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Simultaneous blood perfusion and fluorescence lifetime measurements in skin of healthy volunteers

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Abstract: In this work, we studied the individual variability of parameters measured by fluorescence lifetime spectroscopy simultaneously with the recording of blood tissue perfusion through a fibre-optic probe in the skin of conditionally healthy volunteers.

1. Introduction

The skin plays an essential role as a diagnostic interface for many types of non-invasive measurements. Non-invasive measurements of fluorescence parameters in the skin or mucous membrane are of particular interest for many diagnostic applications and for drug discovery and developing new pharmaceuticals [1, 2]. The group of fluorescence methods based on measuring the parameters of the fluorescence lifetime (FL) have found wide application in the studies of cells and living organisms capable of providing information on the biochemical interactions of fluorescent agents molecular level [3]. Pathological processes in the body lead to a change in tissues' fluorescent activity, which occurs due to a change in the accumulation of endogenous fluorophores [4]. Their quantitative assessment in cells and tissues can serve as a marker for early diagnosis of metabolic disorders leading to various body pathological states.

Among other factors, the level of tissue perfusion with blood also affects the level of endogenous fluorescence. The rate of blood filling changes the optical properties of the tissue, which leads to a change in fluorescence and complicates the interpretation of the data, and affects the metabolism of cells [5]. For reliable measurements, it is necessary to separately monitor the changes of blood flow in the diagnostic volume of the optical probe. The recent development of the wearable LDF monitors [6] and miniaturised SPAD detectors integrated with the semiconductor ps lasers [7] open new opportunities for both blood perfusion monitoring and FL measurements to be implemented as wearable devices. In that respect, the time series analysis of the FL components can be of be of particular interest for the future diagnostics applications.

In this work, we studied the interaction between the FL parameters measured in the skin by the method of time-correlated single-photon counting (TCSPC) and the level of blood perfusion evaluated by laser method Doppler flowmetry (LDF). We have combined both measuring channels in one fibre optic probe to implement the measurements from the same area of interest.

2. Materials and Methods

The experimental setup combined the two channels for the FL measurements and one channel for the blood perfusion recordings. BDS-SM-375-FBC-101 laser source (B&H, Germany) was used for the fluorescence excitation at a wavelength of 375 nm. Two hybrid photodetectors HPM-100-40 (B&H, Germany) with a spectral sensitivity range of 250-720 nm and quantum efficiency of 45% (for 500 nm) were used for the photon counting. Band-pass filters MF 479-40 (ThorLabs, USA) (channel 1) and MF 530-43 (ThorLabs, USA) (channel 2) were used for the selection of the fluorescence emission for the two recording channels. The FL channel in the fibre optic probe consisted of one probing and two collecting multimode fibres. The LDF measurements have been implemented using two multimode collecting fibres and one single-mode fibre for an 850 nm single-mode laser. The source-detector distance between emitting and collecting fibres for both measuring techniques was about 1 mm. The gender-balanced cohort of conditionally healthy volunteers consisted of 8 people with an age of 35 ± 5.6 years. For every volunteer, 5 min recordings were carried out in the skin of forearm with preliminary degreasing with ethanol.

3. Results and Discussion

The FL traces were processed with fitting by two exponential models. Representative traces of the recorded blood perfusion and parameters measured by the FL channel (emission filter 530±43 nm) are shown in Fig 1. Average FL parameters for the skin of forearm measured in the selected emission bands are presented in Table 1. The degree of the mutual relationship between blood perfusion, fluorescence intensity and the calculated FL parameters were evaluated using the Pearson correlation coefficient (Fig. 2).

