Remitted photon path lengths in human skin: *in-vivo* measurement data

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Abstract: The remitted photon path lengths in human skin can be estimated by modelling; however, there are very few experimental data available to validate the simulations. This study exploited the photon time of flight method where picosecond laser pulses at seven wavelength bands in the spectral range 560-800 nm were launched into *in-vivo* forearm skin of 10 volunteers via an optical fiber. The pulses of back-scattered light were detected via another optical fiber placed at variable distance (1, 8, 12, 16 or 20 mm) from the input fiber, with subsequent analysis of their shapes for all 35 spectral-spatial combinations. Using a deconvolution algorithm, the distribution functions of remitted photon arrival times after infinitely narrow input pulse were calculated and transformed into distributions of skin-remitted photon path lengths. Nearly linear dependences of the remitted photon mean path length on inter-fiber distance were obtained for all wavelength bands, while the spectral dependences at fixed inter-fiber distances showed more complicated character, most probably due to absorption of the dermal hemoglobin.

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1. Introduction

Light penetration in tissues and the related total path length of internally scattered photons are important parameters in optical diagnostics and therapy. Composition and density of absorbing chromophores influence the path length of back-scattered (remitted) photons in various tissues, including human skin. Knowledge of the skin-remitted photon path lengths and their distributions at particular wavelengths is crucial for clinical applications, e.g. for reflection pulse oximetry and mapping of the chromophore concentration distribution in skin malformations. Theoretical path length estimations can be based on analytic considerations of the diffusion theory [1] or numerical Monte Carlo model calculations [2–5] However, real skin structure at specific body locations may not correspond to the model assumptions and therefore could lead to mistaken results. Besides, the models consider all launched photons (including those absorbed and forward-scattered) while only the back-scattered photons are detected by the optical contact probes and imaging cameras of the clinical devices. Clearly, directly measured remitted photon path length distributions and mean values are preferable for practical use.

The skin-remitted laser pulse input-output time delays have been recorded previously in classical double-fiber experiments, giving sense about the durations of photon travels in skin structures before remission at some specific wavelengths (783 nm [6], 1064 nm [7], 405 nm and 510 nm [8]). The photon travel time is proportional to its completed distance in the tissue. Consequently, analysis of the remitted pulse temporal features can provide quantitative information on the photon travel lengths in skin before remission.

In this work, an advanced picosecond laser system was used for systematic *in vivo* measurements of healthy human skin. The measurement system allowed changing the wavelength of input pulses and the distance between the emitting and receiving fibers at a constant probe pressure to skin. The remitted pulse parameters at different combinations of wavelengths and the source-detector separations provided information on the distances travelled by photons in skin. Results of the
initial stage of this study [9] were complemented with new experimental data that allowed following the trends of photon mean pathlength dependencies on wavelengths and inter-fiber separations. In particular, the de-convolved functions representing photon travel time distributions after launching an infinitely narrow light pulse into the skin were calculated and their mean values determined for 35 spatial-spectral combinations. These values were further converted into mean values of the remitted photon path lengths in skin; their spatial and spectral dependencies have been obtained and analyzed.

2. Measurement set-up and data processing

The measurement set-up (Fig. 1) has been described in details previously [9]. The time-correlated single photon counting method [10] was used for optical pulse shape measurements. A broadband picosecond laser (White laser micro supercontinuum lasers, Fianium, NKT PHOTONICS, DK, 400-2000nm, pulse full width at half maximum 6 ps, repetition rate 20 MHz) was used as initial light source. Time resolution of the system was 9.7 ps which ensured minimum detectable photon path length ~2 mm. Specific narrow spectral bands were selected by couples of identical interference filters. One of them was filtering the input light while the other was placed in front of the photo-detector (photomultiplier HPM-100-07 combined with the detector controller DCC-100 and data processing card SPC-150, all Becker&Hickl GmbH, DE). According to the HPM-100-07 manufacturer’s data, the FWHM of IRF at 656.5 nm does not exceed 57 ps. Our measured FWHM values of IRFs at all exploited wavelengths were in-between 50+/-2 ps, indicating that temporal response of the photodetector determined the measured shapes of both input and output pulses. As the function $f(t)$ represents the relation of the output pulse shape with respect to the input pulse, its temporal resolution is much better. We estimate it to be ~ 5 ps which causes ~ 1 mm error of the determined photon path lengths. The examined spectral range was 560-800 nm; the spectral bands were selected with a 40 nm step using 10nm half-bandwidth interference filters (Andover Corporation, USA - part numbers 560FS10-12,5; 600FS10-12,5; 640FS10-12,5; 680FS10-12,5; 720FS10-12,5; 760FS10-12,5; 800FS10-12,5).

