Title
Imaging blood flow in human port-wine stain in situ and in real time using optical Doppler tomography.

Journal
JAMA Dermatology, 137(6)

ISSN
2168-6068

Authors
Nelson, JS
Kelly, KM
Zhao, Y
et al.

Publication Date
2001-06-01

OBSERVATION

Imaging Blood Flow in Human Port-wine Stain In Situ and in Real Time Using Optical Doppler Tomography

J. Stuart Nelson, MD, PhD; Kristen M. Kelly, MD; Yonghua Zhao, PhD; Zhongping Chen, PhD

Background: Optical Doppler tomography (ODT) combines laser Doppler flowmetry with optical coherence tomography to obtain high-resolution images of blood flow in human skin in situ and in real time.

Observations: We present a case in which ODT was used on a patient with a port-wine stain (PWS) birthmark to document the change of blood flow in response to laser therapy. It might be possible to use ODT blood flow measurements in situ to assist in assessing the efficacy of laser PWS therapy. If partial restoration of flow occurs immediately or shortly after laser exposure, indicative of reperfusion due to inadequate blood vessel injury, the PWS can be retreated using higher light dosages. Retreatment is continued until the measured Doppler shift is zero due to a permanent reduction in blood flow, indicative of irreversible microthrombus formation in the PWS vessels.

Conclusions: We have demonstrated that ODT may be used for noninvasive imaging of blood vessels in PWS skin. Moreover, ODT will potentially allow laser therapy to be optimized on an individual patient basis by providing a fast, semiquantitative evaluation of the efficacy of PWS laser therapy in situ and in real time.

Arch Dermatol. 2001;137:741-744

TODAY, CLINICIANS rely on many noninvasive biological imaging techniques to diagnose and treat diseases. Noninvasive imaging of the cutaneous microcirculation has many clinical applications related to blood flow monitoring. The ideal imaging system should (1) probe the underlying blood flow with high spatial resolution (10 µm) at user-specified locations in superficial and deep dermal regions; (2) distinguish arterial from venous flow; (3) provide objective imaging information that nurses and other paramedical personnel can easily interpret; (4) be safe and noninvasive; and (5) produce reliable and reproducible results.

Many biomedical researchers have investigated the application of noninvasive optical Doppler techniques, such as laser Doppler flowmetry (LDF), for monitoring blood flow in human skin.[1] The principles of LDF are relatively straightforward. In much the same way that the Doppler effect changes the pitch of a car horn as the car passes an observer, light is frequency shifted when it bounces off moving objects.[2] With LDF, incident light of a single optical frequency enters the skin. A second fiber collects backscattered light, a small fraction of which is frequency (or Doppler) shifted by moving red blood cells; in comparison, light scattered exclusively by static, nonmoving constituents shows no frequency change. The LDF signal is due to multiply-scattered light with a large variance in optical path lengths through the tissue. As a result, spatial resolution is poor (>100 µm), and information relevant to blood flow at a discrete depth is lost.

Optical coherence tomography (OCT) is a recently developed modality for high-resolution (10-µm) noninvasive imaging of living biological tissues.[3-5] Optical coherence tomography is analogous to ultrasound, in which acoustic waves bounce off tissue and are translated into images. Optical coherence tomography fills a valuable niche in biomedical optics by providing accurate in vivo subsurface imaging in 3 dimensions without requiring contact between the probe and the tissue under study.

In its simplest form, OCT uses a broadband near-infrared light source in combination with a Michelson interferometer to produce 2 beams: one beam focuses on the specimen and the other pro-

From the Beckman Laser Institute and Medical Clinic (Drs Nelson, Kelly, Zhao, and Chen) and the Departments of Surgery (Drs Nelson, Zhao, and Chen), Dermatology (Drs Nelson and Kelly), and Biomedical Engineering (Drs Nelson and Chen), University of California, Irvine.

MATERIALS AND METHODS

We developed an ODT instrument[11,12] that uses a fiber-optic Michelson interferometer (**Figure 1**) with a broadband light source (center wavelength, 1300 nm; output power, 5 mW; and bandwidth, 65 nm). Light from the source was coupled into a fiber interferometer using a 2 × 2 coupler and then split equally into reference and target beams. In the reference beam, a rapid-scanning optical delay line was used to change the optical path length for axial scanning. In the reference beam, light backscattered from the skin was coupled back into the fiber and formed interference fringes at the photodetector. Interference fringes were observed only when the optical path length difference between the sample and reference beams was less than the coherence length of the source. The fringe signals were then processed by a computer to generate conventional OCT and ODT images. Images from OCT were obtained by calculating the amplitude of the fringe signals. Optical Doppler tomographic images were obtained by calculating the phase change of the fringe signals.

