Quantification of optical Doppler broadening and optical path lengths of multiply scattered light by phase modulated low coherence interferometry

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Abstract: We show experimental validation of a novel technique to measure optical path length distributions and path length resolved Doppler broadening in turbid media for different reduced scattering coefficients and anisotropies. The technique involves a phase modulated low coherence Mach-Zehnder interferometer, with separate fibers for illumination and detection. Water suspensions of Polystyrene microspheres with high scattering and low absorption levels are used as calibrated scattering phantoms. The path length dependent diffusion broadening or Doppler broadening of scattered light is shown to agree with Diffusive Wave Spectroscopy within 5%. The optical path lengths are determined experimentally from the zero order moment of the phase modulation peak around the modulation frequency in the power spectrum and the results are validated with Monte Carlo simulations.

OCIS codes: (170.3340) Laser Doppler velocimetry; (120.3180) Interferometry; (170.4580) Optical diagnostics for medicine; (170.3890) Medical optics instrumentation

References and links

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we pursue quantitative optical Doppler flowmetry by means of path length selectivity. Prevents calibration from leading to a more quantified assessment of perfusion. In this study, the physiological and anatomical complexity of the microcirculation complicates the noninvasive diagnosis of tissue with light. For example, in laser Doppler blood perfusion monitors (LDPM), the coherent light delivered into the tissue interacts with static as well as moving scatterers, e.g. red blood cells and it records values averaged over different and basically unknown path lengths. This creates an uncertainty in the relation between the measured perfusion signal and the real perfusion [1]. A longer path length will increase the probability that a Doppler shift will occur, thus yielding an overestimation of the blood perfusion, compared to the short path length situation. The distance between illumination and detection fibers also influences the perfusion signal and for large distances the average path length followed by the detected photons through the tissue increases and therefore a larger Doppler shift will be detected [2, 3, 4]. Thus detection of multiple scattered light as function of path length in the scattering medium would result in more quantitative and more reliable tissue perfusion information. Another step in more quantified LDPM would be to develop a calibration procedure. For the calibration of LDPM, different calibration models such as mixed static and dynamic media [5], optoelectronic calibration [6], layered scattering phantom [7] were developed. The present calibration procedure based on motility standard utilizes the Doppler shift imparted by the Brownian motion of polystyrene microspheres in a water suspension. These efforts have been important for the quality assurance of the technique, however the physiological and anatomical complexity of the microcirculation prevents calibration from leading to a more quantified assessment of perfusion. In this study, we pursue quantitative optical Doppler flowmetry by means of path length selectivity.

Path length resolved temporal fluctuations of photon intensity can be measured using amplitude modulation of the light intensity [8], time resolved measurements [9] or recently
developed low coherence interferometry [10-11]. However, for a spatial resolution of 50 micrometers, time resolved and amplitude modulation techniques require either a temporal resolution of 150 fs or electronics working in the GHz range. For this reason, for optical path lengths of only a few millimeters a low coherence interferometric approach is much more suitable. In low coherence interferometry, a user-positioned coherence gate selects the light that has traveled a known optical path length in the medium to interfere with reference light.

Unlike most coherence gated interferometric geometries used in path length resolved measurements such as optical coherence tomography (OCT), utilizing a Michelson interferometer with on axis back reflection [10-11], we use a Mach-Zehnder interferometer with positions for illumination and detection separated by a distance of at least ten times the scattering mean free path [12-13]. In previous studies we validated optical path length distributions by quantifying the Lambert-Beer predictable effect of varying absorption levels [13-14]. Furthermore it was demonstrated that dynamic properties of turbid media can be measured independent of optical absorption, when absorption levels are in the range for biological tissues. For both the Michelson [10-11] and the Mach-Zehnder based interferometric setups [12-13], these low coherence interferometric measurements, however depended on the photons that are Doppler shifted by the Brownian motion of the particles in the medium. Recently we showed that optical path length distributions and path length resolved Doppler shifts of multiply scattered light in both static and dynamic turbid media can be extracted from the spectral peak that was generated by phase modulation of the reference arm [14]. By phase modulation in the reference arm, not only light which has been Doppler shifted in the sample, but also light scattered by static structures will contribute to the interferometric signal. This will enable to extract path length resolved information from mixed static and dynamic turbid media, such as flow of moving red blood cells within static tissue matrices during blood perfusion measurements. Furthermore, phase modulation will enhance the signal-to-noise ratio since the signal component generated by phase modulation can be shifted to higher frequencies than the signal component caused by mutual interference of Doppler shifted light, which occupies the low frequency range of the spectrum. We also showed that the dynamic properties of a medium can be measured independent of its optical absorption properties, at least when absorption levels are applied in the range found for biological tissues.

