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Interstitial Fluorescence Spectroscopy in the Human Prostate During Motexafin Lutetium–Mediated Photodynamic Therapy

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Abstract

The *in vivo* fluorescence emission from human prostates was measured before and after motexafin lutetium (MLu)-mediated photodynamic therapy (PDT). A single side-firing optical fiber was used for both the delivery of 465 nm light-emitting diode excitation light and the collection of emitted fluorescence. It was placed interstitially within the prostate *via* a closed transparent plastic catheter. Fitting of the collected fluorescence emission spectra using the known fluorescence spectrum of 1 mg/kg MLu in an intralipid phantom yields a quantitative measure of the local MLu concentration. We found that an additional correction factor is needed to account for the reduction of the MLu fluorescence intensity measured *in vivo* due to strong optical absorption in the prostate. We have adopted an empirical correction formula given by $C = (3.1 \text{ cm}^{-1} / \mu'_s) \exp(\mu_{\text{eff}} \cdot 0.97 \text{ cm})$, which ranges from approximately 3 to 16, with a mean of 9.3 ± 4.8 . Using a computer-controlled step motor to move the probe incrementally along parallel tracks within the prostate we can determine one-dimensional profiles of the MLu concentration. The absolute MLu concentration and the shape of its distribution are confirmed by *ex vivo* assay and by diffuse absorption measurements, respectively. We find significant heterogeneity in photosensitizer concentration within and among five patients. These variations occur over large enough spatial scales compared with the sampling volume of the fluorescence emission that mapping the distribution in three dimensions is possible.

INTRODUCTION

The development of photodynamic therapy (PDT) as a modality for the treatment of bulky tumors and solid organs has motivated a parallel development in quantitative photodynamic dosimetry. Many of these efforts have been aimed at light dosimetry (1–4). However, it has also been appreciated that the distribution of photosensitizer and its degradation during PDT treatment may significantly effect the outcome of PDT (5–9). Zhou *et al.* (10) have found significant differences in sensitizer uptake among animal tumors, as measured by fluorescence, and in treatment outcome. However, when the PDT light fluence was adjusted

such that the product of drug fluorescence and light fluence was constant among animals, the animal-to-animal variation in treatment response was reduced. This confirms that fluorescence measurements can be used to guide the delivery of a uniform drug-light product among different patients and among tumor sites or regions within a single patient, a strategy referred to as *explicit dosimetry* by Wilson *et al.* (5).

In conjunction with an ongoing Phase I trial of PDT in the human prostate using the investigational agent motexafin lutetium (MLu), we have developed a set of measurements capable of assessing the heterogeneity in light fluence rate, optical properties, and sensitizer concentration during PDT (11). As part of that effort, this paper reports the results of an investigation of the ability of *in vivo* fluorescence spectroscopy to quantify the concentration and spatial heterogeneity of MLu photosensitizer concentration within and among patients. The motivation for this work is the eventual incorporation of the fluorescence-based sensitizer distribution measurements into a spatially resolved dose calculation system.

The measurement of fluorescence emission *in vivo* is complicated by the absorption and scattering of light within the sample being measured. Variations in optical properties 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 (12,13) or by using independent measurements of optical properties to apply a correction to the measured fluorescence signal (14–19). In the current paper, we use a single optical fiber as the source and detector. This reduces the effects of background optical properties by allowing us to primarily collect light that has traveled a short distance in the tissue (12). We have developed an empirical correction method to account for variations due to optical properties on the basis of forward-adjoint fluorescence theory (17,20). The parameters in this model are determined by comparing the spatially resolved drug concentrations obtained from fluorescence spectroscopy with those obtained from spectrally resolved and single-wavelength optical properties measurements. This method provides an approximate correction factor for the position in the prostate at which fluorescence is measured. We provide an assessment of the uncertainty in photosensitizer concentration induced by variations in optical properties.

