Biological Image Segmentation

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Abstract

This paper presents an approach of using multiple image processing techniques including color segmentation, image smoothing, contrast enhancing, edge processing, thresholding and morphological reconstruction processing to identify and count the number of rods and cones in a mouse retina microscope image. Different approaches of image preprocessing have also been tested and the results have also been compared to find an optimal solution for this purpose. An adaptive background area removing method has also been addressed in this paper. This automated counting and adaptive background eliminating algorithm allow the researches to separate the target objects from the background and reduce the rate of overcounting.

KEYWORDS

Image analysis, Image segmentation, Segmentation evaluation, closed bundles detection

INTRODUCTION

Image segmentation is one of the most critical tasks in automatic image analysis. The purpose of this projct is not only to separate the rods and cones from a mouse retina microscope image but also to count the total numbers of the close bundles in the image. Due to the nature of the biological images, the straight lines, real circular shapes, and rectangular shapes are hardly found in this kind of images. Moreover, since this is a microscope image, the original target objects have been stained for some purposes. Both of the two main reasons are making the recognition and localization even more difficult.

In traditional identification methods, scientists in the lab use immunofluorescent staining and fluorescence microscopy for acquiring images from the cell, and the identification works are mostly done by visually inspection via human experts. However, when the target number becomes larger and larger, it is almost impossible to actually manually count them one by one. Besides, miscounting and overcounting are very easily to take place. Therefore, a computer based automated counting algorithm is highly desired for assisting human experts to do their work. A desired algorithm would need the abilities of counting closed bundle shapes targets, and also allowing the scientists to remove unwanted background image.

Although many approaches have been reported and many algorithms have been purposed such as fuzzy C-means, our goal is to find a simple, straight forward, adaptive control, and time efficient method.

METHODS

A straight forward image processing procedure has been performed in this project. The whole procedure can be separated in two stages, which are the preprocessing and the recognizing stages. The preprocessing stage includes color segmentation, image smoothing, and contrast enhancing process. The recognizing stage includes morphological reconstruction and labeling the connected components. The flowing chart is shown in Fig. 1.

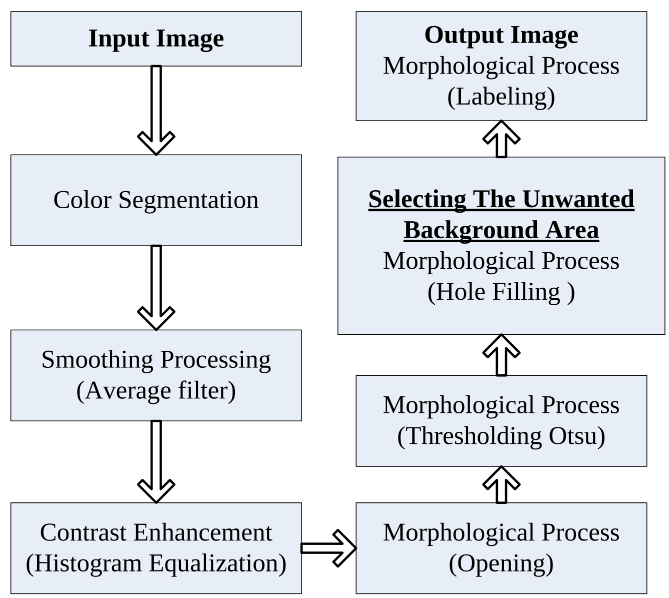


Fig. 1. The flowing chart of this project

The first step in the preprocessing stage is using color segmentation to convert the original RGB image into red, green, blue, and grayscale (R+G+B/3) intensity images for performaing the comparison. This step is very helpful for finding which color has the best response to the edge of the target. The second step is to smooth the raw image to decrease the intensity level of unwanted noise. A 3X3 averaging filter has been selected to use on the images because it has less effect on the adjacent pixels, and is also capable to obscure of the effect caused by noise. Since the noise can be taken as a random event and it is also not connected to its neighbor. Therefore, average filter has the ability to smooth the image without blurring too much on the edges. Histogram equalization has also been used to enhance the contrast of the smoothed image.

