Modeling the local excitation fluence rate and fluorescence emission in absorbing and strongly scattering multilayered media

Dmitry Yudovsky and Laurent Pilon*

Henry Samueli School of Engineering and Applied Science, Department of Mechanical and Aerospace Engineering, Biomedical Inter-Department Program, University of California, Los Angeles, Los Angeles, California 90095-1597, USA

*Corresponding author: pilon@seas.ucla.edu

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We present computationally efficient and accurate semiempirical models of light transfer for real-time analysis of multilayer fluorescing media exposed to normally incident excitation light. The model accounts for absorption and strong forward scattering as well as for internal reflection at the interface between the medium and the surrounding air. The absorption and scattering coefficients are assumed to be constant with depth; the fluorophore concentration is considered piecewise constant. The refractive index ranges from 1.0 to 2.0, and the transport single scattering albedo between 0.50 and 0.99. First, simple analytical expressions for local excitation fluence rate within the medium and surface fluorescence intensity emerging from its surface were derived from the two-flux approximation. Then, parameters appearing in the analytical expression previously derived were fitted to match results from more accurate Monte Carlo simulations. A single semiempirical parameter was sufficient to relate the diffuse reflectance of the medium at the excitation wavelength to the local excitation fluence rate within the medium and to the surface fluorescence emission intensity. The model predictions were compared with Monte Carlo simulations for local fluence rate and total surface fluorescence emission from (i) homogeneous semi-infinite fluorescing media, (ii) media with a semi-infinite fluorescing layer beneath a nonfluorescing layer, and (iii) media with a finite fluorescing layer embedded in a nonfluorescing semi-infinite layer. The model predictions of the local excitation fluence rate and of the total surface fluorescence emission fell to within 5% of predictions by Monte Carlo simulations for single scattering albedo greater than 0.90. The current model can be used for a wide range of applications, including noninvasive diagnosis of biological tissue. © 2010 Optical Society of America

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1. Introduction

Fluorescence is the physical phenomenon in which light is emitted by a substance as a result of excited electrons returning to their ground states after the absorption of excitation light [1,2]. Fluorescing substances (fluorophores) are characterized by their quantum yield, their fluorescence lifetime(s), and their emission wavelengths. Emission occurs over a wide spectral range and at wavelengths longer than the excitation wavelength. The quantum yield is the ratio of the number of photons emitted to the number of photons absorbed, whereas the fluorescence lifetime is the average time the electrons spend in their excited states [1,2]. Biological tissues contain several endogenous fluorophores, such as nicotinamide adenine dinucleotide (NAD or NADH), aromatic amino acids such as tryptophan, and structural proteins, such as collagen and elastin [3]. The optical properties of these fluorophores are sensitive to the environment and the metabolic state of the tissue, making fluorescence spectroscopy a valuable tool to study the health of biological tissues [1,2,4–6].
Steady-state fluorescence spectroscopy has been widely used to monitor biological tissues \cite{1,4–11}. It consists of exposing the medium of interest to collimated or diffuse excitation light (typically UV) and measuring the fluorescence intensity emerging from the tissue surface. Fluorescence spectroscopy typically involves either emission spectra or excitation spectra measurements. Fluorescence emission spectra measurements consist of measuring the fluorescence intensity over a range of wavelengths for a fixed excitation wavelength. On the other hand, excitation spectra measurements consist of measuring the fluorescence intensity at a particular wavelength for a certain range of excitation wavelengths. Fluorophores are typically present throughout biological tissues, and the fluorescent light is absorbed and scattered before emerging from the tissue. Thus, measurements of fluorescence intensity detected at the tissue surface depend on the optical properties of both the fluorophores and the medium. Intrinsic fluorescence is defined as the fluorescence intensity emitted by the tissue’s fluorophores after removing the effects of tissue scattering and absorption.

Analytical approaches to recovering intrinsic fluorescence spectra have been based on approximate solutions of the radiative transfer equation, such as the Beer–Lambert law \cite{12}, diffusion approximation \cite{12–14}, and the two-flux approximation \cite{15,16}. Correction factors for the boundary conditions in the diffusion and two-flux approximations have been developed to account for index mismatch at the boundary between the air and the medium \cite{17,18}. However, the diffusion approximation fails in media when absorption is significant, and two-flux approximation fails if scattering is strongly anisotropic and weak \cite{17,18}. Instead, semiempirical models have been developed that combine the simplicity of approximate solutions and the accuracy of Monte Carlo simulations \cite{11,16,19}. Typically, the algebraic form of an approximate solution is used along with empirical parameters provided in a lookup table obtained from Monte Carlo simulations. Gardner et al. \cite{12} developed a heuristic model for the local fluence rate and fluorescence escape function for a homogeneous, semi-infinite medium with a specified refractive index using six semiempirical parameters expressed as a function of the diffuse reflectance.

Intrinsic fluorescence spectroscopy has been typically performed on internal organs with the assumption that they were homogeneous \cite{4–6}. However, this assumption may not accurately predict fluorescence from tissue in which fluorophore distribution is multilayered. Such an instance can arise if the tissue is stained with a fluorescent dye that (i) significantly alters the fluorescence properties of a layer of tissue but (ii) does not significantly affect tissue scattering or absorption \cite{20–24}. This can be modeled as a two-layer fluorescent medium in which fluorescence is stronger in the top layer than in the bottom. Alternatively, internal malignancies, such as tumors, can be stained intravenously with contrast agents to create a layer of strong fluorescence within healthy tissue that does not fluoresce significantly \cite{25,26}. This situation can be modeled as a three-layer medium such that a strongly fluorescing layer representing the tumor exists between two weakly fluorescing layers. The objective of this study is to develop a simple and accurate expression for the excitation fluence rate and the total fluorescence emission intensity of multilayered fluorescing media. Similar to previous studies, the absorption and scattering coefficients of the medium were assumed to be constant with depth. However, fluorophore concentration was considered to vary stepwise with depth to simulate fluorescence from multilayered tissue. Furthermore, the index of refraction of the medium was treated as a parameter varying between 1.0 and 2.0. This model can be combined with an inverse method to determine the tissue’s intrinsic fluorescence coefficient from the measured fluorescence emission of biological tissue.

