Flow measurement without phase information in optical coherence tomography images

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Abstract: Doppler optical coherence tomography (DOCT) is a valuable tool for depth-resolved flow measurements in tissue. However, DOCT is insensitive to flow in the direction normal to the imaging beam and requires knowledge of the phase of the demodulated signal. We present an alternative method of extracting flow information, using speckle of conventional amplitude optical coherence tomography images. Due to the pixel-by-pixel acquisition scheme of conventional OCT, time-varying speckle is manifested as a change in OCT image spatial speckle frequencies. We tested the ability of speckle to provide quantitative flow information using an Intralipid flow phantom. Over a range of velocities, the ratio of high to low OCT image spatial frequencies was shown to bear a linear relation to flow velocity. With two dimensional imaging, flow in a tube and in vivo hamster skin was visualized. This study shows the feasibility of extracting flow from OCT images in all directions without phase information.

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References and links

1. Introduction

Using phase sensitive detection in an optical coherence tomography (OCT) system, it is possible to measure the Doppler shift caused by moving scatterers. The component of the velocity vector in the plane of the incident beam causes a Doppler shift in the reflected light that can be measured. Doppler OCT (DOCT) has proven useful in mapping blood flow in various tissues, and when the angle of the flow is known it can be calibrated to calculate the actual flow velocity [1,2]. DOCT has proven valuable in measuring blood flow in various tissues including skin [3] and retina [4]. Time varying speckle, while developed separately from Doppler techniques, is essentially an equivalent method for measuring flow for line-of-sight measurements [5]. In OCT images, intensity deviations caused by speckle often degrade an image. However, speckle also carries information about tissue structure and flow, and may be used to differentiate tissues without resolvable anatomical features. Speckle analysis in OCT images could be advantageous to DOCT because it is sensitive to motion normal to the incident beam, and because it eliminates the need for phase-sensitive detection.

Speckle statistics are generally broken into first order and second order statistics. First order statistics describe speckle at a point and zero second order statistics treat the joint statistical properties of speckle at two or more points. Laser speckle statistics have been derived by Goodman [6] and speckle in OCT has been shown to be analogous [7]. This finding suggests that flow measurement techniques developed for laser speckle can be adapted to flow measurement with OCT. In the early eighties it was shown that scattering fluid motion could be measured using single-exposure speckle photography [8]. Over the exposure time of the photograph, motion of scatterers in a fluid cause the speckle pattern in those areas to decorrelate, reducing the local speckle contrast. With the advent of electronic imaging, laser speckle contrast analysis (LASCA) is now performed using a CCD array. LASCA has been used to measure blood flow in tissues such as the retina, skin, and internal organs [9].

Second order temporal speckle statistics have also been shown to carry information about the motion of scatterers. It has been found that the temporal speckle fluctuations at a point show a dependence on the mean velocity of the scatterers. The ratio of high to low frequency components of the power spectrum of speckle intensity has been shown to be a good indicator of average velocity of blood flow in skin. The term “Bio-Speckle” was coined to describe the time varying speckle in living organisms [10]. While techniques such as LASCA rely on taking an image with a finite integration time, usually on the order of a millisecond or more, the pixel-by-pixel acquisition nature of a conventional OCT system does not allow for taking relatively long integration times. For a LASCA-type technique the detector acquisition time would have to be increased and the imaging rate reduced by orders of magnitude. However,
pixels in a conventional OCT system are separated temporally as well as spatially. Therefore, we hypothesize that fluid flow in OCT images can be measured by treating sequential pixels as a sampling of a time varying speckle pattern using a technique similar to the Bio-speckle method. Unlike previous embodiments of this method, OCT offers the capability to provide a depth resolved flow profile.

2. Materials and Methods

The OCT system used in this study was a time domain system with Doppler capability, similar to one described earlier [2]. The light source was a super luminescent diode (SLD-561, Superlum Limited, Moscow, Russia), with a center wavelength of 1293nm and spectral width of 53nm. The reflected signal was sent to a solid state InGaAs detector (2011, New Focus, San Jose, CA). A lock-in amplifier (SR810, Stanford Research Systems, Sunnyvale, CA) was used to demodulate the signal. The amplifier time constant was set to 10 ms as opposed to the optimal setting for stationary samples of 30 ms, to increase the bandwidth around the lock-in frequency and enable larger Doppler shifts to be measured. The analog output from the lock-in amplifier was then converted to a digital signal within the computer. A LabVIEW (National Instruments, Austin, TX) software program coordinated movement of the reference arm, translation of the sample, and data acquisition. The reference arm mirror was a corner cube reflector mounted on a scanner (GSI Lumonics, Billerica, MA).

2.1 Initial flow study

An initial, spatially integrated, study was conducted to determine feasibility of the method. Polymide tubing with an inner diameter of 1.27mm +/- .013 mm, was connected to a syringe pump. The scattering media used was 10% Intralipid solution (Baxter, Deerfield, IL) diluted to 1%. The center of the tube was positioned at the sample arm focus, at an angle of 80.5° with respect to the OCT optical axis. One hundred twenty eight a-scans were obtained through the central axis of the tube. Each a-scan consisted of 512 axial pixels, covering the central 0.39 mm of the tube. Over this region, the flow rate varied less than 10% and was assumed constant. Twelve data sets each were taken at regional flow velocities of 4.9 to 43.6 mm/s. With the utilized system parameters, sequential axial pixels were separated by 1 µm spatially and 36 µs in time. Both amplitude and phase information were saved.