The morphological reconstruction is the first step in the recognizing stage. This process includes the morphologically opening which uses erosion to remove remaining noise like pixels and uses dilation to restore the shape of the target objects. The following step is using Otsu's method to decide the global threshold. Because this method is capable of obtaining a threshold value which has the biggest between-variance and smallest within-variance values to separate a gray intensity image into two clusters. A black and white image can be constructed in this step. However, the empty background may cause a possible overcounting problem in the labeling process. Due to the nature of biological image, the system may take the background as a big closed bundle and also count it into the evaluation. An adaptive background eliminating may be useful for solving this issue.

The adaptive background eliminating method is to apply the concept of holes filling process in morphological reconstruction by visually inspecting and manually selecting the unwanted area. Once the area has been selected, the holes filling process will be filled up automatically by black pixels thereby eliminating the possibility of overccounting caused by background area. Eq. (1) shows the algorithm which is used for Hole filling process.

$$X_k = (X_{k-1} \oplus B) \cap A^c \quad (1)$$

In Matlab, the function "imfill" following the algorithm to fill the selected area.

$$f_m(x,y) = \begin{cases} 1 - f_m(x,y) & \text{If (x,y) is only on the border of f} \\ 0 & \text{Otherwise} \end{cases}$$

$$g = \left[R_{f^c}(f_m) \right]^c \quad (2)$$

After eliminating the background area, the Matlab function "bwlabel" can be used to find the 4-connected or 8-connected components for counting and labeling.

RESULTS AND DISCUSSION

The results of the first step in preprocessing stage are shown in Fig. 2. The original RGB image has been converted into red, green, blue, and grayscale (R+G+B/3) intensity images. The blue intensity image shows the highest contrast ratio in these color segmentation images.

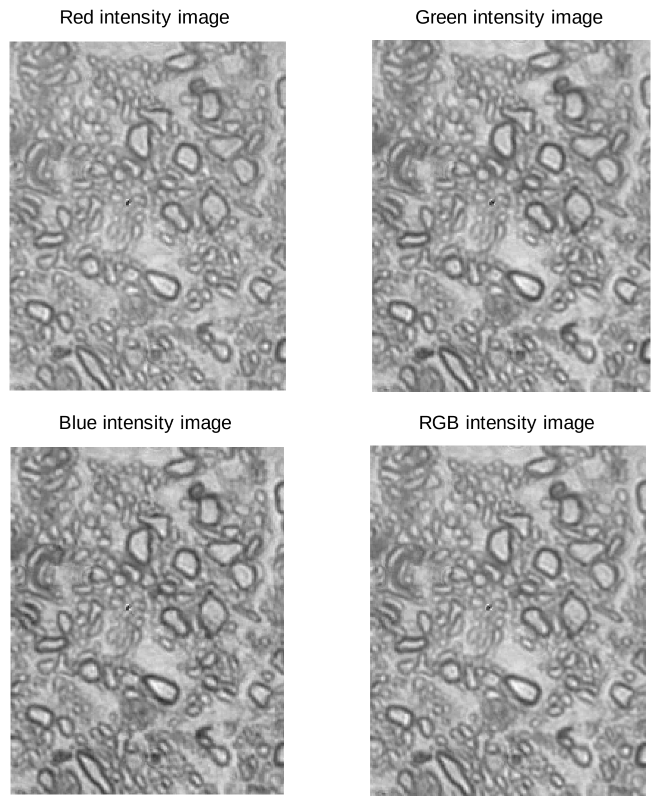


Fig. 2. The results of color segmentation

Although the edge of the rods and cones seems to be more clear in the blue intensity image, the noise still need to be rule out in order to obtain a clean image for further analysis. Therefore, a 3X3 average filter has been used to decrease the noise level. Moreover, circular averaging, and Gaussian lowpass filters have also been used to find out the optimal smoothing method. Fig. 3 shows the results of applying a 3X3 averaging filter on intensity images. The results again show its ability to smooth the image but still keep the edges of target objects. In the comparison of these images, the blue intensity image has once again been chosen for further analysis because of its well performance in the previous steps.

