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Review of the doctoral thesis
of **Saeed Samaei, MSc**
entitled:
"Assessment of depth-resolved blood flow changes in biological tissues based on time-domain diffuse correlation spectroscopy (TD-DCS) technique"

General remarks

The formal legal basis for the review is entrusting me with the role of the reviewer of the doctoral dissertation of Saeed Samaei, entitled: "Assessment of depth-resolved blood flow changes in biological tissues based on time-domain diffuse correlation spectroscopy (TD-DCS) technique" by the Scientific Council of the Nałęcz Institute of Biocybernetics and Biomedical Engineering of the Polish Academy of Sciences at the meeting on September 27, 2022. I received the documentation related to the review on October 17, 2022, in electronic form (I received the doctoral dissertation in paper form on October 25, 2022). The supervisor of the dissertation is Adam Liebert, Prof., PhD, DSc, Eng. and the assistant supervisor is Dawid Borycki, PhD.

The dissertation is written in English. It consists of six chapters preceded by a summary in Polish and English, a list of publications of the author related to the research presented in the thesis, acknowledgments, a list of figures and tables, a table of contents, as well as a declaration that the work was done independently and its results are not have been used by other persons to obtain a degree or academic title or professional qualification. At the end of the dissertation, the author included a bibliography. In total, the dissertation has 83 pages.

The subject of the dissertation

The dissertation of Saeeda Samaei deals with the assessment of changes in blood flow with depth discrimination in biological tissues based on *time-domain diffusion correlation spectroscopy* (TD-DSC).

The nature of the dissertation

The dissertation is mainly analytical and experimental. Theoretical knowledge was used mainly in the description of the propagation of optical radiation pulses in a highly scattering medium to better understand the mechanisms affecting the depth of radiation penetration and the distribution of surface power density of backscattered radiation as a function of the distance from the center's excitation site. Theoretical knowledge was also used to present the dependence of the distribution of the time of flight of photons between two points on the surface of the investigated highly scattering object and to present the autocorrelation of radiation intensity as a function of the time of flight of photons in conditions where the medium contains moving scattering particles.

Research problem and thesis

The introduction, which is the first chapter of the dissertation, describes the importance of assessing blood flow in biological tissues, with particular emphasis on brain tissues. The author presented various methods of measuring blood flow in tissues and found that only a few of them are suitable for continuous monitoring. One of them is transcranial Doppler ultrasonography, the disadvantage of which is that it can only be used in the case of a thin skull and that blood flow can only be measured in large blood vessels. In the small vessels of the brain, blood flow can be measured using *positron emission tomography* (PET), *single-photon emission computed tomography* (SPECT), or *functional magnetic resonance imaging* (fMRI). Unfortunately, the PET and SPECT methods use radioactive substances, which exposes the patient to ionizing radiation, and the fMRI method uses very strong magnetic fields, which means that it cannot be used in patients with a cochlear implant or a pacemaker. In addition, the use of these methods requires patient transport and cannot be used for continuous bedside monitoring. Some methods use sensors to measure the partial pressure of oxygen in brain tissues or the oxygen saturation of the jugular veins, but these are invasive methods that require sensors to be placed directly into the tissue. For these reasons, the author focused on non-invasive optical methods in the further part of the chapter to assess changes in blood flow with depth discrimination in biological tissues. These methods include *laser Doppler flowmetry* (LDF) and *laser speckle contrast imaging* (LSCI). However, LDF and LSCI measurements are limited to the superficial tissue layers. By contrast, the measurement of diffuse-scattered radiation enables the study of changes in the homodynamic state of tissues in the depth range from a few millimeters to several centimeters. This method using *near-infrared spectroscopy* (NIRS) has been used in clinical studies related to the measurement of tissue oxygen saturation and hemoglobin concentration, as well as in determining changes in blood flow.

Another optical method of non-invasive measurement of changes in blood flow in small blood vessels is *diffuse correlation spectroscopy* (DCS) using a monochromatic source. In this method, blood flow is measured based on changes in diffuse-scattered radiation intensity in the time, depending on the radiation propagation time in the tissue, using the measurement of the decay time of the autocorrelation function of the measured optical signal as a function of radiation propagation time.

