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***In vivo* measurement of fluorescence emission in the human prostate during photodynamic therapy**

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Abstract

Among the challenges to the clinical implementation of photodynamic therapy (PDT) is the delivery of a uniform photodynamic dose to induce uniform damage to the target tissue. As the photodynamic dose depends on both the local sensitizer concentration and the local fluence rate of treatment light, knowledge of both of these factors is essential to the delivery of uniform dose. In this paper, we investigate the distribution and kinetics of the photosensitizer motexafin lutetium (MLu, Lutrin®) as revealed by its fluorescence emission. Our current prostate treatment protocol involves interstitial illumination of the organ *via* cylindrical diffusing fibers (CDF's) inserted into the prostate through clear catheters. For planning and treatment purposes, the prostate is divided into 4 quadrants. We use one catheter in each quadrant to place an optical fiber-based fluorescence probe into the prostate. This fiber is terminated in a beveled tip, allowing it to deliver and collect light perpendicular to the fiber axis. Excitation light is provided by a 465 nm light emitting diode (LED) source coupled to a dichroic beamsplitter, which passes the collected fluorescence emission to a CCD spectrograph. Spectra are obtained before and after PDT treatment in each quadrant of the prostate and are analyzed *via* a linear fitting algorithm to separate the MLu fluorescence from the background fluorescence originating in the plastic catheter. A computer-controlled step motor allows the excitation/detection fiber to be moved along the catheter, building up a linear profile of the fluorescence emission spectrum of the tissue as a function of position. We have analyzed spectral fluorescence profiles obtained in 4 patients before and after MLu-mediated PDT. We find significant variation both within individual prostates and among patients. Within a single quadrant, we have observed the fluorescence signal to change by as much as a factor of 3 over a distance of 2 cm. Comparisons of pre- and post-PDT spectra allow a quantification treatment-induced photobleaching. Like the drug distribution, the extent of photobleaching varies widely among patients. In two cases, we observed bleaching of approximately 50% of the drug, while others exhibited negligible photobleaching.

1. INTRODUCTION

A continuing challenge in the clinical administration of photodynamic therapy (PDT) is the initiation of uniform cellular damage. PDT causes cellular damage *via* reactions of singlet oxygen with tissue substrates. The production of singlet oxygen requires a sensitizing drug, light, and available molecular oxygen. Any (or all) of these may be distributed nonuniformly in tissue, resulting in heterogeneities in PDT-induced damage. In an effort to understand the causes of such heterogeneity, we seek to map the distribution of drug in the prostates of patients undergoing motexafin lutetium (MLu)-mediated PDT by measuring the

fluorescence emission of the drug. MLu is a second-generation, water-soluble photosensitizer with an absorption maximum around 732 nm.^{1, 2} The data reported here was acquired from patients enrolled in a Phase I trial of MLu-mediated PDT for recurrent prostate adenocarcinoma. The design of this study was based on a prior feasibility study in a canine model.³

The measurement of fluorescence emission *in vivo* is complicated by the absorption and scattering of light within the sample being measured. Variations in absorption coefficient may be mistaken for variations in fluorophore concentration. Several researchers have developed methods for reducing the effects of background optical properties on the measured fluorescence, either through specially designed optical probes^{4, 5} or by using independent measurements of optical properties to apply a correction to the measured fluorescence signal.^{6–11} In the current paper, we use a single optical fiber as the source and detector. This minimizes the effects of background optical properties by allowing us to primarily collect light that has traveled a short distance in the tissue.

2. METHODS

2.1 Patient preparation and treatment

The *in vivo* results presented here were obtained as part of an ongoing Phase I clinical trial of MLu PDT for the treatment of recurrent prostate carcinoma.¹² Under this protocol, clear brachytherapy catheters are placed in the prostate. Irradiation of the prostate is accomplished by placing cylindrical diffusing fibers (CDF's) of various lengths into the catheters. Several weeks before the planned treatment, patients, having given informed consent, were examined by transrectal ultrasound (TRUS) to determine the size and position of the prostate. For planning purposes, the prostate was divided into four quadrants. The positions of the catheters were chosen to provide uniform illumination of the prostate by maintaining a spacing of 1 cm between them. The length of the CDF in each catheter was chosen to cover the entire length of the prostate.

For the purposes of monitoring the progress of treatment, one additional catheter was inserted in each quadrant. This catheter held an isotropic fiber optic based detector used to monitor the local light fluence during treatment.¹³ This detection catheter was also used for fluorescence measurements before and after PDT treatment, as described below.