Light in the sample path was focused onto a 10-µm spot on the skin surface using a 1:1 magnification gradient index lens (NA = 0.2). The probing beam was aligned with the optical axis oriented at an angle of 5° to 10° from the skin surface so that blood flow parallel to the surface could produce a Doppler frequency shift. Using our instrument, the approximate time to record conventional OCT and ODT blood flow images was 2 seconds, with a velocity sensitivity of 10 µm/s. After scanning, the time required to reconstruct the images was 7 seconds using a workstation platform.

To prevent surface movement, the area imaged was in tight contact with a glass window, and an index-matching oil was inserted between the glass and the PWS to decrease light reflection from the skin surface. The oil also helped to flatten the skin surface so that wavefront distortion of the probing beam at the skin surface was minimized.

A 62-year-old white man with a PWS on the left hand was recruited from the outpatient population of patients available at the Beckman Laser Institute and Medical Clinic, University of California, Irvine; the protocol was approved by the institutional review board. Informed consent was obtained.

To monitor the efficacy of PWS treatment in situ, we constructed a handpiece that combined the laser irradiation with the ODT probe at the same site. The conventional OCT and ODT images were obtained from the exact same site immediately before and after the laser pulse was delivered without moving the probe. The laser used for PWS irradiation was a ScleroPlus (Candela Laser Corp, Wayland, Mass) pulsed dye laser (wavelength, 595 nm; pulse width, 1.5 milliseconds; spot diameter, 7 mm; and fluence, 12 J/cm^2).

After laser irradiation, a 3-mm punch biopsy specimen was obtained from the center of the 7-mm irradiated spot and fixed in 10% formalin. The tissue sample was embedded in paraffin, sectioned, and stained with hematoxylin-eosin. The remaining laser-irradiated area was followed up with a subsequent ODT measurement and evaluation of blanching 3 months after laser exposure.

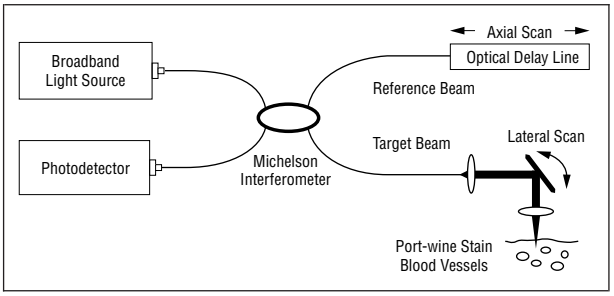

Figure 1. *Schematic of optical Doppler tomographic instrumentation.*

vides a reference. Light reflected from the specimen and reference mirrors is combined at the detector. When the optical path lengths are different between the target and reference beams, no interference is observed and no images are obtained. However, when the optical path lengths are identical for the target and reference beams, interference patterns are formed, and signal-processing algorithms and related hardware translate the interference patterns into images that are displayed as either a 2- or 3-dimensional gray-scale or false-color map. The depth of the structure being imaged in the tissue can be measured by noting the position at which an interference pattern is observed.

Several variations of OCT are now emerging, including optical Doppler tomography (ODT), which combines OCT with LDF to obtain high-resolution tomographic images of static and moving constituents in highly scattering biological tissues.[6-10] The rationale for using ODT to characterize the underlying microvasculature in human skin is that the technique is able to image blood flow with high spatial resolution (10 µm). Furthermore, in contrast to LDF, the overall ODT signal from moving red blood cells is almost entirely due to the Doppler-shifted light. As a result, signal-to-noise ratios are substantially higher.

In this preliminary observation conducted on a patient with a port-wine stain (PWS) birthmark, the ability of ODT to image blood flow with high spatial resolution at discrete locations in highly scattering human skin is demonstrated. Furthermore, we describe how ODT can be used for fast, semiquantitative evaluation of the efficacy of PWS laser therapy in situ and in real time.

