Prof. dr hab. eng. Malgorzata Kujawińska
Warsaw University of Technology
e-mail: malgorzata.kujawinska@pw.edu.pl

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REVIEW
doctoral dissertation by Mr Saeeda Samaei
„Assessment of depth-resolved blood flow changes in biological tissues based on time-domain diffuse correlation spectroscopy (TD-DCS) technique"

The subject of the review is 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", supervised by Prof. Adam Liebert, and Assistant Supervisor by Dr. hab. Dawid Borycki. The review was prepared at the request of the Deputy Director of the Maciej Nałęcz Institute of Biocybernetics and Biomedical Engineering of the Polish Academy of Sciences, Prof. Dorota G. Pijanowska (letter No. SN/003/10/2022 of 14.10.2022).

In accordance with the current regulations of the Law on Higher Education and Science of 20 July 2018 (Journal of Laws of 2021, item 478, as amended), the role of the reviewer is to determine whether the person applying for a doctoral degree has the required scientific achievements (requirement of Article 186), and whether the doctoral dissertation presents the general theoretical knowledge of the candidate in the discipline, as well as the ability to independently conduct scientific work (requirement of Article 187 paragraph 1), and, above all, whether the subject of the doctoral dissertation is an original solution to a scientific problem (requirement of Article 187 paragraph 2).

After studying the dissertation, I have no doubts about the following facts:

1. The subject matter of the dissertation fully corresponds to the area of the discipline of biomedical engineering in the field of engineering and technical sciences.
2. The dissertation is theoretical and experimental, which is reflected in the thesis of the dissertation. The hypothesis (set out on page 8) is clearly formulated and verifiable on the basis of the hearing submitted.
3. Support of the dissertation with three co-authored publications (in two Saeed Samaei is the first author) on the topic of the dissertation, included in the JCR database with an average IF value of 4.63 is the basis for stating that the statutory requirement of Art. 186 has been met, as well as a premise for finding a solution to the original scientific problem.

The doctoral dissertation under review concerns the extension of the functionality of the diffuse correlation spectroscopy (DCS) method used to measure the blood flow index (BFI) in human tissue, in particular cerebral blood flow. The choice of the topic of the work is important from a scientific point of view, but above all from an application/social point of view, because BFI measurements play a critical role in many clinical applications. The doctoral candidate focused on the recently introduced time-domain diffuse correlation spectroscopy (TD-DCS) method, which uses a picosecond light source to separate measured photons based on their time-of-flight.

The layout of the work is quite typical for theoretical-experimental work. In chapter 1, the PhD Candidate motivated the undertaking of the above-mentioned topic of work, presented an extensive literature study (cited 122 literature items) enabling the formulation, on page 8, of a scientific hypothesis. This hypothesis says that the time-gated (TD) function of autocorrelation intensity obtained by the TD-CDS method from a layered scattering medium contains information about the movement of molecules at different depths of this medium. Therefore, it is possible to obtain information about BFI in living tissue by using a comprehensive model determining the relationship between the movement of molecules at different depths and TD-CDS measurement data. To prove the hypothesis, Mr Samaei proposed (Chap. 2.4) and subjected to experimental evaluation (Chapter 4) a new model of the autocorrelation intensity function.

The whole work can be divided into four parts. Chapters 1 and 6 cover the introduction and conclusions respectively. Chapter 2 is devoted to a discussion of the theory of diffuse optics, with particular emphasis on the problems associated with the use of the standard model using the Siegert relationship. Against this background, a modified theoretical model for determining BFI with depth discrimination for ergodic and non-ergodic centers was proposed. The PhD Candidate rightly pointed out two basic limitations of the model: (i) the impact on the measurement results of the actual instrument response function (IRF), which should be as narrow as possible, and (ii) the need to use narrow time gates in the model, which in the experiment is associated with a weak signal and a low CNR (contrast-to-noise ratio).

