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A fresh look at the validity of the diffusion approximation for modeling fluorescence spectroscopy in biological tissue

Chintha Chamalie Handapangoda[1], Malin Premaratne [2] and Saeid Nahavandi[1]

Abstract— **Fluorescence has become a widely used technique for applications in noninvasive diagnostic tissue spectroscopy. The standard model used for characterizing fluorescence photon transport in biological tissue is based on the diffusion approximation. On the premise that the total energy of excitation and fluorescent photon flows must be conserved, we derive the widely used diffusion equations in fluorescence spectroscopy and show that there must be an additional term to account for the transport of fluorescent photons. The significance of this additional term in modeling fluorescence spectroscopy in biological tissue is assessed.**

I. INTRODUCTION

Low-power laser radiation induces tissue fluorescence without tissue damage and thus considered to be a very versatile tool in the diagnostic fluorescence spectroscopy with many applications in medicine [1]–[3]. Some example applications in medicine include the detection of atherosclerosis in the aorta and the coronary artery and dysplasia in the colon and other tissues [1], [4], diagnosis of early stages of cancer, measurement of the concentration of various exogenous agents such as the photosensitizers used in photodynamic therapy [2] and the detection of dental caries [1].

A majority of the diagnostic methods in fluorescence spectroscopy use empirical algorithms derived from studying a limited number of specimens and these algorithms ignore the wealth of biochemical and morphological information of the tissue [4]. Fluorescence spectra observed from optically thick tissue is distorted from the intrinsic spectra of individual fluorescence chromophores and consequently experiments utilizing optical-fibre probes in the clinical setting often yield results that are different from those utilizing a laboratory spectrofluorimeter [4]. Thus, there is a need for better model which enable us to account for intrinsic features of scattering in tissue and facilitate the accurate extraction of the information of physiochemical composition of tissue. Such a model will be able to provide biochemical and morphological information about tissue pathology for tracking the development of disease *in vivo* [4]. These requirements can be met only if such a model at least incorporates effects such as intrinsic fluorescence, scattering, absorption, excitation and collection geometries and the tissue boundary conditions [4].

[1]C. C. Handapangoda and S. Nahavandi are with the Centre for Intelligent Systems Research, Deakin University, Geelong, Victoria 3217, Australia chinthah at gmail.com

[2]M. Premaratne is with the Advanced Computing and Simulation Laboratory ($A\chi L$), Department of Electrical and Computer Systems Engineering, Monash University, Clayton, Victoria 3800, Australia malin.premaratne at monash.edu

Light propagation through tissue is best described by the photon transport theory [5], which is based on the principle of energy conservation. Modeling light propagation through tissue with embedded fluorophores is usually carried out by applying the diffusion approximation to the photon transport theory [2], [6]. However, none of the existing diffusion approximation formalisms systematically considers the simultaneous conservation of energy in both excitation and fluorescent beams and thus phenomenologically arrives at the final equations. The danger in such an approach is that significant contributing terms may escape from the formalism owing to mis-judgement, erroneous assumptions or failed intuition. We show that this is indeed the case with conventional diffusion approximation used in fluorescence spectroscopy and supplement the missing terms to make the coupled equations collectively obey the principle of energy conservation. To arrive at this result, we derived coupled photon transport equations for a tissue medium containing fluorophores. Klose *et al.* [7], [8] presented a tomographic reconstruction algorithm for optical molecular imaging of biological tissue, based on the time-independent (steady state) photon transport equation. Chang *et al.* [9], [10] modeled the migration of excitation and emitted fluorescence photons using two one-speed photon transport equations. We used the coupled time-dependent (transient) photon transport equations to derive the diffusion approximation that describe the distribution of the excitation light beam and the resulting fluorescent light scattered through the medium. To our surprise, the systematic derivation leads to an additional term, which is missing from the conventional usage in fluorescence spectroscopy. Here we show the physical reasons for the appearance of this term and the conditions under which we should retain this additional term to improve the accuracy of calculations.

