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INFLUENCE OF OPTICAL PROPERTIES AND FIBER SEPARATION ON LASER DOPPLER FLOWMETRY

Marcus Larsson\textsuperscript{a,c}, Wiendelt Steenbergen\textsuperscript{b}, Tomas Strömberg\textsuperscript{a}

Abstract

Microcirculatory blood flow can be measured using a laser Doppler flowmetry (LDF) probe. However, the readings are affected by tissue optical properties (absorption and scattering coefficient; $\mu_a$ and $\mu_s$) and probe geometry. In this study the influence of optical properties ($\mu_a \in [0.053, 0.23] \text{ mm}^{-1}; \mu_s \in [14.7, 45.7] \text{ mm}^{-1}$) on LDF perfusion and sampling depth were evaluated for different fiber separations. In-vitro measurements were made on a sophisticated tissue phantom with known optical properties, mimicking blood flow at different depths. Monte Carlo simulations were carried out to extend the geometry of the tissue phantom.

A good correlation between measured and simulated data was found. The simulations showed that, for a fixed flow at a discrete depth, the influence of $\mu_s$ or $\mu_a$ on the LDF perfusion increased with increasing flow depth and decreased with increasing fiber separation. For a homogeneous flow distribution, however, the perfusion varied 40% due to the variations in optical properties, almost independent of fiber separation (0.23-1.61 mm). Therefore, the effect in real tissue is likely to vary due to the unknown heterogeneous blood flow distribution. Further, LDF sampling depth increased with decreasing $\mu_s$ or $\mu_a$ and increasing fiber separation. For a fiber separation of 0.46 mm, the e-1 sampling depth ranged from 0.21-0.39 mm.

Keywords: Doppler effect, fiber optics, laser Doppler flowmetry, Monte Carlo simulations, optical properties, sampling depth.

Introduction

Skin perfusion measurements using the laser Doppler technique depend on how the light interacts with moving blood cells and static tissue. The measurement situation is complex due to the heterogeneous vascular structure. This structure includes both superficial capillaries with relatively low velocity of the blood cells, and larger vessels, deeper in the skin, with higher blood velocities. Therefore, the probability for light interaction with moving blood cells is not only affected by the distribution of blood cells in tissue but also the penetration depth of the detected photons, i.e. sampling depth.\textsuperscript{1} Recently Liebert et al. have experimentally shown, using a layered flow model and a multi channel LDF probe, that the sampling depth increases with increasing separation between emitting and receiving fiber.\textsuperscript{2} They suggest that such a system could be used to discriminate between superficial and deeper lying blood vessels. Similar results regarding the influence of probe geometry on LDF sampling depth was obtained by Johansson et al. during studies of
isolated segments of feline small intestine. The sampling depth has also been studied theoretically using either a Monte Carlo simulation model or a lattice random-walk model. The latter two reports did not only show that the sampling depth increases with fiber separation but also that the it is affected by the optical properties of the tissue. In addition, Weiss et al. shows that the sampling depth decreases with the absorption coefficient. However, their results are restricted to fiber separations larger than 1 mm, a range were LDF is rarely used. It has been observed that LDF perfusion varies both intra and interindividually. The origin of these variations are rather complex and might, as described above, not only depend on the concentration of moving blood cells, blood velocity and vascular structure, but also on the tissue optical properties. To our knowledge, no systematic study on the influence of tissue optical properties, flow depth and probe geometry on LDF perfusion, has previously been presented.

In the present study, we show how LDF perfusion is affected by flow depth, fiber separation and optical properties relevant to skin. A sophisticated tissue phantom, with known geometry and optical properties, and a Monte Carlo model, mimicking the tissue phantom, has been used. Both the tissue phantom and the simulation model consisted of a number of static layers, with different optical properties, separated by moving layers. Thereby a range of different discrete flow depths and optical properties were possible to evaluate. A good agreement between measurements and simulations was found within the geometrical accuracy of the tissue phantom. Since the geometry of a simulation model is easier to control than a physical phantom, the main conclusions of the study were based on a refined and extended simulation model. We also show how changes in the optical properties of a homogeneous flow distribution model affect the LDF perfusion, using Monte Carlo simulations. Further, this paper quantifies and describes how the optical properties and the fiber separation affect the e LDF sampling depth.

