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Original Article

Noninvasive imaging analysis of biological tissue associated with laser thermal injury

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ARTICLE INFO

Article history:
Received 15 September 2014
Accepted 4 October 2016
Available online 4 May 2017

Keywords:
Optical doppler tomography
Laser doppler flowmetry
Optical coherence tomography
Laser thermal injury

ABSTRACT

Background: The purpose of our study is to use a noninvasive tomographic imaging technique with high spatial resolution to characterize and monitor biological tissue responses associated with laser thermal injury.

Methods: Optical doppler tomography (ODT) combines laser doppler flowmetry (LDF) with optical coherence tomography (OCT) to obtain high resolution tomographic velocity and structural images of static and moving constituents in highly scattering biological tissues. A SurgiLase XJ150 carbon dioxide (CO_2) laser using a continuous mode of 3 watts (W) was used to create first, second or third degree burns on anesthetized Sprague–Dawley rats. Additional parameters for laser thermal injury were assessed as well.

Results: The rationale for using ODT in the evaluation of laser thermal injury offers a means of constructing a high resolution tomographic image of the structure and perfusion of laser damaged skin. In the velocity images, the blood flow is coded at 1300 μm/s and 0 velocity, 1000 μm/s and 0 velocity, 700 μm/s and 0 velocity adjacent to the first, second, and third degree injuries, respectively.

Conclusion: ODT produces exceptional spatial resolution while having a non-invasive way of measurement, therefore, ODT is an accurate measuring method for high-resolution fluid flow velocity and structural images for biological tissue with laser thermal injury.

Laser surgery involves high stability and low diffusivity which generates an influx of high energy in a short period of time, and therefore holds a highly important position in studies requiring constancy and precision [1–5]. The increasingly rapid development and progress of laser techniques have been successfully and broadly applied to surgery. However, biological tissues react differently to the absorption and scattering of different light waves, and each wavelength of a laser beam can be used to treat different pathological changes.

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Peer review under responsibility of Chang Gung University.
http://dx.doi.org/10.1016/j.bj.2016.10.004

At a glance commentary

Scientific background on the subject

A noninvasive tomographic imaging technique with high spatial resolution is an accurate measuring method for high-resolution fluid flow velocity and structural images for biological tissue.

What this study adds to the field

In our study on laser thermal injury tissue models, the feasibility and potential application of a noninvasive tomographic imaging technique to characterize and image blood flow has been demonstrated.

Noninvasive techniques for imaging *in vivo* blood flow are of great value for biomedical research and clinical diagnostics [6]. In plastic surgery, the superficial dermal plexus alone is particularly affected by the presence of cutaneous disease (e.g., eczema, scleroderma), vascular lesions (e.g., port-wine stain, hemangioma, telangiectasia), or trauma (e.g., irritation, wound, burn, laser). Local blood flow monitoring is also critical for reconstructive surgery involving rotational or free flaps where vascular occlusion occurs in about 5–10% of cases. Early recognition of vascular compromise is essential for the salvage of failing flaps and planning the most suitable time of replanting [7]. One complication in the form of thermal injury that occurs as a result of laser surgery is scar formation [8,9]. In these situations, it would be most advantageous to the clinician if blood flow and structural features could be isolated and probed at user-specified discrete spatial locations in either the superficial or deep dermis. Numerous approaches have been investigated including angiography, electromagnetic flowmetry, and magnetic resonance imaging (MRI), as well as laser doppler flowmetry (LDF) and doppler ultrasound [10–12]. However, a noninvasive technique for *in vivo* blood flow imaging with high spatial resolution is currently not available as a diagnostic tool in clinical medicine.

