

Multiparameter Spectrophotometry Platform for Turbid Sample Measurement by Robust Solutions of Radiative Transfer Problems

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Abstract—Multiparameter spectrophotometer instruments based on the radiative transfer (RT) theory differ fundamentally from conventional ones in their ability to determine absorption and scattering coefficients and anisotropy factor. Ease of use and robustness of inverse solutions are of necessity for general-purpose applications. We have developed a new instrument platform design incorporating a compact three-photodiode detection configuration without integrating spheres, four-channel signal-processing unit, and Monte Carlo (MC) simulation-based software for accurate and rapid inverse solutions. To verify the platform design, two sphere suspension samples of different size distributions were measured and RT parameters were determined by a sample model with the Henyey and Greenstein (HG) phase function between 460 and 1000 nm. System validation is achieved by comparison of the RT parameters and spectral dependence against the results by Mie theory for single-sized spheres of nominal diameter values. Five wavelengths from 560 to 960 nm were selected for quantitative evaluation of the robustness of the HG sample model and two Mie theory-based sample models assuming light scattering by independent and single spheres. We found that only the HG-based sample model yields high accuracy and strong robustness for inverse determination of RT parameters even for the cases of sphere suspensions in which the assumptions of sample uniformity and scattering by independent and single spheres are not satisfied. These results demonstrate that the new platform with MC simulation-based

inverse algorithm combined with the HG sample model is capable of robust and rapid measurement of RT parameters for turbid samples with optical thickness up to 30.

Index Terms—Light scattering, radiative transfer (RT) theory, spectral measurement, spectrophotometry instrument, turbidity characterization.

I. INTRODUCTION

SPECTROPHOTOMETRY and infrared spectroscopy often serve as the tools of choice for material analysis by their ease of use to acquire spectral data. The data can be presented in the form of either sample absorbance $A(\lambda) = \log(1/T_c(\lambda))$ or attenuation coefficient $\mu_t(\lambda) = 2.30A(\lambda)/D$, where λ is wavelength, T_c the measured signal of collimated transmittance, and D the sample thickness along the direction of incident light beam. Conventional instrument designs for determination of $A(\lambda)$ or $\mu_t(\lambda)$ are founded on the assumption that light attenuation is dominated by atomic and/or molecular absorption [1]–[3]. The assumption, however, breaks down if light scattering is no longer negligible. One must then consider the effect of scattering on measured signals and solve the challenging problems of forward modeling of light scattering and inverse parameter extraction. In return, multiple sample parameters with wavelength dependence enhance markedly the ability to characterize materials by quantification of molecular composition through absorption parameter and wavelength-scaled structures through scattering parameters. Over the past decades, numerous light scattering models of different approximations have been developed and combined with efficient inverse algorithms to retrieve sample parameters from measured signals [4]–[6]. Various spectrophotometric system designs incorporated these methods to determine absorption and scattering parameters in academic laboratory settings. Nearly all designs require integrating spheres for hemispherically integrated measurement of signals and fast parameter extraction from the signals [2], [7]–[13].

We have recently reported a new method for inverse determination of absorption coefficient μ_a , scattering coefficient μ_s , and anisotropy factor g of a turbid sample based on the widely accepted radiative transfer (RT) theory [14]. A three-photodiode configuration was designed to measure diffuse reflectance R_d , diffuse transmittance T_d , and forward transmittance T_f . Without detection of T_c and integrating

Manuscript received June 19, 2020; revised August 28, 2020; accepted October 9, 2020. Date of publication October 19, 2020; date of current version December 31, 2020. This work was supported in part by the Hunan Provincial Natural Science Foundation of China under Grant 2019JJ40110 and Grant 2020JJ4331, in part by the Ministry of Education of China (Science and Technology Development Center) under Grant 2018A02031, and in part by the Scientific Research Fund of Hunan Provincial Education Department under Grant 18B349. The Associate Editor coordinating the review process was Yuhua Cheng. (Corresponding author: Xin-Hua Hu.)

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Digital Object Identifier 10.1109/TIM.2020.3032184

spheres for detection of R_d and T_d , the new design allows measurement of optically thick samples with significantly reduced burdens of sample preparation and system maintenance. Furthermore, a Monte Carlo (MC) method has been applied to ensure accuracy for calculation of signals arising from light radiance $L(\mathbf{r}, \mathbf{s})$ within the sample, which can be understood by the following steady-state RT equation [4]:

$$\mathbf{s} \cdot \nabla L(\mathbf{r}, \mathbf{s}) = -(\mu_a + \mu_s)L(\mathbf{r}, \mathbf{s}) + \mu_s \int_{4\pi} p(\mathbf{s}, \mathbf{s}')L(\mathbf{r}, \mathbf{s}')d\omega' \quad (1)$$

where \mathbf{r} is a vector of location inside the turbid sample, \mathbf{s} and \mathbf{s}' are unit vectors for incident and scattered directions, and $p(\mathbf{s}, \mathbf{s}')$ is the single-scattering phase function. An anisotropy factor g can be defined as the mean value of $\cos\theta = \mathbf{s} \cdot \mathbf{s}'$ or the first moment of $p(\mathbf{s}, \mathbf{s}')$ for quantifying angular distribution of light scattering. Past studies have established that directional distribution of scattered light in term of g presents significant information to distinguish microstructures of materials and tissues for diagnostic purposes [15]–[17]. RT parameters of μ_a , μ_s , and g can be solved inversely as functions of λ by comparing iteratively calculated signals, based on (1) and valid boundary conditions, to the measured ones until they become sufficiently close.

Different from μ_a and μ_s as scalar parameters, the phase function $p(\mathbf{s}, \mathbf{s}')$ distributes on unit sphere embedded in \mathbb{R}^3 and plays a central role in modeling light scattering. Even by assuming axially symmetric scattering to simplify $p(\mathbf{s}, \mathbf{s}')$ into $p(\cos\theta)$, its determination for an unknown sample requires signal measurement at large number of angles. Retrieval of the phase function is obviously impractical for spectroscopic instrumentation. It is thus crucial to identify and validate an effective function for $p(\cos\theta)$ that allows rapid and robust inverse calculations of RT parameters with only g retrieved for quantifying scattering anisotropy. Various parameterized and light scattering model based functions have been investigated for use as $p(\cos\theta)$. These include Mie theory-based ones for sphere suspensions, Rayleigh phase function for particles of small size parameters, and various combined parameterized functions [18]–[23]. Among these choices, the function $p_{HG}(\cos\theta)$ proposed by Henyey and Greenstein (HG) stands out as the most commonly used one [24]. The advantages of $p_{HG}(\cos\theta)$ include insensitivity to detailed angular dependence of scattered signals due to a smooth angular dependence, high computing efficiency in numerical simulations by MC methods, and excellent speed for inverse calculations because only g is needed for $p_{HG}(\cos\theta)$. It should be noted that the smooth angular dependence of $p_{HG}(\cos\theta)$ is of little concern since very few turbid materials are of particulate structures with homogeneous and highly symmetric-shaped particles. Many materials have elementary structures like biological cells or other particles in different shapes, structures, and orientations so that their phase functions exhibit smooth angular dependence due to ensemble averaging of different elements [25]–[28]. The major limitation for use of $p_{HG}(\cos\theta)$ as $p(\cos\theta)$ lies in its monotonic angular dependence for a specific value of g that may differ from angular dependence of certain turbid sample types like biological cells. For example, the HG functions with

