Doppler optical micro-angiography for volumetric imaging of vascular perfusion \textit{in vivo}

Ruikang K Wang* and Lin An
Department of Biomedical Engineering, Oregon Health & Science University, Portland, OR 97237, USA

Abstract

We propose a Doppler optical micro-angiography (DOMAG) method to image flow velocities of the blood flowing in functional vessels within microcirculatory tissue beds \textit{in vivo}. The method takes the advantages of recently developed optical micro-angiography (OMAG) technology, in which the endogenous optical signals backscattered from the moving blood cells are isolated from those originated from the tissue background, i.e., the tissue microstructures. The phase difference between adjacent A scans of OMAG flow signals is used to evaluate the flow velocity, similar to phase-resolved Doppler optical coherence tomography (PRDOCT). To meet the requirement of correlation between adjacent A scans in using the phase resolved technique to evaluate flow velocity, an ideal tissue-sample background (i.e. optically homogeneous tissue sample) is digitally reconstructed to replace the signals that represent the heterogeneous features of the static sample that are rejected in the OMAG flow images. Because of the ideal optical-homogeneous sample, DOMAG is free from the characteristic texture pattern noise due to the heterogeneous property of sample, leading to dramatic improvement of the imaging performance. A series of phantom flow experiments are performed to evaluate quantitatively the improved imaging performance. We then conduct \textit{in vivo} experiments on a mouse brain to demonstrate that DOMAG is capable of quantifying the flow velocities within cerebrovascular network, down to capillary level resolution. Finally, we compare the \textit{in vivo} imaging performance of DOMAG with that of PRDOCT, and show that DOMAG delivers at least 15-fold increase over the PRDOCT method in terms of the lower limit of flow velocity that can be detected.

1. Introduction

Optical coherence tomography (OCT) \cite{1,2} is a non-invasive imaging technology, capable of providing high resolution, depth resolved cross-sectional images of highly scattering sample, such as biological tissue, and is attracting more and more attention for both the medical and non-medical imaging applications since it was first reported in early 1990s \cite{3}. By evaluating phase differences between adjacent A-lines in an OCT B-scan frame, a functional extension of OCT, phase-resolved Doppler OCT (PRDOCT) \cite{4,5}, is developed to extract velocity information of blood flow in functional vessels within the scanned tissue beds. Recent developments in spectral domain optical coherence tomography (SDOCT) \cite{6,7,8,9} have led PRDOCT to \textit{in vivo} imaging of blood flow, particularly in human retina \cite{10,11,12}, thanks to the advantages of the improved imaging speed and sensitivity in SDOCT \cite{13,14}. In spectral domain PRDOCT, the magnitude of Fourier transformation of the spectral interference fringes is used to reconstruct cross-sectional, structural image of the tissue sample, while the phase difference between adjacent A-scans is used to extract the velocity information of blood flow.
within the scanned tissue. Phase resolved method is based on the fact that the phase difference of sequential A-lines is linearly related to the flow velocity; thus, PRDOCT method can be used to obtain quantitative information about the blood flow. Although the PRDOCT method is of high resolution and high sensitivity to the blood flow, its imaging performance is greatly deteriorated by at least two factors: 1) characteristic texture pattern artifact, which is caused by optical heterogeneity of the sample [15], and 2) phase instability that is caused by the sample motion artifacts [16]. A straightforward way to reduce the background characteristic texture pattern in PRDOCT is to use a dense-sampling approach, i.e., to have more A-scans within a B scan than it would be necessary. This dense-sampling approach is effective in reducing the texture pattern artifacts, but it inevitably leads to a significant increase of imaging time, which is not desirable for in vivo imaging applications. To overcome these problems, several methods have been approached to deal with different aspects of these factors. Ren et al. [17] used the delay line filters and Wang et al. [15] used the reverse scanning pattern of the probe beam to suppress the texture pattern artifacts. Another novel spectral method has also been proposed in order to minimize the influence of phase instabilities, called resonant Doppler imaging [18], which extracts the flow information from the intensity signals without extracting the phase. Relying on analyses of the amplitude rather than phase distributions of the OCT signals, a notable method, called joint spectral and time domain OCT [19], was recently developed. This last method, however, requires repeated A scans at the same lateral position, which increases the imaging time.

Most recently, based on full range complex FDOCT [20–22], our group has developed a novel imaging technology, optical microangiography (OMAG) method [23], which unlike the PRDOCT, explores implicitly the phase information embedded in the OCT spectral interferograms. By using heterodyne technology, OMAG method is capable of separating the scattering signals caused by the moving scatters from the scattering signals caused by the static tissue background, i.e. by the tissue microstructures, to achieve imaging of blood perfusion. In addition to its ability to achieve micro-structural imaging, OMAG has shown to provide volumetric vasculature image within the scanned tissue bed, down to capillary level imaging resolution [24–26]. In the past, we have successfully used OMAG to image cerebral blood perfusion in mice [23–25] and blood flows within human retina and choroids [26].

The key advantage of OMAG is that only the signals backscattered by the functional blood appear in the OMAG flow output plane, making blood flow imaging almost free of artifact-induced noises. Such an advantage leads us to ask whether blood flow velocities can be extracted from OMAG blood flow signals. Achieving such an extraction would be a major advance in OMAG imaging of flow in general and of blood flow in particular because, unlike PR-DOCT [4,5], the noise production from the optical heterogeneous properties of the sample will be eliminated, suggesting that a precise quantification of flow will then be possible.

