Morphometric Analysis of Sciatic Nerve Images: A Directional Gradient Approach

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Abstract—The extraction of morphometric features from images of biological structures is a crucial task for the study of several diseases. Particularly, concerning neuropathies, the state of the myelination process is vital for neuronal integrity and may be an indicator of the disease type and state. Few approaches exist to automatically analyse nerve morphometry and assist researchers in this time consuming task.

The aim of this work is to develop an algorithm to detect axons and myelin contours in myelinated fibres of sciatic nerve images, thus allowing the automated assessment and quantification of myelination through the measurement of the g-ratio. The application of a directional gradient together with an active contour algorithm was able to effectively and accurately determine the degree of myelination in an imagiological dataset of sciatic nerves. It was obtained an average error of 1.80%, in comparison with the manual annotation performed by the specialist in all dataset.

I. INTRODUCTION

In nervous tissues the achievement of correct transmission of nerve impulses requires the electrical insulation of the axon by myelin, a highly specialised membrane with a high lipid:protein content that allows efficient saltatory conduction [1]. Oligodendrocytes in the Central Nervous System (CNS) and Schwann cells in the Peripheral Nervous System (PNS) are the glial cells responsible for myelinating axons. The process of myelination, as well as the maintenance of the myelin sheath are crucial for neuronal and axonal integrity and function. Loss of myelin (demyelination) has a vast impact in nervous tissue and in human health. Demyelination in the CNS causes very debilitating diseases (e.g. X-linked Adrenoleukodystrophy, Krabbe’s disease), collectively called leukodystrophies [2]. In the PNS the loss of myelin causes peripheral neuropathies typified by Charcot-Marie-Tooth, one of the most common neurological disorders [3].

The study of the myelination process is of fundamental importance to identify and understand human disorders as well as to investigate putative therapeutic options. A crucial aspect to characterise peripheral neuropathies is to determine the degree of myelination. This is achieved by determining the g-ratio, defined as the ratio of the axon diameter to the fibre (axon and its myelin sheath) diameter. The average g-ratio value of 0.65 represents normal myelination, whereas higher g-ratio values indicate loss of myelin or impaired myelination [4].

The process of counting the number of structures, to measure the g-ratio, perimeter and other important variables is nowadays extremely time consuming and associated to human errors [5]. Researchers select small image regions from each nerve section (Fig. 1), count the axons and measure their g-ratio (using image editing software such as Photoshop). Thus, an automatic and accurate method for this purpose may be the best solution.

Several research groups have made attempts for developing automatic tools for analysing images of nervous structures. The structure of the nervous fibre as well as the method used to obtain the image greatly change the problem and the approach needed for its analysis. Romero et al. [6] suggested a method for segmentation and morphometry, starting with a rough thresholding which is lately improved using connected morphological operators and zonal graphs. This method is applicable to images obtained with electronic microscopy. This images have higher amplification, which leads to much more detail but only capture a small part of the nerve. Silva et al. [5] suggested the use of a software to perform automatic contrast enhancement and a threshold segmentation based on the pixel color and brightness allowing the user to manually separate the fibres that are connected. This algorithm ignores the smaller...
axons, most likely making g-ratio values biased. Zhao et al. [7] suggested an algorithm starting with a coarse segmentation, obtained with a region growing algorithm that combines feature and spacial information together. The segmentation is then improved by identifying axon candidates and removing false axons. Finally, the algorithm separates the fibers where their myelin sheaths are together, using the maximum gradient magnitude. This method was also developed for electron microscopy, and tested with images of the optical nerve. These fibers have a much higher axon density, and their myelin sheaths is usually too close together, which is a different problem than the sciatic nerve.

Although the methods presented before may represent a solution for the problem described in this work, they still present some limitations. Some of them are based on semi-automatic approaches, still requiring input from the user, which could lead to different results depending on the subjectivity of the user. However, the creation of a total automatic tool could cause an additional problem: the presence of false positives and miss detections. Because of this, it is very important to evaluate the intermediate tasks of the process, which are not also normally performed by the researchers.

This work applies a generic biomedical image segmentation approach intended for the assessment of fibre morphometry in sections of sciatic nerves. Our methodology performs firstly a detection of axons’ centroids with a bottom-hat operation and then detects myelin contours combining an active contours algorithm with a directional gradient in order to achieve a successful morphometric analysis. The methodology presented in this work, not only permits to achieve a good g-ratio in comparison to the value measured by the specialist, but also evaluates the performance of the intermediate tasks, i.e. axon contours and the morphometry. The achieved results proved to be accurate for a significant number of fibers in terms of g-ratio measurement and axon contour error.

