Determination of the diameter of simulated human capillaries using shifted position-diffuse reflectance imaging

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Abstract
Multiple diseases are associated with a wide spectrum of microvascular dysfunctions, microangiopathies and microcirculation disorders. Monitoring the microcirculation could thus be useful to diagnose many local and systemic circulatory disorders and to supervise critically ill patients. Many of the scores currently available to help identify the condition of a microcirculation disorder are invasive or leave scope for interpretation. Thus, the present study aims to investigate with Monte-Carlo simulations (as numerical solutions of the radiative transfer equation) whether shifted position-diffuse reflectance imaging (SP-DRI), a non-invasive diagnostic technique, reveals information on the capillary diameter to assess the state of the microcirculation. To quantify the SP-DRI signal, the modulation parameter $K$ is introduced. It proves to correlate almost perfectly with the capillary diameter ($R^2\approx1$), making it a valid parameter for reliably assessing microcirculation. SP-DRI is emerging as an important milestone on the way to early and conveniently diagnosing microcirculation associated diseases.

KEYWORDS
shifted position-diffuse reflectance imaging, microcirculation, critically ill patients, modulation parameter, turbid media, human capillary, Monte-Carlo simulation

1 | INTRODUCTION

Microcirculation describes the blood flow within the terminal section of the vascular system on the level of the capillary bed, the afferent arterioles and the efferent venules. It ensures the basic nutrient supply of the tissues and aims to keep the blood circulation going even in the event of a central change in the blood pressure [1].

In order to cope with these tasks, microcirculation is subject to local and systemic regulatory mechanisms [2]. The capillary bed as the location of the microcirculation thus fulfils two separate functions: On the one hand,
superordinate regulatory mechanisms take effect there, on the other hand, the capillary bed itself influences the overall organism and the global circulatory situation of the individual through local effects. As an example, large quantities of venous blood can be mobilised from the capillary bed or moved thereto in order to change the preload of the heart and thus the cardiac output, if necessary [3, 4].

Changes in capillary blood flow, peripheral resistance and microcirculation, however, do not only occur in physiological metabolic and circulatory states. Multiple diseases — ranging from diabetes mellitus to hypertension and autoimmune diseases — can be associated with a wide spectrum of microvascular dysfunctions, microangiopathies and microcirculatory disorders [2]. Furthermore, pathologic circulatory states such as those associated with hypovolemia can significantly influence the peripheral blood flow and the blood flow in the capillary bed [5]. In the worst case, this results in a generalised circulatory collapse with pathological circulatory dysregulation after the adaptability of the capillary system has been exhausted [6].

This means, in summary, that through adaptive reactions of the capillary bed, it is possible to influence both the local circulatory state of a limited body region as well as the patient's overall blood circulation. Vice versa, local and superordinate processes cause (reactive) changes within the capillary bed. Based on the physiological correlations mentioned before, it can be assumed that monitoring the microcirculation could thus be useful to diagnose various local and systemic circulatory disorders and could be used to monitor critically ill patients [7, 8].

The parameter that enables this regulatory function is the constriction or dilatation of the arterioles or venules. Moreover, there is some evidence that also the capillary bed itself is capable of active and passive changes in calibre [9–14]. This change in diameter causes increased or decreased blood flow in the capillary bed. Thus, in the physiological state the predominant reaction of the capillary bed and its afferent and efferent vessels to different metabolic and circulatory situations is — to put it simply — a change in the capillary blood flow due to a variation in the capillaries' diameter.

Various instrumental, invasive and laboratory tests as well as easy-to-use scores are available to help identify the condition of a microcirculation disorder in a patient. The instruments that can be used range from the easily and quickly ascertainable shock index to pulse oximetry, sublingual pCO₂ tonometry and the assessment of central venous pressure via a vena cava catheter or oesophageal Doppler monitors [15–21]. While the latter techniques are invasive and thus carry the risk of iatrogenic injury, a critical point of all examination methods mentioned is that they leave scope for interpretation and only provide clues for making a diagnosis [18]. Consequently, what is needed is a monitoring approach that makes it possible to obtain reliable information about the state of the microcirculation automatically and in real time.

