PATH LENGTH RESOLVED
OPTICAL DOPPLER PERFUSION MONITORING

Babu Varghese

The work described was carried out at the Biophysical Engineering Group, Faculty of Science and Technology, Institute for Biomedical Technology (BMTI), University of Twente, P. O. Box 217, 7500 AE, Enschede, The Netherlands.

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Path length resolved optical Doppler perfusion monitoring

By Babu Varghese

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Front Cover illustration: Diffusion of light in turbid media like tissue. Scattering of a photon by a moving red blood cell resulting in frequency shift due to Doppler effect.

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DISSERTATION

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on the authority of the rector magnificus,
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on account of the decision of the graduation committee,
to be publicly defended
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by

Babu Varghese
born on 13 May 1974
in Muringoor, India

This dissertation has been approved by:

Promotor	: prof. dr. A. G. J. M. van Leeuwen
Assistant promotor	: dr. Wiendelt Steenbergen

To Indu and my parents

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Chapter 1

Introduction

In this chapter a concise introduction and an overview on the principles and practice of Laser Doppler blood flowmetry are given. Skin perfusion measurements using this technique depend not only on the instrumental factors, but also on the on the extent of interaction of photons with moving red blood cells. Thus for a constant perfusion, the LDF output signal is affected by the variance in individual photon path lengths due to the changes in tissue optical properties and probe geometry. This dependence of the perfusion signal on the photon path length can be solved by the new approach developed in this research project: Path length resolved optical Doppler perfusion monitoring. This chapter describes the purpose of the research and provides an outline of the structure of the thesis.

1.1 Introduction

The invention of lasers in 1960 has introduced new diagnostic and therapeutic applications of light in the field of biomedical optics. The properties of laser light like high intensity, low divergence, and coherence properties are well suited to the needs of the biomedical field. For instance, the unique characteristics of limited temporal coherence of a broadband light source can be utilized to explore information on the sample properties. Techniques for monitoring scattered light with path length sensitivity have potential applications in measuring tissue physiological information. Many of these bio-optical techniques for non-invasive diagnosis deal with interaction of photons with turbid media like tissue and thus several successful attempts were made in measuring path length resolved tissue optical properties.

Path length resolved temporal fluctuations of photon intensity can be measured using amplitude modulation of the light intensity [1], time resolved measurements [2] or recently developed coherence gated [3-6] and wavelength-modulated interferometric systems [7]. 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.

Besides information on photon path lengths, the frequency shift of photons detected from tissue can be of importance. The spectral purity of the laser makes it possible to detect the slight frequency shift produced by the interactions between photons and moving red blood cells in the microcirculation. This led to the development of a new non-invasive diagnostic technique called laser Doppler blood flowmetry, by combining the principles of light beating spectroscopy and the Doppler effect [8,9]. When light with two different frequencies falls on a photodetector, a beat frequency, which is proportional to the difference in the two frequencies, is produced in the detector output. The coherent light that is scattered by static as well as moving scatterers, e.g. red blood cells contains the original light frequency and a slightly different frequency due to the Doppler effect. Thus, when these two fractions of photons are mixed at the detector, a beat frequency equal to the Doppler frequency is produced, from which information regarding the flow of scatterers can be extracted.

Since the description of this frequency shift by Christian Doppler in 1842, it took many technological developments leading to the present art of laser Doppler flowmetry (LDF) to measure blood perfusion. Cummins et al. succeeded in measuring flow velocities by interpreting the Doppler frequency shifted light backscattered from different regions of a fluid undergoing laminar flow [10]. This technique was applied to measure the red blood cell velocities in

the retinal vessels of rabbits [11] and some years later it was used for blood perfusion measurements in microcirculation [12]. Since then a number of technical developments, clinical and experimental applications of LDF have been implemented. Presently used laser Doppler systems can be broadly classified into two categories, namely the laser Doppler perfusion monitors (LDPM) and the laser Doppler perfusion imagers (LDPI). The fibre-optic based Laser Doppler perfusion monitoring introduced in 1978 is used for temporal perfusion measurements in a small sampling volume [13]. For spatial perfusion measurements, Laser Doppler Perfusion Imagers (LDPI) were introduced, which consist of a scanning system that scans the area under investigation and a photo detector capturing the photoelectric current to obtain a perfusion map [14,15]. For real time perfusion imaging, an alternative approach has been developed by the introduction of high speed cameras based on CMOS imaging arrays [16].

'Path length resolved dynamic light scattering,' of which the basic technique is presented in this work, has potential applications in the fields of fundamental as well as applied research in monitoring the spatial and temporal variations in optical properties in turbid media. As a specific biological application of the novel methodology developed in this research project, we translate this technique into a potential clinical tool, 'Path length resolved optical Doppler perfusion monitoring' that can measure perfusion more quantitatively. This will overcome the limitation of conventional laser Doppler techniques, where perfusion signal depends on the optical path length.

1.2 Laser Doppler blood flowmetry

In laser Doppler blood flowmetry, the coherent light delivered to the tissue through an optical fiber, interacts with static as well as moving scatterers, e.g. red blood cells (Fig.1). The light scattered by moving red blood cells receives a slight frequency shift due to the Doppler effect. The Doppler shifted and non-shifted light scattered from the tissue is guided by a second optical fiber, spatially separated from the first fiber to a photodetector where it is mixed, resulting in a speckle pattern. LDF characterizes the time-varying signal arising from the temporal variations in the speckle pattern to estimate the perfusion in biological tissues. There are two procedures that can be used for the characterization of the time-varying signal arising from the temporal variations in the intensity of light falling upon the photodetector: either the comparison of the frequency components of the fluctuating components in the form of a power spectrum, or from the analysis of the intensity autocorrelation function.

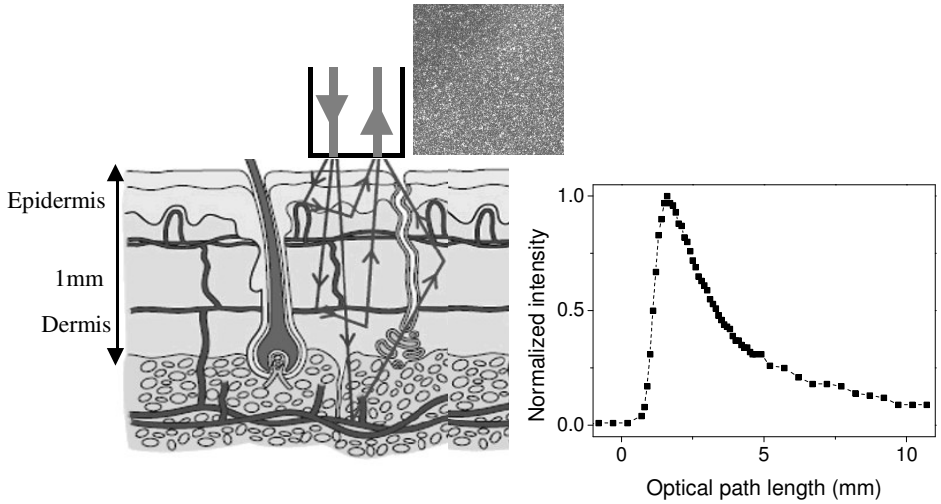

Fig. 1. Principle of laser Doppler blood flowmetry. The coherent beam of light delivered into the skin interacts with moving red blood cells and surrounding static tissue matrices (Left). The speckle pattern resulting from the coherent interference of scattered photons on the detector (Top). Optical path length distributions of multiply scattered (Right).

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 the average photon path length. In laser Doppler blood flowmetry, perfusion values averaged over different and basically unknown path lengths are recorded. A typical optical path length distribution of multiply scattered light from a turbid medium (measured with the new coherence gated interferometric method presented in this thesis) is shown in fig 1. A longer path length will increase the probability of Doppler scattering events, thus yielding a relative overestimation of the blood perfusion, compared to the short path length situation. Hence inter-and intra-individual variations in the LDPM readings will occur for the same perfusion values, introduced by the variance in average optical path length resulting from the changes in tissue optical properties.

Consider a source with frequency f_s incident on an object moving with a velocity $\boldsymbol{v}$, at some angle ϕ to the normal to the direction of motion (Fig. 2), the frequency shift (δf) of the Doppler shifted photon detected in the direction of incident light ($\boldsymbol{k}_i$) becomes [17]:

$$\delta f = f_s - f_d = \frac{2v \sin \varphi}{\lambda} \tag{1}$$

Where f_d is the Doppler shifted frequency, and λ is the wavelength of the light in the medium. Here azimuthal symmetry is assumed, i.e. ζ is uniformly distributed between 0 and 2π.

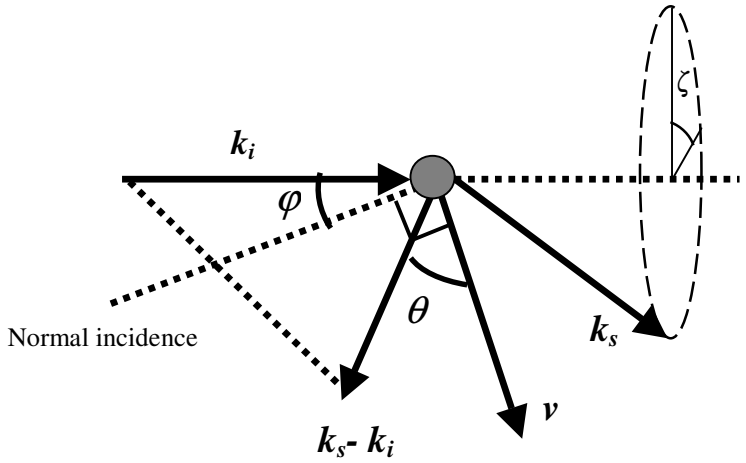

Fig. 2. Scattering of a photon by a moving RBC [17].

If the photon is detected in the direction of $\boldsymbol{k}_s$, the resultant Doppler-shifted frequency becomes:

$$\delta f = \frac{2v \sin\varphi}{\lambda}.\cos\theta \qquad (2)$$

Where θ is the angle between the direction of motion of scattering particle and $\mathbf{k_s}$-$\mathbf{k_i}$. In a system of moving particles, such as red blood cells (RBCs) in tissue, the photons undergo multiple scattering events, and the total Doppler shift is the sum of individual Doppler shift and thus, for *n* events:

$$\delta f = \sum_{i}^{n} \frac{2v_i \sin\varphi_i}{\lambda}.\cos\theta_i \qquad (3)$$

Typical frequency shifts detected by a LDPM instrument using a wavelength of 780 nm in the microcirculation range from 0-20 kHz. The scattering angles are more or less randomized in tissue and a wide range of velocities are present in the microcirculation. Hence instead of a single Doppler frequency, a continuous Doppler frequency spectrum is obtained. Perfusion is measured based on the spectral analysis of the power spectrum.

The fundamental output quantities of a conventional laser Doppler perfusion monitor are the moments M_i of the power spectrum $P(\omega)$; in general the i^{th} moment is defined as

$$M_i = \int_a^b P(\omega)\omega^i d\omega \qquad (4)$$

Here a and b are device dependent low and high cutoff frequencies [18]. Typical values are about 20-50 Hz for the low cut-off frequency, minimizing the effect of bulk motion of tissue relative to the probe and photoplethysmographic effects, and 12-20 kHz for the high cut-off frequency in the case of microcirculation measurements using a wavelength of 780 nm. With $i=0$, a quantity is obtained which is proportional to the concentration of moving red blood cells, for $i=1$ is described red blood cell flux, which is the product of concentration and the root mean square of the red cell velocity, at least for low blood concentrations. Bonner and Nossal showed that the weighted first moment (M_1/M_0) is proportional to the root-mean-square (rms) velocity of the red blood cells [20].

1.3 Clinical applications of LDF

Laser Doppler Flowmetry has been applied to the study of microcirculation over the past three decades and has found many potential clinical and research applications in many fields of medicine [8,9]. A short overview of some of the main applications is summarized below.

Dermatology: LDF is used for the objective measurement of skin lesions, the control of dermatologic therapy, comparison of the reactivity of the cutaneous microcirculation in different ages and ethnic groups, quantification of irritation in patch tests, and erythematous response to irritation or allergic reactions [20].

Pharmacology: LDF is used for measuring cutaneous microcirculation in real time and also allows long terms surveillance of drug action on skin capillaries. Other applications include pharmacodynamics of vasoactive compounds, effects of topical or systemic vasoactive drugs on human tissue blood flow, determination of transcutaneous drug penetration kinetics and evaluation of potency of penetration enhancers [21].

Plastic and reconstructive surgery: By monitoring the blood perfusion in replants and flaps, early signs of impaired circulation can be detected. LDF allows increasing the success rate of flap transplantation from about 50% to 85-90% helping to prevent loss of the replant. LDF is used for the surveillance of free flaps and is used to follow the dynamic variations in blood flow. LDF can be used to distinguish between superficial and deep burns and thus the treatment can be adapted to the type of burn [22,23].

Angiology and vascular surgery: Diabetic microangiopathy can be detected and evaluated by perfusion measurements. This is useful for quantifying the effects of drug therapy and also for defining the need for and level of amputation. In patients with Peripheral Arterial Occlusive Disease, perfusion measurements can be used to locate the site of stenosis [24,25].

Neurosurgery: Laser Doppler assessment of cerebral blood flow is used in conjunction with other techniques for multi modal monitoring of head injured patients. Preoperative measurements can be used to detect changes in the brain microcirculation, which may indicate an impairment of perfusion caused by loss of auto regulation. Early treatment of this impaired circulation will minimize brain damage. By measuring brain blood perfusion for several days post-operatively, along with intracranial and systemic blood pressures new treatment modalities for reducing the recovery period and brain damage can be tested [26,27].

Gastroenterology: The ability to record dynamic circulatory events makes LDF suitable over other available techniques in measuring gastrointestinal blood flow. The assessment of local blood flow regulation within the different layers of the gastrointestinal gives indication of autoregulation, reactive hyperemia and functional hyperemia. Tissue viability can be determined preoperatively by perfusion measurements in the small intestine in ischaemia or following radiation damage. Stable measurements of gastric mucosal blood flow can be obtained by applying LDF through endoscopes. This can be used as a predictor of non-healing of benign gastric ulcers or to measure the effect of treatment intervention on mucosal flow [28,29].

Anesthesiology: LDF is used to monitor the effects of different types of anesthetic analgesic agents on blood perfusion. These effects include sympathetic blockade and changes in peripheral blood perfusion. Early warning of shock during surgery or in intensive care can be obtained, for example by using an intestinal or gastric probe [30].

1.4 Alternative techniques to measure microcirculation

Microcirculation plays a vital role in both health and disease. It supplies oxygen and nutrients to cells, removes the excess heat and waste products, maintains the thermal balance of the body and provides the access for the immune system to defend the body against infectious invasion. Due to this clinical importance of measuring microcirculation, several techniques have been investigated.

Photoplethysmography [31,32]: The intensity fluctuations of light reflected from or transmitted through tissue depend on the blood volume and thus pulsatile changes in tissue blood volume produce corresponding changes in the reflected light. As the arterial flow is pulsatile, and the pulsatility of flow increases with perfusion, the changes in skin perfusion can be studied by

observing the waveform. The waveform demonstrates the inflow and outflow characteristics of blood in the measurement volume. The method is objective, quantitative, non-invasive, not expensive and continuous. However, the method is not useful in darkly pigmented skin and does not measure absolute flow.

Transcutaneous oxygen tension ($tcpO_2$) [33]: This technique uses a probe with an oxygen sensitive electrode and a heating element to assess the oxygen supply to the skin. The amount of oxygen diffusing through the tissue transcutaneously is measured by the electrode in mmHg. But for reliable monitoring, an increased local skin temperature (42 - 45°C) is needed to provoke a local reactive hyperemia. The method is objective, quantitative, non-invasive and continuous. Disadvantages of this technique are that it assesses the microcirculation indirectly, that it is not very sensitive for evaluation of skin microcirculation, and needs very uncomfortable temperatures that cannot be used for a long period of time. Furthermore the method consumes oxygen, and therefore may disturb local metabolism.

Vital capillaroscopy [34,35]: The papillary capillaries can be observed by means of an optical microscope with a magnification of 10-60x in a non-invasive way. This method assesses the anatomy and function of the observed capillaries. The method is subjective, semi qualitative and it does not assess local microcirculation directly, because it establishes morphological changes in the capillaries due to hypocirculation and disease. In dynamic capillaroscopy, a gold standard for assessing the nutritional flow, a video acquisition system is coupled to the microscope and the number of erythrocytes and their speeds are computed for individual capillaries from the video images. This method is non-invasive, quantitative, and continuous and directly measures microcirculation. But this technique is applicable only on intact nailfold capillaries and it gives information on one or few capillaries rather than the microcirculation of the tissue as a whole.

Radioactive microspheres [36]: In this technique, radioactive labeled small plastic spheres are injected into a large vessel and they are lodged in the microvascular network. The size of the microspheres determines the level at which they become lodged. The radioactivity in an excised tissue is taken as assessment of regional blood flow.

Ultrasonic Doppler flowmetry [37]: In this technique, the blood velocities in the single (superficial) blood vessels is obtained from the Doppler shift imparted to the ultrasound frequency (usually 20 MHz) by the moving blood. The method is objective, fairly easy, quantitative, continuous and non-invasive. The probe is highly sensitive to movement and difficult to calibrate. Direct information about local microcirculatory blood flow cannot be obtained with this method.

Fluorescein tracers [38]: In this technique, a fluorescent agent is injected into an artery or vein and when exposed with ultra-violet light, quantitative values can be obtained with a microfluorometer. The technique is

not expensive, easy and useful for demonstrating large changes in skin perfusion. However the method does not give direct quantitative values and cannot be used for studying fast changes in blood flow.

Orthogonal polarization spectral (OPS) imaging [39]: OPS imaging is a recently developed technique, which allows the visualization of superficial blood vessels using a hand-held microscope. The tissue is illuminated with green linearly polarized light for which absorption by blood is maximal. The reflected light from the tissue is detected by a video camera with a polarizing filter oriented 90° to that of the illuminating light. Most of the reflected light keeps its polarization and cannot pass through the orthogonal polarizer (analyzer) to form the image. The light that penetrates the tissue more deeply and undergoes multiple scattering events (more than ten scattering events) becomes depolarized. Hence, only this depolarized scattered light passing through the orthogonal polarizer (analyzer) effectively back-illuminates absorbing material in the foreground. However, current OPS technology can investigate only tissues covered by a thin epithelial layer and therefore internal organs are not accessible, except for preoperative use. Movement artifacts, semi quantitative measure of perfusion, observer-related bias and also inadequate sedation may limit quality of obtained data and its correct analysis.

1.5 Limitations of laser Doppler perfusion monitoring

Laser Doppler perfusion monitoring meets the clinical requirements of a sensitive, continuous, non-invasive method for microvascular blood flow measurements and has been used in clinics for more than 30 years. The important advantages such as high spatial resolution, noninvasiveness and real-time monitoring make this technique a promising noninvasive tool to evaluate microcirculation in both experimental and clinical settings. But in spite of technological improvements throughout the years, LDPM has some critical limitations that restrict its clinical usefulness [40].

(a) *Motion artefact noise* [41]: Motion artifact noise in the perfusion signal are due to the movement of probe head relative to the tissue or due to the different modes passing through the fiber are changed due to the fiber movements. The frequencies produced range from 0 to 3.5 kHz and the amplitude of the signal can be even higher than that of tissue related perfusion signal. This noise could be minimized by the use of small diameter fibers or by using an integrated probe [42] with the diode laser source and detection integrated in the fiber head. Since averaging over long time results in the manifestation of motion artifact noise as apparent increase in the blood flow, the motion artifact noise could be identified in the perfusion signal by keeping the response time settings below 0.2 s.

(b) *Multiple Doppler shifting* [43]: The theoretical framework of perfusion estimation in laser Doppler flowmetry is based on the assumption that the

fraction of photons undergoing multiple scattering events is negligible. Except for highly perfused organs, the multiple Doppler scattering events are significantly lower than the single scattering events and this assumption is valid. But in the case of highly perfused organs, and apical skin (containing shunt vessels involved in thermoregulation) at elevated temperatures, photons will undergo multiple Doppler scattering events. Although Nilsson addressed this issue by using a lineariser, the signal saturates for high flow rates and spectral power density was poor for high concentrations [18].

(c) *Variations in instrument specifications*: In conventional laser Doppler perfusion monitors, the perfusion signal is presented in relative numbers called perfusion units (PU) and this is influenced by a number of instrumental factors:

-wavelength of the light source
-physical and optical properties of the fiber and probe configuration
-detection efficiency and bandwidth
-data acquisition parameters
-calibration parameters.

The three leading manufacturers of LDPM in Europe are Perimed AB, Moor instruments Ltd and Oxford Optronix Ltd. Since different instruments give different readings for the same physiological perfusion values due to the different physical realizations of the instruments, standardization of the procedures and a common calibration device are necessary. In a study performed for the standardization of the laser Doppler method, a linear relationship was observed between the flux-flow ratios of two laser Doppler perfusion monitors (PF5000, Perimed AB, Sweden and Moorlab monitor, Moor instruments Ltd, UK) [44]. Thus in spite of the technical differences between the instruments the practical behavior of the two instruments is similar, which gives promising perspectives for the feasibility of an instrument independent perfusion unit that may help to standardize the laser Doppler method. For the calibration of the LDPM, different calibration models such as mechanical flow model [45], mixed static and dynamic medium [46], layered phantom [47, 49], Optoelectronic calibration [48], motility standard were developed.

(d) *Depth of measurement*: Due to the inter-and intra-individual variations in the heterogeneous microvascular architecture, physiology and optical properties, the knowledge and control of the sampling depth and measurement volume is not possible in conventional laser Doppler flowmetry. Hence it is difficult to interpret whether the perfusion signal is related to the photons that are scattered from superficial or deeper layers of skin. Depth resolved perfusion information could be obtained by varying the distance between transmitting and receiving fibers [50] or by using a multichannel laser Doppler probe [51]. However, these methods require

either a moving laser source or fiber or an array of fibers with separate calibration procedures to compensate for the gains and optical coupling conditions in the photodetector channels. Multiwavelength systems using green, red and near-infrared light utilizing the dependence of light penetration with wavelength in the therapeutic window have been applied in depth resolved measurements, but their use was limited because of the complications with the use of a green laser source [52].

(e) *Instrument Zero/Biological zero* [53, 54]: When the flow of red blood cells is reduced to zero values experimentally by occlusion around the upper arm or by surgical excision of the tissue, the laser Doppler signal is not reduced to absolute zero values. This biological zero level predominantly results from the Brownian motion of macromolecules arising from the interstitial compartment. Furthermore, the red blood cell vasomotion and Brownian motion within the vascular compartment also contribute. While recording low or ischaemic perfusion levels, this biological zero level makes significant contribution. Subtraction of this level from the measured perfusion readings for obtaining more reliable tissue perfusion information is a point of discussion.

(f) *Influence of optical path length* [8, 50, 55, 56]: If red blood cells in vascular blood can be regarded as independent scatterers, the average number of collisions between photons and red blood cells which is used to determine the concentration of blood cells moving in the tissue is given by

$$\overline{m} = \Sigma_{sc}(rbc) * [RBC] * L$$

Where $\Sigma_{sc}(rbc)$ is the scattering cross section of RBCs, $[RBC]$ is the number of moving RBCs in 1 mm^3 of tissue and L is the mean path length of the detected light [8]. For a homogeneous tissue of fixed concentration and blood volume, the value of $\overline{m}$ is proportional to the average path length of the detected light. The average path lengths will be different for different tissue types due to the changes in tissue optical properties in terms of absorption and scattering [43]. Also, the variance in individual photon path lengths (e.g., length and depth) increases with average photon path lengths. For large fiber separations, $\overline{m}$ may be so large that virtually all the detected photons are Doppler-shifted, with a majority of them multiply shifted. As the optical absorption of the tissue increases, the average optical path length and the average depth probed by the emergent photons decreases. If the tissue absorption is predominantly due to absorption by hemoglobin, an increase of blood volume will lead to a decrease in mean path length <n>, which will partially offset the increase of [RBC] on $\overline{m}$. Large differences in perfusion readings will occur when a vascularized pigmented melanoma is compared with normal dermis, since the high absorption coefficient of melanin will greatly reduce the path length, measurement volume and the intensity of detected light. When different tissues are compared, such as

brain (higher than the average scattering coefficient), muscle (lower than average scattering coefficient) and liver (higher than the average absorption coefficient), the measured perfusion levels will vary significantly. This difference must partly be explained from the difference in tissue optical properties rather than real perfusion differences. Therefore, laser Doppler flowmetry provides only a relative measure of the perfusion level.

1.6 Purpose of the research

In spite of many technological improvements throughout the years, laser Doppler flowmetry suffers several serious drawbacks that restrict its clinical applications. One of the important limitations of this technique is the dependence of the perfusion signal on the probe geometry and optical properties of the tissue, i.e. absorption coefficient, scattering coefficient and anisotropy factor. These dependences result from the varying optical path length of detected photons; the longer the optical path length the greater is the probability for Doppler scattering events to occur, thus yielding an overestimation of the blood perfusion, compared to the short path length situation. In skin perfusion measurements using conventional laser Doppler perfusion monitors, the path length of light is unknown and perfusion values averaged over different and basically unknown path lengths are recorded. This will result in inter- and intra-individual variations in the perfusion readings for the same physiological perfusion values.

Path length resolved laser Doppler perfusion monitoring, of which the basic technique is presented in this work, may overcome this limitation and has potential applications in the fields of fundamental as well as applied research in monitoring the spatial and temporal variations in optical properties in turbid media. Path length resolved photon intensities can be measured using amplitude modulation of the light intensity [1] and time resolved measurements [2] for multiply scattered light, or low coherence interferometry [3-6] for imaging based on single backscattered light. However, for path length distributions multiply scattered light with typical path lengths of several millimeter, a spatial resolution of e.g. 50 micrometers is required, for which 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 this research, we have developed an improved method for path length resolved laser Doppler perfusion monitoring, by combining the principles of coherence gated interferometry and laser Doppler blood flowmetry. The method is based on a phase modulated fiber optic low coherence Mach-Zehnder interferometer, in which the limited temporal coherence acts as a band pass filter in selecting the photons that have traveled a specific path length.

1.7 Outline of the thesis.

Chapter 2 describes the development of phase modulated low coherence fiber optic Mach-Zehnder interferometry for path length resolved optical measurements of multiply scattered light. With this setup, path length resolved dynamic light scattering measurements of multiply scattered light were performed in static and dynamic turbid media. The estimated path length distributions are validated by measuring their deformation for increasing absorption and comparing these observations with predictions based on Lambert-Beer's law. The path length distributions are estimated for different absorption levels in the physiological range and these observations are compared with predictions based on Lambert-Beer's law.

In **chapter 3**, we show the experimental validation of optical path length distributions and path length resolved Doppler broadening in turbid media for different reduced scattering coefficients and anisotropies. Water suspensions of Polystyrene microspheres with high scattering and low absorption levels are used as calibrated scattering phantoms. The measured optical path length distributions and path length dependent spectral diffusion broadening of multiple scattered light are validated with Monte Carlo simulations and Diffusive Wave Spectroscopy respectively.

In **chapter 4**, we describe an improved method for coherence domain path length resolved measurements of multiply scattered photons in turbid media based on sinusoidal phase modulation of the optical path length in the reference arm at high phase angles. The path length distributions and path length resolved Doppler spectra of multiply scattered light are obtained from the spectrum of interference peaks. The experimentally measured optical path lengths are validated with the Monte Carlo technique.

Chapter 5 describes a novel technique to distinguish between Doppler-shifted and non-shifted multiply scattered light from a mixed static and dynamic scattering medium. Optical path lengths 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. The experimentally determined thickness of the static layer that overlies the dynamic layer is validated with the Doppler Monte Carlo technique.

In **chapter 6**, the performance of the system is analyzed in terms of its resolution of optical path length assessment, and its ability to represent various flow speeds within the physiological velocity range. The performance of multimode fibers for detection in a coherence gated interferometric scheme is compared with a single mode fiber optic interferometer. From the spectral analysis of moments of the interference peak appearing at the modulation frequency, optical path lengths and path length dependent Doppler shifts are

measured in an aqueous suspension of 10% of Intralipid 20% flowing through a channel in a block of Delrin over a range of average velocities from 0 to 6 mm/s.

In **chapter 7**, we show the first results on path length resolved laser Doppler perfusion measurements in skin and its variations to occlusion and vasodilation. Real time monitoring of perfusion and its variations during occlusion is measured for a given optical path length. Inter-and intra-individual variations in optical path lengths and path length resolved Doppler shifts are measured and the results are compared with a conventional laser Doppler perfusion monitor.

Finally, **chapter 8** describes a summary of the results of this research project and discusses the future applications and potentials of the results described in this thesis.

