Tissue scattering parameter estimation through scattering phase function measurements by goniometer

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An automated optical system is built up to perform goniometric measurement of scattering phase function. Measurements of typical samples including monodisperse polystyrene micro-spheres solution, and mutlidisperse polystyrene micro-spheres solution are carried out in a dark room. The possibility of estimating the average particle size of phantom through analyzing its scattering phase function is demonstrated.

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Light scattering in biological tissue results from the interaction of light with intracellular organelles, extracellular structures and their surrounding media. The scattering spectra of bulk tissues like brain tissue and breast tissue have not been extensively investigated; yet we can know some information about such tissues by getting the scattering spectral features. Mie theory[1−3] is an easy and practical approach to predict the scattering pattern well. By comparing the scattering spectra of different solutions, it is possible to extract some crude information about the average size of scattering centers.

Angular scattering measurement[2,4] is another way to obtain the particle size information. The scattering phase function specifies the angular distribution of the scattered light, and is defined as the differential scattering cross section divided by the total scattering cross section. In Mie theory, the phase function for a single-sized solution is expressed as

$$P(\theta) = \frac{1}{C_{\mathrm{sca}}(m,a,\lambda)} \frac{\mathrm{d}C_{\mathrm{sca}}(m,a,\lambda)}{\mathrm{d}\Omega}, \qquad (1)$$

where λ is the wavelength, a is the radius of the particle, m is the refractive index ratio ($m = n_2/n_1$, where n_1 and n_2 are the refractive indices outside and inside the particle, respectively), $C_{\mathrm{sca}}(m,a,\lambda)$ is the total scattering cross section. The phase function for a multi-sized medium is expressed as

$$p(\theta) = \frac{\sum\limits_i f(a_i)\mu_{\mathrm{s}}(a_i)p_i(\theta)}{\sum\limits_i f(a_i)\mu_{\mathrm{s}}(a_i)}, \qquad (2)$$

where $\mu_{\mathrm{s}}(a_i)$ and $p_i(\theta)$ are the scattering coefficient and scattering phase function for a sphere with radius a_i, $f(a_i)$ is a normalized size distribution function.

From Eq. (2) we can see that there are two parameters to determine the phase function: the shape of the size distribution function, and the average particle size. Taking the assumption about the shape of the size distribution function, only the average particle size is left for estimation. Once we get the angularly resolved scattering data from the experiment, we can get the average particle size information by comparing it with the Mie theory model using least-square minimization method.

An automatic goniometer system is developed which measures the scattering phase function of an optically thin tissue section or dilute cell suspension. The experimental setup is schematically depicted in Fig. 1. The corresponding photograph of the goniometer system is shown in Fig. 2. A 3-mW green laser at 532 nm is adopted as the light source of the system. The output beam from the laser passes through a neutral density (ND) filter before input into a beam splitter (BS), where the input beam is split into reference beam and detection beam. The detection beam passes a spatial filter consisting of two objective lenses and a pinhole. The beam with a diameter of 3 mm after the spatial filter is then redirected to the sample by a mirror. An aperture (not shown in Fig. 1) is placed just before the sample for cleaning up

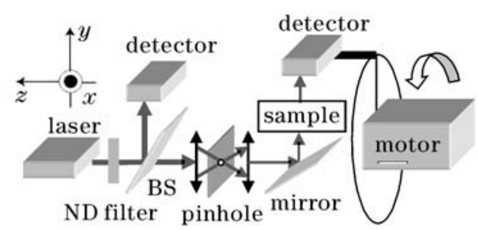

Fig. 1. Schematic of the goniometer used for the measurement of scattering phase function.

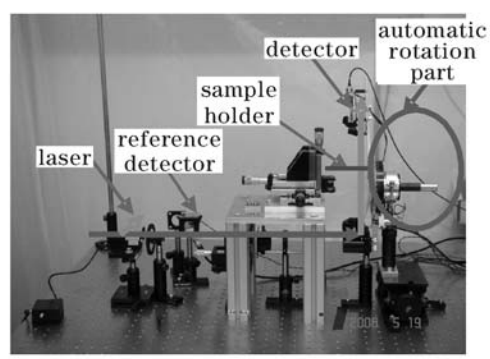

Fig. 2. Photograph of the goniometer system.

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the beam and setting the size of the beam. A detector connected to an automatic rotation unit detects the angular selective scattering light from the sample. Varying the detection angle θ up to $\pm 160°$ at an increment of $1.8°$ relative to the forward direction of the input laser beam upon the sample is achieved by driving the automatic rotation unit. An angular resolution of $0.5°$ is estimated from system parameters including the dimension of the detector, the distance from the detector to the sample, and the angular resolution of the automatic rotation unit[5]. When needed, the output laser is linearly polarized perpendicular or parallel to the surface of the rotation stage.

The reference beam goes to a reference detector after another ND filter (not shown in Fig. 1) for saturation consideration. Because the measuring time of the system usually takes up to 10 to 20 minutes, during which the intensity fluctuation is unavoidable for the green laser source that is implemented in the system, the reference detector is thus configured in the system in order to eliminate the influence of intensity fluctuation of laser source on the measurement accuracy. All the detected signals corresponding to different angular scattering positions are obtained and processed by a special designed divider to get a stabilized scattering phase function with high accuracy.

