Published in final edited form as:

J Biophotonics. 2017 November ; 10(11): 1398–1410. doi:10.1002/jbio.201700034.

Robust numerical phase stabilization for long-range swept-source optical coherence tomography

Shaozhen Song¹, Jingjiang Xu¹, Shaojie Men¹, Tueng T. Shen^{1,2}, and Ruikang K. Wang^{1,2}

¹University of Washington, Department of Bioengineering, Seattle, WA 98195, USA

²University of Washington, Department of Ophthalmology, Seattle, WA 98195, USA

Abstract

A novel phase stabilization technique is demonstrated with significant improvement in the phase stability of a micro-electromechanical (MEMS) vertical cavity surface-emitting laser (VCSEL) based swept-source optical coherence tomography (SS-OCT) system. Without any requirements of hardware modifications, the new fully numerical phase stabilization technique features high tolerance to acquisition jitter, and significantly reduced budget in computational effort. We demonstrate that when measured with biological tissue, this technique enables a phase sensitivity of 89 mrad in highly scattering tissue, with image ranging distance of up to 12.5 mm at A-line scan rate of 100.3 kHz. We further compare the performances delivered by the phase-stabilization approach with conventional numerical approach for accuracy and computational efficiency. Imaging result of complex signal-based optical coherence tomography angiography (OCTA) and Doppler OCTA indicate that the proposed phase stabilization technique is robust, and efficient in improving the image contrast-to-noise ratio and extending OCTA depth range. The proposed technique can be universally applied to improve phase-stability in generic SS-OCT with different scale of scan rates without a need for special treatment.

Graphical Abstract

Keywords

optical coherence tomography; phase-sensitive measurement; swept laser

1. Introduction

Optical coherence tomography (OCT) is a non-invasive, depth-resolved optical imaging modality with high sensitivity and micron-scale resolution [1,2]. Its balance between spatial resolution and penetration depth fills nicely in the gap between confocal microscopy and medical ultrasound sonography, and widely accepted for the structural and functional imaging of various types of biological tissues [3]. During the past decade, the applications of frequency-domain ranging techniques/Fourier-domain OCT (FD-OCT) has made significant improvements in terms of its system sensitivity [4–6] and imaging speed [7]. Moreover, with the inherit advantages of high-speed and solid-state detection, FD-OCT has offered a number of new opportunities, for example label-free functional imaging including angiography [8,9], Doppler velocimetry [10–12], vibrometry [13], elastography [14,15], and birefringent measurement [16]. There are two implementations of FD-OCT by far: 1) a broadband light source with a spectrometer detection is used to spatially resolve the spectral interferograms, termed as Spectral Domain OCT (SD-OCT), and 2) a tunable wavelength-swept light source with a single photo-detector is used to acquire the same spectral interference signal in time domain, which is termed as swept-source OCT (SS-OCT) or optical frequency domain imaging. Due to its outstanding performance of sensitivity roll-off along ranging distance [4,17], easily achievable longer ranging distance [18] and higher imaging speed [19], SS-OCT is gradually becoming the dominant configuration for FD-OCT in experimental research, especially in the applications that requires extended ranging distance [20]. State-of-the-art high-speed SS-OCT based on widely tunable swept vertical-

cavity surface-emitting laser (VCSEL) has even reached a capability of meter-range detection [21], due to its single mode and narrow linewidth emission. VCSEL-SSOCT has been successfully demonstrated in various applications such as full eye imaging [22] and dermatology imaging [23].

While SD-OCT is inherently phase stable since all the spectral sampling points are obtained simultaneously by a line-scan camera, it is generally difficult for SS-OCT to achieve phase-sensitive detection due to two reasons: 1) limitations in wavelength-sweeping repeatability [24] and 2) potential jitter errors in acquiring spectral interferograms [25–27]. Degraded phase-stability of SS-OCT has been the key issue that limits the aforementioned functional imaging applications such as Doppler velocimetry, vibrometry and elastography. In OCT based angiography (OCTA), complex signal based algorithms such as OMAG [28] and phase-variance OCT (PV-OCT) [29] are also proved to deliver superior image quality [30,31] to that of intensity-only algorithms.

A number of phase-stabilization techniques have been reported in the literature in order to enable SS-OCT for functional imaging where phase-sensitive detection is needed. A most common practice to phase-stabilize a SS-OCT is to introduce a calibration mirror signal, upon which to measure the phase error and then to compensate it in the measured signal [25,32]. A more robust approach used two calibration mirror signals so that relatively larger phase error, even with the presence of phase-wrapping, can be coped with [26]. Another hardware-based approach used a Mach-Zehnder interferometer (MZI)-based optical clock (MZI-OC) signal recorded by a separate ADC channel, which can not only generate the equally spaced optical frequency space sampling [33], but also provide the jitter amplitude [34] to compensate for jitter-induced phase error. A recent work introduced a fixed wave-number reference signal in the interferogram, so that the wavelength sweeps can be re-aligned in post-processing [27,35], with a considerable amount of computational cost [35]. Common-path configurations are also reported in literature for phase-stabilization [36]. While the aforementioned techniques demonstrated excellent phase stability and competitive depth ranging distance, they all require additional hardware to implement, making the system more complex, which is often not desirable for real time in vivo applications, particularly when the purpose of the system development is for clinical translation. An impressive work has demonstrated a numerical OCT phase-stabilization method on a 1- μm wavelength SS-OCT [37], however, modifications to the reference beam is still required to provide a common-mode reference signal, which disqualifies it as a fully numerical approach. Moreover, the computational efficiency of this method is challenging since it requires weighted linear-fitting.