In this respect, non-invasive diagnostic techniques based on light are quite promising. In our previous study, we were able to show that it is possible to detect anatomically correctly scaled capillary loops within human skin using the shifted position-diffuse reflectance imaging (SP-DRI) method, an algorithm based on the diffuse reflected light, mathematically equivalent to a computational edge filter (more on that in section 2.3) [22]. As SP-DRI only requires two illumination fibres (or one movable) placed directly next to each other, an alternating illumination of the tissue site and an approach for capturing the diffuse reflection, it is quite easy to be implemented in practice [22].

Based on the earlier findings mentioned above, the present study aims to investigate whether the SP-DRI signal reflects not only the location of the capillary but also information on its diameter. For this purpose, a procedure must first be developed to quantify the SP-DRI signal. Afterwards, the diameter of the capillary loops will be systematically varied in an anatomically reasonable range. Finally, it will be investigated whether there is a correlation between both of these parameters.

This question will be answered by performing extensive Monte-Carlo (MC) simulations, as such simulations provide an environment where all relevant parameters (i.e. in particular geometry and optical parameters) are fully known or can be precisely adjusted — making them ideal for studying such fundamental and yet unknown phenomena. In this respect, this study uses numerical solutions of the radiative transfer equation to simulate the scattering from a physiological model of capillary loops within human skin.

As a MC does not require any significant approximations, it is acknowledged as the gold standard in modelling photon transport in optically heterogeneous media like skin [23].

2 Materials and Methods

2.1 Configuration of the MC simulation

The MC simulation was performed with MCXLAB V2019.4 [24] on computers with MATLAB R2019a. The basic idea and the general structure of the MC simulation are in line with the procedure described in our preceding publication: Haemoglobin-filled capillary loops are embedded in a tissue volume in an anatomically correct depth and size. The capillary loops are connected by the superficial vascular plexus. Details on the five-layered skin model (stratum corneum, epidermis, papillary dermis, upper blood net dermis, reticular dermis) as well as
on the optical properties of the layers and of haemoglobin can be obtained there [22] and also in Tables A1 and A2 (appendix) and Figure 1.

To meet the requirements of the novel research question, however, the geometric parameters of the simulation model were adjusted. The volume used is $1000 \times 1000 \times 2000$ voxels in size and the length of one voxel is 1 $\mu$m. Two capillary loops were incorporated into the volume: The first loop extended from $y = 300$ pixel (px) to $y = 345$ px and the second one from $y = 500$ px to $y = 545$ px, both at $x = 600$ px. In depth, both loops reached from $z = 150$ px to $z = 300$ px. The incorporated superficial vascular plexus ranged from $y = 0$ px to $y = 1000$ px at a depth of $z = 300$ px, also at $x = 600$ px. A graphical illustration of this situation is given in Figure 1.

In accordance with our previous results, illumination wavelengths of $\lambda = 424$ nm and $\lambda = 540$ nm were chosen to meet local maxima of the absorption curve of oxyhaemoglobin [25]. To account for the fact that diffuse reflection is often captured using a fibre bundle, detection fibres with a numerical aperture $A_N = 0.66$ were simulated. During post-processing of the detector data, the diffuse reflection is calculated using Beer's law: For each simulated photon packet detected at the $-z$ boundary, the path lengths that it has travelled in each medium are known, so that its intensity when hitting the detector can
be calculated. If this computation is carried out for all photon packets detected, the diffuse reflection at the \(-z\) boundary can be worked out.

The illumination was realised as a simulated fibre with a core diameter \(\theta_{\text{core}} = 50\mu m\) and a numerical aperture \(A_N = 0.45\). It was located on the \(-z\) boundary \((x = 300 \text{ px} \text{ in all cases}; y = 300 \text{ px} \text{ and } y = 415 \text{ px} \text{ [for } \lambda = 424 \text{ nm}] \text{ or } y = 395 \text{ px} \text{ [for } \lambda = 540 \text{ nm}], \text{ respectively})\) pointing in \(+z\) direction. The incident photon packets were detected on the \(-z\) boundary.

