Phase-sensitive swept source optical coherence tomography for imaging and quantifying of microbubbles in clear and scattering media

Ravi Kiran Manapuram,1 Venu Gopal Reddy Manne,1 and Kirill V. Larin1,2,3,8
1Department of Electrical and Computer Engineering, University of Houston, Texas 77204, USA
2Biomedical Engineering Program, University of Houston, Houston, Texas 77204, USA
3Saratov State University, Saratov 410056, Russia

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A phase resolved system based on swept source optical coherence tomography (SSOCT) called as phase-sensitive SSOCT to detect and quantify gas microbubbles in aqueous and tissue simulated media is developed. The structural images of gas microbubbles are obtained using conventional SSOCT, while common path SSOCT was used to perform the phase-sensitive measurements. The system shows an axial resolution of 10 μm, a phase sensitivity of 0.03 rad, an imaging depth of up to 6 mm in air, and a scanning speed of 20 kHz for a single A-line. The structural images of the bubbles show an accuracy of 10 μm, whereas the temporal phase response show an accuracy of 0.01 μm. Images of rapidly moving bubbles are also presented which indicate that the SSOCT could be ultimately applied for the rapid assessment of microbubbles in biofluids and tissues. © 2009 American Institute of Physics. [DOI: 10.1063/1.3116614]

I. INTRODUCTION

Microbubbles in blood and tissues are one of the root causes for diseases such as decompression sickness, arterial or venous gas embolisms, and barotrauma. The symptoms shown by these diseases include severe pains in joints, pulmonary problems, disorientation and mental dullness, vomiting and skin rash, and usually results from the formation of gas bubbles in blood and other tissues due to rapid changes in barometric pressure (e.g., during scuba or deep-sea diving or rapid changes in altitude). These bubbles can travel to any part of the body, accounting for many serious and sometimes life-threatening disorders.1 For example, gas bubbles in the back or joints can cause localized pain (the bends), while bubbles located in the spinal cord or peripheral nerve tissues may cause paresthesias, neuropaxia, or paralysis. A bubble forming in the circulatory system can lead to pulmonary or cerebral gas emboli. Bubbles may act as emboli and block circulation, as well as cause mechanical compression and stretching of the blood vessels and nerves. Additionally, the blood-bubble interface acts as a foreign surface, activating the early phases of blood coagulation and release of vasoactive substances from cells lining the blood vessels. Formation and/or introduction of gas microbubbles in human blood and tissues remains a serious long-term sequel in patients undergoing cardiac valve replacement (with an annual risk of up to 4%),2,3 high-intensity focused US therapy,4 cesarean section5 and operative hysteroscopy,6 cardiopulmonary bypass and other open-heart surgeries,7 orthopedic surgery, and various laser ablation and laparoscopic surgeries.8 Additionally, gas embolism happens in endoscopy,9 tissue biopsy,10 neurosurgery,11 liver transplantation,12 during central venous line insertion and removal, and even during intravenous antibiotic delivery at home.13 The use of ultrasound bubble contrast media could also lead to gas emboli.14 Thus, formation and introduction of gas microbubbles in human blood and tissues is a significant everyday clinical problem affecting thousands of patients undergoing various surgical and therapeutic procedures. Depending on the clinical situation, the nature of the gas emboli and the number of embolic events can vary greatly. For example, Hills and Butler15 measured intravascular gaseous emboli ranging from 19 up to 700 μm following decompression in dogs. However, several studies suggested that bubbles with a diameter as small as 8 μm could cause blockage and result in the trauma and onset of the symptoms.16,17 Therefore, in order to be effective, an imaging or sensing technique should accurately detect bubbles with a diameter of ≥8 μm. If detection of these bubbles at an early stage is possible, action can be taken to prevent neurological or other complications. Hence, noninvasive functional imaging, monitoring, and quantification of microbubbles forming in blood and tissues have gained importance in searching for effective therapy and early diagnosis.

