Available online at www.sciencedirect.com
SCIENCE DIRECT
Microvascular Research 68 (2004) 143–146
MVR Microvascular Research
www.elsevier.com/locate/ymvre

Brief Communication

Laser speckle imaging for monitoring blood flow dynamics in the in vivo rodent dorsal skin fold model

Bernard Choi,[a,*] Nicole M. Kang,[b] and J. Stuart Nelson[a]

[a] *Beckman Laser Institute, University of California, Irvine, CA 92612, USA*
[b] *Department of Engineering, Harvey Mudd College, Claremont, CA 91711, USA*

Received 19 February 2004
Available online 1 July 2004

Introduction

The rodent dorsal skin fold model is used routinely as a means to study changes in microvasculature flow and architecture (Barton et al., 2001; Friesenecker et al., 1994; Gourgouliatos et al., 1990; Mordon et al., 1997; Tsuzuki et al., 2000; Vargas et al., 2001; Wettstein et al., 2003). A method for high spatial and temporal resolution imaging of blood flow dynamics is required to provide objective evaluation of external stimuli, such as pharmacological intervention, electrical stimulation, or laser irradiation. Existing methods for blood flow imaging are inadequate. Laser Doppler imaging requires mechanical scanning, limiting spatial and temporal resolution (Forrester et al., 2002). Magnetic resonance imaging (Schauble and Cascino, 2003) is impractical for routine use and has very limited temporal resolution. Doppler optical coherence tomography (Li et al., 2001; Rollins et al., 2002; Yang et al., 2003; Zhao et al., 2000) can provide longitudinal cross sections of the microvasculature, but simultaneous high temporal resolution and lateral imaging of blood flow cannot be achieved.

Laser speckle imaging (LSI) (Briers, 2001; Dunn et al., 2001; Forrester et al., 2002) is a technique in which time-integrated speckle patterns generated by low-power laser irradiation are imaged with a CCD camera. A simple, lowpass-filtering algorithm is used to convert raw speckle images to flow images. LSI has several advantages over existing methods, including simultaneous high spatial and temporal resolution, ease of implementation, and relatively low cost. We now report the use of a LSI system that is capable of imaging blood flow dynamics in the in vivo rodent dorsal skin fold microvasculature.

Methods

Laser speckle imaging (LSI) system

A HeNe laser (λ = 633 nm, 30 mW, Edmund Industrial Optics, Barrington, NJ) was used to irradiate the animal under study. A planoconvex lens and optical diffusing substrate were used to irradiate uniformly an area of approximately 2.5 cm in diameter. The speckle pattern was imaged with an 8-bit monochrome CCD camera (Model XC-70, Sony, Japan) equipped with a macro zoom lens. The field of view was set to an area of approximately 1 cm^2. The image integration time was set to 8 ms, and the lens configuration was selected to match approximately the speckle size (approximately 15 μm) with the camera pixel size. Video images acquired at 30 frames per second were transferred from the camera to a PC equipped with a frame grabber (National Instruments, Austin, TX). Custom software written in LabVIEW (Version 7, National Instruments) and MATLAB (Version 6.1, The MathWorks, Inc., Natick, MA) was developed to acquire and process the images. To reduce noise, 10 successive images were processed and averaged.

The image processing algorithm has been previously described in detail (Briers, 2001; Dunn et al., 2001). Briefly, the recorded image sequence was converted to speckle contrast images by applying a 7 × 7 sliding window to each 640 × 480 image. Although the 7 × 7 sliding window reduces the spatial resolution of our system, such a technique is necessary to preserve the assumption of first-order speckle statistics. Because the resultant 640 × 480 speckle contrast image is essentially oversampled, relatively high spatial frequency details remain preserved in the speckle contrast and subsequent flow images. At each window

* Corresponding author. Beckman Laser Institute, University of California, Irvine, 1002 Health Sciences Road East, Irvine, CA 92612. Fax: +1-949-824-6969.
E-mail address: bchoi@laser.bli.uci.edu (B. Choi).

