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The Acousto-Optical Method for Blood Typing (Part 1). Photometric and Statistical Methods for Image Processing

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This work is devoted to the analysis of two possible ways of computer processing of photographic images used in acousto-optical human blood typing. Such analysis is necessary to solve the problem of increasing the time resolution and, as a result, the reliability of blood typing by the acousto-optical method. The results obtained using the suggested statistical approach to image processing are compared with the results obtained using the conventional photometric approach. It is shown that the statistical processing of photographic images provides a 1.5- to 4-fold gain in the resolution of the acousto-optical method in the case of forward typing. For reverse typing, a 2- to 5-fold gain is achieved. This work can be considered as a step towards further improvement of the acousto-optical instrumental method for blood typing.

Introduction

ABO or Rh (rhesus system) blood typing is one of the most frequently performed laboratory tests [1]. The high demand for such tests requires special automatic devices for their implementation (automatic systems for instrumental blood typing) [2-10].

Resolving power (resolution) is a main parameter of such systems. This parameter is differently determined by different authors [6, 7, 11, 12]. For instance, the resolving power is suggested in [11, 12] to be determined as the ratio of the optical signal P_+ of positive agglutination reaction to the signal P_- of negative agglutination reaction (agglutination is present or absent depending on the serum compatibility with blood of the given type). It is obvious that an increase in the resolving power enhances the reliability of blood group determination. It should be stressed that the possibility of errors in blood typing should be completely excluded. However, in cases of insufficient resolving power and variability of blood samples, automatic devices sometimes fail to determine the blood group.

An increase in the resolving power of the device can be attained using the method of standing ultrasonic waves. This method was suggested in [13] and described in more detail in [1, 11-15]. The combination of ultrasonic treatment of the biological object with its optical probing is called the acousto-optical method of blood typing.

The photosignal detection by an analog method based on the use of a photodiode was suggested in [5, 13-15]. Digital detection using a color web camera with further computer estimation of mean pixel brightness in each image within a given area of processing in accordance with the probing beam position was suggested in [11, 12, 16, 17]. It is natural that the maximal resolution of the method is limited by the range of brightness B of image pixels.

It was suggested that the efficiency of the web camera–based acousto-optical method could be increased by statistical processing of the obtained photographic images. This approach is based on the analysis of individual pixel brightness in the processed area of the images obtained in cases of positive and negative agglutination reactions. This does not imply, however, the image brightness averaging over the area.

The method of the statistical pixel-by-pixel processing of photographic images can prove useful not only for increasing the resolving power of the acousto-optical method of blood typing, but also for solving similar medical and technical problems in development of laboratory diagnostic devices, which makes this work fairly promising.

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Materials and Methods

Object of research. The developed acousto-optical method of blood typing was tested within the framework of crossmatching tests involving forward and reverse blood typing. Donor blood of the four ABO groups and corresponding hemagglutinating sera and standard erythrocytes were tested. Samples of the donor blood were centrifuged for 5 min at 3000 rpm. Then, the serum was used for blood typing by the reverse method, while the erythrocyte mass was tested using the forward method of blood typing. The total number of tested samples was 16 in forward typing and 12 in reverse typing. Preliminary experimental tests [1, 16] allowed the optimal conditions of the erythrocyte agglutination reactions to be determined for the forward and reverse methods. The optimal conditions for the forward typing are a 17 : 1 volume ratio of standard serum to tested erythrocytes provided that the erythrocyte mass is diluted by saline (1 : 78). In this case, the volume fraction of the erythrocyte mass in the solution is 1.05%, whereas the volume ratio of the standard serum is 17.8%. For the reverse typing the optimal conditions are a 4.4 : 1 volume ratio of tested plasma to standard erythrocytes provided that the tested plasma is diluted by saline (1 : 4.3). In this case, the volume fraction of the tested plasma in the solution is 17.8%, whereas the volume fraction of the standard erythrocytes is 4.05%. For the forward and reverse typing of human blood using the turbidimetric method a rectangular 2800-μl cuvette with an internal 5-mm gap was used.