A high in-depth, high-speed, and high-sensitive device is required for the imaging, monitoring, and quantification of these microbubbles in epithelial tissues. Previously, several imaging techniques have been proposed and applied to the study of microbubbles in blood including Doppler sonography,18 magnetic resonance imaging,19 and nuclear imaging20 and computer tomography.21 Doppler sonography, the most popular technique since air-bubble interface produces strong ultrasonic reflection in the region of 1–20 MHz, is an ultrasound diagnostic imaging technique. Enhanced with Doppler effect, it is capable of the assessment of moving bubbles by calculating the frequency shift in a particular sample volume.22 However, Doppler sonography can detect only moving intravascular bubbles with a diameter of approximately 50 μm.15 Evidently, this resolution

8Author to whom correspondence should be addressed. Electronic mail: klarin@uh.edu.

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should be improved in order to achieve sensitive imaging and assessment of small microbubbles in blood and tissues.

Optics-based techniques have great intrinsic potential to achieve the goal of noninvasive imaging of microbubbles in tissues and biofluids. Confocal-laser scanning microscopy (CLSM), two-photon fluorescence microscopy (2P-FM), and higher harmonic generation (HHG) microscopy are some examples of optical methods that have been applied in different fields of biological research. CLSM has significant axial and lateral resolutions, but it is limited to \( \sim 100 \, \mu m \) penetration depth due to high attenuation of visible/ultraviolet excitation light. 2P-FM has a higher penetration depth due to near-infrared excitation of fluorophores, but general needs of exogenous fluorophores make this technique not truly “noninvasive.” HHG microscopy does not require application of exogenous fluorophores but is bulky and very costly.

Optical interferometric techniques are extremely sensitive to local changes in scattering, absorption, and refractive indices of the tissues and cells. Since the refractive indices of blood and air are quite different (1.4 and 1.0, respectively), an optical-based sensor will be capable of assessing formation of gas bubbles with ultrahigh sensitivity and accuracy. This motivates the development of a phase sensitive, in-depth imaging device which would detect the microbubbles whose sizes are beyond the imaging capabilities of standard imaging techniques. Phase-sensitive techniques have been already used to detect nanometer-scale motions in the living cells. This paper presents the development and application of phase-stabilized swept source optical coherence tomography (PhS-SSOCT) that could potentially be used for real-time, sensitive, accurate, and noninvasive imaging, monitoring, and quantification of microbubbles in the microvessels of tissues. High in-depth and lateral resolutions of OCT would allow direct monitoring and quantification of microbubbles in circulating blood.

### II. THEORY

OCT is a relatively new noninvasive optical diagnostic technique that provides depth-resolved images of tissues with resolutions of up to a few micrometers to depths of up to several millimeters. This technique was introduced in 1991 to perform tomography imaging of the human eye with 30 \( \mu m \) resolution. Since then, OCT has been actively developed by several research groups for many clinical diagnostic applications.

The basic principle of the OCT is to detect backscattered photons from a tissue of interest within a coherence length of the source using a two-beam interferometer. In time-domain configuration, each depth corresponds to a different time delays, which are measured by moving the reference arm. Each depth information is obtained at different times making the information time encoded. Recently, OCT Fourier detection techniques have emerged which do not require mechanical in-depth scanning and can achieve high speed with improved sensitivity. As different echo time delays of light which produce different frequencies of fringes in the combined interference spectrum, the Fourier domain techniques can measure this time delay by Fourier transforming the interference spectrum of the signal. Fourier domain OCT (FDOCT) offers significantly improved sensitivity and imaging speed compared to time-domain OCT (TD-OCT). FDOCT detection can be performed in two ways: spectral domain OCT (SDOCT), using a broadband light source and a spectrometer with a multichannel analyzer, or swept source OCT (SSOCT), using a broadband narrow-pulse swept-laser source and an InGaAs detector.

Typically, the spectrometers in SDOCT systems employ high-performance charge-coupled devices (CCDs), which are generally available only for wavelengths of up to 1 \( \mu m \). Therefore, the imaging depth in highly scattering tissues is limited due to the wavelength dependence of optical attenuation as well as sensitivity drop off. In contrast, in swept source configuration, the source sends narrow pulses which allow for a slower decay of the coherence function, enabling reduced depth dependent sensitivity drop off compared to SDOCT. Additionally, as the detection could be performed with a single photodetector SSOCT allows using longer wavelength sources and extending imaging depth.

