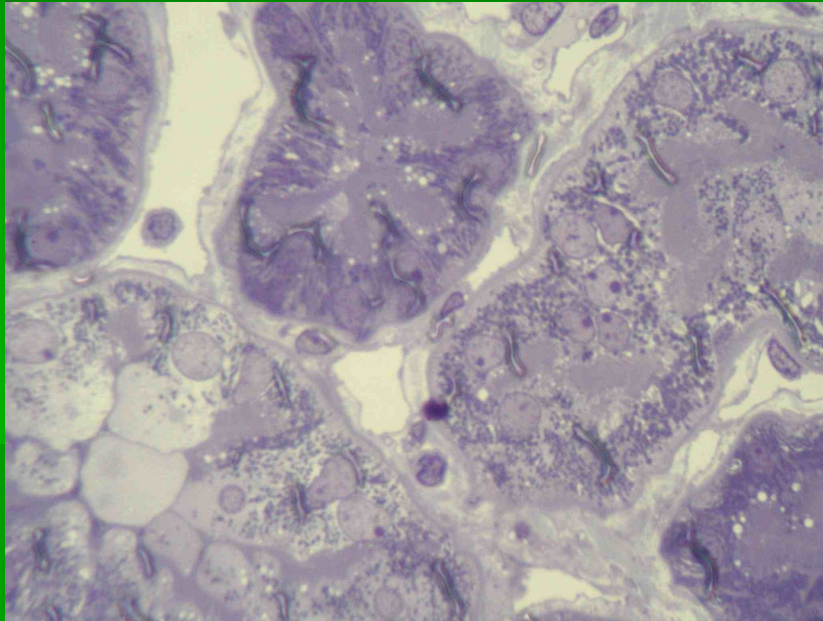
- Dark / overstraining of collagen
- 1% TB in 1% Borax
- Tol. Blue
- ? Pathological
- ? Artefact
- ? Normal



