Discrimination between Doppler-shifted and non-shifted light in coherence domain path length resolved measurements of multiply scattered light

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Abstract: We show a novel technique to distinguish between Doppler shifted and unshifted light in multiple scattering experiments on mixed static and dynamic media. With a phase modulated low coherence Mach-Zehnder interferometer, optical path lengths of shifted and unshifted light and path length dependent Doppler broadening are measured in a two-layer tissue phantom, with a superficial static layer of different thickness covering a semi-infinite dynamic medium having identical optical properties. No Doppler broadening is observed until a certain optical path length depending on the thickness of the superficial static layer. From the minimum optical path length corresponding to the Doppler-shifted light the thickness of the static layer that overlies the dynamic layer can be estimated. Validation of the experimentally determined thickness of the static layer is done with the Doppler Monte Carlo technique. This approach has potential applications in discriminating between statically and dynamically scattered light in the perfusion signal and in determining superficial burn depths.

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

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1. Introduction

Laser Doppler flowmetry (LDF), a non-invasive technique for monitoring blood microcirculation, characterizes the time-varying signal arising from the temporal variations in the speckle pattern to estimate the perfusion in biological tissues [1]. In this technique, the coherent light delivered into the tissue through an optical fiber follows different trajectories and reaches different depths and is guided to the detector through a spatially separated light-collecting fiber. Light which is Doppler shifted by moving red blood cells will be mixed with the non-frequency shifted light scattered from surrounding static tissue matrices, resulting in photodetector current fluctuations. Perfusion is measured based on the spectral analysis of the frequency components of this fluctuating photocurrent. However, skin perfusion measurements are complex not only due to the heterogeneous vascular structure and but also due to the variance in the penetration depth of the detected photons. The depth of light penetration in the tissue depends on the wavelength of the light source, the distance between the transmitting and receiving fibers and the optical properties of the tissue. Thus for a constant perfusion, the LDF output signal is affected by probe induced variations and by the changes in the tissue optical properties in terms of absorption and scattering [2]. This dependence leads to uncertainties in interpreting the Doppler shifted and non-shifted fraction of photons and also in discriminating the fraction of light scattered from superficial (nutrient) and deeper (thermoregulatory) layers of skin. As such, depth resolved perfusion information could be obtained by varying the distance between transmitting and receiving fibers [2] or by using a multichannel laser Doppler probe [3]. However, these methods still give no control over the optical path length traveled by the detected light. Coherence domain path length resolved optical Doppler perfusion monitoring, of which the basic technique is presented in this work, may overcome this limitation of conventional LDF techniques and will enable to correctly interpret the inter-and intra-individual variations in the LDF readings introduced by the variance in individual photon path lengths (e.g., length and depth) due to changes in tissue optical properties.

The technique involves a phase modulated low coherence Mach-Zehnder interferometer with two spatially separated light-delivering and light-collecting fibers as used in conventional laser Doppler perfusion monitors [4]. Compared to both the Michelson [6,7] and the Mach-Zehnder [8,9] based interferometric measurements that depended only on the Doppler shifted fraction of photons, our method is able to measure path length resolved information of non-shifted and Doppler shifted fractions of photons. In a preliminary study, we showed that path length resolved Doppler information from static and dynamic turbid media can be obtained with increased signal-to-noise ratio by sinusoidally modulating the phase of the light in the reference arm [4]. An example of the practical relevance of this approach is in measuring path length resolved information from mixed media, such as flow of moving red blood cells within static tissue matrices. Furthermore, we validated the optical path length distributions and path

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length dependent diffusion broadening of multiple scattered light with Monte Carlo simulations and Diffusive wave spectroscopy respectively [5].

In this study, we will demonstrate that using phase modulated low coherence interferometry, Doppler-shifted and non-shifted multiply scattered light from a mixed static and dynamic scattering medium can be distinguished and the optical path length of each can be measured. The technique was used on a two-layer tissue phantom, with a superficial static layer of different thickness covering a semi-infinite dynamic medium with identical optical properties. From the shape of the Doppler broadened phase modulation peak in the power spectrum, the Doppler shifted fraction of photons and their Doppler distribution can be estimated. Since the photons with large optical path lengths have greater probability to penetrate deep into the dynamic layer and thus to get Doppler shifted, we expect that the shortest optical path length at which the Doppler shifted light can be detected should correlate with the thickness of the static layer. We have performed Monte Carlo simulations to predict this transition optical path length, which is dependent on the thickness of the superficial static layer.