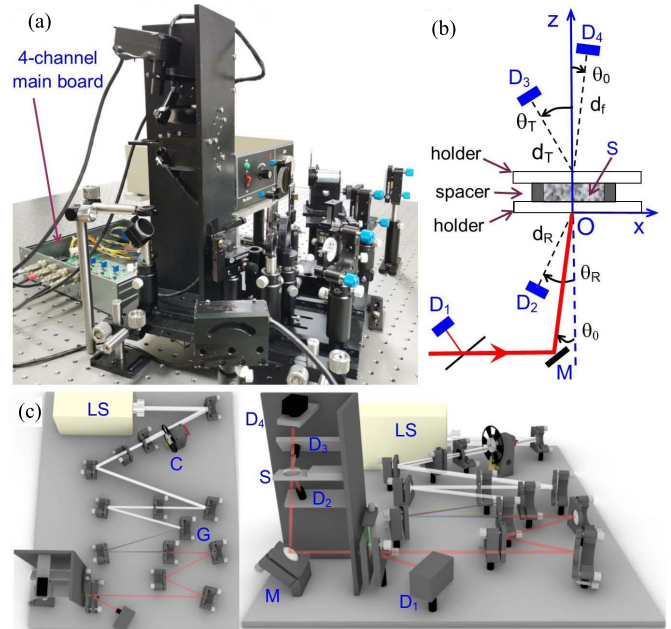


Fig. 1. (a) Photograph of system hardware. (b) Assemblies of sample-holder and three-photodiode detectors: M = mirror, S = sample, θ_0 = incident angle, θ_R and d_R (θ_T and d_T) = angle and distance of D_2 (D_3) for measuring R_d (T_d), d_f = distance of D_4 for measuring T_f . (c) Top and side views of system 3-D rendering: LS = light source, C = chopper, and G = grating.

$g > 0$ leads to underestimation of diffusely reflected light intensity for $\theta > 90^\circ$ and particularly for θ close to 180° [29]. Quantitative evaluation of the HG function and related sample model on solving inverse RT problems, however, remains to be investigated, which is especially important for developing robust multiparameter spectrophotometric instruments.

In this article, we present results of a study to develop a prototype system, validate and evaluate a new instrument platform design for multiparameter spectrophotometric measurement based on the RT theory. The prototype employs the three-photodiode detection configuration without integrating spheres and MC simulation-based inverse algorithm for determination of RT parameters. Two sphere suspension samples were prepared for this study and a sample model with the HG function for $p(\cos\theta)$ was applied for retrieving the RT parameters between 460 and 1000 nm. The system was validated by comparing the above results to those obtained by the Mie theory for single-sized spheres of nominal diameter values. Furthermore, signals measured at five wavelengths were selected to evaluate the robustness of the HG samples models against two other sample models with Mie theory-based phase functions for spheres of different size distributions. By calculating the distribution of an objective function in the parameters space of each sample model, we demonstrate that the HG model exhibits strong robustness for all cases of samples and wavelengths.

II. HARDWARE DESIGN AND SAMPLE PREPARATION

A. System Hardware Design and Signal Measurement

A prototype system has been constructed for this study. Its hardware has four units of light source, monochromator,

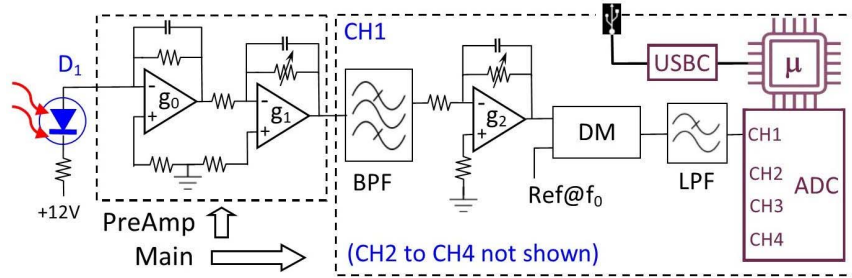


Fig. 2. Schematics of preamplifier and main board of four-channel lock-in circuit for one channel: g_0 , g_1 , g_2 = gains of signal amplifiers; BPF = bandpass filter; Ref@ f_0 = reference signals of frequency f_0 from chopper controller; DM = demodulator; LPF = low-pass filter; ADC = A/D convertor; μ = microprocessor; USBC = USB controller.

sample chamber, and signal processing boards. Fig. 1 shows a system photograph and layout diagrams of signal detection configuration and beam optics. The light source unit includes an 175-W xenon light source (XL1-175-A, WavMed Technologies Corp., Tianjin, China) of wide emission spectrum from 300 to 2500 nm and beam shaping optics. The mirror-based optical layout implements a Kohler illumination design for control of beam size and intensity separately by two diaphragms. A mechanical chopper (SR540, Stanford Research Systems, Sunnyvale, CA, USA) is used to modulate the output beam at a frequency of $f_0 = 385$ Hz before entering into an in-house built monochromator. The Czerny–Turner design-based monochromator supplies a beam of wavelength λ tunable from 460 to 1000 nm and half-maximum bandwidth $\delta\lambda$ of about 4.7 nm by rotating a reflective diffraction grating of 1200 lines/mm (GR1205, Thorlabs, Newton, NJ, USA). The relative large value of $\delta\lambda$ enables an incident beam of adequate intensity with sufficient resolution for our wavelength scanning stepsize $\Delta\lambda$ of 20 nm. The beam is passed through an iris (not shown in Fig. 1) after a turning mirror [M in Fig. 1(b)] to obtain a cylindrical profile on the front surface of assembly with major and minor diameters measured as 10.4 and 4.2 mm, respectively. The half-divergence-angles were found to be 1.3° and 0.21° . A photodiode D_1 is employed to monitor the intensity of incident beam I_0 as shown in Fig. 1(b).