Generally, in SSOCT, the frequency chirped laser source is split into two arms: the reference and the sample arm. The backscattered light from the sample which is the time-delayed copy of the reference at particular depths recombines with the reference to give fringes. The intensity of these interference fringes are detected with respect to time, rf modulated with the envelope of the signal, and then passed through a band-limited filter. Each depth corresponding to a different delay in the backscattered light gives a unique rf signal. All these frequencies are displayed at the same time. Thus, the detector current contains information from all the frequencies which is given by the following equation:

\[
i(t) = \text{Real} \left\{ \int S(\omega - \omega_0) e^{-j \Delta \phi(\omega - \omega_0) d(\omega - \omega_0)} \frac{d}{2\pi} \right\} ,
\]

where \( \Delta \phi(\omega) = (2\pi/\lambda) \Delta \delta(\omega) \) is the phase delay due to the path difference \( \delta(\omega) \) and \( S(\omega) \) is the spectrum of the source. The \( i(t) \) and \( \Delta \phi(\omega) \) are Fourier transform pairs, thus by applying a complex fast Fourier transform (FFT) algorithm to \( i(k) \), which is \( i(t) \) in the \( k \)-space \( k = 2\pi/\lambda \), one can obtain a one-dimensional (1D) OCT depth profile. By generating transversal set of similar 1D depth profiles with a scanning galvo-mounted mirror, a two-dimensional (2D) image could be constructed. Thus, 2D image contains both axial and transverse information.

For a swept source laser, \( k(t) = 2\pi/\lambda t(t) \) is the wave number that does not always obey a linear relation \( k(t) = k_0 + k_1 t \). In theory if it obeys a linear relation, then the laser would have effectively mapped the \( k \)-space to time domain, i.e., \( i(t) = i(k) \). In reality, the frequency sweep of the chirped laser is nonlinear. The \( k(t) \) contains higher order terms and causes nonlinearity in the frequency sweep, which leads to a non-uniform sampling interval. As the Fourier transform is applied to get the depth information, uniformly spaced samples are required. By performing nonuniform Fourier transformation, depth information from the nonuniformly spaced samples can be obtained. Another approach is by using the interference fringes from a Fabry-Pérot interferometer, uni-
formly spaced samples are obtained. The nonlinearity in frequency sweep is analyzed by several groups and proposed different methods to overcome the problem. This paper employs a Mach–Zehnder interferometer-based optical clock (MZI-OC) which generates equally spaced frequency interferogram that range from 12.5 to 200 GHz. All peaks, as well as the zero crossings in the recorded fringes of MZI-OC, are always equally spaced in optical frequency space and are used for recalibration. The samples are collected at these zero crossings, thus eliminating the nonlinearity in the sampling interval. This also serves the purpose of converting into the uniform $k$-space. Without calibrating and remapping to uniform $k$-space, resolution degradation is observed along the $z$-space. In addition to that, this correction offered a significant improvement in the resolution at a particular depth as shown in Fig. 1: the reflectivity profile from a weak reflector without calibrating (dotted) and with calibrating (solid) of the signal.

There are several advantages of SSOCT over SDOCT and TDOCT. In addition to the fact that Fourier domain detection does not need a complex setup for a mechanical scanning of the reference arm, there are sensitivity advantages over TD detection. The SSOCT utilizes a photodetector in a dual-balanced detection mode, in which the common noise is subtracted out of the phase fringes which are then added to enhance the signal strength and sensitivity. In OCT, sensitivity drops off as depth is increased. In SSOCT, however, due to the narrow instantaneous linewidth of the tuning laser source, interference is observed even at deeper depths as the coherence function remains nearly constant. This effect is more distinctly observed in SDOCT as the laser source is not pulsed. The constant coherence function at deeper depths allows the SSOCT to have higher imaging depths. However, SSOCT suffers from a major drawback: the phase is highly unstable from successive $A$-line scans in system utilizing a laser source that is not phase-locked. For a typical SSOCT (without any phase stabilization techniques employed), phase variations from successive $A$-scans for a homogenous media could be as high as $\pi$ radians (usually caused by the bulky movements of the polygon mirror inside the laser).