In this study, a novel fully numerical technique is proposed and demonstrated with significant improvement in the phase stability of a micro-electromechanical (MEMS) vertical cavity surface-emitting laser (VCSEL) based swept-source optical coherence tomography (SS-OCT) system. Without any requirement of hardware modifications, this numerical phase stabilization technique features excellent tolerance to acquisition jitter and without a significant increase of computing budget. We show that the phase-stabilized SSOCT has a measured phase sensitivity of 89 mrad in biological tissue with image ranging distance of up to 12.5 mm at A-line scan rate of 100.3 kHz. We also compare its imaging

performance with conventional numerical approach for accuracy and computational efficiency.

2. Methods

2.1 Hardware setup of SS-OCT system

Figure 1 illustrates the experimental setup of the phase-stabilized SS-OCT system. The light source we employed was a MEMS-VCSEL swept source (SL1310 V1-10048, Thorlabs, Inc.), which operated at 100.3 kHz swept rate with 1305 nm central wavelength and 100 nm spectrum bandwidth. A-line trigger, i.e. wavelength sweep trigger was supplied by the laser source. K-linear sampling clock was provided by the Mach-Zehnder interferometer-based clock module integrated within the laser source. More details of the MZI clock can be found in [38]. We implemented a fiber-based Michelson interferometer by launching the light into a wideband 50:50 coupler. A pair of XY galvanometric mirrors (GVS002, Thorlabs, Inc.) were synchronized with the swept source and controlled by a 12-bit high-speed analog output board (National Instruments, PCI-6713, not shown in Figure 1). An OCT-optimized scanning microscopy lens (Thorlabs, LSM03) was used in the sample arm, with effective focal length of 36 mm, providing a lateral resolution of ~ 19 μm . An adjustable free-space reference arm was used to match the optical delay with sample arm. The backscattered light from the two arms were combined by the 50:50 coupler, and then delivered to a high-speed dual-balanced photodetector with 1.6 GHz bandwidth (PDB480C-AC, Thorlabs, Inc.). A high-speed digitizer (ATS9360, AlazarTech, Inc.) was used to detect the OCT interference signal. The raw OCT data was transmitted to a host computer (Intel Xeon E5-2630v3) through 8-lane PCI-Express Gen2 interface. OCT system control was implemented on LabVIEWTM platform to automatically control all the operations including system calibration, galvo-scanning, system synchronization, real-time imaging preview and data acquisition.

When imaging, the power of the incident light on the sample was 5 mW, giving a measured sensitivity of ~ 105 dB. Benefiting from the excellent instantaneous line width of VCSEL source, the OCT system is proven to possess low sensitivity roll-off. The -3 dB OCT signal roll-off distance was measured to be 7 mm in air.

Note that the hardware setup of this SS-OCT system is similar to commercially available Thorlabs SS-OCT systems, but without any requirement for extra hardware for phase-stabilization. Note that the phase stabilization algorithm proposed below is not only applicable to the SS-OCT system implemented with Thorlabs MEMS-VCSEL source, but also effective for generic swept source OCT systems.

2.2 Characterization of phase signal

There are a number of OCTA algorithms that rely on the phase [29,39,40], or complex signal [28,41–43] variations to achieve angiography of scanned tissue, which have been reported to be more sensitive than intensity-based [44–47] OCTA algorithms. However, there requires some special considerations in the use of complex signal-based algorithms because there are multiple sources that could cause phase changes in the OCT signal. The resultant phase

signal detected by SS-OCT is a composition of phase changes from all the origins including blood flow, tissue bulk motion, and synchronization jitter error. In order to optimize the image quality of OCTA, it is necessary to decompose the detected phase signal in order to eliminate the contributions of phase changes from other origins, leaving only the useful signals induced by blood flow. The following section serves as an analysis of the characteristics of phase changes caused by different origins.

Figure 2 is an illustration of the sources of phase shifts in SS-OCT phase sensitive imaging. It should be noted that since the phase difference (phase shift) signal are usually analyzed for phase-stabilization purposes, conventionally the term “phase” is used for short of “phase difference”. First of all, Doppler effect is a fundamental source of phase shifts. Doppler phase induced by motion can be described as: $\phi_{\text{dop}}(z) = 2k \cdot T \cdot v(z)$, where k is the wavenumber and T is the time interval between two axial scans [11]. Consider an axial scan that contains a functional blood vessel, the phase signal inside the blood vessel would present as a parabolic shape bump, while static tissue should present zero Doppler signal, as shown in Figure 2(b), where magenta line indicates blood flow. In practice, the targeted sample would not be perfectly static due to environmental vibrations and involuntarily motions of the subject. Hence, the bulk motion of tissue would add a constant offset along an axial scan, as illustrated with green line in Figure 2(b).

Unlike Doppler-induced phase shifts, the phase shifts induced by the jitter error in SS-OCT spectrum acquisition is independent of sample motion. However, it is dependent on jitter amplitude and depth position. As an intuitive example shown in Figure 2(a), a scatterer at depth ζ would appear as the interferometric signal with a frequency f , and another scatterer at depth 2ζ gives a frequency $2f$. With a given synchronization time jitter of σ , the resultant phase shifts on the two scatterers are linearly proportional to the interference signal frequency, i.e. depth position of the scatterer.

