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MORPHOLOGICAL SEGMENTATION OF CLUSTERED NUCLEI IN ANALYTICAL CYTOLOGY

Norberto Malpica, Andrés Santos, Carlos Ortiz de Solórzano, Juan José Vaquero, Francisco del Pozo, José Miguel García–Sagredo*
Grupo de Bioingeniería y Telemedicina. E.T.S.I. Telecomunicación (U.P.M.). E-28040 Madrid (Spain)
<andres@die.upm.es>
* Servicio de Genética. Hospital Ramón y Cajal de Madrid (Spain)

Abstract – **Cluster division is a crucial problem in Analytical Image Based Cytology. Existing algorithms often make use either of the image content or its geometric characterization. Both sources of information are needed for a correct segmentation of difficult clusters. Morphological algorithms naturally deal with the object oriented criteria such as shape, size, contrast, connectivity, etc.**

In this paper, we present and evaluate a morphological watershed based algorithm applied to fluorescence stained clustered nuclei division. Results are shown for two different types of samples namely bone marrow and peripheral blood specimens. These results are better than those obtained for other published algorithms. This algorithm can also be easily adapted to different types of specimens.

I. INTRODUCTION

Cluster nuclei division is an important issue in analytical cytology studies where quantitative and morphological information about cells and their component distribution is necessary.

One of the applications of analytical cytometry is the detection of genomic aberrations on malignant or premalignant lesions, correlated with the diagnosis and prognosis of their related diseases. Fluorescence In Situ Hybridization techniques (FISH) provide a tool for this analysis.

Automated Fluorescence Microscopes with high sensitivity, Charge Coupled Devices (CCD) and programmed scanning processes have been developed in the last years to automate the analysis of FISH specimens.

The first stage of the processing of FISH specimens is to determine the location of the counterstained nuclei. Preparation protocols do not allow an appropriate nuclei separation, so an automatic algorithm to segment and define individual nuclei on clusters may greatly improve the throughput of a FISH analysis tool.

In this paper, we present a mathematical morphology based algorithm that uses watershed lines as a unified methodology for the segmentation of different types of clusters. Its results will be shown using two examples: one with a cluster of bone-marrow cells and another with peripheral blood cells. In both cases cells have been counterstained using Propidium Iodide.

II. MATERIALS AND METHODS

A) System Description

The microscope used is a Leitz Ergolux, with a motorized Marzhäuser scanning stage. Images are acquired with a Xillix MicroImager 1400 camera which uses a Kodak KAF 1400 (1,035x1,320 pixels, 6.8x6.8 μm each). The CCD can be clocked in pixel additive mode (binning), in which 4 adjacent pixels are combined for increased sensitivity and frame rate (2x2 binning).

A filter block N2.1 of Leyca (Excitation wavelength BP 515-560, suppression filter LP 580) has been used on the acquisition of the counterstained image, fitting the Propidium Iodide excitation/emission wavelengths (520/610). The objective used is a x63 Fluor, AN 1.30 (oil immersion).

Images are 672x519, and are acquired using the binning facility of the Xillix camera, with an exposure time of 0.3 sec.

B) Algorithm

1. Nuclei and Cluster Identification

After background correction, images are roughly segmented by thresholding the image histogram. An Isodata threshold segments most nuclei, but it is not able to isolate nuclei present on clusters.

After thresholding, resulting objects are labeled and measured in order to reject non-nuclei objects. Features used are object area, perimeter and aspect ratio.

The decision step classifies the different objects into nuclei, debris or clusters of nuclei. These clusters are the input for the proposed algorithm.

2. Watershed algorithm

The watershed algorithm [1] is a morphological operator which permits the detection of crest lines in images. Considering a gray-level image as a topographic surface, water falling on it will crawl down along the walls of the catchment basins corresponding to each minimum. The points where the water can fall to one of two sides are the crest-lines, which are then detected.

The original image has first to be transformed into an image where the boundaries searched are crest-lines and the

objects are valleys surrounded by these crest-lines. Unique markers for each valley have also to be defined.

An incorrect selection of these parameters (image transformation and image markers) would lead to oversegmentation or to a meaningless segmentation.

3. Marker extraction

On bone marrow clusters, nuclei usually have a uniform brightness, with some inter-nuclei gradients. A unique marker per nucleus is obtained as the thresholded domes of height h of the original image.

In peripheral blood, nuclei are granulouse and the previous procedure could lead to non unique markers per nucleus. A different procedure is then needed. We propose to use the maxima of the distance transform of the Isodata thresholded image as markers for the watershed. When clusters are packed, internuclei background markers are useful to obtain an adequate distance transform. These background markers are computed as the inverse morphological top-hat transform of the original image, followed by a binary morphological sequential filter to reduce noise.

Distance transformation of the image is obtained using the erosion transform [2], that is a morphological operation which assigns to each point in an object the maximum number of iterative erosions of the object which would still include the point. The regional maxima of the transform are then extracted.

The concept of immersion that implements the watershed algorithm can be closely simulated making use of a hierarchical queue with priorities defined by the gray level of image points [3].

4. Image Transformation.

On bone marrow specimens, the use of the inverse image has proved to be useful as the input to the watershed algorithm. Gradient (Sobel, morphological, etc.) images could also be used for this purpose.

On peripheral blood, as intranucleus gradients are often present, we propose the inverse of the distance transform as the work image over which to apply the watershed.

III. RESULTS AND CONCLUSIONS

The algorithm has been tested on 200 clustered nuclei. 89% in the bone marrow and 88% in peripheral blood samples were correctly segmented. Figure 1 shows two pairs of original and segmented images for both types of cells.

As it has been shown, the watershed algorithm is a powerful tool for the segmentation of fluorescence stained nuclei. The figures are at least similar to those published for other algorithms [4]. The watershed is a very intuitive algorithm which can be implemented on parallel architectures, offering a methodology that easily adapts to different types of clusters.

IV. ACKNOWLEDGMENTS

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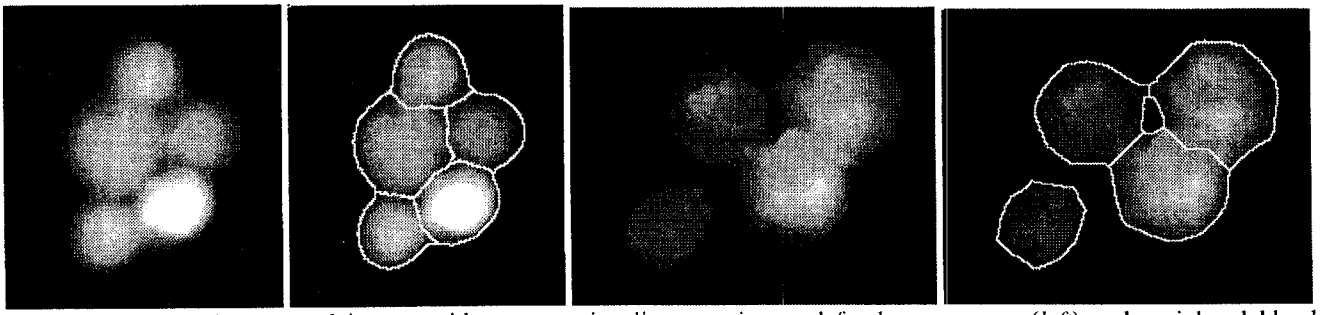

Figure 1: Original images and images with segmentation lines overimposed for bone marrow (*left*) and peripheral blood samples (*right*)