

Establishing Methodology to Study Myelin Involvement in Friedreich's Ataxia

Friedreich's Ataxia (FA)

FA Background

Neurodegenerative disease

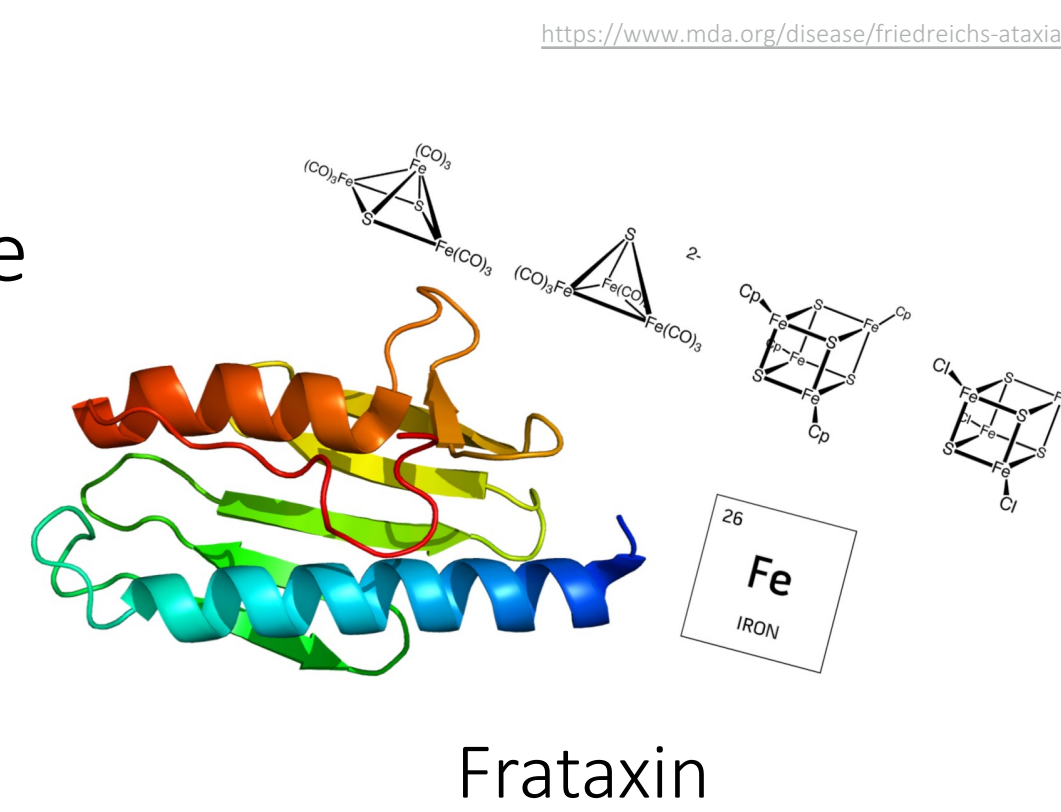
(Perdomini et al. 2013)

1 in 50,000 Americans (NIH)

Autosomal recessive

Mutation of Frataxin [FXN]

(Koeppen 2013)



FXN involved in iron transportation (FXN 2016)

FA Classified as mitochondrial disorder (Perdomini et al. 2013)

Effects of FA

Short life span (NIH)

Multi-systemic disease

(Magrané)

High FXN concentration

(MDA)

Clinical Symptoms

Ataxia (loss of coordination)

Cardiomyopathy (heart disease)

Diabetes (type II)

Peripheral Nervous System

Scoliosis (curvature of spine)

Peripheral Sensory

Neuropathy

Nerve dysfunction

of Peripheral

Nervous System

(PNS)

(Cruz-Martinez et al. 1997)

