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Optical Properties of Human Prostate at 732 nm Measured *In Vivo* During Motexafin Lutetium-mediated Photodynamic Therapy[†]

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Abstract

Characterization of the tissue light penetration in prostate photodynamic therapy (PDT) is important to plan the arrangement and weighting of light sources so that sufficient light fluence is delivered to the treatment volume. The optical properties (absorption [μ_a], transport scattering [μ'_s] and effective attenuation [μ_{eff}] coefficients) of 13 patients with locally recurrent prostate cancer were measured *in situ* using interstitial isotropic detectors. Measurements were made at 732 nm before and after motexafin lutetium (MLu)-mediated PDT in four quadrants. Optical properties were derived by applying the diffusion theory to the fluence rates measured at several distances (0.5–5 cm) from a point source. μ_a and μ'_s varied between 0.07 and 1.62 cm⁻¹ (mean 0.37 ± 0.24 cm⁻¹) and 1.1 and 44 cm⁻¹ (mean 14 ± 11 cm⁻¹), respectively. μ_a was proportional to the concentration of MLu measured by an *ex vivo* fluorescence assay. We have observed, on average, a reduction of the MLu concentration after PDT, presumably due to the PDT consumption of MLu. μ_{eff} varied between 0.91 and 6.7 cm⁻¹ (mean 2.9 ± 0.7 cm⁻¹), corresponding to an optical penetration depth ($\delta = 1/\mu_{\text{eff}}$) of 0.1–1.1 cm (mean 0.4 ± 0.1 cm). The mean penetration depth at 732 nm in human prostate is at least two times smaller than that found in normal canine prostates, which can be explained by a four times increase of the mean value of μ'_s in human prostates. The mean light fluence rate per unit source strength at 0.5 cm from a point source was 1.5 ± 1.1 cm⁻², excluding situations when bleeding occurs. The total number of measurements was $N = 121$ for all mean quantities listed above. This study showed significant inter- and intraprostatic differences in the optical properties, suggesting that a real-time dosimetry measurement and feedback system for monitoring light fluences during treatment should be considered for future PDT studies.

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INTRODUCTION

Photodynamic therapy (PDT) is a treatment modality using light of an appropriate wavelength in the presence of oxygen to activate a photosensitizing drug, which then causes localized cell death or tissue necrosis. Using a surface illumination technique, PDT has been used to treat superficial tumors, including those of the skin, lung, esophagus and bladder (1). This technique is, however, inadequate when applied to large bulky tumors or solid organs because of limited light penetration into tissue. Interstitial light delivery, wherein optical fibers are placed directly into bulky tumors or organs, is a more rational approach in these cases.

The prostate gland is an organ that appears to be a good target for interstitial PDT. Tumors of the prostate are often confined to the prostate itself, and brachytherapy techniques used for the placement of radioactive seed implants can be adapted for the placement of interstitial optical fibers (2). Several preclinical studies have evaluated the feasibility of delivering PDT to the prostate via this interstitial approach (3–8). A trial of interstitial prostate PDT in humans has been reported by Nathan *et al.* (9), who treated 14 men with locally recurrent prostate cancer using *meso*-tetrahydroxy-phenyl chlorin-mediated interstitial PDT. The light treatment was directed toward regions from which biopsies showed cancer or which were suspicious on imaging studies. Because prostate cancer is a multifocal disease, our PDT protocol is designed to ablate the prostate gland completely. The development of a light delivery technique appropriate to this goal has necessitated an improved understanding of light dosimetry, which is critical in planning the configuration of multiple fibers within the organ or tumor.

Several investigators have attempted to characterize the optical properties of prostate tissue in animals (10–12) and in humans (13–15) to predict light dosimetry more reliably. Using diffusion theory for a point source, the absorption (μ_a) and transport scattering (μ_s') coefficients of a particular tissue can be determined yielding the effective attenuation coefficient (μ_{eff}), which provides a measure of light penetration in that tissue (11). This measurement is a critical factor in planning interstitial light source placement. The optical properties of prostate tissue have been most extensively evaluated at a wavelength of 630 nm because it is used to activate the most widely used photosensitizer, Photofrin. However, it has been suggested that a wavelength of 630 nm is suboptimal in achieving uniform and adequate penetration due to the absorption of light by naturally occurring chromophores, such as hemoglobin, melanins, cytochromes and flavoproteins, at that wavelength (10,15). In addition, changing concentrations of hemoglobin (in particular deoxyhemoglobin) during PDT may also alter the optical properties of tissues (11). As a result, Amfield *et al.* (10) investigated the use of longer wavelengths of light beyond the absorption bands of these naturally occurring molecules. A comparison between 630 and 789 nm light revealed a consistent and substantial increase in light penetration with the 789 nm light.

Based on a feasibility and toxicity study in a canine prostate model (7), we have started a Phase I study of motexafin lutetium (MLu)-mediated PDT for prostate cancer. MLu is a water-soluble, second-generation synthetic photoactive drug that has a Q-band absorption peak at 732 nm (16,17). In this study we evaluated the optical properties of the human

prostate by illuminating it with a point source emitting 732 nm light. Differences in optical properties were compared before and after PDT in which the entire prostate was treated. Intraprostatic as well as interprostatic differences were evaluated.

MATERIALS AND METHODS

Patient selection, surgical and PDT procedure

A Phase I clinical trial of MLu-mediated PDT in patients with locally recurrent prostate carcinoma was initiated at the University of Pennsylvania. 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. A total of 14 patients were treated, of which 13 patients have undergone measurement of optical properties. Each patient who signed the informed consent document underwent an evaluation, which included magnetic resonance imaging of the prostate, bone scan, laboratory studies including prostatic specific antigen and a urological evaluation. Approximately 2 weeks before the scheduled treatment, a transrectal ultrasound (TRUS) was performed for treatment planning. An urologist drew the target volume (the prostate) on each slice of the ultrasound images. These images were spaced 0.5 cm apart and were scanned with the same ultrasound unit used for treatment. A built-in template with a 0.5 cm grid projected the locations of possible light sources relative to the prostate. A treatment plan was then prepared to determine the location and length of light sources. Cylindrical diffusing fibers (CDF) with active lengths of 1, 2, 3, 4 and 5 cm were used as light sources. The sources were spaced 1 cm apart, and the light power per unit length was less than or equal to 150 mW/cm² for all optical fibers. The length of the CDF at a particular position within the prostate was selected to cover the full length of the prostate (see Fig. 1a). The final plan often required that the prostate be divided into four quadrants. Four isotropic detectors were used, each placed in the center of one quadrant. A fifth isotropic detector was placed in a urethral catheter to monitor the light fluence in the urethra.

