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ABSTRACT

An experimental method for remitted photon path length measurements in scattering media has been developed and tested on human skin and skin neoplasms, skin phantoms and cell cultures. The photon time-of-flight (PTOF) measurement method was used in this study, where the photon travel time was converted into path length. Remitted light signals were obtained using a picosecond broadband laser and a set of narrowband interference filters in spectral rang 520 – 760 nm. Five different distances of 1, 8, 12, 16 and 20 mm between the source and detector fibers were used. Measurements were performed at different wavelengths and distance combinations; they were taken from human skin and skin malformations, agar-based phantoms with different concentrations of intralipid and hemoglobin, and from cell cultures (DC3F, B16/F10). Parameters related to the remitted photon mean path length will be presented and analyzed.

Keywords: Time-of-flight, photon path length, human skin, skin phantoms.

1. INTRODUCTION

Light penetration depth in tissues and related photon scattering path length are important characteristics in optical diagnostics and therapy. Composition and density of absorbing chromophores influence the scattering path length of diffusely reflected (remitted) photons in various tissues. Due to light scattering, the path length in tissues of each remitted photon can be different. The photon path length in tissues can be estimated theoretically using model calculations, e.g. Monte Carlo method[1,2]. The photon path lengths at specific wavelengths (783 nm[3], 1064 nm[4], 405nm and 510 nm[5]). were measured previously with similar method where two optical fibers were used for input and output signal. Literature sources about experimental studies in a broader visible spectral range for *in-vivo* skin and skin chromophores were not found.

The non-invasive method of photon path length estimation in human skin was created, developed and tested at Biophotonics laboratory of University of Latvia[5,6,7]. Experimental data from human skin, agar-based skin phantoms with intralipid and with/without hemoglobin, as well as from DC3F, B16/F10 cell cultures are presented in current study.

Skin *in vivo* studies are clinically important and can contribute to improvements of algorithms for Monte Carlo simulations[1,8] and for data processing algorithms in skin optical diagnostics[9,10].

2. THE MEASUREMENT SET-UP AND METHOD

The photon path length was measured with TCSPC (Time-correlated single photon counting) system, the setup is illustrated in fig.1. Set-up consists of hybrid photon counting detector HPM-100-07 combined with detector controller DCC-100 (Becker&Hickl, Germany). A broadband pico-second laser (Whitelaser micro supercontinuum lasers, Fianium, NKT Photonics, Denmark; pulse full width at half maximum (FWHM) 6 ps; repetition rate 20 MHz) was used as a light source which emits in the spectral range from 400 nm to 2000 nm.

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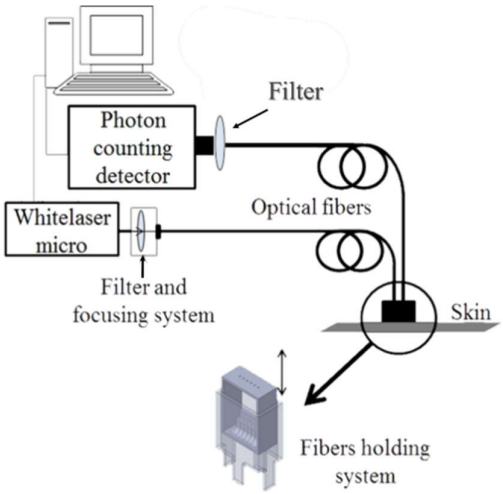

Figure 1. Photon time-of-flight measurements setup.

Spectrally narrowband input light was ensured by a set of two equal interference band filters (Andover Corporation, USA, mod. 520FS10-12.5, 560FS10-12.5, 680FS10-12.5, 760FS10-12.5 USA) with half-bandwidth of 10 nm and central wavelengths at 520, 560, 680 and 760 nm. To ensure optical signal between the input and output fibers (WF-400, Light Guide Optics International, Latvia; silica core diameter, 400 micrometers; and length, 1.05 m) at various distances between them, a special fiber holding system was designed (Fig. 1) with inter-fiber distances of 1, 8, 12, 16, and 20 mm.

The goal of this study was to measure photon path length in human skin, phantoms of skin with intralipid and with/without hemoglobin, and cell cultures in the spectral range of 520-760 nm. The data were collected for each selected wavelength by changing distances between fibers. To ensure equal pressure on the skin surface at all measurements, the probe was designed as a lift where the inside sliding part (with selected distances between the two fibers) lies on the skin providing a pressure determined by its weight, ~35 (g/cm^2); the fiber's holding system is shown in Fig. 1. The outside part of the probe was fixed on the skin or samples during the measurements.

Before the measurements, the skin input pulse shape or instrumental input function (IRF) was recorded by positioning the emitting and receiving fibers onto each other. This was done to determine the time scale for further measurements. Each measurement data was calculated as a mean value of three successively measurements, each ~1 second long.

The measurements were performed on the skin of different body parts of 6 volunteers aged from 25 to 68 with skin photo-type II and III (Fitzpatrick classification) under permission of the local Ethics Committee with written consent of the volunteers. The average spectral power density on skin was ~10 (mW/cm^2), i.e. well below the skin laser safety limit of 200 (mW/cm^2) [11].

