SEGMENTATION OF MICROSCOPE CELL IMAGES VIA ADAPTIVE EIGENFILTERS

S. Kumar[1], S. H. Ong[1], S. Ranganath[1], F. T. Chew[2] , T. C. Ong[2]

[1]Department of Electrical and Computer Engineering, [2]Department of Biological Sciences
National University of Singapore, Singapore

ABSTRACT

This paper presents the use of a PCA based approach to segment cells from RGB light microscope images. The proposed segmentation is accurate and robust under uneven illumination, lighting variation and noise. Principal component analysis (PCA) is first applied to the RGB color bands of the image. The image corresponding to the principal component has significantly better contrast over the original image. A set of eigenfilters is then obtained by applying PCA to local neighborhoods of this image. A pair of filters from this set, corresponding to the second and third largest eigenvalues, resembles ramp edge filters with orientations that adapt to the image. These edge filters are used to obtain the edgemap of the image. We define a criterion that enables accurate detection of valid edges of cells while suppressing noise.

1. INTRODUCTION

Segmentation is an essential step in the automated analysis of microscope cell images [1, 2]. Unfortunately, this procedure in light microscope images is made difficult due to uneven illumination, lighting variation and noise [1-3]. Figure 1 shows two spore images acquired under uneven illumination and distinctly different lighting. The darker regions due to uneven illumination may be falsely detected as cells by automated histogram thresholding techniques such as Otsu's [4]. Shading correction may be employed to solve this problem but this is a tedious task since an empty field is required every time the objective magnification or the microscope illumination setting is changed [3]. Furthermore, intensity and color variation also occurs in microscope images due to changes in intensity of the light source, aperture size or color filter.

Classical edge operators such as Sobel and Prewitt are highly localized in the spatial domain and are therefore relatively insensitive to gradual intensity changes due to uneven illumination and intensity variations. However, they are less suited for processing color images and also produce incomplete contours due to image noise.

(a) (b)

Figure 1: Spore images with (a) uneven illumination and (b) under different lighting conditions.

In this work, an approach based on PCA is proposed for the segmentation of RGB light microscope images. The proposed approach is robust with regards to the above mentioned segmentation problems.

First, the effects of intensity and color variation are reduced via a PCA decorrelation step. The contrast in the original image is enhanced by decorrelating the RGB color bands in the original image using PCA [5]. The decorrelated gray level image corresponds to the principal component.

Next, cells in the decorrelated image are detected using edge filters of localized support. The edge filters are obtained by applying PCA to the set of all local overlapping neighborhoods in the gray level image [6]. The local PCA operation yields a set of eigenfilters where a subset of this function as edge filters since they resemble ramp edge operators. These filters, henceforth called eigenedge filters have orientations that adapt to the image [7].

In order to reduce sensitivity to noise, an edge selection criterion is introduced. Contrary to selecting edges from pixels with high magnitude response, we define a criterion based on the projection angle between eigenedge filters and local neighborhoods of the image [6]. This is done by correlating the eigenedge filters with local neighborhoods after normalizing to unit magnitude. The correlation result is henceforth called normalized gradient map. Finally, binary morphological

0-7803-8554-3/04/$20.00 ©2004 IEEE.

operations are applied so that edge pixels are linked together to form closed boundary contours around each cell.

2. METHODOLOGY

The proposed method comprises the following steps: decorrelating RGB color bands, generating eigenedge filters, computing the normalized gradient map and detecting and combining edge pixels.

2.1. Decorrelating RGB color bands

The original image is basically a cluster of points in 3D RGB color space and PCA is able to determine the principal component eigenvector in this color space along which the scatter of the cluster of points is greatest. The original RGB image is then projected onto this eigenvector such that the contrast of the projected gray level image is enhanced [5].

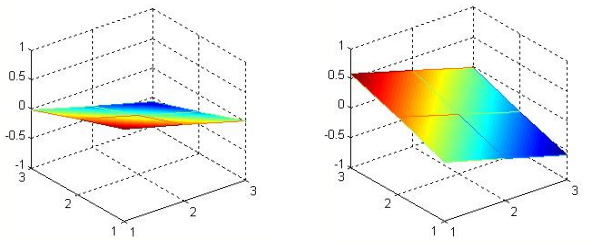
2.2. Generating eigenedge filters

PCA is applied on the projected gray level image from Section 2.1 in order to generate a set of T local filters (eigenfilters) that best represent the local neighborhood data of the image in a least square sense. We call a subset of these eigenfilters as eigenedge filters since they resemble ramp edge operators with orientations that adapt to the image.

