Published in final edited form as:

Lasers Med Sci. 2013 November ; 28(6): 1559–1566. doi:10.1007/s10103-013-1268-7.

Optical and spectroscopic properties of human whole blood and plasma with and without Y_2O_3 and $Nd^{3+}:Y_2O_3$ nanoparticles

Frederick J. Barrera,

Department of Physics and Astronomy, University of Texas at San Antonio, San Antonio, TX 78249-0697, USA

Brian Yust,

Department of Physics and Astronomy, University of Texas at San Antonio, San Antonio, TX 78249-0697, USA

Lawrence C. Mimun,

Department of Physics and Astronomy, University of Texas at San Antonio, San Antonio, TX 78249-0697, USA

Kelly L. Nash,

Department of Physics and Astronomy, University of Texas at San Antonio, San Antonio, TX 78249-0697, USA

Andrew T. Tsin, and

Department of Biology, University of Texas at San Antonio, San Antonio, TX 78249-0697, USA

Dhiraj K. Sardar

Department of Physics and Astronomy, University of Texas at San Antonio, San Antonio, TX 78249-0697, USA

Abstract

The optical properties of human whole blood and blood plasma with and without Y_2O_3 and $Nd^{3+}:Y_2O_3$ nanoparticles are characterized in the near infrared region at 808 nm using a double integrating sphere technique. Using experimentally measured quantities of diffuse reflectance and diffuse transmittance, a computational analysis was conducted utilizing the Kubelka-Munk, the Inverse Adding Doubling, and Magic Light Kubelka-Munk and Monte Carlo Methods to determine optical properties of the absorption and scattering coefficients. Room temperature absorption and emission spectra were also acquired of $Nd^{3+}:Y_2O_3$ nanoparticles elucidating their utility as biological markers. The emission spectra of $Nd^{3+}:Y_2O_3$ were taken by exciting the nanoparticles before and after entering the whole blood sample. The emission from the $^4F_{3/2} \rightarrow ^4I_{11/2}$ manifold transition of $Nd^{3+}:Y_2O_3$ nanoparticles readily propagates through the blood sample at excitation of 808 nm and exhibits a shift in relative intensities of the peaks due to differences in scattering. At 808 nm, in both whole blood and plasma samples, a direct relationship was found with absorption coefficient and Y_2O_3 nanoparticle concentration. Results for the whole blood indicate a small inverse relationship with Y_2O_3 nanoparticle concentration and scattering coefficient and in contrast a direct relation for the plasma.

Keywords

Lasers; Blood; Nanoparticles; Optical properties

Introduction

Near-infrared (NIR) light has become increasingly attractive for biomedical uses due to its weak interaction within the so-called tissue window which allows for deeper penetration in applications such as in photoacoustics, photodynamic therapies, and optical imaging [1, 2]. NIR light is also very attractive for biomedical applications because of increased detection sensitivity in non-invasive modalities that can be enhanced by employing contrast agents which absorb and emit in these NIR regions. Many of the current imaging, sensing, and microscopy modalities in use today utilize either ultraviolet or visible light, but these wavelengths have the drawbacks of possibly damaging the tissue due to higher photon energy and exhibiting poor signal-to-noise ratio due to autofluorescence from cells. In contrast, NIR light is known to be safe for cells, even at higher fluence rates, and NIR light does not induce autofluorescence in cells [3].

In certain cases, using ultraviolet radiation, damage to genetic material may result in cellular damage. Our method uses an excitation wavelength of 808 nm located in the near-infrared region. This allows for probing due to greater penetration depth of tissues than by irradiation with ultraviolet or visible radiation. Also, there are no known strong cellular biological emitters in this region, although oxyhemoglobin and deoxyhemoglobin are known to be significant absorbers in certain intervals of the infrared and near-infrared regions.

Because of the advantages of NIR light in biomedical applications, it is necessary for researchers and practitioners to better understand the optical properties of biological tissues in this regime.

Rare earth ion-doped inorganic crystals have been used in many applications: in glass coloring and as activators or sensitizers for phosphors, light emitters, and in imaging. Rare earth-doped oxides, such as Y_2O_3 , have been shown to be thermally stable [4, 5].