If the distribution of remitted photon propagation times in skin $f(t)$ is measured, the corresponding distribution of photon path lengths can be found as

$$\phi(s) = f(t) \cdot c/n \quad (1),$$

where c is the speed of light in vacuum and n is the mean refraction index of superficial skin tissues (n ~ 1.36-1.4) [3, 12].

The function $f(t)$ could be measured directly if infinitely narrow δ-pulse of input photons would be launched into skin. As laser pulses with tens of picoseconds duration (at half-maximum) are used in our experiments. The skin, skin phantoms and cell sample-remitted pulses are temporally shifted and broadened, but they cannot adequately represent the distribution function $f(t)$. It can be found by de-convolution of the integral (2) [13] which is not a simple task from the mathematical point of view:

$$b(t) = \int_0^t a(t-\tau) f(\tau) d\tau \quad (2),$$

where *a(t)* is the temporal shape of input laser pulse and *b(t)* – the shape of skin output pulse at the same wavelength. Possible solutions using provisional analytic expressions for *f(t*) will be analyzed.

A special program for signal processing and automatization was created in MatLab; the fitting error, converted into path length, is ± 3 mm.

3. RESULTS

Figure 2 illustrates the results of mean photon path length through the skin and neoplasms. The measurements were taken in 5 difference distance between fibers (1, 8, 12, 16, 20 mm) and at four wavelengths 520, 560, 680 and 760 nm. The data was obtained from 6 measurements (skin and neoplasms). The signal for 520 nm and 560 nm for longer distance (16 and 20 mm) was weak and not counted. As expected, increased inter-fiber distance as well as increased wavelength have led to increased photon path length. During the experiment 6 neoplasms with a diameter of 8 to 11 mm were investigated, the measurements were taken at two distance between fibers 1 and 8 mm. The obtained data of mean photon path length in skin and neoplasms are illustrated in fig.2. and in table 1, for neoplasm only at two distances (1 and 8 mm). We observed that photon path length increases on body locations with more fat, which also results in increased error of average data for all measurements at longer wavelengths and larger distances between fibers.

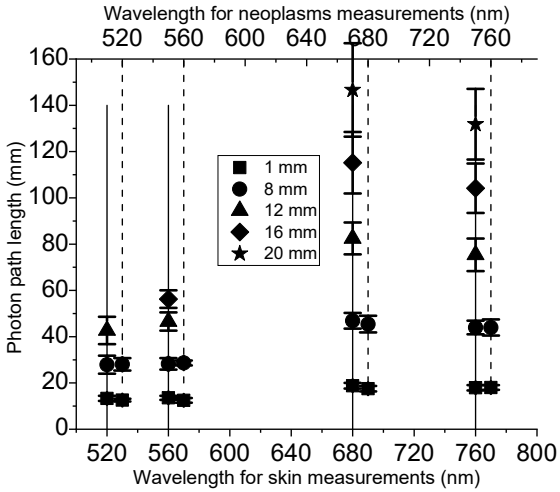

Figure 2. The mean remitted photon path lengths in skin and neoplasms. (–) the data for skin, (--) the data for skin neoplasms.

Table 1. The mean skin- and neoplasm-remitted photon path lengths (in mm, with standard deviation) for all available spectral-spatial combinations. The upper raw represents inter-fiber distances.

Central wavelength, nm	1 mm	8 mm	12 mm	16 mm	20 mm
Skin					
520	13 ± 1	28 ± 4	43 ± 6		
560	14 ± 1	28 ± 2	47 ± 4	56 ± 4	
680	19 ± 1	47 ± 3	82 ± 7	115 ± 13	147 ± 20
760	18 ± 1	44 ± 3	75 ± 7	104 ± 11	132 ± 15
Neoplasms					
520	13 ± 1	28 ± 3			
560	12 ± 1	29 ± 1			
680	18 ± 1	45 ± 4			
760	18 ± 1	44 ± 3			

Figure 3 illustrates the mean photon path length dependencies in phantoms. The phantoms comprised two different concentrations of additives, first was with 1% of intralipid concentration and second with 1% of intralipid and 1% of hemoglobin. The results were obtained for 4 wavelengths (520, 560, 680 and 760 nm) at 5 different distance between fibers (1, 8, 12, 16 and 20 mm). Hemoglobin is the main absorber in human skin below 600 nm. As expected, in phantoms with hemoglobin the mean photon path length is shorter if compared with that in phantoms without hemoglobin. The measurement results are illustrated at figure 3 and table 2.

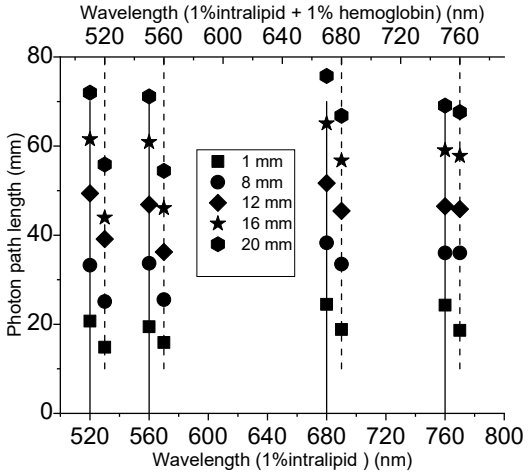

Figure 3. The mean remitted photon path lengths in skin phantoms. (–) the data for phantoms with 1% of intralipid concentration, (--) the data for phantom with 1% of intralipid and 1% of hemoglobin concentration.

