Measurement of particle flux in a static matrix with suppressed influence of optical properties, using low coherence interferometry

Babu Varghese,[1,*] Vinayakrishnan Rajan,[1] Ton G Van Leeuwen[1,2] and Wiendelt Steenbergen[1]

[1]*MIRA Institute for Biomedical Technology and Technical Medicine, Biophysical Engineering Group, University of Twente, P.O Box 217, NL-7500AE Enschede, The Netherlands.*
[2]*Academic Medical Center, Biomedical Engineering and Physics, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands*
*babu.varghese@philips.com

Abstract: Perfusion measurements using conventional laser Doppler techniques are affected by the variations in tissue optical properties. Differences in absorption and scattering will induce different path lengths and consequently will alter the probability that a Doppler shift will occur. In this study, the fraction of Doppler shifted photons and the Doppler broadening of a dynamic medium, are measured with a phase modulated low coherence Mach-Zehnder interferometer. Path length-resolved dynamic light scattering measurements are performed in various media having a constant concentration of dynamic particles inside a static matrix with different scattering properties and the results are compared with a conventional laser Doppler technique, with a simple model and with Monte Carlo simulations. We demonstrate that, for larger optical path lengths, the scattering coefficient of the static matrix in which the moving particles are embedded have a small to minimal effect on the measured fraction of Doppler shifted photons and on the measured average Doppler frequency of the Doppler shifted light. This approach has potential applications in measuring perfusion independent of the influence of optical properties in the static tissue matrix.

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

---

References and links

1. A. P. Shepherd, and P. Å. Öberg, Laser-Doppler Blood Flowmetry (Kluwer Academic, Boston, 1990).
2. M. J. Leahy, F. F. M. de Mul, G. E. Nilsson, and R. Maniewski, "Principles and practice of the laser-Doppler perfusion technique," Technol. Health Care **7**(2-3), 143–162 (1999).
3. A. Liebert, M. Leahy, and R. Maniewski, "Multichannel laser-Doppler probe for blood perfusion measurements with depth discrimination," Med. Biol. Eng. Comput. **36**(6), 740–747 (1998).
4. R. Nossal, R. F. Bonner, and G. H. Weiss, "Influence of path length on remote optical sensing of properties of biological tissue," Appl. Opt. **28**(12), 2238–2244 (1989).
5. A. Liebert, M. Leahy, and R. Maniewski, "A calibration standard for laser-Doppler perfusion measurements," Rev. Sci. Instrum. **66**(11), 5169–5173 (1995).
6. D. Huang, E. A. Swanson, C. P. Lin, J. S. Schuman, W. G. Stinson, W. Chang, M. R. Hee, T. Flotte, K. Gregory, C. A. Puliafito, and J. G. Fujimoto, "Optical coherence tomography," Science **254**(5035), 1178–1181 (1991).
7. K. K. Bizheva, A. M. Siegel, and D. A. Boas, "Path-length resolved dynamic light scattering in highly scattering random media: the transition to diffusing wave spectroscopy," Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics **58**(6), 7664 (1998).
8. A. Wax, C. Yang, R. R. Dasari, and M. S. Feld, "Path-Length-Resolved Dynamic Light Scattering: Modeling the Transition from Single to Diffusive Scattering," Appl. Opt. **40**(24), 4222–4227 (2001).
9. A. L. Petoukhova, W. Steenbergen, and F. F. M. de Mul, "Path-length distribution and path-length-resolved Doppler measurements of multiply scattered photons by use of low-coherence interferometry," Opt. Lett. **26**(19), 1492–1494 (2001).

