OPTICS OF PLATELETS

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Abstract: Optical methods to study platelets with recent application to scanning flow cytometry are presented.

Key words: platelet, spheroid, T-matrix, DDA

1. INTRODUCTION

Platelets are a small subcellular fragment of blood that have an important role in maintaining haemostatic and arterial thrombosis, particularly in fibrillation. Activated platelets adhere to an injury and aggregate to form a haemostatic plug or thrombus. Blood platelet volume, size and shape are markers of their activation and function that can be used to distinguish activated and non-activated platelets. Characterization of human platelets is frequently used as a tool in investigation of disease, such as ischaemic heart disease, cerebrovascular disease, renovascular disease, etc.[1]

Just one characteristic of platelets, platelet volume, is available in instrumental clinical analysis. Unfortunately this instrumental analysis does not help us to clarify optical properties of these cells because these instruments utilize aperture-impedance techniques (e.g. Coulter S Plus).[2] This approach does not take into account the shape variability of platelets. We believe that an enhanced characterization of platelets may have clinical relevance.

With this work we have analyzed results obtained for blood platelets[3] using a Scanning Flow Cytometer (SFC)[4,5] that allows measurement of the

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angular dependence of the light-scattering intensity (the light-scattering profiles, LSP) of individual cells in the region ranging from 5^0 to 120^0. The SFC output signal is proportional to Mueller matrix element S_{11} integrated over the azimuthal angle.

In order to retrieve morphological characteristics from a platelet's LSP we have to solve the inverse light-scattering problem. The obvious and only existing approach to determine characteristics of an arbitrary particle from LSP is the direct comparison of the experimentally measured LSP with the theoretically calculated LSP based on an optical model of the particle. Previously we have demonstrated the validity of this approach for characterization of red blood cells from light scattering.[6]

2. CHARACTERIZATION OF INDIVIDUAL BLOOD PLATELETS FROM LIGHT SCATTERING

2.1 Sample Preparation

Blood was withdrawn from the antecubital vein of normal volunteers with syringe and then put into a tube filled with EDTA as an anticoagulant. Care was taken to ensure a ratio of 1.5 mg EDTA to 1 ml blood in sample. All surfaces in contact with the blood were plastic. The sample was stored at room temperature. The measurement was conducted about 30 minutes after venesection.

2.2 Choice of Shape for Model Platelet: T-Matrix Simulation

A platelet that is a discoid cell with diameter of 2–4 μm and thickness of 0.5–2 μm in the nonactivated state, and it becomes a spicular spheroid in the activated state. Activation of platelets starts when oxygen appears in the blood plasma. The process of platelet activation includes transformation of the cell to aggregate into a thrombus – pseudopodia emerge from a discoid platelet that becomes spicular.[7]

We applied the T-matrix method to simulate light scattering of a platelet modeled as an oblate spheroid, because pseudopodia does not have a substantial effect on the LSP in the current configuration of the SFC.[3] A recent review of the T-matrix approach has been performed by Mishchenko *et al.*[8], and we applied the public-domain T-matrix code from Mishchenko (http://www.giss.nasa.gov/~crmim/) in simulations of light scattering of individual platelets.[9]

2.3 Results

We continuously measured 2000 LSPs of platelets with the SFC and few of them are shown in Figure 1.

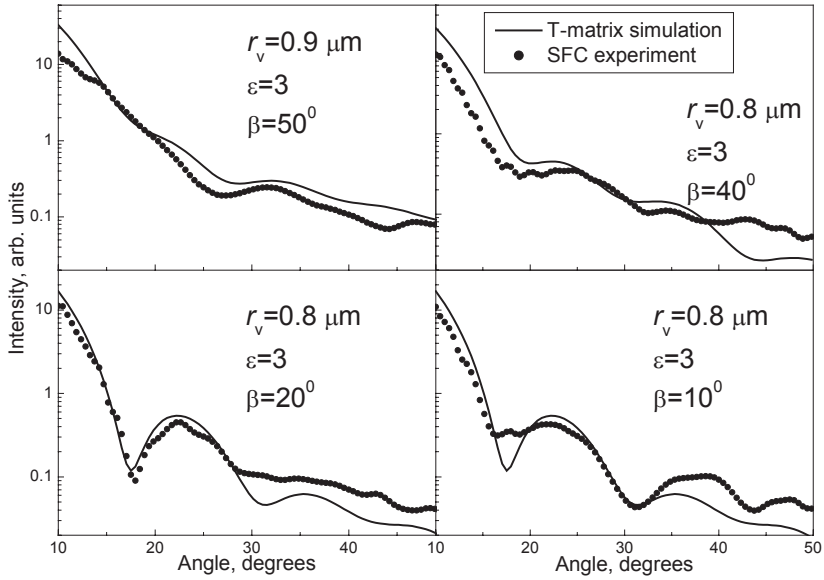

Figure 1. Typical light-scattering profiles of blood platelets measured with SFC (points), and best-fit LSPs of oblate spheroids calculated with T-matrix method (solid lines). The spheroid characteristics, volume-equivalent-sphere-radius r_V, axis ratio ε, polar orientation angle β were used to calculate the best-fit light-scattering profile.