Consider two spectral interferograms, $S_1(k)$ and $S_2(k)$, are repeatedly acquired at a spatial location, where k stands for wavenumber. $S_1(k)$ and $S_2(k)$ would appear as two identical copies of interference signals except that S_2 has a time delay (i.e. a k space shift of σ) relative to S_1 . Here we have $S_2(k-\sigma) = S_1(k)$. According to the translate/time-shifting property of Fourier transform⁴⁸, The Fourier transform of S_2 can be expressed as $\mathcal{F} S_2 = \mathcal{F} S_1 \exp(-i2\pi\sigma z)$. The depth-resolved phase signal induced by jitter error becomes $\phi_{\text{jit}}(z) = -2\pi\sigma z$. Thus, it is clear that the jitter-induced phase error is a ramp signal, with its gradient proportional to the jitter σ , as illustrated in Figure 2(b) with gray line.

Therefore, the ultimate goal of a phase compensation algorithm is to remove the phase error induced by tissue bulk motion and the phase error originated from synchronization jitter. For this purpose, we propose to use the gradient of phase ramp along the depth to extract the jitter amplitude σ . In doing so, however, there are two issues that need be tackled with: phase-wrapping effect that disrupts the linear relationship of phase ramp and depth, and the presence of blood flow that would have an effect on the phase ramp linearity.

2.3 Algorithm of numerical phase stabilization

The process of the numerical phase stabilization algorithm is described in detail in this section, along with the example imaging session conducted on human forearm skin, with an aim to provide high-sensitivity OCTA image.

Imaging protocol and complex data extraction—500 A-lines were acquired for each B-scan, with B-scan repetition rate of 180 Hz, allowing time for fast-axis scanner fly-back. On each cross-sectional position, imaging was repeated 5 times, and the slow scan axis traversed through 500 lateral positions to cover $6 \times 6 \text{ mm}^{-2}$ skin area. The total time for data collection was ~13.9 seconds. For each A-line sweep, 2560 sampling points were acquired to cover the resultant spectral interferogram, providing a depth ranging distance of ~12 mm, even though the penetration depth of OCT beam is typically less than 2 mm. As a pre-processing step to extract complex data, OCT reference spectrum was subtracted from interference signal to remove DC bias [49] and then DFFT was performed with point number of 2560, to extract depth-resolved OCT signal. The top half of Fourier domain data was considered as the valid complex data for further processing.

Phase unwrapping—Phase unwrapping is an essential step for robust estimation of jitter error. However, phase unwrapping for practical real-world applications is a challenging task in digital signal processing due to the noisy wrapped signal. Especially for phase-sensitive OCT (PhS-OCT) imaging, the phase signal-to-noise ratio (SNR) depends on OCT signal strength. Moreover, the presence of functional blood vessels also induces phase shifts that interfere with conventional algorithm where searching for 2π discontinuities along depth is involved. Hence, one essential step in the phase stabilization algorithm is to perform robust and efficient 1-D phase unwrapping in OCT data with the presence of noise and initial phase-discontinuity.

Consider the complex data of two repeated scans $\mathbf{I}(z, t)$ and $\mathbf{I}(z, t+1)$, the phase can be expressed as $\varphi = \angle [\mathbf{I}(z, t) * \text{conj}(\mathbf{I}(z, t+1))]$. In Figure 3(b), the phase data from one representative scan of human skin is visualized. It is apparent that without phase-stabilization, the phase signals suffer from severe instability, as the phase signals on most axial scans drift away from zero, with occasionally phase-wrapping along depth. This observation agrees with the expectations as demonstrated in Figure 2(b).

To demonstrate the phase unwrapping algorithm, a representative phase signal on one cut-out A-scan is plotted in Figure 3(c). The data points with low OCT signal intensity are excluded from data processing. Due to phase-wrapping effect, phase signal along depth splits into two discontinued groups: one positive group and another negative group. Given this regularity of distribution, it is reasonable to conclude that the average value of squared phase signal in one axial scan can be used as a criterion to determine if there is phase wrapping happening on this scan. One condition for this criteria to be valid is that phase-wrapping only happens once along depth. However, since the length of OCT axial scan is short, this condition is always satisfied from our observations of various imaging configurations. Then, an offset of $+2\pi$ can be given to the negative group to reverse the

wrapping effect. In doing so, unwrapped phase is resulted as shown in Figure 3(c), where it can be seen that the ramp trend of the phase data is recovered effectively.

One consideration of the robustness in this phase-unwrapping algorithm is the scenario when the phase values are all distributed closely to $+\pi$ or $-\pi$. The “zero-deviation” criteria would certainly result in a false-positive determination of phase-wrapping. However, in this scenario, the unwrapping procedure would only shift the negative values (whole scan in this case) by 2π , which is a phase ambiguity problem but would not affect the final result for OCTA algorithms due to the help of the following phase disambiguate procedure.

