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Improved calibration procedure for laser Doppler perfusion monitors

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ABSTRACT

Commercial laser Doppler perfusion monitors are calibrated using the perfusion value, i.e. the first order moment of the Doppler power spectrum, from a measurement in a standardized microsphere colloidal suspension under Brownian motion. The calibration perfusion value depends on several parameters of the suspension that are difficult to keep constant with adequate accuracy, such as the concentration, temperature and the microsphere size distribution. The calibration procedure itself may therefore introduce significant errors in the measured values.

An altered calibration procedure, where the zero order moment is used is described and demonstrated in this paper. Since the above mentioned parameters only affect the frequency content of the Doppler power spectrum and not the total power, the zero order moment will be independent of those parameters. It is shown that the variation in the calibration value, as given by measurements on different scattering liquids with a wide range of scattering properties and temperatures, is only a few percent using the proposed method. For the conventional calibration procedure, this variation corresponds to an error introduced by merely a 1°C variation in the reference liquid temperature. The proposed calibration method also enables absolute level comparisons between measured and simulated Doppler power spectra.

Keywords: Laser Doppler flowmetry, calibration, Brownian motion, optical phantoms

1. INTRODUCTION

The calibration procedure of laser Doppler perfusion monitors (LDPM:s) may aim at achieving two different objectives – (1) determining a reference perfusion value so that measurements using different instruments can be compared in a relative manner and (2) to relate the instrument output to a specific blood flow. Attempts at the latter have been carried out [1-3], but as the calibration phantoms used in those studies still fail to mimic microcirculatory blood flow they are not used in any commercial system today. Instead LDPM:s from the two leading manufacturers in the world (Perimed AB and Moor Instruments Ltd.) are calibrated against a standardized colloidal suspension of polystyrene microspheres, i.e. motility standard. Due to Brownian motion, the Doppler power spectrum measured at a certain temperature is repeatable when measuring on this standard. Thus, the perfusion value, i.e. the first order moment of the Doppler power spectrum, can be used to compare perfusion values measured *in vivo* using different instruments. [4, 5]

Unfortunately, this calibration procedure is sensitive to the exact properties of the motility standard which are difficult to keep constant and stable over long time with adequate accuracy. For example, deviation in the temperature, sedimentation effects or aggregation of the microspheres, all have a significant effect on the calibration value. The calibration value can also be affected by small vibrations of the measurement probe or the beaker containing the suspension.

As an alternative, the zero order moment of the Doppler power spectrum above 0 Hz, which is conventionally called the CMBC (concentration of moving blood cells) can be used for the calibration. In contrary to the perfusion value, this measure is only sensitive to the degree of Doppler shifted photons. For a suspension containing only moving scatterers all backscattered photons will be Doppler shifted. Hence, any suspension under Brownian motion should produce a constant CMBC regardless of the properties of the liquid. This would thus eliminate the need for the LDPM users to replace old motility standard. A similar approach has also been used by Serov *et al.* aiming at estimating the absolute fraction of Doppler shifted light. [9] Experienced LDPM users know that the CMBC signal is often unstable, but due to improved signal processing and signal averaging, this is not a problem in the presented procedure.

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The aim of this paper was to propose this improved calibration procedure and to prove its stability. We have already used the procedure in a number of publications [6-8], since it also offers a reliable way to compare the absolute levels of measured and simulated Doppler power spectra, which in turn enables model based signal processing of LDPM measurements. However, the accuracy and stability of the procedure has not previously been fully evaluated.

2. THEORY

The Doppler power spectrum is in principle constituted by the cumulative Doppler shift that each photon undergoes. A single Doppler shift is given by

$$f_{\mathrm{D}} = -2\frac{v}{\lambda}\sin\frac{\theta}{2}\cos\varphi \ , \tag{1}$$

where v is the speed of the scattering particle, λ is the wavelength in the medium, θ is the scattering angle and φ depends on the angle between the particle and the vector describing the scattering. [10] Since λ is constant and $\cos\varphi$ can be assumed to be uniformly distributed between -1 and 1, it can be realized that the Doppler power spectrum from single shifted light only depends on the speed of the scattering particles and their scattering phase function. In practice, most of the Doppler shifted photons are shifted more than once, causing a broadening of the Doppler power spectrum. Note however that single Doppler shifts can be both positive and negative.