In this report, we present a novel method, Doppler OMAG (DOMAG), to evaluate the velocities of OMAG flow signals by measuring the phase difference between adjacent A-lines. The method takes advantage of PRDOCT approach to evaluate the phase difference between adjacent OMAG A-lines. However, the application of the phase-resolved technique requires a correlation between adjacent A scans. This correlation requirement makes extraction of the blood flow velocities in OMAG difficult because in the OMAG flow image, the regions that are occupied by the microstructural signals are rejected by OMAG, leading to that the correlation between adjacent A scans in these regions are totally lost. To overcome this problem, we digitally reconstruct an ideal static background tissue that is totally optically homogeneous to replace the real heterogeneous tissue sample in OMAG. This ideal background tissue provides a constant background signal that makes the adjacent A-scans totally correlated, leading to a dramatic increase of the phase signal to noise ratio (SNR) for the phase-resolved signals that represent flow velocities. This method inherits the advantages of both OMAG and
After introduction of this method, we then demonstrate this technique by using OMAG imaging of flow phantoms to show quantitatively its ability in increasing the phase SNR. We then demonstrate the proposed DOMAG by imaging cerebral blood perfusion in mice in vivo. Finally, we show that the in vivo performance of DOMAG imaging of blood flow is superior to the traditional PRDOCT.

2. System setup

The system used in our experiment is illustrated in Fig. 1. We used a superluminescent diode (SLD, DenseLight, Singapore) with a central wavelength of 1310 nm and a spectral bandwidth of 56 nm as the light source, which provided an axial imaging resolution of ∼13 μm in air. The light from the SLD was coupled into a fiber-based Michelson interferometer, via an optical circulator. In the reference arm, the light was delivered onto a stationary mirror; and in the sample arm, the light was focused into a sample via an objective lens. The zero delay line of the system was set at ∼0.5 mm above the focus spot of sample beam. With a 50 mm focal length of the objective lens in the sample arm, the size of the focus spot on the sample was ∼16 μm, i.e., the lateral resolution of the system was ∼16 μm. The light backscattered from the sample and reflected from the reference mirror were recombined by a 2×2 optical fiber coupler, and then was routed to a home-built high speed spectrometer via the optical circulator. The spectrometer consisted of a collimator of 30 mm focal length, a 1200 lines/mm transmitting grating, an achromatic lens with 100 mm focal length and a 14-bit, 1024 pixels InGaAs line scan camera. The maximum line scan rate of the camera was ∼47 KHz. This spectrometer setup had a designed spectral resolution of 0.141 nm, which gave a measured imaging depth of ∼3.0 mm on the each side of the zero delay line.

To achieve 3D imaging, we used two galvo-scanners to raster-scan the focused beam spot across the sample, with one scanner for X-direction (lateral) scan, and another for Y-direction (elevational) scan. For the experiments presented in this study, the camera integrating time was set at 31 μs for imaging, allowing 1 μs for downloading the spectral data from CCD (1024 pixels, A scan) to the host computer via CameraLink™ and a high-speed frame grabber board (PCI 1428, National Instruments, USA). This configuration determined a line scan rate of ∼31 KHz for the camera. The imaging rate was set at 20 frames (B scan) per second (fps). Each B scan had 2.5 mm span over the sample, consisting of 1500 A-lines. This represents an over sampling factor of ∼10 because the lateral resolution of the system is ∼16 μm. In the elevational direction, there were 500 discrete points along ∼2.5 mm, i.e., 500 B scans. Hence the data cube of each 3D image (C scan) was composed of 1024 by 1500 by 500 (z-x-y) voxels, which took ∼25 s to acquire. The operations for probe beam scanning, data acquisition, data storage and hand-shaking between them were controlled by a custom software package written in C++ language. Note that 500 B scans for a C scan represented over sampling in the elevational direction. In practice, however, 200 B scans would be sufficient to obtain the volumetric images, leading to a temporal resolution of ∼10 s for 3D OMAG imaging using the current setup.

3. Theoretical analyses

The proposed DOMAG is an extension of the OMAG imaging technique to provide the velocity information of the blood perfusion within microcirculatory tissue beds in vivo. DOMAG uses OMAG method to obtain the optical signals backscattered by blood cells in patent blood vessels through rejecting the heterogeneous tissue signals, i.e. the optical signals backscattered by microstructures of the tissue sample. And then, it uses phase resolved DOCT technique [4,5] to extract the flow velocity information. Applying the phase-resolved technique to extract flow velocities from OMAG signals of blood flow seems straightforward. However the technique assumes a correlation between adjacent OCT A scan signals [5]. Unfortunately, such a
correlation is not achievable in the background tissue regions in OMAG, making the extraction of flow velocities difficult. To solve this problem, here we begin presenting the DOMAG method by introduction of OMAG principle.

The spectral interference signal captured by each pixel of the CCD camera in OMAG/SDOCT is essentially the same except the wavelength, \( \lambda \). We assume the wavenumbers of the broadband light source is from \( k_0 \) to \( k_0 + \Delta k \), where \( k_0 = \frac{2 \pi}{\lambda_0} \), and these wavenumbers cover 1024 pixels of the line scan camera respectively. As a consequence, the camera records the spectral interference fringe signal formed between the reference light and the light backscattered from within sample, which can be written as a function of \( k_j \):

\[
I(k_j) = S(k_j) \left( E_\pi \exp(i2k_jr) + \int_{-\infty}^{\infty} a(z) \exp(i2k_j[r+zn]) dz \right)^2 \quad j=1, 2, 3 \ldots 1024
\]

where \( i = \sqrt{-1}, < > \) is the time average, \( k_j \) is the wavenumber of the light captured by the \( j \)th detector (pixel) of the CCD camera, \( I(k_j) \) is the light intensity captured by the \( j \)th detector, \( S(k_j) \) is the spectral density of the light source at \( k_j \), \( r \) is the optical path length for the light traveled in the reference arm, \( n \) is the refractive index of the sample, \( a(z) \) is the magnitude of the light backscattered at depth \( z \). In our system, each B-scan contains 1500 A-lines and covers about 2.5 mm in the lateral direction. So the signal captured by the \( j \)th pixel in each B-scan can be written as a function of time variable \( t \) that relates to the position of focus beam spot on the sample, steered by the X scanning mirror.