Phase disambiguate and jitter compensation—After the above phase-unwrapping procedure, the phase ramp trend is recovered. According to the depth-dependent expression of $\varphi_{jit}(z) = -2\pi\sigma z$, jitter-induced phase change can be compensated if the gradient term ($-2\pi\sigma$) is estimated correctly. This term can be estimated by directly dividing z (depth) for each data point along depth, and taking the median value to exclude the irregularities from noise and blood flow. However, due to 2π ambiguity, the phase signal we obtained from phase-unwrapping could possibly not fit into the description of $\varphi_{jit}(z) = -2\pi\sigma z$. The countermeasure for this is to iterate through a range of ambiguous copies of the phase signal, as shown in Figure 3(d). There will always be one instance of $\varphi(z) + 2\xi\pi$ that complies best with depth gradient. The value of ξ is a random integer within a certain range, depending on the amplitude of synchronization jitter. Our observations indicate the range of ξ is well within $[-4, +4]$, however, if necessary, it can be extended to take account for larger degrees of laser instability. In this case, the phase gradient can be estimated. As illustrated in Figure 3(e), a series of phase gradient results, i.e. $G(z) = (\varphi(z) + 2\xi\pi)/z$ are plotted. The variance of the phase gradient along depth (z) is utilized as the criteria for 2π disambiguate: the group with lowest variance is determined as the correct instance of phase ramp (Figure 3(d)). The median value of this group is determined as the gradient term G . Note that the median estimation is more robust than mean estimation in this scenario since the phase turbulence from blood vessel has minimal effects on the median result, because signal from static tissue is the dominant component. Subsequently, the jitter-induced phase error $\varphi_{jit}(z) = -2\pi\sigma z = G * z$ is estimated for this axial scan, and the corresponding shift is applied to complex OCT data to achieve the jitter-free phase stabilization. The phase-stabilized complex data is then given as $I(z, t+1) = I(z, t) * \exp(-\varphi_{jit}(z))$.

Overview of data-processing—An overview of the procedures for extracting phase-stabilized complex OCT data is given in Figure 4. It demonstrates a roadmap for OCT data extraction and phase-stabilization that can be applied to any imaging protocol that involves temporally repeated sampling, such as A-scan repeated Doppler protocol, and B-scan repeated OCTA protocol. Note that phase difference data is only available for the scans except for the first one. Correspondingly, the final step of phase correction is applied to subsequent scans only, while the first scan serves as initial reference. This procedure can be easily adapted for 3-dimensional scan protocols, simply by the repeated use of the illustrated steps at each spatial locations along y direction (slow axis).

3. Results

3.1 Efficiency and robustness of phase-compensation algorithm

The conventional method to evaluate the phase stability performance of a PhS-OCT system is to use a stationary mirror as sample, and estimate the standard deviation of the phase fluctuations in the OCT signal acquired from the mirror, after taking repeated A-scans [25–27,32–34,36]. However, this method cannot be applied to examine the phase stability enhancement of the proposed algorithm here because the OCT signal from a mirror does not contain depth-dependent information that is required by the algorithm. Instead, we used the standard deviation of phase signal from real tissue acquired from repeated B-scans as a quantification of the phase-stability performance. Naturally, some imaging regions should be excluded in phase stability measurement: 1) regions with low OCT signal (in this study a threshold of 15 dB above noise floor is used) and 2) blood flow regions. Excluding these signal would eliminate the phase changes induced by blood flow, and mitigate the influence of SNR limit. It should be noted that this standard deviation measurement in real tissue would be considerably larger than that measured from a stationary mirror, due to the inevitable motion in tissue, and speckle de-correlation between B-scans.

Figure 5 shows the phase result and cross-sectional OCTA images obtained from repeated B-scans on human forearm skin in vivo. A mask of OCT SNR > 15 dB is applied to all images in Figure 5 to remove invalid phase data. Without any phase compensation procedure, the phase suffers from severe phase noise, as shown in Figure 5(a). As a consequence, any complex-signal-based OCTA algorithm would be affected by the unstable phase, thus, vertical stripe artifacts would appear and deteriorate the image contrast. The OCTA algorithm utilized in this study is complex OMAG [28], and the cross-sectional blood flow image is shown in Figure 5(d).

To demonstrate the performance of the phase-gradient based compensation algorithm, the conventional method modified from [37] that requires weighted linear-fitting is also applied to the same data for comparison. This approach is made possible to work without reference phase signal by extending the length of axial data for linear fitting, and introducing a robust control using MATLAB function “robustfit” [50]. The phase difference result with linear-fitting compensation and phase-gradient compensation are given in Figure 5(b) and (c) respectively. There are a few failure instances observable from the result by the conventional linear fitting compensation method, as indicated by red arrows in Figure 5(b), due to the failure of linear fitting to correctly estimate the gradient. Subsequently, the corresponding complex OMAG result (Figure 5(e)) is contaminated with some apparent artefacts, especially on top of large blood vessels, as indicated by yellow arrows. Since B-scans are repeated 5 times, artifacts can be reduced by averaging, but still noticeable, as indicated by small yellow arrow.

In comparison, the phase-gradient compensation algorithm performs much more robust. In Figure 5(c), the background phase signal is very close to zero, and phase signals induced by blood flow are clear and artifact-free. The corresponding OMAG image (Figure 5(f)) also presents significantly enhanced contrast of blood flow signals compared to Figure 5(d) without phase compensation.

Computational efficiency is an important consideration in the design and evaluation of an algorithm. A benchmark is performed for the above three processing schemes. The statistics of time consumption for computing 5 repeated B-scans of 2560×500 samples are given in Figure 6. Due to the heavy computation demand of iterations in robust fitting, the linear fitting algorithm takes more than 3 seconds to complete the task, while phase gradient compensation algorithm is more than 16 times faster, with even better robustness.

