Axon Detection in Digital Images

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Abstract

This paper presents two algorithms used to successfully identify cellular structures in digital images. More specifically, given digital images of stained and magnified axons from a cross section of an optic nerve, this work applies various digital image processing techniques in order to detect and count the axons. The techniques explored in this paper are Canny edge detection, thresholding (optimum, and hysteresis), morphological pruning, and the Moore boundary tracking algorithm. Algorithms composed of combinations of these techniques are presented along with additional steps used to improve results. Finally, the results obtained are compared to those provided by trained professionals.

KEYWORDS

Digital image processing, Edge detection, Canny, Cellular Structres

INTRODUCTION

Detecting structures in digital images is a problem that can be applied to many fields. Some examples of applications for identifying structures in images are biology, computer vision application in robotics, industrial automation, astronomy, and quality control. A variety of existing image processing techniques can be utilized to assist in identifying structures in digital images.

This work focuses on one specific biological application of structure identification in digital images, namely, that of identifying cellular structures. Biology researchers at CWRU, working with the science of vision, are interested in determining the number of axons in an optic nerve. Simply estimating the number of axons based on taking samples of the density of axons in locales would be prone to error. Manually counting the many axons in a typical digital image representing the full cross section of an optic nerve would take far too much time and the process would also be prone to error. Therefore, researchers desire an automated means of identifying and counting the axons in an image.

Given digital images prepared by Biology researchers, obtained by magnifying the stained cross section of optic nerves, we apply and discuss some techniques involving known digital image processing methods aimed at detecting and counting the number of axons in the images. The results obtained in this work will be compared to sample images that have been processed (counted) by trained professionals.

IMAGE CHARACTERISTICS

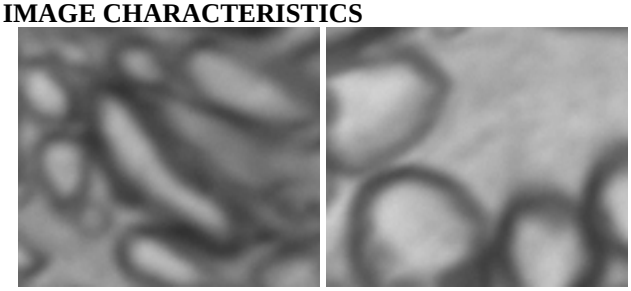

Figure1a and b. Two sample images of axons.

Observe two sample images in Figure1a and Figure1b. The axons are easily identified as the structures surrounded by lower intensity (dark) borders. Figure1a has a number of oblong axons, especially at the center of the image. Figure1a also has very little background, which is characterized by a lack of axons, visible. Figure1b has relatively few but larger axons that are roughly circular in shape. Figure 1b also has much more background visible, particularly in the upper right region of the image.

The variability in the size and shape of axons is a crucial feature of the images. Pattern and shape recognition, a common approach used to separate features in a digital images, was discounted because of the variability of axons. Later, in formulating our axon detection algorithm, we will revisit this feature, using it to make assumptions based on the size of axons from our observations.

Contrast, in the images is relatively low. A low contrast image is one that lacks variability in the intensity of pixels across the entire image. This can easily be observed in noting the compactness in relation to the gray intensity scale of the image's intensity histogram in Figure2. The low contrast of the images is a characteristic that can probably be attributed to the stain used in preparing the images.

Generally, pixels in the digital images of axons can be characterized into three groups based on the regions they represent. The first group of pixels is the low intensity (dark) pixels that compose the axon borders or edges which may correspond to the cell walls. Group two is the set of high intensity pixels that represent the interior of the axon. The last set of pixels is the high intensity background pixels.

Based on a casual visual inspection of the images, observing regions that fall under each of the three pixel groupings, we see that the background pixels have a very similar, if not identical, intensity to axon interior pixels. We also note that the axon borders as the only distinct group in terms of intensity.