Experimental method. After preparation, the sample was exposed to a standing ultrasonic wave. The cuvette with the sample was attached to a piezoelectric converter. The ultrasonic wave was oriented vertically. The piezoceramic converter was powered from a G3-112/1 generator

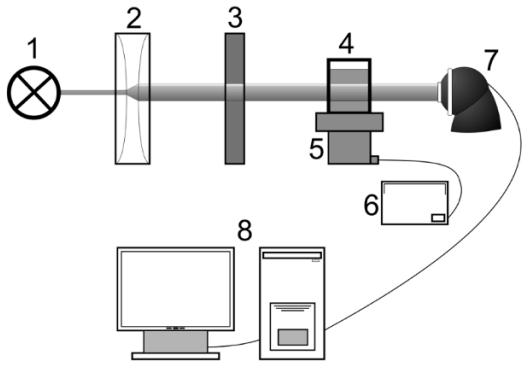

Fig. 1. Device for erythrocyte agglutination recording: 1) LED; 2) condenser; 3) neutral density filter; 4) cuvette; 5) ultrasonic converter; 6) ultrasound generator; 7) 8-bit digital camera; 8) PC.

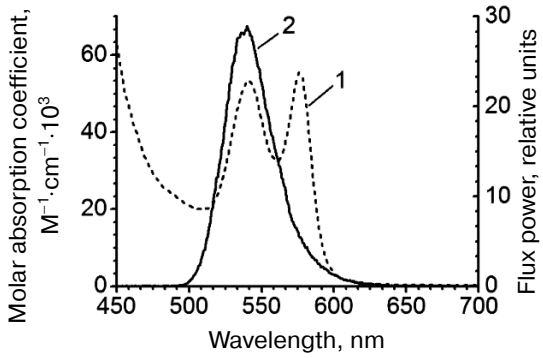

Fig. 2. Spectra: 1) hemoglobin absorption (left Y axis); 2) LED emission (right Y axis).

with an amplifier. Its output voltage was controlled by an S1-79 oscilloscope. The generator was set to resonance with the converter (ν = 2.25 MHz). The output voltage of the generator applied to the piezoceramic element was 15 V. It affected erythrocytes without causing hemolysis. Preliminary tests allowed the optimal ultrasound exposure time to be selected: 90 s both for the forward and for the reverse blood typing [1, 16].

Upon passing through the sample the testing beam was applied to a Logitech QuickCam color web camera. The process of sedimentation of erythrocytes and their aggregates (negative agglutination reaction) or agglutinates (positive agglutination reaction) was photographed using the web camera for 90 s upon switching off of the ultrasound (sample incubation time).

The biological object was tested using the collimated beam of an LXHL-G1S LED (power maximum at λ = 540 nm, see Fig. 1). The LED emission spectrum corresponded to the hemoglobin absorption spectrum in the green range (Fig. 2).

The specially selected light source spectrum (Fig. 2) provided a low level of image brightness in the case of a negative agglutination reaction because hemoglobin of suspended cells absorbed the light. Meanwhile, in the case of a positive agglutination reaction the brightness value was higher because of an increase in the transparency of the medium caused by strong sedimentation of large erythrocyte agglutinates.

Computer processing of photoimages. The two approaches to photographic image processing involve image decomposition into RGB components, the analysis being applied only to the G component [1, 11, 12, 16].

It follows from Fig. 3 that in the case of a negative reaction strong absorption of the probing beam by the erythrocyte hemoglobin is observed. In the case of a positive agglutination reaction, the medium is more trans-

parent because of rapid sedimentation of large agglutinates. Meanwhile, it follows from Fig. 3b that small erythrocyte agglutinates are suspended.

Each digital image (e.g., Fig. 3) has a corresponding characteristic matrix of brightness B in the G channel. The volume of the sample is determined by the number of pixels in the zone of interest with given width w.

