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Changes in relative light fluence measured during laser heating: implications for optical monitoring and modelling of interstitial laser photocoagulation

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Abstract

Dynamic changes in internal light fluence were measured during interstitial laser heating of tissue phantoms and *ex vivo* bovine liver. In albumen phantoms, the results demonstrate an unexpected rise in optical power transmitted ≈1 cm away from the source during laser exposure at low power (0.5–1 W), and a decrease at higher powers (1.5–2.5 W) due to coagulation and possibly charring. Similar trends were observed in liver tissue, with a rise in interstitial fluence observed during 0.5 W exposure and a drop in interstitial fluence seen at higher powers (1–1.5 W) due to tissue coagulation. At 1.5 W irradiation an additional, later decrease was also seen which was most likely due to tissue charring. Independent spectrophotometric studies in Naphthol Green dye indicate the rise in fluence observed in the heated albumen phantoms may have been primarily due to light exposure causing photobleaching of the absorbing chromophore, and not due to heat effects. Experiments in liver tissue demonstrated that the observed rise in fluence is dependent on the starting temperature of the tissue. Correlating changes in light fluence with key clinical endpoints/events such as the onset of tissue coagulation or charring may be useful for on-line monitoring and control of laser thermal therapy via interstitial fluence sensors.

1. Introduction

Interstitial laser photocoagulation (ILP) is a novel procedure for the treatment of solid, unresectable tumours. During ILP, tumours are heated to high temperatures (50–90 °C) by laser energy delivered through optical fibres that are inserted interstitially into the target

site. Confining therapeutic temperatures to the target volume is of particular importance when tissues to be treated are adjacent to vital normal structures. Hence, ILP appears promising as a minimally invasive method for treating tumours in liver (Amin *et al* 1993), brain (Roux *et al* 1992) and prostate (Beerlage *et al* 2000).

Treatment planning models have been developed to gain an understanding of the optical and thermal propagation during ILP and resultant tissue damage (McKenzie 1990, Jacques 1994, Welch and van Gemert 1995, Iizuka *et al* 2000). Such models correlate the effects of laser power, source geometry and dynamic changes in tissue attenuation and blood perfusion during treatment with the final size and geometry of the resulting thermal lesions.

Since the key endpoint of any thermal therapy is tissue damage, experimental validation of most ILP models have focussed on final lesion size or tissue temperature increase generated in either tissue mimicking albumen phantoms (Iizuka *et al* 1999, Halldorsson *et al* 1981) or *ex vivo* tissue (Whelan *et al* 1995, Wyman 1993). This implicitly assumes that validation of lesion size or temperature predictions is also validation of the dynamic light models applied in the simulations. Yet, few if any experiments have attempted to measure such changes.

Using reflectance/transmission techniques, a number of investigators have demonstrated significant increases in the optical scattering properties of albumen (Pickering 1992, Meijerink *et al* 1995) and *ex vivo* liver (Pickering *et al* 1994, Ritz *et al* 2001) due to heating at ILP temperatures. Thomsen *et al* (1989) have demonstrated that these changes are likely due to morphological changes in tissue structure resulting from thermal coagulation. Therefore, interstitially, thermal damage is expected to produce a change in internal light distribution that is related to ILP-induced coagulation of the target site. Although Matthewson *et al* (1987) and van Hillegersberg *et al* (1994) have noted light fluence changes in *in vivo* animal models during ILP, to our knowledge, no systematic experimental studies have examined dynamic *interstitial* light fluence changes during laser thermal therapy. Furthermore, the studies mentioned were performed at low temporal resolutions, which might not allow for a proper evaluation of fluence trends.

Hence, the goal of this work is to experimentally measure the key light trends that occur during ILP and to consolidate our understanding of the biophysical processes that produce these changes. It should be stressed that the purpose of this investigation is not to quantitatively validate the developed mathematical light models of Kim *et al* (1995), Iizuka *et al* (1999) and others. Rather, based on these models and our understanding of tissue optics, we are interested in a qualitative study of the salient features predicted by these models. As a case in point, in a later section of this article we provide a physical mechanism for an unexpected rise (not predicted by previous models) in relative light fluence observed experimentally early in an ILP treatment.

In parallel with the main goal, we examine the relevance of the observed fluence changes in optical phantoms with those of *ex vivo* tissue. Though phantoms provide a homogenous control system to study and validate opto-thermal models, by definition, they are artificially manufactured and might therefore exhibit different optical behaviour than those of biological tissue. The importance of this study is that future validation of optical models with experimentally measured fluence will be important in the correlation of key treatment events for the potential monitoring of ILP using light fluence sensors (Whelan *et al* 2001).

2. Materials and methods

2.1. Albumen phantoms

Dynamic changes in light fluence during ILP were measured in albumen phantoms, as previously described by Iizuka *et al* (1999). The albumen phantoms are composed of

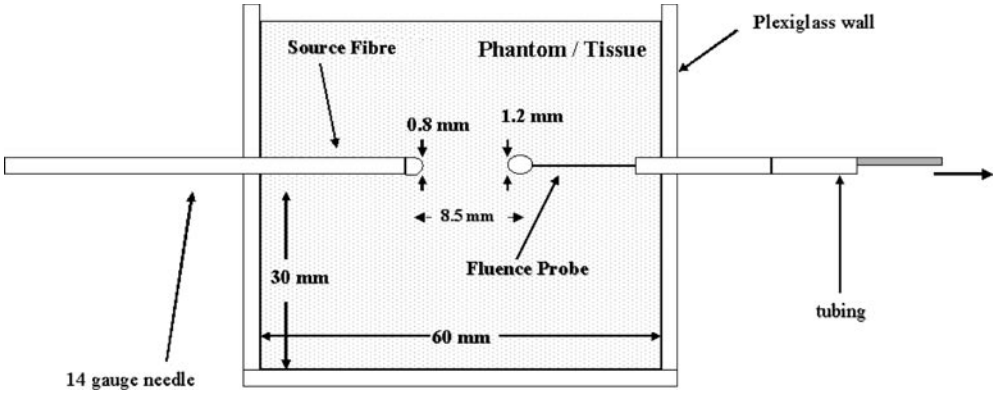

Figure 1. Schematic of light fluence measurement apparatus.

bacteriological agar, Naphthol Green dye and chicken egg albumen. They are simple to construct, model dynamic optical changes in tissues well and allow for reproducible measurements. The method for creating and verifying the optical properties of the phantom, μ_s' (reduced scattering coefficient) and μ_a (absorption coefficient), using the poisoned moderator technique, has been described previously (Iizuka *et al* 1999). For this study, the synthesized phantoms had $\mu_s' = 2.7 \pm 0.1$ cm^{-1} and $\mu_a = 0.5 \pm 0.1$ cm^{-1} (native state), and $\mu_s' = 13.1 \pm 0.5$ cm^{-1} and $\mu_a = 0.7 \pm 0.1$ cm^{-1} (coagulation state). These values are typical of the optical properties of soft tissues in the near-infrared (Cheong *et al* 1990).