When its weight was less than 1% of its initial weight, a photon packet was terminated. To maintain the conservation of energy, the russian roulette approach was used. For each simulation run, \(10^{10}\) photon packets were launched. The details of the simulation runs that are carried out in this study are addressed in section 2.5.

2.2 Variation of the capillary diameter

As the length of one voxel of the simulation volume is 1 \(\mu m\), this corresponds to the smallest possible unit in which the capillary radius can be changed. This resolution thus allows the capillary diameter to be varied in steps of 2 \(\mu m\). Since the diameter of human capillaries in the physiological state can be assumed to be \(\theta_{\text{cap}} = 10\mu m\) [26–28], in this study capillary diameters ranging from \(\theta_{\text{cap}} = 4\mu m\) to \(\theta_{\text{cap}} = 14\mu m\) (in increments of 2 \(\mu m\)) were regarded as reasonable deviations from the norm [10, 29] and simulated accordingly.

The diameter of the superficial vascular plexus was fixed at \(\theta_{\text{exp}} = 30\mu m\). At this point, once again reference should be made to Figure 1.

2.3 SP-DRI normalisation

In our previous paper, we established the novel normalisation method of SP-DRI for the reconstruction of the capillary structure from the simulative data [22]. While the details can be looked up there, the basic principle will be briefly explained once again in the following.

In a first step, two diffuse reflectance data sets have to be created. One data set (matrix 1) differs from the second data set (matrix 2) in that the light source is slightly shifted in \(x\) or \(y\) direction at otherwise identical simulation parameters. Second, the two matrices are shifted against each other so that the relative positions of the light source in both matrices are equal with respect to their \(x\) and \(y\) coordinates. This leads to a relative shift of the capillary structures to each other. Finally, one intensity matrix is divided pixelwise by the other one.

As shown, this makes it possible to identify the location of extremely small structures from diffuse reflectance images. In the present study, SP-DRI method is followed by a filtering with a 2-D Gaussian smoothing kernel with a SD of 10 px.

2.4 Modulation parameter \(K\) and \(K_{\text{norm}}\)

As explained in the introduction, the hypothesis of the present study is that a change in capillary diameter is reflected in the modulation of the SP-DRI signal. It is therefore essential to establish a parameter that represents this behaviour as a quantitative value. For this purpose, in this study the modulation parameter \(K\) is defined as follows.

\[
K = f_{\text{SP-DRI}}(a) \cdot \langle b-a \rangle - \sum_{i=1}^{b} f_{\text{SP-DRI}}(i)
\]

where \(a\) is the position of a local maximum of the SP-DRI signal curve and \(b\) the position of the subsequent local minimum. \(f_{\text{SP-DRI}}(a)\) is the value of the SP-DRI signal at position \(a\), \(f_{\text{SP-DRI}}(i)\) is defined analogously.

The schematic definition of the modulation parameter \(K\) is shown in Figure 2.

The idea behind this modulation parameter \(K\) and the formula describing it is this: In the previous study where a validation of the SP-DRI method was carried out, it was shown that the capillary structure is located at the inflexion point between a local maximum and the subsequent local minimum of the SP-DRI signal. Between these two points, the curve of the SP-DRI signal encloses a specific area that might depend on the amplitude of the signal: According to the hypothesis described above, this area extends with an increasing capillary diameter.
Since different signal curves are to be compared, in the present study the modulation parameter $K$ is normalised at the distance between the respective local maximum and local minimum (distance between $a$ and $b$). Finally, this value is multiplied by 100 for better readability. This normalisation ensures that the $K$ values of all curves can be compared with each other in a meaningful way. For $K_{\text{norm}}$, it therefore follows:

$$K_{\text{norm}} = \frac{K}{b-a} \times 100$$

The local maxima are found with the Matlab command `islocalmax`, where the distance between two maxima must be at least 100 px and exactly two maxima must be found. The same applies to the minima, for which the command `islocalmin` was used. Due to this automated classification there is no subjective influence when determining the extrema.

If the position of the first (second) maximum is larger than that of the first (second) minimum, $\text{NaN}$ (not a number) is set for the corresponding $K$ or $K_{\text{norm}}$ value, respectively.