In this paper, we present results on development of a phase resolved sensing system based on SSOCT and evaluated its performance by quantifying microbubbles of different diameters in clear and scattering tissue-simulating media.

### III. EXPERIMENT

#### A. Setup

The schematic of the system, as shown in the Fig. 2, consists of four main units: the source, the interferometer (Mach–Zehnder in this particular setup), the calibration system, and the data acquisition electronics. The laser source output is split into two arms: one arm containing the interferometer and the other arm containing the recalibration and...
triggering unit. A 90-10 fiber coupler (Thorlabs) is used to send 90% of the light to the interferometer so that the sample arm gets the maximum power. The remaining 10% is further split by a 99-1 fiber coupler with 99% going to a fiber Bragg grating (FBG) and 1% to the MZI-OC. The 99% light goes to the FBG through a three-arm circulator and the reflected pulse is passed to the detector by the third arm of the circulator. This detector outputs a voltage signal which is converted to an electrically tunable transistor-transistor logic (TTL) pulse by a pulse generator (Stanford Research Systems, Inc.). The TTL signal is tuned to a required duty cycle and used to trigger the analog to digital converter (ADC). The other 1% is fed to the MZI-OC whose signal is detected in balanced detection mode, and these electric signals from the detector are acquired by one of the channels of the ADC. In the interferometer arm, the 90% light is split into 1% and 99%, each going to the reference arm and sample arm respectively via circulators. The light coming from the reference arm is passed through an adjustable pinhole to allow attenuation if required. The reflected light from the reference arm and sample arm are coupled into a 50-50 fiber coupler where they recombine and form the interference fringes. These fringes are then detected by a balanced photodetector (BPD), which subtracts the two signals to remove the common mode noise. As the fringes would be out of phase, this BPD is effectively adding the fringes but subtracting the common mode noise. After removing the common mode noise, the fringe encoded voltage is then amplified by a transimpedance amplifier and then rf modulated and acquired by a personal computer (PC) through the other channel of the ADC. Both the information from MZI and the interferometer is acquired simultaneously by the ADC with the receiving of the trigger from the FBG.

1. Data acquisition and signal processing

The fringes are acquired by a 14-bit high-speed digitizer (PCI 5122, National Instruments). The digitizer is operated at 50 Msamples/s and acquires 2500 sample points per A-scan. The sampling rate is chosen by considering the fact that the sampling interval should be smaller than the instantaneous linewidth (0.1 nm); otherwise, a large sensitivity drop off along the depth scan would be observed. Out of these 2500 points, the first 200 and last 252 points are deleted so as to select the data corresponding to the laser wavelength scans. Both the raw signal and the MZI-OC signal will now have 2048 points. After recalibration using MZI-OC, the number of points is decreased due to the fact that the number of peaks and zeros are always less than the total number of points. As a matter of fact, if the nearest neighborhood algorithm is used to find the peaks, which gives 1 point out of 3 points, only one-third of the sample signal would be utilized. Thus, the number of peaks and zeros registered are around 600 indicating the presence of 600 raw data points. Using spline interpolation, three points are inserted between consecutive raw data points thus giving 2400 points. Again, the signal is windowed to have 2048 points. It must be noted that the points that are deleted from the raw signal should not contain any information from the signal. If, in the process of getting 2048 from 2400, any information containing the fringes is lost, then instead of getting 2048 points in the first deletion, more points are retained. The selection of points is made in such a way that the calibrated signal always has 2048 points without losing any fringe information. A complex FFT of this signal gives the depth profile and, due to the symmetry of FFT, each A-line corresponding depth is constructed using 1024 points. It is worth noting that inserting a lower number of points, e.g., two, might result in losing some of the higher frequency components which, in turn, might reduce the imaging depth of the system. Since the backscattered light at a depth greater than the coherence length of the laser source cannot form fringes, the maximum imaging depth would not exceed the coherence length of the laser source.

