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AUTOMATIC CALCULATION OF NUCLEI CHARACTERISTICS IN BIOMEDICAL IMAGES FOR CANCER DIAGNOSTICS¹

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Quantitative measurement is the basic part of cancer diagnostics. Measurement of topological nuclei characteristics and its orientation on images of tissue allows to determine symptoms of diseases. The algorithm for nuclear structure extraction is proposed in the paper. For computing of topological characteristics, nuclei are presented here as a hierarchy of binary images of nucleolus and inclusions. First level contains a binary image of nuclei, the second level - an image of a nucleolus, and the third level is for various nuclear inclusions. Characteristics of nuclei orientation are calculated then from binary images.

Introduction

The objective analysis of cytological and histological images is 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.

Cell is the basic unit of living organisms (both plants and animals) which is capable of reproducing itself. The nucleus is the "Control Center" of each cell. It 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 cell division the chromatin becomes visible as chromosomes. The nucleus also contains ribonucleic acid (RNA), most of which are located in the nucleolus. Nucleolus has dense spherical structure within the nucleus of a cell. It contains RNA for the synthesis of ribosomes and also plays important role in the production of proteins and RNA. Basic characteristic of nucleus images is color of membrane and internal

structure. Complexity of an image of nucleus is characterized by its internal topological 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 significant for analysis of pathological process in histology. It can be divided into four sub-tasks: 1) isolation of nucleus, 2) extraction of nucleus structure, 3) 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].

It is known, that due to a complex nature of histological images, it is practically impossible to develop FULLY 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, algorithms for automatic calculating nucleus characteristics in cells on histological images are presented. Topological nuclei properties and its orientation in tissue images are calculated that

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allows to determine symptoms of cancer diseases and make more precise diagnosis.

Geometrical characteristics of nucleus

Being responsible for controls cell growth and reproduction, nucleus is a membrane bound structure that contains a cell and nuclear and hereditary information. It is commonly the most prominent organelle in a cell. Nucleus (fig. 1) is usually located inside cells.

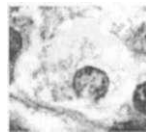


Fig. 1. Color image of cell with nucleus.

Grey-level characteristics of nucleus differ from cell characteristics. In a nucleus, there exist one or more rounded dark particles, which are named nucleolus. Nucleus is bounded by a double membrane called a nuclear envelope. 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 constitutes internal structures without membrane crossing.



Fig. 2. Septa in nucleus.

In spite of the fact that convex form and topological properties are the basic characteristics of nucleus, it is not a crucial factor (e.g. for mitosis or some kind of pathology). Moreover, while coloring a histological preparation (specimen), it is possible that content of nuclei reflects differently on the image. Such a variety results to difficult description of many parameters. Furthermore, the key parameters are: area, diameter, description of topology inside nucleus. Special measurements are applied to calculate these characteristics during identification.

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Detection of nuclei orientation

Morphological changes of standard nuclei of cell in tissues are one of important factors in oncological diagnostics. It may be related to pathological effects caused by cancer.

On the first stage of histological investigations the basic factors for diagnostics are tissues characteristics (nuclei shape, size, orientation and texture description). Instead of using structural analysis of cells populations in tissue, methods of mathematical morphology are often performed here [9,10], particularly by morphological filters for their shape, rather than frequency-oriented operations. These versatile tools can be used on different stages of the automated feature quantification process such as preprocessing, segmentation [11], and feature extraction [6].

The nuclei features are described by many standard geometrical characteristic. But ordinary measurement is not suitable to correct description of nuclei orientation in tissue because simple statistic of it does not correspond to orientation dominant. The goal is to use the fewest necessary measures to characterize an object adequately so that it may be unambiguously classified. When dealing with images containing numerous objects, such as histological or cytological nuclei images, shape descriptors are calculated for all the individual nuclei. Global shape measures can be calculated from the individual image descriptors. For cancer diagnostics, useful object characteristics are: area, major axis, angle, form factors and brightness. The major axis is the longest line that can be drawn through the object (1). The endpoints (x_1, y_1) and (x_2, y_2) (fig.3) are found by computing pixel distance between every combination of border pixels in the object boundary and finding the pair with the maximum length:

$$\text{major axis length} = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2} \quad (1)$$



Fig. 3. Illustration of major axis

The angle is calculated by major-axis too. It is the angle between the major-axis and the x-axis of the image. Its range is from 0° to 180° due to the next formula:

$$angle = \tan^{-1} \left(\frac{y_2 - y_1}{x_2 - x_1} \right) \quad (2)$$

This angle is a measure of object orientation. Result of measuring of binary images of cells is represented in table with geometrical characteristics as shown on fig.4. But characteristics of simple statistics (e.g. mean of angle) not always show dominant orientation of nuclei, because of averaging effect.

We may contrast an orientation by summation of major axis length. Resulting table includes data about orientation angle and total length for every direction. Total length is equal to sum of major axis length:

$$length[orientation] = \begin{cases} \text{major axis, if angle} = \text{orientation} \\ 0, \text{ else} \end{cases} \quad (3)$$



Fig. 4. Fragments of dialog windows for classification/

After recalculation of results which are in the table an orientation diagram can be constructed (fig.5). It is an additional visual method for histological cancer diagnostics. For normal tissue this diagram has only one dominant of nuclei orientation (fig. 5). For cancer case this diagram is similar to star, because nuclei have many orientations (fig. 6). Diseased nuclei change orientation before it

¹ This work is partially supported by ISTC project B-1489.

begin to resize. Therefore this method is useful for early cancer diagnostics.

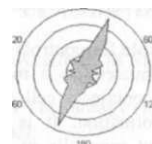


Fig. 5. Orientation diagram of nuclei in normal tissue

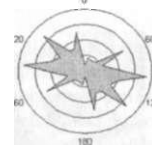


Fig. 6. Orientation diagram of nuclei in tissue for glioblastoma with nuclei polymorphism.

Extraction of structure of nucleus

Extraction of structure of nucleus To calculate topological characteristics, nucleus was presented as a hierarchy of binary images, where the binary image of a nucleus is a root of graph, and next level is the image of a net structure. Nucleolus is a branch of the graph.

First of all, "opening" operation of mathematical morphology is carried out to separate merged close laying objects (fig. 7). Binary images of nucleus structures (fig. 8) are consistently created then. It is necessary to apply "filling" operation for area of nucleus to obtain binary image of a nucleus (fig. 7).



Fig. 7. Binary images of nucleus, spike and nucleolus.

The following stage is required to obtain image of nucleus large structures (septa, inclusions). At this stage the way of coloring of a histological preparation begins to play an important role. Image of nucleus large structure is extracted by method of masking the color image and additional threshold segmentation. To remove noise and dust the morphology "close" operation is carried out then. The objects with a size less than appropriate size of nucleus are removed. Then from this image, borders of nucleus are

subtracted. 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. 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, (e.g. to get full information about nucleus).

Table 1. Hierarchical bytes representation

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



Fig. 8. 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 (table 1). 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 nuclear components and then to analyze them.

Conclusion

The developed algorithm has been realized in the system "Cytron" and tested on many histological images of thyroid gland for diagnostics of thyroid cancer. It allowed to improve a quality of computations of diagnostic characteristics of tissue.

The proposed method allows 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 of presence of nucleus and nucleolus at the multiphase image allows to study structure of a cell and its topological

features. Topological characteristics allow to describe features of histological tissue for cancer diagnostic.

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

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