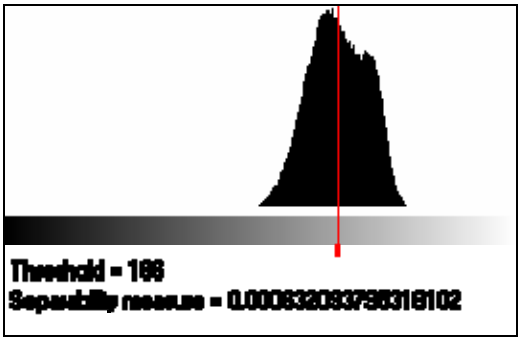

Figure 2. A sample intensity histogram with Optimum Thresholding in red and separability metric indicated.

In addition to depicting the intensity histogram, Figure2 presents some metrics that help us to further characterize the distinct grouping, or lack thereof, based on pixel intensity. In this figure, Optimal Thresholding (OT) was used in an attempt to gain more information on the separability of two (or more) pixel groupings based on intensity. We use OT with only two groupings because of the close intensity similarity of background and interior pixels. Desirable separability measures are close to one. The results obtained in the sample images were rather poor. Separability measures ranged from .002 to .00005. Because of the poor results in using OT, there wasn't much hope for relying on thresholding alone to distinguish the axons.

In our examination of the characteristics of the images, one feature does hold promise for segregating axons. Restating the earlier observation, axons are identified by a dark border in comparison to the lighter axon interior and background. With this distinct feature in mind, we formulate a series of digital image processing steps in hopes of isolating axons.

IMAGE PROCESSING TECHNIQUES

A brief discussion of the digital image processing techniques implemented and used in this work follows.

Canny Edge Detection[1] (CED) is a renowned means of detecting edges in a digital image. CED is an algorithm comprised of four steps.

1. The image is smoothed using a Gaussian filter. As stated in [4], it is sometimes advantageous to omit the Gaussian smoothing phase. This was not the experience encountered in this work. Observe the differences between the center and right images in figure 3. The center image has much truer lines indicating the axon borders in comparison to those in the right image which has many spurious edge lines.

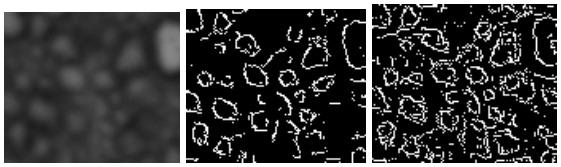

Figure 3a, b and c. The left image is the original, center is the result of CED with Gaussian smoothing, and right is the result of CED without Gaussian smoothing.

2. A derivative operation, such as the Sobel operator, is applied to the image to obtain the edge strengths ad gradients.
3. The gradients and edge strengths from the Sobel operation are then used to apply non-maximal suppression to thin the location of edges.
4. Lastly, Hysteresis Thresholding, which involves using two threshold metrics, is applied to the image to identify the edges as 1s while pixels identified as background are assigned 0s.

Optimal Thresholding (OT) is a statistical processing technique that groups like pixels based on minimizing the between group variance based on intensity. OT using Otsu's method[2] is performed by calculating the normalized histogram and finding the minimum variance for the possible threshold levels.

Morphological Pruning[3] is a technique that allows removal of spurs of a particular length by using spatial masks to group pixels in sets and applying common morphological set operations.

The Moore Boundary Tracking Algorithm[3] (MBTA) involves identifying pixels in the border of a region by following a contiguous sequence of pixels. In this iterative algorithm, we identify pixels that are a high intensity (1 on a scale of 0 to 1) and iterate though that pixel's eight neighbors in a clockwise direction adding the next high intensity pixel to the border grouping, repeating the procedure with that next pixel.

In this work, any color images were converted to grayscale using the following formula for intensity, i, at each pixel where $R, G,$ and B are the red green and blue intensities respectively.

$$i = \frac{R \cdot G \cdot B}{3}$$

The implementation of all techniques herein was in the C# programming language in a managed Microsoft Windows .Net 2.0 application. Source code from the implementation can be downloaded in a Visual Studio 2005 solution from http://filer.case.edu/pcw/eecs490/final/.

AXON DETECTION ALGORITHMS

Algorithm1

Relying on observations that the axon borders are the most distinct features in the images, we approach this problem of

counting axons by detecting borders. CED, the de facto standard for edge detection was implemented and applied.

