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A Theoretical and Experimental Examination of Fluorescence in Enclosed Cavities

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Abstract

Photosensitizer fluorescence emitted during photodynamic therapy (PDT) is of interest for monitoring the local concentration of the photosensitizer and its photobleaching. In this study, we use Monte Carlo (MC) simulations to evaluate the relationship between treatment light and fluorescence, both collected by an isotropic detector placed on the surface of the tissue. In treatment of the thoracic and peritoneal cavities, the light source position changes continually. The MC program is designed to simulate an infinitely broad photon beam incident on the tissue at various angles to determine the effect of angle. For each of the absorbed photons, a fixed number of fluorescence photons are generated and traced. The theoretical results from the MC simulation show that the angle θ has little effect on both the measured fluorescence and the ratio of fluorescence to diffuse reflectance. However, changes in the absorption and scattering coefficients, μ_a and μ'_s , do cause the fluorescence and ratio to change, indicating that a correction for optical properties will be needed for absolute fluorescence quantification. Experiments in tissue-simulating phantoms confirm that an empirical correction can accurately recover the sensitizer concentration over a physiologically relevant range of optical properties.

Keywords

Photodynamic; Fluorescence; Photobleaching; Optical Properties; Monte Carlo; Pleural

1. INTRODUCTION

Photodynamic therapy (PDT) of the thoracic cavity has been investigated in several clinical trials at the University of Pennsylvania.¹ These treatments are performed intraoperatively, following surgical debulking of disease. The goal of these treatments is nominally to deliver a uniform dose of cytotoxic reactive oxygen species to the remaining microscopic disease. The remaining tumor cells are assumed to cover the entire interior surface of the thoracic cavity, including the chestwall, mediastinum, and the exterior surface of the lung, in cases where the lung is left intact. Delivering uniform PDT dose to this entire surface is a significant challenge.

In our current clinical protocols, we deliver light to the cavity *via* an optical fiber inserted into modified endotracheal tube filled with a scattering solution, producing an extended isotropic light source. This light source is moved around the cavity by the physician. The dose delivered to the surface of the cavity is monitored continuously using fiber optic-based isotropic detectors. The position of the light source can be tracked optically, providing real-time feedback to the physician.² Efforts are underway to model the propagation of light with in the cavity and the surrounding tissue.³

Recently, we have developed the ability to collect fluorescence emitted by the photosensitizer (HPPH in this case) during treatment. In this scenario, an optical filter blocks the treatment light collected by an isotropic detector, while letting the slightly longer-wavelength fluorescence emission pass through to a spectrometer for detection. In this paper, we present a theoretical examination of the relationship between the fluorescence collected by an isotropic detector on the surface of the cavity, the fluence rate of treatment light collected by the same detector, and the optical properties of the underlying tissue.

2. METHODS

2.1 Monte Carlo Code

The Monte Carlo algorithm used here was written in Matlab (The Mathworks Inc., Natick, MA.) adapted from the smallmc code previously developed by Prahl et al.⁴ This code uses the implicit capture method described previously⁵ and implemented in the MCML package.⁶ Briefly, this code traces a photon with initial weight of 1 from launch through multiple scattering events. At each scattering event, the photon weight is reduced depending on the optical properties of the medium. Photons are traced until they either escape the medium or fall below a threshold weight, triggering a random 'roulette' process in which a photon has a one in ten chance of surviving with ten times its initial weight and a nine in ten chance of being terminated. The algorithm records the distribution of absorbed light dose in the tissue-simulating medium and the diffuse and specular components of the reflected light.

In this case, we model the tissue in the cavity as a semi-infinite medium. The light source is modeled as an infinitely wide, monodirectional light source. While this is not strictly accurate, it is sufficient to elucidate the basic dynamics of fluorescence detection using an isotropic detector. Photons are launched at the surface with an initial angle determined by Snell's law. Specular reflection at the surface is calculated by the Fresnel reflectance for unpolarized light.

2.2 Fluorescence Modeling

To model fluorescence, at each absorption/scattering event, we launch a separate fluorescence photon with a weight 1/100 the weight of the incoming photon. This ensures that fluorescence photons are generated in proportion to the absorbed excitation light, but reduces the time spent tracking them by forcing many to undergo roulette. In this case, we consider the optical properties to be identical at the absorption and emission wavelengths. We score all photons emitted from the tissue, simulating the signal measured by an isotropic detector.

