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A method to improve reconstruction of the distribution of hemoglobin, oxygenation, and MLu concentration in the human prostate before and after photodynamic therapy

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Abstract

Explicit dosimetry of photodynamic therapy requires detailed knowledge of the light, drug, and oxygenation distributions within the target tissue. We present a method for the optical detection and three-dimensional reconstruction of hemoglobin concentration and oxygenation and sensitizer concentration within the human prostate. Spectrally resolved diffuse transmission measurements were made using a small isotropic fiber-based white light source and an isotropic detector inserted into the prostate via parallel closed transparent catheters. The spectra were modeled using the diffusion approximation appropriate for infinite media. The optical absorption of the prostate was assumed to be a linear combination of the absorption spectra of oxy- and deoxyhemoglobin and MLu, and the scattering was assumed to be of the form $A(\lambda/\lambda_0)^{-b}$. The separation of absorption and scattering coefficients was accomplished based on the spectral shape of the diffuse transmission, rather than the spatial variation in intensity. By making multiple measurements at various source-detector separations, we investigate the signal-to-noise sensitivity of our algorithm. In addition, the redundancy in our source-detector position matrix creates several positions in which the tissue parameters can be reconstructed from multiple independent measurements, allowing an assessment of the repeatability of the algorithm. We find significant heterogeneity in the reconstructed optical properties; however the recovery of spectrally consistent absorption and scattering spectra is improved compared to wavelength-wise reconstruction algorithms.

Keywords

Photodynamic therapy; absorption; spectroscopy; sensitizer; diffusion

1. INTRODUCTION

Dosimetry in photodynamic therapy (PDT) requires knowledge of the basic photophysical and optical parameters of the tissue being treated. From the microscopic point of view, these consist of the local light fluence rate, the local photosensitizer concentration, and the local oxygen concentration. In clinical practice, these local parameters are difficult or impossible to measure. Fortunately, one can determine macroscopic quantities, correlating to volume

averaged microscopic quantities, which may be sufficient to predict clinical outcome. These macroscopic quantities include the optical properties of the tissue, the light source distribution, the macroscopic sensitizer distribution, and the tissue oxygenation as indicated by volume-averaged hemoglobin oxygen saturation. In this paper, we present an improved method for determining the distributions of sensitizer concentration, hemoglobin volume and saturation, and optical properties during interstitial PDT.

We have previously reported the results of a similar study in which the absorption spectrum was determined by making multiple spectral measurements at varying source-detector separations and fitting them with a radiative transport-based analytical theory.¹ When the methods outlined in that paper were applied to a larger patient population, it was found that many of the spectroscopic profiles contained artifacts that made them uninterpretable. In the current paper, we seek to remedy this problem by making use of the known properties of the *in vivo* absorption and scattering spectra and the optical properties measured at a single wavelength.

2. METHODS

2.1 Patient Population

We have previously reported the use of photodynamic therapy (PDT) mediated by the photosensitizer motexafin lutetium (MLu) for recurrent adenocarcinoma of the human prostate.^{2,3} The clinical trial from which the data presented here was taken is a Phase I dose escalation study in which the MLu is administered to the patient at a predetermined interval prior to treatment (3 hours for the patients shown here) and the entire prostate is treated to a pre-determined fluence rate, as monitored by *in vivo* dosimetric probes. The goal of this study is to establish the maximum tolerated dose (MTD) of MLu and light for treatment of the human prostate.

2.2 Single-wavelength and spectroscopic absorption measurement

The apparatus designed for absorption measurements at a single wavelength and for spectrally-resolved measurements of absorption has been described previously.^{1,4,5} A schematic of the setup is shown in figure 1. Both measurements rely on the collection of light emitted by an isotropic 2 mm long cylindrical fiber optic light source by an isotropic fiber optic detector. In the case of single-wavelength measurements, the light source is coupled to the 732-nm treatment laser, and the light is detected by one of several photodiodes incorporated into our *in vivo* dosimetry system. The absorption and scattering coefficients are determined by a nonlinear fitting algorithm that models light propagation with the infinite medium diffusion approximation. The accuracy of this method is determined experimentally to be better than 8% for μ_a and 18 for μ_s' .⁶

For spectroscopic measurements, the laser is replaced with a quartz-tungsten-halogen (QTH) lamp, and the light is detected by a charge-coupled device (CCD) and spectrograph. Both the source and detector fibers are moved along their respective catheters by a computer-controlled step motors (Velmex, Inc., East Bloomfield, NY).