Optical doppler tomography (ODT) combines LDF with optical coherence tomography (OCT) to obtain high resolution tomographic images of static and moving constituents in highly scattering biological tissues [13–16]. The rationale for using ODT to characterize the underlying microvasculature is that the technique is able to probe with high spatial resolution (2–15 μm) at discrete user-specified locations in biological tissues. Such localization is possible because the detected ODT interference fringe intensity gives accurate discrimination of the optical path length of Doppler-shifted and back-scattered light within the coherence length of source. Furthermore, in contrast to LDF, the overall ODT signal caused from moving red blood cells (RBC) is almost entirely due to the Doppler-shifted back-scattered light. As a result, ODT signal-to-noise ratios (SNR) are substantially higher. Inasmuch as tomographic images of blood flow and tissue structure can be obtained simultaneously from a single scan, ODT has shown advantages over existing methodologies. The rationale for using ODT to characterize the underlying microvasculature is that the technique would be able to probe user-specified discrete spatial locations with high spatial resolution. High spatial resolution, noninvasive techniques for *in vivo* blood flow imaging are currently not available as a diagnostic tool in clinical medicine. Such techniques should have a significant impact for biomedical research and clinical diagnosis. The purpose of our study is to assess a noninvasive tomographic imaging technique with high spatial resolution (2–15 μm) to characterize and monitor fluid flow in highly scattering laser thermal injury tissues at user-specified discrete locations.

Methods

The ideal microvascular imaging techniques fulfill several requirements: a) probe the underlying microcirculation at a user-specified depth in both superficial and deep layers; b) distinguish arterial from venous flow; c) detect rapid blood flow changes; d) be safe, noninvasive; and e) produce reliable, and reproducible results. In our study, a high speed ODT based on spectral interferometry is used. The ODT instrument uses a fiber-optic Michelson interferometer with a super luminescent diode (SLD) ($\lambda_0 = 850$ nm, $\Delta\lambda_{FWHM} = 25$ nm) as the light source [14]. The sample and reference mirrors constitute the two arms of the interferometer. Light from the SLD and an aiming beam (He–Ne laser, $\lambda = 633$ nm) are coupled into a fiber interferometer using a 2×1 coupler and then split equally into reference and target arms of the interferometer by a 2×2 fiber coupler. Piezoelectric cylinders are used to modulate the optical path length of light in the reference and target arms by stretching the fiber wrapped around the cylinders. A ramp electrical wave (80 Hz) is used to drive the piezoelectric cylinders to generate optical phase modulation for the interference fringes ($f_o = 1600$ Hz). Light in the sample path is focused onto the turbid sample by a gradient index lens (NA = 0.2) with the optical axis oriented at 15° from the surface. ODT structural and velocity images are obtained by sequential lateral scans of the sample probe (i.e., fiber tip and gradient index lens) at constant horizontal velocity (800 μm/s) followed by a linear incremental movement along the surface.

Light, backscattered from the target, is coupled back into the fiber and forms interference fringes at the photodetector. Temporal interference fringe intensity ($\Gamma_{ODT}(\tau, t)$) is measured by a single element silicon photovoltaic detector, where τ is the time delay between light from the reference and target arms and is related to the optical path length difference (Δ) between the two by $\tau = \Delta/c$. High axial spatial resolution is possible because interference is observed only when τ is within the source coherence time τ_c, or equivalently, when Δ is within the source coherence length ($L_c = \tau_c c$). The interference fringe signal is amplified, high passed, digitized (20 kHz) with a 16-bit analog-to-digital (A/D) converter, and transferred to a computer workstation for data processing [Fig. 1].

The degree of damage is determined by two factors: the temperature and the time period for which the temperature is sustained. A non-linear relationship can exist between the two factors, and thus, even at a lower temperature but with prolonged exposure, thermal damage can still occur. A SurgiLase XJ150 carbon dioxide (CO_2) laser (Sharplan, NJ, USA)