References:

1. E. Gratton and M. Limkerman, "A continuously variable frequency cross-correlation phase fluorometer with picosecond resolution," Biophys. J, **44**, 315-324 (1983).
2. B. Chance, J. S. Leigh, H. Miyake, D. S. Smith, S. Nioka, R. Greenfeld, M. Finander, K. Kaufmann, W. Levy, M. Young, P. Cohen, H. Yoshioka, and R. Boretsky, "Comparison of Time-Resolved and -Unresolved Measurements of Deoxyhemoglobin in Brain," Proc. Natl. Acad. Sci., **85**, 4971-4975 (1988).
3. 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, **58** (1998).
4. 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**, 4222-4227 (2001).
5. 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 interferometer," Opt. Lett.**26**, 1492-1494 (2001).
6. 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**, 595-597 (2002).
7. J. M. Tualle, E. Tinet, S. Avrillier, "A new and easy way to perform time resolved measurements of the light scattered by turbid medium", Optics Communications, **189**, 211-220 (2001).
8. A. P. Shepherd and P. Å. Öberg, Laser-Doppler Blood Flowmetry (Kluwer Academic, Boston, 1990).
9. G. Belcaro, U. Hoffmann, A. Bollinger, A. N. Nicolaides, Laser-Doppler (Med-Orion Publishing Co., London, 1994).

10. H. Z. Cummins, N. Knable, and Y. Yeh, "Observation of Diffusion Broadening of Rayleigh Scattered Light," Phys. Rev. Lett. **12**, 150 (1964).
11. C. Riva, B. Ross, and G.B. Benede, "Laser Doppler measurements of blood flow in capillary tubes and retinal arteries," Invest. Ophthalmol., **11**(11), 936-44 (1972).
12. M. D. Stern, "In vivo evaluation of microcirculation by coherent light scattering," Nature, **254**, 56-58 (1975).
13. D. Watkins and G. A. Holloway, "An instrument to measure cutaneous blood flow using the Doppler shift of laser light," IEEE Trans. Biomed. Eng. BME-**25**, 28-33 (1978).
14. K. Wårdell, A. Jakobsson and G. E. Nilsson, "Laser Doppler perfusion imaging by dynamic light scattering," IEEE Trans. Biomed. Eng. **40**, 309-316 (1993).
15. J. D. Briers, "Laser Doppler, speckle and related techniques for blood perfusion mapping and imaging," Physiol. Meas., **22**, 35-66 (2001).
16. A. Serov, W. Steenbergen, F. F. M. de Mul, "Laser Doppler perfusion imaging with a complimentary metal oxide semiconductor image sensor," Opt. Lett. **25**, 300-302 (2002).
17. M. J. Leahy, F. F. M. de Mul, G. E. Nilsson and R. Maniewski, "Principles and practice of the laser-Doppler perfusion technique," Technology and Health Care, **7**, 143-162 (1999).
18. G. E. Nilsson, "Signal processor for laser Doppler tissue flowmeters," Med. Biol.Eng. Comput, **22**, 343-348 (1984).
19. R. Bonner and R. Nossal, "Model for laser Doppler measurements of blood flow in tissue," Appl. Opt., **20**, 2097-2107 (1981).
20. N. C. Abbot, W. R. Ferrell, J. C. Lockhart and J. G. Lowe, "Laser Doppler Perfusion Imaging of Skin Blood Flow Using Red and Near-Infrared Sources," Journal of Investigative Dermatology, **107**, 882–886 (1996).
21. X. F. Li and Y. P. Wang, "Laser Doppler flowmetry for assessment of myocardial microperfusion in the beating rat heart," Vascular Pharmacology, 46(3), 207-214 (2007).
22. D. H. Park, I. W. Hwang, K.S. Jang, D. G. Han, K. Y. Ahn, "Use of laser Doppler flowmetry for estimation of the depth of burns," Plastic and reconstructive surgery, **101**(6), 1516-1523 (1996).
23. A. E. J. Droog, W. Steenbergen, F. Sjoeberg, "Measurement of depth of burns by laser Doppler perfusion imaging," Burns, **27**(6), 561-568 (2001).
24. A. Becker, W. Paffgen, K.H. Vestweber, "Laser Doppler in angiology: Clinical relevance or expensive game?," Vasa. Supp., **33**, 266-267 (1991).
25. M. Husmann, M. Barton, V. Jacomella, A. Silvestro, B. Amann-Vesti, "Long-term effects of endovascular angioplasty on orthostatic vasocutaneous autoregulation in patients with peripheral atherosclerosis Journal of Vascular Surgery, **44** (5), 993-997 (1989).
26. P. Bolognese, J. I. Miller, I. M. Heger, T. H. Milhorat, "Laser Doppler flowmetry in Neurosurgery," J. Neurosurgical Anesthesiology, **5**(3), 151-158 (1993).

27. E. Arbit, G. R. Diresta, "Application of Laser Doppler flowmetry in Neurosurgery," Neurosurgery Clinics of North America, **7**(4), 741-748 (1996).
28. D. L. Clarke, S. R. Thomson, "Attenuated Gastric Mucosal Blood Flow Predicts Non-Healing of Benign Gastric Ulcers," European Surgical Research, **34**, 6 (2002).
29. K. Johansson, "Gastrointestinal application of laser Doppler flowmetry. An experimental and clinical study in cat and man Acta Chir Scand Suppl., **545**, 1-64.(1988).
30. I. Cederholm, H. Evers, J.B. Löfström, "Skin blood flow after intradermal injection of ropivacaine in various concentrations with and without epinephrine evaluated by laser Doppler flowmetry," Reg Anesth., **17**(6), 322-328 (1992).
31. J. Bhattacharya, P. P. Kanjilal, V. Muralidhar, "Analysis and characterization of Photo-plethysmographic signal," IEEE Trans. Biomed. Eng., **48** (2001).
32. E. R. Sluiter, C. J. Rowaan, J. C. Dorlas, J. A. Nijboer, F. F. Jorritsma, J. A. Blom and J. E. W. Beneken, "Peak-to-peak detector for the arterial pulsations in the plethysmogram II: Results of its Use during anaesthesia," Med. Biol. Eng. Comput, **19**, 267-270 (1981).
33. B. Fagrell, "Advances in microcirculation network evaluation: an update," Int. J. Microcirc. Clin. Exp., **15**, 34-40 (1995).
34. E. J. Abbink, H. Wollersheim, P. M. Netten, P. Smits, "Reproducibility of skin microcirculatory measurements in humans, with special emphasis on capillaroscopy," Vasc. Med., **6**, 203-210 (2001).
35. O. Bongard, B. Fagrell, "Discrepancies between total and nutritional skin microcirculation in patients with peripheral arterial occlusive disease (PAOD)," Vasa. , **19**, 105-111 (1990).
36. A. Holmström, D. H. Lewis, "Regional blood flow in skeletal muscle after high-energy trauma. An experimental study in pigs, using a new laser Doppler technique and radioactive microspheres," Acta. Chir. Scand., **149**(5), 453-458 (1983).
37. W. M. Swartz, N. F. Jones, L. Cherup, A. Klein, "Direct monitoring of microvascular anastomoses with the 20 MHz ultrasonic Doppler probe: an experimental and clinical study," Plast. Reconstr. Surg., **81**(2), 149-161 (1988).
38. D. G. Silverman, F. A. Cedrone, W. E. Hurford, T.G. Bering, and D. D. LaRossa, "Monitoring tissue elimination of fluorescein with the perfusion fluorometer: A new method to assess capillary blood flow," Surgery, **90**: 409 (1981).
39. W. Groner, J.W. Winkelman, A.G. Harris, C. Ince, G.J, K. Messmer, R.G. Nadeau, "Orthogonal polarization spectral imaging: a new method for study of the microcirculation," Nat. Med., **5**, 1209-1212 (1999).
40. M. J. Leahy, F.F.M. de Mul, G.E. Nilsson and R. Maniewski, "Principles and practice of the laser-Doppler perfusion technique," Technology and Health Care, **7**, 143-162 (1999).
41. T. P. Newson, A. N. Obeid, R. S. Wolton, D. Boggett and P. Rolfe, "Laser-Doppler velocimetry: the problem of movement artefact," J. Biomed. Eng. 9 169–172 (1987).

42. F. F. M. de Mul, J. Van Spijker, D. Van der Plas, J. Greve, J. G. Aarndoudse and T.M. Smits, "Mini laser-Doppler (blood) flow monitor with diode laser source and detection integrated in the probe," Appl. Opt., **23**, 2970–2973 (1984).
43. D. Erni, A. Banic, A.M. Wheatley and G.H. Sigurdsson, "Haemorrhage during anaesthesia and surgery: Continuous measurement of microcirculatory blood flow in the kidney, liver, skin and skeletal muscle," European Journal of Anaesthesiology, **12**(4), 423-429 (1995).
44. A. L. Petoukhova, W. Steenbergen, F. Morales, R. Graaff, E.D.de Jong, J.M. Elstrodt, F.F.M. De Mul, G. Rakhorst, "Instrument-independent flux units for laser Doppler perfusion monitoring assessed in a multi-device study on the renal cortex," Microvascular Research, **66**, 83-90 (2003).
45. K. Mito, "Characteristics of laser Doppler flowmeters with differing optical arrangements," J. Med. Eng. 16, 236
46. A. Liebert, M. Leahy and R. Maniewski, "A calibration standard for laser-Doppler perfusion measurements," Rev. Sci. Inst., **66** (11), 5169-5173 (1995). (1992).
47. F. F. M. de Mul, M. H. Koelink, M.L. Kok, P.J. Harmsma, J. Greve, "Laser-Doppler velocimetry and Monte Carlo simulations on models for blood perfusion in tissue," Appl. Opt., **34**(28), 6595–6611 (1995).
48. A. Liebert, P. Lukasiewicz, D. Boggett and R. Maniewski, "Optoelectronic standardization of laser Doppler perfusion monitors," **70** (2), 1352-1354 (1999).
49. W. Steenbergen and F.F.M. de Mul, "New optical tissue phantom and its use for studying laser Doppler blood flowmetry", Proc. SPIE 3196, **12** (1997).
50. M. Larsson, W. Steenbergen, and T. Strömberg, "Influence of optical properties and fibre separation on laser Doppler flowmetry," Journal of Biomedical Optics, **7**, 236-243 (2001).
51. A. Liebert, M. Leahy and R. Maniewski, "Multichannel laser-Doppler probe for blood perfusion measurements with depth discrimination," Med. Bio. Eng. and Comp. **36**, 740-747 (1998).
52. B. Obeid, D. Bogget, N. Barnett, G. Dougherty and P. Rolfe, "Depth discrimination in laser Doppler skin blood flow measurement using different lasers," Medical and Biological Engineering and Computing, **26**, 125-163 (1988).
53. A. Colantuoni, S. Bertuglia and M. Intaglietta, "Biological zero of laser-Doppler fluxmetry: microcirculatory correlates in the hamster cheek pouch during flow and no flow conditions," Int. J. Microcirc. Clin. Exp. **13**(2), 125–136 (1993).
54. D. P. Kernick, J. E. Tooke and A. C. Shore, "The biological zero signal in laser doppler fluximetry-origins and practical implications," Pflügers Arch-Eur. J. Physiol., 437, 624–631 (1999).
55. 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**, 2238-2244 (1989).

56. 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**, 124-134 (2003).

Chapter 2

Path length resolved measurements of multiple scattered photons in static and dynamic turbid media using phase modulated low coherence interferometry*

In optical Doppler measurements, the path length of the light is unknown. To facilitate quantitative measurements, we developed a phase modulated Mach-Zehnder interferometer with separate fibers for illumination and detection. With this setup, path length resolved dynamic light scattering measurements of multiply scattered light in static and dynamic turbid media was performed. Optical path length distributions spanning a range from 0-11 mm are measured from the area under the phase modulation peak around the modulation frequency in the power spectrum. A Doppler broadened phase modulation interference peak is observed that shows an increase in the average Doppler shift with optical path length, independent of absorption. Validation of the estimated path length distributions is done by measuring their deformation for increasing absorption and comparing these observations with predictions based on Lambert-Beer's law.

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2.1 Introduction

Laser Doppler blood flowmetry is a non-invasive technique for monitoring blood microcirculation in biological tissues. The coherent light, which is delivered to the tissue through an optical fiber, interacts with static as well as moving scatterers, e.g. red blood cells. The light scattered by moving red blood cells receives a slight frequency shift due to the Doppler effect. Via a second optical fiber some of the scattered light is guided to the detector [1] where the mixture of Doppler shifted and unshifted light causes photocurrent fluctuations. Based on the power spectrum of the photodetector signal, the conventional Laser Doppler perfusion monitor records perfusion values averaged over different and basically unknown path lengths, which creates an uncertainty in the relation between the measured perfusion signal and the real perfusion. 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 development of techniques for monitoring Doppler shifts with path length information would result in more-quantitative and more-reliable tissue perfusion information.

To obtain path length distributions with widths of a few millimeters several techniques have been explored. Time resolved measurements [2] or amplitude modulation of the light intensity [3] can provide this information. However, for a spatial resolution of 50 micrometers, these techniques require either a temporal resolution of 150 fs or electronics working in the GHz range. To avoid these technical challenges coherence gated [4-8] and wavelength modulated [9] interferometric systems have been applied as an alternative approach to measure path-length-distributions up to 10 times the scattering mean free path length within highly scattering media [4-8]. For both the Michelson [4-6] and the Mach-Zehnder based interferometric setups [7,8], these low coherence interferometric measurements depended on the photons that are Doppler shifted by the Brownian motion of the particles in the medium. In contrast to most single-mode fiber optic Michelson interferometric systems used in Doppler OCT [10], which adopt on axis back reflection , 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 R between the illumination and the detection fiber can be varied, giving one some control over the relative depth sensitivity of the system. Our measurements explore the regime of multiply scattered photons. Furthermore, a large detection window with a sufficiently small modal dispersion without affecting the resolution of the system is achieved by the use of multimode graded-index fibers in the collection of scattered light from the sample. Compared to our previous work [7,8], in this paper we add the feature of phase modulation of the reference beam in the Mach-Zehnder interferometric setup. Low coherence

interferometry with phase modulation of the reference beam has recently been adopted in single scattering spectroscopy to analyze the characteristics of extremely dense colloidal suspensions [11]. The goal of this study is to demonstrate the feasibility of phase modulated low coherence interferometry far beyond the single scattering regime. We show that by this phase modulation, also light that has been scattered by static structures only, will contribute to the interferometric signal. 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.

2.2 Theoretical aspects

The fundamental output quantity of a laser Doppler perfusion monitor is the first moment of the power spectrum $P(\omega)$ of the detector signal; in general, the i^{th} moment is being defined as

$$M_i = \int_a^b P(\omega)\omega^i d\omega \qquad (1)$$

Here a and b are device dependent low and high cut-off frequencies. With i=0, a quantity is obtained which is proportional to the concentration of moving red blood cells, while i=1 describes red blood cell flux, which is the product of concentration and the root mean square of the red cell velocity, at least for low blood concentrations [12].

The method presented here utilizes the values of M_0 and the Lorentzian width of the phase modulation peak appearing in the photodetector signal power spectrum to determine the path length distribution and the Doppler shift of photons as a function of optical path length, respectively. The $zero^{th}$ moment M_0 of the heterodyne spectrum is the total power of the photocurrent fluctuations caused by the interference of Doppler shifted light from the sample and reference light of fixed path length and is proportional to the intensity of photons with a certain path length. The weighted first moment M_1/M_0 is equal to the average Doppler shift of the light in this path length. 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 and 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 Lorentzian line width. For higher peak phase modulation angles, the spectrum of interference signal is composed of frequency sidebands at multiples of the modulation frequency and the path length dependent scattering properties of the medium can be obtained from these interference peaks. For sufficiently small phase modulation angles, higher harmonics are absent in the power

spectrum and the average Doppler shift is be obtained from the width of the resulting phase modulation peak present at the modulation frequency. This can be understood from the phasor description of the interfering fields, as shown in fig. 1.

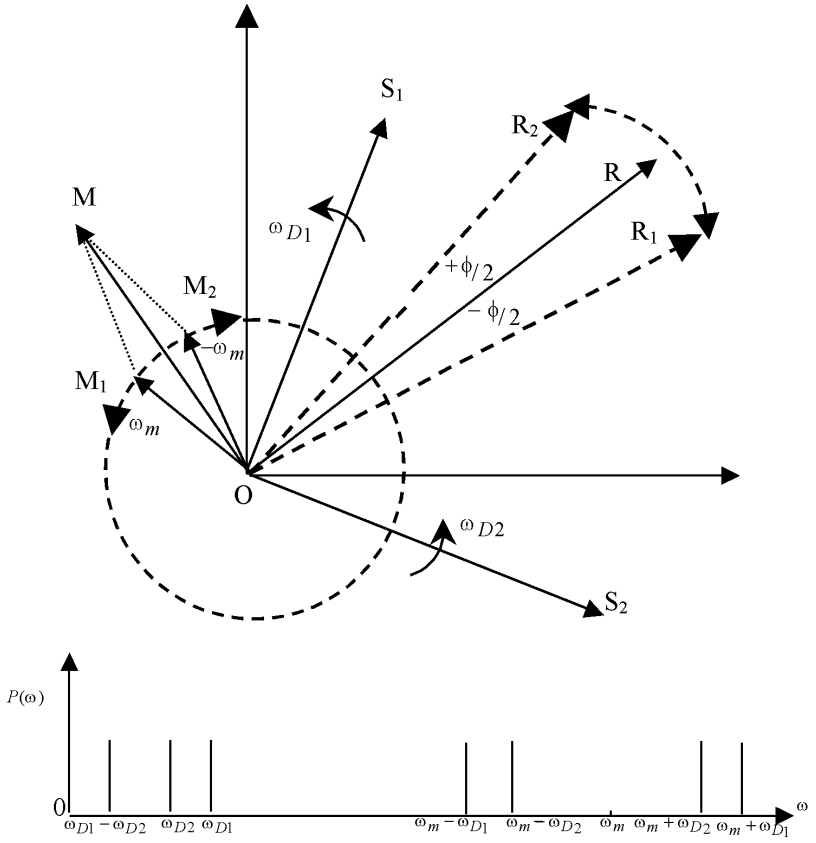

Fig. 1. Phasor diagram for the interfering fields (top) and the resulting power spectrum (bottom).

Here the reference wave and two Doppler shifted sample waves are represented by phasors R, S_1 and S_2, respectively. The angle ϕ is the peak phase deviation due to phase modulation in the reference light, which is depicted by a sinusoidal oscillation of the reference phasor between the extreme phasors R_1 and R_2 on either side of the average phasor R. For small values of ϕ, the oscillation of the reference phasor between R_1 and R_2 is equivalent to the summation of the average reference phasor R, and two phasors M_1 and M_2 of equal length that rotate in opposite directions with a constant angular speed equal to the phase modulation frequency ω_m. The initial phase of M_1 and M_2 should be chosen such that their sum phasor M is perpendicular to R. The

amplitude of M_1 and M_2 is $OM_1=OM_2=1/2.OR.\tan\phi/2$ to achieve the desired phase modulation angle ϕ. (In figure 1 the length of M_1, M_2 and M is enlarged for the sake of clarity). The sample waves S_1 and S_2 with Doppler shifts ω_{D1} and ω_{D2} interfere with both M_1 and M_2. Since only positive frequencies show up in the power spectrum interference peaks are expected at $\omega_m-\omega_{Di}$ and $\omega_m+\omega_{Di}$ $(i=1,2)$. This interference of sample light with reference light will be called 'heterodyne'. In practice from a turbid sample waves are obtained with a distribution of Doppler shifts, leading to a similar distribution of spectral components centered around ω_m. Hence the shape of the peak around ω_m corresponds to the Doppler shift distribution. The component of the reference light represented by the average phasor (OR) that still is at the original light source frequency will also interfere with the scattered light from the sample and thus, apart from the peak around the phase modulation frequency, a heterodyne component will also occur at low frequencies. Finally, the sample phasors S_1 and S_2, will mutually interfere to generate beats at frequency ω_{D1}-ω_{D2}, a component that we call 'homodyne'. Hence, the spectrum at low frequencies is a mixture of homodyne interference of light remitted by the sample, and heterodyne interference between sample light and unmodulated reference light, while the spectral component around the phase modulation frequency is the pure Doppler shift distribution.

2.3 Materials and methods

We use a fiber-optic Mach–Zehnder interferometer (Fig.2) 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. A single mode fiber-optic coupler with a splitting ratio of 90:10 is used to create a reference arm (10%) and a sample arm (90%). 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. The coherence length of the light source, and the intermodal dispersion in the detection fiber, define the path-length resolution of the measurement.

The path length of the reference arm is varied by reflection of the light in a translatable retroreflector and the position of the retroreflector is adjusted to yield an optical path length equal to the optical path length of a certain part of the photons in the sample arm. The reference beam is polarized using a linear polarizer and the phase is sinusoidally modulated at 22kHz using an electro-optic broadband phase modulator (New Focus Model 4002). The sinusoidal phase modulation applied to the light is set to an angle of less than $\pi/2$ radians so that frequency sidebands are absent in the spectra.

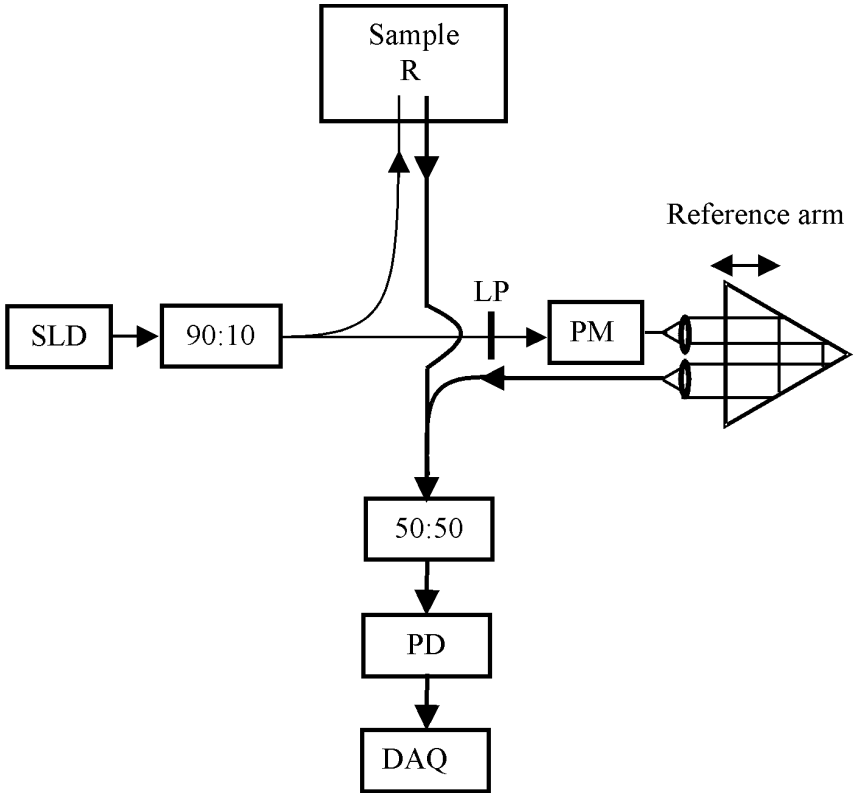

Fig. 2. Schematic of the fiber optic Mach–Zehnder interferometer. Single-mode and gradient index multimode fibers are shown by thin and thick lines, respectively. SLD denotes superluminescent diode, PD is the photodetector, LP is a linear polarizer, PM is the electrooptic phase modulator, 90:10 and 50:50 are single-mode and multimode fiber couplers, respectively.

To validate the obtained optical path length distributions, measurements were performed on three samples with identical scattering properties but increasing absorption levels. The media were an aqueous suspension of 25% of Intralipid 20% [13] and the same suspensions with absorption coefficients of 0.50 mm^{-1} and 0.85 mm^{-1}. For absorption, a black dye (Royal Talens black Ecoline™ Black) was used. The transmission spectrum of a water solution of the black ink in the wavelength range 600-900 nm was measured by a spectrophotometer (Shimadzu UV-2101- PC).

The scattering medium that is used to study the ability of our method to measure on static media is based on 4% (by weight) aqueous solution of poly (vinyl alcohol) (PVA). 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), was used to prepare the aqueous solutions. The desired scattering properties are obtained by adding polystyrene microspheres of ∅ 0.77 μm (μ'_s=2.35mm^{-1}, g=0.85). In this form it is a highly viscous liquid. By adding 4% (by weight) aqueous sodium borate (borax) solution as a cross-linking agent a visco-elastic gel ('slime') is formed with optical properties almost similar to those of the original viscous medium. The amount of borax solution added to the whole volume of the PVA-water-PS suspension (12 ml) was about 1.0 ml to produce a visco-elastic gel medium. Measurements obtained in these viscous and gel media were compared with those obtained in an aqueous polystyrene suspension with equal optical properties.

We measured the path length distribution for a center-to-center fiber distance of 500 μm. The path length distribution is shifted for large fibre distances, which is due to the increase in the length of the shortest path from one fibre to another and is broader due to diffusive light transport. At the detector (New Focus Model 2001 photo receiver) the light that is scattered in the sample and the light from the reference arm after passing through a 50:50 fiber coupler are mixed. The AC photocurrent is measured with a 12 bit analogue to digital converter (National Instruments), sampling at 50 kHz, the signal was sampled for 52 seconds to get an average of 1000 spectra and is Fourier transformed. Squaring this Fourier transform yields the power spectrum of the signal. To get smooth curves we use an average of 1000 spectra for the aqueous and viscous media, and 5000 spectra in the case of the visco-elastic medium.

The position of the retroreflector that corresponds to the zero optical path length in the medium was determined by replacing the sample by a mirror on a distance of approx. 13 mm to the tips of the two fibers. From the position of the retroreflector for which a heterodyne signal was obtained, the position for a zero path length of light probing the scattering samples could be obtained. The power spectra measured with the sample replaced by a reflecting mirror is shown in fig.3. The width of this spectrum is 55.34 Hz.

We measured spectra for a range of positions of the retroreflector in the reference arm. The path length distribution is obtained by subtraction of M_0 of the reference arm noise and of the sample arm 'homodyne' Doppler signal from M_0 of the corresponding spectra around the modulation frequency between frequencies of 20 and 24 kHz. The AC power of the heterodyne spectra is normalized by the DC^2 component to render the results independent of the power of the light source. The variation of the DC value of the reference beam for the whole path length is within 4%. The modulation peak appearing in the power spectra is fitted with a Lorentzian function and the Doppler shift is measured from the corresponding Lorentzian width of the modulation peak.

2.4 Experiments and results

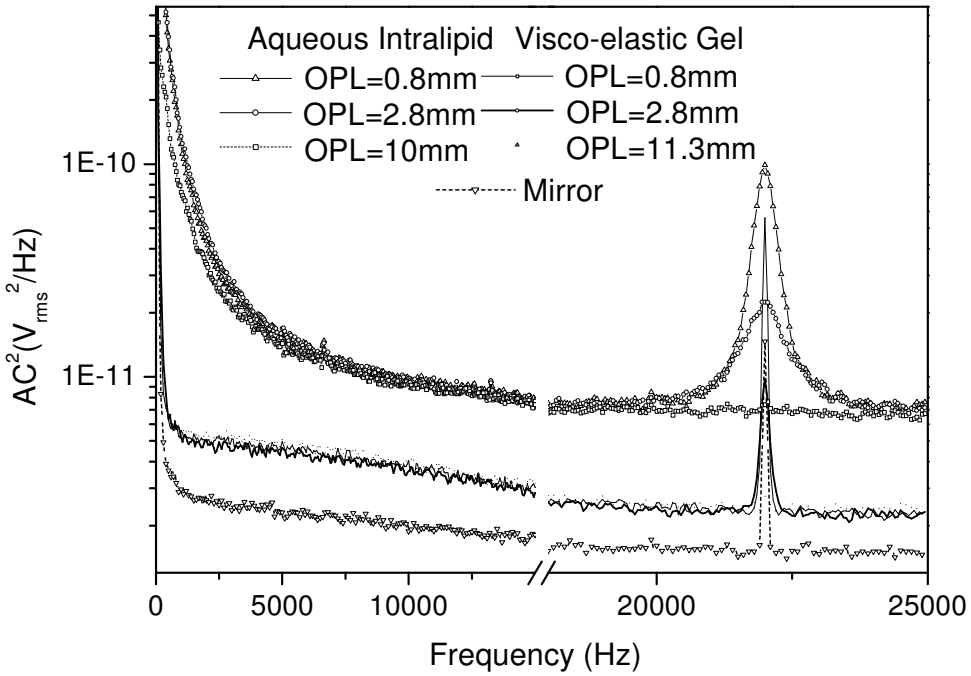

Fig. 3. Power spectra measured for three positions of the reference-arm mirror, corresponding to an optical path length of 0.8, 2.8 and 11.3 mm. An aqueous Intralipid suspension (μ_a = 0.001 mm^{-1}) and a gel medium (μ'_s=2.35mm^{-1}, g=0.85), and mirror are used as the dynamic, static scattering and reflecting media respectively.

