Determination of complex refractive index of polystyrene microspheres from 370 to 1610 nm

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Abstract

We introduce an inverse method for determining simultaneously the real and imaginary refractive indices of microspheres based on integrating sphere measurements of diffuse reflectance and transmittance, and Monte Carlo modelling in conjunction with the Mie theory. The results for polystyrene microspheres suspended in water are presented.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Most soft human tissues are classified as highly turbid media according to their responses to optical radiation in the visible and near-infrared regions. Development of appropriate phantoms is critical to the study of biomedical optics and calibration of optical instrumentation in clinics. Aqueous suspensions of polystyrene microspheres are widely used as phantoms for the studies of tissue and cell optics and benchmarks for calibration of instruments such as flow cytometers (Peters *et al* 1990, Hull *et al* 1998, Shvalov, *et al* 1999, Du *et al* 2001). In addition, polystyrene microspheres are also employed as carriers of different biomedical agents such as antibody markers for a wide range of biomedical research (Kettman *et al* 1998). The attractiveness of polystyrene microspheres as the calibration standard lies in the fact that the scattering and absorption cross sections of the spheres can be accurately calculated with the Mie theory (Bohren and Huffman 1983). However, this is true only if the complex refractive index of polystyrene microspheres is accurately known at the wavelengths of interest. The real refractive index of polystyrene was measured at discrete wavelengths ranging from 442 to 1060 nm using the bulk materials of polystyrene based on a minimum-deviation method (Matheson and Saunderson 1952, Nikolov and Ivanov 2000), however, the imaginary refractive index has not been determined. In this paper, we describe an inverse method for determining the complex refractive index n of polystyrene in the form of microspheres and report the results of n from 370 to 1610 nm.
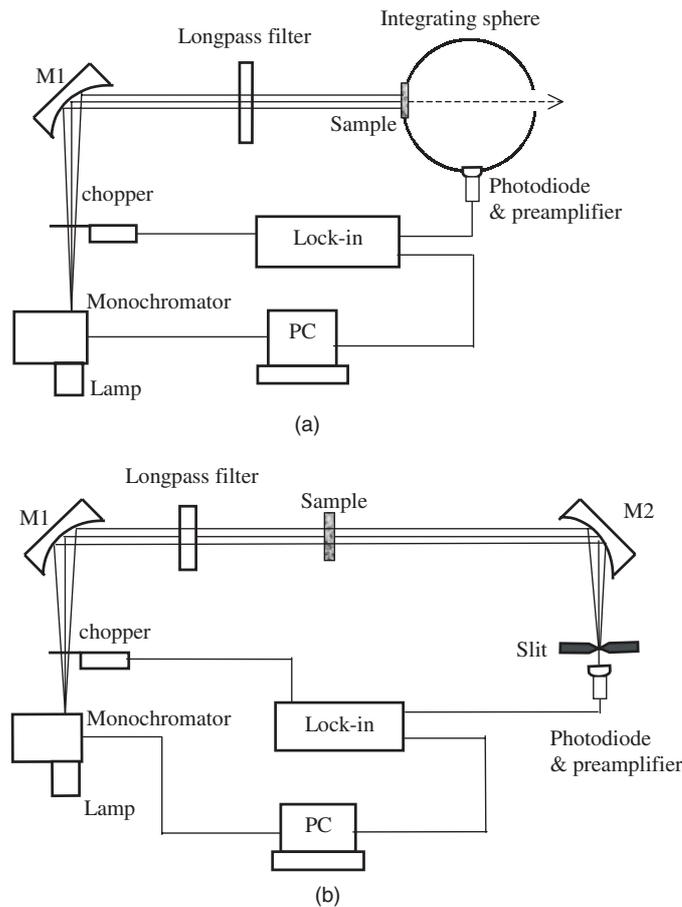
Figure 1. Schematics for measurements of (a) diffuse transmittance T_d and (b) collimated transmittance T_c with concave mirrors M1 and M2.