Stable recording of optical signals via the input and output fibers (WF-400, Light Guide Optics International, LV, silica core diameter 400 microns, length 1,05 m) was ensured by means...
of a custom-made fiber holding probe with inter-fiber distances 1 mm, 8 mm, 12 mm, 16 mm and 20 mm. To provide equal pressure on the skin surface at all measurements, the probe was designed as a lift where the inside sliding part with the couple of fibers lied on the skin, providing a pressure determined by its weight \( \sim 35 \text{ g/cm}^2 \). The outside part of the probe was fixed on skin during the measurements. In order to verify how much variation does probe pressure cause in the mean photon pathlengths, additional series of measurements at two wavelengths (760 nm and 800 nm) and all five inter-fiber separations were taken applying three probe-skin pressures – 35 g/cm\(^2\), 112 g/cm\(^2\) and 224 g/cm\(^2\). The observed variations did not exceed 5%.

Ten volunteers with skin photo-type II or III (Fitzpatrick classification), aged between 25 and 68, were examined with their written consent under permission of the local Ethics Committee. The measurements were taken from healthy skin of the forearm, avoiding contact with large superficial blood vessels. The average spectral power density on skin was \( \sim 10 \text{ mW/cm}^2 \), i.e. well below the skin laser safety limit 200 mW/cm\(^2\) [11].

Processing of the measured data involved comparing the shapes of skin input and output pulses \(- a(t) \) and \( b(t) \), respectively. The temporal distribution function \( f(t) \) of photon arrivals following infinitely narrow \( \delta \)-pulse input were found by de-convolution of the integral

\[
b(t) = \int_0^t a(t - \tau) f(\tau) d\tau.
\]  

This inverse problem was solved using a built-in deconvolution algorithm of Matlab. As the de-convolved function was more noisy than \( b(t) \), original scripts were developed for data smoothing using the Log-normal function, as well as for semi-automatic calculations of the temporal distribution functions and the mean arrival times of skin-scattered photons. In particular, the output signal \( b(t) \) was fitted by Log-normal distribution function using non-linear fitting Matlab \textit{lsqcurvefit} algorithm. This function was selected due to its similarity to the measured data. Next, the input pulse was shifted in time towards output pulse until the rising fronts of pulses coincided at the 5% level. The path length of the first detected photons was obtained as: \( \text{Min path length} = dt \cdot c/n \), where \( dt \) is the time shift towards output pulse. Then the inverse problem \( 1 \) was solved using a built-in Matlab deconvolution algorithm \textit{deconv} and \( f(t) \) was calculated. Finally, the mean arrival time of skin-scattered photons was calculated as the time moment when the area under curve \( f(t) \) equals from left and right side. After restoring \( f(t) \), the corresponding distribution of back-scattered photon path lengths in skin was calculated as

\[
\phi(s) = f(t) \cdot c/n,
\]

where \( c \) is the speed of light in vacuum and \( n \) is the mean refraction index of superficial skin tissues (\( n \sim 1.4 \) [12]). The photon mean path lengths in skin were found as the mean values of integrated path length distribution functions. Eventual error due to different slopes of both rising fronts (after the time-shifting) did not exceed 2 mm.

3. Results

Figure 2 illustrates the measured shapes of input laser pulse (IRF) and the skin-remitted pulses at two wavelength bands and several inter-fiber distances. The signal-to-noise ratio considerably decreased at shorter wavelengths (560 nm, 600 nm) and at longer distances between fibers (16 mm, 20 mm); these spectral-spatial combinations could be recorded only for two volunteers.

Results of deconvolution \( 1 \) are illustrated on Fig. 3 for the case of 760 nm wavelength band and two inter-fiber distances - 8 mm and 20 mm. As expected [1,2], the \( \delta \)-response functions \( f(t) \) are bell-shaped, with some initial time delay that corresponds to travel time of the first detected skin-scattered photons. The obtained photon arrival time distributions were further used to calculate the related mean values of photon travel times and the corresponding mean photon path lengths \( 2 \) for all 35 spectral-spatial combinations; the results are summarized in Table 1.
initial time delay of \( f(t) \) converted into path lengths of the first detected photons varied depending on the wavelength and the inter-fiber distances; all spectral-spatial combinations are illustrated in Table 2.