MLu was administered intravenously to patients at various times before light delivery (depending on the dose level). In the initial six patients, MLu was delivered 24 h before light delivery. The drug-treatment interval and dose of MLu were subsequently changed according to a predetermined scheme dictated by the protocol. The drug-treatment interval and the amount of MLu for the latest patients were 3 h and 2 mg/kg, respectively (see Table 1).

The patients were anesthetized in the operating room with general anesthesia to minimize patient movement during the procedure. TRUS-guided biopsies for MLu measurements were obtained before light delivery. The ultrasound unit was used to guide needle placement in the operating room. A template was attached to the ultrasound unit and was matched to the same 5 mm grid used for treatment planning. Four detector catheters (one for each quadrant) were inserted into the prostate. These detectors were kept in place during the entire procedure of PDT treatment. Four additional preplanned treatment catheters for light sources were then inserted 0.5 or 0.7 cm away from the detector catheters. These source catheters were used for light delivery and optical properties measurements. The optical

properties of the prostate in each quadrant were then measured before light delivery using these existing detectors and a point source inserted into one source catheter. The time for optical property measurements was approximately 5 min. The PDT treatment was then performed one quadrant at a time by inserting the CDF into the source catheters. A 15 W diode laser, model 730 (Diomed, Ltd., Cambridge, UK) was used as the 732 nm light source. The treatment time for each quadrant was dependent on the detector reading in that quadrant. The light was delivered to ensure that the cumulative fluence reading of all four isotropic detectors reached the same prescription fluence. The total treatment time varied but was always less than 1 h. After light delivery, the optical properties of the prostate in all four quadrants were measured again using a point source (for another 5 min). The light sources and detectors were then removed, and posttreatment biopsies were performed.

Diffusion theory and optimization algorithm

μ'_s and μ_a coefficients characterize the scattering and absorption properties of tissues. With the diffusion approximation, the light fluence rate ϕ at a distance r from a point source of source strength, S , can be expressed as (18)

$$\phi = \frac{S\mu_{\text{eff}}^2}{4\pi r\mu_a} e^{-\mu_{\text{eff}}r} = \frac{S3\mu'_s}{4\pi r} e^{-\mu_{\text{eff}}r} \quad (1)$$

where S is the source strength of the point source, $\phi(r)$ is the fluence rate at position r ,

$\mu_{\text{eff}} = \sqrt{3\mu_a\mu'_s}$ is the μ_{eff} in tissues and is applicable for a wider range of μ_a and μ'_s

combinations than the traditional definition of $\mu_{\text{eff}} = \sqrt{3\mu_a(\mu'_s + \mu_a)}$ (19), although it is obtained as an approximation of the latter expression. $r = \sqrt{x^2 + h^2}$, where x and h are the parallel and perpendicular distances from the center of the point source (see Fig. 1). The two free parameters (μ_a and μ'_s) are inherently separable in that for a point source with a given source strength, the magnitude of the fluence rate near the light source ($x = 0$) is determined by μ'_s only and the slope of the spatial decay of the light fluence rate is determined by μ_{eff} only.

In theory, measurements of ϕ at two different distances r from the point source are sufficient to determine both μ_a and μ'_s . In reality, our measurements contain at least four different distances and up to 800 distances for latter measurements. Measurements at multiple sites allow evaluation of the variation of these optical characteristics within the prostate volume.

Because Eq. (1) is a nonlinear equation of two free parameters μ_a and μ'_s , we used a differential evolution algorithm developed by Storn and Price (20). This algorithm is simple and robust, and converges faster than adaptive simulated annealing or the annealed Nelder and Mead approach (20). We modified the algorithm to require that all free parameters (μ_a , μ'_s and h) are positive (21). The deviations between measurement and fit are represented by

standard deviation, $\sum_{i=1}^n \sqrt{((\text{measurement}_i - \text{fit}_i)/\text{fit}_i)^2 / (n - 1)}$, where n is the number of data points needed in the fit.

Calibration of the isotropic detector and phantom verification

Isotropic detectors described by Marijnissen and Star (22) were used for the study. Each isotropic detector was made of an optical fiber with a spherical tip made of TiO_2 (a scattering material). The isotropic detectors were made by Rare Earth Medical (now CardioFocus, Norton, MA) and have an isotropy of better than $\pm 30\%$ from any direction except for angles within 30° of the optical fiber attachment point. Each detector fiber was connected to a photodiode via an SMA connector. The measured photovoltage (V) from the isotropic detector was converted to light fluence rate using:

$$\phi = a(V - b), \quad (2)$$

where a was the conversion factor and b characterized the leakage of the photodiode. This calibration was performed under collimated 732 nm laser light in air. When the isotropic detector was used to measure fluence rate in tissue (or in a liquid optical phantom), a tissue (or water) correction factor of 2.0 was used. This value was determined by measuring the response of the isotropic detectors in and out of water medium for the same incident irradiance (22). The calibration of the isotropic detectors was checked to be accurate to within 5% using an integrating sphere before each individual measurement.