2. Experimental method

A reflective system has been constructed to measure the diffuse reflectance R_d and the diffuse transmittance T_d of a turbid sample with an integrating sphere of 6 inch diameter (IS-060-SF, Labsphere Inc.) and the collimated transmittance T_c with a spatial filtering setup, as shown in figure 1. This system was modified from a previous one with refractive optics (Du *et al* 2001) by replacing lenses with spherical mirrors to eliminate chromatic variation in the system over a wide spectral region. A 1/8 m monochromator of 2 nm resolution (CM110, CVI Laser) with a 30 W tungsten lamp was used as a tunable light source to produce a beam with wavelength λ varying from 370 to 1610 nm. Two sets of photodiode and low-noise, high-gain preamplifier were constructed and used in two separate regions to detect light signals with a lock-in amplifier: a Si photodiode for 370 nm $\leqslant \lambda \leqslant$ 950 nm and a GaAs photodiode for 920 nm $\leqslant \lambda \leqslant$ 1610 nm. In the latter region, a long-pass filter with the cut-off edge at 840 nm was used to remove second-order diffraction from the monochromator.

Polystyrene microspheres of diameter $d = 0.966$ μm were purchased as a suspension in deionized water with a nominal concentration of 10% by weight (5095B, Duke Scientific

Corporation). By diluting with deionized water, we prepared three different microsphere suspensions with nominal number densities of 1.108×10^6 mm^{-3}, 2.056×10^6 mm^{-3} and 5.054×10^6 mm^{-3} based on the nominal concentration of the original suspension from the manufacturer. Since the suspension concentration, c, is a critical factor in determining accurate values of absorption and scattering cross sections of a single microsphere, the value of c has been measured. A small portion of each prepared microsphere suspension was weighed by an electronic balance of resolution 0.1 mg. Then the water in the suspension was evaporated completely at a temperature of 60 °C to obtain dry microspheres with the mass re-measured. The number densities of the three polystyrene suspensions were calculated from the masses of the microspheres and suspensions, the diameter d and mass density of polystyrene $\rho = 1.05$ g cm^{-3}. These were found to be $c = 1.569 \times 10^6$ mm^{-3}, 2.709×10^6 mm^{-3} and 6.186×10^6 mm^{-3}, respectively. These verified values of c were used in the determination of refractive index described below. The increase of the concentration from nominal values to the measured ones was attributed to water evaporation from the container of the original suspension after a long shelf time of about two years.

A suspension sample of polystyrene microspheres was contained between two sapphire windows of 0.5 mm thickness within a spacer of thickness D. The suspension samples were examined under an optical microscope to make sure that the microspheres were not clustered together. Every sample was rigorously shaken before the start of measurement each day to ensure the homogeneity of the suspension. Integrating sphere measurements of R_d and T_d were carried out at wavelengths λ between 370 nm and 530 nm with a step size of 10 nm and between 530 and 1610 nm with a step size of 30 nm for three microsphere samples of different concentrations and fixed thickness at $D = 0.246$ mm. The mean values of the real and imaginary refractive indices of polystyrene microspheres have been determined for each sample of the three concentrations using an inverse method to be discussed below. To validate the integrating sphere measurements and the Monte Carlo based inverse calculations, we determined the attenuation coefficient μ_t from the T_c data measured using the sample with $c = 6.186 \times 10^6$ mm^{-3} (Du *et al* 2001). Three different values of $D = 0.112$ mm, 0.246 mm, 0.463 mm were used to obtain the dependence of $\ln(T_c)$ on D at the same sequence of wavelength steps. The results exhibited an excellent linear relation and μ_t were obtained from the slope at each wavelength

$$\mu_t = -\frac{\Delta(\ln T_c)}{\Delta D}. \tag{1}$$

The experimental errors were estimated to be $\pm 5\%$ for R_d and T_d and $\pm 3\%$ for T_c. All measurements of R_d, T_d and T_c were conducted at a room temperature of about 24 °C.

