Video Article

Toluidine Blue Staining of Resin-Embedded Sections for Evaluation of Peripheral Nerve Morphology

Adel B. Ghnenis[1], Richard E. Czaikowski[1], Zhaojie J. Zhang[2], Jared S. Bushman[1]

[1]School of Pharmacy, University of Wyoming

[2]Department of Zoology and Physiology, University of Wyoming

Correspondence to: Jared S. Bushman at jbushman@uwyo.edu

URL: https://www.jove.com/video/58031
DOI: doi:10.3791/58031

Keywords: Neuroscience, Issue 137, Peripheral nerve, toluidine blue staining, resin embedding, ultramicrotome sectioning, nerve regeneration, nerve injury

Date Published: 7/3/2018

Citation: Ghnenis, A.B., Czaikowski, R.E., Zhang, Z.J., Bushman, J.S. Toluidine Blue Staining of Resin-Embedded Sections for Evaluation of Peripheral Nerve Morphology. *J. Vis. Exp.* (137), e58031, doi:10.3791/58031 (2018).

Abstract

Peripheral nerves extend throughout the body, innervating target tissues with motor or sensory axons. Due to widespread distribution, peripheral nerves are frequently damaged because of trauma or disease. As methods and strategies have been developed to assess peripheral nerve injury in animal models, function and regeneration, analyzing the morphometry of the peripheral nerve has become an essential terminal outcome measurement. Toluidine blue staining of nerve cross sections obtained from resin embedded nerve sections is a reproducible method for qualitative and quantitative assessments of peripheral nerves, enabling visualization of morphology number of axons and degree of myelination. This technique, as with many other histological methods, can be difficult to learn and master using standard written protocols. The intent of this publication is therefore to accentuate written protocols for toluidine blue staining of peripheral nerves with videography of the method, using sciatic nerves harvested from rats. In this protocol, we describe *in vivo* peripheral nerve fixation and collection of the tissue, and post-fixation with 2% osmium tetroxide, embedding of nerves in epoxy resin, and ultramicrotome sectioning of nerves to 1-2µm thickness. Nerve sections then transferred to a glass slide and stained with toluidine blue, after which they are quantitatively and qualitatively assessed. Examples of the most common problems are shown, as well as steps for mitigating these issues.

Video Link

The video component of this article can be found at https://www.jove.com/video/58031/

Introduction

Peripheral nerves extend throughout the body, innervating target tissues with motor or sensory axons[1]. Peripheral nerve defects caused by medical disorders and trauma represent a major public health concern and have large economic impacts[2,3]. Despite the advances in assessing the outcomes of peripheral nerve injuries and understanding nerve regeneration, traditional methods such as nerve histology and staining techniques are essential tools to qualitatively and quantitatively assess nerve health as a terminal outcome measurement in animal models or excised human tissue. This is often paired with electrophysiological measurements of peripheral nerve function, where morphometry can reveal why functional nerve regeneration did or did not occur.

Toluidine blue staining of resin embedded semi-thin peripheral nerve sections is a specialized method for imaging myelinated nerve fibers, providing high quality and clear detailed images of nerve structures[4,5,6]. Toluidine blue is an acidophilic metachromatic stain, discovered by William Henry Perkin in 1856[7], and has been used in several medical applications[8]. Toluidine blue-stained peripheral nerve sections obtained from resin-embedded nerve segments allows for clear visualization of nerve structures. Visualization of myelin sheath structure can be enhanced by the use of osmium tetroxide post-fixation[4,9]. Osmium tetroxide is a toxic oxidant and lipid fixative agent that interacts with the double bonds in lipids, resulting in starkly defined lipid-rich myelin sheaths[10]. However, osmium tetroxide is toxic, expensive, requires a longer incubation of nerve segments, and is not always used.

Alternative methods of processing and staining have been developed for visualization of peripheral nerve morphology; Paraffin, cryogenic sectioning, and epoxy resin-embedded nerve sectioning followed by staining with toluidine blue or phenylenediamine solution has been used to quantify morphological changes of peripheral nerve regeneration [11,12]. These methods each have their advantages and yield essential data on the number of axons, myelin thickness, axon diameter, and axon diameter to myelinated fiber diameter (g-ratio) [11,13,14,15].