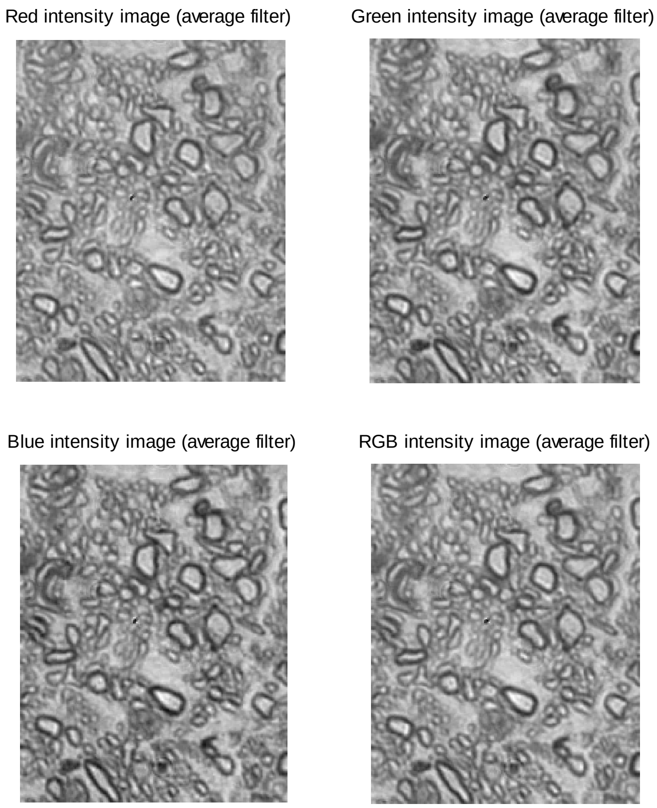


Fig. 3. Applying average filter the intensity images

Fig. 4 shows the enhanced blue intensity image by using the histogram equalization.

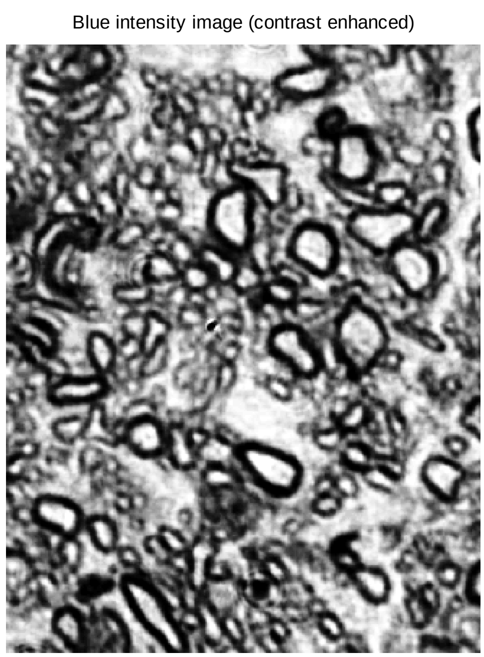


Fig. 4. Image enhancement using histogram equalization

The result of morphological opening is shown in Fig. 5. It is clear to find that the noise level has been further decreasing comparing to Fig. 4.

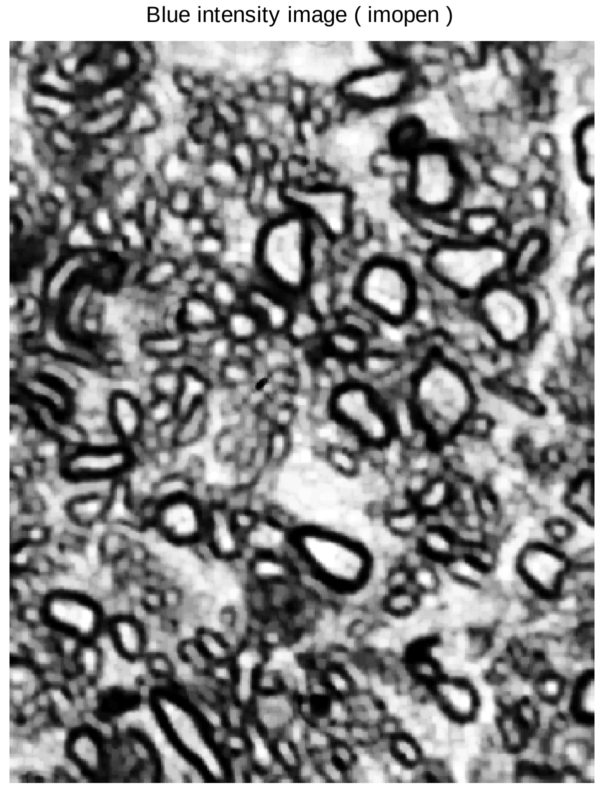


Fig. 5. The result of morphological opening

The adaptive background eliminating selecting window is shown in Fig. 6. The left image shows the image after Otsu's thresholding method. The right figure is the blue intensity image for a reference of the original image. This layout allows the user doing a comparison and then deciding which area needs to be filled. The number of input areas (selecting areas) can be modified in the program.