2. Materials and methods

We use a fiber-optic Mach–Zehnder interferometer with a superluminescent diode (Inject LM2-850,λ=832nm, $\Delta\lambda_{FWHM}$ =17 nm, coherence length L_C =18 μm) that yields 2 mW of power from the single-mode pigtail fiber as the light source. Single mode fibers (mode field diameter=5.3 μm, NA=0.14) are used for illumination, while multimode graded-index fibers (core diameter =100 μm, NA=0.29) are used for detection, providing a large detection window and a small modal dispersion. This scheme offers greater flexibility, since the distance between the illumination and the detection fiber can be varied, giving one some control over the relative depth sensitivity of the system. Since the use of single mode fibers does lead to a too low signal level in a dual fiber geometry as used in conventional laser Doppler perfusion monitors, we take the advantage of multimode fibers to collect sufficient scattered light at large fiber distances. We demonstrated [4-5, 8-9] that use of graded index multimode fibers allows for the detection of optical path length distributions of multiply scattered light without degradation in the path length resolution compared to single mode fibers. Furthermore, the optical path length distributions measured in scattering media with different reduced scattering coefficients and anisotropies did not exhibit significant effect of intermodal dispersion of multimode fibers [5]. 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 AC photocurrent from the detector (New Focus Model 2001 photo receiver) is measured with a 12 bit analogue to digital converter (National Instruments), sampling at 50 kHz for 52 seconds to get an average of 1000 spectra. Fourier transformation and squaring yields the power spectrum of the signal. The setup has been described in more detail elsewhere [4,5].

The scattering medium (Fig. 1) that is used to study the depth sensitivity of our method consists of a semi-infinite dynamic layer of thickness 20 mm under a static layer of varying thickness (0.1-0.9 mm). The static and dynamic scattering phantoms are prepared with polystyrene microspheres of ∅ 0.77 μm to get the same optical properties (μ'_s=2.0 mm^{-1}, g=0.85), based on scattering cross sections following from Mie theory calculations. The static layer is prepared by mixing 4% (by weight) aqueous solution of poly (vinyl alcohol) (PVA) with the water suspension of polystyrene microspheres and 4% (by weight) aqueous sodium borate (borax) solution (cross-linking agent). PVA with a degree of hydrolysis greater than 99%, and an average molecular weight (MW) of 85000–140000 (Sigma-Aldrich, catalog nr 36 314–6), is used to prepare the aqueous solutions. The amount of borax solution added to the whole volume of the PVA-water-PS suspension (12 ml) is about 1.0 ml to produce a static gel medium. The static layer is placed in between two thin glass slides of thickness 150 μm

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and two spacers are placed to get the desired thickness for the static layer. Optical phantoms based on PVA gel are used for tissue mimicking phantoms in diffuse optical tomography [10]. Tissue perfusion within deeper layers was represented by particles in Brownian motion.

The path length distribution and average Doppler shift is measured from the noise corrected zero order moment M_0 (area under the peak) and FWHM of the Doppler broadened interference peak appearing at the modulation frequency in the photodetector signal power spectrum [4,5]. The power spectrum of photocurrent fluctuations of light that is scattered by a monodisperse suspension of particles undergoing Brownian motion and that is heterodyne detected is a Lorentzian distribution. The average Doppler shift, which depends on the scattering properties and photon path length within the strongly multiply scattering medium can be obtained from the FWHM [4,5,11]. The zeroth moment M_0 of the heterodyne spectrum calculated around the modulation frequency of 22 kHz within a bandwidth of 2 kHz is the total power of the photocurrent fluctuations caused by the interference of reference light with the scattered light from the sample and it corresponds to the total intensity of photons with a certain path length [4]. The FWHM of the interference signal is about 50 Hz when a mirror or a statically scattering medium replaces the sample. Thus, the noise corrected M_0 in a bandwidth of 50 Hz at the modulation frequency corresponds to the intensity of non-shifted photons. The fraction of Doppler shifted photons (*f*) can be obtained by filtering out this contribution of non-shifted photons at the modulation frequency from the total intensity of photons. For a given thickness of the superficial static layer, the fraction of Doppler shifted and non-shifted photons for all optical path lengths can be estimated from the area of the corresponding optical path length distributions.