Table 2. The mean remitted photon path lengths in skin phantoms (in mm, with fitting error ± 3 mm) for all available spectral-spatial combinations. The upper raw represents inter-fiber distances.

Central wavelength, nm	1 mm	8 mm	12 mm	16 mm	20 mm
Skin phantom with 1% intralipid concentration					
520	21	33	49	62	72
560	19	34	47	61	71
680	24	38	52	65	76
760	24	36	46	59	69
Skin phantom with 1% intralipid and 1% hemoglobin concentration					
520	15	25	39	44	56
560	16	26	36	46	54
680	19	34	45	57	67
760	19	36	46	58	68

Figure 4 illustrates the mean path length in medium of cell cultures with concentration 6 mill per 1 mL. The measurement was done in two cells cultures, B16/F10 as cancer cells and DC3F as healthy cells. The B16/F10 as cancer cells has more melanin, and measurements were done to determine influence of cell's type and melanin absorption to the mean photon path length. The data are illustrated at the fig. 4 and table 3.

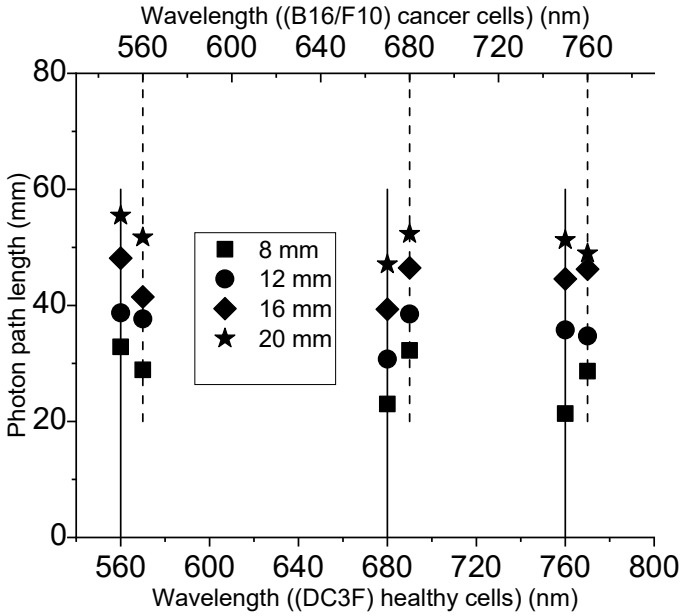

Figure 4. The mean remitted photon path lengths in cells cultures. (–) the data for DC3F cells, (--) the data for B16/F10 cells.

Table 3. The mean remitted photon path lengths in cells culture DC3F and B16/F10 (in mm, with fitting error ± 3 mm) for all available spectral-spatial combinations. The upper raw represents inter-fiber distances.

Central wavelength, nm	8 mm	12 mm	16 mm	20 mm
Healthy cells, DC3F				
560	33	39	48	55
600	27	37	45	53
640	26	41	48	57
680	23	31	39	47
720	26	34	41	50
760	21	36	45	51
800	30	40	48	54
Cancer cells, B161F10				
560	29	38	41	52
600	31	39	47	55
640	28	37	48	59
680	32	39	46	52
720	25	38	45	52
760	29	35	46	49
800	33	41	48	55

4. DISCUSSION

The results for human skin show increase of mean photon path length with increased wavelength, as it was expected, but it is not linear. The same tendency was observed in skin neoplasms - increasing wavelength increases the mean photon path length, but at shorter distances between fibers hemoglobin influence to photon path length was not observed.

The main goal of this study was to determine the photon path length in human skin and influence of skin chromophores. Non-linear photon path length increase most probably is caused by hemoglobin absorption at 760 nm, as that can see at Figure 2. The skin phantom with/without hemoglobin show reduced mean photon path length in phantoms with hemoglobin (Fig.3 and Tab.2). Comparing results of skin phantoms with and without hemoglobin (Fig.2,3 and Tab.1,2), the assumption of hemoglobin influence to photon path length is confirmed. The results of mean photon path length in neoplasms agree with results of skin within the measurement error. The mean photon path lengths in medium of cells with different melanin concentration do not show notable dependency on wavelength (Fig.4 and Tab.3). The unstable increase/decrease of photon path length in cells medium could be result of non-homogeneous distribution of cells.

5. CONCLUSIONS

The main conclusion of this study is that the remitted photon path length does not depend linearly on wavelength. Hemoglobin is the main chromophore which has influence on photon path length in human skin where melanin absorption is insignificant. Our further study will be targeted to measure different body locations at various thicknesses of skin and sub-dermal fat layer, with and without neoplasms.

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