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AUTOMATIC SEGMENTATION OF BIOMEDICAL IMAGES

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ABSTRACT

A new methodology which combines thresholding and probabilistic Relaxation Labelling Process is proposed for automatic segmentation of biomedical electron micrograph images. The image is first thresholded and initial label probabilities of individual pixel are assigned according to the distance of each pixel to the clusters resulted from thresholding. The label probabilities are then estimated and updated iteratively by employing the relaxation labelling process. To reveal local details, the initial labelling process is localized to sub-images of the original image. A heuristic criterion is defined to determine the number of classes that exist in each sub-image. Computing cost and artifacts are greatly reduced if the process is implemented at multiple levels of resolution. The method has been applied to electron micrograph cell images successfully.

I. INTRODUCTION

Automatic segmentation of biomedical images of electron micrograph (EM) images is the most crucial step for their automatic morphological analysis. Such images are difficult to segment due to the shading effects, introduced by sample preparation, electron microscope, photographic printing process, and the acquisition and digitization of EM images. Shading gives poor contrast, non-uniform intensity, among objects and background, and constitutes a major obstacle in automatic analysis of digitized images.

Attempts have been made by a number of researchers to overcome the problem of preprocessing EM images. Ito and Sato [1] selected thresholds manually to extract local line-feature segments [2] in finding DNA information. Lipkin and Lempkin et al [3,4], approached the shading and noise problem by combining the notch filter and thresholding and shrinking techniques in the preprocessing of EM of nucleic acid molecules. In this paper, a new technique is presented which combines thresholding and probabilistic Relaxation Labelling Process (RLP) for automatic segmentation of EM images. In particular, the technique is illustrated by partitioning EM images of cell structures into three classes : hetechromatin, cytoplasm or euchromatin, and background.

The paper begins with a brief discussion of the problems encountered when threshold selection techniques are used for EM cell image segmentation. Problems with published techniques have lead us to the use of a more powerful relaxation algorithm which utilizes the spatial context of each pixel as well as the global statistical information of the images. However, the RLP is inherently quite sensitive to initial probability labelling process [5]. We solved this problem by combining thresholding techniques with the relaxation process. The fine structures of the image can be revealed when the process is localized to sub-images or windows that partition the image. The advantages of this method, in both computing cost and smoothing properties, the efficiency is dramatically increased when multilevel RLP is employed.

II. THRESHOLDING

Thresholding techniques provide fast and simple approaches to image segmentation. Most techniques utilize the shape information of an image histogram, in threshold selection, or try to obtain the best histogram partition with respect to some criteria [6]. However, these methods are quite noise sensitive and hence error-prone, especially when object distributions overlap each other. As an illustration of this, Fig. 1 shows a typical digitized EM cell image that composes of hetechromatin regions (dark), cytoplasm and euchromatin regions (both grey) on a bright background. The image contains 256 x 256 pixels with 256 possible grey levels. Note that no distinct boundary exists between the hetechromatin, euchromatin and cytoplasm regions. Fig. 2 shows the corresponding grey level histogram of this image. The broadening and filling in of the intermodal valleys of the histogram show the effects of shading and noisy characteristics of the image. Fig. 3 shows the thresholded image by employing the recently proposed moment-preserving thresholding method [7]. This method offers several advantages over other popular methods [8,9]:

- no exhaustive optimal search is required;
- no a prior probability distribution is assumed;
- unique optimal thresholds can be obtained.

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As it can be seen, local segmentation errors occur between and within some hetechromatin and euchromatin regions. The connectedness of hetechromatin is not preserved in some regions. The results thus illustrate that grey level thresholding as an algorithm to produce segmentation is ineffective to a large degree due to shading.

To reduce local errors, the raw image can be smoothed before thresholding. Fig. 4 illustrates the segmented results after they have been preprocessed with an adaptive filter [10]. Artifacts within different regions are greatly reduced. However, some fine details are missing, especially in the hetechromatin regions, due to of the blur introduced by filtering. The results show that filtering before thresholding is an inadequate approach for good segmentation.