A homemade, 12-channel light dosimetry system was used for all *in situ* measurements. Five different isotropic detectors were used. The conversion factors were $a = 129\text{--}161$ and $a = 63\text{--}72 \text{ mW/cm}^2/\text{V}$ for the four isotropic detectors with 0.5 mm scattering tips and the isotropic detector with 1 mm scattering tip used in the urethra, respectively. $b = 0.020 \text{ V}$ for all isotropic detectors. The response was found to be linear over the light fluence rate range ($0\text{--}1200 \text{ mW/cm}^2$) to within $\pm 5\%$. The minimum resolution of the dosimetry system was better than 0.1 mW/cm^2 after the introduction of an additional software-controllable analog gain to adjust the dynamic range of the light dosimetry system.

To ensure that the optimization technique was robust and to estimate the accuracy of the optimization algorithm to extrapolate optical properties, we measured the optical properties of an optical phantom with known μ_a and μ'_s using the same methods as those used in human patients. The optical phantom was composed of pure scattering medium (liposyn III, 30% Abbott Lab, North Chicago, IL) and pure absorbing medium (green ink). This type of phantom has been described by Flock *et al.* (23). The concentrations of liposyn used were 0.25% and 0.5% by volume in water. Three green ink concentrations were used: 0%, 0.014% and 0.07%. The optical properties (μ'_s and μ_a) of the liquid phantom were independently characterized by analyzing the decay of intensity along the axis of a broad collimated light beam illuminating the phantom from above (12). They were $\mu'_s = 3.6$ and 7.2 cm^{-1} and $\mu_a = 0.02, 0.1$ and 0.5 cm^{-1} . The phantom was placed in a plastic container, which was painted black and was large enough ($18.2 \times 14.6 \times 7.7 \text{ cm}^3$) to protect the detector from boundary effects. A point light source with an outside diameter of $500 \mu\text{m}$ (Pioneer Optics, Windsor Locks, CT) was used for light delivery, along with a polyurethane template and plastic catheters. Fluence rates were measured at various positions along the length of detector catheters placed at fixed distances ($h = 0.5\text{--}0.7 \text{ cm}$) from the source catheter.

A second set of phantoms was designed to verify that the μ_a of the phantom varies linearly with MLu concentration. These phantoms were constructed with a lipid content of 0.5%. (Note a different batch of Liposyn was used. As a result, $\mu_s' = 4 \text{ cm}^{-1}$ for 0.5% lipid content.) The measurements and data analysis proceeded as above using 732 nm laser and the same setup.

The transmission of the light from the sources through the transparent catheters was measured in a 6 inch diameter integrating sphere (IS60, LabSphere, Inc., North Sutton, NH) as a ratio of the detector reading with and without the catheter. The result was 1.0 for both point and linear sources. The transmission of the catheter for an isotropic detector in air was between 0.95 and 1 depending on the whether the light source was farther away or very close to the detector. The latter was expected in tissue because the light was coming from all directions and was very close to the detector in the catheter. Based on these results no transmission correction was necessary for the transparent catheters for either the light source or the isotropic detectors.

PDT and in vivo light measurements

A total of 14 patients were treated. MLu (Pharmacyclics, Inc., Sunnyvale, CA) injection was administered intravenously (0.5–2 mg/kg) 3–24 h before light administration (16,24). After the patient was anesthetized, the interstitial CDF were placed in the gland using a template with evenly spaced holes, which was attached to the TRUS unit. A 17-gauge plastic catheter (Flexi-needle, Best Industries, Inc., Springfield, VA) containing a metal trocar was placed through the template and into the prostate. The trocar was removed and replaced with the light diffuser. The light energy delivered was prescribed based on *in situ* measured light fluence. Each patient received a light fluence between 25 and 100 J/cm², determined by *in vivo* measurement using isotropic detectors. The maximum unit length source strength in any one fiber was limited to 150 mW/cm. Table 1 summarizes the treatment delivered to all patients.

Measurements were taken at various distances from the light source before and after light treatment in various quadrants of each prostate, measured at 2 cm from the apex of the prostate. For the last two patients, the light sources were moved along the catheter in several locations to also quantify the variation of optical properties in the prostate gland along the catheter.

Ex vivo MLu measurement

In each patient a needle biopsy of prostate tissue from each quadrant was collected before and after light delivery. Biopsies were immediately frozen on dry ice, transported to the laboratory and stored at -80°C until the time of assay. Caution was taken to protect the samples from light. MLu was extracted from the needle biopsies using a procedure based on a previous report (25). Tissue samples were thawed to room temperature, weighed and, depending on the amount of tissue available, two to four of the needle biopsies were combined for analysis. Only biopsies collected at the same time point, *i.e.* either pre- or post-PDT, were combined. A combined tissue weight of $\sim 5\text{--}25 \text{ mg}$ was sought. Specimens were cut into shorter lengths, placed in a 2 mL capped polypropylene tube and homogenized

(Polytron 1200) in 400 μL of phosphate buffer (24 mM, pH 7.5). Homogenates were mixed with 400 μL of chloroform, then 400 μL of methanol was added. After centrifugation (3500 rpm, 15 min) the organic layer was collected and 200 μL was transferred to a cuvette. The fluorescence of the homogenized sample was measured by a spectrofluorometer (FluoroMax-3, Jobin Yvon, Inc., Edison, NJ) with λ_{ex} of 474 nm and λ_{em} of 740 nm (emission scan range from 650 to 850 nm). MLu concentration in the tissue was calculated on the basis of the increase in fluorescence resulting from the addition of a known amount of MLu to each sample after its initial reading. Data are presented as nanograms of MLu per milligram of tissue.

Correlation between μ_a and MLu concentration

To correlate the relationship between μ_a measured *in vivo* with the MLu measured from *ex vivo* biopsy, we plot out the subset of μ_a data (24 points) that had corresponding *ex vivo* biopsies. Notice that although the *ex vivo* biopsies were taken from the same prostate, they are not necessarily at the same location as that used for μ_a measurement because the prostate template was not used for the biopsy. Once the parameters for a linear relationship between μ_a and MLu concentration are established, all measured μ_a data (121 points) were used to examine the variation of MLu concentration intra- and interprostate.

