In vivo burn diagnosis by camera-phone diffuse reflectance laser speckle detection

S. Ragol,1 I. Remer,1 Y. Shoham,2 S. Hazan,1 U. Willenz,3 I. Sinelnikov,4 V. Dronov,4 L. Rosenberg,2 and A. Bilenca1,5,*

1Biomedical Engineering Department, Ben-Gurion University of the Negev, 1 Ben Gurion Blvd, POB 653, Be’er-Sheva 8410501, Israel
2Department of Plastic and Reconstructive Surgery, Soroka University Medical Center, Faculty of Health Sciences, Ben Gurion University of the Negev, Rager Blvd, POB 151, Be’er-Sheva 8410101, Israel
3Lahav CRO Research Unit, POB Negev, Kibbutz Lahav, 8533500, Israel
4Institute of Pathology, Soroka University Medical Center, POB 151, Be’er-Sheva 8410101, Israel
5Ilse Katz Institute for Nanoscale Science and Technology, Ben-Gurion University of the Negev, 1 Ben Gurion Blvd, POB 653, Be’er-Sheva 8410501, Israel

*bielenca@bgu.ac.il

Abstract: Burn diagnosis using laser speckle light typically employs widefield illumination of the burn region to produce two-dimensional speckle patterns from light backscattered from the entire irradiated tissue volume. Analysis of speckle contrast in these time-integrated patterns can then provide information on burn severity. Here, by contrast, we use point illumination to generate diffuse reflectance laser speckle patterns of the burn. By examining spatiotemporal fluctuations in these time-integrated patterns along the radial direction from the incident point beam, we show the ability to distinguish partial-thickness burns in a porcine model in vivo within the first 24 hours post-burn. Furthermore, our findings suggest that time-integrated diffuse reflectance laser speckle can be useful for monitoring burn healing over time post-burn. Unlike conventional diffuse reflectance laser speckle detection systems that utilize scientific or industrial-grade cameras, our system is designed with a camera-phone, demonstrating the potential for burn diagnosis with a simple imager.

©2015 Optical Society of America

OCIS codes: (170.3890) Medical optics instrumentation; (170.4580) Optical diagnostics for medicine; (030.6140) Speckle.

References and links

1. A. Papp, K. Kiraly, M. Härmä, T. Lahtinen, A. Uusaro, and E. Alhava, “The progression of burn depth in experimental burns: a histological and methodological study,” Burns 30(7), 684–690 (2004).
2. S. Monstrey, H. Hoeksema, J. Verbelen, A. Pirayesh, and P. Blondeel, “Assessment of burn depth and burn wound healing potential,” Burns 34(6), 761–769 (2008).
3. K. M. Cross, “Assessment of tissue viability in acute thermal injuries using near infrared point spectroscopy,” (PhD dissertation, University of Toronto, 2010).
4. M. Kaiser, A. Yafi, M. Cinat, B. Choi, and A. J. Durkin, “Noninvasive assessment of burn wound severity using optical technology: a review of current and future modalities,” Burns 37(3), 377–386 (2011).
5. Z. B. Niazi, T. J. Essex, R. Papini, D. Scott, N. R. McLean, and M. J. Black, “New laser Doppler scanner, a valuable adjunct in burn depth assessment,” Burns 19(6), 485–489 (1993).
6. S. A. Pape, C. A. Skouras, and P. O. Byrne, “An audit of the use of laser Doppler imaging (LDI) in the assessment of burns of intermediate depth,” Burns 27(3), 233–239 (2001).
7. H. Hoeksema, K. Van de Sijpe, T. Tondu, M. Hamdi, K. Van Landuyt, P. Blondeel, and S. Monstrey, “Accuracy of early burn depth assessment by laser Doppler imaging on different days post burn,” Burns 35(1), 36–45 (2009).
8. Moor Instruments, “Burn depth assessment using laser Doppler imaging,” http://gb.moorclinical.com/page/professional/faq? (December 17th, 2015).
9. C. J. Stewart, R. Frank, K. R. Forrester, J. Tulip, R. Lindsay, and R. C. Bray, “A comparison of two laser-based methods for determination of burn scar perfusion: laser Doppler versus laser speckle imaging,” Burns 31(6), 744–752 (2005).
10. A. Ponticorvo, D. M. Burmeister, B. Yang, B. Choi, R. J. Christy, and A. J. Durkin, “Quantitative assessment of graded burn wounds in a porcine model using spatial frequency domain imaging (SFDI) and laser speckle imaging (LSI),” Biomed. Opt. Express 5(10), 3467–3481 (2014).
13. D. Jakovels, I. Saknite, G. Krivejna, J. Zaharan, and J. Spigulis, “Mobile phone based laser speckle contrast imager for assessment of skin blood flow,” Proc. SPIE 9421, 94210J (2014).

14. I. Remer and A. Bilenca, “Laser speckle spatiotemporal variance analysis for noninvasive widefield measurements of blood pulsation and pulse rate on a camera-phone,” J. Biophotonics 8(11-12), 902–907 (2015).

15. A. Sadhwani, K. T. Schomacker, G. J. Tearney, and N. S. Nishioka, “Determination of Teflon thickness with laser speckle. I. Potential for burn depth diagnosis,” Appl. Opt. 35(28), 5727–5735 (1996).