The primary distinction of the resin-embedding in this protocol is that it facilitates obtaining 1-2 µm thickness cross-sections due to the hardness of the resin while maintaining the histological qualities of the nerve. These thin sections, as opposed to the 4-5 µm thickness sections obtained from paraffin embedding, provide peripheral nerve sections with higher resolution, allowing for a more accurate quantification of axon myelination, such as the g-ratio, that cannot be obtained from thicker sections[16]. While cryogenic sectioning can be used to obtain 1-2 µm

sections, it has been our experience that it is more difficult to obtain sections without numerous large cracks. Such cracked sections can cause inaccurate counting of the number of axons and aspects of myelination.

In addition to toluidine blue staining[17], a silver staining method[18] and Masson's trichrome staining[4] can also be used to show nerve axons. However, using resin embedding of rat median nerve sections stained with either hematoxylin and eosin or Masson's trichrome showed faint myelin sheaths and unrecognized structures, whereas toluidine blue staining showed clear myelin sheath image and easily can be quantified[4]. Despite some limitations, toluidine blue staining of resin embedded peripheral nerves is a valuable technique that can be used when high resolution images of nerve morphology are required.

The primary disadvantage for resin embedding is that it is time-consuming and does not allow for immunostaining of the same tissue due to the difficulty of antigen retrieval when compared to paraffin and frozen embedded sections techniques. Thus, it is not generally possible to utilize the same tissue for immunostaining that is processed via resin-embedding for toluidine blue staining. Although not used here, if immunohistochemistry is desired in resin embedded sections, the use of glycol methacrylate embedding resins allows for immunohistochemistry to be performed on tissue sections, but it is relatively expensive[19]. This can be somewhat mitigated by cutting the peripheral nerve into separate segments, some for resin-embedding and others for immunostaining directly after fixation.

The process of toluidine blue staining of resin embedded peripheral nerves, as with most histopathological analysis, can be broken up into five stages, including fixation, dehydration, embedding, sectioning, and staining[20]. We aim here to provide a protocol and practical guideline for using resin embedded rat sciatic nerve sections stained with toluidine blue to acquire high quality images.

Protocol

Adult Sprague Dawley rats were used in this project and all procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee.

1. Surgery and In *Vivo* Nerve Fixation

NOTE: Vendor information for all materials and equipment used in this protocol are listed in the **Table of Materials**.

NOTE: *In vivo* nerve fixation is used to preserve the tissue and reduce structural degradation that can occur between the time of death and collection of the nerves. *In vivo* tissue fixation is a standard practice for preparation of nervous system tissue for histology, where perfusion is often a precursor to this. The placement and size of peripheral nerves allowed for in situ fixation. We do not recommend collection and fixation of tissue after euthanasia due to the possibility of tissue degradation.

1. Prepare the rat for general anesthesia by placing it in an induction chamber with 2-3% isoflurane, then place the anesthetized rat in the prone position on the top of a dissection mat and maintain at 1-2% of isoflurane via anesthetic nose cone. To ensure adequate depth of anesthesia, test animals for pedal withdrawal at the hind feet and check for the absence of palpebral reflexes at the eyes by touching the medial canthus of the eye.
   NOTE: Intraperitoneal injection of ketamine is a commonly used alternative to inhaled isoflurane.
2. To expose the sciatic nerve, shave the hair of the hind limb(s) and clean the shaved areas with 70% ethanol. With a scalpel or scissors, make a 2-3 cm incision in the skin along the hind limb from the knee up to the greater trochanter, where palpation of the femur using the sterile scalpel to ensure the proper location of the incision. The femur is located just proximal to the most accessible segment of the sciatic nerve. Even though this is a non-survival surgery, maintain sterile techniques.
3. After making the skin incision, locate the plane between the biceps femoris muscle and the gluteus maximus muscle, and using microdissection scissors separate the underlying fascia and expose 2-3 cm of the underline sciatic nerve. To expose a clear section of the sciatic nerve, use a retractor to widen the gap between the two muscles (**Figure 1A**). With fine forceps and iris scissors, carefully separate the nerve from surrounding connective tissue, taking great care not to compress or cut the nerve.
4. Add sufficient Trump's fixative (4% formaldehyde, 1% glutaraldehyde in 1x PBS (phosphate buffered saline) with 1.16 g of NaH2PO4·H2O per 100 mL) into the cavity containing the exposed nerve to cover the nerve and let sit for 10 min. If necessary, place gauze under the hind leg to improve the angle so that more fixative can be added into the cavity. Remove the fixative after 10 min and repeat this step two more times.
5. Using a dissecting microscope, cut the nerve from both sides using fine dissection scissors, making sure not to stretch or pinch the sciatic nerve, and immediately put the nerve sections in 15 mL tubes containing Trump's fixative at 4 °C for one week, changing the fixative every 48 h.
6. Do not leave animals unattended during any part of the surgical procedure. After removal of the nerve, euthanize animals by cervical dislocation while under anesthesia.