PN Structure

Sensory and Motor Neurons

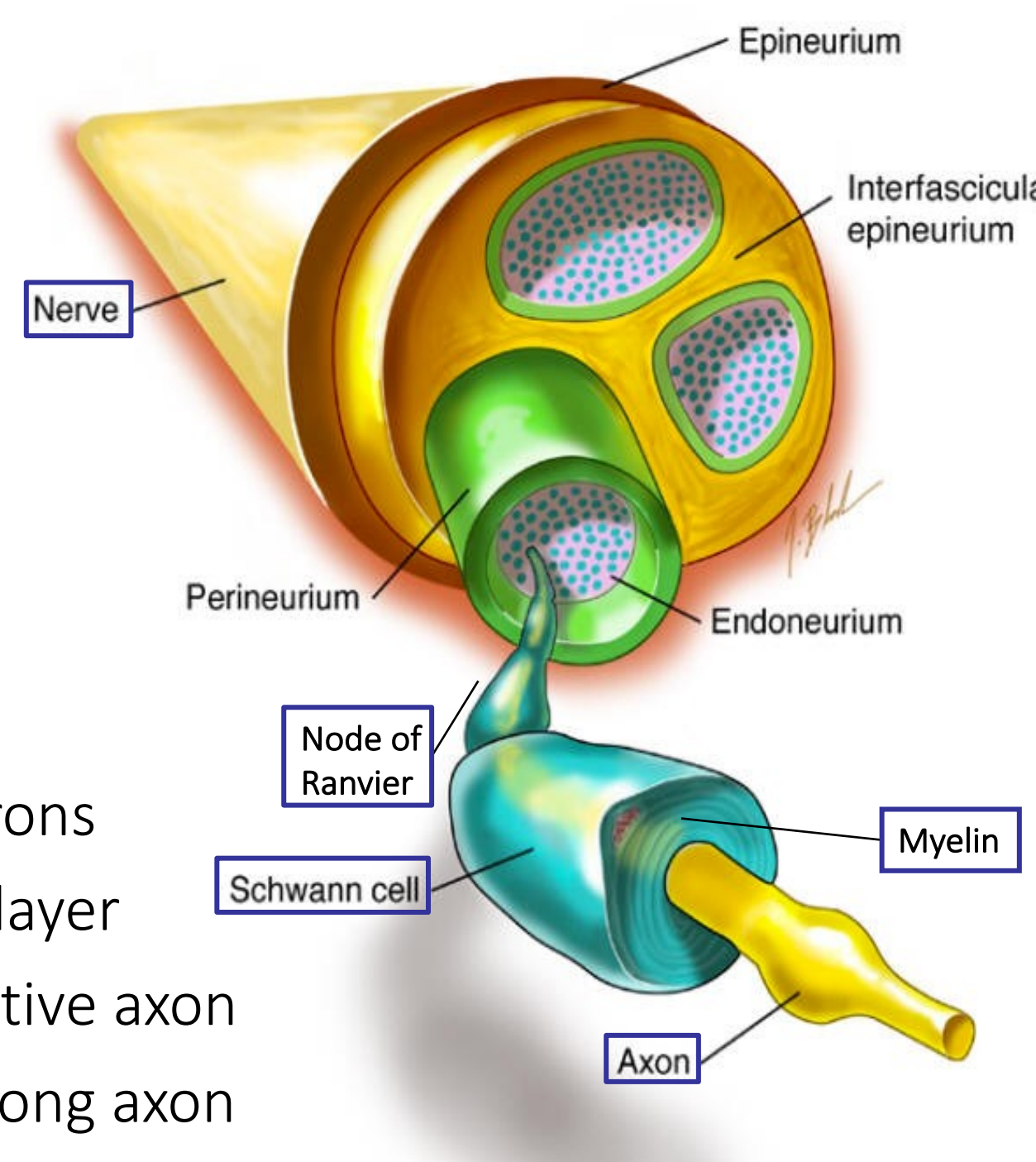
Myelin: insulating outer layer

Node of Ranvier: conductive axon

Current transmitted along axon

Schwann (SC): secrete/maintain myelin

Energetically active