In the dissertation, the author proposed a method of measuring changes in blood flow, also based on correlation spectroscopy, in which a pulsed optical radiation source generating picosecond pulses instead of a continuous radiation source is used. This method is known in the literature as *time-domain diffuse correlation spectroscopy* (TD-DCS). However, previous studies have not been able to isolate blood flow in different layers of tissue with depth discrimination.

In the dissertation, the author formulated the hypothesis that **"the time-gated intensity autocorrelation function obtained from layered multiple scattering media using TD-DCS carries information on the movement of particles contained at different depths in this medium. Therefore, obtaining depth-resolved blood flow information from the living tissue is feasible by employing a comprehensive model describing the relationship between the movement of particles at different depths in the tissue with the measured time-resolved DCS data."** This thesis is formulated clearly.

Solution of the problem

The theoretical foundations of propagation of optical radiation in a highly scattering medium were presented in the second chapter. The first two subchapters of this chapter present the influence of absorption and scattering phenomena on the propagation of radiation in a highly scattering medium as well as absorption coefficient and reduced scattering coefficient as functions of the wavelength of infrared radiation of selected tissues, propagation of optical radiation based on the theory of photon diffusion, and also the influence of boundary conditions on propagation, and the basics of *time-domain near-infrared spectroscopy* (TD-NIRS) based on the measurement of the time of flight of photons. In the next subchapter, the author described the basics of the DSC method, and in the next one – the theoretical basics of the new, proposed method, which is the TD-DCS method. The last section presents the influence of noise on the autocorrelation function determined by the TD-DCS method.

The measurement system developed by the author using the TD-DCS method is presented in the third chapter. In this chapter, the author presented the block diagram of the TD-DCS system, the pulse response of the system, and the methods used for processing measurement data. The measurements were made using three sources of pulsed radiation: a custom-made tunable (wavelength in a range from 700 nm to 1030 nm) mode-locked picosecond titanium-sapphire laser generating pulses (the author did not provide the duration of the pulses) with a frequency of 100 MHz and an average power of 100 mW, a picosecond semiconductor laser (type LDH-PCN-760, PicoQuant GmbH, Germany) generating pulses with a duration of shorter than 90 ps at a frequency of 80 MHz and the average power 12 mW (a wavelength is equal to 760.4 nm), and a fast laser module using a laser diode (type VisIR-765-HP "STED", PicoQuant GmbH, Germany) generating pulses with a duration shorter than 550 ps with a frequency of 80 MHz and an average power of the 560 mW (a wavelength is equal to 765.2 nm). The radiation detector was an avalanche photodiode optimized to detect single photons (PDM type, Micro Photon Devices, Italy). The signal from the photodetector was delivered to the single photons counting with the time correlation system, which was synchronized with the laser pulse triggering system.

Initially, the developed system was used by the author (as part of preliminary tests) to measure the distribution of the time of flight of pulses through a tissue phantom with an absorption coefficient of 0.06 cm^{-1} and a reduced scattering coefficient of 10 cm^{-1} for a wavelength of 760 nm. To make the phantom, the author used a mixture of distilled water, 20% intralipid (B. Braun Melsungen, Germany), and black ink (Rotring, Germany). Optical impulses were delivered from the source to the surface of the phantom and from the surface of the phantom to the photodetector using optical fibers. The distance between the places where the optical radiation was introduced into the phantom and where it was received from the phantom and sent further to the photodetector ranged from 10 mm to 15 mm during the measurements. For the same phantom, the so-called the time-gated coherence coefficient β of the radiation reaching the detector, defined as the time-gated normalized function of the irradiance autocorrelation coefficient for the delay time τ tending to zero minus 1 (definition (2.24) in the dissertation) was measured. Based on the measurements, it was found that the increase in gating time causes a reduction in the coefficient β and an increase in the signal-to-noise ratio. Measurements were made for gate times ranging from 50 ps to 400 ps. The values of the coefficient β were strongly affected by the duration of the laser pulses.

