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Directional blood flow imaging in volumetric optical microangiography achieved by digital frequency modulation

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Abstract

An effective digital frequency modulation approach to achieve directional blood flow imaging within microcirculations in tissue beds *in vivo* for optical microangiography is presented. The method only requires the system to capture one three-dimensional data set within which the interferograms are modulated by a constant frequency modulation that gives one directional flow information. The result is that the imaging speed is doubled and the computational load is halved. The method is experimentally validated by a flow phantom and is tested for imaging of cerebral vascular blood perfusion in a live mouse with the cranium left intact.

Optical microangiography (OMAG) is a recently developed imaging modality that images the volumetric microcirculations within tissue beds up to 2 mm beneath the surface *in vivo* [1,2]. The imaging contrast of blood perfusion in OMAG is based on endogenous light scattering from moving blood cells within biological tissue; thus, no exogenous contrast agents are necessary for imaging. Imaging is achieved by the efficient separation of the moving scattering elements from the static scattering ones within an illuminated tissue through its use of *a constant frequency modulation* f_M in the time-varying spectral interferograms when the probing beam scans the sample. In essence, OMAG mathematically maps the backscattered optical signals from the moving particles into one image—that is, the blood flow image—while it simultaneously maps the backscattered optical signals from the static particles into a second image, which is the microstructural image. The development of OMAG has its origin in Fourier domain optical coherence tomography (FDOCT) [3] and its variation of full range complex FDOCT [4,5]. Since OMAG does not use phases of the optical coherence tomography (OCT) signals to assess the blood flow, OMAG tolerates the inevitable sample movement and tissue optical heterogeneity, thus limiting noise production [1,2].

The original development, however, does not provide the directional capability for OMAG imaging of blood flow, which is a serious drawback in a number of biological and medical applications; for example, in the study of complex flow dynamics in the microfluidic mixers and in the investigation of blood flow involvement in cerebrovascular diseases such as ischemia, hemorrhage, vascular dementia, traumatic brain injury, and seizure disorders. To solve the problem of directional flow imaging using OMAG, Wang [6] recently proposed a method that forces the reference mirror to move back and forth. In such a way, the movement of the reference mirror toward the incident beam images blood flow in one direction, away from the direction of the incidence beam. When the reference mirror moves away from the incident beam, OMAG images blood flow in the opposite direction, toward the direction of the incidence beam. However, the consequence of the mirror moving back and forth is that (1) the OMAG imaging speed is reduced by half and (2) the computational load on OMAG is doubled to obtain meaningful blood flow images because OMAG needs to acquire two three-dimensional (3D) volumetric spectrogram data sets. This multiple imaging is clearly not desirable for fast imaging. An alternative solution to the directional flow imaging using

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mechanical movement of the reference mirror back and forth would represent a major advance to OMAG imaging of blood flow in tissue *in vivo*. In this Letter, we describe a method that only needs to capture one 3D volumetric data set to achieve OMAG imaging of the directional blood flow. The captured 3D data set gives the image of one directional blood flow as described in [1]. Its opposite directional blood flow image, however, is obtained by a digital frequency modulation (DFM) applied to the captured 3D data set.

Following the OMAG method [1], assume the frequency modulation in the interferograms is f_M, a constant frequency. Here f_M can be provided by a number of approaches, for example, moving the reference mirror at a constant velocity in one direction [1,2] or offsetting the sample beam at the scanner that gives the B-scan image [7]. For simplicity, the real function of a spectral interferogram can be expressed by [1]

$$B(t_1,t_2)=\cos[2\pi f_0 t_1+2\pi(f_M-f_D)t_2+\varphi], \tag{1}$$

where f_0 and f_D are the frequency components in the interferogram that represent the microstructural and flow information within a sample and φ is a random phase term. If we construct the analytic function of Eq. (1) by performing the Hilbert transform in terms of t_1 (note that t_2 is constant), then this analytic function is always