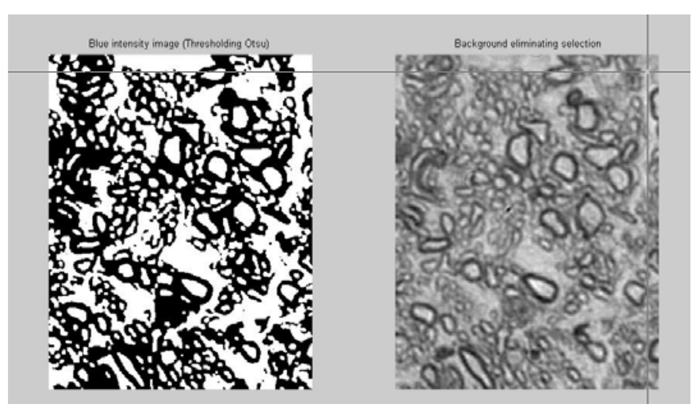


Fig. 6. (Left) Result of image thresholding

(Right) The input selecting image

A demonstration of this program on a sample image is shown in Fig. 7. As shown in these figures, after two unwanted area being selected, the target objects have all been identified.

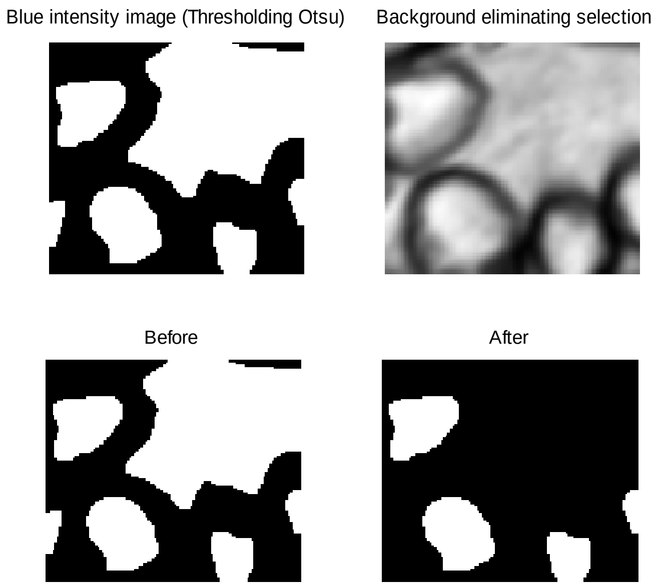


Fig. 7. The demonstration of the purposed algorithm

Fig. 8 shows the background eliminated image of the preprocessed image. Four background areas have been selected to do the holes filling process.

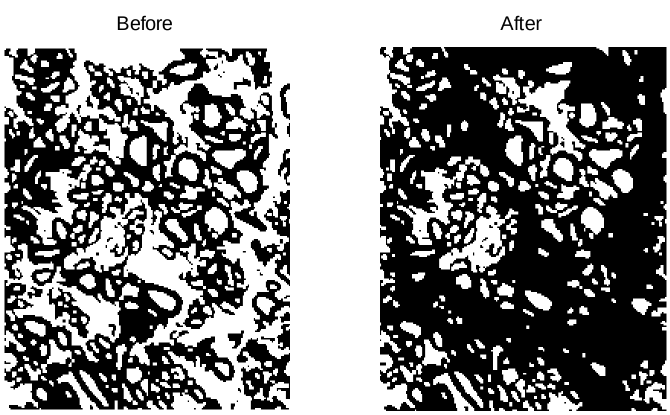


Fig. 8. The result of filling holes

Comparing the two images in Fig. 8, the right image shows obvious larger back area which means more unwanted background areas have been removed. However, some low contrast rods and cones have been taken as the background and being removed. A possible reason for this issue is that only parts of the intensity of these objects can pass the global threshold, therefore, the passed parts are not enough to construct closed bundles. Although it seems eliminate a big part of the target objects, Fig. 9 shows the identified rods and cones on the original image where the percentage of the identification is more than 70%.