with the power of 3, 5, 10, 15 watts (W), and pulse duration of 5, 10, 15, 20 s (s) is used at a room temperature of 25 °C with relative humidity of 82%. Using a continuous mode of 3 watts (W), the first, second and third degree thermal injuries on the back of 20 anesthetized Sprague–Dawley rats respectively are created in our first study. The duration of surface contact exposure is controlled while the temperature is analyzed from 60 to 100 °C by an infrared thermal image instrument (ThermaCAM™S60, FLIR System, Danderyd, Sweden). The degree of thermal injuries is then determined through observation of the skin changes. First degree injuries affect only the outer layer of the skin and cause redness and swelling. Second degree injuries affect both the outer and underlying layer of skin and cause redness, swelling and blistering, and third degree injuries extend into deeper tissues and cause a white or blackened, charred skin appearance. In our following study, twenty Sprague–Dawley rats are included. Each rat have a total of 17 circular areas (each 1 cm in diameter) with 16 circular areas of laser thermal injuries and one area are used as a control without laser illumination, all located on their backs. Laser thermal injury with the parameters of 3, 5, 10, 15 watts (W), and pulse duration of 5, 10, 15, 20 s (s) are assessed. Immediately after laser thermal injury, the rats are brought to the ODT laboratory and the underlying skin microcirculations beneath the treated area are imaged. Biopsies of the burned rat skin are taken over the exact region that are imaged by ODT as well.

Following the biopsy, tissues are stained with Hematoxylin and Eosin-Y stain is processed for light microscopy to determine the depth of laser thermal injury. They are then compared with ODT images taken at the same time as the biopsies. The follow-up images of rat skin are detected 15 min, 30 min, 24 h and 14 days after laser thermal injury as well. All measurements are correlated with clinical measures such as capillary refill and skin color. Safety of the use of ODT is evaluated by searching for adverse effects, which are 1) the depth of penetration caused by different wavelength, 2) use of inadequate intensity of light, and 3) higher resolutions that can cause tissue damage, which are documented by photography and monitored.

Results

In this study, high spatial resolution is achieved because backscattered light from the target recombines with the reference beam and forms interference fringes only when the optical path length difference is within the coherence length of the source light. In the two-dimensional scanning approach, an ODT image of 100 × 100 pixels requires a minimum acquisition time of 90 s. The high speed ODT based on spectral interferometry, which acquires data for ODT structural and velocity images in parallel by using an optical spectrum analyzer at the interferometer output to measure time variation of spectral interference fringe intensity (PODT (υ, t)). In spectral interferometry, modulation of the interference fringe intensity in the spectral domain (P (υ)) is used to determine the location of all scattering objects along the beam propagation direction. It has been shown that spectral interferometry is equivalent to coherent gating. The complex value analytic signal representations of spectral (P (υ)) and temporal Γ (τ) interference fringe intensity are Fourier transform pairs. Measurement of (P (υ)) can be used to determine Γ (τ), which is related to the path length delayed ($\Delta = c\tau$) between light in the biotissue and reference arms. Spectral ODT measures the time variation of spectral interference fringe intensity (PODT (υ, t)). The positions of scattering constituents along the beam propagation axes can be determined by an inverse Fourier transformation of the optical spectrum. The velocity of the blood flow in biotissue can be determined from the oscillation