Methods & Apparatus

Data Acquisition

A digital multi-channel LDF probe system (Figure 1) was used to estimate perfusion generated by a tissue phantom. The LDF system consisted of a 632.8 nm He-Ne laser, connected to the LDF probe via one emitting fiber. Back-scattered light was guided to a detector system via seven receiving fibers arranged in a row. Multi-mode step index fibers (NA=0.37) with a diameter of 230 µm (including 30 µm of cladding) were used. The distances between the center point of the emitting fiber and the center point of the receiving fiber (fiber separation) was 230n µm, n=1,2,3,...,7.

The back-scattered light from each of the seven receiving fibers was detected by two photodetectors. By adding the two photocurrents the light intensity (dc-signal) of the LDF signal was detected. The time varying part (ac-signal) of the LDF signal was identified by subtracting the photocurrents, amplifying and bandpass filtering with a 3:rd order Butterworth filter between 20 Hz and 12.5 kHz. By using a differential detector approach, the common mode noise was reduced. Due to a low SNR for the ac-signals of fiber 6 and fiber 7, these fibers were not used during the measurements in this study.

The filtered ac- and dc-signals were sampled at 50 kHz with a 12-bit data acquisition card (MIO-AT-16-10E, National Instruments Corporation) using the range ±10 V. All data acquisition software was developed using LabVIEW 5.0 (National Instruments Corporation).
with a diameter of 1 µm, served as photon scatterers. In addition, various non-scattering dyes (optical absorbers) were added to the static layers. The anisotropy ($\langle \cos \theta \rangle$, $\theta =$ scattering angle) of the discs and the layers were 0.8-0.83. Scattering coefficient $\mu_s$ and absorption coefficient $\mu_a$ of the tissue phantom, for a wavelength of 633 nm, are shown in Table 1. The scattering coefficient and the absorption coefficient were determined by transmission measurements in a Shimadzu UV-2101PC photospectrometer. The scattering coefficient was determined by measuring the collimated transmission through different numbers of reference samples without dyes, hence with zero absorption. An exponential decrease in the collimated light transmission with total sample thickness was found. Hence, $\mu_s$ could be determined by an exponential fit. A similar procedure, with reference samples that only contained dyes, i.e. with zero scattering, was carried out for determining $\mu_a$. The anisotropy was determined for a wavelength of 636 nm by measuring the angular dependence of the transmission, i.e. the phase function, through

**Tissue Phantom**

The tissue phantom (Figure 2) consisted of a number of parallel rotatable discs, with a thickness of 20-22 µm. Each of these discs was separated from each other by a static layer (approximately 95 µm of thickness), containing four pieces of static structure with different optical properties. These pieces were located at the same radial distance from the centre of the tissue phantom, with an offset of 90°. All together, the static layers were arranged in a way that resulted in four different stacks of static structures, each with the same optical properties. This assembly was placed in a container filled with index matching fluid (Leica index matching oil). The container was covered by a plate provided with four fibre-optic faceplates (windows) through which the laser Doppler probes had optical access to the tissue phantoms. These windows ($w_i - w_n$) provided a mechanical barrier, while not affecting the spatial distribution of both the incoming laser beam and the light escaping the phantom tissue. All static layers belonging to the same window had the same optical properties. The rotating discs and the static layers were made out of a mixture of polyvinyl alcohol and hollow polystyrene microspheres. These microspheres, with a diameter of 1 µm, served as photon scatterers. In addition, various non-scattering dyes (optical absorbers) were added to the static layers. The anisotropy ($\langle \cos \theta \rangle$, $\theta =$ scattering angle) of the discs and the layers were 0.8-0.83. Scattering coefficient $\mu_s$ and absorption coefficient $\mu_a$ of the tissue phantom, for a wavelength of 633 nm, are shown in Table 1. The scattering coefficient and the absorption coefficient were determined by transmission measurements in a Shimadzu UV-2101PC photospectrometer. The scattering coefficient was determined by measuring the collimated transmission through different numbers of reference samples without dyes, hence with zero absorption. An exponential decrease in the collimated light transmission with total sample thickness was found. Hence, $\mu_s$ could be determined by an exponential fit. A similar procedure, with reference samples that only contained dyes, i.e. with zero scattering, was carried out for determining $\mu_a$. The anisotropy was determined for a wavelength of 636 nm by measuring the angular dependence of the transmission, i.e. the phase function, through