2.2. Interstitial measurements in albumen phantoms

Figure 1 is a schematic of the experimental apparatus. Optical signals were measured by an in-house optical detector fibre, constructed using a variation of the method described by Henderson (1991). Orb-tipped fibres (5501 ORB, Surgimedics, Houston, TX) were coated with ultra-violet cured epoxy mixed with titanium dioxide. The titanium dioxide provided scattering around the fibre tip, thereby increasing the effective fibre acceptance angle and approximating an isotropic detection fibre. The source fibre (5601 ORB, Surgimedics, Houston) was aligned facing tip to tip with the detector fibre, along the same line of sight, at a separation distance of $\approx$1 cm. The source/sensor separation is typical of the expected distances used during a clinical ILP procedure. The detector fibre was attached to a micrometre translation stage permitting accurate positioning relative to the source fibre. A 0.05 cm error in source–detector separation was estimated, due mainly to errors associated with fibre alignment. The exact choice of source–detector separation, 0.85 ± 0.05 cm, represented a trade-off between maximizing the signal while minimizing heating of the detector fibre, to prevent erroneous readings and/or sensor damage. The source fibre was coupled to a Diomed-60 laser (Diomed Ltd., Cambridge, UK) operating at 810 nm. Light collected by the optical sensor was converted to a photovoltage signal using a PDA55 photodiode (Thorlabs Inc., Newton, NJ). The photovoltage signal is proportional to the interstitial light fluence and can be equated when scaled by a calibration factor (van Staveren *et al* 1995). Hence, even uncalibrated, photovoltage can still be used for studying relative fluence trends. Voltage output from the photodiode was read into a PC via a multifunction data acquisition system (Labmate Sciemetrics, Nepean, Canada).

Phantom mixtures were solidified in moulds and transferred to the fluence box prior to measurement. To avoid leaving fibre tracks in the phantom, fibres were removed from the

fluence box during transfer, after which source and detection fibres were moved towards each other until a 0.85 cm separation was reached. The agar matrix was sufficiently pliable to permit such a positioning of the fibres.

Phantoms were irradiated using a range of laser powers (0.5–2.5 W) for 600 s. After heating, phantoms were sliced open along the fibre-containing plane and the resulting lesion sizes measured using a ruler. Coagulated phantom appeared as a white, approximately circular region. Multiple samples (2–4) were measured at each input power.

2.3. Interstitial measurements in bovine liver

To test the relevance of the observed optical changes in albumen phantoms with that of tissue, similar measurements were performed in *ex vivo* bovine liver. Liver tissue was measured using two methods. In method 1, the source and detection fibres were aligned, within the fluence box, on the surface of a 2 cm thick slab of liver cut to the dimensions of the fluence box. Following alignment, additional slabs were then placed on top, such that the fibres were sandwiched between the lower and upper layers of tissue (Wyman 1993). A 500 g weight was placed on the top slab to maintain good coupling between the tissue slabs throughout the experiment. This method provided the convenience of simple fibre placement and localization of the generated lesion. In method 2, fibres were inserted into a thick 4–5 cm slab of bovine liver with a 1–2 cm slab of liver placed on top to fill the remainder of the fluence box. As with the albumen phantoms, the fibres were initially aligned and positioned, using the micrometre to the chosen separation distance and retracted prior to transfer of the liver sample into the fluence box. Fibres were then inserted into the liver sample and moved to 0.85 ± 0.05 cm separation. This second method simulates a more clinically relevant condition. For both methods, tissue temperatures were approximately room temperature ($20 \pm 3\ ^{\circ}$C) prior to heating.

Liver tissue was heated for 600 s at laser powers of 0.5–1.5 W. At 0.5 W, irradiation was fractionated (with an approximate 300 s break in between) into two 600 s runs, for a cumulative 1200 s exposure. The purpose of two separate exposures was to test for possible changes in optical properties that may be dependent on irradiation history. At least two separate samples were measured at each power to test for reproducibility of the observed trends.

2.4. Changes in phantom absorption due to heating and laser exposure

As will be described in section 3.1, an unexpected rise in light fluence, particularly with low laser heating powers (0.5–1 W), and at early times with higher powers (1.5–2 W), was observed during the experiments. This change was hypothesized to result from an increase in light penetration due to a decrease in the absorption coefficient of the phantom that results from either thermal heating or photon exposure. Since the absorption properties of the phantoms are determined by Naphthol Green dye, experiments were performed to test for potential changes in dye absorption due to optical effects or thermal effects of ILP.

The absorption spectrum of Naphthol Green dye was measured using a spectrophotometer (Shimadzu, Columbia, MD). Absorption spectra were measured between 600 and 1000 nm, with the wavelength of interest, 810 nm, approximately in the centre of the range. Scans were performed in the original dye solution at room temperature, immediately after irradiation/heating, and following cooling of the samples back to room temperature.

To test for optically induced changes in the dye spectrum, broad beam irradiation was performed on diluted samples at 0.5 W (the lowest power setting on the Diomed 60) for up to 40 min at 810 nm. In addition, a comparison of different irradiation schemes was performed to test for absorption changes as a function of exposure history. Absorption spectra were

measured after 4 × 10 min, 2 × 20 min, 30 + 10 min, and single 40 min exposures. After the initial scan, most samples were re-scanned following a waiting time of ≈5 min to check for 'short term' reversible changes. A single sample was re-scanned ≈3 hours post irradiation to investigate 'long term' reversibility.

