An Automatic Segmentation Approach of Epithelial Cells Nuclei

Claudia Mazo[1], Maria Trujillo[1], and Liliana Salazar[2]

[1] Escuela de Ingeniería de Sistemas y Computación
[2] Departamento de Morfología
[3] Universidad del Valle,
Ciudadela Universitaria Melendez, Cali, Colombia
{claudia.mazo,maria.trujillo,liliana.salazar}@correounivalle.edu.co

Abstract. Histology images are used to identify biological structures present in living organisms — cells, tissues, organs, and parts of organs. E-Learning systems can use images to aid teaching how morphological features relate to function and understanding which features are most diagnostic of organs. The structure of cells varies according to the type and function of the cell. Automatic cell segmentation is one of the challenging tasks in histology image processing. This problem has been addressed using morphological gradient, region-based methods and shape-based method approaches, among others. In this paper, automatic segmentation of nuclei of epithelial cells is addressed by including morphological information. Image segmentation is commonly evaluated in isolation. This is either done by observing results, via manual segmentation or via some other goodness measure that does not rely on ground truth images. Expert criteria along with images manually segmented are used to validate automatic segmentation results. Experimental results show that the proposed approach segments epithelial cells in a close way to expert manual segmentations. An average sensitivity of 76% and an average specificity of 77% were obtained on a selected set of images.

1 Introduction

Tissue samples are used to study biological structures present in living organisms. The samples contain information about cells and their distribution. Tissue samples allow identifying large amount of pathologies by analysing cells normality or abnormality, besides supporting diagnosis in daily medical practice of histologists, biologists, pathologists and related disciplines. Cells are the basic element in histology. The structure of cells varies according to the type and function of the cell. Pathologies are detected by analysing cells, in the daily practice of physicians [16]. Epithelia is one of the four basic body tissues. The lining epithelia have two locations: the lumen — the inner region — of hollow internal organs and external body surfaces coated, epidermis. These locations are always close to areas of light. A cell nucleus is a key part in identifying biological structures. A light region is labeled in Fig. 1 (a). Cell nuclei is enlarged and shown in Fig. 1 (b).

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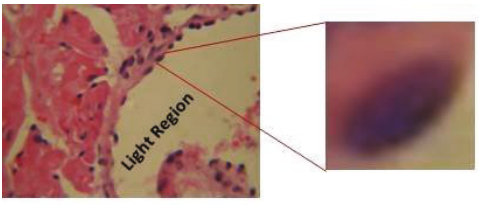

(*a*) (*b*)

Fig. 1. Illustration of Cell Nuclei in Epithelial Tissue. (a) Epithelia Tissue. (b) Cell Nuclei.

On the other hand, digital technologies development has made available, to physicians and biologists, microscopes connected to digital cameras for capturing images in order to preserve information. Different components of a tissue under a microscope are visualised and large repositories of images are gathered to preserve information. The cell image analysis process has two steps: segmenting cell nuclei and measuring morphological descriptions. The segmentation of cell nuclei has been addressed using morphological gradient [8], region-based methods [4], shape-based methods [12], minimax algorithm and thresholding [1], geometric active contours [17] and binary graph cuts [19]. However, these techniques require manual parameters setting. Moreover, histology images contain cell variations, due to photoelectronic and thermal noise, that make the segmentation of cell nuclei an open problem. Automatic segmentation of a cell nuclei involves several problems: the presence of similar cell nuclei that do not belong to the epithelial tissue, image noise acquired by capture devices, cell nuclei size to recognise, and low definition in image areas such as: borders, among others.

In this paper, an automatic segmentation of nuclei of epithelial cells is addressed by including morphological information. As original contribution, segmentation is performed using information in a manner similar to histologists, biologists and pathologists do. The segmentation is addressed in two parts: on the one hand, initially performs the segmentation of cell nuclei and on the other hand, light regions are identified. The distance between those cells and light regions is used to identify epithelia. Experimental results show that the proposed approach correctly segmented epithelial cell nuclei in histological images.

