BIOPHYSICAL STUDIES OF PULSED PHOTOTHERMAL RADIOMETRY
IN TISSUES AND TISSUE-LIKE MEDIA

By

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PULSED PHOTOTHERMAL RADIOMETRY IN TISSUE

Abstract

Pulsed Photothermal Radiometry (PPTR) is a remote sensing technique which monitors the temperature transient at the surface of a sample following the absorption of a brief optical pulse. In this work, it was used to investigate optical and thermal processes occurring in laser-irradiated, turbid, inhomogeneous media such as tissue. An optical diffusion approximation and heat conduction equation were used to derive the turbid PPTR model, for both homogeneous and layered semi-infinite media. These models were tested experimentally in a series of aqueous gel phantoms of known optical and thermal properties, and were found to be in good agreement. A study of discrete optical absorbers in a transparent medium necessitated the inclusion of the thermal radiative effects as an additional mode of heat transfer, and a corresponding PPTR model was derived and tested experimentally. Studies of intact biomolecules and of *in-vivo* tissue have also been performed to test and verify the derived formulations, and to determine the practical utility of PPTR for specific biomedical applications. The theoretical and experimental results presented in the thesis lay the groundwork for further inclusion of PPTR into the diagnostic arsenal of laser medicine.

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- *Felix qui potuit rerum cognoscere causas.*

... *Lucretius*

Четыре дела, однажды начавши, трудно кончить :
1) вкушать хорошую пищу; 2) беседовать с другом;
3) чесать, где чешется; 4) писать диссертацию.

... - Козьма Прутков

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Symbols

A	coefficient of fluence term; cross-sectional area of sphere
a	albedo
a'	transport albedo
a _D	detector area
B	coefficient of fluence term
C	coefficient of fluence term; lumped experimental PPTR parameter; constant in t_{cond}
c	specific heat
D	optical diffusion constant
d	position of buried layer; sphere diameter; total pathlength
E	radiant energy fluence (also H, Φ)
F	radiative flux
f _i	frequency of occurrence of i_n particle
G	Gibbs free energy; scaling constant (similar to K)
g	scattering anisotropy parameter
H	radiant energy fluence (also E, Φ); enthalpy
h	Planck's constant; surface heat transfer coefficient
I	radiance; strength of photometric signal
K	experimental PPTR scaling multiplier
k	thermal conductivity (also κ); semi-empirical fluence build-up factor
k _B	Boltzmann's constant

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θ	polar angle
κ	imaginary part of optical refractive index; thermal conductivity (also k)
λ	wavelength
μ_a	absorption coefficient
μ_s	scattering coefficient
μ_s'	reduced (transport) scattering coefficient
μ_t	total attenuation coefficient
μ_{tr}, μ_t'	transport (reduced) attenuation coefficient
μ_{eff}	effective attenuation coefficient
μ_{IR}	infrared absorption coefficient
ρ	density
σ	Stefan-Boltzmann constant, surface roughness
τ	transmissivity
Φ	radiant energy fluence (also E, H)
Ψ	fractional amount of final product (Arrhenius integral)
Ω, ω	solid angle

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L	sample thickness
N	complex optical refractive index; radiation-to-conduction parameter
n	real part of optical refractive index
{p _i }	properties of the i-th layer
Q	absorption or scattering efficiency; thermal source term
R	residual; universal gas constant
R _D	diffuse reflectance
r	spatial coordinate
S	fluence source term; entropy
S(t)	time-dependent experimental signal
T	temperature
T _D	diffuse transmittance
t	time
t_{cond}	thermal conduction relaxation time
$t_{r/2}$	thermal radiation relaxation time
V	particle volume
W	radiant energy density
x,y,z	spatial coordinates
α	thermal diffusivity
β	non-radiative quantum efficiency
$\Delta l, \Delta x$	element thickness
ϵ	emissivity
η	detector efficiency

x

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following the excitation pulse constitutes the PPTR signal. The essential features of the experimental set-up are shown schematically in Figure 1.

CHAPTER I. Introduction

The main goal of the work presented in this thesis was to extend a particular photothermal-wave, diagnostic technique to investigations of biological tissues and tissue-like media. The technique in question is called pulsed photothermal radiometry (PPTR), and because of the significant amount of optical and thermal information provided by it, such an extension was considered profitable. From the point of view of medical diagnostics, the possible advantages of PPTR studies are twofold: first, such studies may provide a means of non-destructively measuring the optical or the thermal properties of the interrogated material, and/or lead to an estimate of the resulting fluence or temperature fields within it; and second, one may learn about the biologically or medically significant underlying mechanisms of opto-thermal transfer in light-irradiated tissues. We believe that this systematic study lays the groundwork for further inclusion of PPTR into the arsenal of photomedical diagnostic techniques.

The principle of operation of a typical PPTR experiment is as follows. A brief optical pulse is incident on the surface of the interrogated specimen, resulting in a spatially-dependent distribution of absorbed optical energy. In most materials, the de-excitation pathway is thermal, and therefore a corresponding spatially-dependent temperature increase is generated. This in turn causes an increase in the thermal radiative emission from the near-surface regions of the sample, an increase which is monitored with an infrared-sensitive detector viewing the sample surface. The record of this emission as a function of time

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effects when optical absorption occurs at finite-sized discrete sites, for which continuum-based concepts such as volumetric optical coefficients may be inappropriate? Because of the significant temperature excursions at the absorbing sites, we felt that thermal radiative transfer was playing some role in the heat transfer process (in addition to the dominant role played by thermal conduction), and the PPTR model was modified accordingly. In addition to experiments in tissue-simulating phantoms performed to test and verify the above formulations, two biomedical studies were conducted: one involved monitoring exogenous dye uptake in liver tissue following a bolus injection *in vivo* in a rat model, and the second involved determination of optical and thermal properties of intact natural melanin.

The contents of this thesis are divided into three parts. Chapter II deals with applications of lasers in medicine, in particular focusing on interactions of light with tissues, relevant thermal effects, light propagation in tissue, and the issues viz. the biomedical PPTR applications. Chapter III is the crucial part of the thesis, for it contains the published or submitted papers which resulted from the Ph. D. research herein described. Each paper is prefaced with a brief overview which summarizes its salient points and delineates the author's contribution to it. The papers are presented in a thematic rather than chronological order: papers #1, #2, and #3 are somewhat theoretical, #4 and #5 present experimental biophysical applications of the derived theories, and #6 summarizes the state-of-the-art of the biomedical PPTR research as of 1992. Chapter IV contains overall conclusions and future directions. Several miscellaneous issues - use of infrared fibers, effect of surface smoothness, mathematical peculiarity of diffusion theory, approximate solutions in layered media - are discussed in the Appendices.

Figure 1. Principle of operation of a PPTR system.

In our work, we have drawn upon a decade's worth of expertise in PPTR research to specifically address issues relevant to photomedicine. One such issue is the ability to perform absorption spectroscopy in a scattering environment. In other words, can absorption in a turbid medium such as tissue be detected, and the value of the volumetric absorption coefficient determined? Since PPTR is an absorption-based technique, relying as it does on the conversion of the incident optical energy to a thermal transient that is detected with a remote infrared (IR) detector, we felt it was well suited to tackle this problem. When this problem was solved through the application of optical diffusion theory in a homogeneous, semi-infinite medium, we proceeded to examine the case of optically inhomogeneous media. The turbid PPTR model was thus extended to describe the signal behaviour in a two-layer system, with both layers possessing different absorption and scattering parameters. Finally, the issue of discrete optical absorption was examined. That is, what are the opto-thermal

CHAPTER II. Applications of Lasers in Medicine

II.1. Interactions of Light with Tissue and Biomedical Applications

The debate concerning the physical principles, religious meanings, and philosophical implications underlying the nature of light has been ongoing since the dawn of man's recorded history (Asimov 1960; Feyeraabend 1981; Zajonc 1993). It forms a fascinating study in the history and philosophy of human idea development, as can be gauged from a brief perspective of the more recent contributions (Born and Wolf 1981). The present view of the physical nature of light, and of other entities in the universe, is embodied in a concept known as "particle-wave duality". This states that electromagnetic (EM) radiation, of which visible light is but a small portion, can be represented as *either* a collection of particle-like entities (photons), *or* as a wave-like disturbance travelling through space. This apparent dichotomy is somewhat reconciled by the Complementarity Principle, which states that the particle and the wave aspects of a quantum entity are both necessary for a complete description; however, both aspects cannot be revealed simultaneously in a single experiment. In general, it was found that the particle construct was more useful in describing interactions of light with matter (e.g., the photoelectric effect, photochemical absorption), while the wave aspect was more effective in describing propagation of light in space (e.g., Maxwell's equations, antenna theory). The refractive index, which helps explain the absorption and scattering properties of matter, can also be understood on the microscopic scale by the interaction of a transverse electromagnetic wave with orbital electrons of the irradiated material. Often, choosing the particle or the wave description for a particular problem is dictated by the amount of available information, the kinds of answers sought, and the

complexity of the resultant formulation. With few exceptions (Twersky 1962; Twersky 1970; Ishimaru 1978; Fanto 1980), most research in tissue optics has ignored the wave nature of the light field, and treated the optical problem in terms of discrete optical quanta (for example, see discussion in Patterson *et al* 1991a; Wilson 1994).

Light forms an integral part of our natural environment. In fact, the development of vision based on light has had a tremendous evolutionary consequence for the primates, as it allowed large areas of the brain previously devoted to the olfactory sensations to be used for "higher-order" processes, eventually giving rise to abstract thinking (Asimov 1960). Most of the light in our natural environment originates from the nuclear fusion reaction taking place in our sun. The interactions of this light with the objects around us results in some fraction of it impinging on the photochemical receptors in our eyes, enabling us to see the object, and in some cases perceive its colour. In the interaction process itself, light may simply be scattered and eventually remitted from the object without leaving a noticeable mark of its presence; or if some absorption is present, a photochemical or photobiological reaction may be triggered. The visual appearance of a material, and the "what and where" of the photo-interactions within it are largely determined by its optical properties, which can be described in terms of the complex electric permittivity, the complex refractive index, or absorption and scattering coefficients as defined below.

The full explanation of the absorption and scattering mechanisms of EM radiation by matter necessitates the use of quantum mechanics (Cohen-Tannoudji and Diu 1977). Briefly, an absorption event is possible if the photon energy is equal to (or is greater than) some quantum-mechanically-allowed excited state transition of a given molecule. The absorbing molecule is known as the chromophore. Given the necessary information about

delay (with respect to the incident wave) because of the non-instantaneous response of the charges. The resultant EM field is obtained by adding vectorially the incident and the scattered waves. A detailed quantum mechanical analysis yields the probability of such a scattering interaction, and its directional dependence for a given molecule. Macroscopically, when the optical properties of a collection of molecules forming a mass are known, an analysis based on Maxwell's field equations provides analogous results for simple geometric shapes such as spheres and cylinders (Mie 1908; Van de Hulst 1981; Bohren and Huffman 1983). Finally, an operational, particle-like picture envisions scattering as billiard balls collisions, with the probability of collision per unit path length described by the volumetric optical scattering coefficient, μ_s , and the angular direction of the deflected projectile specified mathematically by a probability density distribution known as the phase function. Often, the mean cosine of the scattering angle g (first moment of the phase function) provides adequate description of the directional dependence of the scattered light.

The principal photobiological interactions between non-ionizing EM radiation and living tissue can be classified into three broad and often overlapping categories. The dominant interaction route depends on the optical characteristics of the tissue, the temporal, spatial, and spectral characteristics of the irradiation, and the tissue's biological response mechanisms (Boulois 1985). It is important to realize that these interactions occur only if the photon has been absorbed, for otherwise no appreciable energy is imparted to the medium. Perhaps the most common outcome of medical laser intervention is *photothermal* in nature, with the end result being the localized heating of the irradiated tissue. This forms the basis for much of laser surgery (Dixon 1983), laser hyperthermia (Bown 1983), laser photocoagulation (Matthewson *et al* 1988), selective photothermolysis (Anderson and Parrish 1983), and photothermal diagnostic techniques, one of which is examined in this thesis. In

the photon and the chromophore, quantum mechanics can predict the probability (never a certainty) of an absorbing event. In the process, the photon is "extinguished", and its radiant energy is now manifest in the excited state of the chromophore. In the visible spectral range, the photon energies are on the order of an electron volt, and the molecular transitions are primarily electronic in nature. The subsequent fate of the absorbed radiant energy depends on many factors - the energy spectrum of the chromophore, its interactions with the neighbouring molecules, the lifetime of the excited state, and to some extent its previous history. Some possible routes for de-excitation include photophysical and photochemical transformations, and luminescence (fluorescence or phosphorescence). The most common route in condensed matter is nonradiative de-excitation, whereby the kinetic energy in the immediate neighbourhood of the chromophore is increased. This generally takes the form of increased vibration, rotation, and (sometimes) translation. In other words, the absorbed photon energy has been converted to heat. The macroscopic probability of absorption per unit photon path length is described by the volumetric absorption coefficient, μ_a .

A simple phenomenological explanation of the scattering mechanism invokes the wave-nature of light. In this picture, the transverse electromagnetic wave interacts with electronic charges and dipole moments present in matter. These are set in motion that results in the emission of another electromagnetic disturbance, usually of the same frequency. The scattering process is thus elastic (sometimes termed conservative or coherent), in that the energy of the exiting photon is equal to that of the incident, for any arbitrary direction in space. Inelastic scattering is also possible, whereby the frequency of the scattered photon is different (usually lower) than that of the incident. However, there is a directional dependence to the scattered radiation, both because of the interference effects with the neighbouring charges and because of the physical laws governing EM emission; there may also be a phase

the so-called *photochemical* mode of interaction, absorbed radiant energy is used to initiate chemical reactions. Medical examples of interest include photodynamic therapy, or PDT (Dougherty 1989; Marcus and Dugan 1992; Pass 1993) and psoralen-UVA therapy (Parrish *et al* 1982). Finally, when tightly-focused, high-energy, short laser pulses are used, highly non-equilibrium transient conditions can occur, such as photodissociation *via* bond breakage, plasma formation, non-linear absorption, super- and sub-sonic acoustic transients, and so on. A typical end result may be mechanical rupture or fragmentation of the target or its physical ejection from the irradiated surface *via* ablation (Srinivasan 1986; Walsh and Deutsch 1989). For this reason, the customary name given to this interaction scheme is *photomechanical*. Examples of this mode include urinary and biliary stone fragmentation (Nishioka *et al* 1987), corneal photokeratotomy (Stern *et al* 1989), and atherosclerotic plaque removal (Singleton *et al* 1986; Isner *et al* 1987). Once again, it is important to emphasize the somewhat artificial nature of the above classification, since any particular laser-tissue interaction usually involves a combination of the above effects (sometimes with beneficial, sometimes with deleterious outcomes); however, a judicious choice of the appropriate variables may emphasize one dominant interaction route.

The medicinal uses of light have a long and varied history. Peoples of the Mediterranean basin have been known to treat a skin pigmentation disease known as vitiligo over 2000 years ago by eating a certain plant (a small, flowering weed *Ammi majus L.* that still grows along the Nile river) and then going out into the sun. In the beginning of this century, the connection between sunlight and vitamin D synthesis was established, providing an explanation and a cure for rickets. The use of man-made sources of light began primarily in the 20th century. These early applications include treatment of skin diseases with ultraviolet light (Finsen 1901); light transillumination of the breast for tumour detection

(Cutler 1929); and tumour therapy using drug/light combinations (Policard 1924). Anecdotal evidence for light applications in wound healing, pain relief, and psychiatric therapy has also been plentiful; scientific evaluation of these effects is becoming available (Karu 1987; King 1989). Recent resurgence of pseudo-scientific claims vis a vis stress reduction and smoking cessation using lasers is the modern throwback of this anecdotal trend.

The modern era of laser applications in medicine began shortly after the invention of the ruby laser in 1960. The focused laser beam was effective in coagulating blood vessels in the retina, and in tissue cutting. These early successes were based on thermal effects of laser irradiation, and much of the early work was empirical in nature. As technology improved and the understanding of laser-tissue interactions increased, laser surgery became a viable clinical modality. Besides general-purpose soft-tissue cutting, there are specific surgical laser applications, for example in orthopaedics, neurosurgery, and prostate surgery. The localized laser heating in these surgical procedures enables the surgeon to coagulate blood and close blood vessels, thus preventing excessive bleeding. The eradication of some types of solid tumours via photodynamic therapy is another example of a laser treatment modality currently under active investigation. The photochemistry and the light delivery systems present formidable problems to the chemist, biologist, physicist, and physician; the procedure is currently undergoing late-stage clinical trials in several countries, and has been approved recently in Canada for some forms of bladder cancer (Quadra Logic Technologies, Vancouver - personal communication). Another active area of investigation and treatment relies on the ability of the laser to cause localized mechanical damage such as fracturing and ablation. Clinical applications of photomechanical pressure waves induced by laser pulses delivered through optical fibers include fragmentation of kidney and gallstones, and ablation of calcified plaque in vessels. Although this listing of medicinal uses of lasers is by no

al 1990; Vitkin *et al* 1992; Vitkin *et al*, in press). It appears that this class of techniques is particularly useful for measuring the optical and thermal properties of biological materials that are optically opaque, and / or are difficult to prepare or examine using standard photometric methods.

11.2. Thermal Effects in Light-Tissue Interactions

As the PPTR diagnostic technique used in this thesis is thermal in nature, it is useful to discuss the thermal mode of laser-tissue interactions in greater detail. Depending on the temperature-time history of the tissue, different thermal processes can be delineated. Consider long (i.e., several minutes to several hours) exposure. Up to about 43°C, there is no appreciable irreversible damage. In the interval between 43°C and about 60°C, protein denaturation usually occurs. Laser hyperthermia, photocoagulation, and tissue welding are medical modalities that operate in this interval. Over 60°C, tissue necrosis will result. DNA molecules undergo irreversible damage in the region over 80°C. Beyond 100°C, the tissue will dehydrate as the water boils off, leading to severe necrosis. Because of the various solutes dissolved in tissue water, actual onset of dehydration may as high as ~120°C. Further heating results in charring and carbonization. Both short-term and long-term biological responses, such as haemostasis due to blood coagulation or slow healing rates due to presence of charred tissue must be considered in optimizing the efficiency of the photothermal laser intervention. An interesting issue that has attracted much attention recently is the change in the optical (and thermal) properties of the irradiated tissue during the procedure (Petit *et al* 1993). This will clearly affect the light and heat deposition patterns, and thus the resultant biological response. It seems that denaturation of tissue proteins increases the scattering coefficient (e.g., egg white turning "white" on a frying pan),

means complete, it does indicate the scope and the breadth of the field.

Another important area of the biomedical laser field is tissue diagnostics. The degree of oxygenation of the blood, the blood flow velocity patterns, the concentration of a particular body chromophore, or the distribution of an exogenous drug can be measured noninvasively by detecting diffusely reflected or transmitted photons. Laser-induced fluorescence and Raman spectroscopy (involving photons that have interacted with the vibrational levels of the scatterer, and thus have undergone a slight frequency shift) have been used as a means of distinguishing arterial plaque and tumours from normal tissue (Andersson-Engels 1992; Andersson-Engels *et al* 1990; Liu *et al* 1993). Optical ranging techniques have been used to measure the thickness of skin components, and to demarcate the presence of the different layers of the eye (Huang *et al* 1991). Determination of the optical fluence profiles in irradiated materials, and of their absorption and scattering properties has also received considerable attention (Patterson *et al* 1991b). This information is needed for successful treatment planning, or may be used to diagnose pathological conditions. Optical imaging of tissues is currently being examined. The reduction of contrast due to multiple scattering in most tissues makes this a very difficult problem, and continuous-wave transillumination studies have been inconclusive to date. A more promising alternative, actively pursued at present, is the use of short-pulsed or amplitude-modulated light to perform diffusive wave spectroscopy and imaging (for example, see Chance and Alfano 1993). Various contrast enhancement approaches, for example time-of-flight techniques, multi-phased source and detector arrays, and absorption and spatial filtering methods, are currently being evaluated. Thermal wave techniques, such as photoacoustic spectroscopy and photothermal radiometry, have also been used for medical research (Sennhem *et al* 1988; N'soukpoe-Kosi and Leblanc 1988; Andreoni *et al* 1988; Bernini *et*

whereas carbonization increases the absorption (e.g., a steak turning black as it burns). In both cases, the penetration depth of light will decrease, an effect that needs to be considered in treatment planning.

As noted, the tissue response to a photothermal intervention depends on time as well as on temperature. Experimentally, it has been found that, as the characteristic heating times decrease, higher temperatures are needed to achieve a given end effect (Hermades 1947; Moritz *et al* 1947). Quantitative analysis of this dependence is based on the so-called Arrhenius damage integral (Gladstone *et al* 1941; Birngruber 1979; Stiller 1991). The complex nature of the time-temperature relationship became apparent with the advent of pulsed lasers, when it was found that transient temperatures following a brief laser pulse may reach hundreds of degrees centigrade, sometimes with no appreciable after-effect. In other cases, pulsed laser irradiation results in evaporative or explosive forms of material removal, known as laser ablation. Although often driven by thermal effects, ablation with high-peak-power pulses may also rely on molecular disruption due to bond breaking or plasma formation due to multi-photon absorption. The net result is rapid expansion which will fragment the tissue. This is utilized in pulsed laser surgery for precise cutting and removal of tissue, in bladder and gallstone fragmentation, and in experimental laser coronary angioplasty.

To quantify the thermal denaturation kinetics, the concept of the Arrhenius damage integral needs to be discussed. This arises from the work of Arrhenius (1889) on the optical activity of sugar solutions, who examined their first-order, temperature-dependent, chemical rate processes. Combining rigorous thermodynamic derivations with empirical fits, he found that the fractional amount of the final product (thermally-damaged state in our context),

$\Psi(t, T)$, was predicted by

$$\Psi(t, T) = \int_0^t (k_B T / h) \exp(-\Delta G / RT) dt' \quad (1)$$

where ΔG is the change in the Gibbs free energy on going from the initial to the thermally denatured (damaged) state, k_B is Boltzmann's constant, h is Planck's constant, and R is the universal gas constant. From thermodynamics (Callen 1985), we can express the free energy as the difference between enthalpic and entropic contributions, $\Delta G = \Delta H - T\Delta S$. The Arrhenius integral thus becomes

$$\Psi(t, T) = (k_B / h) \exp(\Delta S / R) \int_0^t T \exp(-\Delta H / RT) dt' \quad (2)$$

which can be evaluated in some circumstances. A common and reasonable approximation is to extract T from the integrand, thereby assuming that the dominant temperature dependence is due to the exponential enthalpy factor. Although not evident in the expression, the temperature is often time-dependent as well, that is $T=f(t)$. The damage integral will likely have a different value at different points in tissue owing to their different time-temperature history, so it must be evaluated at all spatial positions of interest. Finally, the result is very "end-point" dependent, in that the enthalpy change ΔH (and the entropy change ΔS) depend on what condition is taken as the final, thermally-damaged state. For example, thermal denaturation of proteins will have a lower ΔH than that of nucleic acids. There are variations even within similar structure types, for enzymatic proteins possess a lower ΔH for thermal denaturation than do structural proteins such as collagen. Nevertheless, a judicious

closed-form analytical solutions are available for any but the simplest cases, and numerical methods such as finite difference calculations are often invoked (Arapaci 1962).

An interesting and important thermal effect in laser medicine is known as selective photothermolysis, or SP (Anderson and Parrish 1983). It is a scheme of using the optical and thermal properties of the tissues to achieve target selectivity without the need for precise micro-focusing of the light. The idea is to generate and confine heat at certain absorbing targets while limiting the thermal diffusion to surrounding tissues. This has been used clinically primarily in dermatology, for example to treat post-wine-stain lesions, remove tattoos, and cause melanosome specific injuries in skin. Two criteria must be met for SP to succeed: the wavelength of the incident radiation must be chosen such that the target absorption is greater than that of the surroundings, differing by about a factor of two or greater; and the pulse duration must be sufficiently short so that negligible thermal conduction takes place during irradiation. The appropriate time scale is defined by introducing the concept of the thermal conduction relaxation time, which can be defined as "the time required for the central temperature of the Gaussian temperature distribution with a width equal to the particle diameter to decrease by 50%" (Anderson and Parrish 1983). Alternatively, Hu and Barnes (1970) speak of a thermal relaxing time as the time for the temperature at the center of the hot sphere immersed in an infinite medium to relax to e^{-1} times its initial maximum value. Within a geometry-dependent constant, both definitions imply that t_c is equal to the square of the target's characteristic dimension divided by the thermal diffusivity. Thermal diffusivity is defined as $\alpha = k / \rho c$ ($\text{cm}^2 \text{s}^{-1}$). Since this quantity is not very well known for tissue, and is generally dependent on the overall water content, the thermal diffusivity of the target is taken to be approximately equal to that of its surroundings. Thus, there has been no need to specify which α determines t_c ; however, if the

application of the damage integral formalism can result in successful predictions and explanations of the laser-induced thermal damage.

Often, the starting point of thermal modelling in laser biomedicine is the prediction of the spatio-temporal temperature distribution, $T(r, t)$. Provided that heat conduction is the dominant mode of heat transfer *within* the tissue, the temperature function satisfies the thermal diffusion equation:

$$\rho \cdot c \cdot \frac{\partial T(r, t)}{\partial t} = k \cdot \nabla^2 T(r, t) + Q_s - Q_l \quad (3)$$

where ρ is the density (g cm^{-3}), c is the heat capacity ($\text{J g}^{-1} \text{K}^{-1}$), k is the thermal conductivity ($\text{J s}^{-1} \text{cm}^{-1} \text{K}^{-1}$), Q_s is the source term ($\text{J s}^{-1} \text{cm}^{-3}$), and Q_l is the loss term ($\text{J s}^{-1} \text{cm}^{-3}$). In the medical context, the source could be a resistive heater or a hot-tip optical fiber, and the loss may be energy removed through water evaporation or convective heat transfer *via* blood flow. At times, it is possible to represent the gain and the loss terms as boundary conditions, resulting in a significant simplification.

Despite the apparent simplicity of Eq. (3), the solution is generally quite complex. The irregular geometry of the tissue sample and the spatial dependence of the Q_s and Q_l terms can cause major difficulties. In addition, there may be temporal effects, for example in the source term if the optical properties of tissue change during light irradiation (Jaywant *et al* 1993). The temperature dependence of the thermal "constants" may need to be considered (Valvano and Chitsabesan 1987) if significant temperatures are reached, if a phase change takes place, or if a very accurate model is required. As a result, very few

two are different, one should probably use the α of the surroundings. This effect has not been investigated in SP.

It is also important that the temperature of the target does reach the appropriate levels to do the necessary damage to the target, but it should not be allowed to extend much higher, for then the net amount of thermal energy deposited may do damage to the surrounding tissue as well as to the target. Also, mechanical disruption may take place, with undesirable side effects to the surrounding material. Finally, at very high temperatures (say, $> 1000 \text{ K}$), radiative mode of heat transfer may become significant, and conduction-based heat confinement approach may break down (see Paper #3). It may also be that, for some targets, there is no appropriate temperature range that is high enough to do the required target damage, but is low enough to inhibit the initiation of these surrounding processes. In such case, a train of successive pulses may be successful, with pulse duration, repetition rate and energy per pulse suitably optimized.

The state of knowledge of the thermal properties of tissues is not very extensive, especially *in vivo* (Bowman *et al* 1975). Most attention has been paid to specific heat, thermal conductivity, and thermal diffusivity, since the dominant mode of heat transfer in tissues is by conduction. The state of hydration of the tissue plays a large (often dominant) role in determining these properties, partly due to the relatively high specific heat of water. Some measurements of the convective heat transfer coefficient for tissue / air boundary (Colin and Houdas 1967), as well as the radiative grey-body emissivity of some tissue types, has been reported (Steketee 1973).

II.3. Light Propagation in Tissue

In keeping with the particle-wave duality of EM radiation, two corresponding mathematical constructs have been used to model light propagation in scattering and absorbing tissue-like media. The wave description relies on Maxwell's equations and is known as multiple scattering electromagnetic theory; the particle analysis makes use of the Boltzmann transport equation and is called radiative transfer theory. Although the former approach is fundamentally correct (Ishimaru 1978), it has yet to find application in tissue optics because of the stochastic variation in the electrical permittivity of tissue, which is difficult to determine, and which introduces prohibitive complexity into the resultant solutions (Twersky 1970). The more usual particle approach of light propagation is in terms of transport of discrete photons which may be absorbed or scattered without loss of energy (see Patterson *et al* 1991a and references contained therein). Wave phenomena such as polarization, diffraction, and interference are ignored. Light that has been wavelength-shifted after the interaction (e.g., Raman scattering, luminescence emission) is usually neglected but can be modelled (Patterson and Pogue 1994). Electromagnetic interactions between neighbouring scatterers are usually not important (so-called dependent absorption and scattering), but can be incorporated into the formalism by modifying the single-particle properties predicted by Mie theory (Brewster and Tien 1982; Tien 1988; Ma *et al* 1990). Despite these restrictions, experimental data show that transport theory is applicable in many situations. The best method of solution of the radiative transport equation depends on the data available, the information sought, and the accuracy required. In general, exact analytical solutions are rare, and a number of numerical techniques and approximation schemes have been developed (Ozisk 1973; Hansen and Travis 1974; Duderstadt and Hamilton 1976; Patterson *et al* 1991a). In addition, the method of Monte Carlo simulation offers an

the phase function, a term of astrophysical origin. The mean cosine of the scattering angle (also called the anisotropy parameter) is the average value of $(\Omega' \cdot \Omega)$, and is represented by g . $g \rightarrow -1$ implies predominantly backward scattering, $g = 0$ represents scattering symmetric about the forward and backward directions (which may or may not be isotropic), and $g \rightarrow +1$ means that scattering is becoming more forward peaked. The measured scattering anisotropy for most biological tissues in the visible range falls into the forward-scattering regime, with a typical g -value range of -0.70 - 0.98 (Cheong *et al* 1990).

It is not surprising that very few exact solutions of the radiative transfer equation have been found, given its integro-differential nature. Fortunately, it is not always necessary to solve for the radiance. The quantity of interest in many biomedical applications is the amount of light passing through an infinitesimally small sphere of tissue independent of direction, divided by the cross-sectional area of the sphere. This radiant energy fluence rate is calculated by integrating the radiance over all solid angles

$$\Phi(x) = \int_{4\pi} I(x, \Omega) d\Omega \quad (6)$$

Unfortunately, integrating Eq. (4) over the solid angle does not result in appreciable simplification. Although several formal methods of solution of Eq. (4) exist, most require extensive mathematical manipulations and have found limited application. A fruitful practical approach is to obtain approximate analytical solutions to the full equation, or to make reasonable assumptions which will simplify the equation to one which can be solved analytically. As the diffusion approximation falls into the latter category, we will examine that approach in greater detail.

alternative route of light distribution calculation within the particle transport formalism (Prah *et al* 1989; Flock *et al* 1989).

The equation of radiative transfer in the time-independent form is:

$$\hat{\Omega} \cdot \nabla I = -(\mu_a + \mu_s) \cdot I + \int_{4\pi} \frac{\partial \mu_s}{\partial \Omega'} \cdot I \cdot d\Omega' + S \quad (4)$$

where $I = I(r, \Omega)$ is the radiance (energy per unit area per unit solid angle per unit time), r is the spatial coordinate, Ω is the solid angle, μ_a is the linear absorption coefficient that represents the probability per infinitesimal path length that a photon will be absorbed, μ_s is the linear scattering coefficient similarly defined, and S is the source. The equation arises from considering the radiant energy balance in an arbitrary volume of material. Thus, it equates the net change due to energy flow to (i) the loss due to absorption and scattering (first term on right hand side), (ii) the gain due to in-scattering from other directions (second term), and (iii) the gain due to a radiance source (third term). The angular function in the integrand, $\partial \mu_s / \partial \Omega'$, is the differential scattering coefficient, which is the probability per unit pathlength that a photon initially moving in the direction Ω' will be scattered to a new direction Ω (this is sometimes represented as $d\mu_s(\Omega' \rightarrow \Omega)$). It is usually assumed that scattering depends only on the angle between Ω' and Ω , and not on Ω' or Ω individually. Thus,

$$d\mu_s(\Omega' \rightarrow \Omega) = d\mu_s(\Omega' \cdot \Omega) \quad (5)$$

It is customary to define a new function by normalizing the differential scattering coefficient by the total scattering coefficient. The resultant version is, somewhat inappropriately, called

The family of techniques of which the diffusion approximation is a particular case is collectively known as the functional expansion method. The radiance is separated into an unscattered and a scattered component. The photons that have travelled a distance r in tissue satisfy Beer's law,

$$\Phi(r) = \Phi_0 \exp(-\mu_{tr} r) \quad (7)$$

where $\mu_{tr} = \mu_a + \mu_s(1-g)$ is the transport attenuation coefficient. The use of $\mu_s(1-g)$ incorporates the effect of directionally-dependent anisotropic scattering into the diffusion theory formalism (Wyman *et al* 1989), although Steinke and Shepherd (1988) argue that the use of $\mu_a + \mu_s$ may be preferable in some situations. The light that has scattered at least once can be expressed as an infinite sum of orthogonal functions. Legendre polynomials are most often chosen to describe the angular dependence of the radiance. If the expansion is terminated after two terms (an isotropic and a forward-directed term), there results the more tractable diffusion equation (Duderstadt and Hamilton 1976)

$$(D \nabla^2 - \mu_a) \Phi(x) = s(x) \quad (8)$$

where D , the diffusion coefficient, is given by

$$D = \left(3 \cdot [\mu_a + \mu_s(1-g)] \right)^{-1} \quad (9)$$

and $s(r)$ is the general source function. The resulting equation is much more amenable to analytic solution than the parent Eq. (4), but this simplification is achieved with some loss of accuracy. The basic assumption that lead to the termination of the Legendre expansion series after two terms is that the radiance is at most linearly anisotropic. This will be the case far from sources and boundaries, in a medium where scattering events are considerably

more probable than absorption events. However, even in the case with strong absorption, the fluence distribution can be somewhat corrected by the addition of the photons described in the uncollided term of Eq. (7) to the diffuse solution of Eq. (8). Nevertheless, it is best to employ the diffusion approximation in scatter-dominated media, such as most mammalian soft tissues in the red and near-IR portion of the electromagnetic spectrum.

