Cytology 3D structure formation based on optical microscopy images

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Abstract. The article is devoted to optimization of the parameters of imaging of biological preparations in optical microscopy using a multispectral camera in visible range of electromagnetic radiation. A model for the image forming of virtual preparations was proposed. The optimum number of layers was determined for the object scan in depth and holistic perception of its switching according to the results of the experiment.

1. Introduction
Cytological examination is a highly specialized, complete, world-renowned method of morphological verification of diagnosis and at the same time it is one of the least automated and standardized types of laboratory diagnostics. It is well known that in the cytological diagnosis as in any other form of laboratory research, dominated by the subjective factor - the cytoclogic conclusion essentially depends on the skill and experience of the doctor-cytologist. But even experienced professionals sometimes have objective difficulties: in rare or complex in diagnosis tumors and tumor-like lesions; tumors which require additional diagnostic methods to specify histological form; non-neoplastic lesions, morphologically similar to tumors; borderline states, etc. At the same time, cytologic diagnosis is often decisive in determining the nature of the pathological process and the selection of treatment, so its importance for the life and health of the patient can not be overestimated.

Various additional methods of improving the quality of cytological diagnosis are developed to overcome the objective and subjective difficulties encountered in cytology. The doctor-cytologist information technology are actively developed and are successfully putted into practice. The rapid development and distribution of computer information systems, as well as the appearance of scanning microscopes contributes to this [1].

Scanning microscopy solves many problems: raises to a new level capabilities of telepathology (consultations using the Internet), archiving of preparations, improves the quality of intralaboratory and external quality control, cytology training and self-learning [2].

Scanning microscopes allow to automatically input digital image of cytological preparation in the computer. Preparation is scanned at different magnifications, image areas are automatically stitched together, you can view all of the preparation on the screen in the usual form of conventional microscopy, moving in the right areas to a greater magnification [3].
Currently, an increasing number of laboratories apply the preparations made by the method of "liquid-based cytology". These preparations are characterized by a multi-layer structure. In such cases scanning microscopes can not always ensure the completeness of the information of the images if they form an image of cytological preparations using conventional single layer scanning method. In this regard, the development of scanning system of preparations prepared with "liquid-based cytology" is important. This system uses the method based on three-dimensional structure of the objects. [1].

Complexity of preparations prepared by the method of liquid-based cytology lies in the fact, that the investigated cytological structure located in different layers and the focus of the microscope at one of these objects, in other planes are somewhat out of focus (blurred).

In cytological diagnosis color of researched structures is important, so multispectral camera is commonly used for registering microscopic images of cytological preparations. That camera records the electromagnetic radiation in three ranges of the visible spectrum (550-950nm) (400-650nm), (350-550 nm). Accordingly, specified range of recorded signals are indicated by the symbols of RGB (R - Red, G - Green, B - blue). A modern computer monitors (with appropriate color calibration) provided acceptable reproduction color of objects in cytological preparations to diagnose. Determination of the necessary layers of scanning and the distance between the layers remains a problem area [4].

The purpose of this study was to determine the optimum number of scanning layers and the distance between them for preparations prepared by the method of liquid-based cytology, for example preparations of the cervix.

An image forming model on example of one spectral zone for a microscope object located in one layer of the preparation, can be represented by the following equation:

\[
I(x, y) = \int_{-\infty}^{\infty} p(w, h) g(x - w, y - h) dw dh,
\]

where \(I(x, y)\) – illumination function on the photosensitive surface of the camera matrix (recording device), depending on the spatial coordinates \((x, y)\), formed by the optical microscope system for the preparation installed on the objective table;

\(g(x, y)\) – illumination function on the photosensitive surface of the camera matrix formed by the optical system of the "ideal" microscope for preparation mounted on the objective table (here an ideal microscope is the microscope, without distorting the formed image, and obtained image is an accurate likeness of the image of the preparation with an appropriate scaling factor - excluding the diffraction effects);

\(p(w, h)\) – point spread function (PSF) in the plane of the formed image corresponding to the optical system of the "real" microscope, \(w \text{ and } h\) – the spatial coordinates in the plane of the camera recorded image (for the "ideal" microscope \(p(w, h) = \delta(w, h)\), where \(\delta(w, h)\) – delta function).

For preparations in which objects can be in different layers in height, model given above is not acceptable, since different layers correspond to the different shape of the PSF (figure 1). The difference between the PSF for the different layers allows changing the focus to observe the microscopic image of the objects located in different layers in height and having the same coordinates \((x,y)\), while for a fixed position of focusing can be observed only one of those objects.

![Figure 1. Point Spread Function for different layers of the preparation](image)
In the case of an image forming in the preparation of multilayer objects we propose the following model:

$$\text{Im} (H, x, y) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} p(Z - H + H_s, w, h) g(Z, x - w, y - h) dZ dx dy.$$  

Here function $g(Z, x, y)$ represents luminance distribution in the image plane (space coordinates $(x,y)$) for the "ideal" microscope due to the location of objects in the layer of preparation with the vertical coordinate $Z$. $H$ - the distance between the lens and the surface of the microscope stage, $H_s$ - the distance between the lens and the plane, objects in which form a sharp image. Here, the use of characters $Z, H, H_s$ in the capital form reflects the fact, they are measured in the space of the preparation (in the thickness of the preparation), while the lower case characters $x,y,w,h$ correspond to the spatial coordinates in the image plane formed by the microscope on the photosensitive matrix of electronic camera, recording this image. Layer with the coordinate $Z = H - H_s$ corresponds to layer, which forms in the "real" microscope maximally sharp image, the other layers are formed blurry images of objects in these layers. At the same time, the larger the difference $Z$ from $(H - H_s)$, the greater the degree of blur images of objects in the corresponding layer.

Thus, an infinite number of different images Im $(H, x, y)$ can be obtained for preparations with a multilayer structure by changing the value of $H$. The researcher has interest for those images that contain sharp images of objects that represent multi-layered structure. With automatic scanning of the preparation is unknown in advance, which layers contain objects, so before you start scanning, you need to determine how many layers you want to scan and set the step size between the scanned layers so as to "not lose" important objects for the diagnostic conclusion. To solve this problem was an experimental study on real preparations from the cervix, prepared by the method of liquid-based cytology.

Determination of the number of layers to scan carried out on the basis of recording in each position (a step for moving the axis $Z$ equal to 60 nm) for 20 objects in 5 preparations diagnostically significant objects. Then scanning step was selected so, that registration of the most important objects was provided and holistic perception of diagnostically significant objects was remained when viewing layers.

As a result of experiments for cervical preparations prepared by liquid-based cytology, the distance was determined as of not less than 0.6 micrometers, which provides capture of diagnostically significant objects between layers, and the distance between the objects during the experiment was 12 micrometers, which corresponds to 20 layers.

2. Conclusion

The study determined the distance between the layers of automatic microscopic scanning of preparations prepared by the method of liquid-based cytology, and containing multilayer objects (for example, cervical preparations). It was proposed analytical model describing the formation of a microscopic image when observing biological preparations with a multilayer structure in the visible range of the electromagnetic radiation. Method of experimental research scan modes has been described. In results of experiment the distance between the diagnostically relevant objects for automatic scanning cytology preparations prepared by the method of liquid-based cytology (the example of cervical preparations) was determined. Application of the results of the study will allow to form a virtual preparations for multilayer objects without losing the informative part of these objects. This results of work, described in the article, are important both in remote cytological diagnosis and in assessing the quality of cytology laboratories.

Acknowledgments

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