Identification and Tabulation of Axons using Image Processing

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Abstract

This paper presents a method for the automated identification of axons using image processing. Several methods of pre-processing are used in conjunction with watershed image segmentation to estimate and tally the axons. Edge detection, morphological processing, and image thresholding were used to isolate the axons in each image before segmentation. Each method was tuned for different image types; a voting system is used to provide consistent results for a wide variety of images.

KEYWORDS

Image Segmentation, Watershed Transform, Object Detection

INTRODUCTION

One of the professors at CWRU, Dr. Howell, is attempting to count the rods and cones on the retina of mice. Due to the curvature of the retina, it is not feasible to directly count the rods and cones. Instead, the method employed is to cut the optical nerve bundle and examine the number of neural connections (axons). This is a significant image processing problem due to the wide variety of possible axon shapes and sizes.

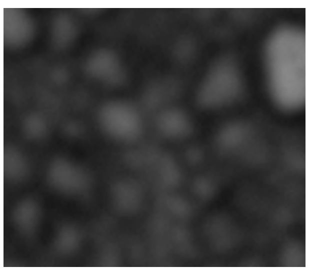

Figure 1: A microscope image of mouse axons

AXON IDENTIFICATION METHODS

Several methods were investigated to pre-process the image, removing background information. Once the image is reduced to axons only, a segmentation method, like the watershed transform, is needed to identify individual axons.

CANNY EDGE DETECTION METHOD

The Canny edge detection method is one of the most popular edge detection algorithms. It uses a Gaussian pre-filter for blurring and noise removal. Four gradient filters are used for horizontal, vertical, and diagonal edge detections. Edge detection with hysteresis thresholding is used for more consistent edge detection. This method comes with a computational penalty, but the improved edge detection is worth it for this non real-time application.

IMAGE THRESHOLDING METHOD

The simplest of the techniques used, image thresholding can be as simple as converting all pixels above a certain grey level to white and setting all others to black. The Matlab implementation of thresholding uses Otsu's method to reduce the variance of black and white pixels. To achieve less noisy and more consistent results, some other operations were also beneficial. Increasing the image contrast before thresholding improved results. Also, the morphological fill and open operators were used to remove smaller objects; this effectively reduces the false positives obtained when counting axons. Of the methods used, this was the most susceptible to noise and was therefore tuned to be less sensitive.

EXTENDED MAXIMA METHOD

Axon identification based on the Extended Maxima transform was attempted, as originally proposed in [1]. This technique identifies the distinct 'bright' areas in an image, removing all background pixels. For most of the axon images, the only bright areas are the axons.

WATERSHED TRANSFORM SEGMENTATION

The watershed transform was used to segment the image after pre-processing. The watershed transform uses the gradient of an image, represented as contour lines to divide an image into regions.

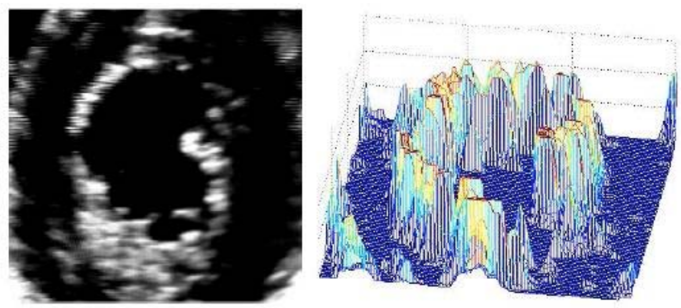

Figure 2: A grayscale image and its representation as a topographic surface [5].

For visualization purposes, an image can be thought of as a series of basins and peaks. The watershed transform segments the image into regions based on these peaks and basins.

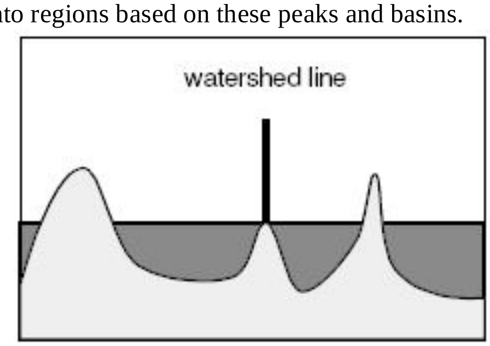

Figure 3: Points where basins meet define a watershed line [5].

The Matlab implementation of the watershed algorithm is described in [2] and [3].

ALGORITHM VOTING

As shown in the results section, it was unfeasible to tune a single method to perform well for all image types. In an attempt to make the counting algorithm as robust as possible, the mean axon count of all three methods was used.

