Preparation of Muscle and Nerve Biopsies for Electron Microscopy

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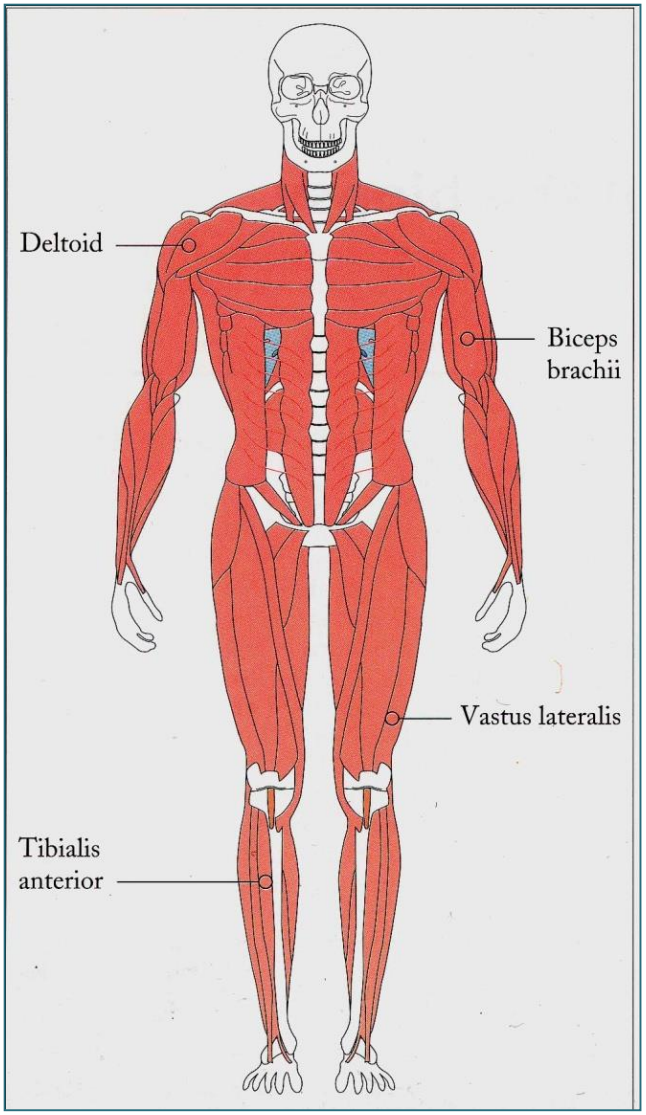
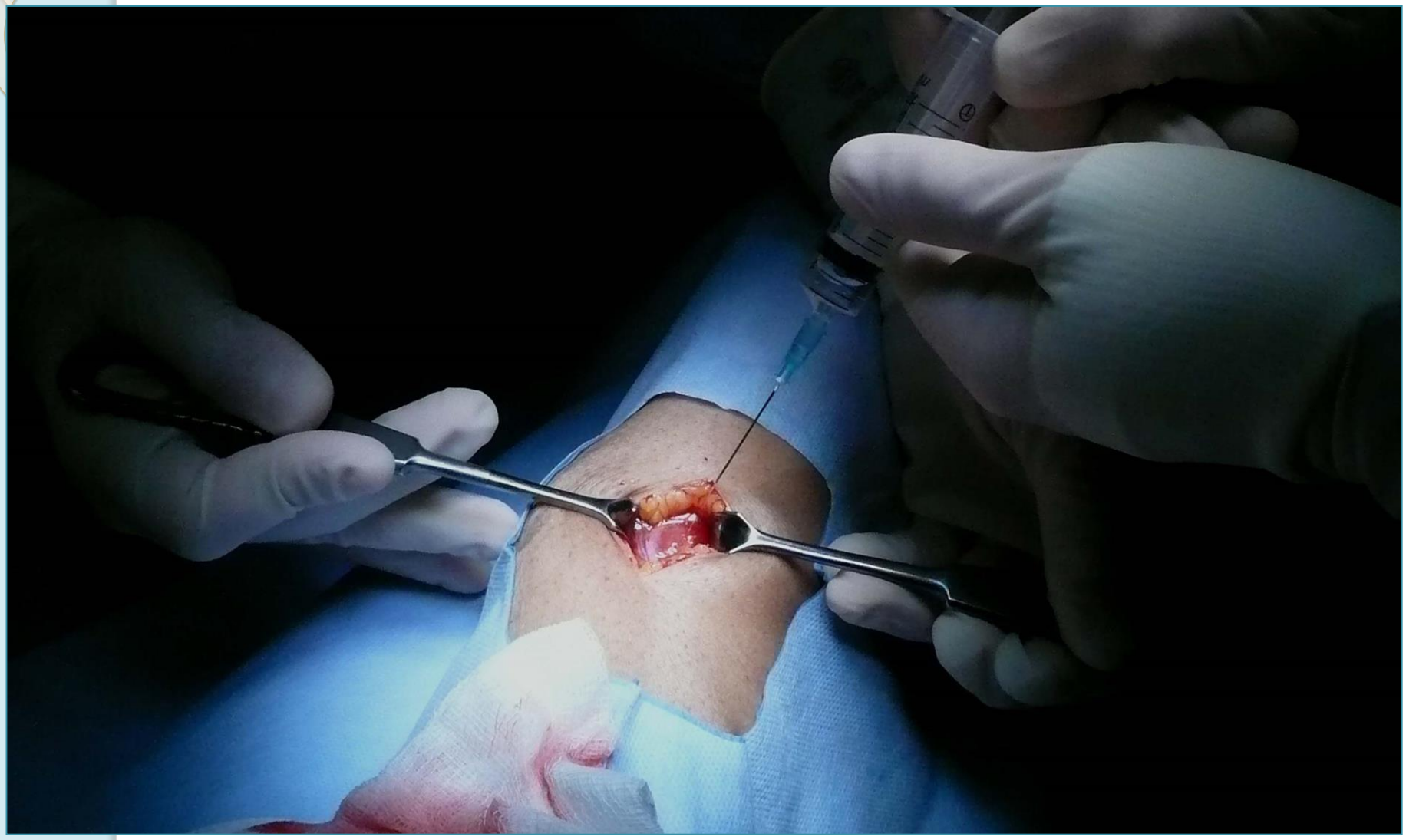
Muscles biopsied



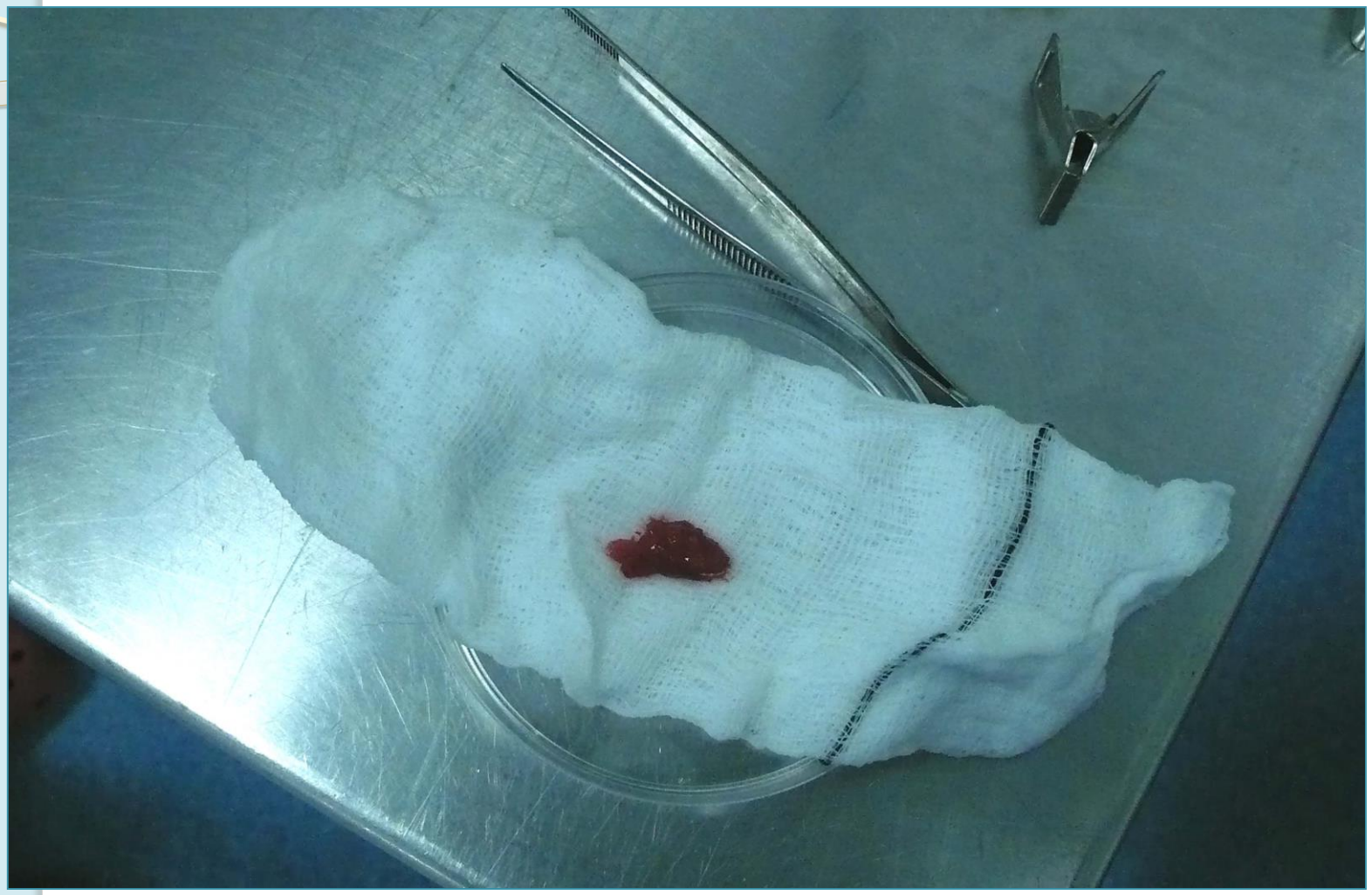
Muscle biopsy



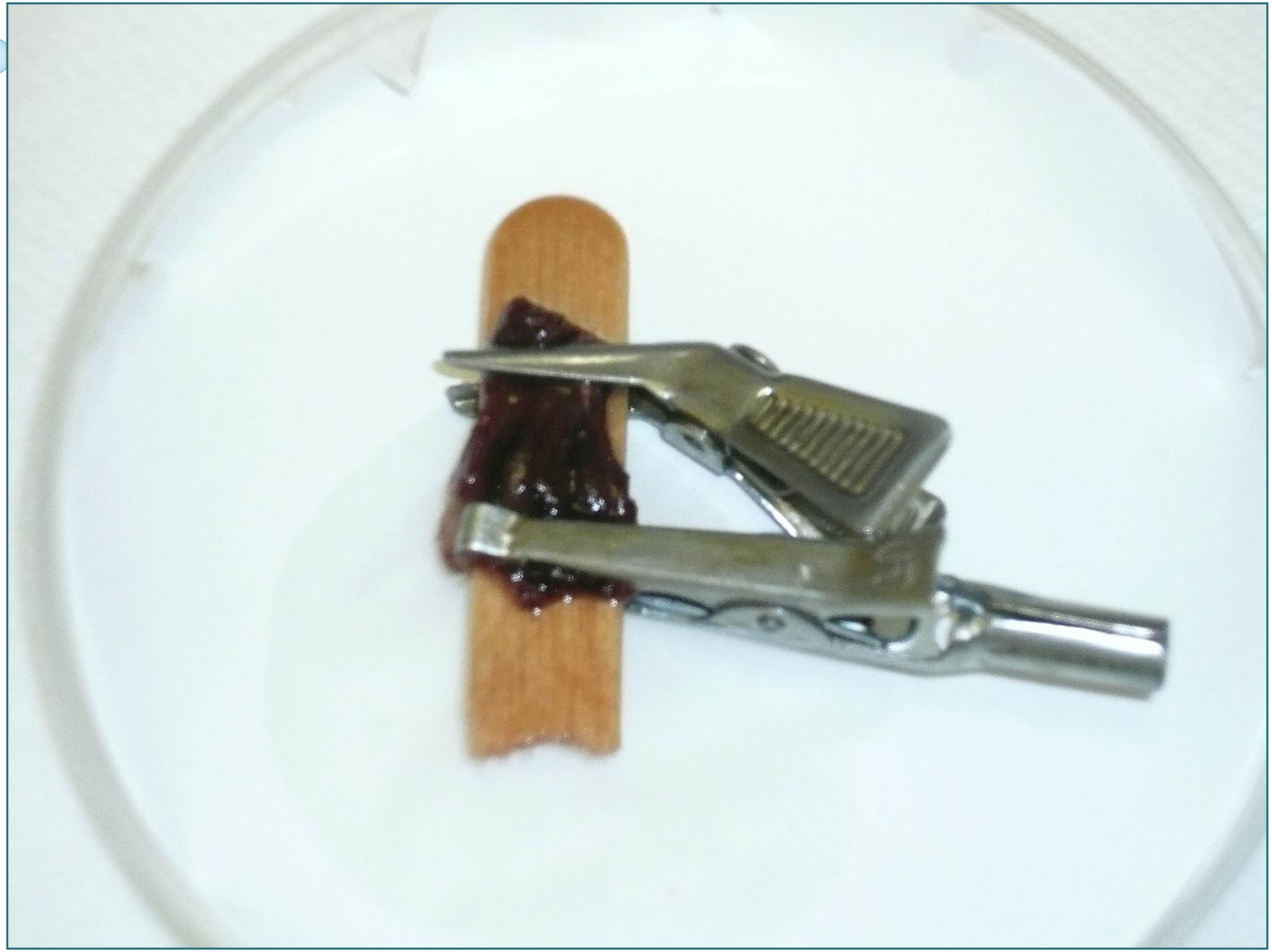
Cutting specimen: muscle

- Divide into two
  - Clamp one piece onto tongue depressor, minimum 1cm between clamps.
  - Immediately immerse in 2.5% buffered glutaraldehyde
- Divide remainder into 0.5-1cm cubes and glue onto cork using gum tragacanth.
  - Freeze in precooled isopentane, stir 30s. Place in precooled labelled pot for storage.
  - .

