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Blood cell sizing with the scanning flow cytometer

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Abstract

The next generation of flow cytometers, Scanning Flow Cytometer (SFC), allows measurement of the angular dependence of light-scattering intensity (indicatrix) of single cells. The light scattering indicatrix contains information about morphological characteristics of cells: size; shape; internal structure; sizes and refractive indices of cytoplasm, nuclear, organelles, et cetera. Basic types of blood cells are platelets, erythrocytes or red blood cells (RBC), and leucocytes or white blood cells (WBC). WBC are divided on three basic classes: lymphocytes, monocytes and granulocytes. In the current study, the light scattering indicatrix of blood cells was measured with SFC. The size of individual platelets, RBC and tree classes of WBC was determined by applying a spectral decomposition of the light scattering indicatrix.

1 Introduction

Blood cell classification and analysis are an important diagnostic technique in the detection of many diseases. An accurate diagnosis could aid in optimal treatment of the disease. The Scanning Flow Cytometer (SFC) [1], allows measurement of angular dependence of light-scattering intensity (indicatrix) of single cells. The light scattering indicatrix contains information about morphological characteristics of cells: size; shape; internal structure; sizes and refractive indices of cytoplasm, nuclear, organelles, et cetera. In order to obtain information about the morphology of a cell it is necessary to solve the inverse light scattering problem. Now, methods for solving the inverse light scattering problem for complex particles as blood cells are insufficiently developed. In the current study, we have presented the approach to a simple method for sizing of complex particles. The spectral decomposition of the light scattering indicatrix provides visual information about the characteristic size of cells [2, 3]. The goal of this paper is to develop an easy and fast tool for blood cell classifications and sizing that may provide a clear view on possible pathologies.

2 Foundations of cell sizing with SFC

The Fourier spectrum of the light-scattering indicatrix of a homogeneous spherical particle contains a pronounced peak. The following empirical equation was derived by Semyanov *et al* [2] to connect the location of the peak in the spectrum of light scattering indicatrix and the size parameter of a homogeneous spherical particle:

$$\alpha = 189.12 \cdot P_f, \qquad (1)$$

where $\alpha = m_0 \pi d/\lambda$, d is the particle diameter, m_0 is the refractive index of the medium, λ is the wavelength of incident light in vacuum, P_f is the position of spectrum peak. This equation has a precision of 3 % accuracy in size of a homogeneous spherical particle with refractive indices in the region from 1.37 to 1.70. This region of refractive indices covers the refractive indices of biological cells.

In order to provide an effective comparison of experimental and theoretical light-scattering data the indicatrices were modified by multiplication with the weighting function $w(\theta)$ defined as:

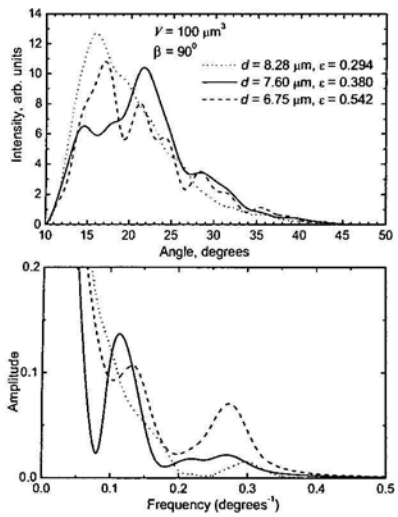

Fig. 1 Modified theoretical light scattering indicatrices (top) and its spectrum (bottom) for erythrocytes of the three different shapes and sizes. V is a volume and d is a diameter of erythrocyte, ε is ratio of erythrocyte's thickness to the diameter d, β is angle between axis of erythrocyte and direction of incident radiation

$$w(\theta) = \sin^2\left(\pi\frac{\theta - 10^\circ}{50^\circ - 10^\circ}\right) \qquad (2).$$

The multiplication corresponds to the standard Hanning window procedure that greatly reduces the effects of the discontinuities at the beginning and the end of the sampling period of the SFC. Moreover this function looks like the SFC instrument function [1] and improves visualization of indicatrices because a logarithmic scale can be substituted with a linear one. We will represent all light scattering indicatrices in modified form below.