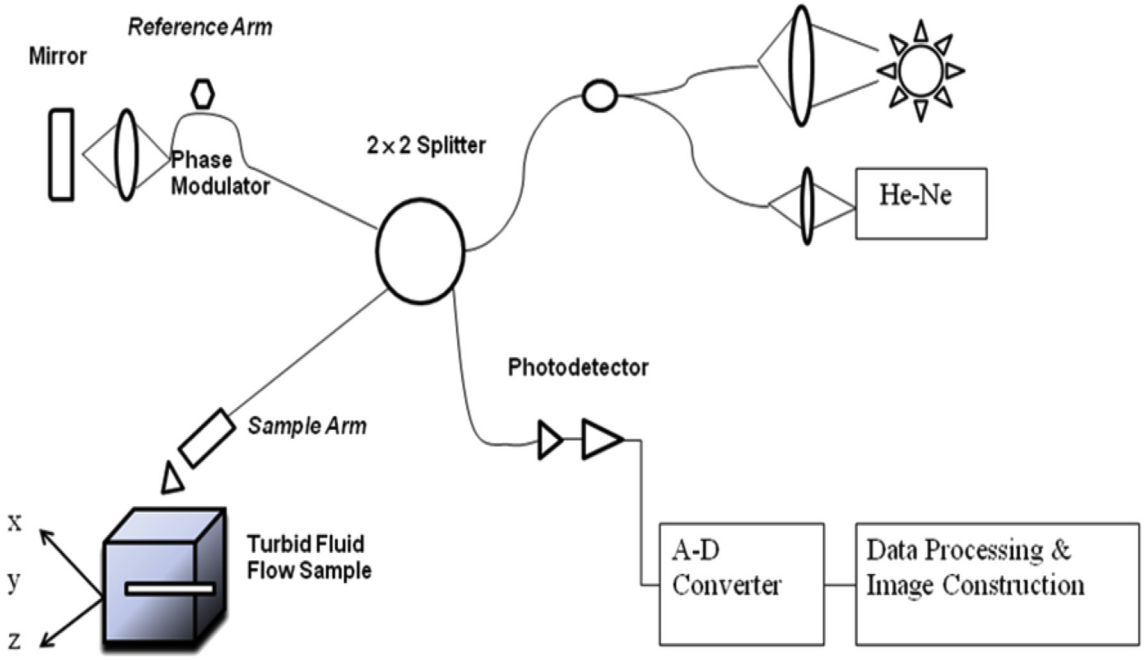

Fig. 1 Schematic Optical doppler tomography instrumentation.

of the optical spectrum. Therefore, from each lateral position of the probe, measurement of (PODT (υ, t)) by an optical spectrum analyzer allows parallel acquisition of position and velocity information over N_2 pixels. Because the entire spectrum of the interference fringe intensity is acquired simultaneously with detector array, image acquisition time is reduced to T = NxΔtp using spectral ODT. An ODT image of 100 lateral pixels with velocity resolution of 100 μm/s can be acquired in a second [21–26].

The duration of surface contact of laser exposure is controlled while the temperature is analyzed by an infrared thermal image instrument. Assuming a body temperature of 37 °C, no measurable effects are observed 5 °C above this. The first mechanism by which tissue is thermally affected can be attributed to conformational changes. These effects are summarized in the single term hyperthermia ranging from approximately 42 °C–50 °C. If hyperthermia lasts for several minutes, a significant percentage of the tissue will already undergo necrosis. Beyond 50 °C, certain repair mechanisms of the cell are disabled. The fraction of surviving cells is further reduced. At 60 °C, denaturation of proteins and collagen occurs which leads to coagulation of tissue and necrosis of cells. The corresponding macroscopic response is visible paling of the tissue. Ta higher temperature (>80 °C) and membrane permeability are drastically increased, thereby destroying the maintained equilibrium of chemical concentration. At 100 °C, water molecules contained in most tissue starts to vaporize. Only if all water molecules have been vaporized and laser exposure is still continuing, does the increase in temperature proceed. At temperatures exceeding 150 °C, carbonization takes place, which is observable by the blackening of adjacent tissue and the escape of smoke. When temperature goes beyond 300 °C, melting can occur depending on the target material.

Thermal injury depth is differentiated into first, second, third or fourth degree injuries. The critical challenge in treating these patients is to assess the depth of thermal injury accurately. An important factor in determining whether the thermal injury could heal without surgical intervention or whether injured tissue must be derided and grafted is the depth of the burn injury. Due to the thin and complex nature of human skin, thermal injury depth variations on the order of 100 μm can make the difference between allowing epithelial regeneration or conducting skin grafting. This decision is particularly crucial on cosmetically and functionally important areas such as the face or hands where the skin is 1–2 mm thick. The images of rat skin with different parameters-immediate and 14 days after laser thermal injury-is detected and analyzed in Tables 1 and 2. Comparing with the ODT and H&E stain histological images, the region underneath the thermal injury appears dark in the structural images. Strong attenuation of back-scattered light from locations deep in the skin surface indicates a high degree of optical scattering. The depth of burn injury related to first, second, and third degree can be detected [Fig. 2]. Dynamic range of the measured back-scattered light in the tomographic structural image was greater than 90 dB. In the velocity image adjacent to the first degree injury [Fig. 2A], the blood flow is coded at 1300 μm/s and 0 velocity. In the velocity image adjacent to the second degree injury [Fig. 2B], the blood flow is coded at 1000 μm/s and 0 velocity. The velocity image adjacent to the third degree injury shows the blood flow coded at 700 μm/s and 0 velocity [Fig. 2C]. Because of the strong attenuation of light by laser thermal injury tissue, the phase modulator is able to detect the doppler shifting of the multiple scattered light out of the detection band, and thus ODT images of laser thermal injury tissues can be produced.

