DOI 10.1007/s10527-016-9592-4
Biomedical Engineering, Vol. 50, No. 2, July, 2016, pp. 78-83. Translated from Meditsinskaya Tekhnika, Vol. 50, No. 2, Mar.-Apr., 2016, pp. 3-7.
Original article submitted February 1, 2016.

The Acousto-Optical Method for Blood Typing Based on Discrete Processing of Photographic Images

V. A. Doubrovski* and M. F. Medvedeva

This work is devoted to the development of the acousto-optical method for blood typing based on analysis of digital photoimages of sedimentation of red blood cells and their aggregates and immune complexes (agglutinates). Two modifications of the discrete method for photographic image processing were suggested and experimentally tested to increase the reliability of instrumental human blood typing. AB0 matching of blood samples was performed. It was shown that the proposed methods for discrete processing of photoimages provided a several hundredfold increase in the resolution of the acousto-optical method for blood typing as compared to the conventional photometric approach. The reliability of blood typing was also considerably improved.

Introduction

Development of methods and devices for instrumental blood typing of donor and recipient blood is an important problem because of the high demand for this hematological test. In contrast to other measuring systems, the notion of relative measurement error can be hardly applied to devices for instrumental blood typing, because the possibility of errors in blood typing should be completely excluded. The reliability of blood typing can be estimated from the resolving power (resolution) R of the method or the device used for blood typing. The resolving power is differently determined by different authors [1-4]. In all cases, it indicates the difference between the values of the selected physical quantity measured in the case of positive agglutination reaction and in the case of negative agglutination reaction. It is obvious that an increase in the resolving power enhances the reliability of blood group determination.

Due to high specificity of blood samples from different donors or recipients the resolution may vary in a broad range even if the samples belong to the same blood type. The specificity is due to many factors, e.g., the agglutination activity of erythrocytes, erythrocyte concentration in the sample, hemoglobin concentration, blood viscosity, etc. The designers of apparatuses for blood typing set certain threshold values $R_{thres}(max)$ and $R_{thres}(min)$ based on the results of technical and biomedical tests. If the measured value of resolution for a given blood sample exceeds $R_{thres}(max)$, the agglutination test is accepted as valid (positive); if $R < R_{thres}(min)$, the test is accepted as invalid (negative). Thus, tests of a given blood sample based on four different sera allow the blood type to be determined. If the value of the resolution R measured in at least one of the tests is within the uncertainty limits $R_{thres}(min) \leqslant R \leqslant R_{thres}(max)$, no definite conclusion about the agglutination reaction is made. In this case, the device indicates that the test failed to determine the blood type. This approach is widely used [5, 6], because any error in blood typing can lead to a fatal outcome of blood transfusion. It is fairly natural that an increase in the resolution of a blood typing device reduces the number of test results falling within the uncertainty limits $R_{thres}(min)$-$R_{thres}(max)$, thus increasing the reliability of blood typing and reducing the number of blood samples with unidentified blood type. Thus, improvement of the resolution of instrumental blood typing is an important task of modern medicine.

The resolution of a device for blood typing can be increased using the acousto-optical method suggested in [7] and considered in more detail in [8, 9]. It was also suggested in [3, 4] to supplement this method with techniques for digital photodetection of erythrocytes and their agglutinates. The photometric and statistical methods of photoimage processing during blood typing using the acousto-optical method were described in [10]. Two new

Saratov State Medical University, Saratov, Russia; E-mail: doubrovski43@yandex.ru
* To whom correspondence should be addressed.

modifications of the photoimage processing technique are suggested in this work. The goal of this work is to test experimentally the suggested technique for discrete processing of photoimages, to elucidate the specific features of this approach, and to estimate the maximum resolution of the acousto-optical method of blood typing based on discrete processing of photoimages.

Materials and Methods

Experimental Technique. 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 AB0 groups and corresponding hemagglutinating sera and standard erythrocytes were tested.