Although the size, shape, and crystalline structure for the doped and undoped yttrium oxide nanoparticles should be similar, the optical properties will be drastically different. Rare earth-doped inorganic nanoparticles (e.g., $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$) are those composed of an inorganic host lattice activated with a trivalent lanthanide. The host lattice provides the structure, and the trivalent ion dopant provides the fluorescence properties. The electronic configuration of the trivalent lanthanides is $4f^n$, with n varying from 1 to 14 from cerium to lutetium, respectively, and the electronic transitions within the 4f shell result in their optical properties. The f electrons are shielded by filled 5s and 5p orbitals and exhibit line-like spectra. The doped $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ nanoparticle allows for emission from the $^4\text{F}_{3/2} \rightarrow ^4\text{I}_{11/2}$ transition while the undoped Y_2O_3 nanoparticle does not.

Yttrium oxide doped with neodymium emits strongly in the infrared region of the electromagnetic spectrum. Although to the authors' present knowledge $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ has not yet been approved for any clinical applications, the infrared emission from this material would allow for greater propagation through biological media than visible fluorescence from other commercially available dyes.

A NIR excitation wavelength at 808 nm results in higher likelihood of longer propagation of radiation through biological media. This wavelength is within the water window, so absorption and scattering is relatively low. In addition, $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ nanoparticles have an intense absorption band in this region. Another accessory aspect for this selection is that oxyhemoglobin and deoxyhemoglobin have isosbestic points near 808 nm [6] in the NIR. This could have application in oximetry or clinical chemistry scenario where devices are used to determine hemoglobin concentration independent of its saturation.

In developing a contrast agent for optical imaging applications, it is ideal to utilize a NIR absorber and emitter which can act without significant interference or quenching from the surrounding biological milieu. Trivalent neodymium doped into nanocrystalline yttrium oxide ($\text{Nd}^{3+}:\text{Y}_2\text{O}_3$) fulfill these requirements, exhibiting strong NIR absorption and emission without being overly sensitive to the outside environment. It is also important to have an understanding of how the host nanoparticle interacts with light in the NIR region, such as how strongly it scatters and absorbs particularly at the excitation wavelength. For this reason, doped ($\text{Nd}^{3+}:\text{Y}_2\text{O}_3$) and undoped (Y_2O_3) nanoparticles were optically characterized.

In our previous work, the various computational methods used to obtain values for the optical properties of biological tissues have been well described [7-10]. The same methods have been employed here to characterize whole human blood and blood plasma with $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ and Y_2O_3 nanoparticles, although we will note that our previously used inverse Monte Carlo (IMC) program has been replaced with another program by the name of Magic Light [11]. An experimental apparatus of two integrating spheres was utilized to measure the diffuse reflectance, diffuse transmittance, and collimated transmittance of our samples with and without the Y_2O_3 nanoparticles under 808 nm illumination. Subsequently, values for the absorption (μ_a) and scattering (μ_s) coefficients were calculated using the two-flux Kubelka–Munk (KM), Magic Light Inverse Monte Carlo (ML_IMC), Magic Light Kubelka–Munk (ML_KM), and Inverse Adding Doubling (IAD) methods. The emission spectrum was also taken for $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ nanoparticles in conjunction with the absorption spectra of the human whole blood to illustrate the practicality of the nanoparticle as a biological contrast agent. The emission spectra were also taken upon exciting $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ with these nanoparticles placed first on the front and then on the back wall of a glass cuvette containing the whole human blood sample. The experiments verified that the nanoparticle fluorescence was attenuated through the whole human blood and elucidated optical differences caused by nanoparticles within the blood in two separate cases.

Materials and preparation

The details of the synthesis and spectroscopic properties of the $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ nanoparticles are given by several authors [12-14]. Whole human blood samples were donated by the South Texas Blood and Tissue Center of San Antonio and separated into the red blood cell and plasma components. The blood constituents were then refrigerated until ready for use. For experiments involving nanoparticles mixed with whole blood, the nanoparticles were added to the plasma and sonicated to ensure uniform dispersion prior to optical measurements. The samples were then reconstituted at a 1:1 ratio of blood cells and plasma.

For the first stage of the study, $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ was synthesized in a 2/98 ratio. The blood sample was not mixed with the $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ nanoparticles so as to adequately control for pathlength by passing through a more uniform layer of material of known thickness as the light traverses the sample. In certain diagnostic modalities, only information relevant to transmission is desired. Furthermore, adding the optically active substance directly to the biological sample for some applications may result in an output signal with an unfavorable signal-to-noise ratio.