10. A. L. Petoukhova, W. Steenbergen, T. G. van Leeuwen, and F. F. M. de Mul, "Effects of absorption on coherence domain path length resolved dynamic light scattering in the diffuse regime," Appl. Phys. Lett. **81**(4), 595–597 (2002).
11. K. Ishii, R. Yoshida, and T. Iwai, "Single-scattering spectroscopy for extremely dense colloidal suspensions by use of a low-coherence interferometer," Opt. Lett. **30**(5), 555–557 (2005).
12. B. Varghese, V. Rajan, T. G. Van Leeuwen, and W. Steenbergen, "Path-length-resolved measurements of multiple scattered photons in static and dynamic turbid media using phase-modulated low-coherence interferometry," J. Biomed. Opt. **12**(2), 024020 (2007).
13. B. Varghese, V. Rajan, T. G. van Leeuwen, and W. Steenbergen, "Quantification of optical Doppler broadening and optical path lengths of multiply scattered light by phase modulated low coherence interferometry," Opt. Express **15**(15), 9157–9165 (2007).
14. B. Varghese, V. Rajan, T. G. Van Leeuwen, and W. Steenbergen, "High angle phase modulated low coherence interferometry for path length resolved Doppler measurements of multiply scattered light," Opt. Commun. **281**(3), 494–498 (2008).
15. G. E. Nilsson, "Signal processor for laser Doppler tissue flowmeters," Med. Biol. Eng. Comput. **22**(4), 343–348 (1984).
16. F. F. M. De Mul, "Monte-Carlo simulation of Light transport in Turbid Media", in: Handbook of Coherent Domain Optical Methods, Biomedical Diagnostics, Environment and Material Science, Tuchin, Valery V. (Ed.), 2004, Kluwer Publishers, 465–533 (2004).
17. M. Larsson, H. Nilsson, and T. Strömberg, "In vivo determination of local skin optical properties and photon path length by use of spatially resolved diffuse reflectance with applications in laser Doppler flowmetry," Appl. Opt. **42**(1), 124–134 (2003).

1. Introduction

The basically unknown scattering and absorption properties of tissue influence noninvasive optical diagnosis in turbid tissues. For instance in laser Doppler blood flowmetry (LDF), a non-invasive technique for monitoring blood microcirculation, the indicated blood flow depends on the number of photons interactions with moving red blood cells and the surrounding static tissue matrices [1–5]. 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 average optical path lengths will be different for different tissue types due to the changes in tissue optical properties in terms of absorption and scattering. Also, the variance in individual photon path lengths (e.g., length and depth) increases with average photon path lengths. Hence laser Doppler perfusion monitoring which records perfusion values averaged over different and basically unknown path lengths, creates an uncertainty in the relation between the measured perfusion signal and the real perfusion. Techniques for the detection of multiple scattered light and its Doppler spectrum as a function of path length may result in more-quantitative and more-reliable tissue information.

Path length resolved photon intensity, and Doppler broadening of completely dynamic media have been measured using low coherence interferometry [6–14]. Earlier we demonstrated that dynamic properties of turbid media can be measured independently of optical *absorption*, for biologically relevant absorption levels [12]. The goal of the present study is to demonstrate that, by path length-resolved dynamic light scattering, information can be obtained regarding the velocity and concentration of dynamic particles within a static medium, independent of the *scattering* properties of the static medium. We will demonstrate that using phase modulated low coherence interferometry, the fraction of Doppler shifted photons f_D and the Doppler broadening of a dynamic medium $\langle \omega_D \rangle$, which are related to the flux of particles, can be measured independently of the optical scattering level of the surrounding static matrix.

2. Methods and materials

The low coherence interferometry setup has been described in detail elsewhere [12–14]. In short, our fiber optic Mach-Zehnder interferometer uses fibers for illumination and detection with a mutual center-to-center distance of 300 µm. The detection fiber is a gradient index multimode fiber (NA=0.29) with a core diameter of 100 µm, while for illumination a single mode fiber (core diameter 5.3 µm, and NA=0.14) was used. The fiber delivered 2 mW of 832 nm light, produced by a superluminescent diode. The reference arm contains an electro-optic broadband phase modulator with a peak optical phase shift of 2.04 radians applied to the

modulator so that the power spectrum contains interference peaks at the phase modulation frequency (and higher harmonics) [14]. The AC photocurrent was measured with a 12 bit analogue-to-digital converter (National Instruments), sampling at 40 kHz. Average power spectra were calculated from 1000 individual spectra with a frequency resolution of 40 Hz, based on 1000 signal samples. The reference arm optical path length was varied in steps of 200 micrometers.

