Doppler imaging with dual-detection full-range frequency domain optical coherence tomography

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Abstract: Most of full-range techniques for Frequency Domain Optical Coherence Tomography (FD-OCT) reported to date utilize the phase relation between consecutive axial lines to reconstruct a complex interference signal and hence may exhibit degradation in either mirror image suppression performance or detectable velocity dynamic range or both when monitoring a moving sample such as flow activity. We have previously reported a technique of mirror image removal by simultaneous detection of the quadrature components of a complex spectral interference called a Dual-Detection Frequency Domain OCT (DD-FD-OCT) [Opt. Lett. **35**, 1058-1060 (2010)]. The technique enables full range imaging without any loss of acquisition speed and is intrinsically less sensitive to phase errors generated by involuntary movements of the subject. In this paper, we demonstrate the application of the DD-FD-OCT to a phase-resolved Doppler imaging without degradation in either mirror image suppression performance or detectable velocity dynamic range that were observed in other full-range Doppler methods. In order to accommodate for Doppler imaging, we have developed a fiber-based DD-FD-OCT that more efficiently utilizes the source power compared with the previous free-space DD-FD-OCT. In addition, the velocity sensitivity of the phase-resolved DD-FD-OCT was investigated, and the relation between the measured Doppler phase shift and set flow velocity of a flow phantom was verified. Finally, we demonstrate the Doppler imaging using the DD-FD-OCT in a biological sample.

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1. Introduction

The Doppler effect was discovered by the Austrian physicist Christian Johann Doppler in the mid 1800s [1,2]. For many years, various Doppler imaging techniques have been developed in the field of ultrasound imaging [3]. Analogous to ultrasonography, optical coherence tomography (OCT) is a non-invasive imaging technology that is capable of depth sectioning of biological tissue, yet at the micrometer scale resolution [4]. Traditionally, OCT imaging contrast relies on the variation of the strength of back-reflected light from a sample that arises from refractive index fluctuation inside a biological sample. In addition to structural mapping, many functional OCT systems have been actively developed in order to gain additional information that will lead to a better understanding of sample properties. Among the many functional OCT systems, Doppler OCT (DOCT) is one of the most useful. It is capable of *in vivo* monitoring of flow activity in biological samples such as blood flow in the human retina [5,6], and the cardiovascular system of animal embryos [7,8]. DOCT provides information about flow location, velocity, direction, and profile that cannot be obtained by intensity mapping alone.

Recent development in DOCT is mostly based on phase sensitive detection so-called phase-resolved DOCT [9]. The early development of phase-resolved DOCT was based on phase sensitive time domain OCT (TD-OCT) that required mechanical scanning of the reference arm and hence limited the maximum acquisition speed to a few kHz regime [10]. Later, the phase-resolved Doppler technique was also extended to frequency domain OCT (FD-OCT), which not only has speed and sensitivity advantages over TD-OCT but also allows direct access to the phase information immediately following the Fourier transform [11]. A spectrometer based frequency domain Doppler OCT (FD-DOCT) utilizing a continuous readout CCD camera and achieving an acquisition speed of 29.3 kHz line rate was reported [12]. Moreover the further increase of imaging speed and the maximum detectable velocity in spectrometer based FD-DOCT, using a high-speed CMOS camera as a detector, was also investigated [5]. However, the increase in maximum detectable velocity accommodated from high-speed acquisition comes with the cost of an increase in the minimum detectable velocity since both of them depend on the acquisition rate. With a camera line rate of 200 kHz, the minimum detectable axial velocities as measured with and without lateral scanning were 800 $\mu\text{m/s}$ and 8.2 mm/s, respectively. Nevertheless, the high speed imaging capability of FD-DOCT is attractive for real time *in vivo* monitoring of flow activity in biological samples as well as for flow segmentation in 3D that provides accurate information of flow angle [7].

One of the main challenges in conventional FD-OCT is the obscured object structure known as a mirror image or ghost image that arises from the Fourier transformation of a real function. Since the demand of high axial-resolution requires the employment of an extremely broadband light source, achieving high axial-resolution at high acquisition speed requires sacrificing spectral resolution that eventually leads to a reduction in the imaging depth range [13]. Therefore, the removal of the mirror image in high resolution FD-OCT is desirable to double the imaging depth range. In addition, it is evident that the performances of phase-resolved DOCT, such as Doppler phase stability and accuracy, highly rely on the signal-to-noise ratio (SNR) of the system [5,10,14]. Therefore, the ability to employ a maximum SNR out of a given phase-resolved DOCT system is desirable. Unlike conventional FD-OCT, full-range FD-OCT allows the use of the region around the zero-delay position, which is the most sensitive region in FD-OCT. Therefore, the combination of Doppler detection and full-range OCT has the promise of improving both structural and Doppler images.