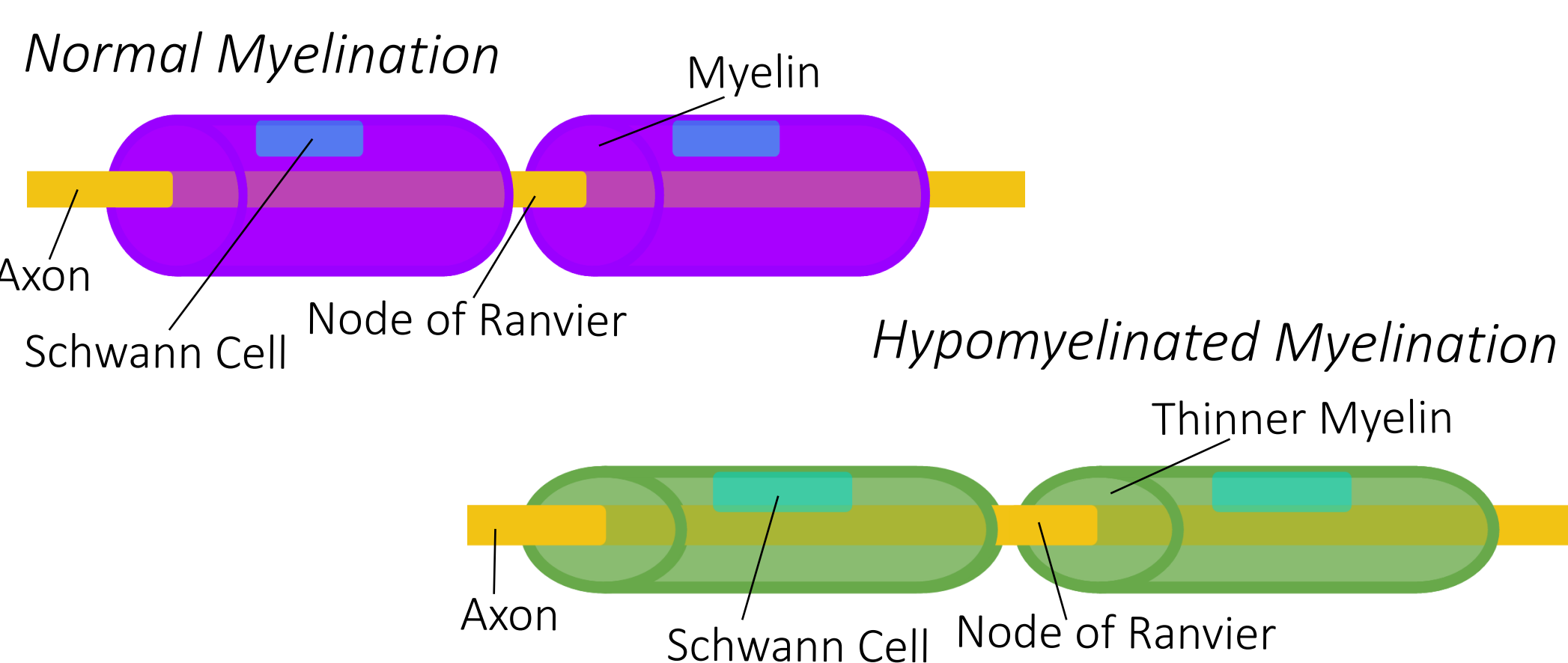
<http://www.newhealthadvisor.com/Peripheral-Nervous-System.html>

Gap in Research

Autopsy Data -> sensory axon hypomyelination (Magrané)

Disintegration/thinner myelin (poor insulation)

Impairs nerve impulse conductance (Tao et al.)