Figure 3 shows the power spectra measured for an aqueous Intralipid solution and visco-elastic gel medium with a fiber distance (R) of 500 micrometer for three positions of the retroreflector, which correspond to optical path lengths of 0.8, 2.8 and 10 mm in the sample. At optical path lengths of 0.8 and 2.8 mm interference peaks are observed around the phase modulation frequency of 22 kHz, while at an optical path length of 10 mm no interference is observed. For the aqueous medium we observe the width of the phase modulation peak to increase with the optical path length. The variation that appears in the power spectra at low frequencies (0-10 kHz) measured for aqueous scattering media (Figure 3) is the result of Doppler broadening of light scattered by particles in Brownian motion. The power spectrum measured for

widely different optical path lengths in the sample and the reference arm, contains the ordinary homodyne signal due to the mutual interference of scattered light over almost equal optical path lengths in the sample. The power spectrum measured when the path length difference between the reference light and the scattered light is within the coherence length of light source, contains a heterodyne component at low frequencies, superimposed on the homodyne spectrum. This is due to the interference of scattered light with that component of the electric field of the reference light which is not phase modulated as explained in the introduction with reference to the phasor diagram shown in figure 1.

For the visco-elastic gel medium, no broadening of the line width is observed in fig.3. Unlike the dynamic media, there is no observable difference at low frequencies between the spectra for the three optical path lengths.

Estimations of path length distributions of photons in the aqueous Intralipid suspension (μ_a = 0.001 mm^{-1}) and for identical suspensions with different absorption levels (0.50 mm^{-1} and 0.85 mm^{-1}) are shown in fig.4. The estimation of the optical path length distribution is obtained for increasing absorption levels. The minimum path length is the same for all absorptions and is related to the fiber distance of 500 micrometer. At this path length all the distributions start to increase independent of the absorption. As the photons with longer path length have a greater probability to be absorbed in an increasingly absorbing medium, M_0 decreases with the absorption. Hence the path length distribution narrows and shows a decrease in the average intensity as the absorption coefficient increases. Lambert-Beer's law can describe the effect of absorption on the path length distribution. According to the law of Lambert–Beer, the light intensity I_0 in an absorbing medium decays exponentially as $I(L)= I_0 \exp(-L. \mu_a/n)$ with L the optical path length, and μ_a and n the absorption coefficient and the refractive index of the medium. To validate that the results shown in figure 4 represent the true optical path length distributions, we verify whether the path length distribution of the original Intralipid and the same suspensions with high absorption coefficients are mutually related by Lambert-Beer's law. The path length distributions of original Intralipid multiplied by the exponential decay function $\exp(-L. \mu_a/n)$ and the experimental data are shown in figure 4 in linear and logarithmic scales. There is a good agreement between the experimental data and the calculated values (for n = 1.33, μ_a = 0.50 and 0.85 mm^{-1}) on the basis of Lambert-Beer's law up to an optical path length of 4.5 mm, which proves that path length distributions have been correctly measured.

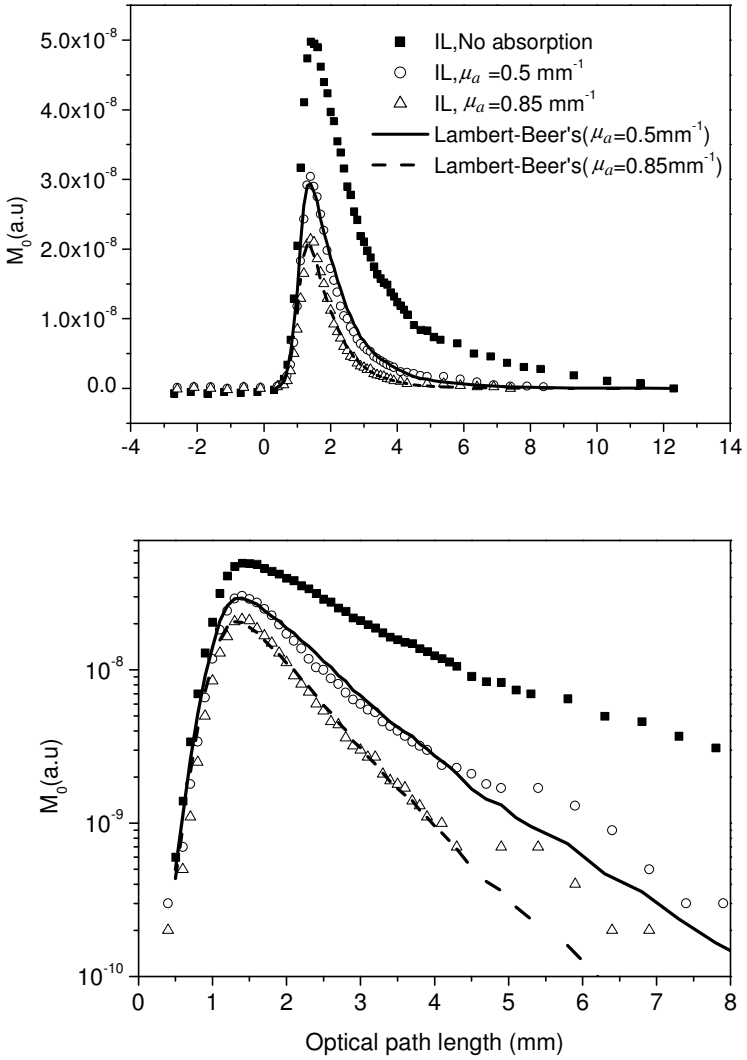

Fig. 4. Optical path length distributions estimated from the zero order moment of the phase modulation peak for an aqueous Intralipid suspension (μ_a = 0.001 mm^{-1}) and for identical suspensions with two different absorption coefficients (0.50 mm^{-1} and 0.85 mm^{-1}), but equal reduced scattering coefficient (linear and logarithmic scales). The lines result from the application of Lambert-Beer's law on the experimental dataset for zero absorption.

The average Doppler shift, measured from the Lorentzian width of Doppler broadened phase modulation interference peaks is represented in fig.5 as a function of the optical path length. The average Doppler shift increases with the optical path length, which can be expected from the increase in the number of scattering events with the optical path length. Further, for a given optical path length the Doppler broadening of the modulation peak is equal for the media with zero absorption and the lowest nonzero absorption coefficient (μ_a = 0.50 mm^{-1}). But in the case of the highest absorption level (μ_a = 0.80 mm^{-1}), the Doppler broadening is equal to those of the other absorption levels for optical path lengths up to 1.1 mm, while for large optical path lengths, the Doppler broadening is found to be decreased, in particular in the optical path length interval between 1.1 and 2 mm.

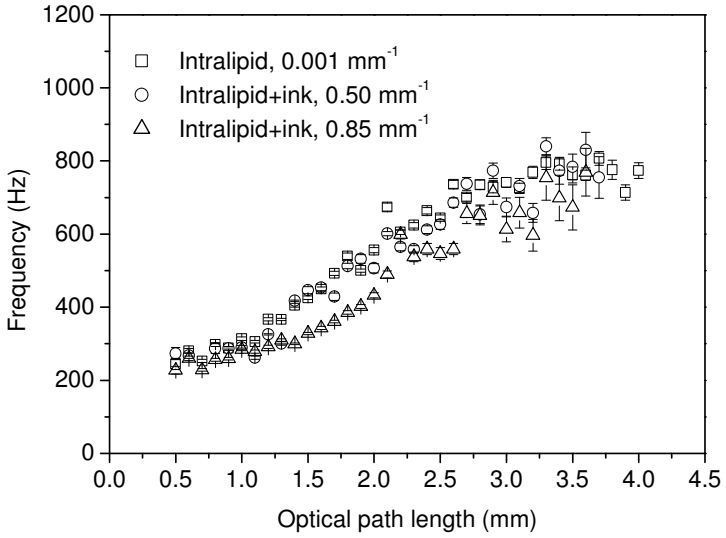

Fig. 5. The average Doppler shift extracted from the phase modulation peak, as a function of the optical path length for an aqueous Intralipid suspension μ_a = 0.001 mm^{-1}) and for identical suspensions with two different absorption coefficients (0.50 mm^{-1} and 0.85 mm^{-1}).

The optical path length distributions measured for the viscous medium, the visco-elastic gel media and the aqueous suspension of Polystyrene microspheres with different dynamical properties and identical optical properties (μ'_s=2.35mm^{-1}, g=0.85), are shown in fig. 6.

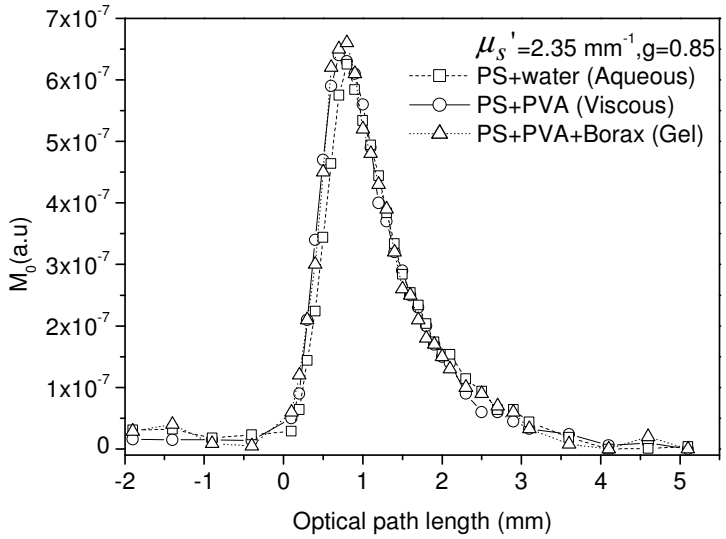

Fig. 6. Optical path length distributions estimated from the zero order moment of the phase modulation peak, for viscous media (PVA+ Polystyrene microspheres), visco-elastic gel media (PVA+ Polystyrene microspheres+Borax) and the aqueous suspension of Polystyrene microspheres with different dynamical properties and identical optical properties (μ'_s=2.35mm^{-1}, g=0.85).

The average Doppler shifts observed for these media, are represented in fig.7 as a function of the optical path length. For the PVA-PS viscous mixture, a decrease in the average Doppler shift is observed compared to the water suspension of polystyrene microspheres with identical optical properties. When Borax was added to this PVA-PS mixture, an almost static gel was formed due to cross-linking and the Lorentzian line width is further reduced for a given optical path length.

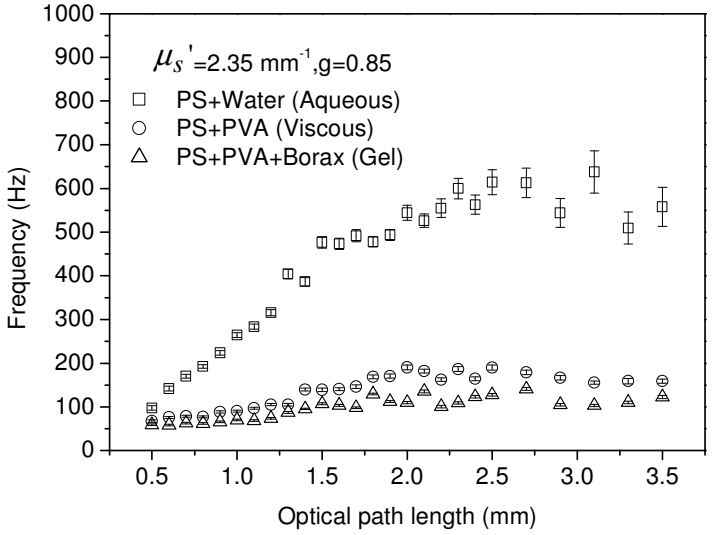

Fig. 7. The average Doppler shift extracted from the phase modulation peak, as a function of the optical path length for viscous media (PVA+ Polystyrene microspheres), visco-elastic gel media (PVA+ Polystyrene microspheres+Borax) and the aqueous suspension of Polystyrene microspheres with different dynamical properties and identical optical properties (μ'_s=2.35mm^{-1}, g=0.85).

2.5 Discussion

In this paper we have presented optical path length distributions and path length resolved Doppler shifts of multiply scattered light, extracted from the spectral peak that was generated by phase modulation of the reference arm in a low coherence Mach Zehnder interferometer. As such, these data can also be obtained without modulation, but then we only can obtain information about photons which where Doppler shifted by the medium. Hence, phase modulation will enable us to measure path length distributions of static, and mixed static and dynamic media. A second advantage of using phase modulation is that the information can be shifted to higher frequencies, where often the noise level is lower and its spectrum is more flat than for low frequencies (Fig.3). Without phase modulation, path length and Doppler broadening information must be retrieved from differences between the homodyne spectrum of photocurrent fluctuations generated by mutual interference of light through the sample, and the spectrum caused by interference of all detected light, which consists of a homodyne and a heterodyne part. Thus, in this case the heterodyne spectrum will occupy the same frequency range as the homodyne spectrum and the

homodyne fluctuations plus real noise will act as noise to the heterodyne spectrum, which contains the path length information. As can be seen in fig.3, with phase modulation the heterodyne spectrum is shifted to a frequency range where the power spectral level of the homodyne fluctuations plus noise is more than one order of magnitude lower, while the level of the noise is a factor 2.5 lower. When the signal-to-noise ratio is expressed as the zero order moment M_0 of the total spectrum for the retroreflector position where maximum interference is measured, divided by the value of M_0 at a position where no interference can be expected, it appears that phase modulation enhances the signal-to-noise ratio by a factor of approximately 30.

The effect of intermodal dispersion of multimode fibers on the path length resolution is reduced by the choice of graded index fibers, with the upper limit of multimode dispersion, which is still of the order of 1mm/m. This latter number suggests that for the fibre length in our setup, dispersion could have a considerable influence on the measured path length distributions. We measured the path length resolution of our setup by replacing the sample arm by a mirror and the position of the retroreflector was translated for which a heterodyne signal was obtained. The path length resolution measured was 50 μm, and this degradation in resolution from 18 μm (as expected from the coherence length of the light source) was due to the modal dispersion of multimode graded index detection fibers. This resolution of 50 μm is sufficient for measuring path length distributions of 10mm and thus do not suggest a significant deteriorating effect of intermodal dispersion in the path length resolved measurements. The results presented here exhibit the trends that must be expected for path length distributions and Doppler shifts of multiply scattered light. For increasing absorption coefficient the estimated path length distributions narrow and show a decrease in the average intensity. This mutual relationship between the path length distributions obtained for various absorption levels through Lambert-Beer's law proofs the correctness of these results.

The observed line width broadening 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. For a given medium with a constant scattering coefficient but absorption coefficients μ_a = 0.001 and 0.50 mm^{-1} the Doppler broadening of path length resolved heterodyne spectra is shown to be independent of the absorption level, for a given optical path length. But in the case of a higher absorption coefficient (μ_a = 0.85 mm^{-1}), for optical path lengths between 1.3 and 2 mm, the Doppler broadening deviates from those obtained for the lower absorption levels. This absorption level would correspond to an unrealistically high relative blood volume of 45%. The nonzero absorption levels used in this study are larger than the values found on average in normally perfused tissue. Therefore, our results indicate that for absorption levels realistic for tissue, our method enables Doppler measurements independent of the absorption level of the medium in which the moving

particles are embedded. A similar behaviour may be obtained for the effect of the scattering coefficient of a static tissue matrix with moving particles.

The path length distributions measured for viscous media, visco-elastic gel media and the aqueous suspension of Polystyrene microspheres (μ'_s=2.35mm^{-1}, g=0.85) are identical and the variation of the DC value for all the above three samples is within 7%. Thus, independent of the Brownian motion of the scatterers, our method can measure realistic optical path length distributions. For a given optical path length, the average Doppler shift is reduced in the case of the viscous water-PVA-PS mixture, compared to the corresponding water suspension of Polystyrene microspheres. In the case of gel, the scatterers can make restricted Brownian motion about their average position and the effect of Doppler broadening due to multiple scattering events is further suppressed. We used an average of 1000 spectra to obtain path length distributions from dynamic samples. However, in the case of a gel medium, an average of 5000 spectra was needed to measure a decent path length distribution. This observation that averaging over a long time was needed in a static medium is similar to the observation by Tualle *et al.* [9] that the path length distributions of multiply scattered light transmitted to a solid scattering medium showed a random shape and they used an ensemble averaging of measurements obtained at 600 positions on the scattering medium. In our visco-elastic gel medium, the slow motion of the scattering particles allowed for averaging of multiple configurations of the medium, which is equivalent to spatial averaging. The background of the apparent inherent noisiness of path length distributions in static media as compared to dynamic media is not clear.

The experimental results presented here are performed on non-flowing samples, in which the Doppler shift was imparted due to Brownian motion of particles. The common method of calibration used in conventional laser Doppler perfusion instruments is based on the Brownian motion of latex spheres in water solution. The calibration signal of these instruments resulting from Brownian motion is within the physiological range. Also, laser Doppler perfusion monitors (Perimed PF5000 and Moorlab) measured similar amounts of Doppler broadening in the motility standard (an aqueous suspension of polystyrene microspheres with a diameter of 320nm) and in vivo on the cortex of pig's kidney [14]. Our preliminary measurements on flowing sample (a rubber tube filled with an aqueous suspension of 20% of Intralipid 20% which was pumped by a peristaltic pump for different flow velocities) indicate that this method has sufficient dynamic range in measuring Doppler shifts from flowing samples. Over a range of velocities (0, 3.68, 5.15 mm/s), the Doppler shift per path length bears a linear relation with the velocity for a range of optical path lengths from 0.4 to 1.4 mm. The average Doppler shift per unit path length measured was 219, 843, 1006 Hz for flow velocities of 0, 3.68, 5.15 mm/s respectively. Also, for a given velocity, the Doppler shift increases with optical

path length due the relative increase in the number of multiple scattering events in the sample arm.

Future experiments on well defined and calibrated samples of polystyrene microspheres with different scattering levels and anisotropies, which are more typical for biological tissue, will reveal the accuracy of our method for Doppler spectra and the results will be compared with the predictions of Diffusive Wave Spectroscopy. With the experimental approach presented here, the dynamic properties of turbid media can be measured independent of optical properties, which is an important condition for absolute perfusion measurements for various tissue types. We aim to extend this method to path length sensitive measurement of tissue perfusion. Furthermore, since for longer optical path lengths the slope of the path length distributions is affected by absorption, differences in path length distribution for various blood concentrations in skin may reveal the hemoglobin content of skin. Determination of blood oxygenation may be an important application of spectroscopic implementation of our technique.

2.6 Conclusion

We have developed an improved method to determine path length distributions of multiple scattered light in static and dynamic turbid media using phase modulated coherence gated interferometry. Optical path length distributions have been measured with optical path length ranges between zero and 11 mm. By relating experimental estimations of optical path length distributions obtained for various absorption coefficients by Lambert-Beer's law, we have proven to be able to measure path length distributions of multiply scattered photons in turbid media. We have shown that path length-resolved dynamic light scattering can measure the dynamic properties of a medium independent of its optical absorption properties, at least when absorption levels are applied in the range found for biological tissues.

Using the fiber-optic Mach-Zehnder interferometer with phase modulation enables us to obtain path length distributions and to extract path length resolved information from mixed static and dynamic turbid media. By its non-invasive nature in measuring path length resolved dynamic light scattering, the method has potential applications in the fields of fundamental as well as applied research in monitoring the spatial and temporal variations in optical properties in turbid media, blood perfusion in tissue and its hemoglobin content.

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References:

1. A. P. Shepherd and P. Å. Öberg, Laser-Doppler Blood Flowmetry (Kluwer Academic, Boston (1990).
2. B. Chance, J. S. Leigh, H. Miyake, D. S. Smith, S. Nioka, R. Greenfeld, M. Finander, K. Kaufmann, W. Levy, M. Young, P. Cohen, H. Yoshioka, and R. Boretsky, "Comparison of Time-Resolved and Unresolved Measurements of Deoxyhemoglobin in Brain", Proc. Natl. Acad. Sci. **85**, 4971-4975 (1988).
3. E. Gratton and M. Limkerman, "A continuously variable frequency cross-correlation phase fluorometer with picosecond resolution," Biophys. J. **44**, 315-324 (1983).
4. 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**, 4222-4227 (2001).
5. G. Popescu, A. Dogariu, "Optical path-length spectroscopy of wave propagation in random media", Opt. Lett. **24**, 442-444, (1999).
6. 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 **58**, 7664-7667 (1998).
7. 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 interferometer", Opt. Lett. **26**, 1492-1494 (2001).
8. 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**, 595-597 (2002).
9. J. M. Tualle, E.Tinet, S.Avrillier, "A new and easy way to perform time resolved measurements of the light scattered by turbid medium", Opt. Comm. **189**, 211-220 (2001).
10. 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**, 1178 (1991).
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**, 555-557 (2005)
12. G. E. Nilsson, "Signal processor for laser Doppler tissue flowmeters, Med. Biol. Eng. comput**.** **22**, 343-348 (1984).
13. S. T. Flock, S. L. Jacques, B. C. Wilson, W. M. Star, and M. J. C. van Gemert, "Optical properties of intralipid: a phantom medium for light propagation studies," Lasers Surgery Med. **12**, 510-519 (1992).
14. A. L. Petoukhova, W. Steenbergen, F. Morales, R. Graaff, E. D.de Jong, J.M. Elstrodt, F. F. M. de Mul and G. Rakhorst, "Instrument independent flux units for laser Doppler perfusion monitoring in a multi-device study on the renal cortex", Micro vascular research, **66**, 83-60 (2003).

Chapter 3

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

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.

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3.1 Introduction

In turbid media, photons follow different trajectories and reach different depths. This 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.

3.2 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. The reference beam is polarized using a linear polarizer and the phase is sinusoidally modulated at 22kHz 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,

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

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, ($D_B = K_B T/3\pi a$) 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), η is the viscosity of the suspending liquid [η =1.0 cps for water] and a is the hydrodynamic diameter (∅0.20 and ∅0.77 μm) of the scattering particles [11,28].

Water suspensions of Polystyrene microspheres (Polysciences Inc) with diameters of ∅0.20 μm (anisotropy factor, g=0.18) and ∅0.77 μm (g=0.85) are used to make calibrated scattering phantoms. Samples with reduced scattering coefficients (μ_s') of 7.00 (g=0.85), 4.95 and 3.25 mm^{-1} (g=0.18) and absorption coefficient (μ_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 (λ =832nm) from a fiber with a diameter of 100 μm is randomly scattered in a layer of water suspension of polystyrene micro spheres (μ_a =0.001 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 (∅0.20 μm) and g=0.85 (∅0.77), reduced scattering coefficients (μ_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 (μ_a =10 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 μm, fiber separation=300 μm, NA=0.29) is assumed to be detected, and its optical path length is recorded.

3.3 Results and discussion

Typical phase modulation peaks appearing in the power spectra measured in our experiments for ∅0.20 μm and ∅0.77 μm 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.

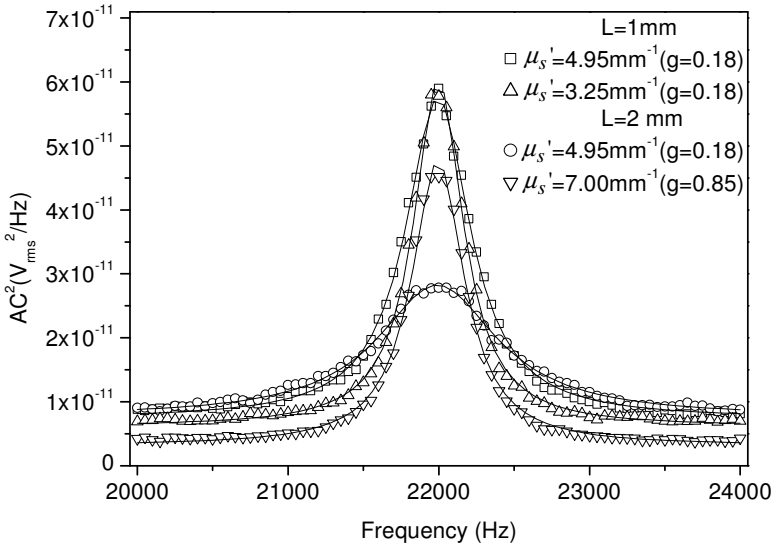

Fig. 1. Phase modulation peaks appearing in the photodetector signal power spectrum fitted with Lorentzian functions.

The experimental optical path length distributions and simulation results for isotropic and anisotropic scatterers are shown in figure 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).

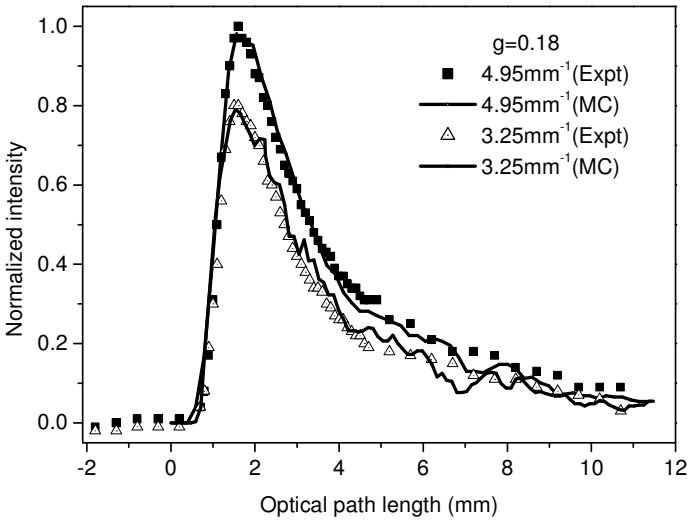

Fig. 2. Measured (points) and Monte Carlo simulated (lines) optical path length distributions for a particle suspension of ∅ 0.20 μm (g=0.18) for two scattering levels.

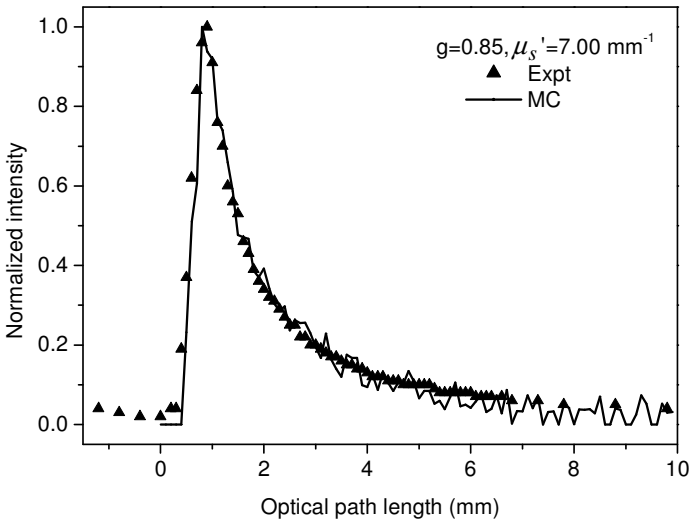

Fig. 3. Measured (points) and Monte Carlo simulated (line) optical path length distributions for a particle suspension of ∅ 0.77 μm (g=0.85).

Figure 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 figure 4 and 5, the average Doppler shift increases with the optical path length and for the suspension with g=0.18 (figure 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 4.5mm. 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 figure 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 $f_0=q^2D_B$, with photon momentum transfer $q = 2k\sin\theta/2$ a function of the scattering angle θ.

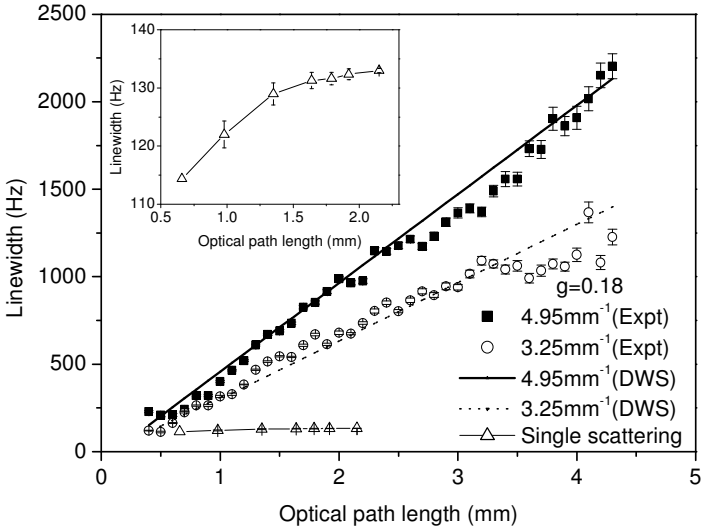

Fig. 4. Experimental (points) and DWS-predicted (lines) average Doppler shift (FWHM of the Lorentzian fit to the phase modulation peak), *vs.* optical path length, for a particle suspension of ∅ 0.20 μm (g=0.18). Inset and lower line in main graph: prediction for single scattering.

