Identification and Characterization of Cell-Derived Microparticles Using Light Scattering

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Abstract

We describe a method for identification and characterization of cell-derived microparticles in plasma, using the scanning flow cytometer, which measures light scattering patterns (LSPs) of individual particles. The identification of MPs in plasma is performed based on shape differences of spherical MPs and other non-spherical plasma particles. Using light-scattering information from LSPs and additionally measured side-scattering we solve the inverse-scattering problem for each measured particle and rigorously characterize it, determining size and refractive index including errors of these estimates. This method allows us to characterize individual MPs larger than 300 nm with median precisions (standard deviations) of 7 nm and 0.0018 for size and refractive index respectively.

1 Introduction

Cell-derived microparticles (MPs) are spherical phospholipid particles, which are present in peripheral blood and body fluids and constitute a heterogeneous population of particles highly variable in size, composition, concentration, cellular origin, and functional properties [1]. Being involved in a variety of physiological processes MPs are considered as potential diagnostic or prognostic biomarkers. That attaches importance to determination of MPs characteristics, which may contain significant clinical information. But detection and analysis of MPs is challenging due to their small sizes (typically defined in ranges between 100 nm and 1 μm) and low (with respect to the medium) refractive indices. It motivates development of new techniques and methods to identify MPs in biological fluids distinguishing them from other constituents of similar size and to characterize MPs both by their size and refractive index.

Scanning flow cytometry is a technique capable of measuring angle-resolved light-scattering patterns (LSPs) for individual particles [2,3]. The LSP contains valuable information, which can be used to deduce particle size, shape, and refractive index. Since a homogeneous sphere is an adequate optical model of MPs a complete characterization (determination of size and refractive index) is possible by solving the inverse light-scattering (ILS) problem using Mie theory for light-scattering simulation. Moreover, it provides unambiguous separation of MPs from other non-spherical plasma constituents on the basis of similarity of their LSPs to that of a sphere. Additional light-scattering data from side-scattering measured using a different wavelength can also be used to improve the accuracy of MPs characterization.

2 Methods

2.1 Light-scattering measurements

Within the Mueller-matrix formalism [4] for description of light scattering by a particle, the LSP and side-scattering intensity (SSC) measured by Scanning Flow Cytometer are expressed as:

$$\mathbf{I}_{\text{th}}^{\text{LSP}}(\theta) = k_1 \int_0^{2\pi} [S_{11}(\theta,\varphi) + S_{14}(\theta,\varphi)] d\varphi \,, \tag{1}$$

$$\mathbf{I}_{\text{th}}^{\text{LSP}}(\theta) = k_2 \int_{apert.} d\varphi d\theta \sin\theta [S_{11}(\theta,\varphi) - S_{12}(\theta,\varphi)\cos(2\varphi) - S_{13}(\theta,\varphi)\sin(2\varphi)] \tag{2}$$

where $S_{ij}(\theta,\varphi)$ are elements of the Mueller matrix, θ and φ are the polar and azimuthal scattering angles, k_1 and k_2 are the scaling coefficients, determined from calibration of the SFC. The LSP is measured at the wavelength of 405 nm in the polar angular range from 5° to 70° and the SSC is measured at the wavelength 488 nm and integrated over circular aperture that is 90±17.5° for both polar and azimuthal diametric angles respectively. SSC is also used to trigger the electronics of SFC and thus its sensitivity determines the detection limit of the instrument.

2.2 Inverse light-scattering problem solution, microparticle identification and characterization

Applying the model of homogeneous sphere to MPs we reduce the solution of the ILS problem to simultaneous fitting of experimental LSPs and SSC signals by theoretical ones. Thus the problem is transformed into the global minimization of the following sum of squares:

$$S(\boldsymbol{\beta}) = \sum_I \left[w(\theta_i)\right]^2 \left[I_{\text{exp}}^{\text{LSP}}(\theta_i) - \eta I_{\text{th}}^{\text{LSP}}(\theta_i, \boldsymbol{\beta})\right]^2 + \\ + \left[I_{\text{exp}}^{\text{SSC}} - I_{\text{exp}}^{\text{SSC}}(\boldsymbol{\beta})\right]^2 \quad (3)$$

where $\boldsymbol{\beta}$ is the vector of characteristics consisting of a diameter d and RI n of a MP, θ_i is the polar scattering angle, $\mathbf{I}_{\text{exp}}^{\text{LSP}}(\theta_i)$ and $\mathbf{I}_{\text{th}}^{\text{LSP}}(\theta_i, \boldsymbol{\beta})$ are the intensities of experimental and theoretical LSP at angle θ_i respectively, $\mathbf{I}_{\text{exp}}^{\text{SSC}}$ and $\mathbf{I}_{\text{th}}^{\text{SSC}}(\boldsymbol{\beta})$ are experimental and theoretical SSC signals respectively, η is the coefficient that compensates an effect of the non-central particle trajectory in the flow cell of the SFC. The theoretical LSP and SSC are calculated from Eqs. (1) and (2) using the Mie theory.

The global minimization is performed by DiRect algorithm [5], which provides an extensive search of global minima $S(\boldsymbol{\beta})$ over the parameter space confined by parameter bounds $d \in [0.1, 2]$ μm, $n \in [1.35, 1.7]$, which amply cover the range of MPs.