III. RELAXATION PROCESS

The probabilistic relaxation labelling process allows the introduction of global contextual information to reduce local ambiguities in pixel labelling. The RLP also provides useful "clean up" for noisy segmentations. The RLP can be defined as follows [11] :

Let (I_i) be the set of pixels and let $(L_1, L_2, \ldots, L_m)$ be the set of possible labels of classes. With each pixel I_i, an m-dimensional probability vector $(p_i(L_1), p_i(L_2), \ldots, p_i(L_m))$ is initially assigned. The component $p_i(L_k)$ indicates the probability that $I_i \in L_k$, where $L_k \in (L_1, L_2, \ldots, L_m)$, and satisfies

$$0 \leq p_i(L_k) \leq 1$$

$$\sum_{j=1}^{m} p_i(L_j) = 1 \qquad (1)$$

The label probabilities are then iteratively updated by independently computing a new $p_i(L_j)$ in the following heuristic manner:

$$p_i^{(n+1)}(L_j) = \frac{p_i^{(n)}(L_j)(1 + q_i^{(n)}(L_j))}{\sum_{k=1}^{m} p_i^{(n)}(L_k)(1 + q_i^{(n)}(L_k))} \qquad (2)$$

where

$$q_i^{(n)}(L_k) = \frac{1}{c} \sum_{k \epsilon N_{(i)}} \sum_{\ell=1}^{m} r_{ij}(L_k, L_\ell) p_j^{(n)}(L_\ell) \qquad (3)$$

where $N(i)$ denotes the 8-neighbourhood associated to the pixel I_i, and c is the number of pixels of such neighbours.

The coefficient $r_{ij}(L_k, L_\ell)$ reflects the degree to which classifying I_i into class L_k is compatible with classifying its neighbouring pixel I_j into class L_ℓ. The $r_{ij}(L_k, L_\ell)$ can be chosen empirically or estimated using mutual information. In our experiment, we defined $r_{ij}(L_k, L_\ell)$ as

$$r_{ij}(L_k, L_\ell) = 1 \text{ if } k = \ell$$

$$\text{else } r_{ij}(L_k, L_\ell) = 0 \qquad (4)$$

The significance of such a definition is that a pair of adjacent pixels having the same labels are assumed to be completely compatible with each other. We found that this arrangement works reasonably well with our images. For a general discussion of the RLP, readers are referred to [12]. The relaxation process, however, is quite sensitive to the initial label assignment. Slow convergence rate and segmentation errors would result from unrealistic initial labelling [5]. To solve such problem, we combined thresholding techniques with the RLP. The image is initially thresholded using [7] and initial probability vectors are estimated according to the Mahalanobis distance between a pixel I_i and class k as

$$p_i^{(0)}(L_k) = \frac{1/d_{ik}}{\sum_{d=1}^{m} 1/d_{i\ell}} \qquad (5)$$

where

$$d_{ik} = \frac{(I_i - \mu_k)^2}{\delta_K^2} \qquad (6)$$

with μ_K and δ_K^2 denote the mean and variance of class k.

As an illustration of the process, Figs. 5 and 6 show the initial labelling and final segmentation respectively. The iterative process stops when 99% of the pixels are labelled with any probability component greater than 0.9. Local segmentation errors are greatly reduced as compared with Figs 3 and 4. However, the morphological structures of the hetechromatin regions are still not preserved. Some fine details are still ignored. The results, as expected, illustrate that global density distribution is often a poor reflection of the local density distribution of an object, since some local information is masked out. Thus, to reveal such details, the initial labelling process can be localized to smaller sub-images or window, that partition the image. Each window w_i is considered to compose of at most three classes, and a lower threshold t_{Li} and a higher threshold t_{Hi} are assigned according to the steps:

step 1: Assume w_i consists of three classes; determine thresholds t_L and t_H for the window. Test the criteria:

$$p_1 p_2 (\mu_1 - \mu_2)^2 > g_1$$
$$p_2 p_3 (\mu_2 - \mu_3)^2 > g_2 \qquad (7)$$

if they are satisfied, then assign

$t_{Li} = t_L$;

$t_{Hi} = t_H$;

and stop.

else go to step 2.

step 2: Assume w_i consists of two classes; determine threshold t for the window. Perform the test as in step 1.

if this is satisfied, then assign

$t_{Li} = t$; $t_{Hi} = t_{gH}$. if $t \leq (t_{gL} + t_{gH})/2$

$t_{Li} = t_{gL}$; $t_{Hi} = t$ if $t > (t_{gL} + t_{gH})/2$

else w_i consists of one class only, and assign

$t_{Li} = t_{gL}$; $t_{Hi} = t_{gH}$.

The simple, but robust, criterion defined in step 1 provides measures of separability between two different classes of object. p_i and μ_i, i = 1, 2 or 3, respectively, denoted the fraction of the pixels and the mean value of the ith class within w_i. t_{gL} and t_{gH} are, respectively, the lower and higher threshold determined for the whole image. g_1 and g_2 are predefined constants.

Once the thresholds for each window are determined, the initial probability vector associated with each pixel in a particular window can be determined as described before. The discontinuities between the boundary of the windows can be eliminated by low pass filtering, with the constraints in (1) being satisfied.