3. Modelling method

The approach of determining inversely the complex refractive index, $n = n_r + in_i$, of polystyrene microspheres from the measured values of R_d and T_d was similar to the previous one used to determine optical parameters from tissue samples (Peters *et al* 1990, Du *et al* 2001). The iteration process was started by calculating the diffuse reflectance and transmittance, $(R_d)_{cal}$ and $(T_d)_{cal}$, using a Monte Carlo code that has been validated extensively (Song *et al* 1999, Du *et al* 2001). With this Monte Carlo code we can accurately track the incident photons and obtain $(R_d)_{cal}$ and $(T_d)_{cal}$ according to the optical parameters of the suspension sample and the geometry of the sample, sapphire windows and integrating sphere. A least-squares method

was used to guide the iteration process with an error function Σ defined as

$$\Sigma = \left(\frac{(R_d)_{cal} - R_d}{R_d} \right)^2 + \left(\frac{(T_d)_{cal} - T_d}{T_d} \right)^2. \tag{2}$$

The iteration process for inverse determination of n stopped when $\Sigma < \Sigma_c$ was satisfied. We employed a value of 4×10^{-4} for Σ_c which corresponds to a relative error of about 1.4% for $(R_d)_{cal}$ and $(T_d)_{cal}$.

The optical response of the sample is characterized by three parameters based on the framework of the radiative transfer theory: the absorption coefficient μ_a, the scattering coefficient μ_s and the scattering phase function $p(\theta)$ where θ is the scattering angle. The attenuation coefficient is defined as $\mu_t = \mu_a + \mu_s$. We assumed that the coefficients of μ_a and μ_s for a suspension sample are related to the total absorption and scattering cross sections, σ_a and σ_s, of a microsphere by the following equation:

$$\mu_a = c\sigma_a + \mu_{aw} \qquad \text{and} \qquad \mu_s = c\sigma_s \tag{3}$$

where $\mu_{aw} = 4\pi n_{iw}/\lambda$ is the absorption coefficient of the water at the wavelength λ, $n_w = n_{rw} + in_{iw}$ is the complex refractive index of water. The above assumption is based on the dominance of the single scattering regime in light transportation through suspension samples of small microsphere concentration. In addition, a phase function calculated from the Mie theory for an unpolarized light beam (Bohren and Huffman 1983) and transparent immersion medium was employed to accurately model the distribution of the scattered light for $370 \text{ nm} \leqslant \lambda \leqslant 950 \text{ nm}$. This was used instead of the Henyey–Greenstein (HG) function employed previously for tissue and other turbid samples (Toublanc 1996, Du *et al* 2001). Using the diameter d of the microsphere and published value of n_w of water (Hale and Querry 1973), we calculated the cross sections and the phase function of a polystyrene microsphere from its assumed values of the complex refractive index. These results in turn provided, from equation (3), the optical parameters of the microsphere suspension sample to be used in the Monte Carlo simulations for obtaining $(R_d)_{cal}$ and $(T_d)_{cal}$. Ten to twenty Monte Carlo simulations were typically used through iteration to inversely determine n_r and n_i. We have verified that n_r and n_i are uniquely determined using Σ as a metric.

For a sphere immersed in an absorbing immersion medium, which is the case for water near $\lambda = 1450 \text{ nm}$, the definition of the scattering cross section σ_s requires careful consideration. Different types of σ_s have been employed and discussed in previous studies (Chylek 1977, Fu and Sun 2001, Yang *et al* 2002). An inherent scattering cross section can be defined in the near-field, i.e., at the sphere's surface, of the scattered light while an apparent scattering cross section can be derived from the asymptotic form of the scattered light fields in the far-field or the radiation zone where light measurements are carried out. Yang *et al* have shown that only the apparent cross section should be used to calculate the optical parameters, as shown in equation (3), because the absorption due to the immersion medium not only attenuates the scattered waves in magnitude but also modulates the wave modes when the waves reach the radiation zone (Yang *et al* 2002). A version of the Mie code for calculating the apparent scattering cross section had been developed (Yang *et al* 2002) and was used in our study to generate μ_s, μ_a and $p(\theta)$ for data in the region of $920 \text{ nm} \leqslant \lambda \leqslant 1610 \text{ nm}$. The Mie code for a transparent immersion medium (Bohren and Huffman 1983) was used for the Monte Carlo calculations in the region of $370 \text{ nm} \leqslant \lambda \leqslant 950 \text{ nm}$ in which the two Mie codes provided nearly identical results on inversely determined n_r and n_i.