Human whole blood was then poured into the cuvettes and refrigerated until use. For the second stage of the study, changes in the optical properties of plasma and whole blood were monitored with the addition of various concentrations of Y_2O_3 . Once the undoped nanoparticles were mixed into the sample, they were injected into Nunc Opticells® for ease of placement within the measurement setup. The concentrations of blood and Y_2O_3 used in this study were 1.0, 0.5, 0.2, 0.1, 0.02, and 0.01 mg/mL with controls of pure human whole

blood and plasma that did not contain any nanoparticles. The plasma samples were set at 1.0, 0.5, and 0.02 mg/mL. The samples were kept on ice in between measurements, and all measurements were completed within 2 h of preparation.

Experimental methods

First, the emission spectrum of $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ nanoparticles was obtained by exciting the sample at 808 nm from a Ti:Sapphire laser at 1 W (SpectraPhysics 3900) and analyzed with a SPEX 1250M monochrometer (Fig. 1). Next, the cuvettes containing human whole blood with $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ nanoparticles were adhered to the front and back respective surfaces (Fig. 2) and were excited with 808 nm light, and the fluorescence spectrum was analyzed by a SPEX 1250M monochrometer for both cases.

Next, optical properties were required for the undoped host nanoparticles within the plasma and blood samples. The samples were placed in between the exit port of the first sphere and the entrance port of the second sphere, and all measurements were performed under 808 nm excitation wavelength. Using the experimental setup delineated in previous work [15], intensity measurements for each sphere were recorded under various conditions so that the radiometric values for diffuse reflectance (R_d) and transmittance (T_d) could be obtained from:

$$R_d = \frac{X_r - C_r}{Z_r - C_r} \text{ and } T_d = \frac{X_t - C_t}{Z_t - C_t} \quad (1)$$

where X_r is the reflected intensity detected in the first integrating sphere with the sample in between the spheres, X_t is the transmitted intensity detected by the second integrating sphere with the sample in between the spheres, Z_r and Z_t are the reflected and transmitted intensities with no sample between the spheres, and C_r and C_t are the correction factors for any ambient light detected. The collimated transmittance, which is measured after moving the second integrating sphere at least 70 cm along the laser propagation pathway while the sample is fixed at the exit port of the first integrating sphere, is determined from the following expression:

$$T_c = \frac{X_c}{Z_c} \quad (2)$$

where X_c is the collimated light intensity detected by the second integrating sphere with the sample at exit port of the first integrating sphere and Z_c is the light intensity detected by the second integrating sphere with no sample at the exit port of the first integrating sphere. Additional details on the experimental design can be found in [16] Sardar et al.

In order to maintain fully oxygenated red blood cells (RBCs), the sample is shaken in room air, and this allows RBCs to swell and not shrink, thereby maintaining a biconcave disk geometry. A distortion in the geometry will cause differences in scattering. However, experimental and theoretical studies using Mie theory have indicated that scattering is minimally affected (~10%) by oxygenated and deoxygenated red blood cells (~7,500 nm) at wavelengths between 600–1,000 nm for approximately spherical particles [17]. In this case, the oxygenated blood scattering is greater than in the deoxygenated blood. The scattering coefficient and scattering anisotropy factor is expected to decrease with increasing wavelength for both oxygenated and deoxygenated red blood cells. Therefore, the oxygenation should not be a large effect in the determination of the scattering.

The measurements necessary to obtain R_d and T_d values were taken at five points per concentration ($n=5$) to obtain a reasonable mean. The refractive indices used for calculations

were 1.37 for human whole blood and 1.345 for human blood plasma, as was previously reported in the literature [8, 18]. After the experimental R_d , T_d , and T_c values were calculated, the data were used in each computational method (KM, IAD, ML_KM, ML_IMC) to obtain values for the coefficients, μ_a and μ_s . The Kubelka–Munk techniques for the two-flux geometry and the details for the iterative Inverse Adding-Doubling and Inverse Monte Carlo methods are available in our previous works [7-10, 19].

Since the nanoparticles act as point sources within the sample, the typical measurements to determine the absorption and scattering coefficients are invalid because they do not allow for a sample with fluorescence. Thus, the absorption and scattering coefficients were not calculated for samples containing the $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ nanoparticles due to its strong emission.

