Comparative Analysis of Cell Segmentation using Absorption and Color Images in Fine Needle Aspiration Cytology

Xuqing Wu and Shishir Shah
University of Houston
Department of Computer Science
Houston, TX 77204-3010, U.S.A.

Abstract—Segmentation of cytological smears plays a critical role in the automated analysis of histological abnormalities by fine needle aspiration cytology. However, smears obtained from fine needle aspiration biopsy are often contaminated with blood. Segmentation of such an image is not a trivial task and the false positive rate could be high if the blood cells cannot be correctly separated from the rest of the sample. Moreover, the fine textured nature of the cell chromatin gives it a non-uniform intensity appearance in both color and gray images. In this paper, we propose an enhanced watershed approach to remove background noise by using short wavelength spectral image and the computed absorption image to improve segmentation accuracy. We also demonstrate a color image segmentation method by applying watershed to the minima imposed aggregation image. Results of segmentation on 20 images of cytological smears are presented and the accuracy compared for the two methods.

Index Terms—Multispectral Image Segmentation, Image Cytology, Fine Needle Aspiration Cytology

I. INTRODUCTION

Image segmentation is a key procedure in automating any computer-aided diagnostic system [1]. Accurate segmentation of the image plays a crucial role because it can ultimately determine the success or failure of computerized analysis procedure. These automated analysis procedures could help to reduce the labor time and increase the detection rate of clinical abnormalities in a multitude of applications [4], [2], [3]. On the other hand, modern diagnostic methods introduce a lot of challenges for the segmentation tasks. For example, fine-needle aspiration is widely used in practice as a safe, inexpensive, minimally invasive, and highly accurate procedure to diagnose a variety of tumors [5]. However, smears obtained from fine-needle aspiration biopsy are usually contaminated with blood. Microscopic images of the smear show that blood cells, in many cases, have similar shape and size as the target cells. In addition to the geometrical similarity, the color of blood cells can also be close to the color of the target cells post-staining due to the non-uniformity of the staining procedure. Without an effective method to exclude blood cells from the target cells, false positive rate of the image segmentation could be very high. Moreover, the fine textured nature of the cell chromatin gives it a non-uniform intensity appearance in both color and gray images. The existence of dense intracellular as well as intercellular matters makes it harder to draw a fine contour along the cell to delineate it without overshooting the cell boundary. In order to overcome these problems, multispectral imaging has attracted much attention since the unique transmission spectra of the biological tissue provides us with additional information that is potentially useful for better classification.

In this paper, we propose a segmentation method which is applied to the multispectral image. In addition, we also demonstrate a color image segmentation procedure using watershed and unsupervised clustering on an edge enhanced aggregation image. Our new algorithm on multispectral image provides a quick solution to the problem of recognizing blood cells by utilizing the knowledge acquired from the spectral information. The comparison of the two methods show that the segmentation based on multispectral image has a slight higher accuracy rate and much lower false positive rate than performing segmentation on the color image alone. The rest of the paper is organized as follows: section II presents the segmentation method applied to the color image. The proposed segmentation algorithm on multispectral image is presented in section III. Section IV compares the experimental results using both color images and multispectral images. The paper is concluded in section V.

II. COLOR IMAGE SEGMENTATION

Watershed based method [6] has been preferred by many researchers and applied to the problem of cell image segmentation because of its ability to deal with touching and overlapped regions. In watersheds, a 2-D image is treated in three dimensions: two spatial coordinates versus gray levels. The gray level of each pixel represents the elevations of the watershed surface. Points at which water would be equally likely to fall into more than one minimum consist of the watershed lines. Points at which a drop of water would fall into a single minimum consist of catchment basin. Watershed lines separate catchment basin into non-overlapped regions [7]. It is a region growing method and groups of pixels called “seeds” are needed to initialize the growing process. The initialization step is deemed as the most critical moment in a growing process [2]. Lezoray [3] suggested a supervised automatic clustering method, in which, each color plane is clustered independently by applying...
watershed operation on the gray-scale histogram. Each section in the cluster corresponds to a representative class of pixels in the image. The clustering information from different color planes is then blended together. However, spatial information is lost because of the use of the histogram. Touched cells are also clustered together in the supervised clustering process. To preserve the intensity variance information in the spatial domain, we apply an unsupervised clustering method to locate the “seed” for each region by using regional minima/maxima extraction.