The sample chamber houses two assemblies of sample-holder and three-photodiode detectors of D_2 to D_4 (FDS100, Thorlabs) to acquire, respectively, the scattered light intensity signals of I_{Rd} , I_{Td} , and I_{Tf} . Another photodiode D_1 is used to monitor incident light intensity I_0 and all photodiodes have the same sensor area of 3.6×3.6 (mm²). The photodiode outputs are measured at each scanned wavelength to determine three measured signals of diffused reflectance $R_d = I_{Rd}/I_0$, diffused transmittance $T_d = I_{Td}/I_0$, and forward transmittance $T_f = I_{Tf}/I_0$. These detectors are installed at the end of black plastic shielding tubes so that their field-of-views are limited to the front or back surface of the sample to prevent receiving light scattered from nonblack surfaces inside the chamber. Detector D_4 is placed along the collimated transmission direction at a larger and adjustable distance d_f ranging from 50 to 90 mm so that T_f could be kept on the same scale as R_d and T_d , which improves convergence of inverse calculations by the HG sample model. The solid angles for acquisition of the R_d and T_d signals are kept in a range of 0.01–0.03 sr to yield sufficient angular integration over the detector sensor surface based on

our earlier experimental results [12]. The detector distances of D_2 to D_4 can be adjusted from the sample assembly for different samples to increase signal-to-noise ratio and avoid detector saturation. The same configurations of sample and detector assemblies are implemented in our MC simulations to accurately determine calculated signals as discussed later.

The signal-processing unit includes four preamplifiers placed close to respective photodiodes and a four-channel lock-in main board connected to a host computer. Fig. 2 presents the schematics of the boards. Each current signal from a negatively biased photodiode is converted into a voltage signal followed by amplification of gain g_1 before output from the preamplifier board. The signal is further bandpass filtered, amplified with gain g_2 , demodulated by a balanced modulator (AD630, Analog Devices, Norwood, MA, USA), and low-pass filtered before digitization by a 24-bit A/D convertor (AD7739, Analog Devices) in the main board. The convertor's output is read by a microprocessor (ATMega32U4, Atmel, San Jose, CA, USA), which handles command and data communications with the host computer via an USB cable and controls amplifier gains of g_1 and g_2 by digital potentiometers.

B. Sample Preparation and Characterization

We chose sphere suspensions as samples for the validation of the platform design and evaluation of the performance of three samples models in inverse determination of RT parameters. Two samples were prepared by diluting purchased polystyrene sphere suspensions with deionized water. The nominal diameters and coefficients of variation (CV) are provided by vendors as $d_s = 0.966$ μm and CV = 1.3% (5095B, Duke Scientific, Fremont, CA, USA) for sample A and $d_s = 11$ μm and CV $\leq 18\%$ (7510B, ThermoFisher Scientific, Waltham, MA, USA) for sample B. Sphere concentrations ρ_s were determined by the masses of suspension and dry sphere component and found to be $(5.37 \pm 0.02) \times 10^6$ mm⁻³ for sample A and $(3.05 \pm 0.02) \times 10^4$ mm⁻³ for sample B using an evaporation method [30]. The front and back slabs of the sample holder were cut from microscope glass slides with thickness around 1.0 mm. The sphere suspension was confined by a ring spacer of 14.3 mm in diameter between the two glass slabs. The sphere size distributions were imaged by a microscope using an objective of 40 \times and 0.65 in NA. The areas of single spheres were measured by a software to determine their diameters and Gaussian curve fitting was applied

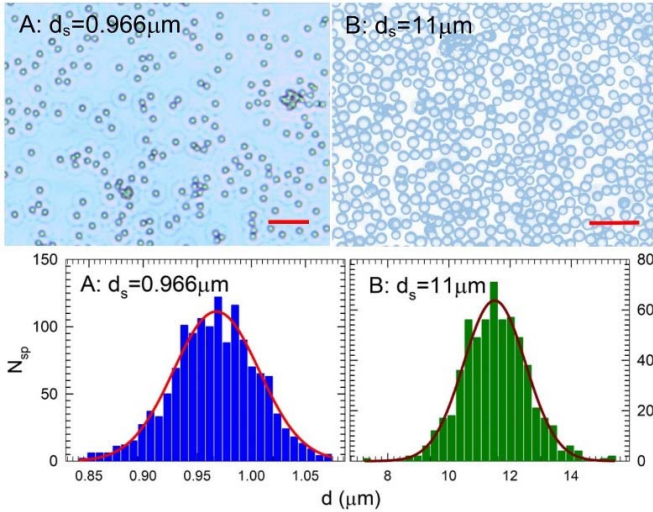


Fig. 3. Images of the sphere suspension sample A with bar = 10 μm and sample B with bar = 50 μm (top row). Histograms with N_{sp} as number of spheres, d as measured diameter values and Gaussian distributions curves by solid lines (bottom row).

to the histogram of measured diameter data. Fig. 3 presents the microscopy images and histograms of size distributions. We determined the mean value and standard deviation of sphere diameters for the sample A as $\mu'_d = 1.80 \mu\text{m}$ and $\sigma'_d = 0.073 \mu\text{m}$. The value of μ'_d was much larger than d_s by a factor of 1.86 that can be attributed to the relative low accuracy in area calculations due to edges blurred by the interference rings. To correct the area measurement errors, the histogram data in Fig. 3 for sample A were scaled by the factor of 1.86 for diameter values so that the scaled values were given by $\mu_d = d_s$ and $\sigma_d = 0.040 \mu\text{m}$. The sphere parameter for sample B was found to be $\mu_d = 11.5 \mu\text{m}$ and $\sigma_d = 1.0 \mu\text{m}$ by the Gaussian curve fitting with no scaling.

III. SYSTEM SOFTWARE DESIGN

The software of prototype system has three components for data acquisition, light scattering simulation, and inverse calculations with Fig. 4 showing flow charts of the first two. Data acquisition was controlled through the microprocessor which synchronizes the wavelength tuning, signal digitization, determination, and export of the measured signals. Simulation of light scattering yields calculated signals from a chosen sample model and RT parameters as input data. The red-lined block in Fig. 4(b) presents the core of light scattering modeling which is the focus of this study for system validation and performance evaluation.