Results and discussion

During the first portion of this study, $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ nanoparticles were excited, and the fluorescence was collected for the two instances: (1) when affixed to the front of a cuvette containing whole human blood and (2) when affixed to the back surface of the same cuvette (Fig. 2). The excitation beam penetrated the blood sample to induce emission from the nanoparticles, which was strong enough to measure without any modifications to the system. By comparing the two cases where the particles are affixed to the front and then the back of the cuvette (Fig. 2), it is evident that the fluorescence from the $^4\text{F}_{3/2} \rightarrow ^4\text{I}_{9/2}$ transition (881 nm) is absorbed by the sample, although the signal is still detectable through 1 cm of blood. The $^4\text{F}_{3/2} \rightarrow ^4\text{I}_{11/2}$ transition (1,052 nm) is more favorable with this excitation wavelength. Therefore, it exhibits a stronger fluorescence intensity overall. Although there is a slight shift in the relative intensities of each peak between the two instances due to slight differences in the scattering strength of the blood across those wavelengths and noting the intensity decrease in the excitation signal on the particles affixed on the exit layer configuration relative to the entrance layer, fluorescence from this manifold propagates well through the blood. By monitoring the entire emission from the $^4\text{F}_{3/2} \rightarrow ^4\text{I}_{11/2}$ transition, this may allow for an estimation of the thickness of a blood sample due to changes in these relative intensities.

For the second portion of the study, plasma and whole human blood with various concentrations of Y_2O_3 nanoparticles were placed in Opticells within the double integrating sphere setup and illuminated with 808 nm light from a Ti:Sapphire laser. The diffuse reflectance and transmittance were monitored for all samples, and the scattering and absorption coefficients were calculated for samples with undoped nanoparticles. In the case of plasma mixed with nanoparticles, the changes in the coefficients as a function of nanoparticle concentration are expressed as a clear trend of substantial increase in the scattering coefficient with a much less severe increase in the absorption coefficient (Fig. 3). The differences between the absorption coefficient values returned by the various computational methods can be attributed to the extremely low absorbing power of the sample at these wavelengths which causes the calculations to be less accurate. Although the difference in absorption coefficients is attributed to the low absorption results presented here, it may be interesting to conduct further studies with increasing concentrations. However, larger concentrations of Y_2O_3 nanoparticles would present other challenges (e.g., maintaining relative monodispersity) for determining the optical properties. Another concern is that, at larger concentrations, at this wavelength, the higher absorption of the sample may render the Kubelka–Munk computations invalid since the absorption is assumed to be significantly greater relative to the scattering.

In the case of whole blood mixed with Y_2O_3 nanoparticles, there is also an increase in the absorption coefficient, but there is a very slight decreasing trend in the scattering coefficient

with increasing nanoparticle concentration (Fig. 4). Although the scattering coefficient values are mostly unchanged, this slight decrease is likely due to the Y_2O_3 nanoparticles scattering more isotropically than red blood cells which are known to be highly forward-scattering. Altogether, these values indicate that the undoped Y_2O_3 particles are not strongly absorbing at the excitation Doubling (*IAD*), Magic Light Kubelka–Munk (*ML_KM*), and the Magic Light Inverse Monte Carlo (*ML_IMC*) wavelength but do contribute to the overall scattering when present in plasma.

The regression analysis (Table 1) exhibits strong correlation for the Kubelka–Munk (both two-flux and Magic Light treatments) for the Y_2O_3 plasma samples in scattering and absorption coefficients. This suggests strong correlation of both increased scattering and absorption with increasing concentration for Y_2O_3 plasma samples for those computational methods. The *IAD* and Magic Light Inverse Monte Carlo methods for Y_2O_3 plasma samples reveal some correlation in the scattering. These experimental consequences are in contrast to the whole blood Y_2O_3 results that lack the strong correlation of some of the blood plasma Y_2O_3 results.

