The Use of Image Segmentation in the Detection of Rods and Cones in the Retina of Mice

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Abstract

Six images of rods and cones in the retina of mice were provided. It was desired that these rods and cones be automatically counted. Several different image processing methods were used to count the number of rods and cones in each image. These methods included adaptive histogram equalization, edge detection, and morphological operations. This paper describes the algorithm used to count the rods and cones in the retina of mice.

Key Words

adaptive histogram equalization, edge detection, image processing, thresholding, contrast adjustment, image segmentation

Introduction

A professor at Case Western Reserve University researches retinal sensing. He is attempting to count the number of rods and cones in the retina of mice. It is very difficult to count these sensors because the retina is curved. Therefore, an image slice of the optical nerve bundle from the retina to the brain has been provided. The closed bundles in the image are the rods and cones and will be counted.

A previous attempt at counting the rods and cones in monkeys has been made [1]. This algorithm uses a Hessian edge detection filter and thresholding using the Fuzzy c-means clustering method. Finally, a decision tree is applied to classify each shape.

This paper will examine a algorithm that can be used to detect the rods and cones in the retina of mice.

Algorithm

Several different image processing methods were used to count the rods and cones in the retina of mice. Six images were used to test the methods. These images can be found in Figure 1. The following is the basic algorithm that was used:

1. Read image
2. Convert image to grayscale
3. Average filter
4. Adjust contrast
5. Average filter
6. Calculate Canny edges
7. Threshold image based upon contrast value at the edge
8. Perform morphological image closing/opening to remove holes
9. Count 8-connected areas in the image
10. Remove background area
11. Output final image

The following sections will discuss the major steps in more detail.

Contrast Adjustment

The contrast adjustment method was the most difficult to determine. First, a histogram of the intensity of each of the images was examined. It was determined that some of the images contained very low contrast, while some of them contained high contrast. Also, the size and shape of the rods and cones varied across images. Figure 1 (a) shows a very low contrast image with small rods and cones; Figure 1 (b) shows a high contrast image with large rods and cones. Therefore, it was difficult to find a contrast adjustment method that worked with both types of images.

Several different methods for adjusting the contrast were examined. These methods included the gamma transform [2], adaptive histogram equalization [3], and the Otsu method [4]. The new images after the gamma transform and adaptive histogram equalization can be found in Figures 2 and 3. The output image of the Otsu method is a black and white image, so no contrast adjusted image is shown. The black and white images that resulted after applying all three methods of contrast adjustment can be found in Figures 4 – 6. It can be seen that the Otsu method picked up the least amount of detail, the adaptive histogram method picked up the most detail, and the gamma transform method was in the middle. The Otsu method was excellent for the large rods and cones, as it did not emphasize contrast differences within rods and cones. This method worked best for the image in Figure 1 (b). The adaptive histogram method was the best for the image shown in Figure 1 (a), which contains low contrast and small rods and cones. The method that was chosen was the adaptive histogram equalization method because it could detect a lot of detail.

In order to reduce contrast differences in large rods and cones, the image was average filtered with a 2 and 3 pixel radius disk before and after histogram equalization, respectively. These small average windows allowed the small rods and cones to be detected but and filtered out some of the contrast differences in large rods and cones.

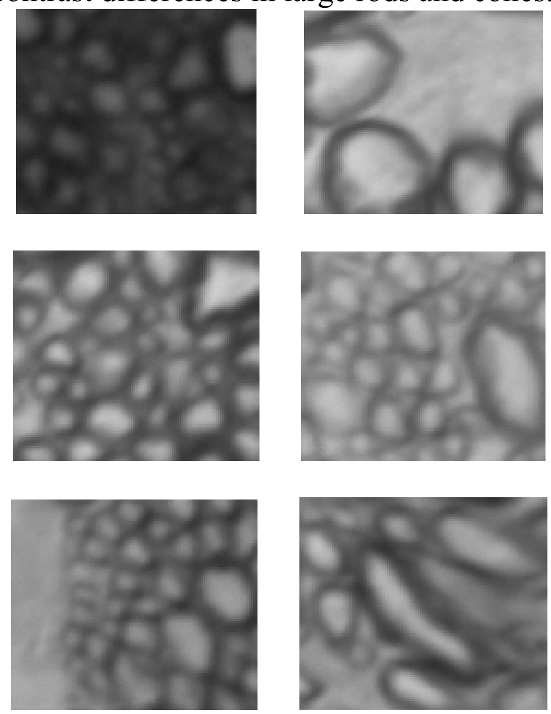

Figure 1: Original images. (a) Low contrast image with small rods and cones. (b) High contrast image with large rods and cones.