2. Osmium Tetroxide Treatment and Resin Embedding

1. Post fixation, carefully remove any remaining fat and connective tissues from the nerve and use a sharp scalpel to cut the nerve into segments approximately 5 mm in length (nerve segments can be separated and labeled in different tubes). Prepare fresh 2% osmium tetroxide diluted in Trump's fixative solution and keep in a glass tube. Immerse nerve segments in 2% osmium tetroxide for 2 h, which can be in plastic tubes for this short period of time.
   NOTE: Some plastic tubes react with osmium tetroxide, causing a darkening of the solution, so prolonged storage of osmium tetroxide in plastic tubes is not recommended.
2. Using an epoxy embedding medium kit, prepare the final embedding formula:

1. Mix the epoxy embedding medium with DDSA solution (dodecenylsuccinic anhydride; mixture A) and mix the epoxy embedding medium with NMA solution (methylnadic anhydride; mixture B). Let both mixtures A and B mix at least 20 min by stirring with a magnetic stirrer.
2. Immediately before use, mix mixture A and B and add the accelerator DPM-30 (2,4,6-tris(dimethylaminomethyl)phenol) in the proportion of 1.5 to 2.0% of the total volume of the mixture. Note that the DPM-30 solution should be measured precisely to prevent the block from becoming dark in color and brittle. All resin chemicals are toxic; take extra care to prevent skin contact or inhalation.

3. Using 1.5 mL tubes, wash nerve segments with PBS and start the dehydration process by placing the nerve segments in different acetone/ distilled water passages: 30%, 60%, 90%, concentrations for 10 min each, then in 100% acetone 3 times for 10 min each. Note that ethanol can be used in the place of acetone.
4. For resin infiltration, place the nerve segments in a mixture of 1 part of the final embedding mixture and 1 part 100% acetone for 30 min, then in a mixture of 2 parts of the final embedding mixture and 1 part 100% acetone for 30 min, and finally place the nerve segments in the final embedding mixture for 30 min.
5. Put the nerve segments into silicone rubber embedding molds (we have used 106 mm length x 71 mm width x 7 mm depth; block size 11 mm length x 6 mm width x 3 mm depth). Gently add the resin on top of the nerves, making sure to cover the whole nerve segments and avoid making air bubbles. Leave the mold containing nerve segments with the resin to polymerize at 60 °C overnight.

3. Sectioning by Ultramicrotome

1. Prepare glass knives using a glass knife maker (**Figure 1B**) and make glass knives with boats(**Figure 1C**), which are used to collect nerve sections floating on the distilled water.
2. Place resin embedded nerve blocks into the ultramicrotome holder with the trapezoidal side facing up. Using the ultramicrotome scope, trim any excess resin surrounding the nerve tissue, and make the block face a trapezoid shape and as close to the longitudinal surface of the nerve as possible using a single-edged blade, such as a razor blade. Do not penetrate the longitudinal surface of the nerve segment, which is recognizable by its dark staining by osmium tetroxide.
   NOTE: Trimming of excess resin will reduce the area of resin to be sectioned and improve blade performance and cutting in subsequent steps.
3. Using the ultramicrotome, make multiple cross sections sufficient to expose a uniform cross section surface of the nerve tissue using a plain glass knife. Once this is done, switch to a glass knife whose boat is filled with distilled water at room temperature and adjust the ultramicrotome to 1-2 µm to cut thin sections.
4. Transfer floating thin sections from the glass knife boat to a drop of deionized water on glass slide using metal loop (**Figure 1D**). Dry slides containing sections by passing several times over a flame for a few seconds, making sure not to overheat the sections. Stain dried sections immediately with toluidine blue or store at room temperature for several days before staining.
   NOTE: Sections can also be dried using a plate warmer at 60 °C for 15 min. A slow drying process makes the sections smooth.