Examples of Doppler power spectra when changing the concentration, speed, and scattering phase function of scattering particles in a medium with both static and moving scattering particles are shown in Figure 1. The spectra originate from Monte Carlo based calculations [7] from a model with blood homogeneously distributed in a static scattering non-absorbing medium. The scattering coefficient of the static medium was $\mu_s = 8.0$ mm^{-1} and the anisotropy factor $g = 0.70$. The scattering coefficient of the blood was set to $\mu_s = 222$ mm^{-1}, the anisotropy factor to $g = 0.991$ and the absorption coefficient to $\mu_a = 0.43$ mm^{-1}. The concentration was 1% in all calculations except in the left figure. The speed was 1 except in the middle figure, and the anisotropy factor equaled 0.991 except in the right figure. For the blood concentration of 0.1%, 18% of the detected photons were Doppler shifted and 66% of these were shifted more than once. Corresponding figures were 74%/86% and 99.9%/99.8% for the concentrations of 1% and 10%, respectively. The anisotropy factor g for motility and milk were 0.53 and 0.81, respectively (see Table 1).

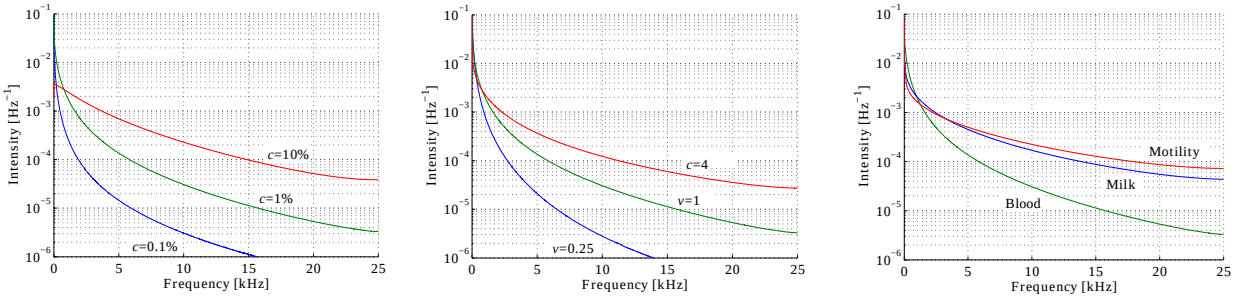

Figure 1 Examples of Doppler power spectra when the concentration (left), speed (middle), and anisotropy factor (right) of the scattering particles are changed. Note that these spectra originate from models with both static and moving scatterers.

Under the assumption that most of the detected Doppler shifted light is shifted only once, it can be realized that the average frequency of the Doppler power spectrum is proportional to the speed, and that the total power for frequencies above 0 Hz is proportional to the concentration of moving scattering particles. Therefore, the CMBC is normally estimated as the zero order moment ($m = 0$) of the spectrum, and the perfusion as the first order moment ($m = 1$). In the digital case with N frequency bins above 0 Hz, this is expressed as

$$\sum_{i=1}^{N} f(i)^m \, p(f(i)) \ . \tag{2}$$

Multiple shifted light introduces a non-linearity to the concentration in both of these values.

3. MATERIAL AND METHODS

A modified two-channel Periflux 5000 instrument (Perimed AB, Järfälla-Stockholm, Sweden), operating at 780 nm, was used throughout this study. The analog first order 7 Hz high pass filter of the system was removed and the analog dc and ac-signals from the two channels were sampled at 50 kHz using a 12-bit AD-converter (DAQpad-6070E, National Instruments Corporation, Austin, Texas). All measurement were performed using a custom made probe with two different distances between the light emitting and light collecting fibers, 0.39 and 1.2 mm, connected to individual measurement channels. These fibers/channels are from now on referred to as the short and long fiber separations, respectively. The optical fibers in the probe had a core diameter of 0.125 mm and NA = 0.37.