Most full-range FD-OCT techniques reported to date share the basic principle of reconstructing a complex spectral interference signal from measurable real signals. The complex spectral interference can be expressed as $\tilde{F}(k) = A(k)\exp\{i\Psi(k)\}$, where $k = 2\pi/\lambda$ is the wave number, and $A(k)$ and $\Psi(k)$ are real functions representing the magnitude and phase of the complex spectral interference $\tilde{F}(k)$, respectively. The early attempts of full-range FD-OCT were based on sequential phase shifting methods, where multiple spectra with a certain phase relation were sequentially acquired and used to reconstruct the complex signal directly, such as the five-step [15], three-step [16], and two-step [17,18] phase shifting methods. The first two techniques directly determined the phase term $\Psi(k)$ of the spectral interference signal from a set of 3 to 5 acquired spectral interference signals. On the other hand, the third method measured the real and imaginary components of the complex spectral interference, (i.e. $\tilde{F}(k) = F(k) + iF(k, \Delta\Psi = \pi/2)$). In all cases, the sequential acquisition of multiple spectra for each axial line lead to a reduction in the frame acquisition speed. Furthermore, the approach is vulnerable to sample movement that occurs during the acquisition of those axial scans used to construct the complex signal. To overcome this limitation, several simultaneous detection schemes, such as the 3x3 coupler [19,20], polarization-based demodulation [21], and dual-detection [22] techniques were proposed. A different approach for retrieving the complex interference signal was based on Hilbert-transform methods such as the carrier frequency modulation [23] and BM-scan [24,25] methods. The Hilbert-transform based methods require no extra acquisition to reconstruct each frame of the full-range image and hence maintain the full acquisition speed of the FD-OCT. Moreover, the acquisition of BM-scan method was further improved by simply offsetting the sampling beam spot away from the pivot point of the scanning mirror to introduce the modulation frequency without additional hardware modification [26–28]. The proposed modulation technique simplifies the acquisition of BM-scan method. Nevertheless in order to obtain depth profiles, the methods require extra processing steps such as forward and backward Fourier transformations as well as band-pass filtering to reconstruct complex spectral interference signals prior to normal Fourier transformation [24].

The combination of full-range FD-OCT and phase-resolved Doppler imaging is challenging because in most cases, both full-range and Doppler capabilities rely on the phase relation between consecutive axial lines. A Hilbert-transform based full-range DOCT using the BM-scan method was demonstrated for imaging of the deep posterior of a human eye [29]. The technique introduced phase modulation during lateral scanning to produce a frequency shift after Fourier transform and then applied band-pass filtering to remove negative frequency components. However, certain amounts of axial movement cause additional frequency shifts in the transformed domain and could lead to unintentional signal loss after band-pass filtering. Therefore, the presence of high axial motion of the sample could affect mirror suppression performance and lead to a reduction in the detectable velocity range of Doppler imaging as compared to what can be achieved by the same system operated in conventional FD-OCT. Recently, a modified BM-scan method based on a parabolic phase modulation technique was proposed to minimize the effect of sample motion and improve the velocity dynamic range [30]. However, an increase in Doppler phase noise was observed.

A different approach to full-range DOCT was based on a time-frequency analysis technique built on a spectrometer-based FD-OCT system called joint spectral and time domain OCT [31]. Contrary to phase sensitive detection, the Doppler phase shift information was determined from the amplitudes of Fourier transformations. The Doppler image determined by the proposed technique was demonstrated to be less sensitive with respect to SNR and more accurate at close to maximum detectable velocity limit than that determined by phase-resolved techniques. Nevertheless, the full range signal was achieved by introducing change in the optical path length in the reference arm at a constant speed that caused a reduction in the detectable velocity dynamic range of the Doppler signal by half when operating in the full-range mode [32]. Moreover, the method employed a large number of

axial scans, for example 16-40 A-scans, and involved 2D Fourier transformation to determine a single line of velocity map that lead to an increase in both acquisition and processing time compare to phase resolved FD-DOCT.

Simultaneous phase shifting is promising for Doppler imaging, nevertheless no experimental confirmation has been reported to date. We recently reported a technique of mirror image removal called Dual-Detection FD-OCT, in which the quadrature components of a complex spectral interference were simultaneously detected [22]. Therefore, the full range signal was obtained without a loss in acquisition speed compared with the conventional FD-OCT. In addition, since the complex interference signal was constructed from two interference signals with a stable $\pi/2$ phase difference simultaneously detected by two independent detectors, any changes in optical path difference during acquisition equally affected the phase change in both detected signals without affecting the $\pi/2$ phase relation between them. Therefore, the mirror suppression performance of DD-FD-OCT was insensitive to sample motion, including large sample movements. One of the advantages of DD-FD-OCT to Doppler imaging is that the full-range signal is achieved without manipulation of the phase relation between consecutive axial lines. Therefore, the phase information of the full-range signal is almost identical to that acquired by the conventional FD-OCT method. Hence the full-range DD-FD-OCT is fully applicable to phase-resolved Doppler detection without reduction in detectable velocity dynamic range. In addition, phase-resolved DOCT can utilize the maximum SNR provided by the full-range capability (i.e. the 10 dB sensitivity fall-off range is doubled, and the most sensitive region around the zero path delay can be used).