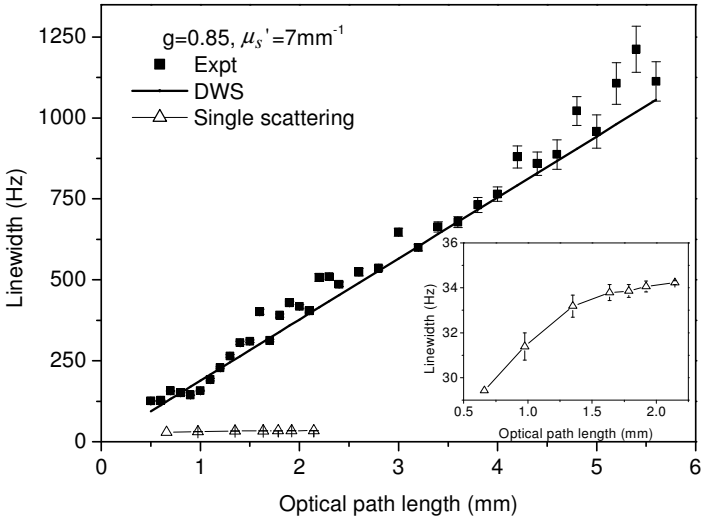

Fig. 5. Experimental (points) and DWS-predicted (line) average Doppler shift (FWHM of the Lorentzian fit to the phase modulation peak) *vs.* optical path length for a particle suspension of ∅ 0.77 μm (g=0.85). . Inset and lower line in main graph: prediction for single scattering.

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 fig. 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 θ will slightly vary, the consequence of which is indicated by the error bars in the single scattering data in fig. 4 and 5. For a particle suspension of ∅ 0.20 μm, 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 (μ_s' =3.25 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 ∅ 0.77 μm, 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.

3.4 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.

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References:

1. A. P. Shepherd and P. Å. Öberg, Laser-Doppler Blood Flowmetry (Kluwer Academic, Boston, 1990).
2. A. Liebert, M. Leahy and R. Maniewski, “Multichannel laser-Doppler probe for blood perfusion measurements with depth discrimination,” Med. Bio. Eng. Comp, **36**, 740-747 (1998).

3. F. Morales, R. Graaff, A. J. Smit, R. Gush, G. Rakhorst, "The influence of probe fibre distance on laser Doppler perfusion monitoring measurements," Microcirculation , **10(5),** 433-441, (2003).
4. M. Larsson, W. Steenbergen, and T. Strömberg, "Influence of optical properties and fibre separation on laser Doppler flowmetry," J.Biomed. Opt, **7**, 236-243 (2001).
5. A. Liebert, M. Leahy and R. Maniewski, "A calibration standard for laser-Doppler perfusion measurements," Rev. Sci. Inst., **66** (11), 5169-5173 (1995).
6. A. Liebert, P. Lukasiewicz, D. Boggett and R. Maniewski, "Optoelectronic standardization of laser Doppler perfusion monitors," Rev. Sci. Inst. **70** (2), 1352-1354 (1999).
7. W. Steenbergen and F.F.M. de Mul, "New optical tissue phantom and its use for studying laser Doppler blood flowmetry", Proc. SPIE **3196**, 12 (1997).
8. E. Gratton and M. Limkerman, "A continuously variable frequency cross-correlation phase fluorometer with picosecond resolution," Biophys. J, **44**, 315-324 (1983).
9. B. Chance, J. S. Leigh, H. Miyake, D. S. Smith, S. Nioka, R. Greenfeld, M. Finander, K. Kaufmann, W. Levy, M. Young, P. Cohen, H.Yoshioka, and R. Boretsky, "Comparison of Time-Resolved and -Unresolved Measurements of Deoxyhemoglobin in Brain," Proc. Natl. Acad. Sci., **85**, 4971-4975 (1988).
10. 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, **58** (1998).
11. 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**, 4222-4227 (2001).
12. 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 interferometer," Opt. Lett.**26**, 1492-1494 (2001).
13. 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**, 595-597 (2002).
14. 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**, 024020 (2007).
15. G. Maret and P. E.Wolf, "Multiple light-scattering from disordered media - the effect of Brownian motion of scatterers," Z. Phys. B **65**, 409 (1987).
16. J. Pine, D. A. Weitz, P.M. Chaikin, and E. Herbolzheimer, "Diffusing-wave spectroscopy," Phys. Rev. Lett. **60**, 1134-1137 (1988).
17. P. J. Berne and R. Pecora, Dynamic Light Scattering (Wiley, New York, 1976).
18. R. Pecora, Dynamic light scattering: Applications of photon correlation spectroscopy (Plenum, New York, 1985).

19. A. Ishimaru, Wave Propagation and Scattering in Random Media (IEEE Press, Piscataway, 1997).
20. J. Durian, “Accuracy of diffusing-wave spectroscopy theories,” Phys. Rev. E **51**, 3350 (1995).
21. P. D. Kaplan, M. H. Kap, A.G.Yodh, and D. J. Pine, “Geometric constraints for the design of diffusing-wave spectroscopy experiments,” Appl. Opt. **32**, 3828 (1993).
22. P.A. Lemieux, M.U. Vera, and D. J. Durian, “Diffusing-light spectroscopies beyond the diffusion limit: The role of ballistic transport and anisotropic scattering,” Phys. Rev. E **57**, 4498 (1998).
23. B. Das, F. Liu, and R. R. Alfano, Vol. 2 of Trends in optics and photonics, 41-44 (1996).
24. I. I. Freund, M. Kaveh, and M. Rosenbluh, "Dynamic multiple scattering: Ballistic photons and the breakdown of the photon-diffusion approximation," Phys. Rev. Lett. **60**,1130-1133 (1988).
25. M. Yoo, F. Liu, and R. R. Alfano, "When does the diffusion approximation fail to describe photon transport in random media," Phys. Rev. Lett. **64**, 2647-2650 (1990).
26. R. Carminati, R. Elaloufi, and J.J. Greffet, “Beyond the Diffusing-Wave Spectroscopy Model for the Temporal Fluctuations of Scattered Light,” Phys. Rev. Lett. **92**, 213903 (2004).
27. F. F. M. de Mul, M. H. Koelink, M. L. Kok, P. J. Harmsma, J. Greve, R. Graaff, and J. G. Aarnoudse, “Laser Doppler velocimetry and Monte Carlo simulations on models for blood perfusion in tissue,” Appl. Opt. , **34**, 6595-6611 (1995).
28. A. G. Yodh, P. D, Kaplan, and D.J.Pine, “Pulsed diffusing-wave spectroscopy: High resolution through nonlinear optical gating,” Phys. Rev. B **42**, 4744 (1990).

Chapter 4

High angle phase modulated low coherence interferometry for path length resolved Doppler measurements of multiply scattered light*

We describe an improved method for coherence domain path length resolved measurements of multiply scattered photons in turbid media. An electro-optic phase modulator sinusoidally modulates the phase in the reference arm of a low coherence fiber optic Mach-Zehnder interferometer, at a high phase modulation angle. For dynamic turbid media this results in Doppler broadened phase modulation interference peaks at the modulation frequency and its multiples. The signal to noise ratio is increased by almost one order or magnitude for large modulation angles and the shape of the spectral peaks resulting from the interference of Doppler shifted sample waves and reference light is not changed. The experimentally measured optical path lengths are validated with the Monte Carlo technique.

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4.1 Introduction

Path length resolved photon intensity in scattering media, and the associated Doppler spectra, can be measured using coherence gated [1-5] and wavelength modulated [6] interferometric systems, in which the limited temporal coherence acts as a band pass filter in selecting the photons that have traveled a specific path length. Michelson [1-2] and Mach-Zehnder based interferometric setups [3-4] for measurements on completely dynamic media have been published. In a preliminary study, we showed that path length distributions in almost static and mixed static-dynamic media can be measured by modulating the optical path length in the reference arm [7]. This will enable path length resolved measurements in mixed media such as tissue perfused with blood.

In phase modulated low coherence interferometry at low modulation angles, the power spectrum shows an interference peak at the modulation frequency only, with a Doppler broadening depending on the dynamic properties of the medium. This technique has been explored in single scattering spectroscopy for analyzing the characteristics of extremely dense colloidal suspensions [8] and in industrial metrology for measuring target displacements [9]. For high peak phase modulation angles, frequency sidebands are observed at multiples of the modulation frequency. Usually, either the phase modulation angle is kept sufficiently small to avoid frequency sidebands [8], or the unwanted high-order harmonics are removed by low-pass filtering [9].

While usually the effect of higher order spectral bands is avoided, in this manuscript we deliberately generate and use them to enhance the signal. We show that the path length distributions and path length resolved Doppler spectra of multiply scattered light can be obtained from the first order peak in the power spectrum corresponding to the phase modulation frequency plus higher order peaks at two or three times the modulation frequency. Optical path length distributions are measured from the area of multiple interference peaks in the power spectrum, in a bandwidth of $\pm$ 2 kHz around these peaks. The average Doppler shift by diffusion broadening is measured from the FWHM of the Doppler broadened phase modulation interference peaks at the modulation frequency and at higher harmonics, appearing in the photodetector signal power spectrum.

4.2 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. A single mode fiber (mode field diameter=5.3 μm, NA=0.14) is used for illumination, while a multimode graded-index fiber (core diameter =100 μm, NA=0.29) is

used for detection, providing a large detection window and a small modal dispersion. The centre-to-centre fibre distance is 300 μm. The reference beam is polarized using a linear polarizer and its phase is sinusoidally modulated at 7 kHz using an electro optic broadband phase modulator (New Focus Model 4002). The peak optical phase shift ($\Delta\phi$) applied to the modulator was increased from 0.51 radians to 2.04 radians so that for the latter phase modulation amplitude frequency sidebands are present 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 [7]. Water suspensions of Polystyrene microspheres (Polysciences Inc) of ∅0.77 μm (g=0.85) are used to make a scattering phantom with a reduced scattering coefficient (μ_s') of 2 mm^{-1}.

Monte Carlo simulations were performed [10] to validate the optical path length distributions. In the simulation, 100000 photons are injected into a simulated medium with scattering properties equal to those of the experimental medium, using Mie scattering functions, and with an absorption coefficient of 0.001 mm^{-1}. Each photon returning to the detection fiber (fiber separation=300 μm, NA=0.29) is assumed to be detected, and its optical path length is recorded.

4.3 Results and discussion

Figure 1 shows power spectra measured for the water suspension of polystyrene microspheres for $\Delta\phi$ =0.51 and 2.04 radians. For the lowest modulation angle, the power spectrum is composed of an interference peak at the phase modulation frequency, and a low frequency component. This low frequency component is composed of interference of the scattered light from the sample and the component of the unmodulated reference light, and mutual interference of light backscattered by the sample. When the phase modulation amplitude is increased, more light is conveyed from the unmodulated to the modulated part of the reference beam. As a consequence the level of the power spectrum at low frequencies (0-5 kHz) is reduced, as can be observed from fig.1 . Apart from an increase of the spectral peak at the modulation frequency, sidebands at higher harmonics *2f* and *3f* (14 kHz and 21kHz) are formed. The shape of the spectral peaks is the same and can be characterized by their amplitudes and line widths. When the signal-to-noise ratio is expressed as the amplitude of the signal minus the noise in the spectrum composed of frequency sidebands at the multiples of the modulation frequency divided by the amplitude of the signal when phase modulation is switched off, for the retroreflector position where maximum interference is measured, it appears that the signal-to-noise ratio is about 4.7

($\Delta\phi$ =0.51 radians) at the modulation frequency. For large phase modulation angles, the amplitudes of the interference signals at the modulation frequency and higher harmonics are increased and thus, when the information from these usually unwanted and often-removed high-order harmonics are utilized by deliberately modulating at large phase modulation angles ($\Delta\phi$ =2.04 radians), the signal noise ratio is increased to 44 (by a factor of 9), as compared to a situation where the peak phase modulation angle is kept lower ($\Delta\phi$ =0.51).

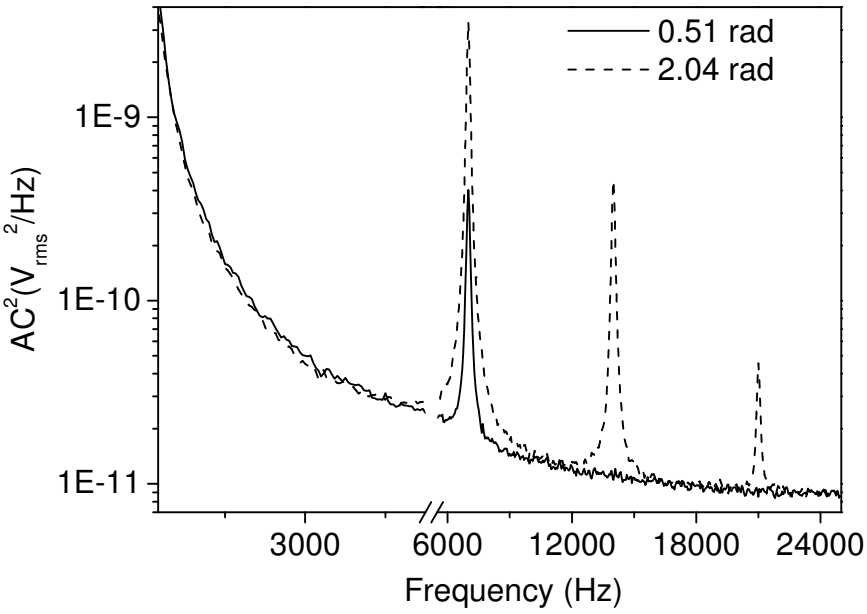

Fig. 1. Power spectra measured for water suspension of Polystyrene microspheres for two different peak optical phase shifts ($\Delta\phi$ =0.51 and 2.04 radians), with the position of the retroreflector corresponding to an optical path length difference of 1.3 mm.

The optical path length distribution obtained by adding the areas of all interference peaks (after subtraction of the background noise, and within a bandwidth of $\pm$ 2 kHz around all centre frequencies) as measured for the large modulation angle is compared with the area of the peak at the modulation frequency for the lower modulation angle in fig. 2. The signal to noise ratio enhancement due to the modulation angle increase is clear from fig. 2, in which the amplitude of the path length distribution measured for large modulation angle is 9 times higher than the low modulation angle case. The distributions are similar when normalized to the maximum value of the path length distribution at the largest phase modulation angle, and smoother curves are

obtained for high phase modulation angles, as shown in the inset of fig. 2. For sinusoidal phase modulation, the phase modulated field amplitude at the k^{th} sideband is given by $J_k(\Delta\phi)$, where J_k is the Bessel function of order k and $\Delta\phi$ is the peak optical phase shift [11]. The theoretically expected increase of the levels of the optical path length distributions is indicated by the solid triangles in figure 2, where the peak amplitudes are indicated relative to the peak amplitude for the highest phase modulation angle. Theoretically, the maximum gain in signal to noise ratio that could be obtained by further increase of peak optical phase shift is 9.4 ($\Delta\phi$ =2.3 radians). In figure 2, the results of Monte Carlo simulations are normalized with the maximum value for comparison with experimental results. There is a good agreement between the experimental data and the simulation results.

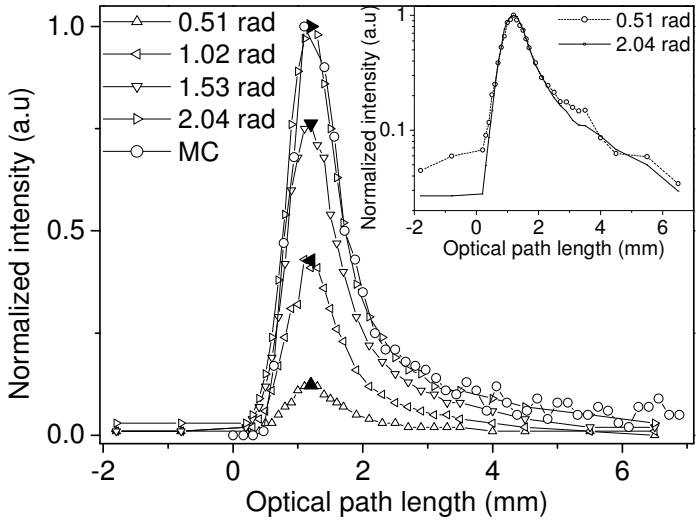

Fig. 2. Optical path length distributions measured for a scattering medium (g=0.85, μ'_s=2.0 mm^{-1}, μ_a = 0.001 mm^{-1}) for different peak optical phase shifts ($\Delta\phi$ =0.51, 1.02, 1.53, 2.04 radians) and the theoretically expected values corresponding to different peak optical phase shifts (solid triangles). Inset: Optical path length distributions normalized with their respective maximum values for $\Delta\phi$ =0.51 and 2.04 radians (logarithmic scale).

The FWHM of the Lorentzian fit to the phase modulation peak at the modulation frequency and higher harmonics for different peak phase modulation angles is shown in fig. 3. The FWHM of the interference signal is about 50 Hz in a static medium. Thus, the bandwidth broadening of the

interference signal for dynamic medium results from the Doppler shift imparted to the interfering photons by the Brownian motion of particles and increases with optical path length due to multiple scattering. The shapes of the interference peaks at the modulation frequency and higher harmonics are similar when normalized to the same maximum values and the FWHM, which for Lorentzian spectra represents the average Doppler shift can be extracted from these interference peaks. For optical path lengths greater than 3.25 mm, the amplitude of the interference signal is low at the higher harmonics and an estimation of width based on the Lorentzian fit to the spectra results in large errors.

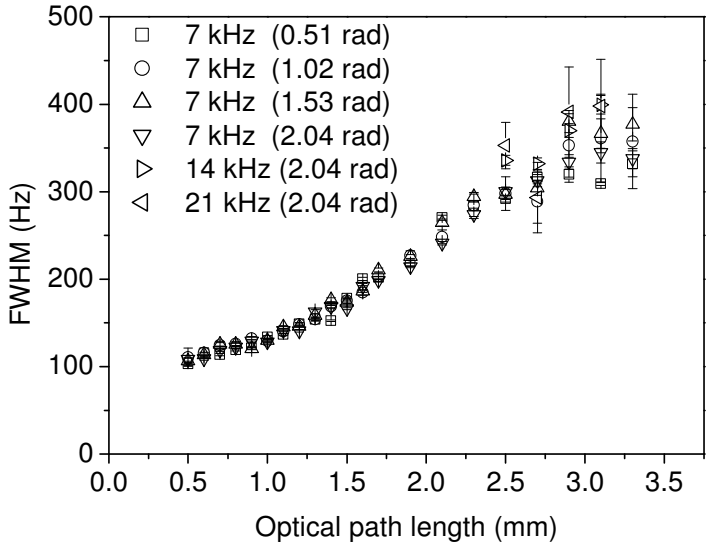

Fig. 3. The FWHM of the spectrum of interference peaks at the modulation frequency and higher harmonics as a function of the path length through the scattering medium (g=0.85, μ'_s=2.0 mm^{-1}, μ_a = 0.001 mm^{-1}) for different peak optical phase shifts ($\Delta\phi$ =0.51, 1.02, 1.53, 2.04 radians).

4.4 Conclusion

To summarize, in this Manuscript we present an improved low coherence interferometry method for measuring path length distributions and path length dependent Doppler broadening of multiple scattered light from turbid media. The improvement is based on sinusoidal phase modulation of the optical path length in the reference arm at high phase angles, leading to interference peaks at both the phase modulation frequency and higher harmonics. Rather than avoiding or neglecting these peaks, path length distributions and path length

resolved Doppler information is obtained from all interference peaks. We showed an enhancement of the signal to noise ratio by a factor of 9 in measured path length distributions and statistical averaging can be done on measured Doppler shifts from the FWHM of spectrum of the sidebands. Good agreement between the experimental path-length distribution and Monte Carlo simulations was found. The modulation frequency can be shifted to higher frequencies, where often the noise level is lower and its spectrum is more flat than for low frequencies. Hence, further increase of the SNR might be obtained by using larger phase modulation frequencies.

We aim to apply this method on living tissue, for instance to perform path length sensitive measurements of tissue perfusion and to measure path length distributions which might reveal information about the tissue structure and function.

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References:

1. 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, **58**, 76647667, 1998
2. 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**, 4222-4227 (2001).
3. 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**, 1492-1494 (2001).
4. 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,” App. Phys. Lett **81**, 595-597 (2002).
5. 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**, 1178-1181 (1991).
6. J. M. Tualle, E. Tinet, S.Avrillier, “A new and easy way to perform time resolved measurements of the light scattered by turbid medium”, Opt. Commu., **189**, 211-220 (2001).
7. 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**, 024020 (2007).
8. 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**, 555-557 (2005).
9. U. Minoni, E. Sardini, E. Gelmini, F. Docchio, and D. Marioli, “A high-frequency sinusoidal phase-modulation interferometer using an electro-optic modulator: Development and evaluation,” Rev. Sci. Instrum. **62**, 2579 (1991).
10. F. F. M. de Mul, M. H. Koelink, M. L. Kok, P. J. Harmsma, J. Greve, R. Graaff, and J. G. Aarnoudse, "Laser Doppler velocimetry and Monte Carlo simulations on models for blood perfusion in tissue," Appl. Opt., **34**, 6595-6611 (1995).
11. A. Yariv, Quantum Electronics, 2nd ed., Wiley, New York, 341-343 (1975).

Chapter 5

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

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.

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5.1 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 [6]. Compared to both the Michelson [4,5] and the Mach-Zehnder [7,8] 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 [6]. 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 length dependent diffusion broadening of multiple scattered light with Monte Carlo simulations and Diffusive wave spectroscopy respectively [9].

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.

5.2 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. 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 [6].

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 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 [6]. 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 [6, 9, 11]. The $zero^{th}$ 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 [6]. 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.

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.

A five-layer system is defined in which light from a fiber 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 (μ'_s =2.0 mm^{-1}, g=0.85, μ_a =0.001 mm^{-1}). 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 thickness of the top static layer was varied from 0.1 mm to 0.9 mm, whereas the thickness of the dynamic layer was 20 mm. 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. In all simulations, $1*10^7$ photons are injected into the sample, and each photon returning to the detection fiber (fiber separation=300 μm, NA=0.29) is assumed to be detected, and its optical path length is recorded.

5.3 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). 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 figure 2 and figure 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.

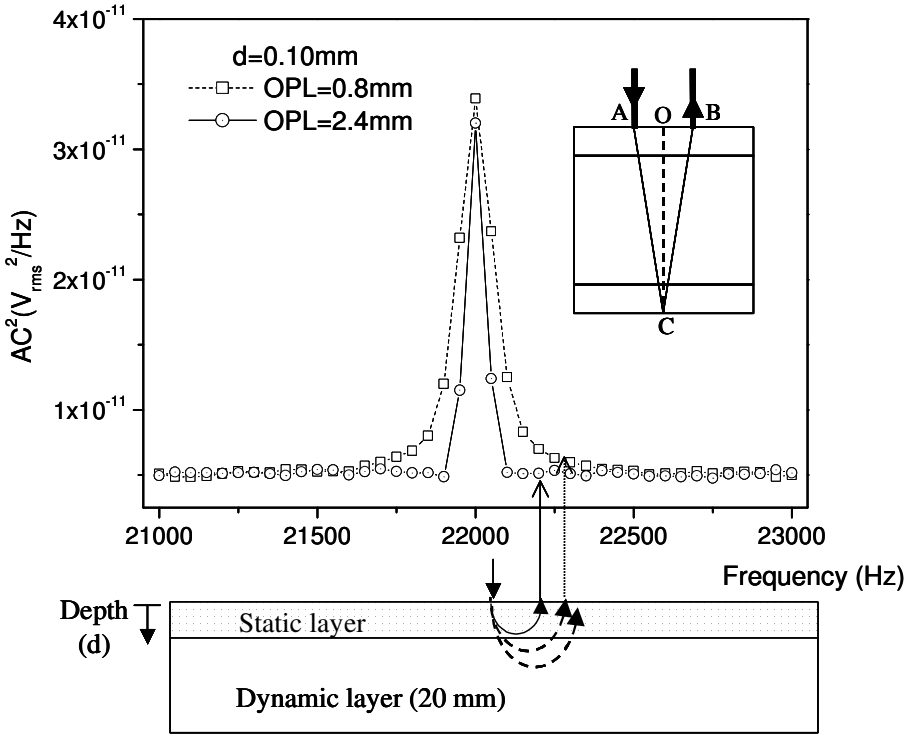

Fig. 1 Typical modulation peak appearing in the power spectra measured for short and large optical path lengths (Top). Schematic diagram of the light propagation through a two-layer scattering phantom, consisting of a top static layer and a bottom dynamic layer with same optical properties (Bottom).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

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 figure 4. As expected, the non-shifted fraction of photons exceeds the Doppler shifted fraction as the thickness of the superficial static layer increases.

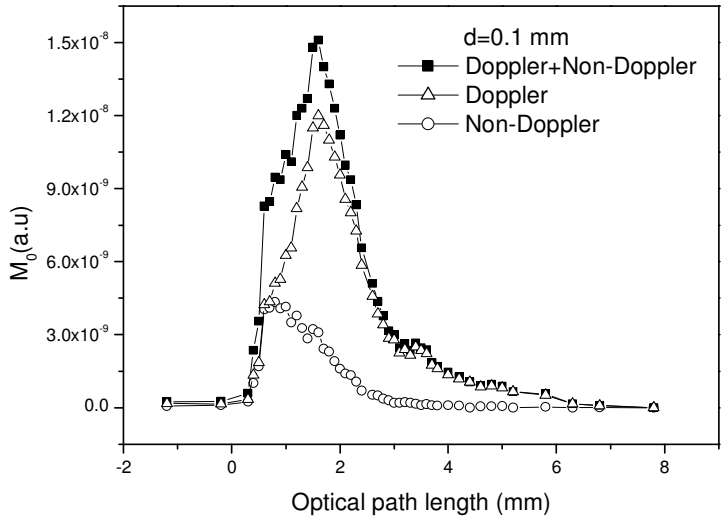

Fig. 2. Optical path length distributions of non-shifted and Doppler shifted photons, measured for a two-layer tissue phantom consisting of a top static layer of thickness 0.1 mm and a bottom dynamic layer of thickness 20mm.

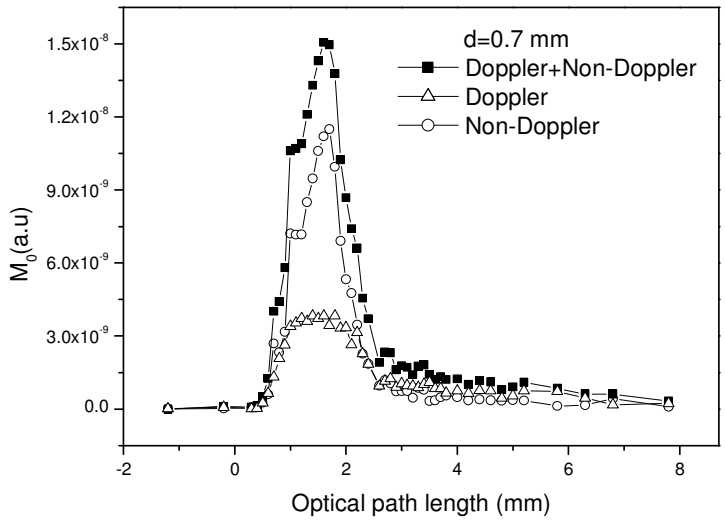

Fig. 3. Optical path length distributions of non-shifted and Doppler shifted photons, measured for a two-layer tissue phantom consisting of a top static layer of thickness 0.7 mm and a bottom dynamic layer of thickness 20mm.

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 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.

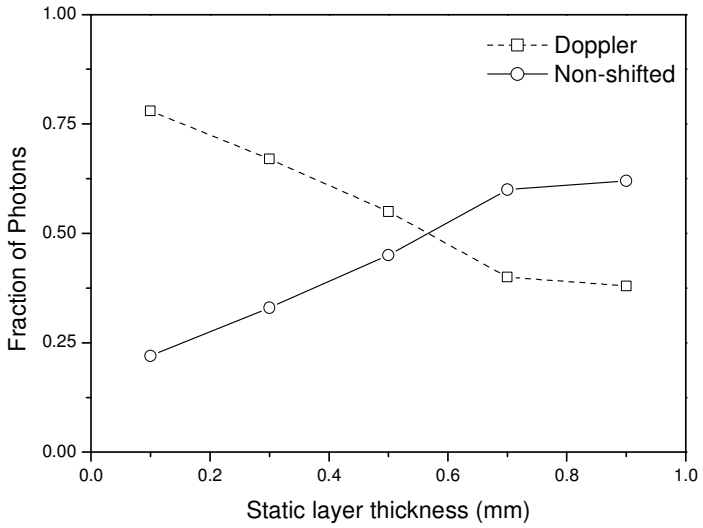

Fig. 4. The fraction of Doppler shifted and non-shifted photons (summed over all optical path lengths) estimated for different thickness of the superficial static layer.