To compare the path-length resolved measurements to standard laser Doppler perfusion monitoring, a PF5000 (Perimed AB, Sweden) with a laser diode operating at 780 nm and a bandwidth of 20 Hz-13 kHz is used. The used fiber-optic probe (Perimed probe 408, standard probe) consisted of two spatially separated fibers (core diameter = 125 μm, NA=0.37) with a center-to-center separation of 250 μm.

In both laser Doppler perfusion monitoring and low coherence interferometry, the calculation of moments of the power spectrum $P(\omega)$ of interference-based photocurrent fluctuations plays a key role. Here we define the spectral moment as

$$M_i(a,b,\omega_m) \equiv \int_{\omega_m+a}^{\omega_m+b} P(\omega)(\omega-\omega_m)^i d\omega \qquad (1)$$

with ω_m the phase modulation frequency (if phase modulation is applied).

Laser Doppler perfusion monitoring, which does not use phase modulation and hence $\omega_m=0$, provides spectral moments $M_i(a,b,0)$ with a and b the above mentioned device dependent low and high cut-off frequencies. For sufficiently low blood concentrations, with $i=0$ a quantity is obtained which represents, along the optical path, the average concentration of moving red blood cells, while $i=1$ describes the average red blood cell flux, which is the product of concentration and the root mean square of the red cell velocity [15].

To determine these parameters path length resolved, we measured the power spectra with the Mach-Zehnder low coherence setup. First, the background noise from the power spectrum around the modulation frequency ($\omega_m = 6$ kHz) is subtracted. The calculated $M_0(0,b,\omega_m)$ of the broadened interference peak (until b=2 kHz from the phase modulation peak) is proportional to the total number of detected photons for that given (by the reference arm) path length [14]. As depicted in Fig. 1, the bandwidths of the detected signals of static and moving particles differ. The full width at half maximum (FWHM) of the interference signal in a statically scattering medium has a value δ_s (δ_s = 50-60 Hz in our system) whereas in the case of dynamic media a Doppler broadened spectral peak around the phase modulation frequency is formed [12]. The area of the Doppler broadened peak, excluding the statically scattered light contribution at the interference peaks, forms an estimation of the amount of the Doppler shifted light at that specific optical path length. For a given optical path length, the fraction of Doppler shifted photons f_D is then given by $f_D= M_0(\delta_s,b,\omega_m)/ M_0(0,b,\omega_m)$. Here we regard the Doppler fraction f_D as a measure of the concentration of particles moving in the static matrix. The average speed of the moving particles is represented by the average Doppler shift of the Doppler shifted fraction of the detected light, which in terms of Eq. (1) is $<\omega_D>=M_1(\delta_s,b,\omega_m)/M_0(\delta_s,b,\omega_m)$ with the frequency ω_D in Hz.

Mixed static-dynamic scattering phantoms were prepared with aqueous suspensions of polystyrene microspheres of $\varnothing$4.7 μm and $\varnothing$0.20 μm respectively. The Brownian motion of particles with $\varnothing$4.7 μm will be sufficiently slow to consider them as effectively static (see below). Three scattering phantoms with the same concentration of particles $\varnothing$0.20 μm ($g=0.18$, μ_s' =0.55 mm^{-1}, μ_s=0.67 mm^{-1}, μ_a=0.001 mm^{-1} and volume concentration=0.30%) were prepared and three scattering levels of the static medium were realized ($g=0.86$, μ_s' =1.4, 0.8, 0.4 mm^{-1}, μ_s=10, 5.7, 2.8 mm^{-1}, μ_a=0.001 mm^{-1} and volume concentration=1.33, 0.77, 0.39%, respectively). The prepared phantoms were contained by a cubic glass cuvette (20*20*20 mm^3).The fiber optic probe was kept inside the medium using a mechanical holder and the holder was mounted on a X-Y-Z translational stage.