2.2 Fluorescence measurement setup

A schematic of the fluorescence spectroscopy measurement system is shown in figure 1. Fluorescence excitation light was provided by a 465-nm light emitting diode (LED) source coupled into an optical fiber. The beam exiting the fiber was collimated and reflected by a dichroic beamsplitter (Chroma Technology, Rockingham, VT) with a cutoff wavelength of 600 nm. The beam was then refocused into a fiber optic based probe. The probe consisted of a single optical fiber terminated in a beveled tip. This fiber emits and collects light at right angles to its axis, allowing it to interrogate tissue adjacent to the catheter in which it is placed. Fluorescence collected by the fiber is collimated and passed through the dichroic beamsplitter. To further discriminate against excitation light, an OG 530 (Schott Glass Technologies, Duryea, PA) glass filter is placed in the beam path, blocking light at

wavelengths shorter than 530 nm. The transmitted light is refocused onto another optical fiber which terminates at the focal plane of a 0.125 m focal length spectrograph (Acton Instruments, Acton, MA). The spectrograph images the fiber onto a liquid nitrogen cooled CCD (Princeton Instruments, Princeton, NJ). The spectrograph and CCD were configured to collect spectra with a pixel width corresponding to 0.43 nm over the range from 440 nm to 940 nm. The actual resolution of the measurement is limited by the spectrograph resolution (~5 nm). The CCD shutter opening and data acquisition were triggered by a transistor-transistor logic (TTL) pulse from the dosimetry computer.

Fluorescence spectra were acquired at intervals of 2 mm along the detection catheter in each quadrant. A total length of 40 mm was sampled in each quadrant. The position of the fluorescence probe was controlled by a step motor-driven positioning stage (Unislide, Velmex, Inc., East Bloomfield, NY) controlled by the same computer that triggered the data acquisition¹⁴. To ensure repeatable positioning of the probe within the catheter, the probe was aligned to the closed end of the catheter prior to the initialization of each scan.

2.3 Data analysis

The spectra acquired by the CCD were digitized and stored in binary format. For each set of measurements, an additional spectrum was acquired with the shutter closed, to account for the dark charge accumulated by the CCD and for the systematic offset present in the CCD's analog-to-digital converter. This signal was subtracted from each frame prior to analysis.

The analysis of data was accomplished using a custom-designed graphical user interface (GUI) written in the Matlab programming environment. The user interface of this program is shown in figure 2. While the essential data analysis and programming could be accomplished in plain text programming, the GUI offers a significant advantage in that it allows the user to see the fitting results as they are generated, and to identify poor fits or spectra that include artifactual data, and to adjust the fitting range to compensate.

The spectra were analyzed using the singular value decomposition (SVD) fitting algorithm described by Finlay *et al.*¹⁵ and included in the GUI. This algorithm requires the selection of basis spectra corresponding to the known components of the fluorescence emission spectrum. In this case, we used the fluorescence of MLu, measured in a Liposyn phantom, as our primary basis spectrum. To isolate the MLu emission, we subtracted the signal measured in the same phantom prior to the addition of MLu. The *in vivo* chemical environment differs from that of our phantom. As a result, we consistently observe a shift in the emission maximum of MLu from approximately 738 nm in the phantom to 745 nm *in vivo*. We have accounted for the wavelength shift by digitally shifting our basis spectrum prior to fitting.

The second component of fluorescence we observe *in vivo* and in phantoms arises from fluorescence in the optical fiber and in the plastic catheter. While this background fluorescence cannot be eliminated given the constraints of our clinical protocol, it can be characterized by measurements in fluorophore-free media, providing a second basis spectrum

The SVD algorithm we employ also includes a 61-term Fourier series¹⁵ to account for fluorescence of unknown origin. The Fourier components are given much lower weight in the fitting routine than the basis spectra of known fluorophores to restrict their application to components of the spectrum that cannot be fit by combinations of these species. In the cases presented here, the Fourier components constitute only a minor contribution to the total fit, indicating that the known fluorophores adequately account for the fluorescence we observe.

The final result of the SVD fitting is an amplitude for each of the known basis spectra, namely MLu and background. Because the background signal originates in the measurement apparatus, its intensity is unchanged from one treatment to the next. We can therefore use the intensity of the background to correct for variations in lamp output, fiber coupling efficiency, and beamsplitter alignment. The MLu amplitude we report has been divided by the background amplitude to account for these variations.

