

Tracking of Oligodendrocyte Remyelinated Axons in Spinal Cords

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Abstract

Demyelination, the loss of the myelin sheath that insulates axons, is a prominent feature in many neurological disorders including multiple sclerosis (MS) and spinal cord injury (SCI) [11, 3]. As a result of demyelination, signals are disrupted within the axons, leading to the loss of motor and other functions. The myelin sheath can be replaced by a process called remyelination, which can be initiated by transplanting stem cell derived oligodendrocyte progenitor cells into the injury site. Animal models have shown the effectiveness of this treatment for partially restoring the myelin sheath and some of the motor function.

We describe a method for automated tracking of the fate of these progenitor cells. The method uses cross-sectional histology, high-resolution imaging, and newly developed digital image processing algorithms to distinguish between regular oligodendrocyte-myelinated axons, newly oligodendrocyte-remyelinated axons and Schwann cell-remyelinated axons, and to track these cell types over time. The method provides an accurate count for each cell type, which is consistent with results obtained from a domain expert by means of cross-verification.

The method, which is based on high-resolution microscopic imagery and multiple time series, can be used for temporal tracking of the amount of newly remyelinated axons after human embryonic stem cell (hESC) treatment.

1. Introduction

After spinal cord injury (SCI), the human body uses two kinds of cells – oligodendrocytes and Schwann cells [12, 2] – to wrap myelin around the demyelinated axons. This process is necessary to restore the conduction of signals within axons. However, animal models of SCI suggest that remyelination is incomplete and that demyelination progressively continues [13]. One of the many therapies being developed to improve the remyelination includes transplantation of oligodendrocyte progenitor cells into the adult spinal cord of rats following SCI.

It is important to identify the remyelination that is attributable to therapies so as to appreciate their effectiveness in addressing the issues of demyelination at the cellular level. Oligodendrocyte-remyelinated axons are identified by their characteristically thin myelin sheaths relative to the diameter of the axons. This ratio of myelin sheath thickness to axon diameter is called the G-ratio. In addition to the G-ratio, the lighter intensity of the myelin sheath of the oligodendrocyte-remyelinated axons is taken into account while distinguishing them from the darker, more compact, Schwann cell-remyelinated axons [13, 7].

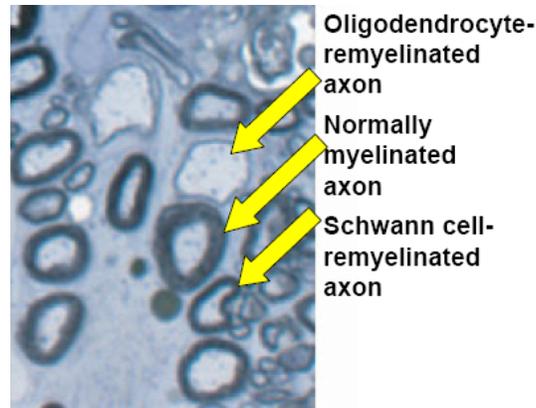


Figure 1. Three types of myelination. The goal of this work is to identify oligodendrocyte-remyelinated axons.

An additional challenge for the image processing algorithm is the size of the images obtained from the microscope (approx. 66 mega-pixels per cross-section = 200 MB of uncompressed RGB image data). Typically, tens of cross-sections are obtained per time step and per animal, and images are taken after one day, after two days, after four days, etc., resulting in more than 100 images for a single study, or multiple giga-bytes of image data.

The amount of image data produced in a single study is prohibitive for accurate manual counting. Therefore, an automated method is required to obtain the specific cell counts necessary for statistical hESC fate tracking.

Figure 1 shows a close-up (229 x 312 pixels = 0.1%), while figure 2 shows an entire cross-section (100%).