The modulation peaks in the power spectra measured in the monodisperse suspensions of polystyrene microspheres of $\varnothing 4.7$ μm (μ_s'=2.75 mm^{-1}, μ_s=18.3 mm^{-1}, volume concentration=2.62%) and $\varnothing 0.20$μm (μ_s'=4.95 mm^{-1}, μ_s=6.04 mm^{-1}, volume concentration=2.67%) for an optical path length of 2 mm in the sample are shown in Fig. 1, along with the peak observed on a static mirror. The FWHM of the interference signal measured in the case of an aqueous suspension of polystyrene microspheres of $\varnothing 4.7$ μm was about 80 Hz. Thus Doppler shifts imparted by the polystyrene microspheres of $\varnothing 4.7$ μm are significantly lower than those imparted by $\varnothing 0.20$ μm and do not lead to considerable broadening compared to the spectral peak observed from the static mirror (50-60 Hz). This indicates that microspheres of $\varnothing 4.7$ μm can be considered as static.
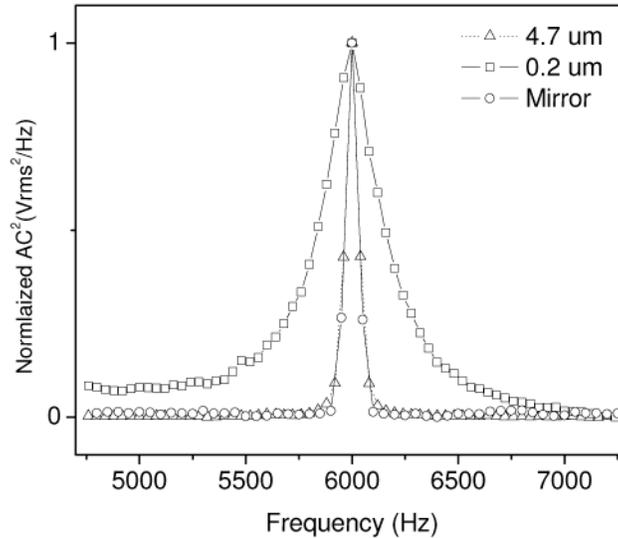

Fig. 1. The heterodyne power spectrum appearing at the modulation frequency measured for monodisperse water suspensions of Polystyrene microspheres ($\varnothing 0.20$ μm and $\varnothing 4.7$ μm) and with a mirror, normalized to their maximum value, for an optical path length of 2 mm.

While we expect that our measurements will yield Doppler fractions and Doppler shifts independent of the scattering level of the static matrix, a longer term goal will be to estimate the flux of particles moving inside static matrices in absolute terms. To this end we need models that relate the outcomes of our measurements to the optical and dynamical properties of the dynamic part of the medium. In this study, we focus on the concentration of moving particles, which may be retrieved from models which relate the measured Doppler fraction f_D to the contribution of the dynamic part of the medium to the total scattering coefficient of the entire medium. We will consider a simple exponential decay model and compare it with the gold standard provided by the Monte Carlo simulation technique. In the exponential decay model we assume that the fraction of *unshifted* light decays exponentially with the traveled optical path length l_{opt}. Consequently, the fraction of Doppler shifted photons will be given by $f_D=1-exp(-\mu_{s,dyn}\ell_{opt}/n)$, with $\mu_{s,dyn}$ the scattering coefficient of the ensemble of moving particles. Monte Carlo simulations were performed with the algorithm and software as described by De Mul [16]. The single mode fiber used in the experiment was modeled as a point source. Photon detection was performed in a ring with inner and outer radius of 0.25 and 0.35 mm (in agreement with the core diameter and position of the real detection fiber), concentric to the light beam for illumination. The simulated numerical apertures for illumination and detection were identical to the experimental values. The three mixed static-dynamic phantoms were exactly mimicked, with the scattering phase functions being

calculated using Mie's theory. Photons which were scattered by the $\varnothing 0.20$ μm particles were given a Doppler label. For each medium and each path length, 20000 photons were detected.