### 2.5 Overview of the simulation runs

It has already been mentioned in the previous sections that two different wavelengths and six different diameters of the capillary loops were considered in the simulations.

Per wavelength and per capillary diameter increment, 10 simulation runs each with $10^{10}$ photon packets were carried out. For each of these simulation runs, the seed was randomly set to a value between 0 and $2^{31}$ (limits excluded).

### 3 RESULTS

In the following, the results of the SP-DRI normalisation for the simulation data with different diameters of the capillary loops are presented. For this, a cross-sectional plane was drawn through the data sets at the region of interest (in this case at $x = 660$ px as the diffuse reflection occurs, as seen from the light source, slightly behind the capillary branch). This procedure is already well documented in earlier publications [22]. The discussion of the results will follow in the next section (section 4).

To get a first idea of the appearance of the data, one data set per simulated capillary diameter is plotted in Figure 3 (ie for each capillary diameter, one data set was randomly selected from the 10 available data sets) for an illumination wavelengths of $\lambda = 424$ nm. When looking at the curves plotted, it is already apparent that their amplitudes differ as a function of the capillary diameter. This applies to both capillary loops present. It is also evident that the classification of the local extrema works well.

To be able to describe this behaviour quantitatively, the modulation parameters $K$ and, in its normalised form,
$K_{\text{norm}}$ were introduced in this publication. While Tables A3 and A4 in the appendix of this publication contain all single $K$ values, the mean values and the respective standard errors of the means, Figure 4 presents the results graphically. In this figure, the means and standard errors are plotted once separately for all left and right capillary loops (compare Figure 3), respectively, and once without such distinction. Looking at that figure, an increase of the normalised modulation parameter $K_{\text{norm}}$ with rising capillary diameters is noticeable for all cases.

In order to support this behaviour statistically, a linear regression analysis was conducted. Each time, the diameter of the capillary loops is set as the independent variable ($x_1$), while the normalised modulation parameter $K_{\text{norm}}$ constitutes the dependent variable (output $Y$). This results in a linear model of the form:

$$Y = \beta_0 + \beta_1 \cdot x_1$$

where $\beta_0$ determines the intercept and $\beta_1$ the slope of the regression curve. The numbers for these regression parameters resulting from the data presented in this study can be found in Figure 4.

To investigate what proportion of the variance of the dependent variable can be predicted by the independent variable, the adjusted coefficient of determination $R^2$ can

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**FIGURE 4** Graphical illustration of the means and standard errors for the different diameters of the capillary loops for an illumination wavelengths of (A) and (B) $\lambda = 424$ nm and (C) and (D) $\lambda = 540$ nm. While in (A) and (C) a distinction is made between the two positions of the capillary loops ($n = 10$ per loop and per $\lambda$), in (B) and (D) the overall result is shown ($n = 20$ per $\lambda$). The corresponding numerical values can be taken from Tables A3 and A4 in the appendix. The dashed lines indicate the corresponding regression curves as they result from the linear regressions computed. The related parameters of this statistical model can also be found in the graphs ($\beta_0$: intercept; $\beta_1$: slope; $R^2$: adjusted coefficient of determination)
be determined. Again, the corresponding results are presented in Figure 4. They suggest that there is an almost perfectly linear relationship between the diameter of the capillary loop and the normalised modulation parameter \( K_{\text{norm}} \) in all three cases.

4 | DISCUSSION

SP-DRI is a non-invasive optical method capable of locating the position of structures such as human capillaries within turbid media. It is a method that is very easy and cost-efficient to implement and that requires little adjustment, which is its inherent advantage [22].

However, it has not yet been investigated whether this technique, beyond this principal ability, is also suitable for reliably providing information on the size of the incorporated structures. With regard to the capillaries already mentioned, this implies: Does the SP-DRI signal reflect diameter variations in the range of only a few micrometres?