\[
I(k_j, t) = S(k_j) \left( E_\pi \exp(i2k_jr) + \int_{-\infty}^{\infty} a(z, t) \exp(i2k_j[r+zn]) dz \right)^2
\]

Because the light backscattered from the sample is quite weak compared to the light reflected from the reference mirror, we do not consider the self cross-correlation between the light backscattered from different positions within the sample. We also do not consider the DC signals because they do not contribute to useful OMAG signals. In these cases, Eq. (2) can be written as:

\[
I(k_j, t) = 2S(k_j) E_\pi \int_{-\infty}^{\infty} a(z, t) \cos(2k_jnz) dz
\]

It is clear that Eq.(3) is constant if the sample is totally optically homogeneous, which means that \( a(z, t) \) and \( n \) do not vary within the entire sample. If this is the case, then the spatial frequency components of the sample in lateral direction presented by Eq.(3) will be a delta function, shown as a red arrow in Fig.2 (A). However in real situations, our imaging sample is often optically heterogeneous, which means that \( a(z, t) \) and \( n \) are functions of time variable \( t \). Thus, Eq.3 can be expressed as:

\[
I(k_j, t) = 2S(k_j) E_\pi \int_{-\infty}^{\infty} a(z, t) \cos(2k_jnz) dz
\]
When there is a patent blood vessel buried within a motionless tissue at position \((z_1, t_1)\), we assume the blood cells (scattering particles) within the vessel move towards the incident beam at a velocity \(v\). The frequency of the light backscattered from these blood cells will be modulated by its velocity. Then, Eq. (4) can be expressed as:

\[
I(k_j, t) = 2S(k_j)E_R \left[ \int_{-\infty}^{\infty} a(z, t) \cos(2k_j n(z, t) z) dz + a(z_1, t_1) \cos(2k_j n(z_1, t_1) (z_1 - vt)) \right]
\]  

(5)

Here we also do not consider the self cross-correlation signal from within the sample. The 1st term on the right side of Eq. (5) represents backscattering signals from a static sample with reflectivity of \(a(z, t)\), while the 2nd term represents backscattering from the moving particles with reflectivity of \(a(z_1, t_1)\) with a velocity of \(v\) at position \((z_1, t_1)\). Moving particles produce a frequency shift caused by the Doppler effect of the moving particles. This is illustrated in Fig. 2 (C), the blue curve is the Doppler beating frequency part. Recall that the phase-resolved technique requires a correlation between adjacent A scans to determine \(v\) of the moving particles [5], and the correlation, in conventional PRDOCT, provided by the 1st term on the right-side of Eq. (5) is also required to suppress the noise signals in the non-flow regions in order to increase the flow imaging contrast. Because of the optical heterogeneity \(n(z, t)\) of a tissue, \(n(z, t)\) imposes a noise background onto the blood flow signals, making it difficult for PR-DOCT to measure precisely the blood flow velocity, particularly in capillaries [24–26]. In contrast, OMAG eliminates the 1st term on the right side of Eq. (5) in order to image blood flow. This elimination renders the noise production due to \(n(z, t)\) to a minimum, but unfortunately, results in OMAG losing its correlation condition between adjacent A scans for the heterogeneous tissue regions. Consequently, the phase-resolved technique cannot be directly applied to OMAG blood flow signals. To solve this problem, our proposed strategy is to digitally re-construct an ideal sample background with a constant backscattering coefficient \(a_0\) and a refractive index \(n_0\) throughout the sample – thus creating a totally homogeneous sample which reinforces a complete correlation among OMAG A scan signals.

\[
I_0(k_j, t) = 2S(k_j)E_R \int_{-\infty}^{\infty} a_0(z, t) \cos(2k_j n_0(z) z) dz
\]

(6)

where \(a_0(z, t) = a_0\) and \(n_0(z, t) = n_0\) throughout the scanned tissue sample. In this paper, \(a_0 = 10^{-6}\), and \(n_0 = 1.35\) when we construct the homogenous tissue background using Eq.(6). These values were taken according to the typical optical properties of biological tissues, i.e. the average reflectivity is between \(10^{-4}\) and \(10^{-7}\) and the average refractive index is 1.35 [28].

The digitally reconstructed homogenous tissue sample subsequently replaces the first term on the right side of Eq.(5). In doing so, OMAG blood flow signal now becomes:

\[
I'(k_j, t) = 2S(k_j)E_R \left[ a_0 \cos(2k_j n_0 z) dz + E_R a(z_1, t_1) \cos(2k_j n_0 (z_1, t_1) (z_1 - vt)) \right]
\]

(7)

The construction of the ideal tissue sample does not affect OMAG signals of blood flow because it only replaces the tissue background signals with a homogeneous background without affecting the blood flow signals within the B scan. If we treat time variable \(t\) as a constant and apply Fourier transform upon wavelength \(k\), then we can obtain:

\[
\tilde{I}(z, t) = FT^{-1} \left[ I(k_j, t) \right] |_{k=A(z, t) exp[i\varphi(z, t)]}
\]

(8)
where $\phi(z, t)$ is the phase of the analytic signal. The phase difference between adjacent A-scans, $n$ and $n-1$, is then evaluated:

$$\Delta \varphi(z, t) = \tan^{-1} \left[ \frac{\text{Im} \left[ \tilde{I}(z, t_n) \bullet \tilde{I}(z, t_{n-1}) \right]}{\text{Re} \left[ \tilde{I}(z, t_n) \bullet \tilde{I}(z, t_{n-1}) \right]} \right].$$ (9)

Based on the linear relationship between phase difference between adjacent A-lines and velocity [4,5], the velocity of flow signal imaged by OMAG can be directly written as:

$$v(z, t) = \frac{\lambda \Delta \varphi(z, t)}{4\pi \Delta t}$$ (10)

where $v(z, t)$ is the flow velocity at depth $z$, $\Delta t$ is the time interval between adjacent A-lines. Note that there is a small constant offset induced by $a_0$ in Eq.(8), which may perturb the evaluated $\Delta \phi(z, t)$. However, the small offset is usually at least 2 orders of magnitude smaller than the OMAG flow signals, leading to a negligible effect on the final evaluated $\Delta \phi(z, t)$.

