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A Mobile Device for Measuring the Size Distribution of Red Blood Cells

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A LED-based optical detection circuit and a principle of analysis of diffraction patterns of wet blood smears are proposed. A compact computerized optical device based on the principles of ektacytometry (diffractometry) has been designed to measure the width of the size distribution of red blood cells.

Introduction

Blood tests are a tool widely used in modern medical diagnostics. Changes in the size distribution of cells in human blood have an effect on blood viscosity [1].

Recent studies have shown that a 1% change in the variance (width) of the size distribution of red blood cells leads to an increase in the risk of death by 14% for patients with cardiovascular diseases [2]. The size distribution of red blood cells is usually measured using microscopic images of blood smears, flow cytometers, and/or Coulter counters. However, modern instruments for measuring the size distribution are still relatively expensive and/or use time-consuming measurement procedures. In this regard, the search for new, more accessible techniques for measuring the width of the size distribution of red blood cells is relevant. These new techniques should provide rapid analysis of a large number of cells.

In laser diffractometry, the size distribution of small particles illuminated with a laser beam is determined from the diffraction pattern [3]. The advantage of this approach is that the laser beam illuminates hundreds of thousands of cells at the same time, which makes it possible to obtain statistically significant results without additional time and computational costs. However, in the classical version of this method, it is necessary to measure the diffraction pattern with an error of less than 1% and solve a large regularized set of simultaneous linear equations. These difficulties cause the high cost of devices of this type.

It was proposed in [4] to determine the width of the size distribution of red blood cells by measuring the visibility of the diffraction pattern. In this method, the red blood cell is approximated as a thin, uniform cylinder with a round base perpendicular to the laser beam direction. It was shown that in this case the visibility depends monotonously on the parameter under study, which makes it possible to determine the distribution width from the visibility using tabulated data.

A technique based on the diffraction pattern visibility was for the first time used to measure the size of small particles in [5]. However, in [5], all particles were individually illuminated by a laser in the same way as in flow cytometers, which led to a significant increase in the cost of the measuring setup.

The use of laser radiation in diffractometers limits the accuracy of determining the contrast of the diffraction pattern because of speckle noise.

The goal of this work was to propose a compact LED-based optical circuit providing detection of the diffraction field in the far zone.

The optical circuit is shown in Fig. 1. The diverging radiation beam of LED 1 passes through a small aper-

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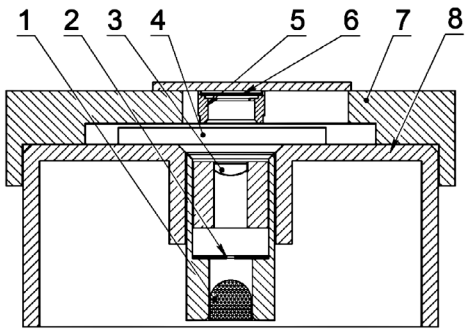

Fig. 1. Optical circuit: 1 – LED; 2 – aperture; 3 – lens; 4 – object glass; 5 – objective; 6 – matrix; 7 – lid; 8 – microscope stage.

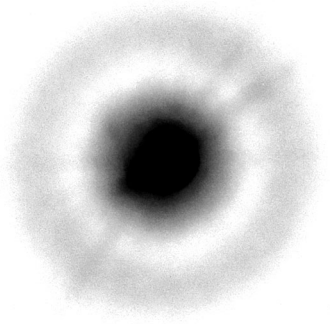

Fig. 2. Typical diffraction pattern of a wet blood smear (negative).

ture 2 and is collimated by a lens 3. Then, it passes through the sample 4 (a blood smear or a transparent channel with blood) and enters the objective 5 of a miniature camera. The objective focuses the radiation transmitted without diffraction into a small spot in the center of a receiving matrix 6 of the camera mounted on a lid 7, which protects the microscope stage 8 from the ambient lighting. A sleeve for the LED is mounted on the microscope stage. The light diffracted on red blood cells forms a diffraction pattern on the matrix around the central spot. This pattern carries information about the geometric parameters of red blood cells.

The source of radiation is a blue LED with a central wavelength of 476 nm. A collimating lens with a focal length of 12 mm forms a parallel beam 3 mm in diameter. Blood samples are placed horizontally between the collimating lens and the camera objective. It is possible to move the sample to select the area of interest or for repeated recording with further averaging of the obtained data. The diameter of the camera lens' aperture (1.5 mm) determines the sample area per frame. A Raspberry compact computer is used as a recorder. It has a 64-bit 4-core processor with a clock frequency of 1.4 GHz, based on the ARM architecture and supporting various Linux OSs. The device has a general-purpose input/output interface, USB 2.0 ports for connecting peripherals, HDMI, Ethernet, Wi-Fi and Bluetooth wireless interfaces, as well as a MIPI CSI port for connecting to the camera.