Laser irradiation of Naphthol Green dye during ILP also produced an increase in sample temperature due to absorption of optical energy. The temperature at the end of irradiation was measured to be $\lesssim$31 °C using a metal thermocouple placed at the centre of the cuvette. Thus, a second test was performed to separate optical and thermal effects by employing a purely thermal method of sample heating using a hot water bath temperature of 45 °C, which was chosen to ensure a significant temperature rise above 31 °C while minimizing vaporization. Prior to heating, cuvettes were covered using a wax slip to minimize water evaporation loss from the sample.

To determine if the nominal 45 °C target temperature used in the water bath tests was of a similar temperature range as during interstitial irradiation, interstitial temperature measurements were performed for comparison. Measurements were made using fluoroptic temperature probes (Luxtron Corporation, Santa Clara, CA) placed parallel and slightly anterior to the tip of the source fibre, the probes were inserted via plastic catheters glued to the source fibre that were positioned at ∼0.5 mm anterior from the fibre tip. Temperature measurements were made just prior to and immediately following the end of laser irradiation at 0.5 W. To avoid self-heating artefacts (Reid *et al* 2001), the probes were retracted within the catheters during irradiation and re-inserted into the tissue following irradiation.

2.5. Changes in liver optical properties due to temperature

Additional tests were performed to elucidate the fluence rise observed in bovine liver. An updated data acquisition system comprised of a National Instruments DAQ card (DAQCARD-AI-16E-4, Austin, TX) was used to measure optical signals. This allowed for improved resolution for real-time optical signal measurements. Unlike albumen phantoms, separation of the scattering and absorbing components of tissue is difficult. However, one possible mechanism for the increase in fluence—the effect of bulk tissue temperature—was explored.

Tissue samples were cooled in a cold storage room to 4 °C. Interstitial fluence measurements were then performed according to method 2 at distances of 0.6–0.85 cm from the source fibre tip. Sample temperatures were measured at the slab centre using digital thermometers before and after irradiation. Interstitial measurements were performed on the same tissue sample with initial tissue temperatures ranging from 6 to 24 °C. The tissue insertion point was rotated to ensure that the same section of tissue was not re-irradiated. To ensure that changes were temperature-related and not due to degradation of the tissue, some samples were cooled back to 4 °C after warming and interstitial fluence measurements repeated.

3. Results

3.1. Changes in internal light fluence of albumen phantoms during ILP

Figure 2 shows typical normalized photovoltage changes measured in albumen phantoms due to heating at a range of source powers and at a fixed source–detector position of 0.85 ± 0.05 cm. For each curve, normalization consisted of dividing photovoltage values by the reading at $t = 1$ s of irradiation, as photovoltage measured at $t = 1$ s should be representative of the native optical properties of the phantom before any changes in properties have occurred. Data collected at 2.5 W only extended to 140 s as the appearance of smoke indicated that charring had occurred during the heating period.

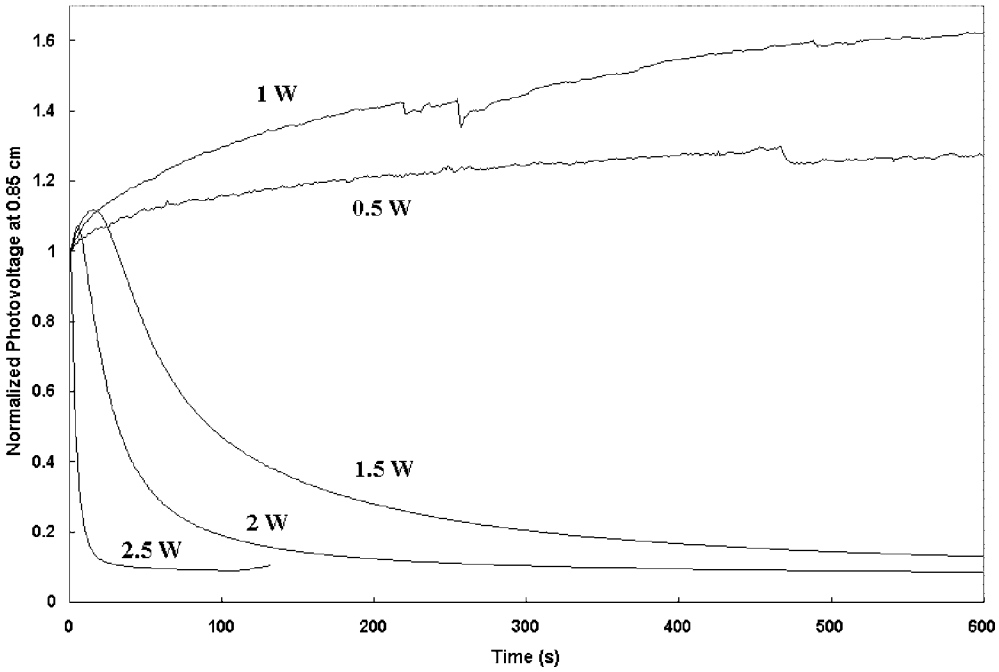

Figure 2. Normalized photovoltages measured by an optical sensor 0.85 cm from the source in albumen phantoms as a function of heating time and laser power.

For heating powers of 0.5–1 W, photovoltage rises steadily throughout the heating period. However, for powers of 1.5 and 2 W, the rise in photovoltage was quickly followed by a subsequent fall some 5–15 s after the start of heating. At 2.5 W applied power, no rise was observed, with a drop in photovoltage occurring almost immediately after the start of irradiation. The rate of signal rise increases with laser power except for the highest power of 2.5 W, where no rise was observed. Similarly, for all phantoms exposed to power >1.5 W, the steepness of photovoltage drop increases with laser power, and eventually plateaus to values of 10–20% of its initial value. For each power, the key features of the photovoltage curves were reproducible though slight variations in the temporal trends (rate of fluence rise and decrease) were observed for different phantoms.

For low heating power (0.5–1 W) no coagulation was observed. At higher powers (1.5–2 W) noticeable coagulation was observed with lesion diameters in the range of 1–2 mm at 1.5 W and 3–4 mm at 2 W. Though there was some variation among phantoms, generated lesions were oval in appearance with final lesion size increasing with laser power.

3.2. Absorption properties of Naphthol Green dye

Interstitial measurements showed only a maximum temperature increase at $\sim$0.5 mm of only 10–11 °C at the end of 10 min irradiation at 0.5 W. Thus, the temperatures near the fibre tip during interstitial irradiation are much less than the 45.5 °C water bath temperature used in the spectrophotometric tests.