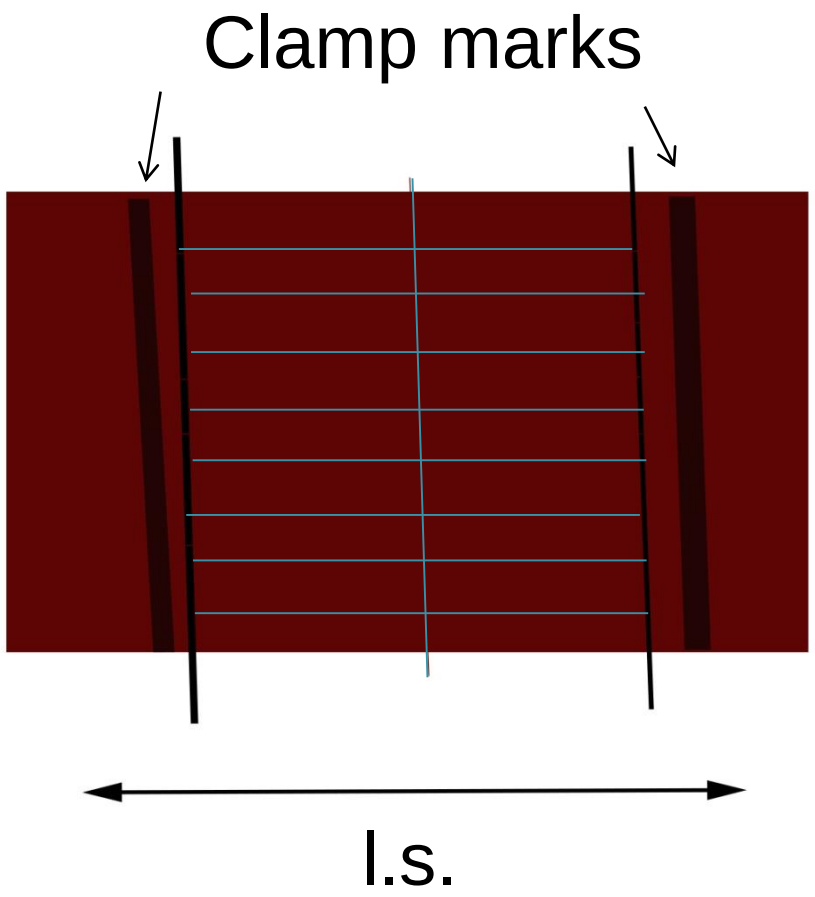
Muscle specimen



Muscle clamps

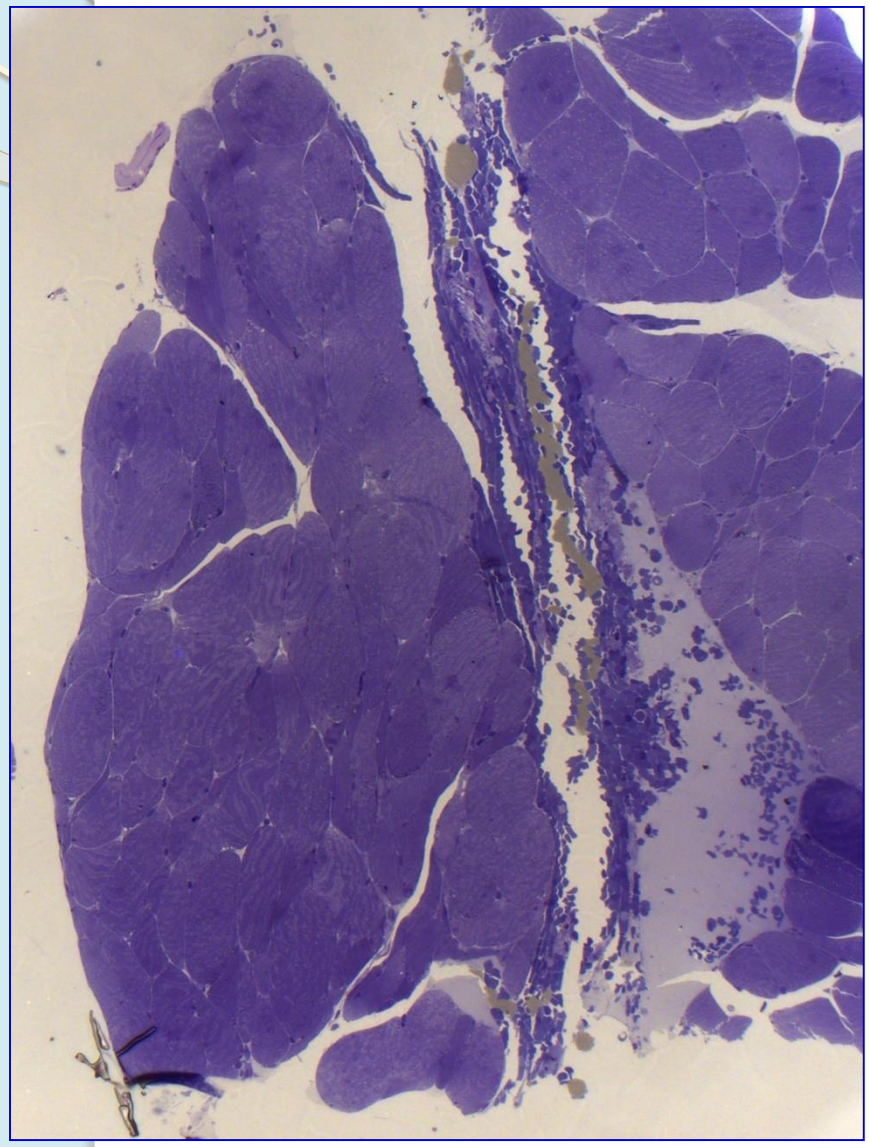


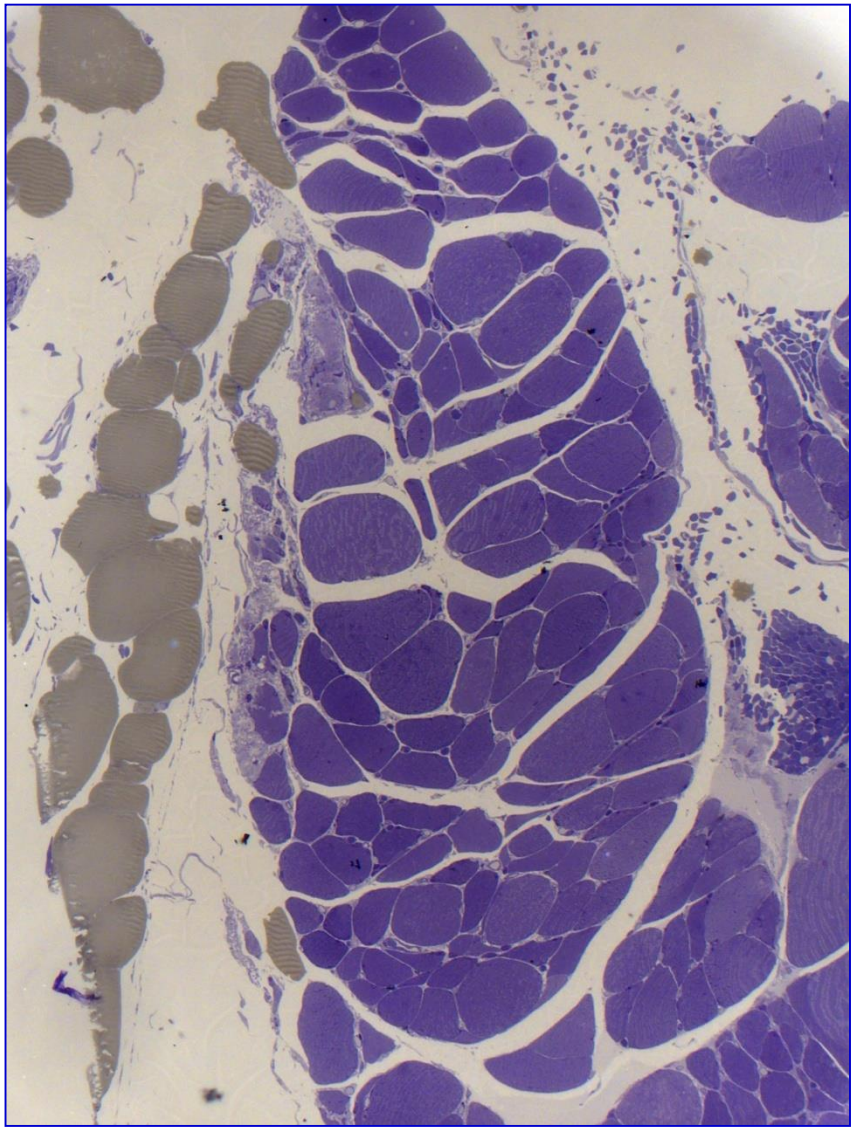
Cutting muscle for EM blocks



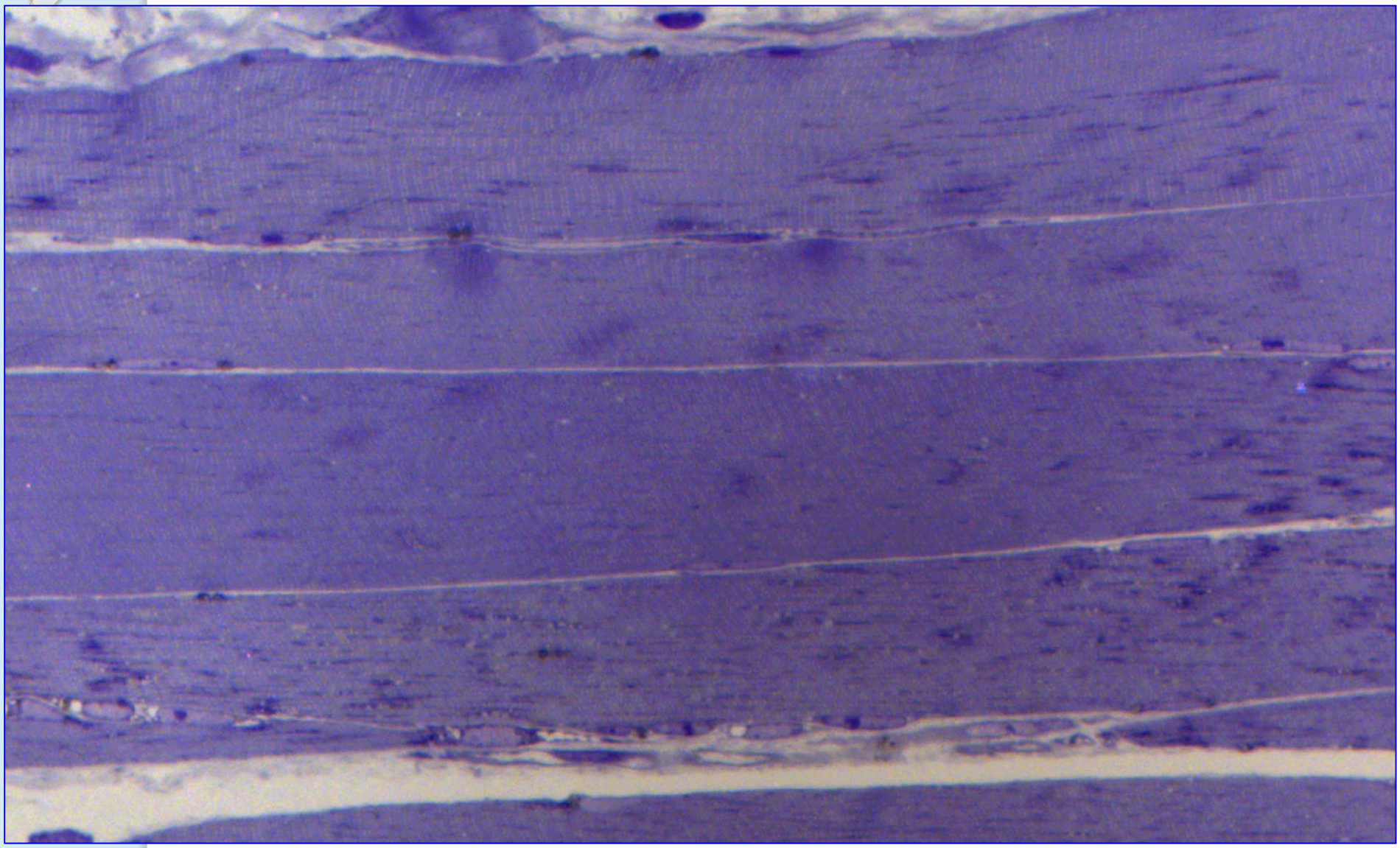
Embedding

Muscle; resin sections

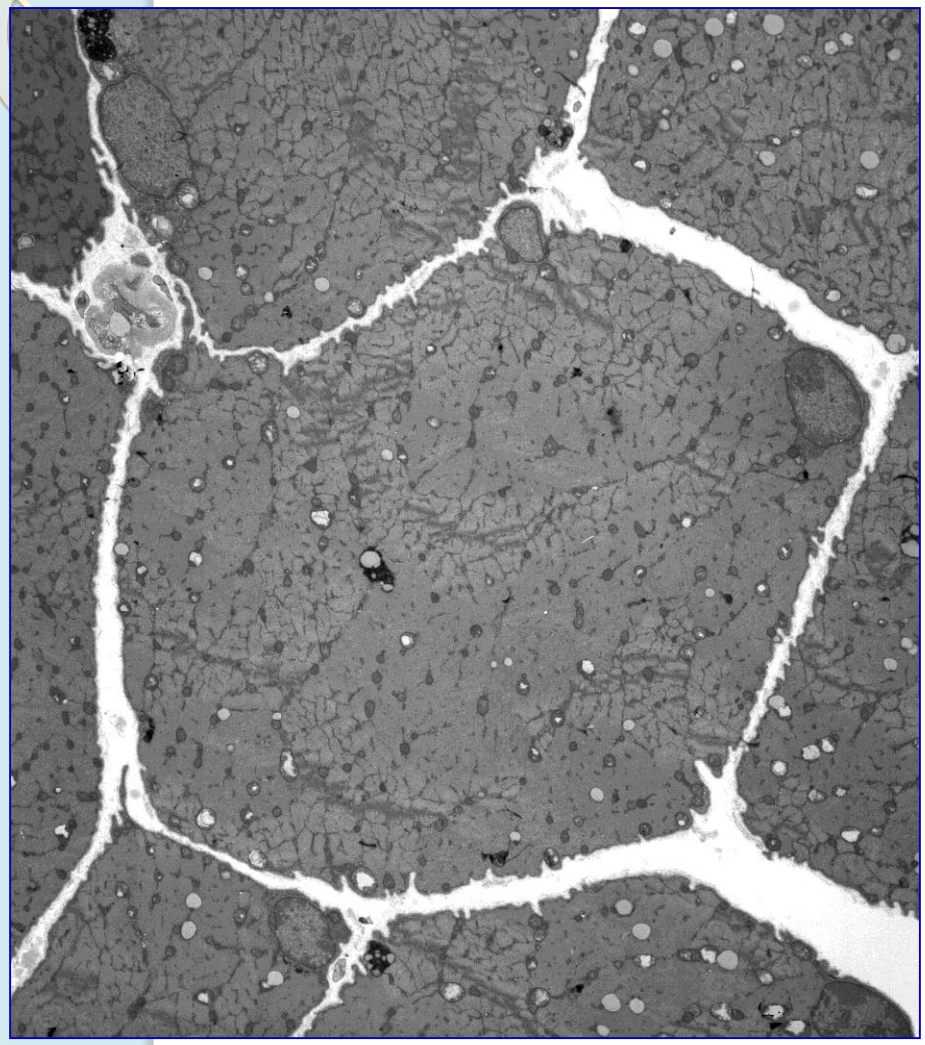


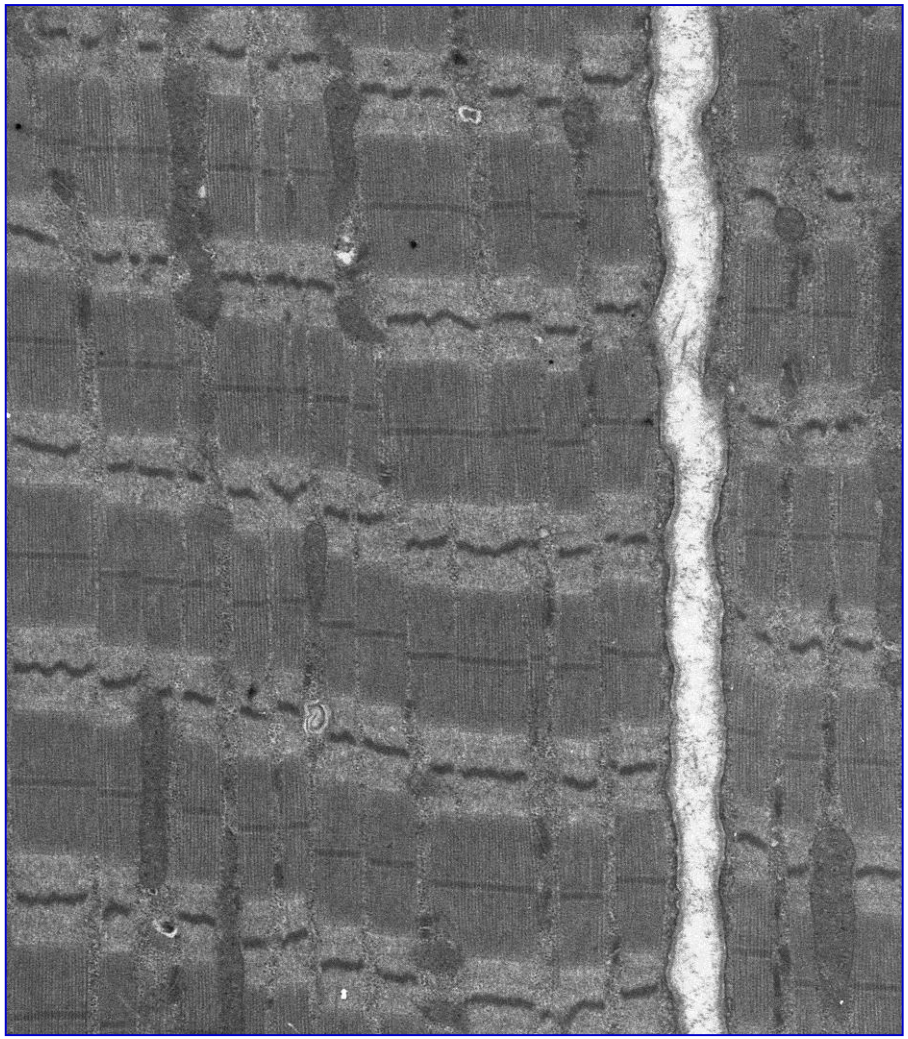


Muscle; resin sections, l.s.

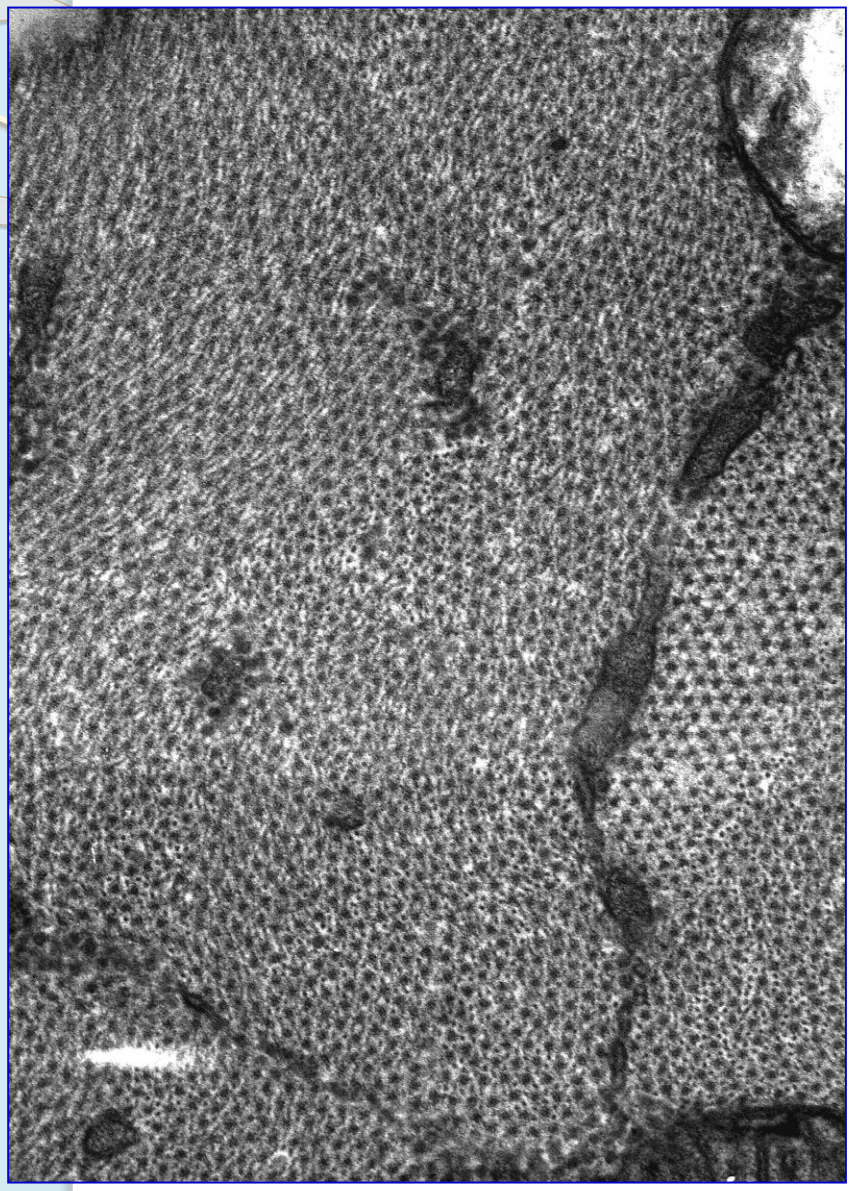


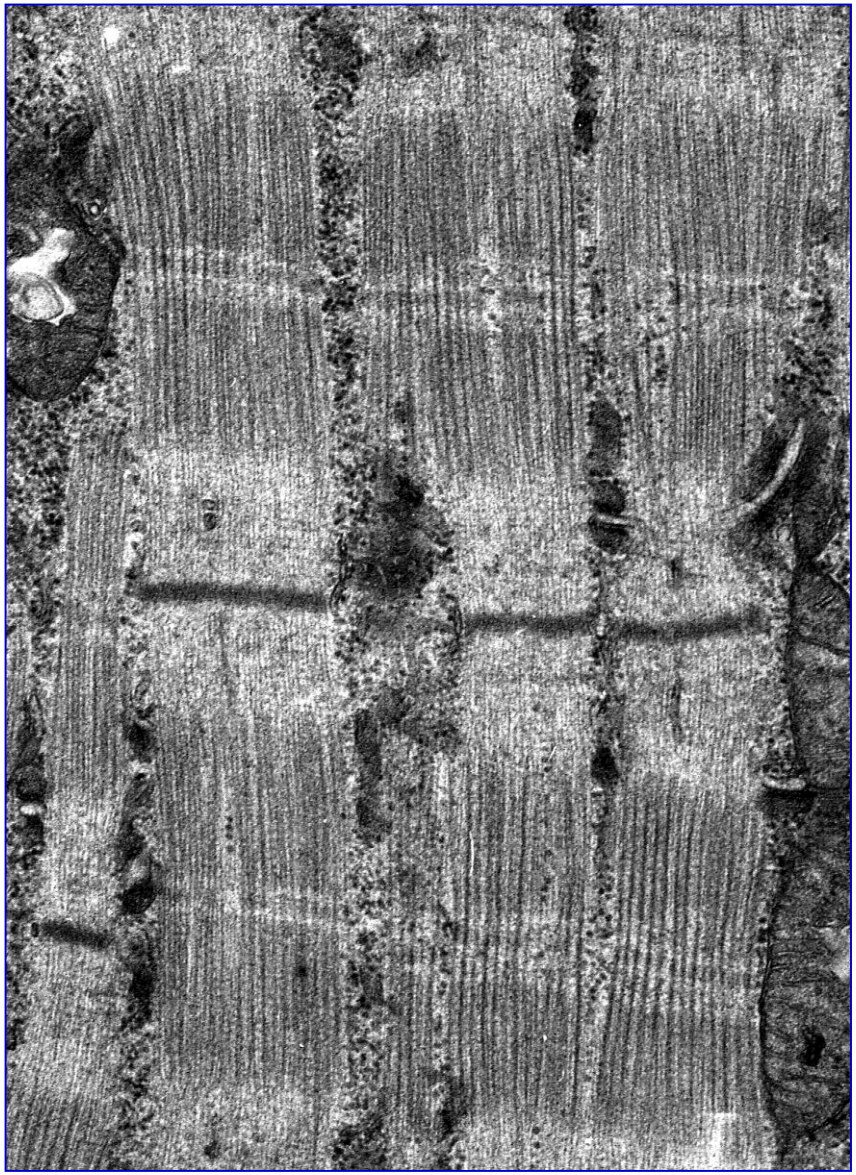
Muscle Structure, E.M.



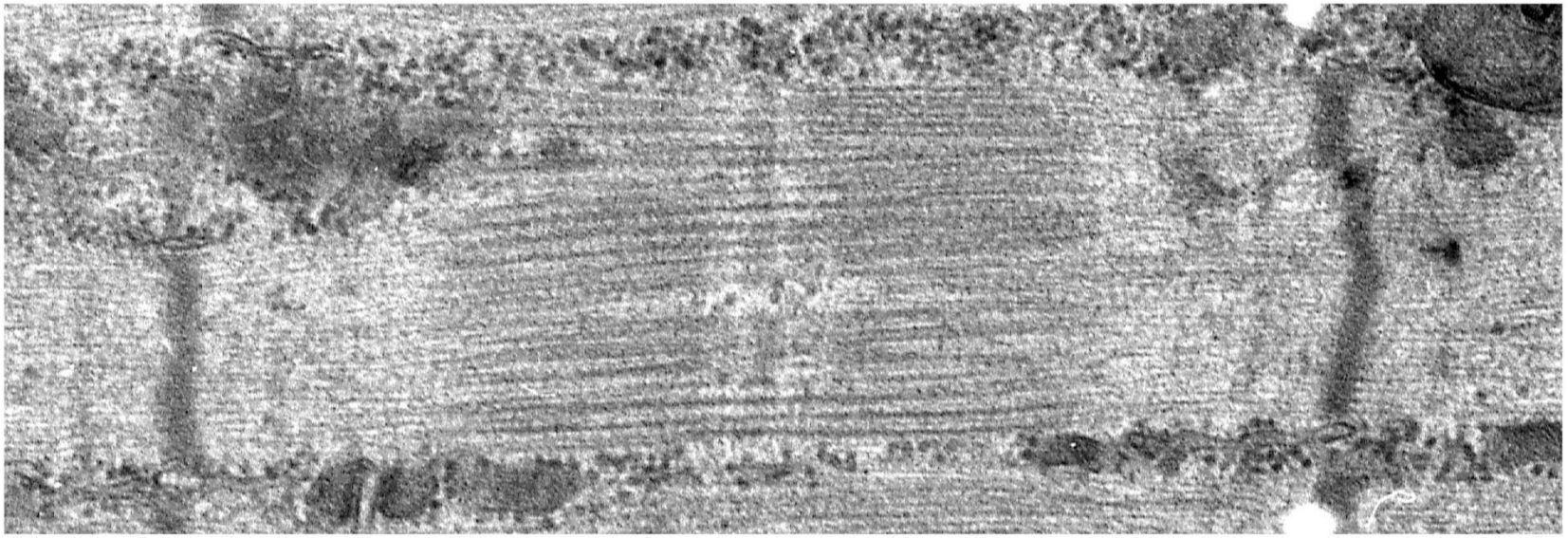


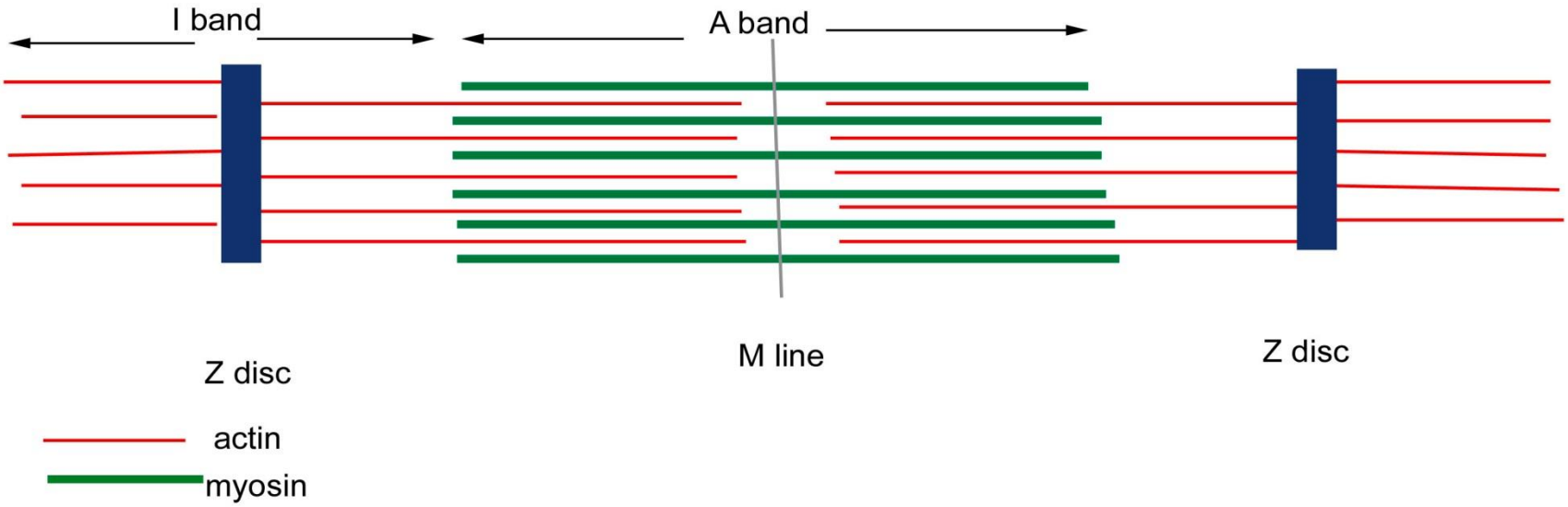
Muscle Structure, E.M.