The Monte Carlo simulation requires that the distribution of randomly selected values of scattering angle θ be governed by the phase function $p(\theta)$. For a numerically obtained Mie phase function, it could be very time consuming to obtain an appropriate distribution of

the angle θ directly from $p(\theta)$ in comparison with the HG function. We adopted a sampling technique (Toublanc 1996) in which a table of Mie phase function values p_i against θ was constructed and normalized by

$$\sum_{i=1}^{N} p_i = 1 \tag{4}$$

where N is the total number of angles equally divided between $0°$ and $180°$. The scattering angle θ was then determined by finding an integer m to satisfy

$$\sum_{i=1}^{m-1} p_i < \text{RND} \leqslant \sum_{i=1}^{m} p_i \tag{5}$$

where RND is a random number uniformly distributed between 0 and 1 and the left sum is set to zero if $m = 1$. We found that $N = 5000$ was sufficiently large to sample the scattering angle θ with an accuracy of $0.036°$ without significantly slowing down the Monte Carlo code. All simulations were carried out on our parallel computing cluster consisting of 32 PCs with celeron CPU of 500 MHz. Each Monte Carlo simulation tracked 3×10^5 photons with statistical fluctuations negligible to the experimental errors of R_d and T_d and took about 5 min to complete on one PC.

4. Results and discussion

The uniqueness of the determined complex refractive index is critical in validating the use of the error function Σ as the metric for guiding iteration. We have studied the dependence of Σ on n_r and n_i at two wavelengths of 950 and 1430 nm with stepsizes of 1×10^{-4} in n_r and 1×10^{-4} in n_i for $\lambda = 950$ nm and 1×10^3 for $\lambda = 1430$ nm, as shown in figure 2 for one sample. These results established that a unique absolute minimum of Σ exists in the ranges of n_r and n_i investigated at both wavelengths of weak and strong absorption. The well-behaved error function Σ demonstrated that the employed inverse algorithm leads to a unique solution of complex refractive index of polystyrene microspheres from the measured data of R_d and T_d. The errors in n_r using the criterion of $\Sigma < 4 \times 10^{-4}$ were estimated to be about 0.002 or less.

The real and imaginary refractive indices of the polystyrene microspheres are presented in figure 3 as functions of the wavelength. The mean values were obtained by averaging over data determined from the three samples of different concentrations with the error bars indicating the standard deviations. For comparison, we included previously reported values of n_r of polystyrene (Matheson and Saunderson 1952, Nikolov and Ivanov 2000) in figure 3(a). For the real refractive index shown in figure 3(a), we observe differences between our data of the microspheres and the early results from bulk samples in the visible region, which increases as the wavelength decreases. It has been reported that the magnitude of strain-induced birefringence in elongated polystyrene films increases as the wavelength decreases from 800 to 400 nm (Inoue *et al* 1998). On the basis of these facts we speculate that the difference in n_r is due to the process related residue strain within the microspheres through the photoelastic effect. The wavelength dependence of n_r has been fitted to the Cauchy dispersion relation (Matheson and Saunderson 1952, Nikolov and Ivanov 2000)

