The method of segmentation of leukocytes in information-measuring systems on the basis of light microscopy

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Abstract. The paper considers the problem of leukocytes segmentation in microscopic images of bone marrow smears for automated diagnosis of the blood system diseases. The method was proposed to solve the problem of segmentation of contacting leukocytes in images of bone marrow smears. The method is based on the analysis of structure of objects of separation and distances filter in combination with the watershed method and distance transformation method.

1. Introduction
Hematology analyzers and flow cytometry technique are widely used for analyzes of blood and bone marrow aspirates in the diagnosis of diseases of the blood system [1-3]. Flow cytometry technique has advantages: high performance, the ability to define immunophenotypical subtype of acute leukemia. But it has the following disadvantages: the complexity of the analysis of the morphology of the cells, the inability to re-sample analysis, an incorrect classification of cells because of the significant variability of the measured characteristics of the cells [3-4].

Currently the automated analysis of microscopic images of peripheral blood and bone marrow smears is actively developing [5]. The procedure of detection of abnormal cells in such systems includes next steps: image enhancement, segmentation of cells, the description of the leukocytes in the language of signs, classification [1-17].

The problem of segmentation of cells in microscopic images of bone marrow smears has particular difficulty [11]. This is due to the fact that in these preparations the leukocytes are often in contact with each other - "stick together", forming chains and conglomerates. In this regard, task of developing segmentation algorithms that allows to separate "sticky" white blood cells is actual.

The purpose of the present paper is to describe results of development and research of a method of segmenting leukocytes on images of blood and bone marrow in large clusters of cells that stick together among themselves.

2. The leukocytes segmentation method
The study of segmentation methods were carried out on 200 microscopic images of bone marrow smears. Leukocytes were present in different quantities (1 to 45) in the one frame field. Images were obtained by the system of computer microscopy(automated Olympus BX43 microscope with camera Imperx IPX-4M1ST-GCFB). Images were saved in BMP format, color-coded RGB24 (~16 million colors). The size of the image 1917x1092 pixels. The color value range of the nuclei by components: R=[47:122], G=[39:114], B=[95:143].
Examples of leukocytes in contact with each other are shown on Figure 1.

![Image of leukocytes](image1)

**Figure 1.** An example images of leukocytes in contact with each other. Images were obtained from bone marrow smears.

Cells separation algorithms were analyzed in according to the publications [6-8] in the first stage of solving of segmentation problem. For example, separation occurs by partitioning of the circuit on the connecting line between the angle points in the algorithms of angles finding. The downside of this algorithm is the case of angles greater than two (Figure 2)

![Image of angles separation](image2)

**Figure 2.** An example of the separation of leukocytes by finding the angles and the problems associated with separation at the corners.

One of the solution of the adherent leukocytes problem is application of algorithms of transformation of distances and algorithm of watersheds. This method is suitable for separating multiple leukocytes, since the watershed algorithm can detect all areas in the transformed image (Figure 3) [7-8]. The disadvantage of this approach is that there are the presence of artifacts, segmentation of voids inside the cells and space in the case of large concentrations of leukocytes. Finding local maxima was used for this problem decision, but that solution spoils the boundaries of the object. In this regard we have proposed an approach for the modernization of the method described above with the addition of filter cavities based on the method of labelling and filtering of the distances in the algorithm of transformation distances.

![Image of watershed and transformation](image3)

**Figure 3.** An example of results of methods of the watershed and transformation of distances for the separation of leukocytes.
A software module with a graphical user interface for computer image processing was developed in C++ with use of Qt library. That allows you to upload images and apply automatic analysis, and display results. An example of applying separation of leukocytes is shown in Figure 4 [9].

![Figure 4](image)

**Figure 4.** An example of segmentation of leukocytes in conditions of contact between the cells without the proposed method of segmentation (A) and using the proposed method (B).

The number of leukocytes was counted manually and programmatically in the performed experiment for each of the 200 frame microscopic images of bone marrow smears. The ratio of the actual number of white blood cells to cells, which were found by software, characterizes the accuracy of the computer system. According to the study, at the stage of using an approach combining the method of the watershed transformation and distance without proposed improvements, the resulting accuracy was 56%, and after the application of our approach accuracy was 91% for white blood cells lined up (Figure 1) and 88% for leukocytes, forming a closed chain (Figure 4).

3. **Conclusion**

The method for segmentation of contacting leukocytes in microscopic images of bone marrow smears was proposed. The method is based on the analysis of structure of objects of a separation and distances filter in combination with the watershed method and distance transformation method. Application of the method improved the accuracy of the segmentation from 56 to 91%.

Testing the proposed method on the extended sample of smears of bone marrow and using the proposed approach in automated systems of the analysis of smears of bone marrow will be further step of our research.

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