$$\begin{aligned}\widehat{B}(t_1,t_2)=&\cos[2\pi f_0 t_1+2\pi(f_M-f_D)t_2+\varphi]\\&+j\sin[2\pi f_0 t_1+2\pi(f_M-f_D)t_2+\varphi],\end{aligned} \tag{2}$$

because f_0 is always >0, which is guaranteed by placing the sample surface below the zero delay line. Thus, given Eq. (2), it becomes a trivial matter, to transform Eq. (1) into

$$B'(t_1,t_2)=\cos[2\pi f_0 t_1-2\pi(f_M+f_D)t_2+\varphi], \tag{3}$$

by digitally multiplying Eq. (2) with a complex function $\exp[-j4\pi f_M t_2]$ and then taking the real part of the results. It is feasible because f_M is known *a priori*. Now, we can construct the analytic function of Eq. (3) by considering t_2 a variable, while t_1 is constant. Then, if $f_M+f_D<0$, the analytic function is

$$\begin{aligned}\widehat{B'}(t_1,t_2)=&\cos[2\pi f_0 t_1-2\pi(f_M+f_D)t_2+\varphi]\\&+j\sin[2\pi f_0 t_1-2\pi(f_M+f_D)t_2+\varphi],\end{aligned} \tag{4}$$

but if $f_M+f_D>0$, the analytic function becomes

$$\begin{aligned}\widehat{B'}(t_1,t_2)=&\cos[2\pi f_0 t_1-2\pi(f_M+f_D)t_2+\varphi]\\&-j\sin[2\pi f_0 t_1-2\pi(f_M+f_D)t_2+\varphi].\end{aligned} \tag{5}$$

Consequently, Eqs. (1)–(5) simulate the exact situation of the mirror moving in an opposite direction to that as described in [1]. This derivation of the analytic function provides a solid mathematical basis for obtaining information on the directions of blood flow from only one

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3D spectrogram data set captured by OMAG. As a result, the directional blood flow imaging can be obtained by the following approach: the analysis detailed in [1] to give one image of blood flow direction when the reference mirror moves *toward* the incident beam, and the analysis described here to give an image of blood flow in the opposite direction, as if the mirror moved *away from* the incident beam.

To verify the DFM method described in this Letter we used an OMAG system that was described in [2] with some modifications. Briefly, the system used a superluminescent diode (Denselight, Singapore) with a central wavelength of 1310 nm and a measured axial resolution of ~12 μm in air. The light emerging at the output of interferometer was sent to a home-built high-speed spectrometer that employed a line scan infrared InGaAs detector to capture the interferograms formed in the system. The spectrometer had a designed full range imaging depth at ~5.8 mm in air. The A-scan rate was set at 17 kHz for this study. The 3D imaging of tissue sample *in vivo* was performed by an *X*–*Y* galvanometer scanner with a scanning priority in the *X* direction (B scan). The *X* scanner was driven by a 16 Hz sawtooth waveform to provide the B scan over ~2.0 mm at the sample, while the *Y* scanner was driven by an ~0.03 Hz sawtooth waveform that provided the beam scanning in the elevational direction of ~2.0 mm as well. To introduce the frequency modulation in the interferograms, we used the beam offset at the *X* scanner in the sampling arm [7], while we kept the reference mirror stationary during imaging. Throughout this study the modulation frequency provided by the beam offset was measured at ~400 Hz, which implies a minimal resolvable flow velocity of ~260 μm/s.

We first conducted a phantom experiment to verify whether the DFM approach is feasible. In the experiment we built a scattering phantom that was made of a gelatin consisting of ~2% milk and ~98% water, which simulated the static scattering elements, within which two parallel capillary tubes with an inner diameter of ~400 μm were submerged. A 2% TiO2 particle solution was flowing through the two capillaries, in which the flow directions were against each other. The maximum flow in the tubes was controlled at ~4 mm/s by a precision dc pump. With the Reynolds number $\ll$1800, the flow was laminar. The angle between the probe beam and the tube was ~70°. The OMAG system was then used to capture one B-scan spectrogram. Figures 1(A) and 1(B) are the resulting microstructural (OCT) and flow images, respectively, directly calculated from the conventional OMAG algorithm [1], where only one directional flow is resolved. Then, we used the DFM approach applied to the same data set. The results are given in Figs. 1(C) and 1(D), where it is evident that the DFM approach is feasible.

