Morphological segmentation of histology cell images

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Abstract

Two algorithms for segmentation of cell images are proposed. They have a unique part that contains computation of morphological gradient to extract object borders and thinning the obtained borders to get a line of one-pixel thickness. For this task, we propose the fast gray-scale thinning algorithm that is based on the idea of the analysis of binary image layers. Then, the obtained one-pixel lines are used to extract cells and compute their characteristics. The algorithms based on morphological and split/merge segmentation are developed and used for this task.

1 Introduction

Extraction and classification of cells in histology images is an important problem that has many applications in practical medicine. The analysis of cell images can be considered as an image segmentation problem where cells or their kernels should be extracted [1]. Being extracted, object characteristics should be computed, analysed and used by doctors.

There were already several attempts to solve this problem. One of the most successful results in this area was obtained by the Kontron company that has realized the analyzer CIRES [2]. They use a controlled file system for the extraction of cells in which cell images are stored. However, the given method is effective only for good quality images, where cells obviously differ from the noise and background. Besides, the extraction of cells is limited by their number in a file system. In [3], an image is processed by the morphological operations of closing and opening with different octagonal structural elements for cell extraction. The algorithm does not always give a satisfactory result. There are other papers considering extraction of neurons, kernels or cells in histology images obtained by electronic microscopy [4].

However, due to the very complex nature of histology images, it is not possible to select or develop automatic segmentation methods that could be applied for any type of such images. This is why most of the papers consider separate features of cell images and methods of their segmentation. The results also depend on a cell image quality. If the difference between cell or kernel and background is small, most of the methods do not work.

From other side, the requirements were passed by medical doctors led to the necessity of the precise computation of the characteristics of cells, kernels and other objects. It made us to develop and apply quite sophisticated technology for extracting objects in histology images. The technology includes edge detection by morphological gradient calculation, thinning of obtained edges to get a one-pixel thick line, image prunning to cut short open branches. Then the technology is separated depending on the processed image types.

In this paper, we propose two algorithms for segmentation of cell images. They have a unique part to compute a morphological gradient for object border extraction and thin the obtained borders to get a line of one-pixel thickness. For this task, we propose the fast gray-scale thinning algorithm that is based on the idea of the analysis of binary image layers. Then, the obtained one-pixel lines are used to extract cells and compute their characteristics. The results were widely tested in BIOSCAN system that has been developed as analyser of medical images.

3 Thinning of histology images

3.1 Main principles of gray-scale thinning algorithm

Most of gray-scale thinning algorithms have been developed to extract image objects and thin them

ignoring image background [5,6]. However, in many images like medical ones, it is quite difficult to separate objects from background. This leads to the necessity to develop an algorithm that allows analyzing entire image and extracting all image ridges. We put the following principles in the thinning algorithm [8]:

1) Layer processing. We consider a gray-scale image as a set of layers and process every layer as a binary image. Previous layers are taken into account when the current layer is processed. If all layers are processed sequentially from top to bottom, then the result of the processing of the last layer will correspond to processing the entire gray-scale image.

2) Image iterations. Image thinning consists of four standard iterations: a) for north, b) for south, c) for east, d) for west edge.

3) Pixel processing. At each thinning step, border pixels decrease to background value, if they are not element of an arc and their removal does not change object connectivity. During pixel processing, we extract two main operations: pixel analysis (how to analyse a pixel and its neighborhood) and pixel value changing (how to change a central pixel value). Subsequently, we consider these operations in more details.

3.2 Pixel analysis

As we defined, the thinning algorithm includes four iterations: a) for north, b) for south, c) for east, d) for west edge. We use the following conditions for the iterations. The pixel is changed if:

a) $p2 < x$ **AND** $p6 \geq x$ **AND** ($p1 < x$ **AND** $p4 \geq x$ **OR** $p3 < x$ **AND** $p0 \geq x$ **OR** $p0 \geq x$ **AND** $p4 \geq x$),

b) $p6 < x$ **AND** $p2 \geq x$ **AND** ($p5 < x$ **AND** $p0 \geq x$ **OR** $p7 < x$ **AND** $p4 \geq x$ **OR** $p4 \geq x$ **AND** $p0 \geq x$),

c) $p4 < x$ **AND** $p0 \geq x$ **AND** ($p3 < x$ **AND** $p6 \geq x$ **OR** $p5 < x$ **AND** $p2 \geq x$ **OR** $p2 \geq x$ **AND** $p6 \geq x$),

d) $p0 < x$ **AND** $p4 \geq x$ **AND** ($p7 < x$ **AND** $p2 \geq x$ **OR** $p1 < x$ **AND** $p6 \geq x$ **OR** $p6 \geq x$ **AND** $p2 \geq x$).

pi are 8-neighbors defined counter clockwise starting from the east pixel (Fig.1).

p3	p2	P1
p4	X	P0
p5	p6	P7

Figure 1: 8-neighbourhood of the central picture element.