### 2. Background

#### A. Radiative Transfer Equation

The transport of excitation light in absorbing and scattering media is governed by the steady-state radiative transfer equation (RTE). The excitation intensity \( I_{x\lambda} \) at wavelength \( \lambda_x \) in direction \( \hat{s} \) and at location \( \hat{r} \) satisfies the steady-state RTE expressed as \cite{27}

\[
\hat{s} \cdot \nabla I_{x\lambda}(\hat{r}, \hat{s}) = -\mu_{a,\lambda} I_{x\lambda}(\hat{r}, \hat{s}) - \mu_{s,\lambda} I_{x\lambda}(\hat{r}, \hat{s}) + \frac{\mu_{s,\lambda}}{4\pi} \times \int_{4\pi} I_{x\lambda}(\hat{r}, \hat{s}_1) \Phi_{x\lambda}(\hat{s}_1, \hat{s}) d\Omega, \tag{1}
\]

where \( \mu_{a,\lambda} \) and \( \mu_{s,\lambda} \) are the linear absorption and scattering coefficients, respectively. The scattering phase function, denoted by \( \Phi_{x\lambda}(\hat{s}_1, \hat{s}) \), represents the probability that radiation propagating into the elementary solid angle \( d\Omega \) around incident direction \( \hat{s}_1 \) will be scattered in direction \( \hat{s} \).

Moreover, a similar equation can be written for the transport of fluorescent light at wavelength \( \lambda_f \) emitted by fluorophores present within the medium \cite{27}:

\[
\hat{s} \cdot \nabla I_{x\lambda}(\hat{r}, \hat{s}) = -\mu_{a,\lambda} I_{x\lambda}(\hat{r}, \hat{s}) - \mu_{s,\lambda} I_{x\lambda}(\hat{r}, \hat{s}) + \frac{\mu_{a,\lambda}}{4\pi} \times \int_{4\pi} I_{x\lambda}(\hat{r}, \hat{s}_1) \Phi_{x\lambda}(\hat{s}_1, \hat{s}) d\Omega + \frac{\gamma_{sf}(\hat{r}) G_{x\lambda}(\hat{r})}{4\pi}. \tag{2}
\]

The last term in Eq. (2) represents fluorescence emission at wavelength \( \lambda_f \) stimulated by the excitation light at wavelength \( \lambda_x \). The term \( G_{x\lambda} \) is the excitation fluence rate expressed as...
where $F_{c,\lambda}(z)$ is a source term associated with the unattenuated collimated incident light, and $F^+_{\lambda}$ and $F^-_{\lambda}$ are the diffuse excitation fluxes propagating into the positive and negative $z$ directions, respectively. Here, $z$ is the depth inside the medium defined from the medium–air interface as illustrated in Fig. 1(a). These diffuse fluxes at arbitrary wavelength $\lambda$ can be expressed in terms of the local intensity $I_{\lambda}(z, \theta)$ as [34]

\[
F^+_{\lambda}(z) = 2\pi \int_0^{\pi/2} I_{\lambda}(z, \theta) \cos \theta \sin \theta d\theta,
\]

\[
F^-_{\lambda}(z) = -2\pi \int_0^{\pi/2} I_{\lambda}(z, \theta) \cos \theta \sin \theta d\theta. \tag{8}
\]

The polar angle $\theta$ was taken relative to the inward normal surface as illustrated in Fig. 1(a). The coefficients $K_{\lambda}$ and $S_{\lambda}$ are the absorption and scattering coefficients for diffuse fluxes, respectively, and $a_{\lambda} = (S_{\lambda} + K_{\lambda})/S_{\lambda}$ [34, 36]. Furthermore, expressions for the backward and forward scattering coefficients for collimated light, respectively, denoted by $S_{1,\lambda}$ and $S_{2,\lambda}$, have been reported in the literature [34]. Expressions for $K_{1,\lambda}, S_{1,\lambda}, S_{1,\lambda},$ and $S_{2,\lambda}$ will be discussed later.

The unattenuated collimated flux that was neither absorbed nor scattered at depth $z$ follows the Beer–Lambert law and is expressed as [27]

\[
F_{c,\lambda}(z) = (1 - \rho_{01})F_{0,\lambda} e^{-K_{c,\lambda}z}, \tag{9}
\]

where $F_{0,\lambda}$ is the incident collimated flux and $K_{c,\lambda}$ is the effective extinction coefficient for the collimated flux at the excitation wavelength $\lambda$. The specular reflectivity for normally incident radiation, denoted by $\rho_{01}$, is given by [27]

\[
\rho_{01} = \left(\frac{n_1 - n_0}{n_1 + n_0}\right)^2, \tag{10}
\]

where $n_1$ and $n_0$ are the refractive indices of the medium and surrounding air, respectively. The boundary condition for the diffuse fluxes in the positive direction $F^+_{\lambda}(0)$ can be expressed as [34]

\[
F^+_{\lambda}(0) = \rho_{10} F^-_{\lambda}(0), \tag{11}
\]

where the hemispherical–hemispherical reflectivity $\rho_{10}$ is the fraction of diffuse flux radiating from within the medium reflected back into the medium due to index mismatch and given by [37]

\[
\rho_{10} = \frac{\pi/2}{\rho^m(\theta_i) \sin 2\theta_i d\theta_i}. \tag{12}
\]

The directional specular reflectivity of the interface for the angle of incidence $\theta_i$ is denoted by $\rho^s(\theta_i)$ and expressed as [27]
\[
\rho n(\theta_i) = \begin{cases} 
\frac{1}{2} \left[ \sin^2(\theta_i) - \theta_i \right] + \tan^2(\theta_i - \theta_c) \\
\tan^2(\theta_i + \theta_c) \end{cases} \quad \text{for } \theta_i \leq \theta_c,
\]
\[
\frac{1}{2} \quad \text{for } \theta_i > \theta_c.
\]  

The angle of transmittance \( \theta_i \) is determined by Snell’s law (i.e., \( n_0 \sin \theta_i = n_1 \sin \theta_c \)), and \( \theta_c \) is the critical angle defined as \( \theta_c = \sin^{-1}(n_0/n_1) \) [27]. Since the negative flux \( F_{-\lambda}^-(z) \) vanishes as \( z \) goes to infinity, the following boundary condition is imposed [34]:
\[
F_{-\lambda}^-(z \to \infty) = 0.
\]  