In this paper, we report on an investigation of the implementation of DD-FD-OCT for phase-resolved Doppler imaging. Since the performance of phase-resolved DOCT highly depends on the SNR of the system, we have also developed an alternative scheme of DD-FD-OCT built on a combination of a fiber-based and free-space setup in a Mach-Zehnder interferometer configuration. The fiber part also adds flexibility to the system enabling integration with handheld [33,34] or endoscopic devices [35,36] while the free-space part provides a stable $\pi/2$ phase relation between the two detected spectral interference signals. To verify the preservation of the velocity dynamic range of phase-resolved DOCT when operating in the full-range DD-FD-OCT setup, the Doppler phase stability and the accuracy of the measured velocities up to the maximum velocity limit were quantified. The accuracy of the velocity measurement was validated through the measurement of the Doppler phase shift of a flow phantom with known flow velocity. The Doppler performance was compared to that achieved by the conventional FD-DOCT processed from the same set of acquired spectra. Finally, *in vivo* Doppler imaging of an African frog tadpole is demonstrated using the full-range DD-FD-OCT.

2. Experimental method

2.1 Fiber-based DD-FD-OCT

In principle, the dual detection technique is applicable to both spectrometer-based FD-OCT and swept-source-based FD-OCT. However, considering cost effectiveness, the swept-source system yields an attractive path since the cost of two photoreceivers is much lower than that of two spectrometers. In this paper, the technique was implemented in a swept-source-based FD-OCT built on a combination of fiber and free-space using a Mach-Zehnder interferometer (MZI) setup as shown in Fig. 1(a). The MZI configuration allowed maximum use of the limited source power as compared with the previously used free-space Michelson interferometer setup in [22]. The light source was a Fourier domain mode locking (FDML) frequency swept laser (Microns Optics) operating at 1320 nm center wavelength with a sweeping range of ~158 nm. The source sweep rate was ~44.6 kHz with 5.6 mW average output power. The output from the light source was coupled into a fiber system and then split by a 20/80, 1x2 fiber coupler. A 20% portion of the power was delivered to a reference arm, in which a Fourier domain optical delay line was implemented in order to compensate for the

overall dispersion [37]. Another portion of the beam was delivered to the sample arm consisting of a collimator, a galvanometer beam steering (VM500, GSI Lumonics), and a 20 mm effective focal length spherical lens. The lateral resolution was quantified through *en face* imaging of a 1951 USAF resolution target (Edmund Optics) as shown in Fig. 1(b). The lateral resolution of less than 15 μm was observed. Both sample and reference beams were then coupled back to the fiber system, delivered through fiber circulators and out-coupled through an adjustable focusers to a free space section.

Fig. 1. (a) Experimental setup of the fiber-based DD-FD-OCT. FC: fiber coupler, PC: polarization controller, BS: beam splitter, COL: collimator, M: mirror, GM: galvo mirror, MZI: Mach-Zehnder interferometer, OBJ: Objective lens, CIR: circulator, FOC: focuser, and BD: balanced detector. (b) An *en face* image acquired over about 4 mm x 4 mm field of view of a calibrated resolution target, where the number of line-pairs per millimeter (lp/mm) and the corresponding lateral resolution in parenthesis of several groups of the target are provided in the red boxes.

In the free-space section, the two beams were superimposed and split at the first beam splitter with a 50/50 split ratio. The interference signals in both paths were detected by two independent 80 MHz balanced photoreceivers (model 1817, New Focus). A $\pi/2$ precise and stable phase relation between the two detected signals was achieved through a slight difference in the alignment of the two detection paths in the free space system so that the two optical path length differences were different by the amount of about a quarter of the center wavelength of the light source [22]. The two detected interference signals were then digitized on each channel of a two-channel, high-speed, 12-bit-resolution analog-to-digital converter operating at 200 Msamples/s (NI PCI 5124, National Instrument). The detected spectra were calibrated to be linear in frequency-space prior to taking a fast Fourier transform (FFT) using the time-frequency relation generated from the position of peaks, valleys, and zero-crossing of an interference signal measured by an additional MZI recorded on another 8-bit-resolution analog-to-digital converter operating at 250 Msamples/s (NI PCI 5114, National Instrument). The spectral interference signals and the MZI calibration signal were measured simultaneously, and the calibration process was done in software [38].

The example of two acquired spectra with quadratic phase relation, when using a single reflector as a sample, is illustrated in Fig. 2(a). The two spectra were almost the same except for a $\pi/2$ phase difference between them. The suppression ratio, which is the ratio between the amplitudes of a signal peak and its mirror counterpart, of about 40 dB [Fig. 2(b)] was observed over a long period of operation. It should be pointed out that, in order to get maximum suppression performance, the magnitude of the two spectra should be ~equal within 2% maximum difference. In this experiment, the 40 dB suppression shown in Fig. 2(b) was achieved by monitoring the plot similar to that in Fig. 2(b), while adjusting the alignment in the free-space section. Once the maximum suppression was achieved, the magnitudes of the two signals were also well matched, unlike that shown in Fig. 2(a). Considering the setup in

Fig. 1, since the two spectra were acquired simultaneously, any movement that occurred in either the reference or the sample arms or both prior to the free-space section equally contributed to the frequency shift in both acquired spectra, and, therefore, did not affect the $\pi/2$ phase relation between them. Furthermore, the full-range signal was achieved without manipulating the phase relation between consecutive axial scans. This capability allowed the ease of implementation of the DD-FD-OCT in phase-resolved Doppler imaging with equivalent velocity dynamic range to the conventional FD-DOCT.