0026-2862/$ - see front matter
doi:10.1016/j.mvr.2004.04.003

position, the mean gray-level intensity ($\langle I \rangle$) and standard deviation (σ) were determined, and the speckle contrast (K) of the center pixel in the window can be computed as (Briers and Webster, 1995):

$$K = \frac{\sigma}{\langle I \rangle} \tag{1}$$

Assuming a flow velocity distribution with a Lorentzian profile (Briers and Webster, 1995), the correlation time (τ_c) of the intensity fluctuations can be calculated from:

$$K = \sqrt{\left\{\frac{\tau_c}{2T}[1 - \exp(-2T/\tau_c)]\right\}} \tag{2}$$

where T is the frame integration time. Maple™ software (Maplesoft, Ontario, Canada) was used to obtain an analytic series expansion for τ_c as a function of K, for the value of T used in this study. Relative flow images were obtained by calculating $1/\tau_c$ at each image pixel; a higher pixel value is analogous to faster blood flow. Because we were not concerned with real-time speckle flow imaging in this study, we performed the image processing off-line.

Animal model

Surgical installation of a dorsal skin fold window permitted observation of full-thickness skin from both the epidermal and subdermal sides (Barton et al., 2001; Gourgouliatos et al., 1990; Mordon et al., 1997; Vargas et al., 2001). The animal was anesthetized with a combination of Ketamine and Xylazine (4:3 ratio, 0.1/100 g body weight) administered by intraperitoneal injection. A section for window placement on the back of the animal was selected, surgically scrubbed, shaved, and depilated. Sutures attached to a temporary mount retracted the dorsal skin away from the animal's body. A circular section with an approximate diameter of 1 cm was cut from one side of the symmetrical skin fold, thus exposing blood vessels in the underlying skin. An aluminum chamber was sutured to both sides of the skin. To prevent dehydration, saline was applied periodically to the subdermal skin. The surgery was performed as defined in a protocol approved by the University of California, Irvine, Animal Use Committee.

Analysis of blood flow in response to laser irradiation

The animal was positioned with the subdermal side facing the LSI system. Selected vessels were irradiated with 0.45-ms, 585-nm pulses from a pulsed dye laser (Model SPTL-1b, Candela Corp., Wayland, MA). Images were acquired before, and at specific time points after, laser irradiation. Blood flow dynamics assessed from analysis

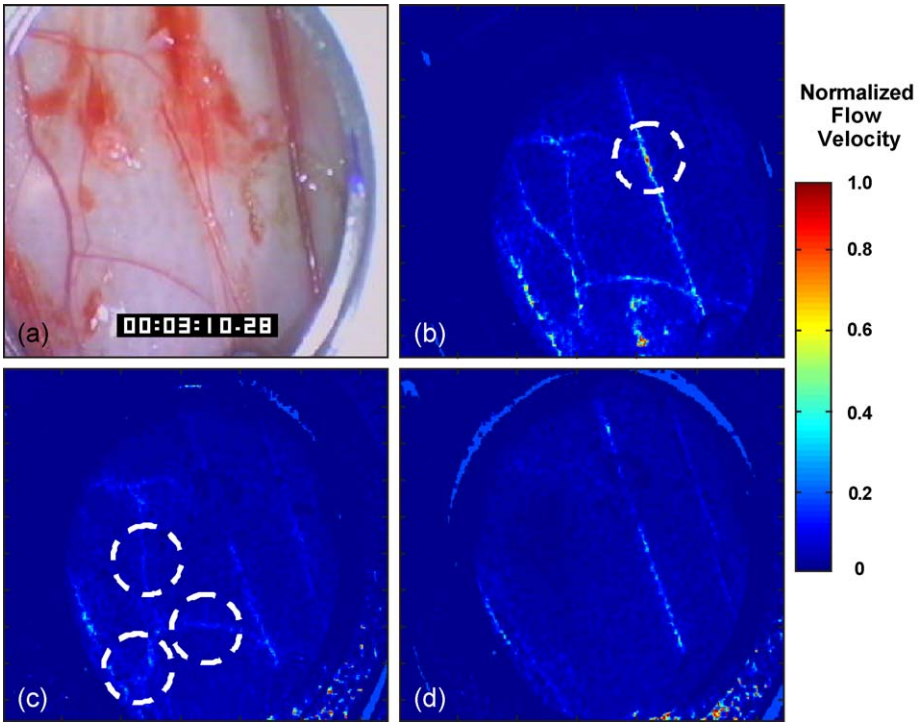

Fig. 1. (a) Exposed microvasculature in the rodent skin fold model. Note the presence of small pieces of attached muscle and fat, slightly obscuring the field of view. (b) Speckle flow image, highlighting regions of blood flow. The encircled region was irradiated with a 585-nm laser pulse, resulting in (c) local flow stoppage in the vessel and reduced flow within the rest of the vessel. The three encircled regions were irradiated next, resulting in (d) complete flow stoppage in those vessels. In d, it was apparent that flow in the first irradiated vessel (i.e., circled region in b) was partially restored. Speckle image dimensions are 1.4-cm width and 1-cm height. The color bar at the right is the normalized flow velocity.

of speckle flow images were compared with qualitative assessments from visual inspection of the vessels.