MATERIALS AND METHODS

Patient preparation and treatment

The *in vivo* results presented here were acquired as part of an ongoing Phase I trial of PDT using the investigational agent motexafin lutetium (MLu, Pharmacyclics, Inc., Sunnyvale, CA) for the treatment of recurrent prostate cancer after radiation therapy at the University of Pennsylvania (21). The protocol was approved by the Institutional Review Board of the University of Pennsylvania, the Clinical Trials and Scientific Monitoring Committee of the University of Pennsylvania Cancer Center and the Cancer Therapy Evaluation Program of the National Cancer Institute. The photosensitizer used in this protocol, MLu, is a second-generation, water-soluble photosensitizer with an absorption maximum around 732 nm (22,23). The design of our protocol involves escalating the administered drug dose and the total light fluence delivered to the prostate, and varying the time between the administration

of the photosensitizer and the beginning of irradiation. The values of each of these parameters for each of the five patients reported here are listed in Table 1. The numbering of patients correlates with that of Zhu *et al.* (4).

Under our protocol, clear brachytherapy catheters are placed in the prostate using a template consisting of holes spaced 0.5 cm apart. Irradiation of the prostate is accomplished by placing cylindrical diffusing fibers (CDFs) of various lengths into the catheters. The range of MLu concentrations investigated and the light source loading pattern used in human patients are determined on the basis of a previously reported study in a canine model (24). Several weeks before the planned treatment, patients, having given informed consent, are examined by transrectal ultrasound to determine the size and position of the prostate. For planning purposes, the prostate is divided into four quadrants. The positions of the catheters are 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 is chosen to cover the entire length of the prostate.

To monitor the local fluence rate during treatment, one additional catheter is inserted in each quadrant. This catheter holds an isotropic, fiber optic-based detector connected to a calibrated photodiode-based dosimetry system that continuously measures and records the fluence rate at the detector position (3,4). This detection catheter is also used for fluorescence measurements before and after PDT treatment (as described below), to measure tissue optical properties at the treatment wavelength of 732 nm (4) and to quantify the absorption and scattering spectra of the tissue (11,25). Biopsy samples are acquired before and after treatment. The absolute MLu concentration of these samples is determined by an *ex vivo* fluorescence assay based on that of Woodburn *et al.* (26). Unlike the optical measurements described below, the biopsies are taken manually, and are not guided by ultrasound. It is therefore impossible to quantitatively relate the biopsy site to the positions of the optical measurements.

Fluorescence spectroscopy setup

Portions of the methods presented here have been reported previously (27). A schematic of the fluorescence spectroscopy measurement system is shown in Fig. 1a. Fluorescence excitation light from a 465 nm light-emitting diode (LED) is collected by an optical fiber, collimated and directed onto a dichroic beamsplitter (Chroma Technology, Rockingham, VT) with a cutoff wavelength of 600 nm. The reflected beam is then refocused onto the proximal end of a probe consisting of a single optical fiber with a 600 μm core, terminated in a beveled tip (FiberOptic Systems, Inc., Simi Valley, CA). 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. The projected area of the emission/ collection region on the outside of the catheter is approximately 1 mm². Fluorescence collected by the fiber is again collimated and, by virtue of its longer wavelength, passes through the dichroic beamsplitter. To further discriminate against excitation light, an OG 530 glass filter (Schott Glass Technologies, Duryea, PA) is placed in the beam path, blocking light at wavelengths shorter than 530 nm. The transmitted light is coupled by an optical fiber and a 0.125 m focal length spectrograph (Acton Instruments, Acton, MA) onto a liquid nitrogen-cooled charge-coupled device

(CCD) (Princeton Instruments, Princeton, NJ). The spectrograph and CCD 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 to approximately 5 nm.