By solving Eqs. (6) and (7) and evaluating the integral in Eq. (3), \( G_{\lambda}(z) \) can be expressed as [34]
\[
G_{\lambda}(z) = 2\pi \left[ F_{+\lambda}^+(z) + F_{-\lambda}^-(z) + F_{c\lambda}(z) \right]
\]
\[
= (1 - \rho_{10}) F_{0\lambda}(k_1 e^{-b_{1\lambda}z} + k_2 e^{-K_{c\lambda}z}),
\]  
where \( b_{1\lambda} = \sqrt{a_{1\lambda}^2 - 1} \) [36]. Expressions for \( k_1 \) and \( k_2 \) can be determined from the boundary conditions given by Eqs. (11) and (14) as [34]
\[
k_1 = \frac{2\pi(a_{1\lambda} - b_{1\lambda} + 1) \{ K_{c\lambda}(\rho_{10} S_{1\lambda} + S_{2\lambda})\} + S_{2\lambda} [ (a_{1\lambda} \rho_{10} - 1) S_{1\lambda} + (\rho_{10} - a_{1\lambda}) S_{2\lambda} ] \} \left[ (a_{1\lambda} + b_{1\lambda}) \rho_{10} - 1 \right] \left[ K_{c\lambda}^2 - b_{1\lambda}^2 S_{1\lambda}^2 \right]^{-1},
\]
\[
k_2 = \frac{2\pi \{ K_{c\lambda}^2 + S_{2\lambda} - S_{1\lambda} \} K_{c\lambda} - S_{2\lambda} [ S_{1\lambda} b_{1\lambda}^0 + (a_{1\lambda} + 1) (S_{1\lambda} + S_{2\lambda}) ] \} \left[ K_{c\lambda}^2 - b_{1\lambda}^2 S_{1\lambda}^2 \right]^{-1}.
\]  

Unlike \( k_1 \), which depends on \( n_1 \) through \( \rho_{10} \) given by Eq. (12), \( k_2 \) is independent of \( n_1 \).

C. Two-Flux Approximation of Fluorescence Light Transport

Fluorescence light transport can be similarly approximated by two fluxes in the positive and negative \( z \) directions, respectively, as illustrated in Fig. 1(b). In this case, Eq. (2) reduces to the following two coupled ordinary differential equations [8,15]:
\[
\frac{dF_{+\lambda}^+(z)}{dz} = -a_{1\lambda} S_{1\lambda} F_{+\lambda}^+ + S_{1\lambda} F_{+\lambda}^- + \frac{1}{2} \gamma_{x\lambda}(z) G_{\lambda}(z),
\]  
\[
\frac{dF_{-\lambda}^+(z)}{dz} = -S_{1\lambda} F_{-\lambda}^- + a_{1\lambda} S_{1\lambda} F_{-\lambda}^+ - \frac{1}{2} \gamma_{x\lambda}(z) G_{\lambda}(z),
\]  
where \( F_{+\lambda}^+ \) and \( F_{-\lambda}^- \) represent the diffuse fluxes at the fluorescence wavelength \( \lambda_f \) propagating in the positive and negative \( z \) directions, respectively. Equations (18) and (19) are coupled to Eqs. (6) and (7) through the isotropic fluorescence emission source term \( \gamma_{x\lambda}(z) \) \( G_{\lambda}(z) \). Finally, boundary conditions for Eqs. (18) and (19) can be stated as [34]
\[
F_{+\lambda}^+(0) = \rho_{10} F_{-\lambda}^+(0), \quad F_{+\lambda}^-(z \to \infty) = 0.
\]  

Various solutions of Eqs. (18) and (19) have been proposed to approximate the fluorescence signal from slabs of finite thickness [8,15,38,39] or semi-infinite homogeneous media [16]. Kokhanovsky [38] derived expressions for the excitation and fluorescence fluorescence rates based on the two-flux approximation for homogeneous slabs and semi-infinite media exposed to diffuse incident irradiation. Furthermore, the author provided relationships between the radiative characteristics of strongly scattering slabs and the two-flux approximation parameters. However, this model assumed the refractive index of the medium and its surroundings to be identical and equal to 1.0. Furthermore, the author did not compare the accuracy of the two-flux approximation with a more accurate solution of the radiative transfer equation and instead suggested that the expressions derived were "only approximately valid" [38]. Similarly, Ramos and Lagorio [8], Shakespeare and Shakespeare [15], and Durkin et al. [16] used the conventional two-flux approximation [35] to analyze fluorescence emission from turbid slabs and semi-infinite media irradiated by diffusely incident light. In each case, the medium was assumed to be homogeneous, to contain a single fluorophore, and to exhibit no index mismatch with the surrounding. Unlike Kokhanovsky [38], these studies also presented inverse methods for determining the intrinsic fluorescence coefficient of media from observed surface fluorescence and reflectance. These models were able to predict the shape of the intrinsic fluorescence spectrum of plant life [8], textiles [15], and human tissue [16] but could not be used to estimate its absolute value. Furthermore, none of these models could be used to analyze fluorescence from multilayer media exposed to normal incident and

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collimated excitation light featuring index mismatched with the surroundings and/or that exhibited depth-dependent fluorophore concentration.

The current study extends the two-flux approximation to absorbing, scattering, and fluorescing multilayer media. It extends the two-flux approximation to accurately model (i) the local excitation fluence rate in semi-infinite media and (ii) the fluorescence emission from media in which fluorophore concentration varies stepwise with depth. Additionally, here the illumination considered was collimated and normally incident as opposed to diffuse. Furthermore, a single semiempirical parameter was fitted to match our model predictions for the excitation fluence rate and fluorescence emission with results from Monte Carlo simulations.

D. Two-Flux Approximation Coefficients

For strongly forward scattering media, the relationship between the effective absorption coefficient for collimated light $K_{a,\lambda}$ appearing in Eq. (9) and $\mu_{a,\lambda}$, $\mu_{s,\lambda}$, and $g_{\lambda}$ can be expressed as [34,40,41]

$$K_{a,\lambda} = \mu_{a,\lambda} + \frac{1}{S_{a,\lambda}} (1 - g_{\lambda}) \mu_{s,\lambda}. \quad (21)$$

Furthermore, the coefficients $K_{a}$ and $S_{a}$ are proportional to $\mu_{a,\lambda}$ and $\mu_{s,\lambda}$ according to [36,42]

$$\mu_{a,\lambda} = \eta_{a} K_{a,\lambda}, \quad \mu_{s,\lambda}(1 - g_{\lambda}) = \chi_{a} S_{a,\lambda}, \quad (22)$$

where $\eta_{a}$ and $\chi_{a}$ are expressed as [36]

$$\eta_{a} = (\phi_{\lambda} - 1)(1 - \omega_{tr,\lambda})/\zeta_{d,\lambda}(\phi_{\lambda} + 1), \quad \chi_{a} = -\omega_{tr,\lambda}(\phi_{\lambda} - \phi_{\lambda}^{-1})/(2\zeta_{d,\lambda}). \quad (23)$$