II. METHODOLOGY

The problem of performing morphometric analysis of images of nerve sections can be separated into two distinct issues: segmentation and feature extraction. The high-level operations that compose the proposed algorithm are presented in Fig. 2.

![Algorithm flowchart.](image)

Center detection was based on a sequence of morphological operations that were applied to the images. The first step was to perform a bottom-hat operation [8] with a disk shaped kernel of 20 pixels in order to enhance contrast. The kernel radius adopted in this task, was defined empirically based on the microscope’s magnification used during image acquisition process.

After applying binarization, several structures appeared to be connected which would compromise the upcoming contour detection phase. To solve this, an Euler Number approach was used. The Euler Number of an image is the number of objects in that region minus the total number of existing holes. Since each object (as binary image) itself can be segmented into an isolated structure composed by a myelin sheat (contour) and an aperture (axon) an Euler Number of zero is expected. If Euler Number is positive then the segmented object has more than one aperture. This happens if upon binarization two or more axons appear connected. For those axons, a morphological erosion operation is applied recursively until the initial linked regions became disconnected (i.e. the Euler Number equals zero) or a predefined number of iterations is achieved. After this iterative process, if any structure still present an Euler Number greater than zero is not considered for the next steps of the algorithm.

Centroids are then defined as the centre of mass of the binary objects. It is important to refer that at this stage it is only important to detect a centroid that is within the edges of the objects, not needing to correspond to the exact geometrical centre.

B. Contours Detection

The problem of detection of closed contours in an image can be solved in different ways: making assumption about the characteristics of the content present in the image; incorporating low-, mid-, and high-level shape priors; or based on contour completion, which is a powerful tool to detect closed regions.

Active contours (or snakes) [9], which are a special case of deformable surfaces, can be seen as a good option for this specific problem. Based on energy minimization, and guided by external and internal forces influenced by the image, the contours are pushed towards nearby edges, allowing accurate edge detection. Many problems have been addressed using active contours such as the detection of edges and lines, motion tracking, and stereo matching [10], [11], [12], [13], [14]; however, they have some limitations. As stated before, snakes are based on energy minimization, thus, gradient information influences directly the performance of the algorithm, and the use of a typical energy function may not achieve the expected results (as will be proved in Section III).

Active contours are represented parametrically as:

$$v(s) = (x(s), y(s))$$

where $x$ and $y$ are the coordinates along the contour, and $s \in [0, 1]$ is the parametric domain. The energy function to be
Fig. 3. Image histograms of the RGB channels of Fig. 1. The histogram of the green channel displays a deeper valley between the background and foreground areas which is an indicator of a higher contrast.

where \( E_{\text{snake}} = \int_0^1 E_{\text{int}}(v(s)) + E_{\text{image}}(v(s)) \, ds \) (2)

de notes the internal energy that controls the arrangement of the snake points, and hence the way the contour can stretch and bend; and \( E_{\text{image}} \) is the edge attraction term. The purpose of \( E_{\text{image}} \) is to attract the snake towards the boundaries of the target object. Typically, \( E_{\text{image}} \) is given by the gradient of the image computed using a traditional convolution 2-D kernel at each contour point [9].

1) A Radial (Directional) Gradient: In this work, a directional gradient to replace the \( E_{\text{image}} \) term of the general equation of active contours model is proposed. Intuitively, axon boundaries manifest themselves as a change in the grey-level values of the pixels, thus originating an edge in the resulting image. Therefore, interpreting the image as a graph with each pixel as a node and edges connecting adjacent pixels, the axon contour corresponds to a low-cost path through edge pixels, with the appropriate weight function. The derivative along each radial line, with the origin of the coordinates in the centres computed before, is given by:

\[
G_\theta(r) = \frac{f(r+h) - f(r-h)}{2h} 
\]

where \( h = 1 \) and \( r \) is the radius.

Depending on inner or outer contour, positive and negative gradient, respectively should be considered. In order to standardise the methodology, the energy information used to compute outer contour is inverted. The result is a specific gradient map for the computation of each contour individually, as illustrated in Fig. 4.

The weight function considered was set as a nonlinear function of the derivative (motivated by previous work on similar settings [15]):

\[
f(d) = f_t + (f_h - f_t) \frac{\exp(\gamma(255 - d)) - 1}{\exp(\gamma 255) - 1}, \quad (4)
\]

with \( f_t = 2, f_h = 32, \gamma = 0.025 \) and \( d \) equal to the magnitude of the derivative.