Noise spectra were recorded at various dc-levels using an internal LED illuminating the detector of each channel. These noise spectra were subtracted from each measured spectrum at the corresponding dc-level to eliminate the effect of the noise. The noise is constituted by shot noise and thermal noise that can be assumed to be white within the frequency interval of interest (0 to 50 kHz). The recorded noise spectra were also used to compensate for the anti-aliasing filter that was added to the Periflux system before the AD-converter, which had a cut-off frequency of 20 kHz. All measured spectra, except the noise spectra, were also dc^2 normalized. Mathematically, the basic signal processing of noise reduction, frequency characteristic compensation and dc^2 normalization of the recorded spectra is expressed as

$$p(f) = \frac{p_{\text{raw}}(f) - p_{\text{noise}}(f, dc)}{p_{\text{noise,norm}}(f, dc) dc^2} \ , \tag{3}$$

where $p_{\text{raw}}(f)$ is the recorded raw spectrum, $p_{\text{noise}}(f,dc)$ is the noise spectrum at the corresponding dc level, and $p_{\text{noise,norm}}(f,dc)$ is the noise spectrum normalized with its mean power between 1 and 3 kHz.

To demonstrate the effect of the phase function, two different scattering liquids were used as calibration agents in the study; motility standard (Periflux 1001, Perimed AB) which is a suspension of latex spheres with an average radius of 160 nm, and homogenized ultra-high temperature (UHT) treated milk (Arla Foods, Sweden), with a fat content of 1.5% and protein content of 3.4%. To demonstrate the effect of different degrees of multiple shifts, measurements in milk were performed in three different concentrations: undiluted (1:1), diluted 1:2, and diluted 1:4. De-ionized water was used to dilute the milk. The scattering coefficient μ_s was measured using a collimated transmission setup [11], with results as shown in Table 1, together with the anisotropy factor g and reduced scattering coefficient $\mu_s' = \mu_s(1 - g)$.

Table 1 Scattering properties at 780 nm for the used liquids.

	μ_s [mm^{-1}]	g [-]	μ_s' [mm^{-1}]
Motility standard	1.6	0.53*	0.85
Milk 1:1	3.7	0.81**	0.70
Milk 1:2	2.2	0.81**	0.42
Milk 1:4	1.1	0.81**	0.21

*Calculated using MIE-theory. **Based on old measurements on same type of milk [12].

Three repeated measurements with the Periflux 5000 system, 20 s long, were performed on each of the four calibration liquids. The probe was fixated in a stand and submerged about 5 mm into the liquid which was kept in a 4 cm diameter beaker of white plastic. At least 35 ml of the liquids was used (corresponding to a depth of about 3 cm). A thermistor connected to a Periflux 5020 temperature unit was attached to the measurement probe using adhesive tape in order to record the temperature of the liquid. The temperature of the liquids in these repeated measurements varied between 20.5 and 21.3 ºC. For the motility standard, three repeated measurements were also recorded when the probe was hand held in order to see the effect of the small movements introduced.

The speed of the Brownian motion is directly correlated to the temperature of the liquid. Therefore, to further explore the effect the speed has on the CMBC and perfusion values, a one and a half hour measurement was performed on the motility standard were the temperature was varied between 3 and 51 ºC. The temperature was varied by placing the beaker in hot water which was slowly cooled using colder water and ice. The CMBC and perfusion were calculated from spectra averaged in 5 s intervals. Measurement points which peaked in the perfusion value, which were obviously caused by movements when adding ice etc., were removed by visual inspection.

4. RESULTS

The average, min and max CMBC and perfusion values from the three repeated measurements on motility standard and milk are shown in Table 2 and 3 for the short and long fiber separations, respectively. The values are normalized with the mean values for motility standard (probe fixated in a stand).