(a) (b)
(c) (d)
(e) (f)

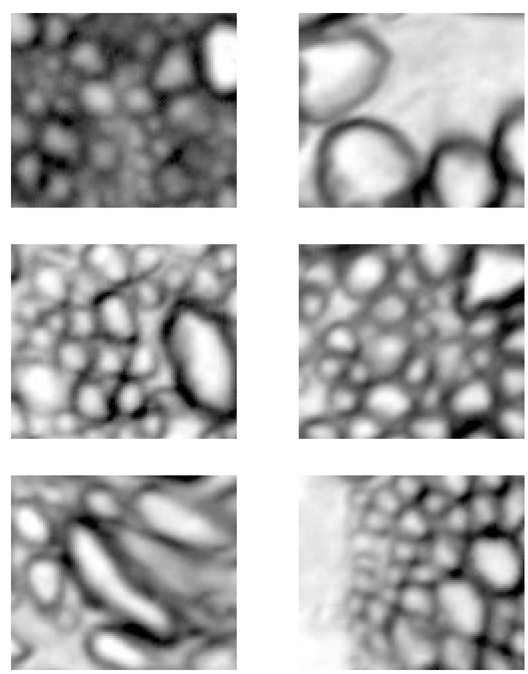

Figure 2: Images after gamma transform.

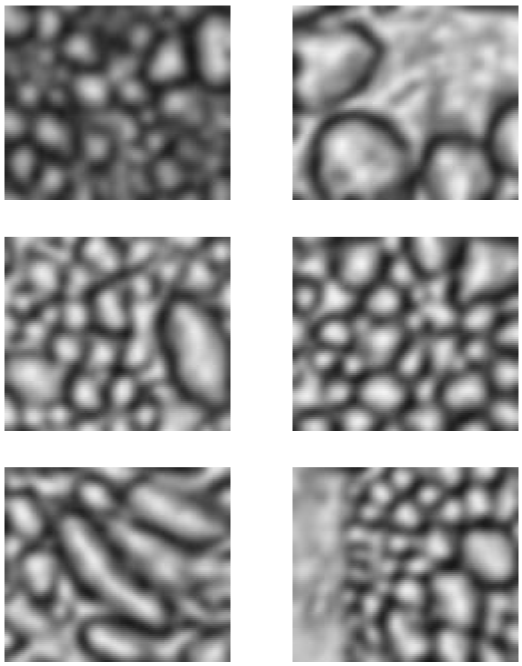

Figure3: Images after adaptive histogram equalization.

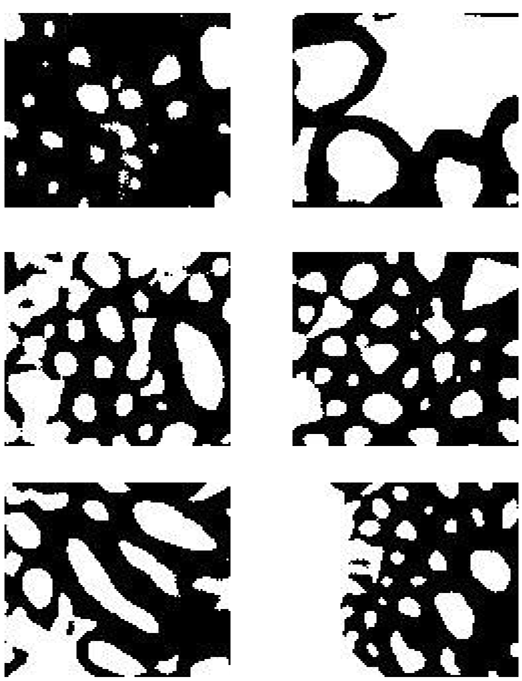

Figure 4: Black and white images thresholded using the Otsu method.