Results and discussion

From a digital photograph of the subdermal side of the rodent skin fold (Fig. 1a), blood vessels are readily evident. In this specific preparation, small pieces of muscle and fat remained attached to the skin, slightly obscuring the field of view. After speckle image processing, the resulting image provided a clear map of relative blood flow (Fig. 1b). The encircled region was irradiated with a laser pulse, resulting in a decrease in blood flow (Fig. 1c). Next, the three encircled regions in Fig. 1c were irradiated in succession with a single laser pulse, resulting in complete flow stoppage within these vessels (Fig. 1d). From the processed images (Figs. 1c,d), it was readily apparent that blood flow was partially restored in the first irradiated region (i.e., circled region in Fig. 1b). This restoration of flow could be identified by careful visual inspection but was readily detected using our LSI system. Longitudinal blood flow profiles (Fig. 2) within the first irradiated region (Fig. 1b) demonstrate the capability of LSI to provide quantitative data of relative blood flow dynamics. Our results indicate that LSI can provide high spatial resolution maps of relative blood flow in a rodent skin fold model. Our system currently can provide a 640 × 480 speckle flow image in approximately 6 s and thus is not optimized yet for near real time imaging. In comparison, Forrester et al. (2002) reported that laser Doppler imaging requires at least 4 min to acquire a 256 × 256 flow image. We believe with implementation of processing algorithms using lower-level programming languages (e.g., C/C++), we can increase dramatically our speckle flow image display rate. An alternate approach is to perform a time-resolved analysis of raw speckle images, which obviates the need for use of a sliding window to process the raw speckle images; such a technique can preserve the high spatial resolution present in the raw images (Forrester et al., 2002).

In highly scattering biological tissues, such as skin, LSI has been used to monitor tissue perfusion dynamics (Briers, 2001; Briers and Webster, 1995; Forrester et al., 2002). Dunn et al. (2001) used LSI to provide cerebral blood flow maps of individual vessels in a relatively small field of view (2–3 mm). With the rodent dorsal skin fold model, relatively large (50–200-μm diameter) vessels are present, allowing for flow monitoring of these vessels over a wider field of view (1-cm diameter in this study). Thus, LSI is well suited for this model, which is a convenient in vivo microvasculature preparation used in diverse applications such as laser therapy (Barton et al., 2001; Gourgouliatos et al., 1990; Mordon et al., 1997; Vargas et al., 2001), oncology (Tsuzuki et al., 2000), critical care (Wettstein et al., 2003), and pharmacokinetics (Friesenecker et al., 1994). We anticipate that LSI can be useful as an objective feedback tool to monitor flow dynamics in other tissues with superficial vessels, such as the chick chorioallantoic membrane (Kimel et al., 2002).