RESULTS

Figure 2 compares the fit and measurement of light fluence rate at different distances from a point source in two optical phantoms made of different concentrations of liposyn and green ink mixtures. The solid lines are the result of nonlinear fitting using the differential evolution algorithm. The resulting optical properties are shown. The extrapolated μ'_s agreed with the true values to within $\pm 6\%$; the extrapolated μ_a agreed with the true values to within $\pm 11\%$.

Figure 3 shows the variation of measured light fluence rate distribution (solid lines) and associated fit (symbols) for (1) different locations in the same prostate gland and (2) the same location in a prostate before and after light delivery. In Fig. 3a, an example of the light fluence rate radial distribution for the right lower (RLQ), right upper (RUQ) and left upper (LUQ) quadrants of one prostate is shown. In this particular patient, the extrapolated optical properties were very different between RLQ ($\mu_a = 0.23 \text{ cm}^{-1}$, $\mu'_s = 7.3 \text{ cm}^{-1}$) and RUQ ($\mu_a = 0.44 \text{ cm}^{-1}$, $\mu'_s = 12 \text{ cm}^{-1}$). A large variation was also observed among different patients (see Fig. 5). When evaluated across all patients, the extrapolated attenuation coefficients varied between 0.9 and 6.7 cm^{-1} , whereas the extrapolated μ'_s varied between 1.1 and 44 cm^{-1} . As a result of the heterogeneity of optical properties, the light fluence rates per unit source strength at 0.5 cm from the point source varied between 0.2 and 4.1 cm^{-2} among the patients. However, no significant change of optical properties was observed before and after PDT treatment in the same site for most but not all patients (Fig. 3b).

Figure 4 shows the temporal dependence of the light fluence rate at the center of the four treatment quadrants. The large variation of light fluence rate was due to the movement of the

isotropic detector. Notice that we did not observe any significant light fluence rate in urethra in all patients treated so far.

The overall variation of optical properties (μ_a , μ_s' and μ_{eff}) and the optical penetration depth ($\delta = 1/\mu_{\text{eff}}$) before (first bar) and after (second bar) PDT for each patient studied is shown in Fig. 5. The error bars represent the standard deviation among the four different quadrants in the same patient. No error bar is present in cases where only one measurement is available. These results are also summarized in Table 2.

The interprostatic heterogeneity of light fluence rate at 0.5 cm from the point source per unit source strength is shown in Fig. 6. The x axis identifies each patient measured. The first bar of the pair was measured before PDT, and the second bar of the pair was measured after PDT. The error bars represent the standard deviation among the four different quadrants in the same patient. The solid line is an average with the range (dashed lines) corresponding to standard deviation. The mean fluence rate per unit source strength at 0.5 cm for all sites (excluding bleeding) was $1.5 \pm 1.1 \text{ cm}^{-2}$ (sample size 121).

The relationship between the measured μ_a and the tissue concentration of MLu, measured *ex vivo* after drug extraction from biopsy specimens is shown in Fig. 7a. μ_a were measured *in situ* and biopsies were collected before (○) and after (*) PDT. For comparison, we have also plotted the measured μ_a of MLu vs MLu concentration (+) in a pure liposyn phantom. Figure 7b shows the relationship between MLu concentration measured from tissue biopsies (bars) and extrapolated from μ_a (symbols) vs the injected MLu dosage to patients. Drug concentration was calculated from μ_a using the linear relationship determined in Fig. 7a: $c \text{ (ng/mg)} = (\mu_a \text{ (cm}^{-1}) - 0.227)/0.0658$. Error bars represent the standard deviation among measurements in different locations within a prostate or different prostates. The correlation coefficient ($R^2 = 0.63$) of the linear relationship between μ_a and tissue concentration of MLu suggests good association, although the exact numerical values for the linear equation may contain error due to the spread of the data.

DISCUSSION

The main objective of this study was to evaluate the optical properties of prostate tissue in the human prostate using 732 nm light. Comparisons were made before, during and after MLu-mediated PDT. Differences within and among patients were also studied.

Intrapatient heterogeneity of optical properties was observed. Figure 3 is a representative example of this heterogeneity at 732 nm. There was a difference in the μ_a ($0.23\text{--}0.4 \text{ cm}^{-1}$) and μ_s' ($6.6\text{--}12 \text{ cm}^{-1}$) values at different locations within the same prostate gland (Fig. 3a). As a result, the fluence rate per source strength at 0.5 cm from the point source varied from 0.8 to 1.3 cm^{-2} . In contrast, the optical properties (μ_a and μ_s') did not change substantially before or after PDT light delivery. Thus, the light fluence rate at the same site of the same prostate did not vary much before and after PDT light delivery (Fig. 3b).

In general, we found a lack of change in the optical properties in a particular site within the prostate before and after PDT. This was confirmed by the absence of any time dependence

of the fluence rate in the middle of the four quadrants (Fig. 4). The results shown in Fig. 4 are typical of temporal fluence rate measurements among patients in the study. It shows very little variation of optical properties for most sites, except for LUQ where the fluence rate seems to decrease. These measurements were performed in such a way that the same site was used before and after PDT. Our general conclusion is consistent with published results at 630 nm for Photofrin-mediated PDT (11) and at 732 nm for MLu-mediated PDT (12) in the canine prostate model, where little time dependence of light fluence rate was observed. However, because of large heterogeneities, variation in optical property measurements before and after PDT was found (see Fig. 5).

There are several potential reasons why no significant light fluence rate was measured in the urethra (Fig. 4). First of all, it could be due to measurement uncertainty. The light treatment was performed one quadrant at a time rather than all four quadrants together. Because the range of the light penetration in human prostate is relatively short (0.4 cm), only those CDF (usually in the RUQ and the LUQ) near the urethra will contribute to the urethra. Because the position of the isotropic detector in urethra was optimized only once during the treatment of one quadrant, it is possible that we had not obtained the peak light fluence rate for the entire treatment. (The position of the urethral isotropic detector could not be optimized multiple times because one can only move the position of the urethra isotropic detector in one direction.) Second, the light fluence could actually be lower in the urethra. Besides the fact that we have avoided placing light sources through the urethra, we have noticed increased absorption in the urethra due to high concentrations of MLu in urine, which will reduce the light fluence rate reaching the isotropic detector and thus, the urethra itself. No serious urethral toxicity has been observed in all the patients treated so far.