The amplitude of the MLu fluorescence obtained by this method *in vivo* is approximately 10 times lower than that obtained by absorption spectroscopy (see section 3.2). This is the result of the fact that our basis spectrum was measured in a phantom where the only absorption was that due to MLu itself. In the *in vivo* environment, other absorbers are present, which leads to a reduction in the measured intensity. It is possible that variations in absorption among patients or among different positions within the same prostate could cause deviations from this ideal correction factor.

One can measure the MLu concentration by an alternate method such as absorption spectroscopy (see section 3.3), and use this value to determine a correction factor for each prostate or even for each quadrant. However, in practice, the availability of reliable absorption data is often limited by localized bleeding and by time constraints. Of the four patients studied thus far, we have reliable absorption and fluorescence data in multiple quadrants both before and after treatment for only one patient, number 13. We have adopted a single correction factor equal to 10 obtained by comparing the fluorescence amplitude of MLu measured *in vivo* in prostate number 13 with absorption measurements at the same position in the same prostate (see section 3.3 below). This conversion yields a concentration of 12.9 mg/kg for a spectrum whose MLu concentration has a peak intensity of 1.0 after normalization. This factor is in approximate agreement with the results of phantom experiments in which ink was added to the phantom to simulate absorption by biological chromophores.

2.4 Monte Carlo Simulation and Analytic Modeling

To assess the effects of optical property variations on measured fluorescence, we have performed Monte Carlo simulations in which the source was modeled as a pencil beam and the detector as a circular aperture with a radius of 500 microns and an acceptance numerical aperture of 0.22. The relative changes in fluorescence resulting from changes in the absorption coefficients at the excitation (μ_{ax}) and emission (μ_{am}) wavelengths and in a simultaneous change in the scattering coefficient (μ_s') are summarized in table 1. In each case, the change shown is the average over several simulations in which one variable was changed and the others were held constant.

These simulations indicate that changes in absorption at the emission wavelength will have negligible effect on the measured fluorescence, since μ_a at the emission wavelength (745 nm) is generally much smaller than that at the excitation wavelength (465 nm). Note that a tenfold increase in μ_{am} caused less than 10% change in fluorescence. Even at the excitation wavelength, a doubling of μ_a caused less than a 20% change in measured fluorescence. Similarly, a doubling of the scattering coefficient at both wavelengths caused only a 22% change in fluorescence. Correction for individual variations in optical properties is therefore unlikely to be necessary unless the variations in μ_a are very great.

Based on these results, we designed a set of experiments in tissue-simulating phantoms containing Liposyn as a scatterer and MLu. In the first, the μ_a of the phantom was varied by varying the MLu concentration ($\mu_{am} = 0\text{--}0.6\text{ cm}^{-1}$, $\mu_{ax} = 0\text{--}1.6\text{ cm}^{-1}$), while μ_s' was held constant ($\mu_{sm}' = 4\text{ cm}^{-1}$, $\mu_{sx}' = 6\text{ cm}^{-1}$). In the second, the μ_a was varied by adding black ink ($\mu_{am} = 0\text{--}0.4\text{ cm}^{-1}$, $\mu_{ax} = 0\text{--}3\text{ cm}^{-1}$, $\mu_{sm}' = 6\text{ cm}^{-1}$, $\mu_{sx}' = 10\text{ cm}^{-1}$). In each case, the fluorescence was analyzed as described above and the resulting MLu signal was normalized to the known MLu concentration. The MLu fluorescence measured in these phantoms is shown in figure 3(a) as function of excitation-wavelength μ_a . The data points from the two phantoms do not overlap because the values of μ_s' and μ_{am} for the two phantoms were different. The change in fluorescence induced by changing μ_a appears to be larger than that predicted by Monte Carlo simulation.

As an alternative to Monte Carlo simulation, the effects of absorption and scattering on measured fluorescence in semi-infinite media can be modeled using the forward-adjoint fluorescence Scheme proposed by Crilly *et al.*¹⁶. Briefly, this method models the forward propagation of excitation light from the source and the time-reversed, or adjoint, propagation of positional importance from the detector. The positional importance is defined as the probability that a photon emitted at a point is eventually captured by a detector. The volume integral of the product of the excitation fluence rate and the importance is proportional to the measured signal. Finlay and Foster⁹ have derived an analytic solution to this model for the case of an isotropic point source and an isotropic detector in an infinite homogeneous medium, which for the diffusion approximation takes the form:

$$F_{\text{det}} = F_0 \frac{1}{4\pi D_x D_m r_{sd}} \left(\frac{\mu_{\text{eff}(x)}^2 - \mu_{\text{eff}(m)}^2}{\mu_{\text{eff}(x)}^2 - \mu_{\text{eff}(m)}^2} \right) (e^{-\mu_{\text{eff}(m)} r_{sd}} - e^{-\mu_{\text{eff}(x)} r_{sd}}), \quad (1)$$

where r_{sd} is the distance between the source and detector, D is the diffusion constant, μ_{eff} is the effective attenuation coefficient, the subscripts x and m denote the excitation and emission wavelengths, respectively, and F_0 is the intrinsic fluorescence of the sample. This formula assumes that both the source and detector are points. In the measurements reported here, a single fiber serves as both source and detector; however it is finite in extent and non-isotropic.

To approximate the true physical situation, we assume that the light beam exiting or entering our probe is a pencil beam, which can be represented by a point source at a distance of 1 transport mean free path in the z direction.¹⁷ To account for the finite extent of the probe, we introduce a lateral shift in the x direction between the source and detector pencil beams. We

have applied equation 1 to the case of the phantoms described above. The results are shown by the solid lines in figure 3(a). In this case we used a lateral shift of 0.45 cm. It should be emphasized that this lateral shift was determined empirically, and does not necessarily correspond to any physical dimension of the probe.

To correct for absorption and scattering effects, we solve equation 1 for F_0 . The data originally shown in figure 3(a), corrected by this method, are shown in figure 3(b). This correction reduces the nearly 10-fold variation seen in figure 3(a) to slightly more than a factor of 2. This method can be used in the phantom case only because the optical properties of the phantom were known *a priori*. To implement such a correction *in vivo* requires an accurate measurement of the local *in vivo* optical properties. This is difficult in a clinical situation because (a) it is often the case that localized bleeding prevents accurate measurement of absorption and (b) when a measurement of μ_a is available, the uncertainty in μ_a may be as large as the correction being made, rendering the uncertainty in the corrected measurement too large to make the correction meaningful.

3. RESULTS

3.1 MLu concentration

We have measured fluorescence spectra for 4 patients thus far. The results of spectral analysis of a typical fluorescence spectrum are shown in figure 4. The noisy line indicates the original data, and the dotted and dashed lines, the components determined by the SVD algorithm. The sum of these is represented by the solid line that closely matches the data. The sum of the contributions of the terms of the Fourier series, labeled 'residual' on the plot, is much smaller in amplitude than the contributions of the background fluorescence and MLu, indicating that these known components accurately model the majority of the measured fluorescence

3.2 Spatial distribution of MLu

Figure 5 illustrates the distributions of MLu within three of the prostates we have measured. Profiles measured immediately before (dashed lines) and immediately after (solid lines) PDT treatment are similar in shape and amplitude. Only the profiles from patient 12 indicate significant reduction in MLu concentration.

In one patient (13), we have obtained fluorescence profiles in all four quadrants of the prostate both before and after PDT treatment. These profiles are shown in figure 6. As in the cases shown in figure 4, the profiles taken before and after treatment show similar shape and concentration, with the exception of the RUQ (panel b), which shows a similar shape but reduced concentration compared to the pre-treatment profile. The measurements in the upper quadrants (LUQ and RUQ) were made 1.5 cm anterior to those in the lower quadrants (LLQ and RLQ, respectively), while those in the right quadrants (RUQ and RLQ) were shifted 2 cm laterally from those in the left quadrants (LUQ and LLQ, respectively).

Among the four prostates we have measured, we consistently observe variations of a factor of 3 in fluorescence signal over distances as short as a few millimeters. In the majority of cases, we find that the spatial distribution of sensitizer measured prior to PDT treatment is

similar to that acquired after treatment. The variation in MLu concentration within individual prostates is as great as the variation among patients, as indicated in Table 2. Each of the four patients listed were given MLu at a concentration of 2 mg/kg body weight 3 hours prior to the first set of measurements.

The maximum fluorescence observed in each patient varied from 5 to 13 mg/kg, significantly higher than the injected concentration. This is consistent with the results of *ex vivo* measurements made on biopsies of the same tissues (data not shown).¹³ In one case (patient #12) we observed significant photobleaching of the sensitizer during treatment. In another (patient # 13), we observe photobleaching by approximately a factor of two in one quadrant, but no significant photobleaching in other quadrants.