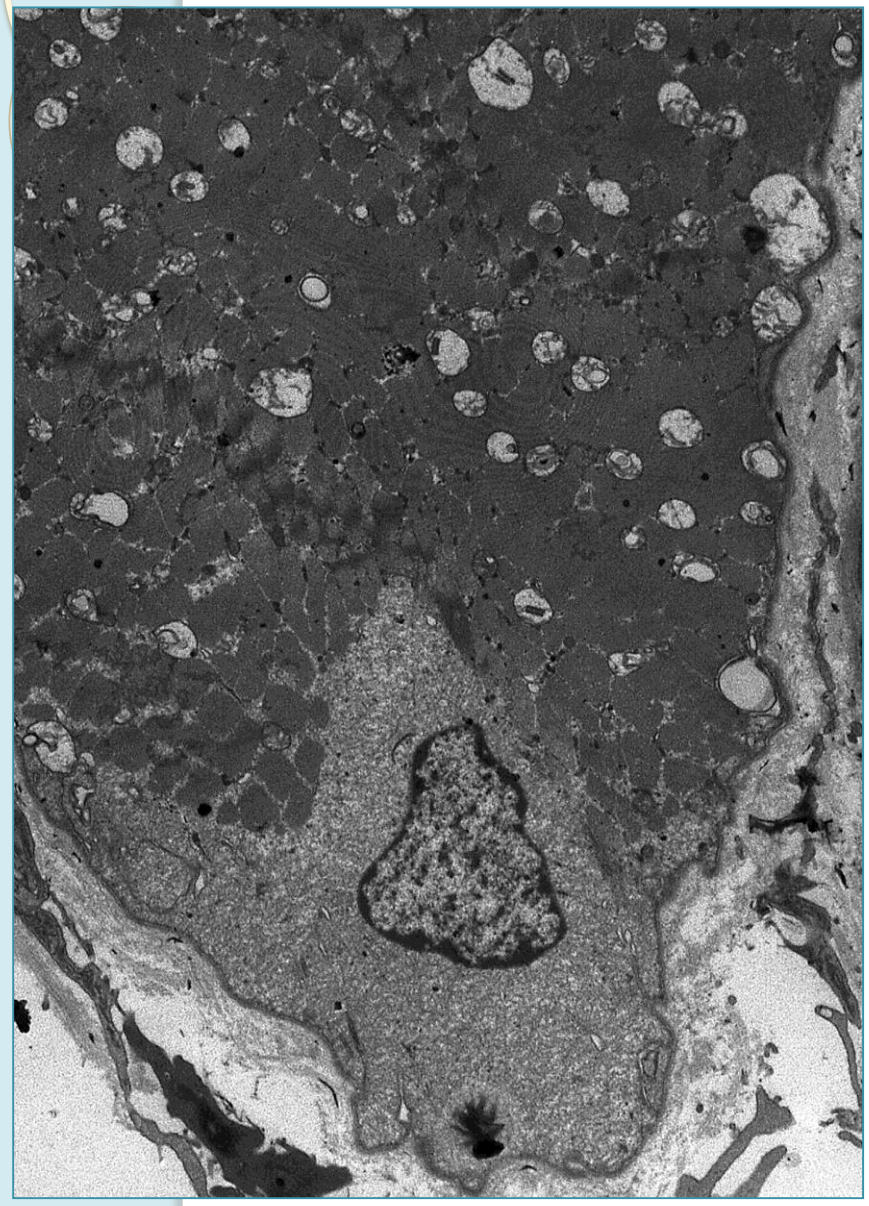


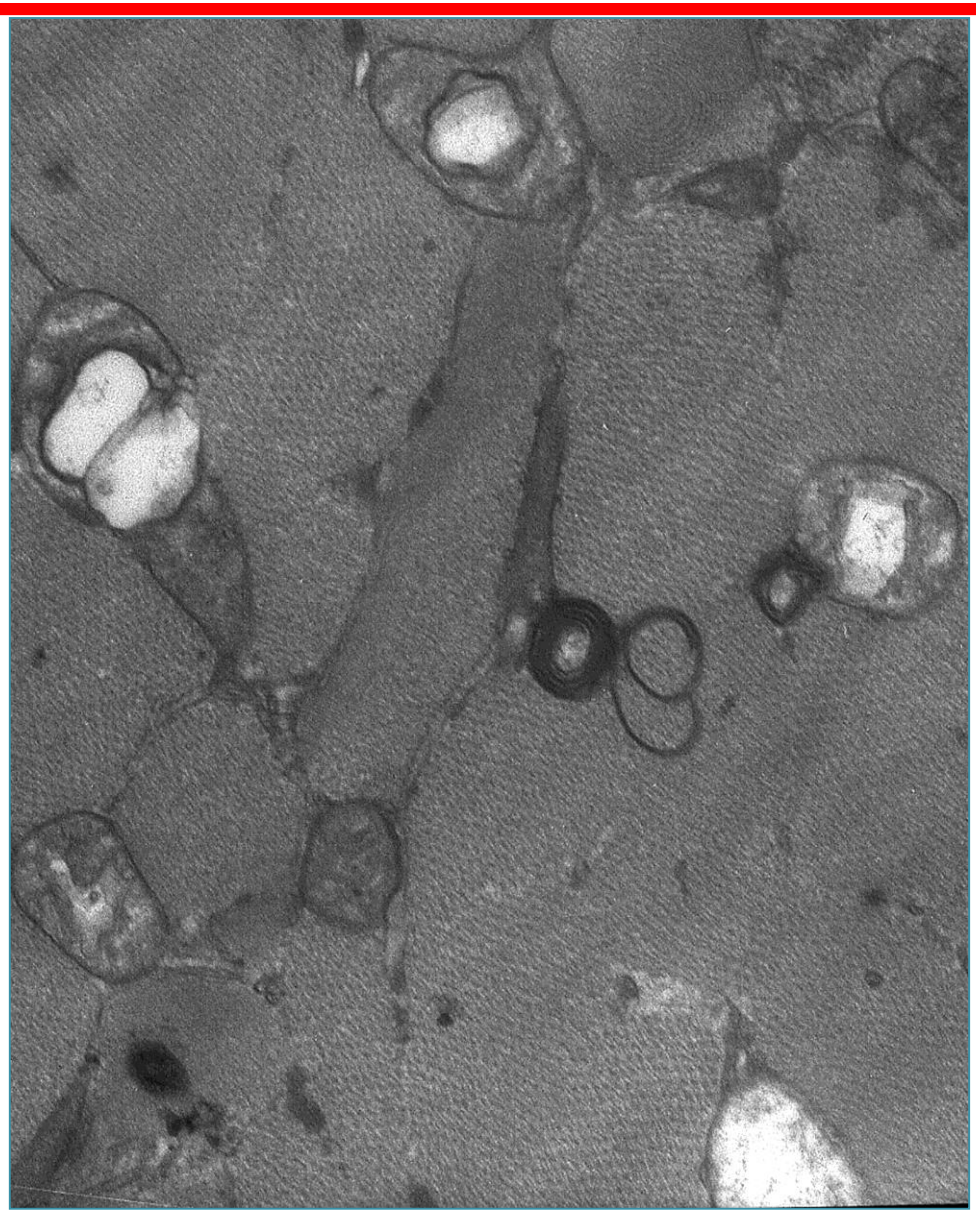
Sarcomere structure



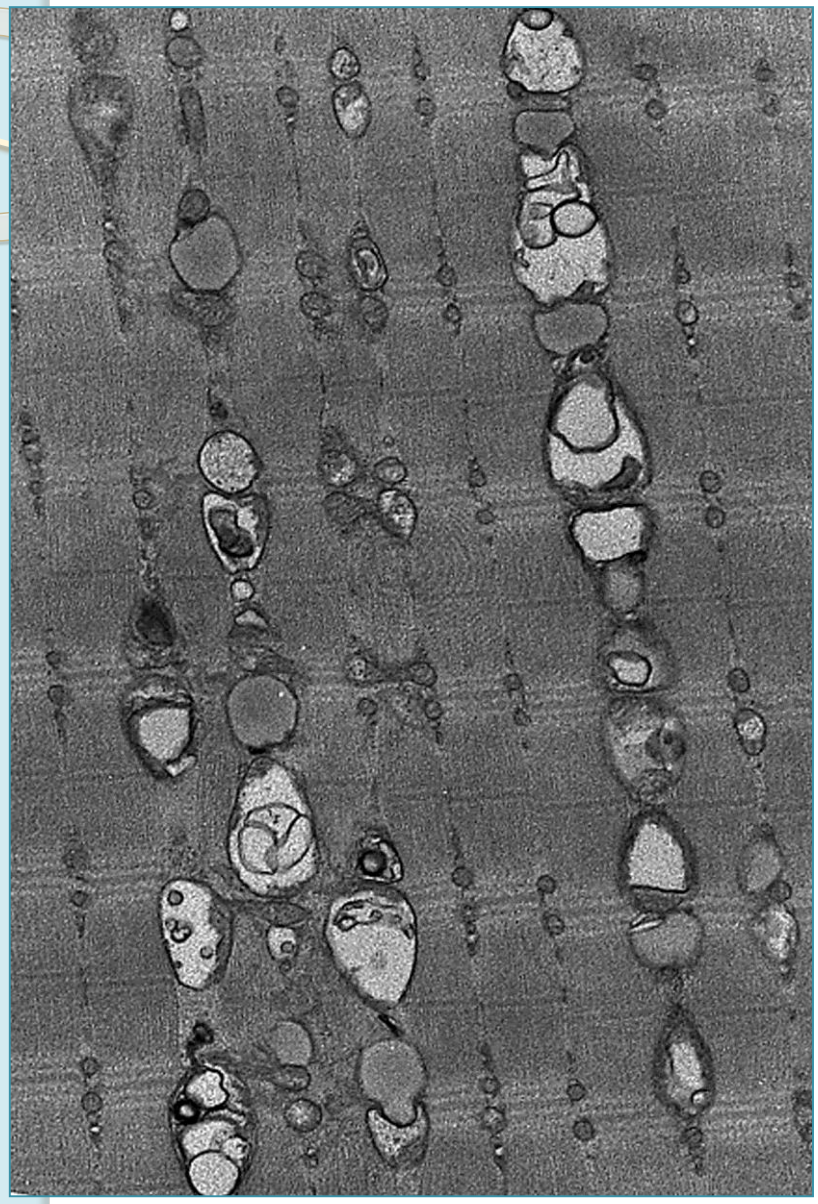


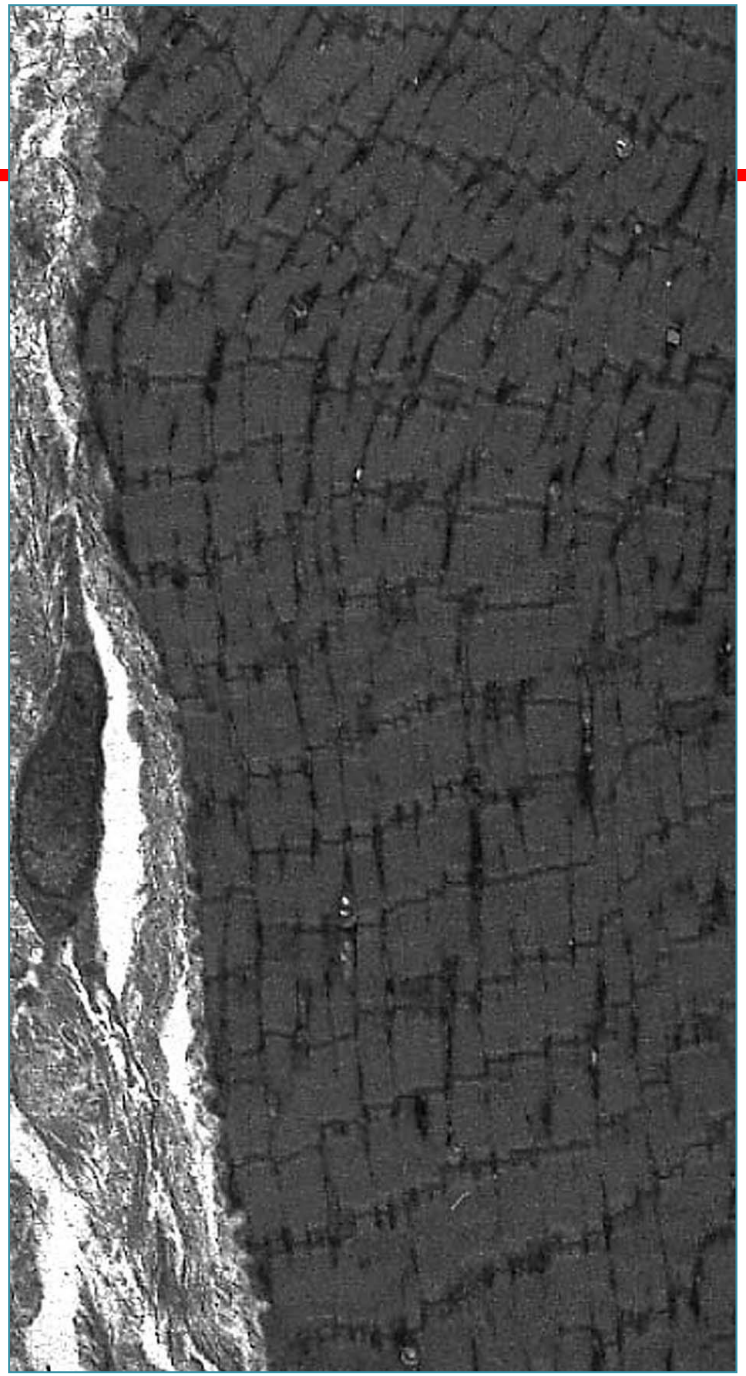
Muscle artefacts



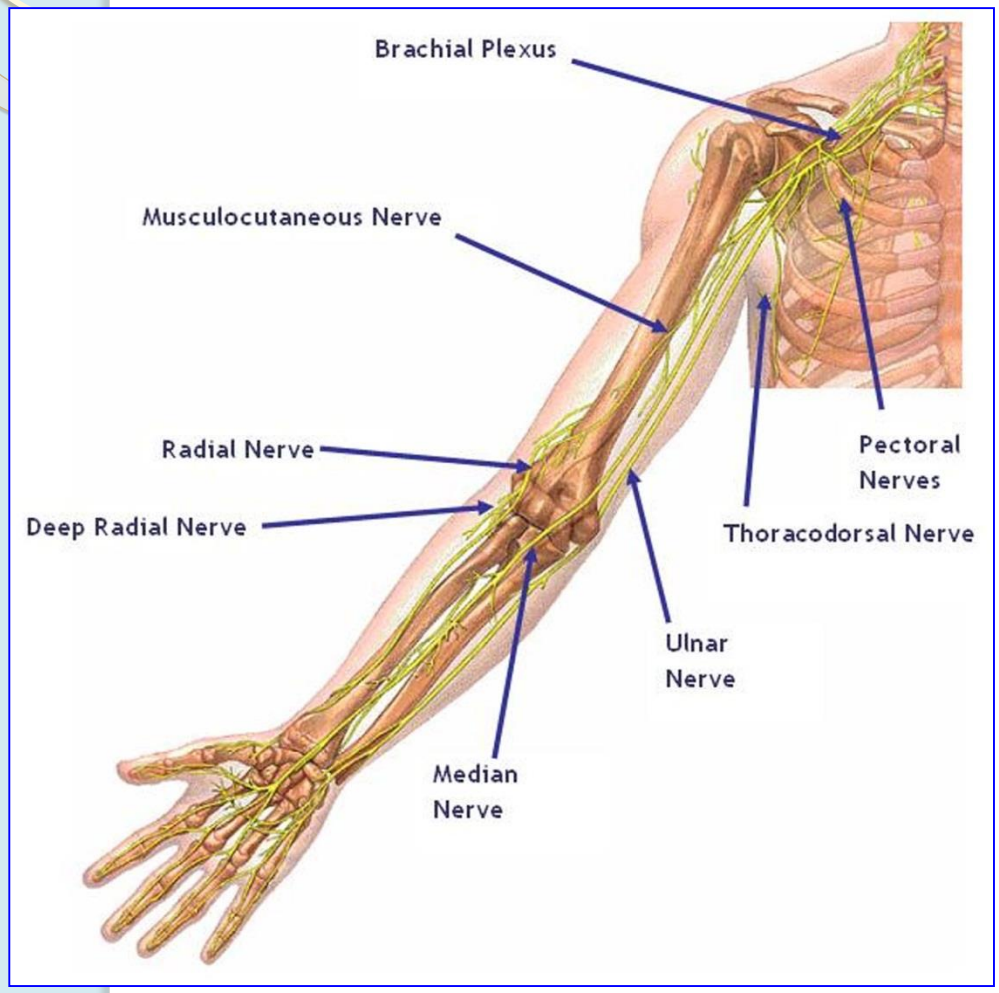


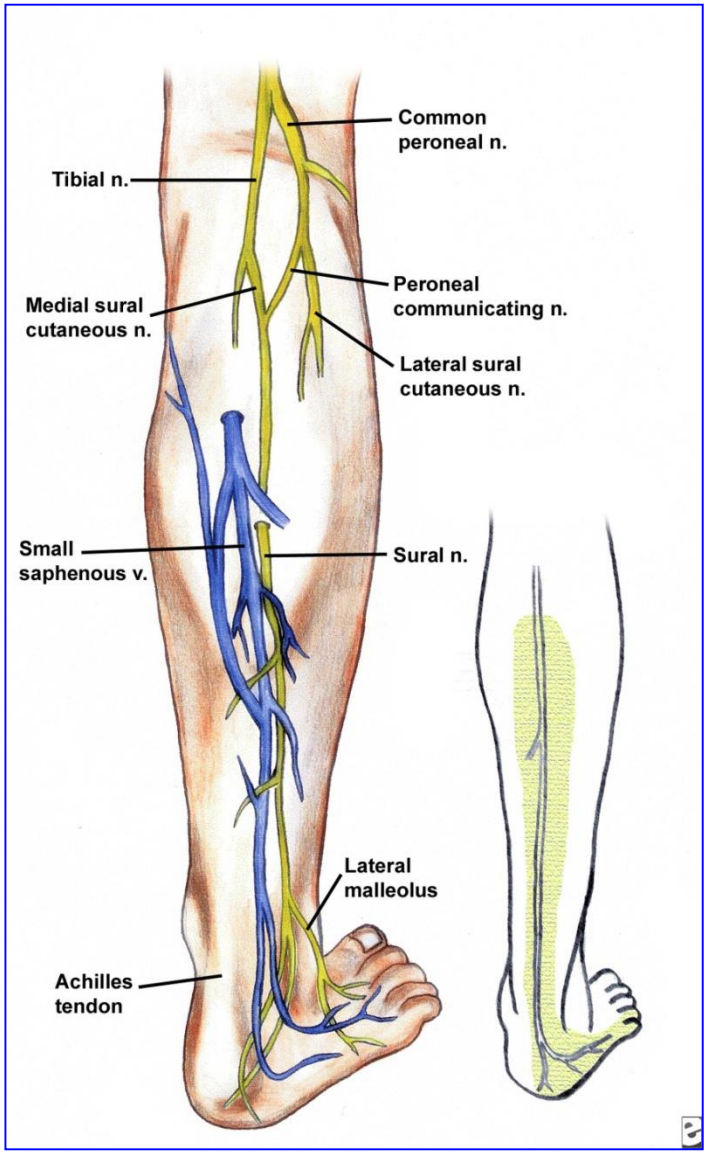
Muscle artefacts



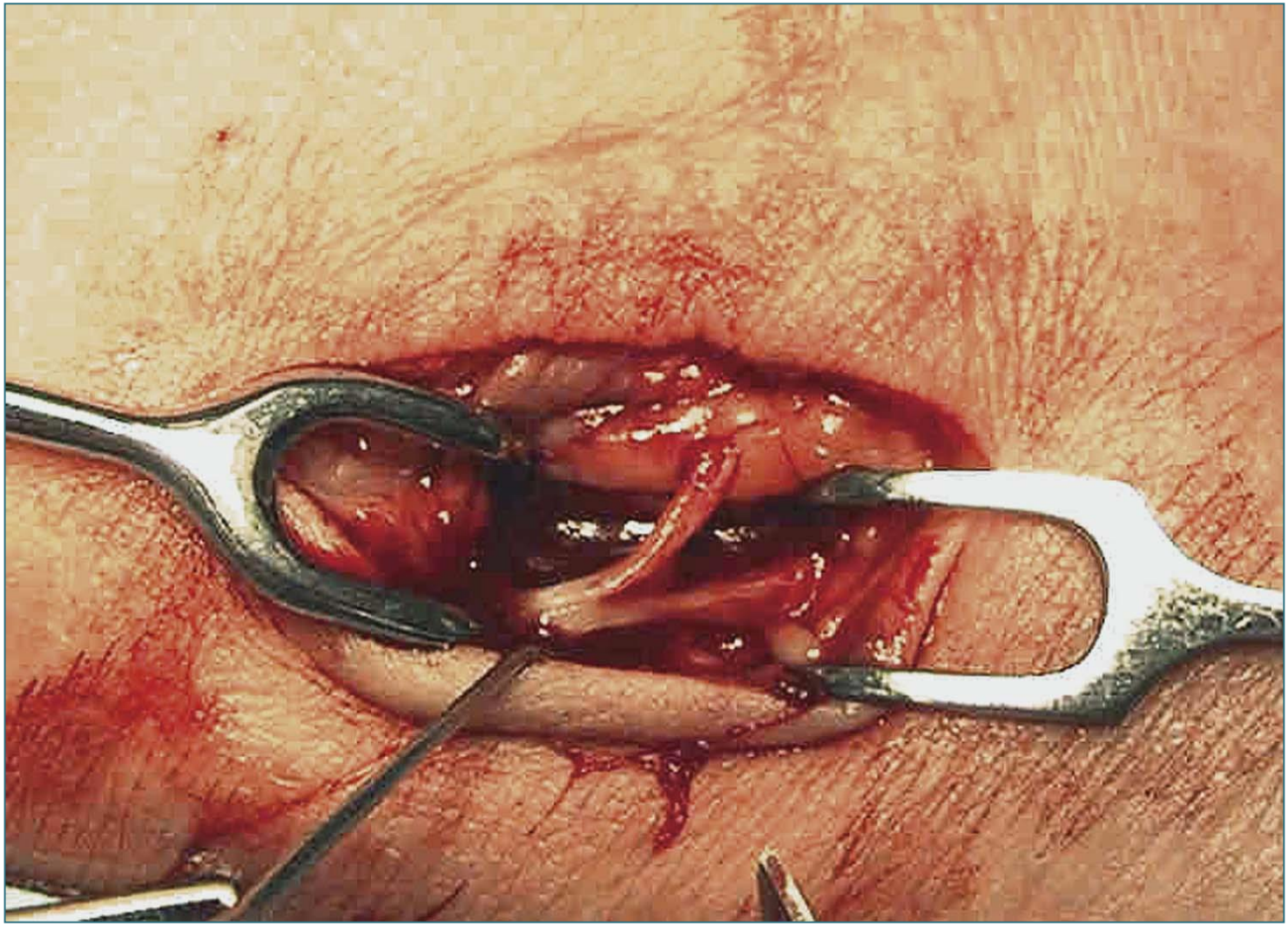


Nerves biopsied



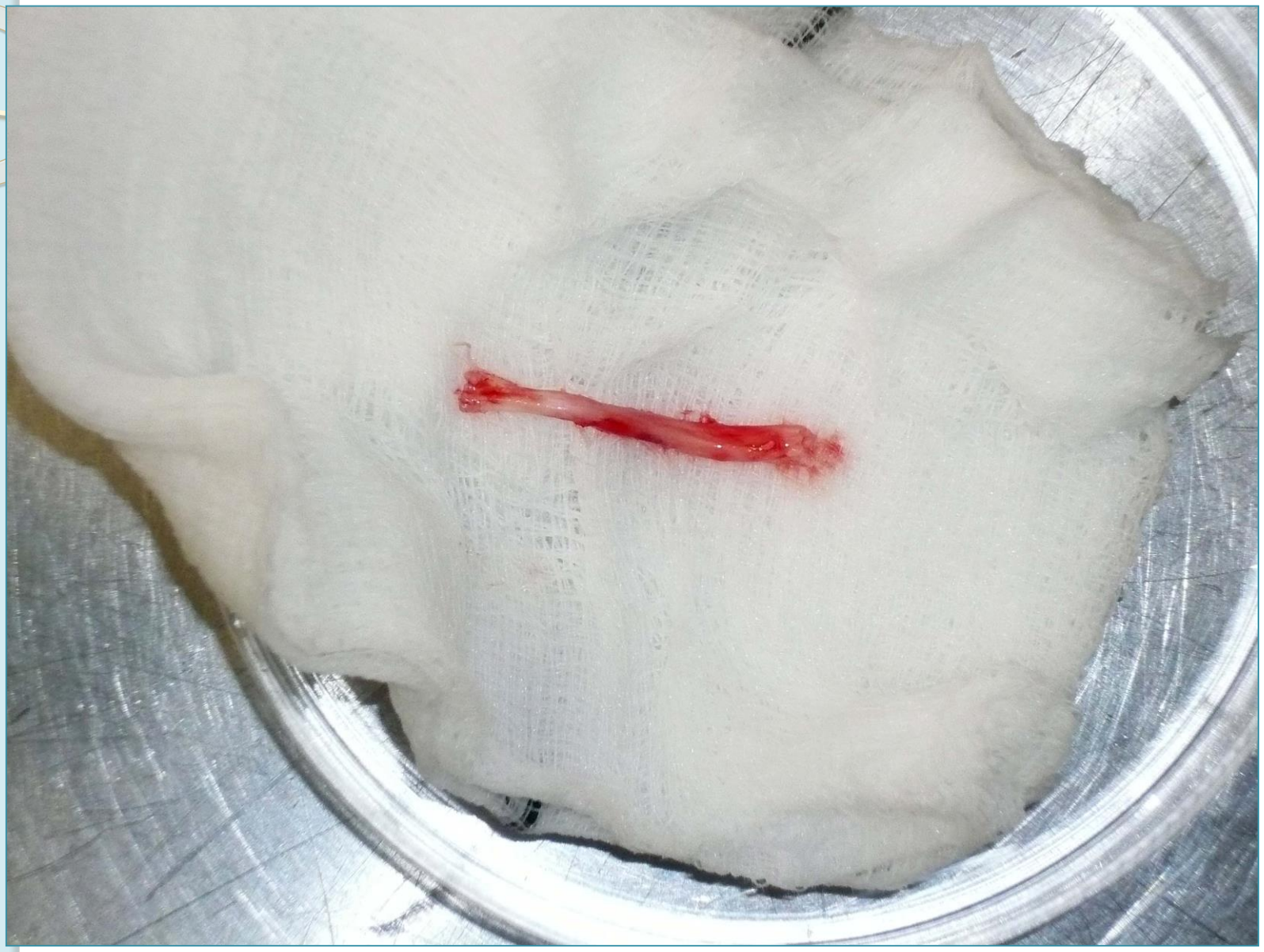


Nerve biopsy

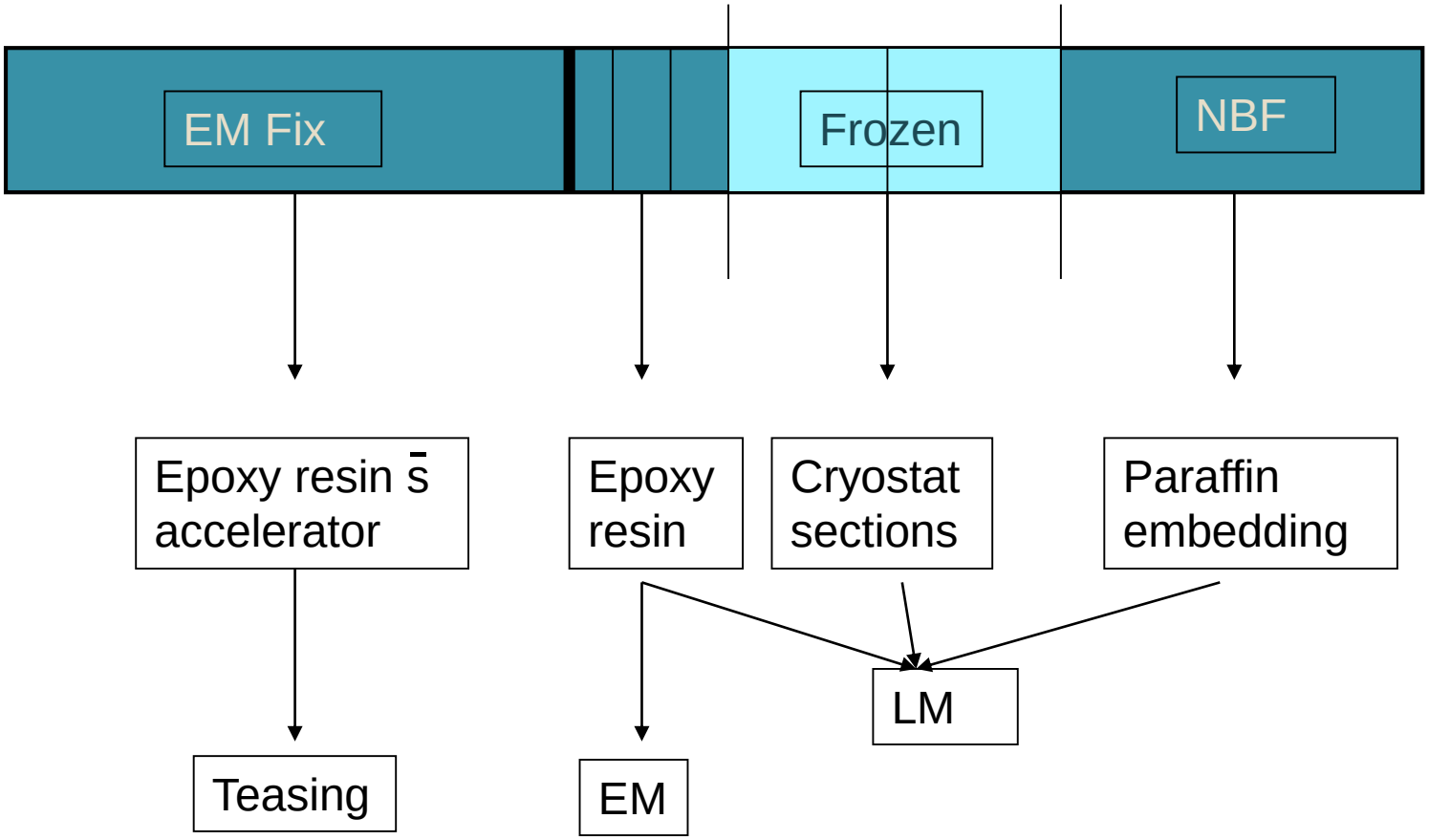


Sural nerve biopsy, at ankle midway between Achilles tendon and lateral malleolus (from Ginsberg L et al. Practical Neurology, 3, 306, 2003)

Nerve biopsy



Cutting specimen: nerve.



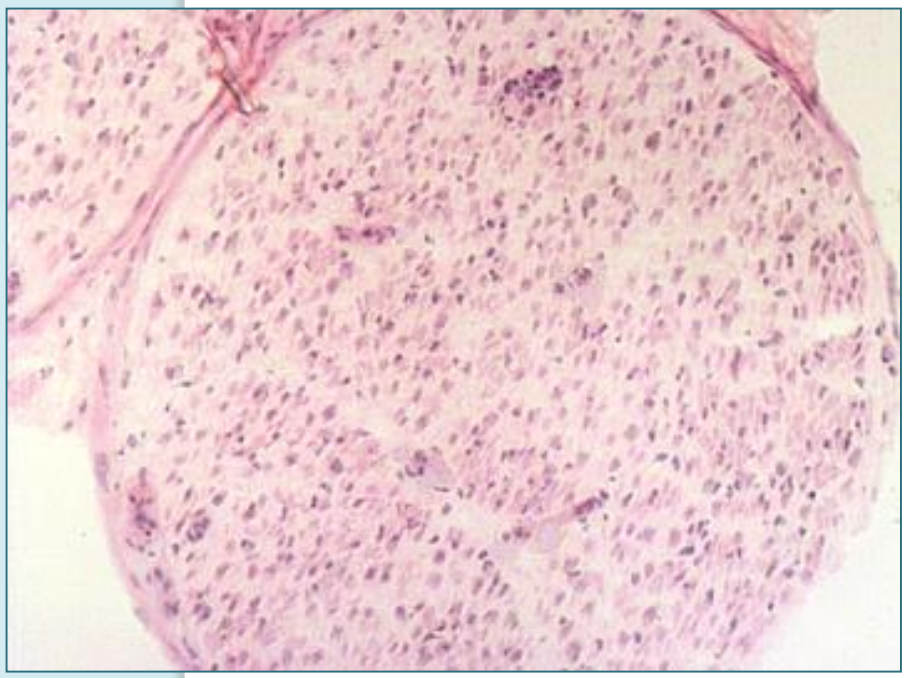
Plan of Campaign

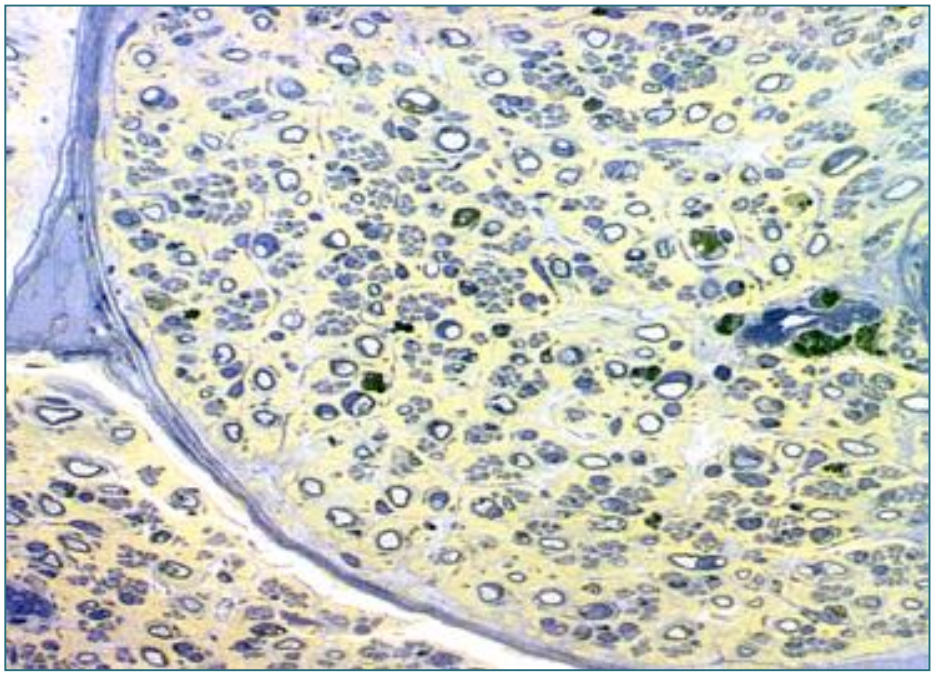
H & E frozen sections,

- Very quick
- Identification of inflammation,
- Myelin difficult to see

Resin sections:

- Several days
- Difficult to identify cells
- Myelin & axons shown





Example: metachromatic leucodystrophy not identifiable on H & E

Recommended Procedure

- **Cut & stain H & E of frozens + Immuno immediately**
- **Cut and stain resin sections for light microscopy as soon as possible**
- **Cut & stain thin sections for EM**
- Do additional immuno after reviewing immuno & resin sections

- Make **teased nerve preparations** if:-
- epoxy shows thin myelin (regeneration or remyelination?)
- epoxy shows possible axonal atrophy
- epoxy shows possible tomaculous changes

- Send **DNA for genetic studies** if indicated

- **Morphometry** of resin section

For best resin processing:-

Glutaraldehyde → Freshly diluted, best quality

Buffer? →
- PO_4 --- May produce EM artefact
- PIPES (piperazine-N-N'-bis (2-ethane sulfonic acid) -- BEST
- Na Cacodylate - Toxic