Fig. 2. (Color online) (a) Two typical spectra with quadratic phase relation simultaneously acquired by the dual detection system when imaging a single reflector; (b) A depth profile demonstrates the suppression performance of the DD-FD-OCT that corresponds to a case of matching amplitudes (unlike that shown in (a)) of the signals shown in (a) within 2%.

2.2 Doppler imaging method

Phase-resolved DOCT relies on the accuracy and stability of the detection of the phase difference between points at the same depth and same lateral position of two consecutive axial scans. Knowing the phase difference, the flow velocity was estimated as

$$V(z) = \frac{\lambda_0 \Delta\phi(z)}{4\pi T n \cos \theta}, \quad (1)$$

where z denotes the axial position, λ_0 is the central wavelength of the source, T is a time interval between the two points used to calculate the phase difference $\Delta\phi(z)$, n is the average sample refractive index, and θ is the angle of the flow direction relative to the propagation axis of the illumination beam [10]. In practice, the calculation of the phase difference $\Delta\phi$ involves the inverse tangential function, and hence exhibits a π phase ambiguity. Moreover, the presence of phase noise imposes a challenge in phase unwrapping of the OCT signal, and could lead to misinterpretation. Therefore, without phase unwrapping, the detectable phase shift of π is maximum, and the maximum detectable *axial velocity* (corresponding to θ equal zero) is given by

$$V_{a,\max}(z) = \frac{\lambda_0}{4Tn}. \quad (2)$$

In our system, the FDML laser was capable of 44.6 kHz sweep rate providing up to 89,200 spectra per second. At the maximum sweep rate of the source, the time interval between two consecutive spectra was 11.2 μ s, corresponding to a maximum detectable axial velocity of ~ 22 mm/s. However, to avoid complexity in data processing and accommodate for real time processing and display, the backward sweep signals were omitted and only forward sweep signals were used in the Doppler phase shift calculation. Therefore, the time interval between two consecutive forward spectra was 22.4 μ s, corresponding to a theoretical maximum detectable axial velocity of about 11 mm/s. Furthermore, we designed a block acquisition scheme for swept-source-based FD-DOCT, where a group of axial profiles used to calculate the Doppler phase shift was acquired at exactly the same lateral position. The technique minimized the effect of lateral scanning to the Doppler phase error [39]. Specifically, the number of sampling points was set so that multiple spectra consisting of both forward and

backward sweep signals were recorded at each lateral position of the sample beam. The block of acquired signal was chopped into M sub-sections containing one forward spectrum per section. Each chopped signal contained 2000 sampled points. After calibration to a linear frequency domain by using the calibration curve obtained by the method previously described in section 2.1, the number of sampling points per spectrum was approximately 1000 points. In order to increase the sampling resolution in the depth profile, a FFT was performed with zero padding to 2048 points. Using the algorithm in [40], the phase shift was then calculated using

$$\Delta\varphi(z) = \tan^{-1} \frac{\sum_{m=1}^{M-1} \text{Im} \{ I_m^*(z) \cdot I_{m+1}(z) \}}{\sum_{m=1}^{M-1} \text{Re} \{ I_m^*(z) \cdot I_{m+1}(z) \}}, \quad (3)$$

where z represents an axial position, $I_m(z)$ is a complex signal achieved from inverse Fourier transform of the m^{th} detected spectral interference, and $I_m^*(z)$ denotes a complex conjugate signal of $I_m(z)$. Using this formalism, the measured phase shift was averaged over the measurements. Therefore, increasing M improves the quality of the detected Doppler phase shift at the cost of a reduction in imaging speed. The influence of the parameter M to the Doppler imaging performance was investigated and will be discussed in section 3.

Two scanning protocols were used in this paper, namely B-mode and M-mode Doppler imaging. In B-mode Doppler imaging, the step-wise function of voltage was applied to the galvo-mirror. At each position of the galvo-mirror, a block of data containing M forward spectra were acquired, and then the galvo-mirror was moved to the next position and another set of spectra was acquired. The process was repeated until the desired amount of lateral pixels per image was achieved. Throughout this paper, a sampling interval of 10 μm was used, (i.e. 200 lateral pixels correspond to a 2 mm physical dimension). The B-mode Doppler was useful for locating the flow.

In M-mode Doppler imaging, the lateral beam position was fixed at a desired lateral position corresponding to the center of the flow location first determined by the B-mode operation. A constant voltage was applied to the galvo-mirror. Without moving the sample beam, a block of data containing M forward spectra was acquired. The acquisition was repeated until the desired amount of lateral pixels per image was achieved. The lateral dimension of the M-mode Doppler image provided information about flow characteristics over time. In M-mode acquisition, the time interval between two consecutive lateral pixels was approximately 2 ms. Therefore, an M-mode Doppler image consisting of 200 lateral pixels represents a depth-resolved flow profile over a 400 ms time window. The M-mode Doppler was used in flow measurement verification in section 3.3.