The spectrum of a spherical particle with two layers has four peaks [3], the locationn of the last peak is proportional to the size of particle. The same phenomenon takes place for a sphere with five layers i.e. the location of last peak is proportional to the size of the particle [3]. For nonspherical particles we assume that the same phenomenon will take place. Fig. 1 shows the modified light-scattering indicatrices of erythrocyte and its spectra as an example of nonspherical particles. We computed this indicatrices using DDA method [3]. We can see that the spectrum of such particles can have one, two or three peaks and the location of the last corresponds to the diameter of the particles. For the sizing of blood cells we have tried to use equation (1) and the position of the last peak for erythrocytes and leucocytes and the position of the maximum peak for the platelets. It is questionable that the coefficient of proportionality is the same as for a homogeneous spherical particle. We will postpone this question to the future work.

3 Blood cell sizing

The light scattering indicatrix of individual blood cells was measured with SFC. Next, the fast Fourier transform (FFT) procedure was applied to the measured and modified light scattering indicatrices. The resulting spectra have multitude peaks. It is necessary to exclude the noise influence. In order to define the essential peak we do the following. First we define the noise of a laser. For interested frequency range the noise of the laser we used was 0.5 % from the total power. Next for weak signals a considerable contribution to the noise is quantization noise of the Analog-to-Digital converter. It can easily be measured in absence of a light scattering signal and was 0.0006 in arbitrary units. Then the

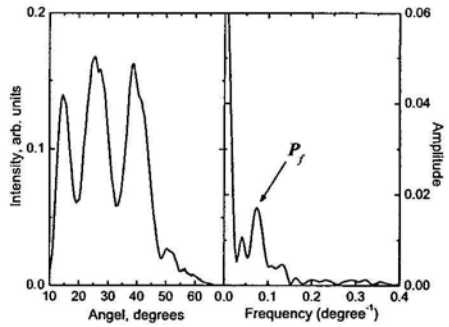

Fig. 3 A typical experimental light scattering indicatrix (left) and its spectrum (right) for single platelets

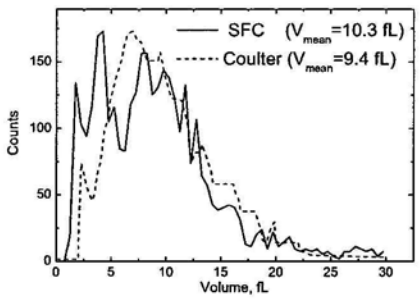

Fig. 2 Comparison volume distributions of platelets for the same blood sample obtained with SFC (solid line) and with Coulter (dash line)

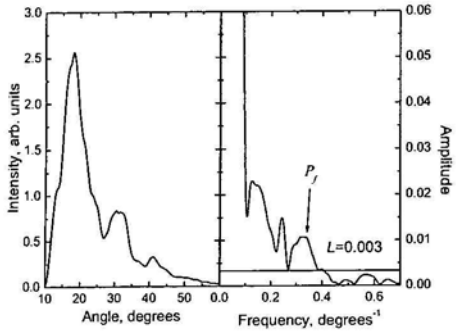

Fig. 6 A typical experimental light scattering indicatrix (left) and its spectrum (right) for single erythrocytes

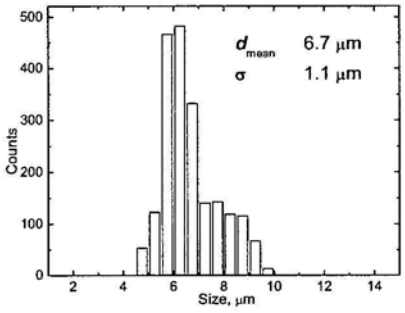

Fig. 4 The diameter distribution of erythrocytes obtained with SFC

noise level can be calculated by $L=0.005\times M+0.0006$, where M is the mean value of measured signal. The essential peaks are defined as those peaks exceeding the noise level. In the case of sizing of erythrocyte and leucocytes the location of the last essential peak is used.

3.1 Platelet sizing

We continuously measured 2000 indicatrices of platelets with the SFC. The FFT was applied to each of them. Fig. 3 shows a typical light scattering indicatrix and its spectrum. The location of the maximum peak (see Fig. 3) has been taken into equation (1) for calculation of the virtual size of platelets, d. Then the volume has been calculated by $V=\pi/6\cdot d^3$ assuming that the d is the diameter of volume-equal sphere. Finally a volume distribution was constructed (solid line Fig. 2).
The volume distribution of platelets of the same blood sample was measured on the hematology analyzer Coulter MAXM (dash line Fig. 2). The result of comparison of two different measurements of platelets volume is presented on Fig. 2. We can see a good agreement between both volume distributions except for small volumes. It is connected with that the rival and locating from the left position peak come into the maximum value. However, we believe that the maximum peak in the spectra of indicatrix for platelets corresponds to the diameter of the volume-equal sphere and that SFC therefore allows for a good sizing of platelets, comparable to results obtained with the well established coulter technique.