Next, we performed an *in vivo* experiment to image the directional microvascular blood flow over the brain cortex in a live mouse with the cranium intact. The experimental protocol was in compliance with the federal guidelines for the care and handling of small rodents and approved by the Institutional Animal Care and Use Committee. The mouse was anesthetized and then the skin on the head was removed to create a window for OMAG imaging through the skull bone. The OMAG system was used to capture one 3D interferogram data set with f_M=400 Hz from which the volumetric microstructural and directional blood flow images were computed using the DFM approach. Figure 2(A) shows the 3D microstructural image rendered *in vivo* for the tissue volume scanned, where the layered structures, including the skull bone, dura matter, and cortex can be demarcated. Figure 2(B) illustrates the directional blood flow map by fusing together two flow images, indicating the blood flow toward (green online) and away from (red online) the incident beam direction. Because of the depth-resolved capability of OMAG imaging, we were able to separately identify blood perfusion in the meninges and the brain cortex. Figures 2(C) and 2(D) show the 2D *x*–*y* projection maps showing, respectively, the directional blood flow within the meninges where the blood vessels were less abundant, and the cerebrovascular flow that maps the detailed blood vessel network, including the capillaries over the cortex. Based on the fact that the blood merges from the smaller vessels into the bigger vessels in venules, while it is the opposite in arteries, the majority of arterioles

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(pointed out by open-tip arrows) and venules (closed-tip arrows) in Fig. 2 could be identified. It has to be mentioned that the direction of flow sensed by OMAG is dependent on the 3D geometry of the vascular network relative to the OMAG probe beam direction, and this dependence complicates the interpretation of blood perfusion. This complication, however, occurs in all the measurement techniques that are based on the Doppler principle, including Doppler OCT [8–10]. The angular dependence of OMAG imaging of the blood flow is still the current subject of study. The ability of OMAG to image the direction of blood flowing in tissue with a resolution down to the capillary level would help researchers understand tissue perfusion in neuropathology, tumor angiogenesis, and sensory stimulations, where the direction of flow may give a clue to decode the neuronal functions that regulate the blood flow and angiogenesis.

In summary, we have presented a digital frequency modulation (DFM) approach to provide the directional flow mapping within a highly scattering medium. The DFM method eliminates the requirement of capturing two 3D interferogram data sets to resolve the directional blood flow information within the scanned tissue volume; thus it reduces the computational load and increases the potential temporal imaging resolution for *in vivo* imaging studies. We have experimentally validated this approach by a flow phantom and shown the potential of OMAG imaging of cerebral vascular blood perfusion in a live mouse with the cranium left intact.

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References

1. Wang RK, Jacques S, Ma Z, Hurst S, Hanson S, Gruber A. Opt Express 2007;15:4083.
2. Wang RK, Hurst S. Opt Express 2007;15:11402.
3. Hausler G, Lindner MW. J Biomed Opt 1998;3:21.
4. Wang RK. Appl Phys Lett 2007;90:054103.
5. Wang RK. Phys Med Biol 2007;52:5897. [PubMed: 17881807]
6. Wang RK. Phys Med Biol 2007;52:N531. [PubMed: 18029974]
7. An L, Wang RK. Opt Lett 2007;32:3423. [PubMed: 18059954]
8. Chen ZP, Milner TE, Srinivas E, Wang X, Malekafzali A, van Gemert MJC, Nelson JS. Opt Lett 1997;22:1119. [PubMed: 18185770]
9. White BR, Pierce MC, Nassif N, Cense B, Park BH, Tearney GJ, Bouma BE, Chen TC, de Boer JF. Opt Express 2003;11:3490.
10. Wang RK, Ma ZH. Opt Lett 2006;31:3001. [PubMed: 17001380]