In Chapter 3, Mr Samaei presented the TD-DCS system he built and the implemented data acquisition and processing process as well as a description of measurement setups for studying the system itself and experiments with phantoms and *in vivo*. Due to the key impact of the instrument response function on the results, a large part of this chapter is devoted to the experimental determination of IRFs for various detectors and radiation sources. For further experiments, the SPAD detector was chosen as giving a narrower IRF. Extensive studies of emitters (spectral range, coherence pathway, IRF, various gating, etc.) showed the greatest usefulness of the LDH emitter, despite the nonlinear distribution of the IRF. The experiments (discussion of the results in Chapter 5) presented in this chapter indicated the possibility of better understanding the results by choosing the width and location of the time gate.

The most important chapters, from the point of view of confirming the hypothesis put forward by the PhD Candidate, are chapters 4 and 5, in which the proposed theoretical model was verified experimentally on several types of phantoms and in *vivo* experiments. (results – chap.4, discussion – ch.5). Studies of the liquid bilayer model have shown that the application of the new model makes it possible to distinguish between particles moving in shallow and deeper layers. The solid-liquid model experiment showed the possibility of obtaining flow information in a liquid sample hidden under a plate with a thickness equal to half the distance between the lighting and detection points. This result was later used in comparative studies in *vivo* experiments. In *in vivo* studies, a new theoretical model was used to quantitatively assess the BFI time courses in an experiment involving forearm occlusion in healthy volunteers. The PhD Candidate showed that changes in BFI in surface tissues (dermis) can be measured separately from muscle BFI, and BFI can be quantified even when a few millimeters static dispersing layer covers the forearm muscle. The final and very important (from the point of view of future applications) verification of the new model was the development of a pressure device to exert controlled pressure on the human forehead *in vivo* and to demonstrate the possibility of determining BFI in the deeper layers (subcranial) of the head.

I believe that the hypothesis put forward at the beginning of the work has been fully proven by the presented research, in which I accept as the originality of the solution of a scientific problem:

1. Proposing and implementing in the TD-CDS system a new, theoretical model for determining the rate of change in blood flow with depth discrimination for multilayer ergodic and non-ergodic centers. The originality of the solution at the world level is evidenced by its publication in *Scientific Reports* 11(1), pp.1-10 (2021) - 22 citations, of which the PhD Candidate is the first co-author.
2. Perform an extensive analysis of the 3 radiation sources used in the TD-DCS and evaluate the influence of source and detector parameters on the impulse response function and measurement capabilities of the TD-CDS. The results of this research were published in the journal *Biomedical Optics Express*, 12(9), pp.5351-5367 (2021) – 4 citations, of which the PhD Candidate is the first author.
3. Building the TD-DCS system together with the development of a set of phantoms that enabled the validation of the proposed model for determining the blood flow change index for various measurement media and conditions (experimental parts in papers in *SR* and *BOE*).
4. Development of a measurement methodology and *in vivo* experiments consisting of (i) forearm occlusion in healthy volunteers and (ii) controlled pressure on the human forehead (in this case also development of a pressure device for the experiment). In both cases, the possibility of discriminating the surface effects of BFI from signals from deep layer signals (described in the experimental parts of Articles *SR* and *BOE*) was demonstrated. These results provide a good prognosis for future activities aimed at implementing the method in clinical trials.
5. In addition, an interesting result obtained during experiments with a bilayer phantom (solid - liquid) was the observation of fluctuations in normalized time-gated autocorrelation curves. This effect suggests the non-ergodic nature of the sample, which helps in choosing the right Siegert relation for analysis. This effect is discussed in much more detail in a publication in the journal *APL Photonics*, 5(7):071301 (2020) - 5 citations, in which the PhD student is the second author.