Figures 3 and 4 plot the absorption spectra of Naphthol Green dye before and after water bath heating and 40 min laser exposure at 0.5 W, respectively. The absorption values presented have been scaled accordingly to values prior to dilution. Qualitatively, several key

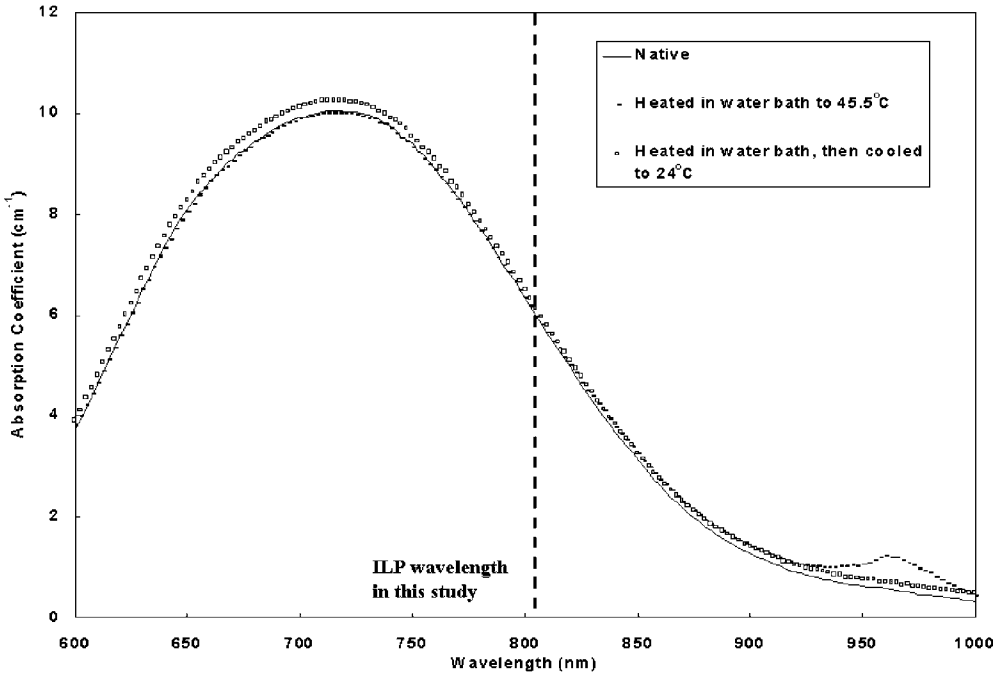

Figure 3. Absorption spectra of Naphthol Green dye following water bath heating and subsequent cooling. For figures 3–5, 0.03870 g of Naphthol Green powder and 1 l of distilled water gives a stock solution with $\mu_a \approx 5.9\ \mathrm{cm}^{-1}$.

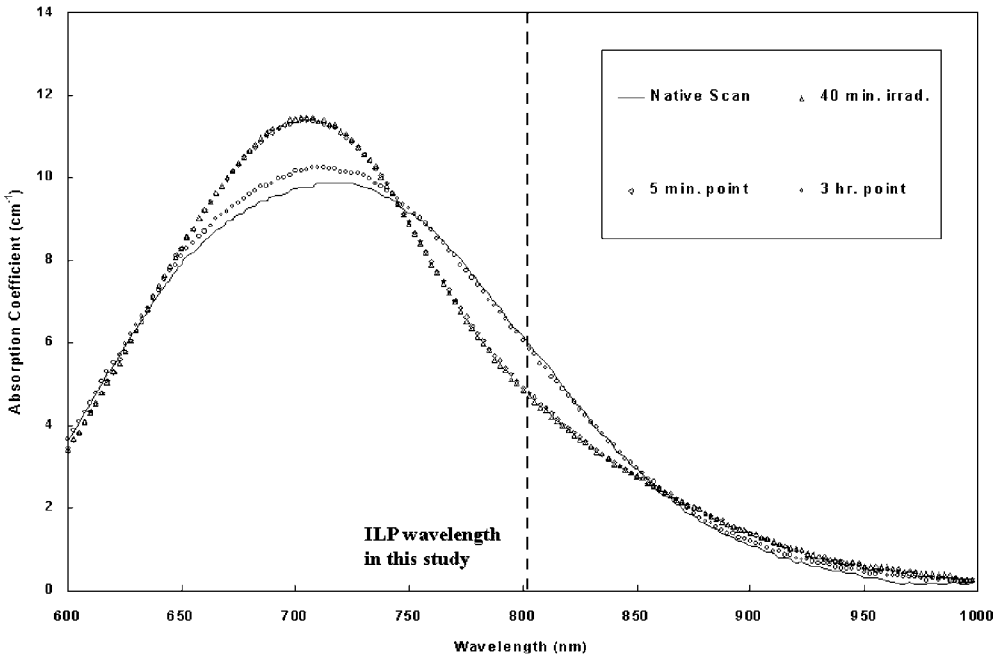

Figure 4. Absorption spectra of Naphthol Green dye following 0.5 W laser exposure at 810 nm for 40 min.