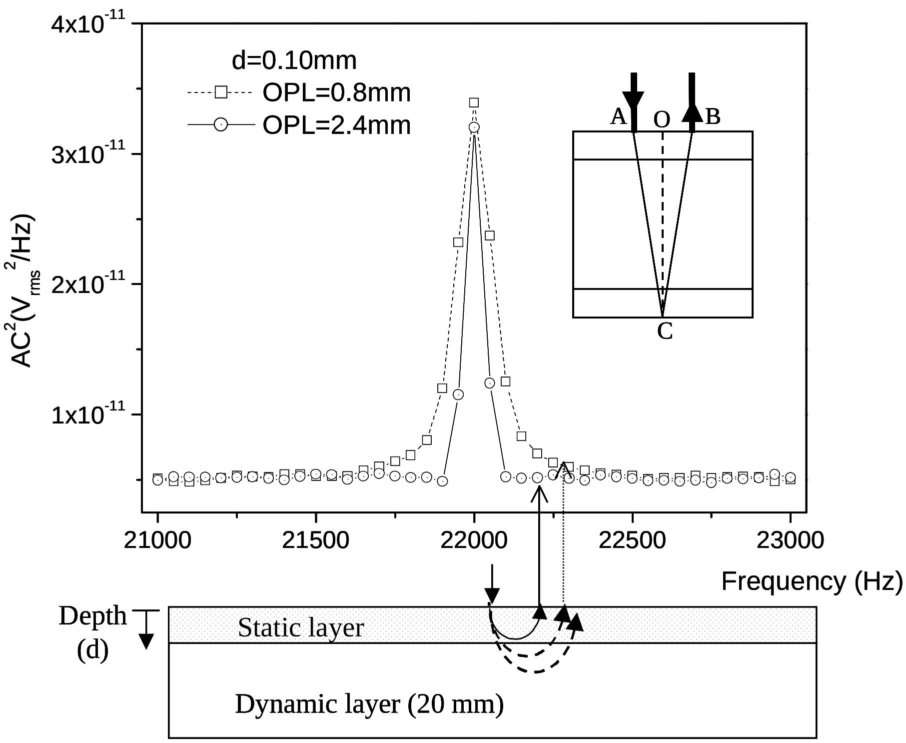

Fig. 1. Typical modulation peak appearing in the power spectra measured for short and large optical path lengths (Top). Schematic diagram of the light propagation through a two-layer scattering phantom, consisting of a top static layer and a bottom dynamic layer with same optical properties (Bottom). The model used for the estimation of the thickness (Inset).

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The photons with large optical path lengths have greater probability to penetrate into the deeper layers corresponding to the dynamic medium and thus will be Doppler shifted. The smallest optical path length corresponding to the Doppler-shifted light is related to the thickness of the static layer and may provide a non-invasive method of estimating the thickness of the static layer. We consider the minimum optical path length (L_{min}) of the Doppler-shifted light to correspond to the shortest possible trajectory of photons between the input fiber and the detection fiber via the top interface of the dynamic layer. Assuming that this shortest path length of Doppler shifted photons corresponds to photons that go in an unscattered or snaky way between two fibers, the thickness of the static layer can be estimated (see inset in fig.1). For simplicity, if the refraction effects through the glass layers of thickness 150 μm are neglected, then the half of the geometric path length corresponding to the observed minimum optical path length (refractive index, n_{medium}=1.33 and n_{glass}=1.5 for two glass plates) corresponds to the hypotenuse (CA) of the assumed right angled triangle. Here the base of the triangle is equal to the half of the fiber separation (r) of 0.15 mm (AO), and the thickness of the static layer can be obtained from the third side (OC). To verify the relation between the thickness of the static layer and this transition optical path length, we have performed Doppler Monte Carlo simulations [12]. We have used an optical path length resolution of 100 and 80 μm in the experiment and the Monte Carlo simulations. The total thickness of the superficial layer is estimated from the average of minimum optical path of Doppler-shifted and maximum optical path length for which only non-shifted light was observed. Thus the variations in the shortest optical path length corresponding to the Doppler-shifted light can be ± 50 and $\pm 40 \mu m$ for experiment and Monte Carlo simulations respectively.