2.3 Flow phantom and pumping system

A flow phantom is illustrated in Fig. 3. The flow speed was set by a computer controlled motorized translation stage (DCX-PCI 100 Controller, Precision MicroControl Corp.) with a resolution of ~ 17 nm per one revolution of the motor head. A syringe ejected 200 mm^3 of contained fluid for every 1 mm translation of the stage. Therefore, knowing the translation speed of the motorized stage, the corresponding total flow rate of the system was determined. By assuming a laminar flow profile, the peak flow velocity (v_m) was estimated through the relation $v_m = 2F/(\text{Flow area})$, where F is the flow rate of the system [41]. A capillary tube was tilted at a fixed angle of 15.12° with respect to the horizontal level as shown in Fig. 3. This produced the angle between the incident beam and the flow direction of 74.88° at the outer surface of the tube, and hence 78.86° at the flow sample after accounting for refraction in the medium ($n = \sim 1.35$) when the illumination beam was perfectly aligned to the vertical direction. This flow angle was chosen to accommodate for the translation range and the maximum speed of the motorized stage so that the flow speed of up to the maximum detectable limit could be observed.

Fig. 3. Illustration of the flow phantom and pumping system.

3. Results and discussion

3.1 Phase stability of the system

The minimum detectable velocity was determined by the phase stability of the system that could be quantified from the temporal fluctuation of the Doppler phase shift ($\Delta\phi_{err}$) while imaging a static structure that ideally should yield zero speed. In practice, there was a small deviation of the phase difference even without the presence of moving scatterers. This served as a theoretical limit in velocity sensitivity for each particular DOCT system. Sequentially, the minimum detectable axial velocity was determined by [10,12]

$$V_{a,min} = \frac{\lambda_0 \Delta\phi_{err}}{4\pi Tn} . \quad (4)$$

We quantified the minimum detectable axial velocity in two scenarios: with lateral scanning (B-mode Doppler) and without lateral scanning (M-mode Doppler). Under each imaging scenario, the stability of the Doppler phase shift was monitored over time when using a fixed mirror as a sample [5,10,12]. In this measurement, the SNR was set to be sufficiently high (i.e. > 60 dB) so that the Doppler phase error was solely dominated by the phase stability of the system as set by, for example, the swept source, the interferometer, the scanning mechanics, and the signal processing. In this experiment, the SNR was defined by $20 \log \left[(S - \mu_{noise}) / \sigma_{noise} \right]$, where S was the signal peak amplitude at the position of the mirror surface averaged across the full lateral dimension of the acquired Doppler image and μ_{noise} and σ_{noise} were the mean and standard deviation of the noise floor measured within the region around the signal peak while the sample beam was blocked, respectively [5]. The measured phase stability under this condition therefore represented the characteristics and performance of the DOCT system. The Doppler phase error was quantified as follow. For each acquisition frame of the Doppler image, the phase shift was averaged over a certain depth range (5 pixels) around the signal peak corresponding with the position of the mirror surface, and then averaged across the full lateral dimension (200 pixels or equivalently 200 positions of the sample beam scanning). The measurement was repeated for 500 frames in both cases. In the literature, two methods of $\Delta\phi_{err}$ quantification were used by calculating a standard deviation (σ) [12] and/or a full width at half maximum (FWHM) of the histogram distribution of the measured Doppler phase shift [5]. Since the two methods yield results that differ by a significant order of magnitude, in this paper, both the standard deviation and the FWHM of the histogram of the phase shift errors were calculated and compared. Moreover, the Doppler phase stabilities at different values of M in Eq. (3) were investigated.

The histogram plots of the measured Doppler phase errors are shown in Fig. 4. The left (a,c) and right (b,d) columns are histograms of phase shift errors when operating in a

conventional FD-DOCT and full-range Doppler imaging using DD-FD-OCT, respectively. To investigate the effect of lateral scanning to the Doppler phase stability, the measurements were performed in both B-mode (a,b) and M-mode (c,d) Doppler imaging. In addition, the minimum detectable axial velocity was calculated by using Eq. (4) as shown in Table 1.

Fig. 4. (Color online) Histogram distributions of measured Doppler phase errors where left (a,c) and right (b,d) columns are corresponding with the measurement data taken with conventional FD-OCT and DD-FD-OCT, and top (a,b) and bottom (c,d) rows are corresponding with B-mode and M-mode operations, respectively; The measurements were conducted at different values of M . The filled square markers and the lines represent measurement values and its Gaussian fit, respectively.