Depending on the number of neighbors (one, two, and more than two), the pixels of the resulting gray-skeleton are respectively classified as end points, normal points and branch points (nodes). End points and branch points are called feature points. Any subset of the skeleton bounded by feature points is called a skeleton branch. If the skeleton branch includes an end point it is called open branch, otherwise it is called closed branch.

When the pixel analysis has been performed, its value should be changed. This can be made in a sequential or parallel way. We consider these posibilities in more detail.

3.3 Pixel changing

3.3.1 Pixel changing in sequential layer processing. In this mode, image is processed from the layer with maximal value to the minimal one. The pixels of each layer having value more than a defined threshold are considered as skeleton pixels, otherwise they belong to background. This algorithm produces a good quality skeleton, but it is slower than the second algorithm type. The result of this algorithm processing is shown in Fig2.
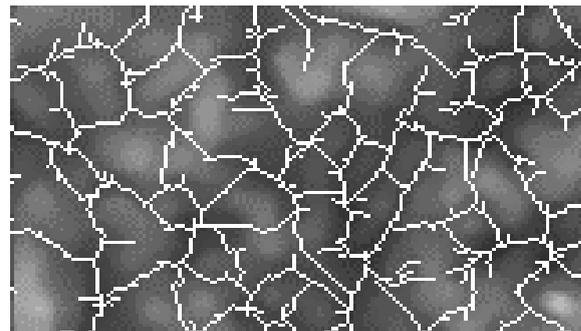
Figure 2. Sequential gray image thinning

3.3.2 Pixel changing in parallel layer processing. The second approach is based on the simultaneous processing of all image layers. In this case, pixels that satisfy the above conditions decrease by 1. It leads to the removal of edge pixels at every iteration. Repetition of these iterations results in decreasing of plates while they are not skeleton elements. The difference from the previous result is that there are more short branches. This effect comes from the different speed of thinning layers. The result of image thinning by this algorithm is shown in Fig.3.
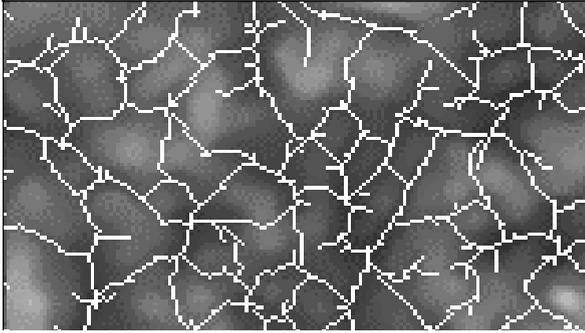
Figure 3: Image thinning by parallel layer processing

3.4 Skeleton binarisation

Binarisation is performed in one image scan. If the analysed pixel has at least one of its 4-neighbors (p0,p2,p4,p6) with value less than its gray value or all 8–neighbors are less then its gray value, then its binary value changes to 1 else its binary value changes to 0.

4 Morphological segmentation of cell images

One of the main features of a cell is its diaphragm, which outlines its boundary. Figure 1a shows a gray scale image of neuron of head brain with indistinct boundaries and noisy background. Our task is to find a cell boundary, which would best correspond to its diaphragm.

a)   b)
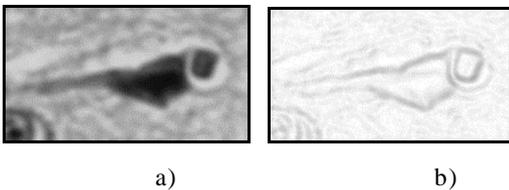
Figure 4: Neuron of head brain: a) gray scale image, b) result of morphological gradient extraction.

According to our approach, at a first stage, the morphological gradient is computed that is the difference of the outputs of erosion and dilation in one iteration (Fig. 4b). Obviously, an optimal border should pass on the sharpest overfall of brightness in a diaphragm, which corresponds to highest gray-scale values on the image obtained with the help of morphological gradient. The obtained borders usually have more than one pixel thickness. Therefore, thinning of border lines is applied.

However, quite many false branches are obtained after thinning that should be deleted. To avoid this problem, we use two possibilities. The first one is to reduce noise at the preprocessing stage. Morphological operations can be used for this aim. For example, closing operation allows smoothing object contours that give

better processing result. The second possibility is that we check and delete false branches after thinning. The result contains only closed contours, where the exterior contour corresponds to the border of a cell (Fig. 5a). To delete interior contours in binary image, contour filling is performed. As a result of all these operations, the binary cell image is obtained (Fig. 5b).

