Multi-functional angiographic OFDI using frequency-multiplexed dual-beam illumination

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Abstract: Detection of blood flow inside the tissue sample can be achieved by measuring the local change of complex signal over time in angiographic optical coherence tomography (OCT). In conventional angiographic OCT, the transverse displacement of the imaging beam during the time interval between a pair of OCT signal measurements must be significantly reduced to minimize the noise due to the beam scanning-induced phase decorrelation at the expense of the imaging speed. Recent introduction of dual-beam scan method either using polarization encoding or two identical imaging systems in spectral-domain (SD) OCT scheme shows potential for high-sensitivity vasculature imaging without suffering from spurious phase noise caused by the beam scanning-induced spatial decorrelation. In this paper, we present multi-functional angiographic optical frequency domain imaging (OFDI) using frequency-multiplexed dual-beam illumination. This frequency multiplexing scheme, utilizing unique features of OFDI, provides spatially separated dual imaging beams occupying distinct electrical frequency bands that can be demultiplexed in the frequency domain processing. We demonstrate the 3D multi-functional imaging of the normal mouse skin in the dorsal skin fold chamber visualizing distinct layer structures from the intensity imaging, information about mechanical integrity from the polarization-sensitive imaging, and depth-resolved microvasculature from the angiographic imaging that are simultaneously acquired and automatically co-registered.

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1. Introduction

Micro-vascular morphology has been considered as one of the major indicators in medical and biological researches characterizing tissue viability and functionality [1]. Angiographic optical coherence tomography (OCT), based on phase-resolved Doppler difference or variance, speckle variance, intensity decorrelation, or complex differential variance has emerged as a promising technique that visualizes three-dimensional (3D) microvascular networks by detecting spatially localized motion within the sample, e.g., blood flow [2–14].

Angiographic OCT measures a change in phase, intensity, or complex OCT signal caused by motion of the light scatterers inside the tissue during the time interval $\tau$, typically between successive A-lines, at the same spatial location. To ensure sufficient correlation between a pair of measurements for high-sensitivity angiographic OCT, the transverse displacement of the imaging beam during $\tau$ must be substantially small relative to the size of the imaging...
beam [15]. In conventional angiographic OCT systems, the transverse beam-scanning rate is intentionally reduced to minimize the decorrelation noise at the expense of the imaging speed.

Several approaches have been introduced to achieve high-speed and high-sensitivity angiographic OCT imaging [11,16–20]. Specially designed transverse sampling patterns were developed to achieve rapid beam scanning across large tissue volumes for angiographic OCT [11,16,17]. Scanning with stepwise or segmented sawtooth (or triangle) waveform along fast scan axis within each B-scan was utilized providing long enough time interval between a pair of OCT signal measurements while minimizing decorrelation noise. A pair of OCT signal measurements between consecutive B-scans was also suggested usually with high-speed OCT system. Recently dual-beam scanning schemes were demonstrated utilizing polarization encoding [18,19] or two identical OCT systems [20] in spectral-domain (SD) OCT systems to achieve high-sensitivity angiographic OCT imaging.

In this manuscript, we present multi-functional optical frequency domain imaging (OFDI) performing angiographic and polarization-sensitive OCT imaging simultaneously. A frequency multiplexing scheme [21] enables the dual imaging beams that are encoded with distinct frequency shifts to perform high-speed and high-sensitivity angiographic OCT imaging. Since the frequency-multiplexed dual beam scheme operates independently from the polarization state of the imaging light, the simultaneous polarization-sensitive imaging was also achieved. We demonstrate the 3D multi-functional imaging of normal mouse skin in the dorsal skin fold chamber. Distinct layer structures from the intensity imaging, information about mechanical integrity from the polarization-sensitive imaging, and depth-resolved microvasculature from angiographic imaging were simultaneously acquired and automatically co-registered.

Fig. 1. Principles of frequency-multiplexed dual-beam illumination. (a) Schematic of the reference arm and the sample arm of the system. (b) Frequency bands occupied by dual-beam OFDI signals: $f_1 = 2f_{\text{FS,ref}}$ and $f_2 = 2(f_{\text{FS,ref}} + f_{\text{FS,s}})$.