RESULTS

Figure 2 shows conventional OCT and ODT images taken in situ from human skin with a PWS. 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 images. The image size is 800 (lateral) by 500 (axial) pixels, with a size of 2.5 µm/pixel. The images in Figure 2 were taken from the palm-side surface of the index finger.

In the conventional OCT image before laser exposure (Figure 2A), the boundary between the stratum corneum and the epidermis is clearly visible, as is an organized network of collagen fibers in the dermis. The conventional OCT image after laser exposure (not shown) did not reveal any notable changes compared with that taken before laser exposure.

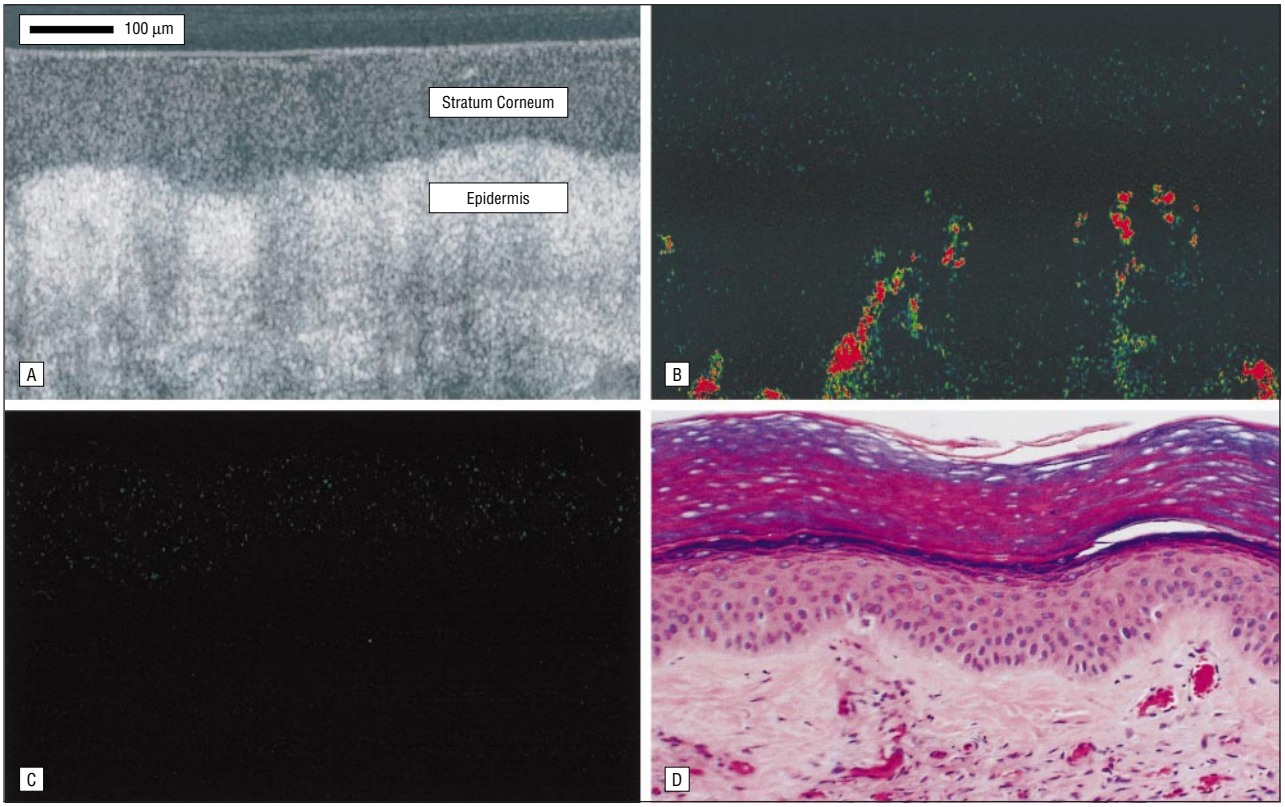

Figure 2. *Tomographic images taken in situ from human skin with a port-wine stain (PWS). A, Conventional optical coherence tomographic (OCT) image before laser exposure. Optical Doppler tomographic (ODT) blood flow images before (B) and immediately after (C) laser exposure. Images A, B, and C were taken from the same skin site without moving the probe beam; ODT images B and C are color-coded tomographic images of blood flow velocity. The boundary between the stratum corneum and the epidermis is clearly visible in the conventional OCT image (A). Many PWS vessels not seen on the conventional OCT image (A) are detected in the dermis up to 500 μm below the skin surface in the ODT image before laser exposure (B). No blood flow is noted in the ODT image immediately after laser exposure (C), indicative of microthrombus formation in the PWS blood vessels. D, Hematoxylin-eosin–stained histologic section from the imaged site (×200). Comparable PWS blood vessels are noted in images B and D.*