Statistics of phase stability is presented in Figure 7. Due to the depth-dependent nature of phase changes, the phase standard deviation is calculated on each depth, excluding low OCT signal and blood flow regions. As shown in Figure 7(a), without phase compensation, phase errors caused by synchronization jitters maintains at a high level of ~1.8 radians throughout all depth beyond 800 μm , indicating the phase noise is close to random white noise within $[-\pi, \pi]$ interval at this depth range. After phase gradient compensation processing, the phase stability is significantly enhanced, with standard deviation down to ~89 mrad. The standard deviation of phase still shows a trend of rising as the position goes deeper, this is again due to the limitation of OCT SNR. The phase-stabilization performance is comparable to that reported in 35, where hardware phase-stabilization is utilized. A histogram plot (Figure 7(b)) of the phase signal has also confirmed the convergence of phase signal towards zero after phase compensation, i.e. the phase stability is significantly enhanced.

Since the phase compensation algorithm is applied on a “per-A-line” basis, the robustness of the algorithm is dependent on the contents of each Aline, particularly, on the signal intensity and ratio of static components. Analysis is performed to investigate the failure rate due to low OCT SNR and the presence of blood vessels. The analysis is based on simulations of a typical A-scan, extracted from experimental data, added with different levels of simulated phase noises, and different ratios of blood vessel components. The phase noise is simulated using the SNR-limited noise equation: $\sigma_{\phi} = 1/\text{SNR}_{\text{OCT}}$ [29]. Algorithm failure is defined as >20 % deviation from correct phase gradient value. Each failure rate is calculated from 800 repeated simulations, and the result is presented in Figure 8. OCT SNR near sample surface (maximum OCT signal on an A-line) is used as a representative index in Figure 8(a). A very low failure rate can be guaranteed while the peak SNR is over 30 dB. The algorithm will lose accuracy in tracking the phase error when SNR on sample surface drops below 30 dB. In Figure 8(b), it can be concluded that the scrambled phase resulted from blood vessels can also lower the accuracy of the algorithm. However, failure rate is only significantly increased when the A-line is mostly composed of blood vessel (>50 %), this scenario is very rare in biological tissue, since the majority component of tissue is static. From these results, it is safe to assume the robustness of the algorithm can satisfy most application scenarios.

3.2 Image quality comparison in OCTA

To evaluate the potential clinical performances of complex signal based OCTA, one typical skin imaging case is presented in Figure 9. Note that for OCT imaging of human skins in vivo, the Institutional Review Board of the University of Washington approved the study and an informed consent was obtained from the subjects. The study was performed in accordance with the tenets of the Declaration of Helsinki and compliant with the Health Insurance Portability and Accountability Act of 1996.

To present the results, *en face* maximum intensity projection (MIP) images are used to represent the 3D data. For the all-depth MIP images (Figure 9(a,b,f,g)), the luminance of pixels is the OCTA flow signal strength, and color represent the depth position of flow signal, using an isoluminescent color map [51]. Grayscale MIP images of certain depth bands (0–300 μm and 300–1200 μm) are also given in Figure 9(c,d,g,h). The MIP image of deeper band (300–1200 μm) is corrected by a projection artifact removal algorithm [52] for accurate presentation of the blood vessel network. Before the projection, the OCTA signal mapping range is determined by automated optimization of image histogram, so that 15 % of data is saturated at low intensities and 1 % saturated at high intensities of input blood flow volume data. In doing so, the contrast of projection image is consistent between different processing algorithms.

Figure 9(a) and (b) shows the OCTA images produced by speckle variance algorithm, which is an algorithm that utilizes OCT intensity signal only. The OCTA result appears identical with or without phase compensation processing as expected, since intensity-based OCTA algorithm are not affected by phase stability. The capillary vessels in the shallow layer is well visualized (Figure 9(c)), and some blood vessels are visible in deep layer (Figure 9(d,e)). In comparison, the image quality is significantly improved using complex-based OCTA algorithm due to the elevated sensitivity, especially the enhancement of deep-layer blood vessels can be observed in Figure 9(i,j), where some large, but deep blood vessels that are invisible in the intensity-only speckle variance results becomes visible in Figure 9(i,j) as indicated by yellow arrows. We also attempted to quantify the contrast-to-noise ratio (CNR) performance of different processing procedures. Here the CNR is calculated by

$C = (\overline{S_{\text{ves}}} - \overline{S_{\text{bck}}}) / \overline{S_{\text{bck}}}$, where S_{ves} and S_{bck} stands for the signal intensity on the blood vessel pixels and background pixels, respectively. Both signals are defined as a subset of pixels, visually selected from the averaged image of the same category, since there is no ground-truth image as standard. Quantitative comparison result of the image CNR is given in Table 1, which agrees with the image quality performance that are assessed from Figure 9.

3.3 Wide field Doppler OCT

Doppler OCT/Doppler OMAG (DOMAG) [53] is another common application of PhS-OCT, to provide quantitative imaging of flow direction and flow velocity *in vivo* without using contrast agent. In this study, we also performed *in vivo* imaging to demonstrate the phase-stabilization of SS-OCT using our algorithm. The subject in this study was the mouse brain with skull left intact, with scalp retracted to expose skull. The mouse is a 3-month old C57/BL6 weighing ~24 g, anesthetized with 1.5 % isoflurane mixed with 0.2 L/min O_2 and 0.8 L/min air flow. All imaging procedures were reviewed and approved by the Institute of Animal Care and Use Committee of University of Washington (Protocol # 4262–01).