The number of photons emitted from the surface and the distribution of light within the tissue are scored separately for fluorescence and reflectance. Because it is not possible for an isotropic detector to separate incident light, diffuse reflectance, and specular reflectance, we add these components together to obtain the treatment wavelength signal. The fluorescence signal is considered separately because it can be separated spectroscopically.

We have simulated two conditions, one simulating a tissue-equivalent medium surrounded by air (representing the phantoms we can measure in the lab) and one simulating the same tissue-equivalent medium surrounded by water (representing the clinical situation, where the cavity is filled with a dilute intralipid solution). Simulations were performed with a μ'_s of 10 cm^{-1} and μ_a ranging from 0.01 to 10 cm^{-1} . The symmetry of the semiinfinite geometry means that other values of μ'_s can be obtained by scaling, as discussed below.

3. RESULTS

3.1 Reflectance

Increasing the angle of incidence from 0 (normal incidence) increases the reflectance, as shown in figure 1. This agrees with previous simulations showing that the fluence rate immediately under the surface is increased in obliquely incident beams because the mean depth of the first scattering event is shifted toward the surface.⁷ This is particularly noticeable in the case of the nearly-index matched case of a water-filled cavity (left panel). In the extreme case of 85° incidence, the diffuse reflectance decreases because of the dramatic increase in specular reflectance. The dotted lines in each figure indicate the reflectance as a fraction of the light that enters the tissue, rather than the incident light. In this case, the increase in diffuse reflectance with angle is clear. In the air-filled cavity (right panel), the increased reflectance of photons exiting the medium, and the resulting increase in multiple scattering near the surface, tends to reduce this effect.

3.2 Fluorescence

The corresponding fluorescence dependence on angle exhibits similar effects, as shown in figure 2. As the angle increases, the point of mean first absorption (and hence the point of first fluorescence) moves toward the surface, increasing the likelihood of fluorescence escaping the tissue. This effect is most prominent in cases of high μ_a . At low μ_a , even relatively long pathlengths exhibit relatively little absorption, so the effect of the depth of fluorescence generation is less important. In these cases, the volume of tissue illuminated by the excitation light becomes more important. At shallow angles of incidence, both the diffuse and specular reflectances increase, and the volume of tissue illuminated effectively decreases, decreasing the fluorescence emission.

In any realistic clinical situation, the quantities we can measure will be the total treatment light (incident light plus diffuse and specular reflectance). In the particular clinical cases we're concerned with, the intensity of the incident treatment light varies with time. Therefore, the quantity of interest will be the fluorescence emission scaled by the total measured treatment light.

Scaling by the measured treatment light will account for variation in treatment source location and intensity, but not necessarily the effects of tissue optical properties or angle of incidence. These effects are summarized in figure 3. While the most extreme effects of angle of incidence (which are common to the reflectance and fluorescence curves) are smoothed out somewhat by taking the ratio, the essential dependence on tissue optical properties remains. This can be seen clearly in figure 4. Except for the extreme case of 85° incidence, a single Fluorescence ratio - μ_a curve describes the behavior under all conditions. This is true for both the air-filled and water-filled cavities, though the detailed shape of the curve differs. This implies that it is not particularly important to account for the angle of incidence. It is, however, important to account for variations in optical properties (μ_a and μ'_s) of tissue, and use a model appropriate for the index of refraction mismatch at the tissue surface.

3.3 Scaling to account for variations in μ'_s

The diffuse reflectance calculated here is a ratio of escaping photon weight to incident photon weight. Therefore, it is scale-invariant. Changing the value of μ'_s will not change the value of reflectance as long as the transport albedo defined as

$$a' = \frac{\mu'_s}{\mu_a + \mu'_s} \quad (1)$$

remains the same. Therefore, the diffuse reflectance collected from a medium with a μ'_s of 10 cm^{-1} and μ_a of 1 cm^{-1} will be the same as that collected from a medium with μ'_s and μ_a of 5 and 0.5 cm^{-1} , respectively.