Samples of the donor blood were centrifuged. 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 allowed the optimal conditions of the erythrocyte agglutination reactions to be determined for the forward and reverse methods. After preparation, the sample was exposed to a standing ultrasonic wave. Preliminary experimental tests 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 included the optimal volume ratio of standard serum to tested erythrocytes. For the reverse typing the optimal conditions included the optimal volume ratio of tested plasma to standard erythrocytes. The ratio of dilution of plasma and erythrocytes with saline was also optimized, as well as the duration of ultrasonic probing, the sample incubation time, etc. [11]. Sample preparation and experimental methods were described in more detail in [10].

The biological object was tested using a collimated beam with a power maximum at $\lambda = 540$ nm. The LED emission spectrum corresponded to the hemoglobin absorption spectrum in the green range. Photographs of the tested sample were taken using a Logitech QuickCam color web camera. The obtained images were transmitted to a PC for processing.

Computer Processing of Photoimages. The methods of discrete processing of photoimages suggested in this work were applied only to the G component of images decomposed into the RGB components. A square zone was selected in each photoimage. The width w of each zone corresponded to the transverse size of the probing light beam and could be varied according to the goal of the test (Fig. 1).

It follows from Fig. 1 that in the case of a negative reaction strong absorption of the probing beam by the erythrocyte hemoglobin is observed (Fig. 1a). In the case of a positive agglutination reaction, the medium is more transparent because of rapid sedimentation of large agglutinates (Fig. 1b). Each digital image has a corresponding characteristic matrix of brightness B.

Estimation of the number of pixels with given brightness. The suggested discrete processing method is based on analysis of the brightness of each pixel. As in [10], the distribution $N(B)$ of pixels in the zone w according to their brightness was determined (Fig. 2).

Then, the number of pixels $N(B \geqslant B_{thres})$ with brightness values above the threshold B_{thres} was determined for the selected zone w using the following equation:

$$N(B \geq B_{\text{thres}}) = \sum_{k=B_{\text{thres}}}^{255} N_k,$$

where k is the current pixel brightness, B_{thres} is the selected brightness threshold, and N_k is the number of pixels with the brightness k. It follows from Fig. 1 that the number of bright pixels is small in the case of a negative agglutination reaction (Fig. 1a), whereas in the case of a positive reaction the number of bright pixels is very high (Fig. 1b). Thus, the resolution of the method can be estimated from the ratio of the numbers of bright pixels for the positive $N_+(B \geqslant B_{thres})$ and negative $N_-(B \geqslant B_{thres})$ agglutination reactions: $R_N = N_+(B \geqslant B_{thres})/N_-(B \geqslant B_{thres})$.

This method provides greater resolution as compared to the photometric approach. Indeed, the mean brightness $\overline{B}$ averaged over the zone w is limited, $0 \leqslant \overline{B} \leqslant 255$ (for

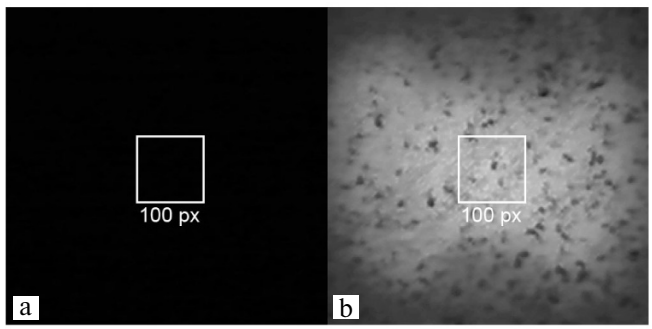

Fig. 1. Typical camera exposure images in the green (G) channel of the RGB decomposition in the case of forward typing: a) negative agglutination reaction; b) positive agglutination reaction. The squares represent the area for statistical processing of the experimental results; the zone width w is 100 pixels.