Table 1 The depth immediately after laser thermal injury in various parameters.

Power(W)/Time(s)	5	10	15	20
3	873 ± 17	1350 ± 23	2344 ± 24	3400 ± 28
5	1250 ± 29	2433 ± 27	3550 ± 29	6422 ± 31
10	2417 ± 31	4550 ± 26	5676 ± 25	6532 ± 27
15	3438 ± 27	6120 ± 29	6553 ± 31	7869 ± 27

Scales: μm.

Table 2 The follow-up depth of laser thermal injury.

Power (τ_p = 5 s)	15 min	30 min	24 h	14 days
3 W (n = 20)	873 ± 17	980 ± 17	1008 ± 23	1050 ± 24
5 W (n = 20)	1250 ± 29	1254 ± 24	1292 ± 24	1302 ± 23
10 W (n = 20)	2417 ± 31	2436 ± 29	2442 ± 27	2460 ± 23
15 W (n = 20)	3438 ± 27	3446 ± 28	3454 ± 24	3455 ± 31

Scales: μm.

Discussion

Numerous approaches for medical diagnosis have been investigated including angiography, electromagnetic flowmetry, and magnetic resonance imaging (MRI) [10]. Inasmuch as all of these techniques have shown limited utility for tomographic imaging of the microcirculation, more recent approaches have incorporated the doppler effect [11,12]. The Doppler ultrasound imaging technique uses the principle that the frequency of ultrasonic waves back-scattered by moving RBC is doppler-shifted [11]. In addition to being noninvasive, the chief advantage of the doppler ultrasound technique is the ability to record images of the heart and large diameter blood vessels (>1 mm). However, the relatively long acoustic wavelengths required for deep tissue penetration limit spatial resolution to approximately 200 μm.

Two fundamental processes govern all interactions of light with matter: absorption and scattering. The probability that absorption will occur depends on specific transitions between allowed electron orbits or molecular vibration modes. On the other hand, scattering occurs when the photon changes its direction of propagation. A small momentum is impaired by scattering, but the photon continues along its way in a different direction. All light returning from skin is scattered light. As light strikes the skin surface, about 5% is reflected due to the sudden change in the refractive index between air (n = 1.0) and stratum corneum (n = 1.55). The remaining 95% of the light can be either absorbed or scattered. Scattering by large particles is rather wavelength independent. However, for particles smaller than the wavelength of light, scattering is much more stronger for short