Table 2 Average (min-max) perfusion and CMBC values from three measurements in each of the scattering liquids for the short fiber separation (0.39 mm). Values are normalized to the mean value of the measurements in motility.

	Perfusion	CMBC
Motility	1.000 (0.965-1.049)	1.000 (0.999-1.001)
Hand held motility	1.365 (1.268-1.464)	1.017 (1.012-1.020)
Milk 1:1	0.818 (0.813-0.823)	1.005 (0.993-1.016)
Milk 1:2	0.920 (0.878-0.976)	0.999 (0.992-1.005)
Milk 1:4	0.823 (0.810-0.839)	0.989 (0.984-0.992)

Table 3 Average (min-max) perfusion and CMBC values from three measurements in each of the scattering liquids for the long fiber separation (1.2 mm). Values are normalized to the mean value of the measurements in motility.

	Perfusion	CMBC
Motility	1.000 (0.971-1.036)	1.000 (0.998-1.002)
Hand held motility	1.250 (1.190-1.311)	1.013 (1.010-1.014)
Milk 1:1	0.946 (0.940-0.958)	0.993 (0.988-0.999)
Milk 1:2	0.936 (0.882-1.011)	1.004 (1.003-1.007)
Milk 1:4	0.744 (0.729-0.755)	1.017 (1.000-1.032)

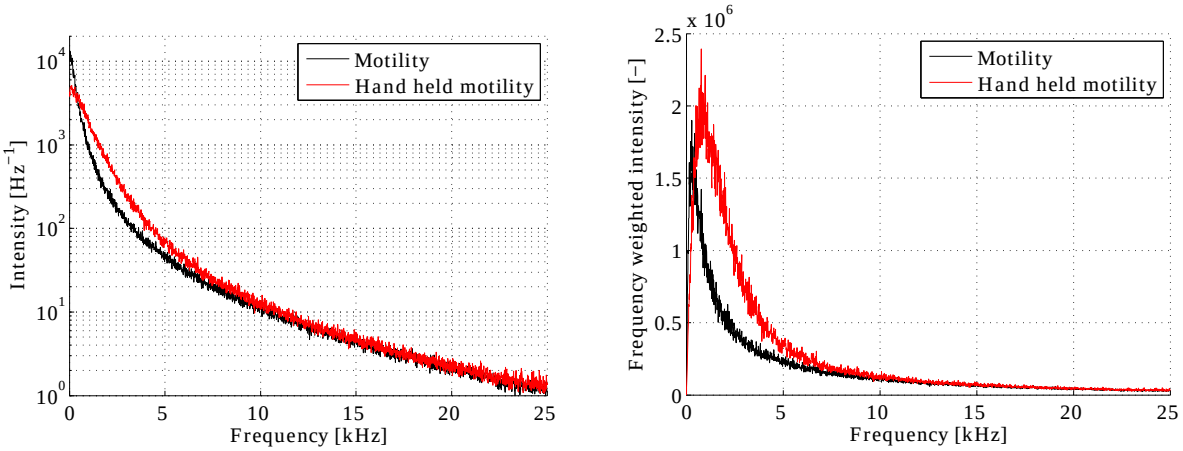

Figure 2 Doppler power spectra of motility standard both when the measurement probe was fixated and when it was hand held. To the left, with logarithmic scale on the y-axis, the normal spectra are shown, whereas frequency weighted spectra with a linear y-axis are shown to the right.