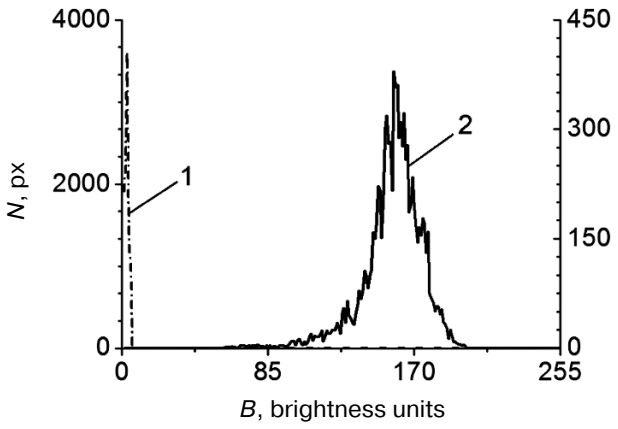

Fig. 2. Typical pixel distribution $N(B)$ over the brightness B in an area w = 100 pixels for negative agglutination reaction $N_{-}(B)$ (curve 1, left Y axis) and for positive agglutination reaction $N_{+}(B)$ (curve 2, right Y axis).

the 8-bit web camera), whereas the number $N(B \geqslant B_{thres})$ of pixels with brightness values exceeding a given threshold (discrete approach) ranges over wider limits: $0 \leqslant N(B \geqslant B_{thres}) \leqslant N_0$, where N_0 is the total number of pixels in the zone w.

It follows from Fig. 3a that $N_{+} = N_{-} = N_0$ at $B_{thres} = 0$. In this case, $R_N = N_{+}/N_{-} = 1$. Therefore, $B_{thres} = 0$ is practically unfeasible within the framework of the approach suggested in this work. In Fig. 3a, the intersection point between the curve 1 and the X axis determines the critical brightness value B_{cr}. It is obvious that in the case of the negative reaction the pixels with the brightness above B_{cr} are completely absent in the image. Therefore, B_{thres} should not exceed B_{cr}, because in this case $N_{-}(B \geqslant B_{thres}) = 0$, so that the value of resolution $R_N(B_{thres}) = N_{+}(B \geqslant B_{thres})/N_{-}(B \geqslant B_{thres})$ becomes senseless (in Fig. 3a, $B_{cr} = 6$). Maximal resolution R_N of the method is reached at $B_{thres} = B_{cr} - 1$, because in the case of negative agglutination reaction $N_{-}(B \geqslant B_{thres})$ monotonically decreases with increasing B_{thres}, while $N_{+}(B \geqslant B_{thres})$ remains constant for a rather long period.

Estimation of total brightness of photoimage pixels. An additional increase in the resolution of the method can be achieved by taking into account both the number of the pixels with brightness above a certain level B_{thres} and the total brightness of these pixels. Indeed, in the case of negative agglutination reaction (Fig. 1a), both the number of pixels with brightness $B \geqslant B_{thres}$ and the brightness of individual pixels are low. As a result, the total brightness S_{-} is also low. On the other hand, in the case of positive agglutination reaction (Fig. 1b), the number of pixels with brightness $B \geqslant B_{thres}$ is high and their brightness is also high. Therefore, in this case, the total brightness of these pixels S_{+} is higher than S_{-}, provided that $B \geqslant B_{thres}$. Thus, the resolution of the acousto-optical method with discrete photoimage processing $R_S = S_{+}/S_{-}$ may be rather high.

For positive agglutination reaction:

$$S_{+} = \sum_{m=1}^{m_0} B_m = \sum_{i=B_{\text{thres}}}^{255} i \cdot N_i \,,$$

where S_{+} is the total brightness of pixels with $B \geqslant B_{thres}$ (positive agglutination reaction), m is the number of pixels, B_m is the brightness of pixel m, m_0 is the total number of pixels with $B \geqslant B_{thres}$, i is the current pixel brightness, and N_i is the number of photoimage pixels with brightness i.