During *in vivo* measurements, the fluorescence probe is placed in one of the clear catheters previously inserted in the prostate for *in vivo* light dosimetry. The arrangement of the catheters used for detection in a typical prostate is shown in Fig. 1b. Fluorescence spectra are acquired at intervals of 0.2 cm along the distal 4 cm of the detection catheter in each quadrant. The position of the fluorescence probe is controlled by a step motor-driven positioning stage (Unislide, Velmex, Inc., East Bloomfield, NY) controlled by the same computer that triggers the data acquisition (11). To ensure repeatable positioning of the probe within the catheter, the probe is aligned to the closed end of the catheter prior to the initialization of each scan. Acquisition of data is coordinated by the light dosimetry computer as follows: First, the light dosimetry system sends a transistor-transistor logic (TTL) pulse *via* the stepper motor controller to the CCD controller. This triggers the acquisition of the first fluorescence spectrum. When the spectrum has been acquired and saved, the CCD controller sends an answering TTL pulse back to the stepper motor controller. Upon receiving this signal, the stepper motor controller moves the optical fiber 0.2 cm, waits for it to come to a complete stop, and triggers the CCD to acquire the next spectrum. This process repeats until the preprogrammed number of spectra has been acquired. Because the TTL triggering is handled within the CCD and motor controllers, the light dosimetry system is not required to monitor the process. At the completion of the process, the light dosimetry system receives a confirmation signal from the motor controller and records the completion time and measurement details in a log file.

Data analysis

The analysis of data is accomplished using a custom-designed graphical user interface (GUI) written in the Matlab (Mathworks, Inc., Natick, MA) programming environment. While the essential data analysis and fitting could be accomplished using batch 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.

Within the GUI program, the spectra acquired by the CCD are corrected for dark current and the offset inherent in the CCD's digitizer, and converted to Matlab matrix format. The spectra are then analyzed using the singular value decomposition (SVD) fitting algorithm described by Finlay *et al.* (28). This algorithm requires the selection of basis spectra corresponding to the known components of the fluorescence emission spectrum. We have constructed two basis spectra, both taken from a Liposyn phantom. The first spectrum is the fluorescence measured in a phantom consisting only of Liposyn dissolved in water to a lipid concentration of 0.5%. This fluorescence signal arises primarily from the plastic catheter and optical components in the beam path. This component is therefore independent of the sample being measured, and can serve as a measure of excitation light intensity. The second

basis spectrum is that of MLu, measured at a concentration of 0.5 mg/kg in the same phantom.

The SVD algorithm we employ also includes a 61-term Fourier series (28) 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 SVD algorithm reduces the measured spectrum to a set of amplitudes, one for the background component, one for MLu and 61 for the Fourier series. To compensate for variations in lamp intensity, we divide the MLu amplitude by the background amplitude obtained from the same spectrum, yielding a normalized MLu amplitude. The scaling of the basis spectra is chosen such that the normalized MLu amplitude obtained from the 0.5 mg/kg MLu phantom is 0.5. Therefore, if the fluorescence is not distorted by sample optical properties (see below), the normalized MLu amplitude is numerically equal to the MLu concentration of the sample in milligrams per kilogram.

The normalized MLu amplitude provides a quantitative measure of the local MLu concentration. Because it is determined by fitting to the entire measured spectrum, this value is less sensitive to noise at individual wavelengths and to the presence of unknown fluorophores with spectra distinct from that of MLu than a single wavelength measurement would be. The relationship between the amplitude and the local concentration of MLu, however, is not necessarily the same as that in the phantom in which the basis spectrum is measured. The *in vivo* case differs from the phantom case in the chemical composition of the medium and in its absorption and scattering coefficients at both the excitation and emission wavelengths of MLu. To take these effects into account, we multiply the measured MLu amplitude by an empirically determined correction factor, as described below.

Effect of optical properties on the measured fluorescence signal

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.* (20). 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 Foster (17) 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:

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

where r_{sd} is the distance between the source and detector, D is the diffusion constant given by $1/(3\mu'_s)$ (29), μ_{eff} is the effective attenuation coefficient equal to $\sqrt{3\mu_a\mu'_s}$ (30), the subscripts x and m denote the excitation and emission wavelengths, respectively, and F_0 is the intrinsic fluorescence of the sample. The diffusion theory fails at small albedos and small source-detector separations. To overcome this, we replace Eq. 1 with the corresponding higher-order P_3 approximation. This approximation has identical form, but four terms rather than one, corresponding to the four combinations of the asymptotic and transient solutions of the P_3 equation at the excitation and emission wavelengths (17). This formula assumes that both the source and detector are point-like. In the measurements reported here, a single fiber serves as both source and detector; however, it is finite in extent and nonisotropic.