4. Toluidine Blue Staining

1. Prepare 1% solution of toluidine blue by dissolving 2 g of sodium borate in 100 mL of deionized water, then add 1 g of toluidine blue and stir until dissolved. Filter the solution using filter paper (pore size: 11 µm) and keep the solution in an opaque bottle at room temperature for up to two weeks.
2. Using a plastic pipette or micro pipettor, add a drop of toluidine blue solution on the top of the nerve sections and leave for 20-30 s.
3. Rinse off all toluidine blue solution excess by gently dipping the slides into deionized water jar and repeat 3-4 times until sections are clear. Dry the slides at least 15 min at 60 °C or overnight at room temperature and then cover the sections with a coverslip using regular mounting medium. Examine the mounted slides immediately or store at room temperature.
4. Examine under a light microscope. A 100X oil immersion lens is recommended for detailed images used to calculate g-ratios.

Representative Results

Resin embedded peripheral nerve sections stained with toluidine blue allow for accurate histological data quantifications. An overview of the procedure is shown in (**Figure 2**). Sciatic nerves sections embedded in resin medium and stained with toluidine blue showed clear images with optimal resolution (**Figures 3**). Nerve damage can cause many changes in nerve morphological structures, for example, changes in nerve fiber, axon diameter, and myelin sheath thickness. This method can preserve nerve structure in its natural form, which facilitates the measurement of several parameters such as the ratio of axon diameter to the total fiber diameter (the g-ratio; **Figure 4**). A variety of factors can lead to less than optimal peripheral nerve sections including the presence of cracks (**Figure 5A**) in the section due to improper handling of the nerve. Holes can also occur in the section (**Figure 5B**) due to insufficient dehydration. Another possible error in the procedure is folding of the sections (**Figure 5C**), which can be remedied by using inoculating loops for section transfer onto the glass slide.

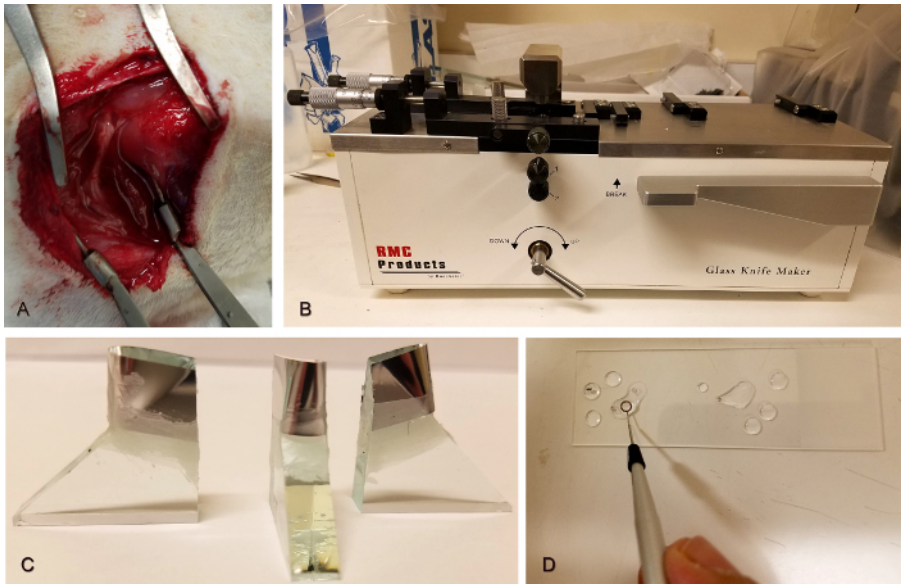


Figure 1: Surgery and sectioning. (A) Rat sciatic nerve during *in vivo* fixation. **(B)** Glass knife maker **(C)** Glass knives with boat. **(D)**Metal loops used to transfer floated thin sections from the glass knives to a drop of deionized water on glass slide. Please click here to view a larger version of this figure.