2 A Segmentation Approach of Epithelial Cell Nuclei

An automatic segmentation approach of epithelial cell nuclei is build using tissue morphological information. The samples were prepared with hematoxylin-eosin staining and by this technique nuclei are distinguished by an intense violet hue, which allows the differentiation of morphological characteristics of this cell structure: the shape, thickness, separation between a cell and other. The segmentation of cell nuclei is addressed in two parts: on the one hand, obtained segmentation with the largest eigenvalue of structure tensor algorithm along with the red and the green color channels are used as input into the K-means algorithm in order to obtain cell nuclei. On the other hand, the green color channel is segmented

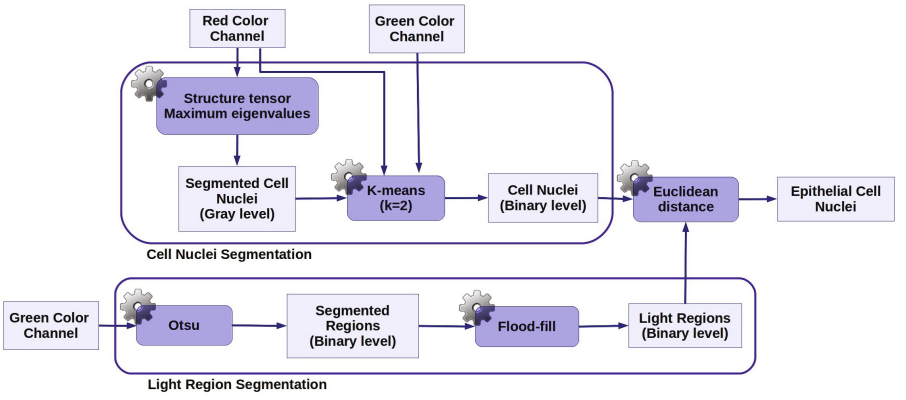

Fig. 2. Diagram of the automatic segmentation Process

using the Otsu algorithm follows by the Flood-fill algorithm in order to identified light regions — regions represented by large intensity values in an image. Finally, distances between cell nuclei and light regions are calculated to identify epithelial cell nuclei. The complete process is illustrated in Fig. 2.

2.1 Cell Nuclei Segmentation

Initially, different segmentation algorithms were evaluated in order to obtain cell nuclei of epithelial tissue; obtained results are: Fig. 3 (a) using the edge segmentation by computing the gradient-magnitude [2], [7]; Fig. 3 (b) using the gradient-magnitude combining with non-maximum gradient suppress [2], [7]; Fig. 3 (c) using the Hessian tensor maximum eigenvalues [5], [6], [9]; Fig. 3 (d) using the Hessian tensor minimum eigenvalues [5], [6], [9]; Fig. 3 (e) using the Structure tensor maximum eigenvalues [3], [10]; Fig. 3 (f) using the Structure tensor minimum eigenvalues [3], [10]; Fig. 3 (g) using the Normalised cut edge detection simple [18]; Fig. 3 (h) using the normalised cut edge detection with T=3000 [18].

The largest eigenvalue of structure tensor was chosen according to the best segmentation results in terms of cell nuclei segmentation, as it can be observed in Fig. 3 (e). The proposed cell nuclei segmentation combines results of the largest eigenvalue of structure tensor with the red and the green color channels into the K-means algorithm.

Largest Eigenvalue of Structure Tensor. The largest eigenvalue of structure tensor [15] is described as follows. Given the red channel of an image the structure tensor J_0 is defined as the outer product of the gradient vector $\bigtriangledown I$:

$$J_0 = \bigtriangledown I \left(\bigtriangledown I\right)^T = \begin{pmatrix} I_x^2 & I_x I_y \\ I_x I_y & I_y^2 \end{pmatrix}, \tag{1}$$

where $\left(\bigtriangledown I\right)^T$ symbolised the transpose of $\bigtriangledown I$. J_0 is extended to the linear structure tensor by a convolution of the components of J_0 with a Gaussian kernel K_p (Gaussian smoothing)in order to consider neighbouring information:

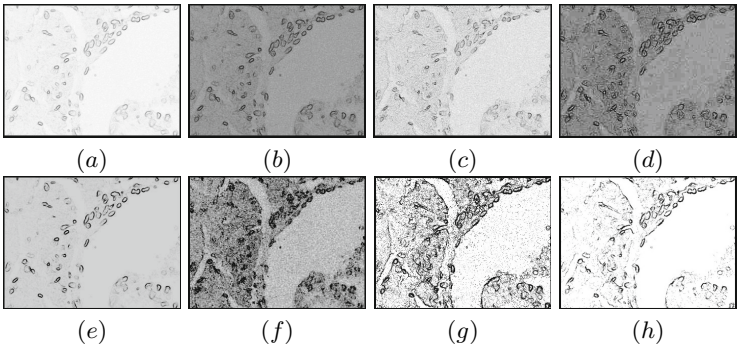

Fig. 3. Segmentation results obtained using Fig. 1 by (a) Using the Gradient-magnitude. (b) Using the gradient-magnitude combining with non-maximum gradient suppress. (c) Using the Hessian tensor maximum eigenvalues. d) Using the Hessian tensor minimum eigenvalues. (e) Using the Structure tensor maximum eigenvalues. (f) Using the Structure tensor minimum eigenvalues. (g) Using the Normalized cut edge detection simple. (h) Using the normalised cut edge detection with T=3000.

