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Noninvasive imaging of *in vivo* blood flow velocity using optical Doppler tomography

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We report the development of an optical technique for noninvasive imaging of *in vivo* blood flow dynamics and tissue structures with high spatial resolution (2–10 μm) in biological systems. The technique is based on optical Doppler tomography (ODT), which combines Doppler velocimetry with optical coherence tomography to measure blood flow velocity at discrete spatial locations. The exceptionally high resolution of ODT permits noninvasive *in vivo* imaging of both blood microcirculation and tissue structures surrounding the vessel, which has significance for biomedical research and clinical applications. Tomographic imaging of *in vivo* blood flow velocity in the chick chorioallantoic membrane and in rodent skin is demonstrated. © 1997 Optical Society of America

High-resolution noninvasive techniques for *in vivo* blood flow imaging are not currently available as a diagnostic tool in medicine. Such a technique could have a significant impact on biomedical research and clinical diagnosis.[1] Numerous approaches have been investigated, including Doppler ultrasound,[2] conventional angiography,[3] laser Doppler flowmetry (LDF),[4] and magnetic-resonance angiography.[3] Each of these techniques has limitations. Conventional LDF, for example, has been used to measure mean blood perfusion in the peripheral microcirculation. However, strong optical scattering in biological tissue limits spatially resolved flow measurements by LDF. Although Doppler ultrasound imaging provides a means to resolve flow velocities at different locations in a scattering medium, the relatively long acoustic wavelength required for deep tissue penetration limits spatial resolution to ~200 μm. Coherence techniques based on detection of the interference-fringe intensity of light backscattered from a sample have been used to image biological samples with micrometer resolution.[5–7] Consequently, flow-velocity sensors based on the coherence interferometry technique are expected to improve spatial resolution.[8,9] In this Letter we report what is believed to be the first noninvasive *in vivo* imaging of blood flow by use of optical Doppler tomography (ODT). Compared with other microvascular imaging techniques, ODT is noninvasive and noncontact, has high spatial resolution (2–10 μm), and provides simultaneous information regarding not only *in vivo* blood flow at discrete locations but also the tissue structure surrounding the vessel.

ODT combines LDF[4] with optical coherence tomography,[5–7,10,11] which uses a Michelson interferometer with a low-coherence light source to obtain sectional images of biological materials. In ODT (Fig. 1), the sample and the reference mirrors constitute the two arms of an interferometer. One determines the amplitude of backscattered light from the sample by measuring the interference-fringe intensity generated between reference and target beams. High axial spatial resolution is possible because light backscattered from the sample recombines with the reference beam in the 2×2 coupler and interferes only when the optical-path-length difference is within the coherence length of the source light. When light backscattered from a moving particle interferes with a reference beam, beating at the Doppler frequency (f_D) occurs, shifting the frequency of the interference-fringe intensity from that of the optical phase modulation by f_D:

$$f_D = \frac{1}{2\pi}(\mathbf{k}_s - \mathbf{k}_i) \cdot \mathbf{V}\,, \tag{1}$$

where $\mathbf{k}_i$ and $\mathbf{k}_s$ are wave vectors of incoming and scattered light, respectively, and $\mathbf{V}$ is the velocity vector of the moving particles.

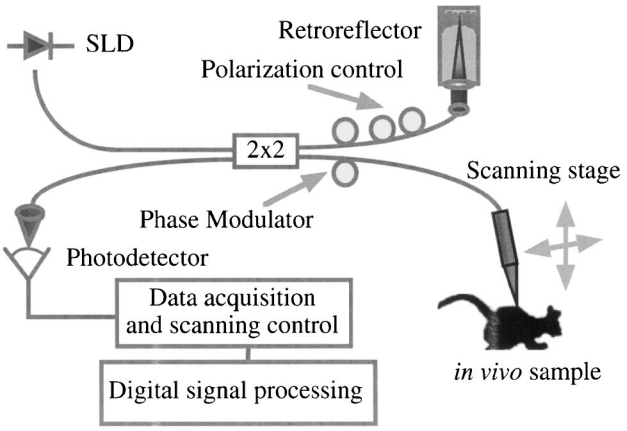

Fig. 1. Schematic diagram of the ODT system. Light emitted from the superluminescent diode ($\lambda_0 = 850$ nm, $\Delta\lambda_{\mathrm{FWHM}} = 25$ nm) is coupled into a single-mode fiber and split into reference and target arms by a 2×2 (50/50) fiber coupler. Stress birefringence is used to match the polarization of reference and sample beams and to optimize fringe contrast. The optical path lengths of light in the reference and the target arms are modulated (1600 Hz) with piezoelectric cylinders electrically driven by a ramp waveform. The target arm is tilted 70–75° with respect to the direction of flow. Optical interference-fringe intensity is measured by a photodetector and digitized with a 16-bit analog–digital converter. Structural and flow-velocity images are calculated from the digitized fringe intensities.