C. Morphometry

The g-ratio, as previously stated, is the ratio between the diameter of the axon (inner diameter) and the diameter of the whole structure, the axon and its involving myelin sheath (outer diameter). The challenge when it comes to analyzing these structures is that they are not circular, which means that the value of the diameter is different depending on the position of the axis system used. On the manual process, researchers just include in the sample the structures that resemble a circle and compute their diameters assuming that approximation. The proposed automatic approach tries to mimic the manual procedure, computing both inner and outer diameters using the area and perimeter of the circle as follows:

\[
\text{Diameter}_P = \frac{P}{\pi} \quad (5)
\]

\[
\text{Diameter}_A = 2 \times \sqrt{\frac{A}{\pi}} \quad (6)
\]

where \( \text{Diameter}_P \) and \( \text{Diameter}_A \) are the diameter computed using the perimeter \( P \) and the area \( A \) of a circle, respectively. The g-ratio value is then compute such as:

\[
g-\text{ratio} = \frac{\sum_{i=1}^N \text{Diameter}_{\text{inner}[i]} / \text{Diameter}_{\text{outer}[i]}}{N} \quad (7)
\]

Since the inner and outer diameter were calculated by two different approaches (one from the area and another from the perimeter) the final g-ratio value is the average of these two. The g-ratio of the whole nerve section is the mean of the g-ratio of all the \( N \) structures in the image.
III. RESULTS

The acquisition and further annotation of sciatic nerve images is not an easy task due to: (1) the amount and diversity of axons that exist in the nerve, (2) the inherent artefacts caused by tissue processing for microscopic analyses, (3) availability of specific, high contrast biological stains that enable automated analyses, (4) the time consuming effort, and (5) the increased number of biological samples needed for a significant evaluation.

In this study Sciatic nerves from 10 adult wild type mice were dissected and fixed by immersion on 4 %glutaraldehyde in 0.1M sodium cacodylate buffer pH7.4 during 7 days. The entire area of the nerve was photographed on an Olympus optical microscope equipped with 40x objective and an Olympus DP 25 camera. Images acquired using Cell B software were aligned and stitched using Photoshop. For automated assessment, the dataset used comprised 10 images of the different sciatic nerves, and two square regions of each image of the nerve section were selected for evaluation purposes.

The evaluation of the algorithm was performed in two steps, supported by manual annotations made by a specialist. First, a comparison between the proposed algorithm and the conventional snakes algorithm was made, in order to evaluate the effect of replacing the conventional gradient by the proposed radial gradient (Section III-A). Then, the validation of the proposed algorithm was performed (Section III-B).

A. Radial versus Conventional Gradient

Concerning the comparison between the proposed algorithm and the conventional snakes method, both algorithms were tested in two regions of a nerve section (Fig. 5). These regions were selected by the specialist in the same way as in the manual process and, then, manually annotated, in terms of the area, perimeter and g-ratio.

The parameters used by both algorithms were empirically defined based on the available image dataset and remained the same in all the experiments. The conventional and the radial snakes were both initialised with a circle with a radius of 2 automatically centred by the values obtained by the method presented in Section II-A. While this circle is used to initialise the evolution of the inner contour, the outer contour starts from the inner contour previously obtained. Therefore, no user intervention is required in the initialisation procedure of both algorithms. Table I summarises all snakes-related parameters used in both algorithms. The parameters were set differently based on the properties of each gradient, so that each one would achieve the best possible results. The gradient map used in the conventional snakes algorithm was computed with the Sobel operator using a $3 \times 3$ kernel.

The comparison results of both approaches are summarised in Table II and the inner and outer contour detections using both our algorithm and conventional snakes are presented in Fig. 5. The proposed algorithm provided g-ratio values very close to the manual g-ratio measurements, with an average error of $0.647\%$ (Region 1) and $0.691\%$ (Region 2) against an average error of $3.980\%$ (Region 1) and $3.789\%$ (Region 2) obtained using the conventional snakes algorithm. The proposed approach outperformed the conventional algorithm regarding area and perimeter measurements. The improved performance of the proposed method is also shown using some illustrative examples (see Fig. 6). As demonstrated in both regions of Fig. 6, there are three main groups of axons in which the proposed approach overcome the conventional gradient algorithm.