The accuracy of the method used to determine optical properties from measurements around a point source was dominated by the uncertainty of the distance between the light source and detector catheters (h , see Fig. 1b). This is because the inserted catheters are not exactly parallel to each other and an error of 1–2 mm is possible, despite the use of ultrasound guidance to position the catheters correctly. A detailed error analysis is included in the Appendix. To reduce the positioning error, the later measurements (starting with Patient 9) were made using a motorized probe, with a positioning accuracy of at worst 0.2 mm. The use of the motorized probe also increased the number of measurement points from typically four points per measurement to approximately 800 points per measurement. We then allowed the distance h to be one of the free parameters of the fit (μ_a , μ_s' and h), and h was allowed to be different from the known separation based on the template by 1–2 mm. Using this method our phantom studies confirmed that one can determine $\phi(0.5)$ and μ_a to within $\pm 20\%$ for most cases. The error for μ_{eff} is much smaller ($\pm 5\%$) and is insensitive to the positioning error in h (see Appendix).

Some of the measurements cannot be used to extrapolate optical properties because of bleeding. This happens when blood surrounding one of the catheters (either source catheter or the detector catheter) blocks the light from reaching the detector. We used a standard of $\phi/S > 0.1 \text{ cm}^{-2}$ to determine if there was sufficient signal to analyze the data. The average success rate for the first seven patients was very poor (approximately 35%). This was

increased to 64% for later patients using a motorized probe, primarily because more measurements can be made in a shorter period of time.

The overall variation of optical properties (μ_a , μ_s' , μ_{eff} and $\delta = 1/\mu_{eff}$) before and after PDT for different patients is shown in Fig. 5 to demonstrate interpatient heterogeneity. The heterogeneity in optical properties was the major reason that the light fluence rate at a fixed distance away from the CDF varied from patient to patient. However, the heterogeneity of optical properties in human prostate is somewhat smaller than that observed in canine prostate at 732 nm (12). Overall μ_a varied between 0.07 and 1.62 cm^{-1} , and μ_s' varied between 1.1 and 44 cm^{-1} . μ_{eff} varied between 0.91 and 6.7 cm^{-1} , corresponding to an optical penetration depth ($\delta = 1/\mu_{eff}$) of 0.2–1.1 cm. The mean values of μ_{eff} and δ were $2.9 \pm 0.7 \text{ cm}^{-1}$ and $0.4 \pm 0.1 \text{ cm}$, respectively. This penetration depth is substantially larger than that of 0.1–0.25 cm predicted for 630 nm (11) but is smaller than 0.5–3 cm observed in normal canine prostate at 732 nm (12). The most probable explanation is that canine prostate has different glandular/structure content than that of human prostate. Whereas the mean reduced scattering coefficient in canine prostate was $3.6 \pm 4.8 \text{ cm}^{-1}$ (12), it was $14 \pm 11 \text{ cm}^{-1}$ in human prostate at the same wavelength (732 nm). The increased reduced scattering coefficient resulted in increased μ_{eff} , or a reduction of optical penetration depth, assuming that the μ_a remains the same. In addition, the increase in μ_s in human prostates compared with that in canine prostates contributed to the reduction of the penetration depth in humans compared with that in canines. Human prostates measured in this study had cancer present and had been subjected to previous radiation therapy. The canine prostate is a normal tissue model. Hypoxia has been described in patients with prostate cancer (26), whereas one would expect normal oxygen levels in normal canine prostate tissue. As a result, there may be more deoxyhemoglobin in these prostates than in normal prostates, resulting in higher μ_a s and shorter penetration depths at the wavelength range 630–800 nm.

The interprostatic difference in fluence rate per unit source strength at 0.5 cm from the point source was shown in Fig. 6. We chose 0.5 cm because this is the middle point for 1 cm spaced interstitial loading. The mean value was $1.5 \pm 1.1 \text{ cm}^{-2}$ for all prostates studied (sample size 121). We observed interpatient fluence rate heterogeneity as a result of heterogeneity of optical properties.

The tissue concentration of MLu and the μ_a in the human prostate at 732 nm as shown in Fig. 7a demonstrates a linear relationship with measured MLu concentration (correlation coefficient $R^2 = 0.63$). This relationship is described by the equation $\mu_a = 0.066c + 0.23$. The relatively large spread of the data can be explained by the large heterogeneity of MLu distribution in different locations of a human prostate (27). Because the location of the *in situ* optical measurements and the tissue biopsies were not exactly the same, some discrepancy between the drug levels measured by the two techniques is expected. Absorption by many naturally occurring chromophores, such as melanins, cytochromes, flavoproteins, and in particular, hemoglobin and deoxyhemoglobin, is small (around 732 nm) (10–13); as such, it is not surprising that the μ_a at 732 nm varies approximately linearly with the MLu concentration. Given the heterogeneity in the patient data, we used a liposyn phantom to examine the relationship between MLu concentration and the μ_a and to confirm

the slope of our clinical observations. Data based on this phantom are shown in Fig. 7a by the “+” symbols. The absence of other absorbers in the phantom decreases μ_a . The slope of the best fit line ($0.062 \text{ cm}^{-1}/(\text{ng/mg})$) is consistent with that observed *in vivo*. The linear relationship between MLu drug concentration and the μ_a at 732 nm is consistent with that observed in canine prostate at 732 nm, but a larger intercept ($\mu_a = 0.23 \text{ cm}^{-1}$) is found in humans than in dogs ($\mu_a = 0.08 \text{ cm}^{-1}$) (12). This may be caused by differences in the oxygenation of cancerous prostate tissue (humans) and normal prostate tissue (dogs) because the attenuation coefficient of oxyhemoglobin at 732 nm is substantially less than that of deoxyhemoglobin. If the tissue is less oxygenated, the attenuation coefficient will increase. The conclusion that more deoxyhemoglobin existed in human prostate is confirmed by *in vivo* diffuse absorption spectroscopy in the same prostate patient population (28). For the two patients studied, the contribution to the μ_a from oxyhemoglobin and deoxyhemoglobin varied between 0.1 and 0.2 cm^{-1} at 732 nm.