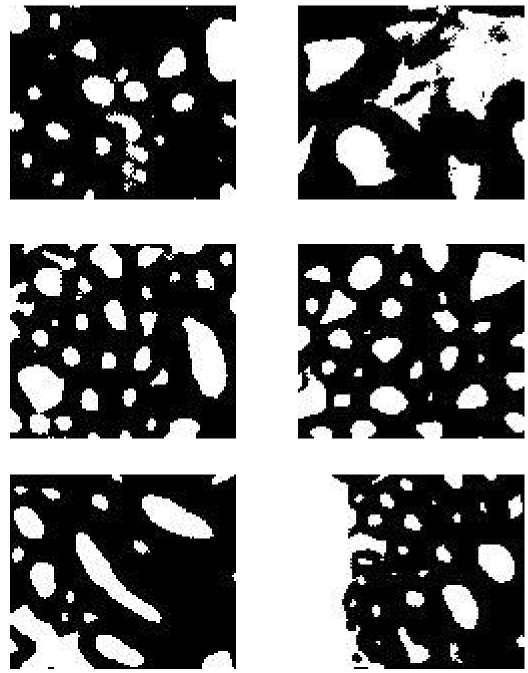

Figure 5: Black and white images thresholded using the gamma transform (gamma = .6).

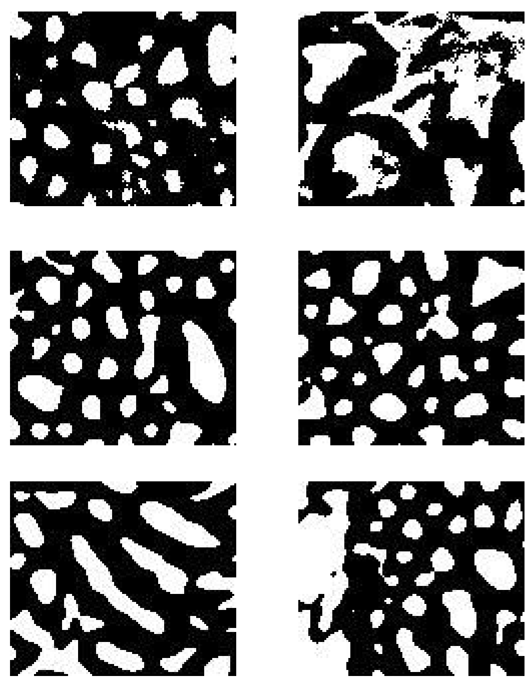

Figure 6: Black and white images thresholded using the adaptive histogram equalization method.

Thesholding using Edge Detection

The Canny [5] edge detection algorithm was used to determine the approximate locations of edges in the images. This algorithm is very sensitive and can detect a lot of edges. Figure 7 shows the edges found in the images after the adaptive histogram equalization and average filtering. These edges do not show clearly separated rods and cones. Therefore, the locations of the Canny edges were used to locate the intensity values at the edges in the original images. The threshold window consisted of one standard deviation on each side of the mean of the intensity at the edges. Intensity values higher than this window became white and intensities lower became black.

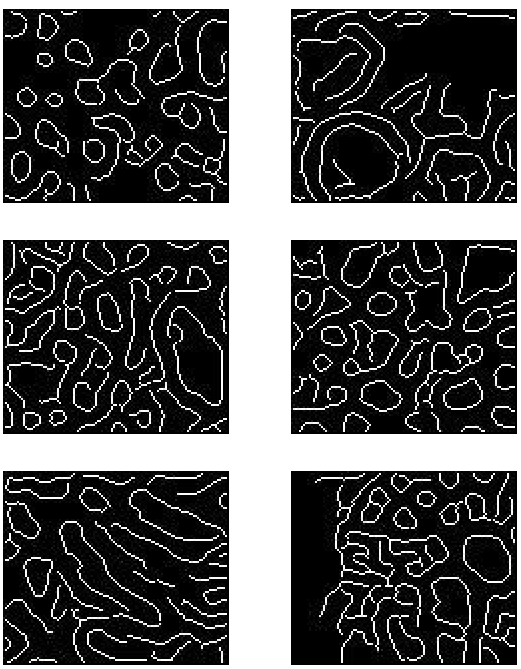

Figure 7: Canny edges.