A single spectrum is acquired at each source-detector position combination. The measured intensity spectrum $I(\lambda)$ is converted to a diffuse fluence rate spectrum using

$$\Phi(\lambda) = C \left(\frac{I_{\text{sample}}(\lambda)}{I_{\text{IS}}(\lambda)} \right), \quad (1)$$

where $I_{\text{IS}}(\lambda)$ is the intensity spectrum measured in a calibrated integrating sphere (Labsphere, North Sutton, NH) using the same source-detector pair used for the sample measurement, and C is a constant relating the source power to the fluence rate in the integrating sphere. The value of C is unique to a given integrating sphere; however for our particular sphere it is independent of wavelength over the range of our measurement. The value of C has been determined independently using a calibrated laser source and isotropic detector. The result of equation 1 is a spectrum representing the fluence rate per unit source power as a function of wavelength. The dependence on instrument response and excitation source spectrum is eliminated.

Data analysis

It is theoretically possible to use a well-calibrated spectrometer to reconstruct the absorption and scattering coefficients at each wavelength independently using the same algorithm we employ in the single-wavelength measurements. In practice, it is helpful to constrain this fit by requiring the scattering spectrum of the sample to take the form

$$\mu'_s = A \left(\frac{\lambda}{\lambda_0} \right)^{-b}. \quad (2)$$

We have previously demonstrated that this method is applicable to patient data in cases of good signal and accurate positioning of the source and detector.¹ However, this approach can fail to correctly interpret *in vivo* data for a number of reasons. First, the acquisition time required for a single spectroscopic measurement is long enough that in a clinical setting it is possible to make spectroscopic measurements at only 8 to 12 detector positions for each source position, in contrast to the 200 to 800 points collected in the single-wavelength method. Second, the absorption coefficient at many of the wavelengths measured is much higher than that at the treatment wavelength. The signal measured at these wavelengths, and therefore the corresponding signal-to-noise ratio, is very low. On the other hand, spectroscopic data has the significant advantage of containing information over a range of wavelengths.

Our correction of the spectroscopic data assumes that the optical properties measured at the treatment wavelength are correct. Using these known optical properties, we reconstruct a theoretical curve describing the dependence of fluence rate on source-detector separation. The theoretical curve is derived from the diffusion approximation to radiative transport for a point source in an infinite medium, which we have also used for our single-wavelength analysis.⁵ Ideally, the spectroscopic data point acquired at the treatment wavelength will fall exactly on this curve. In practice, there are a number of artifacts that can cause disagreement. Our method, which we will refer to as 'position correction', assumes that the

intensity measured is correct, but that its recorded position may be distorted. In this case, the analysis is performed with the position of the spectroscopic data adjusted to match the theoretical curve.

The rationale for this strategy is that the source-detector separation is often difficult to determine in spectroscopic measurements, and small errors in position may cause large errors in intensity in regions of high fluence rate gradient. The corrected spectroscopic data matches the analytic theory exactly at the treatment wavelength, and the continuity of the spectrum is maintained. It is possible that the artifacts are in reality caused by intensity, rather than position, errors. However, over the range of optical properties and source-detector separations we expect to encounter *in vivo*, the shape of the spectrum is not critically dependent on source-detector separation. Therefore the results of position correction and intensity correction are not dramatically different (data not shown).

Once the corrected diffuse transmission spectra are generated, we make use of equation 2, with b set to 1.2, to generate a reduced scattering spectrum given by

$$\mu'_s = \mu'_{s(732)} \left(\frac{\lambda}{732nm} \right)^{-1.2} \quad (3)$$

The selection of a fixed value of b makes the scattering spectrum shape constant, with its value fixed by the single-wavelength data. This makes the fitting algorithm insensitive to changes in the shape of the sample's scattering spectrum, however we expect these changes to be small compared with changes in the amplitude of the scattering spectrum. The absorption spectrum is calculated from the scattering spectrum by inverting the diffusion approximation for fluence rate at a distance r from a point source in an infinite medium, giving

$$\mu_a = \frac{1}{3\mu'_s r^2} \left[\ln \left(\frac{4\pi r}{3\mu'_s} \Phi \right) \right]^2 \quad (4)$$