16. G. J. Tearney and B. E. Bouna, “Atherosclerotic plaque characterization by spatial and temporal speckle pattern analysis,” Opt. Lett. 27(7), 533–535 (2002).

17. S. K. Nadkarni, A. Bilenca, B. E. Bouna, and G. J. Tearney, “Measurement of fibrous cap thickness in atherosclerotic plaques by spatiotemporal analysis of laser speckle images,” J. Biomed. Opt. 11(2), 021006 (2006).

18. R. B., J. Dong, and K. Lee, “Deep tissue flowmetry based on diffuse speckle contrast analysis,” Opt. Lett. 38(9), 1401–1403 (2013).

19. O. Yang and B. Choi, “Laser speckle imaging using a consumer-grade color camera,” Opt. Lett. 37(19), 3957–3959 (2012).

20. L. M. Richards, S. M. Kazmi, J. L. Davis, K. E. Olin, and A. K. Dunn, “Low-cost laser speckle contrast imaging of blood flow using a webcam,” Biomed. Opt. Express 4(10), 2269–2283 (2013).

21. L. Wang and S. L. Jacques, “Use of a laser beam with an oblique angle of incidence to measure the reduced scattering coefficient of a turbid medium,” Appl. Opt. 34(13), 2362–2366 (1995).

22. F. Kamran and P. E. Andersen, “Sensitivity analysis for oblique incidence reflectometry using Monte Carlo simulations,” Appl. Opt. 54(23), 7099–7105 (2015).

23. D. A. Boas and A. G. Yodh, “Spatially varying dynamical properties of turbid media probed with diffusing temporal light correlation,” J. Opt. Soc. Am. A 14(1), 192–215 (1997).

24. ANSI (American National Standards Institute), “American national standard for safe use of lasers,” ANSI Z136.1–2007 (Laser Institute of America, Orlando, 2007).

25. GSE A, “Sony Ericsson XPERIA Arc,” http://www.gsarena.com/sony_ericsson_xperia_arc-3619.php (October 18th, 2015).

26. S. C. Feng, F.-A. Zeng, and B. Chance, “Analytical perturbation theory of photon migration in the presence of a single absorbing or scattering defect sphere,” Proc. SPIE 2389, 54–63 (1995).

27. I. E. Richardson, The H.264 Advanced Video Compression Standard (Wiley, 2010), Chap. 4.

28. S. L. Jacques, “Optical properties of biological tissues: a review,” Phys. Med. Biol. 58(11), R37–R61 (2013).

29. X. Wang and R. M. Kimble, “A review on porcine burn and scar models and their relevance to humans,” Wound Pract Res. 18(1), 41–49 (2010).

30. P. Miao, A. Rege, N. Li, N. V. Thakor, and S. Tong, “High resolution cerebral blood flow imaging by registered laser speckle contrast analysis,” IEEE Trans. Biomed. Eng. 57(5), 1152–1157 (2010).

1. Introduction

Burn injuries represent a major global public health challenge. In 2004, for example, nearly 11 million individuals across the world required medical treatment due to burn injuries. Early and accurate diagnosis of burn wounds is therefore crucial and is particularly essential for determining the most suitable course of treatment that would prevent deterioration of the wounds. The gold standard for burn diagnosis is histopathological analysis of the burn depth by biopsy. However, biopsy and histology of burns is time consuming, invasive and is prone to errors due to sampling bias and the subjective nature of pathological evaluation [1]. Moreover, biopsies are highly impractical for monitoring burn healing progress as they perpetuate the wound presence, thereby increasing the risk of infection. At present, the most widely used diagnostic method for burn injuries is clinical assessment which involves examination of exterior features of the wounds. Although clinical judgment is immediate and inexpensive, it is subjective and has been shown to be inaccurate in 25%–40% of cases regardless of the experience of the burn surgeon [2,3]. As a result, several light-based technologies have been developed over the years to assist in the clinical classification of burns [4].

A well-recognized optical technique for burn diagnosis is laser Doppler perfusion imaging (LDI) [4–6]. LDI is a noncontact scanning method based on a frequency change of laser light upon reflection off moving blood cells. The frequency shift is proportional to the amount of perfusion in tissue, allowing LDI to produce a color-coded map that corresponds to varying burn depths. LDI is considered to be a valid measure of burn...
depth and exhibits high correlation with burn wound histology; yet, current commercially-available laser Doppler imagers are relatively large, require a prolonged scanning time, and are not promoted for early diagnosis during the first 48 hours post-burn (hpb) [4,7,8]. Laser speckle contrast analysis (LASCA) is another optical technique, related to LDI, which has been used for noninvasive scan-free assessment of burn severity [4, 9–11]. In LASCA, a wide-area laser beam illuminates the burn wound to produce speckle from backscatter off the irradiated tissue volume. The volumetric speckle is projected onto a two-dimensional camera sensor to cast a time-integrated speckle image. From this image, a color-coded map that conveys information on burn severity is rapidly generated over a large field-of-view without any scanning by calculating the speckle contrast as the ratio of the standard deviation to the mean speckle intensity over a local sliding window [12]. Recently, LASCA has been shown to provide early assessment of burn severity in a porcine burn model in vivo using a scientific-grade camera [10] and a simple imager comprising a camera-phone [11]. It is worth mentioning that camera-phone-based LASCA imaging has also been demonstrated useful for noninvasive widefield measurements of skin perfusion, blood pulsation and pulse rate [13,14].