Figure 2B-C are color-coded tomographic images of blood flow velocity. In the ODT image (Figure 2B), taken from the exact same site as the conventional OCT image, many PWS vessels are detected in the dermis up to 500 μm below the skin surface before laser exposure. In Figure 2B, static regions appear dark, and blood moving at different velocities is evident. Immediately after pulsed laser exposure using a fluence of 12 J/cm^2, no blood flow is noted in the ODT image, indicative of microthrombus formation in the PWS blood vessels (Figure 2C). Figure 2D is a hematoxylin-eosin–stained section obtained from the punch biopsy specimen taken at the imaged site. Comparable vessels are seen in Figure 2B, D. Furthermore, intravascular microthrombi are visible in the postirradiation biopsy specimen (Figure 2D). Blood flow did not return to pretreatment values, as determined by subsequent ODT scans (not shown) 3 months after laser exposure, indicative of irreversible microthrombus formation. Moreover, clinically significant blanching was noted on the PWS test site 3 months after laser exposure, indicating that revascularization did not occur.

COMMENT

In this observation, we demonstrated how ODT can measure the vascular response of patients with PWSs undergoing laser therapy. Port-wine stain is a congenital, progressive vascular malformation in the dermis; histopathological studies of PWS show an abnormal plexus of layers of dilated blood vessels 150 to 750 μm below the skin surface having diameters varying on an individual patient basis, and even from site to site on the same patient, from 10 to 150 μm.[13]

Use of the pulsed dye laser can induce microthrombus formation[14] and selectively coagulate PWS vessels.[15-17] At low light dosages, pulsed dye laser induces only temporary effects on the PWS vasculature; reperfusion occurs and blood flow returns to preirradiation levels. Higher light dosages might effectively form a totally occluding microthrombus, leading to a reduction in blood flow, which approaches zero immediately after laser exposure and does not return to preirradiation values. It might be possible to use ODT blood flow measurements in situ to assess the efficacy of laser PWS therapy. If partial restoration of flow occurs immediately or shortly after pulsed laser exposure, indicative of reperfusion due to inadequate blood vessel injury, the PWS can be retreated using higher light dosages. Retreatment is continued until the measured Doppler shift is zero because of a permanent reduction in blood flow, indicative of irreversible microthrombus formation in the PWS vessels.

Recently published studies[18,19] have described another modality, confocal microscopy, which can pro-

vide in situ real-time images of trafficking red and white blood cells in human skin. Although this modality has many potential uses not possible with ODT,[20] confocal microscopy does not specifically measure blood flow. Comparatively, ODT images clearly demonstrate flow changes in a distinct false-color map.

In summary, we demonstrated that ODT can be used for noninvasive imaging of blood vessels in patients with PWSs. Moreover, ODT will potentially allow laser therapy to be optimized on an individual basis by providing fast, semiquantitative evaluation of the efficacy of PWS laser therapy in situ and in real time.

Accepted for publication January 22, 2001.

This project was supported by research grant GM-58785 from the Institute of General Medical Sciences (Dr Nelson), grant HL-64218 from the Heart, Lung, and Blood Institute (Dr Chen), and grant BES-86924 from the National Science Foundation, Washington, DC (Dr Chen). Institutional support from the Office of Naval Research, Department of Energy, National Institutes of Health, and the Beckman Laser Institute and Medical Clinic Endowment is gratefully acknowledged.

We thank Thomas E. Milner, PhD, Department of Electrical and Computer Engineering, University of Texas at Austin, for his valuable scientific and technical contributions. The method described in this article is contained within US patent 5991697 (Method and Apparatus for Optical Doppler Tomographic Imaging of Fluid Flow Velocity in Highly Scattering Media) awarded to J. Stuart Nelson, MD, PhD, Thomas E. Milner, PhD, and Zhongping Chen, PhD, and assigned to the Regents of the University of California.