Table 1. The minimum detectable axial velocity at various M values, where the top (without parenthesis) and bottom (in parenthesis) values in each cell corresponded to B-mode and M-mode Doppler imaging, respectively

M	2		3		4		5	
	σ	FWHM	σ	FWHM	σ	FWHM	σ	FWHM
Conventional FD-OCT								
$\Delta\varphi_{err}$ (mrad)	1.04 (0.88)	2.29 (2.11)	0.76 (0.66)	1.75 (1.56)	0.69 (0.59)	1.63 (1.36)	0.61 (0.48)	1.41 (1.13)
$V_{a,min}$ ($\mu\text{m/s}$)	3.62 (3.07)	7.98 (7.35)	2.65 (2.30)	6.10 (5.44)	2.40 (2.06)	5.68 (4.74)	2.13 (1.67)	4.91 (3.94)
Full-range DD-FD-OCT								
$\Delta\varphi_{err}$ (mrad)	0.97 (0.88)	2.33 (1.98)	0.75 (0.63)	1.76 (1.46)	0.69 (0.55)	1.63 (1.28)	0.61 (0.47)	1.40 (1.09)
$V_{a,min}$ ($\mu\text{m/s}$)	3.38 (3.07)	8.12 (6.90)	2.61 (2.20)	6.13 (5.09)	2.40 (1.92)	5.68 (4.46)	2.13 (1.64)	4.88 (3.80)

The results show that the implemented DOCT system exhibits high Doppler phase stability (i.e. the FWHM Doppler phase error between two consecutive axial lines was less than 3 milliradians (mrad) in all cases). Furthermore, the results quantify the improvement in the Doppler phase stability and hence the minimum detectable velocity as a function of M , which was the number of spectra used to calculate the Doppler phase shift. In addition, the phase error was slightly increased when operating in B-mode Doppler compared with the M-mode Doppler demonstrating the effect of scanning mechanics to the system phase stability. In general, one may expect a significant difference in phase errors between M-mode and B-mode operations. In this experiment, the phase errors were only slightly different since the acquisition scheme was designed in the way that all spectra used to determine Doppler phase shift were acquired at the same lateral position and hence the effect of transverse motion was suppressed. In contrary to the B-mode in our experiment, where we acquired two spectra from the same lateral position, in other phase-resolved DOCT techniques where instead raster

scanning is used, the phase estimation is subject to severe degradation in stability unless the ratio between the sample beam width and the lateral sampling interval is sufficiently high. Indeed in raster scanning, the transverse motion of the sample beam during the acquisition period will introduce an additional phase noise in phase-resolved DOCT [14,39]. The block acquisition scheme utilized in this paper does not require oversampling to achieve high phase stability, nevertheless it may be subject to limitation in frame acquisition rate due to the mechanical response of the scanning device and the data readout rate of the detector. Depending on different applications, this trade-off between frame rate and Doppler phase stability should be taken into account when designing a phase-resolved DOCT system.

Moreover, it can be observed from Table 1 that the phase stability in the conventional FD-OCT and DD-FD-OCT were almost the same verifying that the phase stability was not affected by the full-range operation in DD-FD-OCT. It should be pointed out that both full-range and conventional results were calculated from the same set of acquired spectra with and without full-range enabled, respectively. Finally, the phase stability quantified by the two methods, the standard deviation and FWHM, were different by a factor of about two. Therefore, the quantification of Doppler sensitivity by using the standard deviation may have led to an overestimation. The FWHM method should be considered to represent the minimum detectable velocity as was suggested in [5]. The FWHM can be approximated by $2\sqrt{2\ln 2}\sigma$ or 2.3548σ if the measurement data exhibits a Gaussian distribution [42].

3.2 Phase stability in the presence of noise

In the previous experiment, the phase stabilities were measured based on high SNR condition where the noise effect was negligible. Therefore, the phase error was dominated by the system phase error. Under this circumstance, both conventional FD-OCT and full-range DD-FD-OCT exhibited the same phase stability performance. However in the presence of noise, as encountered when imaging biological samples, the phase stability degraded as a function of SNR [5,10,14]. In this experiment, the Doppler phase stability was measured in B-mode Doppler imaging by using stationary diluted milk as a sample. To demonstrate the effect of SNR fall-off to Doppler phase stability of the system, average Doppler phase errors were measured at different locations of the sample relative to the zero-delay position as shown in Fig. 5. The position of the sample relative to the zero-delay position was adjusted by changing the optical path length in the reference arm without any modification of the sample arm. The phase error in each case was averaged over the region of interest (ROI) marked by the white dash box in Fig. 5 with 250 pixels axially and 100 pixels laterally.

The average SNR measured within the ROI was about 20 dB as calculated over the ROI by using the same formula as in section 3.1, where S was the average signal amplitude over the ROI and μ_{noise} and σ_{noise} were the mean and standard deviation of the noise floor measured within the ROI while the sample beam was blocked, respectively. In each scenario, the Doppler phase shift was measured with M equal 5, and the standard deviation σ was calculated from 200 measurements. The phase error was then determined as 2.36σ , assuming a Gaussian distribution. The first scenario, where the zero-delay position was placed below the sample surface that is only available with full-range imaging, provided best phase stability since the SNR was maximum at the zero-delay position in FD-OCT. The phase error at around the zero-delay position measured at averaged SNR of 20 dB was approximately 10 mrad corresponding to a minimum detectable velocity of about 34 $\mu\text{m/s}$. This is about a five times degradation from the case of static mirror measurement. The phase stability degraded as the sample surface was placed further away from the zero-delay position due to the effect of sensitivity fall-off as a function of depth as shown in Fig. 6. The SNR was measured in air by using a mirror as a sample similar to that previously detailed in section 3.1. One can observe that the SNR quickly drops at depth beyond 0.6 mm. The 10 dB SNR fall-off distance was at around 1.4 mm in air corresponding to about 1 mm in tissue.