Signal processing includes several steps, including reference subtraction, recalibrating and resampling to uniform k-space and image construction. The background signal is recorded at the beginning of every acquisition by blocking the sample arm. This signal contains the 1% residual light reflected from the reference arm and from the residual signal in the detectors due to imperfect symmetry of the BPD. Subtracting the reference from the interference signal helps to increase the contrast and remove the artifacts. It removes the low-frequency components introduced by the reference or ambient light. Each depth profile is obtained from the Fourier transform of the interference fringe signal. The logarithm of the absolute value of this complex FFT is mapped to the grayscale to get an image of a single A-scan. By generating a transversal set of similar 1D depth profiles with the scanning galvo-mounted mirror, a 2D image is constructed. Thus, the 2D image contains both axial and transverse information. By scanning the Y-mirror, enface imaging is achieved, thus allowing for three-dimensional imaging.

2. PhS-SSOCT

Jitter present on the output source spectrum causes the acquired fringe data to slowly drift over time. These drifts introduce varying delays between the trigger signal and the subsequent digital fringe data. As the phase is extracted from the complex FFT of the fringe signal, the mismatch in fringe signal and the trigger would result in phase jumps of up to a maximum value of π, as shown in Fig. 3(c). These jumps could be removed by dynamically triggering the ADC using an electrically tunable TTL signal generated from a narrow band (0.1 nm) FBG, as shown in Fig. 2. A reflected optical pulse is generated whenever the source sweeps the FBG reflection wavelength. An optical pulse is detected by the detector every time the laser swept 1315 nm (the reflection wavelength of FBG), thus the frequency of the FBG pulses are also at 20 kHz, as shown in Fig. 3(a). Figure 3(b) depicts the oscilloscope trace showing the TTL trigger signal (second trace), the signal from the sample (third trace), and the MZI-OC signal (fourth trace). This pulse is converted into a TTL signal using a signal generator (Stanford Research Systems). The TTL signal generator generates an optical pulse which can be electrically tunable to get the required duty cycle as shown in the Fig. 3(a). By triggering the ADC with the above TTL signal, the jitter due to electronics is reduced by introducing perfect synchronization between the source...
and data acquisition, which, in turn, reduces the phase variations: standard deviation of 0.016 rad for 512 A-line scans has been achieved, as shown in Fig. 3.

Regardless of the above removal of π jumps, the MZI-OC introduces some phase noise to the system. This is due to the fact that zeros and peaks keep changing with every laser scan due to thermal and mechanical vibrations. Thus, the phase measured from the sampled fringe signal inhibits high phase variations. To avoid this, the MZI-OC is recorded only once and hence the zeros and peaks will not change with every scan and the recalibrated signal will be stable in the phase. The obtained uncertainty in phase is very low and is experimentally verified in the following manner. Phase response of glucose solutions of increasing concentrations increasing by 0.1 M is observed (data not shown). The phase increased in equal steps indicating that the phase measured is indeed the phase introduced by the change in the concentration of the glucose solution with low uncertainty (0.03 rad). With the stabilized phase, the refractive index change as small as $6.3 \times 10^{-6}$ (through 1 mm optical path) can be detected using the PhS-SSOCT.

IV. METHODS

Performance of the developed system was evaluated in water and scattering media containing gas microbubbles of different diameters. Water was injected into a 500 μm flow-through cuvette, and bubbles were generated by introducing different pressures using a peristaltic pump (Fisher Scientific). 1.54% polystyrene spheres were used to simulate the scattering media with a scattering coefficient of 100 cm$^{-1}$ for the 1324 nm wavelength. The beam is scanned across the cuvette as shown in Fig. 4(a). The amplitude of the interference signals in the time-delay domain was recorded from the cuvette. Four characteristic interferometric peaks were observed, corresponding to the interferences between the four surfaces of the cuvette [an example is shown in Fig. 4(b)].