2. Principle of frequency-multiplexed dual-beam angiographic OFDI

A schematic of the frequency-multiplexed dual-beam illumination is described in Fig. 1(a). A free-space acousto-optic frequency shifter in the sample arm angularly separates the zeroth-order (frequency un-shifted) beam and the first-order-diffracted (frequency shifted) beam. An
afocal telescope with a pair of lenses in an infinite-conjugate configuration converts the diverging angularly separated beams into the converging ones. The center of the galvanometric mirror scanner is located at the focal point of the second lens of the telescope. The orientation of the angular separation of the two imaging beams is aligned to match the beam scanning direction of the galvanometer so that the scanning path of the following beam exactly overlaps the trace of the preceding beam. By carefully adjusting the scanning speed of the galvanometer, the two spatially separated beams can illuminate the same location on the sample with a time interval corresponding to exact multiple of the A-line period, effectively removing noise associated with phase decorrelation resulting from the beam scanning. Each beam is encoded with a distinct frequency shift through the use of frequency shifters in both sample and reference arms. Since the two imaging beams occupy different electrical frequency bands as depicted in Fig. 1(b), the corresponding OFDI signals can be demultiplexed in frequency domain and processed to generate angiographic information of the sample, while additionally removing depth degeneracy [22].

3. Imaging system configuration

Figure 2 depicts the configuration of the multi-functional angiographic OFDI system that was built based on the frequency-multiplexed dual-beam illumination scheme. A short cavity length wavelength-swept laser (WSL) [23,24] was constructed using a tunable Fabry-Perot filter (Lambdaquest X1310), providing 123.2 kHz wavelength tuning over 106 nm centered at 1290 nm. The power of the light source was 62 mW at the laser output and 24 mW on the sample. A small portion of the laser output was tapped to generate a trigger signal for each A-line by using a fiber Bragg grating (FBG). A polarization modulator was included for polarization-sensitive imaging. Ninety percent of the laser output was coupled to the sample arm and the remaining ten percent was directed to the reference arm of the system interferometer by a 90/10 fiber directional coupler. In the reference arm, a 21.25 MHz fiber-pigtailed acousto-optic frequency shifter (Brimrose AMF-21.25-1300) was placed at the double pass section providing a frequency shift of 42.5 MHz. A 1% tap coupler directed the sample arm light to the calibration mirror (1% port) [10] and the sample to be imaged through the imaging optics (99% port). A free-space frequency shifter (Brimrose AMF-42.5-1300) in the path of the imaging beam (double pass section) was aligned to provide a diffraction...
efficiency of 50% so that the equal amount of imaging light was separated to both the zeroth-order beam (frequency un-shifted) and the first-order-diffracted beam. The shifting frequency of each acousto-optic frequency shifter was determined to ensure Nyquist sampling and minimum cross-talk between the two OFDI signals occupying two different electrical frequency bands. The beam diameter at the frequency shifter was selected to obtain sufficient optical bandwidth while reducing cross-talk between adjacent diffraction orders at the frequency shifter [21]. The orientation and the scanning speed of the fast axis of the two-dimensional galvanometer mirror (Cambridge Technology 6215H) were carefully aligned and adjusted so that the two spatially separated beams illuminate the same location on the sample with a time interval corresponding to an exact multiple of the A-line period. The interference signal between the reference and the sample arm lights was detected at a pair of balanced receivers (Femto HCA-S, 200 MHz) in the polarization diverse balanced detection arrangement. Each OFDI interference fringe corresponding to each of dual-beam illuminations was acquired over an 85 MHz frequency band centered at 42.5 MHz and 127.5 MHz, respectively, and digitized by a high-speed and high-resolution data acquisition board (Signatec PX14400, 400 MS/s max, 14 bits, 2 channels) at a sampling rate of 340 MHz. To minimize spurious phase noise, the digitizer, the frequency shifters, and the tunable Fabry-Perot filter were phase-locked to each other. A 340 MHz clock and a 10 MHz signal from a single master clock source (Novasource M2) were used as an external clock for data acquisition board and a frequency reference signal to phase-lock the frequency shifters and the tunable Fabry-Perot filter in the WSL. The axial resolution was 10.2 μm and the transverse resolution was 26.6 μm for both imaging beams in tissue. The sensitivity of the system was measured to be 97.3 dB at an A-line rate of 123.2 kHz.