In this study, we used the InGaAs camera to capture the interferograms at 31 KHz A scan rate. Thus, the maximum detectable flow velocity that does not undergo phase-wrapping was 10 mm/s for the OMAG system used (Fig. 1). We selected the modulation frequency $f_c = 400$ Hz for OMAG to filter out the heterogeneous frequencies that represent the static tissue components. This value was empirically determined from the tissue samples used, which corresponded to a minimal flow velocity of $\sim 0.26$ mm/s that can be detected by the system. Figure 3 gives a flow chart on the procedures of how DOMAG works to obtain final velocity images of blood flow of the scanned tissue sample.

4. Results and discussion

4.1 Phantom experiments

To test the performance of the proposed method, we first performed experiments on a flow phantom. The phantom was made from gelatin mixed with 2% milk to simulate the background optical heterogeneity of the tissue in which a capillary tube with an inner diameter of $\sim 200 \mu m$ was submerged and $\sim 2$% TiO$_2$ particle solution was flowing in it. The inclining angle of the tube towards the incident beam, i.e. the Doppler angle, was set at $\sim 85^\circ$. The flow rate of the particle solution was controlled by a precision syringe pump to a range that falls within the detectable range of the OAMG system, i.e. velocity between $0.2$ and $10$ mm/s. In this experiment, each B-scan (lateral direction) contained 1000 A-lines covering 2.5 mm. Thus, the corresponding $\Delta x$ between adjacent A-lines was $2.5 \mu m$. The results are shown in Fig. 4.

Figure 4(A) is the OMAG structural image of the scanned tissue phantom that is identical to the image obtained by conventional SDOCT, while the corresponding OMAG flow image is shown in Fig. 4(B). It can be seen that OMAG successfully delineates the scattering fluid flow within the capillary tube with the background signals from the non-flow region of phantom being rejected. However, OMAG does not provide the velocity information of flowing particles within the capillary tube. The fluid flow velocity information was then evaluated by DOMAG as described in Section 3 [Fig. 4(C)], and PRDOCT [Fig. 4(D)] methods, respectively. Visually, it is clear that DOMAG provides superior imaging performance because the background phase noise is maximally suppressed in Fig. 4(C) when compared to Fig. 4(D). Note that the phase noise suppression occurs in the entire output plane due to the ideal tissue phantom digitally reconstructed in DOMAG. This is an additional advantage for DOMAG because it is now not
necessary to use segmentation method to segment the tissue regions of interest so as to excluding low signal regions for evaluating useful flow velocity signals, as normally done in the conventional PRDOCT, leading to a reduced demand for computing power. To better show the noise suppression by DOMAG, we plotted in Fig. 4(E) the signal profiles across the B scan at depth positions marked in Figs. 4(C) and (D), respectively. The blue curve was extracted from the locations marked with blue line in Fig. 4(C), while the red curve from the same locations marked with the red line in Fig. 4(D). The phase differences caused by the flowing particles are almost the same (the parabolic curve) using these two methods, however the noise background in DOMAG is much smaller than that in PRDOCT.

To quantitatively evaluate the improvement brought by DOMAG method, we calculated the flow velocity signals as well as the phase noise levels. We defined two regions from the structural image [Fig. 4(A)]: flow signal region \(\Omega^S\), marked as the blue circle, and noise region \(\Omega^N\), enclosed by the red lines. The flow signal region was determined by segmenting the lumen of the capillary tube, while the noise region was determined by segmenting the micro-structural signals from the structural image of the scanned phantom. The segmentations are straightforward because in this simple experiment, we knew where the flow is located. In doing so, two masks were produced from the resulted two regions, and used in combination of the DOMAG image [Fig. 4(C)] and PRDOCT image [Fig. 4(D)] to calculate the phase signals in the respective regions. Note that it is not necessary to perform the segmentation to evaluate the phase noises in DOMAG, however, to make it a fair comparison between DOMAG and PRDOCT, the phase differences within the exactly same regions were evaluated for both the methods.

The phase noise level in the velocity image was calculated by evaluating the standard deviation of the phase differences, \(\Delta\phi\), between adjacent A-lines within region \(\Omega^N\),

\[
\sigma_{\Delta\phi} = \sqrt{\frac{1}{M-1} \sum_{\Omega^N} (\Delta\phi - \bar{\Delta}\phi)^2}
\]  

(11)

where \(M\) is the total number of pixels within the region \(\Omega^N\), \(\bar{\Delta}\phi\) is the average value of the phase differences \(\Delta\phi\). The useful flow signals were defined by following algorithm in the flow region \(\Omega^S\):

\[
S = \sum_{\Omega^S} \left(\Delta\phi > \sigma_{\Delta\phi}\right) \times \Delta\phi
\]  

(12)

where the bracket term represents a binary operation for which it returns 1 if \(\Delta\phi\) is larger than \(\sigma_{\Delta\phi}\), else it returns 0. In doing so, the value \(S\) represents the effective detectable signals that are treated as useful signals of flow velocities for the target flow. Finally, the phase SNR is defined by,

\[
\text{Phase SNR} = 20 \times \log \left( \frac{S}{\sigma_{\Delta\phi}} \right)
\]  

(13)

It should be noted that the phase SNR evaluated here indicates a metric for imaging contrast, rather than the phase sensitivity of the system used. The phase sensitivity for both DOMAG and PRDOCT are the same, which is determined by the system setup and the beam scanning pattern over the sample.
The results are tabulated in Table 1 for both the OMAG and PRDOCT methods. Compared to PRDOCT, the phase noise $\sigma_{\Delta \phi}$ was reduced from 0.43 rad to 0.037 rad for DOMAG, which represents more than 11-fold improvement. The detectable effective velocity signals were also improved, from 4198 to 4395. Consequently, the phase SNR was increased by 22 dB, from 79 dB to 101 dB.