3. Results

Figure 2 shows the fraction of Doppler shifted photons f_D as a function of the optical path length, for the three media. As expected, the measured Doppler fraction increases with the optical path length and the confounding influence of the surrounding static matrices is suppressed. Furthermore, Fig. 2 shows the results of Monte Carlo simulations and for the exponential decay model $f_D = 1-exp(-\mu_{s,dyn}\ell_{opt}/n)$. The models in general predict higher values of the the Doppler fraction than the experimental values. Furthermore, the experimental and Monte Carlo results show biphasic behaviour, with a different trend for optical path lengths below and larger than 2 mm.
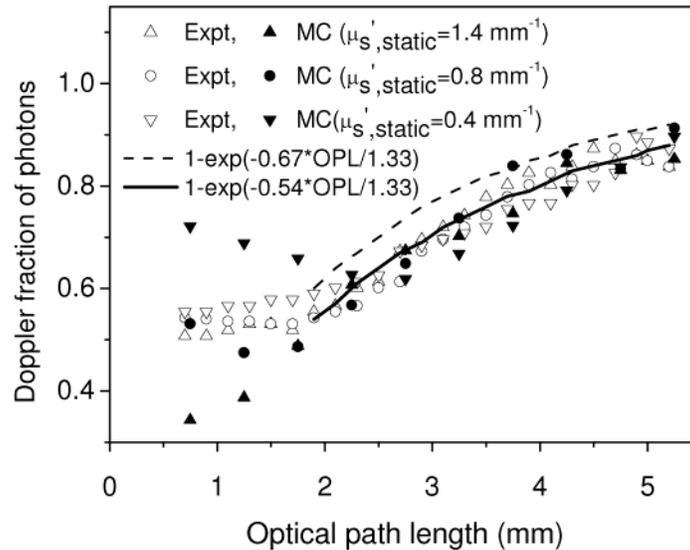

Fig. 2. The fraction of Doppler shifted photons as a function of optical path length, as a result of experiments (open markers), Monte Carlo simulations (filled markers), and experimental decay models, one with a theoretical decay rate (dashed line), and one with the best fit to the experimental results (thick line).

Figure 3 shows the average Doppler shift generated by the moving particles $<\omega_D>=M_1(\delta_s,b,\omega_m)/M_0(\delta_s,b,\omega_m)$ as a function of optical path length. For optical path lengths larger than 2 mm, $<\omega_D>$ increases linearly with optical path length as expected theoretically and experimentally [13]. However, $<\omega_D>$ also shows a different behaviour for optical path lengths smaller than 2mm. Figure 4 shows the average Doppler shift for the three media, as measured with the fiber optic laser Doppler perfusion monitor. Please note that the average Doppler shift decreases with increasing scattering of the static material. The average Doppler shift measured with the low coherence interferometer, averaged over all optical path lengths, is much less sensitive to the influence of the scattering properties of the static medium than when measured with the laser Doppler perfusion monitor.
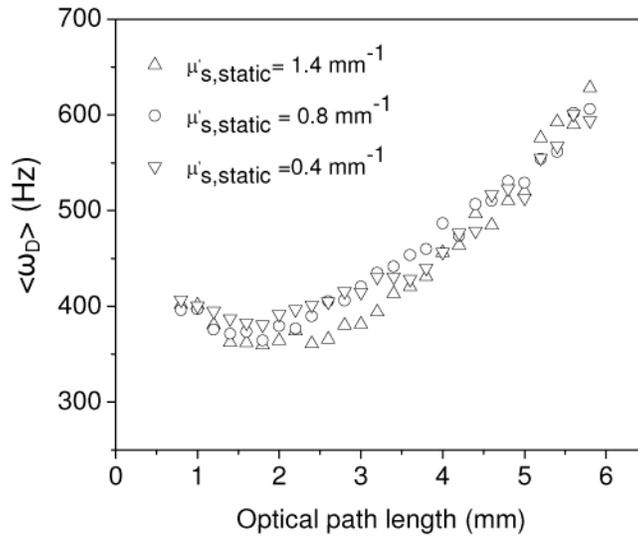
Fig. 3. The Doppler shift measured as a function of optical path length in the medium.