To approximate the true physical situation, we assume that the light beam exiting or entering our probe is a pencil beam. To account for the finite extent of the probe, we represent the excitation source and the detector as two pencil beams separated by an empirically determined shift. We model each pencil beam by a point source at a distance of one transport mean free path in the z direction (31); r_{sd} then becomes the distance between these two virtual sources.

To test the accuracy of this model, 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 from 0 to 10 mg/kg, while μ'_s was held constant. In the second, the μ_a was varied by adding black ink in varying amounts to a phantom with constant μ'_s and constant MLu concentration of 4 mg/kg. The optical properties of each phantom are listed in Table 2. In each case, the fluorescence was analyzed as described above and the resulting MLu signal was divided by the known MLu concentration (in units of milligrams/kilogram).

To account for differences in optical properties between our phantom and the *in vivo* case, we have introduced an empirical correction factor that depends on the measured 732 nm optical properties of the sample. Ideally, the measured optical properties at each position at which fluorescence is measured could be used as inputs for the theoretical expression described in (Eq. 1), and the resulting correction factor could be calculated from first principles. However, such a calculation would require accurate knowledge of the optical properties at the excitation wavelength as well as the emission wavelength, which is beyond the capability of our current measurement system. Instead, we have adopted an empirical, multiplicative correction of the form:

$$C = C_0 \frac{\exp(b\mu_{eff})}{\mu'_s} \quad (2)$$

where the optical properties are those measured at 732 nm. Equation 2 is an approximation of the inverse of Eq. 1, in the case of high albedo and unchanging optical properties at the excitation wavelength.

Absorption measurements

In addition to the fluorescence measurements described above, we measured the MLu concentration using single wavelength (4) and spectrally resolved absorption measurements (23). These were acquired by placing an isotropic fiber-based light source illuminated by the 732 nm treatment laser or by a broad-spectrum lamp in a catheter parallel to the detection catheter and measuring the fluence rate or spectrum in the detection catheter with an isotropic detector, as reported previously (11,25). In the case of spectroscopy, the resulting spectra can be fit using photon diffusion theory to determine the absorption and scattering spectra of the prostate. The absorption spectra can then be fit using the SVD algorithm described above to determine the concentrations of oxy- and deoxy-hemoglobin and MLu. In the single-wavelength case, we assume that the μ_a of the prostate varies linearly with MLu concentration as $\mu_a = ([\text{MLu}] 0.066 \text{ (mg/kg)}^{-1} \text{ cm}^{-1} + B)$, as determined previously from a larger set of patient measurements (4), where B is a constant that accounts for the MLu-independent background absorption of tissue. In the previous work, the best fit value of B was found to be 0.23 cm^{-1} .

To determine the optimal value of B for the patients shown here, we have compared the absorption spectroscopy and 732 nm absorption measurements taken in three quadrants for Patient No. 13, as shown in Fig. 2. A linear fit to the combined data for this patient assuming uniform relative error for all data points yields a B value of 0.11 cm^{-1} , as indicated by the solid line. The minimum and maximum values of B obtained from individual quadrants were 0.16 cm^{-1} and 0.042 cm^{-1} in the right upper and right lower quadrants, respectively, as indicated by the dashed lines in Fig. 2. For all the patients shown here, we have adopted a value of 0.11 cm^{-1} . This value ensures that none of the MLu concentrations derived from 732 nm measurements are negative, and assures good agreement with the spectrally resolved absorption measurements and with the value measured previously in a canine model (32). This relation can then be used to determine the MLu concentration in a particular prostate from that prostate's μ_a .

RESULTS AND DISCUSSION

Phantom verification

The MLu fluorescence measured in the two tissue-simulating phantoms described above is shown in Fig. 3a as function of excitation wavelength μ_{ax} . 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.