A. Signal Calculation by iMC Simulation

An individual photon tracking MC (iMC) code has been developed to calculate signals with the detector configuration illustrated in Fig. 1(b). Details of the code, validation results, and GPU implementation have been published elsewhere and are briefly described below [9], [17], [31]–[33]. At each wavelength, initial or iterated values of RT parameters are imported into the iMC code together with a selected function for $p(\cos\theta)$ according to the selected sample model. An incident light

beam is represented in iMC by N_0 photons on the front surface of sample holder with an incident angle of θ_0 . A total length L_a is determined from μ_a and a random number (RN) uniformly distributed in (0,1) for each tracked photon before it enters the sample assembly. Then each photon propagates through or becomes reflected off various index mismatched interfaces among air, holder glass slab, and sample according to the local Fresnel reflectance. Once a photon moves into the turbid sample, its trajectory evolves as a sequence of segments of pathlengths L_{sj} ($j = 1, 2, \dots$) determined by μ_s and updated scattering angle θ by the selected phase function and RNs. At the end of L_{sj} , the accumulated pathlength $L_s = \sum_{j'=1}^j L_{sj'}$ is compared to L_a . If $L_s > L_a$, the photon is terminated as being absorbed; otherwise it is propagated further until reaching a sample surface. When the photon exits a sample holder glass into air, the code further checks if it hits a detector and increments the related photon counter for affirmative answer. Finally, the calculated signals of R_{dc} , T_{dc} and T_{fc} are obtained by dividing the number of photons reaching a respective detector by N_0 .

For iMC simulations with a selected phase function based on the Mie theory in numerical form only, a lookup table of 5000 elements is compiled and used as input data of iMC for determination of scattering angles. At each scattering site for a tracked photon inside the sample, the following inequalities are evaluated using the lookup table and an RN [34]:

$$2\pi \sum_{k=1}^{K-1} p(x_k) \Delta x_k \leq \text{RN} \leq 2\pi \sum_{k=1}^K p(x_k) \Delta x_k \quad (2)$$

to derive a proper value of x_K and the scattering polar angle $\theta = \cos^{-1}x_K$. The scattering azimuthal angle φ is set to an RN uniformly distributed on $[0, 2\pi]$ for all sample models. The iMC code has been validated against the bidirectional curves of reflection R_b and transmission T_b of turbid samples of an infinite slab with different combinations of RT parameters compiled in [4].

B. Solving Inverse RT Problems

An objective function δ is defined as the squared error between the measured and calculated signals that is given by

$$\delta = \left(\frac{R_d - R_{\text{dc}}}{R_d} \right)^2 + \left(\frac{T_d - T_{\text{dc}}}{T_d} \right)^2 + \left(\frac{T_f - T_{\text{fc}}}{T_f} \right)^2. \quad (3)$$

Inverse determination of the RT parameters μ_a , μ_s , and g from the measured signals at λ is framed as solving an optimization problem toward $\delta \leq \delta_0$ through iteration. For this report, we set the value of δ_0 at 8×10^{-3} which corresponds to about $\pm 5\%$ for averaged relative errors in the measured signals of R_d , T_d , and T_f for the prototype system. A gradient descent-based algorithm was applied to update RT parameters for iMC simulations and to make R_{dc} , T_{dc} , and T_{fc} approach to the measured signals with $\delta \leq \delta_0$ [17].

To speed up inverse calculations, iMC simulations have been implemented for parallel execution on a GPU board (Tesla K20, Nvidia, Santa Clara, CA, USA). The current iMC-GPU code has a speedup of about 70 relative to execution on a CPU of 2.93 GHz (i7-870, Intel, Santa Clara, CA, USA).

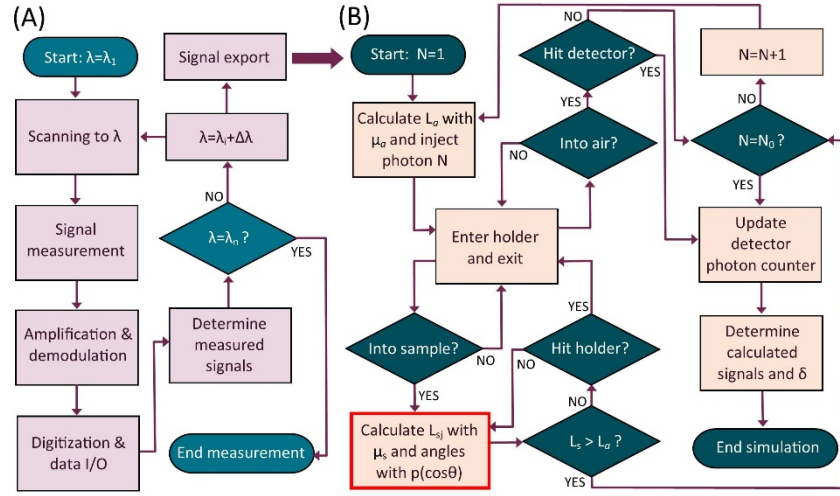


Fig. 4. Flowcharts of software components. (A) Acquisition of measured signals over scanned wavelengths of stepsize $\Delta\lambda$. (B) iMC simulation of calculated signals with N_0 incident photons at a scanned wavelength.

Simulation time depends mainly on the values of μ_a and μ_s for a given sample size and total photon number N_0 . It took 5.2 s to complete an iMC-GPU simulation with $\mu_a = 0.29 \text{ mm}^{-1}$, $\mu_s = 4.74 \text{ mm}^{-1}$, and $N_0 = 5 \times 10^7$ for sample A with averaged statistical fluctuations in calculated signals at about 1.4%. Since much larger fluctuations can be tolerated in the initial search for δ far from δ_0 in parameter space, the value of N_0 for iMC simulations was set to 5×10^6 for initial search and increased to 5×10^7 as δ moves toward δ_0 . Thus, it took about 10 min or less on average to determine RT parameters at the first wavelength of a sample with mean numbers of iterations around 400. The time of inverse solution per wavelength was further decreased to about 5 min or less as a result of closer initial parameter values from the results of last wavelength and smaller μ_s values for longer wavelengths.

C. Modeling of Sphere Sample With Different Phase Functions

For quantitative evaluation of the HG sample model, we constructed two additional sample models by the Mie theory that solves the vector wave equations of light scattering by a homogeneous sphere with a closed-form solution. Different from the HG model with three adjustable parameters of μ_a , μ_s , and g , the two Mie-based models have dissimilar size distributions and number of adjustable parameters. One is for single-sized homogeneous polystyrene spheres in water with $p_{\text{MS}}(\cos\theta, d)$ as the phase function and d as the only adjustable parameter of sphere diameter. The other is for multisized spheres with $p_{\text{MM}}(\cos\theta)$ as the phase function and two adjustable parameters of μ_d as the mean value and σ_d as standard deviation of diameters. The two sample models are denoted, respectively, as the Mie-single-sized (MS) and Mie-multisized (MM) models.