Referring to the editorial side of the dissertation, I state that it is at a high editorial level. The language used is precise, logical and concise. Few comments concern the following aspects:

1. General remark: The complete separation of the discussion of results (Chapter 5) from the extensive experimental part (Chapter 4) makes it much more difficult to read the paper, especially since some choices in the subsequent subsections in Chapter 4 are based on the discussion presented in Chapter 5.
2. In four places in the work, including the list of acronyms (p.xiv) and on page 14 (2times) and p.16), the magnitude "n" was named as *reflective* index instead of *refractive index*.
3. On page 26 the *Novel MSR* nomenclature contains an error that should be "*Novel model with modified Siegert relations...."*
4. On page 68 – 8 line from the top – should be TD-DCS

At the same time, studying the dissertation suggests to the reviewer a set of comments of a twofold nature below. The first of them are substantive comments, which, according to the reviewer, are noticed errors or gaps in the work, while the second are comments of a debatable type. The response to the latter category should be a source of constructive discussion with the PhD student during the public defense. At the same time, due to the desire to limit the volume of reviews, the remarks are not preceded by an introduction and, consequently, can be understood only in the context of the work.

Substantive comments:

1. In the abstract and the Polish title, other terminology is used for the diffuse correlation spectroscopy method *:* " dyfuzyjna spektroskopia korrelacyjna " (title) and " spektroskopia korelacji rozproszonej " (abstract) please explain whether both names are correct or rather the name " dyfuzyjna spektroskopia korrelacyjna " should be used.
2. Section 3.4.1: no justification for the choice of emitters (3 emitters) used for time-domain diffuse correlation spectroscopy (TD-DCS) tests.
3. Determining the length of coherence of emitters based on the width of their spectrum can be subject to significant error. Why was the length of coherence not checked by the interferometric method (mentioned inthe work)?
4. Subsection 3.4.2.1: Can the use of different systems supplying radiation to the sample (including optical fibers) affect the result of the IRF (Instrument Response Function) measurement?
5. The description of the experiment and the premises used in the analysis in chapter 4.2.1.4 is too brief, and therefore difficult to trace, e.g. why in Fig. 4.14 a new SR model with two and three exponential members was used to compare the results (in this experiment, SR with 3 Exp. ACF is used as the only one). Please explain.
6. Chapter 6 (*Conclusions*) is very perfunctory, it does not contain conclusions about the preferred hardware configuration and a critical look at the results obtained (what is still missing, what should be improved) and a look into the future. Please complete during the defense.

Debating notes:

1. Please describe in more detail the application objective related to *in vivo* experiments with additives (solid slab).
2. The paper lacks a description of future work necessary to implement the method in clinical applications. Please comment on this.

The above critical and debatable remarks do not undermine the value of the work, which I evaluate positively. The dissertation documents the PhD Candidate's in-depth knowledge in the field of biomedical engineering regarding the essence of the studied physical phenomena and their modeling, factors affecting their course, as well as the possibility of their practical verification. It is a pleasure to study the methodology of conducting an experiment presented in the dissertation, including such elements as: synthetic mathematical analysis of the problem, design of the research system and detailed analysis of the impact of its modules on the measurement, conducting measurements with proper error analysis and physical interpretation of the obtained results. Thus, I conclude that the doctoral student has demonstrated appropriate knowledge and skills aspiring him to obtain a doctoral degree.

As a summary, I hereby state that the submitted dissertation meets the requirements contained in Articles 186 and 187 of the Law on Higher Education and Science of 20 July 2018 (Journal of Laws of 2021, item 478, as amended), both as regards the required scientific achievements, presentation by the PhD Candidate of general theoretical knowledge in the discipline of biomedical engineering, as well as the ability to independently conduct scientific work, and, above all, as an original solution to a scientific problem. In the latter area, the most important from the point of view of the current regulations, I state that the work represents a significant scientific achievement, which is the proposal of a new theoretical model in the time-domain diffuse correlation spectroscopy method (TD-DCS) and validation of this model through extensive experiments based on the TD-SCS system developed by the PhD Candidate and I put forward a motion for its admission as a doctoral dissertation and admission to public defense.