The imaging protocol for DOMAG is different from the previously described OCTA protocol, because the phase difference needs to be calculated from adjacent A-scans, so that the scan time interval can be reduced to match the requirement to perform DOMAG imaging on mouse brain. Here, 25 repeated A-scans were obtained on the same position, and 400 different positions were scanned to form one B-scan. The slow scan axis traversed through 400 B-scan imaging planes to cover the whole field of view. The total number of A-lines in

this protocol was 4 million. With 100.3 kHz A-line rate, the time consumption for a 3D DOMAG scan was ~42 seconds, including 5 % of the time for scanner fly-back.

From the *en face* MIP DOMAG result in Figure 10(a), the flow velocity map fails to provide any useful information of the blood flow direction and velocity without phase compensation. This can be confirmed by the cross-sectional Doppler phase image – most regions in the image are contaminated by erroneous phase signals due to synchronization jitters. Due to the decrease of phase error amplitude as the signal gets closer to zero-delay line, the Doppler phase signal contrast is slightly better on the apex of the skull, where some faint signal from a vein near the superior sagittal sinus can be observed, as indicated by the arrow in Figure 10(c). In contrast, DOMAG vascular image produced with phase compensation processing shows the entire vascular network on the mouse brain with excellent quantitative contrast of bi-directional blood flow. The Doppler phase signal image reveals the blood vessels not only in the apex region, but also the peripheral regions in Figure 10(d). However, due to the intrinsic limitations of Doppler principle, the DOMAG imaging is only sensitive to the axial velocity, i.e. the projection of the flow velocity vector towards the OCT light direction. The flow velocity denoted by $\otimes$ and $\odot$ symbols in Figure 10 are also limited to axial components away from/towards viewer, respectively. Nevertheless, there are a number of studies aiming to overcome this limitation, e.g. 3-D vessel geometry correction [12] and multi-beam detection [54].

The wide-field DOMAG system performance using the VCSEL swept source is reasonably close to the performance demonstrated in [20], where an perfectly phase-stable akinetic swept source was utilized. It is foreseeable that with the phase compensation algorithm, a conventional, long range SS-OCT system can achieve comparable imaging performance as a phase-stable OCT system, considering less than 1/3 of repetitions were used in this study (4 million A-scans) compared to 12.5 million A-scans in [20].

4. Discussion and conclusion

Although SS-OCT systems generally outperforms SD-OCT in ranging distance and sensitivity roll-off, stability problem has been an ineligible weakness of SS-OCT, especially for OCTA applications where repeatability is essential. Between the two fundamental factors of instability: 1) sweep repeatability, 2) phase stability, we focus on the latter one to optimize the SS-OCT system performance, while the MZI-based optical clock (MZI-OC) solves the sweep repeatability problem. However, we have found that a large degree of phase instability is originated from MZI-OC. To investigate this, an alternative configuration of VCSEL-SS-OCT is tested with and without the MZI-OC. When the MZI-OC is disabled, we used the on-board internal clock for waveform acquisition. A calibration data of the k-space linearity can be obtained *a priori* [18]. With this configuration, the phase stability is measured to be >5 times better than using MZI-OC, without any phase compensation procedure. Our explanation to the phenomenon is that the MZI-OC introduces more synchronization jitters than using sweep start-trigger generated by Fiber Bragg Grating (FBG). At the start of a wavelength sweep, the MZI output is less reliable due to the low amplitude optical energy. Unfortunately, disabling MZI-OC and using internal digitizer clock is not an option for VCSEL-based OCTA and Doppler OCT, due to the poor sweep

repeatability. Since MZI-OC functions as a calibration-on-the-fly for each sweep to achieve k-space linearity, using software k-linearization would result in poor OCT signal repeatability. This again supports our motivation to develop the numerical phase stabilization method.

It is noteworthy that the phase stability reported in our study without phase compensation is significantly worse than some of the literatures about phase stabilization techniques. This is due to the huge differences in the swept laser source, while the sweep repetition rate and coherence length specifications of a swept laser source are the determinant factors of phase stability. Numerical phase stabilization is particularly difficult for long-range SS-OCT like VCSEL based SS-OCT due to large synchronization jitters.

In this study using VCSEL based SS-OCT system, we demonstrated wide-field imaging of complex signal-based OCTA and Doppler OCTA. The comparison of image quality indicate that the proposed phase stabilization technique is robust, and effective in improving the image contrast-to-noise ratio and extending OCTA depth range. The proposed technique can be universally applied to improve phase-stability in generic SS-OCT of different scale of scan rates without special treatment.

Acknowledgments

This work was partially supported by grant from the National Heart, Lung, and Blood Institute (R01 HL093140) and the National Eye Institute (R01 EY024158, R01 EY026532). The content is solely the responsibility of the authors and does not necessarily represent the official views of grant giving bodies.