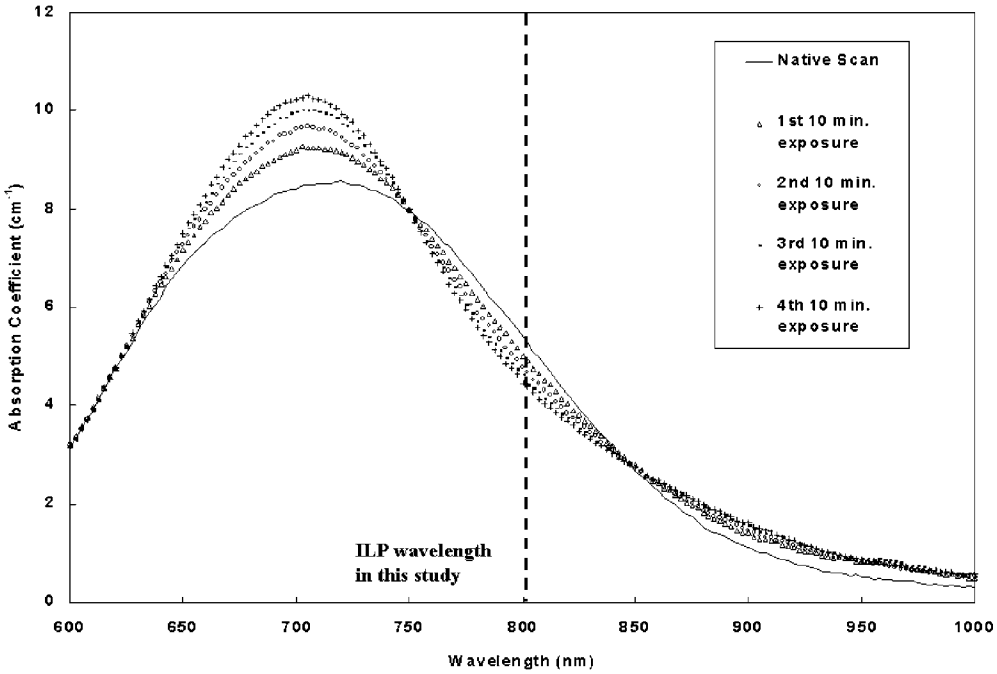

Figure 5. Absorption spectra of Naphthol Green dye following 0.5 W laser exposure at 810 nm fractionated into four 10 min exposures (e.g. run $1 = 1 \times 10$ min exposure, run $2 = 2 \times 10$ min etc.). A delay of approximately 5 min occurred between exposures.

observations can be made from figures 3 and 4. First, little change occurs in the absorption spectrum between 600 and 900 nm due to heating alone (figure 3), particularly at 810 nm. However, water bath heating did result in the appearance of a small peak in the absorption spectrum (figure 3) between 950 and 1000 nm, most likely due to the temperature-dependent overtone and combination spectrum of liquid water (Eisenberg and Kouzmann 1969).

In contrast, the absorption spectra before and after laser exposure are markedly different (figure 4). Laser exposure produced a shift in the absorption spectrum peak of the dye to lower wavelengths and caused a decrease in its absorption properties between 750 and 850 nm. As seen in figure 4, a 5 min wait demonstrated no reversal of these changes. However, an almost complete return to the original spectrum resulted after $\approx$3 hours.

Furthermore, laser-induced changes are also dependent on exposure time. Figure 5 demonstrated a progressive shift in absorption spectrum as the sample was irradiated over four 10 min periods. A similar result was observed for the 2×20 min and $30 + 10$ min treatments. All samples demonstrated an approximately 18% decrease in absorption at 810 nm following a cumulative 40 min exposure, regardless of irradiation history. In all cases, initial irradiation of the dye produced the largest decrease in absorption, with subsequent exposures resulting in progressively smaller effects. This may be due to a gradual depletion or photobleaching of the chromophore concentration due to laser exposure. The photochemistry behind these changes is unknown.

Quantitatively, a comparison of μ_a at 810 nm, before and after laser irradiation demonstrated an 18% decrease due to laser irradiation compared to only a 2% decrease due to water bath heating. Thus, observed absorption changes arise primarily from optical rather than thermal exposure.

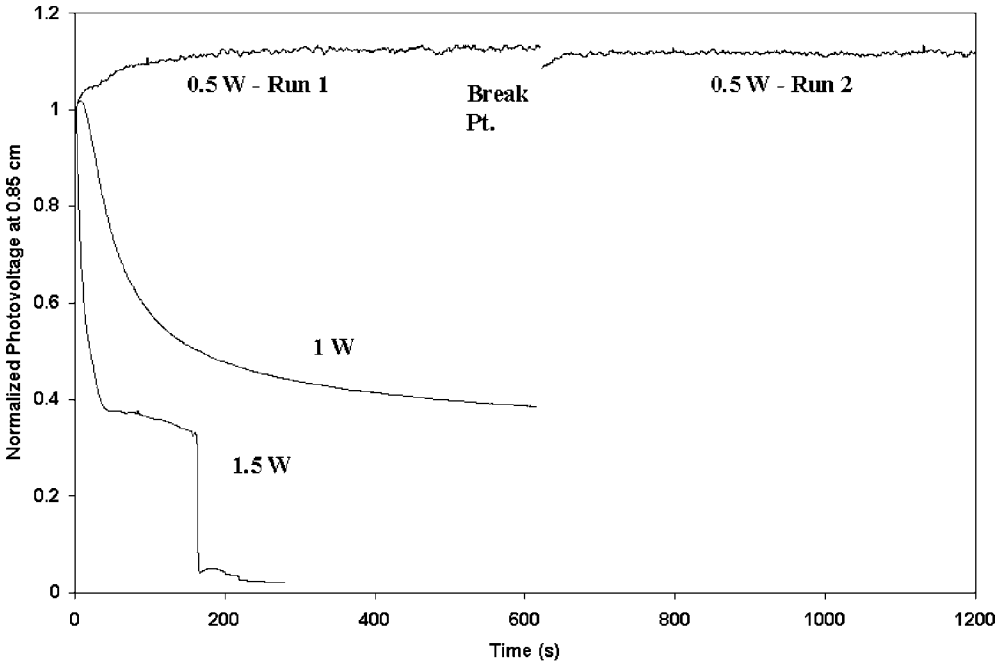

Figure 6. Normalized photovoltages measured by an optical sensor 0.85 cm from the source in bovine liver as a function of heating time and laser power.

3.3. Changes in light fluence of bovine liver during ILP

Trends observed during ILP of albumen phantoms were also detected in bovine liver. Figure 6 demonstrates that for a laser power of 0.5 W, photovoltage increases by approximately 13% after an initial 600 s of irradiation and, after a 5 min break, only 3–4% following a second 600 s exposure. This behaviour is similar to that of Naphthol Green (section 3.2), which demonstrated progressively smaller decreases in absorption following subsequent exposures. No coagulation was observed at this treatment power. It should be noted that a steady increase in photovoltage was only observed when fibres were inserted directly into the liver tissue (section 2.2, method 2) and not when fibres were sandwiched between tissue slabs (section 2.2, method 1). Employing method 1 did not result in noticeable increases in photovoltage during 0.5 W exposure.

At 1 W, the photovoltage initially increased by 2–3%, then decreased to ≈45% of its initial value and remained almost constant for an additional 100 s of exposure. A generated final lesion size of approximately 2 mm diameter was measured following exposure. At 1.5 W, no increase in photovoltage was observed. Instead, the photovoltage decreased at the start of irradiation and settled at approximately 35% of its initial value within the first 50 s. This was followed by a sharp drop in photovoltage at 180 s. Subsequent examination showed a resulting 8–9 mm diameter lesion with a distinct 2 mm diameter charred core. The key trends at each power were found to be reproducible.