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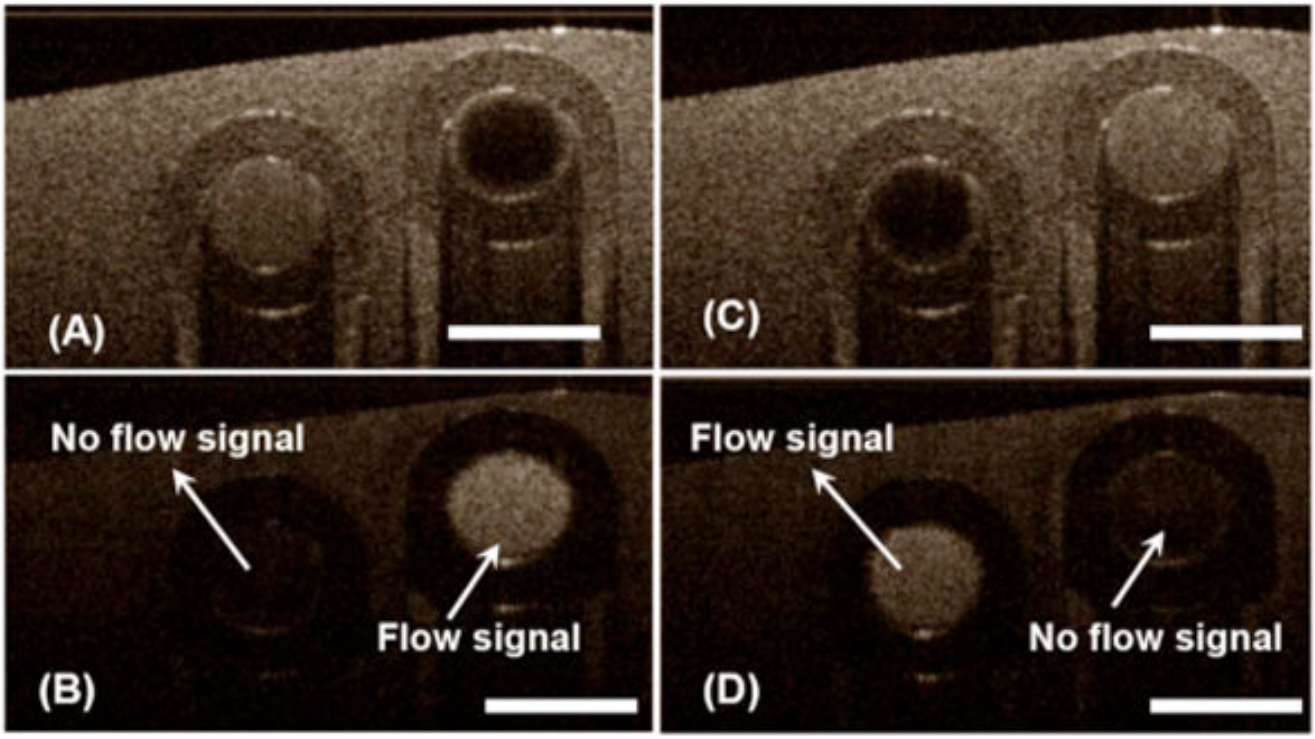

Fig. 1.
(Color online) (A) and (B) are the OMAG structural and flow images calculated from the spectrograms captured when f_M=400 Hz. (C) and (D) are corresponding results computed from the DFM approach applied to the same dataset. Scale bar=500 μm.