$$J_\rho = J_0 * K_\rho = \begin{pmatrix} j_{11}\ j_{12} \\ j_{12}\ j_{22} \end{pmatrix}. \tag{2}$$

The matrix J_p has orthonormal eigenvectors v_1 and v_2 with v_1 parallel to

$$\left(\frac{2j_{11}}{j_{11} + j_{22} - \sqrt{j_{11} - {j_{22}}^2 + 4j_{12}^2}} \right). \tag{3}$$

The eigenvalues are given by

$$\mu_1 = \frac{1}{2}\left[j_{11} + j_{22} + \sqrt{j_{11} - {j_{22}}^2 + 4j_{12}^2} \right], \tag{4}$$

and

$$\mu_2 = \frac{1}{2}\left[j_{11} + j_{22} - \sqrt{j_{11} - {j_{22}}^2 + 4j_{12}^2} \right]. \tag{5}$$

The eigenvalues describe the average contrast in the eigen-directions within a neighbourhood of size (ρ). The vector v_1 indicates the orientation with the highest red value fluctuations, while v_2 gives the preferred local orientation, the coherence direction. Furthermore, μ_1 and μ_2 serve as descriptors of local structure. Isotropic areas are characterised by $\mu_1 \cong \mu_2$, straight edges gives $\mu_1 \gg \mu_2 = 0$ and corner by $\mu_1 \geq \mu_2 \gg 0$ [15]. Fig. 4 (b) shows obtained results using the larger eigenvalues on the red channel.

The K-means Algorithm. It can be observed in Fig. 4 (b) small regions like cells contours. However, the obtained results are in gray level. The K-means algorithm,

with k=2, is used to refine this segmentation. The segmentation of cell nuclei is conducted using the red and the green color channels along with the tensor values — obtained in the previous step — by the K-means algorithm [11].

However, obtained K-means segmentation contains cell nuclei which do not belong to epithelial tissue, it can be observed in Fig. 4 (c). The light regions are commonly used by histologists, biologists and pathologists as information, since epithelial cells are always found close to the light regions. Thus, light regions will be identified in the next section, in order to refine the obtained K-means segmentation.

2.2 Light Regions Segmentation

Light regions are observed in white color in an image. Initially, the Otsu algorithm [14] is used to segment light regions on the green color channel. However, there exit small and large regions identified as light. Thus, segmented regions, using the Otsu algorithm, are not useful. It can be observed in Fig. 4 (d), Otsu segmented many small regions, which are not of interest. The Flood-fill algorithm [13] is used to filter and select larger regions identified as light. Larger regions are identifed using a threshold. Larger regions are shown in Fig. 4 (e) as white areas.

2.3 Combining Segmentation Results

Once light regions are identified and cell nuclei are segmented, distances between cell nuclei and light regions are calculated using the Euclidean measure [20]. A threshold is used to determine if a segmented cell nucleus belongs or not to epithelial tissue. In this way, we keep only segmented cells that are close to light regions. Fig. 4 shows results step by step of the proposed approach.

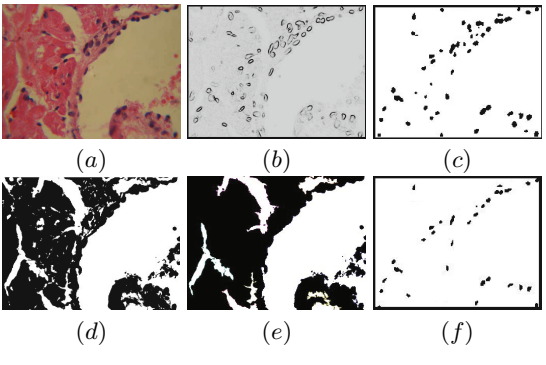

Fig. 4. Illustration of the proposed approach. (a) Original image, (b) Segmented cell nuclei using the largest eigenvalue, (c) Refined segmented cell nuclei by the K-means algorithm, (d) Obtained lighter regions using Otsu's algorithm, (e) Refined lighted regions by the Flood-fill algorithm, (f) Final segmentation of the cell nuclei of epithelial tissue.