A Mie code was utilized to develop a code for calculation of the 4×4 Mueller matrix $[S_{ij}(\cos\theta, d); i, j = 1, \dots, 4]$ as intensities of polarized light scattered by a sphere of diameter d [35]. Other input parameters include the complex refractive index (RI) of sphere n_s , real RI of host medium n_h by water

and λ [30], [36]. The element S_{11} in the output data provides the differential scattering cross section to unpolarized incident light which was normalized as the scattering phase function $p_{\text{MS}}(\cos\theta, d)$ for the MS sample model of single-sized spheres. For the MM model of multisized spheres, we employed a Gaussian distribution function $G(d)$ based on the histogram data in Fig. 3 to obtain a phase function given by [20]

$$p_{\text{MM}}(\cos\theta) = \int_0^\infty G(d) p_{\text{MS}}(\cos\theta, d) d(d) \approx \sum_{q=1}^Q G(d_q) p_{\text{MS}}(\cos\theta, d_q) \quad (4)$$

where

$$G(d) = \frac{1}{\sigma_d \sqrt{2\pi}} e^{-\left(\frac{d-\mu_d}{\sigma_d}\right)^2}. \quad (5)$$

We set $Q = 25$ for numerical calculation of $p_{\text{MM}}(\cos\theta)$ for this study. The RT parameters of μ_a , μ_s , and g were obtained from the size distributions, and the total cross sections of absorption $\sigma_a(d_q)$ and scattering $\sigma_s(d_q)$ are as follows:

$$\begin{aligned} \mu_a &= \sum_{q=1}^Q \rho(d_q) \sigma_a(d_q) = \rho_s \sum_{q=1}^Q G(d_q) \sigma_a(d_q) \\ \mu_s &= \sum_{q=1}^Q \rho(d_q) \sigma_s(d_q) = \rho_s \sum_{q=1}^Q G(d_q) \sigma_s(d_q) \\ g &= 2\pi \int_{-1}^1 x p_{\text{MM}}(x) dx \end{aligned} \quad (6)$$

where $\rho(d_q) = \rho_s G(d_q)$ is the number density of single spheres with diameter d_q and ρ_s as the measured number density of spheres in each sample. For the MS model, the sums in (6) for μ_a and μ_s were reduced to a single term of diameter d and g directly from the Mie code.

IV. SYSTEM VALIDATION AND MODEL EVALUATION

A. Platform Validation

Measurements of nonhemispherical signals of R_d , T_d , and T_f were repeated three times at each wavelength from 460 to

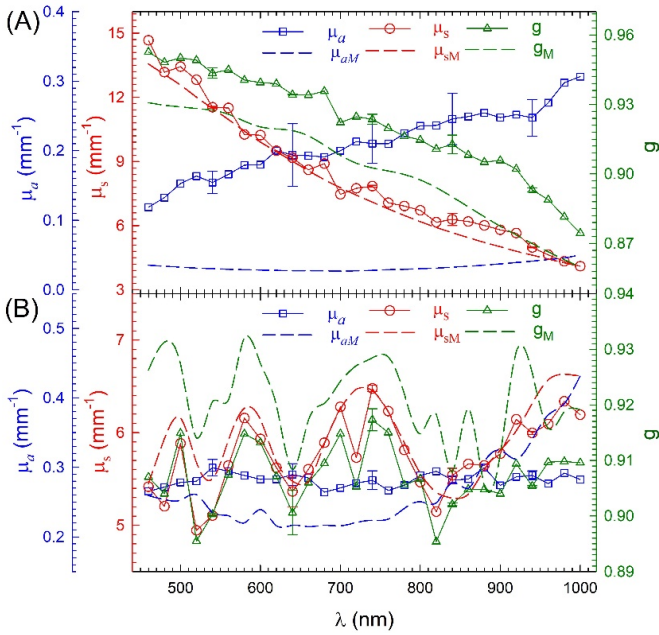


Fig. 5. Wavelength dependence of RT parameters. The symbols and error bars are the mean values and standard deviations determined from three sets of measured signals by the HG sample model that are connected by solid lines. (A) Sample A with $d_s = 0.966 \mu\text{m}$, $\rho_s = (5.37 \pm 0.02) \times 10^6 \text{ mm}^{-3}$, $D = 2.06 \pm 0.01 \text{ mm}$, $\theta_R = 45^\circ$, $d_R = 31.7 \text{ mm}$, $\theta_T = 43^\circ$, $d_T = 44.1 \text{ mm}$, and $d_f = 49.3 \text{ mm}$. (B) Sample B with $d_s = 11 \mu\text{m}$, $\rho_s = (3.05 \pm 0.02) \times 10^4 \text{ mm}^{-3}$, $D = 2.10 \pm 0.01 \text{ mm}$; $\theta_R = 45^\circ$, $d_R = 21.5 \text{ mm}$, $\theta_T = 48^\circ$, $d_T = 35.7 \text{ mm}$, $d_f = 88.4 \text{ mm}$. The distance parameters are defined in Fig. 1(b). The dash lines are RT parameters of sphere suspensions calculated by (6) using the Mie phase function for single-sized spheres with d_s as diameter and ρ_s as concentration.

1000 nm with a stepsize of 20 nm by the three-photodiode configuration as shown in Fig. 1 for the two sphere samples. Experimental parameters of incident beam profile, sizes and distances of sample, holder, and detectors were imported into the iMC code to maximize accuracy of calculated signals. RT parameters were determined at each wavelength by iterating iMC simulations for calculated signals by the HG sample model when $\delta \leq \delta_0$ was reached. To validate the platform design, we also applied the Mie theory to obtain the RT parameters of μ_{aM} , μ_{sM} , and g_M in the same wavelength range according to (6) for the two samples assumed as suspensions of single-sized spheres, where the measured value of sphere concentrations ρ_s and the nominal sphere diameter d_s supplied by vendors were used. The two sets of RT parameters versus wavelength have been previously reported in [14, Fig. 2] and are replotted in Fig. 5 for completeness of discussion.