FIG. 3. (Color online) (a) FBG analog output and the raw signal. (b) Oscilloscope trace showing the synchronization of FBG trigger, raw signal, and MZI-OC. (c) The temporal phase response of 500 μm cuvette at 500 μm depth before stabilization and (d) after stabilization (note the scale).

FIG. 4. (Color online) (a) Orientation of the cuvette with respect to the beam (b) corresponding 1D depth profile (A—glass thickness, B—optical path thickness, C—A+B, 1, 2, 3, 4, are the surfaces of glass cuvette).
In these experiments, the optical delay is calculated as a function of dynamic refractive index modified by the presence/absence of microbubbles from the interferometric peaks that are produced by the reflection from the inner walls of the cuvette in the time-delay domain between the surfaces 3 and 2 or B in Fig. 4. The phase is extracted from the complex Fourier transform of the interference fringes and monitored at the interferometric peak that corresponds to the self-interference between the inner glass surfaces 3 and 2. The signal from surface 2 in Fig. 4 is used as a reference arm in generating the self-interference signal. Phase-sensitive measurements of water are taken before and after injection of the microbubbles. Since the bubbles induced changes in the refractive index, the diameter of the bubble from the phase shift is calculated from

\[ dn = \frac{1}{l} \frac{\lambda}{4\pi} d\phi, \]

where \( dn \) is the refractive index change introduced by the bubble (equal to 0.33 for an air bubble in water), \( l \) is the diameter of the bubble, \( d\phi \) is the bubble-induced phase shift. Phase difference between the A-line corresponding to the center of the bubble (determined as a maximal possible change in the phase) and the A-line outside the bubble are computed to quantify the diameters of the bubbles.

Bubbles are generated by creating a pressure difference and are circulated using the pump. Bubbles of diameters greater than 10 \( \mu m \) were imaged first to test the consistency of the PhS-SSOCT imaging. The images were taken in the inter-interference mode, in which the reference arm can be placed in such a way that all surfaces of the cuvette (Type-48, NSG Precision cells, Inc.) can be shown in real distances as shown in Fig. 4(a). Phase stability was high in self-interference mode compared to the inter-interference mode as discussed earlier. Thus, in all the results presented in this paper, the images were taken in inter-interference mode, and the phase response was measured in self-interference mode.

V. RESULTS AND DISCUSSION

A. Clear media

Figure 5(a) shows the image of a 500 \( \mu m \) cuvette filled with water containing a bubble with a diameter of 224 \( \mu m \) (actual diameter). A high display threshold is selected so as to suppress the self-interference image. The 1D profile of the corresponding self-interference image and the temporal phase response are shown in Figs. 5(b) and 5(c), respectively. It should be noted that the imaging and the phase response were taken one after the other in very quick succession, as the imaging was done in the inter-interference mode and the phase response was studied in self-interference mode.

The reference arm is placed at a distance so that the whole cuvette can be imaged without any negative images that arise due to the symmetry of FFT. Therefore, for Fig. 5(a), all four bright lines correspond to all four surfaces of the cuvette. Each bright line in Figs. 5(a) and 6(a) correspond to each surface of the cuvette (numbered 1–4 in Fig. 4). The missing part in surfaces 3 and 4 is observed only in the case of huge bubbles, indicating that the beam has been refracted so that the reflection from the surface below the...
bubble is not coupled back. In the clear aqueous media, the maximal phase variations were as low as 0.03 rad after a five-point averaging (repeated for at least ten times), which implies that any microbubble that introduces a phase shift greater than 0.03 rad can be detected.