Morphological Opening and Closing

The images in Figure 6 contain a lot of noise. Therefore, in order to eliminate this noise, a closing [6] using a disk with radius 2 pixels was performed. Then an opening [6] using a disk of radius 1 pixel was performed. This eliminated most of the small white pixels and filled in a lot of the white areas. The result of these two operations can be seen in Figure 8.

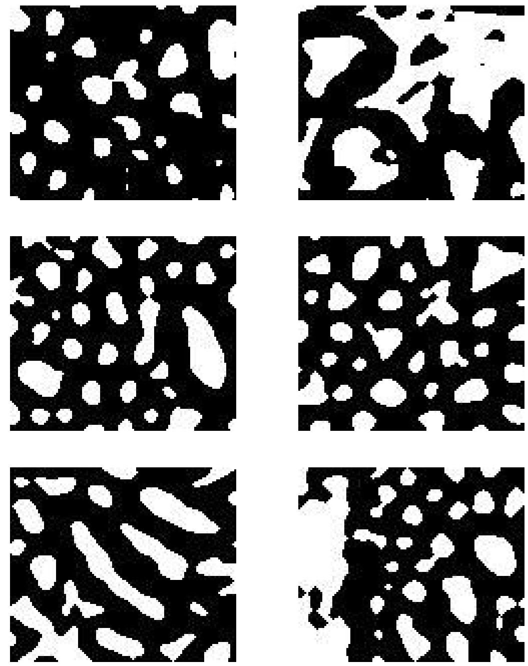

Figure 8: Black and white images after the morphological opening and closing operations.

Background Removal

The background in the original images of the rods and cones was the same color as the rods and cones. Therefore, the thresholded images contained 1's where there were rods, cones, and the image background. However, in most cases, the image background was a significantly larger area than the size of the rods and cones. Therefore, 8-connected areas that were larger than 1000 pixels were removed from the thresholded images. This number was determined by calculating the histogram of the 8-connected areas from all of the images. As can be seen in Figure 9, most of the 8 – connected areas lie below 1000 pixels.

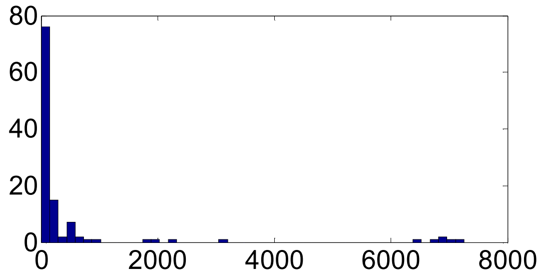

Figure 9: Histogram of 8-connected areas from all 6 images.

Final Results

The number of rods and cones in each image was counted using the same algorithm. The final black and white thresholded images can be seen in Figure 12. Also a large image and the final thresholded black and white image can be found in Figures 10 and 11. A table comparing the calculated and the actual number of rods and cones in the images can be seen in Table 1.

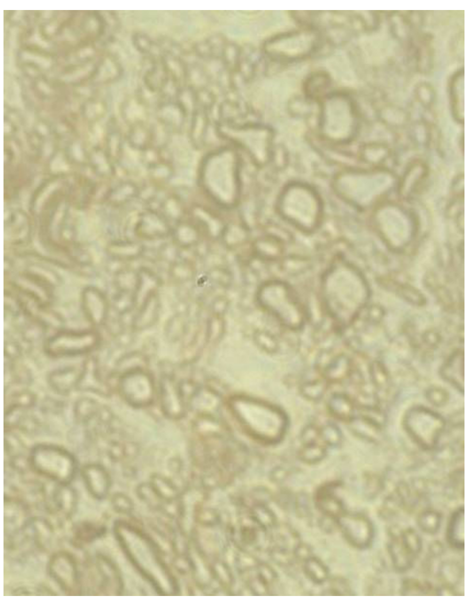

Figure 10: Large image of rods and cones.