Figure 2: Schematic of the method used in this protocol. (A) Adult Sprague Dawley rat is anesthetized, followed by *in vivo* nerve fixation **(B)** and nerve collection **(C)**. The next step is post-fixation with 2% osmium tetroxide **(D)** and resin embedding **(E)**. Resin embedded nerve sections are then trimmed **(F)** and sectioned **(G)** using an ultramicrotome. Finally, nerve sections on glass slide are stained with toluidine blue **(H)**. Please click here to view a larger version of this figure.

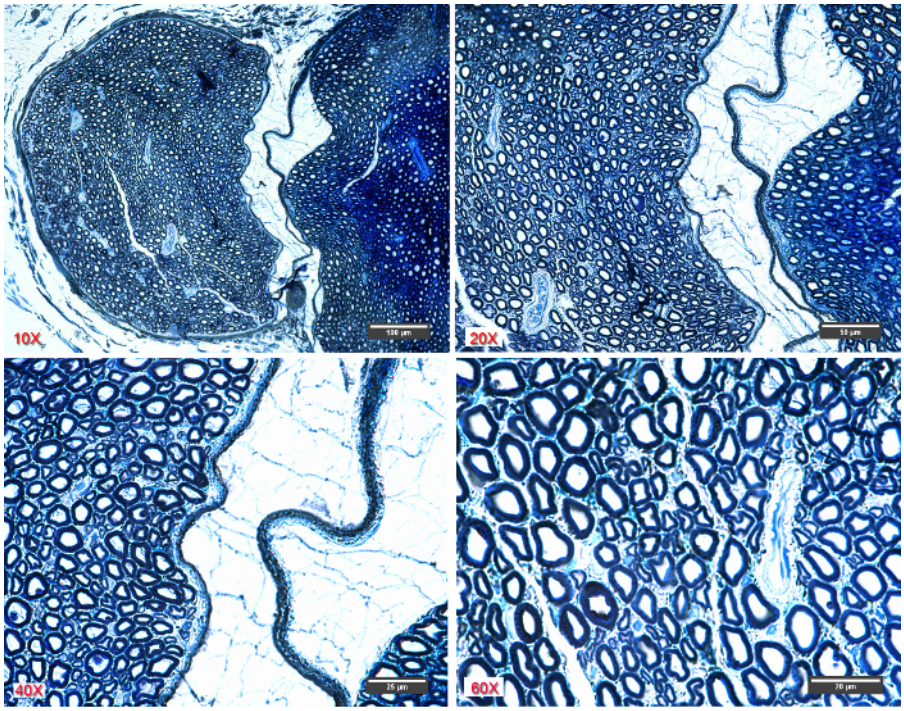
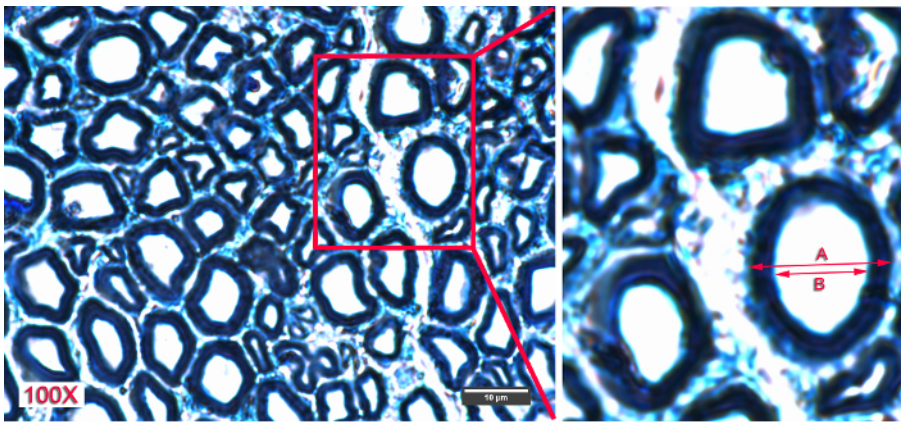


Figure 3: Transverse section of a rat sciatic nerve stained with toluidine blue. Shows sections under different magnifications (10X, 20X, 40X, and 60X) of clear image with optimal resolution. Please click here to view a larger version of this figure.