4. Phase noise of the frequency-multiplexed dual-beam system

To evaluate the sensitivity of the angiographic imaging in the frequency-multiplexed dual-beam system, we performed a phase noise analysis. The sensitivity of the angiographic OCT measurement is fundamentally limited by the phase noise of the system, i.e., the noise in measurement of the phase difference between two time points. The phase noise of the conventional single-beam Doppler OCT system is determined by the signal-to-noise ratio (SNR) and the phase decorrelation noise due to the dither of the closed-loop galvanometric mirror scanner [12,25]. In the frequency-multiplexed dual-beam system, we have an additional decorrelation noise element resulting from the chromatic angular dispersion of the imaging beam diffracted by the frequency shifter as depicted in Fig. 3. Because each wavelength component of the first-order-diffracted imaging beam illuminates slightly different spot on the sample while the zeroth-order imaging beam is focused on a single spot regardless of its wavelength, it induces decorrelation noise in angiographic OCT measurement between the two frequency-multiplexed imaging beams when the following beam reaches the spot where the preceding beam was. The overall phase noise of the frequency-multiplexed dual-beam system is therefore estimated as a combination of these three phase noise elements.
as \( \sigma_{\Delta \phi} = \sqrt{\sigma_{\text{SNR}}^2 + \sigma_{\text{galvo}}^2 + \sigma_{FS}^2} \), where \( \sigma_{\text{SNR}} = \sqrt{SNR} \) [15], \( \sigma_{\text{galvo}} \) is the phase noise due to the galvanometric dithering, and \( \sigma_{FS} \) is the decorrelation noise arisen from the sample arm frequency shifter.

Fig. 4. (a) Phase noise as a function of 3 dB optical bandwidth of the WSL and (b) phase noise as a function of depth in the homogeneous scattering phantom.