The function $\phi_{\lambda}$ is defined as [33]

$$\phi_{\lambda} = \frac{\zeta_{d,\lambda} + \ln(1 - \zeta_{d,\lambda})}{\zeta_{d,\lambda} - \ln(1 + \zeta_{d,\lambda})}. \quad (24)$$

where $\zeta_{d,\lambda}$ is the root of the characteristic equation [33]

$$\omega_{tr,\lambda} = \frac{2\zeta_{d,\lambda}}{\ln[(1 + \zeta_{d,\lambda})/(1 - \zeta_{d,\lambda})]]. \quad (25)$$

An approximate expression for $\zeta_{d,\lambda}^{2}$ as a function of $\omega_{tr,\lambda}$ was found to be [42]

$$\zeta_{d,\lambda}^{2} = \frac{47}{52} + \frac{31}{49} \omega_{tr,\lambda} - \frac{49}{54} \omega_{tr,\lambda}^{2} - \frac{17}{27} \omega_{tr,\lambda}^{3}. \quad (26)$$

The relative difference between Eq. (26) and the exact solution of Eq. (25) was determined to be less than 1% for $0.40 < \omega_{tr,\lambda} < 1.00$ [42].

3. Methods

A. Model Assumptions and Geometry

In this study, biological tissue was approximated as a semi-infinite, one-dimensional, strongly scattering medium. It was characterized by the properties $\mu_{a,\lambda}$, $\mu_{s,\lambda}$, $g_{\lambda}$, and $n_{1}$ at the excitation wavelength $\lambda_{p}$. The incident light source was modeled as a collimated, monochromatic, normally incident beam of infinite radius and intensity $I_{s,\lambda}(0, \theta) = F_{1,\lambda}\delta(\theta)$. All the interfaces were considered smooth and optically flat.

In practice, tissue excitation is typically performed with light between 260 and 500 nm, while fluorescence emission is measured between 350 and 700 nm [1,7]. The ranges for optical properties considered in this study were chosen to include those of human dermis in this wavelength range. Then, the asymmetry factor $g_{\lambda}$ varies between 0.70 and 0.90 [43]. Furthermore, Fig. 2(a) shows the transport single scattering albedo of the human dermis as a function of wavelength between 300 and 700 nm measured in vitro as reported in the literature [43–45]. Optical characteristics of the dermis below 300 nm were not available. While there is disagreement between the values reported in various studies, the single scattering albedo is typically greater than 0.80. Furthermore, tissue fluorescence emission for one-dimensional, homogeneous, semi-infinite media has been shown to depend on $\omega_{tr,\lambda}$ and $\omega_{tr,\lambda}$ as well as on the transport
between 1.00 and 2.00. 

Furthermore, it was assumed that \( \mu_{\text{r}, \lambda_f}, \mu_{s, \lambda_f}, \) and \( g_j \) at the emission wavelength \( \lambda_f \) were independent of location. Then, \( \omega_{\text{tr}, \lambda_f} \) was assumed to vary between 0.50 and 0.99, \( g_j \) between 0.70 and 0.90, and \( r_1 \) between 1.00 and 2.00.

The intrinsic fluorescence coefficient \( \gamma_{\text{sf}} \) was assumed to vary with the optical thickness defined as \( \tau_{\text{tr}, \lambda_f} = \rho_{\text{tr}, \lambda_f} z \). The intrinsic fluorescence coefficient was given by

\[
\gamma_{\text{sf}}(\tau_{\text{tr}, \lambda_f}) = \epsilon_{\text{f}, \lambda_f} QY_{\text{sf}}(\tau_{\text{tr}, \lambda_f}) M(\tau_{\text{tr}, \lambda_f}).
\]

Figure 3 shows the three different fluorophore concentration profiles \( M(\tau_{\text{tr}, \lambda_f}) \) considered in this study. Light regions represent the medium where \( M(\tau_{\text{tr}, \lambda_f}) = 0 \) mole/cm\(^3\), while dark regions represent regions where \( M(\tau_{\text{tr}, \lambda_f}) \) is constant and strictly positive. Figure 3(a) corresponds to a two-layer medium where the fluorophore is uniformly distributed in the medium for \( \tau_{\text{tr}, \lambda_f} > \tau_{\text{tr}, \lambda_f, 1} \) below a nonfluorescing layer. In this case, \( M^S(\tau_{\text{tr}, \lambda_f}) = A u(\tau_{\text{tr}, \lambda_f} - \tau_{\text{tr}, \lambda_f, 1}) \), where \( A \) is an arbitrary constant and \( u(\tau_{\text{tr}, \lambda_f} - \tau_{\text{tr}, \lambda_f, 1}) \) is the step function defined by

\[
u(\tau_{\text{tr}, \lambda_f} - \tau_{\text{tr}, \lambda_f, 1}) = \begin{cases} 1 & \text{if } \tau_{\text{tr}, \lambda_f} \geq \tau_{\text{tr}, \lambda_f, 1} \\ 0 & \text{if } \tau_{\text{tr}, \lambda_f} < \tau_{\text{tr}, \lambda_f, 1} \end{cases}.
\]

Figure 3(b) depicts the concentration profile \( M^H(\tau_{\text{tr}, \lambda_f}) = A u(\tau_{\text{tr}, \lambda_f}) \) corresponding to a homogeneous fluorophore distribution. Finally, Fig. 3(c) depicts the concentration profile \( M^L(\tau_{\text{tr}, \lambda_f}) = A [u(\tau_{\text{tr}, \lambda_f} - \tau_{\text{tr}, \lambda_f, 1}) - u(\tau_{\text{tr}, \lambda_f} - \tau_{\text{tr}, \lambda_f, 2})] \) corresponding to a three-layer system, where the fluorophore is uniformly distributed in the layer between depths \( \tau_{\text{tr}, \lambda_f, 1} \) and \( \tau_{\text{tr}, \lambda_f, 2} \) and surrounded by two nonfluorescing layers.