### A. Morphological Self-dual Reconstruction and Regional Minima Extraction

Direct application of the watershed algorithm generally leads to oversegmentation problem due to noise and other local irregularities, such as the gradient [7]. Morphological reconstruction turns out to be extremely useful for image filtering and segmentation task [8]. Morphological self-dual reconstruction consists of reconstruction by geodesic dilation and erosion. Let \( f \) denote the marker image and \( g \) the mask image. The geodesic dilation of size 1 of the marker image \( f \) with respect to the mask image \( g \) is denoted by \( \delta_g(\cdot) \) and the geodesic erosion is denoted by \( \varepsilon_g(\cdot) \). The dilation process is defined as the point-wise minimum between the mask image and the elementary dilation \( \delta(\cdot) \) of the marker image and the geodesic erosion is the dual transformation of the geodesic dilation:

\[
\begin{align*}
\delta_g(\cdot) &= \delta(\cdot) \land g \\
\varepsilon_g(\cdot) &= \varepsilon(\cdot) \lor g
\end{align*}
\]

The reconstruction by dilation of a mask image \( g \) from a marker image \( f \) is defined as the geodesic dilation of \( f \) with respect to \( g \) iterated until stability is reached. The reconstruction by erosion is the dual transformation of the reconstruction by dilation. Let \( R_g \) denote the reconstruction by dilation and \( R_g^\varepsilon \) denote the reconstruction by erosion, then the overall process is defined as:

\[
\begin{align*}
R_g(f) &= \delta_g(f) \\
R_g^\varepsilon(f) &= \varepsilon_g(f)
\end{align*}
\]

where \( i \) denotes geodesic transformation of a given size \( \cdot \) that can be obtained by iterating \( i \) elementary geodesic dilations/erosions. Thus, the self-dual reconstruction \( R_g(f) \) for a given pixel \( x \) is defined as:

\[
[R_g(f)](x) = \begin{cases} 
[R_g(f)](x) & \text{if } f(x) \leq g(x) \\
[R_g^\varepsilon(f)](x) & \text{otherwise}
\end{cases}
\]

The above process can be easily extended to grayscale images according to [8]. Morphological grayscale reconstruction effectively reduces the intensity variance in a local area while preserving the intensity distribution information across the whole image. After applying morphological grayscale reconstruction to the image in each color plane, the unsupervised clustering process is done by h-extrema extraction. In morphology, a regional minima \( M \) of an image \( f \) at elevation \( t \) is a connected component of pixels with the value \( t \), such that every pixel in the neighborhood of \( M \) has a strictly higher value. To further suppress the irrelevant image features, in this paper, we use \( h \)-minima transformation that will exclude all minima whose depth is lower or equal to a given threshold level \( h \). This transformation is defined as the dual process of \( h \)-maxima extraction which is done by performing the reconstruction by dilation of \( f \) from \( f - h \):

\[
\text{hmin}_h(f) = R^f(\cdot, f + h)
\]

Since the clustering process is applied to each color plane, we need to fuse the regional minima information together. Let \( M_r, M_g \) and \( M_b \) be the binary matrices that hold the regional minima data of red, green and blue color planes. The combination operation is then defined as:

\[
M = M_r \land M_g \land M_b
\]

### B. Edge Enhancement

Before applying watershed method to grow regions based on the “seeds” obtained through regional extremea extraction as section II-A indicates, we want to enhance the dominant feature which is the edge of the image. The task is accomplished by adding the original image with the color gradient image. In this work, the color gradient is estimated using the Di-Zenzo vector-valued gradient [9]. Let \( I \) denote the grayscale image converted from RGB images by eliminating the hue and saturation information while retaining the luminance. The edge enhancement process is defined as:

\[
I_e = (1 - \alpha)I + |\nabla I|
\]

Normalization is applied to \( I \) and \( \nabla I \) before adding them together. The weight \( \alpha \) allows to balance the influence of the global intensity and local gradient value. The edge response is enhanced while the variance in other areas decreases. The gradient information effectively constrains the region growing process and prevents it from overshooting.