In this manuscript, we show the optical path length distributions and spectral diffusion broadening of multiple scattered light measured for calibrated scattering samples with high scattering and low absorption levels. While in ref. [14] we validated the measured optical path length distributions using Lambert-Beer’s law on samples with different absorption levels, in this letter we validate with Monte Carlo simulations for optical path length distributions. In addition the path length dependent diffusion broadening or Doppler broadening is validated with Diffusive Wave Spectroscopy.

2. Materials and method

We use a fiber-optic Mach–Zehnder interferometer with a superluminescent diode (Inject LM2-850, \( \lambda =832\text{nm}, \Delta \lambda_{\text{FWHM}}=17\text{ nm}, \) coherence length \( L_c=18\ \mu\text{m} \)) that yields 2 mW of power from the single-mode pigtail fiber as the light source. Single mode fibers (mode field diameter=5.3 \( \mu\text{m}, \) NA=0.14) are used for illumination, while multimode graded-index fibers (core diameter =100 \( \mu\text{m}, \) NA=0.29) are used for detection, providing a large detection window and a small modal dispersion. The reference beam is polarized using a linear polarizer and the phase is sinusoidally modulated at 22 kHz using an electro optic broadband phase modulator (New Focus Model 4002). The amplitude of sinusoidal phase modulation applied to the modulator is set for a modulation angle of less than 1.57 radians so that frequency sidebands are absent in the spectra. The photodetector signal was sampled for 52 seconds to get an average of 1000 spectra and was measured in steps of 100 microns in air. The setup has been described in more detail elsewhere [14].
In that preliminary study, we showed that the path length distribution can be measured from the area (the zero order moment $M_0$) of the Doppler broadened interference peak appearing at the modulation frequency in the photodetector signal power spectrum [14]. Noise correction is performed by subtraction of $M_0$ of the reference arm noise and of the sample arm Doppler signal from $M_0$ of the corresponding total spectra in the frequency range of 20 kHz to 24 kHz around the phase modulation frequency of 22 kHz. The zeroth moment $M_0$ of the noise corrected heterodyne spectrum is proportional to the intensity of photons with a certain optical path length. The average Doppler shift is measured from the full width at half maximum (FWHM) of a Lorentzian fit of the Doppler broadened phase modulation interference peak appearing in the photodetector signal power spectrum, exhibiting diffusion broadening obeying Einstein-Stokes relation.

Diffusive Wave spectroscopy (DWS), which is an extension of conventional dynamic light scattering (DLS) to the limit of multiply scattering media, relies on the diffusion approximation (DA), to describe the diffusive light transport from the intensity autocorrelation of scattered light [15-19]. DA has been extensively used in characterizing the dynamical properties of physical and biological media [16, 21, 25]. The accuracy and domain validity of the diffusion approximation [20-22] has been studied as regards experimental geometries [21, 23] and fundamental limits [24-25] and the theory has been extended to describe the crossover between the single scattering and the diffusive regimes [26].

According to the fluctuation-dissipation theorem, the power spectrum of light that is scattered by a monodisperse suspension of particles undergoing Brownian motion and is heterodyne detected is a Lorentzian distribution,