The g-ratio = (axon diameter B)/(fiber diameter A)

Figure 4: Transverse section of a rat sciatic nerve stained with toluidine blue. This high-resolution image (100X) can be used to measure the ratio of axon diameter to the total fiber diameter (the g-ratio). Please click here to view a larger version of this figure.

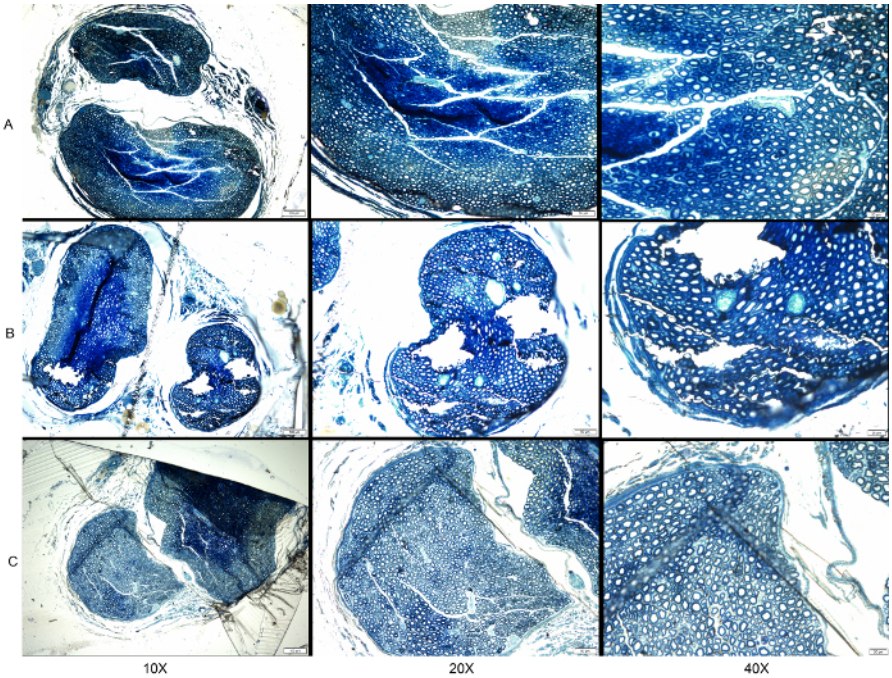


Figure 5: Transverse sections of a rat sciatic nerve stained with toluidine blue under different magnifications. Shown aresome of the problems that may be encountered during performance of this protocol. **(A)** and **(B)**. The presence of cracks and holes inside the nerve section caused by improper handling of the section and insufficient dehydration of the tissue, respectively. **(C)**. Folding of the sections, which may happen during transferring the sections from the glass knives to the slide. Please click here to view a larger version of this figure.

Discussion

Examinations of the morphological structures of peripheral nerve injury and regeneration are frequent subjects of study[13]. In this protocol, we describe the steps to obtain high quality images for histological data quantifications using rat sciatic nerve tissue embedded in resin blocks and stained with toluidine blue. This technique provides an image of nerve morphology in which the nerve regeneration can be quantified by measuring the number of axons, degree of myelination, presence of infiltrative fibrotic tissue, and the health nerves. While the images show sectioning and processing of uninjured nerves as samples, the same steps and materials are applicable to injured nerves and nerves regenerated with conduits or tissue grafts, provided that fixation of the tissue is adequate[20,21].