II. MODIFIED DIFFUSION MODEL FOR FLUORESCENCE

Figure 1 shows a schematic diagram of fluorescence spectroscopy in tissue illustrating a path traced by a photon undergoing a frequency change due to absorption and re-emission by a fluorophore. A fluorophore is a substance that absorbs light and re-emits light of a different frequency with a time delay. We consider fluorophores that absorb light of excitation frequency ν_x and re-emits light of emission frequency ν_m with a time delay τ, which is called the fluorescent lifetime. Let $\sigma_{ax,tiss}$ and $\sigma_{am,tiss}$ be the absorption coefficient of tissue at ν_x and ν_m, respectively; ϕ_{eff} be the quantum efficiency for emission at ν_m given excitation at

ν_x, $\sigma_{ax,fl}$ be the absorption coefficient of fluorophores at ν_x and σ_{sx} and σ_{sm} be the scattering coefficient at ν_x and ν_m, respectively. Our formulation of the photon transport model for excitation and emission light was based on the principle of energy conservation. We derived this model by considering the conservation of the number of photons in an infinitesimal volume of the interacting tissue medium. This model consists of two coupled photon transport equations, each carrying a coupling function that corresponds to the coupling of excitation frequency to emission frequency of fluorophores. In the absence of coupling these two equations reduce to the conventional photon transport equation corresponding to each frequency.

We followed the method presented in [11] to arrive at the diffusion approximation of this photon transport model, systematically. The diffusion model thus derived resulted in the following two diffusion equations:

$$\frac{\partial \Phi_x(\mathbf{r},t)}{\partial t} - D_x c\nabla^2\Phi_x(\mathbf{r},t) + \sigma^0_{Tx} c\Phi_x(\mathbf{r},t) = 0, \tag{1}$$

$$\begin{aligned}&\frac{\partial \Phi_m(\mathbf{r},t)}{\partial t} - D_m c\nabla^2\Phi_m(\mathbf{r},t) + \sigma^0_{Tm} c\Phi_m(\mathbf{r},t)\\ &\underbrace{-3D_xD_m\nabla^2\frac{c}{\tau}\phi_{eff}\sigma_{ax,fl}\int_0^t e^{-(t-t')/\tau}\Phi_x(\mathbf{r},t')dt'}_{\text{new additional term}}\\ &-\frac{c}{\tau}\phi_{eff}\sigma_{ax,fl}\int_0^t e^{-(t-t')/\tau}\Phi_x(\mathbf{r},t')dt' = 0,\end{aligned} \tag{2}$$

where, $D_x = 1/(3\,(\sigma_{ax,fl} + \sigma_{ax,tiss} + (1-g)\sigma_{sx}))$, $D_m = 1/(3\,(\sigma_{am,tiss} + (1-g)\sigma_{sm}))$, $\sigma^0_{Tx} = (\sigma_{ax,fl} + \sigma_{ax,tiss})$ and $\sigma^0_{Tm} = \sigma_{am,tiss}$ with g being the anisotropy factor of the Henyey-Greenstein phase function [12].

Equation (1) is the same as (4a) of [6]. Equation (2) contains all the four terms in (4b) of [6] plus an additional term containing the Laplacian of the energy density corresponding to fluorescence activity. This additional term accounts for the fluorescence photon transport due to the deviation of fluorescence photons density at $\mathbf{r}$ from the average fluorescence density at an infinitesimal neighbourhood surrounding $\mathbf{r}$. Hence, this additional term accounts for the transport of energy density generated by fluorescence photons due to its gradients. That is, it is a diffusion term corresponding to the fluorescence photons.

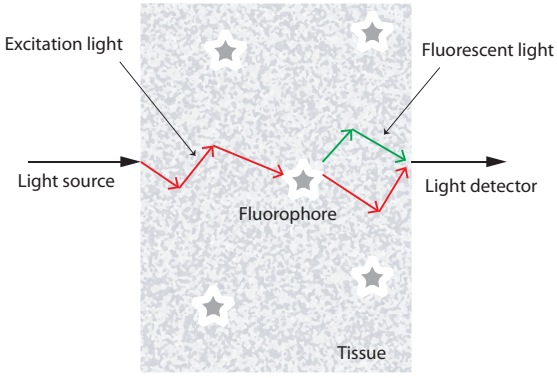

Fig. 1. Schematic diagram of fluorescence spectroscopy in tissue.

