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EXTRACTION OF NUCLEUS TOPOLOGY PROPERTIES IN CELL HISTOLOGICAL IMAGES

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An algorithm for nuclear structure extraction is proposed in the paper. The algorithm consists of two branches. The first one is intended for extraction of cells with an unpainted nucleus, the second one is for a painted nucleus. According to a cell hierarchical structure, binary images of cells, nucleus, nucleolus and inclusions are created. For computing of topological characteristics, cell is presented as a hierarchy of binary images. The first level contains a binary image of cell, the second level contains an image of a nucleus, and the third level contains nucleolus and various cellular inclusions.

Keywords: investigation of cells, nucleus, nucleolus, topological characteristics, image processing

INTRODUCTION

An algorithm for nuclear structure extraction is proposed in the paper. The algorithm consists of two branches. The first one is intended for extraction of cells with an unpainted nucleus, the second one is for a painted nucleus. According to a cell hierarchical structure, binary images of cells, nucleus, nucleolus and inclusions are created. For computing of topological characteristics, cell is presented as a hierarchy of binary images. The first level contains a binary image of cell, the second level contains an image of a nucleus, and the third level contains nucleolus and various cellular inclusions.

The objective analysis of cytological and histological images has been the subject of research for many years. One of the most difficult fields in biomedical image analysis is automated extraction and classification of cells and nucleus.

A cell is the basic unit of living organisms (both plants and animals) that is capable of reproducing itself. The nucleus is the "Control Center" of each cell, which contains DNA (deoxyribonucleic acid, which is genetic information) in the form of genes, and also information for the formation of proteins. DNA, which is combined with protein, is normally dispersed throughout the nucleus as chromatin. During a cell division the chromatin becomes visible as chromosomes. The nucleus also contains ribonucleic acid (RNA), most of which are located in the nucleolus.

The nucleolus is a dense spherical structure within the nucleus of a cell. It contains RNA for the synthesis of ribosomes and also has an important role in the production of proteins and RNA.

Basic characteristic of nucleus images is colour of membrane and internal structure. Complexity of an image of nucleus is characterised by an internal topology structure. Analysis of this structure is very important for pathology diagnoses.

The basic pathology process in cells starts from nucleus. Therefore task of determination of nucleus pattern and its characteristic is important for analysis of pathology process in histology. This task can be divided into four sub-tasks: (1) the isolation of nucleus, (2) extraction of nucleus structure, (3) the extraction of nucleus features such as size and density, grey level extrema, fractal dimension, texture parameters and shape measures, and (4) use of these features to classify the nucleus [1,3]. However, due to a complex nature of histological images, it is practically impossible to select or develop automatic methods that can be applied for solving this task. That is why most of the papers consider task of nucleus image segmentation where only nucleus shape is extracted [4-8].

In this paper, algorithm for nuclear structure extraction is proposed. The algorithm consists of two branches. The first one is intended for extraction of cells with an unpainted nucleus, the second one is for the painted nucleus. According to a cell hierarchical structure, binary images of cells, nucleus, nucleolus and inclusions are created. For computing of topological characteristics, cell is presented as a hierarchy of binary images. The first level contains a binary image of cell, the second level contains an image of a nucleus, and the third level contains nucleolus and various cellular inclusions.

2. THE GEOMETRICAL CHARACTERISTICS OF NUCLEUS

The nucleus is a membrane bound structure that contains a cell hereditary information and controls cell growth and reproduction. It is commonly the most prominent organelle in a cell. Nucleus (fig. 1) is usually located inside cells. The grey-level characteristics of nucleus differ from cell characteristics. In a nucleus, there exist one or some rounded dark particles, that is named nucleolus. The nucleus is bounded by a double membrane called a nuclear envelope. The structure of nucleus can be divided into membrane, septa (fig. 2), inclusions, and nucleolus. Septa and inclusions constitute large structures that are crossing with membrane. Nucleolus constitute internal structures without membrane crossing.