3.3 Comparison with absorption spectra

For one patient, we have measured profiles of MLu concentration using not only fluorescence spectra but also diffuse transmission spectra. These were acquired by placing a white light source in a catheter parallel to the detection catheter and measuring the fluence rate spectrum in the detection catheter with an isotropic detector, as reported previously.^{14, 18} A series of such spectra can be fit using photon diffusion theory to determine the absorption and scattering spectra of the prostate as a function of position along the catheter. The resulting absorption spectra can then be fit using the SVD algorithm described above to determine the concentrations of oxy- and deoxy-hemoglobin and MLu. In figure 7, we plot the concentrations of MLu determined by absorption and fluorescence spectroscopy. The comparison between the MLu fluorescence amplitude and the MLu concentration determined by absorption measurement in this prostate was the basis for the conversion between MLu amplitude and absolute concentration described in section 2.3. It is therefore expected that the two measurements agree in absolute concentration.

The agreement in the spatial distributions measured by absorption and fluorescence is striking, and confirms that the fluorescence and absorption spectroscopy measurements are in fact measuring the same distribution. In the case shown here, the shape of the MLu distribution remains similar before and after PDT, however the MLu concentration as reported by both methods decreases by nearly a factor of two, especially in the region around 2 to 3 cm, in the center of the prostate.

4. DISCUSSION

We have demonstrated the ability of fluorescence spectroscopy, coupled with a linear fitting algorithm, to quantitatively measure the concentration and distribution of MLu in the human prostate. There may be significant variations in optical properties among prostates¹³ and within a single prostate¹⁴. The theoretical work presented in section 2.4 indicates that if the reliability and accuracy of absorption measurements can be improved, these effects can be corrected for each individual prostate. Given the constraints of our current ability to make absorption measurements, however, we have adopted a single, uniform correction factor. The fact that the MLu concentration profiles measured by two independent spectroscopic techniques agree (see figure 7) indicates that this variation will not significantly hinder fluorescence spectroscopy.

While the absorption and fluorescence measurements reported here agree in the MLu concentration, the two measurements are not interchangeable. First, absorption spectroscopy measurements require multiple spectra at each point of interest, and necessarily average over a large volume of tissue to make a measurement.¹⁴ This limits the resolution of absorption spectroscopy, and makes it more time-consuming than fluorescence spectroscopy. While fluorescence spectroscopy lacks the sensitivity of absorption measurements to scattering and hemoglobin dynamics, it offers significant advantages. First, because each measurement requires only the acquisition of a single spectrum, fluorescence measurements can be made very quickly, making it practical to increase the number of measurement points in each scan. This is reflected in figure 6, where the fluorescence measurement points are more densely spaced and cover a larger range than the absorption measurement points. Second, the fluorescence measurement averages a small volume of tissue due to the limited penetration of 465 nm light. This allows a finer resolution than can be achieved using absorption measurement.

Finally and perhaps most importantly from a clinical point of view, the fluorescence measurement requires only a single catheter to acquire a profile, while the absorption measurements require two. In our clinical experience, we have often encountered cases where the pooling of blood around one or more catheters renders the absorption spectroscopy data uninterpretable. In many of these cases, fluorescence spectra could still be measured and analyzed.

The goal of the measurements described here is to map the MLu distribution in the human prostate. The primary motivation for this effort lies in the fact that the effective application of photodynamic therapy depends on the creation of reactive singlet oxygen. This requires sufficient tissue oxygenation, sufficient intensity of treatment light, and sufficient concentration of the sensitizing drug. Our efforts at optimizing PDT delivery have focused on the latter two factors. The goal of light and drug optimization is to deliver an optimal photodynamic dose (defined as the product of light fluence rate and drug concentration) uniformly over the target volume. As the clinician has only limited control over the drug concentration *in vivo* this optimization must be accomplished by adjusting the delivered light intensity. The optimal light intensity distribution can be determined only if the drug distribution can be measured.