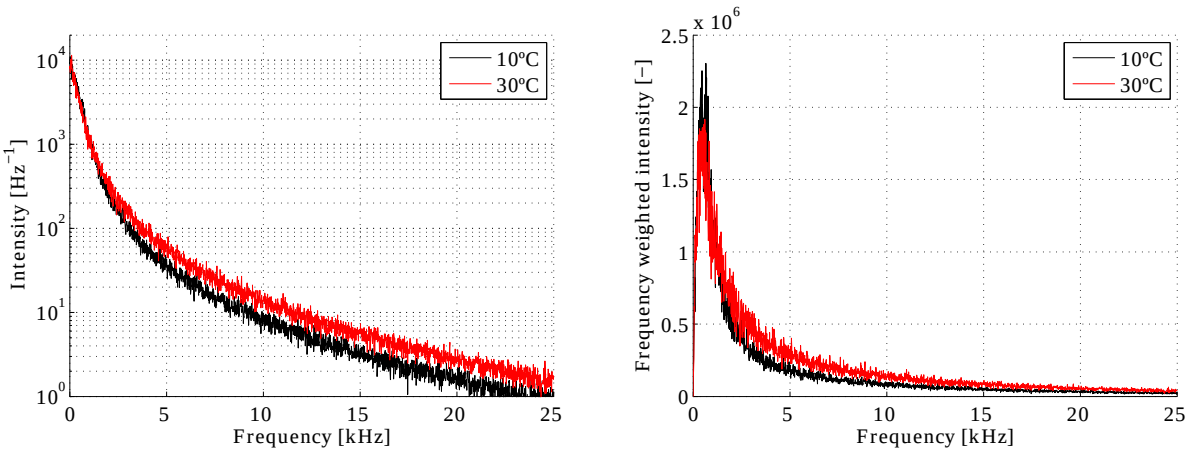

Figure 3 Doppler power spectra of motility standard at 10 and 30ºC. To the left, with logarithmic scale on the y-axis, the normal spectra are shown, whereas frequency weighted spectra with a linear y-axis are shown to the right.

Examples of the Doppler power spectra for the first of the three repeated measurements in motility and hand held motility at the short fiber separation are shown in Figure 2. Both the normal spectra and the frequency weighted spectra are shown, for easier comparison to the CMBC and perfusion values, respectively. The perfusion value of the hand held motility was 52% higher than for the non hand held, whereas the CMBC differed only 1.7%. Corresponding spectra measured at a temperature of 10 and 30ºC are shown in Figure 3. In these measurements, the perfusion values differed 27% whereas the CMBC differed 1.4%.

The relationship between the CMBC and perfusion values is the average frequency of the Doppler power spectrum. For motility (probe fixated in a stand) the average frequency was 0.90 kHz for the short fiber separation and 1.5 kHz for the long. The average frequency for the two fiber separations was unchanged when the light source was changed to another laser diode (same wavelength, different type) and when the detector channels were switched (data not presented).

In Figure 4, the CMBC and perfusion values from the measurement with varied temperature are plotted versus the temperature. The values were gathered into 2ºC wide temperature bins, and the average value of each bin is plotted ± SD. The values were normalized to the average values at 22ºC. The perfusion change per ºC was 2.0% and 1.8% relative to the perfusion at 22 ºC for the short and long fiber separations, respectively (data fitted between 10 and 30ºC).