![Figure 1. The LDF system, including a tissue phantom that simulates flow and a custom made LDF probe with different emitting - receiving fiber separations. The detected light intensity in each receiving fiber was sampled by a data acquisition system (DAQ) in terms of the time varying signal ($ac$) and the light intensity signal ($dc$).](image_url)
thin samples with zero absorption. A sufficiently low scattering level was used to ensure single scattering. The applied method of measuring the scattering phase function has previously been described by Bolt et al. All reference samples where produced from the same batches of the raw materials (polyvinyl alcohol solution, particles and dyes) as the layers and discs of the tissue phantom. Also, the same index matching fluid was used as in the tester. A more thorough description of the optical tissue phantom used in this study has been presented by Steenbergen and De Mul.

![Cross section of the tissue phantom](image)

**Figure 2.** Cross section of the tissue phantom, illustrating two of the four stacks of static structure separated by a number of rotatable discs. All layers are separated by index matching oil. The magnification shows the arrangement of the LDF probe, the fiberoptic faceplate (window), rotatable structures and static layers.

**Table 1.** Optical properties of the static windows and the rotating discs for the tissue phantom and the simulation models.

<table>
<thead>
<tr>
<th></th>
<th>window 1</th>
<th>window 2</th>
<th>window 3</th>
<th>window 4</th>
<th>rotating discs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scattering coefficient $\mu_s$ [mm$^{-1}$]</td>
<td>14.66</td>
<td>44.85</td>
<td>14.80</td>
<td>45.55</td>
<td>7.4±0.1</td>
</tr>
<tr>
<td>Absorption coefficient $\mu_a$ [mm$^{-1}$]</td>
<td>0.212</td>
<td>0.226</td>
<td>0.0405</td>
<td>0.0532</td>
<td>~ 0</td>
</tr>
</tbody>
</table>

Measurements were made using single moving discs at 4 different depths and 11 different velocities, equally distributed in the range 0 to 3.6 mm/s. The disc depth was not the same for window 4 as for window 1 and window 2, where one extra static layer was present between disc 2 and disc 3. All disc depths (distance between phantom surface and center of rotating disc) that were used are shown in Table 2. Due to problems with air bubbles within window 3, LDF measurements were made only on window 1 ($w_1$), window 2 ($w_2$) and window 4 ($w_4$).
Table 2. Average disc depth of rotating discs (and thickness) in µm for the tissue phantom.

<table>
<thead>
<tr>
<th></th>
<th>disc 2</th>
<th>disc 4</th>
<th>disc 6</th>
<th>disc 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>window 1 and window 2 [µm]</td>
<td>190 (21)</td>
<td>421 (22)</td>
<td>653 (22)</td>
<td>885 (22)</td>
</tr>
<tr>
<td>window 4</td>
<td>190 (21)</td>
<td>516 (22)</td>
<td>748 (22)</td>
<td>980 (22)</td>
</tr>
</tbody>
</table>

Signal Processing

According to laser-Doppler theory, measured perfusion, \( Perf_M \), can be estimated as:

\[
Perf_M = \frac{\sum \omega_d \cdot P_M(\omega_d)}{dC_M^2}
\]

(1)