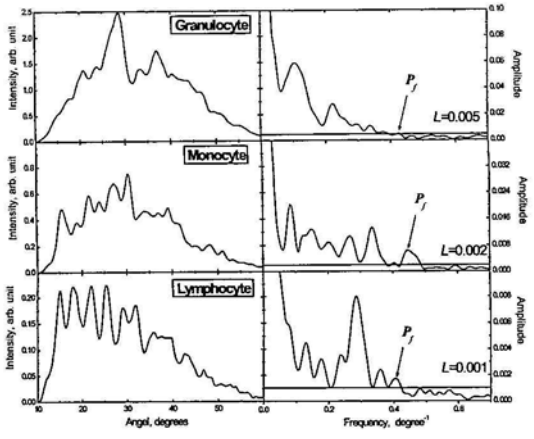

Fig. 5 Typical experimental light scattering indicatrices (left) and their spectra (right) for a single granulocyte (top), monocyte (middle) and lymphocyte (bottom)

3.2 Erythrocyte sizing

We continuously measured 2000 indicatrices of erythrocytes with the SFC. The FFT was applied to each of them. Fig. 6 shows a typical light scattering indicatrix of erythrocytes and its spectrum. The location of the last essential peak (see Fig. 6) has been taken into equation (1) for calculation the virtual diameter of erythrocytes, d. The resulting diameter distribution is shown in Fig. 4. The value of mean diameter is 6.7 μm and the value of standard deviation is 1.1 μm. Clearly, equation (1) is not properly fit for nonspherical

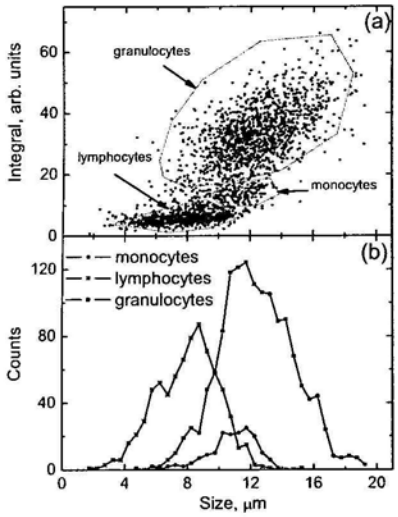

Fig. 7 The integral×size map (a) and size distribution of leucocytes obtained with SFC (b)

particles. Also the shape of the distributions looks strange; it has a shoulder from the right side. This fact should to be investigated in the future using extended set of DDA simulations of erythrocytes.

3.3 Leucocytes sizing

We continuously measured 2500 indicatrices of leucocytes with the SFC. The FFT was applied to each of them. Fig. 5 show typical light scattering indicatrices of three types of leucocytes: granulocytes, monocytes and lymphocytes and their spectra. The locations of the last essential peak (see Fig. 5) have been taken into equation (1) for the calculation of the effective size of leucocytes. We differentiate leukocyte's types accordingly to the integral×size map shown on Fig. 7 (a), where an integral is integral of the light-scattering indicatrix from 10 to 70 degrees. Every point on this map corresponds to a single leukocyte. The resulting size distributions for different types of leucocytes are shown in Fig. 7 (b). The values of mean sizes and standard deviations are d=8.0 μm and σ=2.0 μm for lymphocytes d=10.8 μm and σ=1.5 μm for monocytes, d=12.4 μm and σ=2.3 μm for granulocytes. But the lymphocytes distribution has a shoulder from the right side and granulocytes distribution has a shoulder from the left side. It can be connected with errors in determination of spectrum's peak and should to be investigated in the future.

4 Conclusion

In this study we have demonstrated the feasibility of a simple and fast technique to measure some characteristic of inhomogeneous and nonspherical particles which can be regarded as an effective size. This technique is based on a spectral decomposition of the light scattering indicatrix and was applied to measure effective sizes of blood cells: platelets, erythrocytes, lymphocytes, monocytes, and granulocytes. The spectral decomposition method plays an important role in the commercialization of the SFC, providing a stable and easy-to-use algorithm for different applications with this technique. The application of this technique gives an easy and fast tool for blood cell classification and sizing to provide a clear view to pathologies.

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