3.4. Changes in liver optical properties due to temperature

A noticeable relationship was observed between the extent of fluence increase and bulk tissue temperature. Greater increases in interstitial fluence were observed with tissue samples that

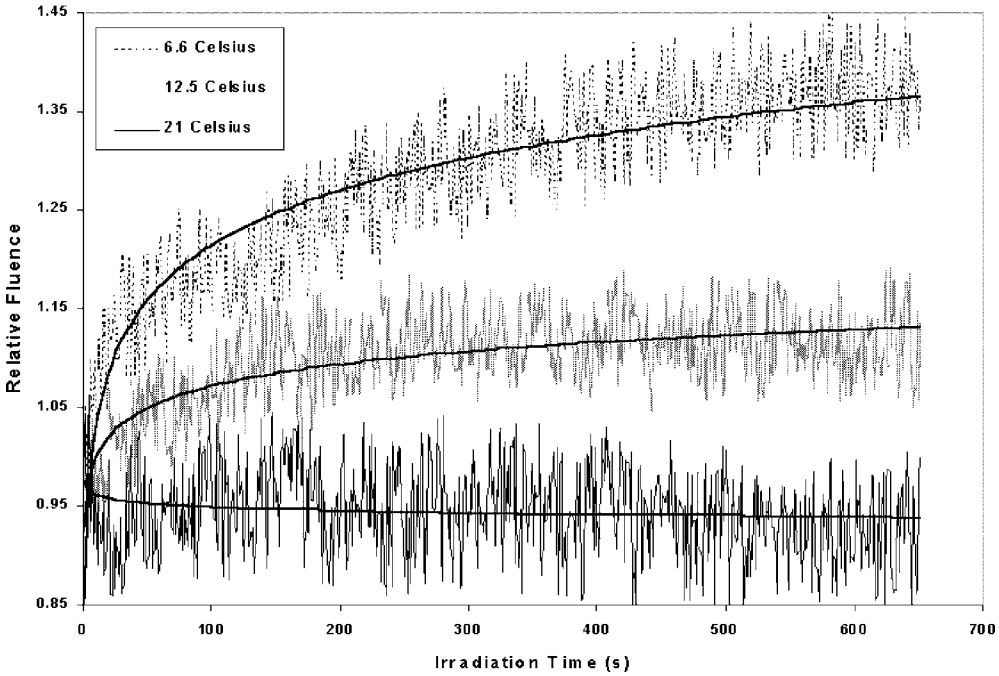

Figure 7. Changes in normalized photovoltage measured by an optical sensor 0.6–0.85 cm from the source in bovine liver for different initial bulk tissue temperatures during 0.5 W irradiation (dotted lines). Samples beginning at low starting temperatures (6.6, 12.5 °C) exhibit a more pronounced fluence increase while samples starting near room temperature (21 °C) demonstrate little to no increase. Solid lines are the logarithmic fits of the raw data.

were cooled, prior to irradiation. Figure 7 is representative data showing changes in measured fluence of ∼35, 10 and 5% in samples with starting tissue temperatures of 6.6, 12.5 and 21 °C, respectively. Data was normalized to the optical signal at 1 s. The strong variation in measured optical signals, compared to figures 2 and 6, is a result of the absence of signal averaging used by the National Instruments acquisition system. In the future, signal averaging will be used as the National Instruments DAQ can sample up to 200 ksamples/s. For this study, to better delineate the optical signal trends, a logarithmic fit was performed on the measured data (solid lines).

Samples beginning at low starting temperatures (6–15 °C) demonstrated increases of between 10 and 35% while samples starting at room temperature (18–24 °C) demonstrated little-to-no increase in measured fluence. In fact, the measured fluence of samples beginning at room temperature actually appeared to decrease slightly (by as much as 10%). Interestingly, by cooling the same sample back to low temperatures, a noticeable fluence increase was once again observed (older samples, greater than 2 days, exhibited a more variable effect).

4. Discussion

Though numerous simulations have been performed to predict the dynamic changes in light distribution during ILP, few, if any, of these simulations have been validated experimentally. We have performed controlled experiments on tissue simulating albumen phantoms and *ex vivo* liver tissues to test for changes in interstitial optical distribution during ILP. Specifically,

we have examined changes observed by an optical sensor located outside a growing region of coagulation. For a sensor located outside a region of coagulated tissue containing the source, a drop in light fluence is expected due to attenuation from the increased scattering coefficient of coagulated tissue (Iizuka *et al* 2000). At low powers (0.5–1 W in phantoms, 0.5 W in liver) in which coagulation was not observed, this drop in fluence was absent. At higher powers (1.5–2 W in phantoms, 1–1.5 W in liver) in which coagulation was observed, a fall in light fluence occurred during heating with the rate of decrease increasing with higher input powers. These results appear to be in good agreement with the predictions of Iizuka *et al* (2000) and Kim *et al* (1995). In addition, in our experiments with bovine liver at 1.5 W of optical power, following the first decrease due to coagulation, a second sharper drop in photovoltage was also observed. This second drop was not observed at 1 W. As the only histological difference between the two experiments was the presence of a charred core, it is likely that the cause of the second drop occurred due to tissue charring. In albumen phantoms, charring occurred for an input power of 2.5 W. However, no associated trend was observed in the photovoltage measurements. This was likely a result of the onset of charring soon after the start of irradiation. This discrepancy between phantom and liver tissue might indicate that albumen phantoms might not exhibit behaviour suitable for light fluence evaluation of tissue charring events.

Though decreases in relative light fluence due to coagulation and/or charring are expected, the observed increase observed particularly at low laser heating powers (0.5–1 W) was surprising. To our knowledge this is the first citing of an observed increase in interstitial light fluence during ILP. We hypothesize that the increase in detected photovoltage was due to an increase in light penetration resulting from changes in the optical properties of the medium. Decreases in either the absorption or reduced scattering coefficient due to thermal heating or photon exposure could result in the observed increase in photovoltage. There is no data in the literature to suggest a decrease in tissue scattering for non-coagulative laser exposures at 810 nm. However, Walsh and Cummings (1994) have demonstrated shifts in the absorption spectrum at mid-infrared wavelengths (1440–2940 nm) due to temperature, while Izatt *et al* (1991) postulated a decrease in absorption due to laser-induced changes or bleaching effects.