In this work, we use point laser illumination of burn wounds (rather than widefield illumination) and camera-phone imaging to detect diffuse reflectance speckle images of the wounds (rather than projected volumetric speckle images). These images are subsequently analyzed for evaluating noninvasively burn severity in tissue phantoms and in a longitudinal in vivo porcine burn study. In particular, we implement a spatiotemporal fluctuation analysis scheme based on the processing scheme proposed by Sadhwani et al. [15] to extract the diameter of the speckle spot, and show that the retrieved diameter agrees well with the thickness of porcine burns of graded severity, enabling not only early discrimination of partial-thickness burns (within the first 24 hpb), but also longitudinal monitoring of the burn healing process (over 104 hpb). Unlike the system devised by Sadhwani et al. and other instruments for diffuse reflectance laser speckle detection using scientific- or industrial-grade cameras [15–18], our apparatus takes advantage of advancements of miniature-camera technology in biomedical sciences [11,13,14,19,20] and employs a camera-phone for diffuse reflectance speckle detection. While the basic setup used here is similar to that of oblique incidence reflectometry (OIR) [21,22], we analyze spatial intensity variations in diffuse reflectance laser speckle patterns rather than OIR distances between the diffusion center and illumination entry point. Note that a technique related to ours, termed diffuse correlation spectroscopy, based on temporal autocorrelation analysis of rapidly fluctuating photon count signals from a single speckle was used to assess burn severity in vivo in a porcine burn model using fiber optics and photon-counting photomultiplier tubes [23]. Finally, it is noteworthy that spatiotemporal pattern analysis of time-varying diffuse reflectance speckle has also been used for measuring biomechanical properties and cap thickness of atherosclerotic plaques [16,17].

The manuscript is organized as follows. Section 2 describes the camera-phone diffuse reflectance laser speckle detection (dr-LSD) system and details methods for analyzing diffuse reflectance laser speckle (dr-LS) patterns, preparing tissue phantoms, using an in vivo porcine burn model, including histopathological and statistical analysis of porcine burn depth. In section 3, we present results and discussion on the instrument performance in phantoms and in a longitudinal porcine burn study. Finally, conclusions are drawn in section 4.

2. Methods and materials

2.1 Camera-phone dr-LSD system

An optical setup comprising a focused red laser for sample illumination and a camera-phone imager for detecting time-integrated dr-LS patterns at the burn surface was designed as illustrated in Fig. 1(a). Concretely, a collimated, linearly polarized He–Ne laser (CVI Melles–Griot) beam with an incident angle of ~25° was focused using an achromatic lens of 300 mm focal length (Thorlabs) to a 64 μm (128 μm) diameter spot on the surface of a tissue phantom (porcine burn). Note that this incident angle was the smallest possible in the current prototype due to the size of optics and optomechanics...
used. Low (high) irradiances of 0.25 W-cm$^{-2}$ (1 W-cm$^{-2}$) and 0.12 W-cm$^{-2}$ (0.77 W-cm$^{-2}$) were used for illuminating the phantoms and animal burns, respectively, consistent with the American National Standard Institute (ANSI) standard for skin exposure limit (1.85 W-cm$^{-1}$ for laser exposure durations of 0.5 s used throughout the experiments [24]). Backscattered diffuse speckle light from the samples was then imaged onto the back-illuminated CMOS sensor of a camera-phone (Sony-Ericsson [25]) through a 10 × zoom lens (Computar) and a polarizer (Thorlabs) whose polarization axis was perpendicular to that of the incident light for minimizing by ~70% glare from the focus point on the sample. The magnification and f-stop of the zoom lens were set to yield ~6-12 red pixels/speckle. Note that we used only the red channel of the interpolated Bayer data provided by the camera-phone sensor. Finally, the instrument was attached to a support arm, allowing convenient orientation of the apparatus over the wounds and stabilizing the system during data recording.

Fig. 1. Camera-phone diffuse reflectance laser speckle detection and analysis (dr-LSD) for burn diagnosis. (a) Experimental setup and tissue phantom schematics. PL$_{1/2}$, crossed polarizers; FL, focusing lens; ZL, 10 × zoom lens; Δz, static-layer thickness; IL, Intralipid perfused layer. (b) Time-integrated dr-LS images of tissue phantoms with Δz$_1$ = 0.1 mm (left panel) and Δz$_2$ = 1 mm (right panel). r$_0$ is the radial transition between sharp and blurred speckle patterns in these images. D = 2r$_0$ defines the spot diameter where deeper burns result in larger D values as illustrated by the tissue light propagation schematics (D$_1$<D$_2$). (c) Spatiotemporal fluctuation analysis of the raw speckle data involved computation of the $\sigma_x(x,y)$ map (left panel), calculation of the $\langle\sigma_x(r,\phi)\rangle_r$ profile (middle panel) and determination of D (right panel).
2.2 Spatiotemporal fluctuation analysis of time-integrated diffuse reflectance laser speckle patterns of burns