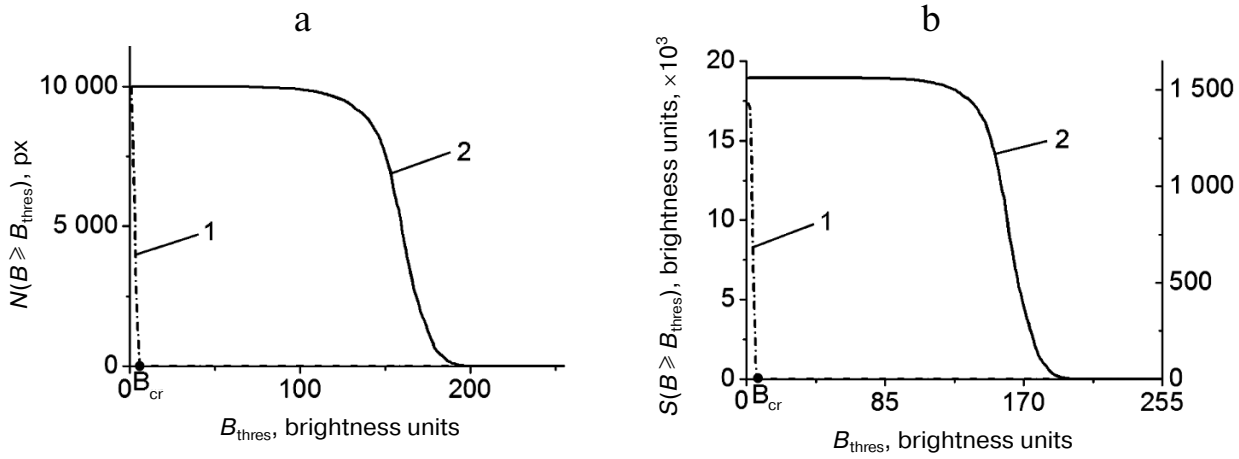

Fig. 3. a) Number of pixels $N(B \geqslant B_{thres})$ with brightness exceeding the threshold value B_{thres} as a function of B_{thres}; b) total brightness of the pixels $S(B \geqslant B_{thres})$ with brightness exceeding the threshold value B_{thres} as a function of B_{thres}. Curves: 1) negative agglutination reaction; 2) positive agglutination reaction; (in b) – right Y axis). The zone width w = 100 pixels.

For negative agglutination reaction:

$$S_- = \sum_{n=1}^{n_o} B_n = \sum_{j=B_{\text{thres}}}^{255} j \cdot N_j ,$$

where S_- is the total brightness of pixels with $B \geqslant B_{thres}$, n is the number of pixels, B_n is the brightness of pixel n, n_0 is the total number of pixels with $B \geqslant B_{thres}$, j is the current pixel brightness, and N_j is the number of photoimage pixels with brightness j.

Figure 3b shows the dependence of the total photoimage pixel brightness $S(B \geqslant B_{thres})$ on the value of B_{thres} for negative (curve 1) and positive (curve 2) agglutination reactions. These curves are similar to the corresponding curves $N(B \geqslant B_{thres})$ for negative and positive agglutination reactions shown in Fig. 3a. However, there is a substantial difference: S_+ and S_- differ from one another even at zero threshold brightness ($B_{thres} = 0$). This allows the resolution of the method $R_S = S_+(B \geqslant B_{thres})/S_-(B \geqslant B_{thres})$ to remain high even at $B_{thres} = 0$, when $N_+ = N_- = N_0$ and $R_N = N_+/N_- = 1$. Similarly to the technique based on estimation of the number of pixels with given brightness, the maximal R_S was observed at $B_{thres} = B_{cr} - 1$. It should be noted that the critical brightness B_{cr} was determined in the same manner as in the technique based on estimation of the number of pixels with given brightness, as the intersection point between the curve $S_-(B \geqslant B_{thres})$ and the X axis (Fig. 3b, $B_{cr} = 6$).