The scaling of the fluorescence for different values of μ'_s is more complicated. The same arguments used to demonstrate scale-invariance for reflectance apply with one exception: We have performed all our simulations with the same concentration of fluorophore (by making the probability of fluorescence equal proportional to the incident photon weight at each step). Consider the case where the value of μ'_s is doubled. Scale invariance holds if μ_a is also doubled. However, this also requires a doubling of the effective μ_a contributed by the fluorophore. To take the previous example, the fluorescence from medium 1 ($\mu'_s = 10 \text{ cm}^{-1}$, $\mu_a = 1 \text{ cm}^{-1}$) would be the same as that from medium 2 ($\mu'_s = 5 \text{ cm}^{-1}$, $\mu_a = 0.5 \text{ cm}^{-1}$) only if the μ_a contribution from the fluorophore, and therefore the fluorophore concentration in medium 1 is twice that in medium 2. Equivalently, if the fluorophore concentration is kept the same and μ'_s and μ_a are doubled, the fluorescence collected will decrease by a factor of two.

3.4 Correction for optical properties

The ultimate goal of the measurements simulated here is to characterize the distribution of, and changes in, sensitizer concentration based on a treatment light-excited fluorescence measurement. Based on the simulated data here, we have developed correction based on a power law of the form

$$C = \frac{A}{\mu_s'} \left(\frac{\mu_a}{\mu_s'} \right)^b, \quad (2)$$

where A and b are constants specific to the index mismatch. The leading μ_s' accounts for the fluorescence scaling described above, while the power law accounts for the effects of absorption. This is a purely empirical correction, however it does meet the basic requirements of scale invariance. The effect of this correction are shown in figure 5. While this correction is adequate to recover the intrinsic fluorescence in the simplified models presented here, it may not be adequate in a realistic clinical situation. Work in experimental validation of this and other corrections is ongoing.⁸

4. DISCUSSION

4.1 Clinical Implementation

The measurements simulated here are part of a larger effort, the goal of which is to predict the tissue response across the entire interior surface of the cavity being treated, and to provide feedback to the clinician to make that response more uniform. Fluorescence measurement can be included in this framework as follows. Prior to the start of treatment, the optical properties of the tissue in the neighborhood of the isotropic detector will be measured by optical methods. As a first approximation, these optical properties can be assumed not to change during treatment. A correction factor will be calculated for each isotropic detector based on the measured optical properties in its local neighborhood. The measured fluorescence will then be monitored in near real-time throughout treatment. The fluorescence of the sensitizer will be corrected for the effects of optical properties, and the sensitizer concentration will be calculated. Changes in sensitizer concentration will inform the calculation of PDT dose and be used for quantification of fluorescence photobleaching.

4.2 Future Directions

In the future, we plan to replace the point measurements described here with fluorescence imaging. The modeling described here will need some modification to accommodate fluorescence imaging. First, the collection angle of an imaging detector will be less than that of an isotropic detector. This may change some of the conclusions presented here. Second, in the current implementation, we have assumed that the optical properties and fluorophore distributions are homogeneous. In the point-measurement case, this is the most conservative assumption. Moving toward a higher resolution measurement will require accommodating spatial variations in both optical properties and fluorophore concentration. Both of these factors will require moving from the 1-D geometry presented here to a full 3-D geometry for calculations of light dose and sensitizer excitation. Work in this area is ongoing³

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Figure 1.

Diffuse reflectance as a function of angle of incidence for semiinfinite media with a surrounding medium of water (left panel) and air (right panel). The value of μ_a is listed in the legend. μ_s' for all simulations. Dotted lines indicate the reflectance as a fraction of light that enters the tissue.

Figure 2.

Emitted fluorescence as a function of angle of incidence for semiinfinite media with a surrounding medium of water (left panel) and air (right panel). The value of μ_a is listed in the legend. μ'_s was cm^{-1} for all simulations. Dotted lines indicate the fluorescence as a fraction of light that enters the tissue.

Figure 3.

Emitted fluorescence as a fraction of total collected treatment light. The conditions are the same as those in figure 1.

Figure 4.

Emitted fluorescence as a fraction of total collected treatment light, plotted as a fraction of μ_a . The conditions are the same as those in figure 1. Angle of incidence is indicated in the legend.

Figure 5.

Effects of the correction factors given in equation 2 on the simulated fluorescence ratio. In each panel, the triangles indicate the uncorrected data, and the circles the data after correction. The data are normalized to show the variation with μ_a .