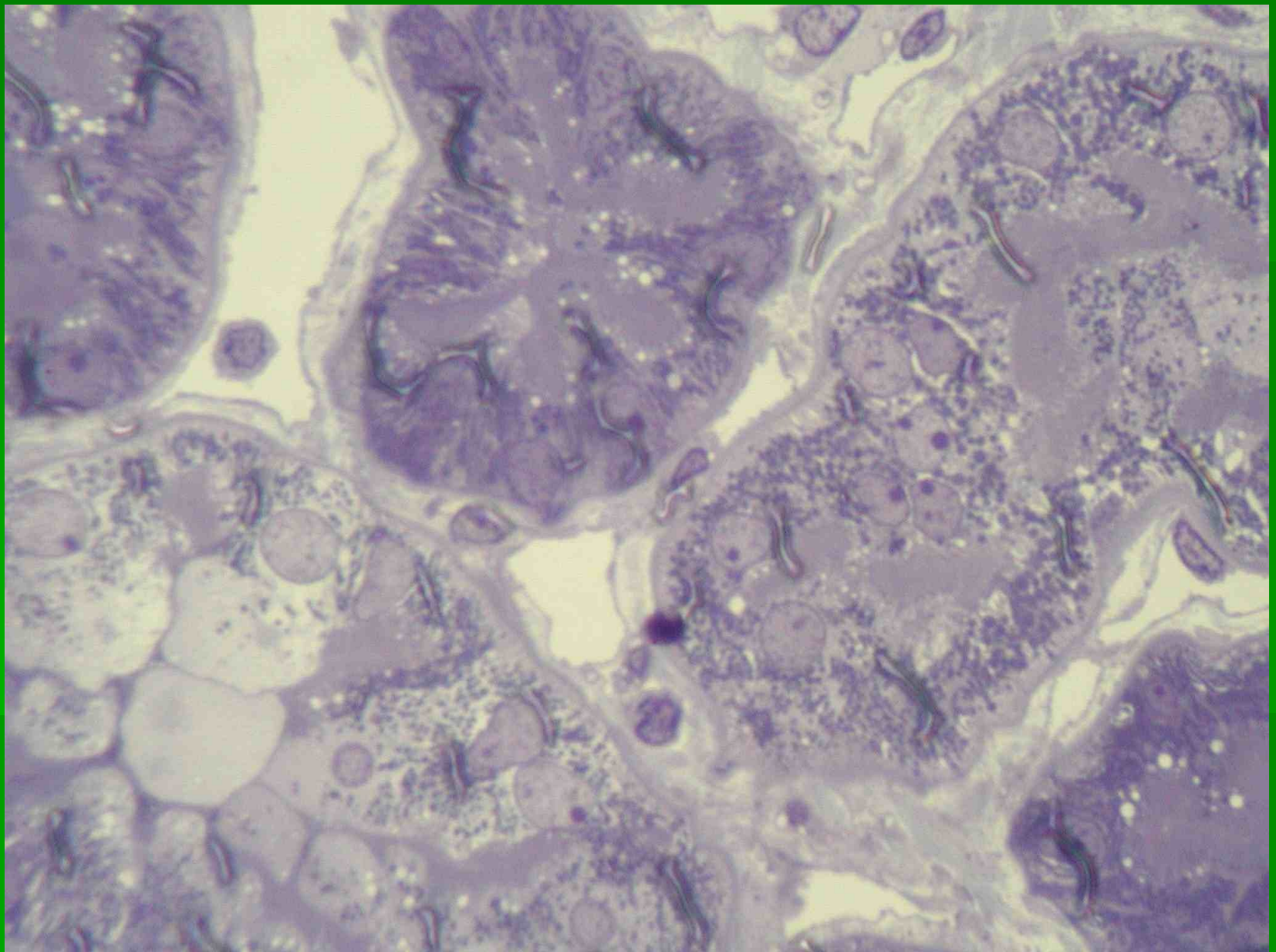


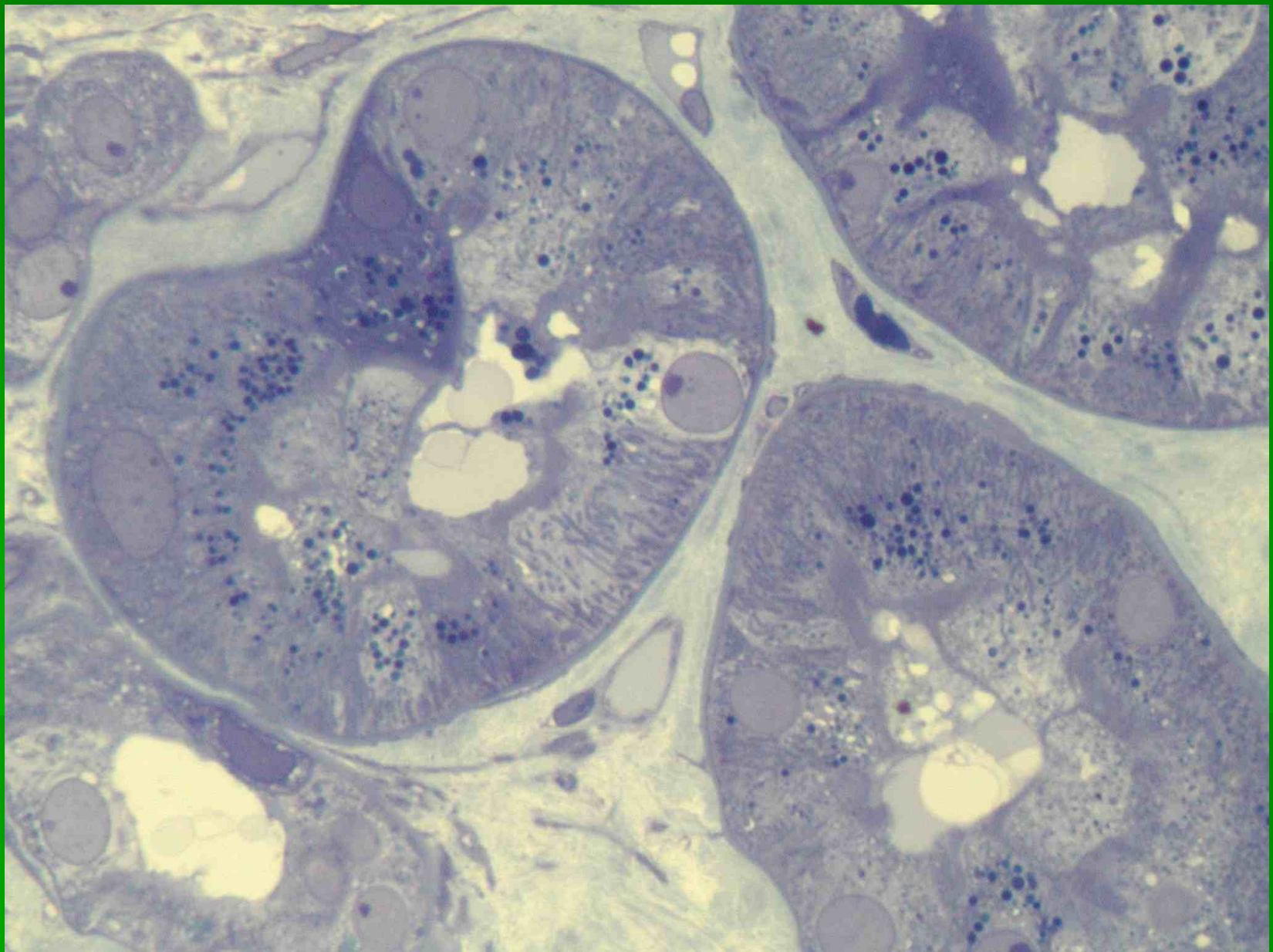


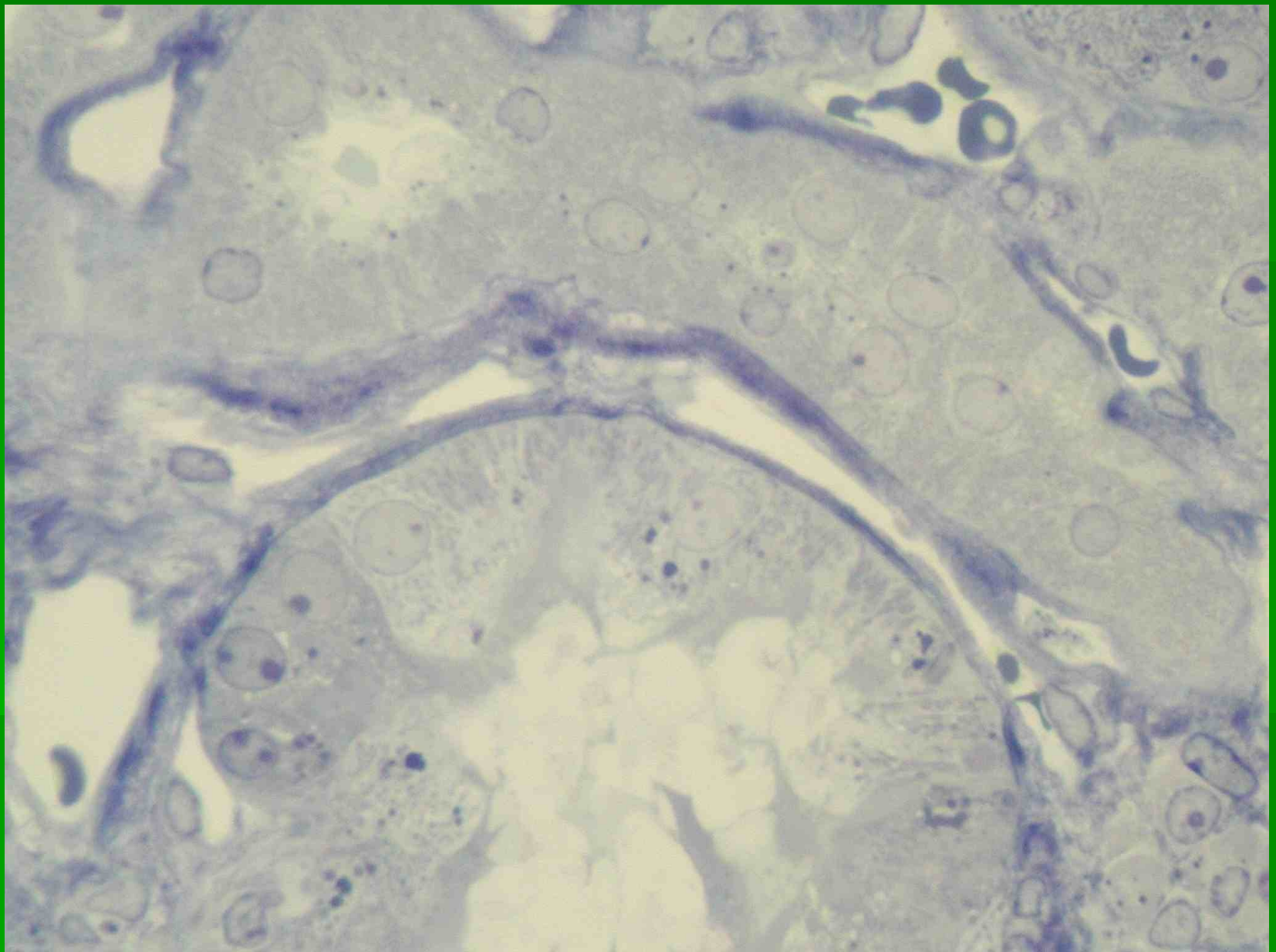
