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Brief Communication

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

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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 ($\lambda = 633$ 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 cm². The image integration time was set to 8 ms, and the lens configuration was selected to match approximately the speckle size (approximately 15 μ m) 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

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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):

$$K = \frac{\sigma}{\langle I \rangle} \quad (1)$$

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:

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

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

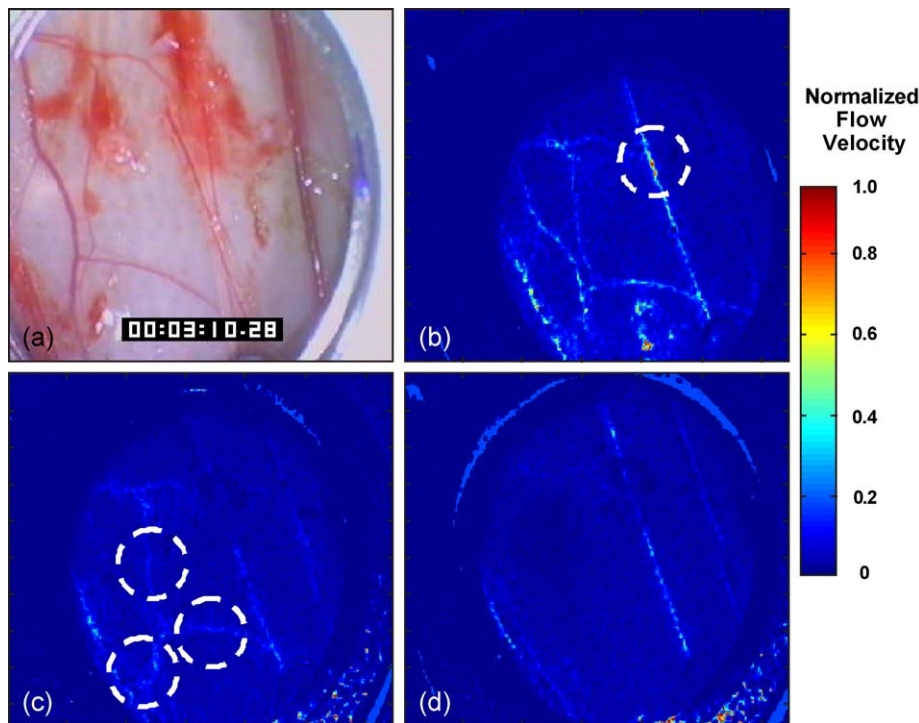


Fig. 1. (a) Exposed microvasculature in the rodent skin fold model. Note the presence of small pieces of attached muscle and fat, slightly obscuring the field of view. (b) Speckle flow image, highlighting regions of blood flow. The encircled region was irradiated with a 585-nm laser pulse, resulting 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

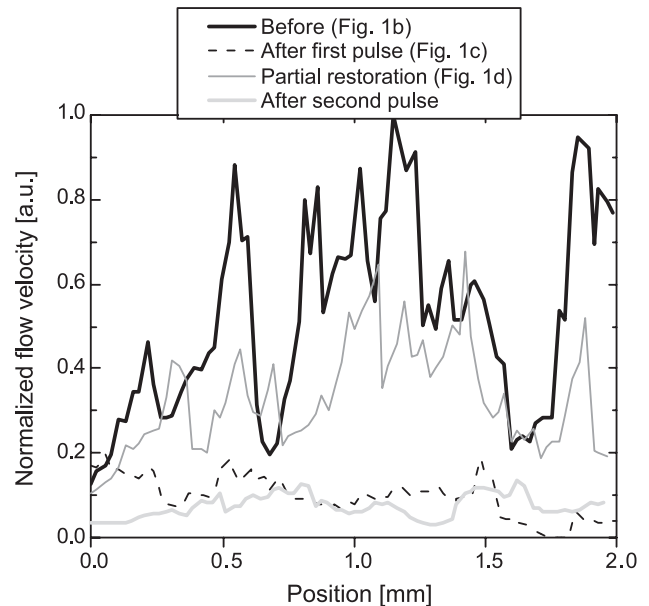


Fig. 2. Normalized blood flow velocity along the length of the blood vessel segment enclosed within the encircled region of the first irradiated vessel (Fig. 1b), demonstrating the flow profile before laser irradiation (black line), and immediately (dashed black line) and 10 min after (solid dark gray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

feedback tool to monitor flow dynamics in other tissues with superficial vessels, such as the chick chorioallantoic membrane (Kimel et al., 2002).

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