The effect of injected drug dose and drug-light interval on tissue concentration of MLu is shown in Fig. 7b. It shows significant intra- and interpatient variation of drug concentration. The extrapolated mean tissue concentration from *in vivo* absorption (symbols) generally agrees with that from *ex vivo* biopsy. Figure 7b was drawn using all 121 absorption measurements available as opposed to Fig. 7a, which used a subset of data (24 points) that had corresponding *ex vivo* biopsies. The large discrepancy between drug concentration measured *in vivo* and *ex vivo* in the two patients receiving 0.5 mg/kg with a 24 h drug-treatment interval is likely a consequence of measurement uncertainties for *in vivo* absorption in this early group of patients. The detector positions for these patients were determined manually using a ruler, which introduced larger errors in the final determination of μ_a . In addition, because μ_a for the earlier patients were small (less MLu), the relative uncertainty in μ_a increased. The large discrepancy in drug concentration measured *in vivo* and *ex vivo* in the group receiving 1 mg/kg with a 6 h drug-treatment interval can be explained by the small number of samples, *i.e.* patients, in this group ($n = 1$). However, some preliminary conclusions can be made from these data. It appears that a reduction in drug concentration is observed after PDT, which may be consistent with photobleaching. The patients who received higher injected MLu doses generally had higher local drug concentrations in the prostate. If one estimates the MLu concentration for 2 mg/kg at 24 h *in vivo* as twice the MLu concentration for 1 mg/kg at 24 h, then the drug concentration seems to decrease systematically with incubation time, *i.e.* drug-treatment interval, by examining the data for 2 mg/kg at 24 h, 2 mg/kg at 6 h and 2 mg/kg at 3 h in Fig. 7b. The heterogeneity in MLu concentration is sufficiently large to potentially require *in situ* monitoring of MLu distribution for future PDT treatments of the human prostate.

When one examines the variation of drug concentration in a single patient, it is clear that the drug concentration can easily change by three times when measured from different locations of the same prostate (data presented in reference [27]). This strong variation suggests that the mean intraprostatic variation is usually larger than the mean interprostatic variation, as shown in Fig. 7b, where the difference between bars (*e.g.* before PDT) for different groups is smaller than the range of the error bar, representing the standard deviation of intraprostatic variation in the same group; most of this variation is caused by intraprostatic variation.

CONCLUSIONS

We have measured the optical properties and MLu concentration of human prostates for 732 nm light. Substantial inter- and intra-patient heterogeneity was observed. The mean light penetration depth in the human prostates is about 0.4 cm, at least two times smaller than those found in the normal canine prostates. The μ_a *in vivo* at 732 nm has been found to be proportional to the tissue concentration of MLu, determined from *ex vivo* biopsies. A realtime dosimetry measurement and feedback system for monitoring light fluences during treatment should be considered for such future PDT studies to account for this heterogeneity.

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Abbreviations

CDF	cylindrical diffusing fiber
LLQ	left lower quadrant
LUQ	left upper quadrant
MLu	motexafin lutetium
PDT	photodynamic therapy
RLQ	right lower quadrant
RUQ	right upper quadrant
TRUS	transrectal ultrasound
μ_a	absorption coefficient
μ_{eff}	effective attenuation coefficient
μ_s'	transport scattering coefficient

APPENDIX

Error analysis of the optimization technique to extrapolate optical properties

There are several uncertainties in determining the optical properties besides uncertainty in measuring the light fluence rate, which accounts for approximately $\pm 5\%$. One is the uncertainty in the position (x) of the detector, which can be 1–2 mm. Another uncertainty is the uncertainty in the distance (h) between the source catheter and the isotropic detector catheter, which could be different from the distances specified on the template by 1–2 mm as well. Both errors introduce an uncertainty in distance (Fig. 1b), $r = \sqrt{x^2 + h^2}$, which can be 1–2 mm. From the phantom measurements, one can estimate the relative uncertainty

introduced by the positioning errors by the ratio of the slope of the fluence rate to fluence rate $d\phi/dr/\phi dr$. Because the fluence rate at distance r from a point source is proportional to $e^{-\mu_{\text{eff}}r}/r$, this value decreases with increasing distance r and follows $(\mu_{\text{eff}}+1/r)dr$. Using this relationship one gets a relative uncertainty in fluence rate of 50% for $r = 5$ mm and 39% for $r = 7$ mm assuming that $dr = 2$ mm and $\mu_{\text{eff}} < 0.5 \text{ cm}^{-1}$. To separate the two sources of errors that contribute to an error in r , one writes $dr = x/r dx + h/r dh$. The first error, x , is a random error and approaches $dr = 0$ for $x = 0$ for any dx because $dr = x dx/r$. Thus, the overall error caused by uncertainty of x for determining μ_a and μ_s' should be small. This is also because it is unlikely that all points are off in the same direction thereby introducing a systematic error. We have minimized this error by using a computerized step motor so that the error in x was less than 0.2 mm. In this case, the error in ϕ should be less than 2% even for $x = r$. The second source of error can be large but it is a systematic error.