![Fig. 2](image)

**Fig. 2.** The 560 nm (a, c) and 800 nm (b, d) input and output pulse shapes at various inter-fiber distances (single volunteer data).

Dependences of the photon mean path length on the distance between fibers at all exploited wavelength bands are presented on Fig. 4. The differences are significant for the 560 nm, 600 nm and 640 nm bands (Fig. 4(a)), whereas in the spectral range 680-800 nm the photon mean path lengths appear to be similar within the error ranges (Fig. 4(b)). As for the 560 nm spectral band, only two volunteers measurements were successful which explains the relatively small dispersion of data (signal-to-noise ratio was close to one and the measurement error could not be properly evaluated).

As follows from Fig. 4, the photon mean path length dependencies on inter-fiber distance at all examined wavelength bands appear to be nearly linear, with the corresponding Pearson
Table 1. The mean skin-remitted photon path lengths (in mm, with standard deviation) for all available spectral-spatial combinations. The upper row represents inter-fiber distances.

| Central wavelength, nm | 1 mm  | 8 mm  | 12 mm | 16 mm | 20 mm |
|------------------------|-------|-------|-------|-------|-------|
| 560                    | 16 ± 3| 27 ± 3| 41 ± 2| 53 ± 5 | 62 ± 1 |
| 600                    | 19 ± 3| 37 ± 3| 53 ± 4| 68 ± 5 | 84 ± 8 |
| 640                    | 21 ± 3| 40 ± 3| 59 ± 5| 75 ± 4 | 94 ± 6 |
| 680                    | 23 ± 4| 41 ± 4| 64 ± 6| 86 ± 10| 110 ± 16|
| 720                    | 22 ± 2| 41 ± 4| 63 ± 4| 85 ± 8 | 106 ± 12|
| 760                    | 22 ± 2| 41 ± 3| 60 ± 3| 78 ± 5 | 96 ± 5 |
| 800                    | 26 ± 3| 42 ± 3| 63 ± 4| 84 ± 8 | 105 ± 10|

*a* data of a single volunteer

Table 2. The shortest path lengths in skin of the first detected photons (in mm).

| Inter-fiber distance (mm) / central wavelength, nm | 560  | 600  | 640  | 680  | 720  | 760  | 800  |
|--------------------------------------------------|------|------|------|------|------|------|------|
| 1                                                | 7 ± 3| 5 ± 2| 7 ± 3| 8 ± 4| 8 ± 4| 7 ± 4| 7 ± 4|
| 8                                                | 15 ± 13| 23 ± 17| 26 ± 21| 27 ± 24| 24 ± 20| 23 ± 19| 22 ± 18|
| 12                                               | 24 ± 15| 35 ± 24| 40 ± 32| 40 ± 30| 37 ± 30| 36 ± 28| 35 ± 28|
| 16                                               | 30 ± 18| 44 ± 28| 52 ± 38| 49 ± 37| 48 ± 36| 47 ± 34| 48 ± 35|
| 20                                               | 24 ± 17| 55 ± 34| 62 ± 43| 67 ± 47| 61 ± 44| 58 ± 39| 59 ± 42|

Fig. 4. The remitted photon mean path length as function of distance between the input and output fibers.

correlation coefficients in the range 0.97…0.99 (Table 3). However, it is not the case for the spectral dependencies (Fig. 5(a)) - at all inter-fiber distances (except for 1 mm) a pronounced maximum around 680-720 nm exhibits, with a following dip at 760 nm. Spectral dependences of the path lengths completed by the first detected photons (Fig. 5(b) and Table 2 show a similar trend. Possible correlation with absorption spectrum of the dermal hemoglobin [10] (marked by the dotted curves) will be discussed in the next paragraph.
4. Discussion

To the best knowledge of authors, this has been the first systematic experimental study of the photon travel times and distances before remission from in-vivo human skin at a relatively broad range of wavelengths and light input-output distances. So far, temporal data were obtained only for a few fixed wavelengths - 1064 nm [7], 405 nm and 510 nm [8]. Our volunteer measurements allowed evaluating the remitted photon mean path length values at all spectral (560, 600, 640, 680, 720, 760 and 800 nm) and spatial (1, 8, 12, 16 and 20 mm) combinations (Table 1) and exploring the trends of their spatial and spectral dependencies (Figs. 4, 5).