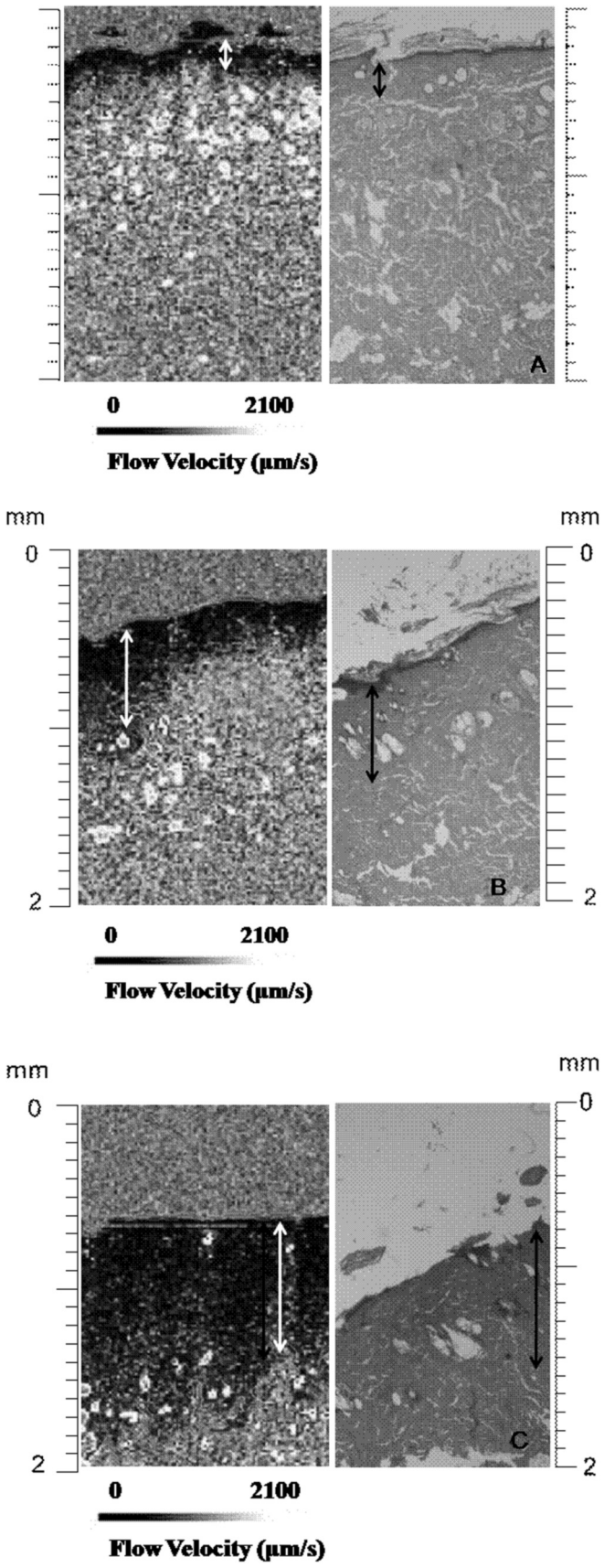

Fig. 2 The transverse scalar bars have been illustrated on the figures 2. In addition, the pathological findings by H&E stain in figures 2A–C have been labeled for correlating with the ODT images of thermal injury zones as well as flow velocities. The dark portions of the image represent injury and display the gradual depth of injury that is exposed to laser. The histological image to the right shows the depth of injury.

wavelengths. Based on this concept, laser doppler flowmetry (LDF) was first described in the 1960's by Yeh and Cummins [17]. However, it was not until 1972 that the first blood flow measurement using LDF was demonstrated [18]. In laser LDF, highly coherent light incidence at a single optical frequency enters the tissue. A second fiber collects the back-scattered light, a small fraction of which by contrast, is doppler-shifted by moving RBC; light scattered exclusively by static constituents has no frequency change. Detection of the doppler shift is founded on the heterodyne beating principle: shifted and non-shifted back-scattered light amplitudes are mixed on the surface of a photoreceiver to produce low frequency (<10 kHz) intensity fluctuations. The power spectrum of such fluctuations is a complex function of RBC velocity distribution and concentration in the microvasculature [12]. Unfortunately, because the LDF signal is due to multiple scattered light with a correspondingly large variance in optical path lengths through the tissue, spatial resolution is poor (>250 μm) and information relevant to blood flow at a discrete location is lost.