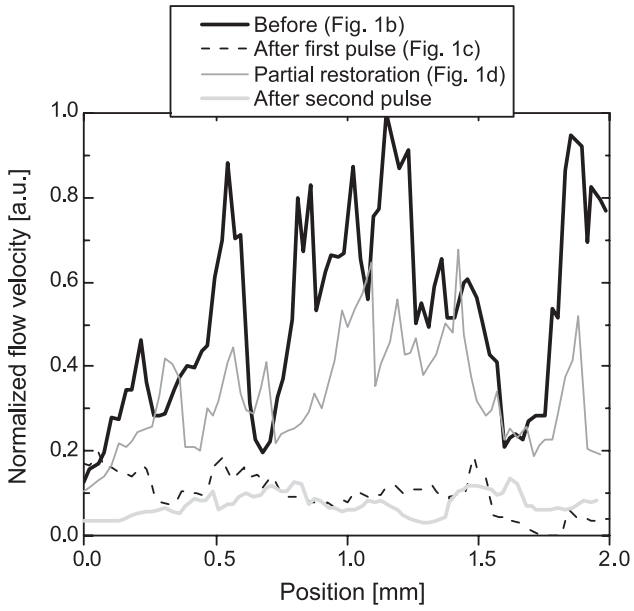

Fig. 2. Normalized blood flow velocity along the length of the blood vessel segment enclosed within the encircled region of the first irradiated vessel (Fig. 1b), demonstrating the flow profile before laser irradiation (black line), and immediately (dashed black line) and 10 min after (solid dark gray line), and immediately after irradiation with a second laser pulse (solid light gray line).

Acknowledgments

The authors thank Cindy Lan and Justin Lotfi for their assistance with the animal surgery and Dr. Julio Ramirez for his assistance with the Maple software. This work was funded in part by the Arnold and Mabel Beckman Fellows Program (BC) and the National Institutes of Health (AR48458, AR47551, and EB2495, awarded to JSN).

References

Barton, J.K., et al., 2001. Cooperative phenomena in two-pulse, two-color laser photocoagulation of cutaneous blood vessels. Photochem. Photobiol. 73, 642–650.

Briers, J.D., 2001. Laser Doppler, speckle and related techniques for blood perfusion mapping and imaging. Physiol. Meas. 22, R35–R66.

Briers, J.D., Webster, S., 1995. Quasi real-time digital version of single-exposure speckle photography for full-field monitoring of velocity or flow-fields. Opt. Commun. 116, 36–42.

Dunn, A.K., et al., 2001. Dynamic imaging of cerebral blood flow using laser speckle. J. Cereb. Blood Flow Metab. 21, 195–201.

Forrester, K.R., et al., 2002. Comparison of laser speckle and laser Doppler perfusion imaging: measurement in human skin and rabbit articular tissue. Med. Biol. Eng. Comput. 40, 687–697.

Friesenecker, B., et al., 1994. Oral administration of purified micronized flavonoid fraction suppresses leukocyte adhesion in ischemia–reperfu-

sion injury—in vivo observations in the hamster skin fold. Int. J. Microcirc.: Clin. Exp. 14, 50–55.

Gourgouliatos, Z., et al., 1990. Laser-irradiation-induced relaxation of blood vessels in vivo. Laser Surg. Med. 10, 524–532.

Kimel, S., et al., 2002. Vascular response to laser photothermolysis as a function of pulse duration, vessel type, and diameter: implications for port wine stain laser therapy. Laser Surg. Med. 30, 160–169.

Li, X.D., et al., 2001. Intraluminal fiber-optic Doppler imaging catheter for structural and functional coherence tomography. Opt. Lett. 26, 1906–1908.

Mordon, S., et al., 1997. Selective laser photocoagulation of blood vessels in a hamster skin flap model using a specific ICG formulation. Laser Surg. Med. 21, 365–373.

Rollins, A.M., et al., 2002. Real-time in vivo colors Doppler optical coherence tomography. J. Biomed. Opt. 7, 123–129.

Schauble, B., Cascino, G.D., 2003. Advances in neuroimaging: management of partial epileptic syndromes. Neurosurg. Rev. 26, 233–246.

Tsuzuki, Y., et al., 2000. Vascular endothelial growth factor (VEGF) modulation by targeting hypoxia-inducible factor-1 alpha → hypoxia response element → VEGF cascade differentially regulates vascular response and growth rate in tumors. Cancer Res. 60, 6248–6252.

Vargas, G., et al., 2001. Use of osmotically active agents to alter optical properties of tissue: effects on the detected fluorescence signal measured through skin. Laser. Surg. Med. 29, 213–220.

Wettstein, R., et al., 2003. Resuscitation with polyethylene glycol-modified human hemoglobin improves microcirculatory blood flow and tissue oxygenation after hemorrhagic shock in awake hamsters. Crit. Care Med. 31, 1824–1830.

Yang, V.X.D., et al., 2003. High speed, wide velocity dynamic range Doppler optical coherence tomography (Part I): system design, signal processing, and performance. Opt. Express 11, 794–809.

Zhao, Y., et al., 2000. Doppler standard deviation imaging for clinical monitoring of in vivo human skin blood flow. Opt. Lett. 25, 1358–1360.