After detecting the edges using CED, we next needed to group contiguous borders. This was implemented using MBTA. Upon observing the results of CED it was apparent that we did not want to count the very small groups that resulted from spurious edges detected by CED from noise in the original image. In this work we assume the smallest axon can be no less than 20 pixels in width and height. Minimum bounding rectangles (MBR) were obtained for each border grouping from MBTA. The MBRs served three purposes, any MBR with a width or height less than 20 pixels was easily omitted as an axon. Additionally, the MBRs were used to format the output for easy comparison to the images that were processed by trained professionals by simply drawing red diagonals through the MBRs. Lastly, MBRs were later used in a novel border aggregation technique that produced mixed results.

Using CED with a Sobel operator and MBTA showed some limitations. Segmentation of the axon borders after CED was causing some axons to be counted more than once, resulting in exaggerated of axon counts. Upon closer examination, it appeared that applying the Sobel operator was causing the borders of the axons to be broken. This was especially evident where the tangent to the border was diagonal. Figure 4 exhibits this segmentation.

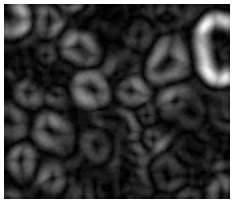

Figure 4. Edge intensities after Gaussian smoothing and applying the Sobel operator. Note the segmented borders, especially on the diagonals.

-2	0	0
0	0	0
0	0	2

0	0	-2
0	0	0
2	0	0

Table 1 and 2. Additional diagonal operators used to remove segmentation from Sobel operators.

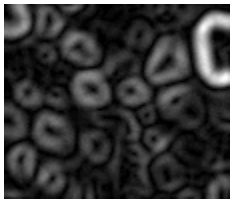

Figure 5. Results of applying Sobel and diagonal operators in tables 1 and 2.

Experimentation with additional diagonal edge detectors resulted in using those shown in tables 1 and 2. These diagonal edge detectors were devised and arrived upon by empirical trials to reduce the segmentation of the borders after edge detection. Results of using the diagonal operators are observed in figure 6. Similar results could have also been achieved by removing the center weighting from the Sobel operators used in CED. (Another potential scheme to alleviate segmentation that went unexplored was increasing the size of the Gaussian smoothing filter beyond 5x5.)

Despite applying the diagonal operators, some of the edges remained segmented, resulting in a small number of axons being counted twice. Three other attempts were made to alleviate segmentation encountered in CED. The first technique was a group aggregating method. The other two techniques involved attempts to fill gaps.

The group aggregation technique utilized the minimum bounding rectangles of the borders to identify borders that had overlapping points. This was easily identified by comparing the corner points of the bounding rectangles for intersection. Aggregation was applied until the groupings no longer changed. Results of applying this technique varied. In images sparsely populated with axons, the results were good. Axons that were previously counted twice due to segmentation in the CED boundary were only counted once. However, in densely populated images the aggregation worsened axon detection. Aggregation often converged to combine all groups into one, only recognizing one axon.

Simple gap filling was implemented by identifying gaps of up to two pixels in the image, characterized by two white pixels separated by two black pixels. The results produced wide axon borders and joining of groups that significantly slowed the MTBA procedure. Figure 6a shows axon edges after CED (note the small gaps in the borders) Figure 6b shows the same after filling gaps of size 2.

Figures 6a and b. Left image is axon edges after CED while right image is after CED then gap filling of size one. Note the undesirable border joining.

The second gap joining technique used morphological closure presented in [5]. Although this technique showed promise, results were far worse than simple gap filling and resulted in gross joining of borders. If more time was available, undoubtedly, shortcomings in the implementation would have been isolated to correct the problems encountered.

Suspecting that the wider axon edge borders, obtained in the simple gap filling procedure, were caused by spurs, one

final effort to correct the CED group segmentation used morphological pruning. The product of pruning then attempting to fill gaps did reduce the border widths observed in Figure 6b, as suspected, but the overall result did not improve the doubly counted axons. As the gap size in simple gap filling was increased, the results dramatically degraded and gross border joining was encountered.

Algorithm2

The second algorithm that was used to detect axons in the digital images departed from the first algorithm at the second step of CED. The images were subjected to a Gaussian smoothing filter then were thresholded using OT. After OT, the remainder of CED was applied followed by MBTA and finally finding MBRs as in the first algorithm.