While all exhibit positive correlation, it is apparent that, for a number of cases, the percent of variation in the scattering or absorption coefficients is not accounted for by the concentrations in a number of the scenarios. In the case of the similarity in the coefficient of determination of the Kubelka–Munk and Magic Light Kubelka–Munk, this may be due in part to the fact that the Magic Light algorithm constructs an initial estimate of sample optical properties from the explicit Kubelka–Munk formulas [11] before progressing to subsequent steps of the algorithm in the case of the Magic Light Kubelka–Munk. The appearance of the strong correlation between concentration and absorption coefficient in the Kubelka–Munk-type models may originate from its similarity to the Beer–Bourger–Lambert relationship.

The consistent relatively moderate to weak correlation among all models in the blood samples is due to the influence of the red blood cells. As noted above, red blood cells have been shown to approximately follow properties as Mie scatterers. The blood medium with its red blood cells moderates the addition of the isotropic Y_2O_3 nanoparticles while, in the case of the plasma, there are no larger scatterers relative to the nanoparticles present in the medium.

Insofar as Kubelka–Munk has been shown to be accurate in highly scattering with low absorption materials, Monte Carlo and *IAD* simulations have been shown to be more accurate than these treatments. The anomalous low correlation in the coefficient of determination may be improved by introducing a nonlinear higher-order fitting to reveal a higher correlation in the variables.

Although there was a lack of correlation found for some of the scattering and absorption coefficients and computational models, we may use these to analyze optical properties under certain axioms. Y_2O_3 fluorescent nanoparticles may be useful in biophotonic applications, and herein, their optical properties with blood have been investigated. In addition, the excitation wavelength propagates through a volume of blood subject to experimental conditions, and the emission is detected through the blood sample from the $^4\text{F}_{3/2}$ $^4\text{I}_{11/2}$ transition of the $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ nanoparticles.

Acknowledgments

This work was supported by partial funding of: National Institutes of Health (NIH)/National Institute of General Medical Sciences (NIGMS) Minority Biomedical Research Support (MBRS) Research Initiative for Scientific Enhancement (RISE) program NIH/NIGMS MBRS-RISE GM6065; National Science Foundation (NSF) sponsored center for Biophotonics and Technology at UC Davis under the Cooperative Agreement No. PHY 0120999; and the

NSF Partnership for Research and Education in Materials (PREM) NSF-PREM Grant No. DMR-0934218. The authors also thank Bagrat Grigoryan for preliminary work and Chris Dennis and Nathan Ray in the synthesis of the nanoparticles.