The final step of color image segmentation is to impose the regional minima \( M \) on the aggregation image \( I_e \). The watershed is then performed on \( I_e \) after the minima imposition.

### III. MULTISPECTRAL IMAGE SEGMENTATION

While the color image segmentation provides satisfactory separation of cells from the background, it does not solve the problem of separating blood cells from the target cells. In this paper, we propose a method which effectively excludes blood cells from the final segmentation by using multispectral images. Our experiments show that the use of multispectral data also improves the detection accuracy rate.

A spectral image consists of a series of images, each acquired by using a narrow band wavelength of light. If we take the wavelength as the third dimension, a spectral image is a 3-dimensional cube with the other two dimensions recording the spatial information of the sample. Studies have shown that biological tissues exhibits unique spectra in transmission [4]. By exploring the spectral differences in tissue pathology, many chemical and physical properties that are not detected under
traditional imaging methods can be revealed and used to aid the segmentation efforts.

In this paper, we use the Olympus BX51 optical microscope and a grating based spectral light source. 2D images are acquired by using a high resolution CCD camera. The Czerny-Turner type monochromator from PTI is used to provide a wavelength range from 400 - 700nm. A wavelength selection consists of two stages. In the first stage, the initial band selection in the window includes only short wavelength selections. In the second stage, the initial band selection is \( \{400 \text{nm}, 410 \text{nm}, 420 \text{nm}, 430 \text{nm}\} \). The band selection begins with letting the right side of the window grow in 10nm intervals until it incorporates the longest wavelength. BD for each growing interval is calculated. Window \( A \) with largest BD value is selected. Next, we let the left side of the window \( A \) shrink in 10nm interval until the window size equals to \( h_{\text{min}} \). Once again, BD for each shrinking interval is calculated and the window with largest BD value is selected.

Applying the above algorithm, the set of wavelengths for separating blood cells from target cells is \( \{400 \text{nm}, 410 \text{nm}\} \). A new image \( G_b \), which takes the average of images in selected bands is defined as:

\[
G_b = \frac{1}{C} \sum_{\lambda} G_{\lambda}
\]

where \( C \) is the cardinality of the set of selected wavelengths. \( G_{\lambda} \) is the spectral image with wavelength of \( \lambda \). Applying Otsu’s thresholding method to the image \( G_b \), we can obtain the optimal threshold which can be used to separate blood cells from the rest of the image. With the Otsu threshold \( t \), the set of pixels belonging to blood cells is defined as:

\[
BC(p) = \{ p \in G_b \mid I(p) \leq t \}
\]

where \( BC \) is the subset of the pixels of the original image whose intensity value of each pixel \( I(p) \) is less or equal than threshold \( t \).

The observation of 31 spectral images (wavelength ranges from 400nm to 700nm) shows that some spectral images play a positive role in differentiating target cells from the intercellular material while others do not (Fig.2). The same band selection algorithm is also applied to find the set of wavelengths that are suitable for separating target cells from intercellular material. The new image that takes the average of images in the selected band is defined as:

\[
G_c = \frac{1}{C} \sum_{\lambda} G_{\lambda}
\]

where \( C \) and \( G_{\lambda} \) have the same meaning as equation 10. With the information of the set of pixels classified as blood cells, a new image \( G \) is defined as:

\[
G(p) = \begin{cases} G_c(p) & \text{if } p \notin BC \\ -1 & \text{if } p \in BC \end{cases}
\]
In spectral imaging, intensities of gray level image are used to determine the proportion of light transmitted by each particle across the exciting spectra. There is an empirical relationship that relates the absorption of light to the properties of the material through which the light is traveling. This relationship is defined by the Lambert-Beer law [10]. According to the Lambert-Beer law, the transmission factor, $T$, is the fraction of incident light at a specified wavelength that passes through a sample:

$$ T = \frac{I_t}{I_i} $$  \tag{14}$$

In Equation 14, $I_t$ is the intensity of the light coming out of the sample and $I_i$ is the intensity of the incident light. By the Lambert-Beer law, transmittance is related to absorbance $A$ as:

$$ A = \log(1/T) $$  \tag{15}$$

One of the major impedances for us to correctly find the contour of target cells is the intensity variation of the cytoplasm. Absorption image, which is in the form of log-transformation, could map a wide range of high gray-level values in the input image into a narrow range of output levels. The opposite is true of lower gray-level values of the input image. Since the target cell areas that we are interested in tend to be located in the low gray-level range, applying log-transformation to its reciprocal will effectively reduce the intensity variance due to the existence of cytoplasm. Let $I_i$ denote the intensity of the incident light. The absorption image is thus defined as:

$$ G_{\text{absorp}} = \log(I_i/G) $$  \tag{16}$$

Fig. 1: Spectrum and Histogram of cell images

**B. Absorption Image**

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**C. Absorption Image Segmentation and Watersheds**

The overall segmentation process by applying watershed method to the absorption image is enumerated as following:
1) Find the set of images suitable for differentiating blood cells and calculate $G_b$ as shown in equation 10.
2) Threshold image $G_b$ and find the blood cell set $BC$ as shown in equation 11.
3) Find the set of images suitable for separating target cells from intercellular material and calculate $G_c$ as shown in equation 12.
4) Exclude the pixels belonging to the set $BC$ from $G_c$ as shown in equation 13.
5) Transform the image $G$ to the absorption image $G_{absorp}$ as shown in equation 16.
6) Apply morphological self-dual reconstruction and h-minima extraction to $G_{absorp}$.
8) Implement edge tracing [15] in each region to find the contour of target cells.

IV. RESULTS

Our experimental image set is comprised of 20 cytological smears stained with the Papanicolaou stain. Each smear is imaged at 40x magnification under both the multispectral imaging system and color imaging system. Images are manually analyzed to establish ground truth and a count of target cells in each image. We perform a comparative analysis between the algorithm developed for the multispectral absorption image and color image to understand the benefit of the proposed approach as well as the benefit of spectral imaging.

In the study, we compare the watershed segmentation result by applying the method to both multispectral absorption images and color images. Figure 3(a), 3(b) and 3(c) show a typical segmentation result obtained by using the absorption image. The result of applying watersheds to the color image is shown in Figure 4(a), 4(b) and 4(c). Segmentation results using both approaches show high detection accuracy. However, segmentation using multispectral absorption image excludes almost all the blood cells while segmentation using color image has difficulties in separating blood cells from target cells. The result of target cell segmentation across the entire dataset using each approach is presented in Table I. For the entire dataset (Fig. 5), the detection accuracy of the proposed method applied to the absorption image is found to be 95.19% with a false positive rate of 2.12%. Accuracy of target cell segmentation increases less than one percentage compared to segmentation using the color image. However, 25.26% decrease in the false positive rate is achieved using the proposed method on the absorption image as compared to the color image.

V. CONCLUSION

In this paper, we have presented a method that improves the accuracy of cell segmentation by exploring the characteristics exposed by specimen structure and composition under spectral imaging techniques. Utilizing the unique intensity distribution in the short wavelength spectral image, background noise is effectively eliminated. The segmentation process is further refined by using absorption image on an optimal set of spectral images. For the comparison purpose, we also develop a color image segmentation method by applying watershed to the minima imposed aggregation image. Segmentation results of both approaches are presented. The two techniques are compared against each other in terms of detection rate and false positive rate.

REFERENCES

**Fig. 4:** Result of applying watersheds segmentation on color images (a)(b)(c).

**Table I:** Comparative result of cell segmentation

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<th>False positive rate using color image %</th>
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**Fig. 5:** Summary of comparative result of cell segmentation