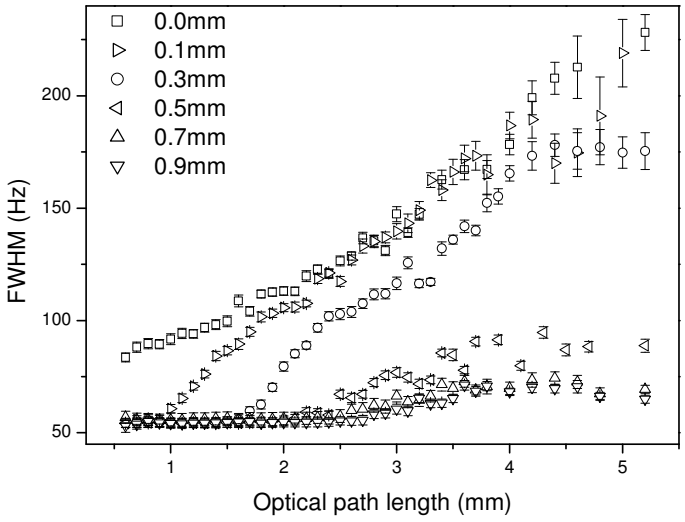

Fig. 5. The average Doppler shift extracted from the FWHM of the phase modulation peak, as a function of the optical path length through the scattering medium, for various thickness of the static layer (0 - 0.9 mm).

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 figure 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 figure 7 indicate the variations in the shortest optical path length that may arise due to the path length resolution of ± 100μm 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.

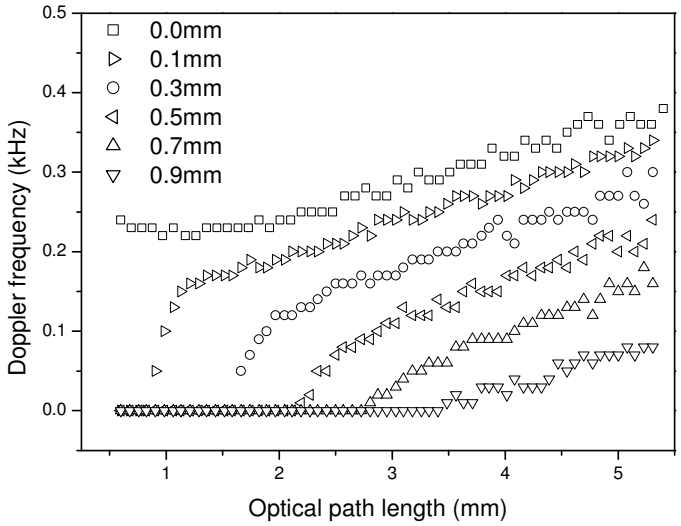

Fig. 6. The path length dependent Doppler broadening of multiply scattered light obtained from Monte Carlo simulations for various static layer thickness (0 - 0.9 mm).

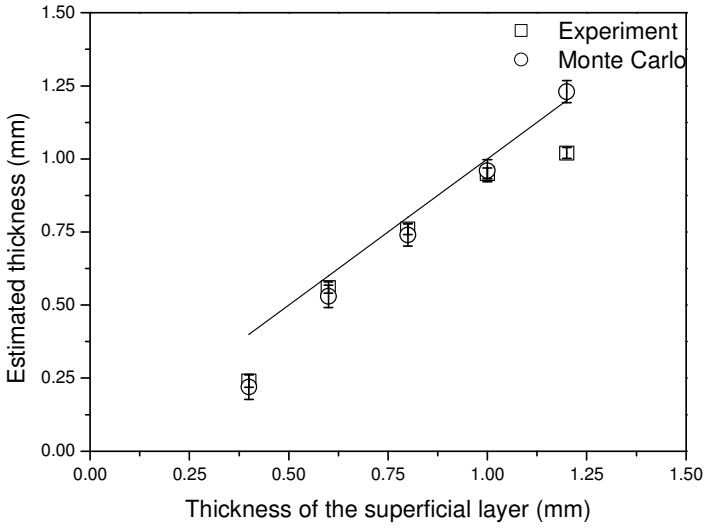

Fig. 7. Thickness of the superficial static layer (including two glass layers) estimated from the path length dependent Doppler broadening. Experimental results (squares) and Monte Carlo simulations (circles).

5.4 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 [6]. 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 figure 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 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.

5.5 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 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. 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.

Acknowledgements

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References:

1. A. P. Shepherd and P. Å. Öberg, Laser-Doppler Blood Flowmetry (Kluwer Academic, Boston, 1990).
2. M. Larsson, W. Steenbergen, and T. Strömberg, "Influence of optical properties and fibre separation on laser Doppler flowmetry," Journal of Biomedical Optics, 7, 236-243 (2001).
3. A. Liebert, M. Leahy and R. Maniewski, "Multichannel laser-Doppler probe for blood perfusion measurements with depth discrimination," Med. Bio. Eng. Comp., **36**, 740-747 (1998).
4. 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**, 4222-4227 (2001).
5. 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, **58**, 7664-7667 (1998).
6. 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**, 024020 (2007).
7. 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 interferometer," Opt. Lett., **26**, 1492-1494 (2001).
8. 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**, 595-597 (2002).
9. 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).

10. J. C. Hebden, B.D. Price, A. P. Gibson and G.Rotle, “A soft deformable tissue-equivalent phantom for diffuse optical tomography,” Phys. Med. Bio., **51**, 5581-5590 (2006).
11. A. G. Yodh, P. D. Kaplan, and D. J. Pine, "Pulsed diffusing-wave spectroscopy - high resolution through nonlinear optical gating," Phys. Rev. B, **42**, 4744-4747 (1990).
12. F. F. M. de Mul, M. H. Koelink, M. L. Kok, P. J. Harmsma, J. Greve, R. Graaff, and J. G. Aarnoudse, “Laser Doppler velocimetry and Monte Carlo simulations on models for blood perfusion in tissue,” Appl. Opt., **34**, 6595-6611 (1995).
13. A. Sadhwani, K. T. Schomacker, G. J. Tearney, and N. S. Nishioka, "Determination of Teflon thickness with laser speckle. I. Potential for burn depth diagnosis," Appl. Opt., **35**, 5727-5735 (1996).
14. A.M.I. Watts, M.P.H. Tyler, M.E. Perry, A.H.N. Roberts, D.A. McGrouther, “Burn depth and its histological measurement,” Burns, **27**, 154-160 (2001).
15. 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**, 1178-1181 (1991).

Chapter 6

Evaluation of a multimode fiber optic low coherence interferometer for path length resolved Doppler measurements of diffuse light*

The performance of a graded index multimode fiber optic low coherence Mach-Zehnder interferometer with phase modulation is analyzed. Investigated aspects were its ability to measure path length distributions and to perform path length resolved Doppler measurements of multiple scattered photons in a turbid suspension of particles undergoing Brownian and translational motion. The path length resolution of this instrument is compared with a system using single mode fibers for illumination and detection. The optical path lengths are determined from the zero order moment of the phase modulation peak in the power spectrum. The weighted first moment, which is equal to the average Doppler shift, shows a linear response for different mean flow velocities within the physiological range.

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6.1 Introduction

Dynamic light scattering (DLS) exploits the temporal fluctuations of scattered waves to extract valuable information about the dynamics of the scattering medium. Optical techniques based on dynamic light scattering have found extensive applications in various disciplines ranging from physics and chemistry to medicine and biology [1]. DLS was originally applied for the characterization of weakly scattering media in which the detected photons are singly scattered before being detected. However, many of the techniques for non-invasive optical diagnosis deal with highly scattering media, in which photons travel along different paths and reach different depths in the sample, resulting in multiple scattering events. Probing these samples with path length sensitivity may improve the quality of the measurements. For example, in conventional Laser Doppler Perfusion Monitors (LDPM), perfusion values averaged over different and basically unknown path lengths are recorded [2]. 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. A longer path length will increase the probability of Doppler scattering events, thus yielding a relative overestimation of the blood perfusion, compared to the short path length situation. This will cause inter-and intra-individual variations in the LDPM readings, for the same physiological perfusion values, introduced by the variance in average optical path length resulting from the changes in tissue optical properties. Thus development of path length resolved light scattering techniques would result in more-quantitative and more-reliable tissue perfusion monitoring and might provide information regarding the structure and function of tissue.

In contrast to low coherence interferometers used in optical coherence tomography (OCT) [3] for path length resolved measurements, adopting on axis back reflection and confocal detection of singly scattered photons, our system uses positions for illumination and detection with a mutual distance of ten to a hundred times the scattering mean-free path length [4-7]. Hence, our measurements explore the regime of multiply scattered photons. The sampling depth will increase for large distances and by changing the fiber distance the light scattered from superficial and deeper layers may be discriminated. Compared to the previously reported low coherence interferometric measurements [4-5, 7-8], which depended on the Doppler shifted photons, recently we showed that the path length resolved information with increased signal to noise ratio could be obtained from mixed static and dynamic turbid media by the addition of phase modulation in the reference arm [6]. Also, these interferometric systems [7-8] employ step-index, single mode fibers. 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 [4-7] that use of graded index multimode fibers allows for the detection of optical path length distributions of multiply scattered light. Furthermore, we validated the optical path length distributions and path length dependent diffusion broadening of multiple scattered light with Monte Carlo simulations and Diffusive wave spectroscopy respectively [7].

The aim of the present work is to establish the performance of the system in terms of its resolution of optical path length assessment, and its ability to represent various flow speeds within the physiological velocity range. In previous reports [4-9], coherence gated optical Doppler measurements are performed on non-flowing samples, in which the Doppler shift was imparted due to Brownian motion of particles. We will demonstrate that our method has sufficient dynamic range in measuring Doppler shifts from flowing samples.

6.2 Materials and methods

The experimental setup is based on a phase modulated low coherence fiber-optic Mach-Zehnder interferometer. A broadband superluminescent diode (Inject LM2-850, λ=832 nm, $\Delta\lambda_{FWHM}$ =17 nm, coherence length L_C =18 μm) that yields 2 mW of power from the single-mode pigtail fiber is used as the light source. A single mode fiber-optic coupler with a splitting ratio of 90:10 is used to create a reference arm (10%) and a sample arm (90%). A single mode fiber (mode field diameter=5.3 μm, NA=0.14) is used for illumination, while a multimode graded-index fiber (core diameter =100 μm, NA=0.29, length=1m) is used for detection, providing a large detection window and a small modal dispersion. For measuring the path length resolution of single mode fiber optic interferometer, single mode fibers are used for detection. The coherence length of the light source, and the intermodal dispersion in the detection fiber, define the path-length resolution of the measurement. The reference beam is polarized using a linear polarizer and the phase is sinusoidally modulated at 17kHz using an electro-optic broadband phase modulator (New Focus Model 4002). The sinusoidal phase modulation applied to the light is set to an angle of less than $\pi/2$ radians so that frequency sidebands are absent in the spectra. The path length of the reference arm is varied by reflection of the light in a translatable retroreflector. To measure the path length resolution of the set-up, the scattering sample was replaced by a mirror at a distance of approximately 1.35 mm from the tips of the two fibers, which are spatially separated by 0.3 mm. The power spectra were measured as a function of the optical path length in the reference arm in steps of 20 microns at positions where the optical path length difference is within the coherence length and in steps of 100 microns for widely different optical path lengths.

For the velocity response test, a scattering model was made of Delrin with a circular straight flow channel (diameter 3.8 mm, length=2.5 cm) at a depth of 0.2 mm under the surface of the Delrin. An aqueous suspension of 10% of Intralipid 20% [9] was used as a scattering medium (μ'_s=5.0 mm^{-1}, μ_a = 0.001 mm^{-1}, g=0.637) and the mean flow through the channel was varied over a range of 0 to 6 mm/s with a peristaltic pump. At the detector (New Focus Model 2001 photo receiver) the light that is scattered in the sample and the light from the reference arm after passing through a 50:50 multimode fiber coupler are mixed. Single mode fiber-optic coupler with a splitting ratio of 50:50 is used in path length resolution measurements using single mode fiber optic interferometer. The AC photocurrent is measured with a 12 bit analogue to digital converter (National Instruments), sampling at 40 kHz, with a number of 1000 samples and is Fourier transformed and squared to yield the power spectrum. The signal was sampled for 58 seconds to get an average of 1000 spectra and power spectra were measured in steps of 100 microns.

In laser Doppler perfusion measurements, the flow parameters are obtained from the spectral analysis of the frequency components in the power spectrum. The fundamental output quantity of a laser Doppler perfusion monitor is the first moment of the power spectrum $P(\omega)$ of the detector signal; in general, the i^{th} moment is being defined as $M_i = \int_a^b P(\omega)\omega^i d\omega$, where a and b are device dependent low and high cut-off frequencies. With i=0, a quantity is obtained which is proportional to the concentration of moving red blood cells, while i=1 describes red blood cell flux, which is the product of concentration and the root mean square of the red cell velocity, at least for low blood concentrations [11]. The method presented here utilizes the area M_0 (zero order moment) and the weighted first moment M_1/M_0 of the phase modulation peak appearing in the photodetector signal power spectrum to determine the intensity and the Doppler shift of photons as a function of optical path length, respectively. The path length distribution is obtained by subtraction of M_0 of the reference arm noise and of the sample arm 'homodyne' Doppler signal from M_0 of the corresponding spectra around the modulation frequency between frequencies of 14 and 20 kHz.

6.3 Results and discussion

6.3.1 Path length resolution of the set-up

The path length resolution was measured by replacing the scattering sample by a reflecting mirror at a distance of 1.35 mm from the fiber tips and the power spectrum was measured as a function of optical path length. When the optical path length in the reference arm was about 2.7 mm, we observed a narrow and strong interference peak at the modulation frequency due to the

interference of reference light with the light reflected from the mirror. This is expected since the light has to pass twice, and this optical path length difference corresponds to 2.7 mm. The M_0 recorded, after correction for the sample arm and reference arm noise, is presented in fig. 1 as a function of optical path length.

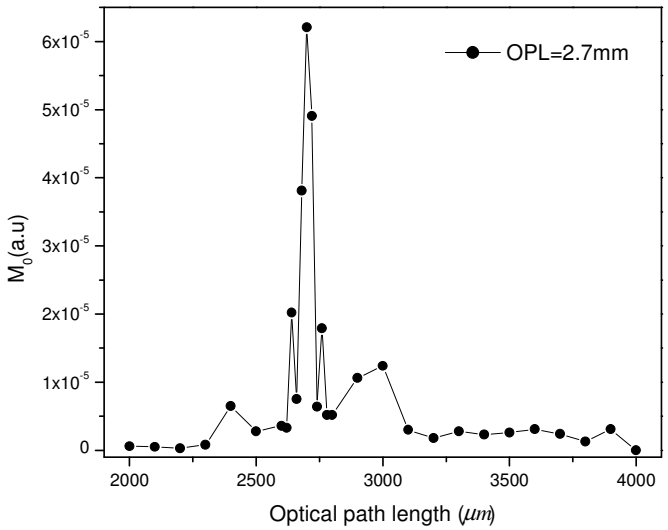

Fig. 1. The dependence of the total AC power of the detector current as a function of the optical path length in the reference arm measured with multimode fiber optic interferometer.

The maximum signal recorded is about a factor of 100 higher than the noise detected when the phase modulation is turned off. We have also observed some satellite peaks at a distance of 60 and 300 μm with respect to the main interference peak which are at least a factor of 3.25 lower than the main peak. The full width at the half maximum of the main peak is about 55 μm. For widely different optical path lengths, the heterodyne signal is reduced to almost zero. For path length resolved Doppler measurements in the scattering media, additional satellite peaks may become evident due to the excitation of other modes. Furthermore, the path length resolution of 100 μm that we used in our measurements may result in suppression of other inherent modes. To account for the origin of these satellite peaks and to evaluate whether any significant degradation in the path length resolution is resulting from multimode fibers, we have compared these results with a single mode fiber optic interferometer. The heterodyne AC power obtained by subtracting the reference arm noise and sample arm noise from the corresponding total spectra in a single mode fiber-optic interferometer is shown as a function of optical path length in fig. 2. The

interference peak is observed for an optical path length of 2.7mm with FWHM ~ 55 μm and no satellite peaks are observed.

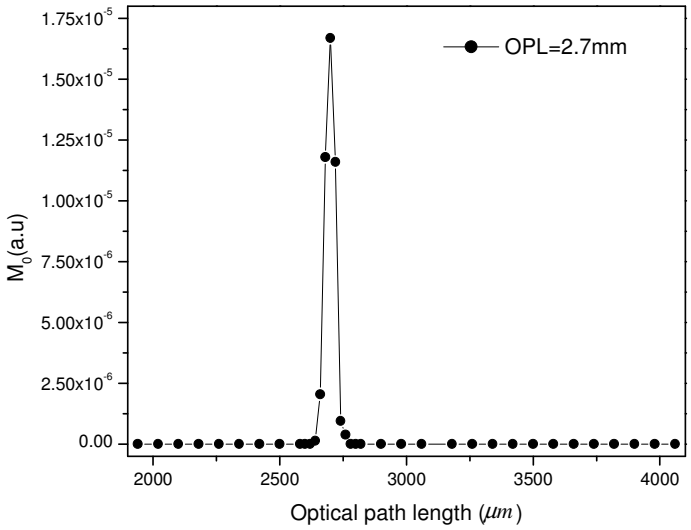

Fig. 2. The dependence of the total AC power of the detector current as a function of the optical path length in the reference arm measured with single mode fiber optic interferometer.

The deterioration of the path length resolution from 18 μm (as expected from the coherence length of the light source) to about 55 μm observed in both single mode and multimode fiber optics interferometers is due to the unbalanced optical dispersion of the reference and sample arms from the electro-optic modulator [12]. The group dispersion of a MgO: $LiNbO_3$ crystal is obtained from the slope of the curve of index of refraction of the crystal simulated with the Sellmeier equation ($n^2(\lambda) = 4.8762 + \frac{0.11554}{(\lambda^2 - 0.04674)} - 0.033119\lambda^2$) as a function of wavelength in the NIR spectral range [12]. In the spectral range of the SLD (815-842 nm), the group dispersion of a MgO: $LiNbO_3$ crystal obtained from the slope is $D_g = 1.1*10^{-4}$ /nm. When the reference beam is passed through the crystal with a geometric length (L_g) of 40 mm, the reduced path length resolution $L = \left[L_c^2 + \left(D_g L_g \Delta\lambda\right)^2\right]^{1/2}$ is about 54 μm, which is in good agreement with the path length resolution measured in our experimental setup [12]. We observe that there is no significant change in the path length resolution when fiber lengths are varied, which indicates that the practical implications of total dispersion mismatch due the presence of air and fiber can be neglected in our

path length measurements having widths of several mms. Even though there is degradation in resolution from 18 μm to about 55 μm, a path length resolution of 55 μm is sufficient for measuring path length distributions of multiply scattered light, which often have widths of 5 mm or larger. But in the case of steep gradients observed for short optical path lengths, higher path length resolution may be required for accurate measurements. The FWHM corresponding to the theoretical value of 18 μm (as expected from the coherence length of the light source) may be obtained by compensating for the optical dispersion in the electro-optic modulator crystal by the introduction of an element with same amount of dispersion in the spectral range of the light source in the sample arm [13].

The above results imply that the use of multimode graded index fibers does not cause serious degradation in the path length resolution compared to single mode fibers. But additional satellite peaks are observed due to the intermodal dispersion in the graded index fiber. The upper limit of multimode dispersion of this fiber is specified as 1mm/m. This value refers to the maximum difference in optical path length between lowest and highest fiber modes and it depends on how higher order modes are excited. The observed distance of the satellite peaks to the main peak is smaller than expected on the basis of the fiber specifications. In our path length resolution measurements with the mirror, probably a much smaller number of modes have been excited. When measured in a scattering medium, higher order modes will be excited in a large core diameter fiber and the output intensity profile measured in the far field of the exit beam is the result of the superposition of all guided modes, resulting in a speckle pattern.

The appearance of satellite peaks may lead to oscillations in the optical path length distributions at locations with strong 2^{nd} and higher order derivatives in the optical path length distribution. Since these peaks are very close to the main peak and the path length resolution is 100 μm, this will not be pronounced in our path length resolved Doppler measurements in the scattering media. The optical path length distributions measured in scattering media with different reduced scattering coefficients and anisotropies did not exhibit the effects of these satellite peaks. Furthermore, the choice of multimode fibers makes it possible to detect multiply scattered light at large fiber distances.

6.3.2 Path length resolved Doppler measurements

The power spectra measured for an aqueous Intralipid suspension flowing through the circular channel (Average flow velocities of 0, 3, 6 mm/s) for two optical path lengths of 0.7 and 1.9 mm are shown in fig. 3 and 4 respectively. The power spectra measured contain contributions from the reference arm (fluctuations of light source power and shot noise), a homodyne Doppler signal from the sample arm and a heterodyne peak at the modulation frequency due to

the interference of the reference light and the sample light. The broadening of the peak results from the Doppler shift imparted to the interfering light by the Brownian and translational motion of particles. The spectrum of the interference signal becomes broad as the flow velocity increases. Since the optical properties of the medium are not changed due to the flow the area of the peak that corresponds to the intensity of photons within a certain optical path length should remain constant.

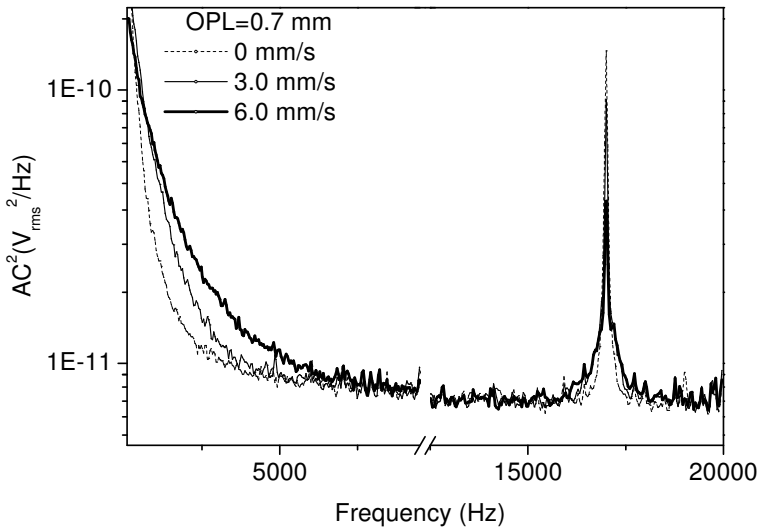

Fig. 3. Power spectra measured for an aqueous suspension of 10% of Intralipid 20% for different flow velocities, for an optical path length of 0.7 mm.

The path length distribution measured 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 around the modulation frequency of 17 kHz (equation 1 with i=0, and with constants a and b 14kHz and 20 kHz respectively) is shown in fig. 5. The zeroth moment M_0 of the heterodyne spectrum equals to the intensity of photons with a certain path length. Optical path length distributions spanning a range from 0-5 mm are measured. The error bars indicate the standard deviation of three measurements. The path length distributions measured for different flow rates are nearly identical and are related to the optical properties of the medium and the detection geometry.

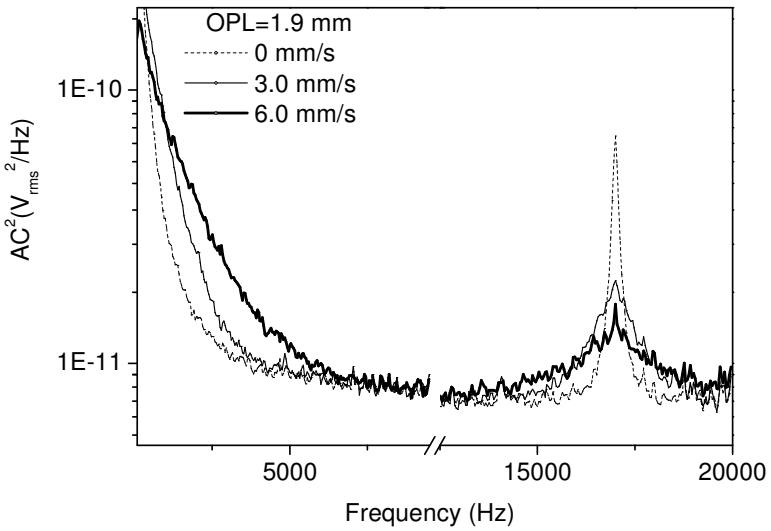

Fig. 4. Power spectra measured for an aqueous suspension of 10% of Intralipid 20% for different flow velocities, for an optical path length of 1.9 mm.

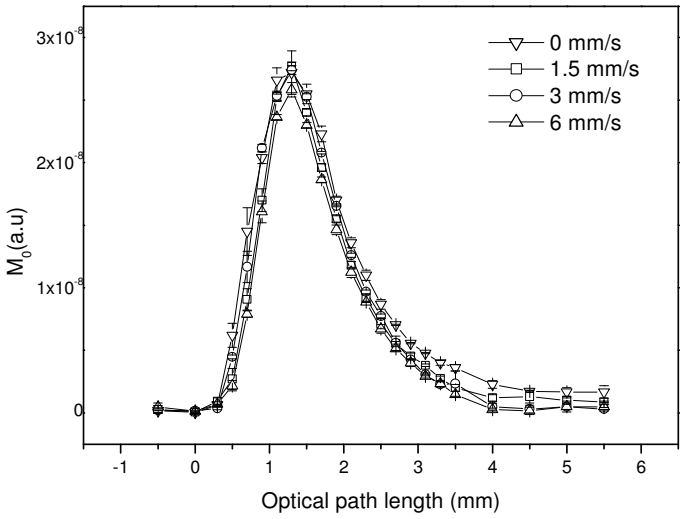

Fig. 5. Optical path length distributions measured for an aqueous suspension of 10% of Intralipid 20% for different flow velocities.

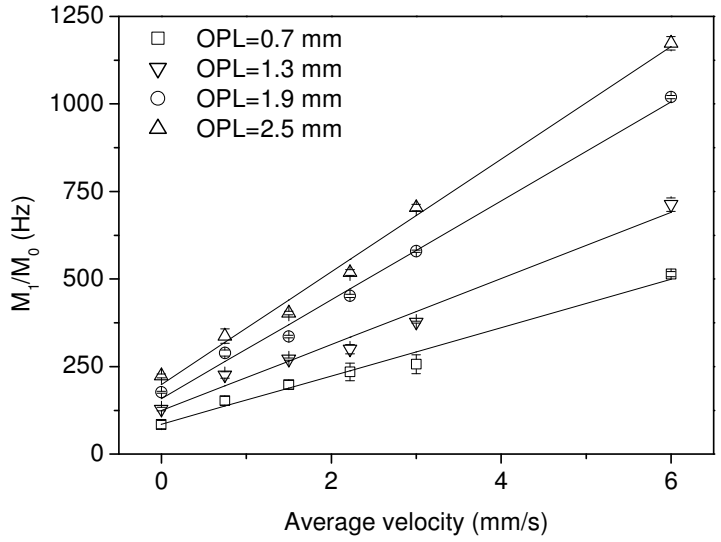

Fig. 6. The average Doppler shift measured from the weighted first moments of the Doppler broadened phase modulation interference peak as a function of optical path length for an aqueous suspension of 10% of Intralipid 20% for different velocities ranging from 0 to 6 mm/s.

The weighted first moments, M_1/M_0 of the heterodyne peak at the modulation frequency (after shifting to zero frequency) in a bandwidth of 3 kHz, after correction for the sample signal and for the reference arm noise are presented in fig.6. The weighted first moment, which represents the average Doppler shift, increases with the optical path length due to the greater probability of interaction of photons with moving scatterers for large optical path lengths in a strongly multiply scattering system. For zero flow velocity, the average Doppler shift is not equal to zero due to the Brownian motion of the scattering particles. For a given optical path length, an increase in the average Doppler shift is observed when the diffusing particles experience translational motion. A linear increase of the average Doppler shift with increase in flow rates is observed. However the flow velocities experienced by the photons will be smaller than that suggested by the average flow values. This is because with a parabolic velocity profile, photons with maximum optical path lengths of 2.5 mm (Fig.6) will have only explored the wall region and not have reached the centre of flow channel, where the particle velocity is maximal. In our previous reports, the Doppler shift was imparted due to the Brownian motion of particles. Since for a given optical path length the broadening of the interference peak appearing at the modulation frequency is accompanied by a decrease in the level of the power spectrum, the peak may get drowned into the background noise for

higher velocities. This may limit the usefulness of our technique for large flow velocities, for instance blood flow measurements in arterioles (6 mm/s) [14]. Secondly, since majority of the photons will be scattered by the surrounding static tissue matrices, less than 10 per cent of the photons are associated with a Doppler shift generated by moving RBCs. In the skin capillary network, the mean flow velocities of red blood cells through the small blood vessels is relatively lower, often in the range of 1 mm/s [14]. In our measurements, for short optical path lengths, significant part of the photons will have traveled only within the static Delrin layer of thickness 0.2 mm, and are thus not Doppler shifted, similar to the case of static tissue with small blood vessels. The Doppler shift estimated for a range of velocities from 0 to 6 mm/s shows a linear response. This is an indication of the practical usability of our method in skin perfusion studies.