$$n(\lambda) = A + \frac{B}{\lambda^2} + \frac{C}{\lambda^4} \tag{6}$$

for the transparent region of λ ranging from 390 to 1310 nm, as shown by the solid line in figure 3(a). With λ in units micrometres, we found $A = 1.5725$, $B = 0.003\,1080$, $C = 0.000\,347\,79$ based on a least-squares fitting.
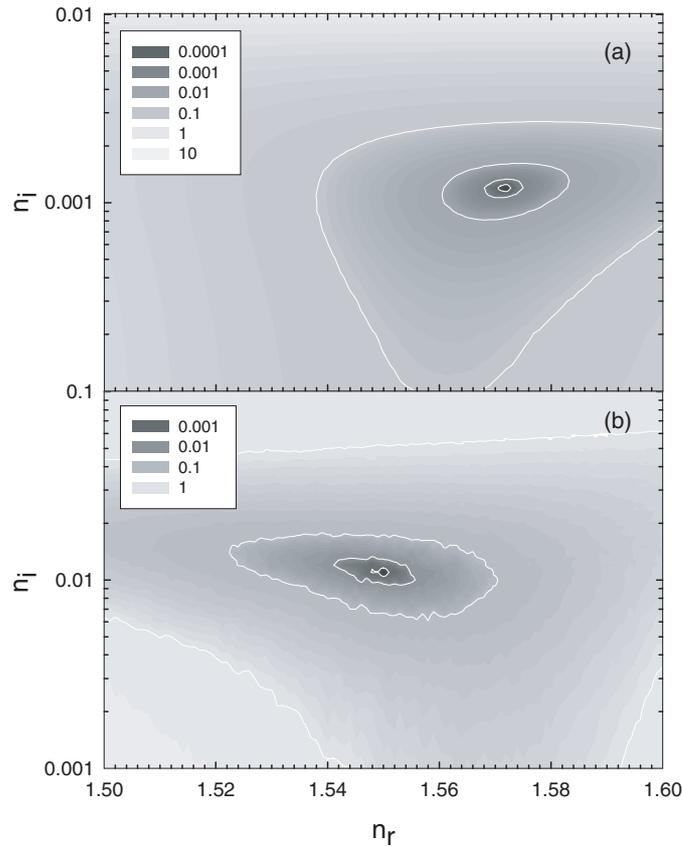
Figure 2. The contour plot of the error function Σ in the plane of n_r and n_i of the polystyrene microspheres in a suspension with $c = 6.186 \times 10^6$ mm^{-3}: (a) $\lambda = 950$ nm; (b) $\lambda = 1430$ nm.

An absorption peak can be seen near 1400 nm in figure 3(b) through the wavelength dependence of the imaginary refractive index and is the cause of the anomalous dispersion in the real refractive index. The increased fluctuation, represented by the large error bars, in the real refractive index in the region near 1400 nm is attributed to the larger errors in R_d and T_d due to the increased absorption of water near 1450 nm (Hale and Querry 1973) and reduced sensitivity of the GaAs photodiode near 1610 nm. We have also found that the absorption of water has a significant effect on the inverse determination of the microsphere index for the near-infrared region and its effect has to be taken into account by using the Mie code for an absorbing medium. For example, the relative difference between the inversely determined indices of microspheres is increased from $\Delta_r = \left| \frac{n_r' - n_r}{n_r} \right| = 0.13\%$ and $\Delta_i = \left| \frac{n_i' - n_i}{n_i} \right| = 37\%$ at $\lambda = 950$ nm to $\Delta_r = 3.0\%$ and $\Delta_i = 90\%$ at $\lambda = 1400$ nm with $n' = n_r' + i n_i'$ as the refractive index determined with the Mie code without considering water absorption.

