Computer microscopy in lymphoma diagnostics

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Abstract. The article describes the application of computer microscopy with multi-spectral camera for the comparative characteristics of normal lymphocytes and lymphoid cells in follicular lymphoma. Wavelet functions are used to quantify parameters of the cells nuclei images.

1. Introduction
The cooperation of doctors, mathematicians and engineers over the last few years has contributed to the development of computer microscopy with using methods of digital image processing of the hematopoietic cells. The emergence of new computer microscopy systems allows to identify structural differences between the nuclei of normal and pathological lymphoid elements quickly and accurately in the analysis of blood smears and aspirates of bone marrow. The data obtained can be applied for the diagnosis of lymphoma and the differential diagnosis with other lymphoproliferative diseases [1-4].

Follicular lymphoma is a malignant indolent b-cell tumor in which metastasis to the bone marrow is determined in 40-70% of patients [5]. Lymphoid pool at follicular lymphoma is heterogeneous. It is presented by two types of cells – centrocyte (morphologically similar to lymphocytes) and centroblasts (larger anaplasticum lymphoid elements). The use of modern optical microscopy with digital image processing enables to estimate the degree of damage bone marrow and characteristics of the pool of lymphoid elements on the basis of specific indexes that is relevant at the present stage [6-13].

The purpose of this work is the study of computer microscopy capabilities with multispectral camera using wavelet analysis to characterize the lymphoid bone marrow pool of follicular lymphoma patients.

2. Materials and methods
A comparative study of the structure of the chromatin of the nuclei of blood lymphocytes and leukemic lymphoid elements was carried out on smears of the peripheral blood of 14 donors and bone marrow smears of 13 patients with follicular lymphoma.

All preparations were fixed and stained by the method of May-Grunwald-Romanovsky. Morphological and immunophenotypic studies of blood and bone marrow was performed by two independent experts in the laboratory of immunology of hematopoiesis of N. N. Blokhin Russian Cancer Scientific Center of the Ministry of Healthcare of the Russian Federation. The diagnosis of
follicular lymphoma was established on the basis of immunohistochemical studies of the biopsy of the lymph nodes of patients.

Cell images were formed using the RGB color model on an automated microscope Olympus BX43 with a multispectral camera Imperx IPX-4M1ST-GCFB. Images were saved in BMP format with a color-coded RGB24 (over 16 million colors). 2764 images were obtained. There were 941 - lymphocytes of blood of 14 donors, 1823 – leukemic lymphoid cells of the bone marrow of 13 patients.

Spatial and chromatic regularity in the chromatin structure of the nuclei of the lymphocytes and lymphoid cells were studied on these images. Features were calculated by using the software developed at the Department "Computer medical systems" of National research nuclear University MEPhI (Moscow Engineering Physics Institute).

The study of the structure of the chromatin of the nuclei was performed with the data conversion to components of the color models XYZ, HSL, Lab, Luv, LCH, HLS, HSV, YUV, YIQ, YCbCr, CMY. Wavelet features were calculated for these components [7-8]. Characteristics of the chromatin of cell nuclei were obtained for the normal cells and for follicular lymphoma cells. Linear classifier with Euclidean, Manhattan and Chebyshev distance functions in two-dimensional feature space were used to separate the data [1-3,6-13].

Chromatin structure of cell nuclei was characterized by 2400 features. They were calculated for the different components of color models presented above [8-9,11-12]. A couple of signs were identified among them so, that all the events were characterized with minimal error. The first sign is PDisp, it is the value of wavelet signs "variance". It was calculated for the wavelet decomposition of the Haar with a color difference component of the Q from color space YIQ. The second sign is PMax, it is the value of wavelet signs "maximum wavelet coefficient". It was calculated for the wavelet decomposition Haar with color-difference component V of color space YUV.

| Donors (D) | Quantity of cells | PMax | Disp |
|-----------|-------------------|------|------|
|           |                   | $\bar{x}$ | S | $S_{x}$ | $\bar{x}$ | S | $S_{x}$ |
| D1        | 78                | 27.3 | 3.3 | 0.3  | 12.8 | 2.1 | 0.2  |
| D2        | 140               | 25.9 | 3.1 | 0.3  | 11.6 | 1.8 | 0.2  |
| D3        | 70                | 29.8 | 2.9 | 0.3  | 11.7 | 2.2 | 0.3  |
| D4        | 111               | 32.6 | 2.8 | 0.3  | 13.8 | 2.5 | 0.2  |
| D5        | 73                | 32.8 | 6.1 | 0.7  | 13.9 | 2.7 | 0.3  |
| D6        | 98                | 26.4 | 2.5 | 0.4  | 12.6 | 2.1 | 0.2  |
| D7        | 26                | 32.3 | 4.1 | 0.8  | 16.6 | 3.5 | 0.7  |
| D8        | 31                | 26.6 | 4.0 | 0.7  | 13.1 | 2.5 | 0.4  |
| D9        | 116               | 27.6 | 3.1 | 0.3  | 13.8 | 1.9 | 0.2  |
| D10       | 39                | 27.6 | 2.7 | 0.4  | 13.1 | 2.2 | 0.3  |
| D11       | 71                | 31.2 | 2.5 | 0.3  | 14.6 | 2.2 | 0.3  |
| D12       | 22                | 31.5 | 6.2 | 1.3  | 16.1 | 4.7 | 1.1  |
| D13       | 22                | 32.4 | 3.4 | 0.5  | 14.8 | 2.1 | 0.3  |
| D14       | 44                | 36.6 | 3.9 | 0.6  | 16.8 | 3.1 | 0.5  |
| the average value | | 30.1 | 3.2 | 0.9 | 14.1 | 1.7 | 0.4 |

3. Results
Features of the spatial brightness structure of nuclei chromatin of blood lymphocytes of 14 donors $P_{Max}$ and $P_{Disp}$ are shown in table 1. The quantity of cells of patient was ranged from 22 to 140. Statistical characteristics $(\bar{x}, S, S_x)$ were calculated for the features $P_{Max}$ and $P_{Disp}$. Here, $\bar{x}$ is the
arithmetic average of the features value, \( S \) is standard deviation, \( S_x \) is standard deviation of the arithmetic mean.