We have applied (Eq. 1) to predict the variation in measured fluorescence resulting from the optical properties of these phantoms. The results are shown by the solid lines in Fig. 3a. In applying (Eq. 1), we used a lateral shift between pencil beams representing the source and detector 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. In Fig. 3b, we plot the factor needed to correct each measurement for the distortion introduced by the difference in optical properties relative to the 0.5 mg/kg phantom. The correction is the inverse of the fluorescence amplitude per unit MLu concentration shown in Fig. 3a. The

correction factors used in analyzing our *in vivo* data correspond to excitation-wavelength absorption coefficients of approximately 1 to 4 cm⁻¹, as indicated by the shaded area.

There may be significant variations in optical properties among prostates (4) and within a single prostate (11). The agreement between theory and experiment indicates that if the reliability and accuracy of absorption measurements can be improved, these effects can be corrected for each individual prostate from first principles. This correction requires accurate knowledge of the absorption and scattering coefficients of the tissue at both the excitation wavelength (465 nm) and the emission wavelength range (approximately 730 to 780 nm). Our current experimental design allows us to measure the optical properties at 732 nm, and our absorption spectroscopy measurements extend over the entire emission range. We do not currently measure the optical properties at the excitation wavelength. Extrapolating the optical properties at 465 nm based on the absorber concentrations obtained from absorption spectroscopy introduces an uncertainty that is likely as large as the correction being made. We have therefore adopted the empirical correction-factor based method described in the Materials and Methods section.

***In vivo* MLu fluorescence**

We have measured *in vivo* fluorescence spectra for five patients thus far. Spectral analysis of a typical fluorescence spectrum is shown in Fig. 4. The noisy line indicates the measured fluorescence emission data, and the dotted and dashed lines, the components determined by the SVD algorithm. We consistently observe a shift in the emission maximum of MLu from approximately 738 nm in the phantom to 745 nm *in vivo*, probably due to differences in chemical microenvironment between the two systems. We have accounted for this wavelength shift by digitally shifting our basis spectrum prior to fitting. The sum of the known components 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.

Determination of parameters for fluorescence correction

In Fig. 5 we plot the ratios of MLu concentration determined by 732 nm absorption (open squares) and by absorption spectroscopy (open triangles) to the corresponding normalized fluorescence amplitude as functions of the measured μ_a at 732 nm. Each value plotted represents the mean from a single quadrant. A total of 13 single wavelength and six spectroscopic measurements from four patients are included. Equation 2 was fit to the data points shown in Fig. 5 with C_0 and b as free parameters, resulting in values of 3.1 cm⁻¹ and 0.97 cm, respectively. It is likely that these values are specific to the probe design we employed. The solution of (Eq. 2) for each data point is indicated by the corresponding filled circles. The points do not lie on a common curve because the value of μ'_s differs from point to point. The correction factor thus determined was applied to all patients for whom 732 nm optical properties measurements were available. Because the fluorescence spectra were measured at more points than the optical properties, the optical properties were interpolated at intermediate points. When the fluorescence measurements extended beyond the range of

the optical properties measurements, the mean optical properties for the quadrant were used. In cases where one quadrant was missing these measurements, the mean optical properties for the patient were substituted. In one case in which reliable measurements were not available for any quadrant, a global mean correction factor of 9.3 was adopted. This value was determined by averaging the measured correction factors based on comparison with values obtained from 732 nm absorption for the patients listed in Table 3.

Spatial distribution of MLu

In one patient (No. 13), we obtained fluorescence profiles in all four quadrants of the prostate both before and after PDT treatment. These profiles, corrected for the effects of optical properties using (Eq. 2), are shown in Fig. 6. The profiles taken before and after treatment show similar shape and concentration, with the exception of the right upper quadrant (RUQ) (Fig. 6b), which shows a similar shape but reduced concentration compared with the pretreatment profile. The measurements in the upper quadrants (left upper quadrant [LUQ] and RUQ) were made 1.5 cm anterior to those in the left and right lower quadrants (LLQ and RLQ, respectively), whereas those in the right quadrants (RUQ and RLQ) were shifted 2 cm laterally from those in the left quadrants (LUQ and LLQ, respectively), as shown in Fig. 1b.