4. Discussion

In this study, we have measured the fraction of Doppler shifted photons and the Doppler broadening of a mixed dynamic and static medium. Whereas the perfusion signal from a laser Doppler monitor depends on the scattering coefficient of the static material, both the fraction and the average Doppler broadening of the Doppler shifted photons measured by a phase modulated low coherence Mach-Zehnder interferometer are independent of the optical properties of the static material. In general, from the results in Figs. 2 and 3, we can observe that these perfusion related parameters as a function of the optical path length are almost identical for the three media, which is a first step towards quantitative perfusion monitoring.

Figure 2 shows that for a given optical path length, f_D is independent of the influence of static matrices, in particular for optical path lengths larger than 2 mm. Furthermore f_D increases with optical path length with a trend that can be depicted by the simple exponential decay model. However, the theoretically predicted Doppler fractions are higher than the experimental values. Nevertheless, if in this model we define scattering coefficient $\mu_{s,dyn}$ as a fitting parameter, it appears that the exponential decay model properly fits the observations for $\ell_{opt} > 2mm$, with $\mu_{s,dyn} = 0.55$ mm^{-1} (see Fig. 2). For $\ell_{opt} > 2mm$, the predicted Doppler fractions by Monte Carlo simulations are similar for the three media and are in good agreement with the experimental results.
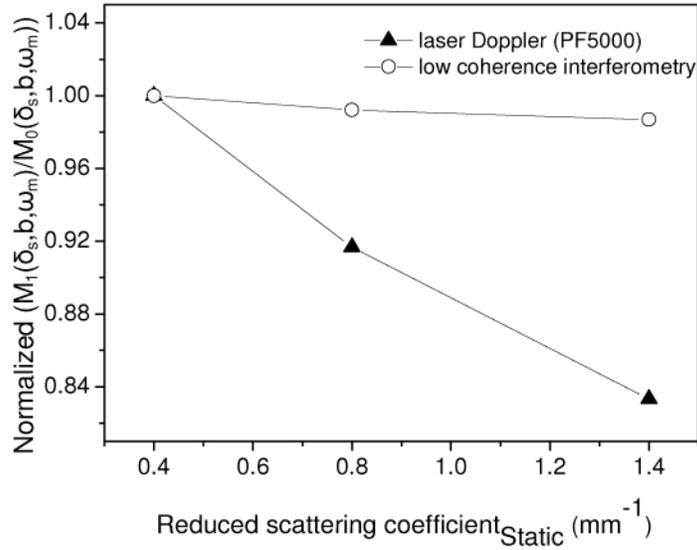
Fig. 4. The average Doppler broadening as represented by M_1/M_0 for the laser Doppler perfusion monitor, and the average value $< M_1/M_0>$ for all optical path lengths for the low coherence interferometer and normalized by the maximum value for the three media.
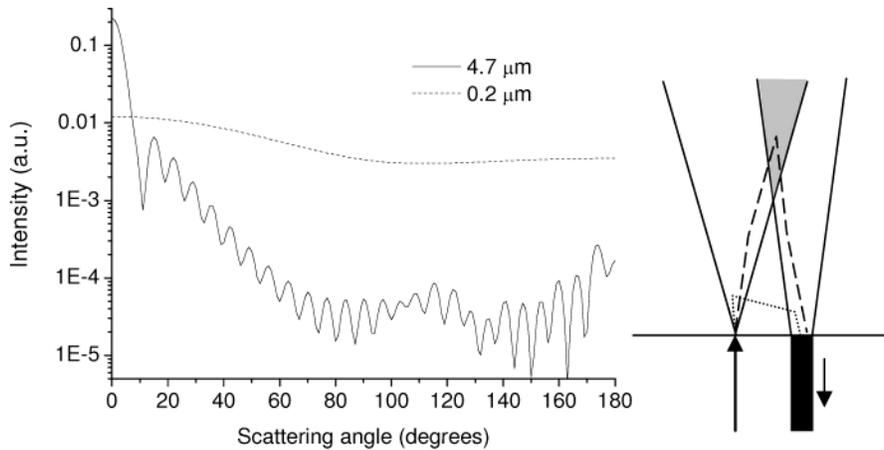