Fuelled by the growing variety of biomedical laser applications, there has been considerable effort to measure tissue optical properties, preferably *in vivo*. A comprehensive compilation of the published optical properties of biological tissues at a variety of wavelengths has appeared recently (Cheong *et al* 1991). Generally, most biomolecules absorb strongly below 300 nm. In the visible, the strong absorbers are the hemoglobins and the melanins; flavins, carotenes, bilirubin and cytochrome aa₃ also display absorption, but these are usually present in smaller concentrations. Above ~ 1.3 μm , water absorption becomes dominant. The absorption spectra of a number of tissue constituents are shown in Figure 2. The spectral region between ~ 650 nm and ~ 1.3 μm is the most transparent, and has been dubbed the "therapeutic window" because of deep light penetration in this wavelength range. Typical values of μ_a are ~ 0.1 - 20 cm^{-1} . Note that, with the exception of melanin, most tissue chromophores display rather structure-rich absorption spectra, with strong spectral dependencies. On the other hand, the scattering properties of tissue do not show much spectral structure, other than a gradual decrease with wavelength. This is probably due to the presence of different sizes and refractive index variations in the tissue structures that cause the scattering, although it has never been determined quantitatively what these specific structures are. However, it is known from multiple scattering theory that size polydispersity and refractive index variations of a scattering species tend to wash out the fine structure of the spectrum (Bohren and Huffman 1983). Typical μ_s values for tissues throughout the

alteration is reversible in a nondestructive evaluation. From the point of view of medical diagnostics, the latter category is obviously preferable. This, and other factors discussed later, help explain the recent emergence of a class of nondestructive methods broadly known as thermal-wave techniques, and of attempts to apply them to the life sciences.

In a typical thermal-wave experiment, the disturbance that provides the information about the unknown material is due to the flow of heat generated by the absorption of incident energy (Rosencwaig 1980). If the incident energy flow is periodic, there results a modulated temperature profile in the material, having the properties of a critically-damped, propagating wave which has been called a *thermal wave*. On the other hand, a single-pulse incident disturbance will result in a single decaying thermal transient, but this scenario often bears the thermal wave designation as well (Mandelis 1987). There are several ways in which these waves can be generated, the two most common being (pulsed or intensity-modulated) optical or electron beams. Several techniques exist for detecting the resulting thermal oscillations in the material. A partial list includes gas-microphone photoacoustic detection (Rosencwaig 1980), optical detection of the thermoelastic deformation of the surface (Olmsted *et al* 1983), piezoelectric detection of the thermoacoustic signal (Hordvick and Schlossberg 1977), optical detection of the refractive index gradient (Ameri *et al* 1981), pyroelectric detection of the refractive index gradient (Power and Mandelis 1987), optical monitoring of the reflectivity change (Opsal *et al* 1983; Vitkin *et al* 1989), and IR measurements of thermal radiative emission (Kanstad and Nordal 1979; Tam and Sullivan 1983; Imhof *et al* 1984). The particular choice of the signal generation and detection technique depends on the type of material under investigation, and the type of information required.

visible are ~ 10 - 200 cm^{-1} , with scattering primarily biased in the forward direction (typical g -values ~ 0.70 - 0.98).

Figure 2. Absorption spectra of some common biomolecules, expressed in terms of the molar extinction coefficient, ϵ ($\epsilon = \log e \cdot \mu_a / C_m$, where C_m is the molar concentration of the chromophore) <From Anderson 1991>.

II.4. PPTR and Photomedical Diagnostics

A useful and informative approach for investigation of a material is to perturb it in a well controlled manner, and to monitor the effect of the induced disturbance. This is the basis of many experimental evaluation techniques, which can be generally grouped into destructive and non-destructive categories. As the name implies, the specimen is irreversibly altered as a result of a destructive measurement (directly as a result of the measurement *per se*, or indirectly as a result of pre- or post-experimental sample preparation), whereas the

The particular thermal wave technique employed for our biomedical studies was PPTR; some of the reasons for this choice are as follows. Since we were primarily interested in the interactions of optical radiation with tissue, optical excitation using a pulsed laser source was selected. For the detection scheme, the monitoring of the IR emission was chosen. This does not require mechanical coupling between the sample and the detecting element; does not rely on the specimen having a well-defined, smooth specularly-reflecting surface; allows fast (μs -ms) temperature measurement with a suitable IR detector and electronics; can be used to examine thick and opaque samples; and does not require special sample preparation either before or after the experiment. As well, some preliminary work (see Appendix A) has shown that some IR-transparent fibers can transmit PPTR signals, thus suggesting a possibility of PPTR applications in remote body organs where a direct line-of sight to the IR detector is unavailable. In short, PPTR seems a good choice from the thermal wave options for diagnostic studies in photomedicine, with potential *in vivo* applications in mind.

The PPTR technique uses radiant energy for both disturbing the material and for monitoring the created disturbance. In a typical PPTR experiment, a light pulse at the incident wavelength of interest impinges upon the sample surface, causing a particular subsurface fluence distribution dependent on the spectral absorption and scattering properties of the sample. If the time duration of the pulse is shorter than the thermal conduction relaxation time of the target, the term $\mu_a \cdot \Phi(x) / \rho c$ is the local temperature excursion at time $t=0$; the temperature field then evolves in accordance with the laws of heat transfer. For the majority of situations, heat conduction describes the thermal evolution adequately; if very large temperatures are involved (greater than several hundred degrees Celsius), radiative transfer within the medium may occur. The elevated temperature distribution in the

subsurface region causes an increase in the blackbody emission across the surface, which is measured with an IR detector viewing the central region of the irradiated spot on the sample surface. Generally, the aim is not to perform optical spectroscopy (thus, the incident probe wavelength is not scanned, but matched to a particular absorption band of a chromophore in the sample), but rather to characterize the spatial, temporal, and thermal dependence of the photo-generated disturbance from the temporal decay of the IR emission. This information may be of value in itself, or it may be used to estimate the incident optical distribution, or the optical and / or thermal properties of the sample.

The reason for the increasing interest in thermal wave techniques as a diagnostic modality in medicine can be understood by comparing its advantages and disadvantages with other medical characterization techniques. Generally, if one is interested in optical dosimetry, optical property determination, or optical imaging (Applied Optics 28(12) 1989; Applied Optics 32(4) 1993; Chance and Alfano 1993), differential diagnosis via fluorescence (Anderson-Engels and Wilson 1992) or the Raman effect (Bagara *et al* 1992), then photometric methods are the techniques of choice in most instances. These have been used for a long time in many other areas of science and technology (e.g., physical chemistry, material science, meteorology, astronomy, microscopy), and the expertise gained in these fields facilitated their adaptation to photobiology and medicine. Of course, the particular problems encountered in photomedicine - turbidity of many tissues, presence of membranes and boundaries, changes due to the cardiac, breathing, and circadian cycles, the ethics of *in-vivo* experiments, the effects of the physiological response, and so on - have necessitated much additional research to ensure successful adaptation of optical technologies. Although a great deal has been accomplished, much work still remains (Parrish and Wilson 1991; Wilson *et al* 1993).

thick samples that are difficult for conventional photometric measurements. These characteristics imply that no special sample preparations or positioning are required in order to perform a PPTR experiment.

Another potential area of interest arises from the thermal content of the PPTR signal. Instead of using the thermal domain information simply as a route to the optical interactions of the experiment, it is sometimes possible and beneficial to study the thermophysical properties of the tissue, their variation with depth, and the specimen's temperature history following the heating pulse. To date, PPTR has been used to determine the thermal diffusivity (Leung and Tam 1984) and thermal conductivity (Leung and Tam 1988), with some depth profiling capabilities (Egee *et al* 1985). PPTR has also been employed to monitor paint degradation (Imhof *et al* 1985), integrity of dielectric coatings (Hiller *et al* 1990), phase transitions in liquid crystals (Imhof *et al* 1989), photosynthetic energy storage in leaves (Kanstad *et al* 1983), and temperature of moving metallic surfaces (Loarer and Greffet 1992). Although this last application is similar to that of a thermal IR camera, the transient nature of the PPTR signal causes less problems with signal drift and stability. Recently, Imhof *et al* (1988, 1990) have reported wavelength-resolved PPTR detection schemes, although the utility of this information remains unclear.

Finally, an interesting thermal inversion scheme is currently being developed (Prah 1992; Jacques *et al* 1993; Milner *et al* 1994). It involves the conversion of the PPTR signal into the depth profile of the initial temperature field. That is, if we assume that the detected IR emission originates from the surface, then the signal, S , is proportional to the time-dependent course of the surface temperature $T(x=0,t)$. Is it then possible to obtain $T(x,t=0)$ from this information? With involved mathematics, these studies seem to answer in the

There are, however, some tissues types which preclude successful applications of photometric spectroscopy. Let us define the product of the optical absorption coefficient and the characteristic geometrical dimension of the sample as the optical absorption thickness, $\tau_a = \mu_a \cdot d$. If the value of τ_a is high (say > 10), then the amount of light transmitted through it, or reflected from it (barring a very high value of the scattering coefficient) will be rather small, and the corresponding measurements difficult if not impossible. In other words, most of the light is absorbed by the sample, and, with the possible exception of radiative deexcitation via luminescence (fluorescence or phosphorescence), its utility for optical diagnostics has been lost. However, the most common fate of the absorbed radiant energy in condensed matter is its vibrational and rotational thermalization with the host lattice, which manifests itself as heat. This is precisely the regime where a photothermal technique such as PPTR is appropriate, due to its ability to detect the resultant temperature rise. In a way, then, photometric and thermal-wave techniques are a complementary diagnostic pair - energy detected by one is not utilized by the other, and *visa versa*. Of course, if light is used to initiate a photochemical reaction, or to cause a mechanical disruption, then heat generation is no longer the only energy de-excitation pathway, and the above complementarity no longer exists. Thermal-wave diagnostics can still be used to examine the thermal effects, and can even be quantified to the point of deducing the non-radiative quantum efficiency of a particular chromophore (Rosenzweig 1980).

Thus, a major niche for PPTR in medical science is the study of optical absorption in optically thick materials and quantifying what, if any, is the effect of optical scattering. Other useful features of this technique include the relative insensitivity of the measurement on sample alignment, the convenience of remote sensing, *retro*-observation geometry (i.e., detector on the same side of the sample as the source), and the ability to work with optically

affirmative. The method works on the physical principle that correlates the depth of a given emitter of heat with the time of the corresponding surface temperature rise as the thermal wave travels to the surface. For example, $S(t=0)$ is due to $T(x=0)$; $S(t=t_1)$ now contains additional contributions from $T(x = \{\alpha_1 t_1\}^{1/2})$, and so on. The accuracy and the maximum depth with which the internal thermal profiles can be determined are presently being evaluated.

Several biophysical and clinical applications of PPTR have appeared over the last decade. Beginning in the early 80's, Nordal and Kanstad (1981), and Bults *et al* (1982) performed optical spectroscopy on green leaves, and reported measurements on human skin using a frequency-domain version of PPTR. Long and Deutsch (1987) investigated optical transfer in normal and diseased human artery, and deduced the attenuation coefficients at several wavelengths in the near UV and visible spectrum. Optical dosimetry in tissue was estimated from the photothermal signal by Jacques *et al* (1987). In the same year, Long, Nishioka and Deutsch (1987) used PPTR to determine the optical attenuation coefficient and thermal diffusivity of opaque specimens of cholesterol and pigment biliary stones, illustrating the technique's unique ability to examine materials inaccessible to conventional spectrophotometric measurements. Also, remote sensing of layered absorbing structures (Long, Anderson, and Deutsch 1987), as applied to human skin, indicated the utility of PPTR for sensing buried absorbers. The sensitivity of PPTR to surface processes was utilized by Imhof *et al* (1990) to monitor *in vivo* skin properties and surface concentrations of topically-applied sunscreen preparations. With the current interest in laser angioplasty for blood vessel recanalization, Obrenski *et al* (1991) employed PPTR to study the optical absorption properties of vascular grafts, sutures, and thrombus. Recently, Jacques *et al* (1993) have used PPTR to study the heat deposition in port-wine-stain lesions and in normal human skin.

Finally, with the appearance of theoretical models applicable to turbid tissues (Anderson *et al* 1989, Prah1 *et al* 1992) it has become possible to quantify the absorption *and* scattering properties of interrogated specimens, as will be presented in this thesis (Vitkin *et al* 1992; Vitkin *et al* 1994).

To summarize, PPRT diagnostics may prove useful in medicine and biophysics for optical and thermal characterization of absorbing tissues which may not be amenable to other characterization techniques. Most of the work in this thesis has been aimed at extending the technique further than has been done previously. The major issues have been the presence of scattering in a homogeneous medium (Paper #1), extension to a layered system (Paper #2), and the possible role of radiative heat transfer in a discretely absorbing medium (Paper #3). Applications of the developed models *in vivo* (Paper #4) and to intact biomolecules (Paper #5) have also been examined. Finally, the current state-of-the-art in PPTR tissue research has been summarized in a book chapter (Paper #6), which echoes some of the comments of this section and discusses the theory and the instrumentation in some detail.

Paper # 1: Prah1 S.A., Vitkin I.A., Bruggemann U., Wilson B.C., and Anderson R.R. (1992). Determination of optical properties of turbid media using pulsed photothermal radiometry. *Physics in Medicine and Biology* 37, 1203-1217.

This paper describes an experimental and theoretical study of PPTR in homogeneous, semi-infinite, turbid media. We were able to derive a PPTR model that includes the effects of scattering, and went on to extract the absorption and scattering coefficients from PPTR spectra of a series of tissue-simulating gel phantoms. *In-vivo* applications of this approach were examined in paper #4. The extension of the theory to a layered case was addressed in paper #2.

The experimental work was carried out at the Wellman Laboratories of Photomedicine, Harvard Medical School, as a collaborative investigation. I joined the collaboration shortly after the start of my Ph.D. studies, conducted all the experiments and the data analysis reported, and derived most of the theory together with Dr. Scott Prah1, the paper's first author (it was his suggestion that a closed-form diffusion theory formulation can be used as a source term in deriving the PPTR signal from a scattering medium). The experimental tasks were greatly simplified by the skilful assistance of Mr. Ulrich Bruggemann, who had assembled most of the PPTR apparatus, wrote the data-collection and conversion software (later modified by Dr. Prah1), and performed some preliminary calibration measurements. The manuscript was written by me, with subsequent augmentations by other authors.

CHAPTER III. Papers

(each preceded by an introduction; copyright permissions in Appendix E)

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Determination of optical properties of turbid media using pulsed photothermal radiometry

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Abstract. Pulsed photothermal radiometry (PPTR) measures blackbody radiation emitted by a sample after absorption of an optical pulse. Three techniques for obtaining the absorption coefficient of absorbing-only, semi-infinite samples are examined and shown to give comparable results. An analytic theory for the time dependence of the PPTR signal in semi-infinite scattering and absorbing media has been derived and tested in a series of controlled gel phantoms. This theory, based on the diffusion approximation of the radiative transport equation, is shown to model the time course of the detected signal accurately. Furthermore, when the incident fluence is known, the theory can be used in a non-linear, two-parameter fitting algorithm to determine the absorption and reduced scattering coefficients of a turbid sample with an accuracy of 10-15% for transport albedos ranging from 0.42-0.88.

1. Introduction

Pulsed photothermal radiometry (PPTR) is a relatively new optical technique based on remote measurement of the temperature changes resulting from absorption of a short light pulse. The temperature is determined by measuring the black-body infrared (IR) emission from the sample under study. By analysing the PPTR signal, information about the thermal or the optical properties of the sample can be obtained. PPTR has been used as a method for depth analysis and non-destructive evaluation of the subsurface thermal properties (Imhof *et al* 1984, Leung and Tam 1984, Tam 1985). In studies of laminated or layered materials, PPTR is used to determine changes in the thermal property of a particular layer (Cielo 1984, Balageas *et al* 1986). Thermal parameters of homogeneous materials have also been determined in samples with known optical properties. The internal distribution light is used as the thermal source, and an analytic expression for the heat diffusion is obtained (Tam 1987). The materials are often coated with a thin, optically absorbing layer so that the heat is deposited exclusively at the surface.

Our goal was to start with the sample's thermal properties and use the thermal signal to determine the absorption and scattering properties. This extends previous PPTR studies that only included absorption in their analysis. For example, successful investigations have yielded the absorption characteristics of biliary calculi (Long *et*

al 1987b), normal and diseased human arteries (Long and Deutsch 1987), and skin (Long et al 1987a). In this paper, we compare various methods for analysing the signal from an absorbing-only medium. One method requires a measurement of the incident fluence and the others use the shape of the temperature decay curve.

At most visible and near-infrared wavelengths, the optical properties of tissue have non-negligible scattering properties (Cheong et al 1990), and consequently, absorbing-only theories are inapplicable. Non-invasive methods for determining the optical absorption and scattering properties of tissues are of particular interest since this information has diagnostic and therapeutic value in photomedicine (Patchan et al 1991, Wilson 1991). Recently, Anderson et al (1989) have developed a model for evaluating the initial temperature rise in PFTR experiments on scattering and absorbing gels. Interpretation of PFTR signals required measurements of either the incident fluence and calibration factors, or the diffuse reflectance from the sample. In this paper, we derive a theory for the infrared emission from a scattering and absorbing (turbid) medium and test it using a series of turbid gel phantoms. We show that the results of Anderson et al (1989) are a special case of the new turbid medium theory. Furthermore, we give two new methods for obtaining optical properties of a turbid material—the first requires an additional measurement of the incident fluence and the second requires an additional diffuse reflectance measurement. Finally, we give evidence that the temporal shape of the PFTR signal is insufficient to determine the optical properties of a turbid material uniquely, and that an additional measurement is necessary to select the correct value.

We used aqueous gel phantoms with known optical properties to test our algorithms. Water was used as the base medium because its thermal diffusivity is well known and is nearly equal to that of most soft tissues (Bowman et al 1975). The techniques for purely absorbing samples had errors less than 10% and those for scattering and absorbing samples had errors of 10–15%.

2. Theory

The photothermal radiometric signal results from the induced temperature rise in the material caused by the absorption of an incident pulse of optical irradiation (Leung and Tam 1984). The optical properties of the material and the irradiation geometry determine the spatial distribution of the optical energy in the material. The medium is assumed to have uniform optical and thermal properties, and all the absorbed optical energy is assumed to be converted directly to heat. In reality, some fraction may be lost to photochemical or photomechanical processes, and these may need to be accounted for in the energy balance, depending on the irradiation wavelength and medium. The optical irradiation is assumed normal to the slab. The irradiation is uniform and sufficiently wide that radial conduction is negligible. The medium is assumed to be semi-infinite, both optically and thermally. The rest of this section follows the work of Leung and Tam (1984).

The one-dimensional heat conduction equation describes the temperature of the sample,

$$\frac{\partial^2 T}{\partial z^2} = \frac{1}{\alpha} \frac{\partial T}{\partial t} \quad (1)$$

where T is the temperature, α is the thermal diffusivity of the sample, t is the time and z is zero at the surface and increases into the material. If we assume negligible

where $f(x) = \exp(-x^2) \operatorname{erfc}(x/\sqrt{2})$, and $\operatorname{erfc}(x)$ is the complementary error function of x (Carslaw and Jaeger 1958). The signal in (8) decays monotonically with time at a rate that depends on the optical and thermal properties of the sample. The IR absorption affects the net infrared emission from the sample and is important for estimating the surface temperature. Specifically, the maximum signal that corresponds to the initial temperature increase at $t = 0$,

$$S(0) = (4\eta\alpha_D\sigma T_0^3) \left(\frac{E_{\text{inc}}\mu_a}{\rho c} \right) \frac{\mu_{\text{IR}}}{\mu_{\text{IR}} + \mu_a} \quad (9)$$

is affected by the relative sizes of μ_{IR} and μ_a . As discussed by Anderson et al (1989), when μ_{IR} is no longer much larger than μ_a , the last term in (9) is no longer unity and the detected signal is reduced accordingly. Furthermore, the signal also depends on the product $\mu_a\mu_{\text{IR}}$, and decreases as either parameter becomes small.

2.2. Homogeneous scattering and absorbing media

Many models of light transport in scattering and absorbing materials have been developed (Hansen and Travis 1974, Patterson et al 1990), varying from accurate numerical models to less accurate closed-form expressions. We shall use the diffusion approximation of the radiative transport equation because it retains the important features of light propagation in tissues (absorption, anisotropic scattering and mismatched boundary conditions) while remaining amenable to analytic manipulation. The diffusion approximation is generally accepted as accurate when scattering dominates absorption (Ishimaru 1978); however, when absorption dominates scattering, the diffusion solution reduces to Beer's law, and the diffusion approximation is again accurate. We proceed to derive the thermal response of a turbid medium assuming the diffusion approximation is valid for intermediate values as well, and will later experimentally test the accuracy of the derived result.

The solution for a one-dimensional, uniform, collimated beam normally incident on a semi-infinite optical slab is (Patterson et al 1990),

$$E(z) = A \exp(-\mu_{\text{eff}} z) + B \exp(-\mu_{\text{tr}} z) \quad (10)$$

with

$$A = \frac{E_{\text{inc}}(9 + 6k)\mu'_s D}{(1 + k\sqrt{4\mu_a D})(1 - 9\mu_a D)} \quad B = \frac{-2E_{\text{inc}}}{1 - 9\mu_a D}$$

where $\mu'_s = \mu_s(1 - g)$ is the reduced scattering coefficient, g is the average cosine of the scattering angle, $\mu_{\text{tr}} = \mu_a + \mu'_s$ is the transport coefficient, k is a semi-empirical constant that depends on the refractive indices of the sample and surrounding medium; $D = (3\mu_{\text{tr}})^{-1}$ is the optical diffusion constant, and $\mu_{\text{eff}} = \sqrt{3\mu_a\mu_{\text{tr}}}$ is the effective attenuation coefficient. Notice that, in the limit of negligible scattering ($\mu_s \rightarrow 0$), $A \rightarrow 0$, $B \rightarrow E_{\text{inc}}$ and $\mu_{\text{tr}} \rightarrow \mu_a$ in agreement with (6).

The PFTR signal for a source corresponding to (10) is

$$S(t) = C \frac{\mu_a}{\rho c} [A f(\mu_{\text{eff}}^2 \alpha t) + B f(\mu_{\text{tr}}^2 \alpha t)] \quad \mu_{\text{IR}} \rightarrow \infty \quad (11)$$

heat loss at the surface, we can use Green's functions and the method of images to solve (1) for the temperature in the sample (Carslaw and Jaeger 1958)

$$T(z, t) = \frac{1}{\sqrt{4\pi\alpha t}} \int_0^\infty T(z', 0) \left[\exp\left(-\frac{(z-z')^2}{4\alpha t}\right) + \exp\left(-\frac{(z+z')^2}{4\alpha t}\right) \right] dz' \quad (2)$$

where $T(z', 0)$ is the initial temperature distribution that depends on the optical and thermal properties of the material, and on the incident light fluence.

The measured radiometric signal is obtained by integrating the Stefan-Boltzmann law over all depths weighted by the infrared attenuation in the material,

$$S(t) = \eta\alpha_D\sigma\mu_{\text{IR}} \int_0^\infty [T(z, t) - T_0] \exp(-\mu_{\text{IR}} z) dz. \quad (3)$$

In this equation, T_0 is the uniform unperturbed sample temperature, η is the detector efficiency (incorporating both the sensitivity and collection efficiency of the detector), α_D is the detector area, ϵ is the emissivity of the sample, σ is the Stefan-Boltzmann constant, and μ_{IR} is the average absorption coefficient of the sample over the range of infrared wavelengths detected. This last quantity determines the depth into the sample from which contributions to the measured signal are obtained: if $\mu_{\text{IR}} \gg 1$, then most of the detected signal is radiated from points close to the surface; as $\mu_{\text{IR}} \rightarrow 0$, the signal disappears because the material is transparent in the infrared. If the temperature rise $(T(z, t) - T_0)$ is small, the bracketed quantity in (3) can be expanded using the binomial theorem. Neglecting terms of $O(T_0^2)$ and lower yields

$$S(t) = 4\eta\alpha_D\sigma\epsilon T_0^3 \int_0^\infty [T(z, t) - T_0] \exp(-\mu_{\text{IR}} z) dz. \quad (4)$$

If $\mu_{\text{IR}} \rightarrow \infty$, then (4) becomes

$$S(t) = 4\eta\alpha_D\sigma\epsilon T_0^3 [T(0, t) - T_0] \quad (5)$$

implying that the signal is a direct measure of the surface temperature increase.

2.1. Homogeneous absorber

The light distribution in a homogeneous absorber is described by Beer's law (Born and Wolf 1980). Assuming normal incidence,

$$E(z) = E_{\text{inc}} \exp(-\mu_a z) \quad (6)$$

where $E(z)$ is the energy fluence, E_{inc} is the light fluence at the surface (the irradiance reduced by the any losses due to specular reflection from the surface), and μ_a is the optical absorption coefficient at the incident light wavelength. If all absorbed light is converted to heat, the initial temperature distribution is

$$T(z, 0) = \frac{E_{\text{inc}}\mu_a}{\rho c} \exp(-\mu_a z) \quad (7)$$

where ρc is the volumetric specific heat of the sample. Substituting this distribution into (2), and using the resulting temperature field to solve (4) yields

$$S(t) = \frac{4\eta\alpha_D\sigma\epsilon T_0^3 E_{\text{inc}}}{\rho c} \frac{\mu_a\mu_{\text{IR}}}{\mu_{\text{IR}}^2 - \mu_a^2} [\mu_{\text{IR}} f(\mu_a^2 \alpha t) - \mu_a f(\mu_{\text{IR}}^2 \alpha t)] \quad (8)$$

where $C = 4\eta\alpha_D\sigma\epsilon T_0^3$ and we have assumed that the IR absorption coefficient is much larger than either μ_{tr} or μ_{eff} . When this is invalid the PFTR signal is given by

$$S(t) = C \frac{\mu_a\mu_{\text{IR}}}{\rho c} \left(\frac{A}{\mu_{\text{IR}}^2 - \mu_{\text{eff}}^2} [\mu_{\text{IR}} f(\mu_{\text{eff}}^2 \alpha t) + \mu_{\text{eff}} f(\mu_{\text{IR}}^2 \alpha t)] + \frac{B}{\mu_{\text{IR}}^2 - \mu_{\text{tr}}^2} [\mu_{\text{IR}} f(\mu_{\text{tr}}^2 \alpha t) + \mu_{\text{tr}} f(\mu_{\text{IR}}^2 \alpha t)] \right) \quad (12)$$

As in the case of a purely absorbing medium (8), the signal vanishes as either $\mu_a \rightarrow 0$ or as $\mu_{\text{IR}} \rightarrow 0$.

The effect of scattering on the PFTR signal is demonstrated in figure 1(a), which shows theoretical signal profiles derived from (12), for a fixed μ_a (47 cm^{-1}) and values of μ'_s ranging from 0 – 137 cm^{-1} . The corresponding fluence profiles, calculated from (10), are shown in figure 1(b). As scattering increases, the fluence near the surface increases and the initial temperature increases. This effect has been explained by Anderson et al (1989) using a heuristic argument based on the internal reflection of backscattered photons at the surface boundary. Their result was

$$E(0) = E_{\text{inc}}(1 + 2kR_d) \quad (13)$$

where R_d is the diffuse reflectance for the refractive index mismatched boundary condition. An equivalent result can be derived rigorously by evaluating (10) at $z = 0$

$$E(0) = A + B = E_{\text{inc}} \left[1 + 2k \left(\frac{\alpha'}{1 + 2k(1 - \alpha') + (2k/3)\sqrt{3(1 - \alpha')}} \right) \right] \quad (14)$$

where the transport albedo is

$$\alpha' = \frac{\mu'_s}{\mu_a + \mu'_s}$$

The quantity in the curly brackets is the diffuse reflectance as predicted by diffusion theory (Flock et al 1989).

Figure 1. (a) The effect of scattering on the PFTR signal. Theoretical profiles for a fixed $\mu_a = 47 \text{ cm}^{-1}$ and various μ'_s obtained using (12) and a fixed incident fluence. (b) The corresponding fluence distributions in the sample.

3. Materials and methods

The turbid samples were collagen gels (Knox Gelatine) whose absorption properties were determined by the concentration of an absorbing dye (Typan Blue, Sigma Chemicals) and whose scattering properties were determined by the concentration of added white polystyrene microspheres (Uniform Latex, Duke Scientific). The gel samples were prepared by combining varying amounts of a Typan Blue dye stock and/or a microsphere colloid with a melted gel solution. Addition of 10% formaldehyde raised the melting point of the gel several degrees by increasing crosslinking between the collagen fibres (Ashtrohan 1979). Uniform mixtures were obtained by vortex mixing with a variable-speed agitator. The mixtures were gelled in 3.5 cm diameter petri dishes. The absorption coefficient of the gels ranged from 20–144 cm⁻¹ at 627 nm. The absorption of the dye stock solution was measured with a spectrophotometer (Hewlett-Packard 8452A) before mixing and after the PFTR experiments (by melting the gels). The scattering properties of the microspheres were calculated using Mie theory (Bohren and Huffman 1983), assuming the manufacturer's data for particle size and refractive index. The refractive index of the gel was taken to be equal to that of water ($n = 1.33$). From the calculated scattering cross-section and anisotropy, quantitative dilution was used to create gels with reduced scattering coefficients ranging from 0–150 cm⁻¹ at 627 nm. Following irradiation, the samples were examined under an optical microscope for any changes resulting from laser irradiation; none were observed.

Figure 2 shows a schematic diagram of the experimental arrangement previously used by Anderson *et al.* (1989). The sample was placed in contact with a metal plate having a 10 mm diameter aperture. The excitation source was a coaxial flashlamp-pumped tunable dye laser (Candela SLL-500), emitting 1 μ s pulses at 627 nm. Output from the laser was focused into a three-meter length of 1 mm diameter silica-core multimode fibre. The emerging light beam was imaged with a standard 5x microscope objective onto the gel sample at an angle of 15° from the normal. This provided a nearly uniform ($\pm 10\%$) beam about 5 mm in diameter. The fluence incident on the sample ranged from 200–400 mJ cm⁻² per pulse.

Figure 2. Experimental set-up for PFTR measurements.

Table 1. Absorption values for absorbing-only samples assuming $\mu_{IR} = 1200$ cm⁻¹. The 'true' values were obtained spectrophotometrically. Column (A) was determined from the analysis of the peak height and the calibration curve information (9); the error is based on uncertainties in the peak height, μ_{IR} , the incident fluence and the calibration slope. Column (B) was determined using (15); the error is the standard deviation on the interval 0–180 ms. Column (C) was determined from the minimum in the response surface; the typical error of 10% is estimated from the surface topology. All values are given in units of cm⁻¹.

'True' μ_a	(A) Peak height	(B) Normalization	(C) Two-parameter
144	137 $\pm$ 23	147 $\pm$ 9	137 $\pm$ 14
117	125 $\pm$ 21	104 $\pm$ 7	104 $\pm$ 10
53	102 $\pm$ 17	92 $\pm$ 5	87 $\pm$ 9
70	73 $\pm$ 12	71 $\pm$ 4	71 $\pm$ 7
46	52 $\pm$ 8	49 $\pm$ 4	49 $\pm$ 5
23	30 $\pm$ 5	21 $\pm$ 6	21 $\pm$ 2

Figure 3. Typical PFTR signal from a homogeneous absorbing-only gel ($\mu_a = 40$ cm⁻¹). The incident fluence was 400 mJ cm⁻². Figure 4. Results using (15) to determine the absorption coefficient from an experimental PFTR profile at any t . The true absorption value is indicated by the horizontal line $\mu_a = 92$ cm⁻¹. The derived value (average over all times) is 92 ± 5 cm⁻¹ for $\mu_{IR} = 1200$ cm⁻¹ and 81 ± 9 cm⁻¹ for $\mu_{IR} = \infty$.

find the absorption coefficient of absorbing-only gels. These eliminated the need for an incident fluence measurement.

The first method will be called the normalization method, because the thermal signal is normalized by its initial value. Dividing the temporal signal in (8) by the initial value from (9) creates a quantity independent of incident fluence and other calibration factors

$$\frac{S(t)}{S(0)} = \frac{\mu_{IR} f(\mu_a^2 \alpha t) - \mu_a f(\mu_{IR}^2 \alpha t)}{\mu_{IR} - \mu_a} \quad (15)$$

The only unknown remaining is the absorption coefficient, which can be determined

The infrared emission from the sample was monitored at normal incidence using a 1 mm² liquid-nitrogen cooled HgCdTe radiation detector (New England MFC 12-1-8) placed at the focal plane of a germanium lens. The detection bandwidth was 3–12 μ m, thus capturing about 40% of the spectral emission emanating from the heated gels (Ozisk 1985). The germanium lens was used in a 1:1 conjugate ratio to provide a 1 mm² detector viewing area at the centre of the irradiated sample surface. For measurements of incident fluence, a known fraction of light exiting the fibre was diverted to an energy meter (Gentec ED200) by a beam splitter. The electrical response of the detector, after pre-amplification, was monitored with a 125 MHz digital oscilloscope (LeCroy 9400A), triggered from the output of a silicon photodiode (EG&G FND-100) which detected the stray light from the dye laser flashlamp. Typically, the sampling rate of the digital oscilloscope was 8 μ s/point, and infrared signals were recorded for 180 ms after the laser pulse. All reported measurements are for a single light pulse. The recorded signal was reduced to 200–400 points by averaging. The stability of the measuring apparatus was checked periodically by observing the signal from a neutral density filter (Schott NG-10).

For system calibration, we measured the detector voltage as a function of the temperature of a copper block coated with lampblack and heated by a resistive heater. The temperature was measured using a precision thermistor (Omega 44031) embedded in the block. For improved signal to noise ratio, lock-in detection was used, with the signal representing the difference between the thermal emission from the room-temperature optical chopper blade and from the heated copper surface. For temperature increases of 5–60 °C, the lock-in amplifier (Stanford Research Systems SR500) voltage was proportional to the copper block temperature, with a ratio of 6.3 ± 0.2 mV °C⁻¹.

4. Results and analysis

4.1. Absorbing gels

A typical thermal response of a purely absorbing gel phantom ($\mu_a = 40$ cm⁻¹) following a 1 μ s, 300 mJ cm⁻² laser pulse at 627 nm is shown in figure 3. A rapid temperature jump followed by a monotonic decay is seen. The initial temperature increase was moderate and in subsequent experiments ranged from 5 °C for the lowest absorbing gels to 15 °C for the highest absorbing and scattering gels.

The absorption coefficient of a purely absorbing gel may be determined from the initial signal, a fluence measurement and several calibration factors by using (9). The bracketed quantities in (9) are known from calibration. The infrared absorption ($\mu_{IR} = 1200$ cm⁻¹) was the average value of the infrared absorption of water weighted by the detector sensitivity. The specific heat of water per unit volume ($\rho c = 4.184$ J cm⁻³ °C⁻¹) was used for the gels. Using these values, PFTR measurements can be interpreted using (9). The results are shown in column A of table 1, with the errors determined from experimental uncertainties. The slight overestimation probably results from errors in measuring the laser spot size.

This initial-temperature method ignores potentially useful information at later times in the temperature signal. Furthermore, additional measurements are needed to interpret the results (calibration curve, fluence measurements). Two alternative approaches, which used the temporal response from the sample, were developed to

for any particular time t . Assuming a thermal diffusivity for the gels which is equal to that of water ($\alpha = 0.0013$ cm² s⁻¹), allows the absorption coefficient to be calculated as a function of time for each sample. Figure 4 shows results for a phantom with a true absorption coefficient of $\mu_a = 93$ cm⁻¹, assuming both finite and infinite values for the infrared absorption coefficient. Surprisingly, the derived values of μ_a are strongly affected by letting $\mu_{IR} \rightarrow \infty$, even though μ_{IR} is larger than μ_a by an order of magnitude in this example. The averaged μ_a values for several gels are displayed in column B of table 1.