The characteristics of platelets were determined from a fit of the experimental and theoretical LSPs. We varied volume-equivalent-sphere-radius r_V, polar orientation angle β of oblate spheroid to find the best-fit LSP for LSP experimentally measured with the SFC. The polar orientation angle is the angle between the direction of the incident radiation and the axis of the oblate spheroid. The axis ratio ε and refractive index *n* of oblate spheroids were fixed at 3 and 1.41, respectively. We were unable to vary all spheroid characteristics because the iteration procedure requires an incredible amount of time for calculations.

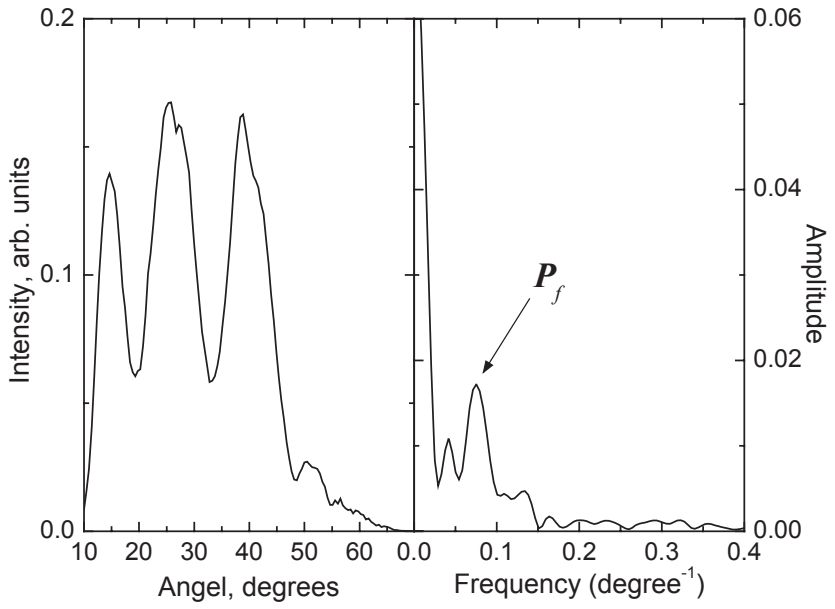

Figure 2. Typical experimental light-scattering profile transformed by Hanning window (left) with spectrum (right) for a single platelet.

In order to determine the volume of measured blood platelets we apply the spectral approach introduced in our previous work.[10] The spectral approach assumes transformation of the LSP with a standard Hanning window procedure and FFT. The typical transformed LSP and spectrum are shown in Figure 2. The location of the maximum peak P_f was used in equation (1) for calculation of the volume-equivalent-sphere-diameter d of platelets:

$$\alpha = 189.12 \cdot P_f , \tag{1}$$

where $\alpha = m_0 \pi d / \lambda$, m_0 is the refractive index of the medium, λ is the wavelength of incident light in vacuum. The spectral approach allows us to form the volume distribution of blood platelets (solid line in Figure 3).

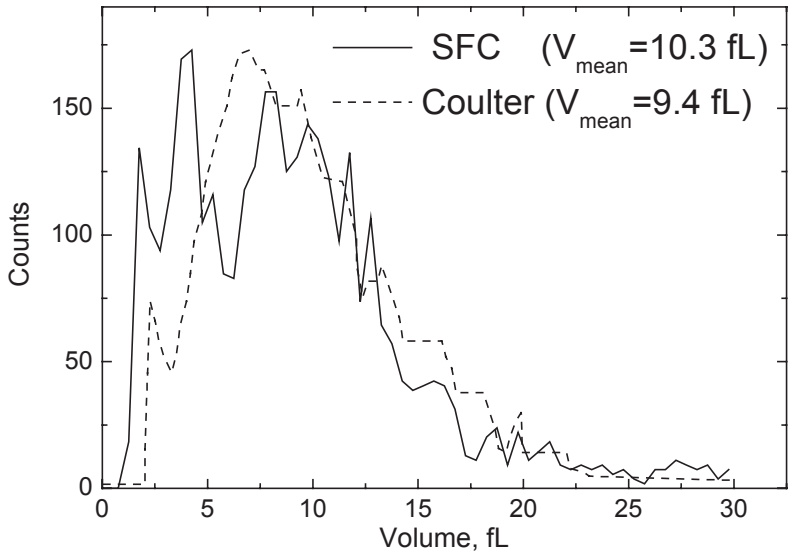

Figure 3. The volume distributions of platelets for the same blood sample obtained with SFC (solid line) and with a Coulter analyzer (dash line).

The volume distribution of platelets of the same blood sample was measured on the hematology analyzer Coulter MAXM (dash line in Figure 3). We found a good agreement between both volume distributions except for small volumes. Our results demonstrate that the maximum peak in the LSP spectrum for individual platelets corresponds to the diameter of the volume-equal sphere and SFC can be used in characterization of blood platelets from light scattering.

3. CONCLUSION

A new method to determine the volume of individual blood platelets has been introduced. The method is based on Scanning Flow Cytometry and the measurement of multi-angle light scattering and a solution of the inverse light-scattering problem with the spectral approach. The parameters of the platelet volume distribution are in good agreement with independent measurements from commercial instruments and literature values.

This work gives access to important hematological parameters and platelet volume, which can be measured using the optical technique. A clinical protocol should be developed for the SFC with the aim of measuring the mean and the width of the distribution of blood platelet volume.

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