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 ($\bar{R}$), and maximal ($R_{\max}$) resolution for the two approaches were estimated for the zone width $w = 100$ as follows: $\bar{R}_N = \bar{N}_+/\bar{N}_-$ ($\bar{R}_S = \bar{S}_+/\bar{S}_-$), where $\bar{N}_+$ and $\bar{N}_-$ ($\bar{S}_+$ and $\bar{S}_-$) are corresponding mean values N_+ and N_- (S_+ and S_-) for all blood samples; $R_{N,\,\min} = N_{+\min}/N_{-\max}$ ($R_{S,\,\min} = S_{+\min}/S_{-\max}$), where $N_{+\min}$ and $N_{-\max}$ ($S_{+\min}$ and $S_{-\max}$) are the minimal value of $N_+(S_+)$ and the maximal value of $N_-(S_-)$ over all results of the tests, respectively; and $R_{N,\,\max} = N_{+\max}/N_{-\min}$ ($R_{S,\,\max} = S_{+\max}/S_{-\min}$), where $N_{+\max}$ and $N_{-\min}$ ($S_{+\max}$ and $S_{-\min}$) are the maximal value of N_+ (S_+) and the minimal value of N_- (S_-) over all results of the tests, respectively. The data obtained using the conventional photometric approach [10] are also given in Table 1 for comparison.

It follows from Table 1 that either of the two modifications of the discrete method of photoimage processing suggested in this work can be used as an alternative to the photometric method. The approach based on the estimation of the number of pixels with a brightness above the threshold value increases the resolution by one to two orders of magnitude at $B_{thres} = 5$. However, at $B_{thres} = 4$, there is already a sharp decrease in $\bar{R}_N$. The approach based on the estimation of the total pixel brightness is the most promising. As shown above, the resolution R_S increases with increasing B_{thres}, while a significant difference between positive and negative reactions is achieved even at zero threshold brightness ($B_{thres} = 0$). However, an increase in B_{thres} leads to an increase in the spread of R_S, thus reducing the reliability of measurements and the repeatability of the results.

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

Photoimage analysis method	Cross-matching component	$R_{\min}$	$\bar{R}$	$R_{\max}$
Photometric	Forward	52	150	319
	Reverse	25	119	262
Estimation of the number of pixels, $B_{\text{thres}} = 5$	Forward	1250	2368	5000
	Reverse	588	1739	10,000
Estimation of the number of pixels, $B_{\text{thres}} = 4$	Forward	4	12	60
	Reverse	3	10	71
Estimation of total brightness, $B_{\text{thres}} = 5$	Forward	21,778	65,452	170,340
	Reverse	6111	41,469	339,855
Estimation of total brightness, $B_{\text{thres}} = 4$	Forward	90	404	2553
	Reverse	44	282	3029

It is obvious that the zone size w influences the resolution of the blood typing method. Calculations demonstrated that for both modifications of the discrete processing method suggested in this work an increase in w did not initially affect the resolutions R_N and R_S. Only when w became comparable to the size of the probing light spot did R_N and R_S begin to decrease. All the while, the ratio $R_S >> R_N$ remained valid. Moreover, if the first modification of the discrete method (estimation of the number of pixels with given brightness) failed to discriminate between positive and negative agglutination reactions, the second modification of the method (estimation of total brightness of photoimage pixels) still provided reliable results. Estimates demonstrated that the optimal zone size w was 50-400 pixels depending on the selected approach to photoimage processing.

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 approach ($R_P = P_+/\bar{P}_-$) [10] and the two modifications of the dis-

TABLE 2. Forward Crossmatching

Serum \ Blood group	0(I)			A(II)			B(III)			AB(IV)		
	R_P	R_N	R_S	R_P	R_N	R_S	R_P	R_N	R_S	R_P	R_N	R_S
0(I)	**1.1**	**0.9**	**0.9**	176	2368	73,991	78	2368	41,263	164	2368	70,790
A(II)	**1.1**	**1.4**	**1.4**	**1.3**	**1.2**	**1.9**	205	2368	80,688	114	2368	55,279
B(III)	**1.5**	**1.9**	**1.9**	124	2,368	58,759	**0.7**	**0.7**	**0.7**	190	2368	77,397
AB(IV)	**1.1**	**1.8**	**1.8**	**0.7**	**0.5**	**0.5**	**1.0**	**0.5**	**0.5**	**0.6**	**0.7**	**0.7**