The normalization method depends on accurate early-time measurements. To avoid this, a more general method based on a non-linear two-parameter fit was developed. The two-parameter method will also be used later in the more challenging turbid-media problem. Equation (8) can be rewritten to show explicitly that the signal only depends on the parameters K and μ_a .

$$S(t) = K \frac{\mu_a \mu_{IR}}{\mu_{IR}^2 - \mu_a^2} [\mu_{IR} f(\mu_a^2 \alpha t) - \mu_a f(\mu_{IR}^2 \alpha t)] \quad (16)$$

Here the scaling multiplier $K = 4\eta\alpha\sigma\epsilon T_{oc}^3 E_{inc}/\rho c$ represents a system calibration constant $4\eta\alpha\sigma\epsilon T_{oc}^3$, multiplied by a sample-specific factor $\epsilon E_{inc}/\rho c$. We again assume that $\alpha = 0.0013$ cm² s⁻¹ and $\mu_{IR} = 1200$ cm⁻¹. The residual for any parameter pair (K, μ_a) is defined as the sum of the squares of the differences between the calculated and measured values of $S(t)$. The minimum residual determines a unique pair (K, μ_a) and therefore the absorption coefficient. A non-linear least-residual search routine with the variables μ_a and K was written, using an algorithm described by Bevington (1969). The convergence criterion was a change in residuals of less than 1 part in 5000. If the best-fit values are now used to calculate a residual for $S(t) \pm \Delta S$ (ΔS being the experimental uncertainty of the signal), then the range of parameter pairs with residuals between the minimum residual and the residual for $S(t) \pm \Delta S$ is an error estimate for the fitted values. For signal errors of $\Delta S = 3$ mV, the error in the fitted values is about 10%.

An example of a least-residuals calculation is displayed in the contour plot of figure 5 for a gel with $\mu_a = 70$ cm⁻¹. The minimum corresponds to $\mu_a = 71$ cm⁻¹. The inner-most contour approximately represents the $S(t) \pm \Delta S$ residual level; the possible range of μ_a is about 66–78 cm⁻¹, which is conservatively specified as $\mu_a = 71 \pm 7$ cm⁻¹. All residuals were calculated over a 3–125 ms time interval; the early times were avoided because of electrical noise from the laser; later times were discarded because the signal was dominated by thermal diffusivity and contains relatively little optical information (Leung and Tam 1984). The use of the full 0–180 ms interval gives (K, μ_a) minima that differ by only 3–4% from the windowed values. The 3–125 ms time interval was used for the results presented in column C of table 1.

The derived values of K (scaled to the appropriate fluence E_{inc}) can be used in the analysis of the turbid gel problem, since K/E_{inc} represents an experimental calibration factor common to all the phantoms. For the gels used in table 1, K/E_{inc} was nearly constant ($\pm 3\%$).

4.2. Absorbing and scattering gels

The PFTR signal for a scattering and absorbing gel is given by (12) and depends on K, μ_a, μ_s, α and μ_{IR} . If we assume constant values for the diffusivity α and

Figure 5. The residual surface used for the two-parameter fit of (16) to PFR signals from an absorbing-only gel ($\mu_a = 70 \text{ cm}^{-1}$). The location with the smallest residual is marked by an asterisk (μ_a, K) = (71 cm^{-1} , 0.593), and represents the best fit to the experimental data.

Figure 6. Theoretical PFR profile, using (12) and the derived optical interaction coefficients (line), and experimental data (symbols). The data are from a gel with $\mu_a = 40 \text{ cm}^{-1}$, $\mu'_s = 150 \text{ cm}^{-1}$; the derived values used to generate the solid line are $\mu_a = 38 \text{ cm}^{-1}$, $\mu'_s = 156 \text{ cm}^{-1}$.

μ_{th} , then the PFR signal only depends on three parameters. The dependence can be reduced to two parameters by using the calibration constant K/E_{inc} obtained from the absorbing-only gels and measuring the incident fluence relative to that used for those gels. The two-parameter algorithm discussed above can now be used for analysing the PFR signal from turbid gels, except that now (μ_a, μ'_s) is varied and (12) is used in place of (9) to find the residuals. In figure 6, the measured PFR signal is shown together with the theoretical prediction using (μ_a, μ'_s) obtained from the two-parameter fit.

The results for a range of turbid gels are displayed in table 2. The 'true' values for the absorption coefficient were determined spectrophotometrically and the 'true' reduced scattering coefficients were calculated from Mie theory. The relative errors between these values and those derived using the non-linear two-parameter fit are ~10–15% and are roughly equal for transport albedos ranging from 0.42–0.88 (figure 7). The small relative errors in the intermediate albedo range ($\mu_a \approx \mu'_s$) are especially encouraging, since present theoretical constructs are generally ineffective in this regime (Wilson 1991).

A sensitivity analysis revealed that the calculated signal $S(t)$ was most sensitive to changes in the absorption coefficient. This is perhaps not surprising: while the optical distribution is sensitive to changes in both μ_a and μ'_s , it is the absorption coefficient that governs the conversion of the optical fluence to heat, which is what is ultimately measured. Systematic changes in the thermal diffusivity and IR absorption coefficient, as well as in the time interval used for analysis, did not alter the location of the minimum residual significantly. Some changes in the μ'_s coordinate of the solution did occur, especially in response to thermal diffusivity variations. The μ_a coordinate of the solution was essentially unaffected. For example, changing the thermal diffusivity from 0.0013 to 0.0010 $\text{cm}^2 \text{ s}^{-1}$ shifted μ'_s by 15%, and μ_a by less

Figure 7. Percentage deviation (from true value) of μ_a and μ'_s derived from the analysis of turbid gels; see also table 2.

Table 2. Results of the two-parameter fit of (12) to PFR signals from various turbid media. Typical errors in the scattering and absorption coefficients of about 10% are estimated from the topology of the least-residual surface near the convergence point. All values are given in units of cm^{-1} .

	μ_a		μ'_s	
	'True'	'Derived'	'True'	'Derived'
20	21	100	102	
20	22	150	159	
40	40	50	51	
40	45	100	110	
40	38	150	156	
70	64	50	51	
70	65	100	99	
70	75	150	173	

than 5%. We thus conclude that the PFR signal is primarily dependent on μ_a , and is affected to a lesser extent by changes in the thermal diffusivity and μ'_s .

If the calibration constant K/E_{inc} is unknown then a three-parameter fit is an obvious choice for finding the three unknown parameters. Unfortunately, an arbitrary PFR signal does not yield a unique triplet (μ_a, μ'_s, K). For example, if the residuals for a fixed point (μ_a, μ'_s) are minimized by optimizing K , then a contour plot similar to figure 8(a) is obtained. The valley, which includes the true value, also permits a multiplicity of solutions (μ_a, μ'_s): each point corresponds to a different value for K . Physically, this implies that different optical distributions will yield similar PFR signals when irradiated by suitably chosen input fluences (figure 8(b)). Thus, it appears that the signal shape alone is insufficient to determine the absorption and the reduced scattering coefficients uniquely. Therefore, if a calibration coefficient is not available, an additional measurement is needed to determine the scattering and absorption coefficients of the material. A convenient additional measurement is the diffuse reflectance of the sample, which specifies the transport albedo and therefore

possibly be used to convert reflection values to transport albedos when the diffusion approximation is valid.

Figure 8. (a) Least-residual contour surface for a turbid gel. The true optical properties, $\mu_a = 20 \text{ cm}^{-1}$ and $\mu'_s = 100 \text{ cm}^{-1}$, are indicated by an asterisk. For each point (μ_a, μ'_s), K is optimized to obtain a minimum residual. No unique minimum residual exists, implying a multiplicity of acceptable solutions. (b) Two theoretical PFR profiles generated using two sets of triplets (μ_a, μ'_s, K_{opt}) from the minimum residual valley of figure 8(a).

5. Discussion

The time profile of the infrared response of a sample to a light impulse is governed by its optical and thermal properties: the former determine the distribution of the optical energy, while the latter dictate the spatial and temporal behaviour of the resultant temperature field. If the thermal characteristics are known, a photothermal technique such as PFR can be a useful non-invasive, non-contact method for probing the optical properties of turbid materials such as biological specimens.

Three methods for obtaining the absorption coefficients in semi-infinite absorbing-only gels are compared. In table 1 the accuracy of the initial peak and normalization methods is as good as that of the two-parameter fit method. This probably indicates consistent experimental technique for this set of absorbing samples. However, any noise in the early signal renders these approaches ineffective, and in such cases the more general and robust two-parameter fit must be used. Such a method does not depend more strongly on the early signals than on later signals, and therefore is relatively insensitive to noise in the early signal.

The PFR response from a scattering medium can be used to determine the absorption and reduced scattering coefficients when the scaled calibration constant K/E_{inc} is known, using (12). This ratio is available from the two-parameter fit analysis using absorbing-only gel phantoms. Alternatively, the ratio could have been determined from the system calibration information, the sample's volumetric specific heat and the incident fluence. However, if this ratio is unknown, some useful optical

information can be obtained from the PFR signal alone, simply by finding the optimum K value for each (μ_a, μ'_s). This approach selects many possible combinations of optical coefficients that lead to a similar minimum residual; additional information is needed to specify them uniquely.

A promising experimental strategy for this purpose is to measure the diffuse reflectance R_d . This measurement, together with the relative increase in the initial signal level, might serve as a check of the diffusion approximation used to derive (12). Such an increase in the initial signal level was observed experimentally, but an independent measurement of R_d would determine if the increase is in quantitative agreement with (13). More importantly, however, knowing the diffuse reflectance will provide the additional optical constraint that, in combination with the least-squares fit to the PFR signal, should lead to a unique pair of (μ_a, μ'_s) values. This would obviate the need to know the system calibration curve and the absolute incident fluence, both awkward measurements to perform accurately.

The turbid medium PFR signal analysis depends on the applicability of the diffusion approximation to the material under study. This is not a restrictive assumption for many homogeneous mammalian tissues (e.g., liver, brain, breast) in the red and near-infrared (600–1110 nm). In this region scattering events are much more probable than absorption events, and the diffusion approximation is appropriate. Given that the optical diffusion model reduces to Beer's law in the limit of negligible scattering, the validity of our formulation can apply to absorption-dominated regions of the optical spectrum as well. This agreement has been verified for transport albedos ranging from 0.42–0.88.

6. Conclusion

Pulsed photothermal radiometry can be used to measure the optical properties of samples with known thermal properties. The analysis of the PFR signals from absorbing-only media has been extended to eliminate the need for the relatively difficult measurement of the absolute value of the fluence of the light pulse. These methods are accurate to about 10% for absorption coefficients ranging from 20–140 cm^{-1} .

An analytic theory for the time dependence of the PFR signal in semi-infinite scattering and absorbing media has been derived and tested in a series of controlled gel phantoms. In materials with scattering, either the incident fluence or another measurement characterizing the optical properties (e.g., diffuse reflectance) is needed to specify uniquely the absorption and scattering coefficients of a turbid medium. We have shown that when the incident fluence is known, the theory can be used in a non-linear two-parameter fitting algorithm to determine the optical properties of a turbid sample with an accuracy of 10–15% for transport albedos ranging from 0.42–0.88. Even in the absence of additional experimental information, the close agreement between theoretical profiles and experimental data suggests that the important physics of PFR in turbid media have been included in our model.

Optothermal techniques should complement purely optical methods because they are sensitive to absorbed radiation (higher absorption means a greater PFR signal). In contrast, diffuse reflection decreases as the sample absorption increases. A combination of diffuse reflectance and PFR measurements may prove useful for non-contact, non-invasive, *in vivo* measurement of the optical properties of scattering and absorbing tissues.

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Résumé

Détermination des propriétés optiques des milieux troubles à l'aide de radiométrie photothermique pulsée. La radiométrie photothermique pulsée (PPTR) mesure l'émission du corps noir issue d'un échantillon après absorption d'une impulsion optique. Les auteurs ont examiné trois techniques utilisables pour l'obtention des coefficients d'absorption d'échantillons uniquement absorbants et semi-infinis, et ont montré qu'elles coïncident à des résultats comparables. Ils en ont déduit une théorie analytique de la variation en fonction de temps du signal PPTR dans les milieux absorbants et diffusants semi-infinis, qu'ils ont testée sur une série de gels fantômes contrôlés. Les auteurs ont montré que cette théorie, reposant sur l'approximation de la diffusion de l'équation du transport radiatif, modélise exactement l'évolution au cours du temps du signal détecté. De plus, quant la fluence incidente est connue, la théorie peut être utilisée dans un algorithme d'ajustement non-linéaire à deux paramètres pour déterminer les coefficients d'absorption et de diffusion réduite d'un échantillon trouble avec une exactitude de 10 à 15% pour des albedos de transport s'étalant de 0.42 à 0.88.

Zusammenfassung

Bestimmung der optischen Eigenschaften dickflüssiger Medien mit Hilfe gepulster photothermischer Radiometrie.

Bei der gepulsten photothermischen Radiometrie (PPTR) wird die von einem schwarzen Körper emittierte Strahlung nach Absorption eines optischen Pulses gemessen. Drei Verfahren zur Ermittlung des Absorptionskoeffizienten von nur-absorbierenden, halb-unendlichen Proben wurden untersucht und führten zu vergleichbaren Ergebnissen. Eine analytische Theorie für die Zeitabhängigkeit des PPTR-Signals in halb-unendlichen Streu- und Absorptionsmedien wurde entwickelt und getestet in einer Reihe spezieller Gel-Phantome. Diese Theorie, die auf der Diffusionsnäherung der Strahlungstransportgleichung basiert, ist in der Lage, den zeitlichen Verlauf des nachgewiesenen Signals genau zu modellieren. Desweiteren kann die Theorie, wenn die einfallende Fluenz bekannt ist, in einem nicht-linearen zwei-parametrischen Anpassungsalgorithmus verwendet werden, um die Absorptions- und reduzierten Streukoeffizienten einer dickflüssigen Probe mit einer Genauigkeit von 10–15% für Transport-Albedos zwischen 0.42 und 0.88 zu bestimmen.

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This paper deals with the theory and practice of PPTR in a turbid, layered medium. Both the optical and the thermal solutions (for fluence distribution and surface temperature, respectively) are new. The PPTR measurements agree well with the predicted surface temperature profiles. The relative simplicity of the closed-form solutions makes for easy implementation of the model for future optical and/or opto-thermal dosimetry studies. The ability of PPTR to detect the presence of a sub-surface layer in the presence of scattering, and to estimate the spatially-varying optical properties has been illustrated and discussed. Further work is required to establish the parameter ranges over which this can be done successfully.

I decided that the extension of the work in paper #1 to the case of a layered medium was worth pursuing. I derived the theory and performed the experiments for this paper. Dr. Patterson made many helpful suggestion during the theory-derivation phase. Drs. Wilson and Anderson made suggestions and corrections to the manuscript.

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ANALYSIS OF LAYERED SCATTERING MATERIALS BY PULSED PHOTOTHERMAL RADIOMETRY: APPLICATION TO PHOTON PROPAGATION IN TISSUE.

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Abstract: A model of Pulsed Photothermal Radiometry (PPTR) based on optical diffusion theory is presented for a turbid, two-layer, semi-infinite medium containing a surface layer whose optical absorption and scattering properties differ from that of the underlying layer. Assuming one-dimensional geometry, we develop expressions for the depth-dependent fluence distributions and radiant energy density profiles, and for the time dependence of the PPTR signal. Experimental tests of the PPTR model in a series of layered phantoms of varying optical properties are described. The results of these tests are consistent with the model predictions.

Keywords: laser, optical diffusion, IR radiometry, two-layer medium

Introduction

Knowledge of the behaviour of light in scattering and absorbing media is essential in many fields of science and technology, such as astrophysics, oceanography, meteorology, microscopy, medicine, and dentistry. Within a medical context, the distribution of light in the various tissue layers can determine the success or failure of therapeutic interventions such as laser surgery or photodynamic therapy.¹ In diagnostic laser medicine, models of light propagation in tissue allow estimations of tissue optical properties, which may be used for treatment planning or may indicate physiological or pathological processes.^{1,2} In these cases, multi-layered tissues are often involved, and light dose estimates must use a model capable of describing photon transfer in optically layered media. At the wavelength ranges of interest (typically visible and near-infrared), tissues are optically turbid, i.e. they exhibit both absorption and elastic scattering of light.

In this paper we will consider the problem of an absorbing and scattering layer in intimate contact with a semi-infinite absorbing and scattering substrate, and derive an expression for the depth-dependent photon fluence as a function of the optical properties of the layer and the substrate, and the top layer thickness. The derivation is based on the diffusion approximation to the radiative transport equation.³ For completeness, we add the contribution of the uncollided flux to the diffusion theory solution in both layers. The resulting expression for the absorbed energy is used as a source term in the heat conduction equation to predict the temperature distribution in the sample, from which the increase in the infrared (IR) emission is derived. We then use pulsed photothermal radiometry (PPTR) in a series of well-characterized layered gel phantoms to test the validity of the derived optical and thermal distributions.

state diffusion equation is

$$-D \cdot \frac{d^2 \phi(x)}{dx^2} + \mu_a \cdot \phi(x) = s(x) \quad (1)$$

where $\phi(x)$ is the depth-dependent diffuse photon fluence (typical units: $J \cdot cm^{-2}$), $s(x)$ is the depth-dependent photon source ($J \cdot cm^{-3}$), μ_a is the linear optical absorption coefficient, and the diffusion coefficient $D = [3 \cdot (\mu_a + \mu'_s)]^{-1}$ where μ'_s is the reduced linear scattering coefficient, defined as $\mu'_s = \mu_s(1-g)$, with μ_s the linear scattering coefficient, and g the average cosine of the scattering angle. The use of μ'_s incorporates the effects of directionally-dependent anisotropic scattering into the diffusion theory formalism.¹⁴ Equation (1) is the time-independent version of the diffusion equation, which is valid here since the PPTR measurements, while time-resolved in the thermal domain, are effectively steady-state in the optical domain. In other words, the laser pulses are long compared to the time required to reach "photon equilibrium".

The solution to Eq. (1) for the model geometry depicted in Fig. 1 is dependent on the source term, $s(x)$. In the case of collimated beam irradiation at the $x=0$ boundary, the source term in the diffusion equation represents the distribution of the first-scattered photons. Note that the remaining collimated photons still contribute to the subsurface fluence, and will be included later. For normally incident collimated irradiance of magnitude Φ_0 (corrected for surface specular reflection), the source function in layer 1 is

$$s_1(x) = \mu'_{s1} \cdot \Phi_0 \cdot \exp(-\mu'_{t1} x) \quad (2)$$

PPTR is an optical and thermal characterization technique that employs a wide-band infrared detector to monitor remotely the transient thermal emission following absorption of an optical radiation pulse.^{4,8} The PPTR signal is proportional to the *product* of the absorption coefficient and the fluence distribution, which accentuates the change in the optical absorption across the layer boundary, as compared with photometric measurements (e.g., diffuse reflectometry), which generally depend on the gradient of the optical fluence only. The theory and applications of PPTR have been well described for homogeneous absorbing and homogeneous turbid media.^{4,5,7} In addition, PPTR signal behaviour has been studied in a two-layer system of different thermal properties,⁹ and with different optical absorption properties, but in the absence of scattering.¹⁰ The derivation here is the first two-layer PPTR theory which accounts for the presence of scattering in both layers and is, therefore, particularly relevant to tissue studies (e.g., skin, retina, blood vessels).

Theory

1. Photon diffusion in a two-layer, turbid medium

The geometry of the two-layer model irradiated with a wide, collimated beam is shown in Fig. 1. One-dimensional approximation is employed throughout the analysis. The governing equation for photon transport in the medium is that of radiative transport,^{11,12} provided that the wave nature of the electro-magnetic light field can be neglected (a common and generally accurate assumption for optical dosimetry in tissue¹³). Unfortunately, exact analytical solutions of this equation have been found for only a few special cases, and simplifying angle-independent approximations have been used by many investigators.^{12,13} If scattering dominates absorption in the medium, the photon propagation can be viewed as a diffusive process, and a simplified governing equation can be derived.^{3,13} This optical steady-

where $\mu'_s = \mu_s + \mu'_a$ is the transport attenuation coefficient. If the refractive index of layer 1 is not equal to that of the overlying medium (e.g., tissue irradiated from air will have an index mismatch of $n_0/n_1 \sim 1.0/1.4$), there will be some scattered photons reflected back into the medium at the $x=0$ surface. In this case, the inwardly directed diffuse photons include the fraction that has reflected at the interface back into the medium. The boundary condition that relates the flow of energy across the interface is

$$\phi_1 - 2 \cdot k \cdot D_1 \cdot \frac{d\phi_1}{dx} = 0 ; \quad x = 0 \quad (3)$$

where k is a parameter that depends on the refractive indices of layer 1 and the surrounding medium.^{15,16,17} For layer 2, the source term is also attenuated by passage through the top layer:

$$s_2(x) = \Phi_0 \cdot \exp(-\mu'_{t1} d) \cdot T \cdot \mu'_{s2} \cdot \exp(-\mu'_{t2} (x-d)) \quad (4)$$

where the parameter T is a function of the refractive indices of layers 1 and 2. A reasonable choice for this parameter arises from Fresnel equations for interface transmissivity, $T = 4n_1^2 / [n_1 \cdot (n_1 + n_2)^2]$ ^{18,19}, although some authors^{20,21} argue that a simpler expression, $T = n_2/n_1$, is appropriate because of the presence of the light velocity ($\sim 1/n$) in the definition of the photon fluence. In our modeling, we will assume a simple and realistic case of the two layers having the same refractive index, so that we will use $T=1$. The boundary conditions at the interface between the layers require that the photon current and the photon fluence be continuous.^{20,23}

$$D_1 \cdot \frac{d\phi_1}{dx} = D_2 \cdot \frac{d\phi_2}{dx} ; \quad x = d \quad (5)$$

and

$$\frac{\phi_1}{\phi_2} = F ; \quad x = d \quad (6)$$

Once again, for index-mismatched layers, there seems to be disagreement in the literature about the value of parameter F . A common choice is $F = (n_1/n_2)^2$ based on the argument that the angular flux density divided by the square of the refractive index is a conserved quantity.^{21,22} However, Keijzer²³ argues convincingly that in going from flux to fluence, the integration over the solid angle effectively removes the n^2 factor, and $F=1$. Since we will be modeling the situation with $n_2=n_1$, F will be set to unity. Eqs. (1)-(6) complete the formulation of the problem.

The complementary and the particular solutions of Eq. (1) with the source functions of Eqs. (2) and (4) can be written as

$$\phi_i(x) = A_i \cdot \exp(-\mu_{effi}x) + B_i \cdot \exp(+\mu_{effi}x) + C_i \cdot \exp(-\mu'_{ei}x) \quad (7)$$

where $\mu_{eff} = (\mu_a / D)^{1/2}$ is the effective attenuation coefficient, and $i = 1, 2$ is the layer subscript. Since the fluence has to remain finite as $x \rightarrow \infty$, $B_2 = 0$. The C 's can be determined from the particular solution of the diffusion equation, by substituting Eq. (7) into Eq. (1), and equating the coefficients of the $\exp(-\mu'_{ei}x)$ terms. We then add the contributions

(6). The algebra is laborious, but the three resulting linear equations can be written in a matrix form, $[A] \cdot [X] = [B]$. $[X]$ is the column matrix containing the unknowns A_1 , B_1 , and A_2 ; $[A]$ is a 3×3 matrix with the entries

$$\begin{aligned} a_{11} &= 1 + 2kD_1\mu_{eff1}, & a_{12} &= 1 - 2kD_1\mu_{eff1}, & a_{13} &= 0, \\ a_{21} &= -D_1\mu_{eff1}\exp(-\mu_{eff1}d), & a_{22} &= D_1\mu_{eff1}\exp(\mu_{eff1}d), & a_{23} &= D_2\mu_{eff2}\exp(-\mu_{eff2}d), \\ a_{31} &= \exp(-\mu_{eff2}d), & a_{32} &= \exp(\mu_{eff2}d), & a_{33} &= -F \exp(-\mu_{eff2}d). \end{aligned} \quad (12)$$

Similarly, the entries of the $[B]$ matrix are

$$\begin{aligned} b_1 &= -C_1 \cdot (1 + 2kD_1\mu'_{e1}), \\ b_2 &= C_1D_1\mu'_{e1}\exp(-\mu'_{e1}d) - C_2D_2\mu'_{e2}\exp(-\mu'_{e2}d), \\ b_3 &= -C_1\exp(-\mu'_{e1}d) + F C_2\exp(-\mu'_{e2}d). \end{aligned} \quad (13)$$

where C_1 and C_2 are the coefficients of the particular solution *without* the contribution of the uncollided flux (Eqs. (10) and (11) without the unity term in brackets). This 3×3 system of linear equations can be solved using Cramer's rule for determinants.

This completes the derivation of the optical distribution in a two-layer turbid system based on the diffusion approximation to the radiative transport equation. Before proceeding to derive the photothermal signal for this system, in Fig. 2 we show some representative fluence profiles for different sets of optical properties in the two layers, illustrating

from the collimated beam (i.e., the uncollided photons) to the coefficients thus determined. For layer 1, this additional term is

$$\phi_{coll1}(x) = \phi_o \cdot \exp(-\mu'_{e1}x) \quad (8)$$

Similarly, for layer 2,

$$\phi_{coll2}(x) = \phi_o \cdot \exp(-\mu'_{e2}d) \cdot T \cdot \exp(-\mu'_{e2}(x-d)) \quad (9)$$

Performing the above operations, we obtain

$$C_1 = \left(\frac{3\mu'_{e1}}{3\mu_{a1}-\mu'_{e1}} + 1 \right) \cdot \phi_o = \frac{2\phi_o}{3\mu_{a1}D_1 - 1} \quad (10)$$

and

$$C_2 = \left(\frac{3\mu'_{e2}}{3\mu_{a2}-\mu'_{e2}} + 1 \right) \cdot \phi_o \cdot T \cdot \exp(\Delta\mu'_{e2}d) = \frac{2\phi_o \cdot T \exp(\Delta\mu'_{e2}d)}{3\mu_{a2}D_2 - 1} \quad (11)$$

where $\Delta\mu'_{e2} = \mu'_{e2} - \mu'_{e1}$, and the unity terms in brackets delineate the contribution of the collimated beam.

To solve for the coefficients A_1 , B_1 , and A_2 of the complimentary equation (right hand side of Eq.(1) = 0), we make use of the three boundary conditions [Eqs. (3), (5), and

particularly the effects of scattering in the top layer. The effect of variable thickness of the top layer is also simulated. In order to approximate subsequent experimental conditions, we assume a refractive index mismatch of 1/1.33 at the $x=0$ boundary, corresponding to a air/water interface, and a matched boundary at $x=d$. The right ordinate axes of Fig. 2 show the product of the absorption coefficient and the depth-dependent fluence, $\mu_a \cdot \phi(x)$, since this quantity is of interest in the photothermal analysis to follow. Physically, it represents the spatially-dependent absorbed energy density (typical units: $J \cdot cm^{-3}$) deposited by the incident light beam. One interesting feature evident from the figure is that, while the fluence distribution is not a very sensitive marker for relative changes in the optical properties of the layers (exhibiting at most a change in the slope for the range of variables examined), the radiant energy density clearly emphasizes the optical property variations when they involve a change in the optical absorption coefficient across the $x=d$ boundary.

Although not directly pertinent to the following photothermal studies, it is also possible to derive the expression for photon current leaving the material at $x=0$. This quantity is called the diffuse reflectance, R_d , and is given as

$$R_d = -D \cdot \frac{d\phi}{dx} ; \quad x=0. \quad (14)$$

Using the expression for the photon fluence, this becomes

$$R_d = D_1 \cdot (\mu_{eff1} A_1 - \mu_{eff1} B_1 - \mu'_{e1} C_1) \quad (15)$$

where C_1 is again the coefficient of the particular solution. After some manipulation, this can be simplified to

$$R_d = \frac{\phi(x=0) - \phi_o}{2k} \quad (16)$$

This form of the expression for the diffuse reflectance has been used previously in PPTR studies^{6,7} to quantify the height of the initial temperature jump in response to a brief laser pulse (i.e., a pulse short enough that no significant heat loss occurs during its duration).

II. Photothermal radiometry in a two-layer, turbid medium

We now derive the PPTR signal from thermally homogeneous but optically layered media as above. The PPTR signal results from absorption of an optical radiation pulse which causes a transient temperature rise and a corresponding increase in emitted blackbody radiation. The radiant energy density distribution acts as the thermal source term (the initial temperature distribution) in the heat conduction equation, which is then solved to obtain the resultant spatially- and temporally- varying temperature field. The accompanying increase in the IR radiative emission is obtained via a depth integral of the emission-weighted fourth power of the temperature distribution (i.e., integrating the escape function of the IR flux over all depth). For a light pulse much shorter than the thermal conduction relaxation time of the target, the theory of PPTR for an optically and thermally homogeneous, semi-infinite, absorbing-only medium shows that^{4,5,7}

$$S(t) = K \cdot \mu_a \phi_o \cdot [\operatorname{erfc}(\sqrt{\mu_a^2 \alpha t}) \cdot \exp(\mu_a^2 \alpha t)] \quad (17)$$

where $\operatorname{erfc}(z) = 1 - \operatorname{erf}(z)$ is the complementary error function, and $\alpha = k/\rho c$ is the thermal diffusivity, with k the thermal conductivity, ρ the density, and c the specific heat. The factor K incorporates the effects of initial sample temperature, sample volumetric specific heat,

$$\begin{aligned} S(t) = K' \cdot [& \mu_{a1} A_1 \exp(\mu_{a1}^2 \alpha t) \cdot [\operatorname{erfc}(\frac{d}{2\sqrt{\alpha t}} + \mu_{a1}\sqrt{\alpha t}) - \operatorname{erfc}(\mu_{a1}\sqrt{\alpha t})] \\ & + \mu_{a1} B_1 \exp(\mu_{a1}^2 \alpha t) \cdot [\operatorname{erfc}(\frac{d}{2\sqrt{\alpha t}} - \mu_{a1}\sqrt{\alpha t}) + \operatorname{erfc}(\mu_{a1}\sqrt{\alpha t})] \\ & + \mu_{a1} C_1 \exp(\mu_{a1}^2 \alpha t) \cdot [\operatorname{erfc}(\frac{d}{2\sqrt{\alpha t}} + \mu_{a1}'\sqrt{\alpha t}) - \operatorname{erfc}(\mu_{a1}'\sqrt{\alpha t})] \\ & + \mu_{a2} A_2 \exp(\mu_{a2}^2 \alpha t) \cdot [\operatorname{erfc}(\frac{d}{2\sqrt{\alpha t}} + \mu_{a2}\sqrt{\alpha t}) \\ & + \mu_{a2} C_2 \exp(\mu_{a2}^2 \alpha t) \cdot [\operatorname{erfc}(\frac{d}{2\sqrt{\alpha t}} + \mu_{a2}'\sqrt{\alpha t})]] \end{aligned} \quad (18)$$

where A_1 , B_1 , C_1 , A_2 , and C_2 are defined above. As expected, for $(\mu_a, \mu_s)_1 = (\mu_a, \mu_s)_2$, Eq. (18) reduces to the optically homogeneous result analogous to Eq. (17).

In Fig. 3 we show representative predicted PPTR signal profiles corresponding to the optical fluence distributions of Fig. 2. Note the characteristic features of most PPTR profiles, namely the delayed rise observed after the initial peak and the overall trend of decreasing signal magnitude at later times. The absorption and scattering properties of both layers, as well as the thickness of the top layer all have a pronounced and complex inter-dependent effect on the signal shape (at a given constant value of thermal diffusivity). Thus, five independent free parameters are needed to specify the PPTR signal (provided the thermal diffusivity and the refractive index are known). In this preliminary study, we will for the most part restrict ourselves to forward modelling. Such an approach may be useful for its predictive value if the optical properties of the layers are known or assumed, for we can then predict the subsurface fluence, the energy density, and the PPTR signal. The problem of inverse fitting to determine the five free parameters will be examined in future work.

emissivity, and various experimental variables. To within a scaling factor, the signal in Eq. (17) describes the temporal dependence of the surface temperature following a brief laser pulse. Eq. (17) is valid for relatively minor temperature excursions (much less than the initial absolute temperature); also, it assumes that the mean IR absorption coefficient, μ_{IR} , is much larger than μ_a . The former assumption is quite often satisfied in practice (typical values: maximum temperature jump $\sim 15^\circ\text{K}$ for a room temperature sample of $T_o \sim 300^\circ\text{K}$). The latter assumption is generally dependent on laser wavelength and tissues properties. In fact, letting $\mu_{IR} = \infty$ may lead to some errors if detailed quantitative information is sought.^{6,7} However, water-based gels (simulating soft tissues) are used in this study, and water is strongly absorbing in mid-infrared ($\mu_{IR} \sim 1200 \text{ cm}^{-1}$ over the $\lambda_{IR} = 7.5\text{--}14 \mu\text{m}$ range of the detector, weighted by its spectral sensitivity), so the condition $\mu_{IR} \gg \mu_a$ is indeed satisfied in our study. Since the resulting simplification in the PPTR signal expression is significant, and the qualitative signal shapes are not substantially distorted, we will assume infinite μ_{IR} in the analysis that follows. In effect, this implies that the detector is monitoring the sample surface only, with no significant contribution from sub-surface heated regions, which is why Eq. (17) is identical in form to the expression for $T_{\text{surf}}(t)$. Methods for incorporating finite μ_{IR} values into the analysis have been described previously.^{4,7}

The PPTR signals in an optically-scattering, homogeneous, semi-infinite medium,⁷ and in a layered non-scattering medium with different absorption properties,¹⁰ have been examined previously. Both the addition of scattering and the presence of layers complicate the analysis because of the additional complexity of the radiant energy density terms. However, the modelling approach is the same as that leading to Eq. (17). Using the solutions of Eq. (7), we obtain

Experimental Methods

The samples consisted of a mixture of gelatin, absorbing dye, and scattering microspheres. They were prepared by combining varying amounts of Trypan Blue (Sigma Chemicals) and latex microspheres of uniform size (0.360 μm diam) (Duke Scientific) with a melted (Knox) gelatine solution. For additional mechanical strength and higher melting point, $\sim 1\%$ of a cross-linking agent (37% formaldehyde solution) were added to the mixture. Compared with the dye, the absorption properties of the other constituents were negligible. The absorption coefficient of the dye solution was measured with a spectrophotometer (8452A, Hewlett-Packard) before mixing; several non-cross-linked gels were melted and measured after the PPTR experiments to verify the absorption values. The scattering properties of the aqueous microsphere suspensions were calculated using Mie theory,²⁴ assuming the manufacturer's data for particle size, refractive index, and concentration. At 628 nm, the absorption coefficient of the final gels ranged from 5–80 cm^{-1} , and the reduced scattering coefficient from 0–120 cm^{-1} . The layered phantoms were formed by spraying liquid gel mixture onto a previously prepared and solidified gel substrate. Smooth thin layers of uniform thickness were achieved with a nebulizer (Model K, Mienhard Associates), operating at 350 kPa with nitrogen gas. Typically, a finished phantom consisted of a $\sim 50\text{-}\mu\text{m}$ film (layer 1) on a $\sim 5\text{-mm}$ substrate (layer 2). The thickness of the top film was measured in two ways: before the PPTR experiments, the phantom was viewed edge-on under an optical microscope. After laser irradiation, the gels were quickly frozen and thawed; this allowed the top layer to be peeled off, which was then placed on a microscope cover slip and again examined edge-on under the optical microscope. For some samples of similar optical properties in the two layers, the second method was the only one that could be used. The accuracy of the thickness measurements was estimated to be $\sim \pm 10\%$. In addition, the uniformity of the top layer thickness did vary at different locations on the phantom surface ($\sim \pm 10\%$), so that the overall uncertainty in the value of d is about $\pm 20\%$.