Contribution of SC & Hypomyelination on PNS demise

Lack of experimental models/tools to study this in FA

Ultrastructural imaging (electron microscopy)

View structural abnormalities

Problem Statement and Objective

Problem: The pathogenesis and consequences of Schwann cell defects in the myelination of Friedreich's Ataxia patients, via ultrastructural imaging, is yet to be extensively researched.

Objective: To establish the protocols for a teased fiber technique along with immunodetection protocols for the imaging of the nerves.

Materials & Methodology

30 day non-tg mouse (WTWT)

Sciatic: longest nerve in PN

Motor/sensory axons (Magrané)

Tuberculin vs Dissecting Needles



http://www.thorlabs.com/objective_microscopy_instructions.html

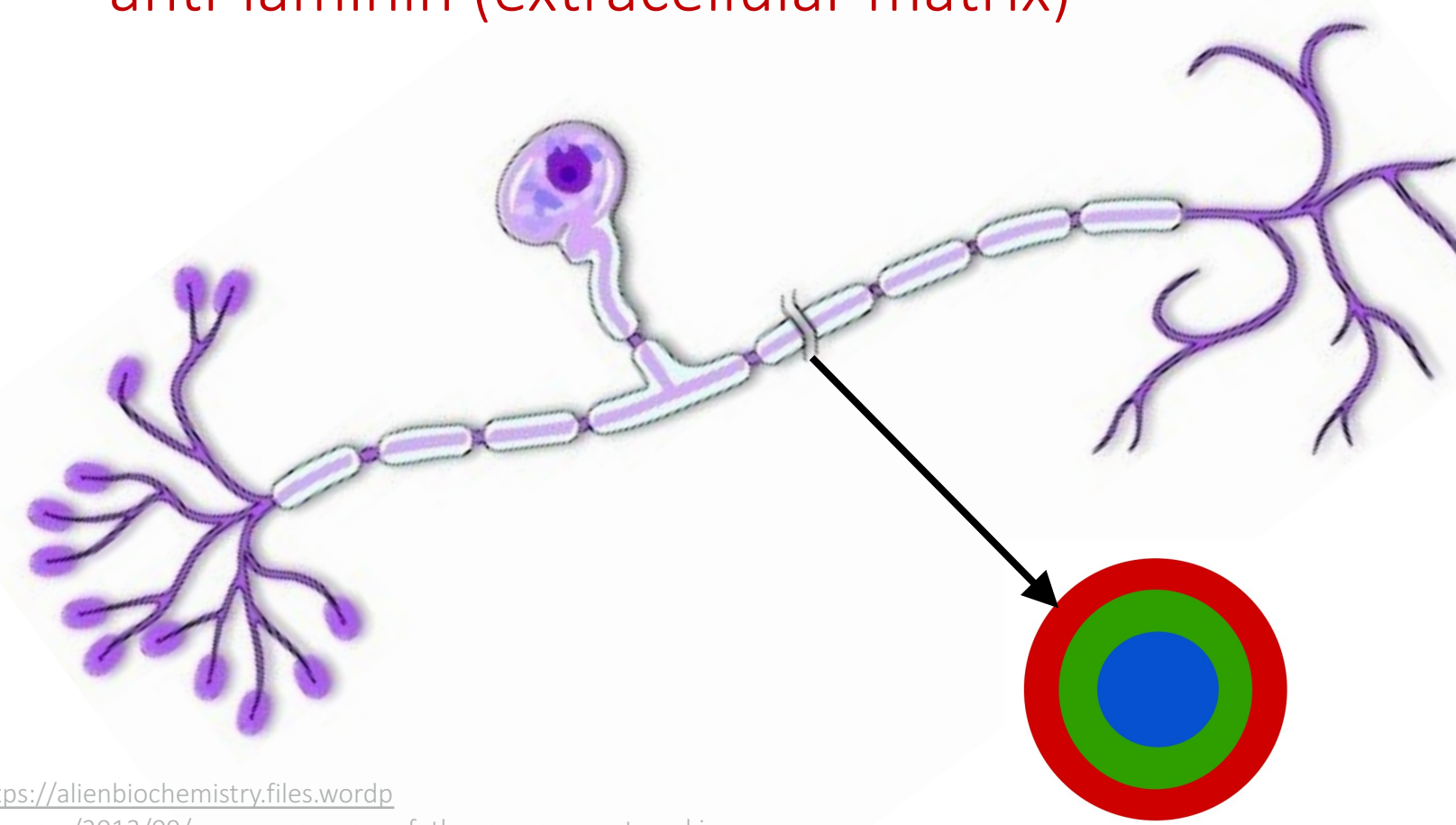
<http://www.casimirohah.com/axonic-gain-presentation-chirurgien/>

