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Doppler standard deviation imaging for clinical monitoring of *in vivo* human skin blood flow

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We used a novel phase-resolved optical Doppler tomographic (ODT) technique with very high flow-velocity sensitivity (10 μm/s) and high spatial resolution (10 μm) to image blood flow in port-wine stain (PWS) birthmarks in human skin. In addition to the regular ODT velocity and structural images, we use the variance of blood flow velocity to map the PWS vessels. Our device combines ODT and therapeutic systems such that PWS blood flow can be monitored *in situ* before and after laser treatment. To the authors' knowledge this is the first clinical application of ODT to provide a fast semiquantitative evaluation of the efficacy of PWS laser therapy *in situ* and in real time. © 2000 Optical Society of America

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Numerous methodologies, including, fluorescein injection, isotopic clearance, angioscopy and angiography, electromagnetic flowmetry, interstitial fluid pressure, reflective photoplethysmography, dermofluorometry, magnetic resonance imaging, and temperature probes, have been investigated in search of the ideal blood flow imaging technique for human skin. Inasmuch as of these methods have shown but limited utility, more-recent approaches have incorporated the Doppler effect.

Optical Doppler tomography[1–3] (ODT) combines Doppler velocimetry with optical coherence tomography (OCT) to measure blood flow velocity at discrete user-specified locations in highly scattering biological tissues. The exceptionally high resolution of ODT permits noninvasive imaging of both *in vivo* blood microcirculation and tissue structures that surround the blood vessels. We recently developed a novel phase-resolved OCT/ODT system that uses phase information derived from a Hilbert transformation to image blood flow in human skin with fast scanning speed and high-velocity sensitivity.[1,4] Our phase-resolved system decouples spatial resolution and velocity sensitivity in flow images and increases imaging speed by more than 2 orders of magnitude without compromising spatial resolution and velocity sensitivity. The minimum blood flow velocity that can be detected in human skin while simultaneously maintaining a spatial resolution of 10 μm is as low as 10 μm/s. The noninvasive nature and high spatial resolution of ODT should be useful in the clinical management of patients for whom imaging blood flow in human skin is required. However, flow velocity imaging is limited by the fact that the Doppler frequency shift depends on the angle between the probe and flow directions and is highly sensitive to the pulsatile nature of the blood flow. In many clinical applications, the location of the microvasculature is more important than the absolute value of the flow velocity. In this Letter we extend our phase-resolved OCT/ODT technique to describe a method that uses variance of the Doppler frequency spectrum to map the microvasculature. This method has the advantage that it is less sensitive to the pulsatile nature of blood flow and provides better mapping of vessel location. In addition, one can also use this method to study turbulence and to separate the Doppler shift that is due to biological flow from the background motion of the tissue under study.

Port-wine stain (PWS) is a congenital, progressive vascular malformation of capillaries in the dermis of human skin that occurs in approximately 0.3% of children.[5] Histopathological studies of PWS show an abnormal plexus of layers of dilated blood vessels located 150–750 μm below the skin surface in the upper dermis that have diameters that vary among patients, and even from site to site on the same patient, over a range of 10–150 μm. The reported mean vascular area measured by histology for PWS patients is 2–8%.[5] The pulsed dye laser can coagulate PWS selectively by inducing microthrombus formation within the targeted blood vessels.[6] From results of these preliminary studies conducted on PWS patients we report the feasibility and potential application of phase resolved OCT/ODT to characterize and image blood flow with high spatial resolution.

The optical device is based on our phase-resolved OCT/ODT system; the experimental setup was described in detail previously.[1,4] Briefly, low-coherence light generated by amplified spontaneous emission of a 1300-nm diode is coupled into the source arm of a fiber-based Michelson interferometer. In the reference arm, a rapid-scanning optical delay line is used for axis scanning; it employs a grating to control the phase and group delays separately and is aligned such that no phase modulation is generated when the group delay is scanned.[7] A fiber-based electro-optic phase modulator is inserted in the reference arm to produce a stable carrier frequency. The rapid-scanning optical delay line for an axial (A) scan is run at 1000 Hz, and the voice-coil stage [for a lateral (L) scan] is driven linearly at a speed of 500 μm/s. The signal is digitized with a 12-bit 5-MHz analog-to-digital converter. In conventional OCT, one uses the fringe signal from each A-line scan to calculate the Doppler shift of the power spectrum with a short-time Fourier-transformation algorithm. Because the time window used in this algorithm is

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inversely proportional to the A-line scanning speed, the velocity sensitivity is limited by A-line scanning speed. In our phase-resolved OCT/ODT we use the phase change of the interference fringe between sequential A-line scans to calculate the Doppler-frequency shift (Fig. 1). Consequently the phase-resolved system decouples spatial resolution and velocity sensitivity in flow images and increases imaging speed by more than 2 orders of magnitude without compromising spatial resolution and velocity sensitivity.