Mitochondria



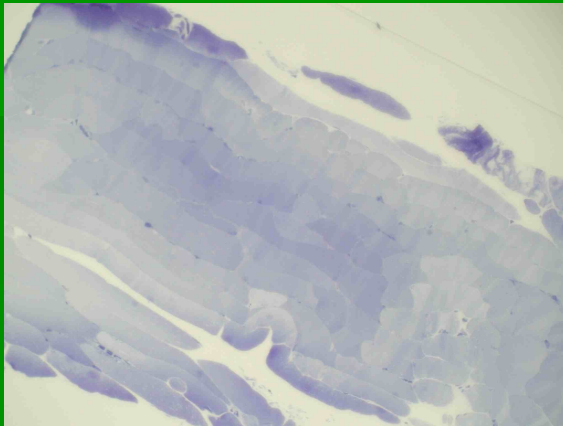
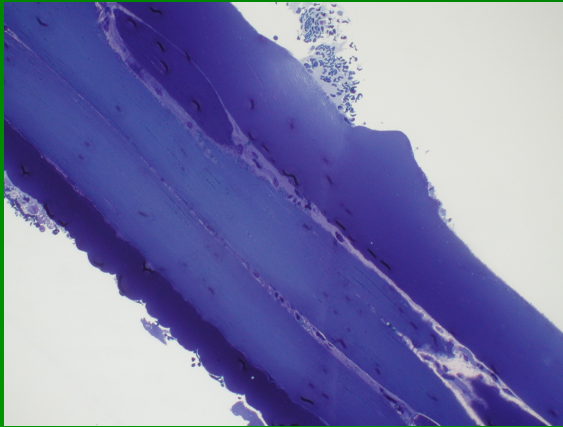
- Preferential staining of mitochondria
- Absent / present
- No pattern
- Useful in mitochondrial myopathies