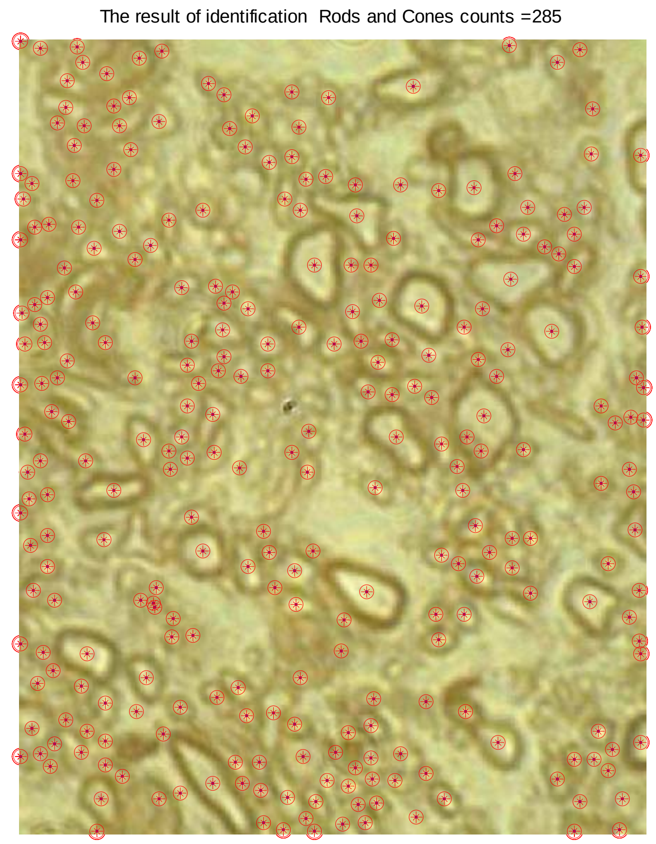
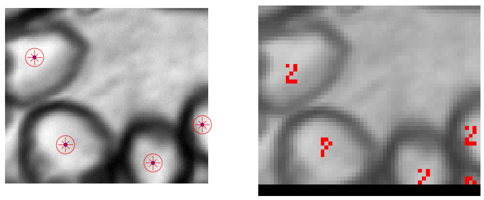


Fig. 9. The result of identification on the target image

EVALUTION OF THE ALGORITHM

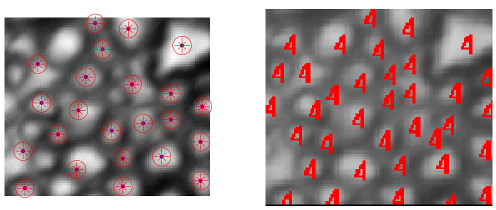
The other sample images have also been used to calculate the efficiency of the purposed algorithm. The number of identified target objects, and the percentage of the success identification are also shown in Fig. 10.

Rods and Cones counts =4



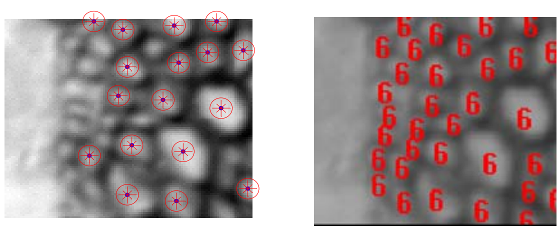
4/5 = 80%

Rods and Cones counts =23



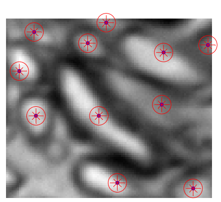
23/36 = 63%

Rods and Cones counts =17



17/33 = 52%

Rods and Cones counts =11



11/14 =79%

Fig. 10. The results of identification on the sample images

From the comparison above, the overall performance percentage of is about 69%. Over counting is barely found in by using the purposed method which is the strength this algorithm. However, since this algorithm is using the holes filling to fill the background to separate the targets and the background, thus, it strongly depends on an optimized pre-processing image to identify as many close bundles as possible. Otherwise the target objects may be removed with the background. Therefore, the effort is still needed to overcome the miscounting of the low contrast objects. The possible solution is to find other thresholding method to obtain the optimal threshold.

SUMMARY

This purposed approach is trying to find a straight forward and time efficient method to separate and count the close bundle target objects. The results show its ability to identify target objects with high true positive rate and acceptable false negative rate. The feature work for this algorithm is to find an optimal threshold deciding method to improve the false negative rate.

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