In our approach, local neighborhood data refers to the set of $W \times W$ neighborhoods at each pixel over the entire projected image [6]. The T eigenfilters are also of size $W \times W$ each, where $T = W^2$. The subset of eigenfilters that function as eigenedge filters correspond to the second and third largest eigenvalues [7]. We set the dimension, W, of these eigenedge filters to 3 for best edge localization.

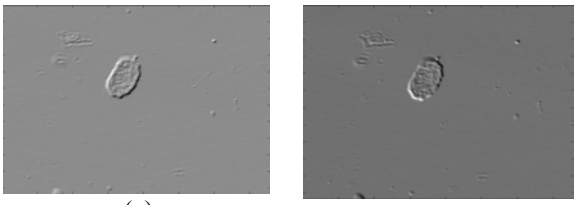
The projection of a set of data vectors onto an eigenvector in PCA is analogous to the correlation of the original image with an eigenfilter. The result of this correlation is a projected image called an eigenimage. The eigenimages corresponding to the eigenedge filters are henceforth referred to as eigenedgemaps.

Figure 2 shows an example of a set of two 3×3 eigenedge filters corresponding to the second and third largest eigenvalues. They are tilted at an angle and appear to extract some ramp edge information from the image. The corresponding eigenedgemaps in Figures 3(a) and (b) display similar edge features but different spatial orientations are emphasized.



(a) (b)

Figure 2. A set of eigenfilters corresponding to (a) Second largest eigenvalue. (b) Third largest eigenvalue.



(a) (b)

Figure 3. The eigenedgemaps corresponding to the eigenedge filters of Figure 2.

2.3. Computing the normalized gradient map

Contrary to the practice of defining pixels with high gradient magnitude response as edge pixels, we aim to detect all true edges, which can have small or large magnitudes [8]. Some true edges on the cell boundary may appear weak due to poor contrast. We propose an alternative criterion, the normalized gradient G_n, that enables detection of all true edges while suppressing noise. The normalized gradient G_n at an arbitrary $W \times W$ local image neighborhood N, can be expressed with respect to an eigenedge filter e_n as follows:

$$G_n(N) = \langle N, e_n \rangle = \frac{\sum_{i=1}^{W}\sum_{j=1}^{W} N(i,j) \cdot e_n(i,j)}{\sqrt{\sum_{i=1}^{W}\sum_{j=1}^{W} N^2(i,j)} \sqrt{\sum_{i=1}^{W}\sum_{j=1}^{W} e_n^2(i,j)}} \quad (1)$$

where the subscript *n* is the index of the eigenedge filter used. Each normalized gradient map, G_n, is further normalized with respect to its global mean, m_n:

$$G_n'(x,y) = |G_n(x,y) - m_n| \quad (2)$$

The normalized gradient G_n' of a pixel indicates the level of similiarity between the pixel's local neighborhood and the edge filter. The use of the normalized gradient map from (2) is similar to Frei-Chen's criterion [9]. It has also been shown to increase the robustness of the result under changes in light intensity [6].

2.4. Detecting and combining edge pixels

The two normalized gradient maps corresponding to the two eigenedge filters are then combined to form an equivalent gradient map G_{eq}. The pixel value at each location of G_{eq} represents the larger of the two normalized gradient maps as follows:

$$G_{eq}(x,y) = \max\left(G_1'(x,y), G_2'(x,y)\right) \quad (3)$$

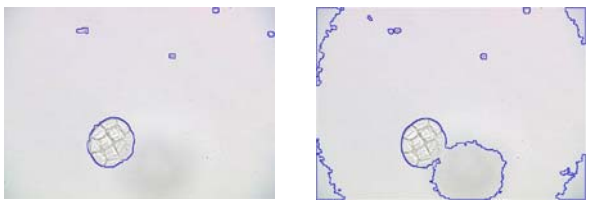
The edges of interest basically represent pixels in G_{eq} with values exceeding a predefined threshold. Poor resolution of cell boundaries may cause the edge pixels in the binary map to form incomplete edge contours. We overcome this problem by applying binary morphological operations to link the edge pixels. The linking of edge pixels is done via a dilation process involving a 5×5 structuring element. Next, the regions within the closed edge contours are morphologically filled. Finally, the filled regions are eroded using the same structuring element to restore the segmented regions to their original sizes.