OsO_4 →
- + 1.5% $K_3Fe(CN)_6.3H_2O$
- + 3% $NaIO_3$

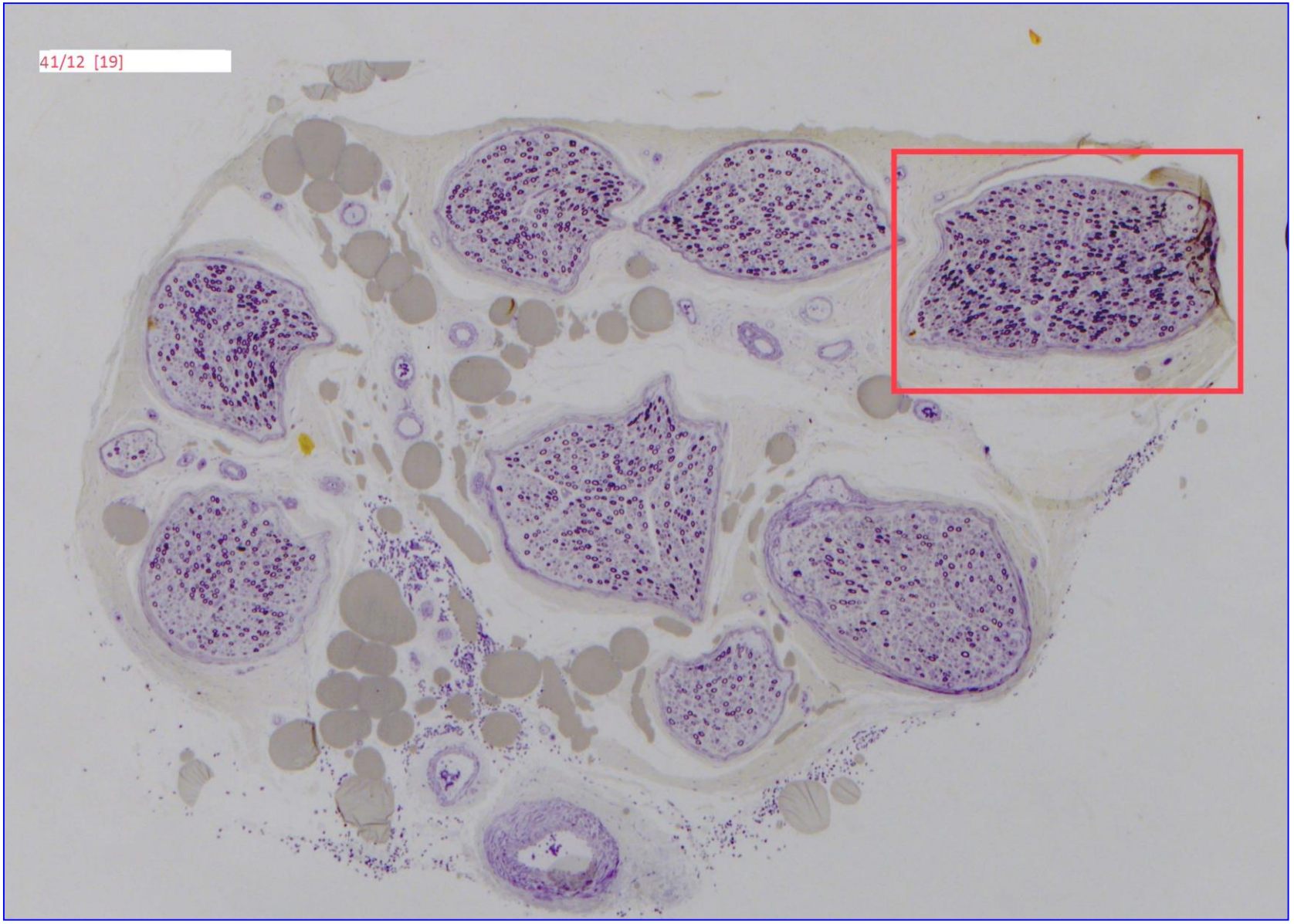
} O/N

Dehydration → Use Dried ethanol

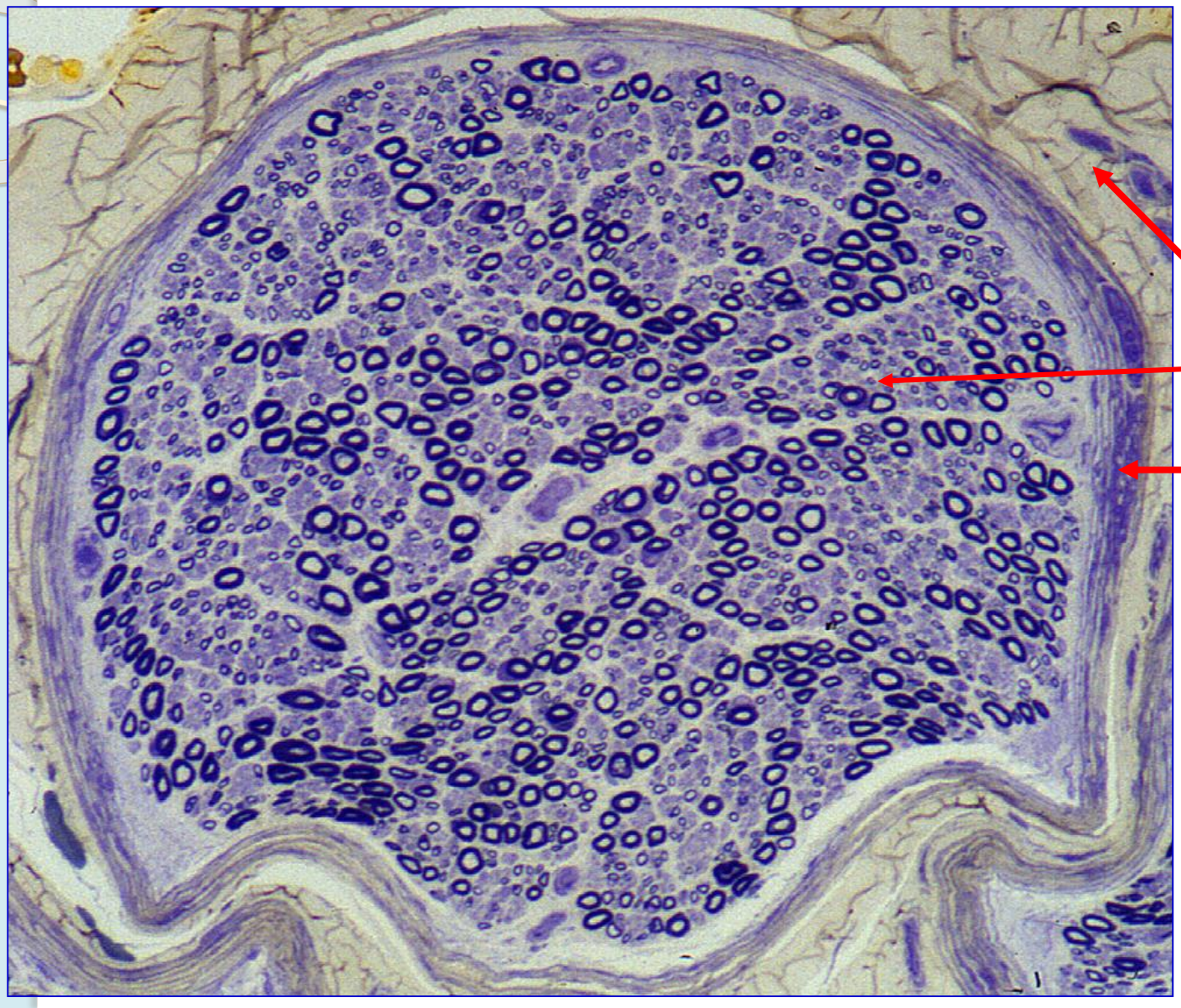
Suggested schedule

2.5% glutaraldehyde/PIPES	**3hr to overnight.**
Wash in PIPES buffer + 2% sucrose	30min
1%OsO_4/PIPES (+1.5%$K_3Fe(CN)_6 3H_2O$ +3%$NaIO_3$)	**3h – overnight**
Dehydrate :-	
15% ethanol	2 x 5 min.
30% ethanol	2 x 10 min.
50% ethanol	2 x 15 min.
70% ethanol	2 x 30 min.
dehydrated absolute ethanol	**3 x 20 min.**
dehydrated absolute ethanol	**2 x 60 min.**
1,2 epoxy propane	rinse
1,2 epoxy propane	2 x 15 min.
epoxy resin +1,2 epoxy propane, 1:1	1 h
epoxy resin +1,2 epoxy propane, 3:1	3 h – overnight
epoxy resin	overnight - 24 h
embed in epoxy resin at 65ºC	at least 24 h.