III. NUMERICAL RESULTS AND DISCUSSION

In order to assess the significance of the new term we will consider the steady state case in which the light source generates continuous wave excitation. For the steady state case the diffusion equations (1) and (2) reduce to

$$-D_x\nabla^2\Phi_x(\mathbf{r}) + \sigma^0_{Tx}\Phi_x(\mathbf{r}) = 0, \tag{3}$$

and

$$\begin{aligned}&-D_m\nabla^2\Phi_m(\mathbf{r}) + \sigma^0_{Tm}\Phi_m(\mathbf{r}) - \phi_{eff}\sigma_{ax,fl}\Phi_x(\mathbf{r})\\ &-3D_xD_m\phi_{eff}\sigma_{ax,fl}\nabla^2\Phi_x(\mathbf{r}) = 0.\end{aligned} \tag{4}$$

From (3) we have

$$\nabla^2\Phi_x(\mathbf{r}) = \frac{1}{D_x}\sigma^0_{Tx}\Phi_x(\mathbf{r}). \tag{5}$$

Using (5) in (4) results in

$$\begin{aligned}&-D_m\nabla^2\Phi_m(\mathbf{r}) + \sigma^0_{Tm}\Phi_m(\mathbf{r})\\ &= \phi_{eff}\sigma_{ax,fl}\left(1 + 3D_m\sigma^0_{Tx}\right)\Phi_x(\mathbf{r}).\end{aligned} \tag{6}$$

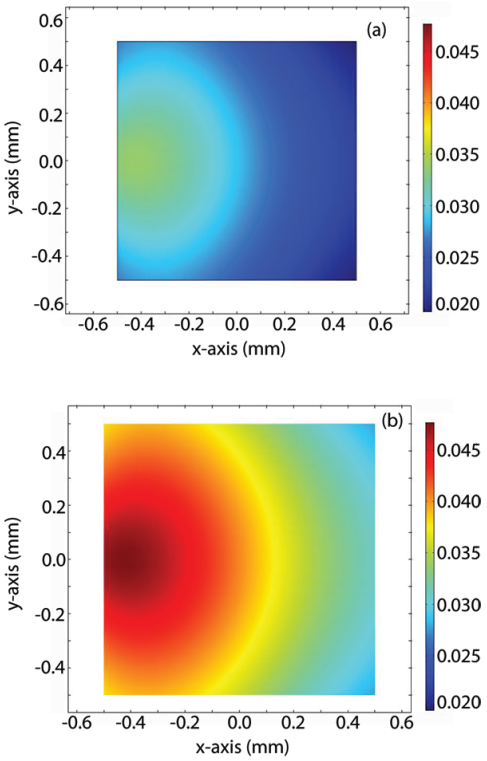

Fig. 2. Fluorescence photon energy density distribution for a line source of 1W power and 0.2mm length at the centre of the left boundary (a) energy density without the new term and (b) energy density increase due to the new term.

From (6) it can be deduced that when the term

$$3D_m\sigma^0_{Tx} = \frac{\sigma_{ax,fl} + \sigma_{ax,tiss}}{\sigma_{am,tiss} + (1-g)\sigma_{sm}} \tag{7}$$

is comparable with 1, the new term provides a significant contribution to the distribution of the fluorescence photon energy density, Φ_m, and cannot be neglected. From (7) we can see that the fluorophore absorption coefficient has a linear relationship with the term on its left hand side. The fluorophore absorption coefficient is linearly proportional to the fluorophore concentration [2]. Therefore, with increasing fluorophore concentration the influence of the new term becomes increasingly significant. From (7) it is evident that with increasing anisotropy factor the significance of the new term increases as well. This effect is shown in Fig. 3 and Fig. 4.