From the above analyses, we can see that the noise level in DOMAG is greatly reduced. It is noted that in PRDOCT, the phase noise level is often termed as the phase sensitivity, which can be determined by the intensity signal to noise ratio of the OCT system, $X$, by the following equation [9]:

$$\sigma_{\Delta \phi}^2 = \left( \frac{1}{X} \right)$$

The phase sensitivity value calculated from Eq. (14) is the upper limit, which the phase resolved method can achieve under the total correlation condition between adjacent A scans, which might be met in PRDOCT by repeated A scans at the same sample position. For the experiments in our study, the OCT intensity SNR for the flow region is $\sim 30$ dB, which is quite common in the case of imaging in vivo. Thus, the corresponding phase sensitivity is $\sim 0.0316$ rad. As we can see, the phase sensitivity of DOMAG evaluated through the B scan is quite close to that of PRDOCT evaluated from the repeated A scans at the same sample position, demonstrating the power of proposed DOMAG method for in vivo imaging of blood flow within the microcirculatory tissue beds.

4.2 In vivo experiments

Next, we performed in vivo experiments to show the potential of Doppler OMAG for non-invasive assessment of microcirculations within tissue beds. Here we demonstrate the capability of DOMAG to image the cerebral blood perfusion in mouse models with the skull left intact. We selected the mouse brain in our experiments, in part because the brain is one of the least accessible organs for non-invasive observations of the blood perfusion (as in the human brain) and because the study of small animal models may prove very useful in developing a novel means of intervening in brain injury in humans. The 3-months old adult mouse, weighing $\sim 25$ g, was shaved to remove hair from the head before optical imaging. The mouse was then anesthetized by using 2% isoflurane (0.2L/min O2, 0.8L/min air), and positioned in a home-made stereotaxic stage to minimize the movement. The body temperature was kept at $\sim 37$ °C throughout the experiments. Before the OMAG data acquisition, a widow on the head was carefully made by removing the overlaying skin to allow OMAG imaging the cerebral blood flow within cortex through the intact skull. The exposed skull was washed by saline to prevent it from dehydrating. The whole imaging session lasted about 30 min, including $\sim 25$ sec for optical imaging data acquisition. After the imaging, the animal was disposed of according to IACUC regulations. The protocol was in compliance with the guidelines of the National Institutes of Health for care and handling of laboratory animals.

Shown in Fig. 5 are the representative results from a single B scan (frame) of a mouse brain. Figure 5(A) is the OMAG structural image, identical to the cross-sectional image obtained from conventional SDOCT, from which the important histological layers were clearly delineated, including the cranium (skull), gray matter (cortex) and white matter. Figure 5(B) shows the corresponding OMAG image of localized blood flow that permeates this cross-section [Fig. 5(A)]. However, this image only provides the backscattered signals from functional blood that does not indicate the flow velocity information, which is needed for quantifying the blood perfusion. Applying the DOMAG method, the velocity information as to the imaged blood flow can be extracted from Fig. 5(B). The result is given in Fig. 5(C),
which represents an image of the Doppler OMAG phase differences, \( \Delta \phi(x, z) \) that can be converted to the velocity values by Eq. (10). It can be seen that the blood flow velocity in capillaries (pointed by white arrows, for example) is imaged by DOMAG.

The 3D imaging in the OMAG system was achieved by scanning the focused sample beam over the skull using the X-Y scanner (Fig. 1). The field of view for our current system was 2.5 mm by 2.5 mm (x-y), which contained 1500 A scans in the x-direction (B scan) and 500 B scans in the y-direction (C scan). The original raw data cube (spectral interferograms) was first processed frame by frame, and then the resulted images, including structural, flow and velocity images, were recombined to produce 3D volumetric visualization of the scanned tissue volume. The results for a typical tissue volume of 2.5x2.5x2.0 mm\(^3\) are given in Fig. 6. Figure 6(A) is a volumetric visualization rendered by merging the micro-structural 3D image (via SDOCT) with the corresponding 3D image of functional blood flows (via OMAG), where the precise locations of blood flow can be identified within microstructures of the sample. In the image, a cut away view is used to appreciate how the blood vessels innervate the tissue volume. Figure 6(B) shows the volumetric network of patent blood vessels within the scanned tissue volume, where the smallest diameter of blood vessels was identified at \(~15\) \(\mu\)m, close to the system spatial resolution \(\sim 16\) \(\mu\)m. The corresponding velocity information for the imaged blood flows is shown in Fig. 6(C), evaluated by use of DOMAG. In Fig. 6(C), the directional flow information is coded with colors, where the red color means the blood moves towards the incident beam direction and the green color otherwise.

To show in detail the blood vessel networks and blood flow velocities within them, the maximum projection approach was used to obtain x-y projection images. Together with the blood vessel perfusion networks [Fig. 7(A)], Doppler OMAG [Fig. 7(B)] provides a potential tool to quantify blood perfusion within the microcirculation tissue beds \textit{in vivo}.

