PROCEEDINGS OF THE
2ND INTERNATIONAL CONGRESS
ON
OPTICAL PARTICLE SIZING

Mechanical and Aerospace Engineering
Arizona State University
Tempe, Arizona

March 5-8, 1990

Congress Chairman
E. Dan Hirleman
Professor of Engineering
Arizona State University

Sponsored by
Arizona State University
National Science Foundation

Published by
Arizona State University
Printing Services

ARBITRARILY-SHAPED PARTICLES MEASURED IN FLOW THROUGH SYSTEMS

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ABSTRACT

Light scattering techniques, including depolarization experiments, applied to micron-sized particles provide a fast non-destructive probe that is very sensitive to small morphological differences. Up till now quantitative measurement of these scattering phenomena were only described for particles in suspension. In this presentation we shall discuss the symmetry conditions applicable to the scattering matrices of polydisperse particles in a flow cytometer. Evidence is provided that quantitative measurement of the elements of these scattering matrices is possible in flow- through systems. Two fundamental extensions to the theoretical description of conventional scattering experiments are introduced: implementation of the localization principle to account for scattering by a sharply focussed laser beam and large cone integration of scattering signals.

INTRODUCTION

Measurement of Elastic Light Scattering (ELS) in flow-through systems is an important diagnostic tool to identify and separate various populations in polydisperse particle suspensions. Flow-through techniques exploit hydrofocussing of a particle suspension by means of a sheath flow. The forced linear array of localized particles is irradiated by a sharply focussed laser beam. The various particles can be identified and isolated with respect to the light scatterin[illegible]nal.

An illustrative example of these types of experiments comes from the biophysical sciences. For instance it was already shown by Salzmann et al., that simultaneous detection of Forward Scattering (FS) and Side Scattering (SS) allows identification of various populations of human peripheral blood cells.[1] Various other light scattering experiments were proposed ever since. It was suggested, for instance, to measure differential light scattering from cells that were simultaneously irradiated by two laser beams tuned to different wavelengths.[2] Other experiments include angular ratio measurement and small angle detection techniques.[3,4] However, due to the short sampling time characteristic for flow-through measurements (typical values are ~ 10 µs per cell), the scope of possible light scattering experiments is limited.[5]

In previous work we proposed a modified Rayleigh-Debye-Gans (mRDG) theory to approximate numerically the light scattering information present in nucleated blood cells such as human peripheral lymphocytes.[6,7,8] It was shown that the simultaneous measurement of FS, SS and Back Scattering (BS) is well suited to characterize various physical properties of biological cells. Parts of these theoretical calculations were recently confirmed by experimental data.[8] Although, theories based on (m)RDG approximations allow rapid calculation of angular light scattering spectra from complex structures, no information on the (de)polarization introduced by the particle is available. It was shown by Bickel et al., that the information content of the scattering matrix elements allows detailed differentiation of various subcellular particles. Especially, minute structural modifications of the scatterer are reflected by the scattering matrix elements.[9,10] These types of experiments however, can not readily be implemented in a flow-through system, since the light scattering signal of each cell (or cell suspension) must be measured over too large a period of time. As a consequence, the measurement of significant Mueller matrix elements in a flow-through system has not attained much attention yet. Despite these experimental complications, implementation of the (de)polarization of the scattered light into a flow-through system is very promising, since the scattering signals can be applied to discriminate and separate various populations of particles. Thus far only one *qualitative* experiment, concerning cross polarization of subsets of human granulocytes in flow, has been reported in literature.[11]

In this presentation we provide evidence that the most significant scattering matrix elements for an ensemble of poly disperse particles (i.e nucleated blood cells) can be obtained from light scattering experiments in a flow-through system.

THEORY

Symmetry Conditions

It can be derived from symmetry conditions that the scattering matrix of biological particles in a flow-through system is be described by[12]:

$$\begin{bmatrix} S_{11} & S_{12} & \text{[illegible]} & 0 \\ S_{12} & S_{22} & 0 & 0 \\ 0 & 0 & S_{33} & S_{34} \\ 0 & 0 & -S_{34} & S_{44} \end{bmatrix}$$

Here it is assumed that each particle from a particular population has both mirror- and reciprocal particles that pass the laserbeam one by one. In addition, this sequential illumination of the particles guarantees that no systematic relation among the individual particles is present. As a consequence, this incoherent scattering allows one to sum all the scattering matrices of the individual particles. Therefore the symmetry conditions of the complete ensemble reduce the number of independent elements in the scattering matrix.

Illumination of Particles in a Flow-Through System

The scattering of a particle in a sharply focused laser beam cannot be described by the conventional planewave equations obtained by Mie. A solution to this problem is formulated by the General Lorenz Mie Theory (GLMT) that has been discussed recently.[13] This theory is well suited to describe the scattering of a Gaussian beam by a spherical, isotropic, homogeneous and nonmagnetic particle with an arbitrary location with respect to the beam axis. The GLMT incorporates the field components of a real laser beam, with a TEM_{00} Gaussian mode, in the amplitude coefficients of the Mie functions. We propose therefore to calculate the elastic light scattering of spheres in flow through equipment by means of this GLMT.

In the GLMT an additional weighting factor g_n is incorporated into the Mie amplitude functions. Due to the complex structure of the formal solution for g_n, computational difficulties may arise.[14] Some simplifications can be obtained if the partial wave components of the incident field (a_n and b_n) are associated with localized incident geometrical rays.[15] This so-called localization principle (LP) associates the partial wave components of order n to a corresponding incident ray, passing the origin of the scatterer at a distance $(n+1/2)\ \lambda/2\pi$. The LP simplifies the computational algorithm for Mie scattering by a homogeneous sphere in an axisymmetric Gaussian beam enormously. The number of terms 'n' that needs to be calculated reduces to approximately '$n \approx \alpha$'.[15]

Both simplifications are implemented in the numerical calculations of the Mueller matrix elements to verify the experimental data.