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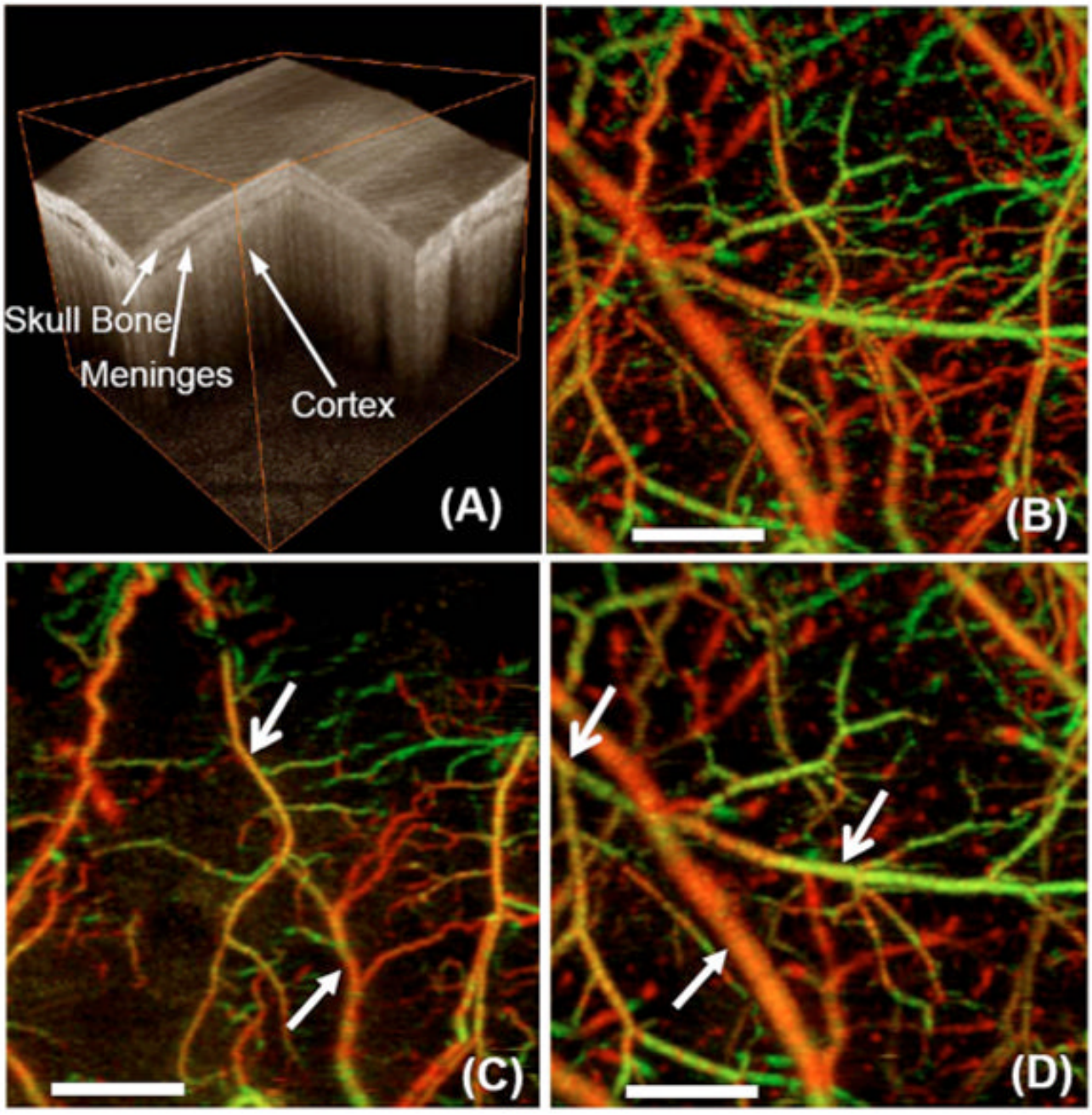

Fig. 2.

(Color online) *In vivo* OMAG imaging results. (A) 3D rendered microstructural image (2 mm × 2 mm × 2 mm) where the physiological layers such as skull bone, meninges, and cortex are delineated. (B) *x*–*y* projection image of directional blood flow network within the scanned volume in (A). Owing to the depth-resolved feature of OMAG imaging, the directional blood flows within the meningeal layer (C), and the cortex layer (D) can be separated. Scale bar=500 μm.