Fig. 5. (Color online) Average phase errors measured from stationary diluted milk when the sample surface was placed at different depth positions by changing the optical path length in the reference, where the white dash box indicates the area that the phase error was averaged over in each case, and the orange dash line denotes the zero-delay position.

Fig. 6. (Color online) Measured SNR (in air) of the system as a function of depth.

3.3 Flow measurement verification

To validate the flow velocity detection capability of the full-range DD-FD-OCT as compared with the conventional FD-OCT, we imaged a flow phantom that was diluted milk pumped through a capillary tube of $\sim 770 \mu\text{m}$ inner diameter by using the pumping system described in section 2.3. Figure 7(a) and 7(d) show cross-sectional intensity images of the flow phantom operated at a flow speed of about 44 mm/s acquired by the conventional FD-OCT and the DD-FD-OCT, respectively. Results demonstrate that the mirror-image removal performance of the full-range DD-FD-OCT was not affected by the sample movement caused by the flow activity. Corresponding with the intensity images in Fig. 7(a) and 7(d), 2D color maps of B-mode Doppler phase shift detected by conventional FD-OCT and the full range DD-FD-OCT are shown in Fig. 7(b) and 7(e), respectively.

The Doppler phase shift is displayed using a color map, where the amounts of Doppler phase shift of $-\pi$, $-\pi/2$, 0, $\pi/2$, and π were mapped to yellow, red, black, blue, and light blue, respectively. In conventional FD-OCT, because of the conjugate relation, the mirror image exhibits the same amount of Doppler phase shift, but with opposite sign, compared with its counterpart corresponding to areas appearing in red and blue colors in Fig. 7(b), respectively. The mirror Doppler signal is successfully invisible in the full range DD-FD-OCT as shown in Fig. 7(e). Furthermore, Doppler phase shifts at various set flow speeds of the flow phantom were measured. The phase shift was determined by using Eq. (3) with M equal 5. The flow angle was set at approximately 78.86 degree after accounting for the refraction of the beam. Since the incident angle was quite wide, the actual illumination power at the sample was dramatically decreased due to the strong reflection at the outer surface of the capillary tube based on Fresnel reflection. This led to the presence of random phase for the background noise over the flow cross-sectional area. To minimize this effect and achieve a smooth flow profile, a 7 points one dimensional median filter was applied to every axial line of the M-

mode Doppler image. The median filter is an efficient method for removing salt-and-pepper noise while minimally altering neighboring pixels [43].

Fig. 7. (Color online) (a) and (d) are intensity images, (b) and (e) are B-mode Doppler images, and (c) and (f) are M-mode Doppler images measured by the conventional FD-OCT and the DD-FD-OCT, respectively, where yellow horizontal dash lines indicate the zero path delay position, a white vertical dash line indicates the lateral position where the M-mode Doppler was operated, and a white solid line at the bottom right of (d) denotes a scale bar that is applied for all images (a-f).

At each set flow velocity, two Doppler images determined by conventional FD-OCT and full-range DD-FD-OCT were calculated from the same set of acquired spectra. All Doppler images acquired with conventional FD-OCT were established from the signal from one of the two detectors since the measured SNR performances of the 1st and 2nd detection systems as well as the full-range system were almost identical given that the difference was less than 1 dB. Therefore, Doppler images obtained by both systems exhibit similar Doppler sensitivity. We decided that it is most important to compare the performances of the conventional FD-OCT and full-range DD-FD-OCT by using the same set of data to ensure that they were measured in exactly the same conditions (i.e. same lateral position of the sample beam and same condition of the flow sample). To measure the peak flow velocity, the center of the flow area, marked as a white dash line in Fig. 7(b) and 7(e), was determined from a B-mode Doppler image [Fig. 7(a)]. Then the lateral position was fixed at that position and an M-mode Doppler image [Fig. 7(c) and 7(f)] was taken. Each M-mode Doppler image consisted of 800 pixels along the depth axis (vertical axis) centered at zero-delay position as indicated by yellow dash lines in Fig. 7 and 200 pixels along the time axis (horizontal axis). The M-mode Doppler color maps corresponding with various flow velocities set by the pump from no flow to 59 mm/s was demonstrated in Fig. 8, where negative and positive velocities represent flow in opposite direction. When operating at a theoretical maximum detectable axial velocity of about 11 mm/s as justified earlier, a maximum absolute flow speed of about 57 mm/s is achievable at the flow angle of 78.86° .

Fig. 8. (Color online) M-mode Doppler images calculated from the full-range signal at various flow velocities set by the pump.