Fig. 7 and Fig. 8 show the initial segmentation and final results respectively. Most of the fine details of the image are revealed and the morphological structure of the hetechromatin regions are now preserved. However, the method becomes more sensitive to shading. Small artifacts are present, especially within the euchromatin regions. The smoothing properties of the RLP are not sufficient to eliminate the errors.

IV. MULTILEVEL RELAXATION PROCESS

The smoothing power of the RLP can be enhanced if the algorithm is processed at multiple levels of resolution. We first perform a small number of relaxation iterations at a finer resolution level. The label estimates are then used as the initial estimates for a coarser level and the estimates are updated iteratively on that level for a few times. Finally, estimates at the coarser level are interpolated back to the finer level and more iterations follow until 99% of the pixels are unambiguously labelled. The basic idea of the process is based on the fact that, for a single level relaxation process, high spatial frequency errors become high frequency error at the coarser level.

Fig. 9 illustrates the final result when the multilevel process is performed at resolution levels of 256 x 256 and 128 x 128. Most of the artifacts are eliminated due to the more efficient smoothing capability of the multilevel approach. Note also that the multilevel process provides a faster convergence rate than standard single-level process, as it propagates contextual information more effectively at the coarser level. In comparison, the single-level algorithm required about 35 minutes to execute, while the multilevel counterpart took 18 minutes. Both algorithms were implemented in C using a 8MHz IBM AT personal computer.

V. CONCLUSION

A method employing thresholding and probabilistic relaxation process was presented for automatic segmentation of biomedical electron micrograph images of cell structures. Dramatic improvement in the quality of the segmentation can be obtained by localizing the labelling process of the system. A simple, but robust, statistical method was defined to determine the number of classes within a sub-image. Efficiencies, in both computing cost and smoothing properties, are greatly increased if multilevel relaxation is employed. The algorithm is local, highly parallel and suitable for implementation by hierarchical computational architecture.

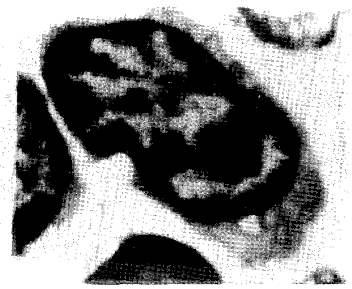

Figure 1

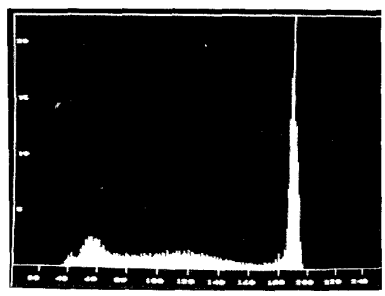

Figure 2

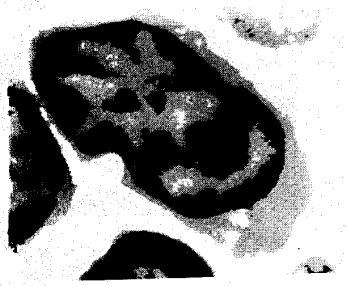

Figure 3

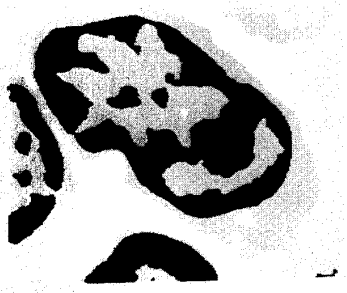

Figure 4

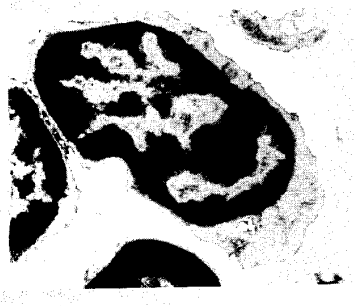

Figure 5

Figure 6

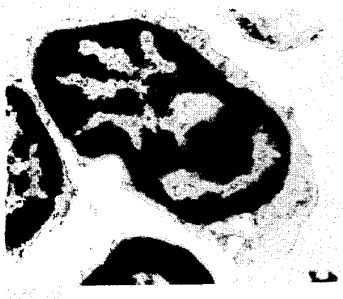

Figure 7

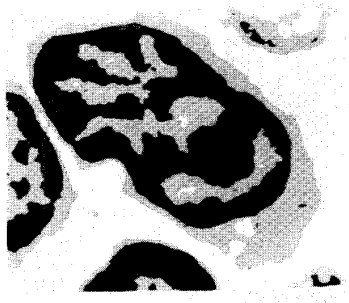

Figure 8

Figure 9

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