Counting Axons in an Optical Nerve Bundle of a Mouse Using the Digital Image Processing Techniques

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Abstract

This paper presents an investigation on using standard digital image processing techniques to quantify the number of axons in an optical nerve bundle of a mouse. The standard methods of processing will include edge enhancement, morphology and thresholding. The inherent limitations of the above methods will be delineated and an alternative method developed. The alternative method demonstrated will be based on statistical methods along with an initial calibration process. Once an image of particular process is calibrated the number of axons can be counted for any nerve bundle image that has been produced from this process. The scope of the defined alternative process will be limited to counting axons without regard to their location in the image.

KEYWORDS

Segmentation, Morphology, Axon, Edge Detection

INTRODUCTION

Counting and characterizing aspects of biological physiology with digital image processing plays a large role in the image processing field today[i]. Morphological methods have been developed because of their ease of use and computational efficiency[iiiiiiv]. The standard methods of image segmentation typically use some combination of edge detection, thresholding and morphology[vvi]. The counting of axons of this study will prove to be unsuccessful using these standard methods. I attempted using Laplacian, gradient, filtering, dilation, erosion, thinning, canny and watershed in combinations.The exemplary images used for counting have the following undesirable characteristics: low contrast, large scaling differences in size and many connected boundaries between groups of axons. The RGB color planes were examined to see if a particular color plane offered a better image for counting axons (Figure 1).

The color in the images did not appear to offer any advantage to the task of axon counting and the decision was made to use the grayscale image for the algorithm development The method proposed in this paper will take advantage of the following characteristics of the exemplary images: the local contrast is consistent throughout the image and the density of axons does not change largely from one part of the image to another. The standard deviations and means of the local contrast have approximately the same value when sections of the image are examined and compared against each other. The image was segmented into nine different histograms (Figure 2) with the local standard deviations, means and errors calculated (Table 1).

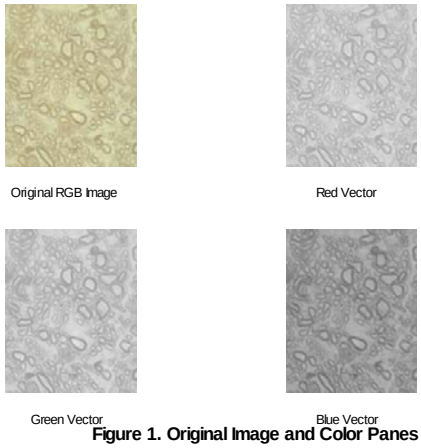

Figure 1. Original Image and Color Panes

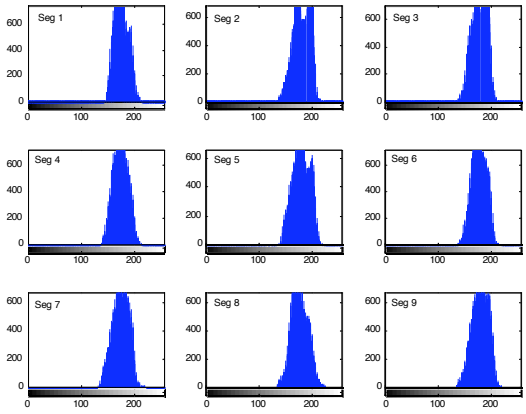

Figure 2. Local Histograms

Segment	Local Mean	Local SD	Global Mean	Global SD	Percent Mean Difference	Percent SD Difference
1	175.3313	13.1348	176.2924	15.0117	0.5451738	12.502914
2	179.1953	15.7297	176.2924	15.0117	-1.646639	-4.782936
3	176.1147	14.4467	176.2924	15.0117	0.1007984	3.763731
4	174.2059	13.9288	176.2924	15.0117	1.1835451	7.2137066
5	178.1861	16.2848	176.2924	15.0117	-1.074181	-8.4807184
6	175.9303	13.8517	176.2924	15.0117	0.2053974	7.727306
7	174.1962	15.3108	176.2924	15.0117	1.1890473	-1.9924459
8	175.409	15.6233	176.2924	15.0117	0.5010993	-4.0741555
9	178.0654	15.6295	176.2924	15.0117	-1.005716	-4.1154566

Table 1

Percent Differences Between Global and Local Statistics

ALGORITHM ('MORPHINE')

The basic steps in the main morphine algorithm are as follows:

1. Convert color image to gray scale
2. Low pass filter image
3. Contrast inversion (makes edges the highest values. This allows the image to be used directly with edge enhancement
4. Enhance edges with blurred Laplacian edge enhancement
5. Dilate image with a disk of radius 3
6. Compute $\sum Pixels \times K + B$, where

   Pixels is grayscale value

   K is the gain from the calibration process

   B is the offset from the calibration process

The steps in the calibration process are similar to the main algorithm

1. Convert multiple subsets of the original color image to gray scale
2. Gaussian low pass filter image
3. Contrast inversion (makes edges the highest values. This allows the image to be used directly with edge enhancement
4. Enhance edges with blurred Laplacian edge enhancement
5. Dilate images with a disk of radius 3
6. Compute the best curve fit for $\sum Pixels \times K + B$. The best value of K and B can be chosen from the calibration data. A function was created that can take the image and return the Axon count. The preprocessed training data before applying the curve fit (Figure 3).