Considering the results presented in this study, this question may be clearly answered in the affirmative. The implemented normalised modulation parameter \( K_{\text{norm}} \) as a metric for the amplitude of the SP-DRI signal clearly varies depending on the diameter of the capillary structure within the tissue. As shown, this relationship is almost perfectly linear in the investigated parameter range from \( \theta_{\text{cap}} = 4 \mu m \) to \( \theta_{\text{cap}} = 14 \mu m \). The calculated adjusted coefficients of determination \( \hat{R}^2 \) confirm that. Consequently, it can be stated that \( K_{\text{norm}} \) increases proportionally with increasing capillary diameter.

This finding implicates a first important conclusion: The normalised modulation parameter \( K_{\text{norm}} \) found in this study seems to be a meaningful and definite index for quantifying the SP-DRI signal. Changes in the signal are reflected in parameter alterations, where the latter may well be interpreted in their direction and magnitude. The SP-DRI technique in combination with the normalised modulation parameter \( K_{\text{norm}} \) is able to effectively quantify capillary diameter changes. Differences in the capillary diameter (at least within the 2-micron increments studied) can be clearly visualised using SP-DRI.

In the present study, the same MC simulation was performed 10 times for each capillary diameter increment (and for each wavelength), with the seed being determined randomly each time to ensure unique simulation results. The presented metrics can thus be interpreted as intra-person variability: The measuring probe is firmly fastened to the subject such that any movements do not influence the measurement. Thus, within this subject the same capillary loops are monitored over a period of time, and changes in the diameters of that capillaries are detected. By always examining the same spot, it is not a fluctuation of the optical properties that significantly influences the SP-DRI signal, but noise. Exactly this noise is present in the data due to the repeated execution of the MC simulation. Consequently, the data presented reflect the important clinical situation of (long-term) patient monitoring.

To verify that the demonstrated relationship is not only the result of a chosen combination of optical properties but that it is present independently of this, the simulations were carried out for different wavelengths (\( \lambda = 424 \) and \( \lambda = 540 \) nm). The first of these two wavelengths allows a maximum imaging contrast due to the very high difference between the absorption coefficient \( \mu_a \) of haemoglobin and the surrounding tissue, making it the optimal wavelength for the later use of the SP-DRI method on a patient [22]. \( \lambda = 540 \) nm, on the other hand, with a smaller difference in the absorption coefficients (as \( \mu_a \) of haemoglobin is lower nearly by a factor of 10, compare Table A2), represents the situation of significant fluctuations in the optical properties and a resulting low imaging contrast. In this study, this wavelength therefore constitutes the worst possible condition for a later examination on a patient.

On the basis of the data introduced in this study, it can be seen that the reported linear relationship between \( K_{\text{norm}} \) and the capillary diameter is present regardless of the particular set of optical properties (ie wavelengths). Although the regression curves for \( \lambda = 424 \) nm show a steeper increase and a better differentiability of the two smallest capillary diameters \( \theta_{\text{cap}} = 4 \mu m \) and \( \theta_{\text{cap}} = 6 \mu m \) - nevertheless, still a reasonable value for the modulation parameter \( K \) can be derived even under such less than optimal conditions that come with the case of \( \lambda = 540 \) nm. This is the second essential conclusion to be drawn from this study.

In the results section, the regression equations are broken down for the three capillary groups distinguished in this study (left capillary loops, right capillary loops, overall). In total, but especially for \( \lambda = 424 \) nm, it is noticeable that the slopes have nearly identical values, while the intercept differs. This means that two emerging effects can be clearly separated: The previously discussed influence of the diameter of the capillary loops is reflected only in the slope of the regression curve (the diameter behaviour is the same in all three cases, leading to comparable slopes), whereas the distance between the illumination and the position of the capillary loop determines the intercept (it differs in the three cases, leading to different intercepts). For \( \lambda = 540 \) nm, this distinction is somewhat blurred due to the fact already discussed above that the two smallest diameters can hardly be differentiated in this case.
The results show that even if the distance just mentioned is not taken into account (cases “overall”), a determination of the diameter of the capillary loops can be made using $K$. However, by knowing this distance (and with it the associated intercept) it is even possible to attribute an absolute diameter value to a value of $K$. Absolute diameter quantifications should therefore be possible even with a large number of capillary loops and thus source detector separations - this is a third important conclusion that can be deduced.