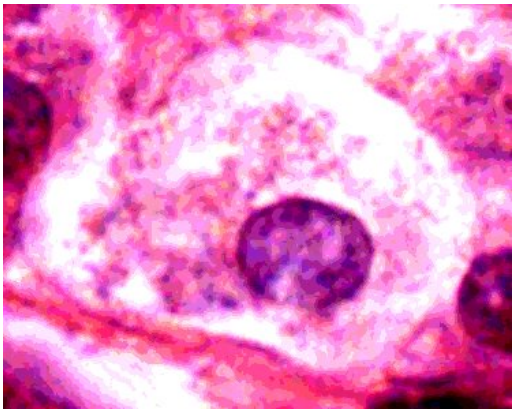


Fig. 1. Color image of cell with nucleus

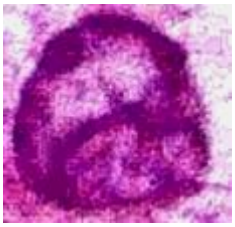


Fig. 2. Septa in nucleus

In spite that the basic characteristic of nucleus is their convex form and topology properties, they are not always the determining factors. For example, in mitosis cases and some kinds of pathology this condition is broken. Besides, on the way of colouring of a histological preparation, the content of cell is reflected differently on the image. In the descriptive pathology process in nucleus, this variety results to difficult description by many parameters. Furthermore, the most important parameters are the area, diameter, description of topology inside nucleus. Special measurements are applied to calculate these characteristics during identification.

3. BINARIZATION OF COLOR IMAGE

To determine nucleus pattern in histology image, a specific permanent colorant is usually used. It posterizes to nucleus by a differing colour. Hematoxylin is the most popular colorant for nucleus. It provides a visualization of nucleus of cells in histologic and cytologic preparations. Hematoxylin is intended for use as dye of nucleus at statement of immunocytochemical reactions in a combination to various types of chromogenes (including with soluble in ethanol), and also for painting hematoxylin and eosin. This colorant paints nucleus by dark blue colour.

To obtain binary image of a nucleus, the threshold segmentation is used in color system coordinates LCH.

$$\begin{cases} L = L \\ C = \sqrt{a^2 + b^2} \\ H = \begin{matrix} \arccos(a/C) & | b \geq 0 \\ 2\pi - \arccos(a/C) & | b < 0 \end{matrix} \end{cases}$$

where L - lightness, C - chromaticity, H - hue.

The basic gray value that we use in processing is lightness (L). Coordinates of hue (H) and chromaticity (C) characterize color connectivity pixel with its environment.

This system is a derivative from the system Lab. It is adapted to orthogonal model of human vision.

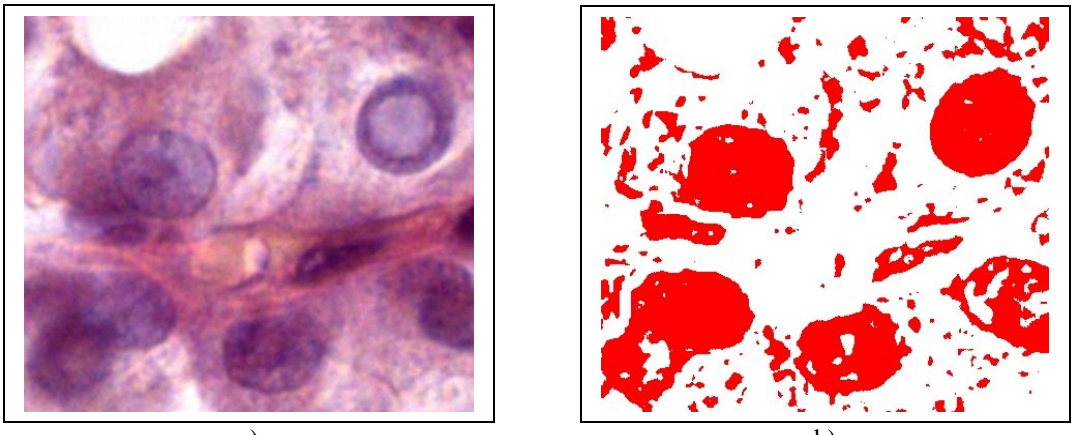
Threshold segmentation for lightness L is performed automatically [2]. The threshold is determined by means of decomposition of the lightness histogram into two parts. Each of them has Gauss-like forms. Then the threshold of chromatic components is determined on the CH coordinates circle of color space. The percentage terms the closest to smaller Gauss-like component of histograms is determined by a sector from the circle. Then, the threshold is exposed by a component of chromaticity H and functionally dependency from saturation C. Difference in color tone is less than $\pi/4$.