$$P(f) = \frac{1}{f_0^2} \frac{A}{1 + \left(\frac{f}{f_0}\right)^2}$$

where $A$ is the amplitude of the power spectrum and $f_0$ is the linewidth [17-18]. According to DWS theory, in the case of diffusive scattering, the power spectrum of diffusive light that is heterodyne detected is Lorentzian and the linewidth, $\pi f_0 = k^2 D_B L (1 - g) / l$ depends on the on the self-diffusion coefficient, ($D_B = K_B T / 6 \pi \eta a$) of the particles in Brownian motion [10]. Here $k$ is the wave number in the scattering medium, $l$ is the photon mean free scattering path, $g = \langle \cos \theta \rangle$ is the scattering anisotropy of the medium, $L$ is the geometrical photon path length (Optical path length/refractive index of water), $K_B$ is the Boltzmann constant, $T$ is the temperature (293 K), $\eta$ is the viscosity of the suspending liquid ($\eta = 1.0$ cps for water) and $a$ is the hydrodynamic diameter ($\varnothing 0.20$ and $\varnothing 0.77$ μm) of the scattering particles [11,28]. Water suspensions of Polystyrene microspheres (Polysciences Inc) with diameters of $\varnothing 0.20$ μm (anisotropy factor, $g=0.18$) and $\varnothing 0.77$ μm ($g=0.85$) are used to make calibrated scattering phantoms. Samples with reduced scattering coefficients ($\mu_s'\) of 7.00 ($g=0.85$), 4.95 and 3.25 mm$^{-1}$ ($g=0.18$) and absorption coefficient ($\mu_a$) of 0.001 mm$^{-1}$, are made from each particle suspension, based on scattering cross sections following from Mie theory calculations, taking into account the wavelength of the laser light of 832 nm and the refractive index of water. This corresponds to photon mean free scattering path of 22, 166 and 252 μm respectively. A cubic glass cuvette (20*20*20 mm) is used as a sample holder. We have used samples with high scattering and low absorption levels so that the medium’s absorption is negligible compared to its scattering level and the propagation of the multiply scattered photons can be described as a diffusion process. This gives DWS maximum possible validity, even for short optical path lengths in our measurements. In DWS measurements, a thick slab of a random medium with sample thickness much greater than the transport mean free path $l^*$ is used so that the number of scattering events is large and the diffusive transport
criteria is satisfied. For smaller thickness, the failure of diffusion theory predictions has been observed experimentally [21, 22, 25].

To verify our path length resolved measurements, we have performed Monte Carlo simulations to predict the optical path length distributions [27]. A two-layer system is defined in which light (\( \lambda = 832 \text{nm} \)) from a fiber with a diameter of 100 \( \mu \text{m} \) is randomly scattered in a layer of water suspension of polystyrene micro spheres (\( \mu_a = 0.001 \text{ mm}^{-1} \)) with a thickness equal to that of the cuvette (20 mm). Since the scattering properties and the particle number density of calibrated polystyrene sphere suspensions as used in this study are well known, it is possible to exactly mimic these properties in simulations. The parameters used for the scattering samples are refractive index, 1.33; absorption coefficient, 0.001 mm\(^{-1}\); Henyey–Greenstein scattering function, g=0.18 (\( \varnothing = 0.20 \mu \text{m} \)) and g=0.85 (\( \varnothing = 0.77 \mu \text{m} \)), reduced scattering coefficients (\( \mu_s' \)) of 7.00 (g=0.85), 4.95 and 3.25 mm\(^{-1}\) (g=0.18). The second layer is defined with a high absorption (\( \mu_a = 10 \text{ mm}^{-1} \)) in order to avoid the possible long path length photons that may penetrate beyond the first layer. But we estimated from the statistics of the maximum scattering depth of Monte Carlo simulated detected photons that there are no photons reaching a depth beyond 10 mm in a scattering sample with lowest scattering level. So in this case there is no influence of highly absorbing second layer on the detected photons. Both the fibers are defined inside the scattering sample. In all simulations, 100000 photons are injected into the sample, and each photon returning to the detection fiber (fiber diameter= 100 \( \mu \text{m} \), fiber separation=300 \( \mu \text{m} \), NA=0.29) is assumed to be detected, and its optical path length is recorded.

### 3. Results and discussion

Typical phase modulation peaks appearing in the power spectra measured in our experiments for \( \varnothing = 0.20 \mu \text{m} \) and \( \varnothing = 0.77 \mu \text{m} \) microspheres for different reduced scattering coefficients (7.00, 4.95 and 3.25 mm\(^{-1}\)) and at two geometrical photon path lengths (1 and 2 mm) are shown in Fig. 1. The interference peak appearing at the modulation frequency is fitted with a Lorentzian function. The line shape is Lorentzian in all cases and it can be characterized by its amplitude and line width.

The experimental optical path length distributions and simulation results for isotropic and anisotropic scatterers are shown in Figs. 2 and 3, respectively. The results are normalized to the maximum value obtained in the sample with higher scattering coefficient. There is a good agreement between the experimental data (squares and triangles) and the simulation results (solid curve).
Fig. 1. Phase modulation peaks appearing in the photodetector signal power spectrum fitted with Lorentzian functions.