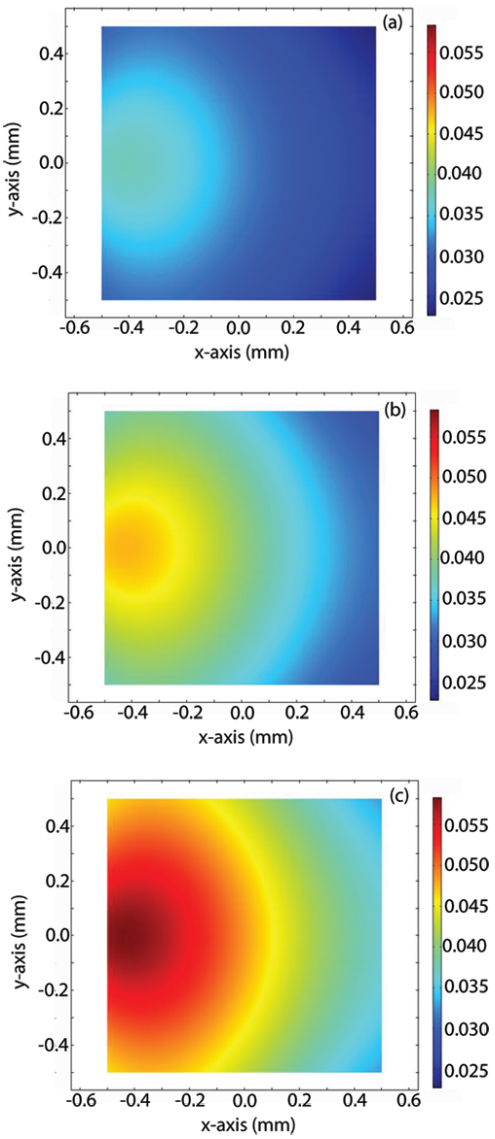

Fig. 3. Variation of the fluorescence photon energy density increase due to the new term with different values of fluorophore absorption coefficient, $\sigma_{ax,fl}$ (a) $\sigma_{ax,fl} = 0.5\text{mm}^{-1}$, (b) $\sigma_{ax,fl} = 0.6\text{mm}^{-1}$ and (c) $\sigma_{ax,fl} = 0.7\text{mm}^{-1}$.

For the simulation results presented in Fig. 2 to Fig. 4 we used the values provided in [13] for the optical properties of human skin in the near infrared wavelength. We have assumed an average absorption coefficient of fluorophores of the same order as that of the tissue.

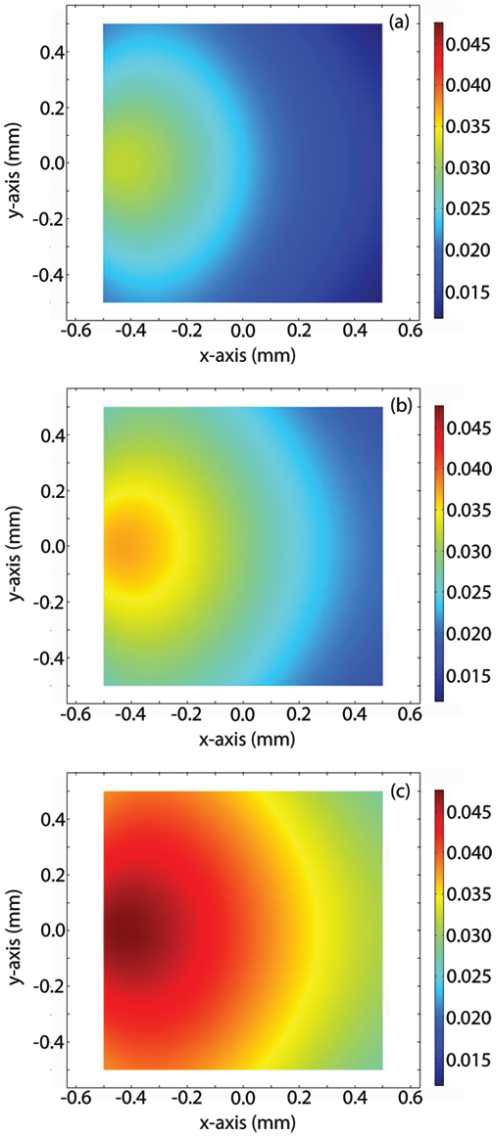

Fig. 4. Variation of the fluorescence photon energy density increase due to the new term with different values of anisotropy factor, g (a) $g = 0.1$, (b) $g = 0.5$ and (c) $g = 0.9$.

Fig. 2 shows the fluorescence photon energy density distribution for a line source of 1W power and 0.2mm length located at the centre of the left boundary. For this simulation we have set $\sigma_{ax,fl} = 0.6\text{mm}^{-1}$, $\sigma_{ax,tiss} = 0.5\text{mm}^{-1}$, $\sigma_{am,tiss} = 0.7\text{mm}^{-1}$, $\sigma_{sx} = \sigma_{sm} = 0.8\text{mm}^{-1}$, $g = 0.9$ and ϕ_{eff} was taken to be 0.58 as in [6]. A tissue refractive index of 1.4 was assumed [14]. Fig. 2(a) shows the fluorescence photon energy density without the new term and Fig. 2(b) shows the fluorescence photon energy density increase due to the new term. Note that the energy density contribution due to the new term is more than twice the previous value.

Fig. 3 shows the variation of the fluorescence photon energy density increase due to the new term with different values of fluorophore absorption coefficient. All the other

simulation parameters were kept constant at the values used for Fig. 2 while the fluorophore absorption coefficient was varied from $0.5\mathrm{mm}^{-1}$ to $0.7\mathrm{mm}^{-1}$. It can be concluded from Fig. 3 that the fluorescence energy density increase due to the new term increases as the fluorophre concentration increases.