Photometric approach. The photometric approach implies correlation of each image with the brightness values in the G channel for positive B_{mean+} and negative B_{mean-} erythrocyte agglutination reactions (mean value over the zone w of processing). The average brightness values B_{mean+} and B_{mean-} were calculated as the power of the light flux incident on the camera using the calibration curve, i.e., the curve of correspondence between the mean incident light flux power P_{mean} and the mean pixel brightness B_{mean} (calibration of the digital photodetectors was described in [1, 16, 17]). In this case, the resolution of the photometric method is calculated as $R_P = P_{mean+}/P_{mean-}$, where P_{mean-} and P_{mean+} are the mean incident light flux powers for negative and positive reactions, respectively. It should be noted that the subscript values (e.g., B_{mean}) correspond to averaging over the zone w of image processing, whereas a horizontal line (macron) above the parameter (e.g., $\overline{B}$) indicates averaging over the experimental results.

Statistical approach. The image processing method is based on analysis of the dependence of the distribution of pixels in the zone w on their brightness $N(B)$. In this case, the distribution variance is the parameter used to differentiate between positive and negative agglutination reactions. The distribution variance also characterizes the intensity of the agglutination reaction. A typical distribution over a zone with w = 100 pixels is shown in Fig. 4 for negative (curve 1) and positive (curve 2) agglutination reactions.

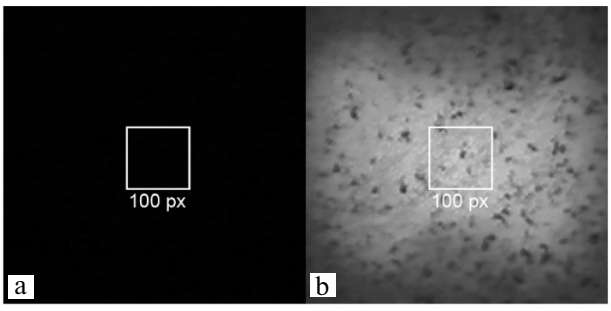

Fig. 3. Typical camera exposure images in the green (G) channel of the RGB decomposition in the case of forward typing: a) negative agglutination reaction; b) positive agglutination reaction. The squares represent the area for statistical processing of the experimental results; the zone width w is 100 pixels.

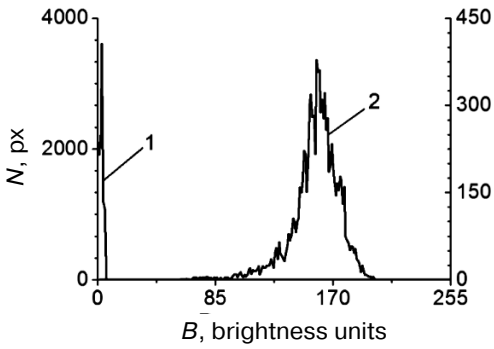

Fig. 4. Typical pixel distribution $N(B)$ over the brightness B in an area w = 100 pixels for negative agglutination reaction $N_-(B)$ (curve 1, left Y axis) and for positive agglutination reaction $N_+(B)$ (curve 2, right Y axis).

The curves shown in Fig. 4 correspond to the photographs of cuvette exposure in Fig. 3. The resolution of the method was calculated as $R_D = D_+/D_-$, where D_+ and D_- are the variances of pixel distribution over the brightness for positive and negative reactions, respectively.

This method under otherwise identical conditions provides greater resolution as compared to the photometric approach. Indeed, the mean brightness B_{mean} averaged over the zone w is limited, $0 \leqslant B_{mean} \leqslant 255$ (for the 8-bit CCD camera), whereas the variance of the distribution $N(B)$ ranges over wider limits.