Among the three RT parameters shown in Fig. 5, the values and λ dependence of μ_s agree quite well with those of μ_{sM} for the two samples of $\mu_s \gg \mu_a$, with better agreement for sample A exhibiting a monotonic dependence. In comparison, the agreements between the absorption coefficients of μ_a and μ_{aM} deteriorate significantly. A closer look of the μ_a differences suggests that the current platform design using the three-photodiode detection configuration is likely to have a lower limit in μ_a determination that likely is around 0.2 mm^{-1} . This conclusion is corroborated by the case of sample B in which the differences reduce considerably for μ_a values

staying above 0.2 mm^{-1} . For anisotropy factor g , the λ dependence agrees well while the values do not. Since the relative differences of g values are opposite in the two samples, it is unlikely that the disagreement is due to underestimate of light scattering for $\theta > 90^\circ$ by the HG model. Rather, the disagreement of both μ_s and g between the HG model and Mie theory could be attributed to sphere settling leading to the failure of Mie theory that assumes uniform suspension and independent single spheres. Sphere settling became especially severe for the sample B of larger volume-to-surface ratios with a settling time less than 5 min and the sample had to be shaken before next wavelength of measurement.

Based on the above observations, one may reach the conclusion that the new platform design can accurately determine scattering coefficient μ_s for homogeneous turbid samples but has a detection floor for absorption coefficient μ_a at about 0.2 mm^{-1} . Without T_c measurement, it is further interesting to observe that optically thick samples can be characterized for optical thickness of $\mu_t D$ up to 30. Inversely determined g values may deviate slightly from “true” values but its wavelength dependence is likely to be accurate for the characterization of scattering anisotropy. In the cases of sample A and B with forwardly peaked scattering patterns, the averaged deviations of g from the Mie predicted values are less than 3%. It should be noted that no adjustable parameters were used in calculation of RT parameters by the Mie theory shown in Fig. 5.

B. Comparison of Measured and Calculated Signals

From the data displayed in Fig. 5, one cannot find out if the Mie phase functions and RT parameters determined by (6) can yield inverse solutions defined by $\delta \leq \delta_0$. For this purpose, we have applied the two Mie-based sample models in iMC simulations to obtain calculated signals on selected wavelengths for quantitative evaluation of their robustness against the HG sample model. Table I lists the mean values and standard deviations of the measured signals together with configuration parameters of the sample and detector assemblies at five wavelengths. The relative errors of the measured signals range from 0.5% to 8.0% with the averaged value at 2.7%. Because of the strongly peaked scattering in the forward direction of both samples, the measured R_d signal is smaller than those of T_d and T_f if the detectors were placed at similar distances from the sample, which led to larger errors for R_d values of the sample A. The design of prototype system was modified to enable adjustment of detector distances for increasing signal intensity and improve significantly the repeatability of the measured data.

The iMC code was used to obtain the calculated signals of R_{dc} , T_{dc} , and T_{fc} for each sample model with the given phase function and trial values of RT parameters. With the measured signals, we have investigated the distribution of objective function δ in the parameter space for robustness evaluation. The parameter space is defined by μ_a , μ_s , and g for the HG model while the corresponding ones are defined by μ_d and σ_d for the MM model and d for the MS model. We determined the objective function δ with fixed step sizes in the respective parameter space around the locations of minimal

TABLE I
MEASURED DATA OF TWO SPHERE SAMPLES AT FIVE WAVELENGTHS^a

λ (nm)	Sample A ($d_s=0.966\mu\text{m}$)			Sample B ($d_s=11\mu\text{m}$)		
	$R_d \times 10^4$	$T_d \times 10^4$	$T_f \times 10^4$	$R_d \times 10^4$	$T_d \times 10^4$	$T_f \times 10^4$
560	3.87 ± 0.18	3.87 ± 0.13	4.79 ± 0.074	4.46 ± 0.063	3.98 ± 0.027	1.47 ± 0.016
660	3.37 ± 0.11	3.78 ± 0.12	4.86 ± 0.028	4.64 ± 0.027	4.03 ± 0.060	1.44 ± 0.036
760	2.99 ± 0.10	3.63 ± 0.14	4.58 ± 0.071	4.84 ± 0.14	4.14 ± 0.073	1.40 ± 0.064
860	2.67 ± 0.080	3.48 ± 0.062	4.36 ± 0.022	4.79 ± 0.057	3.99 ± 0.099	1.33 ± 0.094
960	2.37 ± 0.19	3.39 ± 0.22	4.84 ± 0.052	5.05 ± 0.12	4.05 ± 0.068	1.26 ± 0.064

^aThe mean values and standard deviations of measured data were obtained by 3 measurements.

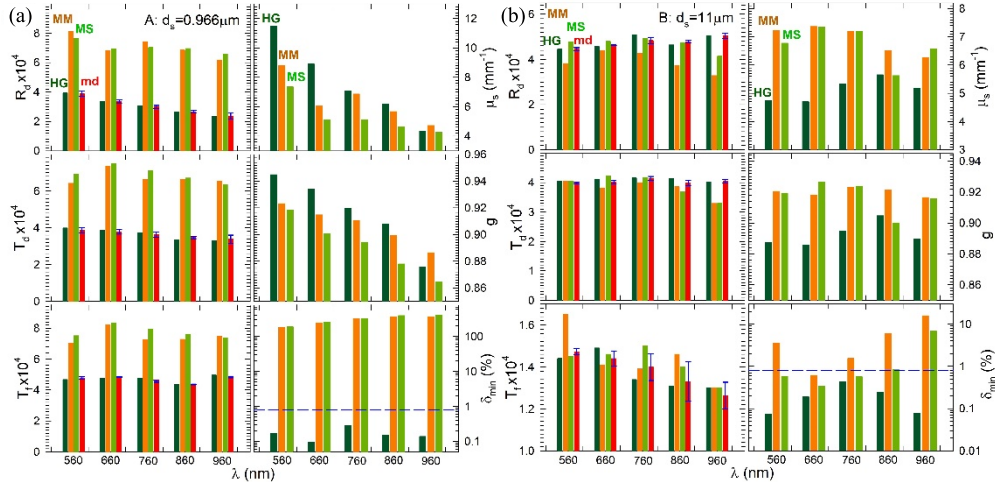


Fig. 6. Wavelength dependence of calculated signals against the mean values and error bars of measured data (md), scattering RT parameters of μ_s and g and δ_{\min} by the HG, MM, and MS models for (a) sample A of $d_s = 0.966 \mu\text{m}$ and (b) sample B of $d_s = 11 \mu\text{m}$. The blue dash lines represent δ_0 used as the threshold for inverse solutions.

value δ_{\min} and examined if $\delta_{\min} \leq \delta_0$ to satisfy the definition of an inverse solution. For the MM and MS sample models, the parameter ranges were set to between 0.6 and $1.2 \mu\text{m}$ for μ_d (or d) and between 0.006 and $0.3 \mu\text{m}$ for σ_d of the sample A based on results of sphere size measurement. For the sample B, the respective ranges were given by between 9.0 and $14 \mu\text{m}$ and between 0.8 and $2.5 \mu\text{m}$. The RT parameters were calculated for the MM and MS models by (6) after the sphere size parameters were determined at δ_{\min} for comparison to those of the HG model.