In order to assess the possibility of measuring the speed of scattering particles in a medium of different viscosity, measurements of the distribution of the time of flight of photons through tissue phantoms having different viscosity were performed. Viscosity changes were obtained by using phantoms that contained glycerol with different concentrations (0%, 10%, 30%, and 50%). The absorption coefficient of these phantoms was 0.02 cm^{-1} and the reduced scattering coefficient was 11 cm^{-1}. Changes in the position of the scattering particles were caused by Brownian motion. Differences in the distributions of the time of flight of photons through these media were, as expected, insignificant, but the influence of the speed distribution of the scattering particles (depending on the viscosity of the phantoms) on the time-gated intensity autocorrelation function, and, more specifically, on the decay time of the autocorrelation, turned out to be very large. Thus, changes in this time can be correlated with changes in the velocity distributions of scattering particles in the phantom.

Next, this system was used to measure the distribution of the time of flight of photons through the tissues *in vivo* – through the skin located above the flexor carpi radialis muscle of the upper arm. Measurements were carried out in conditions of free blood flow through the examined tissue and in conditions when this flow was blocked by a tourniquet placed on the arm. Based on the time-gated intensity autocorrelation function, a strong relationship between the relative *blood flow index* (BFI) and states when the blood flow was free and when it was blocked with a tourniquet was obtained. This strong relationship occurred when the relative BFI was determined from the measured intensity autocorrelation function for a delay of 390 ps from the time at which the measured impulse response of the systems reaches a maximum value and for a 100-ps gating interval. By carrying out this experiment, the author proved that BFI changes can be detected based on the measurement of the time-gated intensity autocorrelation function.

The method of determining the BFI with depth discrimination in biological tissues based on measurement data from the TD-DCS system is described in detail in the fourth chapter. To experimentally verify the validity of the thesis, the author developed phantoms in the form of a cube-shaped cuvette with a side length of 6 cm and whose walls were made of a black material that strongly absorbs optical radiation. The inside of the cuvette was filled with a homogeneous, highly scattering medium in a liquid state, where the movement of particles occurs under the influence of Brownian motion. The author also developed phantoms in which the cuvette contained two layers of medium with the same absorption coefficient and reduced scattering coefficient as in the phantom with a homogeneous medium. The upper layer was the medium in a liquid state with a higher viscosity than the lower one, i.e. a liquid in which the movement of scattering particles took place at a lower speed than in the layer below, or the medium in a solid state, in which the movement of scattering particles did not take place. The thickness of the upper layer was 5 mm. In the case when both layers were a liquid medium, they were separated from each other by a thin transparent mylar film with a thickness of 23 μm. In the upper wall of the cuvette, there were two holes 10 mm apart, covered with a thin transparent foil to prevent the liquid filling the cuvette from leaking out. Through these holes, optical radiation pulses were supplied to and removed from the phantom by optical fibers. As a liquid medium, the author used a mixture of distilled water, milk (3.2% fat content) or intralipid (in the case of a phantom with an upper layer in the solid state), glycerol with a purity >99.5%, and diluted black ink. Diluted black ink was added to the phantoms in such an amount that the liquid absorption coefficient was 0.06 cm^{-1}. Three phantoms in the form of a homogeneous liquid were prepared, in which the reduced scattering coefficient was 7.5 cm^{-1}, 10 cm^{-1}, or 12.5 cm^{-1}. In phantoms using two layers of liquid, the top layer contained 30% glycerol to increase viscosity.

For such phantoms, the author measured the distribution of the time-of-flight of photons between the holes located on the upper wall of the phantoms and determined the time-gated normalized function of the irradiance autocorrelation coefficient $g_2(t_s, \tau)$ for various time gate opening delays t_s from the time of phantom excitation and different delays τ. For these functions, the author determined the decay time of the autocorrelation curve $\xi(t_s)$ by fitting the theoretical decay curve to the curve obtained as a result of the measurement. The model in which the decay time of the autocorrelation function is determined in this way was named by the author as the standard model. In this model, a good fit can be obtained for phantoms that are a homogeneous strongly scattering medium and for two-layer phantoms for small time gate opening delays t_s (i.e. when photons propagated mainly in the upper layer are recorded) – the author presented the function $g_2(t_s, \tau)$ for $t_s = 0.3$ ns on the graph. For average delays ($t_s = 0.6$ ns), when trying to fit the theoretical curves to the curves obtained by measurement for two-layer phantoms, we obtain a situation in which these curves cannot be matched. This is because for medium delays photons that propagated both in a medium with a low (for a liquid medium with increased viscosity by glycerol) or zero (for a solid-state medium) speed of scattering particles and through a medium with a relatively high speed of scattering particles are recorded. We obtain a similar situation for long delays ($t_s = 0.9$ ns), but we obtain a slightly better fit of these curves due to the relatively smaller number of photons that propagated only in the upper layer.