B. Monte Carlo Simulations

The RTE for excitation light given by Eq. (1) was solved using the Monte Carlo simulation software developed by Wang and Jacques [46,47] for light transfer through nonemitting, absorbing, and scattering media. This software was modified according to Welch et al. [6] to solve the RTE for fluorescence emission in the medium given by Eq. (2). The Henyey–Greenstein scattering phase function given

![Fig. 3. Schematic of (a) step, (b) homogeneous, and (c) layered fluorophore concentration profiles \( M^S(\tau_{\text{tr}, \lambda_f}) \), \( M^H(\tau_{\text{tr}, \lambda_f}) \), and \( M^L(\tau_{\text{tr}, \lambda_f}) \), respectively. Light gray represents the medium without fluorophore \( [M(\tau_{\text{tr}, \lambda_f}) = 0 \) mole/cm\(^3\)], while dark gray represents regions with fluorophore \( [M(\tau_{\text{tr}, \lambda_f}) = A \) mole/cm\(^3\)].](image)
by Eq. (4) was used to account for anisotropic scattering. The variance in the prediction of fluorescence intensity emerging at the surface of the medium increased as the transport single scattering albedo $\omega_{tr,\lambda}$ decreased, because absorption by the medium dominated over scattering resulting in fewer backscattered fluorescence photons. Thus, the number of simulated photon packets per simulation was increased until the variance associated with the fluorescence emission fell below 1% for the most stringent case of a semi-infinite homogeneous medium with $\omega_{tr,\lambda} = 0.50$. The variance for the most stringent case was calculated from ten repetitions of the same simulation. Each simulation required 100,000 photon packets or less to achieve the convergence criteria.

4. Analysis

A. Semiempirical Model of Excitation Fluence

The solution to Eqs. (6) and (7) given by Eqs. (15)–(17) has been shown to give poor estimates of the excitation fluence rate $G_{\lambda}(z)$ for media with index mismatch at the media–air boundary or when the semi-infinite medium is weakly scattering and the excitation source is collimated [17,48]. In the current study, the coefficients $k_1$ and $k_2$ in Eq. (15) were determined by fitting the local fluence rate $G_{\lambda}(z)$ predicted by Eq. (15) to that obtained by Monte Carlo simulations. By rearranging Eqs. (22)–(25), the product $b_{\lambda}G_{\lambda}(z)$ in the first term of Eq. (15) can be rewritten as $\zeta_{d,\lambda}^2\beta_{tr,\lambda} = \zeta_{d,\lambda}^2\tau_{tr,\lambda}$ [36,42]. Furthermore, by taking a polynomial Taylor series expansion of $K_c,\lambda$ defined by Eq. (21) with respect to $g_{\lambda}$ about $g_{\lambda} = 1$, the coefficient $K_c,\lambda$ can be expressed approximately as a function of $\beta_{tr,\lambda}$:

$$K_c,\lambda = \mu_{a,\lambda} + 2(1 - g_{\lambda})\mu_{\lambda} = \beta_{tr,\lambda}(1 + \omega_{tr,\lambda}).$$

Then, by introducing the coefficient $\xi_{c,\lambda} = (1 + \omega_{tr,\lambda})$, Eq. (15) can be rewritten exclusively as a function of $\tau_{tr,\lambda}$:

$$G_{\lambda}(\tau_{tr,\lambda}) = (1 - \rho_{01})F_{0,\lambda}(k_1e^{-\zeta_{d,\lambda}^2\tau_{tr,\lambda}} + k_2e^{-\zeta_{\lambda}^d\tau_{tr,\lambda}}).$$

Equation (30) can be further simplified by considering conservation of excitation energy. Indeed, the amount of excitation energy absorbed by the medium in a differential element $dz$ is $\mu_{a,\lambda}G_{\lambda}(z)dz$ [27]. Thus, the sum of the energy absorbed and the total energy reflected by the medium must be equal to the incident excitation energy $F_{0,\lambda}$. This can be expressed as [27]

$$F_{0,\lambda} = \int_{0}^{\infty} \mu_{a,\lambda}G_{\lambda}(z)dz + F_{0,\lambda}(R_{d,\lambda} + \rho_{01}),$$

where the integral term represents the excitation energy absorbed throughout the medium, and $F_{0,\lambda}R_{d,\lambda}$ and $F_{0,\lambda}/\rho_{01}$ represent the energy diffusely and specularly reflected by the medium, respectively. The quantity $F_{0,\lambda}(R_{d,\lambda} + \rho_{01})$ can be measured experimentally or calculated analytically [27,42]. By substituting Eq. (30) into Eq. (31), $k_1$ can be expressed as

$$k_1 = \zeta_{d,\lambda}^2\left(1 - \frac{R_{d,\lambda} - \rho_{01}}{1 - \omega_{tr,\lambda}} - \frac{k_2}{1 + \omega_{tr,\lambda}}\right).$$

Therefore, calculating $G_{\lambda}(\tau_{tr,\lambda})$ given by Eq. (15) for any value of $\omega_{tr,\lambda}$, $\beta_{tr,\lambda}$, and $n_1$ required the knowledge of a single empirical fitting parameter, namely, $k_2$.

B. Fluorescence Emission

In this section, an expression for the fluorescence flux emerging from the tissue and given by $(1 - \rho_{01})F_{\lambda}^e(0)$ is developed. To do so, the local excitation fluence rate given by Eq. (30) is used to solve Eqs. (18) and (19) with various fluorophore concentration profiles to yield

$$(1 - \rho_{10})F_{\lambda}^e(0) = (1 - \rho_{10})F_{0,\lambda}\xi_{f,\lambda}Q_{Y_f}A_T^{\lambda}.\tag{33}$$

The quantity $T_{xf}$ is a dimensionless function and will be referred to as the transfer function.