Fig. 6 compares the calculated signals against the measured data by the left-side bar diagrams of (a) and (b) for sample A and B at five wavelengths. The right-side diagrams show the scattering RT parameters of μ_s and g determined at δ_{\min} among the sample models. These data show clearly that the HG model can yield calculated signals well matched to the measured ones in all cases with the adjustable parameters of μ_a , μ_s , and g . The other two models, however, yield inverse solutions with $\delta_{\min} \leq \delta_0$ satisfied only in certain cases in the space of μ_d and σ_d or d . For sample A, the values of μ_s and g and their λ dependence exhibit fairly good agreement among the three models despite the fact of δ_{\min} being significantly larger than δ_0 for the MM and MS models. For sample B, the Mie-based models improve markedly with δ_{\min} less than or close to δ_0 except the case of $\lambda = 960 \text{ nm}$. It is in this case,

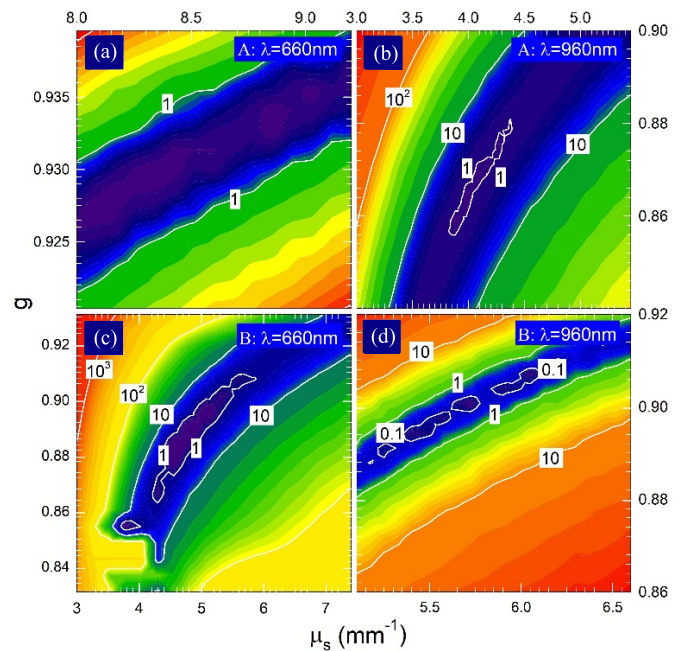


Fig. 7. Contour plots of δ values in % in the μ_s - g plane of parameter space for the HG model with sample ID and λ values as marked for different μ_a values. (a) $\mu_a = 0.193 \text{ mm}^{-1}$. (b) $\mu_a = 0.269 \text{ mm}^{-1}$. (c) $\mu_a = 0.285 \text{ mm}^{-1}$. (d) $\mu_a = 0.277 \text{ mm}^{-1}$. All iMC simulations were performed with $N_0 = 1 \times 10^8$.

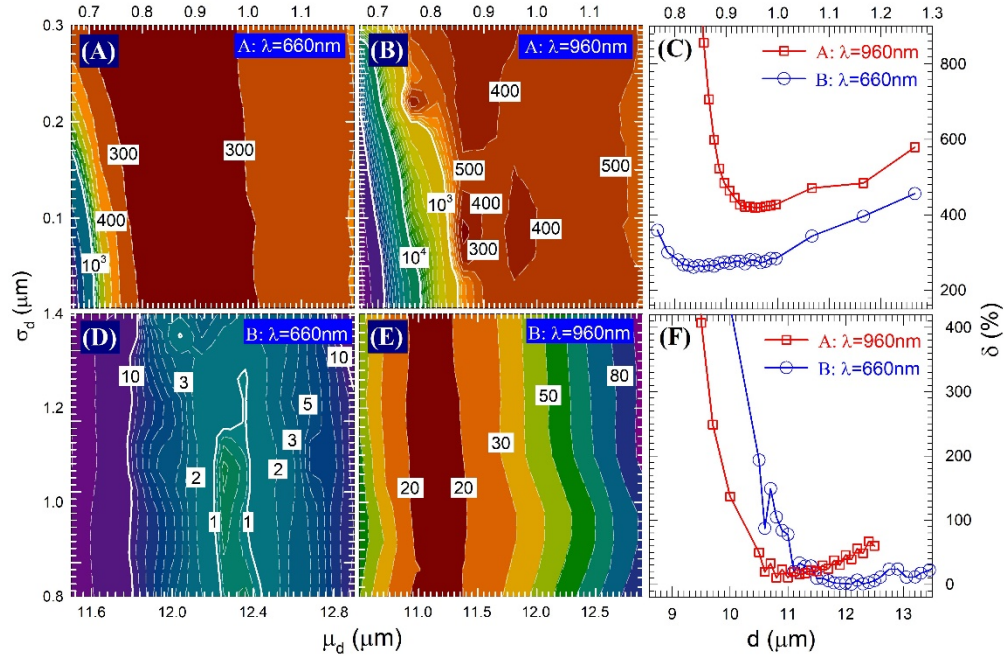


Fig. 8. Contour plots of δ values in % with sample ID and λ values marked for different Mie-based sample models. (A) MM. (B) MM. (C) MS. (D) MM. (E) MM. (F) MS. All iMC simulations were performed with $N_0 = 1 \times 10^8$.

very interestingly, that the two Mie-based models can match T_{fc} to T_f with μ_s and g larger than those of the HG model but yield R_{dc} and T_{dc} significantly smaller than the measured data. This can be explained by the fact that either $p_{MS}(\cos\theta, d)$ or $p_{MM}(\cos\theta)$ for spheres of $d \sim 11 \mu\text{m}$ is of a very high peak value for forward scattering of $\theta \sim 0$ in comparison to $p_{HG}(\cos\theta)$ and smaller values in side scatter of R_{dc} and T_{dc} (see [14, Fig. 3]). It is also worth noting that the measured values of sphere density ρ_s for both samples can produce RT parameters for the Mie-based models in the same ranges of those used for searching inverse solutions by the HG model through (6).