In the Monte Carlo simulation [12], a five-layer system is defined in which light (λ =832nm) from a fiber with a diameter of 100 μm is delivered into a scattering medium consisting of a glass layer, a static scattering layer, a glass layer, and a dynamic layer with the same optical properties as the static scattering layer. 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 static and dynamic scattering samples are refractive index, 1.33; absorption coefficient, 0.001 mm^{-1}; Henyey–Greenstein scattering function, g=0.85 (∅0.77), reduced scattering coefficients (μ_s') of 2.0 mm^{-1} (g=0.85). The thickness of the two thin glass slides and the semi-infinite dynamic media were 150 μm and 20 mm respectively. The thickness of the superficial static layer was varied from 0.1 to 0.9 mm. Brownian motion of the particles within the dynamic layer was modeled by assigning a Gaussian velocity distribution with an average velocity of 0.1mm/s with random direction. The fifth layer is defined with a high absorption ($\mu_a = 10$ mm^{-1}) to increase the simulation speed by removing from the simulation a minority of long path length photons. 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, $1*10^7$ photons are injected into the sample, and each photon returning to the detection fiber (fiber diameter= 100 μm, fiber separation=300 μm, NA=0.29) is assumed to be detected, and its optical path length is recorded.

3. Experiments and results

Figure 1 shows the typical modulation peak appearing in the power spectra measured with a fiber distance of 300 μm for optical path lengths of 0.8 and 2.4 mm in the sample. The scattering medium consists of a static layer of thickness 0.1 mm overlying a dynamic layer of thickness 20 mm. For short optical path lengths, the reference light interferes with the photons scattered from the superficial static layer and are not Doppler shifted (circles and solid line).

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For large optical path length, the width of the peak is broadened due to the Doppler shifts imparted to the deeply penetrated multiply scattered photons by the Brownian motion of particles (squares and dashed line).

The optical path length distributions measured for the scattering media consisting of a dynamic layer (20 mm) under a static layer of thickness 0.1 and 0.7 mm with identical optical properties are shown in fig. 2 and fig. 3, respectively. By separating the 50 Hz bandwidth signal from the 2 kHz bandwidth signal, the optical path length distributions of the Doppler and non-Doppler shifted photons could be determined. For a static layer thickness of 0.1 mm, for optical path lengths up to 0.7 mm, the contributions of Doppler-shifted and non-shifted fractions of photons are equally strong, whereas the majority of the photons will be Doppler shifted for larger optical path lengths (Fig. 2). This is expected, since the photon mean free scattering path of 78 μm indicates that 28% of the photons will be scattered for the first time in the dynamic layer and thus will get Doppler-shifted. Many photons scattered for the first time in the static layer will be forwardly scattered into the dynamic layer. For a sample with a static layer thickness of 0.7 mm, the optical path length distributions of the Doppler and non-Doppler shifted photons are changed (Fig. 3). For this sample, the non-shifted fraction of photons exceeds the Doppler-shifted fraction up to 2.1 mm. For larger optical path lengths the majority of the light is Doppler shifted. When both fractions of photons averaged over all optical path lengths are estimated, the Doppler-shifted photons is decreased from 0.78 to 0.40 when the thickness of the static layer is increased from 0.1 to 0.7 mm. The fractions of Doppler shifted and non-shifted photons estimated for different thicknesses of the static layer are shown in fig. 4. As expected, the non-shifted fraction of photons exceeds the Doppler shifted fraction as the thickness of the superficial static layer increases.