The corner of close color perception grows with reduction of color saturation, therefore it is meaningful to enter color pseudo-angle for the analysis:

$$\mathbf{H = H_{old}\, C_{max}/C,}$$

where H - color pseudo-angle, H_{old} - real hue, C_{max} - the maximal change of a chromaticity (saturation), C - chromaticity (saturation). Thus, region in circle-like line of color psevdo-angle determinate pattern of nucleus.

In the result, the binary image of a nucleus (fig. 3) is obtained.



a) b)

Fig. 3. Fragment of histology image: a) original, b) result of binarization

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4. EXTRACTION OF STRUCTURE OF NUCLEUS

In an ideal case, the cell should be distinguished by presence of a nucleus and nucleolus. To obtain the topological characteristics, nucleus is presented as a hierarchy of binary images, where the binary image of a nucleus is a root of graph, at the following level is the image of a net structure, the nucleolus is branches.

First of all, “opening” operation of mathematical morphology is carried out for separation of the merged close laying objects (fig. 4). Binary images of nucleus structures (fig. 5) are consistently created. To obtain binary image of a nucleus (fig. 4), it is necessary to execute “filling” operation to area appropriate for a nucleus.

The following stage is necessary to obtain the image of nucleus large structures (septa, inclusions). At this stage the way of colouring of a histology preparation begins to play an important role. An image of nucleus large structure is extracted by means of masking the color image and additional threshold segmentation. For removal of noise and dust the morphology “close” operation is carried out. The objects with a size less than the established size of nucleus are removed. Then from this image, borders of nucleus are subtracted.

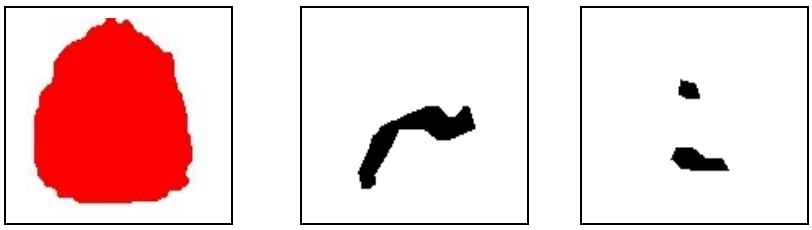

Fig. 4. Binary images of nucleus, spike and nucleolus

When binary images of nucleus, spike and nucleolus are determined, they constitute multiphase hierarchical image.

The idea of hierarchy of areas is easily realized in computer facilities using bits.

Object	Operation	Separate value	Result of association
Nucleus	1 < < 0	1	1
Nucleus structure	1 < < 1	2	3
Nucleolus	1 < < 2	4	7

For analysis of various cellular organelles this table can be continued. As each byte has eight bits, eight levels of hierarchy are optimum to allocate. Advantage of this method consists of sufficiency of one multiphase image used to draw conclusions on all levels of classification. (for example to get full information about cell).

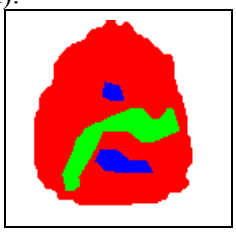

Fig. 4. Hierarchical image of nucleus

Thus, pixel value of the nucleus in this point corresponds to 1. Pixel of nucleolus in a nucleus the pixel corresponds to 7. In case of presence of inclusion, which does not lay in a nucleus, value of pixel is 5. Therefore it is easy to operate with various cellular components and then to analyze them.

CONCLUSION

The proposed method allows to modify result by removing the objects which are not belonging to nucleus and to construct the multiphase (hierarchical) image. This image reflects the hierarchy of nucleus structures. The obtained multiphase image of a nucleus can be used for subsequent analysis and measurements. Besides presence of nucleus and nucleolus at the multiphase image allows to study structure of a cell and its topological features.

Special case is presence of organelles or any nucleus inclusions. If the object with a level

appropriate the second bit does not satisfy the geometrical characteristics to a nucleus then pixel value is changed to value (XOR1ANDX) > > 1) AND 1. In the case when pixel is equal 7, it needs to be reduced up to 1 as it belongs only to a cell. Pixels with value 2,4,6, obviously are noise, therefore they are destroyed. When characteristics of areas are collected, among them it is easy to determine the objects appropriate to cells.

The proposed algorithm may be applied to extract any elongated nucleus and is tested on histology and cytology preparations.

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