TABLE 3. Reverse Crossmatching

Standard erythrocytes \ Blood group	0(I)			A(II)			B(III)			AB(IV)		
	R_P	R_N	R_S	R_P	R_N	R_S	R_P	R_N	R_S	R_P	R_N	R_S
0(I)	**1.0**	**0.97**	**0.9**	**0.7**	**0.2**	**0.2**	**0.7**	**0.2**	**0.2**	**1.1**	**1.0**	**1.0**
A(II)	185	1739	59,105	**1.7**	**3.0**	**3.0**	44	1739	20,136	**0.9**	**1.6**	**1.6**
B(III)	65	1739	28,206	182	1738	58,428	**0.7**	**0.2**	**0.2**	**1.1**	**1.0**	**1.0**

crete method suggested in this work ($R_N = N_+/\overline{N}_-$ and $R_S = S_+/\overline{S}_-$). Values of $\overline{P}_-$, $\overline{N}_-$, and $\overline{S}_-$ were estimated by averaging of P, N, and S in the samples with a negative agglutination reaction. The zone size was assumed to be $w = 100$; for the discrete methods B_{thres} was assumed to be 5. The semibold letters indicate a negative agglutination reaction.

It follows from Tables 2 and 3 that the discrete approach to photoimage processing provides a considerable increase in the resolution of the acousto-optical method of blood typing as compared to conventional photometry: a 10- to 40-fold increase in the case of estimation of the number of pixels with given brightness ($R_N = N_+/\overline{N}_-$) and a 300- to 500-fold increase in the case of estimation of total brightness of photoimage pixels ($R_S = S_+/\overline{S}_-$).

Conclusion

A new discrete method for photoimage processing within the framework of the acousto-optical method of blood typing was suggested and tested. This approach provides a substantial (up to 500-fold) increase in the resolution as compared to conventional photometry and, therefore, improves the reliability of determination of human blood type. The high resolution of the acousto-optical method attained using the suggested approach allows the blood type to be determined even in the case of weak erythrocyte agglutination. It also allows an algorithm for estimating the intensity of erythrocyte agglutination to be developed. It is important to note that an actual device for blood typing can implement a combination of the photoimage processing methods suggested in this work and in [10]. Such a combination significantly increases the reliability of blood typing. From the technical point of view, the discrete approach to photoimage processing suggested in this work allows all advantages of a photodetector based on a web camera to be used fully. This becomes possible due to the pixel-by-pixel analysis of images. In general, the development of the discrete approach to processing of experimental results can be regarded as a further step in progress of the acousto-optical method of human blood typing.

REFERENCES

1. Steven R.A., A Simplified Visible/Near-Infrared Spectrophotometric Approach to Blood Typing for Automated Transfusion

Safety: Thesis presented to North Carolina State University, Raleigh (2005).
2. Lambert J.B., A Miniaturized Device for Blood Typing Using a Simplified Spectrophotometric Approach: Thesis submitted to North Carolina State University, Raleigh (2006).
3. Ganilova Yu.A., Doubrovski V.A., Zabenkov I.V., Proc. SPIE, **7999**, 799903 (2011).
4. Doubrovski V.A., Dolmashkin A.A., Opt. Spectrosc., **109**, No. 2, 263-267 (2010).
5. Chung A., Birch P., Ilagan K., Transfusion, **33**, No. 5, 384-388 (1993).
6. Mintz P.D., Anderson G., Barasso C., Sorenson E., Immunohematology, **10**, No. 2, 60-63 (1994).
7. Alipov A.N., Vaninskii V.Z., Denisov L.B., Donskov S.I., Doubrovskii V.A., Zav'yalov E.N., Knyaz'kov N.N., "The Method for Determining the Agglutination Reaction", USSR Invention Certificate No. 1683760 (1987).
8. Doubrovski V.A., Dvoretski K.N., Ultrasound Med. Biol., **26**, No. 4, 655 (2000).
9. Doubrovski V.A., Dvoretskii K.N., Balaev A.E., Acoust. Phys., **50**, No. 2, pp. 146-155 (2004).
10. Doubrovski V.A., Medvedeva M.F., Biomed. Eng., No. 1, 58-64 (2016).
11. Doubrovski V.A., Medvedeva M.F., In: Problems of Optical Physics and Biophotonics. SFM-2013 [in Russian] (2013), pp. 34-42.