Table A shows a numerical simulation of the errors in μ_a and μ_s' caused by the errors in h for two conditions of μ_a and μ_s' . The standard deviation of the fit is calculated by

$\sum_{i=1}^n \sqrt{((\text{measurement}_i - \text{fit}_i)/\text{fit}_i)^2/(n-1)}$. One can use the minimum error to determine the correct h value to within 0.05 cm. In Table A, this corresponds to the fit for $h = 0.55$ cm. The physical separation is $h = 0.6$ cm. We do not understand why the optimal h , which gives the best μ_a and μ_s' , is about 0.05 cm shorter than the physical separation of $h = 0.6$ cm. But we have demonstrated that one can extract h to within 0.05 cm. Using this additional constraint and allowing h to deviate from the nominal value will reduce the final uncertainty to less than $\pm 6\%$ for μ_a and less than $\pm 11\%$ for μ_s' . Assuming that the h can still deviate from the true h (0.55 cm) by ± 0.05 cm, we estimated that one can determine μ_a to an uncertainty of less than $\pm 20\%$ and μ_s' to an uncertainty of less than $\pm 40\%$, provided that the optimal h is within 0.5 mm of the true value of h . Clearly, if one allows up to 2 mm uncertainty in h , then one can introduce an unacceptable error for μ_a and μ_s' (see Table A) with up to $\pm 215\%$ and $\pm 450\%$ errors, respectively. The error in μ_{eff} is much smaller ($\pm 30\%$) over the same range of h . To reduce this error further, we have used the value of the standard deviation of the fit as a guide to determine the optimal separation h .

Table A

Error analysis of deviations in μ_a , μ_s' , μ_{eff} and $\phi/S(0.5 \text{ cm})$ caused by the uncertainty of h (nominal $h = 0.6$ cm)

h (cm)	μ_a (cm^{-1})	μ_s' (cm^{-1})	μ_{eff} (cm^{-1})	ϕ/S (0.5 cm)	Standard deviation of fit (%)
$\mu_a = 0.5 \text{ cm}^{-1}$, $\mu_s' = 3.6 \text{ cm}^{-1}$, $\mu_{\text{eff}} = 2.3 \text{ cm}^{-1}$					
0.40	1.07	1.17	1.93	0.28	27.6
0.45	0.81	1.76	2.07	0.35	17.2
0.50	0.65	2.48	2.20	0.43	11.9
0.55	0.53	3.37	2.32	0.53	10.9

h (cm)	μ_a (cm ⁻¹)	μ_s' (cm ⁻¹)	μ_{eff} (cm ⁻¹)	ϕ/S (0.5 cm)	Standard deviation of fit(%)
0.60	0.44	4.57	2.45	0.66	12.7
0.65	0.37	5.99	2.57	0.81	15.2
0.70	0.30	7.98	2.69	1.00	17.8
0.75	0.25	10.65	2.81	1.26	20.3
0.80	0.20	14.30	2.93	1.58	22.4
$\mu_a = 0.4 \text{ cm}^{-1}, \mu_s' = 7.2 \text{ cm}^{-1}, \mu_{eff} = 2.9 \text{ cm}^{-1}$					
0.40	0.70	2.75	2.41	0.43	15.2
0.45	0.56	3.91	2.57	0.54	8.7
0.50	0.46	5.41	2.73	0.68	5.1
0.55	0.38	7.34	2.90	0.83	4.8
0.60	0.30	10.33	3.04	1.09	7.0
0.65	0.24	14.31	3.19	1.39	9.6
0.70	0.19	19.96	3.34	1.80	12.1
0.75	0.14	28.09	3.49	2.35	14.3
0.80	0.11	39.95	3.64	3.10	16.3

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(a)

(b)

Figure 1.

(a) Transrectal ultrasound image of a human prostate, showing the position of source (closed circle) and detector (x) fibers. The source positions labeled 1, 2, 3 and 4 were used for optical properties measurement for the RUQ, LUQ, RLQ and LLQ, respectively, with the detector placed in a separate catheter in each quadrant. The open circle on the right upper quadrant is for a linear source that passed through the position but is too short to have active light component in the cross-section plane. The grid on the template (“+”) is 0.5 cm apart.

(b) Schematic of the measurement geometry, illustrating the coordinates used to determine the source–detector distance.

Figure 2.

Measurement of light fluence rate (mW/cm^2) at different distances, x , from a point source in liquid optical phantoms: (a) $\mu'_s = 3.6 \text{ cm}^{-1}$ and $\mu_a = 0.02, 0.1$ and 0.5 cm^{-1} and (b) $\mu'_s = 7.2 \text{ cm}^{-1}$, $\mu_a = 0.02, 0.08$ and 0.40 cm^{-1} . Symbols represent measurements with an isotropic detector. The solid lines are the best fit with the resulting fit optical properties (from top to bottom): (a) $\mu'_s = 3.7, 3.8$ and 3.4 cm^{-1} and $\mu_a = 0.03, 0.097$ and 0.53 cm^{-1} and (b) $\mu'_s = 7.5, 7.1$ and 7.3 cm^{-1} , $\mu_a = 0.022, 0.074$ and 0.38 cm^{-1} , respectively.

Figure 3.

Measured light fluence rate per unit source strength (ϕ/S) at distances along the catheter, x , from the point source measured *in vivo* in human prostate gland for Patient 13. Lines are measured data and symbols are fits. (There are too many measured points to express the measured data clearly as symbols.) (a) Light fluence rates in the right lower quadrant ($\circ$), right upper quadrant ($\times$) and left upper quadrant ($*$) of the same prostate before PDT. (b) Light fluence rates before ($\circ$) and after (+) light treatment in the right lower quadrant of the prostate gland. The optical properties are $\circ$ — $\mu_a = 0.23 \text{ cm}^{-1}$, $\mu'_s = 7.3 \text{ cm}^{-1}$, $\phi(0.5)/S = 1.1$

cm^{-2} and $h = 0.5$; x — $\mu_a = 0.44 \text{ cm}^{-1}$, $\mu'_s = 12.0 \text{ cm}^{-1}$, $\phi(0.5)/S = 0.78 \text{ cm}^{-2}$ and $h = 0.7$; *
— $\mu_a = 0.25 \text{ cm}^{-1}$, $\mu'_s = 11.6 \text{ cm}^{-1}$, $\phi(0.5)/S = 1.3 \text{ cm}^{-2}$ and $h = 0.7$; +— $\mu_a = 0.25 \text{ cm}^{-1}$, $\mu'_s =$
 6.6 cm^{-1} , $\phi(0.5)/S = 1.0 \text{ cm}^{-2}$ and $h = 0.5 \text{ cm}$.