While it was pointed out in the introduction to this publication that a crucial aspect of current clinical procedures for monitoring the state of the microcirculation is that these methods are invasive, leave scope for interpretation or only provide clues for making a diagnosis [18], this study was able to demonstrate that SP-DRI is an excellent approach to solving this issue: Reliable information can be obtained in real time with an easy to implement setup.

It is therefore the fourth important finding of this study that SP-DRI, in combination with the modulation parameter $K$, may be an important milestone on the way to early and conveniently diagnosing diseases associated with the constriction or dilation of the arterioles, venules or the capillary bed itself (microvascular dysfunctions, microangiopathies, microcirculation disorders). As already mentioned initially, this covers many diseases, among them diabetes mellitus, hypertension and autoimmune diseases [2].

In summary, this study has succeeded in further developing SP-DRI; the basic functionality of this method has already been described in an earlier publication [22]. For this purpose, the corresponding algorithm for the calculation of the modulation parameter $K$ was presented. A proof of concept was carried out - it is now the next step to validate this approach at first on optical phantoms and thereafter in vivo (which will also make it possible to finally include significantly more than two capillary loops in the investigation). Since, however, the SP-DRI method has already been successfully validated on the phantom, it can be assumed that the application of the modulation parameter $K$ will also prove successful beyond this simulation study.

Beyond that, with the present study it has now been possible to demonstrate that the SP-DRI signal also reflects information on the diameter of the capillary loops. For this purpose, the modulation parameter $K$ was introduced which is derived from the amplitude of the SP-DRI signal. It was found that there is an almost perfectly linear relationship between $K$ and the diameter of the capillary structure within the tissue. For both wavelengths investigated ($\lambda = 424$ and $\lambda = 540$ nm), the calculated adjusted coefficient of determination $R^2$ confirms the plausibility of the model ($R^2 \approx 1$).

The findings of the present investigation indicate that SP-DRI may be a suitable technique for monitoring the state of the human microcirculation as it is related to the capillary diameter. With this, SP-DRI may be an important milestone on the way to early and conveniently diagnosing microvascular dysfunctions, microangiopathies or microcirculation disorders.

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CONFLICTS OF INTEREST
The authors declare that there are no competing financial or non-financial interests in relation to the work described.

AUTHOR CONTRIBUTIONS
Moritz Späth, MSc, designed the various simulation runs and its underlying structure, he conducted the simulations and wrote the MATLAB scripts to evaluate the data. He did the data evaluation and interpretation. He prepared the manuscript and drafted it. Dipl.-Phys. Martin Hohmann and Benjamin Lengenfelder, MSc, helped to establish the data evaluation methods. Dr. Maximilian Rohde helped to embed the findings in a medical context. Prof. Dr. Dr. Florian Stelzle and Dr.-Ing. Florian Klämpfl guided the general research strategy and gave a critical revision of this manuscript. All authors read and approved this manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX

TABLE A1  Skin layers considered in the MC simulations

| Tissue layer                  | Thickness along z axis | Vasculature (diameter) |
|-------------------------------|------------------------|------------------------|
| Stratum corneum              | 20 μm                  | —                      |
| Epidermis                    | 80 μm                  | —                      |
| Papillary dermis             | 150 μm                 | Loop (10 μm)           |
| Upper blood net dermis       | 80 μm                  | Horizontal cylinder (30 μm) |
| Reticular dermis             | 1500 μm                | —                      |
| Deep blood net dermis        | 80 μm                  | —                      |
| Subcutaneous tissue          | 6000 μm                | —                      |

Note: This table is taken from the reference [22].