Generally, this algorithm produced much more crisp and distinct edges compared to algorithm1. Overall, results were very favorable. Minimal segmentation was observed. Single axons that were previously characterized as two due to segmentation in algorithm1 were observed as one.

One caveat of this algorithm concerned results obtained in very low contrast and high axon density images. In these cases, OT would group very close axons together resulting in too few axons being counted.

With more time spent on this algorithm, experimentation with segmentation techniques to apply the OT in locales may have yielded still better results.

RESULTS

Of the two algorithms presented in this work, the second involving Gaussian Smoothing, OT, CED, and MBTA, performed more accurately in comparison to algorithm one. The gold standard images processed by trained observers detected axons that were much smaller than obtained in either of the two algorithms. This was partially due to assumptions made regarding the minimum axon size and the performance of OT and CED in areas of low contrast.

Because of the problems encountered with edge segmentation and the assumptions made on the minimum axon sizes, the first algorithm overestimated axon counts in all of the sample images and the second algorithm under estimated the number of axons in comparison to the gold standards, with exceptions observed in figures 8 and 11 (which was due to the large borders of the axons resulting in a double border appearing after processing).

The actual number of axons in an image, as indicated in gold standards, should fall between the two algorithms with a tendency toward the second.

Performance of the algorithms using the small images was very fast, each operation took less than a few sends. Processing an image of the entire optic nerve failed due to memory constraints on the 32-bit 2GB desktop system used.

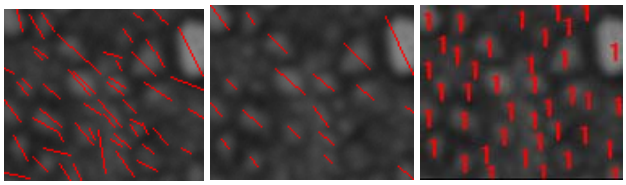

Figure 7. Left are results of algorithm 1, center are the results of algorithm 2, and right are from the gold standard results of processing by a trained professional.

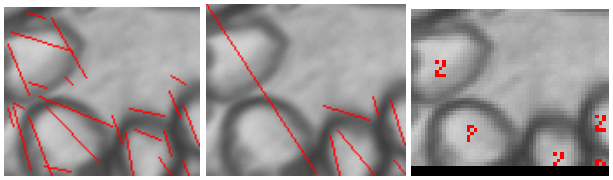

Figure 8. Algorithm 1, 2 and gold standard respectively.

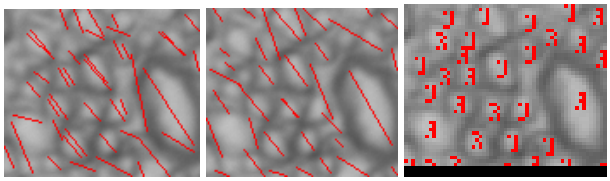

Figure 9. Algorithm 1, 2, and gold standard respectively.

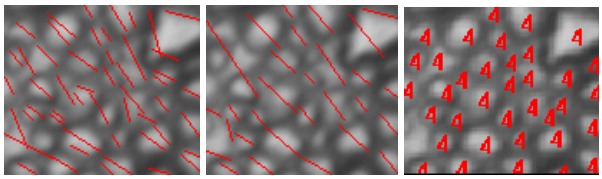

Figure 10, Algorithm 1, 2, and gold standard respectively.

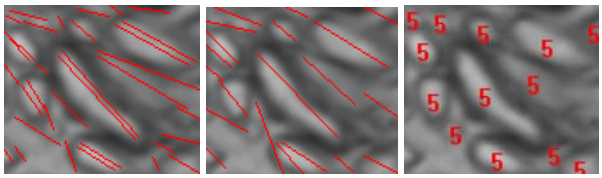

Figure 11, Algorithm 1, 2, and gold standard respectively.

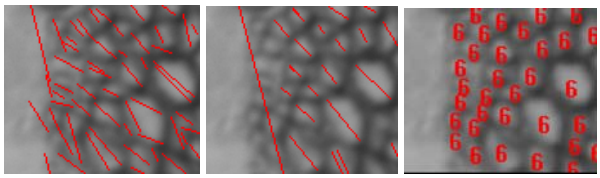

Figure 12, Algorithm 1, 2, and gold standard respectively.

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