One group corresponds to the axons with a thick myelin sheat, as illustrated in the middle images of Fig. 6(a). In this case, the outer contour of the axon was just correctly detected using the proposed approach due to the larger capture range of the radial gradient when compared to the conventional gradient.

The second group corresponds to the axons in which there is a smooth transition between the axon and the inner contour (see upper and bottom images of Fig. 6(a)). This happens because, in the conventional gradient map, the gradient magnitude of the outer contour is much larger than gradient magnitude of the inner contour. The result is an incorrect

![Fig. 5. Overview of the detection of centres, inner and outer contours of axons from a sciatic nerve section. The regions considered were extracted from the nerve section shown on Fig. 1. A and B correspond to a contour detection using the snakes algorithm while C and D using the proposed method.](image)
evolution of the snake from the inner contour to the outer contour. Consequently, the outer contour is not also correctly segmented as its segmentation depends on the detection of the inner contour. On the other hand, the radial gradient map does not demonstrate this limitation since the inner and outer contours regions have different opposite gradient values.

The third group correspond to the segmentation of adjacent axons. As illustrated on the bottom images of Fig. 6(b), while the radial gradient approach provides a correct contour detection even in these cases, the outer contour was not correctly segmented using the conventional gradient since it evolved to the contour of the adjacent axon. The better performance of the radial gradient approach, when it comes to the segmentation of adjacent contours, is mainly due to the fact that the radial gradient map just promotes the enhancement of the outer contour of the axon that is being segmented, while suppresses the gradient in the outer contours of its adjacent axons (see Fig. 4).

Fig. 7 shows some examples of axons from both regions 1 and 2 in which the contours, obtained with our methodology, were not correctly detected. This occurs mainly, as it is visible, in axons with more irregular shapes along with pronounced concavities. As the directional gradient is computed making use of a set of radial lines from the centre of the axon to the outside, the regions that cannot be entirely covered by this radial lines will have a low gradient value, leading to an incorrect contour detection.

Overall, the inner contour average error, regarding both area and perimeter, was always larger than the outer contour average error independently of which kind of gradient was used. This is interpreted as due to the irregularity and pronounced concavities that the inner contour often presents, along with the known weakness of snakes segmentation approaches in the evolution into boundary concavities.

B. Validation of the Proposed Algorithm

The validation of the proposed morphometry methodology was made using the remaining 9 images of the database. To accomplish that, the average g-ratio value of each image was computed through the automatic method to be compared with the g-ratio values manually annotated. As stated before, the average g-ratio of an image is given by the mean of the individual g-ratios of all the N axons present in that image. These results are summarised in Table III, in which both automated and manual average g-ratio measurements along with the absolute error rates are presented.

In general, the automatic process results were very close to the ones obtained in the manual process, with an average absolute error of 1.80%. The differences between the proposed automatic approach and the manual process were never greater than 3.60%. These results suggest that the proposed approach is useful and robust enough to reliably extract morphometric features from the images under analysis and, hence, replace the manual process.

IV. CONCLUSIONS

Images of biological tissues are usually unique in terms of structure and constitute therefore different challenges when it comes to performing automatic morphometry. In this paper we present an approach specifically designed for nerve section images from which myelinization is to be assessed with the purpose of studying neuropathies. The goal is to extract g-ratio values from neural structures of axon and involving myelin sheath. This algorithm, with a good performance comparing to manual analysis, finds centroids of the structures and detects myelin contours using an active contours algorithm combined with an innovative directional gradient. The obtained results were validated by comparing the algorithm’s performance with an existing method, which confirmed its robustness. Generally, this automatic algorithm is reproducible and constitutes an objective tool capable of extracting features from several sciatic nerve images.

An approach like the proposed one is of great value for formerly mentioned research groups. The extraction of morphometric features from images like the ones analysed in this study is a time consuming task (if performed manually) and likely to be a subject of many sources of error such as: fatigue of the analyser, expertise performing the analysis and the usage
of small regions instead of the image of the whole nerve section. With this automatic technique it is possible to mislead those sources of error by patronizing the analysis and also to greatly reduce the execution time.

As future work, a validation step based on the topological properties of the axons (i.e. circularity) will be introduced before the contours detection phase. The purpose of this step is to maximize the quality of the detected structures through the rejection of atypical axons. In addition, it is intended to extend this method to images obtained by different microscopy techniques along with the creation of a customized plugin for ImageJ ¹ so that the research community can easily use the proposed morphometric methodology.

REFERENCES


¹ImageJ webpage: http://imagej.nih.gov/ij/