The digitized fringe signal $\Gamma_j(t)$ is first passed through a digital bandpass filter to increase the signal-to-noise ratio. The complex function $\tilde{\Gamma}_j(t)$ is then determined through an analytic continuation by use of the Hilbert transformation[4]:

$$\tilde{\Gamma}_j(t) = \Gamma_j(t) + \frac{i}{\pi} P \int_{-\infty}^{\infty} \frac{\Gamma_j(\tau)}{\tau - t} \mathrm{d}\tau, \tag{1}$$

where j denotes the jth A-line scan and P denotes the Cauchy principle value of the integral. The Doppler-frequency shift is determined from the average phase shift between sequential A scans from the following equation:

$$\Delta f = \frac{1}{2\pi T} \tan^{-1}\left[\frac{\mathrm{Im}\left(\sum_{j=1}^{n} \tilde{\Gamma}_j \tilde{\Gamma}_{j+1}^{*} \right)}{\mathrm{Re}\left(\sum_{j=1}^{n} \tilde{\Gamma}_j \tilde{\Gamma}_{j+1}^{*} \right)} \right], \tag{2}$$

where T is the time interval between sequential scans and n is the number of sequential scans that are averaged. To increase the signal-to-noise ratio in ODT images, we average eight sequential A-line scans. The standard deviation of the Doppler-frequency spectrum, σ, is calculated as follows:

$$\sigma^2 = \frac{\int_{-\infty}^{\infty} (\omega - \overline{\omega})^2 P(\omega) \mathrm{d}\omega}{\int_{-\infty}^{\infty} P(\omega) \mathrm{d}\omega} = \frac{1}{T^2} \left(1 - \frac{\left| \sum_{j=1}^{n} \tilde{\Gamma}_j \tilde{\Gamma}_{j+1}^{*} \right|}{\sum_{j=1}^{n} \tilde{\Gamma}_j \tilde{\Gamma}_j^{*}} \right), \tag{3}$$

where $P(\omega)$ is the Doppler power spectrum and $\overline{\omega}$ is the centroid value of the Doppler frequency shift. The value of σ depends on the flow velocity distribution. Variations in flow velocity will broaden the Doppler-frequency spectrum and result in a larger σ.

Cross-sectional structural and velocity images of a PWS located on the left upper extremity of a human volunteer as obtained by our system are shown in Fig. 2. The scanning range is 2 mm (lateral) by 2 mm (axial), but only the linear part (1.25 mm) of the axial scan is shown in the figure. The image size is 800 (lateral) by 500 (axial) pixels, with a pixel size of 2.5 μm, which keeps the image resolution consistent with the coherence length of the light source (10 μm). To prevent surface movement we placed the area imaged in contact with a glass window and inserted index-matching oil between the glass and the PWS to decrease reflection of light from the skin surface. The index-matching oil also helped to flatten the skin surface such that the wave-front distortion of the probe beam at the skin surface was minimized. Figures 2A (structural image), 2B (velocity image), and 2C (variance image) were taken from the palm-side surface of the ring finger. Figures 2D–2F were taken from the forearm. In addition to an organized network of collagen fibers in the dermis, one can clearly see the boundary between the stratum corneum and the epidermis in the structural images (Figs. 2A and 2D). Many PWS vessels are detected 400 μm to

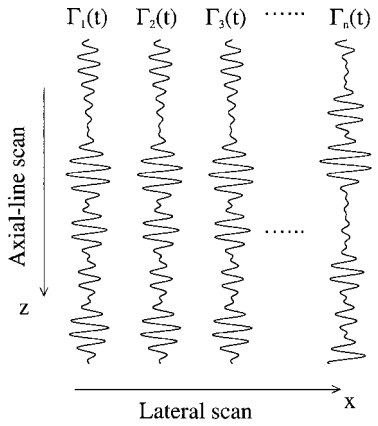

Fig. 1. Schematic signal-processing diagram for the phase-resolved OCT/ODT system.

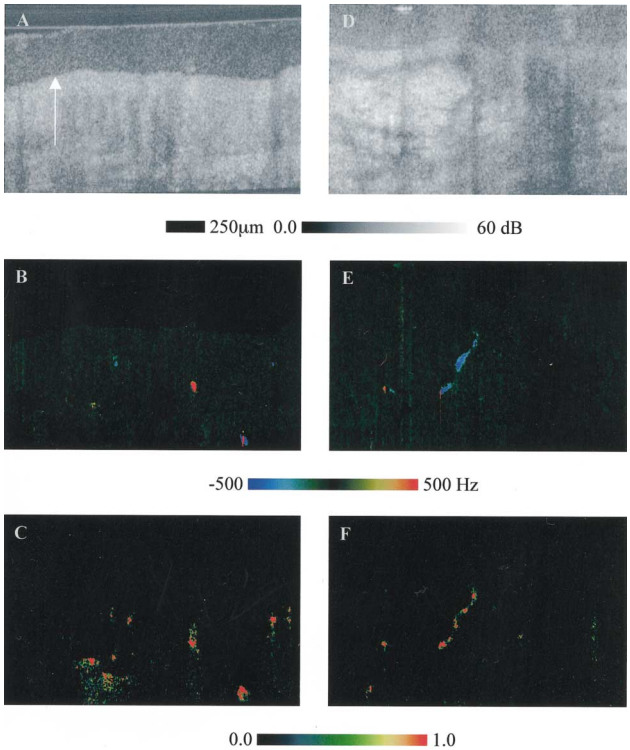

Fig. 2. Tomographic images of a left upper extremity PWS. A, D, Structural images; B, E, Doppler-shift (blood flow velocity) images; C, and F, normalized variance of flow velocity. Images A–C are taken from the palm-side surface of the ring finger. Images D–F are taken from the forearm. The arrow in image A indicates the boundary between the stratum corneum and the epidermis.