To evaluate burn severity (i.e., depth) from time-integrated dr-LS images of a burn, we used a processing scheme based on that of Sadhwani et al. [15]. In brief, assuming that a burn wound can be simplified as a two-layered medium with a static (or low-perfusion) burn layer overlying a perfused tissue, Sadhwani et al. showed that the radial transition between sharp and blurred speckle patterns in time-integrated dr-LS spots of burn phantoms agrees well with burn depth as illustrated by the point $r_0$ in Fig. 1(b). This agreement is due to the semi-oval shape of diffuse reflectance light distributions in tissue [26] and the (quasi)static versus dynamic coherent light scattering from the burn layer and the underlying perfused tissue, respectively. As a result, the diameter of diffuse reflectance speckle spots of burn wounds, defined as $D = 2r_0$, can provide a noninvasive means for estimating burn severity.

To determine $r_0$ (and hence $D$), we first acquired with a camera-phone imager a data set of 15 raw diffuse reflectance speckle frames of the burn wound with 720p resolution and H.264 compression at 30 frames-s$^{-1}$. Note that the H.264 format is a lossy video compression method that attempts to remove duplicate image data between frames (e.g., background information) while estimating image motion data from neighboring frames [27]. Next, the following processing steps were applied to the raw data set using Matlab software (Mathworks) on a standard personal computer: (i) Conversion of each of the 15 dr-LS frames to standard deviation images by calculating the square root of the sample variance over a sliding window of $5 \times 5$ pixels, and calculation of a 15-frame-averaged standard deviation image, $\sigma_r(x,y)$, as depicted in the left panel of Fig. 1(c). (ii) Computation of an angularly-averaged radial standard deviation profile, $\langle \sigma_r \rangle_{r_0}$, by constructing standard deviation profiles along the radial direction of $\sigma_r(x,y)$ at $N$ uniformly distributed azimuthal angles, $\{\phi_\alpha\}$, in the interval $[0^\circ,180^\circ)$ using bilinear interpolation, and averaging the resulting profiles over the azimuthal angle as shown in the middle panel of Fig. 1(c). The number of angles $N$ used throughout this work was 72. (iii) Thresholding of $\langle \sigma_r \rangle_{r_0}$ at a value $\sigma_T$, that defines the domain of radii $[0, r_0]$ for which the local speckle pattern is considered to be sufficiently sharp with $\langle \sigma_r \rangle_{r_0} \geq \sigma_T$. The threshold $\sigma_T$ was selected to be half the maximum value recorded for a $\langle \sigma_r \rangle_{r}$ profile of normal skin. Finally, the speckle spot diameter $D = 2r_0$ was determined as the distance between the two interceptions of the $\langle \sigma_r \rangle_{r_0}$ profile with $\sigma_T$, defining a measure for the burn thickness, $\Delta z$, as indicated in the right panel of Fig. 1(c).

2.3 Tissue phantoms

We produced two-layered tissue phantoms consisting of a statically-scattering Teflon layer overlying a perfused Intralipid region as illustrated in Fig. 1(a). Mimicking of normal skin and skin burns of different depth was obtained by using Teflon sheets (Pronat Industries) with thickness of $\Delta z = 0.1$ mm and $\Delta z = 0.25, 0.5,$ and $1$ mm for the upper phantom layer, respectively, whereas simulation of different blood perfusion conditions within tissue was accomplished by flowing 9% Intralipid (Sigma) at translational speeds of $v = 0, 1,$ and $3$ mm-s$^{-1}$ in the lower perfused layer using a digital syringe pump (New Era Pump Systems). Note that Intralipid perfusion is purely Brownian when $v = 0$ mm-s$^{-1}$. Also, reduced scattering coefficients of Teflon and 9% Intralipid were measured to be $35$ cm$^{-1}$ and $25$ cm$^{-1}$ [15, 28], respectively, using oblique incidence reflectometry [21,22].

2.4 Porcine burn model

An in vivo animal experiment was performed using a porcine skin burn model. The experiment was in compliance with protocols approved by the National Animal Care and Use Committee (#IL-13-05-098) and was conducted in agreement with the Guide for the Care and Use of Laboratory Animals at the Lahav C.R.O research unit (Kibbutz Lahav, Israel). Full details of the experiment are described in our previous publication [11]. In short, one female pig (~2 months old, body weight of 33 kg) was individually acclimated to housing facilities for 5 days before use. The animal was fasted 12 hours prior to
anesthesia and hair was clipped from its dorsum at the beginning of the experiment. General anesthesia, endotracheal tubing and monitoring of oxygen saturation, body temperature, electrocardiogram and heart rate were maintained continuously throughout the experiment. Partial-thickness burns (3 × 3 cm² in area) were created on the pig’s dorsum by placing a brass block heated to 100°C (in boiling water) on the porcine skin for a predetermined contact time [1]. Four groups of 12 burns were inflicted, each with a different probe-skin contact time of 10, 20, 30, and 40 s, resulting in a total of 48 burn sites (see Fig. 2(a) in [1]). Temperature was monitored using a thermocouple threaded into the bottom of the block. Only the weight of the block was applied with no extra pressure (see Fig. 2(b) in [1]). For minimizing variations in producing the burns, only one person (YS) created all burns.