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**TABLE A2** Optical properties of the skin layers and the microvasculature ($\kappa$ = haemoglobin)

| Element                | 424 nm $\mu_a$ [mm$^{-1}$] | 424 nm $\mu_s$ [mm$^{-1}$] | 540 nm $\mu_a$ [mm$^{-1}$] | 540 nm $\mu_s$ [mm$^{-1}$] | g   | n   |
|------------------------|----------------------------|----------------------------|----------------------------|----------------------------|-----|-----|
| Stratum corneum        | 1.46                       | 50.00                      | 1.00                       | 50.00                      | 0.90| 1.53|
| Epidermis              | 3.19                       | 13.96                      | 1.81                       | 7.84                       | 0.85| 1.34|
| Papillary dermis       | 0.80                       | 13.36                      | 0.45                       | 11.09                      | 0.80| 1.40|
| Upper blood net dermis| 1.14                       | 13.36                      | 0.65                       | 11.09                      | 0.90| 1.39|
| Reticular dermis       | 0.68                       | 13.36                      | 0.39                       | 11.09                      | 0.76| 1.40|
| Deep blood net dermis  | 1.37                       | 13.36                      | 0.78                       | 11.09                      | 0.95| 1.39|
| Subcutaneous tissue    | 0.64                       | 7.00                       | 0.36                       | 7.00                       | 0.80| 1.44|
| Microvasculature       | 203.17                     | 8.00                       | 28.75                      | 8.00                       | 0.96| 1.36|

Note: This table is a shortened version of the table from the reference [22].

**TABLE A3** Modulation parameter $K_{\text{norm}}$ as a function of the diameter of the capillary loops for an illumination wavelength of $\lambda = 424$ nm

| # measurement | $K_{\text{norm}}$ [a.u.] for a $\Theta_{\text{cap}}$ of 4 $\mu$m | $K_{\text{norm}}$ [a.u.] for a $\Theta_{\text{cap}}$ of 6 $\mu$m | $K_{\text{norm}}$ [a.u.] for a $\Theta_{\text{cap}}$ of 8 $\mu$m | $K_{\text{norm}}$ [a.u.] for a $\Theta_{\text{cap}}$ of 10 $\mu$m | $K_{\text{norm}}$ [a.u.] for a $\Theta_{\text{cap}}$ of 12 $\mu$m | $K_{\text{norm}}$ [a.u.] for a $\Theta_{\text{cap}}$ of 14 $\mu$m |
|---------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| Left capillary loops | 1 0.2000 0.7298 1.3269 1.9510 3.0574 3.8418 | 2 NaN 0.7090 1.4892 1.4186 3.2636 4.1750 | 3 NaN 0.6244 1.5013 2.1069 3.1547 3.7898 | 4 0.1899 0.7138 1.2942 2.4296 2.4860 3.3602 | 5 0.2827 0.6067 1.2156 2.0603 2.7734 3.0634 | 6 0.0831 0.4952 1.4458 2.0699 2.4860 3.7913 | 7 0.2655 1.2291 1.6051 2.7098 2.9851 3.4383 | 8 0.1588 0.6520 1.4812 2.5564 2.9884 3.4075 | 9 0.3408 0.6683 1.6348 2.3690 2.6086 3.3989 | 10 0.3088 1.0820 1.5278 2.0757 3.0397 3.7029 | Mean 0.2287 0.7510 1.4522 2.1747 2.8843 3.5969 | SE 0.0304 0.0715 0.0427 0.1152 0.0880 0.1012 |
| Right capillary loops | 11 0.4603 1.1336 1.9484 2.3061 3.5585 3.5855 | 12 NaN 1.5242 2.0100 2.6976 3.1038 4.0475 | 13 0.2544 1.3423 1.6992 2.6239 3.8874 4.1885 | 14 0.6640 1.2649 1.8652 2.2892 2.8022 3.6852 | 15 0.3937 0.9400 1.8055 2.2763 2.8516 4.0677 | 16 0.8692 1.7224 1.7973 2.5466 3.6329 3.7886 | 17 0.5717 1.1824 2.0546 2.7546 3.9456 4.2378 | 18 0.4141 0.7933 1.9054 2.8587 3.8637 3.6426 | 19 0.4697 1.2184 2.1023 2.6618 3.4410 4.3138 | 20 0.3258 1.6242 2.0219 2.8618 3.1168 4.2788 | Mean 0.4914 1.2746 1.9210 2.5876 3.4203 3.9836 | SE 0.0623 0.0922 0.0409 0.0717 0.1355 0.0892 |
| Overall Mean | 0.3678 1.0128 1.6866 2.3812 3.1523 3.7902 | SE 0.0479 0.0827 0.0610 0.0813 0.0998 0.0792 |