Digital diffraction patterns were recorded using a RaspberryPiCameraModule v2.1 camera equipped with a Sony IMX219PQ CMOS sensor with a diagonal of 4.6 mm (1/4.0) and an effective resolution of 3280 × 2464 pixels. The exposure time per frame was 5 ms.

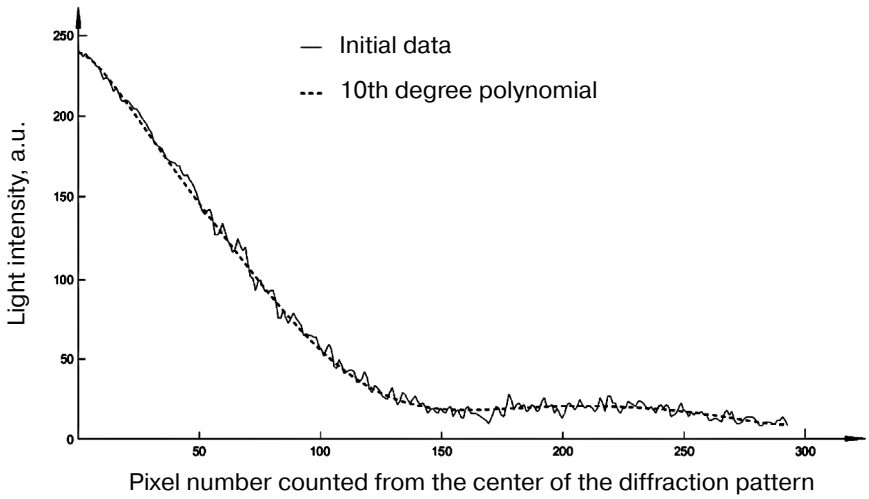

Fig. 3. Typical intensity distribution along the diffraction pattern radius.

Figure 2 shows the diffraction pattern of a wet blood smear detected using the developed optical system. Special software was developed in MATLAB to analyze the diffraction patterns. The algorithm for diffraction pattern processing provides the extraction of the monochrome image component. Then, the center of the diffraction pattern is determined as the center of mass of the set of points with brightness exceeding half the maximum. Secant rays are drawn from the center at angles in the range 0-360° with a step of 1°. These rays are used to construct the intensity profile. In the case of round particles, the diffraction pattern has circular symmetry, which makes it possible to average the profiles over all rays, reducing thereby the speckle noise and the receiving matrix noise. Then, to smooth out the remaining fluctuations, the averaged profile is approximated by a tenth degree polynomial (Fig. 3).

The intensities I_{min} and I_{max} at the first intensity minimum and maximum, respectively, are determined from the obtained smooth curve. The visibility v of the diffraction pattern is calculated as $v = (I_{max} - I_{min})/(I_{max} + I_{min})$. Calibration experiments gave a typical visibility value of about 7%. Tabular data was used to determine the cell size spread from the diffraction pattern visibility. The relative cell spread was found to be 18%. It should be noted that red blood cells have to be fixed with glutaraldehyde. In our experiments, we used the blood sample preparation protocol from [6].

Conclusions

A diffractometry technique for measuring the width of the size distribution of red blood cells has been proposed. A compact device has been developed for detecting diffraction patterns of red blood cells illuminated using a low-coherence light source (LED). The computing power of the device and its input/output interfaces provides for detection of diffraction patterns and allows the cell size distribution to be calculated from the diffraction pattern visibility. In our opinion, the proposed prototype device holds much promise for the use in clinical practice to detect deviations from the norm in the size distribution of red blood cells.

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REFERENCES

1. Kozinets, G. I. and Makarova, V. A., Examination of the Blood System in Clinical Practice [in Russian], Triada-X, Moscow (1997).
2. Patel, K. V., Ferrucci, L., Ershler, W.,B., Longo, D. L., and Guralnik, J. M., "Red blood cell distribution width and the risk of death in middle-aged and older adults," Arch. Int. Med., **169**, No. 5, 515-523 (2009).
3. Black, D. L., McQuay, M. Q., and Bonin, M. P., "Laser-based techniques for particle-size measurement: A review of sizing methods and their industrial applications," Progr. Energy Comb. Sci., **22**, No. 3, 267-306 (1996).
4. Nikitin, S. Y. et al., "Relation between the diffraction pattern visibility and dispersion of particle sizes in an ektacytometer," Quantum Electron., **41**, No. 9, 843-846 (2011).
5. Farmer, W. M., "Measurement of particle size, number density, and velocity using a laser interferometer," Appl. Opt., **11**, No. 11, 2603-2612 (1972).
6. Baskurt, O. K. et al., "Comparison of three commercially available ektacytometers with different shearing geometries," Biorheology, **46**, No. 3, 251-264 (2009).