2.5 Histopathology

Histopathology of porcine burn biopsies is detailed in our previous publication [11]. In brief, four 4-mm punch biopsies were removed from each group of burns of probe-skin contact times of 10, 20, 30, and 40 s at 8, 32, and 104 hpb. Biopsied sites were excluded from subsequent dr-LSD measurements. Blinded burn thickness assessment from hematoxylin-and-eosin (H&E) slides of the burn biopsies was conducted independently by two pathologists, with joint re-assessment performed for >0.1-mm discrepancy between estimations. Burn thickness was determined by the deepest predetermined histopathological feature identified in the slides. These features included, for instance, vascular dilation and congestion, separation of collagen fibers (edema), eosinophilia of collagen fibers and empty cavity of the pilosebaceous unit as shown in representative digital images of H&E slides of the different burn groups in Fig. 2(a)-2(d).

![Fig. 2. Representative digital images of H&E-stained slides of (a) 10-s, (b) 20-s, (c) 30-s, and (d) 40-s burns. Burn depth, indicated by the two-sided arrow, was evaluated by the deepest predetermined histopathological criteria identified in the slides. The single asterisk denotes empty cavity of the pilosebaceous unit, the diamond indicates separation of collagen fibers (edema), double asterisks identify vascular congestion, and the triangle points to eosinophilia of collagen fibers through the dermis. The scale bar is 1 mm.](image)

2.6 Statistical analysis

For camera-phone dr-LSD of burn phantoms, we tested significance of speckle spot diameter data using two-way analysis of variance (ANOVA) with factors of static-layer thickness (Δz = 0.1, 0.25, 0.5, and 1 mm) and translational perfusion speed (v = 0, 1, and 3 mm·s⁻¹). Tukey’s post-hoc analysis and Pearson’s correlations were calculated when appropriate.

For histopathological assessment of porcine burn thickness, significance of estimated burn depth data was tested by two-way ANOVA with factors of probe-skin contact time (10, 20, 30, and 40 s) and time post-burn (8, 32, and 104 hpb). Tukey’s post-hoc tests and Pearson’s correlations were calculated between histopathological burn depth and contact time. In addition, one-way ANOVA followed by Tukey’s post-hoc analysis was performed on biopsy data collected at 8 hpb.
For in vivo camera-phone dr-LSD of porcine burns, statistical significance of speckle spot diameter data was determined via repeated-measures ANOVA with factors of probe-skin contact time (10, 20, 30, and 40 s) and time post-burn (8, 32, and 104 hpb). Tukey’s post-hoc analysis and Pearson’s correlations were further used to study the relationship between speckle spot diameter and significant factors. In addition, one-way ANOVA followed by Tukey’s post-hoc analysis was performed on speckle spot diameter data collected at 8 hpb.

3. Results and discussion

3.1 Selection of threshold \( \bar{\sigma}_r \) and number of azimuthal angles \( N \) in camera-phone dr-LSD of burns

To select the threshold value of \( \bar{\sigma}_r \) and the number of azimuthal angles \( N \) for determining the speckle spot diameter of burns (and hence burn depth and severity), we produced \( \langle \bar{\sigma}_r (r, \varphi) \rangle \) profiles (averaged over \( N \) angles) from camera-phone dr-LSD images of purely Brownian tissue phantoms with static-layer thicknesses of \( \Delta z = 0.1-1 \) mm (see sections 2.2, 2.3 in Methods and Materials). Note that \( \Delta z \leq 1 \) mm was selected to enable measurements of large speckle spot diameters (with tolerable camera sensor saturation) using a maximal illumination power similar to that planned for the in vivo experiments. We tested various threshold values and number of angles for optimal distinguishability between the phantoms. This selection procedure is similar to that in [15], but here threshold values were chosen to be fractions of the maximum value recorded for a \( \langle \bar{\sigma}_r \rangle \) profile of normal skin phantom, referencing speckle spot diameter data of burns to that of normal skin. Figure 3(a) shows typical \( \langle \bar{\sigma}_r \rangle \) profiles of the phantoms measured at incident intensity of 1 W-cm\(^{-2}\) and calculated using 72 azimuthal angles. The different symbols in the figure indicate threshold values of 100, 75, 50, and 25% of the maximum value of a \( \langle \bar{\sigma}_r \rangle \) profile of normal skin phantom (blue curve). Note the central region in these profiles where a reduction in the speckle standard deviation occurred (due to partial saturation of the camera-phone sensor). As exemplified in the figure, the distance between the two interceptions of a \( \langle \bar{\sigma}_r \rangle \) profile with a fixed threshold defines the speckle spot diameter, \( D \). Figure 3(b) shows the behavior of \( D \) versus \( \Delta z \) for the different thresholds alongside with the corresponding linear fits. Linear fit slope, coefficient of variation and Pearson’s correlation values were respectively 2.35, 141%, and 0.9 for 100% threshold; 2.67, 80%, and 0.96 for 75% threshold; 2.9, 19%, and 0.99 for 50% threshold; 2.26, 91%, and 0.95 for 25% threshold. From this data, we see a positive linear relationship of \( D \) with \( \Delta z \) for all thresholds \((r \geq 0.9)\). In addition, we see that a threshold of half the maximum of the \( \langle \bar{\sigma}_r \rangle \) profile of normal skin phantom resulted in the highest sensitivity (largest slope) and lowest dispersion (smallest coefficient of variation), best distinguishing between all tissue phantoms. As a result, we chose a threshold of half the maximum of a \( \langle \bar{\sigma}_r \rangle \) profile of normal skin phantom for the burn phantom experiments, and a threshold of half the maximum of a \( \langle \bar{\sigma}_r \rangle \) profile of in vivo normal porcine skin for the in vivo porcine experiments. Note that \( \langle \bar{\sigma}_r \rangle \) profiles of normal skin should be taken at the same intensity as for the burn wounds.