The collected information was processed by two methods. First a DOCT velocity image was formed using the method described earlier [2]. A complex short time Fourier transform was calculated using an axial sliding window 64 data points long. The centroid of the power spectrum was taken as the Doppler frequency, and flow velocities calculated. The velocity magnitudes were averaged to obtain a single value. Second, speckle analysis was performed using the amplitude information only. Four sequential axial pixels blocks were averaged to mitigate the effects of noise and single-pixel speckle caused by wide-angle multiple scattering. A MatLab (The MathWorks, Natick, MA) program was written to plot the frequency content of this time- and space-varying signal. First all a-scans were concatenated and the discrete Fourier transform taken. Each a-scan took 1/54.3 seconds to acquire so there was a strong frequency component at 54.3 Hz due to concatenation. This frequency component as well as the strong DC component were removed. Since the speckle pattern was sampled at 6944 Hz (1/(4*36µs)) the highest frequency that could be detected was 3472 Hz. The frequency range was divided evenly into 5 bins, and into each bin was placed the magnitude of the Fourier transform integrated over the respective range. Similar to the technique used in time-varying speckle [11] the ratio of the value in the forth highest frequency bin divided by the lowest frequency bin (high to low ratio, or HLR) was calculated.

2.2 Imaging flow study

The feasibility of performing speckle flow measurements using a two-dimensional phantom and in vivo OCT images was determined in a second study. The phantom was a glass capillary tube with an inner diameter of 0.73 mm, placed at an angle of 80° with respect to the optical axis. The tube was submerged in 0.5% intralipid solution, and the same solution was pumped...
through the tube with average velocities ranging from 0 to 17.5 mm/s. Images were obtained with an optical image depth of 1.5 mm, lateral range of 2 mm, 1500 pixels per a-scan, and 500 a-scans/image. Amplitude and phase data were processed as with the one-dimensional study to produce a Doppler velocity image. For the speckle flow image, sequential pixels in the amplitude image were averaged together in groups of four. This resulted in an image with 4 µm square pixels. A Matlab program was written that used a 64 x 64 pixel sliding window to compute the HLR and plot the result into a new speckle flow image. To minimize erroneous velocity values due to noise in low-signal regions of the image, a threshold was applied. To compensate for non-uniform beam diameter (focus effects), the HLR values for a known static section of the image (the first 30 a-scans) formed a correction factor that was subtracted from each a-scan in the image.

An in vivo image of hamster skin flap was also evaluated. The image was obtained from the epidermal side. The image was 1.5 mm in depth and 2.0 mm in lateral range, with 750 pixels per a-scan and 1000 a-scans per image. Two by two pixel blocks were averaged to create an image with 4 µm square pixels. In this case, the algorithm was modified slightly so that the Fourier transform was taken across, rather than down, the a-scans. This enabled approximately two orders of magnitude greater flow sensitivity.

3. Results

Changes in speckle pattern with increased flow were visible to the eye, and apparent in both Doppler and Speckle flow measurements. Figure 1 shows the computed average Doppler velocity and speckle HLR for the first study. Doppler velocity measurements saturate at higher velocities. HLR values are approximately linear for flow rates above 15 mm/s.

![Fig. 1. Graph of calculated Doppler velocity and high-low ratio (HLR) calculated from phantom experiments.](image)

Results from the two-dimensional phantom study are shown in Fig. 2. An example amplitude image (at a flow rate of 15 mm/s) is shown on the left-hand side. Processed speckle flow and Doppler images at flow rates of 0, 10, 15, and 17.5 mm/s are shown on the right. In the speckle flow images, at zero flow, the stationary Intralipid is blue (zero flow), as is the capillary tube wall due to the thresholding algorithm. As the flow velocity of the Intralipid is increased, signal is seen starting near the center of the tube, and at 17.5 mm/s, a parabolic flow profile is realized. In the Doppler velocity images, the stationary Intralipid is yellow (zero flow), and the capillary tube is seen in red. The tube is represented as a high velocity because of the low signal to noise ratio in the glass region; no thresholding algorithm was applied in the Doppler velocity algorithm. Figure 3 shows the results of applying the speckle flow algorithm to a hamster skin image. The amplitude image is shown on the left and the speckle flow image in the middle. The corresponding histology image (right) shows blood vessels in locations corresponding to high HLR in the speckle flow image.
4. Discussion

Presented here is the first application to our knowledge, of speckle analysis in OCT images to determine depth-resolved flow. OCT speckle has been used for other purposes. Gossage et al. found that speckle texture could be used to differentiate tissues even if their OCT images lacked visible feature [12]. Yu et al. used holographic OCT to measure healthy, necrotic, and poisoned tumor spheroids and noted a difference in speckle correlation time, with the autocorrelation function decaying most rapidly in the healthy tissue [13]. Qualitatively, changes in speckle in regions of flowing blood have been described earlier [14].