Antibodies (Melendez-Vasquez)

anti-neurofilament (NFM) (axon/cytoskeleton)

anti-myelin binding protein (MBP) (myelin)

anti-laminin (extracellular matrix)



https://allenbiochemistry.files.wordpress.com/2013/09/sensory_neuron_of_the_nervous_system.jpg

1. Perfused mouse nerves
2. Utilizing needles, teased nerves

Established Dissecting Technique

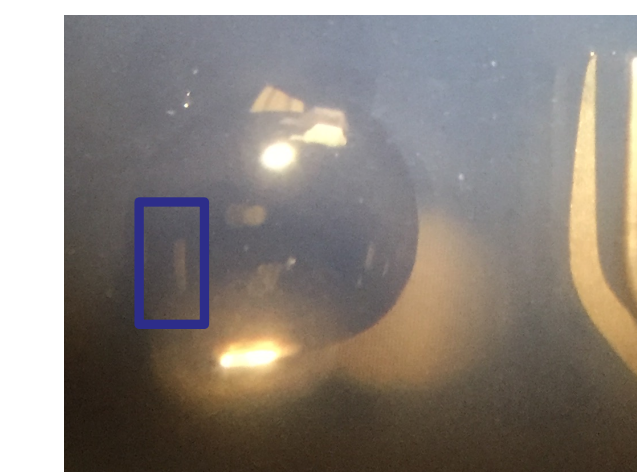
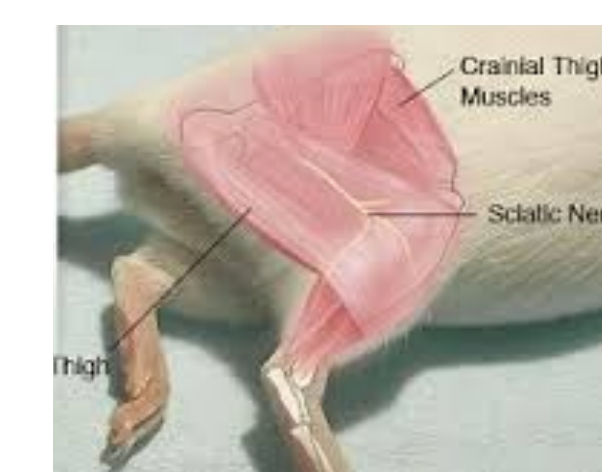
Literature Review