\( P_M(\omega_d) \): Measured Doppler power spectrum

\( \omega_d \): Angular frequency of Doppler shift (\( \omega_d : 0 \rightarrow 12500 \text{ Hz} \))

\( DC_M \): Measured total light intensity

The Doppler spectrum was calculated as a mean over 50 power spectra, where each power spectrum was estimated from 4096 samples of the corresponding ac-signal. dc-values were calculated as a mean over 50 200 samples. Calibration of all dc values were carried out by normalizing each channel with its relative amplification factor derived by emitting a constant amount of light into each fiber. This procedure made it possible to compare simulated with measured dc decay as a function of fiber separation.

Since the tissue phantom uses a rotating rigid lattice of scattering and absorbing particles to simulate flow, the number of moving scatters is constant. Therefore, as long as the maximal Doppler frequency of the back-scattered light is lower than the cut off frequency of the low pass filter (12500 Hz), perfusion will vary linearly with the disc velocity \( v_{disc} \):

\[
Perf_M (v_{disc}) = k \cdot v_{disc} + U_{bias}
\]

(2)

The slope \( k \), quantified with linear regression, was used as the measured perfusion value for a disc velocity of 1 mm/s. The perfusion bias \( U_{bias} \) compensates for noise and bandwidth limitations. All calculations were made using Matlab 5.2 (The MathWorks, Inc.).

Simulation Model

To further understand how photons migrate through a turbid medium, such as the tissue phantom used in this study, a mathematical model is needed. For simplicity, mainly due to the need of registering the photon Doppler shifts, the complexity of the measurement setup and the tissue phantom structure, a Monte Carlo simulation approach was chosen. With this technique, it is possible to simulate the pathway of individual photons if the scattering-coefficient, absorption-coefficient and the phase function of the medium are known. A short review and comparison of the Monte Carlo technique with measurements and diffusion theory has been presented by Flock et al.\(^{15,16}\)

For the Monte Carlo simulations, the same geometrical configuration and optical characteristics as for the tissue phantom were modeled using a disc velocity of 1 mm/s.
The probability distribution of the photon scattering angle was modeled using the Henyey-Greenstein phase function \( g = 0.815 \). To compare LDF spectra, Monte Carlo simulations using one set of optical properties (window 1) and one single rotating disc (disc 2) were carried out for two of the velocities (0.7 and 2.2 mm/s) that were used in the LDF measurements. Furthermore, an extended simulation model (model 2) with the same geometric configuration for all windows and the same optical properties as the tissue phantom, was designed. This model was evaluated for both single moving discs and multiple moving discs (all discs), using a disc velocity of 1 mm/s. Simulated disc depths using model 2 are shown in Table 3. Each simulation was carried out for a fixed number of detected photons (500000).

### Table 3. Average disc depth (in µm) of rotating discs for the extended simulation model (model 2). The thickness of all rotating discs was 21 µm.

<table>
<thead>
<tr>
<th>Disc number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disc depth [µm]</td>
<td>71</td>
<td>187</td>
<td>303</td>
<td>419</td>
<td>535</td>
<td>651</td>
<td>767</td>
<td>883</td>
<td>999</td>
<td>1115</td>
<td>1231</td>
</tr>
</tbody>
</table>

Since a ring shaped detector was used in the simulations, all photons had to be weighted differently depending on their detection radius (Appendix). The photon weight factor or intensity \( p_i \) is given by:

\[
p_i = \frac{1}{\pi} \cdot \cos^{-1}\left( \frac{r_i^2 + L(r_i)^2 - R_{\text{fiber}}^2}{2 \cdot r_i \cdot L(r_i)} \right)
\]  

- \( p_i \): Intensity of photon \( i \)
- \( r_i \): Detection radius of photon \( i \)
- \( L(r_i) \): Emitting - receiving fiber separation (Depends on \( r_i \))
- \( R_{\text{fiber}} \): Radius of receiving fiber core (100 µm)