RESULTS AND DISCUSSION

Six sample images with known axon counts were used to benchmark each counting technique. Significant tuning and experimentation with each pre-filter attempted to get the best performance possible.

Each technique was not tuned for each individual image; overall performance was valued since the goal of the project is to automate axon counting for a wide variety of images.

CANNY EDGE DETECTION METHOD

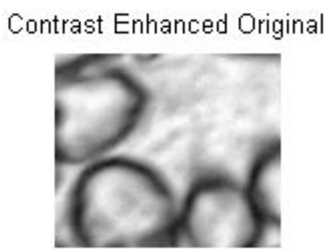

Figure 4: Sample Image 2: a sparse image with a large, bright background. This image was problematic for the Canny and Extended Maxima methods.

The difficult part of tuning the Canny method pre-filter was how to condition the image before using edge detection. Low axon density images, like Figure 4, yielded many false positives when simply using the Canny method without other morphological methods.

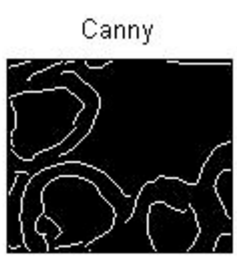

Figure 5: The results of Canny Edge Detection for Sample Image 2.

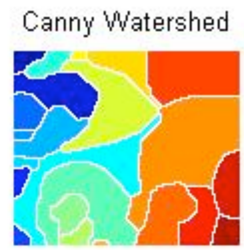

Figure 6: Watershed output from Canny edge detection for Sample Image 2.

Notice all of the falsely identified axons in Figure 6. Methods to improve the performance for the Canny method, such as dilating or blurring the image before calculating edges, were explored. Unfortunately, they resulted in too much performance loss for the more common high axon density images, such as Figure 7.

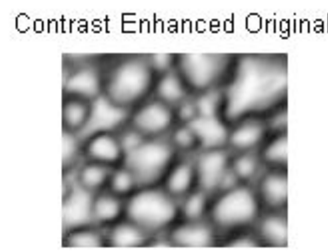

Figure 7: Sample Image 4: A more typical Axon density image.

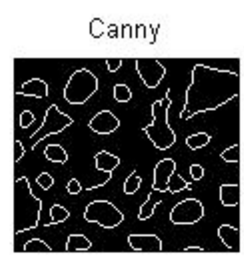

Figure 8: The results of Canny edge detection for Sample Image 4.

Figures 8 and 9 illustrate more typical results for using Canny edge detection: an accurate axon count for high density images. The edge detection is sensitive enough to pick out subtle axons with no false positives for these high density images. As shown in Tables 1 and 2, the Canny method yielded the best results for Images 1, 3, 4, and 6; all the high axon density images.

Figure 9: Watershed output from Canny edge detection for Sample Image 4.

SIMPLE THRESHOLD METHOD

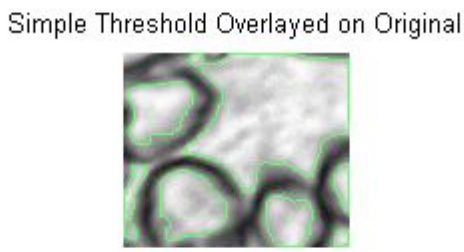

Figure 10: Image Thresholding for Sample Image 2.

Thresholding the image yielded excellent results for the low axon density images like Sample Image 2. The image background was properly identified with only one false axon identification due to the background being divided into two sections.

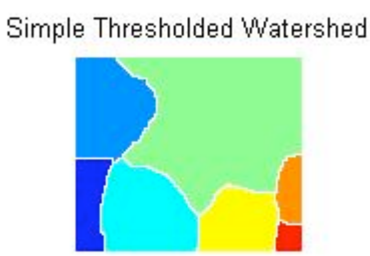

Figure 11: Watershed output for Image Thresholding for Sample Image 2.

Unfortunately, well tuned image thresholding was not sensitive enough for high axon density images like Figure 12. Numerous axons are either lumped together or missed for these images. Changing the sensitivity of the thresholding did not improve results.

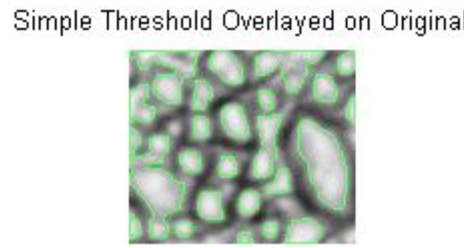

Figure 12: Image Thresholding for Sample Image 3.

EXTENDED MAXIMA METHOD

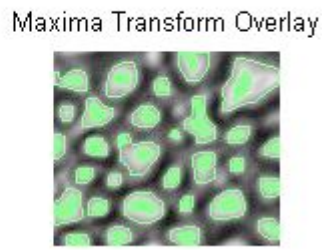

Figure 13: Extended Maxima Transform for Sample Image 4.