As expected, we see significant variation among the four quadrants. However, the general features; namely, a peak in MLu concentration around 0.5 to 1.5 cm and a shallow minimum around 3.0 cm, 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. The origin of this fluorescence variation is not clear; however, the phenomenon of increased fluorescence near the periphery of an organ or tumor has been observed in animal models (33).

We have compared spatial distributions obtained by fluorescence measurements with single-wavelength and spectrally resolved absorption measurements from 16 scans in four patients to determine the relation between the optimal correction factor and the measured μ_a at 732 nm, as described in Materials and Methods. The mean calculated correction factor used for each patient is listed in Table 3. For comparison, we also list the mean values of ratio of MLu concentrations determined by absorption spectroscopy and 732 nm μ_a to that determined by uncorrected fluorescence spectroscopy. These values are in rough agreement with the calculated values for each patient.

The relatively small variation in the correction factor, given the large variation in tissue optical properties (4,11) is likely explained by a combination of several factors. First, the μ_a of hemoglobin at the excitation wavelength is an order of magnitude greater than in the emission window, and that of MLu is greater by a factor of two or more. The result is that optical properties at the excitation wavelength have a much greater effect on the fluorescence than those at the emission wavelengths. At the excitation wavelength, however, the absorption in tissue is dominated by hemoglobin, so variations in MLu concentration will have relatively little effect.

Second, single-wavelength absorption measurements of a larger set of human prostates performed as part of our clinical protocol indicate that the majority of the interpatient variation in absorption coefficient at 732 nm arises from variations in MLu concentration. This indicates that the effect of absorption at 732 nm will be to reduce the measured fluorescence in cases of high MLu concentration, and to increase it in cases of low MLu concentration (4). Our fluorescence measurements may therefore underestimate the heterogeneity in MLu; however, that our correction factor is determined empirically ensures that the mean MLu concentration will be recovered accurately.

In Fig. 7, we plot the concentrations of MLu determined by single-wavelength absorption measurements, absorption spectroscopy and fluorescence spectroscopy measured in the RUQ of Patient No. 13. Because comparisons of the type shown in Fig. 7 were the basis for the patient-specific factor used to correct the fluorescence, it is expected that the three measurements agree in absolute concentration. The single-wavelength and spectrally resolved absorption measurements, however, are independent and have not been scaled or normalized to match one another.

The agreement in the spatial distributions measured by absorption and fluorescence is good, particularly in the post-PDT case (Fig. 5b), and confirms that the fluorescence and absorption 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 all three methods decreases by nearly a factor of two, especially in the region around 2 to 3 cm, in the center of the prostate. Despite this local photobleaching, the mean MLu concentration in the quadrant as a whole decreased by only 20%, because the concentration outside this region remained unchanged.

Comparison of mean MLu concentration among patients

In Fig. 8 we plot the mean, standard and maximum deviations of MLu concentration in each quadrant. The samples are restricted to points within 2.5 cm of the distal end of the prostate to prevent including measurements from other structures. The data are grouped by patient. Within each patient, the quadrants are plotted in order from RUQ to LLQ as indicated. Only for Patient No. 13 did we make measurements before and after treatment in all four quadrants. The mean MLu concentration obtained from fluorescence measured *in vivo* is plotted as a function of the corresponding value measured *ex vivo* from biopsies in Fig. 9. Both the pre-PDT (open squares) and post-PDT (filled squares) data are plotted. The error bars indicate the range in MLu concentration measured *in vivo*. The dashed lines connect each pre-PDT measurement with its corresponding post-PDT measurement. For comparison, the solid line has a slope of 1, indicating perfect agreement. Although the biopsy-determined value is within the range of the fluorescence measurements for the majority of cases, the uncertainties in the measurements and the sample size make it impossible to draw any statistically significant conclusions based on this comparison.