Speckle analysis of flow in OCT images offers an advantage over DOCT because it does not require phase sensitive demodulation. Logarithmic amplifiers and other types of envelope detection schemes are often used in OCT systems, normally with loss of phase. Therefore, speckle analysis would be useful for analyzing motion with these types of instruments. Speckle analysis also has the added benefit of being able to detect motion normal to the OCT optical axis, although it is still about an order of magnitude more sensitive to motion parallel to the optical axis. This disparity occurs because only subwavelength motion of a scatterer along the axial dimension is necessary to influence the phase of the signal, whereas to a first approximation a scatterer needs to enter or leave the focal volume in the lateral dimension to influence the phase. We have demonstrated (data not shown) that flow measurement with speckle is possible with the flow normal to the optical axis, but with reduced sensitivity.

Although time varying speckle and Doppler techniques are essentially equivalent [5] and thus should have equivalent spatial/velocity resolution and accuracy, the presented results were affected by the instrumentation and algorithms utilized. For example with HLR, attempts to use a 64 x 1 pixel window (as used for Doppler velocity measurements) were unsuccessful in producing a smooth flow profile. Thus, a 64 x 64 pixel window was used which lead to decreased spatial resolution in the flow images. Also, Fig. 2 appears to show that speckle flow provides a higher fidelity representation of the parabolic flow profile in the phantom.
experiments. However, this result is most likely due to our experimental setup and Doppler analysis technique. In addition to the limited demodulation electronic bandwidth attenuating the large Doppler-shifted frequency components, the centroid algorithm is flawed as shown by Kulkarni et al. [15]; the asymmetrical distribution of noise around the Doppler-shifted spectra causes the velocity to be underestimated making the flow profile in the tube appear flatter. These shortcomings are also the cause of saturation in computed flow velocity seen in Fig. 1.

Speckle flow measurement has some disadvantages. Most obviously, it cannot determine the direction of flow, as Doppler measurements can. Note that in Fig. 2 the speckle flow image shows only positive values, however in the Doppler image, the velocity is correctly shown to negative. Both Doppler and speckle flow techniques have an inherent tradeoff in flow sensitivity with acquisition time. For a fixed number of pixels analyzed, the Doppler frequency resolution increases with longer time between successive pixels. Similarly, the longer the time between pixels, the more motion of scatterers occurs creating more high spatial frequency content. Thus measurement of slower flows is possible. Measurement across a-scans, rather than down each a-scan, can be implemented to achieve high flow sensitivity, as was done to create the image in Fig. 3. As an alternative, in fast OCT systems successive OCT images could be averaged together then the local contrast computed in a method similar to LASCA [11].

Both Doppler and speckle analysis techniques are sensitive to non-linear motion of the reference arm mirror and to movement of the sample. The techniques developed to overcome these confounding factors in DOCT should be applicable to speckle flow measurements as well [16]. In the in vivo images, speckle flow was measured beneath the blood vessels. This may be due to the large scattering coefficient and anisotropy of blood (estimated to be 650 cm$^{-1}$ and 0.995, respectively at 1293 nm by extrapolation from Yaroslavsky et al. [17]) causing multiple forward scattering of photons, in turn causing a distortion of the deep speckle pattern. A similar effect can be seen in Doppler images. Images taken in Intralipid (0.5%), which has a smaller scattering coefficient and anisotropy (estimated to be 4.4 cm$^{-1}$ and 0.35, respectively by extrapolation from van Staveren et al. [18]), do not exhibit this effect.

Theoretically, the axial speckle size should remain constant in static media determined only by the coherence length of the source [19]. However, we found as a practical matter that the axial speckle frequency content appeared shift to lower values out of focus, and that the speckle contrast decreased. This could be due to an increased numbers of scatterers in the larger sampled volume, or to a greater amount of wide-angle scattering. Hardware changes that could eliminate focus effects are the use of dynamic focus (with or without lateral priority scanning such as is used in optical coherence microscopy) and axicon lenses. If a region of zero flow is known, calibration as performed in this study worked well to mitigate focus effects. It may be that to a first order, focus effects could be calibrated out of the system using a standard of similar optical properties to the tissue being investigated.

There are a number of future studies that should be done to study potential of this new technique. It has been found when using second order temporal statistics in direct imaging medical applications that different types of tissue have different speckle frequency distributions. For instance, the calibration of HLR to flow rate shown in Fig. 1 could not be applied to blood flow in hamster skin. Individual calibration could be performed for tissue of particular optical properties and blood of a given hematocrit. HLR has proven to work well in direct imaging of skin blood flow, but it has been shown that a different quantity, the mean frequency of the spectral distribution, was a better indicator of blood flow in internal organs [10]. Since there is limited power in the high frequency bins at low flow rates, HLR is non-linear under these conditions, as seen in Fig. 1. Therefore, when applying speckle analysis to OCT images of biological samples it may be necessary to find what feature of the spectral distribution is the best indicator of velocity for the particular tissue being imaged. Future studies will perform a calibration for blood flowing in biological tissue phantoms.

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