An 2.0 Version of Automated Peripheral Nerve Image Analysis Protocol for Axon and Myelin Sheath Determination and Evaluation.

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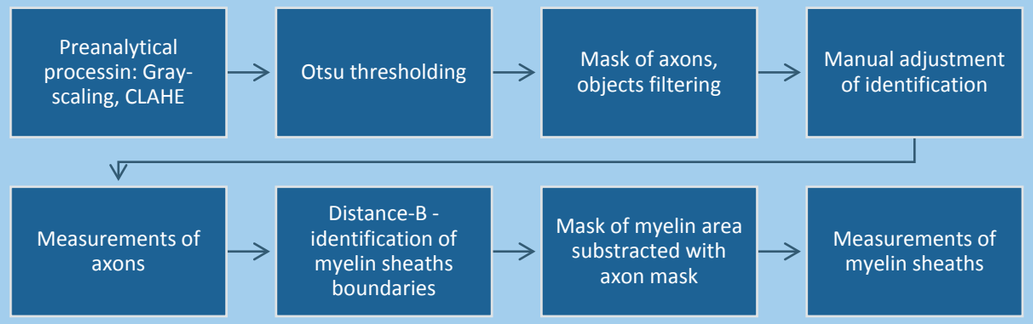
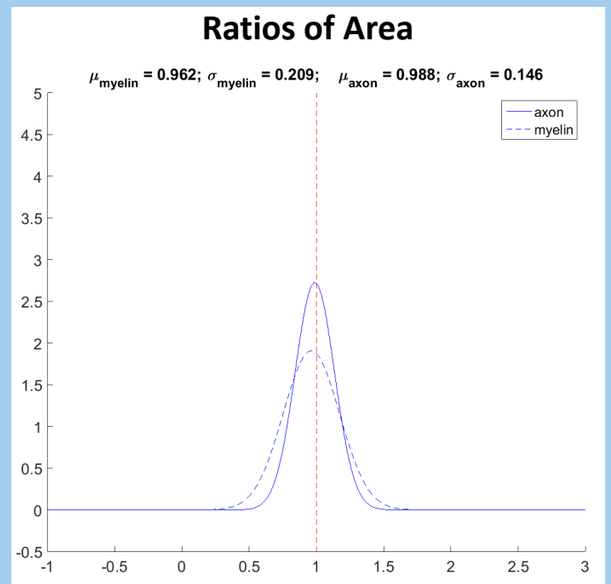
Introduction

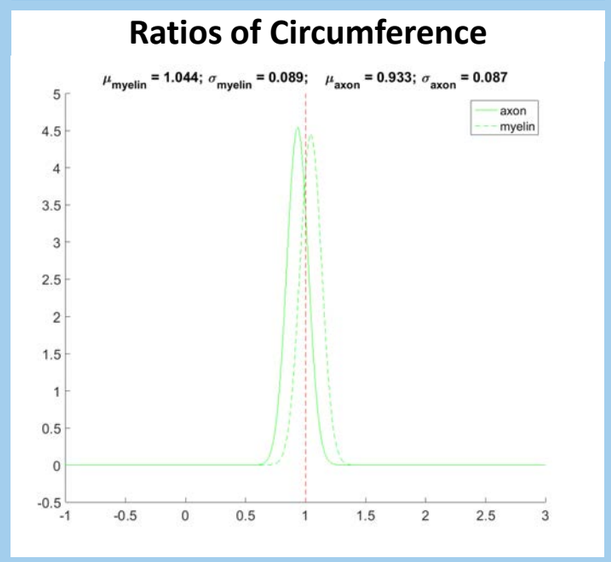
Histologic analysis of nerve tissue remains a crucial method of evaluating research on peripheral nerves. Histomorphometric nerve cross-section analysis is considered a gold standard that enables comparison between different studies. Precise identification of nerve fiber elements: axons and myelin sheaths is a key step, that determines proper of parameters as axon count and density, G ratio and more. Manual methods are time-consuming and prone to human error. We have developed and optimized a nerve cross-section scan analysis protocol.

Materials & Methods

Rat sciatic nerve fragments were harvested and fixed according to standard preparation protocol for scanning electron microscopy. Semithin sections were stained with toluidine blue and captured at 40× magnification. Images were uploaded in self-design image processing macro in FIJI and optimized pipeline for image analysis in CellProfiler (as shwon in flowchart). A module for manual error and artifact removal was added.. Sets of parameters were measured for both structures: area, perimeter, mean radius, G ratio and shape indexes. Results were compared with manual determination and measurements of 30 random nerve images assessed by 3 blinded researchers in FIJI.

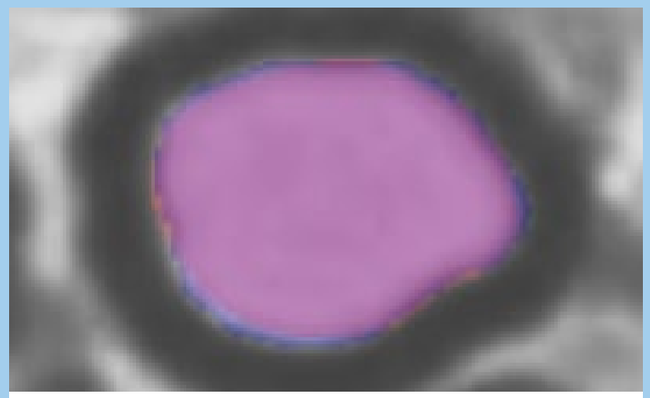






Results

The designed protocol enables determination of axons with 99,5% coverage and myelin sheath with 98% coverage with manual technique ($p<0,05$). The time needed to perform one image analysis in case of automated protocol oscillates around 2 minutes and manual technique takes ~ 76 minutes.



Representative example of an axon area recognition pattern:
Red – human markings
Blue – Protocol markings

Conclusions

An updated version of the protocol was optimized and enhanced with intuitive module for easy removal of artifacts and errors of automated analysis. Further protocol evolution involves neural net learning.