In the model proposed by the author, two decay times of the autocorrelation curve, corresponding to fast and slow-moving scattering particles, were introduced. The appearance of a sharp increase in the component of the autocorrelation function associated with fast-moving particles for a time gate opening delay greater than 600 ps (i.e. for a delay after which the share of photons that propagated in the lower layers of two-layer phantoms rapidly increases) allows speed differences in each of the phantom layers to be detected. By fitting the theoretical autocorrelation curve, proposed by the author, to the experimental data, the author obtained a good fit for both small and large time gate opening delays t_s, which allows determining the value of the product of the share of moving scattering particles in the total number of scattering particles and the diffusion coefficient associated with Brownian motion. In the case where the speed of the moving scattering particles corresponds to the blood flow in the tissue vessels, the product determined in this way is named the blood flow index. Based on the experiment, the author showed that the time-gated autocorrelation function of the intensity of propagating radiation in a multilayer scattering medium, obtained using the TD-DCS method, provides information about the movement of particles at different depths of this medium, **and thus proves the thesis of the dissertation**.

To prove that obtaining information on blood flow at different depths of living tissue is possible by the use of a comprehensive model describing the relationship between the movement of particles at different depths in the tissue and measurement data obtained by a system using the time-resolved diffusion correlation spectroscopy method, the author used the method for phantoms *in vivo* measurements. In these measurements, the author determined changes in the relative blood flow index in the forearm after the end of the occlusion using a tourniquet placed on the arm and, in another experiment, changes in this index on the skin on the frontal surface of the head and in the brain tissues when the skin was subjected to pressure. In the case of the forearm, thanks to the new method, it was possible to distinguish the changes in relative blood flow index depending on the depth of the flow. The experiment, repeated three times on head tissues, was performed on a healthy 29-year-old man. The author determined changes in the relative blood flow index in the outer layers of the head tissues depending on the depth induced by the application of pressure with a device pressing the skin on the frontal surface. Compression was applied using a profiled plate with a curvature matching the curvature of the head. The force applied to this plate came from a movable piston placed in a cylinder to which air was supplied under controlled pressure. The measurement was made under the place where the pressure was applied – the distance between the places where the optical radiation was introduced into the head tissues and where it was received from the head tissues and sent further to the photodetector was 1 cm. The autocorrelation function of the radiation intensity propagating in the head tissues, obtained using the TD-DCS method, was determined for the pressure values affecting the skin surface of 0 mmHg, 150 mmHg, 200 mmHg, and 250 mmHg. Using the method developed by the author, he showed that with the increase of this pressure, the calculated relative blood flow index in the skin decreases, while under the surface of the skull bones it remains constant within the margin of error. In the standard method, such dependence was not obtained. Thus, the **author proved that obtaining information about blood flow at different depths of living tissue is possible by the use of a comprehensive model describing the relationship between the movement of particles at different depths in the tissue and measurement data obtained by a system using the time-resolved diffusion correlation spectroscopy method**.

When proving the thesis and solving the encountered problems, the author used the right methods, and the assumptions made can be considered valid – some critical remarks can be made to *in vivo* experiments and to the interpretation of measurement data (see the section "Substantive remarks, weaknesses of the thesis").

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Author's knowledge and knowledge of literature

The contents of the doctoral dissertation allow concluding that the author has a good knowledge of conducting biomedical research and of defining and effectively solving problems occurring on the border of medicine, optoelectronics, and advanced diagnostic techniques. Knowledge of literature is good and its use is appropriate. Mostly, the author cites literature from the last decade, coming from renowned scientific journals with a global reach. However, a certain insufficiency is related to the lack of reference to the literature on the description of propagation of radiation in a highly scattering medium using the time-dependent Boltzmann transport equation and the differences in predicting the distribution of the time of flight of photons in a strongly scattering medium using the diffusion method and the method using the solution of the aforementioned Boltzmann equation (see the section "Substantive remarks, weaknesses of the thesis"). The bibliography of the dissertation includes 171 items.