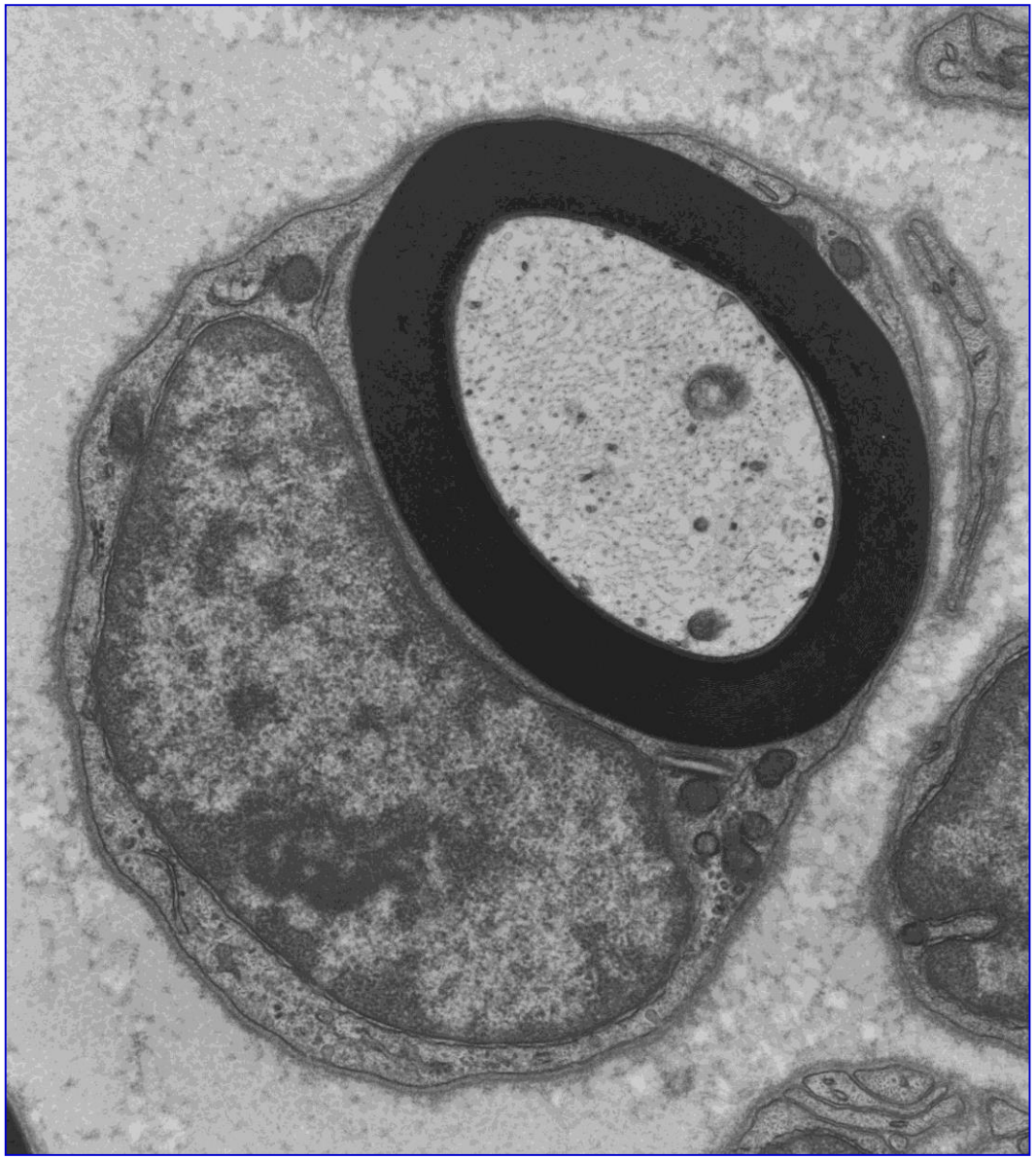
Trimming for EM



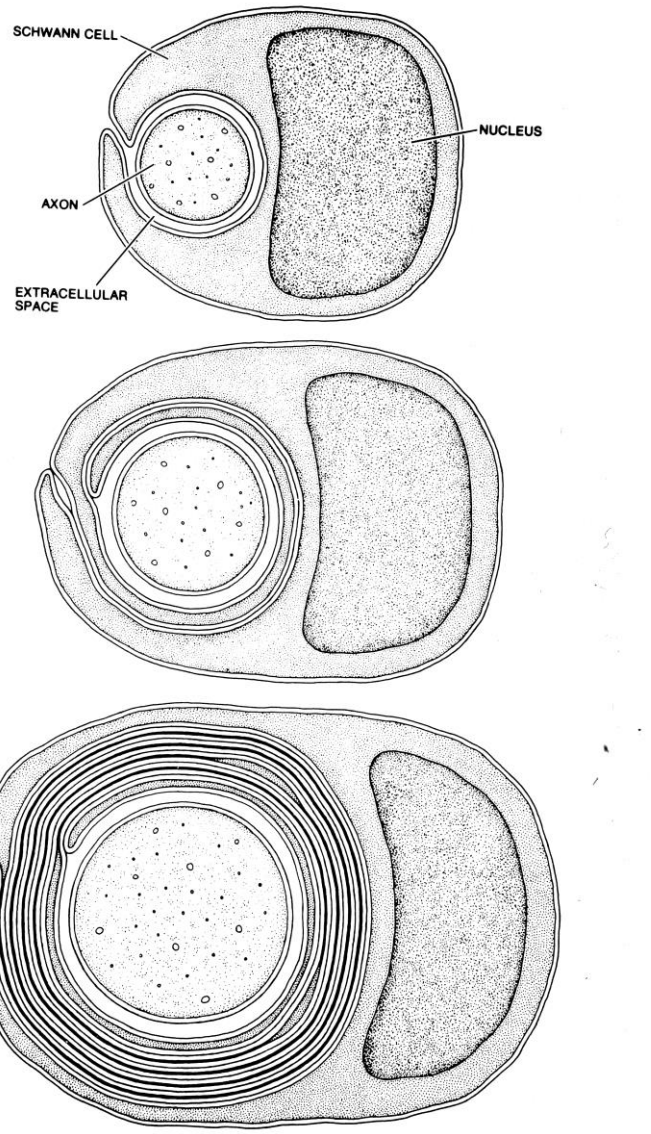
Nerve Structure

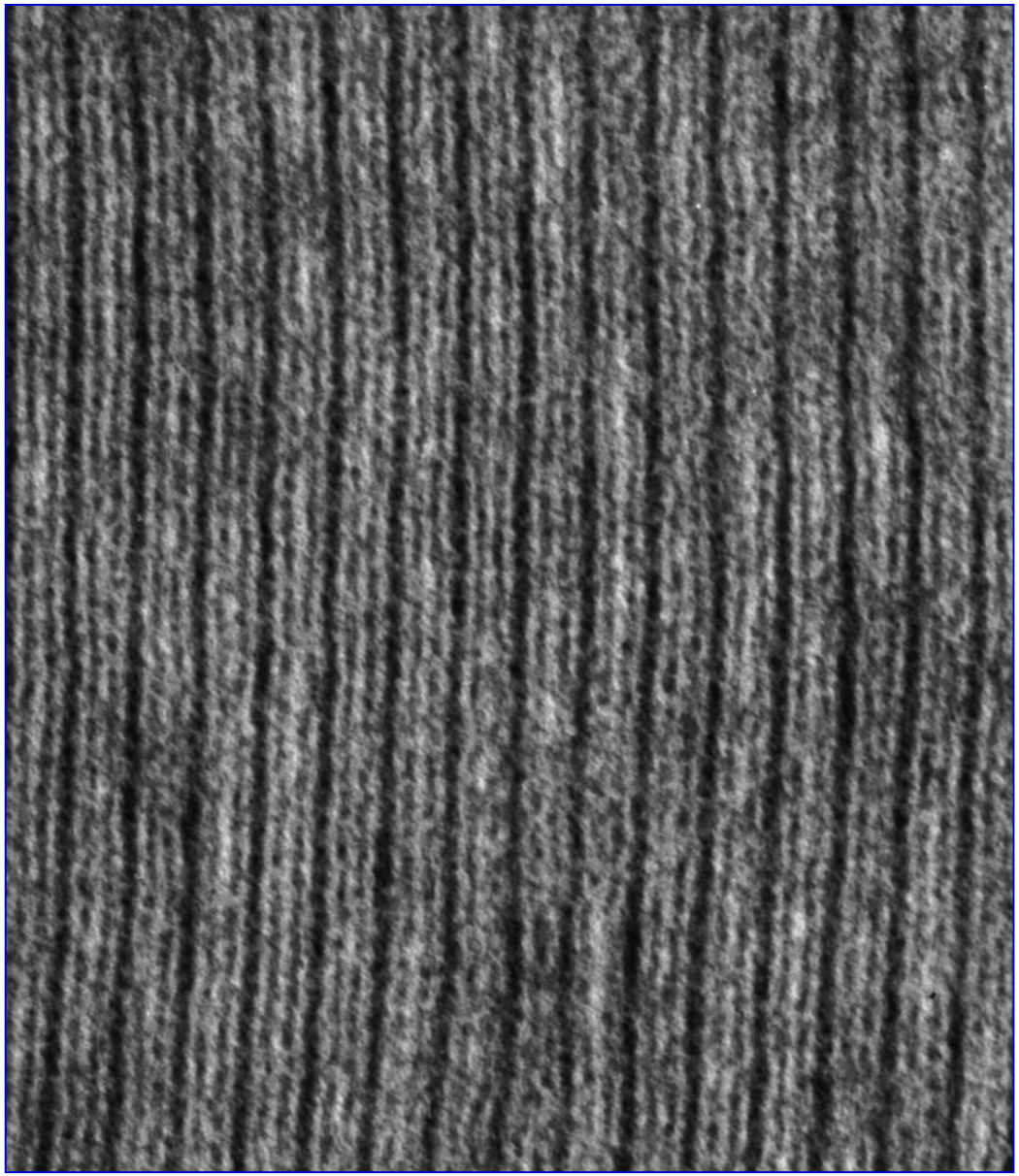


Normal Myelinated Nerve Fibre

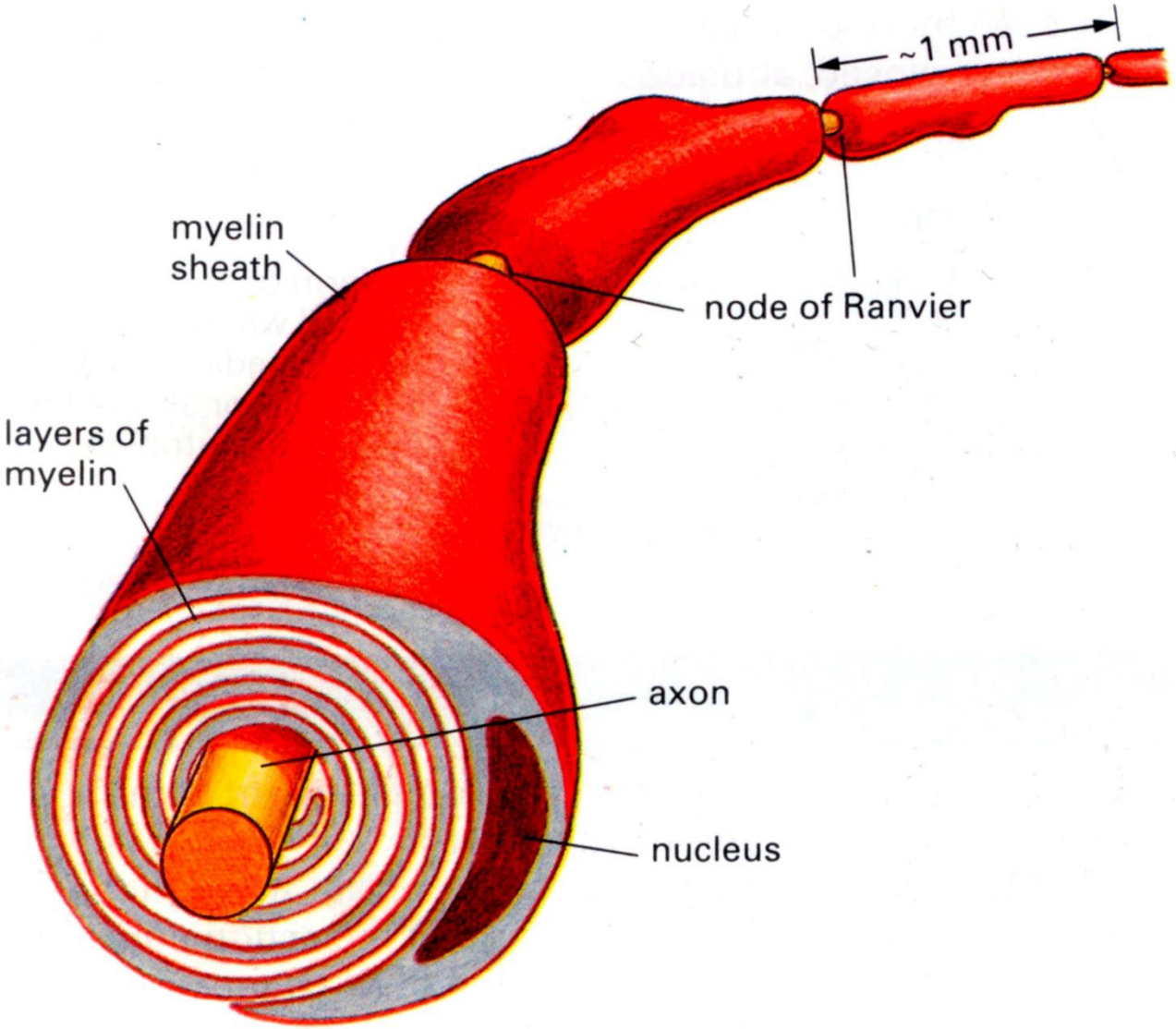


Nerve Structure

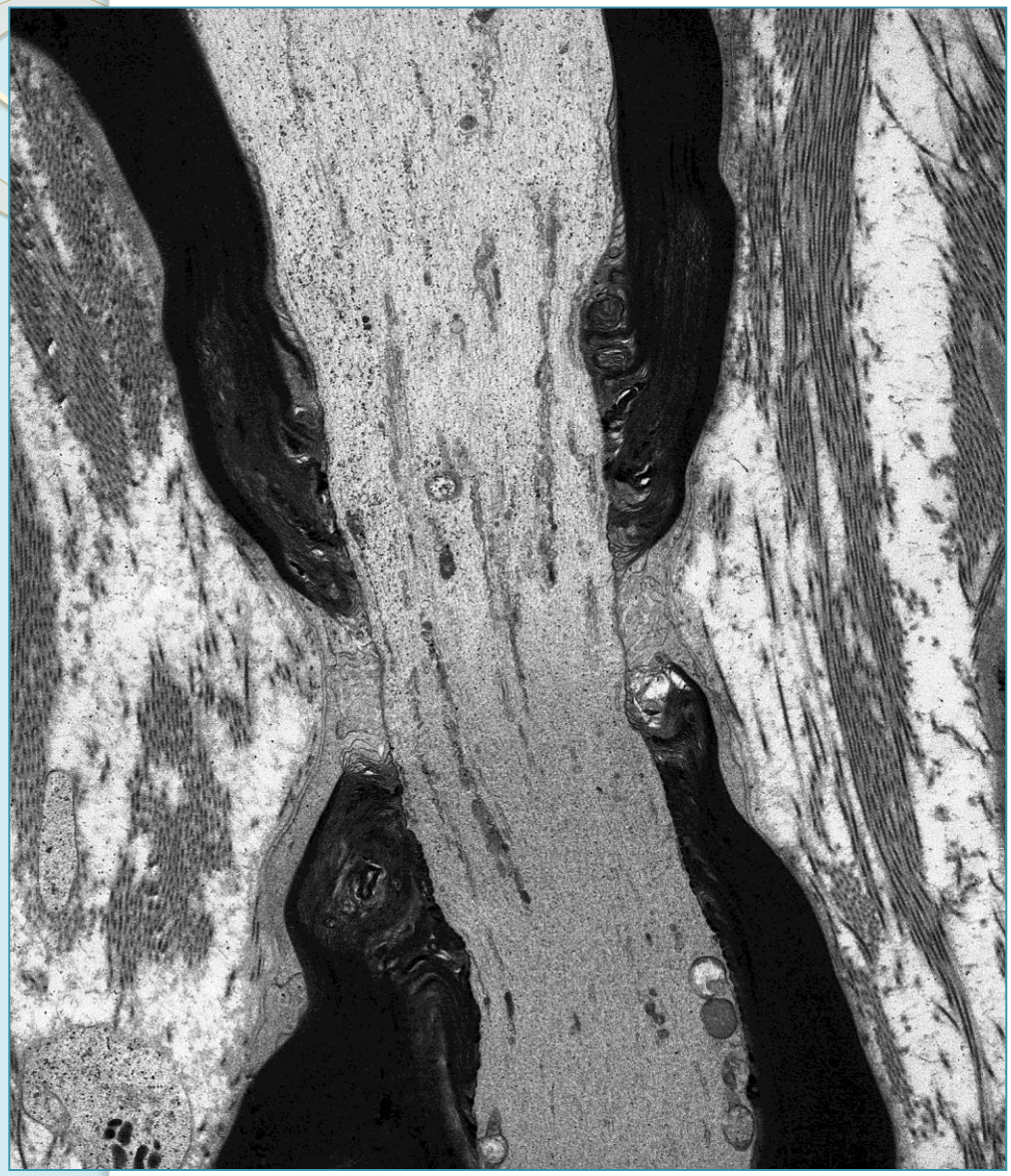


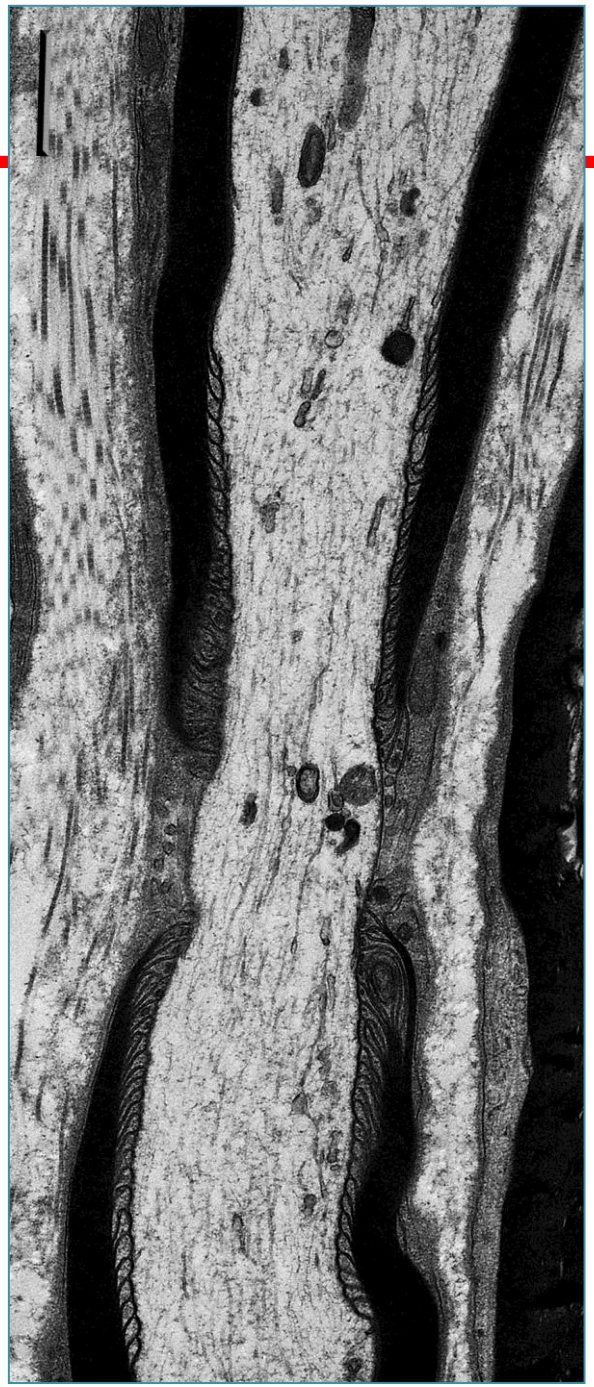


Myelin Structure, 3d

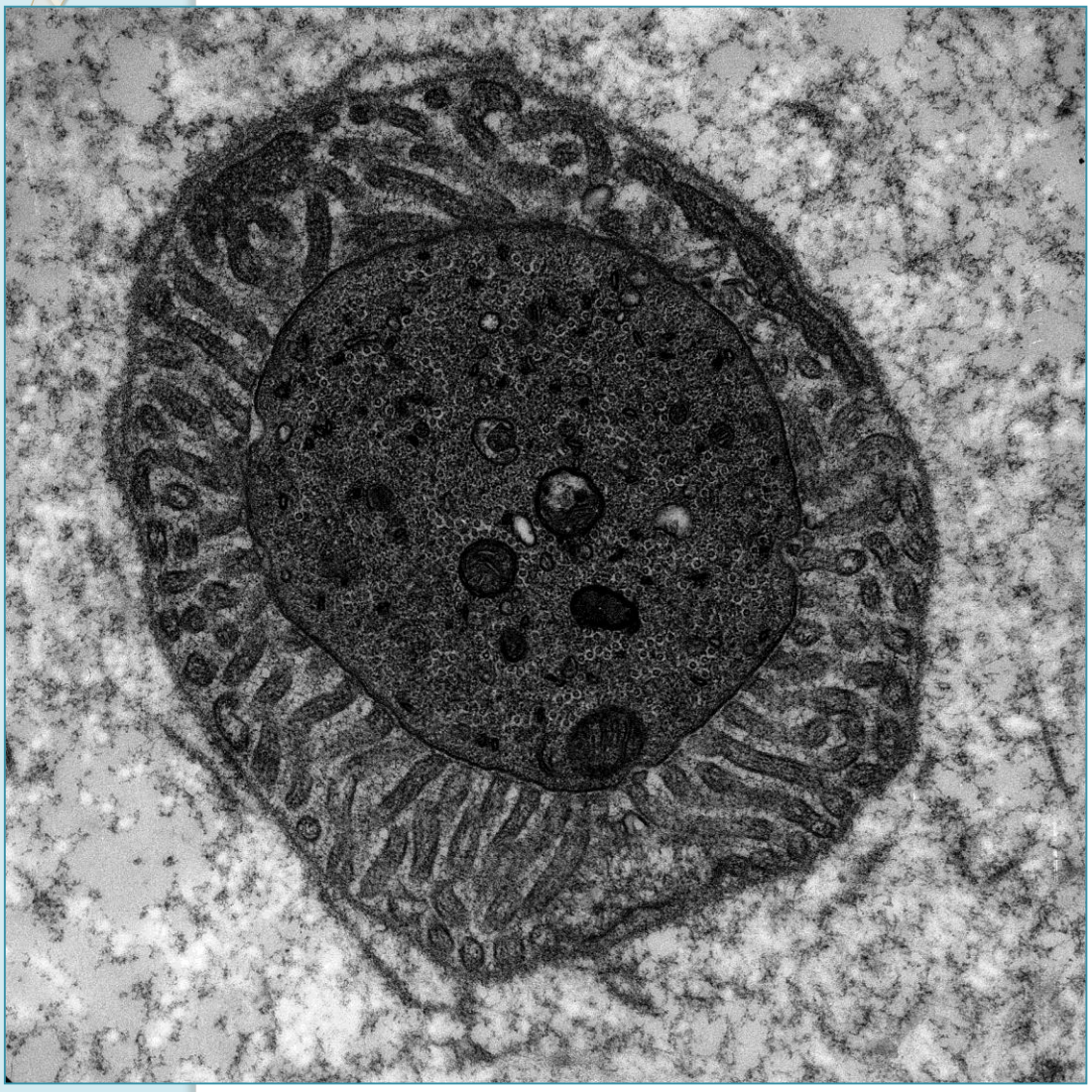


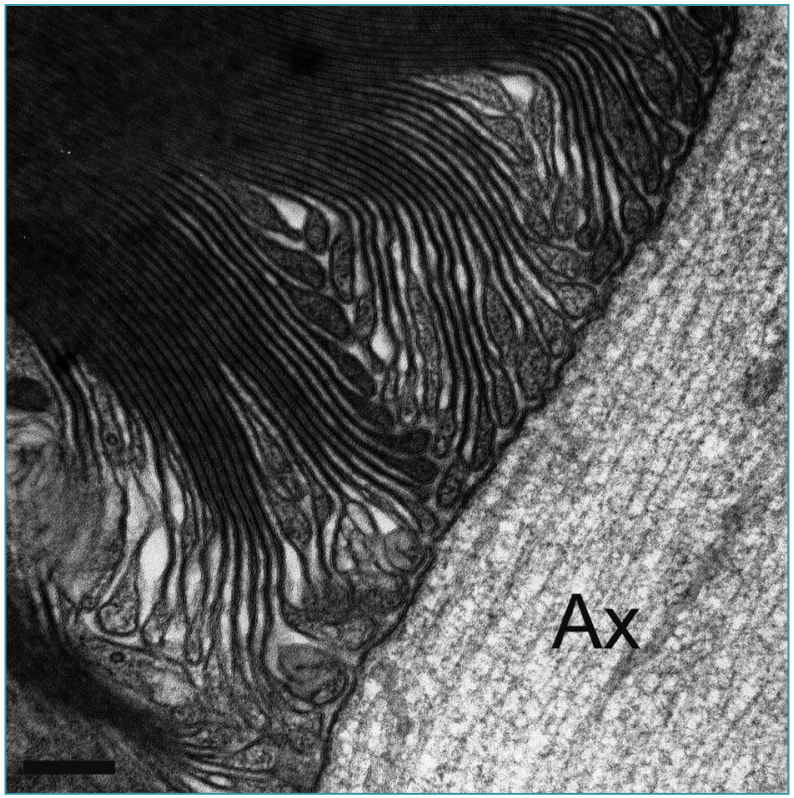
Node of Ranvier



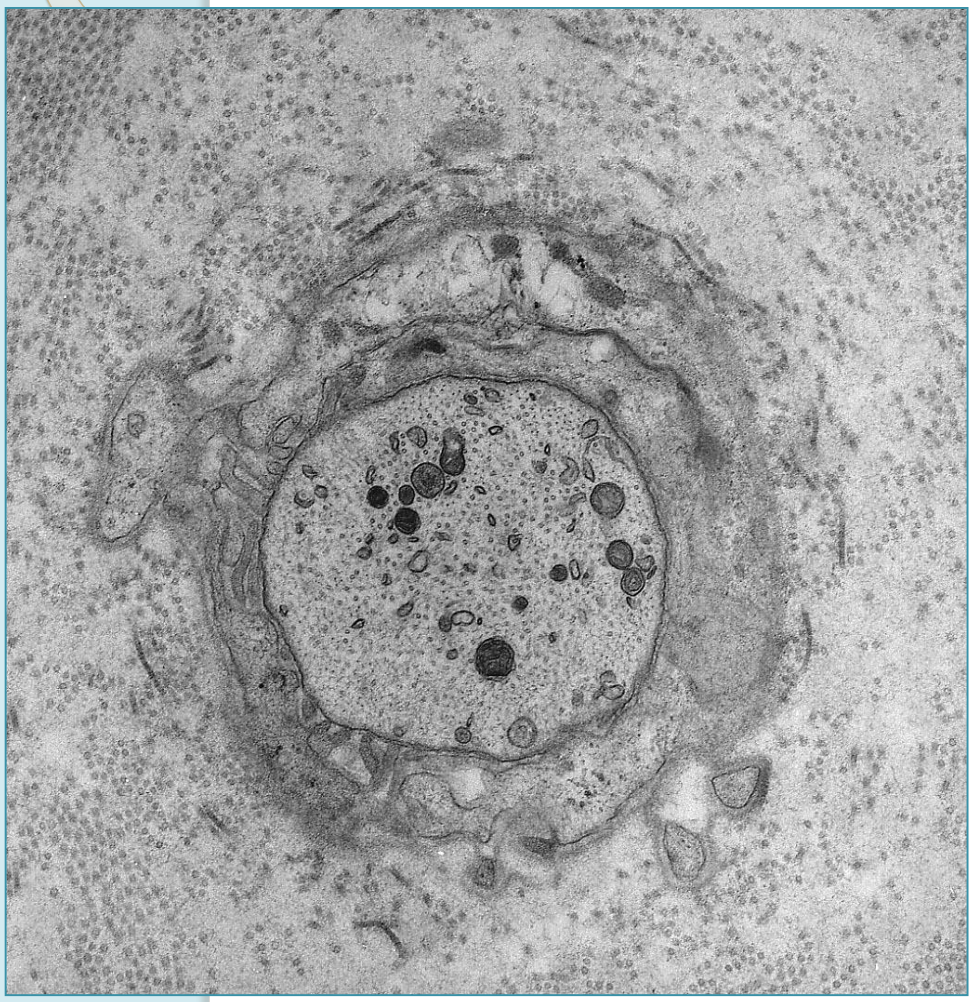


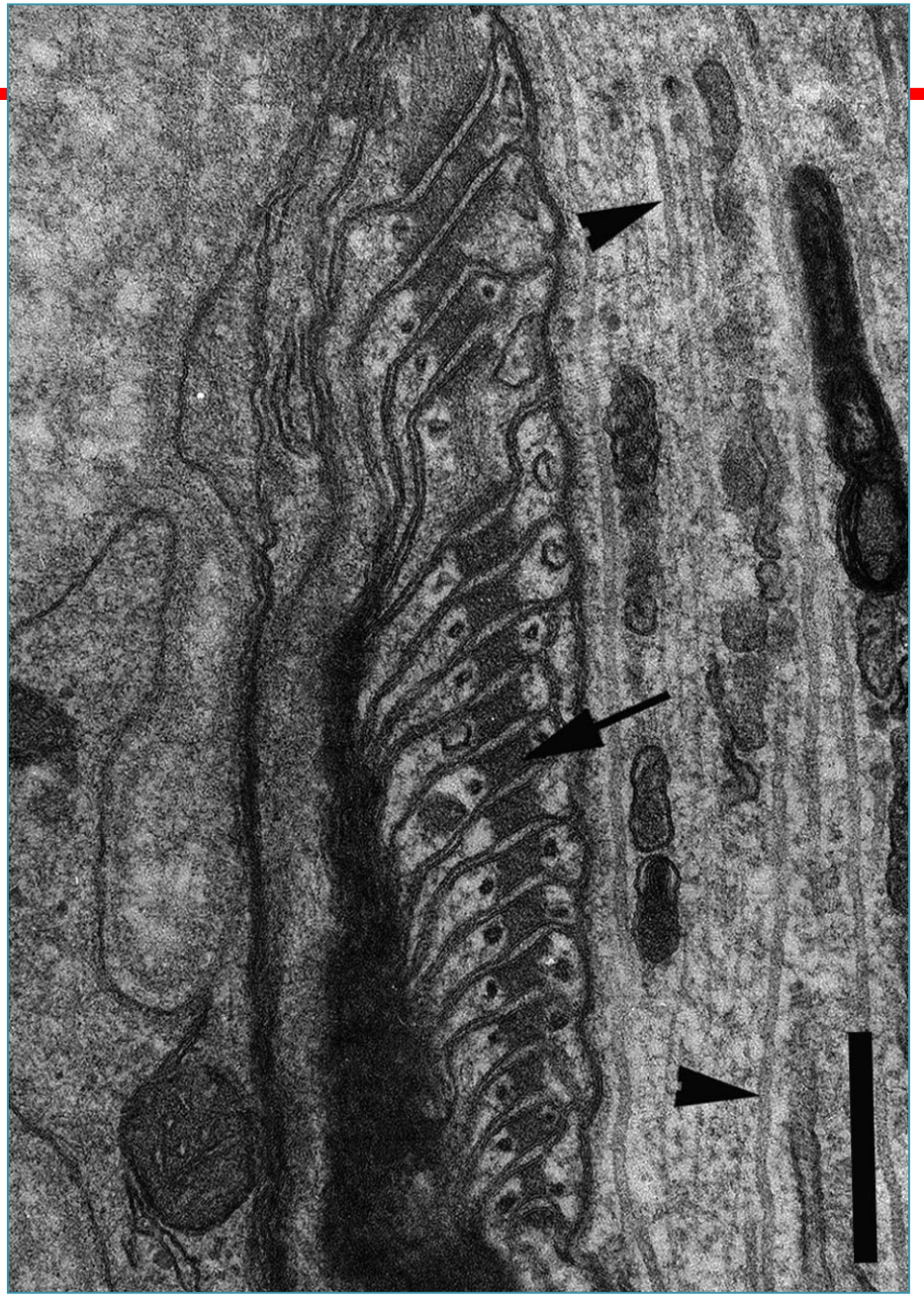
Node of Ranvier



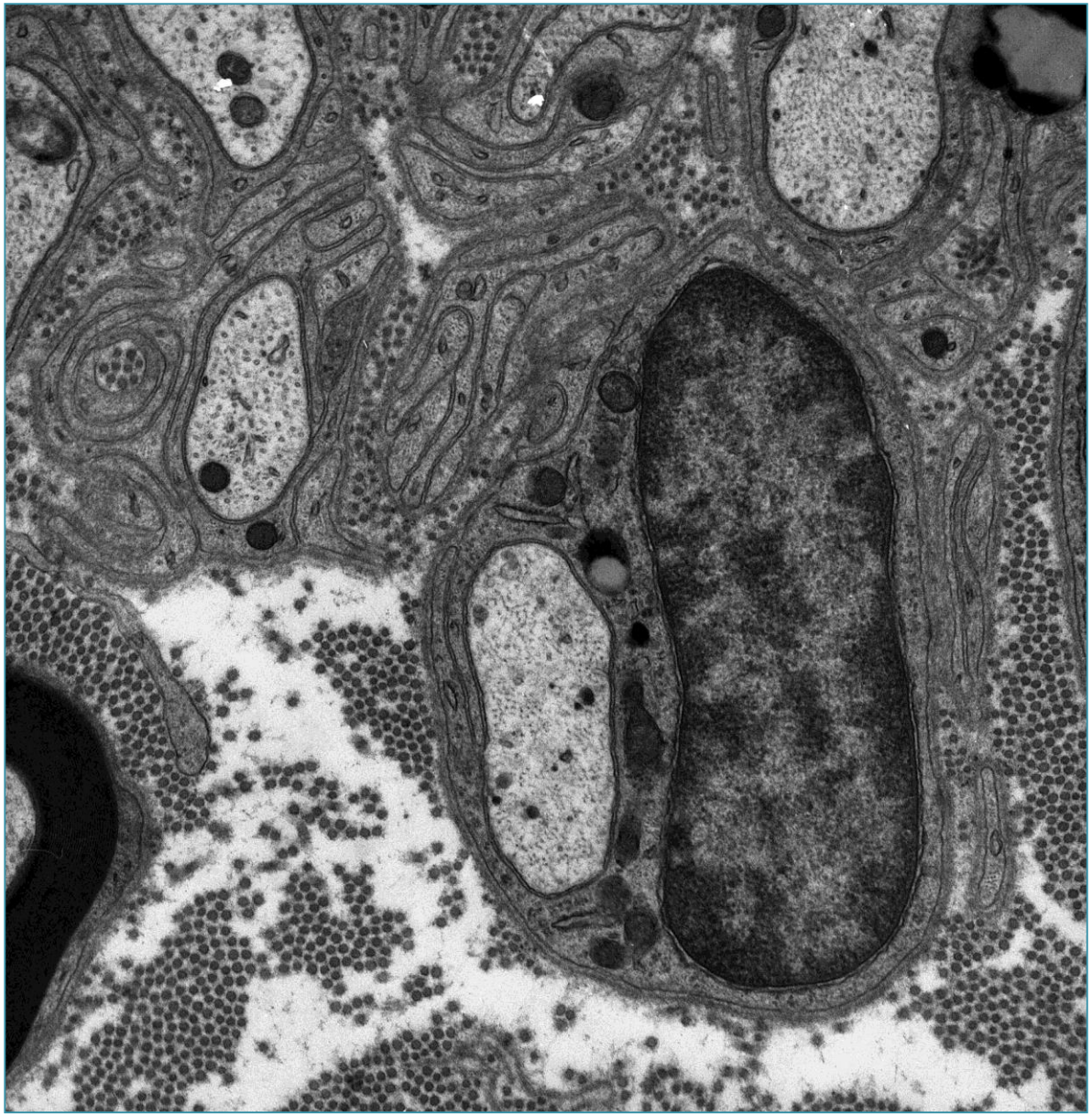


Node of Ranvier

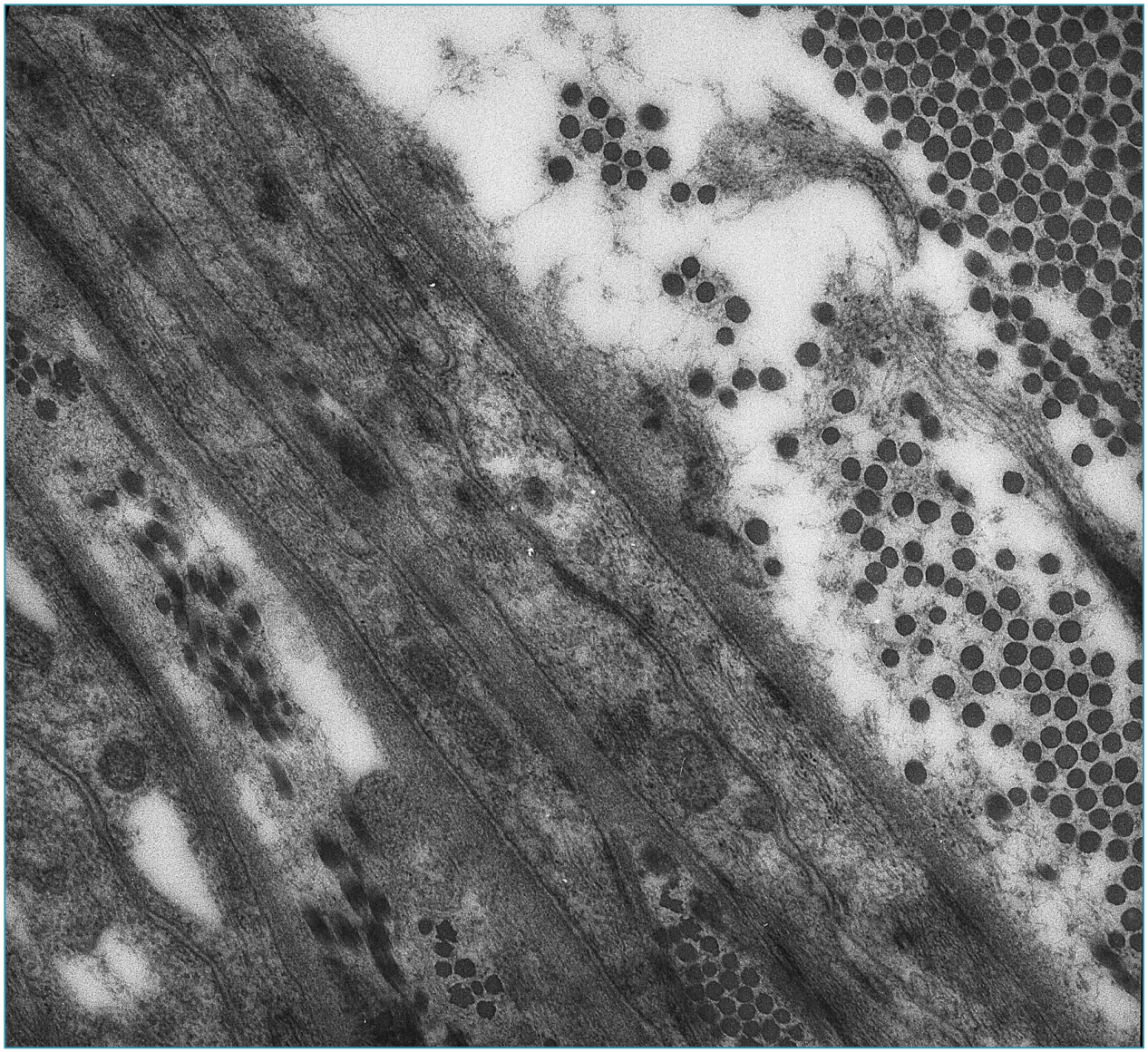




Remak fibres



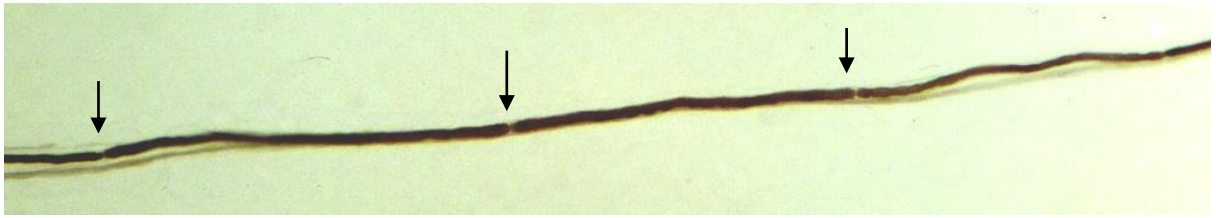
Perineurium



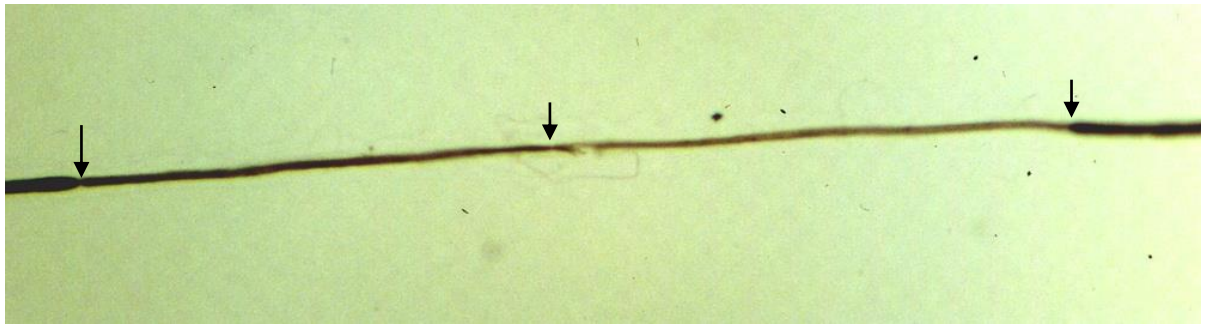
Teased Nerve Fibres

Axonal degeneration

Axonal regeneration



Remyelination



Teasing Schedule

- As before but:

- Don't add $K_3Fe(CN)_6 3H_2O$ to OsO_4
- Use Toluine instead of propylene oxide
- Do NOT add accelerator to the resin mix

- Store samples in fridge or freezer
- For teasing use finest needles obtainable
- Gently pull perineurium apart
- Separate into smaller bundles of fibres
- Separate individual nerve fibres
- Pick up carefully, lay out as straight as possible on a clean slide
- Cover with fresh complete resin mix