6.4 Conclusions

In this paper, we presented path length resolved Doppler measurements of diffusely scattered light in an aqueous suspension of lipid particles undergoing Brownian and translational motion, using a phase modulated low coherence Mach Zehnder interferometer with graded index multimode fiber for detection. To evaluate the effect of intermodal dispersion of multimode fibers in low coherence interferometry, we replaced the scattering medium by a mirror and the position of the retroreflector was translated. A sharp interference peak with a width of 55 μm has been observed in the power spectrum, along with weak additional satellite peaks. The performance of multimode fibers for detection in a coherence gated interferometric scheme is compared with a single mode fiber optic interferometer and the results indicate that there is no significant effect of intermodal dispersion in the path length resolution of our experimental setup. Theoretical calculations indicate that the degradation in the path length resolution from 18 μm (as expected from the coherence length of the superluminescent diode) to 55 μm is due to the unbalanced optical dispersion of the reference and sample arms due to the electro-optic modulator. From the spectral analysis of moments of the interference peak appearing at the modulation frequency, optical path lengths and path length dependent Doppler shifts are measured in an aqueous suspension of 10% of Intralipid 20% flowing through a channel in a block of Delrin over a range of average velocities from 0 to 6 mm/s. The optical path lengths are measured from zero order moments of the Doppler broadened heterodyne peak observed at the modulation frequency. The average Doppler shift was estimated from the weighted first moment M_1/M_0 of this peak for different flow velocities ranging from zero to 6 mm/s. The average Doppler shift increases with the optical path length, due to multiple scattering. The measured Doppler shift shows a linear response with velocity for different flow velocities in the physiological range (1 mm/s in capillaries to 6

mm/s in arterioles). The path length distributions measured in scattering media (fig. 5) suggest that the effect of intermodal dispersion is very weak and the effect of additional satellite peaks that may result from the intermodal dispersion (fig. 1) are not evident. Thus, multimode graded-index fibers provide a large detection window with a small modal dispersion in path length resolved dynamic light scattering.

Acknowledgements

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References:

1. B. J. Berne and R. Pecora, Dynamic Light Scattering with applications to Chemistry, Biology and Physics (John Wiley & Sons, Inc., NewYork, 1976).
2. A. P. Shepherd and P. Å. Öberg, Laser-Doppler Blood Flowmetry (Kluwer Academic, Boston, 1990).
3. 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**, 1178 (1991).
4. 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 interferometer", Opt. Lett., **26**, 1492-1494 (2001).
5. 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", Applied Physics Letters, **81**, 595-597(2002).
6. 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**, 024020 (2007).
7. 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).
8. 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 , **58**, 7664-7667 (1998).
9. 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**, 4222-4227 (2001).
10. H. J. van Staveren, C.J.M. Moes, J. van Marle, S. A. Prahl, and M.J.C. van Gemert, "Light scattering in Intralipid-10% in the wavelength range of 400-1100 nm,"Appl. Opt., **30**, 4507-4514 (1991).

11. G. E. Nilsson, "Signal processor for laser Doppler tissue flow. meters," Med. Biol. Eng. Comput, **22**, 343-348 (1984).
12. C. K. Hitzenberger, A. Baumgartner, and A. F. Fercher, "Dispersion induced multiple signal peak splitting in partial coherence interferometry," Opt. Comm., **154**, 179-185 (1998).
13. X. C. Yao, A. Yamauchi, B. Perry, and J. S. George, Rapid optical coherence tomography and recording functional scattering changes from activated frog retina," Appl. Opt. **44**, 2019-2023 (2005).
14. A. C. Guyton, Textbook of Medical Physiology (W.B. Saunders company, Philadelphia, 1991).

Chapter 7

Path length resolved optical Doppler perfusion monitoring*

In laser Doppler measurements, perfusion values averaged over different and basically unknown path lengths are recorded. To facilitate quantitative path length resolved perfusion measurements, we developed a phase modulated Mach-Zehnder interferometer with spatially separated fibers for illumination and detection. With this setup, we report the first path length resolved perfusion measurements on human skin and its variations to external stimuli. Perfusion and its variations during occlusion are measured real time for a given optical path length. Inter-and intra-individual variations in optical path length distributions and path length resolved Doppler shifts are measured, which indicates that these variations should be taken into account while comparing the perfusion readings from comparable sites between individuals and from different sites of the same individual. Path length resolved perfusion measurements are compared with the perfusion signal obtained with a conventional laser Doppler perfusion monitor.

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*Part of this chapter has been submitted for publication in Microcirculation as: Babu Varghese, Vinayakrishnan Rajan, Ton G. van Leeuwen, and Wiendelt Steenbergen, "*In vivo* optical path lengths and path length resolved Doppler shifts of multiply scattered light".

7.1 Introduction

Laser Doppler blood flowmetry is a non-invasive technique for monitoring blood microcirculation in biological tissues [1]. However, perfusion measurements using these conventional laser Doppler techniques are affected by probe induced variations [2] and variations in tissue optical properties in terms of absorption and scattering [1,3]. The physical explanation of this dependence is the variance in the optical path lengths of the photon migration in tissue. In conventional laser Doppler techniques, perfusion values averaged over different and basically unknown path lengths are recorded, which creates an uncertainty in the relation between the measured perfusion signal and the real perfusion. 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. Techniques for monitoring perfusion with path length selectivity may overcome this limitation and enable to correctly interpret or counter-act the inter-and intra-individual variations in the perfusion readings introduced by the tissue optical properties.

The path length specific technique used in this study 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 [4,5,6]. Compared to both the Michelson [7-9] and the Mach-Zehnder [10,11] based interferometric measurements that could only record the Doppler shifted photons, our method is able to measure path length resolved information from mixed media [4], such as flow of moving red blood cells within static tissue matrices. The path length distributions and path length dependent diffusion broadening of multiple scattered light from turbid media were validated with Monte Carlo simulations and Diffusive wave spectroscopy respectively [5].

The goal of this study is to demonstrate the feasibility of phase modulated low coherence interferometry in measuring *in vivo* optical path lengths and path length resolved Doppler shifts. In this study, we show the first results on path length resolved laser Doppler perfusion measurements in skin and its variations to occlusion and Capsicum cream provocation. For a given optical path length, perfusion was measured in real time before, during and after an artery occlusion of the upper arm. The method was evaluated in three human subjects on the same measurement location to establish inter-individual variations. Measurements were performed at three different locations of one individual for observing intra-individual variations resulting from the heterogeneity of the tissue, both in the static matrix and in the microvascular architecture of the skin. In all measurements, perfusion was simultaneously measured with a conventional fiber-optic laser Doppler perfusion monitor.

7.2 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. The fibers are spatially separated by a centre to centre distance of 300 μm and are embedded in a black epoxy resin surrounded by a metal tube of internal diameter 6 mm. The reference beam is polarized using a linear polarizer and the phase is sinusoidally modulated at 6 kHz using an electro optic broadband phase modulator (New Focus Model 4002) with a peak optical phase shift of 2.04 radians applied to the modulator. The AC photocurrent is measured with a 12 bit analogue to digital converter (National Instruments), sampling at 40 kHz, averaged over 1000 spectra and was measured in steps of 200 microns in air. The setup has been described in more detail elsewhere [4,5,6].

The fundamental output quantity of a laser Doppler perfusion monitor is the first moment of the power spectrum $P(\omega)$ of the detector signal; in general, the i^{th} moment is being defined as

$$M_i = \int_a^b P(\omega)\omega^i d\omega \qquad (1)$$

Here *a* and *b* are device dependent low and high cut-off frequencies. With *i*=0, a quantity is obtained which is proportional to the concentration of moving red blood cells, while i=1 describes red blood cell flux, which is the product of concentration and the root mean square of the red cell velocity, at least for low blood concentrations [12].

In our instrument, for large phase modulation angles ($\Delta\phi$ =2.04 radians) the power spectra contain interference peaks at both the phase modulation frequency and higher harmonics (Fig.1). When path length resolved information from these high-order harmonics is utilized, the signal to noise ratio is increased by almost one order or magnitude, as compared to a situation where the peak phase modulation angle is kept lower to avoid higher harmonic peaks ($\Delta\phi$ =0.51). Optical path length distributions are obtained by adding the areas of all interference peaks (after subtraction of the background noise, and within a bandwidth of $\pm$ 2 kHz around all center frequencies) in the power spectrum [6]. The FWHM of the interference signal is about 50 Hz in a statically scattering medium 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 interference peaks, forms an estimation of the amount of Doppler shifted light at that specific optical path length. The average Doppler shift corresponding to the Doppler shifted light is calculated from the weighted first moments (M_1/M_0) of the heterodyne peak at the modulation frequency, after correction for the sample signal and for the reference arm noise (in a bandwidth of 50 Hz-2 kHz close to the phase modulation frequency and its higher harmonics, indicated by *a* and *b* in Eq.2)

$$M_i = \sum_{j=1}^{3} \int_{j\omega_m+a}^{j\omega_m+b} P(\omega)(\omega - j\omega_m)^i \, d\omega \qquad (2)$$

The Doppler shift measured from the weighted first moments averaged over all optical path lengths will lead to an overestimation for long path lengths. To avoid this overestimation in the perfusion signal and to measure flux in absolute units, the Doppler shift for a given optical path length is weighted with the corresponding optical path length distribution of Doppler shifted photons. The flux (M_1) is defined as the product of Doppler fraction of photons in the perfusion signal and the weighted average Doppler shift. The conventional laser Doppler perfusion monitor used in our measurements is a PF5000 monitor (Perimed AB, Sweden) with a laser diode (780 nm) as the light source. The PF5000 has a bandwidth of 20 Hz -13 kHz (*a* and *b* in Eq.1) and a time constant of 0.2 s was used for monitoring. The fiber-optic probe (Probe 408, standard probe) consists of two spatially separated fibers (core diameter =125 μm, NA=0.37) with a center-to-center separation of 250 μm. The calibration of the PF5000 was done with motility standard (water suspension of polystyrene microspheres with a diameter of 320 nm). After the calibration, PF5000 displayed a standard reading of 250 perfusion units when measuring in the motility standard.

7.2.1 Optical path lengths and path length resolved Doppler shift measurements

Measurements were performed on the skin of the dorsal side of the right forearm of a healthy human volunteer (Skin type- Type II) in the sitting position. A probe holder (PH 08) was attached to the skin with a double-sided adhesive tape. The subject rested approximately 10 minutes prior to the measurements. The fiber optic probe of the PF5000 was inserted into the probe holder and the perfusion was measured for comparison with the succeeding measurements with our developed probe. Measurements were performed on unprovoked skin and perfusion variations due to occlusion (for 50 seconds) were measured for a given optical path length and this was repeated for all optical path lengths. To measure the changes in perfusion during occlusion, arterial occlusion of the upper arm was done with a cuff inflated to a pressure of 170 mm of Hg. After this, we removed the probe holder and 0.5 g of Capsicum cream (Midalgan, 15 mg methylnicotinaat and 50 mg glycolmonosalicylaat per

gram, Remark Groep BV, Meppel, The Netherlands) was applied over an area of 9 cm^2 to the same measurement location and we measured variations in perfusion after 5 minutes. Capsicum cream increases thermoregulating activity of the skin by increasing the mean concentration and mean velocity of red blood cells in few minutes on the area where the cream is applied [13]. Skin sites were avoided with visible large superficial blood vessels, hair and pigment variations.

7.2.2 Real time monitoring of blood flow

For monitoring the perfusion changes in real time, measurements were performed on the dorsal side of the forearm with the length of the reference arm tuned such that the optical path length in the tissue was 1.7 mm in the sample arm. The signal was sampled at 40 kHz for 2.6 seconds to get an average of 100 spectra. Simultaneous measurement of perfusion was performed with the PF5000 at a position approximately 3 cm distant with respect to the probe of the low coherence interferometer. The perfusion was recorded for 30 seconds before occlusion, 90 seconds during occlusion, 105 seconds between occlusions, second occlusion for another 90 seconds and final measurements for 30 seconds.

7.2.3 Inter-and intra-individual variations in optical path lengths and path length resolved Doppler shifts

For measuring inter-individual variations, measurements were performed on the dorsal side of the forearm of three human subjects (A, B, C), one of which female (B). From visible appearance, the skin type of person A (aged 25) belongs to type II whereas skin type of person B (aged 30) and person C (aged 28) belong to type IV. To assess the intra-individual variations, measurements were performed at three positions, viz. fingertip, dorsal and palmar side of forearm of the same individual. Before the measurements with our setup, perfusion readings for one minute were taken at the same locations with the PF5000.

7. 3 Experiments and results

7.3.1. Optical path lengths and path length resolved Doppler shift measurements

Figure 1 shows the power spectra measured on the forearm skin for an optical path length of 1 mm in the tissue. The power spectrum contains interference peaks at the modulation frequency (6 kHz) and at its multiples (12 and 18 kHz). The broadening of the peak resulting from the Doppler shift imparted by the moving red blood cells to the multiply scattered photons is reduced during the arterial occlusion of the upper arm. The area under the peaks, which represents

the intensity of detected photons within a certain optical path length, does not change during occlusion. The DC value of the photocurrent generated by the light detected from the sample was same (160 mV) before and during occlusion. This indicates that the blood volume does not change due to occlusion, but the mean velocity of moving scatterers is decreased. Thus for a given optical path length, the Doppler shifted fraction of photons is reduced during occlusion.

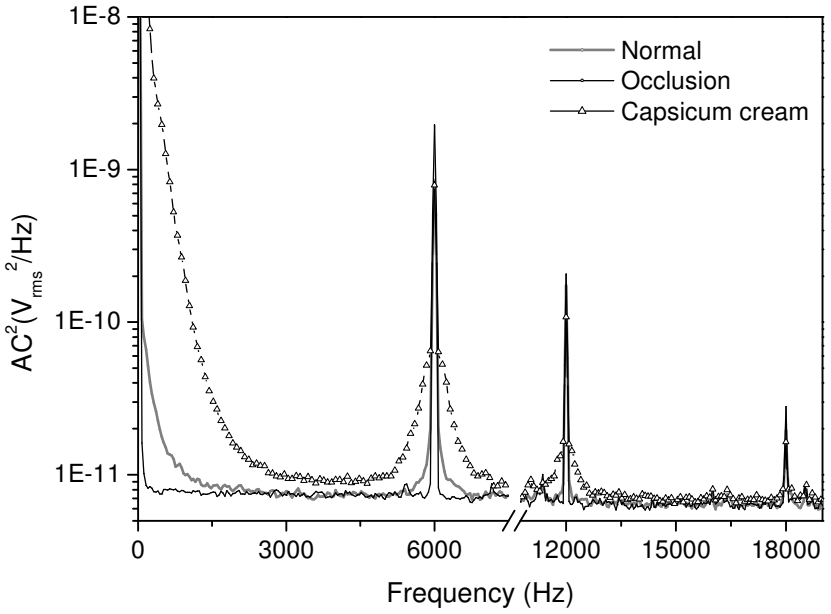

Fig. 1. Power spectra measured on the dorsal side of the forearm and without and with external stimuli: occlusion and capsicum cream provocation.

The optical path length distribution of photons measured in skin before and during occlusion is shown in figure 2 and 3 respectively. With a fiber distance of 300 μm, optical path lengths spanning a range of zero to 6 mm are measured. The fraction of Doppler shifted photons averaged over the entire optical path length measured from the respective areas of the optical path lengths is decreased from 28 to 18% during occlusion. When capsicum cream was applied to the skin, both the intensity and the average Doppler shift of scattered photons, represented by the area and the width of the peak increased (Fig.1). In this case, the DC photocurrent value generated by the light detected from the tissue increased to 280 mV. The optical path length distributions for Doppler and non-Doppler shifted light after application of capsicum cream are shown in figure 4. As expected, the Doppler-shifted fraction of photons (41 %) increased after application of capsicum cream.

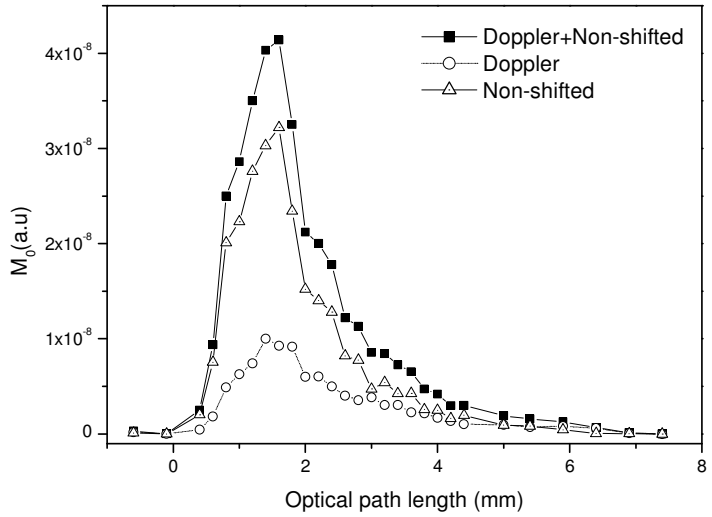

Fig. 2. Optical path length distributions of Doppler shifted and non-shifted photons measured before occlusion.

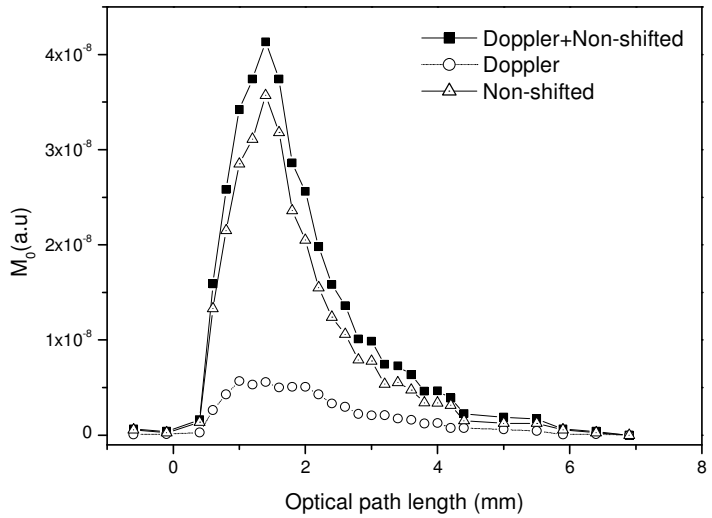

Fig. 3. Optical path length distributions of Doppler shifted and non-shifted photons measured in skin during occlusion.

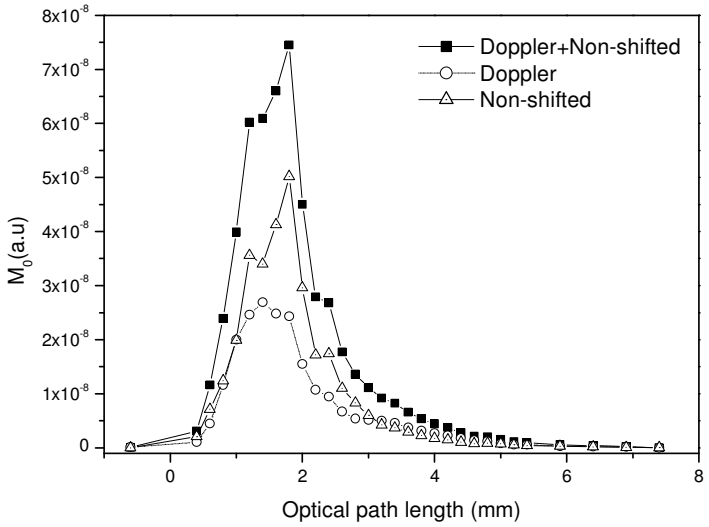

Fig. 4. Optical path length distributions of Doppler shifted and non-shifted photons measured after capsicum cream provocation.

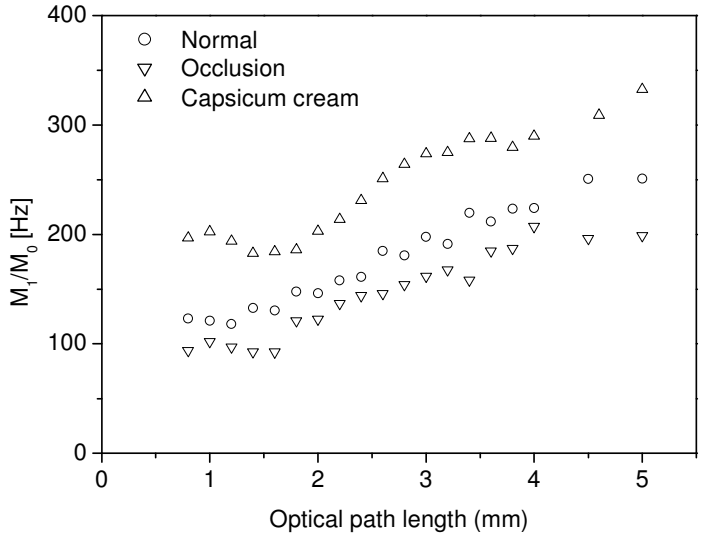

Fig. 5. Weighted first moments, which are equal to the average Doppler shift as a function of optical path length, without and with external stimuli: occlusion and capsicum cream provocation.

As shown in figure 5, the weighted first moment M_1/M_0 of the Doppler shifted light, which represents the average Doppler shift, increased with the optical path length due to the greater probability of interaction of photons with moving scatterers for large optical path lengths. The observed decrease in average Doppler shift during occlusion is due to the decrease in the mean velocity of RBCs. For a given optical path length, an increase in the average Doppler shift is observed with capsicum cream provocation. This could be explained by the increase in the velocity of red blood cells and also by the increase in successive Doppler shifts (multiple scattering) imparted to the photons by the richly perfused skin, resulting from an increased concentration of red blood cells.

7.3.2 Real time monitoring of blood flow

Figure 6 shows the perfusion signal measured in real time for an optical path length of 1.7 mm in skin. At an optical path length of 1.7 mm the intensity of multiply scattered photons is nearly maximal. The occlusion results in the suppression of mean flow velocity of red blood cells to nearly zero values (biological zero). The Doppler shifted fraction of photons estimated from the area of the Doppler broadened peak, and excluding the statically scattered light from the analysis, normalized by the total intensity of both fractions of photons for the same optical path length is reduced due to occlusion (Fig. 6, Left, Top). The flux of red blood cells measured from the first moment also decreased during occlusion (Left, Bottom). Since the realization of occlusion takes some time, the perfusion decreases gradually and finally drops to biological zero values. After the occlusion is released, the perfusion signal increases above the normal value, an effect called Post Occlusive Reactive Hyperemia (PORH). PF5000 traces recorded with a time constant of 0.2 seconds are averaged for the same measurement time used in our measurements (2.6 seconds). Real time measurements performed with our setup show similar trends as the perfusion readings measured with the PF5000. However, smoother perfusion signals are shown from the PF5000 compared to the fluctuating perfusion signal obtained with our LCI set up. The lowest value recorded with PF5000 is relatively lower during occlusion and the PORH peak with LDF is shorter in time than with LCI. These variations could be resulting from the fact that both techniques probe different positions (spatial separation of 3 cm) and our probe measures physiological perfusion in the superficial layers (with an optical path length of 1.7 mm) compared to the perfusion averaged over all depths.

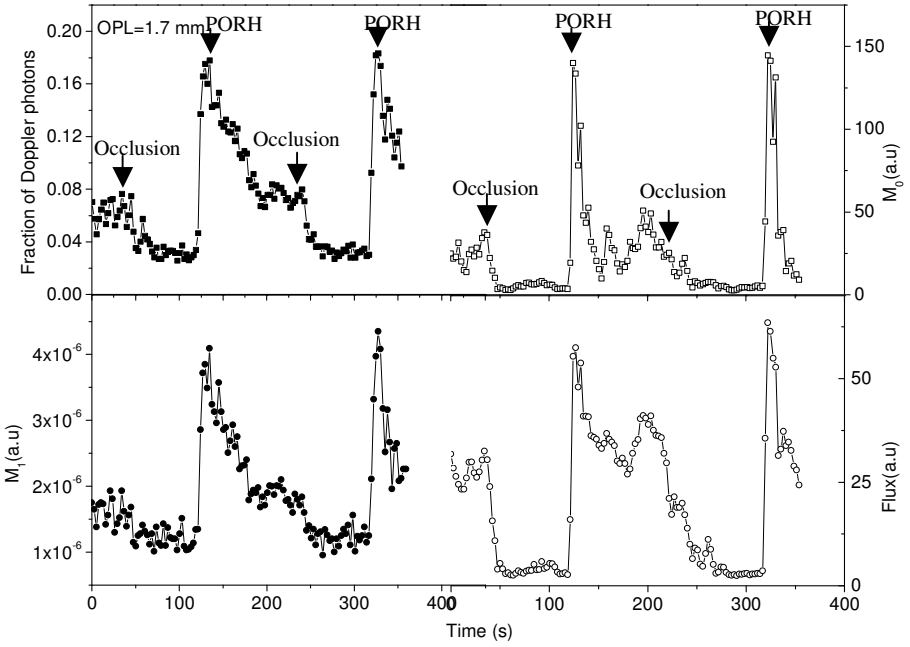

Fig. 6. Real time monitoring of the Doppler-shifted fraction of photons and the weighted first moments measured with our setup (Left) for an optical path length of 1.7 mm. Simultaneous perfusion measurements recorded with PF5000 at an adjacent position (Right).

7.3.3 Inter-and intra-individual variations

Optical path length distributions and the corresponding path length resolved Doppler shifts measured on the dorsal side of the forearm of three individuals are shown in figure 7 and figure 8 respectively. The optical path length of the peak of the distribution is shifted and the distributions is relatively broader for subject A, indicating that the scattered photons appear to emanate from a slightly deeper region in the skin. The fraction of Doppler shifted photons, which provides information about the blood volume is lower (28%) for subject A than for subject B (45%) and C (44%). The path length dependent average Doppler shift measured for subject C is higher for short optical path lengths, whereas that measured in subject B is consistently lower. But for large optical path lengths, the same amount of Doppler shift is measured for subject A and C.

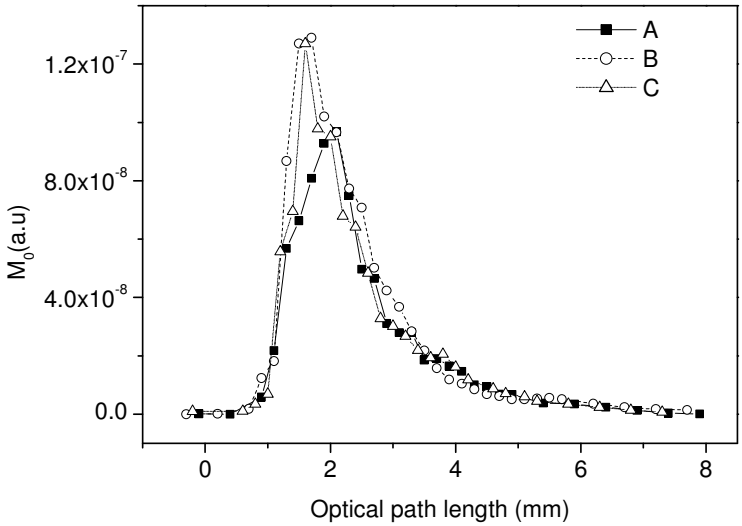

Fig. 7. Inter-individual variations in optical path length distributions of multiply scattered light measured on comparable sites (dorsal side of the forearm) of three individuals.

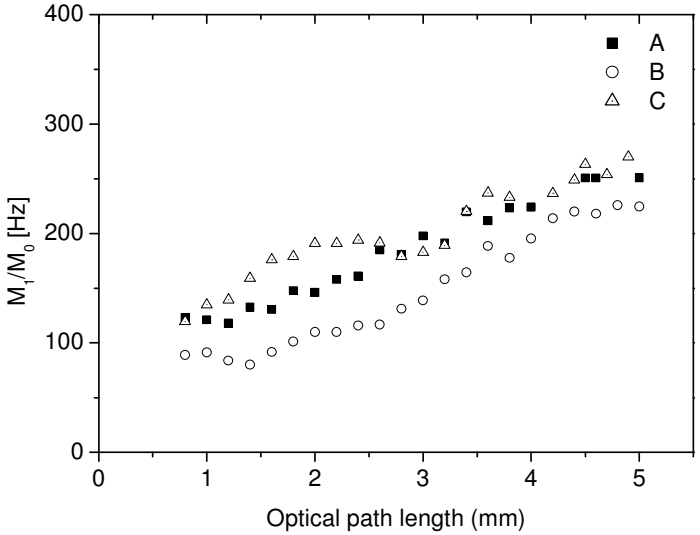

Fig. 8. Inter-individual variations in path-length resolved average Doppler shifts measured on comparable sites (dorsal side of the forearm) between individuals.