Fig. 4 shows the variation of the fluorescence photon energy density increase due to the new term with different values of anisotropy factor, g, of the Henyey-Greenstein phase function. We kept all the other parameters constant at the values used for Fig. 2 while the anisotropy factor was varied from 0.1 to 0.9. It can be seen from Fig. 4 that the fluorescence photon energy density increase due to the new term increases as the anisotropy factor increases. When modeling biological tissue with the Henyey-Greenstein phase function, the anisotropy factor is usually taken to be around 0.9 due to the highly forward-peaked nature of scattering. It can be seen from Fig. 4(c) that for this value of the anisotropy factor, the contribution of the additional term is quite significant. Therefore, neglecting the additional term may result in significant errors in fluorescence based imaging of biological tissue.

IV. CONCLUSIONS

In this paper we presented a modified diffusion approximation for fluorescence in biological tissue based on a more accurate photon transport model. Our simulations based on realistic biological tissue parameters showed that the new additional term in the modified diffusion model is significant and cannot be neglected for fluorescence spectroscopy in medical applications. We also showed that with increasing uneven fluorophore concentration and increasing anisotropy factor the significance of this new term increases. Hence we recommend this revised diffusion model for modeling fluorescence spectroscopy in biological tissue to prevent significant errors.

REFERENCES

[1] L. I. Deckelbaum, J. K. Lam, H. S. Cabin, K. S. Clubb, and M. B. Long, Discrimination of normal and atherosclerotic aorta by laser induced fluorescence, Laser Surg. Med., vol. 7, pp. 330–335, 1987.

[2] M. S. Patterson and B. W. Pogue, Mathematical model for time-resolved and frequency-domain fluorescence spectroscopy in biological tissues, Appl. Opt., vol. 33, no. 10, pp. 1963–1974, 1994.

[3] S. A. Engels and B. C. Wilson, *In vivo* fluorescence in clinical oncology: fundamental and practical issues, J. Cell. Pharmacol., vol. 3, pp. 66–79, 1992.

[4] J. Wu, M. S. Feld, and R. P. Rava, Analytical model for extracting intrinsic fluorescence in turbid media, Appl. Opt., vol. 32, no. 19, pp. 3585–3595, 1993.

[5] M. Premaratne, E. Premaratne, and A. J. Lowery, The photon transport equation for turbid biological media with spatially varying isotropic refractive index, Opt. Express, vol. 13, no. 2, pp. 389–399, 2005.

[6] C. L. Hutchinson, J. R. Lakowicz, and E. M. Sevick-Muraca, Fluorescence lifetime-based sensing in tissues: A computational study, Biophys. J., vol. 68, pp. 1574–1582, 1995.

[7] A. D. Klose, and A. H. Hielscher, Fluorescence tomography with simulated data based on the equation of radiative transfer, Opt. Lett., vol. 28, pp. 1019–1021, 2003.

[8] A. D. Klose, V. Ntziachristos, and A. H. Hielscher, The inverse source problem based on the radiative transfer equation in optical molecular imaging, J. Comput. Phys., vol. 202, pp. 323–345, 2005.

[9] J. Chang, H. L. Graber, and R. L. Barbour, Imaging of flourescence in highly scattering media, IEEE Trans. Biomed. Eng., vol. 44, pp. 810–822, 1997.

[10] J. Chang, R. L. Barbour, H. Graber and R. Aronson, Fluorescence optical tomography, Experimental and Numerical Methods for Solving Ill-posed Inverse Problems: Medical and Nonmedical Applications, SPIE vol. 2570, pp. 59–72, 1995.

[11] T. Khan and A. Thomas, On derivation of the radiative transfer equation and its spherical harmonics approximation for scattering media with spatially varying refractive indices, Clemson University Mathematical Sciences, Tech. Rep. TR2004-12-KT:149, 2004.

[12] G. E. Thomas and K. Stamnes, Radiative transfer in the atmosphere and ocean, Cambridge: Cambridge University Press, 1999.

[13] T. Troy and S. N. Thennadil, Optical properties of human skin in the near infrared wavelength range of 1000 to 2200 nm, J. Biomed. Opt., vol. 6, pp. 167–176, 2001.

[14] J. J. J. Dirckx, L. C. Kuypers, and W. F. Decraemer, Refractive index of tissue measured with confocal microscopy, J. Biomed. Opt., vol. 10, pp. 044014, 2005.