While all steps of the protocol are essential, some of these steps are more likely to lead to poor quality sections or images. Insufficient tissue dehydration may lead to holes in the nerve sections (**Figure 5B**). One possible explanation is the immiscibility of resin with water, and excess water in the tissue prevents resin from infiltrating the tissue. Therefore, allowing sufficient time and the use of absolute acetone is essential to ensure proper dehydration step. Although we used acetone in this protocol, ethanol can also be used. Many resins, however, are not reactive with ethanol, so the tissue must be treated with propylene oxide to serve as a transition between the ethanol dehydration and resin embedding.

Due to the thinness of the sections, transferring nerve sections from the glass knife to the glass slide should be done with great care (**Figure 5C**). 1-2 µm sections are very fragile, and improper transfer of the section could cause the section to break. If the section is picked up in the central portion of the section with a needle, the section is prone to folding in on itself and often will be difficult to unfold when transferred to the slide. Different tools can be used for the transfer of the section to the glass slide, including needles and loops, and each should be tested for the specific user to determine which will yield the best transfer results. For our purposes, using a loop that could encompass the entire section greatly reduced the folding of sections when they were transferred from the glass knife to the microscope slide (**Figure 1D**).

In general, proper handling of the nerves is essential as compression of the nerves can cause cracks to occur in nerve sections (**Figure 5A**). Nerve tissue compression can happen during transfer of nerve segments in the processes of exposure in the animal, fixation, dehydration, and resin embedding. To avoid nerve tissue compression, nerve segments should be transferred by fine forceps and picked up on one end of the segment ideally by just the epineurial layer, so the entire nerve will not be affected. The presence of cracks and lines (knife marks) in the nerve sections may also occur due to dull or overused glass knives. To guarantee optimal sectioning, we recommend making fresh glass knives and changing the glass knife every 25-30 cuts, fewer if sectioning through nerve encased within a conduit.

Resins can be relatively expensive, are inadequate when longitudinal nerve sections are required, and can require expensive tools such as an ultramicrotome and a glass knife maker. Despite these limitations, toluidine blue staining of resin embedded peripheral nerve sections is still considered the gold standard for visualization of cross sections of peripheral nerve morphometry[4,5]. Longitudinal sections can also reveal important features of peripheral nerves, such as axonal continuity and nodes of Ranvier, but is most useful with immunohistochemistry. In such cases it is the general practice to differentially process two sections of the same nerve, one for toluidine blue cross sections and the other for longitudinal immunohistochemistry. Due to increased incidences of peripheral nerve trauma and injuries and the needs of a better way of assessing nerve morphological structures, this method can continue to be applied as an essential tool to gain histological data of nerve damage and regeneration.

Disclosures

The authors declare that they have no conflicts of interest.

Acknowledgements

The authors would like to thankthe Jenkins Microscopy Facility at the University of Wyoming for their help, and the members of the Bushman lab, Kelly Roballo, Hayden True, Wupu Osimanjiang and Subash Dhunghana, for assistance in animal care. This publication was made possible by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grant # 2P20GM103432.