Calculation of the FS, SS and BS Intensities

In contrast to conventional scattering equipment, the detectors in a typical flow through system are located close to the scattering entity. As a consequence, relatively large numerical apertures are involved. Therefore, the calculated scattering intensities must be integrated over a large cone in the FS, SS and BS directions. The total intensity measured by a detector surface element $R^2 \sin\theta d\theta d\phi$ is:

$$I_{FS,BS} = \frac{I_0}{k^2R^2} \int_{\phi}\int_{\theta} R^2\, I(\theta)\, \sin(\theta)\, d\theta\, d\phi$$

where $I(\theta)$ is the calculated intensity from the GLMT program.

ϕ ranges from 0 to 2π for both the FS and the BS. A beam stop was inserted to remove the primary beam from the sensitive area of the FS detector, whereas in the BS direction a hole in the detector partially removed the backward scattered light. In the SS direction however, $\Delta\phi = \Delta\theta$, and it follows that:

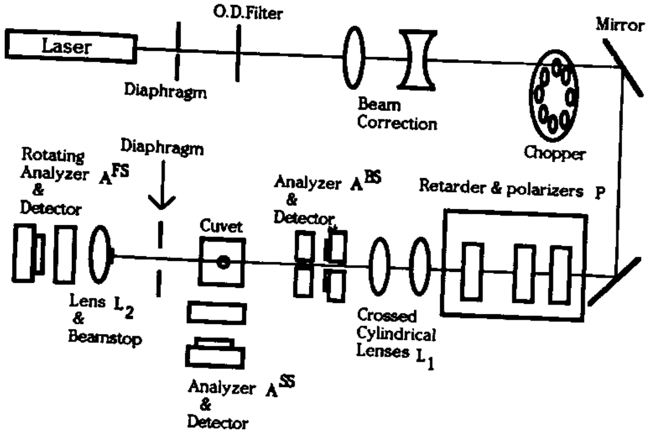

figure 1: Outline of the optical configuration for polarization experiments in a flow through system

$$I_{SS} = \int_{90-\Delta\theta}^{90+\Delta\theta} I(\theta) r(\theta,\phi) d\phi$$

where $r(\theta,\phi) = 2.0 \left\{ (\Delta\phi)^2 - (90-\theta) \right\}^{1/2}$. These intensities were calculated for all scattering matrix elements.

EXPERIMENTAL METHODS

Experiments are performed to investigate whether flow-through equipment is suited for *quantitative* determination of the scattering matrices of particles in flow. The arrangement of the optical elements is shown in figure 1.

To interpret the experimental data, we applied the Generalized Lorenz Mie Theory (GLMT)[13] and simultaneously calculated the integrated light scattering intensities (where polystyreen spheres were used as testparticles). In addition a calibration technique for the special purpose optics is applied, where the commutativity of the Mueller matrices of the optics and the particles is exploited.[12]

RESULTS

Four different values of α ($2\pi r/\lambda$) in the range $15<\alpha<100$ were studied at different wavelengths (λ = 476, 488, 632 nm). The normalized S_{12}, S_{33} and S_{34} elements were measured in the FS, SS and BS directions and compared with calculated elements for various beamwaist radii. A typical example is shown in figure 2.

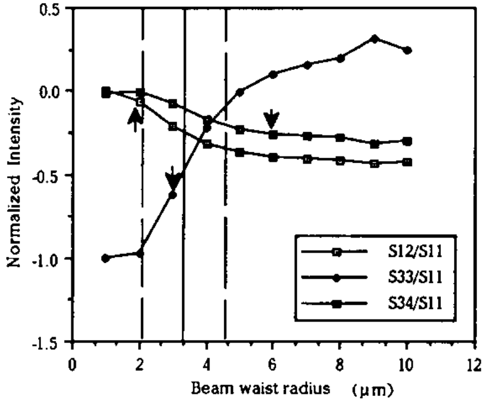

Figure 2: Numerical simulation of the influence of beamwaist radius on the normalized scattering elements in the BS direction for $\alpha = 43.36$. The arrows indicate the measured values obtained with our flow-through equipment. The solid vertical line indicates the mean measured beam waist whereas the broken vertical lines are determined by the corresponding standarddeviation.

DISCUSSION

To investigate the possibility of application of a flow-through system to measure the 6 relevant scattering matrix elements of micron sized particles (S_{11}, S_{12}, S_{22}, S_{33}, S_{34}, S_{44}), a test system with exactly known scattering characteristics must be studied.

We investigated the influence of the sharply focussed laser beam on the calculated scattering profile by means of numerical simulations. An example of this simulation was depicted in figure 2. Insertion of the measured values showed that the corresponding beam waist radii are grouped around 3.5 μm .The estimated beam waist radius and the numerically calculated beam waist radius are approximately equal. We therefore conclude that after implementation of the beam waist radius the measured terms of the scattering matrix correspond *quantitatively* with the calculated values.

CONCLUSIONS

In this study we demonstrate that quantitative measurement of the scattering matrix elements of arbitrarily shaped particles can be performed in flow-through equipment. A reduced scattering matrix for arbitrarily shaped particles in a flow through system was derived from symmetry conditions. It was shown that theoretical simulation of this type of experiment requires implementation of the beamshape into the Mie scattering functions. In addition large cone integration must be applied to account for the relative large detector surfaces.

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