Simulated light intensity, \( d_{c,n} \), was estimated by summation of all photon intensities detected by fiber \( n \):

\[
d_{c,n} = \sum_{i_n}^{N_n} p_i
\]

- \( i_n \): All photons detected by fiber \( n \)
- \( p_i \): Intensity of photon \( i \)
- \( N_n \): Number of photons detected by fiber \( n \)

The simulated Doppler power spectrum was estimated by first calculating the frequency distribution \( H_{c,n}(k) \) of the simulated photons, detected by fiber \( n \). A fixed frequency resolution \( \Delta f \) of \( 12500/512 \approx 24.4 \text{ Hz} \) and a frequency interval of \([-12500, 12500] \text{ Hz} \) were used. Hence, \( H_{c,n}(k) \) is given by:
The power spectrum $P_{S,n}(k)$ was obtained by convoluting the frequency distribution $H_{S,a}$ with itself. The corresponding perfusion value was estimated as:

$$\text{Perf}_{S,a} = \frac{\sum_k 2\pi k \cdot \Delta f \cdot P_{S,n}(k)}{d_{c_{S,a}}^2}$$

### Influence of Optical Properties

The effect of differences in optical properties on LDF perfusion was evaluated by comparing the two perfusion values $\text{Perf}(\text{OP}_a, d, l)$ and $\text{Perf}(\text{OP}_b, d, l)$. Both these values have been simulated using the same disc depth ($d$) and fiber separation ($l$), but two different sets of optical properties ($\text{OP}_a$ and $\text{OP}_b$). The ratio, denoted as $\text{Perf}(\text{OP}_a, \text{OP}_b, d, l)$, between these two perfusion values was defined as:

$$\Delta \text{Perf}(\text{OP}_a, \text{OP}_b, d, l) = \frac{\text{Perf}(\text{OP}_a, d, l)}{\text{Perf}(\text{OP}_b, d, l)}$$

This ratio indicates how perfusion, generated at different disc depths and measured using different fiber separations, is altered when the optical properties change from $\text{OP}_a$ to $\text{OP}_b$. By selecting $\text{OP}_a$ and $\text{OP}_b$ wisely, the influence of either absorption or scattering coefficient could be evaluated.

Maximal influence of optical properties on LDF perfusion, i.e. maximal perfusion variation, was evaluated for each fiber separation between 0.23 and 1.61 mm by calculating the ratio:

$$\max_{\text{OP}} \left( \frac{\text{Perf}(\text{OP}, d, l)}{\min_{\text{OP}} \text{Perf}(\text{OP}, d, l)} \right)$$

Both the homogeneous flow distribution model (simulation model 2 with all discs moving) and the discrete flow depth model (simulation model 2 with single discs moving) was evaluated by calculating the maximal perfusion variation.

### Sampling Depth

LDF sampling depth, as a function of optical properties and fiber separation, was defined as the disc depth where the perfusion had decayed to $e^{-1}$ of the maximal perfusion. Due to a limited number of perfusion values, linear interpolation was used to estimate the sampling depths.
Results

**dc Decay – Measured and Simulated**

A good correlation between measured and simulated (model 1) dc decay was found (Figure 3). This indicates that the optical properties and the Monte Carlo model were correct. Both measured and simulated dc values were normalized against the dc value of fiber 3 and window 2, thereby eliminating differences in amplification between the measurements and the simulations.

![Figure 3. Simulated (model 1; solid lines and empty markers) and measured (dashed lines and filled markers) dc decay as a function of fiber separation for the optical properties of window 1, 2 and 4 (S = simulated; M = measured).](image)

**Doppler Power Spectra – Measured and Simulated**

For the two simulated velocities, a good agreement between the frequency distribution of measured and simulated Doppler power spectra was found (Figure 4). This indicates that the Henyey-Greenstein phase function and the anisotropy (g=0.815) of the simulation models is appropriate.