### 4.3 Comparison between DOMAG and PRDOCT for \textit{in vivo} imaging

Because both the DOMAG and PRDOCT methods are capable of providing the velocity information for the blood flows within the living biological tissue, here we provide a comparison between these two techniques for the cases of \textit{in vivo} imaging. It is noted that the final imaging results can be different under the different system setups, for example the setups for the imaging speed and the number of A scans used in a single B scan. Thus for a fair comparison, we used the same data set, obtained from a mouse brain with the skull left intact under the exactly same system configurations, to obtain the DOMAG and PRDOCT images of cerebral blood flow. Note that for this set of experiments, the imaging speed was 20 KHz A scan rate. Figure 8 shows results from a typical B scan obtained from the cortical brain of a mouse. The OMAG method obtained the images of microstructures via SDOCT [Fig. 7(A)], blood flow via OMAG [Fig. 8(B)] and the corresponding velocities of blood flow via DOMAG [Fig. 8(C)]. DOMAG calculated the velocities of blood flow in functional vessels, including capillaries (white arrows for example), and even in the vessels \(~1.5\) mm deep below the bone surface (red arrow). However, the PRDOCT result [Fig. 8(D)] indicates that conventional DOCT fails to provide detailed velocities of blood flows in this case. The level of background phase-noise is an important metric when quantifying blood flow, particularly in capillaries, because this metric affects our ability to extract useful flow signals from the noisy background. Using the method described in Section 4.1, the noise level for PRDOCT was typically 0.5 rad, largely due to the heterogeneous property of the tissue sample as seen in Fig. 8(D) as discussed in Section 3, suggesting that PRDOCT might not be able to measure blood flow velocities \(< 1.1\) mm/s when the A scan rate is at \(~20\) kHz. However, DOMAG was able to reduce this noise level to 0.034 rad that is comparable to the phantom experiments shown in Section 4.1, suggesting an approximate 15-fold improvement in imaging blood flow velocities over...
conventional PRDOCT in this in vivo imaging case. Thus, we expect that DOMAG will be a good candidate of tool to quantify blood flow within a perfused tissue.

To further show the advantages of DOMAG in imaging the blood flow velocities over PRDOCT, we compared 3D DOMAG and PRDOCT images evaluated from a scanned tissue volume from the mouse brain cortex with the skull left intact. Fig. 9 (shown as projection images to x-y) illustrates the difference between OMAG, DOMAG, and PRDOCT imaging of cerebral blood flow in mice under the same experimental conditions. To obtain the PRDOCT flow image, we had to use algorithms established in [26,27] to reduce the noise artifacts, where algorithms for minimization of the sample motion artifacts, segmentation of regions of interest and correction of phase-wrapping errors were implemented. Note that it did not require performing the segmentations in DOMAG in order to render the 3D image as the phase noise level was low in the entire 3D space. These results indicate that DOMAG [Fig. 9(B)] could reliably determine the velocities of blood flows within almost all vessels in the scanned tissue. Not surprisingly, PRDOCT [Fig. 9(C)] can be erroneous in quantifying blood flows within the scanned tissue, due to noise produced in PRDOCT, which masks slow flows (<1.1mm/s) in small vessels. Furthermore, blood vessel diameters seen in the DOMAG and OMAG images were significantly larger than those in the DOCT images, suggesting a clear advantage of DOMAG over DOCT in quantifying blood flow in the scanned tissue.

The reason for these differences is that the performance of conventional PRDOCT is limited by the background texture noise pattern that caused by the optical heterogeneity, i.e., microstructures, of the tissue sample [15]. On the other hand, Doppler OMAG uses an ideal reconstructed sample as the tissue background, which makes the adjacent OMAG A scans totally correlated, maximally satisfying the correlation requirement for the phase-resolved technique. As a consequence, DOMAG reduces the background phase noise to a minimum. This improves the capability of DOMAG to detect low blood velocity near the wall of the blood vessel, resulting in that the diameter of blood vessel detected by DOMAG is larger than that by PRDOCT, as we seen in Fig. 9.

An alternative way to better illustrate the phase noise levels is to use 3D plots of cross-sectional images (B scans), e.g., Fig. 8. Shown in Fig. 10 is such an illustration for a typical B scan from the cortical brain in mice. Figure 9(A) is the conventional PRDOCT flow velocity plot, without applying the segmentation approach to eliminate the random phases in low signal regions. The flow signals are pointed by the black arrows and the noise in useful signal region is pointed with red arrow. In the low OCT signal regions, for example the region above tissue surface where there is no light reflectivity and the region deep in the tissue where the detected optical signal is low due to the light attenuation, the evaluated phases will exhibit random phase noise signals in PRDOCT. From Fig. 10(A), it is clear that the noise in the low-signal region overwhelmed the useful flow signals, thus the segmentation is often needed in PRDOCT to exclude these random noises. After segmentation of tissue region of interests using the methods in [26,27], a better view is given in Fig. 10(B) to show the effects of background noise in the tissue region. In Fig. 10(B), the noise pointed with red arrow is so high that it prevents the small blood vessel signals from detecting. Especially, we should pay attention to those pointed by blue arrows, which show difficulty in distinguishing whether they are blood signals or noise. For DOMAG, shown in Fig. 10(C), the background noise is very small compared to the blood vessel signals, which improves significantly the imaging performance for DOMAG.

It should be noted that Ren et al previously proposed a moving-scatter-sensitive optical Doppler OCT (MSS-DOCT) technique for in vivo blood flow imaging using SDOCT [17,29]. In their method, a delayed-line filter was used to suppress the influence of stationary scatters through subtracting adjacent complex axial scans before applying the phase resolved method to calculate the Doppler frequency shift. There are distinct differences between DOMAG and
MSS-DOCT in that 1) the former operates on the wavenumber domain signals, i.e., interferograms, while MSS-DOCT operates on the time domain signals, i.e. complex OCT images; 2) DOMAG filters out the signals originated by the static scattering components from the entire B scan signals, while the latter uses a delayed line filters applied to the neighborhood A scans to suppress the static signals.