References

1. Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, Hee MR, Flotte T, Gregory K, Puliafito CA. Science. 1991; 254:1178–1181. [PubMed: 1957169]
2. Tomlins PH, Wang RK. J Phys D Appl Phys. 2005; 38:2519.
3. Drexler W, Liu M, Kumar A, Kamali T, Unterhuber A, Leitgeb RA. J Biomed Opt. 2014; 19:071412. [PubMed: 25079820]
4. Choma MA, Sarunic MV, Yang C, Izatt JA. Opt Express. 2003; 11:2183–2189. [PubMed: 19466106]
5. De Boer JF, Cense B, Park BH, Pierce MC, Tearney GJ, Bouma BE. Opt Lett. 2003; 28:2067–2069. [PubMed: 14587817]
6. An L, Li P, Shen TT, Wang R. Biomed Opt Express. 2011; 2:2770–2783. [PubMed: 22025983]
7. Wojtkowski M, Srinivasan VJ, Ko TH, Fujimoto JG, Kowalczyk A, Duker JS. Opt Express. 2004; 12:2404–2422. [PubMed: 19475077]
8. Spaide RF, Klancnik JM, Cooney MJ. JAMA Ophthalmol. 2015; 133:45–50. [PubMed: 25317632]
9. Xu J, Wei W, Song S, Qi X, Wang RK. Biomed Opt Express. 2016; 7:1905–1919. [PubMed: 27231630]
10. Yang V, Gordon M, Qi B, Pekar J, Lo S, Seng-Yue E, Mok A, Wilson B, Vitkin I. Opt Express. 2003; 11:794–809. [PubMed: 19461792]
11. Chen Z, Milner TE, Srinivas S, Wang X, Malekafzali A, van Gemert MJ, Nelson JS. Opt Lett. 1997; 22:1119–1121. [PubMed: 18185770]
12. You J, Du C, Volkow ND, Pan Y. Biomed Opt Express. 2014; 5:3217–3230. [PubMed: 25401033]
13. Wang RK, Nuttall AL. J Biomed Opt. 2010; 15:056005. [PubMed: 21054099]
14. Kennedy KM, Chin L, McLaughlin RA, Latham B, Saunders CM, Sampson DD, Kennedy BF. Sci Rep. 2015; 5

15. Song S, Huang Z, Nguyen T-M, Wong EY, Arnal B, O'Donnell M, Wang RK. J Biomed Opt. 2013; 18:121509. [PubMed: 24213539]
16. De Boer JF, Milner TE, van Gemert MJ, Nelson JS. Opt Lett. 1997; 22:934–936. [PubMed: 18185711]
17. Yun S, Tearney G, de Boer J, Iftimia N, Bouma B. Opt Express. 2003; 11:2953–2963. [PubMed: 19471415]
18. Song S, Xu J, Wang RK. Biomed Opt Express. 2016; 7:4734–4748. [PubMed: 27896012]
19. Huber R, Adler DC, Fujimoto JG. Opt Lett. 2006; 31:2975–2977. [PubMed: 17001371]
20. Xu J, Song S, Wei W, Wang RK. B Biomed Opt Express. 2017; 8:420–435.
21. Wang Z, Potsaid B, Chen L, Doerr C, Lee H-C, Nielson T, Jayaraman V, Cable AE, Swanson E, Fujimoto JG. Optica. 2016; 3:1496–1503. [PubMed: 28239628]
22. Grulkowski I, Liu JJ, Potsaid B, Jayaraman V, Lu CD, Jiang J, Cable AE, Duker JS, Fujimoto JG. Biomed Opt Express. 2012; 3:2733–2751. [PubMed: 23162712]
23. Wang H, Baran U, Wang RK. J Biophotonics. 2015; 8:265–272. [PubMed: 24659511]
24. Xi J, Huo L, Li J, Li X. Opt Express. 2010; 18:9511–9517. [PubMed: 20588797]
25. Vakoc B, Yun S, De Boer J, Tearney G, Bouma B. Opt Express. 2005; 13:5483–5493. [PubMed: 19498543]
26. Baumann B, Potsaid B, Kraus MF, Liu JJ, Huang D, Hornegger J, Cable AE, Duker JS, Fujimoto JG. Biomed Opt Express. 2011; 2:1539–1552. [PubMed: 21698017]
27. Choi W, Potsaid B, Jayaraman V, Baumann B, Grulkowski I, Liu JJ, Lu CD, Cable AE, Huang D, Duker JS. Opt Lett. 2013; 38:338–340. [PubMed: 23381430]
28. An L, Qin J, Wang RK. Opt Express. 2010; 18:8220–8228. [PubMed: 20588668]
29. Fingler J, Schwartz D, Yang C, Fraser SE. Opt Express. 2007; 15:12636–12653. [PubMed: 19550532]
30. Zhang A, Zhang Q, Chen C-L, Wang RK. J Biomed Opt. 2015; 20:100901. [PubMed: 26473588]
31. Mahmud MS, Cadotte DW, Vuong B, Sun C, Luk TW, Mariampillai A, Yang VX. J Biomed Opt. 2013; 18:050901.
32. Zhang J, Chen Z. Opt Express. 2005; 13:7449–7457. [PubMed: 19498770]
33. Manapuram R, Manne V, Larin K. Laser Phys. 2008; 18:1080–1086.
34. Braaf B, Vermeer KA, Sicam VADP, van Zeeburg E, van Meurs JC, de Boer JF. Opt Express. 2011; 19:20886–20903. [PubMed: 21997098]
35. Chen W, You J, Gu X, Du C, Pan Y. Sci Rep. 2016; 6
36. Adler DC, Huber R, Fujimoto JG. Opt Lett. 2007; 32:626–628. [PubMed: 17308582]
37. Hong Y-J, Makita S, Jaillon F, Ju MJ, Min EJ, Lee BH, Itoh M, Miura M, Yasuno Y. Opt Express. 2012; 20:2740–2760. [PubMed: 22330511]
38. Ahsen OO, Tao YK, Potsaid BM, Sheikine Y, Jiang J, Grulkowski I, Tsai T-H, Jayaraman V, Kraus MF, Connolly JL. Opt Express. 2013; 21:18021–18033. [PubMed: 23938673]
39. Fingler J, Zawadzki RJ, Werner JS, Schwartz D, Fraser SE. Opt Express. 2009; 17:22190–22200. [PubMed: 19997465]
40. Kim DY, Fingler J, Werner JS, Schwartz DM, Fraser SE, Zawadzki RJ. Biomed Opt Express. 2011; 2:1504–1513. [PubMed: 21698014]
41. Wang RK, Jacques SL, Ma Z, Hurst S, Hanson SR, Gruber A. Opt Express. 2007; 15:4083–4097. [PubMed: 19532651]
42. Srinivasan VJ, Jiang JY, Yaseen MA, Radhakrishnan H, Wu W, Barry S, Cable AE, Boas DA. Opt Lett. 2010; 35:43–45. [PubMed: 20664667]
43. Vakoc BJ, Lanning RM, Tyrrell JA, Padera TP, Bartlett LA, Stylianopoulos T, Munn LL, Tearney GJ, Fukumura D, Jain RK. Nat Med. 2009; 15:1219–1223. [PubMed: 19749772]
44. Mariampillai A, Leung MK, Jarvi M, Standish BA, Lee K, Wilson BC, Vitkin A, Yang VX. Opt Lett. 2010; 35:1257–1259. [PubMed: 20410985]
45. Barton JK, Stromski S. Opt Express. 2005; 13:5234–5239. [PubMed: 19498514]
46. Jonathan E, Enfield J, Leahy MJ. J Biophotonics. 2011; 4:583–587. [PubMed: 21887769]