Generally, for an optical path difference of one wavelength, the phase shift would be $2\pi$ in a homogenous medium. Equation (2) describes a relationship between the changes in the refractive index and the phase of PhS-SSOCT. For an air bubble in water, the change in the refractive index would be 0.33 which translates to a minimum bubble size of 2 $\mu$m for the phase to be unwrapped by one $2\pi$ jump. Since the system’s resolution is 10 $\mu$m, bubbles with diameters greater than 10 $\mu$m can be clearly seen in the structural image, as shown in Fig. 5(a). When there is no bubble, the optical path length between the inner surfaces of the cuvette is 665 $\mu$m (refractive index of water is 1.33, so 500 $\mu$m $\times$ 1.33=665 $\mu$m), which is observed as a peak at 665 $\mu$m in the corresponding 1D depth profile. As the beam interacts with the bubble, the optical path length keeps decreasing until it reaches the center of the bubble and increases again to the original value. This change in the optical path length is reflected as a shift in the peak in the corresponding 1D profile, as shown in Fig. 5(b). The larger the bubble, the greater is the decrease in the optical path length, and hence the greater is the shift. The number of $2\pi$ jumps by which the phase to be unwrapped is then calculated from the peak shifted in the 1D profile. This unwrapped phase is then added to the PhS-SSOCT phase response to get the true phase response. For the clear media, each depth pixel corresponds to a one way physical path difference of 5.88 $\mu$m, which is equal to 2.94 “$2\pi$” jumps. Thus, for the bubble shown in Fig. 5(a), the number of $2\pi$ jumps would be 69/2 $\times$ 2.94=113 as the peak shifts by 69 depth pixels [Fig. 5(b)]. By plugging this true phase in Eq. (2), the size of the bubble obtained is 219 $\mu$m. The actual diameter of the bubble measured is 224 $\mu$m. The true phase and the PhS-SSOCT phase (phase before adding required $2\pi$ jumps) is plotted in the same graph with two different scales on the right and left of the Y-axis.

Similarly, several bubbles with different diameters (52 $\mu$m, 94 $\mu$m, 160 $\mu$m, etc.) were taken and quantified. The obtained error ranged from 0.19 to 10 $\mu$m, which can be attributed to the $2\pi$ ambiguity. The change in the path differences of less than 10 $\mu$m is not reflected in the 1D profile (due to limited imaging resolution of 10 $\mu$m). In this case, the number of $2\pi$ jumps by which the phase should be unwrapped cannot be determined and can take any integer value between 1 and 5. This means that microbubbles with diameters with multiples of 2 $\mu$m (up to 10 $\mu$m) would show the same phase response. For instance, if the number of $2\pi$ jumps calculated from the phase shift is 60, the actual number of $2\pi$ jumps can be any number between 60 and 65. Likewise, if the measured diameter of a bubble is 120 $\mu$m, then the actual bubble diameter could be 120, 122, 124, 126, or 128 $\mu$m. However, this ambiguity can be resolved by implementing a fast real-time continuous unwrapping algorithm which will be developed in our future studies. This algorithm would acquire multiple phase recordings between consecutive changes in the path difference of less than 2 $\mu$m and continuously unwrap the phase information. It would also allow the exact quantification of microbubbles that are beyond the imaging capabilities of the system.

Figure 6(a) shows an example of small bubbles which cannot be resolved from SSOCT structural imaging. These bubbles can, however, be detected by the PhS-SSOCT. The sizes of the bubbles were estimated to be 1.9 $\mu$m (but could be 3.9, 5.9, 7.9, or 9.9 $\mu$m due to the ambiguity discussed above) for Fig. 6(a). Therefore, PhS-SSOCT is an effective device for ultrasensitive quantification of microbubbles with diameters significantly less than imaging capabilities of the employed system. However, the error due to ambiguity can still be decreased by increasing the number of A-line scans with in the same frame and by reducing the focused beam diameter. For instance, as the beam propagates across the bubble, it interacts more and more of the air/gas volumes in the bubble indicating the increase in diameter. If a 10 $\mu$m bubble is scanned in five steps of 2 $\mu$m (the difference between two consecutive A-line diameters) each for half the bubble, then the phase would be needed to unwrap only once. Hence, no $2\pi$ ambiguities would be observed. To achieve that, a 3 mm transverse length should be scanned with 1500 A-lines as opposed to 512 A-lines. Thus, to obtain 1500 A-lines in the same time, the speed of the system should be enhanced by about three times, which can be achieved by using Fourier domain mode locked laser as described in Ref. 28.