To examine the consistency of the inverse calculations, we compared the attenuation coefficients $\mu_t = \mu_a + \mu_s$ determined from the measurements of T_c, based on equation (1), and from the refractive indices of microsphere and water, based on equation (3), for one sample with the microsphere concentration of $c = 6.186 \times 10^6$ mm^{-3}. The results are shown in figure 4 which demonstrate the excellent agreement between the two methods of determining μ_t. The signal-to-noise ratio of the T_c data became less than 10 for wavelengths between 370 and
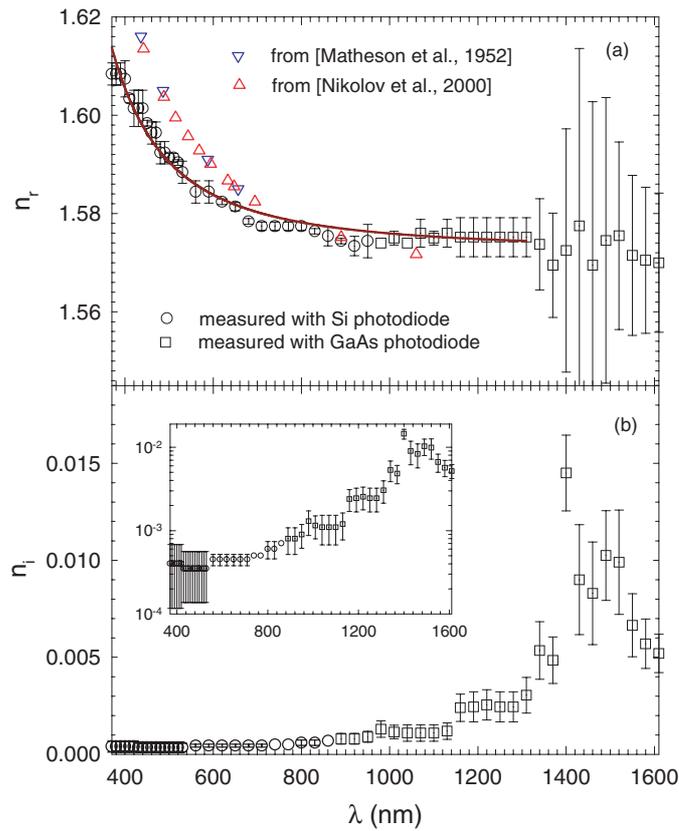
Figure 3. Inversely determined refractive indices of the polystyrene microspheres as functions of wavelength: (a) n_r with solid line as the fitting curve based on the Cauchy dispersion formula; (b) n_i, inset: n_i on a log-scale.
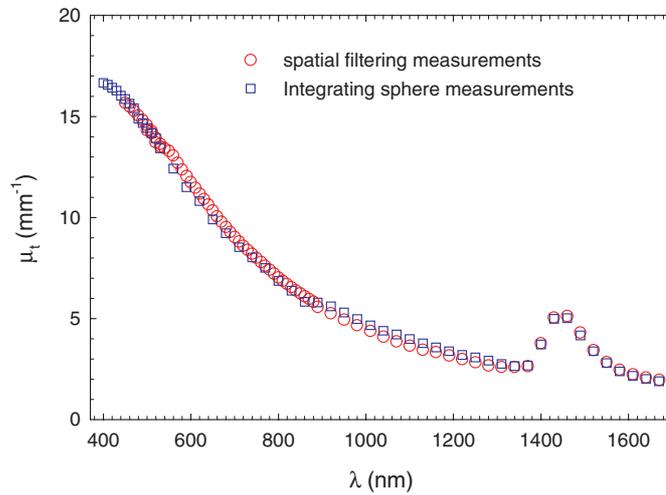
Figure 4. Comparison of the attenuation coefficients determined by T_c from the spatial filtering measurements and by R_d and T_d from the integrating sphere measurements for a suspension sample with concentration $c = 6.186 \times 10^6 \text{ mm}^{-3}$.

450 nm because of the reduced light intensity and sensitivity of the Si photodiode and thus no μ_t data were derived from T_c.

In summary we have introduced an inverse method for determining the complex refractive index of microspheres suspended in an immersion liquid of water based on integrating sphere measurements and Mie-based Monte Carlo modelling. This method provides a means to measure the complex refractive index of materials in spherical shapes in addition to the conventional methods of refraction-based minimum-deviation and total-reflection-based refractometry. Furthermore, this method using the integrating sphere has the advantage of large signal-to-noise ratios that is important in determining the complex refractive index quasi-continuously over a wide spectral region.

Acknowledgments

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