Resin Staining

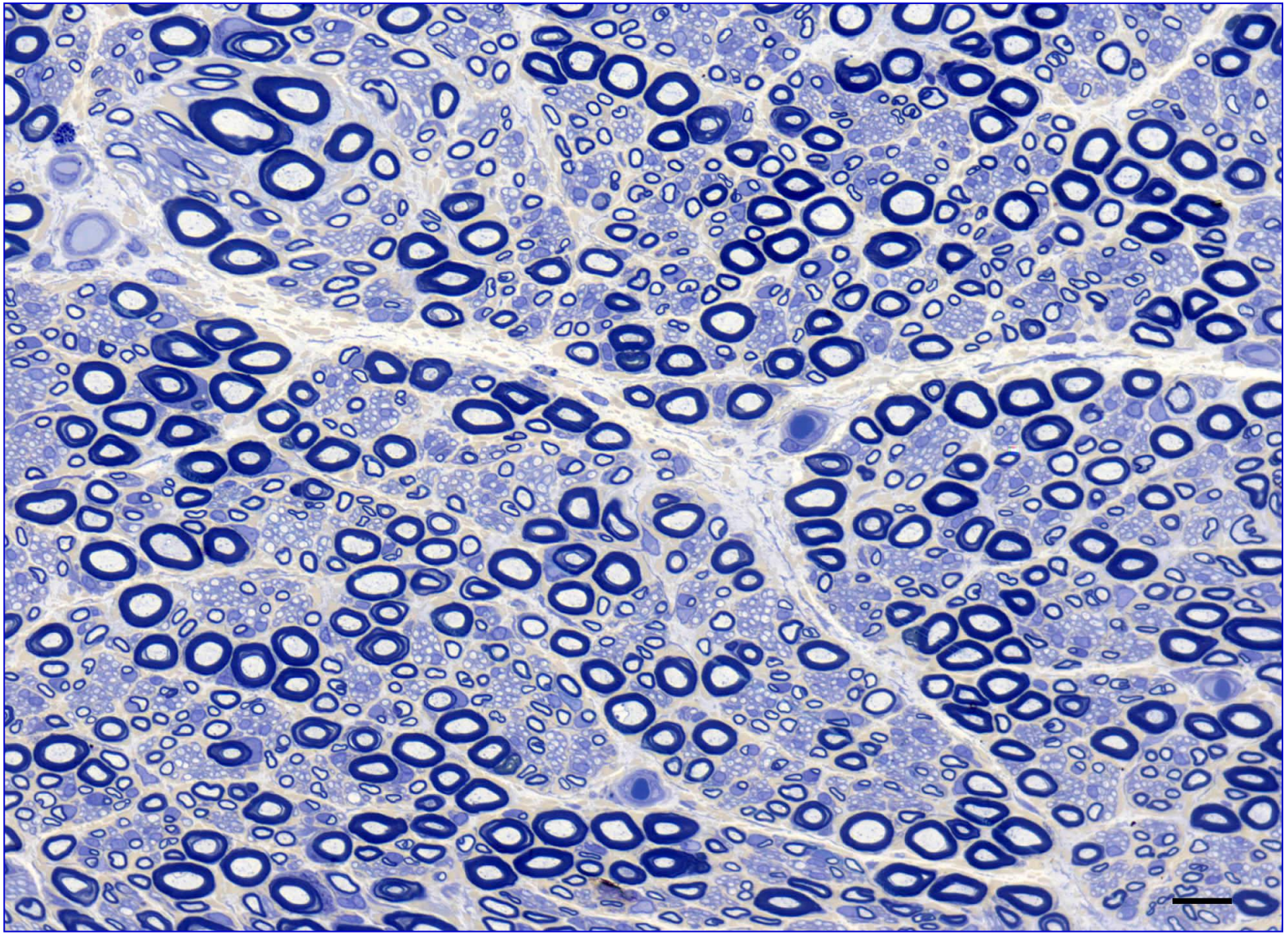
- Thionine & Acridine orange
  - 0.1% thionine in 90% ethanol
  - 1% acridine orange aqueous
    - Both alkaline solutions

- Procedure
  - Sections collected on slide in 10% acetone
  - Thionine placed on, heated, washed off; dried
  - AO placed on, heated, washed off, dried.
  - Coverslipped with epoxy resin
    - Ref. Sievers J. 1971, Stain Technol 46, 195-9

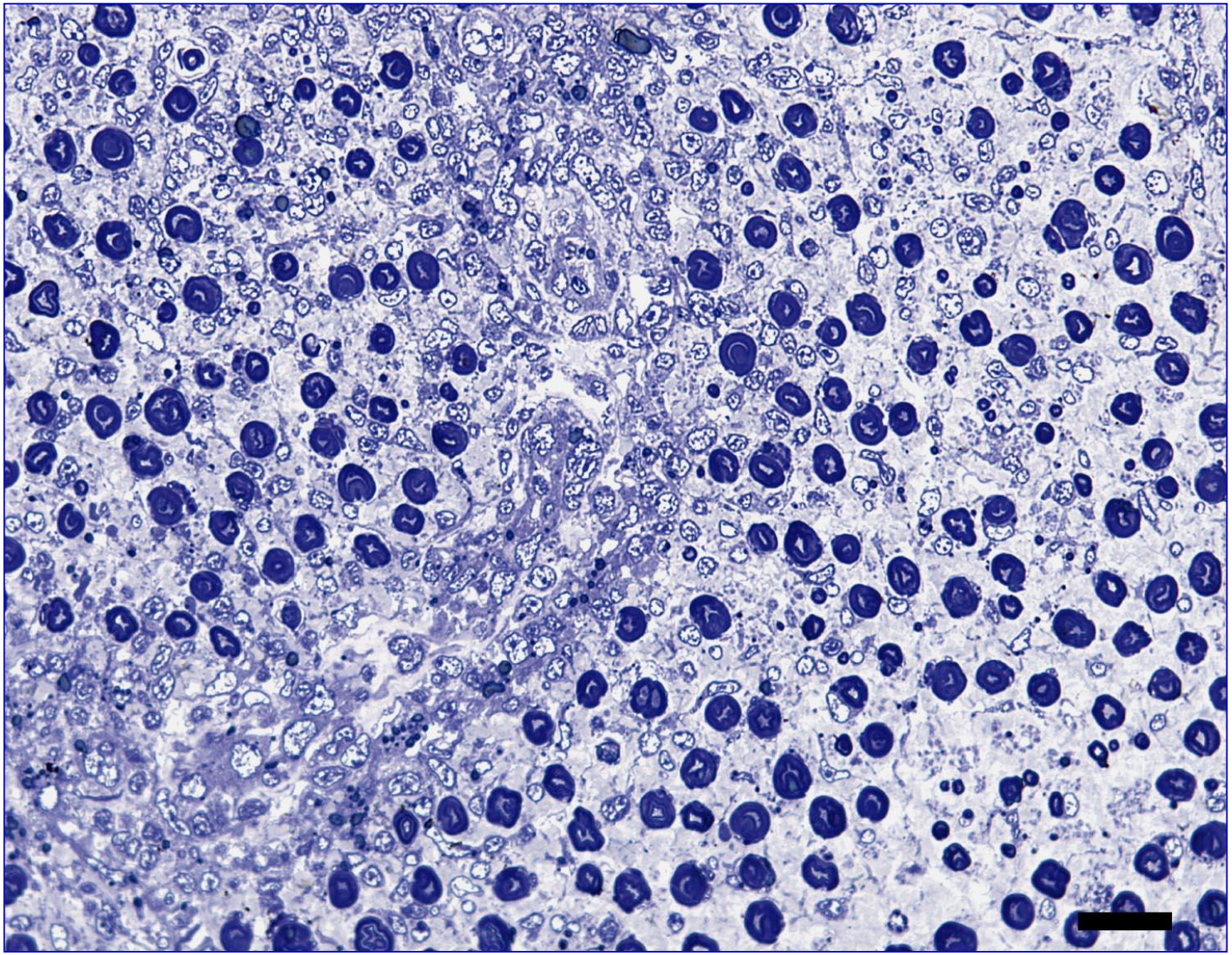
Avoiding Artefacts

- Handle nerve very carefully;
  - Do not stretch, cut carelessly, twist or compress
- Fix immediately
  - Use fresh fix (or keep frozen)
- Process carefully
  - Dehydrate ethanol (Linde sieves)
- Cut perfect sections
- Stain correctly