Example Techniques

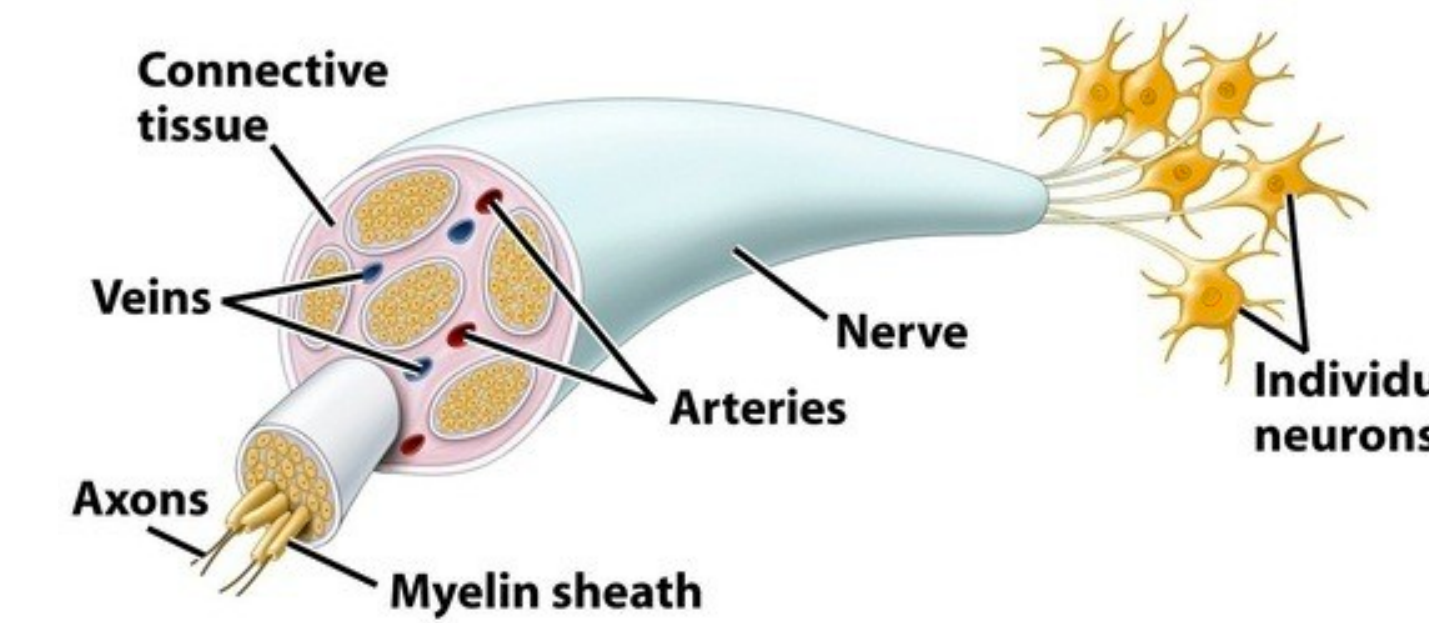
Trial and Error

Tuberculin > Dissecting

3. Performed Immunohistochemistry
- Fixed dissected nerves (Magrané)
- Protein intact + maintaining outer structure
- Permeabilize outer membrane (controlled setting)
- Incubate with antibodies + fix samples to a cover slip
4. Confocal Microscopic Imaging



Sciatic nerve from dissecting microscope (Desiraju)



<https://www.quora.com/Whats-the-difference-between-sensory-neurons-sensory-cells-and-sensory-neurons>



Disorderly teased sciatic (Desiraju)

Discussion

Ultrastructure visible of individual nerve fiber

Antibodies and dilutions stained cells

Neurofilament detection → labeled the axonal network

Myelin detection → SC differentiation and myelin formation

Laminin detection → maturity of culture, secreted by SCs

Conclusion

Successful ultrastructural imaging established teased fiber technique & immunodetection methods

Nerves adequately teased

Antibody dilutions sufficient

→ Evaluate hypomyelination contribution to sensory neuron dysfunction and FA pathogenesis

Significance

Ultrastructural imaging evaluates abnormalities and disease progression

Diseases with peripheral sensory neuropathy or mitochondrial disorders

20,000,000 Americans suffer from peripheral neuropathy (NIH 2016)

1,000-4,000 Americans born annually diagnosed (UMDF 2016)