To determine \( \langle \bar{\sigma}_r \rangle \), whether 72 azimuthal angles is an adequate number of angles, \( N \), for computing \( \langle \bar{\sigma}_r \rangle \) profiles (from which speckle spot diameters, \( D \), are retrieved), we studied the dependence of \( D \) on \( N \) using a purely Brownian 1-mm-thick burn phantom as presented in the top panel of Fig. 3(c). The figure shows that while \( D \) maintained a similar level at different \( N \) values, a reduction in the dispersion of \( D \) occurred with increasing \( N \) as quantified by the coefficient of variation of \( D \) in the bottom panel of Fig. 3(c). Accordingly and similarly to [15], for all experiments in this work, \( \langle \bar{\sigma}_r \rangle \) profiles were produced by averaging radial standard deviation profiles calculated at 72 azimuthal angles.

Received 19 Oct 2015; revised 17 Dec 2015; accepted 18 Dec 2015; published 23 Dec 2015
(C) 2016 OSA
1 Jan 2016 | Vol. 7, No. 1 | DOI:10.1364/BOE.7.000225 | BIOMEDICAL OPTICS EXPRESS 23
3.2 Camera-phone dr-LSD of burn phantoms

To investigate the capability of camera-phone dr-LSD for early burn diagnosis, we used burn phantoms of different depth and blood perfusion conditions as detailed in section 2.3 of Methods and Materials. Figure 4(a), 4(b) presents bar plots of the mean and standard error of the speckle spot diameter, \( D \), measured for the various phantoms under low and high irradiances of 0.25 and 1 W-cm\(^{-2} \), respectively. Results for normal skin phantom (with minimal static-layer thickness of \( \Delta z = 0.1 \) mm) are also displayed for comparison.

The figure shows that speckle spot diameter increased linearly with static-layer thickness for both low and high irradiance levels at all flow speeds, \( v \), in the perfused layer of the phantoms \( (r>0.97 \text{ with } P<0.05) \). However, by comparing Fig. 4(a), 4(b), we see that under high irradiance, diameter measurements appeared to be unable to resolve between thin tissue phantoms (in any flow speed) as opposed to the results obtained under low irradiance. This conclusion is supported by finding that, under irradiance of 0.25 W-cm\(^{-2} \), speckle spot diameter statistically distinguished \( (P<0.01) \) between phantoms of different static-layer thickness regardless of flow speed, but could not discriminate between phantoms with static-layer thicknesses closer than 0.25 mm under the higher...
irradiance of 1 W-cm\(^{-2}\) due to stronger saturation of the camera-phone sensor by brighter illumination). In addition, regression slopes for the \(D-\Delta z\) data (marked with triangles in Fig. 4(a), 4(b)) were larger for the high than for the low illumination level at all flow speeds, showing that speckle spot diameter was more sensitive to thicker burn phantoms under high irradiance than under low irradiance regardless of flow speed (due to increased number of photons backscattered from deeper in the phantom and exiting farther from the illumination entry point). These results show the possible tradeoff between thickness-resolution and depth-of-sensitivity of dr-LSD at different irradiance levels on the burn, and point to the potential utility of camera-phone dr-LSD for assessing burn severity during the early period post burn where blood perfusion irregularities often occur [4].

3.3 In vivo camera-phone dr-LSD of porcine burns

In this section, we demonstrate the utility of dr-LSD in a longitudinal in vivo animal burn study. To this end, a porcine burn model was used owing to its high resemblance to human skin in terms of wound healing and structure [29] (see section 2.4 of Methods and Materials). By histopathological analysis, we first assessed the thickness of burns in the four groups of probe-skin contact times of 10, 20, 30, and 40 s as described in section 2.5 of Methods and Materials. Figure 2(a)-2(d) shows typical images of burn biopsy slides of the different burn groups. Two-sided arrows in this figure indicate the histopathologically evaluated burn thickness (see legend of Fig. 2 for details). The results for the histopathological assessment of burn depth are summarized in Fig. 5 in bar plots of the mean and standard error of the histopathological burn depth by the four burn groups at 8, 32, and 104 hpb.