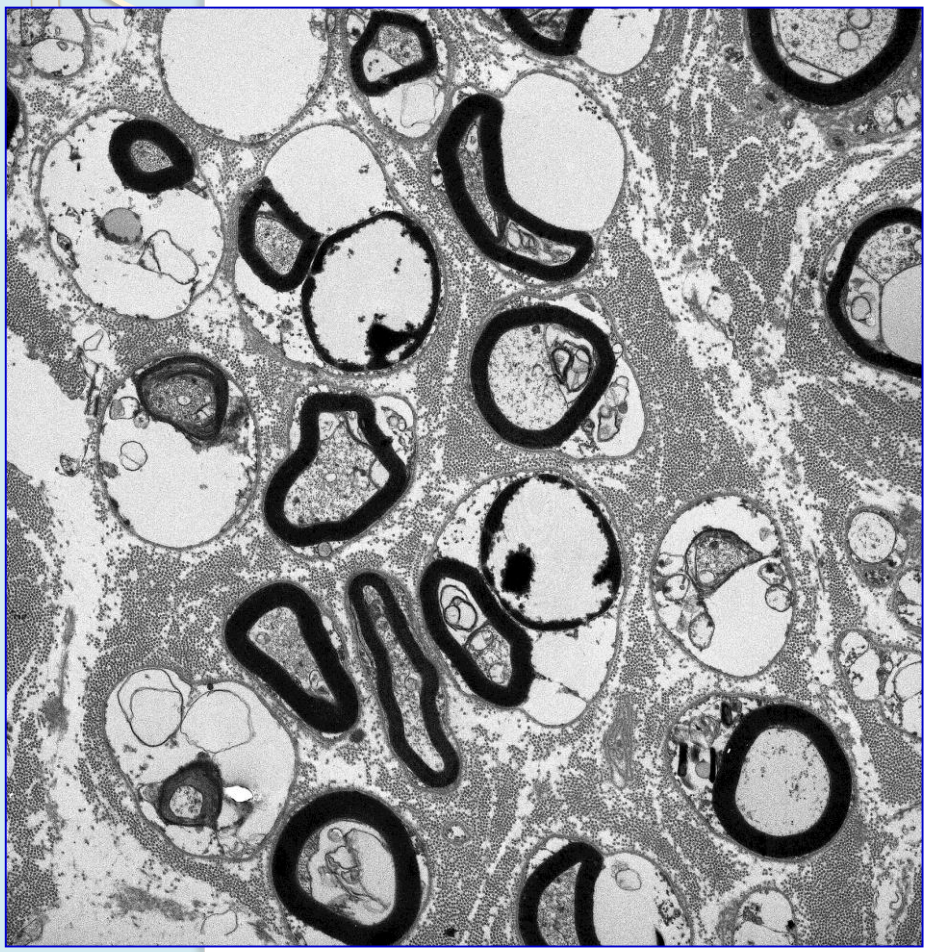
Normal Nerve

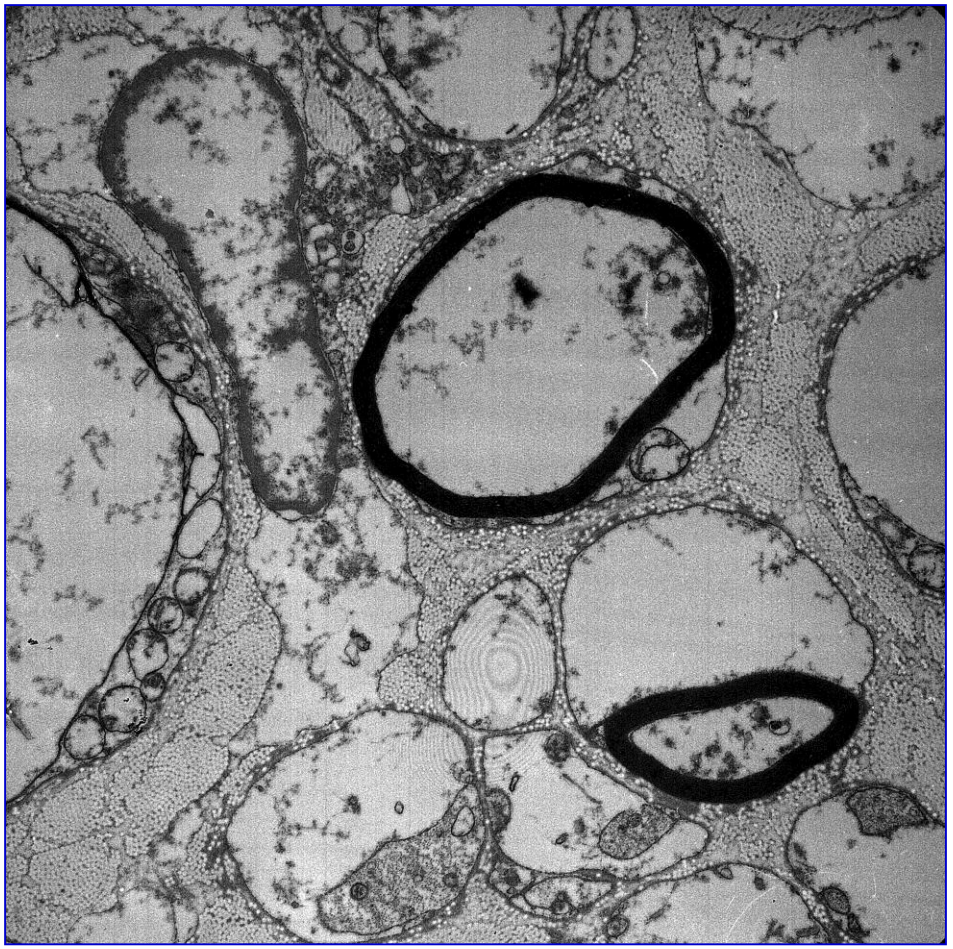


Handling + PM Artefact

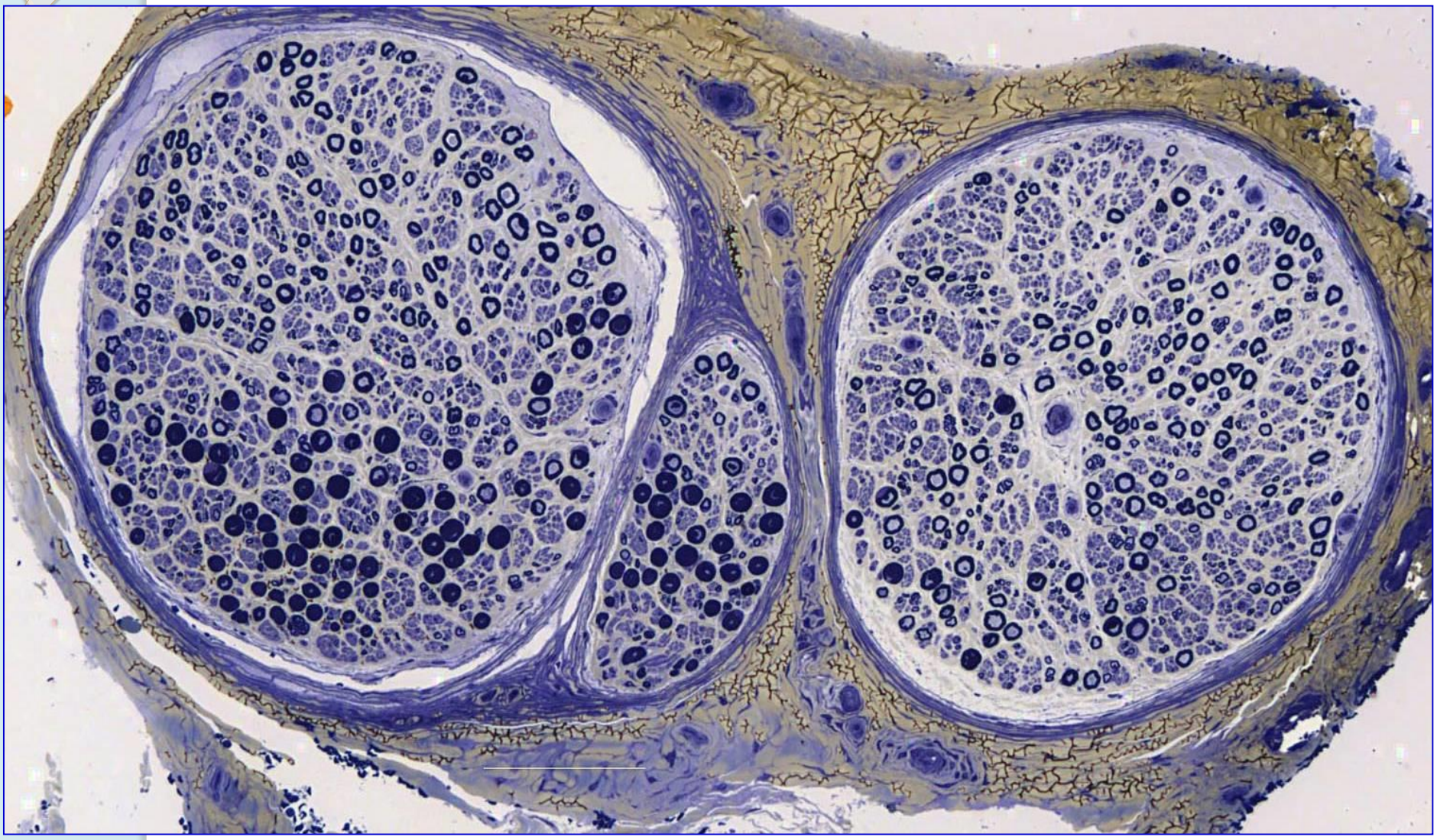


PM Artefact

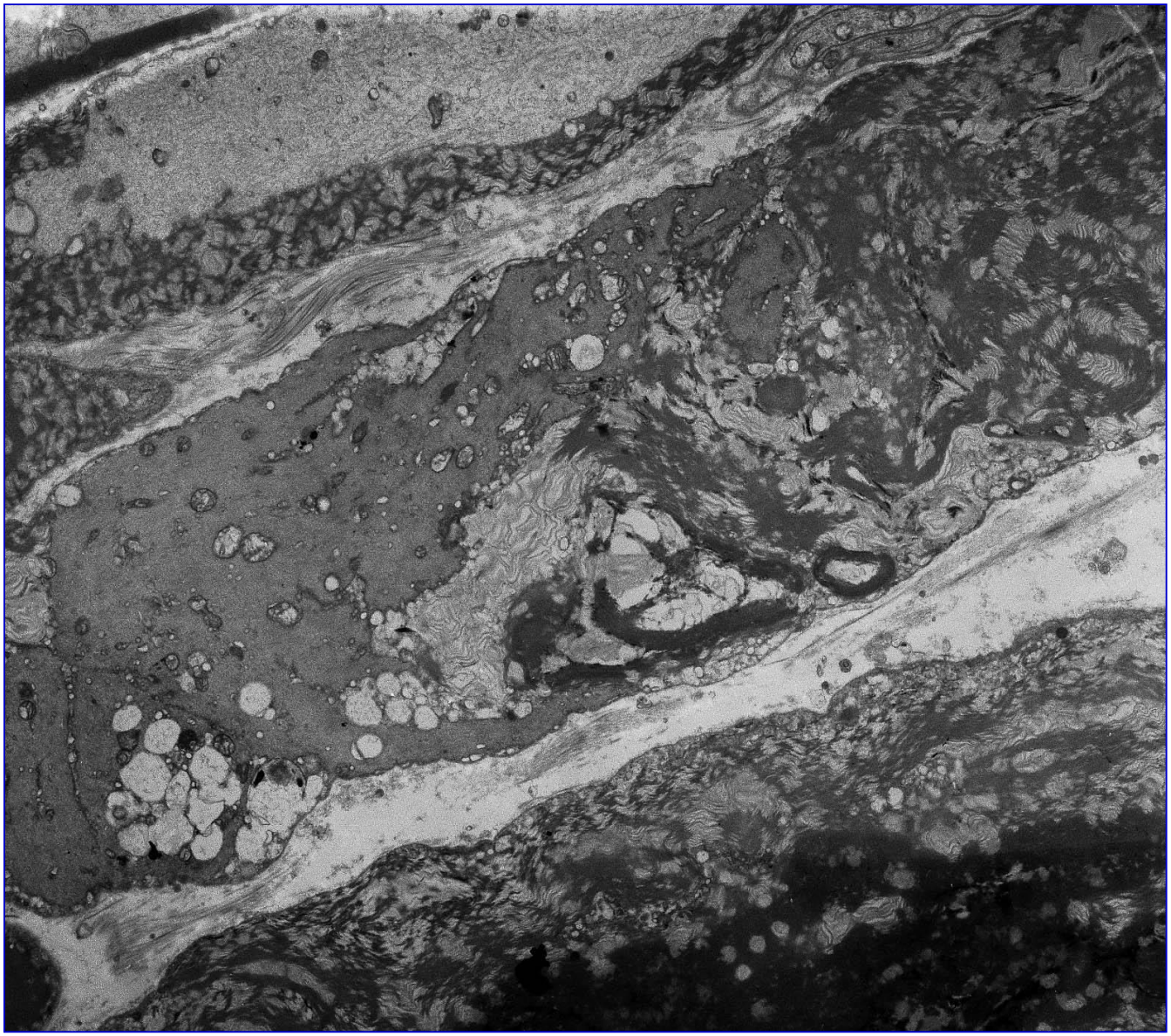




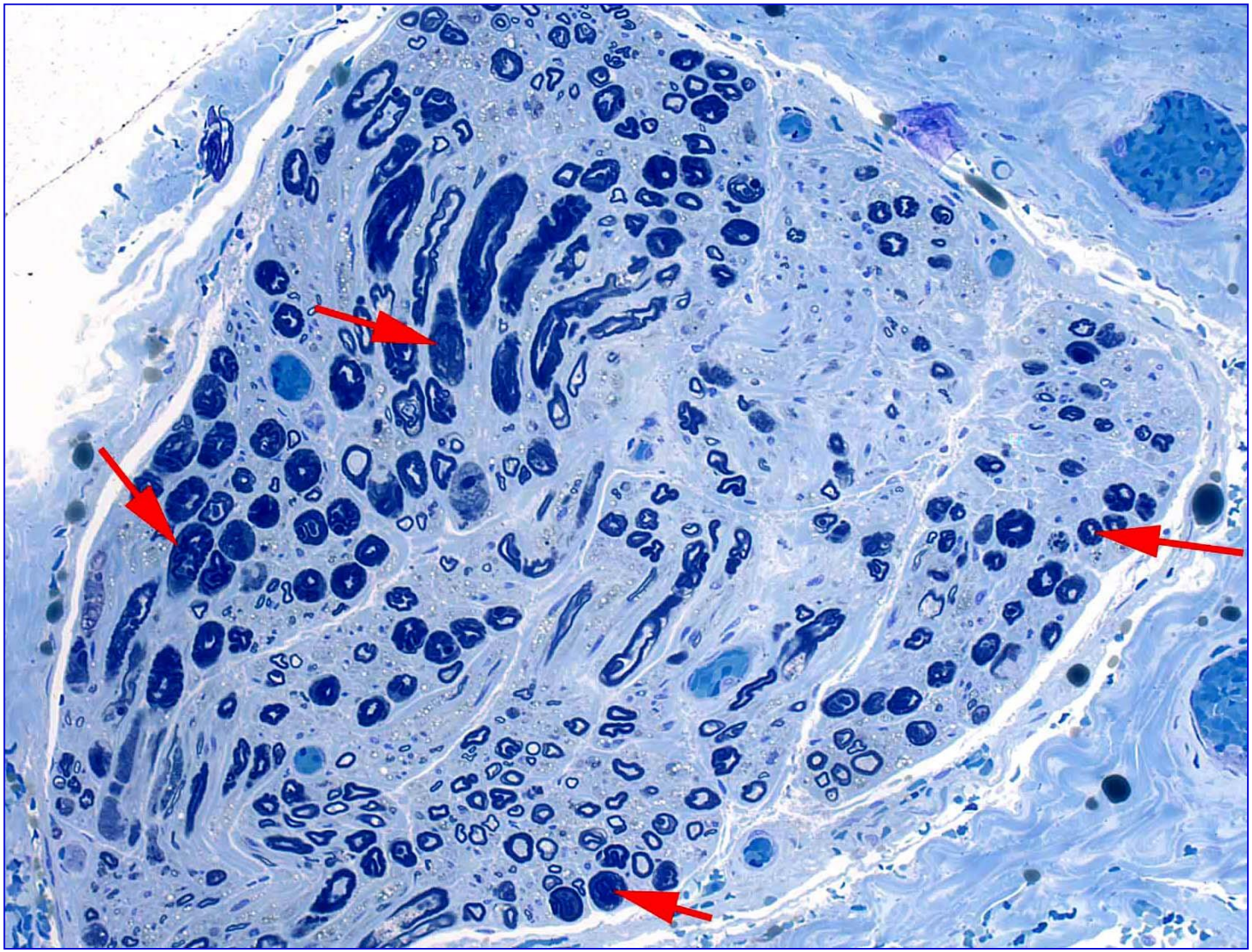
Handling Artefact



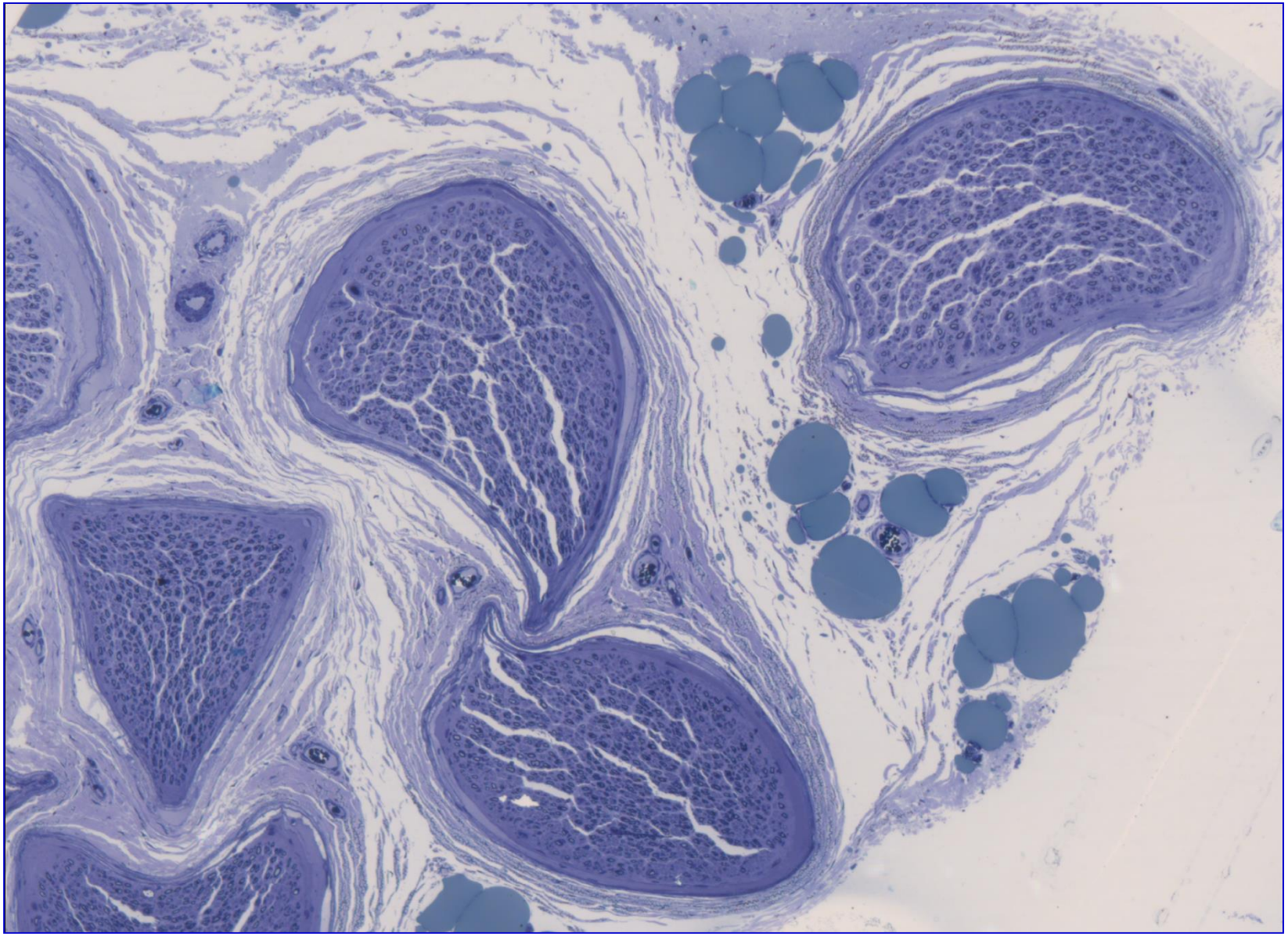
EM Handling Artefact



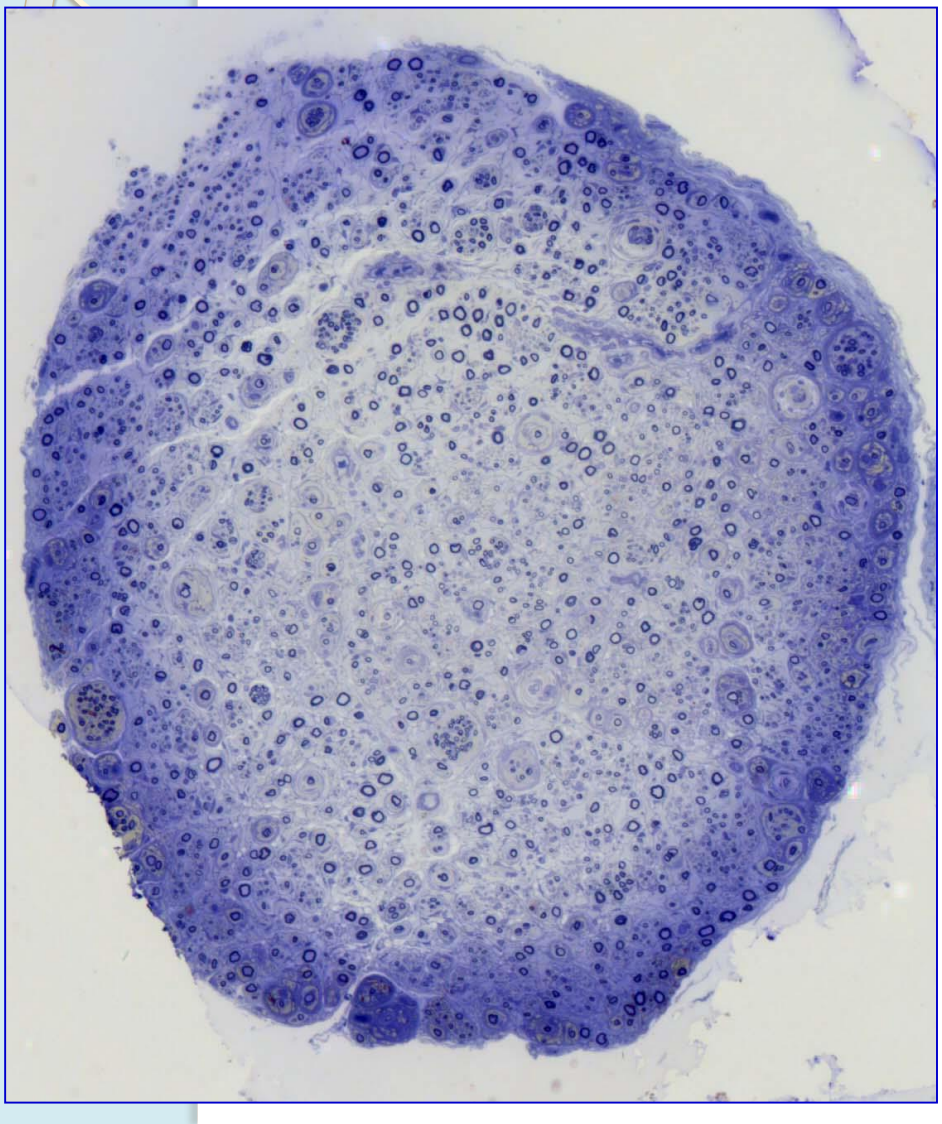
Handling + poor staining

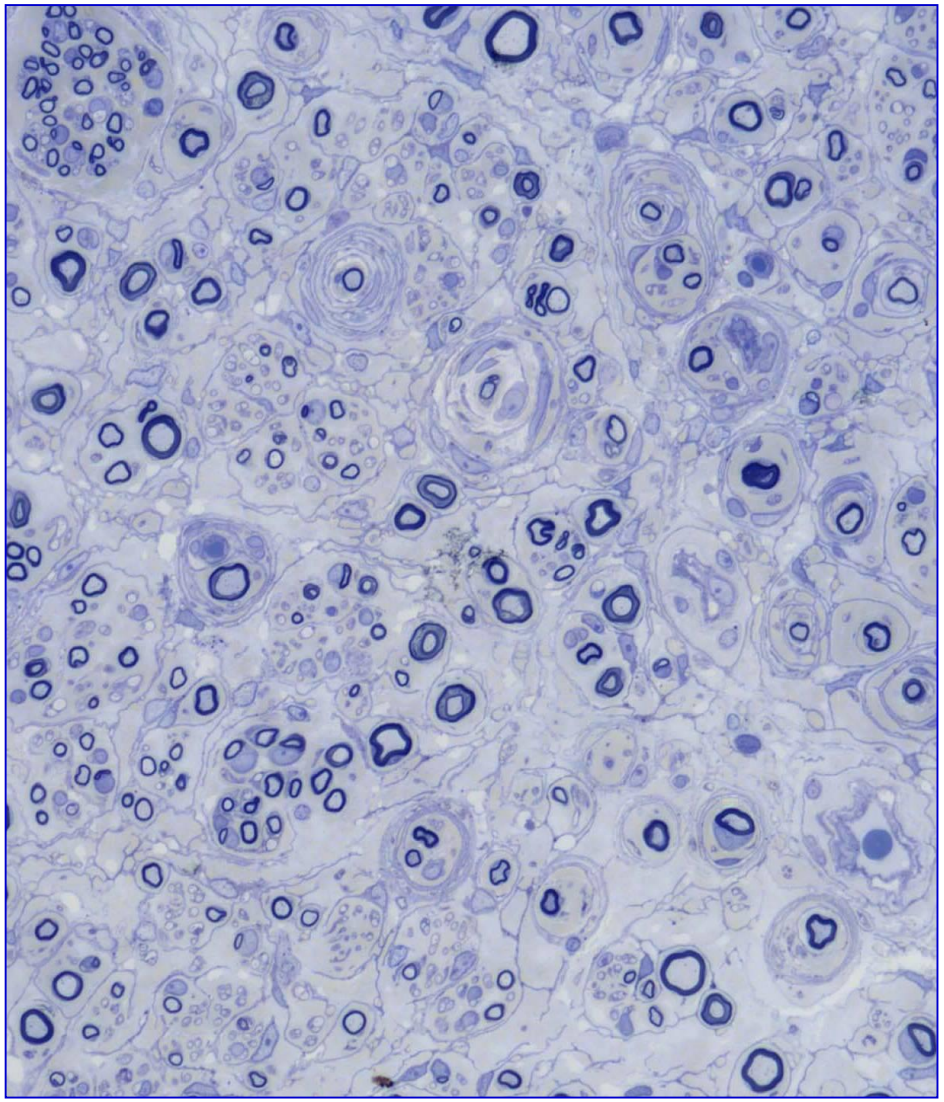


Shrinkage (drying out or hyperosmol?)

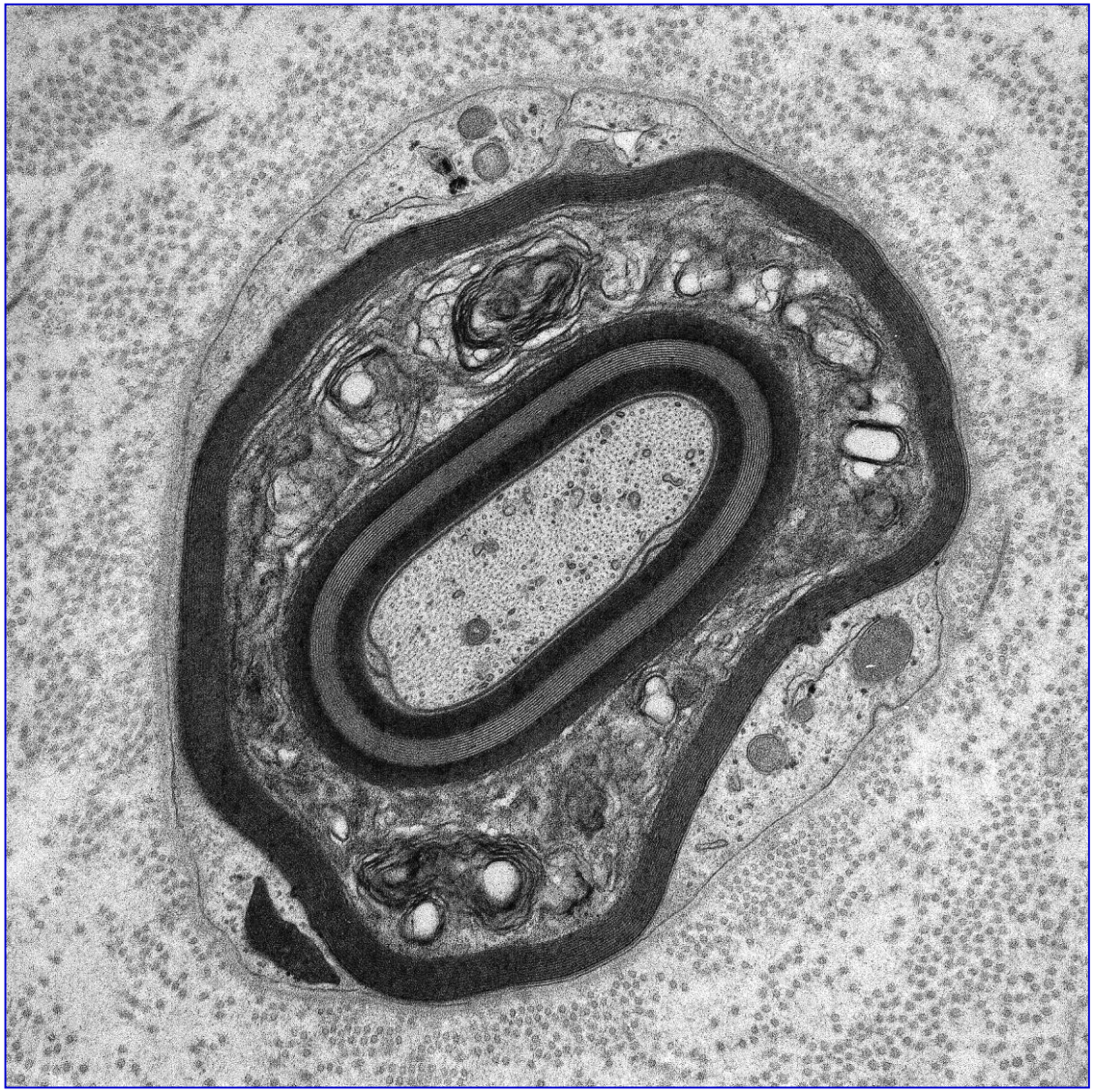


Inadequate Fixation





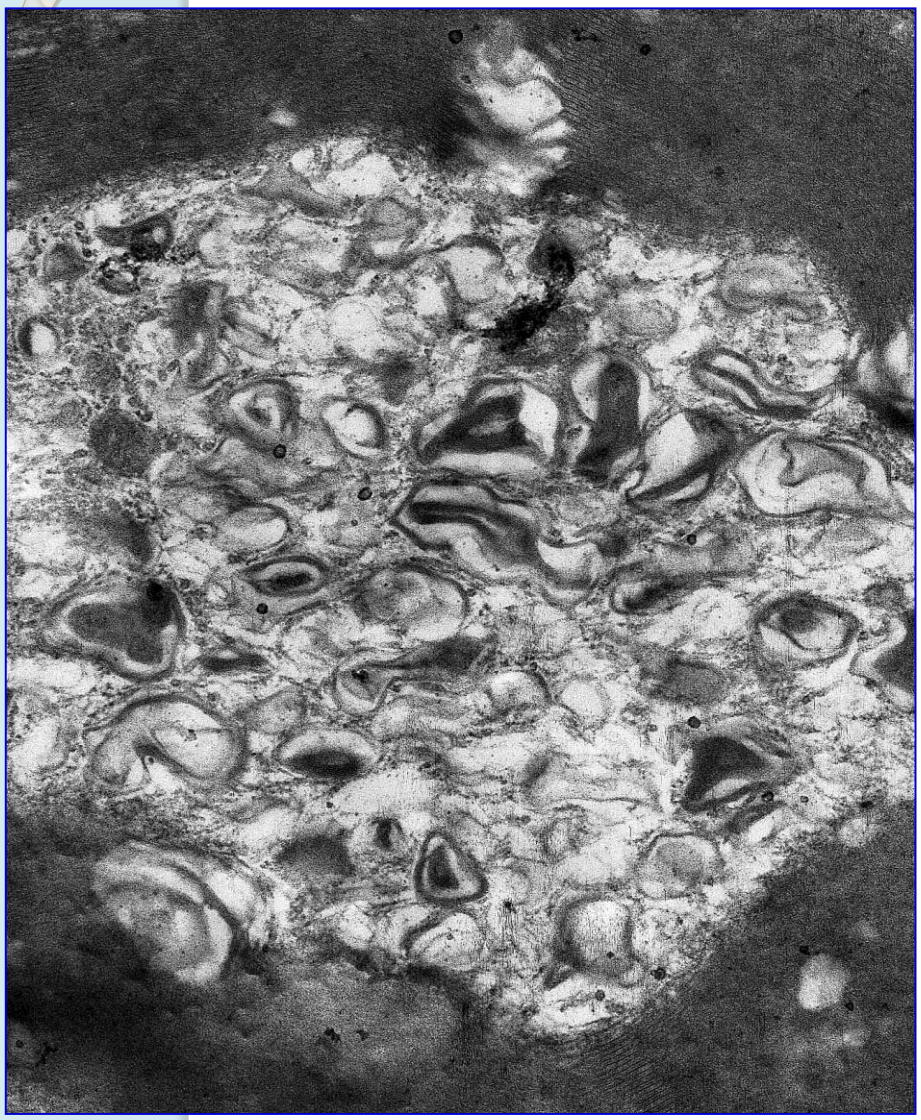
Inadequate fixation

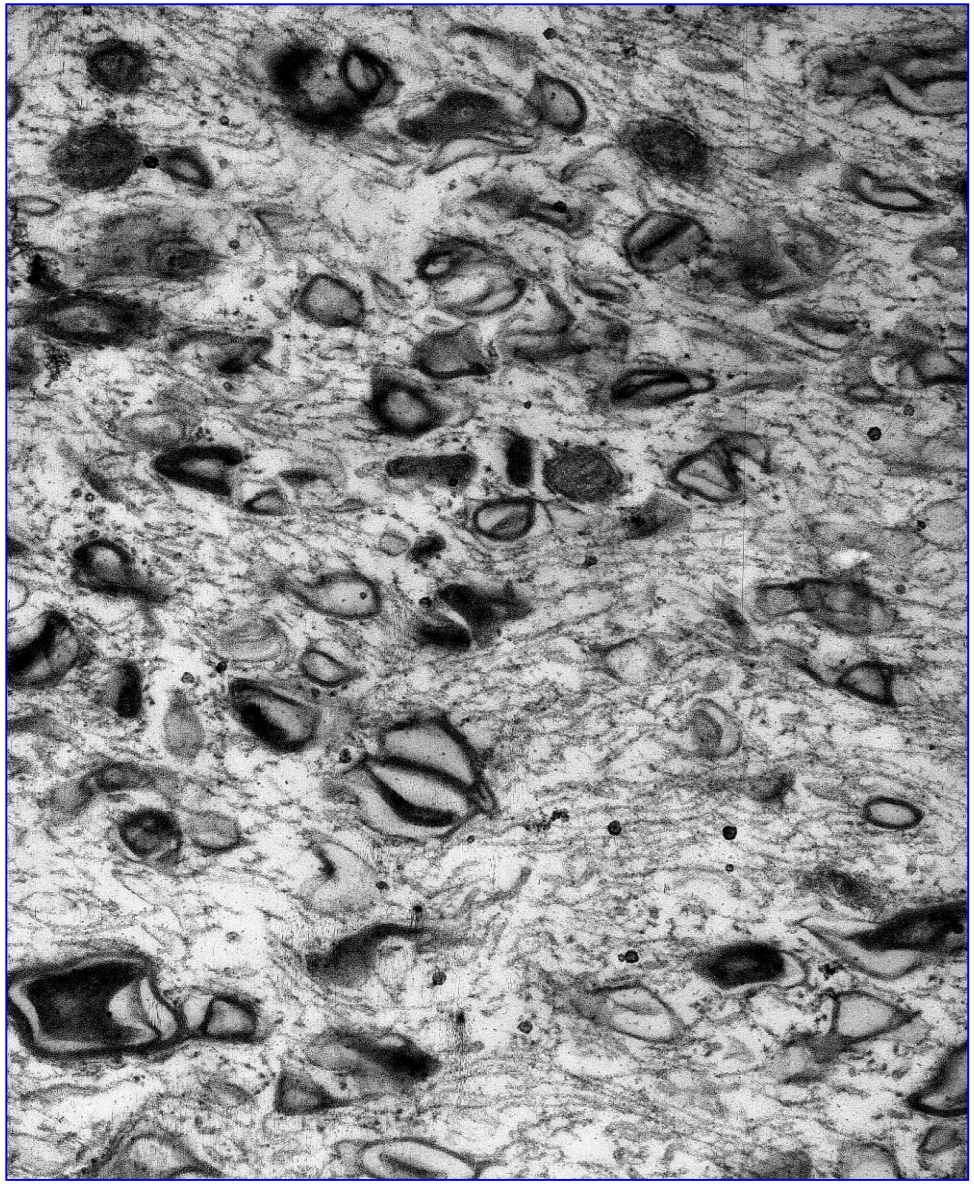


Unsatisfactory Schedule

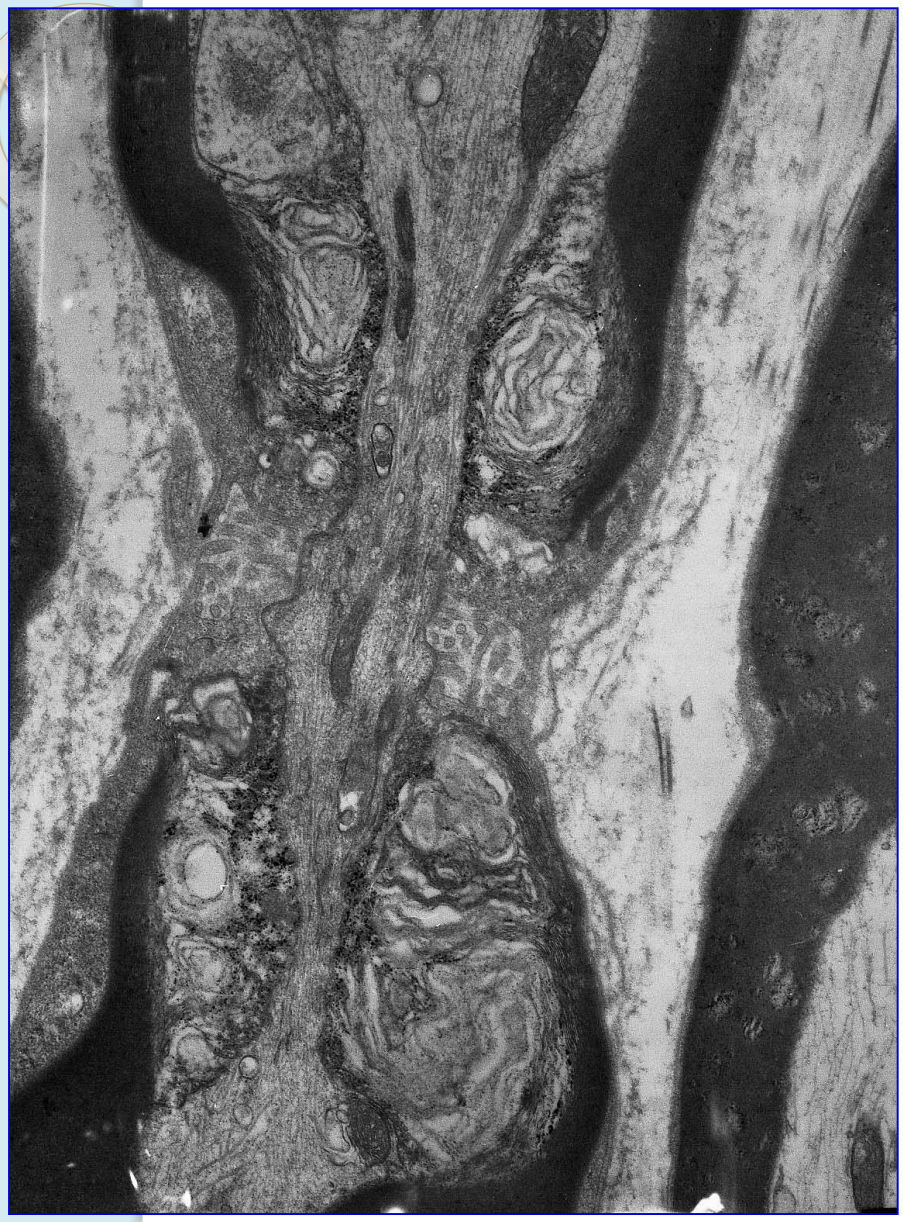
• Glutaraldehyde		3hr
• Sucrose cacodylate buffer x6		O/N
• 1% osmium tetroxide		3hr
• 50% ethanol		10 min
• 70% ethanol		10 min
• Absolute ethanol	x2	10min ea
• Propylene oxide	x2	15min ea
• PO:epoxy resin	1:1	1hr
• PO:epoxy resin	1:3	O/N
• Final resin		