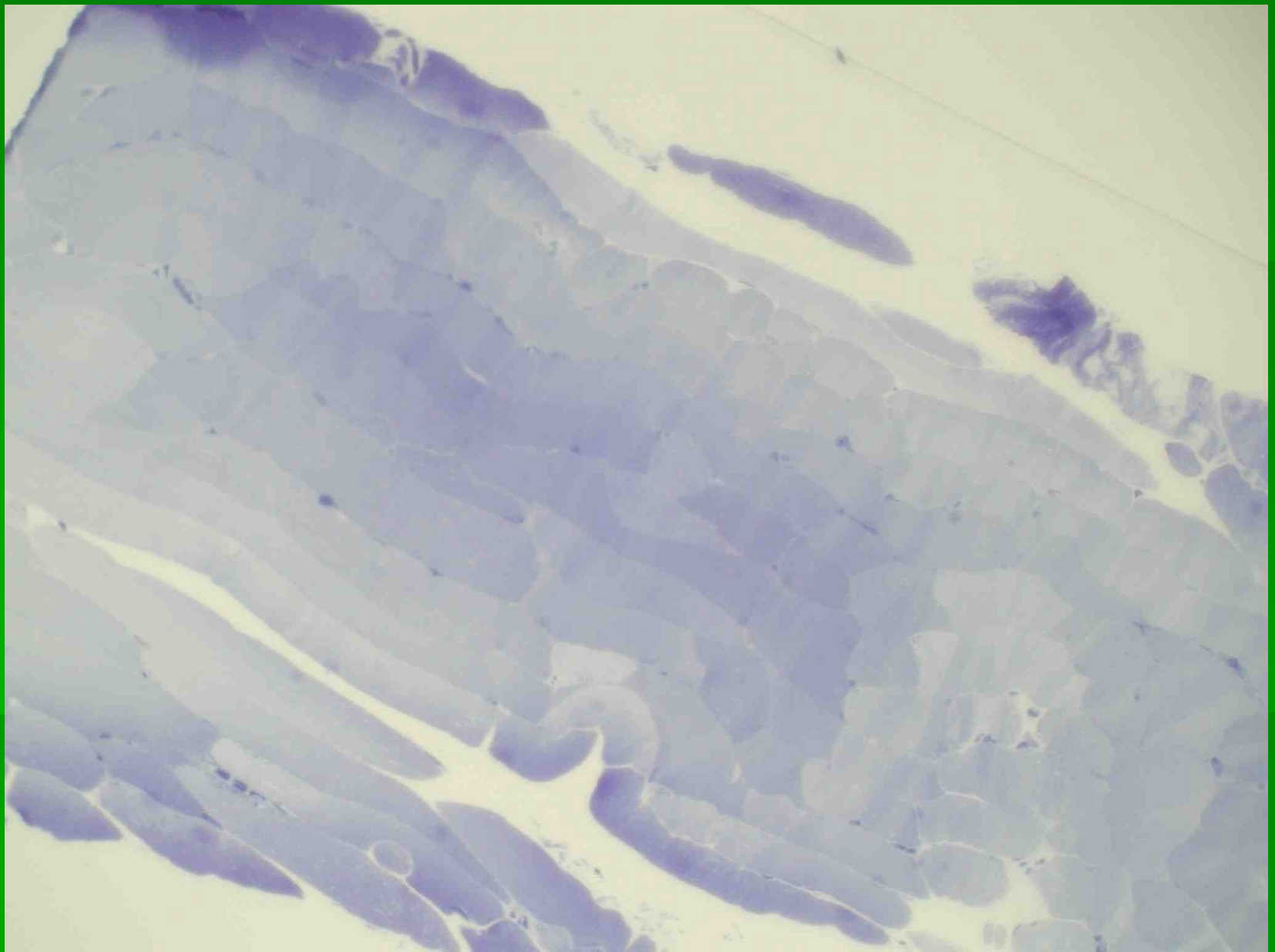


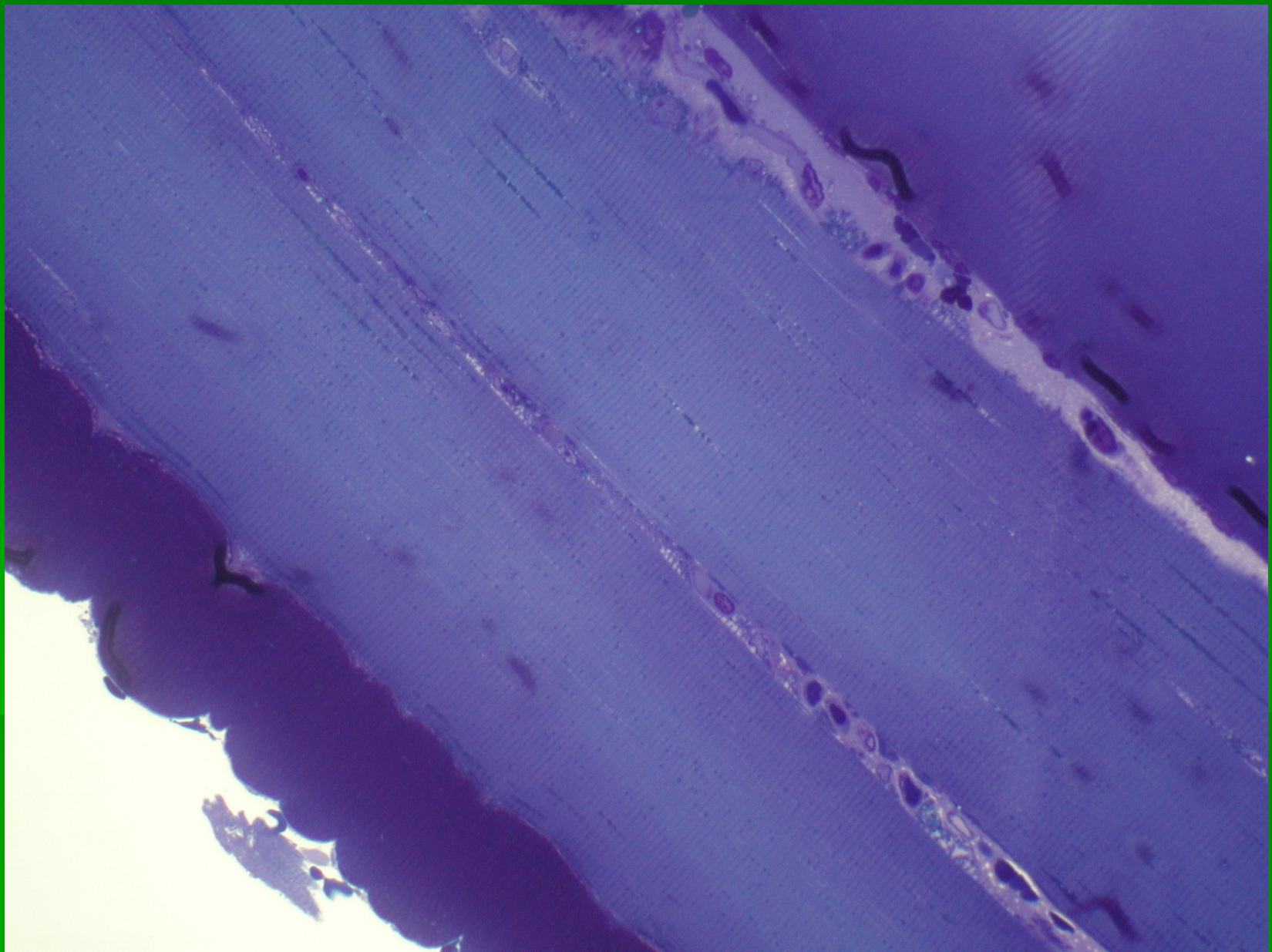