Using this solution we separate MPs from other plasma constituents, such as platelets and cellular debris, supposed to have non-spherical shape, by similarity of their LSPs structure to that of sphere, and perform residual correlation analysis to quantitatively describe these differences. In particular, number of intersections of experimental and best-fit theoretical LSPs is used, which has smaller values for non-spherical particles due to larger correlation between neighboring residuals [6]. Also based on the solution of the ILS problem we rrigorously characterize each measured particle, determining its size and refractive index including errors of these estimates.

3 Results

The developed method was applied to analyse MPs populations in three differently prepared samples of untreated, centrifuged and filtered trough 1.2 μm pore filter platelet plasma. Typical results of individual MPs characterization with determined characteristics and their uncertainties are shown in Figure 1.

Polystyrene microspheres of 400 nm and 1 μm were also measured and analyzed, showing a good agreement in their size and refractive index determination and thus confirming the method adequacy. The MPs and polystyrene microspheres distributions over size and refractive index are shown in Figure 2. The major part of detected MPs falls in the range of 400 – 800 nm for size and 1.46–1.50 for the refractive index, and the median precisions in determination of each parameter for single measured particle are 7 nm and 0.0018, respectively.

Details of the obtained results are described in [7].

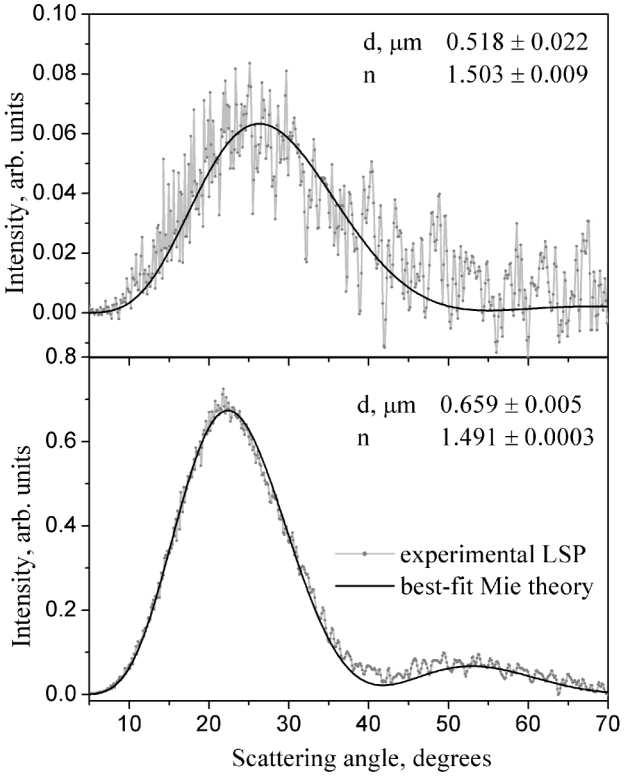

Figure 1 *Typical results of characterization of single MPs, depicting weighted experimental and best-fit Mie theory LSPs. Estimates of particles size d and refractive index n (mathematical expectation ± standard deviation) are also shown.*

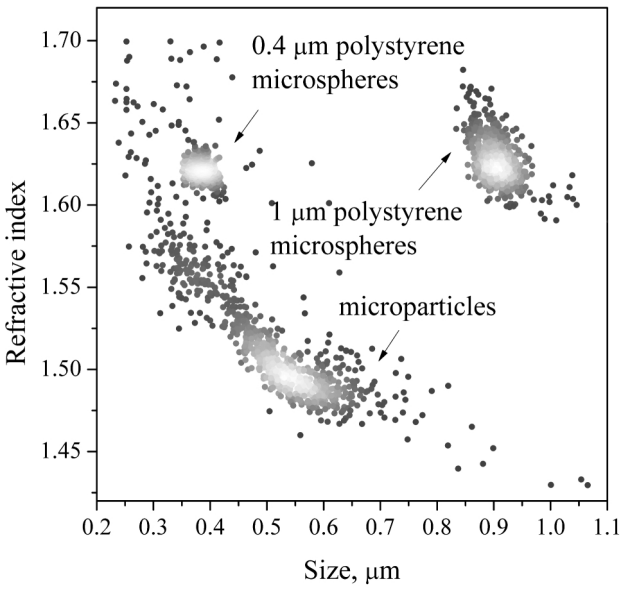

Figure 2 *Distributions over size and refractive index for MPs and polystyrene microspheres of 0.4 and 1 μm.*

4 Conclusion

Here we describe a method for identification and characterization of blood microparticles using angle-resolved light scattering measured with the SFC. The identification of spherical MPs among other measured non-spherical sample constituents, including platelets and cellular debris, is based on the structure of their LSPs, that could be described by Mie theory for MPs, and shows large disagreement with theory for other particles. Each MPs is characterized by size and refractive index obtained from the inverse light-scattering problem solution with uncertainties of these parameter estimations. The accuracy of particle parameter estimations is improved by using an additional to LSP scattering information from side-scattering measured on the other laser wavelength and in different angular range.

The only limitation of this approach is size detection limit - the current optical set-up of the SFC can reliably analyze MPs larger than 400 nm. But the detection limit could be further decreased to 300 nm using higher-power lasers and middle-angle light scattering instead of SSC.

5 References.

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