3. RESULTS AND DISCUSSION

The algorithm is applied to a set of test images to demonstrate its robustness with respect to uneven illumination, lighting variation and noise.

3.1. Uneven illumination

Figure 4 compares the segmentation performance of the proposed method against Otsu under uneven illumination. The object of interest is circular in shape with a mesh-like texture. Otsu's method falsely segments the unevenly illuminated region at the corners of the image as well as the dark area located next to the object.



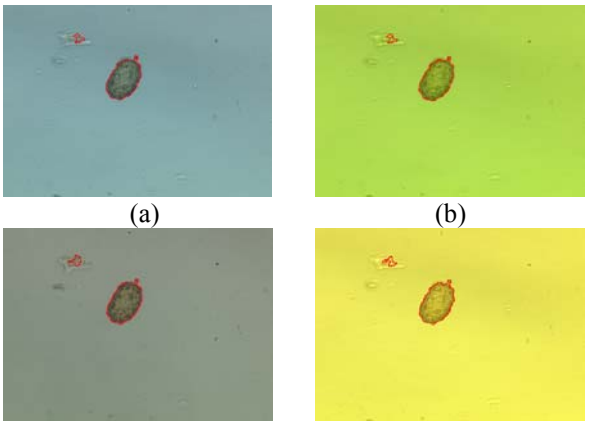
(a) (b)

Figure 4. Segmentation results of pollen image under uneven illumination. (a) Proposed method. (b) Otsu's method.

The proposed method gives accurate results since the small window size of the eigenedge filters makes them relatively insensitive to the gradual intensity changes due to non uniform illumination.

3.2. Lighting color variation

Figure 5 shows the segmentation results of the proposed method under four differently colored microscope filters. As observed, the spore (large particle) is accurately detected in all four cases. The robustness is due to contrast enhancement when the image is projected from RGB to gray level space.



(a) (b)

(c) (d)

Figure 5. Segmentation results of fern spore image with different microscope filters. (a) Blue. (b) Green. (c) Gray. (d) Yellow.

3.3. Lighting intensity variation

Figure 6 shows the same field of view as in Figure 5 but taken under very low light intensity. Although it is not realistic to operate a microscope in this manner, the example shows the robustness of the proposed method under extreme conditions. This method could prove useful in other application domains such as motion tracking in the dark.

(a) (b)

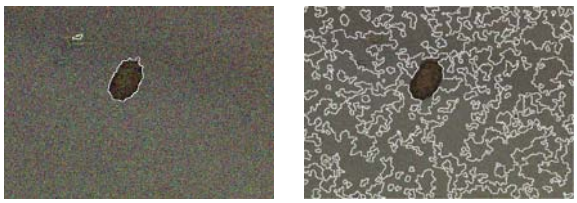
Figure 6. Segmentation results under very low light source intensity. (a) Proposed method. (b) Canny's method.

The proposed method was compared against Canny's edge detector. The hysteresis threshold parameters in Canny's method were tuned to give the best overall results for a large set of spore images. The standard deviation of Canny's operator was set to 0.76 based on Haralick's regression model [10]. Canny's method performs poorly, as observed in Figure 6(b)

since the image contrast is very poor. The accurate segmentation in Figure 6(a) by the proposed method is due to the contrast enhancing step of Section 2.1 and the lighting insensitivity of the eigenedge filters [6].