Section Thickness, Over / Under staining



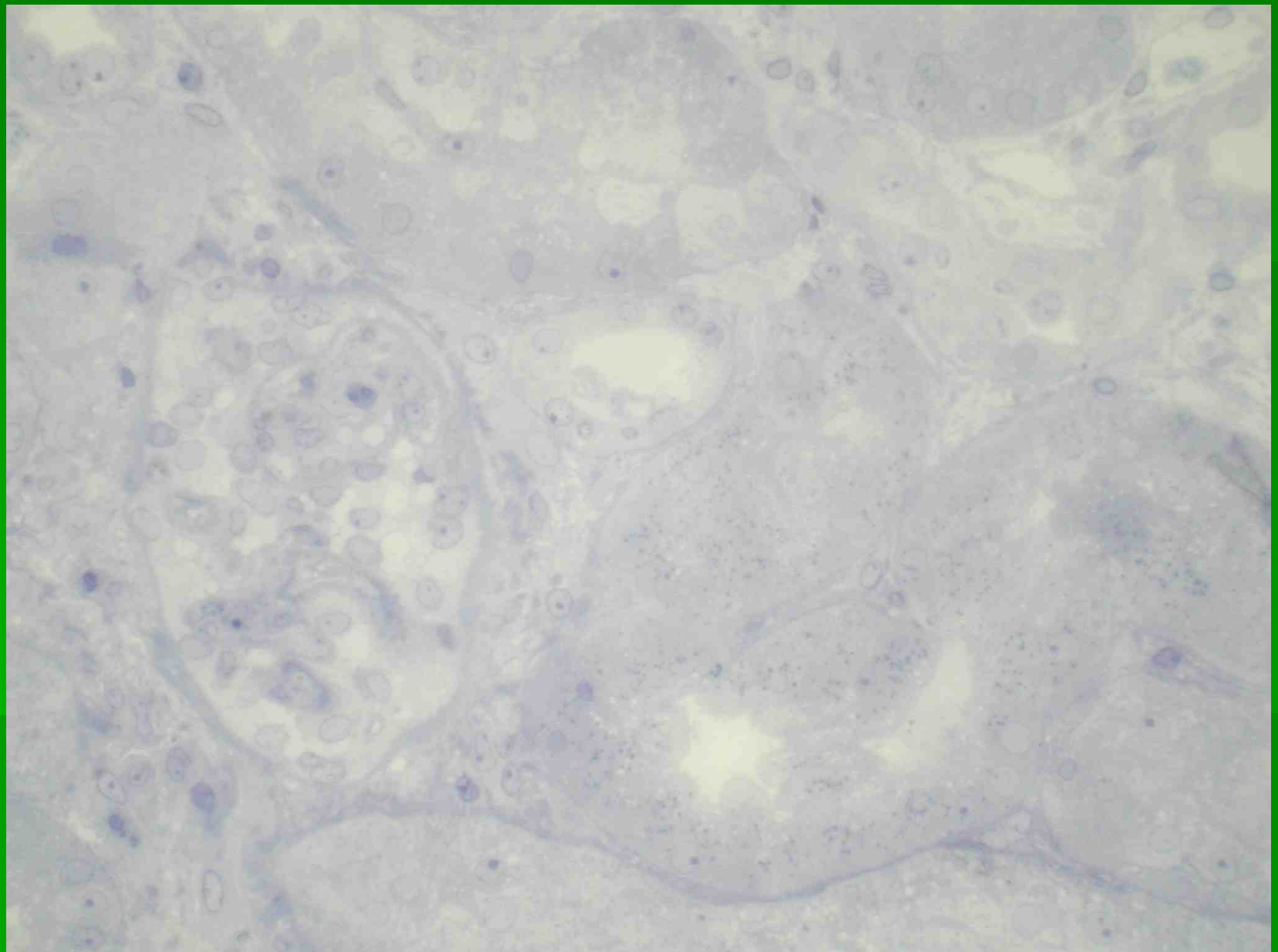
- In both cases loss of detail / contrast
- Is thickness important?
- Or is it staining intensity alone?