Fig. 5. *Left*: Mie scattering phase functions for $\varnothing 4.7$ and $\varnothing 0.2$ micrometer polystyrene particles (n=1.56) in water (n=1.33). *Right*: configuration, drawn on scale, of illumination and detection fibers (spacing 300 micrometers), and their numerical apertures (0.29 and 0.15 respectively) with the common region shaded grey. The dotted and dashed photon trajectories are discussed in the main text.

For larger optical path lengths, the average Doppler shift increases with the optical path length, as expected. The overall dependence on the static matrix optical properties on the Doppler shift is small, as depicted in Fig. 3. In the case of a higher scattering coefficient ($\mu_s'=1.4mm^{-1}$), for optical path lengths between 2.5 and 3.5 mm, the Doppler broadening is lower in comparison with those obtained for the lower scattering levels. We may express the overall dependence of the measured concentration, represented by f_D, and the particle velocity, represented by M_1/M_0, by their average value. This yields average Doppler fractions $<f_D>$ of 0.692, 0.685 and 0.694, and average Doppler shifts $<M_1/M_0>$, of 447.7, 447.9 and 442.2 Hz, for $\mu_s' =0.4$, 0.8 and 1.4 mm^{-1}, respectively. Measurements at a single optical path length may be more suitable in practice. For single path lengths, Figs. 2 and 3 feature maximum variations of 10% for both f_D and M_1/M_0. In contrast, the laser Doppler perfusion

monitor readings of M_1/M_0 vary by 20% for the involved range of μ_s' values. These values, along with $<M_1/M_0>$ obtained with low coherence interferometry, are shown in Fig. 4. These results clearly illustrate the suppression of the influence of the scattering matrix, compared to its influence for a laser Doppler perfusion monitor.