In Fig. 10, we plot the mean concentration of MLu in each prostate measured by *in vivo* fluorescence, the *ex vivo* fluorescence assay, and single-wavelength and spectrally resolved absorption before (open symbols) and after (filled symbols) irradiation. For Patient No. 16, we measured 732 nm absorption along four catheters in each quadrant. The mean reported

here is the mean of all measurements in the entire prostate. Several anomalous values of μ_a larger than 2.5 cm^{-1} were discarded as outliers. The dotted lines show the mean of the four measurements in each patient, and the shaded region extends one standard deviation on each side of the mean.

The light-induced destruction of photosensitizer, or photo-bleaching, during PDT has been observed for several sensitizers. *In vivo* measurements of photobleaching of MLu, however, have not been previously reported. In one case (Patient No. 12) we observed significant photobleaching of the sensitizer during treatment. In another (Patient No. 13), we observe some photobleaching in the RUQ, but no significant photobleaching in other quadrants. In general, the variation in MLu concentration within a given patient is greater than the change induced by irradiation, making a generalization with respect to the photobleaching behavior *in vivo* impossible in the present study.

While the absorption and fluorescence measurements reported here agree in the MLu concentration, the two measurements are not interchangeable. Absorption measurements, because they allow characterization of fluorescent and nonfluorescent absorbers, can provide information about hemoglobin concentration and oxygen saturation, in addition to drug concentration measurement. Fluorescence measurement requires only a single catheter to measure the drug distribution, while the absorption measurements require two. In our clinical experience, we have often encountered cases in which the pooling of blood around one or more catheters renders the absorption spectroscopy data uninterpretable. In many of these cases, fluorescence spectra, which require only one catheter to be blood-free, could still be measured and analyzed.

We anticipate that fluorescence measurements similar to those shown here will be useful for PDT dosimetry. They allow rapid acquisition of drug distribution information at high resolution with far fewer scans than would be required for an absorption spectroscopy measurement of comparable resolution. It is true that the fluorescence measurement requires a corresponding optical property measurement for optical properties correction, which adds to the complexity and acquisition time. Ongoing work in our laboratory aims to develop a method for determining absorption and scattering coefficients from a single scan of a single-wavelength linear light source such as that used for treatment. We anticipate that this will greatly improve the efficiency of data acquisition, and make fluorescence spectroscopy practical as part of a near-real time dosimetry system.

CONCLUSIONS

We have demonstrated the ability of fluorescence spectroscopy, coupled with an empirical, model-based fitting algorithm, to quantitatively measure the concentration and distribution of MLu in the human prostate. The variation in MLu concentration among prostates makes individual measurement necessary. In addition, we observe variations in MLu fluorescence within individual prostates that is equal to or greater than the variation among prostates of different patients. This indicates that to optimize treatment for the entire prostate, it will be necessary to compile a map of the MLu concentration in three dimensions. 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 (Fig. 4). Our current work indicates that the spatial variation of MLu concentration occurs on a scale of approximately 1.5–2 cm in all three dimensions. This can be achieved in our current clinical protocol by using the catheters currently reserved for treatment (at 1 cm spacing), in addition to the four dedicated detector catheters for fluorescence measurement. Work in this area is ongoing.

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Figure 1.

(a) 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. (b) Arrangement of the fluorescence detection catheters in a typical human prostate.

Figure 2.

Comparison of the 732 nm absorption coefficient with the MLu concentration determined by absorption spectroscopy. The quadrant from which each data point was taken is indicated in the legend. The solid line indicates the best fit used to determine the offset B , as described in the text.

Figure 3.

(a) MLu fluorescence measured in two sets of phantoms, plotted as a function of excitation-wavelength absorption coefficient. See the text for details. The solid lines indicate the values predicted by (Eq. 1), and (b) the corresponding correction factor required to recover (F_0) as determined from (Eq. 1). The shaded area indicates the range of correction factors determined by comparing *in vivo* scans, and the dashed line indicates the mean *in vivo* correction factor. In both cases, the correction factor was normalized to 1.0 for the case of the 0.5 mg/kg MLu phantom.

Figure 4.

SVD analysis of a typical fluorescence emission spectrum (reproduced with permission from reference [27]). The spectrum is separated into MLu and background components and a small residual composed of a Fourier series.