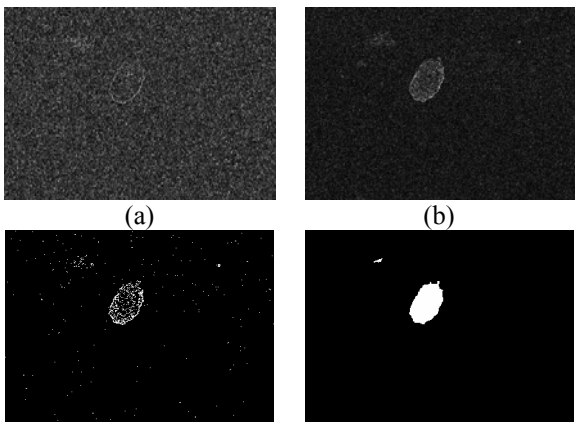
3.4. Additive Gaussian noise

Figure 7 shows that the proposed method is more robust than Canny's method under additive Gaussian noise. The accuracy of the proposed method (Figure 7(a)) is significantly better than Canny's results (Figure 7(b)). The corresponding Canny's gradient magnitude map in Figure 8(a) is significantly noisier compared to the equivalent gradient map G_{eq} in 8(b). Figures 8(b) to (d) show the sequence of processed images leading to accurate cell detection by the proposed method. The robustness under noisy conditions is due to the accurate discrimination between true edges and noise by G_{eq}.



(a) (b)

Figure 7. Segmentation results under additive Gaussian noise (mean = 0, variance = 0.01, SNR = -9.6dB): (a) Proposed method. (b) Canny's method.



(a) (b)

(c) (d)

Figure 8. Comparison between Canny's and the proposed method (8(a) and (b)). Processed images in proposed method (8(b)-(d)). (a) Canny's gradient magnitude map. (b) Equivalent gradient map G_{eq}. (c) Edge pixels (d) Detected cell region

4. CONCLUSION

A novel PCA based approach has been proposed for segmentation of cells from RGB light microscope images. This approach is robust under uneven illumination, lighting variation and noise. It presents novel ways of taking advantage of PCA firstly, in a point wise operation to obtain a contrast enhanced image and secondly, in a local neighborhood operation to obtain the eigenedge filters. The eigenedge filters resemble ramp edges and approximate the orientation and profile of natural edges in the image in the least square sense. The localized support of the filters makes the edge detection results relatively insensitive to gradual intensity changes in uneven illumination. The proposed edge resemblance criterion, expressed by the equivalent map G_{eq}, enables the accurate detection of all true edges of the cells in the image and suppresses noise. Relative insensitivity of eigenedge filters to uneven illumination and lighting contribute towards the robustness of the method. Contrast enhancement also diminishes the intensity and color variation effect. The proposed approach is simple to implement and computationally efficient as it requires an inversion of only a 3×3 covariance matrix for the decorrelation step and a 9×9 matrix for the generation of eigenfilters. The approach gives significantly better segmentation results compared to Canny's method for the set of light microscope cell based images considered in this paper.

5. REFERENCES

[1] L.P. Dzung, X. Chenyang, L.P. Jerry, "A survey of current methods in medical image segmentation", *Technical Report*, Department of ECE, Johns Hopkins Univ., USA, 1999.

[2] J. Bollmann, P. Quinn, M. Vela *et al.*, "Automated particle analysis: Calcareous Microfossils", *Image Analysis, Sediments and Paleoenvironments*, Kluwer, Netherlands, 2003.

[3] F.J. W-M Leong, M. Brady, J.O'D. McGee, "Correction of uneven illumination (vignetting) in digital microscopy images", *J Clin. Pathol*, 56, pp. 619–621, 2003.

[4] N. Otsu, "A thresholding selection method from gray level histogram", *IEEE Trans. Sys., Man Cyber.*, 9(1), pp. 62–66, 1979.

[5] M. Ledwith, "Land cover classification using SPOT Vegetation 10-day composite images – Baltic Sea Catchment basin", *GLC2000 – "First Results Workshop" JRC-Ispra,* 2002.

[6] V. Colin de Verdi`ere, J.L. Crowley, "Visual recognition using local appearance", *Fifth Euro. Conf. on Comp. Vision*, 1, pp. 640–654, 1998.

[7] P.J.B. Hancock, R.J. Baddeley, L.S. Smith, "The principal components of natural images", *Network Comp. in Neural Sys.*, 3(1), pp. 61-70, 1991.

[8] J. Canny, "A computational approach to edge detection", *IEEE. Trans. Patt. Anal. Machine Intell.*, PAMI-8, pp. 679–698, 1986.

[9] W. Frei, C.C. Chen, "Fast boundary detection: A generalization and a new algorithm", *IEEE Trans. Computers*, C-26(10), pp. 988-998, 1977.

[10] G. Liu, R.M. Haralick, "Two practical issues in Canny's edge detector implementation", *Int. Conf. on Patt. Recog.*, 3, pp. 676-678, 2000.