Figure 9 depicts the path-length variations in the three measurement sites (Finger, dorsal and palmar side of the forearm) of one individual C. The distributions normalized with their respective maximum values are shown in the inset of fig. 9. Optical path lengths and path length resolved Doppler shifts measured on the dorsal and palmar side of the forearm do not show significant variations. For a given optical path length, the average Doppler shift measured on the fingertip is higher than that measured in the forearm skin (Fig. 10). Optical path lengths measured on the fingertip are 40% longer as compared to forearm measurements. The Doppler shifted fraction of photons measured on the fingertip is about 38 % and that measured on the dorsal and palmar sides of the forearm are 32% and 17% respectively.

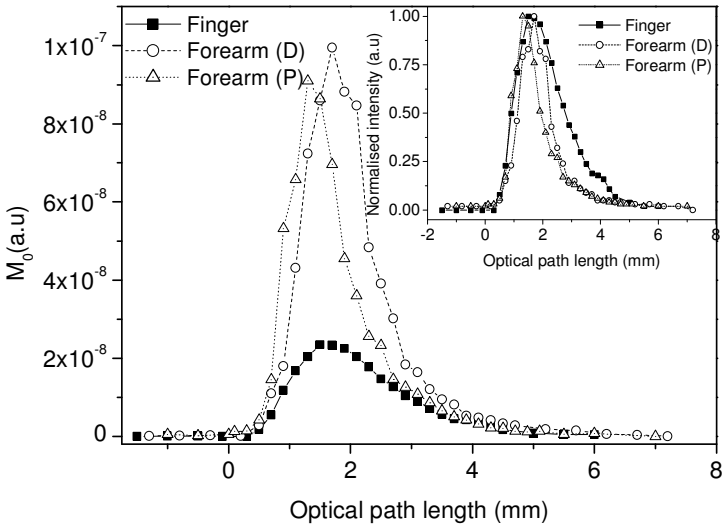

Fig. 9. Intra-individual variations in optical path length distributions measured on different sites (Finger, dorsal and palmar side of the forearm) of the same individual. Inset: Optical path length distributions normalized with their respective maximum values.

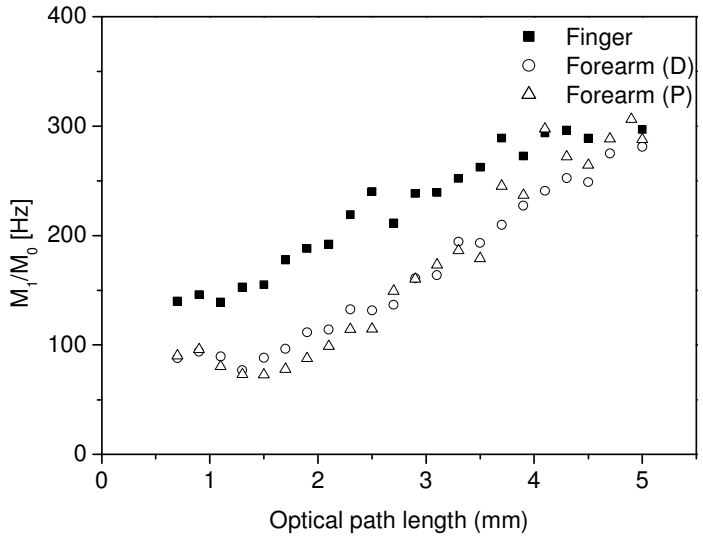

Fig. 10. Intra-individual variations in path-length resolved Doppler shifts measured on different sites (Finger, dorsal and palmar side of the forearm) of the same individual.

In table 1, the first moment of Doppler shifted photons, the Doppler shifted fraction of photons and the weighted average Doppler shift measured with our LCI setup are compared with the perfusion readings recorded with the PF5000. The Doppler shift for a given optical path length is weighted with the corresponding optical path length distribution of Doppler shifted photons to calculate the weighted average Doppler shift. While comparing our results with the perfusion readings of the PF5000, it has to be noted that the differences in the wavelength and numerical aperture will affect the overall sampling depth and measurement volume. As expected, there is a decrease in the measured concentration and velocity during occlusion. Similar decreasing trends are shown qualitatively in the Doppler shifted fraction of photons and the weighted first moments which is measured with our setup. The increase in the concentration and velocity shown in PF5000 readings by the capsicum cream activity is similar to the increase in the Doppler shifted fraction of photons and the average Doppler shift measured with our setup. The average Doppler shift measured with our LCI setup and the velocity measured with PF5000 shows similar trends in inter and intra individual measurements and also during occlusion and capsicum cream provocation. The velocity of subject B measured with PF5000 is lower than that of subjects A and C, which is similar to the observation that the weighted first moments measured with our setup is lower.

	Perimed (PF 5000)			LCI		
	Flux (a.u)	Concentration (a.u.)	Velocity (a.u.)	M_1	Doppler fraction of photons (%)	Average weighted Doppler shift (Hz)
Normal	28 (±4)	45 (±7)	61 (±10)	1792	28	151
Occlusion	5 (±1)	8 (±3)	43 (±9)	1026	18	122
Capsicum cream	90 (±9)	68 (±4)	234 (±15)	3034	41	210
A	28 (±4)	45 (±7)	61 (±10)	1792	28	151
B	4 (±1)	12 (±4)	32 (±6)	1980	45	112
C	57 (±5)	31 (±4)	74(±13)	3168	44	179
Finger	230 (±18)	109 (±21)	224 (±39)	4066	38	211
Forearm (D)	12 (±1.7)	7.4 (±1.9)	155 (±27.6)	1472	32	122
Forearm (P)	10 (±1.2)	9.7 (±2.1)	120 (±17.3)	595	17	109

Table 1: Comparison of perfusion readings measured with LCI set up and PF5000.

7.4 Discussion

In this paper we have presented path length resolved Doppler measurements of multiply scattered light from skin and the results are compared with a conventional laser Doppler technique. In general, the influence of photon path lengths on the measured perfusion signal is overcome by this technique. Also, our method allows us to discriminate between the Doppler-shifted and non-shifted fraction of photons in the detected photodetector signal. However, direct comparison of our results with the perfusion signals of the PF5000 is difficult. In our heterodyne detection technique, the power spectrum of photocurrent fluctuations results from the interference between Doppler shifted light and unshifted reference light, whereas conventional laser Doppler techniques are based on mutual interference between all components of the light detected from the tissue only, either Doppler shifted or unshifted, hence without reference beam. This leads to an extra mechanism of broadening of the power spectrum, depending on the fraction of Doppler shifted photons. Furthermore, due to this mutual interference in LDF the power in the fluctuations saturates for large fractions of Doppler shifted light. Secondly, both techniques sample different parts of the vascular bed. This difference can mainly be attributed to the longer coherence length of the light source used in the PF5000, measuring the perfusion averaged over all path length compared to the path length resolved perfusion measurements that are performed with our low coherent light source. The wavelength of the light source and the differences between the two fiber

optic probes will also influence the measurement volume, but probably only to a small extent. Furthermore, since relatively longer times are required for measuring the complete optical path length distributions, the recordings are affected by the temporal variations in the physiological circumstances of the measurement site. Real time monitoring of perfusion for a given path length is achieved with a temporal resolution of 2.6 seconds and this make it difficult to follow all physiologic changes. For instance, path length resolved Doppler measurements should be obtained in 0.1 second in order to follow the cardiac cycle and reactive hyperaemia responses.

7.4.1 Optical path lengths and path length resolved Doppler shift measurements

Compared to the estimation of the Doppler-shifted fraction of photons based on the first-order [15] or second-order statistics [16] of the fluctuating speckle pattern, the presented approach provided path length resolved information about the probed region. Using this speckle approach, the fraction of Doppler-shifted photons estimated in the light backscattered from the human skin of forearm was 15% (fiber distance= 250 μm, fiber core diameter =125 μm), similar to our observation that most of the scattered light in the perfusion signal is scattered from the surrounding static tissue matrices. However, their path length averaged approach does not provide information about the probed region and this will lead to uncertainties in interpreting the physiological information, when measured on tissue with different extent of perfusion at different depths.

7.4.2 Real time monitoring of blood flow

During occlusion, the observed discrepancy between the calibrated zero of the instrument and the "biological zero" level is attributed to the fact that though the blood perfusion is arrested by the inflated cuff, minor Doppler components are recorded by the instrument due to the no-flow laser Doppler signal from vasomotion, Brownian motion from within the vascular compartment and of macromolecules in the inter- and intracellular fluids, and the effects of cuff compression [17]. The time traces measured with the PF5000 are relatively smooth in comparison with the readings obtained with our setup for a given optical path length. This is because, in conventional laser Doppler instruments such as PF5000, perfusion values averaged over all optical path lengths are measured, which often spans a range of zero to 6 mm. In our setup, the coherence length of the light source acts as a bandpass filter in selecting the photons that have travelled a specific optical path length (for instance 1.7 mm) in the skin with a tolerance of ± 50 μm, as defined by the path length resolution of the setup.

7.4.3 Inter-and intra-individual variations

The optical path lengths measured with fiber distances typical for LDPMs showed inter-and-intra individual variations, which is contradictory to the observations made by Bonner and Nossal that the path length variation is not a large factor in typical LDPMs [1]. This clearly shows that these variations should be taken into account while comparing the perfusion readings from comparable sites between individuals and from different sites of the same individual. The major drawback of LDF resulting from significant variations in optical path lengths has been pointed out by Tenland et al. [18] and Braverman et al. [19]. Larsson et al. observed that average path length varied up to ~40 % (max/min) between individuals and the average intra-site variability is less than the inter-site variability [14]. Methods for the local photon path length estimation based on the diffuse reflectance approach can eliminate this influence, but their results showed non-linearities for short optical path lengths due to the fact that they considered the average photon path length and not the actual path length distribution [14]. In our coherence-gated approach, we estimate the actual path length distribution rather than the average photon path length. The preliminary results presented in this manuscript indicate that the path length distributions are very sensitive to the skin site that is being probed and the intra-individual variability is higher than the inter-individual variability measured on comparable sites between different individuals. In general, fingertips have lower absorption and reduced scattering coefficients than the forearm skin resulting in an increase of optical path lengths [14]. This increased lengths that the photons travel in the finger are evident from the normalized distributions measured with our setup (Inset of Fig. 9). Assuming a homogenous tissue perfusion, the conventional LDPM readings are thus expected to show a relative overestimation on fingertips compared to the forearm readings due to the linear dependence of perfusion on average photon path length [14]. This is shown in the flux readings obtained with PF5000, which showed an increase (factor of 23) in the flux recorded on finger as compared to the forearm recordings. With LCI, this factor is reduced to 7. Since small source-detector separations of 0.3 mm result in small sampling volume and superficial sampling depth, the perfusion readings will be sensitive to the variations in the microvascular architecture.

7.5 Conclusion

In this manuscript, we for the first time report on path length resolved Doppler measurements of diffusely scattered light from skin, using a phase modulated low coherence Mach Zehnder interferometer with spatially separated fibers for illumination and detection. Optical path length distributions of multiply scattered light, spanning a range of 0-6 mm, and their response to external

stimuli such as occlusion and capsicum cream provocation have been measured. The Doppler shifted fraction of photons, which is related to the blood volume and the path length dependent average Doppler shift, which is related to the mean velocities of red blood cells, are decreased during occlusion. Both quantities are significantly increased when capsicum cream was applied. The path length resolved perfusion measured in real time with our setup, for an optical path length of 1.7 mm, and its variations during artery occlusion showed correlation with the perfusion signal measured using a conventional LDPM. Measurements were performed on the dorsal side of the forearm of three human subjects and on different skin locations of the same individual to compare the inter-and intra-individual variations. The results indicate that that these variations should be taken into account while comparing the perfusion readings from comparable sites between individuals and from different sites of the same individual.

The path length resolved perfusion measurements presented here may overcome the inherent limitation of conventional LDPM that restrict its clinical usefulness, where the perfusion signal depends on an unknown photon path length. This will enable to correctly interpret or counter-act the inter-and intra-individual variations in the LDF readings introduced by the variance in tissue optical properties. This approach enables to discriminate between the Doppler-shifted photons resulting from interaction with the moving red blood cells and the non-shifted light scattered only by the surrounding static tissue matrices. Another important feature of this approach is the tunable depth resolved perfusion information that can be achieved. By changing 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 the perfusion signal from superficial and deeper layers of tissue. However, further developments and fundamental research are required in developing this into a tool that is suitable for use in a clinical environment, with acceptable measurement times and suitable patient interfaces.

Acknowledgements

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References:

1. A. P. Shepherd and P. Å. Öberg, Laser-Doppler Blood Flowmetry (Kluwer Academic, Boston (1990).
2. F. Morales, R. Graaff, A. J. Smit, R. Gush, G. Rakhorst, “The influence of probe fiber distance on laser Doppler perfusion monitoring measurements,” Microcirculation, 10(5), 433-441 (2003).
3. M. Larsson, W. Steenbergen, and T. Strömberg, "Influence of optical properties and fibre separation on laser Doppler flowmetry," J. Biomed. Opt., **7**, 236-243 (2001).
4. 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, 024020 (2007).
5. 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).
6. 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. Communications, 2007 (In press).
7. 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**, 1178 (1991).
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**, 4222-4227 (2001).
9. 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, **58**, 7664-7667 (1998).
10. 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 interferometer”, Opt. Lett., **26**, 1492-1494 (2001).
11. 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**, 595-597(2002).
12. G. E. Nilsson, “Signal processor for laser Doppler tissue flowmeters,” Med. Biol.Eng. Comput, **22**, 343-348 (1984).
13. A.N. Serov, J. Nieland, S. Oosterbaan, F.F.M. de Mul, H. van Kranenburg, H.H. Bekman, and W. Steenbergen “Integrated optoelectronic probe including a vertical cavity surface emitting laser for laser Doppler perfusion monitoring,” IEEE Trans Biomed Eng., **53**(10), 2067-74 (2006).

14. 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**, 124-134 (2003).
15. J. D. Briers, "The statistics of fluctuating speckle patterns produced by a mixture of moving and stationary scatterers," Opt. Quant. Elect., **10**, 364-366 (1978).
16. A. Serov, W. Steenbergen, and F. de Mul, "Prediction of the photodetector signal generated by Doppler-induced speckle fluctuations: theory and some validations," JOSA A **18**, 622-630 (2001).
17. P. Kernick, J. E. Tooke and A. C. Shore, "The biological zero signal in laser doppler fluximetry-origins and practical implications," Pflügers Arch-Eur.J.Physiol., **437**(4),624-631 (1999).
18. T. Tenland, E. G. Salerud, G. E. Nilsson, and P. Å. Öberg, "Spatial and temporal variations in human skin blood flow", Int. J. Microcirc. Clin. Exp., **2**, 81-90 (1983).
19. M. Braverman, A. Keh, and D. Goldminz, "Correlation of laser Doppler wave patterns with underlying microvascular anatomy", J. Invest. Dermatol., **95**, 283-286 (1990).

Chapter 8

Summary and outlook

In this chapter a summary of the results and conclusions are given. An outlook to future developments is presented, which will translate the novel methodology developed in this project, 'Path length resolved optical Doppler perfusion monitoring' into a potential clinical tool that can bring more quantitatively reliable tissue perfusion information.

8.1 Summary

Laser Doppler blood flowmetry is a non-invasive technique for monitoring blood microcirculation in biological tissues and has been used in clinics for last three decades [1]. In spite of its important advantages such as high spatial resolution, noninvasiveness and real-time monitoring, this technique has several problems limiting its use in clinical practice [2,3]. This is partly caused by the fact that LDPM cannot measure in absolute flow units, by the large flux-signal fluctuations and by the lack of standardization of laser Doppler perfusion monitors. The complexity and randomness of the microvascular network further complicates the measurement situation. An important limitation of this technique is the dependence of perfusion signal on the photon path lengths [4-6]. Coherence domain path length resolved optical Doppler perfusion monitoring, of which the basic technique is developed in this thesis, will overcome this limitation. This will enable to correctly interpret the inter-and intra-individual variations in the LDF readings introduced by the variance in individual photon path lengths due to changes in tissue optical properties and probe geometry.

In **chapter 2**, we present an improved coherence gated interferometric technique for path length resolved measurements of diffusively scattered light. The principle of this method and its physical realization based on phase modulated low coherence fiber optic Mach-Zehnder interferometer is described. 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. With a center-to-center fiber distance of 500 μm, optical path length distributions spanning a range from 0-11 mm were measured from the area under the phase modulation peak. We validated the optical path length distributions by quantifying the predictable effect of varying absorption levels using Lambert-Beer's law. The average Doppler shift increased with the optical path length, which can be expected from the increase in the number of scattering events with the optical path length. 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. The path length distributions measured for viscous media, visco-elastic gel media and the aqueous suspension of Polystyrene microspheres ($\mu'_{s=}$2.35mm^{-1}, g=0.85) were identical and the variation of the DC value for all the above three samples was within 7%. Thus, independent of the Brownian motion of the scatterers, our method can measure realistic optical path length distributions.

In **chapter 3**, we show the optical path length distributions and spectral diffusion broadening of multiple scattered light measured for calibrated scattering samples with different reduced scattering coefficients and

anisotropies. In the multiple scattering regimes, the diffusive light transport through a turbid medium can be described by Diffusive Wave spectroscopy (DWS), which is an extension of the conventional dynamic light scattering (DLS) technique. Water suspensions of polystyrene microspheres with diameters of ∅0.20 μm (anisotropy factor, g=0.18) and ∅0.77 μm (g=0.85) are used to make scattering phantoms 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. Samples with reduced scattering coefficients (μ_s') of 7.00 (g=0.85), 4.95 and 3.25 mm^{-1} (g=0.18) and absorption coefficient (μ_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 light and the refractive index of water. Good agreement between experimental path-length distributions and Monte Carlo simulations was found. The path length dependent diffusion broadening or Doppler broadening of scattered light is shown to agree with Diffusive Wave Spectroscopy within 5%.

In **chapter 4**, we describe an improved low coherence interferometry method for measuring path length distributions and path length dependent Doppler broadening of multiple scattered light from turbid media. The improvement is based on sinusoidal phase modulation of the optical path length in the reference arm at high phase angles ($\Delta\phi$ =2.04 radians), leading to interference peaks at both the phase modulation frequency and higher harmonics. We showed an enhancement of the signal to noise ratio by a factor of 9 in measured path length distributions at high phase angles as compared to a situation where the peak phase modulation angle is kept lower ($\Delta\phi$ =0.51). The distributions are similar when normalized to the maximum value of the path length distribution at all phase modulation angles, and smoother curves are obtained for high phase modulation angles. Furthermore, statistical averaging can be done on measured Doppler shifts from the FWHM of spectrum of the sidebands. Good agreement between the experimental path-length distribution and Monte Carlo simulations was found. Further increase of the SNR might be obtained by shifting the modulation frequency to higher frequencies, where often the noise level is lower and spectrum is more flat than for low frequencies.

In **chapter 5**, 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 thickness (0.1-0.9 mm) covering a dynamic medium with identical optical properties (μ'_s=2.0 mm^{-1}, g=0.85). By separating the 50 Hz bandwidth signal corresponding to the statically scattered light

contribution at the modulation frequency from the total intensity, the optical path length distributions of the Doppler and non-Doppler shifted photons is determined. 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. A remarkable observation is that no Doppler broadening of the interference peak is observed until a certain optical path length, which is in turn related to the thickness of the static layer. Assuming ballistic or snaky photon paths between the fiber tips, via the dynamic layer, the thickness of the static superficial static layer can be estimated from the minimum optical path length of Doppler shifted light. Good agreement between the experimentally determined thickness of the static layer and Monte Carlo simulations was found. This approach has potential applications in determining burn depths and in discriminating between statically and dynamically scattered light in the perfusion signal.

In **chapter 6**, the performance of multimode graded index fibers for detection in a coherence gated interferometric scheme is compared with a single mode fiber optic interferometer. To evaluate the effect of intermodal dispersion of multimode fibers in low coherence interferometry, we replaced the scattering medium by a mirror and the length of the reference arm was varied. A sharp interference peak was observed at the modulation frequency due to the interference of reference light with the light reflected from the mirror. When the zero order moment (area of the heterodyne peak corresponding to the intensity of photons) is recorded as a function of optical path length, we have observed a main peak with FWHM=55 μm and some additional satellite peaks at a distance of 60 and 300 μm with respect to the main interference peak. These peaks are at least a factor of 3.25 lower than the main peak. With a single mode fiber optic interferometer, full width at the half maximum of the main peak was about 55 μm and no satellite peaks were observed. The deterioration of the path length resolution from 18 μm (as expected from the coherence length of the light source) to about 55 μm observed in both single mode and multimode fiber optics interferometers is due to the unbalanced optical dispersion of the reference and sample arms from the electro-optic modulator. The results indicate that there is no significant effect of intermodal dispersion in the path length resolution of our experimental setup. With a graded index multimode fiber for detection, we have performed path length resolved Doppler measurements of diffusely scattered light in an aqueous suspension of lipid particles undergoing Brownian motion and pressure driven flow. From the spectral analysis of moments of the interference peak appearing at the modulation frequency, optical path lengths and path length dependent Doppler shifts were measured in an aqueous suspension of 10% of Intralipid 20% flowing through a channel in a block of Delrin over a range of average velocities from 0 to 6 mm/s. The average Doppler shift estimated from the weighted first moment M_1/M_0 of the interference peak, excluding the central

part representing the statically scattered light, showed a linear response with velocity for different mean flow velocities in the physiological range (0 to 6 mm/s). The path length distributions measured in scattering media suggest that the effect of intermodal dispersion is weak and the effects of additional satellite peaks that may result from the intermodal dispersion are not evident. Thus, multimode graded-index fibers provide a large detection window with a small modal dispersion in path length resolved dynamic light scattering.

In **chapter 7**, we present a first report on path length resolved Doppler measurements of diffusely scattered light from skin and its response to stimuli such as occlusion and vasodilation. The Doppler shifted fraction of photons, which is related to the blood volume and the path length dependent average Doppler shift, which is related to the mean velocities of red blood cells, were decreased during occlusion, as expected. Both quantities are significantly increased when capsicum cream provocation was applied. The path length resolved perfusion changes measured in real time (averaged over 2.6 s) with our setup and its variations during artery occlusion showed correlation with the perfusion signal measured using a conventional LDPM device. Measurements were performed on the dorsal side of the forearm of three human subjects and on different skin locations of the same individual to compare the inter-and intra-individual variations. Inter-and intra-individual variations in optical path lengths and path length resolved Doppler shifts are measured, which indicates that these variations should be taken into account while comparing the perfusion readings from comparable sites between individuals and from different sites of the same individual.

The path length resolved perfusion measurements presented here will overcome the inherent limitation of conventional LDPM that restrict its clinical usefulness, where the perfusion signal depends on the photon path length. This will enable to correctly interpret the inter-and intra-individual variations in the LDF readings introduced by the variance in individual photon path lengths.. This approach also enables to discriminate between the Doppler-shifted photons that interacted with the moving red blood cells and the non-shifted light scattered from the surrounding static tissue matrices. Another important feature of this approach is the tunable depth resolved perfusion information that can be achieved. By increasing the optical path length of 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.

By its non-invasive nature in measuring path length resolved dynamic light scattering, the method has potential applications in the fields of fundamental as well as applied research in monitoring the spatial and temporal variations in optical properties in turbid media and blood perfusion in tissue with path length sensitivity.

8.2 Outlook

Laser Doppler perfusion monitoring is used in clinics and in vascular research as a potential tool for assessing microcirculation of the skin in a continuous and non-invasive way. However, due to the technical limitations of LDPM and complexity and randomness of the microvascular network, it has not been successfully applied in clinics, for instance like pulse oximetry [4-6]. Hence this technique has great potential for further developments and creates a fertile ground for fundamental research. The results of this research project suggest several promising directions for future investigations.

Development of path length resolved optical Doppler perfusion monitoring, of which the laboratory prototype was developed in this project, translated the research concept of measuring perfusion with path length sensitivity into a physical realization [7, 8]. However, further developments and fundamental research are required in developing this into a tool that is suitable for use in a clinical environment, with acceptable measurement times and suitable patient interfaces, and the ability to measure blood perfusion in absolute units.

8.2.1 Development and refinement of the technique

In the present stage of the development of low coherence interferometry, perfusion measurements can be obtained for a specified optical path length within 2.6 seconds (100 spectral averages). In the ideal case, perfusion measurements for other optical path lengths can be simultaneously obtained by constructing a fiber optic probe with multiple detection fibers arranged symmetrically with respect to the launching fiber with deliberately varied fiber lengths in the sample arm. Also, when a conventional LDPM is used for perfusion measurements, this instrument can be used at the measurement site for estimating path length distributions and path length resolved Doppler information at a given wavelength.

In our measurements, a path length resolution of 200 μm would be sufficient for measuring path length distributions of multiply scattered light, which often have widths of 5 mm or larger. This could be obtained by using a light source of larger coherence length than the currently used 18 μm, which will also result in signal to noise ratio enhancement. Further improvements could be obtained by using a balanced detector and a lock in detection system. An automated system with motorized scanning of the reference path is an important step to make it more clinically viable. Optical phase modulation of the reference light using a piezoelectric modulator will make the system more compact and economically feasible.

8.2.2 Measuring flux of red blood cells independent of optical properties of surrounding static tissue matrices

Our preliminary results indicate that for absorption levels realistic for tissue, our method enables Doppler measurements independent of the absorption level of the medium in which the moving particles are embedded [7]. Further measurements on mixed static and dynamic turbid media, with tunable optical properties of the surrounding static tissue matrices will clearly demonstrate the advantage of this method over conventional LDPM.

8.2.3 Towards absolute perfusion measurements

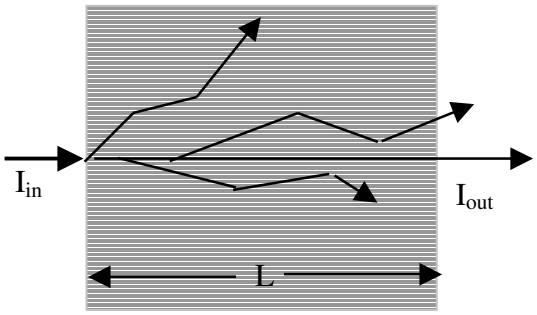

Fig. 1. Collimated light transmission through a cuvette of geometrical length *L* filled with blood.

One of the biggest challenges that we face is to measure red cell flux in absolute units. Here, a simple approach will be presented which uses the fact that we can measure the fraction of Doppler shifted photons f_D and the average Doppler shift M_1/M_0 for a given optical path length. In this approach, for simplicity red blood cells are considered to be independent scatterers, and tissue is regarded as a homogeneously perfused medium. The Doppler fraction of photons obtained by normalizing the intensity of Doppler shifted photons with the total intensity at a specific optical path length will saturate for very large optical path lengths because in that case all the photons will be Doppler shifted. For a given geometrical path length (*L*) associated with the selected optical path length, the concentration of moving red blood cells can be obtained by measuring the fraction of Doppler shifted photons for that geometrical path length, and by the application of Lambert-Beer's law. The situation is analogous to the collimated light transmission through a cuvette of geometrical thickness *L* (Fig. 1) only filled with blood, in which the transmitted light intensity decays exponentially with optical path length as:

$$\frac{I_{out}}{I_{in}} = \exp(-LC\sigma) = (1 - f_D) \qquad (1)$$

where I_{in} and I_{out} are incident and transmitted collimated light intensities, C and σ are the concentration and scattering cross section of moving red blood cells and f_D is the fraction of Doppler shifted photons. Here the transmitted intensity (ballistic component) is analogous to the non-shifted fraction of photons ($1-f_d$) that would have escaped after traveling a geometrical path length L through a tissue with an equal concentration of red blood cells. The weighted first moment (M_1/M_0), which represents the average Doppler shift, increases with optical path length due to multiple scattering. Flux of red blood cells can be measured from the product of concentration measured for a given optical path length (eqn.1) and the corresponding average Doppler shift for that optical path length as. We present the following tentative formula:

$$Flux \propto -\frac{1}{L\sigma}\ln(1-f_D)\frac{M_1}{M_0} \qquad (2)$$

The proportionally constant that should complete eq.(2) must establish the relationship between the observed average Doppler shift M_1/M_0 and the mean velocity of red blood cells. Here, the following quantities will play a role:

- the single scattering phase function, which will determine the Doppler shift distribution for single scattering by flowing blood
- the extent of multiple Doppler scattering, which will depend on L and the average concentration of blood.