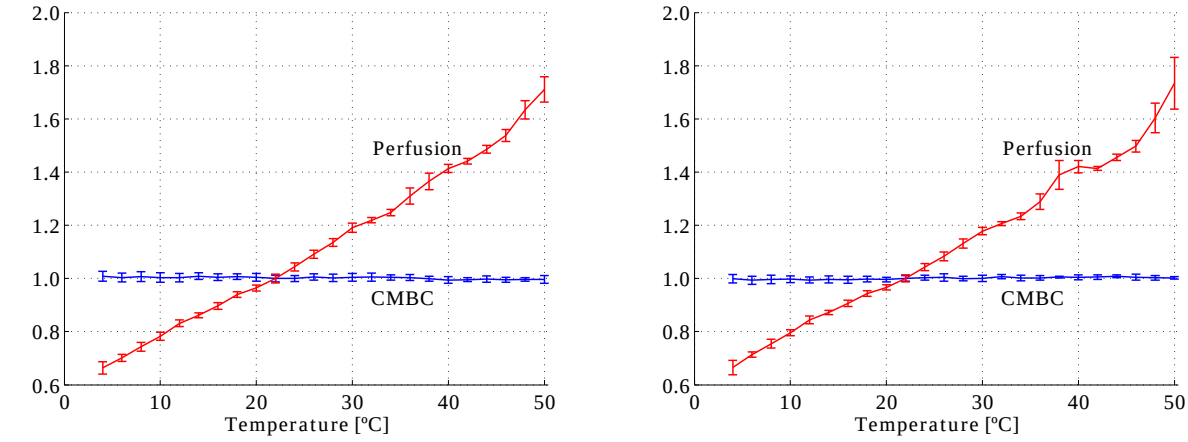

Figure 4 Calculated perfusion and CMBC values as a function of liquid temperature for short (left panel) and long (right panel) fiber separations, respectively.

5. DISCUSSION

We have experienced both sedimentation and aggregation of motility standard in our previous work with LDF instruments. The effect of sedimentation is easily reverted by shaking the beaker, but aggregation may both be difficult to observe and can not easily be reverted. The increased perfusion values in motility when the probe was hand held originate from the small movements from the hand, and the increase was more than 20% although the hand was tried to be kept steady. Placing the beaker on a surface with small vibrations, e.g. caused by a computer fan, can possibly introduce similar increases in the perfusion. Furthermore, it is not at all certain that the temperature of the motility standard is always at 22ºC when the instrument is calibrated, and a 10% variation merely due to different temperatures (± 2.5ºC) in various measurement settings is not unlikely. Added together, these effects lead to significant calibration divergences using the conventional calibration procedure. The proposed improved calibration procedure effectively eliminates these faults. Tables 2 and 3 and Figure 4 clearly show the superior stability of the CMBC compared to the perfusion value when changing liquid properties as well as when introducing movements. Figures 2 and 3 also demonstrate that the CMBC is stable when spectra differ both at low and high frequencies.

For obvious reasons, the procedure fails when a significant part of the Doppler power spectrum falls above the cut-off frequency of the low pass filter of the system or when the detected light levels fall out of the range of the system. This sets some limitations to the liquids used. Since the CMBC is relatively more sensitive to a narrow interval of low frequencies of the Doppler power spectrum than is the case of the perfusion value, the requirements of the system and the signal processing is higher than for the conventional calibration procedure and a somewhat longer signal averaging may be required for stable results. In the system we used, the first order high pass filter with cut-off frequency of 7 Hz was also removed which improved the stability of the CMBC signal considerably. Unfortunately, the advantages of the proposed procedure are reduced when it comes to laser Doppler perfusion imaging since the speckle size is affected by the optical properties of the calibration liquid which also affects the zero order moment of the Doppler power spectrum. [13]

It may be surprising that the perfusion value does not differ more between the various milk concentrations considering the large differences in the concentration and scattering coefficient. It is logical to assume that the detected light is multiple shifted to a higher degree when increasing the scattering coefficient, causing a broadening of the Doppler power spectrum and increased perfusion value. This is contradictory to the fact that the perfusion value is lower for the undiluted milk than for the milk diluted 1:2 for the short fiber separation (Table 2). However, the measurement volume is also affected by the optical properties, and it is generally decreased with an increased scattering, especially for small source detector separations. [14] A smaller measurement volume decreases the degree of multiple shifts which is one explanation for this behavior. For high concentrations, the size distribution of the scattering particles may also change, and the optical properties are further affected when the scattering particles are close to each other. [15] This is observed in Table 1 where the scattering coefficient is not twice as high for undiluted milk as for milk diluted 1:2, as expected. The measurement volume is also affected by the scattering phase function. Therefore, a change in the scattering phase function affects booth the degree of multiple Doppler shifts and the size of each single Doppler shift.

For “backward compatibility” reasons, it is feasible to be able to convert a calibrated CMBC value into the conventional calibrated perfusion value in motility standard at 22ºC. For a specific probe setup and operating wavelength, the conversion factor is simply the average frequency of the Doppler power spectrum, which may be measured under controlled conditions by the manufacturer.