![Figure 4. Simulated (model 1; empty markers) and measured (filled markers) Doppler power spectra for two different rotating disc velocities (0.7 and 2.2 mm/s), using window 1, disc 2 and fiber 1.](image)
Perfusion Decay – Measured and Simulated

Simulated and measured perfusion as a function of disc depth is presented in Figure 5 for fiber 1 and fiber 4. To eliminate differences in amplification all values were normalized against the maximum perfusion detected by each fiber. The result shows that the perfusion decay is faster for fiber 1 than for fiber 4. There is also an increase in perfusion decay with increasing $\mu_s$ ($OP_{w1} \rightarrow OP_{w2}$) or increasing $\mu_a$ ($OP_{w1} \rightarrow OP_{w2}$). Comparing simulated perfusion decay with measured perfusion decay indicates a small but systematic discrepancy that increases with the disc depth.

![Image of Figure 5](https://example.com/image.png)

**Figure 5.** Simulated (model 1; solid lines and empty markers) and measured (dashed lines and filled markers) perfusion as a function of disc depth for fiber 1 (a) and fiber 4 (b) ($S =$ simulated; $M =$ measured).

Influence of Optical Properties on Simulated LDF Perfusion

Model 2 was used to evaluate the influence of optical properties on LDF perfusion. The result, presented in Figure 6, indicates that the influence of either $\mu_s$ or $\mu_a$ on LDF perfusion increased with increasing flow depth and decreased with increasing fiber separation. Figure 6 also shows that the influence of $\mu_s$ on LDF perfusion increased with the magnitude of $\mu_a$ (Figure 6a vs. 6b), the influence of $\mu_a$ on LDF perfusion increased with the magnitude of $\mu_s$ (Figure 6c vs. 6d).
Figure 6. Influence of optical properties on simulated LDF perfusion (model 2) for a range of rotating disc depths. The relative perfusion change ($\Delta$Perf) due to variations in $\mu_a$ (a and b) or $\mu_s$ (c and d) is presented for fiber 1, 4 and 7:

a) $\mu_a$: 0.0405 → 0.212 mm$^{-1}$, $\mu_s \approx$ 15 mm$^{-1}$;

b) $\mu_a$: 0.0532 → 0.226 mm$^{-1}$, $\mu_s \approx$ 45 mm$^{-1}$;

c) $\mu_s$: 14.80 → 45.55 mm$^{-1}$, $\mu_a \approx$ 0.05 mm$^{-1}$;

d) $\mu_s$: 14.66 → 44.85 mm$^{-1}$, $\mu_a \approx$ 0.2 mm$^{-1}$.

The maximal perfusion variation in the simulation data (model 2 using both single and multiple moving discs), as a function of fiber separation, is presented in Figure 7. For the homogeneously distributed flow model (all discs moving) the maximum perfusion variation was on average 40% and ranging [22, 50]%, for fiber separations between 0.23 and 1.61 mm. In general, all single moving disc models resulted in larger variations than the model with all discs moving. There was also an increase in the maximal perfusion variation with increasing disc depth. However, a decreased variation with increased fiber separation could be noticed for disc 3 and below (>0.3 mm).
Figure 7. Simulated maximal perfusion variation, due to the variations in optical properties, for a homogeneous distributed flow model (model 2 with all discs moving) and a discrete flow model (model 2 with single discs moving).

Sampling depth - Simulated

The LDF sampling depth has been evaluated using simulation model 2. The result, shown in Figure 8, indicates that LDF sampling depth increases with decreasing μs (w1, w2, w3, and w4) or μa (w1, w2, w3, and w4). The sampling depth also tends to increase linearly with fiber separation. According to the simulations the sampling depth for fiber 2 (0.46 mm of fiber separation) is 0.21-0.39 mm.

Figure 8. Simulated (model 2) e^−1 LDF sampling depth for a range of fiber separations (0.23-1.61 mm) and optical properties (w1s-w4s).