![Fig. 5. Bar graphs of mean and standard error of histopathological burn depth by probe-skin contact time at 8, 32, and 104 hpb.](image)

From the figure, we see a relatively linear positive relationship between histopathological burn thickness and probe-skin contact time at 8, 32, and 104 hpb (\(r = 0.75, 0.7, \) and 0.66, respectively, all with \(P<0.05\)). Two-way ANOVA on the data showed that histopathological burn depth significantly increased (\(P<0.01\)) with contact time, regardless of time post-burn. Tukey’s post-hoc tests then revealed statistically significant differences in the histopathological depth of the 10-s and 40-s burns, 10-s and 30-s burns, and 20-s and 40-s burns, regardless of time post-burn. Other burn groups closer in contact time were not statistically distinguished by their histopathological burn thickness. To further check for statistical difference in the histopathological depth of the different burn groups during the first 24 hpb only, we performed one-way ANOVA followed by Tukey’s post-hoc analysis for burn biopsy data collected at 8 hpb, and found that histopathological burn thickness discriminated statistically between the 10-s and 40-s...
burns only. These post-hoc results may stem from biopsy sampling errors and from the method used for inflicting burn wounds (which yielded limited-thickness burns with relatively large thickness dispersion in each of the four burn groups). Use of other probes for creating burns and techniques for collecting biopsies in a porcine model could possibly improve the statistical distinction between burn groups of different contact time by histopathological analysis [10]. Importantly, the effect of time post-burn on histopathological burn thickness was found to be slightly insignificant ($P = 0.059$); nevertheless, a clear decreasing trend of the thickness of the different burn groups over the period of the experiment is observed from Fig. 5, indicating a possible process of burn healing over time post-burn. Finally, we note that differences in burn depth assessed here compared to other porcine burn studies (e.g., [1,10]) may be associated with variations in the animal skin thickness (due to different age, weight and sex of the pigs used), variations in pressure exerted by the brass block on skin, variations in infliction time and brass block temperature, and the different histopathological criteria defined for estimating burn thickness. Particularly, in our work, the thickness of the pig’s dermis was assessed to be 2-3 mm (larger than that reported in [1]) and the pressure exerted by the brass block on skin was not controlled (in contrast to [10]).

To explore the ability of the camera-phone dr-LSD prototype for in vivo burn diagnosis and monitoring, we collected dr-LS images of porcine burns at 8, 32, and 104 hpb by properly orienting the system over the burn sites during $\sim$1 min and then acquiring 15 images during exhalation intervals of $\sim$1.25 s over 0.5 s at 30 frames-s$^{-1}$. Note that this procedure assisted in minimizing breathing artifacts [11]. To retrieve speckle spot diameters of the different burns, the raw dr-LS image data was subsequently exported for processing in Matlab at a rate of a few seconds per burn site using a non-optimized code running on a standard personal computer. Figure 6(a)-6(d) displays representative dr-LS images of the four burn groups recorded at 8 hpb under high irradiance. The red circles in the images correspond to the retrieved speckle spot diameters of the burn wounds and can be seen to expand outward with burn thickness (i.e., severity). As exemplified in Fig. 6(e), diameters were recovered from $\langle \sigma \rangle_s$ profiles of the different burns at a fixed 50% threshold calculated from in vivo normal porcine skin under high irradiance (marked with dashed line).

---

![Fig. 6. Representative dr-LS images of porcine burn wounds with probe-skin contact times of (a) 10 s, (b) 20 s, (c) 30 s, and (d) 40 s. The images were recorded at 8 hpb under irradiance of 0.77 W-cm$^{-2}$. The red circles mark the retrieved speckle spot diameters of the burns. The scale bar is 2 mm. (e) $\langle \sigma \rangle_s$ profiles for burns of different contact time. The dashed line indicates the fixed 50% threshold used to recover the speckle spot diameters. The 50% threshold value was equal to half the maximum value recorded for a $\langle \sigma \rangle_s$ profile of in vivo normal porcine skin under high irradiance.](image-url)
Figure 7(a), 7(b) presents bar plots of the mean and standard error of speckle spot diameter by porcine burn groups of contact time of 10, 20, 30 and 40 s obtained at 8, 32, and 104 hpb under low and high irradiances, respectively. Statistical analyses of the ability of the speckle spot diameter to discriminate between the different burn groups showed the following points. (i) Under low irradiance, a relatively linear behavior of speckle spot diameter with probe-skin contact time was obtained at 8 hpb ($r = 0.76$ with $P < 0.05$), whereas weak correlations were observed at 32 and 104 hpb ($r = 0.4$ and 0.5, respectively, both with $P < 0.05$). ANOVA tests on the low irradiance speckle data confirmed a significant increase of speckle spot diameter with contact time regardless of time post-burn. However, subsequent Tukey’s post-hoc comparisons showed that speckle spot diameter statistically distinguished only the 10-s burns from the 40-s burns regardless of time post-burn (crosses in Table 1, left panel) and in addition, the 10-s burns from the 30-s burns and the 20-s burns from the 40-s burns during the first 24 hpb (circles in Table 1, left panel). (ii) Under high irradiance, speckle spot diameter significantly increased ($P < 0.01$) with contact time regardless of time post-burn. The increase was relatively linear at all times post-burn ($r = 0.84, 0.75, 0.73$ at 8, 32, 104 hpb, respectively, all with $P < 0.01$) and agreed well with the histopathological burn depth results. Moreover, Tukey’s post-hoc associations discovered that, regardless of time post-burn, speckle spot diameter measurements at high illumination intensity significantly discriminated between all burn groups, except between the 20-s and 30-s burns and the 30-s and 40-s burns (crosses in Table 1, middle panel). When analyzing high irradiance speckle data of the first 24 hpb only (circles in Table 1, middle panel), similar significant burn groups were identified with the exception that the 20-s and 30-s burns were also statistically different and the 30-s and 40-s burns were only slightly insignificant ($P = 0.087$). These results demonstrate that under adequate illumination levels dr-LSD can achieve satisfactory thickness-resolution and depth-of-sensitivity performance, providing useful information on burn severity of partial-thickness skin burns even at very early times post-injury. (iii) Regardless of burn group and illumination level on the burns, speckle spot diameter significantly decreased ($P < 0.01$) with time post-burn. Subsequent Tukey’s post-hoc comparisons showed that speckle spot diameter significantly distinguished burns measured at 8 or 32 hpb from burns probed at 104 hpb for both low and high irradiances (squares in Table 1, right panel). These findings suggest that dr-LSD can be suitable for monitoring burn healing over time post-burn.