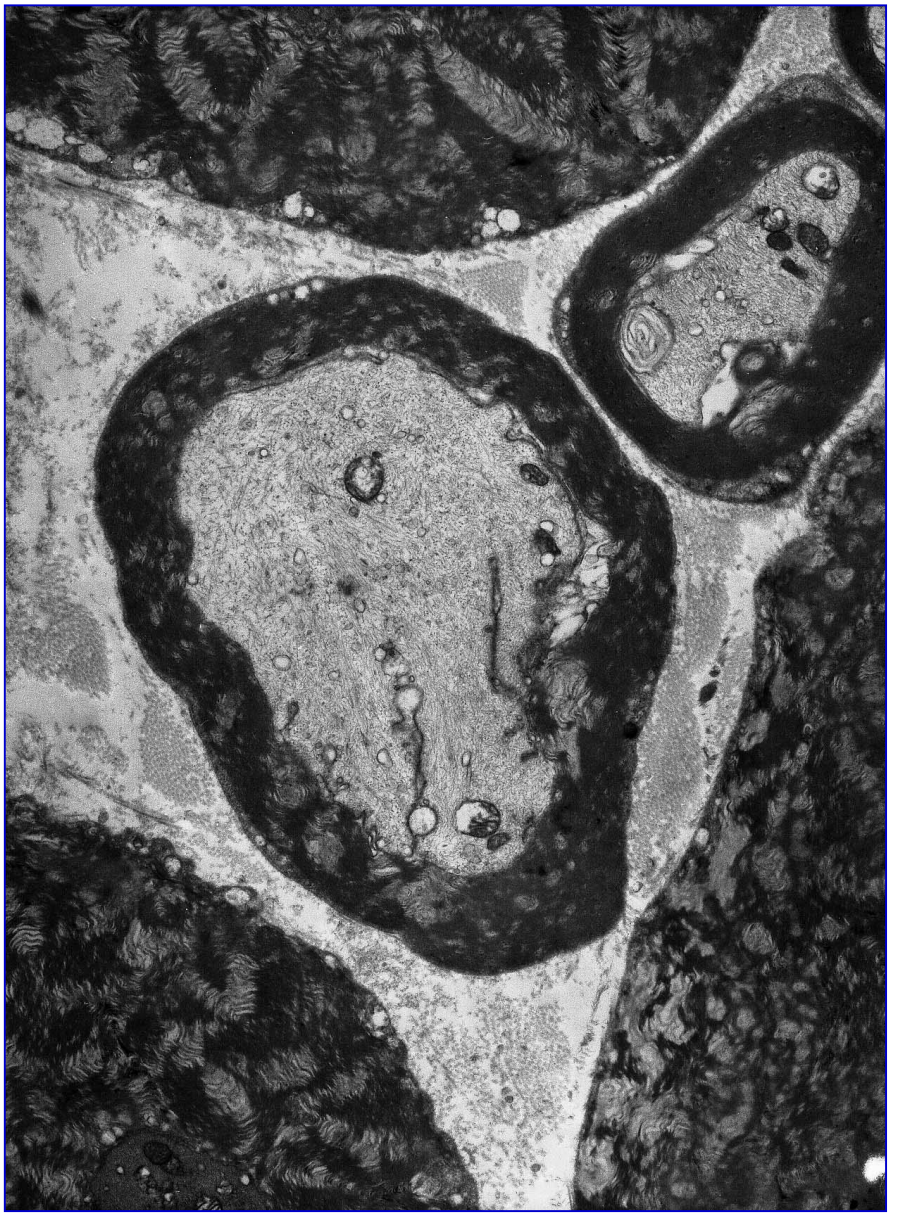
Processing Artefacts



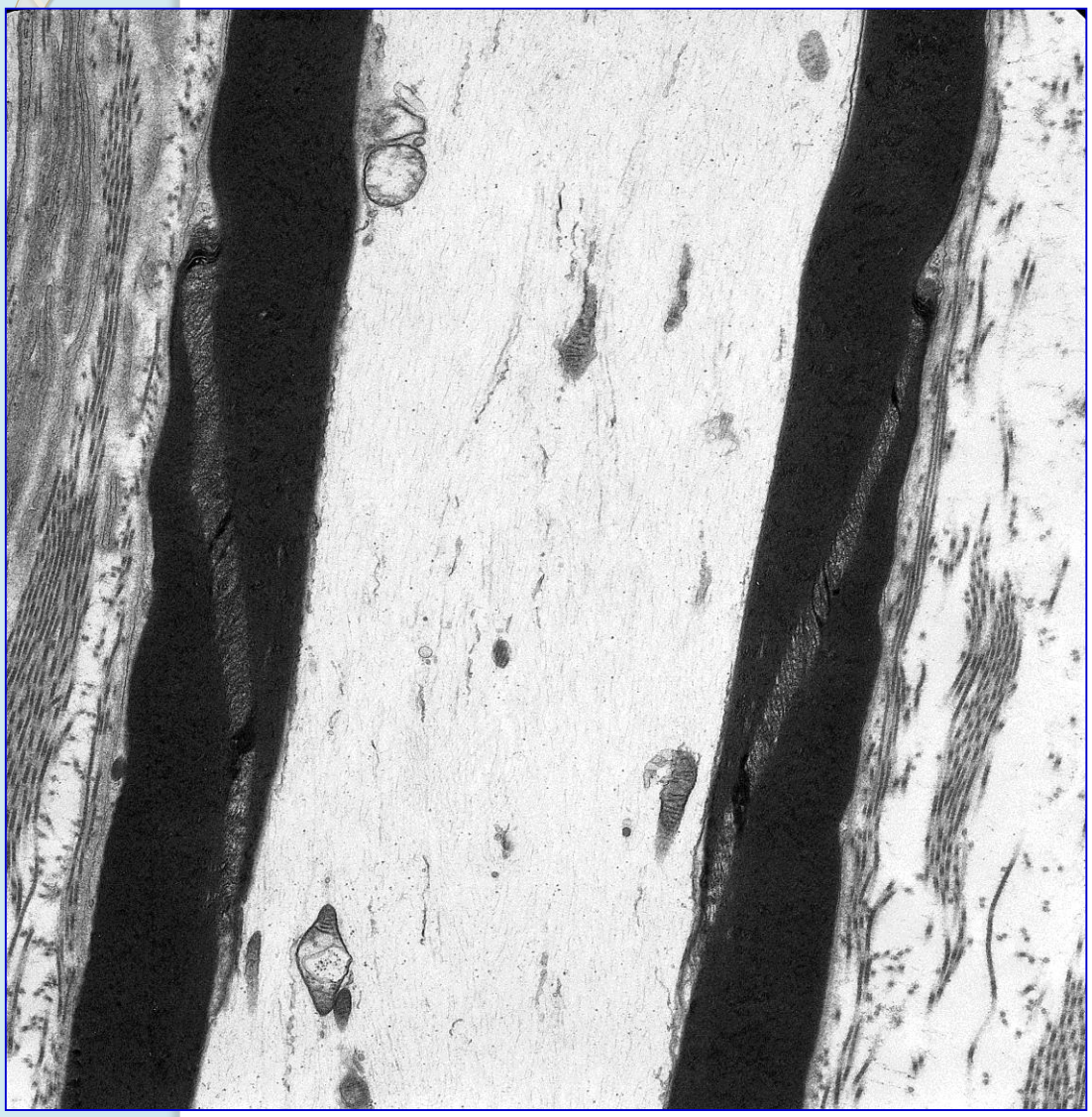


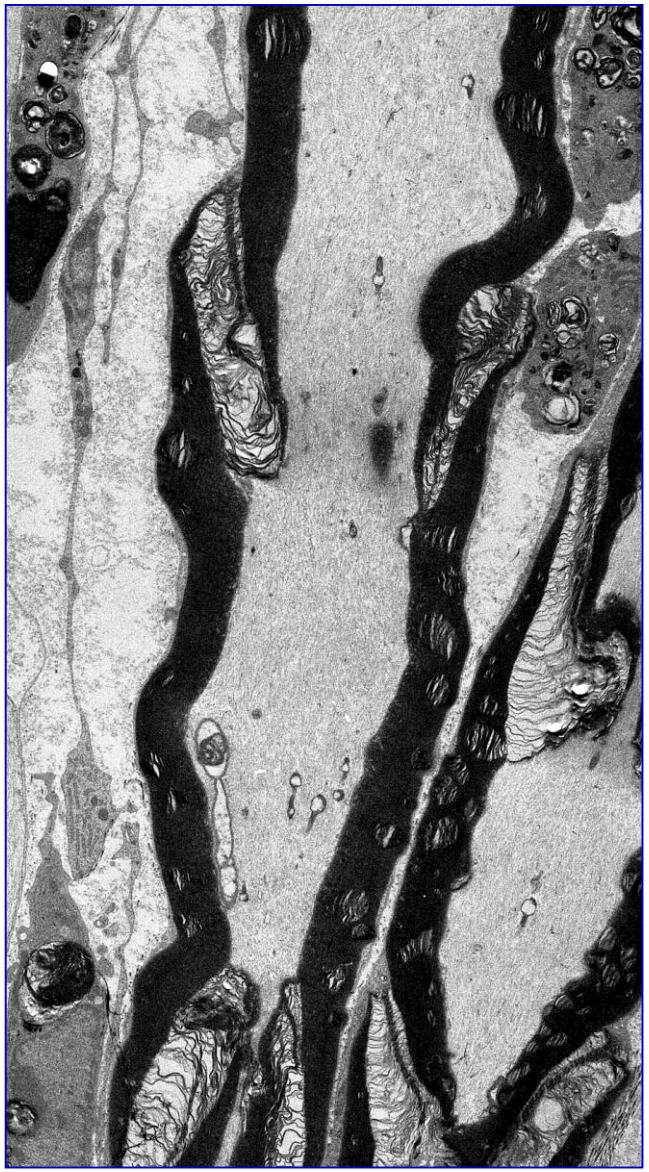
Processing Artefact



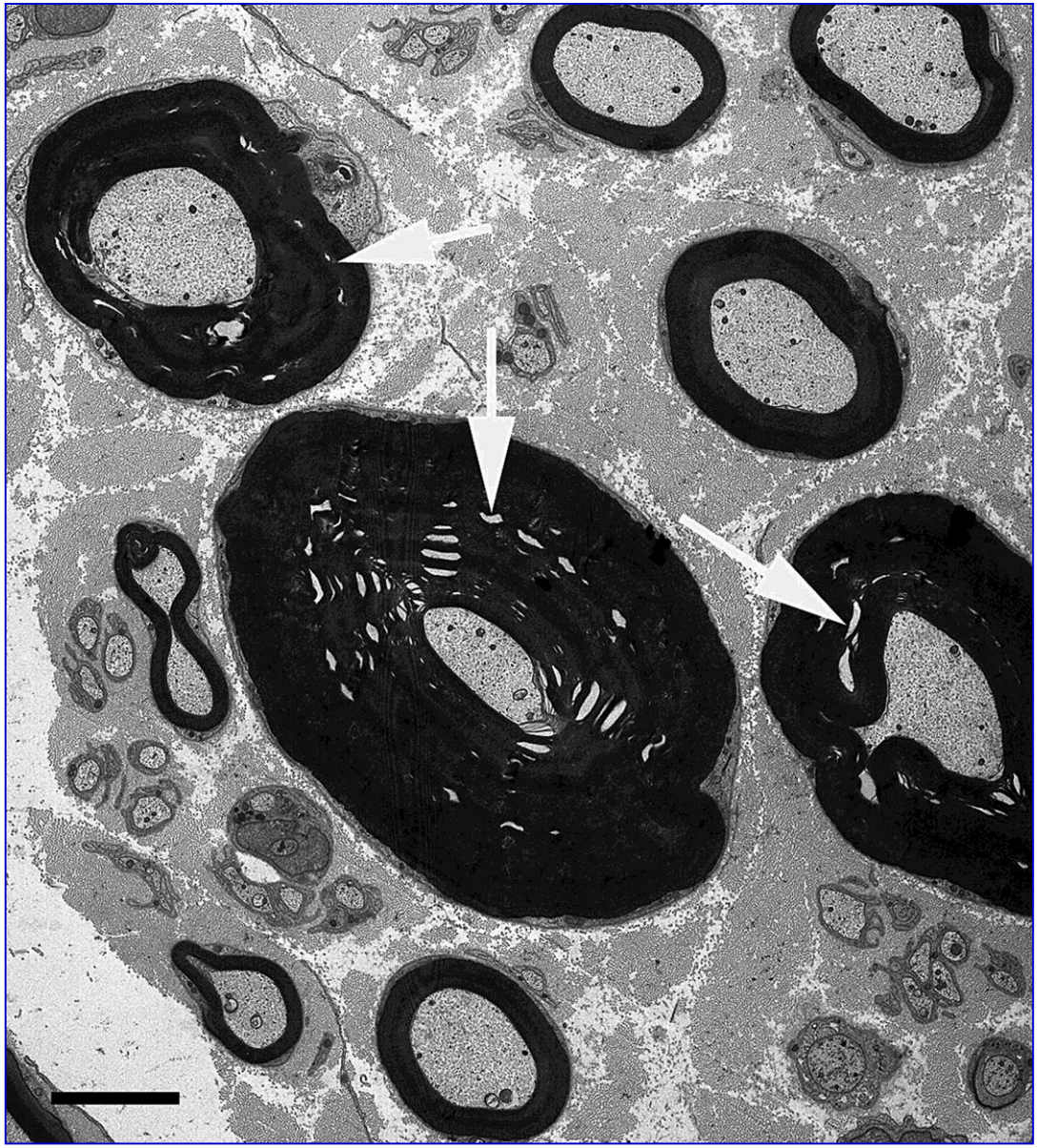


Processing artefact: Schmidt-Lanterman Incisures

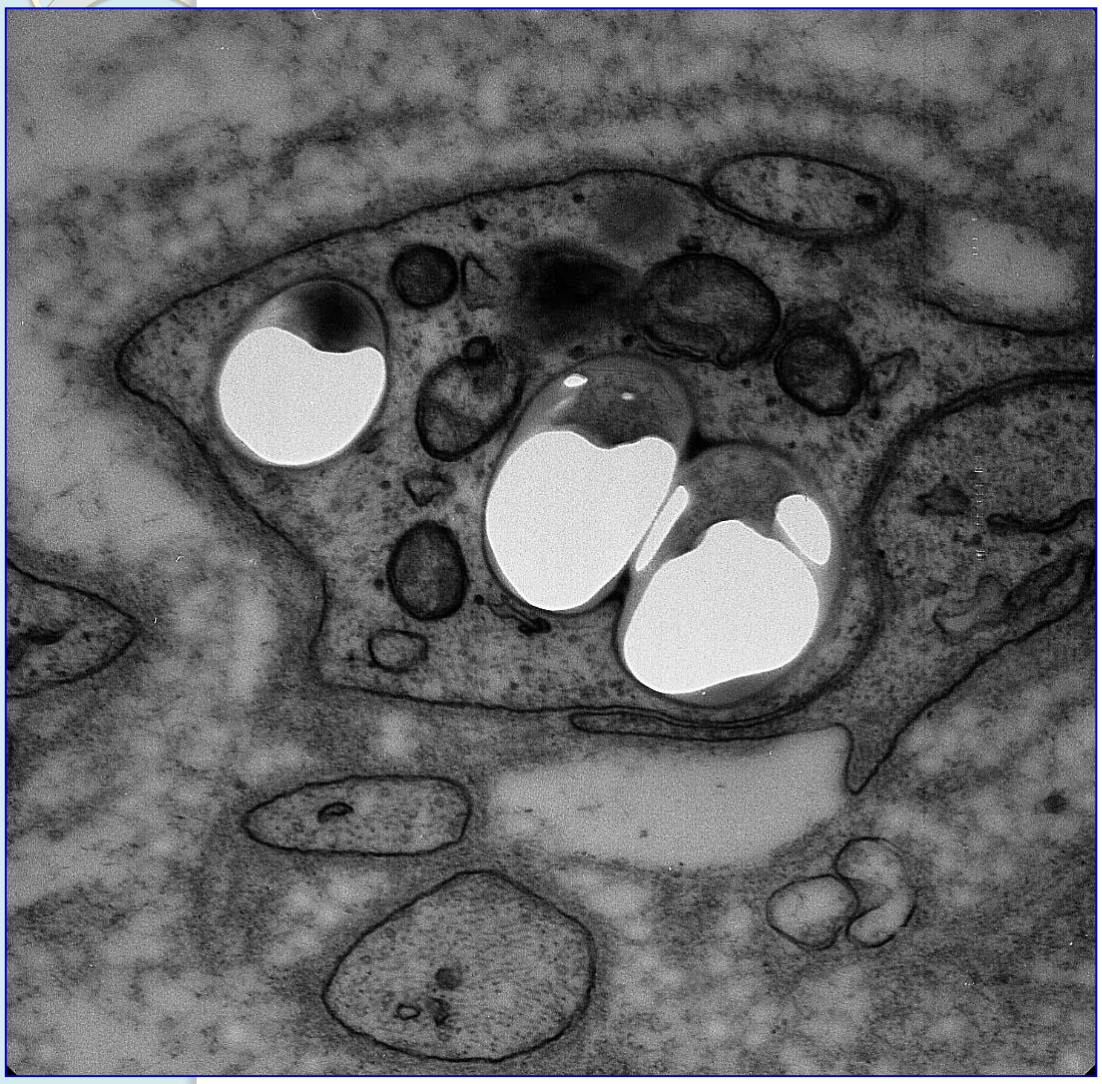


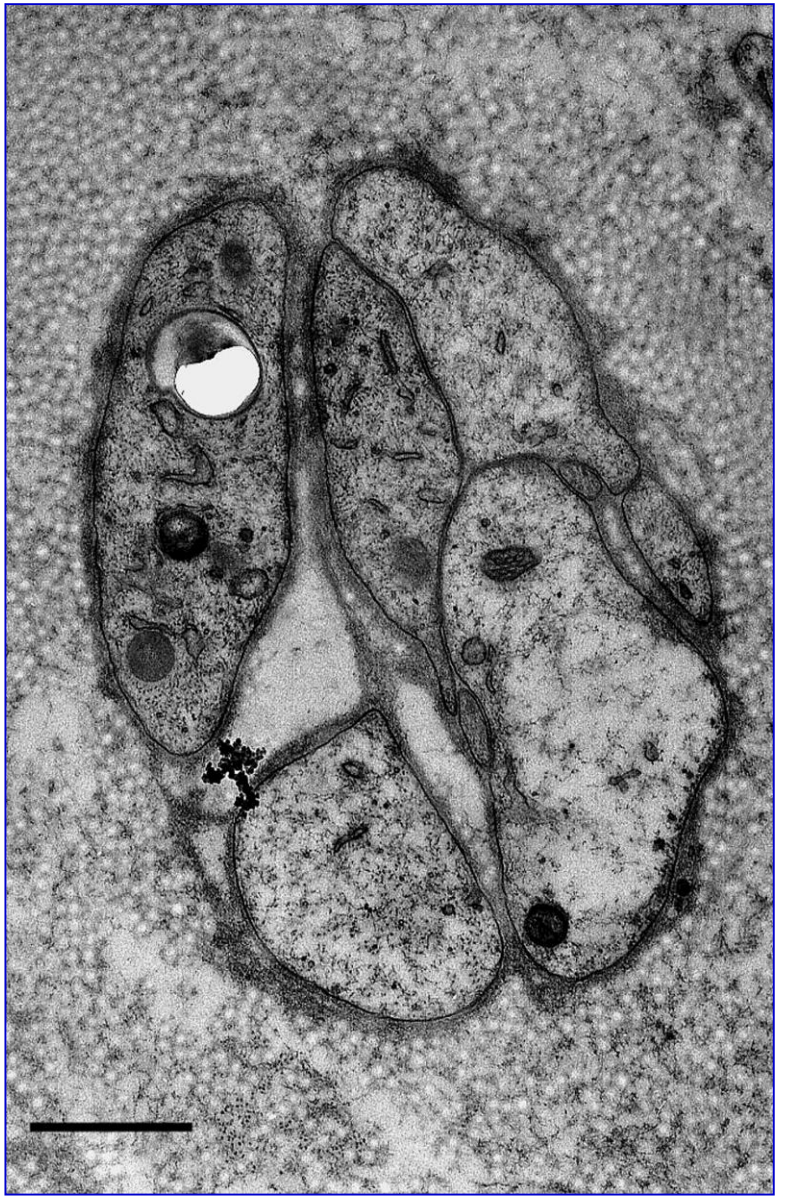


Processing Artefact



Useful Artefact

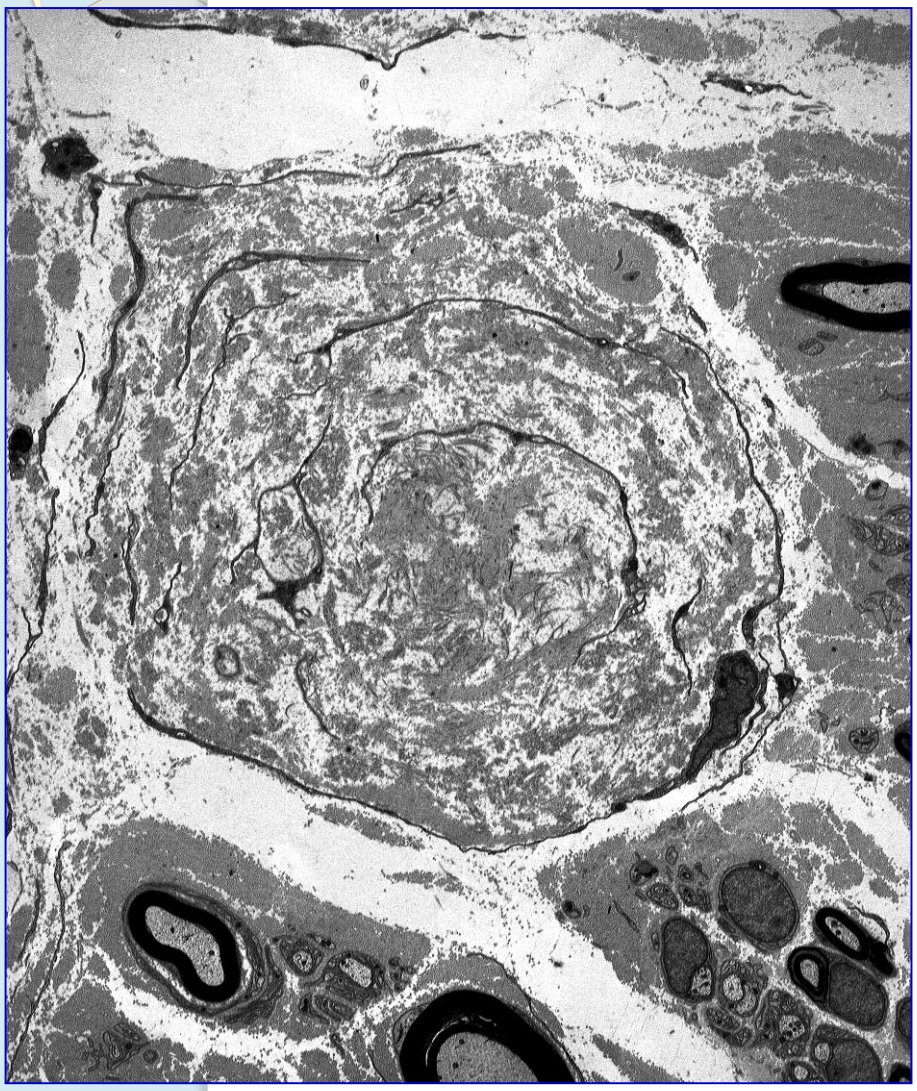




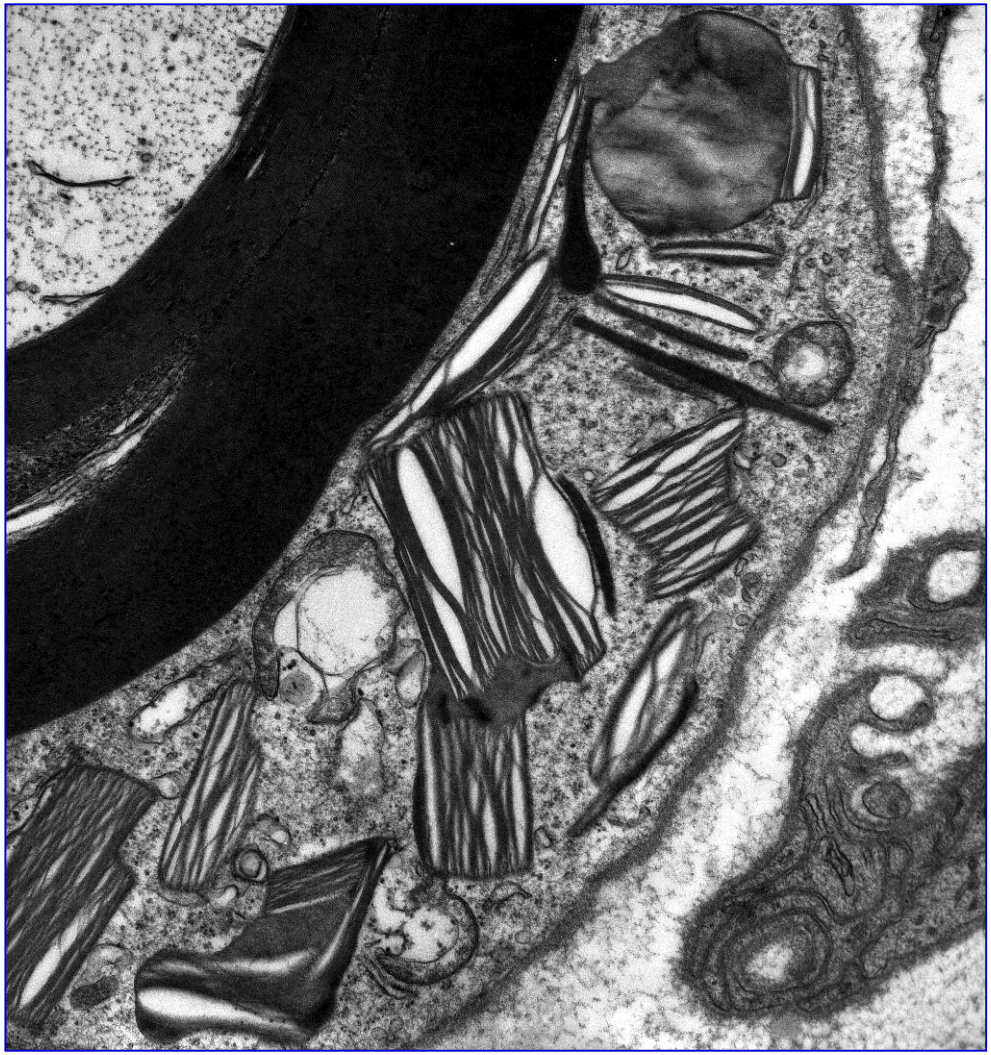
Normal ‘Abnormalities’

- Age changes (>55yrs)
- Renaut bodies
- Reich granules
- TS through node of Ranvier
- Artefacts

Normal!

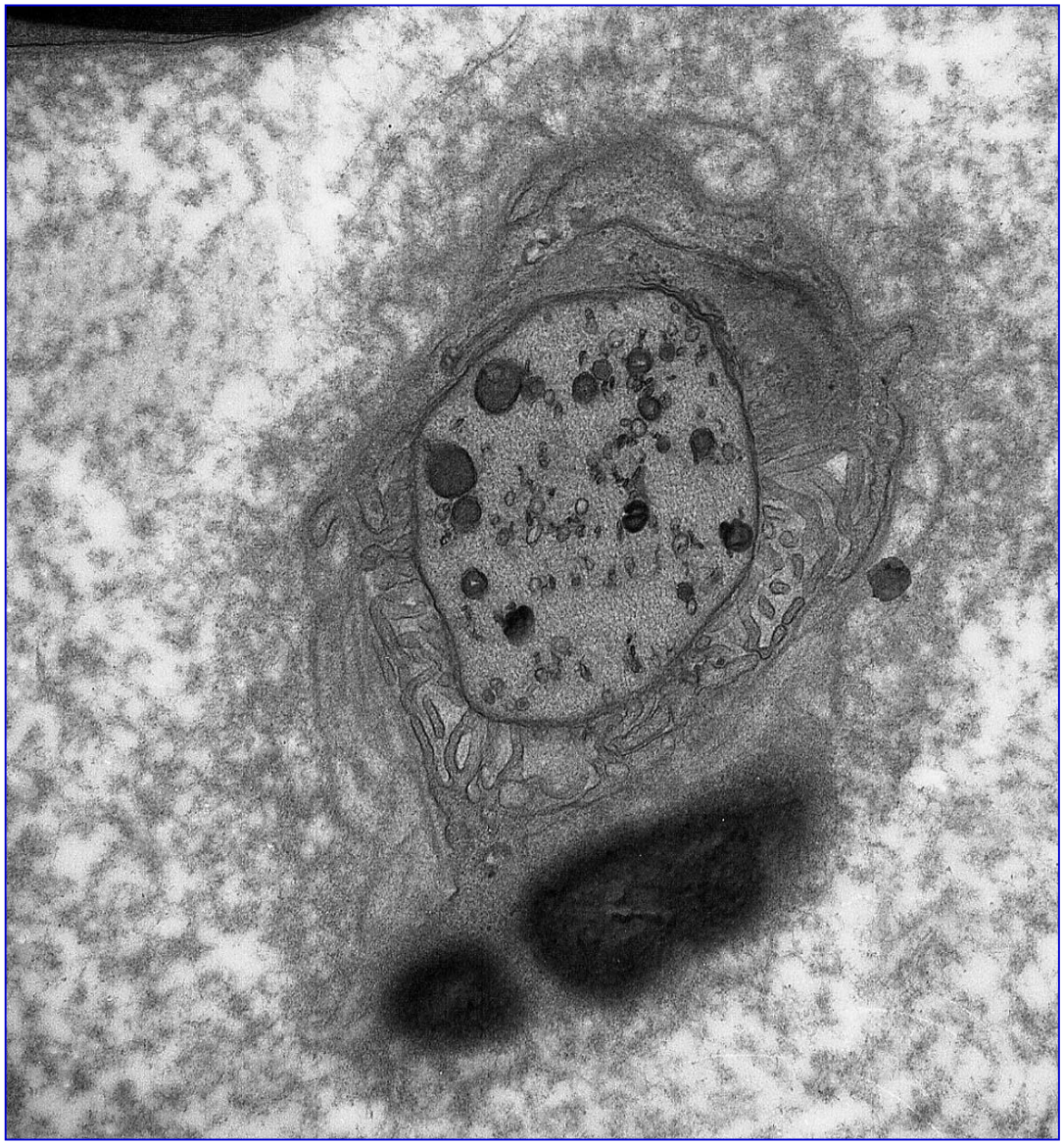


Renaut body



Reich granules acid ptase +ve, =lysosomes,

Node



AND FINALLY:

.**Vital C, Vallat J-M.** *Ultrastructural Study of the Human Diseased Peripheral Nerve.* Amsterdam: Elsevier, 1987.

Midroni G, Bilbao JM, Cohen SM. *Biopsy diagnosis of peripheral neuropathy*. Boston: Butterworth-Heinemann, 1995.

King RHM. *Atlas of Peripheral Nerve Pathology*. London: Arnold, 1999.

Cumming,WJK, Fulthorpe, J, Hudgson, P, Mahon, M. *Colour Atlas of Muscle Pathology*, Mosby-Wolf, London, 1994

Dawson TP, Neal JW, Llewellyn L, Thomas C *Neuropathology Techniques.* Hodder Arnold 2003

King, RHM, *The Diagnostic Electron Microscopy of Nerve. In Diagnostic Electron Microscopy; A Practical Guide to Interpretation and Technique.* Eds Sterling J.W. & Eyden, B. Wiley, 2012

King,R.H.M. *Microscopic Anatomy of Peripheral nerve, Chap 2 Peripheral nerve disorders (Hand book of Clinical Neurology).* Eds Said, G. & Krarup,C. Elsevier, 2012??

King, R.H.M. & Ginsberg,L. *Nerve Biopsy. Chapter 5 As above*