Hence, quantitative flux measurements require prior knowledge concerning the optical properties of blood. Here we are facing complications, which have to be taken into account. For low hematocrit (Hct) values the red blood cells do not influence each other's optical properties, and the attenuation coefficient will be proportional with Hct. For high hematocrit values however, red blood cells can not be regarded as individual scattering centres, and the attenuation coefficient will be nonlinear with Hct. From hemorheological studies it is known that the local Hct value depends on the blood vessel diameter and the local shear rate [9]. This illustrates to complexity of the interaction of light with flowing blood. The consequence is that the sensitivity of optical Doppler techniques to red cell flux may be different for vessels of different size. Furthermore, the scattering phase function that defines the angular dependence of light scattering and the collimated transmission are influenced by the rheology of blood, for instance in response to shear rate [10]. Hence, while Doppler information for fixed and known path lengths can be measured based on the methodology presented in this thesis, the next problem posed by the complex optical behaviour of flowing blood has to be tackled. However, the complex behaviour of flowing blood also affects traditional laser Doppler techniques, which nevertheless have been proven to give output signals linear to flux. This gives us confidence that flux measurements in absolute units will be feasible.

8.2.4 Clinical applications

This technique may lead to new diagnostic tools, which are noninvasive or minimally invasive. The tissue optical properties of the medium cannot be directly measured from the optical path length distributions. Our method is capable of measuring optical path length of multiply scattered photons that have traveled optical path length of 5 mm or larger. This implies that our system is capable of measuring physiological information from deeper layers of tissue, which is not possible with OCT techniques. Measuring large optical path lengths is important for determining absorption level variations in the physiological range that are associated with changes in [Hb]. The average Hemoglobin-level may be obtained from the differences in path length distributions obtained for various blood concentrations in skin by the application of Lambert-Beers law.

Optical tissue characterization for a range of wavelengths strongly enriches the information on the tissue condition. For instance, tissue oxygenation based on spectroscopic application of our technique can be done by using a stable and versatile source of low coherence light, such as the Ti: Sapphire laser. This may lead to a combined non-invasive instrument for the measurement of microvascular perfusion and oxygen saturation. Recently Amelink et al. showed the feasibility of differential path-length spectroscopy (DPS) to determine the local tissue optical properties and to discriminate the local morphological and physiological changes occurring during malignant transformations [11]. An important potential application of our approach is to perform absolute perfusion measurements, which will open new possibilities and clinical applications. Potential applications include determination of superficial burn depth [12], observation of the tissue condition in the follow-up stage and discrimination between malignant and benign lesions [13].

8.2.5 Spatial perfusion measurements

While the fiber optic based configuration is ideal for temporal perfusion measurements in a small sampling volume with a high spatial resolution, the combination of low coherence interferometry with an imaging feature would be highly desirable for spatial perfusion measurements over a large area. A scanning system with a laser beam of limited diameter [14] or a wide beam in combination with a CMOS detection array offers further possibilities for noncontact imaging [15].

8.2.6 Alternate approach: Swept source interferometry

An alternative approach to obtain path length resolved Doppler information is based on swept source interferometry with a wavelength-swept laser source of narrow bandwidth [16]. In this method, the wavelength of a light source (center wave length λ_c) is scanned continuously over a certain wavelength range at a given tuning speed ($d\lambda/dt$), and the power spectrum of the interference signal due to the wavelength scanning can be directly related to the path length distribution, without the need of scanning a reference mirror. When the wavelength of the light source is continuously tuned, and multiply scattered light is detected, two photon fractions having a certain optical path length difference (Δl) which represent light that has been produced at different instants and hence different field frequencies will cause a beat frequency

$$\omega_b = -\frac{1}{\lambda_c{}^2}\frac{d\lambda}{dt}\Delta l \qquad (3)$$

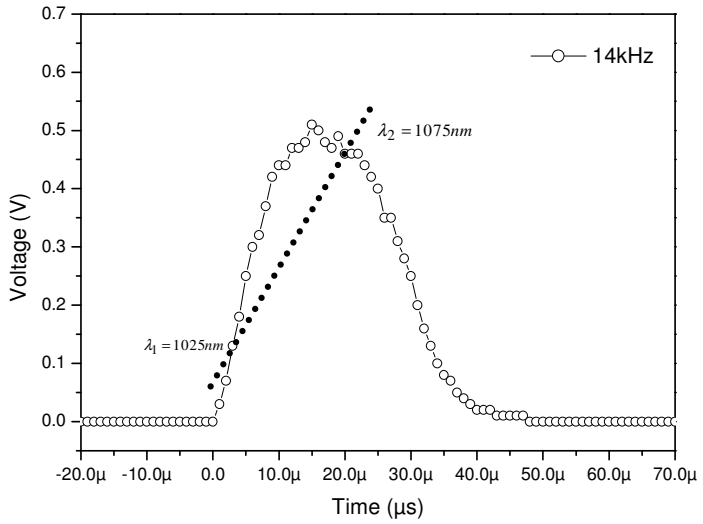

Fig. 2. The instantaneous output power (circles) and wavelength (dotted line) of a wavelength-swept laser (Santec HSL2000, tuning range=50 nm @14 kHz, center wavelength=1050 nm, optical output power=3 mW) that can be used for path length resolved measurements.

For a single optical path length difference between two arms this leads to a single beat frequency. The optical properties of the sample generate a distribution of optical path lengths in the sample arm and this will lead to a

distribution of beat frequencies whose shape is closely related to the optical path length distribution. This will increase the speed of measurement since mechanical scanning of the reference arm mirror is not required. In contrast to the coherence gated technique where the short coherence length of the light source acts as a bandpass filter in selecting the photons that have travelled a specific optical path length in the medium, all photons will contribute to the interference signal in swept source approach, resulting in signal to noise ratio enhancement. In this technique, the wavelength range will determine the resolution of the path length discrimination. For a center wavelength of 1050 nm, and a required path length resolution of 100 μm, the light source requires a tuning range of approximately 11 nm. In fig. 2 the instantaneous output power (circles) and wavelength (dotted line) of a typical swept source (Santec HSL 2000, tuning range=50 nm @14 kHz, center wavelength=1050 nm, optical output power=3 mW, coherence length=6mm, tuning speed=16 nm /10 μs) is shown. With this tuning speed, for an optical length distribution of 0-10 mm, the beat frequencies will be 0-15 MHz using eqn. 3. The shortest optical path length of the detected photons depends on the fiber geometry and typically this is about 600 μm, corresponding to a spatial separation of 300 μm between the fibers. Furthermore, the beat frequencies can be tuned by changing the optical path length of the reference arm. Thus an optical path length distribution spanning a range of 0.6 to 10 mm will lead to beat frequencies in the range of 0.8 to 10 MHz, which is well measurable. Hence a typical swept source such as Santec HSL 2000 can be used for path length resolved measurements of multiply scattered light. From the utilization point of view, wavelength modulation of the laser diode used in conventional LDPMs might be an interesting option.

In conclusion, our studies on path length resolved optical Doppler perfusion monitoring provides more qualitatively and more quantitative reliable tissue perfusion information. Further research in this direction will improve the clinical applicability of LDPM.

References:

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**, 143-162 (1999).
3. T. Tenland, E.G. Salerud, G.E. Nilsson and P. Å. Öberg, “Spatial and temporal variations in human skin blood flow,” Int. J. Microcirc. Clin. Exp., **2**(2), 81-90 (1983).

4. M. Larsson, W. Steenbergen, and T. Strömberg, "Influence of optical properties and fibre separation on laser Doppler flowmetry," J. Biomed. Opt., **7**, 236-243 (2001).
5. 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**, 2238-2244 (1989).
6. 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**, 124-134 (2003).
7. 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**, 024020 (2007).
8. 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).
9. H. L. Goldsmith, G. R. Cokelet, P. Gaehtgens, P. R. Fåhraeus, "Evolution of his concepts in cardiovascular physiology," Am. J. Physiol., **257** (3 Pt. 2), 1005-1015 (1989).
10. H. Schmidt-Schonbein, E. Volger, H. J. Klose, "Microrheology and light transmission of blood II. The photometric quantification of red cell aggregate formation and dispersion in flow," Pflugers Arch., **333**, 140–155 (1972).
11. A. Amelink, H. J. C. M. Sterenborg, M. P. L. Bard, and S. A. Burgers, "*In vivo* measurement of the local optical properties of tissue by use of differential path-length spectroscopy," Opt. Lett. **29**, 1087-1089 (2004).
12. A. Sadhwani, K. T. Schomacker, G. J. Tearney, and N. S. Nishioka, "Determination of Teflon thickness with laser speckle.I. Potential for burn depth diagnosis," Appl. Opt., **35**, 5727-5735 (1996).
13. P. Carmeliet, "Mechanisms of angiogenesis and arteriogenesis," Nat. Med., **6**(4), 389-395 (2000).
14. K. Wårdell, A. Jakobsson and G.E. Nilsson, "Laser Doppler perfusion imaging by dynamic light scattering," IEEE Trans. Biomed. Eng., **40**, 309-316 (1993).
15. A. Serov, W. Steenbergen, F.F.M. de Mul, "Laser Doppler perfusion imaging with a complimentary metal oxide semiconductor image sensor," Opt. Lett., **25**, 300-302 (2002).
16. J. M. Schmitt, "Optical Coherence Tomography (OCT): A Review", IEEE Selected Topics in Quantum Electronics, **5**(4), 1205-1215 (1999).

Samenvatting

Laser Doppler Perfusie Meting (LDPM) is een niet-invasieve techniek voor het meten van de doorbloeding van de microcirculatie in biologische weefsels en wordt sinds een aantal decennia toegepast in de kliniek [1]. Ondanks voordelen als hoge spatiele resolutie, niet-invasief en 'real time' meten, zijn er een aantal problemen die de klinische toepassing beperken [2,3]. Dit wordt voor een deel veroorzaakt door het feit dat LDPM de doorbloeding niet kan meten in absolute flow-eenheden, door grote fluctuaties in het flux-signaal en doordat de LDPM systemen niet zijn gestandaardiseerd. De meetsituatie wordt verder bemoeilijkt door complexiteit en willekeur van het microvasculaire netwerk. Daarnaast is een andere belangrijke beperking van deze techniek dat het gemeten perfusie-signaal afhankelijk is van de weglengte die de fotonen hebben afgelegd [4-6]. Deze laatste beperking kan worden opgelost door gebruik te maken van coherentie-domein weglengte opgeloste optische Doppler perfusie monitoring. De ontwikkeling van de basis van deze techniek is beschreven in dit proefschrift. Deze methode maakt het mogelijk om de inter- en intra-individuele variaties in de LDPM waarden, door variatie in de individuele foton weglengtes veroorzaakt door variaties in optische eigenschappen en probe geometrie, te correct te interpreteren.

In **hoofdstuk 2** presenteren we een verbeterde "coherentie-gated" interferometrische techniek voor weglengte-afhankelijke meting van diffuus verstrooid licht. Hoofdstuk 2 bevat een beschrijving van het principe van deze methode en de realisatie van het systeem gebaseerd op een fase gemoduleerde laag-coherente fiber optische Mach-Zehner interferometer. We laten zien dat optische weglengte-verdelingen en weglengte afhankelijke Doppler verschuivingen van meervoudig verstrooid licht in zowel statische als dynamische verstrooiende modelsystemen kunnen worden bepaald uit de spectrale piek die wordt gegenereerd door fase-modulatie van de referentie arm. Met een afstand tussen de centra van de twee fibers van 500 μm zijn weglengte-verdelingen gemeten over een bereik van 0-11 mm door analyse van het oppervlak onder de fase-modulatie piek. De weglengte-verdelingen zijn gevalideerd met behulp van de wet van Lambert-Beer door de absorptie te variëren. De gemiddelde Doppler verschuiving nam toe met toenemende weglengte, wat kan worden verklaard aan de hand van een toename in het aantal verstrooiings-gebeurtenissen bij een toenemende weglengte. Daarnaast hebben we laten zien dat voor fysiologische waarden van de absorptie, de dynamische

eigenschappen van een verstrooiend medium kunnen worden bepaald onafhankelijk van de absorptie. De weglengte-verdelingen die gemeten zijn aan viskeuze media, visco-elastische gelen en waterachtige suspensies ($\mu'_{s=}$2.35mm^{-1}, g=0.85) waren identiek en de variatie in DC-waarde voor deze drie media was minder dan 7%. Dit betekent dat onze methode realistische optische weglengteverdelingen kan meten onafhankelijk van de Brownse beweging van de verstrooiiers.

In **hoofdstuk 3** laten we spectrale diffusie verbreding en optische weglengte verdelingen zien, die gemeten zijn aan meervoudig verstrooid licht voor gekalibreerde verstrooiende media met verschillende anisotropiewaarden en gereduceerde verstrooiingscoëfficiënten. Voor meervoudige verstrooiing kan het lichttransport door het weefsel worden beschreven met *Diffusive Wave spectroscopy* (DWS). Dit is een uitbreiding van het gebruikelijke *Dynamic Light Scattering* (DLS) model. Waterige suspensies van polystyreen microbolletjes met een diameter van 0.20 μm (anisotropiewaarde g=0.18) and 0.77 μm (g=0.85) zijn gebruikt om verstrooiende media te maken waarbij de absorptie van het medium te verwaarlozen is ten opzichte van de absorptie, zodat de voortplanting van de meervoudig verstrooide fotonen kan worden beschreven als een diffusieproces. Dit zorgt voor een maximale geldigheid van DWS, zelfs voor korte optische weglengtes in onze metingen. Van bovengenoemde suspensies zijn media gemaakt met een gereduceerde verstrooiingscoëfficiënt (μ_s') van 7.00 (g=0.85), 4.95 en 3.25 mm^{-1} (g=0.18) en met een absorptie coëfficiënt (μ_a) van 0.001 mm^{-1}. Deze waarden zijn gebaseerd op verstrooiings-doorsnedes zoals deze met behulp van Mie-theorie zijn berekend waarbij rekening is gehouden met de golflengte van het licht en de brekingsindex van water. Er is een goede overeenkomst gevonden tussen de experimentele weglengte-verdelingen en Monte-Carlo simulaties. De weglengte afhankelijke *diffusion broadening* ofwel Doppler verbreding van het verstrooide licht komt binnen 5% overeen met de waarden die worden berekend met de DWS theorie.

In **hoofdstuk 4** beschrijven we een verbeterde laagcoherente interferometrische methode voor het meten van weglengte-verdelingen en weglengte afhankelijke Doppler verbreding van meervoudig verstrooid licht aan verstrooiende media. De verbetering is gebaseerd op sinus-vormige fase modulatie van de optische weglengte in de referentie-arm bij hoge fase-hoeken ($\Delta\phi$ =2.04 radialen), wat leidt tot interferentie-pieken bij zowel de modulatie frequentie als bij hogere harmonischen. We laten een verbetering zien van de signaal-ruis verhouding met een factor 9 in gemeten weglengte verdelingen bij hoge fase-hoeken vergeleken met een situatie met een lage fase-modulatie-hoek ($\Delta\phi$ =0.51). De verdelingen zijn vergelijkbaar als ze worden genormaliseerd op de maximum waarde van de weglengte-verdeling voor alle fase-modulatie hoeken, maar gladdere curves worden verkregen voor de hogere fase-modulatie

hoeken. Verder kunnen de uit de spectrale verbreding (breedte op halve hoogte) berekende Dopper-verschuivingen van alle pieken worden gemiddeld. Een goede overeenkomst is gevonden tussen de experimentele weglengteverdelingen en Monte-Carlo simulaties. Een verdere toename van de signaal-ruis verhouding kan worden verkregen door de modulatie-frequentie verder te verhogen, omdat bij hogere frequenties het ruis-niveau lager is en het spectrum veel vlakker is dan voor lage frequenties.

In **hoofdstuk 5** laten we weglengte-verdelingen zien van meervoudig verstrooid licht gemeten aan modelsystemen met daarin zowel statische als dynamische verstrooiers. Optische weglengte-verdelingen en weglengte-afhankelijke Dopplerverschuivingen van meervoudig verstrooid licht zijn gemeten aan een model-systeem bestaande uit twee lagen: een bovenste laag met een variërende dikte (0.1-0.9 mm), bestaande uit statische verstrooiers met daaronder een laag met dynamische verstrooiers met dezelfde optische eigenschappen (μ'_s=2.0 mm^{-1}, g=0.85) als de bovenste laag. De optische weglengte-verdeling van de Doppler- en niet-Doppler verschoven fotonen is bepaald door het signaal van het statisch verstrooide licht in een bandbreedte van 50 Hz rond de modulatiefrequentie te scheiden van de totale intensiteit. De fractie Dopplerverschoven fotonen overschrijdt de complementaire fractie niet-Doppler verschoven fotonen voor grote optische weglengte. Voor toenemende dikte van de bovenste statische laag, neemt de fractie niet-Doppler verschoven fotonen toe. Een opmerkelijke waarneming is dat geen Doppler-verbreding van de interferentie-piek wordt waargenomen tot een bepaalde optische weglengte, welke is gerelateerd aan de dikte van de statische laag. Aanname van ballistische paden tussen de fiber uiteindes maakt het mogelijk om de dikte van de statische bovenste laag te bepalen uit de minimum weglengte van het Doppler verschoven licht. Er is een goede overeenkomst gevonden tussen de experimenteel bepaalde dikte van de statische laag en Monte-Carlo simulaties. Een mogelijke toepassing van deze benadering is het bepalen van de diepte van brandwonden en het scheiden van statisch en dynamisch verstrooid licht in het doorbloedingssignaal.

In **hoofdstuk 6** is het gebruik van multimodale graded index fibers voor de detectie in de "coherence gated" interferometer vergeleken met het gebruik van single mode fibers. Om het effect van intermodale dispersie in een multimodale fiber in een laag-coherente interferometer te evalueren, hebben we het verstrooiende medium vervangen door een spiegel en vervolgens hebben we de weglengte van de referentie-arm gevarieerd. Een scherpe interferentie piek werd waargenomen op de modulatie frequentie door interferentie van licht uit de referentie arm met licht gereflecteerd aan de spiegel. Meting van het nulde-orde moment (oppervlak onder de heterodyne piek, overeenkomend met de intensiteit van de fotonen) als functie van de optische weglengte gaf een hoofdpiek met breedte op halve hoogte van 55 μm met daarnaast een aantal zij-pieken op een afstand van 60 en 300 μm ten opzichte van de hoofd-piek. Deze

pieken waren tenminste een factor 3.25 lager dan de hoofdpiek. Met een single-mode fiberoptische interferometer was de breedte op halve hoogte van de hoofdpiek ongeveer 55μm en werden er geen zij-pieken waargenomen. De verslechtering van de weglengteresolutie van 18 μm (zoals verwacht op basis van de coherentie-lengte van de lichtbron) naar 55 μm, waargenomen bij zowel de single-mode als de multi-mode fiberoptische interferometer, is veroorzaakt door niet afgestemde optische dispersie in de referentie-arm en sample-arm van de electro-optische modulator. De resultaten laten zien dat er geen significant effect is van intermodale dispersie op de weglengte resolutie van onze experimentele opstelling. Met een graded index multimodale fiber voor detectie hebben we weglengte-afhankelijke Doppler metingen gedaan aan diffuus verstrooid licht van een waterige suspensie van vetdeeltjes waarbij de vetdeeltjes bewegen door Brownse beweging of door druk-gedreven stroming. Met spectrale analyse van de interferentie piek bij de modulatie frequentie zijn weglengte verdelingen en weglengte afhankelijke Doppler verschuivingen gemeten aan waterige suspensies van 10% Intralipid-20% terwijl dit met gemiddelde snelheden van 0 – 6mm/s stroomde door een kanaal in een blok Delrin. De gemiddelde Dopplerverschuiving, zoals bepaald uit het gewogen eerste-orde moment M_1/M_0 van de interferentie piek, waarbij het centrale deel dat afkomstig is van statisch verstrooid licht niet is meegenomen in de berekening, liet een lineair verband zien met de snelheid in het fysiologische bereik (0 tot 6 mm/s). De weglengte verdeling gemeten in verstrooiende media suggereert dat het effect van intermodale dispersie zwak is en de extra zij-pieken die voortkomen uit de intermodale dispersie zijn niet waargenomen. Multimodale graded-index fibers zorgen dus voor een groot detectie-apertuur met een kleine modale-dispersie in weglengte-afhankelijke dynamische lichtverstrooiing.

In **hoofdstuk 7** laten we eerste resultaten zien van weglengte-afhankelijke Doppler metingen van diffuus verstrooid licht in de huid terwijl er stimuli zoals occlusie en vaso-dilatatie worden aangebracht. De Doppler-verschoven fractie fotonen, welke gerelateerd is aan het bloed volume, en de weglengte-afhankelijke gemiddelde Doppler verschuiving, welke gerelateerd is aan de gemiddelde snelheid van de rode bloedcellen, namen zoals verwacht af gedurende de occlusie. Beide grootheden namen toe wanneer een doorbloedings-stimulerende crème op de huid werd gesmeerd. De weglengte-afhankelijke doorbloedingsveranderingen gemeten met ons systeem in real-time (gemiddeld over een periode van 2.6 s) en de variaties gedurende een occlusie lieten een correlatie zien met de signalen gemeten met een conventionele laser-Doppler perfusie monitor. Metingen zijn uitgevoerd op de dorsale zijde van de onderarm van drie vrijwilligers en daarnaast is de meting uitgevoerd op verschillende locaties voor dezelfde persoon om de inter- en intra-individuele variaties te kunnen vergelijken. Er zijn inter- en intra- individuele variaties in optische weglengtes en weglengte-afhankelijke Doppler verschuivingen

waargenomen. Dit wijst erop dat deze variaties moeten worden meegenomen bij de vergelijking van perfusie-waarden gemeten op vergelijkbare plekken bij verschillende personen en voor metingen op verschillende plekken bij dezelfde persoon.

De weglengte-afhankelijke perfusie metingen zoals we die hier hebben laten zien kunnen worden gebruikt om de beperking van de conventionele laser Doppler perfusie monitor voor klinisch gebruik te vermijden omdat daar de gemeten perfusie afhankelijk is van de weglengte van de fotonen. Dit maakt het mogelijk om de inter- en intra- individuele variaties in perfusie-uitlezing, veroorzaakt door variatie in individuele foton-weglengtes, correct te interpreteren. Deze benadering maakt het ook mogelijk om onderscheid te maken tussen Dopplerverschoven fotonen die een interactie hebben ondergaan met bewegende rode bloedcellen en niet-Doppler verschoven fotonen die zijn verstrooid aan de omringende statische weefsel matrix. Een andere belangrijke eigenschap van deze benadering is dat diepte afhankelijke perfusie-informatie kan worden verkregen. Door de optische weglengte in de referentie-arm groter te maken wordt ervoor gezorgd dat fotonen die dieper in het weefsel zijn geweest interfereren met de referentie-fotonen, zodat er dus een onderscheid kan worden gemaakt tussen oppervlakkige en diepe perfusie in het weefsel.

Doordat de techniek in staat is om niet-invasief weglengte-afhankelijke informatie over dynamische lichtverstrooiing te meten, zijn er potentiële toepassingen in het monitoren van ruimteopgeloste en tijdsopgeloste variaties in optische eigenschappen van verstrooiende media en doorbloeding van weefsel.

Publications

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," Journal of Biomedical Optics, 12 (2), 024020 (2007).

B. Varghese, V. Rajan, T. G. van Leeuwen and W. Steenbergen, "Quantification of Optical Doppler broadening and optical path lengths of multiply scattered light with phase modulated low coherence interferometry," Optics Express, 15 (15), 9157-9165 (2007).

B. Varghese, V. Rajan, T. G. van Leeuwen and W. Steenbergen, "Discrimination between Doppler-shifted and non-shifted light in coherence domain path length resolved measurements of multiply scattered light," Optics Express, 15 (20), 13340-13350 (2007).

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," Optics Communications, (In press).

B. Varghese, V. Rajan, T. G. van Leeuwen and W. Steenbergen, "Evaluation of a multimode fiber optic low coherence interferometer for path length resolved Doppler measurements of diffuse light," Review of Scientific Instruments (In press).

B. Varghese, V. Rajan, T. G. van Leeuwen and W. Steenbergen, "Path length resolved optical Doppler perfusion monitoring," Journal of Biomedical Optics Letters (In review).

B. Varghese, V. Rajan, T. G. van Leeuwen and W. Steenbergen, "In vivo optical path lengths and path length resolved Doppler shifts of multiply scattered light," Microcirculation (In review).

B. Varghese, V. Rajan, T. G. van Leeuwen and W. Steenbergen, "Influence of static scattering matrices on coherence domain path length resolved Doppler measurements of multiply scattered light," Optics Letters (to be submitted).

V. Rajan, B. Varghese, T. G. van Leeuwen, and W. Steenbergen, "Speckles in laser Doppler perfusion imaging," Optics Letters, 31, 468-470 (2006).

V. Rajan, B. Varghese, T. G. van Leeuwen, and W. Steenbergen, "Quantification of spatial intensity correlations and photodetector intensity fluctuations of coherent light reflected from turbid particle suspensions," Physical Review E, 75, 060901-4 (2007).

V. Rajan, B. Varghese, T. G. van Leeuwen, and W. Steenbergen, "Effect of speckles on the depth sensitivity of laser Doppler perfusion imaging," Optics Express 15, 10911-10919 (2007).

V. Rajan, B. Varghese, T. G. van Leeuwen, and W. Steenbergen, "Review of methodological developments in laser Doppler flowmetry," Lasers in Medical Science (In press)

V. Rajan, B. Varghese, T. G. van Leeuwen, and W. Steenbergen, "Influence of tissue optical properties on laser Doppler perfusion imaging, accounting for photon penetration depth and the laser speckle phenomenon," Journal of Biomedical Optics (In review).

V. Rajan, B. Varghese, T. G. van Leeuwen, and W. Steenbergen, "Speckle size and decorrelation time; space-time correlation analysis of coherent light dynamically scattered from turbid media," Optics communications (In review).

Conference proceedings

B. Varghese, V. Rajan, T. G. van Leeuwen and W. Steenbergen, "Quantification of Doppler broadening in path length resolved diffusive light scattering using phase modulated low-coherence interferometry," Biomedical Applications of Light Scattering. Edited by Wax, Adam; Backman, Vadim. Proceedings of the SPIE, Volume 6446, pp. 644603, 2007 (Oral paper presentation).

V. Rajan, B. Varghese, T. G. van Leeuwen, and W. Steenbergen, "Depth sensitivity of laser Doppler perfusion imager: quantification based on experiments and Monte Carlo simulations on static and dynamic scattering phantoms," Optical Diagnostics and Sensing VII. Edited by Coté, Gerard L.; Priezzhev, Alexander V. Proceedings of the SPIE, Volume 6445, pp. 644506, 2007 (Oral paper presentation).

B. Varghese, V. Rajan, T. G. van Leeuwen and W. Steenbergen, "Path length resolved Doppler measurements of multiple scattered photons in turbid media for various absorptions using phase modulated low-coherence interferometry," Biophotonics and New Therapy Frontiers. Edited by Grzymala, Romualda.

Proceedings of the SPIE, Volume 6191, pp. 322-329, 2006 (Oral paper presentation).

V. Rajan, B. Varghese, T. G. van Leeuwen, and W. Steenbergen, "Role of speckles in laser Doppler perfusion imaging: an investigation on particle suspensions," Biophotonics and New Therapy Frontiers. Edited by Grzymala, Romualda. Proceedings of the SPIE, Volume 6191, pp. 281-288, 2006 (Oral paper presentation).

B. Varghese, T. G. van Leeuwen and W. Steenbergen, "Optical probing of turbid media using a path length resolved low coherence Mach Zehnder interferometer", Proceedings of 2nd International Graduate summer school, Biophotonics '05, Ven, Sweden, 2005 (Poster presentation).

B. Varghese, T. G. van Leeuwen and W. Steenbergen, "Optical probing of living tissue structure using low coherence interferometry," Proceedings of Dutch Annual Conference on Biomedical Engineering, Papendal, Netherlands, 176-177, 2004 (Poster presentation).

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Babu Varghese

Curriculum Vitae

Babu Varghese was born in Muringoor, Kerala, India on May 13, 1974. He obtained Bachelor of Science (B.Sc.) and Master of Science (M.Sc.) degrees in Physics in 1994 and 1997 respectively from the University of Calicut, Kerala. In 2000 he completed the Master of Technology (M.Tech) study in Optoelectronics and Laser Technology from the International School of Photonics, Cochin University, Kerala. As a part of his M.Tech course he carried out a research project on "Laser beam deflection technique for the study of stratified fluids using lateral effect position sensitive photodetectors" at the Naval Physical and Oceanographic Laboratory, Defence Research and Development Organisation, Kochi, India. After his M.Tech he worked as project Engineer (2001-2002) at Deposition Sciences Inc, CA, USA and Lecturer in Physics (2002-2003) at Sahrdaya College of Engineering and Technology, Kerala, India.

From December 2003 until October 2007 he performed research towards his Ph.D thesis at the Biophysical Engineering Group (BPE) of the University of Twente, The Netherlands under the supervision of Prof. Dr. Ton G. van Leeuwen and Dr. Wiendelt Steenbergen. During his Ph.D research, he developed a new bio-optical technique "Path length resolved optical Doppler perfusion monitoring," the results of this research are presented in this thesis. In October 2007, he joined Philips Research in Eindhoven, The Netherlands as Senior Scientist in the department of Care and Health Applications.