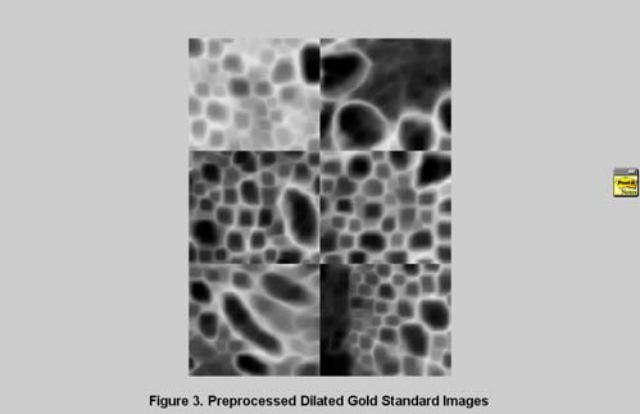

Figure 3. Preprocessed Dilated Gold Standard Images

RESULTS AND DISCUSSION

The proposed method was applied to 6 images that where used as gold standards for testing the algorithm. The results are summarized in Table 2.

Test Case	Expert Count	Morphine Algorithm	Percent Difference
Gold 1	40	40	0.00
Gold 2	4	31	-675.00
Gold 3	30	33	-10.00
Gold 4	36	35.5	1.39
Gold 5	13	35.2	-170.77
Gold 6	33	33	0.00
Project Img	?	170	

Table 2
Comparison Standard to Morphine Algorithm

The result is encouraging but does not appear to be very effective when the images are small with a small count. The basic assumption for the algorithm is that the data is statistically large enough across all the size scales of the axons that are to be counted. If I throw out the data that I would consider insignificant because of the small sample count the results are all within 10% for the number of axons counted. I am not sure if all the images are of the same scale. The image with the axon count of 4 appears to maybe have been scaled to fit the image space. When trying morphological methods I ran into dead ends when trying to separate clear boundaries and objects. I tried to get the objects separated to facilitate a filling operation that could

then be thinned to single pixels so that the axons could easily be counted by counting the pixels. It was after struggling with the first method without much success I decided to change my approach. The morphine algorithm gave good results when the axon count in the image was above 30 (Figure 4)

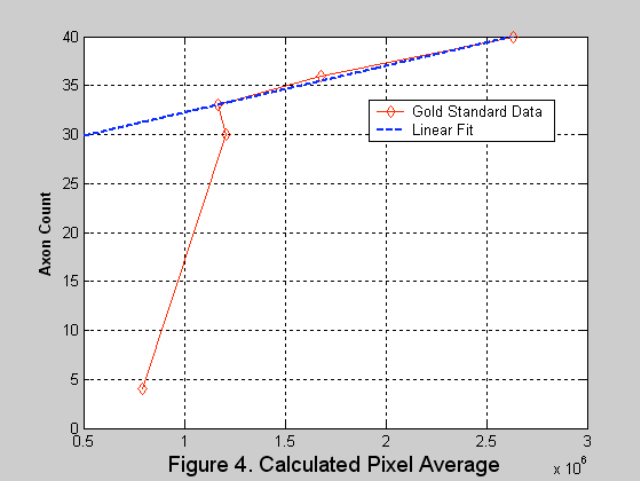

Figure 4. Calculated Pixel Average

The usefulness of the morphine method will depend on other factors that were basic assumptions for the premise of the algorithm. From observing the original image it looks like the total axon density was consistent when viewing from one area of the nerve to another providing the area of interest is large enough. It is important that the method of taking the image of the cross section of the nerve bundle provides a consistent contrast from a given nerve bundle to another. If it is important to know something about the size and shape distributions of the nerve bundle the morphine algorithm will not work. To improve the accuracy of the morphine method it is important to use larger portions of the images for the calibration images. The calibration images should be large enough to include at least 50 axons. The calibration images should include cases where there is a small density of axons and a large density of axons.

SUMMARY

A method was proposed that could be used to automate the counting of axons in the optical nerve bundle of a mouse. The measured axons agree to the prediction that was calculated with a linear model using the design parameters for axon counts larger than 30. The proposed method is fast and does not use any image processing besides edge enhancement and basic morphological processing.

A future study would need to look at the effects of using large calibration images and the repeatability of creating images of the optical nerve bundles.

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