A schematic diagram of the PPTR apparatus is shown in Fig. 4. The output of a

coaxial flashlamp-pumped tunable dye laser (Candella SSL-500), producing 1- μ s long pulses at 628 nm, was focused into a 3 m length of 1 mm diameter silica-core multimode fibre, which served to homogenize the beam. The output face of the fibre was imaged to a spot about 5 mm in diameter, providing uniform irradiance ($\pm 10\%$) of 100-400 mJ $\cdot$ cm $^{-2}$ per pulse. The latter was measured by diverting a known fraction of light exiting the fibre to an energy meter (ED200, Gentec). A liquid nitrogen cooled HgCdTe blackbody radiation detector (MCP-12-1-8, New England) viewed the central 1 mm 2 zone of the irradiance spot through a 1:1 conjugate ratio germanium lens ($f/2$, 2.54 cm diameter). A band-pass germanium filter (7.5-14 μ m) prevented any unwanted visible or near-IR light from reaching the detector. The detector signal was amplified with a compensated 0-10 MHz dc-coupled preamplifier, and acquired by a 350 MHz digital oscilloscope (9420, Lecroy). The oscilloscope acquisition cycle was triggered by the output of a silicon photodiode (FND-100, EG&G) which detected the stray light from the dye laser flashlamp. All reported measurements are for a single light pulse. The PPTR signals from the oscilloscope were transmitted to a personal computer, and the recorded signal was reduced to 100-400 points by averaging. The detector and electronics were rigidly mounted in a 0.6 cm thick stainless steel box to increase mechanical stability and to shield from electromagnetic interference. The stability of the overall system was checked periodically by observing the signal from a neutral density filter (NG-9, Schott Glass Technologies Inc).

Results

The PPTR signal from a layered phantom with top layer properties (μ_a , μ_s') $_1 = 30$, 65 cm $^{-1}$, and bottom layer (μ_a , μ_s') $_2 = 70$, 110 cm $^{-1}$ is shown in Fig. 5. The top layer was peeled off after a freezing and thawing cycle, and its thickness was measured to be 90 ± 15 μ m. Also shown is the predicted signal profile, obtained using Eq. (18) with the above parameters. Both experimental data and model plot have been normalized to their respective peaks for ease of comparison. The qualitative agreement between the two curves is evident, although quantitative differences are present. For example, the model prediction falls $\sim 5\%$ below the

layers. For example, the presence of a delayed PPTR peak [as in Fig. 6(b)] is manifest only if $\mu_{a2} > \mu_{a1}$ and the top layer is no deeper than several hundred micrometers (depending on its optical and thermal properties). The presence of scattering in either or both layers confounds simple conclusions, sometimes washing out the absorption and resultant radiant energy density contrast, while at other times demarcating it further.

Discussion

The structure and pigmentation of tissues commonly encountered in laser photomedicine show considerable variability, and the assumption of spatial homogeneity of optical properties may be too restrictive. The optical model developed in this study examines the effects of these variations when they can be confined to two discrete layers in intimate contact with each other. The model may be of use in computing optical dosimetry in layered structures such as the skin, and pathological conditions such as pigmented lesions. Incorporation of the scattering properties into the analytical framework of the model will be of particular importance when analyzing scatter-dominated situations, such as most soft tissues in the red and near-infrared region of the electromagnetic spectrum. The relatively simple, closed-form expressions for the fluence distributions allow for easy inclusion of the scattering effects.

The derivation the PPTR signal from the resultant optical distribution offers a means of interpreting the time course of the heat propagation within surface regions. By virtue of its dependence on the *product* of the absorption coefficient and the fluence field, the shape of the PPTR signal may reveal the presence of a buried structure where a photometric technique would be insensitive, for example in a scattering medium where both layers show appreciable absorption. The model can be used predictively by generating a set of PPTR curves of systematically varying absorption, scattering, and thickness in the two layer system; measured PPTR spectra may then be matched to the theoretical curves, providing

experimental signal for the first 80 milliseconds, indicating perhaps a slightly greater than predicted radiant energy density close to the surface. If the interface at $x=d$ is not truly continuous (both optically and thermally), for example due to minute air bubbles trapped during the deposition of the top layer, this may explain the increased signal magnitude at these early times (although not at very early times, e.g., for $t < 5$ ms). This possibility was tested by examining a phantom in which both layers were made from the same gel stock, and thus had identical optical properties. Its PPTR signal agreed well with the model predictions for a homogeneous turbid medium, without any noticeable discrepancy at early times, and no delayed peak corresponding to the location of the boundary. However, it is not possible to rule out the possibility that layers of different optical properties, synthesized as they are from different stock materials, possess a discontinuity at the interface *ipso facto*. The addition of the microspheres may also cause a slight drop in the ρ_c value of the composite phantom (about 1-3%, as $\rho_{c_{\text{microspheres}}} \sim 0.35\rho_{c_{\text{H}_2\text{O}}}$, and they account for $< 5\%$ of the phantom volume), increasing the signal as observed.

The effect of varying the thickness of the top layer is shown in Fig. 6. Again, each data set has been normalized to its peak value for ease of comparison. The graphs display general agreement between the model and normalized experimental data. This provides a particularly good test of the model since the curves exhibit major shape changes as a function of the thickness of the top layer: (a) a rounded peak at low d [Fig. 6(a)], due to the heat flow from the second layer before the signal due to the top layer had decayed; (b) a sharp peak followed by a delayed peak at medium values of d [Fig. 6(b)], which shows a delayed thermal wave from the buried layer arriving after the top layer signal has decreased; and (c) a featureless monotonic decay (similar to the PPTR from a homogeneous medium) at large d [Fig. 6(c)], indicating that the influence of the deeper layer is diminished (in fact, only the slight displacement of the initial peak to $t \sim 3$ ms suggests the presence of a layered structure). It is important to note that these features are not simply dependent on the thickness of the top layer alone; they are also influenced by the optical properties of both

an estimate of the spatial structure and optical properties of the layers. Such data can guide the planning of the treatment protocol, or be used as a diagnostic marker for a particular condition. The solution of the full inverse problem (that is, obtaining the optical properties of the two layers, as well as the interface depth) should also be possible in principle.

The good agreement between theoretical and experimental PPTR signals from layered, scattering media suggests that the diffusion theory model adequately describes the time course of the surface temperature. The normalized amplitudes were generally consistent with the model predictions, although more experimental data and more accurate fluence measurements are required to fully support this, and to further explore predicted results. The importance of the scattering process in determining the fluence distribution and the radiant energy density profile is evident from the simulations. If absorption does not dominate, and scattering is not highly anisotropic in the forward direction, the backscattered light will increase the sub-surface fluence, thus modifying the two characteristic aspects of the PPTR signal, namely: (1) the initial temperature rise in the superficial layer, which will increase because of the backscattered light, especially in the presence of the refractive index mismatch at the $x=0$ surface, and (2) the magnitude and arrival time of the delayed thermal wave due to the second layer, both of which depend on μ_{a1}' and μ_{a2}' in a complicated way. It is also apparent that the buried layer will not be detected via PPTR if it is situated more than a few hundred microns below the surface: the exact limit of depth detectivity is dependent on the optical and thermal properties of both layers.

There may be several reasons for the very slight disagreements between model predictions and experimental results seen in Figs. 5 and 6. In addition to the possible lack of intimate contact at the layer interface mentioned earlier, the consequences of the infinite μ_{R1} assumption may need further examination. Also, the top layers are optically thin (in the order of the transport mean free path), so that the photons may not be truly diffuse. A further possible source of discrepancy could be the validity of Eqs. (7) and (18). In the optical

regime where scattering dominates absorption, the photons are predominantly diffuse, and diffusion-based descriptions are certainly applicable. At the other extreme, the limit of negligible scattering is also well handled in the model through the inclusion of the uncollided flux in the two layers, so that Eq. (7) collapses to Beers' law as the coefficients A and B become zero.⁹ In the intermediate regime of comparable scattering and absorption, the situation is less clear. Although the fluence distribution, $\phi(x)$, predicted by diffusion theory may be inaccurate in this regime, it is the radiant energy density distribution $\mu_a \cdot \phi(x)$ that is convolved with Green's function solutions of the heat conduction equation to determine the temperature field, and the qualitative features of the temporal dependence of the surface temperature may still be described adequately. Quantitatively, the validity of the PPTR model based on the diffusion approximation has been verified in semi-infinite homogeneous phantoms in the range $\mu_a/\mu_s \approx 0.7 - 5.0$.⁷ Although not specifically examined in this study, the applicability of the derived PPTR model in the intermediate optical regime (one or both layers) deserves further investigation.

It is interesting to compare the theoretical predictions derived above to *in-vivo* experimental data available in the literature. As an illustration, Figure 7 shows a result from a recent study of port-wine-stain lesions at 577 nm wavelength,⁸ together with the model prediction from Eq. (18), assuming the following properties for the skin: $\mu_a \sim 8 \text{ cm}^{-1}$ (highly pigmented epidermis), $\mu_s' \sim 102 \text{ cm}^{-1}$, $\mu_s \sim 38 \text{ cm}^{-1}$ (assuming 10% dermal blood content), $\mu_a' \sim 45 \text{ cm}^{-1}$, $n_1 = n_2 \sim 1.37$,²² $\alpha \sim 0.00108 \text{ cm}^2 \text{ s}^{-1}$ (75% tissue water content).⁸ The depth of the port-wine-stain, d , was adjusted to obtain the best least-squares fit to the experimental data. The resultant value of d , 135 μm , is in good agreement with the approximate depth estimated by the authors, $\sim 150 \mu\text{m}$. As seen, the model predicts the important features of the measured signal reasonably well.

Conclusions

In this paper, we have developed a PPTR theory applicable to a scattering and absorbing

two-layer medium, and verified it by comparison with experimental results from a set of well-characterized gel phantoms. The model is based on diffusion theory approximation to the radiative transfer equation and, while the optical solution has been presented previously (Ref. 21, and reference therein), its extension to PPTR is new. Since PPTR is an absorption-based technique, it may prove useful in situations where conventional photometric techniques are restrictive, for example in non-invasive depth profiling of highly absorbing species in a turbid medium. Despite the simplifications inherent in the diffusion theory formulation, the optical and thermal models presented here should be useful for quantitative assessment of light and heat propagation in light irradiated tissue.

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Fig. 1. Geometry of the semi-infinite, 2-layer model.

Fig. 2. Predicted fluence distribution profiles (solid curves), showing (a-c) the effect of scattering in the top layer, and (d-f) the effect of top layer thickness. The right ordinate of each graph shows the corresponding radiant energy density profiles (dashed curves). All curves were generated with a refractive index mismatch at $x=0$ ($n_2/n_1 = 1/1.33$), and an index match at $x=d$ ($n_2/n_2 = 1$). The optical coefficients have units of cm^{-1} . Fig. 3. PPTR signal profiles obtained from Eq. (18). (a) PPTR signals for the fluence curves of Fig. 2(a-c). (b) PPTR signals for the fluence curves of Fig. 2(d-f). For these and subsequent model curves, the thermal diffusivity is equal to $0.0013 \text{ cm}^2 \text{ s}^{-1}$.

Fig. 4. Diagram of the experimental PPTR apparatus.

Fig. 5. Comparison of experimental and predicted PPTR signals for a two layered turbid medium.
 $d = 90 \mu\text{m}$.

Fig. 7. Comparison of theoretical model with *in-vivo* PPTR data, from Ref. 8. The data (points) are from a subsurface skin lesion known as port-wine-stain. The fit (curve) was generated assuming optical and thermal properties from Refs. 8 and 20 (see text). The data and the fit were independently normalized to their respective delayed peaks for ease of comparison (normalization to the $t=0$ peak was not used because of uncertainty in the data at early times).

Fig. 6. Experimental and theoretical PPTR signals as a function of the thickness of the top layer. Theoretical PPTR profiles are obtained from Eq. (18), with $(\mu_a, \mu_s)_1 = 30, 65 \text{ cm}^{-1}$, and $(\mu_a, \mu_s)_2 = 50, 90 \text{ cm}^{-1}$.

Paper # 3: Vitkin I.A., Wilson B.C., Anderson R.R., and Prahl S.A. (1994) Pulsed photothermal radiometry in optically transparent media containing discrete optical absorbers. *Physics in Medicine and Biology* 39, in press (this paper is included in galley proof form, so the journal page numbers will be different at publication).

This paper discusses PPTR studies in a medium with discrete absorbing centres, a so-called "hot-spot" model, which may be applicable in tissues such as skin or the pigmented choroidal epithelium in the retina. The localized temperature jumps during pulsed laser irradiation of these tissues may be very high. An unexpected but highly reproducible feature in PPTR spectra from a "hot-spot" gel model has lead to the intriguing suggestion that intra-medium radiant energy transfer may contribute to the cooling of the absorbing centres, in addition to the more usual conductive losses. The paper deals with this problem of simultaneous conductive-radiative transfer in a participating (i.e., absorbing and emitting) medium, both theoretically and experimentally. Although intra-medium radiation affected the PPTR signals, its influence on the internal temperature field was slight, at least for the range of examined conditions. In the context of laser medicine, however, it is possible that radiative heat transfer may become important when localized transient treatment temperatures reach still higher levels (~ thousands of degrees centigrade).

The interest in studying the details of optical and thermal transfer in particulate media using PPTR has existed at the Wellman Labs before I joined the collaboration. Subsequently, all the work for the paper was done by me, both the theoretical modelling and experimental verification. The manuscript was written by me, and edited by other authors.

continued on and off for about two years (and the debates are still continuing!).

Note: there is a lengthy addendum to this paper, containing material that was not crucial for submission to a journal, but may be appropriate in a more detailed examination such as this. It is presented in the form of footnotes, the corresponding locations in the manuscript being marked with a double-dagger (') symbol.

jumps in bulk media with uniformly dispersed absorption are limited to ~ 10 – 20°C , and radiative transfer need not be invoked (Tom *et al* 1982).

In this study, we have investigated a system whose PPTR signals differ greatly from those previously reported in the literature, namely one in which the optical energy absorption is localized at discrete centres within the medium, resulting in large localized temperature jumps. By suspending highly absorbing carbon-black particles in an optically transparent water-based gel, and irradiating the composite phantom with a brief laser pulse, the generated temperature differences between the absorbing particles and the transparent surrounding gel are shown to be at least several hundred degrees Celsius. Hence, radiative heat transfer influences the heat transfer dynamics within the medium, and affects the shape of the PPTR signal. Such models may be relevant, for example, to specific biomedical applications of pulsed lasers, such as ocular interventions involving the retinal pigment epithelium (Roider *et al* 1992) or the trabecular meshwork (Park and Lotina 1993), and melanosome targeting and tattoo removal in dermatology (Polla *et al* 1987, Vance *et al* 1985, Taylor *et al* 1990). These medical procedures are often optimized using the concept of selective photothermolysis (Anderson and Parrish 1983), which achieves target selectivity by using a preferentially absorbed laser pulse shorter than the thermal relaxation time of the absorber. This time is calculated based on heat conduction effects only. However, with very small discrete absorbers such as melanosomes or pigmented particles, extremely high spatially localized temperatures are produced following irradiation with sub-microsecond pulses. In this setting, it is important to understand the potential role played by thermal radiative transfer within the medium. The model developed in this paper is intended to examine this issue, and to enable quantitative PPTR investigations in cases where this role may be significant.

Our general theoretical approach is to model the spatio-temporal dependence of the temperature field, $T(\mathbf{r}, t)$, in a medium with both conductive and radiative heat transfer mechanisms. We initiate the calculations at the time immediately following the absorption of a brief laser pulse by the particulate absorbers. From $T(\mathbf{r}, t)$, we then calculate the IR emission from the medium as a function of time, and compare these results with PPTR signals measured experimentally by an IR detector viewing the centre of the irradiated area on the surface of the medium.

2. Theory

2.1. Basic equations

Analysis of combined transient conduction and radiation transfer following a brief laser pulse involves two coupled differential equations. First, at any point in the medium, energy conservation gives

$$\rho c \partial T / \partial t = -\nabla \cdot (-k \nabla T + F) \quad (1)$$

where ρ is the density, c is the specific heat, k is the thermal conductivity, t is the time, T is the temperature, and F is the thermal radiative flux (power per unit area). F can be obtained by integrating the radiative intensity distribution I (power per unit area per solid angle) over the appropriate direction in space:

$$F = \int_{4\pi} I \cos \theta \, d\Omega \quad (2)$$

Pulsed photothermal radiometry in optically transparent media containing discrete optical absorbers

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Abstract. A description of heat transport by conduction and radiation in inhomogeneous materials following absorption of a brief optical pulse is presented, and investigated experimentally using pulsed photothermal radiometry (PPTR). The model indicates that the role of radiation as an intramedium heat transfer modality increases with increasing temperatures and decreasing infrared (IR) absorption of the medium. However, for the range of conditions analysed in this study, conductive transfer dominates. Thus, the inclusion of radiation does not significantly perturb the internal temperature profiles, although it does influence the radiometric emission from the sample, and hence the PPTR signal. The thermal confinement effects described in this study may be relevant in photomedicine, for example in pulsed laser irradiation of tissues containing small absorbing targets.

1. Introduction

Pulsed photothermal radiometry (PPTR) is a remote sensing technique that uses an infrared (IR) detector to monitor the transient increase in thermal radiative emission induced by absorption of pulsed optical radiation (Tam and Sullivan 1983, Imhof *et al* 1984). Physical parameters such as optical absorption and scattering coefficients (Prahl *et al* 1992), thermal diffusivity (Leung and Tam 1983), and thermal conductivity (Leung and Tam 1988) can be obtained by analysing the shape of the emission-time curve. Because of its non-contact nature, relative insensitivity to geometrical alignment of the sample, and ability to examine opaque materials, PPTR has seen some biomedical applications (see, for example, Vitkin *et al* (1994a) and references therein). Typically, theoretical models of PPTR use conductive heat transfer to describe the evolution of the temperature field in the sample following the optical pulse. However, when the locally induced temperature excursions become significant (greater than several hundred degrees Celsius) thermal radiation becomes increasingly efficient as a mode of heat transfer, and the temperature field in the material may be influenced by its presence. Quantitative analysis of this effect is very challenging, involving simultaneous conduction and radiation heat transfer. In most circumstances, PPTR is primarily used as a non-destructive characterization technique, the transient temperature

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where θ and Ω are the polar and the solid angle, respectively. The intensity, in turn, is obtained from the solution of the radiative transfer equation (Chandrasekhar 1954, Ozisik 1973), an integro-differential equation that contains a temperature-dependent black-body term as a source:

$$\nabla \cdot \nabla I = -(\mu_a + \mu_s)I + \int \mu_s(\Omega' \rightarrow \Omega) I \, d\Omega' + \mu_a I_{\text{BB}}(T^4). \quad (3)$$

In (3), the term on the right-hand side represents the loss due to absorption and scattering, with μ_a the linear absorption coefficient of the medium and μ_s the linear scattering coefficient, both averaged over the (primarily IR) wavelengths of the radiative intensity distribution. The second term is the gain due to in-scattering, and the last term is the augmentation of intensity due to the emission of thermal IR radiation by the material in the direction Ω . For an ideal black body, this emission varies as the fourth power of absolute temperature when integrated over all energies; for a real emitter, the integration is more problematic because of the presence of absorbing and transmitting bands in its energy spectrum, detailed knowledge of which is generally inadequate. Nevertheless, the result is still taken to be proportional to the fourth power of temperature, with emissivity, $\epsilon < 1$, introduced to account for the decreased emission compared to the ideal (black-body) radiator. Thus, determining the temperature field in the material under the conditions of simultaneous conductive and radiative transfer requires solving the two coupled equations (1) and (3). Although some approximate solutions have been obtained (Ozisik 1973, Viskanta and Hirtleman 1978, Siegel and Howell 1988, Brewster 1992), the transient solution in an arbitrary geometry has proven very difficult. Thus, we simplify the formulation by assuming that the scattering of thermal radiation is negligible compared to absorption, a common and accurate assumption in systems where the scattering coefficient is smaller or even equal to the absorption coefficient (Goody and Yung 1989). This is certainly applicable to an IR absorber as strong as water and, hence, probably true of most soft tissues. Then, (3) predicts that the local spatial gradient of radiative intensity is equal to the difference between IR emission and absorption (the former depends on the local temperature; the latter depends on the thermal emission and, therefore, temperature of the surrounding medium). This treatment of the radiative term enables the numerical solution of the energy equation using a finite-difference formulation, as below.

For algebraic simplicity and clarity of presentation, we make a number of assumptions. Firstly, instead of working in three dimensions as suggested in the experimental case, figure 1(a), we assume that the incident light, and the conductive and thermal radiative fluxes, depend on depth in the medium only. This amounts to replacing the 'hot centres' of figure 1(a) with the 'hot planes' of figure 1(b), and assuming that heat transfer is in the x direction only. Secondly, as mentioned above, we ignore scattering since $\mu_a \gg \mu_s$ for tissue in the spectral region of greatest IR emission. Thirdly, we assume that most of the material properties (except for the thermal conductivity of water and the heat transfer coefficient at the medium-air interface) are temperature independent.¹¹

The method of solution consists of dividing the medium into a number of smaller slabs of thickness Δx , as in the classical finite-difference formulation. By virtue of its temperature, each element emits a specified amount of radiative energy, split equally into the positive and negative x directions. It also received radiant emission from all other elements on both sides, attenuated by the absorption in the medium by the factor $\exp(-\mu_a d)$, where d is the distance from the origin of the radiant flux to the element in question, and μ_a is the absorption coefficient of the medium. Note that we have also changed the subscript on the absorption coefficient to emphasize the fact that the absorption and emission phenomena

Figure 1. (a) A schematic diagram of the experimental phasmon containing discrete absorbing centres in an optically transparent medium, subject to wide-beam irradiation at the top surface. The dotted horizontal arrow denotes the central region whose thermal emission is monitored by the IR detector. (b) The corresponding one-dimensional geometry used in the theoretical model.

under discussion occur primarily in the IR region of the electromagnetic spectrum. Rewriting (1) in finite-difference form, we have the following relation for the temperature T at time t :

$$\rho c(T_i' - T_i)/\Delta t = (k/\Delta x^2)[T_{i-1} - 2T_i + T_{i+1}] + (1/\Delta x)[F_{ab}(i) - F_{em}(i)] \quad (4)$$

where $i = 1, 2, 3, \dots$ refers to the element number, Δt is the time increment, and T' is the temperature at time $t + \Delta t$. The net radiative flux emitted from the i th element is (Sparrow and Cess 1978, Al Abed and Sacadura 1983, Atalla and Hasan 1986)

$$F_{em}(i) = 4\pi[\mu_R(i)\Delta x\sigma T_i^4 n_R^2]/\pi = 4\mu_R(i)\Delta x\sigma n_R^2 T_i^4 \quad (5)$$

to the granule diameter, and its magnitude is T_0 . Thus, the initial temperature field has a picket-fence profile, with the absorbing centres accounting for the height and the location of the pickets. The location of the first picket and the repeat distance (corresponding to the percentage loading of the absorbing granules) are specified at the start of the simulation. Light scattering and heat loss during the laser pulse will influence the initial temperature field; these effects will be described later.

2.2. Numerical solution

The formulation presented above was simulated on a digital computer (IBM 486) using the values of the parameters summarized in table 1. As the volume content of the absorbing pigment was very small ($< 3\%$), the bulk properties of the medium were assumed to be equal to those of water. (4) was solved using an explicitly finite-difference scheme. This is best suited for predictions of early-time behaviour. Unfortunately, it is difficult for any given method to be well suited for both early-time and late-time calculations (Sutton 1986), but we are interested in signal dependence for all times. Thus, the time step chosen (viz. $1 \mu s$) was close to the maximum allowed by stability considerations (Gerald and Wheatley 1984, Arpaio 1962), and was confirmed by halving the time step and ensuring the solution results did not change. The slab was divided into 1000 evenly spaced elements (1001 nodes). In addition to the variables listed in table 1, the input to a particular simulation specified the location of the first hot centre (at position x_1), the relative abundance of the hot centres (a pigment layer every P elements), the size of the hot centre (Q elements), the magnitude of T_0 , and the value of μ_R . The element at position x_1 and $Q/2$ of its neighbours on both sides were assigned the temperature T_0 (see figure 1(b)). This procedure was repeated every P elements until $i = 1000$ was reached. All other locations in the medium had initial temperature T_0 . Then, the absorption matrix was calculated using (7) for $i = 1, 2, \dots, 950$, where we assume that events deeper in the medium will not be manifest at the surface. The value of N in that equation depends on the product $\mu_R \Delta x$, and was generally ~ 30 . It was selected so that the sum

$$\sum_{m=j-N}^N e^{-m(\mu_R \Delta x)} \quad (10)$$

was within 0.3% of its value when $N = \infty$. Then, (4) was solved throughout the medium for the temperatures at the next time step, the radiation escaping from the surface was tabulated as $S(t)$ according to (9), and the entire procedure repeated. As a consistency check, T_0 was put equal to T_0 for all elements, and the simulation executed for 5000 time steps. The temperature everywhere remained equal to T_0 within the double-precision limit of the computer.

Figure 2 shows an example of the thermal radiative emission from the sample surface (left ordinate), to be compared to the measured PPTR signals described later. Also plotted is the temperature at the centre of an absorbing layer at a position deep within the medium (right ordinate). Examining the signal behaviour first, we note the very sharp peak at early time (height ~ 321 units at $t \sim 1 \mu s$, FWHM $\sim 5 \mu s$) followed by a fast decrease, then a more gradual rise to a second peak at ~ 3 ms, and a slow monotonic decay thereafter. The last feature is reminiscent of conductive heat transfer as confirmed by numerous PPTR measurements (Tam and Sullivan 1983, Pahl et al 1992), and is probably indicative of the cooling of the entire layer once the extreme temperature gradients within it have been smoothed out. We postulate that the initial sharp peak is caused by the direct thermal radiation from the heated granules close to the surface, the extent of the depth contribution

where n_R is the infrared index of refraction of the medium surrounding the element in question. Similarly, the flux absorbed by the i th element is due to the emissions of all its neighbours:

$$F_{ab}(i) = \mu_R(i)\Delta x \sum_{j=-\infty}^{j=+\infty} F_{em}(j)e^{-\mu_R(j)|i-j|\Delta x} \quad j \neq i \quad (6)$$

where $\mu_R(i, j)$ is the spatially dependent absorption coefficient between nodes i and j . The use of different values of μ_R allows us to incorporate the different radiative properties of the particulates and of the surrounding media. However, since the samples examined in this study were $\sim 98\%$ water, and the IR properties of the particulate inclusions are assumed to be similar to it, we will take the IR absorption coefficient as constant throughout the medium for most simulations. An exception will be made when we examine the effect of the radiative properties of the medium, which are varied while μ_R of the inclusions is kept fixed (see the discussion concerning figure 5). Then, using the definition of the transmissivity function along a line of sight ab , $\tau(a, b) = \exp(-\mu_R[a - b])$, the last equation can be rewritten in a computationally convenient form as (Brewster 1992)

$$F_{ab}(i) = (\mu_R(i)\Delta x) \sum_{j=-N}^{j=i+N} 4\sigma T_j^4 n_R^2 [\tau(j, i) - \tau(j-1, i)] \\ = (\mu_R(i)\Delta x) \left\{ \sum_{j=-N}^i 2\sigma T_j^4 n_R^2 [\tau(j, i) - \tau(j-1, i)] \right. \\ \left. + \sum_{j=i+1}^{i+N} 2\sigma T_j^4 n_R^2 [\tau(j, i) - \tau(j-1, i)] \right\} \quad (7)$$

where $N = 0, 1, 2, \dots$ is the number of elements that separate the emission node j from the absorption node i . The resulting equations describe the simultaneous conductive and radiative transfer in the medium.

In order to complete the formulation, we need to specify the boundary and the initial conditions. At the sample-air interface, energy is transferred by convection and radiation. The instantaneous energy balance at the interface can be expressed as

$$k \frac{\delta T}{\delta x} = h(T_1 - T_a) - \left(\sum_{j=1}^N F_{em}(j) [\tau(j, 1) - \tau(j-1, 1)] - \sigma T_1^4 \right) \quad x = 0 \quad (8)$$

where h is the convective heat transfer coefficient, and T_1 and T_a are the sample surface and the ambient air temperatures, respectively. The radiative term includes the flux emitted from subsurface elements, attenuated according to their distance from the surface. This is more accurate than having the outgoing emitted flux simply originate at the surface ($i = 1$) element. The radiometric signal seen by the IR detector will be proportional to this term, that is

$$S(t) \sim \sum_{j=1}^N F_{em}(j, t) [\tau(j, 1) - \tau(j-1, 1)]. \quad (9)$$

The initial temperature, $T(x, t = 0)$, is assumed to be equal to T_0 throughout the medium, except at the location of the heated absorbing granules. There, its spatial extent is equal

Figure 2. (a) The predicted thermal radiative emission (left ordinate) and the temperature at the mid-point of a centrally located hot centre (right ordinate) as functions of cooling time: Initial hot-centre temperature, 3000 K; IR absorption coefficient, 1000 cm^{-1} . All other parameters used in the simulation are as in table 1. (b) An expanded view of the initial signal peak. (c) An expanded view of the initial temperature.

Figure 3. (a) The effect of neglecting intramedium radiative transfer. The conditions are the same as in figure 2, except that heat exchange is by conduction only. (b) An expanded view of the initial signal peak. (c) An expanded view of the initial temperature.

Figure 5. The effect of varying the medium absorption coefficient, μ_R , with conductive-radiative transfer. $T_h = 5000$ K, $\mu_R(\text{hot centre}) = 1000$ cm^{-1} .

Table 1. Parameter values used in generating figures 2-5. The thermophysical and optical properties are those of water.

Thermal conductivity, k	$\sim 0.0056 \text{ W cm}^{-1} \text{ K}^{-1} / (T/T_0)^2$
Density, ρ	1 g cm^{-3}
Specific heat, c	$4.184 \text{ J g}^{-1} \text{ K}^{-1}$
Surface heat transfer coefficient, h	$\sim 0.001 \text{ W cm}^{-2} \text{ K}^{-1} / (T_{\text{surface}})^{0.5}$
IR refractive index, n_{IR}	1.3
Ambient temperature, T_0	300 K
Initial medium temperature, T_0	300 K
Time increment, Δt	10^{-6} s
Element thickness, Δx	10^{-4} cm
Initial width of a hot centre	3 μm (three elements wide)
Relative abundance of hot centres	10% (three μm wide hot centre every 30 μm)
Initial hot-centre temperature, T_h	1000–5000 K
IR absorption coefficient of medium, μ_R	200–3000 cm^{-1}

* For $T < 600$ K, $k(T) = -5.39 \times 10^{-3} + 6.38 \times 10^{-5}T - 7.99 \times 10^{-8}T^2$ (least-squares fit to data tables of Weast and Astle (1983)). For $T > 600$ K, $k(T) = 0.006$.

* $h(T_0 < 400 \text{ K}) = 0.0006$; $h(T_0 > 400 \text{ K}) = 0.0008$; $h(T_0 > 500 \text{ K}) = 0.001$; $h(T_0 > 600 \text{ K}) = 0.004$, where T_0 is the temperature of the surface node (Weast and Astle 1983).

relatively slow (of the order of milliseconds) conduction process. What is interesting is the role played by intramedium thermal radiative transfer in the overall heat transfer process. The thermal history of the hot centre also shows the fast peak followed by a more gradual

Figure 4. The effect of varying the initial hot-centre temperature, T_h , with conductive-radiative transfer. (a) A comparison of IR emissions, with $T_h = 1000$ K (top) and $T_h = 5000$ K (bottom). (b) The temperature of a deeply located hot centre with and without radiative transfer, with $T_h = 1000$ K (top) and $T_h = 5000$ K (bottom). $\mu_R = 1000 \text{ cm}^{-1}$.

being dictated by the magnitude of the absorption coefficient μ_R . The appearance of the second peak is more puzzling, for it seems to suggest an arrival of a delayed thermal wave. In this model, such a wave cannot be due to scattering effects or layered structures, two instances that can cause a delayed PPTR peak (Vitkin et al. 1994b). We interpret it as indicating that the heated particles close to the surface have been quickly (of the order of microseconds) and efficiently cooled by radiative emission to the environment, whereas the emissions from the deeper layers have been reabsorbed in the medium. In such a scenario, the effective temperature of the deeper layers is higher than that of the radiatively cooled surface layers shortly after the pulse. This borne out by the fact that the centrally positioned granule is always at a higher temperature than the one close to the surface during the simulations. Thus, there results a time-delayed thermal wave arriving at the surface by a

monotonic decay. The abrupt transition from a fast-cooling to a slow-cooling regime (at $t \sim 20 \mu\text{s}$, and $T \sim 1660$ K) is due to the presence of the other hot centres in the vicinity of the particle; in their absence, the hot centre continues its fast monotonic decay (confirmed by simulations with only one hot-centre particle present).

In order to ascertain the role of radiative heat transfer in the cooling process of the hot centre, we repeated the simulation under identical conditions, with heat transfer occurring by conduction only. The radiative term was retained only near the boundary, where it was used to calculate the signal magnitude via (9). Figure 3 demonstrates the resultant signal and the temperature history of a central hot node. The signal and temperature profiles are qualitatively similar to figure 2, but differ in quantitative detail. Notice, for example, that the hot-centre temperature profile is slightly lower under the conduction-radiation conditions than under the conduction-only scenario. This is due to the additional heat lost by radiation. As for the signal comparison, there is a significant difference in the magnitude and in the arrival time of the delayed thermal wave. The conduction-only signal is generally higher and arrives later at the surface than if radiative transfer is included. This is probably because the temperature distribution in the sample with radiative transfer becomes uniform somewhat faster than by conduction alone. Thus the top layer, whose temperature is primarily responsible for the thermal IR emission, registers the presence of the deeper hot spots faster in figure 2 than in figure 3, and its second-peak signal and the hot-spot temperatures are lower (indicative of a more uniform temperature profile in the medium).