Note: The respective means and standard errors (SE) are also shown. The values refer to the curves shown in Figure 4.
| # measurement | &nbsp; | 4 µm | 6 µm | 8 µm | 10 µm | 12 µm | 14 µm |
|---------------|------|------|------|------|------|------|------|
| Left capillary loops | 1 | 0.1628 | 0.1733 | 0.5101 | 0.7162 | 0.6063 | 1.1892 |
| &nbsp; | 2 | 0.1839 | 0.1384 | 0.3315 | 0.7217 | 1.0111 | 1.0916 |
| &nbsp; | 3 | 0.3247 | 0.4103 | 0.2303 | 0.3613 | 1.3029 | 0.9120 |
| &nbsp; | 4 | 0.2726 | 0.2417 | 0.1681 | 0.9210 | 0.9564 | 1.0023 |
| &nbsp; | 5 | 0.2790 | 0.1131 | 0.4993 | 0.4911 | 0.9866 | 1.2957 |
| &nbsp; | 6 | 0.1155 | NaN | 0.7590 | 0.5912 | 0.9873 | 0.9773 |
| &nbsp; | 7 | 0.0862 | 0.0945 | 0.3341 | 0.7629 | 0.9494 | 1.1607 |
| &nbsp; | 8 | NaN | NaN | 0.2040 | 0.5411 | 1.0619 | 1.3467 |
| &nbsp; | 9 | 0.0846 | 0.1997 | 0.4898 | 0.4293 | 0.8090 | 1.2465 |
| &nbsp; | 10 | NaN | 0.1983 | 0.2568 | 0.5019 | 0.9528 | 1.2918 |
| Mean | &nbsp; | 0.1887 | 0.1962 | 0.3783 | 0.6038 | 0.9624 | 1.1514 |
| SE | &nbsp; | 0.0330 | 0.0351 | 0.0582 | 0.0547 | 0.0558 | 0.0475 |
| Right capillary loops | 11 | 0.2192 | 0.3884 | 0.9913 | 1.0581 | 1.3937 | 2.1029 |
| &nbsp; | 12 | 0.3789 | 0.1184 | 1.2289 | 1.2976 | 1.4810 | 1.8482 |
| &nbsp; | 13 | 0.1924 | 0.1542 | 0.5423 | 0.8383 | 1.2007 | 1.7890 |
| &nbsp; | 14 | 0.1432 | 0.1987 | 0.5126 | 0.6175 | 0.8018 | 1.4773 |
| &nbsp; | 15 | 0.2507 | 0.4561 | 1.1078 | 1.0319 | 1.5471 | 1.7177 |
| &nbsp; | 16 | 0.3279 | 0.2466 | 0.6024 | 0.5567 | 1.0905 | 1.8632 |
| &nbsp; | 17 | 0.3449 | 0.4831 | 0.9459 | 0.6183 | 0.7906 | 2.0395 |
| &nbsp; | 18 | 0.2061 | 0.2223 | 0.2809 | 1.2639 | 1.3621 | 1.5631 |
| &nbsp; | 19 | 0.1684 | 0.4372 | 0.3047 | 0.9123 | 1.0338 | 1.6220 |
| &nbsp; | 20 | NaN | 0.5908 | 0.8134 | 0.9493 | 1.3616 | 1.7050 |
| Mean | &nbsp; | 0.2480 | 0.3296 | 0.7330 | 0.9144 | 1.2063 | 1.7728 |
| SE | &nbsp; | 0.0279 | 0.0510 | 0.1051 | 0.0826 | 0.0853 | 0.0629 |
| Overall Mean | &nbsp; | 0.2201 | 0.2703 | 0.5557 | 0.7591 | 1.0843 | 1.4621 |
| SE | &nbsp; | 0.0220 | 0.0353 | 0.0712 | 0.0599 | 0.0569 | 0.0809 |

**Note:** The respective means and standard errors (SE) are also shown. The values refer to the curves shown in Figure 4.