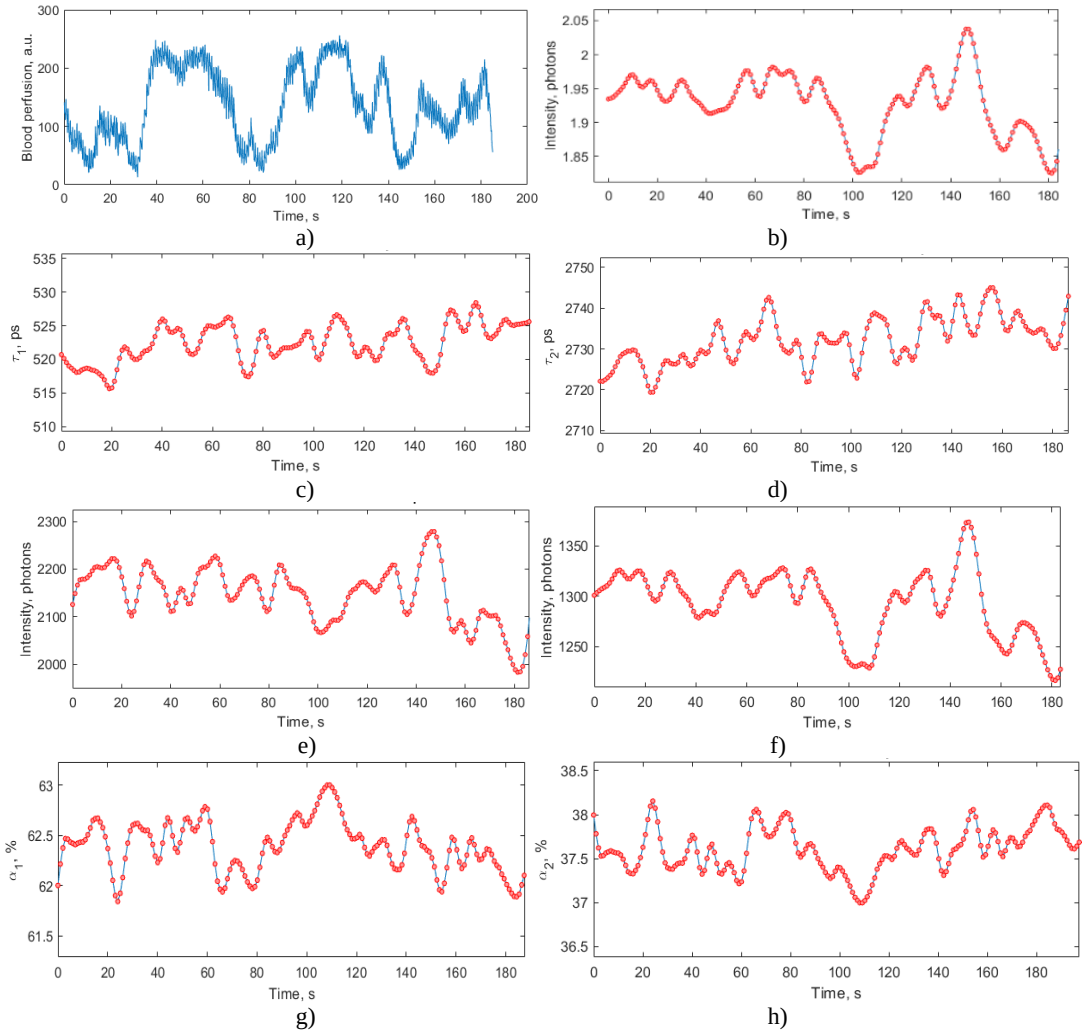

Fig. 1. Representative traces of the recorded blood perfusion and parameters measured by FL channel (with the emission filter at 530±43 nm for this example): a) LDF blood perfusion; b) fluorescence intensity; c) lifetime of the first component; d) lifetime of the second component; e) intensity of the first component; f) intensity of the second component; g) fraction of the first component in total fluorescence; h) fraction of the second component in total fluorescence.

Table 1. Average FL parameters for the skin of forearm measured in the selected emission bands.

Parameter	Emission band	Value
τ_1, ps	479±40 nm	551±41
	530±43 nm	505±62
τ_2, ps	479±40 nm	2904±59
	530±43 nm	2927±79
α_1, %	479±40 nm	56±2
	530±43 nm	57±2
α_2, %	479±40 nm	43±2
	530±43 nm	42±2

The absolute values of the measured FL times point out that the main detected in the measurements fluorescence agents are NADH and FAD both free and bound with protein. The dynamics and shape of the time series traces for channel two (530±43 nm) differs from the data from channel one (479 ± 40 nm). That might be explained by the stronger influence of the cellular fractions of FAD in the spectral band of emission. The intensity of fluorescence demonstrated a high degree of correlation with the blood perfusion. Whereas the lifetime of the first and the second components and their relative fraction in the total fluorescence, have shown a minor degree of linear relationship with LDF data.

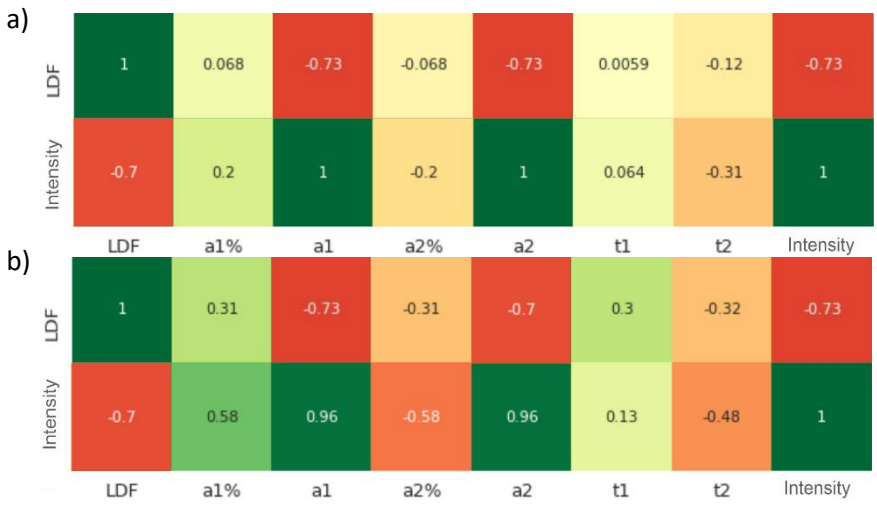

Fig. 2. Pearson correlation coefficients for LDF blood perfusion, fluorescence intensity and the calculated FL parameters: a) FL channel 1 - 479 ± 40 nm, b) FL channel 2 - 530 ± 43 nm.

The high correlation of the fluorescence intensity parameters with blood perfusion in significant degrees can be explained by the absorption of the fluorescence emission and excitation radiation by the blood. Nevertheless, the influence of the blood perfusion level on the processes in cellular tissue metabolism should also be taken into account.

4. Conclusion

In the talk, we present the setup for the multimodal recordings of the time series and results for the recorded traces' comparative analysis. Using the approach, we have assessed the individual variability of the FL parameters measured in healthy volunteers' skin with excitation at 375 nm and emission detection in two wavelength ranges (479 ± 40 nm and 530 ± 53 nm). The recordings were annotated by simultaneous traces of blood perfusion measured through the same fibre optic probe. The presented approach paves the way to the time series analysis of the FL data and blood perfusion for advanced physiological studies and the development of new diagnostic criteria for medicine.

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