Spectrophotometric experiments were performed in an attempt to determine the cause of the 'photobleaching' effect observed in albumen phantoms and bovine liver. A particular advantage of albumen phantoms is the simplicity of their composition allowing for easy separation of its absorbing and scattering components. In contrast, given the structural complexity of tissue, a rigorous analysis of the optical contributions from its various components is difficult.

Our experiments suggest that the increase in measured fluence observed in phantoms at low powers was likely due to a decrease in the absorption coefficient of Naphthol Green dye arising from exposure to laser irradiation and not due to thermal heating. The biomolecular mechanism behind this effect is not clearly understood and is outside the scope of this investigation. To test if the fluence behaviour observed in albumen phantoms was an artefact of the phantom materials or has relevance to real tissues, interstitial light fluence experiments were also performed on *ex vivo* bovine liver. Interestingly, a rise in photovoltage at $\leqslant$1 W was also observed in liver and the albumen phantoms. This is surprising as synthetic chromophores such as Naphthol Green dye might be expected to exhibit different photoexcitation properties than those of biological chromophores. However, the mechanisms behind the fluence rise in liver might follow a more temperature-dependent mechanism than the laser-induced mechanisms associated with albumen phantoms.

When liver samples were cooled to low temperature (6–15 °C), the fluence rose by 10–35% (after 0.5 W irradiation for 650 s), compared to little or no increase when liver

samples were at room temperature (18–24 °C). Furthermore, re-cooling samples caused the fluence increase to manifest again. Hence, unlike the dye phantom model, this effect in liver tissue appears to be a thermally induced change due to cooling than a 'photobleaching' effect. A temperature-related increase in fluence might explain why it was only observed when sensors were positioned interstitially and not when placed between two liver slabs. Tissue near the surface will quickly equilibrate to room temperature, while tissue within bulk liver will warm at a much slower rate. As a result, in method 1, the interface between tissue slabs was likely at room temperature and the fluence increase was not observed. In method 2, the tissue at the sample centre was approximately 10–15 °C below room temperature due to thermal insulation by the surrounding tissue, thereby allowing the fluence rise to manifest. Another possibility is the effect of air gaps formed between slabs.

Unfortunately, a complete histological analysis of the contributing chromophores in *ex vivo* bovine liver is difficult and beyond the scope of this article. However, we note that a previous study by Laufer *et al* (1998) has indicated that changes in tissue scattering has been observed in tissue at temperature below the coagulation threshold (25–40 °C). These tests were performed on *ex vivo* dermis and subdermis tissue and at a higher temperature range than that employed in our experiments (6–24 °C). However, our results indicate that tissue optical property changes (below the coagulation threshold) can also occur in *ex vivo* bovine liver and at a lower temperature range. Further studies are required to determine the exact cause of the rise observed in bovine liver.

The dynamic changes in light fluence may have important implications for treatment planning, on-line monitoring and control of ILP and other biophotonic procedures. For example, the observed photobleaching in optical dye-containing phantoms should be included in optical and opto-thermal models. Since the effect is due to laser irradiation and not temperature, it may also impact photodynamic therapy (PDT) modelling and measurement. The physical state of the material (solid versus gel versus liquid) containing the photobleachable absorber may also influence the magnitude of the fluence rise, being least noticeable in liquid because of dye convection. In the future, other near-infrared dyes rather than Naphthol Green might be required for the construction of optically stable PDT and ILP phantoms.

Our results also indicate that measurements of interstitial fluence might offer a means of inferring tissue structure changes during ILP. Evidently, for a sensor placed outside a region of coagulation, a decrease in light fluence marks the onset of tissue coagulation. However, as indicated in the measurements of bovine liver, changes in light fluence might serve as potential indicators not only for the onset of tissue coagulation, but for tissue charring as well. Tissue charring is associated with bubble formation and smoke production, and for ILP of organs such as brain or prostate, should be avoided. Time delays associated with temperature sensors provide insufficient feedback response to prevent the onset of the charring process. Here we observed an almost instantaneous drop in light intensity likely associated with the onset of tissue charring. Hence, fluence sensors might offer an alternate, more rapid and accurate means of feedback monitoring.

In addition, the dynamic changes observed in our experiments might offer predictive capabilities. As shown in the albumen phantom experiments, photobleaching produces a non-coagulative increase in photovoltage. This rise was shown to increase with increasing laser power and may provide a means of preventing tissue charring and controlling early lesion growth. As a possible example, we note that no rise in light fluence was observed in albumen phantoms or liver tissue during ILP that leads to charring (2.5 W in albumen phantoms, 1.5 W in liver tissue). A potential inference from this result is that the absence of an initial rise in light fluence might provide an on-line indication of the forthcoming tissue charring process, such that laser powers should be reduced, proactively.

Even if correlation of fluence rise and tissue heating proves difficult, on-line monitoring techniques might simply focus on time of initial decrease in fluence. Assuming the threshold temperature for tissue coagulation is $\approx$60 °C, a drop in light fluence not only infers coagulation, but indicates that tissue temperatures near the source have likely reached $\geqslant$60 °C. Thus, an early decrease in relative light fluence would indicate a high rate of power deposition while a later drop in relative fluence would likely be associated with a low power deposition rate. Light fluence might therefore be used to approximate the maximum temperature near the source.

Pre-photobleaching of tissue (at non-coagulative source powers) near the source fibre might also be incorporated into ILP protocols to strategically avoid tissue charring. Charring near the source fibre occurs due to the high power deposition, a product of absorption and fluence. Pre-bleaching could lower the absorption coefficient of the tissue and thereby decrease the power deposition near the source.

Finally, an unexpected advantage in the low temperature dependence of the fluence rise observed in liver tissue is the increased penetration of light in cooled tissues. Such an effect might provide a synergistic effect during ILP involving cooling catheters, with cooled tissue both minimizing the potential for charring and allowing for greater light penetration to generate larger regions of thermal damage.

5. Conclusions

We have investigated the dynamic changes in the relative fluence distribution of light during ILP of albumen phantoms and *ex vivo* liver tissue as an initial investigation of using such measurements for monitoring tissue structure changes during ILP. Synthesized albumen phantoms demonstrated very similar trends as those of *ex vivo* liver tissue.