3 Experimental Validation

In order to assess the proposed approach, epithelial tissue samples were processed with hematoxylin-eosin staining to highlight the cell nuclei. A set of 30 images were obtained and used to validate the proposed approach. Automatic segmentation results are evaluated by experts in a qualitative way. Also, expert manual segmentations are used as ground-truth to calculate the sensitivity and specificity measures. Sensitivity relates to the ability to segment epithelial cell nuclei and specificity relates to the ability not to segment non-epithelial cell nuclei.

Automatic segmentation and manual segmentation results of selected images are in Fig. 5. It can be observed that the proposed approach segmented all epithelial cell nuclei. However, non-epithelial cell nuclei are segmented as well, when they are close to light regions. Those cells have additional features that we are not taken into account, yet. A quantitative evaluation of the obtained results is in Table 1. The automatic segmentation results are compared with the manual segmentation – as ground truth.

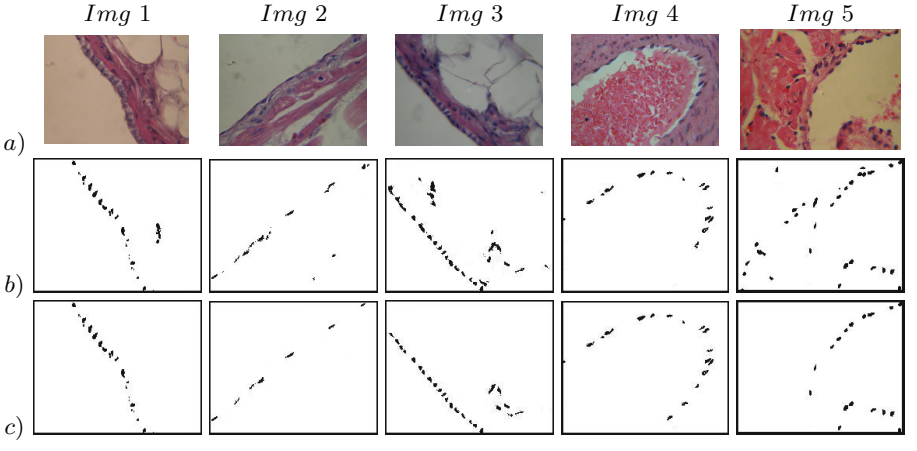

Fig. 5. Results obtained using automatic and manual segmentation of the epithelial cell nuclei. (a) Original images. (b) Automatic segmentation results. (c)Expert manual segmentation results.

Table 1. Performance evaluation of selected images

Confusion Matrix	Img 1	Img 2	Img 3	Img 4	Img 5	Avg 30 Imgs
True Positive	18	6	22	17	17	19
False Negative	6	6	9	0	15	6
False Positive	3	7	7	0	13	7
True Negative	25	18	23	54	30	24
Sensitivity	0.75	0.50	0.71	1	0.53	0.76
Specificity	0.89	0.72	0.77	1	0.30	0.77

Expert criteria are used to validate the automatic segmentation results. Table 2 contains the expert judgements using poor, average, good, very good and excellent in the evaluation of the automatic segmentation results of the images in Fig. 5. The evaluation of the expert 1 was based on observing nuclei, not morphology. The evaluation of the expert 2 took into account morphology, shape of nuclei and location these aspects are not taked into account in the proposed approach. According to the experts, the proposed approach is a promising tool since there is a discrimination of nuclei by their location to recognise epithelial tissue.

Table 2. Performance evaluation of selected images by expert

Expert	Img 1	Img 2	Img 3	Img 4	Img 5
Expert 1	Very good	Excelent	Good	Excelent	Good
Expert 2	Good	Good	Good	Very good	Good

Summarising, the segmentation results are guided by light regions as histologists, biologists and pathologists do. Images 1, 2, 3 and 5 contain non-epithelial cells close to light regions which mislead the segmentation. Those cells and enclosing areas contain additional information that histologists, biologists and pathologists use during the analysis. This information will be included as a constraint.

4 Conclusions

Cells are the foundation for recognising tissues present in an organ. Once cells are segmented, it is possible to identify a tissue or tissues. Segmentation is an initial step in a cell analysis process. In this paper, an automatic segmentation of epithelial cell nuclei was presented. The cell nuclei segmentation combines different source of information as input to the K-means algorithm. The proposed epithelial cell nuclei segmentation is based on combining segmented cell nuclei and identified light regions. Obtained results provided a closer segmentation to the expert-eye segmentation, according to the expert opinions.

As original contribution, the segmentation is performed using information in a manner similar to histologists, biologists and pathologists do. That is, perform the segmentation of cell nuclei, identify light regions, and calculate the distance between those cells and light regions to identify epithelia.

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