![Fig. 7. Bar graphs of mean and standard error of speckle spot diameter by probe-skin contact time at 8, 32, and 104 hpb and under (a) low and (b) high incident irradiance.](image-url)
Note that despite burns imaged at 8 and 32 hpb were not statistically distinct by their speckle spot diameters, a much lower p-value was obtained for high irradiance ($P = 0.146$ versus 0.988 at low irradiance), indicating that higher signal-to-noise ratios could potentially have improved burn healing monitoring by the prototype.

As a final remark, it is interesting to examine the speckle spot diameter results obtained for the porcine burn study in this work to the speckle contrast results measured on the same burns by camera-phone LASCA imaging (see Fig. 7 here and Fig. 6 in [11]). While speckle spot diameter and speckle contrast showed an increasing trend with probe-skin contact time at 32 and 104 hpb, speckle spot diameter exhibited such a trend also at 8 hpb, indicating on the improved capability of camera-phone dr-LSD for early burn diagnosis. Furthermore, differences in the mean speckle spot diameter of the different burns between 32 and 104 hpb were overall more apparent than those in the speckle contrast, implying that monitoring of the progress of burn healing could possibly be improved by using camera-phone dr-LSD. The enhanced performance of camera-phone dr-LSD over camera-phone LASCA imaging probably stems from a more depth-selective sampling of the burn tissue volume by diffuse reflectance laser speckle than by projected volumetric speckle light.

Table 1. Tukey’s post-hoc pairwise comparison matrices of burn groups and hours post-burn

| burn groups, 0.12 W-cm$^{-2}$ | burn groups, 0.77 W-cm$^{-2}$ | hours post-burn, 0.12/0.77 W-cm$^{-2}$ |
|--------------------------------|--------------------------------|--------------------------------------|
| 10 s  | 20 s  | 30 s  | 40 s  | 10 s  | 20 s  | 30 s  | 40 s  | 8 h   | 32 h  | 104 h |
| o     | 0     | 0     | 0     | ×     | o     | 0     | 0     | o     | 0     | 0     |
| 0     | o     | 0     | 0     | 0     | o     | 0     | 0     | 0     | 0     | 0     |
| ×     | o     | 0     | 0     | ×     | o     | 0     | 0     | ×     | o     | 0     |

*Circles and crosses mark pairs of burn groups that were significantly different ($P<0.05$) by their speckle spot diameters measured at 8 hpb and averaged over time post-burn, respectively. Squares marks pairs of hours post-burn at which burns were significantly different ($P<0.05$) by their mean speckle spot diameters averaged over burn group.

4. Conclusions

We have developed a noninvasive and contact-free diffuse reflectance laser speckle detection (dr-LSD) instrument for burn diagnosis. The system employed point illumination of the burn area and a camera-phone imager for detecting time-integrated diffuse reflectance speckle spots at the burn surface. A subsequent radial analysis of intensity fluctuations in these speckle spots retrieved their diameter. The speckle spot diameter was then shown to increase with deeper burns using burn phantoms and in a porcine burn model in vivo. Furthermore, we showed the dependence of thickness-resolution and depth-of-sensitivity of dr-LSD on the incident irradiance on the burn area. Finally, we demonstrated that the camera-phone dr-LSD prototype can significantly distinguish partial-thickness burns in the early period post-burn (first 24 hpb) and monitor burn recovery over time.

While we used a large red laser and an industrial zoom lens in the current camera-phone dr-LSD system design, future instruments could employ compact laser diode modules and detachable camera-phone zoom lenses [13,20]. Moreover, handheld use of camera-phone dr-LSD devices might be achieved by implementing numerical image stabilization techniques [30] and fast speckle image processing on mobile phones. Finally, use of longer wavelengths for burn illumination (e.g., 780 nm) would allow deeper penetration into skin burns, enabling evaluation of deeper burns. For the same magnification and f-stop used here at 633 nm, longer wavelengths would require a larger field-of-view for detecting the larger resulting speckle spots and would increase the size of single speckles, decreasing spatial sampling of the speckle spot diameter and potentially reducing the resolution at which burn depth is assessed.
Acknowledgments

This work was financially supported by the Israel Ministry of Economy through the Kamin program. IR gratefully acknowledges the support of the Azrieli Foundation under the Israeli graduate studies fellowship program.