The proposed improved calibration procedure does not solve the problem of relating the output from the LDPM:s to any specific blood flow. However, the procedure performs an absolute calibration of the total power of the Doppler power spectrum where all detected light is Doppler shifted. This absolute calibration can be utilized to compare measured and simulated spectra in an absolute manner which opens up for new model based signal analyses, which in turn can give absolute flow levels in physiologically relevant units. [7]

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REFERENCES

[1] Liebert, A., Leahy, M., and Maniewski, R., "A calibration standard for laser-Doppler perfusion measurements," Review of Scientific Instruments 66(11), 5169-5173 (1995)
[2] Steenbergen, W. and Mul, F.F.M.d., "New optical tissue phantom and its use for studying laser Doppler blood flowmetry," Proc. SPIE 3196, 12-23 (1998).
[3] Steenbergen, W. and Mul, F.F.M.d., "Description of an improved laser Doppler calibrator," Proc. SPIE 3599, 68-75 (1999).
[4] Leahy, M.J., de Mul, F.F., Nilsson, G.E., and Maniewski, R., "Principles and practice of the laser-Doppler perfusion technique," Technology and Health Care 7(2), 143-62 (1999)
[5] Nilsson, G.E., Salerud, E.G., Strömberg, N.O.T., and Wårdell, K., "Laser Doppler Perfusion Monitoring and Imaging", in [Biomedical photonics handbook], Vo-Dinh, T., Editor, CRC Press: Boca Raton, FL. 15-1 - 15-24. (2003)
[6] Fredriksson, I., Larsson, M., and Strömberg, T., "Optical microcirculatory skin model: assessed by Monte Carlo simulations paired with in vivo laser Doppler flowmetry," Journal of Biomedical Optics 13(1), 014015 (2008)
[7] Fredriksson, I., Larsson, M., and Strömberg, T., "Model-based quantitative laser Doppler flowmetry in skin," Journal of Biomedical Optics 15(5), 057002 (2010)
[8] Fredriksson, I., Larsson, M., Nyström, F.H., Länne, T., Östgren, C.J., and Strömberg, T., "Reduced Arteriovenous Shunting Capacity After Local Heating and Redistribution of Baseline Skin Blood Flow in Type 2 Diabetes Assessed With Velocity-Resolved Quantitative Laser Doppler Flowmetry," Diabetes 59(7), 1578-1584 (2010)
[9] Serov, A.N., Steenbergen, W., and Mul, F.F.M.d., "Method for estimation of the fraction of Doppler-shifted photons in light scattered by a mixture of moving and stationary scatterers," Proc. SPIE 4001, 178-189 (2000).
[10] Fredriksson, I., [Quantitative laser doppler flowmetry], PhD Thesis, Department of Biomedical Engineering, Linköpings universitet. Linköping. http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-19947, 27-40 (2009)
[11] Wang, L.H. and Jacques, S.L., "Error Estimation of Measuring Total Interaction Coefficients of Turbid Media Using Collimated Light Transmission," Physics in Medicine and Biology 39(12), 2349-2354 (1994)
[12] Lindbergh, T., Fredriksson, I., Larsson, M., and Strömberg, T., "Spectral determination of a two-parametric phase function for polydispersive scattering liquids," Optics Express 17(3), 1610-1621 (2009)
[13] Rajan, V., Varghese, B., van Leeuwen, T.G., and Steenbergen, W., "Speckles in laser Doppler perfusion imaging," Optics Letters 31(4), 468-470 (2006)
[14] Fredriksson, I., Larsson, M., and Strömberg, T., "Measurement depth and volume in laser Doppler flowmetry," Microvascular Research 78(1), 4-13 (2009)
[15] Zaccanti, G., Del Bianco, S., and Martelli, F., "Measurements of optical properties of high-density media," Applied Optics 42(19), 4023-4030 (2003)