Our results indicate that, during irradiation at 810 nm, dynamic changes in optical properties result in three important trends: (1) an increase in light fluence, (2) a decrease in light fluence due to coagulation of tissue and (3) a sharp second drop in light fluence due to the onset of tissue charring. Results 2 and 3 agree with current ILP light models and predictions regarding fluence changes due to coagulation and charring. However, the increase in fluence has not been reported previously in albumen phantoms or in tissues. In albumen phantoms, this appears to be caused by a decrease in absorption properties of Naphthol Green dye due to the optical effects of laser irradiation. Such photobleaching phenomena could have important implications in modelling and experimental validation of optical and opto-thermal procedures. In bovine liver, the increase in fluence appears to be related to a change in optical attenuation at low tissue temperatures. The cause of this effect is currently unclear. A low temperature effect might carry implications in ILP applications with cooling catheters.

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References

Amin Z, Bown S G and Lees W R 1993 Liver tumor ablation by interstitial lasers photocoagulation: review of experimental and clinical studies *Sem. Interventional Radiol.* **10** 88–100

Beerlage H P, Thuroff S, Madersbacher S, Zlotta A R, Aus G, de Reijke T M and de la Rosette J J 2000 Current status of minimally invasive treatment options for localized prostate carcinoma *Eur. Urol.* **37** 2–13

Cheong W F, Prahl S A and Welch A J 1990 A review of the optical properties of biological tissues *IEEE J. Quantum Electron.* **26** 2166–85

Eisenberg D and Kouzmann W 1969 *The Structure and Properties of Water* (Oxford: Clarendon) pp 295–6

Halldorsson T, Langerholc J, Senatori L and Funk H 1981 Thermal action of lasers irradiation in biological material monitored by egg-white coagulation *Appl. Opt.* **20** 882–5

Henderson B 1991 An isotropic dosimetry probe for monitoring light in tissue, theoretical and experimental assesment *Ph.D. Thesis* (Edinburgh: Heriot Watt University)

Iizuka M, Sherar M D and Vitkin I A 1999 Optical phantom materials for near infrared lasers photocoagulation studies *Lasers Surg. Med.* **25** 159–69

Iizuka M, Vitkin I A, Kolios M C and Sherar M D 2000 The effects of dynamic optical properties during interstitial lasers photocoagulation *Phys. Med. Biol.* **45** 1335–57

Izatt J A, Albagli D, Britton M, Jubas J M, Itzkan I and Feld M S 1991 Wavelength dependence of pulsed lasers ablation of calcified tissue *Lasers Surg. Med.* **11** 238–49

Jacques S L 1994 Experiments and modeling of the dynamic nature of continuous lasers ablation of tissue *Proc. SPIE* **2323** 273–83

Kim B, Jacques S L, Rastergar S, Thomsen S and Motamedi M 1995 The role of dynamic changes in blood perfusion and optical properties in thermal coagulation of the prostate *Proc. SPIE* **2391** 443–50

Laufer J, Simpson R, Kohl M, Essenpreis M and Cope M 1998 Effect of temperature on the optical properties of *ex vivo* human dermis and subdermis *Phys. Med. Biol.* **43** 2479–89

Mackenzie A L 1990 Physics of thermal processes in lasers-tissue interaction *Phys. Med. Biol.* **35** 1175–1209

Matthewson K, Coleridge-Smith P, O'Sullivan J P, Northfield T C and Bown S G 1987 Biological effects of intrahepatic Neodymium:Yttrium-Aluminum-Garnet lasers photocoagulation in rats *Gastroenterology* **93** 550–7

Meijerink R, Essenpreis M, Pickering J W, Massen C H, Mills T N and van Gemert M J C 1995 Rate process parameters of egg white measured by light scattering *Lasers Induced Interstitial Thermotherapy* ed G Muller and A Roggan (Bellingham, WA: SPIE Optical Engineering Press) pp 66–80

Pickering J W 1992 Optical property changes as a result of protein denature in albumen and yolk *J. Photochem. Photobiol.* B **16** 101–11

Pickering J W, Posthumus P and van Gemert M J C 1994 Continuous measurement of the heat-induced changes in the optical properties (at 1,064) of rat liver *Lasers Surg. Med.* **15** 200–5

Reid A D, Gertner M R and Sherar M D 2001 Temperature measurement artefacts of thermocouples and fluoroptic probes during lasers irradiation at 810 nm *Phys. Med. Biol.* **46** N149–N157

Ritz J P, Roggan A, Germer C T, Isbert C, Muller G and Buhr H J 2001 Continuous changes in the optical properties of liver tissue during lasers-induced interstitial thermotherapy *Lasers Surg. Med.* **28** 307–12

Roux F X, Merienne L, Leriche B, Lucerna S, Turak B, Devaux B and Chodkiewixz J P 1992 Lasers interstitial thermotherapy in stereotactical neurosurgery *Lasers Med. Sci.* **7** 121–6

Thomsen S, Pearce J A and Cheong W F 1989 Changes in birefringence as markers of thermal damage in tissues *IEEE Trans. Biomed. Eng.* **36** 1174–9

van Hillegersberg R, van Staveren H J, Kort W J, Zondervan P E and Terpstra O T 1994 Interstitial Nd:YAG lasers coagulation with a cylindrical diffusing fiber tip in liver metastases *Lasers Surg. Med.* **14** 124–38

van Staveren H J, Jarijinisson J P A, Aalders M C G and Star W M 1995 Construction, quality assurance and calibration of spherical isotropic fiber optic light diffusers *Lasers Med. Sci.* **10** 137–47

Walsh Jr J T and Cummings J P 1994 Effect of the dynamic optical properties of water on midinfrared lasers ablation *Lasers Surg. Med.* **15** 295–305

Welch A and van Gemert M(ed) 1995 *Optical-Thermal Response of Lasers-Irradiated Tissue* (New York: Plenum)

Whelan W M, Chun P, Chin L C L, Sherar M D and Vitkin I A 2001 Lasers thermal therapy: utility of interstitial fluence monitoring for locating optical sensors *Phys. Med. Biol.* **46** N91–N96

Whelan W M, Wyman D R and Wilson B C 1995 Investigations of large vessel cooling during interstitial lasers heating *Med. Phys.* **22** 105–15

Wyman D 1993 Selecting source locations in multifiber interstitial lasers photocoagulation *Lasers Surg. Med.* **13** 656–63