We first measured \( \sigma_{\text{galvo}} \) by imaging a homogeneous scattering phantom with the conventional single-beam method at the exact same location without scanning the imaging beam but with the galvanometer servo driver turned on. From the measurement of 10,240 consecutive A-lines, phase noises with different time intervals were calculated. After subtracting the contribution of \( \sigma_{\text{SNR}} \), the \( \sigma_{\text{galvo}} \) was measured to be 0.12 ± 0.011 rad over time interval of 0.1 – 8.5 ms. Assuming Gaussian beam envelope, the phase noise due to the imaging spot displacement between the two beams for phase difference measurement is given by \( \sigma_{\Delta \phi} = \sqrt{\frac{4\pi}{3} \left(1 - \exp\left(-\frac{2\left(\frac{\Delta x}{d}\right)^2}{\exp(\alpha)}\right)\right)} \) [15], where \( \Delta x \) is the spot displacement and \( d \) is the \( 1/e^2 \) width of the imaging beam spot at the focus. The spot size of the imaging beam on the sample is given by \( d = \frac{4F_1F_2\lambda}{F_2\pi \omega_0} \), where \( F_1, F_2 \) and \( F_3 \) are the focal lengths of the lenses in the imaging optics, \( \lambda_0 \) is the center wavelength of the WSL, and \( \omega_0 \) is the \( 1/e^2 \) beam width at the fiber-optic collimator. The spot displacement for the wavelength component with \( \lambda = \lambda_0 + \Delta \lambda \) due to the sample arm frequency shifter can be given by \( \Delta x = \frac{F_1F_2(\lambda - \lambda_0)}{F_2p \cos \beta_0} \), where \( p \) is the pitch of the acoustic grating formed in the frequency shifter that can be calculated from the velocity and the frequency of the acoustic wave, and \( \beta_0 \) is the beam incident angle to the frequency shifter. With the Gaussian spectral profile, the mean spot displacement can be given by \( \left| \Delta x_{FS} \right| = \frac{\int d\lambda |\Delta x| \exp\left[-\alpha(\lambda - \lambda_0)^2\right]}{\int d\lambda \exp\left[-\alpha(\lambda - \lambda_0)^2\right]} \), and the decorrelation noise caused by the sample arm frequency shifter is then given by \( \sigma_{FS} = \sqrt{\frac{4\pi}{3} \left(1 - \exp\left(-\frac{2\left(\frac{\Delta x_{FS}}{d}\right)^2}{\exp(\alpha)}\right)\right)} \). In the system, we used optical components with the
following parameters: $F_1 = 25 \text{ mm}$, $F_2 = 100 \text{ mm}$, $F_3 = 35 \text{ mm}$, $\alpha_0 = 0.54 \text{ mm}$, $p = 58.5 \text{ \mu m}$, and $\beta_0 = 11 \text{ mrad}$. The theoretically estimated overall phase noise and the standard deviation of the phase difference measured with a homogeneous scattering phantom as a function 3 dB optical bandwidth of the WSL are plotted in Fig. 4(a). The average SNR of the region of interest was 27.7 dB, which gives $\sigma_{\text{SNR}} = 0.041$. The standard deviations of the phase difference measurement with different spectral widths were obtained by applying the Gaussian window with different width (different $\alpha$) to the same fringe data. The theoretical estimation shows good agreement with the experimental data in the regime where the 3 dB bandwidth of the light source is broader than 30 nm. Figure 4(b) plots phase noise measured and calculated as a function of depth in the homogeneous phantom using a WSL with 3 dB bandwidth of 50 nm (106 nm before application of the Gaussian window). The theoretical curve that was obtained by using the average SNR value at each depth fits well with the experimentally measured phase noise. Experimentally acquired phase noise shown in Fig. 4 was measured from a B-scan (1,024 A-lines). The measurement was repeated over 10 frames and the mean ± standard deviation of the phase noise is presented.

5. Imaging

![Fig. 5. 3D vasculatures of normal mouse skin in the dorsal skin fold chamber acquired with time interval of (a) 0.26 ms, (b) 2.08 ms, and (c) 8.32 ms between the two measurements by the frequency-multiplexed dual imaging beams. Depth is denoted by color: yellow (superficial) to red (deep). Scale bar, 500 \text{ \mu m}.](image)

We imaged the vascular network of normal mouse skin in the dorsal skin fold chamber using the frequency-multiplexed dual-beam system. The dorsal skinfold chamber window model was prepared in normal skin of the BALB/c mouse. After anesthesia with zolazepam/tiletamine and xylazine, the dorsal skinfold chamber window was fixed to the custom-made stereotactic frame for imaging. Three-dimensional (3D) angiographic data set acquired from the system was processed by using the complex differential variance (CDV) algorithm [11,26]. Figure 5 shows en face vascular projections using color to encode depth (yellow (superficial) to red (deep)). Figure 5(a) shows the 3D vasculature over a volume of 6 mm (X: 1024 lines) x 6 mm (Y: 1024 lines) x 4 mm (Z: depth) acquired in 8.5 s. The time interval between the two measurements by the frequency-multiplexed imaging beams was 0.26 ms, which corresponds to the 32 A-line periods. As we increase time interval by reducing the fast-axis scanning speed, we were able to image smaller vessels in the dorsal skin of a mouse as shown in Figs. 5(b) and 5(c) with increased imaging time of 68 s and 272 s, respectively. The arterioles, venules, and capillary networks are visualized with depth-resolved color-coding.

For multi-functional imaging that provides intensity, angiographic, and polarization-sensitive (PS) images, we performed the frequency-multiplexed dual beam imaging with the polarization modulator turned on (Fig. 2). Simultaneous acquisition of all three-types of data
ensured absolute registration of these three image sets. Figure 6 shows the depth-projected vasculature in the dorsal skin fold of another mouse in the middle, and the cross-sectional intensity and local birefringence images at four different locations of the mouse skin on both sides of the angiographic image. While the angiographic projection visualizes the 3D microvascular networks, the intensity cross-sections show distinct layer structures of the mouse skin, such as muscle and dermis layers, and the corresponding local birefringence (local phase retardation) cross-sectional images show strong birefringence in the muscle layer and typical transversely varying birefringence in the dermis.