Discussion & Conclusions

Validity of Simulations

There was a good agreement between measured and simulated dec decay (Figure 3) and LDF power spectrum (Figure 4). This strengthens the validity of the optical properties and the Monte Carlo simulation model. However, perfusion as a function of flow depth (Figure 5) showed a small systematic mismatch between measured and simulated values. The difference tended to increase almost linearly with flow depth. The most likely explanation for this is that the presented disc depths of the tissue phantom are slightly
smaller than the actual disc depths due to the matching oil between the rotating discs and the static windows. The agreement between measured and simulated data indicates that the results from the expanded Monte Carlo simulations (model 2) reflect measurements with an ideal geometry.

**Influence of optical properties**

The influence of optical properties on LDF perfusion was studied in a more general way by evaluating $\Delta \text{Perf}$ (eq. 7), using perfusion data from the expanded simulations (model 2). Figure 6a-6d indicates that an increase in either scattering or absorption coefficient will result in a decreased LDF perfusion. In detail, the findings show that the influence of the change in absorption coefficient on the LDF perfusion is rather weak for the most superficial discs, but increase rapidly for larger disc depths (Figure 6a vs. 6b). According to Figure 6c and 6d, the influence of the change in scattering coefficient on the LDF perfusion increases almost linearly with increasing disc depth. Figure 6a-6d also indicates that the simulated LDF perfusion readings seem to be slightly more affected by the changes in scattering coefficient than to the changes in absorption coefficient, at least for the most superficial discs. It is also clear that the influence of optical properties on LDF perfusion is not only affected by the change in scattering or absorption coefficient, but also by the magnitude of the non-changed coefficient. For example, a change in absorption coefficient has a larger impact on the LDF perfusion when the scattering coefficient is relatively large (Figure 6a vs. 6b). This can be understood when realizing that a larger scattering level will increase the path length of the detected photons, thus making the effect of absorption and changes in absorption larger. The same is valid for a change in scattering coefficient (Figure 6c vs. 6d). These results show that it is not only the optical properties or the actual flow depth that affects the LDF perfusion, but rather a combination of both. One way of interpret this result is that the optical properties affects the sampling depth and thereby makes the LDF readings sensitive to the actual flow depth.

By increasing the fiber separation the influence of optical properties on LDF perfusion decreased, when measuring at discrete disc depths. This effect is most pronounced when a change in the scattering coefficient occurs (Figure 6c vs. 6d). However, all perfusion readings on human skin originate from photons that have been Doppler shifted at different skin depths. This means that the depth distribution of the detected photons scattering events, i.e. sampling depth, will affect the measured perfusion. This distribution is not only determined by the optical properties of the sampling volume but also by the fiber separation; an increase in fiber separation will increase the sampling depth. As mentioned before, the influence of optical properties on LDF perfusion is higher for a deep located blood flow than a superficial one. Therefore, an increase in fiber separation does not guarantee that the influence of optical properties on LDF perfusion decreases when measuring a distributed blood flow (e.g. human skin). This is illustrated in Figure 7, where both a homogeneous and discreate flow distribution model is assumed. In the case where all discs move, the maximum perfusion variation (eq. 7) is rather independent of the fiber separation, in average 40% and ranging [22, 50]%. However, an increasing trend could be recognized, most probably as an effect of increasing sampling depth with increasing fiber separation. Figure 7 also indicates that the maximum perfusion variation increases with flow depth. Therefor, the increasing trend is probably even more pronounced when studying real tissue, where the unknown heterogeneous blood perfusion distribution is assumed to increase with skin depth.
Sampling Depth
Assume a homogeneous blood flow distribution, no multiple Doppler shifts and that the distribution of the detected photons scattering events decreases exponentially with depth. The used definition of sampling depth then implicates that ~63% of the perfusion signal is generated in the region above this depth.