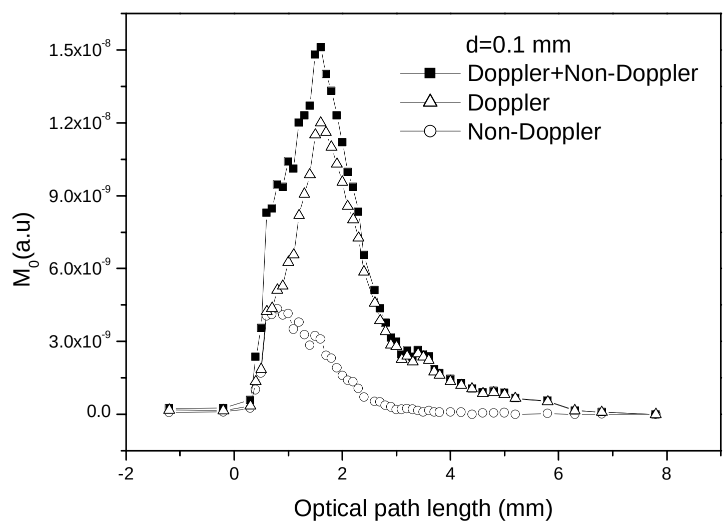

Fig. 2. Optical path length distributions of non-shifted and Doppler shifted photons, measured for a two-layer tissue phantom consisting of a top static layer of thickness 0.1 mm and a bottom dynamic layer of thickness 20mm.

The average Doppler shift, measured from the FWHM of Doppler broadened phase modulation interference peak is represented in fig. 5 as a function of the optical path length. As depicted in fig. 5, in dynamic media, the average Doppler shift increases with the optical path length. But, when a thin layer of statically scattering medium is introduced on top of the dynamic media, the width measured for shorter optical path lengths corresponds to statically scattered light. For longer optical path lengths, the width of the peak increases with path

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length as the photons scattered from the dynamically scattering layer contribute to the interference signal. The optical path length at which the transition from the statically scattered to dynamically scattered light is detected depends on the thickness of the static layer.

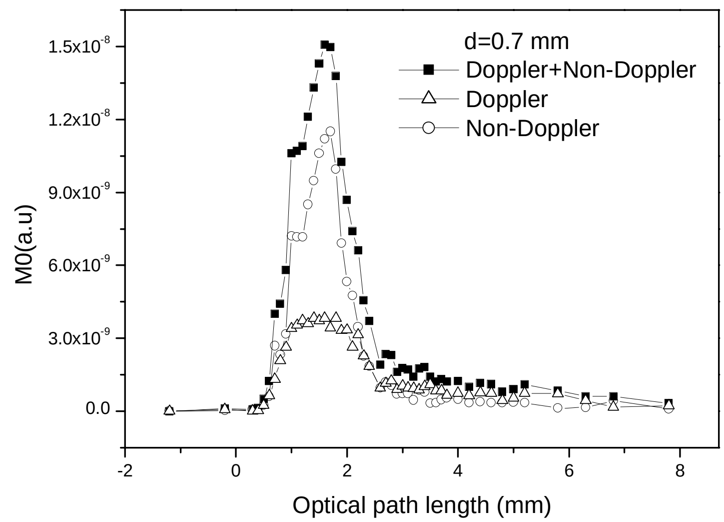

Fig. 3. Optical path length distributions of non-shifted and Doppler shifted photons, measured for a two-layer tissue phantom consisting of a top static layer of thickness 0.7 mm and a bottom dynamic layer of thickness 20mm.