Corresponding author and reprints: J. Stuart Nelson, MD, PhD, Beckman Laser Institute and Medical Clinic, University of California, Irvine, 1002 Health Sciences Rd E, Irvine, CA 92612 (e-mail: snelson@laser.bli.uci.edu).

REFERENCES

1. Bonner RF, Nossal R. Principles of laser Doppler blood flowmetry. In: Shepherd AP, Oberg PA, eds. *Laser-Doppler Blood Flowmetry.* Dordrecht, the Netherlands: Kluwer Academic Publishers Group; 1990:15-41.
2. Miller F. Wave motions. In: *College Physics.* 3rd ed. New York, NY: Harcourt Brace Jovanovich; 1972:233.
3. Huang D, Swanson EA, Lin CP, et al. Optical coherence tomography. *Science.* 1991;254:1178-1181.
4. Fercher AF. Optical coherence tomography. *J Biomed Opt.* 1996;1:157-173.
5. Izatt JA, Hee MR, Swanson EA, et al. Micrometer-scale resolution imaging of the anterior eye in vivo with optical coherence tomography. *Arch Ophthalmol.* 1994; 112:1584-1589.
6. Chen Z, Milner TE, Dave D, Nelson JS. Optical Doppler tomographic imaging of fluid flow velocity in highly scattering media. *Opt Lett.* 1997;22:64-66.
7. Chen Z, Milner TE, Srinivas S, et al. Noninvasive imaging of in vivo blood flow velocity using optical Doppler tomography. *Opt Lett.* 1997;22:1119-1121.
8. Chen Z, Milner TE, Wang XJ, Srinivas S, Nelson JS. Optical Doppler tomography: imaging in vivo blood flow dynamics following pharmacological intervention and photodynamic therapy. *Photochem Photobiol.* 1997;67:56-60.
9. Barton JK, Milner TE, Pfefer TJ, Nelson JS, Welch AJ. Optical low coherence reflectometry to enhance Monte Carlo modeling of skin. *J Biomed Opt.* 1997;2:226-234.
10. Barton JK, Welch AJ, Izatt JA. Investigating pulsed dye laser–blood vessel interaction with color Doppler optical coherence tomography. *Opt Express.* 1998; 6:251-256.
11. Zhao Y, Chen Z, Saxer C, Xiang S, deBoer JF, Nelson JS. Phase resolved optical coherence tomography and optical Doppler tomography for imaging blood flow in human skin with fast scanning speed and high velocity sensitivity. *Opt Lett.* 2000;25:114-117.
12. Zhao Y, Chen Z, Saxer C, et al. Doppler standard deviation imaging for clinical monitoring of in vivo human skin blood flow. *Opt Lett.* 2000;25:1358-1360.
13. Barsky SH, Rosen S, Geer DE, Noe JM. The nature and evolution of port-wine stains: a computer-assisted study. *J Invest Dermatol.* 1980;74:154-157.
14. Kimel S, Svaasand LO, Hammer-Wilson M, et al. Differential vascular response to laser photothermolysis. *J Invest Dermatol.* 1994;103:693-700.
15. Anderson RR, Parrish JA. Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. *Science.* 1983;220:524-527.
16. Garden JM, Polla LL, Tan OT. The treatment of port-wine stains by the pulsed dye laser: analysis of pulse duration and long-term therapy. *Arch Dermatol.* 1988; 124:889-896.
17. Geronemus RG. Pulsed dye laser treatment of vascular lesions in children. *J Dermatol Surg Oncol.* 1993;19:303-310.
18. Aghassi D, Anderson RR, Gonzalez S. Time-sequence histologic imaging of laser-treated cherry angiomas with in vivo confocal microscopy. *J Am Acad Dermatol.* 2000;43:37-41.
19. Aghassi D, Gonzalez E, Anderson RR, Rajadhyaksha M, Gonzalez S. Elucidating the pulsed-dye laser treatment of sebaceous hyperplasia in vivo with real-time confocal scanning laser microscopy. *J Am Acad Dermatol.* 2000;43:49-53.
20. Aghassi D, Anderson RR, Gonzalez S. Confocal laser microscopic imaging of actinic keratoses in vivo: a preliminary report. *J Am Acad Dermatol.* 2000;43:42-48.