According to Figure 8 the sampling depth in LDF increases with increasing fiber separation. It is also obvious that the optical properties have a strong influence; an increase in either scattering or absorption coefficient will result in a decreased sampling depth. Similar trends regarding the absorption coefficient for fiber separations larger than 1 mm have previously been reported by Weiss et al. However, their predictions of the sampling depth were larger than ours, probably because they used a lattice random-walk model with a point source and a point detector. The influence of the probe geometry, using Monte Carlo simulations, has previously been investigated by Jakobsson et al. They found similar trends regarding fiber separation but a more superficial sampling depth than we found, probably because they studied the median depth of the detected photons pathways.

Conclusions
In conclusion, there was a good agreement between measured and simulated data, indicating that the results from the expanded Monte Carlo simulations (model 2) reflect measurements with an ideal geometry. The simulations showed that an increase in either scattering or absorption coefficient of the static tissue resulted in a decreased LDF perfusion and LDF sampling depth. Further, the simulations of a single rotating disc at a discrete disc depth showed that the influence of the scattering coefficient and the absorption coefficient on the LDF perfusion increased with increasing disc depth. By increasing the fiber separation the influence of optical properties on LDF perfusion decreased, when simulating a single rotating disc. However, the sampling depth increased with fiber separation. This means that, for a distributed flow model, a large fiber separation will result in an larger influence from the deepest lying flows that generates LDF perfusion values that are more sensitive to the optical properties, compared to a small fiber separation. Therefore, an increased fiber separation will not guarantee a decreased influence from the optical properties on LDF perfusion, when measuring at a distributed blood flow. For a homogeneous flow distribution model, the maximum perfusion variation due to the different optical properties was in average 40%, almost independent of fiber separation.

Acknowledgements
This research was supported by the European Commission through the SMT4-CT97-2148 contract. Under this contract, a cooperation runs between the Universities of Twente and Groningen (the Netherlands), Linköping and Malmö (Sweden), Toulouse (France), the companies Perimed AB (Sweden), Moor Instruments and Oxford Optronix (UK), and the Institute of Biocybernetics and Biomedical Engineering in Warsaw (Poland).
Appendix

Conversion of Ring to Fiber Detector

In contrast to the measurements on the tissue phantom, a ring shaped detector (NA=1) was used to speed up the simulations. Therefore, to be able to compare the in-vitro measurements with the simulations, all simulated photons had to be weighted differently depending on where they were detected. A photon, detected at distance $r_i$ from the center of the emitting fiber (Figure 9), was weighted by $p_i$, defined as:

$$p_i = \frac{dC}{2 \cdot r_i} \cdot \pi \cdot r_i^2$$  \hspace{1cm} (9)

where $dC$ is the fractional circumference given by $r_i$ and limited by the border of the detecting fiber:

$$dC = 2 \cdot r_i \cdot \theta$$ \hspace{1cm} (10)

The law of cosines gives:

$$R_{fiber}^2 = L(r_i)^2 + r_i^2 - 2 \cdot L(r_i) \cdot r_i \cdot \cos(\theta)$$ \hspace{1cm} (11)

Combining equation 9-11 results in:

$$p_i = \frac{1}{\pi} \cdot \cos^{-1}\left(\frac{r_i^2 + L(r_i)^2 - R_{fiber}^2}{2 \cdot r_i \cdot L(r_i)}\right)$$ \hspace{1cm} (12)

This weight factor is only defined when the detection radius falls inside the core of one of the receiving fibers ($r_i \in [n \cdot 0.23 - 0.1, n \cdot 0.23 + 0.1]; \ n = 1, 2, 3, ..., 7$). Photons falling outside this range were assigned a weight factor of zero. The distance between emitting and receiving fiber ($L$) depends on the simulated detection radius ($r_i$) and is given by:

$$L(r_i) = n \cdot 0.230 \text{ mm} \ , \ n = \text{round} \left(\frac{r_i}{0.23}\right)$$ \hspace{1cm} (13)

Figure 9. Photon weights, when converting a ring-shaped detector to a fiber detector (see Appendix), can be calculated if the detection radius $r_i$, fiber separation $L(r_i)$ and fiber radius $R_{fiber}$ is known.
References