5. Conclusions

We have demonstrated a novel method, Doppler OMAG, for imaging flow velocity in the functional blood vessels within the microcirculation tissue beds in vivo. The method takes the advantages of both OMAG and phase-resolved method. By introducing a digitally reconstructed tissue background that is of optically homogeneous and no attenuation into DOMAG, the texture noise background caused by the optical heterogeneity of the real tissue sample can be eliminated, leading to a dramatic improvement of DOMAG imaging of blood perfusion in the highly scattering tissue. We have quantitatively shown, through phantom experiments, that DOMAG method improved the phase SNR by 22 dB, from 79 dB to 101 dB. In addition, DOMAG automatically eliminated the random phase noises within the regions of low reflectivity, including the region above the tissue surface. Note however that the use of segmentation method in order to exclude the phase-noise in low signal regions is often needed in the conventional PRAOCT. This advantage makes DOMAG method segmentation free for many applications, leading to reduced demand for computing power. We also demonstrated the potential of DOMAG imaging of cerebral blood flow in the cortical brain of mice, achieved by shining the light through the intact skull. When compared to PRAOCT, we showed that DOMAG delivers superior in vivo imaging performances in providing the velocity information within the patent vessels.

6. Acknowledgement

The authors would like to thank Mrs Yali Jia who assisted in the animal experiments for the results presented in this article. The work was supported in part by research grants from the National Heart, Lung, and Blood Institute (R01 HL093140) and the American Heart Association Grant-in-Aid (0855733G). The content is solely the responsibility of the authors and does not necessarily represent the official views of grant giving bodies. The authors also acknowledge the generous support from DenseLight Semiconductors Pte Ltd, Singapore.

References and links

1. Fercher AF, Drexler W, Hitzenberger CK, Lasser T. Optical coherence tomography – principles and applications. Rep. Prog. Phys 2003;66:239–303.
2. Tomolins PH, Wang RK. Theory, development and applications of optical coherence tomography. J Phys. D: Appl. Phys 2005;38:2519–2535.
3. Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, Hee MR, Flotte T, Gregory K, Puliafito CA, Fujimoto JG. Optical coherence tomography. Science 1991;254:1178–1181. [PubMed: 1957169]
4. Chen ZP, Milner TE, Srinivas S, Wang X, Malekañzali A, van Gemert MJC, Nelson JS. Noninvasive imaging of in vivo blood flow velocity using optical Doppler tomography. Opt. Lett 1997;22:1119–1121. [PubMed: 18185770]
5. Zhao YH, Chen ZP, Ding ZH, Ren H, Nelson JS. Real-time phase-resolved functional optical coherence tomography by use of optical Hilbert transformation. Opt. Lett 2002;25:98–100. [PubMed: 18007724]
6. Hausler G, Lindner MW. Coherence radar and Spectral radar- new tools for dermatological diagnosis. J. Biomed. Opt 1998;3:21–31.
7. Leitgeb RA, Schmetterer L, Hitzenberger CK, Fercher AF, Berisha F, Wojtkowski M, Bajraszewski T. Real-time measurement of in vitro flow by Fourier domain color Doppler optical coherence tomography. Opt. Lett 2004;29:171–173. [PubMed: 14744000]
8. Zhang J, Chen ZP. In vivo blood flow imaging by a swept laser source based Fourier domain optical Doppler tomography. Opt. Express 2005;13:7449–7459. [PubMed: 19498770]

9. Vakoc BJ, Yun SH, de Boer JF, Tearney GJ, Bouma BE. Phase-resolved optical frequency domain imaging. Opt. Express 2005;13:5483–5492. [PubMed: 19498543]

10. Leitgeb RA, Schmetterer L, Drexler W, Fercher AF, Zawadzki RJ, Bajraszewski T. Real-time assessment of retinal blood flow with ultrafast acquisition by color Doppler Fourier domain optical coherence tomography. Opt. Express 2003;11:3116–3121. [PubMed: 19471434]

11. White BR, Pierce MC, Nassif N. In vivo dynamic human retinal blood flow imaging using ultra-high-speed spectral domain optical Doppler tomography. Opt. Express 2003;11:3490–3497. [PubMed: 19471483]

12. Makita M, Hong Y, Yatagai MYT, Yasuno Y. Optical coherence angiography. Opt. Express 2006;14:7821–7840. [PubMed: 19529151]

13. Leitgeb R, Hitzenberger CK, Fercher AF. Performance of Fourier domain vs. time domain optical coherence tomography. Opt. Express 2003;11:889–894. [PubMed: 19461802]

14. Choma MA, Sarunic MV, Yang CH, Izatt JA. Sensitivity advantage of swept source and Fourier domain optical coherence tomography. Optics Express 2003;11(18):2183–2189. [PubMed: 19466106]

15. Wang RK, Ma ZH. Real-time flow imaging by removing texture pattern artifacts in spectral-domain optical Doppler tomography. Opt. Lett 2006;31:3001–3003. [PubMed: 17001380]

16. Yun SH, Tearney GJ, de Boer JF, Bouma BE. Motion artifacts in optical coherence tomography with frequency-domain ranging. Opt. Express 2004;12:2977–2998. [PubMed: 19483816]

17. Ren HW, Sun T, MacDonald DJ, Cobb MJ, Li XD. Real-time in vivo blood-flow imaging by moving scatterer-sensitive spectral-domain optical Doppler tomography. Opt. Lett 2006;31:927–929. [PubMed: 16599214]

18. Bachmann AH, Villiger ML, Blatter C, Lasser T, Leitgeb RA. Resonant Doppler flow imaging and optical vivisection of retinal blood vessels. Opt. Express 2007;15:408–422. [PubMed: 19532258]

19. Szkulmowski M, Szkulmowska A, Bajraszewski T, Kowalczyk A, Wojtkowski M. Flow velocity estimation using joint Spectral and Time domain Optical Coherence Tomography. Opt. Express 2008;16:6008–6025. [PubMed: 18545302]