The obtained results qualitatively agree with theoretical assumptions that the tissue-scattered photon path lengths increase with the light input-output distance and with wavelength in the 560-800 nm range [14,15]. The obtained numerical values, however, appear to be somewhat higher than those predicted by modelling. In particular, at 1 mm inter-fiber distance for the 680 nm band our mean value is $23 \pm 4$ mm and $22 \pm 3$ mm for the 640 nm band, while the Monte Carlo (MC) simulation at similar conditions (659 nm, 0 mm) resulted in a lower mean value, $\sim 3$ mm [2]. It may seem surprising at the first sight; however, the most probable reason for such difference is the design of the MCML model which considers travels of all launched photons, both absorbed and scattered ones. If the absorbance in skin is high, path lengths of the absorbed photons are very short and, consequently, the total mean value of all photon path lengths has to be much lower than that of the back-scattered photons only (which were detected in this experiment). By using the MCML-based modeling approach, the systematic error in the photon pathlengths distribution can be extremely critical for the predictive quantitative assessment of...
photon pathlengths in the skin tissues. Eventually, some alternative Monte Carlo model, such as on-line Monte Carlo [4] could be used more successfully; in frame of this model the photon pathlengths are counted based on the Beer-Lambert law and the uncertainties with the variations of the photon paths are avoided. In this case the photon pathlengths distribution within the skin tissues are presented in terms of sampling volume [3,5]. Besides, the MC-models usually do not count the numerical aperture of the receiving fiber – if the fiber tip is in good contact with skin, mainly those photons travelling collinear to the fiber axis (i.e. orthogonal to the skin surface) are detected, and their path lengths certainly are longer than the statistically averaged pathlength of all launched photons. For longer wavelengths and inter-fiber distances our data (Table 1) generally agreed with the results of previous laboratory measurements on animal tissues [6] where the mean remitted photon path length was estimated to be \( \sim 5 \) times larger than the inter-fiber distance.

Within the measurement error range, practically linear dependencies of the photon mean path length in skin on the inter-fiber distance were obtained (Fig. 4) which generally agrees with the model-based expectations [16]. The differences in photon path length values are significant for the wavelength bands centered at 560 nm, 600 nm and 640 nm (Fig. 4(a)), while those at 680 nm – 800 nm intersect within the error range. Most probably, it reflects the dermal hemoglobin absorption which considerably decreases in the 560-640 nm spectral range, with less pronounced variations in the 680-800 nm range [13]. Impact of hemoglobin is also supported by the observed correlation of the hemoglobin absorption spectrum with the observed non-linear spectral dependencies of the photon mean path length in skin (Fig. 5(a)) and of the shortest path lengths related to the first detected photons (Fig. 5(b)). One can see that the maximum values of the photon mean path length are located in the 680-720 nm spectral range where the total hemoglobin absorption reaches its minimum; besides, in this spectral range light efficiently penetrates in the blood-rich dermal layer [16]. At 1 mm inter-fiber separation penetration of the detected light in skin is much lower than at separation of 8 mm or longer, therefore the spectral dependence at 1 mm is close to linear without a pronounced maximum.

Clearly, this study had certain limitations to determine the remitted photon path lengths in skin with higher accuracy. According to our estimates, the used set-up and signal processing technique allowed establishing the mean photon path lengths with accuracy of about 5 mm; the statistical spread of data between volunteers was of the same order. Using in future faster photodetectors and more advanced deconvolution algorithms may help to reduce the measurement/calculation error probably down to 1 mm; however, the statistical spread between individual humans most probably would remain on the same level.

5. Summary

Results of systematic experimental study on the back-scattered photon travel distances in healthy human skin were reported for the spectral range 560-800 nm and the interval of light input-output distances 1-20 mm. Mean values of the skin-remitted photon path lengths have been obtained at 35 spectral-spatial combinations, along with graphical representations of their spatial and spectral dependencies. These dependencies qualitatively agreed with the theoretical expectations while the obtained numerical values were considerably higher than those obtained by MC-modelling. This difference can be explained by involvement of absorbed photons in the model calculations while experimentally only the back-scattered photons were collected and analyzed. Impact of absorption by dermal hemoglobin on the spectral dependencies of the photon mean path length has been observed and discussed. The exploited methodology based on time-correlated single photon counting proved to be efficient for this kind of studies; higher accuracy of data might be achieved in future by using the “white” laser with higher output power and pulse repetition rate.
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Disclosures

The authors declare that there are no conflicts of interest related to this article.

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