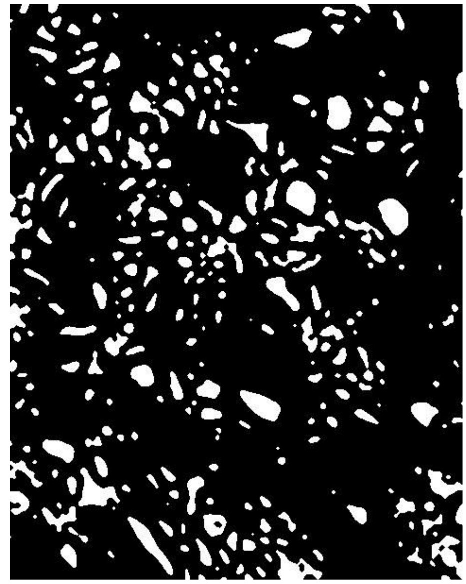

Figure 11: Back and white image of Figure 10 showing rods and cones in white. 325 rods and cones were counted in this image.

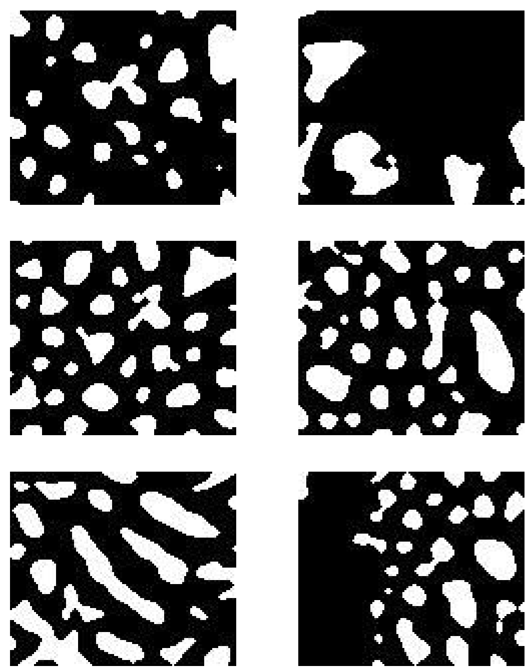

Figure 12: Final black and white images showing rods and cones in white. These images correspond to originals in Figure 1.

(a) (b)
(c) (d)
(e) (f)

Table 1: Actual and calculated rods and cones for all 6 images.

Image	Actual	Calculated
1	40	26
2	5	7
3	32	35
4	36	34
5	13	21
6	33	28
large image	325	n/a

Discussion

This algorithm worked fairly well in detecting the rods and cones. It worked the best for high contrast images with small rods and cones. This can be seen in Figure 12 (c), (d), and (f). The algorithm did not work as well for the images with large rods and cones. This can be seen in Figure 12 (b) and (e). The image in Figure 12 (a) was a low contrast image, and the algorithm did a decent job in detecting the rods and cones.

There are two areas for improvement for this algorithm. First, there needs to be a method for automatically determining if histogram equalization needs to be done. In some cases (such as the image in Figure 1 (b), the image already contains plenty of contrast and increasing the contrast resulted in less desirable results.

Next there needs to be a method for determining the average size of the rods and cones in the image. This algorithm works very well for images with small rods and cones. However, in images with large rods and cones, the histogram equalization actually divided rods and cones into pieces. This problem could be solved by either a larger average filter, or by decreasing the contrast in the histogram equalization.

Summary

An algorithm for detecting rods and cones was developed. This algorithm averaged the image and then performed adaptive histogram equalization. Next, the image was thresholded using the Canny edge operator to detect edges. Morphological closing and opening operations were performed to clean up the black and white image. Finally, the background was eliminated. The number of rods and cones was counted and compared with the actual number. It was found that this algorithm works best on high contrast images with small rods and cones. However, adjustments can be made to improve the output for images with large rods and cones.

References

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