Contribution of the dissertation to the development of optical diagnostic techniques

The method developed by the author is a good basis for further research, the aim of which is to implement the new method of assessing the blood flow index in clinical practice, where such an assessment is required not only in the subsurface layer of the tissue. Such an implementation would be advisable not only to assess the blood flow index in brain tissues but also in other organs, e.g. skin after burns, skin after transplantation, and heart after implantation of bypasses or stents.

The original work of the author

The original achievements of the author include:

1. Proof of a thesis of great practical importance, i.e. showing that the non-invasive optical method, which is time-resolved diffusion correlation spectroscopy, can assess changes in blood flow with depth discrimination in biological tissues.
2. Showing that time-resolved diffusion correlation spectroscopy can be used to determine the blood flow index in tissues as a function of depth.
3. Construction of an optode which, using a new method of data analysis from the time-resolved diffusion correlation spectroscopy system, can estimate the blood flow index from a depth greater than 6 mm.
4. Publications of research results related to the doctoral dissertation in three articles in journals from the JCR list: i.e. in APL Photonics (in which the author is the second co-author), in Biomedical Optics Express (the first co-author), and in Scientific Reports (the first co-author), as well as in one post-conference article from the congress organized by Optica (formerly OSA) (in which the author is the first co-author). The author also has achievements related to a scientific activity that was not directly related to the dissertation: he is the co-author of four post-conference papers in Proceedings of SPIE and an article from conferences organized by Optica (in which the author is the first co-author).

Substantive remarks, weaknesses of the thesis

1. Chapter 2 presents the theory of propagation of optical radiation in a highly scattering medium based on the diffusion method. However, it should be noted that the time-dependent solution of the diffusion equation (equation (2.5)) for short pulses of optical radiation is characterized by relatively large errors for early photons, e.g. the time-dependent reflectance (equation (2.10)) takes non-zero values for each time $t > 0$, which is not true, because it would mean that part of the radiation in a highly scattering medium propagates at a speed higher than the speed of light. Also, the maximum value of the

time-dependent reflectance calculated based on equation (2.10) appears earlier than in reality or when we determine it from the solution of the time-dependent Boltzmann transport equation, which describes the propagation of optical radiation in a highly scattering medium much more accurately than-dependent diffusion equation (see e.g. M.S. Patterson *et al.*, Appl. Opt., 1989, 28(12), 2331–2336, or S.L. Jacques, IEEE Trans. Biomed. Eng., 1989, 36(12), 1155–1161). Both equations give practically the same solutions for times of flight of photons three times longer than the time for which there is a maximum of time-dependent reflectance. Hence, the question arises: how accurate are the decay time-based blood flow indices when we use equation (2.27) derived from the model based on the time-dependent diffusion equation?

2. Changes in the relative blood flow index obtained for a long delay in opening the time gate t_s may raise doubts, because in the case of the brain, the observed movement of scattering particles comes not only from the blood flow, but also (probably to a much greater extent) from the surface of the brain moving under the influence of heart rate (see e.g. D. Greitz *et al.*, Neuroradiology, 1992, 34(5), 370–380), as well as breathing. Considering that there are many more scattering particles in the brain than in the blood, the interpretation of the measurement data may be incorrect. According to this reviewer, it would be better to perform a similar experiment (obviously after obtaining the approval of the relevant ethics committee) on a patient with cerebral edema, when, as a result of high intracranial pressure, the width of the subarachnoid width is zero, and thus the surface of the brain is immobile relative to the skull bones.
3. What is the reason that in the case of *in vivo* blood flow studies in the head tissues, the relative blood flow index, shown in Fig. 4.18 c), determined for the long time gate delay for the pressures applied to the skin of 150 mmHg, 200 mmHg, and 250 mmHg, is about 0.4–0.5, since this pressure should not affect the blood flow in the brain? Why is this ratio not around 1?
4. What was the person's systolic blood pressure during the head tissue blood flow experiment? If the pressure applied to the surface of the skin is greater than the systolic pressure, the skin blood flow should cease. Assuming (as the author stated in the dissertation) that the person was healthy, the relative rate of blood flow in the skin for the applied pressure of 200 mmHg and 250 mmHg should be the same. If the subject was rested and not suffering from hypertension, also the relative skin blood flow index for the applied pressure of 150 mmHg should be the same as for the pressures of 200 mmHg and 250 mmHg.
5. The thesis lacks a broader discussion on the selection of sources of pulse radiation from the point of view of its coherence length. What is the influence of the coherence length of the optical radiation source in the time-resolved diffusion correlation spectroscopy used to measure the blood flow index? What are the requirements for this parameter? Would it be advisable to use a source with a limited coherence length – what would be the optimal value of this length?
6. What prevented more people from taking place *in vivo* experiments? The proof of the thesis related to blood flow at different depths of living tissue for more people would be stronger.