For optical path lengths smaller than 2 mm, the measured behaviour of fD differs significantly from the trend measured and predicted by the simple exponential model. Also for these short optical path lengths, the Doppler fractions predicted by the Monte Carlo simulations show biphasic behaviour and the three media show completely different trends in the Monte Carlo predicted Doppler fractions. But for optical path lengths larger than 2 mm, the experimental results, the exponential model and Monte Carlo results agree. In previous reports on path-length resolved DLS spectroscopy, singly scattered light was observed for short optical path lengths and the transition from single scattering to multiple scattering was exemplified though the increase in Doppler broadening after a minimal number of scattering events [7,8,13]. However, a simple distinction between regimes for single and multiple scattering does not hold in the present case of different fibers for illumination and detection. Their configuration is shown on scale in Fig. 5. Single scattering is only possible within the common volume of the aperture cones, with as consequence a minimum optical path length for single scattering of 1.5 mm. However, light has been detected for much shorter optical path lengths, as shown by Figs. 2 and 3. These short path lengths are only possible for trajectories with a few scattering events at angles around 90 degrees, for instance the dotted trajectory in Fig. 5. As shown by the phase functions in Fig. 5 (both normalized to have a total area of 1) for the two types of particles, this is most likely to occur through multiple scattering by the $\varnothing 0.2$ micrometer particles. Larger path lengths however, might also include a few forward scattering events and a single large angle scattering event. Therefore in these larger trajectories the 4.7 micrometer particles will have a relatively larger contribution. Since the scattering probability of this particle increases by a factor of 4 for the scattering angle increasing from 90 to 180 degrees (while the phase function for the small particle remains almost constant in this range of scattering angles), and since the contribution of both types of particles to the total scattering coefficient has the same order of magnitude in our experiment, the probability of scattering by large particles only will increase. This explains the decrease of f_D with the optical path length as observed in the simulations for the most diluted medium, and the only very slow increase of f_D with the optical path length in the experiment. Also the small initial decrease of the measured average Doppler shift as shown in Fig. 3, may be explained by this. Hence, rather than selecting single scattered photons by considering short path lengths, in this case the short path lengths select photons which have been mainly scattered by isotropically scattering particles, compared to the large photon path lengths.

Our experimental results do not show the different behaviour of the Doppler fraction in the single scattering regime between the three media. Clearly, our system shows the best quantitative behaviour for longer optical path lengths, while for short optical path lengths it has difficulties in distinguishing between Doppler shifted and statically scattered light.

Another limitation of our experimental data might be formed by the mixed static dynamic phantom used in this study, where we assume that the Brownian motion of the $\varnothing 0.20$ μm particles is not impeded by the presence of large particles. Clearly, a phantom with a really rigid static matrix would be more favorable. Furthermore, only a limited range of total reduced scattering coefficients is considered. The main limitation here was formed by the scattering coefficients of the undiluted particle suspensions. The considered range between 0.95 and 1.95 mm^{-1} will include many important tissues. However, the performance of our method at higher scattering coefficients needs to be established.

5. Conclusion

To summarize, the low coherence interferometry scheme in the fiber optic Mach-Zehnder configuration enables optical Doppler or dynamic light scattering measurements of dynamic media embedded in a static medium. We demonstrate that, for larger optical path lengths, the scattering coefficient of the static matrix in which the moving particles are embedded have a

small to minimal effect on the measured fraction of Doppler shifted photons, which represents the concentration of moving particles, and on the measured average Doppler frequency of the Doppler shifted light, which describes particle speed. Although for the multiple scattering regimes this quantity agrees with an exponential decay model, the decay rate differs from the expected ones. Also there is a discrepancy, although smaller, with the results of Monte Carlo simulations.

Nevertheless, with the application of tissue perfusion monitoring in mind, our results promise the technique to render perfusion measurements independent of the type of static tissue, provided that the vascular bed is anatomically and physiologically homogeneous within the measurement volume. This ability of our technique is an important condition for absolute perfusion measurements. When different tissues are compared, such as brain (high scattering), muscle (low scattering) and liver (high absorption), the measured perfusion levels will vary significantly [1]. In general, fingertip skin has a lower scattering than forearm skin, resulting in an increase of optical path lengths [17]. Laser Doppler flux signals are generally higher in finger tips than in forearm skin. This difference must partly be explained from the difference in tissue optical properties rather than real perfusion differences. Path length resolved optical Doppler perfusion monitoring, of which the basic technique is presented in this work, may overcome this limitation and enable to measure blood perfusion in tissue with suppression of the confounding influence of optical properties in the tissue matrix.

In future work, suppression of tissue optical effects will have to be considered for a larger range of reduced scattering coefficients. Also, the methodology will have to be further developed with respect to measurement speed, and also from the economic point of view. Eventually, clinical introduction of low coherence interferometry will depend on the diagnostic advantages balanced against other aspects such as measurement speed and price.