In theory, a generic drug distribution could be developed and used for all patients. However, the variation among prostates makes this impractical. In addition, we observe variations in MLu fluorescence within individual prostates that is equal to or greater than the variation among different patients' prostates. This indicates that characterization of the tissue MLu concentration for each patient is necessary, but will only result in optimal treatment of the point of measurement. To optimize treatment for the entire prostate, it will be necessary to build up a map of the MLu concentration in three dimensions. This, coupled with a similar map of tissue optical properties, will allow the optimization of the light source distribution to provide uniform photodynamic dose. As a first step to creating a map of MLu distribution, we have acquired fluorescence profiles in all four quadrants of the prostate of one patient, shown in figure 6. As expected, we see significant variation among the four quadrants. However, the general features, namely a peak in MLu concentration around 5 to

15 mm and a shallow minimum around 30 mm, are reproduced in three out of the four quadrants. This indicates that the variations in MLu concentration occur on a scale of approximately 1.5 to 2 cm (the spacing of the catheters used to make these measurements) in all three dimensions. We therefore expect a set of measurements with a catheter spacing of 1 cm to be sufficient to characterize the MLu distribution in a typical prostate. This can be achieved in our current clinical protocol by using the catheters currently reserved for treatment, in addition to the four dedicated detector catheters, for fluorescence measurement. Work in this area is ongoing.

The observation of MLu photobleaching may have significant implications for photodynamic dosimetry. It has long been appreciated that photobleaching may reduce the photodynamic dose by reducing the sensitizer concentration as time progresses.¹⁹ More recently, it has been suggested that photobleaching itself can be used to monitor the progress of photodynamic therapy.²⁰ For the case of singlet-oxygen-mediated photobleaching, photodynamic destruction of target tissues and photobleaching of the drug are inseparably related.²¹ If this is the case for MLu, it is possible that the quadrants where we observe significant photobleaching are those where the greatest dose of singlet oxygen is delivered. An alternative explanation is that those quadrants where no photobleaching or a slight increase in MLu concentration is observed are better vascularized, and have the bleached sensitizer replaced from the circulating pool or from adjacent tissue. These two explanations make opposite predictions concerning the effectiveness of treatment in those quadrants that exhibit photobleaching versus those that do not. Further research into the photobleaching behavior and *in vivo* vascular effects of MLu is needed to resolve the underlying dynamics responsible for the changes in concentration, or lack thereof, that we observe.

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Figure 1.

Fluorescence spectroscopy setup. The computer that acquires and stores fluorescence spectra also controls the position of the detection fiber via a step-motor positioner (not shown). The dichroic allows a single fiber to deliver excitation light and collect emitted fluorescence.

Figure 2.

Graphical user interface (GUI) used to analyze fluorescence emission spectra. The GUI provides a front end for the SVD algorithm and gives real-time feedback, allowing interactive fitting.

Figure 3.

(a) MLu fluorescence measured in two sets of phantoms, plotted as a function of excitation-wavelength absorption coefficient. See text for details. The solid lines indicate the values predicted by eq. 1, and (b) the corresponding fluorescence corrected for optical effects (F_0) found from eq. 1.

Figure 4. SVD analysis of a typical fluorescence emission spectrum. The spectrum is separated into MLu and background components and a small residual composed of a Fourier series.

Figure 5.

MLu fluorescence profile as a function of position within the prostate for patients 12 (a), 15 (b and c) and 17 (d). The profiles measured before (dashed line) and after (solid line) PDT treatment are similar in shape and amplitude. The patient number and quadrant from which each data set was taken are indicated in the title of each frame.

Figure 6.

MLu fluorescence profiles acquired in the four quadrants of a single prostate before (dashed lines) and after (solid lines) PDT treatment. The quadrant from which the data was taken is indicated in the title of each frame.

Figure 7.

MLu fluorescence (open circles) and absorption (filled squares) profiles as functions of position within the prostate of patient #13. The profiles measured before (panel a) and after (panel b) PDT treatment are similar in shape, but indicate some photobleaching, especially in the center of the prostate.

Table 1

Effect of changing optical properties on the measured fluorescence signal in a pencil-beam geometry in an infinite medium.

Parameter	Change	Change in fluorescence
$\mu_{ax} \text{ (cm}^{-1}\text{)}$	2→4	−19 %
$\mu_{am} \text{ (cm}^{-1}\text{)}$	0.05→0.5	−8.1%
$\mu_{sx}' \text{ (cm}^{-1}\text{)}$	10→20	+ 22 %
$\mu_{sx}' \text{ (cm}^{-1}\text{)}$	5→10	

Table 2

Range in MLu concentration measured by fluorescence spectroscopy before and after PDT treatment. Patient #13 exhibited photobleaching in one quadrant only.

Patient	MLu Concentration	
	Before (mg/kg)	After (mg/kg)
#12	2.5 – 5.0	0.4–1.7
#13	2–10.5	1–11
#15	1–16	2–13
#17	3.4–9.4	0.7–12.4