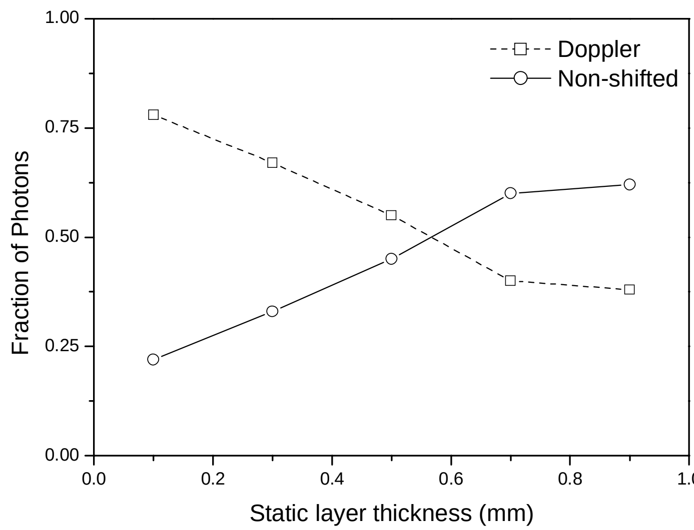

Fig. 4. The fraction of Doppler shifted and non-shifted photons (summed over all optical path lengths) estimated for different thickness of the superficial static layer.

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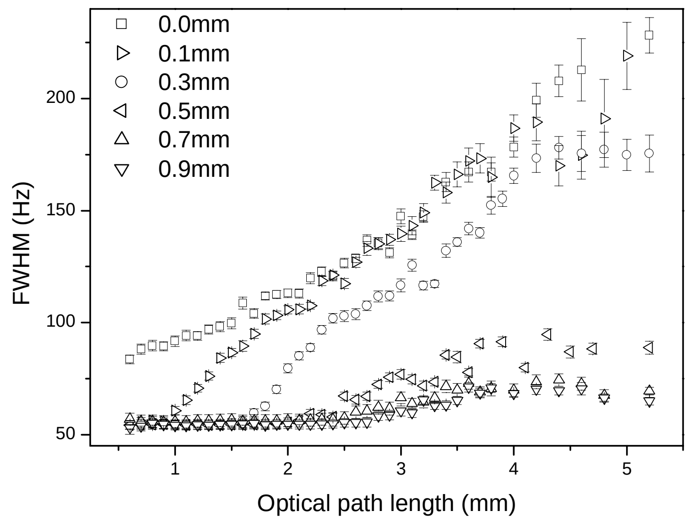

Fig. 5. The average Doppler shift extracted from the FWHM of the phase modulation peak, as a function of the optical path length through the scattering medium, for various thickness of the static layer (0-.0.9 mm).

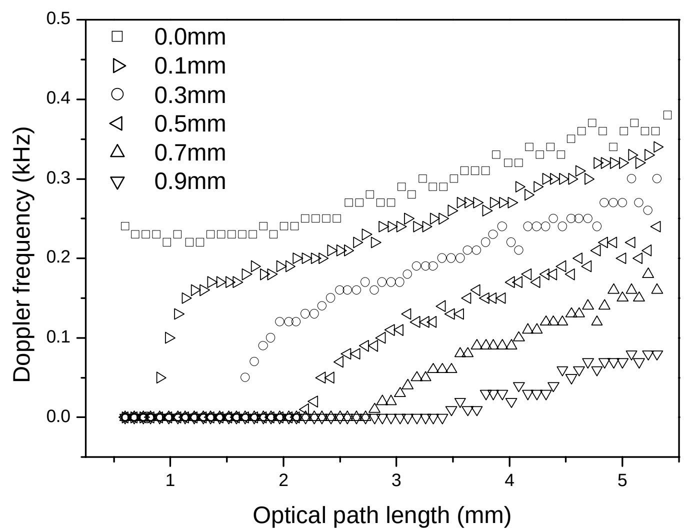

Fig. 6. The path length dependent Doppler broadening of multiply scattered light obtained from Monte Carlo simulations for various static layer thickness (0-0.9 mm).