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Fluid-flow velocity at each pixel is determined by measurement of the Doppler frequency shift, which is defined as the difference between the carrier frequency established by the optical phase modulation and the centroid of the measured power spectrum at each pixel. Two-dimensional images are formed by sequential lateral scans at a constant horizontal velocity of 800 μm/s, followed by incremental probe movements (10 μm) in the vertical (axial) direction. In addition to the flow-velocity image, one can obtain a tomographic structural image from the same scan simultaneously by calculating at each pixel the magnitude of the power spectrum at the carrier frequency established by the optical phase modulation.

To demonstrate the ability of ODT to image *in vivo* blood flow, we studied two biological models, the chick chorioallantoic membrane (CAM) and rodent skin. The CAM is a well-established model for studying microvasculature and has been used extensively to investigate the effects of vasoactive drugs as well as optical and thermal processes in blood vessels.[12] Because the CAM microvasculature is located in a transparent matrix, direct viewing and noninvasive imaging of the blood vessels are possible after the apex of the chick egg shell is removed. The effects of optical scattering in biological tissues are investigated in the rodent skin model.

In the CAM model, blood flow in a vein is imaged to minimize the effect of pulsation. Structural (Fig. 2A) and velocity (Fig. 2B) images of CAM blood flow are obtained simultaneously. The blood vessel wall, chorion membrane, and yolk sac membrane are evident in the structural image. In the velocity image, static regions ($V = 0$) in the CAM appear dark, and blood moving at different velocities is evident. The magnitude of blood flow velocity is maximal at the vessel center and decreases monotonically toward the peripheral wall. A horizontal cross section of the velocity image near the vessel center is shown in Fig. 2C. The excellent fit of the velocity profile to a parabolic function indicates that blood flow in the CAM vein is laminar.

In the rodent (Sprague-Dawley) skin model, *in vivo* blood flow in both veins and arteries is imaged by ODT. Cross-sectional structural (Fig. 3A) and velocity (Figs. 3B and 3C) images are obtained simultaneously in a single two-dimensional scan. The presence of vessel-like circular features can be observed in the structural image (Fig. 3A). Strong attenuation of the light backscattered from locations deep in the skin indicates a high degree of optical scattering. The dynamic range of the measured backscattered light in Fig. 3A is ~45 dB. Velocity images of blood flow moving in opposite directions, as determined by the sign of the f_D [Eq. (1)], are shown in Figs. 3B and 3C. Blood flow in two small veins (Fig. 3B) and an artery (Fig. 3C) is clearly identified. To demonstrate the versatility of ODT, we also recorded *en-face* structural (Fig. 4A) and velocity (Fig. 4B) images by scanning with the target beam focused 200 μm below the skin surface. Blood flow in a branching vessel is clearly evident. These results demonstrate that ODT can be used for noninvasive imaging of *in vivo* blood flow velocity in highly scattering biological tissues.

The lateral and the axial spatial resolutions of our ODT system are limited by the beam spot size and the coherence length of the light source to 5

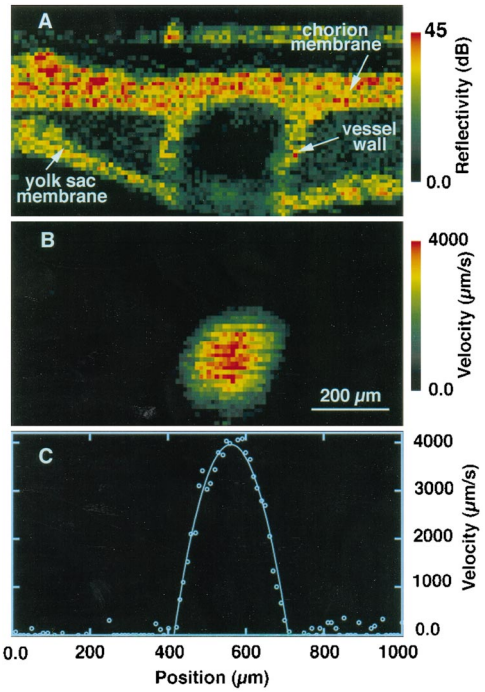

Fig. 2. ODT images of *in vivo* blood flow in a CAM vein. A, color-coded structural image. B, color-coded velocity image. C, velocity profile along a horizontal cross section passing through the center of the vein. Open circles, experimental data; solid curve, fit to a parabolic function.