Results and Discussion

The mean values averaged over the samples and the resolution spread for the two approaches to photoimage processing used in the acousto-optical blood typing are given in Table 1. The values of the minimal (R_{min}), mean ($\overline{R}$), and maximal (R_{max}) resolution for the two approaches were estimated for the zone width w = 100 as follows: $\overline{R}_P = \overline{P}_+/\overline{P}_-$ ($\overline{R}_D = \overline{D}_+/\overline{D}_-$), where $\overline{P}_+$ and $\overline{P}_-$ ($\overline{D}_+$ and $\overline{D}_-$) are corresponding mean values P_+ and P_- (D_+ and D_-) for all blood samples; $R_{P,\,min} = P_{+min}/P_{-max}$ ($R_{D,\,min} = D_{+min}/D_{-max}$), where D_{+min} and D_{-max} are the minimal value of $P_+(D_+)$ and the maximal value of $P_-(D_-)$ over all results of the tests, respectively; and $R_{P,\,max} = P_{+max}/P_{-min}$ ($R_{D,\,max} = D_{+max}/D_{-min}$), where D_{+max} and D_{-min} are the maximal value of $P_+(D_+)$ and the minimal value of $P_-(D_-)$ over all results of the tests, respectively.

The results given in Table 1 show that the statistical method of photoimage processing leads to a 2- to 4-fold improvement in the image resolution as compared to conventional photometry. Meanwhile, the two approach-

TABLE 1. Mean Values Averaged over the Samples and Resolution Spread of the Blood Typing Method

Photoimage analysis method	Crossmatching component	R_{min}	$\bar{R}$	R_{max}
Photometric	Forward	52	150	319
	Reverse	25	119	262
Statistical	Forward	118	328	857
	Reverse	93	399	938

es demonstrate a significant spread of the resolution values.

It is natural that the zone size w influences the resolution of the blood typing method. For example, the maximal resolution of the photometric method $\bar{R}_P$ is observed at w ranging from 0 to 100, whereas for the statistical method $\bar{R}_D$ is maximal at 450-650 pixels.

Values of resolution R of the acousto-optical method of forward and reverse blood typing are compared in Tables 2 and 3 for the photometric ($R_P = P_+/\bar{P}_-$) and statistical ($R_D = D_+/\bar{D}_-$) approaches. Values of $\bar{P}_-$ and $\bar{D}_-$ were estimated by averaging of P and D in the samples with a negative agglutination reaction. The zone size was assumed to be $w = 100$. The semibold numbers indicate a negative agglutination reaction.

It follows from Tables 2 and 3 that the statistical approach to photoimage processing provides an increase in the resolution of the acousto-optical method of blood typing as compared to conventional photometry: a 1.5- to 4-fold increase in the case of forward blood typing and a 2- to 5-fold increase for reverse blood typing, depending on the interacting blood–agglutinating serum (forward method) or plasma–standard erythrocytes (reverse method) pairs.

Conclusion

A statistical approach to photoimage processing within the framework of the acousto-optical method of blood typing was suggested and tested. This approach provides a substantial increase in the resolution as compared to conventional photometry and, therefore, improves the reliability of the instrumental method of determination of human blood group.

TABLE 2. Forward Crossmatching

Serum \ Blood group	0 (I)		A (II)		B (III)		AB (IV)	
	R_P	R_D	R_P	R_D	R_P	R_D	R_P	R_D
$0_{\alpha,\beta}$ (I)	**1.1**	**1.2**	176.2	201.7	77.7	173.6	163.9	291.6
A_β (II)	**1.1**	**1.3**	**1.3**	**1.0**	204.9	344.1	113.7	430.5
B_α (III)	**1.5**	**1.5**	123.8	563.7	**0.7**	**0.9**	190.2	289.9
AB_0 (IV)	**1.1**	**1.1**	**0.7**	**0.7**	**1.0**	**0.7**	**0.6**	**0.7**

TABLE 3. Reverse Crossmatching

Standard erythrocytes \ Blood group	0 (I)		A (II)		B (III)		AB (IV)	
	R_P	R_D	R_P	R_D	R_P	R_D	R_P	R_D
0 (I)	**1.0**	**1.0**	**0.7**	**0.8**	**0.7**	**0.8**	**1.1**	**1.1**
A (II)	185.3	719.7	**1.7**	**1.1**	43.5	170.0	**0.9**	**0.9**
B (III)	64.6	132.2	181.5	572.8	**0.7**	**0.8**	**1.1**	**1.4**

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