Figure 4 demonstrates the effect of the variable T_h on the IR signal. Since the magnitude of the thermal emission is highly dependent on temperature ($\sim T^4$), the radiative-emission signal is higher for the higher-temperature simulation. In fact, if we compare the peak values of figure 4(a), we find $(321/0.6)^{1/4} \sim 5$, as expected from the ratio of the initial hot-centre temperatures. Moreover, the thermal gradients in the medium become more pronounced with higher hot-centre temperatures, so the importance of radiative transfer in homogenizing the temperature distribution increases. This was evident from comparing temperatures of the hot centres in the absence of radiation for the two T_h cases (part (b) of figure 4): the radiation caused a larger increase in the cooling rate for higher initial temperature, although the effect was relatively minor in both instances.

Figure 5 shows the effects of varying μ_R of the medium. For these simulations, the IR absorption coefficient of the hot centres was kept constant at 1000 cm^{-1} to ensure that their thermal emission was constant, and the effects observed were entirely due to the IR absorption properties of the surrounding medium. The hot centre cools faster with decreasing absorption coefficient of the surrounding medium, indicating that more energy is lost to the surrounding medium/external environment via thermal emission. This is because a small absorption coefficient represents a large radiative interaction distance; i.e., as the medium becomes more transparent in the IR, radiative exchange between the emitting centres is less impeded by the intervening medium, and their temperature drops. On the other hand, large μ_R means short reabsorption distance, so the medium offers a large effective resistance to radiative cooling. It should also be noted that the simulation times increase significantly for small μ_R values, since more terms must be evaluated in the radiative emission sum (see (10)).

In general, the relative importance of the two modes of energy transport can be expressed by a non-dimensional quantity known as the radiation-to-conduction parameter, M_i . Consider two emitting surfaces at temperatures T_1 and T_2 , separated by a medium with properties k , μ_R , and n_{IR} . For a radiatively thick medium, M_i is given as (Ozisik 1973, Brewster 1992)

$$M_i = 4\pi\epsilon_0\sigma T_i^3 / k\mu_{IR} \quad (11)$$

where the subscript is based on a particular temperature T_i (either T_1 or T_2). For $M_i \rightarrow \infty$ radiative energy transfer dominates (radiation alone establishes the temperature field), and for $M_i \rightarrow 0$ conduction dominates. In figures 2-5, M_i ranged from ~ 0.007 to ~ 1.3 , with the most common set of examined conditions ($T_b = 5000$ K, $\mu_{IR} = 1000$ cm $^{-1}$) yielding $M_i \sim 0.9$. As (11) and figures 4 and 5 demonstrate, the importance of the radiative contribution increases with increasing temperatures and decreasing IR absorption. (In analogy with thermal conductivity, radiative conductivity can be defined for radiatively thick media, $k_{rad} = 16\pi^2\sigma T^3/3\mu_{IR}$ (Ozisik 1973), which summarizes the importance of temperature and IR absorption coefficient for effective radiative contribution to heat transfer). Likewise, the role of conduction diminishes with decreasing values of thermal conductivity.

Finally, we examine the temperatures and the IR emission when thermal transfer occurs in three dimensions, using Cartesian coordinates (x, y, z), in order to ascertain whether the simplified 1D representation is adequate for comparison with PPTR experiments. In three dimensions (4) takes the form

$$\rho c l_{ijk}^4 \frac{dT_{ijk}}{\Delta t} = (k/\Delta l^2) [T_{i-1,j,k} + T_{i+1,j,k} + T_{i,j-1,k} + T_{i,j+1,k} + T_{i,j,k-1} + T_{i,j,k+1} - 6T_{i,j,k}] + (1/\Delta l) [F_{ab}(i, j, k) - F_{em}(i, j, k)] \quad (12)$$

where $\Delta l = \Delta x = \Delta y = \Delta z$ is the element length. The directional emission terms now become

$$F_{em}(i, j, k) = [4\mu_{IR}(i, j, k)\Delta l\sigma n_{IR}^2 T_{i,j,k}^4]/3 \quad (13)$$

in the x direction, and similarly in the y and z directions. The absorption term is similarly modified to account for the six fluxes incident on a given volume element. The boundary formulation remains similar to that in (8). Analogous to the 1D case, (13) was solved using an explicit finite-difference scheme. The simulation was performed on a Micro-VAX 4400. The matrix size was $70 \times 70 \times 70$ nodal points. The coordinates of the central nodes of hot centres were selected randomly in the x - y - z space, subject to the constraint that they be nine nodes apart; then the six adjacent nodes are also assigned the temperature T_h , and all other nodes are initiated with the temperature 300 K. This corresponds to $\sim 1\%$ vol/vol loading of $3 \mu\text{m}$ diameter particles, to be compared directly with subsequent experimental PPTR results. Other inputs were $\mu_{IR} = 1000$ cm $^{-1}$ and $T_b = 5000$ K. The tabulated IR signal represents the emission from a $20 \text{ nodes} \times 20 \text{ nodes}$ node area centrally located at the surface. The results in figure 6 show the same signal features as seen previously, illustrating that 1D modelling is adequate for qualitative comparison with the experiments.

3. Experimental details

The details of the photothermal transfer dynamics were studied using PPTR. Figure 7 shows a schematic diagram of the experimental arrangement, which has been described previously (Prah et al 1992). Briefly, a Q-switched ruby laser (Lasermetrics 939R3L) delivered 694 nm pulses of 50 ns duration. The emerging light was directed through a 7 mm diameter aperture for spatial filtering, passed through a confocal doublet lens pair (Galilean telescope), and was focused onto the sample surface at 10° from the normal. This resulted in a nearly uniform beam ($\pm 15\%$) of about 5 mm diameter. The incident fluence was

varied by insertion of volume-absorbing neutral-density filters in the beam path (~ 100 – 700 mJ cm $^{-2}$ per pulse). A liquid-nitrogen-cooled HgCdTe detector (New England MPC 12-1-8) was placed at the focal plane of a Ge lens to detect the 7.5–14 μm IR emission from a 1 mm 2 area at the centre of the sample's front surface. For measurement of incident fluence, a known portion of light was diverted to an energy meter (Scientech 365) by a beam splitter. The electrical response of the detector, after preamplification, was monitored with a fast digital oscilloscope (LeCroy 9420A), triggered from the output of an Si photodiode (EG&G FND-100) that detected the stray light from the Q-switched laser pulse. The sampling rate of the digital scope was ~ 5 ns/point for early-time studies, and $\sim 8 \mu\text{s}$ /point to examine the long-time effects; data were recorded for 30 000–40 000 points after the trigger. The recorded PPTR signal was then reduced to 200–400 points by averaging. The stability of the system was checked periodically by observing the signal from a neutral-density filter (Schott NG-10) placed at the sample position.

Figure 7. A schematic diagram of the experimental PPTR apparatus. The laser light is incident at $\sim 10^\circ$ from the normal, as is the beam splitter.

Three different stock dispersions were formed to prepare samples for PPTR measurements. Two consisted of different lots of carbon-black pigment (Ashbury Graphite) mixed with deionized water. The two lots differed primarily in particle size. Lot 1 ('large') had $d_{ave} \sim 3.5 \mu\text{m}$, and a log-normal size distribution with 75% of particle diameters in the 1.9–4.4 μm range, and lot 2 ('small') had $d_{ave} \sim 0.9 \mu\text{m}$, and 75% of the particles within 0.6–1.6 μm . Recommended amounts of dispersant (Marasperse BCA-1, Lingotech) were used. The third stock was dry natural melanin powder (Sigma Chemicals). A given stock was gently mixed with a melted collagen gel solution (Knox Gelatine). Small amounts ($\sim 0.2\%$ vol/vol) of formalin were added to the gel mixture to induce cross-linking in the gel, so that its mechanical strength and melting point were increased. The mixture

Figure 6. Results of the 3D treatment of the simultaneous conduction-radiation problem, with 1% vol/vol hot-centre loading. $T_b = 5000$ K, $\mu_{IR} = 1000$ cm $^{-1}$. (a) Details of the initial peak.

was poured into 3.5 cm Petri dishes and allowed to solidify. The finished samples had a smooth-surface black appearance, and were ~ 6 mm thick. Optical transmission, confocal reflectance, and TEM microscopy revealed uniform pigment distribution throughout the gel matrix, with no evidence of settling or conglomeration. The resultant solid concentration in the samples varied from 0.45 to 2.35% (wt/vol).¹²

4. Results and discussion

4.1. Results from carbon-black samples ('large' particles)

Figure 8 shows a typical PPTR signal from a cross-linked collagen gel containing 2.35% of 3.5 μm diameter carbon granules. The detected signal is qualitatively similar to the theoretical predictions of figures 2–6. We can estimate the maximum temperature reached by the carbon particles following the 50 ns laser pulse as follows. Assuming no thermal losses during the pulse duration, the maximum temperature excursion will be

$$T_h \sim \Phi_0 Q_s A / \rho c V \quad (14)$$

where Φ_0 is the incident fluence, A and V are the particle cross-sectional area and volume, and Q_s is the absorption efficiency of the particle. The latter quantity can be calculated using Mie theory (Bohren and Huffman 1983) if the optical constants of bulk carbon are known. Most literature values indicate that $n_{carbon} \sim (1.5$ – $2.0) + (0.5$ – $1.0)i$ in the visible (Janzen 1979, Pluchino et al 1980). Using these values, and $n_{vac} = 1.33$ and $\lambda = 694$ nm, Mie theory predicts $Q_s \sim 1.0$ for all the particle diameters in this sample, so the particle absorbs all the optical energy incident on it. Using $\Phi_0 \sim 400$ mJ cm $^{-2}$, $\rho \sim 2$ g cm $^{-3}$, $c \sim 0.70$ J g $^{-1}$ K $^{-1}$, the expected range of maximum temperature excursions is $T_h \sim 2100$ K ($d = 1.9 \mu\text{m}$), 1150 K ($d = 3.5 \mu\text{m}$), and 900 K ($d = 4.4 \mu\text{m}$). Thus the maximum attainable temperature is inversely proportional to the particle diameter; since the thermal radiative emission depends on the product of particle surface area and the fourth power of temperature, we expect IR emission $\propto d^{-2}$. The dependence of the PPTR signal on the size of the absorbing centres will be discussed later.

The effect of increasing the incident fluence by 50% is shown in figure 9. Compared with figure 8, the initial peak is significantly higher (~ 35 units against 11 units) and somewhat broader (FWHM $\sim 10 \mu\text{s}$ against $\sim 6 \mu\text{s}$). Given that $T_h \propto \Phi_0$, and IR emission $\propto T_h^4$, the experimentally observed increase in the height of the first peak is lower than expected ($11 \times (1.5)^4 \sim 55$). Perhaps there are inaccuracies in using (14) to estimate T_h , or experimental problems in detecting such a large transient signal (for example, photons with $\lambda < 7.5 \mu\text{m}$ will not be detected because of the Ge filter, and the fraction cut off will be greater at higher fluences because of Wien's Law (Planck 1959)).¹³

4.2. Effect of absorber particle size

Figure 10 shows a PPTR signal using small-diameter carbon granules. Compared with the spectrum of figure 8, the first peak is higher (~ 19 units against 11 units) and narrower (FWHM $\sim 4.5 \mu\text{s}$ against $6 \mu\text{s}$). Moreover, the second peak is much less pronounced, and occurs at an earlier time (~ 1 ms). The two features of the first peak are generally consistent with the theoretical predictions for the radiative cooling of a small particle, although a much higher peak would have been expected if indeed IR emission $\propto d^{-2}$ ($11 \times (3.5/0.9)^2 \sim 160$). To examine the cooling mechanism at early times in greater detail, we investigated the PPTR

Figure 8. (a) A typical PPTR signal from a water gel phantom containing 2.35% (wt/vol) 'large' carbon-black granules, $d_{ave} \sim 3.5 \mu\text{m}$. The incident fluence was $\sim 400 \text{ mJ cm}^{-2}$. (b) Details of the initial peak.

cooling conditions unimpeded by interactions with other particles or by reabsorption of IR radiation. The results are shown in figure 11. Again, it is seen that the smaller $0.9 \mu\text{m}$ particles reach a higher initial temperature and cool faster than the larger $3.5 \mu\text{m}$ particles, but the observed peak height difference (~ 0.5 against 0.2) again seems relatively slight. One possible reason may be the wide particle-size polydispersity in both carbon samples decreasing the difference between their signals relative to theoretical predictions based on their respective average diameters.

4.3. Possible inaccuracies in estimating the maximum temperature T_b

The early PPTR signal appears to depend less strongly on fluence and on particle size than expected theoretically, suggesting a lower actual maximum temperature level. We therefore examine the validity of our T_b estimate. There are two assumptions inherent in (14): that there is no heat loss from the particle during the heating pulse, and that the absorbed energy is instantaneously distributed throughout the particle. For the first assumption, if the heat loss occurs by conduction, the characteristic time for the cooling process is approximately

$$t_{\text{cond}} = d^2 / C\alpha \quad (15)$$

where $\alpha = k/\rho c$ is the thermal diffusivity of the surrounding medium, and C is a constant in the range 2–27, depending on the dimensionality of the problem and the definition of the end-point temperature (Hu and Barnes 1970, Anderson and Parrish 1983). Substituting $\alpha_{\text{water}} = 0.0013 \text{ cm}^2 \text{ s}^{-1}$, the typical cooling times for the three diameters above are ~ 1 – $6 \mu\text{s}$ for $C = 27$. Thus, significant conductive cooling does not occur during the duration of the 50 ns laser pulse. However, there is also radiative cooling, which may prevent the absorbing centres from reaching the thermal extremes predicted by (14). The importance of this effect is much more difficult to quantify because of its highly non-linear temperature dependence. Extremely rapid cooling (from $\sim 100 \text{ K } \mu\text{s}^{-1}$ to $\sim 500 \text{ K } \mu\text{s}^{-1}$, estimated from the first microsecond or so of the early peaks of figures 8–10, and from simulation results) implies that the radiative loss also occurs during the laser pulse, and the predictions of (14) will be an over-estimate.

The second assumption of (14) is that the energy absorbed within the particle is uniformly and instantaneously distributed throughout its volume. In modelling 'opaque' absorbers, where characteristic dimensions are greater than the optical absorption length, an absorbed energy gradient results. This may produce higher local temperatures than predicted by (14) on the side of the particle facing the incident light. However, if the thermal conductivity and diffusivity of the particle are high, and radiative exchange within it is efficient, the subsequent redistribution and equalization of energy is rapid, especially for micrometre-sized particles (of the order of nanoseconds to microseconds). This absorptive shielding effect underestimates T_b , unlike the above radiative cooling effect (which overestimates T_b), and is also very difficult to quantify. However, the measurements imply lower temperatures than expected, so absorptive shielding does not appear to dominate.

A further possible source of error is the use of constant fluence throughout the medium, and therefore constant T_b , since some of the deeper particles may be partially screened by the overlying absorbers. We, therefore, examined a gel containing 2.35% (wt/vol) of 'large' carbon pigment to which scattering polystyrene microspheres (1.0% wt/vol) were added as light scatterers; these alter the fluence distribution within the gel. The gel phantom appeared cloudy grey, with a similarly smooth surface as seen without the microspheres. There was no significant difference in the PPTR signal from this scattering-absorbing combination,

Figure 9. The effect of incident fluence on the PPTR signal. The conditions were as in figure 8, except the incident fluence was $\sim 600 \text{ mJ cm}^{-2}$. (b) Details of the initial peak.

signal from a water smear containing 0.001% wt/vol carbon powder. The smear was placed on a smooth surface of an IR-transparent window of zinc selenide. The choice of this window material, and of the low pigment loading, was made to simulate single-particle

Figure 10. A PPTR signal from a water phantom containing 2.35% (wt/vol) 'small' carbon-black granules, $d_{ave} \sim 0.9 \mu\text{m}$. (b) Details of the initial peak.

implying that alterations in subsurface fluence do not play a major role in the subsequent conduction-radiation transfer, or at least do not influence the radiometric emission from the

material. Thus, to first order, our assumption of equal T_h at all granule locations throughout the volume is adequate for the PPTR model.

Figure 11. A comparison of the early-time PPTR signals from two wet monolayers containing very tenuous suspensions ($\sim 0.001\%$ wt/vol) of each of the two carbon particle sizes.

4.4. Results from melanin samples

Figure 12 shows a PPTR signal from an aqueous gel phantom containing melanin powder. The particle size distribution is similar to the 'small' carbon-black samples examined above. However, the signal is dramatically different—the double-peak structure seen before has been replaced by a gradual monotonic decay from the initial maximum. This signal behaviour is characteristic of materials with volumetrically distributed optical absorbers, such as neutral-density filter or a molecular dye solution (Tam and Sullivan 1983, Prah et al 1992). Since the melanin sample is clearly a particulate suspension, there must be some significant differences between the two particle types to account for the different PPTR signals. The thermal properties of the two are similar, within about a factor of ~ 8 (Crippa and Viapiani 1990, Vitkin et al 1994c). There is a significant difference between the optical constants in the visible, particularly in the imaginary part of the refractive index: whereas $n_{\text{carbon}} \sim 2.0 + 1.0i$, $n_{\text{melanin}} \sim 1.6 + 0.1i$ (Kurtz et al 1987). However, for the particle sizes tested here, even melanin yields $Q_a \sim 1$. An essential difference is that carbon absorption remains high in the IR (Janzen 1969, Pluchino et al 1980, Brewster and Toyomoto 1986), while melanin is essentially IR transparent ($\mu_{IR} \sim 0$) (Wolbarsht 1982). Thus, its radiative emission term becomes inefficient (5), so the heat initially deposited in the melanin

Figure 13. The effect of IR transparency of the hot centres. $T_h = 5000$ K, $\mu_{IR}(\text{hot centres}) = 0 \text{ cm}^{-1}$, $\mu_{IR}(\text{medium}) = 1000 \text{ cm}^{-1}$. Other parameters are as in figure 2.

values of T_h , the spectrally resolved μ_{IR} of the medium (appropriate to the spectrum emitted from the hot granules) is probably smaller than the wavelength-averaged μ_{IR} employed in the simulations.^{14†}

5. Summary and conclusions

We have studied the transient behaviour of the temperature field in a medium with discrete absorbing centres following a brief laser pulse. The PPTR signal from such a system is so unlike previous published results from experiments with volumetrically distributed absorption that an additional heat transfer mechanism of radiative transfer has been invoked. While radiative transfer has been previously used in PPTR models to account for IR emission from the sample surface, the intermedium heat transfer has always been assumed to follow the laws of heat conduction. Experimental results and theoretical predictions have been compared for a range of particle types, sizes, and initial temperatures. It is seen that radiative transfer becomes more significant with increasing temperatures of the hot centres, with decreasing IR absorption of the medium, and with increasing IR absorption of the particles, implying that thermal confinement becomes difficult for small hot objects. The overall radiative contribution to intermedium heat transfer, and therefore its perturbation of the internal temperature field, appears relatively minor for the range of the examined parameters. It does, however, strongly influence the radiometric emission from the sample, and hence the PPTR signal.

Figure 12. A typical PPTR signal from a water phantom containing 2.35% (wt/vol) melanin powder. The incident fluence is $\sim 400 \text{ mJ cm}^{-2}$.

granules is not emitted radiatively, either to the IR detector to give the initial PPTR spike, or, more importantly, to the surrounding medium to cool the granule. The dominant cooling must take place by the slower mechanism of conduction to surrounding water matrix. We modelled this situation in figure 13, where the results do indeed display the experimentally seen behaviour. These findings confirm the importance of radiative transfer during the early phase of cooling by IR-emitting particles.

4.5. Effect of medium properties

The assumption of temperature- and wavelength-independent optical and thermal properties of the medium and of the hot centres may be inaccurate in some cases. The highest inaccuracy is likely to result from ignoring the temperature-wavelength dependence of the emission spectrum and of the IR absorption coefficient. The wavelength of the black-body emission maximum is described by Wien's law (Planck 1959); for example, at $T = 300$ K, $\lambda_{\text{max}} \sim 9.7 \mu\text{m}$, and for $T = 3000$ K, $\lambda_{\text{max}} \sim 1 \mu\text{m}$. The μ_{IR} values used above correspond to the absorption properties of water in the ~ 2 – $15 \mu\text{m}$ range. However, as the emission maximum shifts to lower wavelengths at higher temperatures, water transparency below $\lambda \sim 1.3 \mu\text{m}$ may become significant. Thus, if the local temperature transient results in a significant visible incandescence flash, this energy fraction will be lost from the sample. Such flashes were observed with the carbon samples but not with the melanin samples. Nevertheless, this is unlikely to be the dominant cooling mechanism because of the high temperatures needed to enable a significant fraction of the energy to be emitted in the visible-to-near-IR range (Ozisk 1973). However, at very early times in samples with high

Detailed knowledge of the optical and thermal transfer dynamics is important in modelling and optimizing laser-material interaction in fields such as laser fusion and photomedicine. Pulsed photothermal radiometry, PPTR, a non-contact measurement technique, is well suited to supply this information. The mechanism investigated in this paper to explain some features of the PPTR signals, that of radiative heat transfer within the medium, may become important if the temperature transients reach thousands of kelvin. Application where this may occur include laser ablation of materials, tattoo and pigmented skin lesion removal in dermatology, and pulsed laser therapies in ophthalmology.

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Numerical integrations of the digitized signals was carried out ($t = 0 - 50 \mu\text{s}$, step size = $0.3 \mu\text{s}$; $t = 50 \mu\text{s} - 90 \text{ ms}$, step size = 0.3 ms). From Figs. 8 and 9, respectively, this yielded (normalized to the fast peak of Fig. 8): radiative integrals ~ 1 and 5, conductive integrals ~ 40 and 60. Thus, conductive transfer is approximately proportional to fluence (and therefore T_0), as expected, and the total radiative transfer is supralinearly dependent (although not as T_0^2). Note that in both cases, the percentage of heat lost from the medium by direct radiation to the environment is fairly small, of the order of a few percent. Of course, this may not be indicative of the true contribution of intra-medium radiative transfer, as the detected early signal is only sensitive to the outward emission of relatively few hot centres within an IR absorption depth or so of the surface.

In general, it is quite difficult to demarcate the regime of significant radiative contribution to the energy transfer mechanism within the medium. From the previous discussions, it is clear that the contribution increases with increasing temperature levels in the medium. As well, it seems that decreasing the magnitude of μ_{IR} increases the role of radiative transfer in levelling out the temperature gradients in the medium. In order to further quantify when radiation becomes important, and how important it becomes, one approach is to estimate the areas under the two PPTR signal peaks as above. However, both mechanisms contribute to varying degrees to the entire signal, so to associate a given peak with a single heat transfer mode exclusively is an oversimplification. As well, the PPTR measurement samples $\sim 1/\mu_{\text{IR}}$ depth of the surface, so inferring events deeper within the medium may be difficult. Probably the simplest approach would be to estimate the conduction-to-radiation parameter as indicated in Eq. (11). It is important to realize, however, that μ_{IR} may be temperature-dependent because the emission wavelength spectrum depends on temperature via the Planck function, and most materials have $\mu_{\text{IR}} = f(\lambda)$. As well,

Addendum to paper #3

¹¹¹ These assumptions are probably justified for our modelling purposes. Similar 1-D approaches have been shown to yield accurate temperature profiles and radiative fluxes (for example, Al Abed and Sacadura 1983; Arai et al 1984; also results in Fig. 6). As well, such a simple approximation is used because uncertainty in the radiative optical properties of most materials does not warrant a more detailed treatment. The assumed independence of μ_{IR} on temperature significantly reduces the complexity of the model, and is unlikely to cause serious errors given the relatively short times and the relatively small regions that sustain significantly elevated temperatures; however, some recent work indicates that the IR absorption properties of water may be temperature-dependent (Cummings and Walsh 1993).

¹²¹ Two additional samples using carbon particles were made. The procedure for the first was same as above, except that the suspension liquid was heavy water (D_2O). The second utilized clear optical epoxy (Epoteck - 310) as the pigment-containing medium. These samples were used to check the effects of varying the thermophysical and IR optical properties of the medium on the PPTR signal.

¹²² Presently, we can estimate the relative efficacy of the two cooling mechanisms as follows. The PPTR signal is proportional to the emissive power from the sample. Thus, the time integral of the signal is proportional to the net emitted energy. We may interpret the time integral of the initial sharp peak as representing the heat loss due to direct radiative transfer from superficial hot centres to the environment; and the time integral of the second broad peak as representing the heat lost as a result of conductive transfer within the medium.

the temperature will be a function of position and time, resulting in a spatio-temporal dependence of the radiation-to-conduction parameter. Finally, this simplified estimate does not take into account the finite volume effects, that is, the effects of the size of the emitter and of the nature and location of the medium boundaries within which the energy transfer is occurring.

¹⁴¹ We attempted to examine the effect of different μ_{IR} values experimentally by investigating the PPTR behaviour of a heavy water gel, since D_2O is transparent from the visible to $\sim 2.5 \mu\text{m}$, as opposed to H_2O which begins to absorb at a shorter wavelength of $\sim 1.3 \mu\text{m}$. However, we did not see significant differences, probably because the spectral differences between the two types of water are not very large, and the fractions of energy lost in the visible to near-IR range were insufficient to alter the PPTR signals.

In addition, we investigated the possibility of transient changes of state of the medium occurring and affecting the signals, for example, gel melting, cross-links breaking, or water boiling in the vicinity of the absorbers in the few nanoseconds during/after the pulse. We used clear optical epoxy as the imbedding medium for the pigment granules; its boiling point is at a considerably higher temperature than water, $\sim 700\text{K}$. Its PPTR spectrum looked very similar to the water-based phantoms. We also examined the PPTR response at lower fluences, where the local temperatures jumps are definitely lower than the melting and the boiling points of the epoxy. Although this does not prove that there is no change of phase in the water-based phantoms, we do conclude that the essential features of the PPTR signal are not due boiling of the water or decomposition of the cross-links in the gel matrix.

The major cause of complexity in the coupled conduction-radiation modelling lies in mathematical description of the absorption and re-emission of radiative energy throughout the medium. A much simpler analytical solution results if the particle emits IR energy only from its surface, and the surrounding medium is IR-transparent. The results may be useful as a first-order estimate of radiative cooling rates once the importance of radiative transfer has been ascertained (for example, from Eq. (11)). This simpler problem is solved below.

To estimate the radiative cooling rates (in absence of conduction) of an IR-opaque object in an IR-transparent medium, consider first a small object of finite IR absorption such that part of the emission from its center does escape to the surrounding matrix (as in the simulations here). In such a scenario, the radiative transfer depends on the volume of the emitter. However, with increasing particle size and/or μ_{IR} , only radiation from its superficial surface layers reaches the outside medium, and radiative exchange now depends on the surface area of the emitter. This area-dependent cooling will be slower and less effective than the volumetric cooling of small particles. It can be analyzed using the so-called "lumped" formulation to determine the radiative contribution to cooling (Arpaci 1962; Lienhard 1983), as follows. A sphere of diameter d and initial uniform temperature T_h subject to heat transfer by radiation only will cool according to

$$T(t) = \frac{T_h}{(1 + 3 \cdot K \cdot T_h \cdot t)^{1/3}}, \quad K = \frac{6 \cdot \sigma \cdot \epsilon}{\rho \cdot c \cdot d} \quad (3.1)$$

where ϵ is the average surface emissivity of the sphere. Analogous to conductive cooling described by Eq. (15), it is possible to estimate the characteristic radiative cooling time:

Figure 3. An isolated sphere cooling by radiation only, according to the lumped-analysis predictions.

Additional References for the Addendum

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$$\tau_{r,1/2} = \frac{7}{3 \cdot K \cdot T_h^3} \quad (3.2)$$

where $\tau_{r,1/2}$ is the time in which the average temperature of an isolated sphere falls by 50% through radiative cooling. Notice that, unlike the conductive case, the radiative cooling time depends not only on the physical and geometrical variables, but also on the initial temperature. This is because the conductive flux is proportional to the difference in the first powers of temperature (i.e., the temperature gradient), whereas the radiative flux is proportional to the difference in the fourth powers of the temperature. Eqs. (3.1) and (3.2) were derived assuming the ambient temperature $T_a = 0K$. For the more general case:

$$4 \cdot K \cdot T_h^3 \cdot t = 2 \cdot \left(\tan^{-1} \frac{T}{T_a} - \tan^{-1} \frac{T_h}{T_a} \right) + \ln \left(\frac{T + T_a}{T_h + T_a} \right) - \ln \left(\frac{T - T_a}{T_h - T_a} \right) \quad (3.3)$$

Figure 3 shows the graphical results of the lumped radiative analysis, starting with the initial sphere temperature of $T_h = 1000K$. Notice that, although the initial cooling is rapid, there is a marked decrease in the cooling rate as the asymptotic limit of the ambient temperature is approached. In further refinement of the lumped analysis modelling, one should use energy balance analysis to allow the ambient temperature to increase, concomitant with the temperature decrease of the cooling sphere. This will result in slower cooling by reducing the effective temperature differences between the inside and the outside of the sphere.

Paper # 4: Vitkin I.A., Wilson B.C., Kaplan R.S., and Anderson R.R. (1992) The feasibility of monitoring exogenous dye uptake in tissue *in vivo* using pulsed photothermal radiometry. *Journal of Photochemistry and Photobiology B: Biology* 16, 235-239.

This paper describes an *in-vivo* application of the PPTR model (paper #1) in turbid media. The experiment involved monitoring the pharmacokinetics of Evan's Blue dye in a rat liver model, following its *i.v.* administration. Although the physiological interpretation of results was not clear, the ability of PPTR to follow the changes in the amount of dye solution present in the liver was demonstrated. This was only a proof-of-concept study, and no attempt of quantifying the absolute dye concentration was made.

Having conceived the idea of this *in-vivo* PPTR study, I spent some time writing the experimental protocol that was approved by the Massachusetts General Hospital Animal Experimentation Panel. The experimental work was done by me at the Wellman Labs, with some assistance from Mr. Ross Kaplan. The manuscript was written by me, and edited by all the co-authors.

Preliminary Note

The feasibility of monitoring exogenous dye uptake in tissue *in vivo* using pulsed photothermal radiometryI. A. Vitkin^a, B. C. Wilson^{a,b,*}, R. S. Kaplan^b and R. R. Anderson^b^aHamilton Regional Cancer Centre / McMaster University, 699 Concession St., Hamilton, Ont., L8V 5C2 (Canada)^bWellman Research Laboratories of Photomedicine, Massachusetts General Hospital, Boston, MA 02114 (USA)

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1. Introduction

There are a variety of procedures in photomedicine which rely on preferential absorption and retention of exogenous dyes in tissue [1], for example using light-activated photosensitizers to treat solid tumors or the use of photothermal dyes to enhance local laser energy deposition. For optimum treatment delivery, it is of value to determine the concentration of the dye in target tissue. Ideally, this should be done by a minimally-invasive, *in vivo* technique. Fluorescence and diffuse reflectance spectroscopy have been used for this purpose [2-4]. Here we report a preliminary study to determine the feasibility of using pulsed photothermal radiometry (PPTR) to monitor uptake of an exogenous dye in tissue *in vivo*. Normal rat liver was chosen as a model tissue since it is optically homogeneous, easily accessible and well perfused. Evan's Blue was used as a simple dye having a high and well-determined molar extinction coefficient. An analysis of the PPTR signal, based on optical diffusion theory, was used to derive the relative absorption-time curve and the transport scattering coefficient of the dye-containing tissue.

Pulsed photothermal radiometry is a non-contact, non-destructive optical diagnostic technique [5] which has been widely used in materials science but used only to a limited extent for studies in tissue (e.g. ref. 6). A short, low-fluence pulse of laser light is incident on the sample under study, and the heat generated through optical absorption is monitored with an infrared (IR) detector, which measures the black-body emission from the irradiated surface. As the sample returns to thermal equilibrium,

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irradiated with a known incident fluence. For each experimental PPTR signal, a non-linear, least-squares fit was performed over the time interval 3-150 ms with μ_a and μ'_s as the variables.

3. Results and discussion

Figure 1 shows the typical PPTR measurements from liver following Evan's Blue injection. The data at $t=0.5$ min showed a signal maximum several milliseconds after the laser pulse, indicative of a delayed thermal wave arriving at the tissue surface. This could be due to a depth concentration gradient in the liver, with the superficial tissues containing less dye than deeper layers. This apparent inhomogeneity was not seen at 2-35 min after injection. The maximum uptake, as indicated by the largest initial PPTR signal, was at 20 min after injection. The *post mortem* measurement shows further decrease in PPTR signal intensity, which may be due to rapid blood loss from the tissue, as has been observed by other optical techniques [10].

The fitted absorption and transport scattering coefficients are shown in Table 1. The derived values shown in the table indicate that the uptake of the dye increases the absorption coefficient of the tissue without appreciably affecting its scattering properties, indicating that the model used does properly separate the absorption and scattering components. With the high values of absorption used here, the fitting was not very sensitive to the exact μ'_s value; nevertheless, these values were reasonably consistent with the μ'_s values reported in the literature at this wavelength [11], which, with the exception of an outlying measurement at 100 cm^{-1} , are in the range 5-20 cm^{-1} . This is encouraging, since the validity of the diffusion-theory model (eqn. (1)) could be in doubt for this tissue where the optical transport albedo (μ'_s/μ_a) is low (0.18-0.37, Table 1). The model has been confirmed previously, however, in turbid gels for transport albedo down to 0.4 [8], and also eqn. (1) reduces to the correct

Fig. 1. PPTR signals from rat liver *in vivo* before and at 0.5-35 min following injection of 4 ml kg^{-1} 2% Evan's Blue, and immediately *post mortem*.

the time dependence of the detected IR signal is governed primarily by the optical and thermal properties of the sample. PPTR is well suited to study optical absorption in an absorbing and scattering (turbid) medium, as only the absorbed fraction of the optical field contributes to the thermal signal. For an optically and thermally homogeneous tissue, the change in light absorption owing to uptake of an exogenous absorber alters the PPTR signal and thereby, in principle, its concentration can be quantified.

2. Materials and methods

The PPTR system has been described previously [7, 8]. Briefly, a 1 μs pulse of 630 nm light from a tunable pulsed dye laser was delivered to the tissue surface, via a multimode fibre with a distal lens, as a uniform 5 mm diameter spot. The incident irradiance was typically 400 mJ cm^{-2} , corresponding to an initial surface temperature rise of 5-10 $^{\circ}\text{C}$. The central 1 mm^2 of the illuminated area was viewed through a germanium lens by a fast HgCdTe infrared detector, the amplified output of which was stored on a digital oscilloscope.