To delete objects not being cells, the computation of their area parameters is performed. The objects that do not satisfy apriori given geometric or optical parameters are deleted from the image (Fig. 5c).

a)   b)

c)
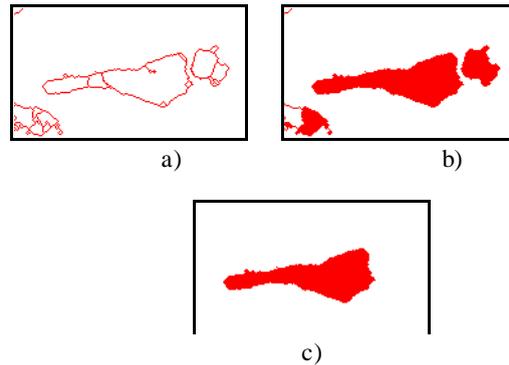
Figure 5: Extraction of a neuron: a) contour extraction after thinning operation, b) object filling, c) a binary image of a neuron obtained in the result of area measurements.

5 Split/merge segmentation of cell images

Very often a fabric image structure is understood as an image background and it is very difficult to extract a cell, as its gray values are similar to background gray values (Fig. 6a). In this case, the split/merge segmentation algorithm can be considered as the most suitable one [7]. The problem in this algorithm is a choosing initial regions "atoms" at the initialization stage.

We propose to use as "atoms" regions in the binary image obtained in the result of sequential realization of morphological gradient (Fig. 6b), thinning and binarization (Fig. 6c).

3
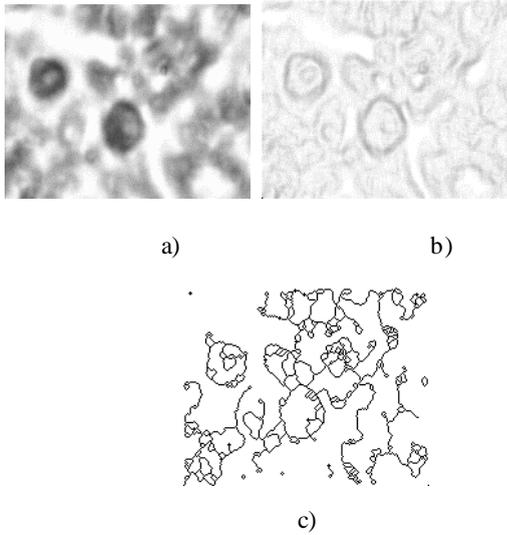
Figure 6: Images of a human liver cells: a) an initial gray scale image, b) result of morphological gradient, c) line extraction by thinning and binarization.

After these steps, we have binary image consisting of densely laying regions that are separated by lines of one pixel thickness. Region merging is performed by computing the region characteristics: Average and Variance [7].

The region merging procedure is as follows. Let us call the region the identified first, as a parent region. Then, the adjacent regions are extracted and their characteristics are compared with the parent region by the following conditions:

1. The difference of a variance for gray-scale values should not exceed a given value defining the difference of a cell from a fabric.

2. The average value of gray-scale value of each region should not fall outside the limits of the variance of the other region.

Under these conditions peformance, regions are merged and the new region is colored by a color of parent region. Then the characteristics of new integrated region are calculated:

$$
\begin{cases}
M_{12} = \dfrac{A_1 \cdot M_1 + A_2 \cdot M_2}{A_1 \cdot A_2} \\
s_{12} = \dfrac{1}{A_1 + A_2}\left(A_1 \cdot s_1^2 + \dfrac{A_1 \cdot A_2 \cdot (M_1 - M_2)^2}{A_1 + A_2} + A_2 \cdot s_2^2\right)
\end{cases}
$$

where M1, M2 are the average gray-scale values for parent and adjacent region, s1, s2 are the variance of gray-scale values for parent and adjacent region, A1, A2 are the squares of parent and adjacent regions, M12 is the average gray-scale value for integrated region, and s12 is the variance of gray-scale value for integrated region.

Then, the image is scanned to find new adjacent regions. If all regions are treated, one fom adjacent regions is considered as parent and the process is continued.

In the end, a set of regions corresponding to different image parts is obtained (Fig. 7a). To match the extracted regions with cells, geometric characteristics of the regions are computed and compared with apriori known ones. Regions that do not correspond to cells are deleted and remained regions are filled by separate color. In the end, we obtain a binary image containing only cells (Fig.7b).
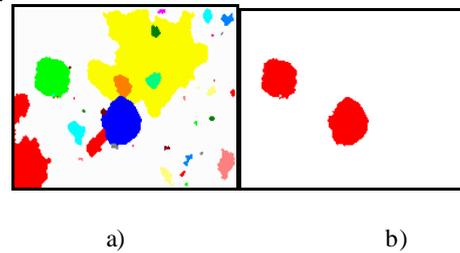
Figure 7. a) Result of region merging with a threshold value of a variance of gray-scale value 2,8, b) Binary images of cells chosen with the help of comparisons of squares.

6 Conclusion

In this paper, we proposed segmentation algorithms for histology cell images by means of mathematical morphology and split/merge operations. The results were widely tested in the BIOSCAN system that has been developed as analyser of medical images [9]. The performed experiments show that the proposed algorithms produce good results for most of the images that are used in medical practice. We should also note that the segmentation result depends on the quality of preparation and on the colouring of histology images. The obtained binary images can be used for measurements of cell characteristis for scientific and diagnostic purposes.

7 References

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