Using a noninvasive tomographic imaging technique, ODT makes characterizing and monitoring blood flow with high spatial resolution (2–15 μm) at discrete user-specified locations in highly scattering biological tissues possible [19,20]. The exceptionally high spatial resolution of ODT has broad implications for the clinical management of patients where blood flow monitoring is essential. Information provided by ODT could be used, for example, to monitor perfusion and viability of skin flap before, during, and after reconstructive procedures; to determine the efficacy of pharmacological intervention in salvaging surgical skin flaps or replants; to image microcirculation during sepsis; and to evaluate tissue necrosis. In clinical applications, preoperatively, ODT can assist surgeons in deciding on one of two possible treatment plans for thermal injury cases: (a) concentrate on patient's support while the wound evolves and healing begins without surgery; or (b) immediate debridement to excise necrotic skins followed by skin grafting. Intraoperatively, ODT can assist surgeons in determining the optimal depth for debridement prior to definitive closure. Postoperatively, ODT can be used to monitor physiologically significant healing events including neovascularization. In addition, the ability of ODT to detect tissue changes of the thermal injury provides surgeons with information that previously were not attainable unless a biopsy was taken.

In proper application of ODT, measurements are recommended for proper dosimetry, although calculations are useful in defining the protocol for treatment, consistent with the capabilities of the equipment, the desired duration of irradiation of the patient, and one's knowledge of the optical properties of the tissues. Anisotropic scattering has to be included in calculations of the transport of light in tissue. Anisotropy is, of course, included in measurements of the

diffusion coefficient and diffusion length. It is important to recognize that the absolute magnitude, as well as the shape, of the spatial distribution of the radiant energy fluence is affected by the anisotropic scattering. Meanwhile, the character of reflection, absorption, and scattering by biotissues can be effectively changed with the help of different artificial methods. For example, reflection and absorption spectra can be changed laser thermal injury. On the other hand, the radiant energy fluence and transmitted radiance are decreased only within about three diffusion lengths of the absorbing lesion. Thus, if the depth of laser thermal injury is more than a few millimeters below the skin surface, contrast will be relatively low, and the depth may not be detected precisely or even at all.

The rationale for using ODT in the clinical management of laser thermal injury is that this technique offers a method of constructing a high resolution tomographic image of the structure and perfusion of thermal injury of human skin. The potential to rapidly image and accurately distinguish viable from nonviable tissue over large areas is greatly valuable to laser surgeons. As achieving such accuracy became possible, only the avascular necrotic skin would be removed, thereby preserving underlying tissue and decreasing the need for reconstruction. In our ODT findings, the region underneath the laser thermal injury (from first to third degree) appears dark in the structural image. This can be due to the strong attenuation of light by injured tissue and doppler shifting of the multiple scattered light out of the detection band established by the phase modulator [27,28]. In the velocity image adjacent to the first degree injury, the blood flow is coded at 1300 μm/s and 0 velocity. Reepithilization without scar formation is possible and follow-up observation and wound care is indicated. In the velocity image adjacent to the second degree injury, the blood flow is coded at 1000 μm/s and 0 velocity. Dermal regeneration with minimal scar formation is possible. Meticulous wound care and prevention of wound infection is indicated. Finally in the velocity image adjacent to the third degree injury, the blood flow is coded at 700 μm/s and 0 velocity. Although our preliminary studies suggest a depth of 1–2 mm may be successfully imaged, the development of ODT systems using high power low coherence sources at a longer wavelength (~1300 nm) has the potential to probe deeper into highly scattering biological tissue [29,30].

In conclusion, our results have demonstrated in preliminary *in vivo* studies on laser thermal injury tissue models the feasibility and potential application of ODT to characterize and image blood flow with high spatial resolution at discrete user-specified locations in highly scattering biological tissues. Thus a study with more samples should be carried out to confirm our findings. Given the noninvasive nature of the measurement, exceptional spatial resolution, simple hardware requirements, and relatively compact size, ODT should be a promising technique for both basic research and clinical medicine.

Conflicts of interest

There is no conflicts of interest.

Acknowledgement

This study was supported by the Chang Gung Memorial Hospital, grant CMRPG 3E0221, CMRPG 3E1151, CMRPG 3E0581, CMRPG 32001. We would also like to thank Professor Chih-Chung Yang of Graduate Institute of Electro-Optical Engineering, National Taiwan University, and Professor Zhong-Ping Chen, Beckman Laser Institute, University of California Irvine, Irvine, CA, USA, for their technical support for this study.

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