Before considering the different fluorophore concentration profiles depicted in Fig. 3, Eqs. (18) and (19) were solved for an isotropic emission source of unit intensity at an arbitrary optical depth $\tau_{tr,0}$. This so-called impulse response was found by replacing $\gamma_{\lambda}(z)G_{\lambda}(z)$ with $\delta(z - z_0)$ in Eqs. (18) and (19). The corresponding impulse transfer function $T_{xf}^0(\tau_{tr,0})$ can be expressed as

$$T_{xf}^0(\tau_{tr,0}) = \frac{(1 - \rho_{10})(a_{\lambda} + b_{\lambda} + 1)e^{-\zeta_{d,\lambda}^2\tau_{tr,0} - b_{\lambda} - \rho_{10}}}{2(a_{\lambda} + b_{\lambda} - \rho_{10})}.$$

Then, for any arbitrary concentration profile $M(\tau_{tr,\lambda})$, the fluorescence emission transfer function is given by

$$T_{xf} = \frac{1}{\beta_{tr,\lambda}} \int_{0}^{\infty} M(\tau_{tr,\lambda})G_{\lambda}(\tau_{tr,\lambda})T_{xf}^0(\tau_{tr,\lambda})d\tau_{tr,\lambda}.\tag{35}$$

Evaluating Eq. (35) for the two-layer concentration profile $M^S(\tau_{tr,\lambda}) = A_{\lambda}(\tau_{tr,\lambda} - \tau_{tr,\lambda-1})$ [Fig. 3(a)] yields

$$T_{xf}^S(\tau_{tr,\lambda-1}) = \frac{k_1e^{-\zeta_{d,\lambda}^2\tau_{tr,\lambda} + \zeta_{\lambda}^d\tau_{tr,\lambda}}}{\beta_{tr,\lambda}} + \frac{k_2e^{-\zeta_{d,\lambda}^2\tau_{tr,\lambda} - \zeta_{\lambda}^d\tau_{tr,\lambda-1}}}{\beta_{tr,\lambda}} T_{xf}^0(0).\tag{36}$$

Moreover, the transfer function for a homogeneous medium $T_{xf}^H$ [Fig. 3(b)] can be determined by setting
\( \tau_{tr, \lambda, 1} \) to zero in Eq. (36):

\[
T^H_{xf} = T^S_{xf}(0) = \left( \frac{k_1}{\zeta_{d, \lambda} r_{sf} + \zeta_{d, \lambda}} + \frac{k_2}{\zeta_{e, \lambda} r_{sf} + \zeta_{e, \lambda}} \right) \frac{T^S_{xf}(0)}{\beta_{tr, \lambda}}.
\]  

(37)

Finally, the transfer function due to the three-layer concentration profile depicted in Fig. 3(c) and given by

\[ M^L(\tau_{tr, \lambda, 1}, \tau_{tr, \lambda, 2}) = A[u(\tau_{tr, \lambda} - \tau_{tr, \lambda, 1}) - u(\tau_{tr, \lambda} - \tau_{tr, \lambda, 2})] \]

can be expressed as

\[
T^d_{xf}(\tau_{tr, \lambda, 1}, \tau_{tr, \lambda, 2}) = T^S_{xf}(\tau_{tr, \lambda, 1}) - T^S_{xf}(\tau_{tr, \lambda, 2}).
\]  

(38)

where \( T^S_{xf}(\tau_{tr, \lambda}) \) is defined by Eq. (36).

5. Results and Discussion

A. Local Excitation Fluence

Monte Carlo simulations were performed to calculate the normalized excitation fluence rate \( G_{\lambda, \lambda}^{MC}(\tau_{tr, \lambda, 1})/F_{0, \lambda, 1} \) for ten values of \( \omega_{tr, \lambda} \) between 0.50 and 0.99 and four values of \( n_1 \) between 1.00 and 2.00. The function \( G_{\lambda, \lambda}^{MC}(\tau_{tr, \lambda, 1})/F_{0, \lambda, 1} \) was observed to be self-similar with respect to \( \rho_{tr, \lambda, 1} \) so \( \rho_{tr, \lambda, 1} \) was set to unity for each simulation. A semi-infinite medium was simulated with a finite numerical grid of length \( L_{\text{grid}} \) in the z-direction set to be ten times the penetration depth for collimated light derived from diffusion theory so that \( L_{\text{grid}} = 10(3 \beta_{o,a, \lambda})^{-1/2} \) [40]. For this grid length, the finite slab was effectively semi-infinite, since all the energy of the collimated beam reaching \( z = L_{\text{grid}} \) vanished, thus satisfying the boundary condition given by Eq. (14). The grid consisted of \( N_z \) elements of length \( \Delta z = L_{\text{grid}}/N_z \). The number of grid elements \( N_z \) was set to be 200 as a compromise (i) to make \( \Delta z \) sufficiently small so as to capture the effects of internal reflectance on \( G_{\lambda, \lambda}^{MC}(z) \) near the medium’s surface and (ii) to ensure that the variance associated with the local fluence rate obtained by Monte Carlo simulations in each grid element was less than 1%.

The values of parameters \( k_1 \) and \( k_2 \) necessary in Eq. (30) were determined by minimizing the cost function (CF) defined as

\[
\text{CF} = \sqrt{\frac{1}{N_z} \sum_{i=1}^{N_z} [G_{\lambda, \lambda}^{MC}(i\Delta z) - G_{\lambda, \lambda}(i\Delta z)]^2},
\]  

(39)

corresponding to the root-mean-square error between the estimate of the excitation fluence rate predicted by Monte Carlo simulations \( G_{\lambda, \lambda}^{MC}(z) \) and \( G_{\lambda, \lambda}(z) \) predicted by Eq. (30). Figure 4 shows \( k_1 \) and \( k_2 \) found by minimizing the CF as functions of the diffuse reflectance \( R_{d, \lambda, x} \) for \( n_1 \), equal to 1.33, 1.44, 1.77, and 2.00 and \( \omega_{tr, \lambda, 1} \) ranging between 0.50 and 0.99. These quantities were plotted as functions of \( R_{d, \lambda, x} \) in order to provide a simple relationship between the diffuse reflectance \( R_{d, \lambda, x} \), which can be measured experimentally, and the local excitation fluence rate, which cannot. The value of \( k_1 \) increased with increasing \( R_{d, \lambda, x} \) and \( n_1 \), while the value of \( k_2 \) decreased with increasing \( R_{d, \lambda, x} \) and was approximately invariant with \( n_1 \). In fact, regardless of the value of \( n_1 \), \( k_2 \) could be approximated by a linear relationship with the logarithm of \( R_{d, \lambda, x} \), namely,

\[
k_2 = -0.137 \log_{10}(R_{d, \lambda, x}) - 1.357.
\]  

(40)

Values of \( k_1 \) computed from Eqs. (30) and (40) and those obtained by minimizing the CF agreed with each other to within 0.1%. In addition, the independence of \( k_2 \) with respect to \( n_1 \) was suggested by Eq. (17) by virtue of the fact that \( k_2 \) is independent of \( \rho_{o, \lambda} \). Note that Gardner et al. [12] used a similar approach by fitting six semiempirical parameters expressed as a function of the diffuse reflectance \( R_{d, \lambda, x} \). The authors considered a semi-infinite and homogeneous medium with \( n_1 = 1.33 \) or 1.38, \( g \) ranging from 0.7 and 0.9, and \( \omega_{tr} \) between 0.5 and 1.0. In contrast, Eqs. (30) and (32) predict the excitation fluence rate for (i) any values of \( n_1 \) between 1.0 and 2.0, (ii) \( g \) ranging from 0.7 and 0.9, and (iii) \( \omega_{tr} \) between 0.5 and 0.99. The model depends on a single semiempirical parameter \( k_2 \), depending on only the diffuse reflectance, and it ensures energy conservation.