47. Jia Y, Bailey ST, Hwang TS, McClintic SM, Gao SS, Pennesi ME, Flaxel CJ, Lauer AK, Wilson DJ, Hornegger J. *Proc Natl Sci.* 2015; 112:E2395–E2402.
48. Pinsky, MA. *Introduction to Fourier Analysis and Wavelets.* American Mathematical Society; 2002.
49. Wang RK, Ma Z. *Phys Med Biol.* 2006; 51:3231. [PubMed: 16757873]
50. Holland PW, Welsch RE. *Commun Stat Theory Methods.* 1977; 6:813–827.
51. Geissbuehler M, Lasser T. *Opt Express.* 2013; 21:9862–9874. [PubMed: 23609692]
52. Zhang A, Zhang Q, Wang RK. *Biomed Opt Express.* 2015; 6:4130–4143. [PubMed: 26504660]
53. Wang RK, An L. *Opt Express.* 2009; 17:8926–8940. [PubMed: 19466142]
54. Trasischker W, Werkmeister RM, Zotter S, Baumann B, Torzicky T, Pircher M, Hitzenberger CK. *J Biomed Opt.* 2013; 18:116010. [PubMed: 24247747]

Figure 1.
Schematic of the SS-OCT system based on VCSEL swept source.

Figure 2.

Demonstration of phase error sources in SS-OCT (a) example of phase errors introduced to different interference frequencies (b) Decomposition of phase signal from simulated tissue with presence of blood vessel.

Figure 3.

Visual demonstration of phase compensation algorithm using one example A-scan. (a) OCT intensity image of a B-scan on human forearm skin. (b) Phase results calculated from two repeated B-scans. (c) Phase result on an A-scan, demonstrates phase wrapping that happens around depth of 200 pixels. (d) Unwrapped phase signal and four of its ambiguous copies. (e) Phase gradient (ϕ/z) plot, dashed line indicates the determination result of phase gradient.

Figure 4.

Flowchart of exemplar phase-stabilized OCT data processing. Here k stands for optical wavenumber, x is lateral position, z is depth position, and t is the index of repeated scans. G stands for phase gradient.

Figure 5. Phase stability and complex OCTA performance comparisons. (a–c) Phase results with no phase compensation (a), linear fitting compensation (b), and phase gradient compensation (c). (d–f) Complex OCTA results with no phase compensation, linear fitting compensation, and phase gradient compensation. All scale bars =10 mm.

Figure 6.
Computation budget of phase compensation algorithm (Computing platform: MATLAB 2016b, single thread on Intel Xeon E3-1225v3 CPU).

Figure 7. Phase stability statistics in static tissue regions. (a) Phase stability on different depth. (b) Histogram of phase signal.

Figure 8.

Failure rate simulation results. (a) Failure rate at different SNR scenarios, here SNR stands for the SNR at the sample surface. (b) Failure rate at different blood vessel ratios.

Figure 9.

En face MIP images of OCTA volume results from human forearm skin. (a–e) Speckle variance OCTA result, (a) without and (b) with phase compensation. (c–d) Speckle variance OCTA results from two depth ranges as shown. (e) deep layer vessels processed by projection artefacts removal. (f–j) Complex OMAG result, (f) without and (g) with phase compensation. (h–i) Complex OMAG results from two depth ranges as shown. (j) deep layer vessels processed by projection artefacts removal.

Figure 10. Comparison of widefield DOMAG imaging of mouse entire brain. (a–b) DOMAG *en face* MIP image (a) without phase compensation (b) with phase compensation. (c–d) Cross-section images of Doppler phase on a selected B-scan at the position indicated by white arrow in (b), (c) without phase compensation, (d) with phase compensation. (e) OCT structural image at the same B-scan position.

Table 1

Image contrast-to-noise ratio comparisons

	No Compensation	With Phase Compensation		
	All depth	All depth	0–300 μm	300–1200 μm
Speckle variance	7.41	7.41	7.54	1.22
Complex OMAG	0.234	7.50	11.5	5.25