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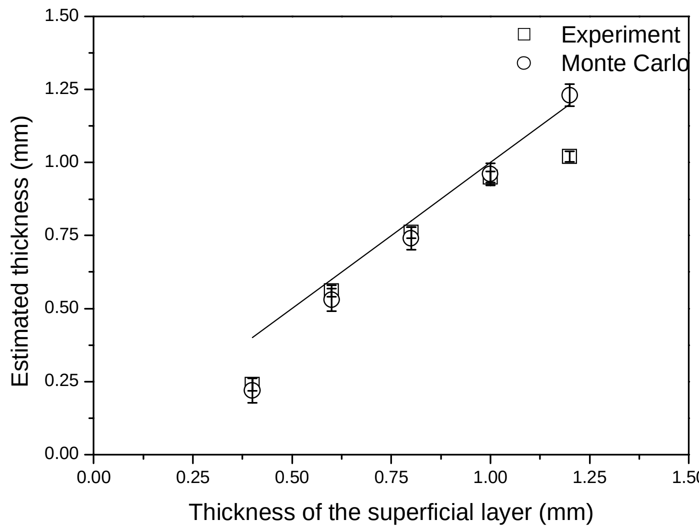

Fig. 7. Thickness of the superficial static layer (including two glass layers) estimated from the path length dependent Doppler broadening. Experimental results (squares) and Monte Carlo simulations (circles).

To verify the dependence of minimum optical path length of Doppler-shifted photons on the thickness of the superficial static layer, we have performed Doppler Monte Carlo simulations. The results of simulations are shown in fig. 6. For short optical path lengths, no Doppler shifted light is measured and the minimum optical path lengths corresponding to the dynamically scattered light increases with increasing thickness of the static layer. The total thickness of the static layer and two glass slides estimated from the minimum optical path length corresponding to the Doppler shifted light is in good agreement with the results of Monte Carlo simulations (fig. 7). The error bars in fig. 7 indicate the variations in the shortest optical path length that may arise due to the path length resolution of $\pm$ 100 μm used in the experiment and Monte Carlo simulations. The straight line represents the real total layer thickness of the static layer and two glass slides.

4. Discussion

In this paper we have presented path length resolved measurements of multiply scattered light from mixed static and dynamic turbid media. Optical path length distributions and path length resolved Doppler shifts of multiply scattered light are measured from a two-layer scattering phantom, with a superficial static layer of various thicknesses covering a dynamic medium with identical optical properties. In the case of a static medium like a visco-elastic gel the width of the interference peak does not broaden with optical path length, whereas in the case of dynamic media more and more power is set to frequencies around the phase modulation frequency, resulting in Doppler broadening [4]. Thus, the area of the Doppler broadened peak, excluding the statically scattered light contribution at the modulation frequency, forms an estimation of the fraction of Doppler shifted light. As expected, the Doppler fraction exceeds the complimentary non-shifted part for large optical path lengths. For increasing thickness of the superficial static layer, the estimated non-shifted fraction of photons increases.

The observed path length dependent line width broadening shown in fig. 5 results from the detection of multiply scattered photons and as the number of scattering events increases, the width of the peak increases with optical path length. However, the average Doppler shift

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per unit optical path length decreases with increase in the thickness of the static layer. For optical path lengths greater than 2.5 mm, the average Doppler shift measured with a static layer of thickness 0.1 mm is nearly equal to that measured in a completely dynamic medium, indicating that the contribution from the statically scattered light to the interference signal is significantly lower for large optical path lengths. A remarkable observation is that an average Doppler shift equivalent to the static medium is measured until a certain optical path length, which in turn depends on the thickness of the static layer. This corresponds to the photons that are scattered from the superficial static layer. After this transition optical path length, Doppler broadening of the peak is observed, since the photons with large optical path lengths penetrate into the dynamic layer. From the minimum optical path length (L_{min}) of the Doppler-shifted light, we have estimated the thickness of the superficial static layer.

We validated the estimated thickness of the superficial static layer with Monte Carlo simulations. The experimentally determined transition optical path length and the thickness of the static layer estimated from this optical path length are in good agreement with the simulation results. The estimated thickness is marginally lower than the real one, except for the smallest and largest thickness where a larger difference is found. The marginal difference can be explained from the methodological accuracy. The assumed relation between the position of the retroreflector and the actual optical path length in the sample arm is based on the zero optical length determined with a reflecting mirror with accuracy $\pm 50\,\mu$m. Another source of error might be in the simplification of assuming linear photon paths without taking refraction into account. However, when the refraction effects though glass layers are taken into account, the estimated shift is about 2 μm in a glass layer of $150\,\mu$m, which is not significant. The larger differences for the smallest and largest layer thickness cannot be explained from the applied method.