Fig. 2. Measured (points) and Monte Carlo simulated (lines) optical path length distributions for a particle suspension of $\varnothing 0.20 \mu m$ ($g=0.18$) for two scattering levels.
Fig. 3. Measured (points) and Monte Carlo simulated (line) optical path length distributions for a particle suspension of $\varnothing 0.77 \mu m$ ($g=0.85$).

Fig. 4. Experimental (points) and DWS-predicted (lines) average Doppler shift (FWHM of the Lorentzian fit to the phase modulation peak), vs. optical path length, for a particle suspension of $\varnothing 0.20 \mu m$ ($g=0.18$). Inset and lower line in main graph: prediction for single scattering.

Figures 4 and 5 show the measured FWHM of the fitted Lorentzian spectra vs. the optical path length of the multiple scattered light, compared with the predicted linewidth based on Diffusive Wave Spectroscopy. The data points are the experimentally measured Doppler shift and the lines indicate the path length dependent Doppler broadening predicted by Diffusive Wave Spectroscopy. As depicted in Figs. 4 and 5, the average Doppler shift increases with the
optical path length and for the suspension with g=0.18 (Fig. 4), the average Doppler shift decreases with a decrease in reduced scattering coefficient. This can be attributed to the decrease in the number of scattering events per unit optical path length. The experimental results are in good agreement with the predictions of DWS for optical path lengths up to 4.5 mm. For large optical path lengths, the amplitude of the interference signal is low and an estimation of the line width based on the Lorentzian fit to the spectra results in significant errors. In Figs. 4 and 5, theoretical predictions are given of the linewidth broadening for single scattering as a function of the optical path length, where we used the expression $f_0 = q^2 D_0$, with photon momentum transfer $q = 2k \sin \theta / 2$ a function of the scattering angle $\theta$.

In our dual fibre geometry, the value of $\sin(\theta/2)$ for singly scattered light increases with the optical path length, causing the single scattering Doppler broadening to increase with the optical path length, which is shown in Figs. 4 and 5. Assuming a uniform index of refraction and neglecting the dimensions of the fiber facets, a given optical path length is realized for single scattering in all positions on an ellipsoidal surface of which the focal points coincide with the fiber tips. Only that part on the ellipsoidal surface which is within the common volume of the angular apertures of both fiber will contribute to single scattering. On a given ellipsoid the value of $\theta$ will slightly vary, the consequence of which is indicated by the error bars in the single scattering data in Figs. 4 and 5. For a particle suspension of $\varnothing 0.20 \mu m$, the power spectra measured in the regime of shorter optical path lengths up to three scattering mean free paths have a linewidth that agrees with single scattering (136 Hz) for the scattering medium with lower reduced scattering coefficient ($\mu_s' = 3.25 \text{ mm}^{-1}$). In previous reports on path-length resolved DLS spectroscopy, singly scattered light has been observed for short optical path lengths [10-11] and they showed the dependence of detection of multiply scattered light on the geometry of the detection optics and on the anisotropy of the scattering [10] and a theoretical model was developed to predict this transition regime across the full range of path lengths from single scattering through diffusive transport [11]. In the case of a particle suspension of $\varnothing 0.77 \mu m$, we observe that the measured linewidth for short optical
path lengths is above the calculated linewidth for singly scattered light (35Hz). This is expected, since the photon mean free scattering path of 22 μm indicates that light is scattered multiple times (~20) before being detected and can be described as a diffusion process even for the shortest optical path length of 0.5 mm.

4. Conclusion

To summarize, in this manuscript we present path length distributions and path length dependent diffusion broadening of multiple scattered light from turbid media for different reduced scattering coefficients and anisotropies, where the particle dynamics are governed by Brownian motion. The path length dependent diffusion broadening of scattered light showed good agreement with the predictions of Diffusive Wave Spectroscopy. Good agreement between experimental path-length distributions and Monte Carlo simulations were found. Hence, we can use this method to measure optical path lengths and path length resolved Doppler information from general turbid media. The experimental approach presented here is not restricted to the study of Brownian motion or to completely dynamic samples. In an earlier study, we demonstrated that the path length distributions could be obtained from mixed static and dynamic turbid media [14] and path length resolved Doppler information obtained from the width of the modulation peak can be used to determine the Brownian and translational movement of moving particles within static matrices, such as microcirculatory blood flow in tissue.

Acknowledgments

This work was sponsored by the Netherlands Technology Foundation STW (Grant TTF 5840) and the Biomedical Technology Institute of the University of Twente.