Thus, a single measurement of the absorption and reduced scattering coefficients of tissue and a single diffuse transmission spectrum allow a complete reconstruction of the absorption and reduced scattering spectra of the sample. The scattering spectrum gives no new information about the sample. Its amplitude is known from the single-wavelength measurement, and while the scattering shape may be indicative of morphological changes in the tissue,⁷⁻⁹ our assumption of a fixed shape renders our algorithm insensitive to such changes. The absorption spectrum of the tissue, on the other hand, contains a great deal of information. We assume that the absorption spectrum of tissue is the linear combination of the absorption spectra of a set of known basis spectra,

$$\mu_a(\lambda) = \sum c_i \varepsilon_i(\lambda) + F(\lambda), \quad (5)$$

where c_i is the concentration of the i^{th} absorber, ε_i is its absorption spectrum, and F is an exponentially-weighted Fourier series designed to account for the absorption by unknown

components. The values of the various c_i 's are determined using a singular value decomposition (SVD) algorithm. This algorithm has been used previously for the fitting of absorption and fluorescence spectra.^{1,10,11} The amplitude assigned to the Fourier series can serve as a measure of the goodness of the fit: a large Fourier component indicates either the presence of unknown absorbers or errors in the determination of the absorption spectrum.

For ease of implementation and use, we have designed a Matlab-based graphical user interface (GUI) to run the fitting algorithm. The interface presented to the user by this program is shown in figure 2. This program includes subroutines for reading in the data files from the spectrometry system, processing the data, and performing various fits.

3. RESULTS

3.1 Spectral Fitting

A typical set of diffuse fluence rate spectra is shown in figure 3. The thin lines superimposed on the data represent the best fit using a wavelength-by-wavelength fitting algorithm reported previously.¹ The corresponding profile at 732 nm is shown in figure 3b. In this case, the data is data on the left side of the plot (open circles) are artificially high. The corresponding spectra have not been included in the fitting. One source of uncertainty in the analysis of such a set of spectra is the difficulty in determining the position of the center of the curve, *i.e.* the position at which the detector is closest to the source. In wavelength-by-wavelength fitting, the outcome can be critically dependent on this position. The absorption spectrum recovered by the fit shown in figure 3 is shown in figure 4, along with the best fit with a set of known basis spectra. The relatively large amplitude of the residual, or Fourier series, contribution is indicative of the artifacts introduced by the fitting algorithm.

Figure 5 shows the data points shown in figure 3b along with the corresponding curve predicted by the diffusion approximation informed by the single-wavelength-determined optical properties ($\mu_a = 1.14 \text{ cm}^{-1}$, $\mu_s' = 1.69 \text{ cm}^{-1}$).

The symbols show the positions of the data points as measured (squares) and corrected (circles). The absorption spectra derived from this fit at each source-detector separation are shown in figure 6. All the absorption spectra agree at 732 nm, a consequence of the assumptions of the correction method. The agreement at other wavelengths is a confirmation that any inaccuracies in the correction method have relatively little effect on the shape of the spectrum.

The SVD analysis of one of these spectra is shown in figure 7. Compared to the fit to the uncorrected spectrum (figure 4), this fit exhibits a much smaller residual, indicating that the derived spectrum is consistent with that expected from a sample whose absorption is dominated by the known absorbers used in our basis set. The fact that the residual take the form of a monotonically sloping, short-wavelength component may indicate that the tissue's scattering function does not exactly match that specified in equation 3.

3.2 Spatial distributions

By combining the fits at multiple source and detector positions, we can generate a set of overlapping line scans through the prostate. One such set is shown in figure 8. Here, the position plotted is the mean of the source and detector position. We have plotted the concentrations of oxy- and deoxyhemoglobin and MLu in panel (a). In panel (b), we plot the total hemoglobin concentration and the hemoglobin oxygen saturation. The agreement in profiles among scans taken at different source-detector positions confirms the physical consistency of our correction model.

The continuity of the hemoglobin oxygen saturation is preserved from one source position to the next better than is the total hemoglobin concentration. This may be a result of the fact that the total hemoglobin concentration depends linearly on the absorption coefficient used to reconstruct the spectrum, and is therefore subject to tradeoff between μ_a and μ_s' in the initial single-wavelength fitting. The fact that the total hemoglobin and MLu concentrations are both elevated at the first source position and reduced at the third provides evidence that at least some of the effect we see may be due to such a tradeoff.