References

1. Chatterjee DK, Ruffailah AJ, Zhang Y. Upconversion fluorescence imaging of cells and small animals using lanthanide doped nanocrystals. *Biomaterials*. 2008; 29(7):937–943. [PubMed: 18061257]
2. Sardar DK, Nash KL, Yow RM, Gruber JB, Sayka A. Rare-earth doped nanocrystals for biosensing and imaging. *Biophotonics International*. 2008; 15:45–48.
3. Wang F, Tan WB, Zhang Y, Fan X, Wang M. Luminescent materials for biological labeling. *Nanotechnology*. 2006; 17(1):R1–R13.
4. Boisseau, P.; Lahmani, M. *Nanoscience: nanotechnology and nanobiology*. Springer; Berlin: 2009.
5. Petermann K, Huber G, Fornasiero L, Kuch S, Mix E, Peters V, Basun SA. Rare-earth-doped sesquioxides. *J Lumin*. 2000; 87-89:973–975.
6. Horecker BL. The absorption spectra of hemoglobin and its derivatives in the visible and near infrared regions. *J Biol Chem*. 1943; 148:173–183.
7. Sardar DK, Mayo ML. Optical characterization of melanin. *J Biomed Opt*. 2001; 6(4):404–411. [PubMed: 11728198]
8. Sardar DK, Levy LB. Optical properties of whole blood. *Lasers Med Sci*. 1998; 13:106–111.
9. Sardar DK, Yust BG, Barrera FJ, Mimun LC, Tsin ATC. Optical absorption and scattering of bovine cornea, lens, and retina in the visible region. *Lasers Med Sci*. 2009; 24:839–847. [PubMed: 19495828]
10. Sardar DK, Salinas FS, Perez JJ. Optical characterization of bovine retinal tissues. *J Biomed Opt*. 2004; 9(3):624–631. [PubMed: 15189102]
11. Yaroslavsky IV, Yaroslavsky AN, Goldbach T, Schwarzmaier HJ. Inverse hybrid technique for determining the optical properties of turbid media from integrating-sphere measurements. *J Appl Phys*. 1996; 35(34):6797–6809.
12. Sardar DK, Dee DM, Nash KL, Yow RL, Gruber JB. Optical absorption intensity analysis and emission cross sections for the intermanifold and the inter-Stark transitions of $\text{Nd}^{3+}(4\text{f}^3)$ in polycrystalline ceramic Y_2O_3 . *J Appl Phys*. 2006; 100(12):123106–123107.
13. Nash KL, Dennis RC, Gruber JB, Sardar DK. Intensity analysis and energy-level modeling of Nd^{3+} in $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ nanocrystals in polymeric hosts. *J Appl Phys*. 2009; 105(3):033102–033106.
14. Nash KL, Dennis RC, Ray NJ, Gruber JB, Sardar DK. Absorption intensities, emission cross sections, and crystal field analysis of selected intermanifold transitions of Ho^{3+} in $\text{Ho}^{3+}:\text{Y}_2\text{O}_3$ nanocrystals. *J Appl Phys*. 2009; 106(6):063117–063118.
15. Yust BG, Mimun LC, Sardar DK. Optical absorption and scattering of bovine cornea, lens, and retina in the near-infrared region. *Laser Med Sci*. 2011 doi:10.1007/s10103-011-0927-9.
16. Sardar DK, Salinas FS. Optical properties of a laser dye in a solid-state polymeric host. *J Appl Phys*. 2002; 91(12):9598–9602.
17. Faber DJ, Aalders MCG, Mik EG, Hooper BA, van Gemert MJC, van Leeuwen TG. Oxygen saturation-dependent absorption and scattering of blood. *Phys Rev Lett*. 2004; 93(2):028102–028104. [PubMed: 15323954]
18. Li H, Lin L, Xie S. Refractive index of human whole blood with different types in the visible and near-infrared ranges. *Proc SPIE*. 2000; 3914:517–521.
19. Sardar DK, Swanland GY, Yow RY, Thomas RJ, Tsin ATC. Optical properties of ocular tissues in the near infrared region. *Lasers Med Sci*. 2007; 22:46–52. [PubMed: 17143656]

Fig. 1. Absorption spectrum of human whole blood from 330 to 2,550 nm and the fluorescence spectrum of Nd³⁺:Y₂O₃ from 850 to 1,500 nm with an excitation wavelength of 808 nm

Fig. 2. (Top) Fluorescence spectrum of $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ excited at 808 nm as the signal passes through human whole blood. (Bottom) Fluorescence spectrum of $\text{Nd}^{3+}:\text{Y}_2\text{O}_3$ excited at 808 nm as the excitation light passes through human whole blood

Fig. 3. Coefficients of human blood plasma versus concentration of nanoparticle from computational methods (Kubelka-Munk (*KM method*), Inverse Adding Doubling (*IAD method*), Magic Light Kubelka-Munk (*ML_KM method*), and the Magic Light Inverse Monte Carlo (*ML_IMC method*)). (Top) Absorption coefficient; (bottom) scattering coefficient

Fig. 4. Coefficients of human whole blood versus concentration of nanoparticle from computational methods (Kubelka-Munk (*KM method*), Inverse Adding Doubling (*IAD method*), Magic Light Kubelka-Munk (*ML_KM method*), and the Magic Light Inverse Monte Carlo (*ML_IMC method*)). (*Top*) Absorption coefficient; (*bottom*) scattering coefficient

Table 1

Regression table for absorption (μ_a) and scattering (μ_s) coefficients of human blood plasma (*top*) and whole blood (*bottom*) from computational methods: Kubelka-Munk (*KM*), Inverse Adding Doubling (*IAD*), Magic Light Kubelka-Munk (*ML_KM*), and the Magic Light Inverse Monte Carlo (*ML_IMC*)

R square (R^2) values	KM		IAD		ML_KM		ML_IMC	
Blood plasma	μ_a	μ_s	μ_a	μ_s	μ_a	μ_s	μ_a	μ_s
	0.9801	0.9992	0.02581	0.9939	0.9993	0.9976	0.1455	0.7040
Whole blood	μ_a	μ_s	μ_a	μ_s	μ_a	μ_s	μ_a	μ_s
	0.3145	0.3989	0.0665	0.1853	0.3925	0.4122	0.2394	0.2527