2. Background

Digital image cytometry, the analysis of cell attributes from microscopy images, serves as an essential component of biomedical research. Cytometry can be carried out *manually* or by *automated* algorithms. One of the current methods for identifying the cells wrapped around remyelinated axons is the line sampling technique [3] that is achieved *manually* by computer-assisted microscopy. There are two kinds of error introduced in manually estimating and classifying the remyelinated axons. Remyelinated axons are analyzed on $5 \times 625 \mu\text{m}^2$ areas aligned on a radial oriented line that originates from the central canal of the spinal cord and extends to the outermost limit of the spinal cord cross section.

The remyelinated axons are identified in small areas and estimated for a larger total area of pathology. On average, manual identification of occurs only in approximately 15.6% of the actual area of pathology. This leaves out a high percentage of estimation in which a large amount of error – the estimation error – can be introduced to the data. In addition to the estimation error, there is classification error which is because of the subjectivity involved in the manual classification of the axons. Basically, distinguishing oligodendrocyte-remyelinated axons is subjective. It is not known how much error is introduced to the data when there are multiple examiners counting these axons. As a result, it is important to be able to normalize the error across examiners with an automated method of identification.

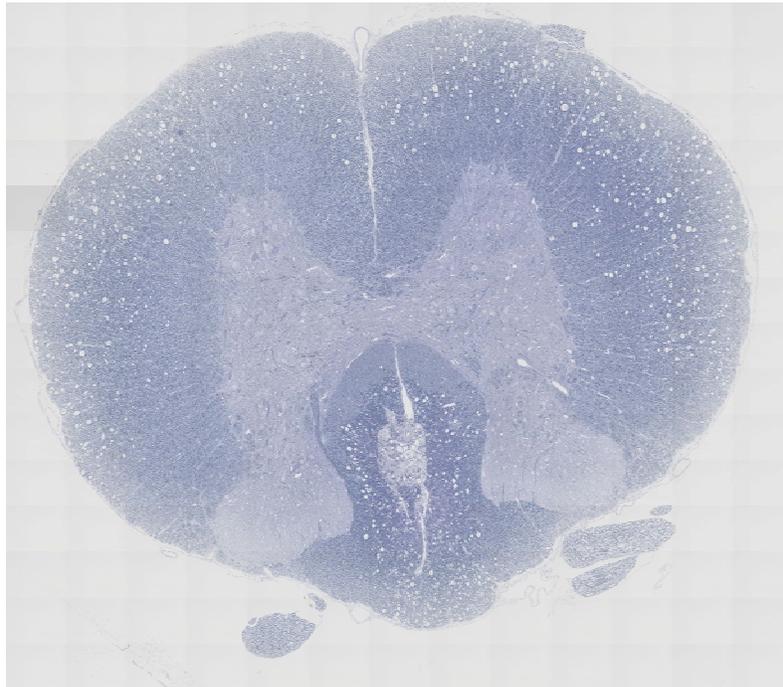
In addition to these errors, the G-ratios, when manually measured and calculated are influenced by the examiners subjectivity. Moreover the G-ratio is not calculated for every

single axon because this would be a tedious and time intensive work. This again leads to more classification errors.

It is difficult to reduce these errors by increasing the percentage of the actual area of pathology. Typically, axons are identified in a $1\mu\text{m}$ thick slice every 2mm per animal. Therefore, in projects involving many groups, identifying can take several weeks. An increase in percentage of counted area would not only increase the analysis time, but might also increase the error due to human factors including fatigue. With an automated system, a higher percentage of area of pathology can be analyzed at a more frequent interval for higher accuracy, with less subjective classification, and with less human fatigue.

Thus, it is evident that *automated* recognition and classification of the axons would be imminent in reducing the turn-around-time and the subjective error in this research. Current automated approaches include shape-based analysis of cells [14, 5, 1] and the Watershed method as a shape-independent classifier, which is very susceptible to noise [6, 10]. Separation of cell boundaries to detect individual cells is an active area of research [9, 4, 8, 15]. Jones et al. [8] used a non-Euclidean Voronoi diagram on Riemannian manifolds to detect cell boundaries and segment cells based on known cell nuclei.