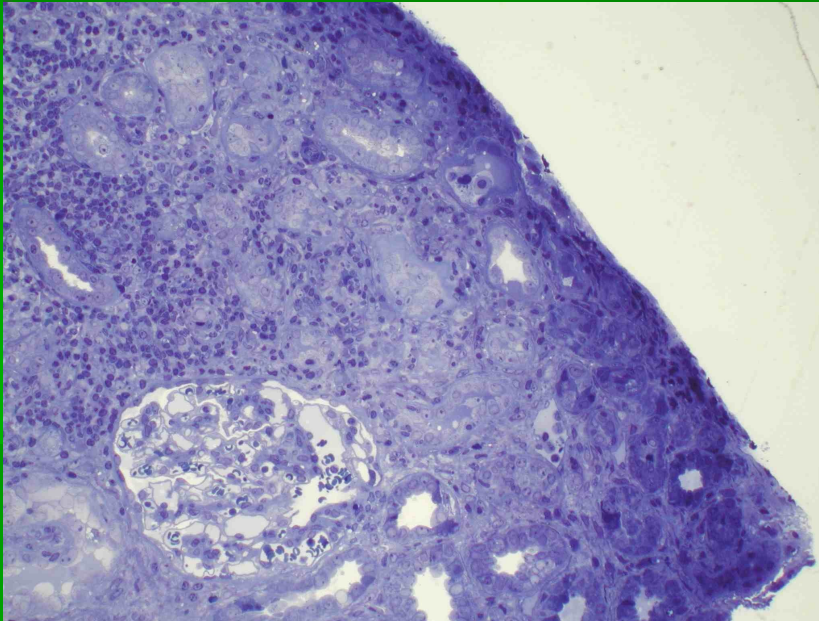


Coverslipping

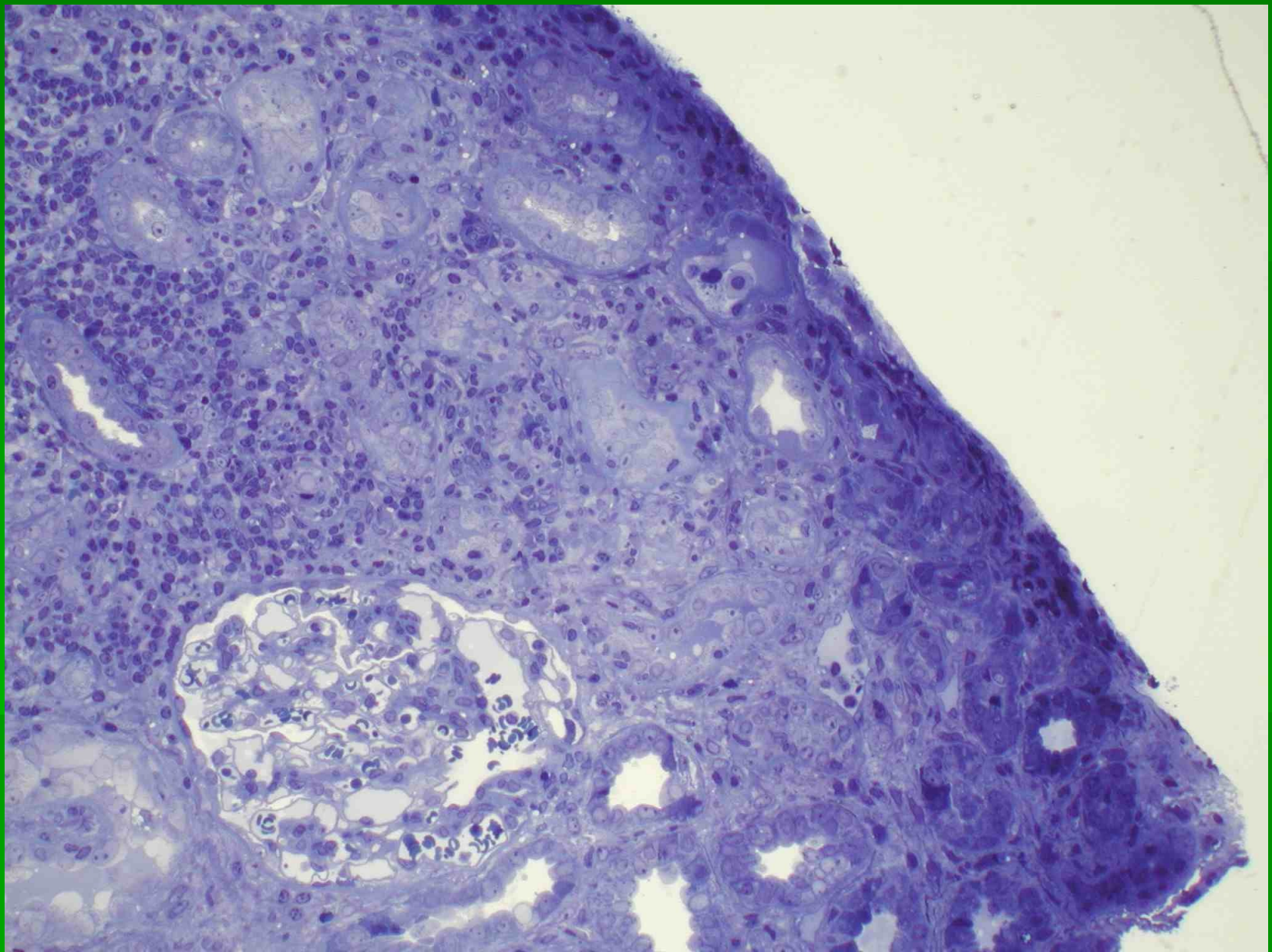
- Several slides received without coverslips
- Limits resolution
- Reduces contrast
- “Fuzziness” on photomicrographs
- Also 1 submission received with section sandwiched between 2 slides, made high power examination impossible



Edge Effect



- We saw a few examples of edge effect
- Cause?
 - Uneven thickness
 - Insufficient drying time
 - Differential impregnation of solvents, resin etc



Method for Best Technique

- Thionin stain
 - Dissolve 0.05g Thionin in 22.5ml of absolute alcohol on a magnetic stirrer. Then add 22.5ml of distilled water followed by 5ml of 0.1M NaOH
 - Filter the resulting solution, should be reddish in colour rather than purple
 - Keeps at room temp. for about a week

Brief Method

- Place dry sections on 70-80 deg C hotplate
- Cover with Thionin solution for 15-30 seconds
- Wash slide well in distilled water
- Full method will be posted with EQA results on the ACEM website