For illustration purposes, Fig. 5(a) shows the normalized excitation fluence rate \( G_{\lambda, \lambda}^{MC}/F_{0, \lambda, 1} \) calculated by Monte Carlo simulations and predicted by Eqs. (30), (32), and (40) as a function of \( \zeta_{d, \lambda} \tau_{tr, \lambda} \) for \( \omega_{tr, \lambda} \) between 0.50 and 0.99 and \( n_1 = 1.44 \). The index of refraction \( n_1 \) was chosen to be 1.44 to represent the human dermis in the visible range [29]. In practice, the index of refraction of the medium under investigation would need to be determined separately. Furthermore, tissue is typically excited at a wavelength where absorption is strong. Thus, the transport single scattering albedo at the excitation wavelength \( \omega_{tr, \lambda} \) was chosen to be 0.700. We also
showed the cases when the transport single scattering albedo at the emission wavelength $\omega_{tr,\lambda}$ is close to and much larger than that at the excitation wavelength $\omega_{tr,\lambda}$. The relative deviation between the model predictions and the Monte Carlo simulation was less than 5% for all values of $\zeta_{d,\lambda} \tau_{tr,\lambda}$ and $\omega_{tr,\lambda}$ greater than 0.90 typical of biological tissues [29]. For $\omega_{tr,\lambda} \leq 0.90$ and $\zeta_{d,\lambda} \tau_{tr,\lambda} < 1$, the model predictions of $G_{x}(\tau_{tr,\lambda})$ deviated from Monte Carlo simulations by approximately 5%. For larger values of $\zeta_{d,\lambda} \tau_{tr,\lambda}$, model predictions and Monte Carlo simulations fell to within 1% of each other for all $\omega_{tr,\lambda}$. Figure 5(b) shows the CF for $n_1$ between 1.33 and 2.00 and $\omega_{tr,\lambda}$ between 0.50 and 0.99. The absolute root-mean-square error was less than 4% for all values of $n_1$ and $\omega_{tr,\lambda}$ considered.

B. Fluorescence Emission from One- and Two-Layer Media

Figures 6(a) and 6(b) show $T_S^{\text{sf}}$ estimated from a Monte Carlo simulation as a function of the transport optical thickness of the nonfluorescing layer $\tau_{tr,\lambda,1}$ associated with concentration profile $M^S(\tau_{tr,\lambda,1})$ for $\omega_{tr,\lambda}$ equal to 0.70, $r_{xf}$ varying between 0.20 and 5.00, for $\omega_{tr,\lambda}$ equal to 0.750 and 0.900, respectively, and $n_1 = 1.44$. These values are representative of biological tissues [29]. Predictions by Eqs. (32), (36), and (40) are also plotted for comparison. Figure 6 indicates that $T_S^{\text{sf}}$ decreased with increasing $\tau_{tr,\lambda,1}$. This can be attributed to the fact that the thickness of the top nonfluorescing layer increased. Thus, the fluorescence emission took place deeper inside the medium,
where the excitation fluence rate $G_{\lambda}(z)$ was reduced significantly. Additionally, the attenuation, or self-absorption [1], experienced by the fluorescent light as it traveled to the medium’s surface was larger. Similarly, as the ratio of extinction coefficients $r_{xf} = \beta_{tr,\lambda f}/\beta_{tr,\lambda x}$ increased, $T^S_{xf}$ decreased due to stronger attenuation by the medium at the fluorescent wavelength. For all cases considered, predictions by Eq. (36) followed the trend and magnitude of $T^S_{xf}$. In addition, prediction accuracy increased with increasing $r_{xf}$ and $\omega_{tr,\lambda x}$. This is due to the fact that the accuracy of the two-flux approximation with the boundary condition given by Eq. (20) diminishes for optically thin media and improves for strongly scattering media [17]. For the sake of clarity, only results for $\omega_{tr,\lambda x} = 0.70$ were shown. However, Fig. 6 is representative of the accuracy of Eq. (36) in predicting $T^S_{xf}$ for all the values of $\omega_{tr,\lambda x}$ considered in this study.

In order to compare model predictions of $T^S_{xf}$ used in Eq. (33) and Monte Carlo simulations for the fluorescence emission, the relative prediction error was defined as

\[
E(T_{xf}) = \left| \frac{T^S_{xf} - T^{MC}_{xf}}{T^{MC}_{xf}} \right|,
\]

where $T^{MC}_{xf}$ is the transfer function predicted by Monte Carlo simulations. Figure 7 shows the relative prediction error $E(T^S_{xf})$ for concentration profile $M^S(t_{tr,1})$ as a function of $\omega_{tr,\lambda x}$ for $\omega_{tr,\lambda f}$ equal to 0.700, $r_{xf}$ between 0.20 and 5.00, and $t_{tr,1}$ between 0 and 1.0. The relative error $T^S_{xf}$ was found to in-

Fig. 7. Relative error between prediction of $T^S_{xf}$ by Monte Carlo simulations and predicted by Eq. (36) averaged over $t_{tr,1}$ between 0 and 1 for $A = 1$ mole/cm$^2$, $\omega_{tr,\lambda x} = 0.70$, $n = 1.44$, and $r_{xf}$ between 0.20 and 5.00.

Fig. 8. Relative error between predictions of $T^S_{xf}$ by Monte Carlo simulations and by Eq. (37) as a function of $\omega_{tr,\lambda x}$ averaged over $\omega_{tr,\lambda f}$ between 0.50 and 1.00 for $r_{xf}$ between 0.20 and 5.00, $A = 1$ mole/cm$^2$, and $n_1 = 1.44$.