In this work we introduce robust, shape-independent algorithmic solutions to automatically detect and classify the oligodendrocyte-remyelinated axons, and to track their fate over time. Since we are using different animals, we cannot track the fate of individual cells. This can only be done in an in-vivo study, for instance, using fluorescent or radionucleotide markers. We are using a statistical approach instead.



**Figure 2. Histology seven days after hESC injection.
(8,944 x 7,785 pixels)**

3. Challenges in Automated Remyelination Type Classification

Several artifacts in the images make automated remyelination type classification challenging. The fact that the images are usually littered with large amounts of cellular debris, i.e., proteins and other cell bodies, require the algorithm to be robust in terms of accurate cell identification. On top of this, the classifier must be shape-independent as Schwann cells and oligodendrocytes do not conform to any particular shape. In particular, axons are not necessarily convex.

Other artifacts, such as varying intensity levels across the image, also need to be addressed. The latter can be resolved by introducing a local gradient based method to identify cell boundaries.

4. Image Segmentation and Classification

Our technique first detects the axon boundaries progressively using isocontours with varying gradient levels. Compared to an intensity-based method, the gradient-based method makes the algorithm robust to changes in intensity levels caused by different illumination, slice thickness, staining, stray light, and other factors. Gradient-based isocontouring is independent of shape and can be computed for all myelin sheath types regardless of their absolute intensity values.

The detected structures are subsequently analyzed for unwanted features including cellular debris, which are then removed. Finally, the remyelinated axon parameters including the G-ratio are calculated. The oligodendrocyte-remyelinated axons are then detected by evaluating this quantity.

5. Results

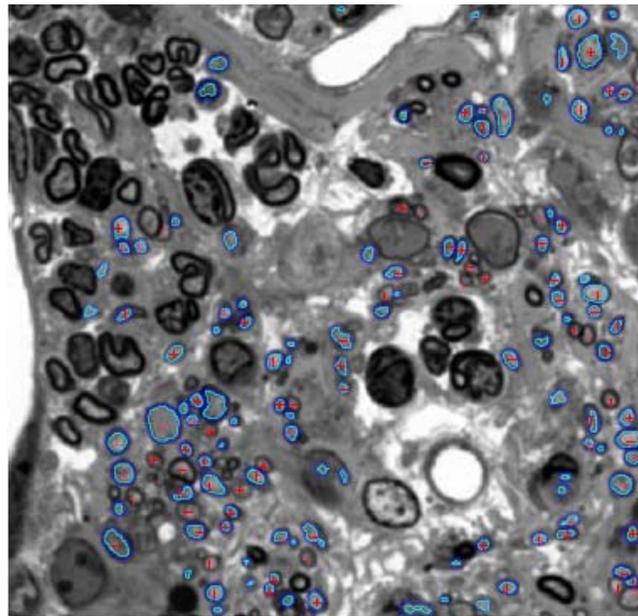


Figure 3. Manual (red) vs. automated (cyan) classification.

Figure 3 shows the results of manual vs. automated classification. The red markers correspond to the manual mark-up of the image. The cyan contours refer to the automated

segmentation and classification. The images (488 x 470 pixels, total: 255 axons) exposed a good correlation between the manual (count: 92 axons) and the automated method (count: 100 axons) for identifying oligodendrocyte-remyelinated axons. These results have been confirmed on other similar-sized and larger images which cannot be shown here.

6. Conclusions

The gradient-based progressive isocontouring for cell detection is a generic pre-processing method to detect closed shapes. We have developed a robust method for stem cell fate tracking based on a G-ratio classification.

Our success in 2D geometry based post-processing of structures for axon-separation and noise removal might evoke great interest in the newly developing bio-geometry community. This automated classification of oligodendrocyte-remyelinated axons has also injected a lot of excitement among the neurobiologist collaborators. This project will relieve them of several weeks of pain-staking routine, repetitive, and mundane task that consumes several hours of trained people power. The accuracy of our detection and classification results have been corroborated, appreciated, and accepted by them and on account of its reliability, we are sure these methods will find widespread applicability reducing the turnaround time of their research.

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