Adult male Wistar rats (≈ 600 g) were anaesthetized (8% ketamine, 12% xylazine, 1% atropine injected i.p. at 0.6 ml kg^{-1}), and the liver exposed through an abdominal incision. An initial PPTR signal from the liver was collected as a reference, prior to injection of dye. Two different dyes (Evan's Blue and Trypan Blue) were used initially in five animals to check that the changes in the PPTR signal were qualitatively consistent with increasing concentration in the liver. For this report, the data from only one animal are presented and analyzed in detail, for the purpose of illustrating the feasibility of the technique.

Evan's Blue dye (2% in saline; absorption coefficient = 3097 cm^{-1} at 630 nm, measured with a spectrophotometer) was then injected at 4 ml per kg body weight via the penile vein (time $t=0$), and PPTR signals collected at several times thereafter. Three to four signals were obtained from different locations on the liver at each time, and the last signal was stored after a stable signal was obtained. The tissue surface was kept moist with physiological saline between measurements. This procedure was continued to $t=35$ min, after which the animal was sacrificed in a carbon monoxide chamber and a *post mortem* signal collected.

The PPTR signals were analyzed as follows. In a semi-infinite, thermally- and optically-homogeneous turbid medium, the PPTR signal, $S(t)$, is given by [8]

$$S(t) = K \cdot \mu_a \mu_{tr} \left\{ \frac{A}{\mu_{tr}^2 - \mu_a^2} [\mu_{tr} f(\mu_{tr}^2 \alpha) - \mu_a f(\mu_a^2 \alpha)] + \frac{B}{\mu_{tr}^2 - \mu_a^2} [\mu_{tr} f(\mu_{tr}^2 \alpha) - \mu'_s f(\mu_{tr}^2 \alpha)] \right\} \quad (1)$$

where A and B are functions of the optical absorption and transport scattering coefficients, μ_a and μ_s , and absorption and transport scattering coefficients, μ_a and μ_s , and $\mu_{tr} = \mu_s / (1 + \mu_s / \mu_a)$ is the transport attenuation coefficient, $\mu_{tr} = (3\mu_s / \mu_a)^{1/2}$ is the effective attenuation coefficient, μ_{tr} is the infrared absorption coefficient, α is the thermal diffusivity, and $f(x) = \exp(-x) \cdot \text{erfc}(x^{1/2})$. The optical distribution that leads to eqn. (1) is derived from diffusion theory, and is expressed as a sum of uncollided and diffuse fluxes. Except for the overall scaling factor K and the optical coefficients to be determined, the values of all other parameters were known or assumed. The K value was determined using a reference signal from a well-characterized neutral density filter

TABLE 1

Results of the two-parameter fit of eqn. (1) to the PPTR signals shown in Fig. 1. The uncertainties correspond to a 5% increase in the least-squares value of the fit, defined as the sum of the squared differences between the experimental PPTR signal and the prediction of eqn. (1) over the 3-150 ms time interval

Time after injection (min)	Absorption coefficient $\pm 6\%$ (cm^{-1})	Transport scattering coefficient $\pm 11\%$ (cm^{-1})
0.5	46	27
2	63	30
4.5	75	26
10	93	33
20	174	38
35	120	27
Post mortem (40)	105	25

Beer's Law form, applicable to a pure absorber, as μ'_s tends to zero. Further rigorous testing in the transport albedo range below 0.4 is required to ensure that the derived optical coefficients are accurate.

It was not possible in this preliminary study to confirm the PPTR values measured *in vivo* with the absolute concentration of the dye in tissue, for example, by spectrophotometry of extracted dye. However, we note that, assuming a 5.5% blood/body weight ratio, the absorption coefficient of circulating dye should be about 205 cm^{-1} . Since the blood perfusion of the liver is 30-50% [12], and Evan's Blue binds strongly to serum albumin and remains in circulation for some hours [13], we are likely monitoring primarily the kinetics of liver perfusion with dyed blood as a function of time. There may also be a small component of dye accumulation in liver tissue, perhaps via staining of the intra- or extracellular matrix. Given that the majority of the absorbing dye is in the circulation in this study, the applicability of the homogeneous tissue model described by eqn. (1) may be questioned. However, studies in inhomogeneous gels containing discrete absorbing particles yield PPTR signals which show specific features not observed in the rat liver. It is likely, therefore, that the amount and the microdistribution of the absorbing dye in the liver tissue does not violate the homogeneous PPTR diffusion theory model.

Finally, regarding the sensitivity and the signal-to-noise ratio of the technique, calculations indicate that μ_a changes of approximately 4 cm^{-1} , and μ'_s changes of approximately 7 cm^{-1} could be resolved for this albedo range. The sensitivity should improve for lighter pigmented soft tissues with higher transport albedo. Experimental improvements such as signal averaging may further increase the signal-to-noise ratio, and allow smaller changes in optical properties to be quantified.

4. Conclusions

We have shown that the uptake of an exogenous dye in tissue can be monitored remotely *in vivo* using PPTR. Employing optical and thermal diffusion theory, the PPTR signals can be analyzed to determine the optical absorption and transport scattering coefficients of tissue. Changes in the absorption relate to dye uptake, and

the *in vivo* dye concentration can be inferred. Further work will investigate the advantages and limitations of this method compared with other optical techniques such as fluorescence or reflectance spectrophotometry, and determine the sensitivity, accuracy, and time resolution for relative or absolute concentration measurements *in vivo*.

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OPTICAL AND THERMAL CHARACTERIZATION OF NATURAL (*Sepia officinalis*) MELANIN

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Abstract—The optical properties and the thermal diffusivity of natural cuttlefish (*Sepia officinalis*) melanin have been measured. The optical absorption and scattering properties of melanin particles were determined at 580 nm and 633 nm, using photometric and photothermal techniques. For the photometric studies, the absorption and the transport scattering coefficients were determined from the measurements of diffuse reflectance and transmittance. The scattering anisotropy was obtained from an additional measurement of the total attenuation coefficient and independently obtained by goniometry. For photothermal studies, pulsed photothermal radiometry was used to deduce the absorption and transport scattering coefficients via a model based on optical diffusion theory. Pulsed photothermal radiometry was also used to provide the thermal diffusivity of solid melanin pressed pellets.

INTRODUCTION

Melanins are a class of biological pigments found in many species. Their functions vary,^{1,2} but a major one in human skin is solar photoprotection, provided via optical absorption and scattering by epidermal melanosomes. In characterizing melanin, one must be aware of its biologic variability, the problems involved in its isolation, purification and inevitable alteration, and its granular nature, which often makes the measurement and the data analysis difficult and ambiguous. Specifically, various photometric studies of melanin have led to a wide reported range of optical properties,³ especially in its granular form. This is partly because the optical absorption (or transmission) spectrum of the particulate medium will often bear little resemblance to that of the bulk parent material, depending on its state of aggregation. (A case in point is the natural color variations of the ink that result from different pigment size, spatial distribution and relative abundance of ocular melanin.) The difficulties involved in isolating, purifying and handling natural melanins may also contribute to the variable and often contradictory optical data obtained from melanin studies. To avoid these difficulties, "synthetic" melanins have been used in place of the natural ones, but the validity of the synthetic model is at times questionable. Thermal properties of melanin have been less extensively studied, although the dissipation of heat from melanin particles is an important aspect of laser surgery for pigmented skin lesions.

In this paper, we report photometric and photothermal measurements of optical absorption and scattering properties of a well-characterized suspension of natural melanin particles at two representative wavelengths in the visible and

measurements of thermal diffusivity of a pressed melanin pellet.

The current state of knowledge of the structure and function of epidermal melanin has been summarized recently;⁴ other varieties, such as ocular melanin, have also been studied⁵ and reviewed.⁶ A better model system seems to be melanin obtained from the ink sac of the cuttlefish *Sepia officinalis*.

MATERIALS AND METHODS

Melanin material. The melanin samples used were Melanin (Molten, CA), which consist of primary cuttlefish granules isolated and purified from the ink sacs of the cuttlefish *S. officinalis*, and suspended in distilled water at 7% w/w concentration. Several procedures for isolation and handling have been described that yield *S. officinalis* melanin powder with highly reproducible analytical parameters.⁷ The particles are roughly spherical in shape, as seen in a typical transmission electron micrograph (TEM) photomicrograph of Fig. 1a, a histogram of particle diameter based on 124 particles is shown in Fig. 1b. The granules are composed primarily of the pigment, with an additional amino acid content of about 5%. The particle density is 1.65 g cm^{-3} .

Sample preparation. For photometric studies, the Molten stock solution was diluted with distilled deionized water to the appropriate concentration and placed into a glass cuvette. For goniometric measurements, the cuvette consisted of two 0.15 mm-thick glass microscope slides separated by 1 mm-thick Teflon spacers. To ensure single scattering, this experiment was performed at 0.009% and 0.006% solids by weight. A well-characterized suspension of polystyrene microspheres (refractive index $n = 1.59$, particle diameter $d = 0.36 \mu\text{m}$) was used to test the accuracy of the goniometry. For the measurements of diffuse reflectance and transmittance, the cuvette consisted of two plates of 2 mm-thick pyrex glass separated by 2 mm, and the range of concentrations used was 0.07-0.12%. For the calculated transmission experiment, the same form of sample of 1 mm.

The sample preparation for photothermal measurements was more involved. For time-resolved optical pulsed photothermal radiometry (PPTR) measurements, the Molten stock was gently mixed with a methylcellulose gel solution (Keweenaw, 76-w/w). The mixture was poured into 3.5 cm petri dishes and allowed to solidify. The

Paper # 5: Vitkin I.A., Woolsey J., Wilson B.C., and Anderson R.R. (1994) Optical and thermal characterization of natural (*Sepia officinalis*) melanin. *Photochemistry and Photobiology* 59, 455-462.

This paper deals with the optical and thermal characterization of cuttlefish melanin.

Although camouflage and photoprotection are the primary functions of this class of biological pigments, the data on their optical properties is surprisingly rare. This study was undertaken to fill this void, and may perhaps explain the different pigmentation (i.e., colour) shown by different skin diseases. Optical transfer in a well-characterized, nearly monodispersed suspension of melanin microgranules was studied using a goniometric and an integrating-sphere apparatus. Comparison of Monte Carlo simulation results with the data yielded the optical properties of the microgranules; these were confirmed with a PPTR measurement. To facilitate understanding of the thermal transients in melanosomes during pulsed laser surgery, the thermal diffusivity of melanin was also measured using PPTR.

The idea of quantifying optical and thermal properties of particulate melanin originated with me, and the details of the experimental approach were clarified in subsequent discussions with Drs. Wilson, Anderson, and Kollias; the subject was also discussed during my Feb / 1993 Committee Meeting. All the photothermal work for the paper was done by me, at the Wellman Laboratories. I also performed the goniometric measurements. The integrating sphere measurements and simulations were performed by Joshua Woolsey at the Hamilton Regional Cancer Centre. The manuscript was written by me, and corrected by other authors.

Figure 1. (a) A TEM photomicrograph of a Molten melanin sample. Magnification = 140,000x. Black bar represents 0.1 μm . (b) Histogram of the particle size distribution of a typical sample, sized into 10 nm-wide bins. The sample size is 124 particles.

finished samples had a smooth-surface black appearance. Optical microscopy and TEM revealed uniform pigment distribution throughout the gel matrix, with no evidence of settling or conglomeration. The melanin concentration in the samples varied from 0.45 to 1.12%. A reference gel phantom was made using a known amount of trypan blue dye to form a homogeneous absorbing sample. For back-scattered PPTR measurements, the water in the stock solution was allowed to evaporate. The remaining dry melanin powder was placed in the hollow chamber of a cylindrical stainless steel shell ($d = 7 \text{ mm}$) and compressed from both ends at a pressure of about $3.4 \times 10^4 \text{ kPa}$ (measured with an MTS-310 extensometer). The resultant melanin pellet had a typical thickness of about 2.0 mm, and a matte appearance similar to the original powder. The PPTR measurements were insensitive to the exact applied pressure, provided that it was great enough that the pellet held together. The measured thermal diffusivity in pellets subject to repeated compressions were within 10% of one another. Six different pellets (three each from two different starting batches of stock solution) were prepared to ensure the reproducibility of the deduced thermal diffusivity value. Finally, the measurement technique was tested with a painted glass cuvette and with a pellet of pressed carbon black.

Scattering phase function measurements. The angular dependence of light scattering by the melanin granules was determined with the goniometer shown in Fig. 2. The sample cuvette described above was placed at the center of a 30 cm-diameter cylindrical tank filled with filtered, deionized water. The top of the cuvette was 3 cm below the water surface to minimize the internal reflection of the scattered light from the air-water interface. The output of a 5 mW 592 nm HeNe laser (PMS Electro-Optics LHVR-01004), reflected off two mirrors for ease of beam positioning, was directed through a 2 mm-

Figure 2. Schematic of the apparatus used to measure the scattering phase function. M = positioning mirror.

diameter iris aperture to reduce off-axis light stray reflections from the tank. A 3 mm $\times$ 3 mm piece of matte black tape was attached to the inside of the tank as the $\theta = 0^\circ$ position as a beam stop. The light was modulated by a mechanical chopper (Stanford Research Systems SR400), and lock-in detection was used to improve the signal-to-noise ratio. The detector arm, manually rotated about the tank circumference in the plane of the laser beam, contained a fast PIN photodiode detector (Newport 873) oriented tangentially to the tank surface. The detector active area was 0.051 cm^2 . The total angular uncertainty in the scattering angle value, including the angular resolution and the position of the detector arm, was estimated to be $\Delta\theta = \pm 0.2^\circ$. The output of the photodiode was fed into the lock-in amplifier (Stanford Research Systems SR850) for synchronous detection of the scattered light.

The smallest angle at which measurements were made was 4° in order to avoid contributions from the scattered beam, the largest was 170° because of the physical size of the detector arm. The measurements were recorded at 2° intervals, with the first step used in the angular regions where the reading varied most generally, for $\theta \leq 30^\circ$. The sample was rotated as required ($< 2^\circ$) in the θ range between 60° and 120° to minimize the effect of the cuvette edges and internal reflections within the cuvette. As a null sample, the same cuvette was filled with water and the procedure repeated. The result was subtracted from the sample measurements. As a calibration check, the scattering phase function of a monodispersed water suspension of polystyrene microspheres of known size and refractive index was measured and compared with Mie theory calculations.⁸ The melanin measurements were then repeated at a lower concentration of 0.0005%. Finally, the whole measurement procedure was repeated at 633 nm, using light from a different HeNe laser (Spectra Physics 100-2).

Absorption, scattering and total attenuation coefficients—photometric measurement. A double integrating sphere system was used to measure the diffuse reflectance and transmittance of melanin samples prepared as above. The experimental arrangement is depicted in Fig. 3. The light source was a 75 W xenon arc lamp (PTI Inc. LSP 500X), coupled to a computer-controlled monochromator for wavelength selection. A 530 nm long-pass filter was placed at the exit of the monochromator to eliminate the second-order diffraction lines. The source beam, after being mechanically chopped (Stanford Research Systems SR450), was focused onto the sample with a 2 mm-diameter spot size. Two photodiodes (Hamamatsu S1330) were placed in the 90° detector ports of the 6" integrating spheres (Lab-sphere), and their output was fed to two lock-in amplifiers (Stanford Research Systems SR850). The spheres had baffles to prevent the photodiode detector from having a view of the irradiated spot. The modulator circuit provided the reference signal to both lock-in amplifiers. The specular reflectance returned into the front entry port of the first sphere, and the direct transmittance exited through the back exit port of the second sphere. The voltages corresponding to diffuse reflectance R_d and diffuse transmittance T_d were stored

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†Abbreviations: PPTR, pulsed photothermal radiometry; TEM, transmission electron microscopy.

Figure 3. Schematic of the apparatus used to measure the diffuse reflectance and transmittance. $P = \text{flux}$, $I = \text{loss}$, $A = \text{aperture}$.

directly in the computer. Measurements at three different concentrations of the melanin stock solution were performed at $\lambda = 580$ nm, and then repeated at $\lambda = 633$ nm. The throughput of the integrating sphere was typically 10–20% for the reflection measurement, and 50–70% for transmission. After accounting for the signals from a water null sample, the measurements were compared with R_{∞} and T_{∞} pairs generated by a Monte Carlo simulation to derive the absorption coefficient, μ_a , and the transport scattering coefficient, μ_s (see below).

In order to measure the total attenuation coefficient μ_t , a narrow-beam transmission was used. The collimated light beam from the arc lamp-monochromator assembly was incident directly on the photodiode of a 4 mm² active area, coupled to a preamplifier. The sample cuvette was positioned about 100 cm in front of the photodiode, and a 1 mm-diameter circular aperture between the sample and the diode was used to reject off-axis scattered light. The solid angle subtended by the detector was thus $\approx 10^{-4}$ sr. The signal from a water-filled cuvette was subtracted from the sample measurements at each of three different melanin concentrations.

Absorption and scattering coefficients and thermal diffusivity—photothermal measurements. Figure 4 shows a schematic diagram of the experimental PPTF arrangement. The mode of operation for front-ended studies (i.e., illumination from the same side as the detector) has been described previously.¹¹ Briefly, a coaxial flashlamp-pumped tunable dye laser (Candell SL-500) emitted 1 ns pulses that were coupled into a 3 m length of 1 mm-diameter silica core multimode fiber. The emerging light beam was directed through a standard 5× microscope objective at the sample at 15° from the normal, providing a near-Gaussian spot of about 5 mm diameter (700–400 nm cm² per pulse). The two laser dyes used were rhodamine-590 for $\lambda = 592$ nm and rhodamine-6G for $\lambda = 633$ nm. For back-ended measurements, the optical fiber was brought around to the back of the sample, and a 3× microscope objective focused to form a 5 mm-diameter spot on the rear surface of the melanin pellet. A liquid-nitrogen-cooled HgCdTe radiation detector (New England MPC 12-1-S) was placed on the focal plane of a paraxial lens to detect the 7–14 μ m IR emission from a 1 mm² area at the center of the sample. A portion of light exiting the fiber was diverted to an energy meter (Gentec ED000) by a beam splitter. The electrical response of the detector, after preamplification, was monitored with a fast digital oscilloscope (LeCroy 920A), triggered from the output of a silicon photodiode (EG&G FDN-1000) that detected the stray light from the dye laser pulse. For a typical trace, the sampling rate of the digital wave form was 100 ns. The data were recorded for 50–300 ms after the trigger. The recorded PPTF signal was then reduced to 200 data points by averaging. The stability of the system was checked periodically by observing the signal from a neutral density filter (Schott NG-10) placed at the sample position.

Photometric studies

The gonimetric data sets for 4–170° range were analyzed as follows. Assuming azimuthal symmetry and single scat-

Figure 4. Schematic of the apparatus for pulsed photothermal radiometry measurements. Fiber 1 is used for front-ended optical measurements, and fiber 2 for back-ended thermal studies. BS = beam splitter, L = microscope objective lens.

$$I = \int_{-\pi/2}^{\pi/2} \int_0^{\pi} I(\theta, \phi) \sin \theta d\theta d\phi \quad (1)$$

where $d\Omega$ is the differential solid angle. Linear interpolation at the two angular extremes was used to extend the data to the full 0–180° range. Numerical integration was performed to handle the unevenly spaced angular readings.

In order to calculate the absorption coefficient μ_a and the transport scattering coefficient μ_s ($\mu_t = \mu_a + \mu_s$) from the diffuse reflectance and transmittance data, a model of radiative transfer is needed.¹² Monte Carlo simulation was used to generate a look-up table for (R_{∞}, T_{∞}) at different values of (μ_a, μ_s) , covering the expected range of experimental values.¹³ The input to the simulation included the various geometrical parameters that define the experimental setup. As the sample was a very dilute suspension of melanin in water, its refractive index was assumed to be $n = 1.33$. The reflections at the air-glass and glass-water interfaces (see Fig. 3) were accounted for by using a Russian roulette¹⁴ algorithm based on the Fresnel reflection coefficients for randomly polarized light. At each intersection site, the scattering angle ϕ was randomly sampled from the Henyey-Greenstein angular function¹⁵ using the average g -value derived from the gonimetric measurements. The use of the Henyey-Greenstein phase function was preferred because its relatively simple analytical form was easy to incorporate into the Monte Carlo simulation and because it fitted the scattering data reasonably well (see Fig. 5). Upon reaching either side of the sample, the "weight" of each photon was scored according to the total distance l travelled in the sample via exponential absorption ($1 - e^{-\mu_t l}$) and μ_s/μ_t . With this procedure, reflectance and transmittance for a range of μ_a values were calculated at each μ_s value, resulting in significant decrease in computer simulation time for generating the necessary look-up table. The photons were discarded after they reached a sufficiently small weight (0.00001) and a number of photons used in each run (78 values of μ_a per μ_s value) was 500,000. The experimentally

DATA ANALYSIS

The gonimetric data sets for 4–170° range were analyzed as follows. Assuming azimuthal symmetry and single scat-

Wave-length	Optical property	Experimental results	Mie theory
580 nm	μ_a (cm ⁻¹ /%)	249 ± 34	263
	μ_s (cm ⁻¹ /%)	182 ± 23	199
	μ_t (cm ⁻¹ /%)	30 ± 7	31
	$g = 1 - \mu_a/\mu_t$	0.66 ± 0.30	0.62
	g_{exp} (592 nm)	0.67 ± 0.07	0.61
633 nm	μ_a (cm ⁻¹ /%)	207 ± 28	194
	μ_s (cm ⁻¹ /%)	127 ± 19	146
	μ_t (cm ⁻¹ /%)	66 ± 26	66
	$g = 1 - \mu_a/\mu_t$	0.65 ± 0.29	0.58
	g_{exp}	0.65 ± 0.07	0.58

^aThe coefficients are calculated via $\mu_{\text{eff}} = (2/Q)A(\rho/2V)$, where Q is the efficiency, A the cross-sectional area and V the volume of the i th particle, evaluated at the average diameter of the intervals of Fig. 1b. ρ is the density of melanin as depicted on the ordinate of that figure; ρ is the melanin density. Similarly, the scattering anisotropy is calculated from $g = (2/Q)A(\rho/2V)Q_{11}/Q_{\text{ext}}$. The optical constants for the calculations are $n = 1.625 + ik$, with $k(580) = 0.158$, $k(592) = 0.148$, and $k(633) = 0.120$.¹⁶ The uncertainties in the optical coefficients are from the uncertainties in the slopes of the corresponding regression lines (see Fig. 6); the uncertainty in the calculated g -value is from the uncertainties in the optical coefficients; the uncertainty in the gonimetric g -value is estimated from the experimental errors and its numerical integration used in its calculation.

RESULTS AND DISCUSSION

Photometric studies

As a gonimetric control, measurements of the scattering angular distribution of a 0.02% polystyrene microsphere suspension (corrected for a water null) were analyzed in accordance with Eq. 4. At 592 nm, this yielded $g = 0.70$. Using manufacturer's data for refractive index (1.59 ± 0.005) and particle radius ($0.18 \mu\text{m}$), a Mie scattering calculation¹⁷ for polystyrene microspheres in water predicted a g -value of 0.71. Figure 5 shows the angular dependence of the scattering phase function; the experimental data have been normalized by the $1/\sin^2 \theta$ factor for comparison with the Henyey-Greenstein function.¹⁵ At 592 nm and 0.009% melanin concentration, the result was $g = 0.67$. Data from 0.006% melanin sample yielded $g = 0.66$, indicating that single-scattering conditions were likely met. Using the g -value from the narrow beam measurement (see next paragraph), the optical thicknesses for the gonimetric measurement is $(207 \text{ cm}^{-1}/\%) \times 0.009\% \times 0.2 \text{ cm} = 0.4$, so that the necessary condition of single scattering is indeed satisfied. The gonimetric results are summarized in Table 1.

Figure 6 shows the dependence of μ_a and μ_s on melanin concentration at 633 nm, as determined from the (R_{∞}, T_{∞}) data. The g -value used to generate the Monte Carlo look-up tables for this was 0.65. The slope of the regression lines yielded $\mu_a = 127 \pm 19 \text{ cm}^{-1}/\%$ and $\mu_s = 28 \pm 7 \text{ cm}^{-1}/\%$. The total attenuation coefficient from the narrow beam measurement was $\mu_t = 207 \pm 28 \text{ cm}^{-1}/\%$. We may combine these results to determine the g -value via $g = 1 - \mu_a/\mu_t$ and compare it with the gonimetric measurement. As displayed

Figure 6. Optical properties of melanin suspension at three different concentrations. The average of three measurements was used at each point for comparison with the Monte Carlo simulation results. The error bars correspond to an independent change in each coefficient that changes the last significant value of the Monte Carlo fit to the data by less than 10%.

In Table 1, this yields $g_{\text{exp}} = 0.65 \pm 0.29$. Also displayed are the corresponding results obtained at 580 nm wavelength. The agreement between the directly measured and the derived melanin anisotropy values is largely within experimental error, indicating the self-consistency of the experimental methods. We have also reanalyzed the (R_{∞}, T_{∞}) data by generating Monte Carlo tables at slightly different g -values (0.6 and 0.7 at 633 nm) and found the (μ_a, μ_s) results changed very little ($< 5\%$). It appears that the gonimetric result is the most accurate estimate of g , whereas the value calculated from μ_a and μ_s could only serve as a check of the experimental techniques. Also notice that although the results at 633 nm can be compared directly, the other comparison is somewhat hindered by the disparity in the measuring wavelengths (580 nm vs 592 nm). However, this is unlikely to lead to a serious discrepancy, as the particle size is considerably smaller than the wavelength so that the scattering phase function will vary little with wavelength, as confirmed in Table 1 ($g_{\text{exp}} = g_{\text{exp}}$).

The rather forward-peaked nature of melanin scattering implied by these results may appear at odds with predictions of the Mie scattering theory, where particles much smaller than the incident wavelength are expected to scatter isotropically (i.e., $g = 0$). However, a closer examination of the size distribution in Fig. 1b indicates some particles with ~ 150 – 300 nm diameter. These larger particles exhibit more forward-peaked scattering and generally have higher scattering efficiencies; both of these factors will bias the scattering pattern into the forward direction, compared with the nominal particle size. The significant contribution of even a small number of relatively large particles has recently been observed in ink.¹⁸ Using the refractive index data from melanin free acid and melanin ammonium salt^{19,20} we have

Figure 5. Average of the scattering phase functions of melanin in water at 592 nm. Six measurements have been performed, three at 0.006% and three at 0.009% melanin concentration. Error bars are $\pm 5\%$. The solid line shows the theoretical Henyey-Greenstein function with $g = 0.67$, as calculated from the experimental average. The theoretical and the experimental values are independently normalized so that $f(0, 580) \times \sin^2 \theta \times d\theta = 1$ for both.

measured (R_{∞}, T_{∞}) values at three different melanin concentrations were then compared with the values predicted by Monte Carlo. The (μ_a, μ_s) pairs that yielded best agreement between theory and experiment were selected using linear interpolation between closest simulation points, and plotted separately against the experimental melanin concentration (Fig. 7). Linear regression through the origin was used to estimate the μ_a and the μ_s dependence on melanin concentration.

The total attenuation coefficient μ_t was derived from the narrow beam transmission measurement in accordance with Beer's Law:

$$\mu_t = -\ln I / L \times \ln(I_0/I) \quad (2)$$

where I is the signal with the melanin suspension sample of thickness L , I_0 is the detected signal with a water sample. From several measurements at three different melanin concentrations, the total attenuation coefficient was plotted as a function of concentration. The points were fitted to a straight line through the origin, and μ_t determined from the slope.

Photothermal studies

As the name implies, photothermal techniques can be used to study both optical and thermal processes in materials. To tackle the problem first, we analyzed the front-ended PPTF data with a closed-form expression that depends on μ_a and μ_s ^{11,21}

$$S(t) = C \frac{\mu_a}{\mu_t} \frac{1}{\sqrt{\pi}} \frac{1}{\sqrt{t}} \left[\frac{1}{\mu_a} \left(1 - \frac{\mu_a}{\mu_t} \right) \exp(-\mu_a^2 t) + \frac{1}{\mu_s} \left(1 - \frac{\mu_s}{\mu_t} \right) \exp(-\mu_s^2 t) \right] \quad (3)$$

where C is an experimental constant, ρ is the density, c the specific heat and α the thermal diffusivity of the sample, θ_s is the surface fluence (incident irradiance reduced by the specular reflection loss at the boundary) and $f(\theta) = \exp(-\theta^2/2)$ with $\text{erfc}(x)$ being the complementary error function. μ_a is the average absorption coefficient of the sample over the IR detection wavelength range. The parameters A , B , μ_a and μ_s are functions of the optical properties μ_a and μ_s ($\mu_t = \mu_a + \mu_s$, $\mu_s = [\mu_t(1 - g)]$). Thus, front-ended PPTF can be used to determine μ_a and μ_s of an unknown material if all other parameters in Eq. 3 are known or assumed. Because the melanin-gel samples were $\sim 99\%$ water, we used $\rho = 0.0013 \text{ cm}^{-3}$, $c = 1200 \text{ cal}^{-1}$, and $N = 1.33$. This still leaves three unknowns, namely C , μ_a and μ_s . To simplify the fitting procedure, we chose to calculate C by measuring the PPTF signal from a homogeneous gel with a known added amount of tyrosin blue dye. Because μ_a is known in this case (and was confirmed by an independent spectrophotometric measurement), and $\mu_s = 0$, Eq. 3 can be used directly to estimate C . This overall constant was then applied to the analysis of the melanin samples. Thus, we calculated residuals for a range of (μ_a, μ_s) pairs at a fixed α , where a residual is defined as the sum of the squares of the difference between the calculated and the measured values of $S(t)$. The (μ_a, μ_s) pair that yields the minimum residual was taken as the best solution.

The versatility of PPTF allows in addition for studies of sample thermal properties. If the radiative emission from the rear surface of a thin slice is measured following laser heating of the front surface, the arrival of the heat pulse is largely determined by the material's thermal diffusivity, α . If the slice is optically thick both at the excitation and the detection wavelengths, then the signal at the rear surface is given by^{22,23}

$$S(t) = G \left[1 + 2 \sum_{n=1}^{\infty} (-1)^{n+1} \exp(-n^2 \pi^2 \alpha t / L^2) \right] \quad (4)$$

where G is a scaling constant similar to C and L is the sample thickness. It has been pointed out^{22,23} that the signal reaches 50% of its asymptotic maximum when $\text{erfc}(\sqrt{\pi \alpha t / L^2}) = 1/2$. Instead of a complete fit to Eq. 4 to determine the best value of thermal diffusivity, α , can be obtained by measuring the time it takes $S(t)$ to reach 50% of maximum. We used this time to get a starting estimate for α , and then fitted the entire normalized back-ended PPTF signal to get a more accurate estimate of the thermal diffusivity. The value that yielded the smallest residual (defined as above) over the 0–300 ms PPTF signal time interval was selected as the best.

It should be pointed out that both the thermal and the absorption of the incident light pulse and the IR emission occur in a thin surface layer ($\ll L$) adjacent to the sample surface. With an excellent absorber such as melanin pressed into a thin pellet ($\mu_a = 10^4 \text{ cm}^{-1}$ in the visible range²⁴), this is true. The same cannot be said with certainty about μ_s , as it is known that melanin absorption decreases in the near infrared range.²⁵ In the absence of quantitative information, we will, however, assume that the IR absorption length at $\lambda = 10 \mu\text{m}$ is also smaller than the thickness of the pellet L , so that Eq. 4 is valid.

Figure 7. Front-ended PPTF signal of a 1.33% melanin gel. The irradiation wavelength is 633 nm. The solid line is the best least-squares fit to the data using Eq. 3 with $(\mu_a, \mu_s) = (233, 30) \text{ cm}^{-1}$.

calculated the size-weighted optical properties of our melanin mixture. As seen in Table 1, the measured optical properties are mostly within the experimental uncertainties of the Mie theory predictions. The different contributions of differently sized particles emphasize the importance of the melanin granule size in determining the outcome of the light-particle interaction. Given the natural size variation of epidermal melanosomes in humans,²⁶ this point may be particularly relevant for optical dosimetry in skin.²⁷

Photothermal studies

Figure 7 shows a typical front-ended PPTF signal from a collagen gel with 1.33% melanin particles at 633 nm. The (μ_a, μ_s) pairs yielding the minimum residual when fitted to Eq. 3 were determined for this data and for 0.50% and 0.45% gels. The μ_a and μ_s values are displayed in Table 2.

As seen from Table 2, the optical coefficients determined by PPTF are similar to the photometric results. However, some systematic differences do emerge. For example, the photometrically determined absorption is somewhat higher and the transport scattering somewhat lower than the corresponding results from the integrating spheres. This is possibly due to the limitations of the diffusion theory-based PPTF model in this low absorbance regime; however, the validity of the model will improve at very low absorbance because of the inclusion of the unscattered photon flux in the derivation of Eq. 3 (in the limit of no scattering, the optical distribution becomes equal to the familiar Beer-Lambert law²⁸). Despite these differences both the photothermal and photometric data imply that the melanin granules are not perfect black bodies but also significantly scatter light in this wavelength range. This effect is likely to be pronounced in human skin, where a large fraction of epidermal melanosomes have larger particle diameters (avoid, 0.4–0.8 μm in the longitudinal²⁶). The thermal diffusivity was obtained from the back-ended

Table 2. Optical and thermal properties of melanin derived from the photothermal measurements^a

Wave-length	Property measured	Experimental results
580 nm	μ_a (cm ⁻¹ /%)	176 ± 29
	μ_s (cm ⁻¹ /%)	28 ± 8
633 nm	μ_a (cm ⁻¹ /%)	137 ± 34
	μ_s (cm ⁻¹ /%)	24 ± 7
	α , in units of 10 ⁻⁴ cm ² /s ^b	1.42 (450)
		1.34 (380)
		1.38 (380)
		1.33 (283)
		1.22 (353)
	Ave. $\pm 1.37 \pm 0.13$	

^aThe bracketed numbers in the bottom row represent the pellet thickness in μm . The uncertainties in the optical coefficients are derived as in Table 1. The uncertainty in the average thermal diffusivity value is the standard deviation of the individual measurements.

PPTF measurements and Eq. 4. To test the validity of the method, we examined the back-ended PPTF spectra of a painted glass slide and of a pressed pellet of carbon black. The two diffusivity values obtained (0.0032 and 0.0123 cm²/s) are in excellent agreement with the tabulated thermal properties of glass and of amorphous carbon (0.0031 and 0.0118 cm²/s²⁹).