Figure 5. Correction factor (ratio of fluorescence amplitude to concentration) determined using Eq. 2 (calculated C).

Figure 6. MLu fluorescence profiles acquired in the four quadrants of the prostate for Patient No. 13 before (dashed lines) and after (solid lines) PDT treatment. Frames (a) through (d) depict the results from the right upper, left upper, left lower and right lower quadrants, respectively, as indicated in the panel titles. Measurements in all four quadrants were corrected for optical properties using the correction factor appropriate to this patient.

Figure 7.

MLu fluorescence (filled circles), absorption spectroscopy (triangles) and 732 nm absorption (squares) profiles as functions of position within the right upper quadrant of Patient No. 13. The profiles measured before (a) and after (b) PDT treatment are similar in shape, but indicate some photobleaching, especially in the center of the prostate.

Figure 8.

Concentration of MLu measured by fluorescence spectroscopy for each patient. Within each patient, the concentration from each quadrant is plotted separately, as indicated. The solid and dashed error bars represent the standard and maximum deviations of measurements within each quadrant, respectively. The concentrations measured before treatment (open circles) are grouped with the corresponding measurements made after treatment (solid diamonds), and all are corrected using the factors listed in Table 3.

Figure 9.

Mean MLu concentration determined by *in vivo* fluorescence plotted as a function of the concentration determined by an independent *ex vivo* assay. The dotted and solid error bars indicate the maximum and standard deviations, respectively, of measurements within each prostate. Dashed lines connect the pre-PDT measurements with their corresponding post-PDT measurements. The solid line indicates exact agreement.

Figure 10.

MLu concentration in five human prostates measured by fluorescence spectroscopy (circles), *ex vivo* fluoroscopy (squares), absorption spectroscopy (diamonds) and 732 nm absorption (triangles). Both pre-PDT (open symbols) and post-PDT data (filled symbols) are plotted. The dotted lines indicate the mean of all measurements in each patient, and the shaded areas include one standard deviation. The mean fluorescence correction factor for each patient is given in Table 3.

Table 1

Treatment parameters for each patient, including MLu dose, interval between drug administration and beginning of irradiation and total fluence prescribed to the prostate

Patient number	MLu dose (mg/kg)	Interval (hours)	Total fluence (J/cm ²)
12	2	3	50
13	2	3	100
14	2	3	100
15	2	3	100
16	2	3	150

Table 2

Constituents and optical properties of the two sets of phantoms used to investigate the effect of absorption on fluorescence measurements. In each case, the absorption coefficient due to MLu ($\mu_{a\text{ MLu}}$) and the total absorption coefficient ($\mu_{a\text{ Total}}$) are listed separately

Phantom	1	2
Liposyn (%)	0.5	0.75
MLu (mg/kg)	0 to 10	4
Ink (%)	0	0 to 0.5
465 nm		
$\mu_{a\text{ MLu}}(\text{cm}^{-1})$	0 to 1.6	0.63
$\mu_{a\text{ Total}}(\text{cm}^{-1})$	0 to 1.6	0.63 to 5.0
$\mu'_s(\text{cm}^{-1})$	6.8	10.2
732 nm		
$\mu_{a\text{ MLu}}(\text{cm}^{-1})$	0 to 0.63	0.25
$\mu_{a\text{ Total}}(\text{cm}^{-1})$	0 to 0.63	0.25 to 0.39
$\mu'_s(\text{cm}^{-1})$	4.0	6.0
Symbol in Fig. 3	○	■

Table 3

Fluorescence correction factors determined by comparing spatially resolved fluorescence, absorption spectroscopy and 732 nm absorption measurements in four patients and the correction factor determined using (Eq. 2)

Patient	Absorption spectroscopy/ fluorescence		732 nm/fluorescence	
	No. of scans	Mean	No. of scans	Mean
12			1	7.3
13	6	5.8	6	6.4
15			1	11.4
16			3	12.1
Overall	6	5.8	11	9.3 ± 4.8
				9.4 ± 2.7