As shown in Fig. 3, the automatic rotation unit consists of a large gear, a rheostat (wire wound type), and a motor mounted together with a small gear. When the motor is driven through a direct current (DC) input controlled by a computer, the smaller gear forces the larger one to rotate in the opposite direction. Since the detector is connected to the large gear, it begins to rotate as well. At the same time, the large gear drives the rheostat to work, that means, when the large gear turns around, output resistance of the rheostat varies accordingly. Setting the half resistance corresponding to the position at zero degree, and applying 10 V to the whole resistance of rheostat, the detector angular position can be determined from the output resistance voltage by

$$\theta = (V - 5) \times \pi / 5 \text{ (rad)}. \quad (3)$$

Before the measurement, the suspension should be dilute enough to satisfy the condition which ensures only single scattering[6]. To remove artifacts caused by stray reflections, background measurement of distilled water in the tank is taken. This background so measured should be subtracted from the signals from solution measurement.

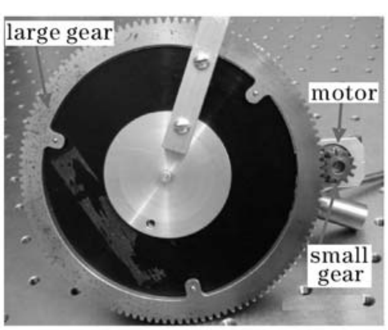

Fig. 3. Automatic rotation unit for angular scattering detection.

We studied two phantoms in experiment they are suspensions of monodispersed polystyrene micro-spheres with $m = 1.2$ and size a of 35 and 100 nm[1].

All the solutions of polystyrene micro-spheres were measured at the angle from $10°$ to $160°$, and the measurements took for about 20 minutes. The data gotten at very oblique angles near $90°$ were unreliable since the

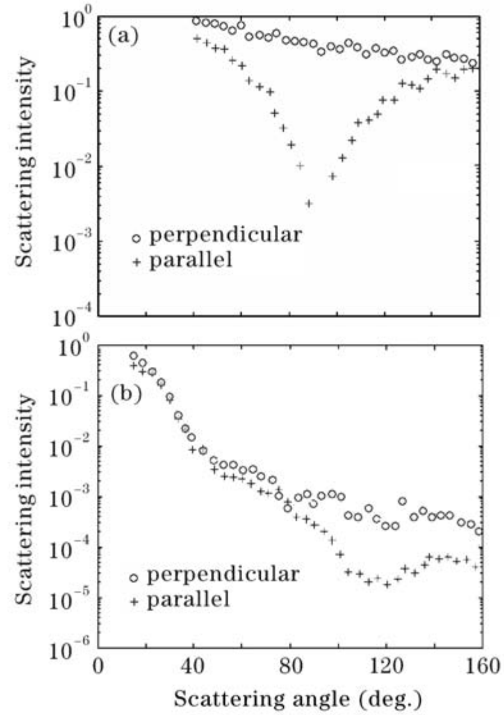

Fig. 4. Angular scattering measurement results for polystyrene micro-spheres with size (a) $a = 35$ nm and (b) $a = 100$ nm for different polarizations.

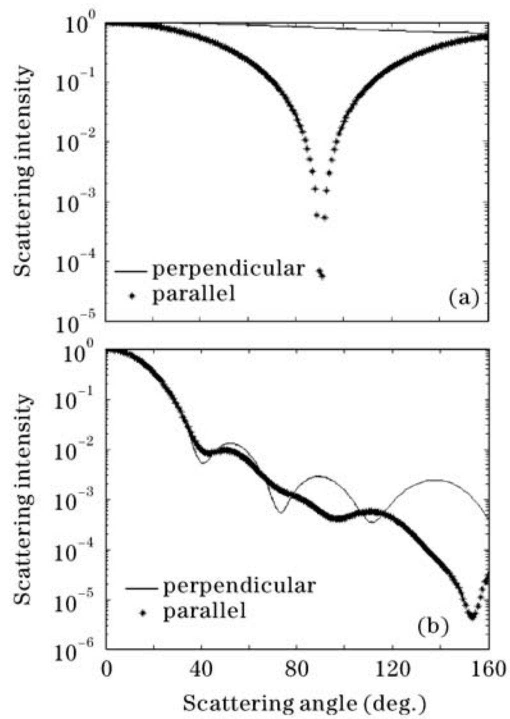

Fig. 5. Phase functions calculated from Mie theory from scattering media with single size at (a) $a = 35$ nm and (b) $a = 100$ nm for different polarizations.

effect of glass plate, so we interpolated these data. The angular scattering results gotten from our goniometer system are shown in Fig. 4. Figure 5 shows the corresponding simulation results by Mie theory.

As can be seen from Figs. 4 and 5, a good match is evident between phase function curves obtained by experiment and theoretical simulation. From these results, we can conclude that under the same conditions, as the particle size becomes larger there are more oscillatory components in the phase function, and more light is scattered in the forward direction. And for different media, the relationships between the phase function from the two polarizations are different.

In summary, we have developed an automatic goniometric apparatus for the measurement of scattering phase function. And from the scattering phase function, we can deduce some information about the particle size of tissue or phantoms. So, when defining the particle size distribution from beginning, we can solve the mean particle size by measuring the angular scattering pattern. This is useful when applied to get the scatterer size information of some tissues that are not well investigated.

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