Figure 4.

Light fluence rate vs time measured by the *in vivo* dosimetry system for the four quadrants for Patient 7 at 732 nm wavelength. Large disturbances of light fluence rate were caused by movement of detector positions, per observation by the operators.

Figure 5.

Variation of (a) μ_a , (b) μ'_s , (c) μ_{eff} and (d) δ in patients before (open bars) and after (solid bars) PDT. The height of each bar is the mean value measured in each patient. The error bars specify the standard deviation among the four quadrants of a prostate. The solid horizontal line is the average of all measurements, and the dashed lines are the standard deviation of the average.

Figure 6.

Measured mean fluence rate per power, ϕ/S , at 0.5 cm from the point source at 732 nm in different patients before (open bars) and after (solid bars) PDT. The error bars reflect intraprostate variation of ϕ/S . The solid horizontal line is the average of all measurements, and the dashed lines are the standard deviation of average: 1.5 ± 1.1 cm $^{-2}$.

Figure 7.

(a) *In vivo* absorption coefficient (μ_a) at 732 nm as a function of measured *ex vivo* MLu concentration (in ng/mg) from the same human prostate. The symbols are measured points *in vivo*: ○—before PDT, *—after PDT. The solid line is a linear fit to the measured *in vivo* data. The symbol “+” represents the measured μ_a of MLu vs MLu concentration in a pure liposyn phantom. The dashed line is a linear fit to the phantom data with $\mu'_s = 4 \text{ cm}^{-1}$, (b) Extrapolated MLu concentration from *ex vivo* fluorescence (bars) and from μ_a (symbols) as a function of injected MLu concentration: open bars (○)—before PDT, solid bar (□)—after

PDT. Drug concentration calculated from μ_a uses the linear relationship determined in (a): c (ng/mg) = $(\mu_a \text{ (cm}^{-1}) - 0.227)/0.0658$. The thin and thick error bars correspond to the standard deviation of the *ex vivo* biopsy and *in vivo* absorption measurements, respectively. Notice that (b) used all measured μ_a data (121 points), whereas (a) used a subset of μ_a data (24 points) that had corresponding *ex vivo* biopsies. (Insufficient data existed to extrapolate tissue concentration from *in vivo* absorption measurement for 1 mg/kg MLu injection at 6 h drug-treatment time interval before PDT due to bleeding.)

Table 1

Distribution of patients for measurements of optical properties. The number in parentheses is the number of valid measurements that is not affected by bleeding

Patient Number	MLu dose (mg/kg)	Drug-treatment interval (h)	Light fluence (J/cm ²)	No. measured before PDT	No. measured after PDT	Optical properties along a catheter?
2	0.5	24	25	4 (1)	4 (1)	No
3	0.5	24	25	4 (1)	1 (1)	No
4	1	24	25	4 (3)	1 (1)	No
5	1	24	25	4 (1)	1 (1)	No
6	1	24	25	3 (2)	3 (2)	No
7	1	6	25	4 (0)	4 (2)	No
8	2	6	25	4 (0)	4 (0)	No
9*	2	6	50	7 (6)	7 (7)	No
10*	2	6	100	8 (6)	7 (5)	No
11*	2	3	25	8 (7)	7 (5)	No
12*	2	3	50	21 (20)	9 (7)	Yes
13*	2	3	100	28 (20)	20 (20)	Yes
14*	2	3	100	29 (2)	29 (0)	Yes

* Measurements made with a motorized probe.

Table 2

Summary of optical properties before and after PDT in human prostates. The values in the parentheses are the standard deviation of the mean values measured from different locations of the same prostate. No standard deviation is listed if only one data point is available

Patient number	Before PDT				After PDT			
	μ_a (cm ⁻¹)	μ'_s (cm ⁻¹)	δ (cm)	ϕ/S (cm ⁻²)	μ_a (cm ⁻¹)	μ'_s (cm ⁻¹)	δ (cm)	ϕ/S (cm ⁻²)
2	0.09	29.8	0.34	3.34	0.09	43.7	0.29	3.78
3	0.15	22.0	0.31	2.15	0.07	33.4	0.37	4.07
4	0.43 (0.28)	7.69 (4.76)	0.41 (0.14)	0.97 (0.81)	0.51	1.67	0.63	0.36
5	0.21	11.8	0.37	1.44	0.13	7.18	0.60	1.48
6	0.27 (0.27)	10.5 (11.2)	0.50 (0.05)	1.74 (1.78)	0.19 (0.20)	18.9 (18.4)	0.45 (0.06)	3.18 (3.32)
7	—	—	—	—	0.30 (0.08)	23.7 (13.9)	0.24 (0.11)	1.09 (0.40)
9	0.53 (0.36)	6.61 (4.51)	0.41 (0.09)	0.77 (0.42)	0.64 (0.25)	7.00 (5.59)	0.33 (0.10)	0.54 (0.23)
10	0.63 (0.32)	4.62 (2.87)	0.42 (0.10)	0.56 (0.29)	0.19 (0.05)	9.27 (4.47)	0.54 (0.31)	1.33 (0.64)
11	0.67 (0.17)	6.39 (3.18)	0.32 (0.10)	0.51 (0.18)	0.83 (0.45)	5.45 (3.89)	0.38 (0.16)	0.47 (0.30)
12	0.71 (0.43)	8.99 (6.51)	0.32 (0.12)	0.61 (0.35)	0.30 (0.06)	20.2 (4.8)	0.28 (0.08)	0.98 (0.05)
13	0.27 (0.14)	18.5 (11.6)	0.30 (0.07)	1.46 (0.72)	0.26 (0.09)	17.0 (8.8)	0.31 (0.07)	1.42 (0.56)
14	0.72 (0.11)	3.37 (1.37)	0.39 (0.11)	0.40 (0.01)	—	—	—	—