![Fig. 6. 3D multi-functional imaging. (a) Depth-projected vasculature in the dorsal skin fold of another mouse acquired with the time interval between the dual beams of 4.16 ms. (b)-(e) Cross-sectional intensity and local birefringence images at four different locations of the mouse skin on both sides of the angiographic image. Scale bar, 500 μm.](image)

6. Discussion

One of the shortcomings of our frequency-multiplexed dual-beam system is 6 dB sensitivity penalty caused by the frequency shifter at the sample arm. Since the light incident on the frequency shifter is split into two imaging beams and only 50% of the returning light from the sample is coupled back to the system through the frequency shifter, the system suffers sensitivity penalty of 6 dB compared with the conventional single-beam system.

Another limitation of the proposed system is relatively short time interval between the two frequency-multiplexed imaging beams. For visualization of small vessels and capillaries, long time interval of typically several milliseconds, which is at least several times longer than the interval that the current frequency-multiplexed dual beam provides, is necessary. To obtain long time, we need large separation between the two frequency-multiplexed imaging beams relative to the beam width on the sample. Increasing the beam separation on the sample by modifying the magnification of the sample arm imaging optics does not help to increase the time interval because it also increases the beam spot size. While the separation between the two beams is given by $s = \frac{F_1 F_2}{F_2} \theta_0$, the size of the beam spot on the sample is given by

$$d = \frac{4 F_1 F_2 \lambda}{F_2 \pi \omega_0}.$$  

The beam separation relative to the spot size is, therefore, given by

$$\frac{s}{d} = \frac{\pi \omega_0 \theta_0}{4 \lambda},$$

which we cannot change by modifying the magnification of the imaging optics. Reducing the spot size by increasing the beam diameter at the fiber-optic collimator in front of the frequency shifter can slightly increase the time interval, but the larger beam size limits the optical bandwidth at the frequency shifter.

Although the short time interval limits angiographic visualization of small vessels, it can be effectively utilized to quantitatively visualize the fast flows. Our frequency-multiplexed...
dual-beam system can easily provide short time interval of < 0.3 ms. Sub-millisecond time interval is necessary to selectively image the large vessels with fast flow, which cannot be achieved in the single-beam systems due to the limitation of the scan speed of the galvanometric mirror scanner [12]. Although the conventional method utilizing measurements between successive A-lines can provide very short time interval simply by slowly scanning the fast-axis galvanometric mirror allowing oversampling, it is usually too short even for most of the very fast blood flow (e.g. 0.01 ms with 100 kHz A-line rate). Smart combination of the intra-B-scan dual-beam and inter-B-scan single-beam schemes may provide quantitative angiographic imaging over wide range of flow speed by efficiently utilizing short time interval of the proposed dual-beam system.

7. Conclusion

We have demonstrated the multi-functional angiographic OFDI system using frequency-multiplexed dual-beam illumination. A frequency shifter in the sample arm provides two spatially separated imaging beams occupying distinct electrical frequency bands that can be demultiplexed in the frequency domain processing. Since the frequency-multiplexing scheme operates independently from the polarization state of the imaging light, we have achieved simultaneous polarization sensitive imaging in this system. The proposed multi-functional dual-beam OFDI system provides automatically co-registered three-dimensional intensity, polarization-sensitive, and angiographic images. The multi-functional imaging of the normal mouse skin in the dorsal skin fold chamber visualized distinct layer structures in the intensity image, information about mechanical integrity in the polarization-sensitive image, and depth-resolved microvasculature in the angiographic image that are acquired simultaneously. Smart combination of the intra-B-scan dual-beam and inter-B-scan single-beam schemes may provide quantitative angiographic imaging over wide flow rate range by efficiently utilizing short time interval of the proposed frequency-multiplexed dual-beam system.

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