The PPTF signal from a 211 μm pressed melanin pellet, heated by 633 nm laser pulse at the front surface, is depicted in Fig. 8. The best fit of this signal to Eq. 4 is obtained with $\alpha = .00124 \text{ cm}^2/\text{s}$. The thermal diffusivity results of six different pressed pellets of melanin (along with the final pellet thickness in μm), are shown in the bottom row of Table 2. The average thermal diffusivity was $0.00137 \pm 0.00013 \text{ cm}^2/\text{s}$. This result differs somewhat from the reported value for synthetic melanin gels of $\alpha = 0.0016 \text{ cm}^2/\text{s}$, but this could be due to the different nature of the two sample types. The fact that our results showed relative insensitivity to the degree of compression (just the minimum pressure required to keep the pellet intact) warranted further investigation. One possibility is that all the air pockets and pores have been collapsed, so that the further compression has no effect. However, additional pressure did lead to $\sim 5\%$ reduction in film thickness, implying the presence of some voids in the sample and a slight increase in density. Because there was no corresponding change in thermal diffusivity (within the precision of the measurement), the thermal conductivity k of the pellet must increase. As $\alpha = k/\rho c$ is constant, it is also possible that the specific heat is decreasing with increased applied pressure; however, this is unlikely because a solid block of material contains more atoms per unit volume than a porous one and thus should have a higher specific heat. What likely happens is that the number and the arrangement of the remaining pores is relatively unimportant, as the thermal conductivity of air is low. (An analogy could be drawn with two unequal resistors connected in parallel, whereby the effective

Figure 8. Back-ended PPTR signal of a pressed melanin pellet of 211 μm thickness. The heating laser pulse ($\lambda = 633 \text{ nm}$) arrives at the front surface of the pellet at time $t = 0$. The solid line is the best fit from Eq. 4, using the least-mean-square thermal diffusivity value of $\alpha = 0.00124 \text{ cm}^2 \text{ s}^{-1}$. The search was initiated at $\alpha = 0.00135 \text{ cm}^2 \text{ s}^{-1}$, which was estimated from the time to reach 50% of the maximum signal.

resistance of the combination is largely determined by the one with the smallest resistance.) Thus, once the asymptotic thermal conductivity is nearly attained, further compression may increase it a small amount (by bringing more melanin particles into intimate contact), but this will be accompanied by a counteracting increase in density.

CONCLUSIONS

It is ironic that melanins, which are widely employed in nature for their optical properties (camouflage, photoprotection, coloration, retinal light absorption), have been difficult to characterize optically. Detailed knowledge of the optical and thermal properties of melanin is also important in modeling and optimizing light-tissue interaction such as melanosome photodisruption.^{2,22} Special attention must be paid to the particulate nature of the melanin pigment, especially the optical properties for which granular powder may differ significantly from the parent bulk material.

The photometrically measured optical absorption and scattering properties of cuttlefish melanin were consistent with Mie theory predictions, with strong dependence on the size of the melanin particle. This underscores further the importance of the granular nature of the melanin pigment, whereby seemingly minor differences in particle size can lead to large differences in its interaction with light. The finite size of the scattering coefficient implies that the common assumption of black-body behavior of the melanin granule is somewhat in error.

The application of the results presented here to studies of human epidermal melanin should be considered. The human melanosomes differ somewhat in composition, shape and size from the cuttlefish melanin model: they have a lower

melanin content (about 30–70%), and they are ovoid rather than spheroidal in shape, with the long dimension of 0.4–0.8 μm . Their larger size implies that epidermal melanosomes should have an even higher albedo (and more forward-peaked scattering phase function) than found in this study and cannot be regarded as pure absorbers, all the more so because their pigment content is lower. The substantial scattering may be important when estimating the amount of light absorbed by a melanosome during a laser treatment, as the absorption efficiency is not unity as often assumed, and thus the local temperature rise may be lower than calculated. As well, the skin pigmentation of some pathological conditions may depend on the scattering by melanosomes and/or melanin aggregates. The relevance of the thermal diffusivity result presented here is difficult to ascertain, but the α of human melanosomes is likely to be similar, based on their significant melanin content.

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Note: Several inaccuracies were discovered in the published version of this paper by the

thesis committee members. These are dealt with in the footnotes below, the

corresponding locations in the manuscript being marked with a double-dagger (^{††})

symbol.

^{††} -- An optical thickness of 0.4 does not ensure single scattering. Given the experimental data, it is more correct to say that the constancy of the derived g-value at two different concentrations (0.66 at 0.006%, 0.67 at 0.009%) seems to imply predominantly single scattering.

^{††} -- Particles much smaller than the wavelength should scatter symmetrically, *not* isotropically, although $g \sim 0$ for both. In the limit of a single charged particle, an isotropic phase function would have a non-zero intensity along the vibration axis, a physically impossible situation. The actual 3-D phase function would look like a surface of revolution obtained by rotating a sideways "figure-8" about its centre.

PULSED PHOTOTHERMAL RADIOMETRY STUDIES IN TISSUE OPTICS

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1. INTRODUCTION

The phenomenon of thermal radiative emission is the basis of a materials evaluation technique known as photothermal radiometry. This technique involves irradiation of the sample with monochromatic light which is absorbed, causing a temperature rise. The increase in the black-body radiative emission due to this rise is recorded with an infrared detector that views the sample surface. The detected signal contains information about the optical and thermal properties of the sample. Previous studies have used either modulated or pulsed light excitation methods; in this chapter, we will examine the applicability of the latter approach, known as pulsed photothermal radiometry (PPTR), to the study of optical properties of tissue.

Unlike many photometric techniques described elsewhere in this volume, PPTR does not operate directly in the optical domain, but rather relies on detection of heat that results from light absorption followed by non-radiative deexcitation. As shown below, assuming a

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I was asked by the editors to summarize the growing field of biomedical applications of PPTR, particularly with reference to tissue optics. The book is a review of opto-thermal interactions in laser photomedicine, so the inclusion of PPTR was *apropos*. The chapter is a review of the biomedical PPTR literature. It was written by me, with inputs and corrections from Drs. Wilson and Anderson. It is included in the thesis because it contains several original points (for example, the discussion on the effects of thermal diffusivity and of IR absorption coefficient on the PPTR signal).

constant irradiance over a surface, the photothermal signal depends on the product $\mu_a \cdot H(z)$, where μ_a is the absorption coefficient and $H(z)$ is the spatial fluence profile with depth which depends on tissue optical properties. Provided that a suitable optical propagation model is chosen to predict $H(z)$, and several thermodynamic parameters of the tissue are known, PPTR can then be used to find the tissue optical scattering and absorption properties. Due to its sensitivity to *absorbed* radiation, PPTR has shown promise in turbid materials that exhibit significant absorption, an optical regime generally inaccessible to purely photometric techniques. PPTR's ability to study thermal events occurring in the light-irradiated sample is also of value in itself, as many laser-tissue interactions involve a complex interplay of optical and thermal phenomena. Given these potential applications, and its inherent non-contact *in-vivo* capability, PPTR may become a useful tissue characterization tool in the biomedical laser community.

A general theoretical approach to modelling the PPTR effect involves solving for the spatial optical distribution $H(z)$ following the light pulse, converting this to a thermal source, and using the heat conduction equation to obtain the temperature field in the sample as a function of position and time. Then, using the Stefan-Boltzmann Law and accounting for sub- as well as surface radiation that can reach the detector, one obtains an expression for the infrared signal as a function of time. This approach has been pioneered by Leung and Tam [1], and independently by Imhof *et al.* [2], to generate a PPTR model that is widely used today by various researchers. However, this model treats absorbing materials only, the resulting one-dimensional optical distribution being simply described by Beer's Law [3]. The assumption of negligible optical scattering has not been restrictive for many applications, and an impressive array of studies have employed this model, for example, to monitor paint degradation [4], examine structural integrity of laminated materials [5], determine the

to be accounted for in the energy balance. The starting point of the analysis is then the one-dimensional heat conduction equation:

$$\frac{\partial^2 T}{\partial z^2} = \frac{1}{\alpha} \frac{\partial T}{\partial t} \quad (1)$$

where T is the temperature, α is the thermal diffusivity ($\alpha = k/\rho c$, with k the thermal conductivity, ρ the density, and c the specific heat), t is the time, and z is the direction along which the conduction process occurs. Assuming a semi-infinite solid in the z -direction and negligible heat loss at the $z=0$ boundary, Eq. (1) can be solved to give the spatio-temporal temperature field [14], [15]

$$T(z, t) = \frac{1}{\sqrt{4\pi\alpha t}} \int_0^\infty T(z', 0) \cdot \left[\exp\left(-\frac{(z-z')^2}{4\alpha t}\right) + \exp\left(-\frac{(z+z')^2}{4\alpha t}\right) \right] \cdot dz' \quad (2)$$

In (2), z' is an integration variable that covers the spatial extent of the distributed heat source, and $T(z', 0)$ is the initial temperature distribution that depends on the optical and thermal properties of the material, and incident light fluence. Assuming negligible heat conduction during the optical pulse duration (pulse length $\ll$ thermal conduction relaxation time), the initial temperature distribution is

$$\begin{aligned} T(z, 0) &= \beta \cdot \mu_a \cdot H(z) / \rho c \\ &= \beta \cdot W(z) / \rho c \end{aligned} \quad (3)$$

where $H(z)$ is the spatial fluence, $W(z)$ is the absorbed energy density, ρc is the volume specific heat, and β is the non-radiative deexcitation quantum efficiency (effectively the ratio of heat energy generated to light energy absorbed). In many cases of interest, absorbed light-to-heat conversion is the dominant deexcitation pathway, so β approaches unity; for brevity, we assume $\beta=1$ in the remainder of the analysis.

thermal diffusivity of thin films [6], study thermal conduction at an interface [7]. In the life sciences, PPTR applications have included determination of μ_a of human arteries [8], optical studies of biliary calculi [9], and examination of optical absorption in skin [10]. However, many biological materials at most visible and near-infrared wavelength scatter as well as absorb light, creating a subsurface fluence distribution that can deviate significantly from the monotonic exponential decay predicted by Beer's Law. A PPTR examination of such media may be misleading if the data analysis is performed using an absorbing-only PPTR model.

In this chapter, we shall present a PPTR theory that includes the effect of optical scattering, discuss the instrumentation necessary to perform a PPTR experiment, summarize its biomedical applications to date, and demonstrate its experimental applications with some results from recent turbid media studies [11],[12],[13]. The advantages and limitations of this photothermal technique will then be discussed.

1. THEORY

The mechanism of generation of the photothermal radiometry signal can be understood in terms of the induced temperature excursion in the material caused by the incident optical irradiation pulse [1]. The optical constants of the material and the irradiation geometry determine the spatial distribution of the optical energy, which is delivered, scattered, and absorbed in the material on the picosecond time scale. It is assumed here that the medium is uniform in both optical and thermal properties. We will also assume that all the energy absorbed is converted to heat via non-radiative deexcitation: in reality, some fraction may be lost to luminescent, photochemical, or photomechanical processes, and these may need

The measured radiometric signal is then given by integrating the Stefan-Boltzmann law over all depth [16]:

$$S(t) = \eta a \sigma \mu_{IR} \int_0^\infty [T(z, t)^4 - T_0^4] \exp(-\mu_{IR} z) dz \quad (4)$$

In Eq. (4), T_0 is the uniform unperturbed sample temperature, η is an efficiency factor (incorporating the sensitivity and the collection efficiency of the detector), a is the detection area, σ is the Stefan-Boltzmann constant, and μ_{IR} is the average absorption coefficient of the sample at the wavelengths of IR detection. Note that the surface emissivity, usually quoted when calculating the black-body radiant flux from a real body at a given temperature, is not explicitly present in the expression; rather, its effect is accounted for by the depth integral. The μ_{IR} parameter determines the depth into the sample that contributes to the signal, as most of the emission from depth greater than $\approx 1/\mu_{IR}$ does not reach the surface but rather gets reabsorbed in the sample bulk. Thus, for a very large IR absorption coefficient, the detected signal is indicative of only the surface temperature, whereas a small μ_{IR} (IR transparency) implies a large detection depth. As will be evident below, a small μ_{IR} value decreases the PPTR signal, and in the limit of $\mu_{IR} = 0$ the signal disappears since neither absorption nor emission of the IR radiation is possible. For thermal emission in the 3 - 13 μm band, a typical $1/\mu_{IR}$ depth for biological materials is about 10 - 30 μm . Note that in the above discussion we have assumed that the irradiating pulse duration is small (so that negligible heat conduction takes place *during* the pulse), and the IR detector risetime is fast, a condition readily satisfied by typical experimental systems. When the induced temperature rise $[T(z, t) - T_0]$ is much smaller than T_0 , we can expand the integrand by the binomial theorem and neglect terms of the order of T_0^{-2} and lower, leading to

$$S(t) = C \mu_{IR} \int_0^\infty (T(z, t) - T_o) \exp(-\mu_{IR} z) dz \quad (5)$$

where $C = 4\eta\sigma T_o^3$. In the case of very large or infinite infrared absorption coefficient μ_{IR} , the temperature term is constant over the depth of finite integrand (i.e., the negative exponential will quickly approach zero for anything but the smallest z), and can be taken outside the integral. Eq. (5) then reduces to

$$S(t) = C \cdot [T(z=0, t) - T_o] \quad (6)$$

implying that the signal is a direct measure of the surface temperature transient. In practice, the assumption of an infinite μ_{IR} is appropriate when neither the optical nor the thermal distribution vary significantly in a depth $1/\mu_{IR}$, as discussed below. We now turn to the optical analysis to derive expressions for $H(z)$ and hence for the term $T(z, 0)$ in Eq. (2).

II.A Homogeneous Absorber

We first examine the relatively simple case of a homogeneous absorbing medium in which the light distribution is described by Beer's law [3]. Assuming normal incidence, the spatial fluence $H(z)$ is

$$H(z) = H_o \cdot \exp(-\mu_a z) \quad (7)$$

where H_o is the light fluence at $z=0$ (incident fluence reduced by the loss due to surface specular reflection). Combining Eqs.(2), (3), (5), and (7), without assuming a large μ_{IR} , the PPTR signal is

II.B Homogeneous Turbid (Scattering and Absorbing) Media

The distribution of light in a scattering and absorbing medium has been the subject of intense theoretical and experimental investigations, and several models of varying complexity have been developed [17], [18], as discussed in detail in Chapters A - B of this volume. In the present formulation, only the diffusion approximation will be employed, as it provides one of the simplest closed-form expressions for the photon fluence distribution, and its range of validity in tissue optics has been established. As discussed extensively in the literature [19], the diffusion approximation is generally valid far from sources and boundaries in highly scattering media, i.e., media in which the scattering coefficient $\mu_s \gg$ absorption coefficient μ_a , or, equivalently, the single scattering albedo $a = \mu_s/(\mu_s + \mu_a)$ is high. Although this condition may not be strictly true for a given light wavelength and tissue sample, we shall choose a solution to the diffusion equation that reduces to the Beer's-law behavior when absorption dominates scattering. We proceed to derive the PPTR response of a turbid medium assuming the diffusion approximation is valid for intermediate values as well, and will later present experimental results to justify this assumption.

The derivation of the diffusion equation and its solutions under different sets of conditions has been presented in *Chapter XX* (this volume) and appears in numerous references [17], [18]. We can write the solution for a one-dimensional, uniform, collimated beam normally incident on a semi-infinite optical slab in a convenient form as a sum of two exponentials [20]:

$$S(t) = \frac{4\eta\sigma T_o^3 H_o}{\rho C} \cdot \frac{\mu_a \mu_{IR}}{\mu_{IR}^2 - \mu_a^2} \cdot [\mu_{IR} \cdot F(\mu_a^2 \alpha t) - \mu_a \cdot F(\mu_{IR}^2 \alpha t)] \quad (8)$$

where $f(x) = \exp(x) \cdot \text{erfc}(\sqrt{x})$, and $\text{erfc}(x) = 1 - \text{erf}(x)$ is the complementary error function [14]. The functional form of Eq. (8) represents a monotonic decay of signal with time, with the rate dependent on the optical and thermal properties of the sample. This is illustrated in Fig. 1, which plots theoretical PPTR profiles for some typical parameter values appearing in Eq. (8). In 1(a), we demonstrate the effect of varying the optical absorption coefficient, while 1(b) shows the effect of variable thermal diffusivity. Note that the IR optical absorption coefficient, μ_{IR} , plays a significant role in the PPTR expression by determining the net IR emission from the sample for a given temperature distribution. This is especially important in estimating the measured temperature jump; as seen from Eq. (8), the maximum signal corresponding to the initial temperature excursion occurs at $t=0$:

$$S(t=0) = S_o = 4\eta\sigma T_o^3 \cdot \frac{H_o}{\rho C} \cdot \left(\frac{\mu_a \mu_{IR}}{\mu_{IR}^2 - \mu_a^2} \right) \quad (9)$$

Thus, the measured maximum corresponds to the initial temperature jump ($= H_o/\rho C$), reduced by a factor $\mu_{IR}/(\mu_{IR} + \mu_a)$. As discussed by Anderson *et al.* [11], this reduction is significant when the approximation $\mu_{IR} \gg \mu_a$ is not valid. This is illustrated graphically in Fig. 1(c). In effect, the important optical parameter controlling the signal magnitude (" μ_{IR} ", the bracketed term in Eq. (9)) depends on the optical absorption coefficients at *both* the excitation *and* the detection wavelengths through the inverse relation $1/\mu_{IR} = 1/\mu_a + 1/\mu_{IR}$. As well, it is evident that the signal depends on the product $(\mu_a \mu_{IR})$, and decreases as either parameter becomes small.

$$H(z) = A \cdot \exp(-\mu_{eff} z) + B \cdot \exp(-\mu_{tr} z) \quad (10)$$

$$A = \frac{H_o (9 + 6K) \mu_s' D}{(1 + K\sqrt{4\mu_a D}) (1 - 9\mu_a D)} \quad , \quad B = \frac{-2H_o}{1 - 9\mu_a D}$$

where $\mu_s' = \mu_s(1-g)$ is the reduced scattering coefficient, g is the average cosine of the scattering angle (1st moment of the scattering phase function), $\mu_a = \mu_a + \mu_s'$ is the transport coefficient, H_o is the surface fluence, K is a semi-empirical constant that depends on the refractive indices of the sample and surrounding medium [21] (it is a monotonically increasing function of the index mismatch, equal to 3.25 for a typical mismatch ratio of 1.4/1), D is the optical diffusion constant $[3(\mu_a + \mu_s')]^{-1}$, and $\mu_{eff} = (\mu_a/D)^{1/2}$ is the effective attenuation coefficient. Notice that, in the limit of negligible scattering, $A \rightarrow 0$, $B \rightarrow H_o$, and $\mu_a + \mu_s' \rightarrow \mu_a$, in agreement with Eq. (7); this is because the solution in Eq. (10) includes the contribution from the uncollided (collimated) flux. Thermal analysis using the source function in Eq. (10) results in

$$S(t) = C \cdot \frac{\mu_a}{\rho C} \cdot [A \cdot F(\mu_{eff}^2 \alpha t) + B \cdot F(\mu_{tr}^2 \alpha t)] \quad (11)$$

where we have assumed an IR absorption coefficient much larger than either μ_a or μ_{eff} (using Eq. (6)). In this expression, the two unknowns that determine the rate of signal decay are the optical absorption and reduced scattering coefficients, μ_a and μ_s' . In the general case of comparable μ_{IR} , μ_a , and μ_{eff} , Eq. (5) yields

$$S(t) = C \cdot \frac{\mu_a \mu_{IR}}{\rho C} \cdot \left[\frac{A}{\mu_{IR}^2 - \mu_{eff}^2} \{ \mu_{IR} \cdot F(\mu_{eff}^2 \alpha t) - \mu_{eff} \cdot F(\mu_{IR}^2 \alpha t) \} \right. \\ \left. + \frac{B}{\mu_{IR}^2 - \mu_{tr}^2} \{ \mu_{IR} \cdot F(\mu_{tr}^2 \alpha t) - \mu_{tr} \cdot F(\mu_{IR}^2 \alpha t) \} \right] \quad (12)$$

As in the case of a purely absorbing medium (Eq. (8)), the signal vanishes as either μ_a or μ_{tr} $\rightarrow 0$.

The effect of scattering on the PPTR signal is demonstrated in Fig. 2(a), which shows theoretical signal profiles derived from Eq. (12), for a fixed μ_a ($= 30 \text{ cm}^{-1}$) and a range of μ_s from 10 to 50 cm^{-1} . The absorption coefficient and the thermal diffusivity are chosen to facilitate easy comparison with Fig. 1. The highest albedo sample exhibits the largest percentage signal drop from the initial jump. The corresponding fluence profiles, calculated from Eq. (10), are shown in part (b) of the Figure. The increase in the subsurface fluence accounts for the increasing initial temperature excursion as the scattering coefficient is increased. This effect has been explained by Anderson *et al.* [11] using a heuristic argument based on the total internal reflection of backscattered photons at the surface boundary. The result was

$$H(z=0) = H_0 \cdot (1 + 2kR_d) \quad (13)$$

where R_d is the diffuse reflectance of the material which has a refractive index-mismatched boundary condition. An equivalent result can be derived rigorously by evaluating Eq. (10) at $z=0$:

$$H(z=0) = A + B = H_0 \left[\frac{(9+6k)\mu_s' D}{(1+k\sqrt{4\mu_s D})(1-9\mu_s D)} + \frac{-2}{1-9\mu_s D} \right] \quad (14)$$

which can be expressed as

centered at 5 μm ; the latter two detector types are best for room-temperature work of the type described here, with broad-band response typically centered at about 10 μm . Cryogenic cooling may be necessary to minimize the noise in the detection system. The impulse response of the complete PPTR set-up should usually be a few μs or less (i.e., a bandwidth of 0 to about 1-10 MHz), so that the excitation and the detection are "instantaneous" on the heat transfer time scale, and the finite response times of the experimental components need not be included in the model.

Fig. 3 shows an example of an experimental PPTR system used for tissue studies. The sample is placed in contact with a metal sample-mounting plate with optical access through a circular aperture. The excitation source in this experiment is a coaxial flashlamp-pumped tunable dye laser, emitting 700 ns pulses at 627 nm. The excitation fluence incident on the sample is typically 300 $\text{mJ} \cdot \text{cm}^{-2}$ per pulse. In order to obtain a wide uniform beam profile, the output from the laser is focused into a 1 mm diameter silica core multi-mode fiber, and the emerging light beam is directed through a standard microscope objective at the sample at $\sim 15^\circ$ from the normal. A real, magnified image of the fiber tip is projected onto the sample surface. This provides a nearly uniform ($\pm 10\%$) round exposure area several mm in diameter. Beam homogenizing is also possible with a lens-aperture combination, especially if the laser output is not too noisy. If the uniformity of the incident irradiation is not assured, the validity of the one-dimensional analysis described earlier may be questioned.

The IR emission is monitored at normal incidence to the sample surface by a liquid nitrogen cooled HgCdTe radiation detector placed at the focal plane of a germanium lens. The interference filter / detector combination used yields an effective IR detection bandwidth from 7.5 to 14 μm . For measurement of incident fluence, a known portion of light exiting

$$H(z=0) = H_0 \cdot \left[1 + 2k \cdot \left\{ \frac{a'}{1 + 2k(1-a') + \left(1 + \frac{2k}{3}\right) \sqrt{3(1-a')}} \right\} \right] \quad (15)$$

where the quantity in the curly brackets is the diffuse reflectance as predicted by the diffusion theory [22], for reduced albedo $a' = \mu_s' / (\mu_a + \mu_s')$.

III. INSTRUMENTATION

In this section, we discuss the characteristics of the experimental system for PPTR investigation of biological media. The central components are an excitation source to create the initial temperature field, and an IR detector to monitor the resultant thermal emission. Various pulsed light sources can be used, for example a nitrogen, an excimer, a dye, or a Q-switched ruby laser with pulse durations on the order of nanoseconds to milliseconds. Desired pulse energies depend on the irradiation spot size, detector sensitivity, and absorption of the sample, but generally 10-30 mJ per pulse should suffice. It is desirable to limit the maximum sample heating to about 10 $^\circ\text{C}$ to avoid any sample damage or any nonlinear radiometric effects, as the PPTR is linear in the temperature rise ΔT (Eq. (5)) only for small ΔT . As well, temperature rises above 20 $^\circ\text{C}$ should be avoided to prevent excessive heat loss at boundary. Averaging of PPTR signals from multiple optical pulses is often unnecessary, although the signal-to-noise may be improved this way if needed; in using signal averaging, the repetition rate should not exceed $\sim 1 \text{ Hz}$ to allow temperature relaxation between pulses. Common IR detectors are liquid nitrogen cooled InSb, PbSnTe, or HgCdTe systems, with the last being the most common. The InSb detector may be preferable when working in a high temperature environment, as its peak sensitivity is

the fiber is diverted to an energy meter by a beam splitter. The electrical response of the detector, after pre-amplification, is monitored with a fast digital oscilloscope, triggered by a silicon photodiode that detects the onset of the dye laser pulse. The stability of the measuring apparatus is checked periodically by observing the signal from a stable, absorption-type glass neutral density filter.

IV. RESULTS AND ANALYSIS

IV.A Absorbing Media

Beginning in the early 80's, Nordal and Kanstad [23],[24] performed optical spectroscopy on green leaves, and reported measurements on human skin using a frequency-domain version of PPTR. Following the appearance of the PPTR theory several years later [1],[2], and further preliminary examinations of skin [4], the first detailed biomedical study of Long and Deutsch [8] investigated optical transfer in normal and diseased human artery at several excitation wavelengths in the near UV and visible spectrum. These were opaque, optically thick samples, which were destroyed by attempting to make thin samples of known thickness for photometric measurements. Due to the possible weak presence of scattering, the authors were careful to state that the derived optical parameter may not be the absorption coefficient but is more properly termed the attenuation (or extinction) coefficient. The high values of the derived attenuation coefficients (25 - 180 cm^{-1}), and the ill-defined geometry of the specimens clearly demonstrated the main advantages of PPTR as an alternative non-contact method to measure the optical properties of tissues which are not amenable to conventional

optical techniques.

In a similar application, Long *et al.* [9] reported PPTR measurements of optical and thermal properties of opaque specimens. They derived values of the optical attenuation coefficient and of thermal diffusivity of cholesterol and pigment biliary stones. As conventional spectrophotometric methods necessitated optically thin stone slices, PPTR was chosen because no special sample preparations are needed, and the technique was well suited for measurement of optically thick materials. Assuming $\mu_{TR} \gg \mu_a$, the authors analyzed the measured spectra with a simplified form of Eq. (8); as the time decay of the signal is specified by the argument of the first complimentary error function in that equation, they determined the best-fit value of μ_a^2/α for the different stone types.

Another very important application of potential value to the characterization of biological structures is the remote sensing of layered absorbing structures by PPTR, as reported by Long *et al.* [10]. A buried blood layer at the epidermal/dermal boundary of human skin is manifest as a delayed peak in the IR signal. The irradiation wavelength was 577 nm, coinciding with one of major oxyhemoglobin absorption bands. Knowing the thermal diffusivity of skin, and, with less precision assuming typical absorption coefficients of the epidermis and the dermis, these authors [10] have calculated the depth of the buried absorber from the delay time of the maximum signal. The results are illustrated in Fig. 4. Note the non-zero initial signal (at $t=0$), thought to be due to melanin absorption in the epidermis and stratum corneum. No delayed thermal wave was seen at 632 nm excitation, as there is relatively little absorption by oxyhemoglobin at that wavelength. Again, possible presence of scattering interactions must be accounted for in the model if the results are to be quantitatively consistent. This study illustrates the use of PPTR in characterizing an optically

of determining μ_a ignores the potentially useful information in the rest of the time signal.

To circumvent these drawbacks, Prah *et al.* have developed two alternative approaches that use the temporal structure of the PPTR response. The first, called the normalization method because the entire signal is divided by the initial (peak) value, obviates the necessity to know the incident fluence and the calibration factors by writing the signal as a function of the measured value $S(t=0)$. From Eqs. (8) and (9),

$$\frac{S(t)}{S(t=0)} = \frac{\mu_{TR} \cdot F(\mu_a^2 \alpha t) - \mu_a \cdot F(\mu_{TR}^2 \alpha t)}{\mu_{TR} - \mu_a} \quad (16)$$

The only unknown in Eq. (16) is the absorption coefficient, which is chosen for any time t to satisfy the equality. The α value used in the fit is $0.0013 \text{ cm}^2 \cdot \text{s}^{-1}$, equal to that of water. The results of the calculation are shown in column B of Table I. The errors are the standard deviations of the individual μ_a values in the 0-180 ms time interval. Although this method also relies heavily on the accuracy of the $t=0$ signal value, the results obtained are in close agreement with the known absorption coefficients.

In order to avoid the dependence on the accuracy of the $S(t=0)$ measurement that is inherent in the last two approaches, and to develop a general method that is applicable to the more challenging turbid media problem, Prah *et al.* chose to fit the complete time signal using a non-linear two-parameter fit. Eq. (8) can be re-written to display the explicit dependence of the signal on the two parameters, K and μ_a :

$$S(t) = K \cdot \frac{\mu_a \mu_{TR}}{\mu_{TR}^2 - \mu_a^2} \cdot [\mu_{TR} \cdot F(\mu_a^2 \alpha t) - \mu_a \cdot F(\mu_{TR}^2 \alpha t)] \quad (17)$$

where $K (=4\eta\sigma\sigma T_s^3 H/\rho c)$, the scaling multiplier, represents a system calibration constant

transparent but thermally detectable overlayer, a concept that may be useful in biomedical diagnostics.

Finally, for the purpose of illustrating the quantitative application of the theory derived earlier, we now review, in some detail, results from a control study of Prah *et al.* [12] in which tissue phantoms of known optical properties were characterized by PPTR. The phantoms were 5 mm thick aqueous collagen gels with varying amounts of trypan blue dye as the absorber and polystyrene microspheres as scatterer. Fig. 5 shows a typical thermal response of a non-scattering absorbing gel phantom following a single 1 μs , 300 $\text{mJ} \cdot \text{cm}^{-2}$ dye laser pulse at 627 nm. A rapid temperature jump followed by a monotonic decay is seen, in agreement with the theoretical signal profiles shown in Figs. 1 and 2. The estimated initial temperature excursion was approximately 7 °C, based on the calibration of the detector with a blackbody temperature source; this is well within the range of validity of the PPTR theory.

To quantify the PPTR method, one must solve the inverse problem, i.e., to obtain the optical coefficients from the experimental signals. The μ_a of a purely absorbing sample may be determined from the initial signal magnitude, a fluence measurement, and several calibration factors by using Eq. (9) [12], [13]. The results are shown in Column A of Table I, with the errors determined from experimental uncertainties. The 'true' values for the absorption coefficient μ_a were determined with an independent spectrophotometric measurement of the absorbing gel phantoms. Although the results are in reasonable agreement with the true sample values, the peak PPTR signal is difficult to measure reliably because it is sensitive to any coincident noise present in the early time signal, such as stray IR radiation from the excitation source or electromagnetic noise resulting from the laser pulse; besides, this method

$4\eta\sigma\sigma T_s^3$, multiplied by a sample-specific factor $H/\rho c$. Using Eq. (17) with $\alpha = .0013 \text{ cm}^2 \cdot \text{s}^{-1}$ and $\mu_{TR} = 1200 \text{ cm}^{-1}$, one can calculate the residuals in the (K, μ_a) space, where the residual is the sum of the squares of the difference between the predicted and measured values of $S(t)$ over the time interval of interest. The absorption coefficient is then estimated from the minimum in the residual response surface. If the best-fit values are now used to calculate the residual for $S(t) \pm \Delta S$ (ΔS is the average experimental uncertainty in the signal), then the range of parameter pairs with the residuals within this contour gives an error estimate for the fitted values. The results are presented in column C of Table I. Again, the agreement between known values and ones derived from the measurement is quite good.

The most striking feature of the results of Prah *et al.* is that all three methods are very successful in determining the absorption coefficient of the absorbing samples. Note that photometric techniques would be very difficult to implement for such highly absorbing non-scattering thick samples. Specifically, from Table I, the reconstruction accuracy of the first and second method (columns A and B) is as high or somewhat higher than that of the least-residual response surface approach (column C). However, any early-time noise coincident with the optical pulse renders the first two approaches ineffective. Under more challenging experimental conditions, the more general and robust (K, μ_a) search must be used, since the peak signal value is no more crucial than any other $S(t)$ in the fitting time interval.

IV.B Absorbing and Scattering (Turbid) Media

PPTR studies of absorbing and scattering materials have been few to date. Although scattering is present in varying degrees in most biological samples, the first PPTR study aimed specifically at quantifying its effect was performed by Anderson *et al.* [11].

Comparison of the initial temperature jumps in homogeneous absorbing media with and without scattering showed the dependence of the PPTR signal on the fluence distribution. By varying the refractive index mismatch at the boundary, the authors tested the relationship between the diffuse reflectance and the peak of the PPTR signal, and found it in good quantitative agreement with the predictions of Eq. (13). Their study thus showed that differences in the subsurface fluence profiles, caused by different amounts of scattering, can be monitored remotely by PPTR. This study also directly confirmed the values of k inherent in the diffusion theory given in Eq. (15).

With the current interest in laser angioplasty for blood vessel recanalization, Obremski *et al.* [25] employed PPTR to study light interaction with vascular grafts, sutures, and thrombus. Analysis of the peak height, based on Eq. (13) and supplemented with an independent measurement of R_d and μ_{tr} , yielded absorption values for thrombus that were significantly higher than for the artificial materials, at a wavelength of 480 nm. The μ_a values calculated with the Kubelka-Munk analysis of integrating sphere data on thin films of the same samples were consistently higher than PPTR absorption results, but the same trends in both studies were seen.

The sensitivity of PPTR to surface processes was utilized by Imhof *et al.* [26] to monitor *in-vivo* skin properties and surface concentrations of topically applied sunscreen preparations. Because of the presence of scattering, the absorption model of Eq. (8) did not apply, and the authors parametrized the PPTR signal in terms of a phenomenological opto-thermal decay time parameter. Estimates of relative surface concentrations and characteristic residence periods of three different sunscreens were obtained, and possible future applications such as monitoring of transdermal drug delivery were noted.

parameter fit. Instead of optimizing each parameter independently, it is useful to minimize the residuals by optimizing K at any (μ_a, μ_s') point. Rewriting Eq. (12) as $S(t) = K \cdot \text{Fit}(\mu_a, \mu_s')$, we can minimize the residuals explicitly with respect to K :

$$\frac{\delta R}{\delta K} = \frac{\delta}{\delta K} \sum (S_e - K \cdot \text{Fit}(t))^2 = 0 \quad (18)$$

where S_e is the experimentally measured signal, and the summation is taken over the appropriate time window. The K that will minimize the residual at a particular (μ_a, μ_s') pair is thus

$$K_{\text{opt}} = \frac{\sum [S_e \cdot \text{Fit}(t)]}{\sum [\text{Fit}(t)^2]} \quad (19)$$

Unfortunately, a given PPTR signal does not necessarily yield a unique minima, and sets of (μ_a, μ_s') pairs can be found that fit the measurement equally well. This is illustrated in the contour plot Fig. 7(a), where we find a minima valley in the (μ_a, μ_s') space, each point corresponding to a different value of K_{opt} . The valley, which includes the true value of the coefficients, implies a multiplicity of acceptable solutions. Physically, this says that different optical distributions can yield similar PPTR signals when irradiated by suitably chosen input fluences (Fig. 7(b)). Thus, it appears that the signal shape alone is insufficient to determine the absorption and the reduced scattering coefficients uniquely. In order to select a particular (μ_a, μ_s') pair from the contour plot in the absence of the K -value, one needs an extra piece of (optical) information to act as "additional coordinate" along the valley. Experimentally, a convenient way to resolve the ambiguity is to measure the diffuse reflectance of the sample. This will specify the transport albedo within the range of validity of diffusion theory (since $R_d = f(\alpha')$ - see discussion following Eq. (15)), and thus provide an additional constraint on the optical coefficients that should collapse the minimum valley of Fig. 7(a) to a single point.