Future Research

Presence of ultrastructural abnormalities

FA mouse model, control and positive control

Presence and frequency of the nodes of Ranvier

Mitochondrial distribution around these nodes

Extend studies to older mice

References

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Results

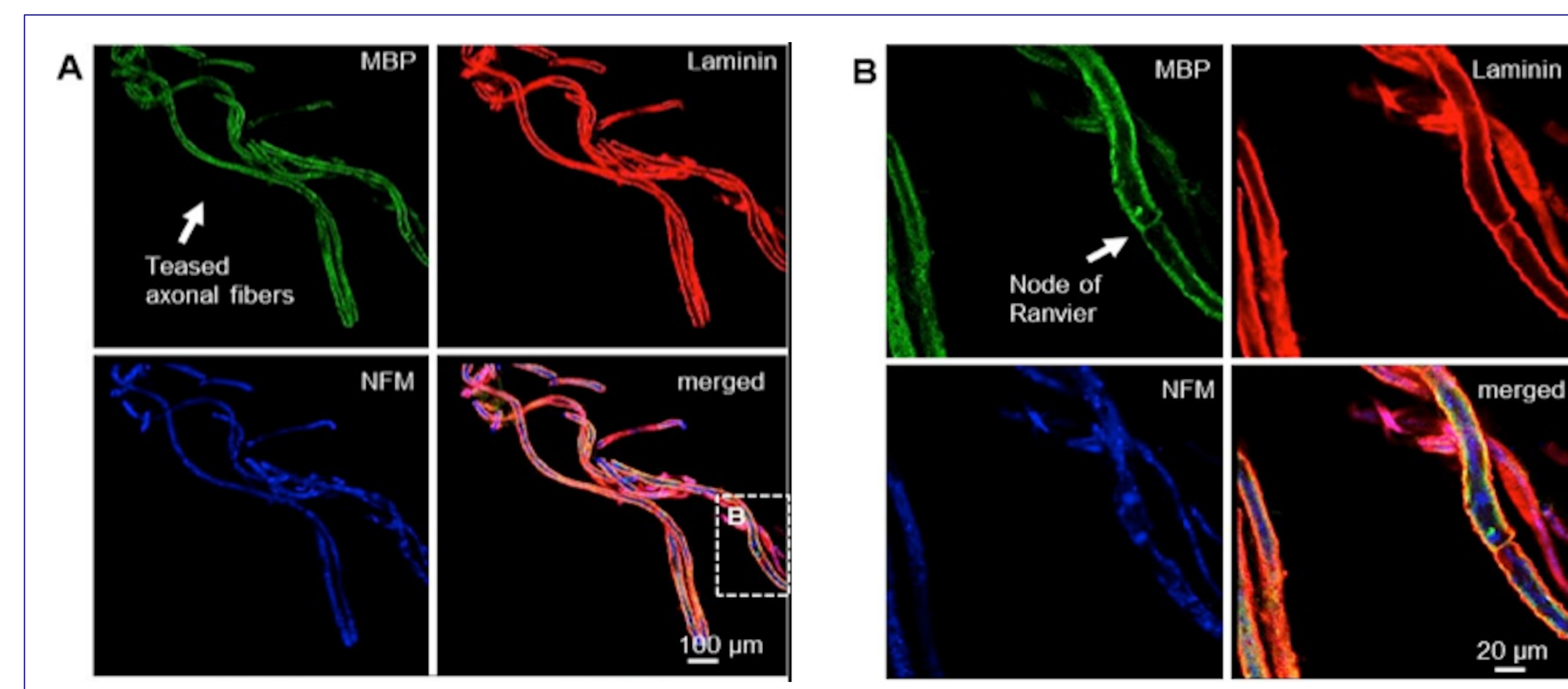


Figure 1. Teased axonal fibers from sciatic nerves of 30 day old mouse. Three antibodies were used, as indicated: anti-MBP, anti-laminin, and anti-NFM. Merge represents the three antibody images together. **A.** low magnification of the teased axonal fibers. **B.** higher magnification of the dotted area shown in A (merged) to better visualize the different nerve structures.