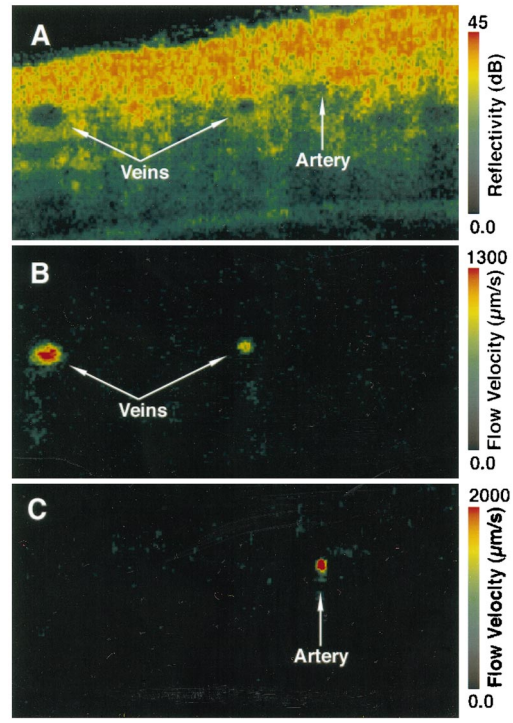

Fig. 3. ODT images of *in vivo* blood flow in rodent skin. A, color-coded tomographic structural image. B, color-coded velocity image of venous blood flow (into the page). C, color-coded velocity image of arterial blood flow (out of the page). Structural and velocity images are obtained simultaneously from a single two-dimensional scan.

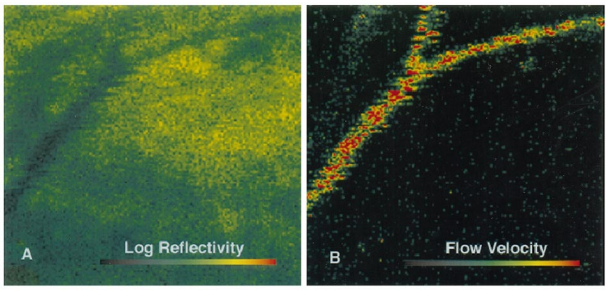

Fig. 4. A, *en-face* structural and B, velocity images of blood flow in a vessel 200 μm below the skin surface.

and 13 μm, respectively.[6] Higher axial resolution can be achieved if a source with a broader spectral emission is used. Velocity resolution in our current system is $\sim$100 μm/s. This resoluton depends on the data-acquisition time at each pixel and the angle between the flow direction and the target beam. Resolution can be improved if a smaller angle and (or) a longer acquistion time is used. With our current scanning speed of 800 μm/s, data-acquisition time for an ODT image of 1 mm $\times$ 1 mm with 10-μm spatial resolution is $\sim$3 min. The scanning speed is ultimately limited by the data-acquisition time at each pixel, which affects the detection sensitivity and the velocity resolution. If we assume that detection of the Doppler shift requires that one sample the interference-fringe intensity over at least one oscillation cycle, pixel-acquisition time varies inversely with f_D. For example, resolving a 1-kHz Doppler shift, which corresponds to a velocity of 1000 μm/s if the angle between the probe beam and the flow direction is 70°, means that the minimum data-acquisition time at each pixel is $\sim$1 ms. When time variation of the interference-fringe intensity data corresponding to each pixel is acquired sequentially, a 100 $\times$ 100 pixel ODT image can be acquired in 10 s. However, *in vivo* blood flow at user-specified locations can be probed with a response time of $<$1 ms for a single pixel. The imaging speed in our current ODT system is limited because temporal interference-fringe intensity data are acquired serially. Shorter image acquisition time ($<$1 s) is possible if other scanning techniques are implemented, for example, if the time variation of fringe intensity data at multiple pixels is recorded in parallel.

ODT has great potential for use in the clinical management of patients who can benefit from microvascular monitoring. The probing depth of ODT in tissue is similar to that of optical coherence tomography and is of the order of 1–2 mm, which covers the epidermal and the dermal ranges. Information provided by ODT could be used to monitor perfusion and viability before, during, and after reconstructive procedures, determine the efficacy of pharmacological intervention for failing surgical skin flaps or replants,[13] image microcirculation during sepsis, assess burn depth,[14] diagnose atherosclerotic disease, and investigate the mechanisms of photodynamic therapy for cancer treatment.[15] Although clinical applications of ODT remain untested, we have used this technique to monitor changes in blood flow dynamics and vessel structure following photodynamic therapy.[16] ODT could also be applied to other nonmedical areas in which rapid noninvasive imaging of turbulent or laminar flow is required. Given the noninvasive nature of the measurement, exceptional spatial resolution, simple hardware requirements, and relatively compact size, ODT is a promising technique for both basic research and clinical medicine.

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