The full solution of the inverse problem of PPTR in turbid media has recently been described by Prah *et al.* [12] who used the least-residual method to derive the values of μ_a and μ_s' . They have assumed constant values for α and μ_{tr} , and have used the value of K derived from the corresponding absorbing phantoms and scaled by incident fluence; then the PPTR signal described by Eq. (12) depends only on μ_a and μ_s' . The same search algorithm as used for absorbing-only samples, now with the optical coefficients as the fitted parameters, has yielded encouraging results; the % deviation from true sample values is shown in Fig. 6. The 'true' reduced scattering coefficients have been calculated from Mie theory. The agreement between the true μ_a and μ_s' values and those derived from the fit is within $\sim 10 - 15\%$ for the range of transport albedo of 0.42 to 0.88. It should be noticed from Fig. 6 that the agreement appears equally good for the entire albedo range, indicating the applicability of the PPTR theory based on the diffusion approximation. While the validity of the diffusion approximation is well established in highly scattering media ($\alpha' > 0.9$), its accuracy is unclear in some of the less scattering gels of this study set. However, good agreement has been found. In the absorption-dominated regime, this is due to the inclusion of uncollided flux contribution in the derivation of Eq. (10). The good agreement in the intermediate albedo range ($\mu_a \approx \mu_s'$) is especially encouraging, as present analytic solutions of the transport equation are generally inaccurate in this regime [27].

In some application of PPTR, there may not be enough information to determine the value of the scaling factor K . Additional experimentation to determine the instrument calibration curve, the incident fluence, and estimate of the sample's thermal properties can then be used to estimate K , as demonstrated by the *in-vivo* example described below [13]. Alternatively, in principle one may forego additional experiments and thermal properties estimation in favour of a slightly more complicated mathematical procedure that involves a three-

Finally, we conclude this section with a recent example of an *in-vivo* PPTR study in turbid media [13]. Fig. 8 shows several spectra collected at different times (in minutes) in rat liver following an *i.v.* injection of an absorbing dye solution, including a *post mortem* signal. The laser output (energy = 60-100 mJ, pulse length = 1 μ s, wavelength = 630 nm), after passing through a homogenizing length of 1-mm optical fiber, was imaged to a 5-mm-diam uniform spot on the liver surface, with the HgCdTe detector viewing the central 1 mm² of the illuminated area. The uptake of the dye is manifest as a change in absorption, and thus the PPTR signal, so its time course in the tissue can be monitored using PPTR. Results can be further quantified by an analysis described above, namely by measuring some (K) , and estimating other ($\mu_{tr} = 1200 \text{ cm}^{-1}$, $\alpha = 0.0015 \text{ cm}^2 \text{ s}^{-1}$, index mismatch = 1.38) variables in Eq. (12), and then performing a non-linear least-squares two-parameter fit to deduce μ_a and μ_s' . K was determined using a reference measurement from a well-characterized neutral density filter irradiated with a known incident fluence, scaled to account for the differences in thermal properties of tissue and glass. The (μ_a, μ_s') results indicate that the uptake of the dye increases the absorption coefficient of the tissue without appreciably affecting its scattering properties, as expected. The outcome of this feasibility study, namely the reasonable range of the deduced values, and the ability of the model to properly separate the absorption and scattering components is encouraging for future applications of PPTR. These applications will likely involve tissues with significant (endogenous or exogenous) absorption, where the use of purely photometric techniques is problematic.

In working with tissue volumes of finite dimensions like liver, it is prudent to question the assumptions of the 1-D, semi-infinite geometry that were used to derive the PPTR theory. For the optical part of the problem, the uniform beam width (5 mm), and the thickness of the liver lobe ($\approx 4 \text{ mm}$) are both greater than the effective optical penetration depth of 630

nm light in soft tissue (≈ 1 mm), validating the 1-D, semi-infinite (optically thick) geometry formulations, respectively. For the thermal analysis, the relevant dimension is the thermal diffusion length, $l_{th} = \sqrt{4 \cdot \alpha \cdot t}$. At the end of our observation interval, $t = 200$ ms, which corresponds to $l_{th} = 0.35$ mm. This is again much smaller than the other characteristic lengths (i.e., the radial extent of the beam and the tissue thickness), so the formulation is applicable in the thermal domain as well. In addition, the IR detector is viewing the center of the irradiated spot, where any possible deviation due to radial light and heat propagation is least manifest.

To estimate the sensitivity of the PPTR technique, we note that the detection system described in Fig. 3 shows a linear response to the temperature of the blackbody source, with a calibration slope of roughly $9.0 \text{ mV} \cdot ^\circ\text{C}^{-1}$. The background rms noise signal is about 3mV peak-to-peak, implying that the NEP for this system would translate into a minimum resolvable temperature rise of 0.3°C . This forms a rough estimate of the PPTR sensitivity to temperature changes, although some improvement is possible, for example by signal averaging or by overfilling the blackbody detector to maximize the signal-to-noise ratio. We may proceed further and determine the minimum resolvable changes in the optical absorption and transport scattering coefficients of a turbid medium provided that the model of Eq. (12) applies. Since the signal is directly proportional to incident fluence H_0 , one may simply increase the incident pulse energy until a detectable signal appears. Most previously reported studies have operated at irradiance levels of $1 \text{ J} \cdot \text{cm}^{-2}$ or less; using typical values for the parameters in Eq. (12), we estimate the minimum detectable changes in optical properties to be $\Delta\mu_a = 2.5 \text{ cm}^{-1}$ and $\Delta\mu_s' = 5 \text{ cm}^{-1}$. However, in weakly absorbing samples, or in samples with very similar optical properties, higher H_0 values (perhaps $10\text{--}100 \text{ J} \cdot \text{cm}^{-2}$) will generate stronger signals and will increase the resolution in μ_a and μ_s' , and increase the

optical spectrum as well. This agreement has been verified in the gel phantom study for the transport albedo range of 0.42 to 0.88. The μ_a and μ_s' values derived from the PPTR data of the rat liver study suggest that the theory may be applicable in lower albedo regime as well. Further rigorous testing in the transport albedo range below 0.4 is required to ensure that the derived optical coefficients are accurate.

An additional issue in PPTR studies in turbid media is the best strategy for selecting the true μ_a and μ_s' values from the multiplicity of acceptable solution pairs. The ambiguity may be somewhat circumvented by using experimental calibration values and an estimate of other parameters that are contained in the factor K of Eq. (12) [13]. An alternative promising experimental strategy is an independent measurement of the diffuse reflectance R_d from which an albedo value could be deduced. Experimental measurement of R_d can be performed simultaneously with PPTR, by the simple addition of a photodetector for reflected light.

There are many interesting biological materials which can be, and have been to a limited extent, examined experimentally with PPTR. However, for studies of striated (e.g. skin, muscle) or optically inhomogeneous media (e.g. retinal pigment epithelium), for which the non-contact non-invasive nature of PPTR is well suited, the present models are not quantitatively applicable. The extension of the PPTR theory to layered turbid structures is fairly straight forward, and would involve using an appropriate $H(z)$ fluence functions and carrying out the integrations outlined in Eqs. (2) through (5). This is fact has already been done for the simpler case of layered absorbers [10]. The analysis of the optically inhomogeneous media (such as a transparent matrix loaded with discrete absorbers) is a more formidable task which may require a different formulation of the thermal as well as the optical analysis. PPTR investigations in such tissues may elucidate the mechanisms of photo-

minimum detectable value of μ_a . At still higher irradiance levels, one may monitor the onset of non-linear optical processes, such as two-photon absorption or dielectric breakdown through their effect on the PPTR signal.

V. FUTURE TRENDS AND CONCLUDING REMARKS

Pulsed photothermal radiometry is used to study optothermal transfer dynamics in a variety of materials. In this chapter we have outlined the basis of PPTR measurements for turbid biological samples. The time profile of a sample's response to a surface optical impulse is governed by its optical and thermal properties: the former determine the distribution of the optical energy, while the latter dictate the spatial and temporal behavior of the resultant temperature field. For absorbing, and for absorbing and scattering media we have shown how to get the optical interaction coefficients if the thermal properties are known. The theoretical derivations are illustrated with experimental results from biological PPTR studies. The common theme emerging from these investigations is that PPTR is a useful probe for determining optical properties and layered structure in tissues.

While the use of PPTR in absorbing media has been well established in both inert and biological materials, the range of validity of the turbid medium PPTR analysis presented here depends on the applicability of the diffusion approximation to the material under study. This does not present a problem for many homogeneous mammalian tissues (e.g. liver, brain, breast) in the red and near infrared (600 - 1110 nm). In this region, scattering events are much more probable than absorption events, and the diffusion approximation is appropriate. Given that the derived turbid media solution reduces to Beer's law in the limit of negligible scattering, the validity of the formulation applies to absorption-dominated regions of the

thermal transfer that occur during pulsed laser procedures, for example the details of transient heating and cooling of targeted chromophores during selective photothermolysis [28].

To broaden the scope of clinical applications of PPTR, one would prefer to use an infrared optical fiber to collect the blackbody emission so as to examine tissues and organs that are not directly accessible with a lens-based detection system currently in use. At present, *in vivo* measurements are limited to tissues that can be placed in the line-of-sight of the IR detector (e.g., skin, teeth, surgically exposed internal organs). Some recent work with hollow waveguides [29] and IR transparent glasses [30],[31], appears promising in this respect. Depending on the source and fiber geometry, the 1-D theory described in this chapter may need modification, especially if the excitation and detection fiber are radially offset from each other.

Optothermal techniques should complement purely optical methods in the studies of optically opaque materials, because they are sensitive to *absorbed* radiation (higher absorption means higher PPTR signal); in contrast, photometric experiments become more difficult as absorption increases (more light lost in the sample). The thickness of the sample (provided that it is optically and thermally thick) and the precise orientation of the surface relative to the source and the detector (provided that it is within $\sim 20^\circ$ from normal) do not seem to influence the PPTR signal. This relative insensitivity to the precise geometry of the specimen is another advantageous feature of the method. In summary, PPTR is an attractive tool for optical and thermal investigations of biomedical materials which are not easily characterized by other techniques.

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Fig. 1.

(a) The effect of absorption on the PPTR signal. Theoretical profiles obtained by varying μ_a in Eq. (8) at fixed incident fluence. Other relevant parameters have the values shown above. (b) The effect of varying the thermal diffusivity $\alpha = k/\rho c$. The change in α is assumed to arise from the change in the volumetric specific heat. If ρc is held constant, and k varies, the curves start off from a common maximum at $t=0$, and the one with the largest α decays the fastest. (c) The importance of the infrared absorption coefficient, μ_R , in measuring the peak surface temperature. Note that the true and the measured temperatures are nearly equal only if $\mu_a \ll \mu_R$.

Fig. 2. (a) The effect of scattering on the PPTR signal. Theoretical profiles obtained by varying μ_s' in Eq. (12) at a fixed incident fluence. (b) The corresponding fluence distributions in the sample, calculated from Eq. (10). The $\mu_s' = 0$ curve is described by Beer's law.

Fig. 3. Schematic diagram of the experimental PPTR apparatus currently in use for tissue optics research (with permission [12]).

Fig. 4. PPTR signal from *in vivo* human skin containing a subsurface blood layer at the epidermis/dermis boundary. The excitation wavelength is 577 nm (with permission [10]).

Fig. 5. Typical PPTR signal from a homogeneous absorbing-only gel phantom. The incident fluence is $300 \text{ mJ} \cdot \text{cm}^{-2}$ (with permission [12]).

Fig. 6. Percentage deviation from true values of μ_a and μ'_s derived from the analysis of turbid gels; see text for details (with permission [12]).

Fig. 7.

(a) Least-residual response surface for a turbid phantom. The true optical properties of this sample are indicated by the asterisk. For each (μ_a, μ_s) point, the optimum K is found from Eq. (19). No unique minimum residual exists. (b) Two theoretical PPTR profiles generated using two sets of triplets (μ_a, μ_s, K_{opt}) from the minimum residual valley of Fig. 7(a) (with permission [12]).

Fig. 8.

PPTR signals from rat liver *in vivo* before, at 0.5 - 35 minutes after injection of an absorbing dye solution, and immediately *post-mortem* (with permission [13]).

True μ_a	(A) Peak height	(B) Normalization	(C) Two-parameter
144	137 $\pm$ 23	147 $\pm$ 9	137 $\pm$ 14
117	125 $\pm$ 21	104 $\pm$ 7	104 $\pm$ 10
93	102 $\pm$ 17	92 $\pm$ 5	87 $\pm$ 9
70	73 $\pm$ 12	71 $\pm$ 4	71 $\pm$ 7
46	52 $\pm$ 8	49 $\pm$ 4	49 $\pm$ 5
23	30 $\pm$ 5	21 $\pm$ 6	21 $\pm$ 2

TABLE I. Summary of the results of the three methods used to find the absorption coefficients of absorbing samples. Column (A) - from peak height analysis and calibration curve information. (B) - from a fit to Eq. (16). (C) - from the minimum in the response surface. All values are in units cm^{-1} (with permission [12]).

CHAPTER IV. Conclusions and Future Directions

In this chapter we will briefly summarize the experimental and theoretical findings of our PPTR investigations, and will recommend some possible directions for future work. Specific conclusions have already appeared at the end of the corresponding papers.

In Paper #1, we examined the application of PPTR to studies in turbid, semi-infinite media. An analytic theory for the time dependence of the signal was derived and tested in a series of gel phantoms with well-defined optical and thermal properties. The theory is based on the solutions of the optical diffusion and the heat conduction equations. It can be used in a non-linear, two-parameter fitting routine to determine the absorption and the reduced scattering coefficients of the turbid sample with $\sim 10\%$ accuracy; however, an additional piece of optical information is required to ensure the convergence of the fit. In our experiments, the additional information was the overall scaling factor obtained from a PPTR-spectrum of an absorbing-only phantom. Another possibility is to use the transport albedo from a diffuse reflectance measurement. In addition, three different methods for obtaining the absorption coefficient in the absence of scattering have been outlined.

Several specific directions for future investigation became evident as a result of this study:

- The optimum experimental and theoretical strategy for obtaining the unique (μ_a, μ_s') pair from a single PPTR signal needs to be specified.

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distributions, similar energy densities, or similar initial temperature patterns? It may then be that the PPTR signal is a function of some combined group of optical/thermal/geometrical parameters, more than of the individual parameter values. The predictive ability of the model to determine the fundamental tissue properties may be restricted, but the model should still be useful for determining optical and thermal distributions in laser irradiated tissues.

- The parameter range over which the model is sensitive to the presence of two layers of different optical properties needs to be examined.

- In order to arrive at a closed-form analytical solution, we assumed that the IR absorption coefficient is much larger than any visible optical coefficient in the medium. This was certainly true for the water-based phantoms, but may or may not be true in some tissues. To arrive at a more rigorous solution, one needs to re-derive the final expression for $S(t)$ retaining all the terms containing μ_{IR} . This can probably be done at the expense of simplicity; the final expression will contain ten terms instead of the five now present (see Eq. (18) of Paper #2).

- The presence of the factors T in the source term [Eq. (4)] and F in the fluence boundary condition [Eq. (6)] remains controversial. There is definite disagreement about these factors in the literature, and it is very difficult to decide which approximation is consistent with diffusion theory. For the purposes of the paper, this effect is irrelevant (we used $n_1=n_2$, both theoretically and experimentally, so that $T=F=1$), but the correct expression must be determined for future use.

Paper #3 dealt with opto-thermal transfer in a transparent medium containing

- The range of validity of the derived theory needs to be examined over a wider albedo range. As well, it would be interesting to see if two different scattering materials with different μ_s and g values, but with identical $\mu_s'=\mu_s(1-g)$, will yield similar PPTR signals. In other words, does the optical Similarity Principle carry over into the photothermal domain?

- The effects of variable thermal diffusivity can be examined further. For example, if it is used as a free fitting variable along with μ_a and μ_s' , how is the minimum residual valley in (μ_a, μ_s') space affected? The response surfaces in (μ_a, μ_s') space looked similar for 5-10% variations in the thermal diffusivity, but this effect was not evaluated rigorously.

Theoretical and experimental analyses of layered, semi-infinite, turbid media using PPTR were presented in Paper #2. The solution of the one-dimensional optical diffusion equation in a two-layered system was used in the heat conduction equation to derive the temporal dependence of the surface temperature following a brief optical pulse. The predicted thermal transient was found to agree well with the PPTR signals from a series of layered phantoms of varying optical properties. The derived model may be useful for depth profiling in layered media, particularly in the presence of significant absorption in either or both layers.

Some issues deserving further examination include:

- It should be determined if the problem is ill-defined. That is, is it possible for two layered materials of different optical, thermal, and geometric properties to yield identical PPTR spectra (within the experimental uncertainty)? If so, does that imply similar fluence

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discrete optical absorbers. As the local temperature transients following a brief laser pulse can exceed several hundred degrees centigrade, thermal radiative heat transfer may begin to play a role in energy redistribution in the sample. We developed a model that incorporates simultaneous conduction and radiation, and tested it with PPTR in a series of pigment-containing phantoms. Results indicated the increasing importance of thermal radiation in reducing the thermal gradients as the temperatures increased and as the infrared absorption coefficient decreased.

Several unresolved questions remain:

- To what extent is radiative transfer relevant in tissue under realistic biological conditions? Do the local temperatures ever attain sufficiently high levels to evoke it? PPTR signals from melanin-loaded gels did *not* show the initial fast peak suggestive of radiative transfer in the carbon-loaded phantoms. Thus, it seems unlikely that the radiative mechanism would be important in present clinical applications of pulsed lasers involving melanosomes (or any other endogenous chromophore). However, in the case of laser tattoo removal, the exogenous ink pigments are more similar to carbon than to melanin (in black tattoos, they often are carbon blacks), and significant radiation heat transfer may exist. Experiment with tattoo inks are needed to test this speculation.

- Is it possible to specify unequivocally (i.e., quantitatively) the relative importance of conductive *versus* radiative heat transfer for a given set of conditions? Although there are qualitative trends (such as the increasing importance of radiative transfer with temperature), a very large number of parameters (thermal, optical, geometric - of the target as well as of the surrounding medium) is involved. Although we made some attempt to delineate the

dominant regimes, further work is clearly needed.

In Paper #4, we reported a preliminary study to determine the feasibility of using PPTR to monitor the uptake of an exogenous dye in tissue *in vivo*. Using the analysis outline in Paper #1, we were able to quantify the observed changes in the PPTR signals by calculating the absorption and the reduced scattering coefficients of liver as a function of time following the injection of the dye. It appears that we were primarily monitoring the kinetics of liver perfusion with dyed blood. The uptake caused an increase in the absorption coefficient without appreciably affecting the scattering properties: this indicated that the model was properly separating the absorption and scattering components.

At the conclusion of this study, several moot points remained to be addressed:

- A detailed physiological interpretation of the experimental results is clearly lacking. For example, the maximum PPTR signal was obtained at ~ 20 minutes after injection, and then decreased slightly. This pattern was seen in two more animals injected with the same dye (Evan's Blue), and two others injected with a lower concentration of Trypan Blue. Does this imply that most plasma-bound drugs reach their maximum concentration in the liver after this time interval?
- Is it valid to assume a homogeneous medium described by volumetric absorption and reduced scattering coefficients? If the dye does indeed stay in circulation (bound, say, to serum albumin), then we may have a spatially-inhomogeneous medium consisting of an unstained cellular matrix permeated by the numerous dye-containing capillaries. However,

APPENDICES

Appendix A. IR fiber study

The range of biomedical applications of PPTR could be greatly extended if a suitable optical fiber could be found that would transmit the IR emission from the irradiated site to a remote detector. This would eliminate the necessity of establishing a direct line-of-sight between the interrogated tissue and the blackbody detector, a difficult (or impossible) requirement to fulfil in some important clinical cases. This way, both the exciting visible photons and the detecting IR photons would be delivered *via* optical fibers. Either a contact probe for surface studies, or an endoscopic probe for examinations inside the body could be constructed, both allowing greater flexibility for biomedical uses.

The search for a suitable delivery system in the IR has been fraught with difficulties (Merberg 1993). Conventional silica fibers do not transmit above ~ 1.8 μm . Some of the IR-transmitting materials cannot be drawn into fibers; others are very hydrophobic and cannot be used in wet or humid environments typically found *in vivo*. The issues of sensitivity to ultraviolet exposure and excessive transmission losses below a large minimum bend radius have been problematic for some fiber types. Nevertheless, some potentially suitable IR optical fibers have recently been developed. These include heavy-metal fluoride glasses, single-crystal sapphire, chalcogenide glasses, polycrystalline silver halides, and hollow waveguide structures. Most of these new IR delivery systems do, however, require more careful handling than the conventional silica fiber optics.

diffusion of the various blood components through the capillary walls will stain both intra- and extra-cellular matrix, thus making it more homogeneous optically. Our histological analysis was not adequate to resolve this issue.

Determination of optical and thermal properties of melanin granules from cuttlefish *Sepia Officinalis* was the subject of Paper #5. Unexpectedly, we measured a significant scattering component, both photometrically and photothermally. The measured thermal diffusivity of the granules agreed well with a previously reported literature value for synthetic melanin. The importance of the granular nature of melanin, in particular the particle size distribution, in dictating its optical properties became evident throughout this study. One wonders if the particle size distribution of the cuttlefish melanin offers its owner the best "smoke-screen" for escape. Is the opacity (or hiding power) of this melanin suspension at a maximum? Can the scattering contribution become significant relative to absorptive attenuation?

Paper #6 summarized the state-of-the-art of biomedical PPTR research as of 1992. The recurrent theme for future applications of PPTR was its ability to perform optical and thermal characterization in materials with significant optical absorption. The development of IR-transmitting fibers, as investigated briefly (Appendix A) will likely widen the scope of potential clinical utility of the technique.

For the preliminary PPTR investigation reported here, we used a silver halide fiber graciously supplied by Dr. Abraham Katzir, of Tel Aviv University in Israel. The fiber was 0.9 mm in outside diameter, 0.8 m in length, and its composition was 50% AgCl, 50% AgBr. Although not directly measured, the fiber transmission loss was estimated to be ~1 dB/m over the 4-12 μm wavelength range, and its numerical aperture was ~ 0.6. To implement its use within the existing PPTR system, the distal end was placed at the focus of the IR detector (i.e., in the aperture plane, where the sample was positioned in the previous PPTR experiments). The positioning was accomplished using an x-y-z translation stage, by maximizing the signal while the proximal end of the fiber viewed a hot object. Once the distal end position was fixed, the proximal end of the IR fiber and the proximal end of the visible-light delivery fiber were arranged to view a common point on the surface of a neutral density filter. Figure A1 depicts the resultant experimental arrangement. It was found that the PPTR signal transmitted through the IR fiber was rather sensitive to the precise geometrical arrangement of the proximal ends of the two fibers, and to their distance from the irradiated/emitting surface. For the purposes of this preliminary study, we chose a particular geometric arrangement that was easily reproducible and that yielded clean and strong PPTR signals. As shown in the inset of Figure A1, the proximal ends of the fibers were positioned at 45° to each other, with their tips touching. The sample was placed at normal incidence to the visible-light fiber, at a distance of 1.92 mm (the thickness of a double glass slide, used here as a convenient spacer).

Typical PPTR spectra from a series of neutral density filters are shown in Figure A2. The incident spot size was 1.5 mm in diameter, corresponding to an incident fluence of ~ 1.75 J/cm². While this irradiance is likely too high for any practical diagnostic application, it was adequate for the present proof-of-concept study. The fluence value is ~ 5X that of

typical lens-based PPTR experiments reported in this work, and the corresponding signals (compared at $t=0$) are $\sim 2X$ larger. Thus, there is about a factor of two loss in sensitivity, due to transmission, coupling, and detection losses. The latter two types could probably be reduced by a more refined optimization of relative positions of the object, the two fibers, and the IR detector.

Figure A1. Schematic illustration of PPTR using an IR-transmitting fiber. The inset shows the detail of fiber arrangement near the sample surface.

Figure A2. PPTR signals from several neutral density filters detected through an IR fiber.

interact very differently with incident and/or emitted radiation. This leads to classification of surfaces as specular or diffuse. The optical 'smoothness' or 'roughness' is not absolute, but, rather, is measured with respect to the radiation wavelength. It is usually characterized by the dimensionless parameter $\sigma_{\text{rms}}/\lambda$, where σ_{rms} is the root-mean-square surface roughness, and λ is the wavelength in question. To study the effect of surface topology experimentally, we compared the PPTR signal from an optically-smooth neutral density filter with that from a roughened one (the damage inflicted by a grinding wheel, brush roughness gauge = 38 mils). Typical spectra are shown in Figure B1.

As can be seen, the PPTR signal from rough filters is considerably greater than from a smooth one. This is probably due to multiple reflections of the incident optical radiation at the surface, which increases the probability of surface absorption, and, therefore, leads to a larger PPTR signal by maximizing the deposition of incident radiant energy in the superficial layers of the material. This increase in energy density due to the increased surface pathlength of incident light more than compensates for the increased amount of light escaping from the material via diffuse reflectance. Indeed, this is analogous to elevating the PPTR signal level by adding scatterers to an absorbing sample - again, the additional loss of light due to increased diffuse reflectance is overshadowed by raising the fluence values in the near-surface region (Paper #1). Thus, contrary to some general claims of thermal-wave literature, the degree of optical smoothness of the interrogated surface can significantly influence the PPTR signal.

It is likely that the pores and facets of the rough sample affect the IR emission as well. The majority of the photons involved have a correspondingly longer wavelengths ($\sim 10 \mu\text{m}$), and thus the characteristic feature size on the roughened surface needs to be of this

To make sure that the detected signal was not caused by spurious reflections, but was due to the true IR emission from the heated sample, a clear glass slide was placed at the sample position. No signal was evident in this arrangement, except for an occasional spike at early times (height ~ 0.01 units on the scale of Figure A2). The spike would be present about 20% of the time: it may have been due to dust on the irradiated glass slide.

To conclude, it appears that PPTR signals can be successfully transmitted via silver halide fibers. Once the relative position of the two fiber ends has been optimized and fixed in a suitable probe arrangement, possible endoscopic applications are evident. Although the presence of the 45° angle between the two fibers at the proximal end may make it seem like the probe will be rather bulky, the pliability of silver halide would allow the two fibers to be side-by-side (parallel) for most of their length, with the angled portion only in the last several mm. Alternatively, one may use a micro-prism or a gradient-index lens at the tip of the visible-light fiber. If the resulting bulge could be kept sufficiently small, the ability to perform PPTR through a catheter at a remote organ site could significantly increase the biomedical utility of the technique.

Appendix B. Blackbody cavity effect

Much has been said in the thermal-wave literature about the relative insensitivity of the photothermal techniques to the surface conditions of the interrogated sample (e.g., Rosencwaig 1980). In fact, this relative insensitivity is often quoted as one of the major advantages of PPTR, as it eliminates the need for careful and laborious sample preparation methods that are necessary with some other techniques. On the other hand, it is well known from the radiative transfer literature that an optically smooth and an optically rough surface

dimension or larger to be noticeable. This is likely the case for the 'very rough' filter of Fig. B1. As in the case of the optical photons, there will be an increase in multiple reflection between surface asperities, and this will cause an increase in emissivity (and absorptivity, by Kirchhoff's Law). This is known as the blackbody cavity effect, which can be used to explain why the emissivity of polished metals is $\sim 5\%$, while of oxidized metals is $\sim 70\%$.

Figure B1. Effect of surface roughness on the PPTR signal. Viewed with a dark-field reflectance microscope, the "rough" sample showed surface grooves of $\sim 15 \mu\text{m}$ in width, while the "very rough" had grooves $\sim 30 \mu\text{m}$ wide. Their depth is probably smaller than the width.

Thus, it appears that surface roughness will influence the PPTR results by altering the interactions with both the incident and the emitted radiation. This has broader implication

in reference to optical modelling in tissues, where a smooth boundary is generally assumed (although this is seldom checked experimentally). The degree of deviation from smooth-boundary behaviour can be quantified via a nondimensional parameter $\sigma_{\text{med}}/\lambda$. This effect was not investigated further in this thesis.

Appendix C. Mathematics of the diffusion theory - a quirk

A curious point arose in the computations of the fluence distributions using the diffusion approximation (see Paper #1 and Paper #2 for further details). There appears to be a problem in evaluating the expression for the fluence (Eq. 10 of paper #1) when the transport albedo $a' = 2/3$, i.e., when $\mu_a = \mu_s/2$. At this point, $\mu_{\text{eff}} = \mu_a = 3\mu_s/2$, and the two exponential terms collapse into one. Adding the coefficients A and B, (i.e., evaluating the fluence at $x=0$) we obtain

$$\begin{aligned} A + B &= \Phi_{\text{inc}} \cdot \left[\frac{(9+6k)\mu_s D}{(1+k\sqrt{4\mu_a D})(1-9\mu_a D)} - \frac{2}{1-9\mu_a D} \right] \\ &= \Phi_{\text{inc}} \cdot \left[\frac{(9+6k)\mu_s D - 2(1+k\sqrt{4\mu_a D})}{(1+k\sqrt{4\mu_a D})(1-9\mu_a D)} \right] \end{aligned} \quad (\text{C1})$$

Evaluating the above expression at $\mu_a = \mu_s/2$ gives

$$A + B = \Phi_{\text{inc}} \cdot \left[\frac{(1/9)(9+6k) - 2(1+2k/3)}{(1+2k/3)(1-1)} \right] = \frac{0}{0} \quad (\text{C2})$$

Thus, the fluence is undefined at this particular value of the albedo. It is interesting to note

Appendix D. Layered media approximations using the homogeneous solution

(helpful discussions with Dr. D.R. Wyman are greatly appreciated)

The relative simplicity of the diffusion theory solution in a homogeneous semi-infinite medium (paper #1), and its relative complexity in a two-layer medium (paper #2) makes it tempting to approximate the latter using the former. One may envision some method of successive approximations based on progressively more refined combinations of the simpler (homogeneous, semi-infinite) solution to improve accuracy in the layered case, perhaps similar to the first-order perturbation theory in quantum mechanics (Anderson 1971). For example, consider a one-dimensional object with properties $\{p_1\}$ for $x < d$, and properties $\{p_2\}$ for $x > d$ (i.e., a two-layer model). Suppose we ignore the presence of the internal boundary, and solve for fluence $\phi(x)$ based on properties $\{p_1\}$ only assuming a semi-infinite medium without an internal boundary; then, in evaluating $\phi(x > d)$, we "cheat" and replace all $\{p_1\}$ parameters appearing in the solution with $\{p_2\}$. This approach is certainly simpler than obtaining the exact solution following the method in paper #2, and if the error introduced is acceptable, it may be preferable. Of course, the error needs to be quantified, the term "acceptable" must be better defined, and the end use of the solution should be considered (Will it be used to evaluate the fluence at depth? Or to calculate the surface remittance? Or serve as a source in the heat conduction equation? The definition of acceptable error will probably be different for each of these cases). Then, a different approximation may be tried to reduce the error, or perhaps an iterative scheme could be worked out whereby the next approximation make use of the results of the previous. If the range of validity of a particular approximation method could be demarcated as a function

that the fluence function is well-behaved on both sides of this singular point (to within an arbitrarily small amount - even using $a' = 2/3 \pm 10^{-12}$ did not cause a computational problem). This is illustrated in Figure C1. It is not immediately obvious why this specific value of the albedo leads to this singularity, which appears to be model artifact rather than representing the physical reality. In all calculations, we avoided the $a' = 2/3$ point.

To investigate the limit of the subsurface fluence as $a' \rightarrow 2/3$, we used L'Hospital's rule. Thus,

$$\frac{d}{da'} (\text{numerator}) / \frac{d}{da'} (\text{denominator}) = \frac{3\Phi_{\text{inc}}}{3+2k}, \quad a' = 2/3. \quad (\text{C3})$$

For an index-matched boundary ($k = 1$), this yields $\Phi_{x=0} = 0.6 \cdot \Phi_{\text{inc}}$, a smaller value results for an index-mismatched boundary where $k > 1$. The significance of these numbers is unclear.

Figure C1. Subsurface fluence as a function of transport albedo, evaluated using diffusion theory solution.

of $\{p_1\}$, $\{p_2\}$, and d , it may then be possible to try to extend this approach to an n -layered system, or to apply it to a different governing equation (e.g., heat conduction or wave equations). None of these generalizations and extensions were attempted, but some preliminary simulations were done for the specific case of approximating the fluence in a two-layered medium from the diffusion theory solution in a (non-layered) semi-infinite model. Some results are presented below.

The fluence in a homogeneous, semi-infinite medium includes contributions from diffuse and collimated photons, and is given as (see Paper #1):

$$\phi(x) = A \cdot \exp(-\mu_{\text{eff}} x) + B \cdot \exp(-\mu_{\text{tr}} x) \quad (\text{D1})$$

where A and B depend on the medium properties (Paper #1, also Eq. (C1)). Note that in the region $x < d$, the exact solution in a two-layer medium (Eq. (7) of paper #2) contains an additional term with μ_{eff} in the exponent. Next, in a two-layered system, it would be convenient to write the fluence as

$$\begin{aligned} \phi(x > d) &= [A_1 \cdot \exp(-\mu_{\text{eff}1}(x-d)) + B_1 \cdot \exp(-\mu_{\text{tr}1}(x-d))] \\ &\quad [A_2 \cdot \exp(-\mu_{\text{eff}2}x) + B_2 \cdot \exp(-\mu_{\text{tr}2}x)] \end{aligned} \quad (\text{D2})$$

where the parameters with subscript i need to be specified. The easiest selection would be simply $i=2$, i.e., evaluate all the parameters using the properties of the second layer (case I - see Figure D1). It is also possible to use μ_{eff} and μ_{tr} , but to leave the coefficients as A_1 and B_1 throughout (case II). Another option would be to use μ_{eff} and μ_{tr} , but to improve on A_1 and B_1 by using the continuity of fluence and current at $x=d$ (similar to the exact approach of Paper #2 - see Eq. (5) and (6)). Of course, since the entire appeal of this whole

methodology is in its simplicity, the use of exact boundary conditions with the corresponding increase in mathematical complexity may be counterproductive. We label this approach as Case III in Figure D1. Clearly, other improvements on these approximations are possible, including the possibility of successive iterations, or a spatial transform of the form $d \rightarrow d + \Delta d$, where $\Delta d = f([p_1], [p_2])$. Clearly, more modelling is needed to choose the best approximation strategy for this problem, and to determine if a more general methodology is possible.

Figure D1. Fluence distributions in a two-layer, turbid medium. An exact (diffusion theory) solution and three approximate results are shown (see text).

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