References

1. Zacchigna, S., Ruiz de Almodovar, C., Carmeliet, P. Similarities between angiogenesis and neural development: What small animal models can tell us. *Current Topics in Developmental Biology.* **80**, 1-55 (2008).
2. Grinsell, D., Keating, C. P. Peripheral nerve reconstruction after injury: A review of clinical and experimental therapies. *BioMed Research International.* **2014**, 1-13 (2014).
3. Chen, M.B., Zhang, F., Lineaweaver, W.C. Luminal fillers in nerve conduits for peripheral nerve repair. *Annals of Plastic Surgery.* **57** (4), 462-471 (2006).
4. Scipio, F., Raimondo, S., Tos, P. Geuna, S. A simple protocol for paraffin-embedded myelin sheath staining with osmium tetroxide for light microscope observation. *Microscopy Research and Technique.* **71**, 497-502 (2008).
5. Raimondo, S., Fornaro, M., Di Scipio, F., Ronchi, G., Giacobini-Robecchi, M. G., Geuna, S. Chapter 5: Methods and protocols in peripheral nerve regeneration experimental research: part II-morphological techniques. *International Review of Neurobioly.* **87**, 81-103 (2009).
6. Carriel, V., Garzon, I., Alaminos, M., Campos, A. Evaluation of myelin sheath and collagen reorganization pattern in a model of peripheral nerve regeneration using an integrated histochemical approach. *Histochemistry and Cell Biology.* **136**, 709-717 (2011).
7. Sridharan, G., Shankar, A. A. Toluidine blue: A review of its chemistry and clinical utility. *Journal of Oral and Maxillofacial Pathology.* **16**, 251-255 (2012).
8. Epstein, J.B., Scully, C., Spinelli, J. Toluidine blue and Lugol's iodine application in the assessment of oral malignant disease and lesions at risk of malignancy. *Journal of Oral Pathology & Medicine.* **21**, 160-163 (1992).
9. Carriel, V., Garzon, I., Alaminos, M., Cornelissen, M. Histological assessment in peripheral nerve tissue engineering. *Neural Regeneration Research.* **9,** 1657-1660 (2014).
10. Dykstra, M. J. *A manual of applied techniques for biological electron microscopy.* New York: Plenum. 257 pp (1993).
11. Vleggeert-Lankamp, C. L. The role of evaluation methods in the assessment of peripheral nerve regeneration through synthetic conduits: A systematic review. *Journal of Neurosurgery.* **107** (6), 1168-1189 (2007).
12. Williams, P., Wendell-Smith, C.P., Finch, A., Stevens, G. Further uses and methods of processing of fresh frozen sections of peripheral nerve. *Journal of Cell Science.* **3** (69), 99-105 (1964).
13. Castro, J., Negredo, P., Avendaño, C. Fiber composition of the rat sciatic nerve and its modification during regeneration through a sieve electrode. *Brain research.* **1190**, 65-77 (2008).
14. Raimondo, S., Fornaro, M., Di Scipio, F., Ronchi, G., Giacobini-Robecchi, M.G., Geuna, S. Methods and protocols in peripheral nerve regeneration experimental research: Part II-morphological techniques. *International review of neurobiology.* **87**, 81-103 (2009).
15. Bozkurt, A., Lassner, F., O'Dey, D., Deumens, R., Böcker, A., Schwendt, T., Janzen, C., Suschek, C.V., Tolba, R., Kobayashi, E., Sellhaus, B. The role of microstructured and interconnected pore channels in a collagen-based nerve guide on axonal regeneration in peripheral nerves. *Biomaterials.* **33** (5), 1363-1375 (2012).
16. Weis, J., Brandner, S., Lammens, M., Sommer, C., Vallat, J. M. Processing of nerve biopsies: A practical guide for neuropathologists. *Clinical Neuropathology.* **31**(1), 7.-23 (2012).
17. Battiston, B., Tos, P., Geuna, S., Giacobini-Robecchi, M. G., Guglielmone, R. Nerve repair by means of vein filled with muscle grafts. II. Morphological analysis of regeneration. *Microsurgery.* **20** (1), 37-41 (2000).
18. Bhattacharyya, T.K., Thomas, J. R. Comparison of staining methods for resin-embedded peripheral nerve. *Journal of Histotechnology.* **27**, 161-164 (2004).
19. Zbaeren, J., Zbaeren-Colbourn, D., Haeberli, A. High-resolution immunohistochemistry on improved glycol methacrylate-resin sections. *Journal of Histotechnology.* **30** (1), 27-33 (2007).
20. Alturkistani, H.A., Tashkandi, F. M., Mohammedsaleh, Z. M. Histological stains: A literature review and case study. *Global Journal of Health Science.* **8** (3), 72-79 (2016).
21. Ezra, M., Bushman, J., Shreiber, D., Schachner, M., Kohn, J. Porous and nonporous nerve conduits: the effects of a hydrogel luminal filler with and without a neurite-promoting moiety. *Tissue Engineering Part A.* **22**(9-10), pp.818-826. (2016).
22. Bhatnagar, D., Bushman, J.S., Murthy, N.S., Merolli, A., Kaplan, H.M., Kohn, J. Fibrin glue as a stabilization strategy in peripheral nerve repair when using porous nerve guidance conduits. *Journal of Materials Science: Materials in Medicine.* **28**(5), p.79. (2017).