20. Wang RK. In vivo full rang complex Fourier domain optical coherence tomography. Appl. Phys. Lett 2007;90:054103.

21. Wang RK. Fourier domain optical coherence tomography achieves full range complex imaging in vivo by introducing a carrier frequency during scanning. Phys. Med. Biol 2007;52:5897–5907. [PubMed: 17881807]

22. An L, Wang RK. Use of scanner to modulate spatial interferogram for in vivo full range Fourier domain optical coherence tomography. Opt. Lett 2007;32:3423–25. [PubMed: 18059954]

23. Wang RK, Jacques SL, Ma ZH, Hanson S, Gruber A. Three Dimensional Optical Angiography. Opt. Express 2007;15:4083–4097. [PubMed: 19532651]

24. Wang RK, Hurst S. Mapping of cerebrovascular blood perfusion in mice with skin and cranium intact by Optical Micro-AngioGraphy at 1300nm wavelength. Opt. Express 2007;15:11402–11412. [PubMed: 19547498]

25. Wang RK. Three dimensional optical angiography maps directional blood perfusion deep within microcirculation tissue beds in vivo. Phys. Med. Biol 2007;52:N531–N537. [PubMed: 18029974]

26. An L, Wang RK. In vivo volumetric imaging of vascular perfusion within human retina and choroids with optical micro-angiography. Opt. Express 2008;16:11438–11452. [PubMed: 18648464]

27. Mujat M, Chan RC, Cense B, Park BH, Joo C, Akkin T, Chen TC, de Boer JF. Retinal nerve fiber layer thickness map determined from optical coherence tomography images. Opt. Express 2005;13:9480–9491. [PubMed: 19503151]

28. Tuchin V. Tissue Optics : Light Scattering Methods and Instruments for Medical Diagnosis. 2007ISBN number: 0819464333. SPIE

29. Ren H, Li X. Clutter rejection filters for optical Doppler tomography. Opt. Express 2006;14:6103–6112. [PubMed: 19516783]
Fig. 1.
Schematic of the OMAG system used in this study to image the velocities of blood flow, where PC represents the polarization controller and CCD the charged coupled device. The laser diode emitting the light at 633 nm was used for aiming purposes during imaging.
Fig. 2.
Diagram of frequency components for different tissue sample: (A) an ideal tissue sample (optically homogeneous sample) with no moving particles; (B) a real tissue sample (optically heterogeneous sample) with no moving particles; (C) a real tissue sample (optically heterogeneous sample) with moving particles.
Fig. 3.
Flow chart showing the steps for DOMAG to evaluate the velocities of blood flow from a B scan dataset, $l(k,t)$. The data coordinates are indicated in the lower right corner of each data block, where $t$ is the time variable of probe beam scanning over a sample, $k$ is the wavenumber, $f$ is the spatial frequency, and $z$ is the imaging depth. $\text{FT}|_t$ represents the Fourier transform (FT) against the time variable $t$ in the B scan, $\text{FT}^{-1}|_f$ indicates the inverse FT against the spatial frequency, $f$, and $\text{FT}|_k$ is FT against the wavenumber $k$. 
Fig. 4. Flow Phantom experiment results. (A), OMAG structural image; (B) OMAG flow image; (C), DOMAG velocity image; (D) PRDOCT velocity image; and (E) flow signal profiles extracted from the positions marked in (C) and (D). See text for the marked regions in (A).
Fig. 5. In vivo OMAG imaging results for a typical B scan of a mouse brain with the skull left intact. (A) OMAG image of microstructures, identical to conventional SDOCT image; (B) the corresponding OMAG image of blood flow; and (C) the corresponding DOMAG image of velocities of the blood flow.
Fig. 6.

*In vivo* 3D OMAG imaging of the cortical brain of a mouse with the skull left intact. The volumetric visualization was rendered by (A) merging the 3D micro-structural image with the 3D cerebral blood flow image, (B) the 3D signals of cerebral blood flow only, and (C) the corresponding DOMAG imaging of velocities within the 3D blood flow network in (B). The red color in (C) represents the blood moves towards the incident probe beam, and otherwise the green color. The physical image size was 2.5×2.5×2.0 (x-y-z) mm³.
Fig. 7.
Maximum projection view (x-y) of (A) OMAG and (B) DOMAG of the cerebral blood flow in the cortical brain of the mouse shown in Fig. 6.
Fig. 8.
Comparison between OMAG and PRDOCT B scan imaging of the cortical brain in mice \textit{in vivo}. (A) OMAG structural image (i.e. SDOCT) and the corresponding (B) OMAG flow image, (C) DOMAG flow velocity image, and (D) PRDOCT flow velocity image, respectively. See the text for explanation of the arrows.
Fig. 9.
Comparison between 3D OMAG and PRDOCT imaging of the cortical brain in mice with the intact skull in vivo. Shown are the maximum x-y projection views of (A) OMAG cerebral blood flow image, (B) DOMAG flow velocity image and (C) PRDOCT flow velocity image, respectively. The physical size of scanned tissue volume was 2.5×2.5×2.0 mm$^3$. White bar = 500 μm.
Fig. 10.
3D plot of a typical B scan of flow images. (A) Conventional PRDOCT flow image without segmentation; (B) conventional PRDOCT flow image with segmentation; and (C) Doppler OMAG flow image.
Table 1
Evaluated phase noise, effective signal and phase SNR of phase differences for DOMAG and PRDOCT

|         | $\sigma_{\Delta \phi}$ | $S$     | Phase SNR |
|---------|-------------------------|---------|-----------|
| DOMAG   | 0.037 rad               | 4395 rad| 101 dB    |
| PRDOCT  | 0.43 rad                | 4189 rad| 79 dB     |
| Improvement | 11.6 (times)             | 4% (unitless) | 22 dB     |