Fig. 9. Transfer function $T^L_{xf}$ versus the optical depth predicted by Monte Carlo simulations and by Eq. (38) with $\omega_{tr,\lambda x} = 0.700$, $A = 1$ mole/cm$^2$, $n_1 = 1.44$, $r_{xf}$ between 0.20 and 5.00, and $t_{tr,1} = 1$ cm for $\omega_{tr,\lambda f}$ equal to (a) 0.750 and (b) 0.900.
and greater than 0.44 and for \( \omega_{tr,\lambda} = \frac{70}{70} \), \( A = 1 \) mole/cm², \( n_1 = 1.44 \), and \( r_{sf} \) between 0.20 and 5.0.

Fig. 10. Relative error between prediction of \( T_{sf}^{\omega} \) by Monte Carlo simulations and by Eq. (35) averaged over \( \tau_{tr,\lambda} \), between 0 and 1 for \( \omega_{tr,\lambda} = 0.70, A = 1 \) mole/cm², \( n_1 = 1.44 \), and \( r_{sf} \) between 0.20 and 5.0.

Increase with decreasing \( \omega_{tr,\lambda} \) and \( r_{sf} \) for the same reasons as those previously discussed. For example, it was less than 5% for \( r_{sf} = 0.44 \) and \( \omega_{tr,\lambda} \) greater than 0.87. Furthermore, the relative error was less than 5% for all \( r_{sf} \) if \( \omega_{tr,\lambda} \) was greater than 0.95.

Similar results were found for the concentration profile \( M_{H}(\omega_{tr,\lambda}) \) when \( \tau_{tr,\lambda} = 0 \). Figure 8 shows the relative prediction error \( E(T_{sf}^{H}) \) as a function of \( \omega_{tr,\lambda} \) between 0.5 and 1.0 and \( r_{sf} \) between 0.2 and 5.0. In this case, the relative error was less than 5% for all values of \( r_{sf} \) considered and for \( \omega_{tr,\lambda} \) greater than 0.92. For \( \omega_{tr,\lambda} \) less than 0.70, the relative prediction error was larger than 10%, so these values were not reported.

C. Fluorescence Emission from Three-Layer Media

Figures 9(a) and 9(b) show the transfer function \( T_{sf}^{\omega} \) estimated from Monte Carlo simulations as a function of the transport optical thickness of the top nonfluorescing layer \( \tau_{tr,\lambda,1} \) for \( \omega_{tr,\lambda} \) equal to 0.70, \( r_{sf} \) between 0.20 and 5.00, and for \( \omega_{tr,\lambda} \) equal to 0.750 and 0.900, respectively. In each case, the thickness of the concentration layer was arbitrarily chosen such that \( \tau_{tr,\lambda,1} = L/\omega_{tr,\lambda} \), with \( L = 0.1 \) cm. Predictions by Eq. (35) using Eqs. (32) and (40) are also plotted for comparison. As \( \tau_{tr,\lambda,1} \) increased, the fluorescence emission occurred deeper within the medium, and thus, \( T_{sf}^{\omega} \) decreased, as was the case with \( T_{sf}^{\omega} \).

Figure 10 shows the relative prediction error \( E(T_{sf}^{\omega}) \) as a function of \( \omega_{tr,\lambda} \) for \( \omega_{tr,\lambda} \) between 0.50 and 1.00, and \( r_{sf} \) between 0.20 and 5.00. Here, also, the relative error increased with decreasing \( \omega_{tr,\lambda} \) and \( r_{sf} \). For \( r_{sf} = 0.44 \), the prediction error was less than 5% for \( \omega_{tr,\lambda} \) greater than 0.87. Furthermore, the prediction error was typically less than 5% for all the values of \( r_{sf} \) greater than 0.44 and for \( \omega_{tr,\lambda} \) greater than 0.97.

D. Model Limitations and Applicability

The current diffuse reflectance and fluorescence emission models are valid to semi-infinite media with optically smooth surfaces illuminated by uniform, collimated, and normally incident light and featuring uniform absorption and scattering coefficients and stepwise fluorophore concentration. They can be used to analyze reflectance measured by normal–normal optical probes, provided that a correction factor is determined to relate the normal–normal to the normal–hemispherical reflectance and fluorescence emission. The estimate of the fluorescence emission given by Eq. (33) is the integral of the emitted fluorescence intensity over the upper hemisphere. Similarly, the diffuse reflectance \( R_{d,u} \) used in Eq. (31) is the integral of the diffusely reflected intensity over the upper hemisphere. These quantities can be measured in practice with an integrating sphere [1, 29, 30]. The current model can be used in an inverse method to retrieve the optical properties of tissues and the fluorophore concentration.

6. Conclusion

A model of excitation fluence rate and surface fluorescence emission from turbid media was developed for semi-infinite, absorption, and strongly forward scattering media. The model accounts for index mismatch between the medium (\( n_2 \) between 1.0 and 2.0) and the surrounding air (\( n_0 = 1 \)). Using the two-flux approximation and invoking the energy conservation principle, the local excitation fluence rate was successfully modeled by Eqs. (30) and (32) with a single semiempirical parameter, depending only on diffuse reflectance [Eq. (40)]. Then, the total surface fluorescence emission was simulated for (i) homogeneous semi-infinite media, (ii) media with a semi-infinite fluorescing layer underneath a nonfluorescing layer, and (iii) media with a discrete fluorescing layer embedded in a nonfluorescing semi-infinite layer. For \( \omega_{tr,\lambda} \) greater than 0.90 and \( r_{sf} \) greater than 1, model predictions of the surface fluorescence emission were within 5% of results by Monte Carlo simulations for all values of \( \omega_{tr,\lambda}, \beta_{tr,\lambda}, \), and \( n_1 \) considered. The current model can be used to quickly and accurately simulate the local fluence rate and surface fluorescence emission of biological media, such as the skin, or to analyze fluorescence spectroscopy data gathered from in vivo biological samples to recover intrinsic fluorescence spectra using an inverse method.

Nomenclature

\( A \): fluorophore concentration, mole/cm³
\( a, b \): two-flux dimensionless parameters
\( \text{CF} \): cost function
\( F^+, F^- \): diffuse forward and backward fluxes, W/cm² · nm
\( F_0 \): radiative flux of incident beam, W/cm² · nm
\( g \): Henyey–Greenstein asymmetry factor
\( G \): local fluence rate, W/cm² · nm
\( I \): radiation intensity, W/cm² · sr · nm
\( r \): position vector, cm
\( k_1, k_2 \): fitting coefficients appearing in Eq. (15)
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