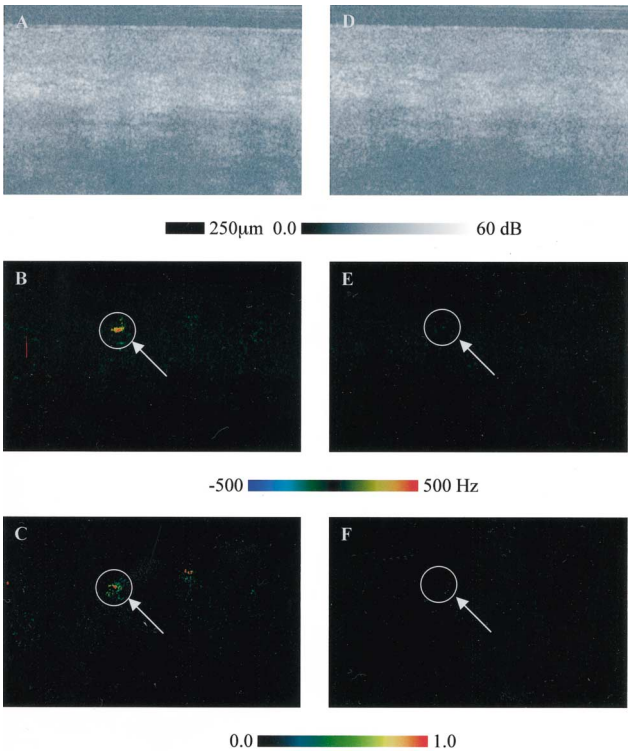

Fig. 3. Tomographic images of identical PWS sites before (A–C) and after (D–F) laser treatment. A, D, Structural images; B, E, Doppler-shift (blood flow velocity) images; C, and F, normalized variance of flow velocity. The vessel areas are marked by circles and arrows in the velocity and variance images.

1 mm below the skin surface in the velocity images (Figs. 2B and 2E).

In addition, flow turbulence, which is determined by the standard deviation σ of the Doppler spectrum, is shown as two-dimensional images in Figs. 2C and 2F. As can be seen, it is much easier to identify the PWS blood vessels in the σ images (Figs. 2C and 2F) than in the velocity images (Figs. 2B and 2E). This phenomenon can be attributed to the pulsatile nature of blood flow. Given the fact that blood flow turbulence is determined only by the physical characteristics of the blood–vessel structure, determining the variance provides a much more accurate mapping of the subsurface microvasculaure in human skin.

To monitor the efficacy of PWS laser treatment *in situ*, we constructed a device that combines a therapeutic beam with the OCT–ODT beam. The OCT–ODT image can be taken immediately after the therapeutic laser pulse without the need to replace the probe beam. The laser used for PWS treatment is a ScleroPlus pulsed dye laser (Candela Laser Corporation, Wayland, Mass.) with a wavelength of 595 nm and a pulse width of 1.5 ms. Figure 3 shows the response to a 12-J/cm^2 therapeutic laser pulse. In the structural images (Figs. 3A and 3D) there is no visible difference before and after laser exposure, which implies that the adjacent skin structures were not affected by the treatment. In the velocity (Figs. 3B and 3E) and the variance (Figs. 3C and 3F) images, however, no blood flow is noted after laser exposure, which is indicative of irreversible microthrombus formation in the PWS blood vessels. Blood flow did not return to pretreatment values, as determined by subsequent scans made as much as 24 h after laser exposure.

The rationale for using ODT in the clinical management of PWS is that the technique offers a means of providing a fast semiquantitative evaluation of the efficacy of laser therapy in real time. If partial restoration of flow occurs immediately or shortly after pulsed laser exposure, which would indicate reperfusion owing to inadequate blood-vessel injury, the PWS can be re-treated with higher dosages of light. Retreatment is continued until the measured Doppler shift is zero owing to a permanent reduction in blood flow, which is indicative of irreversible microthrombus formation in the PWS vessels.

In summary, we have developed a new imaging method that uses velocity variance to determine the location and shape of blood vessels below the skin surface. We have shown that phase-resolved OCT/ODT can be used to image blood flow in PWS human skin by providing a fast semiquantitative evaluation of the efficacy of PWS laser therapy *in situ* and in real time on an individual-patient basis.

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