In spite of its simplicity, the accuracy of our static layer estimation is good. When this approach is extended to in vivo measurements, it seems to be justified to regard the tissue as a single static layer of thickness d with homogeneous average refractive index (n_{tissue}) and from geometrical considerations, the thickness of the superficial static layer can be calculated. Sadhwani et al. reported an estimation of superficial static layer thickness that overlies a dynamic layer, by monitoring the speckle pattern at the surface as light propagates radially away from the point where the tissue was illuminated with a focused beam [13]. They showed that the diameter of the static speckle pattern increases linearly with the static layer thickness and the thickness of the static layer was empirically calculated from the speckle lobe size obtained by the convolution of a step function of the detector and a Gaussian function of the speckle lobe. As an application Sadhwani et al. mentioned the assessment of the depth of burn wounds. They modeled a burn wound as a superficial static layer with little or no blood flow overlying a perfused tissue and the quantitative estimation of the depth of the burn can be made from the radial extent of a speckle pattern that arises from burned tissue. Watts et al. showed the correlation between the laser Doppler measurements of dermal flow and the burn depth measured based on the histological assessment [14]. In the histological measurements, they studied the interface of the deepest blocked vessels and the most superficial patent as a demarcation of damaged and healthy tissue. However, burn wounds are much more complex than the two-layer model used in our study, and the perfusion in the tissue layer underlying the burn will cause less dynamic light scattering than the particle suspension used in this study. Hence, practical use of this approach in determining the depth of burn requires further in vivo measurements.

5. Conclusion

We have developed a new method to discriminate between Doppler shifted and non-shifted multiply scattered light using phase-modulated coherence gated interferometry. Optical path length distributions of Doppler shifted and unshifted light, spanning a range from 0 to 6 mm are measured in a two layer static and dynamic turbid phantom, with a superficial static layer placed on top of a dynamic turbid medium having identical optical properties. Until a certain optical path length, no Doppler broadening of the interference peak is observed, which is in

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turn related to the thickness of the static layer. From the minimum optical path length of Doppler shifted light, we have proven to be able to estimate the thickness of the static superficial static layer. Here we assumed ballistic or snaky photon paths between the fiber tips, via the dynamic layer. Good agreement between experimentally determined thickness of the static layer and Monte Carlo simulations was found.

We aim to apply this method to perform path length resolved perfusion measurements in skin, which will overcome the inherent limitation of conventional LDPM that restrict its clinical usefulness, where perfusion signal depends on photon path length. In contrast to Doppler OCT systems measuring singly scattered photons [15], we aim for multiply scattered light having traveled few millimeters through the tissue, and we quantitatively analyze the path length dependent Doppler broadening in a non-confocal two-fiber configuration as used in conventional LDPMs. Single-mode fiber optic Michelson interferometric systems used in Doppler OCT [15] adopt on axis back reflection, whereas we use a multimode fiber-optic Mach–Zehnder interferometer, with positions for illumination and detection separated by a distance of ten to a hundred times the scattering mean free path length. This scheme offers greater flexibility, since the distance between the illumination and the detection fiber can be varied, giving one some control over the relative depth sensitivity of the system. Thus our measurements explore the regime of multiply scattered photons. In a normal skin, less than 10% of the photons are Doppler shifted due to the relative lower amount of red blood cells in the total skin volume. However, in richly perfused tissues this fraction of Doppler shifted photons increases due to the higher amount of red blood cells present in the skin capillary network. The results presented here show that this approach has potential applications in discriminating between statically and dynamically scattered light in the perfusion signal. Also, the path length resolved Doppler approach presented here will enable to correctly interpret the inter-and intra-individual variations in the LDF readings introduced by the variance in individual photon path lengths. Another important feature of this approach is the tunable depth resolved perfusion information that can be achieved. By translating the optical path length in the reference arm, the photons migrated deeper into the tissue can be made to interfere with the reference light and thus enable to discriminate between perfusion signal from superficial and deeper layers of tissue. Determination of superficial burn depth may be an important application of our technique.

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