It is also possible that the local hemoglobin concentration really does vary significantly on the spatial scales shown here. Because each scan is taken at a different source position, it is possible that a vessel or area of increased vascularization near one source position could increase the measured total hemoglobin concentration for all the measurements taken with the source at that position. The opposite trends in MLu and total hemoglobin observed in the second, fourth and fifth source positions cannot be explained simply by μ_a/μ_s' tradeoff, and indicate real spatial variation in vascular structure and drug distribution.

The hemoglobin oxygen saturation is determined primarily by the shape of the absorption spectrum, and is therefore less sensitive to the μ_a/μ_s' tradeoff. This particular patient's SO_2 varies from 50 to 80% over the sampled region, indicating that the prostate is less well-oxygenated than normal tissue, but not hypoxic. In contrast, the prostate from which the spectra shown in figure 7 were taken showed almost no evidence of oxyhemoglobin, indicating significant hypoxia.

4. DISCUSSION

4.1 Implications for *in vivo* dosimetry

The ability to construct a complete map of the optical properties of the human prostate will allow us to reconstruct the 3-D fluence rate in the prostate given a predetermined light source arrangement¹² and to develop 3-D treatment planning¹³. The current results indicate that we will in the future be able to generate 3-D maps of sensitizer concentration and tissue oxygenation as well. This will give us, in theory, all the inputs required to model the deposition of singlet oxygen dose in the target tissue, the *in vivo* implementation of explicit dosimetry.¹⁴ The variation in drug and oxygen supply observed within and among patients indicates the potential problem with dosimetry based solely on light dose, and the potential gain to be achieved by 3-D model-based dosimetry.

4.2 Future work

The fact that our algorithm allows the generation of an absorption and scattering spectrum for each diffuse transmission spectrum recorded allows us to reconstruct a significantly over sampled map of optical properties of the human prostate. This allows improvements in two directions. First, a map of sufficient resolution can be reconstructed from a far smaller number of measured spectra than we currently employ. This will allow faster sampling and therefore less interruption of treatment. Conversely, the over sampling may allow us to reconstruct the optical properties of the prostate at a resolution finer than our source-detector spacing. The resolution of the sensitizer concentration map can be further improved by incorporating the results of a previously reported fluorescence detection system.¹⁵ This will require a 3-dimensional model of optical heterogeneity, rather than the piece-wise homogeneous model we've presented here. Work is currently underway to develop fully 3-dimensional models based on the finite element method (FEM),¹² and to adapt them to spectroscopy in the human prostate.

From the point of view of instrumentation development, the greatest challenge in high-resolution mapping of the prostate using interstitial fibers is the time required for data acquisition. In addition to the developments in the fitting algorithm outlines above, which are aimed at reducing the number of data points needed, we are developing a computer-controlled system of variable attenuators and beamsplitters to allow the acquisition of data from multiple quadrants with minimal switchover time. These improvements have the potential to generate a complete 3-D map of the prostate in a matter of minutes. This, combined with real-time treatment planning¹³ will allow individualization of PDT treatment to each patient.

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Figure 1. Setup for single wavelength (solid) and spectroscopic (dashed) diffuse transmission measurements in the human prostate. Only one set of optical fibers is connected at a time.

Figure 2.
Graphical user interface (GUI) for analysis of prostate absorption spectra.

Figure 3.

Typical set of diffuse transmission data acquired *in vivo* from a human prostate, shown as (a) spectra and (b) the radial profile at 732 nm.

Figure 4.

Absorption spectrum resulting from the fits shown in figure 3. The fit residual (heavy dotted line) is large compared to other components.

Figure 5.

Comparison of the intensity of the diffuse transmission spectra at 732 nm (squares) with the positions derived from diffusion theory (line and circles).

Figure 6. Absorption spectra derived from the spectra shown in figure 3 after correction and fitting using equation 4.

Figure 7.

SVD fit to one of the spectra shown in figure 6. After correction, the residual is reduced compared to that shown in figure 4.

Figure 8.

(a) Concentrations of the major absorbers measured along a line in a human prostate using SVD analysis of the corrected spectra and (b) the corresponding total hemoglobin concentration and hemoglobin oxygen saturation.