Editorial notes

1. The blood flow rate shown in Fig. 4.16 b) and c) as well as in Fig. 4.17 b) and c) begins to drop rapidly even before occlusion – this should not be possible. The gray area showing the occlusion should start slightly earlier in the drawings.
2. Fig. 4.18 – in the legend, there is an undefined term "Exp ACFs". One can only guess that this is a model in which two components and two decay times were used to describe the autocorrelation function.

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3. Function names should not be italicized, e.g. the function “exp” in formulas (2.8)–(2.10), (2.17), (2.26), (2.28), (2.29), (2.31)–(2.33), and (2.38) is misspelled in italics.
4. In formulas, symbols that are not variables should not be italicized. This applies primarily to indices. Similar shortcomings are found in the work outside the formulas.
5. Formulas should be treated as parts of sentences to which rules related to punctuation should be applied. Therefore, if, for example, the next sentence begins after the formula, then the formula should be followed by a period. If we continue a complex sentence after the formula, e.g. when the continuation begins with the word “where”, then there should be a comma after the formula.
6. Physical units used in the work, such as mW, m, cm, mm, µm, nm, ns, ps, and cps are very often written incorrectly in italics.

Summary

The presented doctoral dissertation contains a description of an attempt to solve a very important problem in the field of medical diagnostics, which is the measurement of blood flow in tissues, taking into account depth. To solve this problem, the author proposed the use of time-resolved diffusion correlation spectroscopy, for which he developed a new method of measurement data processing, enabling the differentiation of layers in which blood flow takes place and the assessment of the blood flow index. The new method was verified experimentally for phantoms and *in vivo* for living tissues for two layers. In the proposed method, the extension for a larger number of layers seems to be possible by increasing the number of exponential terms with different decay times. To solve the problem formulated in the thesis, the author conducted literature studies related to the modeling of propagation optical radiation in a highly scattering medium based on the time-resolved diffusion equation and presented the influence of moving scattering particles on the autocorrelation of the intensity of radiation propagating in such a medium. Based on the conclusions resulting from this model, he developed the TD-DSC system to measure the movement of these particles. He used this system to measure the movement of scattering particles under the influence of Brownian motion in phantoms containing layers of a highly scattering medium with different viscosities and to measure blood flow in tissues *in vivo*. Thanks to the use of a new method of processing measurement data, the author showed the possibility of evaluating the movement of scattering particles as a function of depth, and thus proved the thesis put forward in the thesis.

It deserves the recognition that the research results presented in the doctoral dissertation were published, among others, in reputable, highly rated journals from the JCR list: in APL Photonics (impact factor in 2021 $\mathrm{IF}_{2021} = 6.382$, 100 points of the Ministry of Science and Education of Poland list), in Biomedical Optics Express ($\mathrm{IF}_{2021} = 3.562$, 140 points), and in Scientific Reports ($\mathrm{IF}_{2021} = 4.996$, 140 points).

In conclusion, I state that the **work presented by Saeed Samaei, MSc meets the requirements for doctoral theses** specified in art. 187 of the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended), and thus **I recommend the thesis for public defense**. Also, taking into account the complexity of the tests performed, their great practical importance in medical diagnostics, as well as the publication of the results of work in reputable journals on the JCR list, of which he is the first co-author in 2 items, **I include the doctoral dissertation of Saeed Samaei, MSc to the category of outstanding, deserving of distinction**.

J. Pluciński