C. Evaluation of Robustness by δ Distributions

To better understand the influence of sample model on solving inverse RT problems, we have obtained the maps of δ functions in the parameter spaces of the three sample models in two cases of $\lambda = 660$ and 960 nm for the two samples. Fig. 7 presents the contour plots of δ as functions of μ_s and g with μ_a kept at the constant value for $\delta = \delta_{\min}$ by the HG model in the four cases of different samples and wavelengths. We note that the numbers of detected photons for the calculated signals fluctuate as a result of statistical variance in iMC simulations. Even with N_0 increased to 1×10^8 , these variances can still reach to 1% for calculated signals because the number of detected photons are about 10^4 . This leads to fluctuation of δ around 0.1% when the measured and calculated signals are very close to each other or δ becomes close to or less than δ_0 . Consequently, multiple local minima are visible, as shown in Fig. 7(a) and (d), by the contour plot software interpolating the fluctuating values of $\delta < 1\%$ in these regions of μ_s and g . Despite these artifacts, the results in Fig. 7 clearly demonstrate that the RT problems defined

by the HG sample model form convex optimization problems. Thus, a global minimum exists that enables inverse solutions by a gradient descent-based algorithm for all cases using $p_{HG}(\cos\theta)$ as the phase function.

Compared to the HG model, the two Mie based models vary significantly in the stability and feasibility for inverse solutions among the different cases of samples and wavelengths. The plots presented in Fig. 8(A)–(C) shows clearly that neither of the MM and MS model can produce calculated signals close to the measured data for sample A satisfying $\delta_{\min} \leq \delta_0$. Furthermore, the values of sphere parameters determined at δ_{\min} were found to be $\mu_d = 0.76 \mu\text{m}$ and $\sigma_d = 0.30 \mu\text{m}$ by the MM model and $d = 0.84 \mu\text{m}$ by the MS model. These values deviate considerably from the measured values before or after scaling as shown in Fig. 3. For sample B, both Mie-based models can produce calculated signals much closer to the measured data with $\delta_{\min} \leq \delta_0$ as illustrated in Fig. 8(D) and (F) for $\lambda = 660$ nm. Still the sphere diameter values of μ_d and d are significantly larger than d_s or μ_d determined by microscopy. For the case of $\lambda = 960$ nm, both models failed to yield inverse solutions even though the d values for $\delta = \delta_{\min}$ are fairly close to d_s for both samples.

The results presented from Figs. 6–8 demonstrate clearly that iMC simulations combined with the HG sample model provide a robust approach for inverse solutions of RT parameters from the measured signals of R_d , T_d , and T_f . In contrast, the Mie theory solves rigorously a light-scattering problem only for uniform sphere suspension samples under the assumption of independent and single spheres. The assumptions of suspension uniformity and independent single spheres, however, can be very difficult to be realized in samples, which should cause the inability of MM or MS model to match calculated signals to measured signals in most cases. Additionally, the sphere concentration ρ_s of sample A was set

high to have sufficient signal-to-noise ratios for the R_d and T_d signals measured by photodiodes. The sample A appears like milk for the high ρ_s value and presented clearly a problem in which the electromagnetic fields scattered by other spheres must be considered together with those of the incident light for the considered sphere. Therefore, the failure of MM and MS models to yield inverse solutions defined by $\delta < \delta_0$ for sample A can be attributed to violation of the assumptions for sample uniformity and independent single spheres. Based on these considerations, the improvement of the Mie-based models in the case of sample B can be regarded as a consequence of decreased ρ_s as shown in Figs. 6 and 8. Even with these improvements, the two Mie-based models are not as stable for gradient descent-based search as the HG model. Also notable is the inversely determined values of μ_d and d at δ_{\min} being significantly different from the microscopically measured values, which may be due to the aggregation and settlement of the 11- μm spheres during signal measurement.

Finally, we have investigated the effect of uncertainty in measured signals on the RT parameters inversely determined by the HG sample mode. The values of the measured signals listed in Table I show that the relative errors of these signals average at about 2.7%. The RT parameters of the two sphere samples were determined by the inverse algorithm with the measured signals of R_d , T_d , and T_f increased by 3.0% from their mean values in Table I. The percentage change varies from -2.7% to 3.0% for μ_s and from -0.3% to 0.3% for g from their values shown in Fig. 6 among the five wavelengths in the case of sample A. But the relative changes of μ_a are much larger, ranging from -80% to 0%, which indicates indeed a detection floor of μ_a around 0.2 mm^{-1} for the prototype system. In comparison, the relative changes of μ_s and g are similar in the case of sample B but those of μ_a decrease to a range of -48% to 0% since their values are between 0.27 and 0.42 mm^{-1} . It is clear from these results that the presented method of multiparameter spectrophotometry can yield accurate values of μ_s and g but has reduced sensitivity and accuracy for μ_a when its values are close to the detection floor and orders of magnitude smaller than those of μ_s .

V. CONCLUSION

We have developed a prototype system to test a new platform design for multiparameter spectrophotometric instruments for accurate characterization of homogeneous turbid samples by the RT theory. Two sphere suspension samples have been measured to determine inversely their RT parameters from 460 to 1000 nm and the system validation has been achieved by comparison to the RT parameters determined by the Mie theory for independent and single spheres. We focused on evaluation of the platform performance to solve inversely the RT parameters from the measured signals with three sample models. With an objective function δ and the criterion of $\delta \leq \delta_0$ for inverse solution, we have shown quantitatively that the HG sample model outperforms the two Mie-based sample models for all cases of the two samples at five wavelengths. These results demonstrate clearly that the HG function parameterized by g exhibits strong robustness in solving inverse light-scattering problems and extracting

RT parameters for spectroscopic analysis of turbid samples. By comparison, Mie-based phase functions and sample models are applicable only to problems in which light transport in a uniform suspension is dominated by scattering by independent and single spheres that are rarely the case for practical applications. Our results further demonstrate for the prototype system that the uncertainties in the measured signals and the inversely determined scattering parameters of μ_s and g can be kept to about 5% or less, while a detection floor of about 0.2 mm^{-1} exists for μ_a in the cases of strongly turbid samples.

ACKNOWLEDGMENT

The authors thank Dr. Kenneth M. Jacobs for his helps on design and construction of the prototype system.

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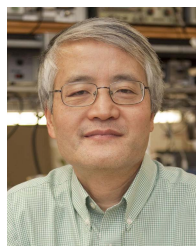
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