As can be seen from table 1, the mean values of the structure characteristics of the nuclei of lymphocytes changes from 25.9 to 36.6 for \( P_{\text{Max}} \) and from 11.6 to 16.8 for \( P_{\text{Disp}} \). Average features for \( P_{\text{Max}} = 30.1 \pm 0.9 \), \( P_{\text{Disp}} = 14.1 \pm 0.4 \).

Features of the structure of the chromatin of leukemic lymphoid cells in 13 patients are presented in table 2. Scatter of values was from 27.3 to 38.4 for \( P_{\text{Max}} \) and from 15.9 to 22.8 for \( P_{\text{Disp}} \) for features of lymphoid cells. Average features for \( P_{\text{Max}} = 28 \pm 1 \) and for \( P_{\text{Disp}} = 19.3 \pm 0.8 \).

**Table 2.** The mean values of the characteristics \( P_{\text{Max}} \) and \( P_{\text{Disp}} \) for cells of 13 patients with follicular lymphoma.

| Patients with the follicular lymphoma (FL) | Quantity of cells | \( \bar{P}_{\text{Max}} \) | \( S_x \) | \( S_{\bar{P}_{\text{Max}}} \) | \( \bar{P}_{\text{Disp}} \) | \( S_x \) | \( S_{\bar{P}_{\text{Disp}}} \) |
|------------------------------------------|-------------------|-----------------|------|-----------------|-----------------|------|-----------------|
| FL 1                                     | 98                | 31.6            | 3.1  | 0.3             | 17.1            | 3.7  | 0.4             |
| FL 2                                     | 146               | 30.2            | 3.2  | 0.2             | 16.8            | 2.8  | 0.3             |
| FL 3                                     | 124               | 27.9            | 2.3  | 0.2             | 22.2            | 5.1  | 0.4             |
| FL 4                                     | 176               | 30.9            | 2.9  | 0.2             | 22.7            | 4.5  | 0.3             |
| FL 5                                     | 61                | 25.8            | 3.2  | 0.4             | 22.8            | 6.1  | 0.8             |
| FL 6                                     | 46                | 27.3            | 2.8  | 0.4             | 16.5            | 3.1  | 0.4             |
| FL 7                                     | 145               | 27.4            | 1.7  | 0.1             | 20.9            | 3.7  | 0.3             |
| FL 8                                     | 39                | 38.4            | 2.0  | 0.3             | 20.6            | 5.6  | 0.9             |
| FL 9                                     | 175               | 29.1            | 5.3  | 0.4             | 18.6            | 3.8  | 0.3             |
| FL 10                                    | 376               | 26.4            | 3.9  | 0.2             | 21.5            | 5.9  | 0.3             |
| FL 11                                    | 215               | 23.6            | 4.2  | 0.3             | 20.9            | 5.7  | 0.4             |
| FL 12                                    | 95                | 24.5            | 3.1  | 0.3             | 15.1            | 2.5  | 0.3             |
| FL 13                                    | 127               | 24.1            | 2.9  | 0.3             | 15.2            | 2.9  | 0.2             |
| the average value                         |                   | 28.2            | 4.1  | 1.1             | 19.3            | 2.9  | 0.8             |

As can be seen from the data presented in table 3, the characteristics of the average values of \( P_{\text{Disp}} \) of the donors and patients with follicular lymphoma differs from normal values of lymphocytes for the significance level \( p=0.05 \).

Thus, the study of the structure of the chromatin of the nuclei of the lymphocytes and leukemic lymphoid cells with the use of computer microscopy allows to establish the difference between normal and follicular lymphoma.

**Table 3.** The mean values of the characteristics \( P_{\text{Max}} \) and \( P_{\text{Disp}} \) for patients.

| Patient (sample) | Quantity of patients | \( \bar{P}_{\text{Max}} \) | \( S_x \) | \( S_{\bar{P}_{\text{Max}}} \) | \( \bar{P}_{\text{Disp}} \) | \( S_x \) | \( S_{\bar{P}_{\text{Disp}}} \) | \( p \) |
|-----------------|----------------------|-----------------|------|-----------------|-----------------|------|-----------------|---|
| Donors          | 14                   | 30.1            | 3.2  | 0.9             | 14.1            | 1.7  | 0.4             | 0.05 |
| Patients with the follicular lymphoma | 13                   | 28.2            | 4.1  | 1.1             | 19.3            | 2.9  | 0.8             | 0.05 |

\( p \) – the level of significance, it is calculated for student's t – test [14].

The distribution of average values of the features of donors and patients with the follicular lymphoma are represented in Figure 1.
Figure 1. The distribution of the two classes lymphoid cells and lymphocytes in a two-dimensional feature space. The X-axis shows the feature $P_{\text{Max}}$, Y-axis shows the feature $P_{\text{Disp}}$.

The results indicate the applicability of separation of lymphoid cells and lymphocytes through wavelet analysis of images obtained by optical microscopy with multispectral camera.

4. Conclusion
The study showed the applicability of wavelet analysis in the computer microscopy systems with the aim of obtaining of quantitative characteristics of lymphoid cells for follicular lymphoma diagnosis.

Planned step for further research is to determine the differences of lymphoid cells from other bone marrow cells and increase of volume of the experimental sample.

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