Sequentially, the peak flow velocity from each acquired M-mode Doppler image was determined. The phase shift was first averaged along the time axis yielding an averaged phase-depth profile, and then averaged from 5 pixels around the peak of the profile. To minimize the effect over time of slow fluctuation of the flow speed intrinsic to the characteristic of the pump, the measurement was repeated for 200 frames of M-mode Doppler images acquired at 2 fps. This frame rate provided measurement data that included several cycles of the slow flow fluctuation over time. The mean and standard deviation were computed representing a Doppler phase shift at the peak of the flow profile for each set flow speed of the flow phantom. Finally, the measured peak velocities were calculated and compared to those estimated from the pump's parameters. The absolute flow velocities were determined by using Eq. (1). Using the system's parameters that were $\lambda_0 = 1320$ nm, $T = 22.4$ μ s, $\theta = 78.86$ degree, and $n = \sim 1.35$, Doppler velocity corresponding to each set velocity of the pump was calculated and compared as shown in Fig. 9.

Fig. 9. Plots between the measured velocity at the peak of the flow profile and the set flow velocity measured by the conventional FD-OCT (left) and the full-range DD-FD-OCT (right). 200 measurements were performed at each set flow velocity. Each data point corresponds to a mean value, and the size of the error bar at each measurement point represents the FWHM of the distribution of the measured Doppler phase shift estimated by 2.36σ assuming a Gaussian distribution.

From Table 1, at M equal 5, the measured Doppler phase error was ~ 1 mrad, and the minimum detectable axial velocity was computed to be ~ 4 μ m/s corresponding to an absolute flow speed of 21 μ m/s at 78.86 degree. In this measurement, the average SNR at around peak flow location was about 15 dB. When imaging such a weak backscattering sample, the presence of noise degraded the Doppler phase sensitivity to about 24 mrad (FWHM) that corresponded with the minimum detectable absolute flow velocity of about 0.45 mm/s at the flow angle of 78.86 degree in both cases of the conventional and the full-range operations as determined by 2.36σ of the measurement data at no flow (see Fig. 9). Furthermore, as the flow speed was increased, the standard deviation of the measured Doppler phase was further broadened particularly at high flow velocity from 30 to 59 mm/s. This broadening was induced by flow fluctuation caused by the pump especially when traveling over a long distance of the motorized stage. This small fluctuation was intrinsic to the characteristic of the pump and hence affected both the conventional and the full-range measurements by almost the same amount as shown in Fig. 9. Nevertheless, this fluctuation tended to occur in cycles and therefore the measured flow speed was still acceptable compared to the set flow speed after averaging over a long period of time except for the last measurement at ~ 59 mm/s, which

is beyond the maximum detectable velocity of the system as limited by the π phase ambiguity. At the set flow speed of ~ 59 mm/s, the fluctuation tended to occasionally cross the π phase ambiguity boundary, which corresponded to an absolute flow velocity of ~ 57 mm/s, hence suffered from phase wrapping and could not provide a correct measurement as can be observed in both plots in Fig. 9.

Finally, we demonstrate Doppler imaging of blood flow inside the heart of an African frog tadpole (*Xenopus Laevis*) using the full-range DD-FD-OCT system. Two intensity images of the tadpole heart with both the conventional FD-OCT and the full-range DD-FD-OCT processed from the same set of acquired spectra are shown in Fig. 10(a) and 10(b), respectively. The flow activity can be observed in the corresponding Doppler maps as shown in Fig. 10(c) and 10(d), where the mirror Doppler is completely suppressed in the full-range mode [Fig. 10(d)] as compared with that processed in the conventional mode [Fig. 10(c)]. The flow activity was observed at the location indicated by a white arrow in Fig. 10(b). With the full-range capability, one can place the region of interest close to the zero-delay position and utilize the high SNR around this region to improve Doppler sensitivity.

Fig. 10. (Color online) (a) and (b) are intensity images and (c) and (d) are corresponding Doppler images of the heart of an African frog tadpole processed with and without full-range enabled, respectively. A white arrow in (b) indicates location of the flow activity displayed in (d).

4. Summary

In summary, we have developed a full range DD-FD-OCT system to accommodate for Doppler imaging by using a combination of free-space and fiber-based in an MZI configuration with the benefits of flexibility, power efficiency, easy alignment, and stable full-range performance. We demonstrated the feasibility of the full-range DD-FD-OCT method to achieve Doppler detection without degradation of the mirror image suppression performance and without any loss in velocity dynamic range compared with the conventional FD-OCT. The Doppler phase stability was quantified and compared with that achieved by the conventional method. In addition, the Doppler phase shifts corresponding to different flow velocities produced by the flow phantom were measured and verified. The system was implemented in a swept-source based FD-OCT capable of an acquisition speed of up to 22,000 A-lines/s for Doppler imaging. In addition, we have designed a block acquisition technique for swept source based FD-OCT that allows Doppler determination at precisely the same lateral position, and hence provide high Doppler phase stability. Finally, the Doppler image of blood flow inside the heart chambers of an African frog tadpole using the full-range DD-FD-OCT was demonstrated. The full-range capability allows for the placement of the

flow region close to the zero-delay position, and hence improves flow visibility and sensitivity that is particularly useful for imaging of weak scattering sample such as biological samples.

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