The Extended Maxima results were second best for every sample image. High axon density images, like Figure 13, yielded several missed axons but no false positives.

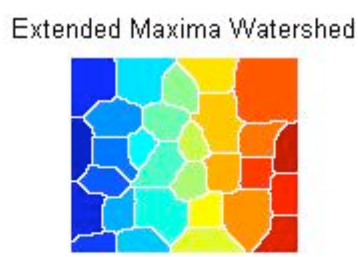

Figure 14: Watershed output for Extended Maxima Transform for Sample Image 4.

The Extended Maxima technique was particularly vulnerable to misidentification of bright backgrounds as axons, since no shape analysis was taken into account. Figure 15 shows a poor result for the Extended Maxima transform; much of the bright background is improperly identified as axons.

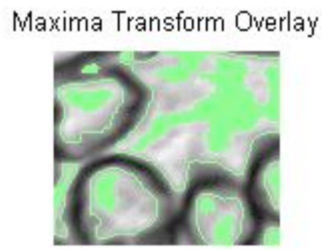

Figure 15: Extended Maxima for Sample Image 2.

Table 1: Results for Axon Counting Techniques on Sample Images

Results for Axon Counting Techniques					
Sample Image #	Analyst	Canny	Image Threshold	Maxima Transform	Average
1	39	35	20	26	27
2	5	22	6	10	13
3	32	33	23	29	28
4	36	33	28	28	30
5	13	22	16	19	19
6	33	36	19	28	28

ALGORITHM VOTING

Each explored method provided good performance for certain image types. A simple to improve axon count estimates is to average the three pre-filter methods axon counts. As shown in Table 2, this resulted in similar mean errors as the Canny and Extended Maxima methods, but with significantly less variance in the results. This represented an improvement due to the desirability of consistent results and removal of outliers.

Table 2: Axon Counting Technique Errors and Statistics

Automated Axon Counting Technique Error Metrics				
Sample Image #	Canny	Image Threshold	Maxima Transform	Average
1	4	19	13	12
2	17	1	5	8
3	1	9	3	4
4	3	8	8	6
5	9	3	6	6
6	3	14	5	5
Mean Error	**6.17**	**9.00**	**6.67**	**6.83**
Error Standard Deviation	**5.95**	**6.72**	**3.50**	**2.86**

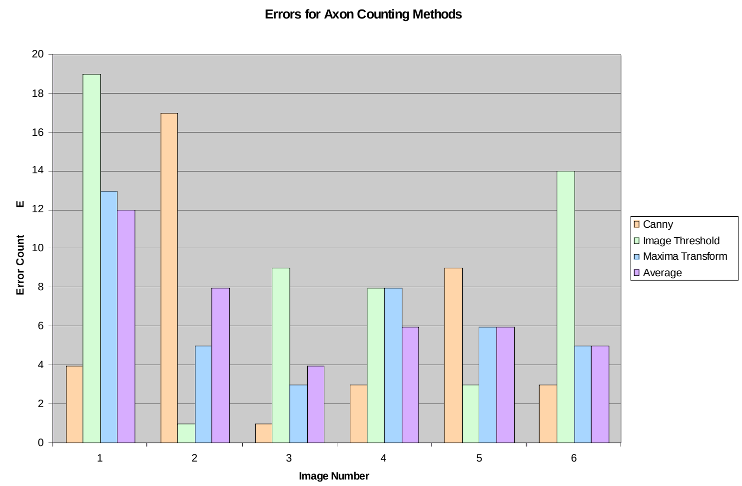

Figure 16: Axon Counting Errors for each Technique

Another potential improvement would be to classify images into high and low axon density images, either automatically or manually. Each group could then be processed with the best method for that image type. Figure 3 shows errors if the Canny method were used for high axon density images and Image Thresholding for low axon density images. As the table shows, errors could be reduced by almost a factor of 3 over the averaging method presented, with improved variance as well.

Table 3: Image Classification Errors and Statistics

Image Classification Error Metrics		
Sample Image #	Canny	Image Threshold
1	4	19
2	17	1
3	1	9
4	3	8
5	9	3
6	3	14
Classified Mean Error		**2.50**
Error Standard Deviation		**1.22**

SUMMARY

Several methods for image processing based mouse axon counting were presented. The performance strengths and weaknesses of each method were discussed; as were several methods for improve results and using the correct algorithm for each image.

A simple add-on to this project would be to automate the image classification, perhaps using a neural networks approach. This could result in a fully automated counting program that provides results superior to analyst capabilities.

REFERENCES

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