B. Scattering media

The detection, imaging, and quantification of microbubbles in scattering media were also performed on a similar note. Figure 7(a) depicts the image of the cuvette when filled with scattering media. A high dynamic range is chosen to identify if any self-interference lines are observed. The phase is monitored at the peak corresponding to 705 $\mu$m path length. The phase variations obtained were 0.04 rad (the same phase sensitivity is obtained even if milk is used as scattering media), indicating that the minimum size of the bubble that could be detected is 0.01 $\mu$m. Figure 7(b) shows the image of a bubble with an actual diameter of 142 $\mu$m, and the diameter obtained from PhS-SSOCT measurements.
was 143 μm from Fig. 7(d). Note that the phase response in Fig. 7(d) is shown for few A-lines so that the phase response could be clearly displayed.

The bubbles that are beyond the imaging capabilities of SSOCT are shown in Fig. 8. The portion circled in blue in the image is the place where the phase indicates that there could be three bubbles of diameters all around 0.8 μm (but that could be 2.8, 4.8, 6.8, or 8.8 μm). As the sizes of the bubbles are smaller than the focused beam spot (~25 μm), they act as a hindrance to the beam passage and create a shadow on the other interface as shown in Fig. 8(a).

According to our previous animal studies, whole images of the capillaries (<100 μm, e.g., capillaries in sclera are around 30 μm (Ref. 50)) could be constructed with OCT, including using in the common path mode. Moreover, to perform in vivo measurements for quantifying bubbles in highly scattering media, conventional SSOCT must be used. Hence, it is required to have a stable phase in the inter-interference mode which could be achieved, e.g., using a calibration arm as discussed in Ref. 51. The results in common path mode suggest that the same methodology can be extended to the inter-interference mode for quantifying the size of the microbubbles. For samples in which any two surfaces where the phase is measured are changing (e.g., during pulsatile blood flow), a new algorithm could be developed for the correction of the wall movement. However, in inter-interference mode, it is not a necessity for the physical distance between the surfaces to remain constant. This is because the phase at all depths are measured, and the depth containing the bubble should have a refractive index change of 0.43 assuming the average refractive index of a tissue is 1.43 which would reflect in the phase; however, experimental verification is required. The tissue is not homogeneous and the sensitivity of measurements could be affected.

The PhS-SSOCT is also tested for the detection of fast moving large bubbles. Figure 9 depicts the M-mode image of air bubbles and air gaps that encountered the beam. The sizes of the bubbles can be predicted from the depth scan (Y-axis) and the speed of the bubbles from the transverse scan.

FIG. 7. (Color online) Image of the cuvette with scattering media (a) without any bubble, (c) with a bubble, and their corresponding temporal phase response at the self-interference peak in (b) and (c).

FIG. 8. (Color online) (a) Cuvette with very small bubbles and (b) the corresponding temporal phase response.
the PhS-SSOCT to quantify microbubbles in tissues in vivo.

VI. CONCLUSIONS

In this paper, we demonstrated that the PhS-SSOCT is capable of imaging, detecting, and quantifying of large and small microbubbles in clear and scattering media. The images shown were taken using the conventional SS-OCT and the phase results were obtained using common path SSOCT. The results suggest that small microbubbles with diameter beyond imaging capabilities of the system can be detected and quantified using the PhS-SSOCT. Potentially, microbubbles with diameters of as small as 0.01 μm (that introduce a phase shift of 0.03 rad) can be detected and quantified with this method. Our future studies will focus on the development of the effective phase unwrapping algorithm that will quantify the microbubbles in both clear and blood simulated media without any 2π ambiguities. A phase stability of 0.03 rad in the inter-interference mode can be achieved by incorporating a calibration mirror in the sample arm as discussed in the reference will allow the PhS-SSOCT to quantify microbubbles in tissues in vivo.
50 M. F. Mafee, G. E. Valbasson, M. Becker et al., Imaging of Head and Neck (Thieme Medical, Stuttgart, Germany, 2004).